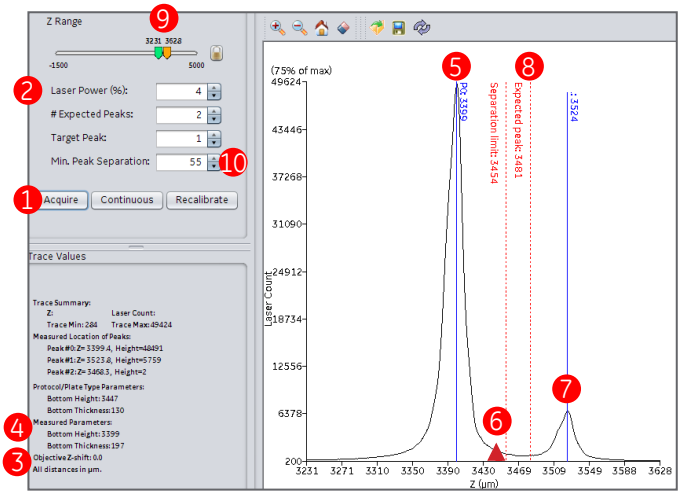
GE Healthcare

IN Cell Analyzer

Adding a plate map

Adding or modifying a plate map

1. In the ***Setup*** tab, click the ***Eject*** icon to open the door and load sample.
   Note: A dry plate with no cellular sample may be used in this procedure.
2. Click the ***Eject*** icon to close the door. Use Plate View to navigate to a well in the middle of the plate.
3. In the ***Objective*** drop-down, select 10×.
4. Press ***Ctrl + L*** to open the ***Laser Autofocus*** (LAF) tool.
5. Click ***Acquire*** (1). If the first peak reaches saturation 65535, reduce laser power (2). If you see two distinct and clearly separated peaks, proceed to step 6. If not, see "Interpreting LAF traces and troubleshooting tips."
6. In the ***Application*** menu, select ***Plate/Slide Manager***. If you are adding a plate, select the ***Create new*** icon, then ***New Plate***, then select your plate type. If you are editing a plate, select the plate map, then select ***Edit*** icon.
7. If you are adding a plate, in the ***Plate Editor***, edit the ***Plate Name*** to include unique identifiers (e.g., manufacturer and model number, your name).
8. In the ***Plate Editor***, enter the ***Measured Parameters*** values for ***Bottom Thickness*** (3) and ***Bottom Height*** (4) from the ***LAF*** tool.
9. Set the ***Bottom Height Variability*** according to your ***Bottom Thickness*** as follows:
   - Plates ~200 µm, use 25 µm
   - Plate 200-500 µm, use 50 µm
   - Plates >500 µm, use 100 µm
10. If you know the well parameter specifications of your plate, enter them here.
    - Adjust ***Well Layout*** to change number of rows and columns or spacing between adjacent wells
    - Adjust ***Well Parameters*** to change well shape and size
    - Adjust ***Well Offset*** to change the distance between the edge of the plate and well A1/upper left well of sample



11. Click ***Apply*** and ***OK***. Close ***LAF*** and ***Plate/Slide Manager*** tools.
12. Select your new or edited plate in the Plate/Slide drop-down and click ***Verify***. Confirm that the z value of ***P0*** (5) is within 10% of red arrowhead (6) and that ***P1*** (7) is within 10% of ***Expected Peak*** (8).

Interpreting LAF traces and troubleshooting tips

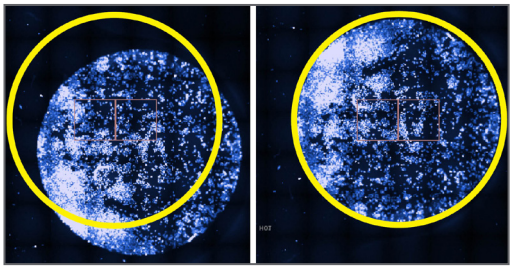
The ***Laser Autofocus*** tool uses a near infrared laser to locate areas of significant refractive index difference between the objective and the sample. When looking at the trace:

- ***P0*** (5) represents the interface between air and the bottom of the plate/sample.
- ***P1*** (7) represents the interface between the bottom of the plate and the sample.
- The x-axis represents distance in the z dimension, the y-axis represents laser counts.
- The red arrowhead (6) indicates the ***Expected Bottom Height*** of the plate.
- If you see only one peak, increase the range of the z-range sliders (9). Be conservative to avoid hitting the plate with the objective.
- If you still see only one peak, increase ***Laser Power*** (2) until a second peak is detected. If a second peak is not detected with maximum laser power, select another well or position within the well.
- ***Min Peak Separation*** (10) is defined as the distance between the z location of ***P0*** and where the tool starts to look for ***P1***.
- If the peaks are not defined correctly, decrease the ***Min Peak Separation*** (10).

Using preview scan to fine tune well parameters

1. In the ***Channels*** menu, select the channel to use for ***Preview Scan***.
2. In ***Plate View***, click the ***Preview Scan*** icon and draw a rectangle around the top left corner of the plate/sample.
3. Click ***Preview***.
4. After the scan completes, use ***Plate View*** to verify that the plate map and sample wells overlap completely. Use the ***Zoom*** tool if required.
   Note: If it is hard to visualize sample location, use the ***Palette*** icon to adjust contrast.
5. In ***Plate View***, select the ***Gear*** icon and use the arrows to align the plate map with the Upper Left well.
6. Repeat at Upper Right and Lower Left corners of sample.
7. In the ***Options*** menu of the ***Gear*** tool, you can ***Save*** the current Fine Tuning Parameters to a file, ***Load*** a previous set of Fine Tuning Parameters, or ***Reset*** the well grid to the default layout.

Adjusting well alignment



Before After

Troubleshooting focusing

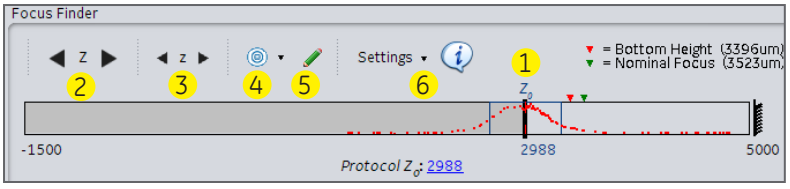
After adding and verifying a plate map, if not all images are in focus, follow these steps.

1. If using a cellular sample, confirm that the wells are at least ½ full of liquid. If not, fill wells and run the "Adding or modifying a plate map" procedure.
2. If using a cellular sample, confirm that there are cells within the field of view (FOV).
3. If you are using an ASAC objective, in the ***Objective Lens*** card, confirm the collar setting matches plate ***Bottom Thickness***.
4. Re-acquire offsets using ***Auto Offset***.
5. Confirm that ***Software Autofocus*** is turned off.
6. Repeat the "Adding or modifying a plate map" procedure.

Focus finder tool

The ***Focus Finder*** tool provides a way to manually find or adjust focus.

1. In the ***Setup*** tab, select the ***Focus Finder*** icon.
2. Drag the black bar (1) through z, noting the positions of the red dots in the graph. When the red dots move higher in the y-axis, you are getting closer to focus.
3. When you are close to focus, use the large ***Z*** (2) and small ***z*** (3) buttons to refine focus.
   Note: The large ***Z*** adjusts in 5 µm steps and the small ***Z*** adjusts in 1 µm steps.
4. Click the ***Software Autofocus*** (4) icon to further refine focus.
5. If desired, click the ***Green Pencil*** (5) icon to mark your z position and update the ***Initial Focus (Z_0)*** position in the ***Dashboard***.
   Note: If you cannot find focus, increase Exposure time in the ***Settings*** (6) drop-down, change the FOV or change channels.



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