IN Cell Analyzer 6000/6500*HS* Maintenance

Routine laser maintenance

IN Cell Analyzer 6000 and 6500*HS* require routine laser maintenance to ensure they perform optimally and consistently. Here, we describe laser homing and aperture procedures. You will learn what each procedure is, why it is important, and how and when to perform it.

For optimal system performance, run both procedures at least monthly or any time the temperature in the room differs by greater than 2°C from the temperature at which the procedure was previously performed.

Laser homing

What is laser homing?

Laser homing is the process of restoring the internal position of the lasers within the multi-laser engine (MLE) to the position defined when the system was manufactured. In this homing routine, the present location of each beam is measured and used to determine the necessary adjustment. Laser position can drift from the home position due to shipment, temperature changes in the environment, or opto-mechanical change in the instrument. This homing procedure does not apply to the 405 nm laser because this laser is not contained within the MLE.

Why should I perform laser homing?

If lasers drift from their home positions, the change in fluorescence intensity can vary by a factor of four due to the internal laser alignment within the MLE. This will result in increased exposure times and an increased risk of photobleaching or phototoxicity within the sample.

Aperture calibration

What is aperture calibration?

The aperture calibration routine is used to map the excitation laser line to the active portion of the detector on the sCMOS camera. To align the laser line precisely with the imaging rows of the detector, a galvanometer (galvo) controlled mirror position is adjusted systematically in fine steps until optimal positioning is achieved. Galvo positions are susceptible to drift due to temperature fluctuations in the environment. Running this aperture calibration at appropriate intervals will ensure your IN Cell Analyzer continues operating at peak performance.

Why should I perform aperture calibration?

If the laser line position is not completely synchronized with the rolling shutter readout mode of the camera, the images will not capture the emitted fluorophores. This will result in unnecessarily increased exposure times and the risk of increased photobleaching or phototoxicity within the sample. EDGE confocal imaging on the IN Cell Analyzer 6500*HS* is especially dependent on aperture calibration.

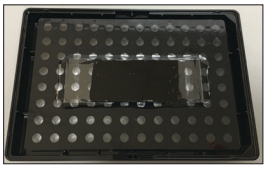
Software required for laser maintenance

- IN Cell Analyzer acquisition software 7.1 or higher
  - For IN Cell Analyzer 6000, download: http://incelldownload.gehealthcare.com/download_data/incell/6000/incell_6000_download_page.htm
  - For IN Cell Analyzer 6500*HS*, download: http://incelldownload.gehealthcare.com/download_data/incell/6500/incell_6500_download_page.htm
- For assistance with installation, download “IN Cell Analyzer Installation” 29285558AA: http://incelldownload.gehealthcare.com/download_data/incell/incell_docs/incell_docs.htm

Tool required for laser maintenance

- IN Cell Analyzer Aperture Calibration Plate Part #29268527
  - IN Cell Analyzer 6000 customers: Order this part from your local Sales Team.
  - IN Cell Analyzer 6500*HS* customers: This part is included with purchase of the IN Cell Analyzer system.

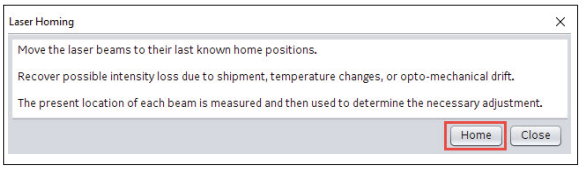
Top of Aperture Calibration Plate


Bottom of Aperture Calibration Plate

Laser homing procedure

1. From the IN Cell Analyzer acquisition software, select ***Application|Hardware|Laser homing.***
2. In the Laser Homing window, select ***Home***.

   Note: Laser homing is a process internal to the multi laser engine (MLE) and it is not necessary to load a sample into the instrument.



3. In the ***Home All Lasers?*** window, select ***OK***.
4. When the ***Laser Homing*** window states "Laser homing complete," select ***Close***.

5. Record the room temperature at the day and time of laser homing in a publicly posted log. This will enable other users to decide if laser homing is necessary prior to their imaging session.

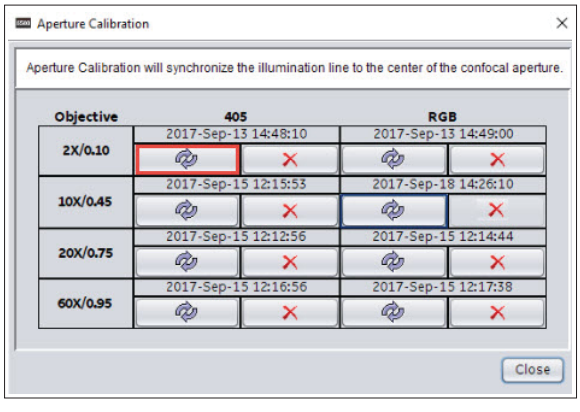
Aperture calibration procedure

Load the plate

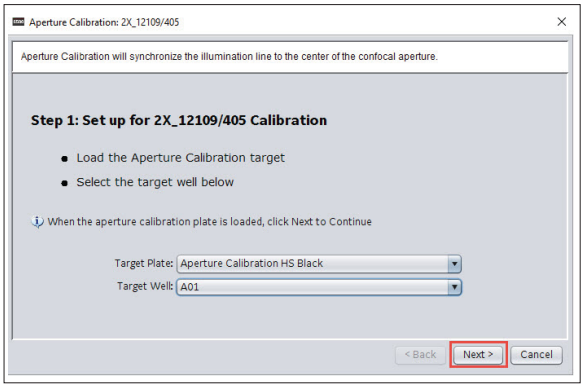
1. Remove the cover from the bottom of the IN Cell Analyzer Aperture Calibration Plate.
2. Inspect the bottom of the Calibration Plate and confirm that the slide is free from dust and fingerprints. If the plate needs to be cleaned, use an optical lens cleaner and lens tissue to clean the tool.
3. From the IN Cell Analyzer acquisition software, select ***Eject*** to open the sample door.
4. Load the Calibration Plate into the IN Cell Analyzer 6000/6500*HS* system.
5. Select ***Load*** from the toolbar to close the door.

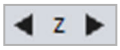
Refresh aperture calibration

1. From the IN Cell Acquisition software, select ***Application| Hardware|Refresh*** aperture calibration.
2. If prompted, select **Yes** to save any changes to the current protocol.
3. The Aperture Calibration table lists the ***Objectives*** and lasers available on your system. Each objective must be calibrated with the ***405*** nm laser and the ***RGB*** lasers separately (488, 561, and 642 nm). The date and time at which the previous calibration was last performed are displayed in the table. If it has been a month or more since calibration was performed, or if the room temperature has changed by greater than 2°C from which the temperature of calibration, proceed to the next step.
4. Select the ***Refresh***  button to start the ***405*** nm laser calibration for the lowest magnification lens.



5. For ***Step 1*** of the calibration process, select the ***Aperture Calibration HS Black*** for the ***Target Plate*** and ***A01*** for the ***Target Well***. Select ***Next***.



6. When performing ***Step 2***:
   - If the laser line is close to in focus, confirm that the ***Max*** intensity is less than 50 000 and proceed to ***Step 7***.
   - If the laser line is far from focus, use the ***Focus Finder*** to bring it into focus.
     a. In the ***FocusFinder*** tool, use the **Z**  buttons to adjust the ***Z position*** until the laser line is in focus.
     b. Use the mouse scroll wheel to zoom in and confirm focus is optimized.
     c. Examine the ***Max*** *i*ntensity displayed in the image window in the lower left corner. If the value is above 50 000 counts, reduce the ***Exposure*** or ***Laser Power*** in the Dashboard until the ***Max*** intensity is below 50 000 counts.
     Note: When the ***Z position*** is adjusted using the ***FocusFinder***, or when the ***Exposure*** or ***Laser Power*** are adjusted in the Dashboard, the values are automatically updated in the Aperture Calibration window.
7. Click the ***Software Autofocus***  icon in the ***FocusFinder*** and then select ***Calibrate***.
8. When the ***Aperture Calibration Results*** window appears, the calibration is complete. Select ***Yes*** to save these results and then ***OK***. The Aperture Calibration table will be updated to reflect the current date and time of the calibration.
9. Repeat steps 3-8 for both ***405*** and ***RGB*** lasers for all remaining objectives.
10. Record the room temperature at the time of calibration in a publicly posted log. This will enable other users to decide if aperture calibration is necessary prior to their imaging session.
11. Select ***Eject*** and remove the calibration plate from the instrument.
12. Secure the cover to the bottom of the plate using lab tape to keep it clean.

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

www.gelifesciences.com

GE Healthcare Bio-Sciences AB, Björkgatan 30, SE-751 84 Uppsala, Sweden
GE Healthcare UK Ltd, Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK
HyClone Laboratories, Inc., 925 W 1800 S, Logan, UT 84321, USA
GE Healthcare Europe GmbH, Munzinger Strasse 5, D-79111 Freiburg, Germany
GE Healthcare Japan Corporation, Sanken Bldg. 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073, Japan
For local office contact information, visit: www.gelifesciences.com/contact
KA924121117TN 12/2017