Applied Scientific Instrumentation
29391 W. Enid Rd
Eugene, OR 97404

Ultimate Resolution of Optical Microscopes and Video Systems Related to Requirements for X,Y,Z Stage Resolution and Repeatability

The wave nature of light imposes fundamental limitations on the resolution of an optical system. In visible light optical microscopy, the resolving power of the optical system is often described by the Rayleigh criterion. A point source can be just resolved from a neighboring points source when it is separated from the other by the radius of the first zero of the diffraction limited Airy disk. For a self-luminous body (as in flourecsence microscopy) this distance, *d* is given by

$$d = 0.61\,\lambda_0 / NA_{obj}$$

where λ_0 is the wavelength of the light and NA_{obj} is the numerical objective of the objective lens. For transmitted light microscopy, the resolving power is also affected by the *NA* of the illumination optics. In this case the resolving power, *d* is given by

$$d = 1.22\,\lambda_0 /(NA_{obj} + NA_{cond})$$

where the *NA* of the condenser is taken into consideration. These expressions for the resolving power give us a good idea of the physical limits of the microscope system for the x,y plane.

In the z-direction, the depth-of-focus is an important quantity of interest. The depth-of-focus, d_f for high NA objectives can be given approximately as

$$d_f = \lambda_0 n / 2NA^2$$

where n is the index of refraction of the medium the object is embedded in.

Table 1 below shows the resolving power and depth-of-field for typical high quality microscope objectives.

Table 1: Resolution limits for various microscope objectives

Magnification	Immersion Substance	NA_{obj}	Depth of Focus	Resolving power (NA_{cond} = 0.55; λ=500 nm)
×40	Air (n=1.0)	0.65	0.59 µm	0.51 µm
×100 oil	Oil (n=1.5)	1.25	0.24 µm	0.34 µm
×40 oil	Oil (n=1.5)	1.4	0.21 µm	0.31 µm
×40 water	Water (n=1.3)	1.2	0.24 µm	0.35 µm

When images are captured with a CCD camera, the camera's pixel size will also impact the ultimate resolution of the imaging system. Most scientific CCD cameras have pixel sizes that are a few microns across. Table 2 show the pixel resolution for typical CCD sensors and objective lens combinations, assuming a magnification of ×1 in front of the camera.

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Table 2: CCD camera resolution

Microscope Objective	Pixel Size	Pixel Resolution
× 40	15 µm	0.38 µm
× 40	10 µm	0.25 µm
× 100	10 µm	0.10 µm
× 40	6.8 µm	0.17 µm
× 100	6.8 µm	0.068 µm

Choosing the appropriate microscope stage resolution for your application

Some automated applications require very high accuracy stages to keep the overall imaging system resolution at a high level. For X, Y stages some applications require that the system to be able to return to a previously visited location such that acquired images will overlap pixel to pixel. An example of such an application would be if a time lapse "movie" were to be made of cells at several sites on a slide. In this application, the stage would move to each site sequentially and snap a picture. When the images are collated, and the movies played back, there should be no "jitter" of the images due to stage positioning errors. This will require stage repeatability that is of the same order, or better than the system optical resolution. Ensuring very tight requirements, with high optical resolution systems, will require stages equipped with linear feedback encoders.

Examples of application requiring somewhat less accuracy include sampling for stereology, and mapping applications. For these applications linear encoders are not needed for the x,y stage.

Accuracy for the z-axis focus control requires other considerations. Optical serial sectioning and 3-D reconstruction using deconvolution algorithms require collecting images at many closely spaced z-intervals. One may wish to sample at near the system optical z-axis resolution in order to obtain the maximum information. This will require samples every 0.2µm or so. To ensure the best possible results when requiring consistent sub-micron movements, linear encoders on the z-axis with 50nm resolution are the best cost-effective choice.

Another application requiring high z-accuracy and measurement repeatability is stereology. In this application, the measurement of the sample volume requires accurate z-axis information. Small errors in repeatability or relative position errors will skew the statistics of the sample. The smaller the sample thickness used, the better that the z-accuracy needs to be. The minimum requirement for such an application is a high quality, closed loop z-axis drive. The addition of linear encoders with <0.1µm resolution ensures that the z-axis drive does not contribute to systematic errors in stereology measurements.

For most visible optical applications there is little reason to need resolution or repeatability of an x,y, or z stage much better than the optical resolution of the system. Usually the added cost of extremely fine encoder resolution would be better spent on better optics, since that is where the primary resolution limit exists.