Resolution Limits of Optical Microscopes and Related Requirements for Mechanical Stages

Optical Resolution

The wave nature of light imposes fundamental limitations on the resolution of an optical system. For a self-luminous body, as in fluorescence microscopy, the resolving ability is commonly defined using the Rayleigh criterion: a point source of light can barely be resolved from a neighboring point source when spaced by the Airy disk radius. It can be shown that this distance d_{xy} is given by

\begin{equation} d_{xy}={0.61λ \over \mathrm{NA}_{obj}} \end{equation}

where $λ$ is the wavelength of the light and NA_{obj} is the numerical objective of the imaging objective lens. The prefactor varies with the criteria to define resolution: 0.61 is the prefactor for the Rayleigh criterion which is the most common, 0.515 is the prefactor for FWHM of point source, 0.5 is the prefactor for Abbe resolution, and 0.47 is the Sparrow resolution limit. Lateral resolution is synonymous with d_{xy}.

For transmitted light microscopy, the resolving power is also affected by the numerical aperture of the illumination optics.[1)] For transmitted light using a condenser with numerical aperture NA_{cond} the lateral resolution is given by

\begin{equation} d_{xy,trans}={1.22λ \over (\mathrm{NA}_{obj}+\mathrm{NA}_{cond})} \end{equation}

In the z-direction, the objective lens' resolving power or axial resolution is equivalent to the depth of field[2)]. The most common expression for the depth of field d_z is

\begin{equation} d_z={2λn \over \mathrm{NA}_{obj}^2} \end{equation}

where n is the index of refraction of the medium in which the object is embedded. Some versions of this equation include a term for the effects of lateral sampling, which we omit for the optic-limited case. The prefactor varies with the criteria to define resolution: the factor of 1.0 or 2.0 is the Abbe resolution most often used, a prefactor of 1.772 is for FWHM of a point source, and a prefactor of 1.22 is occasionally used. Axial resolution is almost always worse than to the lateral resolution, and the asymmetry is especially pronounced at low NA_{obj}.

Table 1 shows the resolving power and depth-of-field for some example microscope objectives. Note that the resolution does not depend on the magnification laterally or axially and mainly depends on NA_{obj}.

Table 1: Resolution limits for various microscopes when $λ$ = 520 nm and NA_{cond} = 0.55

Magnification	Medium	NA_{obj}	d_z	d_{xy}	$ d_{xy,trans}$
x10	Air (n=1.0)	0.4	6.50 µm	0.79 µm	0.67 µm
x40	Air (n=1.0)	0.65	2.46 µm	0.48 µm	0.53 µm
x40 water	Water (n=1.33)	0.8	2.16 µm	0.40 µm	0.47 µm

Table 1: Resolution limits for various microscopes when $λ$ = 520 nm and NA_{cond} = 0.55					
Magnification	**Medium**	**NA_{obj}**	**d_z**	**d_{xy}**	**$ d_{xy,trans}$**
x40 oil	Oil (n=1.51)	1.4	0.80 µm	0.23 µm	0.33 µm
x100 oil	Oil (n=1.51)	1.4	0.80 µm	0.23 µm	0.33 µm

These above expressions for diffraction-limited lateral (d_{xy}) and axial (d_z) resolution give us a good idea of the physical limits of the microscope system. Deviations in positions smaller than these resolution limits will be rendered undetectable by diffraction. The resolution obtained in practice can be worse than the diffraction limit due to optical aberrations or improper sampling.

“Super-resolution” microscopy techniques allow one to surpass the diffraction limit, but they incur significant trade-offs and further discussion is a separate topic. Very briefly, super-resolution methods fall into two categories: (1) localization techniques which determine the center point of isolated fluorophores (e.g. STED, PALM, STORM) and (2) structured illumination techniques in which the illumination pattern has a fine structure which is moved, either a periodic grid patterns or a scanned excitation point. Localization methods can achieve resolution in 10s of nanometers but require special efforts and long exposures to isolate fluorophores. Structured illumination in contrast can win at most a factor of 2 in resolution (excepting nonlinear methods) but otherwise are more like traditional fluorescence microscopy.

Spatial Sampling

When images are captured with a digital camera, the size of the camera's dexel (detection pixel) size will also impact the ultimate resolution of the imaging system. Most scientific cameras have dexels a few microns across. The pixel size in the resulting image p is given by

$$p={d \over M}$$

where d is the dexel size and M is the total magnification. For infinity microscopes (near-universal and industry standard), the magnification M is given by the ratio of the tube lens and objective lens' focal lengths. [3)]

Table 2 show the resulting pixel size for some example sensors and objective lens combinations, assuming nameplate magnification.

Table 2: Digital camera resolution			
Magnification	**Dexel Size**	**Pixel Size**	**Nyquist-limited d_{xy}**
x40	16 µm	0.40 µm	0.80 µm
x100	16 µm	0.16 µm	0.32 µm
x40	10 µm	0.25 µm	0.50 µm
x100	10 µm	0.10 µm	0.20 µm
x40	6.5 µm	0.163 µm	0.33 µm
x100	6.5 µm	0.065 µm	0.13 µm

The Nyquist criterion says the resulting resolution can be no more than twice the spatial sampling. However, excessive oversampling will increase the data size without adding additional true information. For example, using an objective with NA 1.0 and light with a wavelength of 520 nm, d_{xy} is ~320 nm. Thus if the pixel size is larger than 160 nm in the final image then the sampling

will limit the resolution instead of the optics. Suppose further the camera dexel is 6.5 µm (typical sCMOS), then with a 40x magnification (162.5 nm pixels) there will be slight undersampling, with 60x magnification (108 nm pixels) there is 50% oversampling, and using a 100x magnification (65 nm pixels) there is huge oversampling. Similarly, when collecting 3D stacks the z-step should be less than half the depth of field (d_z); otherwise the attained axial resolution will be limited by sampling instead of the optics.

Impacts on Stage Specifications

For most visible optical applications there is no reason to require resolution or repeatability of a stage significantly better than the optical resolution of the system. Instead money would be better spent on addressing the fundamental limitation of the optics.

When it comes to stages, there are subtle but important differences between the definitions of resolution, accuracy, and repeatability. *Resolution* typically means the smallest possible move and/or the fundamental unit of measured position. With motorized stages, the smallest possible motion is usually a about two counts on the rotary encoder (even if linear encoders are used) and the encoder unit is the measurement resolution. *Accuracy* is knowing exactly where you are in absolute or relative terms; subtle sources of error such as leadscrew pitch variability and mechanical backlash typically limit accuracy unless linear encoders are used. *Repeatability* quantifies how well you can return to the exact same position over and over again. The relative importance of resolution, accuracy, and repeatability depends on the application.

Resolution is often the most important specification for the Z-axis focus control. Optical serial sectioning and 3D reconstruction using deconvolution algorithms require collecting images at many closely spaced z-intervals. Spacing is usually chosen to meet the Nyquist sampling criterion based on the optical axial resolution, so step sizes may be as small as 0.3 µm. Stereology is an example application requiring both excellent resolution and repeatability in the Z-axis. For the most consistent sub-micron focus movements, use either a piezo stage or motorized stage with linear encoders. ASI's LS-50 with 16 TPI leadscrew (without linear encoders) is a cost-effective focus device and perfectly adequate for many applications. Adding a linear encoder to the LS-50 more than doubles the price but improves accuracy and repeatability and is recommended for applications where the LS-50 is tasked with performing Z-stacks. Piezo stages are significantly more expensive and have limited travel, but have the fastest possible step/settle times.

Repeatability is usually the most important specification of XY stages. An example of such an application is making a time lapse “movie” of cells at several sites on a slide, where the stage moves to each site sequentially in a repeating fashion. When the images from a single site are collated there ideally would be no “spatial jitter” of the images due to stage positioning errors. This requires repeatability comparable to (or better then) than the system's optical resolution. On the other hand, applications such as stereology and mapping generally do not require exquisite XY repeatability. Using linear encoders instead of rotary encoders improves repeatability somewhat (especially for larger moves) and also improves accuracy. ASI's controllers include automatic backlash correction that practically eliminates the effect of mechanical backlash.

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1)

A similar situation occurs in confocal microscopy where the same numerical aperture appears on both the illumination and detection paths, but it only applies in the case of an in the limit of an infinitesimally small pinhole.

2)

This is commonly confused with depth of focus, which is a similar measure defined at the sensor instead of at the sample.

3)

For any given objective lens, the nameplate magnification assumes a tube lens with a particular standard focal length, and the standard varies by manufacturer. See our page on infinity microscope basics.