

SUPPLEMENTARY INFORMATION

EVALUATION OF THE SEGMENTATION METHOD ON A SIMULATED OBJECT

To get a better understanding of the behavior of the proposed segmentation method and in particular on the influence of missing wedge artifacts on the results, simulations have been performed. For this purpose we compared the gradient watershed segmentation algorithm to segmentation using absolute threshold values.

For the simulations a two-dimensional phantom object with a size of 512*512 pixels has been created. The phantom is shown in Supplementary Fig. 1a. It consists of a grey circle (pixel value = 152) on a black background (pixel value = 0). Inside the grey circle we created 13 “precipitates” represented as white circles and ellipses (pixel value = 255) of different sizes. Ellipses are oriented either horizontally or vertically. The horizontally oriented ellipses will be affected strongest by missing wedge artifacts.

Two datasets of one-dimensional projections of the object were calculated. The first dataset for a tilt range from -78° to +78° with a step of 1° corresponding to the tilt range used in the experiments, the second dataset for a full tilt range of -90° to +89° again with a step of 1°. The second dataset will therefore not suffer from missing wedge artifacts. To the projections white Gaussian noise was added. The two sets of projections with and without missing wedge are shown in Supplementary Fig. 1b and 1c respectively.

Each of the two datasets is reconstructed using a SIRT algorithm with 20 iterations. The reconstruction of the dataset with missing wedge is shown in Supplementary Fig. 2a, without missing wedge in Supplementary Fig. 2b. Before segmentation of the reconstructions anisotropic diffusion filtering is applied on the reconstructions, the filtered reconstructions are shown in Supplementary Fig. 2c&d. On the filtered reconstructions watershed segmentation and segmentation using an absolute threshold value are performed. Watershed segmentation was done as described in the main text. For the segmentation using absolute threshold values

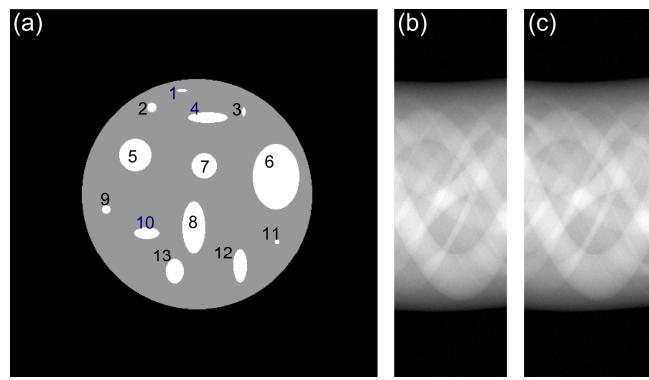
the threshold was set manually. It was chosen low enough to allow segmentation of the smallest precipitates, which have lost contrast in the reconstruction due to resolution limits imposed by the number of projections.

Supplementary Figs. 2e&f show the results of the segmentation for both datasets using an absolute threshold, Supplementary Figs. 2g&h show images with erroneous pixels for both segmentations. Supplementary Figs. 2i-k show results of the watershed segmentation for both datasets and the corresponding images with erroneous pixels. For segmentation using an absolute threshold value, we observe a systematic overestimation of the size of the precipitates, which increases with particle size, for both datasets, with and without missing wedge. This is due to the chosen threshold value, which was selected to include also small precipitates. Using a lower threshold would reduce this effect for large precipitates, but would lead to the loss of the smallest precipitates in the segmentation. The errors using watershed segmentation are significantly lower, in particular for large particles. For the dataset without missing wedge erroneous pixels are constrained to very thin layers on the surface of the precipitates. Such errors are to be expected due to discrete steps at interfaces of the phantom object. Concerning missing wedge artifacts, the ellipses oriented horizontally are affected by such artifacts for both segmentation methods, though the impact is limited due to the relatively large tilt range of +/- 78°. Missing wedge artifacts can be seen for example precipitate #4: Pixels are missing in the segmentation on two sides of the precipitates, while the precipitate is elongated on the two other sides. Though these artifacts are visible for both segmentation methods, their impact is significantly smaller using watershed segmentation.

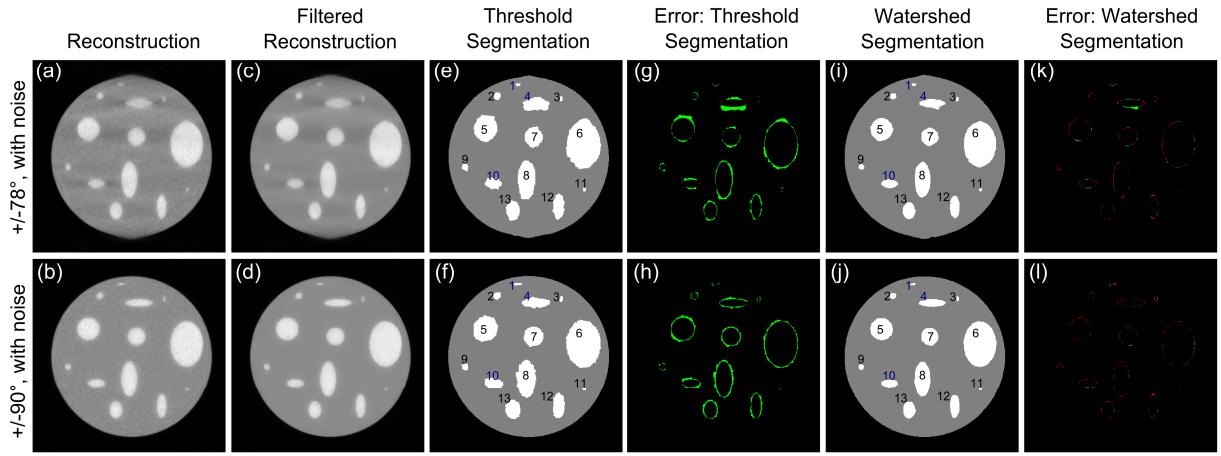
In Supplementary Table 1 and Supplementary Fig. 3 measurements and measurement errors for all precipitates for each segmentation are given. The results show a smaller error for the watershed algorithm compared to segmentation using a threshold value. While in these measurements the particles affected by missing wedge artifacts do not show systematically a

larger error for the reduced dataset, we note that this is also due to a partial compensation of errors from the different sides of the particles. This can however not be generally expected for all particle shapes.

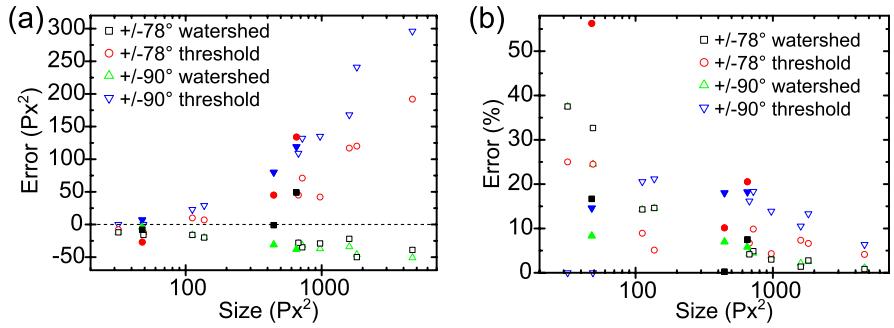
These simulations indicate that while the missing wedge has an impact on the measurement results its effect is limited by the relatively small missing tilt range. For the simulated object the watershed segmentation method performs significantly better than thresholding of the reconstruction. The segmentation method also limits the effects of missing wedge artifacts on the results. For larger particles the error using the proposed method is well below 10% for datasets with and without missing wedge. For smaller particles the ratio of surface pixels compared to the total area of the precipitates increases and leads to a larger error in the simulations for both datasets with and without missing wedge.



Supplementary Figure 1. (a) Simulated phantom with 13 precipitates. Precipitates are indexed from 1 to 13. Numbers of precipitates, which are prone to missing wedge artifacts (1, 4, 10) are colored blue. (b) Noisy projections of the phantom for a tilt range from -78° to $+78^\circ$. (c) Noisy projections of the phantom for a tilt range of -90° to $+89^\circ$.



Supplementary Figure 2. Reconstruction and segmentation of the phantom from noisy datasets with and without missing wedge: Original reconstruction (a) with missing wedge and (b) without missing wedge. Filtered reconstruction using anisotropic diffusion filtering (c) with missing wedge and (d) without missing wedge. Results from the segmentation using an absolute threshold (e) with missing wedge and (f) without missing wedge. (g) Erroneous pixels of the segmentation shown in (e). (h) Erroneous pixels of the segmentation shown in (f). Results using watershed segmentation (i) with missing wedge and (j) without missing wedge. (k) Erroneous pixels of the segmentation shown in (i). (l) Erroneous pixels of the segmentation shown in (l). In the error images pixels missing in the segmentation are shown in red, while additional pixels are shown in green.



Supplementary Figure 3. (a) Absolute error for the different segmentation methods with and without missing wedge for all particles as a function of particle size. (b) Relative error for the different segmentation methods with and without missing wedge for all particles. The error of precipitates prone to missing wedge artifacts is displayed with filled symbols.

Supplementary Table 1. Measured size of precipitates and relative error for the noisy phantom with and without missing wedge, using gradient watershed segmentation and thresholding. The precipitates are sorted by size. Values for precipitates prone to missing wedge artifacts are shown in bold font.

Index	Size (px²)	+/-78° watershed	+/-78° threshold	+/-90° watershed	+/-90° threshold
11	32	20 (-37.5%)	24 (-25.0%)	20 (-37.5%)	32 (0.0%)
1	48	40 (-16.7%)	21 (-56.3%)	44 (-8.3%)	55 (+14.6%)
3	49	33 (-32.7%)	37 (-24.5%)	37 (-24.6%)	49 (0.0%)
9	112	96 (-14.3%)	122 (+8.9%)	96 (-14.3%)	135 (+20.5%)
2	137	117 (-14.6%)	144 (+5.1%)	117 (-14.6%)	166 (+21.2%)
10	444	443 (-0.2%)	489 (+10.1%)	413 (-7.0%)	524 (+18.0%)
4	653	702 (+7.5%)	787 (+20.5%)	615 (-5.8%)	772 (+18.2%)
13	674	646 (-4.2%)	719 (+6.7%)	645 (-4.3%)	783 (+16.2%)
12	720	685 (-4.9%)	791 (+9.9%)	688 (-4.4%)	852 (+18.3%)
7	973	944 (-3.0%)	1015 (+4.3%)	936 (-3.8%)	1108 (+13.9%)
5	1597	1575 (-1.4%)	1714 (+7.3%)	1563 (-2.1%)	1765 (+10.5%)
8	1812	1762 (-2.8%)	1932 (+6.6%)	1766 (-2.5%)	2053 (+13.3%)
6	4647	4608 (-0.8%)	4839 (+4.1%)	4596 (-1.1%)	4943 (+6.4%)