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Original Article

IP8-FLUORESCE: A Prospective Paired Cohort Study Evaluating the Diagnostic Accuracy of Fluorescence Confocal Microscopy for Real-time Assessment of Surgical Margins in Radical Prostatectomy

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Abstract

Background and objective: Positive surgical margins (PSMs) following radical prostatectomy (RP) are linked to adverse oncological outcomes. Intraoperative margin assessment facilitates immediate secondary resection, enabling more men to undergo "nerve-sparing" RP and improving functional outcomes. Existing techniques, however, have not been adopted widely due to inherent limitations. Fluorescence confocal microscopy (FCM) is a more feasible alternative, offering rapid, high-resolution imaging of unprocessed tissue. This study evaluates the diagnostic performance of FCM for detecting PSMs during RP.
Methods: In this multicentre, prospective, blinded, paired cohort study, men undergoing RP for localised or locally advanced prostate cancer were enrolled across three UK uro-oncology centres between August 17, 2023, and September 23, 2024. FCM was performed on fresh prostatectomy specimens using the Histolog scanner. The whole specimen was examined en face with no tissue resection. Final histopathology served as the reference standard. The primary outcome was the diagnostic performance of FCM for PSM detection on a per-patient level, assessed by sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Multiple definitions of PSMs were evaluated. This study was prospectively registered on ISRCTN (21536411).

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Key findings and limitations: A total of 156 patients were recruited. The prevalence of all PSMs was 30.8% (48/156). For all lengths of PSMs, including focally positive and <1 mm margins, sensitivity, specificity, PPV, and NPV were 48% (95% confidence interval 33–63%), 94% (88–98%), 79% (60–92%), and 80% (72–87%), respectively. For PSMs of ≥3 mm, FCM demonstrated sensitivity of 79% (54–94%), specificity of 94% (89–97%), PPV of 71% (48–89%), and NPV of 96% (91–99%). Of the false negative cases, 84% were ≤2 mm; 52% were at the apex.

Conclusions and clinical implications: FCM is a feasible, rapid technique for intraoperative margin assessment in RP. Its diagnostic accuracy is reasonable for clinically significant, longer PSMs, but limited for shorter margins, particularly at the apex. These findings support further evaluation in a clinical utility study to determine whether intraoperative FCM can guide surgical decision-making, optimise nerve sparing, and ultimately improve oncological and functional outcomes.

ADVANCING PRACTICE

What does this study add?

The use of frozen section in radical prostatectomy to guide intraoperative decision-making can reduce positive surgical margins by facilitating secondary resection where necessary, improving functional outcomes for prostate cancer patients. However, access to the technique remains limited. Fluorescence confocal microscopy (FCM) is a more feasible alternative, but has yet to be evaluated prospectively in a well-powered study against histopathology. To our knowledge, the IP8-FLUORESCE study is the first prospective and blinded study to assess the diagnostic accuracy of this technique, and the first to examine the entire prostate surface. We show that FCM is most accurate for detecting positive surgical margins that measure ≥3 mm, but its performance is likely poorer than techniques using frozen section. A utility study assessing FCM to guide intraoperative decision-making is now required.

Clinical Relevance

This prospective, multicentre, blinded study provides the first robust evaluation of fluorescence confocal microscopy for real-time assessment of surgical margins during radical prostatectomy, demonstrating high specificity and reasonable sensitivity for margins >/=3 mm, especially located in the posterolateral surface. The technique represents a rapid, tissue-preserving alternative to frozen section analysis, with potential to support real-time surgical decision-making and broaden nerve-sparing opportunities. However, diagnostic sensitivity for smaller or apical positive margins was sub-optimal, underscoring the need for refinement of the method and clinical utility trials to determine its impact on both oncological safety and functional outcomes before widespread implementation. Associate Editor: Gianluca Giannarini, MD.

Patient Summary

Prostate cancer that is contained to the prostate can be cured by a surgical technique called radical prostatectomy. However, in approximately one-third of cases, a small rim of cancer is left behind: this is known as a positive surgical margin. In this study, we tested a technology called fluorescence confocal microscopy to see whether it could accurately identify positive surgical margins during prostate cancer surgery whilst the patient is still asleep. We showed that the technology can be used in the operating theatre to produce results in a matter of minutes. Whilst it can detect the most serious margins (measuring ≥3 mm) in four out every five cases, it was prone to miss smaller areas. A future study is now required to assess whether acting on the findings of fluorescence confocal microscopy could improve outcomes for patients undergoing surgery.

1. Introduction

Radical prostatectomy (RP) is the gold-standard surgical treatment recommendation for localised and locally advanced prostate cancer. However, positive surgical margins (PSMs) after RP can be associated with adverse long-term oncological outcomes [1]. Intraoperative margin assessment offers the potential to mitigate this risk by enabling immediate secondary resection when a PSM is identified [2]. Additionally, it can increase the proportion of patients eligible for nerve-sparing surgery and improve postoperative functional outcomes without oncological compromise [3–5].

Neurovascular structure-adjacent frozen-section examination (NeuroSAFE) is the most established method, employing a frozen section analysis of the posterolateral prostate margins to guide nerve-sparing decisions [5]. Whilst the use of the NeuroSAFE technique facilitates an increase in nerve preservation and improvement in erectile function, it is labour intensive and reliant on dedicated on-site pathology support [3,6–8]. Further, margin assessment with NeuroSAFE typically prolongs RP by up to 1 h [3]. As a result, its implementation has been restricted [9]. With recent high-level evidence suggesting that intraoperative margin assessment with NeuroSAFE can appreciably improve the rates of postoperative erectile function, there is now a requirement for a faster, more feasible tool.

Fluorescence confocal microscopy (FCM) is an emerging alternative, offering rapid, high-resolution imaging of fresh, unprocessed tissue [10–13]. Unlike frozen sectioning, FCM preserves specimen integrity for subsequent histopathological evaluation because it does not require specimen cut up [14]. Early-phase studies have demonstrated its capability for scanning prostate biopsy tissue, and small unblinded series suggest comparable diagnostic accuracy to intraoperative frozen-section RP margin analysis when examining the posterolateral aspect of the prostate [8,13–18]. Despite these preliminary studies, robust evidence supporting its accuracy is lacking, and to date, no prospective, blinded evaluations of FCM for margin status assessment in RP have been conducted. This study aimed to assess the diagnostic performance of FCM for detecting PSMs in RP by analysing the whole prostate surface, with formalin-fixed paraffin-embedded final histopathological evaluation as the reference standard.

2. Patients and methods

2.1. Study design

IP8-FLUORESCE was a multicentre, prospective, and blinded, paired cohort study. The primary objective was to evaluate the accuracy of digital FCM for the detection of PSMs in RP. Consecutive patients undergoing RP for localised or locally advanced prostate cancer without previous prostate cancer treatments were enrolled across three regional academic uro-oncology centres in the UK. The exclusion criteria included previous treatment for prostate cancer and patients who did not provide written informed consent. The use of any robotic system or surgical approach was permitted. Both nerve-sparing and non–nerve-sparing cases were included.

The IP8-FLUORESCE study protocol has been published previously [19] and was approved by the institutional review boards at each site [20]. The study was registered on August 1, 2023, prior to enrolment of the first patient (ISRCTN21536411).

2.2. Procedures

Robotic-assisted RP was performed in all cases, and surgical technique was left to the preference of the surgeons in each participating institution. Immediately after extraction from the abdominopelvic cavity, RP specimens were scanned on the Histolog scanner (SamanTree Medical SA, Lausanne, Switzerland) using the technique we described previously [19]. In brief, after immersion in nuclear reagent liquid for 10 s, six surfaces of the intact RP specimen were scanned in the following sequence: apex, base, left posterolateral, anterior, right posterolateral, and posterior (Supplementary Fig. 1). Images were pseudonymised at the point of scanning. Specimens then underwent formalin fixation and paraffin embedding for standard-of-care whole-mount histological assessment [20]. Immunohistochemistry was permitted at the request of the pathologist. Histopathology slides were pseudonymised and reported by an independent histopathologist (A.S.) once recruitment was complete. For the final histological assessment, the pathologist was provided with basic clinical data including prostate-specific antigen (PSA) level, prostate magnetic resonance imaging report, and biopsy results. After a 6-wk washout period, two expert uropathologists (A.S. and A.H.) reported the FCM images, blinded to the final RP histopathology results. As the primary objective of this study was to evaluate the diagnostic performance of FCM for PSM detection, and all FCM images were reported after recruitment had been complete, no intraoperative action (eg, secondary resection) was taken based upon the findings of the FCM scan. Basic preoperative clinical data were again made available whilst reporting the deidentified FCM images. Both histopathologists undertook a confocal imaging training module developed by the device manufacturer as well as reviewing 31 cases with histological verification from a pilot study [14]. To minimise the learning curve effect for interpretation of FCM margin images, the first 74 FCM cases (phase 1) were double reported, with both histopathologists evaluating the images together and reaching a consensus on margin status. The remaining cases (phase 2) were reported independently by a single histopathologist (A.S.). In phase 2, if a margin was deemed indeterminate or positive, the case was re-reviewed by both the histopathologists before a final consensus was reached.

2.3. Outcomes

The primary outcome was the diagnostic performance of FCM for the detection of PSMs compared with the final histopathological reference standard (Fig. 1). Diagnostic accuracy was assessed by calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), on a per-patient level. FCM performance was tested against four definitions of PSMs on the final margin assessment:

Any PSM:

Definition 1: any length of prostate cancer of any grade (including focal and <1 mm PSMs) touching the inked surface.

"Clinically significant" PSM:

1. Definition 2: ≥ 3 mm of any Gleason pattern.
2. Definition 3: primary Gleason pattern ≥ 4 of any length.
3. Definition 4: multifocal positivity of any grade or length.

Three millimetres of cancer at the inked surgical margin on histopathology was chosen as the cut-off length for a clinically significant margin based on expert pathologist consensus and the existing evidence [21]. Additionally, this was the threshold at which secondary resection was

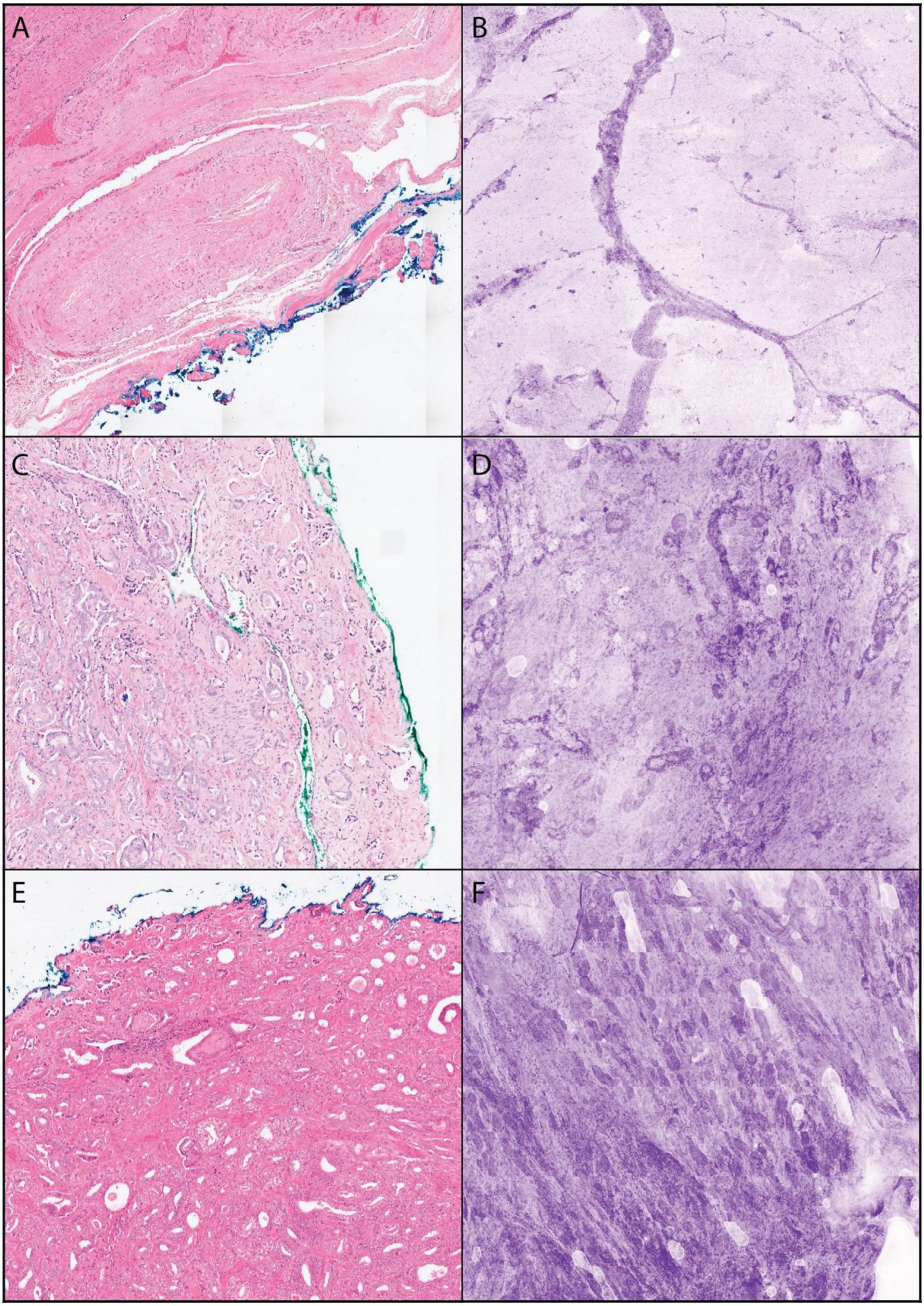

Fig. 1 – Comparison of histopathology and fluorescence confocal microscopy (FCM) images at the prostate margin. (A and B) Negative surgical margin at the left posterolateral surface. Histopathology (A) shows extraprostatic fibroadipose connective tissue at the inked margin, with traversing blood vessels and nerves, but no glandular elements. The corresponding en face FCM scan (B) demonstrates similar stromal and neurovascular features, consistent with a negative margin. (C and D) Positive surgical margin at the posterior surface. Histopathology (C) reveals invasive malignant glands abutting the inked surface. Corresponding FCM image (D) shows abnormal cellular architecture consistent with a malignant focus at the surface. (E and F) Positive surgical margin at the prostate apex. Histopathology (E) shows malignant cells at the inked margin. The corresponding FCM image (F) reveals densely packed and infiltrative glandular structures indicative of malignancy at the apical margin.

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performed in the NeuroSAFE PROOF study [3]. A multifocal PSM was defined as cancer at the inked surgical margin of more than one scanned prostate surface, for example, at the left posterolateral circumferential surface and the prostate base.

Based on a pilot study and discussion with the device manufacturers, it was noted that the quality of FCM images of the base was highly variable due to the presence of diathermy artefact (Supplementary Fig. 2). The percentage of image completeness for scans of each prostate base surface was therefore reported prospectively. Images with <50% completeness were classed as nondiagnostic and subsequently excluded from the analysis.

2.4. Statistical analysis

The sample size calculation was determined a priori and updated twice following two prespecified interim reviews of event rates. These reviews were conducted by an independent administrator using standard-of-care histopathology reports to assess the prevalence of PSMs after enrolment of 50 and 100 cases. For any length of a PSM (definition 1), the prevalence varied between 41% and 43%, and for clinically significant PSMs (definitions 2–4), the prevalence was 18% at both interim reviews. The sample size was based on the precision (half-width of the confidence interval [CI]) of the assumed estimate of the sensitivity (85%), consistent with a single-proportion CI width calculation. The full list of assumptions for the sample size calculations are included in Supplementary Table 1. The final recruitment target covering all definitions and following the two interim analyses was set at 153 patients, with a target of 49 PSMs.

Descriptive statistics are presented as medians, with interquartile ranges (IQRs) for non-normally distributed data and means with standard deviations for normally distributed data. Contingency tables were used to calculate sensitivity, specificity, PPV, and NPV, comparing FCM with final histopathology for the detection of PSMs according to the four definitions. Exact two-sided 95% CIs were calculated using the Clopper-Pearson method. All statistical analyses were conducted using R (version 4.4.0). The study is reported in accordance with the STARD guidelines [22].

2.5. Post hoc analysis

To further characterise the performance of FCM, a post hoc analysis was conducted evaluating diagnostic accuracy across increasing lengths of PSM involvement, from 1 to 5 mm.

Additionally, false negative cases with PSMs of $\geq$3 mm and all false positive cases were re-reviewed, unblinded to the histopathology results, by the two study pathologists after completion of the main analysis. This review did not influence the primary results, but aimed to offer greater insight into sources of diagnostic discrepancy and potential limitations of the technology.

To assess for a learning curve effect, a subgroup analysis was conducted comparing diagnostic performance for PSMs of $\geq$3 mm between the two reporting phases. Sensitivity, specificity, PPV, and NPV were calculated for each phase with 95% CIs. The Method of Variance Estimates Recovery (MOVER) approach was applied to generate a 95% CI for the absolute difference in proportions.

2.6. Role of the funding source

This study was generously funded by The John Black Charitable Foundation and The Urology Foundation (Innovation and Research Award 2023). The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

3. Results

Between August 17, 2023 and September 23, 2024, 156 men underwent RP and intraoperative scanning with the Histolog scanner. In 67/156 (42.9%) cases, the basal surface FCM scan was considered nondiagnostic. In these cases, the basal FCM scan and the basal result on final histology were excluded, leaving a total of 869 surfaces for review.

3.1. Baseline characteristics

The median age and PSA were 64 yr (IQR 58–69) and 7.4 ng/ml (5.3–10.8), respectively. Most patients were considered high risk (64.7%) according to European Association of Urology risk groups, and 21.2% had locally advanced disease (Table 1).

3.2. Final histology PSM characteristics

On final histopathology, the overall prevalence of a PSM of any length on a per-patient level was 30.8% (48/156). The PSM rate was lower than predicted in the interim review of event rates, owing to significant variability in reporting of margin outcomes when histopathology slides underwent a central review compared with the standard-of-care reports used for the interim reviews. Nevertheless, the actual number of PSMs (48) was only one less than the prespecified target (49).

The prevalence of clinically significant PSMs ($\geq$3 mm, primary Gleason pattern 4, multifocal margins) was 18.6% (29/156), aligning with the interim event rate reviews. The median PSM length on histopathology was 2 mm (IQR 1–5 mm). Of 869 surfaces included, there were 67 (7.7%) PSMs. PSMs were most common at the apex (39.8%; 26/67) and least common on the posterior surface (4.5%; three of 67; Supplementary Table 2).

3.3. Diagnostic performance of FCM

Performance characteristics of FCM for detecting PSMs according to the four prespecified definitions are summarised in Table 2. Sensitivity was 48% (95% CI 33–63%) for definition 1 (all PSM lengths, including focal positivity) but was higher for clinically significant PSMs, reaching 79% (95% CI 54–94%) for definition 2 (PSMs of $\geq$3 mm). Across all definitions, specificity was high (>90%). PPV was highest for definition 1 (79%; 95% CI 60–92%). NPV was consistently high, particularly for clinically significant PSMs (definitions 2–4).

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Table 1 – Baseline patient, tumour, and surgical characteristics

Characteristic	*N* = 156
Age (yr), median (IQR)	64 (58–69)
Ethnicity, *n* (%)	
Asian	17 (10.9)
Black	37 (23.7)
Mixed	3 (1.9)
White	72 (46.2)
Other	12 (7.7)
Unknown	15 (9.6)
Charlson comorbidity score, median (IQR)	4 (4–4)
First degree relative with PCa, *n* (%)	
Yes	31 (19.9)
No	121 (77.6)
Not recorded	4 (2.6)
PSA (ng/ml), median (IQR)	7.4 (5.3–10.8)
PVol (ml), median (IQR)	37.0 (28.3–45.8)
Extraprostatic extension on MRI, *n* (%)	
Yes	13 (8.3)
Suspicious	32 (20.5)
No	111 (71.2)
Cambridge prognostic group, *n* (%)	
2	55 (35.3)
3	59 (37.8)
4	27 (17.3)
5	15 (9.6)
EAU risk category, *n* (%)	
Intermediate	22 (14.1)
High	101 (64.7)
Locally advanced	33 (21.2)
ISUP grade group (final pathology), *n* (%)	
2	83 (53.2)
3	52 (33.3)
4	3 (1.9)
5	18 (11.5)
T stage (final pathology), *n* (%)	
2a	2 (1.3)
2b	3 (1.9)
2c	61 (39.1)
3a	75 (48.1)
3b	15 (9.6)
Robotic system, *n* (%)	
Da Vinci X/Xi	151 (96.8)
Hugo	5 (3.2)
Nerve sparing, *n* (%)	
Unilateral	27 (17.3)
Bilateral	119 (76.3)
Non–nerve sparing	10 (6.4)

EUA = European Association of Urology; IQR = interquartile range; ISUP = International Society of Urological Pathology; MRI = magnetic resonance imaging; PCa = prostate cancer; PSA = prostate-specific antigen; PVol = prostate volume.

3.4. *Post hoc analyses*

In the post hoc per-patient analysis, FCM was more accurate for detecting longer lengths of PSM. For example, sensitivity for detecting PSMs of >3, 4, and 5 mm was 79% (54–94%), 83% (59–96%), and 81% (54–96%), respectively (Supplementary Table 3).

3.5. *False negative classifications*

There were 25 false negative cases. Of these 25 cases, 15 (60.0%) were ≤1 mm and 21 (84.0%) were ≤2 mm; 13 of 25 (52.0%) false negative cases were at the apex. The four cases with missed PSMs of length ≥3 mm were due to incomplete image acquisition (two cases), motion artefact (one case), and diathermy artefact (one case; Supplementary Fig. 3).

3.6. *False positive classifications*

Of the 29 patients who were called as positive on FCM, six were false positive cases. Justifications for false positive cases could be divided into two categories:

1. Close surgical margins (one case): a 3 mm margin was identified on FCM. On histopathology, tumour was found at <0.1 mm from the inked margin at the corresponding region.
2. Misclassification (five cases): a 10 mm anterior margin, 1 and 8 mm apical margins, a 1 mm posterior margin, and an 8 mm basal margin were called positive on FCM. On unblinded review, these areas were reclassified as benign glands or stroma.

3.7. *Learning curve*

The absolute differences in the performance characteristics between phase 1 (in which all FCM images were dual reported) and phase 2 (in which dual reporting occurred only for indeterminate or suspected positive cases) are summarised in Supplementary Table 4. Whilst a trend towards improved performance in phase 2 was observed, particularly for PPV, the wide CIs indicate substantial uncertainty in the estimates, precluding a definitive assessment of the learning curve effect.

4. Discussion

4.1. *Summary of main results*

In this prospective, blinded diagnostic accuracy study, FCM demonstrated adequate performance for detecting and ruling out PSMs of ≥3 mm in length in fresh RP specimens compared with final histopathology. FCM was less accurate for assessing focal PSMs or those 1–2 mm in length, with sensitivity of <50% when including all lengths of PSMs. Specificity remained above 90%, reflecting the large number of negative margins in the study. Nevertheless, for the most clinically relevant margins, FCM showed reasonable discriminative ability.

Table 2 – Performance characteristics of FCM on a per-patient level

Definition	Description	n	Number of PSMs	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
1	All	156	48	0.48 (0.33–0.63)	0.94 (0.88–0.98)	0.79 (0.60–0.92)	0.80 (0.72–0.87)
2	≥3 mm	127	19	0.79 (0.54–0.94)	0.94 (0.88–0.98)	0.71 (0.48–0.89)	0.96 (0.91–0.99)
3	Primary Gleason ≥4	146	12	0.58 (0.28–0.85)	0.94 (0.88–0.98)	0.54 (0.25–0.81)	0.95 (0.89–0.98)
4	Multifocal	122	14	0.64 (0.35–0.87)	0.94 (0.88–0.98)	0.60 (0.32–0.84)	0.95 (0.89–0.98)

CI = confidence interval; FCM = fluorescence confocal microscopy; NPV = negative predictive value; PPV = positive predictive value; PSM = positive surgical margin.

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The superior performance of FCM for longer PSMs can likely be attributed to the imaging characteristics of the technology. Whilst FCM offers high-resolution imaging, its ability to capture cytological detail in comparison with histopathology is limited. The histopathological diagnosis of prostatic adenocarcinoma relies on the careful evaluation of a range of cytological, both nuclear and cytoplasmic, and architectural features. The reliance of FCM on predominantly architectural features, compounded by a lack of adjunct techniques such as immunohistochemistry, limits the diagnostic accuracy for small tumour foci. As the technology develops over time, the resolution of FCM will likely improve, allowing superior discrimination of nuclear features and greater diagnostic capability for small foci. However, the clinical significance of such small regions is uncertain. Evidence from large retrospective cohorts and meta-analyses of smaller cohorts with long-term follow-up suggests that unifocal PSMs under 3 mm in length are unlikely to correlate with long-term oncological outcomes such metastasis-free or prostate cancer–specific survival, and may not necessitate additional intervention [1,23,24]. However, prospective evidence for the impact of PSMs on prostate cancer–specific survival is lacking. In our practice with the NeuroSAFE procedure, we do not perform secondary resection for PSMs of $\leq$2 mm.

The false positive rate was higher than expected (six of 29; 20.7%). False positive cases must be minimised as far as possible in intraoperative margin assessment to reduce the risk of performing unnecessary secondary resection resulting in adverse functional consequences. In one case, a PSM was identified on FCM but found to be a close surgical margin on histology, with tumour cells <0.1 mm from the inked surgical margin. It is possible that the 30 µm scanning depth of the confocal microscope detected cancer a few micrometres below the prostate surface. The clinical relevance of such cases is unknown and requires further evaluation. The remaining five false positive cases were reassigned as benign tissue on unblinded post hoc review, suggesting that the learning curve effect may not have been completely mitigated against. Whilst FCM images share features with conventional histology, our findings underscore the importance of robust training for those interpreting FCM images, even experienced pathologists. Another consideration is the difference in imaging planes between histopathology and FCM. As histopathological assessment necessitates sectioning prostate specimens at approximately 5 mm intervals, small regions of cancer falling between these sections may be missed. It is possible that the en face scanning method used with FCM could detect such cases when pathology does not.

The en face scanning technique allowed us to perform FCM scans with specimens completely intact, without any tissue sectioning. This not only reduced the time taken for the FCM scan, but also preserved tissue integrity for a subsequent histopathological analysis. Further, performing FCM in the operating theatre with the operating surgeon present may provide a better impression of exactly where a PSM lies in the prostate bed compared with a frozen section analysis, where the specimen is sectioned and assessed in a laboratory. This may facilitate more accurate secondary resection when required and is particularly relevant as detection rates of cancer in secondary resection specimens from intraoperative frozen section range from 0% to 42% [25].

4.2. *Strengths and weaknesses*

To the best of our knowledge, this is the first blinded, prospective, multicentre study to evaluate FCM for margin assessment in RP. We additionally published our protocol and sample size calculation a priori [19]. The multicentre approach across a diverse patient population enhances generalisability, whilst the inclusion of two inbuilt event rate reviews allowed for adjustment of recruitment targets based on an interim assessment of PSM rates at prespecified stages of recruitment. Such an approach was critical in attempting to mitigate one of the key challenges in diagnostic accuracy studies: the uncertainty of event rates.

A notable limitation was the high attrition rate for PSMs, primarily due to discrepancies between standard-of-care histopathology reports used in interim event rate reviews and blinded central reporting of histology slides by an independent uropathologist. Interobserver reproducibility remains highly variable in prostate biopsy, and here we highlight the significant variability amongst pathologists in reporting surgical margin status in RP [26–31]. In our a priori sample size calculation, we estimated that 49 PSMs would be required to robustly assess FCM for PSM detection. Based on an expected prevalence rate of 40% from the second interim event rate review, we calculated that a sample size of 140 patients would be sufficient. By over-recruiting to 156 patients to cover all definitions, we fortuitously compensated for the lower-than-expected final prevalence (30.8%), with 48 PSMs in total.

Our experience highlights the challenge of interpreting basal, anterior, and apical margins on FCM compared with posterolateral surfaces, largely due to artefact and anatomical complexity. Nevertheless, an advantage of FCM is that it enables assessment of all prostatic surfaces. This broader application may provide more comprehensive oncological assessment, although it may come at the cost of reduced accuracy in anatomically complex regions such as the prostate apex, where the majority of missed PSMs occurred in our study. Future work should explore whether limiting FCM to posterolateral surfaces, where image quality is highest and the clinical utility of secondary resection has been evaluated most extensively, or only to suspected cancer-bearing surfaces could yield the greatest diagnostic accuracy.

4.3. *Comparison with existing research*

Previous studies of FCM on RP specimens have been limited by small sample sizes and a lack of histopathologist blinding [8,14,16,32]. The largest prospective study from Baas et al [8] included 96 posterolateral surfaces sectioned from the 50 specimens scanned on the same confocal microscope used in the present study. The reported sensitivity and specificity on a per-surface level were 86% and 99%, respectively, though CIs were not reported. Whilst these figures were higher than those reported here, the performance

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characteristics were calculated from only 15 PSMs and the pathologist was unblinded to the location of the PSMs on histopathology, likely leading to inflated estimates of FCM performance. A blinded analysis of the posterolateral surfaces of 31 RP specimens by Almeida-Magana et al [33] reported sensitivity of 73–91% and specificity of 94–100%, though the median PSM length was nearly triple that in this study. Our study is the first to apply intraoperative margin assessment to regions other than the posterolateral surfaces, and the lower sensitivity reported here was likely driven by the high proportion of false negative cases at the apex and the short median overall PSM length. The sample size and number of PSMs assessed with FCM in our study are larger than those reported in all published series combined.

The only widely studied method for intraoperative margin assessment in prostate cancer is the NeuroSAFE technique. Schlomm et al [2], in their seminal observational cohort including 5392 patients undergoing RP with NeuroSAFE, reported sensitivity of 93.5% and specificity of 98.8%. Other contemporary series report sensitivity and specificity ranging from 76.8% to 100.0% and 92.7% to 97.4%, respectively [34–36]. We found similar specificity (94%) for FCM but considerably lower sensitivity (48% for all margins). Sensitivity increased substantially for PSMs of ≥3 mm (79%) but was still inferior to that reported in the largest NeuroSAFE series, reflecting the known limitations of FCM in identifying small tumour foci and highlighting the importance of selecting a clinically meaningful margin length threshold. As experience grows with FCM in prostate cancer surgery and the technique matures, it is likely that diagnostic accuracy will reach comparable levels, especially for longer PSMs. Indeed, in the present study, over 80% of the missed PSMs were "clinically insignificant" (≤2 mm). The four missed PSMs of length ≥3 mm were subject to presence of artefact. In our experience, artefact is rarely present at the posterolateral surfaces where FCM is most likely to be employed. Nevertheless, our reported sensitivity of 48–79% is suboptimal.

The NeuroSAFE PROOF randomised controlled trial provides the first high-quality evidence that intraoperative margin assessment with secondary resection for PSMs of ≥3 mm improves postoperative functional outcomes in men undergoing RP [3]. Whilst not powered for oncological outcomes, at 1-yr follow-up, there were no significant differences between the NeuroSAFE and standard-of-care arms [7,32,33]. However, the technique has failed to gain wider traction outside of a handful of high-volume centres due to the high cost of the required equipment such as cryotomes, complex tissue preparation, and the unacceptable time taken for results to be conveyed to the operating surgeon [9]. Although FCM procedure times were not formally recorded in our study, scanning and reporting typically took around 10 min in total. Baas et al [8] reported a median FCM procedural time of just 8 min compared with 50 min for NeuroSAFE in a cohort undergoing both techniques. FCM has the advantage of using a single, mobile scanner that can be kept in the operating theatre or nearby office, requiring only one technician or trained surgeon/nurse to prepare and scan the specimen. Whilst the upfront cost of the Histolog scanner is approximately £250 000 (€290 000/$340 000), the overall per-case expenditure is lower than that of NeuroSAFE due to the simplified workflow. The operational simplicity and scalability of FCM offer a more economically viable pathway to intraoperative margin assessment across a broader range of surgical centres. Additionally, the pathologist can report the images from a remote location, a valuable option for centres where laboratory services are centralised. Unlike NeuroSAFE, FCM produces high-resolution images without the need for tissue sectioning, leaving the capsule intact for histopathological assessment.

4.4. *Implications for practice*

Although FCM demonstrated high specificity, overall sensitivity ranged from 48% for any margin to 79% for clinically significant margins, which is insufficient to support immediate change to clinical practice and is lower than reported in studies employing a frozen section analysis [2,34–36]. We included all-comers, with a median PSM length of only 2 mm, and scanned the entire prostate surface, including the apex and base where image artefact is more common. These factors likely contributed to lower sensitivity than reported in smaller, unpowered series. However, our findings establish a robust foundation for further research, which should focus on patient groups most likely to benefit from intraoperative margin assessment, such as those not otherwise considered candidates for nerve sparing, and restrict scanning to posterolateral surfaces where image quality is most reliable. Future utility studies should compare RP with FCM-guided secondary resection, limited to PSMs of ≥3 mm, to standard-of-care RP without intraoperative margin assessment. Such a study would be feasible to run due to the characteristics of confocal microscopy with the Histolog scanner and the capacity for FCM scans to be reported centrally.

5. Conclusions

This study is the first prospective, multicentre, blinded evaluation of FCM for intraoperative margin assessment in RP. FCM during RP is feasible, with reasonable performance for PSMs of ≥3 mm, which are most clinically relevant. However, refinement of the technique and further evaluation specifically focusing on the examination of the posterolateral surfaces is required before change in practice.

Author contributions: Nikhil Mayor had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Mayor, Winkler, Ahmed, Shah.
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Supervision: Winkler, Ahmed, Shah, Connor.
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Supplementary data

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