Cool high resolution Techniques

Recall STORM/PALM...

1. STED—Form a laser from a few dyes!
2. EM—go to shorter wavelengths
3. EM + X-rays—amazing! Overcomes problems from both techniques
Cool video: (remember GFP?)

Fluorescent protein from jelly fish

https://www.youtube.com/watch?v=sXM_WmwT2v4
On class of Super-Resolution Microscopy
Inherently a single-molecule technique
that relies on blinking or on/off behavior

Huang, Annu. Rev. Biochem, 2009

STORM
STochastic Optical Reconstruction Microscopy

PALM
PhotoActivation Localization Microscopy (Photoactivatable GFP)

Bates*, 2007 Science
(Won 2014 Nobel Prize)
More STORM Images

Figure 2. 3D STORM Images of Synapses

Dani, Zhuang, 2010, Neuron
STimulated Emission Depletion (STED)

Recent development in super-resolution microscopy  S. Hell  
(2014 Nobel Prize)

Net result is a smaller (than $\lambda/2\text{NA}$) Point Spread Function

Sharpen the fluorescence focal spot is to selectively inhibit the fluorescence at its outer part.

Huang, Annu. Rev. Biochem, 2009  
http://www.mpibpc.gwdg.de/groups/hell/
Biological Example of STED

The transient receptor potential channel M5

Analysis of spot size for **Confocal** (A) and **STED** (B) images of TRPM5 immunofluorescence layer of the olfactory epithelium. (A, C Inset) Confocal image at a lower (higher; box) magnification taken with a confocal microscope. (B) STED image. Effective point-spread function in the **confocal (189 nm)** and **STED (35 nm)** imaging modes.

Hell, PNAS, 2007
Microscopes
Cells discovered with invention of microscope.

Or with CCD

1000x, 0.2um

$10^6$x, 2 nm

20,000, 10 nm (3-d)
Techniques for measuring distances
(where physicists have made a big impact on bio.)

X-ray *diffraction* (atomic resolution)
Electron (*Imaging*) Microscopy (nm-scale)
Visible (*Imaging*) Microscopy (nm - µm)

Bacteria on head of a pin at different magnifications
What relationship between wavelength, \( \lambda \), and energy, \( E \), and momentum, \( p \), does this correspond to?

\[
E = h \nu = hc/\lambda; \quad p = h/\lambda
\]

Where does Planck's constant come from?

The Planck constant came from law of black body radiation: that the electromagnetic radiation emitted by a black body could be modeled as a set of harmonic oscillators with quantized energy of the form: \( E = h \nu \)

http://en.wikipedia.org/wiki/Black-body

Relationship between radiation of an object and its temperature
Resolution of Electron Microscope

Given electron 100 KeV, (typical upper-value for electron microscope) what is $\lambda$?

$E_{100\text{keV}} = 0.004 \text{ nm (really short!)}$

In reality, because not perfect electron lenses, resolution is $\sim 1 \text{ nm}$.

**E.M. are far from ideal.**
Transmission electron microscope
Mitochondria (home of $F_1F_0$ATPase)

http://www.cas.miamioh.edu/mbiws/microscopes/types.html

http://faculty.ccbcmd.edu/~gkaiser/SoftChalk/%20BIOL%20230/Prokaryotic%20Cell%20Anatomy/proeu/proeu/proeu_print.html
DNA replication

Transmission electron micrograph of a mitochondrial DNA molecule

http://www.gettyimages.co.uk/detail/photo/replication-transmission-electron-high-res-stock-photography/126725319
Scanning Tunneling Microscopes

Coloured scanning electron micrograph of a cat flea

http://www.topdesignmag.com/
25-amazing-electron-microscope-images/

http://remf.dartmouth.edu/images/
insectPart3SEM/source/30.html
The larva of a bluebottle fly

Common housefly
The head of a human flea
50x zoom of human eyelash hairs
Plus and minuses of techniques

**Electron Microscopy** is great -- get molecular resolution
Can get in vivo by going to very cold temperatures
(*Cryo Electron Microscopy*)
However,
doesn’t get atomic resolution,
Doesn’t work on live samples.

**X-ray Crystallography** is great – get atomic resolution
However,
Requires highly artificial buffer conditions, not in vivo.

**Cryo-Electron Microscopy & X-ray:** can get E.M.
images in vivo and compare atomic resolution images with x-rays.
Recent Revolution: Single Particle E.M.

When combined with x-ray crystallography, the most amazing technique! Atomic resolution on samples which you can’t crystallize the whole thing!
3-D map of the *T. thermophilus* ATP synthase

Class evaluation

1. What was the most interesting thing you learned in class today?

2. What are you confused about?

3. Related to today’s subject, what would you like to know more about?

4. Any helpful comments.

   Answer, and turn in at the end of class.