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ISBN
9781957171210

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Publication Date
2023

DOI
10.1364/ntm.2023.nw4c.3

Intravital multiphoton microscopy of human skin with label-free molecular contrast

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Abstract. This presentation will highlight the most recent results of exploring the feasibility of a custom designed clinical multiphoton imaging platform to identify and distinguish immune cell populations in human skin, based on label-free molecular contrast.

The study of mammalian immune cells and their interactions with tissue in situ is critical for understanding autoimmune disease initiation and designing better therapeutic strategies. Intravital multiphoton microscopy (MPM), fluorescent reporter mouse models and *in vivo* cell and tissue labeling techniques have made possible the investigation of immune cells interactions at a cellular/subcellular level in their native environment. These approaches remain limited to animal models as translation to humans requires label-free imaging based on endogenous signals. Several key challenges hinder the advance of MPM as a tool for intravital label-free imaging in humans: 1) high scanning rates are required to capture fast dynamics and minimize motion artifacts; 2) endogenous signals are weak and difficult to detect; 3) correlative methods are not available to reliably identify cell types.

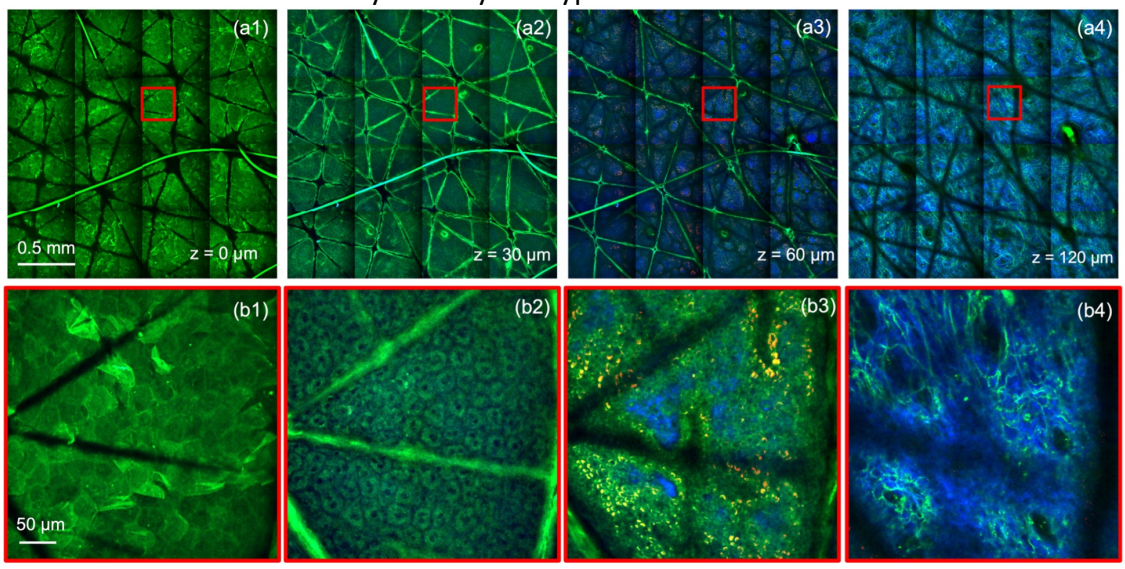

Figure 1. *In vivo* depth-resolved FLAME images acquired on the face of a volunteer. The time-resolved macroscopic (a1-a4) and close-up images (b1-b4) show the keratinized stratum corneum (a1, b1), epidermal keratinocytes (a2-a3, b2-b3) as non-pigmented (TPEF, green) and pigmented (TPEF, red) cells, collagen (SHG, blue) and elastin (TPEF, green) fibers (a4, b4). Each 16 Mpx macroscopic image (a1-a4) was acquired in ~ 40 s. Images are color-coded by short-lifetime (<1.6ps) TPEF (red) and long-lifetime (>1.6ps) TPEF (green). FLAME features a stable patient-imaging head interface attached to a miniature translational stage that enable image acquisition over large areas (few mm^2) and long periods of time without significant lateral or axial drifting caused by motion. In current pilot studies, the team has demonstrated stable time-laps images over mm^2 area and over time periods of 20 minutes.

Our group has recently developed a fast large area multiphoton exoscope (FLAME) for *in vivo* imaging of human skin to rapidly map out macroscopic tissue areas (cm-scale) with microscopic resolution and enhanced chemical contrast. FLAME has the ability to generate *in vivo* 3D images of human skin (Figure 1) over macroscopic areas (up to 8x8 mm^2) with microscopic resolution (0.5-1 μm) at fast acquisition rates (tens of seconds).[1] FLAME features label-free specificity for melanin and NADH based on their spectral and rapid time-resolved endogenous fluorescence detection. Selective detection of melanin allows for imaging of pigment-rich cells such as melanophages with high specificity, while time-resolved NADH fluorescence detection reports on the protein binding activity of these molecules within the immune cells and on their metabolic heterogeneity.

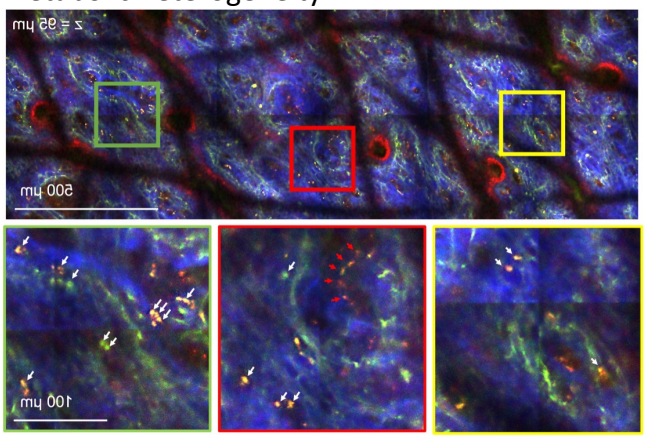

Figure 2. *In vivo* FLAME imaging of dermal cell populations in human skin. Representative mm-scale MPM image acquired *in vivo* from the dermis of a volunteer's forearm. The image shows collagen (blue) and elastin (green) fibers surrounding pigmented keratinocytes (bright red) around hair follicles. The dark lines represent skin folds. The close-up images show red blood cells along a blood vessel (red arrows), and several populations of immune cells (white arrows) that can be distinguished based on their different fluorescence lifetime.

We tested the feasibility of FLAME to detect immune cells in human skin by *in vivo* imaging of the normal and injured human skin in 10 volunteers. The representative images (Figures 2 and 3) show that FLAME has the ability to detect dermal cells, including resident immune cells based on time-resolved single photon counting detection of the NADH fluorescence. The cells are identified based on fresh skin tissue labeling antibodies against cell surface markers on macrophages, tissue-resident memory CD8+ T cells and other immune cells of interest.

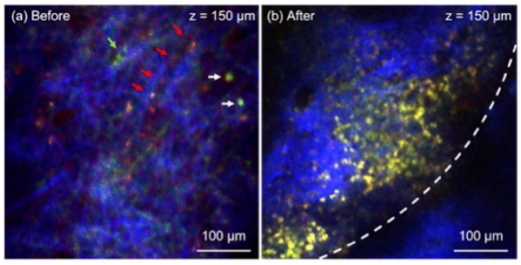

Figure 3. *In vivo* FLAME imaging captures immune cells recruited at an injury area in human skin. (a) Image of the dermis before the injury showing different dermal cell populations such as red blood cells along a blood vessel (red arrows), fibroblasts (green arrow) and immune cells (white arrows) surrounded by collagen fibers (blue). (b) Image of the dermis acquired from the same area after an injury showing immune cells infiltrates (yellow). The dashed line delineates a partial area of the injury generated by removal of the epidermis following suction blistering.

If successfully validated in a larger clinical study, this approach would be a critical first step in making it possible to characterize cellular-level immune responses in human skin at the bedside, with broad applications ranging from detecting early immune reactions to developing improved cancer treatments.

References

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