International Confocal Group

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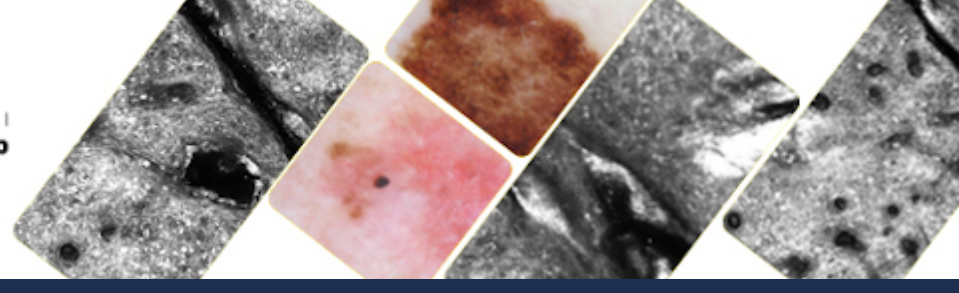

Ex vivo confocal laser scanning microscopy of 83 facial basal cell carcinomas: A single-centre prospective one year study of real-world Mohs experience

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1. Introduction

- Ex vivo confocal laser scanning microscopy (CLSM) is a novel integrative non-invasive imaging tool that can be used within Mohs micrographic surgery (MMS), for tumour margin assessment.
- Studies have reported up to 88% sensitivity and 99% specificity (Bennassar A et al, 2014) with CLSM in diagnosing basal cell carcinomas (BCCs), compared to conventional histology.
- Its real-world utility in the literature has triggered variable opinion among practising Mohs surgeons.
- The fourth-generation Vivascope-2500, currently integrated within our Mohs unit, allows fresh tissue scanning with minimal preparation with acetic acid and acridine orange, creating digitally coloured mosaic images comparable to gold standard frozen sections stained with H&E, within minutes.
- This has potential for enhancing the patient experience, allowing multiple stages and repair in a single session, and improve overall efficiency.

2. Methods

- We aimed to characterise our Mohs experience over the past 12 months.
- We prospectively analysed 83 consecutive MMS 1st stage specimens over 1 year, involving head and neck BCCs, using the Vivascope-2500 on one Mohs list.
- 83 head and neck BCCs were processed with the Vivascope-2500 and then with frozen sectioning.
- CLSM is currently used by one surgeon and biomedical scientists (BMS) trained in interpretation of digital images.
- We characterised the real-world sensitivity and specificity of CLSM in our cohort.

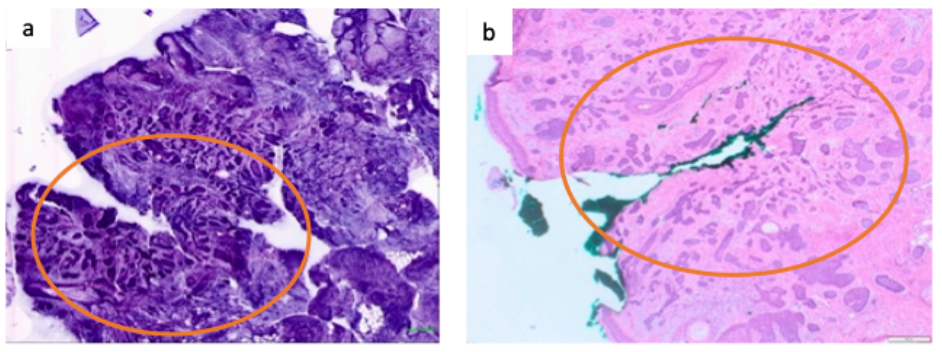

Fig 1. Example of concordance in CLSM and frozen sectioning *Comparable CLSM (a) and frozen section H&E (b) highlighting BCC in red showing* concordance in images.

3. Results

- 83 facial BCCs (54 nodular, 12 infiltrative, 12 nodular and infiltrative, 3 nodular and micronodular, 1 micronodular and infiltrative and 1 micronodular)
- Concordance in tumour mapping for 77 cases between CLSM and frozen sections *(Fig 1)*. Basal budding appreciated well in both modalities
- 6 cases of non-correlation: suspected tumour on CLSM differed from true tissue margins on frozen sections. 3 cases due to false positive inflammation *(Fig 2)*. Other cases due to misinterpretation of follicular structures *(Fig 3)* and artefact/ floaters.
- Our cohort had 78.3% sensitivity and 91.7% specificity for CLSM compared to frozen sections and standard histology.

Unique finding: No tumour seen on CLSM but the initial cut via microtome for frozen section of a thin conchal bowl specimen, showed a nodular BCC → *We suggest the microtome initially cut into tumour and highlight a diagnostic conundrum.*

Has CLSM imaging minimised tissue loss with narrowly clear margins? Or has a BCC been potentially missed i.e. false negative vs. true negative *(Fig 4)*.

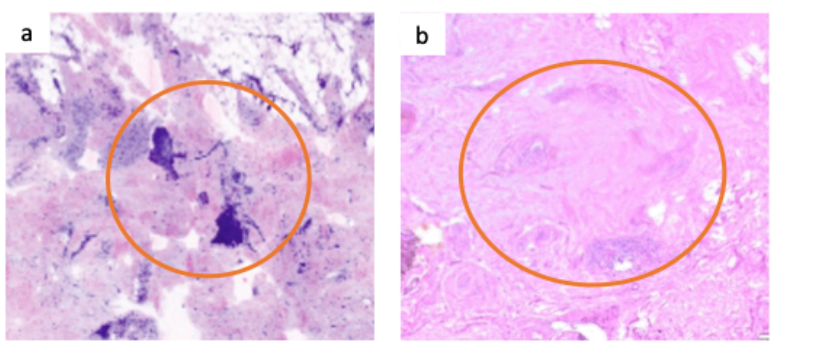

Fig 2. False positive: inflammation *Focus of inflammation in deep CLSM image (a) was clear on frozen section (b).*

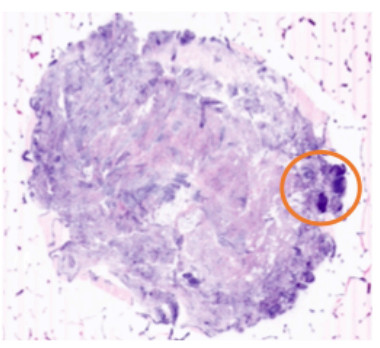

Fig 3. Follicular structures misread as BCC *Follicular structure causing false positive CLSM.*

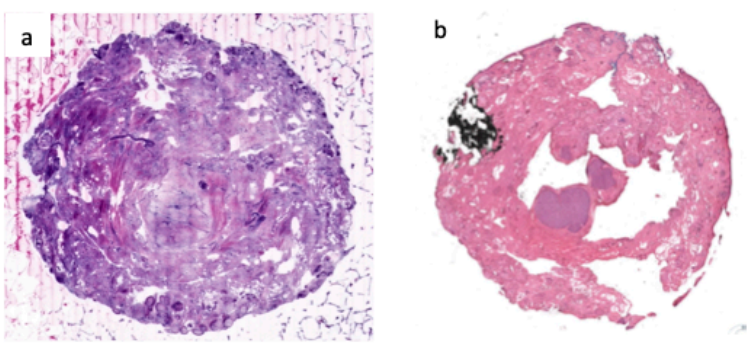

Fig 4. True negative or false negative? *No tumor seen in CLSM (a) however processing of thin conchal bowl specimen shows nodular BCC in frozen section (b)*

4. Conclusion

- Vivascope-2500 CLSM shows promise in MMS. From our experience. Training in CLSM image interpretation is important for Mohs surgeons and BMS.
- Suboptimal image quality and issues with tissue flattening may lead to false positive results and reduce the reliability of CLSM images.
- Identifying skin structures may be challenging and cause false positive tissue margins e.g. follicular structures misinterpreted as tumour islands. Inflammatory infiltrate can mimic BCC-like features.
- Our experience highlights important caveats with CLSM, when utilised in MMS. Regular pathology reporting, improved flattening techniques and appropriate sample selection, can potentially improve image interpretation with CLSM and minimise future errors.
- Enhanced AI algorithms and integrating digital real-time pathologist review, could allow for rapid diagnostic pickup.