

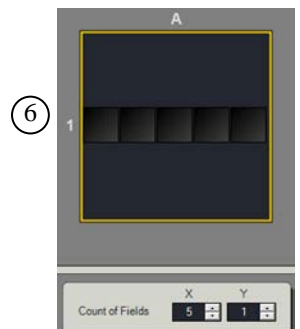
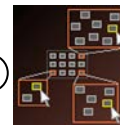
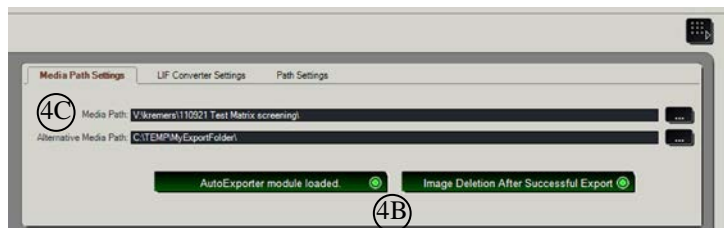


# Matrix Screening Wizard: Mark and Find

This manual will help you to set up a multiple position time lapse imaging experiment in a single dish or well. It will help you to set up a reflection-based autofocus routine.

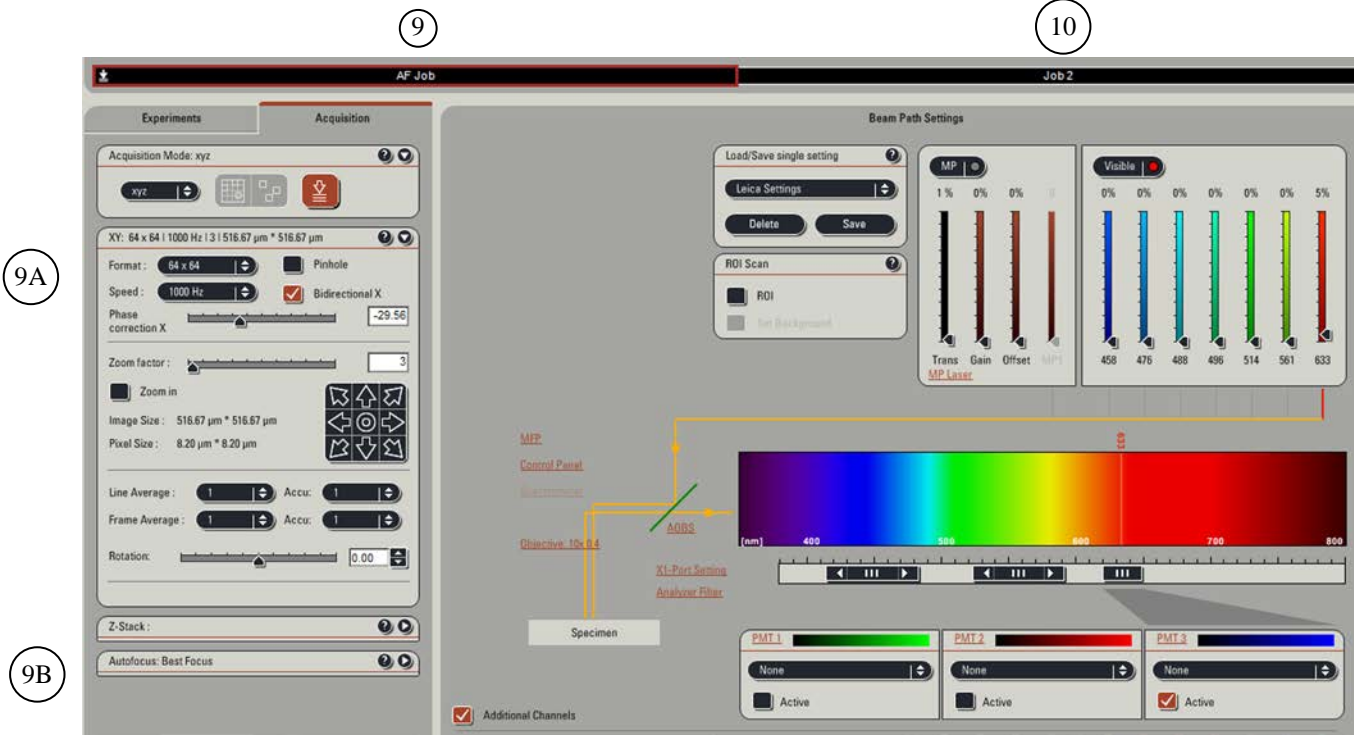
1. Start the LASAF software and **initialize the microscope stage**.
2. Setup the imaging parameters (lasers, detectors, etc) and focus your sample.
3. Select the Matrix Screening Wizard from the top left corner of your screen.
4. Setup Data Export
  - A. Click on  in the top right corner.
  - B. Activate the “AutoExporter module” and “Image deletion after successful export”.
  - C. Specify the directory on the V: network drive where your data should be stored. **Do not change the “Alternative Media path”.**
  - D. Click  in the top right corner to go back to the Matrix Screening Wizard.



5. Select “Mark and Find mode” (lower right).
6. Go to Setup Template tab (bottom left corner)
7. Enter the number of scanfields you want to image in “count of fields”.

Remark: you need to click enter after every change

8. Go to Setup Jobs tab (bottom left corner)
9. Set up Autofocus Job:
  - A. 64x64 pixels; Scan speed 1000Hz; Bidirectional X; Zoom 30; No averaging.
  - B. Go to “Autofocus: Best Focus” tab and select Analyze type = Reflection Based Method.
  - C. Activate only the 633nm laser and set the laser to 0.5%.
  - D. Set AOBS to reflection.
  - E. Enable PMT 3 to image the laser beam (625-640nm). Set gain between 600 and 900V.
  - F. Manually focus to find the reflection at the coverslip. Adjust gain if necessary.

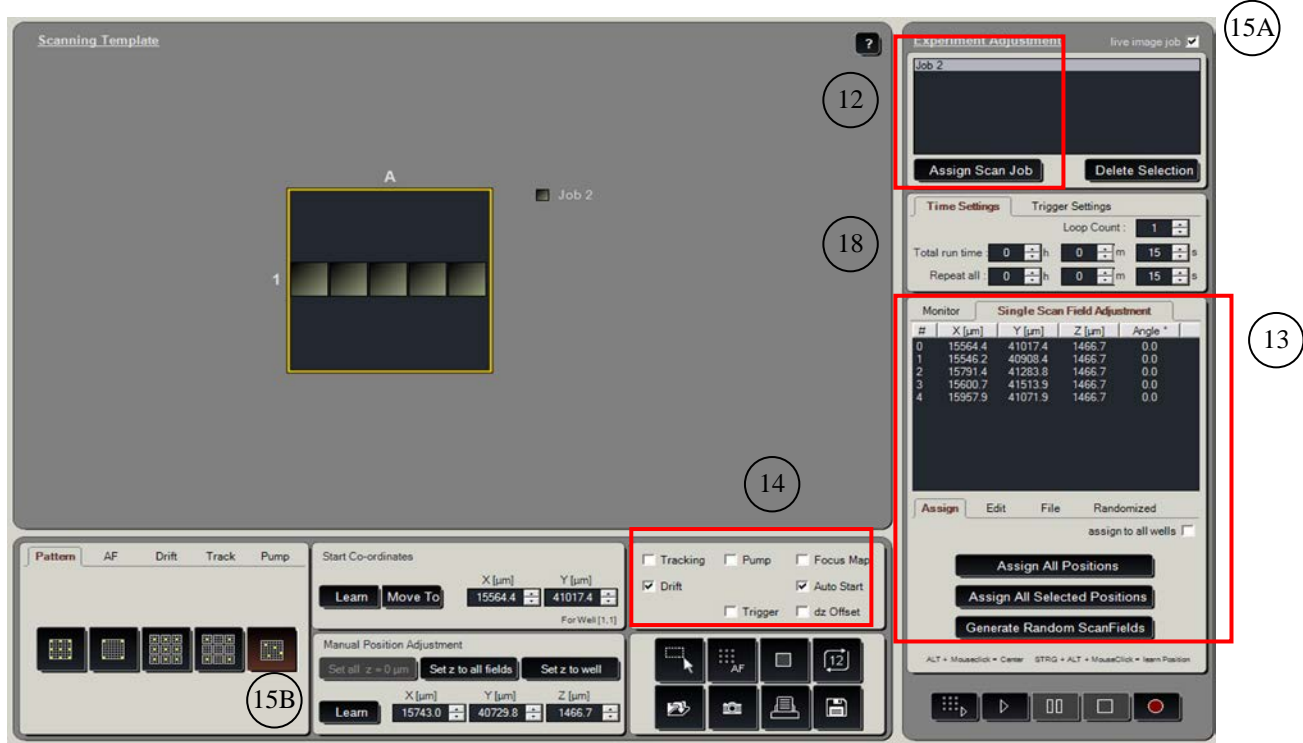


10. Select the imaging Job (Job 2) and set up your imaging parameters, including Z-stack settings (range and number of slices).


*Remark: If you want to use sequential imaging, you need to add a sequential imaging job (click "add" in top left corner).*

11. Go to Setup Experiment tab (bottom left corner).

12. Select all scan fields and click "Assign Scan Job" in top right panel.



13. Assign stage positions to scan fields.

Use live imaging (  ) to find cells. First center a cell of interest on the screen and then use Ctrl+Alt+mouseclick to store the location of the cell. After all positions are stored click “Assign all positions” to assign the positions to scan fields.

Remark: The maximum number of saved locations is equal to the number of scan fields.


14. Activate Drift control, dz Offset, and Auto Start.

Remarks: Autofocus is performed once at the beginning of the experiment. Use Drift control to repeat the autofocus routine during a time-lapse experiment.


“Auto start” will start the time lapse experiment immediately after the autofocus routine has finished.

15. Setup Autofocus.

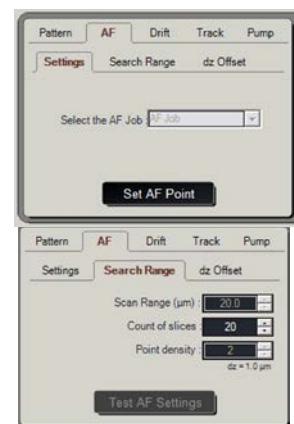
A. Unmark “Live image job” in the top right corner.

B. Select random autofocus pattern (  ) in lower left panel.

C. Go to “AF” tab and click on “Settings” tab. Select all scan fields and click on “Set AF Point”.

D. Start live imaging (  ) and click on the first scan field. Focus until you find the reflection at the coverslip. Stop live imaging.

E. Go to “Search Range” tab and set the scan range to 20, the “Count of Slices” to 40, and the Point density to 1.



15C

15E

16. Setup dz Offset.

A. Go to “dz Offset” tab. Make sure “Live image job” in the top right corner is not checked. Start live imaging, click on the first scan field and make sure you’re focused at the coverslip. Click “Set focus plane”.

B. Now activate “Live image job” again in the top right corner. Focus at the middle of the cell and click “Set image plane”.



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17. Setup Drift control (only for time-lapse imaging).

A. Go to the “Drift” tab (lower right corner). Select all scan fields and click “Set DC Point”. Set the scan range to 5 µm and the “count of slices” to 15.



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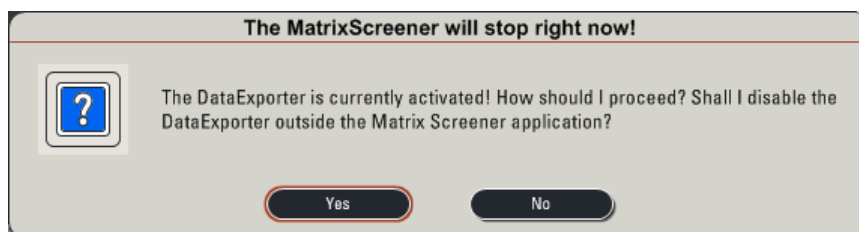
18. Set the number of loops or total duration and time delay (repeat all) of the experiment (**only for time-lapse imaging**).

19. Click (  ) to start the experiment.

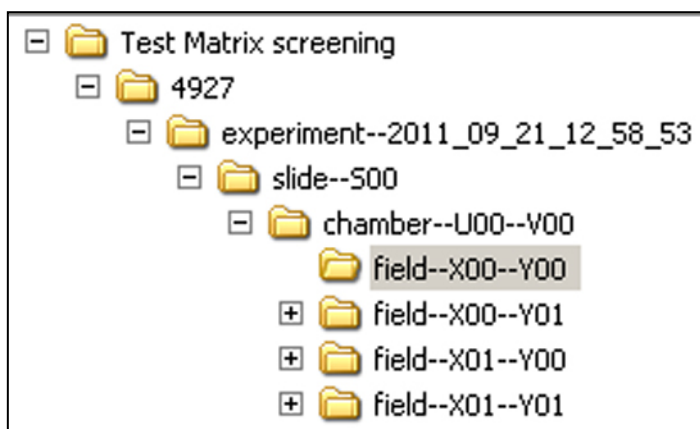
*Remark: "Auto Start" must be enabled to begin time lapse imaging immediately after autofocus is finished. Otherwise only the autofocus routine will run.*

### **Important!!!**

After finishing your experiment, close the Matrix screening wizard (bottom right corner) and **disable** the DataExporter when this message appears.



### **Export data folder structure:**



### **Structure of file names:**

image--L0000--S00--U00--V00--J14--E00--O01--X00--Y00--T0000--Z00--C00.ome.tif

Image Job                      Scan field                      Time point                      Z-slice                      Channel

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