Supplement #13 to MorphCol:

Improvement of Autofocus for AMOR

By Michael Knappertsbusch, 27 April 2009

1.) Introduction and problem.

The autofocus of AMOR was originally implemented by Herzig and Schmutz (2007). Tests have shown, that the autofocus operated only well in the case of large specimens. With smaller specimens most specimens could not be automatically focused. It was only later discovered, that focus and tilt operations did not sufficiently cooperate: During pitch and roll movements there was no interspersed focus and with every further tilt movement the specimen moeved further out of the focal plane. The result were unsharp images during automatic orientation of the specimen (specimens in this version were only seen in the red LUT image window, so that the defocused state was not easily recognized). This failure was less severe in large specimens but it led to notorious misfunctioning with small specimens in the automatic mode. Autotilt has also led to misfunctioning in the single-specimen mode at higher magnifications. In addition, during roll movement (=left right tilting) the specimen remained longer in focus than during pitch movement (=backwards-forwards tilting), which makes a correction unavoidable especially for the pitch movement. The reason is because of the slight deviation of the pitch axis from a true eucentric axis. This misfunctioning of the autofocus during the autotilt cycles resulted in poor efficiency and even caused the entire program to freeze, especially if the burred specimen started to touch the border of the imaging frame.

2.) Correction of the autofocus and autotilt of AMOR:

2.1.) Influence of the tilt or roll movement on the sharpness of the image

In order to test the defocussing-effect during tilting operations, the stage was tilted in single specimen mode using mouse clicks. During Pitch movement 15 mouse clicks were necessary for a tilt by 22.5°. During Roll movement 15 mouseclicks caused a tilt by 22.5°. In both cases sensibility was set to ”normal”. In summary, one mouseclick in sensibility=”normal” results in a pitch or roll movement by 1.5°.
Note: Sensibility=”fine” are 10 motorsteps, sensibility=”normal” are 100 motorsteps; this applies to the pitch and roll movenet and also for the autofocus.

A defocusing-experiment was then carried out using slide Downwind HG-82, 4cm, Slide a (code DWHG82_4_aK), using first a large menardiform specimen (field #16) and then a small specimen (field #43). For each setting of magnification Pitch- and Roll movements were activated using mouse clicks in single specimen mode and with sensibility set to ”normal” or “fine”. The number of mouse-clicks were recorded until the specimen started to become out of focus (see table I and II below).

Table I: Protocol for the large specimen (field #16)

Mag (reading in AMOR)	Number of mouseclicks Pitch	Number of mouseclicks Roll
0.63x	16 (sensibility=”normal”)	20 (sensibility=”normal”)
0.79x	9 (sensibility=”normal”)	15 (sensibility=”normal”)
1.00x	6 (sensibility=”normal”)	12 (sensibility=”normal”)
1.25x	4 (sensibility=”normal”)	6 (sensibility=”normal”)
1.57x	3 (sensibility=”normal”)	3 (sensibility=”normal”)
1.98x	2 (sensibility=”normal”)	3 (sensibility=”normal”)

Table II: Protocol for the small specimen (field #43)

Mag (reading in AMOR)	Number of mouseclicks Pitch	Number of mouseclicks Roll
1.98x	2 (sensibility=”normal”)	1 (”normal”), 6 (“fine”)
2.49x	2 (”normal”)	1 (”normal”), 6 (“fine”)
3.13x	1 (”normal”), 6 (“fine”)	1 (”normal”), 4 (“fine”)
3.89x	1 (”normal”), 4 (“fine”)	1 (”normal”), 10 (“fine”)
3.93x	1 (”normal”), 4 (“fine”)	1 (”normal”), 11 (“fine”)

Thus, using a large specimen at magnification set to 1.00x ended in a 6 * 1.5°=9° pitch movement until the specimen became unsharp and in a 12 * 1.5° = 18° roll movement until the specimen became unsharp.

2.2.) Improvement of autotilt:

In order to eliminate the misfunctioning AMOR was modified to version AMOR 3.2. This was done by Richard Schorpp at FHNW Brugg/Windisch. In version 3.2 a separate autofocus cycle was implemented after each pitch- and roll interval. Because every autofocus cycle was the followed by a complete focus scan of the specimen, the entire process became very long. The modification, however, resulted in improved behaviour and images were mostly in focus, even with small specimens. In order to accelerate autotilt, the autofocus and center functions are only activated when the tilting (pitch or roll) angles are larger than 1 degree. This eliminates unnecessary autofocus loops at very small pitch and roll movements, where focussing is no longer necessary (see experiments described under section 2.1).

In the original version of Herzig and Schmutz (2007) Autotilt is programmed so that the first tilt movement realizes 500 motorsteps. During the following intervals always half of the previous number of steps are realized, i.e. 250 steps, then 125 steps, then 62 steps, 31 steps, 15 steps, 7 steps, etc. If the increment is less than 10 steps tilting stops (7 steps and less are no longer realized). Experimentation with AMOR in single specimen mode has shown, that pitch and roll movements of less than 62 steps do no longer influence the focus of the image. Therefore, in the modified version of AMOR (version 3.2) autofocus and autocenter functions are no longer applied if the tilt movement is less than 62 steps.

3. Evaluation of the ExtendFocus function in AMOR

The ExtendFocus function was implemented by Sebastian Stapfer (2008). While in principle this function works, it does not (yet) produce sufficient image quality: Very often the function gives somewhat defocused images. Richard Schorpp gives a good summary of the subprogram, as follows:

Movement away from the focused image:
- 250 steps focus vertically upwards (image completely out of focus).
9 images taken at every -50 steps downwards until position -200.
Launching of program CombineZ5 and generation of an extended focus image.

The focus motor of the microscope has a resolution of 1000 steps per mm (1 step=1 micrometer). However, it is not clear how many motorsteps are moved when activated by mouse-click. In order to evaluate the vertical steps per single mouse-click in AMOR a NaCl crystal was mounted in a slide and was used to measure the length of each vertical step per mouseclick. The height of the crystal was first measured by imaging the crystal with AMOR in side-view at a magnification of 0.79x (reading in AMOR), see Figure 1. Pixels were converted into micrometers applying first the necessary post-correction with MagCorr2.f and then convertion of pixels into micrometers as implemented in Trace_AMOR1_batch.f. The crystal was then turned in upside position, placed in the stage and focused at the top plane of the crystal (magnification set to 1.98x). The microscope was then lowered using mouse-clicks until the bottom of the slide appeared in focus. These operations were done in single specimen mode and a sensibility=”fine”. For the height difference of 3142.8 micrometers of the crystal 31 mouse clicks were applied, which results in a resolution of 101.4 micrometers per mouseclick during sensibility=”fine” (corresponds to 10 motorsteps).

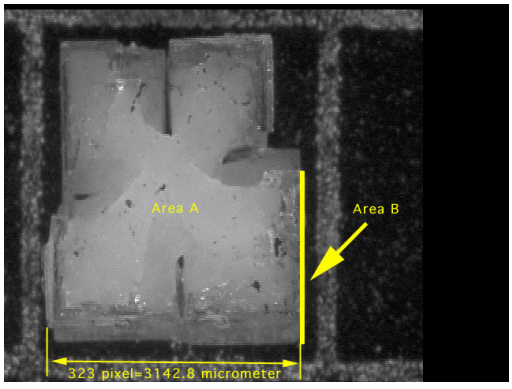

Figure 1. NaCl crystal in side view at magnification of 0.79x (reading in AMOR). The length of the crystal (at the basis, in x-direction) is 323 pixels, which corresponds to 3142.8 micrometers. Area B is facing to the right in this perspective. The image size is 640x480 pixels.

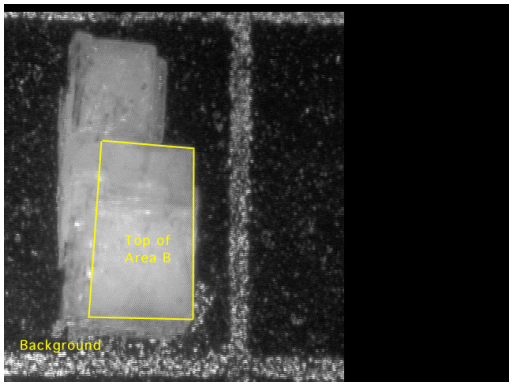

Figure 2. Same NaCl crystal as illustrated in Figure 1, but in upright position. The area B (uppermost crystal facet) shown in Figure 1 is now directed upwards. The vertical distance from the bottom of the slide to area B measures 3142.8 micrometers. To move the focus from the bottom to the top of the crystal required 31 mouse clicks in AMOR (single measurement mode, sensibility=”fine”). The image size is 640x480 pixels.

4.) Summary

The corrected program AMOR 3.2 was supplied on DVD and could easily be installed on our machine. Until present, the new version has a much better performance, and the program becomes no longer freezed at every other occasion. Implementation of a user-defined magnification during Autotilt will further improve the performance, especially for small specimens.