

MorphCol supplement # 29

# AMOR - System AMOR2 Intercalibration Experiment

4-22 June 2021, by Michael Knappertsbusch

## 1. Introduction

With the completion of System AMOR2 in March 2021 there exist now at the Natural History Museum Basel two systems for automated orientation and imaging of foraminiferal shells, e.g. the old **AMOR**, and the new **System AMOR2**. While AMOR was developed using LabView version 8.5 and employing a 3CCD camera (model DXC-390P) from Sony the new System AMOR2 has different hardware components including a truly digital camera (KY-F75U from JVC), and was developed under LabView version 14. With these modifications an intercalibration experiment is now timely and necessary in order to check precision and inter-comparability of morphometric measurements on foraminifera, that were collected using either the old or the new of the two machines.

## 2. Setup of the Experiment

Tests were run on both devices, AMOR (running under program AMOR v. 3.28) and System AMO2 (running under program AMOR v. 4.2) and using the same test specimen. This test specimen is a *G. tumida* mounted in keel view and comes from sample DSDP 68-502-1H-CC, 0-4cm, 500-1000 $\mu$ m, 1/128 split. In the original slide the specimen was the first in field no. 2 (encoded as specimen 0201, hence its full specimen name is 502\_0100CCK0201). Specimen 502\_0100CCK0201 was designated as the standard for all such tests, being on the ancient Macintosh imaging system, its successor AMOR, or the latest System AMOR2. For this purpose the specimen was permanently fixed in a separate plummer cell, slide 502\_0100CCK0201, into field no. 30. It is deposited at the NMB in the collection to the MorphCol cocumentation (colln. Knappertsbusch, 2015).

Two tests runs (1 and 2) on each device (AMOR, System AMOR2) were run for this analysis, all in the single specimen mode. Test 1 investigates the influence of changing magnification on the test specimen, Test 2 is a repeatability test for AutoTilt if the magnification remains constant at zoom=2.49x. The results of tests 1 and 2 serve for documentation of *intra*-machine variation and for *inter*-machine variation. For *intra*-machine variation the so-called  $\xi$  value was estimated, which describes the mean difference between maximum and minimum ray-lengths of the outline points in polar coordinate notation (see section 5.1 further below). For *inter*-machine variation, the so-called  $\Delta$  value was defined, which is the mean difference of mean ray-lengths (R) from tests 1 and 2 performed with AMOR and System AMOR2 (see section 5.2 further below).

### 2.1. Settings and test runs on old AMOR (Sony camera, objective 1.0x, empty Cmount):

Slide 502\_0100CCK0201

Single object mode, symmetric, sensibility = normal, *no* AutoRotate applied, use Slide 60x as slide calibration.

Diaphragm fully open, maximum cross-polarization.

Test 1:

- 1.) Goto field 30, focus, AutoCenter, zoom to 1.98x (all zoom readings from AMOR program).
- 2.) AutoTilt.
- 3.) zoom to 0.63x (all zoom readings from AMOR program).
- 4.) AutoCenter, focus manually if necessary.
- 5.) Capture (Tiff image).
- 6.) Goto 3, next zoom position, repeat 4-6 until object does no longer fit in the imaging window (at zoom > 2.49x).

This loop results in 7 images at

zoom = 0.63x  
zoom = 0.79x  
zoom = 1.00x  
zoom = 1.25x  
zoom = 1.57x  
zoom = 1.98x  
zoom = 2.49x

Finalize test 1: Return to field 1 (zoom goes automatically back to 1.00x), move to field 30 (zoom=1.00).  
Now ready for test 2.

Test 2:

- 1.) Goto field 30, focus, AutoCenter, zoom to 2.49x (all zoom readings from AMOR program), Autocenter, focus manually.
- 2.) AutoTilt.
- 3.) zoom to 2.49x (all zoom readings from AMOR program).
- 4.) AutoCenter, focus manually if necessary.
- 5.) Capture (Tiff image).
- 6.) Goto 1, repeat 14 times.

This loop results in 15 images, each at  $zoom = 2.49x$  (as read in the AMOR program). Images are saved as 0201@2.49x, 0202@2.49x, . . . , 0215@2.49x

The time for completing the AutoTilt cycle was measured for each runs (in seconds): 125, 135, 123, 143, 145, 131, 143, 125, 120, 130, 121, 120, 121, 130. From these 14 observations the cycle length for AutoTilt varied between 120 and 145 seconds, the average was 129 seconds per cycle.

Test 2 finished.

## **2.2. Settings and test runs on System AMOR2 (JVC camera, objective 1.0x, empty Cmount):**

Slide 502\_0100CCK0201

Single object mode, symmetric, sensibility = normal, *no* AutoRotate applied, use Slide 60x as slide calibration.

Diaphragm halfway open (at position 3), maximum cross-polarization.

Test 1:

- 1.) Goto field 30, focus, AutoCenter, zoom to 1.55x (all zoom readings from AMOR program; the zoom=1.55x in program AMOR version 4.2 program corresponds to the physical position of the microscope at the 1.60x mark). A larger magnification (1.98x as in old AMOR) does not work because otherwise the specimen becomes larger than the video window).
- 2.) AutoTilt (sensibility=normal); *no* autorotate applied.
- 3.) zoom to 0.63x (all zoom readings from AMOR program).
- 4.) AutoCenter, focus manually if necessary.
- 5.) Capture (Tiff image).
- 6.) Goto 3, next zoom position, repeat 4-6 until object does no longer fit in the imaging window (at zoom > 1.93x).

This loop results in only 6 images at

zoom = 0.63x  
zoom = 0.79x  
zoom = 0.99x  
zoom = 1.23x  
zoom = 1.55x  
zoom = 1.93x

Finalize test 1: Return to field 1 (zoom goes automatically back to 0.99x), move to field 30 (zoom=0.99x).  
Now ready for test 2.

Test 2:

- 1.) Goto field 1 (zoom goes to 0.99x), goto field 30, zoom to 1.55x (all zoom readings from AMOR program), AutoCenter, focus manually, *no* AutoRotate applied.
- 2.) AutoTilt (sensibility=normal).
- 3.) zoom to 1.55x (all zoom readings from AMOR program).
- 4.) AutoCenter, focus manually if necessary.
- 5.) Capture (Tiff image).
- 6.) Goto 1, repeat 14 times.

This loop results in 15 images, each at  $zoom = 1.55x$ . Images are saved as 0201@1.55x, 0202@1.55x, . . . , 0215@1.55x.

Also here, the time needed for completing an AutoTilt cycle was measured (sensibility=normal, in seconds): 170, 135, 170, 130, 143, 170, 175, 170, 165, 165, 175, 162, 170, 170, 166. From these 15 observations the cycle length for AutoTilt varied between 130 and 175 seconds, the average was 162.4 seconds per cycle.

Test 2 finished.

### 3. Pre-processing of images:

Processing and preparation of images for outline extraction was done on a PC (Windows) with ImageJ 1.48 from Wayne Rsband: The Tiff images created during each test were saved in directory "Tiff files". For each test Tiff images were loaded to stack, select all, smooth, sharpen, smooth, sharpen, smooth, multiply by 1.6. Segmentation from background with wand tool (8 connected, tolerance=50.0; method=default, background=white): Manually slice through stack and adjust for every image the limits between particle and background, then click apply. Check visually if separation between background and particle was good. Then make stack binary. Slice manually through stack and close single pixel embayments of particle with the close command. Manually remove black areas between particle and left border of image if necessary. Save as image sequence in Tiff format into directory "B\_W\_Tiff", and in raw format into directory "Raw files". For outline extraction and analysis the raw files were transferred to iMac. Images were renamed accordingly so that they can be processed on the iMac with the outline extraction programs Trace\_AMOR2\_batch.out and Trace\_AMOR3\_batch.out and subsequent programs.

#### 4. Generation of particle outlines:

Extraction of cartesian coordinates from the images and all subsequent analysis was done on a iMac (MacOs System 9.2). For the old AMOR device (with the Sony camera) the correct outline extraction program is Trace\_AMOR2\_batch.out, in combination with a correction of magnifications. This correction is done with program MagCorr2.out. For System AMOR2 (with the JVC camera) the correct outline extraction program is Trace\_AMOR3\_batch.out and no correction for magnification is necessary (see MorphCol supplements #26 and #27). Cartesian outline coordinates generated with the two trace programs are stored in files ending with \_T (for "Traced"). The sequence of analytical steps for images created with AMOR and System AMOR2 are given in Table 1 below:

|                             | AMOR                     | System AMOR2             |
|-----------------------------|--------------------------|--------------------------|
| Correction of magnification | MagCorr2.out             | none                     |
| Outline extraction          | Trace AMOR2_batch.out    | Trace AMOR3_batch.out    |
| Interpolation               | Sprep60.out (250 points) | Sprep60.out (250 points) |

**Table 1.** Programs used for outline analysis for images generated with old AMOR and System AMOR2.

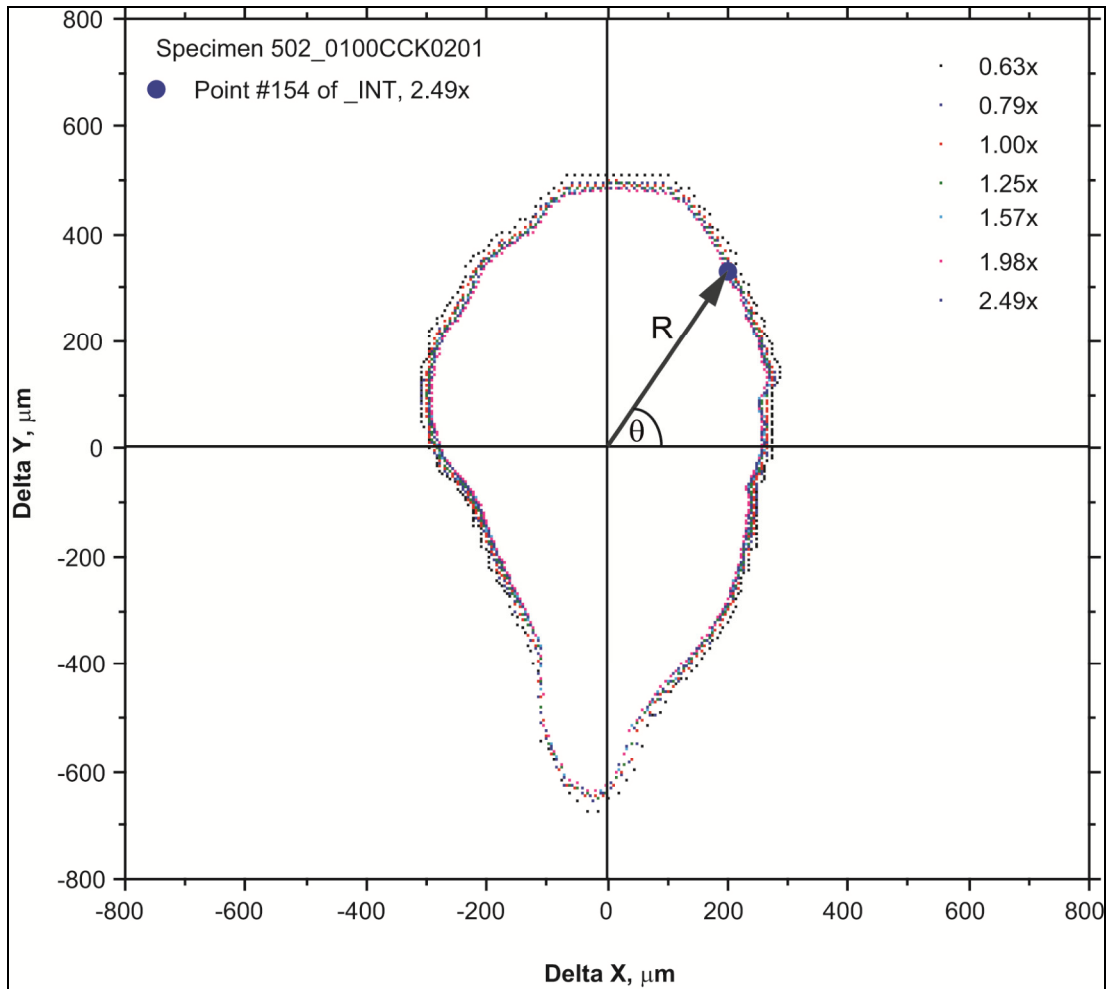
For equiangular interpolation of outline coordinates the recently expanded program version Sprep60.out was applied. Sprep60.out generates interpolated coordinates from centered outlines in form of cartesian and polar coordinates (in its previous version Sprep53.out interpolated outlines are only generated in form of cartesian coordinates). All outlines were interpolated to 250 points at equiangular distances of 1.44 degrees.

#### 5. Error analysis from outlines generated with AMOR and System AMOR2:

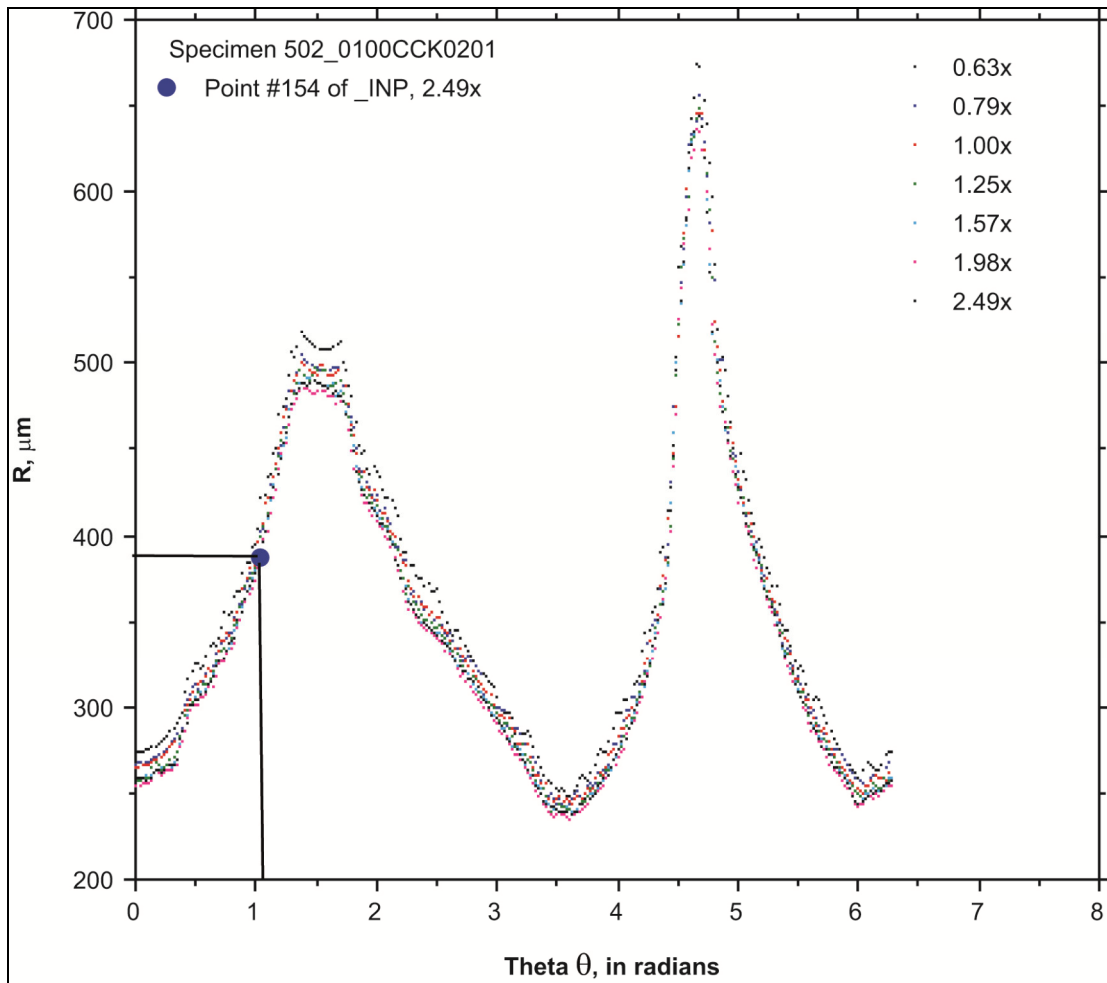
From the \_T files program Sprep60.out creates three different output files with interpolated outline coordinates:

- \_INT files contain the *cartesian* coordinates of the centered, interpolated outlines.
- \_INP files contain the *polar* coordinates of the centered, interpolated outlines.
- \_POL files contain the polar coordinates of the centered, original outlines.

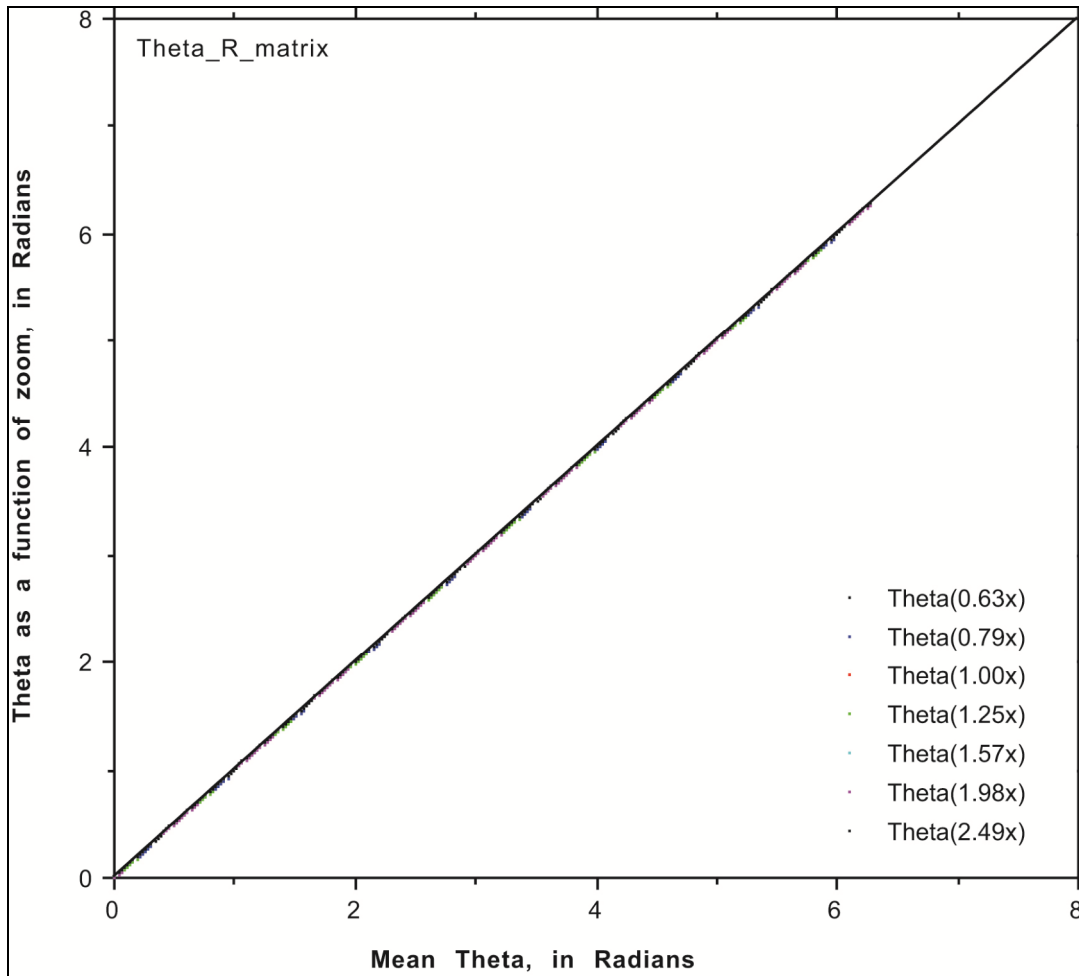
Centered means that the origin of the outline was set to the centroid. For the present experiment only \_INT and \_INP files were used. Variation of outlines between the tests can be visualized by plotting the cartesian coordinates from \_INT files, but estimation of precision and repeatability parameters is easier when using polar coordinates (\_INP files). For example, Figure 1 illustrates the 7 interpolated outlines in X,Y cartesian form (in micrometers) from Test 1 with old AMOR (Test for variation at changing magnifications). Figure 2 shows the same 7 outlines in polar coordinates (with the angles Theta in radians and R in micrometers). The variation of Theta of the seven outlines is truly negligible between changing magnifications (see Figure 3).



**Figure 1.** Centered and interpolated (250 equiangular points) outlines of specimen 502\_0100CCK0201 in cartesian form, that were oriented and imaged with old AMOR at 7 magnifications from 0.63x through 2.49x (old AMOR, Test 1,  $\delta X$  versus  $\delta Y$  plotted from \_INT files from program Sprep60.out). The indicated blue point ( $\Delta X=200.62 / \Delta Y=331.52$ ) is point No. 154 in the \_INT) file taken at zoom=2.49x and is the same point as illustrated in Figure 2 in polar coordinates.



**Figure 2.** Centered and interpolated (250 equiangular points) outlines of specimen 502\_0100CCK0201 in polar notation, that were oriented and imaged with old AMOR at 7 magnifications from 0.63x through 2.49x (old AMOR, Test 1, R versus Theta plotted from \_INP files from program Sprep60.out). The blue point at Theta=1.027 / R=387.499 is point no. 154 in the \_INP file for zoom=2.49x and is the same point in polar coordinates as indicated in Figure 1.



**Figure 3.** Plot of the Theta for each outline (250 points) against the average Theta value from the seven outlines between 0,63x and 2.49x (old AMOR, Test 1, Theta values plotted from \_INP files generated with program Sprep60.out). The variation of Theta between the 7 outlines is truly negligible and does not deviate from the diagonal line.

It becomes obvious, that the polar notation is more advantageous for quantification of variation parameters than the cartesian notation, because error ranges between different outlines are directional (e.g. they vary from one angle Theta to the next). For example, variation between outlines are relatively large in the intervals of maxima and minima of R, and maximal in R close to  $\Theta = 4.5$  radians.

For better overview each test (e.g. Test1\_Varying\_magnifications, and Test2\_Repeatability) with old AMOR and System AMOR2 the results were organized in subdirectories, e.g.

- [Trace\_AMOR2\_batch]
- [Sprep60]
- [Raw Images]
- [Graphs\_INT]
- [Graphs\_INP]

### 5.1. Intra-machine variation

The outlines given in subdirectories [Graphs\_INP] for each test contain a text matrix called Theta\_R\_matrix, from which the deviation between outlines in each test and between the devices could easily be calculated. The intra-machine variation is here defined as  $\xi$  e.g.,

$$\xi = (\text{Sum } \xi_i)/250, \text{ with}$$

$$\xi_i = \pm (\text{Max } R_i - \text{Min } R_i)/2$$

$R_i$  is the ray length at angle  $\text{Theta}_i$ .  $\xi_i$  is the center between the maximum ray and the minimum ray at position  $\text{Theta}_i$ . And  $\xi$  is the arithmetic mean of all 250 values of  $\xi_i$ .

This  $\xi$  intra-machine variation was calculated for Test 1 and 2 for the two devices AMOR and System AMOR 2 and are summarized in Table 2. The relative error of  $\xi$  is also expressed as percentage of  $\xi$  from the average ray length (Mean R) over all curves in a particular test.

|                     | <b>Test 1</b><br>(Influence of magnific.) | <b>Test 2</b><br>(Repeatability)        |
|---------------------|---|---|
| <b>AMOR</b>         | $\xi = 11.3898895$<br>(3.21% of Mean R)   | $\xi = 6.65502627$<br>(1.91% of Mean R) |
| <b>System AMOR2</b> | $\xi = 4.4000919$<br>(1.25% of Mean R)    | $\xi = 5.93324104$<br>(1.70% of Mean R) |

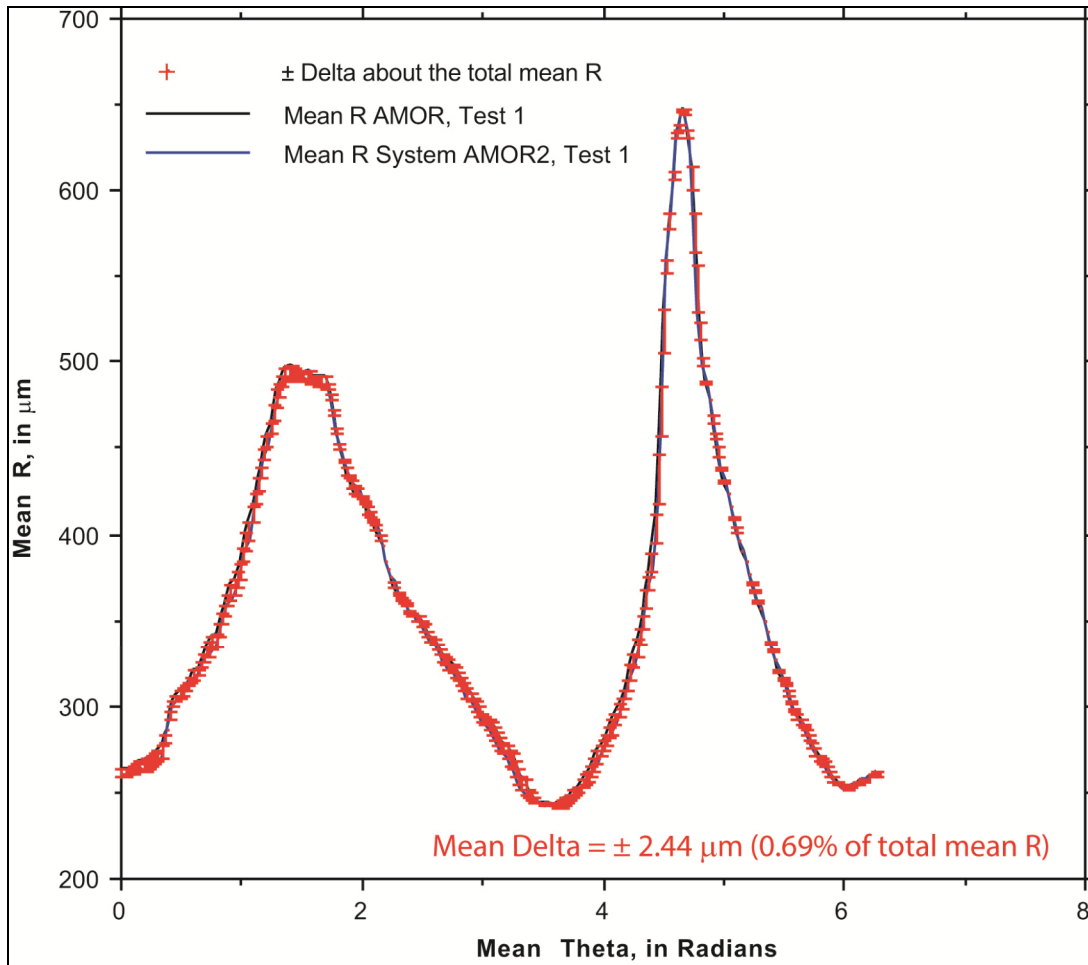
**Table 2.** Intra-machine variation for AMOR and System AMOR2. Test 1 illustrates the influence of changing magnification during automatic orientation. Test 2 illustrates the repeatability of automated orientation at constant magnifications of 2.49x (old AMOR) and 1.55x (SystemAMOR2).

### 5.2. Inter-machine variation between AMOR and System AMOR2:

For estimation of the inter-machine variation of outlines taken with AMOR and System AMOR2 the means of  $R_i$  and  $\text{Theta}_i$  for *Test 1* were calculated from both machines, and then the half absolute differences between the two ( $\Delta_i$ ) were plotted in Figure 4.

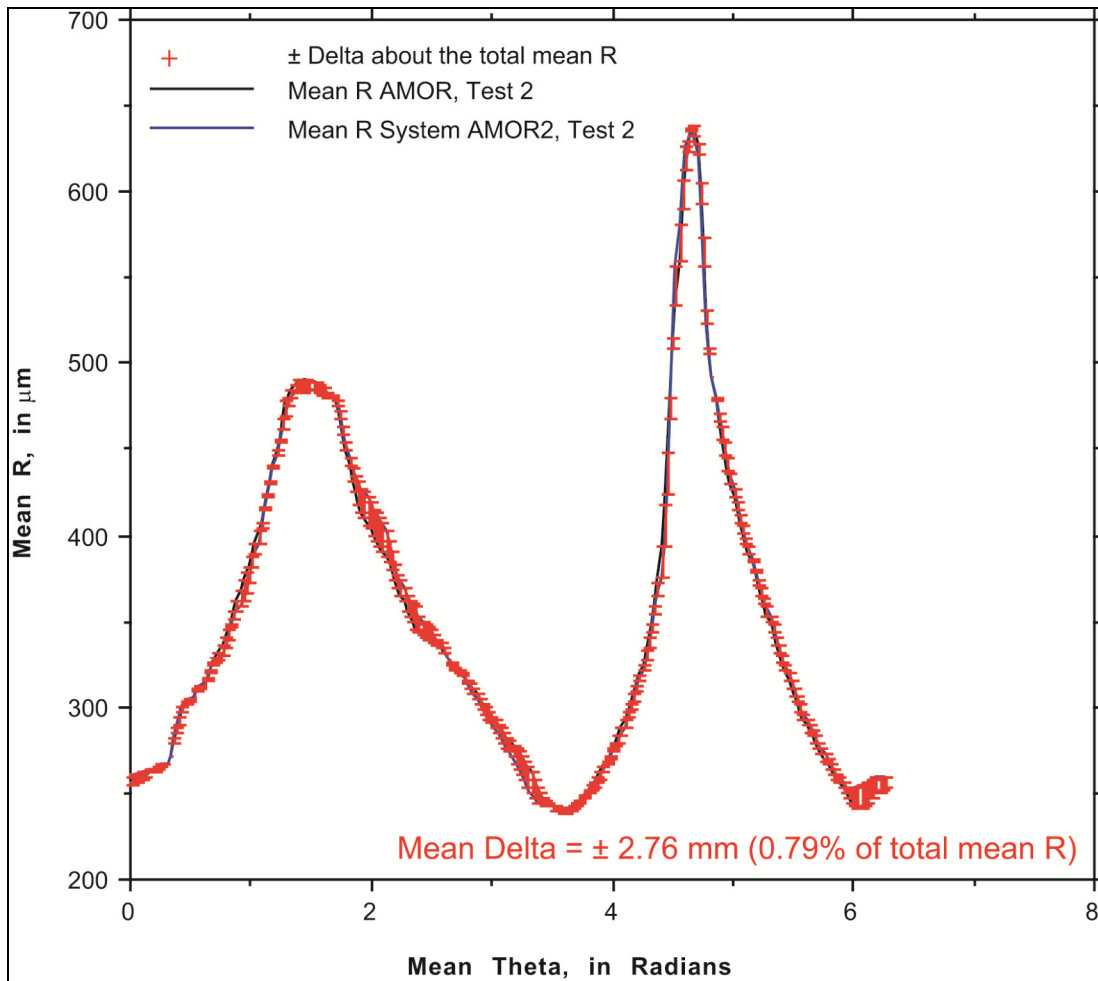
$$\Delta_i = \text{Abs}[\text{Mean}R_i (\text{AMOR}) - \text{Mean}R_i (\text{System AMOR2})]/2$$





**Figure 4.** Inter-machine variation of mean outlines in polar coordinates between AMOR and System AMOR2 for Test 1 (e.g., changing magnifications, polar coordinates from \_INP files generated with program Sprep60.out). The black and blue lines represent the means of ray  $R_i$  at angle  $\Theta_{i_j}$  obtained with AMOR and System AMOR2, respectively. The red error bars represent the inter-machine variation expressed as  $\pm\Delta$  about the average of the black and blue lines. The integral variation of R between AMOR and System AMOR2 is  $\pm 2.438\mu\text{m}$  or 0.69% of the mean over all  $R_i$  values.

The same approach was applied for estimation of inter-machine variation between AMOR and System AMOR2 for *Test 2* (repeatability), and illustrated in Figure 5 and summarized in Table 3.



**Figure 5.** Inter-machine variation of mean outlines in polar coordinates between AMOR and System AMOR2 for *Test 2* (e.g., Repeatability, polar coordinates from \_INP files generated with program Sprep60.out). The black and blue lines represent the means of ray  $R_i$  at angle  $\Theta_i$  obtained with AMOR and System AMOR2, respectively. The red error bars represent the inter-machine variation expressed as  $\pm\Delta$  about the average of the black and blue lines. The integral variation of  $R$  between AMOR and System AMOR2 is  $\pm 2.76\mu\text{m}$  or 0.79% of the mean over all  $R_i$  values.

|                             | <b>Test 1</b><br>(Influence of magnific.)                  | <b>Test 2</b><br>(Repeatability)                           |
|-----------------------------|--|--|
| <b>AMOR vs System AMOR2</b> | $\Delta = \pm 2.43817152 \mu\text{m}$<br>(0.69% of Mean R) | $\Delta = \pm 2.75787448 \mu\text{m}$<br>(0.79% of Mean R) |

**Table 3.** Inter-machine variation for AMOR and System AMOR2. Test 1 illustrates the influence of changing magnification during automatic orientation. Test 2 illustrates the repeatability of automated orientation at constant magnifications of 2.49x (old AMOR) and 1.55x (SystemAMOR2).

## 6. Conclusions:

- With old AMOR, Trace\_AMOR2\_batch.out needs to be combined with MagCorr2.out. When using System AMOR2, outlines can be determined using Trace\_AMOR3\_batch.out without prior correction of the magnification.

- Analysis of orientation is ideally done using polar coordinates of outlines (ray length  $R_i$  versus angle  $\Theta_{\theta_i}$ ).

- Comparison of orientation tests on the same specimen shows, that the deviation between outlines is directional. It is largest in Theta intervals, where the R curve attains its extrema, but also in a Theta interval between about 4.5-4.8 radians (258°-275°).

- Automated orientation and imaging of the same specimen with AMOR and System AMOR2 shows generally excellent matching.

- During Test 2 (repeatability test) the average time to complete a single AutoTilt cycle on old AMOR is about 129 seconds. With System AMOR2 this time is somewhat longer, i.e. 162 seconds.

- In old AMOR, intra-machine variation shows an average deviation of  $\xi = 1 \pm 1.4 \mu\text{m}$  (=3.21% from the mean radius) if magnification is changed. Repeatability (n=15 repetitions, constant magnification) shows a deviation of  $\xi = \pm 6.7 \mu\text{m}$  (=1.91% from the mean radius).

- In System AMOR2, the average intra-machine variation  $\xi$  is slightly better (lower) than with old AMOR, showing values of  $\xi = \pm 4.4 \mu\text{m}$  (=1.25 % from the mean radius) if magnification is changed, and with  $\xi = \pm 5.93 \mu\text{m}$  (=1.70% from the mean radius) when orientation and imaging is 15 times repeated at constant magnification).

- The inter-machine variation shows excellent match of outlines from the same specimen and obtained with the two devices: When the magnification was changed the average deviation of outlines between old AMOR and System AMOR2 was  $\Delta = \pm 2.44 \mu\text{m}$  (0.69% of the mean radius R). Repeatability between old AMOR and System AMOR2 (n=15, constant magnification) is even better with  $\Delta = \pm 2.76 \mu\text{m}$  or an average deviation of 0.79% from the mean radius R.

## 7. Data repository

All images and derived data to this report are electronically stored on the internal Media server of the Natural History Museum Basel. A pdf version of this report and program codes are part of the MorphCol documentation, also on the above Media-server, but see also .Knappertsbusch (2015) for earlier versions of MorphCol documentations.

## 8. Literature cited

**Knappertsbusch, M. (2015).** MorphCol 2004-2013. A collection of Fortran 77 programs for geometric morphometry. Unpublished Technical Report, 1st Edition. Naturhistorisches Museum Basel, Augustinerstrasse 2, 4001-Basel, Switzerland. Zip archive available from <http://doi.pangaea.de/10.1594/PANGAEA.848773>.

For later editions see <http://micropal-basel.unibas.ch/Research/MORPHCOL/Start.html>. A mirror of these later MorphCol editions is also deposited on the internal Media server of the Natural History Museum Basel.

**Knappertsbusch, M. (2018).** MorphCol supplement#26. Korrekturen fehlerhaftes Trace\_AMOR1\_batch.out Programm. Part of later MorphCol edition, 7 p. <http://micropal-basel.unibas.ch/Research/MORPHCOL/Start.html>

**Knappertsbusch, M. (2019).** MorphCol supplement#27. Calibration for System AMOR2 with JVC camera. Part of later MorphCol edition, 10 p. <http://micropal-basel.unibas.ch/Research/MORPHCOL/Start.html>