Clinical Study Protocol

FLUORESCE

Full Study Title

Imperial Prostate 8 - **Fluo**rescence confocal microscopy for **r**apid **e**valuation of **s**urgical **c**ancer **e**xcision

Short Study Title

IP8-FLUORESCE

A prospective cohort study to assess the accuracy of digital fluorescence confocal microscopy for assessment of surgical margins in radical prostatectomy specimens

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This protocol describes the IP8-FLUORESCE study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the UK Policy Frame Work for Health and Social Care Research. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

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GLOSSARY OF ABBREVIATIONS

AE	Adverse effect
AUC	Area under the curve
BCC	Basal cell carcinoma
BCR	Biochemical recurrence
BRC	Biomedical Research Centre
CE	Conformité Européene
CRF	Case report form
ECG	Electrocardiogram
FCM	Fluorescence confocal microscopy
H&E	Haematoxylin & eosin
HTA	Human Tissue Act
ICHTB	Imperial College Healthcare Tissue Bank
ISRCTN	International Standard Randomised Controlled Trial Number
ISUP	International Society of Urological Pathology
NHS	National Health Service
NS	Nerve sparing
NVB	Neurovascular bundle
PSM	Positive surgical margin
REC	Research ethics committee
RP	Radical prostatectomy
SAE	Serious adverse effect
SOP	Standard operating procedure
TMG	Trial management group

STUDY SUMMARY

TITLE	A prospective cohort study to assess the accuracy of digital fluorescence confocal microscopy for assessment of surgical margins in radical prostatectomy specimens (IP8-FLUORESCE).
DESIGN	Prospective, multicentre, blinded, cohort design (ex vivo).
OBJECTIVES	**Primary** To determine the accuracy of digital FCM in detecting and ruling out positive surgical margins in prostate specimens on a patient level, in patients undergoing radical prostatectomy for prostate cancer. **Secondary** To determine the accuracy of digital FCM in detecting and ruling out positive surgical margins on an individual margin/image level. To evaluate the classification performance of FCM in differentiating between low- and high-grade prostate cancer. To assess the interobserver variability in reporting of digital FCM images.
OUTCOME MEASURES	**Primary** Sensitivity, specificity, positive and negative predictive value of digital FCM for detection of clinically significant prostate cancer at surgical margins, with traditional H&E histopathology as the reference standard, on a per-patient basis. Clinically significant cancer is defined as Gleason score >/=7 (ISUP Grade Group >/=2). **Secondary** Sensitivity, specificity, positive and negative predictive value of digital FCM for detection of cancer at surgical margins, with traditional H&E histopathology as the reference standard, on a per-image/margin level. Area under the receiver operating characteristic curve (AUC) for cancer detection of digital FCM with traditional H&E histopathology as the reference standard.

	Agreement of digital FCM with the pathology report for cancer length at margin (mm) on a margin and patient level. Agreement of digital FCM with the pathology report for cancer grade at margin (mm) on a margin and patient level. Cohen's kappa coefficient for agreement between readers (two individual histopathologists, histopathologist vs trainee histopathologist, histopathologist vs urologist).
POPULATION	Men undergoing radical prostatectomy for prostate cancer.
ELIGIBILITY	**Inclusion Criteria** Men undergoing radical prostatectomy at any of the study centres during the study period. All nerve sparing and non-nerve sparing cases will be included consecutively. **Exclusion Criteria** Men who do not consent for ex vivo tissue research. Men who are listed for salvage prostatectomy and had a previous prostate cancer treatment (radiotherapy, brachytherapy, focal therapy).
DURATION	The recruitment period will begin in August 2023 and run until the required number of cases to reach statistical power have been recruited.

1. INTRODUCTION

1.1. BACKGROUND

Fluorescence confocal microscopy (FCM) is an imaging technique whereby digital images of fresh tissues are produced almost instantly, without the need for time-consuming fixation techniques[1]. It has been evaluated across a number of different organ systems and can accurately diagnose prostate cancer when compared to conventional histopathology[2–5]. Intraoperative analysis of surgical margins during prostatectomy using frozen sections (NeuroSAFE)[6] has not been widely adopted in the UK due to a number of major obstacles. FCM is cheaper, quicker, and requires less resources than NeuroSAFE and may represent a more feasible alternative. Two small, underpowered and unblinded studies have evaluated FCM for intraoperative analysis of surgical margins with promising results[7,8]. The IP8-FLUORESCE study aims to robustly evaluate FCM in the real-time identification of positive surgical margins in radical prostatectomy specimens using a prospective, blinded, paired-cohort confirmatory design.

1.2. RATIONALE FOR CURRENT STUDY

Radical prostatectomy (RP) is an effective oncological and widely proffered approach to treating prostate cancer. There is reasonable evidence that for men with the correct indication a small to moderate survival increase is seen as long as the life expectancy is 10 to 15 years[9].Functional outcomes are variable and high long-term rates of urinary incontinence and erectile dysfunction have been reported in the UK[10]. In potent patients undergoing RP, preservation of the neurovascular bundles (NVBs) may spare erectile function in 50% to 80%[11]. Nerve-sparing RP, however, results in higher rates of positive surgical margins (PSMs), specifically in men with early extracapsular extension that is often not seen on pre-operative MRI staging[11]. Up to 50% of men with a PSM will experience biochemical recurrence (BCR) which in turn may require additional adjuvant treatment[12]. PSMs have not been shown to be an independent predictor of overall survival but are considered an important surrogate for complete cancer excision. Ex vivo intra-operative assessment of PSMs using frozen section in the NeuroSAFE procedure has been shown to reduce PSMs[6]. Whether this translates to a better oncological outcome is unknown. The NeuroSAFE method may also increase the number of men suitable for nerve-sparing surgery but we do not know if this leads to improved erectile function rates. The unproven benefits of NeuroSAFE and the major cost implications of longer operating times by 60-70 minutes has prevented mainstream adoption. This is compounded by the UK-wide shortage of histopathologists[13]. While concordance between NeuroSAFE and conventional histopathology is excellent, the procedure is time-consuming, laborious, and costly, restricting its use in UK clinical practice[14]. This calls for a cheaper, faster and similarly accurate method of real-time PSM assessment during prostate cancer surgery: FCM is the foremost candidate of all real-time tissue imaging technologies.

What is FCM?

Confocal laser microscopy uses pinhole apertures in the microscope detector to reject out-of-focus light, reducing blur and producing high-resolution imaging in thick tissue samples. Fluorescence confocal microscopy uses fluorochromes to increase the cell-to-stroma contrast. Depending on the laser wavelength, reflectance (488 nm) and fluorescence (785 nm) can be applied[15]. Novel confocal microscopes allow visualisation with reflectance and fluorescence simultaneously and the traditional greyscale is digitally enhanced to provide a two-colour scale which resembles haematoxylin and eosin (H&E) staining. FCM requires minimal tissue preparation (typically staining samples in Acridine Orange for 10 seconds then washing with saline) and can produce high-resolution images in less than a minute. Scanners are portable and require only one square metre of space, and can therefore be kept in the operating theatre or an office. Critically, the samples can be re-examined with conventional techniques after FCM as the fluorescent dye does not alter the specimen and tissue fixation is not required. Images can be directly uploaded to electronic patient records for review by pathologists or trained urologists[7].

Summary of prostate applications

FCM appears to be highly accurate when compared to traditional histological analysis across multiple organ systems[16]. In the setting of basal cell carcinoma (BCC), the technique is well-established, allowing for rapid identification of margins with high sensitivity and specificity in Mohs samples[17]. Whilst there is no Level 1a/b evidence available, a number of prospective studies have shown that FCM works in principle for the purpose of real-time prostate cancer diagnosis on biopsy core tissue[2–5] and should be further evaluated for its application in a wider patient population. The potential application in intra-operative real-time analysis of surgical margins has only been analysed in two small studies with high probability of bias.

Rocco et al[8] reported the first proof-of-concept study assessing FCM in determining PSMs. This study was underpowered and not blinded. It only included 24 patients of which 50% were lost to follow up. Mohs sections at the NV-adjacent-posterolateral surface on eight patients with intermediate or high-risk prostate cancer patients undergoing full-bilateral-NS robot-assisted RP (RARP) were assessed with FCM by two uro-pathologists. The samples were subsequently fixed in formalin and embedded in paraffin and analysed with H&E staining. Seven of the eight patients had negative surgical margins with 100% agreement between FCM and traditional histopathology, and complete inter-rater agreement for FCM between the two pathologists. For the patient with a PSM, a focal secondary resection was performed, allowing complete resection of the tumour.

The second study directly compared NeuroSAFE to FCM in determining PSM rates[7]. Surgical margins were assessed during 50 NS-RARP procedures in patients scheduled for NeuroSAFE and the accuracy of FCM and NeuroSAFE were compared with conventional histopathology. 96 posterolateral sections of RP specimens were evaluated. FCM identified 15 (16%) PSMs and NeuroSAFE identified 14 (15%). There was a substantial level of agreement between

FCM and NeuroSAFE, and the ability of both techniques to identify PSMs compared to histopathology was excellent, with high overall diagnostic accuracy. Critically, FCM required a significantly shorter procedure time than NeuroSAFE (8 vs 50 minutes). Whilst a larger sample size than the first proof-of-concept study, it was not powered to draw key conclusions. A further significant limitation of this study was the non-blinded assessment of the FCM images.

2. STUDY OBJECTIVES

Primary

To determine the accuracy of digital FCM in detecting and ruling out positive surgical margins in prostate specimens on a patient level, in patients undergoing radical prostatectomy for prostate cancer.

Secondary

To determine the accuracy of digital FCM in detecting and ruling out positive surgical margins on an individual margin/image level.

To evaluate the classification performance of FCM in differentiating between low- and high-grade prostate cancer.

To assess interobserver variability in reporting of digital FCM images.

3. STUDY DESIGN

IP8-FLUORESCE is prospective, multicentre, blinded, cohort study performed ex vivo using prostate specimens obtained during radical prostatectomy to evaluate the accuracy of FCM in determining surgical margin status. The reference standard will be an independent histology report. The Histolog® Scanner (SamanTree Medical SA, Lausanne, Switzerland) will be used to obtain FCM images. A pilot phase consisting of 5 patients will be performed to define the exact standard operating procedure (SOP) for handling and scanning of tissues.

Men undergoing RP for localised prostate cancer will have both FCM and histopathological evaluation of surgical margins. Once recruitment is complete, images will be reported blinded by an independent histopathologist trained in interpretation of FCM images.

The study is ex vivo and no deviations from standard of care will take place during the study.

3.1. PRELIMINARY SOP

Surgical procedure

Patients with prostate cancer diagnosed based upon suspicious MRI with associated confirmatory prostate biopsy whose case has been discussed in the locoregional multidisciplinary meeting will undergo robotic prostatectomy (RP). All cases will be robotic-assisted using the Da Vinci Xi surgical system (Intuitive Surgical, Sunnyvale, USA). Both nerve-sparing (bilateral or unilateral) and non-nerve sparing procedures will be performed depending on individual surgeon assessment of each case. RP will be performed by experienced urological surgeons across three major tertiary referral study centres. Men who consent to ex vivo tissue research through will be included.

FCM technique

The Histolog® scanner is a CE-IVD-certified scanning device with a wide-field-of-view confocal laser scanning microscope, designed for scanning large biological specimens in a clinical setting. Tissue fluorescence is excited by a laser at the wavelength of 488 nm and fluorescence emission is collected at a wavelength >500 nm. Additional post-processing is not required though an inbuilt system automatically converts them from monochromatic to purple in order to increase readability and align more closely with traditional H&E-stained images.

Prostate specimens will be removed from the abdominopelvic cavity and photographed to obtain views from each aspect in order to retain orientation during the scanning procedure. The specimens will be immersed whole in the Histolog® Dip (a fluorescent histological stain) for 10 seconds, then rinsed in 0.9% saline and transferred to the Histolog® Dish.

The specimens will be scanned whole, without the need for any tissue resection. They will be placed on the Histolog® Scanner and weighed down with a small bag of sand (process TBC during pilot phase) to increase tissue contact with scanner.
A low-resolution preview image will be acquired in 5 seconds to make a technical judgement of the completeness of the image, the presence of excess tissue, and the absence of air bubbles. If necessary, the specimen will be repositioned and a high-resolution digital image will be acquired within 50 seconds. The procedure will be performed for six margins in total for each specimen:

- Base
- Apex
- Left lateral
- Right lateral
- Posterior
- Anterior

The specimen will then be formalin-fixed and paraffin-embedded before undergoing formal histological assessment for margin status by a histopathologist. Clinical management of these patients will follow current standard-of-care pathways.

Outcome reporting

The histopathology slides for each patient will be digitally scanned and uploaded to a secure cloud-based server alongside the FCM images. All files will be pseudoanonymised and the server locked until the recruitment target has been reached. Once recruitment is complete, histopathology slides will be re-reported by expert independent uro-pathologists who will also report the margin status for the FCM images. They will be blinded to the margin status and the patient details in the histology report when making the FCM report, and vice versa. The histopathologist will provide the overall margin status on a per-patient level, as well as a report on a margin level, for length of cancer at the margin and highest grade (Table 1).

Table 1. Example case report form for margin status for a single patient.

	FCM Margin Status			**Histopathology Margin Status**		
	(0=negative, 1=positive, 2=indeterminate)	*Length (mm)*	*Grade (highest; 3, 4, 5)*	*(0=negative, 1=positive, 2=indeterminate)*	*Length (mm)*	*Grade (highest; 3, 4, 5)*
Base						
Apex						
Lateral (left)						
Lateral (right)						
Anterior						
Posterior						
Overall						

The recruitment target is 5 patients for the pilot phase, followed by 100 patients for the main phase. The study will run for 6 months or until the recruitment target is reached.

3.2. STUDY OUTCOME MEASURES

Primary

Sensitivity, specificity, positive and negative predictive value of digital FCM for detection of clinically significant prostate cancer at surgical margins, with traditional H&E histopathology as the reference standard, on a per-patient basis. Clinically significant cancer is defined as Gleason score >/=7 (ISUP Grade Group >/=2).

Secondary

Sensitivity, specificity, positive and negative predictive value of digital FCM for detection of cancer at surgical margins, with traditional H&E histopathology as the reference standard, on a per-image/margin level.

Area under the receiver operating characteristic curve (AUC) for cancer detection of digital FCM with traditional H&E histopathology as the reference standard.

Agreement of digital FCM with the pathology report for cancer length at margin (mm) on a margin and patient level.

Agreement of digital FCM with the pathology report for cancer grade at margin (mm) on a margin and patient level.

Cohen's kappa coefficient for agreement between readers (two individual histopathologists, histopathologist vs trainee histopathologist, histopathologist vs urologist)

4. PARTICIPANT ENTRY

4.1. PRE-REGISTRATION EVALUATIONS

All participants scheduled for RP undergo routine history taking, examination, diagnostic workup, and staging to ensure suitability for the procedure. All patients will undergo standard of care preoperative workup including renal and liver function testing, ECG, and anaesthetic review.

Due to the ex vivo design of the study, no screening visits are required. Further, patients will not have any contact, undergo any tests, or require any follow-up out of line with standard of care management other than the digital FCM scan intraoperatively.

4.2. INCLUSION CRITERIA

Any person over the age of 18 with a prostate undergoing radical prostatectomy at Imperial College Healthcare NHS Trust during the study period.

All nerve sparing and non-nerve sparing cases will be included consecutively.

4.3. EXCLUSION CRITERIA

Subjects who are unwilling or unable to consent for ex vivo tissue research through Imperial College Healthcare Tissue Bank (ICHTB).

Subjects scheduled for salvage RARP

Subjects enrolled in concurrent clinical trials requiring ex vivo prostatic tissue for research.

4.4. WITHDRAWAL CRITERIA

Patients may decide to opt out of IP8-FLUORESCE at any time. This is entirely within their right to do so. Such cases will be reported to the research team so that no further data are entered onto the database. Data captured before consent was withdrawn will be used in the study, but no further data, beyond this date will be collected or used in any analysis. Reason for withdrawal must be recorded in the CRF/eCRF and medical records. We will continue to recruit patients until the target number for study is met.

5. ADVERSE EVENTS

5.1. DEFINITIONS

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward medical occurrence or effect that:

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*

- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

5.2. REPORTING PROCEDURES

This is an ex vivo study. Any AEs that occur as a result of the RP procedure will be reported in line with usual clinical practice. It is not a requirement for the CI to be informed.

6. ASSESSMENT AND FOLLOW-UP

No follow-up or follow-up visits are required for this ex vivo study.

7. STATISTICS AND DATA ANALYSIS

7.1. Sample size calculation

The sample size for diagnostic studies incorporating the prevalence of the PSM was calculated based on expected sensitivity of 0.86 and specificity of 0.96 reported by Baas et al[7]. The authors included 49 patients and analysed 96 prostate sides (a per patient analysis was not carried out). The prevalence of a PSM as detected by standard pathology analysis was 15%. The study did not have an a priori sample size estimation and was not blinded.

The sample size for the planned study was calculated to power a sensitivity analysis in line with the practical application of the new test i.e. that detection of a PSM by FCM would be a safe trigger for a change of intra-operative management.

The following assumptions were made:

- Sensitivity 0.85
- Specificity 0.95
- Prevalence of PSM: 30%. The assumption is based upon a review of 768 historical RPs performed at our centre. The over-all PSM rate was 41% due to high admixture of high-risk patients from the local unscreened population, 47% for high-risk cancers, 37% for intermediate risk cancers and 34% for low-risk cancers.
- Precision: +/- 14 points (a variability deemed sufficient for a pilot study)
- Confidence level: 95%
- Expected dropout rate for poor scans: 10%

Estimated sample size for sensitivity is n=27 PSM patients, inflated by 10% to n=30 for expected drop out. Adjusting for the prevalence of 30% a final sample size of 100 patients is deemed sufficient.

Since the true prevalence of PSMs for the study cohort is not known, an interim assessment of observed PSM rate is planned a priori. This will be used to adjust the sample size accordingly.

Assessment of the interim PSM rate is planned after 50 patients by a pre-specified independent assessor nominated before the start of the study. The assessment is based on the clinical histopathology reports for each patient which includes PSM status.

Example for sample size adjustment after interim analysis of prevalence are presented as follows: If the observed PSM rate is 25% the sample size would need to be increased to 120 patients. Conversely, if the observed PSM rate is 35% the sample size would decrease to 86 patients.

Full details of planned statistical summaries and analyses will be outlined in a pre-defined, signed and dated statistical analysis plan before any analyses are performed. Any deviations from the analysis plan will be fully documented with reasons for doing so.

Data and all appropriate documentation will be stored for 10 years after the completion of the study.

8. REGULATORY ISSUES

8.1. ETHICS APPROVAL

This study was granted ethical approval via the Imperial College Healthcare Tissue Bank (ICHTB) which is approved by Wales REC3 to release human material for research. The Human Tissue Authority (HTA) licence reference is 12275. Research ethics committee (REC) approval is granted under reference 22/WA/0214. ICHTB is supported by the National Institute for Health Research (NIHR) Biomedical Research Centre (BRC) based at Imperial College Healthcare NHS Trust and Imperial College London. Further information on Imperial's BRC can be found here:

https://imperialbrc.nihr.ac.uk/about-us/acknowledgement/.

Ethics for tissue collection from University College London Hospital was granted on 12th March 2024 by the UCL/UCLH Biobank for Studying Health and Disease (Project reference NC36.24).

Ethics for tissue collection from Guy's and St Thomas' NHS Foundation Trust was granted on 26th February 2024 by the King's Health Partners Cancer Biobank (HTA Licence No: 12121, REC No: 23-EE-0005).

The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

8.2. TRIAL REGISTRATION

The study will be registered on on the ISRCTN trial registry in accordance with requirements of the International Committee of Medical Journal Editors (ICMJE) regulations.

8.3. CONSENT

Consent for use of human tissue for medical research is part of the standard consent process for patients undergoing surgery or biopsy at Imperial College Healthcare NHS Trust. Patients receive an information leaflet via the ICHTB prior to signing consent. Only those patients who have signed this subsection of the surgical consent form will be included.

8.4. CONFIDENTIALITY

The investigator must ensure that the subject's confidentiality is maintained. On the CRF or other documents submitted to the Sponsors, subjects will be identified by a subject ID number only. Documents that are not submitted to the Sponsor (e.g. signed informed consent form) should be kept in a strictly confidential file by the investigator.

The investigator shall permit direct access to subjects' records and source document for the purposes of monitoring, auditing, or inspection by the Sponsor, authorised representatives of the Sponsor, NHS, Regulatory Authorities and REC.

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

8.5. INDEMNITY

Imperial College Healthcare NHS Trust holds standard NHS Hospital Indemnity and insurance cover with NHS Resolution for NHS Trusts in England, which apply to this study.

8.6. SPONSOR

Imperial College Healthcare NHS Trust will act as the main Sponsor for this study.

8.7. FUNDING

A grant of £60,000.00 for this study was generously awarded by The Urology Foundation (TUF) through the Innovation and Research Award 2023, funded by the John Black Charitable Foundation.

9. STUDY MANAGEMENT

A trial management group (TMG) including the Chief Investigator, co-investigators and key collaborators, trial statistician and trial manager. The TMG will be responsible for day-to-day conduct of the trial and operational issues. Details of membership, responsibilities and frequency of meetings will be defined separately. A lay person will be included.

10. PUBLICATION POLICY

Information concerning the study, patent applications, processes, scientific data or other pertinent information is confidential and remains the property of the Sponsor. The investigator may use this information for the purposes of the study only. It is understood by the investigator that the Sponsor will use information developed in this clinical study and, therefore, may disclose it as required to other clinical investigators. In order to allow the use of the information derived from this clinical study, the investigator understands that he/she has an obligation to provide complete test results and all data developed during this study to the Sponsor. Verbal or written discussion of results prior to study completion and full reporting should only be undertaken with written consent from the Sponsor. Therefore, all information obtained as a result of the study will be regarded as CONFIDENTIAL, at least until appropriate analysis and review by the investigator(s) are completed. A Clinical Study Report summarising the study results will be prepared and submitted to the ICHTB within a year of the end of study.

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