Which Magnification Should I Choose? Practical Considerations for Whole Slide Scanning (and Other Large Datasets)

Most people know what stains or fluorophores are in their sample, and they usually know what each stain or fluorophore should look like and roughly where in the tissue it should appear, so they choose one that is present across most of the tissue (like DAPI) to use as the focus channel.

However, many people with both histology stain and fluorescence get stuck choosing a magnification, especially now that we have more magnifications available.

Honestly though, the choice isn't magnification but pixel size, which is related to magnification but is much more informative.

The pixel is the smallest possible unit of your image; pixel size determines the level of detail visible in the final image.

Table of pixel sizes for each objective

CRi Pannoramic MIDI
20x – 0.3225um (323nm)

CRi Pannoramic SCAN
40x – 0.1644um (165nm)

Olympus VS200
2x – 3.274um (3274nm)
4x – 1.637um (1637nm)
10x – 0.6504um (650nm)
20x – 0.3251um (325nm)
40x – 0.1626um (163nm)
60x – 0.1084um (108nm)

But how do you know what pixel size you need?

Well, let's ask a few questions:

- What are you looking for in this sample?
- How big is your smallest object of interest?
- How much detail do I need in that object in order to analyze it?

How big are your smallest objects of interest?

Your smallest object of interest may be different from the smallest object you have stained for. If you have stained with an antibody to a cell surface receptor to identify cells of a certain type, for example, those receptors are probably less than 200nm in size, but your object(s) of interest are the cells or cell clusters identified by that marker. So you can base your decision about pixel size on the size of the cell or cell cluster rather than the receptor.

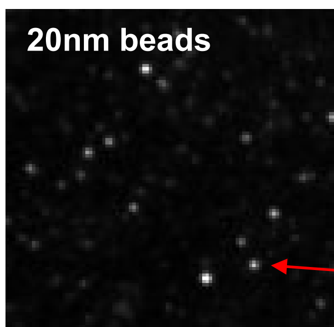
If you're not sure how big your smallest object of interest is, here are some examples: a typical eukaryotic cell nucleus is 5-20um (5,000 – 10,000 nm) in diameter. Mitochondria range in size from 0.5um (500nm) to 3um (3000 nm). Vesicles range between 0.1um (100nm) up to 1um (1000nm) and larger for apoptotic bodies. However, there is considerable variation in size both within these categories and within a given sample. The best way to know how big your objects of interest are is to know your particular sample.

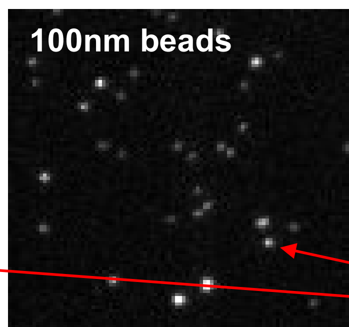
According to the Nyquist/Shannon Sampling Theorem, for accurate reconstruction of an object from pixels, the **pixel size of the image should be *no larger than half* the size of your smallest object of interest**.

For example, if you're interested in mitochondria, they are approximately 500nm at their smallest. Half of that is 250nm, so choose a magnification (40x) that creates a pixel size smaller than that (163-165nm) for adequate sampling.

There are no pixel sizes smaller than 100nm. What about objects smaller than 200nm?

Well, there is some interesting news for you.





Because we're imaging with waves of visible light, there's a lower limit to the size of the objects we can see. This limit is known as the Abbe diffraction limit and it is approximately 200nm.

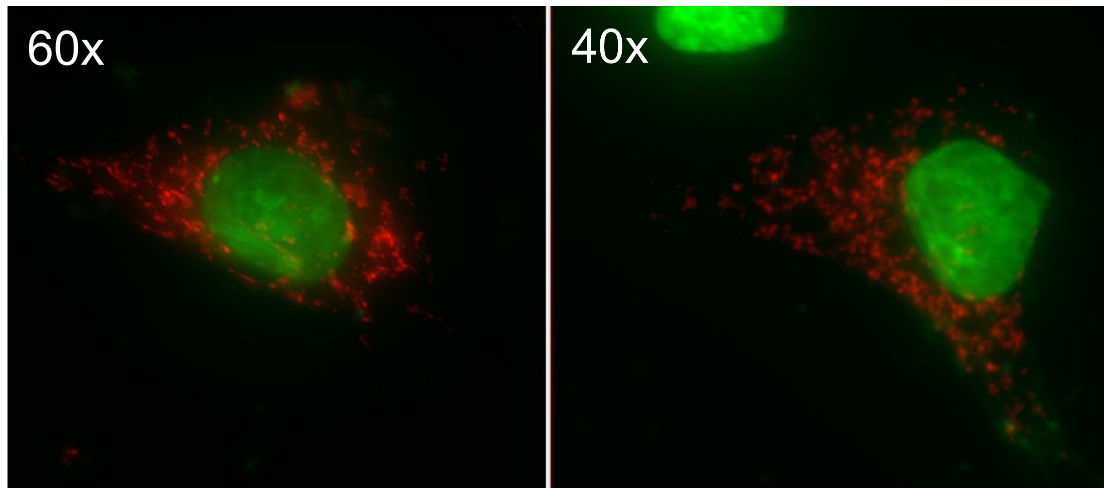
This means that objects that are smaller than 200nm will appear larger than their true size, and always as 200nm spots.

Yes, there are ways around the diffraction limit, but whole slide scanning with a widefield microscope is not one of them. So **there are effectively no objects smaller than 200nm as far as users of the whole slide scanners are concerned**.

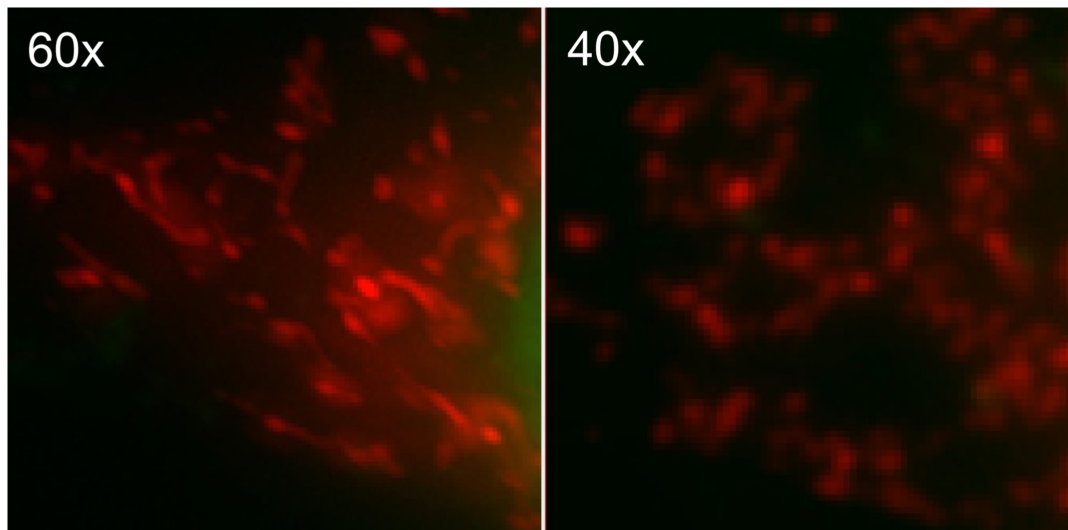
How much detail do I need in my objects in order to analyze them?

The answer to this question may lead you to a larger or smaller pixel size / higher or lower magnification than you would have chosen based purely on Nyquist-Shannon.

Using our mitochondria example above, at the whole cell level the mitochondria are well defined with both 40x (right) and 60x (left) scanning. For visualization and measurements of total mitochondria area, both magnifications are adequate and 40x would allow for larger scan areas to be created before file size gets too large to handle.



However, at the level of the individual mitochondria, the organelles are much smoother and better defined with 60x scanning (left) that with 40x (right) scanning. Measurements of individual mitochondria size will be more accurate with the 60x scan.



Why not just choose the smallest pixel size all the time?

Good question. If small is good then smaller is better. Right? Well, no. We do not necessarily want to oversample for two reasons:

- Oversampling can make your sample look dimmer, as the light from the sample is spread over a larger number of pixels, so each pixel gets less light / less signal
- The file size can grow to the point where the file is too big to open and the data is unusable. **For every 2x increase in magnification there is a 4x increase in data size!**