Standardizing the resolution claims for coherent microscopy

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The definition and reporting of spatial resolution for coherent imaging methods has varied widely in the imaging community. We advocate the use of a pattern of spokes as a standard target that is straightforward to measure and the mandatory inclusion of information about underlying a priori assumptions.

Scientific development is founded upon 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 [1]. As such, resolution can be quantified by measuring the intensity point spread function (iPSF) of the microscope [2] and stating well-known features of it, such as the distance to the first minimum (Rayleigh), the distance at which two adjacent iPSFs show no intermediate dip (Sparrow), or the highest spatial frequency of the sample captured (Abbe). The Fourier transform of the iPSF, known as the optical transfer function (OTF), is another common measure.

The situation is more complex for coherent imaging, since 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 [1, 3].

Consider two point sources separated by a small distance and emitting mutually incoherent light, which are resolved at the Rayleigh limit (i.e. just separated by a clear dip) when imaged by a specific microscope. The same point sources in the

same locations will not be resolved by the same microscope (i.e. 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 (i.e., shifted by π 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. Further compounding the issue, many recent coherent imaging methods rely upon computational post-processing, with a digitally tunable 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 [4], but is challenging to encompass within a single measurement or scalar performance metric.

With this context in mind, we believe that the establishment of an unambiguous practical resolution standard is highly desirable and would allow all users to transparently and reliably assess the merits of newly developed coherent microscope techniques. The proposed standard described here 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 large class of coherent imaging systems in terms of a transfer function that relates input and output complex fields of the imaging system:

$$\mathcal{F}[U_i(x,y)] = C\left(f_x, f_y\right)\mathcal{F}[U_s(x',y')]. \quad (1)$$

Here, $C\left(f_x, f_y\right)$ is the imaging system coherent transfer function (CTF) for spatial frequencies $\left(f_x, f_y\right)$, $U_s(x',y')$ and $U_i(x,y)$ are the 2D scalar monochromatic optical fields at the sample and image planes, respectively, and $\mathcal{F}$ 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 $\left(k_x, k_y\right)$, for example, and is the quantity of interest in our discussion of resolution.

Originally, Eq. (1) was used as a general linear model for analog coherent imaging systems [1]. In such a setup, $U_i(x,y)$ denoted the optical field physically at the image plane. By placing an ideal source at the sample plane and recording the complex field at the microscope back focal plane, one could also measure $C\left(f_x, f_y\right)$ for direct system characterization (i.e., 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, $C\left(f_x, f_y\right)$ 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.

Standard part 1: spatial frequency coverage

Since it is increasingly common to emphasize or deemphasize 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 upon 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") [5, 6, 7], the distance between two feature edges [8] ("edge-to-edge", whose value can vary with feature width), or simply the width of a single feature [9] (whose value can vary significantly with contrast). Spatial frequencies are connected to a full grating period (i.e., a full-pitch distance), so we suggest this distance in a periodic pattern as a standard metric. Furthermore, many different measures of contrast are widely used, including those based on image intensity, magnitude or phase angle that vary nonlinearly with the field at the sample plane, like intensity, should be avoided (see cautions in [10]).

Standard part 2: 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 [11] or a particular sample model [12]. A similar argument applies to compressive sensing-based reconstructions, which typically assume the sample field or its gradient is sparse [13, 14]. Alternatively, an image deconvolution might

help to fill in missing information by adding a positivity constraint or a regularization term [15].

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 [16], should clearly state their 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 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.

Standard part 3: 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 (e.g. 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 (e.g., see [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 [18, 19, 20, 21]. At least one prior work has suggested the star standard to jointly verify different microscope modalities [22].

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 (i.e., to mathematically map from square wave to sinusoidal contrast) [23]. In situations where image pixilation is significant, we recommend using methods such as that reported in [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 (i.e., 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 centered at different sample locations, as demonstrated in Figure 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 Oldenbourg et al.), and reporting the minimum spoke periodicity at which phase contrast is still observable (see Figure 3).

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 (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.

TEXT BOX: GOOD PRACTICE SUMMARIZED

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 (e.g. sparsity) and post-processing steps applied to the data (e.g., 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

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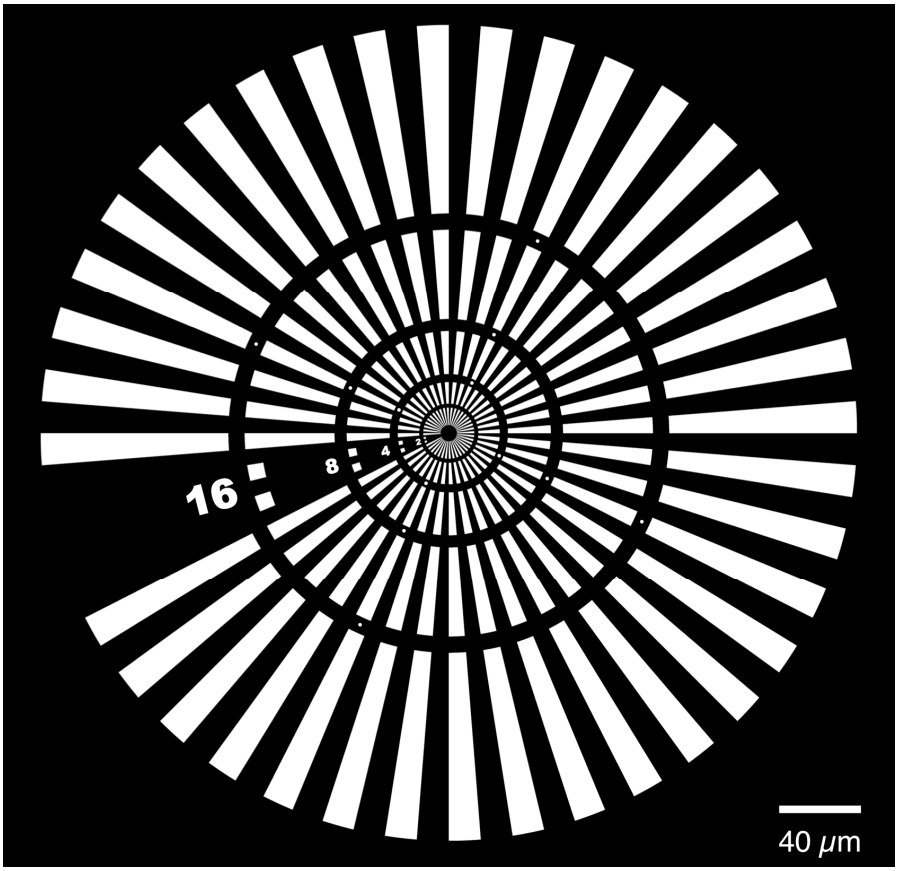

Figure 1: The “Siemens star” target for characterizing resolution in a coherent microscope. This standard includes small fiduciary markers for accurate identification of the target center, as well as numerical marks to denote the full-pitch periodicity (units: μm) along the inner radius of each concentric ring. These numbers also denote the width of each fractional spoke (measured along the radial dimension), which are included as a means to calibrate magnification, and are not to be used for reporting resolution. Instead, contrast should be measured radially and reported as a function of spoke periodicity, and described in the text.

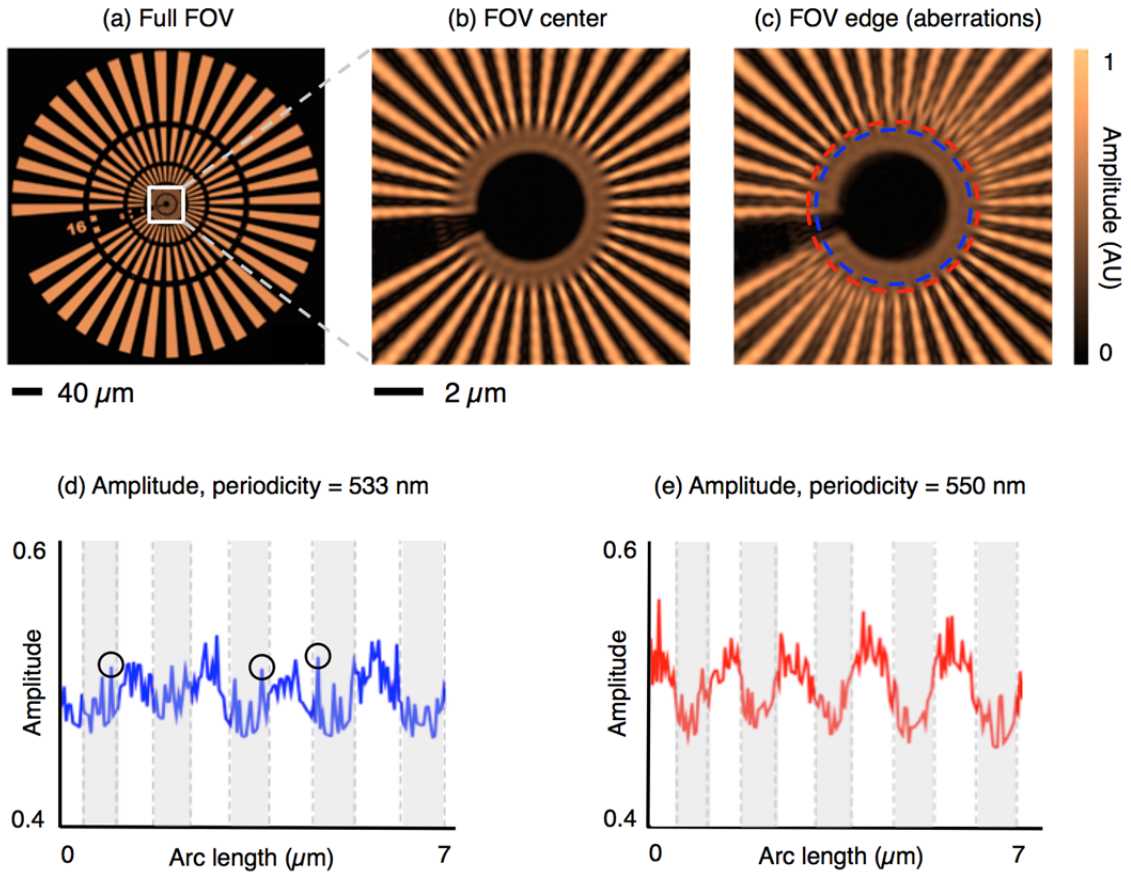

Figure 2: Simulated example of a resolution report with the Siemens star for a coherent microscope. (a) Amplitude image of the target with zoom in to center (region in white box) shown in (b), from a simulated microscope under coherent illumination (λ = 0.40 μm, 100× 0.8 NA objective, pixel size = 1.3 μm, with Poisson noise). (c) Re-imaged target center after moving it to the edge of the sensor field-of-view (FOV, re-centered 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. Since 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 red circle in (d), showing that spokes at a periodicity of 550 nm are unambiguously resolved (verified for all spokes).

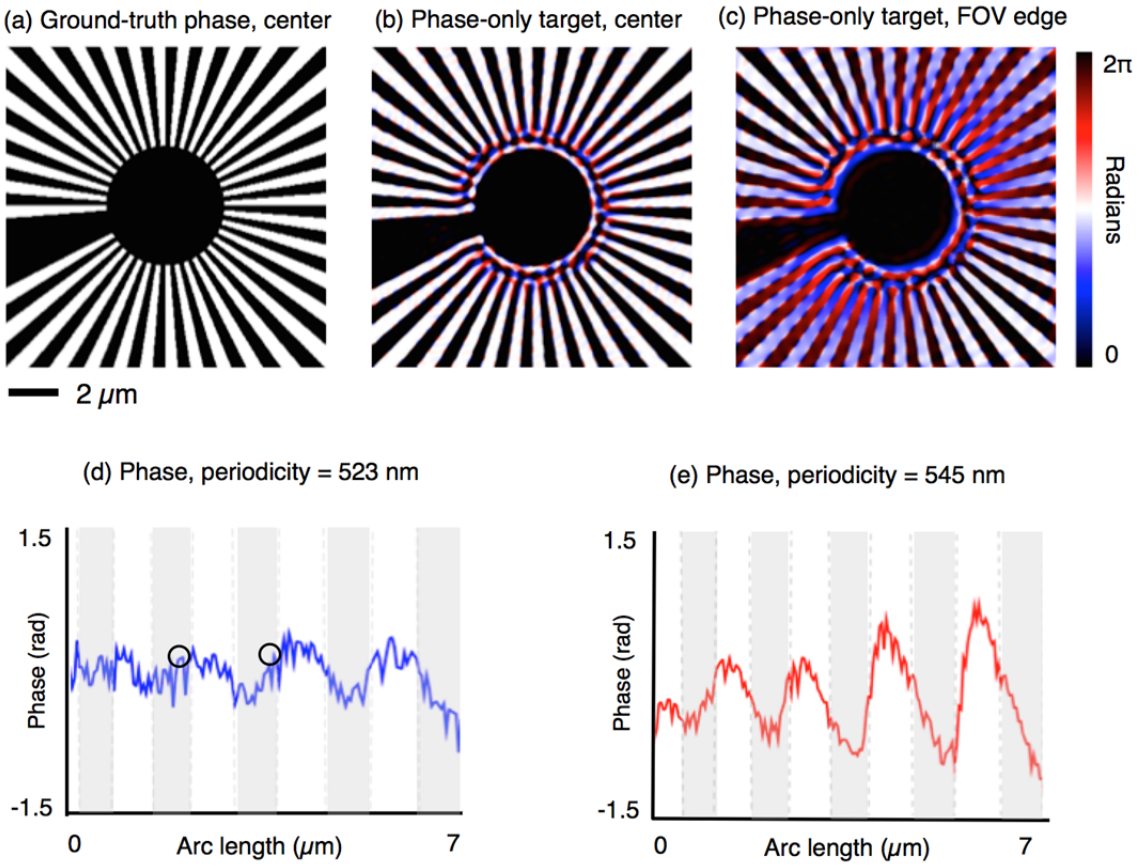

Figure 3: Simulated resolution report with a phase-only Siemens star (similar system specifications and noise as Figure 2). (a) Ground-truth phase target center. (b) Example phase map from the center FOV. (c) Re-imaged target center after moving it to the edge of the sensor FOV, showing the influence of aberrations. (d) Plot of phase values (re-centered at 0) along a segment of a circular trace at 523 nm spoke periodicity, similar to Figure 2(d), with problematic values circled. (e) Plot along a circular trace at a slightly larger periodicity (545 nm) where spokes are unambiguously resolved (verified for all spokes).