

A METHOD FOR ESTIMATING THE RESOLUTION OF REAL IMAGES

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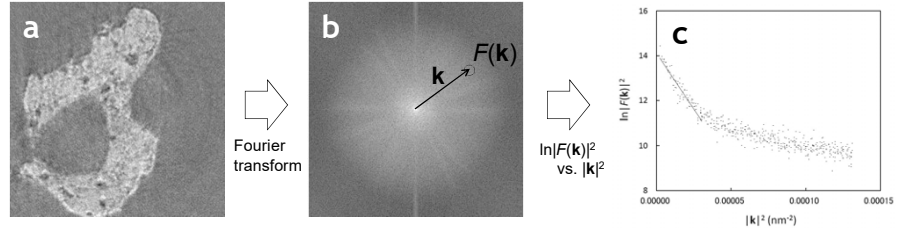
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Estimating the resolution of real images

The resolution of sample image depends on a number of factors, including X-ray optics, stage drift and sample condition. The resolvability of the sample image should therefore be determined from the sample image itself. Here we report a method for estimating the spatial resolution of real images from a logarithmic intensity plot in the Fourier domain [1].

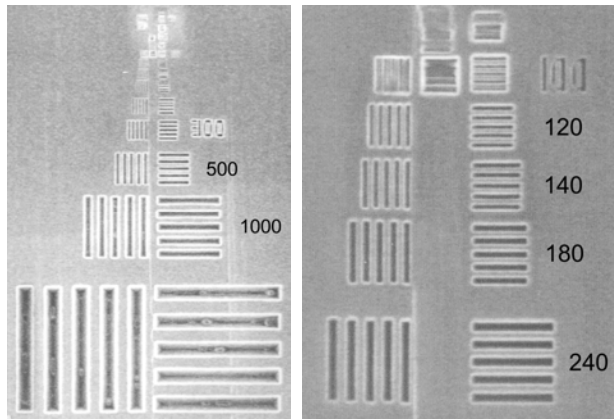
The analyses of test images indicated that the full width at half maximum (FWHM) of a Gaussian point-spread function applied to the original image can be estimated from the logarithmic intensity plots. The spatial resolution of imaging X-ray microtomography using Fresnel zone-plate optics was estimated with this method. The FWHM of the point spread function estimated from a cross section of human brain neuron coincided with the resolution determined from the test object.

These results indicated that the logarithmic intensity plot in the Fourier domain provides an alternative measure of spatial resolution without explicitly defining a noise criterion.



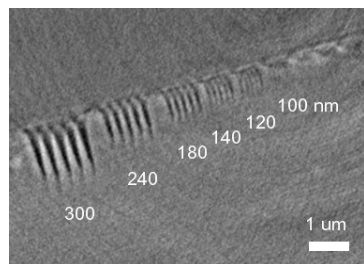
Estimation of spatial resolution from logarithmic intensity plot in the Fourier domain ↑

First, original image (panel a) was subjected to Fourier transform, giving a frequency-domain pattern (b). Then, the logarithm of squared norm of the Fourier transform was plotted against squared distance from the origin (c). Spatial resolution was estimated from the linear correlation observed at the left end of the plot. The slope of the rest of this plot represents the Nyquist limit of digitization.



An aluminum test object with square-wave patterns ↑

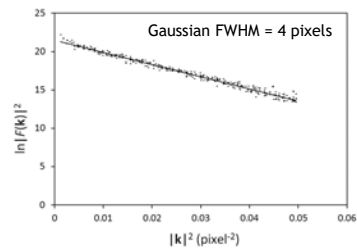
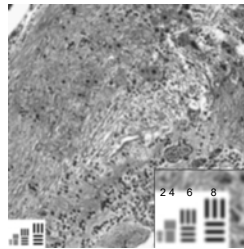
An aluminum rod with approximate dimensions of 120 x 120 x 2000 μm was subjected to ion-beam milling using a focused ion-beam (FIB) apparatus [1-3]. A series of square wells was carved on the surface. Each pattern was composed of half-pitch wells and half-pitch intervals, i.e., a 500-nm well and 500-nm interval for a 1000-nm pitch.



Microtomography with FZP optics

Imaging microtomography was performed at the BL37XU beamline of SPring-8. A Fresnel zone plate with an outermost zone width of 50 nm was used as an X-ray objective lens, and an X-ray guide tube as a beam condenser. Transmission images produced by 8-keV X-rays were recorded using a CMOS-based detector placed at 27.5 m from the sample. The cross section indicated that the patterns up to 120 nm pitches were clearly resolved. Some structures of the 100-nm pitch pattern were also visualized, indicating that the resolution is about 100-120 nm.

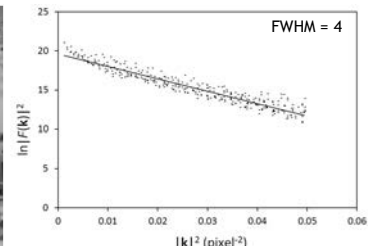
The lower panel shows a cross section of a human pyramidal neuron taken with the same imaging optics. Cellular nucleus, dendrite, and cytosolic structures are visualized. This section was used for the resolution estimation with logarithmic intensity plot.



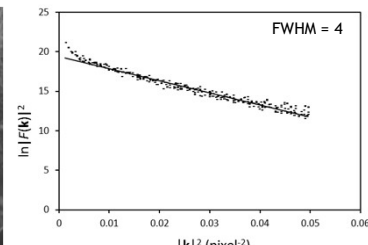
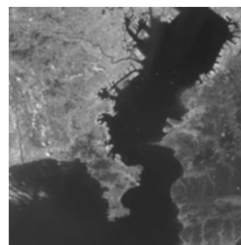
Plots of test images

Test images were generated by convolving the Gaussian point-spread function and the original image (left panels). The appearance of bar patterns embedded in the original image proved that the Gaussian function applied to the original image introduced blurring corresponding to the Gaussian FWHM.

These test images were analyzed with the logarithmic intensity plot in the Fourier domain (right panels). Linear correlations corresponding to the Gaussian were observed at the left ends of the plots. These indicated that the Gaussian point-spread function can be extracted from the image.

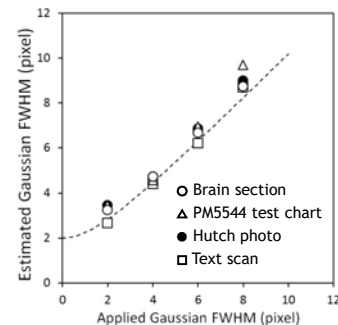


The same slope was identified when the same Gaussian blurring was introduced. This is independent of the type of original image, including a satellite image of Tokyo Bay (©JAXA). Therefore, this method can provide a resolution measure that is directly related to the FWHM of the point spread function. The primary advantage of this method is that the resolution can be estimated without explicitly defining a noise criterion.

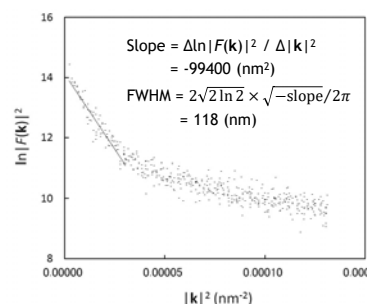


→The FWHM of the point spread function estimated from the logarithmic intensity plot coincided with those of convolved Gaussians, indicating that the spatial resolution of any type of image can be estimated with this method.

←The spatial resolution of a neuron cross section (left) was estimated from the logarithmic intensity plot in the Fourier domain. The plot was calculated from the boxed area. The FWHM estimated from this plot was 118 nm, which coincides with the resolution (100-120 nm) determined from the test object. We suggest that the logarithmic intensity plot in the Fourier domain provides an alternative measure of image resolvability regardless of visualization modality.



[1] Mizutani et al. (2016) *J. Microscopy* **261**, 57-66.
[2] Mizutani et al. (2010) *Nucl. Instrum. Meth. A* **621**, 615-619.
[3] Mizutani et al. (2010) *Micron* **41**, 90-95.



Resolution estimation with logarithmic intensity plot

The structure $\rho(\mathbf{r})$ observed in the image can be expressed as the sum of the point spread functions (PSF) $\rho_i(\mathbf{r})$ of each structural constituent i ,

$$\rho(\mathbf{r}) = \sum_i \rho_i(\mathbf{r}) = \sum_i \rho_i(\mathbf{r}_i + \mathbf{r}'),$$

where \mathbf{r}_i is the coordinate of the center of PSF ρ_i , and \mathbf{r}' the local coordinate within ρ_i . If we assume that each PSF ρ_i with amplitude a_i is proportional to the standard PSF $\rho_o(\mathbf{r}')$ with unit amplitude, each PSF can be written as

$$\rho_i(\mathbf{r}_i + \mathbf{r}') = a_i \rho_o(\mathbf{r}').$$

Then, the Fourier transform $F(\mathbf{k})$ of the structure $\rho(\mathbf{r})$ is calculated as

$$\begin{aligned} F(\mathbf{k}) &= \int \rho(\mathbf{r}) \exp(2\pi i \mathbf{r} \cdot \mathbf{k}) d\mathbf{v} \\ &= \sum_i \int \rho_i(\mathbf{r}_i + \mathbf{r}') \exp[2\pi i (\mathbf{r}_i + \mathbf{r}') \cdot \mathbf{k}] d\mathbf{v} \\ &= \sum_i a_i \exp(2\pi i \mathbf{r}_i \cdot \mathbf{k}) \int \rho_o(\mathbf{r}') \exp(2\pi i \mathbf{r}' \cdot \mathbf{k}) d\mathbf{v}'. \end{aligned}$$

Here, we define the Fourier transform of the standard PSF as

$$f_o(\mathbf{k}) = \int \rho_o(\mathbf{r}') \exp(2\pi i \mathbf{r}' \cdot \mathbf{k}) d\mathbf{v}'.$$

The square norm of $F(\mathbf{k})$ is

$$\begin{aligned} |F(\mathbf{k})|^2 &= F(\mathbf{k}) F^*(\mathbf{k}) \\ &= \sum_i a_i \exp(2\pi i \mathbf{r}_i \cdot \mathbf{k}) f_o(\mathbf{k}) \cdot \sum_j a_j \exp(-2\pi i \mathbf{r}_j \cdot \mathbf{k}) f_o^*(\mathbf{k}) \\ &= \sum_i \sum_j a_i a_j f_o(\mathbf{k}) f_o^*(\mathbf{k}) \exp[2\pi i (\mathbf{r}_i - \mathbf{r}_j) \cdot \mathbf{k}] \\ &= |f_o(\mathbf{k})|^2 \left[\sum_i a_i^2 + \sum_i \sum_{j \neq i} a_i a_j \exp[2\pi i (\mathbf{r}_i - \mathbf{r}_j) \cdot \mathbf{k}] \right]. \end{aligned}$$

If the positional relationship $(\mathbf{r}_i - \mathbf{r}_j)$ can be regarded as being random and if each a_i has comparable amplitude (corresponding to a homogeneous random structure), the second term in the parentheses can be ignored. Therefore, the square norm logarithm can be approximated with

$$\ln |F(\mathbf{k})|^2 \cong 2 \ln |f_o(\mathbf{k})| + \ln \left(\sum_i a_i^2 \right).$$

The PSF constituting the image should be expressed with a certain function for the resolution estimation. The PSF of the real image depends not only

on the optical system but also on the mechanical drift and sample deformation. Though it is difficult to represent all these blurring effects with a simple model function, a PSF approximated with a Gaussian can be easily identified by drawing a logarithmic plot in the Fourier domain. Therefore, we approximated the standard PSF with a Gaussian:

$$\rho_o(\mathbf{r}') = \exp \left(-\frac{|\mathbf{r}'|^2}{2\sigma^2} \right).$$

This assumption was proven appropriate in the resolution estimation of a sample image. The Fourier transform of this standard PSF in the n -dimensional space is expressed as

$$f_o(\mathbf{k}) = (\sqrt{2\pi}\sigma)^n \exp(-2\pi^2\sigma^2|\mathbf{k}|^2).$$

This gives us the relationship,

$$\ln |F(\mathbf{k})|^2 \cong -4\pi^2\sigma^2|\mathbf{k}|^2 + \ln \left(\sum_i a_i^2 \right) (2\pi\sigma^2)^n,$$

indicating that the width σ of the standard PSF $\rho_o(\mathbf{r}')$ can be determined by plotting $\ln |F(\mathbf{k})|^2$ as a function of $|\mathbf{k}|^2$. A similar plot in crystallography is known as the Wilson plot (Wilson, 1942).

The Fourier transform calculations were performed with the RecView program. The logarithm of the average squared norm in each 5×5 pixel bin of the Fourier transform was plotted against the squared distance from the origin. The region along the reciprocal coordinate axes was not included in the plot. The spatial resolution was then estimated from the linear correlation representing the FWHM of point spread function.

See also:

Mizutani *et al.* (2016) *J. Microsc.* **261**, 57-66.