Optical Imaging for Neuroscience and Developmental Biology

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Bioengineering
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Outline

• Introduction
  ➢ Biomedical Imaging Modalities
  ➢ Why Optical Imaging?
  ➢ Optical Biopsy with Optical Coherence Tomography (OCT) and Microscopy (OCM)

• Applications in Neuroscience and Developmental Biology
  ➢ 3D imaging of brain slices
  ➢ Evaluate heart function in fruit flies

• Summary
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• Summary
BIOMEDICAL IMAGING MODALITIES

- X-Ray
- Computed Tomography (CT)
- Positron Emission Tomography (PET)
- Magnetic Resonance Imaging (MRI)
- Ultrasonography (US)
- Optical Imaging
X-RAY

✓ Discovered in 1895 by Wilhelm Conrad Röntgen, who received the first Nobel Prize in Physics in 1901.
X-RAY
X-RAY

Ionizing radiation
COMPUTED TOMOGRAPHY (CT)

✓ Invented in 1971 by Allan Cormack and Godfrey Hounsfield, who shared the 1979 Nobel Prize for Physiology or Medicine
COMPUTED TOMOGRAPHY (CT)
POSITRON EMISSION TOMOGRAPHY (PET)

- Concept was introduced by David E. Kuhl, Luke Chapman and Roy Edwards in the late 1950s.
- Was further developed by Michel Ter-Pogossian, Michael E. Phelps and others.
POSITRON EMISSION TOMOGRAPHY (PET)

- Inject radioactive tracer, Fluorodeoxy-D-glucose (FDG), an analogue of glucose.
- Pairs of gamma rays emitted by the tracer were detected.
- The concentrations of tracer give tissue metabolic activity proportional to tissue glucose uptake.
POSITRON EMISSION TOMOGRAPHY (PET)
MAGNETIC RESONANCE IMAGING (MRI)

- Paul Lauterbur (University of Illinois) demonstrated first MRI image in living mouse in 1974.
- Peter Mansfield (University of Nottingham) demonstrated first MRI image in human in 1977.
- They won the Nobel Prize for Physiology or Medicine in 2003.
MAGNETIC RESONANCE IMAGING (MRI)
ULTRASONOGRAPHY (US)

- First applied to the human body by Dr. George Ludwig at the Naval Medical Research Institute in 1940s.
- Typically, 2 to 18 megahertz, though frequencies up to 50–100 megahertz have been used experimentally.
ULTRASONOGRAPHY (US)
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## WHY OPTICAL IMAGING?

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>X-Ray</th>
<th>CT</th>
<th>PET</th>
<th>MRI</th>
<th>US</th>
<th>Optical Imaging</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionizing Radiation</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Spatial Resolution</td>
<td>mm-cm</td>
<td>mm-cm</td>
<td>cm</td>
<td>mm</td>
<td>100um - mm</td>
<td>Um to sub-um</td>
</tr>
<tr>
<td>Temporal Resolution</td>
<td>second</td>
<td>min</td>
<td>Tens of min</td>
<td>min</td>
<td>Sub-second</td>
<td>Sub-second</td>
</tr>
<tr>
<td>Contrast</td>
<td>Tissue density</td>
<td>Tissue density</td>
<td>Contrast agents</td>
<td>Tissue parametric property</td>
<td>Tissue mechanical properties</td>
<td>Intrinsic contrast / contrast agents</td>
</tr>
<tr>
<td>3D capability</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Cost</td>
<td>$</td>
<td>$$$</td>
<td>$$$</td>
<td>$$$</td>
<td>$$</td>
<td>$</td>
</tr>
</tbody>
</table>

**Characteristics Summary:**
- **Ionizing Radiation:**
  - X-Ray: Yes
  - CT: Yes
  - PET: Yes
  - MRI: No
  - US: No
  - Optical Imaging: No
- **Spatial Resolution:**
  - X-Ray: mm-cm
  - CT: mm-cm
  - PET: cm
  - MRI: mm
  - US: 100um - mm
  - Optical Imaging: Um to sub-um
- **Temporal Resolution:**
  - X-Ray: second
  - CT: min
  - PET: Tens of min
  - MRI: min
  - US: Sub-second
  - Optical Imaging: Sub-second
- **Contrast:**
  - X-Ray: Tissue density
  - CT: Tissue density
  - PET: Contrast agents
  - MRI: Tissue parametric property
  - US: Tissue mechanical properties
  - Optical Imaging: Intrinsic contrast / contrast agents
- **Imaging Depth:**
  - X-Ray: Deep
  - CT: Deep
  - PET: Deep
  - MRI: Deep
  - US: Deep
  - Optical Imaging: Shallow
- **3D capability:**
  - X-Ray: No
  - CT: Yes
  - PET: Yes
  - MRI: Yes
  - US: Yes
  - Optical Imaging: Yes
- **Cost:**
  - X-Ray: $ 
  - CT: $$$
  - PET: $$$
  - MRI: $$$
  - US: $$
  - Optical Imaging: $
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BIOPSY

Benign

Several days to weeks!!!

Tumor
In situ, real-time imaging of tissue microstructure with a resolution approaching that of histology, without the need for tissue excision and processing.

Especially important in situations where excisional biopsy is either hazardous or impossible, e.g., in ophthalmic or cardiovascular applications, neuroscience and developmental biology.

OBJECTIVE: OPTICAL BIOPSY
HIGH RESOLUTION SUBSURFACE IMAGING

- Standard Clinical
- High Frequency
- Optical Coherence Tomography
- Confocal Microscopy
- Optical Coherence Microscopy

RESOLUTION (log)

IMAGE PENETRATION (log)
OPTICAL COHERENCE TOMOGRAPHY (OCT)

OCT IN OPHTHALMOLOGY

Fovea to optic disc

HIGH SPEED, ULTRAHIGH RESOLUTION OCT
(250,000 – 400,000 A-lines/s)

High Speed, Ultrahigh Resolution OCT of Human Retina

High Speed OCT of Human Anterior Segment

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Organotypic Hippocampal Slice Cultures

Characteristics:

a. Hippocampus of 7-day old Sprague-Dawley rats

b. ~300µm thick

Cheung and Cardinal *BMC Neuroscience* 2005

Collaboration with Dr. Yevgeny Berdichevsky at Lehigh
Comparison of OCM and Confocal Images

Nuclei: anti-NeuN

F. Li, et al, Neurophotonics, 2014
Quantify Neurons in 3D

CA3: Cornuammonis III
CA1: Cornuammonis I
DG: Dentate gyrus

Chu et al., Journal of Molecular Histology, 2007
Evaluation of seizures-induced neuronal injury as days *in vitro* increased using OCM

****: p < 0.01 and ***: p < 0.001

DIV7: n=18
DIV14: n=11
DIV21: n=18
DIV28: n=9
Slice thickness measurement as days *in vitro* increased using OCM.

The cross-sectional images are from CA3 region.

**p < 0.01 and ***p < 0.001**

DIV7: n=18; DIV14: n=11; DIV21: n=18; DIV28: n=9
Neuroprotective Effects of KYNA

- CTRL
- KYNA

400 µm

200 µm

DIV7 DIV21

DIV21

*: p < 0.05 and ***: p < 0.001

n = 9 in each group
Slice thickness in control and KYNA group

The cross-sectional images are from CA3 region

DIV7
CTRL

CA1 CTRL CA1 KYNA CA3 CTRL CA3 KYNA

*: p < 0.05 and ***: p < 0.001
n = 9 in each group
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OCT IMAGING OF THE DROSOPHILA HEART

Collaboration with Drs. Rudolph Tanzi and Airong Li at MGH
OCT IMAGING OF THE DROSOPHILA HEART

Longitudinal View

Cross-sectional View
- Diastolic Phase

Cross-sectional View
- Systolic Phase
OCT Imaging of the Drosophila Heart

24B-GAL4/+ (Control)

UAS-dPsn; 24B-GAL4 (Over-expression of dPsn)

UAS-dPsnRNAi; 24B-GAL4 (Silencing of dPsn)

M-Mode Imaging
<table>
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<tr>
<th>Parameters</th>
<th>7 Day old</th>
<th>30 Day old</th>
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<tbody>
<tr>
<td></td>
<td>24B-GAL4/+ (Control) N=31</td>
<td>UAS-dPsn; 24B-GAL4 N=31</td>
</tr>
<tr>
<td>HR (BPM)</td>
<td>262 ± 10</td>
<td>307 ± 11</td>
</tr>
<tr>
<td></td>
<td>***↑</td>
<td>*↓</td>
</tr>
<tr>
<td>ESD (µm)</td>
<td>20 ± 2</td>
<td>14 ± 2</td>
</tr>
<tr>
<td></td>
<td>17 ± 2</td>
<td>17 ± 1</td>
</tr>
<tr>
<td>EDD (µm)</td>
<td>67 ± 2</td>
<td>56 ± 3</td>
</tr>
<tr>
<td></td>
<td>*↓</td>
<td>**↓</td>
</tr>
<tr>
<td>FS (%)</td>
<td>69 ± 4</td>
<td>76 ± 3</td>
</tr>
<tr>
<td></td>
<td>67 ± 4</td>
<td>71 ± 2</td>
</tr>
</tbody>
</table>

HR: Heart rate; EDD: End-diastolic dimension; ESD: End-systolic dimension; FS: Fractional shortening

*p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001: vs. Age-matched controls;
#p<0.05, ## p<0.01, ### p<0.001, #### p<0.0001: vs. 7-day old age group
↑ shows significant increase;  ↓ shows significant decrease

DROSOPHILA HEART DEVELOPMENT

2nd instar larva – L2
DROSOPHILA HEART DEVELOPMENT

3rd instar larva – L3
DROSOPHILA HEART DEVELOPMENT

Pupa day 1, 8hr – PD1
DROSOPHILA HEART DEVELOPMENT

Pupa day 2, 32hr – PD2
DROSO PHILA HEART DEVELOPMENT

Pupa day 3, 72hr – PD3
DROSOPHILA HEART DEVELOPMENT

Pupa day 4, 88hr – PD4
DROSOPHILA HEART DEVELOPMENT

Adult day 1
DROSOPHILA HEART DEVELOPMENT
DROSOPHILA HEART RATE

A. Alex, et al, under review
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Various imaging modalities can be used for clinical and research applications

Optical imaging provides unique advantages (resolution, contrast, etc.)

Optical biopsy can be achieved by OCT and OCM

None-invasive evaluation of epilepsy models in rat brain slices

None-invasive characterization of heart function in fruit flies
ECE 368/468, BioE 368/468
Introduction to Biophotonics / Optical Biomedical Imaging

Spring, 2015
Thank you!