

Image-Pro[®] Plus Product Note

Co-localization

Introduction

In fluorescence microscopy, co-localization is a useful tool for determining the levels of two fluorescent probes binding at the same apparent physical location within a cell or subcellular structure. Obtaining relevant qualitative information in the form of overlapping regions is of interest to an investigator, as is the quantitative aspect of that overlap. Reporting data in a commonly understood and accepted format is of great benefit to those who may need to submit data to publications for review and publication.

Applications and Examples

Image-Pro Plus contains a co-localization function that provides you with the tools to display both qualitative and quantitative results for co-localization analyses. It is used primarily in traditional co-localization studies measuring the degree of overlap of fluorescent signals, but may also be used in Fluorescence Resonance Energy Transfer (FRET) applications as well.

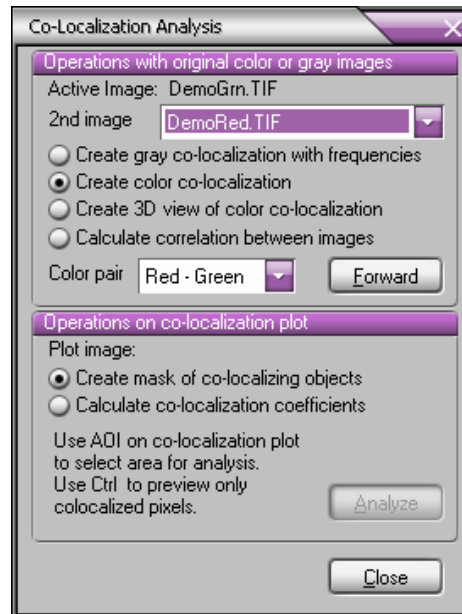
This function requires two independently acquired images labeled with different fluorochromes. The following example uses a mouse fibroblast cell triple-labeled with FITC (microtubules), MitoTracker (mitochondria) and DAPI (nuclei), acquired under typical fluorescence microscopy conditions. The degree of co-localization between the mitochondria and microtubules is measured and displayed both visually and numerically. In this case, colocalization helps in determining whether there is any signal overlap between the fluorescent labels, allowing the investigator to decide whether or not to use emission/excitation filter sets that more stringently separate the color wavelengths from one another.

Implementation

Measure|Co-Localization...

- Acquire individually-labeled fluorescent images.

- Select Measure|Co-Localization... the following dialog box appears:



- To view the quantitative overlap between the two images, select 'Calculate correlation between images' and click 'Forward'. A table similar to the one below will appear:

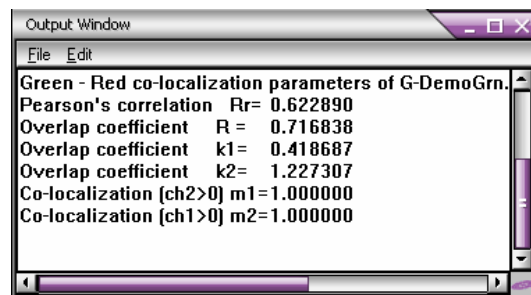


Fig. 1 Image Correlation Table

- Select 'Create Color Co-localization' from the color-pair to use and click 'Forward'. A similar plot appears:

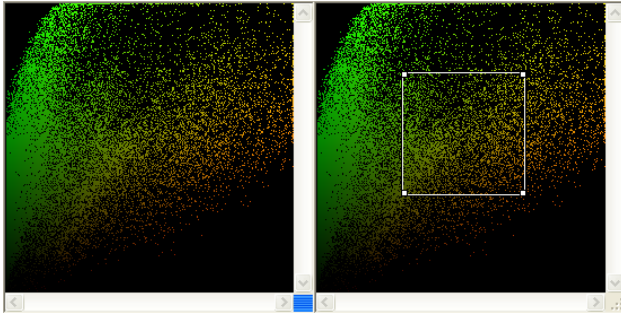


Fig. 2 Co-localization Plot

Fig 3. Area of Interest Within Plot

- Place an Area of Interest on the co-localization to exclude background and other unwanted signal (Fig 3.)
- To create a co-localization mask of the two fluorochromes, select 'Create mask of co-localizing objects' and click 'Analyze' (Fig. 4)
- To create co-localization coefficients, select 'Calculate co-localization coefficients' and click 'Analyze' (Fig. 5)

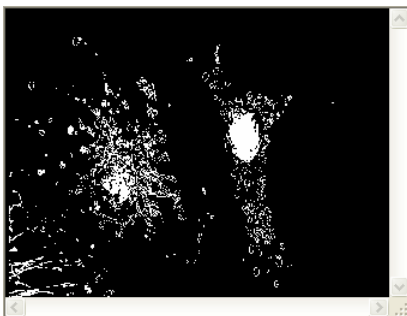


Fig 4. Co-localization Mask

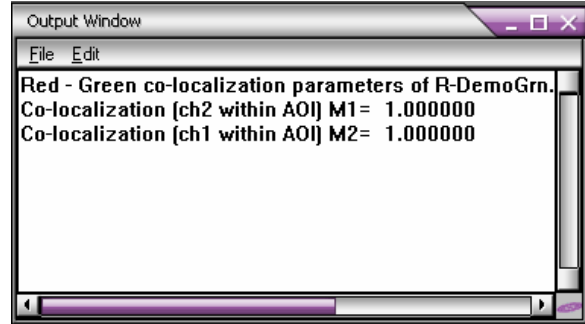


Fig. 5 Co-localization Coefficients

Fig 4. shows the extent of co-localization between the red (MitoTracker) and green (FITC) channels. Fig 5. displays the actual degree of localization within the masked area, indicating the amounts of red (M1) and green (M2) in the co-localized areas.

See Also

Media Cybernetics Application Note- "Co-localization of Fluorescent Probes"

Media Cybernetics Solution Note- "Three Dimensional Co-localization"

Media Cybernetics Product Note- "Color Composite"

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.

Media Cybernetics, Inc.
8484 Georgia Avenue
Silver Spring, MD 20910 USA
Phone: +1-301-495-3305
Fax: +1-301-495-5964

Email: info@mediacy.com
Web: www.mediacy.com

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