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REVIEW

Intraoperative margin assessment during radical prostatectomy: is microscopy frozen in time or ready for digital defrost?

Eoin Dinneen,[1,2] Ricardo Almeida-Magana,[1,2] Tarek Al-Hammouri,[1,3] Iona Fernandes,[2] Nikhil Mayor,[4,5] Larissa Mendes,[6] Mathias Winkler,[4,5] Anna Silvanto,[6] Aiman Haider,[6] Alex Freeman[6] & Greg Shaw[1,2]

[1]*Division of Surgery & Interventional Science, University College London,* [2]*Department of Urology, Westmoreland Street Hospital, University College Hospital London,* [3]*Centre for Medical Imaging, University College London,* [4]*Department of Urology, Imperial College NHS Healthcare Trust, Charing Cross Hospital,* [5]*Imperial Prostate, Division of Surgery, Imperial College London and* [6]*Department of Histopathology, University College Hospital London, London, UK*

Dinneen E, Almeida-Magana R, Al-Hammouri T, Fernandes I, Mayor N, Mendes L, Winkler M, Silvanto A, Haider A, Freeman A & Shaw G

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Intraoperative margin assessment during radical prostatectomy: is microscopy frozen in time or ready for digital defrost?

Intraoperative frozen section (IFS) is used with the intention to improve functional and oncological outcomes for patients undergoing radical prostatectomy (RP). High resource requirements of IFS techniques such as NeuroSAFE may preclude widespread adoption, even if there are benefits to patients. Recent advances in fresh-tissue microscopic digital imaging technologies may offer an attractive alternative, and there is a growing body of evidence regarding these technologies. In this narrative review, we discuss some of the familiar limitations of IFS and compare these to the attractive counterpoints of modern digital imaging technologies such as the speed and ease of image generation, the locality of equipment within (or near) the operating room, the ability to maintain tissue integrity, and digital transfer of images. Confocal laser microscopy (CLM) is the modality most frequently reported in the literature for margin assessment during RP. We discuss several imitations and obstacles to widespread dissemination of digital imaging technologies. Among these, we consider how the '*en-face*' margin perspective will challenge urologists and pathologists to understand afresh the meaning of positive margin significance. As a part of this, discussions on how to describe, categorize, react to, and evaluate these technologies are needed to

Address for correspondence: Eoin Dinneen, Division of Surgery and Interventional Science, University College London, Charles Bell House, 43-45 Foley Street, London W1W 7TS, UK. e-mail: eoin.dinneen@nhs.net

Abbreviations: BCR, biochemical recurrence; CLM, confocal laser microscopy; CT, computed tomography; EF, erectile function; FFPE, formalin-fixed paraffin embedded; H&E, haematoxylin & eosin; IFS, intraoperative frozen section; iPSM, intraprostatic positive surgical; ISUP, International Society for Urological; MRI, magnetic resonance imaging; NS, nerve-sparing; NSM, negative surgical margin; NVB, neurovascular bundle; OCT, optical coherence tomography; PC, prostate cancer; PDD, photodynamic diagnosis; PET, positron emission tomography; PROMS, patient-reported outcome measures; PSM, positive surgical margin; PSMA, prostate-specific membrane antigen; RARP, robot-assisted radical prostatectomy; RCT, randomized controlled trial; RP, radical prostatectomy; SR, secondary resection margin; UC, urinary continence.

improve patient outcomes. Limitations of this review include its narrative structure and that the evidence base in this field is relatively immature but developing at pace.

Keywords: confocal laser microscopy, digital imaging, frozen section, NeuroSAFE, prostate cancer, radical prostatectomy, surgical margins

Introduction

In radical prostatectomy (RP), the path between maximal preservation of nerve-rich periprostatic tissue and excision of all prostate cancer (PC) is a challenging balancing act, particularly in patients with aggressive PC features such as extraprostatic extension or high-volume, high-grade tumours. Tissue-sparing techniques such as nerve-sparing (NS), bladder neck preservation, complete urethral preservation, apical urethral preservation, and Retzius-sparing RP aim to improve postoperative erectile function (EF) and urinary continence (UC). However, if this results in a positive surgical margin (PSM) oncological outcomes can suffer, with evidence that PSMs are associated with biochemical recurrence (BCR), metastases, cancer-specific mortality, and overall mortality, particularly in high-risk groups.[1]

Pathologists can play a significant role in helping surgeons achieve this delicate balance through intraoperative frozen section (IFS) microscopic margin assessment.[2] The NeuroSAFE technique, developed in the Martini Klinik, Germany (Figure 1), is a standardized IFS technique that evaluates the posterolateral prostate surface to confirm that nerve-sparing has not incurred a PSM that would necessitate a secondary resection (SR). It allows surgeons to perform more NS whilst decreasing PSM rates.[3]

The accuracy of NeuroSAFE compared to final (i.e. formal-fixed paraffin-embedded [FFPE]) prostatectomy specimen margin status is well established.[4,5] Furthermore, several observational studies have demonstrated an increase in NS rates,[6] improved urinary continence and sexual function patients who had RP conducted with the assistance of the NeuroSAFE technique when compared to historical and contemporary standard of care RP cohorts.[7–9] Notably, Fossa *et al.* showed, using patient-reported outcome measures (PROMS), that in patients with good EF prior to RP, the NeuroSAFE technique permitted preservation of EF in 28% more patients.[10] However, as noted in the 2024 updated EAU prostate cancer guidelines,[11] prospective randomized comparative studies are still lacking[12] and until now IFS (including NeuroSAFE) during RP is not considered the standard of care.

Despite these reported benefits of NeuroSAFE, many centres have been unable to adopt the technique, as it relies upon IFS performed in the laboratory with expensive prerequisite equipment and skilled staff to process the sample. Further, it requires the availability of an experienced histopathologist for immediate reporting of the slides in the midst of a global pathology workforce shortage.[13] In the NeuroSAFE PROOF feasibility randomized clinical trial (RCT), our analysis showed that the NeuroSAFE technique added 1 hour to the length of robot-assisted RP (RARP)[5] and cost £625.10 per case.[12] Other authors have similar findings.[14]

With the advent of digital histopathological imaging, there is burgeoning interest in new technologies that can generate high-quality microscopic images within minutes. In this narrative review article, we outline the practical attractions of these novel imaging modalities, we describe deficiencies in the existing evidence, and we will consider limitations that may need to be overcome for these technologies to become game-changing.

NOVEL MICROSCOPIC DIGITAL IMAGING – WHAT IS OUT THERE?

In 2021, a systematic review identified five new imaging modalities with possible applications in real-time margin assessment during RP.[15] These were: optical coherence tomography (OCT), photodynamic diagnosis (PDD), light reflectance spectroscopy, and confocal laser microscopy (CLM). Other novel imaging modalities for surgical margin status in general include structured illumination microscopy,[16] nonlinear microscopy,[17] RP specimen positron emission tomography (PET) computed tomography (CT) imaging,[18] intraoperative fluorescence detection,[19] and prostate-specific membrane antigen (PSMA) antibody fluorescence detection.[20] Until now, none of these modalities have become mainstream in real-time surgical PC treatment, although *ex vivo*

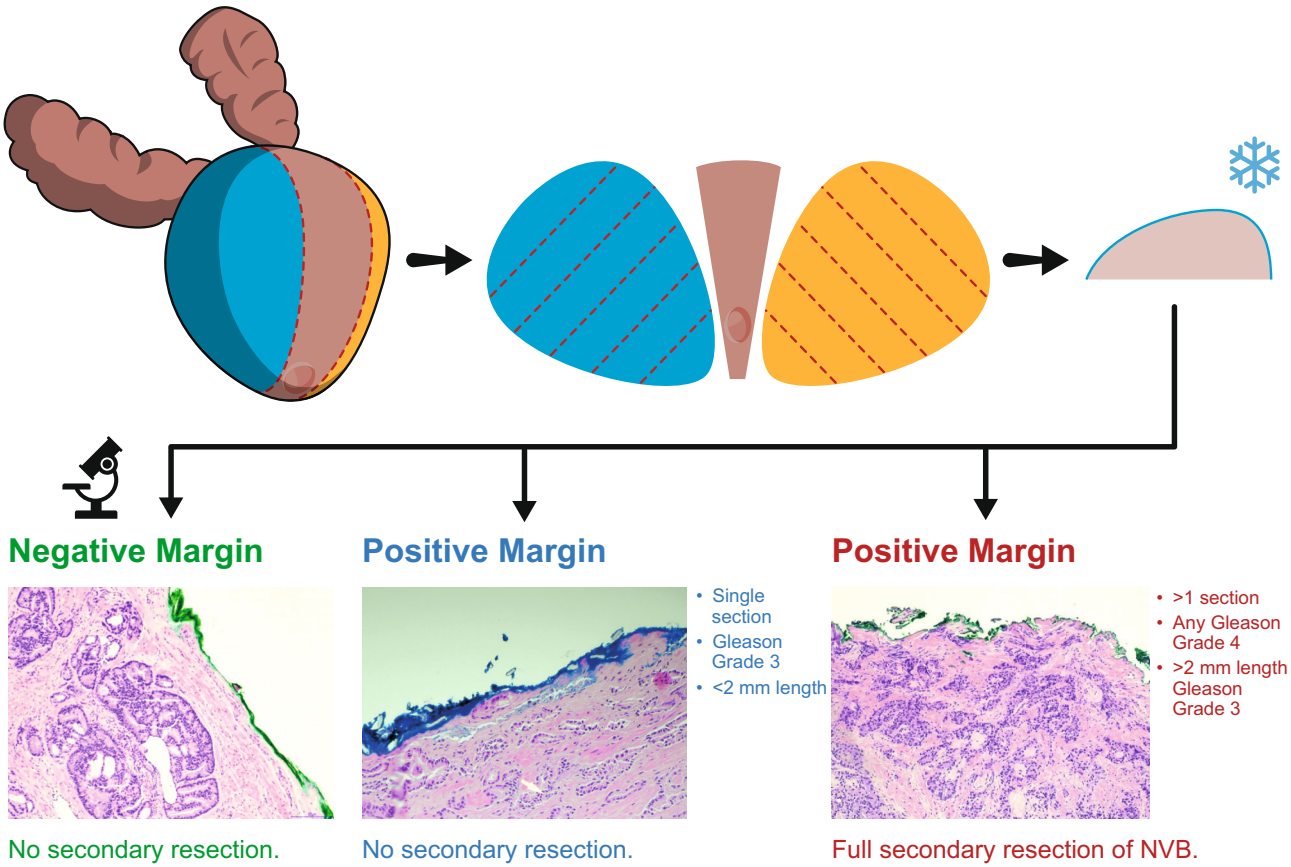

Figure 1. The NeuroSAFE technique. Schematic diagram demonstrating painting, cut-up, and processing of the prostate during the NeuroSAFE technique and the panels demonstrate examples of different outcomes including the intraoperative surgical response (i.e. secondary resection or not according to the margin status. Reproduced with permission of the authors and BMC from Dinneen *et al.*[12]

fluorescence CLM is the technology with the most ongoing interest and representation in the literature. This review, therefore, will focus on *ex vivo* CLM. We will also discuss the concept of evaluating specimen margins '*en-face*', where an entire surface area of the margin is inspected instead of cross-sections through the margin at intervals, as is the conventional approach (Figure 2).

CLM produces high-resolution images of fresh specimens with cellular-level detail using photo-reactive dyes. The technique works by shining a point laser onto the specimen that reflects to a laser light detector positioned immediately behind a pinhole aperture positioned in an optically conjugate plane, thus eliminating the out-of-focus signal (hence the name confocal). As only reflected light produced by fluorescence very close to the focal plane is detected, the image's optical resolution is very high. Data from returning fluorescent light is collected and collated to construct a high-resolution digital image.

FASTER REAL-TIME IMAGING – THE NEW PROMISE

Available machines

This review will consider two commercially available machines; the Histolog (SamanTree Medical, Lausanne, Switzerland) and the Vivascope (Vivascope, Munich, Germany), as these have been used and published in the surgical oncology literature, including prostate cancer margin assessment. The Histolog excites tissue fluorescence with a laser at 488 nm and fluorescence emission with a wavelength of >500 nm is collected, producing a toluidine-blue mono stain-like image. The penetration depth is fixed at 30 μm. The scanner tray of Histolog microscope (see Figure 3) allows a larger scan area (48 × 36 mm) and takes 50 s per image. By contrast, the VivaScope system employs a dual laser system, with a reflectance mode at a wavelength of 785 nm and fluorescence at 488 nm, enabling dual contrast (pseudo-H&E) and high-resolution images in

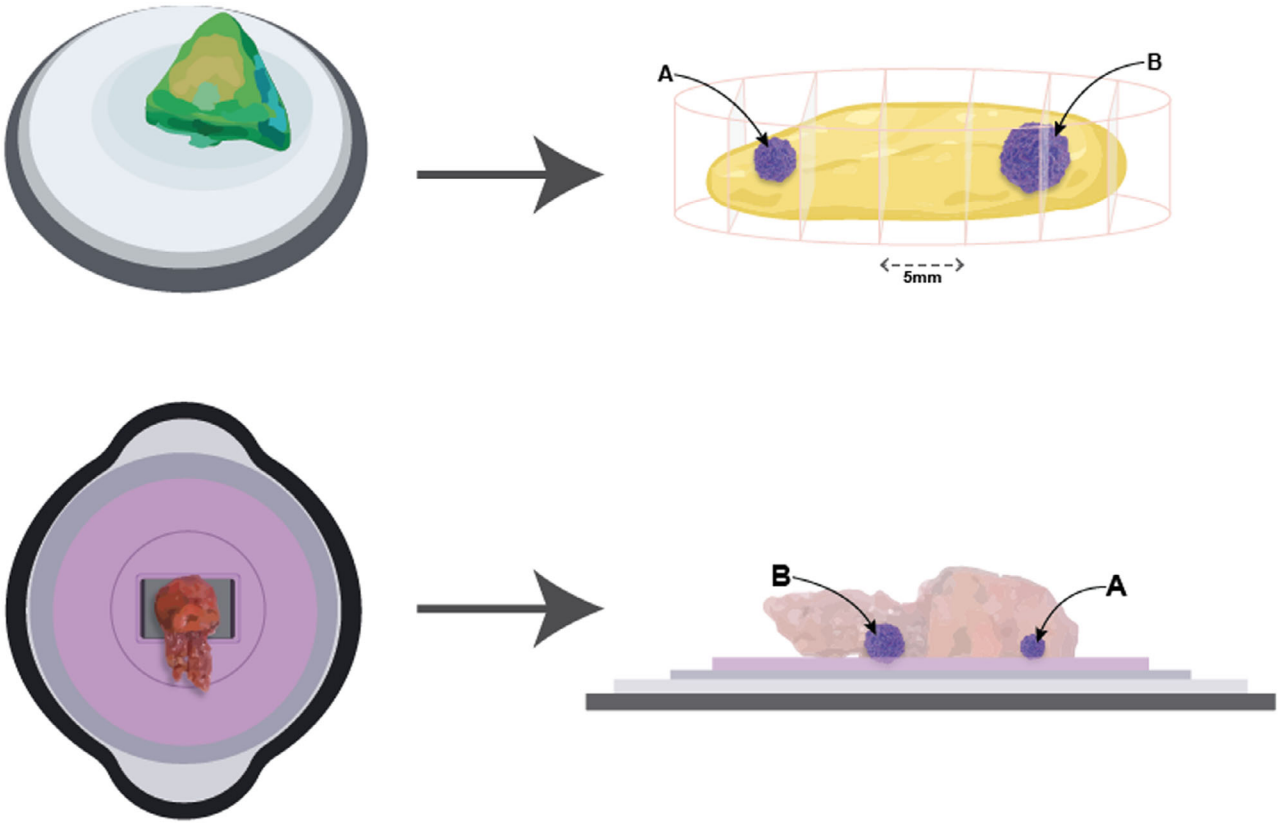

Figure 2. Cross-sectional versus *en-face* margin assessment. Top: Illustration of the NeuroSAFE technique. Cross-sections of posterolateral margins are taken at 5-mm intervals meaning tumours at margins measuring <5 mm (A) can be missed, whereas tumours >5 mm (B) are more likely to be detected. Bottom: In CLM imaging, margins are scanned *en-face*. Any length of tumour at the margin can potentially be detected, but the clinical significance of PSM (particularly smaller PSM) detected from this perspective remains unknown.

various tissue depths ranging from 4 to 200 μm. The VivaScope provides a maximum total scan area of 25 × 25 mm, allows magnification up to ×550, performs vertical scanning of tissues (allowing for different levels to be visualized), and requires 4 min per sample of this size.

Location of the machine

Whereas IFS must take place in a laboratory with a cryostat, staining and slide mounting equipment, and a microscope, CLM microscopes can work off relatively compact computers and thus can easily be situated in or nearby the operating room (Figure 3). This is more time-efficient, as there is no need to transport the specimen. Additionally, either the principal surgeon or a member of the surgical team can prepare and image the specimen, which is particularly valuable when areas of concern have been identified through surgeon suspicion or preoperative imaging (magnetic resonance imaging [MRI] or PSMA-PET).[21,22]

Rapid real-time processing

A key advantage of CLM is the speed and ease with which samples can be processed; timing is critical because the patient remains under general anaesthetic whilst the results are awaited. Rocco *et al.* reported proof-of-concept, scanning *en-face* periprostatic tissue taking just 1–2 min per sample.[23] Baas *et al.* performed a direct comparison between CLM and IFS. Posterolateral tissue sections from 50 patients were analysed with CLM with a median procedural time of 8 min (interquartile range [IQR]: 5–20) then submitted for IFS, which took a median of 50 min (IQR: 45–59).[24] Almeida *et al.* were the first to report CLM in RP specimens without the need for any tissue sectioning. Their evaluation of CLM in 31 patients evaluated the posterolateral margins *en-face* in completely intact RP specimens.[25] This technique reduces preparation time but, critically, also preserves tissue integrity for subsequent conventional histopathological analysis.

Another potentially important benefit of the aforementioned speed of histological feedback is that it

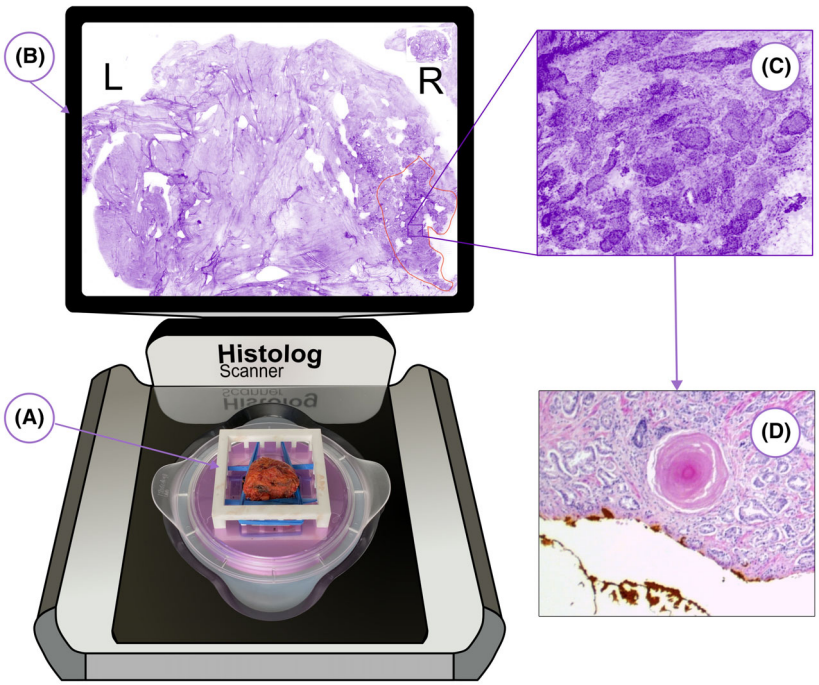

Figure 3. Setup for intraoperative CLM *en-face* margin assessment of the prostate. (A) Prostate specimen mounted in a 3D printed holder. (B) Digital image of the whole posterolateral surface displayed on a monitor for immediate visualization and analysis. (C) High-power digital magnification of malignant glands. (D) Corresponding histology slide stained with haematoxylin and eosin confirming a PSM. Reproduced with permission of the authors and publishers from Almeida *et al.*[25]

may allow surgeons to expedite their learning curve and reduce PSM rates quicker. To the best of our knowledge, there are few other histological feedback mechanisms that provide this level of detailed information so quickly to the operating team. Indeed, lowering PSM rates is often considered one of the big challenges in prostate cancer surgery and can take a long time to improve significantly.[26]

Larger surgical margin surface area

Hitherto, IFS of prostate margins has been performed in a cross-sectional manner, allowing visualization of the inked margin in one plane at repeated intervals (typically 5 mm) (see Figure 1). This cross-sectional approach to specimen cut-up emulates the same method used for the FFPE histopathological analysis, and relies upon the assumption that sampling is representative of the larger surgical margin surface despite less than 1% of the total surface area being analysed[27] (which may explain why local recurrence sometimes occurs in the context of negative surgical margins [NSM], and indeed why a close surgical margin is an independent predictor for BCR[28]). Although *en-face* prostate margin analysis was mooted by Oxley *et al.* (emulating the Mohs technique) in 2018,[29] technical difficulties particular to specimen preparation and orientation on a single cryostat chuck rendered this impractical. However, novel digital imaging modalities permit this approach. Examining the entire margin may identify PSM missed by conventional whole-mount FFPE margin evaluation as observed by Wang *et al.* using *en-face* Giga-pixel structured illumination microscopy[16] and our studies using the Histolog Scanner.[25]

Novel imaging technologies are not tissue-destructive

IFS requires the RP specimen to be sampled through cut-up, which carries the risk of ink spillage or capsular retraction that might alter final FFPE margin assessment. In contrast, CLM and other digital imaging methods can provide images of the specimen margin without cut-up.[30] Acridine orange staining does not affect subsequent painting, processing, and reviewing of the RP specimen, nor indeed immunohistochemistry, should it be required.[31]

Digital pictures

Electronic transfer of full-size images that can be zoomed in to magnifications similarly to high-power light microscopy is an appealing characteristic of

digital imaging modalities. This function is already possible on the Histolog scanner. In the future, triage by surgeon or artificial intelligence supported by pathologist review where required may reduce the burden on pathologists.

Novel Digital Imaging – Current Issues and Uncertainties

Despite these possibilities, to the best of our knowledge, neither CLM nor any other novel digital imaging platform has been adopted as the standard of care in *ex-vivo en-face* margin assessment during RP to date, nor in any surgical oncology with the exception of skin cancer. Although the field is evolving, most published studies are at IDEAL stages 1 and 2[30] or exploratory studies embedded into trials that focus on IFS.[32,33] With the possible benefits outlined in detail above, it is adroit to examine the factors hindering these technologies from becoming widely accepted for real-time RP margin assessment.

IS *EN-FACE* NOVEL IMAGING RELIABLE FOR MARGIN ASSESSMENT?

First, it is important to acknowledge the relative lack of evidence currently. Per our group's 2023 systematic review, CLM demonstrated good diagnostic accuracy (>80%) for margin assessment.[34] However, this was based on only four studies (146 prostate specimen evaluated), of which three reports were from the same group (using the Vivascope where the combined number of PSMs in the study populations was relatively scarce [total; 5 of 38 13.1%]), meaning that robust statements on sensitivity cannot yet be made.[23,35,36] The prospective multicentre diagnostic study, IP8-FLUORESCE (ISRCTN: 21536411), will have a much larger sample size and a standardized protocol for assessment of PSM diagnostic accuracy against traditional histopathological specimen assessment.

Given that CLM and other digital imaging technologies provide high-quality microscopic pictures (Figure 4), they should be able to perform well in *en-face* PSM detection, and indeed there is growing evidence to support this. *Ex vivo* CLM provides the same high accuracy as final pathology in the prostate adenocarcinoma biopsy setting.[37,38] More relevant, Baas *et al.* evaluated the performance of the Histolog CLM machine using an *en-face* technique and found a high sensitivity (86%) and specificity (96%) against FFPE.[24] Further evidence can be seen in the pictorial atlas articles from both Rocco and Panarello and their colleagues who found high levels of agreement between CLM and final pathology identification of both prostatic and periprostatic structures.[39,40] Panarello *et al.*[40] used the Cellvizio confocal laser endomicroscope (Manua Kea Technologies, Paris, France) in the *ex vivo* setting and demonstrated a high proportion of 'highly informative' images. Bertoni et al. using the Vivascope found CLM to FFPE concordance of 97.14% for muscular, 97.14% for nervous, 97.14% for vascular, and 94.2% for fatty tissue.[39] A very recent and innovative study by Musi *et al.*[32,33] demonstrated 100% concordance with IFS and FFPE when the two pathologists found the CLM *en-face* image of the prostate margin as either 'strongly positive' or 'strongly negative.' However, these two categories represented 35 and 30 of the 54 margins analysed by CLM overall, and when all CLM margins were taken into consideration, sensitivity compared to the reference standard ranged from 64.7% to 70%.[33]

There are several challenges of demonstrating diagnostic equivalence between *en-face* digital imaging modalities (such as CLM) and traditional cross-sectional final FFPE histopathology reports. First, the change in perspective from cross-sectional to *en-face* analysis necessarily involves a change in view for pathologists and there may be an associated learning curve. Second, since FFPE samples are taken every 5 mm, false-negatives might spuriously reduce CLM specificity (such as represented diagrammatically in Figure 2). Third, in some instances there may be difficulty differentiating between malignant glands actually touching the surface or being exceedingly close to the surface but not touching. This can occur when the absence of an inked margin makes it difficult to assess the margin exactly. Fourth (and related), different machines provide images with different levels of tissue depth focus. For instance, the Histolog depth focus is fixed at 30 μm, which is a little greater than the typical epithelial cell length, whereas the Vivascope depth focus can be altered from 4 to 200 μm. Interestingly, Musi *et al.* set their depth of focus using the Vivascope to the 'lowest thickness optical set-up' but still found that in approximately half of cases where the CLM was 'probably positive' the same margin was negative IFS and FFPE.[33] Further innovative and detailed histopathological diagnostic studies, such as the work described by Musi *et al*,[33] Almeida *et al*[41] and IP8-FLUORESCE will need to be conducted to highlight these fundamental considerations.

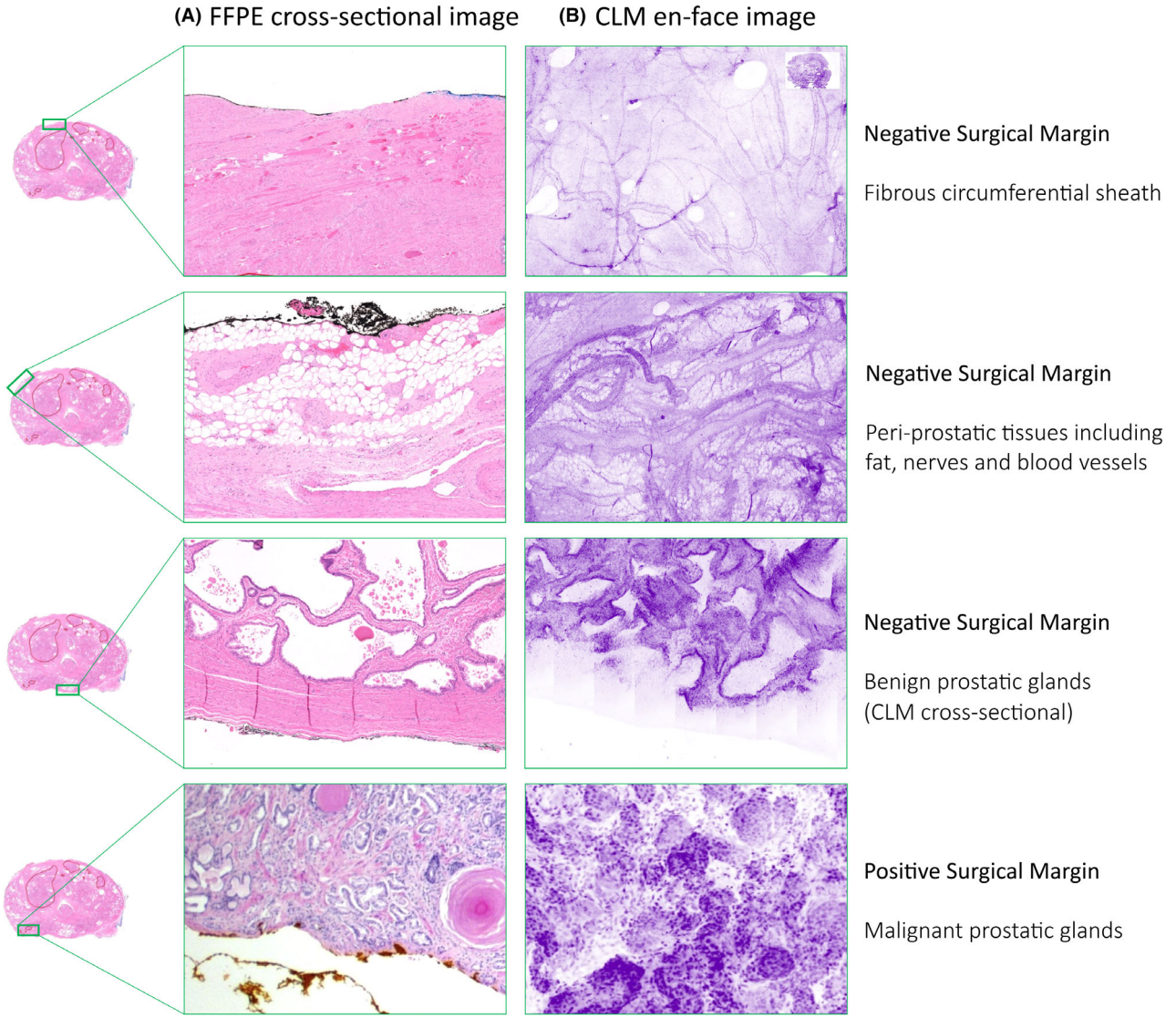

Figure 4. Comparison of corresponding prostate margin images generated by (A) traditional cross-sectional formalin-fixed paraffin-embedded (FFPE) processes and (B) new *en-face ex vivo* confocal laser microscopy (CLM) including; Top Row, fibrous prostate sheath = negative surgical margins(NSM), Second Row, periprostatic structures = NSM, Third Row, benign acinar glands = NSM, Bottom Row, malignant acinar glands = PSM.

THE LONG-TERM ONCOLOGICAL SIGNIFICANCE OF *EN-FACE* PSM

Although the analysis of a larger surface area from novel digital imaging is a possible benefit, we should consider the change in possible oncological significance of the PSM. As per the International Society for Urological Pathology (ISUP) guidelines on the cut-up of the RP specimen, parallel slices should be taken in the plane perpendicular to the rectum.[42] PSM is then classified by the length of the tumour touching the ink.[43] Consequently, the oncological significance of PSM is understood in relation to BCR only by measuring PSM length in this fashion. Measured in this manner (although possibly analysed in different statistical ways, such as dichotomised or continuous), length of PSM appears to be independently prognostic for BCR,[44] although there are numerous studies that suggest PSM </= 3 mm are not associated with a higher risk of BCR compared to NSM.[45,46] Thus, when deciding how to respond to the IFS PSM and protocolise the performance of SR, the understanding of the importance circumferential PSM and length of PSM in the urological literature

has been cited by previous groups to establish their practice.[4,5] On the other hand, to the best of our knowledge, no group has linked any oncological outcomes and PSM measurement provided from an *en-face* perspective, and such information would evidently be pertinent and informative for the intraoperative decision-making when a PSM is encountered.

Given the protracted lead time from RP to BCR with and without PSM and the relative scarcity of PSM (~12–16% in large contemporary series[1,47,48]), it may be some years before urologists and pathologists have a definitive answer to the question: 'what amount of *en-face* PSM is significant?' However, some important questions to consider in subsequent studies are:

- Should the greatest diameter of PSM be used, or could the surface area measurement in mm^2 be a better predictor of BCR?
- Can the Gleason pattern at the margin be distinguished and reported on CLM? The ability to report this may differ according to which part of the prostate is analysed, the nature/size of the PSM, and the technology or even machine used. The importance of this aspect of intraoperative assessment is underlined by various studies that demonstrate the association of Gleason pattern 4 at the margin and risk of BCR,[46] and indeed the NeuroSAFE PROOF trial protocol instructs SR when any pattern 4 is noted at the margin.[12]
- What are the artefacts associated with inadequate imaging, e.g. excessive pressure, incomplete or prolonged staining, movement artefacts, and air bubbles? An exhaustive list should be created and the frequency of problems reported in early-stage reports.
- Can capsular incision (also known as intraprostatic) PSM (iPSM) be properly assessed, given that these crevices within the prostate may retract away from the surface that is visualized? If it is not feasible to image iPSM, what are the implications, given that these can still contribute to an increased risk of BCR[49]?
- Should different levels of *en-face* PSM prompt different types of SR response; focal/partial SR for small low-grade margins vs. complete ipsilateral NVB SR for large or high-grade PSM, or in men with higher-risk preoperative disease characteristics. The proportion of cases with prostate cancer identified within the SR specimen varies greatly in the published literature from 0% to 42%.[2] Sharing data on cancer rates within SR tissue among different centres will be important for optimizing protocols in the future.

WHAT AREAS OF THE PROSTATE SHOULD BE EXAMINED *EN-FACE*?

Previous groups who advocate IFS have focussed on the posterolateral prostate margin, as the NS status is unequivocally related to EF and UC after RP. Further, NeuroSAFE has a well-described methodology of SR for conversion of a PSM to a NSM[3] with BCR rates equivalent to that of patients with initial NSM.[6] Faster digital imaging technologies may enable assessment of more of the RP specimen. One option is to guide assessment by preoperative imaging and augmented-reality image overlay as described by Petralia and colleagues.[21,32] Alternatively, implementing standardized protocols to image certain aspects of the prostate (including apex and base) during all procedures could be employed as described by von Bodman,[50] Tully,[51] and Pak.[52]

The problem of correlating areas for SR in the surgical bed based on identification of a PSM in a prostate that has been excised remains unsolved. Rocco *et al.* have suggested measuring the distance of PSM on CLM digital image and then using the same measurement by placing a measuring tape briefly inside the patient to determine where the tissue may harbour cancer.[35] Augmented-reality console image overlay with IFS/CLM of at-risk margins may also help direct targeted partial SR when intraoperative assessment reveals a PSM.[33,53] The possibility of leaving coloured clips (for example) as fiducial markers during the original dissection strategy is an interesting proposition that remains unexplored. All of these strategies try to overcome the issue that after the prostate is excised there is considerable deformation of the periprostatic tissues within the fossa that render accurate SR excision of the corresponding tissue a challenge.

COST OF MACHINES & DIFFERENT MACHINES

The availability of equipment for *en-face* novel digital imaging is not widespread. The cost of consumables is low (particularly when compared to IFS); however, the initial price for these machines are considerable currently; for instance, the Histolog Scanner costs > £200,000. Integrating health economic analysis in studies evaluating these technologies will be important and should take account of ancillary savings to the hospital, such as consumables, technician training, turnaround times, and the potential for use in multiple tissue types. Ultimately, the value of this additional cost will depend upon and be measured against the benefits to patients. Currently, the

evidence on the benefit of intraoperative margin assessment is largely limited to retrospective studies examining IFS of the prostate, although, as mentioned, many of these demonstrate patient benefits. The results of the NeuroSAFE PROOF RCT will also help to inform the degree of patient benefit. In the future, the cost of CLM technology is likely to decrease, and indeed its speed and versatility may mean improved or widened intraoperative applications, but, at the time of writing, this remains work for the future. As with any new methodology, user groups will need to become familiar with their own equipment and develop standard operating procedures[39] to optimize image generation for interpretation. Provision of training and annotated image libraries by manufacturers and expert mentoring will be key to this process.

Conclusion

This narrative review article highlights the promise of *en-face* novel microscopic digital imaging modalities to replace and potentially surpass IFS during RP. However, as with all complex surgical innovations, these new strategies need thorough evaluation to ensure that they are reliable and improve patient outcomes. Whilst further expansion of the literature on these techniques is expected, some of the functional and oncological outcomes pertinent to RP will take time to mature and be reported. In the meantime, detailed prospective pathological studies comparing *en-face* PSM to traditional PSM are essential for clinicians to integrate these technologies into their practice. Collaboration between experts, high-volume centres, and industry will be key to addressing the knowledge gaps described above, with the aim to improve outcomes for men undergoing RP in the modern era.

Author contributions

ED wrote the article. ED, GS, RA, TAH, AF, NM, and AH were involved in the concept for the article. ED, GS, RA, TAH, AF, AH, IF, MW, and NM performed critical review and editing of the article. ED, RA, TAH, and NM prepared the figures for the article.

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Conflict of Interests

There are no other conflicts of interest to report. No commercial parties, nor study sponsors, had any involvement nor influence in the study design, in the collection, analysis and interpretation of data, in the writing of the review and in the decision to submit for publication.

Data availability statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Patient consent

Where histological images of patients have been used in this article, full written consent for such was obtained prior to image preparation.

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