MetaXpress® 6 Software Guide

Configuring Image-Based Autofocus During Acquisition

Date Revised 06/09/15 Version B

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Chapter Purpose

The purpose of this chapter is to guide the user through enabling and configuring an **Image-based autofocus** routine on the **Acquisition** tab.

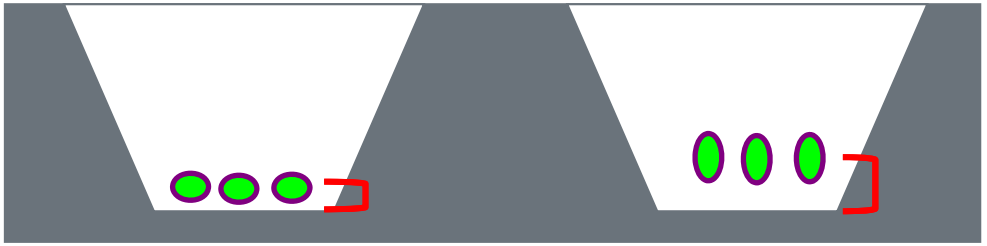
Image-based autofocus is a software focus routine used when the post-laser offset changes significantly from site to site or well to well. This can be due to variation in sample (i.e. suspension cells; cells dying and changing thickness; cells grown in a coating or gel; cells growing on top of each other; tissue sections; and whole organisms such as zebrafish and C. elegans) as well as plate variations (i.e. plate bottom thickness, coatings).

The image-based autofocus routine acquires a Z Series of images (not saved), determines the optimal focal plane and snaps an image at that position.

When Do You Add in Image Based Focus?

In most assays, all wells have the same relative offset from the laser focus (typically well bottom).

Cases where adding in a narrow range image based focus may help:



If the sample phenotype changes, such as in cases where cell goes from flat to round, the offset may vary per well.

If cells are growing on or in a surface such as a gel or coating and the amount of gel varies, the offset may vary per well.

Advantages and Disadvantages

Advantages

- Sample flexibility: Samples do not have to be adherent cell monolayers (i.e. suspension cells, tissue sections, cells grown in a matrix, or whole organisms)
- Minimize or eliminate out of focus images
- In case of laser autofocus failure, image-based autofocus can be implemented as a recovery method to acquire an in-focus image

Disadvantages

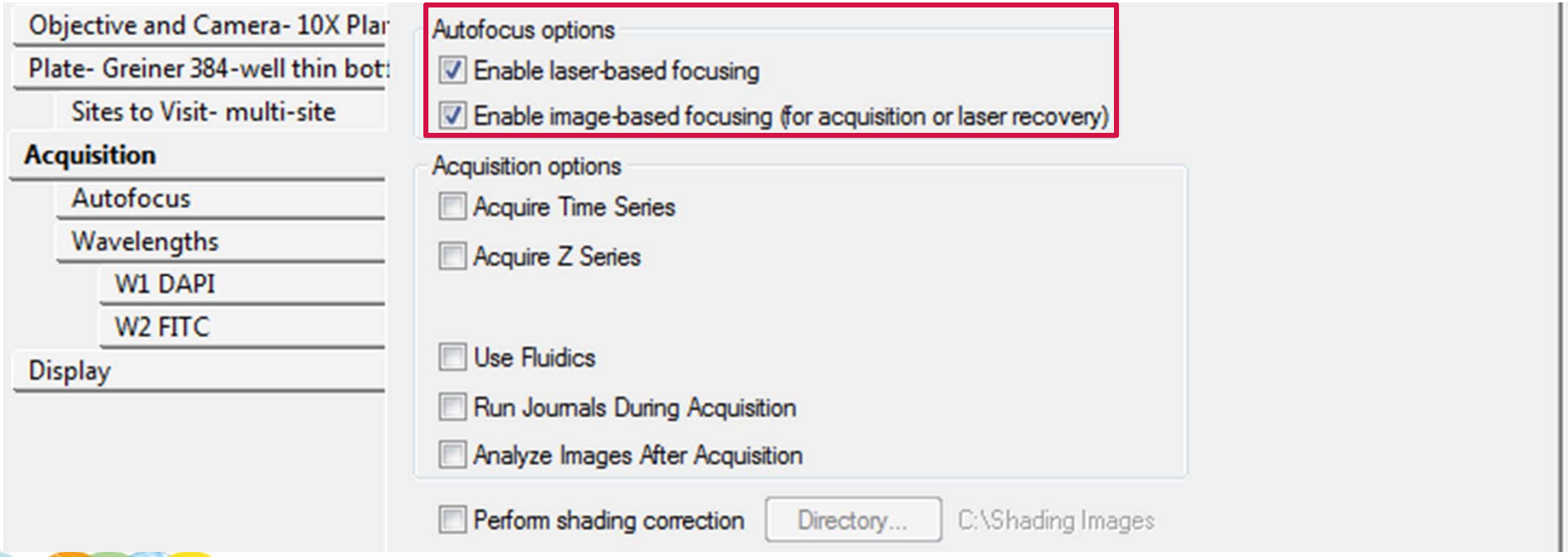
- Slower than laser autofocus: focus time increases with the number of Z steps
- Photobleaching and phototoxicity: An image is acquired for each Z step, exposing the sample to the excitation light each time. Sensitive dyes may bleach and sensitive cells may be damaged.

Enabling Image-Based Autofocus

1. Begin with setting up acquisition settings as you would normally. Refer to corresponding chapters for more details.

2. Select the **Acquisition** tab
   - Molecular Devices recommends to always **Enable laser-based focusing**
   - **Enable image-based focusing**

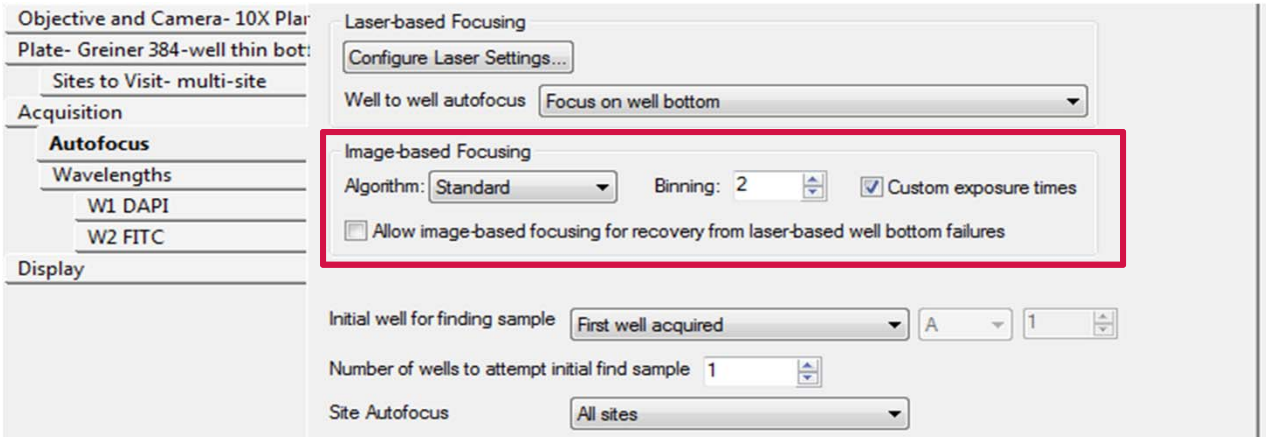
NOTE Some of the choices shown below may not appear in your version of MetaXpress



Enabling Image-Based Autofocus

3. Select the **Autofocus** tab

4. Under the **Image-based Focusing** section:
   - Set **Algorithm** to **Standard**
   - Select binning (to speed up the image-based focusing routine, set binning ≥ 2)
   - Enable **Custom exposure times** (optional): Uses a different exposure time for the focusing routine than that used for acquiring images; this is entered on the **W** tabs
   - Optionally enable **Allow image-based focusing for recovery from laser-based well bottom failures**: this option will run image-based autofocus and try to acquire an in-focus image if the laser fails to find a focal position and can be set on the **W** tabs

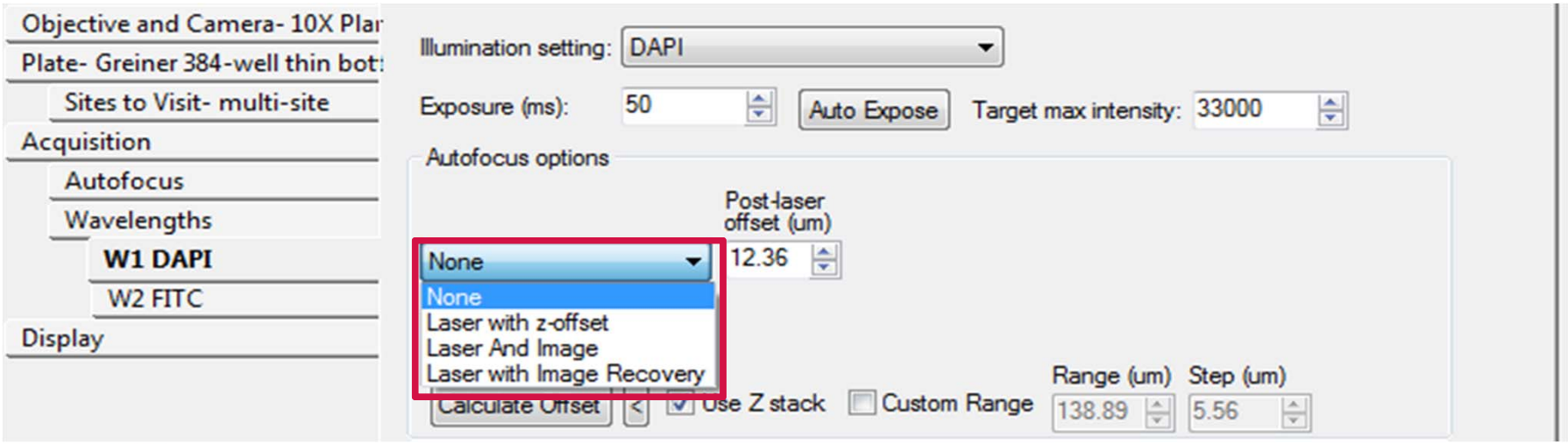


Enabling Image-Based Autofocus

5. Select the **W1** tab

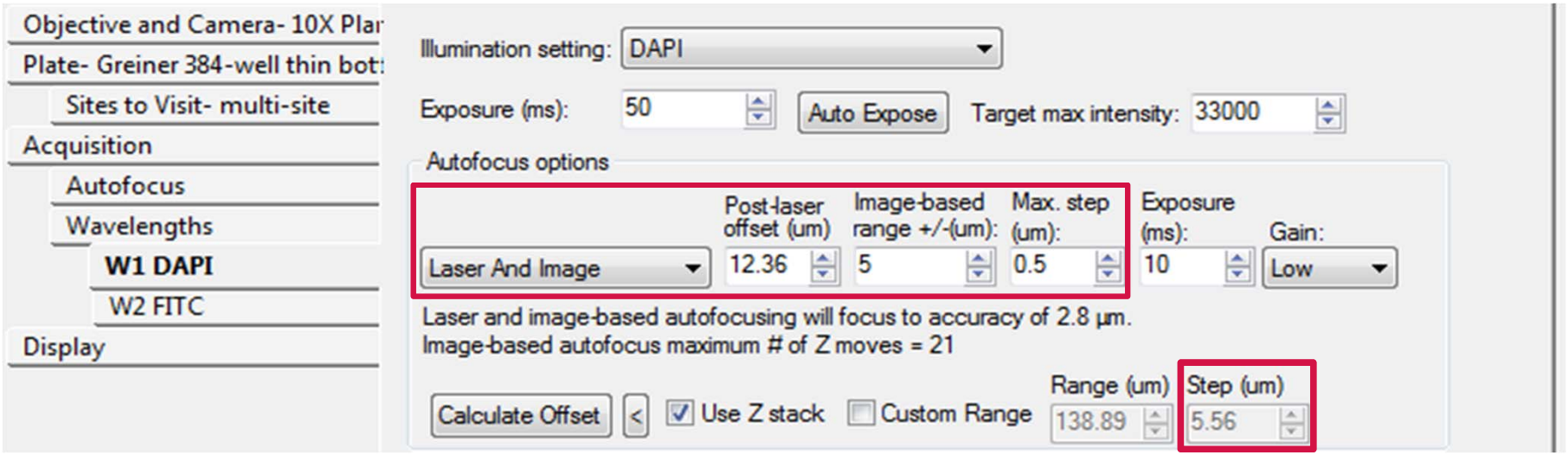
6. Under the **Autofocus Options** section
   - Select **Laser and Image** when you cannot determine a single post-laser offset that works for all positions in your plate. The software will run laser autofocus, apply the post-laser offset, then run image-based autofocus to search for the optimal focal position

NOTE Do not select **Laser with Image Recovery** if your plate or samples can be imaged with a single post-laser offset value. This option is only applied as a backup if the laser autofocus routine fails. If the laser autofocus successfully finds a the plate, then image-based autofocus will not run



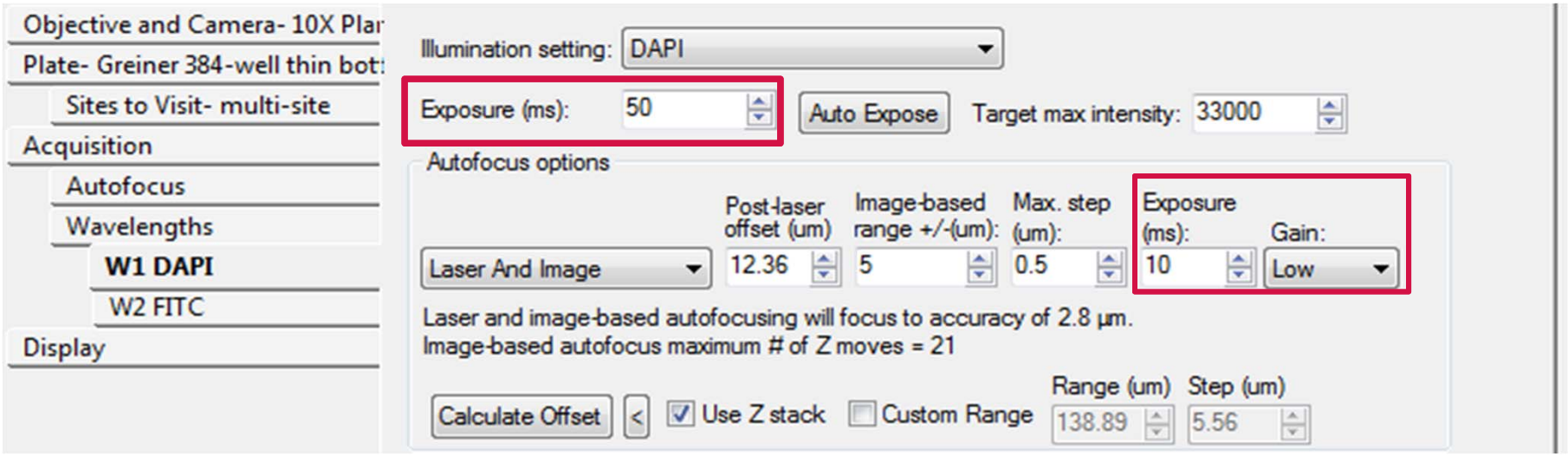
Setting Focus Range, Step Size, and Exposure

7. On the **W1** tab
   - Determine post-laser offset using the **Calculate Offset** button to get within the range of focus for the sample. If the optimal value changes significantly across a sample, set the post-laser offset to the average of the different values
   - Set **Image-based range** as the distance above and below the Post-laser offset for the software to search for focus.
   - The **Max. step** is the distance between each step in the Z search range. Start with the default value shown for the selected objective (i.e., 10X = 5.56). Use smaller values for higher magnification. Do not exceed 25 µm.



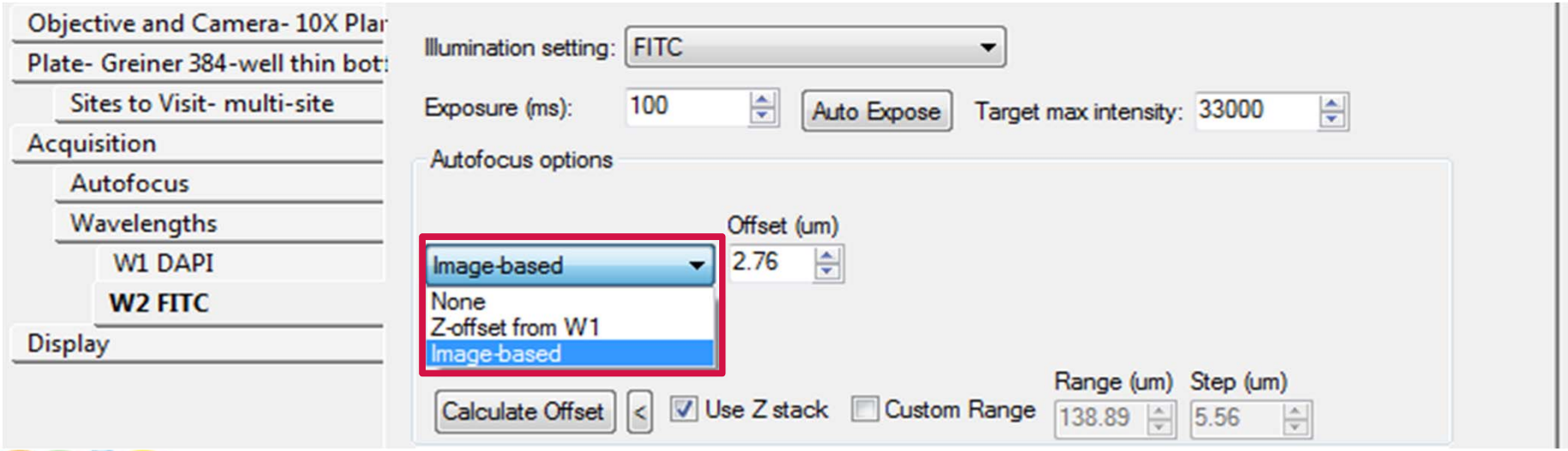
W1 Focus Exposure and Gain

8. On the **W1** tab
   - Under the **Autofocus Options** section, set the **Exposure** to a value lower than the exposure used for image acquisition in order to speed up the image-based autofocus routine
   - If the image acquisition exposure ≤ 50 ms, Molecular Devices recommends using the same exposure time for image-based focus. Disable **Custom exposure times** on the **Autofocus** tab.
   - Set **Gain** to the **High** in order to speed up the autofocus routine. This option not available if you disable custom exposure times.



Subsequent W tabs – Imaged Based Focus

9. Select **W2** tab (and subsequent wavelength tabs)
   - In many cases, image-based autofocus is only needed for wavelength 1.
   - For subsequent wavelengths, it may be possible to determine a single Offset value for all wells or the entire sample.
   - To do this, select **Z-offset from W1** from the drop-down menus and use the **Calculate Offset** function to determine focus offset
   - Select **Image-based** when you cannot determine a single post-laser offset that works for all positions in your plate or sample. Configure image-based autofocus parameters as described for wavelength 1.

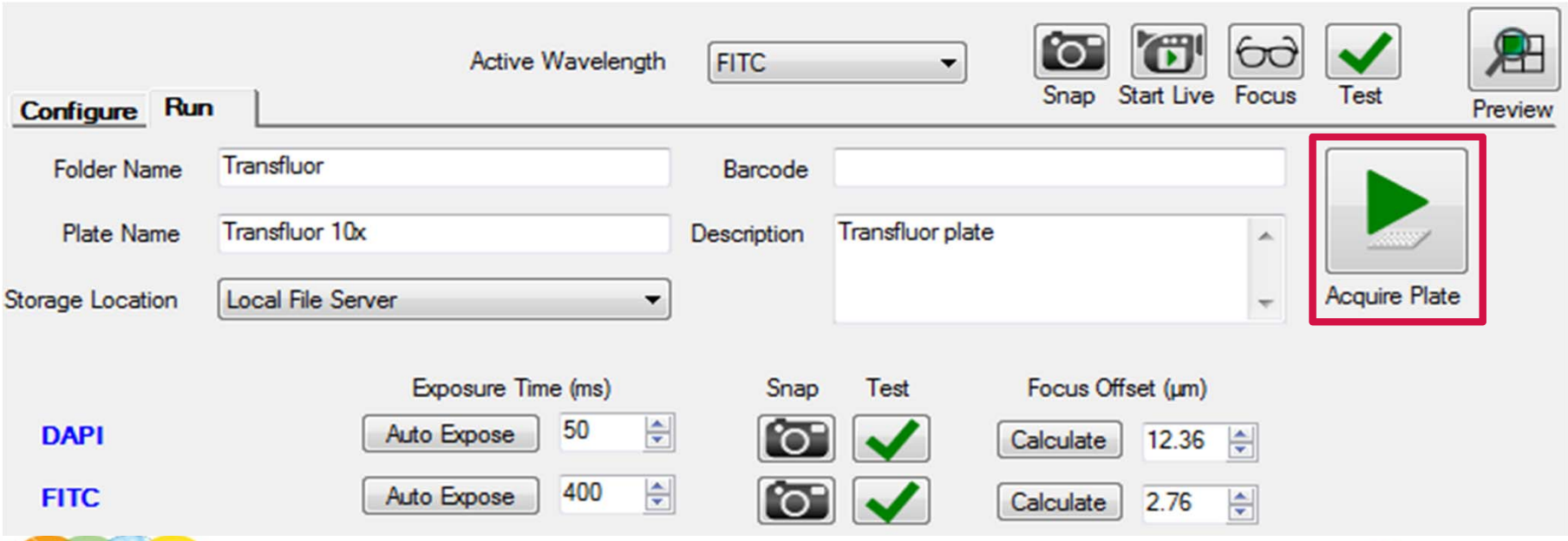


Acquiring the Plate

10. Set up acquisition parameters for the rest of the plate as you would normally

11. Click on the **Save Protocol** button to save settings. Molecular Devices recommends to save to file rather than database.

12. Click on the **Acquire Plate** button to begin acquisition of the plate



Support Resources

- F1 / HELP within MetaXpress® Software
- Support and Knowledge Base: http://mdc.custhelp.com/
- User Forum: http://metamorph.moleculardevices.com/forum/
- Request Support: http://mdc.custhelp.com/app/ask
- Technical Support can also be reached by telephone:
  - 1 (800) 635-5577
  - Select options for Tech Support → Cellular Imaging Products → ImageXpress Instruments

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