

Calibration Protocol

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CALIBRATION SHOULD BE DONE *BEFORE* YOU START TRACING.

If you trace first and then calibrate, *all the traces will be wrong*. So calibrate first!
(If you accidentally make a lot of traces before calibrating, there is a protocol to fix it. But it's best to calibrate first!)

Calibrating a new series

1. Go to the calibration grid (typically section 0).

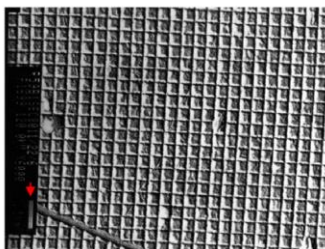


Figure 9.1. Image of calibration grid. An electron micrograph of a diffraction grating replica with a known line spacing of 0.463 microns. The scale bar at the left (below the arrow) was generated by the instrument at a nominal length of 1 micron.

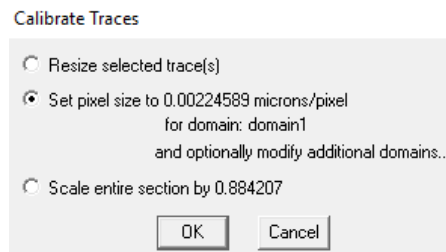
2. Name two palette squares `calibrate_x$+` and `calibrate_y$+`. This will ensure that each line has a different number associated with it (e.g. `calibrate_x1`, `calibrate_x2`, etc.).
3. Zoom in till you see only about a dozen boxes. That's about how close you should be in order to accurately trace across 10 boxes from corner to corner.
4. Using the "Draw Line" tool, trace the length of 10 boxes, 5 traces in the x direction (with `calibrate_x$+`) and 5 traces in the y direction (with `calibrate_y$+`), spread out evenly across the grid. (Avoid the extreme edges and far corners, as that is where the most distortion occurs.)
5. When your traces are completed, select all of the traces (Ctrl-S).
6. Trace > Calibrate > "Enter New Length" of **4.63 μm** > OK. Do this for each calibration trace. (Note: Each box on the calibration grid is 0.463 μm to a side, so 10 boxes is 4.63 μm .)

Enter New Length

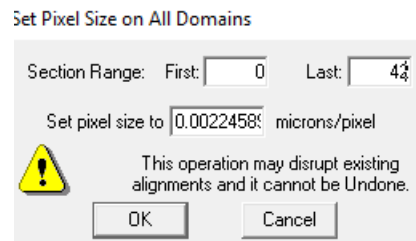
Length of calibrate_x1 in microns:

OK Cancel

7. Calibrate Traces: Select “Set pixel size to...” It will list the pixel size based on the average of the lengths of your calibration traces. OK.



8. Set Pixel Size on All Domains: After “Last,” enter the highest section number in your series to give the full Section Range > OK. The series is now calibrated.



9. The calibration traces (calibrate_x\$+ and calibrate_y\$+) will not be correctly sized after the pixel size adjustment. They can be left as a visual indicator that the series has been calibrated.

10. To verify that a series is calibrated, draw lines on the calibration grid and check their length compared to known length of the calibration grid (0.463µm to a side). You can also use the 1µm scale bar on the cal grid.

Changing pixel size in a series

If a series has been previously calibrated and then a new crop of that series is made, it might just be quicker to copy the pixel size from the previous calibration. If this makes sense for your project, you can go to Section List > highlight all sections > Modify > Pixel Size > input the new pixel size.

Note: The default pixel size for a new series is 0.002540.

Fig. 9.1 above from the [Reconstruct User Manual](#). For more info, read the Reconstruct User Manual (Ch. 9, p. 96-98) and p. 4 (below).

From [Reconstruct User Manual](#) (Fiala, Harris, & Sorra 2009), p. 4:

Calibrate Pixel Size

An important parameter during image import is the pixel size, which we ignored in the previous section. This parameter specifies the size of image picture elements (pixels) in terms of the units of measure of the series (Chapter 9). If the pixel size in the Import Images dialog was accurate, no further calibration is required. However in most cases the pixel size should be adjusted using the image of a calibration grid or scale that was photographed with the series. This is best done before any alignments or tracing, since changing the pixel size later can invalidate these adjustments.

The process of calibration in RECONSTRUCT™ involves drawing traces of known length and then entering that length value into the program. Select the Line Tool (Fig. 1.2) from the Tools window and draw a line trace of known length on a section containing a calibration grid image. (For more details on calibration grids and lines see Chapter 9.)

After the calibration trace is drawn, select the *Calibrate...* item from the Trace menu. A dialog box will appear for each calibration trace displaying the current length of the trace. Enter the actual length of the trace in place of the current length.

When the lengths for all selected traces have been entered, a Calibrate Traces dialog will appear (Fig. 1.3) offering three options. Select the second option to set the correct pixel size for the current section. When the "Set Pixel Size on All Domains?" dialog appears, set the First Section to the first section of the series and the Last Section to the last section. Verify the value in the "Set pixel size to..." box and click the OK button. This will set the pixel size of all images in the series to the value determined from the calibration image. The pixel size for any image can be verified by using the Domain List while viewing the section in the main window.



Figure 1.3. The Calibrate dialogs as filled out for a global calibration of the pixel size of images. Top: The first dialog requests that a pixel size calibration be done for the image on which the calibration lines were traced. Bottom: The next dialog allows this same pixel size to be applied to every image in every section of the series.

Note that after calibration of pixel size, the calibration lines are no longer accurately matched to the calibration image on which they were drawn. This is to be expected. Since these calibration lines are no longer useful they can be deleted from the calibration section. An additional check on the calibration would be to redraw one or two calibration lines and verify that their length is now correct.