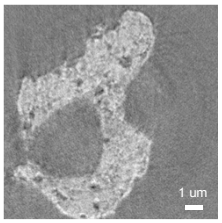
Spatial Resolution of Pre-reconstruction Raw Images and their Nano-CT Slices

Ryuta Mizutani[1]*, Rino Saiga[1], Susumu Takekoshi[2], Chie Inomoto[2], Naoya Nakamura[2], Makoto Arai[3], Kenichi Oshima[3], Masanari Itokawa[3], Vincent De Andrade[4], Francesco De Carlo[4], Akihisa Takeuchi[5], Kentaro Uesugi[5], Yasuko Terada[5] and Yoshio Suzuki[6]

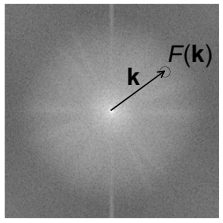
[1]Tokai Univ; [2]Tokai Univ Sch of Medicine; [3]Tokyo Metropolitan Inst of Medical Sci; [4]Advanced Photon Source, Argonne National Lab; [5]JASRI/SPring-8; [6]Univ of Tokyo; *ryuta@tokai.ac.jp

Estimating the resolution of raw images and tomographic slices

Image resolvability is the primary concern in microscopy. In microtomography and nanotomography, a series of raw images are taken while rotating the sample. The resultant raw images are subjected to the tomographic reconstruction to visualize sample cross sections. Therefore, the resolution of the tomographic slice depends on the raw images. However, the resolutions of the pre-reconstruction raw images and their relationship with tomographic slices have not been delineated. Here, we report analyses of the resolution of pre-reconstruction raw images of human neurons and its reconstructed slices.



Fourier transform



Plot $\ln|F(\mathbf{k})|^2$ vs. $|\mathbf{k}|^2$

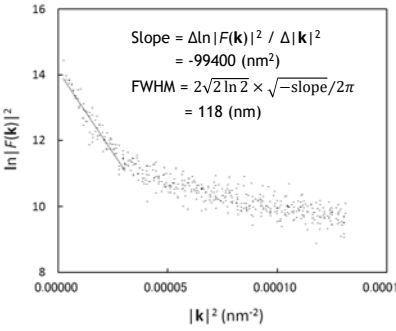
Resolution estimation in Fourier domain

In crystallography, spatial resolution is defined from the detection limit of high-angle diffraction, which primarily depends on the spatiotemporal displacement of atoms. The atomic displacement broadens the apparent distribution of the electron density of each atom, causing the high-angle diffractions to have lower intensities. This relationship can be illustrated as a linear correlation in a logarithmic intensity plot in reciprocal space. We applied this method to nanotomography.

First, sample image (upper panel) was subjected to the Fourier transformation, giving its frequency-domain pattern (middle). Then, the logarithm of squared norm of the Fourier transform was plotted against the squared distance from the origin (lower). If we assume a Gaussian point spread function (PSF), the logarithmic square norm of the Fourier transform $F(\mathbf{k})$ is proportional to the square distance from the origin [1]:

$$\ln|F(\mathbf{k})|^2 = -4\pi^2\sigma^2|\mathbf{k}|^2 + \text{constant},$$

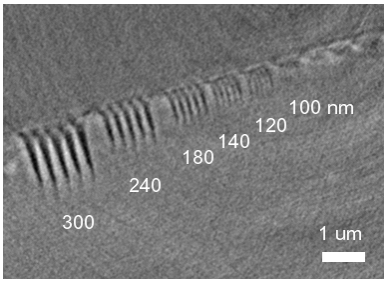
where $\mathbf{k}$ is the Fourier domain coordinate and σ is the standard deviation of the Gaussian PSF. Therefore, spatial resolution can be estimated from the linear correlation in this plot. The full width at half maximum (FWHM) of the PSF estimated from this image was 118 nm.

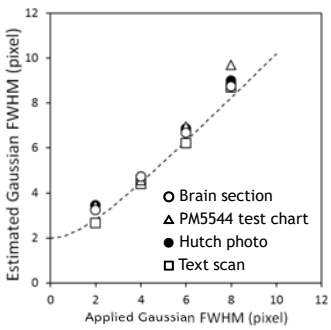


Comparison with a test object

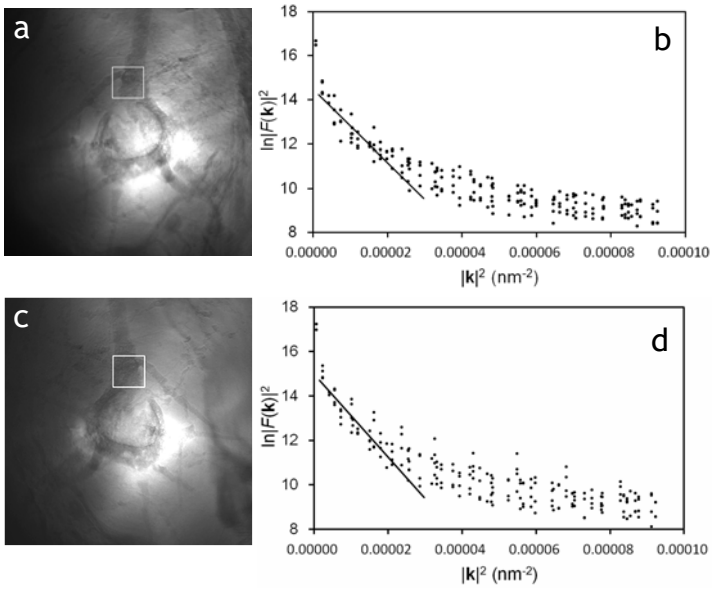
X-ray nanotomography was performed at the BL37XU beamline of the SPring-8 synchrotron radiation facility. A Fresnel zone plate with an outermost zone width of 50 nm was used as an x-ray objective lens, and an x-ray guide tube as a beam condenser. Transmission images produced by 8-keV x-rays were recorded using a CMOS-based detector placed at 27.5 m from the sample.

A cross section of an aluminum test object [2,3] indicated that patterns up to 120 nm pitch were clearly resolved (left). Some structures of the 100-nm pitch pattern were also visualized, indicating that the resolution is 100-120 nm. This coincides with the resolution (118 nm) estimated from the logarithmic intensity plot in the Fourier domain . We suggest that the logarithmic plot provides an alternative measure of image resolvability regardless of visualization modality.

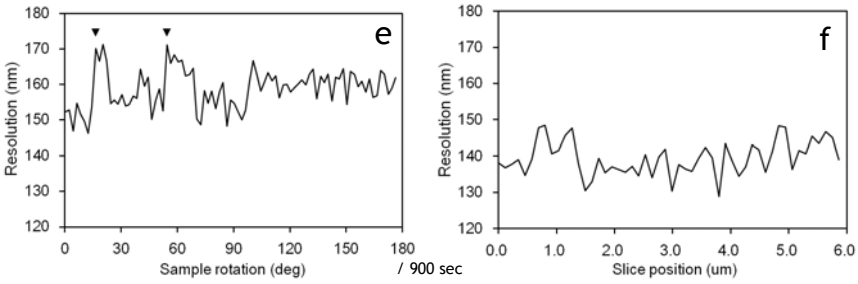




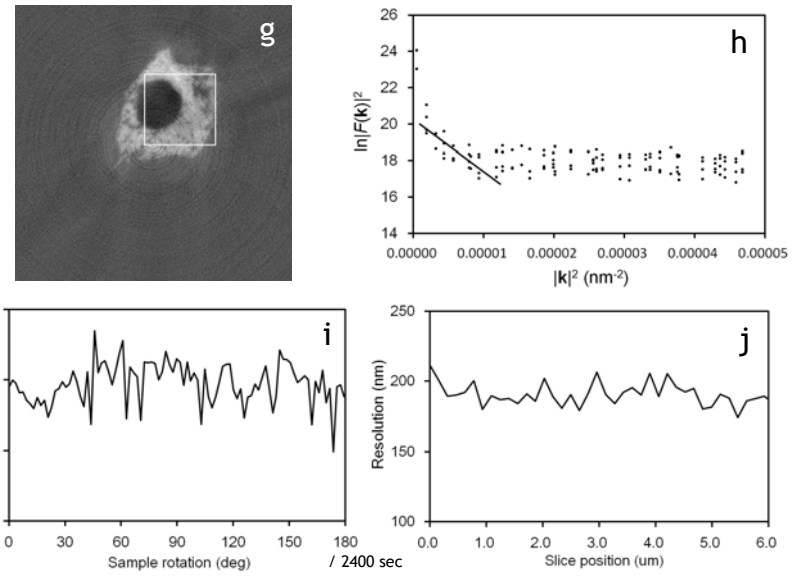
← Test images were generated by convolving Gaussian PSF with a number of different images [1] including a picture taken with a cellphone camera. FWHM of the PSF estimated from logarithmic intensity plots of the test images coincided with FWHMs of convolved Gaussians independently of image types. This indicates that the spatial resolution of any image can be estimated with this method [1,4].



← Panels **a** and **c** show first and last raw frames of a dataset of a human cerebral neuron of a schizophrenia case taken at the BL37XU beamline of SPring-8. Cell body apex indicated with white boxes (256 × 256 pixel; 5.9 × 5.9 μm²) was used for the resolution estimation. Panels **b** and **d** show their logarithmic intensity plots [1]. PSF of region of interest (ROI) can be extracted using this plot independently of structures in the ROI [1,4]. The FWHM of the PSF was estimated from the left end slope. Linear regression was performed by using data between 0.2 × 10^{-5} nm^{-2} and 2.0 × 10^{-5} nm^{-2}. Drawing a line in these plots correspond to approximating the PSF with Gaussians.



The resolutions estimated from raw images showed a few jumps (panel **e**; indicated with triangles) in the first half of the raw image dataset, suggesting that sample drift occurred during the image acquisition. The resolutions of the latter half showed a plateau indicating that sample deformation and x-ray irradiation reached some sort of equilibrium. The resolution fluctuations are also ascribable to other factors, such as optics instability or nanometer glitches of the rotation stage. Tomographic slices were reconstructed from this dataset. Resolutions of the tomographic slices were estimated every 5 slices and plotted against the position along the sample rotation axis (**f**). A 200 × 200 pixel region of the cross section of the cell body apex was used for the estimation. The resolutions of the tomographic slices were mostly better than those of the raw images. This is ascribable to the dose fractionation theorem. The resolution plot against the rotation axis showed no apparent trend, indicating that the resolution has no dependence on the position along the rotation axis.



Panel **g** shows a nanotomography section of a human cerebral neuron of a schizophrenia case taken at the 32-ID beamline of Advanced Photon Source of Argonne National Laboratory. Cellular nucleus and organelles in the cytoplasm were visualized. A 256 × 256 pixel area (6.7 × 6.7 μm²) of a neuronal soma indicated with a white box was used for the resolution estimation. Panel **h** shows its logarithmic intensity plot [1]. Linear regression was performed by using data between 0.10 × 10^{-5} nm^{-2} and 1.0 × 10^{-5} nm^{-2}.

Resolutions of raw images of this dataset showed spikes (panel **i**) toward the high resolution direction, suggesting that the best resolution should be 150 nm or higher though some fluctuations perturbed the image quality. In panel **j**, resolutions of tomographic slices were estimated every 6 slices and plotted against the slice position. The slice-by-slice resolution was almost constant and coincided with the average of the frame-by-frame resolution. These results indicate that the resolution of nanotomography depends not only on the optics performance, but also on many other factors including sample deformation, stage precision, and mechanical/thermal instability.

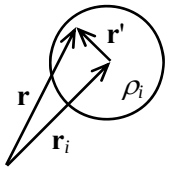
[1] Mizutani *et al*. (2016) *J. Microsc*opy **261**, 57-66.
[2] Mizutani *et al*. (2010) *Nucl. Instrum. Meth. A* **621**, 615-619.
[3] Mizutani *et al*. (2010) *Micron* **41**, 90-95.
[4] Saiga *et al*. (2018) *Micron* **105**, 64-69.
https://mizutanilab.github.io/

Resolution estimation with logarithmic intensity plot

The image $\rho(\mathbf{r})$ can be expressed as the sum of the point spread functions (PSF) $\rho_i(\mathbf{r})$ of each image element *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 basis PSF $\rho_o(\mathbf{r}')$ with unit amplitude, each PSF can be written as

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

The Fourier transform $F(\mathbf{k})$ of the image $\rho(\mathbf{r})$ is calculated as

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

Hereafter, we represent the Fourier transform of the basis PSF $\rho_o(\mathbf{r}')$ with

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

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

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

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 logarithm of $|F(\mathbf{k})|^2$ 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 math function in order to evaluate the image numerically. The PSF of the real image depends not only on the optics performance but also on many other factors including mechanical drift and sample deformation. Though it is difficult to represent all blurring effects with a simple model, Gaussian approximation is widely used and can be easily identified as a linear correlation in the logarithmic plot. Therefore, we approximated the 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 real sample images. The Fourier transform of the Gaussian PSF in the *n*-dimensional space is expressed as

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

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 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).

This method is implemented in the RecView software available from https://mizutanilab.github.io/. The average squared norm in each 5 × 5 pixel bin of the Fourier transform is used for the plot in order to reduce plot data. The region along the reciprocal coordinate axes is not included in the plot, since some cameras give streaks along the axes. The resolution can be estimated with the linear regression of the left end slope, though the linearity is not essential. Drawing a line in this plot just corresponds to a Gaussian approximation of PSF.

See also:

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

Saiga *et al*. (2018) *Micron* **105**, 64-69.