The new LSM Software from Carl Zeiss sets a standard in the quantitative colocalization analysis of multichannel images. Colocalized regions can be analyzed in a simple and intuitive way. The corresponding pixels are selectively displayed in the image window.

Colocalization – Analysis and Visualization

Colocalization is the analysis of the raw data, and how colocalization can be quantified reproducibly. The Release 3.2 of the Carl Zeiss LSM Software for the first time provides a comprehensive, integrated solution for colocalization analysis. Colocalization parameters can be determined automatically or interactively with the aid of software tools and used for the comparative analysis of different specimens. Specimen-wise analyses can be tested for reproducibility, and colocalization structures can be visualized. Various numerical colocalization parameters are available for quantitative analysis.

Colocalization – the Principles

In the analysis of multilabel specimens, the colocalization of labeled structures in a channel subjected to different emission conditions is of scientific interest. Frequently, colocalization data in an image is an indication of an expected functional interaction. While the acquisition of multichannel images in this kind of studies usually is associated with technical and methodological difficulties such as higher image acquisition times and problems with the acquisition of simultaneous signals, the colocalization grades of different specimens have to be compared.

The LSM PASCAL, this functionality is also available in the Multitracking mode.

The term used for the specimen whose photometric emission is registered by the detector. The term is a functional term for the photometric detection of multichannel images. Where a combination of emission and excitation crosstalk is present, the Multitracking mode of the LSM 5 family can be depended on to be free from this kind of crosstalk.
Functions at a Glance

Scattergram: A graph in which each channel of a multichannel image is assigned to a quadrant. Pixel pairs from both channels are divided into quadrants. The distribution of pixel pairs in each quadrant is displayed in the image window.

Image Window: A window in which the multichannel image that corresponds to the selected channel is displayed. The regions of interest, defined in the scattergram, are visualized in the image window.

Crosshair Function: A tool that allows the user to select and mark regions of interest in the multichannel image. The selection is displayed in the scattergram and the image window.

Drawing Tools: A variety of drawing and masking tools, such as freehand lines and mask inversions, are available for defining regions of interest (ROIs) in the multichannel image.

Tool Box: The Tool Box includes all tools required for analysis.

Colocal subdialog: The Coloc subdialog provides all tools necessary for colocalization analysis.

Add to this the unique functions for crosstalk-free multichannel image acquisition, such as Multitracking mode and Emission Fingerprinting, and it becomes clear why the LSM 5 family is a comprehensive, integrated solution for colocalization analysis. Colocalization parameters can be calculated and visualized for the comparative analysis of different specimens. Selected image areas of interest can be bleached without the use of chlorine.

Various numerical colocalization parameters are available for quantitative analysis. The Release 3.2 of the Carl Zeiss LSM Software for the first time provides a comprehensive, integrated solution for colocalization analysis. Colocalization parameters can be determined automatically or interactively with the aid of software. A comprehensive analysis of colocalization parameters selected by the user can be bleached without the use of chlorine. Selected image areas of interest can be bleached without the use of chlorine. Selected image areas of interest can be bleached without the use of chlorine.

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Scissors

Multichannel: The image has more than two channels. This function allows you to select the specific channel in the red-quadrant window.
Analysis and Visualization of Colocalized marker proteins for functional focal adhesions is vinculin. Cell One of the essential intracellular structural proteins for focal contacts is vinculin. Cell orients itself along the focal adhesions. more than 100 different proteins while it hesions are dynamic structures, at which position according to the back- ROIs determines the crosshair window, the position of the crosshair can be edited. close to the coordinate origin ponding pixels are displayed with the background. The corres- tagged with Cy3). The Image with Alexa 488; red: vinculin vinculin (green: actin tagged Fig. 1a: Colocalization analysis described above, the single-tagged pixels of the two channels pixels over the four quadrants of the scattergram. With the position of the crosshair corresponds to the sum of the two defined in the image window. For either channel then, the automated method computes, channel by channel, the average object information and no background information. The crosshair position, this method is particularly well suited to the standardized function again permits direct access to the colocalized area, the degree of colocalization, and other quantitative parameters. Thanks to its capability to visualize intensity clouds excellently suited to the analysis of the ratio of fluorescence tags, e.g., as an indication of the expression levels of fusion proteins. It is not necessary to know all details of the expression vectors. Alternatively, the Scatter ROI mode to label colocalization conditions that selectively consider the cell boundary: the single-tagged pixels of the two channels are selected, the respective quadrants of the scattergram. The scattergram represents all pixels of the image. The area pixels are then displayed selectively in the Image ROI. For either channel then, the crosshair function again permits the direct access to the colocalized area, the degree of colocalization, and other quantitative parameters. Thanks to its capability to visualize intensity clouds.
Colocalization Analysis with the LSM 5 Family

Where Do Actin and Vinculin Colocalize?

Visualization of Colocalized Image Regions

Cells growing adherently form plasma membrane contacts between the cell membrane and the extracellular matrix. These focal contacts are sites of signal transduction, at which the cell interacts in a highly dynamic manner with its environment. They are responsible for the organization of the cytoskeleton, which is a network of intracellular actin filaments that are often associated with vinculin and other proteins. These interactions of proteins are crucial for functional cell adhesions. For example, in fibroblasts, vinculin can bind to actin filaments and thereby stabilize them, and it also binds to the integrins that dimerize to create focal contacts. Vinculin is therefore a key player in the function of focal contacts, and its binding to the cytoskeleton is responsible for the assembly of functional focal adhesions.

The Crosshair Function

The Crosshair function provides a handle in the visualization of colocalized image regions. It is a tool that aids in the identification and analysis of colocalized regions in the image. To analyze the colocalization of two different proteins, the user can use the Crosshair function to select regions of interest. The crosshair is positioned on the scattergram, which displays the distribution of pixel intensities. The crosshair position can be adjusted by the interactive method, shift the crosshair in the scattergram, so that the bottom left quadrant represents all non-matched pixels, whereas the top left and top right quadrants represent matched or non-matched pixels, respectively.

The Scatter ROI Mode

The Scatter ROI mode is used for the selection of regions of interest, and the analysis of colocalized structures. This mode allows the user to select regions of interest in the image, and to analyze the distribution of pixel intensities. The Scatter ROI mode is just as important as the Crosshair function. It allows the user to select regions of interest in the image, and to analyze the distribution of pixel intensities.

Expression Analysis

For the expression analysis, the LSM 5 provides different software tools for the analysis of expression ratios. Trasfers are digitally registered, i.e., with low gray levels. The single-tagged pixels are displayed in the scattergram, with the corresponding pixels being maintained. On switching the scattergram mode to Scatter ROI, the pixels in the Image window now selectively show the focal adhesions (white). The Image window now selectively shows the focal adhesions, and the respective cloud in the scattergram. The area and the position of the image pixels are then displayed and analyzed.

Colocalization Conditions

In the example below, COS-7 cells were transfected with constructs that are excellently suited to the analysis of the ratio of fluorescence tags, e.g., as an indication of the expression levels of certain intracellular proteins. The scattergram is depicted as a scattergram, and the position of the image pixels are then displayed and analyzed. The area, position, and ratio of the colocalized pixel clouds are shown, and the evaluation of the ratio of fluorescence tags, e.g., as an indication of the expression levels of certain intracellular proteins.
Cells growing adherently form specialized focal contacts, which are sites of cell adhesion. These focal contacts are highly dynamic structures, at which the cell attaches and detaches from the extracellular matrix. They consist of a basement membrane, ECM, fibronectin, and several proteins of the focal adhesion complex.

**Where Do Actin and Vinculin Colocalize?**

Vinculin and actin are proteins for focal contacts. Vinculin is a protein that is specifically enriched at focal contacts. It is involved in many cellular processes such as adhesion, migration, and signaling. Actin, on the other hand, forms an extensive network of filaments that contribute to cell shape, motility, and force generation.

**Analysis and Visualization of Colocalized Image Regions**

Vinculin and actin both have a prominent role in the visualization of colocalized image regions. They are localized near the cell membrane and cell nucleus. The intensity of vinculin and actin fluorescence can be used to analyze the localization of these proteins.

**The Crosshair Function**

The crosshair function has a wide role in the visualization of colocalized image regions. The crosshair function can be used to analyze the localization of proteins at focal contacts. The crosshair function can be used to analyze the localization of proteins at focal contacts.

**The Scatter ROI Mode**

The Scatter ROI mode is used to analyze the localization of proteins at focal contacts. The Scatter ROI mode is used to analyze the localization of proteins at focal contacts.

**Expression Level Analysis with the Scattergram Function**

Expression level analysis with the scattergram function can be used to analyze the localization of proteins at focal contacts. Expression level analysis with the scattergram function can be used to analyze the localization of proteins at focal contacts.

**Are GFP and YFP Expressed in a Constant Ratio?**

Expression level analysis with the scattergram function can be used to analyze the localization of proteins at focal contacts. Expression level analysis with the scattergram function can be used to analyze the localization of proteins at focal contacts.
Analysis and Visualization of Colocalized markers for functional focal adhesions in which vinculin and actin are One of the essential intracellular structural orients itself along the focal adhesions.

The Crosshair Function

The Crosshair function has a pivotal role in the visualization of colocalized image regions, as it is a tool to manipulate the pixel information. To analyze the arbitrarily selected plane of view, the Crosshair function allows for the intuitive definition of a background ROI which should be defined in the image window. For each channel, the function can be used separately or together with the other channels. Each of the two channels can be set to different display and gray levels. The background pixel set can be selected interactively, with a crosshair function, the image intensity, and the standard deviation of a background ROI defined in the image window. The parameters of the channel correspond to the sum of the two channels. Each method leads to a background ROI that can be used in the next step to analyze the selected regions of the image. The background ROI allows the study of the cell periphery. Quantitative data such as the area of colocalized structures near the cell nucleus as areas of colocalized signals of the actin and vinculin tags are marked blue. The CFP intensity distribution of the actin and vinculin channels is show blue in the scattergram. The background pixels of the ROI in the bottom right part of the scattergram are then color-coded to allow fast identification of single-tagged pixels of the same color, i.e., with low gray levels. The single-tagged pixels of quadrants 1 and 2 are shown red and green, respectively. Colocalized pixels of quadrant 3 are shown blue. The reference regions are marked with the Crosshair ROI.

The Scatter ROI Mode

The Scatter ROI mode is depicted in the figure below. The Scatter ROI is a region of interest that is defined in the image window. It allows for the interactive definition of reference areas in the scattergram. In the example, areas with colocalized pixels are selected interactively, with the background pixel set being maintained. On switching to the Scatter ROI mode, the pixels in the Image window displays all pixels within the Scatter ROI. After the Image ROI has been deleted, the reference area are selected with a freehand ROI (Fig. 2b). The corresponding area is then displayed in the scattergram. The area of the plot which is excised by the Scatter ROI is shown blue. The CFP expression levels can be assigned a cell in the Image window. With this procedure, each of the three pixel clouds can be correspond with the pixels of Scatter ROI. The position of the clouds correlates with the intensity of the CFP expression. The Scatter ROI can be used to selectively display the CFP intensity distribution of certain subcellular structures. The Scatter ROI is excellently suited to the analysis of the ratio of fluorescent tags, e.g., as an indication of the expression ratio of certain intracellular proteins. If cells are transfected with constructs that are tagged with fluorescent proteins, the ratio of fluorescence intensity is frequently used to determine whether or not the corresponding biochemical function will be established. In other cases, the main point of interest is the relative abundance of different subcellular molecules and a background ROI can be used.

The Scatter ROI mode allows for the interactive definition of the CFP intensity distribution of certain subcellular structures. The Scatter ROI can be used to selectively display the CFP intensity distribution of certain subcellular structures. The Scatter ROI is excellently suited to the analysis of the ratio of fluorescent tags, e.g., as an indication of the expression ratio of certain intracellular proteins. If cells are transfected with constructs that are tagged with fluorescent proteins, the ratio of fluorescence intensity is frequently used to determine whether or not the corresponding biochemical function will be established. In other cases, the main point of interest is the relative abundance of different subcellular molecules and a background ROI can be used. Thanks to its capability to visualize intensity distributions, the many scattered spots in the CFP expression levels can be assigned a cell in the Image window. This feature allows the analysis of the ratio of fluorescent tags, e.g., as an indication of the expression ratio of certain intracellular proteins. If cells are transfected with constructs that are tagged with fluorescent proteins, the ratio of fluorescence intensity is frequently used to determine whether or not the corresponding biochemical function will be established. In other cases, the main point of interest is the relative abundance of different subcellular molecules and a background ROI can be used. Thanks to its capability to visualize intensity distributions, the many scattered spots in the CFP expression levels can be assigned a cell in the Image window. This feature allows the analysis of the ratio of fluorescent tags, e.g., as an indication of the expression ratio of certain intracellular proteins. 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If cells are transfected with constructs that are tagged with fluorescent proteins, the ratio of fluorescence intensity is frequently used to determine whether or not the corresponding biochemical function will be established. In other cases, the main point of interest is the relative abundance of different subcellular molecules and a background ROI can be used.
Functions at a Glance

- Scattered ROI Mode: To create a tool for selecting two channels from the total number of image channels. This function allows you to define regions of interest (ROIs) in the scattergram.
- Scatter ROI Mode: To measure the colocalization coefficient, Pearson’s correlation coefficient, colocalization parameters (e.g., area and mean intensity), and Emission Fingerprinting, and it becomes clear that the LSM PASCAL, this functionality is also available in the Multiscan mode.
- Collector: A general indication for colocalization is the acquisition of emission and excitation crosstalk in combination with emission fingerprinting.
- Collector: The term used for the emission spectrum of the confocal detector, while the term used for the emission spectrum is the excitation spectrum. Images acquired in the Multiscan mode of the LSM 5 META system is required. By means of a dummy-pseudofingerprinting feature, it can reveal overlap-free multichannel images, even if the emission spectra of the dye pairs overlap completely. [see references].
- Collector: The term used for the excitation spectrum of the confocal detector, while the term used for the emission spectrum is the excitation spectrum. Images acquired in the Multiscan mode of the LSM 5 META system is required. By means of a dummy-pseudofingerprinting feature, it can reveal overlap-free multichannel images, even if the emission spectra of the dye pairs overlap completely. [see references].
- Collector: A general indication for colocalization is the acquisition of emission and excitation crosstalk in combination with emission fingerprinting.
- Collector: The term used for the emission spectrum of the confocal detector, while the term used for the emission spectrum is the excitation spectrum. Images acquired in the Multiscan mode of the LSM 5 META system is required. By means of a dummy-pseudofingerprinting feature, it can reveal overlap-free multichannel images, even if the emission spectra of the dye pairs overlap completely. [see references].

Colocalization – Analysis and Visualization

In the analysis of high-magnification specimen, the colocalization of labeled structures is of central importance. Frequently, colocalization serves as an indication of a suspected functional interaction. While the acquisition of multicolor images in this kind of specimen, users are often uncertain about how to proceed with the analysis of the raw data, and how colocalization can be quantified reproducibly. The LSM 5 META system is required. By means of a dummy-pseudofingerprinting feature, it can reveal overlap-free multichannel images, even if the emission spectra of the dye pairs overlap completely. [see references].

Polychromatic Scanning

The LSM PASCAL confocal scanning system allows you to define regions of interest (ROIs) in the scattergram.

Colocalization – the Principles

- The Coloc subdialog provides all tools required for analysis.
- The new LSM Software from Carl Zeiss sets a standard for biomedical research.
- For further information, please contact www.zeiss.de/lsm
- E-mail: micro@zeiss.de
- Telefax: ++49 36 4164 31 44
- 07740 Jena
- Carl Zeiss
- Labels with the LSM 510 META. 40-546 e/05.02
- [see references].
Functions at a Glance

45-0012 e/11.02

For further information, please contact:
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Colocalization - Analysis and Visualization

In the analysis of multifluorescent specimens, the colocalization of labeled structures is a problem of central importance. Frequently, colocalization serves as an indication of a suspected functional interaction. While the acquisition of multichannel images is a basic requirement, users are often uncertain about how to proceed with the analysis of these images. The new LSM Software from Carl Zeiss sets a standard for the comparative analysis of different specimens. Selected image areas of interest can be tested for colocalization separately, and colocalized structures can be visualized.

Various numerical colocalization parameters are available for quantitative analysis. They can be tested for colocalization separately, and colocalized structures can be visualized. Various numerical colocalization parameters are available for quantitative analysis. They can be tested for colocalization separately, and colocalized structures can be visualized.

Multichannel images are compared with one another. The intensities of the two channels are each pixel pair has two intensities - one for either channel. The intensities of the two channels are each pixel pair has two intensities - one for either channel.

A graph in which two channels of a multichannel image are compared with one another. The intensities of the two channels are each pixel pair has two intensities - one for either channel. The intensities of the two channels are each pixel pair has two intensities - one for either channel.

Functions at a Glance

Scale Bar Mode: This mode displays subpopulations in a scattergram. The corresponding quadrants are printed in the text.

Image ROI Mode: This function permits the extraction of colocalized image areas. The precise regions containing exclusively the colocalized regions.

Show Table: This function allows access to numerical colocalization parameters.

 histo image

The image is divided into four quadrants: Background, Single-tagged, Double-tagged, and Confocal. The quadrants are printed in the text.

Colocalization – the Principles

A general requirement for colocalization analyses is the acquisition of multichannel images. This means that one of the laser lines used excites two or more dyes (Excitation Crosstalk). Where a combination of emission and excitation crosstalk is present, the multitracking mode of the LSM 5 family can be depended on to be free from this kind of crosstalk. Multitracking mode.

For the comparative analysis of different specimens, colocalization parameters can be determined automatically. Simultaneously, the acquired confocal detection volumes are recorded simultaneously in different channels. This means that one of the laser lines excites two or more dyes (Excitation Crosstalk). Where a combination of emission and excitation crosstalk is present, the Multitracking mode of the LSM 5 family can be depended on to be free from this kind of crosstalk.