

Follow our guidelines carefully to ensure proper system function.

EQUIPMENT

- A microscope system (camera, stage, objectives, computer etc.)
- Calibration grid slide
- The latest version of MBF software.
- Optional: Allen wrenches for aligning camera and stage.

BEFORE YOU START

1. Turn on the microscope system and the computer.
2. Start the software.
3. Load the calibration grid slide onto the microscope.

OVERVIEW OF THE CALIBRATION PROCESS

- A. Add and tighten the objectives.
- B. Verify the camera to stage alignment.
- C. Calibrate lenses.
- D. Perform parcentric/parfocal calibration.
- E. Check the accuracy of the calibration.

A. Add and tighten the objectives

- Tighten all the objectives by hand to make sure they have not come loose.
- Add any new objectives to the microscope BEFORE calibrating the system.

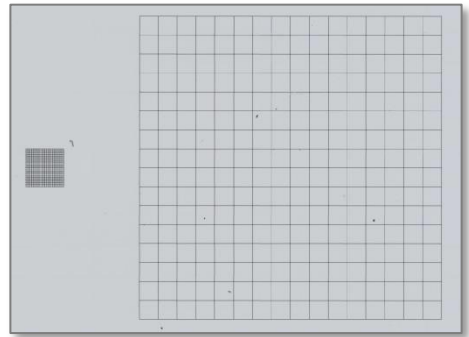
B. Verify the camera to stage alignment

Verify that the camera is oriented in the proper direction.

What appears through the eyepieces should be identical in orientation to what appears on the screen.

Example with the MBF calibration grid slide →

If the small grid is to the left of the large grid when looking through the eyepieces, then it should appear the same way on the computer screen. If it appears backwards, you need to rotate the camera 180°.

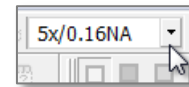


- Loosen the set screw and rotate the camera. Tighten the set screw when finished.

C. Calibrate lenses

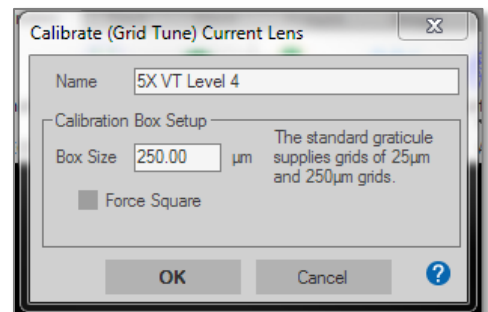
1. Select the lowest power objective (5x in this tutorial) on the microscope.
2. Enable **Joy Track** to locate the 250 μm (large) grid.
3. Turn off **Joy Track**.
4. Verify that the software lens is set to 5x to match the objective.

If there is no software lens defined for the objective, see [Calibration: Creating a Lens](#).



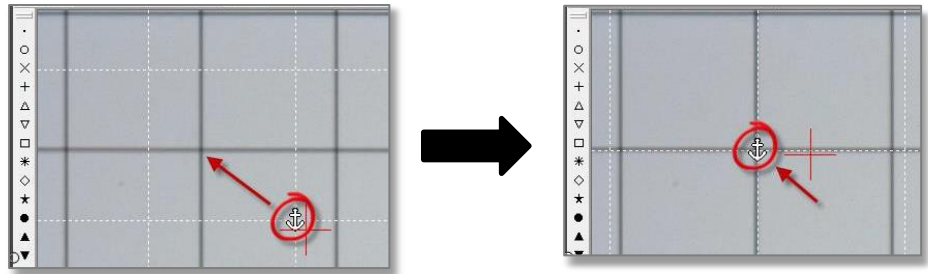
5. Go to **File>Calibration>Recalibrate**.

- ▶ Enter the box size that corresponds to the grid you're using: 250 μm for 5x

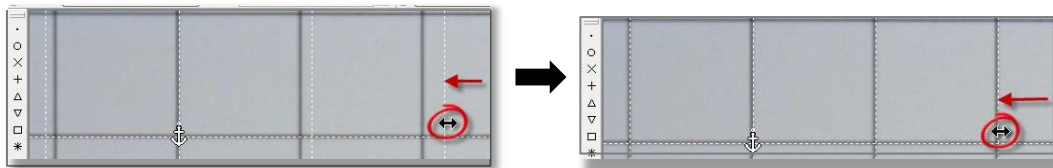


6. A white grid with dotted lines appears. Align the white grid with the slide grid (black lines).
If the slide grid appears to be lopsided compared to the white grid on the screen, see [Troubleshooting](#).

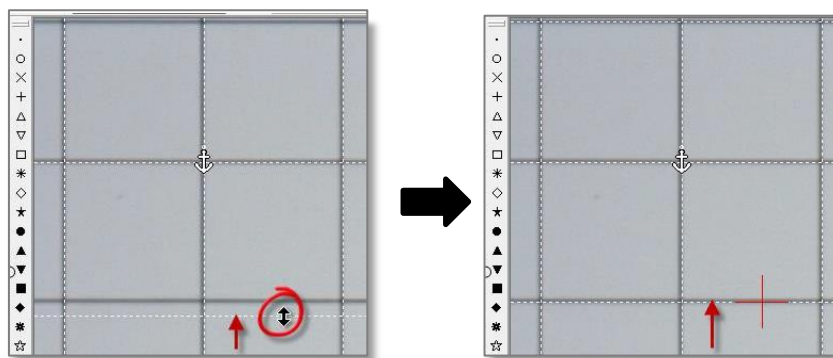
7. Drag the anchor to the corner of a cell located in the top left of the screen.



- a. Align the vertical lines.
 - i. Hover the mouse over a white vertical line until the cursor changes to a double arrow.
 - ii. Drag the white dotted line to align it with the closest vertical slide grid line.
 - iii. Repeat i-ii until all vertical lines are aligned.

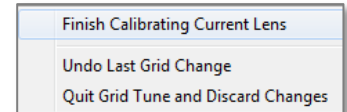


- b. Align the horizontal lines.
 - i. Hover the mouse over a white horizontal line until the cursor changes to a double arrow.
 - ii. Drag to align with the closest horizontal slide grid line.
 - iii. Repeat i-ii until all horizontal lines are aligned.



8. When the white grid is aligned as accurately as possible with the slide grid, right-click and select **Finish Calibrating Current Lens**.

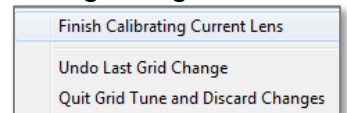
The 5x lens is calibrated.



9. Enable **Joy Track** to locate the 25 μm (small) grid, then turn off **Joy Track** .
10. Select the next objective (40x in this tutorial) on the microscope and verify that the software lens is set to 40x to match the objective.
11. Enable **Joy Track** to move inside the 25 μm grid toward the top left corner, then turn off **Joy Track**.
12. Select **File>Calibration>Recalibrate**.
- ▶ Enter the box size that corresponds to the grid you're using: 25 μm for 40x
13. Another white grid with dotted lines appears. Align the white grid with the slide grid (black lines).
- a. Drag the anchor to the corner of a cell located in the top left of the screen.
 - b. Align the vertical lines.
 - c. Align the horizontal lines.

14. When the white grid is aligned as accurately as possible with the slide grid, right-click and select **Finish Calibrating Current Lens**.

The 40x lens is calibrated.



15. Select the next objective (63x in this tutorial) on the microscope and verify that the software lens is set to 43x to match the objective.

Don't use oil.

16. Select **File>Calibration>Calibration>Recalibrate**.
- ▶ Enter the box size that corresponds to the grid you're using: 25 μm for 63x

15. Repeat steps 13-14.

D. Perform parcentric/parfocal calibration

Don't move the stage at any time; don't use oil with oil objectives.

1. The 100x (highest power) objective is in position and the corresponding software lens is selected.
2. The top left corner of the 25 μm (small) grid is in view.

3. **Optional:** Increase the size of the cursor for a more accurate alignment for the **parcentric** calibration.

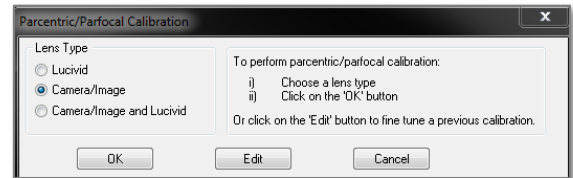
Use the **File>Preferences>General Preferences> Cursor** panel to increase the **Crosshair Size**
(recommended: 75 px)

OR

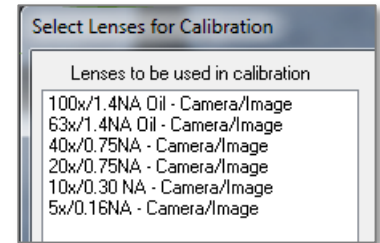
Hold down the **CTRL** key while pressing the **Up** or **Down** arrow keys.

4. Click **File>Calibration/ Parcentric Parfocal**.

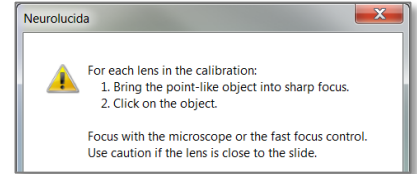
- a. In the **Parcentric/Parfocal Calibration** window, select **Camera/Image** under **Lens Type**.



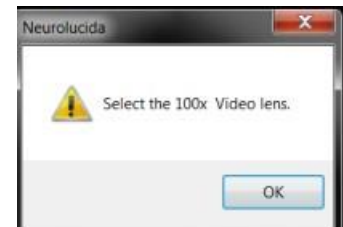
- b. In the **Select Lenses for Calibration** window, verify that the lenses are listed in descending order, from highest to lowest power then click **OK**.



5. An alert is displayed. This is a reminder that, for each lens, you need to click the **EXACT SAME POINT** on the grid, regardless of where it appears on the screen.



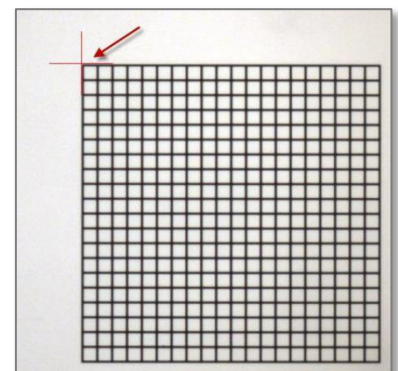
6. Follow the prompt: Verify that the 100x objective (called video lens here) is in position.



7. Bring the top left corner of the grid into focus.

8. Click the outer edge of the top left corner of the grid.

Align the crosshairs of the cursor with the edges of the grid as precisely as possible. You'll click the same point with the other objectives.



9. The program prompts you to switch to the next highest power objective. Don't use oil.

Optional: Before switching objectives, adjust the light level on the microscope to see the grid clearly enough.

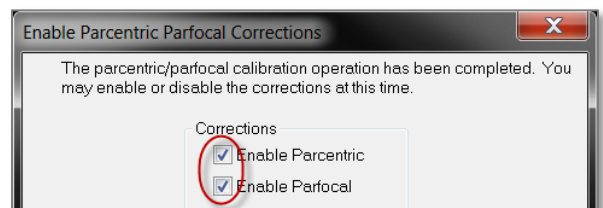
When you switch objectives on the microscope, the program automatically selects the corresponding software lens.

- a. Bring the top left corner of the grid into focus.
- b. Click the outer edge of the top left corner of the grid.

10. Repeat step 9 for the remaining lenses (40x, 20x, 10x, 5x).

11. Once all lenses have been calibrated, the **Enable Parcentric Parfocal Corrections** window is displayed. Check BOTH the **Enable Parcentric** box and the **Enable Parfocal** box.

Don't check ENABLE PARFOCAL if your microscope has an internal motorized Z axis.



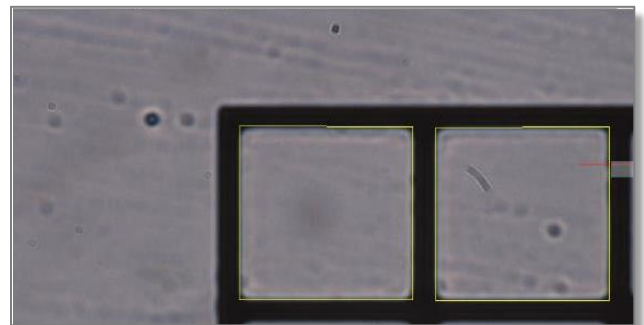
E. Check the accuracy of the calibration

1. Move the 100x (highest power) objective in position and select the corresponding software lens.

2. Trace the contours of two adjacent cells in the top left corner of the grid.

3. Switch to the next lower power objective (63x here) and select the corresponding software lens.

4. Verify that the contours remain aligned with the grid cells, and that the grid is automatically brought into focus.



- If the objects are not automatically brought into focus when switching between objectives, perform the parfocal calibration again.
- If you can't obtain a suitable parcentric or parfocal calibration, consult the [Troubleshooting](#) section.

5. Repeat steps 3-4 for all the remaining objectives, from highest power to lowest power (40x, 20x, 10x, and 5x).