Magnification, Resolution and Sampling Rates

Resolution is the ability of an objective to definitively resolve an object, or group of objects, as a single entity(s). Magnification plays no part in the ability of an objective to resolve an object of a given size. While magnification will make an image bigger, it will not necessarily make it clearer. Resolution is governed solely by the Numerical Aperture (NA) of the objective, the wavelength of light and for axial resolution, the refractive index of the sample.

Magnification will affect how much light the objective can collect. Higher magnification objectives collect less light, so where possible use the highest NA, lowest magnification objective available.

It is important when discriminating small structures that the Nyquist Sampling Criteria is satisfied. Nyquist Sampling dictates that to optimally represent an analogue signal in digital space, the analogue signal needs to be sampled at least 2.3 times. In microscopy terms this means that the pixel size of an image needs to be at least 2.3 times smaller than the object that is being resolved.

To use an example: if the object you are trying to resolve is 1µm across, the size of the pixel will need to be ~0.43µm (2.3 times smaller than 1µm). If the pixel is bigger than this, there is no way of knowing that what looks like a single object is actually only one and not many.

If you are attempting to capture the highest resolution image possible with a given microscope configuration (objective, camera/scanner, excitation and emission wavelength etc) then you must ensure that the pixel size is atleast 2.3 times smaller than the calculated resolution of the objective.

On a wide field microscope equipped with a digital camera the pixel size is usually fixed for each objective and cannot be adjusted. The software used to take an image with the camera will show what the pixel size is, usually it is small enough to achieve the theoretical maximum resolution of the objective. It is important to make sure you are aware of the pixel size for widefield imaging. To use the example from above, if a certain camera/objective combination gave a pixel size of 0.6µm it would not be possible to fully resolve a 1µm mitochondria.

On a confocal microscope the pixel size can be adjusted. This is achieved by scanning at a higher image resolution (e.g. 1024x1024 instead of 512x512). If there are more pixels in the image each one has to represent a smaller area. Adjusting the zoom will result in the pixel being smaller as well, at the cost of a smaller sample area imaged.

It is also important to apply Nyquist sampling rates to Z-stacks and timelapse imaging. For z-stacks, it is important that the user sets the z-step size to 2.3 times smaller than the Z-resolution of the objective. For timelapse imaging the time interval of a timelapse should be set to 2.3 times smaller than the time it takes for the smallest object you wish to resolve to travel it's own diameter.

Refer to the Nyquist calculator on this website to determine the correct XYZ sampling rates (pixel sizes) for your images.

Ref: Handbook of biological confocal microscopy / edited by James B. Pawley.