

General Tools for use with Screening Modules

Abstract

This document describes common tools and procedures for use with screening application modules when using either the non-database or database version of the Discovery-1 System. This application notice applies to Discovery-1 version 6.2 and later.

Note: For more information about the procedures and dialog boxes described in this document, refer to the Discovery-1 online help system. Press [F1] to access help for the active dialog box.

Instructions

This document describes the following procedures:

- Opening an image using
 - The Open command on the File menu
 - The Review Screen Data dialog box
- Opening an assay
- Configuring an Assay and Saving Settings, including
 - Using tools to determine object widths, areas, and intensity over background
 - Logging data to Excel or a text file and/or database
 - Testing settings
 - Saving settings
- Once the parameters are known, running an assay on
 - An entire plate
 - Multiple plates
 - The current image

Note: Configuring and saving settings must be completed before you run an assay for the first time. After settings are saved, they can be loaded as needed without further configuration.

This document assumes the following:

- MetaMorph/Discovery-1 Version 6.2 is loaded
- The modules to be used have been installed with the software (see the System Administration guide for more details)

Keywords: application modules v6 v6.2

Issue Type: mods

Document ID
D50025

Product
Discovery-1, version 6.2

Created
01-Oct-2004

Last Reviewed
01-Oct-2004

Loading Images

The following procedures describe the steps that you need to complete to load

- Individual images
- A plate of images

Loading Individual Images - Database or Non-database

To load individual images, complete the following procedure:

1. From the Windows Start menu, choose Programs>Meta Imaging Series 6.1>MetaMorph icon or the Discovery-1 icon to open MetaMorph/ Discovery-1.
2. From the File Menu, click *Open*; the Open dialog box appears.
3. Select the appropriate folder, then select the image name and click *Open*. Your selected image will open in a Discovery-1 image window. You can also highlight the image files with your mouse and drag them into the MetaMorph/ Discovery-1 window.
4. Repeat step 2 and 3 until all needed images are open.

Loading a Plate of Images - Database or Non-database

This procedure assumes that your images were acquired using the Screen Acquisition dialog box. To load your images, complete the following steps:

Non database

1. From the Windows Start menu, select Programs> Meta Imaging Series 6.1> Discovery-1 to open Discovery-1.
2. From the Apps Menu, choose *Review Screen Data*; the Review Screen Data dialog box opens.
3. Click *Select Plate*. The Screen Data Utilities dialog box opens, and the Review Screen Data dialog box temporarily closes.
4. If no plate data sets are shown in the Plates (*.htd) box, click *Select Directory*. The Browse for Folder dialog box opens.
5. Select the folder (directory) containing the screen data set that you want to view, and click *OK*. The names of the data sets contained in the folder are displayed in the Plates (*.htd) box.
6. In the Plates box, highlight the data set you want to view, then click *View*. The Screen Data Utilities dialog box closes, and the Review Screen Data dialog box reopens.
7. Go to step 7 under “Database.”

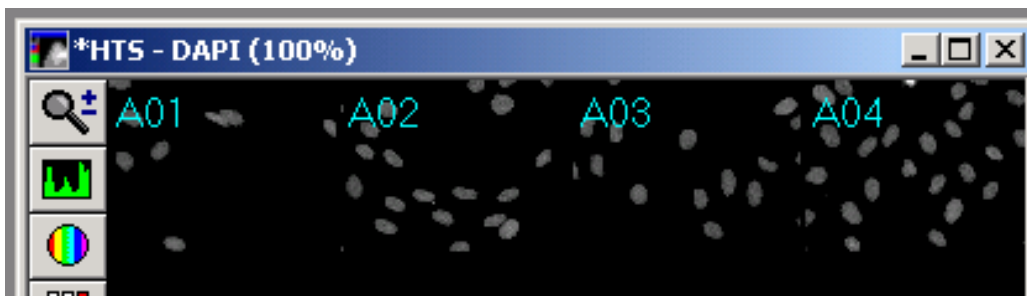
Database

1. From the Windows Start menu, select Programs> Meta Imaging Series 6.1> Discovery-1 to open Discovery-1.
2. Enter the Database login information
3. From the Apps Menu, choose *Review Screen Data [DB]*; the Review Screen Data dialog box opens.
4. Click *Select Plate*. The Screen Data Utilities dialog box opens, and the Review Screen Data dialog box temporarily closes.
5. If no plate data sets are shown in the Plates box, then select for Show plates: All Plates

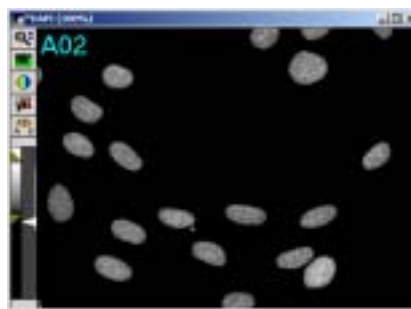
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6. In the Plates box, highlight the data set you want to view, then click *View*. The Screen Data Utilities dialog box closes, and the Review Screen Data dialog box reopens.
7. In the Wavelengths box, click the check box next to wavelength(s) that you want to use for your analysis.
8. Click *Apply* to view a montage window (“HTS-Wavelength”) of the selected sites.
9. Click a site within the montage window to open a new image windows of the selected site, as shown in Figure 1:

Figure 1
Opening an Image of a Site



In this example, thumbnail A02 was clicked, and the following image window opened:



Opening an Assay to be configured

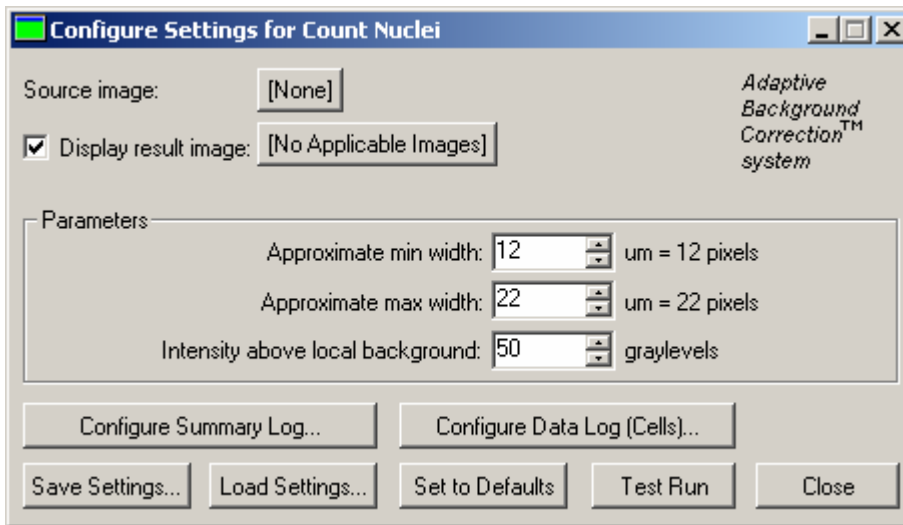
To use the assay interactively or within a journal:

From the Main Menu click Apps, then click the Module that you want to run, such as Count Nuclei, Translocation, and so on. Figure 2 shows the dialog box for Count Nuclei.

To use the assay directly in Review Screen Data or Review Screen Data[DB]

1. From the Review Screen Data dialog box, select the Assay tab.
2. Select the appropriate assay from the Assay drop-down list (for example, Count Nuclei).
3. Non database only: Click *Location* and choose the folder for storing and retrieving assay settings. (Suggested location: C:\Assay\)
4. Database: the settings will be stored in the database that you are logged into.
5. Click *Configure Settings*. The Configure Settings dialog box for that assay will open. Figure 2 shows the dialog box for Count Nuclei.

Figure 2
Count Nuclei Configure Dialog Box



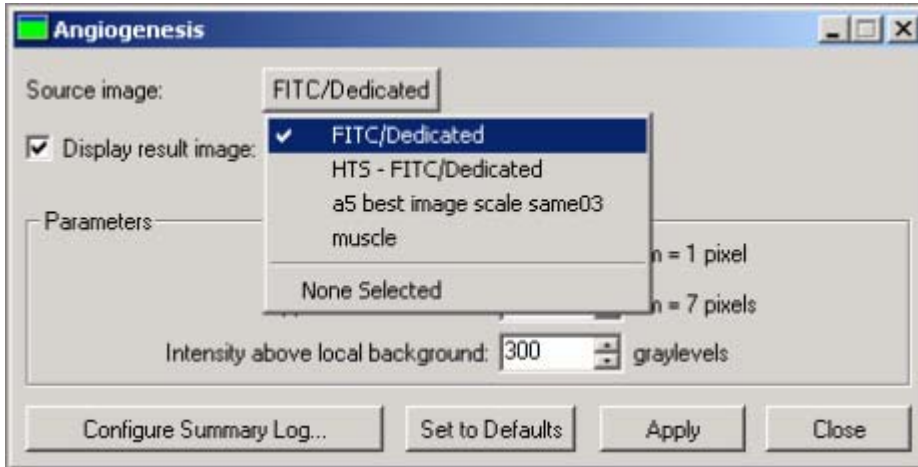
Configuring the Assay

Image selectors

Source image: Source images are indicated with the following names:

- Count nuclei: Source image
 - Angiogenesis: Source image
 - Translocation: Compartment image and Translocation Probe image
 - Neurite: Neurite image and Nuclear Image
1. Click *Source image* and select the appropriate image name from the drop down list. Refer to the example in figure 3.
Note: when using Review Screen Data select the image that just gives the wavelength name, not the thumbnail preview window (the image with HTS-wavelength).
 2. Repeat this for all the image selectors needed.

Figure 3
Selecting a source image via the drop down list




Checking your results

Overlay image: An overlay on the main image is created. You can toggle this setting on or off with the Show/Hide overlay button on the side of your image window.

Result image: All of the modules can produce a result image depicting what was measured.

Note: To display a result image a check box option must be checked and an image must be selected, otherwise an overlay on the main image is created.

Using Image overlay

1. With the *Display result image* deselected select Apply / Test Run
2. Go to your main image and toggle the Show/ Hide overlay button , which is the second button on the side of the image. If no overlay is shown check the other wavelength images or change the settings.

Using Result image

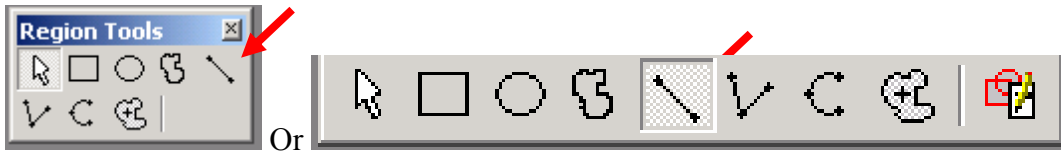
1. Check *Display result image*.
2. Open the image selector for *Display result image* and if needed change it from None to New, Overwrite, or Add to. (Recommended selection is Overwrite.)
 - New: creates a new image every time the assay is run
 - Overwrite: overwrites the selected image or creates a new image if one does not exist
 - Add to: adds a plane to a stack
3. Click on the image name (typically indicated with [Source]).
4. Select Specified and give the image an appropriate name, such as Result.
5. Repeat steps 1-4 for all needed Result images
6. Select Apply/ Test run to check your result image

Determining the width of objects:

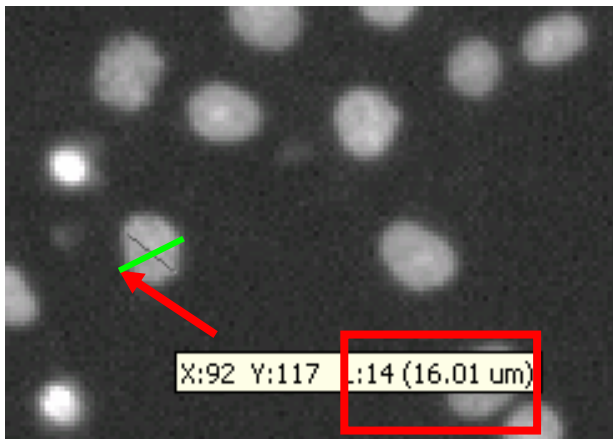
Many of the modules require you to specify a minimum, average, and/or maximum width. Use one of the following methods to determine the width of objects.

Determining the width using region tools:

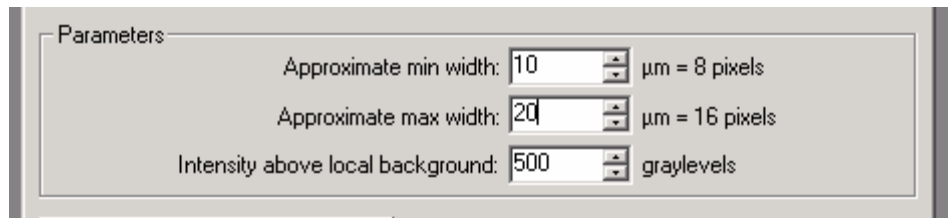
1. Select Region from the main toolbar, then Region Tools
2. The following toolbar should appear



3. Select the Line Tool.
4. Place your mouse pointer on the image and locate the object that you want to measure.
5. Move the mouse pointer to one of the edges and click.
6. Move the mouse pointer to the opposite edge and read of the number next to the L: In the following example, the value is 16. This number represents the cell width in pixels.



7. Type or select a value in microns in the Approximate Width box. In the following example, the value is 16. The dialog box accepts a single decimal place number.

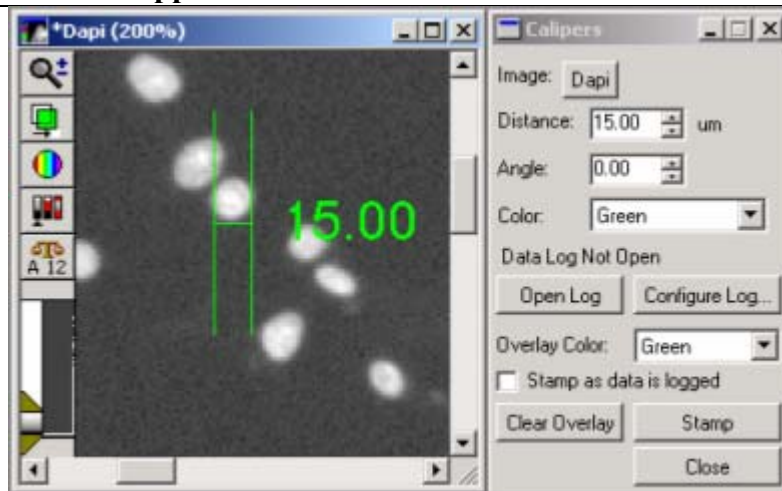


8. You can remove the region lines by selecting Regions, Clear regions from the main toolbar, or right mouse click on region and select delete region

Determining the width using calipers

Note: the caliper function may need to be loaded into the software. If the command is not present in the Measure menu please see your administrator to have this drop-in loaded.

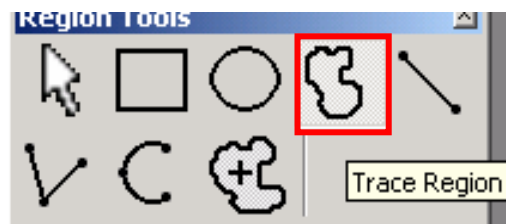
1. Select Caliper from the Measure menu: The following dialog box should opens.



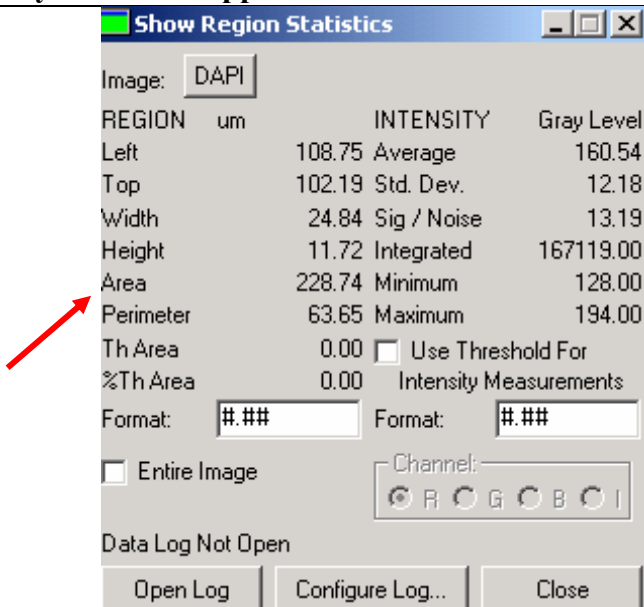
2. Move the pointer to the caliper in the image; keep the right mouse button pressed until a appears. Place your mouse pointer on the object to be measured keeping the right button depressed.
3. Reduce the size of the caliper by double-clicking the right mouse button on the caliper line until the caliper line “flashes.” Use one of the markers at the end of one of the lines to increase or decrease the size of the caliper line.
4. The size of the line is displayed in the image as well as in the Caliper dialog. **Note:** This image is calibrated in microns, and not in pixels.
5. Type or select the value that is shown in the image window in the dialog box.

Determining the Area or Width of an object using Show Region Statistics


1. Select the Trace Region tool from the region menu



2. Draw a region of interest around a typical compartment
3. Open Show Region Statistics (Measure menu)
4. Uncheck “Entire Image.”
5. Read the Area and/or Width measurement.



Determining the intensity above background

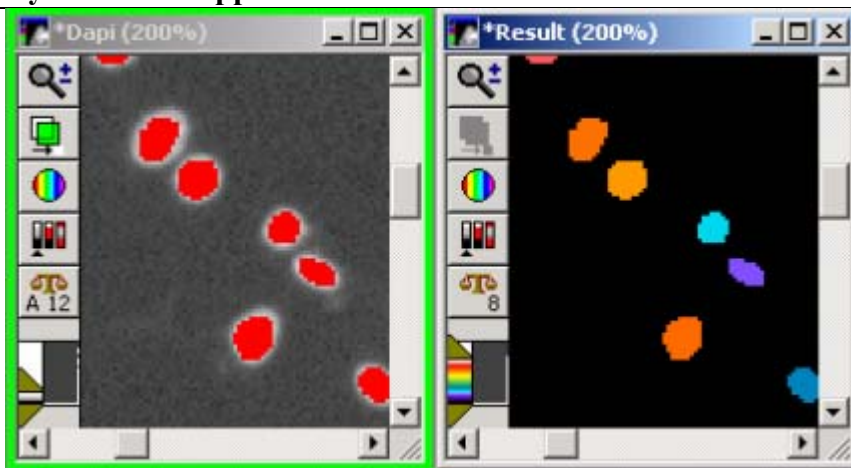
1. Select the Arrow on the region tool bar. 
2. Find one of the dimmest objects.
3. Move your mouse over the object and read of the grey value for this cell. This is the number that is displayed at the middle of bottom of the screen after the →. In the example below, this value is 162.



4. Move the mouse just outside the cell to measure the background.
5. Calculate the difference between the cell signal and the background. For the example image, the calculation would be as follows: $162 - 130 = 32$
6. Enter a slightly lower value in the Intensity above local background. In this example, a value of approximately 25 might be appropriate.

Testing your settings

1. Once all the settings have been entered, click either *Apply* or *Test Run* to use them for a measurement. An overlay image will appear. If selected, a result image will also appear.



Source image with an overlay

Result image with the cells individually colored

2. Compare the result image with the original to see if everything that you want has been detected. If not lower the intensity above background parameter first before modifying the other parameters.

Configure Log

1. Click Configure Summary Log. The Configure Log dialog box opens. Check and/or uncheck individual parameter configuration settings. Refer to the description of these settings under Dialog Box Options: Configure Summary Log in the Help file under F1.
2. Repeat the same for the Data Log if this option is present

Save settings

Single images: Module accessed directly from the Apps menu. The settings can not be saved if the assay is being run interactively or within a journal.

Review Screen Data:

- **Non database.** Click Save Settings to name and the settings are saved in the directory that is displayed on the Review Screen Data dialog on the Assay tab behind Location.
- **Database:** Click Save Settings to name and the settings are saved in the database that you are currently logged into.

Running the assay

If you are running the assay interactively or recording it in a journal, complete the following steps:

1. Open a summary log from the Log menu of Discovery-1. (Log, Open Summary Log, Select *DDE* if using Excel, change the sheet name if needed, followed by *OK*) and data log if needed (change the sheet name otherwise it will overwrite the summary data)
2. From the Apps menu select your assay (for example, Count Nuclei)
3. Set the appropriate parameters and select the images as described earlier in this document
4. Select *Apply*, the data is exported to Excel
5. Select the next images in the set and repeat steps 3 through 5.

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If you are running the assay through Review Screen Data or Screen Data Utilities, complete the following steps:

Non database

1. Open a summary log from the Log menu of Discovery-1. (Log, Open Summary Log, Select *DDE* if using Excel, change the sheet name if needed, followed by *OK*) and data log if needed (change the sheet name otherwise it will overwrite the summary data)
2. From the Assay tab of the Review Screen Data dialog box, select your assay (for example, Count Nuclei).
3. Select a setting from the *Settings* drop-down list.
4. Click *Run Assay for All Positions* to run the assay for all position on the plate.

OR

Click *Run Assay for Selections* to run the assay for the selected sites (select sites by clicking on different data points with right mouse).

OR

Click *Run Assay for Site* to run the assay for the current displayed image.

Database

You can choose to log into Excel and/ or the Database.

1. To log into Excel: Open a summary log from the Log menu of Discovery-1. (Log, Open Summary Log, Select *DDE* if using Excel, change the sheet name if needed, followed by *OK*) and data log if needed (change the sheet name otherwise it will overwrite the summary data)
2. To log into the Database, select the option Log into Database on the Assay tab of review Screen Data. Deselecting this option will omit logging into the Database.
3. From the Assay tab of the Review Screen Data dialog box, select your assay (for example, Count Nuclei).
4. Select a setting from the *Settings* drop-down list.
5. Click *Run Assay for All Positions* to run the assay for all position on the plate.

OR

Click *Run Assay for Selections* to run the assay for the selected sites (select sites by clicking on different data points with right mouse).

OR

Click *Run Assay for Site* to run the assay for the current displayed image.

Viewing your data

Excel: To view your data in excel, switch to the excel file by selecting the icon at the bottom of the window.

Database:

1. Switch to the Measurements Tab on review Screen data
2. From the dropdown list after measurements select the measurement that you want displayed in the window

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- To display all the measurements in the window, Change Data View to *Measurements vs Well*

Data view: Measurement vs Well

	Total Nuclei	Total Area	Mean Area	Integrat
A01	126.00	19160.94	152.07	5321
A02				
A03	265.00	40051.56	151.14	1131
A04	196.00	34453.13	175.78	105E
A05	86.00	13573.44	157.83	523:
A06	76.00	10835.94	142.58	481:
A07	59.00	9285.94	157.39	438:

- To display your individual cell data (data log), select site details at the bottom of review screen data.

Note: For more information on displaying data in the Database please select F1 (help) and enter the word database under the index tab.