Fluorescence imaging of cancer using dual-axis confocal microscopy and wide-field endoscopy

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SCIT Program Seminar
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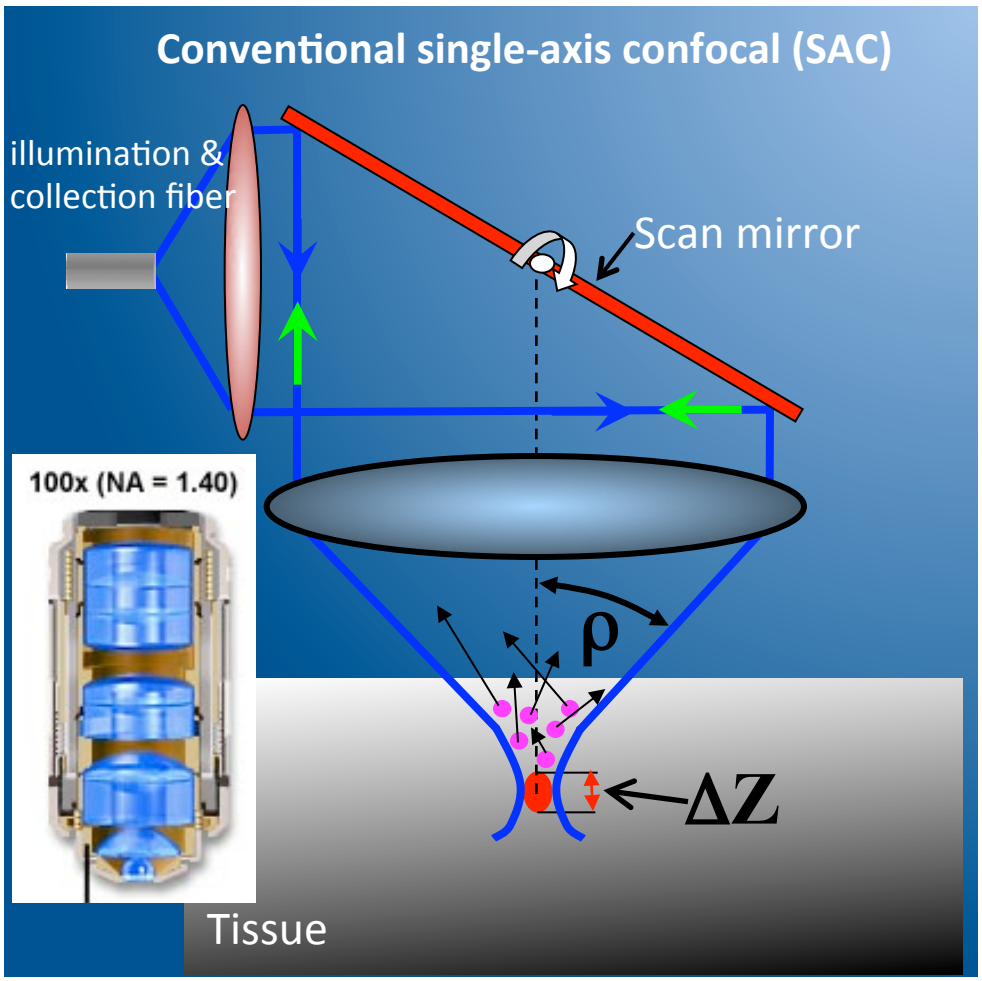
Outline

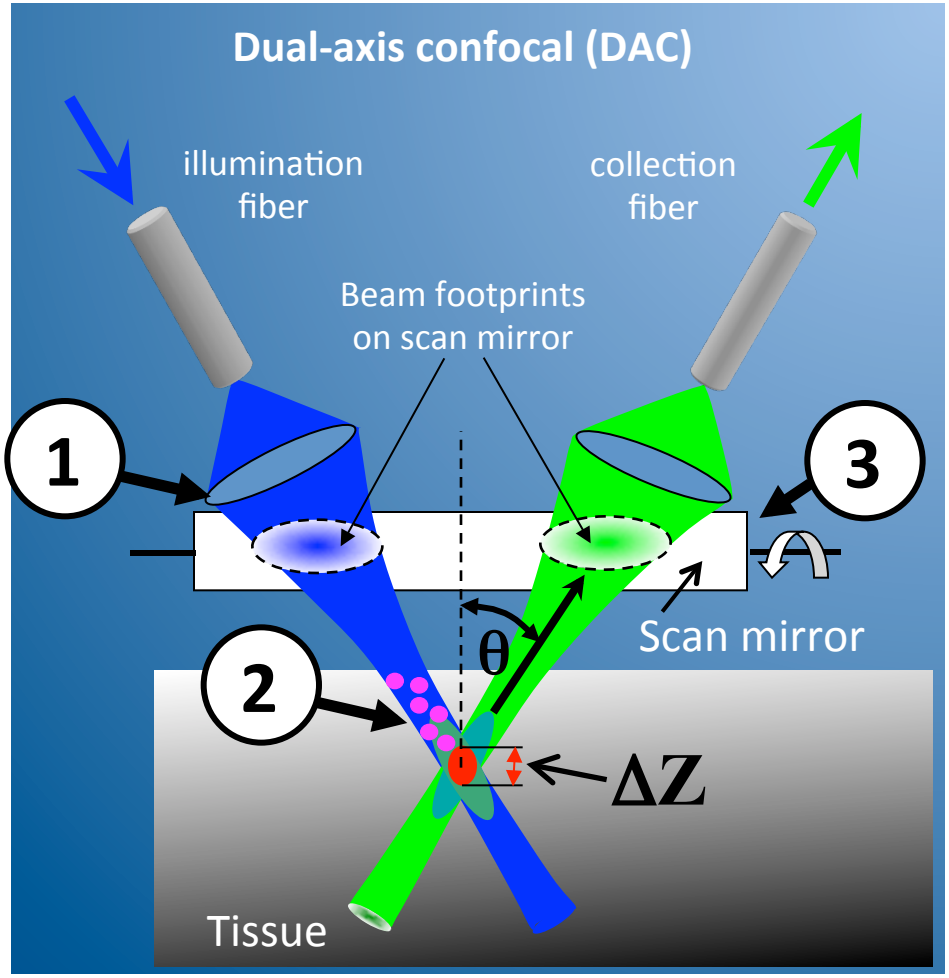
- Technology overview
  - Dual-axis confocal microscopy
  - Wide-field fluorescence imaging with the SpyGlass fiberscope
- Colon cancer detection using a targeted molecular probe in the Apc^{Min} mouse
- Brain cancer detection in collagen tissue phantoms using SpyGlass/DAC and RGD knottin peptide

Technology Overview

Dual-Axis Confocal (DAC) Microscopy

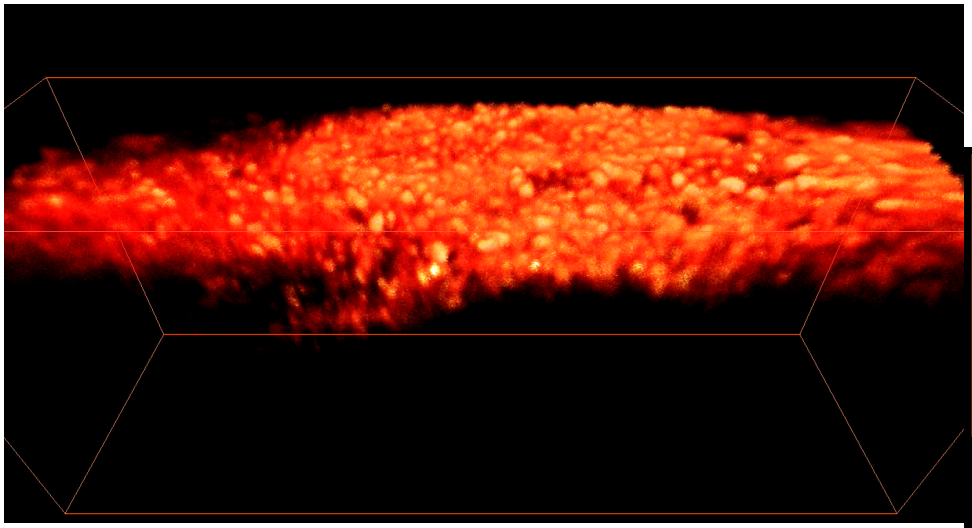
Comparing two confocal scanning microscope architectures:

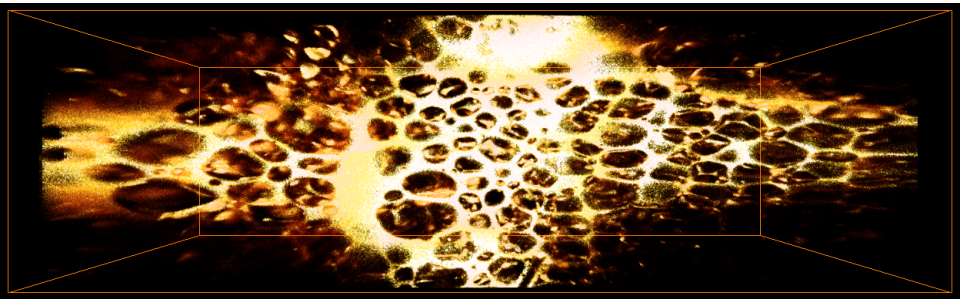


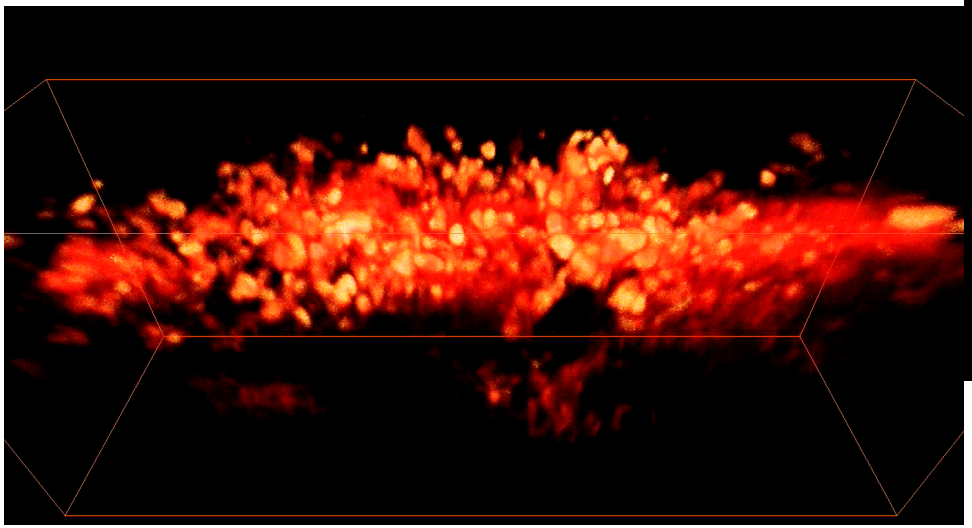


1) Uses simple low-NA optics to obtain thin optical-section thickness ΔZ
2) Reduces scattering noise in 3-D volume images
3) Easier to miniaturize using a MEMS scanner

Volumetric Imaging with Cellular Resolution



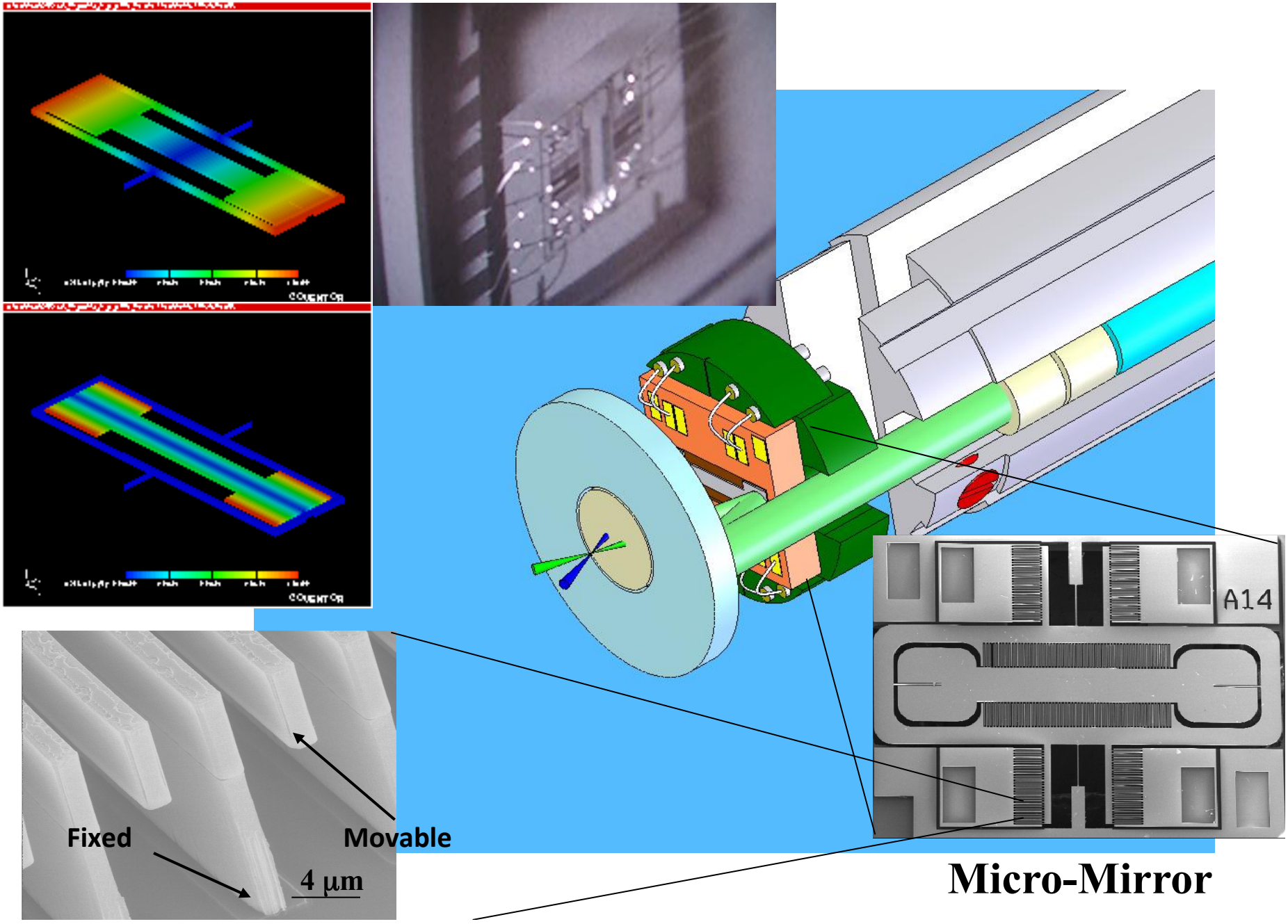




Colon Tissue

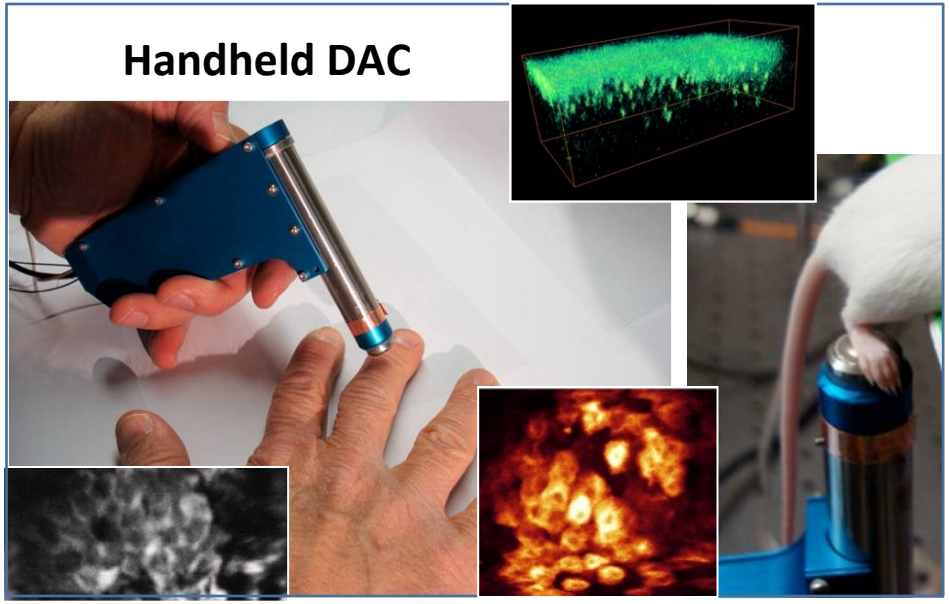
Medulloblastoma

MEMS Core for Miniaturization

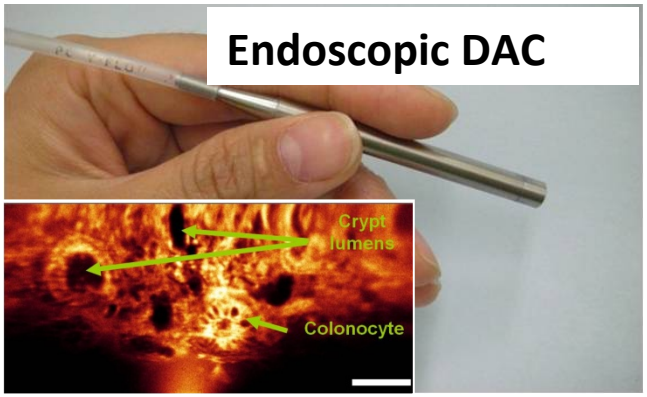


Current DAC Microscopes

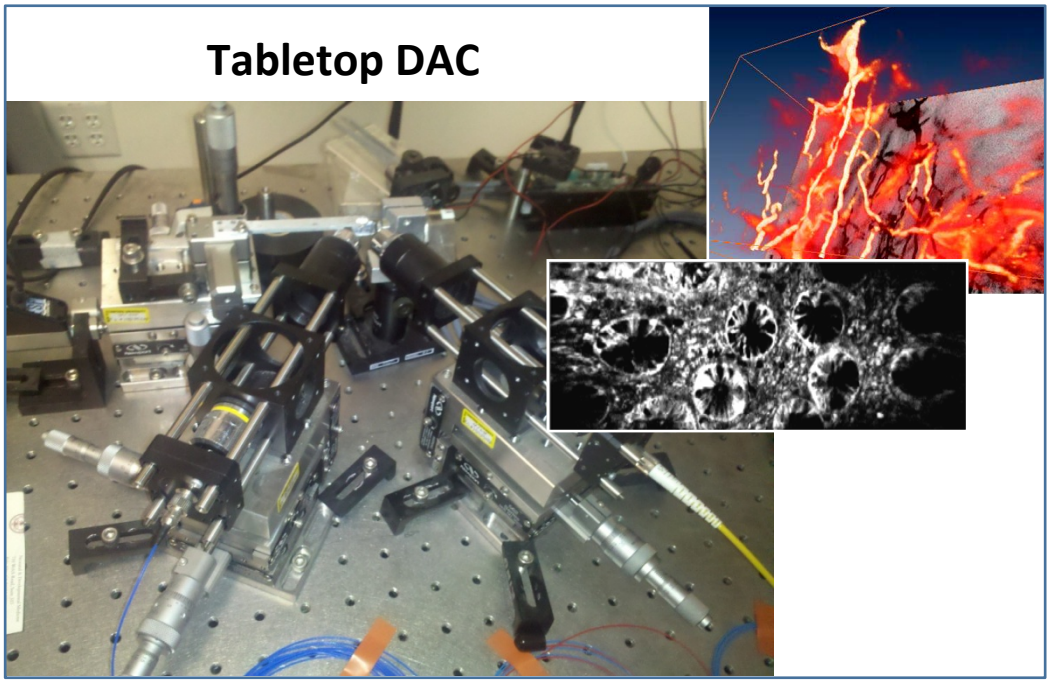
Handheld DAC



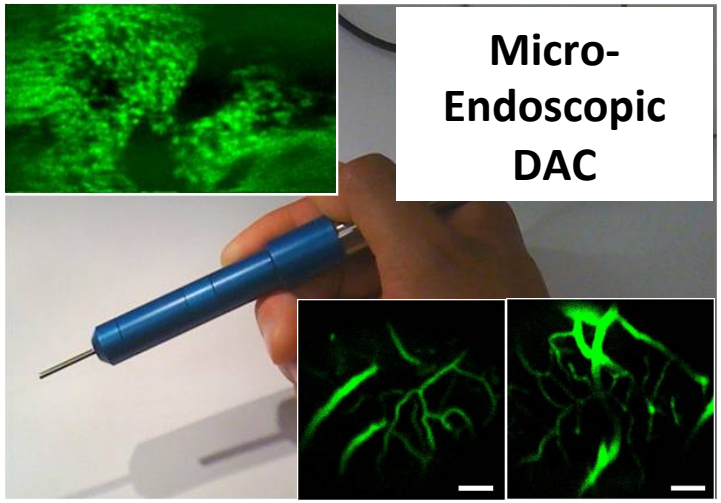
Endoscopic DAC



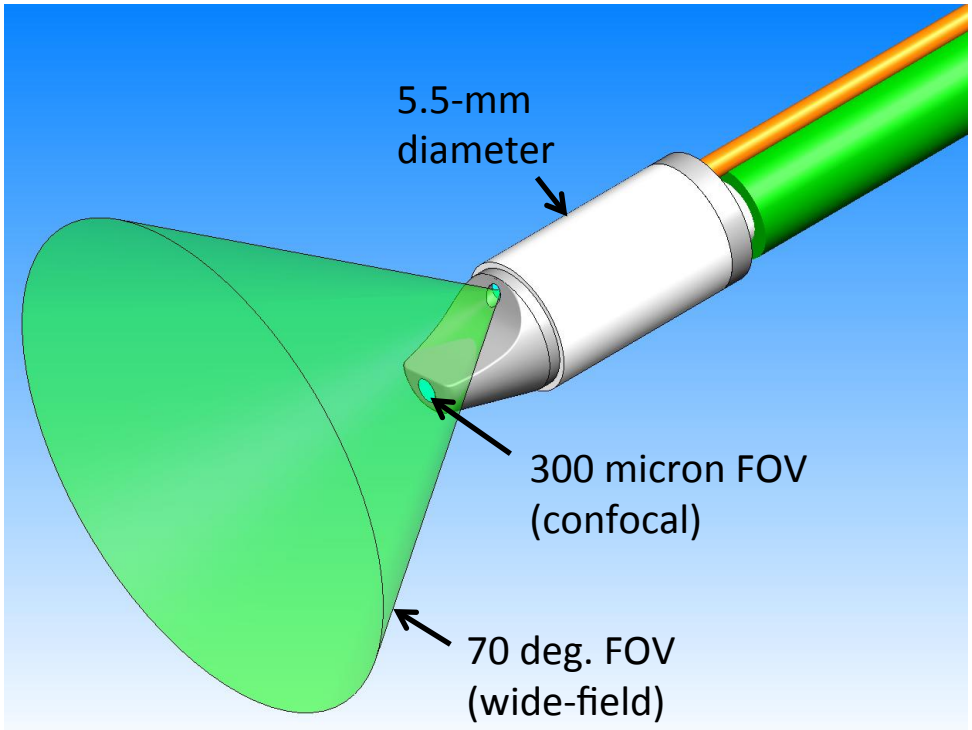
Tabletop DAC

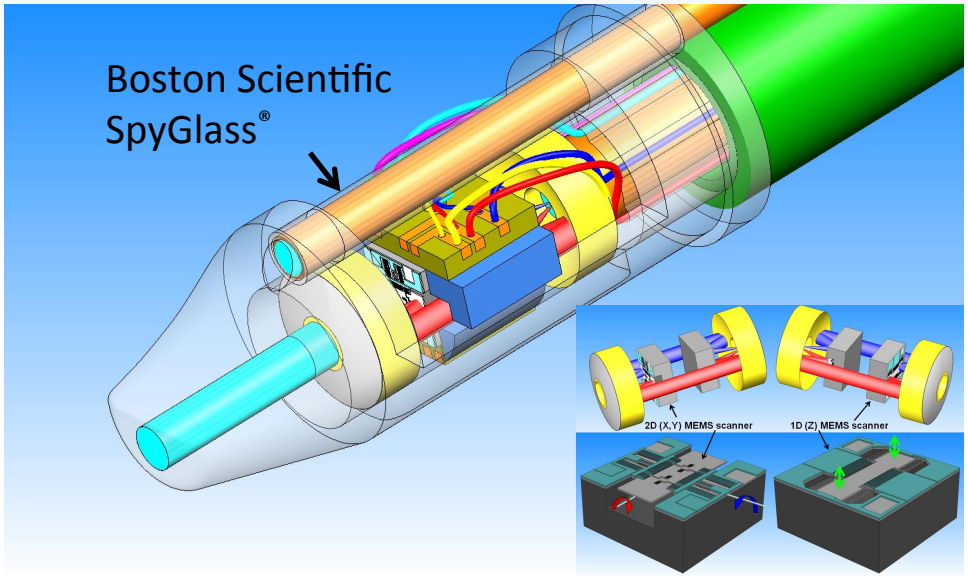


Micro-Endoscopic DAC

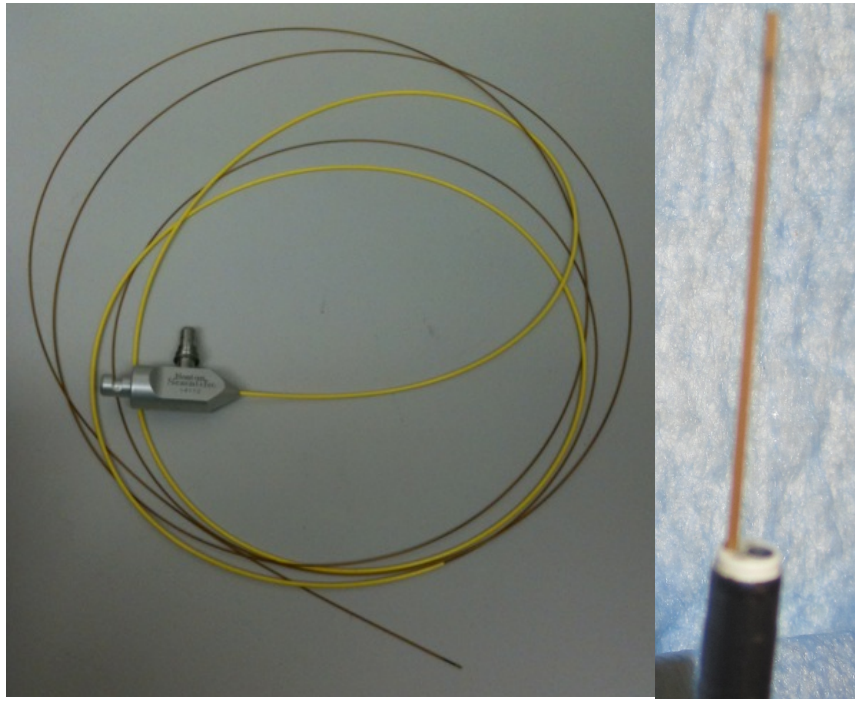


Next Generation DAC Microscope



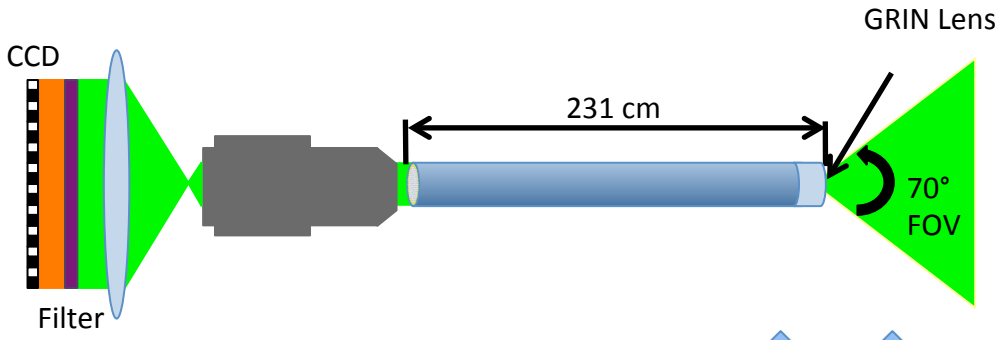


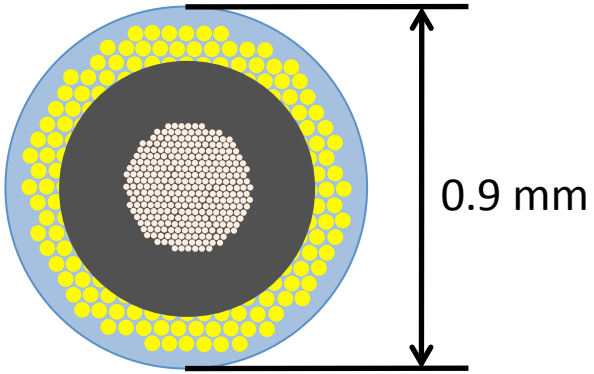
- 3.5-mm DAC
- All-MEMS scanning
- Combined with SpyGlass



Boston Scientific SpyGlass Fiberscope

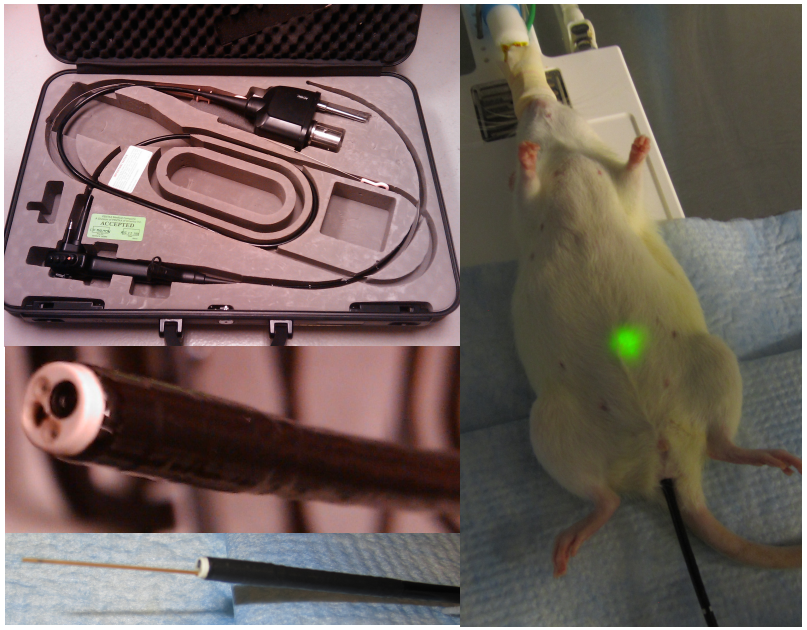
SpyGlass Fiberscope Overview

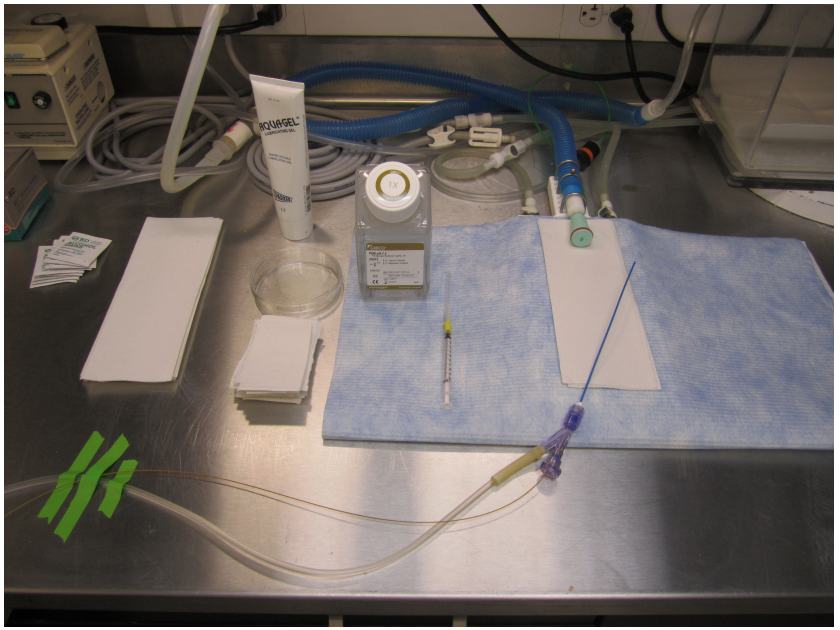




Commercial bronchoscope (3.8-mm diameter) for rats

Custom endoscope (1.8-mm diameter) for mice

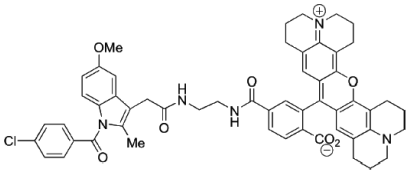




Colon Cancer Detection using a Targeted Molecular Probe in Apc^{Min} Mice

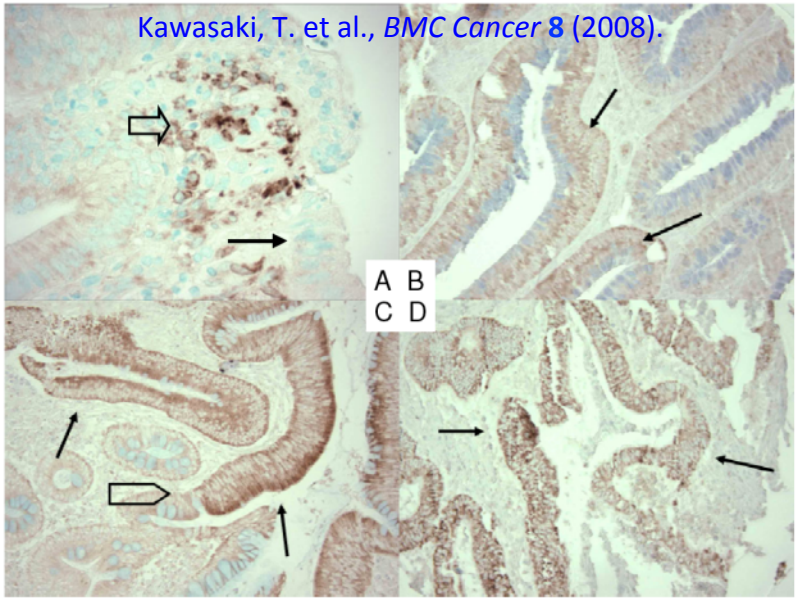
Targeting Colon Cancer in Small Animals with Fluorocoxib

The Probe



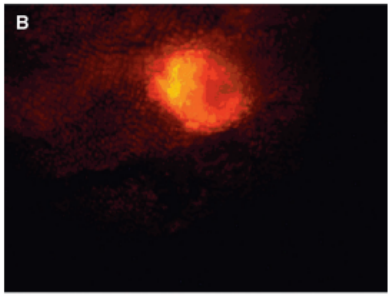
Uddin MJ, et al. *Cancer Res.* **70**, 3618 (2010).

- **Fluorocoxib** targets COX-2 enzyme over-expressed in cancer
- Developed by the Marnett lab at Vanderbilt

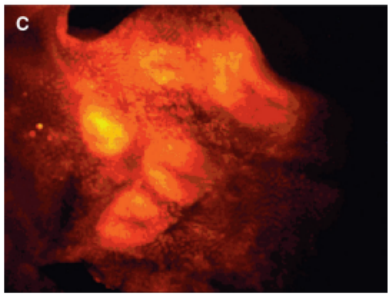


Kawasaki, T. et al., *BMC Cancer* **8** (2008).

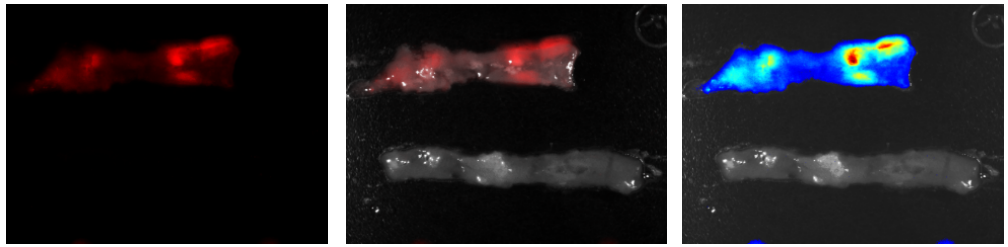
The Animal Model



Apc^{Min} Mouse

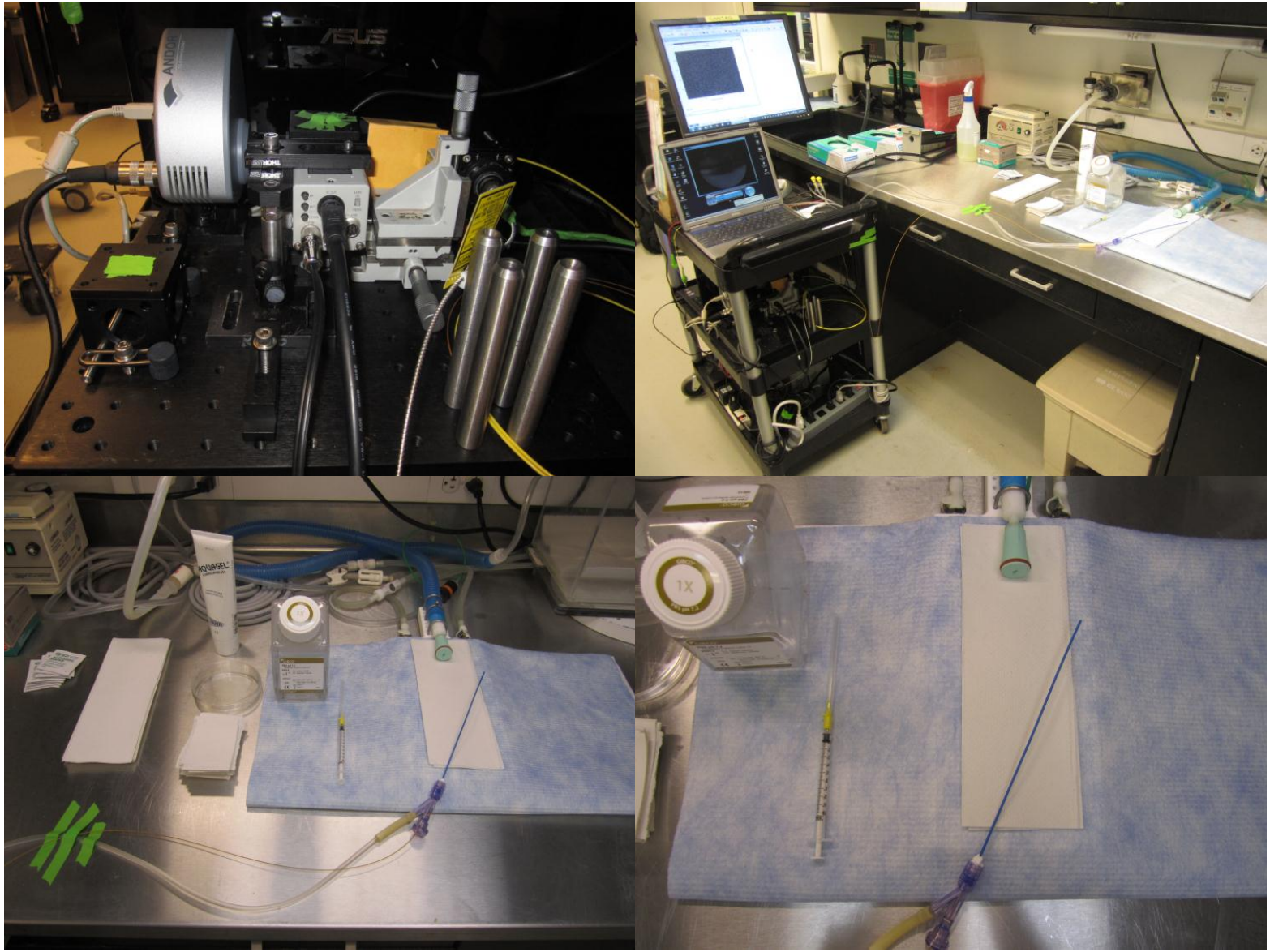


Fluorescence microscope images showing 50:1 contrast with fluorocoxib in small intestinal polyps (Marnett lab)

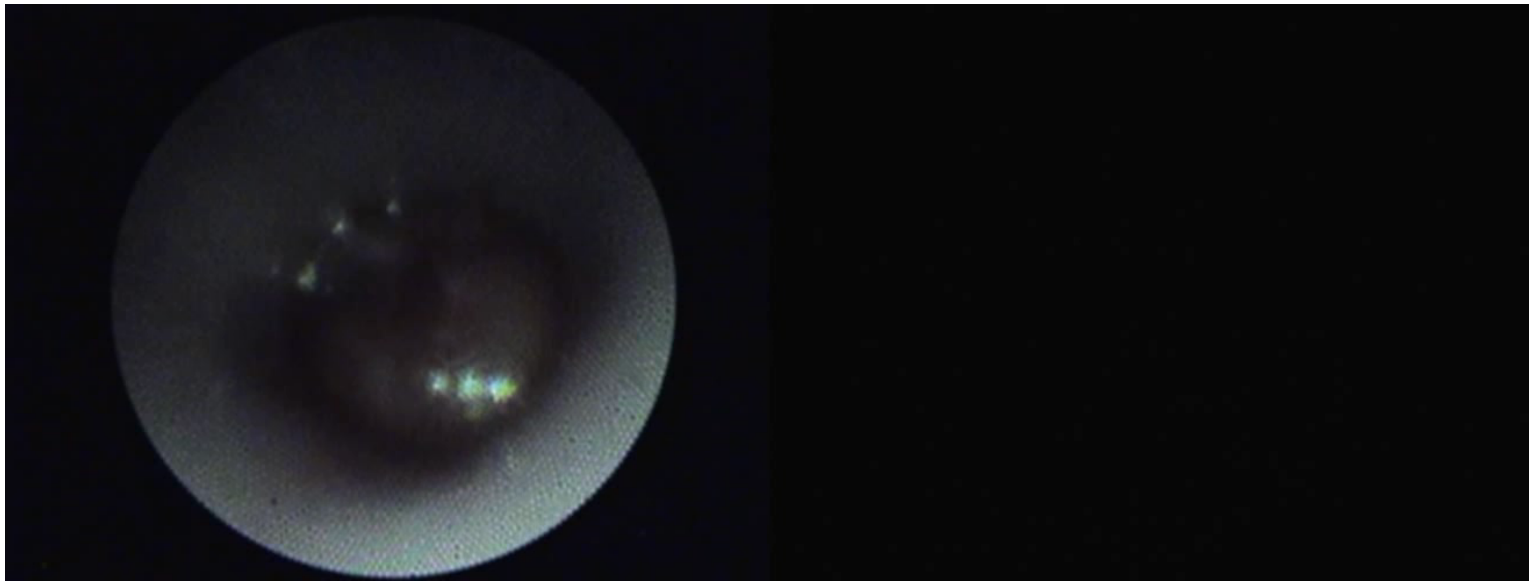


Wide-field fluorescence (CRI Maestro) images of intestinal polyps (Contag lab)

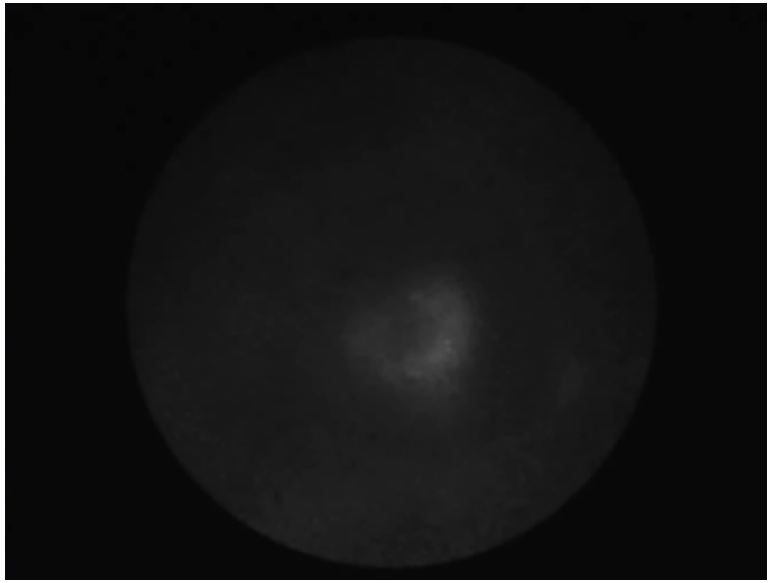
Mouse Endoscopy System



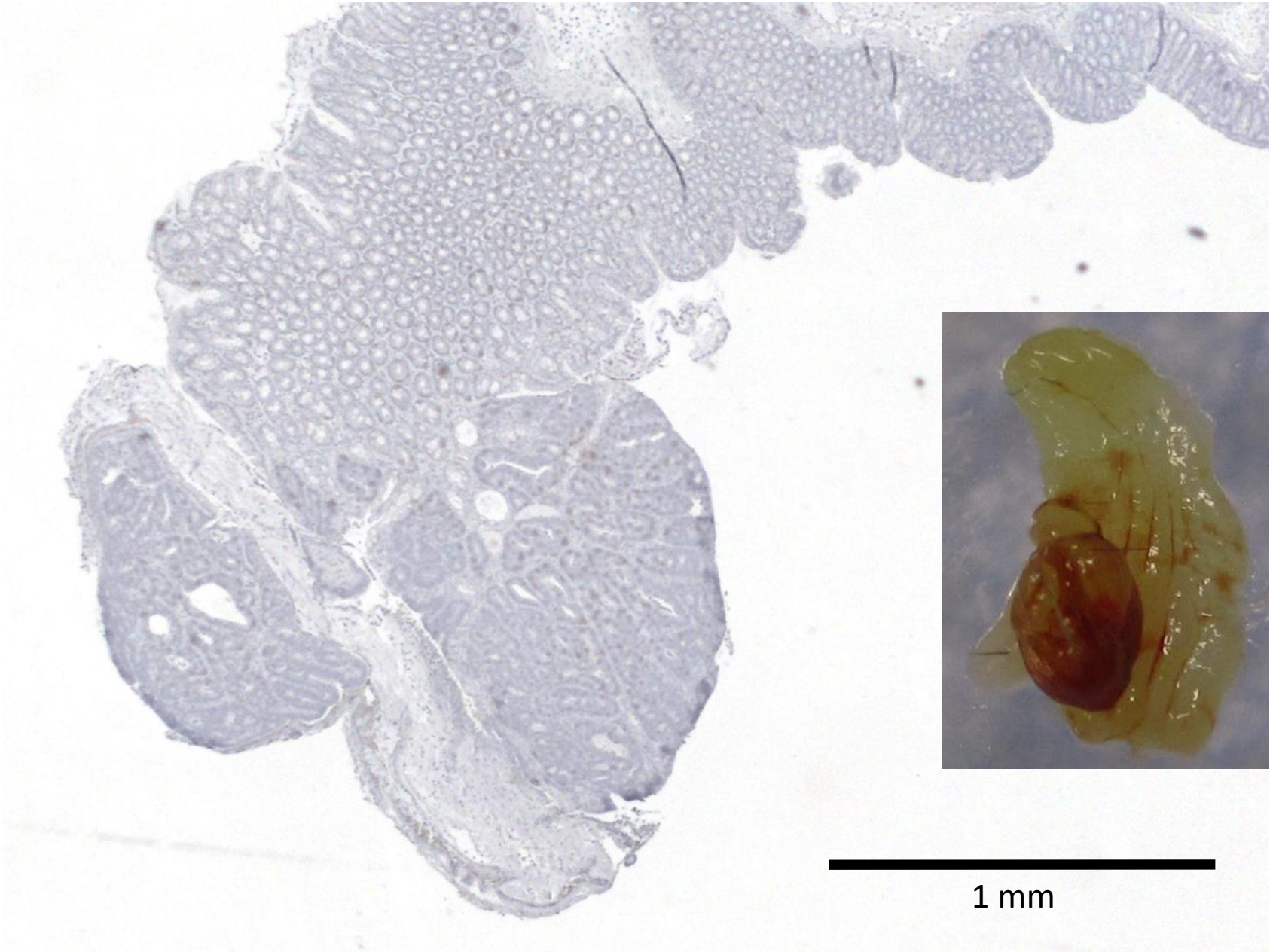
Tumor-Specific Labeling with Fluorocoxib



- Screened 5 Apc^{Min} mice for polyps
- 1 mouse had a polyp in the distal colon
- Mouse was injected i.v. with 1 mg/kg fluorocoxib
- Fluorescence imaged 3 hours after injection



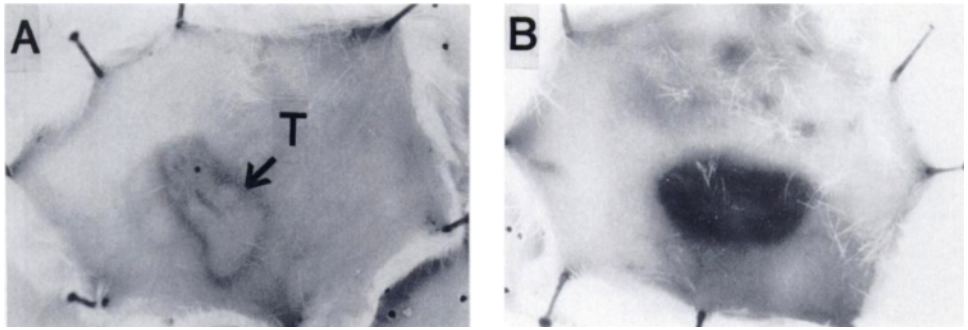
Antibody Staining: No COX-2, Benign Polyp



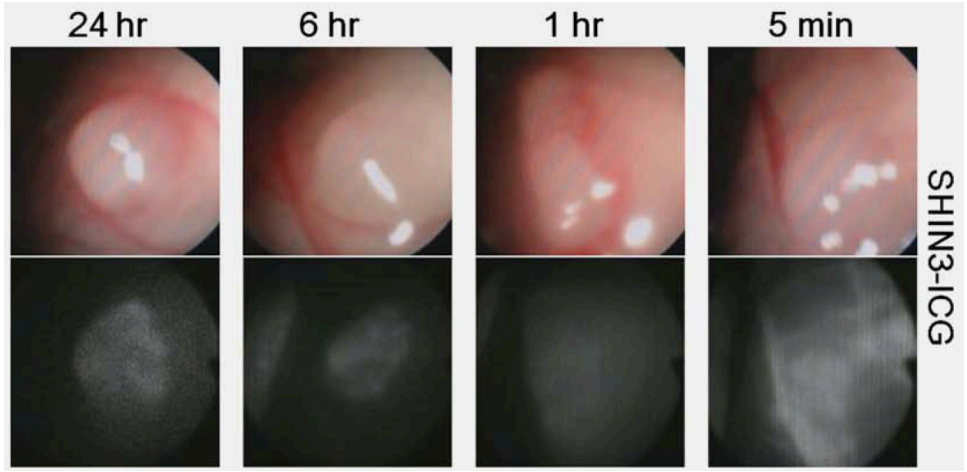
EPR Effect

How did we get tumor-specific signal if COX-2 is not over-expressed in the tumor?

- "Enhanced Permeability and Retention"
- Tumors have porous vasculature and lack lymphatic access; molecular probes leak into the tumor and get trapped
- More significant for larger molecules (macromolecules, m.w. > 40 kDa) and nanoparticles, but could still occur for small molecules
- **Inject fluorocoxib and ICG (1 mg/kg each) into Apc^{Min} mouse and image intestine on CRI Maestro**

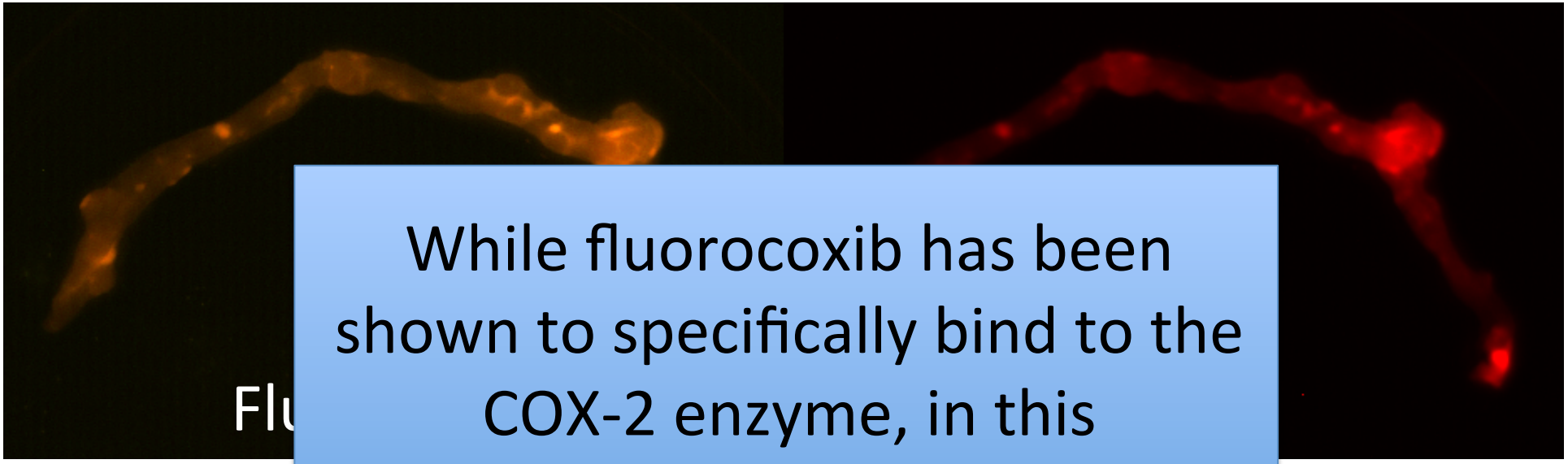


Accumulation of Evans blue-albumin complex in intracutaneous skin tumor 0 (A) and 6 hrs (B) after i.v. injection in a mouse.
Matsumara Y and Maeda H, *Cancer Res.* **46**, 6387 (1986).



ICG labeling of ovarian cancer (i.p.-injected) in the abdominal cavity of a mouse.
Kosaka N, Mitsunaga M, et al., *Int. J. Cancer* **129**, 1671 (2011).

Fluorescence Comparison: Fluorocoxib vs. ICG



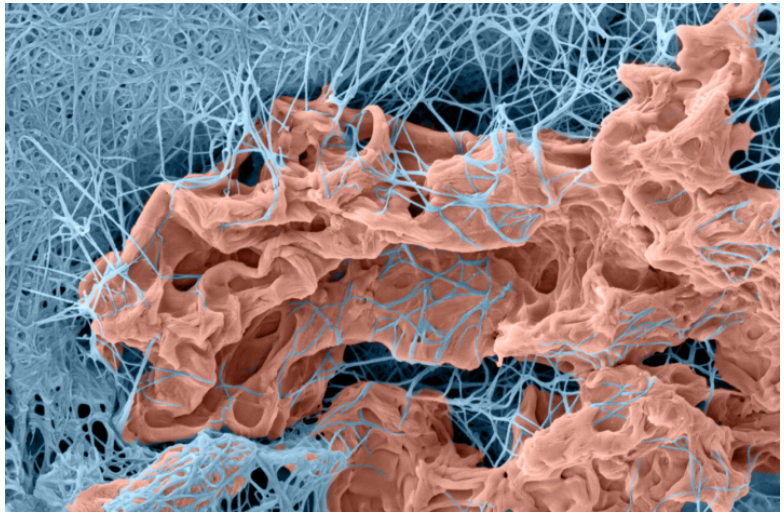
While fluorocoxib has been shown to specifically bind to the COX-2 enzyme, in this experiment, the dominant mechanism of tumor labeling is the EPR effect

- Fluorocox
  identical
  intestine
- Probe mi
  leaked out of the polyps into adjacent tissue
- Leakage also observed in excised colon tissue imaged with SpyGlass

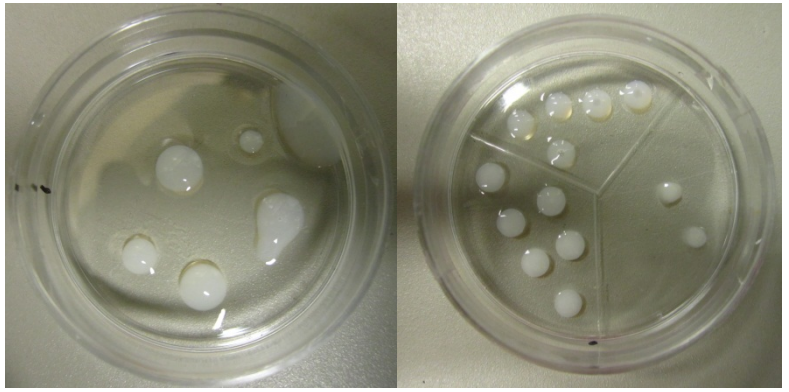
Brain Cancer Detection in Collagen Tissue Phantoms using SpyGlass/DAC and RGD Knottin Peptide

Collagen Tissue Phantoms

- Collagen is the main component in connective tissue (skin, tendon, vascular ligature, organs, organic bone)
- Density of collagen fibers and cross-linking determine tissue strength (e.g. “areolar tissue” connecting skin to muscle vs. skin or tendons)



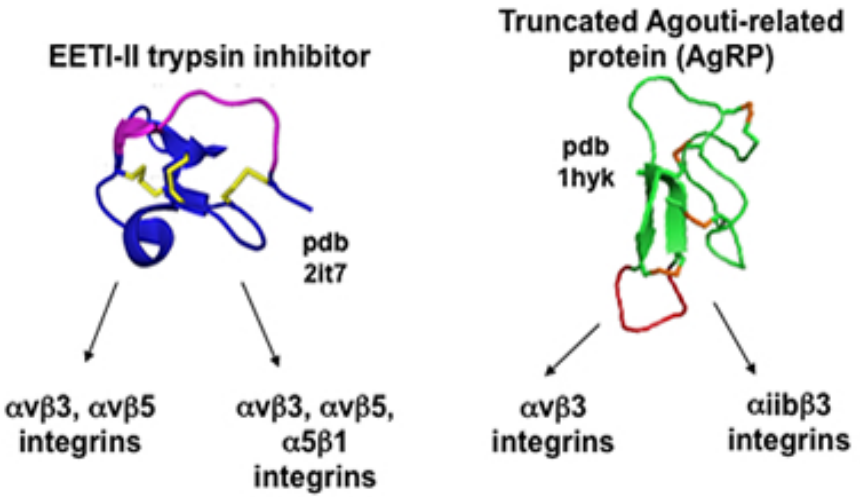
Fibrosarcoma infiltrating surrounding collagen extracellular matrix.
Sabeh et al., *J. Cell Biol.* **185,** 11 (2009).



Collagen phantoms containing various cancer cell types.

RGD Knottin Peptide

- Engineered by the Cochran lab at Stanford
- Cystine knot peptides target $\alpha_v\beta_3$, $\alpha_v\beta_5$, and $\alpha_5\beta_1$ integrin receptors over-expressed by cancer cells
- Labeled with Alexa Fluor 555 (555/565-nm excitation/emission)

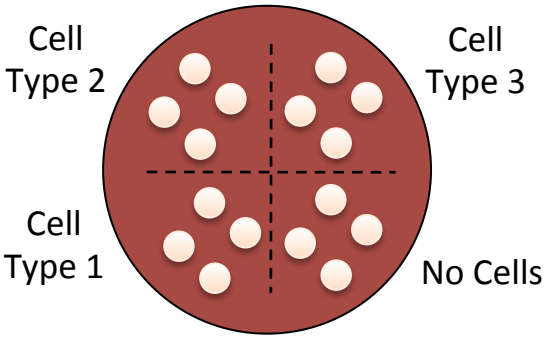


http://cochranlab.net/research/knottins.shtml

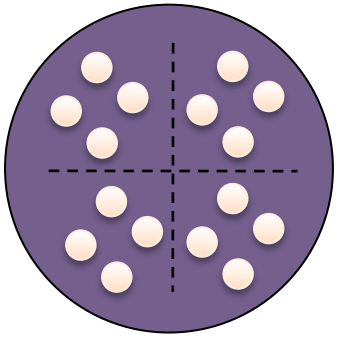
Features:

- Single-digit nM affinity
- Stable at 4 C for months
- Soluble in water/media (great for *in-vivo* use)

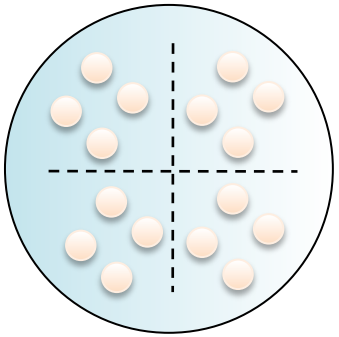
Labeling Procedure



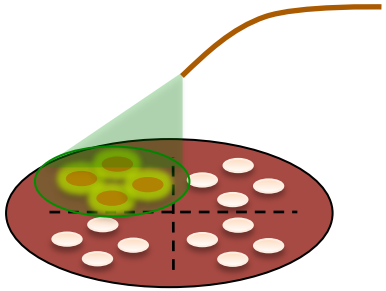
Prepare collagen and incubate 24 hrs in media



Treat phantoms with RGD knottin for 2 hrs



Wash phantoms in PBS on rocker



Survey dish for bound peptide using SpyGlass

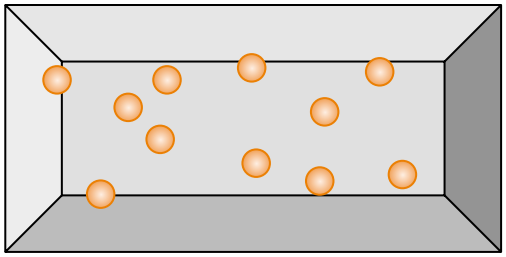
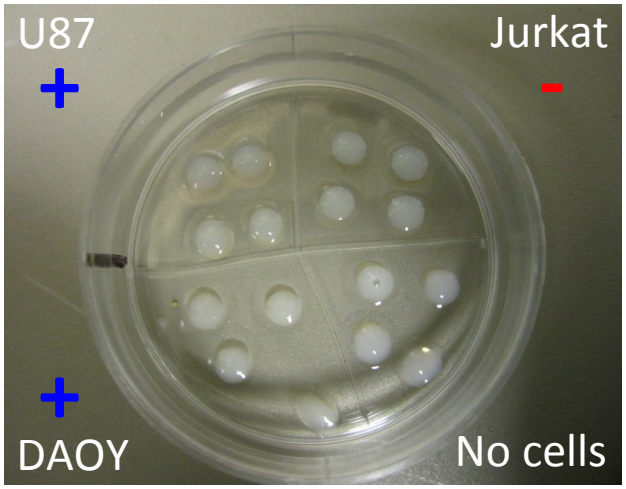


Image phantoms with DAC to confirm cell labeling

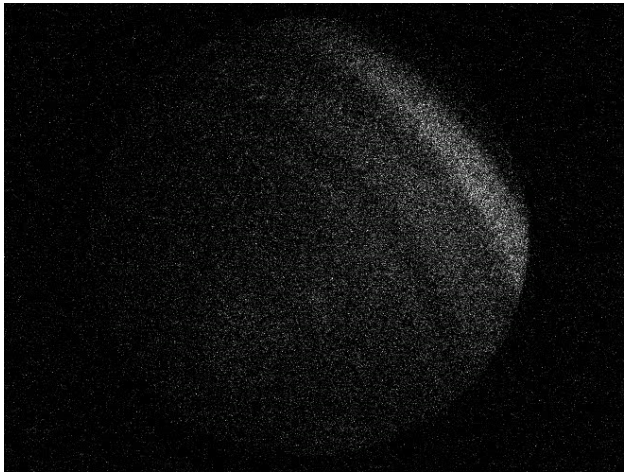
Imaging Results with SpyGlass and DAC

Glioblastoma

T-Cell Lymphoma



Medulloblastoma



SpyGlass Video

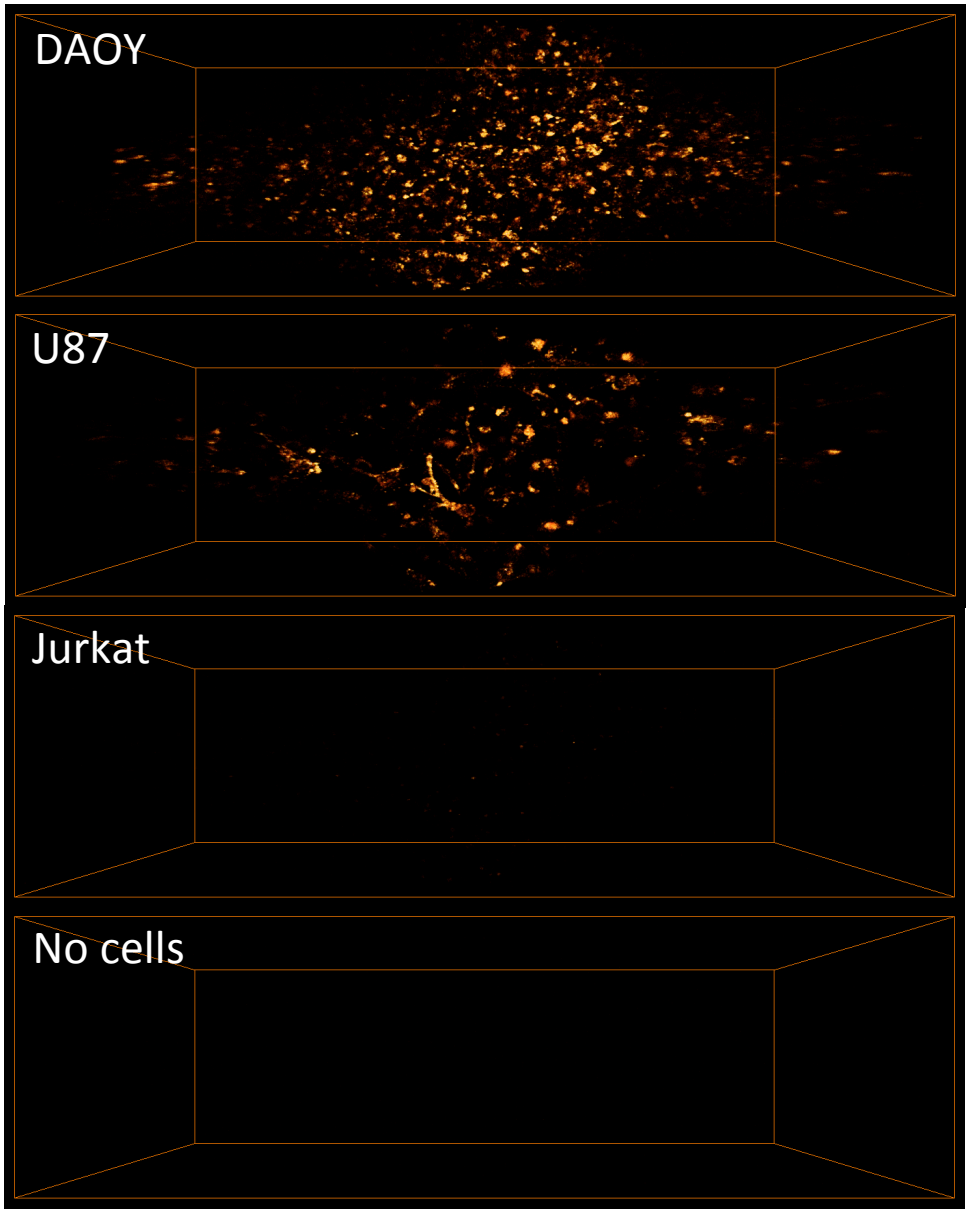
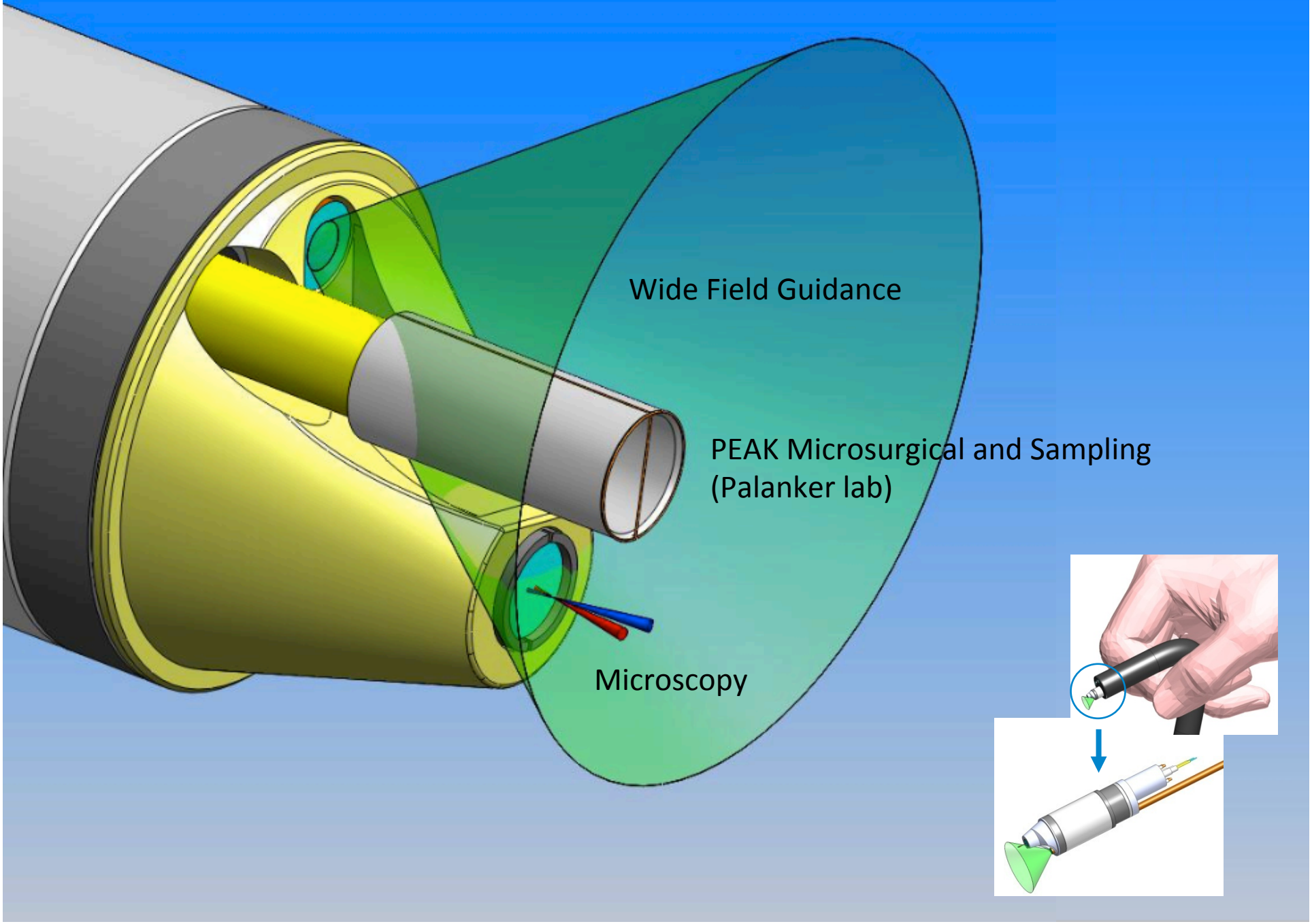


Image-guided Tissue Sampling and Multiplexed Molecular Analysis



Summary

- A miniature, multi-modal device combining a dual-axis confocal microscope with the SpyGlass can aid in cancer detection with minimal interference with existing instrumentation
- The SpyGlass is useful for probe testing in small animals and will be (relatively) easy to translate into the clinic
- Collagen phantoms provide convenient 3D tissue models for probe testing and motivate the combined use of the SpyGlass and DAC for cancer margin detection during surgery

Acknowledgements

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  - Contag Lab
    - Jonathan Liu
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  - Marnett Lab
    - Jashim Uddin
  - Histology
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  - Stanford Photomedicine Center