

# Standardizing the resolution claims for coherent microscopy

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The definition and reporting of spatial resolution for coherent imaging methods varies widely in the imaging community. We advocate the use of a standard spoke-pattern imaging target and the mandatory inclusion of information about underlying *a priori* assumptions.

Scientific development is founded on the use of precisely defined units and metrics. In microscopy, important metrics include the imaging system's magnification, field of view, depth of field, and spatial resolution. While many of these are easy to define in an unambiguous manner, the measurement of resolution can be problematic. In this Commentary, we propose the adoption of a standard imaging target and outline good practice for reporting the spatial resolution of a coherent optical microscope (a system where the light emitted from the sample retains phase information with respect to the illumination).

In an incoherent microscope, such as a fluorescence microscope, defining and reporting resolution is fairly straightforward. The connection between the optical intensity emitted from the sample and the intensity detected at the image plane is linear for incoherent systems<sup>1</sup>. As such, resolution can be quantified by measuring the intensity point spread function (iPSF) of the microscope<sup>2</sup> and stating well-known features of it, such as the distance to the first minimum (Rayleigh limit), the distance at which two adjacent iPSFs show no intermediate dip (Sparrow limit), or the highest spatial frequency of the sample captured (Abbe limit). The Fourier transform of the iPSF, known as the optical transfer function, is another common measure.

The situation is more complex for coherent imaging, as the microscope now has a linear response to the optical field and not its optical intensity, which is the usually measured quantity. This has led to ambiguity and widespread confusion about (1) the type of sample that should be used to accurately report coherent system resolution and (2) the type of measurement that should be reported.

The ambiguity that arises in coherent imaging is well illustrated by

the famous case of imaging two closely spaced features<sup>1,3</sup>.

Consider two point sources separated by a small distance and emitting mutually incoherent light, which are resolved at the Rayleigh limit (that is, just separated by a clear dip in intensity) when imaged by a specific microscope. The same point sources in the same locations will not be resolved by the same microscope (that is, will appear as only one large spot) if the sources are instead emitting light that is coherent and in-phase. However, the two sources become fully 'resolved' when their emissions are in anti-phase (that is, shifted by  $\pi$  radians) to each other.

This means that the intensity profile of an image rendered by a coherent imaging system is phase dependent. As such, simply measuring and reporting its intensity response is an unsuitable means to characterize resolution in an unambiguous manner. Further compounding the issue, many recent coherent imaging methods rely on computational post-processing, with a digitally manipulated image formation pipeline. Another factor that needs to be taken into account is the presence of noise, which can impact image quality and resolution limits<sup>4</sup>, but is challenging to encompass within a single measurement or scalar performance metric.

With this context in mind, we believe that the establishment of a practical unambiguous resolution standard is highly desirable and would allow all users to transparently and reliably assess the merits of coherent microscope techniques. The proposed standard and guidelines described here (summarized in Box 1) can form a baseline criterion for imaging system characterization.

Consideration of the following mathematical model is helpful to understand our suggested standard. It is possible to express the behaviour of a

## Box 1 | Good practice guidelines.

1. Report the predicted non-zero area of the coherent transfer function based on system specifications.
2. Explicitly provide information on any *a priori* assumptions applied to the sample (for example, sparsity) and post-processing steps applied to the data (for example, deconvolution).
3. Experimentally image the Siemens star target and report its amplitude and/or phase.
4. Summarize performance with the minimum periodicity metric, if desired (see text).
5. For wide-field systems, present data for different locations across the sample plane.

large class of coherent imaging systems in terms of a transfer function that relates input and output complex fields of the imaging system:

$$F[U_i(x, y)] = C(f_x, f_y)F[U_s(x', y')] \quad (1)$$

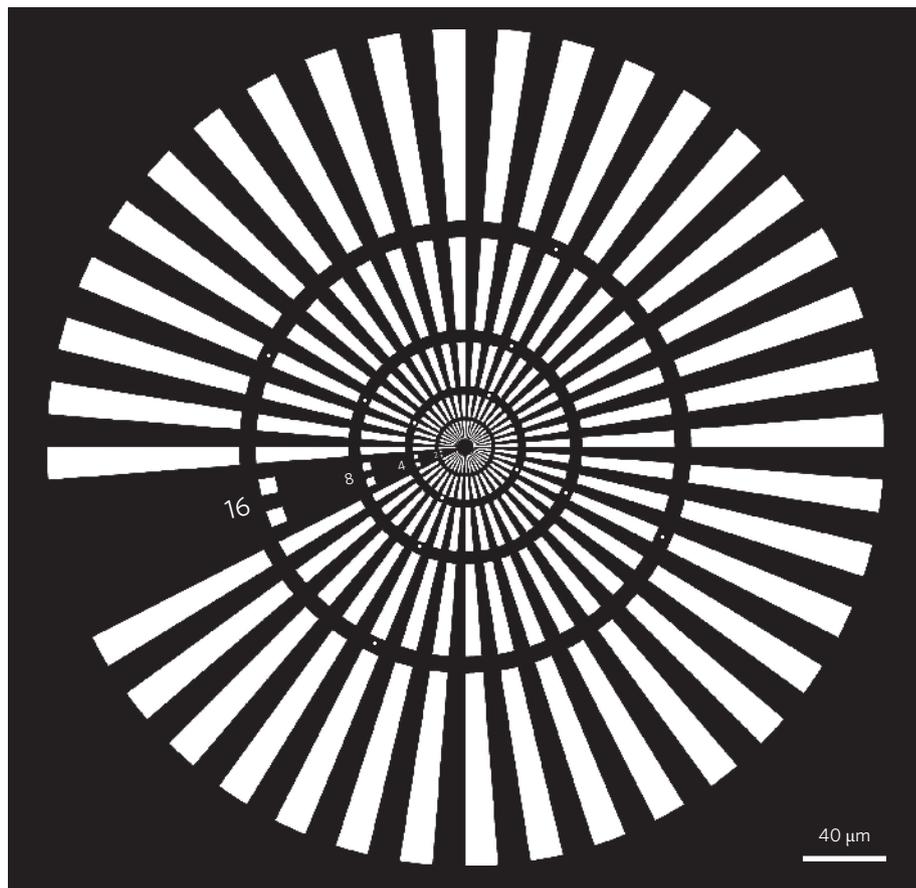
Here,  $C(f_x, f_y)$  is the imaging system coherent transfer function (CTF) for spatial frequencies  $(f_x, f_y)$ ,  $U_s(x', y')$  and  $U_i(x, y)$  are the 2D scalar monochromatic optical fields at the sample and image planes, respectively, and  $F$  denotes a 2D Fourier transform. All quantities here are complex valued, because the system is linear in complex field (not intensity). Embedded within this equation could be an unknown thin sample transmission function,  $t(x', y')$ , which defines the sample field via  $U_s(x', y') = t(x', y')e^{i(k_x x' + k_y y')}$  when illuminated by a plane wave with wavevector  $(k_x, k_y)$ , for example, and is the quantity of interest in our discussion of resolution.

Originally, equation (1) was used as a general linear model for analog coherent imaging systems<sup>1</sup>. In such a set-up,  $U_i(x, y)$  denoted the optical field physically at the image plane. By placing an ideal point source at the sample plane and recording the complex field at the microscope back focal plane, one could also measure  $C(f_x, f_y)$  for direct system characterization (that is, for a direct link between  $U_i(x, y)$  and the sample,  $t(x', y')$ ). Now with some computationally driven microscopes,  $U_i(x, y)$  may no longer represent a physical field. Instead, it is often synthesized from a set of measurements. Likewise,  $C(f_x, f_y)$  may no longer be directly connected to optical hardware, but is instead partially defined through software, and thus may contain values that can be varied after data capture.

### Spatial frequency coverage

Since it is increasingly common to emphasize or de-emphasize certain image features through digital adjustment, we believe that it is less critical to precisely report the complex values of the CTF. Instead, the presence of a non-zero CTF entry simply states that the imaging system can observe an amplitude grating of a certain spatial frequency. A binary plot of these non-zero transmittance locations directly reports the set of spatial frequencies that can physically transfer through the system, offering an objective map of detectability. A standard report of the expected performance of a coherent microscope should thus include, at a minimum, the non-zero transmission area of  $C(f_x, f_y)$  for the proposed specifications. This report also directly connects to the useful theoretical measure of a 'cutoff' resolution, which is the minimum resolvable full grating period. If the support of  $C(f_x, f_y)$  reduces to a simple disk, then it is sufficient to report its expected radius. Otherwise, one should describe its expected coverage across two dimensions.

Thus far, many publications concerning coherent imaging do not report the non-zero spatial frequency transmittance of the system. Instead, it is common to simply report the minimum resolved distance between two (or more) sample features, based on some contrast measure. A wide variety of distance metrics can be found in the literature, including the distance between maximum and minimum contrast ('half pitch')<sup>5-7</sup>, the distance between two feature edges<sup>8</sup> ('edge to edge', whose value can vary with feature width), or simply the width of a single feature<sup>9</sup> (whose value can vary significantly with contrast). Spatial frequencies are connected to a full grating period (that is, a full-pitch distance), so we suggest this distance in a periodic pattern



**Figure 1** | The 'Siemens star' target for characterizing resolution in a coherent microscope. This standard includes small fiduciary markers for accurate identification of the target centre, as well as numerical marks to denote the full-pitch periodicity (units:  $\mu\text{m}$ ) along the inner radius of each concentric ring. These numbers and the fractional spokes are included as a means to calibrate magnification, and are not to be used to directly report resolution. Instead, contrast should be measured radially and reported as a function of spoke periodicity, as described in the text.

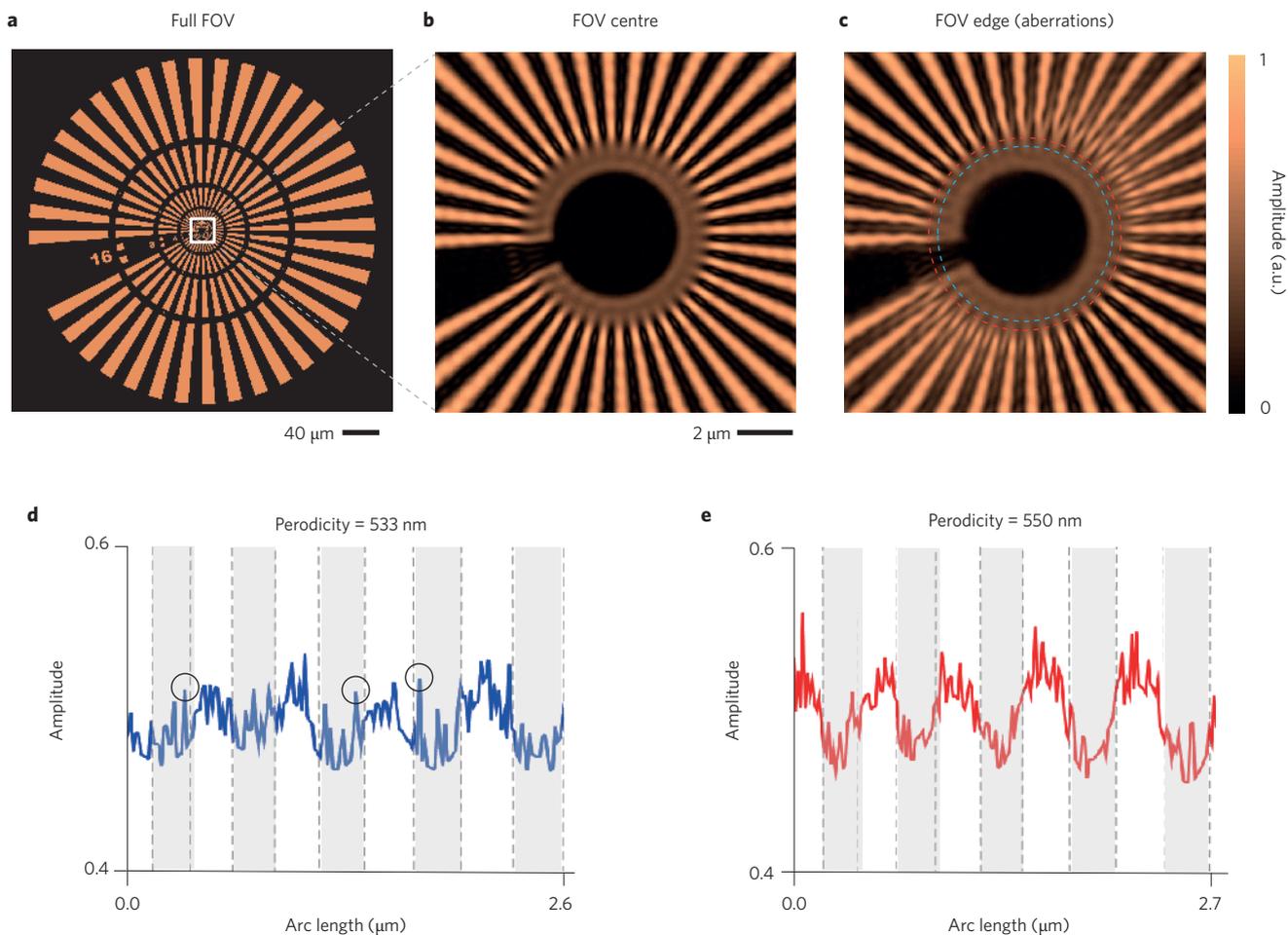
as a standard metric. Furthermore, many different measures of contrast are widely used, including those based on image intensity, magnitude, and phase angle, and those that vary nonlinearly with the field at the sample plane, such as intensity, should be avoided (see cautions in ref. 10).

### Computationally enhanced resolution

Often, the computational procedure that reconstructs images from a coherent microscope makes use of *a priori* knowledge of the sample to improve performance. For example, many well-known 'super-resolution' or 'bandwidth extrapolation' techniques can extend resolution beyond the physically enforced spatial frequency limit of the microscope, by assuming a spatially bounded sample<sup>11</sup> or a particular sample model<sup>12</sup>. A similar argument applies to compressive sensing-based reconstructions, which typically assume the sample field or its gradient is sparse<sup>13,14</sup>. Alternatively, an

image deconvolution might help to fill in missing information by adding a positivity constraint or a regularization term<sup>15</sup>.

Of course, it can be beneficial to incorporate such procedures into an image formation pipeline. However, it is important in the interests of transparency that one clearly state any *a priori* assumptions used when reporting system resolution. This allows others in the coherent imaging community to understand and properly assess the utility and limitations of a reported method and thus the scenarios and samples that it is applicable to. For example, methods that achieve resolution beyond the diffraction limit, but assume a specific sample geometry or property<sup>16</sup>, should clearly state the assumed model. This level of information greatly helps microscope users who may wish to employ a specific method to avoid false expectations about its level of performance or applicability. For example, assuming



**Figure 2** | Simulated example of a resolution report with the Siemens star for a coherent microscope. **a,b**, Amplitude image of the target (**a**) with zoom in to centre (region in white box; **b**) from a simulated microscope under coherent illumination ( $\lambda = 0.40 \mu\text{m}$ ,  $100\times 0.8$  numerical aperture objective, pixel size =  $1.3 \mu\text{m}$ , with Poisson noise). **c**, Re-imaged target centre after moving it to the edge of the sensor field of view (FOV; re-centred in this figure), where aberrations further limit effective resolution. **d**, Plot of amplitude values along a segment of the blue circle in **c** at 533 nm spoke periodicity. As noisy values within 'dark' spokes (circled) exceed values within 'bright' spokes, it is not possible to unambiguously claim a resolution of 533 nm. **e**, Similar plot along the red circle in **c**, showing that spokes at a periodicity of 550 nm are unambiguously resolved (verified for all spokes).

a sparse sample will likely help with the localization of a small number of scatterers, but will not be helpful in digital pathology, where the samples often lack a predefined structure.

All techniques, whether they exploit *a priori* knowledge or not, are ultimately limited by the presence of noise and aberrations and this should not be forgotten or neglected. Next, we propose and describe the use of a standard imaging target and its experimental measurement, which will offer a realistic, noise-dependent map of system performance.

### Experimental target

We propose the collective use of a 'Siemens star' target (a pattern of spokes; Fig. 1) for experimentally reporting coherent system resolution. This target approximates a radially varying measure of

spatial frequency contrast in the presence of noise and system aberrations. Unlike reporting just a single contrast measure between two nearby features, an image of the Siemens target (or a region of it) simultaneously provides multiple contrast measurements from a wide range of spatial frequencies. This helps to avoid the danger of only reporting the highest spatial frequency present in the intensity image (for example, reporting image features such as the intensity width of a point object), and minimizes possible misinterpretations of intensity information, such as in the two-point example discussed above. In addition, the effects of aberrations or an angled illumination source are readily observed in the Siemens star target (for example, see ref. 17), and will thus be more evident in this experimental demonstration of system performance.

Many coherent imaging experiments, with resolution down to the nanometre scale, have successfully adopted this convenient pattern<sup>18–21</sup>. At least one previous work has suggested the star standard to jointly verify different microscope modalities<sup>22</sup>.

If a CTF quantification is desired, the Coltman series may be used to convert the measured contrast from each periodicity within this binary mask pattern to an appropriate CTF value (that is, to mathematically map from square wave to sinusoidal contrast)<sup>23</sup>. In situations where image pixelation is significant, we recommend using methods such as that reported in ref. 24 to perform automated pixel extraction. We advocate reporting the achieved system resolution in a single metric, if desired, by stating the minimum spoke periodicity at which contrast is

still observed (that is, is above the noise floor). If contrast is unobservable above a periodicity limit, then one should report the highest periodicity at which contrast can be detected. To fairly report resolution variations across a larger field of view, one should also ideally capture and report multiple images with the target centred at different sample locations, as demonstrated in Fig. 2. Caution must be taken when identifying resolvable target areas — unacceptable amounts of shift, distortion, or noise should not be ignored when making a claim of successful imaging.

If the coherent microscope under test is designed for measuring phase, then we suggest imaging a binary phase-only Siemens star pattern (as in ref. 22), and reporting the minimum spoke periodicity at which phase contrast is still observable.

Siemens star targets with micrometre-scale resolution are currently available through Edmund optics (part no. 58-833) and with nanometre-scale resolution through NTT-AT ([www.ntt-at.com/product/x-ray\\_chart/](http://www.ntt-at.com/product/x-ray_chart/)). One should attempt to use targets with many spokes, as these are better approximations to the plane-grating

situation. A size and format ideal for the optical microscope will soon be available through Ready Optics.

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