1192
doi:10.1017/S1431927622004986

Microsc. Microanal. 28 (Suppl 1), 2022
© Microscopy Society of America 2022

Dose-efficient tcBF-STEM with Information Retrieval Beyond the Scan Sampling Rate for Imaging Frozen-Hydrated Biological Specimens

Yue Yu[1], Michael Colletta[1], Katherine A. Spoth[1,2] David A. Muller[1,3], and Lena F. Kourkoutis[1,3]*

1. School of Applied and Engineering Physics, Cornell University, Ithaca, New York, USA.
2. Cornell Center for Materials Research, Cornell University, Ithaca, New York, USA.
3. Kavli Institute at Cornell for Nanoscale Science, Ithaca, New York, USA.
*Corresponding author: lena.f.kourkoutis@cornell.edu

Tilt-corrected bright-field (tcBF) STEM, a dose-efficient coherent imaging technique, has been applied to various frozen-hydrated biological systems and outperformed conventional TEM and energy-filtered TEM (EFTEM) for relatively thick specimens [1, 2]. In tcBF-STEM, the resolution limits set by the scan step size can be overcome by making use of redundant information in diffraction space. This allows us to reduce the sampling in real space and still recover information up to a limit set by the probe-forming aperture semi-angle, α [2]. Here we present an effective upsampling procedure for tcBF-STEM and demonstrate information transfer up to 7 times the real-space Nyquist sampling limit: For data collected at a Nyquist frequency (f_N) of 1/16 $Å^{-1}$, the power spectrum of the resulting tcBF-STEM image shows information transfer up to 1/2.3 $Å^{-1}$. Employing fewer scan positions in STEM imaging reduces effects due to the finite detector read-out time which is important for dose-sensitive specimens.

In tcBF-STEM nearly all the incident electron dose is used by collecting the entire convergent beam electron diffraction (CBED) pattern on a pixelated detector [1] such as the electron microscope pixel array detector (EMPAD) [3,4] used here. We combine images formed from individual pixels in the bright-field (BF) disk after correcting for image shifts induced by probe aberrations and the equivalent of beam tilt in TEM. Upsampling is accomplished by determining and applying sub-pixel image shifts even at extremely low doses. Images of frozen-hydrated apoferritin proteins were successfully shift-corrected with only ~0.03 e-/$Å^2$ in a single image by leveraging the probe aberration function [2].

Padding the original image is necessary for sub-pixel shifting and is crucial for artifact-free upsampling. To investigate the effects of different padding methods on upsampling, a 256×256 pixels tcBF-STEM image was obtained on a gold test specimen with an 8-Å step size and a 5-mrad semi-convergence angle (Fig. 1a). Figure 1b shows the same data but upsampled to 2048×2048 pixels using duplication padding and subsequent sub-pixel shifting. Duplication padding copies the original value of a pixel to the neighboring inserted pixels as schematically illustrated in Fig. 1i. As a direct outcome of duplication padding, information transfer at $2n \cdot f_N$ ($n \in Z^+$) is suppressed as reflected in Fig. 1f. To correct for this information suppression, zero padding is instead applied by inserting pixels with zero intensity (Fig. 1i), and the resulting image is shown in Fig. 1c. The FFT (Fig. 1g) of the image shows improved information transfer, however, artifacts visible at specific frequencies ($f_x = 2n \cdot f_N$, $f_y = 2m \cdot f_N$; $n, m \in Z^+$) remain. As a result, the real-space image (Fig. 1c) displays periodic intensity variations caused by a non-uniform distribution of sub-pixel shifts. By tracking the sub-pixel shift distribution and applying an intensity normalization based on this distribution, the periodic artifacts can be corrected. The final artifact-free upsampled image is shown in Fig. 1d. The 2.3-Å gold diffraction ring in the FFT (Fig. 1h) is retrieved from the original dataset collected at a Nyquist frequency of 1/16 $Å^{-1}$.

Upsampling with zero padding and normalized shifting enables artifact-free information retrieval well beyond the Nyquist limit. This procedure provides a distinct improvement in upsampled low-dose tcBF-

CrossMark

https://doi.org/10.1017/S1431927622004986 Published online by Cambridge University Press

STEM images of frozen-hydrated biological specimens. Here, we demonstrate the advantages of this process on cryo-tcBF-STEM images of frozen-hydrated *E. coli* (Fig. 2a) and horse spleen apoferritin (Fig. 2e). Comparison of the upsampled images of *E. coli* and apoferritin using duplication padding (Fig. 2c, Fig. 2g) and zero padding plus normalization (Fig. 2d, Fig. 2h) shows marked improvements with the latter method. Combining tcBF-STEM imaging with the artifact-free upsampling approach presented here offers a robust imaging method for any low-contrast frozen-hydrated biological specimens and is particularly beneficial for relatively thick systems where the conventional TEM imaging is hampered by blurring due to chromatic aberrations and EFTEM imaging where the electrons that contribute to blur are removed is inhibited by low signal [5].

Reference:

[1] KA Spoth et al., Microscopy and Microanalysis **23** (2017), p. 804.
[2] Y Yu et al., Microscopy and Microanalysis **27**(S1) (2021), p. 758.
[3] MW Tate et al., Microscopy and Microanalysis **22** (2016), p. 237.
[4] HT Phillipp et al., arXiv:2111.05889 (2021).
[5] This work is supported by NSF (DMR-1654596, DMR-1429155, DMR-1719875) and the Packard Foundation.

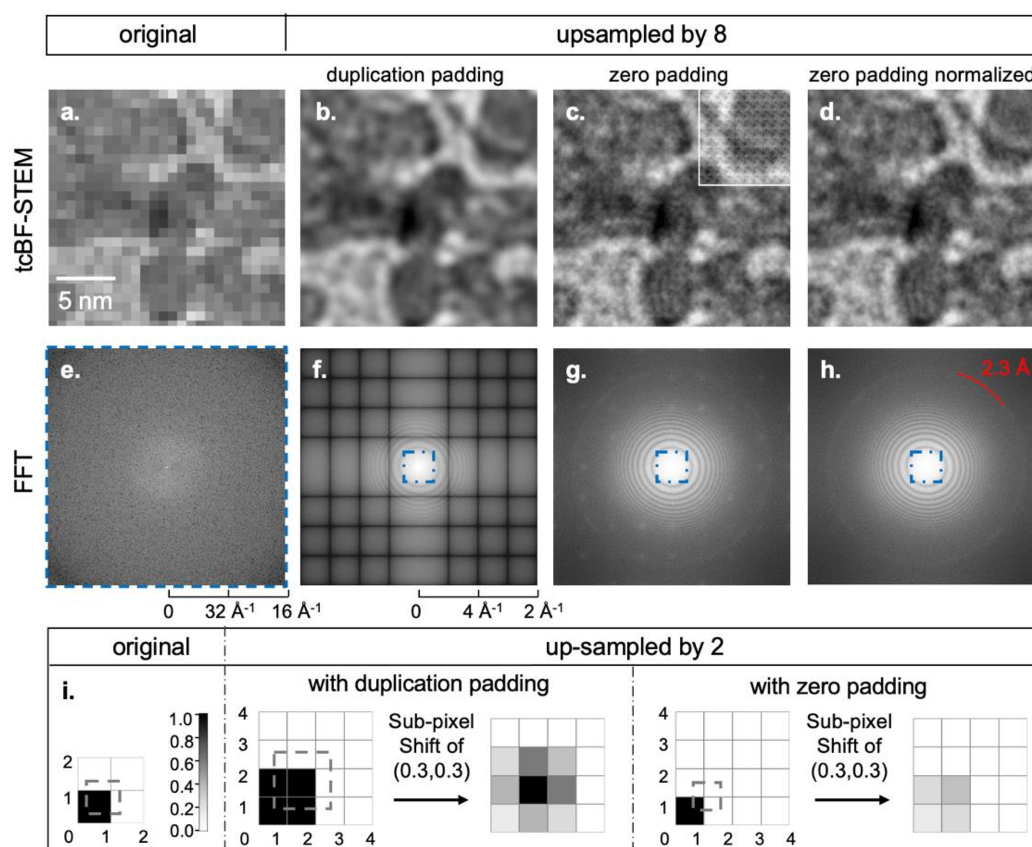

Figure 1. Artifact-free upsampling of tcBF-STEM is accomplished with zero padding and normalized sub-pixel shifting. **(a)** and **(e)** is the 256×256 pixels tcBF-STEM image and its FFT of a gold test specimen with 8-Å scan pixels. Upsampling is achieved by sub-pixel shifting, which requires padding the original

https://doi.org/10.1017/S1431927622004986 Published online by Cambridge University Press

image. **(b)** and **(f)** are the results of upsampling to 2048×2048 pixels with duplication padding. **(c)** and **(g)** are with zero padding. The periodic pattern due to the nonuniform sub-pixel shift distribution is enhanced for better visibility in **(c, inset)**. **(d)** and **(h)** are the results after correcting the periodic artifacts by tracking and normalizing the shift distribution. In **(h)** the 2.3-Å gold diffraction ring is detected even with the original dataset being collected at a 1/16 $Å^{-1}$ Nyquist frequency. **(i)** Schematic of the two different padding approaches for sub-pixel shifting of a 2×2 pixels test image.

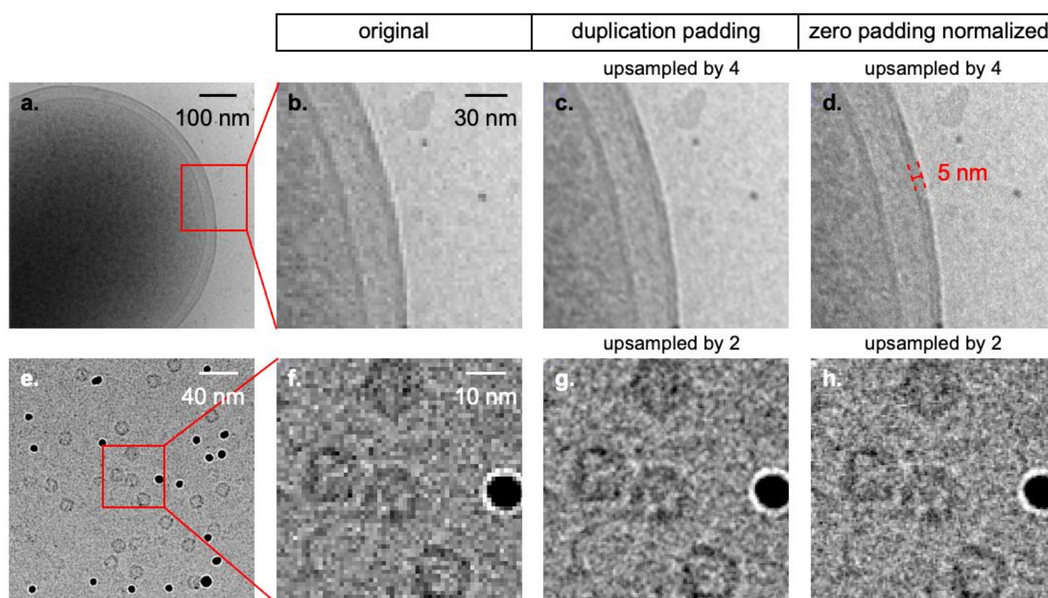

Figure 2. Low-dose tcBF-STEM images of frozen-hydrated biological specimens show marked improvements by optimization of the upsampling procedure. **(a-d)** and **(e-h)** are cryo-tcBF-STEM images of frozen-hydrated *E. coli* and horse spleen apoferritin. Upsampled images of *E. coli* with duplication-padding and normalized zero-padding are presented in **(c)** and **(d)**. **(g)** and **(h)** are upsampled tcBF-STEM images of **(f)** with duplication padding and normalized zero-padding, respectively.

https://doi.org/10.1017/S1431927622004986 Published online by Cambridge University Press