7.016 Recitation 19 – Fall 2018

*(**Note:** The recitation summary should NOT be regarded as the substitute for lectures)*

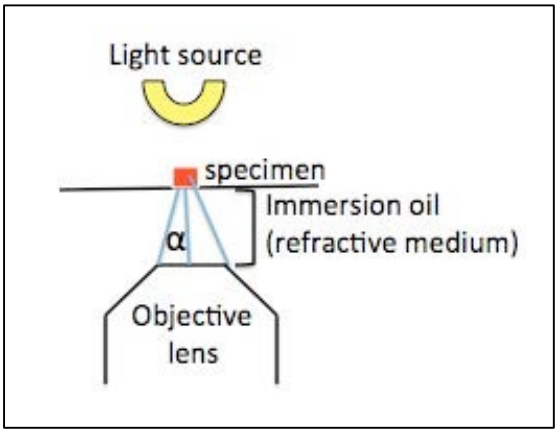
Summary of Lecture 29 (11/26):

Microscopy is a technique to view objects, living organisms, cells, subcellular structures or macromolecules within a cell that cannot be seen by the naked eye. The lecture focused on three branches of microscopy: Optical microscopy, electron microscopy and the newly emerging field of super-resolution microscopy.

Our ability to see a structure depends on …

- A **light source**, which is used to generate **contrast** that allows you to differentiate an object/ specimen from the background.
- The **size** of the object / specimen.
- **Resolution** (structures need to be far enough apart so we can resolve them). The human eye can resolve objects down to 100-200 microns, light microscopes can resolve objects down to 200nm by using light and electron microscopes can resolve objects at sub nanometer levels. However, to view a specimen using electron microscope, you have to fix (or kill) the specimen, which may result in artifacts.
- **Sample preservation:** This is how well the specimen to be viewed is preserved while preparing the specimen to achieve optimal resolution, magnification and contrast.

What determines resolution? This is the smallest distance between two points on a specimen that can still be distinguished by the observer or camera system as two separate entities.



d_{min} = Distance between two points that can be resolved.

λ = Wavelength of excitation light

NA (Numerical aperture) = Range of angles of light that the objective lens collects) or **NSinα (N** represents the refractive index of the medium that the light is going through, air, N=1; water, N=1.33; glass, N=1.5**)**

d_{min} = 1.22λ / 2 (NA) = λ / 2

So, the lower the d_{min}, the greater you will be able to resolve the object and vice versa.

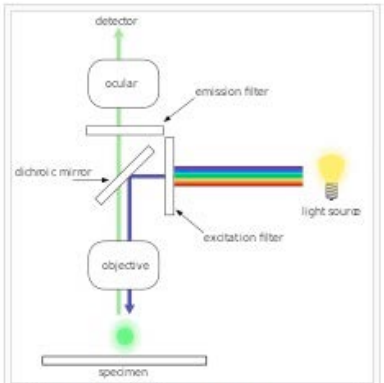
Related link: https://www.microscopyu.com/microscopy-basics/resolution

In electron microscope, you allow electron beam to pass through thin cross sections (that are 30-60nm in diameter). In 2D, if you observe the optical slice of endoplasmic reticulum (ER) then it appears as a stack of pancakes on top of each other. But if you make a 3D reconstruction of serial optical slices of ER, then it appears a helicoid, spiral structure.

What determines Contrast? Native structures absorb or refract light to generate a contrast that allows us to see the specimen and distinguish it from the background.

Adding dyes can further enhance contrast. For example, uranyl acetate is a dye used for electron microscope. This is an electron dense dye, which bends electron beams to generate an image.

Fluorescence microscopy: This is an optical microscope that uses fluorescence to generate an image.



Schematic of a fluorescence microscope.

Wikipedia.com

Here, the specimen to be examined is illuminated by light of a specific wavelength. This light is absorbed by the fluorophore in the specimen allowing them to emit light of a longer wavelength. The excitation light is separated from the much weaker emitted fluorescence through the use of a spectral emission filter.

Super-resolution microscopies (STORM and PALM): This applies novel approaches to circumvent the diffraction barrier, allowing users to acquire nanoscale information with optical systems. This helps…

- Single fluorescent molecules (which might label a protein of interest) can be imaged and their centers can be localized to a fraction of the size of the fuzzy spot that corresponds to their image.

- Closely spaced, optically overlapping fluorescent molecules can be separated, and each can be localized if there is a distinguishing characteristic. For example, if two molecules light up separately in different image frames, the center of each can be inferred to a fraction of the spot sizes.

- A new class of fluorescent proteins has been developed in the past several years, and a distinguishing subset of these proteins can be turned into a fluorescing state while the remainder remains dark. This last insight led to a new kind of microscope dubbed PALM (photoactivated localization microscopy) by Eric Betzig and his team. This microscope can activate, sample, and localize the centers of a very small subset of closely spaced label molecules. After bleaching the first subset, this process is repeated for a new sparse subset to collect new centroid locations, and iterated thousands of times until a significant fraction of fluorescent label molecules have been sampled. Please see the movie for an illustration of this principle.

- Eric Betzig's Nobel lecture: https://www.nobelprize.org/prizes/chemistry/2014/betzig/lecture/

Questions

1. Which of the following options allow you to see mitochondria within **a living cell**?

- ☐ Optical microscope with a resolving power of 200nm
- ☐ Electron microscope
- ☐ Fluorescence microscope using antibodies specific to mitochondrial protein
- ☐ Fluorescence microscope to see cells that express mitochondrial-GFP fusion protein

2. Explain how the medium that the light goes through between the objective lens and the specimen can impact the resolution.

3. What type of immersion media would give you the greatest resolution on a light microsope?

- ☐ No media (air), refractive index of 1.00
- ☐ Water, refractive index of 1.33
- ☐ Oil, refractive index of 1.51

Solution key

1. Which of the following options allow you to see mitochondria within **a living cell**?

   a. Optical microscope with a resolving power of 200nm
   b. Electron microscope
   c. Fluorescence microscope using antibodies specific to mitochondrial protein
   d. Fluorescence microscope to see cells that express mitochondrial-GFP fusion protein

2. Explain how the medium that the light goes through between the objective lens and the specimen can impact the resolution.
The refractive index of the media that the light goes through can impact the numerical aperture and therefore the d_{min} and resolution.

3. What type of immersion media would give you the greatest resolution on a light microsope?

   e. No media (air), refractive index of 1.00
   f. Water, refractive index of 1.33
   g. Oil, refractive index of 1.51

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