A handheld optical-sectioning microscope for cancer detection and surgical guidance

C. Yin[1], A.K. Glaser[1], Y. Chen[1], L. Wei[1], S. Abeytunge[2], G. Peterson[2], C. Glazowski[2], N. Sanai[4], M.J. Mandella[3], M. Rajadhyaksha[2] & J.T.C. Liu[1]

1 University of Washington, Department of Mechanical Engineering, Seattle, WA 98195, USA; 2 Memorial Sloan-Kettering Cancer Center, Dermatology Services, Department of Medicine, New York, NY 10010, USA; 3 Stanford University School of Medicine, Department of Pediatrics, Stanford, CA 94305, USA; 4 Barrow Neurological Institute, St. Joseph's Hospital and Medical Center, Phoenix, AZ 85013, USA

❶ Clinical needs

Early detection of oral cancers

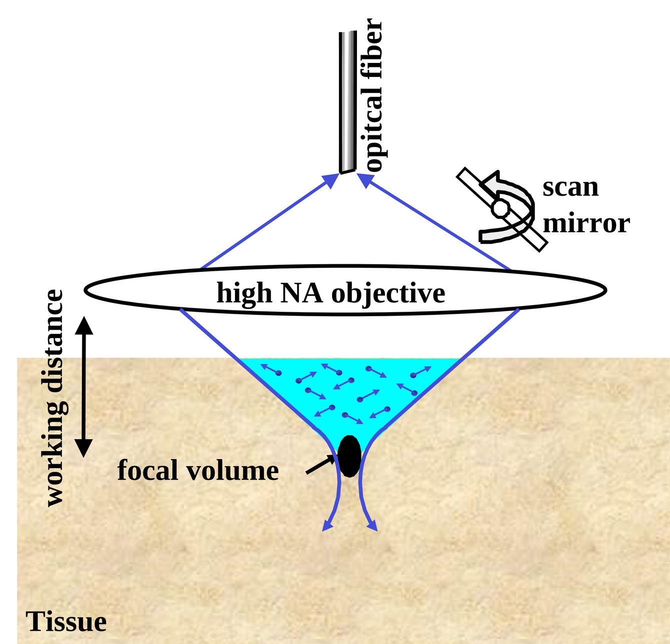
- Existing screening tools have high sensitivity but poor specificity (many false positives).
- Gold standard diagnosis requires physical biopsy of suspicious lesions (many of which are benign), which is slow, expensive, and invasive.
- **Real-time non-invasive "optical biopsy" or "point-of-care pathology" would help to examine suspicious lesions for early detection.**

High-resolution image-guided resection of brain tumors

- Current imaging modalities (MRI, CT, and wide-field fluorescence) often lack the resolution, sensitivity, and contrast to accurately delineate tumor margins, especially for diffuse gliomas.
- 5-ALA-induced PpIX fluorescence is often undetectable in low grade gliomas via wide-field fluorescence imaging (see panel ❻).
- **High-resolution fluorescence microscopy has the resolution and sensitivity to quantify sparse PpIX expression in low-grade gliomas and guide tumor removal.**

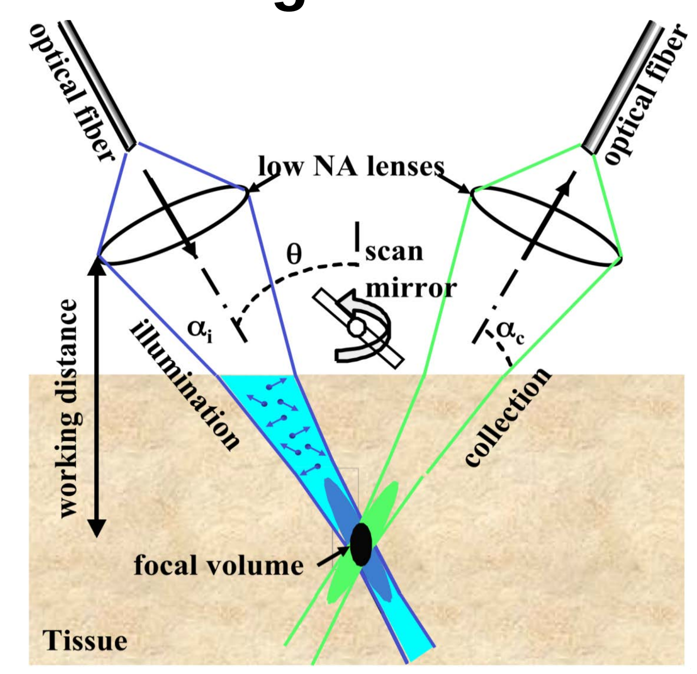
❷ Handheld line-scanned (LS) dual-axis confocal (DAC) microscope

The dual-axis advantage



Single-axis confocal

- High-NA focusing = short working distance
- **More background noise from scattered light**

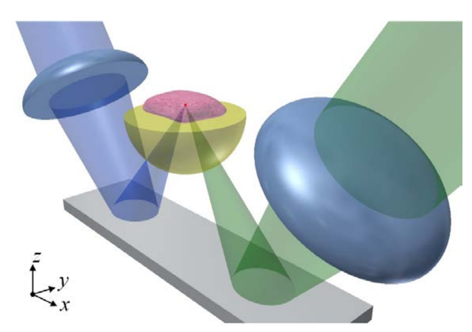


Dual-axis confocal

- Low-NA focusing = long working distance
- **Less noise from scattered light**

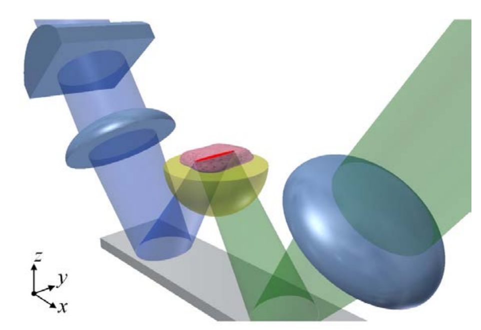
The line-scan advantage

Point-scanned DAC

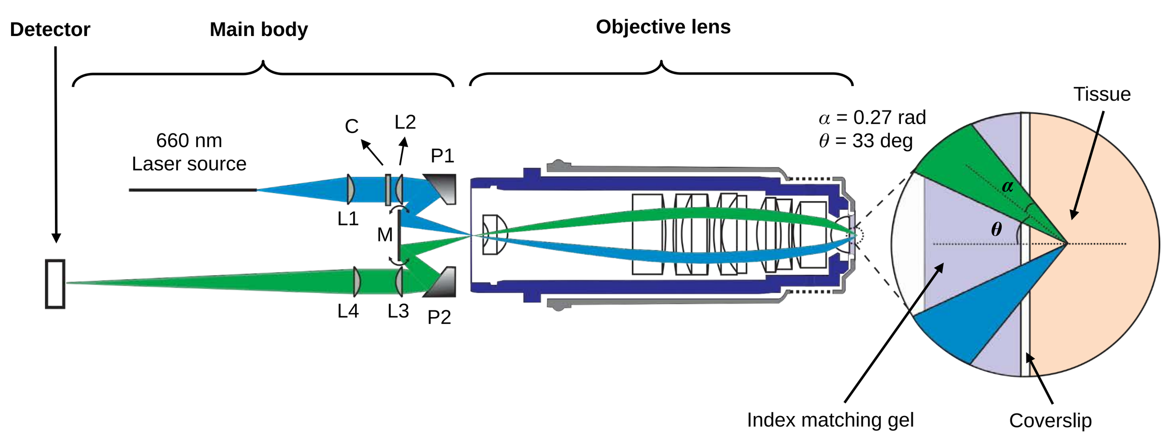


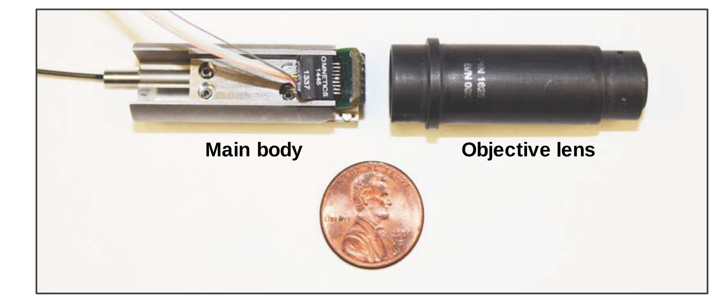
- High contrast and imaging depth
- Slow pixel-by-pixel scanning is required to construct an image, which leads to motion artifacts.

Line-scanned DAC

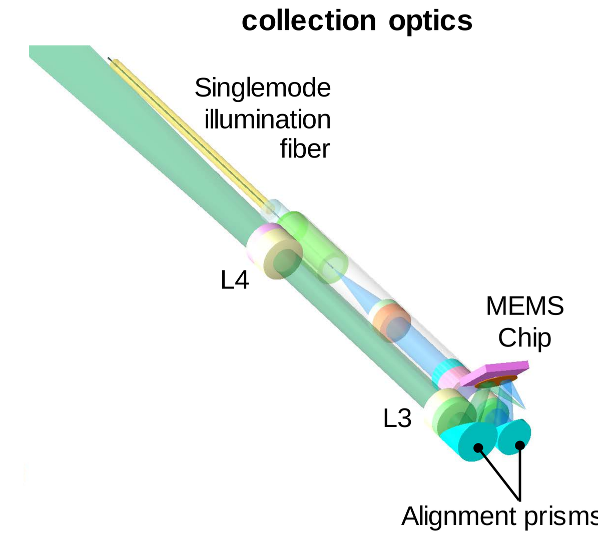


- High contrast at shallow depths only
- **Fast line-by-line scanning to minimize motion artifacts during handheld use.**

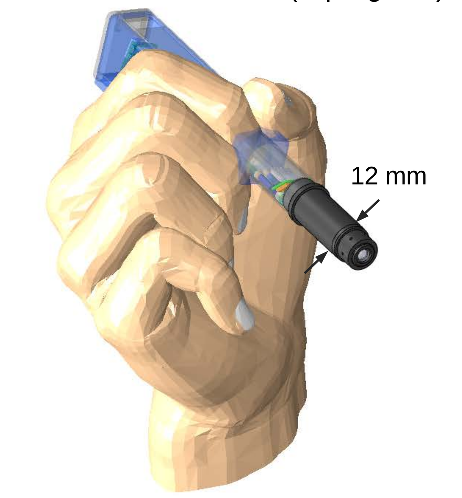




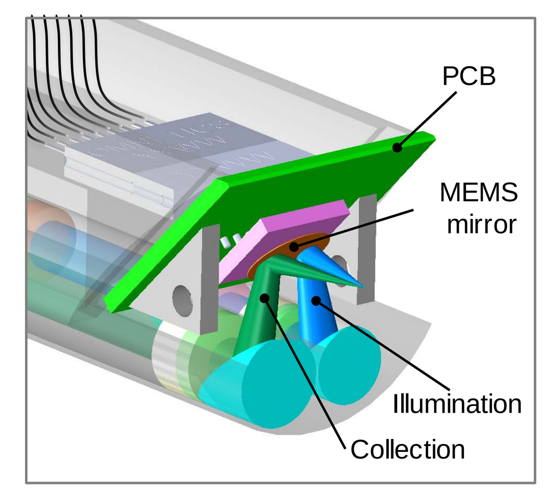
Illumination and collection optics

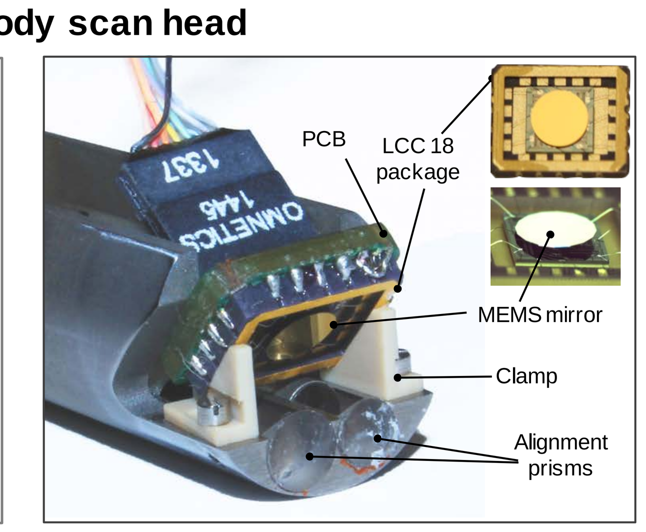


Handheld device (in progress)

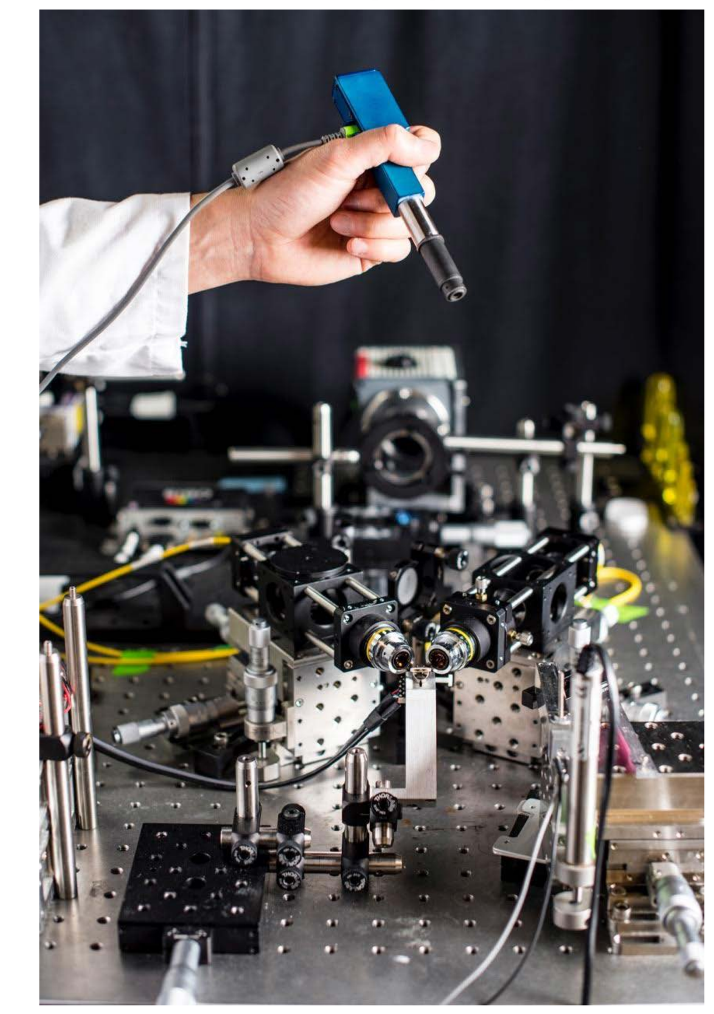


Main body scan head

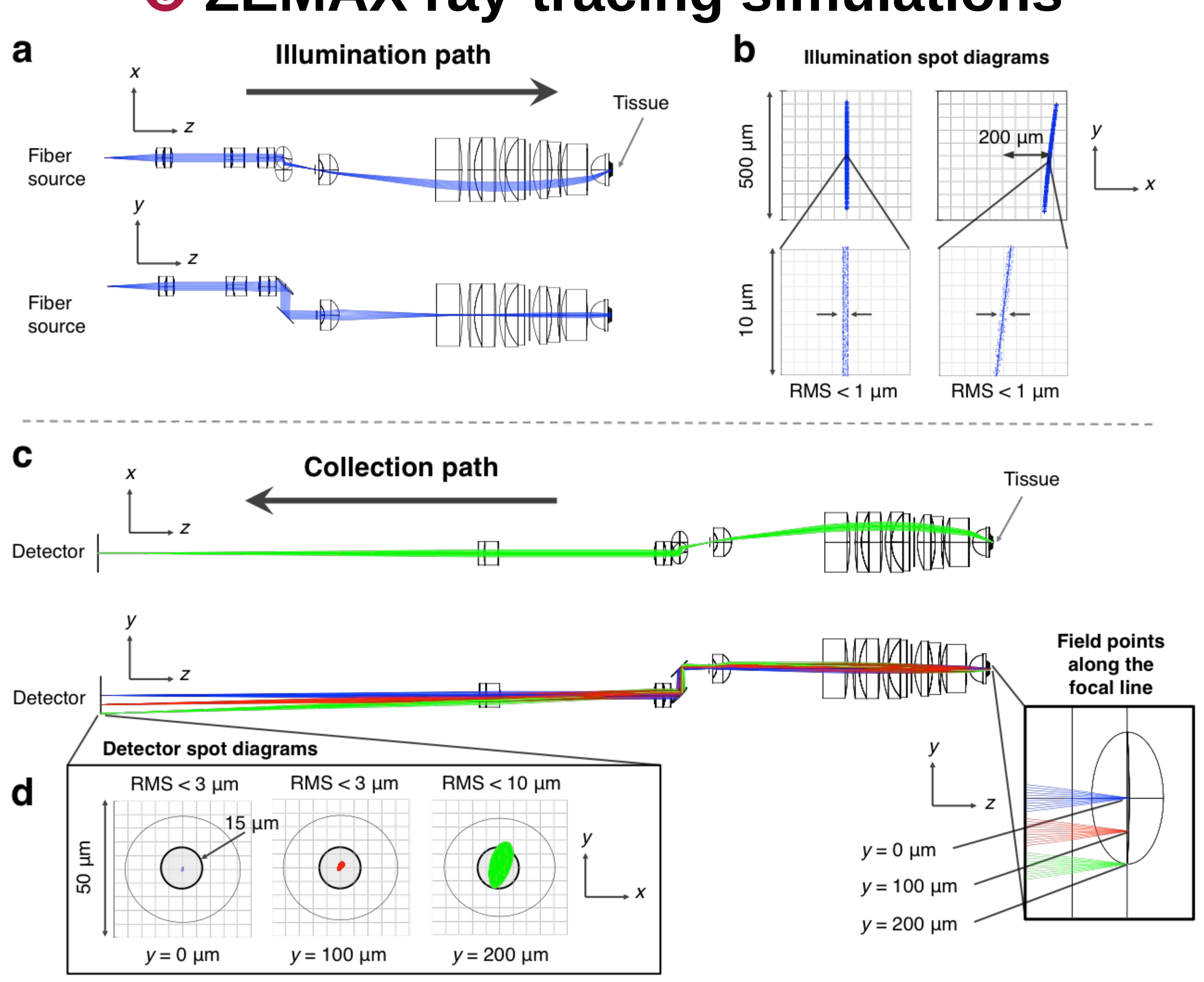




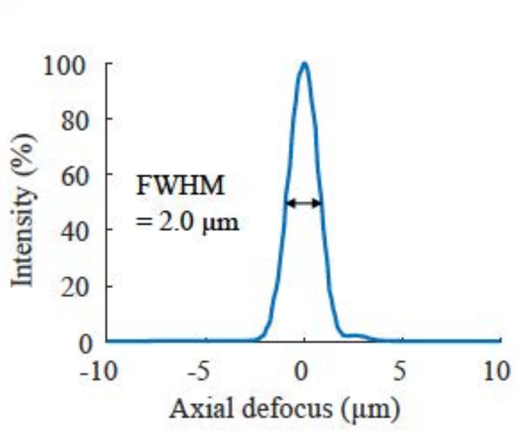
Photograph of miniature device held above a large tabletop DAC microscope prototype

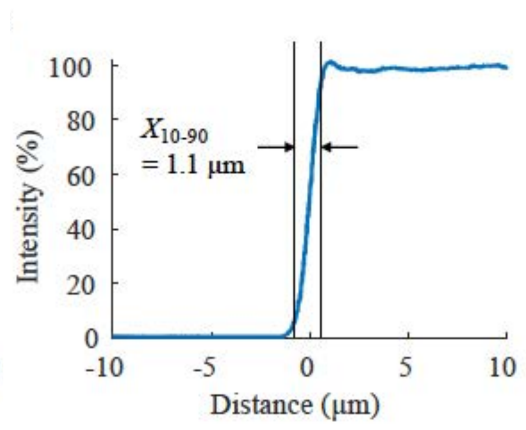


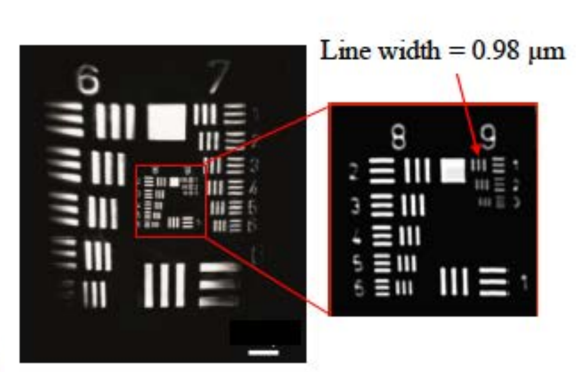
❸ ZEMAX ray-tracing simulations



❹ Axial and Lateral resolution

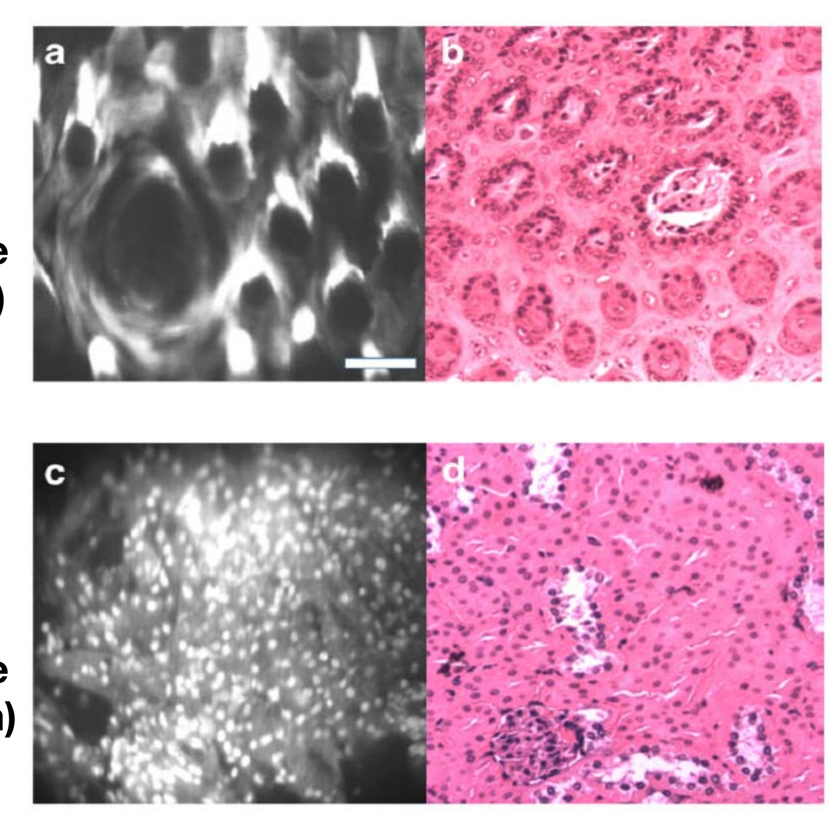




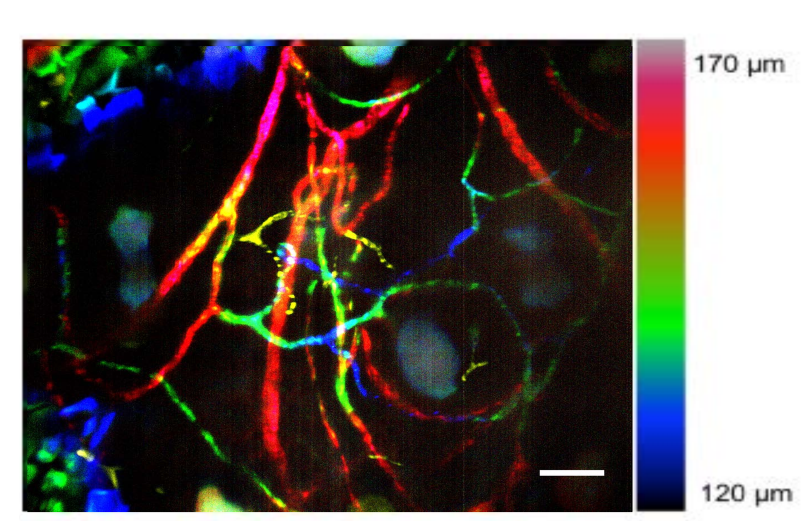


❺ Images of fluorescently labeled fresh tissues

	Images from handheld LS-DAC	Histology
Mouse tongue stained with methylene blue (depth ~50 µm)	a	b
Mouse kidney stained with methylene blue (depth ~100 µm)	c	d



Mouse ear vasculature imaged at 16 frames/sec and color coded for imaging depth



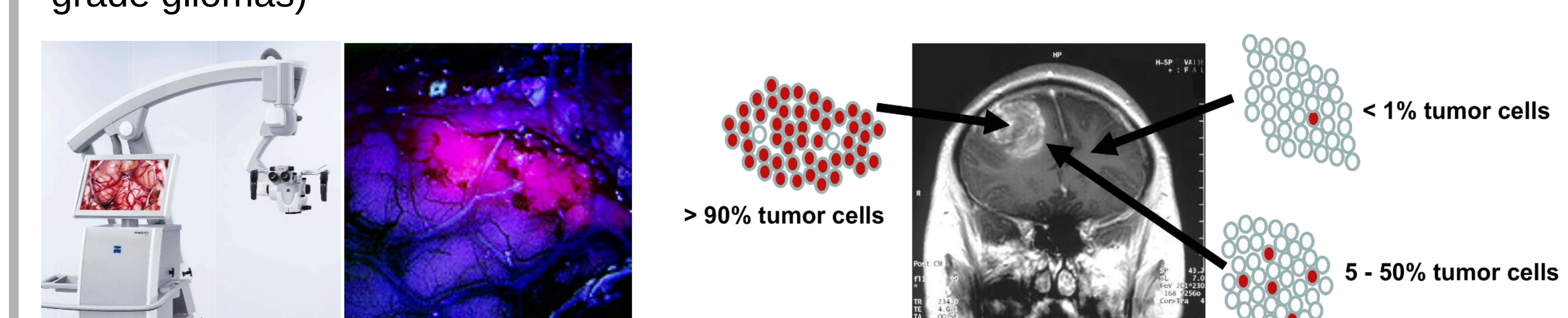
Next steps: utilize handheld LS-DAC microscope in oral-cancer patients at the Memorial Sloan-Kettering Cancer Center

❻ Clinical: 5-ALA-induced PpIX for low-grade glioma resection

Why high-resolution microscopy?

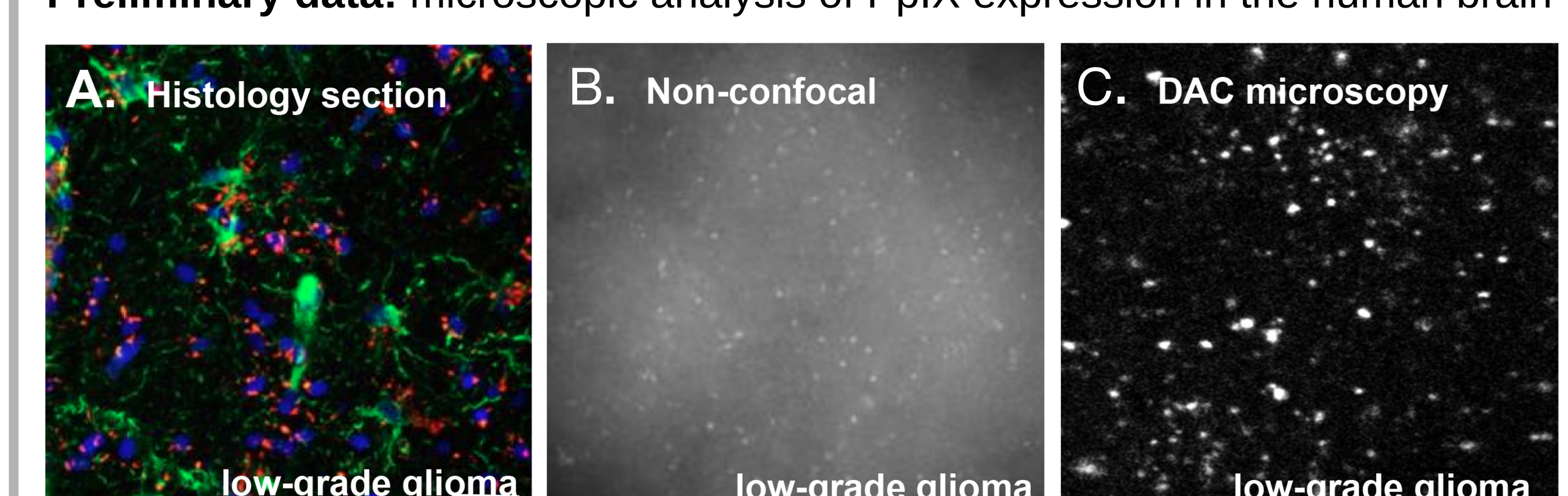
Fact: Wide-field fluorescence image-guided surgery with 5-ALA-induced PpIX has improved outcomes for patients with high-grade gliomas. [Stummer et al., *Lancet Oncology*, 2006].

Shortcomings: 1) Image intensity is subjective, especially at the diffuse margins. 2) Poor sensitivity to detect sparse tumor cell populations (e.g., diffuse margins & low-grade gliomas)



Solution: Intraoperative confocal microscopy has the resolution/sensitivity to detect sparse and disseminated fluorescence from tumor cells.
[Sanai et al., *J. Neurosurg.* 2011, Liu, Meza, & Sanai, *Neurosurgery* 2014]

Preliminary data: microscopic analysis of PpIX expression in the human brain



Next steps: develop image processing algorithm to quantify the density and intensity of sub-cellular PpIX fluorescence in human low-grade glioma tissues

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