High dose efficiency atomic resolution imaging via electron ptychography

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Abstract

Radiation damage places a fundamental limitation on the ability of microscopy to resolve many types of materials at high resolution. Here we evaluate the dose efficiency of phase contrast imaging with electron ptychography. The method is found to be far more resilient to temporal incoherence than conventional and spherical aberration optimized phase contrast imaging, resulting in significantly greater clarity at a given dose. This robustness is explained by the presence of achromatic lines in the four dimensional ptychographic dataset.

1. Introduction

Energetic electrons, X-rays, and neutrons all cause radiation damage, making dose efficiency an important topic in a broad range of imaging applications, from healthcare and biology to chemistry, physics and materials science. Making the most of the signal available from finite doses can be crucial to seeing the subject clearly before significantly damaging or destroying it. For beam sensitive subjects, the practical resolving power of an imaging system can depend as much on the signal to noise obtained before the sample is damaged as on the imaging optics. Phase contrast imaging can provide significant advantages in this context [1]. For example, phase retrieval in X-ray computed tomography can reduce radiation doses by factors of a thousand or more [2]. Phase retrieval methods also enable lensless coherent X-ray diffractive imaging by solving the so called phase problem computationally [3]. Removing the lens also removes lens aberrations, but radiation damage still limits the resolution of the technique to around 1–10 \( \text{nm} \) depending on the sensitivity of the sample [4]. Ptychography [5–8], a related advanced wavefield reconstruction technique, allows for scanned lensless imaging with a shifting aperture [9]. Ptychography has also been combined with scanning transmission X-ray microscopy to provide imaging of extended specimens with much improved resolution and signal to noise [10,11]. Ptychographic X-ray tomography is now a popular and routinely used technique at beamlines around the world with up to 16 \( \text{nm} \) isotropic 3D resolution [12]. However, for imaging at atomic resolution phase contrast imaging with electrons provides the greatest amount of information for a given amount of radiation damage [13].

To achieve atomic resolution with electrons, either transmission electron microscopy (TEM) or scanning TEM (STEM) are employed. High resolution TEM (HRTEM) employs phase contrast imaging in which plane wave illumination is approximated and contrast is formed via the interference of diffracted beams. In the absence of either aberrations or a phase plate to introduce phase differences in the diffracted beams, little to no contrast is obtained. Therefore imaging is typically performed with a combination of defocus and spherical aberration that optimizes contrast. In STEM, however, aberrations are generally removed to form as fine a probe as possible from a convergent electron beam. This probe is then scanned across the sample and the scattering recorded as a function of position. The analytical capabilities this provides, such as the annular dark field (ADF) and spectroscopic signals, have made STEM a popular tool in physics and materials science. However in fields such as molecular biology where specimens have very low dose tolerances indeed, HRTEM still dominates due to the efficiency of its phase contrast imaging. The rise of cryo-EM, highlighted by the 2017 Nobel Prize in Chemistry, has spurred a search for means of improving phase contrast imaging such as phase plates [14]. Recent technological advances have also greatly enhanced the capability to record the four dimensional datasets ptychography requires in STEM [15–24], however its performance relative to HRTEM has remained an open question.

Here we evaluate the dose efficiency of ptychographic phase contrast imaging in STEM in comparison to HRTEM for weakly scattering specimens. Image simulations allow us to control and analyse the influence of the factors affecting image contrast. We use graphene as an ideal simple evaluation specimen, and also an adenosine triphosphate (ATP) molecule as an example of imaging small molecules. We find that the greatest difference between the dose efficiencies of phase contrast...
HRTEM and STEM ptychography is that the latter is far less susceptible to temporal incoherence. Temporal incoherence and lens fluctuations lead to defocus spread which can significantly reduce image quality. With typical levels of temporal incoherence, the clarity of the ptychographic phase images is far higher than the equivalent HRTEM images. This can be understood in terms of the presence of achronic lines in the double disk overlap regions in the probe reciprocal space of the four dimensional ptychographic dataset. In addition, ptychographic phase images are readily interpretable, and provide images at twice the resolution of HRTEM images under equivalent conditions. The results show that, at least for weakly scattering specimens, such ptychographic imaging can provide superior dose efficiency to HRTEM.

2. Material and methods

The MULTEM package [25] was used to simulate both HRTEM images and convergent beam electron diffraction (CBED) patterns. The CBED patterns were then processed using the single side band (SSB) method of ptychography [18] with an in house produced MATLAB code. Finite doses were simulated using a Poisson noise generator. The ability to apply defocus spread is built into the MULTEM package for HRTEM. For the STEM ptychography, defocus spread was implemented by simulating the CBED patterns across the desired range of defocus values and averaging, simply weighting each slice equally. Care was taken to ensure the data was normalized such that sum of each CBED pattern was equal to one before multiplying by the dose and inputting to the noise generator. Similarly the HRTEM images were normalized such that the incoming wave is an array of ones, and the output image averages to one, before applying the dose.

3. Results and discussion

Fig. 1 compares HRTEM and STEM images simulated with a dose of 20,000 e/Å², an accelerating voltage of 80 kV and 35 mrad semi-angle apertures. A second order Butterworth low pass filter [26] with a cutoff frequency corresponding to just outside the third ring of spots of graphene (1.07 Å) has been applied to the bottom of each image. HRTEM imaging conditions in Fig. 1 were chosen as Cs = 20 μm, and 9 nm defocus which optimizes the graphene contrast in the partially coherent case, and corresponds to the values used in previous HRTEM experiments [27]. The STEM data was aberration free. With the exception of the perfectly coherent HRTEM image, a defocus spread of 3.2 nm was employed. The effect of phonons was checked with up to 1024 phonon configurations and an RMS value of 0.039 Å [28], and found to be insignificant for the present comparison.

The lattice is visible in the partially coherent HRTEM image, but the carbon atoms themselves are barely resolved even when low pass filtered. Filtering cannot bring back the information lost to the noise. The ptychographic image produced with the same defocus spread and noise level however shows very clearly resolved atoms. In fact the ptychographic image more closely resembles the perfectly coherent HRTEM image, suggesting that the ptychographic method is relatively insensitive to the effects of partial temporal coherence. For comparison, a medium angle ADF (MAADF) image, using a 40–240 mrad detector, is also shown. Such MAADF imaging is standard in STEM for imaging weakly scattering light 2D materials. The image includes a Gaussian blur to account for finite source size, with parameters set such that the image resembled experimental images at the higher dose of 10⁶ e/Å² [29]. At the much lower dose of 20,000 e/Å² used in Fig. 1 the lattice is barely visible, with only the first ring of spots (2.13 Å) present in the FFT of the image, as expected for this dose [30].

A significant difference between the ptychographic and the coherent HRTEM images of graphene in Fig. 1 is that the HRTEM image shows fictitious atomic contrast in the center of every carbon hexagon. These arise from the oscillations that occur in the contrast transfer function (CTF). Such contrast can be misleading, and it is beneficial that the ptychographic images do not exhibit it. This is because the ptychographic CTF is a simple, single signed curve increasing in magnitude continuously from zero frequency until peaking at a and slowly falling to zero again at 2a where a is the probe convergence semiangle [19]. This passband CTF can be seen in the intensity of the FFT of the ptychographic images in Fig. 1. Very low spatial frequencies have a low intensity, and there is a clear cutoff at around 0.67 Å beyond which noise and signal are suppressed. The upper cutoff is at a slightly lower frequency than expected as for the present conditions λ/2a = 0.60 Å, where λ is the wavelength of the fast electrons. However this turns out to be the result of partial coherence, as for the defocus spread free ptychographic data at the same dose (Fig. 2) the cutoff indeed appears at 0.60 Å.

Passband CTF conditions can also be created in HRTEM by using Cs correction to push the first CTF crossover out to correspond with the aperture limit, cutting off the oscillations at higher frequencies as illustrated in Fig. 2. This provides HRTEM images that lack spurious contrast and are therefore easier to interpret. However the maximum frequency passed will correspond to a, rather than the 2a of ptychography. Thus for equally capable aberration correctors, ptychography will provide twice the resolution. Furthermore, as shown in Fig. 2, such passband conditions are still limited by the coherence envelope. For example, using a 70 mrad aperture the resolution of the coherent HRTEM is equal to that of the 35 mrad ptychographic image, but once partial temporal coherence is included the HRTEM resolution decreases severely, with spots only up to 1.23 Å visible in the FFT. In contrast, partial temporal coherence only slightly reduces the maximum resolution clearly present in the ptychographic image from 0.7 Å to 0.8 Å.

A second comparison between HRTEM and ptychographic phase contrast imaging is shown in Fig. 3 using the small, easy to simulate, ATP molecule. The molecule is largely only a single atom thick in the projection we have used, and is thus too thin to be visible with current methods at doses it can survive. We again chose a dose of 20,000 e/Å² for the finite dose simulations as it facilitates comparison of the dose efficiencies of HRTEM and the ptychography. Although this dose is far
Simulated passband imaging of graphene at 80 kV and 20,000 e−/Å². Pink overlays on the CTF plots indicate the oscillations at higher spatial frequencies removed by the aperture. The images are shown at the same scale as in Fig. 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Phase contrast imaging of an ATP molecule at a dose of 20,000 e−/Å² except where noted. A Butterworth low pass (LP) filter has been applied to the HRTEM images on the right. A model of the structure in the inset with P pink, O red, N light blue and C brown. The scale bar indicates 0.5 nm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

higher than the molecule can survive, the results nevertheless demonstrate that the advantages of ptychography also apply to non-periodic specimens such biological molecules. The defocus was set to 2 nm and C₀ to zero for the HRTEM simulations as these parameters were found to maximize the contrast. The STEM images were again simulated assuming an aberration free probe. All the images in this figure were simulated using still atoms. Both ptychographic images and the partially coherent HRTEM images use a defocus spread of 3.2 nm. Again the ptychographic images are far clearer than the equivalent HRTEM, and more closely resemble the clarity of the completely coherent HRTEM. While the Butterworth filter despeckles the images, the detrimental effect of partial coherence on HRTEM imaging cannot be removed by low pass filtering.

Pelz et al. have compared the performance of ptychography using a defocused probe iterative Bayesian approach to HRTEM for biological molecules with simulations [31]. They used far larger molecules, 64 kDa to 4 MDa, allowing them to use doses typical for cryo-EM. The iterative Bayesian ptychography clearly far outperformed conventional HRTEM. Their simulations also showed the ptychography performing significantly better than in focus HRTEM using a Volta phase plate at higher spatial frequencies, with up two orders of magnitude higher signal to noise. Interestingly Pelz et al. did not consider partial coherence. The present results show that with a realistic defocus spread ptychography can be expected to further outperform HRTEM in cryo-EM, and mostly likely also when using a phase plate. Furthermore, although defocused probe techniques allow for easier access to low doses in experimental ptychography, the defocus is detrimental to or completely precludes simultaneous imaging with ADF or spectroscopic techniques. It will be interesting to compare iterative methods such as the Bayesian approach used with an in focus probe to direct methods such as the SSB method in future studies. Although the SSB ptychographic method employed here imparts a degree of band pass filtering due to the shape of its CTF, the reasons for its efficiency and resilience to partial coherence are more complex. In the weak phase approximation, the Fourier transform of the intensity in the Ronchigram with respect to probe position can be written

\[
G(K_f, Q_p) = |A(K_f)|^2 \delta(Q_p) + A(K_f)A^*(K_f + Q_p)\Psi^*(-Q_p) + A^*(K_f)A(K_f - Q_p)\Psi(Q_p),
\]

(1)

where \(\Psi(Q_p)\) is the Fourier transform of the object transmission function at a spatial frequency of \(Q_p\), \(K_f\) is the position on the detector or scattering angle, and \(A(K_f) = |A(K_f)| \exp(i \chi(K_f))\) is an aperture function describing the extent of the aperture via a top hat function, \(a(K_f)\), and the aberrations present in the probe via the aberration function \(\chi(K_f)\) [6]. Due to the multiplication of the top hat functions in \(G(K_f, Q_p)\), transfer of the object transmission function for a given spatial frequency occurs only where the unscattered disk, \(A(K_f)\), and the diffraction disks, \(A(K_f \pm Q_p)\), overlap. Furthermore, because \(\Psi^*(-Q_p)\) and \(\Psi^*(-Q_p)\) have the same amplitude but are \(\pi\) radians out of phase, where the pairs of diffraction disks overlap, they cancel out. Therefore, without aberrations, transfer of the transmission function occurs only in regions of double disk overlap, where the diffracted disks overlap the central disk but not each other. For each spatial frequency the position in \(K_f\) of these double overlap regions is different, as illustrated in Fig. 4. It is by separately extracting the phase and amplitude from the double overlap regions in \(G(K_f, Q_p)\) of each spatial frequency that ptychography maximises the signal to noise ratio. This is not possible with a detector that simply integrates over fixed areas of the scattering. Subtracting opposing quadrants of the bright field disk as in differential phase contrast also does not adjust the integration regions with respect to frequency resulting in lower efficiency [18, 19, 22].

Fig. 5 shows the amplitude of \(G(K_f, Q_p)\), plotted for a single \(Q_p\) with a strong amplitude, simulated with various defocus spreads, \(\Delta\). With \(\Delta = 0\) the amplitude is high throughout the double disk overlap regions, but as the defocus spread is increased lines of increasing sharpness appear along the centers of the double disk overlaps. Along these so called achromatic lines, \(G(K_f, Q_p)\) is immune to aberrations such as defocus [7]. This is the physical origin of the robustness of ptychography to defocus spread. In the presence of significant defocus spread, the shape of the amplitude acts as a filter. Regions strongly affected by
The combination of robustness to partial coherence, interpretability and double resolution relative to HRTEM mean that STEM ptychography offers very substantial benefits for low dose imaging. The greatest barrier to reaping these benefits experimentally is at present the speed of pixelated detectors. So far the speed of the camera has limited the rate the STEM probe can be scanned in every STEM ptychography experiment. While the probe current can be reduced somewhat, slower scans also mean greater susceptibility to drift, probe jitter, and environmental noise, and at some point reducing the probe current becomes impractical. Fortunately, cameras are improving rapidly and pixelated detectors are becoming available that should allow ptychography to be performed with the microsecond dwell times typical in STEM imaging. The weak phase approximation used in the SSB method of ptychography employed here quickly breaks down for thicker heavier samples, and methods to account for the effects of dynamical scattering will be highly desirable for such samples. However, the present results already indicate that with a sufficiently fast detector, single particle type analysis of light beam sensitive samples could benefit greatly from STEM ptychography. Despite the immense recent progress in cryo-EM, resolution and signal to noise in structural biology is still limited by radiation damage. An increased dose efficiency might not only further improve resolution, but also permit obtaining the same resolution with a smaller number of particles. Furthermore, experimentally both the SSB and the more sophisticated Wigner-distribution deconvolution method, which relies on a multiplicative approximation, retain remarkable imaging efficiency even for much thicker and heavier crystals than these approximations apply, revealing light elements in a heavy lattice at least on par with negative Cs HRTEM. Understanding this will take further study, however Plamann and Rodenburg have already shown how highly related ptychographic methods can be described in terms that go beyond the weak phase approximation and provide accurate imaging of Si \langle110\rangle up to about 25 nm in thickness. Furthermore, methods to explicitly account for dynamical scattering in ptychography are already being developed.

4. Conclusion

In conclusion, we have demonstrated that STEM ptychography is capable of providing superior dose-efficiency to conventional HRTEM for light materials. The achromatic lines in the double disk overlap regions of the ptychographic datasets provide far higher resilience to partial coherence than conventional HRTEM. Although monochromators and chromatic aberration correctors are now available to mitigate this sensitivity of HRTEM, STEM ptychography offers phase images with inbuilt interpretability and double resolution. Further research into optimizing ptychography for thick and heavy specimens will be needed, but with detectors becoming available that enable truly...
rapid acquisition of the four dimensional datasets required, the future is looking bright for low-dose ptychographic STEM.

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References

[1] T.E. Gureyev, Y.I. Nesterets, F. de Hoog, G. Schmalz, S.C. Mayo, S. Mohammadi, G. Tromba, Duality between noise and spatial resolution in linear systems, Opt. Express 22 (8) (2014) 9087–9094.

[2] M.J. Kitchen, G.A. Buckley, T.E. Gureyev, M.J. Wallace, N. Andress-Thin, K. Uesugi, N. Yagi, S.B. Hooper, CT dose reduction factors in the thousands using X-ray phase contrast, Sci. Rep. 7 (1) (2017) 15953.

[3] H.N. Chapman, A. Barry, S. Marchesini, A. Noy, S.P. Hau-Riege, C. Csi, M.R. Howells, R. Rosen, H. He, J.C.H. Spence, U. Weierstall, T. Bezt, J. Jacobsen, D. Shapiro, High-resolution AB initiates three-dimensional X-ray diffraction microscopy, J. Opt. Soc. Am. A 23 (5) (2006) 1179–1200.

[4] S. Marchesini, H.N. Chapman, S.P. Hau-Riege, R.A. Lexmond, A. Sroke, H. He, M.R. Howells, H. Padmore, R. Rosen, J.C.H. Spence, U. Weierstall, Coherent X-ray diffractive imaging: applications and limitations, Opt. Express 11 (19) (2003) 2344–2353.

[5] W. Hoppe, Diffraction in inhomogeneous primary wave fields 0.1. principle of phase determination from electron diffraction interference, Acta Crystallogr. Sect. A Crystal Phys. Diff. Theor. Gen. Crystallogr. A 25 (4) (1969) 495–501.

[6] J.M. Rodenburg, B.C. McCallum, P.D. Nellist, Experimental tests on double-resolution coherent imaging via STEM, Ultramicroscopy 48 (3) (1993) 304–314.

[7] P.D. Nellist, J.M. Rodenburg, Beyond the conventional information limit: the relevant coherence function, Ultramicroscopy 54 (1) (1994) 61–74.

[8] P.D. Nellist, B.C. McCallum, J.M. Rodenburg, Resolution beyond the ‘information limit’ in transmission electron microscopy, Nature 374 (1995) 630.

[9] H.M.L. Faulkner, J.M. Rodenburg, Movable aperture lensless transmission microscopy: a novel phase retrieval algorithm, Phys. Rev. Lett. 93 (2004) 023903.

[10] F. Pfeiffer, X-Ray ptychography, Nat. Photonics 12 (1) (2018) 9–17.

[11] R. Henderson, The potential and limitations of neutrons, electrons and X-Rays for atomic-resolution microscopy of unstained biological molecules, Q. Rev. Biophys. 40 (2007) 135–193.

[12] R. Daney, B. Bujise, M. Khoshouei, J.M. Piltzko, W. Baumeister, Volta potential and GTM acknowledge funding from the EPSRC (grant EP/M010708/1).