# MorphCol Supplement #11 – Magnification Test with AMOR3

**Michael Knappertsbusch**
9. to 23. September, 2008

This report replaces the part about the conversion of magnifications in MorphCol Supplement #8.

## History

AMOR2 was extended to AMOR3 by implementation of a slide calibration and number-detection algorithm. The program was adapted to LabView version 8.5 (FHNW Bachelor thesis of Lukas Widmer, 2008), and the flexible coupling and the old clamp holding the motor-zoom were replaced and improved by a solid mechanical fixation of the motor zoom with the microscope stand, respectively. The lower and upper limits of the motor zoom were re-adjusted using the new Limit.vi o program of Lukas Widmer. These modifications needed re-calibration and testing of the magnification function in AMOR3 and an adaption of the conversion program MagCorr, which corrects the magnification values exported by AMOR3 to the real magnifications at the binocular microscope. The results of these tests are described in the present report.

## Experimental setup:

Specimen: 502_0100CCK0201 was used for this experiment (=same specimen as in MorphCol supplement #3, #5, #7 and #8). The specimen was still remounted in keel view into the center of field 30 of a 60 fields slide from the previous tests. The imaging set up was the following:

AMOR2
Parameters a and b in file Programme/AMOR3/settings.ini were a=0.64732, b=0.0039901 (same as evaluated in MorphCol suppl. #5 and #8).
Camera: Sony DXC-390P
Cmount 1x
Leica MZ 6 binocular microscope using motorized zoom (zoom range from 0.63x to 4x).
Diaphragma opening at microscope set at 3
Achromat 1x objective
Cross-polarized light (new cross-polarizer from Volpi)
Illumination using Volpi 6000-1, light at 4 (fully open)

## Fine-calibration of magnification for AMOR3, single mode

After initialization [microscope moves to magnification 1.00 (value returned from AMOR3]) the motorzoom was moved to the individual standard magnifications at the microscope (readings at the mm-scale of the zoom wheel of the microscope, i.e. 0mm (=0.63x), 8mm (=0.80x), 16mm (=1.00x), 23.5mm (=1.25x), 31.5mm (=1.60x), 39mm (=2.00x), 47.5mm (=2.50x), 55mm (=3.20x), and 63mm (=4.00x) in single-measurement mode, and the corresponding magnifications indicated in the zoom-display of the AMOR3 program were recorded (see Table 1 and Figure 1).

| Modus | Standard Mag | Standard mm | Mag AMOR3 | LED Limit.vi | X control pts | Y control pts |
|---|---|---|---|---|---|---|
| single, fine<br>single, fine | 0.63x<br>0.63x | 0 mm<br>0 mm | 0.63x 0.63x<br>0.64x 0.63x | on<br>on | 0.635 | 0.630 |
| single, fine<br>single, fine | 0.80x<br>0.80x | 8 mm<br>8 mm | 0.80x 0.80x<br>0.80x 0.80x | off<br>off | 0.80 | 0.80 |
| single, fine<br>single, fine | 1.00x<br>1.00x | 16 mm<br>16 mm | 1.01x 1.01x<br>1.01x 1.01x | off<br>off | 1.01 | 1.00 |
| single, fine<br>single, fine | 1.25x<br>1.25x | 23.5 mm<br>23.5 mm | 1.25x 1.25x<br>1.25x 1.25x | off<br>off | 1.25 | 1.25 |
| single, fine | 1.60x | 31.5 mm | 1.59x 1.59x | off | 1.59 | 1.60 |

| | | | | | | |
|---|---|---|---|---|---|---|
| single, fine | 1.60x | 31.5 mm | 1.59x 1.59x | off | | |
| single, fine | 2.00x | 39 mm | 1.96x 1.98x | off | 1.975 | 2.00 |
| single, fine | 2.00x | 39 mm | 1.98x 1.98x | off | | |
| single, fine | 2.50x | 47.5 mm | 2.51x 2.51x | off | 2.505 | 2.50 |
| single, fine | 2.50x | 47.5 mm | 2.49x 2.51x | off | | |
| single, fine | 3.20x | 55 mm | 3.13x 3.13x | off | 3.13 | 3.20 |
| single, fine | 3.20x | 55 mm | 3.13x 3.13x | off | | |
| single, fine | 4.00x | 63 mm | 3.97x 3.93x | on | 3.94 | 4.00 |
| single, fine | 4.00x | 63 mm | 3.93x 3.93x | on | | |

Table 1: Evaluation of magnification values returned from AMOR3 at positions of standard magnification as read from the microscope. Modus: single=single-measurement mode, fine=sensibility “fine”. Standard Mag = magnification numbers at raster positions of the microscope zoom wheel. Standard mm = millimetre readings at standard magnification positions of zoom wheel. Mag AMOR3 = magnification readings returned by the AMOR3 program (=same values returned to List_of_files in auto-measurement mode). LED = lighting at upper and lower stop positions of motor zoom. X control pts, Y control pts = averaged x,y coordinates of Mag AMOR3 / Standard Mag pairs.

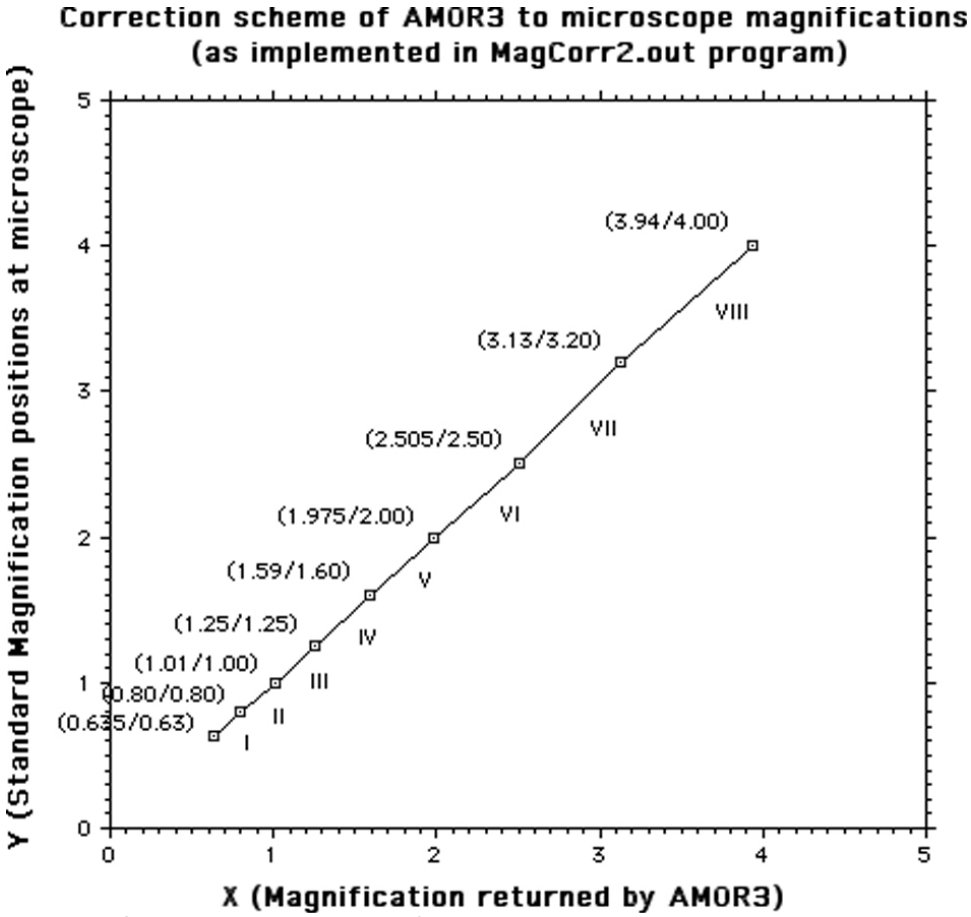


Figure 1. Correction scheme of AMOR3 magnifications to magnifications at microscope zoom wheel. X values are the X control pts and Y values are the Y control pts given in Table 1. Roman numerals indicate the segments for linear interpolation as implemented in MagCorr2.out program.

Because the magnifications, that were returned by the AMOR3 program, do not exactly match with the corresponding physical magnification positions at the microscope zoom wheel (readings in mm), a correction was necessary prior to outline extraction from images with the Trace_AMOR1_batch.out program. This

correction is done with program MagCorr2.out (see Appendix for listing) by segmentwise linear interpolation along the line shown in Figure 1. Input are the magnification values provided by the AMOR3 program (either read from the display in the single-measurement mode or by using the values in the List_of_files file, that is produced in the automeasurement mode). Output are the corrected magnifications ready for use with the outline extraction program Trace_AMOR1_batch.out.
The equations for the linear segments I through VIII are as follows:

| | |
|---|---|
| Segment I [ X<0.8 ]: | Y = 1.030303 * X – 0.0242424 |
| Segment II [ 0.8 <= X < 1.01 ]: | Y = 0.9523809 * X + 0.0380953 |
| Segment III [ 1.01 <= X < 1.25 ]: | Y = 1.0416666 * X – 0.0520833 |
| Segment IV [ 1.25 <= X < 1.59 ]: | Y = 1.0294117 * X – 0.0367647 |
| Segment V: [ 1.59 <= X < 1.975 ]: | Y = 1.038961 * X – 0.051948 |
| Segment VI [ 1.975 <= X < 2.505 ]: | Y = 0.9433962 * X + 0.1367925 |
| Segment VII [ 2.505 <= X < 3.13 ]: | Y = 1.12 * X – 0.3056 |
| Segment VIII [ x >= 3.13 ]: | Y= 0.9876543 * X + 0.108642 |

## Testing of the new calibration

1. For testing the magnification conversion specimen: 502_0100CCK0201 was used (see Figures 2 and 3).

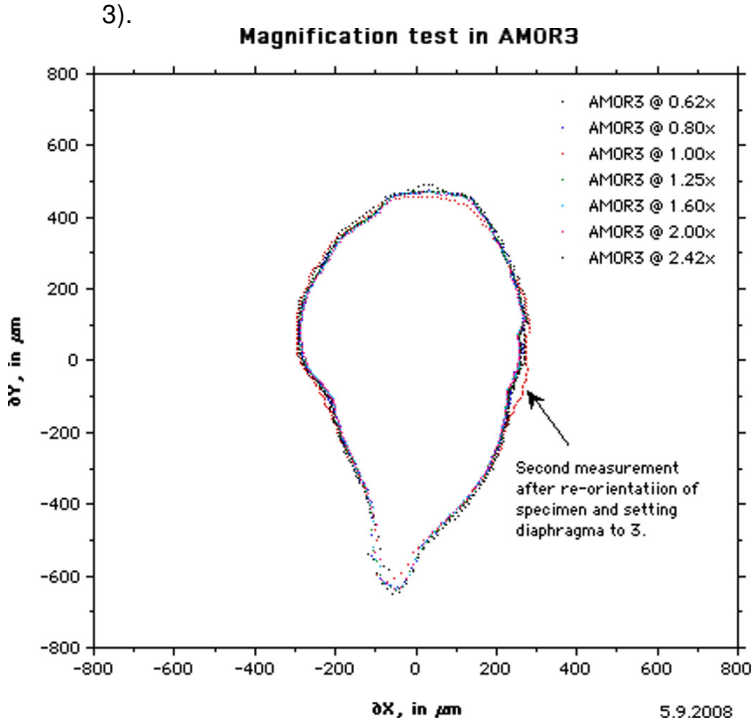


Figure 2: Interpolated (250 points) outlines of specimen 502_0100CCK0201 after tilting and capturing at magnifications from 0.63x through 2.42x using AMOR3. There is good coincidence among the outlines. The red case was after re-initialization of AMOR and re-imaging at a slightly different diaphragma position.

The already mounted test specimen in keel view in the center of field No. 30 of a 60 fields slide was used, as was already done so during previous tests. In AMOR3 the single specimen mode was set and then the specimen was then autocentered and autofocused. For tilting the magnification was set to 1.59x (as read in the AMOR3 program). The specimen was focused again, and the autotilted, autofocused and autorotated at this magnification. Thereafter, Tiff images were captured in this positions at magnifications of 0.63x, 0.80x, 1.01x, 1.25x, 1.59x, 1.98x, and 2.42x (Mag readings from AMOR3 program).
The images were then copied to Macintosh and processed there. Processing was as follows:

2. Binarization and conversion from Tiff to Raw images using Nih-Image macro “automation”.

3. Saving as raw files with Adobe Photoshop (images 3001r, 3002r, …,3007r).
4. Correction of AMOR3 magnifications (0.63x, 0.80x, 1.01x, 1.25x, 1.59x, 1.98x, and 2.42x) using MagCorr2.out program.
5. Outline extraction from raw images using Trace_AMOR1_batch.out and applying the corrected magnifications to produce sequence _T files.
6. Generating interpolated outlines with 250 points using the Sprep53.out program to produce sequence of _INT files.

Re-formatting _INT files and plotting _INT outlines on top of outlines illustrated in MorphCol Supplemets #3 and #8 for comparison (see Figures 2 and 3). Figure 3 illustrates, that the fine-calibration and implementation in MagCorr2.out works reasonably well.

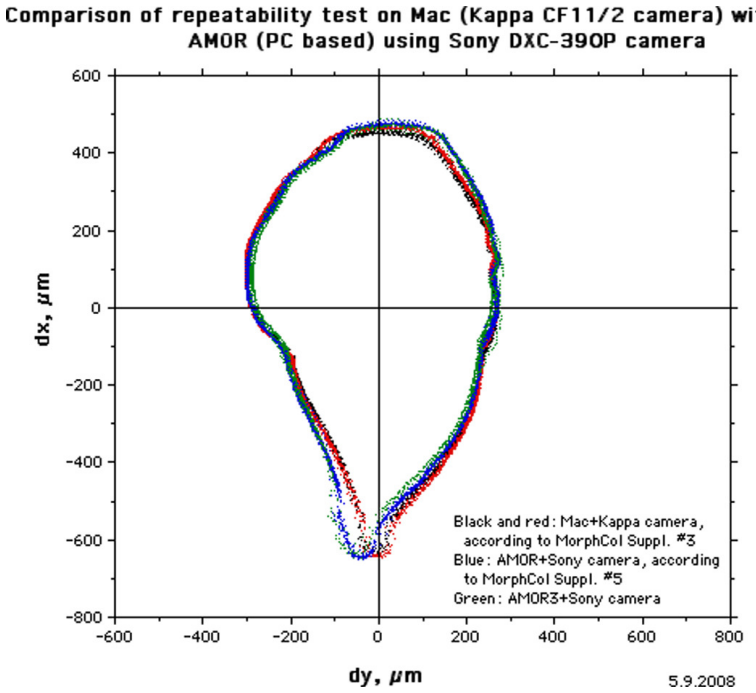


Figure 3: Overlay of magnification test from AMOR3 experiment (in green) with earlier tests using the same specimen (in blue: using AMOR2 on PC, also with Sony camera; in red and black: using Macintosh-based system and Kappa camera).

Because in the above experiment the specimen was too big for testing the functionality at higher magnifications, s second test was done for testing the full magnification range of AMOR3 using the smaller specimen ODP 154-925B-1-1, 15-16cm, >63µm, 1/8 split, Slide b, specimen No. 3501, see Table 2.

| No | Image file | Mag@tilt | Mag@capture | LED | Mag (mm) |
|---|---|---|---|---|---|
| 1 | 3501r,0.63x | 2.49x | 0.63x | On | 0 mm |
| 2 | 3501r,0.79x | 2.49x | 0.79x | Off | 8 mm |
| 3 | 3501r,1.00x | 2.49x | 1.00x | Off | 16 mm |
| 4 | 3501r,1.25x | 2.49x | 1.25x | Off | 24 mm |
| 5 | 3501r,1.57x | 2.49x | 1.57x | Off | 32 mm |
| 6 | 3501r,1.98x | 2.49x | 1.98x | Off | 40 mm |
| 7 | 3501r,2.49x | 2.49x | 2.49x | Off | 47.5 mm |
| 8 | 3501r,3.13x | 2.49x | 3.13x | Off | 56 mm |
| 9 | 3501r,3.89x | 2.49x | 3.89x | on | 62.5 mm |

Table 2: All magnification readings are from AMOR3 program.

The new specimen was positioned at constant 2.49x magnification, then autocentered, autofocused, autorotated as before. In this position images were captured from 0.63x through 3.89x (all magnification readings from the AMOR3 program). Images were then transferred to Mac, binarized using the Nih-Image "automation" macro, saved as raw file. The magnifications were corrected using MagCorr2.out program, then the raw images were traced using Trace_AMOR1_batch.out, processed using Sprep53.out, and then plotted (see Figure 4).

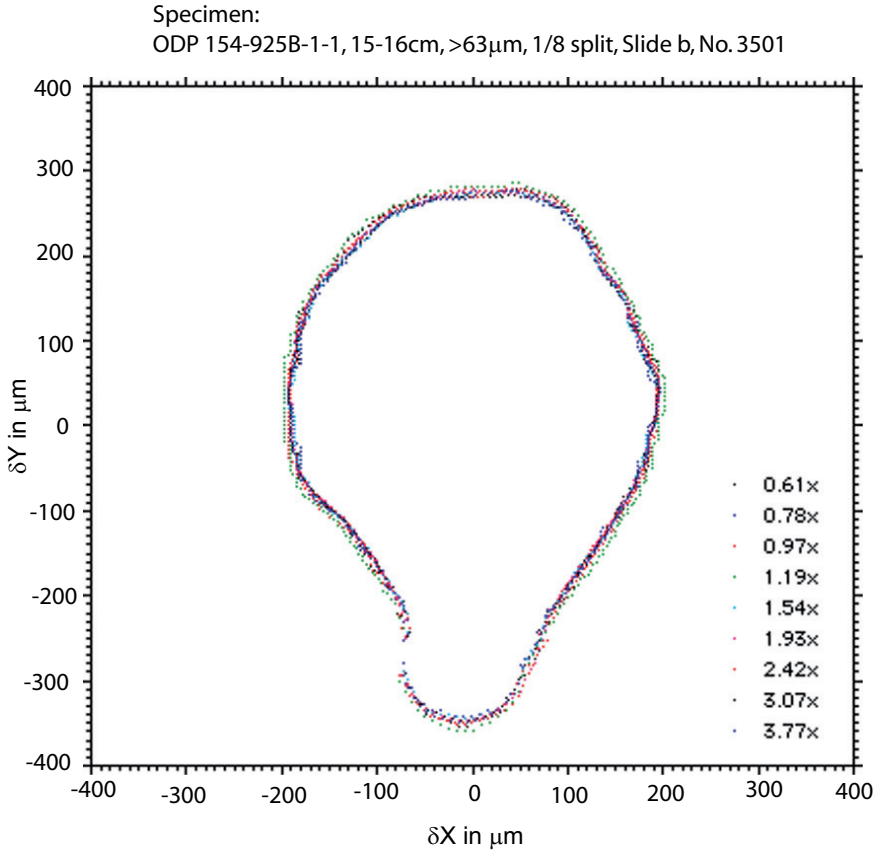


Figure 4: Results from Test 2. In the legend, image names were converted to 3501 for 0.63x, 3502 for 0.79x, …, to 3.89x.

Figure 4 shows that there is variation of the outlines from the same specimen at the same position under changing magnifications. However, the overlap of the outlines is reasonably good for shape and size analysis, the latter showing larger variations than these influences. The reason for the slight variations is, that extraction of outlines and interpolation to 250 points lead to differences in Cartesian coordinates between small magnifications and larger magnifications because of changing resolution. Secondly, light intensities differ at changing magnifications (much light at low magnification, less light at high magnification), which causes different grey-level gradients across the edge (but constant threshold during outline extraction). Thirdly, the different grey-level gradients across the edge of the shell at different magnifications influences the appearance of the binarized image and so also causes variation in edge-detection ("outward migration of edge" as illumination increases, erosion of edge and appearance of "pixel-embayments" as illumination decreases. For routine-measurements it is therefore important to keep illumination and diaphragma constant across series of measurements

In Figure 5 a flow-scheme of all the post-processing is shown.

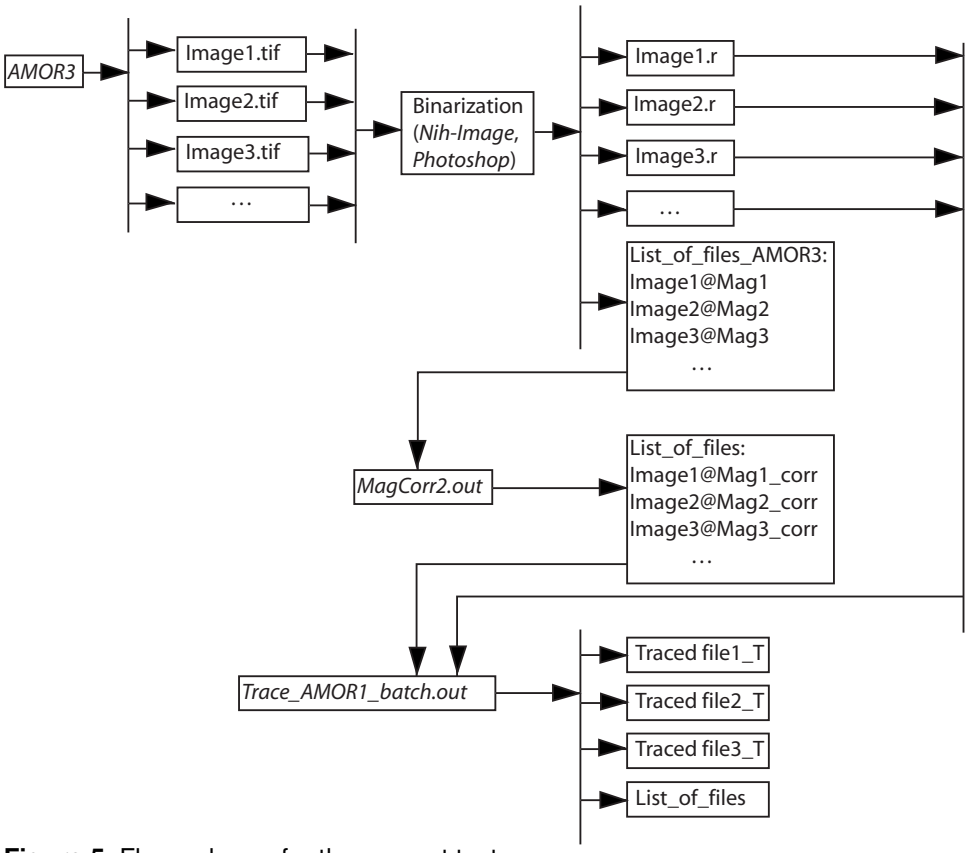


**Figure 5**. Flow-scheme for the present test.

## Conclusions

The tests have shown that with the adapted programs of MagCorr2.out specimens show good coincidence at all magnifications with previously generated outlines from the same specimen, so that the AMOR3 system (single specimen mode) is ready for routine application.

## Appendix

```
      Program MagCorr
C
C     By Michael Knappertsbusch
C     Version 2.0, 4.9.2008, modified from version 1
C     For AMOR3 from bachelor thesis of Lukas Widmer (2008).
C
C     This program corrects the magnifications, that are determined and displayed
C     in AMOR3 (formula file Programme/AMOR3/settins.ini), into the correct values
C     at the zoom wheel of the Leica MZ6 microscope. The method is by segment-wise
C     linear interpolation (segments I through VIII) as described in section 3 of
C     Knappertsbusch (2008). Fine calibration of AMOR3. MorphCol Supplement #11,
C     September, 2008.
C
C
C     Input is file INPUT (i.e. an ascii text file called list_of_files, which AMOR3
C     generates and which contains the name of each image taken together with the
C     corresponding (AMOR3 calculated) magnification. The format of this information
C     is explained in the following example:
```

```
C
C            3001r,1.90
C
C      The first 4 characters indicate the specimen number in that slide (characters 1-2
C      are the field number, characters 3-4 are the specimen [usually 01 when working
C      with AMOR3]). The fifth character is a lowercase r, indicating a raw file format
C      (required by the program Trace_AMOR1_batch.out).
C      The sixth charcter is a comma (can be used herein as a delimiter between specimen
C      number and magnification). The following four characters indicate the
C       magnification as calculated from AMOR3.
C
C
C      Output is file OUTPUT (the file containing the image file names and corresponding
C      corrected magnifications). The output file can the be imported into
C      program Trace_AMOR1_batch.out for outline extraction.

C      Variables:
C      X = Magnification returned by AMOR3
C      Y = Corrected magnification
C
C
       INTEGER N
       DOUBLE PRECISION X,Y
       CHARACTER*13 INPUT
       CHARACTER*20 OUTPUT
       CHARACTER*5 IMAGE
C
       WRITE(9,*) '************************************'
       WRITE(9,*) '*                                  *'
       WRITE(9,*) '*           MagCorr                *'
       WRITE(9,*) '* Corrects the magnification       *'
       WRITE(9,*) '*        from AMOR3                *'
       WRITE(9,*) '*                                  *'
       WRITE(9,*) '* Version 2 from 4 September 2008 *'
       WRITE(9,*) '*    by Michael Knappertsbusch     *'
       WRITE(9,*) '*                                  *'
       WRITE(9,*) '************************************'
       WRITE(9,*) ' '
       WRITE(9,*) '. . .Enter the "list_of_files" from AMOR3. . .'
       READ(9,5) INPUT
 5     FORMAT(A13)
C
C
C***   Generating an output file for the corrected magnifications:
C
       WRITE(9,*) '. . .Enter an output name. . .'
       WRITE(9,*) '              (max 20 chars)'
       READ(9,6) OUTPUT
 6     FORMAT(A20)
       OPEN(15,FILE=OUTPUT,STATUS='NEW')
C
C
C***   Write a header line for the output to the screen:
C
       WRITE(9,*) 'SPECIMEN #, MagAMOR3, Corrected Mag'
C
C
C
C***   Opening and reading the input file:
C
       OPEN(14,FILE=INPUT,STATUS='OLD')
       N=0                                  ! N=Counter for the files in list_of_files
 10    READ(14,*,END=100) IMAGE,X
       N=N+1
C
C
       IF (X.LT.0.8) THEN                   ! [X < 0.80; segment I ]
         Y=1.030303*X-0.0242424
       ELSE IF ((X.GE.0.8).AND.(X.LT.1.01)) THEN      ! [0.80 • X < 1.01; segment II ]
         Y=0.9523809*X+0.0380953
       ELSE IF ((X.GE.1.01).AND.(X.LT.1.25)) THEN     ! [1.01 • X < 1.25; segment III ]
         Y=1.0416666*X-0.0520833
       ELSE IF ((X.GE.1.25).AND.(X.LT.1.59)) THEN     ! [1.25 • X < 1.59; segment IV ]
```

```
        Y=1.0294117*X-0.0367647
      ELSE IF ((X.GE.1.59).AND.(X.LT.1.975)) THEN    ! [1.59 • X < 1.975; segment V ]
        Y=1.038961*X-0.051948
      ELSE IF ((X.GE.1.975).AND.(X.LT.2.505)) THEN   ! [1.975 • X < 2.505; segment VI ]
        Y=0.9433962*X+0.1367925
      ELSE IF ((X.GE.2.505).AND.(X.LT.3.13)) THEN    ! [2.505 • X < 3.13; segment VII ]
        Y=1.12*X-0.3056
      ELSE IF (X.GE.3.13) THEN                ! [3.13 • X; segment VIII ]
        Y=0.9876543*X+0.108642
      END IF
C
C
C***  Output:
C
      WRITE(9,50) IMAGE,X,Y                        ! Output of results to screen
      WRITE(15,60) IMAGE,Y                    ! Output of results to file
 50   FORMAT(A5,'      ,   ',F4.2,'  ,   ',F4.2)
 60   FORMAT(A5,',',F4.2)
      GOTO 10
C
C
 100  WRITE(9,*) ' '
      WRITE(9,*) '. . .',N,' magnifications corrected. . .'
      PAUSE 101
 101  CONTINUE
C
      STOP
      END
```

Macro „automation“ for Nih-Image:

macro 'Auto processing_kevins [A]';
{Macro for processing foraminifers in reflected light,using the Leica binocular, and with polarizers. Published in: Brown, K. 2007. *Biogeographic and morphological variation in late Pleistocene to Holocene globorotalid foraminifera*. Ph. D. Thesis, University of Basel, Switzerland. Pdf file available at http://pages.unibas.ch/diss/2007/DissB_8290.htm}

Var
counter,x,y: integer;
begin;
AverageFrames('Average',16);

```
for counter:=1 to 3 do begin
Sharpen;
Smooth;
end;
MultiplyByConstant(1.25);
end;


macro 'Saving as Tiffimage [T]';
begin;
SetSaveAs('TIFF');
SaveAs('name');
end;


macro 'Foram Processing For Single File [P]';
{for a single file}
Var
x,y:integer;
beginX:=GetNumber('Enter width of left black border (number of pixels (55)):',x);
y:=640;
SetDensitySlice(1,160);
MakeBinary;
Invert;
Erode;
Dilate;
Erode;
Dilate;
MakeROI(0,0,x,y);
Fill;
KillROI;
MakeROI(350,0,480,640);
Fill;
KillROI;
Invert;
end;


macro 'Export as RAWfile [R]';
begin;
SetExport('RAW');
Export('name');
StartCapturing;
end;


macro 'Rotate left [N]';
var
xscale,yscale,angle: integer
begin;
MakeROI(0,0,640,480);
xscale:=1;
yscale:=1;
angle:=-1;
ScaleAndRotate(xscale,yscale,angle);
end;
```

```
macro 'Rotate Right [M]';
var
xscale,yscale,angle:integer
begin;
MakeROI(0,0,640,480);
xscale:=1;
yscale:=1;
angle:=1;
ScaleAndRotate(xscale,yscale,angle);
end;
```