MetaMorph® Software Basic Analysis Guide
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Section I: How Measure Functions Operate

1. Selected images

All measurement functions require at least one image to be selected. The dialog box has an image selector that allows the choice of which image to measure.

Two commonly used measurements require two selected images for proper evaluation; Measure Colocalization and Correlation Plot.

2. Thresholding

Most of the measurement functions require that the selected image “be thresholded.” An image can be in one of three possible states – Threshold OFF, Inclusive Threshold, or Exclusive Threshold. For an image to “be thresholded” means that the image is in the Inclusive Threshold or Exclusive Threshold state. Inclusive Thresholds means that parts of an image that have gray values between Low and High limits will be measured. Exclusive Thresholds means that parts of an image with gray values outside of the Low and High limits will be measured.

Thresholds can either be set manually in a journal/macro or by using a built-in Auto Threshold function. Auto Threshold for Dark objects or Auto Threshold for Light objects uses the histogram of gray values within the image to set the Low and High limits, assuming that the background gray values are High or Low (respectively).

Depending upon the type of question being asked and the variance in the background between images a fixed threshold range or the Auto Threshold function can be used. The Auto Threshold for light objects should be used for fluorescent images if the background varies between images since fluorescent specimens are always brighter than the background.

3. Regions of interest (ROI’s)

- Creating Regions of interest

MetaMorph® software supports several types of ROI’s as well as multiple ROI’s per image. ROI’s may be rectangles, ellipses, manually traced, or linear. An image may contain multiple ROI’s, but only one may be active at any one time. ROI’s may be saved to a file, loaded from a file, cleared from an image or transferred from one image to another using similarly named functions of the Region menu. The most common method for creating ROI’s is to use the Create Regions Around Objects function (Region menu). The image is first segmented (thresholded) and
measured using the *Integrated Morphometry Analysis* (IMA) function (Measure menu). (See technical notes T20025, T20054, T20074) The IMA function allows for exclusion of objects based upon size, position, shape and other classification criteria. ROI’s can be created around all measured objects. ROI’s may be loaded from a file ROI may be drawn manually on an image, but this is seldom used for screening applications.

- **Using Regions of interest**

Under most situations if there is an active ROI on an image, the image outside of the ROI will be ignored. There are three measurement functions that are exceptions to this rule. The function *Show Region Statistics* will measure the entire image if *Entire Image* is checked, regardless of ROI’s present.

The function *Region Measurements* allows the choice of *Active Region*, *All Regions* or the *Entire Image* when making measurements.

*Integrated Morphometry and Analysis* has a preference that allows the measurement of all regions. When this preference is checked Integrated Morphometry and Analysis will measure all ROI’s simultaneously.

More often than not, regions of interest will be used to loop a journal (Journals/macros are discussed in the next section) so that individual objects (usually cells) can be analyzed.

4. **Which Measurement function should I use?**

- **Measuring total intensity**

Two functions can be used to measure the total intensity on an image. *Show Region Statistics* and *Region Measurements*. *Show Region Statistics* will measure the total intensity, average intensity, maximum and / or minimum intensity of the entire image or a region of interest. It can also measure the same parameters looking at only thresholded pixels. *Region Measurements* offers similar capabilities.
• **Measuring total thresholded area**

*Show Region Statistics and Region Measurement* can both be used to measure the thresholded area of the entire image or a region of interest. If the image is calibrated in microns the area measurements will be in units of square microns.

• **Measuring localization of multiple probes**

*Measure Colocalization* can be used to measure the area or integrated intensity of two fluorescent probes and can also measure how much of either probe does or does not overlap the other probe. If more than two probes are used, masking and *Show Region Statistic* should be used. When the fluorescent signal is not easily thresholded, the *Correlation Plot* function can be used to calculate the correlation coefficient between two probes.

• **Counting and / or measuring individual objects**

Individual objects can be counted using the *Integrated Morphometry and Analysis* function, or the *Count Cells* function. *Integrated Morphometry and Analysis* is used more often because it provides tools for classifying objects (throwing out unwanted objects) based upon size, shape, position (e.g., touching the edge of the image), texture, and a variety of other parameters. Usually, objects are counted using Standard Area. The Total Count of Standard Area listed in the *Summary* data would be equal to the total number of cells. Standard Areas need to be determined prior to the measurement using the *Configure Object Standards* function (*Measure* menu). Individual objects/cells can also be logged using *Integrated Morphometry Analysis*. Individual object data would be similar to FACS data and could include fluorescence intensity, size, shape, texture or other data.

• **Measuring levels of Calcium, pH or other ions.**

Although single wavelength ion indicators are easy to use, differences in cell loading and cell shape/path length make ratiometric indicators the more common choice for screening applications. In order to measure ion levels on a cell-by-cell basis, the following protocol is suggested. Correct all images for background and shading artifacts using *Correct Shading* and/or *Arithmetic* (process menu). Use the *Arithmetic* or the *Ratio Images* functions (Process menu) to calculate the division between the two images. Use one wavelength image to define regions of interest around all suitable cells using the *Create Regions Around Objects* function (Region menu) and then transfer the regions to the ratio image. Then set up a conversion between gray values and concentration using the *Calibrate Gray Levels* (Measure menu) to make all subsequent measurements on the ratio image in calibrated units.
5. How to record measurements

Measurements made within MetaMorph® software can be stored via dynamic data exchange to a spreadsheet program or to a text file. The act of saving the measured data is referred to as ‘logging’ and the data files are called ‘log files’.

- Configure Log

Before measurements are stored, the user must configure what information is to be stored. All measurement functions work through a common Configure Log dialog. The first eight parameters remain the same for all measurement functions as they refer to information from the active image. The following parameters are especially pertinent for screening:

- Image name refers to the image window name.
- Image Date and Time refers to when the image was originally collected.
- Stage label refers to the well and site within the well for the measurement.

Parameters following the first eight are specific to each measurement function.
• Logging Data

Before a measurement can be saved to either the spreadsheet or the text file the ‘log’ must be established. Many of the measurement functions contain a button that says Open Log (the button will change if the log connection has been opened), or the Open XXX Log function can be selected from the Log menu.
• Different types of Logs

MetaMorph® software divides the data that is measured into different classes that are stored in different logs. Most all measurement functions and some non-measurement functions used for screening save their data in the “Data Log”. This includes measurements of regions, colocalization, thresholds and variables (discussed later). The exception to this rule is Integrated Morphometry and Analysis.

IMA can measure a variety of types of data. IMA sends individual object information to an Object log, and also sends a summary of the measurements made to a Summary log. Integrated Morphometry and Analysis sends histogram or scatter plot information to a Data log. These different logs could be connected to the same text file or spreadsheet, but this is not the preferred technique as it is possible for the data from one log to overwrite the data from another.

• Viewing Data that has been logged

Data that has been sent to a spreadsheet may be viewed by typing ALT-Tab until the spreadsheet program is the active application. (Do not attempt to edit the spreadsheet while the MetaMorph® program is sending data to the spreadsheet because the MetaMorph® program will be unable to send future information to the spreadsheet or to the correct position on the spreadsheet.) Data that has been sent to a text file can be displayed using the View Current (type) Log function on the Log menu. Text files may be viewed while data is being sent without any interruption of the transfer.

Section II: How Journaling Works

What is a journal?

Journals are macros in a language specific to Meta Imaging Series® Software and are stored as files with the extension “.JNL”. Many other software programs utilize macros, but there are certain features that make Journals special and will be discussed in this section.
Why are journals used?

Journals are used to simplify complex tasks. For example a journal could be used to:

→ correct the background of an image
→ calculate a threshold level
→ create a binary mask of the image
→ manipulate the binary mask to remove extensions
→ apply the mask to the original image
→ count the number and length of extensions

Journals can also be used to simplify repetitive tasks. For example a journal could be used to:

→ measure the thresholded area of multiple images
→ count the cells in multiple images
→ measure the intensity at a certain distance from the centroid of each object in an image

Journals can be executed:

→ for every position in a screening sample
→ for every image in a directory
→ for every plane in a stack
→ for every region on an image
→ a specified number of times
→ until a condition is found to be either true or false

Journals can be used to collect important information from the user about the experiment. This information could then be added to image annotations and stored with the experimental data.

How are journals executed?

Journals may be executed by pushing a button on a taskbar. A custom taskbar is configured using the Taskbar Editor so that a button is assigned to the journal. This operation is often used to run a journal that needs to be run once before a full assay (journal) is executed in Plate Data Utilities or Review Plate Data. A taskbar may also be used to walk the user through multiple journals in a set specific order. Other methods of executing journals include the Run Journal function or one of the Loop functions in the Journal menu.

How are journals created and edited

Journals are recorded by placing the software into ‘Record Mode’, using the function Start Recording in the Journal menu.
When this occurs, the top of the application window will indicate that a journal is being recorded by showing [Recording]. Nearly all of the subsequent operations will be recorded into the journal until either Pause Recording, or Stop Recording is chosen. When journal recording is finished (by choosing Stop Recording in the Journal menu) a dialog will ask whether to save the journal and add it to a taskbar. Once journals are created they may be edited using the Edit Journal function in the Journal menu.

This function opens a journal file to look at in the Journal Editor.

The right side of the dialog box contains the list of functions in that journal, while the left side of the dialog box contains built-in functions, other journals, or specified actions that can be dragged from the lists into the journal. Standard control character shortcuts work for cut, copy, and paste between parts of a journal or between journals. One box makes a journal function interactive (allows user modification at the time of execution), while another check box allows inactivates a function in the journal without removing it from the journal file. Buttons to handle journal opening, saving and closing can be found on the bottom of the dialog. Choosing View Function List by Description provides an alternative display format that shows many of the parameters used by the journal functions. When variables are used (see below) assignments appear as equations as shown in the last line of the example below.

```
1: Integrated Morphometry - Measure("DAPI")
2: Integrated Morphometry - Log Data([Last Result], SUMMARY, ACCUMULATEDDATA, 28, 2)
```
Image selectors

• **Names**
Image selectors are the mechanism by which journals determine which image or image window will be operated on and where results should be stored. There are several possible ways in which image selectors in journals might function.

→ Journal functions might operate on specifically named images. However, as soon as the names of the images are changed that journal would cease to function.

→ Journal functions might operate on the active image when that journal starts. This would not require the specific names of images to match for a journal to function, but could not be used if a function required more than one image at a time.

→ Journal functions might operate on the last result from a previous operation. This may be useful if the user interactively changes the name of the image. It may also be useful if the name of the starting image is unknown and therefore the name of subsequent images that result from processing the initial image is unknown. An example of this is the Duplicate function in which the resulting image name is “Copy of x” (where x is the starting image name).

→ A journal function might be used to select an image at the time the journal is executed.

MetaMorph® software image selectors support the following methods: Specified image names, Current at Start, Last Result and Interactive image selector. For obvious reasons the interactive image selector is not often used for HTS assays.

• **New, Add to, Overwrite and plane selectors**
Many processing and graphics operations produce one or more result images. The software provides the ability to create a new image in memory, to overwrite an existing image in memory, or the ability to add to an existing image in memory thereby creating a stack of images. If the image is added to a stack of images, to the image can be added to the currently active plane, or the top or bottom of that stack. This does not affect the ability to record a journal that overwrites or adds to an image that does not exist in memory.

• **Caveats to image selectors**

When running or looping a journal, the active image at the time of execution becomes the current image at start. This is true even when running a journal from within another journal. Users commonly run into problems after editing a journal in which Last Result is used. If a journal is edited there may no longer be a last result image to refer to. The best way to avoid image selector problems is to use Specified image names or Current at start.

When no image matches the image selector in a journal, the journal function will often call up an interactive dialog that asks for the source image. If this problem occurs, edit the journal in the
Journal Editor and look at the image selector for the function causing the problem. Typical troubleshooting for this type of problem is to make functions in the journal interactive or comment them out to figure out which function is specifically causing the problem.

When using specified image names, mention the specific names in the documentation for the journal. For example, if a journal operates on a DNA image and uses the name DAPI, the same journal will not run if someone else configures an experiment to use Hoechst or any other name, despite the fact that the two fluorophores are nearly identical.

**Running sub-journals, looping and branching journals**

*(When a journal is run from within a second journal it is referred to as a sub-journal.)*

- **Why run a journal from another journal?**

Sub-journals are used to reduce repetition and complexity in a journal. If several steps are repeated in a procedure placing those steps in a second sub-journal reduces the complexity of the original journal. For example, if a journal makes a measurement for every region on an image this could be written to select region 1, make the measurements, log the results, select region 2, make the measurements, log the results..., for as many regions as there may be. It becomes a great deal simpler to use a built in journal function that “Loops a journal for all regions in an image” to run a second journal. The second journal makes the measurements on the active region of interest and logs the data. The second journal can be modified to change what information is being logged.

Sub-journals are also used to make the use of image selectors easier. If the user is asked to pick an image there are four choices to use the newly active image for other journal operations:

1) Use an interactive rename command to allow the user to choose an active image to rename to a pre-selected specified image name (losing the original image name).
2) Duplicate the image (requiring more memory) and rename the “Last Result” to a specified image name.
3) Have the user make the image active interactively every time the image is needed.
4) Have the user make the image active interactively once, and then run a sub-journal.

When the sub-journal is run, the active image window becomes “Current at Start” while it is running and is often the simplest. Sub-journals also allow for branching. Using the function *Branch on Variable* (see below) allows for one set of steps or another to be carried out depending upon the conditions.

- **Types of loops**

MetaMorph® software supports a variety of ways to loop and run a journal.

→ Loop a Journal
→ Loop for all regions
→ Loop for all planes of the Stack
→ Loop variable

The loop functions are all similar in that they execute a second journal multiple times. The loop commands differ by how many times they execute and whether a specified image and specified image plane continues to be “Current at Start” throughout the execution.
Variables

- Assign Variable

Assign Variable is equivalent to the “=” function in Basic. This dialog selects a variable and assigns a value to the variable. Once a value is assigned to the variable, it maintains the value until it is either deleted (using Delete Variable function in the Journal menu), assigned a new value (using Assign Variable or Enter Variable), or the program is finished. There are some built-in program variables that are exceptions to this rule. These variables may update either when hardware is manipulated, the active image is modified, the active region of interest is modified or a new measurement is made.

A variable has no value before that value is assigned. Attempting to use a variable without a value assigns the value “###ERROR###” to the string and may cause problems if the variable is expected to contain a numeric value. After a measurement is made, the measurement becomes accessible using Assign Variable. The exception to this is Integrated Morphometry and Analysis where the measurement has to be made and logged before it is accessible. The measurement variables are listed in the measurement section of Assign Variable.

Variable assignments accessing a measurement can be made as many times as needed. However the assignments will access only the last measurement made and can be a problem with the measurement function Integrated Morphometry and Analysis. For example, if two parameters were measured, Area and Standard Area Count, the example above returns the Total - Standard Area Count because the parameter Standard Area Count is measured after the parameter Area. The only way to guarantee the parameter returned in the Assign Variable is to make that parameter the only one measured. If individual objects are to be put into variables a similar strategy should be followed.

→ Measure the image using Integrated Morphometry and Analysis
→ Use the function Create Regions Around Objects to create multiple regions on the image – one per object
→ Loop a journal for all regions

The Loop for all Regions functions will select each of the regions of interest making the region active and running the journal. Since there will be only one object per region, only one measurement will be mapped to the Assign Variable.

- Branch on Variable

Branch on Variable is equivalent to the function “if X then run journal A, else run journal B” where X is a logical statement and the variable is compared to another variable or to a constant value. If the comparison is True one journal is run, otherwise a second journal may be run (the second journal selector may be left blank).

- Loop Variable

Loop Variable supports For/Next loops, equivalent to “For i = X to Y step Z run journal A”. Journal A is executed a specific number of times and the variable i is initially set to the value X and is sequentially incremented by the value Z. Loop Variable also supports the function “While X= Y run journal A” and “Run journal A until X is True (or False). These loops execute a journal
until a condition is either True or False. Within the While loop, the condition is checked before the first execution of the journal.

- **Enter Variable**

*Enter Variable* is an interactive function that allows the user to enter a value into a variable. This function permits the user to enter numerical values, text, Yes/No, a specific selection, or multiple selections. The entered value is then assigned to a variable similar to the *Assign Variable* function. This function would normally be used to ask for set-up information from the user. For example, if a screen is used for multiple cell types but each cell type has individual limits for positive and negative results, it would be possible to run a setup journal and ask the user for the limits using *Enter Variable*. The actual journal used for the screen could then use the variables in its calculations.

**Strategy for constructing assay journals**

>The following section describes the recommended parts of journals for a screening assay.

**The setup journal**

The main purpose for a setup journal is to resolve the unknowns and execute operations that may only need to be carried out once at the beginning of the assay.

**Indicate to the user what the journal does**

It is extremely useful to document journals and it should pursue become normal practice. The function *Show Message and Wait* can be used to communicate instructions to the user and can also be used to document a journal by commenting out the function in the journal editor.

**Preferences**

The Preferences function (Edit menu) offers several options that should be set in the journal / assay to guarantee reproducibility of measurements. The *Measure Objects* tab has three options that can directly affect measurements:

- Fill holes in objects includes object holes in measurements when checked
- Exclude objects if centroid is not in current region and
- Exclude objects that touch the edge of the image will exclude objects depending upon their location

Turn off the option *Warn user when measurement data will be erased*. Otherwise the user will have to interactively select OK every time a new measurement is made with *Integrated Morphometry and Analysis*.
Open logs
In order to guarantee that measurements are saved it is a good idea to include functions to open the corresponding Log to a file or spreadsheet. If the Log is already open there will not be an error message and the old Log will be used. To guarantee that a new log is used close the corresponding Log (there will not be an error message if one is not open) before opening a new Log connection.

Load State files and Configure Logs
For screening applications, an IMA state file is commonly used to set up the classifiers (the definition of what is a valid object) and the measurement parameters. The IMA state file also includes the configuration for logging data. If more than one state file is to be used in an assay, the state files should be loaded within the main assay journal so that the proper state file is loaded just prior to making the measurement with IMA. Each measurement function has a configure log function that sets up which parameters should be saved. For most applications this step can be carried out once in the setup journal.

Clear up other unknowns
By the time the main assay journal is run all of the unknowns should be resolved. This means that if *Standard Area Count* is to be measured the standard area parameter should be set. If a threshold value is used that may vary from experiment to experiment, the threshold value should be set and saved. All interactive journal commands should be found here and there should be no interactive journal commands within the main assay journal.¹

The main assay journal

Correct images
Image processing may be able to enhance images if the system has not been configured optimally, or if there is extensive light fluctuations, or if the signal is weak².

Shading and Background Correction
MetaMorph® software has a variety of tools available for decreasing the effects of shading or background.

- The *Background and Shading Correction* dialog (Process menu) provides four tools for correcting or removing background:
  → Subtract background can be used to remove either an image or a set grey value from an image. This works well if shading is not an issue and backgrounds remain relatively constant.
  → Statistical correction uses the maximum, minimum or average of a region of interest as a calculated background. This works well when shading is not an issue but the background level changes. For this function to work it is important

¹ If a user is entering or choosing a value that will affect the measurement results it is good laboratory practice to log this parameter with the results.
² When problems arise, image processing should always be considered a last resort compared to optical or experimental modifications.
that the region of interest used be background in all situations i.e. no cell moves into the area used.

→ Correct shading provides for a background and a shading image to be to correct for both background and shading. Note that the “Shading image” should have its background removed prior to use in this dialog.

→ Flatten background can be used to even out the shading by calculating a false shading image. For each image using a kernel operation. This is not as accurate as Correct Shading but often is satisfactory for counting of objects.

- Often a false background image can be calculated by taking the grayscale minimum for multiple images. Images can be loaded into a stack and a minimum calculated using the Stack Arithmetic function (Process menu). This assumes that every pixel in the image has at least one frame in which it reaches a background / minimum level.

Other methods of image enhancement

MetaMorph® software comes with a variety of other image processing tools including Morphology Filters, 2D Deconvolution, FFT and multiple Basic Filters, all functions found in the Process menu.

Segment the image

Individual images

Segmentation of individual images involves thresholding. The most common methods are shown below.

1. The first method involves the Auto Threshold for Light Objects function found in the image toolbar. This function works well for images with high signal-to-noise such as DAPI stained nuclei. This method can be used when counting objects, but do not use this method for measuring the thresholded intensity, as the threshold level will vary with the data.

2. A second method of thresholding involves specifying the threshold to be used using the Threshold Image function or the Set Color Threshold functions in the Measure menu. Both of these functions specify a threshold range to be used for the images in the assay.
3. A third method involves creating a mask that is applied to a second image. Masks are often made using IMA to filter objects or an application module result image. In most situations masks are binary images created using the Binarize operation of the Binary Operations function (Process menu).

![Binary Operations](image)

An example of the use of masks might be to measure the signal of one probe that is adjacent to but outside the area of a second probe. A binary mask is made from the first probe. The mask is dilated one or more times producing a dilated mask. The original binary mask is subtracted from the dilated mask producing a donut shaped mask:

![Mask Example](image)

This final mask result is applied to the image of a second probe using Logical AND in the Arithmetic function (Process menu).

**Store and or display the data**

Data is typically stored by logging to either a spreadsheet program or to a text file. The data logging can be carried out in one of two ways.

1) The measurement functions used in the previous steps both measure and log the data.
2) The measurement functions used in the previous steps measure the data, but variables are used to log data.

The first method makes use of the Configure Log function found in all measurement dialogs. Configure Log is journalizable and can be executed in a setup journal. The second method involves making measurements, assigning the measurements to variables, and logging variables using the Log Variable function. The advantage of this method is that multiple variables can be manipulated using arithmetic operations prior to logging. A typical use of this method would be to log the ratio of two different measurements (for example total signal intensity normalized to the number of cells).
Sample journals

- Measure thresholded area

The following sample journal measures the thresholded area and percent thresholded area.

The set up journal shown below describes to the user what the assay will be doing. The journal then opens a data log and configures Show Region Statistics to log the appropriate parameters. In the last three steps the user:

- interactively sets the image threshold limits
- stores the threshold limits in a file and
- stores the threshold limits in the data log

The assay journal shown below loads the threshold from the stored file and measures using Show Region Statistics.

---

**Version 1.0.2**
• **Count number of cells**

The following sample journal counts the number of nuclei in the field to determine the number of cells present.

→ The set up journal shown below describes to the user what the assay will be doing
→ The journal then
  1) sets the preferences
  2) opens a summary log and
  3) loads an IMA state file
→ The IMA state file specifies that *Standard Area Count* will be measured
→ sets minimum and maximum sizes for valid objects
→ Finally, the user is asked to interactively set the standard area parameter

C:\ assay\Count Nuclei\Count Nuclei_setup.JNL

<table>
<thead>
<tr>
<th>Functions</th>
<th>Descriptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count the number of nuclei</td>
<td></td>
</tr>
<tr>
<td>1: Show Message and 'Wait(&quot;The following jou...&quot;, NO TIMEOUT)</td>
<td></td>
</tr>
<tr>
<td>2: Preferences</td>
<td></td>
</tr>
<tr>
<td>3: Open Summary Log(OPENDDE and ASKMODE, &quot;&quot;)</td>
<td></td>
</tr>
<tr>
<td>4: Integrated Morphometry - Load State(&quot;Nuclei&quot;)</td>
<td></td>
</tr>
<tr>
<td>5: Object Standards - Set Area(Standard Area = 310)</td>
<td></td>
</tr>
<tr>
<td>End of Journal</td>
<td></td>
</tr>
</tbody>
</table>

→ The assay journal shown below autothresholds an image named ‘DAPI’
→ The journal then measures the image
→ Then the journal logs the current results to a summary log

C:\ assay\Count Nuclei\Count Nuclei.JNL

<table>
<thead>
<tr>
<th>Functions</th>
<th>Descriptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count Nuclei : autothreshold and count using IMA</td>
<td></td>
</tr>
<tr>
<td>1: Auto Threshold for Light Objects (Legacy heuristic algorithm)</td>
<td></td>
</tr>
<tr>
<td>2: Integrated Morphometry - Measure(&quot;DAPI&quot;)</td>
<td></td>
</tr>
<tr>
<td>3: Integrated Morphometry - Log Data(&quot;DAPI&quot;, SUMMARY, CURRENTDATA, 1, 2)</td>
<td></td>
</tr>
<tr>
<td>End of Journal</td>
<td></td>
</tr>
</tbody>
</table>
**Measure Overlap**

The following sample journal measures the area and integrated intensity of overlap between two images.

- The setup journal shown below describes to the user what the assay will be doing.
- The journal then opens a data log and configures which measurement will be logged by the Measure Colocalization function.
- One image will be autothresholded, but the other will have a specified threshold.
- In the last three steps the user interactively sets the threshold limits for the second image and the threshold limits are stored in a file and in the data log.

```
C:\assay\Measure Overlap\Measure Overlap_setup.JNL
```

<table>
<thead>
<tr>
<th>Functions</th>
<th>Descriptions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Measure the overlap between the DAPI and the FITC_GFP image</strong></td>
<td></td>
</tr>
<tr>
<td>1: Show Message and Wait(&quot;The following jou...&quot;, NO TIMEOUT)</td>
<td></td>
</tr>
<tr>
<td>2: Open Data Log(OPENMODE and OPENWFITEMODE, &quot;&quot;)</td>
<td></td>
</tr>
<tr>
<td>3: Configure Measure Colocalization Log()</td>
<td></td>
</tr>
<tr>
<td>4: Threshold Image(&quot;FITC_GFP&quot;, 718, 4095, Inclusive)</td>
<td></td>
</tr>
<tr>
<td>5: Save Gray Threshold(&quot;FITC_GFP&quot;, &quot;threshold&quot;)</td>
<td></td>
</tr>
<tr>
<td>6: Log Gray Threshold(&quot;FITC_GFP&quot;)</td>
<td></td>
</tr>
</tbody>
</table>

- The assay journal shown below selects and autthresholds an image named 'DAPI'.
- The journal then selects and loads a threshold for an image named 'FITC/GFP'.
- The thresholded areas and integrated intensities are measured along with measurements of overlap between the images.
- The results are then automatically logged to a data log.

```
C:\assay\Measure Overlap\Measure Overlap.jnl
```

<table>
<thead>
<tr>
<th>Functions</th>
<th>Descriptions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Measure Overlap between DAPI and FITC_GFP</strong></td>
<td></td>
</tr>
<tr>
<td>1: Auto Threshold for Light Objects(Legacy heuristic algorithm)</td>
<td></td>
</tr>
<tr>
<td>2: Integrated Morphometry · Measure(&quot;DAPI&quot;)</td>
<td></td>
</tr>
<tr>
<td>3: Integrated Morphometry · Log Data(&quot;DAPI&quot;, SUMMARY, CURRENTDATA, 1, 2)</td>
<td></td>
</tr>
<tr>
<td>4: Integrated Morphometry · Load State(&quot;bacteria&quot;)</td>
<td></td>
</tr>
</tbody>
</table>

- **End of Journal**
Section III: MetaMorph® Program Organization

The key programs of the Meta Imaging Series® Software:

1. MetaMorph®/MetaXpress® Software
The MetaMorph®/MetaXpress® programs are imaging toolboxes containing many functions for image collection, microscope automation, image processing, and image analysis. MetaMorph® software is the core program. MetaXpress® software is a version of the MetaMorph® program designed to do high content screening with MDS Analytical Technologies high content imaging hardware. The MetaXpress® program will automatically start up by logging into the MDCStore™ database. The MetaVue™ software contains only limited functionality for image acquisition, processing and analysis.

2. Meta Imaging Series® Updater
The Meta Imaging Series® Updater program is used to update software to newer versions. These versions usually contain updates to software problems and additional functionality. Software updates may be found and downloaded from the MDS Analytical Technologies MetaMorph® software update website - www.meta.moleculardevices.com/updates.

3. Meta Imaging Series® Administrator
The Meta Imaging Series® Administrator (MISA) utility is used to configure or modify the settings of all of hardware for all of Meta Imaging Series® applications. It will be used during installation of a system by the system administrator and should not be modified by untrained individuals unless directed by MDS Analytical Technologies or one of its authorized dealers. The MISA utility allows applications to be run in Single-User or Multi-User mode. In either mode the MISA utility contains tools for setting up one or more hardware configurations including the camera or video card. It also contains tools for setting up one or more hardware configurations. It is also used to add or remove software modules (“drop ins”), from the program. A drop-in manages one or more functions in the software. Obviously, adding drop-ins gives additional functionality. However some drop-ins require hardware that might not be present or provide functionality that is seldom used. To minimize confusion and to maximize the speed of the program, not all drop-ins are loaded by default. Not all users are alike and it is up to the system administrator to determine which drop-ins should be loaded for optimal use of the system.