

Chip Insert Calibration Quick Guide

Below are the guidelines describing how to calibrate the chip insert on the ZellScanner ONE.

Note: Calibration is necessary whenever the insert is removed. This may happen for cleaning purposes or when misalignment is observed.

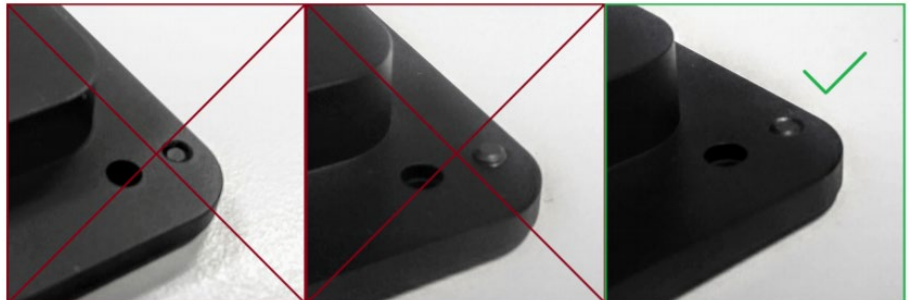
A. Preparing the Stage Insert

1. Remove the stage insert from the microscope stage.
2. Clean both the stage insert (Fig. 1) and the ledge of the opening for the stage insert with distilled water and, if necessary, a mild detergent. Assure all precipitated buffer or dust is thoroughly removed as it can slant the stage insert.
3. Use the hex key (in calibration slide box) to level the stage insert grub screws with the bottom of the insert (Fig. 2)
4. Check the alignment of the insert. The insert should be level with the top of the microscope table.



Fig. 1 | Chip Insert on Stage

Fig. 2 | Detail of the grub screw alignment
Left: too far in
Middle: too far out
Right: level



5. Open the ZKW ScanApp.
6. Place a ZellSafe™ cell ship loaded with PBMCs at an adequate density into the slide holder on the ZellScanner ONE.

B. Checking the Focus

1. Use the coaxial drive to move the stage until the chip channel is directly above the objective.
2. Use the coarse focus drive to move the objective up until the cell layer becomes visible in the ScanApp but is still out of focus.

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B. Checking the Focus Continued...

3. Concentrating on the top left corner of the ScanApp view, use the fine focus drive to focus the cell layer. (Fig. 3)

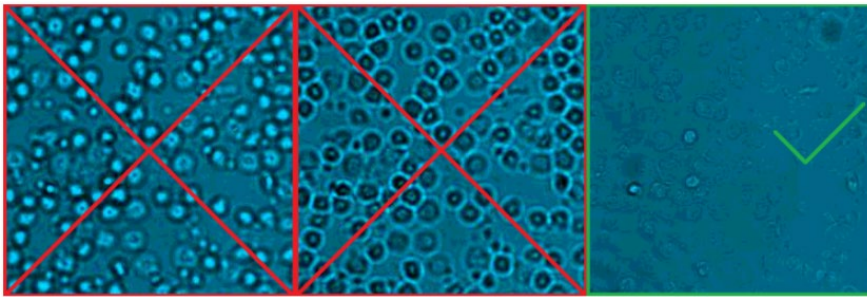


Fig. 3 | Focus examples
Left: z-axis too low
Middle: z-axis too high
Right: proper focus

4. Note down the value of Focus in the “Scanner Controls” window.
5. Repeat steps B2-B4 for the three remaining corners: Top Right, Bottom Left, and Bottom Right. (Fig. 4)

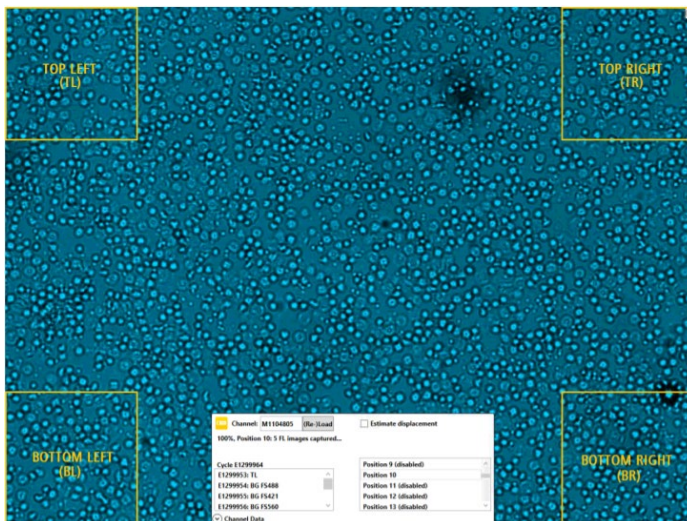


Fig. 4 | Example view of ScanApp with focus zones

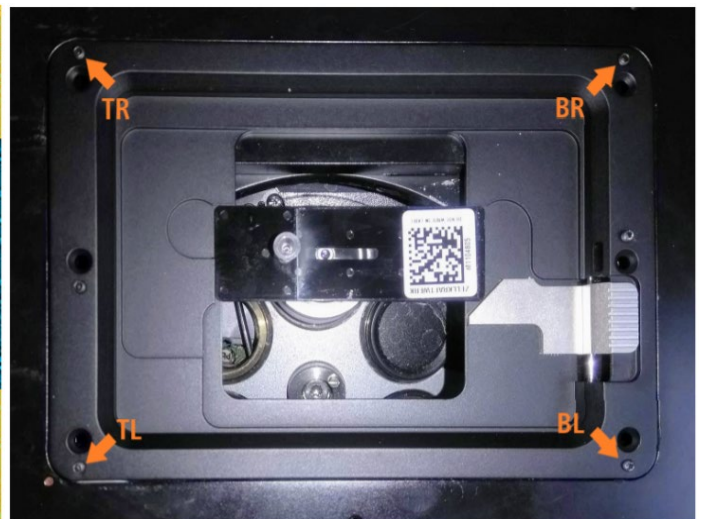


Fig. 5 | Spacer screws in the corners of the chip insert as designated

Note: The configuration is rotated 90° anti-clockwise relative to the configuration shown in the ScanApp.

6. If the focus difference between every possible pair of corners is $<1\mu\text{m}$, the image is considered plane.
7. If there are two or more corners with a focus difference $\geq 1\mu\text{m}$, adjust the spacer screws as described in Section C.

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C. Adjusting the Chip Insert

1. Determine which corner has the lowest focus height.
2. Refer to Fig. 5 to determine which spacer screw corresponds to the corner with the lowest focus height.
3. Use the hex key to turn that spacer screw clockwise:
 - i. 1 full turn; if the difference between any two corner focus heights is above 10 μ m
 - ii. $\frac{1}{2}$ turn; if the highest difference between any two corner focus heights is between 4.0 μ m and 2.0 μ m
 - iii. $\frac{1}{4}$ turn; if the highest difference between any two corner focus heights is between 2.0 μ m and 1.0 μ m
4. Place your index fingers on the corners adjacent to the one you adjusted and alternately apply gentle pressure to them.
5. If the insert tilts from one side to the other, turn the screw in the corner with the second-lowest focus clockwise in 1/16 turns.
6. Repeat steps C4 and C5 until the tilting stops.
7. Repeat sections A and B as needed.
8. Proceed with calibration using the ABC-slide according to the ABC-Calibration of ZellScanner One Protocol.