Introduction to Computational Fluorescence Microscopy

EE367/CS448I: Computational Imaging and Display
stanford.edu/class/ee367
Winter 2016, Lecture 14

Gordon Wetzstein
Stanford University
H. Rankin, transgenic *xenopus laevis* (African Clawed Toad) tadpole neurons (green); technique: confocal 10x
M. Kandasamy, stained cells: actin (pink), DNA (yellow), mitochondria (green); technique: super resolution microscopy
M. Boyle, larva of nephasoma pellucidum (peanut worm); technique: confocal 40X
D. Burnette, osteosarcoma cell (bone cancer) showing actin (purple), mitochondria (yellow), DNA (blue); technique: structured illumination microscopy (SIM)
T. Deerinck, HeLa cells with microtubules; technique: 2-photon microscopy 300X
Nikon Small World Competition

- annual photography competition, see www.microscopyu.com/smallworld/gallery/
- showed only fluorescent samples (many others in the gallery)
- this lecture: overview of fluorescence microscopy techniques
OBAMA'S BRAIN
The White House has set lofty objectives for its BRAIN Initiative. Now it is up to the participants (purple) to develop a strategy for the programme.

PRIVATE RESEARCH
- The Allen Institute for Brain Science $60 million annually
- Howard Hughes Medical Institute $30 million annually
- Kavli Foundation $4 million annually for 10 years
- Salk Institute for Biological Studies $28 million

FEDERAL AGENCIES
(First year funding)
- Defense Advanced Research Projects Agency $50 million
- National Institutes of Health $40 million
- National Science Foundation $20 million

OBJECTIVES
- Provide the knowledge for addressing debilitating disorders.
- Develop new imaging technologies and understand how information is stored and processed in neural networks.
- Understand how brain activity leads to perception, decision-making and, ultimately, action.
- Produce a sophisticated understanding of the brain, from individual genes to neuronal circuits to behaviour.

RESEARCH COMMUNITY
- The National Science Foundation convened workshops to solicit ideas.
- The National Institutes of Health set up a ‘dream team’ of 15 scientists that is producing reports based on community feedback.

source: white house & nature
Brain Initiative

• frontier of science (past frontiers: fly to moon, decode human genome)

• two key factors: fluorescence microscopy & computational illumination
Widefield Microscopy

![Diagram of Widefield Microscopy](source: microscopyu)
Microscope Objective

Anatomy of the Microscope Objective

- Decorative Barrel
- Manufacturer
- Specifications
- Immersion Adjustment Collar
- Magnification and Immersion Color Codes
- Threads
- Stop Face
- Spring System
- Lens Error Correction Lens Group
- Correction Collar
- Front Lens

source: Zeiss
The Diffraction Limit

Ernst Abbe, 1905

source: wikipedia
The Diffraction Limit

\[ d = \frac{\lambda}{2n \sin \alpha} = \frac{\lambda}{2NA} \]
The Diffraction Limit

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The Diffraction Limit

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Airy disk
The Diffraction Limit

\[ d = \frac{\lambda}{2n \sin \alpha} = \frac{\lambda}{2 NA} \]
Lateral and Axial Resolution & Missing Cone
Fluorescence Microscopy

- excitation and emission
- coherence / incoherence
- fluorescent labels
- calcium imaging
Fluorescence Microscopy (epi setup)
Fluorescence Microscopy (epi setup)

source: Nikon MicroscopyU

source: wikipedia
Sensors used in Microscopy

- e.g., Andor iXon Ultra 897: cooled to -100° C or Hamamatsu Ocra Flash4.0 V2
- scientific CMOS & CCD (~20-50K)
- reduce pretty much all noise, except for photon or shot noise
Fluorescence Microscopy - Challenges

- inherently 2D – need 3D for active brain imaging
- higher-resolution in 2D and 3D
- scattering
- larger fields of view, bleaching
- solution: engineer detection and illumination optics, algorithms, chemistry
Fluorescence Microscopy - Challenges

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Superresolution Fluorescence Microscopy

- stimulated emission-depletion (STED) microscopy

- localization microscopy
  - 2D: STORM/PALM etc.
  - 3D: double helix PSF
  - localization algorithms

- structured illumination microscopy (SIM)
2014 Nobel Price in Chemistry: super-resolved fluorescence microscopy

Eric Betzig
(Howard Hughes Institute)

Stefan Hell
(Max Planck Institute)

W. E. Moerner
(Stanford)
Stimulated Emission-Depletion (STED) Microscopy

Excitation spot  De-excitation spot  Emitted spot

source: wikipedia
Stimulated Emission-Depletion (STED) Microscopy
Localization Microscopy: PALM / STORM

Figure 1: Basic Principle of STORM Superresolution Imaging

(a) Original image
(b) Focused image
(c) Combined image
(d) Focused image
(e) Combined image
(f) Focused image

Figure 4: Superresolution Imaging of Microtubules with STORM

(a) 3 μm
(b) 500 nm
(c) 3 μm
(d) 500 nm
Structured Illumination Microscopy (SIM)

Resolution Enhancement by Structured Illumination Microscopy

Figure 8

... whiteboard ...
3D Fluorescence Microscopy

- confocal microscopy
- 2 photon microscopy
- light sheet microscopy
- 3D deconvolution microscopy / focal stacks
- others: spinning disk confocal, aperture correlation, …
Confocal Microscopy

FIG. 3.

INVENTOR.

BY

1957
Confocal Microscopy
Widefield vs Confocal – Thin Sample

source: http://microscopysolutions.ca/
Widefield vs Confocal – Thick Sample

source: http://microscopysolutions.ca/
2-Photon Microscopy

2-Photon Microscopy

3D Deconvolution Microscopy

Fluorescent Beads Focused and Defocused

\[
f = H g
\]

\[
N_p \times 1
\]

\[
N_p \times N_e
\]

\[
N_e \times 1
\]

\[
\text{index}
\]

\[
n_D = 1.5140
\]
3D Deconvolution Microscopy
Light Sheet Microscopy

- invented by R. Zsigmondy, Nobel price in 1925
- Nature Method of the Year 2014
Light Field Microscopy

- can do refocus, but more interesting: instantaneous 3D volume (for fluorescence)!
- diffraction becomes an issue

[Levoy et al. 2006]
Light Field Microscopy

(a) 

(b) 
Native Image Plane  
Tube Lens  
Objective  
Native Object Plane  

(c) 

(d) 
Experimental light field  

Levoy Group, Stanford
Light Field Microscopy

\[
\begin{align*}
\text{Light Field} & \quad \begin{pmatrix}
\vdots \\
\vdots \\
\vdots \\
\end{pmatrix} \\
N_p \times 1 & \quad \begin{pmatrix}
\vdots \\
\vdots \\
\vdots \\
\end{pmatrix} \\
\text{Measurement Matrix} & \quad \begin{pmatrix}
\vdots \\
\vdots \\
\vdots \\
\end{pmatrix} \\
N_p \times N_v & \quad \begin{pmatrix}
\vdots \\
\vdots \\
\vdots \\
\end{pmatrix} \\
\text{Volume} & \quad \begin{pmatrix}
\vdots \\
\vdots \\
\vdots \\
\end{pmatrix} \\
N_v \times 1 & \quad \begin{pmatrix}
\vdots \\
\vdots \\
\vdots \\
\end{pmatrix}
\end{align*}
\]

Levoy Group, Stanford
3D Light Field Deconvolution

- light field contains aliasing
- use 3D deconvolution to get higher resolution

[Broxton et al. 2013]
3D Light Field Deconvolution

- lateral resolution is depth dependent!

[Broxton et al. 2013]
Functional 3D Brain Imaging

C. elegans

[Prevedel et al. 2014]
Functional 3D Brain Imaging

Captured Light Field

optics design by Marc Levoy

[Prevedel et al. 2014]
maximum intensity projection of volume
350um x 350 um x 24 um at 50Hz
~70 neurons in head region

[Prevedel et al. 2014]
Next: Midterm!

• bring laptop or calculator!
• bring slides (download on your computer beforehand)
• focus on classes 2-11
• don’t be late! we’ll start at 11:30 pm and you have until 12:50pm
• good luck!