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

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#### **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



## **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 <u>early and minimal residual</u> <u>disease</u>, understand the origins of disease, develop novel targets, and refine our selection of drug candidates









#### Trade Offs in Resolution and Depth Optical and non-optical imaging modalities





#### **Endoscopy: Imaging and Therapy**



### **Endoscopy Limitations**

- Relies on structural information only.
- Difficult to visualize adenomas (polyps) < 2mm.<sup>1</sup>



• Difficult to detect flat lesions regardless of size.<sup>2,3</sup>



- Random biopsies often taken in suspect areas.<sup>5</sup>
- Difficult to differentiate types of adenomas and carcinomas from inflammation.<sup>6</sup>
- Skill/Experience dependent

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



#### **Dual Axis Confocal Microscope**





#### **Clinical Objectives for Miniaturization** Optical sectioning with histopathologic resolution and molecular contrast

- 1) Specifications 3.2 mm x 10 mm (endoscope compatible)
- Multispectral 50-800 nm
   Dual modality (wide field and vertical scanning
   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)
- As training (vice rate diging)
   Manufacturability (silicon processing)
   Jual-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



Mild adenocarcinoma-table top DAC



# Performance of Dual-Axis Microscopes Anatomic imaging of Barrett's to Squamous Junction—excised tissue



Vertical





## Integrated Macro- and Microscopic Imaging



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

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





Local and global optimization of mosaicing-preserves data quality and enables visualization of larger fields of view.

3-axis scanning using two MEMS scanners









Machined parts for Clinical Multi-Modality DAC Systems





# **Multiplexing with Fluorophores**



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





Multiplexing with Nanoparticles: Surface Enhan	ced
Raman Scattering (SERS)	-



#### Tools to Enable the Transition from Mouse to Man







# Assembly and Use of Scanning Raman Endoscope



# **Circumferential Scanning Raman Endoscope**







**Raman Spectra Acquisition** 









## <u>Multiplexing</u> Molecular Endoscopy: Scanning Raman Endoscope and SERS





## **Spectral Phantom**







**Data Reconstruction & User Display** 





### **Internal Control & Ratiometric Value**







## **Real-time Ratiometric Imaging**



Detection Limit: 56 nanoparticles/cell

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

## **Real-time Ratiometric Imaging**



Account for non-specific accumulation of nanoparticles
 Account for variable working distance

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



# **Mapping Signals in The Colon**

#### Advances in Instrumentation for In Vivo Systems Biology



Multispectral dual axis confocal Wide-field fluorescence Pulsed electron avalanche knife (PEAK) In line microfluidics for rapid multiplexed molecular assays

#### **Optical Imaging of Three Areas**

- Accessible organs
- Skin, oropharynx
- Hollow organs

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- Esophagus, stomach, colon, rectum, intestine, ampulla of vater, uterine cervix, bladder, lungs
   Surgically-accessed organs
- Prostate, ovaries, muscle, bone, breast, brain



#### Lab Members and Collaborators

Contag Lab			
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#### 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

no. Regressed "Tumor"

- Normal liver morphology
- Decreased cancer markers
- Restoration of liver markers
- Genomic stabilization
- Appear metabolically silent

Shachaf et al Nature, 431:1112-1117









# <u>Vision</u>: Scanning Raman Microendoscope for Multiplexed Cancer Screening



## Raman Microendoscope



#### 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



## **Cancer Therapies**

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





Near infra-red (NIR) Window





Imaging over a Range of Scales With Optics











## Components



**Functional Scanning Raman Endoscope** 









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





























































# MEMS Scanners for Smaller and more Versatile DAC Microscopes





A grain of ri

### **3-Axis MEMS Scanner Assembly**





## **3-D MEMS Scanning Module**











**Evolution of DAC Microscopes** 









## **Gastrointestinal Cancers**

- Cancer of the stomach, colon and rectum account for 35% of cancer in Korean men and 28% of cancer in Korean women (health care costs: 659 billion won)
- These three cancers are on the rise in Korea in men and women
- Account for 23% of all cancer deaths in Korea.
- Affects men and women nearly equally and in the next three years there
  is a projected 9% increase in cancer deaths in Korea
- Cancer screening in increasing in Korea—good for prevention but puts stress on the health care system ( colon screening went from 25% to 70% over last 10 years)



Cancer Facts and Figures 2014 in the Republic of Korea-National Cancer Center



#### **Microenvironment: Immune Cells**



MRI-Compatible Implantable DAC Microscope to Examine the Tumor Microenvironment





# Performance of the DAC Microscope Visualizing the Vasculature





Normal

Tumor

Mouse Ear Tumor Model

Jon Liu





## **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





100 ms exposure 500 ms exposure

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

#### Molecular Probe for Cox-2 Enzyme



## **Basal Cell Carcinoma (BCC)**

- Basal Cell Nevus Syndrome
- Mouse Model
  - Ptch1+/- K14Cre-ER2 p53fl/fl
  - Treated with tamoxifen at age 6 weeks and IR (ionizing radiation) treated at age 8 weeks
  - Macroscopic BCCs develop at age 5 months





asal Cell Nevus Syndro (many BCCs)

#### **Detection of Microscopic Skin Cancers**



Area 3 suspected, 10x





Microscopic BCC





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

## Widefield Guidance: SpyGlass System



Boston Scientific SpyGlass







# Spyglass with LED and Laser Excitation



# **Mouse Study**

- Inject Apc<sup>Min</sup> mice with fluorocoxib molecular imaging agent
- Fluorescence signal accumulates in polyps in the intestine and colon





Maestro images of intestinal polyps taken by Hyejun Ra

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showing 50:1 contrast
with fluorocoxib in
small intestinal polyps



Video clips from Apc02 mouse showing uptake of fluorocoxib in a

#### **Rationale for Point-of-care Pathology**

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- Early lesions are small and require microscopic detection Currently diagnosis is made with microscopic analyses of a limited number of biopsies; sampling error Microscopic detection of molecular markers aids in early diagnosis, guides biopsy, enables staging and guides therapy. The time and distance between the patient and the diagnosic event are too great, and the amount of tissue sampled too small Biopsies are taken with limited image guidance in the absence of molecular markers Reimbursement for pathology's restrictive .
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CEA

MIB-1 p53

#### Next Generation DAC Designs



## **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.