



MetaXpress® 6 Software Guide

Creating Color Overlay Images up to 7 Colors

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Chapter Purpose

The purpose of this chapter is to guide the user in creating a color overlay image with up to 7 individual wavelength channels, including transmitted light images.

To create color overlays (up to 3 colors-RGB) for multiple selected wells in **Review Plate Data**, refer to corresponding chapter.



Creating Color Overlay Images in Overlay Images

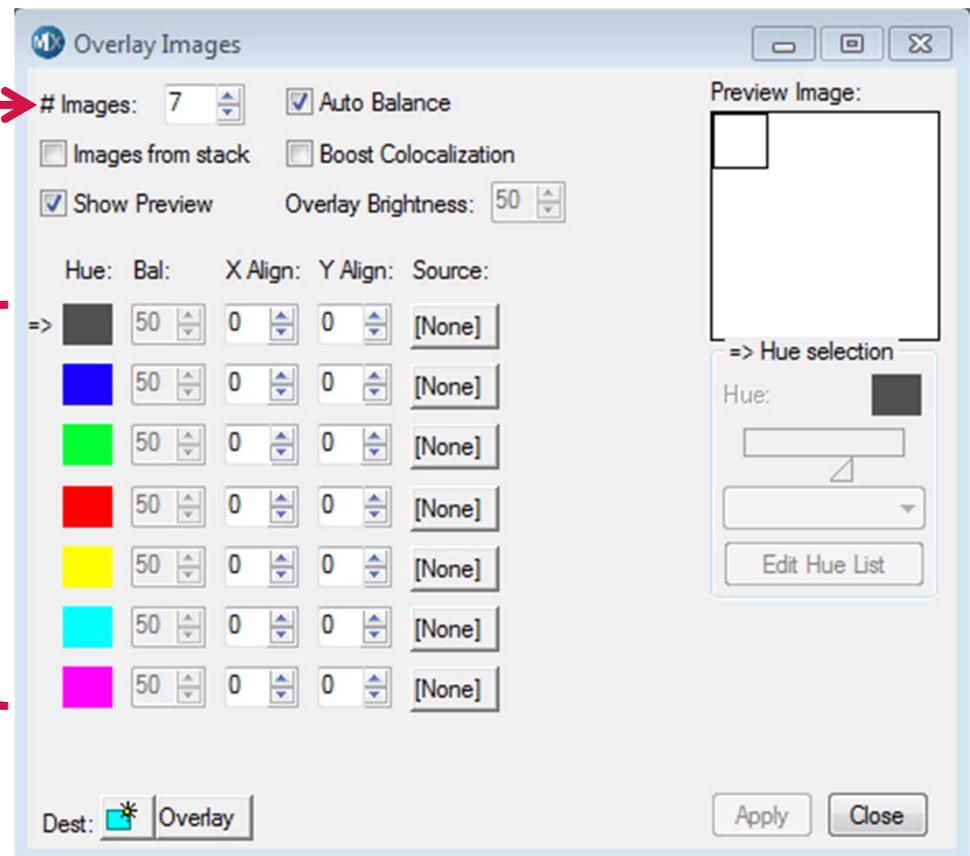
1. Open the **Overlay Images** dialog from the main menu: select **Edit > Display > Overlay Images**

- To use this dialog, source images must be either 8-bit or 16-bit images

- Enter **# Images** to overlay (max 7)

- The first color shown is always a greyscale image (transmitted light image), followed by up to 6 fluorescence images

- Example: For a 2 color fluorescence experiment, set **# Images** to 3

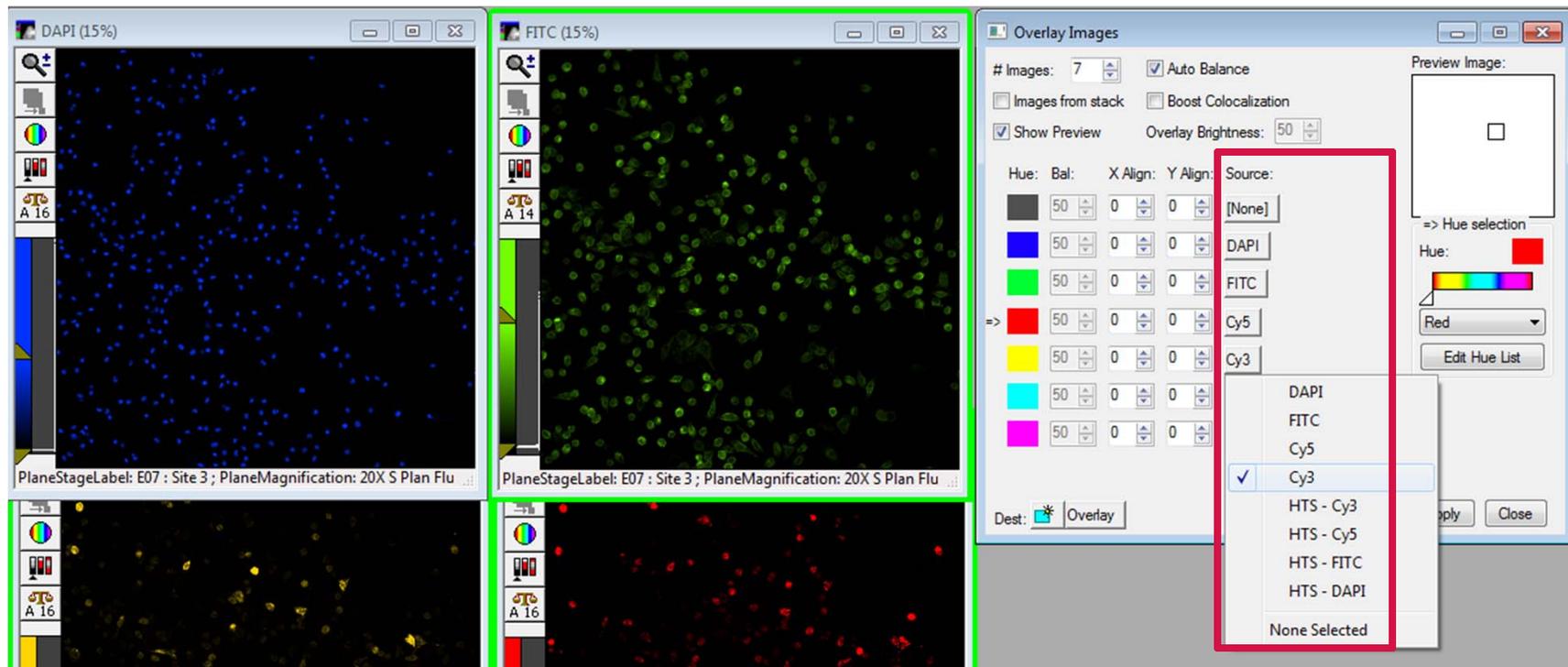


Creating Color Overlay Images in Overlay Images

2. Under **Source**, click on the button next to each color and select the image representing that color

NOTE Take note of the image names. Some images start with “HTS”; these refer to the thumbnail montages

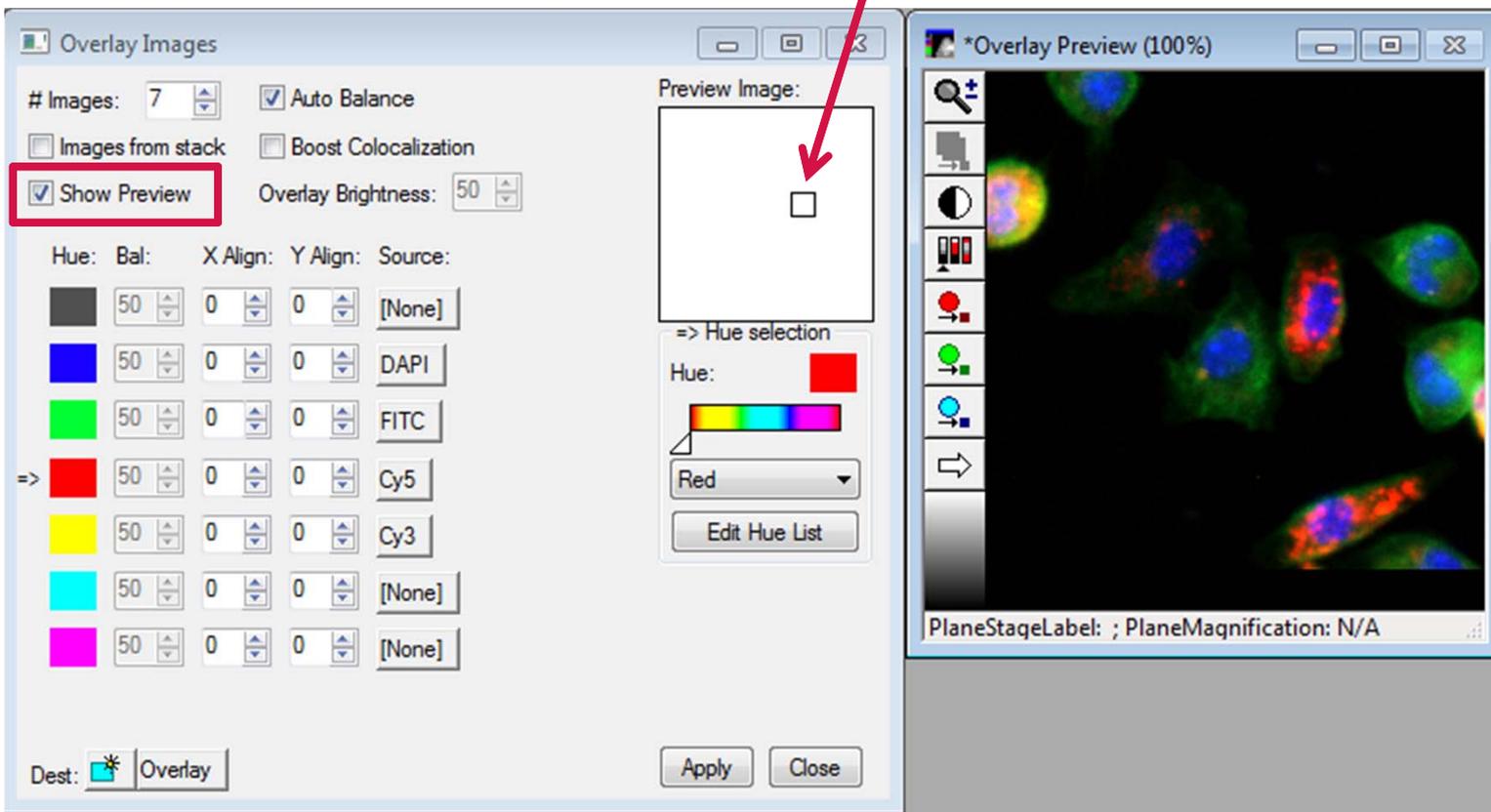
3. If you don't have a transmitted light (brightfield) image, then set **Source** to **None** next to the grey hue



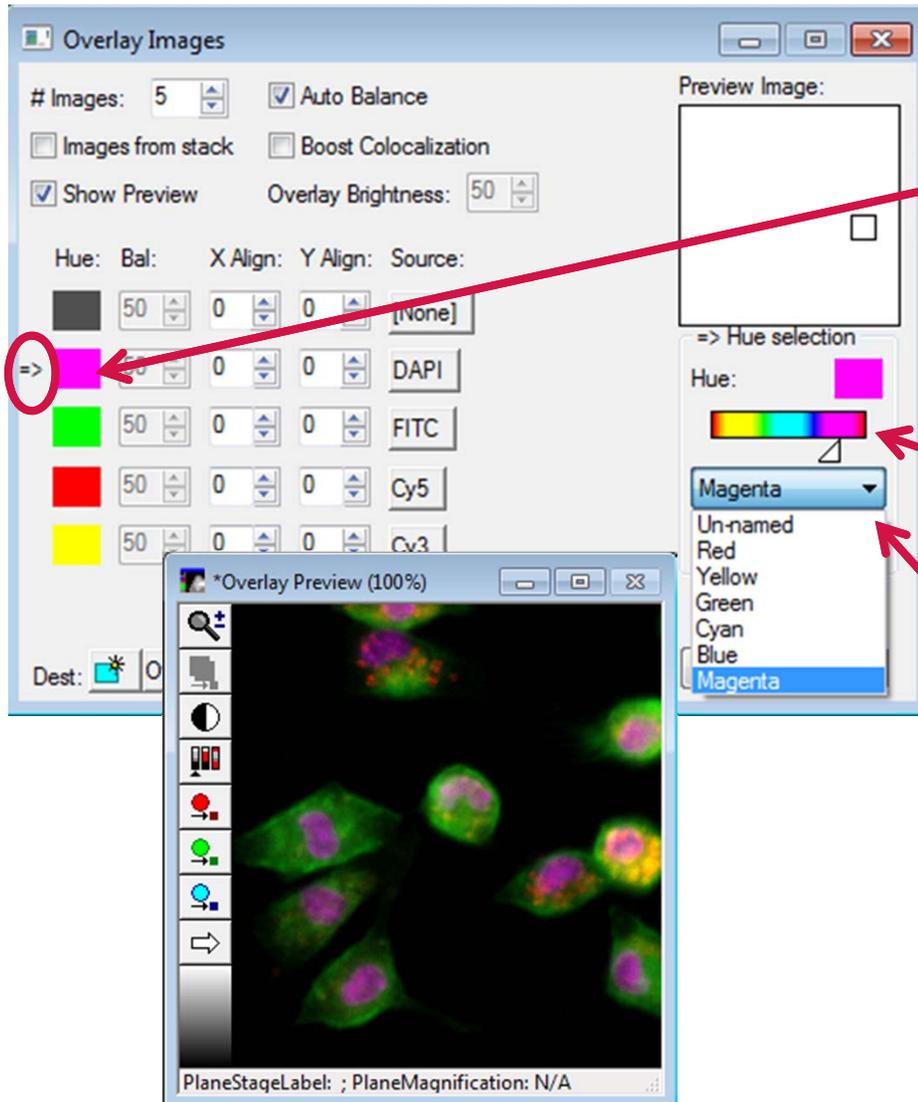
Creating Color Overlay Images in Overlay Images

4. Enable **Show Preview** to display an overlay preview image

Move the square to preview zoomed areas



Creating Color Overlay Images in Overlay Images



5. To change the displayed colors:

- Left-click mouse on the **Hue** to assign a different color
- The arrow indicates the selected color to be changed

- Choose the color by moving the slider
- Choose the color by moving the slider

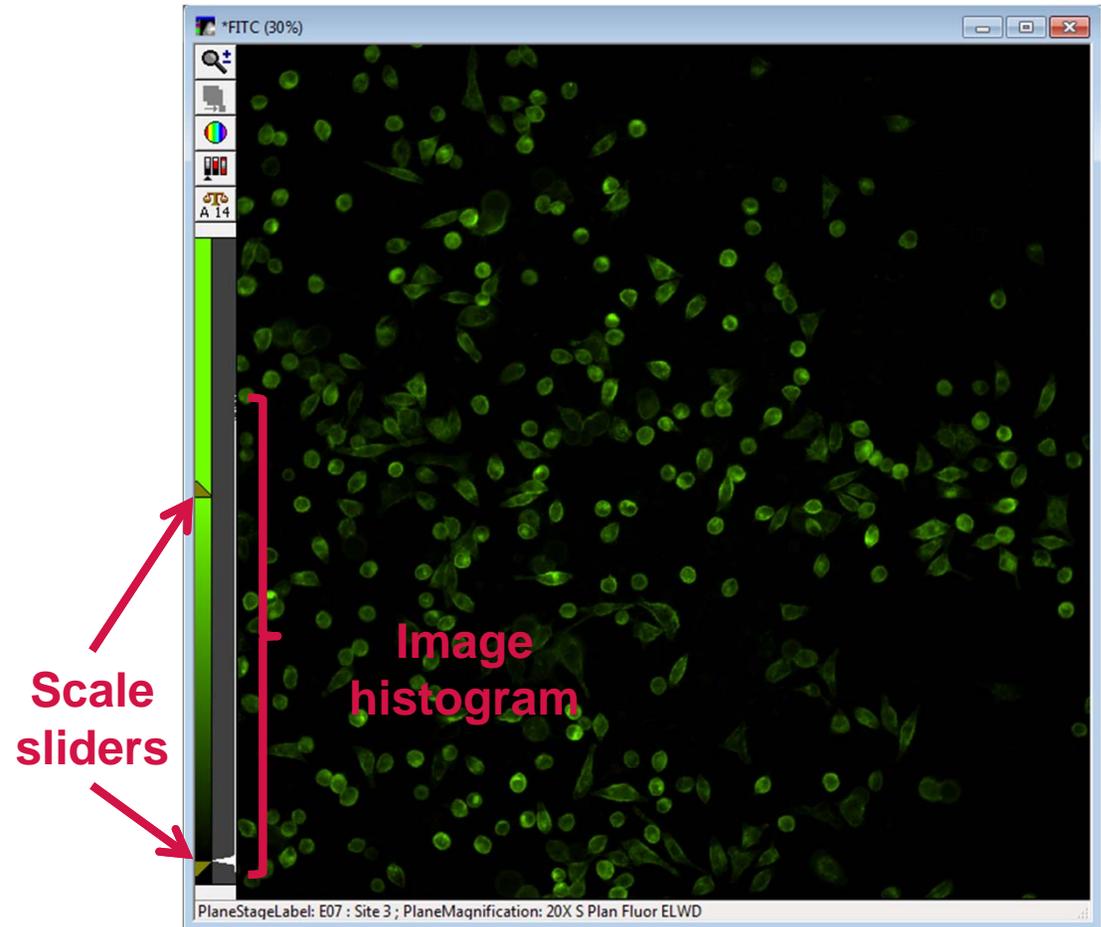
OR

- Select the color in the drop-down list
- Color changes are automatically updated in the **Overlay Preview** window



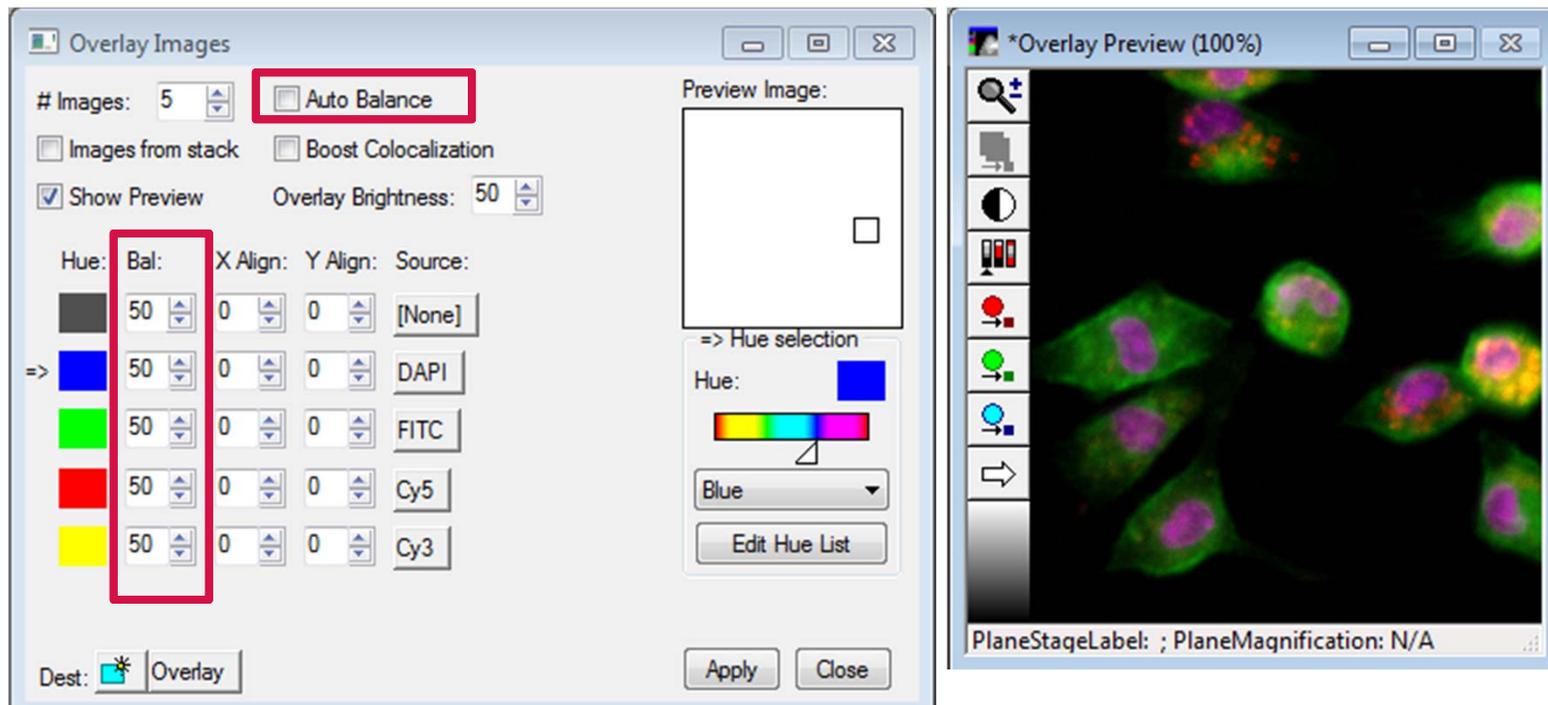
Creating Color Overlay Images in Overlay Images

- The resulting overlay image will have the image characteristics of the source images
- For example, increasing the brightness and contrast in the source image will also do so in the overlay image
- If **Show Preview** is enable, you can perform any adjustments to the source images, such as increasing contrast or scaling of the intensity range, and it will update in the Preview.



Creating Color Overlay Images in Overlay Images

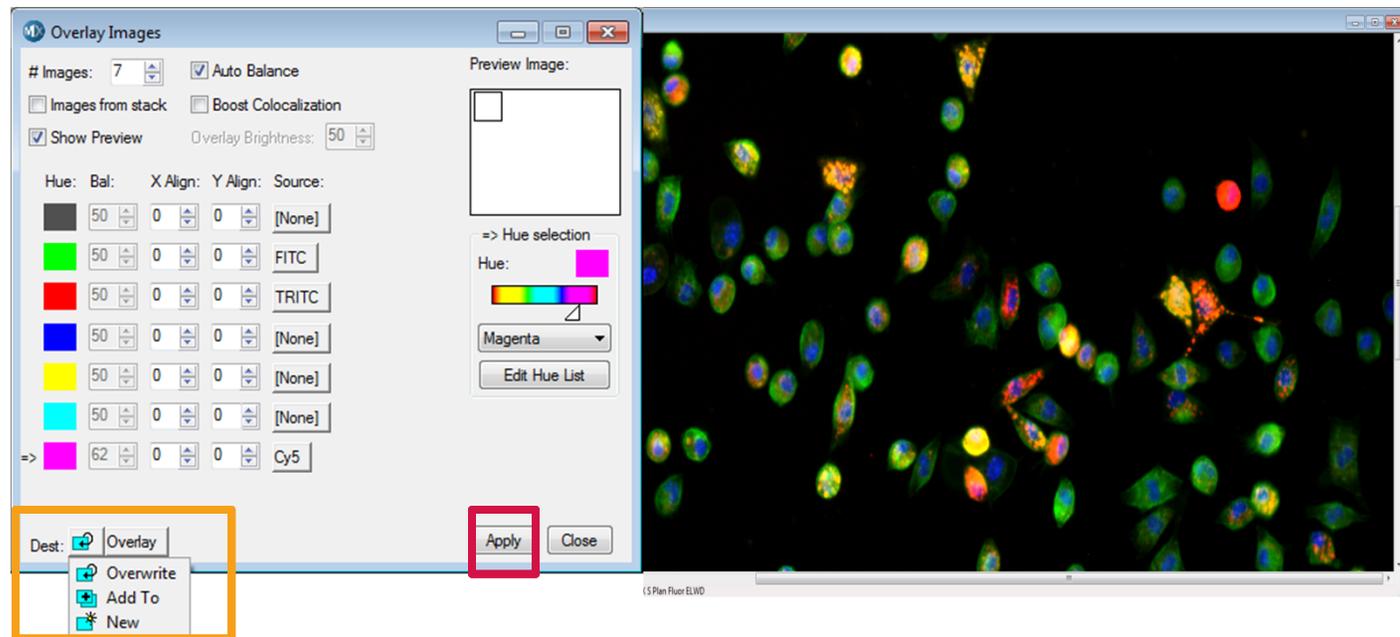
- The **Balance** (relative contribution of each image) to the final overlay image can be adjusted (optional)
 - Uncheck **Auto Balance**
 - Adjust balance under the **Bal:** column
 - Monitor the adjustments on **Overlay Preview** window



Saving Color Overlay Images

7. Click on the **Apply** button to generate the overlay image. The resulting image will be a 24-bit color image (can not be used for most image analyses)
8. To save the overlay image, right-click on the image, select **Save As**.

NOTE Everytime the user clicks on **Apply**, a new overlay image will be generated. In order to overwrite the displayed image, click on the icon next to **Dest** in the lower left hand corner and select **Overwrite**



Example: Color Overlay with Transmitted Light

The screenshot displays a microscopy software interface with three main image windows at the top: 'Transmitted Light (15%)', 'FITC (15%)', and 'TRITC (15%)'. Below these is an 'Overlay Images' control panel and two preview windows.

Overlay Images Control Panel:

- # Images: 3
- Auto Balance
- Images from stack
- Boost Colocalization
- Show Preview
- Overlay Brightness: 50
- Hue: Bal: X Align: Y Align: Source:
- Transmitted Light: Hue 44, Bal 0, X Align 0, Y Align 0
- FITC: Hue 50, Bal 0, X Align 0, Y Align 0
- TRITC: Hue 50, Bal 0, X Align 0, Y Align 0
- Dest: Overlay
- Buttons: Apply, Close

Preview Windows:

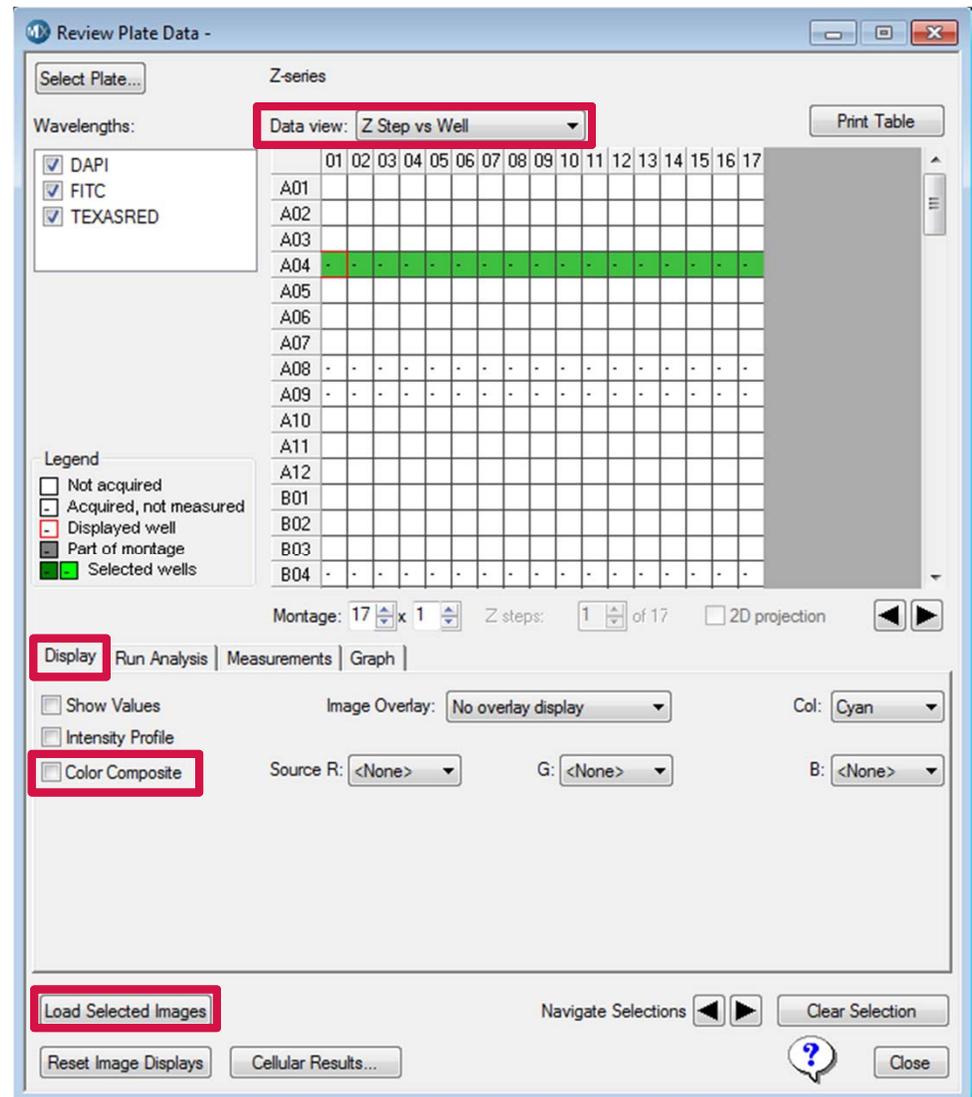
- *Overlay Preview (120%) - Auto Balance OFF:** Balance adjusted. The overlay shows significant background noise and uneven brightness.
- *Overlay Preview (120%) - Auto Balance ON:** Automatic balance. The overlay shows a much cleaner and more uniform image.



Creating Color Overlay of Image Stacks

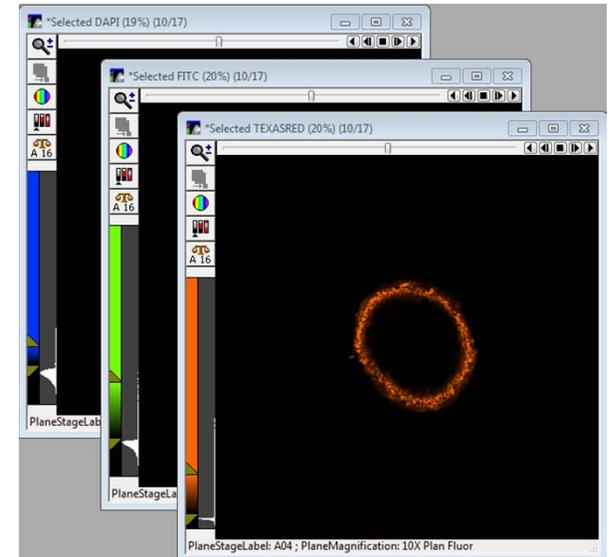
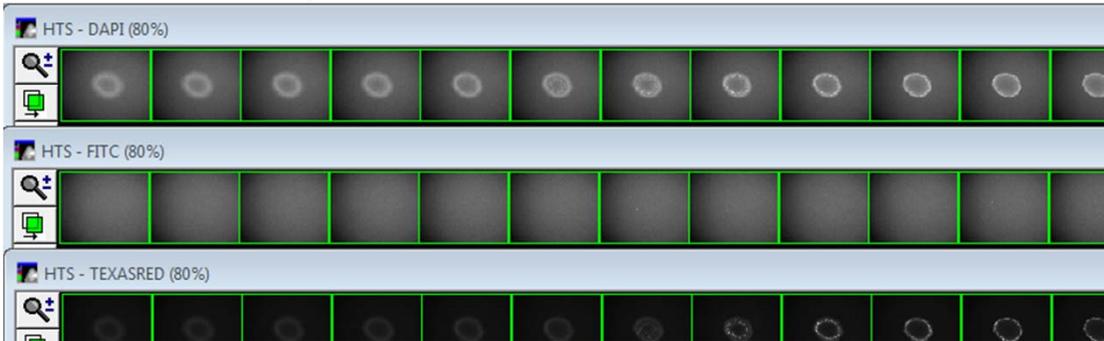
1. To overlay image stacks, open **Review Plate Data** and select the plate of interest by clicking on the **Select Plate** button
2. In **Data view**, select **Z Step vs Well** or **Time vs Well**
3. Select the well of interest
 - Right-click within plate view to select all Z steps or Time points of one well

Note* If multiple sites have been acquired, de-select **All Sites and click on the site of interest*
4. On the **Display** tab, uncheck **Color Composite**
5. Click on the **Load Selected Images** button to create a stack of all Z-steps or Time points

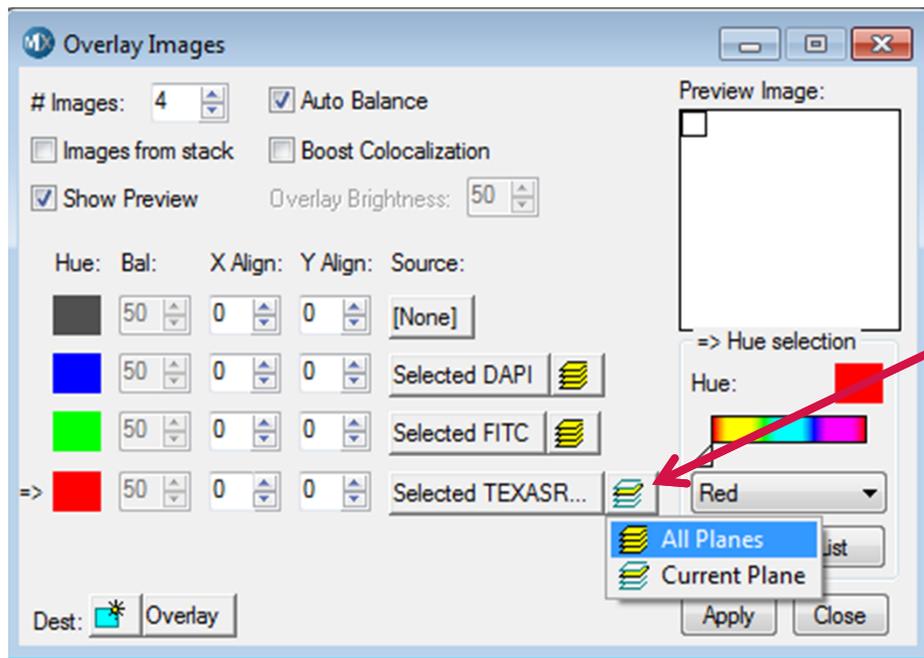


Creating Color Overlay of Image Stacks

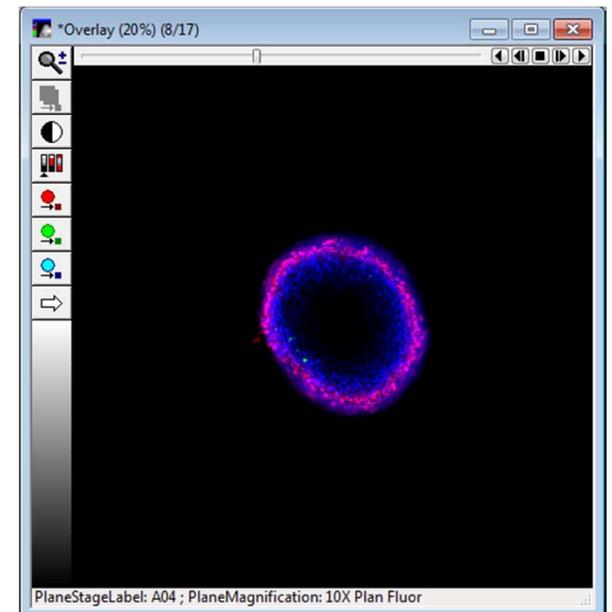
Images to be loaded: Green outline



All planes loaded



Click here to select **All Planes** to overlay whole stack



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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