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Quantitative Measurement of Resolution as a Function of Defocus in Different Microscopy Modalities Using a Simplified Technique

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The distance over which the resolution of a microscope image changes appreciably, related to the depth of field, is an important parameter. This value determines the height of an object that can be imaged and said to be "in focus". Although this distance is important in microscopy, it is typically quoted from calculations based on simplified assumptions (such as perfect optics) [1] or presented qualitatively [2]. In order to measure the effects of defocussing in different microscopies, we use the logistic equation to determine image resolution from light-to-dark edge transitions [3]. The logistic equation is given as:

$$S(x) = \frac{a}{1 + e^{-(x-x0)/b}} + c$$ where the relevant fit parameter is b, and resolution is defined to be $3.33b$.

The characteristic distance over which the light-to-dark transition occurs has been related to the resolution of a spatially calibrated scanning transmission electron image of the Si<110> lattice. An example of an image analyzed by this method is presented in Figure 1. Images like this provide many different light-to-dark transitions that provide a robust measurement of resolution. Here we use this definition of resolution to measure resolution at different values of defocus in different types of microscopy.

Our method compares changes in resolution with changes in focus for different instruments, different imaging modes, and for the same instrument in different configurations. For example, characterizing optical microscope objectives of different magnifications and numerical apertures is accomplished by repeatedly imaging while changing the sample-objective distance. An identical region of these images containing many light-to-dark transitions is then statistically described at each stage height by the mean and standard deviation of the resolutions derived from each line fit in the region of interest (see Figure 2).

This method can also be used to relate image resolution to defocus in electron and ion micrographs. In electron and ion microscopy, the focus of the objective lens is varied providing defocused images with varied reported working distance. A region of interest is then fit in an identical manner, allowing us to determine the effective depth of field for a specific configuration of apertures, detectors, and lens values (see Figure 2).

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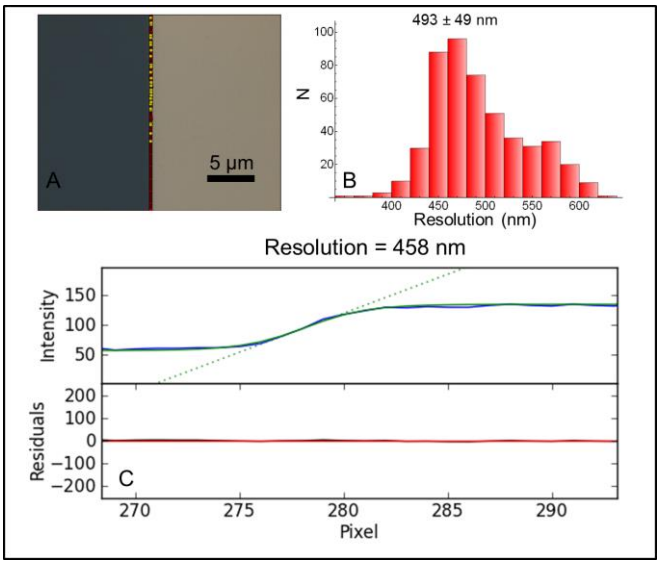

Figure 1. Measurement of resolution using a sigmoidal fit. (a) Spatially calibrated optical bright field image. This region of interest includes 248 light-to-dark transitions, each of which is fit separately resulting in the histogram in (b). (c) A single fit, showing the intensity profile (black), the resultant fit (blue), the tangent line at midpoint (green dashed line), and the residuals of the fit (red) .

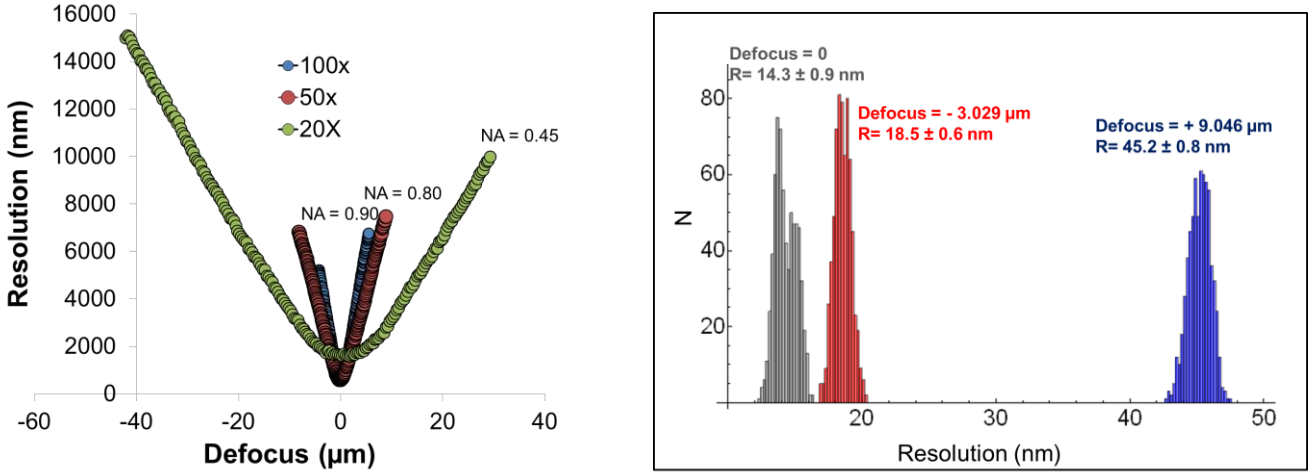

Figure 2. Resolution versus defocus in optical and scanning electron micrographs. Left, resolution as function of defocus for multiple optical microscope objectives in bright field reflection imaging. Right, histograms of analyzed field emission SEM images showing the change in resolution of an edge imaged at 5 kV with a 30 µm aperture, using an Everhart-Thornley detector at a working distance of 5 mm.

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