MorphCol Supplement 5

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3.2.1.1 Analysis of variation in shape and size due to repeated automatic positioning of a single microfossil into the same position using AMOR Stage V

The same experiment as described in MorphCol supplement #3 was done but using the AMOR Stage V, which is PC based, and the Sony DXC-390P video camera. The goal was to find the precision of automated positioning the same specimen 30 times into the same keel position.

Experimental setup:
Specimen: 502_0100CCK0201 was used for this experiment (=same specimen as in MorphCol supplement #3). The specimen was remounted in keel view into the center of field 12 of a new, empty slide (36 fields) for this test. Because during automated tilting the specimen ran partially out of the imaging window at a magnification of 2.5x (=magnification used in MorphCol supplement #3), the orientation part of the experiment was performed at 2.0x magnification. Final imaging was then performed at 2.50x.

Magnification at orientation: 2.0x (specimen ran partially out of window at 2.5x)
Magnification at final imaging: 2.50x

Camera: Sony DXC-390P
Cmount 1x
Leica MZ 6 binocular microscope using zoom body (zoom from 0.63x to 4x).
Diaphragma opening at microscope set at 3
Achromat 1x objective
Cross-polarized light (swan-neck with polarizer caps on and pol-filter on objective)
Illumination using Volpi 4000-1, light at 4 (fully open)

Imaging and orientation with AMOR Stage V
Operation was done in single mode.

Sequence of operations:
1.) Set MAG to 1.25 in programme and on Microscope
2.) Enter field No (for example 12, where specimen was placed in) and hit return
3.) Autocenter
4.) Change magnification to 1.6x in Program and on microscope
5.) Autocenter
6.) Change magnification to 2.0x in program and on microscope (=optimum mag for orientation)
7.) Autocenter
8.) Autofocus
9.) Auto tilt (at MAG=2.0x)
10.) Change MAG to 2.50x
11.) Autocenter
12.) Autofocus

13.) Autorotate
14.) Capture (Expand to 640x480 pixels, shift to 60 pixels from left)
15.) Goto 1

Post-Processing:
Image convertion from Tiff files to raw files was done using Nih-Image 1.61 macro *Automate* (P for processing, R for saving to raw file format). [Note, that in the previous repeatability experiment with the Macintosh and with the Kappa camera there was no cross polarized illumination, and Tiff to Raw image convertion was done manually using Adobe Photoshop].

Outline extraction and processing of outline coordinates:
Outline extraction on the black and white images was done using program Trace_AMOR1_batch.out, which was previously calibrated for the AMOR-Sony camera system. This calibration uses the following equations for pixel to µm conversion (see MorphCol Supplement #4):

$$XPrec = 0.12986 * MAG + 0.000223 \qquad r^2=1.000$$
$$YPrec = 0.15742 * MAG - 0.00023756 \qquad r^2=1.000$$

Thereafter the outlines were interpolated to 250 points (cartesian coordinates) using program Sprep53.out, and the conversion to polar coordinaes was done with program XY_to_PhiD1.out, as was done in the repeatability test on the Macintosh plus Kappa camera system.

Results: Image quality:
Images from the two imaging systems provided different quality: The AMOR-Sony system ended in a image showing an elongated (distorted) shell (Figure 1) at lower contrast. On the Macintosh-Kappa camera system, the distortion is much less, and the sharpness (variation) is higher (Figure 2).

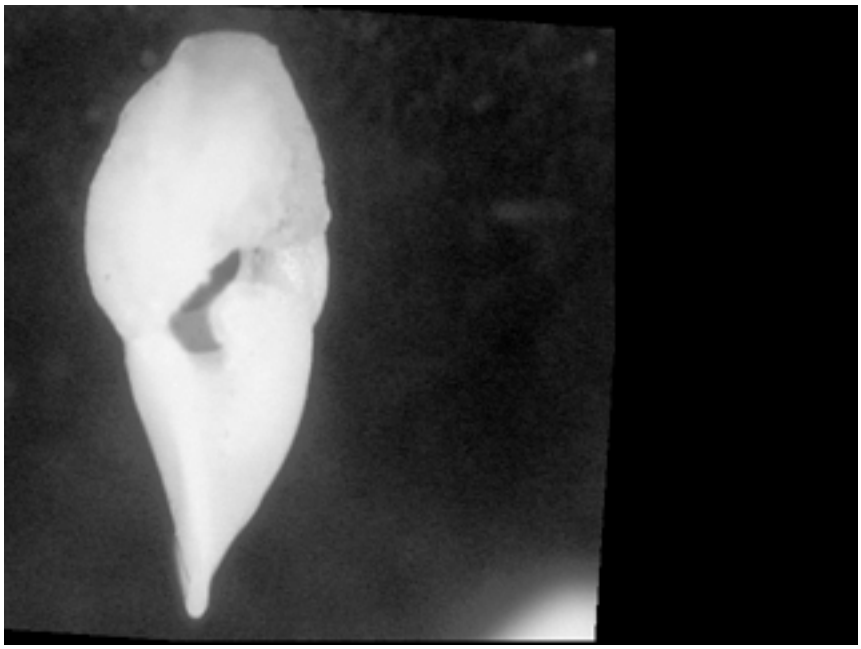


Figure 1.
Tiff image of specimen 502_0100CCK0201 after the Sony DXC-390P camera and AMOR Stage V.

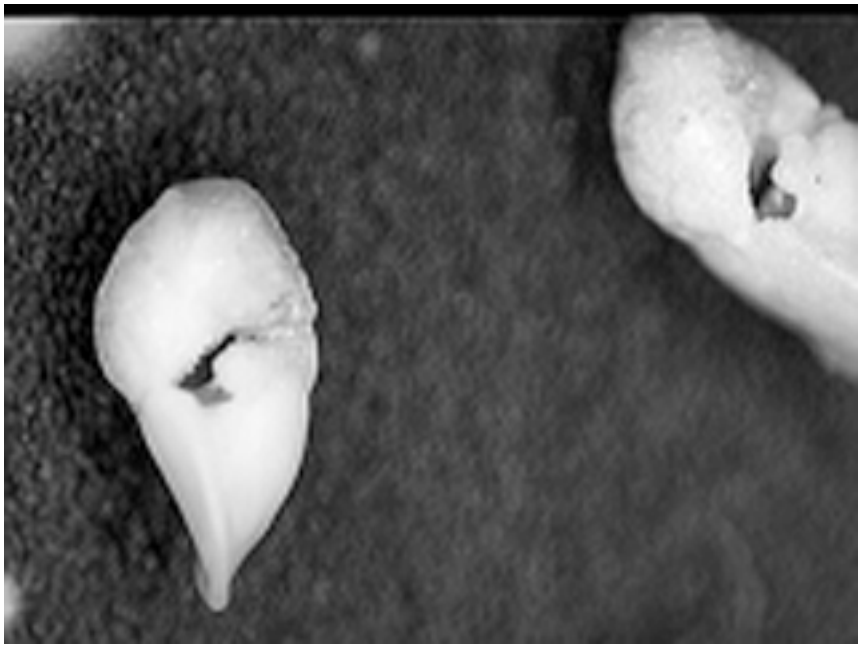


Figure 2.
Tiff image of specimen 502_0100CCK0201 after using the Kappa CF 11/2 camera and the Macintosh based imaging system.

The distortion of the image when using AMOR is, however, completely eliminated after outline extraction with program Trace_AMOR1_batch.out (see Figure 3). This figure

confirms, that the pixel to µm conversion of the distorted images from the Sony camera in program Trace_AMOR1_batch.out works correctly. Note, that application of the Autorotate function in AMOR resulted in a slight rotation to the left when compared with the manually oriented specimen in MorphCol supplement #3. This angular offset is due to the rotation method, which is based on the calculation of the momentum of inertia in every specimen to find the vertical placement of the shell on the computer monitor (in manual orientation this was just done by visual judgement).

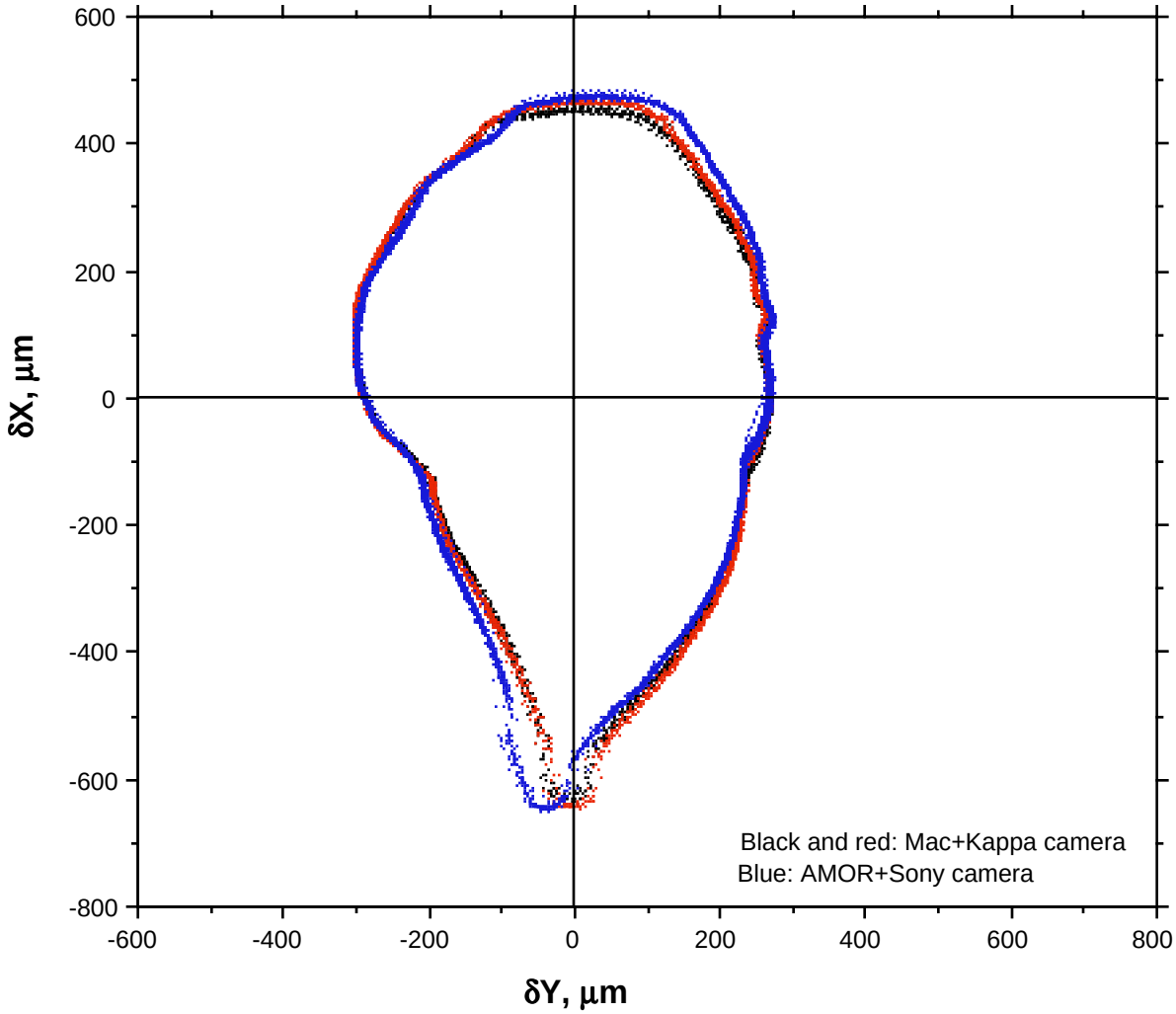
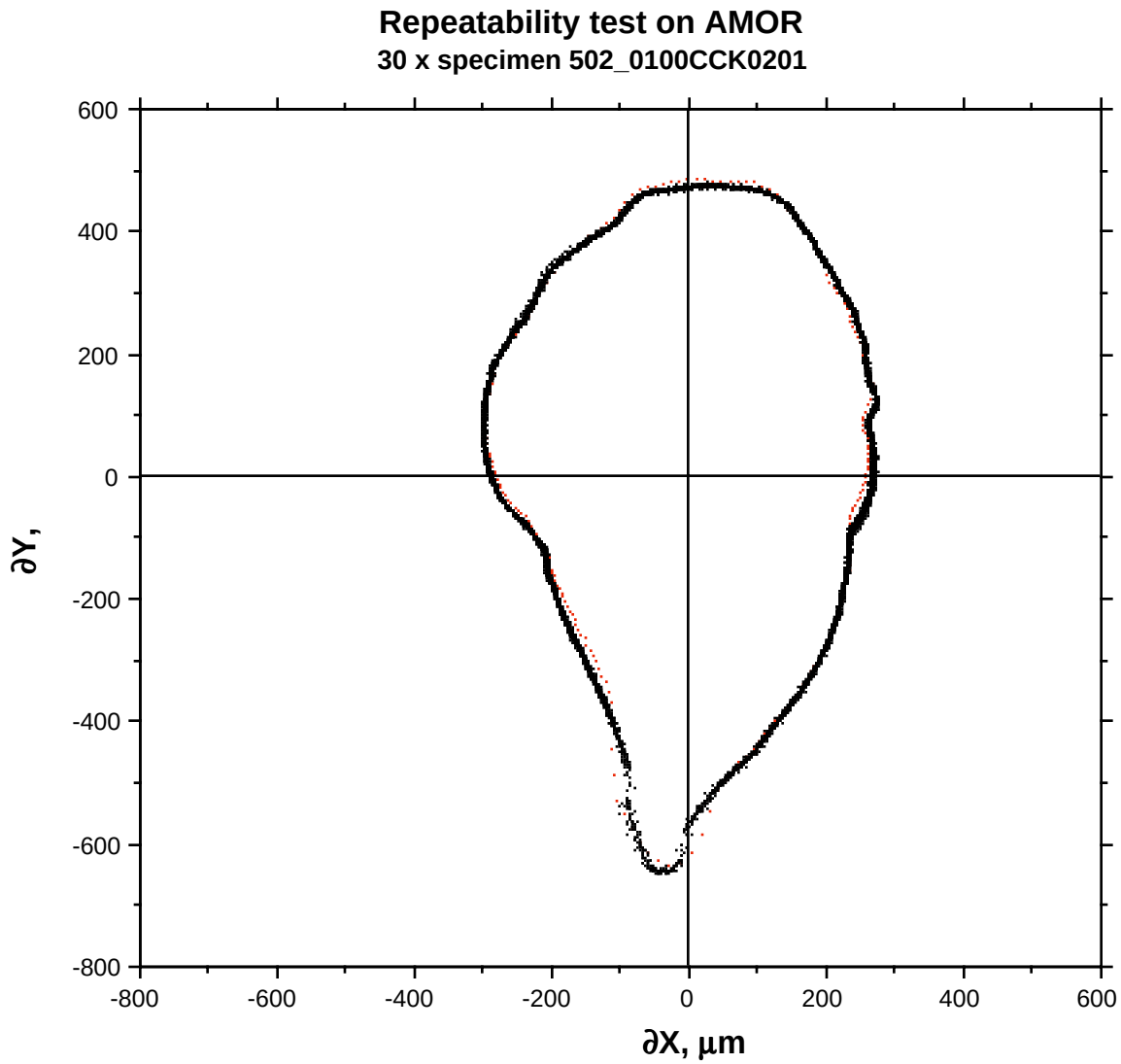


Figure 3.
Comparison of outlines (interpolated to 250 points using Sprep53.out) when using the Macintosh-based imaging system with the Kappa CF11/2 camera (15 black and 15 red outlines) against the outlines after using AMOR (PC) based system with the Sony DXC-390P camera (30 blue outlines). The blue outlines are rotated to a small amount to the left because of the Autorotate operation after automated tilt.

Precision of automated orientation using AMOR Stage V:
Figure 4 illustrates, that the degree of overlap after orientating the same specimen 30 times into the same orientation using AMOR Stage V is nearly perfect. There was only 1 outline (measurement No. 3) out of the 30 outlines, that is a bit off.



The somewhat off specimen (red outline) is measurement no. 0003

Figure 4.
Outlines of specimen No. 502_0100CCK0201 after 30 x orientation using AMOR Stage V.

Analysis of polar coordinates of the outline

As was done in the case of the manual positioning test the polar form of the outlines is analyzed in order to quantify the variation in shape change (for methods see MorphCol Supplement No. 3). A plot of ray length (Rho) and the associated angular argument (Theta) for every value of Theta and for each of the 30 outlines is illustrated in Figures 5 and 6.

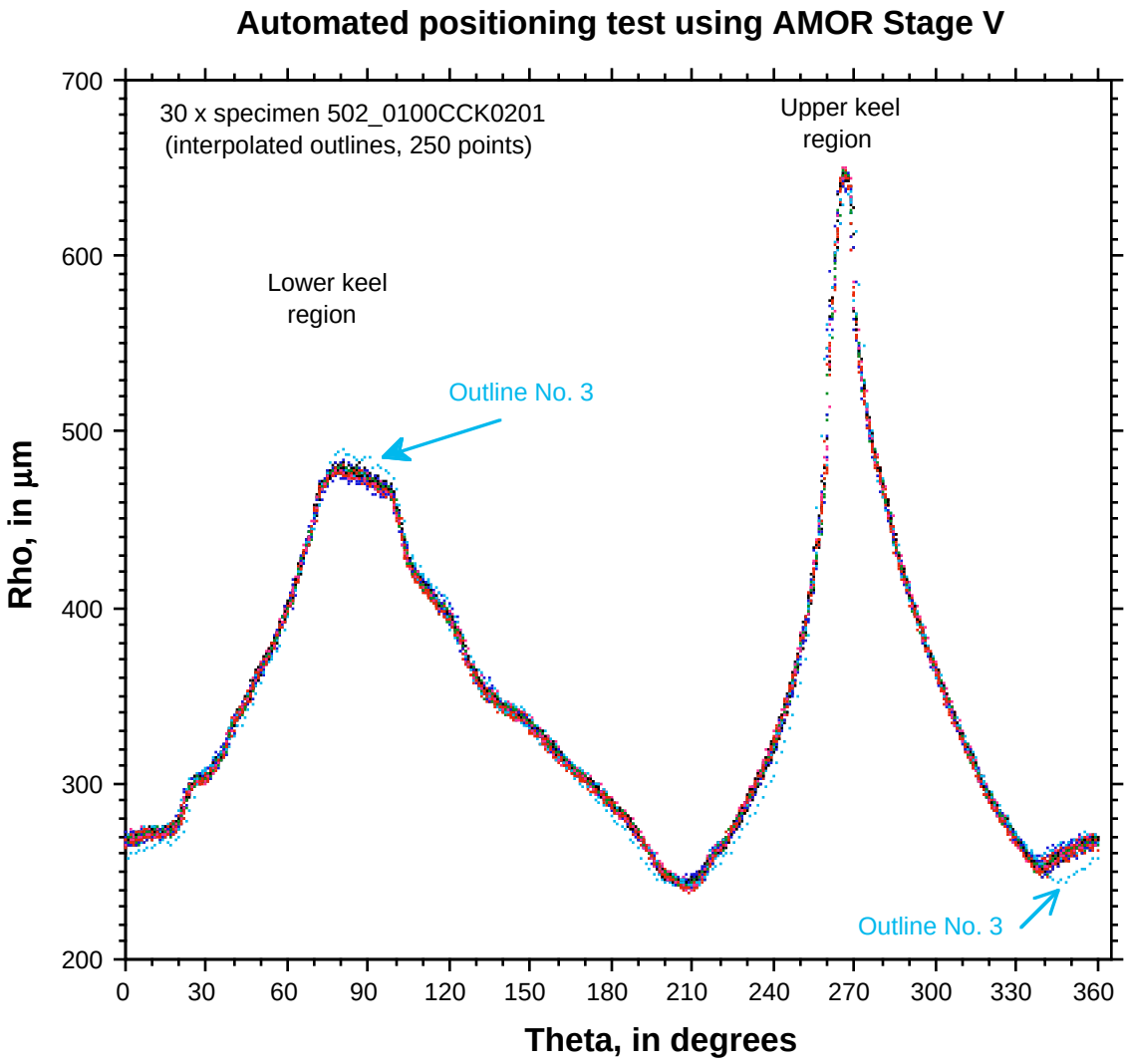


Figure 5.
Plot of the ray lengths (Rho) versus Theta for the 30 outlines produced with AMOR Stage V. Already from visual inspection and comparison with Figure 3 in MorphCol Supplemet No. 3 it is obvious, that the precision has improved, particularly in the keel region.

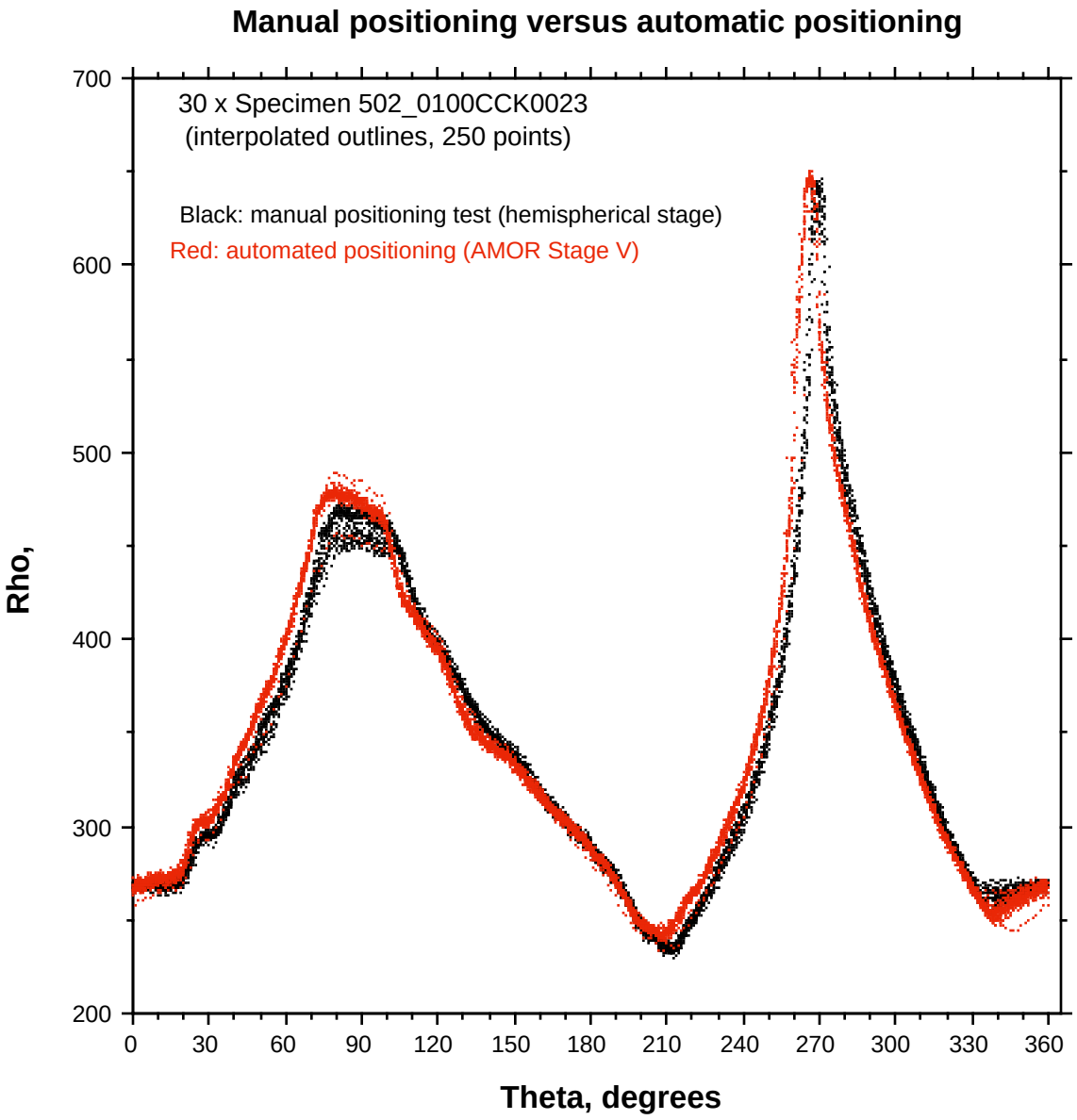


Figure 6.
Comparison of positioning tests on hemisphaerical stage (manual, black outlines) with those made with AMOR Stage 5 (red outlines). The small shift in Theta of the two data sets is due to the Autorotate function applied to the shells when using AMOR Stage V (see Figure 3 above).

Polar ray analysis (see Figure 7)
With the automated orientation the average 95% confidence interval about the means is now ±0.0291 µm (in manual orientation it was ±1.6503). The average range of variation in Rho is 6.127µm (=1.76% of the mean ray length. The mean ray length over the 30 specimens in this experiment is 349.114 µm. In comparison, in the manual orientation the average range of variation in Rho was as large as 18.263 µm. The maximum absolute error is 61.98 µm at n=199 (in manual orientation this value was 117 µm).

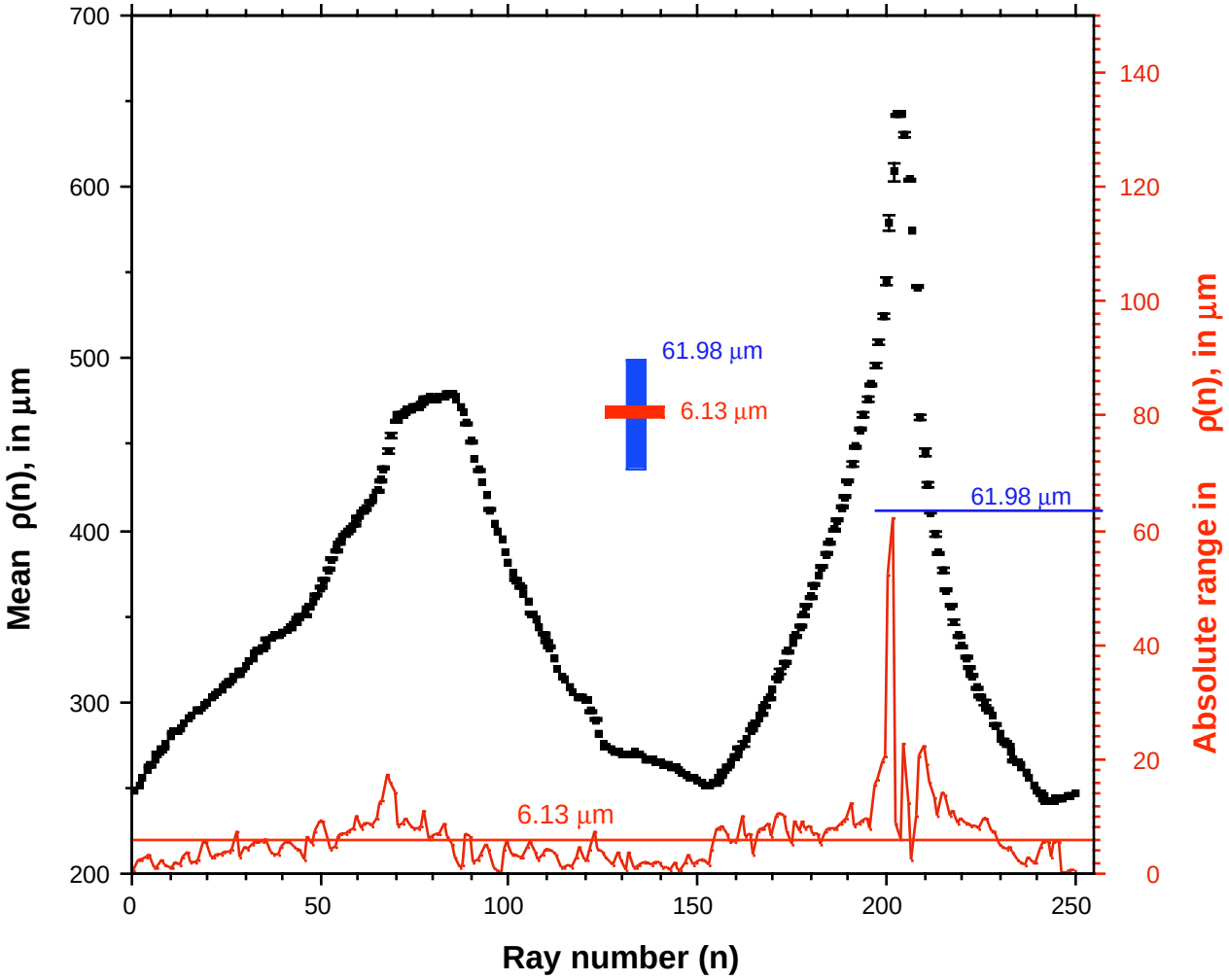


Figure 7.
Results of the polar analysis of outlines obtained with AMOR Stage V. Black curve = mean ray length at each position n versus ray number (n=1 to 250), averaged over 30 outlines. Vertical bars indicate ±95% confidence intervals about the mean at each ray position. Red curve: Absolute range in Rho(n) in µm at every n'th ray position. The absolute range was determined by $[Max(\rho_{1,n}, \rho_{30,n}) - Min(\rho_{1,n}, \rho_{30,n})]_{n=1,...,250}$. The overall mean variation in ray length for all 30 outlines and averaged over all 250 rays is 6.13 µm (=red horizontal line).

Conclusions

1.) The precision of automated orientation of specimens with AMOR Stage V has improved from 18.26 µm to 6.13 µm. In relative terms, i.e. with respect to the average ray length, this is an improvement from to 1.6% in manual orientation to 1.76% in automated orientation.

2.) The variation in the keel region is much more stable when using automated orientation than in manual orientation.

3.) Due to the momentum of inertia method applied in the AMOR Stage V a slight deviation in the rotational position by about -3.5° occurs in comparison to manually

oriented speciemens. This deviation must be compensated for when combined measurements with manual and automatic orientation are combined for analysis.