In vivo longitudinal deep tissue depth-wise imaging by side-view confocal endomicroscopy

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Micro-optics have been broadly applied to visualize the deep tissue of live animals as they have enabled us to characterize morphological and functional features in biological system. Still there are unmet needs to monitor long-term cellular activities or wide-range morphogenesis with high spatiotemporal resolution. To perform a longitudinal and repetitive depth-wise intravital imaging of deep tissue, herein, we established a small diameter needle-shaped side-view confocal endomicroscope that can be deeply inserted into live tissue in a minimally-invasive manner *in vivo* (Fig 1.(a)). By inserting the side-view endomicroscope consisting of triplet GRIN lens into the breast tumor over three weeks, we successfully visualized the cellular-level tumor microenvironment deeply buried in the solid tumor including dynamic interaction between proliferating population and their surrounding vasculature (Fig 1.(b)). Furthermore, the endoscopic probe was implemented into deep brain tissue, thereupon structural features (e.g. neuroanatomical pathways connected to the hypothalamus) were clearly demonstrated in a depth-wise manner (Fig 1.(c-g)), all of which were subsequently validated with 3D-reconstruction analysis (Fig 1.(h)). Collectively, the customized side-view confocal endomicroscopy would be a versatile platform to investigate cellular dynamics and connectivity underlying deep tissue *in vivo*.

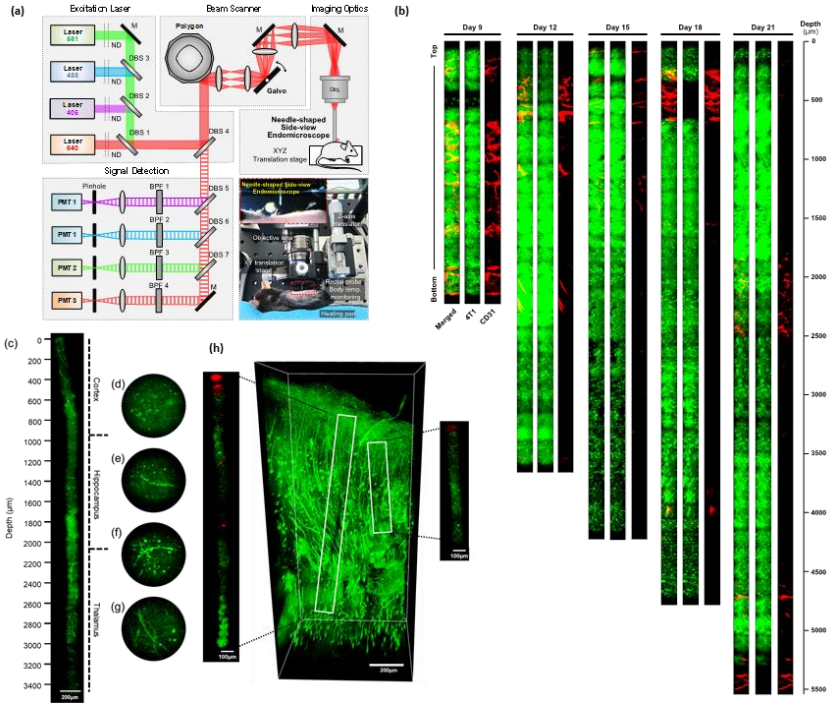

Figure 1. (a) Schematic of the fabricated needle-shaped side-view endomicroscope packaged into a 22G hypodermal needle tip. (b) Depth-wise mosaic images of the growing tumor obtained from a single mouse from day 9 to 21 with 3-day intervals after the inoculation of the cancer cells with continuous insertion of the needle-shaped side-view endomicroscope (c-g) Depth-wise reconstructed images of deep brain tissue of Thy1-YFP-H transgenic mouse and (h) comparative analysis with 3D structure of CLARITY-processed specimen.

Reference

[1] Kim et al, "In vivo wide-area cellular imaging by side-view endomicroscopy", *Nature Methods*, 7(4):303-305 (2010)

[2] Ahn and Kong et al., "In vivo longitudinal depth-wise visualization of tumorigenesis by needle-shaped side-view confocal endomicroscopy", *Biomedical Optics Express*, 10(6): 2719-2729 (2019)