Method of Procedure

Nanoro Manual Calibration

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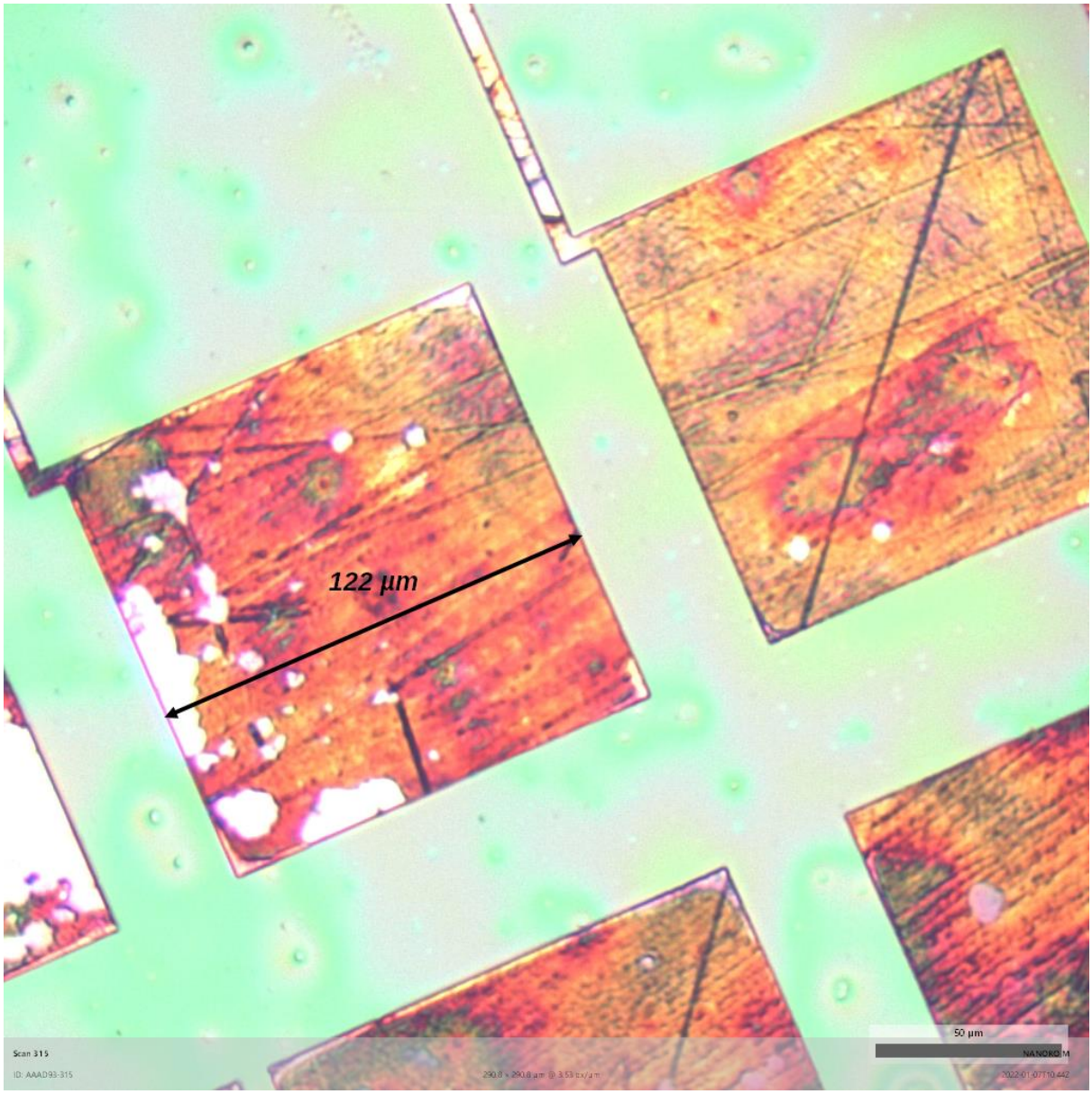
Illustration

Microscopes can be used for metrology (i.e. measuring), but need to be calibrated first, either by using an accurately calibrated lens, or by calibration performed using the lens in situ. The distance below is estimated by the software to be 122 µm. However the accuracy of the measurement will depend on the accuracy of the calibration. Calibration is the theme covered here.



Quick guide to manual scale calibration

This is a quick guide to the steps required for manual scale calibration using a calibrated slide. You may want to skip past this on first reading, for an introduction and detailed explanation, and for alternative uses of this calibration feature, e.g. for camera angle calibration.

Manual Scale Calibration Icon

1. Open the manual scale calibration dialog box
    a. From the menus: Lens – Manual Scale, or
    b. By clicking on the icon in the Lens Panel
2. With a calibration slide under the microscope click on the button “use Calibrated Sample” in the “setup” tab.
3. With an image on screen containing the two calibration points with known deparation, click “CAPTURE IMAGE” IN THE Capture tab.
4. In the “Point 1” tab, click on the first point. Its coordinates should be shown.
    a. Note that it is more precise if you click “EXPAND” and select the point that way, see detailed description later for details.
5. In the “Point 2” tab, click on the second point. Its coordinates should be shown.
    a. As above
6. In the Lens Magnification tab, enter the calibrated distance between the points.
7. Still in the Lens Magnification tab, confirm the value entered by clicking “OK”.
8. Note: values are backed up when the program closes. You may want to do that now.

Why manual calibration?

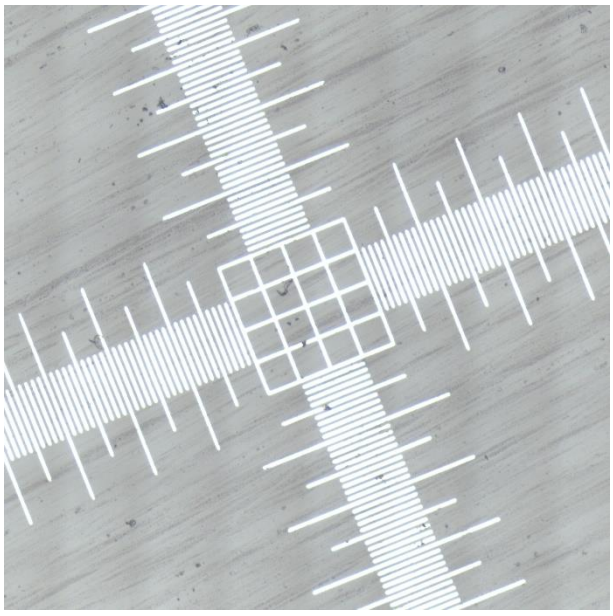
Nanoro-M is a microscope designed for large area scanning of flat surfaces. Since its introduction, its Super-resolution Microsphere Amplifying Lens (SMAL) has been adapted for use in other microscopes and development of a bio-imaging microscope is underway.

Nanoro-M uses an accurate robotic X-Y stage for positioning and the accuracy of this stage is also used as part of the calibration process. However, the possibility of using a manual X-Y stage or a less accurate (but cheaper) X-Y stage, means an alternative method of calibration is desirable.

A common method of calibrating microscopes is to use a calibration slide (or sample), see the illustration below. This doesn't require an accurate robotic X-Y stage. This document describes software introduced to calibrate

1. the camera angle, and
2. the scale factor of a lens

without necessarily needing an accurate robotic X-Y stage.

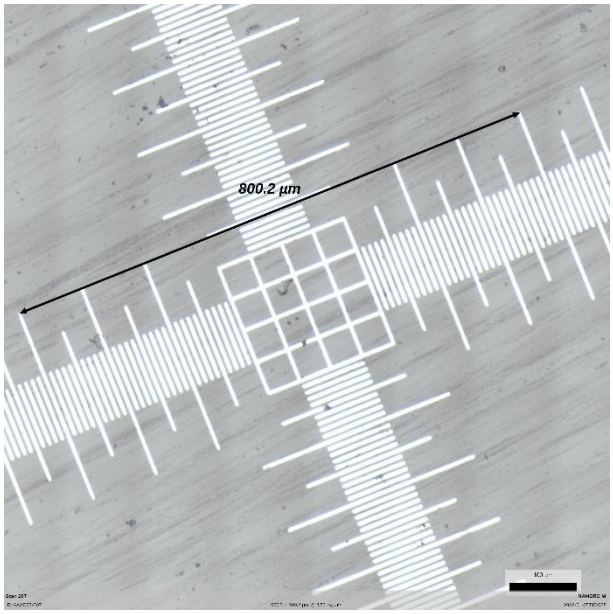


A calibration slide viewed under a microscope

Scale Calibration Introduction

In the text below, note the repeated referral to ***two image points*** in the different calibrations.

Method 1: Calibrated slide.



Calibration process

The calibration process is fundamentally as simple as clicking on ***two points in an image*** and typing in the known separation between the two points.

Clicking the points determines the pixel coordinates of each point and the length of the vector between the two points gives the pixel distance between them. The calibration scale is used to determine physical distance on the sample. For example with the slide shown, the major lines are 100 µm apart and the chosen points are therefore 800 µm apart.

Method 2: Calibrated dial.

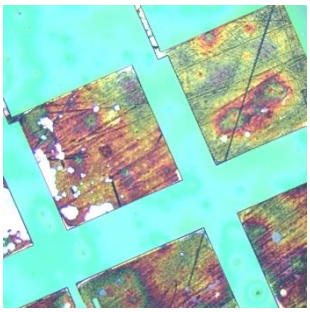
Calibrated dial

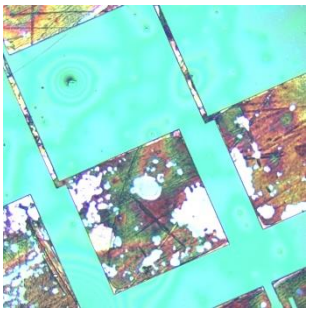
A microscope may have calibrated dials for the X and Y displacements.

Rather than knowing the distance between two points on a sample, the known distance when turning the dial, is between a point on the slide/sample before and after movement: where the point was and where the point moves to.

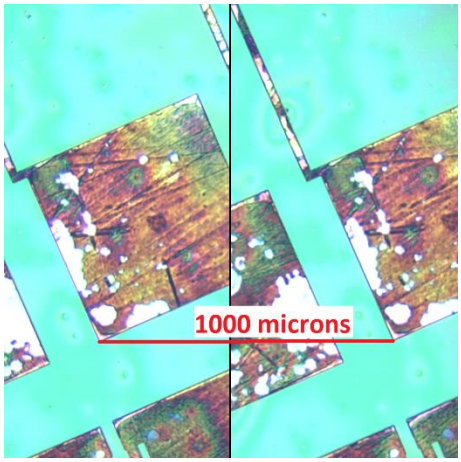
Again, the calibration process is fundamentally as simple as clicking on ***two points in images*** and typing in the known separation between the two points. The known separation is the distance given by how for the dial is turned.

For example, suppose the two images below are captured before and after moving an X distance of 1000 microns.





Then clicking on matching points in each image and typing in the distance of 1000, allows us to calibrate the scale. This is best illustrated by combining the images, as shown below.



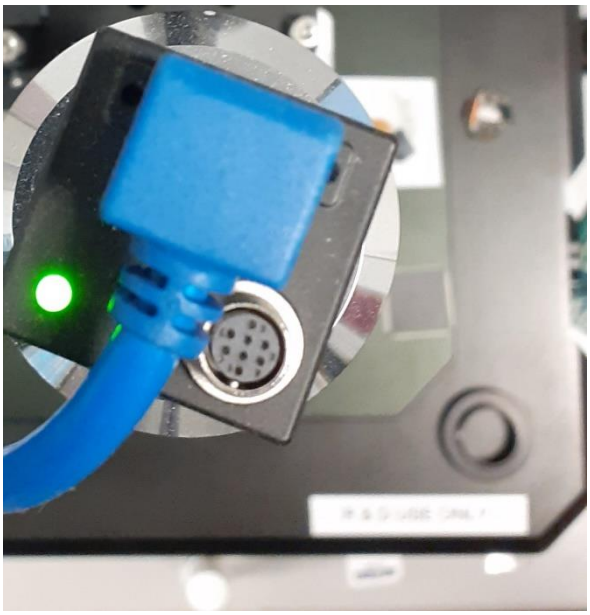
Camera Calibration Introduction

The camera angle is the clockwise rotation needed on the camera to straighten it.

Equivalently …

The camera angle is the amount of anti-clockwise rotation if currently has from straight.

So in the image below the camera angle is approximately -20 °.

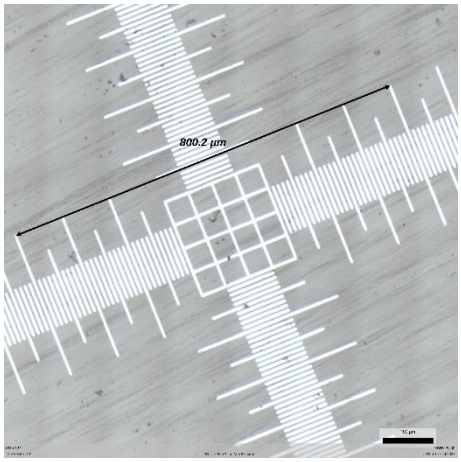


Camera Angle

In the previous example, the X-Y stage was moved along the X axis, and the image translated perfectly along the X axis too.

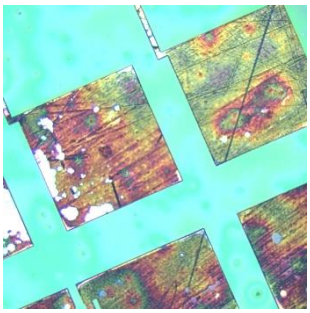
However, the X axis of the X-Y stage and the axis of the camera are not necessarily in perfect alignment. The discrepancy is known as the camera angle. The camera is in blue above.

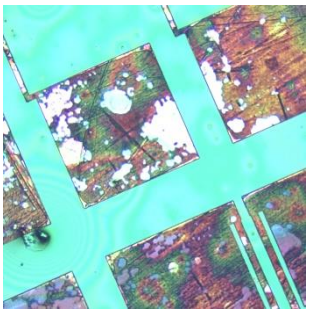
Note that a camera twisted clockwise will make the image appear rotated anticlockwise, and vice versa.



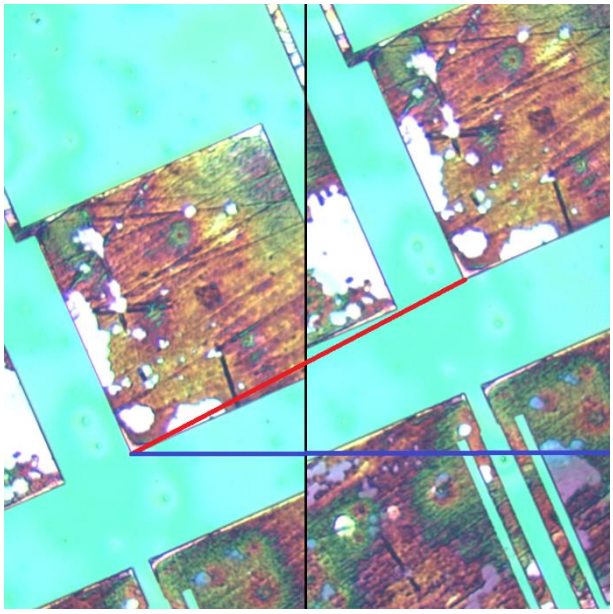
Method 3: Camera Angle Calibration

Suppose the two images below are captured before and after moving the stage solely in the X direction.





Despite moving the XY stage only in the X direction, the image is translated horizontally and vertically, due to the camera angle. Combining half of each image as before, we can illustrate how the camera angle can be calculated, using ***two points: a reference point from each image***.



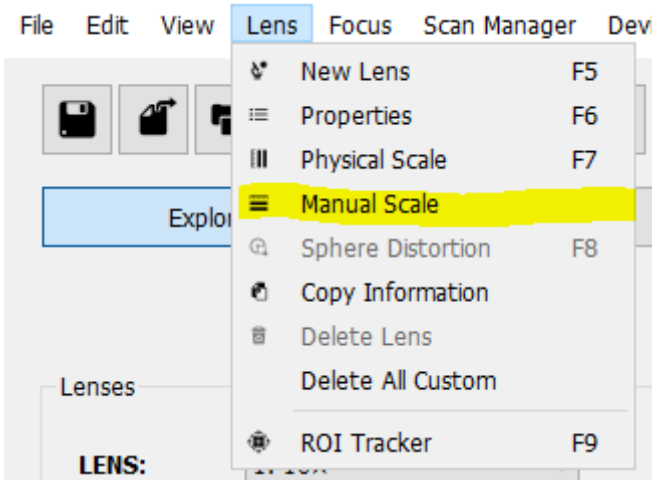
The blue line represents the trajectory of the point if the camera were perfectly aligned. The red line represents the direction the reference point moved across the image. The angle between these two lines is the camera angle.

Invoking Calibration

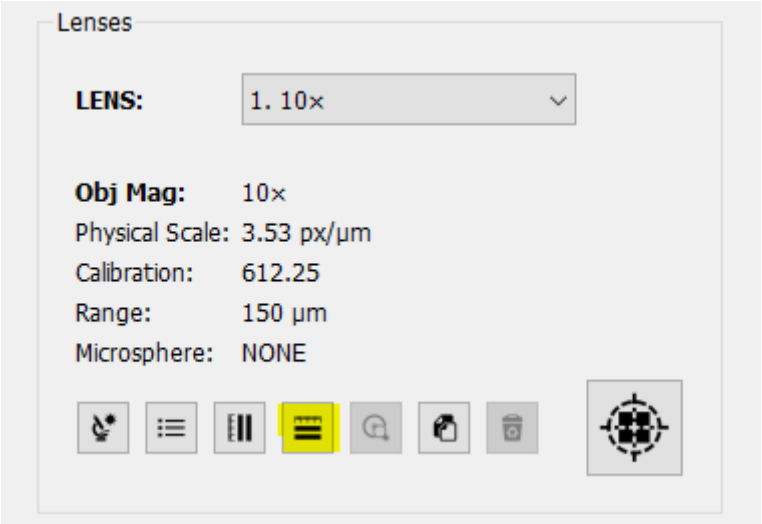
Invoking Scale Calibration

Each lens has its own scale and should be calibrated separately. Scale calibration can be invoked from:

- The lens menu, or
- The lens panel

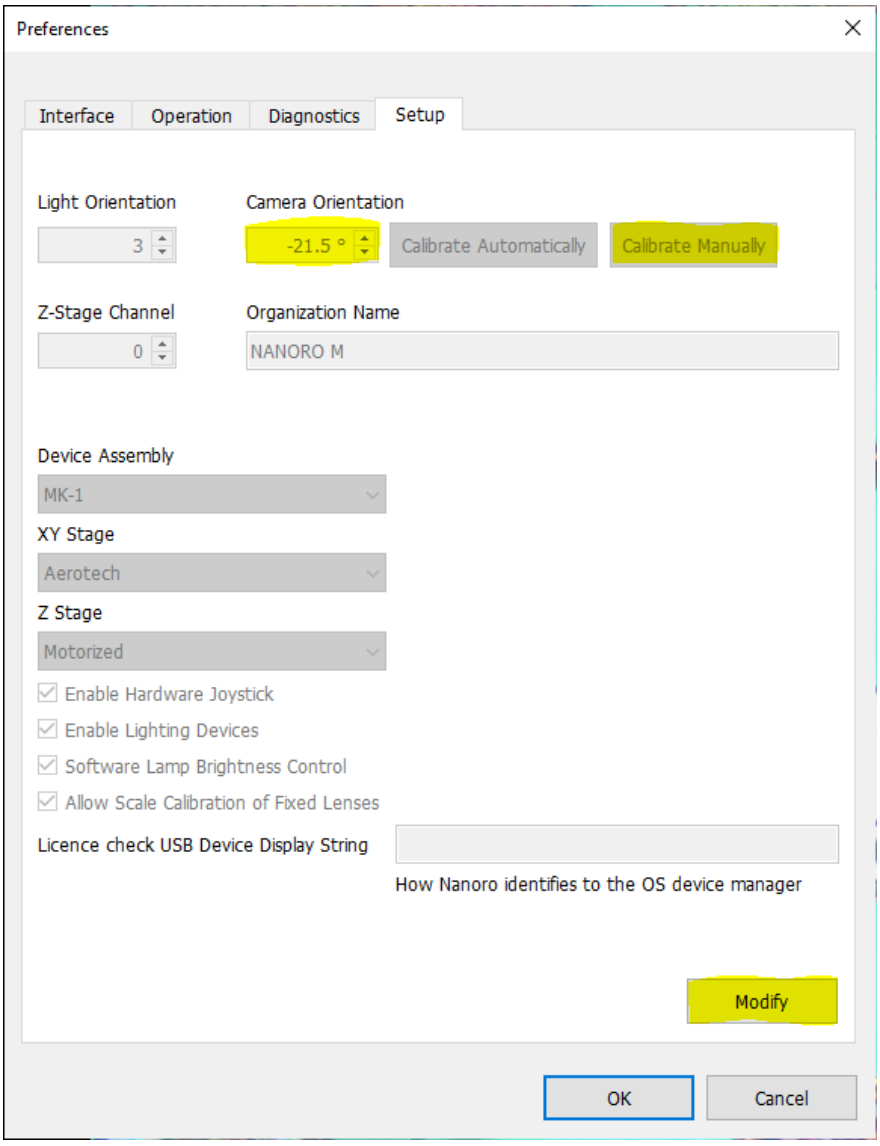


The lens menu



The lens panel

Invoking Camera Angle Calibration

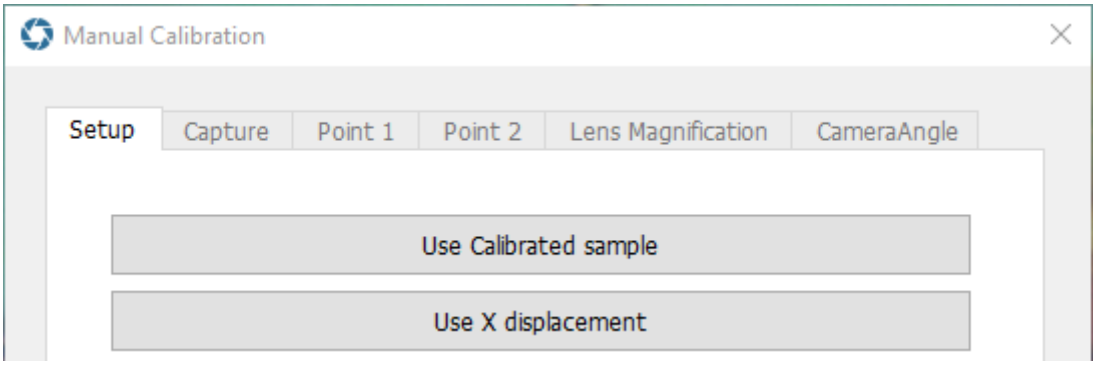


It is rarely necessary to change the camera, and so the settings are protected by requiring the Modify button to be pressed, to enable the calibration button to be pressed.

Since camera angle calibration requires movement in the X direction only, ***when using microscopes with robotic X-Y stages, the user may wish to first set the camera orientation to zero degrees***. This makes it easier to move the XY-stage in the X direction only, since the joystick controls movement in camera coordinates.

The Manual Calibration dialog box

The manual calibration dialog box is a tabbed widget, where the tabs allow data to be entered and are expected to be completed in order.



Manual Calibration Dialog Tabs

Here is an overview of the tabs. They are described in detail later.

Setup

This tab allows the user to choose between calibration methods.

It is not required for camera angle calculation is this is always performed using the **X displacement** method.

Capture

This tab allows images from the microscope camera to be captured for the determination of the two required calibration points.

Point 1

… and …

Point 2

These two tabs work identically and allow a single calibration point to be selected. The Calibration dialog box has a fixed size, but for greater precision, a button allows a resizable image to be opened and enlarged for increased accuracy. In the future this may become zoomable for even further accuracy, via a mouse wheel button.

Lens Magnification

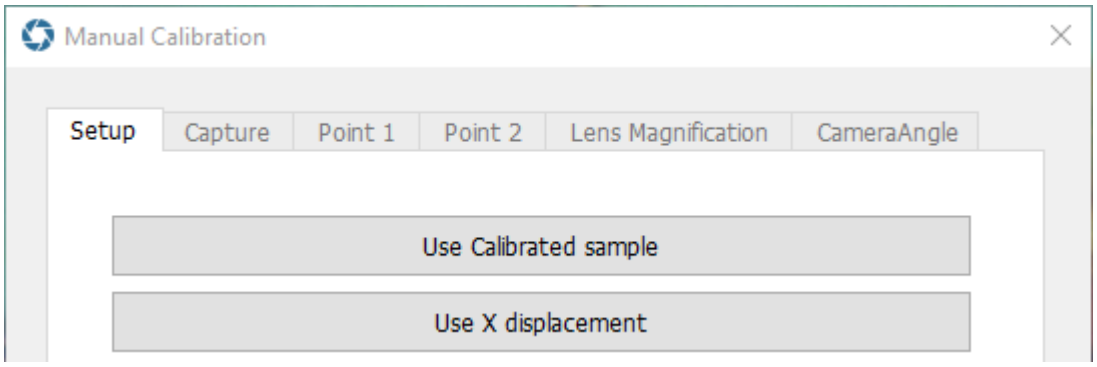
Once the selected points are chosen, calibration becomes possible and a tab for the calibration should become enabled. For lens calibration this will be the Lens Magnification tab.

Camera Angle

If the dialog box is invoked from “Settings”, then the dialog box is used for camera angle calibration and the “Camera Angle” tab becomes active when the points are chosen, rather than the “Lens Magnification” tab.

These tabs will now be discussed in further detail.

Setup tab



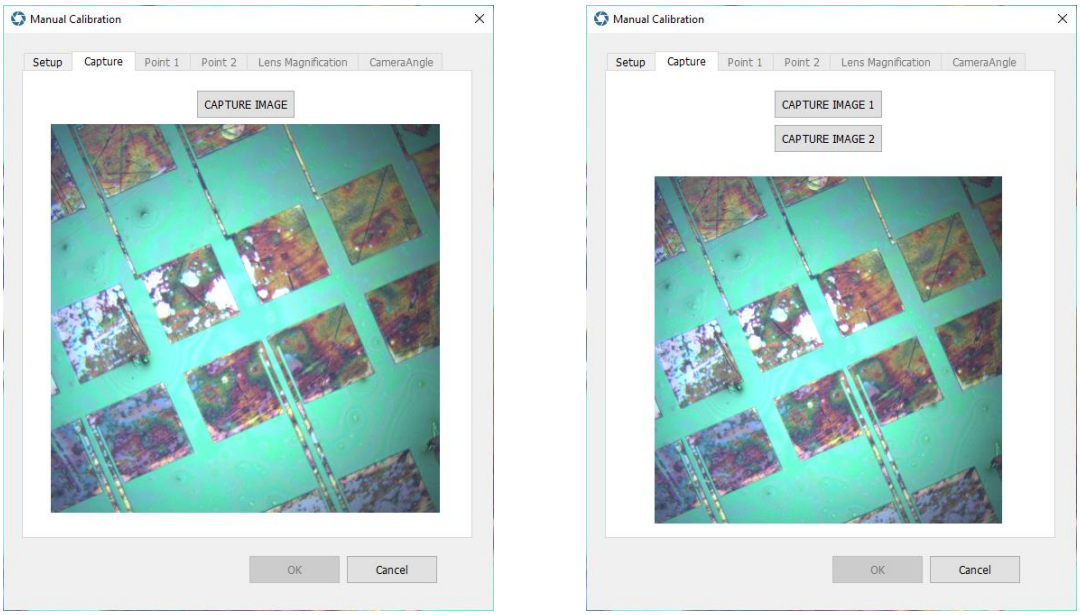
Setup tab

Using a calibrated sample, just one image is required. Two different points will be selected from the same image. The distance between the points will be entered and determines the scale.

Using the X displacement method, two images are required. The same point will be selected from the two images. The distance moved between images will be entered and determines the scale.

Clicking on either button chooses the method used for calibration and takes the user to the capture tab.

Capture Tab



Two versions of the capture tab, for comparison.

Here we see two versions of the capture tab.

- The illustration on the left is for capturing the single image required when using a calibrated sample.
- The illustration on the right is for capturing two images required when using the X displacement method of scale calibration.

Point1 and Point 2

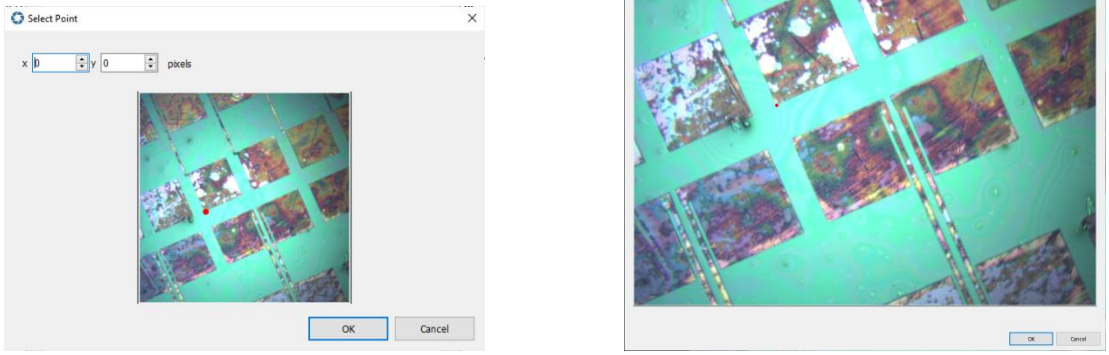
Point selection

This illustration shows the tab for selection of a single point from the captured image.

The user will click on a recognisable feature of the image and the coordinates of that point will be captured by the software. In this example, the user has clicked at the location of the red dot, which is 674 pixels across and 1209 pixels down ***on the original captured image***.

The accuracy is given by the displayed image. For improved accuracy, click “Expand”.

Expanded Point Selection

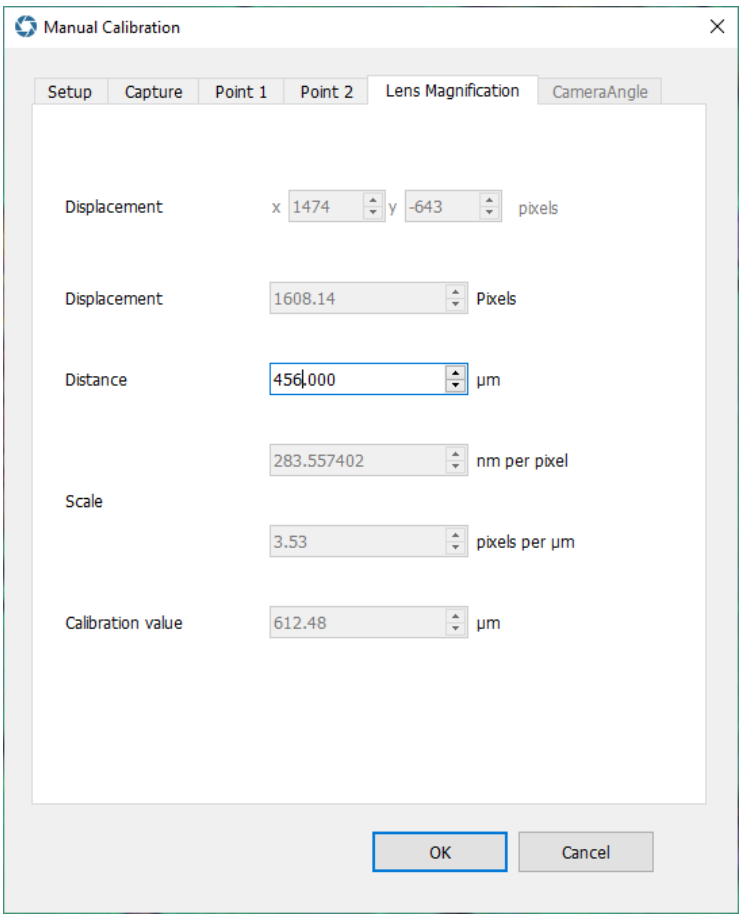


Different size and shape point selection dialog boxes.

This shows the point selection dialog box in two different sizes and shapes.

The captured image is shown inside the dialog box and is rescaled to fit nicely, maintaining its aspect ratio to prevent it looking distorted. Computer screens vary, and the initial size should fit the screen being used. The accuracy of point selection will be increased by using a larger image, so the dialog box should be enlarged to nearly fill the available screen.

Lens Magnification Tab



Lens Magnification Tab

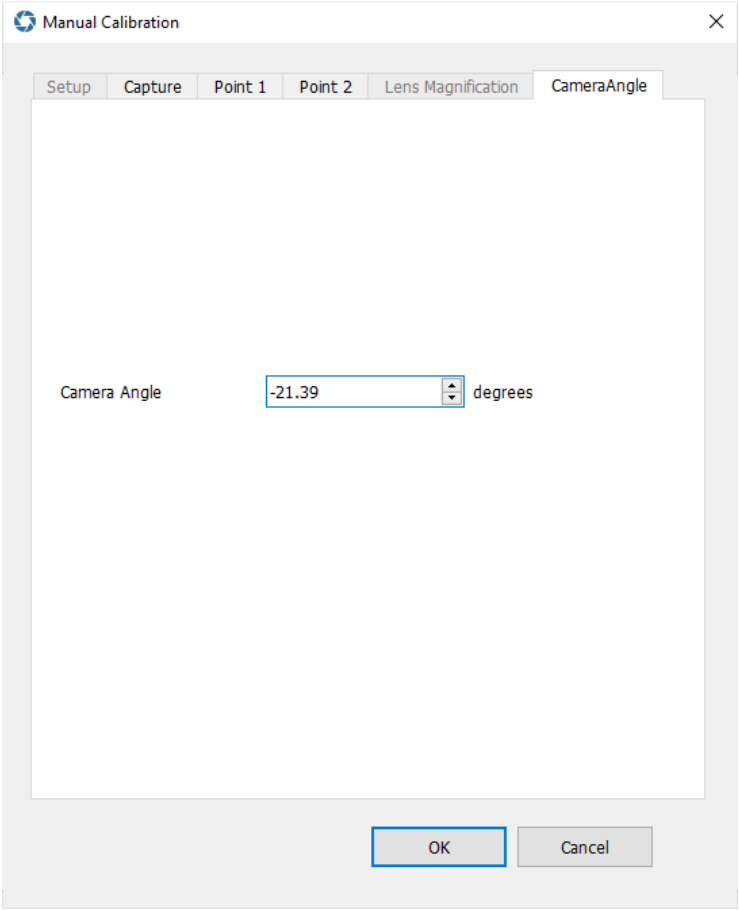
The lens magnification tab calculates the lens scaling in pixels per micron.

Click “OK” if you are happy with the data entered and for the calculated value to be stored.

The displacement is the vector from point 1 to point 2. Using Pythagoras theorem this is calculated as a pixel distance between the points.

Enter the known distance between the points for the scale to be calculated. This is displayed in multiple formats, for easier checking, depending on what the user is familiar with.

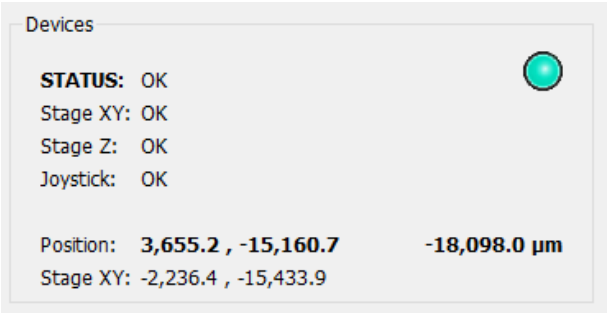
Camera Angle Tab



Camera Angle Tab

The camera angle tab displays the camera angle calculated from the displacement vector from point 1 to point 2. It is the clockwise rotation required to bring the camera into alignment with the XY-stage. Visually check the value is sensible before clicking “OK”.

Note. Depending on the software version, the device status panel may show current coordinates in XY stage coordinates in addition to in camera coordinates (used for scanning and stitching).



Device Status Panel