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Research Letter

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Improving fluorescence confocal microscopy for margin assessment during robot-assisted radical prostatectomy: The LaserSAFE technique

Several studies have demonstrated that the NeuroSAFE technique is safe and accurate in guiding nerve-sparing decisions during robot-assisted radical prostatectomy (RARP) [1,2]. However, it remains uncertain whether this translates to better functional outcomes. The NeuroSAFE PROOF trial (https://www.neurosafeproof.com/) will generate Level 1 evidence to answer this question [3]. Recruitment is complete, and the study is in the follow-up phase with results expected in mid-2024. In identifying centres for this study, logistic concerns owing to the time- and resource-intensive nature of NeuroSAFE were found to be prohibitive for many NHS centres where RARP is performed in volume [4]. Therefore, we were interested in exploring potential alternatives to replace frozen section, such as ex vivo fluorescence confocal microscopy (FCM), as it can quickly generate digital scans of unfixed specimens, requiring minimal tissue preparation and consumables [5].

The first report to evaluate FCM as a tool for analysing positive surgical margins (PSMs) in RARP specimens described a Mohs-like technique to sample the posterolateral surface of the prostate [6]. Concordance with final pathology was almost perfect; however, the procedure required up to 25 min to perform [7]. Recently in this journal, Baas et al. [8] described the results of analysing en face shavings of the posterolateral surface of the prostate using the Histolog® Scanner (SamanTree Medical SA, Lausanne, Switzerland), a commercially available, portable, laser confocal microscope with a large objective lens that is capable of scanning an area of 48 × 36 mm in 50 s. They found a sensitivity of 86% and specificity of 96% compared to the final pathology analysis (FPA), and a substantial agreement with NeuroSAFE ($K = 0.80$). Nevertheless, both techniques still require the surgeon or pathologist to cut the area of interest to be analysed. This has limitations such as possible capsule retraction at the borders of the specimen leading to false positive margins, and the need for expertise in sample processing to avoid affecting final pathology results.

To address these limitations, we have developed the LaserSAFE technique to process intact RARP specimens using the Histolog Scanner to scan the entire posterolateral surface en face. This paper describes the LaserSAFE technique and reports its preliminary accuracy performance metrics.

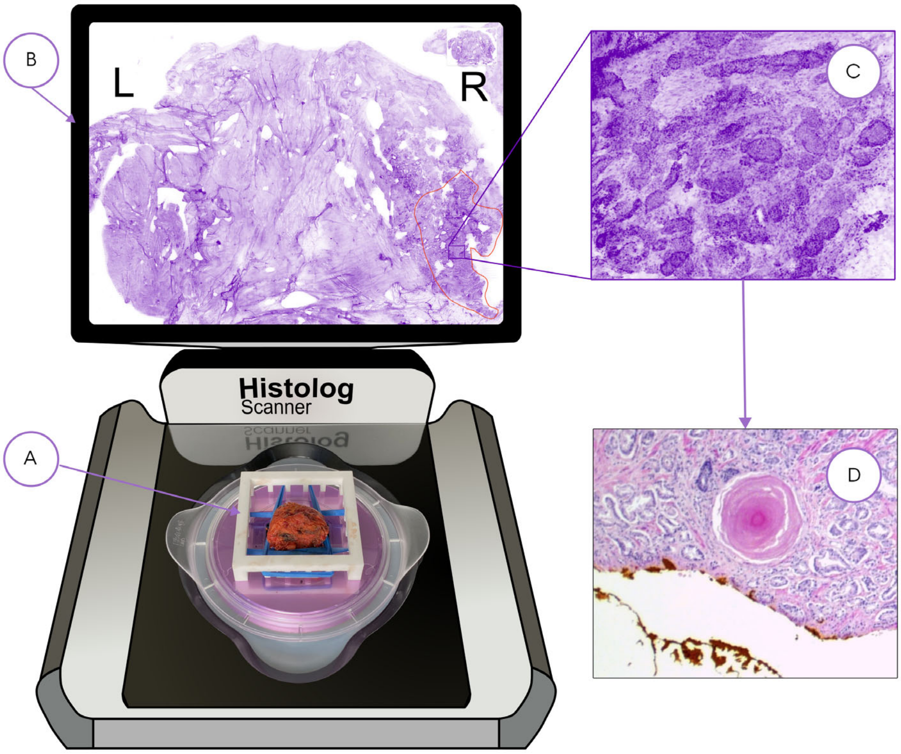
The RARP is performed using a transperitoneal anterior standard approach. Following completion of the surgery, the specimen is extracted though a supraumbilical incision. The prostate is cleaned using a damp gauze and completely submerged into Histolog Dip, a photoreactive solution, for 10 s. The specimen is rinsed with 0.9% NaCl solution before placing it on the microscope objective. A live view mode offered by the Histolog® Scanner can then confirm adequate positioning and stability of the specimen. By rotating the prostate three images are obtained: the posterior surface, left posterior and right posterior borders. Some overlap between them ensures that the whole prostatic surface adjacent to the neurovascular bundle is scanned. If required, gentle compression with a saline bag can increase the surface area in contact with the objective. As movement artefacts are common and can heavily disrupt image quality, we designed a three-dimensional (3D) printed nylon-based specimen holder with disposable rubber bands to optimise specimen positioning and minimise specimen movement, thus reducing the need for repeated scans (Fig. 1 and Fig. S1). The high-resolution digital images can then be stored in the microscope hard drive or transmitted to the pathologist for remote analysis.

To evaluate the accuracy of LaserSAFE, we processed RARP specimens from patients enrolled in the control arm of the NeuroSAFE PROOF (NCT03317990) study [3] who signed an optional informed consent form, from April 2022 to February 2023. All patients underwent RARP using the DaVinci X Surgical System® (Intuitive Surgical Inc., Sunnyvale, CA, USA). The results of the LaserSAFE analysis did not affect subsequent specimen processing or inform clinical care in accordance with the proof-of-concept nature of the study.

A training platform provided by the microscope manufacturer allowed a consultant uropathologist (A.H.) to gain experience with en face FCM image interpretation. Study images were then reviewed retrospectively, using a purpose-designed data collection sheet. Parameters recorded include image completeness (yes/no), staining quality (good/poor), nuclear clarity (good/poor), presence of cancer (yes/no), International Society of Urological Pathology Grade (1–5) and margin status (PSM/negative surgical margin). As the objective of this initial phase was to develop the methodology and understand the learning curve, FPA reports and images were available to the pathologist for comparison.

We collected 31 RARP specimens with a median prostate volume of 34.5 mL, with a tumour volume of 3.25 mL, 26

Fig. 1 Setup for LaserSAFE analysis. (**A**) Prostate specimen mounted in a 3D printed holder. (**B**) Digital image of the whole posterolateral surface displayed on a monitor for immediate visualisation and analysis. (**C**) High-power digital magnification of malignant glands. (**D**) Corresponding histology slide stained with haematoxylin and eosin confirms a PSM.



of which were graded as Gleason (3 + 4) and five as (4 + 3). It took one operator <10 min to image both posterolateral surfaces of each specimen. In total, 60 FCM images were acquired, 59 were graded as having good quality and contrast, and >90% of the specimen surface was analysable in 56. The FPA confirmed eight PSMs, whilst LaserSAFE correctly identified seven. In this false-negative case, no glands were identifiable on the FCM image and a Gleason (4 + 3) 3-mm PSM was detected on the surface of a 127 g specimen.

Interestingly, glands visible in FCM images prompted the request for additional FPA levels in two cases. One was confirmed as a malignant 1.6-mm PSM initially missed on FPA the other case was considered a false positive as only benign glands were present at the margin. This finding can be explained by the fact that FPA cuts sections every 5 mm potentially resulting in the missed detection of PSMs that are apparent when analysed en face.

Our results showed a sensitivity of 87.5% (95% CI 86.4–88.6%) and specificity of 98.1% (95% CI 97.6–98.5%) for diagnosis of a PSM. The Cohen's kappa coefficient for concordance between LaserSAFE and FPA was 0.86, indicating almost perfect agreement. It is important to note these preliminary results are limited to a single consultant unblinded pathologist.

To ensure the external validity of our findings and provide a more objective evaluation of the effectiveness of LaserSAFE, we are currently awaiting the results from blinded assessments conducted by three consultant uropathologists and two pathology trainees. Furthermore, a feasibility study to evaluate the implementation of LaserSAFE in clinical practice and compare the results to NeuroSAFE is in development.

We believe the advantages of this new technique, including its speed and the fact that it does not damage the specimen, will make it possible to perform real-time analysis of PSMs in centres that currently lack the necessary resources to perform NeuroSAFE and in doing so, allow safe nerve sparing for more patients undergoing RARP.

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Disclosure of Interests

Ricardo Almeida-Magana receives a salary from NIHR RFPB and Prostate Cancer UK (PCUK) grants; Greg Shaw receives consulting fees from Angle plc and has received travel expenses from Janssen and Johnson and Johnson (not related to the present manuscript); Matthew Au, Tarek Al-Hammouri, Alex Freeman, Aiman Haider and Kate Dinneen report no conflict of interest. Samantree Medical SA (Lausanne, Switzerland) provided the Histolog® Scanner, training, and consumables but was not involved in the study design, execution, results or writing of the manuscript.

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Abbreviations: 3D, three-dimensional; FCM, fluorescence confocal microscopy; FPA, final pathology analysis; PSM, positive surgical margin; RARP, robot-assisted radical prostatectomy.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 LaserSAFE 3D specimen holder description and 3D printer files.