What Makes a 'Good' Image? Part II: Sampling Rate

Given a very high signal to noise ratio (SNR), the level of sample details (resolution) that can be observed are fundamentally limited by the numerical aperture (NA) the objective. High numerical aperture objectives collect/focus a larger solid angle (cone) of light and so can achieve higher optical resolutions. However, for a single objective, this solid angle cannot exceed 180^0 and so optical resolution is limited to details about 200 nm size.

However, an objective's optical resolution and the resolution present in a captured image are often not the same and are not even related. This disconnect arises during the image recording process, when the optical image is sampled by a discrete array of detector elements called pixels. If the **pixel size** is much larger than the smallest features in the optical image after magnification and projection onto the detector, then some optical resolution will be lost and the resulting image pixelated (termed **under-sampling**). Conversely, if the pixels are too small, the full optical resolution is captured, but smaller pixels have other costs in terms of acquisition time, file size, SNR, and field-of-view (termed **over-sampling**). From this perspective a 'good' image is one that is sampled appropriately.

The happy medium pixel size (or equivalently, scan spacing on a confocal) can be easily calculated from the system's total magnification and optical resolution. For example, if the optical resolution is 200 nm and total magnification is 100x, a 200 nm feature in the sample will be 20,000 nm (20 um) after magnification and projection onto the detector. The most appropriate pixel size is then one-half of the magnified, smallest-feature size, or 10 um in this example. The factor of one-half is a consequence of the **Nyquist sampling theorem**, whose details are beyond the scope of this summary. In practice, camera pixel sizes usually range between 4 um to 16 um, depending on the type and model of camera. **Be sure to choose a magnification that is appropriate for the camera** in order to capture the full optical resolution. In the case of confocal imaging, unlike the physical pixels of a camera, scan spacing is determined by the motion of mirrors and so is user-adjustable as needed.

The above calculation is an approximation and there are many reasons why slightly larger or smaller pixels could be justified. For example, due to shot noise low, SNR images contain less optical resolution than implied by the objective's NA. In this case, a somewhat larger pixel size is reasonable. Larger pixels will also collect more light, thereby helping to increase the SNR. Given high SNR, perhaps smaller pixels can be justified: Since pixels are square and their size is defined as the length of an edge, pixel size along the diagonal is actually larger by a factor of $\sqrt{2}$. Thus, resolution may be limited along the image diagonals due to under-sampling. For certain types of sparse or highly structured samples (e.g. crystals or packed arrays) pixels larger than calculated above may be sufficient due to nuances of Nyquist's sampling theorem (termed compressed sensing). Finally, there are many instances when the experiment simply does not require capturing he finest optical details present in the sample.

Please contact us (microscopy@umich.edu) to help you find the right resolution for your imaging experiments. We look forward to working with you!

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