MetaXpress® 6 FAQ

HELP! I Do Not See My Sample...

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MOLECULAR DEVICES
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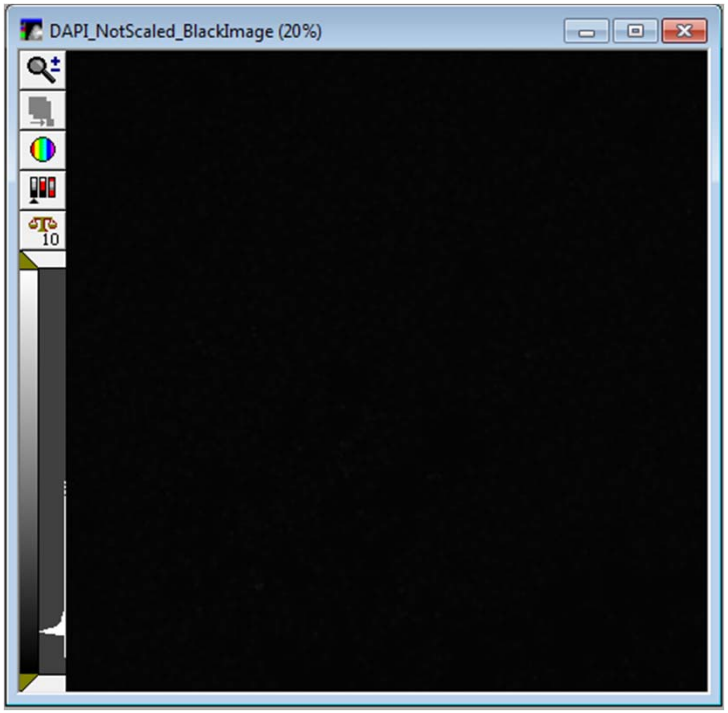
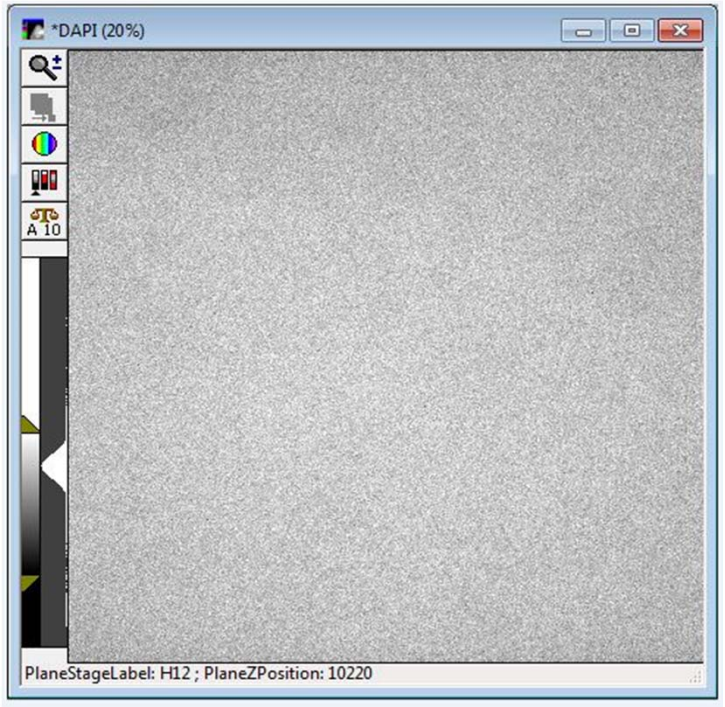
Frequently **A**sked **Q**uestions Overview

The purpose of this FAQ guide is to help the user troubleshoot image acquisition while configuring acquisition settings, such as not seeing an image, seeing out of focus images, and adjusting brightness and contrast.

Help! I Do Not See My Sample

1. While testing your sample through **Plate Acquisition Setup**, you may see a
   - Completely black, white, or solid-colored image
   - Snowy image





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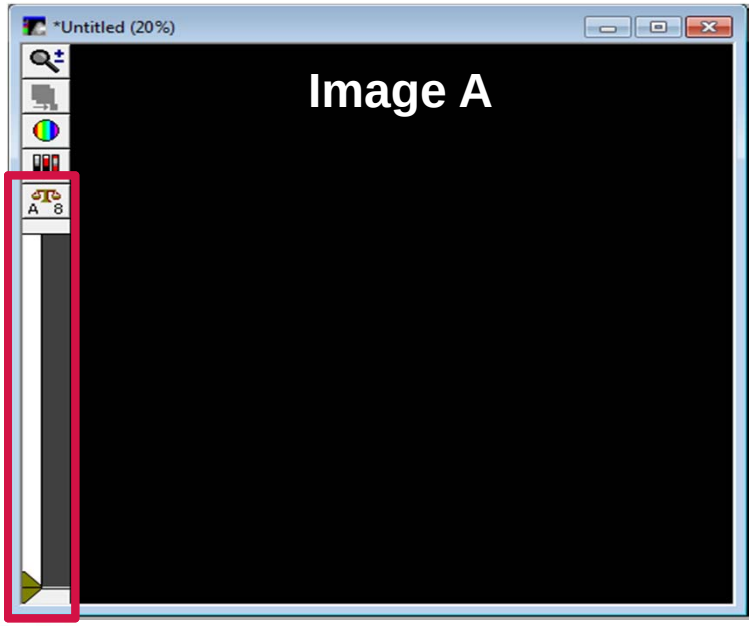
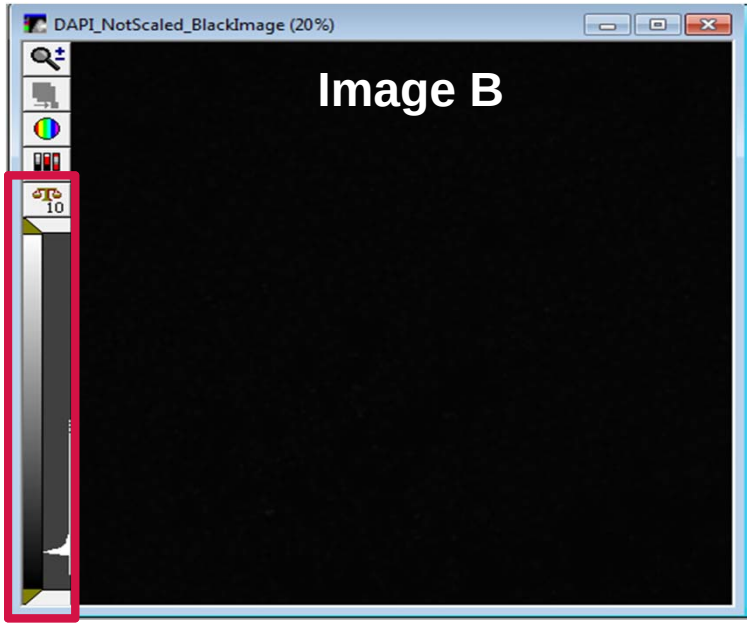
2. Examine the **Scale** icon  on the image window
   - The **Scale** icon indicates the bit range of pixel intensities in the image. **Image A** below shows the pixel intensities in an 8 bit range (< 255), while **Image B** shows pixel intensities are in a 10 bit range (< 1023). Ideally, the image should be at least 10 bit or higher. If it is not, increase the **Exposure time**.
   - The **A** next to bit range indicates **Auto Scaling** is turned on. With Auto Scale on, MetaXpress limits the image display to the lowest and highest gray levels acquired in the image.





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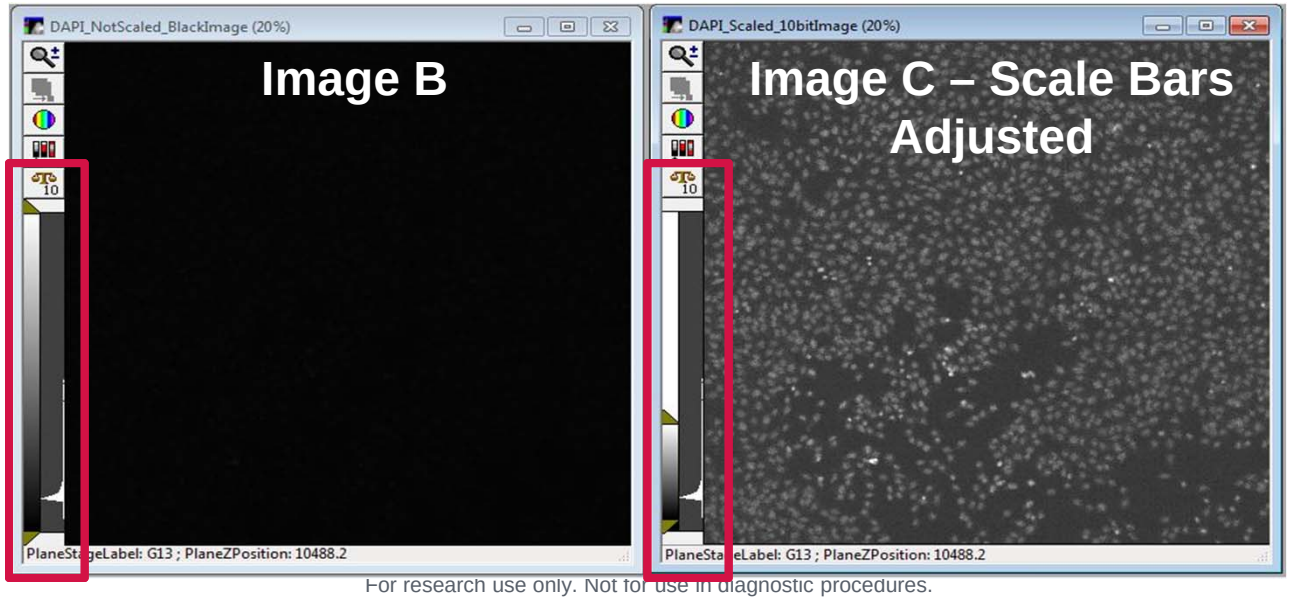
3. Examine the white histogram (vertical white graph below the Scale icon and next to the scale bars) on the image window as indicated below
   - The Histogram indicates the range of pixel intensities in the image, low intensities at the bottom and high intensities at the top. The histogram on **Image B** shows there are a range of pixel intensities in the image, however the scale sliders are set so that brightest pixels are not visible.
   - Adjust the scale sliders by enabling **Auto Scale** or using your mouse to drag the upper bar downward until you see your sample (**Image C**). Make sure the lower scale bar is below the peak at the bottom of the histogram.
   - If a histogram only contains the peak at bottom, increase the **Exposure time**



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4. If you still do not see your sample, check the following:

   - Check the bottom of the plate for fingerprints, scratches, or debris
   - Confirm the plate is in the system and loaded in the correct orientation
   - Verify you are in a site/well containing sample with signal (right-click in the Plate Map)
   - Check that the light source is on
     - Check that the physical On/Off button on the light source is set to On
     - Check that light is reaching the sample by opening the side panel and clicking the **Start Live** button. You should see light hitting the bottom of the plate above the objective.
     - If light is not reaching the sample, restart the system and computer. If this does not resolve the problem, contact technical support
   - Check that the correct filter(s) and objectives are in the system and selected

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4. If you still do not see your sample, check the following (cont.):

- If using Transmitted light (with tower)
  - Verify that the Options Controller box is turned on
  - Make sure the plate does not have an opaque seal/lid
  - Check that light is reaching the sample by placing a white piece of paper or tissue under the condenser (select the highest light settings from the illumination menu in **Plat Acquisition Setup** and click **Start Live)**
  - Confirm that shutter on the system door opens upon clicking **Focus, Test, or Preview** buttons in the **Plate Acquisition Setup** dialog
  - Verify that both the Aperture adjustment ring and Field-stop slider are open *NOTE* Refer to pages 57-58 of the **ImageXpress® High Content Screening System Options User Guide**
  - Verify that the correct phase ring in place on the TL tower for the objective selected. If not using a phase contrast objective, the ring should be rotated so that **A** faces forward.

- If using LED transmitted light
  - In **Plate Acquisition Setup,** select the appropriate illumination and click the **Start Live** button. Verify that the light is by opening the side panel and looking at the plate door (above the objective).

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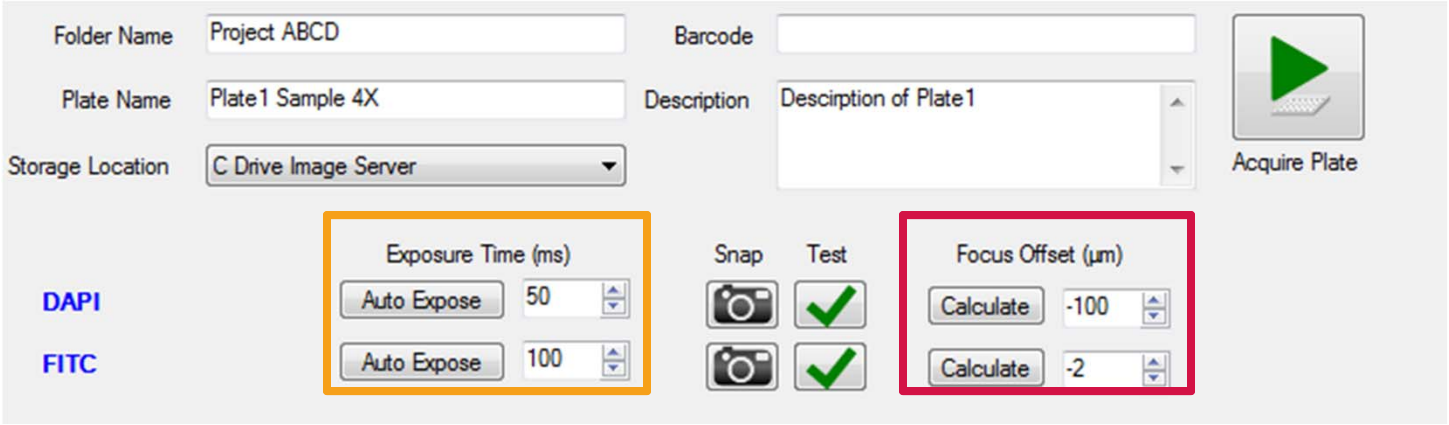
5. If the image still appears to be blank, completely out of focus or snowy:

   - Right-click on another well/site containing sample in the **Plate Map** section (ideally a well with the highest fluorescent signal)

   - Check the value of the laser Focus Offset (post-laser offset) either on the **Run** tab on the corresponding **W** tab
     - If this value is very large (positive or negative), change it to 0, click the **Calculate Offset** button and determine if your sample can be seen in the resulting image stack



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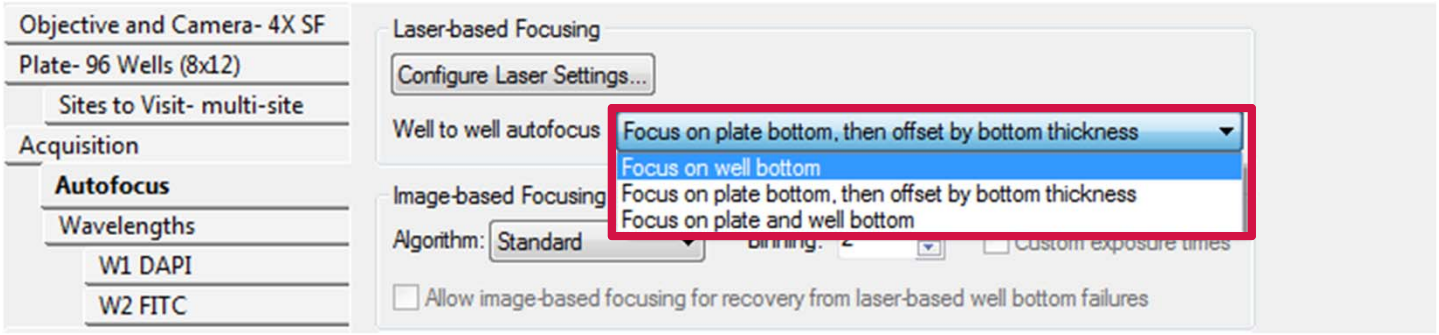
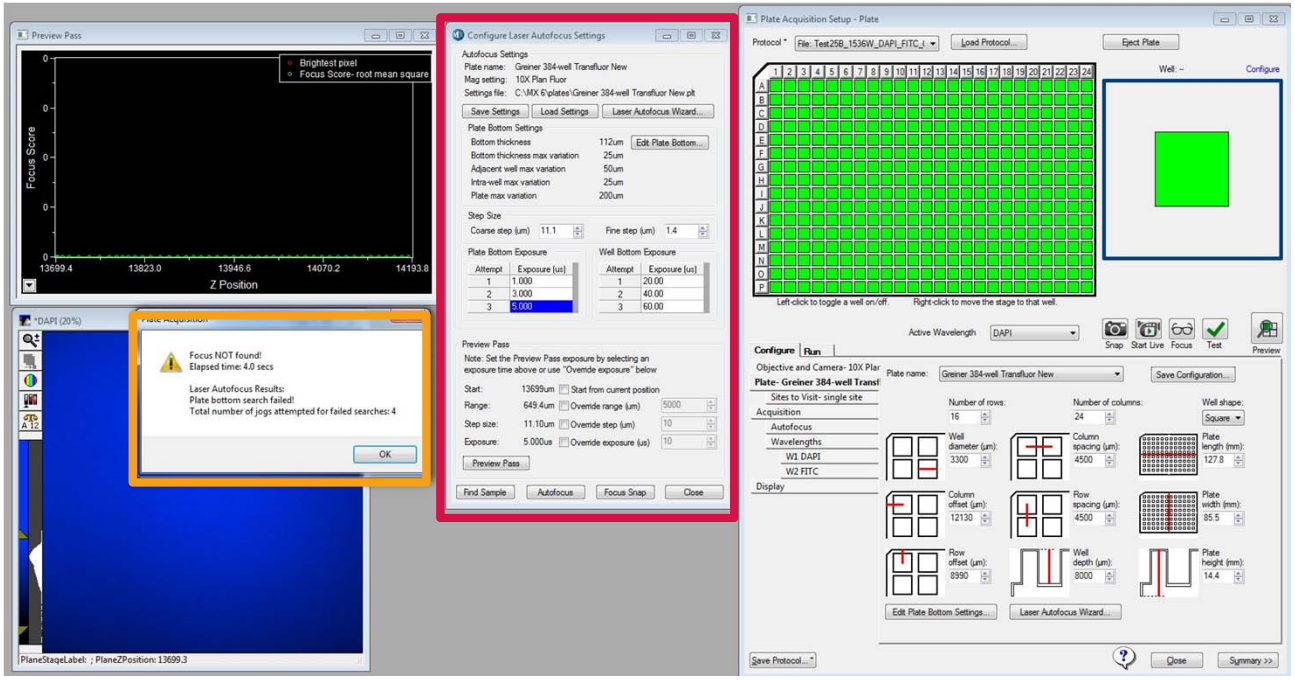
5. If the image still appears to be blank, completely out of focus or snowy:

   - Check the laser autofocus settings on the **Autofocus** tab
     - Make sure the **Well to well autofocus** settings are appropriate for the magnification and plate type chosen. Change the setting and test again.
     - Otherwise, click on the **Configure Laser Settings** button. Refer to the next section for further instructions.



Testing Laser Autofocus Settings

- Right-click on a site/well containing sample in the Plate Map section
- Click on the **Find Sample** button in the **Configure Laser Autofocus** Settings dialog
- If you get a “Focus NOT Found!” message, this indicates that either:
  - The plate type you are using and the plate type chosen on the **Plate** tab are not the same
  - Laser autofocus settings may need to be adjusted. Refer to chapter **Advance Laser Autofocus Settings and Troubleshooting** for more details.



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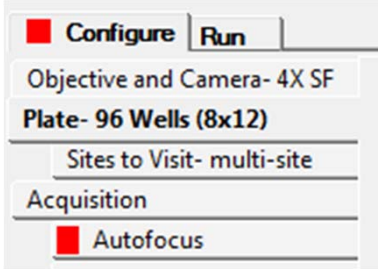
6. Continue to check the following:
   - Under the **Plate** tab in **Plate Acquisition Setup**, make sure the correct plate type is chosen
   - Make sure that a red box does not appear next to the **Configure** tab and **Autofocus** tab. This indicates that you need to run **Laser Autofocus Wizard** for this plate type and objective
   - Try a different wavelength
   - Use a stand alone microscope to verify that you have cells and/or fluorescent signal in the wells



7. If all of the above still fails, contact technical support. Please have your instrument serial number available.

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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