Electronic Supplementary Information for:

A new approach to high resolution, high contrast electron microscopy of macromolecular block copolymer assemblies.

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Exit wave reconstruction

Exit wave reconstruction (EWR) is a process in which the phase of the electrons after they have passed through the sample can be recovered by analyzing information from a series of differently aberrated images. This is most commonly implemented by recording multiple images with a varying defocus. There are several such methods for performing exit-wave reconstruction, some such as the focal and tilt series reconstruction (FTSR) algorithm developed by Kirkland[1] and co-workers produce a result in one application, and other techniques like iterative wave function restoration and the maximum likelihood method by Allen[2] and Coene[3] respectively, require numerous iterations before reaching convergence and as such would be difficult to implement into a fast EWR scheme like the one used here.

The basic principle of reconstruction from multiple images is that each image will have a different transfer function due to the different aberrations and that the gaps in the information transfer from one image can be filled by information from another image. Where the information is present in multiple images a better estimate can be achieved by averaging over the different values. Additionally once the complex exit wave has been restored, higher order aberrations can be removed computationally if they have been accurately determined via other methods.

In FTSR the complex exit-wave ψ can be can be calculated as in Equation 1 where the wave transfer function for each image w_i is known, c_i is the Fourier transform of the image contrast, and υ represents a noise to signal ratio to prevent a zero denominator when there is no transfer from any image.

$$\psi = \frac{(\sum_i |w_{i-}|^2 + \upsilon)\sum_i w_{i-}{}^* c_i - \sum_i w_{i-} w_i{}^* \sum_i w_{i-} c_i}{(\sum_i |w_{i-}|^2 + \upsilon)(\sum_i |w_i|^2 + \upsilon) - |\sum_i w_{i-} w_i|^2} \quad (1)$$

This requires knowledge of the aberrations (at least the defocus) for each image in order to calculate the wave transfer functions. The relative focus difference between any two images can be calculated by a phase compensated phase correlation function (PCPCF) at which point an exit wave restoration can be produced at some arbitrary focus level relative to the image series.[4] The absolute focal level of this restored exit wave can then be accurately determined by a phase contrast index (PCI) approach[4] which allows an exit wave at the correct focus level to be produced. This exit wave can also be compensated for the effects of other aberrations such as spherical aberration without any reliance on expensive hardware aberration correctors. Additionally the effects of the CCD camera can be compensated for with the inclusion of a modulation transfer function (MTF)

and noise power spectrum (NPS). The MTF and NPS in this work were both determined using the FTSR package from HREM Research Inc.

Rapid EWR in Digital Micrograph

The reconstruction implemented in Gatan's Digital Micrograph software was developed to produce a restored exit-wave from a focal series of images in as little time as possible in order to develop the procedure as a technique that could be performed routinely at the microscope without requiring extensive offline processing. A general reconstruction for a focal series of 20 images of 2048 by 2048 pixels can be performed in around 2 minutes on a typical quad-core workstation. Where additional hardware such as a powerful GPU is present this time can be reduced to around 10 seconds, (not including image acquisition). This is partly due to developing a parallelized code that can be accelerated on suitable computers, and partly due to the omission of some refinements included in other reconstruction software (the focal step between images is assumed to be exactly as specified during acquisition with no compensation for focal drift). This quicker reconstruction enables rapid evaluation of experimental results, whilst a full reconstruction using a package such as FTSR from HREM Research Inc. can be used to refine specific results in a more complete manner.

The DMG plugin also contains the necessary functionality to control the microscope and record the focal series of images (provided the minimum focal step of the microscope has previously been calibrated). It can be seen in Fig. S4 that the results from this method compare well with those using the more complete reconstruction procedure. This plugin is available on request from m.a.dyson@warwick.ac.uk.

Supplementary Figure S1

Fig. S1 shows the final 20 images from a full 40 image series of the polyacrylic acid-b-polystyrene polymersome (4008 by 2672 pixels at 0.5s exposure time) after the images have been corrected for specimen drift. The improvement in contrast with increasing defocus is readily apparent, as is the stability of the polymersome throughout the entire series.

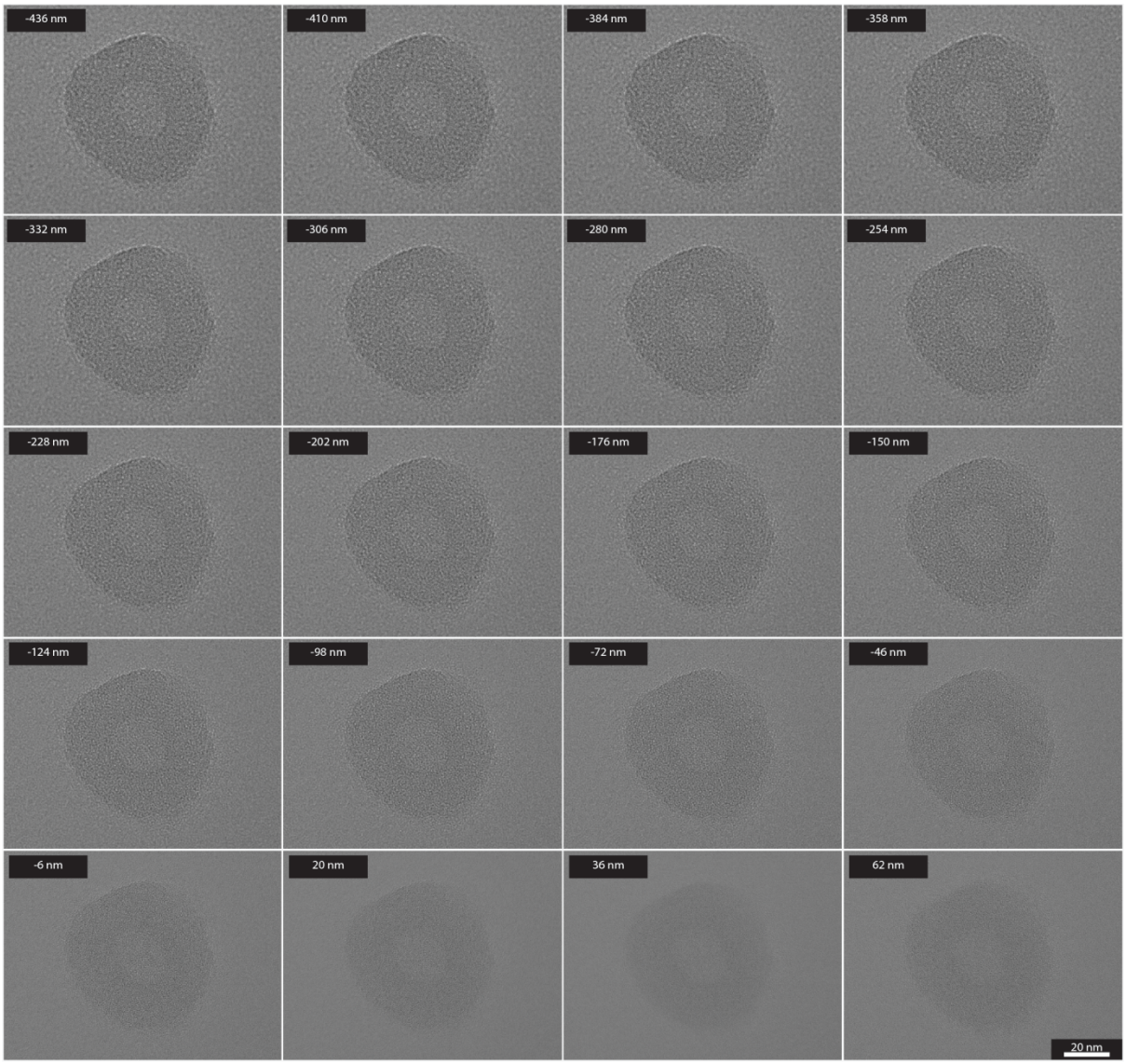

Figure S1. Drift corrected focal series of bright field TEM images of the polyacrylic acid-b-polystyrene polymersome starting from most underfocus. The focal variation between images was determined to be 26 nm.

Supplementary Figure S2

To further test the applicability of this approach, EWR of the cylindrical micelle supported on an ultra-thin amorphous carbon support with a nominal thickness of 6 nm was performed. Whilst sufficient contrast is observed to locate the micelle, the phase reconstruction is dominated by contributions from the amorphous support material. This demonstrates the necessity in using extremely low-contrast supports such as graphene oxide for EWR of the cylindrical micelle.

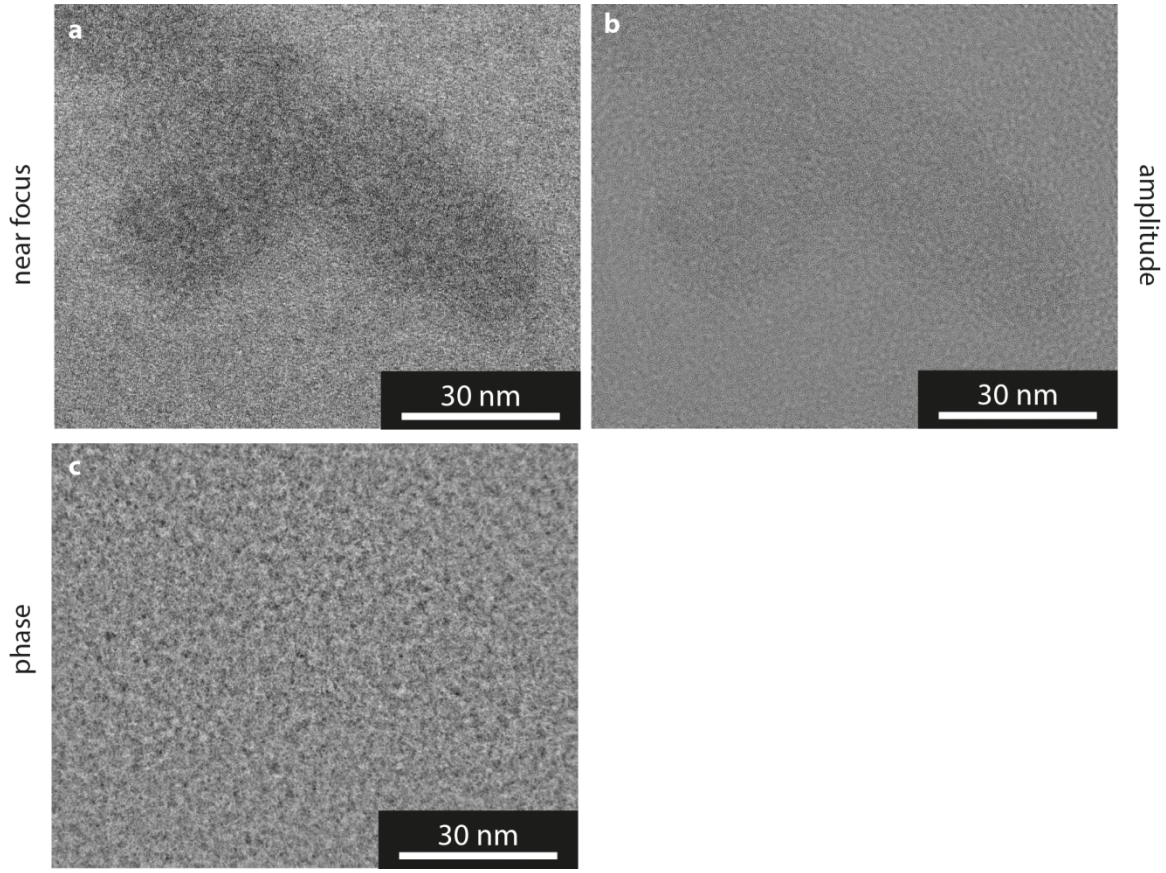

Figure S2. EWR of a cylindrical micelle on ultra-thin (6 nm) amorphous carbon support: (a) near focus, (b) EWR amplitude (c) EWR phase image. The near focus image was taken with focus of -25nm.

Supplementary Figure S3

The reconstructions in Fig. S3 were generated from two non-overlapping subsets of images from the series shown in Fig. S1. Fig. S3a was reconstructed using only the even numbered images, and Fig. S2b was reconstructed using only the odd numbered images. It can clearly be seen that the two independent results both produce remarkably similar contrast features and that in this case the result was over-determined by the number of images in the complete series.

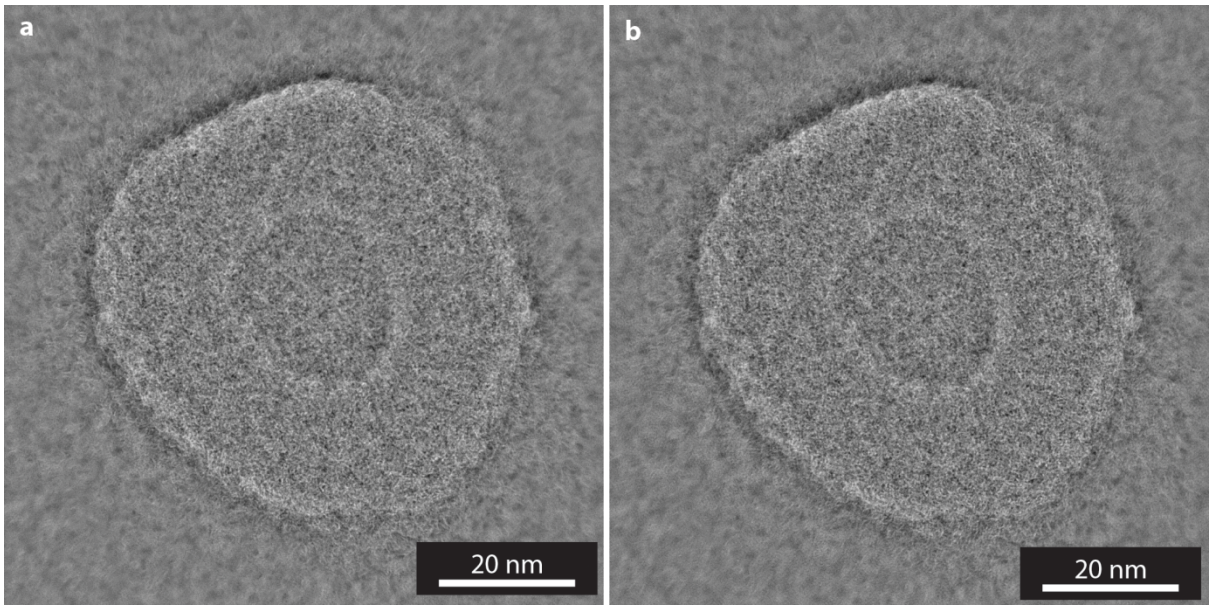

Figure S3. Phase image reconstructions of the polyacrylic acid-b-polystyrene polymersome using only half of the images from the complete focal series. (a) even numbered images only and (b) odd numbered images.

Supplementary Figure S4

EWR can also be performed starting from a focal series using as few as 3 images. Fig. S4 shows the results of EWR from a focal series as the number of images used in the reconstruction is increased.

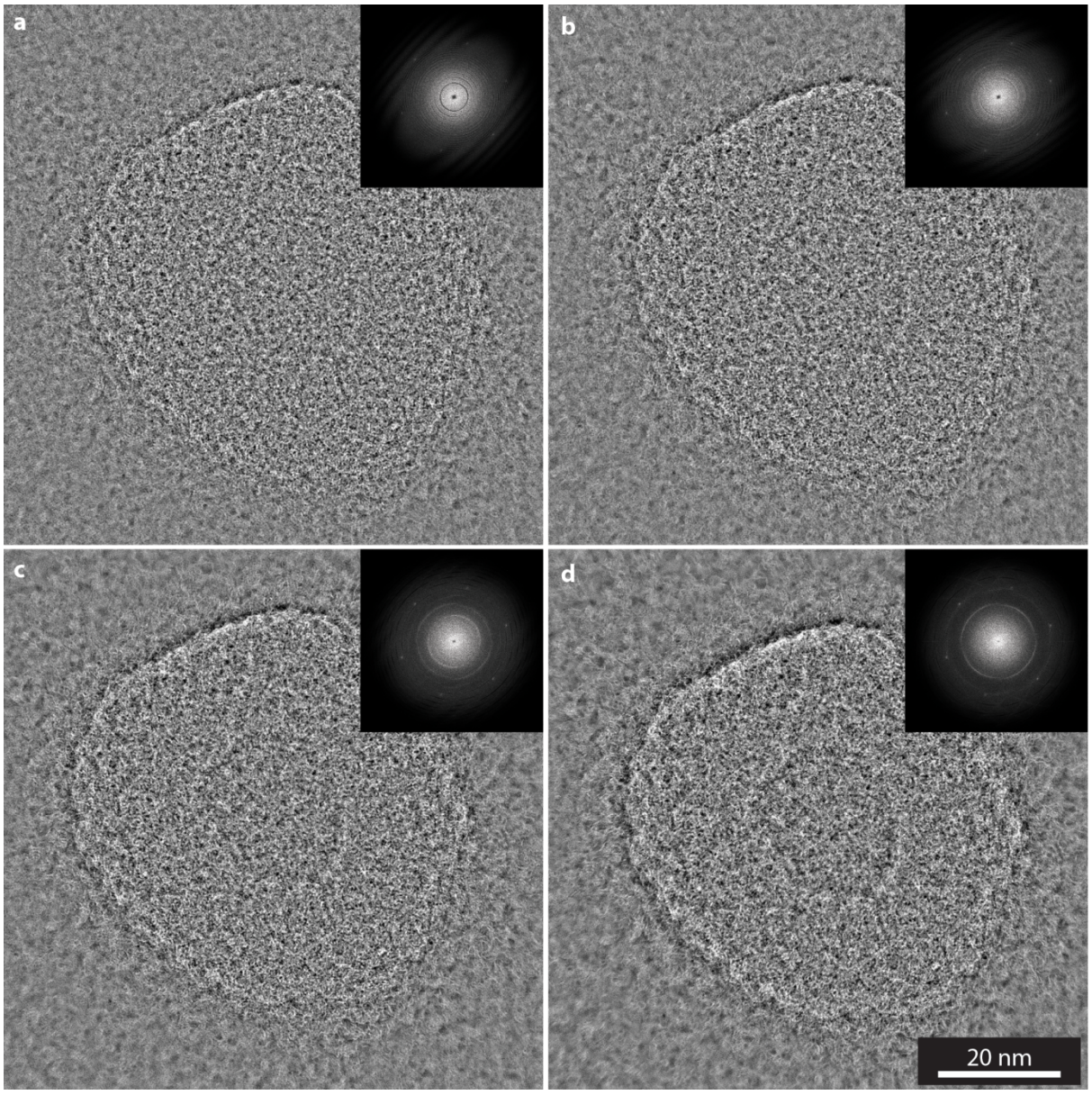

Figure S4. Phase image reconstructions of the polyacrylic acid-b-polystyrene polymersome using different numbers of images evenly spaced from the focal series. (a) reconstruction from 3 images (b) reconstruction from 5 images (c) reconstruction from 10 images and (d) reconstruction from 20 images. FFTs of the reconstructed phase image are inset.

Supplementary Figure S5

The same focal series has been used to perform EWR using a number of different algorithms to check the consistency of the results. Three of the reconstructions were performed using custom implementations of the EWR algorithms proposed by Coene[3], Allen[2] and Kirkland[1] and one was performed using a commercially available software package (FTSR for Digital Micrograph from HREM Research Inc.). The results are qualitatively similar in each case.

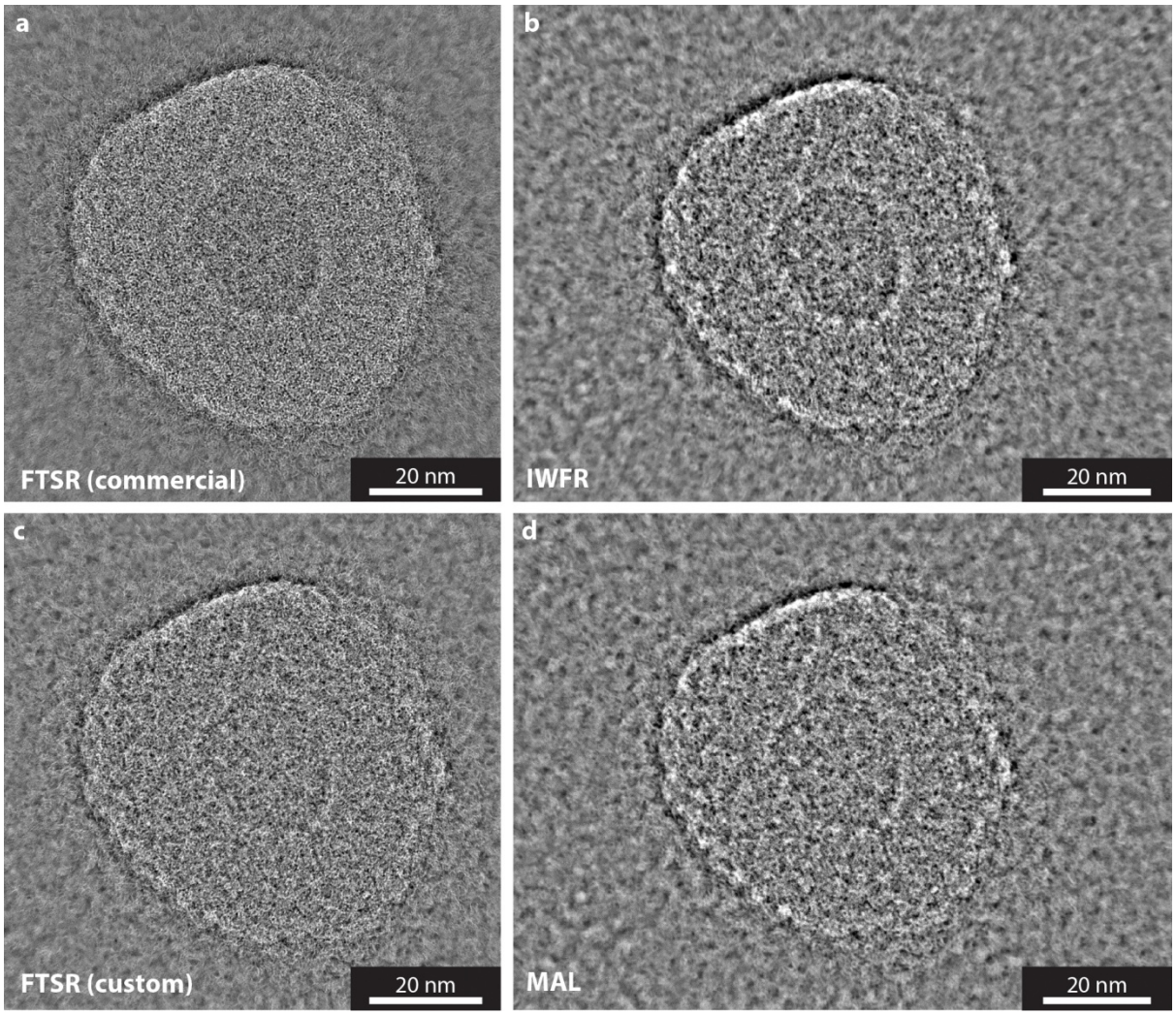

Figure S5. Phase image reconstructions of the polyacrylic acid-b-polystyrene polymersome using various exit-wave reconstruction algorithms on the same image series using the same determined parameters (where possible). (a) Commercial FTSR package from HREM Research Inc. (b) IWFR, (c) fast custom FTSR implementation and (d) MAL.

Supplementary Figure S6

From a restored exit wave it is possible to simulate images at any given focus level with the aberrations reintroduced. This method has been used to simulate the images in the experimental focal series from the restored exit wave. This internal verification procedure can be used to test the validity of the reconstruction by looking at its ability to reproduce the experimentally observed intensities for the entire series of images.

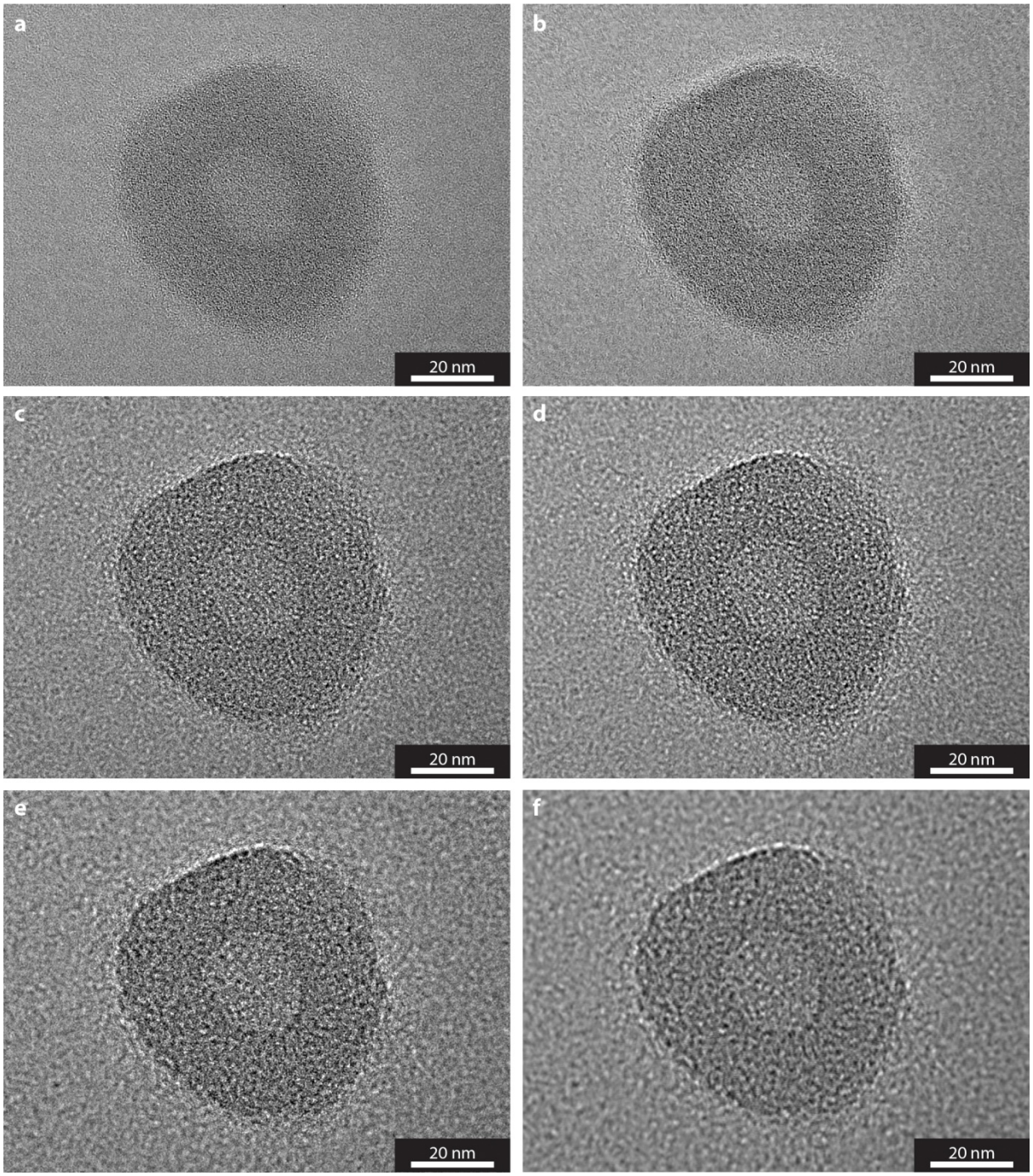

Figure S6. Comparison between experimental bright field TEM images of the polyacrylic acid-b-polystyrene polymersome (left) and simulated images generated from the restored exit wave (right). (a) + (b) Slight overfocus 62nm, (c) + (d) underfocus -202nm and (e) + (f) underfocus -436nm.

References

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4. R. R. Meyer, A. I. Kirkland and W. O. Saxton, *Ultramicroscopy*, 2002, **92**, 89-109.