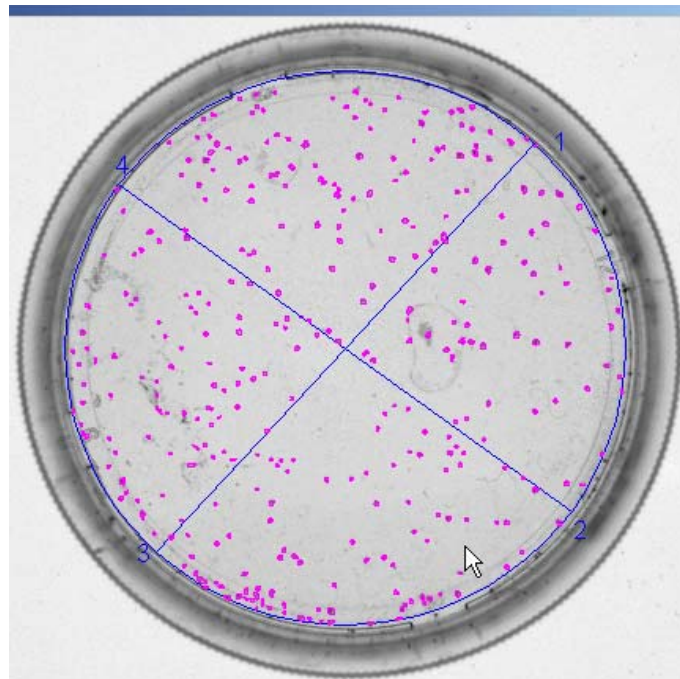




# *AIDA Colony Counting for Windows*

*User's Manual*



Part Number 2005 08 1.0



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# Introduction

1

## Overview

The AIDA Colony Counting module provides an automatic counting of colonies in a circular or rectangular petri dish and 24 or 96 wells micro titer plates. It needs an image of the over named objects as input.



## Starting Colony Counting

To select the **Colony Counting** mode:

- 1 On the tool bar, click the **Evaluation** button.

A dialog box appears offering you a list of available evaluation modes.

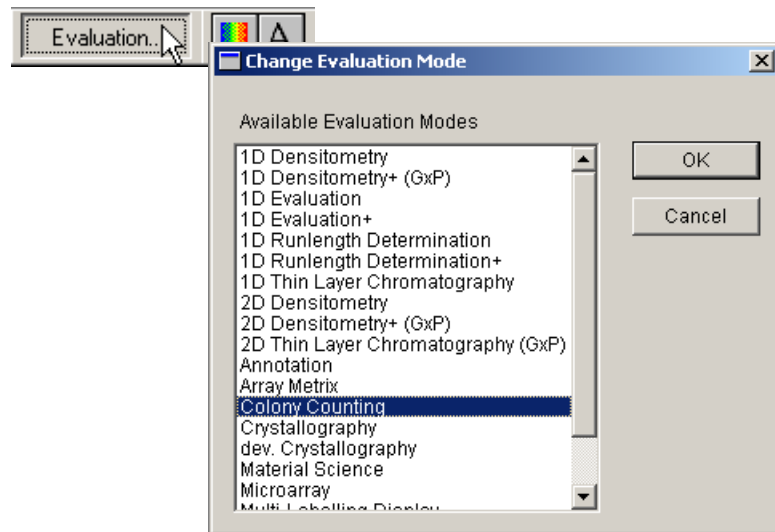


Figure 1-1 Changing Evaluation Mode

The list entries reflect the installed AIDA application modules.

- 2 Select the **Colony Counting** list item and click **OK**.

---

**Note** – This evaluation mode is only available if you purchased the AIDA Colony Counting module.

---



## Using Colony Counting

## 2

### Data Windows in Colony Counting

In addition to the AIDA standard data windows **Image** and **Histogram** the **Colony Report** data windows is available in the **Colony Counting** mode.

You can access this data windows by choosing the entry **Colony Report** from the **View** menu

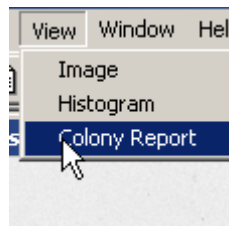
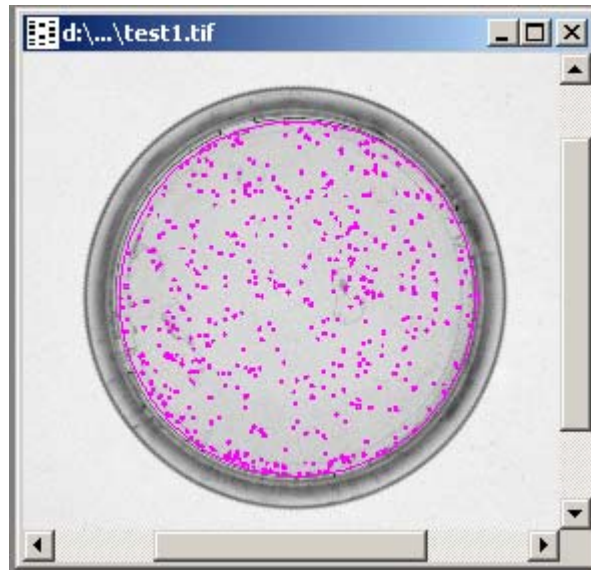


Figure 2-1 View Menu

## Image Window in Colony Counting

All data are evaluated from the **Image** window. Although it might not always be on top, it is always open. The title bar of the **Image** window shows the full file path of the image



You can copy the content of the **Image** window to the clipboard using the **Copy Content** command of the **Edit** menu. See the Export Image section in the AIDA Image Analyzer Basic Concepts and Features User's Manual for further explanation.



## Contextual Menu of the Image Window

In the contextual menu of the **Image** window in Colony Counting you can select all found colonies or delete selected colonies:

### Select All Colonies:

If there are some not selected colonies, the contextual menu, that opens after right-clicking, provides you with the commands Select All and delete if there are selected colonies for the selection.

### Delete Selected Colonies:

If you have selected some of colonies or all of them, the contextual menu, that opens after right-clicking, provides you with the delete command for the selection.

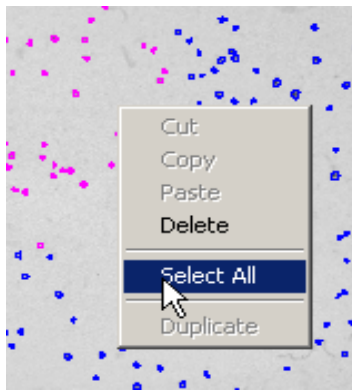


Figure 2-2 Contextual Menu (Select All)

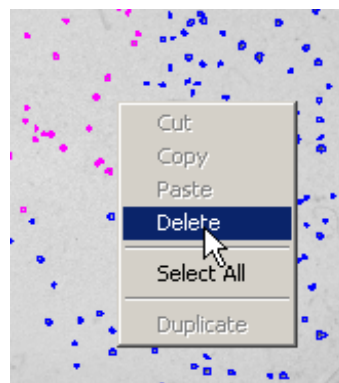


Figure 2-3 Contextual Menu (Delete)

## Image Overlays

You can specify the label type and font you want to apply to the overlays (Segments of Search Area) using the **Image Overlays** command of the **Options** menu.

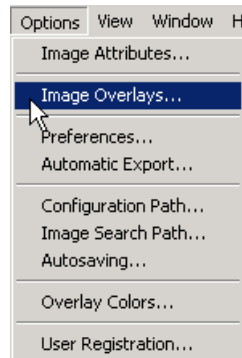


Figure 2-4 Options Menu: Opening the Image Overlays Dialog Box

After choosing **Image Overlays** from the **Options** menu, the **Image Overlays** dialog box opens allowing you to set the font, font size, and font style (bold, italic, roman typeface on white background) as well as the rotation (0°, 45°, 90°) for the labels. The detected lanes can be surrounded by a frame (**Border Lines**) and tracked by a **Middle Line**.

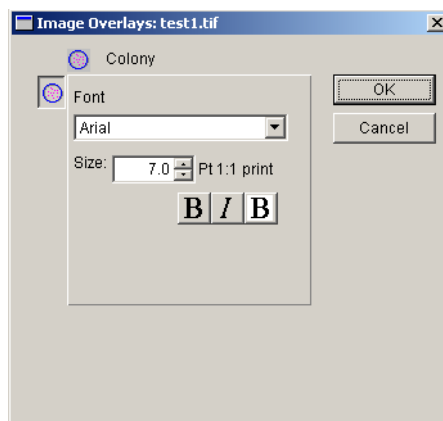


Figure 2-5 Image Overlays Dialog Box



## Colony Report Window

The **Colony Report** window consist of tables for each segment of Search Area, listing the area, circularity, integral and maximal intensity of particular colony.

### Data Description

Available **Columns** in the **Result Table**:

Table 2-1 *Columns Available in the Result Table of the Profiles Window*

Column	Short Description
Number	The number of the colony.
Area	The area of the colony.
circularity	The circularity of colony in range of [0.000-1.000].
Position. [ <i>length unit</i> ]	The position of the band in the image measured from the edges of the image.
Integral [ <i>signal unit</i> ]	The integrated colony intensity.
Max Intensity[ <i>signal unit</i> ]	The maximal Intensity in the region of colony.

*signal unit* and *length unit* = units of measurement, depending on the type of the imaging device used to obtain the image and the options set in the **Image Attributes** dialog box.

The following additional rows can be found or created exclusively in the **Colony Report**:

Table 2-2 *Additional Columns in the Region Report*

Total Number	Displays the total number of found and created colonies in all Segments.
--------------	--

You can select the number and type of columns you want to display using the contextual menu of any column in the window (see below).

#### To change the column order:

- 1 Click the heading of the column to be moved.
- 2 Press the mouse button and drag to the desired position in the table.



3 Release the mouse button.

No	Circularity	Area [pixel]
<b>20: 6 colonies found</b>		
1	0.986	15.000

No	Circularity	Area [pixel]
<b>20: 6 colonies found</b>		
1	0.986	15.000

No	Area [pixel]	Circularity
<b>20: 6 colonies found</b>		
1	15.000	0.986

Figure 2-6 Changing Column Order

**To change the column width:**

- 1 Position the mouse pointer over the border of the column whose width you want to change.
- 2 Click and drag the border in the appropriate direction.
- 3 Release the mouse button:

No	Area [pixel]	Circularity
<b>20: 6 colonies found</b>		
1	15.000	0.986

No	Area [pixel]	Circularity	
<b>20: 6 colonies found</b>			
1	15.000	0.986	177

No	Area [pixel]	Circularity
<b>20: 6 colonies found</b>		
1	15.000	0.986

Figure 2-7 Changing Column Width



## Contextual Menu of the Colony Report Window (Result Table)

Each column in the **Result Table** has a contextual menu attached. To open the contextual menu, right-click on the column heading (gray area).

No	Area [pixel]	Circularity	Position	Integral [QL]	Max. Intens [QL]
6	38.00		84.000	83341.0	2610.
7	118.00		61.000	435975.0	8967.
8	147.00		82.000	421373.0	4324.
9	13.00		94.000	30146.0	2777.
10	22.00		23.000	36411.0	1791.
11	10.000	1.000	687.000 , 823.000	16613.0	1748.
12	125.000	0.503	689.000 , 844.000	394514.0	6241.
13	12.000	0.560	655.000 , 809.000	24815.0	2243.
14	10.000	1.000	640.000 , 817.000	20157.0	2062.
15	269.000	0.600	657.000 , 828.000	1149074.0	8330.
16	97.000	0.539	658.000 , 855.000	425008.0	9821.

Figure 2-8 Contextual Menu of the Profiles Window's Result Table

With the first two commands of the contextual menu, you can sort the table of the **Result Table** by any value column in ascending order (A to Z or zero to 9) or descending order (Z to A or 9 to zero). Just choose the appropriate menu item (**Sort Ascending by/Sort Descending by**).

The **Select/Unselect** commands of the contextual menu are used to select/deselect a column to export. The column heading of selected columns are highlighted.

To hide a column, choose **Hide** command from its contextual menu. You can recover hidden columns using the **Select Columns** command (see below).

To specify the decimal digits for the value output in a column, choose **Settings of** from the contextual menu and enter the value for the decimal digits in the dialog box displayed.

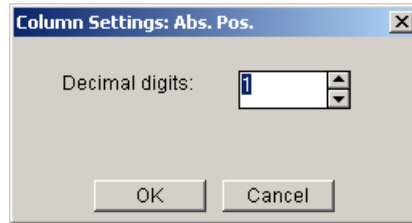


Figure 2-9 Setting Output Format for Columns

Use the **One-lined Header/Two-lined Header** command to reduce or expand the column heading by one line (the second line indicates the units of measurement).

**To select/deselect the columns that should be displayed in the Result Table:**

- 1 Choose **Select Columns**.  
A dialog box appears displaying two lists (**Show Columns/Hide Columns**).
- 2 Select the columns you want to show or hide and click the appropriate arrow symbol to transfer the items between the two lists.
- 3 To deselect a particular item, just click it again.

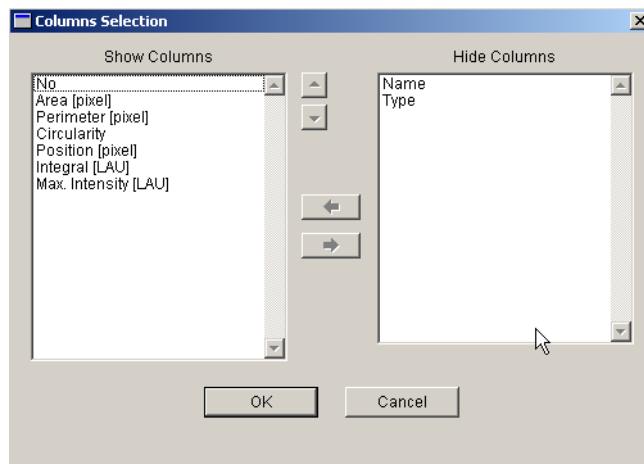


Figure 2-10 Columns Selection Dialog Box



## Toolboxes in Colony Counting

In addition to the standard AIDA toolboxes **Display Control** and **Publishing Layout**, the **Colony Determination** toolboxes becomes available after selecting the **Colony Counting** mode.

You can access Colony Determination toolbox from the **Evaluation** menu or by clicking the appropriate button on the too bar.

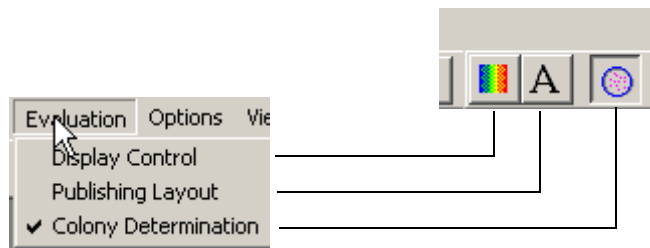


Figure 2-11 Mapping between Evaluation Menu Entries and Toolbar Buttons

## Colony Determination Toolbox

The controls of the **Colony Determination** toolbox allow you to define Search Area, set search parameters and edit new colonies.

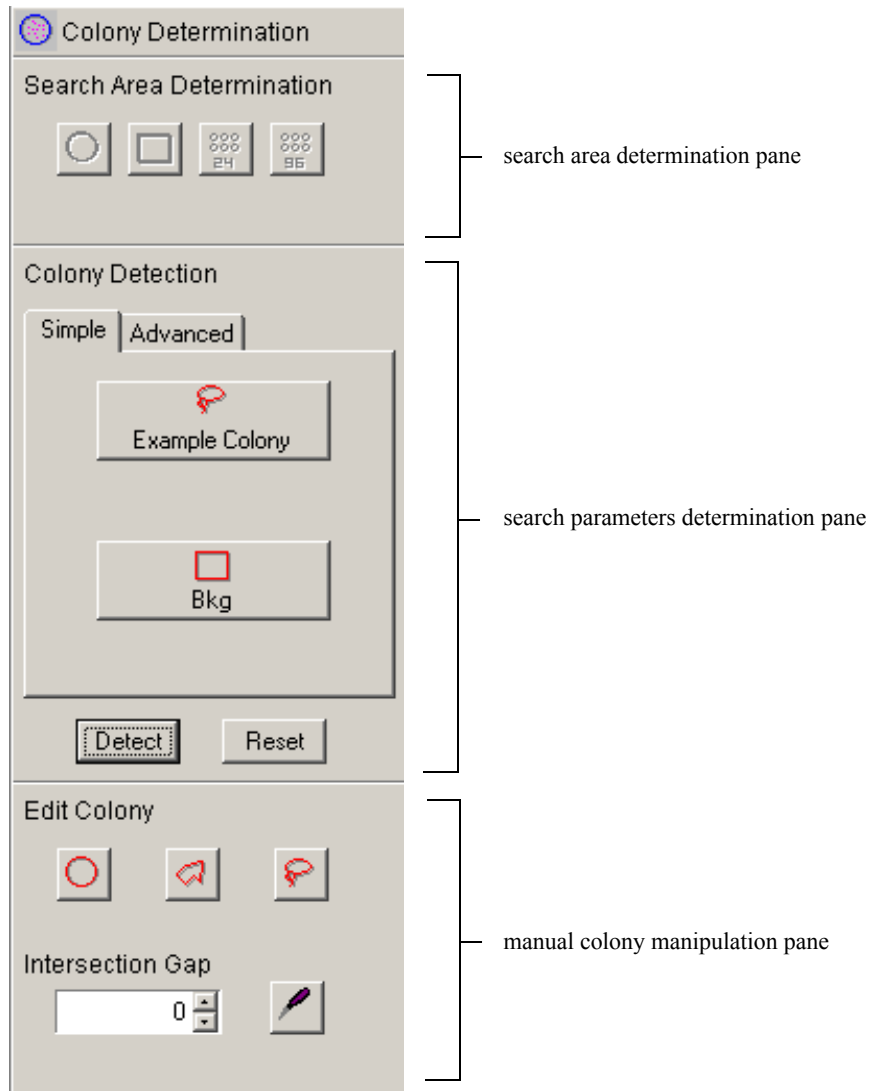


Figure 2-12 Colony Determination Toolbox (Overview)



### To display the Colony Determination toolbox:

- On the main menu, click **Evaluation** and choose **Colony Determination**.

Alternatively you can click the **Colony Determination** button on the toolbar.

### Search Area Determination Pane

The first step in the analysis of a Colony Counting-application is the determination of the Search Area.

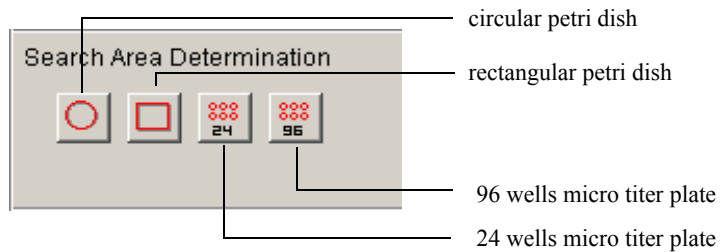


Figure 2-13 Search Area Determination Pane (Overview)

Once you determined an overlay of your search area, you can modify it before there are colony detected or created respectively the same way as described chapter Performing Colony Counting.

## Colony Detection Pane

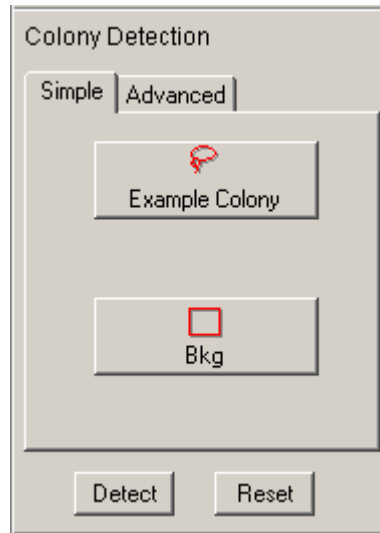


Figure 2-14 Search Parameters Determination Pane (Simple Mode)

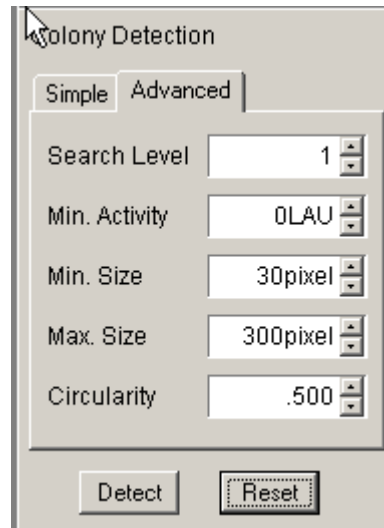


Figure 2-15 Search Parameters Determination Pane (Simple Mode)

This pane will be needed to determine the search parameters and start the search process. You can switch between the simple and advanced mode. In simple mode you have to create at least one example colony to let the program determine the appropriate parameters set while in advance mode you can set the parameters explicitly. Setting the search parameters is very important to obtain good results.

Clicking the **Reset** button set the parameters to the default values. Click the **Detect** button to start the detection process.

## Colony Edit Pane

This pane will be needed to create undetected colonies and disjoin connected colonies manually. Select the upper three boxes to create colonies with appropriate shape. For more details see chapter **Performing Colony Counting**.

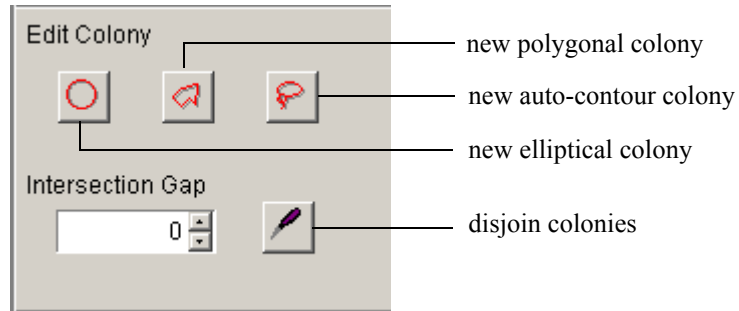


Figure 2-16 Edit Colonies Pane



## Publishing Layout Toolbox in Colony Counting

In AIDA Colony Counting the standard AIDA **Publishing Layout** toolbox is expanded by an additional button for specifying the print options for the **Colony Report**:

### Print Options for Colony Report

Using the **Colony Report** button you can configure layout setting for the **Report Table** window.

- 1 Click the **Print Options: Colony Report** button.  
The appearance of the **Protocol Print** pane changes.

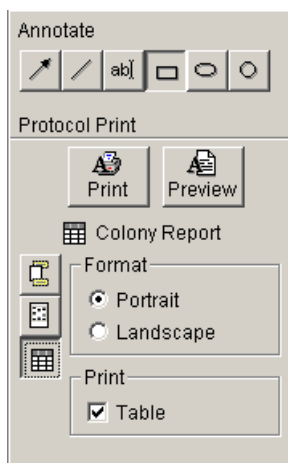


Figure 2-17 Print Options for the Profiles Window

- 2 Select the appropriate options:

In the **Format** pane select the paper orientation (**Portrait** or **Landscape**) by clicking the appropriate radio button.

In the **Print** pane you can specify to print the table or not.

## Print

In AIDA there are two ways for printing your image and evaluation data:

- Choose **Print** from the **File** menu,
- or*
- Use the **Protocol Print** pane on the **Publishing Layout** toolbox.

The **Print** command of the **File** menu allows you to print your data 'on the fly' without permanently storing the layout/printing settings, whereas the **Protocol Print** pane provides you with a complete set of printing features which includes saving the setting with the data file for reasons of reproducibility.

Choosing the **Print** from the **File** menu opens the **Print** dialog box, allowing you to configure the printout to contain only the overlays and results that are currently needed. Click the check boxes for the various items to turn them on or off. Select the number of copies, you want to print.

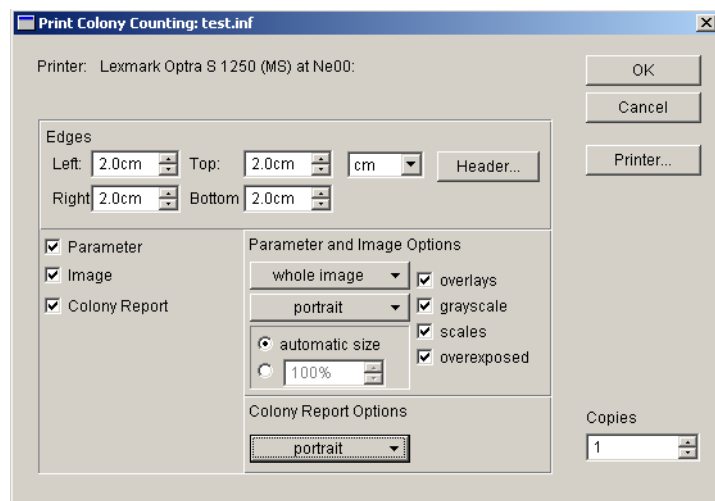


Figure 2-18 Print Dialog Box



The margins of the printout can be selected in the **Edges** part of the dialog box. The **Header** button displays the following dialog box:

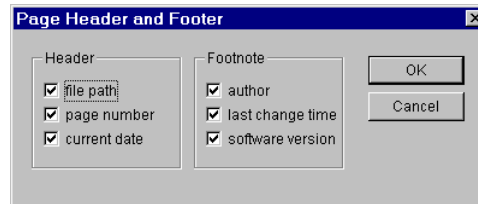


Figure 2-19 Page Header and Footer Dialog Box

Here the **Header** and **Footer** of the report can be configured to contain various image and evaluation specific data.

The **Parameter** (data overlays), the **Image** and **Colony Report** information can be selected in the left part of the **Print** dialog box.

In the right part of the dialog box (**Parameter and Image Options**) you can select **Whole Image**, or **Window Content** from the first drop-down menu. **Landscape** or **Portrait** format can be selected from the second drop-down menu. The printing of **Overlays**, **Grayscales**, **Scales**, and **Overexposed** can be selected. The printout can be automatically fitted to the page, or the relative size of the image representation can be selected (**in %**).

In the **Colony Report Options** pane you can specify the paper orientation (**Landscape** or **Portrait**) for the printout.

## Preview

As for printing, in AIDA there are two ways of previewing pages before they are actually printed:

- Using the **Print Preview** option of the **File** menu, or
- Using the **Preview** button on the **Publishing Layout** toolbox.

The **Print Preview** option of the **File** menu opens the **Preview** dialog box, which allows you to specify the layout settings for the preview. The options presented here are the same as in the **Print** dialog box and those selectable from the various panes of the **Publishing Layout** toolbox. However, they are not stored with the data file.



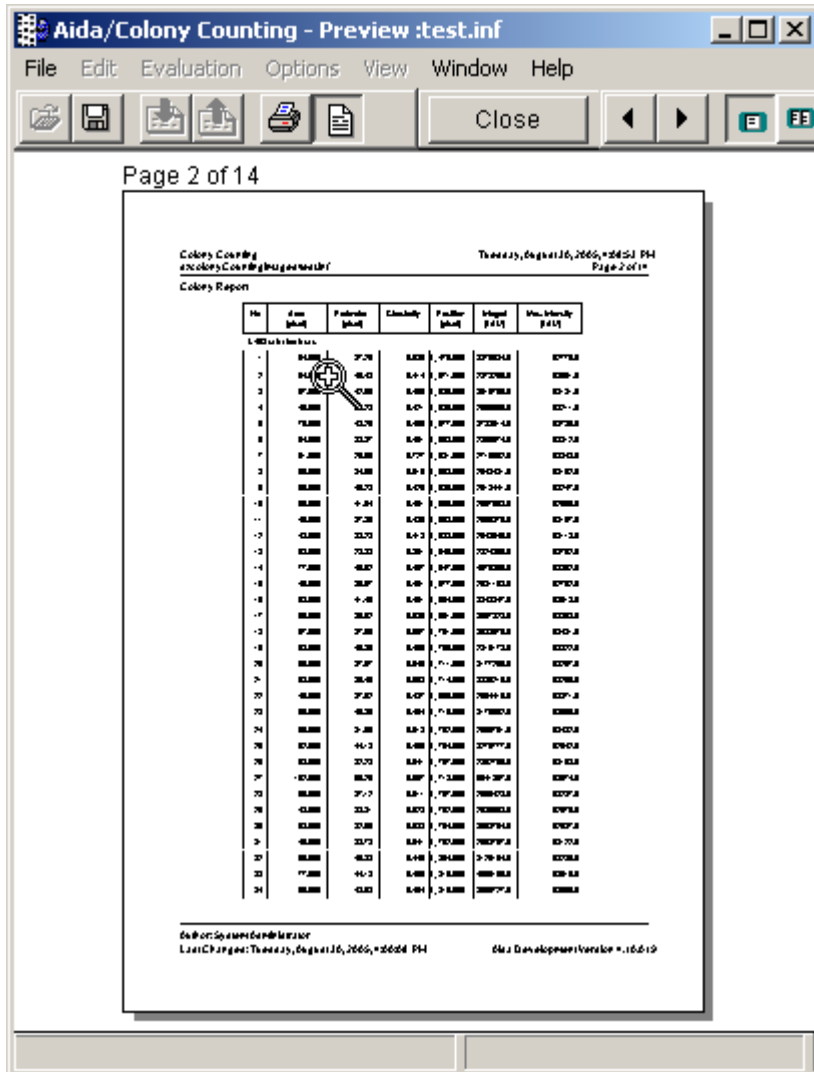


Figure 2-21 Preview Example Part 2

## Export and Import

In AIDA Colony Counting **Colony Report** table can be exported to be imported in other documents or programs. In addition the templates can be exported to be applied to other images. The various functions can be found in the **Export** and **Import** submenus of the **File** menu.

### Exporting Templates

To export your own evaluation templates:

- 1 On the **File** menu, click the **Export** menu item and then choose **Template** from the submenu.
- 2 In the **Export Template** dialog box that appears type a name for the export file and confirm the action by clicking **Save**,

or

- 1 Click the **Export Template** button on the tool bar to open the **Export Template** dialog box



Figure 2-22 Main Toolbar (Export Template Button)

- 2 Type a name for the export file in the **File Name** text box and complete the export action by clicking **Save**.

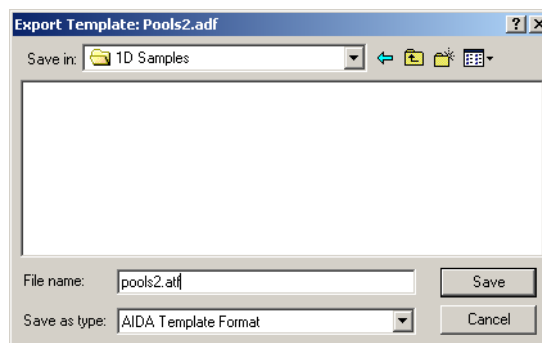


Figure 2-23 Export Template Dialog Box



## Exporting Colony Counting Report Table

To export the Colony counting Report Table:

- 1 On the **File** menu, click the **Export** menu item and then choose **Colony Report** from the submenu.
- 2 In the **Colony Report Export** dialog box that appears type a name for the export file.

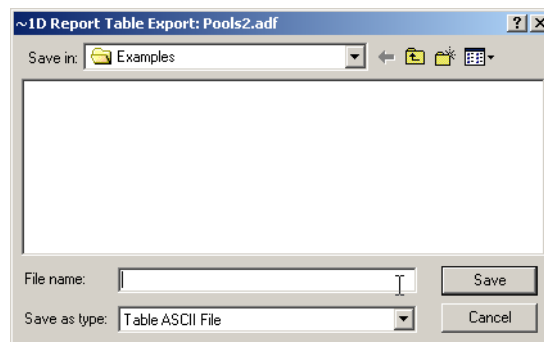


Figure 2-24 Colony Counting Report Table Export Dialog Box

- 3 Click **Save** to confirm the export.  
You can specify the **Range** of the exported rows (**Header** only, **All Rows** or **Selected Rows** only) as well as the **List Delimiter** and **Decimal Delimiter** in the respective panes of the dialog box. Selecting the **System Defaults** button applies the Windows default settings to **List Delimiter** and **Decimal Delimiter**.
- 4 Complete the action by clicking **OK**.  
The data are saved to a simple ASCII (text) file.

## Importing Templates

To import previously saved templates to another equivalent image:

- 1 On the **File** menu, click the **Import** menu item and then choose **Templates** from the submenu.



- 2 In the **Import** dialog box that appears select the template to be imported and click **Open**.

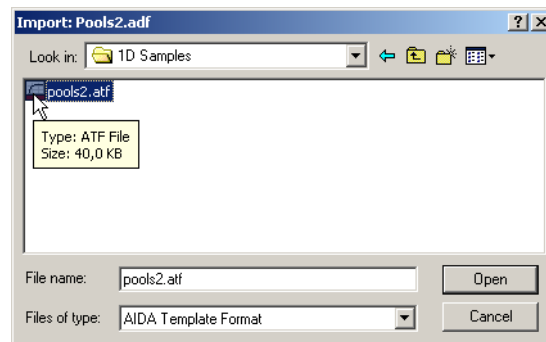


Figure 2-25 Import Template Dialog Box

The imported Overlays can be arranged to fit the image by moving the whole overlay set using the AIDA standard overlay editing functions.



## Performing Colony Counting 3

### Assigning Search Area and its Segmentation

The first step in the Colony Counting is the determination of the search area and its segmentation. The Search Area tells the module where to search for colonies and its segmentation organizes the Colony Report table in sub tables. The result is a fully evaluated image where all the information can be collected in a comprehensive printout.

With AIDA Colony Counting it is usually preferable to select all parameters that are necessary for an automatic detection of colonies. When all parameters are well chosen, further colonies can be automatically selected and assigned. The detected colonies can be manipulated in shape and size and even divided if some connected colonies detected as one. Further it is a manual creating of colonies which does not fulfill the search parameter conditions.

To begin with the evaluation of Image it is important to set the **Image Attributes** via the **Options** menu first (see “AIDA Image Analyzer Basic Concepts and Features User's Manual” for details). If these settings are not correct further image evaluation will not produce meaningful results.

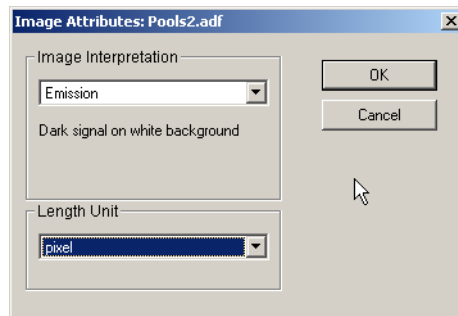


Figure 3-1 Image Attributes Dialog Box

The first step in the analysis of a Colony Counting-application is the determination of the Search Area.

## Search Area Determination

Select appropriate tool from the Search Area Determination pane to create a the Search Area for your application.

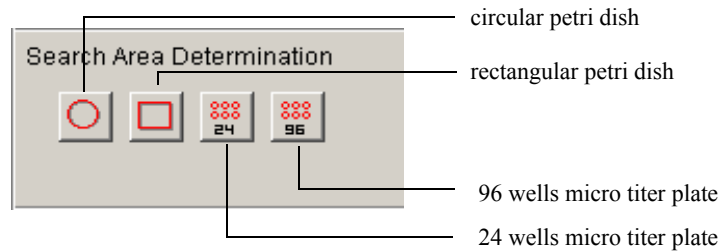


Figure 3-2 Search Area Determination Pane (Overview)

Once you determined an overlay of your search area, you can modify it before there are colonies detected or created respectively the same way as described below. Double click the confirmed overlay to enter the edit mode.

**Circular Petri Dish Tool:** Select this tool to create an overlay to determine the search area on a petri dish Image and its intersection if necessary.

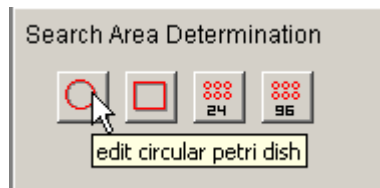


Figure 3-3 Circular Petri Dish Selection

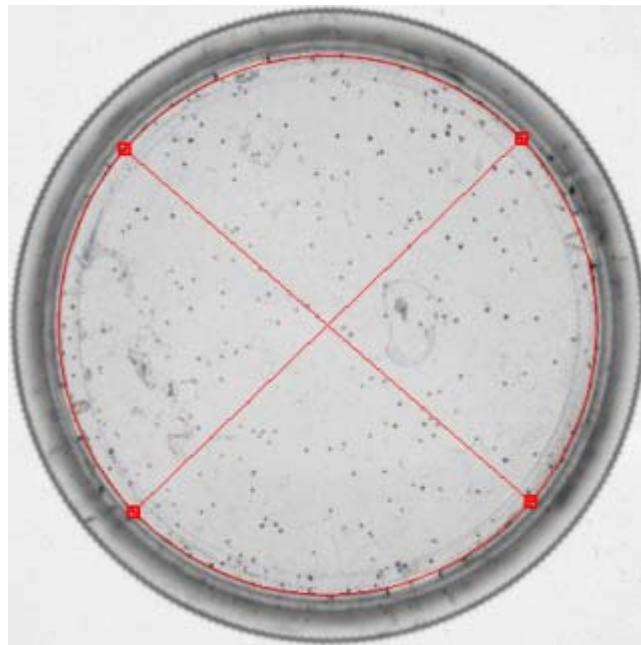
### To create an overlay on a circular petri dish:

- 1 Select petri dish box by a mouse click.
- 2 Press the mouse left button on an arbitrary point on the boundary of desired search area and drag to the opposite point on the other side of the boundary.
- 3 Release the mouse button. You are now in “create” mode.
- 4 Press the mouse left button and drag it to move the search area to a desired position and release it
- 5 Double click on the boundary of the search Area intersect the search area to two segments. You can repeat this step till you have the appropriate segmentation of the search area. Once you create an intersection line, you can move this line by clicking



the mouse left button on an end of intersection line and drag it to a desired position and release it. Move the cursor to an end of an intersection line and press the delete button to remove it.

- 6 you can change to “scale”, “rotate” and again to “create” modes by clicking the mouse left button once. In these mode you can change the size and the position of the search area.
- 7 Once you have created an appropriate search area with or without a segmentation, click the mouse right button to confirm your search area.



*Figure 3-4 Editing Circular Petri Dish with Segmentation*

**Rectangular Petri Dish Tool:** Select this tool to create an overlay to determine the search area on a petri dish Image and its intersection if necessary.

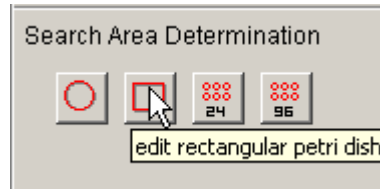


Figure 3-5 Rectangular Petri Dish Selection

**To create an overlay on a rectangular petri dish: Select rectangular petri dish box by a mouse click.**

- 1 Select petri dish box by a mouse click.
- 2 Press the mouse left button on the upper left edge of the petri dish and drag to its bottom right edge. The connection line of this points describe the diagonal of rectangular overlay, so you can choose these points in other ways.
- 3 Release the mouse button. You are now in “create” mode.
- 4 Press the mouse left button and drag it to move the search area to a desired position and release it
- 5 Double click on the boundary of the search Area create an intersection line between the click position and the a point on the opposite edge with the same width or height respectively. You can repeat this step till you have the appropriate segmentation of the search area. Once you create an intersection line, you can move this line by clicking the end of the intersection line and drag it to a desired position and release it. Move the cursor to an end of an intersection line and press the delete button to remove it.
- 6 you can change to “scale”, “rotate” and again to “create” modes by clicking the mouse left button once. In these mode you can change the size and the position of the search area.
- 7 Once you have created an appropriate search area with or without a segmentation, click the mouse right button to confirm your search area.

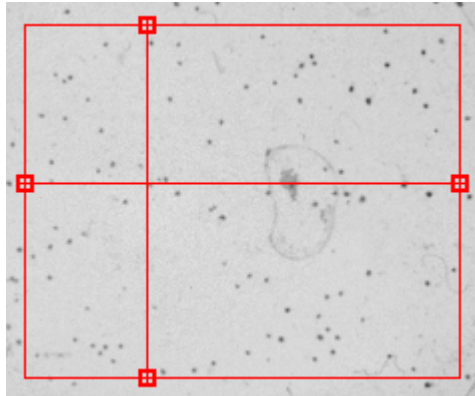


Figure 3-6 Editing Rectangular Petri Dish with Segmentation

**Rectangular Petri Dish Tool:** Select this tool to create an overlay to determine the search area on a petri dish Image and its intersection if necessary.



Figure 3-7 Micro TiterPlate with 24 Wells Selection

**To create an overlay for a micro titer plate:**

- 1 Select on available micro titer plate. You can edit both available plates the same way.
- 2 Press the mouse left button on the upper left edge of the plate and drag to its bottom right edge. The connection line of this points describe the diagonal of the plate overlay, so you can choose these points in other ways.
- 3 Release the mouse button. You are now in “create” mode.
- 4 Press the mouse left button in the overlay but outside of the wells and drag it to move the whole search area to a desired position and release it.
- 5 Click inside a particular well to adjust its position.

- 6 you can change to “scale”, “rotate” and again to “create” modes by clicking the mouse left button once. In these mode you can change the size and the position of the search area.
- 7 Once you have created an appropriate overlay of the plate, click the mouse right button to confirm your search area.

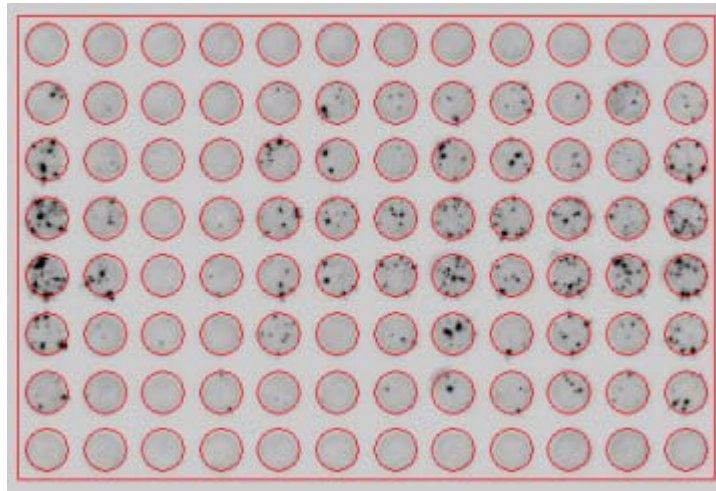


Figure 3-8 Editing Rectangular Petri Dish with Segmentation

### Setting Colony Detection Parameters

This pane will be needed to determine the search parameters and start the search process. You can switch between the simple and advanced mode. In simple mode you have to create at least one example colony to let the program determine the appropriate parameters set while in advance mode you can set the parameters explicitly. Setting the search parameters is very important to get good results. Clicking the **Reset** button set the parameters to the default values. Click the **Reset** button to start the detection process.



Using simple mode to determine appropriate search parameters:.

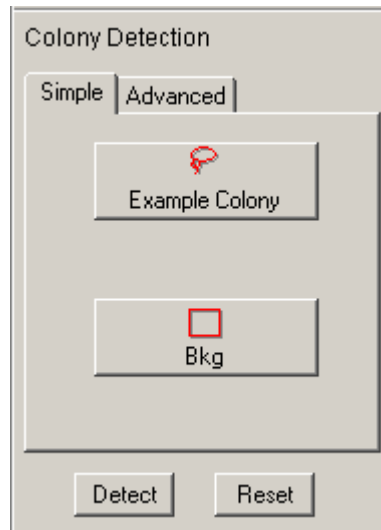


Figure 3-9 Search Parameters Determination Pane (Simple Mode)

- 1 Click the **Example Colony** tool box, to create example colonies.
- 2 Press the mouse left button on a colony and drag around. An overall will be automatically created. You can drag around till you get the desired shape of the example colony and release the button. A right click confirm the creation of example colony. You can repeat this step several times. It is recommended to do this at least twice, one colony with the weakest intensity and one with the minimal allowed size. In this way you set implicitly the minimal intensity and the minimal allowed size of colonies. It is also recommended to create one or more large colonies if the size of large colonies is approximately larger than twice of the minimum.
- 3 You can create a rectangular **Background Region** the same way as **Example Colony**. This set the minimal intensity in the parameters set. The current value of **Min. Activity** will be replaced with the average intensity in the background region if it is more than the current value of **Min. Activity**. Use advanced mode to set search parameters manually:

### Using advanced mode to set the search parameters:.

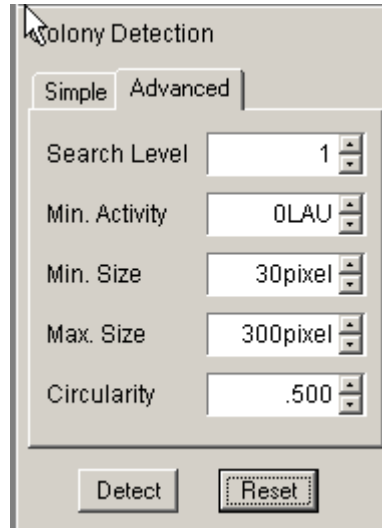


Figure 3-10 Search Parameters Determination Pane (advanced Mode)

In this pane you can set the search parameters explicitly.

- 1 You can determine the **Search Level** between 1-10. **Search Level** stays unchanged while you are in simple mode. Increasing this parameters increases the sensitivity of search algorithm. But a higher search level causes sometimes a longer search time, so it is recommended not to choose high levels if not necessary.
- 2 Trough **Min. Activity** you can set the minimal intensity which have to be considered as a valid signal.
- 3 **Min. Size** determines the smallest allowed size for the colonies.
- 4 **Max. Size** determines the largest allowed size for the colonies.
- 5 **Circularity** can be set in range of 0.001-1.000. The higher this value the rounder the allowed colonies.

### Manual Editing of Colonies

This pane will be needed to create undetected colonies and disjoin connected colonies manually. Select the upper three boxes to create colonies with appropriate shape.



### Using Edit colony pane:.

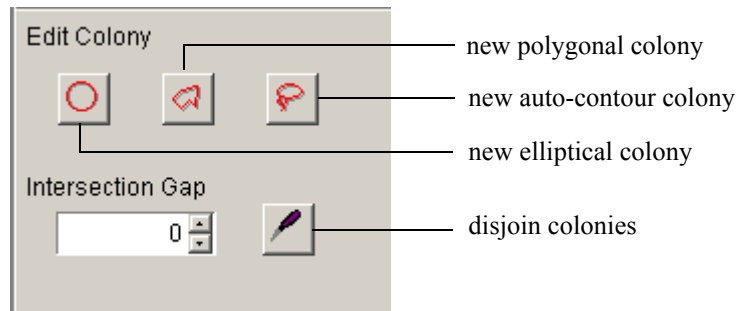


Figure 3-11 Edit Colonies Pane (Simple Mode)

- 1 Select the new elliptical colony by clicking to create an elliptical colony. Once you have selected this box, press the mouse left button and drag it diagonally till you get the desired shape and release it. Clicking the mouse right button confirms your new colony.
- 2 Select the new polygonal colony by clicking to create a polygonal colony. Once you have selected this box, press the mouse left button and drag it to the second vertex of your polygon and release it. The next vertices can be determined by a mouse click on the desired position. Repeat this till you achieve the desired form and confirm it with a mouse right button click.
- 3 Select the new auto-contour colony by clicking to create a colony consisting of pixels with similar intensity. Once you have selected this box, press the mouse left button and drag it around to get a desired form and release it. This can be confirmed by a mouse right button click.
- 4 In the **Intersection Gap** field you can set the distance between two connected colonies, which will be disjoined as described below.

- 5 Select the disjoin colony box divide two connected colonies. Press the mouse left button and drag it to draw an intersection curve and release it. A right click divide the colony.

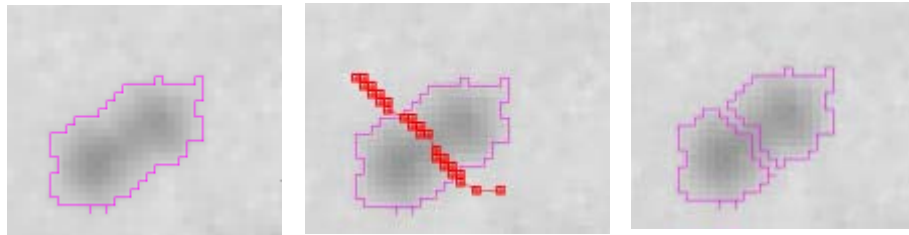


Figure 3-12 Disjoin Connected Colonies

In addition to tools in **Edit Colony Pane**, you can change the form of colonies directly on image. All colonies are polygons unless they are created as elliptical colonies.

**To manipulate the shape and size of a colony:** Double click the colony, which must be changed. You can now see the vertices of colony as little red squares. you can change their position by drag and drop or delete them by pointing them with the mouse curser and pressing the delete button. Once you get your desired shape and size, cavern your changes by a right click.

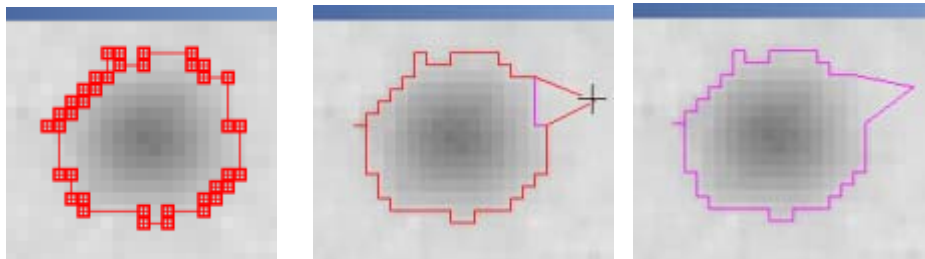


Figure 3-13 Manual Colony Manipulating