INTERSTITIAL DIAGNOSIS AND TREATMENT OF BREAST TUMOURS

Thesis submitted for the degree of

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by

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ABSTRACT

INTERSTITIAL DIAGNOSIS AND TREATMENT OF BREAST TUMOURS

This thesis exploits the interaction of light with breast tissue for diagnosis and therapy.

Optical biopsy is an experimental technique, based on Elastic Scattering Spectroscopy (ESS), being developed for characterising breast tissue. An optical probe interrogates tissue with a white light pulse, with spectral analysis of the reflected light. 264 spectral measurements (50 patients) were obtained from a range of breast tissues and axillary lymph nodes and correlated with conventional histology of biopsies from the same sites. Algorithms for spectral analysis were developed using ANN (Artificial Neural Network), HCA (Hierarchical Cluster Analysis) and MBA (Model Based Analysis). The sensitivity and specificity for cancer detection in breast and lymph nodes were:

<table>
<thead>
<tr>
<th></th>
<th>Breast cancer</th>
<th>Lymph nodes</th>
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<tbody>
<tr>
<td></td>
<td>ANN</td>
<td>HCA</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>69%</td>
<td>67%</td>
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<tr>
<td>Specificity</td>
<td>85%</td>
<td>79%</td>
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Interstitial Laser Photocoagulation (ILP) involves image guided, thermal coagulation of lesions within the breast using laser energy delivered via optical fibres positioned percutaneously under local anaesthetic. Two groups were studied:

1) Nineteen patients with benign fibroadenomas underwent ILP and the results compared with 11 treated conservatively. Thirteen ILP patients (14 fibroadenomas) and 6 controls (11 fibroadenomas) have reached their one-year review:
<table>
<thead>
<tr>
<th>Patient group</th>
<th>ILP treated</th>
<th>Non treated controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average percentage of original volume at 12 months</td>
<td>18%</td>
<td>64%</td>
</tr>
<tr>
<td>Patient perception at 12 months</td>
<td>Larger = 0%</td>
<td>Larger = 29%</td>
</tr>
<tr>
<td></td>
<td>Same size = 7%</td>
<td>Same size = 57%</td>
</tr>
<tr>
<td></td>
<td>Smaller = 36%</td>
<td>Smaller = 0%</td>
</tr>
<tr>
<td></td>
<td>Gone = 57%</td>
<td>Gone = 14%</td>
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</table>

These differences are statistically significant (P<0.001).

2) Six patients with primary breast cancers underwent ILP (with pre- and post-ILP contrast enhanced MRI) within 3 weeks of diagnosis and were then treated with Tamoxifen. Four underwent surgery at 3 months, two showing complete tumour ablation. MRI was reasonably accurate at detecting residual tumour.

In conclusion: a) optical biopsy is a promising ‘real time’ diagnostic tool for breast disease.

b) ILP could provide a simple and safe alternative to surgery for fibroadenomas.

c) ILP with MRI monitoring may be an alternative to surgery in the management of some patients with localised primary breast cancer
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DEDICATION

To my wife Caroline
CHAPTER 1  BREAST CANCER

1.1  INTRODUCTION

There are around 570,000 new cases of breast cancer in the world each year, of which over 35,000 are in the United Kingdom [Office for national statistics, 1996]. In the UK, incidence is the highest in the world [McPherson et al., 1994] with breast cancer being the leading cause of death in women [Cancer research campaign, 1996]. Breast cancer survival rates are also extremely low in the UK, with a current 5-year survival of 68%. This compares poorly with the rest of Europe (average 73%) [Anonymous, 1999] and the US (average 84%) [Office for national statistics, 1999]. No other cancer produces such an emotive response amongst the general public, due mainly to increased public education and high profile coverage in the popular press of the deaths of not just well known celebrities but also the 'average mother'. With NHS reforms recently announced by the government [Department of Health, 2000] and ever increasing activity of patient advocate groups, attention has been re-focused on all aspects of breast cancer care, causing renewed efforts to improve prevention, detection and treatment. Despite many recent advances however, the cure for breast cancer is still some way off.

1.2  A BRIEF HISTORY

Mention of breast cancer has been found in written texts from as early as 3000 BC (The Edwin Smith Surgical Papyrus) [Breasted, 1930] in which was stated that there was no known treatment for the disease. This sentiment was shared by Hippocrates (484-425 BC) who went so far as to say that treatment actually hastened death. It was not until the second century that thoughts about both the cause and effective treatment of breast cancer where first voiced. Galen (129-200 AD) believed that cancer was due to an excess of black bile in the body, which he called "melancholia". He also was first to liken its appearance to that of a crab with leg-like extensions. Despite his views about a systemic cause, Galen was a proponent of surgical excision of tumours as was Leonidus (180 AD) who described a knife and cautery technique.
Little changed for the next 1000 years until the involvement of the church to discourage surgery (Council of Tours 1162). This lead to a plethora of alternative treatments including compression of the breast with lead plates, laying on of hands, application of goats dung and bleeding from the basilic vein [Donegan, 1995]. The period from the 16th to the 18th century however, saw a move back to surgical treatment. In an age before anaesthesia, efforts where concentrated on a swift amputation, and a variety of traction and cutting instruments were devised.

Along with renewed enthusiasm for surgery, thoughts returned to a possible cause for cancer. In the early 17th century, Descartes (1596-1650) first put forward a lymphatic theory as the origin of breast cancer. Hunter (1728-1793) took up this idea and taught that a systemic injury caused a defect in lymph leading to coagulation. He postulated that from this coagulated lymph, arose breast cancer. It was LeDran however who in 1757, was first to produce the more modern theory that breast cancer began as a local disease and then spread via the lymphatics to regional nodes. This was a major shift from the ‘systemic’ theories and lead to the belief that a cure could be reached by early surgical intervention. Attention was therefore focused on gaining an adequate excision of the tumour and also of the axillary glands. Such surgery was standardised by Halsted who described the radical mastectomy in 1894 [Halsted, 1894]. This included excision of not only the breast and axillary nodes, but also overlying skin and most of the pectoralis major muscle. This was shown to dramatically reduce local recurrence rates and was widely accepted as the operation of choice until as recently as the 1970's when less radical surgery became popular once more.

During the 19th century, various discoveries achieved a greater understanding of the natural history of breast cancer, leading to the introduction of adjuvant treatments and refinement of surgical techniques. In the mid 19th century, the advent of general anaesthetic and antisepsis, radically changed the success of surgery although overwhelming sepsis still caused major mortality. It was not until the mid 20th century however, with the introduction of antibiotics, was sepsis finally reduced to a minimum. Of equal importance was enthusiasm for microscopic and macroscopic pathological assessment, championed by Muller and Virchow. The latter concluded in 1858 that cancer arose from epithelial cells and spread along fascial planes and lymphatics. This added weight to Ledran's local disease theory. Astley Cooper made
another important observation in 1836 when he noted that the growth of breast cancers sometimes fluctuated with the menstrual cycle. It was not until 1896 however that Thomas Beatson reported regression of breast cancers in two women following surgical castration. This was the first systemic treatment for this disease. The last important discoveries of the 19th century were X-rays by Wilhelm Roentgen and other sources of ionising radiation such as uranium by Becquerel, and radium by Pierre and Marie Curie. Such was the rapid investigation of their use, that by 1912, radiotherapy was established treatment both for reducing the incidence of recurrence and also for palliation in advanced disease.

The 20th century saw a gradual move away from radical surgery and also introduction of more sophisticated systemic treatments. It was realised that even with the most extensive extirpative surgery, at least one third of patients still died from their disease. It was evident therefore that some tumours metastasised long before they were clinically evident and that surgery was only useful to gain local control of disease in these patients. This lead to the re-introduction of the “modified” radical mastectomy by Patey [Patey and Dyson, 1948] which spared the pectoralis muscle. With greater sophistication of adjuvant radiotherapy techniques, even this procedure has been superseded by breast conservation surgery with the aim of achieving adequate removal of the tumour with a margin of surrounding ‘healthy’ tissue. Various trials have shown that local excision with radiotherapy does not increase mortality when compared to mastectomy alone [Fisher et al., 1995;Jacobson et al., 1995;Veronesi et al., 1994], although recurrence rates are higher.

The second major advance in treatment in the latter half of the last century was the introduction of adjuvant therapies. Both chemotherapy and endocrine therapies are now standard treatment, and each has made an impact on breast cancer survival. With the explosion of research focused on unravelling the biological complexities of cancer development, growth and metastasis, there is no doubt that this century will bring us ever closer to the ultimate cure.
1.3 Natural History

Despite a rapid expansion in our knowledge of cancer we are still very ignorant of the precise biology of trigger factors, growth, metastasis and recurrence. Even after nearly 2500 years of philosophy, the question still remains as to whether breast cancer, or any cancer for that matter, is a local or a systemic disease. As mentioned in the preceding section, views on this subject have repeatedly swung one way and then the other. To try and answer this question we need to look at the available evidence.

Most people believe that a percentage of women are effectively cured following surgical excision of their breast cancer. This would therefore favour the ‘local’ theory if this statement were correct. Where this argument falls down however is the fact that there is no clear consensus on a definition of the word “cured”. Some would claim that a woman is ‘personally’ cured if she dies from other causes, whereas a ‘clinical’ cure can only be defined as when the mortality rate of a breast cancer population equals that of an age-matched normal population. The problem with this hypothesis however is that probably every clinician involved in the care of patients with breast cancer will remember someone who presented with recurrence 30 or 40 years after the primary tumour. These patients are few and far between, probably because they have died from other causes. Never the less, there is evidence of a time lag between surgery and recurrence which cannot simply be explained by slow tumour growth [Demicheli et al., 1998]. Also longitudinal studies have shown a persistent increased risk in breast cancer death even after a time period of more than thirty years [Brinkley and Haybittle, 1984]. Whether this latency is due to ‘dormant’ tumour cells, or other causes, such as phenotypic drift, genetic predisposition, internal environment or external influences, is open for conjecture and many theories have been postulated [Baum, 1993; Devitt, 1994].

Bernard Fisher has been one of the main proponents of a systemic cause over the past 40 years, and his arguments, through interpretation of large randomised trials, make compelling reading [Fisher, 1996]. There are several conclusions that suggest that breast cancer is a systemic disease at least from its outset, if not before. Each is considered below:
• *Survival does not depend on the extent of surgery:* Several trials investigated the outcomes following mastectomy compared to lumpectomy with or without radiotherapy [Fisher et al., 1995; Jacobson et al., 1995; Veronesi et al., 1994]. The conclusion was that although there was a progressive increase in the risk of recurrence from mastectomy to lumpectomy with radiotherapy, to lumpectomy alone, long-term disease free survival rates were similar. This suggested that death from metastasis, without systemic therapy, was inevitable in a certain group of patients regardless of the local treatment. It was also noted that recurrence almost inevitably led to the appearance of metastasis. The conclusion was therefore that recurrence was a predictor for disease progression but not a harbourer of malignant cells, which could metastasise at a later date. The metastatic deposits must have been already there from the time of the primary tumour.

• *Early versus late surgery to the axilla did not effect survival:* In the NSABP-B04 trial part of the randomisation included either immediate axillary clearance or delayed until disease became evident [Fisher et al., 1985]. Again, no difference in survival was seen, suggesting a predictor role only.

• *Use of systemic treatments increased survival:* Unlike differences in surgical techniques, introduction of systemic treatments did actually produce a survival benefit in the long term, however modest [Anonymous, 1992]. Although even current endocrine and chemotherapy techniques are relatively crude, they have demonstrated that treating breast cancer as a systemic disease from the outset must be the way forward.

• *Node negative patients still die from breast cancer:* Up to 30% of node negative patients will ultimately die from distant metastases [Jatoi, 1999]. Despite some arguments as to micrometastases being missed by conventional histology, it is unlikely that this can account for such a high figure. It is again more likely that distant deposits were present at the outset of the cancer, especially when point 2 (above) is taken into consideration.
The proponents of the old Halstedian view that breast cancer starts locally and spreads in a centrifugal and contiguous fashion, initially found their champion in the results from the screening trials where there was an increase in survival in those screened compared to controls [Kerlikowske et al., 1995]. The argument that long-term breast cancer mortality could be reduced by detecting and treating breast cancers at an early stage seemed to prove the theory that there is a time lag between a cancer developing and subsequently metastasising. Some have argued however that this conclusion does not take into account the increased detection and treatment of in-situ disease amongst the screened population [van Netten et al., 1995]. Theoretically breast cancer may not metastasise until it becomes invasive, therefore the reduction in mortality may simply be due to treating pre-invasive and hence pre-metastatic disease.

Until we have a better understanding of the true biology of cancer, there is no doubt that the argument will continue. Hopefully however, the era of implementing toxic and mutilating treatments without a sound scientific basis, will be left in the past.

1.4 Epidemiology and Risk Factors

The United Kingdom has the highest incidence of breast cancer in the world. Of the 35,000+ new cases in the UK each year, approximately 15,000 will eventually die from their disease. At age 50, the incidence of breast cancer is almost 2 per 1000 women per year [McPherson et al., 1994] and it is estimated that a women’s lifetime risk of developing breast cancer is between 1 in 8 and 1 in 10 in Western countries [Bryant and Brasher, 1994;Feuer et al., 1993]. These staggering statistics are a solemn message not only to those providing breast cancer care but also to the lay population.

There are many risk factors that increase the chance of developing breast cancer. Some of these are pre-determined but some can be altered by the individual. Examples of fixed risk factors include age, family history, age at menarche and menopause, geographical variation and previous benign breast disease. Flexible factors however, are age at first pregnancy, breast-feeding, hormonal manipulation,
diet, weight, exercise, alcohol intake and exposure to ionising radiation. Each will be considered separately below:

- **Age:** Of all risk factors, age has the greatest effect. Breast cancer incidence rises sharply, doubling every ten years until the menopause when the increase begins to slow a little [Feuer et al., 1993]. This means that despite the media coverage of young women with breast cancer, around half of all cases present after the age of 65.

- **Family History:** Around 5-10% of breast cancers arise in those with a family predisposition of which around half are thought to be due to BRCA1 or BRCA2 mutations. Despite these relatively low figures, many attendances to the breast clinic are by concerned women with a family history of breast cancer. Most genetic breast cancers occur before the age of 65, which can help in determining risk for an individual. It is generally accepted that there is a 2-3 fold increased lifetime risk for a woman with a first degree relative who developed breast cancer before the age of 50. This risk is inversely proportional to the age at onset of cancer in the affected relative. Also if 2 first-degree relatives are affected, this risk increases by 4 to 6 times [McPherson et al., 1994].

- **Menstrual history:** Early onset of menarche and late menopause both increase risk [Tavani et al., 1999]. This is thought to be due to the general principle that risk is directly proportional to the oestrogen load, especially when cyclical, as this increases the number of proliferative cycles that the breast epithelium undergoes and hence increases the chance of mutation [Fentiman, 1998a]. Although generally a fixed risk factor, menopause can be influenced by oophorectomy, where the younger the iatrogenic menopause the lesser the risk of later developing breast cancer [Schairer et al., 1997].

- **Geographical variation:** Eastern countries, such as Japan, have approximately a 5-fold lower incidence than Western Countries. Part of this is known to be environmental, as studies of Japanese migrants to Western countries have shown an increase towards the incidence of native Westerners [Buell, 1973].
• **Benign breast disease:** There are many conditions that fall under the umbrella of 'benign breast disease', most of which have no bearing on cancer risk [Devitt, 1981]. Two exceptions however are breast cysts and atypical ductal hyperplasia (ADH), both of which increase risk significantly [Dixon et al., 1999; Dupont and Page, 1985].

• **Age at first pregnancy:** Both nulliparity and older age at first pregnancy increase the risk of breast cancer [Chie et al., 2000]. This was first noticed by the high incidence of breast cancer amongst celibate nuns. There is approximately a 2 times increase in risk after the age of 30 which continues to rise. Interestingly this protective factor has not been found for women with BRCA1 or 2 mutations [Jernstrom et al., 1999] although numbers in this study were small.

• **Breast feeding:** As well as the proven benefits to their child, women who breast feed can benefit themselves by reducing their own risk of breast cancer. This reduction however only seems to last until they reach the menopause [Newcomb et al., 1994].

• **Hormonal manipulation:** The use of combined oral oestrogen and progesterone for contraception has been shown to increase risk, especially if taken by younger women before their fist pregnancy. This increase has however not been seen in women using injectable progesterone only preparations [Shapiro et al., 2000] which may actually confer a benefit. Recent studies have suggested a risk increase for patients taking hormone replacement therapy (HRT) although tumours tend to be of lower grade [Bilimoria et al., 1999; Gapstur et al., 1999]. The question that hasn’t been answered to date however, is if the overall mortality rate for those taking HRT is higher than a control group, especially when the benefits are taken into account. Recent evidence however has shown that contrary to what was previously believed, HRT actually increases the risk of cardiovascular disease as well as the known increase in breast cancer and thrombo-embolism [Writing Group for the Women’s Health Initiative
Investigators 2002]. On the positive side, hip fractures and colon cancer ore reduced.

- **Diet:** Much has been written about dietary influences on breast cancer. Some have suggested that increased dietary fat increases risk although a recent large cohort study [Holmes et al., 1999] and overview [Hunter et al., 1996], have contradicted this theory. What may actually increase risk is excess energy intake, especially from refined bread and pasta [Franceschi and Favero, 1999]. Soya intake in early life, vegetables, beta-carotene, vitamin E, calcium and polyunsaturated fatty acids have all been associated with reducing risk but it is difficult however to determine which compounds in each food actually confer the positive benefit.

- **Weight:** In postmenopausal women, obesity is associated with an increased risk whereas for pre-menopausal women, the opposite is true [London et al., 1989]. What may be more important is recent weight gain rather than static weight [Kumar et al., 1995]. Some have suggested that this increased risk may be due to increased endogenous oestrogens derived from aromatisation of adrenal oestrogens in fat [Fentiman, 1998b].

- **Exercise:** General exercise throughout life will obviously have an effect on weight gain, and hence reduce risk, although there has been an association found between exercise in early life and a reduction in lifetime risk [Marcus et al., 1999].

- **Alcohol intake:** Moderate to excessive alcohol intake is associated with a higher risk although this finding has not been consistent [McPherson et al., 1994]. If a risk exists however, it may be reduced by adequate folate intake which is involved in DNA synthesis [McPherson et al., 1994].

- **Smoking:** There is some debate within the literature with some studies suggesting an increased risk [Band et al. 2002 ][Kropp & Chang 2001] whereas others did not [Lash & Aschengrau 2001]. A recent meta-analysis suggested only a small
increased risk, particularly for pre-menopausal cancers (Relative risk 1.2), and with increasing risk with earlier onset of smoking [Khuder et al. 2001].

- **Ionising radiation and electromagnetic fields:** Increased breast cancer incidence has been found in patients who were exposed to ionising radiation either through multiple medical x-rays for chest disease, or after the Hiroshima and Nagasaki bombs [Fentiman, 1998b]. Although less conclusive, there is some evidence that women who have increased exposure to electromagnetic fields, may have an increased risk [Caplan et al., 2000]. This could have implications for women who are exposed to EMF’s through their occupation e.g. electricity or telecommunications workers.

There are many more, less well-established risk factors, which are too numerous to list in this text. It is evident however, that although statistical significances can be demonstrated, for some of the lifestyle associated risk factors it is difficult to determine which is important, due to much overlap. Women are therefore often given conflicting messages about how best to improve their risk. Ultimately, most minor changes in lifestyle will have minimal benefit compared to the overriding impact of fixed risk factors such as age, genetic predisposition or previous significant benign breast disease.

### 1.5 Pathology

Since the middle of the 19th century, there has been interest in the histological appearance of breast tumours. Initially, pathologists relied on the simple microscope, but now with sophisticated laboratory tests, which can identify specific cellular markers, the accuracy of the histological assessment and the information gained, is rapidly improving. Not only do pathologists assess the breast tumour, but also the metastatic status of the axillary nodes, for which routine excision and sampling still remains standard surgical practice.

The breast is a secretory organ composed of around 15-20 distinct glandular units, each of which has a ductal orifice on the nipple. Each duct branches several times and ends as a terminal ducto-lobular unit. In the non-lactating breast, the ducts and
lobules are lined by cuboidal epithelium that is surrounded by myoepithelial cells. These two layers are separated by a basement membrane from the surrounding connective tissue, fat, lymphatics and blood supply. With each menstrual cycle, a proliferation of the cuboidal cells is seen which subsides following menstruation. During pregnancy however, the epithelial cells of the lobules undergo marked hypertrophy in preparation for milk production. After the menopause, diffuse atrophy of the lobular units occurs to the point where the breast consists almost entirely of fat and loose connective tissue. The timing and extent of this involution is however extremely variable between individuals and with the widespread use of hormone replacement therapy is often arrested, or even reversed.

Many different types of breast malignancy have been identified and described, from in-situ disease to aggressive, invasive cancer. Most breast cancers (approximately 90%) are either lobular or ductal in origin of which the latter makes up the greatest proportion (around 80-90%). The remaining 10% of malignant types are a collection of rare pathologies and will be considered briefly at the end of this section. Each tumour is further subdivided into either invasive or in-situ cancer and graded according to mitotic counts and pleomorphism. Many cancers however are composed of more than one tissue type and often in-situ and invasive cancer co-exists.

The importance of histopathological assessment of breast tumours is two-fold. Firstly, to keep recurrence to a minimum, adequate resection must be reached and if tumour margins are positive i.e. the tumour extends to the resection margin, further surgery can be directed to remove more tissue in a certain plain. Secondly, the tumour type, grade and lymph node status has a bearing on prognosis and whether any adjuvant treatments are appropriate.

1.5.1 In-situ cancer
These are areas within the breast, composed of cancerous cells that have not yet invaded through the basement membrane. Before the introduction of mammographic screening, these tumours comprised around 5% of the total, as they are usually impalpable and hence asymptomatic. They now however comprise around 15-20% of all patients with breast cancer as their presence is often only discovered by the
presence of microcalcification. Two main types exist; Ductal (DCIS) or Lobular carcinoma *in situ* (LCIS):

DCIS arises in the terminal duct lobular unit distending the ducts as it grows. There are two sub-types, comedo or non-comedo, which may again be interspersed. Comedo type DCIS grows in a solid manner and is characterised by central necrosis. It often also incites a surrounding inflammatory response, sometimes producing a palpable lump. Non-comedo type tends to have cells that are less irregular and have smaller nuclei i.e. lower grade. Necrosis is typically absent and overall this type is less aggressive and is therefore less likely to recur if surgically resected. The natural history of DCIS is still poorly understood as but it is evident that not all DCIS becomes invasive. This makes treatment options difficult, especially as it is often extensive, covering a large portion of the breast, which therefore necessitates mastectomy.

LCIS tends to have cells that are smaller and more regular than its ductal counterpart, and generally does not produce an inflammatory reaction. Microcalcification is also less common and it is therefore generally only found as an incidental finding in pathological specimens. Its natural history is even less well known and is generally considered a risk factor rather than a precursor for malignant disease [Simpson and Page, 1992] and extensive surgery is therefore deemed inappropriate.

### 1.5.2 Invasive Carcinoma

As the name suggests, invasive or infiltrating carcinoma extends through the basement membrane into the surrounding tissues. It also has gained the ability to metastasise although the point at which this happens is hotly contested (see above). There are two main types, again ductal and lobular.

Infiltrating ductal carcinoma (IDC) is by far the most common of the two and is composed of irregular nests of epithelial cells, often with surrounding fibroblastic proliferation. These tumours therefore often present as a palpable lump. Cytologically the cells are no different to DCIS and also vary in their level of
differentiation. Necrosis is not a common feature except in poorly differentiated, rapidly growing tumours.

Invasive lobular carcinoma (ILC) may occur alone or mixed with IDC. The amount of fibrosis is less variable. The characteristic microscopic features are of single strands of malignant cells, infiltrating between stromal fibres. This feature is termed 'Indian filing'. A fibrotic reaction is not as common and therefore it often presents more as a diffuse, indurated area. This can sometimes make it difficult to pick up clinically. Biologically, it is just as aggressive as IDC, but this also depends on grading.

1.5.3 Less common types of invasive carcinoma

There are several other types of invasive breast cancer that are generally referred to by a name describing their microscopic appearance.

- **Colloid (mucinous) carcinoma**: This tends to occur in older women and is composed of clusters of epithelial cells, surrounded by extracellular mucin.

- **Tubular carcinoma**: Composed of well-differentiated cells only one or two layers thick, which form infiltrating duct-like structures.

- **Medullary carcinoma**: Macroscopically it appears as a well-circumscribed, pale grey mass. Microscopically it is composed of sheets of highly pleomorphic cells with a lymphoid infiltrate at the periphery.

- **Metaplastic carcinoma**: This very rare tumour is composed of malignant epithelial cells that have undergone partial differentiation and may therefore show areas of malignant squamous, fibrous, cartilaginous or bony tissue, mixed with a malignant glandular component.

- **Malignant Phyllodes**: This is a malignant variant of a benign tumour which resembles a fibroadenoma (see chapter 6). The distinction is made by a hypercellular stroma with a high mitotic count. Sometimes a distinction between
benign and malignant can be difficult as there is wide spectrum in mitotic activity, but when there is an obviously sarcomatous stroma, it is deemed malignant.

Other miscellaneous tumours are true sarcomas such as angiosarcoma, fibrosarcoma and malignant fibrous histiocytoma. Rarely, lymphoma or secondary tumours appear in the breast. For most of the tumours outlined above, the prognosis is generally better than for IDC or ILC and often an 'apparent cure' can be reached with surgery alone [Simpson and Page, 1992].

1.5.4 Prognostic factors

Through pathological examination of excised tumours it is possible to reach a prognosis for an individual patient although there are many aspects that can help to decide this. By far the most important in breast cancer is the stage, or extent of spread of a tumour. Standard staging is from I to IV, which encompasses tumour size and extent of local, regional or distant spread. The higher the stage, the worse the prognosis (table 1.1).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>5 year survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Tumour &lt; 2cm with no direct extension or nodal metastases</td>
<td>84%</td>
</tr>
<tr>
<td>II</td>
<td>Tumour 2 to 5cm, with or without nodal metastases</td>
<td>71%</td>
</tr>
<tr>
<td>III</td>
<td>Tumour &gt; 5cm, or fixed to pectoralis muscle or fixed nodes</td>
<td>48%</td>
</tr>
<tr>
<td>IV</td>
<td>Any tumour fixed to chest wall (ribs or intercostal muscles) or involving skin, or distant metastases</td>
<td>18%</td>
</tr>
</tbody>
</table>

Table 1.1 – Simplified staging based on TNM classification where T= tumour size, N= presence of axillary nodes and M= presence of metastases. [Miller et al., 1994]
There have been many attempts at producing a more accurate prognosis through incorporation of other factors such as histological grade, oestrogen receptor status, proliferative capacity, and lymphatic or vascular invasion. The best validated of these is the Nottingham prognostic index which uses a scoring system for histological grade, oestrogen receptor status, site of initial metastasis (SIMD) and disease-free interval [Robertson et al., 1992]. More recently, there has been much interest in identifying certain genetic abnormalities that have a bearing on tumour growth, such as C-erb B2 (Her-2), epidermal growth factor receptor (EGFR) or p53.

Histological grade is however more widely accepted and understood, and is a measure of the differentiation of a tumour i.e. its resemblance to the original cell type. It is based on quantification of three factors: (1) the degree of glandular differentiation, (2) the degree of nuclear atypia and (3) the mitotic index [Elston, 1987]. Each is scored out of three and the sum of which gives an overall grade where 3 to 5 is well differentiated, 6 to 7 is moderate, and 8 to 9 is poorly differentiated. In general, the more undifferentiated, the more aggressive the tumour, and hence the worse the prognosis.

As pathological analysis becomes ever more sophisticated, further subtyping of tumours is set to continue. Prognostic indicators are undoubtedly interesting from an academic point of view, and are sometimes helpful in directing treatments. However, percentage survival figures for an individual patient based on her tumour characteristics often become meaningless as ultimately at present, survival time depends on good luck rather than any other factor.

1.6 Sentinel Nodes

It has been well validated that the presence of axillary lymph node metastases is the single most important prognostic factor in women with breast cancer with the overall incidence of axillary metastatic disease approaching 50% [Carter et al., 1989]. This incidence has however been shown to be directly related to the size of the tumour [Walls et al., 1993]. The optimal treatment of axillary disease has always been controversial but through large randomised trials such as NSABP-B04, delayed treatment has been shown to have no effect on overall survival [Fisher et al., 1985].
It is therefore now accepted by most, that surgical removal of non-clinically apparent metastatic nodes is purely for prognostic purposes only.

Axillary lymph node clearance has been the mainstay of surgical treatment for breast cancer until relatively recently. Unfortunately it carries significant morbidity especially when combined with radiotherapy. Such complications range from the relatively minor, such as haematoma, stiff shoulder or paraesthesia, to the more debilitating arm lymphoedema. There has therefore been a move to try and avoid such surgery for the 50%+ of patients who are lymph node negative. Initially an effort to achieve this was through lymph node sampling, where only a few lymph nodes were removed (usually 4 or more) from the lower axilla to achieve staging [Steele et al., 1985]. The proponents of this technique demonstrated it to be reliable both for initial assessment of the axilla and also for controlling local disease through the addition of axillary radiotherapy when lymph node metastases were found [Forrest et al., 1995]. Even more recently however, interest has been directed towards sentinel lymph node biopsy, which aims to detect, remove and histopathologically assess the first node to which a particular breast cancer is most likely to metastasise.

The concept of the sentinel lymph node (SLN) was first introduced by Cabanas in 1977 for the management of penile cancer [Cabanas, 1977] and later by Morton for melanoma [Morton et al., 1992]. The theory is that the first lymph node to receive lymphatic drainage from a tumour should be the first site of lymphatic spread and therefore removal of this node alone should serve as an indicator of lymph node status. Only relatively recently has research been directed into its use for the management of breast cancer. Three main techniques for identifying the SLN in breast cancer have been described:

- Injection of visible dye such as patent blue or isosulphan blue to outline the lymphatic vessels and lymph nodes during surgery.

- Lymphoscintigraphy following local injection of radionuclide (usually technetium-99m-labelled antimony sulphate or colloidal albumin) to pre-operatively map lymphatic drainage.
- Local injection of radionuclide and use of a hand held gamma probe during surgery to help direct dissection.

The main concerns with sentinel lymph node biopsy (SLNB) are the possibilities of failed detection of the correct lymph node and therefore false negative biopsies i.e. the sentinel node is negative but other axillary nodes are positive. It also cannot be assumed that the if the sentinel node is negative, the remainder of the nodes will be negative, as the presence of 'skip lesions' have been well documented [Roche et al., 1997] i.e. normal level 1 nodes but metastatic level 2 or 3 nodes. This may simply be due to abnormal lymphatic pathways. Such aberrant drainage channels may become better understood as experience in lymphoscintigraphy accrues. To test the efficacy of each method of detection, initial study designs included routine axillary dissection following SLNB. Some investigators have suggested that using a combination of both blue dye and radioisotope can increase the yield and accuracy [Hill et al., 1999]. A recent review of various trials however, suggested that scintigraphy and/or use of a gamma probe was much more accurate than blue dye alone. The optimum method however was a combination of dye and radioisotope techniques [McIntosh and Purushotham, 1998]. Numbers of patients however were generally smaller than in the Hill study. The findings of this review are summarised in table 1.2. Experience of all of these techniques is still fairly limited and it is clear that there is a definite learning curve for the surgeons involved, but undoubtedly with further experience, these figures can only improve further.

Further support for SLNB lies within the ability to target the most important nodes for thorough histological analysis. Detection of lymph node metastases has until recently relied mainly on standard single sectioning and H&E staining. It is clear however that multi-sectioning and the addition of immunohistochemistry techniques can identify otherwise occult metastases. The importance of such micrometastases has been hotly debated but a recent review article concluded that conventional techniques will underestimate nodal involvement and patients with micrometastases have a reduced overall survival [Dowlatshahi et al., 1997]. As these techniques are expensive and laborious, it is impractical to assess every excised axillary lymph node in this way. SLNB therefore offers the chance to focus valuable resources at
thorough assessment of one or two nodes only. The small false negative rate of SLNB may therefore be compensated for by the increased detection of otherwise occult disease.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Number of studies</th>
<th>Detection rate (mean)</th>
<th>False negative rate (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue dye alone</td>
<td>5</td>
<td>66-92% (73.8%)</td>
<td>0-17% (11.4%)</td>
</tr>
<tr>
<td>Gamma probe alone</td>
<td>2</td>
<td>82-98% (90%)</td>
<td>0% (0%)</td>
</tr>
<tr>
<td>Scintigraphy and gamma probe</td>
<td>4</td>
<td>69-98% (88.3%)</td>
<td>0-5% (2.8%)</td>
</tr>
<tr>
<td>Scintigraphy and/or gamma probe</td>
<td>6</td>
<td>92-100% (93.7%)</td>
<td>0-15% (4%)</td>
</tr>
</tbody>
</table>

Table 1.2 - summary of SLNB studies

At present, no centres in the United Kingdom are routinely using SLNB. However, a substantial number of breast surgeons have gained enough experience in the technique to progress past the learning curve, with detection rates approaching 100% and 1% or less false negative rates. The next stage therefore is to test the safety of treating patients on the basis of SLNB alone. To this end, randomised trials are shortly about to commence where adjuvant treatment will be based either on SLNB or standard axillary node dissection. Providing there is no difference in disease free or overall survival between the two groups, SLNB will undoubtedly become routine, especially as the morbidity from this procedure should be considerably less when compared to a full axillary dissection.
CHAPTER 2  CANCER DIAGNOSIS

2.1 INTRODUCTION

Breast cancer patients present themselves to the clinician with a variety of different symptoms. Far and away the most common of these is the presence of a breast lump, although other presenting complaints include nipple discharge, skin rash, pain or symptoms from metastatic disease. It is generally accepted however that only one in ten patients attending a breast clinic in the UK, with any of the above symptoms, will have breast cancer. Although better awareness of breast cancer through implementation of public education projects is desirable, this has inevitably led in the short term to increased patient anxiety and therefore greater numbers of women presenting to their doctor with breast problems. This has undoubtedly increased the ratio of benign to malignant disease presenting to specialist breast clinics adding pressure to the already over-stretched service. With individual counselling and further drives on health education, patients will hopefully become even better informed and perhaps will be able to discriminate between sinister and non-sinister symptoms.

This level of public education is however some way off and therefore in an attempt to keep pace with the increasing number of referrals, most breast clinics in the UK are striving to increase efficiency and still provide an ever-improving service to the patient. This has been achieved by a well-established multi-disciplinary approach to the diagnosis and treatment of breast cancer and the creation of ‘one-stop clinics’. At such clinics, in the space of a morning or afternoon, patients can see a specialist, be examined, have appropriate investigations and be given a diagnosis. They then can either reassured if benign, informed of further investigations such as excision biopsy, or for the unfortunate few with breast cancer, be given preliminary counselling with a plan for surgery and other possible treatments. Such a service relies on input from several medical disciplines including surgeons, radiologists and pathologists. Also, in a central role, is the clinical nurse specialist who will provide not only advice and comfort but also be an important contact at all stages during treatment.
2.2 CLINICAL PRESENTATION

As mentioned above, patients present to their doctor with a variety of symptoms. As with any patient, a careful history is important as the first stage of the consultation. Particular questioning about menstrual history, parity, breast-feeding, hormone replacement therapy and oral contraceptives are always obtained as these have some bearing on the risk of breast cancer (see chapter 1). Perhaps however of greatest importance is a family history of breast cancer, especially first-degree relatives who developed the disease before the menopause. This has by far the greatest bearing on a patient’s lifetime risk of developing breast cancer.

With regards to symptoms, elaboration in the history can often help to reach a level of suspicion even before examination and investigation takes place. Each presenting complaint is considered below:

- **Breast lump:**
Most women will develop a breast lump at some time in their life. The vast majority of these however are thankfully benign. Most commonly, patients present with an area of "lumpiness" rather than an individual lump. This often becomes more prominent pre-menstrually and is usually situated in the retro-areolar region or the upper outer quadrant of the breast [Haagensen, 1986]. Examination reveals an area of nodularity, which is of different consistency to the rest of the breast and is frequently bilateral.

Another common cause for seeking medical advice is during pregnancy, especially for the primigravida, when the breast can become very unfamiliar with increased volume and firmness. Different parts of the breast can hypertrophy to differing extents and this can often give the impression of a lump. Most of the benign conditions (see below) can also become more prominent as they are often hormonally dependent. Also related to pregnancy, in post-partum patients who are breast-feeding, development of a painful lump, with overlying erythema of the skin, is almost invariably a breast abscess.

The finding of a discrete lump should always raise suspicion although in pre-menopausal patients, most of these are also non-malignant. For common benign
conditions, the age of the patient has a bearing on the likelihood of a particular diagnosis. Fibroadenomas, which are benign condensations of glandular and connective tissue, are prevalent up to the age of 35. Breast cysts however, are more common after this age and until the menopause. Both of these conditions classically present as a smooth mobile lump, most of which are between 2 and 4 centimetres in diameter. Occasionally however they can become much larger than this, and may increase in size at an alarming rate. There is also a myriad of other benign conditions, detailed description of which lies beyond the scope of this thesis. These range from what are thought to be normal variations in breast development, often with non-scientific names such as ‘fibroadenosis’, ‘sclerotic breast disease’ or ‘fibro-cystic disease’, to conditions that predispose to breast cancer such as ‘atypical ductal hyperplasia’. Often these diagnoses are not reached until after excision biopsy.

In post-menopausal ladies, the presence of a new discrete lump should always be considered to be malignant until proven otherwise. Despite the constant reports in the popular press of young (and often famous) patients dying from breast cancer, it remains very much a disease of the elderly [Feuer et al., 1993]. However, with the increased prescribing of hormone replacement therapy, benign breast lumps may become more common in post-menopausal patients.

Despite confidence in a benign diagnosis, any patient presenting to a breast clinic with a discrete breast lump should have further investigation by means of ‘triple assessment’. This combines clinical examination, radiological imaging, and histological assessment either by fine needle aspiration cytology (FNAC) or core-cut biopsy. Even those patients with what may be considered to be benign nodularity should at least have either ultrasound or mammographic assessment to exclude an underlying discrete mass.

• **Nipple discharge:**
Most nipple discharges are considered to be ‘physiological’ and can range from being serous and relatively clear, to viscous and creamy. Occasionally patients present with a green, foul smelling discharge, which is usually due to intra- or peri-ductal infection. Most of these will settle down by themselves or after a short course of antibiotics. Any persistent discharge needs further investigation as outlined below.
Blood-stained discharge however, raises the possibility of an underlying malignancy and every effort should be made to exclude this including mammography and cytological assessment of a smear taken from the nipple following expression of the fluid. Thankfully, the underlying cause is commonly a benign intra-duct papilloma although this diagnosis is often not reached until after surgical exploration of the ducts.

- **Changes to the skin or nipple:**

  Patients are often incorrectly referred to a breast clinic with simple dermatitic rashes or other epidermal lesions such as skin tags or change in a mole. Also they may present with intra-dermal lesions such as sebaceous cysts. For the experienced breast clinician these can easily be dealt with and do not require the full gamut of breast investigations. Features which can however signify an underlying breast cancer are puckering or dimpling of the skin and ‘peau d'orange’. This is where the skin is oedematous and slightly discoloured resembling the skin of an orange, hence the name. Occasionally patients present very late with a fungating breast cancer, which either appears as a skin ulcer with an underlying breast lump, or as an irregular, discoloured (and often bleeding) mass protruding through the skin. Diagnosis on these occasions is straightforward.

  An area on the breast where an otherwise innocuous rash does cause concern is on the nipple. ‘Paget’s disease’ is a scaly rash radiating from the nipple onto the areola and is due to an underlying intra-ductal carcinoma spreading along the milk ducts and onto the skin. The rash often bleeds and can be pruritic. Although a nipple rash is still most commonly due to benign skin conditions such as eczema, all patients need thorough investigation. This includes mammography and incision biopsy of a wedge of nipple, or adjacent skin containing the rash, for histological investigation.

  Change in the size or shape of the nipple can also be due to breast cancer, most commonly nipple retraction or inversion. This is due to the fibrotic response around a cancer that pulls the nipple into the breast. An ‘inverted nipple’ can however be an entirely normal finding in around 5% of the population. It is only when a normally everted nipple, becomes inverted ‘de novo’ that investigation is necessary with
mammography or ultrasound. Often no cause is found for the retraction although occasionally it can be due to a fibrotic reaction following a mild peri-ductal mastitis. Enquiry of the patient about recent weight gain can also provide an explanation as when the breast enlarges with fat, the nipple can sometimes become enveloped and give the impression of retraction.

- **Breast Pain:**

  Despite popular opinion, most breast cancers are painless, but the common misconception that cancer causes pain leads to many consultations. The most common presentation of breast pain is a young, pre-menopausal woman with cyclical breast pain, which is worst just before menstruation. Rarely any investigation other than clinical examination is necessary and patients can generally be reassured. The majority of these patients will respond to Evening Primrose Oil, which has been shown to be successful in up to 97% of patients with no serious side effects [Cheung, 1999; Gateley et al., 1992].

  Despite the above statements, any patient with non-cyclical breast pain, especially when unilateral and in a post-menopausal woman, needs investigation for underlying malignancy. If no breast lump is palpable, mammography is all that is required and thankfully for the majority of patients no cause can be found and the pain is usually self limiting, especially after reassurance.

- **Metastatic symptoms:**

  The metastatic course of breast cancer is outlined in detail in chapter 1 but in summary, a breast cancer either spreads through local lymphatics, especially to the axillary nodes, or by haematogeneous spread, most commonly to bone, brain, lung or liver. Patients presenting to their General Practitioners with symptoms relating to these other organs should be referred to the appropriate specialities for initial investigation but it is appropriate to refer female patients with axillary lymphadenopathy to a breast clinic. The only proviso to this is in patients with bilateral axillary nodes, widespread enlarged nodes in the neck or groin, patients who have recently had a viral illness, or patients with signs and symptoms of haematological pathology such as fatigue, weight loss or an enlarged spleen or liver.
Initial consultation should enquire about recent viral illness, trauma to the breast or arm, or any new skin lesions on the breast or arm. Investigation includes breast examination, examination of the relevant upper limb for unnoticed injury or suspicious skin lesions, mammography in the older patient, and FNAC of the lymph node. Occasionally excision biopsy is warranted especially if the node continues to enlarge. If an occult breast lesion is found, this should obviously be investigated further.

Rarely a metastatic lymph node is found without any obvious primary tumour. This can cause great difficulty not only as a diagnostic challenge but also understandably for the patient who is as much in the dark as the clinician. This can be very unsatisfactory and rigorous investigation in search of a primary should be implemented quickly. Such investigations can include chest X-ray, whole body CT scanning and more recently Magnetic Resonance imaging of the breast and Positron Emission Tomography (PET). These will be discussed in more detail below.

2.3 Radiological imaging

Most patients attending the breast clinic with any of the above symptoms will require some form of breast imaging following the initial history and examination, as even the most experienced clinician will not have 100% diagnostic accuracy by examination alone. Also, somewhat unscientifically, a negative ultrasound or mammogram often serves as an added ‘reassurance’ to the patient. Although Magnetic resonance imaging (MRI) and Scintimammography are becoming more commonplace with an explosion of research concentrating on their merits, these are still very much second line investigations. At present however, for the routine investigation of breast symptoms, the mainstays remain mammography and ultrasound scanning. Each of these modalities has their relative merits and limitations and will be considered separately.

2.3.1 Mammography

Radiography of the breast, or mammography, was first described in Germany in 1913 [Salomon, 1913] but was not routinely employed until the early 70’s following
introduction of low-dose, film screen technology [Gold, 1992]. The principle mammographic views used today are mediolateral oblique (MLO) and cranio-caudal (CC). Although not precisely perpendicular to each other, they are complimentary by giving information in two planes. An MLO view also causes the breast to fall forward allowing examination of the axillary tail and deep portion of the breast. During mammography, compression is used to hold the breast motionless, separate parenchymal densities and reduce radiation dose by decreasing the breast thickness. Further views can be obtained if necessary including a true lateral, magnification views or spot compression. This last technique is used to further separate tissues allowing differentiation between a true mass or a composite shadow i.e. an apparent mass created by several areas of slightly denser breast tissue lining up in the plane of view. Although most patients admit to some discomfort during the procedure (caused by compression) rarely is it frankly painful, and with modern techniques radiation dose is kept to a minimum (2.3-4.6 mGy per breast) [Burch and Goodman, 1998; Suleiman et al., 1999].

Interpretation of mammograms is a skilled procedure and accuracy of diagnosis has been shown to be directly related to experience [Ciatto et al., 1996; Elmore et al., 1998; Nodine et al., 1999]. The main disadvantage of mammography is that interpretation becomes more difficult when the breast consists mainly of fibro-glandular tissue i.e. younger women. For most patients under 50 years old, the breasts tend to be more radio-opaque making mammography less accurate at detecting cancer [Edeiken, 1988]. For older women however, mammographic accuracy in experienced hands has been reported to be as high as 93% [Eltahir et al., 1999]. Two-view mammography also increases diagnostic accuracy although for breast screening, single MLO views are often used with a subsequent CC view obtained only if there is any suspicion on the former (see section 2.5).

Radiologically, breast cancer appears as a mass, clustered microcalcifications or both. The mass is almost invariably irregular with a spiculated margin due to the infiltrative process. There is often associated distortion of the surrounding parenchyma which can occasionally be the only indication of underlying pathology in the absence of a mass. On the other hand, well-circumscribed masses are nearly always benign, especially if surrounded by a radiolucent halo. Scattered calcification
seen on mammography is also generally benign, however when clustered, and irregular in shape, margin and density, malignancy must be excluded by biopsy. Occasionally, calcification can appear to follow the line of the ducts often branching as it maps out a breast lobule. This appearance usually indicates ductal carcinoma in situ (DCIS). Of all microcalcification associated with malignancy, many are due to DCIS, therefore it is considered an important sign for early, and potentially curable, breast cancer.

2.3.2 Breast ultrasound

Over the past decade, the use of breast ultrasound (US) has increased dramatically and is now an integral part of routine investigation of breast disease. It was first described by Wild and Neal in 1951 [Wild and Neal, 1951], although was initially thought of only as a tool to discriminate between solid and cystic lesions [Jackson, 1990]. However, with the introduction of high-frequency linear-array transducers and computer enhanced imaging, ultrasound is becoming increasingly recognised as having potential beyond its originally intended use [Staren and O'Neill, 1998]. It is widely accepted however that in it’s present form, it has no potential as a tool for screening. The main reasons for this are that it is time consuming to scan both breasts in their entirety, is costly and impractical to produce a comprehensive set of ‘hard copies’ and it does not easily pick up microcalcification. It is also highly operator dependent and not only has a higher false negative rate compared to mammography but also a high false positive rate leading to unnecessary extra, and often invasive investigations [Teh and Wilson, 1998].

Ultrasound does however have many important uses, with its main role still remaining the further evaluation of clinical or mammographic abnormalities, especially delineating between solid or cystic lesions. This is especially true for younger women who have mammographically dense breasts where ultrasound is often the only imaging modality used to investigate clinical abnormalities. In patients who have diffuse nodularity that appears only as a dense region on mammography, ultrasound can exclude an underlying discrete mass if the area in question is found to be homogeneous. Another useful application is in the assessment of a nipple discharge where fluid filled, dilated ducts can be visualised leading to the diagnosis
of duct ectasia. Occasionally, in experienced hands, intra-ductal lesions can sometimes be visualised. Ultrasound has also been evaluated for investigation of women with possible rupture of breast implants, with some success [Azavedo and Bone, 1999], although MRI is felt to be the most useful for this purpose (see below). Despite the varied uses of ultrasound outlined above, probably the greatest advantage over mammography is in image-guided biopsy where it has become the method of choice. This is due to several factors including ease of use, patient comfort and real time imaging. This is discussed further below (section 2.4.4).

Over the past few years much has been written about the ever-increasing ability of ultrasound to discriminate between benign and malignant lesions. These include the use of doppler US to determine patterns of vascular distribution [Raza and Baum, 1997] and improvements in imaging allowing discrimination by certain characteristics such as tumour margin, depth/width ratio and acoustic shadowing or enhancement [Lamb et al., 2000]. Some investigators have looked at the impact these discriminatory factors have on achieving an added benefit to mammography in the diagnosis of breast cancer. A recent paper found that ultrasound correctly diagnosed cancer in over 40% of patients who had inconclusive findings on mammography [Skaane, 1999]. Work is ongoing to further validate these methods and also the relatively recent introduction of vascular enhancing agents such as micobubbles [Kedar et al., 1996], may help improve sensitivity and specificity for lesions which are difficult to assess with current methods.

There is no doubt that ultrasound has made a huge impact on the investigation of breast disease over the past decade. With rapid development of equipment leading to improved image quality, it is set to have an expanding role. Further training of not just radiologists, but also breast surgeons and specialist radiographers, will inevitably continue to improve the level of service given to patients due to the speed and versatility of ultrasound in both diagnosis and as an aid to treatment.

2.3.3 Magnetic Resonance Imaging

The use of Magnetic Resonance Imaging (MRI) of the breast is gaining popular support, but is still mainly limited to large research centres in the UK and is not yet
considered ‘standard’ investigation. Over the past few years however, it has
developed an increasing role for investigating the ‘difficult breast’ i.e. a patient who
has equivocal findings using conventional methods. There has also recently been a
drive to try and standardise breast MRI with the aim of introducing some quality
assurance measures and guidelines with regards to its use [Anonymous, 1999b].

Unlike mammography and ultrasound that rely mainly on variable densities to
differentiate between normal, benign and malignant tissue, breast MRI utilises the
difference in vascularity between such tissues following the administration of an
intravenous contrast agent such as dimeglumine gadopentate (Magnevist®). It is well
validated that invasive tumours rely on new vessel growth (neovascularisation), in
order to increase in size [Folkman et al., 1989]. These new vessels develop under the
influence of various angiogenic growth factors e.g. vascular endothelial growth
factor, VEGF [Locopo et al., 1998]. By introducing an agent into the circulation
which produces an increased signal intensity in tissue that has a rich vascularity e.g.
breast cancer, it is possible to make tumours ‘stand out’ from the background. Breast
cancers therefore generally appear bright on contrast enhanced T1-weighted images.
Unfortunately however, some benign lesions, such as fibroadenomas, also have
increased vascularity and therefore can also appear as an enhanced lesion.

There has therefore been much work aimed at increasing the accuracy of MRI not
only for the initial detection of breast lesions but also in differentiating between
benign and malignant tumours. A plethora of MR techniques have been tried and
there is still much discussion as to which imaging parameters are best [Weinreb and
Newstead, 1995]. Opinions are divided in a variety of areas including choice of
contrast agent, pulse sequences, orientation of plane, post processing techniques and
whether both breasts are imaged together or individually. There is also variation in
imaging equipment including magnet field strength and use of dedicated breast coils.

Despite these ‘differences in opinion’ there is no doubt that breast MRI is extremely
good at detecting breast cancer with most studies reporting a sensitivity of over 95%
[Davis and McCarty, Jr., 1997;Ercolani et al., 1998;Heywang-Kobrunner et al.,
1997]. Specificity can however vary greatly and has been reported as anywhere
between 37% and 91% [Drew et al., 1999;Harms et al., 1993]. With further
advancements in both hardware and software to improve image quality and increasing experience of radiologists, these figures may further improve.

There are several clinical situations where interest in the possible use of breast MRI is most concentrated. These indications are however undoubtedly set to expand as further validation is achieved:

- **Investigation of women with equivocal findings on mammography or ultrasound:** Occasionally, a 'suspicious' area is seen on mammography in only one view therefore making conventional biopsy impossible. Where ultrasound is unhelpful, MR imaging may have a role in helping to either confirm or exclude an underlying breast cancer or other breast pathology [Lee et al., 1999].

- **Follow up of breast cancer patients who have undergone breast conservation surgery and radiotherapy:** In this situation, both clinical examination and mammography have traditionally been difficult to interpret due to scarring and radiation change. MRI may be more sensitive in picking up recurrence in patients where there is clinical suspicion [Mumtaz et al., 1997], but its use as a blanket screening tool has been questioned [Coulthard et al., 1999].

- **Evaluation of the breasts of women with silicon implants:** With the increased popularity of breast augmentation and reconstructive procedures following breast cancer surgery, the incidence of breast cancer patients with silicon implants is set to rise. Mammography in these patients is generally difficult and also less accurate than for the general population as the implants are radio-opaque causing some of the breast to be obscured. Ultrasound has been found to be a useful adjunct to mammography in these patients [Carlson et al., 1993]. With MR imaging creating a cross sectional view, the implant can easily be differentiated from the 'natural' breast. MR also has an increasing role in assessing implants for possible rupture [Harms, 1998; Middleton, 1998].

- **Screening of high risk women:** There has been for a long time, much debate as to the best way to achieve early and reliable detection of breast cancers in young
women at high risk due to family history. This is especially true for those women
carrying a predisposing genetic mutation such as BRCA1 or BRCA2. This debate
has mainly been fuelled by the low sensitivity of mammography in the younger
patient due to the relative density of the breast caused by glandular tissue
[Edeiken, 1988]. MR imaging has therefore been proposed as a possible method
of screening these women in an attempt to detect early and therefore potentially
curable cancers [Orel and Schnall, 1999]. Multi-centre trials to test this
hypothesis are currently underway in England, the United States, Canada and
Holland. A recent report has shown that MR imaging is more accurate in
detecting breast cancer than mammography in these ‘high risk’ patients [Kuhl et
al., 2000], although it will be some time before it is known if there is a true
survival benefit. Also there is a very real question as to whether the results of
these trials can be transferred to the general population.

- **Evaluation of extent of disease:** Although triple assessment has a high sensitivity
  for diagnosing breast cancer, mammography and/or ultrasound is not reliably
  accurate in determining the extent of the tumour. MR imaging may therefore
  have a role in evaluating multifocality or multicentricity in patients selected for
  breast conservation surgery. This may have an impact on therapeutic approach
  [Conrad et al., 1999; Fischer et al., 1999], the subsequent incidence of local
  recurrence [Davidson et al., 1997] or re-excision rates due to positive tumour
  margins.

- **Determining the early effect of primary chemotherapy:** For patients who are
  undergoing primary (neoadjuvant) chemotherapy, it is sometimes difficult to
  assess the clinical response with either clinical examination or mammography.
  Some researchers have claimed good accuracy in determining the extent of
  residual disease following such treatment [Abraham et al., 1996]. This may be of
  use in determining the subsequent surgical approach i.e. mastectomy versus
  breast conservation surgery.

There is little doubt that breast MR imaging will continue to be used in the
evaluation of breast cancer for a selected group of patients. At present however, there
are many questions still to be answered with regards to its true accuracy in some of
the clinical situations mentioned above. Also standardisation of not only equipment and imaging protocols but also (and probably most importantly) the training and subsequent experience of radiologists, is still a long way off. Another major problem is development of MR guided biopsy systems for sampling areas of enhancement that cannot be seen with either mammography or ultrasound. Only recently have ‘open access’ breast coils become more readily available with the newer generation magnets. Such attachments however are not available for every existing MRI. There are also still problems with needle artefacts and rapid loss of contrast enhancement [Heywang-Kobrunner et al., 2000]. Once these problems have been ironed out, and the cost effectiveness calculated, MR imaging of the breast may become as routine an investigation as the other imaging modalities. At present however, the high accuracy of mammography and ultrasound will continue to be invaluable in the detection and evaluation of breast cancer.

2.4 BREAST BIOPSY

Along with clinical examination and radiological imaging, biopsy makes up the third part of the so-called ‘triple assessment’, which is routinely performed on all patients with a palpable breast lump. With the introduction of screening however, the need to develop ever more accurate methods of gaining a sample of non-palpable lesions, has lead to the development of more sophisticated biopsy methods. For palpable lesions, controversy exists between the use of fine needle aspiration cytology versus core-cut biopsy. The relative merits of each are discussed below followed by a brief description of new technologies and image-guided biopsy.

2.4.1 Fine needle aspiration cytology (FNAC)

Diagnosis of palpable or image detected breast lesions by FNAC is currently the most widely used technique in the UK. It involves inserting a small-bore cannula attached to a syringe into the area of tissue to be sampled. Withdrawing the plunger of the syringe creates a vacuum and several ‘passes’ through the lesion are made. This draws small clumps of cells into the needle, which are then ejected onto a glass slide by passing some air back through the needle from the syringe. Following
staining, these cells can then be examined microscopically. The standard reporting of the findings are on a five-point scale as outlined below:

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>inadequate i.e. insufficient cells for diagnosis</td>
</tr>
<tr>
<td>C2</td>
<td>benign</td>
</tr>
<tr>
<td>C3</td>
<td>atypia, probably benign</td>
</tr>
<tr>
<td>C4</td>
<td>suspicious of malignancy</td>
</tr>
<tr>
<td>C5</td>
<td>malignant</td>
</tr>
</tbody>
</table>

**Figure 2.1: Cytological grading of aspirate.**

The main advantages of FNAC are that it is minimally invasive, causes only slight discomfort, can be performed quickly in the out-patient setting, results can be obtained almost immediately (providing a cytopathologist is present at the clinic) and it is relatively inexpensive. The main disadvantage however is that without architectural information, classification of the type of tumour can be difficult which may have a bearing on treatment option. Also assessment of the histological grade is unreliable [Robinson et al., 1994].

The accuracy of cytological examination of cells aspirated through small-bore cannulae has been well validated with most recent studies reporting less than 10% false negative rate for breast cancer [Ciatto et al., 1995;Cook and Robinson, 1991;Eltahir et al., 1999]. The main problem however is that accuracy is decreased in patients with in-situ and lobular carcinomas due to the higher proportion of normal to malignant cells in these lesions [Ciatto et al., 1993;Sadler et al., 1994]. There is some evidence that accuracy is improved, and inadequate samples are less, when the aspiration is performed by a cytolopathologist [Fentiman, 1998]. This is probably due to the experience of the cytologist and also the chance to quickly check the sample and repeat if necessary. To try and minimise the discomfort to the patient, some researchers have compared the accuracy of the technique using different sizes of needle (21 versus 23 gauge) or the use of local anaesthetic [Daltrey et al., 1999;Walker, 1998]. They found that there was no statistical difference between the techniques although numbers were relatively small.
Despite a relatively high false negative rate compared to other biopsy techniques the merits of FNAC are considerable, especially in the era of one-stop-clinics (see below, section 2.6.1). Also when combined with clinical examination and imaging, overall sensitivity approaches 100% [Drew et al., 1999; Eltahir et al., 1999]. It should be borne in mind however that there is significant variation in the reported accuracy of FNAC in the medical literature. This is best summarised in a review by Giard and Hermans [Giard and Hermans, 1992]. The main conclusion however is that it is important for every breast cancer unit providing this service, to conduct an honest audit of their own practice, ensuring that diagnostic accuracy remains high.

2.4.2 Core-cut needle biopsy

Core-cut needle biopsy (CNB) involves sampling a larger portion of breast tissue than FNAC. For breast biopsies, most would use a 14 gauge needle fitted to an automatic firing device as smaller gauge needles are less reliable [Nath et al., 1995]. After infiltration with local anaesthetic, a small incision is made in the skin (2mm) that allows easy passage of the needle into the breast. The action of the needle and how the sample is obtained is described diagrammatically below (section 9.2.3).

CNB has several advantages over FNAC that rely on the fact that a larger tissue sample is obtained retaining the architectural structure of the breast lesion being sampled. This allows true histological interpretation. It is therefore more reliable at differentiating the type of tumour in question, estimation of grading is improved, and overall diagnostic accuracy is better than that of FNAC especially for in-situ cancers. The main disadvantages however are that it causes more trauma, and subsequently more pain to the breast, and can have serious complications if misused e.g. damage to vessels or nerves if sampling from the axillary tail, or pneumothorax if angled towards the chest wall. In experienced hands however, with awareness of these possible pitfalls, these complications should not arise.

Although CNB can be used for ‘blind’ biopsy of palpable lesions, a move towards image-guided biopsy has resulted in improved accuracy with some now recommending this as a routine alternative to surgical biopsy. A recent paper by
Nguyen et al., reported a sensitivity of 99% and a specificity of 100% for detection of breast cancer and included a short review of 10 similar studies giving a range of 93-100% and 85-100% respectively [Nguyen et al., 1996]. Interestingly there were almost no complications except in one large study [Parker et al., 1994], which reported minor complications in 0.2% of patients (haematoma or infection).

Whether CNB will replace FNAC is open for debate. In Europe, aspiration cytology remains the investigation of choice, whereas in the US, core-cut is becoming the standard. This may be due to the North American medico-legal climate, where false negative investigations are less well tolerated. There is no doubt however that FNAC when part of a triple assessment brings the overall sensitivity to almost 100% with less patient discomfort and at less expense than CNB. Some have suggested that core-cut biopsy could perhaps be reserved for those patients where initial FNAC is inadequate or where the result conflicts with the findings on imaging and/or clinical examination [Carty et al., 1994]. CNB will however remain the first line technique for biopsy of most mammographically detected lesions, especially for the assessment of micro-calcifications (see below, section 2.4.4).

2.4.3 New technologies

In the effort to obtain larger and more accurate biopsies, new devices have recently been developed, some of which are becoming accepted into general usage. Two worth mention are the vacuum assisted biopsy device (Mammotome) and the Advanced Breast Biopsy System (ABBI). The former uses suction, to pull the tissue to be biopsied into a side channel [Berg et al., 1997]. A rotating knife subsequently cuts the tissue, and the specimen is transported to the back of the needle where it can be removed. Rotating the instrument along its axis, and repeating this process can sample a large amount of tissue in a circumferential manner around the needle. As the outer diameter of the needle is around 3mm (11 gauge), and often large areas of tissue are removed, bleeding or haematoma can be a problem although this has a reported incidence of around 1% [Burbank, 1997].

The ABBI system can remove much larger amounts of tissue and involves a tube up to 20mm in diameter with a rotating cutting edge [Liberman, 1999]. As the device is
passed into the breast, a large core of tissue is removed. Some have suggested this as a method for complete local excision of both benign and malignant lesions [Damascelli et al., 1998;LaRaja et al., 1999], but the incidence of positive margins is greater [Velanovich et al., 1999], and its role has been questioned [Liberman, 1999].

Both of these systems rely on image guidance for accurate positioning. Whether they are more sensitive than core-cut biopsy at diagnosing breast cancer remains to be seen, particularly as the increased cost of these disposable systems is significant and the complication rates are higher [Leibman et al., 1999].

2.4.4 Image guided biopsy

With the introduction of screening mammography came the problem of accurately obtaining a biopsy of lesions which where impalpable and could only be seen on the mammogram. The greater use of ultrasound for solid lesions has reduced the need for mammographically directed biopsy, but there still remains a proportion of lesions that cannot be visualised on ultrasound, especially microcalcifications.

Ultrasound guided biopsy is the simplest way to obtain a tissue sample from a suspicious area [Klijanienko et al., 1998] as most solid lesions on mammography will be visible with ultrasound. The main advantages are that it allows real time placement of the biopsy needle into the correct position and it is relatively cheap. Some manual dexterity is necessary by the operator as well as the ability to work in 3 dimensions from 2 dimensional images, but with adequate training and practice, this becomes a relatively simple procedure. Both FNA and core-cut biopsy can be utilised, with the added safety of watching the track of the needle to prevent untoward injury to other structures.

As mentioned above however, not all mammographically detected lesions can be seen on ultrasound, which has therefore prompted the introduction of mammographically guided stereotaxic biopsy. Initially this was achieved by the use of ‘add-on’ equipment connected to an existing mammography unit [Ward et al., 2000] but now dedicated breast biopsy equipment is becoming more commonplace. Patients can either be sitting in the upright position or lie prone, with the breast
protruding through a hole in the table. A perforated plate allowing access to the breast achieves compression of the breast in the cranio-caudal plane. Two mammographic views are obtained, usually at 15° either side of the midline to give views differing by 30°. These are then digitised and recorded onto a computer. The radiologist marks the site of the lesion onto the screen in each view, and co-ordinates are then calculated in three planes. These are next programmed into the biopsy device, which automatically obtains the tissue sample from the correct area. With the introduction of digital mammography and with increasing sophistication of both the computer software and biopsy devices, the speed and accuracy of these systems are improving. There is some debate as to which biopsy device is most accurate [Velanovich et al., 1999] although at present most breast units in the UK would use either core-cut or mammotome for obtaining stereotaxic biopsies.

Magnetic resonance (MR) image guided biopsy is still at an early stage but systems are just starting to become commercially available. This will hopefully solve the problem of being able to biopsy areas only seen using this imaging modality. There are several problems however, which are specific to MR that have had to be overcome. These include inaccessibility to the patient while in the magnet, patient movement, development of non-magnetic instruments and rapid loss of enhancement of the lesion.

To obtain the best quality images, high field strength, closed bore magnets are needed which prevent access to the patient whilst imaging is taking place. Some investigators have used a 0.5 Tesla open configuration interventional MR system that allows freehand placement of the biopsy needle [Daniel et al., 1998]. Images are updated every few seconds and the angle of insertion of the needle is monitored using infrared receivers. All other techniques rely on imaging first, and then bringing the patient out of the magnet to perform the biopsy. This has been possible using a freehand technique as for the open magnet, but most investigators are now moving towards stereotaxic localisation using a perforated compression plate and fiducial markers. The best validated of these methods has been developed by Heywang-Köbrunner and co-workers in conjunction with Siemans (Siemans, Erlangen, Germany; Epoxonic, Munich, Germany) [Heywang-Kobrunner et al., 2000]. In this system, the patient lies in the prone system, with the breast protruding through a hole.
in the table. A customised surface coil and breast immobilisation device allows breast compression in the medio-lateral plane by a series of flexible bars that are spaced to allow access to the breast. Following contrast enhanced 3D imaging, the patient is removed from the magnet and co-ordinates are calculated. A vacuum biopsy system is used in exactly the same way as for mammographically guided biopsies. The patient is then re-imaged to ensure that part, if not all, of the enhancing lesion has been removed. This system has several advantages:

- With the patient lying prone, movement due to respiration is dramatically reduced.
- Use of existing, non-magnet compatible biopsy systems can be used.
- A large enough sample can be obtained which compensates for any slight discrepancy in positioning. As a small cavity is left following biopsy, this can be visualised in the post procedure images allowing easier confirmation of adequate sampling.
- Compression is kept to a minimum – as contrast enhancement relies on the increased vascularity of the tumour, breast compression can theoretically interfere with perfusion of these lesions.
- The system is flexible enough to allow access to any part of the breast from either a medial or lateral approach.

There is no doubt that an improvement in all forms of breast imaging has lead to smaller and smaller breast cancers being identified. The mechanisms to obtain accurate localisation and sampling of these lesions are already available. Whether detection at such an early stage will prove to confer a survival benefit to patients with breast cancer, is however still very much open for debate.

2.5 BREAST SCREENING

When considering a certain illness or disease for screening, the longstanding principles listed below, developed by the World Health Organisation, should always be carefully analysed:

- The condition should pose an important health problem
• The natural history of the disease should be well understood
• There should be a recognisable early stage
• The treatment of the disease at an early stage should be of more benefit than treatment started at a later stage
• There should be a suitable test
• The test should be acceptable to the population
• There should be adequate facilities for the diagnosis and treatment of abnormalities detected
• Screening should be repeated at intervals determined by the natural history of the disease
• The chance of physical or psychological harm to those screened should be less than the chance of benefit
• The cost of a screening programme should be balanced against the benefit it provides

The main goal of breast cancer screening is that it should reduce the overall mortality from breast cancer compared to an unscreened population. There has however been much debate and controversy in recent years, not only regarding this endpoint but also many of the other screening criteria. Various trials have looked at the screening test itself although general consensus at present is that clinical breast examination, breast self examination and breast ultrasound are ineffective screening tools [Sirovich and Sox, Jr., 1999; Teh and Wilson, 1998]. At present this leaves mammography as the best method for breast cancer screening. Recent interest in MR imaging for screening of high-risk women has resulted in the commencement of several trials but the outcomes in terms of mortality reduction will not be available some time [Orel and Schnall, 1999]. Whether MR will be cost effective as a general screening test is however doubtful.

With mammographic screening, most would agree that for an unselected, population-based programme to be both cost effective and produce a worthwhile reduction in cancer mortality, it is essential to maintain as high a percentage of participation as possible. Despite many interventions through health promotion, published studies from the UK have reported screening attendance rates, depending on the region, of
between 60-72% [Anonymous, 1999a]. In the Swedish studies however, this figure was much higher (74-89%) [Nystrom et al., 1993]. It has been suggested that for nation-wide implementation, a figure of 70% compliance would be necessary to achieve a worthwhile benefit to the population as a whole [Blamey et al., 1994].

The first trial for mammographic breast cancer screening began in the early 60's in New York and was a randomised controlled trial (RCT) of mammographic screening and clinical examination versus neither in women aged 40-64 years [Shapiro, 1997]. Since then other RCT's have taken place in Sweden, the UK, and Canada. Each trial was slightly different in design with variation between age range of study population, interval of screening and inclusion of other variables such as clinician or patient breast examination and levels of information. Most of these studies began between 1965 and 1980. A recent meta-analysis of these trials concluded that the reduction in breast cancer mortality for women aged 50 to 74 years was 26% (significant) but for women aged 40-49 this reduction was only 7% (non-significant) [Kerlikowske et al., 1995]. For the older age group this reduction was irrespective of number of mammographic views, screening interval or length of follow up. This, and previous analyses, has led to general acceptance of national screening in most developed countries. Some criticism has recently been expressed about the set-up of the trials [Gotzsche and Olsen, 2000], but most believe that any randomisation bias can be explained or adjusted for.

For women aged 40-49 years there have been some interesting observations leading to much controversy. Both the meta-analysis above, and independent analyses of the Swedish [Nystrom et al., 1993] and HIP (New York) trials [Chu et al., 1988] have shown that the non-statistical trend for reduced mortality in this age group does not become evident until around 10 years or more after the commencement of screening. Before this time period, there is little if any reduction at all. The reasons for this are still unclear. Possible factors however may be the smaller overall breast cancer rate in this population, the increased likelihood of an aggressive tumour or the fact that after 10 years, this population group are all above 50 and therefore mimic the results from the older age groups. Whether or not any mortality reduction will become significant with longer follow up, or larger numbers due to population screening, there are other end points which may be valid reasons for continued screening of
these patients. These include detection of smaller tumours leading to increased breast conservation surgery and the reduced need for axillary dissection or adjuvant chemotherapy. All of these factors could substantially increase a patient’s quality, if not quantity, of life.

Despite the proven advantages of screening, one must always bear in mind the disadvantages. These include discomfort to the patient due to breast compression, false positive and negative results and cost implications. For every 1000 women screened, around 5-10% will be recalled due to an abnormal finding on their mammogram. Around one quarter of these will undergo some form of biopsy of which the vast majority will be benign [Kerlikowske et al., 1993]. Not only does this carry a significant risk of morbidity from unnecessary invasive procedures but also causes much anxiety for those patients who are recalled. False reassurance has the opposite effect by creating a false sense of security for patients who develop symptoms from a breast cancer between screens. Finally, in today’s climate of health care rationing, the significant cost of whole population screening may deprive other areas of breast cancer care from making improvements such as research, provision for ‘one-stop’ breast clinics or reductions in waiting time to be assessed and have treatment.

There is no doubt that for the foreseeable future population based mammographic screening will continue. Fine-tuning of the process will undoubtedly continue, as will the controversies. Unfortunately we are unlikely to ever again have the opportunity to repeat the randomised-controlled trials of the 70’s and 80’s for the over 50’s. For patients younger than this, the jury is still out!

2.6 SUMMARY

The diagnostic armoury available to the clinician is ever increasing. With universal use of new image guided techniques, the need for a “diagnostic lumpectomy” should now be a thing of the past for the routine investigation of a breast lump. Mammography and ultrasound are now well-validated techniques although the role for MR imaging is yet to be fully evaluated. Certainly with the increasing availability of MR guided biopsy systems, this modality will become more the routine, rather
than the exception. With regards to tissue biopsy, there is still some controversy between the relative merits of FNAC versus core-cut biopsy. Both have their pros and cons although ultimately, the continued success of FNAC relies on the experience of the cytopathologist. Certainly without it, the ability to run 'one-stop clinics' will be severely hampered (see section 3.8.1). The real goal for diagnosis is to achieve as near as possible 100% sensitivity for detecting breast cancer and without a concurrent decrease in specificity. Also of equal importance is the accurate diagnosis of benign conditions, such as fibroadenomas, that can simply be observed. There is however the need to keep the level of unnecessary invasive diagnostic procedures to a minimum, which is where new imaging modalities such as MRI may have a role. With the techniques available today, these ideals are not too far away.
CHAPTER 3 CANCER TREATMENT

3.1 INTRODUCTION

Compared to Halsted’s era, there now is an armory of treatment regimes for patients with breast cancer. These include endocrine therapies to manipulate the hormonal environment, chemotherapy and radiotherapy as cytotoxic agents, reconstructive techniques to help maintain body image and improve quality of life, and also finally, but not least, palliative care for those patients where cure is not possible. Although most patients will have at least two lines of attack, surgery still remains the mainstay for the treatment of local disease, which offers hope of a relative cure for the majority of patients, that is if ‘cure’ is possible at all!

3.2 SURGERY

Little has really changed over the past century in the surgical treatment of breast cancer in terms of technology. Most surgeons still perform the operation with a scalpel, cautery and sutures. What has changed however is a move towards more conservative, breast preserving surgery. This shift however has only really taken place in the past 20 years following the publication of several large studies which evaluated the safety in terms of overall mortality of performing such surgery in conjunction with radiotherapy [Fisher et al., 1995; Jacobson et al., 1995; Veronesi et al., 1994].

Breast conservation surgery generally means the excision of the breast tumour with a surrounding rim of tissue. Most would accept that a 1cm margin around the tumour is adequate although there is still much debate about this. It is however clear that although local recurrence rates are higher with lumpectomy compared to wide local excision or quadrantectomy, the overall survival remains the same [Veronesi et al., 1994]. There is however a play off between removing enough tissue to keep local recurrence to a minimum against removing too much, with a resultant poor cosmetic outcome.
Lesion size is the main predictor of whether breast conservation surgery is possible. An arbitrary cut off figure of less than 4cm is often quoted but it is highly dependent on the relative size of the breast and the position of the lesion i.e. excision of a moderate sized tumour in a small breast may have a worse outcome than a larger lesion in a large breast. Also, excision of tumours in the lower aspect of the breast often have a poorer cosmetic outcome compared to those in the upper part due to a pulling down of the nipple through removal of supporting tissue and cicatrization of the scar.

Mastectomy however is still an important and useful operation for many patients and constitutes around a third of all operations for primary breast cancer. The indications for performing extirpation of the breast are again relative to the individual patient but broad guidelines are listed below:

I. Patients with large tumours or extensive in-situ disease.

II. Multifocal disease

III. Patient request – this may be due to a genetic predisposition, for ease of follow up, or simply the fear of recurrence.

IV. Cosmetic outcome – Where this is likely to be poor, some patients may opt for mastectomy with reconstruction.

V. Younger patient – Although this is a relative indication, there is evidence that the chance of local recurrence following breast conservation surgery is inversely proportional to age. Therefore armed with this information, some patients may request mastectomy [Sainsbury et al., 2000].

Some form of axillary surgery is also necessary for patients with invasive carcinomas. Not only does this help control local disease where metastasis is present but probably more importantly it provides information for staging and hence prognosis. The status of the axillary lymph nodes will dictate whether chemotherapy
is indicated for some patients. The relative merits of sentinel node biopsy, axillary node sampling or clearance are discussed in greater detail in chapter 1 (section 1.6).

3.3 **Endocrine Therapy**

In 1895 the first surgical oophorectomy was described as a treatment for breast cancer which followed on from Cooper's observations earlier in the Century. Surgical castration was superseded by irradiation of the ovaries which continued until fairly recently. It was in fact not until the 1970’s, that the oestrogen receptor was discovered, and medical manipulation thus considered. At that time however, combination chemotherapy was in vogue, which dampened the interest in hormonal therapies. Over the past decade however, endocrine manipulation has gained renewed interest, as the effects from chemotherapy seem to have reached a relative plateau.

The mainstay of adjuvant hormonal treatment for breast cancer over the past 15-20 years has been tamoxifen. This non-steroidal anti-oestrogen competes with naturally occurring oestrogen for the oestrogen receptor sites, so preventing oestrogen 'drive' to the breast cancer cells. Its precise sub-cellular effects are still being unravelled but it is also thought to have an effect on the production of certain growth factors. The latest overview by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG), of all the large tamoxifen trials, illustrated the outstanding role that this drug plays in the treatment of breast cancer [Anonymous, 1998b]. Their findings are summarised in table 3.1.

The conclusions of this overview were that tamoxifen produces a statistically significant reduction in breast cancer recurrence and mortality for oestrogen receptor positive patients irrespective of age, nodal status or concurrent use of other adjuvant treatments. Although it found indirect evidence of 5 years treatment being more beneficial than 1 or 2 years it recommended waiting for results of trials specifically designed to answer this question. For oestrogen receptor negative or poor tumours, the benefits were less clear (although still marginally statistically significant), with the recommendation that such patients should be entered into trials.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hormone receptors</strong></td>
<td>34% recurrence reduction, and 20% mortality reduction for ER-positive or ER unknown tumours.</td>
</tr>
<tr>
<td></td>
<td>10% recurrence reduction, and 6% mortality reduction for ER-poor tumours.</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>Statistically significant benefit for all age groups but increasing benefit with age.</td>
</tr>
<tr>
<td><strong>Duration of treatment</strong></td>
<td>5 years was more beneficial than 1 or 2 years using indirect evidence between different trials.</td>
</tr>
<tr>
<td><strong>Nodal status</strong></td>
<td>Benefit is similar for both node-negative and positive patients.</td>
</tr>
<tr>
<td><strong>Addition to chemotherapy</strong></td>
<td>Some benefit but recommend commencement after chemotherapy</td>
</tr>
<tr>
<td><strong>Side effects</strong></td>
<td>Significant increased incidence of endometrial cancer but far outweighed by benefits on breast cancer mortality.</td>
</tr>
<tr>
<td></td>
<td>No statistical increase in colorectal cancer, pulmonary embolus or hepatoma.</td>
</tr>
</tbody>
</table>

Table 3.1 – Summary of the findings of the 1998 EBCTCG overview of adjuvant tamoxifen trials [Anonymous, 1998b].

Other methods of hormone manipulation are currently under scrutiny, such as aromatase inhibitors which block the synthesis of oestradiol, and gonadotrophin releasing hormone analogues which block the production of follicle-stimulating hormone and luteinising hormone which stimulate oestrogen production by the ovary. A detailed discussion of these treatments is beyond the scope of this thesis.

There is no doubt however, that hormonal manipulation has contributed greatly to improving the survival rates of patients with breast cancer and is sure to remain a mainstay of adjuvant treatment for some time to come.
3.4 CHEMOTHERAPY

It is accepted that for a certain group of patients, surgery will not achieve cure despite how radical the resection, but for these patients, the presence of distant metastases will ultimately lead to death. It is evident therefore that any major breakthrough in increasing survival for patients with breast cancer must rely on eradicating, or at least arresting the proliferation of metastatic deposits. Early work in the 1960's utilised mono-chemotherapy as adjuvant therapy to surgery with modest results. It was not until the widespread use of combination chemotherapy regimes in the 1980's that the benefits of such treatment became more obvious. Since then there have been many attempts to improve survival by altering both the combinations of chemotherapeutic drugs and changing the time scale for treatment. Such tinkering has achieved only modest improvements if at all.

In a similar manner to their work on tamoxifen, the Early Breast Cancer Trialists' Collaborative Group have also addressed the issue of adjuvant chemotherapy. Their latest overview of randomised trials for polychemotherapy for early breast cancer was published in 1998 [Anonymous, 1998a]. They evaluated the outcomes for patients both below and over 50 years of age at presentation. The results and their recommendations are summarised in table 3.2.

In conclusion they suggest that adjuvant polychemotherapy is beneficial for most patients but this benefit decreased with increasing age. The proportional benefits were unaffected by menopausal status, ER status, tamoxifen use or most surprisingly nodal status. The 10 year mortality reduction was marginally better for node positive patients, 11% versus 7% in the under 50's and 3% versus 2% in the over 50's, but this difference was not statistically significant.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Statistically significant benefit for all age groups but decreasing benefit with increasing age. Under 50’s RR = 35% MR = 27% Over 50’s RR = 20% MR = 11%</td>
</tr>
<tr>
<td>Nodal status</td>
<td>Benefit is similar for both node-negative and positive patients.</td>
</tr>
<tr>
<td>Duration of treatment</td>
<td>3-6 months polychemotherapy as effective as longer regimes.</td>
</tr>
<tr>
<td>Choice of chemotherapy agent(s)</td>
<td>Evidence of added benefit with anthracycline-containing regimes but suggest waiting for the results of further trials</td>
</tr>
<tr>
<td>Addition to tamoxifen</td>
<td>Some benefit independent of tamoxifen use i.e. chemotherapy and tamoxifen are complementary, not competing.</td>
</tr>
<tr>
<td>Hormonal receptor status</td>
<td>No difference in benefit for ER positive compared to ER negative tumours.</td>
</tr>
<tr>
<td>Adverse effects</td>
<td>No statistical evidence of increase in non-breast cancer deaths in those receiving chemotherapy.</td>
</tr>
</tbody>
</table>

Table 3.2 – Summary of the findings of the 1998 EBCTCG overview of randomised trials of polychemotherapy for early breast cancer [Anonymous, 1998a].

RR – recurrence reduction  MR – mortality reduction

Despite this ‘benefit for all’, it should be remembered that although chemotherapy does not cause excess non-cancer mortality, it is not without significant side effects. Alopecia, nausea, vomiting, fatigue and lethargy are all common problems experienced by women, which should not be disregarded. As the benefits significantly reduce with age, adjuvant chemotherapy tends to be most appropriate for the younger patient where side effects and the time spent undergoing treatment may be more acceptable. Age alone however should not be a barrier to offering such treatment to patients.
3.5 Radiotherapy

Radiotherapy for the treatment of breast cancer was introduced around the turn of the last century following the discovery of x-rays, gamma radiation and radium by Röentgen, Becquerel and Curie respectively. Its main use today is for control and prevention of local recurrence. The effect of radiotherapy on long-term survival is less well proven as although there is a reduction in breast cancer death, there is a corresponding increase in non-breast cancer mortality [Anonymous, 2000].

The main group of patients undergoing radiotherapy are those having breast-conserving surgery, where it is routinely used as an adjuvant treatment. Typical doses are 40-50 Gy to the breast with 10-20 Gy boost to the excision site. This dose is fractionated with daily treatment for 3-5 weeks commencing around 4 weeks following surgery. Such combined therapy has been shown to dramatically reduce the local recurrence rate compared to breast conserving surgery alone, and without compromising mortality [Fisher et al., 1995;Forrest et al., 1996].

Other situations where radiotherapy is considered is for patients with large aggressive tumours, involvement of the chest wall or axillary irradiation for patients with positive lymph nodes who have not undergone complete axillary clearance.

As mentioned above, radiotherapy has minimal, if any effect on overall mortality. This has been due in part to the significant side effects of older radiotherapy regimes, which often caused damage to the left anterior descending coronary artery. This inevitably led to increased cardiac deaths. Other side effects included radiation pneumonitis, cutaneous radionecrosis, osteoradionecrosis and brachial plexopathy with axillary irradiation. With more modern machinery, using tangential and more controllable fields, such side effects have diminished, and hopefully a survival benefit will emerge.
3.6 AESTHETIC SURGERY

3.6.1 Introduction, history and contemporary procedures

Body image is the second greatest concern, after that from cancer, of women undergoing breast surgery. Once the remit of plastic surgeons, breast reconstruction is today becoming ever more commonplace, and with increasing sub-specialist training is now performed by many general surgeons with an interest in breast disease. It includes post-mastectomy and post-conservative surgical reconstruction, contra-lateral surgery and nipple reconstruction as well as purely cosmetic augmentation, reduction and mastopexy.

The concept of breast reconstruction was first introduced around the time Halsted described his radical mastectomy in 1894 [Halsted, 1894]. Initial attempts in the late 19th century using fat grafts failed due to lack of blood supply. In 1906 Tanzini performed the first autologous latissimus dorsi flap. This however did not gain acceptance nor did various other attempts using tubed pedical flaps from the contra-lateral breast or other sites. This was mainly due to an insufficient volume of tissue to produce an adequate breast mound.

In the early 60's, the first silicone implant was introduced by Cronin and Gerow [Cronin and Gerow, 1963]. Due to lack of tissue coverage following radical mastectomy, this method was not totally successful until the popularisation of the modified radical mastectomy in the early 70's. This allowed sub-pectoral insertion of the implant, which produced better coverage and lowered the risk of capsular contraction [Gruber et al., 1981]. Further improvement with implants came with the development of a chest wall tissue expander [Radovan, 1982]. This allowed for the subsequent placement of a larger implant which also gave the opportunity to produce ptosis of the reconstructed breast by initial overinflation. Radovan’s technique was quickly superseded by Becker who introduced a permanent expandable implant which could be inflated after insertion [Becker, 1984]. This one-stage technique is still commonplace today.
One of the major problems with the early implants was that of capsular contracture, leading to a painful, overly firm breast. This problem however has to some extent been reduced by the use of textured implants [Maxwell and Falcone, 1992].

Latissimus dorsi flaps alone had been tried and largely abandoned during the first half of the 20th century, but were re-introduced in the late 1970's used in combination with a silicon implant to achieve a breast mound [Bostwick et al., 1979; Schneider et al., 1977]. Interestingly in some third world countries e.g. India, LD flap is a popular 'stand alone' reconstruction as it is relatively cheap compared to silicon. More recently, certain groups have employed fat harvesting techniques to provide enough autologous tissue to reconstruct a breast of sufficient volume without the need for implants [Delay et al., 1998].

The transverse abdominus musculocutaneous (TRAM) flap was first introduced by Hartrampf in 1982 [Hartrampf et al., 1982] and since then has come to be the 'gold standard'. Debate remains as to the benefits of free flaps over pedicled rotation flaps [Grotting et al., 1989; Larson et al., 1999] but overall, the TRAM flap gives an adequate volume of tissue for most breast reconstructions and produces an aesthetically pleasing breast of correct consistency.

3.6.2 Psychological considerations:

There is no doubt that for women and men, the breast is one of the most important external features of femininity. Any surgery therefore, from lumpectomy to mastectomy, naturally raises great concern about body image to women with breast cancer. Common worries range from the ability to wear certain clothes, such as tight fitting garments or swimwear, to fears about a change of attitude by partners, especially with regards to sexual appreciation of their body [Schain et al., 1985].

There has been a major shift in the last decade in the attitudes of both the surgeon [Spyrou et al., 1998] and the patient towards breast reconstruction [Trabulsy et al., 1994]. This has been due to several factors including the greater availability of such procedures, increasing patient awareness and a rapidly expanding wealth of evidence.
showing the psychological benefits and improved quality of life following breast reconstruction [Pusic et al., 1999; Schain et al., 1985; Stevens et al., 1984].

Many studies have looked at patient’s satisfaction following breast surgery [Berry et al., 1998; Mansel et al., 1986; Pusic et al., 1999; Ramon et al., 1997]. The general consensus is that immediate reconstruction offers the best possible psychological outcome for the majority of patients [Noone et al., 1985; Schain et al., 1985; Stevens et al., 1984]. Unfortunately however, not all patients are suitable for such surgery either due to physical reasons e.g. co-existing medical disease, or psychological reasons. Also those who do not necessarily receive consistent information from their carers [Handel et al., 1990; Iscoe et al., 1994; Pusic et al., 1999]. Morbidity from all breast reconstructive procedures is not insignificant and itself may have psychological repercussions.

With the ever-growing trend towards breast conservation surgery, mastectomy is being performed less commonly. There will always however be a significant minority of women, for whom mastectomy is the only option. As the psychological aspects of losing a breast are now appreciated much more than in the past, it is appropriate to offer all women some form of reconstruction. The final decision of whether to have a reconstruction or not, following full counselling, is therefore left to the patient rather than their carers, which has been for so long the case.

3.7 Palliative Care

Despite the best medical care possible, and all the recent advances outlined above, women (and men) are still dying from breast cancer. Care of these patients is just as important, if not more so, than for the majority who are long term survivors.

The principles of palliation are concerned with control of symptoms of both the primary disease and metastases. Prolongation of life should also be a consideration but must not be the primary goal. Common sites and corresponding symptoms of advanced breast cancer with relevant treatments are discussed below.
- **Local progression; skin ulceration, lymphoedema, brachial plexopathy**: High energy radiotherapy, systemic chemo/endocrine therapy. Manual massage and compression for arm oedema. Surgery is usually avoided as progression may be rapid with recurrence, which may more difficult to control.

- **Bone metastasis; pain and/or fracture**: Local radiotherapy, analgesics. Bisphosphonates suppress osteoclast activity leading to slowing of progression and also for control of malignant hypercalcaemia.

- **Liver metastases; nausea, anorexia, weight loss, general fatigue**: Local radiotherapy, corticosteroids.

- **Lung metastases, pleural effusion; shortness of breath**: Pleural drainage, pleurodesis with talc or tetracycline.

- **Cerebral metastases; headache, neurological symptoms**: Dexamethasone, radiotherapy.

- **General symptoms; pain, nausea, constipation, insomnia**: Medical management.

The hospice movement has done much to improve palliative care over the past 40 years. This was pioneered by Dame Cicely Saunders in the early 1960’s at St Christopher’s Hospice, South London whose ethos has now been adopted throughout the world. The holistic approach to patient care is central to its philosophy with not only care for the physical effects of the disease but also the emotional and spiritual aspects for both the patient and family alike. Gone should be the days where patients were left to suffer, isolated and in pain, with little regard for the non-physical aspects of the illness. The fear of such a demise is however still ingrained upon many, a belief which is slowly being dispelled. The right to die with dignity, accompanied by family with adequate control of symptoms, must surely be respected and achieved for all.
3.8 CURRENT PROBLEMS IN BREAST CANCER CARE AND FUTURE IMPROVEMENTS

The United Kingdom has one of the worst figures for breast cancer survival in Europe [Anonymous, 1999]. The reasons for this are not fully understood but may be due to a composite of factors such as genetic predisposition for aggressive tumours, training of medical and nursing staff, lack of a co-ordinated approach to cancer care in every region or simply under-funding of the NHS. Whichever of these deficiencies is the case, it is important that there is a concerted effort to improve the quality of service given to patients with breast cancer in order to maximise outcomes and hopefully increase survival rates for these unfortunate women.

In 1995, the previous Chief Medical officer, Sir Kenneth Calman, published his report into the provision of cancer services in the United Kingdom [Calman and Hine, 1995]. In it was set out a blueprint for how patients with all types of cancer, not just of the breast, should be referred, assessed and treated. The report contained many recommendations but these were based upon three basic principles:

- Standardisation of cancer services throughout the UK with improvements centred on the needs of the patients and their families.
- Encouragement of sub-specialisation in all oncology disciplines.
- Pooling of expertise by encouraging a multi-disciplinary approach to cancer care and also creating an environment for clinical research to flourish.

How they described achieving this was to limit the provision of cancer care in any one region to a few large district general hospitals. Some would be nominated as cancer-units, which would assess and treat all common and uncomplicated cancer cases, with one or two large cancer-centres (generally teaching hospitals) that would act as tertiary referral centres for rarer or more complicated cases. Over the past few years implementation of this strategy has been taking place.

The theory behind such a plan is the evidence that a subspecialty approach to cancer care, where large numbers of patients with a particular disease are treated by a multi-disciplinary hospital team, is beneficial to the patient in terms of reduction in
morbidity and mortality. This is generally true, but like so many things in medicine is highly dependent on the individual abilities of the medical and nursing staff in any one hospital, the resources available to them, and the working relationship of all parties involved. In fact, some evidence would suggest that results from cancer treatment might not depend on the caseload of the hospital or the number of patients a particular surgeon treats per year [Kee et al., 1999].

For breast cancer care however, the evidence is stronger for the belief that if a patient is referred to a specialist breast cancer centre, their outcome will be better [Gillis and Hole, 1996; Sainsbury et al., 1995]. Some have suggested that increased sub-specialisation of surgeons dealing with breast cancer reduces mortality rates [Gillis and Hole, 1996]. Many more have however pointed out that it is probably greater embracement of the multi-disciplinary approach and subsequent increased use of adjuvant chemo- and endocrine therapy that conveys a survival benefit for patients treated in specialist centres [Sainsbury et al., 1995; Sikora, 1996]. It is on this basis that improvements to breast cancer services in the UK should be directed.

3.8.1 Referrals, ‘one-stop clinics’ and waiting lists

There is some evidence that the time between a patient with breast cancer finding a lump, or developing another symptom, and having definitive treatment, may have a bearing on their outcome in terms of survival. One large overview suggested a reduction in survival with delays over 3 months [Richards et al., 1999]). This however was not confirmed by a large retrospective study [Sainsbury et al., 1999], which found no difference between patients who had delays of over 60 days. This may however be explained by the fact that patients with clinically suspicious signs, and hence more aggressive disease, tend to be referred more urgently, therefore creating bias between the two groups being studied. Despite this conflicting evidence, there have been many new initiatives to improve the time taken to see a specialist, be accurately diagnosed and have subsequent treatment. It is often overlooked however that delays are not only due to the medical providers, but may be caused by a patient ignoring symptoms or being too frightened to seek medical advice [Nosarti et al., 2000]. A real effort therefore needs to be made in public education as well as improvement to the existing cancer services if this overall delay
time is to be diminished. Despite many government edicts and patient promises, improvements are unlikely in an already over-stretched NHS without additional, ring-fenced funding.

With regards to the time between a patient seeing her GP, and attending a breast assessment clinic, an ideal figure of less than 2 weeks is often quoted [Department of Health, 2000]. This figure is however arbitrary and when compared to the United States, where patients are usually seen the following day, it seems a long time to wait for the anxious patient. Certainly, the introduction of electronic booking systems where GP’s can access the hospital appointment system and arrange a time convenient for the patient to be seen within the next few days is some way off for the whole population. Such a system would however cut out the inevitable delay of paper posting of appointments. Whether the difference between one day and two weeks is clinically significant is unlikely (especially when the outcomes of screening are taken into account). This delay is however significant to a women who is waiting anxiously with a lump in her breast.

Over the past few years, most large breast cancer centres have introduced ‘one stop clinics’ for patients with breast symptoms [Eltahir et al., 1999]. In the space of a few hours, women can be seen by a specialist and have appropriate investigations and given the results. Usually the patient is initially seen by a surgeon, who obtains a history and conducts an examination. Next, appropriate imaging is organised, either mammography, ultrasound or both. Finally, when necessary, FNAC is performed and immediately examined by a cytopathologist in the clinic. The main advantage of this system is that for the majority of women with normal or benign findings, they can be reassured immediately and discharged with advice. This cuts down on further anxiety waiting for the results of investigations. The second advantage is that for patients with breast cancer, after appropriate counselling by the surgeon and breast care nurse, a treatment plan can be made including arrangement of a mutually suitable date for surgery. This again cuts down on the time from symptoms to surgery.

The final area for improvement is in shortening the time between diagnosis and treatment. In the UK this is highly variable depending on the resources of an individual region [Department of Health, 2000]. With the increase in the numbers of
patients requesting immediate breast reconstruction putting pressure on already overbooked operating lists, and the current bed shortage leading to cancelled operations, lengthening of the time gap set to continue. The only real way to tackle this problem is with protected beds, and increased provision of medical and nursing staff.

When planning improvements to provision of cancer services the needs and psychological well-being of the patient must not be overlooked. Whether a reduction in waiting times for hospital appointments and subsequent surgery have any survival benefit is unclear, although the difference of a few weeks is realistically not going to make a difference. What does matter however is the reduced anxiety to patients and the overall standard of service they receive, which always must be regarded of equal, if not primary, importance.

3.8.2 Research and new treatments

Even if all patients are seen in a specialist unit, diagnosed and operated on in the space of a day, this is unlikely to have any dramatic effect on overall survival figures, as in most units in the UK, patients with breast cancer are assessed and treated within 4-6 weeks. The real improvements in reducing breast cancer mortality must therefore come through advances in prevention and treatment.

Over the past 40 years, the increasing use of chemo- and endocrine therapies has made a dramatic impact on breast cancer survival (see section 3.3 & 3.4). With the movement from mono- to polychemotherapies in the 70’s, the introduction of anthracylines in the 80’s, and with Taxanxes in the 90’s, gradual reduction in overall mortality has been reached [Nabholtz et al., 1999]. Also we are now seeing the benefits of the introduction of Tamoxifen some 15 years previously. Certainly the scope for improvements for such treatments will continue, especially with regards to endocrine therapy, although these will ultimately reach an optimum effect.

The main problem in the treatment of breast cancer, or any cancer for that matter, is that we are still very ignorant of the biology of malignant transformation of cells, growth and metastasis. Certainly some steps towards unravelling the process have occurred over the past few decades but we are still not much further on towards a
cure than Halsted was with his radical mastectomy over 100 years ago. There is no
doubt that this century will inevitably produce ‘breakthroughs’, but where the real
difference will happen is still open for debate.

Advances in chemotherapy and endocrine manipulation are extremely unlikely to
make a huge difference. With better understanding of the genetic influence on cancer
development, as seen in BRCA1 and BRCA2, new therapeutic strategies will
undoubtedly develop. Another ‘hot’ area of research is the production and influence
of growth factors, antagonists of which are already being tested. Mounting evidence
of environmental factors may also help solve the puzzle. It may however be that the
answer will come through prevention rather than cure, with some form of genetic
vaccine. The possibilities of all of these modalities are certainly exciting but perhaps
manipulation of some, as yet of undiscovered, biological process will be discovered?
It may however be a combination of various lines of attack that will achieve 100% survival, with individual patient tailoring of treatment for their specific tumour, if
such a goal is possible?

Until such a time we must strive to implement the armoury we have already. There
are huge differences in overall mortality rates for breast cancer between different
countries. Some of this is undoubtedly due to genetic and environmental factors, but
certainly there are huge discrepancies in the way patients are treated throughout the
world. It is the responsibility of everyone caring for patients with any disease
process, whether cancer or not, to utilise the available resources to the best of their
ability, based on a sound understanding of the current scientific evidence.
CHAPTER 4 OPTICAL BIOPSY

4.1 INTRODUCTION:
Various types of optical spectroscopy have been evaluated for tissue diagnosis including fluorescence, Raman and near-infrared (NIR) spectroscopy. Each of these will be considered briefly below. This thesis however concentrates on a fourth type, known as elastic scattering spectroscopy (ESS). All types of tissue spectroscopy are similar in that they have the ability to assess the biochemical and/or structural composition of tissue. By characterising such variables in living tissue, it is therefore theoretically possible to utilise such knowledge in the detection of abnormal tissue types such as cancer or dysplasia. The term ‘Optical Biopsy’ has been coined which describes the process of evaluating the optical properties of tissue with subsequent analysis of the received data. Such a system based on ESS, has the potential to become a clinically useful diagnostic aid, which could have wide-ranging application.

![The Visible Spectrum](image)

*Figure 4.1 – Diagrammatic representation of the visible spectrum demonstrating colour versus wavelength in nanometers*

4.2 SPECTROSCOPY
A ‘spectrum’ is defined as “a charted band of wavelengths of electromagnetic radiation obtained by refraction or diffraction”. ‘Spectroscopy’ is “examination by means of an instrument for developing and analysing spectra”. In other words, spectroscopy involves passing some form of electromagnetic radiation into a
substance (in medical terms the substance is human tissue), and characterising the spectra received. This is the basis for all types of spectroscopy. The only difference is the nature of the ‘input’ and ‘output’ radiation. All four types of spectroscopy that are mentioned above utilise ‘light’ from within or just outside the visible range of electromagnetic radiation (Fig 4.1).

4.2.1 Fluorescence Spectroscopy:

Laser induced fluorescent spectroscopy relies on the presence of fluorescent chromophores (fluorophores) within the tissues being investigated. Laser light is absorbed by these fluorophores and then after an amount of time is re-emitted as photons with a lower energy and hence of longer wavelength. The input light therefore has to be relatively high energy e.g. blue or ultraviolet, and the spectra emitted is often characteristic of the material being investigated. The fluorophores can either be endogenous such as collagen, elastin, NADH and flavins (known as autofluorescence) or externally administered exogenous fluorescent drugs that are designed to concentrate in the tissue in question e.g. malignant or pre-malignant tissue. The main problems with fluorescent measurements in tissue are the presence of several non-fluorescing chromophores such as haemoglobin, and the high levels of scattering. These two factors cause absorption and distortion of the fluorescent signal that can make interpretation and reproducibility difficult. Other technical problems include the need for relatively short wavelength laser light and sophisticated detectors due to the low emission levels.

There have however been many studies evaluating the accuracy of diagnostic fluoroscopy in a variety of clinical situations. These have included colonic, oesophageal, cervical and dermatological pathologies. These are summarised by Bigio and Mourant [Bigio and Mourant, 1997], with the general trend being a reduction in autofluorescence from abnormal tissue compared to normal. There have been two reports of its use in the diagnosis of breast cancer [Gupta et al., 1997; Tang et al., 1989] with reasonable results. Tang and colleagues introduced a technique for detecting both lung and breast cancer tissue but the numbers were too small to gain meaningful statistics. In the more recent paper by Gupta and colleagues, readings were obtained form 63 patients, 28 of whom had invasive breast carcinomas and the
remaining with benign breast pathology. They reached an overall accuracy of 98% in discriminating between malignant from benign or normal breast tissue. The main drawback however was that the measurements were obtained from ex-vivo samples so reducing the variables caused by absorption, especially from haemoglobin. It will be interesting to see if in-vivo studies produce similar results!

4.2.2 Raman Spectroscopy:
Raman spectroscopy also uses a laser light source but the way the returning signal is produced is however slightly different. The Raman effect arises when the incident light excites molecules in the sample that subsequently scatter the light. While most of this scattered light is at the same wavelength as the incident light, some is scattered at a different wavelength. This inelastically scattered light is called Raman scatter (Fig 4.2).

![Raman scatter and Elastic scatter](image)

*Figure 4.2 - Raman spectroscopy: scattered light of different wavelength to incident light*

This results in some of the light energy being converted into vibrations in the material. The nature of the returning photons depend on the tissue being interrogated and therefore have wavelength peaks which are characteristic of the vibrations. Raman can give chemically specific information, but as only a small amount of light is scattered in this way and complex molecules like proteins have so many vibrational modes, the spectra can take a long time to acquire and the forest of lines
produced can be hard to interpret. Some work has been reviewed on the ability of Raman to detect abnormal breast tissue [Manoharan et al., 1998]. The authors also presented some results of their own trial with encouraging results. The main flaws however were similar to above, in that the tissue samples were studied ex-vivo, the numbers were small (13 patients) and during analysis the same ‘training’ and ‘testing data sets’ were used (see section 8.4 for further explanation of analysis techniques). The authors however have outlined future work with in-vivo testing and greater patient numbers.

4.2.3 Near-Infrared Spectroscopy:

Near-infrared (NIR) spectroscopy relies on the absorption and scattering properties of tissues to modify a transilluminated beam of NIR photons. Unlike light in the visible spectrum, infrared light has the ability to pass through a significant depth of tissue. There has been renewed interest recently in the use of NIR spectroscopy for the detection of breast tumours [Hawrysz and Sevick-Muraca, 2000; Pogue et al., 2001; Tromberg et al., 2000; Zhu et al., 2000]. By using a NIR emitter on one side of the breast, and a detector on the opposite side, intensity values can be obtained at various wavelengths for a particular region of the breast (Fig 4.3). Most investigators have concentrated on the absorptive characteristics of haemoglobin to develop ‘haemoglobin maps’. Using the hypothesis that angiogenesis is essential for tumour growth [Folkman et al., 1989], the aim is to create an image of the relative haemoglobin concentrations within the breast by obtaining transmission intensities at different points on the breast. Tumours will therefore appear as areas of increased haemoglobin concentration.

Pogue and colleagues [Pogue et al., 2001] described a tomographic system where the breast is encircled inside a ring of 16 NIR emitters and receivers. They had some success in detecting breast lesions in 2 patients, although one was a fibroadenoma and the second, a malignant tumour. Other investigators have suggested that the relative increase in haemoglobin concentration may differentiate benign and malignant lesions [Zhu et al., 2000].
The main problem with NIR spectroscopy for characterising breast tumours is that the information gained is based on the scattering and absorptive properties of a depth of normal tissue either side of the lesion, and not just the tumour itself. This may lead to problems with spurious results from surface blood vessels, differences in the density of breast tissue between patients or even skin colour.

4.2.4 Elastic scattering and Mie theory:

Elastic Scattering Spectroscopy (ESS), refers to the spectral classification of a substance due to the refraction and absorption of photons of light without change in wavelength (hence the term 'elastic'). This is in direct comparison to Raman or fluorescent spectroscopy where the wavelength of the received photons is changed. In general, ESS has a much stronger signal i.e. total amount of returning light, than either of the above. Investigation of the spectral properties of a substance over a greater wavelength range is also achievable. The advantage ESS has over NIR spectroscopy is the sensitivity to scattering and absorption in relatively equal amounts compared to NIR where absorption is by far the predominant feature. Thus much information about the scattering properties of tissue can be gained which may have important discriminating features between different tissue types.
According to Mie theory, the degree of scattering of light increases directly with the size of the scattering particles and inversely with the wavelength. A technique, which is sensitive to the wavelength dependence of scattering, may therefore be able to detect this difference [Perelman et al., 1998]. Elastic scattering spectroscopy, sometimes referred to as diffuse reflectance spectroscopy, has gained recent interest for clinical diagnostic applications [Bigio and Mourant, 1997; Ge et al., 1998; Knoefel et al., 1996; Mourant et al., 1995; Mourant et al., 1996; Mourant et al., 1998].

When light enters tissue, it can be elastically scattered, inelastically scattered or absorbed. Scattering within tissue depends on 2 factors:

- Wavelength of light: photons with a shorter wavelength i.e. towards the blue part of the visible spectrum have a greater scattering potential than those with a longer wavelength i.e. red light [Mourant et al., 1995].
• Tissue properties: cellular structure and cellular matrix architecture, intracellular organelles, nuclear structure, hydration of intra- and extra-cellular compartments and temperature [Bigio and Mourant, 1997] all influence scattering (Fig 4.4).

As the majority of refractive surfaces in tissue are at a cellular or subcellular level, differences of these components will theoretically produce different scattering spectra. Therefore it should be possible to discriminate between cancerous tissue, where the cells and nuclei are in general larger and less uniform, and normal or benign tissue. Absorption is also a feature of an elastic scattering spectrum although the extent of the influence of absorption on the overall spectrum depends on the optical geometry of the system employed. A more detailed description of the system design is described in chapter 8.

### 4.3 Conclusion

There is much excitement about the clinical possibilities of all such spectroscopic biopsy techniques, with many researchers presenting encouraging results in a variety of anatomical areas. Elastic Scattering Spectroscopy however has several advantages compared to the others as outlined above. Further benefits of a system such as the one described here, include use of a non-laser light source, which reduces the cost, and near instantaneous readings. The difficulties in interpreting the large amount of data from a broad spectrum and a multitude of absorbers may prove difficult, but also may ultimately lead to the best diagnostic accuracy by incorporating information about a variety of tissue components.
CHAPTER 5 THERMAL ABLATIVE TECHNIQUES

5.1 INTRODUCTION

The use of extreme heat to treat breast cancer has been documented as far back as 3000-2500 BC with mention of "cauterization with a fire stick" [Breasted, 1930]. As heat has the ability to destroy tissue, as evidenced by a simple skin burn, the early physicians saw its potential to destroy cancerous lesions, with the added benefit of achieving cautery to blood vessels simultaneously. Thermal destruction of tissue can occur in two ways. Traditionally, any thermal ablative technique has relied on explosive vaporisation (true ablation) by using a high energy source to cause intracellular water to reach 100°C and therefore vaporise, causing rupture of the cell membrane. The second method is by protein denaturing (thermal coagulation) which utilises a lower energy source to raise the temperature above 50°C for a set period of time. It has been shown that temperatures as low as 41°C will cause cell death if exposure is prolonged [Overgaard and Suit, 1979], but as the temperature is increased the time to achieve this decreases in a linear fashion [Borrelli et al., 1990].

The mechanism of tumour cell destruction is complex but most probably is due to reduced pH, hypoglycaemia, vasoconstriction, increased capillary permeability and oedema [Vaupel et al., 1988]. It should be noted however that it is not only high temperatures which can destroy tissue, extreme cold has also been used for many years as a method for destroying tissue [Gage, 1998]. Indeed ‘frostbite’ can be just as damaging to tissue, if not more so, than a burn!

Following the relatively recent development of new methods of achieving interstitial thermal ablation, such ‘thermal destructive’ methods are now gaining interest for the treatment of a variety of benign and malignant tumours where evaporative ablation is undesirable. This has however only come about relatively recently, through the advent of more sophisticated imaging, and has lead to ever increasing evidence of its efficacy along with a rapid expansion of its possible applications [Lamb and Gedroyc, 1997].
5.2 THERMAL ABLATIVE TECHNIQUES

There are five main methods of thermal ablation, all of which can be adapted for minimally invasive therapy. Three of these; laser photocoagulation, radiofrequency and microwaves, utilise energy from different areas of the electromagnetic spectrum. The other two are ultrasound (pressure waves) and cryotherapy (direct damage by freezing). The main goal with any thermal treatment is to achieve local destruction of solid tumours, with minimal destruction of surrounding tissues and equal or less morbidity compared to conventional surgery. Laser photocoagulation is considered in detail in section 5.3. The other four methods are also discussed individually below. Another important factor is accurate assessment of the extent of tumour destruction, either at the time of treatment or shortly afterwards. To this end, various imaging modalities have been tested including ultrasound scanning, CT and MRI. These will be discussed further in section 5.5.

5.2.1 Radiofrequency (RF)

RF thermal ablation of tissue utilises a high frequency, alternating current, to cause local heating of tissue through resistance. This heating is caused by friction due to the excitation and motion of intracellular ions in a similar manner to electrosurgical coagulation used for cautery. Various possible uses have been described including the treatment of liver tumours [Rhim and Dodd, 1999], benign bone tumours [Barei et al., 2000], prostatic hyperplasia [Chapple et al., 1999] and breast carcinoma [Jeffrey et al., 1999].

For interstitial treatments various probe designs are available, although the basic concept remains constant with a shielded shaft and an exposed tip that acts as the active electrode. A further dispersive electrode pad is placed on the patient’s body to complete the circuit. Electrical current flows from the tip of the probe into the surrounding tissue with the size of the coagulated area (thermal lesion) depending on the power output, the length of exposed electrode and the duration of treatment [Goldberg et al., 1995]. The frequency used is typically around 500KHz with power outputs of up to 200 Watts (usually around 50 Watts). Probes are 2-3mm in diameter and can be placed into the target lesion under x-ray, ultrasound or MR guidance.
Some investigators have demonstrated that a larger thermal lesion can be produced with the use of a cooled probe [Goldberg et al., 1996] (Fig 5.1). Cold saline is pumped through a channel in the probe to maintain a temperature at the exposed tip of 20-25°C. This reduces charring at the end of the electrode, which can hinder dissipation of the electrical energy into the surrounding tissues. At the completion of the treatment there is enough latent heat within the surrounding area to increase the temperature of the tissue in direct contact with the probe to around 60°C [Solbiati et al., 1997]. This is sufficient to cause thermal coagulation of any cells that may have escaped damage due to the direct cooling effect of the probe. Another suggested method of increasing the thermal lesion size is by the use of bipolar technique where two interstitial probes are placed a short distance apart (2cm) within the tissue to be treated. Lesions of up to 8 cm$^3$ compared to 1.0 to 1.8 cm$^3$ with a monopolar method, have been demonstrated [Rossi et al., 1996].

The main drawback is that temperature monitoring with MRI poses difficulties because of the interference of the RF therapy waves with the MR image, although techniques such as RF switching [Zhang et al., 1998] have helped to eliminate this problem. Also MR compatible probes are not yet commercially available. Such monitoring techniques are however currently being evaluated [Boaz et al., 1998].
5.2.2 Percutaneous microwave coagulation therapy (PMCT)

PMCT has been mostly investigated for the destruction of both primary and secondary liver tumours [Hamazoe et al., 1995; Matsukawa et al., 1997; Seki et al., 1999]. The microwaves are generated by a magnetron in a microwave generator and passed through a high frequency coaxial cable and via a probe to an electrode tip. The microwaves pass from the end into the surrounding tissues where the energy is absorbed, producing heat by molecular vibration of dipoles. The typical frequency used is 2450 MHz with power outputs of around 60-80 Watts [Matsukawa et al., 1997]. The probes are generally placed through the centre of a percutaneous ‘guide needle’ which is initially placed into the centre of the tumour under ultrasound guidance. The extent of tissue damage has been found to relate to the power output of the microwave generator although thermal lesions of up to 3.6cm in maximal diameter have been produced using a power output of 80 Watts for 60 seconds [Seki et al., 1999]. The maximum power output is however limited by the diameter of the electrode, therefore probes with greater diameters are needed to achieve larger thermal lesions. Some authors have suggested that treating several overlapping areas for shorter times e.g. 20 seconds, 5mm apart, will reduce the incidence of charring. Similar to radiofrequency, this may interfere with penetrance of the microwaves into the surrounding tissues [Hamazoe et al., 1995].

MR thermal imaging has also been investigated for this modality [Moriarty et al., 1998] with the use of MR compatible probes constructed from non-ferromagnetic materials. This is also possible, as the frequency of the microwaves do not interfere with the MR signal.

5.2.3 High Intensity Focused ultrasound (HIFU)

HIFU is another method under current investigation, although the principle was described as far back as 1942 [Lynn et al., 1942]. There has however been a recent resurgence of research into its possible use for the treatment of a variety of benign and malignant conditions [Chen et al., 1999]. The major advantage of this technique over the other modalities is that it is completely non-invasive i.e. no skin incision or puncture is necessary. The theory is relatively straightforward: Medical ultrasound mainly operates at frequencies between 0.5 and 10MHz giving wavelengths of 0.15-
3mm. These short wavelengths can therefore be focused into an area less than 1mm\(^2\). By increasing the power output, local heating occurs to the tissue within this focal area by absorption of the energy waves. Once the threshold for protein denaturing has been reached, i.e. around 60°C for a few seconds, irreversible tissue damage occurs. It has been demonstrated that the level of heating, and hence tissue damage, can be directly related to the power output of the US system [Hynynen et al., 1997]. The area of heated tissue is very localised with a sharp demarcation between heat damaged and normal tissue [ter Haar, 1998]. This is especially attractive in areas where it is desirable to minimise destruction of adjacent healthy tissue e.g. within the brain.

Most currently available focused ultrasound systems are spherically curved with a small fixed focus. As the mechanical wave does not interfere with MR imaging and systems can be made which are non-magnetic, focused single transducers have been developed which are MR compatible [Cline et al., 1995]. Both targeting and monitoring of treatments with real time temperature mapping is therefore possible [Hynynen et al., 1997; McDannold et al., 1998].

The main disadvantage however is that not all tissues are accessible as there must be an acoustic window between the ultrasound transducer and the target lesion i.e. US passes poorly through bone or gas due to absorption or reflection respectively. It is also at present quite time consuming to treat large volumes of tissue as only a few cubic millimetres are destroyed with each sonication. This problem may however be solved with the use of phased array systems.

### 5.2.4 Cryotherapy

The use of cryotherapy for the destruction of tumours was first described in the mid 19th Century by James Arnott by using iced saline solution at -20°C to treat locally advanced cancer [Gage, 1998]. In recent times however it's main use has been in treating skin lesions. With the advent of interstitial probes however, other possible applications are currently under investigation [Gage, 1992], especially for the treatment of solid tumours.
Cryotherapy relies on freezing of tissues beyond a threshold for cellular destruction (<50°C). With a rapid cooling rate, intracellular ice causes direct damage to the intracellular structures. With slower cooling, extracellular ice causes dehydration of adjacent cells due to the resulting osmotic shift. Also thrombosis of small vessels leads to ischaemic damage. The probes themselves consist of an insulated shaft and exposed tip through which liquid nitrogen at -195°C is circulated. During treatment, tissues surrounding the tip freeze in a fairly uniform manner giving the characteristic ‘ice-ball’. Optimum treatment parameters have not yet been calculated but rapidity of freezing, lower temperatures and slow thawing all increase its effectiveness [Gage and Baust, 1998].

One of the problems encountered however is the unpredictability of cellular death at the periphery of the ice-ball, as some viable cells have been found in this region in experimental studies [Gage and Baust, 1998]. This has lead to some investigators employing a freeze-thaw, refreeze cycle [Staren et al., 1997]. An alternative approach would be to extend the ice-ball well outside the boundaries of the tumour but this may result in excess damage to healthy surrounding tissue. Another problem is the size of the probe has to be relatively large (3mm diameter) in order to produce sufficient cooling. This makes minimally invasive, interstitial treatment more difficult, especially for less accessible areas. Cited complications have been in the treatment of liver tumours where the degree of haemorrhage and thrombocytopenia has been found to be greater than for other techniques e.g. radiofrequency ablation [Bilchik et al., 2000].

Most researchers have employed ultrasound to determine the extent of the ice-ball as reflection of the ultrasound waves at the interface between non-frozen tissue and the ice-ball produces a hyperechoic rim with posterior acoustic shadowing. More recently, following the development of glass probes, the use of MR imaging has been investigated as this has multiplanar imaging capabilities. Frozen tissue has a dramatically reduced T2 time which leads to a signal void within the ice-ball [Matsumoto et al., 1992]. With gradient echo sequences, the extent of freezing can be monitored in real-time, allowing any adjustment of the probe position to be carried out.
5.3 **INTERSTITIAL LASER PHOTOCOAGULATION (ILP)**

5.3.1 **Introduction**

Interstitial laser photocoagulation relies on delivery of laser light via optic fibres placed directly into the centre of tumours. The energy is absorbed within the surrounding tissues causing a local thermal necrosis [Bown, 1983]. This 'dead' tissue is subsequently resorbed by the body's normal healing process [Lai et al., 1999; Steger et al., 1992]. There have been several reports in the recent literature of the use of ILP in different clinical situations but most have concentrated on liver, prostate and breast [Bown, 1998].

The fibres used are very fine (400 microns) and can be placed down small gauge cannula (typically 18 gauge). Various lasers have been tried but most investigators now use either a semiconductor diode laser or Nd:Yag at wavelengths between 805 and 1064nm. Laser heat delivery is particularly useful in the MR setting as neither interferes with each other. Also necrosis seen on post treatment MR scans has been correlated accurately with histological lesions [Mumtaz et al., 1996], which therefore creates a useful method of determining the extent of thermal damage.

The largest reported group of patients treated with ILP has been for liver metastases. Amin and co-workers at our own institution described their experience in the treatment of 55 liver tumours in 21 patients and found 82% of patients fulfilled the UICC criteria for at least a partial response i.e. >50% reduction in tumour volume [Amin et al., 1993]. Recent results from the same group have concentrated on long-term outcome in over 500 treated patients. This has reported a median survival of 27 months and a 5-year survival rate of 26%. This is compared to patient outcomes following operative treatment for metastases at the same institution, of 33 months and 30% respectively [Dodd et al., 2000]. Another group reported a mean survival of 35 months [Vogl et al., 1997]. Five-year survival was however not presented.

Some work has been published on the treatment of benign prostatic hyperplasia. One study has shown marked improvement to patients' symptoms and uroflowmetry following treatment [de la Rosette et al., 1997]. Another possible clinical use has been suggested in the treatment of benign uterine fibroids. Law et al demonstrated a
mean reduction in tumour volume following ILP of 37.5% at 3 months in 12 female volunteers [Law et al., 1999]. All patients reported an improvement in symptoms of abdominal discomfort, urinary frequency and dysmenorrhea.

5.2.2 Principles of ILP in Breast Disease

For treatment of tumours of the breast, the procedure can be carried out under local anaesthetic and mild sedation. Up to 4 needles can be placed directly into the tumour either under ultrasound or MR guidance. Optic fibres are then placed down the centre of the cannula. Once in place the needle is withdrawn slightly so the bare ends of the fibre lie within the tumour. Previous work has demonstrated that the size of the laser induced necrosis is more predictable when the bare end of the fibre is pre-charred before insertion [Harries et al., 1994]. This in effect causes a deposit of carbon particles at the tip of the fibre, which absorb the laser energy to produce heat. Tissue damage is therefore produced solely by a thermal mechanism, rather than by direct interaction of laser light photons with tissue. Without charring, some patients have shown to have no discernible treatment effect. The reasons for this are unclear but may relate to the absorption of the available energy by a larger volume of tissue so there is not enough energy per unit volume of tissue to produce a biological effect.

At our unit we use a semiconductor diode laser which creates a laser light source at 805nm. This is just beyond the visible spectrum in the near infrared. Once the fibres are safely secured in position the laser is activated for 10 minutes at 2-3 watts per fibre. This gives a characteristic pathological lesion with central charring and liquifactive necrosis, a surrounding area of heat fixed tissue consistent with coagulative necrosis and an outer haemorrhagic rim (Fig 5.2).

Microscopically, the central area contains carbonised and necrotic debris. Within the surrounding heat fixed zone, the features are of morphologically normal cells but with featureless, hyperchromatic, smeared nuclei and a hypereosinophilic cytoplasm consistent with coagulative necrosis. Within the haemorrhagic peripheral zone the cells are less severely disrupted, with the only indication of cell damage being slightly hyperchromatic nuclei. Also within this zone are proliferating fibroblasts and
damaged blood vessels with extravasation of red blood cells. It has been shown using immunohistochemical staining (NADH-diaphorase) that all these areas contain dead tissue [Mumtaz et al., 1996]. This finding has been recently confirmed by a group in Stanford who are evaluating radiofrequency ablation of breast tumours [Jeffrey et al., 1999].

Figure 5.2 – macroscopic specimen of laser treated lesion demonstrating 3 'thermal zones'.

5.4 Current status of ILP and other thermal techniques in breast

Conservative treatment for breast cancer is now well established, with long term outcome similar to patients treated by mastectomy [Veronesi et al., 1994]. It is therefore recognised by most, that lumpectomy or wide local excision serves only to control local disease (see chapter 1). Such evidence gives support to the use of other modalities for local destruction of breast tumours. The aim of thermal ablation is
therefore to destroy all viable tumour cells along with a reasonable surrounding rim of normal tissue.

The earliest reported case of a laser treated breast tumour was by Steger in 1989 [Steger et al., 1989]. Since then there have only been a few reports of preliminary studies investigating the feasibility of treating breast cancers in this way [Akimov et al., 1998; Dowlatshahi et al., 2000; Harries et al., 1994; Mumtaz et al., 1996]. These are summarised below.

Harries et al, at our own institution, treated 44 patients with breast cancer prior to having routine surgical excision. Predictable areas of tumour necrosis (median 14mm) were achieved using a pre-charred fibre. Only one minor complication occurred which was a slight haemorrhage from the puncture site. Mumtaz reported the outcomes of a further 20 patients with similar results but no complications. Ultrasound, CT and MRI assessment of tumour ablation was also explored. This is discussed further in section 5.5.

A second group in Ukraine (Akimov et al) more recently presented their results in the treatment of 35 breast cancer patients. Their technique was slightly different in that they used a Nd:YAG laser and at slightly higher power outputs (2.5-6 Watts vs 2-2.5 Watts). Twenty-eight patients underwent laser therapy prior to surgery and a further 7 had ILP as their only invasive treatment. All tumours again showed laser induced necrosis with increasing diameter of destruction with increasing power. There were slightly more complications however with 4 minor skin burns and one gaseous rupture. These are probably related to the increased Wattage employed. As the first two studies have shown, 2.5 Watts is adequate to produce cell death without the fear of evaporative ablation. Of the 7 patients who did not undergo surgery, 3 were post-menopausal with severe co-existing medical disease. Following ILP they all showed a good response to treatment with tumour shrinkage. A further 3 patients with metastases at the time of diagnosis underwent palliative ILP. Two of the three showed a reasonable local response to treatment. The third however showed no change in tumour size. The final patient was a young woman who refused surgery. Unfortunately she progressed quickly with aggressive metastatic disease. Overall however their results were again very encouraging with minimum complication.
The most recent study by Dowlatshahi et al, presented the results of 36 patients with tumours less than 2cm treated with an 805nm Diode laser. They employed a water-cooled diffusing fibre that allows light to spread from the tip equally in all directions and also maintains the temperature below 100°C. All patients subsequently underwent surgery 8 weeks later. Overall results showed complete tumour ablation in 66% using a single probe. Only 2 minor skin burns were encountered and no serious complications.

The only other report of thermal ablation of human breast tumours in-vivo was by Jeffrey and co-workers who described the effects of radiofrequency ablation prior to routine surgery in 5 patients [Jeffrey et al., 1999]. Using a single 15-gauge electrode they demonstrated tumour ablation in all patients with no complication. The aim was to assess the feasibility of destroying breast tumours by this method and they intentionally left part of the tumour untreated to evaluate the margin between ablated and non-ablated tissue. The size of necrosis was similar to laser therapy although the treatment times were much longer, 30 minutes versus an average of 10 minutes for ILP.

The above studies have demonstrated the feasibility of producing local destruction of breast cancers using thermal ablative techniques. None of the studies however set out to completely destroy breast tumours, although this was achieved in many cases. The main challenge however is to produce a means of assessing residual tumour in-vivo. Several imaging methods have been evaluated to achieve this. These are outlined in the following section.

5.5 IMAGING FOR ILP

To date all studies have relied on histological assessment for definitive detection of residual tumour following thermal ablation. Looking into the future however, for ILP to be an accepted alternative treatment for breast cancer, or any other tumour for that matter, there must be a verifiable and accurate means of assessing the extent of tumour necrosis. An ideal imaging modality would allow real-time assessment of the extent of tissue destruction. This would allow adjustment of treatment parameters or
re-positioning of probes/fibres to ensure that there has been complete ablation of the
tumour, with a 'safe' surrounding rim of tissue. Furthermore it is also important to be
able to accurately detect any residual tumour or recurrence at any point in time
following ILP. To date, four imaging modalities have been evaluated for this role;
ultrasound, CT, MRI and PET.

Some of the earliest work with ILP for liver and breast tumours investigated the
accuracy of ultrasound and CT at determining tissue necrosis [Amin et al.,
1993;Harries et al., 1994;Steger et al., 1989]. Steger found ultrasound useful at
directing and placing the fibres prior to treatment but did not find it accurate at
determining the extent of thermal damage. The two studies by Amin and Harries
reported similar findings. In the more recent study however by Dowlatshahi, they
suggested that the use of an ultrasound intravenous contrast agent (Optison) could
detect the loss of blood flow to coagulated tissue [Dowlatshahi et al., 2000]. Data
regarding precise histopathological correlation was however not presented and this
method may therefore not be accurate enough to detect small areas of residual
tumour. The same study presented the data on the use of PET scanning, pre- and
post- laser treatment. They found reasonable correlation between isotope uptake and
residual tumour but this was only performed in a limited group of 4 patients.

Before the widespread availability of MRI, dynamic enhanced CT was investigated
for determining the extent of tissue destruction and residual tumour [Amin et al.,
1993;Harries et al., 1994]. Characteristically the necrotic tissue appeared as
completely avascular areas. This was confirmed by core-cut biopsy, which did not
find any residual viable tumour in these areas. In the paper by Harries, 6 patients had
CT with subsequent histopathological correlation to the resected specimen. This
showed reasonable accuracy at detecting the volume of treated tissue. There have
however been no larger trials evaluating CT in the breast. Also, as CT is not a
conventional imaging modality for breast cancer, its role is of doubtful certainty.

It is now widely accepted that MRI has the best chance of achieving the above goals
for several reasons:
1) Thermally destroyed tumours show signal change on both T1 and T2 MR sequences [Klotz et al., 1997; Solbiati et al., 1997] and also lose their uptake of enhancement agents such as gadolinium [Mumtaz et al., 1996].

2) MR uses non-ionising radiation and therefore reduces radiation exposure to patients and staff as compared with CT.

3) Real time temperature mapping during treatment is possible through several temperature dependent MR parameters. These are signal intensity (SI) change due to T1 effects [Cline et al., 1994], SI change due to diffusion [Bleier et al., 1991] and signal phase change due to Proton frequency shift [de Poorter et al., 1995].

There have been a multitude of papers in the recent literature exploring the possibilities of using MR to guide, monitor and follow up thermally ablated tumours, some of which are mentioned above. Only one study however has specifically assessed its accuracy following ILP in breast cancer [Mumtaz et al., 1996]. Pre- and post-treatment scans were performed and compared to the pathological specimen following routine surgical excision. The correlation coefficients for the diameter of tissue necrosis and residual tumour were 0.80 and 0.86 respectively. Immediate imaging (within 24 hours following ILP) did not however show good correlation with the final histology, whereas delayed imaging i.e. >24 hours, was much more accurate. The reasons for this were unclear. Taking into account slight discrepancies in measurement between in-vivo images and ex-vivo specimens, and also a reported maximum discrepancy in any measured diameter of 3mm, these results show that MR has great potential in achieving the goals set out above. With increasing interest in minimally invasive thermal ablation for a variety of tumours, these results should hopefully be given further support. Once fully verified as an accurate means of at least initially detecting residual tumour, ILP will surely gain wider acceptance as a viable alternative to surgery for some patients with breast cancer.
CHAPTER 6  FIBROADENOMAS

6.1 Natural history

The benign fibroadenoma of the breast has probably caused more unnecessary anguish to young women than any other breast symptom. They are by far the most common cause of a discrete breast lump in young women and reach a peak incidence between the ages of 20 and 30 [Dixon et al., 1996]. They are however not an uncommon finding in older women and are often only discovered through routine screening mammography, where they appear as well circumscribed lesions, often with characteristic “popcorn calcification”. Originally classified as a true benign neoplasm (hence -adenoma), they are now regarded as an aberration in the normal changes that occur in the breast throughout life. This conclusion has partly been due to the increased detection of occult lesions following the widespread introduction of ultrasound for breast imaging, which can detect impalpable fibroadenomas as small as a few millimetres in diameter. This has lead to introduction of the term ANDI (aberrations of normal development and involution) which provides a framework for a new classification system of many benign breast changes including fibroadenomas [Hughes et al., 1987]. Much debate has arisen in recent years about the natural history of fibroadenomas as some will inevitably regress spontaneously, raising the question of whether all fibroadenomas should be excised or whether it is safe to treat conservatively, by observation alone.

Several studies have attempted to determine the outcome of fibroadenomas if left in-situ [Carty et al., 1995; Dent and Cant, 1989; Dixon et al., 1996; Sainsbury et al., 1988; Smallwood, 1989; Wilkinson et al., 1989]. The results of these studies are summarised in table 6.1.

The studies by Sainsbury and Carty were however biased towards regression as some patients whose lesions were enlarging had excision and were subsequently excluded from the analysis. The findings were however similar and in that they all demonstrated that some will regress, some will remain static and some will enlarge. The individual outcome of an untreated fibroadenoma is however still unpredictable.
Table 6.1 - Outcome of conservative treatment of fibroadenomas.

There has been much interest in assessing any possible risk factors associated with an increased chance of developing fibroadenomas. Suggested predisposing factors have been as varied (and predictable) as social class, to the much maligned caffeine [Boyle et al., 1984], which unsurprisingly does not increase risk. Fentiman summarises the evidence for a variety of factors [Fentiman, 1990]. Family history of breast cancer, use of oral contraceptives, endocrine abnormalities and reproductive factors such as age at menarche or first pregnancy, nulliparity or early menopause, do not influence the risk of developing fibroadenomas. There is however some evidence that decreasing weight and high socio-economic status confer an increased risk. It has been postulated however that fibroadenomas are more prominent, and hence more easily detectable in a non-fatty breast. Also, wealthier women are more likely to be health conscious and therefore will seek medical opinion more readily. The main conclusions therefore are that there seem to be no definite predisposing factors for the development of these benign lesions.

Growth of a fibroadenoma is generally slow but may be rapid during pregnancy. Rarely they can infarct, mimicking a malignant process, and this is thought to be due to either outstripping of the blood supply or by thrombosis of a feeding vessel [Silverberg and Masood, 1997]. There is much controversy about the possibility of malignant change within a fibroadenoma. Although there have been case reports of
carcinoma arising from within a fibroadenoma, as there is no strong epidemiological evidence that a history of fibroadenoma predisposes to malignancy, most have concluded that malignant change occurs simply by chance and is no more likely than in completely normal breast tissue [Dixon, 1991; Ozzello and Gump, 1985]. After all, both fibroadenomas and carcinomas are both relatively common, so the chance of them co-existing is not unreasonable.

6.2 Pathology

Fibroadenomas, as mentioned above, are now generally regarded as an aberration of normal development rather than a true neoplasm. There is histological evidence that they arise from the breast lobule and this is further supported by several features of fibroadenomas i.e. they arise at a time when lobular development is maximal, they enlarge during lactation and secrete milk, and involute during the menopause. Further evidence as to their origin is that proliferation of stroma within a lobule is hormone dependent whereas that of the remainder of the breast parenchyma is not. Also, on the rare occasions when carcinomas are found within a fibroadenoma at histology, they have a high incidence of lobular carcinoma in-situ [Silverberg and Masood, 1997].

Macroscopically, the lesion is a firm, rubbery mass and is sharply demarcated from the surrounding breast tissue by a white, glistening capsule. The cut surface reveals pink or grey bulging tissue, which is separated into lobules by fibrous septa.

Microscopically, they consist of a benign local proliferation of epithelial cells and fibroblasts. Classically two histological types are described but these are now thought to be irrelevant as both can co-exist in the same lesion. The pericanalicular form consists of well preserved glandular structures within a loosely surrounding connective tissue stroma. Glandular proliferation with moderate atypia is often seen but this is readily distinguishable from carcinoma. In the intracanalicular form, a hyperplastic fibrous stroma causes characteristic elongation and compression of the glandular structures, thus giving the appearance of distortion or atrophy. Growth of a fibroadenoma is due to stromal proliferation with the secretary cells constituting only a single layer.
Two other variations of fibroadenomas are often described in the literature. *Giant fibroadenoma* is generally regarded as a rapidly growing type that is over 5cm in diameter. Histologically they are usually similar to the common type as described above and are therefore diagnosed by clinical size alone. *Juvenile fibroadenoma* is a rapidly growing, and often bilateral, tumour of teenage girls. Although there is very active proliferation of both the epithelial and connective tissue elements, again the diagnosis is often only reached from clinical grounds i.e. the age of the patient and the rapidity of growth [Silverberg and Masood, 1997].

### 6.3 Diagnosis

The pre-operative diagnosis of a fibroadenoma can only be confidently reached by “triple assessment” as outlined in greater detail in chapter 2. In summary this involves clinical examination, radiological imaging (usually ultrasound) and biopsy. It has been shown that if each of these three diagnostic modalities is confidently positive for fibroadenoma, then the combined accuracy approaches 100% [Drew et al., 1999; Eltahir et al., 1999].

On examination, fibroadenomas are described as firm, smooth, rubbery and extremely mobile. It is this latter characteristic that has earned them the colloquial terminology “breast mouse”. They are generally round but are often found to be lobulated. Frequently more than one lesion is found at examination but this should not cause a premature diagnosis of a benign lesion as cancer can co-exist. Each palpable lump should therefore be considered separately.

Ultrasound is generally the imaging modality of choice as most patients with fibroadenomas are young and therefore have dense glandular breasts, making interpretation of mammograms difficult. Characteristically they appear as well circumscribed, slightly hypoechoic, homogeneous structures. Often they are slightly lobulated and may appear as two or more apparently coalesced lesions. There may also be evidence of slight distortion of the surrounding stroma with the impression of tissues being gently pushed apart. This is in contrast to a carcinoma, which may have associated irregular distortion of the surrounding breast tissue. Another clue to the
diagnosis is a slightly oval shape with the long axis perpendicular to the chest wall. There has been some investigation into the accuracy of MR imaging for assessment of fibroadenomas but this modality is still in its relative infancy and has had varying success [el Yousef et al., 1985; Hochman et al., 1997; Weinstein et al., 1999].

In the UK most centres use fine needle aspiration cytology (FNAC) for confirmation of the clinical and ultrasound findings. This is a well-validated technique although is operator dependent (see section 2.4.1). The aspirates from fibroadenomas are usually cellular with two cell types corresponding to the epithelial and stromal elements. The epithelial cells characteristically cluster into monolayered sheets with finger-like projections. The stromal elements appear as small cells with naked nuclei either mixed with the epithelial cells or lying freely in the background. Occasionally atypia is seen leading to a label of C3. Rarely this can be significant enough to mimic carcinoma. In North America, core-cut biopsy is more readily carried out which inevitably increases accuracy due to structural information. When combined with examination and ultrasound however, there is little evidence that the overall combined accuracy is superior to FNAC, with the added morbidity of increased trauma to the breast and therefore increased patient discomfort.

6.4 TREATMENT

Traditional treatment for all fibroadenomas has been surgical excision. This however carries with it the risks of general anaesthetic, morbidity from the surgical procedure and subsequent scarring to the breast, which can be aesthetically important to young women. Now that it is accepted that these lesions are not true neoplasms, but are in fact probably an exaggeration of normal development, the necessity for excision is being increasingly questioned. Also reassuringly they do not appear to carry an increased risk of malignancy. In fact, most clinicians would now agree that for most women, excision is not warranted and they should be actively discouraged from undergoing such treatment. The debate therefore has now centred on the ability of aforementioned diagnostic techniques to rule out malignancy. This is very much age dependant in that the prevalence of breast cancer and fibroadenomas are with age, directly and indirectly proportional respectively.
Several studies have aimed to determine the accuracy of diagnosis, and therefore the relative safety of leaving a fibroadenoma untreated [Sainsbury, 1988 #182; Carty, 1995 #185; Cant, 1995 #829; Dent, 1989 #831; Smallwood, 1989 #848; Wilkinson, 1989 #850; Dixon, 1996 #179]. Some have also, on the basis of their findings, recommended an age cut off for conservative treatment over which the risks of missing malignancy are unacceptably high. The results of these studies are summarised in Table 6.2.

<table>
<thead>
<tr>
<th>Author</th>
<th>No. of patients</th>
<th>Diagnosis:</th>
<th>False negative rate</th>
<th>Recommended age cut off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sainsbury</td>
<td>102</td>
<td>Examination &amp; FNAC only</td>
<td>0%</td>
<td>35</td>
</tr>
<tr>
<td>Carty</td>
<td>70</td>
<td>Triple assessment</td>
<td>0%</td>
<td>30</td>
</tr>
<tr>
<td>Cant</td>
<td>321</td>
<td>Examination &amp; FNAC only</td>
<td>0%</td>
<td>25</td>
</tr>
<tr>
<td>Dixon</td>
<td>202</td>
<td>Triple assessment</td>
<td>0%</td>
<td>40</td>
</tr>
<tr>
<td>Smallwood</td>
<td>95</td>
<td>Triple assessment</td>
<td>0%</td>
<td>35</td>
</tr>
<tr>
<td>Wilkinson</td>
<td>110</td>
<td>Examination &amp; FNAC only</td>
<td>0%</td>
<td>35</td>
</tr>
</tbody>
</table>

Table 6.2 – Safety of conservative management of fibroadenoma

There is no doubt that for some women, non-surgical management of a fibroadenoma is an option. It should be pointed out however that in several of the aforementioned studies, accuracy rates for the specific diagnosis of a fibroadenoma ranged from 68 to 100%. Those studies employing ultrasound, which is now standard practice, generally had better diagnostic accuracy. Certainly other benign lesions were misdiagnosed but encouragingly no cancers were missed. Another factor that some authors pointed out was patient acceptability of conservative treatment. This ranged from 21 to 92% [Cant et al., 1995; Dixon et al., 1996]. This may have been due to the way in which information was presented to the patient.
In summary, current evidence would suggest that conservative management for fibroadenomas diagnosed by triple assessment is safe for most women, probably with a cut off age of 35 years. Such management is still however in its infancy and it will be important for all surgical teams advocating leaving fibroadenomas in-situ, to continually audit their own practice. There certainly is a case for pooling of data on a national basis to ensure the safety of women going down this line. Undoubtedly, there will be the rare patient whose carcinoma will slip through the net. Sunday tabloid coverage of such cases should however not discourage those caring for these patients to return to “excision for all”. It should always be emphasised that surgical management is also not without risk. Despite ongoing patient education, there will always be some who will still request excision. This should always be respected. With laser photocoagulation however, they may have another option!
CHAPTER 7 AIMS OF THESIS

This thesis is an investigation and evaluation of the ‘optical diagnosis and treatment of breast tumours’. This statement incorporates two distinct new technologies, namely ‘Optical Biopsy’ (diagnosis) and 'Interstitial Laser Photocoagulation' (treatment). Although the common theme is the use of ‘light’, as there are two main studies, it is appropriate to consider the aims of each part of the thesis separately.

7.1 OPTICAL BIOPSY

Optical biopsy (OB) is a novel new way of evaluating various types of tissue, with the intention of producing a diagnostic tool. The principles of OB, including data from previous work, are described in detail in chapter 4.

The aim of this part of the thesis is to evaluate the ability of an optical system, based on the theory of elastic scattering spectroscopy, to discriminate between different types of breast tissue and to determine the metastatic status of axillary lymph nodes in patients with breast cancer. Two main challenges were faced:

1) To accurately collect data from a variety of breast tissues, including axillary lymph nodes, and precisely correlate the optical readings with a conventional histological specimen.

2) To characterise the optical data, using computer assisted analysis programmes, produce subsequent algorithms for analysis of new data, and ultimately test these algorithms with ‘unknown’ data, to determine the accuracy of the entire system.

It is envisaged that the ultimate goal is to produce a commercially viable, fully validated diagnostic aid, which has the ability to characterise unknown breast tissue in a variety of clinical settings. The main advantage of such a system is the ability to achieve ‘real time’ analysis. This commercial aspect however does not constitute part of the aims of this thesis.
7.2 INTERSTITIAL LASER PHOTOCOAGULATION

Interstitial Laser Photocoagulation (ILP) utilises laser energy, to produce local heating of tissue, causing thermal necrosis of a target lesion. The principles and design of the system are described in chapter 5 (section 5.3).

The aim of this second part of the thesis is to evaluate the safety and efficacy of treating both benign and malignant tumours of the breast. Two main studies with slightly different aims were designed to achieve this.

1) ILP of Fibroadenomas:
   a) To determine the long term healing of laser treated breast tumours, by treating patients with benign fibroadenomas of the breast with ILP.
   b) A second aim of this study is to evaluate the ability of ILP as stand alone treatment for such benign tumours.

2) ILP for Breast Cancers:
   a) To investigate the ability of ILP to completely destroy a primary breast cancer in-situ.
   b) Evaluation of Magnetic Resonance Imaging as a method of determining the extent of laser induced necrosis and the presence of any residual viable tumour.

The main endpoint of ILP is that it must be less traumatic to the patient, cosmetically superior, and equally as safe with similar or less morbidity and no added mortality. If this can be achieved, then ILP may become a viable alternative to surgery for both benign and malignant tumours of the breast in a selected group of patients.
CHAPTER 8
OPTICAL BIOPSY - TECHNICAL DEVELOPMENT

8.1 INTRODUCTION
The main challenge of this thesis was to progress from preliminary ex-vivo studies to development of systems both for the in-vivo measurement of breast tissue, and also the subsequent analysis of the collected data. There were however several technical questions that needed to be answered, some of which were known about at the outset, but some of which were only encountered as the research progressed. Each individual problem, its investigation and subsequent solution are outlined below. In chapter eight, the methods, results and conclusions of the actual in-vivo testing of the system will be presented.

8.2 SYSTEM DESIGN:
The optical biopsy system consists of a pulsed white light source, delivery and return fibres, and a spectrometer interfaced to a laptop computer (fig 8.1).

Figure 8.1 – Optical biopsy system with schematic representation
The light source employed is a xenon arc lamp (EG&G) with a wavelength range of 300 to 900nm (fig 8.2). Short pulses of light (10 msec) are focused and then coupled to a fibre-optic ‘delivery’ fibre, the distal end of which is placed in gentle contact with the tissue to be interrogated. Scattered light is collected and passed to the spectrometer (Ocean Optics Dunedin, Florida) via the ‘return’ fibre, which is adjacent to the delivery fibre (see chapter 4 - fig 4.4). The spectrometer consists of a diffraction grating and linear CCD array that detects the intensity of the received light for each individual wavelength. This information is relayed to the laptop computer (Compaq Armada) via a cable link and PCMCIA card (DAQ700). Custom software integrates firing of the lamp and collection of the data. There is also a feedback mechanism that stops the pulses of light when the number of returning photons for any wavelength reaches 3000 (the CCD has a maximum range of 4000 counts). The returning light signal is presented in graphical form as a function of wavelength verses intensity.

![Figure 8.2 - spectral characteristics of xenon lamp](image)

Prior to any clinical measurements the ESS system is calibrated with a reflectance standard (Spectralon™, Labsphere, Inc., North Sutton, New Hampshire). This has a diffuse reflectance >98%, over the entire wavelength range of the system and is considered to be ‘spectrally flat’ i.e. reflects all wavelengths equally. Such
referencing of the system to a known standard allows the normalisation of spectral data between 'optical biopsies'. Variations within the system such as different probes, coupling to the xenon lamp or spectrometer, overall temperature, transmission along the fibres, spectrometer drifts etc., are therefore minimised. All components of the system which are either in direct contact with the patient, such as the probes and optic fibres, or indirectly i.e. the reference standard, are sterilised by autoclave prior to any procedure. The spectralon itself is also housed in a chamber which protects it against air-borne contaminants (fig 8.3).

8.2.1 Spectral acquisition:
In order to obtain an optical reading, the fibre probe is placed in contact with the tissue to be interrogated. The measurement is activated, either using the keyboard or with a foot-pedal. The system automatically takes a background measurement without firing the lamp to quantify the amount of ambient light reaching the 'receive' fibre from room lights. This is followed immediately (within 100 msec) by an ESS measurement with the pulsed lamp being triggered. The system then subtracts the background spectrum from the ESS spectrum. Typical 'trigger' to display time is less than 1 second for each individual measurement.
8.2.2 Optical Geometry:

The proximal ends of the fibres are connected to the lamp and spectrometer by SMA connectors. A number of short pulses of light (typically 5-10) are sent through the delivery fibre. Following elastic scattering as described above, only a small fraction of the scattered light from the tissue reaches the receive fibre. The collected light is then guided to the spectrometer where an optical spectrum is generated for further processing.

The core diameters of the 'delivery' and 'receive' fibres are 400μm and 200μm respectively. Both fibres lie adjacent to each other and at the distal end are encased in a metal sheath. The centre-to-centre fibre separation within this sheath is set at 340 μm. The external diameter of the metal casing in different probes varies between 0.8mm and 4mm depending on the situation that optical readings are being taken. For this probe geometry, the volume of tissue visited by the collected photons has been postulated to occupy a zone approximately 500 μm long, 300 μm wide and 300 μm deep. This has been determined from computational simulations using a Monte-Carlo code, which incorporates Mie theory for the details of the scattering events (Mourant, Boyer, et al. 1996 861 /id). The separation of the fibres also has a profound bearing on the character of the received signal. For narrow separation, as with this design, the reflectance spectrum depends mainly on the scattering properties of tissue although some absorptive features also influence the signal. For a probe design with wider separation, the reverse is true.

The geometry described above has been developed and used for several years by our co-workers in Los Alamos. The sizes of the send and receive fibres are partly determined by the constraints of the connections to the xenon lamp and spectrometer and also have been selected for their broadband light transmission over the spectral range used in this study. The precise diameters have however been optimised to allow an adequate amount of light to enter the tissue but not to overpower the spectrometer with the received light. The separation of the fibres is also standardised by these fibre sizes when they are abutted together. This separation is conveniently a suitable distance apart to optimise signal due to the scattering properties of tissue.
Furthermore, by using such slender fibres, the overall diameter of the entire probe can be kept small enough to pass through narrow gauge cannula. It would be feasible to produce a system that has a single send and receive fibre, which could be made even smaller. This however would have limitations, as a significant proportion of the signal would be determined by reflection from the tissue surface. Also absorption would play a greater role and the technical considerations of rapid switching between send and receive modes would be difficult. For all these reasons, the current probe geometry offers the best compromise between size, simplicity of construction, and predominance of scattering within the returning signal.

8.3 CLINICAL PROBE DESIGN

Several methods of collecting data were investigated (see section 9.2). Preliminary ex-vivo work used hand held probes approximately 4mm in diameter but it was obvious that it would be necessary to produce probes of much smaller calibre in order to access tissue within the breast. Although it would be feasible to take in-vivo optical measurements from the cut surface of benign breast tumours during an operation, this is not possible for breast cancers. It is a strict surgical edict, to where possible, not incise through breast cancer tissue as this can cause seeding of cancer cells to the surgical wound or deep within the tumour bed.

As it has been speculated that the probe was only obtaining information from tissue to a depth of 1mm from its tip (see above), the main difficulty was to obtain a tissue sample for histological analysis from precisely the same point. It was therefore evident that a probe capable of passing through a standard biopsy needle would need to be developed so an initial optical reading could be obtained before taking a standard tissue biopsy for comparison. It was therefore decided to develop a probe small enough to pass through a standard 14-gauge core-cut biopsy needle. In conjunction with the collaborators in Los Alamos, an initial flexible probe was built with a diameter of 0.8mm. This was the smallest possible diameter due to the size of the optical fibres within the probe. It was found however that this allowed too much blood to gather within the bore of the core-cut needle which interfered with the optical reading due to excessive absorption by haemoglobin (Fig 8.8). A metal clad
optical probe was subsequently manufactured with a diameter of 1.85mm. This created a snug fit within the bore of the outer part of the core-cut needle, which minimised the amount of blood present at the tip giving better optical readings (Fig 8.4).

![Image of probes](image_url)

*Figure 8.4 Rigid probe (b) for using with core cut needle (a), and flexible probes (c)*

A second new demand on the design of the probe was the ability to withstand adequate sterilisation. Initial in-vivo measurements used probes sterilised with gluteraldehyde in a manner similar to that used for endoscopic instruments. The main drawback however was that the adhesive used at the SMA connector end would eventually degrade under these conditions so therefore only the distal end could be sterilised. It was felt however that a fully autoclavable probe would be beneficial as the whole probe could be packaged and sterilised similar to other operating instruments. This would allow the surgeon to handle the whole probe and then ‘hand off’ the SMA connectors to be attached to the rest of the optical biopsy system. Several probe designs were tested. Initial problems with partial melting of the outer plastic sheath were overcome by using a polyamide coating. This allowed sterilisation at 126°C for 11mins, without damage. This is a standard regime for similar instruments used during laparoscopy and was implemented following
direction from the sterile services department at the Middlesex Hospital. This inevitably has lead to a more reproducible and verifiable sterilisation process. Another sterilisation method that has been employed more recently is the use of ethylene oxide. This avoids high temperatures that may have a long-term effect on the probes. Both techniques are however adequate to fulfil ‘operating theatre standards’.

8.4 EX-VIVO STUDIES

In order to gain a better understanding of the optical readings that were being obtained, and also to assist in the ultimate analysis of this data, several experiments were designed. These are outlined below:

8.4.1 Volume of tissue

_AIM:_

To determine experimentally the volume of tissue that the optical probe was interrogating.

BACKGROUND:

Computerised simulation models using a Monte-Carlo method had previously calculated that with the present configuration of the probe, the majority of information collected was from tissue surrounding the tip of the probe to a depth of around 0.3mm, and width of between 0.3 and 0.5mm (Mourant, Boyer, et al. 1996 861 /id)(fig 8.5 – personal communication, Dr Paul Ripley). It was important however, to confirm this volume of interrogation (VOI) experimentally, for two reasons:

I. In order to ensure good correlation with the histological specimen, we needed to know how ‘point-specific’ the optical system was. Although the implications of having a large VOI would make it less essential to be millimetre specific in obtaining a tissue sample from exactly the same spot as the optical biopsy, it would however create a problem in heterogeneous tissue (as breast tissue invariably is). The data from the optical biopsies would
therefore be a composite of more than one tissue type making interpretation very difficult, if not almost impossible! If however we could show that the VOI was indeed less than 1mm, the interpretation would be clearer, as local histological variations would be less. This however would make precise optical/histological correlation of paramount importance.

II. If we were to ultimately develop a fully validated diagnostic tool, it would be essential to know if the optical system could detect pathological tissue at distances remote from the tip of the probe. If however there was only a small VOI, it would be necessary to obtain multiple measurements in different areas, in order to ‘biopsy’ a lesion thoroughly (as is currently the case with conventional biopsy.

Figure 8.5 – Monte Carlo simulation of the depth of interrogation of photons returning to the ‘receive’ fibre. Brighter red/yellow squares indicate increasing numbers of photons passing through this area. Yellow line indicates ‘threshold level’.
**METHODS / RESULTS:**

A standard pork chop (5oz) was purchased from the local butcher's shop and allowed to reach room temperature. This provided a sharp interface between two distinct types of tissue i.e. muscle and fat. Following standard calibration of the optical system, measurements were taken in incremental steps of one millimetre across the muscle/fat interface, over a total distance of 3cm i.e. 15mm either side (Figure 8.6).

The optical readings are shown in figure 8.7. For clarity only the readings from 3 millimeters either side of the interface are included. These show that there is very little difference in the graphs until the probe reached a distance of less than 1mm from the tissue interface. This was most easily visible as a change in gradient between 320 and 380nm. There was also a general reduction in the total haemoglobin absorption (see section 8.4.3). The experiment was repeated with the probe angled at 30 degrees to the vertical plane, towards the tissue interface. Similar results were obtained.

**CONCLUSION / DISCUSSION:**

Information is only gained to a depth of 1mm or less from the tip of the probe. This correlated accurately with the theoretical value obtained by Mourant {Mourant, Boyer, et al. 1996 861 /id}. As discussed above, this made it essential to take accurate tissue samples. How this was achieved is discussed below (section 9.2). The small VOI however afforded a better chance of accurate analysis and interpretation of the results by reducing the degree of heterogeneity of the tissue.
Figure 8.6 - Volume of interest readings – the probe was gradually moved across the muscle/fat interface

Figure 8.7 - Volume of interest results: sudden change of curve less than 1mm from muscle/fat interchange
8.4.2 Pressure effects

AIM:
To determine the affect of pressure applied to the probe on the character of the optical spectra.

BACKGROUND:
It is known that the density of tissue has an effect on scattering and absorption, and therefore it was theoretically possible that different pressures applied through the probe to the tissue, may have an effect on the resulting spectra. As there was no simple method of standardising the force applied to the probe, it was important to determine if different pressures had any effect on the spectra and if so, could this be quantified.

METHODS/RESULTS:
A series of optical readings were obtained from a single point on a pork chop by first placing the probe on the surface lightly and then gradually increasing the pressure manually. Two spectra were obtained for each pressure, which were subsequently averaged. This was repeated three times on different areas of the pork.

With increasing pressure the overall amplitude of the spectral curve increased slightly. This was due mainly to an increase in the number of pulses of light due to feedback mechanism of the system. The average returning number of photons per pulse however decreased with increasing pressure. When standardised at 800nm however, the relative values and shape of the curve remained relatively constant (Fig 8.8).

CONCLUSION/DISCUSSION:
Although it was not possible to quantify the amount of pressure being applied for each reading, the general trend revealed that the overall shape of the curve did not vary with different levels of tissue compression. If there had been a significant
change in the spectra, it would have been possible for research purposes to quantify and standardise the pressure being applied for each reading by developing a probe with an attached pressure monitor. Thankfully however, this was not necessary as not only would it have added an extra dimension to be taken into account for the analysis, but also it would have had significant implications for the design and manufacture of a commercial system providing validation is achieved.

The implications of this experiment were that the simple design of the probe could be maintained. As the shape of the spectra was unchanged it is relatively simple to standardise the data at a certain point to account for a change in the overall amplitude of the curve. From a practical point of view however, all spectra were obtained by the same operator (the author of this thesis) and a very similar pressure was applied in every case. It was evident following this experiment, that a significant change in manual pressure was required to have any visible effect on the overall amplitude of the spectra, or indeed the number of pulses produced by the feedback system. Any

\[\text{Figure 8.8 - Pressure effects on optical spectra showing no significant change in spectral characteristics with increasing pressure when normalised at 800nm}\]
variations in pressure between spectra were therefore negligible although in the analysis would be accounted for anyway (see section 8.5).

8.4.3 Blood absorption effects

AIM:
To characterise the absorption properties of blood as an aid to data analysis.

BACKGROUND:
The main absorber of light in blood is the chromophore, haemoglobin. It was evident early into the study that if too much blood was present, the optical signature was dramatically affected with absorption of a large amount of light in the blue/yellow part of the spectrum. There was however a range of absorption and it was not clear to what extent this would affect the subsequent analysis of the data. It was therefore decided that it was important to characterise the absorption pattern of haemoglobin for two reasons:

I. To ascertain a threshold level for blood contamination below which data could be deemed reliable.

II. To determine if haemoglobin concentration could be quantified and subsequently be standardised within the analysis process.

It is known that there are three main absorption peaks for oxygenated haemoglobin, 415nm, 542nm and 577nm (Knoefel, Kollias, et al. 1996 873 /id). There are also separate absorption peaks for deoxygenated haemoglobin at 430nm and 555nm. The combined absorption band at 415-430nm is known as the ‘Soret Band’, and the range between 542 and 577 are known as the ‘Q’ bands. It was decided therefore to attempt to both qualify and quantify the absorption characteristics of haemoglobin for the optical biopsy system.
METHODS / RESULTS:

A sample of venous blood was obtained from the medial cubital vein of a healthy male volunteer. Both the needle and syringe were previously heparinized to prevent clotting of the sample. A series of dilutions were made with normal saline in 2ml plastic flasks by a double dilution technique. These ranged from 1:0 i.e. whole blood, to 1:16384 / blood:saline.

The optical biopsy system was calibrated in the normal way. Readings were obtained from each solution with the flasks seated within the calibration pot i.e. with the base on the spectralon to ensure flat reflectance/absorption of any escaped light from the solution (Fig 8.9).

Figure 8.9 Haemoglobin dilution – 1 part blood to x part saline. Only every third dilution has been plotted for clarity.
The main absorption peak at 577nm was used and the level of absorption from the 1:16384 dilution used as a baseline i.e. each spectra was standardised at 800nm (see below). From this the absorption levels were calculated for each dilution and plotted on a linear scale of dilution against absorption (Fig 8.10). It was found that the degree of absorption by haemoglobin was relatively linear in relation to the concentration until the 1:512 dilution. This is equivalent to around a ten-fold reduction in returning light at the absorption peaks. Above this level, the graph became super-linear, and increased exponentially. Any spectra with haemoglobin absorption levels lying above this point would therefore be unreliable.

![Figure 8.10](image)

*Figure 8.10 – Haemoglobin dilution showing roughly initial linear response with loss of linearity beyond 1:512. (Dilutions above 1:64 have not been included as values are too large). Semi-log scale.*

Following on from this initial investigation, a second experiment was designed to determine the relative contribution of oxygenated versus deoxygenated haemoglobin. A further sample a venous blood was obtained and heparinized. A dilution of 1:1024 was produced from the sample as this fell within the linear range but still had sufficient absorption to assess all 5 peaks. The solution was placed in a 10ml glass flask and 100% oxygen was bubbled through the solution for 5 minutes to produce
fully oxygenated haemoglobin (Figure 8.11). An optical reading was taken immediately after cessation of the bubbling. Next, 100% nitrogen was passed through the diluted blood for a further 5 minutes, which causes the oxygen to dissociate into solution thereby creating maximally deoxygenated haemoglobin. This effect is achieved by decreasing the partial pressure of oxygen in solution. Again a reading was obtained immediately after cessation of the gas. The two curves are illustrated in Figure 8.12 and correspond almost identically to those by Knoefel et al [Knoefel, Kollias, et al. 1996 873 /id]. The only difference is the overall amplitude of the two bands where Knoefel found a ten-fold difference in relative absorption between the Soret and Q bands whereas with our system there was only a four-fold difference. The reasons for this are unclear but it is most probably due to the optical biopsy system relying on scattering to obtain a spectra, rather than a transmissive signal with Knoefel’s technique that is mainly due to absorption.
Figure 8.12 - Haemoglobin absorption: $HbO_2$ - oxygenated $Hb$
$Hb$ - deoxygenated $Hb$

From these two spectra, it is evident that the absorption peak at 415-430nm corresponds to the level of oxygenation, with a shift of the peak to the right with increasing deoxygenation. There was also marked difference in the shape of the absorption curve between 542 and 577nm which depends on the absorption due to deoxygenated haemoglobin at 555nm i.e. a single absorption peak with deoxygenated haemoglobin. Although at present it not possible to accurately quantify the absolute levels of oxygenation of haemoglobin, it is possible to identify the spectral characteristics of grossly oxygenated or deoxygenated blood.

**CONCLUSION / DISCUSSION:**

Haemoglobin, being a major chromophore, has a large influence on *in-vivo* spectra. It was recognised early into the study that it would be useful to obtain its characteristics within the optical biopsy system. Although it had been studied using a transmissive system, i.e. light passed through a thin film of blood and the absorption spectra collected on the other side, the characteristics due to both absorption and scattering within a system such as the ‘optical biopsy’ had not been documented.
These experiments therefore answered two important questions. Firstly, there was a threshold level for the amount of haemoglobin absorption allowable before the readings became unreliable as the plot of absorption versus dilution described a straight line above a dilution of 1 in 512. Below this dilution level, the graph becomes non-linear. It had been observed from some of the earliest *in-vivo* optical readings (see chapter 9), that blood on the surface of the tissue in question produced dramatic absorption in the wavelength regions outlined above. It was therefore important to quantify what level of blood contamination was acceptable with regards to obtaining the training spectra needed to assess the system i.e. if too much blood is present between the probe and the tissue being interrogated, this data should be excluded from the final analysis. Alternatively the surface of the tissue and the probe could be cleaned with a sterile swab to remove excess blood and the reading repeated. It became evident however that almost none of the *in-vivo* readings contained levels of haemoglobin absorption above the cut-off level. This was slightly unexpected, although pleasing, as it was originally thought that a lot of spectra contained too much blood.

Secondly, it was now possible to roughly quantify the level of oxygenation within tissue in general terms i.e. poorly or richly oxygenated. It was evident during the experiment that there was a marked change in colour during de-oxygenation (as evident when comparing dark red venous blood to bright red arterial blood). All the optical biopsy system is doing is quantifying this change that is visible to the naked eye. Such information may also be of benefit in the subsequent analysis of the data (see below, section 8.5).
8.4.4 Blue dye

**AIM:**
To determine the optical characteristics of blue dye within sentinel lymph nodes and produce a compensatory algorithm during data analysis.

**BACKGROUND:**
One of the early problems encountered with evaluation of sentinel nodes was the distortion of the spectra by blue dye within sentinel nodes (see figure 8.13). In our unit, lymphoscintigraphy, using technetium radio-labelled colloid, and patent blue dye (food colouring E151) are used for sentinel node identification. This is described in greater detail above (section 1.6). Although some sentinel nodes had only a small amount of dye within the cortex, this was highly variable and unless this could be quantified and compensated for, the computer analysis of the data would be affected. It was postulated that the particles within patent blue would be too small to produce any appreciable scattering effect therefore it would act as a pure absorber of light. If this was the case, the absorption should vary in a linear fashion regardless of the concentration.

![Figure 8.13 – Effect of blue dye on spectra](image_url)
METHODS / RESULTS:

Standard dilutions of patent blue dye in saline were made using a x10 dilution technique. A 0.1ml drop of each dilution was placed separately on a piece of pork fat at room temperature (figure 8.14). This was allowed to equilibrate with the fat for 10 minutes. After this time, optical readings were obtained from each dilution site as well as an area with no dye present, which acted as a baseline standard. The optical spectra are demonstrated in figure 8.15. Absorption is evident in the red and yellow parts of the spectrum, which corresponds to the blue reflectance of the dye. This is maximal at 655nm. Interestingly a further absorption peak was observed at 415nm, which was not expected. A second plot was made of absorption at 655nm versus dilution (figure 8.16 – log scale). The relative absorption was calculated by subtracting the value for each dilution from the ‘baseline value’ of fat. The overall absorption by the blue dye was again found to vary in a linear fashion with dye concentration when compared to the standard. This approximate linearity however continued throughout the range of dilutions with no cut off point.
Figure 8.15 – Relative intensity curves of blue dye absorption with increasing x10 dilutions

Figure 8.16 – Blue dye absorption curve at 655nm (log scale): roughly linear plot with decreasing dilution.
CONCLUSION / DISCUSSION:

In a similar fashion to haemoglobin, the absorption characteristics of patent blue dye within tissue can be characterised and vary in an approximate linear fashion with concentration (Figure 8.16). Unlike haemoglobin however there was no cut off point for an ‘allowable’ level of blue dye. This confirms the hypothesis that blue dye exerts only an absorption effect on the spectra with no appreciable scattering. Also it was possible to fully characterise the exact characteristics of patent blue absorption by subtracting a spectra containing blue dye from the baseline fat measurement (figure 8.17). Using this data, the interference of blue dye with the optical signature from sentinel nodes can therefore be compensated for in subsequent analysis. This will be discussed further in section 8.5.

Figure 8.17 – Absorption characteristics of Patent Blue dye
8.5 Principles of Spectral Classification

Three methods of classification of the spectral data have been developed. All are based on Artificial-intelligence pattern-recognition (AI-PR) but vary in their methods and the amount of 'human input'. Artificial neural networks and hierarchical cluster analysis were evaluated by our collaborators in Los Alamos whereas model based analysis was developed in London. An in-depth discussion of the intricacies of artificial intelligence methods is beyond the scope of this thesis however each will be considered separately in broad terms, in an attempt to give some background to how the results presented below (chapter 9) were reached.

8.5.1 Artificial Neural Networks (ANN)

This method of classification consists of several input parameters and two output parameters. Essentially this means that the spectra are condensed into 20 points by taking an average value within wavelength bands of 20nm width, over the range of 330-750nm (Fig 8.18).

Each spectra was characterised as either malignant or non-malignant. This compacted data is then entered into an ANN computer programme, which looks for similarities between the two histologically grouped sets of data i.e. it compares all the data for the cancer spectra, all the non-cancer spectra and then contrasts the two. The programme then produces an algorithm for classification of unknown spectra from this ‘training set’ of data. To verify the reliability of this algorithm, a ‘testing set’ of data is entered, and the computer is asked to characterise each spectra i.e. tries to determine whether the spectra is from malignant tissue or not. By repeating this process several times, the accuracy of the system can be assessed. Such a method relies solely on statistical probabilities and has no ‘human’ input into classification.

8.5.2 Hierarchical Cluster Analysis (HCA)

The broad principles of HCA are essentially similar to ANN in that the spectral data is condensed in a similar manner and the analysis is purely statistical. It differs however in that it can have more than two outputs i.e. several broad histological
subsets can be classified e.g. malignant, suspicious, benign, normal breast tissue. HCA also has the ability to have an unclassified group when it comes to the ‘testing set’. This is probably more realistic as it corresponds better to clinical realities where it is not always possible to classify a histological biopsy with 100% confidence. An ANN system on the other hand will always ‘force’ a definite answer, which is more likely to lead to inaccuracies. After all, breast tissue is wonderfully heterogeneous, even within a particular histological subset.

Figure 8.18 – ANN, compaction of spectra: average is taken within every 20nm block

8.5.3 Model Based Analysis (MBA)

Model based analysis differs from the first two in that there is a certain amount of ‘human’ classification, or pre-processing, of the data before it is entered into a AI-PR programme. This has two main advantages over entirely computerised, statistical based analysis:

I. With greater understanding of the various characteristics of the spectra such as β-carotene absorption in fat (Fig 8.19), or high deoxyhaemoglobin levels within necrotic tumours (Fig 8.12), such characteristics can be given greater ‘weight’ than would be normally assigned on a purely statistical basis. The dangers of this however are that some tumours are highly vascular and
therefore have high levels of oxyhaemoglobin or a rare liposarcoma could be mistaken for normal fatty breast tissue and thus caution must be observed in order not to produce too much “human influence”.

II. Potentially more useful is standardisation of the spectra at certain set points and quantification of the gradients between two points rather than the simple input of an average value for every 20nm width. The examples below indicate ways of improving the pre-processing in order to account for any variables that are not directly related to the tissue type e.g. blood contamination. In this way it should be possible to accentuate the differences between various tissues and at the same time reduce the amount of input information. This should have the overall effect of improving the accuracy of the system, as with purely statistical based analysis, the variables due to factors other than differences in tissue type will have some influence.

\[ \text{Figure 8.19 - } \beta\text{-carotene absorption (arrow)} \]
STANDARDISATION OF THE SPECTRA AT 800NM

Depending on the overall density/absorption of the tissue, the optical biopsy system automatically decides the number of pulses of light needed to produce an 'adequate' spectrum. This is achieved by a negative feedback system during the acquisition of the spectra. The overall intensity therefore is a little variable. In order to account for this difference, all the spectra are normalized at 800nm (fig 8.20). This value was chosen for two reasons. Firstly beyond this point, the noise within the signal becomes very large and hence standardisation further into the near infrared would become less reliable. Secondly, there are no influences from known chromophores within this region which could be affecting the spectra.

LEVELLING OF SLOPES IN NEAR INFRARED

The overall gradient of the curve was found to be variable in different regions and between different tissue types e.g. fibroadenomas tended to have a greater overall slope from 320 to 800nm than tumours. Another important feature however was that in general, the slope between 650nm and 800nm was found to be linear. It was realised therefore that if the gradients were levelled to a horizontal line between these
two values, this again would reduce the number of input data by reducing the variable spectral range to only 320 – 650nm. Also, as the spectral information above 650 is 'transferred' to the shorter wavelengths, this could possibly increase the differences observed in this region of the spectrum (fig. 8.21). Such standardisation also allows easier quantification of haemoglobin absorption as all spectra are levelled to a straight horizontal line within this linear region. By extrapolating from this line, haemoglobin absorption, at the various aforementioned absorption peaks, can be calculated.

Figure 8.21 – Levelling of gradient in near infrared.

USE OF GRADIENT VALUES

After ‘human’ interpretation of representative spectra from different types of tissue it became obvious that certain areas of the spectral curves varied more than others and that the overall general trend of the slope of each spectra was quite different. In order to add increased weight to such features, the use of gradient values between two different points was employed. Such values can then be used as input parameters for final statistical analysis. An example of how such gradients are achieved and their possible use as a strong differentiating feature between different tissue types is displayed in figure 8.22. The gradients shown are purely for demonstration and only serve to represent how such differences can be used. In the real analysis however,
many different gradients have been tested which are chosen by manually looking for patterns between various groups of tissue types.

![Graph showing calculated and used gradient values](image)

Figure 8.22 – Calculation and use of gradient values: line drawn between points at 360 and 620nm

8.6 DISCUSSION / CONCLUSION

By accounting for and normalising such known variables, the unknown factors which contribute to the shape of the curve, can be analysed by the AI-PR packages. This reduces the number of variables and therefore affords a better chance of achieving greater accuracy. It should be noted however that the examples in the above figures have been chosen to accentuate the differences between the spectra from different tissues. In reality the differences are much less obvious and there is also considerable overlap. At present, little is known about the effects of different types of tissue on the spectral characteristics at a cellular or sub-cellular level. Work is ongoing however, to achieve a greater understanding of such effects. This new knowledge can then be used to further pre-characterise the spectra.
CHAPTER 9 OPTICAL BIOPSY – CLINICAL STUDIES

9.1 INTRODUCTION

The aims of the clinical studies presented in this chapter are twofold. Firstly, to evaluate the Optical Biopsy System (OBS) in an in-vivo setting, and secondly, to develop the computer algorithms for analysis of subsequent ‘unknown’ data i.e. to produce a prototype diagnostic tool. Two studies were undertaken simultaneously at the Middlesex Hospital London: In-vivo optical analysis of breast tissue, and ex-vivo analysis of axillary lymph nodes.

9.2 IN-VIVO: BREAST TISSUE

9.2.1 Introduction

One of the main problems faced when trying to develop any diagnostic instrument for the breast, is the enormous diversity of different types of breast tissue ranging from normal glandular or fatty tissue, through benign breast diseases, to in-situ, and ultimately invasive, carcinomas. Even within each broad histological heading, many varieties exist, often with more than one histological subgroup of cells being identified within a ‘traditional’ tissue biopsy. The challenge therefore was not only to evaluate the OBS, but also to try and categorize the corresponding histological samples that were taken. From earlier experiments that confirmed the OBS was only ‘sampling’ tissue within 1mm of the tip of the probe, it became paramount to obtain a tissue biopsy for histological comparison from precisely the same area of breast tissue. For the purposes of simplicity, these paired optical and histological biopsies will be referred to as “data sets”. Several methods were devised to achieve this.

9.2.2 Data acquisition

Three methods of obtaining data sets from a variety of different types of breast tissue were devised to take advantage of all possible scenarios where biopsies could be obtained with least patient discomfort. All patients received written and oral information regarding the study (Appendix 1), which outlined the aims of the study
and advised of any risks. Full written informed consent was obtained from all patients. All volunteers were patients undergoing breast surgery under general anaesthetic for a variety of reasons. All data sets were obtained while the patient was anaesthetized to avoid unnecessary additional discomfort. Each method of biopsy will be discussed separately:

**TUMOUR BED MEASUREMENTS**

The simplest method that was first tested was to obtain data sets from the tumour bed tissue following wide local excision of a breast lump. Following adequate haemostasis, an area of breast tissue on the tumour bed was marked with blue ink in a diamond pattern (Figure 9.1). First two optical readings were obtained from the centre of the diamond followed immediately by a ‘pinch’ biopsy approximately 2mm in diameter. This was achieved by lifting the tissue with a pair of surgical forceps and incising around the forceps with a scalpel. After the first few occasions, it was realised that following the optical reading, a small mark could be made on the tissue surface by applying downwards pressure to the probe. This was sufficient to direct the tissue biopsy.

**CORE-CUT BIOPSY**

The disadvantage with the tumour bed method was that the majority of biopsies were taken from normal surrounding tissue and subsequently very few spectra from malignant tissue were likely to be obtained. To increase the numbers of cancer spectra, it was evident that optical biopsies would have to be taken in a similar
manner to conventional biopsies, as cutting into a breast cancer in-vivo during an operation is not possible, due to the significant risk of tumour cell seeding into the wound. This led to subsequent development and design of a probe, small enough to pass through a standard 14-gauge core cut biopsy needle. The logistics of this are discussed further in chapters 4 and 8.

Biopsies were obtained from patients undergoing either mastectomy or wide local excision for breast cancer. Directly following induction of anaesthesia, before surgery commenced, several data sets were obtained, first from tissue surrounding the tumour and then from the tumour itself. Care was taken to limit the biopsy field only to tissue that was subsequently being removed as part of the standard resection. This avoided the problem of possible seeding of breast cancer cells into surrounding healthy tissue by the passage of the biopsy needle through the far side of the lesion. This however was less of a concern for the mastectomy patients in whom the entire breast was subsequently removed. From these patients multiple biopsies were obtained from ‘normal’ breast tissue.

The method of obtaining the data sets was simple. First a 14-guage Automatic Cutting Needle (Manan) was inserted into the required area within the breast. Once in position, the depth was noted by using the graduations on the outer sheath of the needle, and the inner stylet was removed. Following calibration, the optical probe was inserted through the needle until it was felt to come in contact with the tissue (Figure 8.2). Gentle pressure was applied and two optical readings were obtained. The optical probe was then removed and the inner stylet of the biopsy needle re-inserted. This was next placed in a Pro-Mag™ automatic biopsy system (Manan). The depth of the needle was checked, adjusted where necessary, and the gun fired to obtain a core of tissue for routine histological assessment.
Figure 9.2 – Method of obtaining an optical biopsy through a core-cut needle

**BENIGN LESIONS**

In order to obtain spectra from a range of benign breast pathologies, especially those that present with a discrete breast mass, a third method was devised. Patients were recruited from the day surgical ward who were undergoing excision biopsy of a breast lump which had been deemed by triple assessment to be benign or probably benign.
During surgery, the surface of the lesion was exposed and adequate haemostasis achieved. Before the blood supply to the lesion was compromised, several readings were taken from its surface. Following this, a small stab incision with a number 15 scalpel was made through the capsule (or on the surface where no capsule was present). The optical probe was then inserted into the centre of the lesion. Again, 2 or more readings were obtained and the lesion was immediately removed and sent for histological assessment.

9.2.3 Histopathological correlation

Following optical readings, all tissue samples were sent for histopathological assessment. A single consultant pathologist with a specialist interest in breast disease (SL) examined all optical biopsy specific biopsies. In order to achieve exact correlation of the data sets, close collaboration with the pathologist was essential, with specific marking techniques employed for the core-cut biopsies. This supplied additional information in order to delineate the region of interest i.e. the position on tissue biopsy from where the optical biopsy had been obtained. As the length of tissue from a core-cut was typically 2cm long, it was important to determine which part of the ‘worm’ corresponded to the optical reading. An experiment was therefore devised to determine the extent of protrusion of the optical biopsy probe from the end of the outer sheath of the core cut biopsy needle.

![Figure 9.3 - Measurement and correlation of data sets for core-cut biopsies](image-url)
A standard 14-gauge core-cut needle was inserted freehand into a large chicken breast. The inner stylet was removed and the optical probe inserted until it came in contact with the tissue. Standard pressure was applied and the length of the optical biopsy probe protruding from the proximal end was marked. Both the core-cut needle and the probe were then removed, keeping the mark on the probe level with the proximal end of the core-cut needle. The distance the probe protruded from the distal end was then measured. This was repeated three times and an average obtained. The distance of protrusion was found to be 2mm on all three occasions.

Next the core-cut needle was reassembled and placed in the Pro-mag biopsy gun. The mechanism was triggered and then the outer sheath withdrawn to allow measurement of the relative positions of the end of the outer sheath compared to the groove in the inner sheath, which contains the biopsy (Fig 9.3). It was found that the 2mm protrusion of the optical probe, corresponded the proximal extremity of the tissue sample.

The results of this experiment indicated that we needed to gain information of the histological nature of the proximal end of the core of tissue. To achieve this, the distal end of each core was marked with India ink before fixing in formalin. The pathologist was then able to give specific information about the tissue at the opposite end to the ink. In addition, unless a good core of tissue was obtained, the biopsy was discarded, as accurate correlation would not therefore be possible.

For the tumour bed biopsies, only a small biopsy was obtained (2mm diameter). All such biopsies contained relatively homogenous tissue therefore specific marking was not necessary. Also for benign lesions it was decided that unless the tissue was relatively heterogeneous within the lesion itself, this too would be discarded. Most of lesions however were relatively homogeneous fibroadenomas.
9.2.4 Data Processing and Analysis Results

**DATA NUMBERS:**

50 female patients were recruited into the study, all of which were undergoing breast surgery under general anaesthetic. There were a total of 147 data sets collected, which included most of the common types of normal and pathological breast tissue. The total numbers for each are presented in table 9.1.

<table>
<thead>
<tr>
<th>Histological Type</th>
<th>Number of data sets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal breast tissue</td>
<td>86</td>
</tr>
<tr>
<td>Fibroadenoma</td>
<td>13</td>
</tr>
<tr>
<td>Other benign breast disease</td>
<td>19</td>
</tr>
<tr>
<td>Carcinoma-in-situ</td>
<td>6</td>
</tr>
<tr>
<td>Invasive carcinoma</td>
<td>23</td>
</tr>
</tbody>
</table>

*Table 9.1 – Total numbers of data sets for each tissue type*

For the purpose of clarity of presentation the wide variety of histological subsets have been condensed into broad headings. Normal breast tissue was either fibro-fatty or glandular. Benign breast tissue incorporated a variety of tissue types ranging from sclerosing adenosis to atypical ductal hyperplasia. The breast cancer spectra incorporated both ductal and lobular types. No other, less common type of breast cancer was encountered. It is evident that the numbers of non-cancer data are greater than for both in-situ and invasive cancer. This simply reflects the bias towards obtaining benign or normal spectra from the methods of biopsy as outlined above. The cancer spectra were all obtained via the core-cut method which was used approximately 40% of the time (table 9.2).

Two spectra were obtained for each ‘optical biopsy’. These were compared to each other in order to ensure that there was reproducibility at each biopsy site. All these dual spectra were compared using a ‘Pearson Correlation’. No two spectra were
however found to be statistically different. An average was therefore obtained before input into the various analysis techniques outlined below.

<table>
<thead>
<tr>
<th>Type of biopsy</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour bed</td>
<td>64</td>
<td>43.5%</td>
</tr>
<tr>
<td>Core-cut</td>
<td>58</td>
<td>39.5%</td>
</tr>
<tr>
<td>'Open' biopsy for benign lesions</td>
<td>25</td>
<td>17%</td>
</tr>
</tbody>
</table>

*Table 9.2 - Numbers for each type of biopsy*

**DATA PROCESSING:**

For the pure computer based analysis methods (ANN and HCA), all spectra were first normalised to the same total value i.e. area under the curve. This eliminated variations due to optical coupling, transmission via the different optical probes and fibre or the number of light pulses emitted to obtain the spectrum. The normalized spectra were next divided into 21 wavelength bands of 20nm width between the spectral range of 330-750nm. From these bands, an average value (or intensity) was calculated. In addition to the averaged intensity values, a series of gradient values were also used. This was achieved by determining the slope of the curve between various set points. A total of 10 gradients were calculated which along with the intensity values, comprised all of the input parameters for the Artificial Intelligence analysis.

For the artificial neural network analysis, the training and testing data sets were split into two groups of 80% and 20% respectively. This was repeated five times to ensure that all spectra were tested. The output parameters were either malignant (positive) or non-malignant (negative). A commercially available neural net package was used (‘Brain Maker Pro’, California Scientific Software). Results are presented below.

Hierarchical cluster analysis relies on large data sets to develop testing algorithms so therefore to maximise the training data, a 'leave-one-out' method was employed i.e. all but one of the data sets were used for training and the final spectra then tested.
This was repeated for all spectra and the statistics determined from the sum of all the tests. The software used was also commercially available ('VERI', Sandia National Laboratories). As mentioned in chapter 8, HCA differs from ANN in that more than 2 outputs can be achieved. Although it would have been possible to attempt to classify all different histological subtypes, this would require far more data than we have collected so far. It was therefore decided to simplify the system with only three possible outcomes for the test spectra i.e. malignant, non-malignant and unclassified. This third parameter simply means a spectrum that does not fall into a recognisable pattern based on the training set. Again, results are presented below.

The model-based analysis differed from the other two in that a certain amount of pre-processing was employed. This is outlined in detail in section 8.4.3. In effect the spectra were standardised to the same value at 800nm and also the gradient between 650 and 800nm. Also they were corrected for levels of haemoglobin absorption and for the sentinel nodes, blue dye contamination. These allowed easier identification of the true differences due to elastic scattering rather than absorption or the set up of the system. Following this pre-processing, a significantly reduced set of input parameters were then easily assessed using a linear discriminant analysis software package (Systat 9.01 – SPSS Software). An 80/20 split was again employed for the training and testing.

**Analysis Results:**

In this study, the aim was to evaluate the ability of the OB system to discriminate between cancerous and non-cancerous tissue, with development of the subsequent analysis methods. Therefore for the purpose of testing the system, both in-situ and invasive cancer spectra were classified as ‘malignant’ and all other spectra classified ‘non-malignant’. Table 9.3 below illustrates the results of the in-vivo testing of breast tissue with separate figures given for each type of analysis. The percentage figures represent the ability to determine if a test spectrum was malignant or not i.e. a malignant spectrum was deemed positive, and a non-malignant spectrum, negative. In presenting the statistical results, sensitivity and specificity are presented in the standard way.
Sensitivity $= TP / (TP + FN)$
Specificity $= TN / (TN + FP)$

Where TP, TN, FP, FN represent true positives, true negatives, false positives and false negatives respectively, as determined by the corresponding histopathology.

<table>
<thead>
<tr>
<th></th>
<th>ANN</th>
<th>HCA</th>
<th>MBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>69%</td>
<td>67%</td>
<td>94%</td>
</tr>
<tr>
<td>Specificity</td>
<td>85%</td>
<td>79%</td>
<td>92%</td>
</tr>
<tr>
<td>% classified (HCA only)</td>
<td></td>
<td></td>
<td>91.5%</td>
</tr>
</tbody>
</table>

Table 9.3 – Results for classification of spectra by different analysis techniques

ANN – Artificial Neural Network; HCA – Hierarchical Cluster Analysis; MBA – Model Based Analysis

9.2.5 Discussion

It is evident that by far the best results have been attained from the model based analysis. This is probably because variability in haemoglobin absorption was only partially compensated for by the AI-PR systems and therefore variations in haemoglobin were falsely used as criteria for developing the algorithms. It is self evident that the amount of blood present between the probe tip and the tissue is dependent on the type of biopsy being performed and the chance of causing haemorrhage from passage of the biopsy needle. Such variables cannot be compensated for within totally computer based pattern recognition programmes.

Although the results for ANN and HCA have been disappointing, comparison of the three techniques have further emphasised the need for continuing work on understanding characterising the spectra that are obtained.
9.2.6 Conclusion

The results presented represent the first time such an optical biopsy method, based on elastic-scattering spectroscopy, has been assessed for the classification of breast tissue. The accuracy of the system when analysed by the model-based system has been very encouraging with an overall accuracy for discriminating between malignant and non-malignant tissue of over 90%. With further validation of the system through collection of more data sets, the accuracy can only improve further. Overall, these early results show real promise towards the development of a clinically useful, real-time, diagnostic tool.

9.3 EX-VIVO: AXILLARY LYMPH NODES

9.3.1 Introduction

The aim of this study was to determine the accuracy of the optical biopsy system at determining the metastatic state of an axillary lymph node. The main advantage of such a system would be in the sentinel lymph node setting where immediate analysis of a sentinel node could decide whether or not to proceed with a full axillary node clearance. This would be a great advance on the current system of frozen section analysis, which at best takes 45 minutes to 1 hour, and itself is less than reliable [Dixon 1998][Veronesi et al. 1997] the main problem being a high false negative rate. There is no doubt that a fast, reliable, real time diagnostic tool would certainly be invaluable to avoid unnecessary delay in the operating theatre.

9.3.2 Methods

34 female volunteers were recruited from patients undergoing axillary lymph node excision as part of surgery for primary breast cancer. Of these patients, there were a total 117 data sets from 62 lymph nodes. Some of these patients were undergoing sentinel lymph node biopsy (SLNB) as part of a separate research project at the Middlesex Hospital (see acknowledgements). The remaining patients were undergoing standard routine axillary lymph node clearance (ALNC). Table 9.4 illustrates the numbers in each group.
<table>
<thead>
<tr>
<th>Type of biopsy</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLNB</td>
<td>11</td>
</tr>
<tr>
<td>SLNB &amp; ALNC</td>
<td>2</td>
</tr>
<tr>
<td>ALNC</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
</tr>
</tbody>
</table>

Table 9.4 – breakdown according to type of operation

SLNB = sentinel lymph node biopsy  ALNC = axillary lymph node clearance

Figure 9.4 – optical biopsy from a bi-valved axillary lymph node

In all patients the node was dissected free of the surrounding tissue either in- or ex-vivo. The node was then bivalved, centrally along its long axis with a scalpel blade. Up to three spectra were obtained from the cortex of each node depending on its size (Fig 9.4).
All spectra were obtained within 10 minutes of excision. For small nodes, a single reading was obtained centrally within the cortex. This allowed the subsequent localisation of the point on the histology specimen which corresponded to the optical biopsy. It was realised that in some nodes there may only partial replacement with metastatic deposit and it was important therefore for these nodes to ensure that an accurate correlation could be achieved. For larger nodes which were obviously completely replaced with metastasis macroscopically, several spectra were obtained at different points. The completeness of replacement was subsequently confirmed microscopically in all cases. There were however some lymph nodes which showed only partial replacement by metastatic tumour.

All patients undergoing SLNB had lymphoscintigraphy with technetium labelled colloid in conjunction with injection of patent blue dye subcutaneously, 5-10 minutes prior to dissection. Initial localisation with a collimated gamma probe directed the surgeon to the correct site for the skin incision overlying the node. Subsequent visual determination of the node was achieved by the blue dye. Following dissection, the sentinel node was immediately double checked using the gamma probe, with a positive result being determined by a gamma count greater than ten times the background reading in the axilla. Confirmation by this node to background radiation ratio is the standard practice at our institution.

9.3.3 Results

The aim of this study was to determine the metastatic status of an axillary lymph node in patients with primary breast cancer. The analysis performed was in a similar manner to that described above for breast tissue. There was no discrimination made between metastatic nodes from different types of primary tumours. The majority however were from invasive ductal carcinoma, the remainder from invasive lobular carcinoma. In those patients with sentinel lymph nodes, some had strong absorption bands due to the blue dye which altered the spectra considerably (Fig 8.4). This however could be compensated for using a previously determined algorithm (see section 7.3.4) before continuing with the computer analysis. For the purposes of the analysis, a node containing any focus of malignant metastatic cells was deemed
positive and all others regarded as negative regardless of whether they were completely normal or reactive. A total of 14 positive nodes and 48 negative nodes were submitted for analysis. Sensitivity and specificity is presented below for the determination of a positive node in the test set (Table 9.5).

<table>
<thead>
<tr>
<th></th>
<th>ANN</th>
<th>HCA</th>
<th>MBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>58%</td>
<td>91%</td>
<td>57%</td>
</tr>
<tr>
<td>Specificity</td>
<td>93%</td>
<td>76%</td>
<td>85%</td>
</tr>
<tr>
<td>% classified (HCA only)</td>
<td></td>
<td></td>
<td>83.3%</td>
</tr>
</tbody>
</table>

Table 9.5 – Results for determining a positive lymph node
ANN – Artificial Neural Network; HCA – Hierarchical Cluster Analysis; MBA – Model Based Analysis

9.3.4 Discussion

Comparable to the results from the breast tissue spectra, there was again a wide variation in the sensitivities and specificities between the three different analysis techniques. No one method was particularly better than another although generally model based analysis fared worse than the other two, which was in marked contrast to the results for breast tissue. Why there is such discrepancy is unclear although it may be that some of the spectra from the lymph nodes were affected by the fibrous capsule which when tested from the surface of the node, produced a very different spectra compared to the cortex. Certainly, for some of the smaller nodes (<5mm) it was technically difficult to place the probe in the cortex in a way not to receive information from either the capsule or the medulla of the node, which contains vascular tissue. It may be that there is a cut off in size of node that produces unreliable results. A further possible explanation may be the discriminatory finding of β-carotene in fat, which has a high (almost 100%) specificity for normal breast tissue. This factor could not be used for lymph nodes which are relatively devoid of fat within the cortex, although even within malignant nodes, may have fat within the medulla. Another problem was where only partial metastatic infiltration occurred (n=2). As these nodes were again generally much smaller than nodes that were completely replaced by tumour, it was difficult to achieve accurate correlation...
between the site of optical biopsy and histological examination. These questions however will be addressed as the study continues.

9.3.5 Conclusion

The results for lymph nodes were less impressive than for breast tissue however, depending on the type of analysis employed, sensitivity of up to 91% (HCA) and specificity of up to 93% (ANN) was achieved. This is encouraging enough to continue with the study as a fully validated system has a useful and exciting clinical application. There is no doubt that within this pilot study there has been a steep learning curve. However, with better validation of the data sets in terms of position of the optical probe on the surface of the lymph node, and ongoing work on the analysis methods, we believe that the results can only improve.
CHAPTER 10  ILP FOR BREAST CANCERS

10.1 INTRODUCTION

Interstitial Laser Photocoagulation (ILP) of malignant breast tumours has been described in previous publications [Akimov et al. 1998][Mumtaz et al. 1996] (see chapter 5). These studies have had reasonable success in exploring the feasibility of this procedure. They did not attempt however to destroy tumours completely. Two important questions have also yet to be fully answered. What is the natural history of tumours treated in this way and how are such tumours best followed up? It is only by attempting to answer these questions that such a treatment can become accepted as an alternative to surgery. To achieve this, two studies were designed based on work previously carried out at the Middlesex Hospital, London. One study concentrated on the treatment of breast cancer with subsequent follow up by magnetic resonance imaging. The results of this study are presented in this chapter. A second study investigating the efficacy of ILP at treating benign breast fibroadenomas is discussed in chapter 11.

10.2 AIMS

The aim of this study was threefold:

- To study the effects of ILP in the treatment of malignant breast tumours over a three-month period.

- To relate these findings to serial MR images of the tumours, both pre- and post-treatment.

- To attempt to treat the whole tumour with a surrounding rim of normal tissue.
10.3 Patients and Methods

10.3.1 Patient selection

Patients were recruited from surgical clinics at the Middlesex Hospital, London and the Royal Sussex County Hospital, Brighton. Approval was obtained from the appropriate local ethics committees. Certain inclusion criteria were stipulated:

- Patients should be post-menopausal, specifically older than 64 years.
- The tumours should be primary breast carcinomas less than 3cm in maximum diameter as assessed by magnetic resonance imaging.
- The tumours should be oestrogen receptor positive.

Patients who fulfilled these criteria were identified and approached after discussion with the surgeon in charge of the patient’s care. All participants received an extensive information sheet regarding the nature of the study (Appendix 2). In addition the aims of the research and the nature of the laser treatment were explained in depth both by the investigators and the respective breast care nurses at both hospitals. These discussions were recorded by audiotape and this was given to the patient as further permanent information about the study. A two-way discussion about the nature of the treatment was encouraged and the patient was continually assured that they could withdraw from the study at any time, at which stage standard surgical and medical treatment would take place in line with national guidelines. It was also stressed that such withdrawal would in no way be detrimental to their future care. After full written informed consent, those willing to participate were entered into the study.

All patients underwent full routine work up including mammography, fine needle aspiration cytology and/or core-cut biopsy. Although not routine for all patients, core biopsy was deemed essential for all patients entering the trial in order to assess accurately tumour type, grade and oestrogen receptor status, as such information would not be obtainable after laser ILP treatment. All patients received ILP within 2 weeks of deciding to enter the trial and were placed on Tamoxifen 20mg daily. They were also given a daytime contact number should problems arise and were reviewed.
at 4-6 weeks at the outpatient clinic. Routine surgery was subsequently performed 3 months after ILP.

10.3.2 Treatment protocols

Laser treatment was performed exclusively in the Middlesex Hospital as an outpatient procedure. All patients received intravenous sedation and analgesia, typically midazolam 5mg and pethidine 50mg. In view of this sedation, oxygen was administered in low dose via nasal specula. A trained member of the nursing staff carried out routine observations including electrocardiograph, blood pressure and pulse oximetry monitoring throughout the procedure.

All laser treatments were carried out in conjunction with the same consultant radiologist (MAH-C). Localisation of the tumour was achieved by ultrasound guidance. Up to 4, 18 gauge needles were inserted into the tumour (Figure 10.1), the number varying according to tumour size and shape. In one patient, magnetic resonance localisation was employed, as the tumour was not visible on ultrasound. This was achieved using a 0.2Tesla open configuration magnet.

Figure 10.1 – Needles and optic fibres in place just prior to treatment
Once the needles were correctly positioned, optic fibres attached to the laser source were passed through the cannulae to protrude 3mm from the end of the needle into the tumour tissue. These were secured to the external end of the cannula in order to prevent them inadvertently slipping out of position. All staff and the patients were also required to wear laser safety goggles for the duration of the laser treatment. The laser was then activated for a standard 10 minutes at 2.5 Watts per fibre. During treatment, the skin was continually cooled by cold saline. Careful attention was also paid to the skin around the entry points of the cannula to ensure that over heating did not occur which could lead to skin burning. A further preventative measure was to use an oblique approach for superficial tumours in order to have a reasonable length of needle in the breast tissue i.e. the skin puncture site was slightly remote from the point directly superficial to the breast tumour. Any heat conducted along the needle should therefore have time to dissipate before reaching the skin surface.

The laser source employed for all patients was a semi-conductor diode operating at 805 nanometers (Diomed, Cambridge). Optic fibres were 400 micrometers in

*Figure 10.2 – Use of a beam splitter to allow simultaneous usage of four optic fibres*
diameter with a polyamide cladding. Before each treatment the laser was calibrated using an in-built power level detector. In practice this involves placing the end of the fibre into the detector and firing at the required energy output for a set period (usually 1 second). The actual output was then measured and a true level adjustment was made automatically. Where more than one fibre was used, a beam-splitter was employed which allowed up to 4 fibres to be activated simultaneously (figure 10.2). This therefore significantly shortened the treatment time for multi-fibre treatments.

Following treatment, patients were taken to an observation ward where they remained under supervision of a trained nurse for 1-2 hours until the effects of the sedation wore off. At this stage they were allowed to return home under the care of a friend or relative. Routine follow up was arranged at a time convenient for the patient at around 4-6 weeks following ILP.

10.3.3 Imaging protocols

Magnetic resonance imaging was performed on all patients on 3 separate occasions: immediately pre-ILP, immediately pre-surgery i.e. at 3 months, and an interim scan at 4-6 weeks. Scans were performed in both participating hospitals. In the Middlesex Hospital this is a 1.5Tesla closed bore magnet (Siemens) and in Brighton, a 1Tesla magnet (Philips). All scans were performed according to a similar protocol with both breasts imaged simultaneously using a dedicated breast surface coil. T1 weighted, gradient echo, dynamic contrast enhanced scans were obtained. Patients received intravenous Magnevist™ (dimeglumine gadopentate) at the recommended dose of 0.2 mmol/Kg body weight. Initially scans were performed in the axial plane but in later imaging, coronal scanning was employed similar to all other breast MR imaging performed at the Middlesex Hospital. Post-imaging subtraction scans were also produced. All images were reported by a consultant radiologist with a specialist interest in breast magnetic resonance imaging (MAH-C).

Pre-and post treatment scans were subsequently compared. The interim scan was performed as a further safeguard against any residual tumour growth. The final, pre-operative scan was used to assess the accuracy of MRI at determining the extent of tumour ablation. This was achieved by ensuring that the pathological specimen was
orientated and sectioned in the correct plane, which corresponded to the magnetic resonance images. Direct comparison between the two could therefore be achieved.

10.4 RESULTS

A total of six patients were enrolled into the study. In four patients, the full protocol was followed. In two patients however, due to co-existing medical ailments, ILP only was performed, as the patients were deemed unfit for surgery. This decision was explained to the patients before entering the trial and it was stressed that the laser treatment would therefore be considered part of their palliative care. The clinical situation, treatment and subsequent outcome for each patient will be discussed separately below.
**Patient 1**

Age: 68

**Size of tumour:** 10 x 10 mm

**Core biopsy:** Grade 1, invasive ductal carcinoma.

**No. of ILP needles:** 1

**MRI findings:** Initial scans pre-ILP demonstrated a well-defined enhancing tumour consistent with the mammographic findings of a small carcinoma. The pre-surgery scans revealed complete loss of signal from the tumour site with a surrounding rim of enhancement (Fig 10.3). The assumption from the scan was complete tumour destruction with a surrounding inflammatory response.

![Figure 10.3 - Pre and Post laser MRI scans showing signal loss within the region of the original tumour with a surrounding rim of enhancement.](image)

**Histology findings:** Breast tissue measuring 70 x 60 x 20 mm. On sectioning there was a well circumscribed yellow and brown lesion measuring 25 x 20 x 15 mm. Microscopically, the specimen revealed an area of coagulative necrosis surrounded by an area of fat necrosis. No viable tumour was identified within the specimen (fig
Four axillary lymph nodes from sampling showed reactive change only with no evidence of metastatic carcinoma.

Figure 10.4 – Histology showing (a) medium power view of coagulative necrosis and remnants of dead tumour cells and (b) low power view showing dead tumour (arrow)

**PATIENT 2**

Age: 66  
Size of tumour: 22 x 20 mm  
Core biopsy: Grade 2, mixed invasive ductal and lobular carcinoma.  
No. of ILP needles: 2

MRI findings: Initial scans pre-ILP again demonstrated a well-defined enhancing tumour consistent with the mammographic findings of a carcinoma. Pre-surgery scans showed a similar post-ILP appearance to patient 1 (figure 10.5).
Figure 10.5 – Pre and Post ILP MR scans showing complete loss of enhancement corresponding to complete tumour necrosis

**Histology findings:** Breast tissue measuring 75 x 70 x 20mm. On sectioning there was a yellow lesion measuring 40 x 30 x 15 mm. Microscopically, there was surrounding fat necrosis with central coagulative necrosis (fig 10.6). No viable tumour was identified. Four sampled axillary nodes had reactive change only.

Figure 10.6 – Low power (a) and high power (b) views of laser treated tumour showing complete tumour cell death with loss of nuclei and cellular architecture
**PATIENT 3**

Age: 84
Size of tumour: 21 x 19 mm
Core biopsy: Grade 2, mucinous carcinoma.
No. of ILP needles: 2

MRI findings: Initial scans revealed an irregular enhancing tumour adherent to the chest wall musculature. Pre-surgery scans showed loss of signal within most of the tumour but a small area of enhancement remained (3mm) (Figure 10.7). This was felt to be residual tumour as the enhancement characteristics were similar to the original tumour.

![MRI scans](image)

*Figure 10.7 – Pre and Post ILP MR scans suggesting small area of residual tumour (arrow)*

Histology findings: Mastectomy specimen measuring 19 x 13 x 3 cm. On sectioning there was a lesion measuring 45 x 25 x 20 mm. Microscopically, there was an infarcted mucinous carcinoma. The majority of the tumour showed evidence of coagulative necrosis, however there were pockets of morphologically viable tumour, the largest measuring 3 mm. None of 12 excised lymph nodes showed evidence of metastatic carcinoma.
**Patient 4**

Age: 67

Size of tumour: 12 x 12 mm

Core biopsy: Grade 1, invasive ductal carcinoma with areas of DCIS.

No. of ILP needles: 1

**MRI findings:** Initial scans showed a typically enhancing tumour consistent with the mammographic findings. Pre-surgery scans showed virtually no residual enhancement (figure 10.8). There was however faint enhancement in the region of the laser treated tumour. The enhancement characteristics of this residual area were however different to the original pre-ILP treated tumour i.e. the uptake curves were less steep and overall enhancement was less intense (see below). It was uncertain whether therefore from the MR scans alone, whether this represented a mild residual inflammatory reaction or residual tumour.

*Figure 10.8 – Pre and Post ILP MR scans showing faint residual enhancement*
**Histology findings:** Breast tissue measuring 65 x 60 x 20mm. On sectioning there was a lesion measuring 35 x 25 x 12 mm. Macroscopically, there was fat necrosis surrounding a 12mm central area suggestive of residual tumour. Microscopically, residual tumour was confirmed (figure 10.9). There however was no evidence of coagulative necrosis. Four axillary nodes showed no evidence of metastatic carcinoma.

![Histology showing residual tumour](image1)

Figure 10.9 – Histology showing residual tumour
a) low and b) high magnification

**Clinical follow up:** All of the first four patients are alive and well with no evidence of local or distant recurrence with a minimum follow up time of 2 years.
**PATIENT 5**

Age: 85  
Size of tumour: 15 x 21 mm  
Core biopsy: Grade 2 invasive ductal carcinoma  
No. of ILP needles: 2  

MRI findings: Pre and post ILP scans were performed. In the post treatment scans contrast enhancement was lost in the area of the treated tumour consistent with a complete ablation.  

Clinical follow up: The patient was alive and well 19 months following ILP. Only a tiny palpable nodule remained (1cm x 0.5cm). There was no evidence of metastasis.

**PATIENT 6**

Age: 84  
Size of tumour: 25 x 20 mm  
Core biopsy: Grade 2 invasive ductal carcinoma  
No. of ILP needles: 2  

MRI findings: Pre and post ILP scans were performed. The initial enhancing tumour showed complete loss of enhancement following laser treatment suggesting complete ablation.  

Clinical follow up: The last patient review was 8 months following ILP. The palpable tumour was reduced in size although was still palpable

No patients had any significant side effects except for a little discomfort in the breast for a few days following ILP. This was relieved by simple analgesia such as paracodol. All patients were able to continue with day-to-day activities immediately following discharge.
10.5 DISCUSSION

The main shortfall of this study was undoubtedly the small number of patients recruited. Despite this, there was still much important information gained about several aspects of this treatment. There has only been one report to date of laser treated breast tumours with observation of their subsequent progress for any longer than a few days [Akimov et al. 1998]. No one however has evaluated such longer-term progress with MR imaging of laser treated tumours.

Results from the first few patients were very encouraging. In the first two, laser ablation was complete, which correlated with the MRI findings. In the third patient, although there was some residual tumour, this was detected with MRI. The one case (patient 4) where treatment was not as successful was disappointing and the reasons for this are unclear. One possible explanation is a problem that was encountered midway through the trial of ILP for fibroadenomas that was running concurrently (see chapter 11). A technical problem arose with the laser source where it was recognised that the calibration was not functioning accurately. This may have lead to undertreatment of the tumour. Another theory, again originating from the fibroadenoma study, was that the extent of pre-charring of the fibre tip was not adequate. This could also result in unpredictable and inadequate treatment [Harries et al. 1994]. These theories are further supported by the observation that there was no central charring/coagulative necrosis, which is seen with the higher temperatures around the tip of the laser fibre. The question of why there was marked reduction of enhancement in the MR images, despite apparent viable residual tumour, is also uncertain. It may be that although treatment was not complete, ILP may have caused impairment of the tumour blood supply. This in turn would lead to loss of enhancement on MR. If this was the case, the viability of such a tumour in the longer term is uncertain.

The use of dynamic fast sequence scanning allows the enhancement characteristics of a certain area to be studied. There has been much debate about the ability of signal intensity time course data to differentiate between benign and malignant enhancing lesions. To obtain such data, a rapid sequence of images (usually every 60 seconds) is
obtained before and after gadolinium enhancement. By taking a region of interest within an enhancing lesion, a plot of signal intensity versus time can be achieved. Various authors have presented data comparing enhancement curves with histopathology of the excised lesions [Orel 1999]. Criteria for differentiation between benign and malignant lesions have however been varied and include gradient of the initial enhancement slope, maximum increase in enhancement and the overall shape of the curve. Generally a steep initial enhancement gradient and a high maximum intensity are associated with carcinoma. Kuhl and colleagues investigated the overall shape of the curve [Kuhl et al. 1999]. They found 3 broad enhancement patterns. Type 1 was a gradual steady increase of enhancement over time, type 2 reached a steady plateau and type 3 showed an initial plateau and then a drop in enhancement (fig 10.10).

They found that type 1 curves were associated with benign disease in 83% and type 3 was more likely to be malignant (57%). For type 2 curves there was significant overlap and these were therefore classed as indeterminate.

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Figure 10.10 – Diagrammatic representation of time intensity curves as proposed by Kuhl et al [Kuhl et al., 1999].
The exact nature of gadolinium enhancement is still a little uncertain but it is accepted that areas of increased vascularity will enhance i.e. tumours that exhibit angiogenesis. The problem however is that there is significant overlap with all the above criteria and such data is only useful in conjunction with the morphological characteristics of an enhancing lesion. With regards to ILP, it is difficult to know if enhancement characteristics will have any role to play in determining the nature of residual enhancement. The main theory for this statement is based on the fact that the thermal effects will undoubtedly have some effect on the vascular supply to the tumour as seen with patient 4. This could lead to false negative scans for residual tumour. It is more likely therefore that the absolute presence or absence of enhancement will be a better indicator of residual disease.

One area which has not been explored in this thesis but would further help to ensure adequate treatment at the time of ILP would be the use of MR thermometry. Temperature sensitive sequences have been touched upon in chapter 5 (section 5.5). Although several methods of temperature mapping have been postulated, most investigators have concentrated on T1 signal intensity change and Proton Resonance Frequency Shift (PRFS). Both have their relative merits but also certain disadvantages.

With increasing temperature, T1 relaxation time lengthens therefore signal intensity decreases. This occurs in a relatively linear fashion until the temperature reaches around 60°C when a plateau is reached [Lamb & Gedroyc 1997]. Acquisition times are fast (around 3 seconds per slice) and thermal colour coded images can be obtained (Thermo-TurboFLASH, Siemens). The main disadvantage with this method is however the antagonistic decrease in T1 relaxation time within coagulated tissue. This fact has lead some investigators to concentrate on frequency shift imaging as this is dependent on temperature change only [Graham et al. 1999]. PRFS has also been shown to be more accurate than signal intensity change for temperature mapping [Moriarty et al. 1998]. The main drawbacks are slow acquisition times (up to 1 minute per slice) and marked sensitivity to motion artefacts. These problems can however be partly overcome with faster imaging (spoiled gradient echo – 20 seconds per slice) and immobilisation of the tissue in question. The need for sub 20 second imaging is also questionable as most coagulation methods have treatment times
extending over several minutes. Of greater problem however is the difficulty of using this technique in tissues containing fat, due to temperature-induced susceptibility changes [de Poorter et al. 1995]. This may make signal intensity change more suitable for monitoring thermal ablative methods within the breast. In conclusion, MRI of the breast shows much promise for both real time and post treatment imaging following ILP. The best sequence protocols are however still open for debate.

One criticism of the trial was the administration of Tamoxifen during the 3-month period between ILP and surgery. The rational behind placing these patients on tamoxifen was twofold. Firstly it is routine to prescribe anti-oestrogen therapy to all post-menopausal women following "surgery" for breast cancer, especially if the tumours are oestrogen receptor positive. As ILP can be considered an alternative method of tumour mass removal, it would not therefore be ethical to withhold this treatment until after conventional surgery at 3 months post diagnosis. Secondly, as we had no definite proof that MR imaging could accurately assess whether complete tumour ablation had taken place, it was again ethically important to ensure that any possible residual tumour growth was suppressed. Tamoxifen, being considered a tumorostatic agent for the majority of post-menopausal patients with oestrogen receptor positive tumours all patients, was again the obvious choice.

The main concern with concurrent administration of Tamoxifen was the possibility of tumour regression causing "clouding" of the results. Some would argue that Tamoxifen itself could achieve complete tumour regression. A review of the literature however clearly demonstrates that such a response is by far in the minority ranging from 8-39%. Several studies are summarised in table 10.1 [Bates et al. 1991][Gaskell et al. 1992][Horobin et al. 1991][McDonald et al. 1990][Robertson et al. 1988]. The results are all based on the Union Internacional Contra la Cancerum critria (UICC) where partial response is when the sum of the two maximum diameters a tumour, reaches less than 50% of the original size [Hayward & Rubens 1977].
### Table 10.1

<table>
<thead>
<tr>
<th>Study</th>
<th>Complete response(%)</th>
<th>Partial response(%)</th>
<th>Static (%)</th>
<th>Progression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaskell</td>
<td>8</td>
<td>24</td>
<td>30</td>
<td>27</td>
</tr>
<tr>
<td>McDonald</td>
<td>17</td>
<td>17</td>
<td>34</td>
<td>31</td>
</tr>
<tr>
<td>Robertson</td>
<td>39</td>
<td>16</td>
<td>16</td>
<td>30</td>
</tr>
<tr>
<td>Bates</td>
<td>16</td>
<td>9</td>
<td>62</td>
<td>12</td>
</tr>
<tr>
<td>Horobin</td>
<td>34</td>
<td>15</td>
<td>30</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>555</td>
<td>24%</td>
<td>15%</td>
<td>38%</td>
</tr>
</tbody>
</table>

Table 10.1 – Summary of the results of several trials evaluating the response of primary breast cancer to Tamoxifen (numbers are in percentages) Totals are adjusted according to study size.


The time to regression is also quite clearly time dependent, in that maximum response generally takes longer than 6 months to reach this stage. In view of this evidence it was felt that it was unlikely that a significant proportion of patients entering the trial would get a significant response to the tamoxifen alone, within the 3-month time scale. Therefore any observed tumour cell death could be attributed mainly to the laser effects.

### 10.6 Conclusion

This pilot study has demonstrated that ILP continues to be a safe treatment for breast cancer with no reported serious complications in our cohort. Despite the small numbers, the majority of patients had a good response to treatment and it is clear that complete tumour ablation is achievable. For the first time, by allowing a time lag between ILP and surgery, histopathology has confirmed that the tumour ablation appears permanent. It has also been shown that magnetic resonance imaging is relatively accurate at detecting residual tumour. Although still at an early stage of evaluation, ILP could be an alternative to surgery, for a selected group of patients.
What is needed is a trial of long-term outcome of ILP versus surgery in combination with standard adjuvant treatment. As both treatments have the aim of achieving only local control of disease, mortality rates should theoretically be similar. What would be interesting however would be the comparison of morbidity and local relapse. What is also interesting, although anecdotal, is that all the patients involved with the trial commented on the relative ease of undergoing ILP compared to surgery and also without exception would much prefer ILP should a further tumour arise in the future.
CHAPTER 11  ILP OF BENIGN BREAST FIBROADENOMAS

11.1 INTRODUCTION

The natural history of fibroadenomas of the breast is reasonably well validated (see section 6.1). In broad terms, around a third will get larger, a third smaller and a third will remain the same size. In order however to study the efficacy of ILP for fibroadenomas, it is important to compare laser treated patients with a group who have no treatment.

It can be argued that all but the most rapidly growing fibroadenomas should receive a trial of conservative management, and this certainly is becoming normal practice in the UK (see chapter 6). There are however a sub-group of women who will always request excision due to fear of leaving a lump in-situ. There are also others who will request surgical treatment at some period down the line, should their lump fail to resolve. Indeed few women would admit to be totally happy about leaving a fibroadenoma indefinitely, especially if it is particularly prominent. With ILP, there possibly exists a viable alternative to traditional surgical excision.

There have only been two published studies to date where laser photocoagulation has been employed as a treatment for fibroadenomas[Basu et al., 1999;Lai et al., 1999]. In the paper by Lai et al (National Medical Laser Centre), 29 fibroadenomas with a mean diameter of 25mm were treated (range 14-35mm in max diameter). Patients were offered ILP in the interim between diagnosis and surgery (1-6 months). Ninety seven percent decreased in size on serial ultrasound. Only 6 patients continued with the original plan of surgery. The remainder were happy with the reduction in size following ILP and declined further intervention. Of the 14 patients followed up for one year, all were clinical impalpable, with only one fibroadenoma detectable on ultrasound (9mm). In general there was a gradual reduction in size over the year with an average decrease of 38%, 60% and 92% at 3, 6 and 12 months respectively. No significant complications were recorded. Three patients however had minor skin burns around the puncture site.
Basu et al used a very similar method of ILP, the only real difference being the use of a Nd:YAG laser operating at 1064nm as opposed to a semi conductor diode laser (805nm) at our institution. The design of the study was also relatively similar. Twenty seven patients were treated with mean two dimensional size of 2.6cm² (approx. 1.6cm in average diameter i.e. smaller than Lai’s trial). Follow up was shorter (8 weeks) but there was a larger average decrease (60%). Ten patients with residual lumps however underwent excision biopsy. Again no significant complications were observed although 8 patients had minor skin burns around the puncture sites and two had a sterile, self limiting discharge.

Both these papers have demonstrated the safety and efficacy of this technique. The smaller number of skin burns in the first study is probably attributable to an oblique course used for needle placement when the lumps are superficial, rather than going directly through the skin overlying the lesion. Also cooling of the breast with cold saline may also play a positive role in preventing this complication. Both studies demonstrated an initial increase in size over the first two weeks, presumably due to an inflammatory response, before a gradual decrease in size over the following months. It is therefore important to warn patients undergoing ILP that the lump will initially become larger before it gets smaller.

11.2 AIMS

The aims of this study were twofold. Firstly to follow the natural history of laser treated breast tissue, as further support for the use of ILP for fibroadenomas and also for breast cancer patients. Secondly, to prove that ILP is a safe and effective alternative for the treatment of benign breast fibroadenomas.

11.3 PATIENTS AND METHODS

Patients were recruited from the surgical symptomatic breast clinic at the Elizabeth Garrett Hospital for Women, following approval from the local ethics committee. The study was designed as a comparative trial between two groups of patients. One
group underwent ILP and the other had continued observation of their fibroadenoma but no treatment. Inclusion criteria were age under 35 and a fibroadenoma diagnosed by triple assessment (clinical examination, FNAC or core-biopsy, and ultrasound). All three parts of the assessment had to be definitive for fibroadenoma i.e. U2 for ultrasound, C2 for cytology and the characteristic findings of mobility and rubbery consistency on examination.

Suitable patients were approached following diagnosis. All received a full written and verbal explanation of the study (Appendix 3). Those willing to participate, self-selected into one of the two groups. Again reassurance was given that they could withdraw from the study at any time, at which stage treatment of their choice would be undertaken e.g. surgical excision or discharge to the general practitioner.

Patients who opted for observation were reviewed over the course of the ensuing year at 3, 6 and 12 months. At each review they underwent clinical examination and ultrasound measurement of their fibroadenoma. The maximum diameter was recorded in three planes to allow volume measurement. Perhaps most importantly, the patient own opinion about change in size was also noted i.e. larger, smaller or similar size. Some patients requested further follow up at the end of the planned review period and this was accommodated at 6 or 12 monthly intervals.

In the group who elected for ILP, this was carried out as soon as possible, on a date convenient for the patient. The procedure was exactly the same as for the breast cancer patients (section 10.3.2). The patients were contacted by telephone after a few days to enquire about any possible problems. Following this, review was similar to the non-treated group i.e. 3, 6 and 12 months.

11.4 RESULTS

Patient demographics are presented in table 11.1. In summary however, a total of 30 patients entered the study, 19 in the treatment group and 11 in the control group. All patients were pre-menopausal. The total number of fibroadenomas for each group was however equal, numbering 20. One patient in the treatment group had 2
fibroadenomas; the remainder had only 1. The number of fibroadenomas in the control group however ranged from 1 to 9!

<table>
<thead>
<tr>
<th>Patient group</th>
<th>ILP treated</th>
<th>Non treated controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>Average age</td>
<td>27.3</td>
<td>30.7</td>
</tr>
<tr>
<td>Total number of fibroadenomas</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Average volume</td>
<td>5.03cm³ (3.52)</td>
<td>1.77cm³</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White = 16</td>
<td>White = 10</td>
<td></td>
</tr>
<tr>
<td>Afro-Caribbean = 2</td>
<td>Asian = 1</td>
<td></td>
</tr>
<tr>
<td>Asian = 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current oral contraception usage</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Position of lesion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UOQ = 9</td>
<td>UOQ = 12</td>
<td></td>
</tr>
<tr>
<td>UIQ = 3</td>
<td>UIQ = 4</td>
<td></td>
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<td>LIQ = 2</td>
<td>LIQ = 2</td>
<td></td>
</tr>
<tr>
<td>LOQ = 4</td>
<td>LOQ = 3</td>
<td></td>
</tr>
<tr>
<td>Retro-areolar = 3</td>
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<td></td>
</tr>
<tr>
<td>Side of lesion</td>
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<td></td>
</tr>
<tr>
<td>Right = 9</td>
<td>Right = 11</td>
<td></td>
</tr>
<tr>
<td>Left = 12</td>
<td>Left = 10</td>
<td></td>
</tr>
</tbody>
</table>

Table 11.1 – Patient demographics. UOQ = upper outer quadrant, UIQ = upper inner quadrant, LOQ = lower outer quadrant, LIQ = lower inner quadrant.

The average initial volume was markedly different between the two groups, 5.03cm³ vs 1.77cm³. This represented a bias towards patients with smaller lesions being happier to observe them rather than to proceed straight to surgery or ILP. There were however two very large lesions in the treatment group which skewed the mean significantly. If these two patients are left out, the average volume is only 3.52cm³ in the treatment group. This however is still double the average size of the controls. Ethnic mix and oral contraceptive use were similar. The position of the lesions were
also similar and agree with previous published reports where the upper outer quadrant is the most common site (around 50% of patients).

Within the treatment group, the number of fibres used varied from 1 to 4, with an average of 2 fibres per fibroadenoma. The total laser energy ranged from 1000 to 5400 Joules (average 2560J). One patient lay outside the upper age limit of 35 years. She had a long history of multiple fibroadenomas and the current lump had been present, unchanged, for more than 1 year. At her request, and in agreement with the consultant surgeon in charge of her case, ILP was performed. Most patients described a little discomfort during the procedure. In one patient however the procedure was discontinued after 350 seconds due to excess pain. This however subsided immediately on cessation of treatment. There were no serious complications, however the patient whose treatment was cut short, experienced a small skin burn (which healed with a small 2mm scar) and a sterile serous discharge which resolved spontaneously. This complication however was believed to be the result of an extremely superficial lesion.

Follow up of patients was as outlined above. The findings on ultrasound scanning and patient perception about change in size of the lump are presented in table 11.2. Within the ILP treated group, three patients were lost to follow up during the course of the 12 months. This has been taken into account in the analysis. Also a further 3 patients had only reached their 6 month review. This left a total of 13 patients (14 fibroadenomas) reaching the full 12-month review stage. Within the control group, 1 patient was lost to follow up and in one patient the fibroadenoma had disappeared at the 6-month review and she did not request any further review. A further 3 patients had only reached their 6 month review. This left 6 patients reaching the 12-month review (10 fibroadenomas). Percentages however are based on each patient’s individual fibroadenoma and then averaged according to the number having reached each review date. A graphical representation of the percentage change in volume for all the fibroadenomas followed up for one year are presented in figures 11.1 and 11.2.
<table>
<thead>
<tr>
<th>Patient group</th>
<th>ILP treated</th>
<th>Non treated controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average initial size</td>
<td>5.03cm³</td>
<td>1.77cm³</td>
</tr>
<tr>
<td>Percentage of original volume at 3 months</td>
<td>Mean = 78.7</td>
<td>Mean = 114.8</td>
</tr>
<tr>
<td></td>
<td>Median = 77.3</td>
<td>Median = 116.1</td>
</tr>
<tr>
<td></td>
<td>Range = 2-220</td>
<td>Range = 13-571</td>
</tr>
<tr>
<td></td>
<td>(n=17)</td>
<td>n = 19</td>
</tr>
<tr>
<td>Patient perception at 3 months</td>
<td>Larger = 5.5%</td>
<td>Larger = 21%</td>
</tr>
<tr>
<td></td>
<td>Same size = 11%</td>
<td>Same size = 71%</td>
</tr>
<tr>
<td></td>
<td>Smaller = 78%</td>
<td>Smaller = 7%</td>
</tr>
<tr>
<td></td>
<td>Gone = 5.5%</td>
<td>Gone = 0%</td>
</tr>
<tr>
<td>Percentage of original volume at 6 months</td>
<td>Mean = 44.2</td>
<td>Mean = 134.6</td>
</tr>
<tr>
<td></td>
<td>Median = 33.8</td>
<td>Median = 85.7</td>
</tr>
<tr>
<td></td>
<td>Range = 0-135</td>
<td>Range = 29-478</td>
</tr>
<tr>
<td></td>
<td>n = 17</td>
<td>n = 16</td>
</tr>
<tr>
<td>Patient perception at 6 months</td>
<td>Larger = 0%</td>
<td>Larger = 18%</td>
</tr>
<tr>
<td></td>
<td>Same size = 11%</td>
<td>Same size = 82%</td>
</tr>
<tr>
<td></td>
<td>Smaller = 55%</td>
<td>Smaller = 0%</td>
</tr>
<tr>
<td></td>
<td>Gone = 34%</td>
<td>Gone = 0%</td>
</tr>
<tr>
<td>Percentage of original volume at 12 months</td>
<td>Mean = 17.8</td>
<td>Mean = 64.1</td>
</tr>
<tr>
<td></td>
<td>Median = 20.2</td>
<td>Median = 83.5</td>
</tr>
<tr>
<td></td>
<td>Range = 0 – 45</td>
<td>Range = 13 – 430</td>
</tr>
<tr>
<td></td>
<td>n = 14</td>
<td>n = 11</td>
</tr>
<tr>
<td>Patient perception at 12 months</td>
<td>Larger = 0%</td>
<td>Larger = 29%</td>
</tr>
<tr>
<td></td>
<td>Same size = 7%</td>
<td>Same size = 57%</td>
</tr>
<tr>
<td></td>
<td>Smaller = 36%</td>
<td>Smaller = 0%</td>
</tr>
<tr>
<td></td>
<td>Gone = 57%</td>
<td>Gone = 14%</td>
</tr>
</tbody>
</table>

*Table 11.2 – Average volume change on ultrasound measurement and patient perception of change in size (n = number of fibroadenomas in each group reaching review points)*
These graphs illustrate that although some ILP treated fibroadenomas initially became a little larger, all gradually reduced in size over the 12 month period. Conversely, in the untreated control group, the fibroadenomas generally stayed of
similar size with a only a few showing quite erratic changes in size, both larger and smaller.

In summary there was an expected gradual reduction in the average size of fibroadenomas in the ILP treated group. The average decrease in volume was 78.7%, 44.2% and 17.8% at 3, 6 and 12 months respectively. This is presented in figure 11.3.

![Graph](image-url)

*Figure 11.3 – Average percentage volume compared to original size over time*

In the control group, results are similar to those previously published in that some will enlarge, some remain static and some will become smaller. The apparent sharp decrease in the average size between 6 and 12 months is due to several factors. One patient with a rapidly growing fibroadenoma requested surgery and did not reach her 12-month review. A further 2 patients with enlarging fibroadenomas had only been followed up for 6 months. Bias was therefore artificially towards those patients with smaller or static lumps. If assumption was made that their fibroadenomas would remain at least the same size, a more realistic figure at 12 months would be 108% of original. This however is purely conjectural and the actual figure will be achieved when these patients are reviewed. None the less, there still remains a marked
statistical difference between the two groups (p<0.001; Students t-test for unpaired groups).

### 11.5 Ultrasound Characteristics

In contrast to the breast cancer patients presented in chapter 10, ultrasound was felt to be a suitable method for assessment and follow up of the fibroadenomas. The reasons for this are twofold. Firstly it is not as essential to be precisely accurate about the extent of treated tissue, as any residual fibroadenoma does not pose any danger to the patient. Despite this it is however desirable to be able to assess the extent of coagulation with reasonable certainty, in order to ensure that at least the majority of the tumour has been treated. Secondly, should ILP become an accepted method of routine treatment of fibroadenomas, ultrasound is a much more readily available diagnostic tool. Ultrasound also plays an integral role in the positioning of the laser fibres for the actual treatment (fig 11.4).

![Figure 11.4](image)

*Figure 11.4 – single needle placed in the centre of the fibroadenoma prior to treatment.*
In addition to measuring the change in size of a fibroadenoma, the characteristics of laser treated tissue were also evaluated. Typically, ILP treated areas become hyperechoic compared to the normally hypoechoic fibroadenoma (fig 11.5).

Figure 11.5 – Pre (a) and post (b) ILP of a lobulated fibroadenoma 6 months following ILP. The overall size is reduced and also the centre of the tumour has become hyperechoic consistent with coagulated tissue.
Some of the smaller fibroadenomas became quite indistinct and difficult to separate from the normal surrounding breast tissue. This was presumably because of the area of coagulation extending a little way past the capsule of the tumour (fig 11.6).

Figure 11.6 – Pre (a) and post (b) ILP of a small fibroadenoma 6 months following ILP. The centre is hyperechoic and the margins are indistinct suggesting coagulation slightly outside the tumour.
For larger fibroadenomas multiple fibres were used (maximum 4). These were spaced as equally as possible within the tumour (fig 11.7). It was important each time to check the position of such needles in two planes as although a needle could appear central on one view, it could in fact be near the edge of the tumour in another plane.

![Figure 11.7 - Three needles spaced relatively equally throughout a larger tumour (arrows). The orientation is transversely across the needle rather than longitudinally.](image)

For the very large tumours, it was not possible to coagulate all of the tissue at one sitting. Areas of coagulated fibroadenoma could however be distinguished from non-treated areas (fig 11.8). This is useful for planning and positioning of fibres for further ILP of the same fibroadenoma. In this way it would be feasible to treat larger tumours.
The above images demonstrate the effectiveness of ultrasound for the assessment, guidance of treatment, and follow up of ILP treated fibroadenomas. In a time where health resources are limited, ultrasound appears to be a reasonable alternative to MRI in these patients.

11.6 DISCUSSION

This study has found similar results to previous work in that all fibroadenomas decreased in size with the majority disappearing completely. What it also adds is comparison to a control group. Unlike Lai et al, there were however 6 patients whose fibroadenoma did not completely disappear clinically following ILP. They all however decreased in size. In a further two patients there was a small impalpable remnant, only detectable on ultrasound. There are however probable reasons for this. Of the 6 patients who had palpable remnants, four had maximum diameters greater
than 3cm (range 31-49mm; average volume 13.6cm³). In fact only two other patients had fibroadenomas of this size that did completely resolve (max diameter 31mm; average volume 3.6cm³). Both of these patients had discus shaped, long and flat fibroadenomas rather than the more usual spherical type. In the fifth patient there was apparent failure of the laser as no characteristic treatment effect seen on follow up ultrasound (see below). In this patient, repeat ILP achieved 67% reduction at the latest 6-month review. In the final patient, although the lesion reduced in volume by 71% at 12 months, because of a superficial position, it was still palpable.

Two other minor technical problems were encountered during the study. Following the patient with no observed treatment effect, the internal laser calibration system was checked using an independent power detector. This revealed a problem with inaccurate calibration tending to cause a reduction in the true wattage being produced. This problem was therefore corrected and rechecked. A second observation was a reduction in charring around the fibre tip following treatment. It is our normal practice to pre-char the optic fibre tips before insertion. The theory is that the laser energy is absorbed by the carbon particles and is emitted as heat. This therefore gives a more predictable size of coagulation. Pre-charring was originally achieved by placing a small drop of the patients own blood on the tip of the probe and firing the laser at high power (10 watts) for a few seconds until smoking was seen. This method was replaced by using a match or cigarette lighter to place soot on the end. It was felt however, during the study, that this method was perhaps not as efficient and a return to the original practice took place. This lead to much better post treatment effects visible on ultrasound.

11.7 CONCLUSION

Despite the technical problems, ILP was still shown to be effective as a treatment for benign fibroadenomas of the breast. This is further supported by the comparison to a control group of untreated patients. There does however seem to be a cut off in the size of fibroadenoma that can be treated which is probably more volume related than simply due to maximum diameter. Certainly however, fibroadenomas that are greater than 3cm and are more spherical rather than flat, are unlikely to completely disappear
with a single treatment. There is no reason however that patients cannot undergo 2 or more treatments, with targeting of any untreated areas.

There is pleasing proof of the continuing safety of this technique. Also this study gives support to the assumption that ILP treated tissue gradually undergoes resorption through normal healing and repair mechanisms. These observations add further evidence for treatment of breast cancers in this way. With regards to the treatment of fibroadenomas, there are increasing numbers of patients entering the trial with further encouraging results. Recruitment continues and we hope, in the not too distant future, to be at a point where this treatment may be offered as a reliable alternative to surgery.
CHAPTER 12
DISCUSSION, SUMMARY AND FUTURE DIRECTIONS

The aim of this thesis was to evaluate the interaction of light with breast tissue in order to develop new techniques for diagnosis and therapy of breast tumours. To achieve this, several studies were undertaken. These however fell broadly under the headings of 'Optical Biopsy' and 'Interstitial Laser Photocoagulation'. Throughout this thesis each of the two main studies have been considered in their own right. This division is continued below.

12.1 OPTICAL BIOPSY

The concept of optical biopsy is relatively simple. Light entering tissue is either scattered or absorbed. The degree of scattering depends on the nature of the tissue being interrogated. A system that is sensitive to such scattering could therefore have the potential to differentiate between various types of breast tissue i.e. breast cancer versus benign or normal breast tissue. Such a system, if fully validated, could have several clinical applications.

Experimental studies confirmed the calculated assumption that the optical system was only characterising tissue at a depth of less than 1mm from the tip. Although this made precise correlation with the histological sample more of a technical challenge, it does however mean that in the clinical setting, the optical biopsy is point specific, which ensures that only the area of tissue in question is being interrogated, and not a large volume of surrounding tissue that could give erroneous results. In practice, the analysis of spectra from a large volume of tissue may also have proved impossible due to the heterogeneity of breast tissue. The experimental confirmation of a small area of biopsy was therefore vitally important for progression of the project.

The key to optical biopsy lies firstly in precise optical and histological correlation and secondly, in the analysis of the data. Algorithms for spectral analysis were developed using ANN (Artificial Neural Network), HCA (Hierarchical Cluster Analysis) and MBA (Model Based Analysis). To help in the MBA, several ex-vivo experiments were devised. This helped to characterise the known spectral
characteristics of haemoglobin within blood, β-carotene in fat, and blue dye within ‘sentinel’ lymph nodes. By accounting for such variables, the true differences in the spectra due to the scattering properties of malignant breast tissue would be clearer. Indeed, for the detection of breast cancer versus normal or benign breast tissue, the model-based analysis was found to be extremely accurate with both sensitivity and specificity greater than 90%. MBA was however less accurate in the differentiation between positive and negative lymph nodes. The reasons for this are unclear but may be a reflection of the technical difficulties in obtaining consistent readings from the cortex of small (<1cm) lymph nodes. It is generally accepted however that MBA probably has the greatest promise of achieving even better detection rates compared to the purely statistical analysis methods of ANN and HCA. Further development of MBA is ongoing.

Applications of a fully validated system for the characterisation of breast disease are exciting. Such an instrument would be capable of giving a ‘real time’ diagnosis in an outpatient setting. This would be equivalent to having a cytopathologist available for immediate inspection of an FNAC, thus freeing up this valuable resource. Immediate \textit{in-situ} assessment also has its advantages, as multiple areas can be “biopsied” in a short period of time to decrease the chance of sampling error. Also if an indeterminate signal is obtained, the clinician can immediately proceed to core biopsy. Similar to FNAC, optical biopsy may not be able to characterise every conceivable type of normal, benign or malignant breast tissue. Another possible use includes the assessment of resection margins immediately following wide local excision rather than waiting for histopathological examination. This would have a dramatic impact by reducing the need for ‘re-excision’ operations for positive margins. Not only would this have an impact on optimising the scarce resource of operating theatre time, but also and perhaps more importantly, would reduce unnecessary discomfort and anxiety for patients. Furthermore, should sentinel node biopsy become accepted and routine practice, immediate differentiation between positive or negative lymph node status would enable the surgeon to proceed to full axillary clearance, again obviating the need for a second surgical procedure. Another interesting and novel use for this system is for the differentiation between Paget’s disease of the nipple and simple dermatitis or eczema. This is always a difficult diagnosis to make clinically and usually results in an incision biopsy. This can be
quite uncomfortable for the patient, as the nipple is a particularly sensitive area. Certainly, an optical biopsy where the probe is simply placed in direct contact with the skin would be quick, simple and most importantly painless. Some optical biopsies of Paget's disease, normal nipple skin and dermatitis have already been obtained from patients at the NMLC with obvious differences being noted. Numbers however are few but work is ongoing.

Despite concentrating on the breast, optical biopsy has limitless application in many clinical settings. Other possible areas that are already being investigated at NMLC are its use for melanoma, Barrett's oesophagus and colonic polyps. For melanoma, the challenge is to differentiate between benign naevae and malignant melanoma. Another aim would be to detect a dysplastic naevus before it becomes malignant. This would be of particular benefit to patients with dysplastic naevus syndrome who undergo numerous excision biopsies for suspicious lesions every year. This is not to mention the thousands of patients who attend dermatological outpatient clinics in the UK each year with a "suspicious mole". The ability to characterise multiple naevae in seconds has its obvious benefits. For both Barrett's oesophagus, and colonic polyps, probes have been manufactured that can pass down the biopsy channel of an endoscope. Again, immediate characterisation of suspicious areas would be extremely useful. Other obvious clinical uses are for cervical dysplasia, oral malignancy and other dermatological conditions. Theoretically however, optical biopsy could be used to characterise any abnormality, whether detected clinically, or radiologically. Using image guidance, such as ultrasound, CT or MRI, any area of the body is potentially reachable.
12.2 **INTERSTITIAL LASER PHOTOCOAGULATION**

Interstitial laser photocoagulation (ILP) is a method of causing thermal necrosis of tissue. Two studies were undertaken concurrently at the National Medical Laser Centre. These were designed with three main aims:

1) To further assess the feasibility and safety of treating breast tumours in this way.
2) To determine the long term sequelae of ILP treated breast tissue.
3) To develop a means of accurately assessing the extent of thermal necrosis in-situ.

12.2.1 **ILP for the treatment of benign fibroadenomas of the breast**

Patients with benign fibroadenomas diagnosed by triple assessment were invited to enter the study. Those willing to participate, self selected either ILP or no treatment. Nineteen patients underwent ILP and were compared to a control group of 11 patients who elected to have conservative treatment with ultrasound follow up. Following ILP there was a general reduction in size within all fibroadenomas with the majority completely disappearing after 12 months. This reduction was statistically significant compared to an untreated control group. It also confirmed previous findings of a gradual reduction in size over time presumably due to macrophagic ingestion of necrotic tissue. The results from this study demonstrated the efficacy of ILP for fibroadenomas. Those patients who had residual lumps generally had larger lesions (>3cm in diameter). All patients were generally happy with the outcome of treatment and no serious complications occurred.

ILP is an ideal technology for the treatment of this benign condition. Although it is safe to leave fibroadenomas untreated, as discussed previously, many women still request treatment as they do not like having a ‘lump’ in their breast. ILP therefore offers a viable alternative to surgery. The main advantages over surgery are the lack of scarring and the avoidance of general anaesthetic and its related risks. The only drawback is the time lag between ILP and the disappearance of the lump. This may
not suit all women, but certainly no women who entered the trial stated this to be a concern.

12.2.2 ILP for the treatment of malignant breast cancers

Inclusion criteria were age over 64 with malignant, oestrogen receptor (ER) positive, primary breast tumours less than 3cm in maximum diameter. All underwent ILP within 3 weeks of diagnosis, were placed on Tamoxifen, and had definitive surgery at 3 months. Contrast enhanced MRI scans were obtained pre- and post-ILP and pre-operatively. Six patients were recruited, 4 of which underwent surgery. Of these, two patients showed complete ablation of their tumour. Two others showed partial ablation. MRI was shown to be reasonably accurate at determining the presence of residual tumour, i.e. loss of contrast enhancement following ILP correlates with no remaining viable tumour. The remaining 2 patients were deemed unfit for surgery due to co-existing medical disease. Both however obtained a good clinical response with a marked reduction in the size of the palpable lump and loss of enhancement on MR imaging.

Although numbers were small, the results have been encouraging. One cited problem is the loss of histological assessment of the treated tumours that may have an impact on further therapies such as chemo- or radiotherapy. This however can be solved by obtaining several core-biopsies from the tumour prior to ILP (as was the case with this study). Certainly we have proven that ILP can completely destroy small breast cancers. With the now widespread acceptance that the aim of breast cancer surgery is to control local disease, as evidenced by the wide local excision trials, there is no reason that ILP cannot also fulfil this goal. Similar to the benefits stated in the treatment of fibroadenomas, cosmesis and general simplicity of the procedure are compelling arguments for it actually having certain advantages when compared to traditional surgery.
12.3 CONCLUSION AND FUTURE DIRECTIONS

Both studies had overall positive results. These were however pilot studies and as such have identified several areas were further work is needed. What has been shown however is that Optical Biopsy is relatively accurate at differentiating between benign and malignant breast tissue. Our understanding of model based analysis is still however at an early stage but certainly a better understanding of the various factors which make up the spectral components of an 'optical biopsy' can only further improve sensitivity and specificity. Although work to date has only concentrated on the difference between malignant and non-malignant tissue, from the preliminary results so far, it should be possible for optical biopsy to characterise various types of tissue e.g. normal, fibroadenomas, sclerotic breast tissue, atypical ductal hyperplasia etc. This would obviously be desirable in the initial diagnosis of a breast lump as a definitive diagnosis of a fibroadenoma obviates the need for any further intervention.

With increasing knowledge about the biology of cancer, it may be possible to incorporate a 'dye' to a genetic based carrier compound that specifically targets and concentrates in malignant tissue. It would then be a simple matter to spectrally detect the increased levels of such a substance within a suspicious area of breast tissue. Such 'enhancement' of the optical biopsy would inevitably lead to increased accuracy. One possible carrier would be an antibody to endothelial growth factors that are known to be of increased concentration within tumour tissue. The limits of such ideas are only as narrow as the imagination.

The next step in this research programme is to dramatically increase the numbers of biopsies. This work is ongoing. Once sufficient data has been accumulated to further train and test the analysis methods, it is envisaged that the system will be tested against conventional cytology and histology. Initially a trial comparing the accuracy of optical biopsy to detect positive resection margins in-vivo versus the histopathological assessment would be relatively simple to perform. Such a trial would have immediate benefits if the system were validated. In such a setting 100% accuracy would not be as important (although desirable), as all specimens would continue to be examined conventionally. Any ‘missed’ positive margins would then
be detected and further treatment planned. Certainly if accuracies of 90% were maintained, this would avoid a second operation for 9 out of 10 women with positive margins. The published positive margin rate following wide local excision is variable but usually is between 25 and 50% [Luu et al., ;Mai et al., ;Sauter et al., 1994]. Even with a conservative figure of 25% (which is more the accepted norm) this reduction on operation numbers would be substantial. To conduct such a trial would probably involve collaboration with a number of research groups. At present the equipment used is relatively large and expensive i.e. a laptop computer linked to the lamp and spectrometer. The hardware however could be substantially reduced in size and complexity once definitive analysis algorithms have been produced. At present the laptop interface is required as it also performs all the analysis. It would however be feasible to run the software from a small palmtop sized computer and also build a compacted version of the lamp and spectrometer (at present these are housed in a large case to allow room for any future adjustments). Such a system would therefore also be substantially cheaper to manufacture.

With regards to ILP, it has been shown to be a viable and safe alternative to surgery for fibroadenomas and may be an alternative for a selected group of patients with primary breast cancer who are either unsuitable for, or refuse surgery. Also in this small series, MRI was relatively accurate at detecting the effect of ILP and also the presence of any residual tumour. What is required however is further validation of MR with the recruitment and treatment of more patients. If it can continually be shown to reliably detect residual viable tumour and also provide longer term follow up in terms of recurrence, then ILP treated breast cancers can be safely treated and left in-situ. What would be even more desirable would be the ability to assess the extent of tumour necrosis in ‘real time’. Temperature sensitive MR sequences have the promise to achieve this. With the increasing availability of open access breast coils where laser treatment can be occur while the patient is within the MR scanner, the possibility of exploring this technique is now feasible. This would allow repositioning of the fibres to ensure complete tumour destruction at the one sitting, rather than having to wait for the results of post treatment scans. There is no reason however that repeat ILP treatments cannot be used for patients with larger tumours, either benign or malignant. Again, this is dependant on validating reliable imaging methods for determining the extent of tumour necrosis.
Similar to optical biopsy, ILP has many applications outside the breast. The treatment of hepatic tumours for palliation of metastatic disease has already been discussed. Other areas showing promise are for the treatment of benign uterine fibroids and osteoid osteomas. Potentially many other benign or malignant tumours could be treated in this way.

The next step in this research programme would be to set up a clinical trial of ILP with MR follow up versus conventional surgery. If long-term outcomes are similar, the argument for one over the other rests on patient's acceptability and experience of each treatment. It is likely however that any procedure that leaves no visible cosmetic defect and is performed under local anaesthetic will have an advantage with regards to overall patient satisfaction. The design of such a trial would again have certain resource implications if it were to be extended outside of the NMLC. Certainly there is the expertise amongst radiologists to position the fibres as it is no more difficult than performing an ultrasound guided biopsy, which is standard practice throughout the UK. Laser sources are a relatively cheap capital outlay and certainly are well within the budget of a modest research grant. As this pilot study is still ongoing, the evidence of the accuracy of MR will hopefully shortly reach a stage where such a trial could be considered.

There is no doubt that both ‘Optical Biopsy’ and ‘Interstitial Laser Photocoagulation’ have real promise. What is more encouraging is that they both have a clear clinical application which would give further armoury to the clinician, investigating and treating breast cancer. This thesis therefore gives support to further investigation of these exciting new technologies.
CONFIDENTIAL
PATIENT INFORMATION SHEET

OPTICAL BIOPSY FOR DIAGNOSIS OF LESIONS IN THE BREAST

Ms C Saunders, Dr M Hall-Craggs, Mr G Briggs, Prof S G Bown

We are sorry you have developed an area in your breast that needs investigation. The normal way to do this is to insert a thin needle into the suspicious area under local anaesthetic and remove a tiny piece of tissue to examine under the microscope. Sometimes this is just done in the outpatient clinic and on other occasions some form of imaging (usually an ultrasound scanner or a magnetic resonance (MR) scanner) is used to be sure the needle is in the right place. The tissue specimen is then processed and examined under a microscope. We are assessing a new technique known as "optical biopsy" which in due course, may be able to give an immediate answer, but at present we need to study how best to use it. We should like to ask you if we could take optical biopsy measurements on your breast at the same time as a conventional biopsy so the results from the two can be compared.

The optical biopsy system is very simple in concept. A thin optical fibre (about the thickness of a piece of thread) is put down the needle used for the conventional biopsy and a short burst of white light passed down the fibre. The diagnostic information is obtained by analysing the light that is reflected back up the fibre. The measurement only takes a few seconds and the power of the light used is so low that it will not affect you in any way. Thus the optical biopsy will only increase the time of your routine biopsy procedure by a few minutes and will not cause any additional discomfort.

If it is decided that you need an operation on your breast or your axilla (the area under your arm next to the breast), we should also like to take optical biopsy measurements during surgery on the tissues seen during the operation that are going to be removed, and then compare the results with microscopic examination of the tissues in the laboratory. One of our research team will explain to you which areas we would like to measure in your case.

You do not have to take part in this study if you do not want to. Please discuss it with anyone you wish before making a decision. If you decide to take part, you may still withdraw at any time without giving a reason. Your decision whether to take part will not affect the rest of your care and management in any way. If you agree to take part, we will ask you to sign a consent form. All proposals for research using human subjects are reviewed by an ethics committee before they can proceed. This proposal was reviewed by the joint UCL/UCLH committees on the ethics of Human Research. The investigators can be contacted on 0171-380-9060 (or outside office hours, through the Middlesex Hospital, 0171-636-8333).

Initialled by Subject............................................... Initialled by Witness..............................................
Date..........................................................
Prepared by the National Medical Laser Center

Last modified on 05 January, 2003
Supplementary information sheet

Principal investigator: Prof SG Bown.
Other investigators: Ms C Saunders, Dr MA Hall-Craggs, Mr G Briggs.

As this research programme is being funded by the United States Army Medical Research and Materiel Command (USAMRMC) as part of a major research programme on the diagnosis and treatment of breast cancer, we are asked to provide further specific information to you before you agree to participate in the study.

Naturally, you will not be asked to pay any costs in relation to this study. We are not able to pay you for your participation, but we will reimburse you for any out of pocket travel expenses incurred if you make additional visits to this hospital solely related to your participation in this study. Should you be injured as a direct result of participating in this research project, you will be provided medical care, at no cost to you, for that injury. You will not receive any injury compensation, only medical care. You should also understand that this is not a waiver of your legal rights. You should discuss this issue thoroughly with the principal investigator before you enroll in this study. If there is any possibility that you might be pregnant, you should not volunteer for this study. A urine pregnancy test will be done prior to treatment, and you will be excluded if the results are positive.

Representatives from the US Army Medical Research and Materiel Command or the Food and Drug Administration may inspect the records of the research in their duty to protect human subjects in research. By enrolling in this study, you should understand that the United States Army Medical Research and Materiel Command (USAMRMC) will collect certain information about you, including your name, address, study name and dates. The purpose is, first, to readily answer an individual's questions about their participation in research sponsored the USAMRMC; and second, to ensure that the USAMRMC can exercise its obligation to ensure research volunteers are adequately warned of risks and to provide new information as it becomes available. The information will be retained in this database for a minimum of 75 years. All information obtained in this database is protected under the Privacy Act of 1974. Personal identifying information may not and will not be released unless the subject (or legal guardian) provides written approval of such disclosure. Each subject on whom data are collected, upon written request to Human Subjects Protection Division, Office of the Deputy Chief of Staff for Regulatory Compliance and Quality, USAMRMC may have access to their record, and only their record, contained in the database.

In the event of a research related injury, you should contact any of the investigators at the National Medical Laser Centre, Institute of Surgical Studies, 67-73 Riding House Street, London W1P 7LD. Telephone no. 0171-504-9060 (outside working hours via the Middlesex Hospital Switchboard 0171-636-8333). Any questions on subjects rights should be addressed to UCL/UCLH Committee on the Ethics of Human Research, Chairman - Professor A Maclean, 9th Floor, St Martin's House, 140 Tottenham Court Road, London W1P 9LN. Telephone no. 0171-380-9579

Initialled by Subject................................. Initialled by Witness.................................
Date.............................................

Prepared by the National Medical Laser Center

Last modified on 05 January, 2003
CONFIDENTIAL
PATIENT CONSENT FORM

OPTICAL BIOPSY FOR DIAGNOSIS OF LESIONS IN THE BREAST

Miss C Saunders, Dr S Lakhani, Mr G Briggs, Dr MA Hall-Craggs, Prof S G Bown,

Have you read the information sheet about this study? YES/NO
Have you had the opportunity to ask questions and discuss this study? YES/NO
Have you received satisfactory answers to all your questions? YES/NO
Have you received enough information about this study? YES/NO

My questions concerning the study has been answered by

...............................................................................................................................................(Doctor's name)

I understand that I am free to withdraw from the study at any time without giving a reason and withdrawal from the study will not affect my routine care and management. I agree to take part in this study.

Patient signature:.............................................................Date.................................................................

Please print name and Address......................................................................................................................................................

Witnessed: .............................................................Date.................................................................

Please print name (doctor)......................................................................................................................................................

Investigator: .................................................................................................................................................................

Date: ...........................................................................

Prepared by the National Medical Laser Center

Last modified on 05 January, 2003
We are sorry that a tumour has been found in your breast and that an operation is necessary. We understand that you have been recommended to take the drug tamoxifen for 3 months to shrink the tumour before surgery. We are assessing a new treatment called Interstitial Laser Photocoagulation (ILP) which in the future, may be able to treat tumours like yours without surgery. However, at present, we cannot be sure that ILP can completely destroy tumours. We should like to ask you to help us to assess this new treatment by permitting us to use it on your tumour prior to surgery. Taking part in this research programme will not help in the treatment of your tumour now, but it may help patients with similar problems in the future. It could help you should you be unlucky enough to develop another tumour in your breasts at a later date.

The concept of ILP is simple. Under local anaesthetic, one or a small number of thin needles are inserted through the skin into the tumour. An optical fibre (about as thick as a piece of thread) is then passed through each needle and laser energy used to gently coagulate and destroy the tumour without damaging overlying tissues. Dead tumour tissue is removed by normal body healing mechanisms. Treatment is carried out in an interventional magnetic resonance (MR) scanner to locate the needles correctly. To judge whether the treatment has destroyed the entire tumour, we need to ask you to have 3 conventional MR scans, one as part of your initial assessment at the time of diagnosis, one a few days after ILP and a final one after 3 months of tamoxifen, just before your surgery. For these scans, you will need to lie on your tummy with the breasts resting in specially shaped cups (a "breast coil") and then slide into the scanner. You will not feel anything during the scan though it may take 20-30 minutes and you will hear some rattle like noises. The laser treatment and scans are performed as outpatient procedures. We will check the accuracy of the scans and efficacy of the laser treatment by examination of the tissue removed at your operation. More than 100 patients have had ILP prior to breast surgery in this hospital and so far there have been no injuries associated with this procedure. Occasionally, there is discomfort when the laser is on. We can give you a sedative and pain killer if required, or the treatment can be stopped at any time, when the discomfort will cease immediately. There is a very small risk of a tiny burn on the skin if the fibre is not in deep enough, but this is minimised by careful placement of the fibres and cooling the skin during treatment.

You do not have to take part in this study if you do not want to. Please discuss it with anyone you wish before deciding. If you decide to take part, you may still withdraw at any time without giving a reason. Your decision whether to take part will not affect the rest of your care and management in any way. If you agree to take part, we will ask you to sign a consent form. All proposals for research using human subjects are reviewed by an ethics committee before they can proceed. This proposal was reviewed by the joint UCL/UCLH committees on the ethics of Human Research.
Supplementary information sheet

Principal investigator: Prof SG Bown.
Other investigators: Ms C Saunders, Dr MA Hall-Craggs, Mr G Briggs, Dr S. Lakhani

As this research programme is being funded by the United States Army Medical Research and Materiel Command (USAMRMC) as part of a major research programme on the diagnosis and treatment of breast cancer, we are asked to provide further specific information to you before you agree to participate in the study.

In general, this is a simple and safe procedure. If the fibres are not positioned correctly in your breast, there is a chance of a small skin burn, but this has only happened twice in using this laser technique to treat more than 100 patients with breast lesions, and on both occasions, the resulting scar has been smaller than would have been produced if the lesion had been removed by conventional surgery. The risk has been further reduced by improved imaging techniques and by cooling the overlying skin during treatment.

Naturally, you will not be asked to pay any costs in relation to this study. We are not able to pay you for your participation, but we will reimburse you for any out of pocket travel expenses incurred if you make additional visits to this hospital solely related to your participation in this study. Should you be injured as a direct result of participating in this research project, you will be provided medical care, at no cost to you, for that injury. You will not receive any injury compensation, only medical care. You should also understand that this is not a waiver of your legal rights. You should discuss this issue thoroughly with the principal investigator before you enroll in this study.

Representatives from the US Army Medical Research and Materiel Command or the Food and Drug Administration may inspect the records of the research in their duty to protect human subjects in research. By enrolling in this study, you should understand that the United States Army Medical Research and Materiel Command (USAMRMC) will collect certain information about you, including your name, address, study name and dates. The purpose is, first, to readily answer an individual's questions about their participation in research sponsored the USAMRMC; and second, to ensure that the USAMRMC can exercise its obligation to ensure research volunteers are adequately warned of risks and to provide new information as it becomes available. The information will be retained in this database for a minimum of 75 years. All information obtained in this database is protected under the Privacy Act of 1974. Personal identifying information may not and will not be released unless the subject (or legal guardian) provides written approval of such disclosure. Each subject on whom data are collected, upon written request to Human Subjects Protection Division, Office of the Deputy Chief of Staff for Regulatory Compliance and Quality, USAMRMC may have access to their record, and only their record, contained in the database.

In the event of a research related injury, you should contact any of the investigators at the National Medical Laser Centre, Institute of Surgical Studies, 67-73 Riding House Street, London W1P 7LD. Telephone no. 0171-504-9060 (outside working hours via the Middlesex Hospital Switchboard 0171-636-8333). Any questions on subjects right should be addressed to UCL/UCLH Committee on the Ethics of Human Research, Chairman - Professor A Maclean, 9th Floor, St Martin's House, 140 Tottenham Court Road, London W1P 9LN. Telephone no.0171-380-957.

Initialled by Subject.................................................... Initialled by Witness...........................................
Date.....................................
Prepared by the National Medical Laser Center

Last modified on 05 January, 2003
CONFIDENTIAL
PATIENT CONSENT FORM

INTERSTITIAL LASER PHOTOCOAGULATION (ILP)
AND TAMOXIFEN FOR THE TREATMENT OF BREAST TUMOURS

Ms C Saunders, Mr G Briggs, Dr M Hall-Craggs, Dr S. Lakhani, Prof S G Bown

Have you read the information sheet about this study? YES/NO
Have you had the opportunity to ask questions and discuss this study? YES/NO
Have you received satisfactory answers to all your questions? YES/NO
Have you received enough information about this study? YES/NO

My questions concerning the study has been answered by

...........................................................................................................(Doctor's name)

I understand that I am free to withdraw from the study at any time without giving a reason and withdrawal from the study will not affect my routine care and management. I agree to take part in this study.

Patient signature:..............................................................

Date..............................................................

Please print name and Address..............................................................

Witnessed: ..............................................................

Date..............................................................

Please print name (doctor)..............................................................

Investigator:..............................................................

Date: ..............................................................

Prepared by the National Medical Laser Center

Last modified on 05 January, 2003
INTERSTITIAL LASER PHOTOCOAGULATION (ILP) FOR FIBROADENOMAS OF THE BREAST

Mr Gavin Briggs, Mr M Keshtgar, Dr M Hall-Craggs, Prof S G Bown

We are sorry that you have developed a lump in your breast but are glad that tests have shown that it is not cancer. The conventional treatment options for a benign breast lump such as yours are either to do nothing and just follow you up regularly in the outpatient clinic to be sure it does not get any larger or cause any other problems, or remove it by a small operation as a day case procedure under general anaesthesia. Surgical removal leaves a small scar. This will always be in an area that is as hidden as possible, but may occasionally cause alteration of the breast shape. We are assessing a new treatment for these breast lesions known as Interstitial Laser Photocoagulation (ILP) and would like to ask you to take part in a study comparing ILP with surgery and with no active treatment. We feel that it is important for you to decide which treatment option you prefer after the merits and problems of each have been explained to you and you have had a chance to discuss them. Our aim is to collect information from patients who have been treated in each way to assess the ease, comfort and effectiveness of each approach to decide when ILP may be the most appropriate option for patients in the future.

The technique of ILP is simple in concept. Local anaesthetic is applied to the skin and one or more small needles are inserted into the breast lump under guidance from an ultrasound scanner (or occasionally a magnetic resonance scanner). A thin optical fibre (about as thick as a piece of thread) is passed through each needle into the lump and the laser activated for a few minutes to gently coagulate the lesion. During laser treatment you may experience a feeling of warmth or occasionally pain within the breast. If necessary, some sedation and a pain killer will be given. There may be some soreness in the treated breast for a few days, but you should be able to resume your normal activities the following day. The lump slowly disappears over a period of several months as the normal healing process in your body removes the area of dead tissue destroyed by the laser treatment. There is a small chance that ILP could change the contour of the breast, which could be disfiguring, but this is less likely than after surgery.

If you agree to participate in this research study, whichever approach you choose, an ultrasound or magnetic resonance scan will be done prior to treatment, if it was not done for diagnosis. We should like to see you again after 3, 6 and 12 months to examine and do an ultrasound scan of any residual lump and to take a photograph to see if the size or shape of the breast has changed. If you choose surgery or ILP, you will be seen a few days after treatment to be sure that healing is proceeding satisfactorily and that there are no complications.

You do not have to take part in this study if you do not want to. Please discuss it with anyone you wish before making a decision. If you decide to take part, you may still withdraw at any time without giving a reason. Your decision whether to take part will not affect the rest of your care and management in any way. If you agree to take part, we will ask you to sign a consent form. All proposals for research using human subjects are reviewed by an ethics committee before they can proceed. This proposal was reviewed by the joint UCL/UCLH committees on the ethics of Human Research.

Prepared by the National Medical Laser Center

Last modified on 05 January, 2003
Supplementary information sheet for patients who choose to have treatment with ILP

Principal investigator: Prof SG Bown.  
Other investigators: Mr M Keshtgar, Dr MA Hall-Craggs, Mr G Briggs

As this research programme is being funded by the United States Army Medical Research and Materiel Command (USAMRMC) as part of a major research programme on the diagnosis and treatment of breast cancer, we are asked to provide further specific information to you before you agree to participate in the study.

In general, this is a simple and safe procedure. If the fibres are not positioned correctly in your breast, there is a chance of a small skin burn, but this has only happened twice in using this laser technique to treat more than 100 patients with breast lesions, and on both occasions, the resulting scar has been smaller than would have been produced if the lesion had been removed by conventional surgery. The risk has been further reduced by improved imaging techniques and by cooling the overlying skin during treatment. If there is any possibility that you might be pregnant, you should not volunteer for this study. A urine pregnancy test will be done prior to treatment, and you will be excluded if the results are positive.

Naturally, you will not be asked to pay any costs in relation to this study. We are not able to pay you for your participation, but we will reimburse you for any out of pocket travel expenses incurred if you make additional visits to this hospital solely related to your participation in this study.

The United States Department of Defense is funding this research project. Should you be injured as a direct result of participating in this research project, you will be provided medical care, at no cost to you, for that injury. You will not receive any injury compensation, only medical care. You should also understand that this is not a waiver of your legal rights. You should discuss this issue thoroughly with the principal investigator before you enroll in this study.

Representatives from the US Army Medical Research and Materiel Command or the Food and Drug Administration may inspect the records of the research in their duty to protect human subjects in research. By enrolling in this study, you should understand that the United States Army Medical Research and Materiel Command (USAMRMC) will collect certain information about you, including your name, address, study name and dates. The purpose is, first, to readily answer an individual’s questions about their participation in research sponsored the USAMRMC; and second, to ensure that the USAMRMC can exercise its obligation to ensure research volunteers are adequately warned of risks and to provide new information as it becomes available. The information will be retained in this database for a minimum of 75 years. All information obtained in this database is protected under the Privacy Act of 1974. Personal identifying information may not and will not be released unless the subject (or legal guardian) provides written approval of such disclosure. Each subject on whom data are collected, upon written request to Human Subjects Protection Division, Office of the Deputy Chief of Staff for Regulatory Compliance and Quality, USAMRMC may have access to their record, and only their record, contained in the database.

In the event of a research related injury, you should contact any of the investigators at the National Medical Laser Centre, Institute of Surgical Studies, 67-73 Riding House Street, London W1P 7LD. Telephone no. 0171-504-9060 (outside working hours via the Middlesex Hospital Switchboard 0171-636-8333). Any questions on subjects rights should be addressed to UCL/UCLH Committee on the Ethics of Human Research, Chairman - Professor A Maclean, 9th Floor, St Martin’s House, 140 Tottenham Court Road, London W1P 9LN. Telephone no. 0171-380-9579

Initiated by Subject...................................................... Initialled by Witness................................................
Date...................................................

Prepared by the National Medical Laser Center

Last modified on 05 January, 2003
Have you read the information sheet about this study?  YES/NO

Have you had the opportunity to ask questions and discuss this study? YES/NO

Have you received satisfactory answers to all your questions? YES/NO

Have you received enough information about this study? YES/NO

My questions concerning the study has been answered by

........................................................................................................(Doctor's name)

I understand that I am free to withdraw from the study at any time without giving a reason and withdrawal from the study will not affect my routine care and management. I agree to take part in this study.

Patient signature:........................................... Date..............................................

Please print name and Address........................................................................................................

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Witnessed: .............................................. Date..............................................

Please print name (doctor)...........................................................................................................

Investigator: ..........................................................................................................................

Date: ............................................................

Prepared by the National Medical Laser Center

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