MorphCol Supplement 4

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3.1.1 Calibrations for Trace on different imaging systems

The imaging system with which the microfossils are recorded influences the quality of the digital images. In particular, the brand of the frame grabber (and so, the computer platform for which the framegrabber works), the video camera, and the optics used on the microscope affect the calibration curve, that is implemented in the Trace program for outline extraction. In the following, three different systems are tested:

1.) Imaging system is Macintosh based, using camera CF 11/2 from Kappa;
2.) Imaging system is AMOR (PC based), using camera CF 11/2 from Kappa;
3.) Imaging system is AMOR (PC based), using Sony camera DXC-390P.

The AMOR (**A**utomated **M**easurement system for m**OR**phometry of microfossils) system is a PC based orientation and imaging device for microfossils and will be described elswhere.
All three systems have different characteristics and provide different calibration curves, which are developed in the following sections. The calibration was done by repeated measurements of a standard ruler (1mm scale with 0.01mm subdivisions) in horozontal and vertical directions were performed, and the pixel readings were converted into micrometers by graphical correlation.

3.1.1.1 Macintosh based imaging system using CF11/2 camera from Kappa

Experimental setup:
Leica MZ6 binocular microscope.
Kappa CF 11/2 camera.
Cmount 1x.
Power Macintosh 8500 (built-in QuickTime framegrabber).
Nih-Image 1.61 software from Wayne Rasband.
Achromat 1x and 2x objectives.
Planapochromat 1x lobjective.

Different versions of Trace with different calibrations were developed, e.g.:

Trace32_batch.out:
(Calibrated using 1mm/0.01mm scale, using Achromat 1x and 2x objectives).

XPREC = 0.11829 * MAG + 0.00201 (in pixel/µm)
YPREC = 0.11792 * MAG + 0.00221 (in pixel/µm)

Trace33_batch.out, Trace34_batch.out, Trace35_batch.out and option 1 in Trace36_batch.out:
(Calibrated using the 0.01mm and the 0.1mm scale, using Achromat 1x and 2x objectives, and also tested with polarizer plate inserted in objective lense. This calibration is a newer one and replaces that used in Trace32_batch.out. For data on these calibrations see MorphCol Supl. #1).

XPREC = 0.11984 * MAG + 0.00048188 (in pixel/µm)
YPREC = 0.11926 * MAG + 0.00074223 (in pixel/µm)

Trace36_batch.out:
(Calibration as above in option 1, and for Planapo 1x objective in option 2):

For Achromat 1x or 2x objectives (=Option 1):
XPREC = 0.11984 * MAG + 0.00048188 (in pixel/µm)
YPREC = 0.11926 * MAG + 0.00074223 (in pixel/µm)

For Planapo 1x objective (=Option 2):
XPREC = 0.14823 * MAG - 0.00057757 (in pixel/µm)
YPREC = 0.14735 * MAG - 0.00051501 (in pixel/µm)

3.1.1.2 AMOR (PC) based imaging system using CF11/2 camera from Kappa

Experimental setup:
Leica MZ 6, Kappa CF 11/2 camera. Camera connected to "black box" and black box connected to framegrabber (using BNC connector at black box).
Camera settings (switches at back side of camera): Exposure fix or Auto, Video, white set manual.
Microscope-Camera connection: 1x Cmount.
Imaging system: PC, using AMOR program and IMAQ 1405 framegrabber from NI.
Calibration scale: 1mm scale with 0.01mm subdivisons.

Capturing images in program AMOR (single mode):
Images saved as tiff files, 72 dpi, all expanded to 640x480 pixels, and without any horizontal shift (shift 0 pixels from left border).

Optics used:
Achromat 1x and 2x objectives, measurements horizontally and vertically at every magnification.

Two types of illumination were checked:
1.) Folders /Achromat 1x, Horizontal, Pol/ and /Achromat 1x, Vertical, Pol/:
Achromat 1x objectives, measured horizontally and vertically, illumination with ring light from above, polarizer filter inserted.

2.) Folders /Achromat 1x, Horizontal/, /Achromat 1x, Vertical/, /Achromat 2x, Horizontal/ and /Achromat 2x, Vertical/: Illumination was transmitted light, e.g. the micrometer scale was placed on the mirror-holder (with a CD placed in between, so that the light came from beneath through the hole of the CD). No polarized light was applied here. This illumination provided better images at high and low magnifications.
In Table 4.1 the values of XPrec (horizontal) and YPrec (vertical) are given as a function of the magnification and shown in Figure 4.1.

These results show, that

a.) insertion of the polarizer plate in front of the bjective lense has no influence on the calibration (as was already demonstrated in the Mac based system.

b.) Calibration of AMOR + Kappa, using 1x and 2x objectives gives the following equations, that need to be implemented in the Trace program:

XPrec [pixel/µm] = 0.098274 * MAG + 0.00015617 r^2=1.000
YPrec [pixel/µm] = 0.11825 * MAG + 0.00054792 r^2=0.999

c.) The image quality using the Kappa camera on AMOR was not really good (low resolution).

Table 4.1:

Magnification (Reading at microscope)	XPrec (pixel/µm) (1x and 2x objective)	YPrec (pixel/µm) (1x or 2x objective)	Polarizer used
0,63	0,061	0,077	No
0,80	0,078	0,095	No
1,00	0,099	0,121	No
1,25	0,124	0,150	No
1,60	0,156	0,188	No
2,00	0,198	0,236	No
2,50	0,248	0,301	No
3,20	0,313	0,379	No
4,00	0,395	0,477	No
1,26	0,123	0,149	No
1,60	0,157	0,186	No
2,00	0,196	0,239	No
2,50	0,247	0,298	No
3,20	0,312	0,376	No
4,00	0,392	0,4737	No
5,00	0,495	0,5987	No
6,40	0,624	0,755	No
8,00	0,788	0,9511	No
0,63	0,062	0,073	Yes
0,80	0,078	0,094	Yes
1,00	0,099	0,119	Yes
1,25	0,125	0,151	Yes
1,60	0,156	0,190	Yes
2,00	0,197	0,239	Yes
2,50	0,248	0,301	Yes
3,20	0,313	0,377	Yes
4,00	0,395	0,452	Yes

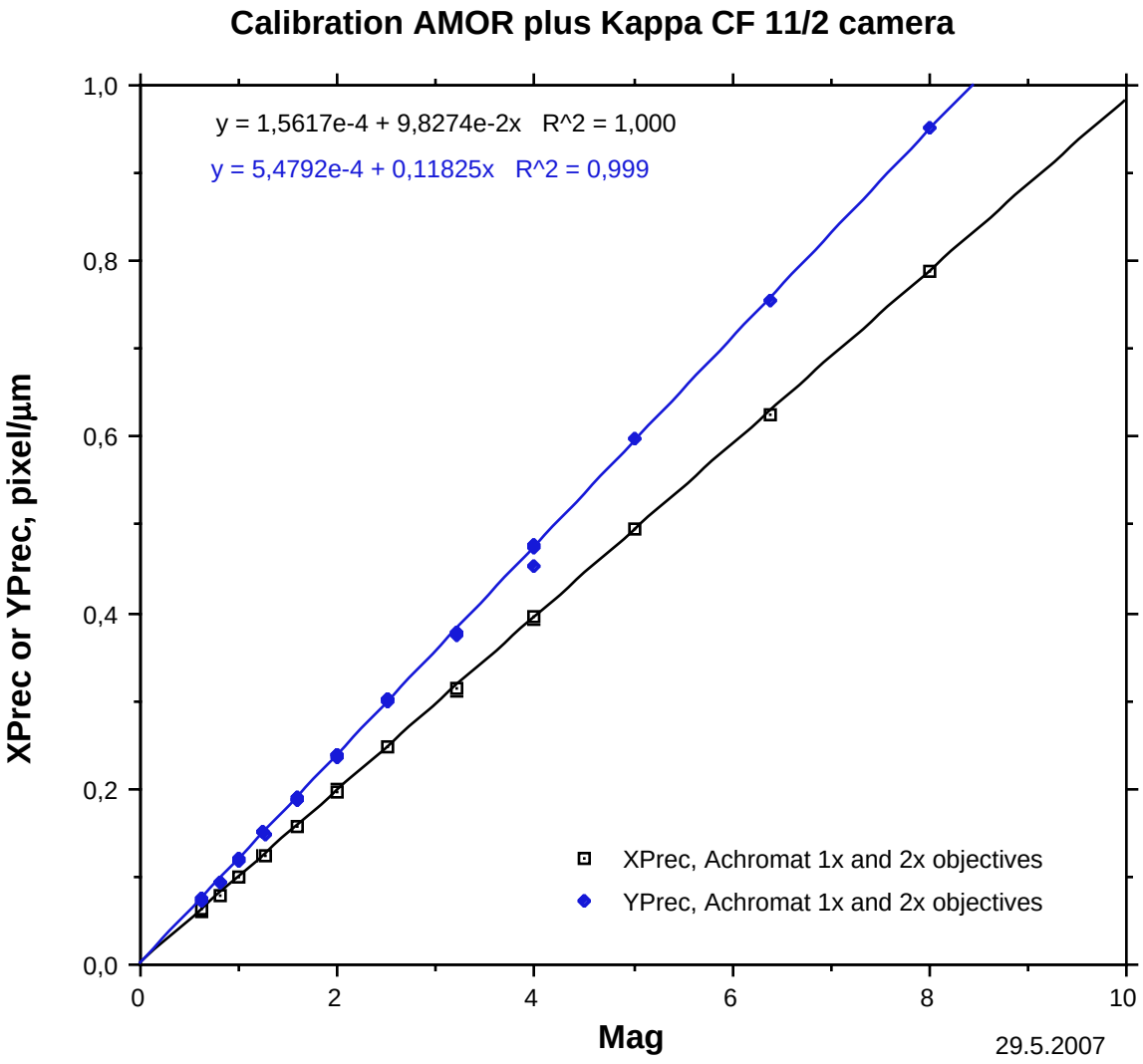


Figure 4.1

3.1.1.3 AMOR (PC) based imaging system using Sony DXC-390P camera

Experimental setup:

AMOR System using Leica MZ 6 binocular microscope, Sony DXC-390P camera, frame grabber used is IMAQ 1405 from National Instruments, Cmount 1x, Ring-Illumination, Pol filter inserted, PC, Monitor from Hitachi, Program AMOR, calibration scale 1mm, subdivisions 0.01 mm.

Images saved as Tiff images, 640x480 pixels (using expand to 640x480 pixels option in AMOR). Software used for reading pixel values in images: Nih-Image 1.62b 7

Objectives	Zoom settings
Achromat 1x,	0.63x, 0.8x, 1.0x, 1.25x, 1.6x, 2.0x, 2.5x, 3.2x, 4.0x
Achromat 2x,	0.63x, 0.8x, 1.0x, 1.25x, 1.6x, 2.0x, 2.5x, 3.2x, 4.0x
Planapo 1x,	0.63x, 0.8x, 1.0x, 1.25x, 1.6x, 2.0x, 2.5x, 3.2x, 4.0x

All measurements were done in horizontal (XPrec, in pixel/µm) and vertical (YPrec, in pixel/µm) direction (see Table 4.2). The resulting correlation lines are shown in Figure 4.2.

Results:

a.) Calibration of AMOR + Sony, using 1x and 2x objectives gives the following equations, that need to be implemented in the Trace program:

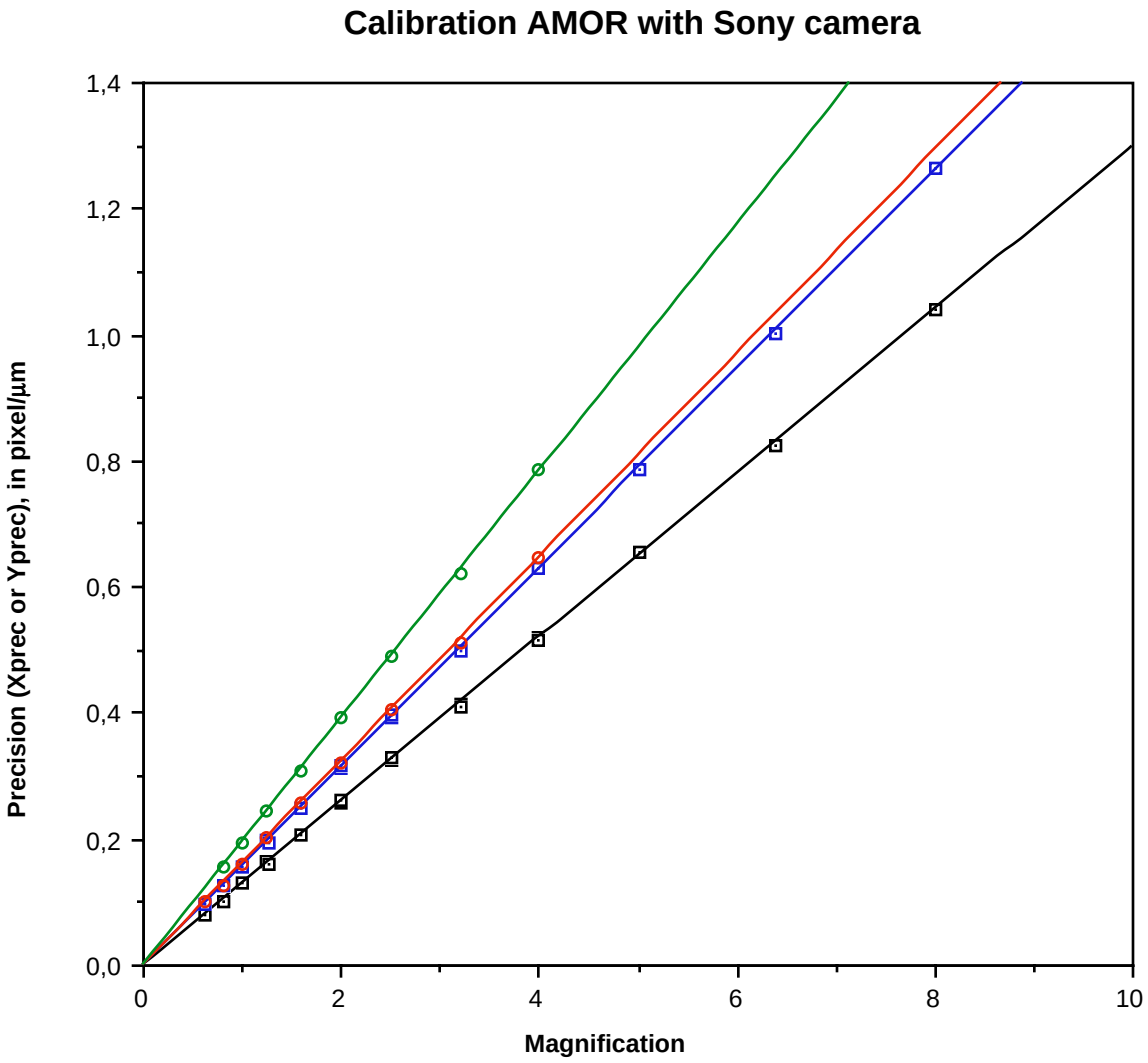
XPrec [pixel/µm] = 0.12986 * MAG - 0.00022300 r^2=1.000
YPrec [pixel/µm] = 0.15742 * MAG - 0.00023756 r^2=1.000

b.) Calibration of AMOR + Sony, using the 1x Planapo objective gives the following equations, that need to be implemented in the Trace program:

XPrec [pixel/µm] = 0.16153 * MAG - 0.00011242 r^2=1.000
YPrec [pixel/µm] = 0.19574 * MAG - 0.00047040 r^2=1.000

Table 4.2

Magnification	XPrec (Achromat 1x&2x)	YPrec (Achromat 1x&2x)	Prec (Planapo 1x)	YPrec (Planapo 1x)
0,6300	0,0810	0,0988	0,1010	
0,8000	0,1030	0,1270	0,1280	0,1570
1,0000	0,1300	0,1580	0,1620	0,1940
1,2500	0,1640	0,1980	0,2040	0,2460
1,6000	0,2070	0,2500	0,2570	0,3100
2,0000	0,2610	0,3170	0,3230	0,3930
2,5000	0,3300	0,3980	0,4070	0,4911
3,2000	0,4150	0,5000	0,5120	0,6200
4,0000	0,5220	0,6314	0,6478	0,7855
1,2600	0,1620	0,1960		
1,6000	0,2060	0,2500		
2,0000	0,2600	0,3140		
2,5000	0,3270	0,3950		
3,2000	0,4120	0,5000		
4,0000	0,5180	0,6286		
5,0000	0,6567	0,7880		
6,4000	0,8243	1,0022		
8,0000	1,0400	1,2629		



Achromat 1x or 2x, horizontal (X)
y = 2,2300e-4 + 0,12986x R^2 = 1,000

Achromat 1x or 2x, vertical (Y)
y = - 2,3756e-4 + 0,15742x R^2 = 1,000

Planapochromat 1x, horizontal (X)
y = - 1,1242e-4 + 0,16153x R^2 = 1,000

Planapochromat 1x, vertical (Y)
y = - 4,7040e-4 + 0,19574x R^2 = 1,000

Figure 4.2

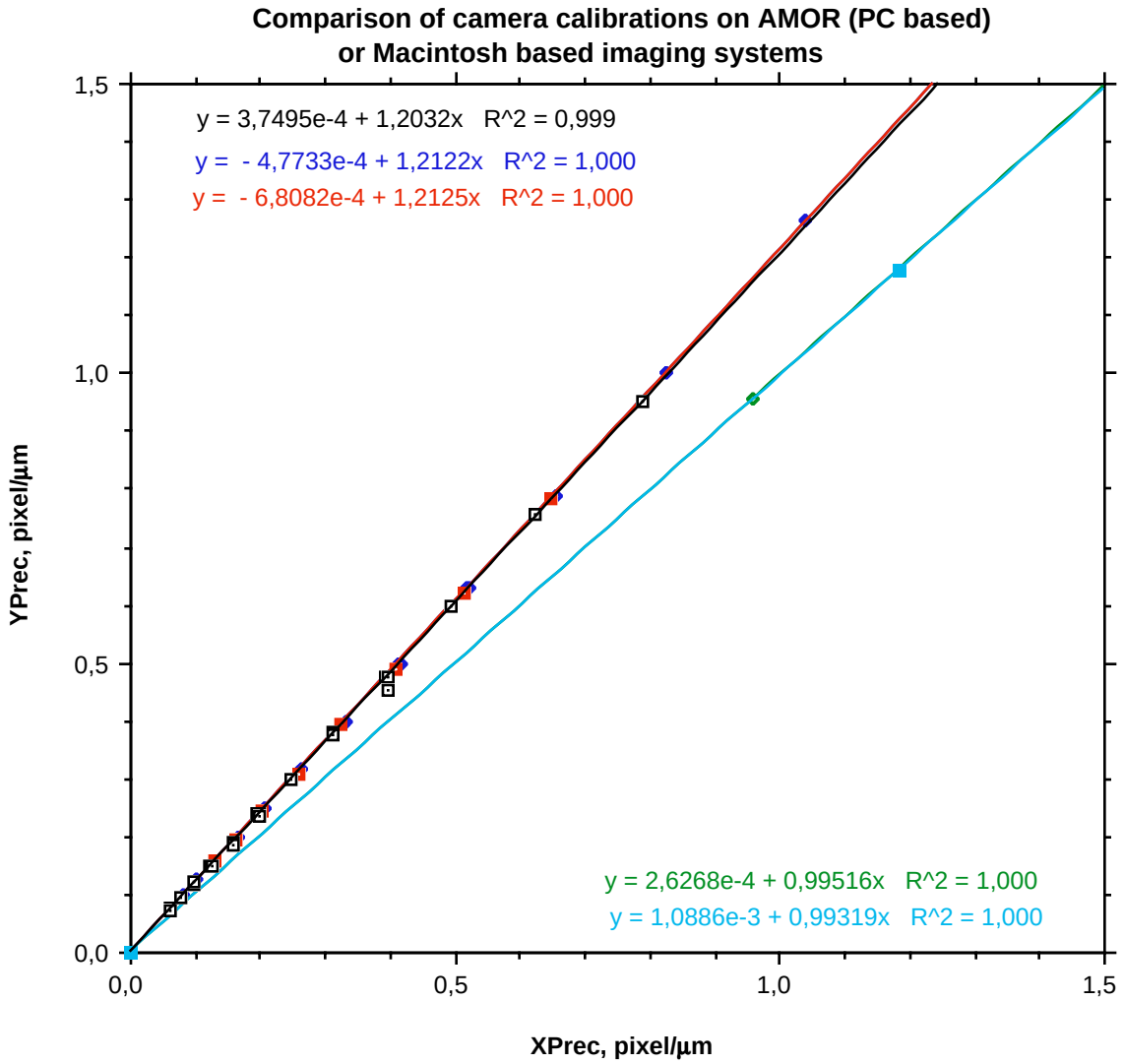

Comparison of camera calibrations on AMOR (PC based)
or Macintosh based imaging systems
y = 3,7495e-4 + 1,2032x R^2 = 0,999
y = - 4,7733e-4 + 1,2122x R^2 = 1,000
y = - 6,8082e-4 + 1,2125x R^2 = 1,000
y = 2,6268e-4 + 0,99516x R^2 = 1,000
y = 1,0886e-3 + 0,99319x R^2 = 1,000
YPrec, pixel/µm
XPrec, pixel/µm
0,0
0,5
1,0
1,5
PC using IMAQ 1405
framegrabber from NI
AMOR+Kappa camera, Achromat 1x+2x
AMOR+Sony camera, Planapo
AMOR+Sony camera, Achromat 1x+2x
Mac using builtin
QuickTime framegrabber
Mac+Kappa, Achromat 1x+2x
Mac+Kappa, Planapo
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Figure 4.3

Conclusions from the calibration experiment

1.) The Kappa CF11/2 camera works also with the AMOR based system.
2.) The Kappa camera on the AMOR system results in lower resolution images. Therefore, better use the Sony camera together with AMOR for imaging an measuring microfossils.
3.) When using AMOR both, the Kappa and Sony cameras end in distorted images (circle becomes an oval). The distortion (ratio of YPrec/XPrec=1.21) is almost identical with both cameras (see Figure 4.3).
4.) On the Macintosh based system the distortion of the image is much less (YPrec/XPrec=0.993, see Figure 4.3), which is close to 1 (no distortion).
5.) The above observations demonstrate, that the framegrabber used in AMOR is responsible for the strong distortion on AMOR system.
6.) When measuring microfossils with the AMOR based system, it is recommended to use the Sony camera.
7.) Using the Sony camera on AMOR the distortion must be compensated for by implementing new equations in Trace:

For Achromat 1x and 2x objectives:

XPrec = 0.12986 * MAG + 0.000223 r^2=1.000

YPrec = 0.15742 * MAG - 0.00023756 r^2=1.000

For the Planapo 1x objective:

XPrec = 0.16153 * MAG - 0.00011242 r^2 = 1.000

YPrec = 0.19574 * MAG - 0.0004704 r^2 = 1.000