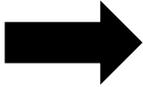## OVERVIEW OF THE CALIBRATION PROCESS

A. Add and tighten all the objectives.

B. Verify the camera to stage alignment.

C. Calibrate lenses with calibration grids.

D. Perform parcentric/parfocal calibration.

E. Check the accuracy of the calibration.

In this tutorial, we demonstrate how to calibrate 5x, 40x, and 63x lenses:

CALIBRATE LENSES WITH CALIBRATION GRIDS

1. Load the calibration grid slide onto the microscope.

2. Select the lowest power objective (5x in this tutorial) on the microscope.

3. Enable **Joy Track** (click the **Joy Track** icon) to locate the 250 µm (large) grid.

4. Turn off **Joy Track** (click the **Joy Track** icon again).

5. 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.*
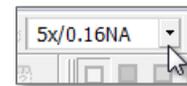
6. Select **Tools>Grid Tune Current Lens** to open the **Grid Tune Current Lens** window.

    ▸ Enter the box size that corresponds to the
   grid you're using: 250 µm for 5x
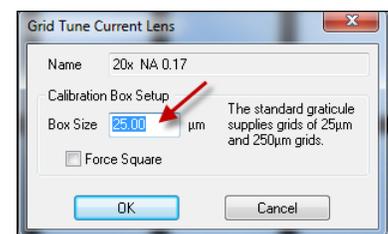
7. A white grid with dotted lines appears. Align the white grid with the slide grid (black lines).

    *If the slide grid appears to be lopsided compared to the white grid on the screen, see
    Troubleshooting.*

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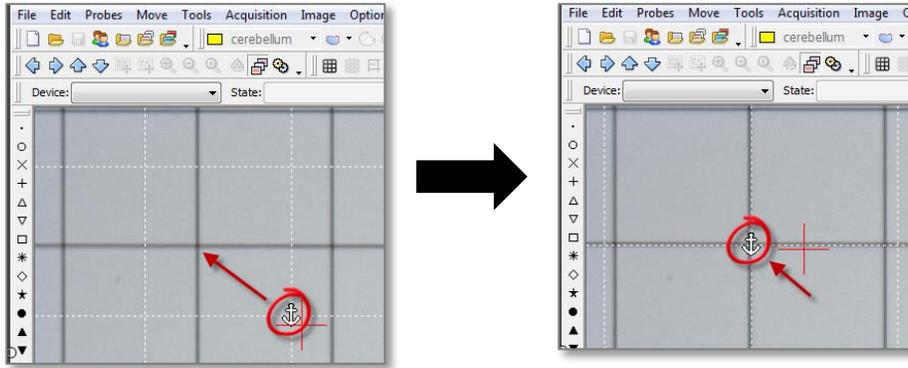

b. Align the vertical lines.
    i. Hover the mouse over a white vertical line until the cursor changes to a double arrow.
    ii. Drag the white dotted line to align it with the closest vertical slide grid line.
    iii. Repeat i-ii until all vertical lines are aligned.
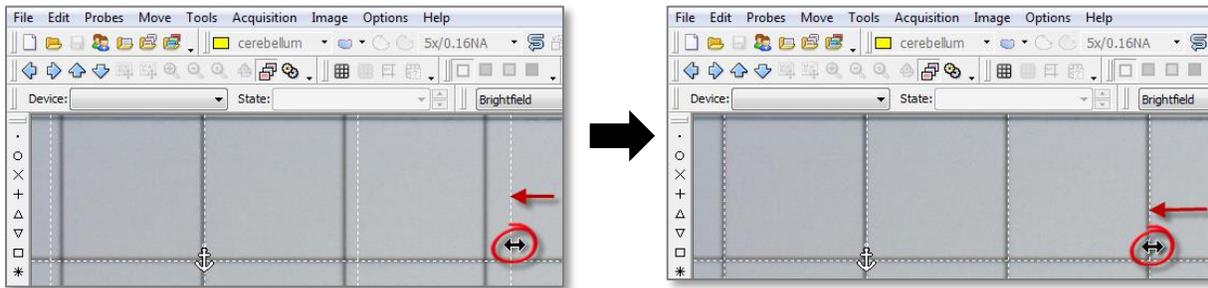
c. Align the horizontal lines.
    i. Hover the mouse over a white horizontal line until the cursor changes to a double arrow.
    ii. Drag to align with the closest horizontal slide grid line.
    iii. Repeat i-ii until all horizontal lines are aligned.
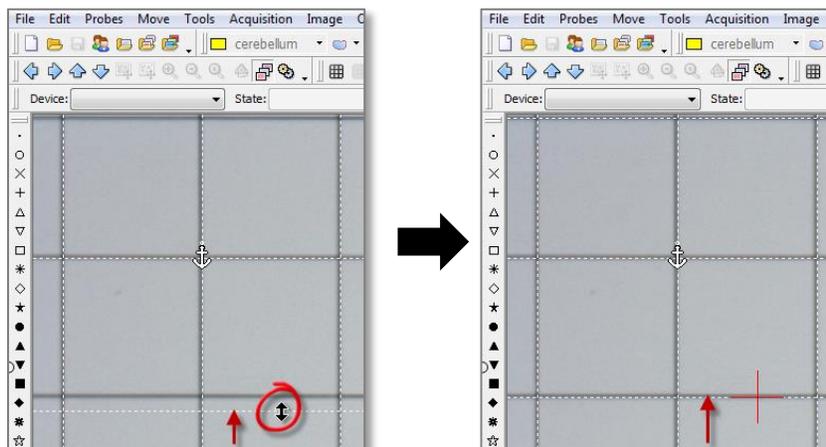
8. When the white grid is aligned as accurately as possible with the slide grid, right-click and select **Finish Calibrating Current Lens**.

    The 5x lens is calibrated.

    | Finish Calibrating Current Lens |
    | Undo Last Grid Change |
    | Quit Grid Tune and Discard Changes |

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 **Tools>Grid Tune Current Lens** to open the **Grid Tune Current Lens** window.

    ‣ 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.

    | Finish Calibrating Current Lens |
    | Undo Last Grid Change |
    | Quit Grid Tune and Discard Changes |

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 **Tools>Grid Tune Current Lens** to open the **Grid Tune Current Lens** window.

    ‣ Enter the box size that corresponds to the grid you're using: 25 µm for 63x

15. Repeat steps 13-14.

> Once the lenses are calibrated, you're ready to perform the parcentric/parfocal calibration.