INTTEGRAGTED OPTICAL MICROSCOPY FOR IMAGING MEMBRANE FENESTRATION OF LIVER CELL

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Liver sinusoidal scavenger endothelial cells (LSECs) are engaged in blood clearance activity. LSECs are uniquely characterized structurally with fenestration (nano-holes of ~50-200 nm diameter) grouped in sieve plates, which allows small soluble material, but not larger particles to pass through the sinusoidal wall. As fenestrations and sieve plates are present only on the cell membrane, TIRF (total internal reflection microscopy) configuration is preferred over epi-fluorescence. Here, we use the evanescent field on top of a waveguide surface to image liver cell membranes. The primary LSEC are immobilized directly on top of waveguide, fixed within 3 hours after the isolation from the rat and stained with Cell Mask Orange (excitation with 532 nm). A thin layer of fibronectin is coated onto the waveguide to help immobilization of LSECs on the waveguide surface. Fig.1a shows a bright field image of LSECs and Fig1.b) shows cell imaged using the evanescent field of the waveguide. The cell membrane network and sieve-plates (dark holes in the membrane) are imaged, while Fig. 1.c shows an epi-fluorescence image of the same cell. In epi-fluorescence excitation the cell nucleus becomes emphasized as a consequence of the top-illumination, and as illumination power increases the signals from the nucleus saturate the image before membrane fenestrations can be imaged. In waveguide excitation, the evanescent field is dominant only near the surface (~100-150 nm) providing default optical sectioning and illuminating fluorophores that are in close proximity to the surface and thus benefiting higher signal-to-noise ratio. Optical waveguides provide a uniform excitation over large sample area and the method represents an integrated, on-chip approach for fluorescent imaging, with a possible extension towards super-resolution imaging methods.

Fig.1 (a) Bright field image of LSECs on top of waveguide. (b) Imaging LSEC membrane using evanescent field excitation from the waveguide, (c) standard epi-fluorescence image of same cell.