Making Sense of Optical Contrast For Point-of-Care Pathology

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Outline

• Current paradigm of histology for cancer diagnosis
• Dual-axis confocal (DAC) fluorescence microscopy
• Emulating H&E (hematoxylin & eosin) tissue staining with DAC microscopy
• DAC imaging and machine learning to identify cancer
Current Paradigm of Histology for Cancer Diagnosis
Key Limitations of Current State-of-the-Art Tissue Microscopy

• Conventional hematoxylin and eosin (H&E) stained pathology slides take 6-8 hours to prepare
• They require a multi-step process with skilled but repetitive manual steps
• They result in a 5 micron thick section

Slide courtesy of David Rimm, Yale
Tissue removed from patient

Tissue placed in transport media (fixative)

Gross examination and description

Dissection and preparation of sample cassettes according to protocol

Further fixation and processing included paraffin embedding

Mounting at embedding station and microtome sectioning of tissue blocks

Deparaffinization, staining of tissue and coverslipping slides

To Pathologist

From Fresh Tissue to Glass Slide

Takes 6-8 hours

Slide courtesy of David Rimm, Yale
Dual-Axis Confocal Microscopy Benefits

• Optical sectioning without cutting tissue
• Image stacks: hundreds of tissue sections in one 3D acquisition
• With miniature microscopes, tissue sections can be analyzed in the operating room or gross pathology room
Dual-Axis Confocal Fluorescence Microscopy
Dual-Axis Confocal (DAC) Microscopy

Comparing two confocal scanning microscope architectures:

1. Uses simple low-NA optics to obtain thin optical-section thickness $\Delta Z$
2. Reduces scattering noise in 3-D volume image
3. Easy to miniaturize using a MEMS scanner

Miniaturized systems enable confocal microscopy at the point of care

MEMS mirrors developed by the Solgaard Lab at Stanford
Current DAC Microscopes

Handheld DAC

Tabletop DAC

Endoscopic DAC

Micro-Endoscopic DAC
Image-guided Tissue Sampling and Multiplexed Molecular Analysis

Wide Field Guidance

PEAK Microsurgical and Sampling (Palanker lab)

Microscopy
Emulating H&E (Hematoxylin & Eosin) Staining with DAC Microscopy
Volumetric Imaging of Colon Tissue

- Standard H&E stain
- **Hematoxylin**: stains nucleic acids (blue-purple)
- **Eosin**: stains proteins (pink)

- DAC imaging requires fluorescent dyes
- FDA-approved clinical dyes:
  - **Indocyanine green (ICG)**: stains membrane & extracellular matrix (ECM)
  - **Methylene blue**: stains cytoplasm & nucleus
- Nuclear-specific dyes for ex-vivo use
  - **DRAQ5**
  - **DAPI** (requires UV light)
Collaboration with David Rimm, Yale

Volumetric Imaging of Colon Tissue
Image Processing to Emulate H&E Staining

- 2x2x9 averaging (XxYxZ) to improve SNR
- Start with blank (white) image
- Invert grayscale images and color map (785/ICG => magenta, 660/DRAQ5 => blue). In inverted images, darker => more fluorescence
- Use non-inverted grayscale images for alpha channel (transparency). For transparency mask, darker => more transparent
- Overlay both inverted, color-mapped images onto the white background using respective transparency masks
DRAQ5 + methylene blue + ICG

Colon Tissues

DRAQ5 + ICG
Vision: DAC Array in the Gross Pathology Room

"Google Maps"-like pan and zoom
DAC Imaging and Machine Learning to Identify Cancer
Guided Resection by Morphological Analysis

- After bulk resection, the DAC microscope can image margins with FDA-approved dyes, 300-um deep in tissue
- Machine learning algorithms can be trained to identify cancerous margins

Northcott et al., 2012
Machine Learning to Identify Cancer

• Developed by Kirk Gossage, PhD
• Texture-based and neural-network-derived segmentation algorithm
• Contag Lab collaborates with Levenson Lab at UC Davis to apply the algorithm to DAC images

• Breast cancer tissue stained with hematoxylin and p53 immunostain
• **Hematoxylin channel alone** (unmixed) used to identify cancer regions (pink overlay)

Entire process takes less than 30 s

Slide courtesy of Richard Levenson, UC Davis
Proof-of-Concept Experiment with DAC Microscope

- SCID mice with MDA-MB-231 human breast cancer xenografts in the mammary fat pad were sacrificed from another study.
- Tumors were collected along with normal brain tissue (cerebellum). Breast cancer and brain tissue should be easy for a computer to distinguish.
- 3 tumor and 3 normal cerebellum samples were soaked in ICG and imaged on the DAC microscope.
- One tumor/normal pair imaged twice for a total of 4 tumor and 4 normal image stacks.
- 63 optical sections were hand-picked from the data sets for computer analysis.
Tumors

Cerebellum

Breast cancer vs. normal cerebellum, ICG staining
Machine Learning: Flank Tumors vs. Cerebellum

- Normal cerebellum
- MDA-MB-231 tumors

- Collaboration with Richard Levenson (UC Davis)

- 24 normal cerebellum and 39 breast cancer flank tumor sections

- About 15 of the images were used as a training set
Larger Data Set for Medulloblastoma Detection

8 ul of concentrated GFP-luc DAOY cells were injected into 8 weeks or older nude mice under anaesthesia. Examples show imaging of orthotropic and flank models 10 minutes after mice were injected IP with a 100 ul of luciferin.

Slide courtesy of Markus Deutschmann
Summary

• Current histological analysis is labor-intensive and time consuming
• DAC microscopy offers rapid optical sectioning and may emulate traditional H&E staining or guide selection of tissues for traditional processing
• DAC imaging with FDA-approved dyes and computer vision analysis may guide surgical resection by identifying cancer margins at the point of care
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