

# Changing the Diagnostic Paradigm: Imaging with Molecular Specificity and Cellular Resolution

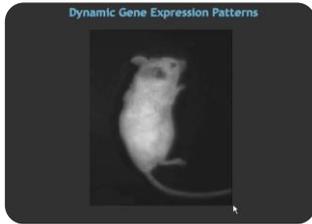
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Departments of Pediatrics, Radiology and  
Microbiology & Immunology  
Molecular Imaging Program at Stanford (MIPS)  
Stanford University



## Imaging Refines Animal Models

Enabling discovery beyond what has previously been possible

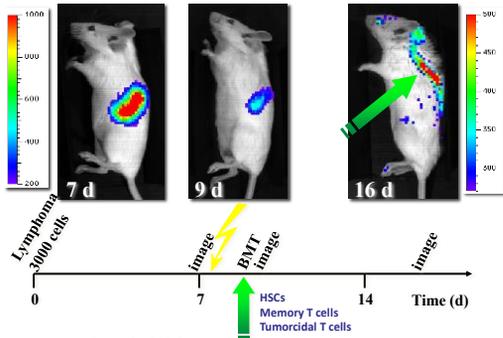
- Access to new information because the contextual influences of the host are intact
- Increased data per animal
  - Whole body scans
  - Temporal changes
  - Ease of use permits fine temporal resolution
- Improve statistics--"Built-in" internal controls
- Image-guidance for tissue sampling--the correct tissue at the right time
- Track labeled therapeutic or target
- Image-guided "Omics"
- Cell culture to in vivo links



Dynamic in vivo measures of gene expression



## Molecular Imaging in Animal Models of Early, and Minimal Residual, Disease



Edinger et al. *Blood* 15:640-8

## Imaging in Cancer Biology

The nearly two decades Molecular Imaging research has led to advances in reporters, probes and instrumentation that have served to **refine and accelerate the study of in vivo biology and drug development**—however, it is time to use molecular imaging approaches to dramatically **change paradigms**.

Preclinical imaging over the entire disease course will enable us to target **early and minimal residual disease**, understand the origins of disease, develop novel targets, and refine our selection of drug candidates

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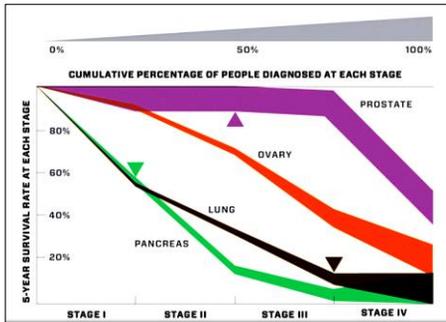
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## Cancer: Early Diagnosis and Prognosis



WIRPD

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School of Medicine

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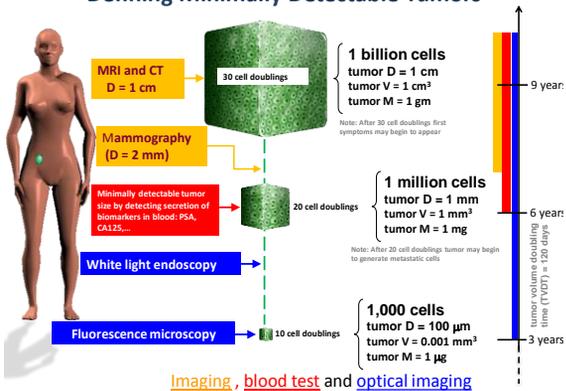
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## Defining Minimally Detectable Tumors




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## Miniaturization of the Confocal Microscope For Point-of-care Pathology

### Tabletop Microscope




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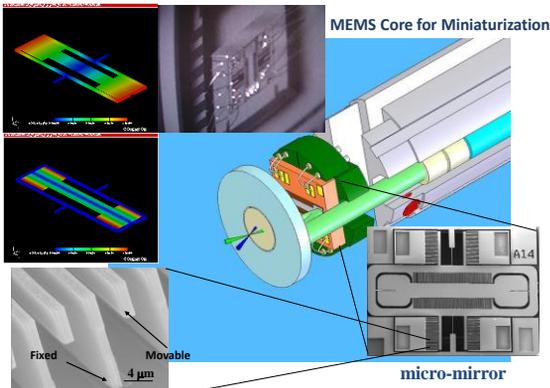
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### Dual Axis Confocal Microscope




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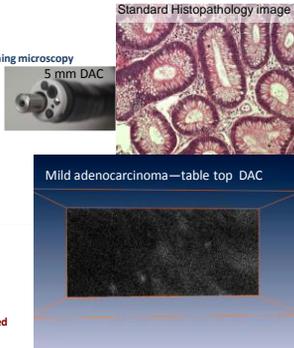
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### Clinical Objectives for Miniaturization

Optical sectioning with histopathologic resolution and molecular contrast

- 1) Specifications
  - 3.2 mm x 10 mm (endoscope compatible)
  - Multispectral 500-800 nm
  - Dual modality (wide field and vertical scanning microscopy)
- 2) Confocal optics + fluorescence provides
  - Dynamic range
  - Large field-of-view (0.3-0.5 mm)
  - Deep working distance (0.3-0.5 mm)
  - High contrast images (molecular probes)
- 3) MEMS scanner provides:
  - Small size (endoscope-compatible)
  - Fast scanning (video rate imaging)
  - Manufacturability (silicon processing)
- 4) Dual-axis confocal (DAC) optics allows:
  - Simple, inexpensive optics (low-NA)
  - Three-dimensional imaging (efficient optical sectioning)
  - Scalable
- 5) Differences
  - In vivo, 3D, contextual influences intact,
  - Real-time—point-of-care microscopy—need pathologist to be real time




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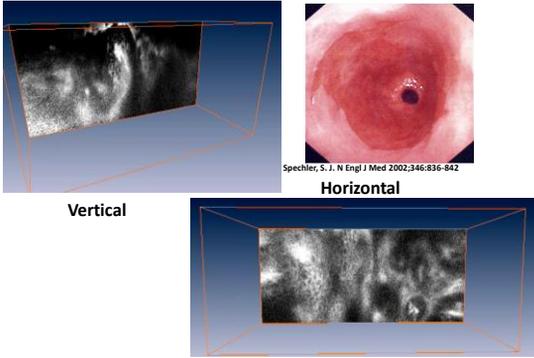
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### Performance of Dual-Axis Microscopes

Anatomic imaging of Barrett's to Squamous Junction—excised tissue



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### Contast Agents



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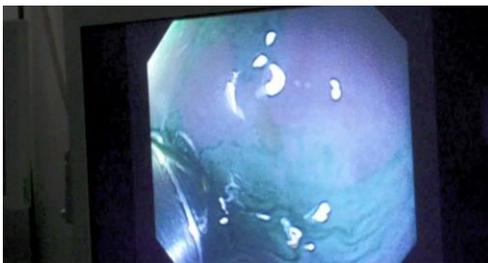
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### Integrated Macro- and Microscopic Imaging



Topical ICG  
Colorimetric and fluorescent contrast for macro- and microscopic imaging

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### Towards Larger Fields of View: Mosaics of Clinical DAC Data



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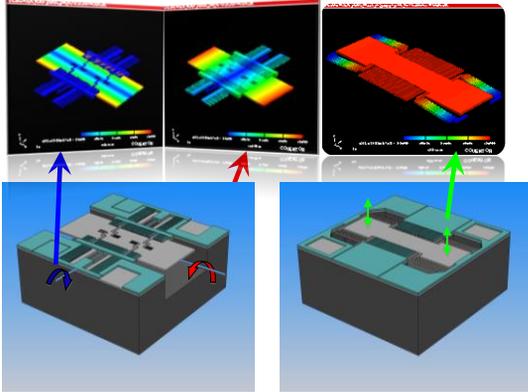
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### 3-axis scanning using two MEMS scanners



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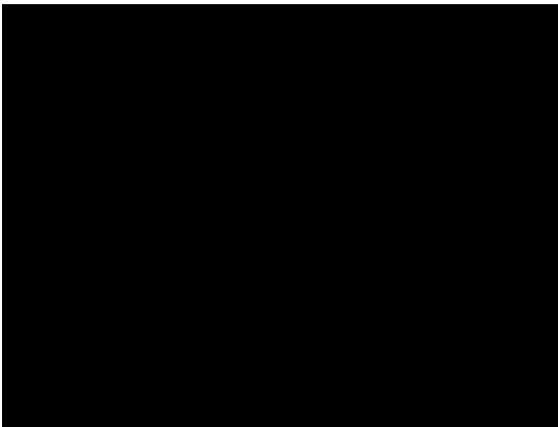
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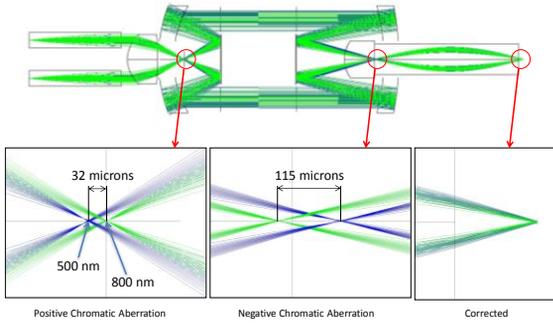
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Achromatic Optical System Allows **Multispectral** Operation over Range of 500 nm – 800 nm



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Machined parts for Clinical Multi-Modality DAC Systems



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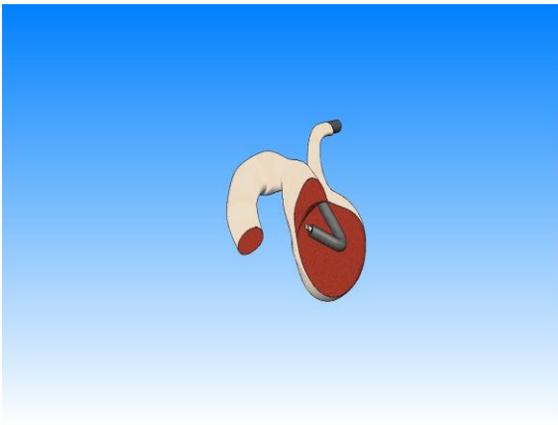
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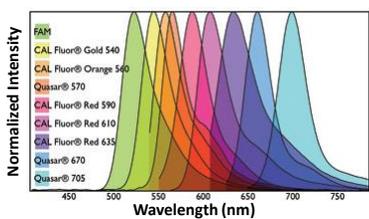
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## Multiplexing with Fluorophores

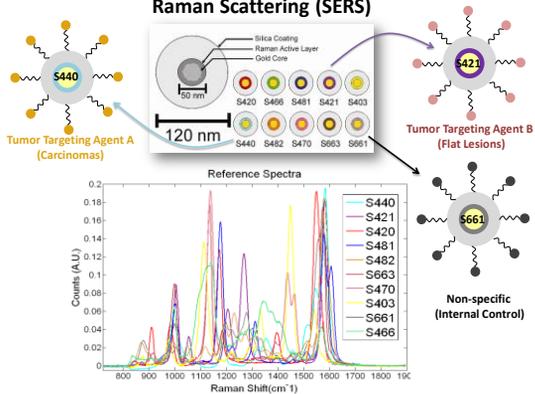


When multiplexing, the fluorescent signal from one reporter can bleed into adjacent channels.

<https://www.biosearchtech.com/display.aspx?catid=226,234&pageid=53>

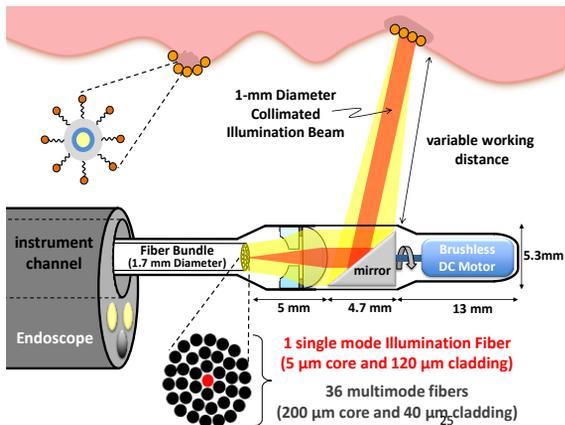
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## Multiplexing with Nanoparticles: Surface Enhanced Raman Scattering (SERS)



## Tools to Enable the Transition from Mouse to Man





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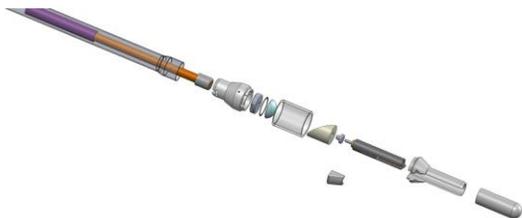
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### Assembly and Use of Scanning Raman Endoscope



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### Circumferential Scanning Raman Endoscope



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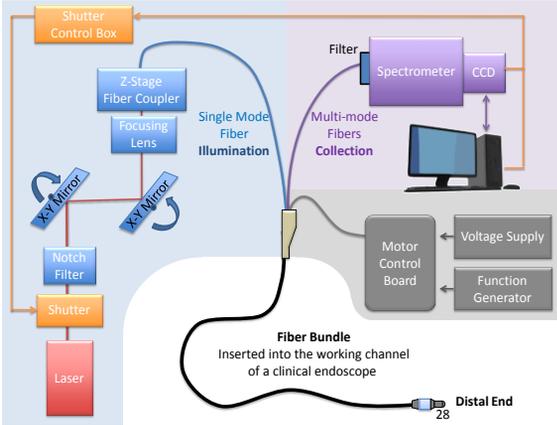
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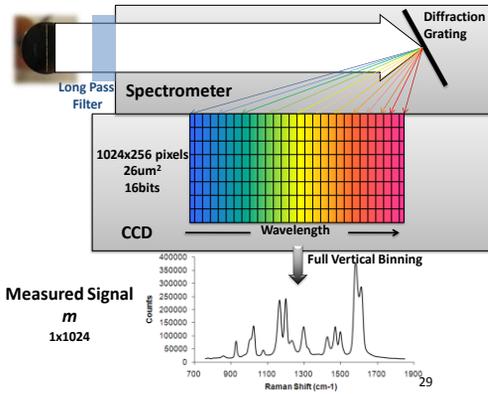
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### Raman Spectra Acquisition



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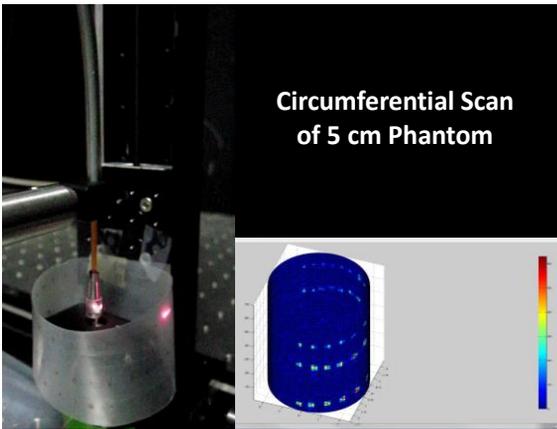
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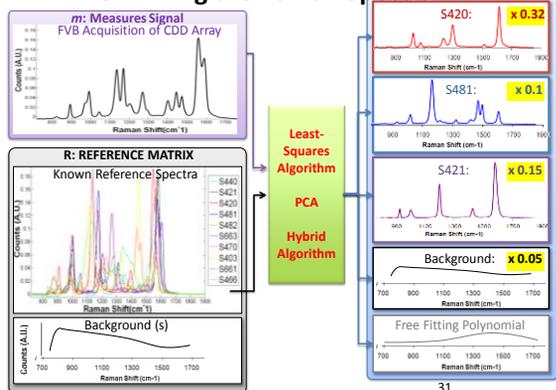
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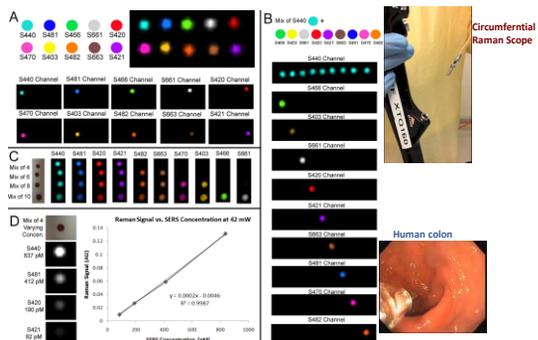
### Unmixing the Raman Spectra



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### Multiplexing Molecular Endoscopy: Scanning Raman Endoscope and SERS



### Spectral Phantom

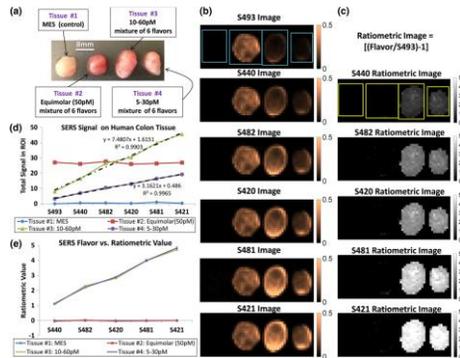






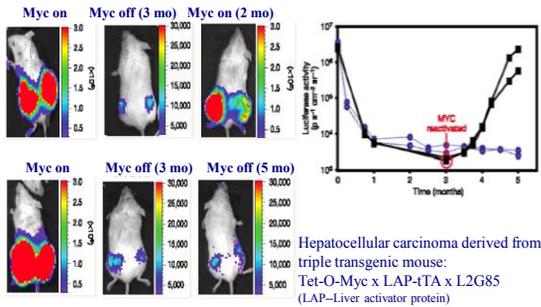


### Ratiometric Imaging



Garai, Ellis, et al. "High-sensitivity, real-time, ratiometric imaging of surface-enhanced Raman scattering nanoparticles with a clinically translatable Raman endoscope device." *Journal of Biomedical Optics* 2013

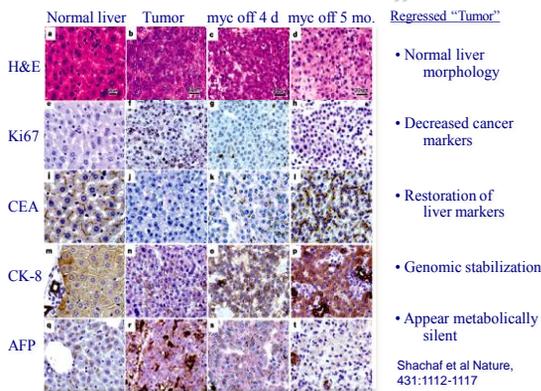
### Model of Minimal Residual Disease and Targeted Therapy Ectopic Regulation of the Myc Oncogene

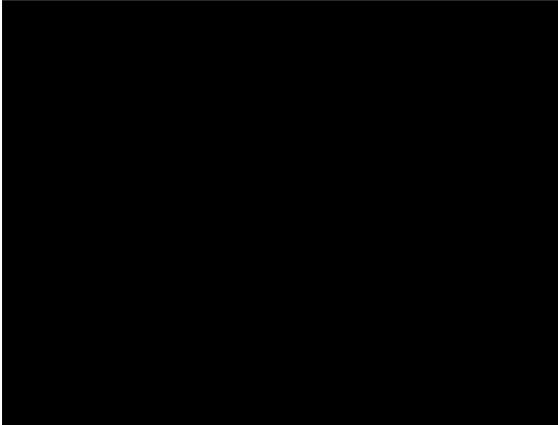


Stanford University School of Medicine

Shachaf et al Nature, 431:1112-1117

### Reversion to a "Normal" Phenotype





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Circumferential Raman Endoscope for GI Endoscopy



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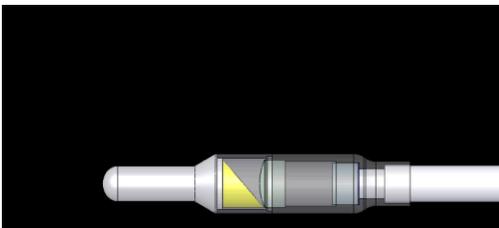
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Vision: Scanning Raman Microendoscope for Multiplexed Cancer Screening



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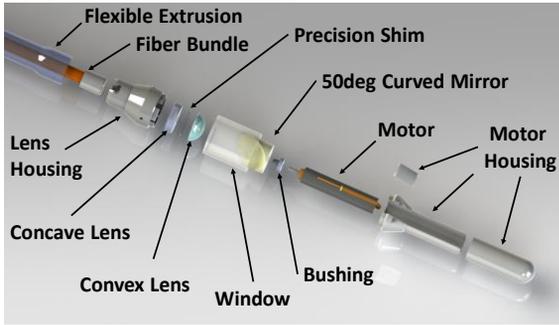
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### Raman Microendoscope



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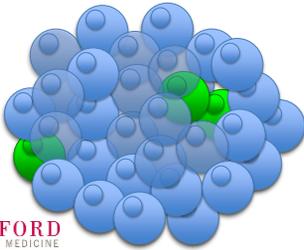
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### Rationale: Cancer Initiating Cells—Stem Cells— as an Emerging Paradigm

- Cancer initiating and cancer sustaining cells
- Root of the disease—Characterized, in part, an absence of markers or shared stem cell markers, metabolically less active, and may be more “like” normal cells




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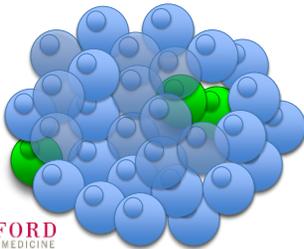
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### Cancer Therapies

- Targeted bulk of disease
- Therapies developed with animal models of late stage disease
- May leave minimal residual disease—clearance by immune response
- Need to visualize cancer stem cells in order to devise effective therapies




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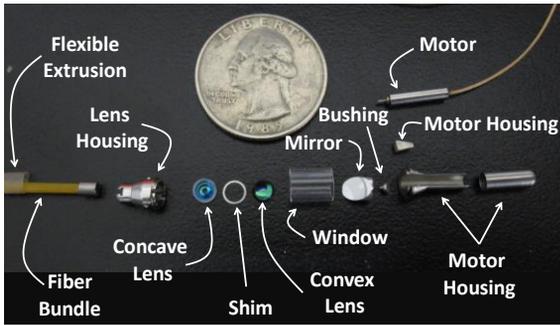
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### Components



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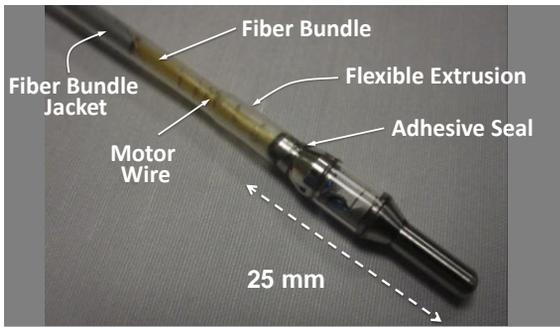
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### Functional Scanning Raman Endoscope



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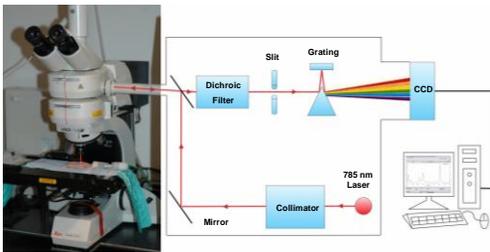
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### Renishaw inVia Raman Microscope



<http://www.renishaw.com/en/invia-raman-microscope-6260>

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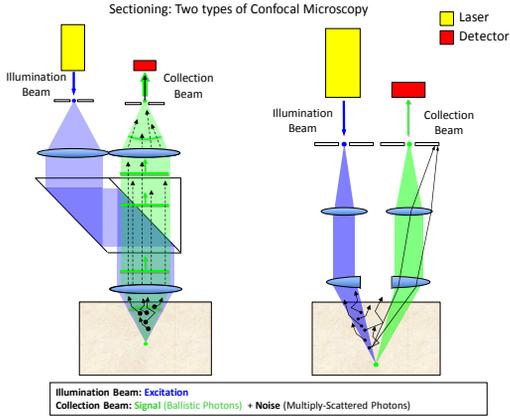
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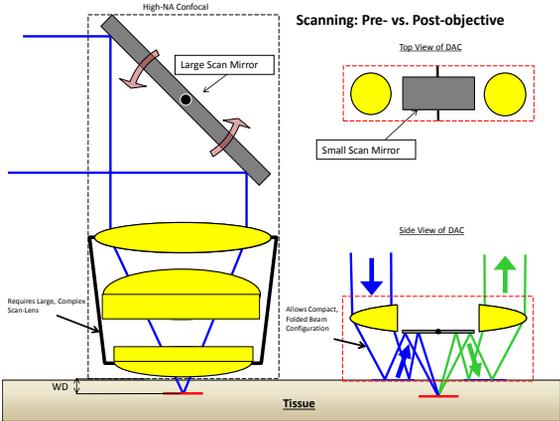
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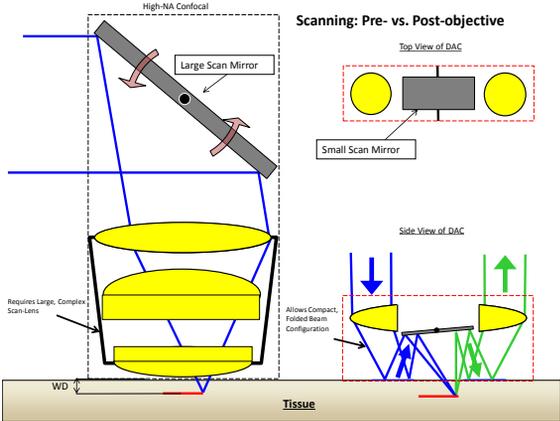
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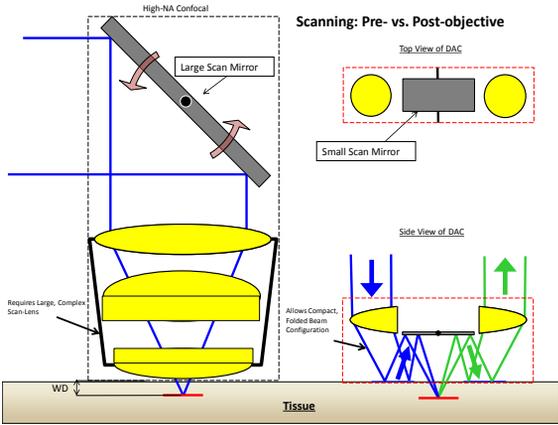
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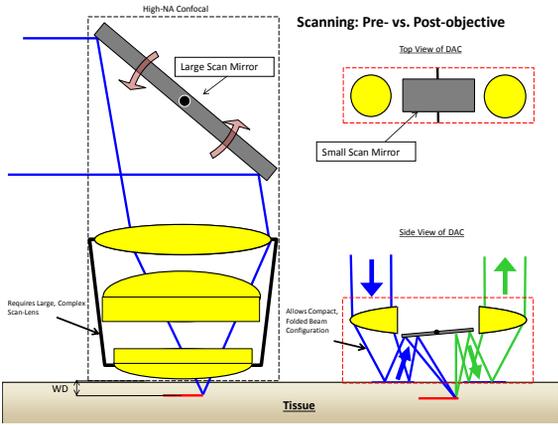
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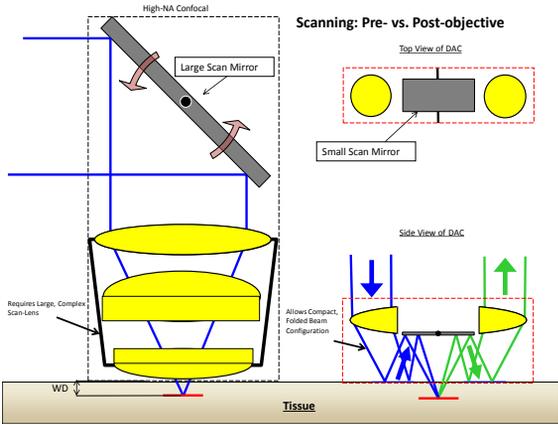
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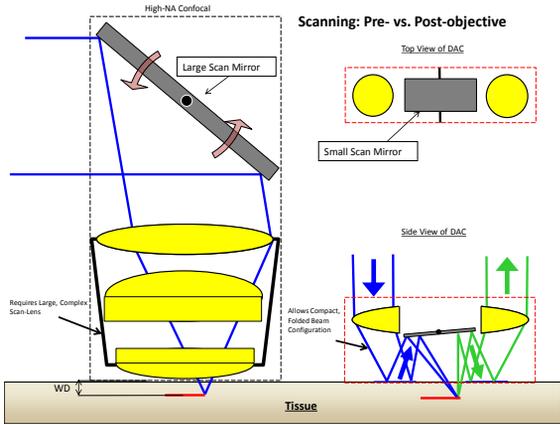
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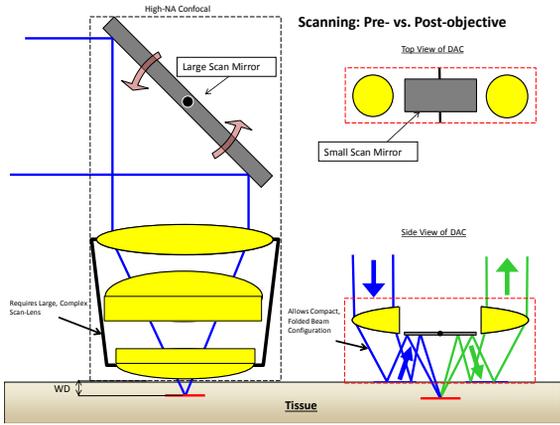
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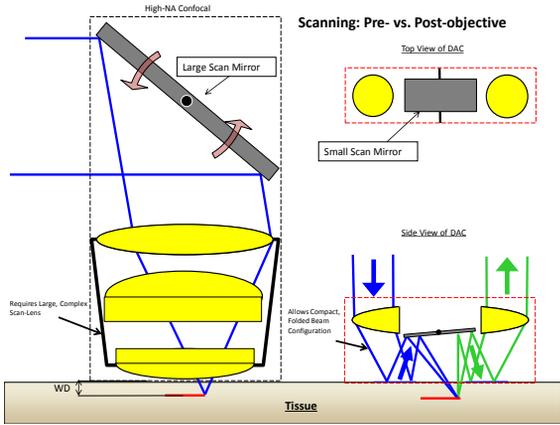
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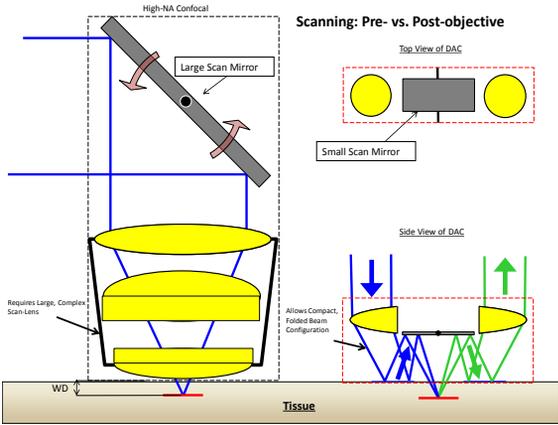
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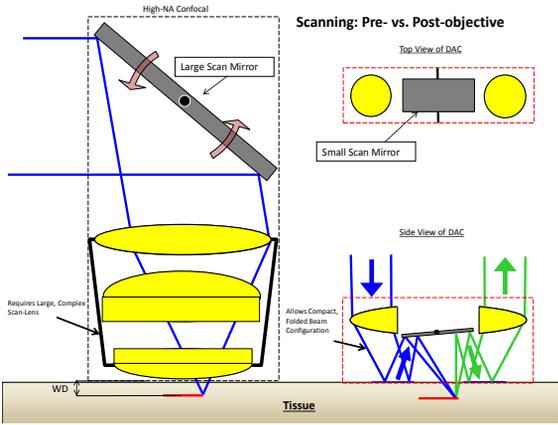
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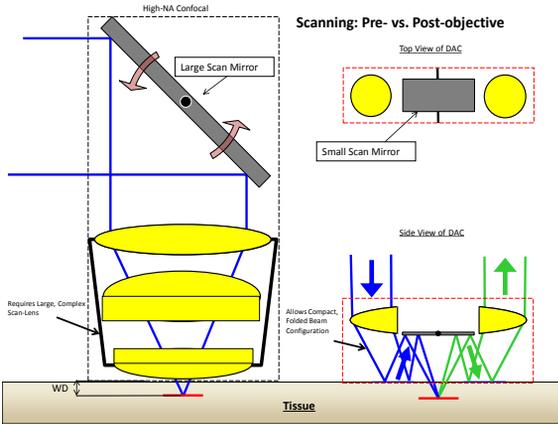
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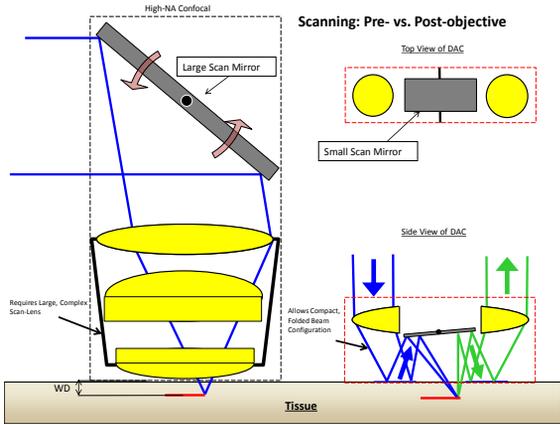
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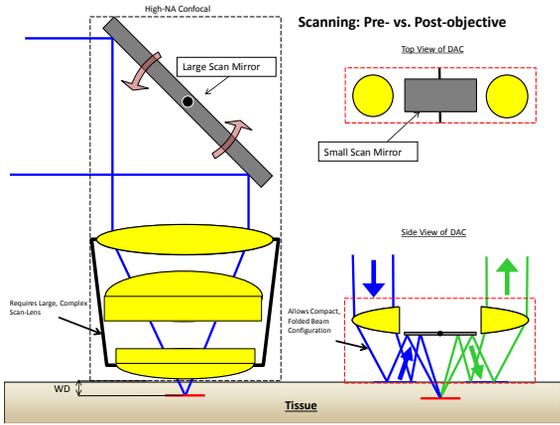
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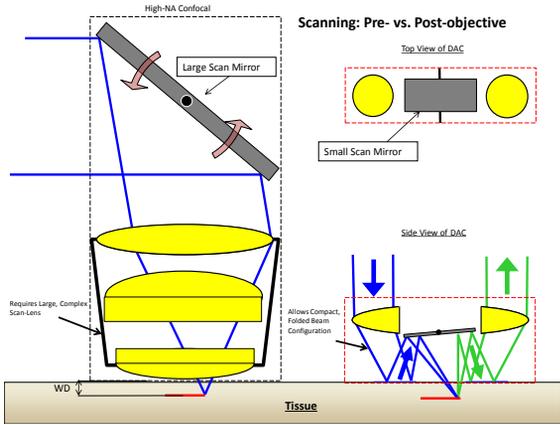
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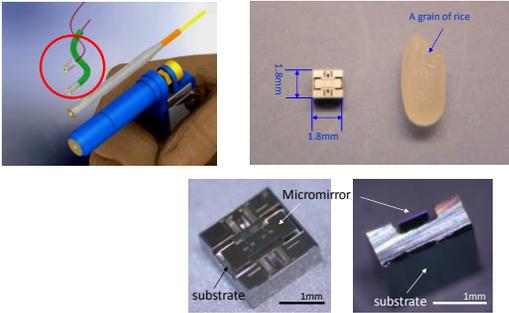
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### MEMS Scanners for Smaller and more Versatile DAC Microscopes



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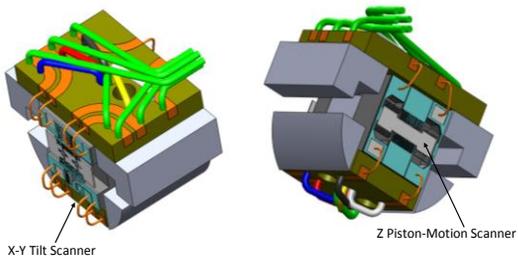
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### 3-Axis MEMS Scanner Assembly



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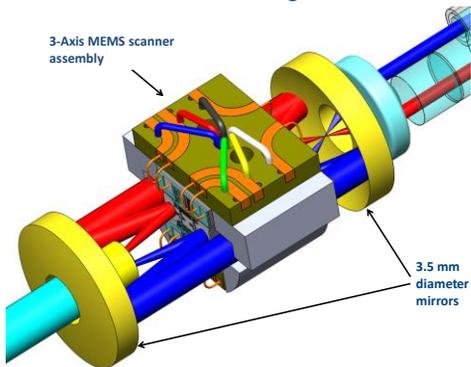
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### 3-D MEMS Scanning Module



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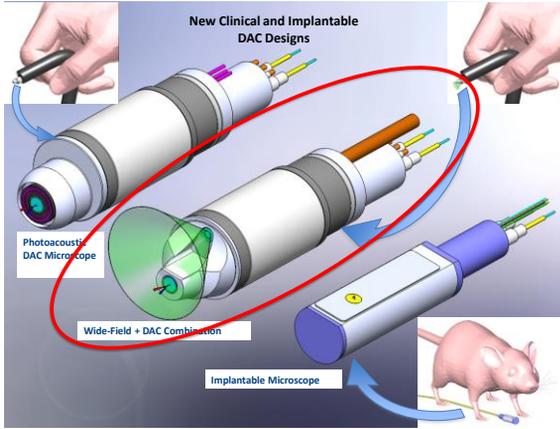
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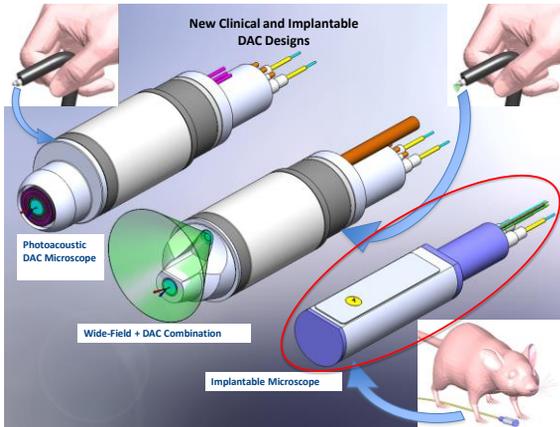
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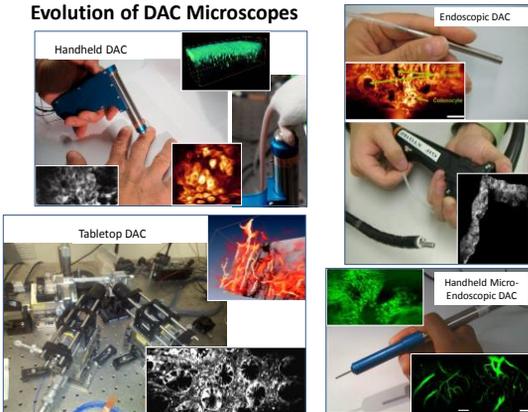
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### Evolution of DAC Microscopes



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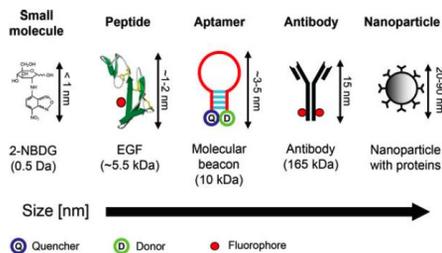
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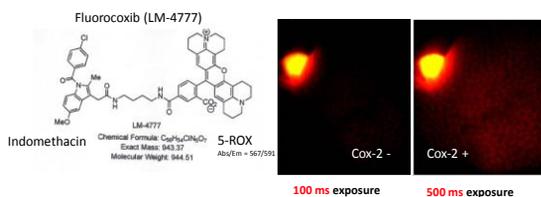
## Molecular Probes for Specific Contrast



Rebecca Richards-Kortum

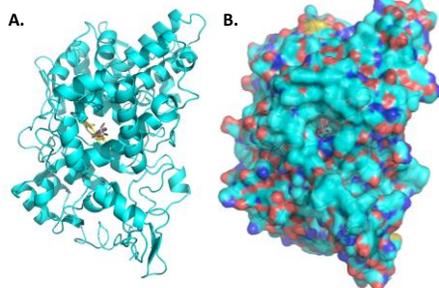
## Fluorocoxib for *In Vivo* Imaging Colon Cancer Cells in Liver of Nude Mouse

- Fluorocoxib (LM-4777) preferentially binds COX-2
- Detection of cancer and inflammation



Uddin, Marnett et al., 2010 (Vanderbilt University)

## Molecular Probe for Cox-2 Enzyme

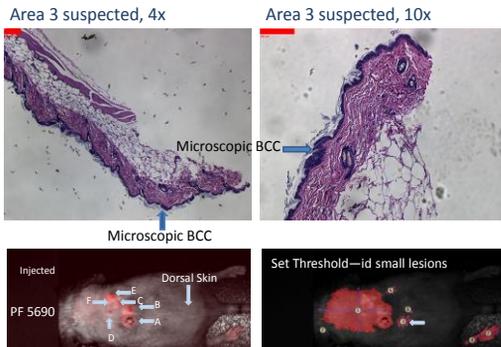


## Basal Cell Carcinoma (BCC)

- Basal Cell Nevus Syndrome
- Mouse Model
  - Ptch1<sup>+/-</sup> K14Cre-ER2 p53<sup>fl/fl</sup>
  - Treated with tamoxifen at age 6 weeks and IR (ionizing radiation) treated at age 8 weeks
  - Macroscopic BCCs develop at age 5 months



## Detection of Microscopic Skin Cancers



Imaging enables the *in vivo* study of cell biology, and when integrated with thorough studies in culture and *ex vivo*, can reveal the **nuances of disease mechanisms** and of **subtlety of therapeutic responses**.

**Visible animal models** of human biology and disease comprise one of the most important contributions of molecular imaging to human health since they have accelerated and refined the analyses of mammalian biology by offering a rapid readout for the development of new therapies.

The challenge now is to use imaging for **early detection**, to target the molecular basis of early disease and to improve prevention.

### Interpretation of In Vivo Data



Intravascular imaging of atherosclerosis—ex vivo

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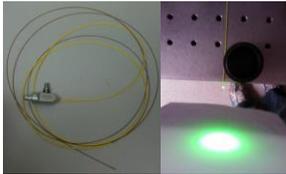
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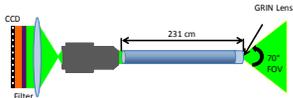
### Widefield Guidance: SpyGlass System



Boston Scientific SpyGlass



PENTAX EB-1170K bronchoscope



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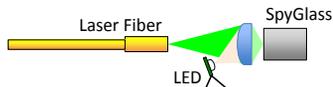
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### Spyglass with LED and Laser Excitation



A high-power white LED was placed beside the laser beam path and coupled off-axis through the lens. The laser and LED can be independently switched on and off.



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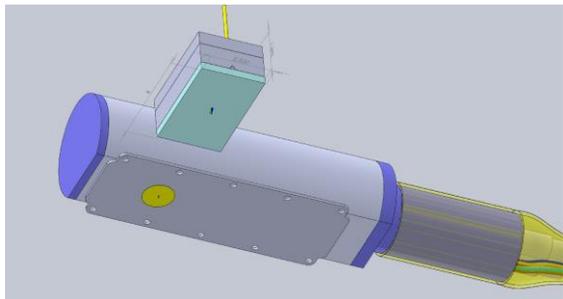
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### Next Generation DAC Designs



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### Developments in Imaging

The nearly two decades Molecular Imaging research has led to advances in reporters, probes and instrumentation that have served to **refine and accelerate studies of mammalian biology**— however, it is time to use imaging approaches to **dramatically change paradigms in medicine and biomedical research.**

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