MetaXpress® 5 Software Guide

Creating a New Multi-well Plate Template

MOLECULAR DEVICES
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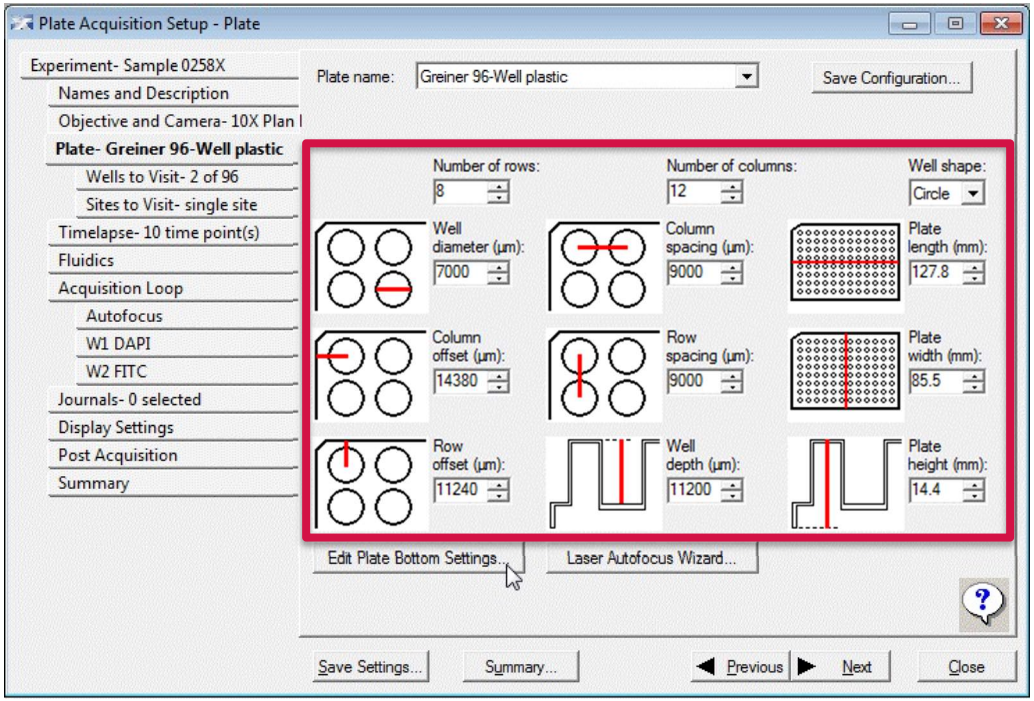
Chapter Purpose

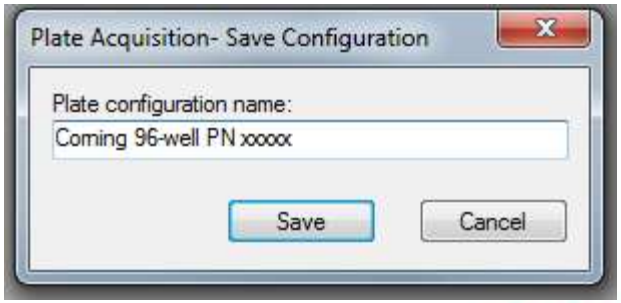
The purpose of this chapter is to guide the user in creating and configuring new multi-well plate templates. This process includes entering and testing the plate dimensions.

This chapter will not cover running the **Laser Autofocus Wizard** and troubleshooting laser autofocus failures. Refer to corresponding chapters for details on these procedures.

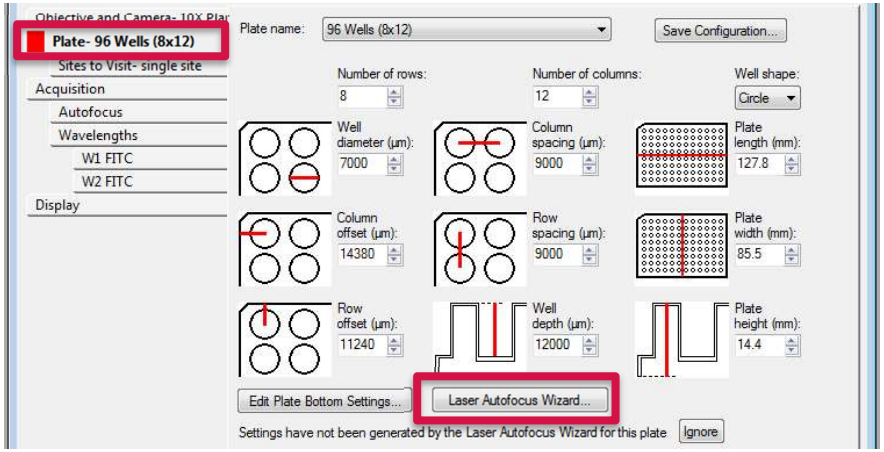
Creating a New Multi-Well Plate Template

1. In the **Plate Acquisition Setup** dialog
   - Select the **Plate** tab
     i. Select a **Plate Name** in the drop down menu that is similar to your plate (i.e. plate type, manufacturer, well format)
     ii. Enter the manufacturer's plate dimensions into each of the boxes
     iii. Click on the **Save Configuration** button
     iv. In **Save Configuration** window, change the plate name, ideally including brand, well format and part number, then click **Save**





Running the Laser Autofocus Wizard

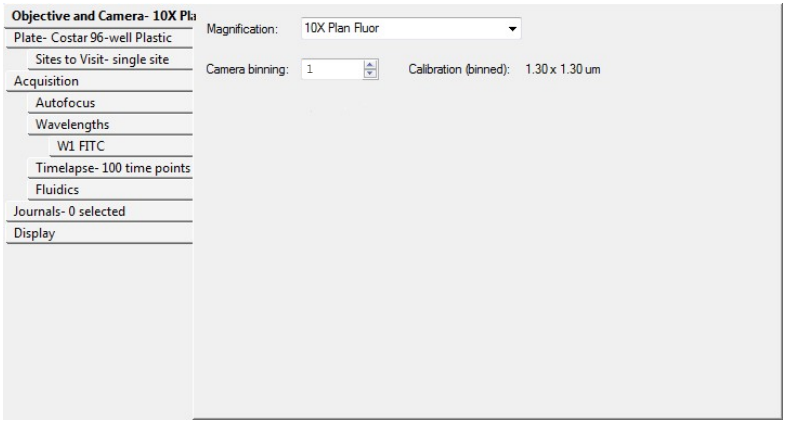


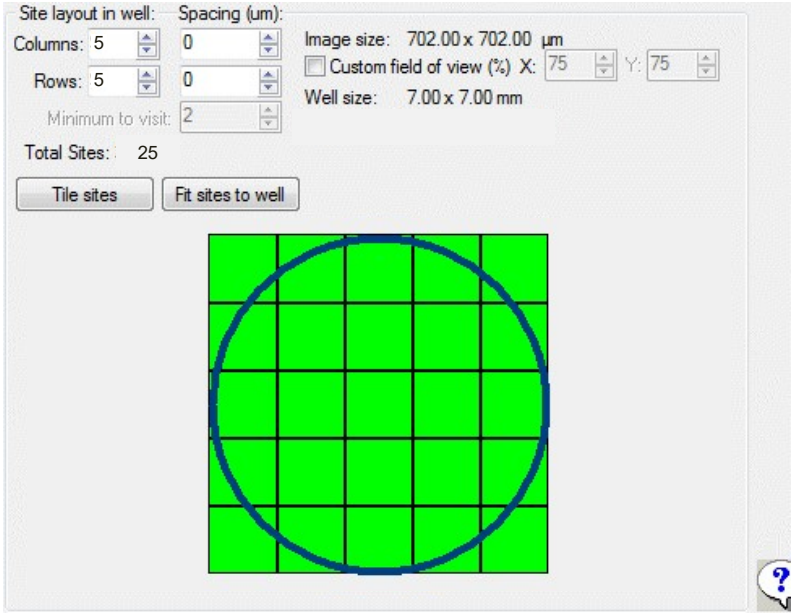
2. Refer to chapter **Running the Laser Autofocus Wizard for New Multi-Well Plates** for details
   - If Laser Autofocus settings have never been configured for the plate, a red box should appear on the **Plate** tab
   - If Laser Autofocus settings have not been configured for objective chosen, a red box should appear on the **Autofocus** tab

NOTE Laser Autofocus settings are copied from the original template to the new template; Molecular Devices recommends to always run **Laser Autofocus Wizard** on new templates.

NOTE The behavior may be different in older versions of software. In older versions, Laser Autofocus settings were not copied to the new plate file, and no red warning was shown.

Making Sure Plate Dimensions are Correct





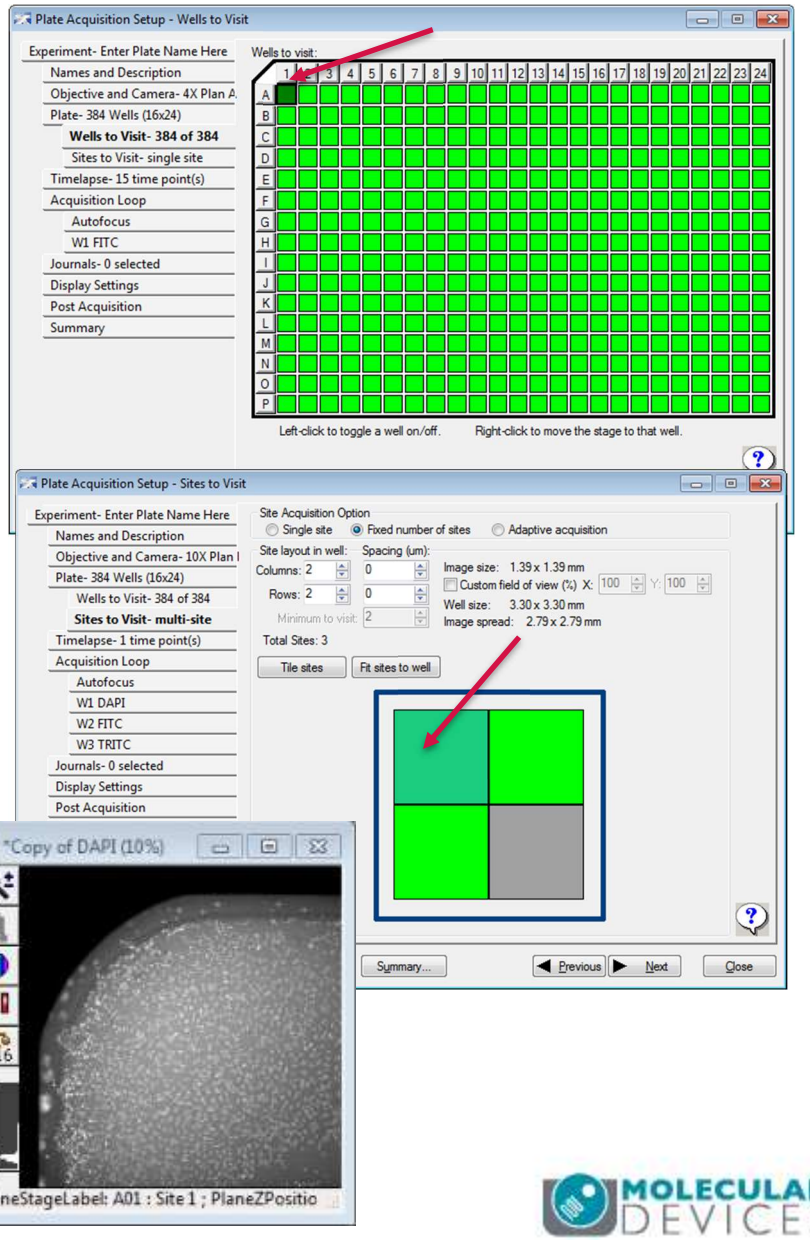
After LAF settings have been created, it may be necessary to check that the plate dimensions are correct on the **Plate** tab. Molecular Devices recommends to have a plate with sample in the 4 corners of the plate, however, not necessary.

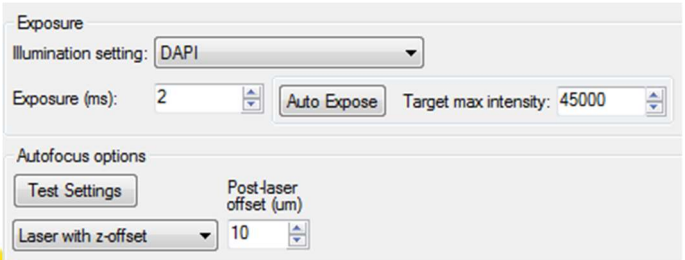
1. On the **Objective and Camera** tab:
   - Select the 10x objective
   - Set **Camera Binning** to 1

2. On the **Sites to Visit** tab:
   - Select **Fixed Number of Sites**
   - Set **Columns** and **Rows** so that the total number of sites cover the entire well. Typically four (2 X 2) sites for 384-well and twenty-five (5 X 5) for 96-well plates at 10X.
   - Set **Spacing** to 0

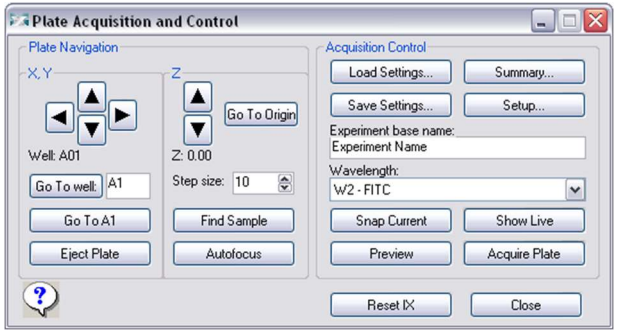
Making Sure Plate Dimensions are Correct

3. On the **Wells to Visit** tab, right-click on well A1 to move to that well. It should be highlighted in black.
4. On the **Sites to Visit** tab, right-click on site 1 to move to that site. It should turn dark green
5. On Plate Acquisition Setup, go to the **W1** tab:
   - If there is sample on the plate, verify that the appropriate filter set is selected from the **Illumination setting** drop-down menu. Otherwise, select **DAPI**
   - Click **Test Settings** to focus and snap an image
   - Adjust the **Exposure** and **Post-laser offset** as needed so that the borders of the well are visible in the image.





Making Sure Plate Dimensions are Correct

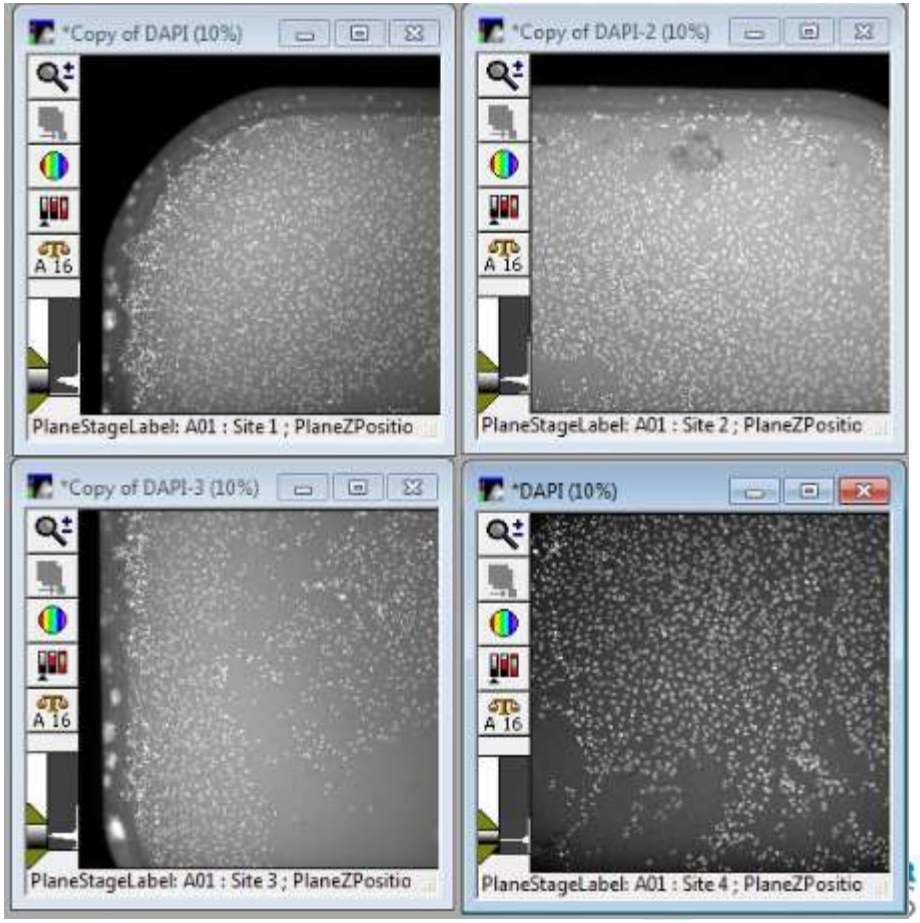


6. Go to **Screening > Plate Acquisition and Control**

7. Click on the **Show Live** button

8. Move to each of the corner sites of the well (right click in site map) and verify that you can see the well edges

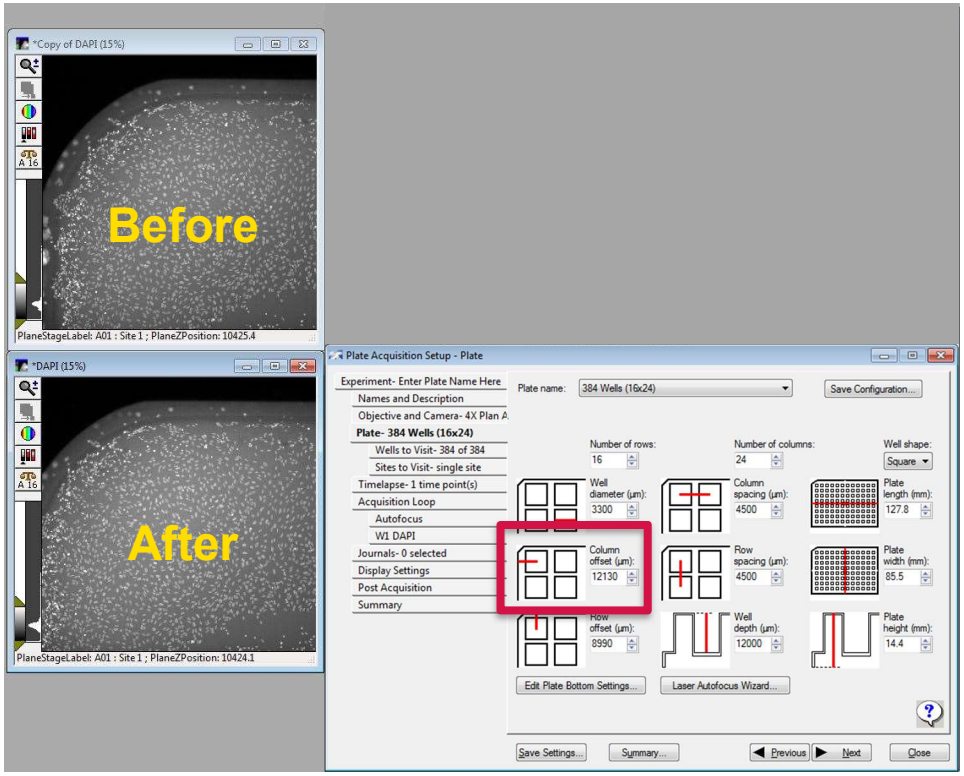
9. For example, in the images to the right, we see that the well A1 of this 384-well plate is shifted down and to the right



Making Sure Plate Dimensions are Correct

8. If the wells are shifted right or left, the **Column offset** needs to be adjusted on the **Plate** tab

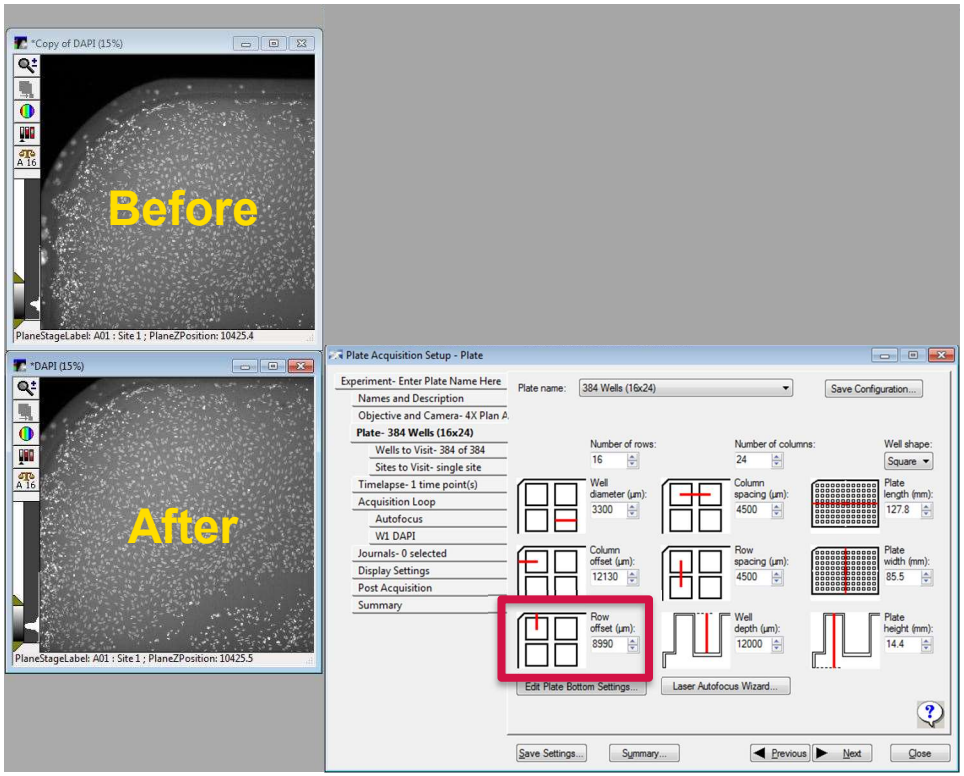
- Increase this number to shift the well left, Decrease this number to shift the well to the right
- Adjust the number and click the **Save Configuration** button
- Right-click on any other well in the plate and right-click again on **A1** for the settings to take affect
- Repeat the above steps until the well appears to be centered
- You can use the **Line region tool** to measure distances
- Begin by drawing a line from the image edge to the nearest well border. Divide this number by two and adjust the **Column offset** by this amount
- In the example to the right, Column offset was increased by 200 µm in order to shift the well left.



Making Sure Plate Dimensions are Correct

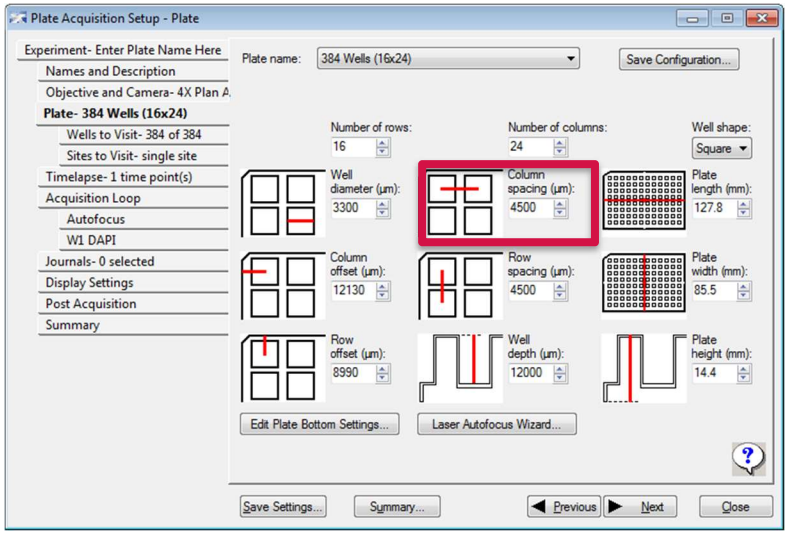
9. If the wells are shifted up or down, the **Row offset** needs to be adjusted on the **Plate** tab

- Increase this number to shift the well down, Decrease this number to shift the well up
- Adjust the number and click the **Save Configuration** button
- Right-click on any other well in the plate and right-click again on **A1** for the settings to take affect
- Repeat the above steps until the well appears to be centered
- You can use the **Line region tool** to measure distances
- Begin by drawing a line from the top of the image to the border of the well. Divide this number by two and adjust the **Row offset** by this amount
- In the example to the right, Row offset was decreased by 200 µm in order to shift the well up



Making Sure Plate Dimensions are Correct

10. After A1 has been centered, check the upper right hand corner of the plate
    - Move to the upper-right hand corner well (i.e. A12 for 96-well and A24 for 384-well)
    - On Plate Acquisition and Control, click on the **Find Sample** button, then the **Show Live** button
    - Right-click on each corner site and examine the well
        - If the well is shifted right or left, **Column spacing** will need to be adjusted



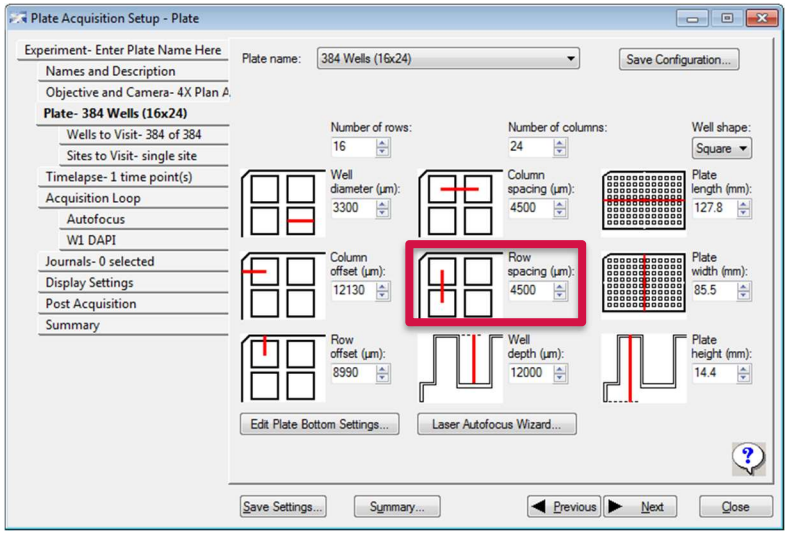
NOTE Remember to right-click on another well and then right-click on the well of interest after saving changes to the plate in order to activate the new plate dimension settings

Making Sure Plate Dimensions are Correct

12. Check the bottom corners of the plate

    - Move to the bottom left (i.e. H1 for 96-well or P1 for 384-well)
    - On Plate Acquisition and Control, click on the **Find Sample** button, then the **Show Live** button
    - Right click on each corner site and examine the well
        - If the well is shifted up or down, **Row Spacing** will need to be adjusted
    - Lastly, check the bottom right well (i.e. H12 for 96-well or P24 for 384-well)
    - If all corner wells are centered, click on the **Save Configuration** button one more time to confirm settings have been saved.

NOTE Remember to right-click on another well and then right-click on the well of interest after saving changes to the plate in order to activate the new plate dimension settings



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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