



July 5, 2010

ImageFocus/CMEX Calibration

*This document supersedes all previous versions of this topic*

## Introduction

In order to perform accurate measurements, all errors caused by aberrations from all optical elements and CMOS camera sensor must be eliminated. Therefore the CMEX camera and microscope must be calibrated with the ImageFocus 2.5 program at least once at installation, prior to any measurement



It's strongly recommended to verify periodically the accuracy of the calibration



*For the calibration procedure you need:*

- a micrometer slide like the AE.1110 (1mm/100) or AE.1111 (2mm/100) for calibration of compound microscopes
- an AE.1112 (50mm/500) micrometer slide for stereomicroscopes
- ImageFocus software v2.5 and camera drivers installed

## Calibration procedure

- 1- Install the CMEX camera on the microscope

*It's preferable to use the phototube of the microscope*

*If a phototube is not available, remove a eyepiece and insert the CMEX camera*

- 2- Plug the USB plug of the CMEX camera into a free USB-2 port of the computer

- 3- Position the slide micrometer under the objective with the lowest magnification

For compound microscopes (biological or material microscopes), use the AE.1110 (1mm/100) or AE.1111 (2mm/100) slide micrometer ( 10  $\mu$ m divisions )

For stereo microscopes - with lower magnifications -, use the AE.1112 (50mm/500) slide micrometer ( 100  $\mu$ m division )

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-4- Adjust focus till you obtained a sharp image of the micrometer scale

-5 - Launch the ImageFocus software



-6- Click the selection button (button with 3 dots) in the right upper corner of the software.

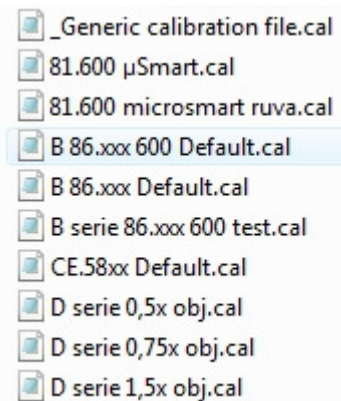
Calibration file:  ... Camera: CMEX 1300x

Select from the list:

- a preconfigured calibration file by Euromex microscope model e.g. "B 86.xxx Default" for a standard Novex B serie microscope

or

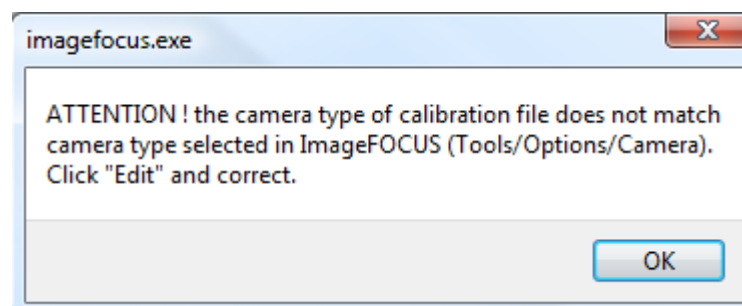
- the universal \_Generic calibration file.cal



- \_Generic calibration file.cal
- 81.600 µSmart.cal
- 81.600 microsmart ruva.cal
- B 86.xxx 600 Default.cal
- B 86.xxx Default.cal
- B serie 86.xxx 600 test.cal
- CE.58xx Default.cal
- D serie 0,5x obj.cal
- D serie 0,75x obj.cal
- D serie 1,5x obj.cal



Remark. It could be that the following warning message appears, just click OK

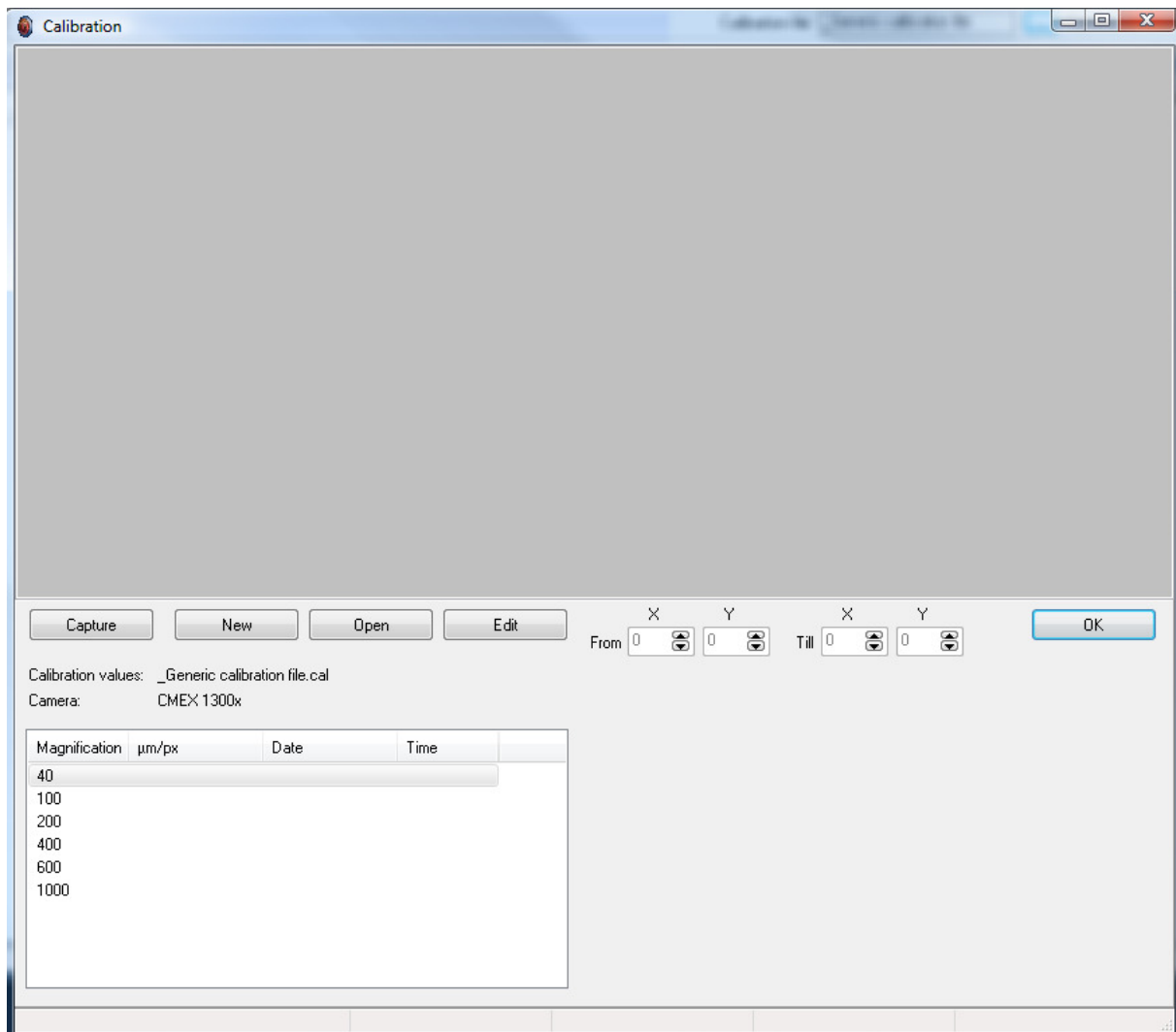


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-7- Click from the menu TOOLS / CALIBRATION.

The calibration window appears:



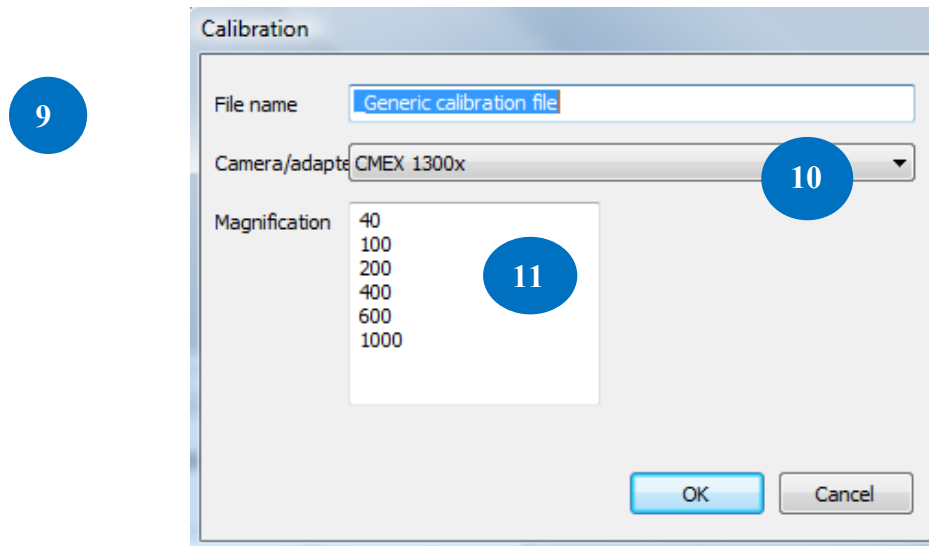
The "Edit" button allows you to change the magnification list of the calibration file

The "Open" button allows you to edit a previously saved calibration file

The "New" button allows you to create a new calibration file

Click on the OK button to save the calibration file with current settings

- 8- Click on the  icon



- 9- Rename the calibration file with a suitable name, e.g. "Lab microscope"

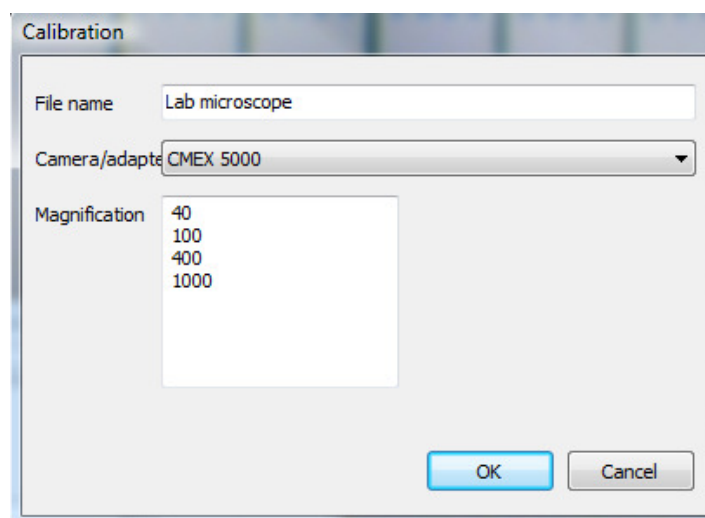
*If you opened a previously saved calibration file for modifying, you don't need to rename the calibration file.*

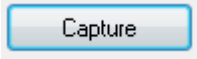
- 10- Select the camera used from the listbox (e.g CMEX 1300x)

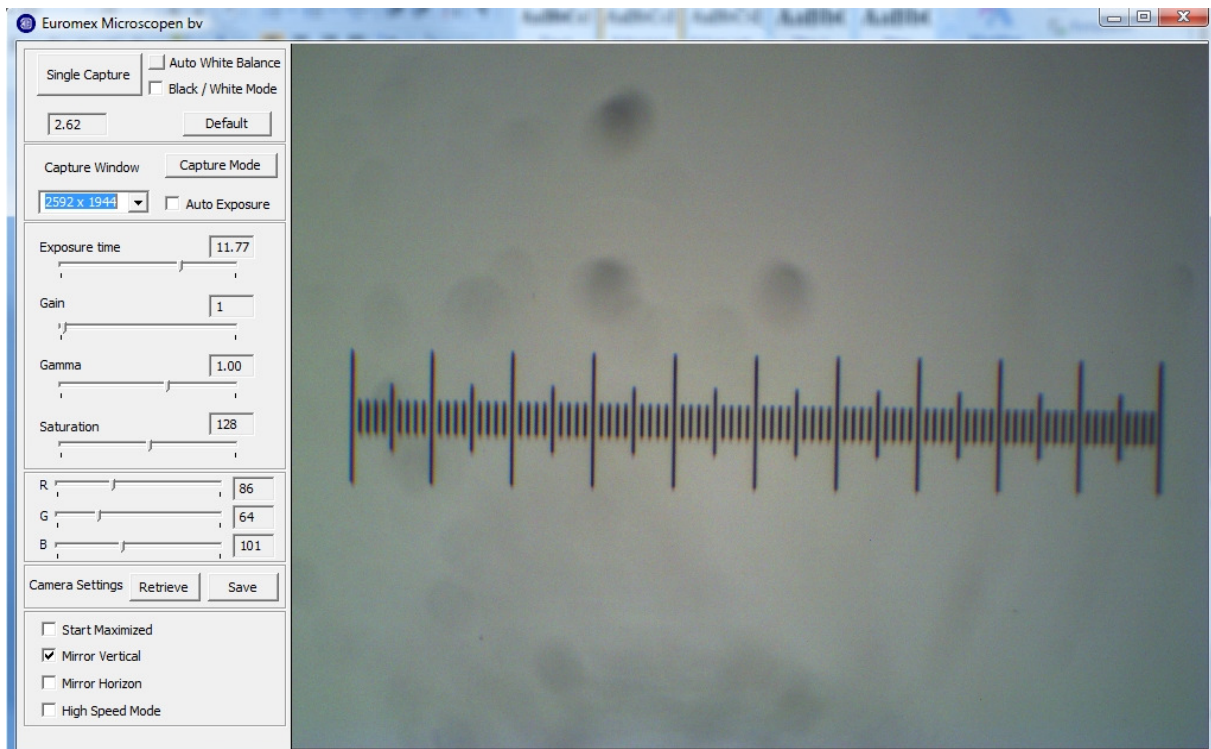


Verify the available magnifications of your microscope

- 11- Add / Remove magnification(s) from the list by clicking with the mouse in the box and make the changes as necessary, e.g. 40 100 400 and 1000x



- 12- Click the OK button to save the calibration file
- 13- Position a objective to calibrate, e.g. the 10x magnification objective
- 14- Click the  button to have live images of the calibration slide



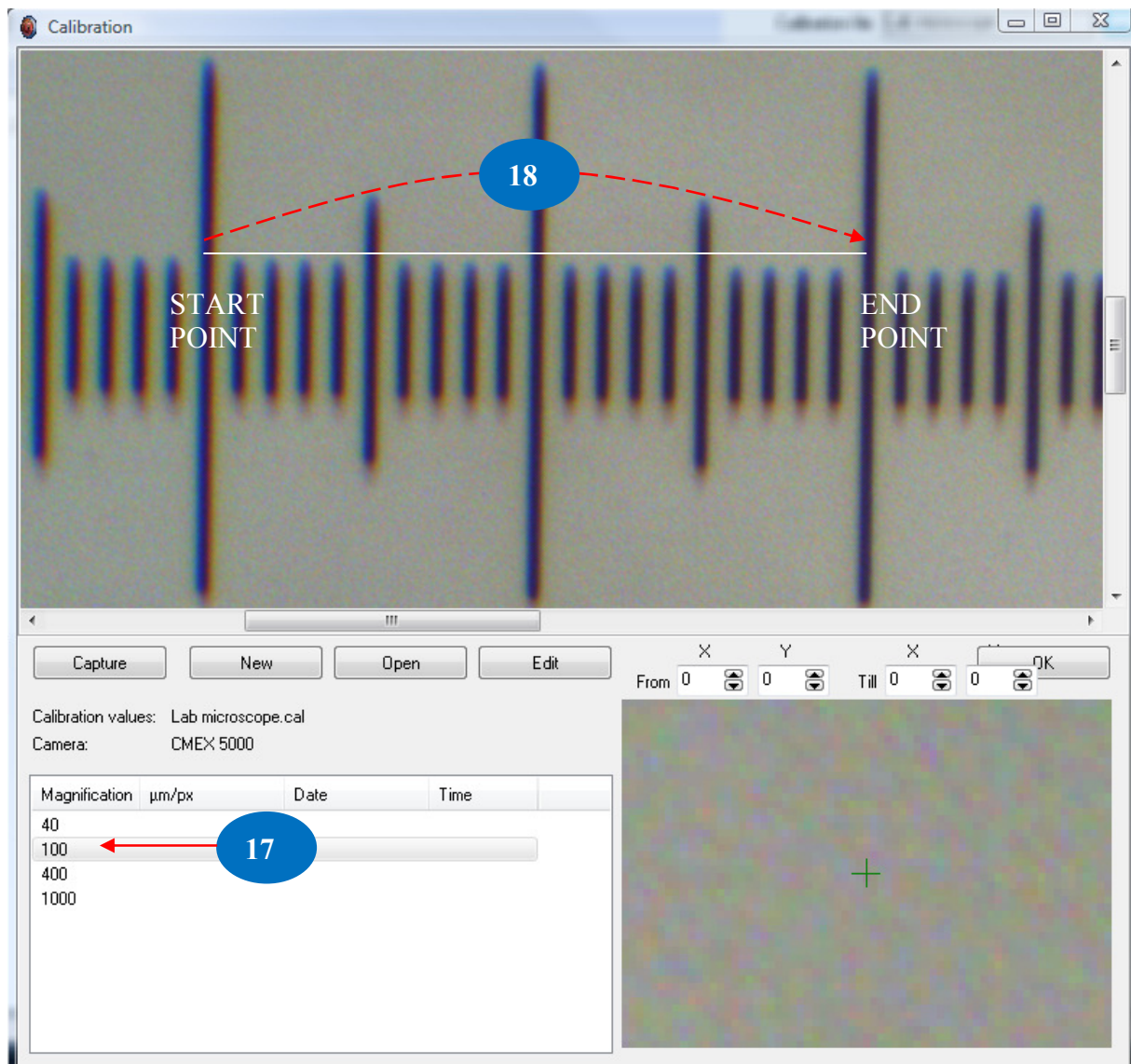
- 14- Adjust camera settings and focusing in order to have a sharpest image as possible
- 15- Adjust camera settings and focusing in order to have a sharpest image as possible
- 16- Click "Single capture" to take a image of the micrometer slide

The image of the micrometer slide will appear in the calibration screen

Use the horizontal and vertical sliders to position the micrometer slide in the middle  
Of the calibration screen



- 17- Select from the list the corresponding magnification to the objective selected, e.g. 10x objective → 100x magnification



- 18- Select a start point, hold right mouse button across the micrometer ruler, than release at a chosen end point (a multiple of divisions as large as possible). Keep the traced line along the micrometer scale. You can Modify start and End points by clicking the "From XY" and "Till XY" controls. The points will be adjusted.

*The left bottom screen shows a detailed view of the mouse pointer in order to help selecting the Start and End points*

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- 19-** Count the number of divisions selected and multiply with the scale corresponding to the micrometer slide uses

Distance in  $\mu\text{m}$ :

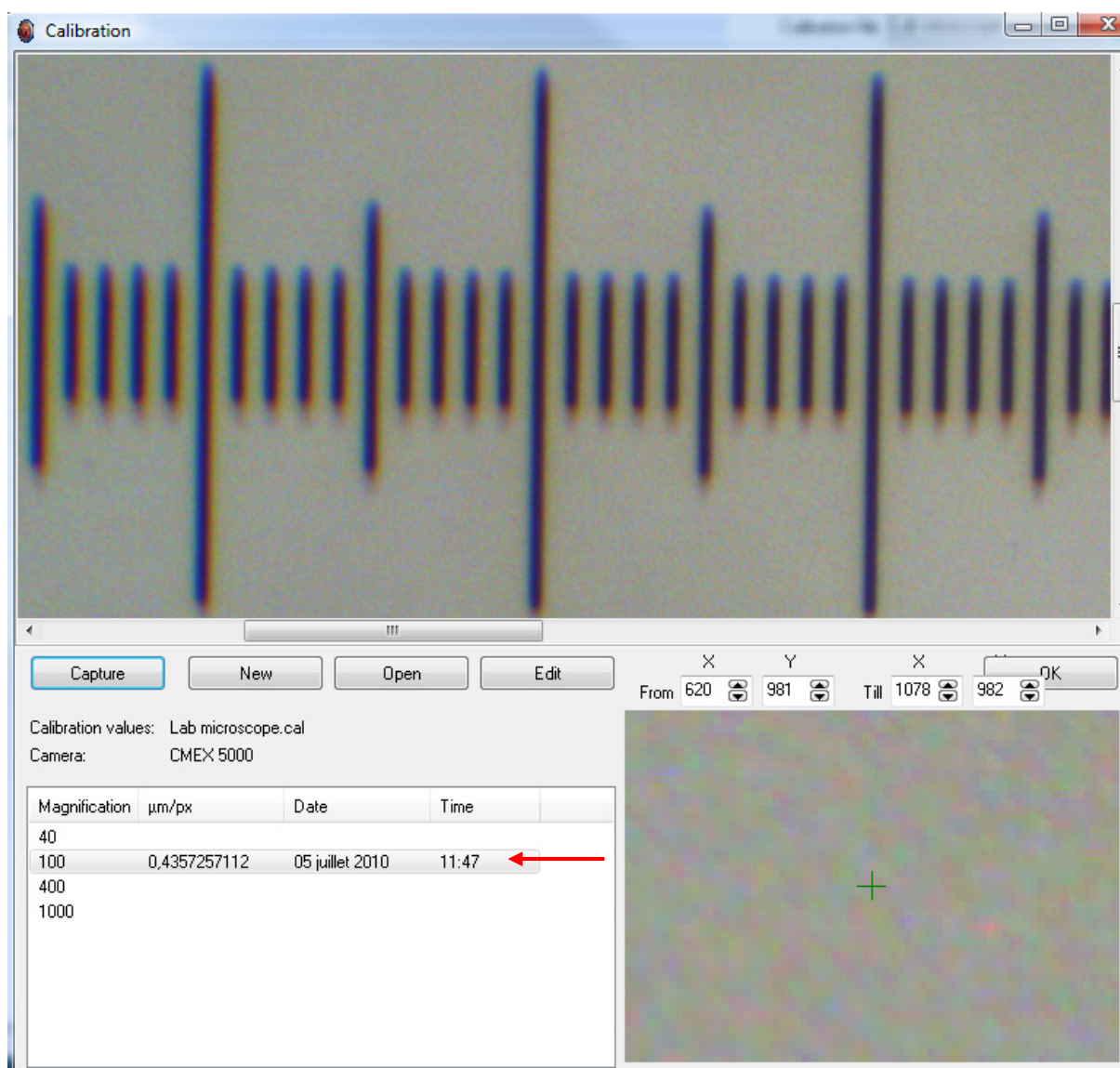


Reminder:

AE.1110 (1mm/100) = 10  $\mu\text{m}$  per division, AE.1111 (2mm/100) = 10  $\mu\text{m}$  per division  
and AE.1112 (50mm/500) = 100  $\mu\text{m}$  per division )

Enter this number in the dialog box and press OK (e.g. 20 divisions  $\times$  10  $\mu\text{m}$  = 200  $\mu\text{m}$ )

The calibration value (e.g. 0.4357..  $\mu\text{m}/\text{pixel}$ ) will appear in the calibration screen



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- 20- Repeat the calibration from steps 14 to 19 and verify if listed calibration values of of selected magnification stay stable

Magnifica...	µm/px	Date	Time
40	2,6702245894	25 maart 2008	19:46
100			
400			
1000			

Magnifica...	µm/px	Date	Time
40	2,673794402	25 maart 2008	19:50
100			
400			
1000			



*with a correct executed calibration the difference between two calibrations values should not exceed 1/10 µm*

- 20- Calibrate all objectives that need calibration by repeating steps 13 to 19
- 22- Save the calibration file by clicking the OK button