GE Healthcare

IN Cell Analyzer

Start-up and basic acquisition

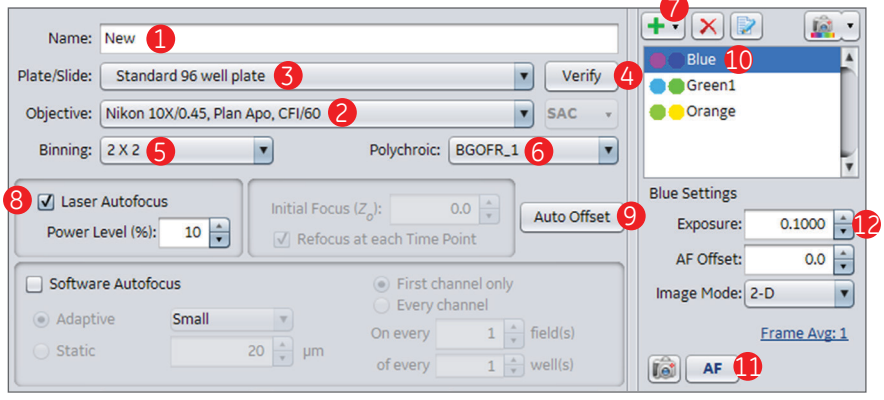
System start-up

1. Turn on IN Cell Analyzer using button located on right side of system. Turn on workstation. The order in which they are turned on does not matter.
2. Wait until the system is fully initialized as indicated by a solid green light.
3. Double-click the ***IN Cell Analyzer*** icon.

Load sample and start a new protocol

1. In the ***Setup*** tab, click the ***Eject*** icon to open the door and load sample.
2. Click the ***Eject*** icon to close the door and move the stage to well A1.
3. Click the ***Start a new protocol*** icon, then enter a unique protocol name 1. Click ***Save***.
   Note: Name should be 16 characters or less. Do not use special characters.

Verify plate map



1. In the ***Objective*** 2 drop-down, select 10× objective.
2. In the ***Plate/Side*** 3 drop-down, select correct plate map (manufacturer and catalogue number).
3. Click ***Verify*** 4 to confirm that your plate type matches the map selected. Compare the position of Peak 0 (P0) with the red arrowhead and compare Peak 1 (P1) with the dashed red line.
   - If the expected values differ from the measured values by more than 10%, it is recommended to create a new plate map, as defined in the application guide “Adding a plate map” (29258821).
   - If expected value differs from the measured value by less than 10%, no changes are required. Close ***Laser Autofocus*** (LAF) tool. For more information, see “Interpreting LAF traces”.
4. In the ***Objective*** 2 drop-down, select objective needed for your assay.

Create protocol

1. In the ***Binning*** 5 drop-down, set binning to 2 × 2.
   Note: Binning reduces image file sizes dramatically, that is, at 1 × 1 binning a single channel image is 8 MB, at 2 × 2 binning it is 2 MB. Set binning to 1 × 1 only when maximal image resolution is required.
2. On the IN Cell Analyzer 2200 and 2500*HS*, select appropriate ***Polychroic*** 6.
3. Click the ***Add New Channel*** 7 icon and add channels required for your experiment.
4. Check ***Laser Autofocus*** 8 box and click ***Auto Offset*** 9. This will use both LAF and ***Software Autofocus*** to find the focal plane for each channel. Click ***OK*** to apply the offsets.
5. Highlight a channel 10 and click ***AF*** 11.
6. Adjust ***Exposure*** 12 to target 10000 to 20000 counts for fixed cells or approximately five times the background for live cells. Repeat for all additional channels.
   Note: Use the ***Min/Max*** values at the bottom of the image panel or hover over the image to optimize ***Exposure***. Keep ***Max*** value below 45000 counts to prevent saturation (65535 counts).
7. On the IN Cell Analyzer 6000 and 6500*HS*, if your sample is thick or has high background, try imaging in confocal mode. Uncheck the ***Open Aperture*** box and adjust ***Aperture*** (AU) as needed on a channel-by-channel basis. Smaller values are more confocal while larger values are closer to ***Open Aperture*** mode.
   Note: You might need to refine exposure after adjusting aperture settings.

8. Click the ***Fields*** card to set number and placement of fields.
9. In ***Plate View***, click and drag while holding down ***Shift*** to deselect wells. To reselect, click and drag while holding down ***Ctrl***.
10. To designate control wells, right-click a well and select ***Mark as Control Well/Positive or Negative***.
11. Visit several wells in the plate and confirm that imaging conditions (exposure time, confocality, focus, etc.) are appropriate.
12. Click ***Save***.

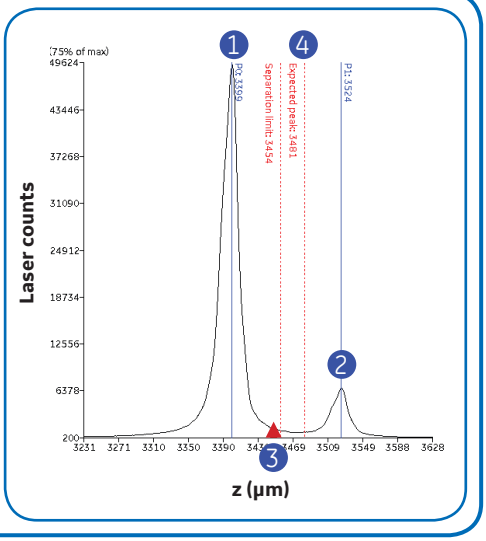
Start the scan

1. Click ***Scan***.
2. Enter an ***Annotation/PlateID***, select your file naming method and click ***Run***.

Interpreting LAF traces

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:

- Peak 0 (1) represents the interface between air and the bottom of the plate/sample
- Peak 1 (2) 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 (3) indicates the ***Expected Bottom Height*** of the plate
- The red dotted line (4) indicates the ***Expected Peak*** from the plate/sample interface



GE Healthcare Bio-Sciences Corp.
100 Results Way
Marlborough, MA 01752
USA

www.gelifesciences.com

29258710AA 04/2017