Supplemet #7 to MorphCol:

Fine calibration of AMOR2

By Michael Knappertsbusch, 4.2.2008

1.) Introduction and problem.

After addition of the motor-zoom (Diploma-thesis Stapfer 2007), the evaluation by Knappertsbusch (2007a) has shown, that a number of features needed to be improved before the system can be used for routine imaging. These works were performed by Stapfer during two-weeks long post-diploma thesis project. At the end of the year Stapfer returned an improved system of AMOR Version 2 (briefly AMOR2).

In the present report AMOR2 is tested again for the performance of the corrected automated magnification. The tests show, that still a fine correction needs to be included in the work process in order to have full coincidence of the magnification calculated by AMOR2 and the true magnification set at the microscope wheel.

The magnification, that is returned from the program (calculated from the motorstep-to-magnification curve, see equation [I] below), does not exactly coincide with the magnification indicated on the mechanical zoom wheel (mm readings of the calibration curve). This deviation must be fixed.

In the following these tests are described, a solution for a fine calibration is presented, and a repeatability test is provided for the single mode and automated mode of AMOR2 using this fine calibration.

2.) Testing the precision of Auto-Zoom in AMOR2.

2.1.) Comparison of calculated magnifications provided by AMOR2 versus manually calibrated magnifications

Experimental set up:
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 (new polarizing set from Volpi).
Illumination using Volpi 4000-1, light at 4 (fully open).

The specifications for conversion of zoom-motor steps into the magnification at the microscope wheel are those evaluated in Figure 3 of Knappertsbusch (2007a), i.e.

$$\text{Mag (at microscope)} = 0.64732 * 10^{\text{Exp}\,(0.0039901 * \text{Steps})} \qquad \text{equation [I]}$$

[Note: these parameters are written in file Programme/AMOR2/settings.ini].

Tests were done using specimen 502_0100CCK0201 from the study of Knappertsbusch (2007b). The specimen was transferred in a new multifield slide, in keel-position.

AMOR2 was started up, the single mode operation (sensibility=fine) was chosen.
The motorzoom is then automatically positioned to magnification (Mag)=1.00x.
In this position, the program returns a magnification value of 1.02 (seen in the window, this value is then also written to the list_of_files, if a picture is saved.

The motorzoom was then changed to 0.63x (switching to sensibility="normal" and cklicking on the "-" button triggers the motor to run into the 0.63x position). By switching back to sensibility ="fine" the motor was then fine adjusted until the zoom position concided with 0.00 mm, which corresponds to the exact magnification of 0.63x (= calibration curve). However, the magnification read in the program shows then a slight deviation from the ideal value (0.65x instead of 0.63x).

These steps were repeated for every standard calibration point of the zoom (i.e. at 0.63x, 0.80x, 1.00x, 1.25x, 1.60x, 2.00x, 2.50x, 3.20x, and 4.00x). This series was measured two times. The mechanical magnifications, the corresponding millimeter readings at the zoom wheel, and the values for the magnifications, that were returned by the AMOR2 program were then noted and plotted (see Figures 1 and 2, and Table 1).

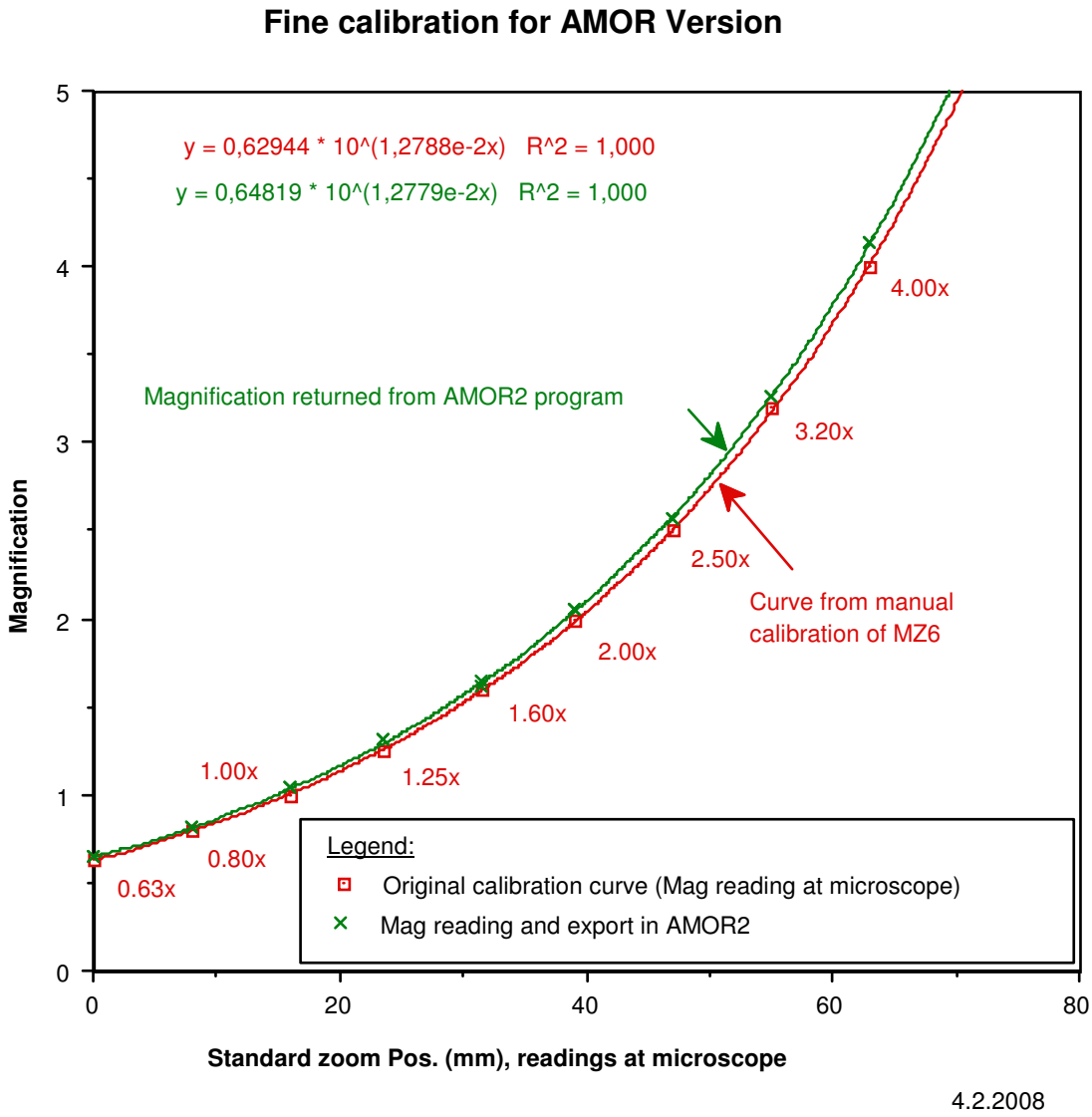

Figure 1. Magnification versus mechanical zoom position (mm reading) for the Leica MZ6. Red curve shows values for the manual calibration, green curve shows the magnification value returned by the AMOR2 program as a function of the standard zoom positions. The numbers along the red curve indicate the magnifications at standard zoom positions.

Table 1

Mag reading at microscope	Mag reading in AMOR2	Zoom pos. (mm)
0.63	0.65	0.00
0.63	0.65	0.00
0.80	0.81	8.00
0.80	0.81	8.00
1.00	1.04	16.0
1.00	1.04	16.0
1.25	1.31	23.5
1.25	1.31	23.5
1.60	1.64	31.5
1.60	1.62	31.5
2.00	2.06	39.0
2.00	2.06	39.0
2.50	2.57	47.0
2.50	2.57	47.0
3.20	3.26	55.0
3.20	3.26	55.0
4.00	4.14	63.0
4.00	4.14	63.0

Explanation of Table 1. The magnifications in the first column are the standard positions and correspond to the original manual calibration curve for the Leica MZ6 as illustrated in Table 1 and Figure 1 of Knappertsbusch (2007a). The magnifications in the second column are the values, that are returned from the AMOR2 program (single mode). These values are exported and written to the List_of_files. Note, that they deviate from the manual calibration curve. In the third column are the mm readings of the standard zoom positions, that correspond to the manual calibration curve.

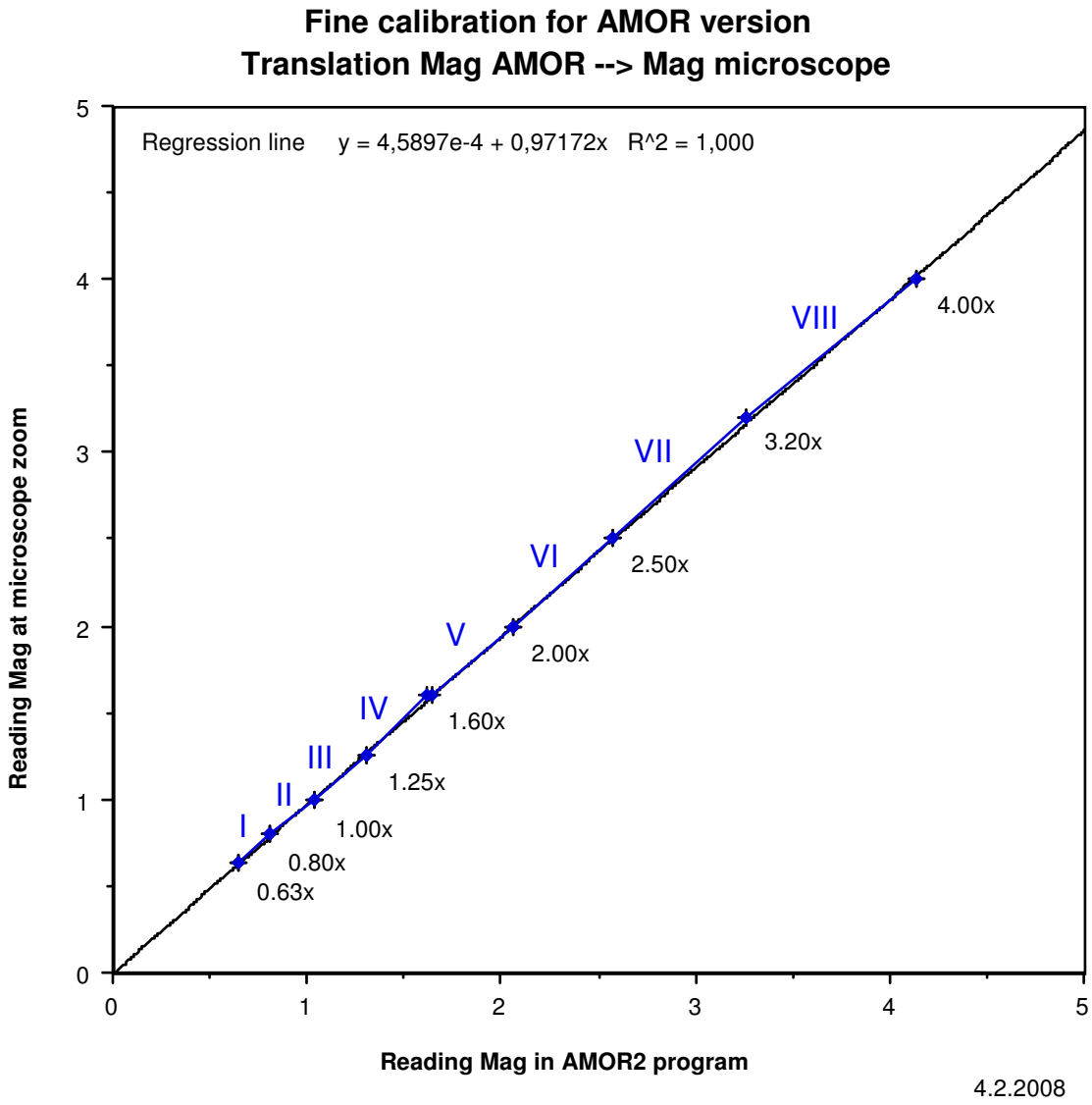

Figure 2. Plot of the calibrated magnifications read at the microscope (column 1 in Table 1; i.e. the value as it should be) versus the magnification value calculated and exported by the AMOR2 program (column 2 in Table 1; i.e. the deviating value) for each standard position. The linear regression line is in black (=regression model for fine correction of the magnifications). The blue line segments connect each point with the next one in the sequence (segment-wise linear interpolation model for fine correction of the magnification). The numbers along the line indicate the magnifications at standard zoom positions. Blue roman numeral indicate segments used in program MagCorr1.out (see section 3).

2.2.) Re-checking the conversion of motor steps into standard zoom positions for AMOR2

In AMOR2 the exponential law

$$Mag = a * Exp(b * Steps) \qquad \text{equation [II]}$$

is applied to translate motor steps into magnifications, which are then displayed in the single operation/fine resolution mode of the program and exported to list_of_files during the single and automatic mode of AMOR2.

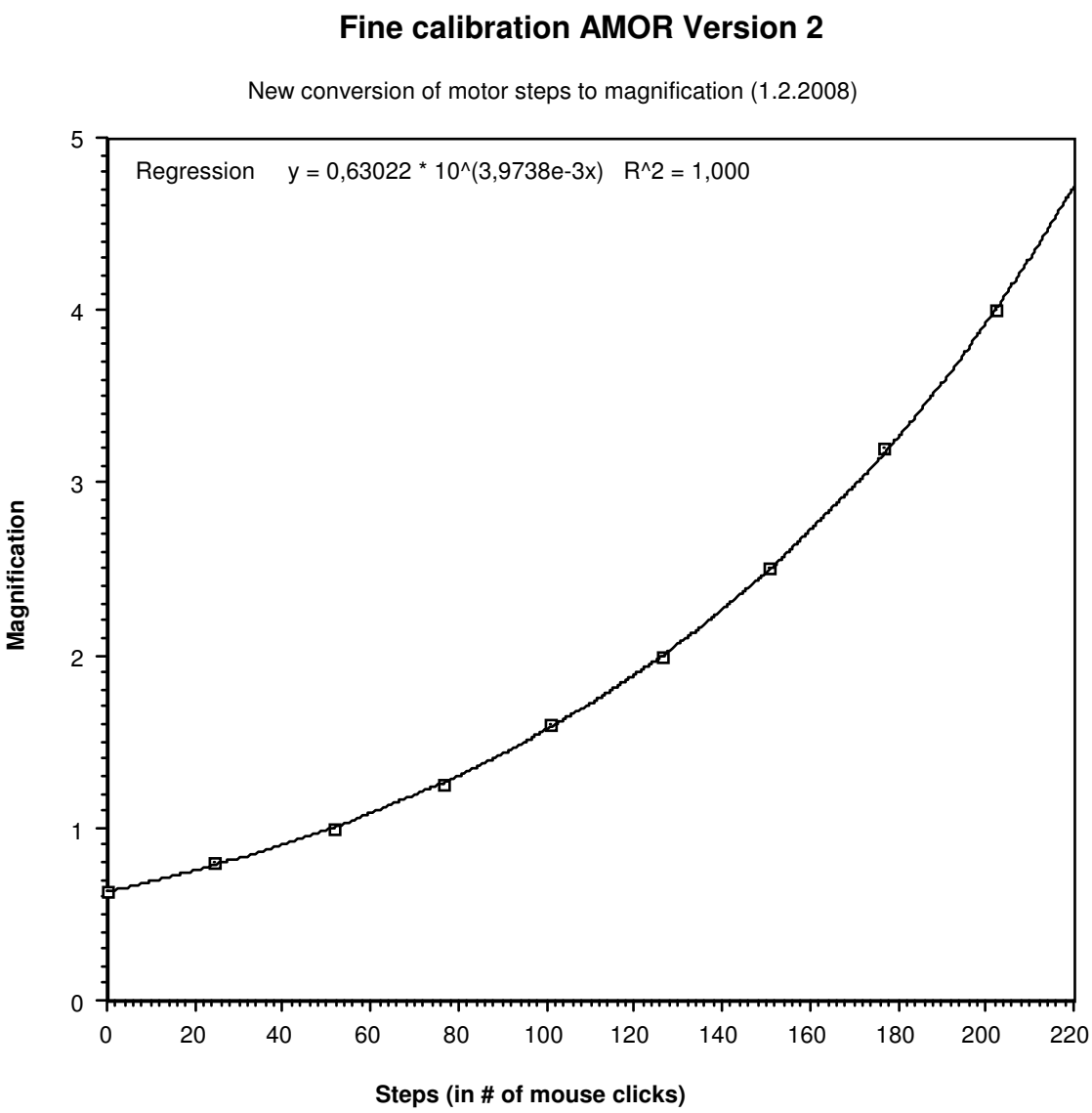

Figure 3. Plot of the number of motor steps versus standard magnifications (i.e. column 3 versus column 1 from Table 2) for AMOR2. The exponential regression line follows a slightly different formula with a=0.63022 and b=0.0039738.

The values for a and b are written in file Programme/AMOR2/settings.ini. For the improved AMOR2 version provided by Stapfer these values were a=0.64732, and b=0.0039901. These values were determined in a test in section 3 of Knappertsbusch (2007a). One possibility to eliminate the deviations of calculated magnifications from the manual calibration curve is to re-evaluate the coefficients a and b in equation [II]. The observations for this re-evaluation are presented in Figure 3 and in Table 2 below.

Standard Mag at microscope	Zoom position in mm	Motor steps # of clicks	Cumulat. steps	Cumulat. steps	Cumulat. steps
Forward			Forward	Backward	Average
1.00x	16 mm [1.04x]	0			
0.80x	8 mm [0.81x]	27			
0.63x	0 mm [0.65x]	25	0	0	0
0.80x	8 mm [0.81x]	24	24	25	24.5
1.00x	16 mm [1.04x]	27	51	52	51.5
1.25x	23.5 mm [1.31x]	25	76	77	76.5
1.60x	31.5 mm [1.64x]	24	100	102	101
2.00x	39 mm [2.06x]	25	125	128	126.5
2.50x	47 mm [2.57x]	24	149	152	150.5
3.20x	55 mm [3.26x]	26	175	178	176.5
4.00x	63 mm [4.14x]	26	201	204	202.5
Backward					
4.00x	63 mm [4.14x]	0		204	
3.20x	55 mm [3.26x]	26		178	
2.50x	47 mm [2.57x]	26		152	
2.00x	39 mm [2.06x]	24		128	
1.60x	31.5 mm [1.64x]	26		102	
1.25x	23.5 mm [1.31x]	25		77	
1.00x	16 mm [1.04x]	25		52	
0.80x	8 mm [0.81x]	27		25	
0.63x	0 mm [0.65x]	25		0	

Table 3. Measurements of the re-evaluation of motor-steps to magnification conversion. In the first column are the standard magnifications for the Leica MZ6. In column 2 are the mm readings from the zoom-wheel, that correspond to the standard magnifications. The values in parentheses are those returned by the AMOR2 program (see section 2.1). In column 3 are the number of steps of the motor (=number of mouse clicks). In column 4 and 5 are the cumulative motor steps. In column 6 are the motor steps averaged from the forward and backward movement of the motor zoom.

The result from this evaluation is a slightly different exponential law for the improved AMOR2 version than it was before [compare with evaluation in Knappertsbusch (2007a)], with a more precise steps-to-magnification conversion. However, at some positions along the forward and backward movements (which cover the same distance) provided slightly different steps. Especially at the upper limit (magnification 4.00x) the precise mechanical position of the zoom is only arrived after two extra clicks, which leads to a somewhat unfavorable performance. After substitution of the previous coefficients for a and b in equation (II) by the new values, the program returned almost ideal values within the lower range of the magnification. Approaching higher magnifications (3.20x and higher) the returned magnifications become less precise (because of the power-law). In addition, there are "inactive steps", i.e. making a single mouse-click, the magnifications are calculated, but the motor does not advance by a step. The exact reason for this behaviour is not clear (this has led to the "half steps" in column 6 of Table 2). At the magnification of 4.00x, further difficulties appear, which sometimes lead to hanging the program.

In conclusion, the direct conversion of steps-to-magnification leads to a lower performance of the program, than post-correction of returned magnifications by using the regression line from section 1.2.

3.) Implementation of a model for fine calibration in AMOR2

Correct magnifications from AMOR2 can be obtained in 3 ways:

a.) Adjustment of the calculated magnification by a segment-wise linear interpolation between standard magnifications, i.e. using the segments I through VIII of the blue line indicated in Figure 2.

b.) Adjustment of the calculated magnifications by the regression line found in section 2.1.

c.) Modification of a and b in equation (II) in the settings.ini file.

Method c.) is less ideal, because of the lower performance of AMOR2 when the new constants a=0.63022 and b=0.0039738 are inserted in equation (II).

Method b.) is possible, but gives somewhat less precise correction factors than method a.).

Method a.) is therefore chosen for the fine calibration of AMOR2. This method is nothing else than applying the exponential law of

$$Mag = 0.64732 * 10 Exp(0.0039901 * Steps)$$

as indicated in the settings.ini file, and then correcting the magnification values to the standard curve by a segment-wise linear interpolation between control points (see segments I through VIII in Figure 2).

The equations for these segments are the following:

Segment (I): Interval [$X < 0.81$], coordinates (0.65/0.63) and (0.81/0.80)
$Y = 1.0625 * X - 0.060625$

Segment (II): Interval [$0.81 \leq X < 1.04$], coords (0.81/0.80 and (1.04/1.00)
$Y= 0.8695652 * X + 0.0956521$

Segment (III): Interval [$1.04 \leq X < 1.31$], coords (1.04/1.00) and (1.31/1.25)
$Y = 0.9259259 * X + 0.037037$

Segment (IV): Interval [$1.31 \leq X < 1.63$], coords (1.31/1.25) and (1.63/1.60)
$Y = 1.1290323 * X - 0.2290322$

Segment (V): Interval [$1.63 \leq X < 2.06$], coords (1.63/1.60) and (2.06/2.00)
$Y = 0.9302325 * X + 0.0837209$

Segment (VI): Interval [$2.06 \leq X < 2.57$], coords (2.06/2.00) and (2.57/2.50)
$Y = 0.9803921 * X - 0.0196078$

Segment (VII): Interval [$2.57 \leq X < 3.26$], coords (2.57/2.50) and (3.26/3.20)
$Y = 1.0144928 * X - 0.1072463$

Segment (VIII): Interval [$X \geq 3.26$], coords (3.26/3.20) and (4.14/4.00)
$Y = 0.9090909 * X + 0.2363636$

These equations are implemented in Fortran program MagCorr1.f (see listin in section 3.1 below). Input are the magnifications given in file "list_of_files", which is generated during the automatic modus of AMOR2.(see flow diagram in Figure 4).

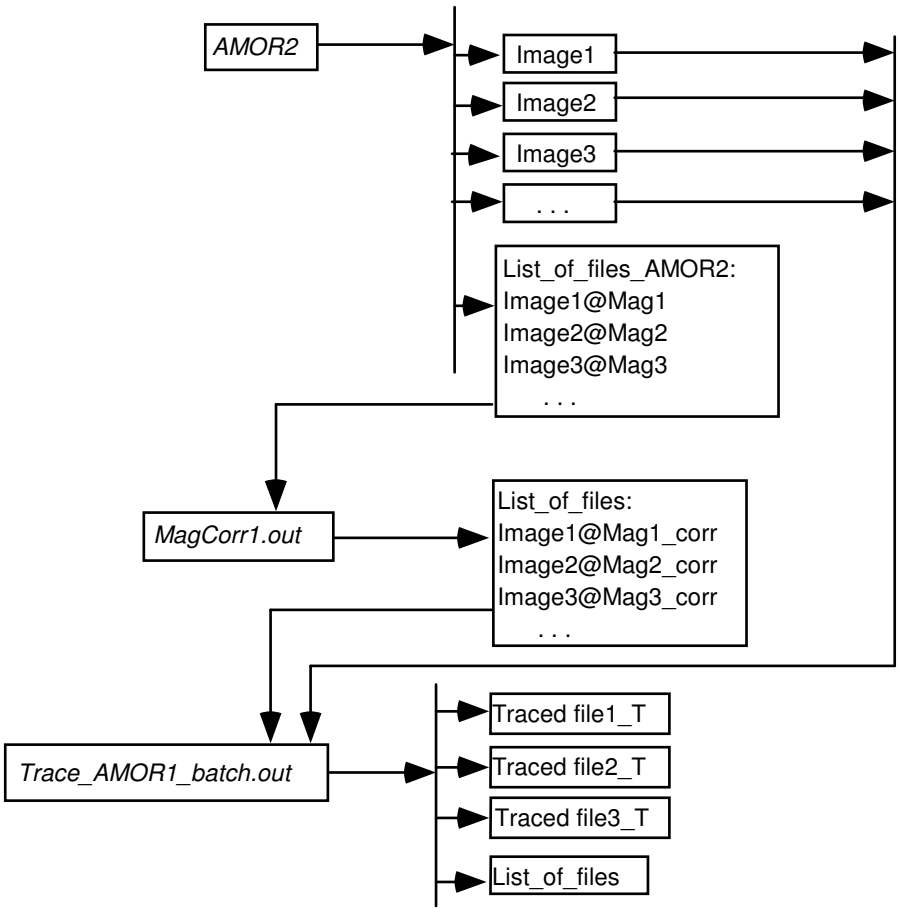

Figure 4. Flow-diagram showing the sequence of working steps and the position of program MagCorr1,out. Program Trace_AMOR1_batch.out must be used because AMOR2 uses the Sony camera (see Knappertsbusch, 2007c). For application of MagCorr1.out, it is necessary, that in the settings.ini file the values of a=0.64732, and b=0.0039901 are written (if another steps-to-magnification model is applied, the set of linear equations for segments I through VIII must be modified as well). Please note also, that Trace_AMOR1_batch.out is applied, which uses the camera calibration for the Sony DXC-390P camera. If another camera type is used, then the calibration curve in the tracing program must be adapted too.

3.1.) Code for MagCorr

```
      Program MagCorr
C
C     By Michael Knappertsbusch
C     Version 1.0, 5.2.2008
C
C     This program corrects the magnifications, that are determined and
C     displayed in AMOR2 (formula file Programme/AMOR2/settins.ini),
C     into the correct values at the zoom wheel of the Leica MZ6 microscope.
C     The method is by segment-wise linear interpolation (segments I
C     through VIII) as described in section 3 of Knappertsbusch (2008).
C     Fine calibration of AMOR2. MorphCol Supplement #7, 4 February, 2008.
C
C
C     Input is file INPUT (i.e. an ascii text file called list_of_files, which
```

```
C       AMOR2 generates and which contains the name of each image taken
C       together with the corresponding (AMOR2 calculated) magnification.
C       The format of this information is explained in the following example:
C
C                   3001r,1.90
C
C       The first 4 characters indicate the specimen number in that slide
C       (characters 1-2 are the field number, characters 3-4 are the specimen
C       [usually 01 when working with AMOR2]). The fifth character is a
C       lowercase r, indicating a raw C       file format (required by the program
C       Trace_AMOR1_batch.out). The sixth charcter is a comma (can be used
C       herein as a delimiter between specimen number and magnification).
C       The following four characters indicate the magnification as calculated
C       from AMOR2.
C
C
C       Output is file OUTPUT (the file containing the image file names and
C       corresponding corrected magnifications). The output file can the be
C       imported into program Trace_AMOR1_batch.out for outline extraction.
C
C       Variables:
C       X = Magnification returned by AMOR2
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 AMOR2               *'
        WRITE(9,*) '*                                *'
        WRITE(9,*) '* Version 1 from 2 February 2008  *'
        WRITE(9,*) '*    by Michael Knappertsbusch    *'
        WRITE(9,*) '*                                *'
        WRITE(9,*) '**********************************'
        WRITE(9,*) ' '
        WRITE(9,*) '. . .Enter the "list_of_files" from AMOR2. . .'
        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 #, MagAMOR2, 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
C                                                                    list_of_files
 10     READ(14,*,END=100) IMAGE,X
        N=N+1
C
C
        IF (X.LT.0.81) THEN                          ! [X < 0.81; segment I ]
          Y=1.0625*X-0.060625
        ELSE IF ((X.GE.0.81).AND.(X.LT.1.04)) THEN   ! [0.81 ≤ X < 1.04; II ]
          Y=0.8695652*X+0.0956521
        ELSE IF ((X.GE.1.04).AND.(X.LT.1.31)) THEN   ! [1.04 ≤ X < 1.31; III ]
          Y=0.9259259*X+0.037037
        ELSE IF ((X.GE.1.31).AND.(X.LT.1.63)) THEN   ! [1.31 ≤ X < 1.63; IV ]
          Y=1.1290323*X-0.2290322
        ELSE IF ((X.GE.1.63).AND.(X.LT.2.06)) THEN   ! [1.63 ≤ X < 2.06; V ]
          Y=0.9302325*X+0.0837209
        ELSE IF ((X.GE.2.06).AND.(X.LT.2.57)) THEN   ! [2.06 ≤ X < 2.57; VI ]
          Y=0.9803921*X-0.0196078
        ELSE IF ((X.GE.2.57).AND.(X.LT.3.26)) THEN   ! [2.57 ≤ X < 3.26; VII ]
          Y=1.0144928*X-0.1072463
        ELSE IF (X.GE.3.26) THEN                     ! [3.26 ≤ X; VIII ]
          Y=0.9090909*X+0.2363636
        END IF
C
C
C***    Output:
C
        WRITE(9,50) IMAGE,X,Y                              ! Output of results to
C                                                                        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
        STOP
        END
```

References

Stapfer. S. (2007). Automatisierter Vergrösserungswechsler (Auto-Zoom) und erweiterte Fokussierung für AMOR. Fachhochschule Nordwestschweiz. 44 p. Betreuung: Prof. Dr. J. Eisenecker.. FHNW Muttenz. Expertin: Frau M. Lefèbvre. Auftraggeber: PD M. Knappertsbusch.

Knappertsbusch. M. (2007a). Evaluation des Zoom-Motors (Diplomarbeit S. Stapfer. 2007). MorphCol/Supplement #6. 7 p.. dated from 19.11.2007 (unpublished pdf document). For example. an upper range motor stop needed to be installed. a smoother motor movement was needed. the range and conversion of motor-steps into a magnification needed to be corrected and refined. and the display of the actual magnification in the single mode. fine sensibility needed a correction.

Knappertsbusch. M. (2007b). Morphological variability of Globorotalia menardii (planktonic foraminifera) in two DSDP cores from the Caribbean Sea and the eastern Equatorial Pacific. Carnets de Geologie /Notebooks on Geology. Brest. Article 2007/04. URL http://paleopolis.rediris.es/CG2007_A04/index.html

Knappertsbusch. M. (2007c). Analysis of variation in shape and size due to repeated automatic positioning of a single microfossil into the same position using AMOR Stage V. MorphCol Supplement 5, 31.5.2007.