Motivation Outline Tissue Optics Optical Coherence Tomography Architectonics Structures Connectivity

Optical Coherence Tomography
Inferring architectonic structures and connectivity in the brain

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Great interest in architectonic structures

- diseases and disorders
- fMRI
- connectivity

⇒ Projects on *ex vivo* imaging

MRI (sequences, post processing, registration to *in vivo*)
- + whole brain
- + higher resolution
- + some architectonic structures revealed
- - brain dependent (aging, fixation, PMI...)
- - a lot of cortical areas can’t be delimited

⇒ New direction: OCT

Histology (validation for MRI, guide)
- + whole brain
- + cellular resolution
- + gold standard for architectonic structures
- - labor intensive
- - distortions /deformations due to cutting, mounting and staining
- - different dyes for different interests (cells, myelinated fibers)
NEW DIRECTION

- What is OCT?
  - Optical: use of light
  - Coherence: use of low coherence interferometry
  - Tomography: 3D volume

- Why use OCT?
  - high resolution: up to 1 \( \mu \text{m} \)
    \( \Rightarrow \) cells (neurons): cytoarchitecture
    \( \Rightarrow \) fibers: myelooarchitecture & connectivity
  - relies on intrinsic optical properties
    \( \Rightarrow \) no staining
  - image the fixed blockface
    \( \Rightarrow \) no or less deformation (compare to histology)
1. TISSUE OPTICS
   - Basics
   - Tissue Optical Window

2. OPTICAL COHERENCE TOMOGRAPHY
   - Principle
   - Spectral Domain OCT/OCM
   - Image Processing

3. ARCHITECTONICS STRUCTURES
   - Ex vivo MRI vs. OCT
   - Cortical boundary
   - Nissl Stain vs. OCT
   - Assessment of deformations

4. CONNECTIVITY
   - Principle and Process
   - DTI vs. OCT
   - Future work
Motivation Outline

Tissue Optics

Optical Coherence Tomography

Architectonics Structures

Connectivity

Tissue Optics: Basics

- Cells (e.g. neurons)
- Myelinated fibers (the myelin sheath has a high refractive index)
Tissue Optical Window

Motivation Outline

Tissue Optics

Optical Coherence Tomography

Architectonics Structures

Connectivity

Tissue Optical Window

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Optical Coherence Tomography

1. Principle
2. Spectral Domain OCT/OCM
3. Image Processing
Principle

- Introduced by Fujimoto ¹
- 3D technique with high resolution
- Analogue to UltraSound

\[
\text{Speed of sound} : \quad 1480 \text{ m.s}^{-1} \quad (\text{in water})
\]
\[
\text{Speed of light} : \quad 3 \times 10^8 \text{ m.s}^{-1}
\]

**PRINCIPLE: RESOLUTION / PENETRATION**

- **Resolution (log)**: OCT, Confocal microscopy, Ultrasound, Standard clinical.
- **Penetration depth (log)**: 1 mm, 1 cm, 10 cm.

- OCT: 10 μm, 100 μm, 1 mm.
- Confocal microscopy: 1 μm.
- Ultrasound: High frequency, Standard clinical, 1 cm, 10 cm.

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**Optical Coherence Tomography**
**Principle: Interferometry**

Light is too fast ⇒ Interference between the reflected light and a reference light

- **High Coherent source**
  - (e.g. monochromatic laser)

- **Low coherent source**
  - (e.g. superluminescent diode)

[Diagram of Michelson interferometer with light source and detector]
**PRINCIPLE:** **TIME DOMAIN OCT (TD-OCT)**

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- **Optical Coherence Tomography**
SPECTRAL DOMAIN OCT (SD-OCT)$^2$

- No movement of the reference mirror
- Faster (1 spectra = 1 depth profile)
- Reduced losses

$^2$V. J. Srinivasan, et al., "Optical coherence microscopy for deep tissue imaging of the cerebral cortex with intrinsic contrast", Optics Express, 20(3), 2012
SD-OCT vs TD-OCT

**TD-OCT**
- axial resolution FWHM
- intensity vs time

**FD-OCT**
- bandwidth FWHM
- intensity vs optical frequency

**Depth profile**
- Z-axis with interfaces z_1, z_2, z_3

**I(σ)**
- z_0, z_1, z_2, z_3
- Partially reflecting interfaces

**FT**
- Fourier transform of the intensity signals

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Optical Coherence Tomography
EXAMPLE
Motivation Outline Tissue Optics **Optical Coherence Tomography** Architectonics Structures Connectivity

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# Resolutions and Depth of Focus

- Axial Resolution: depends on light source bandwidth \( \Delta \lambda \)

\[
l_c = \frac{2 \ln 2}{\pi} \frac{\lambda_0^2}{\Delta \lambda}
\]

- Lateral Resolution: depends on the objective (Numerical Aperture)

\[
\Delta x = \frac{2\lambda_0}{\pi NA}
\]

- Depth of focus: depends on the objective (Numerical Aperture)

\[
b = \pi \frac{\Delta x^2}{2\lambda_0}
\]
**Example: Medulla (Brain Stem)**

SuperLuminescent Diode (SLD)

- $\lambda_0 = 1310\text{nm}$
- $\Delta\lambda = 170\text{nm}$

**Obj. 10x, NA 0.3**
- Axial res. 3.5 $\mu$m
- Lateral res. 3 $\mu$m
- Depth of focus 20 $\mu$m

**Obj. 40x, NA 0.8**
- Axial res. 3.5 $\mu$m
- Lateral res. 1 $\mu$m
- Depth of focus 3 $\mu$m
Image Processing

- 1 acquired volume = 1.5 mm × 1.5 mm × 1.5 mm (10x obj.)
- Average Intensity Projection over 400 µm below the surface
- Maximum Intensity Projection over 400 µm below the surface
- XY translation stage
- Stitching: Fiji plug-in based on Fourier shift theorem \(^3\)

\(^3\) S. Preibisch, S. Saalfeld, P. Tomancak, ”Globally optimal stitching of tiled 3D microscopic image acquisitions”, Bioinformatics, 25(11), 2009
Isocortex, Brodmann areas 36 and 20

Average Intensity Projection

Maximum Intensity Projection

Cytoarchitecture

Myeloarchitecture
Architectonics Structures

1. ex vivo MRI vs. OCT
2. Cortical boundary: EC / PC
3. Nissl stain vs. OCT: isocortex
4. Assessment of deformations
ex vivo MRI vs. OCT: hippocampus

7T, FLASH, 60 µm³, TE=22ms, TR=55ms, FA=25°, 1 run

OCT
lateral resolution: 3 µm

73 y.o., Male, PMI < 24hrs, length of fixation 4 months

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ENTORHINAL / PERIRHINAL CORTEX BOUNDARY

7T, FLASH, 100 µm³, TE=20ms, TR=40ms, FA=20°

67 y.o., Male, PMI 12hrs
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**Entorhinal / Perirhinal Cortex boundary**

**Average Intensity Projection**

- dysgranular IV starts
- 35 oblique layer
- 35b
- 35a
- PC
- EC

**Maximum Intensity Projection**

- oblique layer in 35
- EC/PC border
- EC layer IV
- EC Layer II
Entorhinal / Perirhinal Cortex boundary

- Oblique layer in 35
- Layer IV abruptly ends
- EC layer II
- Columns

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Nissl Stain vs. OCT: isocortex

Gold standard: Nissl stain

OCT (Average Intensity)
Deformations: registration to the blockface

Blockface
Connectivity

OCT-based Tractography

1. Principle and Process
2. Comparison with DTI
INFLUENCE OF RADIUS: SIMPLE CASE

Area size 151 μm
FT, pad 500 pix
FT ODF

Area size 299 μm
FT, pad 500 pix
FT ODF

Area size 1001 μm
FT, pad 500 pix
FT ODF
## MULTIPLE DIRECTIONS

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<th>Area Size</th>
<th>FT, pad 500 pix</th>
<th>FT ODF</th>
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Optical Coherence Tomography
DTI vs. OCT

DTI: 4.7T, 300µm³, 2 low-b img, 20 dir, 
b=2048, TE=28ms, TR=320ms, FA=180°

OCT: lateral resolution = 3µm
ODF :area 300µm, step 150µm
Future work

- 3D reconstruction / 3D ODF
  - Vibrotome + XYZ Translation Stages
    - Tim Ragan, Phil Knodle (Tissue Vision) \(^5\)
    - Octopus
  - Imaging a volume of human brain (several cm\(^3\))

- Registration with MRI
- Comparison with DTI and DSI
- Polarization-sensitive OCT \(^6\)

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