Technical Note for microsphere imaging.

Resolution

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Introduction

Several standard methods to define and measure optical resolution exist today. Optical resolution can be defined as **angular optical resolution** (the ability to separate objects in close angular proximity) or **spatial optical resolution** (the ability to separate objects in close physical proximity). These definitions are of course related. However, the relationship between definitions and practical measurement is not so easily described. Ideally, one wants a quick, simple, reliable and repeatable method that allows the user to understand the instrument resolution. This is the purpose of this technical note; to give a brief overview of concepts and methods before outlining a simple procedure to make an indicative measurement of optical resolution.

Background to resolution

The ability of traditional brightfield microscopes to resolve features is generally limited in three areas: those relating to the system, those relating to the sample and those dictated by the natural laws that describe the fundamental optical limit.

Sample limitations affect how *easy* it is to resolve features, but they do not define the fundamental resolution of the microscope. Difficult samples can limit the ability to resolve features due to low signal levels (e.g. transparency or high absorbency) that reduce the signal-to-noise to a level where the features are indistinct and difficult to reliably attribute a measurement to.

Systematic limits come from the system design, tolerances and element quality. For the optical elements, items such as aberration (spherical, coma and astigmatism amongst others) and chromatic dependency for the desired wavelengths are all important. Similarly, the choice and placement of the optical elements can make a large difference to the result, as do elements placed in the optical path such as an iris or an image sensor.

Resolution

Finally, for good high-contrast samples in a high-quality traditional microscope**, the resolving limit is fundamentally defined by the diffraction limit of the light** employed to illuminate the sample and the aperture within the design. This final limit comes from the answer to the question, “when is something resolved?”. The answer most readily expressed and accepted as the definition is that of the Rayleigh criterion, where two Airy discs generated from sources of equal intensity are overlapping with the peak intensity of one being placed at the first minima of the other[1] (See fig. 1 below). This precise definition can be expressed as the angular resolution limit and is described by formula (1), and can be approximated by formula (2) for spatial resolution, where θ is the angular resolution (radians), λ is the wavelength of light, D is the diameter of the aperture, f is the focal length of the lens and Δl is the spatial separation in the imaging plane. Separation of the Airy discs in this way creates an overlap of intensities that when summed, creates a 26% dip in intensity between the two peaks[2].

$$\theta = 1.22\,\frac{\lambda}{D} \qquad (1)$$

$$\Delta l \cong 1.22\,\frac{f\lambda}{D} \qquad (2)$$

The real-world combination of all the above described limits describes a feature of an optical system referred to as **the Point Spread Function (PSF). The PSF combines all the limitations of the microscope to describe how a hypothetical two-dimensional x-y delta-function would be spread-out across the image plane.** In an idealised single circular lens example, the PSF will be the Airy disc when a point source is placed on-axis in the object plane. In a microscope, all the affects described above contribute to a transfer function that can be quite complex, resulting in a PSF that varies in x and y dimensions. This will of course and have implications for the resolution.

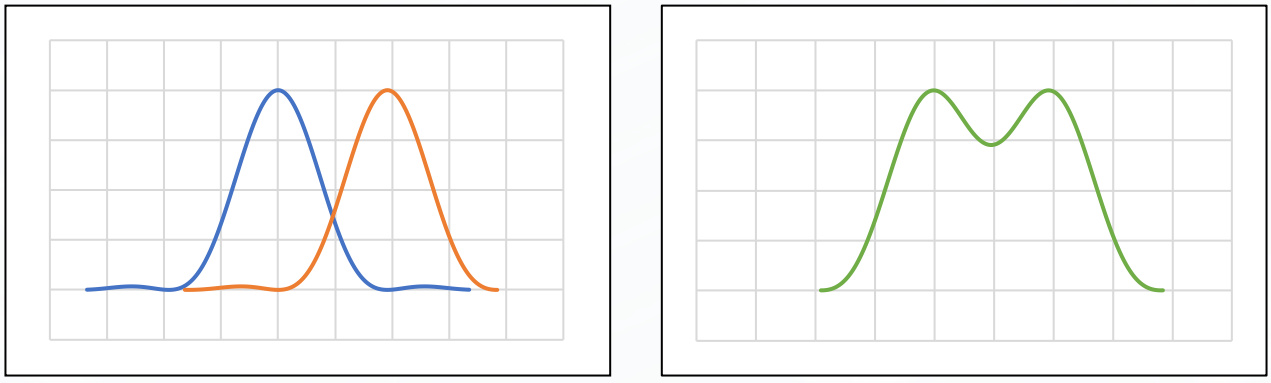


Figure 1. A graphical indication of the Rayleigh limit of resolution. Left: Overlapping Airy disc functions with the first minimum of orange overlapping the main peak of the blue trace. Right: Summation of the intensities, demonstrating a 26% intensity drop in the centre. Axes scales are arbitrary in these examples.

Resolution

Practical Measurements: The Point Spread Function.

The PSF is a complex quantity to understand in a real physical instrument as the “spread” in one axis can be different to the spread in another. To complicate matters further, what the PSF is in one area of the image plane will be different to other areas of the image plane as the optical paths traversed will be different. Finally, representing a point source in the object plane is, in itself, a non-trivial task and can never be an ideal point source with zero dimension in x and y as well as having optical intensity. Due to the small dimensions of any source that attempts to replicate this, the actual optical intensity is likely to be quite small resulting in difficulties with regards to signal-to-noise.

Consequently, a full definition of a microscope’s PSF can become unpractical to measure with point source approximations, and in the end, only provide a PSF approximation.

One simple practical method to understand the resolution of the image being viewed is to move away from PSF to the concept of the Line Spread Function (LSF). The LSF is the equivalent of the PSF, but in one dimension rather than two. This has a huge effect on the practicality of the measurement as we only require an edge that shows a sharp step in contrast; something that is relatively easy to create and to measure. Graphical illustration of a LSF, an ideal edge and an edge response are shown in Figure 2. As a line is the derivative of an edge, so is the LSF the derivative of the edge response[3].

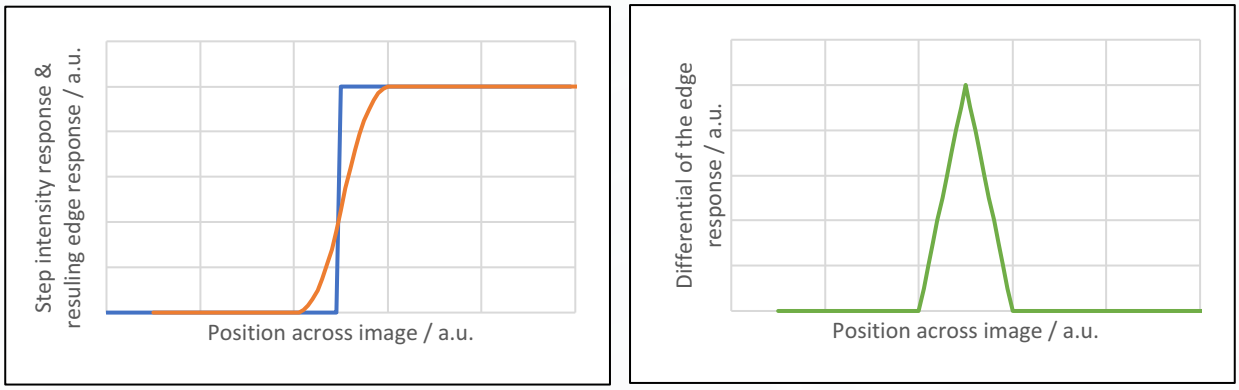


Figure 2. Left: Idealised step change (blue) and resulting edge response (orange) due to convolution with a LSF. Right: Differential of the resulting edge response (this is the LSF).

Resolution

There are two common ways to quote the resolution from the figures shown above: the 10 – 90% intensity transition distance taken from the edge response (orange) and the Full-Width-Half-Maximum (FWHM) distance taken from the LSF (green)[4]. In the case of the triangular LSF, the 10 – 90% and the FWHM measurements agree closely.

Example

Figure 3 shows images of a common high-density, high-performance microprocessor taken by the SMAL lens. A cross-section through these features is extracted and the LSF calculated, then finally the resolution of the features is determined using the FWHM measurement.

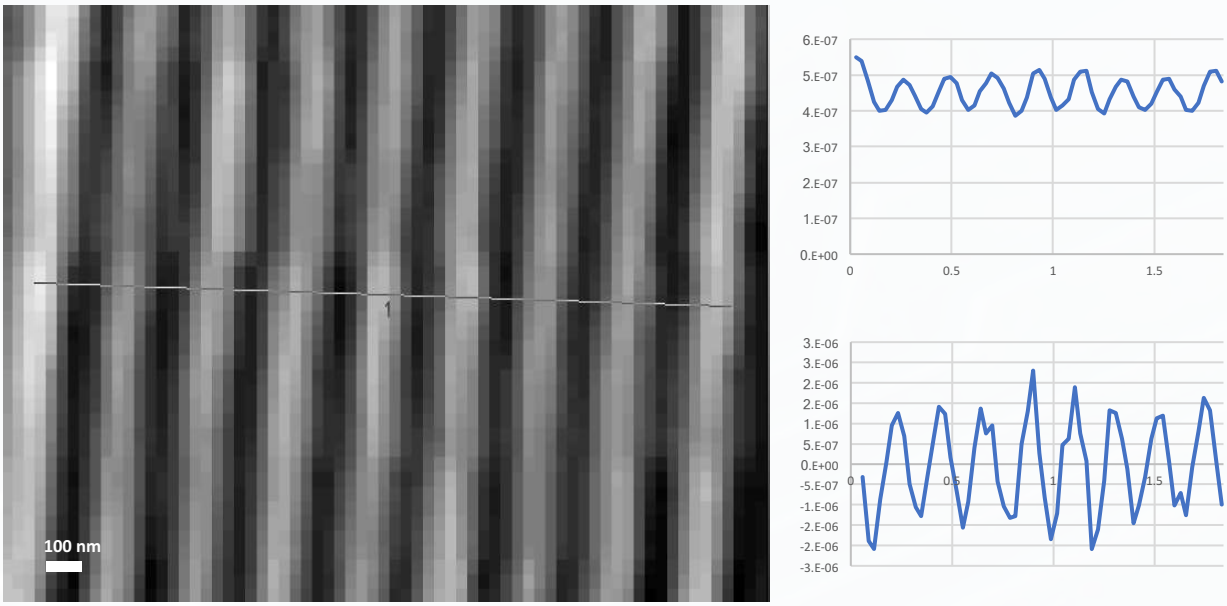


Figure 3. Left: Analysis on the red spectral component of a SMAL lens full colour image of a common high-density, high-performance microprocessor. The horizontal line marked “1” indicates the cross-section region for data analysis. Upper right: Numerical values of the image cross-section taken from line marked “1”. Lower right: LSF calculated from the differential of the upper right data. Scales of upper and lower right indicate a total cross-section range of 1.84 µm with around nine 100 nm features.

Resolution

From figure 3, sixteen transitions from light to dark, or dark to light can be seen. Each one provides an indication of the resolution where we assume a near-perfect step-response transition between light and dark (or vice-versa). The resolutions determined from the transitions are averaged and indicate an approximate resolution of 80nm.

Conclusion

The line spread function is a powerful mechanism to quickly understand instrument resolution and avoid complicated measurement, analysis or sample preparation. The example application shown in this technical note demonstrates that it is applicable to super-resolution bright field microscopy and suggests resolution in the range of 80nm can be achieved using this sample.

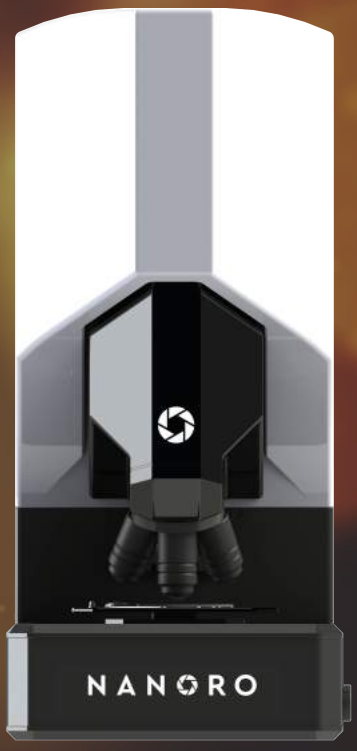
References

[1] "Principles of Optics.", Born, M.; Wolf, E. (1999). Cambridge University Press. p. 461. ISBN 0-521-64222-

[2] "Using photon statistics to boost microscopy resolution", Xavier Michalet and Shimon Weiss, Proc Natl Acad Sci U S A. 2006 Mar 28; 103(13): 4797–4798.

[3] "Edge response revisited", Lashansky, Shimshon & Mansbach, Shlomo & J. Berger, Michael & Karasik, Tehila & Bin-Nun, Moran. (2008). Proceedings of SPIE - The International Society for Optical Engineering. 10.1117/12.777957.

[4] "The Scientist and Engineer's Guide to Digital Signal Processing", Steven W. Smith, ISBN-10: 0966017633.



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