

Using Integrated Morphometry Analysis (IMA) to select parts of an image for further analysis

Abstract

There are many ways to process an image, but there are only a few primary ways to segment¹ an image. The following paragraphs discuss some of the available methods.

Instructions

Thresholding of gray values

Thresholding an image identifies pixels of a specific range of gray values to which one or more selected processes can be applied. Usually high gray values are used for fluorescence and low gray values for transmitted light. Many measurement functions such as Measure Colocalization, Integrated Morphometry and Analysis will measure only the pixels selected by thresholding. Other measurement functions such as Show Region Statistics, Region Measurements, and Correlation Plot have options to use only the thresholded pixels for measurement.

Binarizing Images

Binary images are usually made by setting a threshold range and using the Binary Image operations function in the Process menu. Binary images can be processed using functions not available for 8 or 16 bit images. Binary images are then used with the Logical And option in the Arithmetic function (Process menu) to select the white pixels in the second image and setting the other pixels to gray value 0. The binary image is used as a mask. **Note:** When using this function, make sure that the Result Depth is set to 8 or 16 bits.

Identifying Regions of interest

If there is an active region of interest, most processing operations and measurements will operate on only the pixels within the active region. Within a journal, each region of interest on the image could specifically be made active and a second journal run. This is often used to make measurements on individual objects on an image, each object being bounded by a region of interest.

Using Integrated Morphometry and Analysis to select parts of an image that pass your classification

Integrated Morphometry and Analysis (IMA) measures any set of thresholded pixels on an image that passes classification. These sets of pixels are called "objects." IMA also contains a list of classifications that can be turned on. Classifications are measurements for which you set limits. For example, if you turned on the Area classification, you could select only objects of a specific size range. Using Preferences, you can also exclude objects that touch the image border.

As previously discussed, regions of interest can be used to select specific parts of an image. These regions are most often created using the Create Regions Around Objects function located on the Region Menu. The Create Regions Around Objects function creates regions of interest around "Objects". If the image has never been measured using IMA, all groups of thresholded pixels are treated as objects. Therefore,

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any group of thresholded pixels (at least two pixels touching each other) would be an object. However, if you measure an image using IMA, you have defined objects that pass your classification settings. Thus, regions of interest are only created around your "objects."

Example 1

The following steps are an example of a typical usage, but not necessarily in this exact order:

1. Set Preferences to exclude or not exclude objects that touch the edge of the image using the preferences function under the Edit menu and choosing the Measure Objects tab.
2. Threshold an image (use autothreshold or a specific threshold range).
3. Load an IMA State file containing the classification settings for objects of interest. Typically area, shape factor and average or total gray value are used to find valid cells, nuclei or other structures.
4. Measure the thresholded image using IMA.
5. Create regions using the Create Regions Around Objects function found on the Region Menu.

You can now transfer the regions to another image, measure all of the regions simultaneously using Region Measurements or IMA (If the Measure All Regions preference is checked) or use the Loop for all Regions function to make each region of interest in turn active and run a journal (found in the Journal menu Loop submenu). **Note:** This procedure required an IMA measure command but did not require logging of the measured results.

IMA can also be used to create a binary image in which only the valid objects are positive (white). The function to do this is not yet available through the IMA dialog or menu, but may be added to a journal in the journal editor. The journal function is Integrated Morphometry - Create Object Mask. The Create Object Mask function creates a binary image named IMA Object Mask in which the white areas correspond to all of the green and yellow areas seen when the image was measured using IMA. If no objects were green or yellow the image will be black.

Note: If you use this function frequently, you should create a journal specifically containing this function that can be called when recording a journal.

Example 2

The following steps are an example of a typical usage, but not necessarily in this exact order:

1. Set Preferences to exclude or not exclude objects that touch the edge of the image using the preferences function under the Edit menu and choosing the Measure Objects tab.
2. Threshold an image (use autothreshold or a specific threshold range).
3. Load an IMA State file containing the classification settings for objects of interest. Typically area, shape factor and average or total gray value are used to find valid cells, nuclei or other structures.
4. Measure the thresholded image using IMA.
5. Create regions using the IMA - Create Object Mask command available through the journal editor.

You can now use binary operations on the image. The binary image can be used as a mask on another image using the Arithmetic dialog (Process menu) and the Logical And option.

¹ To segment an image is to decide what pixels of an image are of interest and what pixels of the image are not. For example accepting pixels with gray levels greater than 500 but rejecting pixels with gray levels less than 500.