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Line-scan Confocal Endomicroscopy for Rapid Digital Histology of Early Breast Cancer

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Abstract: We present a high-speed line-scan confocal laser endomicroscope, which enables digital histopathology of freshly excised un-fixed breast tissue specimens in real-time.
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1. Introduction

There are over 55,000 breast cancers diagnosed per year in the United Kingdom alone [1]. Breast cancer and pre-cancer are thought to originate from cells lining the milk ducts/lobules, however, standard radiologic examinations of breast duct lesions can only provide indirect information. Fibre based ductoscopy is an emerging technique that allows real-time visual access of the breast ductal system to help in-vivo diagnostics, avoid exploratory breast tissue resections and facilitate minimally invasive interventions. [2]. However, large rigid size of the fibre probe, low resolution and navigation difficulties limit their applicability in actual clinical practice. The CRUK-EPSRC "MAMMOBOT" project has recently been presented to address these limitations by developing a millimeter scale steering soft robot integrated with flexible endomicroscope for real-time virtual histology of the ductal system [3].

In this paper, we present the design of an in-house developed miniaturised flexible fibre-bundle probe integrated with high-speed line-scan confocal laser endomicrosocpe (LS-CLE) system that can pass through the working channel of the MAMMOBOT platform. We demonstrate the utility of LS-CLE to provide cellular resolution images of breast ducts, progressing from normal ducts to ductal carcinoma in-situ (DCIS) and invasive ductal carcinoma (IDC) in real-time by comparison to conventional histopathology which is the gold standard.

2. Experimental set-up

The LS-CLE system is compact and portable and can be easily used in the operating theatre and positioned close to the patient [4, 5]. A cylindrical lens (f = 50 mm) is used to create a focused line from a 50 mW, 488 nm laser (Vortran Stradus, 488). A galvo-mirror (Thorlabs, GVS001) sweeps the line across the proximal end of the fibre-bundle in a direction perpendicular to the line. A high resolution pre-clinical fibre-bundle imaging probe ColoFlex UHD from Cellvizio (Mauna Kea Technologies, France) with 2.5 mm outer diameter, FOV of 240 μm and Nyquist sampling resolution of 2.2 μm was integrated with the LS-CLE system and used for imaging.The bundle relays the line to the tissue via the distal micro- objective and returns the collected fluorescence from all the points along the line. The fluorescence is imaged onto a monochrome rolling-shutter CMOS camera (Flea 3, FL3-U3-13S2MCS). The rolling shutter of the CMOS camera operates as a virtual detector slit that rejects most of the out-of-focus light leading to optical sectioning at frame rates of 120 Hz.

For this study, freshly excised breast tissues were acquired from consented patients who underwent breast surgery between November 2020 and October, 2021, under an Imperial College tissue bank license (Project R12047). Several small tissue cut-outs, approximately 5 mm in size, were taken from normal and diseased sections (as subsequently confirmed by histology). The cut-outs were topically stained with 0.01% acriflavine hydrochloride solution for 1 minute followed by a gentle wash with PBS saline to remove excess stain. The tip of the endomicroscope probe was gently applied to the tissue surface and scanning videos and images were acquired using a custom labview GUI. The images were processed to remove the fibre pixelation artefacts and a video mosaicking algorithm was implemented to reconstruct large-area mosaics of the scanned margin surface in real-time. At the end of the imaging procedure, excess dye was gently wiped off the surface of the tissue, and the specimen was returned to histology for routine H&E analysis. The reconstructed mosaics (on an average 3 per scan) from each margin were later correlated with histology slides acquired from the same region and visual comparisons were made to evaluate tissue morphology and discernible features.

3. Results

For this preliminary study, tissue samples from 15 patients receiving breast cancer surgery have been evaluated as 5 normal glandular tissue, 5 fibroadenoma (FAD), and 5 ductal carcinoma in situ (DCIS) with concurrent progressive invasive ductal carcinoma (IDC). Histopathological analysis of excised tissue was performed during oncological resection. For each tissue 3 videos each of about 60 seconds duration were acquired from different scanned sites on the tissue surface. Representative LS-CLE images of normal lobule, FAD and IDC and their corresponding histopathology are presented in Figure-1. Normal tissue constituted a honeycomb arrangement of adipose cells with sheets of parallel collagen fibres constituting stroma and blood vessels. Ducts were visualised with clearly stained and organised nuclei along with breast lobules with clusters of acini. Clear fluorescent borders between the cells containing interspersed nuclei are seen. FAD was classified by the appearance of stromal and epithelial hyperplasia. Stromal and lobular proliferation is also seen, compared to normal tissue. A slit like lobular appearance is featured, where branching of the ducts into the stroma are observed. DCIS was reported in all malignant breast tissue patients at the peripheries of IDC. In DCIS, we observed disorganised appearances of the cells lining the ductal epithelium, strongly fluorescent non-uniform cell nucleus aggregates, but importantly, there was no stromal invasion and a clear border is visualised. In IDC, its distinction from DCIS, was the poor fluorescence of cell nuclei, disorganisation of ductal architecture and cell nucleus aggregate infiltration within poorly organised collagen sheets (stroma).

4. Summary

The LS-CLE system allows for non-invasive real-time 'virtual' histology imaging of whole freshly excised breast tissue specimens without having to section and fix them. This work demonstrates the effectiveness of LS-CLE as a flexible micro-ductoscope in identifying discernible features corresponding to normal ducts, DCIS and distinguishing them from IDC at sub-cellular scale. Such a miniaturized tool could be very useful as the next generation mammary ductoscope for full duct outline mapping by detecting cancer or pre-cancerous lesions in the milk ducts in real-time, thus minimising the risk of re-operation, morbidity and costs associated with surgical interventions.

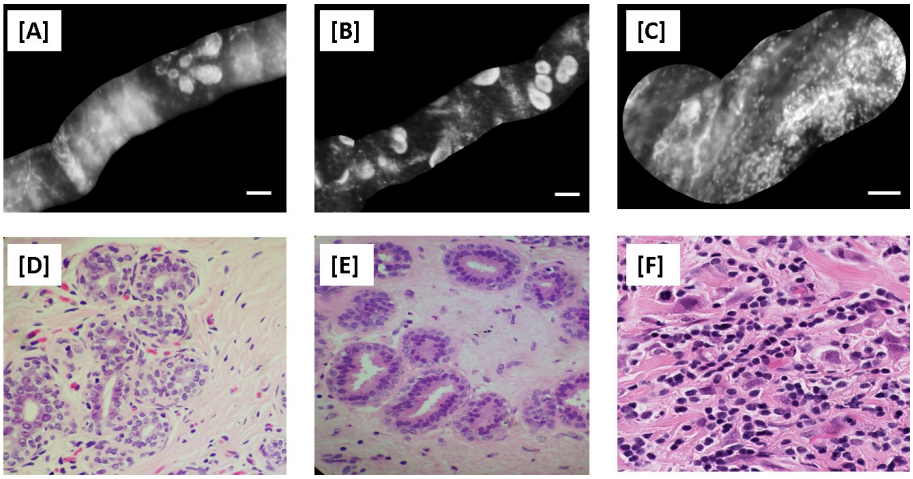

Fig. 1. Representative LS-CLE images of [A] Normal lobule, [B] fibroadenoma and [C] IDC and their corresponding H&E stained histopathology images [D-F] respectively. Scale bar is 100μm.

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