Molecular Probes and Instrumentation for Early Detection of Colon Cancer

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Molecular Imaging Program at Stanford (MIPS)
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Visualizing Small Numbers of Tumor Cells
Development of Novel Therapies Targeting **Minimal Residual Disease**

Edinger et al. *Blood*
Model of Minimal Residual Disease and Targeted Therapy
Ectopic Regulation of the Myc Oncogene

Hepatocellular carcinoma derived from triple transgenic mouse:
Tet-O-Myc x LAP-tTA x L2G85
(LAP--Liver activator protein)

Shachaf et al Nature, 431:1112-1117
Reversion to a “Normal” Phenotype

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

Cancer Initiating Cells—Stem Cells

- Cancer initiating and cancer sustaining cells
- Root of the disease—Characterized, in part, by 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 minimal residual disease—clearance by immune response
- Need to visualize cancer stem cells in order to devise effective therapies
Defining Minimally Detectable Tumors

- Imaging, blood test and optical imaging

MRI and CT
D = 1 cm

Mammography
(D = 2 mm)

Minimally detectable tumor size by detecting secretion of biomarkers in blood: PSA, CA125, ...

White light endoscopy

Fluorescence microscopy

30 cell doublings

1 billion cells
- tumor D = 1 cm
- tumor V = 1 cm³
- tumor M = 1 gm

Note: After 30 cell doublings first symptoms may begin to appear

20 cell doublings

1 million cells
- tumor D = 1 mm
- tumor V = 1 mm³
- tumor M = 1 mg

Note: After 20 cell doublings tumor may begin to generate metastatic cells

10 cell doublings

1,000 cells
- tumor D = 100 μm
- tumor V = 0.001 mm³
- tumor M = 1 μg

3 years

6 years:
tumor volume doubling time (TVDT) = 120 days

9 years:
Miniaturization of the Confocal Microscope

Tabletop Microscope

Miniaturization
What is the Smallest Possible Lens?:
This Aeschna dragonfly has over 28,000 lenses in her compound eyes

- Small eye volume/low mass,
- Large field-of-view,
- 1 optical channel (pixel) per lens,
- Low data processing requirements (high flicker rate),
- Low energy requirement (metabolism)
What is the “Price” for a Fully Functional Optical Design?

This is the size of lens needed for achieving the FOV of a fly and also the resolution of the human eye.
High-NA Confocal

Requires Large, Complex Scan-Lens

Side View of DAC

Top View of DAC

Scanning: Pre- vs. Post-objective

Allows Compact, Folded Beam Configuration

Large Scan Mirror

Small Scan Mirror

Tissue
Scanning: Pre- vs. Post-objective

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Tissue
Evolution of DAC Microscopes

First Handheld DAC

Tabletop DAC

Endoscopic DAC

Handheld Micro-DAC for guided brain tumor resection
MEMS Core for Miniaturization

Fixed
Movable

4 µm

micro-mirror
Contrast Agents

Colon

Esophagus

Topical ICG
Colorimetric and fluorescent contrast for macro- and microscopic imaging
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.
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
Performance of Dual-Axis Microscopes
Anatomic imaging of Barrett’s to Squamous Junction—excised tissue


Vertical

Horizontal
MEMS Scanners for Smaller and more Versatile DAC Microscopes
3-axis scanning using two MEMS scanners
3-D MEMS Scanning Module

3-Axis MEMS scanner assembly

3.5 mm diameter mirrors
Achromatic Optical System Allows Operation over Range of 500 nm – 800 nm

Positive Chromatic Aberration

Negative Chromatic Aberration

Corrected
Molecular Probes for Specific Contrast

Rebecca Richards-Kortum
Pan-Cathepsin Probe: BMV-109

- High cathepsin-protease enzyme activity in inflammation- and tumor-associated macrophages
- BMV-109 targets enzyme, covalently binds and de-quenches after cleavage by protease

Ex vivo staining of human polypoid tumor

Sample of healthy human intestine tissue
Sample of human intestinal tumor
In Vivo Sequential Dual-Mode Imaging
Human Tumor in Mouse Colon
Topical Application of Cathepsin probe (BMV109)

White Light

Fluorescence

Boston Scientific SpyGlass
Laser Fiber

GRIN Lens

CCD

LED

70° FOV
Validation: Probe is Bound to Enzymes in Excised Tissue Samples

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<th></th>
<th>Control</th>
<th>Probe (topical)</th>
<th>Probe (i.v.)</th>
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<tbody>
<tr>
<td>Tumor</td>
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After in vivo imaging, covalent binding of the probe to cathepsins can be confirmed with **fluorescent** detection of labeled proteins from the target tissues.
Multiplexing with Fluorophores

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

Multiplexing with Nanoparticles: Surface Enhanced Raman Scattering (SERS)

Tumor Targeting Agent A (Carcinomas)

Tumor Targeting Agent B (Flat Lesions)

Non-specific (Internal Control)

Reference Spectra
Tools to Enable the Transition from Mouse to Man

Miniaturization

instrument channel

Endoscope

Fiber Bundle (1.7 mm Diameter)

1-mm Diameter Collimated Illumination Beam

variable working distance

1 single mode Illumination Fiber (5 µm core and 120 µm cladding)

36 multimode fibers (200 µm core and 40 µm cladding)
Components

- Flexible Extrusion
- Lens Housing
- Fiber Bundle
- Concave Lens
- Convex Lens
- Window
- Shim
- Mirror
- Motor Housing
- Motor
- Convex Lens Shim
Multiplexing Molecular Endoscopy: Scanning Raman Endoscope and SERS

A

Multiplexing Molecular Endoscopy: Scanning Raman Endoscope and SERS

B

Mix of S440 +

C

Mix of 4
Mix of 6
Mix of 8
Mix of 10

D

Mix of 4 Varying Concent.

Raman Signal vs. SERS Concentration at 42 mW

y = 0.00002x - 0.0046
R² = 0.9987

Human colon
Spectral Phantom

S493 S440 S482 S420 S481 S421 All 6 Flavors
Simulated Tumor Hunt—Real time
Mapping Signals in The Colon

**STRUCTURE**
Surface Topology From Intrinsic Raman
- Human

**FUNCTION**
Ratiometric Image: \[
\frac{(S420)}{(S493)} - 1
\]
- Porcine
## Lab Members and Collaborators

### Contag Lab

<table>
<thead>
<tr>
<th>Name</th>
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### Collaborators

#### Mini-microscopes
- Gordon Kino, Stanford
- Olav Solgaard, Stanford
- Pierre Khuri-Yakub, Stanford

#### Pathology
- Jim Crawford, Hofstra School of Med.
- David Rimm, Yale University

#### Clinical
- Jacques Van Dam, USC
- Shai Friedland, Palo Alto VA
- Jean Tang, Stanford

#### siRNA and skin delivery
- Roger Kaspar

#### Probe Chemistry
- Paul Wender, Stanford
- Christina Zavaletta, Stanford
- Matt Bogyo, Stanford
- Larry Marnett, Vanderbilt
- Dave Ostrov, Univ. of Florida
- Sam Gambhir, Stanford