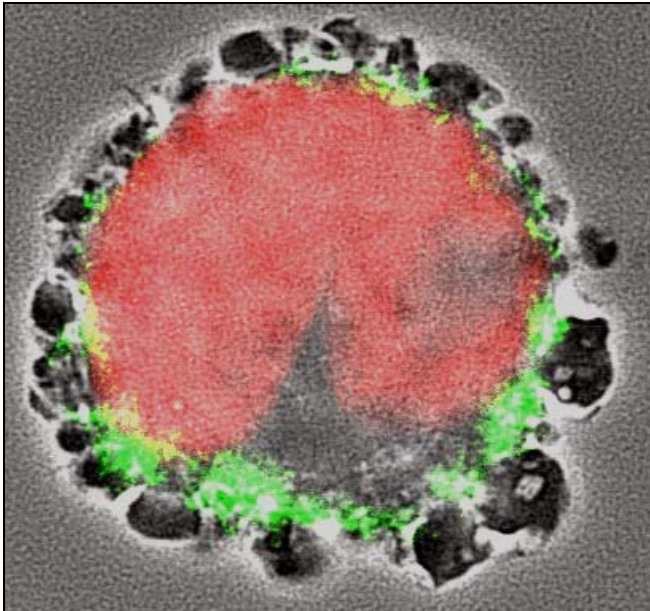


# Image-Pro<sup>®</sup> Plus Product Note

## Color Composite



Phase Contrast/FITC/Rhodamine Composite of an MEG-01 cell.  
(Courtesy of Doug Franks- University of Ottawa)

### Introduction

Combining multiple fluorescent images into a composite is a common practice. Usually, fluorescent wavelengths are acquired individually and then merged together. And often times it is necessary to acquire and merge a fluorescent image and an image acquired through brightfield techniques. For a variety of reasons it is not always possible to acquire the images simultaneously, so image processing tools are necessary to present a complete and accurate qualitative picture.

Several issues need to be considered when combining fluorescent images. Since images of this type are typically acquired in a monochrome environment, it is necessary to have tools to colorize the individual images. Registration issues need to be considered as well, as chromatic shift and other effects may be revealed in the composite image. Finally, three dimensional fluorescence stacks may need to be merged together into a composite image to view the interactions of the various fluorochromes with one another.

### Applications and Examples

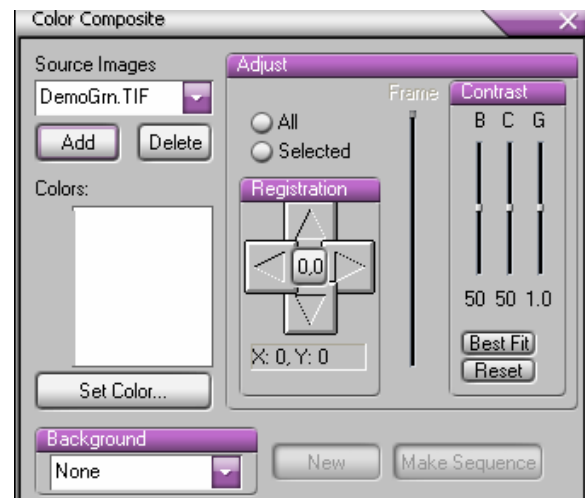
The Color Composite feature is found in Image-Pro Plus v. 4.5 and higher. It contains functions for the creation, coloration, registration and contrast enhancement of composite images. Additional features are present to merge image stacks into three-dimensional and composite images as well as methods to subtract background.

The Color Composite tool is used primarily to merge and register multiple fluorescent images acquired as monochrome single wavelengths into a color composite image. Two useful tools for acquiring these images are AFA<sup>™</sup> and Scope-Pro<sup>®</sup>, modules designed to work as plug-ins for Image-Pro Plus. Afterward, the image may then be evaluated through the **Measure|Colocalization...** command for the presence of colocalized signal. It is also an excellent tool for merging brightfield/fluorescence combinations such as those created using Green Fluorescent Protein (GFP) and Differential Interference Contrast (DIC) images.

It is important to note **Process|Color Composite...** returns a 24-bit image. It has been designed as a visualization tool, and is not intended for quantitative analysis.

### Implementation

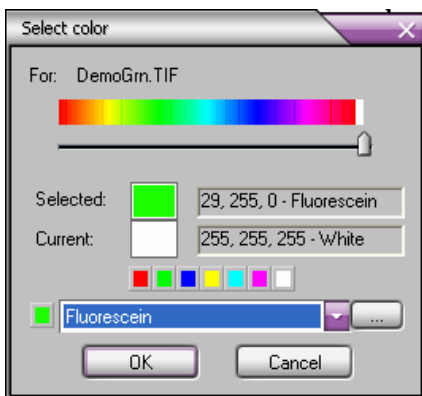
Process|Color Composite...



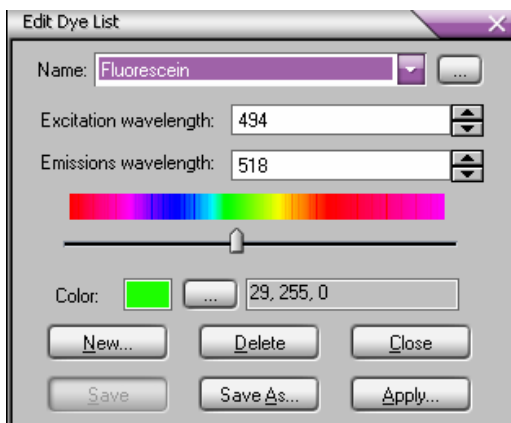
The Color Composite Window

*Source Images*- This box allows you to select an image to be added to the composite image. Note that once any image has been chosen for the colors, only images of the same size will be listed in the Source Images list.

- Click 'Add' to add an image shown in the Source Images drop-down box to the composite image.
- Click 'Delete' to remove the currently selected image from the composite.
- After image selection, apply a color to the image using the 'Select Hue' slider or by selecting one of the predefined fluorescent dyes in the drop-down list, then click 'OK'.



- For brightfield images, simply click 'OK' without selecting any color value.
- To create a custom-defined LUT for a non-listed fluorochrome, click '...' The following box appears:



- The dropdown list contains the list of currently available dyes.
- Click 'New' to create a new dye.

- Click 'Delete' to remove the highlighted dye from the list.
- Click 'Close' to close the Edit Dye List dialog.
- Click 'Save' to save a particular lookup table. Alternatively you may click 'Save As...' to save a revised LUT to a new file.
- Click 'Apply' to place the selected lookup table on the image.

*Colors* - These are the images that have been added from *Source Images*. The 'Set Hue' button at the bottom of the list allows you to change the look-up table information for selected images.

- Once Source Images have been added to the list, color adjustment may be necessary. Select an image by highlighting it. Click 'Set Hue' and the Select Hue slider will reappear. Make any color adjustment and click 'OK'.

The **Background** grouping is used to select a background image from one of the source images. All other images are subtracted from the background prior to display, allowing them to show through. This is particularly useful for combining brightfield images with fluorescent probes. For example, a DIC image can be combined easily with a GFP image by selecting the DIC image as the background, allowing the GFP image to show through. Another example would be to select a DAPI image as a background, allowing gene probes to show within the nuclei without color mixing.

The **Adjust** grouping permits contrast adjustment of individual image planes, stacks of images or composite images through the 'Contrast' feature. Use these controls along with highlighting source images in the Colors window to choose which image you are adjusting.

The **Registration** feature permits you to move one source image or stack of images relative to the other(s) present.

- Click 'Selected' and highlight the image in the Color Composite list. Use the Registration arrow buttons to shift the image in the appropriate direction(s).
- Click 'All' and use the Registration arrow buttons to shift the image in the appropriate direction(s).

The **Frame** slider allows you to move to different positions within a stack image.

- Click and hold the frame slider to move to different points in the image sequence. The sequence may consist of either a time-lapse or through focus series of frames.

The **Contrast** grouping allows you to change contrast settings for one or all of the images in the composite.

- Click 'Selected' and highlight an image from the Source Images list to adjust the brightness, contrast and gamma for the selected image.
- Click 'All' to adjust brightness, contrast and gamma for the entire image.
- 'Best Fit' performs a histogram stretch of the selected image plane or entire image.

The 'New' button allows you to create a new composite image from the images and stacks open in the Image-Pro workspace.

- Click 'New'. The images that are currently open will appear in the Source Image list.

The 'Make Sequence' button creates a composite stack from individual stack images added through

*Source Images.* This tool is especially useful for combining multiple single-wavelength stacks into a composite image. The composite image stack may also be opened in 3D Constructor™, an Image-Pro Plug-In module.

## See Also

Applications Note- Colocalization of Fluorescent Probes

Solutions Note #1275- Three-Dimensional Fluorescence Colocalization

Image-Pro Plus Product Note- Colocalization

## Related Products

- Advanced Fluorescence Acquisition (AFA™) Microscope and Peripheral Automation Plug-In
- Scope-Pro® Plug-In
- SharpStack™ Deconvolution Plug-In
- 3D Constructor Three Dimensional Display and Rendering Plug-In

## How To Order

For more information on Image-Pro Plus, and to locate a Media Cybernetics' reseller in your area, visit our website at [www.mediacy.com](http://www.mediacy.com).

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