









All parameters and calculations are the same as the corresponding images of Figs. 3(a)–3(c). Again, the aperture-matched case yields a higher resolution, at the cost of a lower contrast.

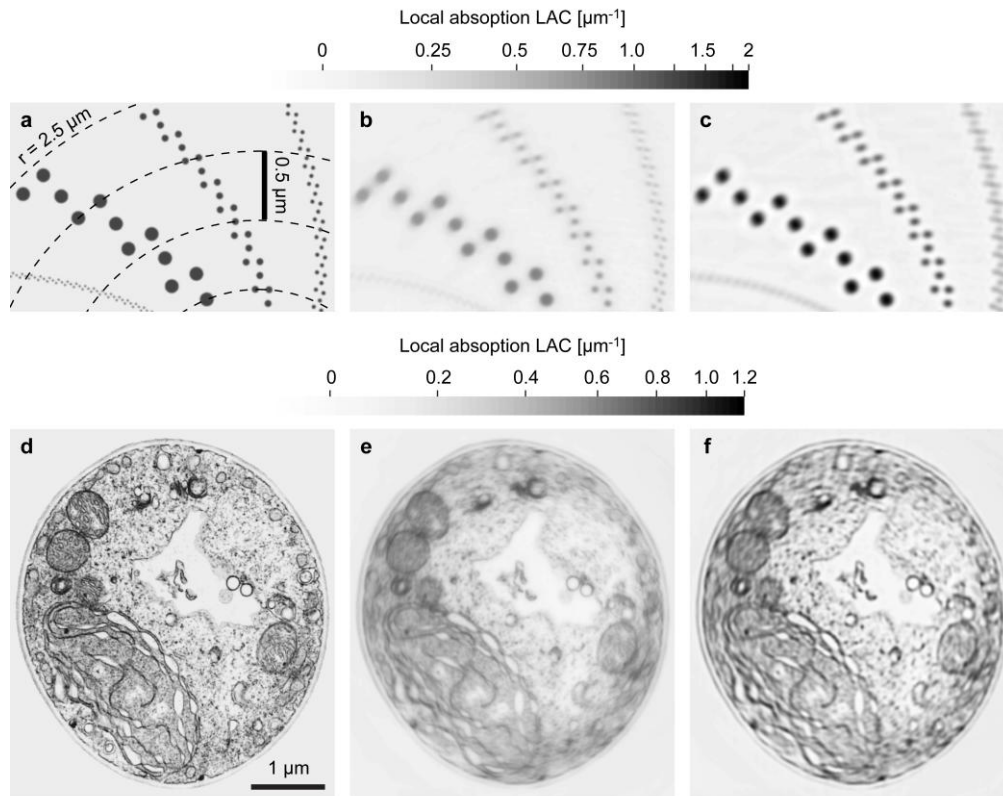


Fig. 3. Slices from reconstructed tomograms based on simulated images. (a) The resolution phantom contains Mylar features (15, 30, 50, and 100 nm in size and separated by two diameters) in water. The two cases of aperture-matched illumination (b) and partially coherent illumination (c) provide different contrast mechanisms and resolution. Note that the object is not shown in its whole. The grey levels correspond to the LAC and comparisons with the phantom indicate the error in the reconstructions. The realistic cell phantom (d) is simulated in (e-f) with the same coherence parameters as in (b-c), again showing the role of coherence in the illumination on tomographic reconstructions.

Thus the visual impression of Fig. 3(f) is preferred although the resolution is lower than in Fig. 3(e).

### 5. Stray light analysis

Ray-trace simulations of x-ray microscope systems complement the wave-propagation model by calculating stray light. The stray light is produced by other orders than the +1st order of the zone plate, with the -1st order being the main contributor. The ray-trace simulations were made in three dimensions by tracing a fan of 250 rays from each detector pixel, through the zone plate and back to the x-ray source. This procedure is equally accurate but computationally much faster than simulating from the source. The 1st and -1st diffraction order intensities were modeled, although the simulation can also include higher orders. The model includes aberrations of the condenser optic and simulations are made in the absence of an object. Just like the wave propagation model and the tomographic reconstructions, these calculations were performed using MATLAB.

Figure 4(a) shows the results of the ray-tracing model for the +1st order image intensity,  $I_1$ , of the source and the -1st order stray-light intensity,  $I_s$  on the detector for the microscope

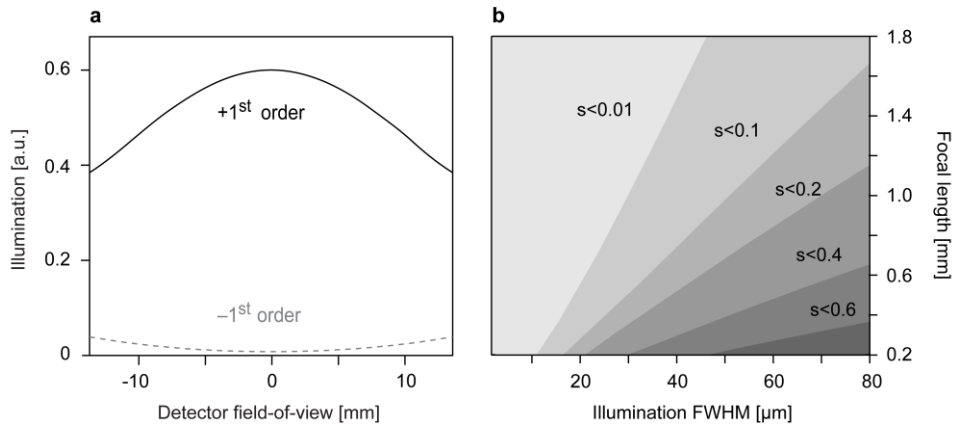


Fig. 4. Results of stray-light simulations under aperture-matched conditions. (a) Illumination light and stray light shown over the field-of-view for the 99  $\mu\text{m}$  diam, 30 nm outermost zone width zone plate. (b) Stray-light ratio as a function of the source size in the object plane. The stray light increases for shorter focal lengths and for larger source sizes.

arrangement given earlier and a source size of 40  $\mu\text{m}$  in the object plane. For this arrangement, the stray light is low in the center but increases off-axis. The stray light ratio, defined as  $I_s/I_1$ , in the centre of the field of view is shown in Fig. 4(b) as a function of the object-plane source size and zone plate focal length. Clearly, the stray-light ratio increases with the source size and decreases with focal length. The stray light impairs contrast in the 2D microscope images, which results in an underestimation of the LAC in the 3D tomograms. The effect is stronger when studying highly absorbing specimens, since the resulting error in the LAC will be large. Furthermore, as x-ray microscopes push the resolution to below 10 nm, stray light will become more dominant as the focal lengths will be very small. This leads to longer exposure times and a higher deposited dose, potentially limiting the resolution by increasing noise. Note that systems using low-brightness large-size sources need to reduce stray light by apertures or source demagnification, potentially prohibiting tomographic image acquisition or increasing exposure times.

## 6. Conclusions

In summary, we have presented a numerical model for tomographic image formation of thick samples in transmission x-ray microscopes. The model was applied to study the influence of partial coherence on DOF and on the accuracy of tomographic reconstructions. We have also presented results from ray-trace simulations, which show that transmission x-ray microscopes may suffer from detrimental stray light. Both the degree of partial coherence and the stray light affects the accuracy of the reconstructed LAC, making it more challenging to categorize structures within cells. From the model we estimate that a resolution approaching 30 nm (half-period) over full 5- $\mu\text{m}$  diameter objects should be achievable with a 30 nm outermost-zone-width zone plate. Higher resolution is obtained for smaller objects or smaller-diameter regions within larger objects. Such high-resolution quantitative 3D imaging of intact cells in their hydrated near-native state is of significant importance for studies of the function and structure of biological material on the nanoscale.

## Acknowledgments

This work was supported by the Swedish Research Council, the Swedish Foundation for Strategic Research, and the Wallenberg Foundation.