Confocal Cross-Polarized Imaging of Skin Cancers to Potentially Guide Mohs Micrographic Surgery

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Precise microsurgical excision of epithelial cancers in high-risk anatomical sites is guided by the frozen histology examination of each excision during the surgery. A well-known example is Mohs **(AQ:1)** micrographic surgery for the excision of basal- and squamous-cell cancers (BCCs, SCCs) that occur most frequently on older people's faces.[1] Since frozen histology preparation requires 20-45 minutes per excision, a Mohs **(AQ:1)** procedure typically lasts several hours. Confocal reflectance imaging offers a method to avoid frozen histology. Using acetowhitening and crossed polarization to enhance the contrast of the cancers, confocal reflectance imaging enables the rapid noninvasive examination of BCCs and SCCs in surgical excisions.

In confocal brightfield reflectance images of human epidermis, the cytoplasm appears bright and the nuclei dark in basal and squamous cells.[2] The nuclei appear dark due to weak light back-scattering from the 30-100 nm thin chromatin filaments **(AQ:2)**. The underlying dermis consists of collagen bundles and also appears bright with dark spaces in-between. Thus, when cancerous epidermal cells invade the dermis (as in BCCs and SCCs), confocal detection of the cancer is not possible because the cells and nuclei lack contrast relative to the surrounding normal dermis.

To enhance the contrast of the nuclei within the BCCs and SCCs, we wash the surgical skin excisions with 5% acetic acid. Acetic acid makes the nuclei appear bright in confocal brightfield images.[3-4] The acetic acid causes chromatin compaction into 1-5 μm-thick **(AQ:3)** strands with an increase in refractive index. Subsequently, light back-scatter increases and makes the nuclei appear bright and easily detectable.[5] We observe that the brightness of the acetic acid-washed nuclei does not vary much when illuminated with linearly polarized light and imaged through a rotating analyzer. On the contrary, the brightness of the collagen varies from maximum to minimum (dark). The significant depolarization of the compacted chromatin's back-scattered light most likely occurs due to the multiple scatterings within the chromatin's structure **(AQ:4)**. Whereas, the _____ from the collagen preserves the illumination polarization due to single-back scatter **(AQ:5)**. Between crossed polarizers, we see bright nuclei in the BCCs and SCCs in strong contrast against a dark background of surrounding normal dermis (see Fig. 1).

Cancer nests are first detected at low-resolution (0.3 NA, 30 μm-thin sections) in 1-2 mm-large fields-of-view (Figure 1a, b). Initial detection is followed by careful high-resolution (0.9 NA, 3 μm-thin sections) **(AQ:3)** examination of nuclear morphology in 0.25-0.50 mm-small fields-of-view (Fig. 1c). This process is similar to the histology exam procedure. To examine the typically 2-20 mm-large excisions, individual images are tiled in software to create a mosaic (Fig. 1d). We create mosaics of 20 x 20 mm-excisions in five minutes. Thus, the Mohs **(AQ:1)** surgery may proceed faster, saving both the patient and surgeon several hours per day. Rapid confocal cross-polarized microscopic-examination of excisions may guide the microsurgery of any human epithelial tissue and improve the management of surgical pathology.

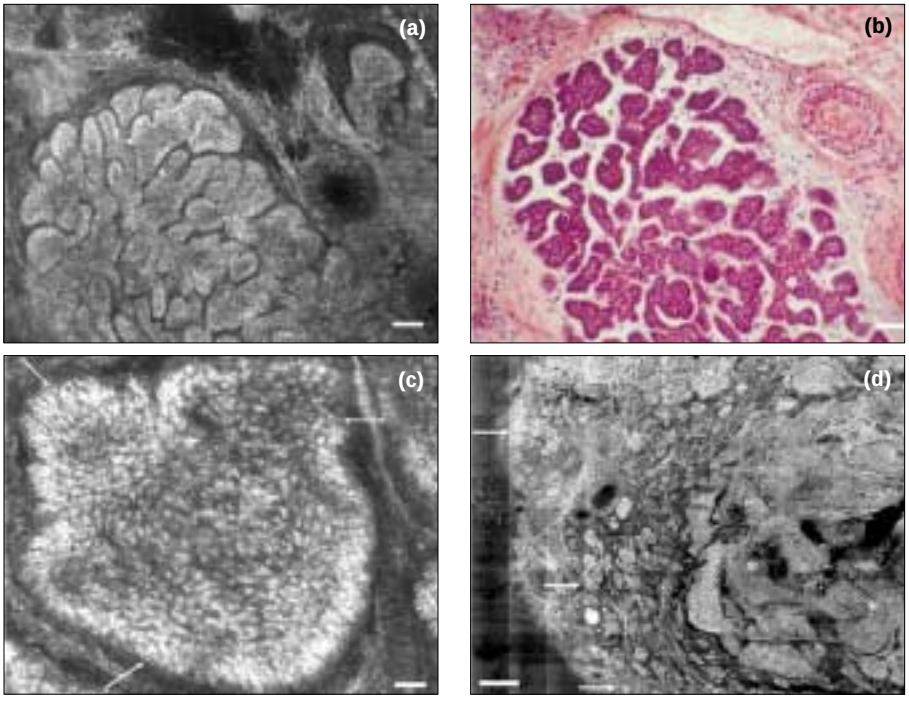

Figure 1. Confocal cross-polarized images of acetic acid-washed surgical skin excisions of a BCC show bright nests (* in a) that correlate well to the purple-stained nests (* in b) in the corresponding histology; (c) abnormal nuclei appearing as bright oval shapes within (*) and along the periphery (arrows) of a nest, surrounded by dark collagen; and (d) confocal mosaic in which the location, shape, size and morphology of bright nests (* and arrows) are clearly detected in a large field-of-view. Scale bar = 100 μm (a, b), 25 μm (c), 1 mm (d).

References

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Author queries

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2. Edit OK?
3. Is "?" the right measurement? Your symbol was not legible in our original Word document.
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5. Please insert a specific term before "from the collagen"