cellAnalyst™

User's Manual

Version 2.0

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User Notes…

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Introduction

cellAnalyst analyzes images and builds a database of cell information captured from microscopy images. It has the following capabilities:

- Creates hierarchical albums/image structure. Images from hard disk can be imported into albums.
- Analyzes images (counts cells) and stores the output in a database.
- Enables users to query the database and retrieve images with relevant cell information.

Hierarchical Albums/Images Structure

cellAnalyst enables users to create an image library and within it a hierarchy of albums and images. The concept of albums and images is similar to the concept of folder and files of a hard disk. Just like a folder may contain other folders and/or files, an album may contain other albums and/or images. The difference is that each image or album can be contained in more than one album.

The following commands are supported:

Library	Album	Image
Create library Open library Delete library	Create album Add images from hard disk to an album Add a complete folder of images from hard disk to an album Delete album Rename album Link album to another album (possibly more than one)	Copy/Move images from one album to another using drag and drop Link image to an album (an image can belong to more than one album) Delete image from an album Rename image

cellAnalyst allows user to add annotation to any image. Later these annotations can be used to search images.

Analysis of Images & The Stored Data

cellAnalyst analyzes images and computes the number of objects of different kinds found in each image. In biological images objects found in an image are referred to as cells.

The user can vary the following parameters of the objects to get the optimal analysis of an image:

- Size
- Contrast

Since it can take a little effort to determine the optimal settings, they are preserved in the database. The analysis data about the objects found in the image is displayed and stored in the database. This data includes the following:

Descriptor	Definition
Type	Dark or light object
Location	The X, Y-coordinates of the geometric center of mass of the object
Size	The area of the object measured in pixels
Perimeter	The length of the boundary of the object
Roundness	A perfect circle will have a value of 100
Intensity	The highest or the lowest intensity of the pixels for light or dark objects respectively
Contrast	The difference between the intensity of the object and the intensity of the surrounding area
Saliency	The combination of size and contrast; indicates how important this object is relative to other objects
Center of Mass	If the object intensity values are not homogeneous, this indicates the true center of mass
Average Contrast	The difference between the average intensity of the object and the intensity of the surrounding area

Querying the Database

After images have been analyzed and the information about the cells has been stored in the database, it can be queried by the user. Some examples of queries are as follows:

- Retrieve all images where dark-cell count is between 100 and 200.
- Retrieve all images that contain cells between 200 and 300 pixels in size.
- Retrieve all the information about all the cells found in a particular image.
- Retrieve the optimal settings for analysis of a given image.
- Retrieve all images that have been analyzed with size settings between 200 and 250 and contrast setting between 20 and 50.

- Retrieve all images with “red blood cells” in their annotation.

Open Source Software

cellAnalyst uses the following open source software:

- SQLite Relational database management system (www.sqlite.org). SQLite is used for storing album/image hierarchy and image analysis data.

- ITK Library (www.itk.org). ITK library is used for applying filters to an image.

Getting Started

Installing cellAnalyst

cellAnalyst runs on the Microsoft Windows platform. Before installing this software make sure that Microsoft .NET Framework 3.0 Package is installed on your PC. Microsoft Windows/Vista OS comes with .NET 3.0. However, if you are using Windows/XP OS, it will be necessary to download .NET 3.0 Framework and install it on your PC. It is available free of charge from the Microsoft web site, www.microsoft.com. The minimum display resolution of your PC monitor should be 1280 by 720 pixels.

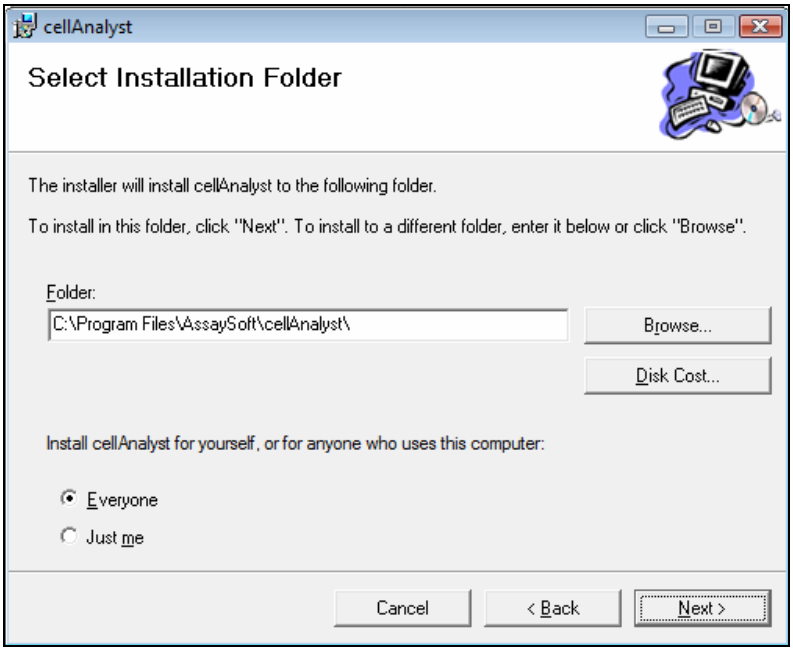
The *cellAnalyst* software package contains the following files:

- setup.exe
- cellAnalystSetup.msi

Run the "setup.exe" and the software will get installed in the following folder.

- C:\Program Files\AssaySoft\cellAnalyst

If you would like to install the software at a different location, please specify the new location during installation.

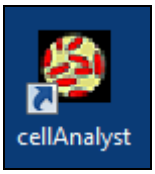


Accept the software license agreement and select "Next" button. After the installation an icon of the *cellAnalyst* software will appear on the desktop.

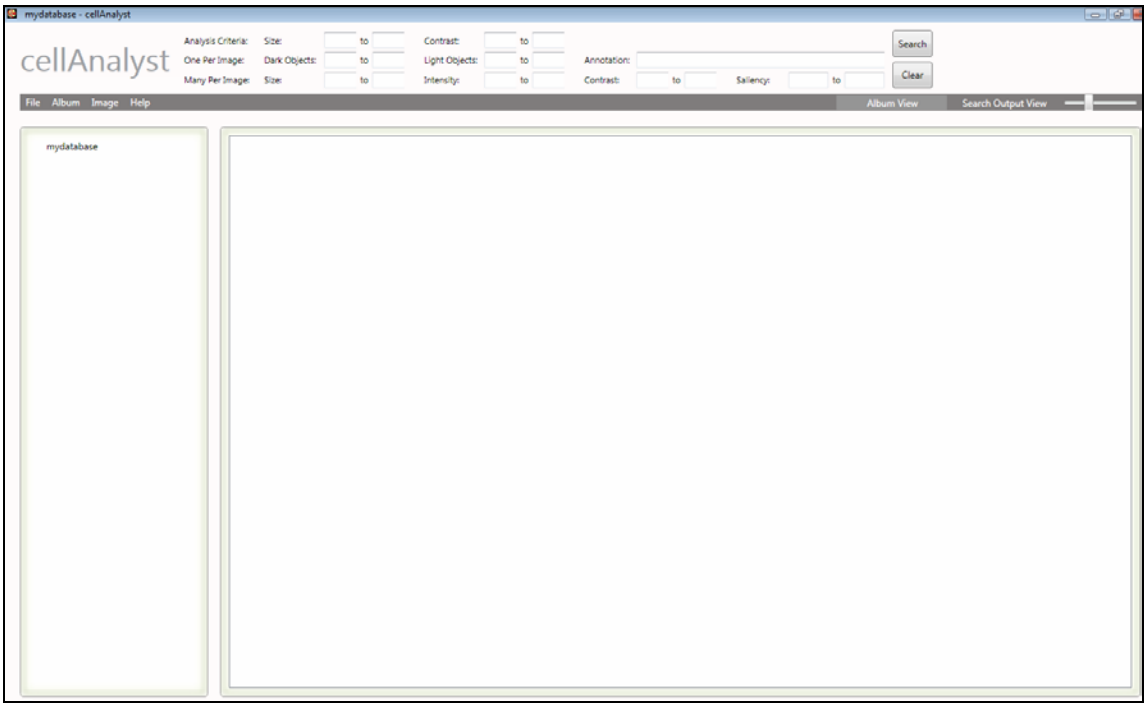
To uninstall *cellAnalyst*, select the "Uninstall cellAnalyst" icon from the program files.

Starting cellAnalyst

Start *cellAnalyst* by double clicking on the desktop *cellAnalyst* icon or by selecting it from the list of programs.



The following user interface will be displayed.



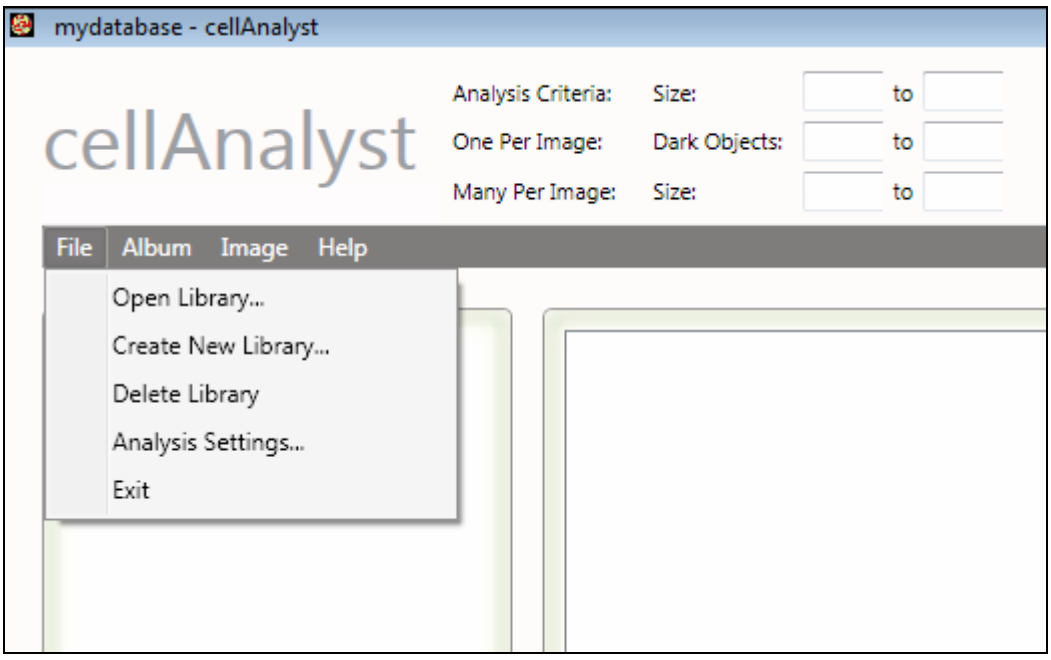
A default library called “mydatabase” will be automatically created. The “File” menu commands allow you to create a new library, open another existing library and delete the current library. Libraries will contain albums and images.

Using Images, Albums, & Libraries

Create a Library

If you want to create a new library, select the menu item "File" and select the command "Create New Library". A dialog box will appear that will allow you to browse files on your hard disk. Select the folder in which you would like to create your library and specify the library name (for example "My Library"). A new library is created and the new library name is displayed in the left panel.

Next time you run *cellAnalyst*, the last library you used will be opened.



Open a Library

If you want to open another existing library, select the menu item "File" and select the command "Open Library". A dialog box will appear which will allow you to browse image files. Select the folder in which the library is located. Select the library file and the new library name will be displayed in the left panel.

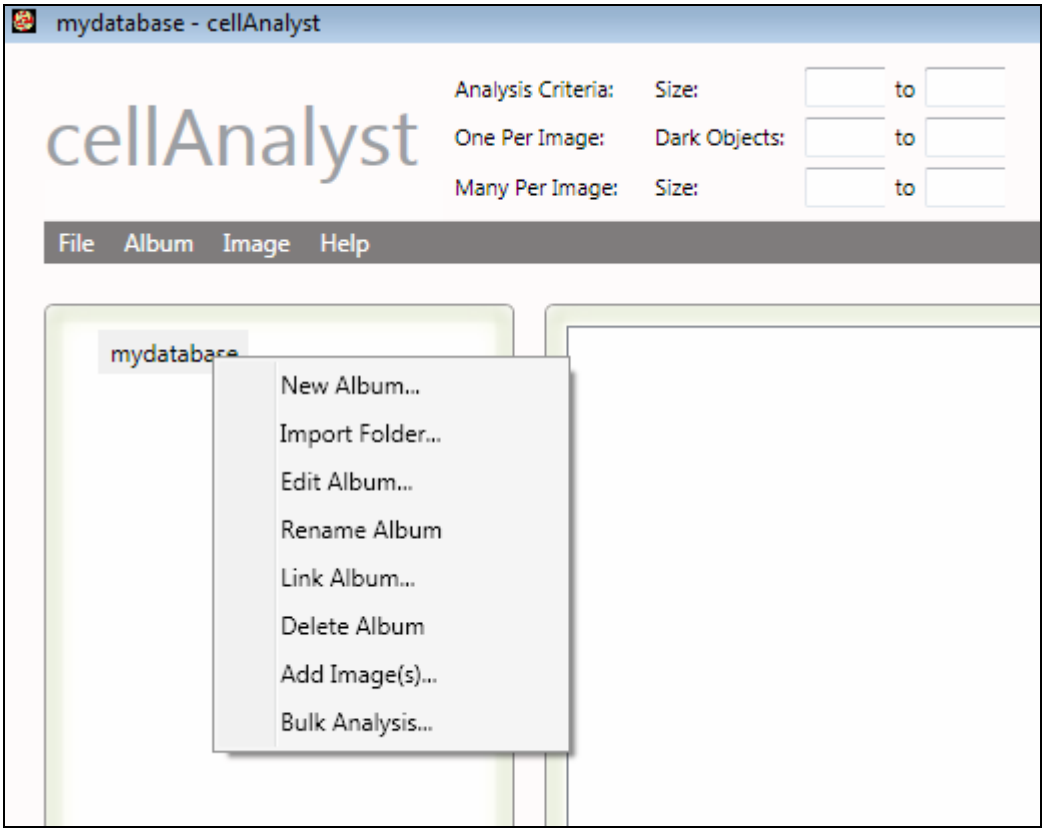
Delete a Library

If you want to delete the current library, select the menu item "File" and select the command "Delete Library". The current library will be deleted and the *cellAnalyst* application will close. Next time you start the software, the default "mydatabase" library will be opened.

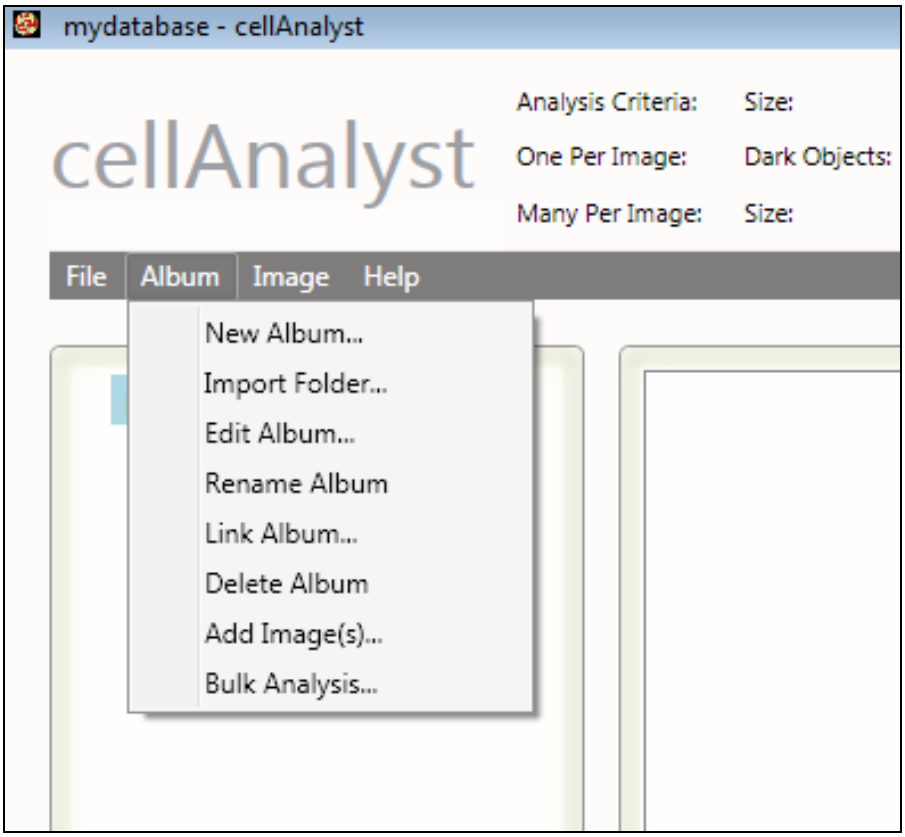
After a library is opened (or created), you can add albums and images to that library.

Create an Album

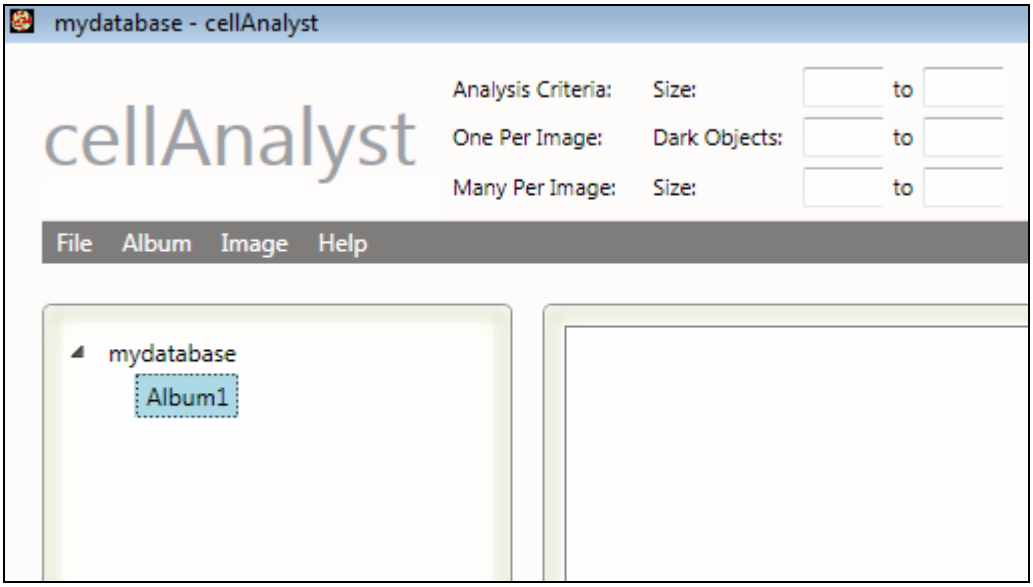
At the top of your album/image hierarchy you will see the name of the library. This is an analogue of your root folder. To add an album, right click on your library. Select “New Album” from the menu. A blank album with “New Album” name is added. Replace “New Album” with an appropriate name for this album (for example “Microscopy images”).



All album-related commands can be found in a context sensitive menu (right click the object and all available commands are displayed) or under the “Album” menu.



You can create as many albums as you want in the current album. Album names will always remain sorted except when a new album is added.

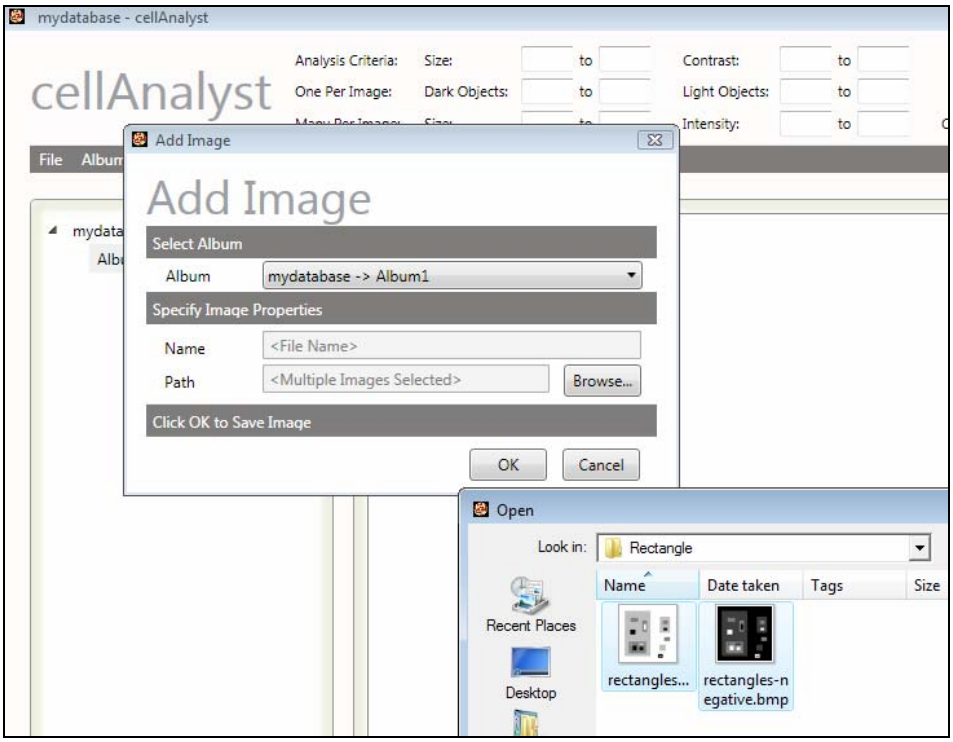


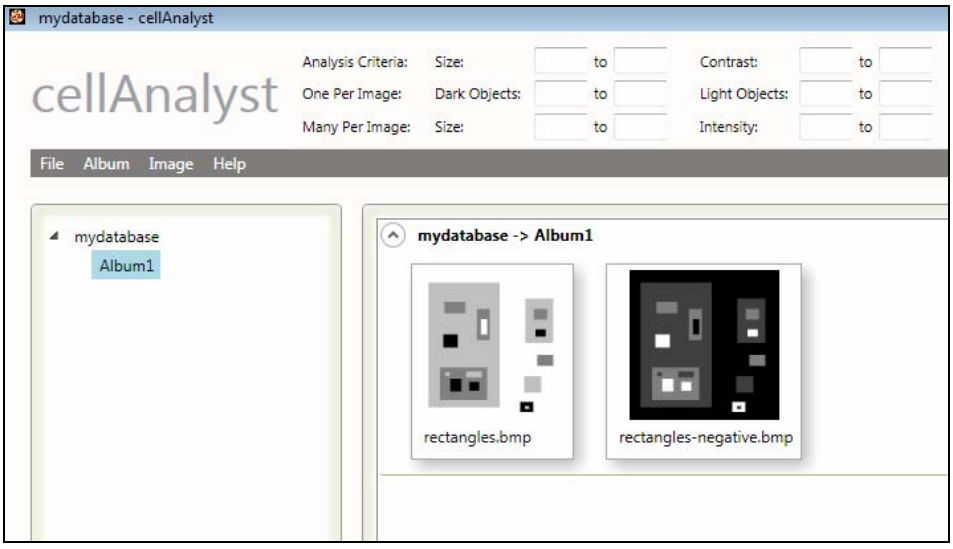
The procedure for adding an album to any sub-album is the same. Right click on the name of the album and select "New Album." A blank album with "New Album" name is added. Edit "New Album" and replace it with an appropriate name.

Add Images to an Album

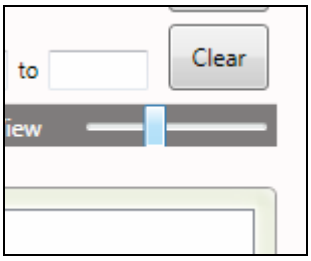
Right click on any album. Select the "Add Image(s)" command. At this stage a dialog box is opened and you can browse your hard disk and select the images to be added to this album. Several images can be added at a time.

Once the images are selected, they are added to the library and their thumbnails are displayed on the right. The name of the image is displayed under the thumbnail. Image name should be unique within an album.

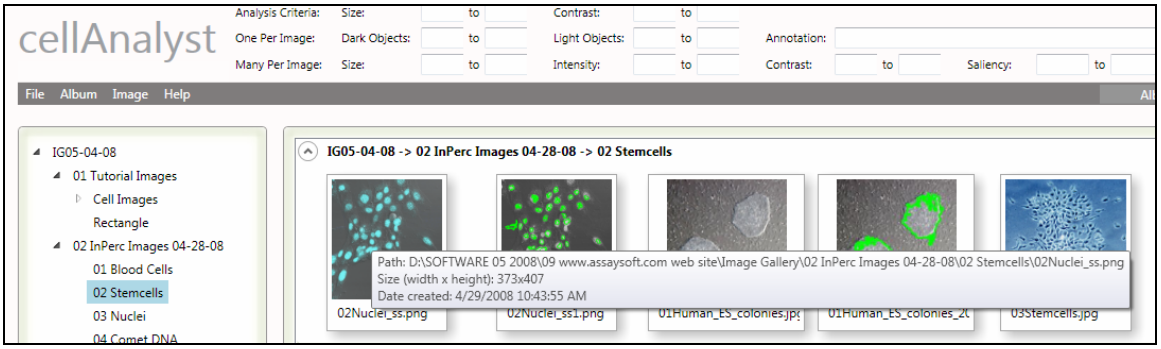




The slider located on the right side of the command bar allows you to control the size of the thumbnails.



To view the path of the image file, hover the mouse over the thumbnail and it will be displayed as a tool-tip.



The image size (width x height) and the date when the image file is created are also displayed as a tooltip.

Any size image can be added; however, only images with less than 2 million pixels can be analyzed.

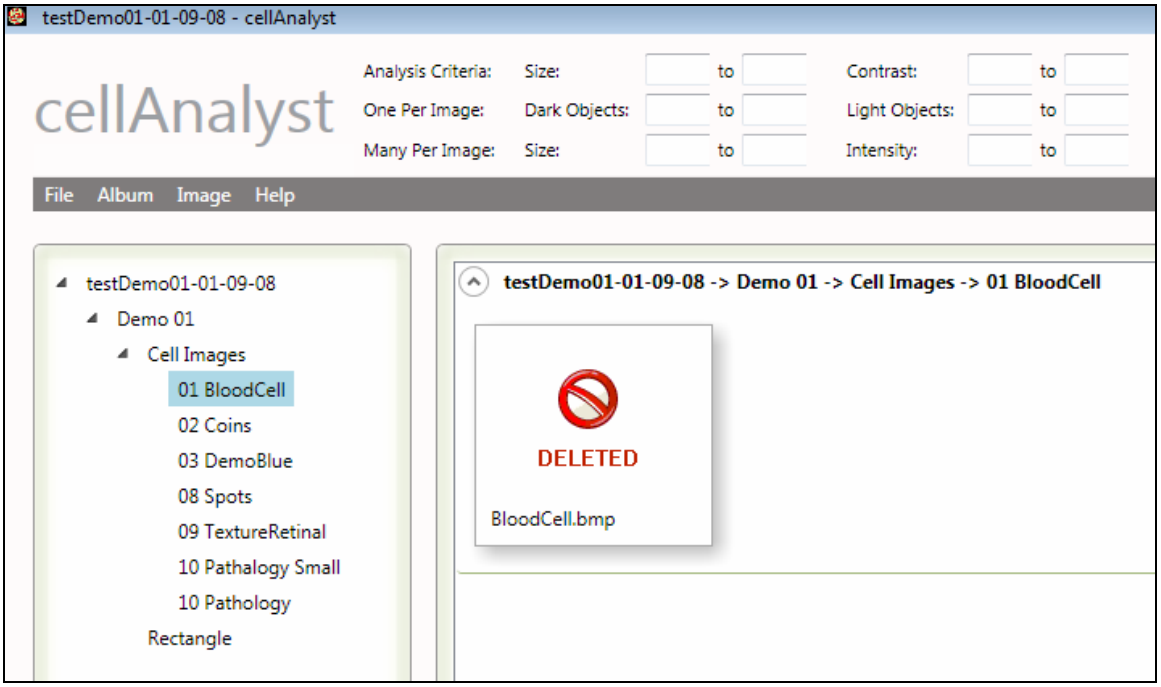
Example

Images that *can* be analyzed	Images than *cannot* be analyzed
W x H = 1,000 x 1,000 = 1,000,000 pixels	W x H = 1,000 x 2,000 = 2,000,000 pixels
W x H = 500 x 500 = 250,000 pixels	W x H = 2,000 x 1,000 = 2,000,000 pixels
W x H = 1,400 x 1,400 = 1,960,000 pixels	W x H = 1,500 x 1,500 = 2,250,000 pixels

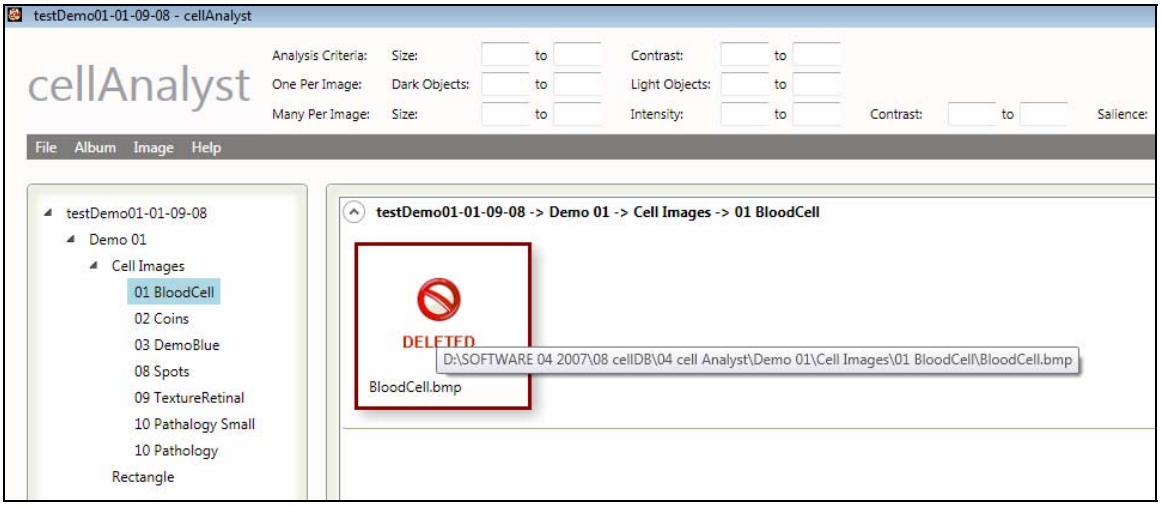
If an Image File is Deleted from Hard Disk

When an image file is copied into the *cellAnalyst* album system, the software does not maintain a copy of that image in its archives. If the user deletes that image file from hard disk after copying in the *cellAnalyst* system, *cellAnalyst* will not be able to retrieve it.

For example, if the user deletes the image file "BloodCell.bmp" from hard disk, cellAnalyst will display "DELETED" as its thumbnail.

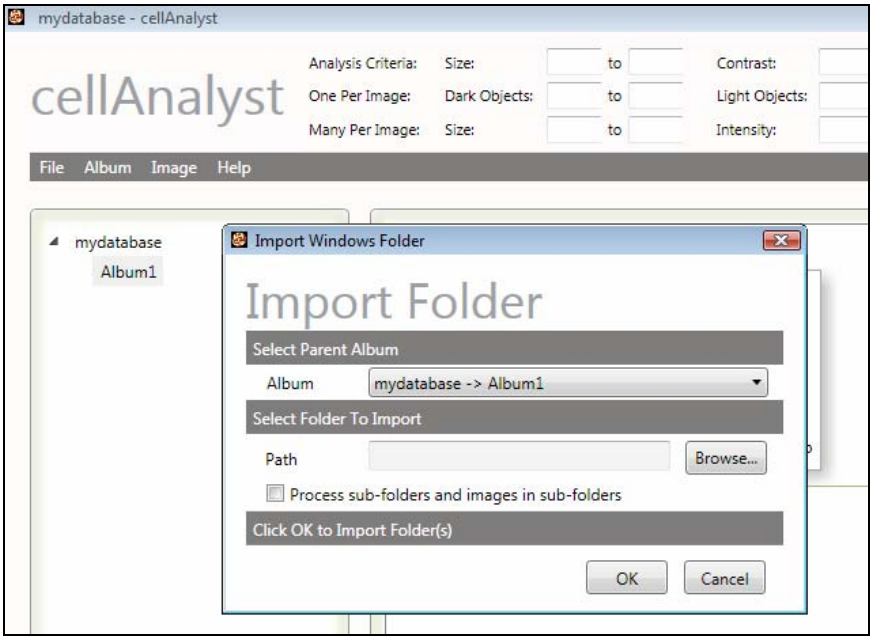


To fix this problem, hover the mouse over the thumbnail and the path of the image file will be displayed. Copy the missing image file at that location.



Add an Entire Folder of Images to an Album

Right click on any album. Select “Import Folder” command. At this stage a dialog box is opened and you can browse your hard disk and select a folder. This folder will become an album in *cellAnalyst* and all images in the folder will be added to this album. If you want all the sub-folders to be included as well, check the “sub-folder” box.



Delete an Album

Right click on the album to be deleted and select the “Delete Album” command from the menu. Before that album is deleted, user will be asked to confirm the delete operation.

Rename an Album

Select the desired album, and click it one more time. The album name becomes editable. Type in the new name. Alternatively, right click on the album and select the “Rename Album” command from the menu. Type in the new name. Push “Enter.”

Link an Album to Another Album

Linking means that an album can have more than one parent and it can be found via different paths. Select an album. Select the menu “Album” and command “Link Album.” Specify the destination album. There will be one physical copy of this album visible from the parent albums.

Move Images from One Album to Another Using Drag-and-Drop

Images can be moved from one album to another using drag-and-drop. Select an image in an album. Drag the image to another album in the "Album panel" and then release it. The image now belongs to the new album.

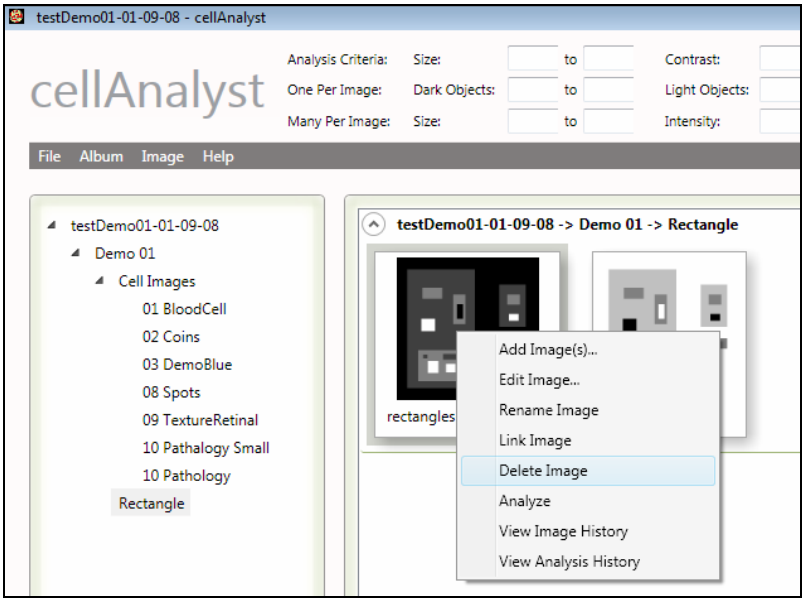
Link Images in one Album to Another

Linking means that an image can have more than one parent and it can be found via different paths. Select an image in an album. Select the menu "Image" and command "Link Image". Specify the destination album. At this time there will be one physical copy of the image visible from either of the parent albums.

When you delete a linked image from one album, it will only be deleted from that album. If there are other albums to which it is linked, the image will still physically exist in the database. Only when a linked image is not linked to any album, it will be deleted from the database.

Delete Images from an Album

To delete an image from an album, select an image and right click. In the context menu select the "Delete Image" command. Before that image is deleted, user will be asked to confirm the delete operation.



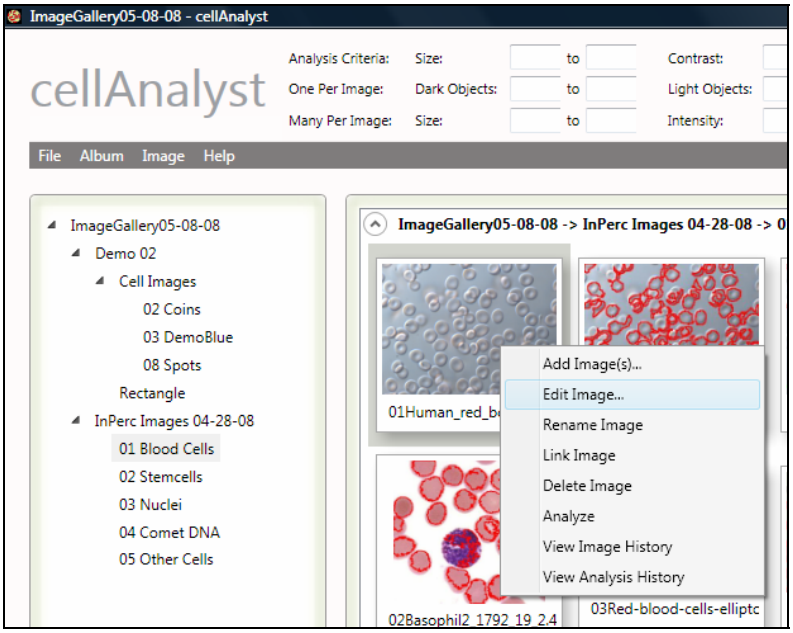
Rename an Image

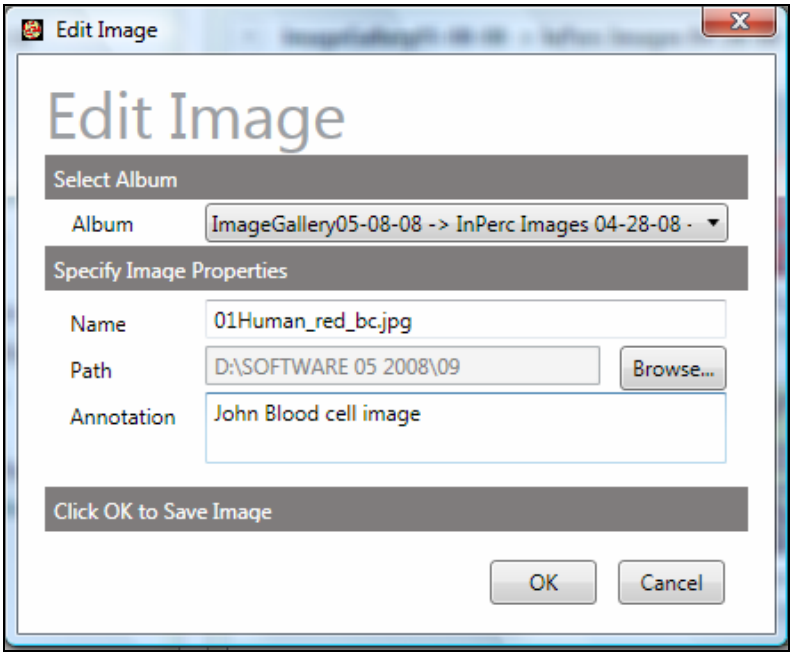
Select the image to be modified, and click on its name (*not* the thumbnail) one more time. The image name becomes editable. Type in the new name. Push "Enter."

Adding Annotation to an Image

User can add annotation to any image. Later these annotations can be used to search images.

Select the image that needs annotation, and click on the “Image/Edit Image” menu command (or right click on the image and select “Edit Image” command).



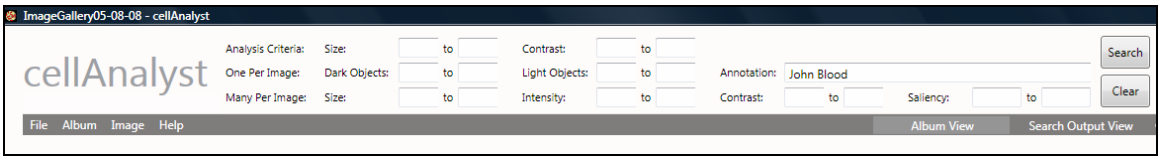


Enter any text in the “Annotation” text box. For example, user may enter “John Blood cell image.” Select “OK” button.

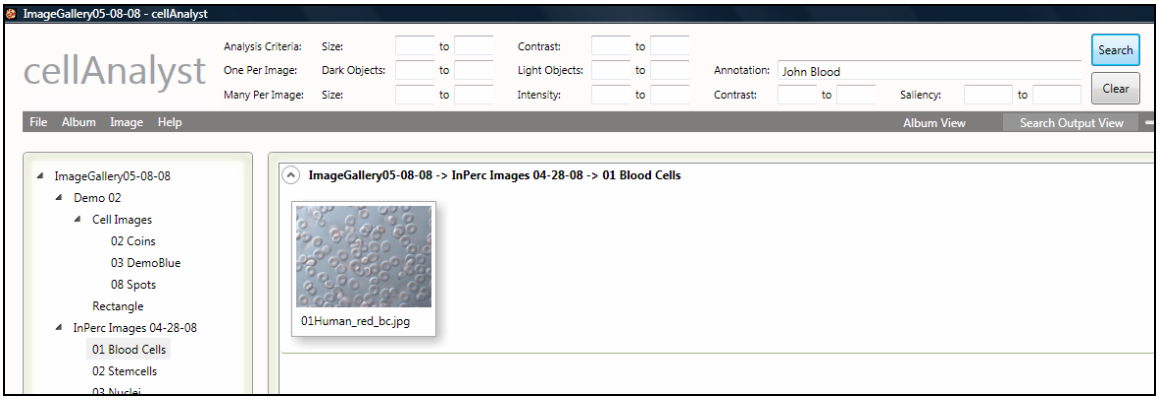
Searching Images using Annotation

Once annotation is saved in the database, user can find this image by using the search capability.

For example, the user can enter the string "John Blood" in the search window and push the "Search" button.



The "Search Output View" would display the image that satisfied the search criteria.

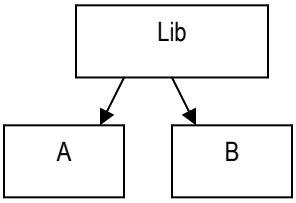


Examples

Suppose there are no albums in library “Lib”.

Add album A
Right click library “Lib”, choose “New Album”. Type “A”.

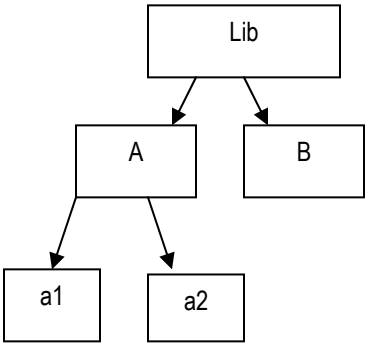
Add album B
Right click library “Lib”, choose “New Album”. Type “B”.



Add images a1 and a2 to album A

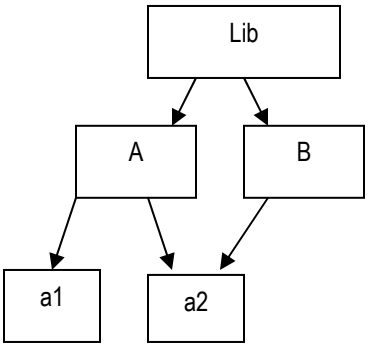
Add image a1
Right click album A “Add Image(s)”.
Select image a1 from hard disk.

Add image a2
Right click album A “Add Image(s)”.
Select image a2 from hard disk.



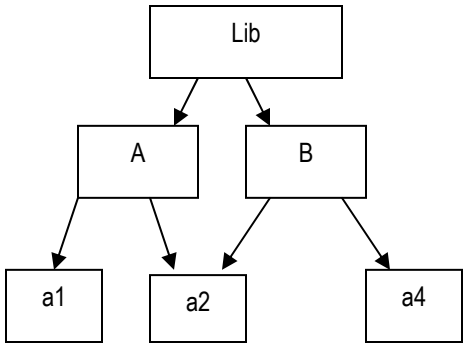
Link image a2 to album B

Select image a2.
In menu “Image” choose command “Link Image”.
Select album Lib/B.



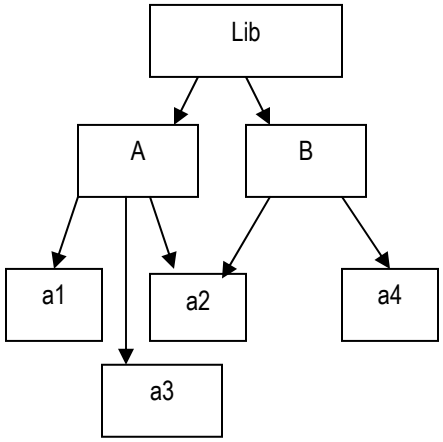
Add image a4 to album B

Right click album B “Add Image(s)”.
Select image a4 from hard disk.



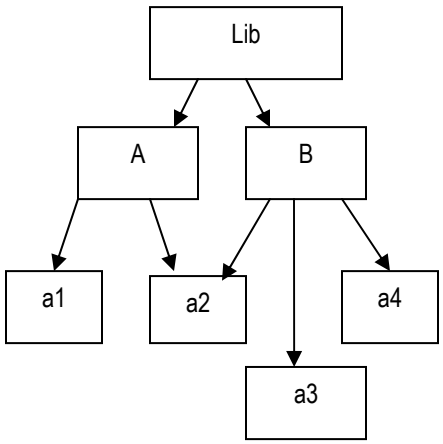
Add image a3 to album A

Right click album A “Add Image(s)”.
Select image a3 from hard disk.



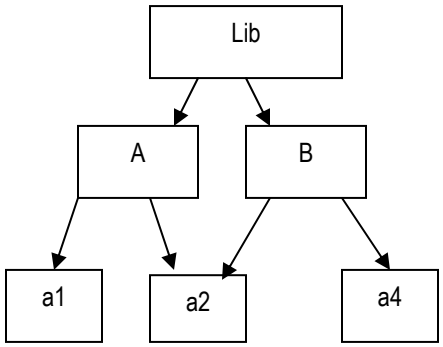
Move image a3 to album B

Drag and drop image a3 to album B.



Delete image a3 from album B

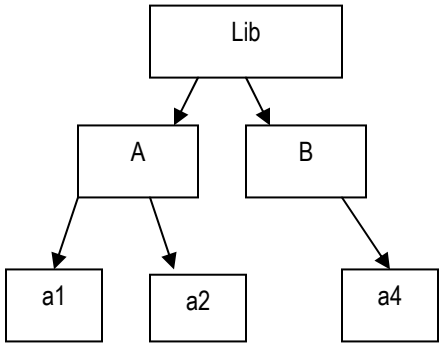
Select image a3 and right click.
Select “Delete Image” command.



Delete image a2 from album B

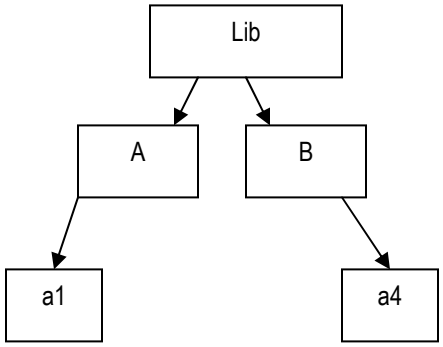
Select album B.
Select image a2 and right click.
Select “Delete Image” command.

Since image a2 was linked to two albums, after being deleted from album B, it will still exist in the library because it is linked to album A.



Delete image a2 from album A

Select album A.
Select image a2 and right click.
Select “Delete Image” command.



Visualizing & Manipulating Images

Visualization of Biological 2D, 3D, 4D and 5D Images

cellAnalyst provides tools to visualize 2D, 3D, 4D and 5D images.

cellAnalyst can visualize all the standard image formats: TIFF, JPG, BMP, PNG etc. These images are called 2D images (2-dimensional images). The reason for the name is that the intensity value of the pixel is a function of its location represented by its X, Y coordinates in the image. For color images the intensity value consists of the intensity values of Red, Green, and Blue. However, it is still a function of the X, Y coordinates of the pixel.

In addition to these standard 2D formats, *cellAnalyst* can also visualize biological 3D, 4D and 5D images.

3D images are used in biology when more than one image of a specimen is taken. For example, one may take an image of every slice of a specimen. Suppose a specimen is 1 inch thick. An image of this specimen is taken and then the specimen is cut (sliced) by 1/10th of an inch. Another image of the specimen is taken. Once again specimen is sliced by 1/10th of an inch and another image is taken. This way 10 images of the same specimen are captured. This set of 10 images will be called a 3D image. As you can see, the 3rd dimension represents the depth of the specimen.

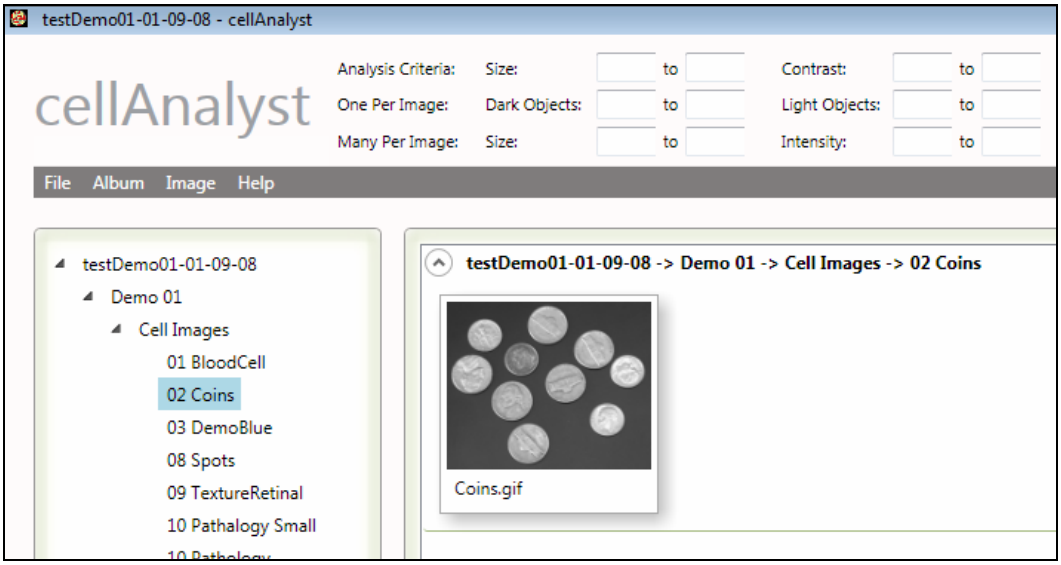
4D images are like 3D images with one more dimension added—time. At time "0" a set of 3D images is taken. Then at time "1" (when the specimen may have changed slightly) another set of 3D images is taken. Then this process is repeated multiple times. All these images are stored in a single file and this file is called a 4D image. As you can see, a 4D image is just like video.

5D images add a 5th dimension—the channel sequence. A grayscale image has just one channel. A color image has 3 channels—red, green, and blue. Images taken under different frequencies provide more channels.

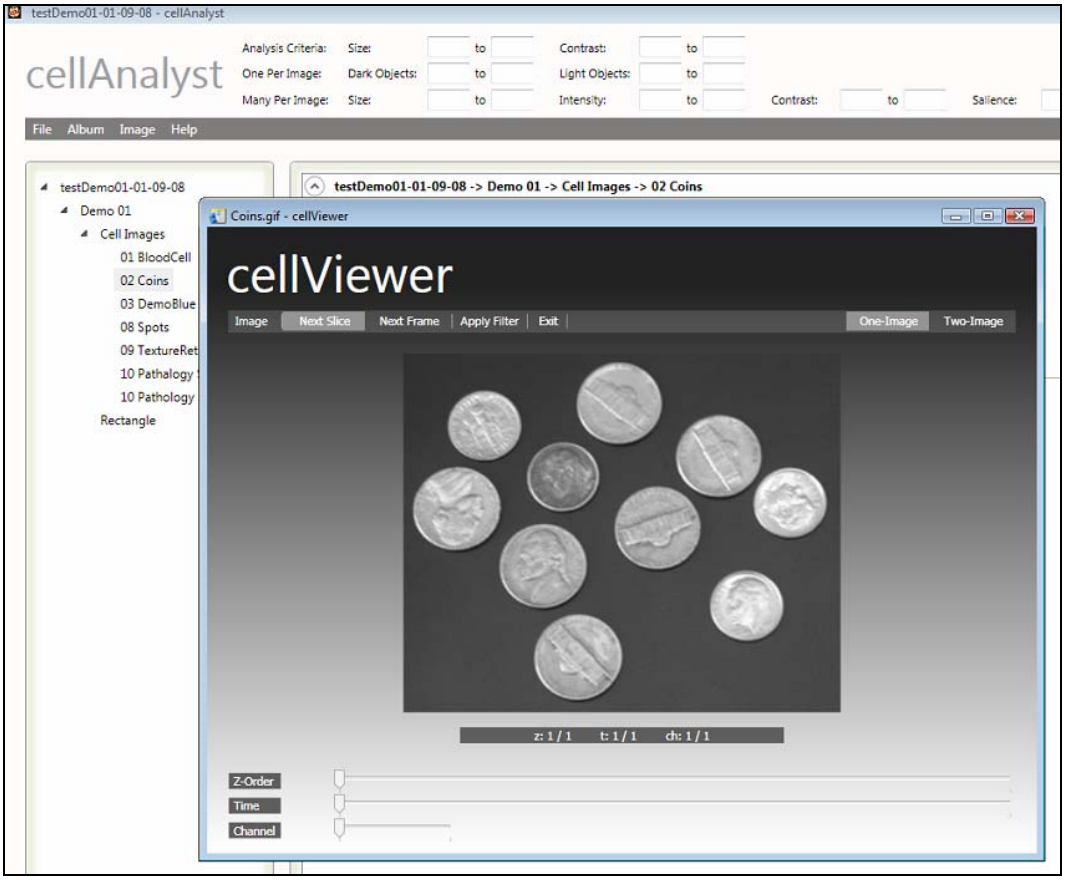
Currently, all 2D, 3D, 4D and 5D images can be viewed but only 2D images can be analyzed.

Visualize Images

To visualize an image in a larger window double click its thumbnail.

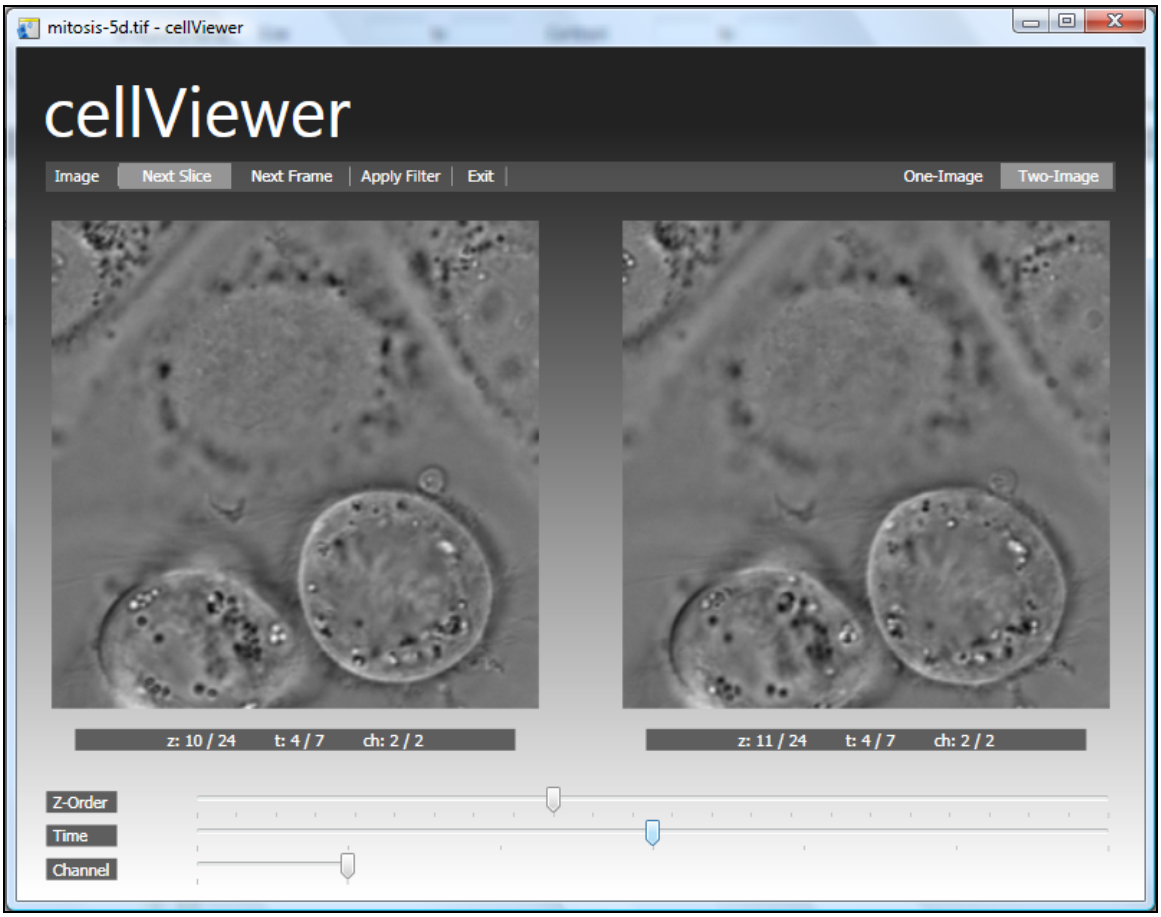


The *cellViewer* application starts and full size version of the selected image is displayed.



The *cellViewer* is a 5D image viewer. If the image is a 2D image (X, Y coordinates and the intensity value of the pixel only), the complete image is displayed with the following parameters: "Z-Order" (depth) = 1, Time = 1, and Channel = 1.

The following figure shows how a 5D image is visualized. The image can be displayed in "One-Image" or "Two-Image" mode. In the "One-Image" mode, only one frame is displayed, and the "Two-Image" mode the current frame and the adjacent frame are displayed.



There are 3 sliders at the bottom of the window. They are used to display the other frames of the 5D image. The "Z-Order" slider will vary the depth of an image. The "Time" slider will vary the time of the image, and the Channel slider will vary the channels in the image.

In the "Two-Image" mode, the *cellViewer* will display the current and the adjacent frames of a 5D image depending upon which slider is used to display that image. A label under an image displays the current "Z-Order", "Time" and "Channel."

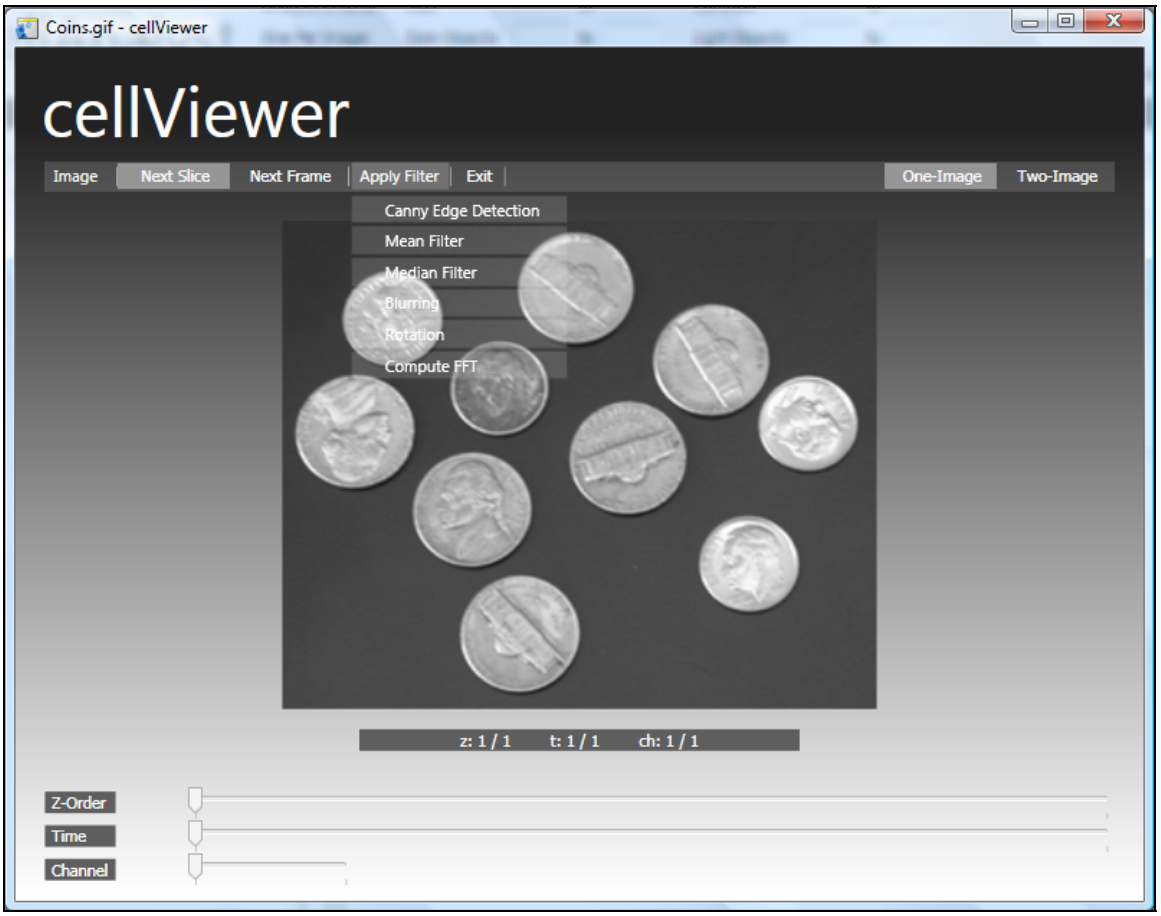
The above figure shows an image that has a depth of 24 and time span of 7, and has two channels. Currently the two adjacent frames are displayed with Z-Order = "10 of 24" and "11 of 24" time = "4 of 7" and Channel = "2 of 2".

Manipulate Images

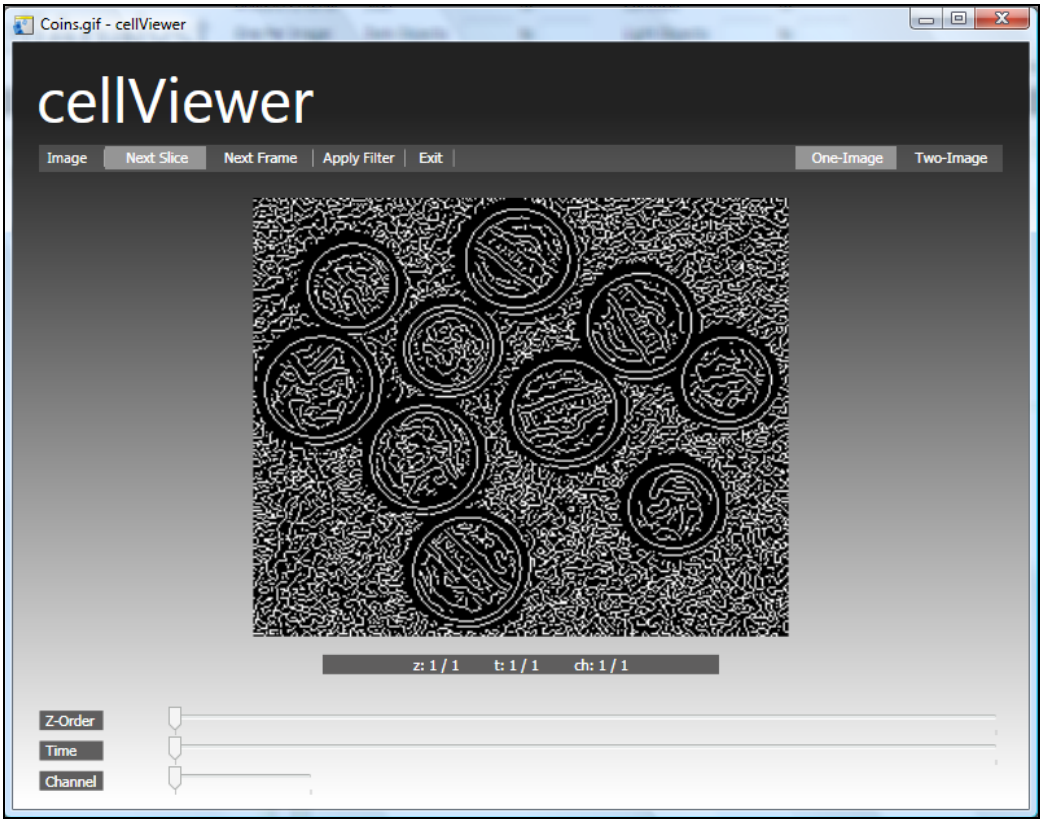
cellViewer contains several special effects that can be applied to the image. Select "Apply Filter" menu command. The following filters appear.

- Canny Edge Detection
- Mean Filter
- Median Filter
- Blurring
- Rotation

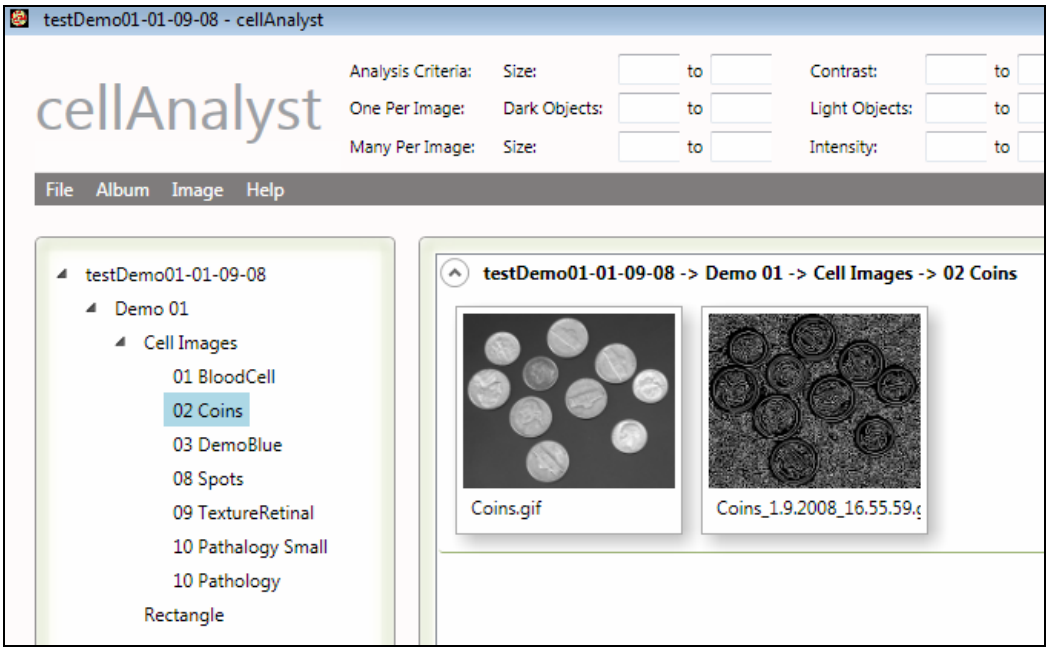
The following figure shows the original image before filters are applied.



The next figure shows the image after the “Canny Edge Detector” filter has been applied.



After a filter is applied, the modified image can be saved. To save the modified image, select the “Image/Save” menu command. The new image is now located in the same album as the original.



Maintain Version Control

As a filter is applied to an image and the modified image is saved, version control is maintained. The system will store the following information in the database.

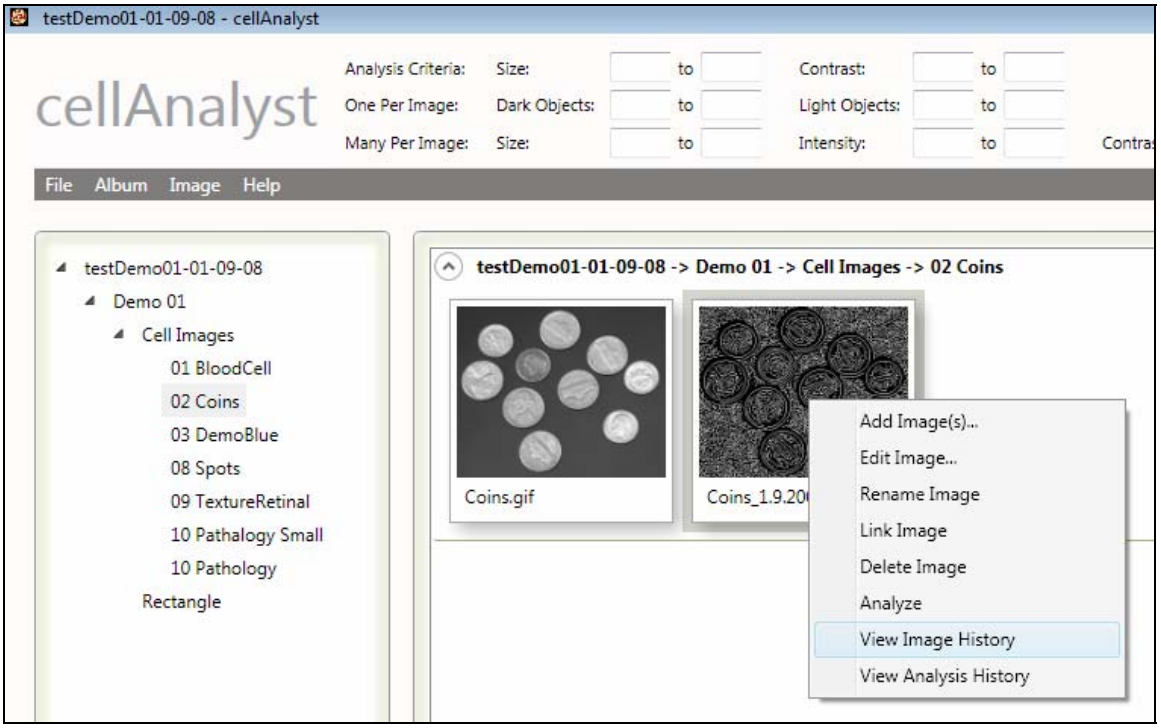
- The names of the new and original images and the indication that the new image was created from the original image.
- The specific command(s) that modified the original image.
- The date/time the image was modified.

As a result, the user can see the complete pedigree of the image.

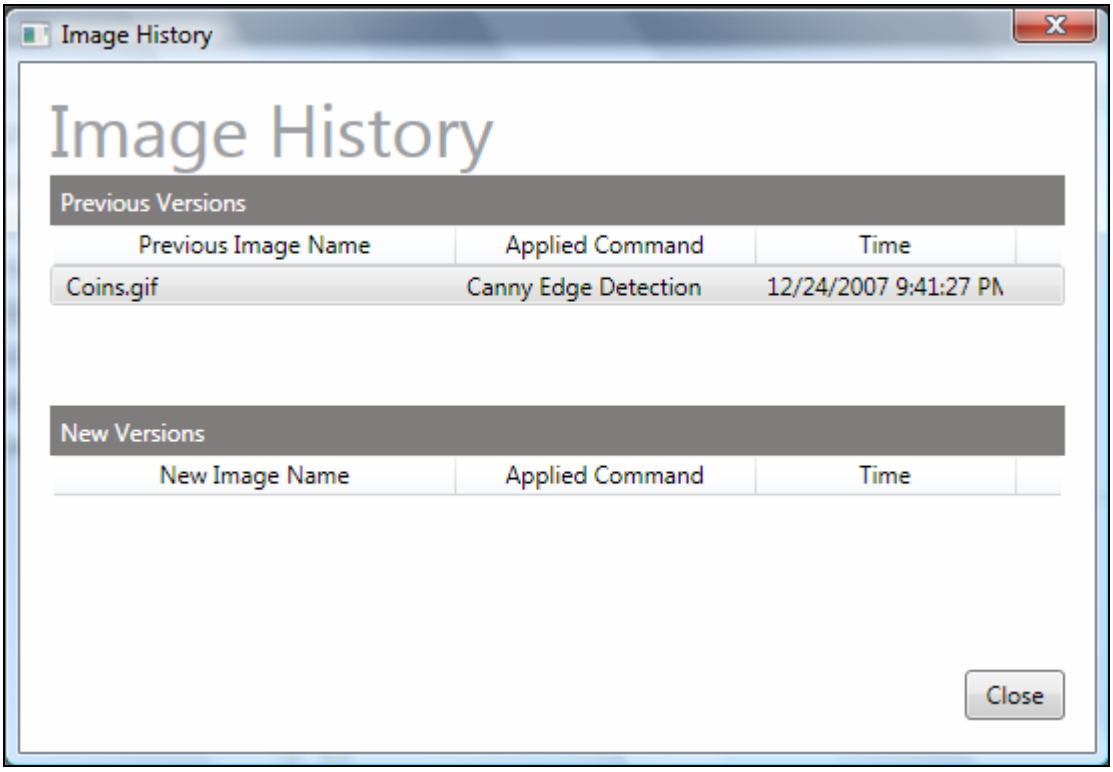
Let's consider an example. Suppose the user starts with image A. Image B is created by applying the "edge detection" filter to Image A. Next image C is created by applying rotation to Image B. The user can now display the history of Image C, as follows:

A
--B (apply edge detection on A)
----C (apply rotation to B)

To see the complete pedigree of the image, right click the image and select the "View Image History" command.



The system will display the parent image (image name from which the current was derived), the name of the filter that was used to modify the image, and the date and time when that image was modified.



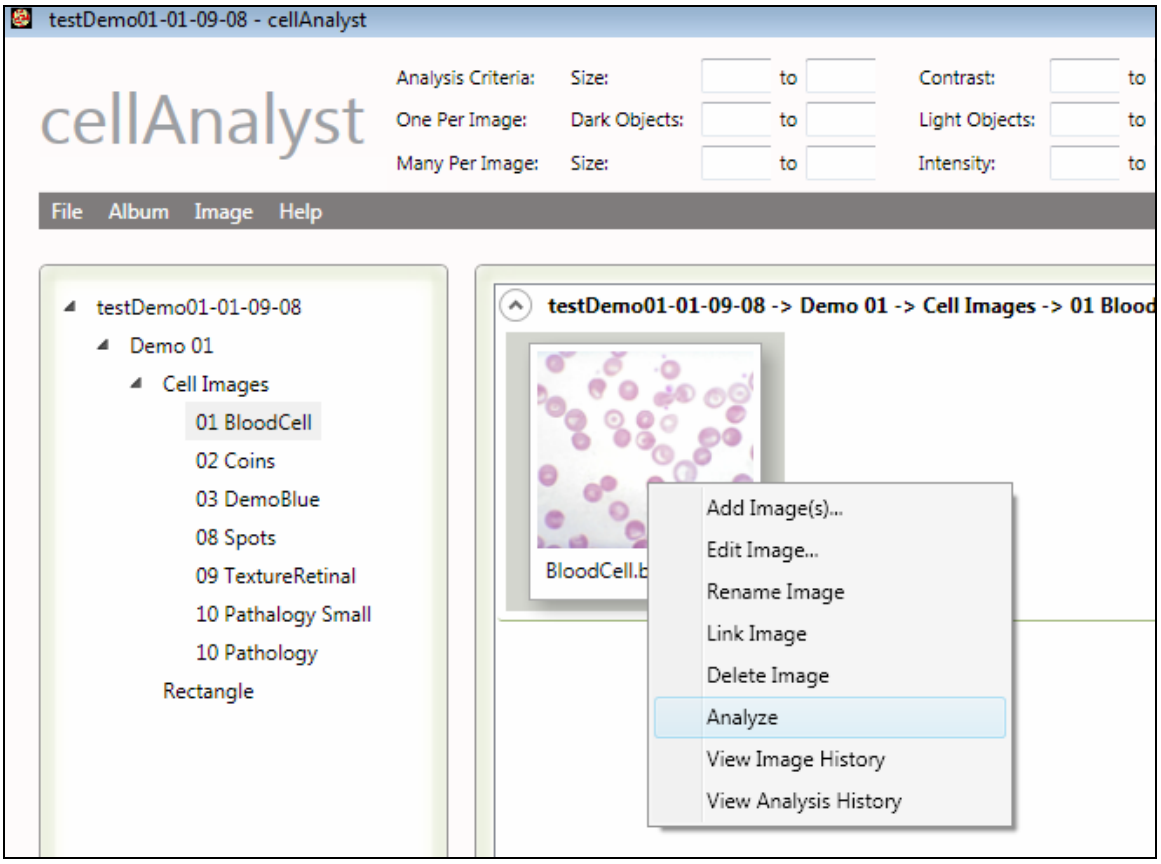
Analyzing Images & Storing Analysis Data

Start Analysis Engine

cellAnalyst analyzes the image and finds the number of cells (objects) of different kinds found in the image. Only 2D images with less than 2 million pixels can be analyzed.

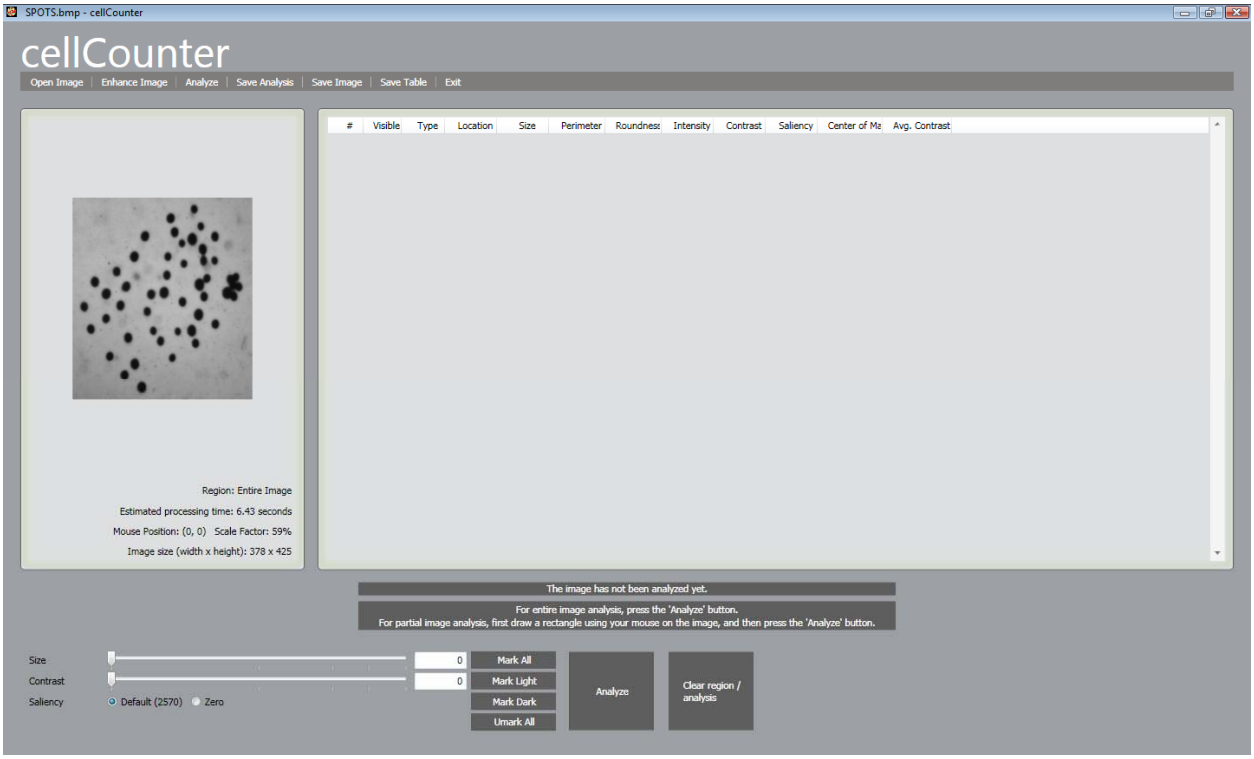
Images that *can* be analyzed	Images than *cannot* be analyzed
W x H = 1,000 x 1,000 = 1,000,000 pixels	W x H = 1,000 x 2,000 = 2,000,000 pixels
W x H = 500 x 500 = 250,000 pixels	W x H = 2,000 x 1,000 = 2,000,000 pixels
W x H = 1,400 x 1,400 = 1,960,000 pixels	W x H = 1,500 x 1,500 = 2,250,000 pixels

To start analysis, right click on an image. Select the "Analyze" context sensitive command from the menu. The "Analyze" command can also be accessed from the menu command "Image/Analyze."



Graphical User Interface

For image analysis the following user interface is initially displayed.



The left pane displays the image and the right pane is for the output of the analysis. The center divider can be moved in either left or right direction to enlarge or shrink the panes.

Place the mouse over the image. By rolling the mouse wheel the user can zoom in/out. As the mouse moves over the image, the mouse location is displayed below the image.

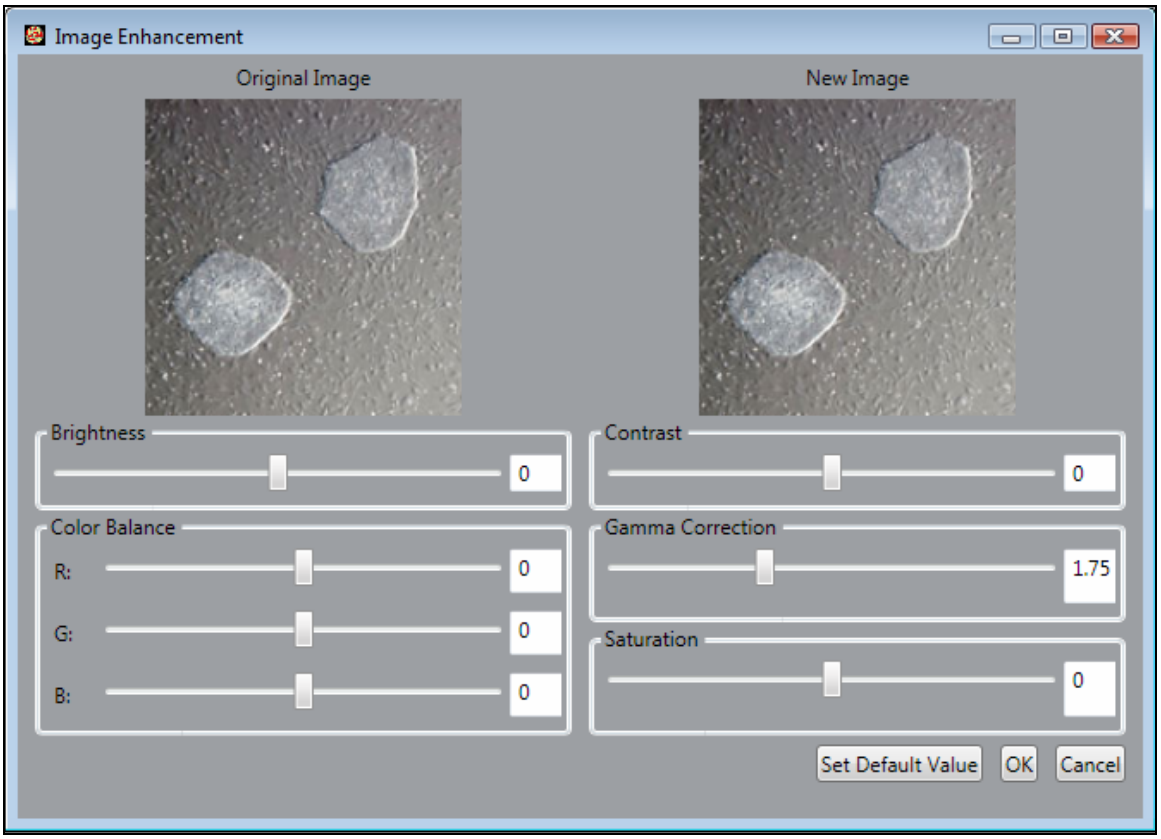
The following information is displayed below the image.

- Mouse location
- Scaling factor (zoom factor)
- Dimensions of the image (width x height)

The table columns, the buttons, and the sliders all have tool-tips associated with them. Hovering the mouse over these items will reveal the tool-tips.

Enhance Image

Before image analysis you have an option to enhance the image. The menu command "Enhance Image" provide these tools.



You can change the following parameters of an image.

- Brightness
- Contrast
- Color Balance (R, G, B)
- Gamma Correction
- Saturation

As you change these parameters, the changes are reflected in the "New Image." Once you are satisfied with the changes, select the "OK" button and the changes will be applied to the image.

If the image is modified by using these operators, and the user chooses to save the analysis, the modified image is saved in the same album as the original image and the analysis is associated with the modified image.

Complete Image Analysis

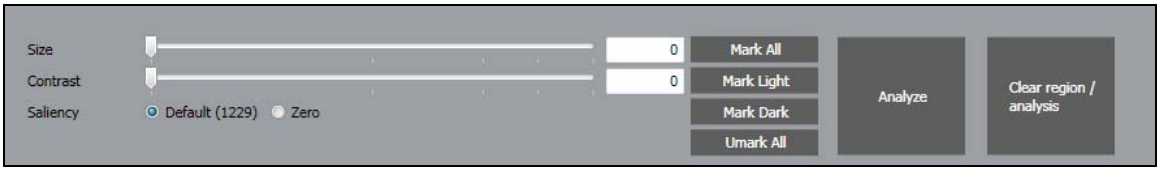
Image analysis is carried out on a single color channel data. For colored images, cellAnalyst computes the single channel data by combining all the three channels (Red, Green, Blue). For gray scale (single channel) images only the available channel data is analyzed.

Move the slider for "Size", "Contrast" to choose settings for analysis.

To make the initial choices for these parameters (Size and Contrast) the user can rely on any *a priori* information about the image and the cells. For example, if the user knows that the cells in the image can't be less than 100 pixels in size, make the "Size" setting equal 100. Don't worry if this data is scarce or approximate—you will have an opportunity to adjust these settings later.

Both sliders are logarithmic in the sense that there is more sensitivity at the lower end of the scale.

Saliency is a combination of size and contrast. Use the "default value" for saliency to start the analysis. If you need to capture extremely small objects, set the saliency to zero.

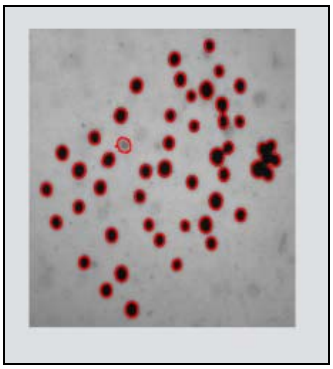


Click the "Analyze" button (located to the right of the sliders) or the "Analyze" menu command.

The only objects captured will be the ones satisfying the restrictions set by the sliders. They will have large size than the value of the "Size" slider and higher contrast than the value of the "Contrast" slider.

On the left, contours appear around the cells. If the cells are dark, the contours are red; if they are light, the contours are green. Light areas inside dark cells will also have green contours, while dark areas inside light cell will have red contours.

One indication that you may have reached the optimal settings is when you see contours tightly hugging the objects (cells).



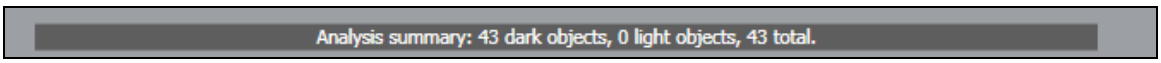
It is possible that on the first try some cells don't have contours and some meaningless spots are captured as if they were cells. You can fix that later—see the next section "Analysis Strategy."

On the right the data about these objects is displayed. The table contains the list of all objects in the image satisfying the analysis restrictions. To reiterate, for each objects the following data is displayed:

- Type (dark or light object)
- Location (the X, Y-coordinates of the geometric center of mass of the object)
- Size (the area of the object measured in pixels)
- Perimeter (the length of the boundary of the object)
- Roundness (a perfect circle will have a value of 100)
- Intensity (the highest or the lowest intensity of the pixels for light or dark objects respectively)
- Contrast (the difference between the intensity of the object and the intensity of the surrounding area)
- Saliency (the combination of size and contrast; indicates how important this object is relative to other objects)
- Center of Mass (if the object intensity values are not homogeneous, this indicates the true center of mass)
- Average Contrast (the difference between the average intensity of the object and the intensity of the surrounding area)

#	Visible	Type	Location	Size	Perimeter	Roundness	Intensity	Contrast	Saliency	Center of Ma	Avg. Contrast
1	☑	Dark	134,165	315	75	70	111	53	7163	135,165	22
2	☑	Dark	221,280	135	43	88	20	51	4672	221,280	34
3	☑	Dark	157,239	183	52	85	20	52	6714	157,238	36
4	☑	Dark	234,138	146	45	90	19	51	5154	234,138	35
5	☑	Dark	165,203	223	55	91	19	51	8291	165,203	37
6	☑	Dark	298,132	148	46	86	18	52	5324	298,132	35
7	☑	Dark	112,186	250	59	88	18	52	9361	112,186	37
8	☑	Dark	169,279	154	47	86	18	52	5584	170,279	36
9	☑	Dark	231,218	204	54	87	18	52	7580	231,218	37
10	☑	Dark	198,162	197	54	83	18	52	7306	198,162	37
11	☑	Dark	92,155	213	56	84	18	52	7622	93,155	35
12	☑	Dark	209,336	156	48	85	17	51	5527	209,336	35
13	☑	Dark	185,301	195	54	83	17	53	7250	185,301	37

When the analysis is finished *cellAnalyst* displays below the table the total number of objects (cells) found in the image. The count is broken down into "dark" or "light" objects.



User can repeatedly analyze images using different slider settings. To start a new "Complete" or "Partial" image analysis, select the "Clear region/analysis" button, select new slider values, and press the "Analyze" button.

Analysis Strategy

Do not expect to get perfect results in the first attempt. To get good results you may have to analyze an image under several different settings. It is an iterative process and after each attempt you will come closer to your goal.

For example: Suppose you have an image with several larger and several smaller objects and several objects with higher contrast and several with lower contrast. Now, suppose you know *a priori* that the larger objects with higher contrast are cells and the rest is noise. Then the strategy is as follows. Start with the size value that is about the average of the sizes of the larger and the smaller cells and the contrast value that is about the average of higher and lower contrast. Run analysis and observe the contours. If some of the noise is captured, increase the thresholds. If some cells aren't captured, decrease the thresholds.

One indication that you may have reached the optimal settings is when you see contours tightly hugging the objects (cells).

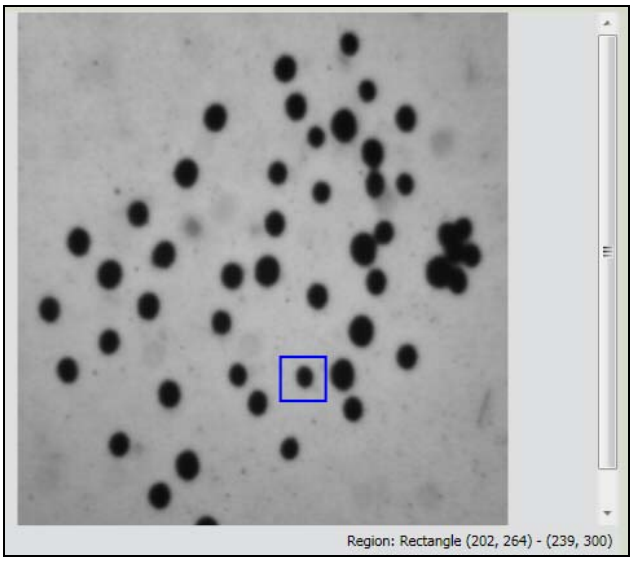
Try to get as close as possible to the optimum segmentation. It may take several attempts.

To get the best results, go beyond the optimum segmentation (in the automatic mode) and then de-select the objects (in the manual mode) that you know are not cells.

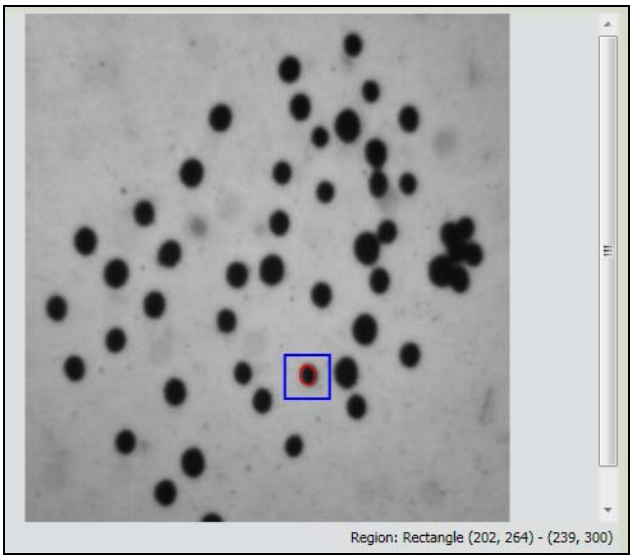
Partial Image Analysis

The quality of analysis depends upon the slider settings. For a novice user it would take many attempts to find good settings. While the analysis of the entire image may be long (depending on its size), using the partial image analysis will help find the optimal values of the sliders much more quickly.

Draw a Region of Interest (ROI) in a shape of rectangle on the image using your mouse and press the "Analyze" button.



The Region of Interest (ROI) dimensions will be displayed below the image. Only the ROI portion of the image is analyzed. If the ROI is small compared to the entire image, the analysis results can be achieved instantly.



The following is a step-by-step procedure to get optimum values of the sliders (Size and Contrast) using partial image analysis.

1. Size slider setting

    - Draw a square (ROI) around one of the smallest cells. Analyze.
    - Move the Size slider to the right in small increments and reanalyze until the contour around the cell looks tight.
    - Go to the table and make a note of the size of this cell. In our example, it is 200.
    - Reduce this number by 10-25%. Set the Size slider to that value. In our example, it is 150.

2. Contrast slider setting

    - Clear the ROI and draw a new ROI around one of the cells with lowest contrast. Analyze.
    - Move the Contrast slider to the right in small increments and reanalyze until the contour around the cell looks tight.
    - Go to the table and make a note of the contrast of this cell. In our example, it is 80.
    - Reduce this number by 10-25%. Set the Contrast slider to that value. In our example, it is 70.

3. Entire image

    - Analyze the Entire image with these settings of the sliders for Size and Contrast. In our example, these are 150 for Size and 70 for Contrast.
    - Move the sliders to the right in small increments and reanalyze until the contours around the cells look tight.

Manual Mode

Next, in the manual mode the user can also exclude noise and irrelevant details from the analysis one by one. There are two approaches.

To mark and unmark a row in the table, check and uncheck the square in the beginning of the row. If an object is marked or unmarked in the table on the right, a contour appears or disappears around the corresponding cell in the image on the left.

To unmark a cell in the image, press and hold the "Control" key and select that unwanted cell on the image with a mouse click. The contour around this cell will disappear and the square in the beginning of the corresponding row will be unchecked.

Mark & Unmark Groups of Objects

You can mark and unmark groups of objects. Select the "Mark Light" or "Mark Dark" button to display only the light or the dark objects found in the image. Either may be appropriate when you have no interest in features inside cells. These choices will also help to deal with noise.

Note: "Unmark All" will allow you to view the original image without contours.

Performance

The time to analyze an image is a function of its size. The analysis time for an image of size 300 x 300 pixels will take as little as a few seconds on a Pentium Duo 2GHz machine. As the image size increases, the processing time will also increase. The processing time for an image of 1000 x 1000 pixels may take as much as 40 seconds. If the processing is taking too long, the user has an option to cancel the analysis.

cellAnalyst estimates the processing time and displays that figure under the image.

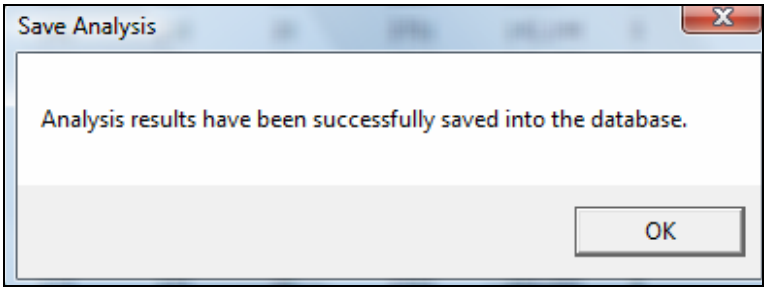
For large images (1000 x 1000 pixels or larger), it is recommended that partial analysis is used first.

The maximum image size allowed is 2 million pixels (width x height: 1400 x 1400).

Save Analysis Results

To save the analysis results select the "Save Analysis" command. The slider setting values and the parameters of objects found in the image are saved in the library along with the time stamp.

To re-analyze the image, select different settings for the sliders and click the "Analyze" button (or the menu command). To save the results of the current analysis select the "Save Analysis" command. Since a time stamp is associated with each analysis, user can analyze an image as many number of times as desired. All analysis results along with the slider settings are saved in the library.



If the image is modified by using “Image Enhance” operators, and the user chooses to save the analysis, the modified image is saved in the same album as the original image and the analysis is associated with the modified image.

Save the Analyzed Image

The segmented image displayed in the left pane including contours can be saved as a simple image file in one of the common formats. Select the “Save Image” menu command.

The default segmented image file name would be as follows.
“<original image file name>_<value of Size slider>_<value of Contrast slider>_<date>_<time>.<file format>”.

For example, if the original image file name is “Human_red_bc.bmp”, and the value of the “Size” and “Contrast” sliders are “25” and “0” respectively. The default segmented image file name would be “Human_red_bc_25_0_5.20.2008_11.22.6.bmp”.

Save the Output Table

The table displayed in the right pane with all the information about the objects can be imported into a Microsoft Excel spreadsheet format. Select the “Save Table” menu command.

The default Excel spreadsheet file name would be as follows.
“<original image file name>_<value of Size slider>_<value of Contrast slider>_<date>_<time>.xml”.

For example, if the original image file name is “Human_red_bc.bmp”, and the value of the “Size” and “Contrast” sliders are “25” and “0” respectively. The default spreadsheet file name would be “Human_red_bc_25_0_5.20.2008_11.29.54.xml”

Exit

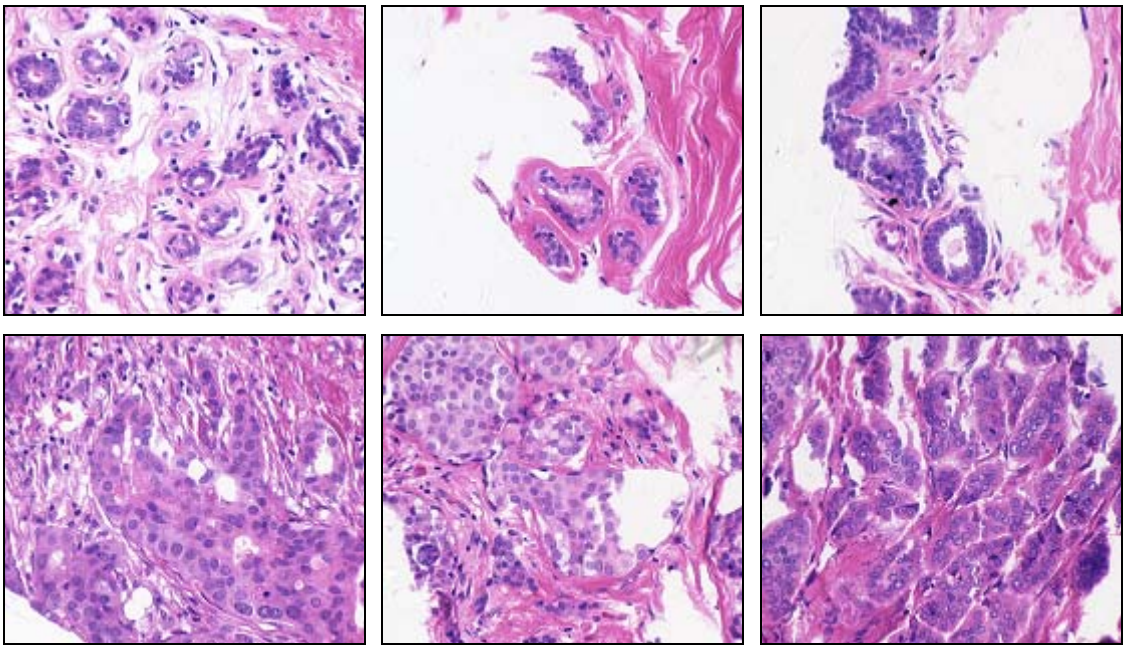
Exit the analysis screen by selecting the “Exit” menu command or close the window.

Bulk/Batch Analysis

Every image may require different values for “Size” and “Contrast” to be optimally analyzed. Therefore, user should analyze images one at a time to get the best results.

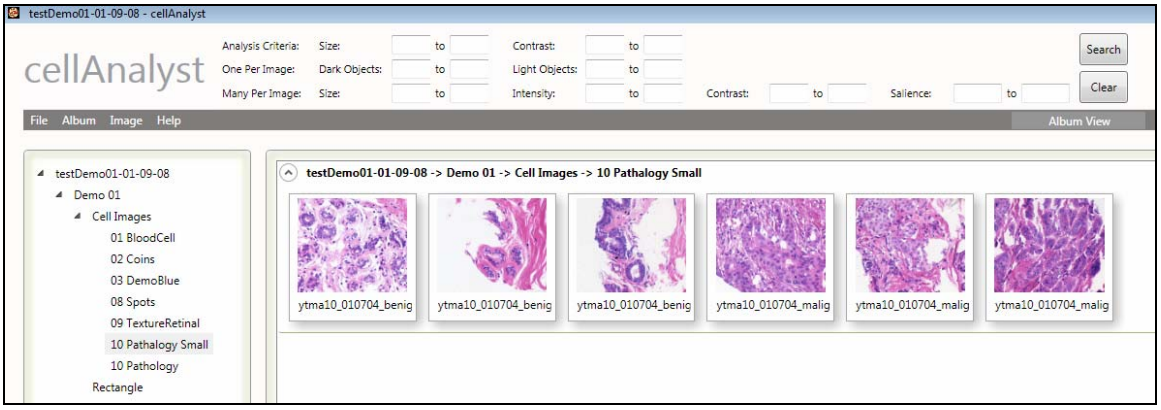
Sometimes, however, images come from the same source and have similar texture, color and pattern. Such sets of images can be analyzed in one step using the “Bulk Processing” command.

Suppose we have the following 6 images with similar texture, color and pattern.

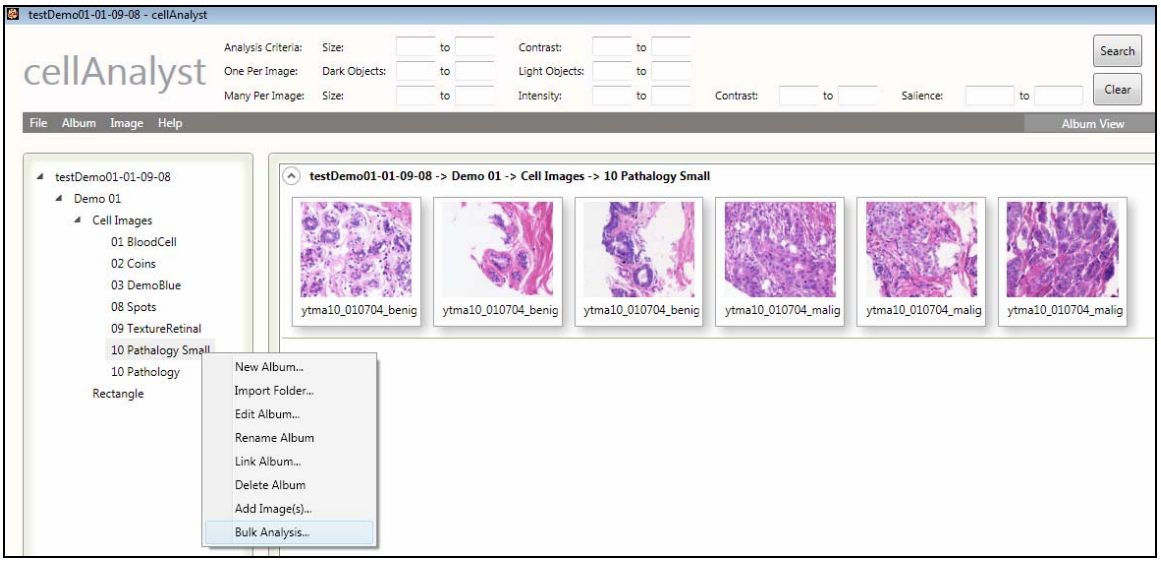


All of these 6 images can be analyzed using a single command.

Suppose these images are located in a single album.

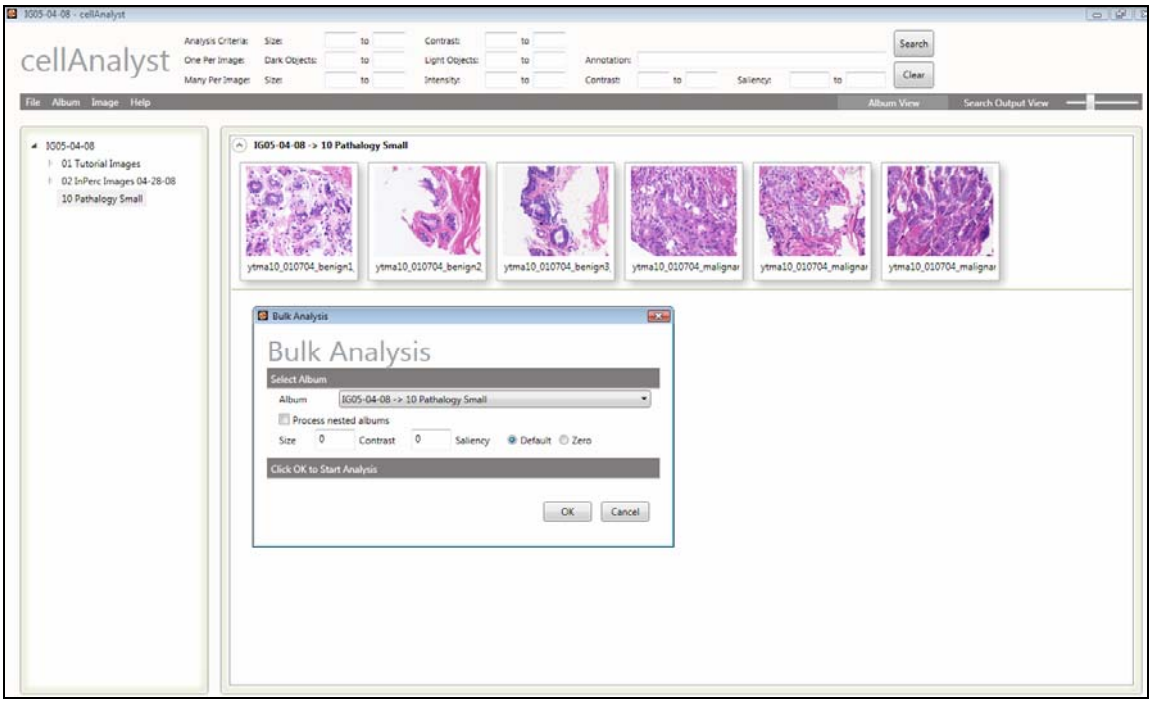


Select the album name and right click on it. Select the “Bulk Analysis” command.



Specify the analysis parameter values of "Size" and "Contrast." Check the "Process Nested Album" box if the album contains sub-albums and you want to process them using the same analysis parameters. Select the "OK" button.

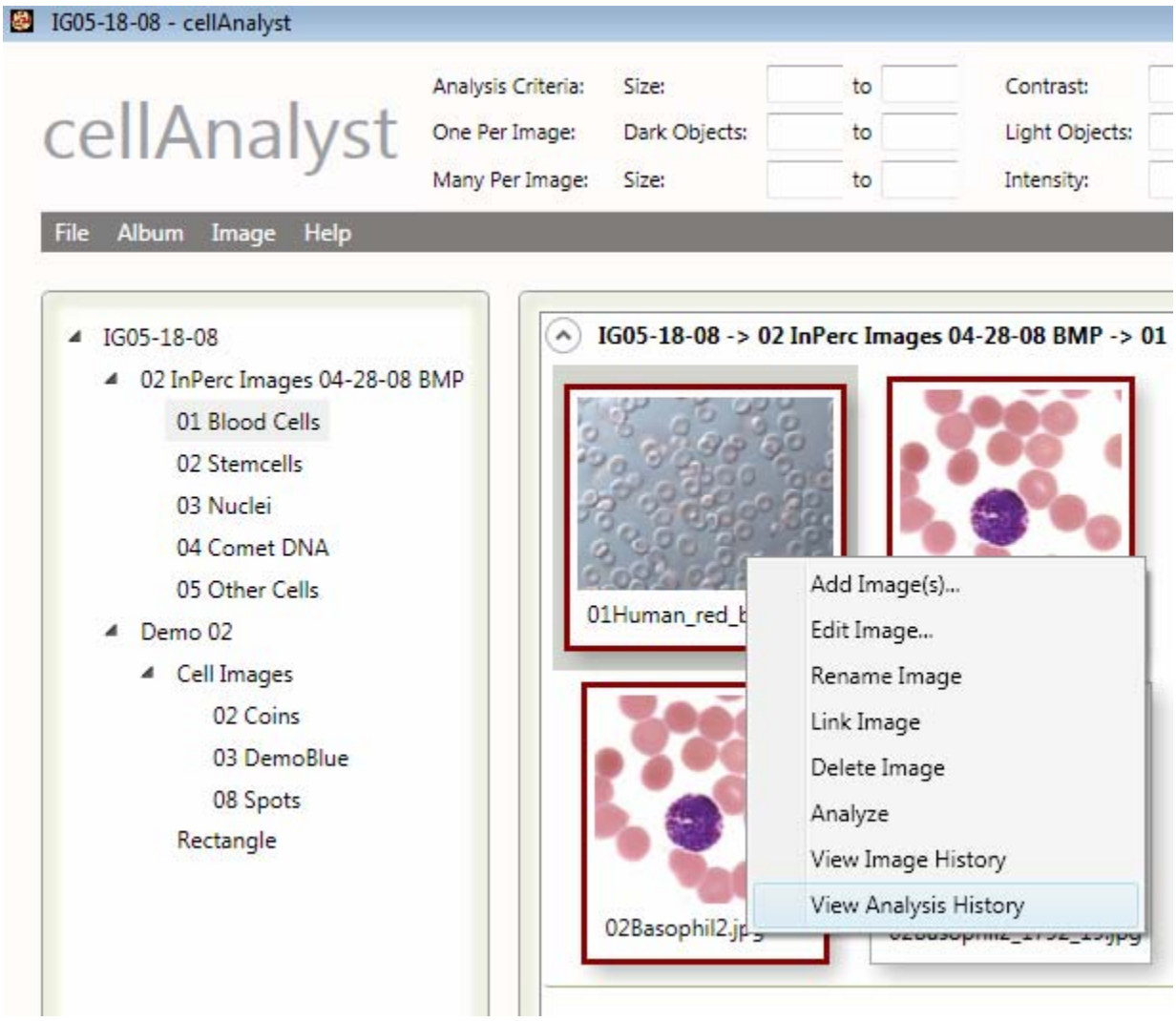
All the images will be processed and the analysis data for each image will be stored in the library as before. Later that data can be visualized using the "View Analysis History" command.



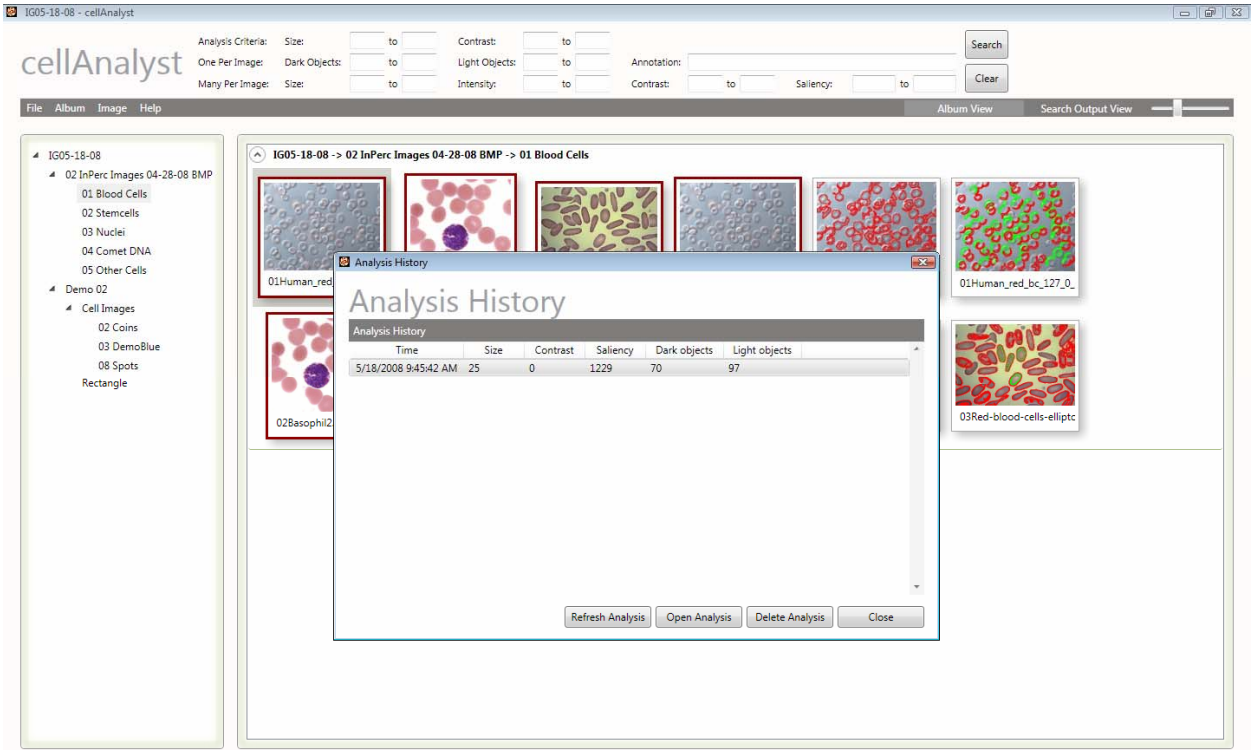
Querying The Database

Retrieve Analysis Results

Images that have already been analyzed will have red border around their thumbnails. To retrieve the analysis results, right click an image and select “View Analysis History.”



You will see a table with the summary of the analysis data and a time stamp.



Retrieve the analysis results by selecting the desired analysis and clicking the "Open Analysis" button. You will see the analysis results retrieved from the database in the same format as before.

If you choose to delete an analysis, click the "Delete Analysis" button.

The 'Analysis History' dialog box needs to be refreshed to see the most current analysis. If the most recent analysis is not displayed, press the 'Refresh Analysis' button.

Search Images

Images can be searched according to the analysis data saved in the database. The selection criteria can be the analysis parameters (for example, size and contrast), or information about the objects found in the image.

The selection criteria are first entered in the search pane at the top of the window and the "Search" button is pushed. Images that satisfy the selection criteria are displayed in the "Search Output View."

Examples

Suppose we have the following 4 images in a library:

Image 1

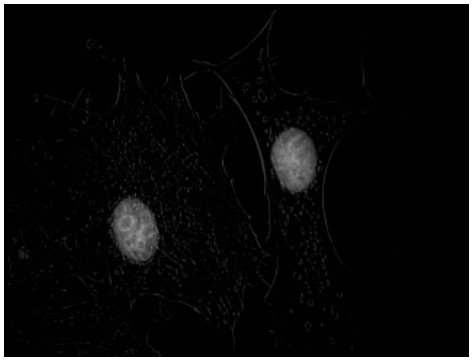


Image 2

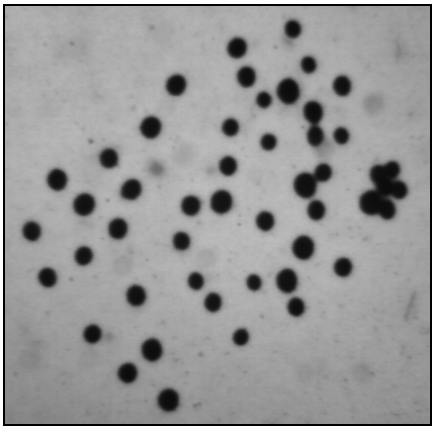


Image 3

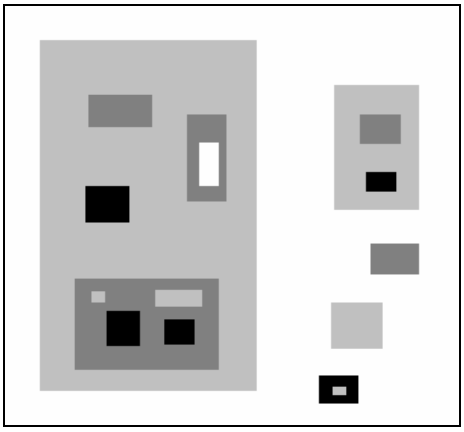
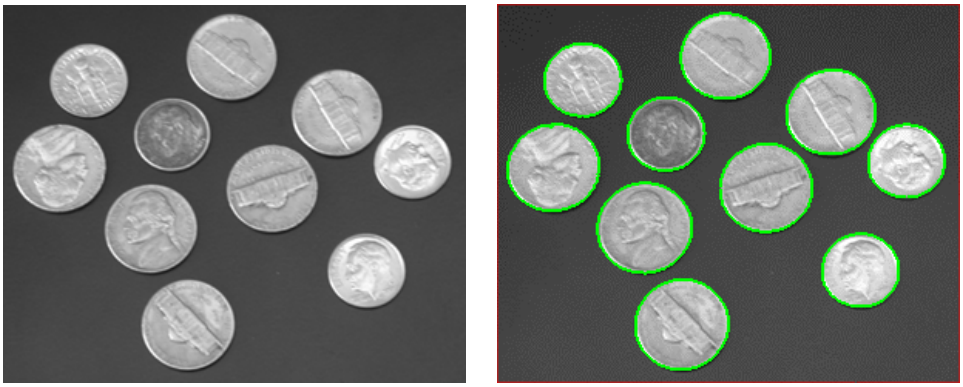


Image 4

Suppose analysis of these images produced the following results:

Image 1

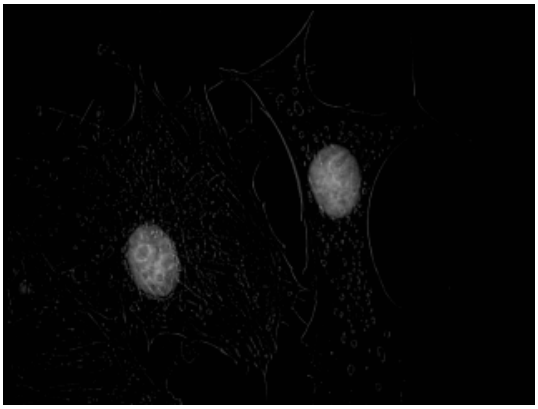


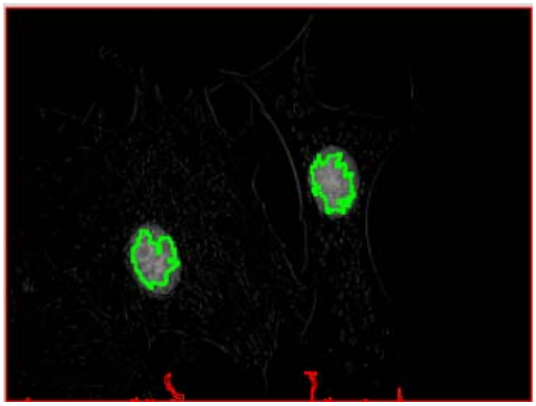
- Image Size = 300 x 246 pixels: 8 bits per pixel
- Analysis settings: Size = 1838, Contrast = 102
- Output: Dark Objects = 1, Light Objects = 10, Total Objects = 11

The table for Image 1 is below.

Objects	Light/Dark	Location (X, Y)	Size	Contrast	Border	Intensity	Saliency	Alt. location (X, Y)	Avg. contrast
1	Dark	(150, 123)	73800	232	256	24	13980204	(149, 120)	189
2	Light	(95, 145)	2797	103	72	175	279561	(95, 145)	99
3	Light	(119, 208)	2761	103	120	223	174504	(120, 206)	63
4	Light	(174, 119)	2671	103	88	191	229190	(174, 119)	85
5	Light	(36, 106)	2614	103	141	244	127609	(36, 105)	48
6	Light	(148, 33)	2512	104	124	228	146147	(147, 33)	58
7	Light	(216, 70)	2481	104	151	255	92576	(216, 69)	37
8	Light	(109, 84)	1855	103	90	193	89585	(110, 84)	48
9	Light	(235, 172)	1846	103	149	252	120709	(235, 172)	65
10	Light	(55, 49)	1843	104	131	235	106076	(55, 47)	57
11	Light	(265, 102)	1838	114	141	255	135178	(264, 101)	73

Image 2



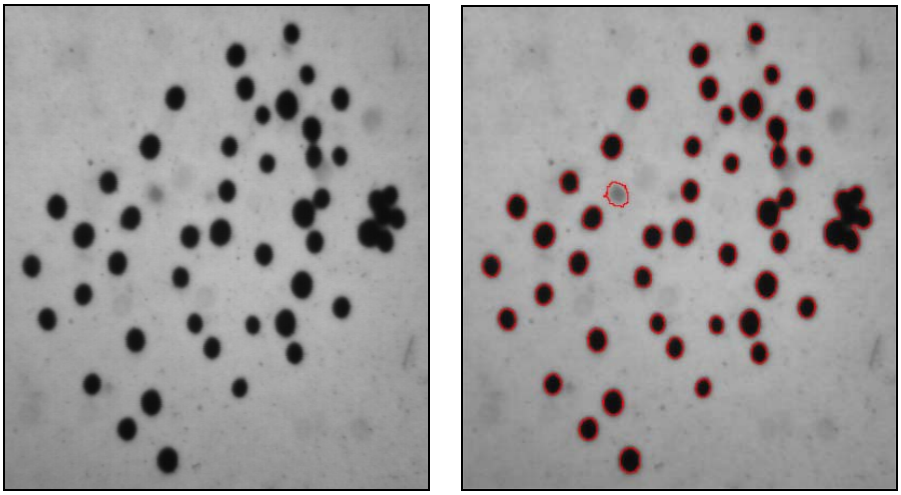


- Image Size = 320 x 240 pixels: 8 bits per pixel
- Analysis settings: Size = 563, Contrast = 0
- Output: Dark Objects = 1, Light Objects = 2, Total Objects = 3

The table for Image 2 is below.

Objects	Light/Dark	Location (X, Y)	Size	Contrast	Border	Intensity	Saliency	Alt. location (X, Y)	Avg. contrast
1	Dark	(160, 119)	76703	1	2	1	76703	(160, 119)	1
2	Light	(89, 153)	577	66	85	151	12941	(89, 153)	22
3	Light	(198, 106)	568	25	87	112	12148	(198, 106)	21

Image 3



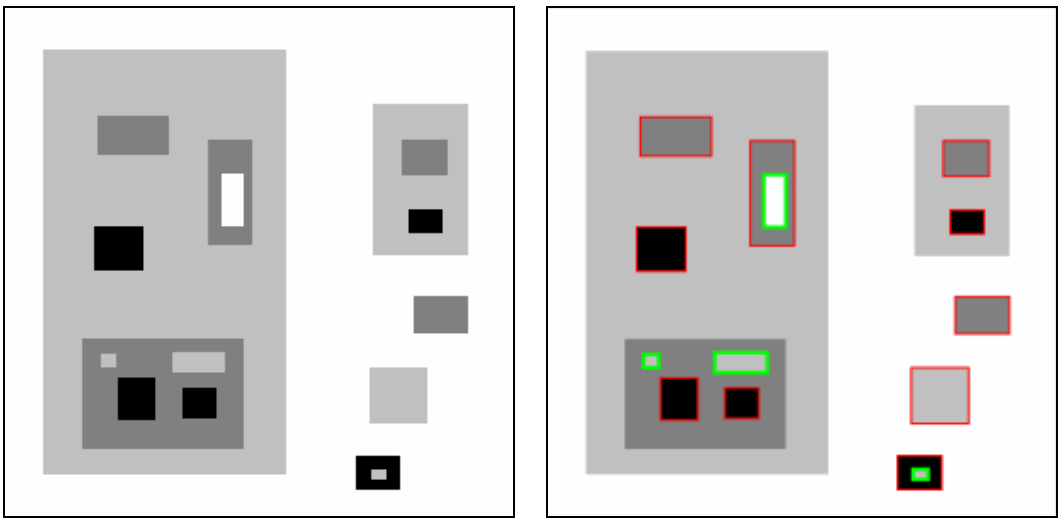
- Image Size = 378 x 425 pixels: 8 bits per pixel
- Analysis settings: Size = 0, Contrast = 50
- Output: Dark Objects = 43, Light Objects = 0, Total Objects = 43

The table for Image 3 is below.

Objects	Light/Dark	Location (X, Y)	Size	Contrast	Border	Intensity	Saliency	Alt. location (X, Y)	Avg. contrast
1	Dark	(336, 188)	1373	51	61	10	51362	(336, 188)	37
2	Dark	(272, 178)	565	53	67	14	21654	(272, 178)	38
3	Dark	(274, 118)	473	51	61	10	15311	(274, 117)	32
4	Dark	(251, 87)	348	51	61	10	12810	(251, 87)	36
5	Dark	(265, 245)	318	52	67	15	12268	(265, 245)	38
6	Dark	(134, 165)	315	53	164	111	7163	(135, 165)	22
7	Dark	(192, 199)	312	51	68	17	11687	(192, 199)	37
8	Dark	(250, 279)	310	52	67	15	11872	(250, 279)	38
9	Dark	(145, 399)	306	51	61	10	11251	(145, 400)	36
10	Dark	(131, 349)	292	52	64	12	11034	(131, 349)	37
11	Dark	(71, 202)	291	52	67	15	10743	(71, 202)	36
12	Dark	(129, 123)	270	51	67	16	10250	(130, 123)	37
13	Dark	(47, 176)	253	52	67	15	9426	(47, 176)	37
14	Dark	(112, 186)	250	52	70	18	9361	(112, 186)	37
15	Dark	(152, 81)	249	53	67	14	9587	(152, 81)	38
16	Dark	(117, 294)	248	53	67	14	9284	(117, 294)	37
17	Dark	(100, 226)	239	52	67	15	8544	(101, 226)	35
18	Dark	(109, 372)	235	51	61	10	8318	(109, 372)	35
19	Dark	(206, 43)	228	51	63	12	8361	(206, 43)	36

20	Dark	(165, 203)	223	51	70	19	8291	(165, 203)	37
21	Dark	(214, 72)	222	51	64	13	8067	(214, 72)	36
22	Dark	(92, 155)	213	52	70	18	7622	(93, 155)	35
23	Dark	(38, 275)	212	53	67	14	7734	(38, 275)	36
24	Dark	(299, 81)	211	54	68	14	8119	(299, 81)	38
25	Dark	(24, 228)	210	51	68	17	7180	(24, 229)	34
26	Dark	(276, 207)	204	51	67	16	7347	(276, 207)	36
27	Dark	(231, 218)	204	52	70	18	7580	(231, 218)	37
28	Dark	(78, 333)	204	51	64	13	7205	(78, 333)	35
29	Dark	(70, 253)	201	53	70	17	7507	(70, 253)	37
30	Dark	(300, 265)	197	52	67	15	7271	(300, 265)	36
31	Dark	(198, 162)	197	52	70	18	7306	(198, 162)	37
32	Dark	(185, 301)	195	53	70	17	7250	(185, 301)	37
33	Dark	(258, 305)	190	53	67	14	7182	(258, 305)	37
34	Dark	(157, 239)	183	52	72	20	6714	(157, 238)	36
35	Dark	(200, 123)	167	51	68	17	5856	(200, 123)	35
36	Dark	(255, 24)	165	51	64	13	5810	(255, 24)	35
37	Dark	(209, 336)	156	51	68	17	5527	(209, 336)	35
38	Dark	(169, 279)	154	52	70	18	5584	(170, 279)	36
39	Dark	(298, 132)	148	52	70	18	5324	(298, 132)	35
40	Dark	(269, 60)	146	54	68	14	5392	(269, 60)	36
41	Dark	(234, 138)	146	51	70	19	5154	(234, 138)	35
42	Dark	(221, 280)	135	51	71	20	4672	(221, 280)	34
43	Dark	(230, 95)	135	51	67	16	4696	(230, 95)	34

Image 4



- Image Size = 300 x 300 pixels: 8 bits per pixel
- Analysis settings: Size = 0, Contrast = 0
- Output: Dark Objects = 10, Light Objects = 4, Total Objects = 14

The table for Image 4 is below.

Objects	Light/Dark	Location (X, Y)	Size	Contrast	Border	Intensity	Saliency	Alt. location (X, Y)	Avg. contrast
1	Dark	(133, 109)	1612	64	192	128	103168	(133, 109)	64
2	Dark	(232, 228)	1122	63	255	192	70686	(232, 228)	63
3	Dark	(76, 75)	966	64	192	128	61824	(76, 75)	64
4	Dark	(67, 142)	754	192	192	0	144768	(67, 142)	192
5	Dark	(257, 181)	704	127	255	128	89408	(257, 181)	127
6	Dark	(247, 88)	567	64	192	128	36288	(247, 88)	64
7	Dark	(78, 230)	550	128	128	0	70400	(78, 230)	128
8	Dark	(220, 274)	520	255	255	0	132600	(220, 274)	255
9	Light	(134, 113)	403	127	128	255	51181	(134, 113)	127
10	Light	(114, 209)	372	64	128	192	23808	(114, 209)	64
11	Dark	(115, 233)	360	128	128	0	46080	(115, 233)	128
12	Dark	(248, 126)	280	192	192	0	53760	(248, 126)	192
13	Light	(61, 208)	72	64	128	192	4608	(61, 208)	64
14	Light	(220, 275)	54	192	0	192	10368	(220, 275)	192

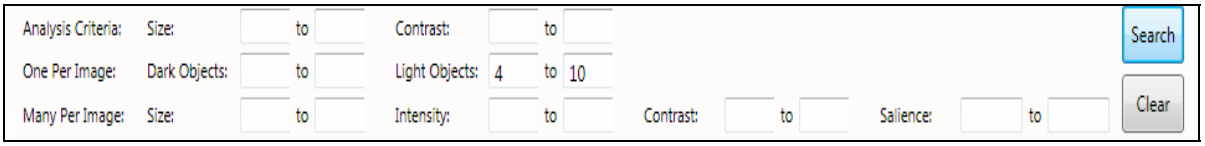
All the analysis data is stored in the database. The database can be searched according to any combination of queries that can be given. There are 3 search criteria—"Analysis Criteria", "One Per Image", and "Many Per Image" each containing more several fields.

Let's consider a few examples.

Query 1

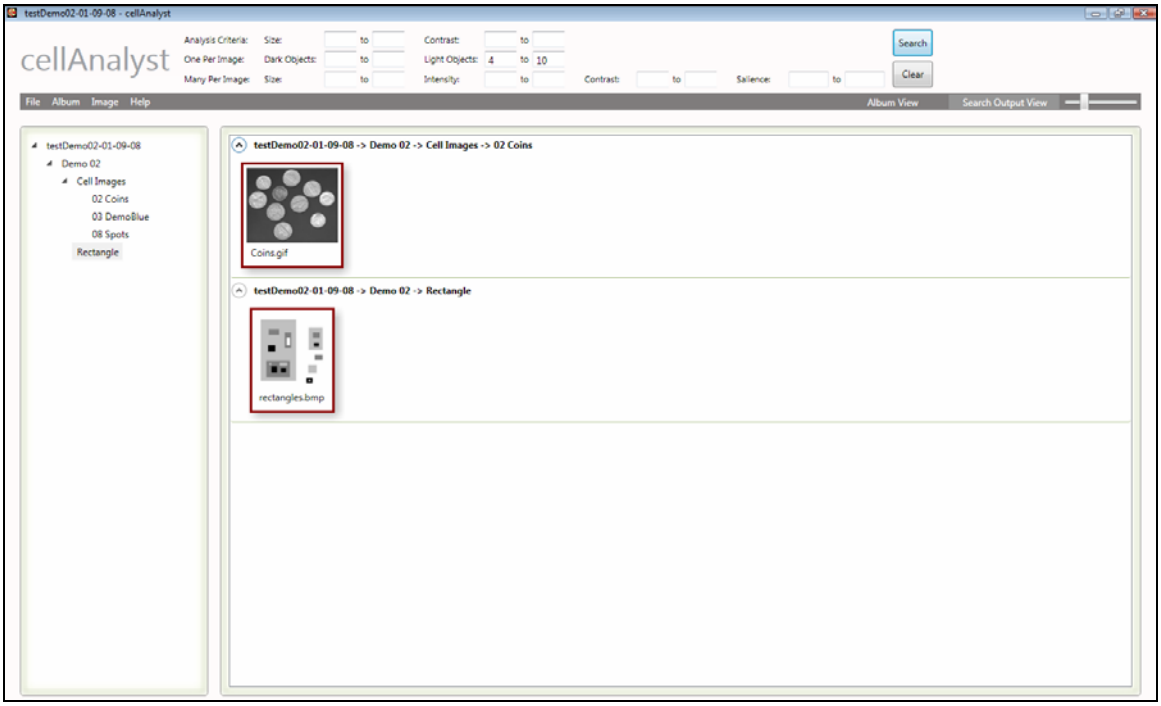
Retrieve images that contain between 4 and 10 light objects.

The answer for this query is image 1 and image 4.



The right pane of the user interface works in two different modes. The first one is the "Album View" in which the thumbnails of all the images stored in the current album are displayed. The second one is the "Search Output View" that displays the output of a search query.

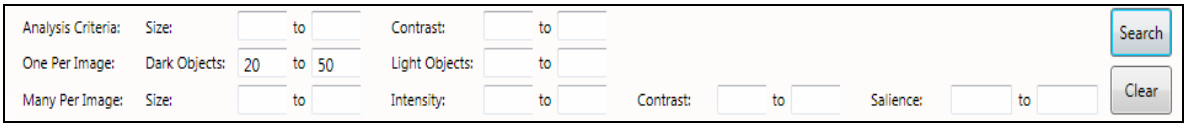
Enter the values 4 and 10 in the "One Per Image/Light Objects" boxes and push the "Search" button. The program will display Images 1 and 4 in the "Search Output View."



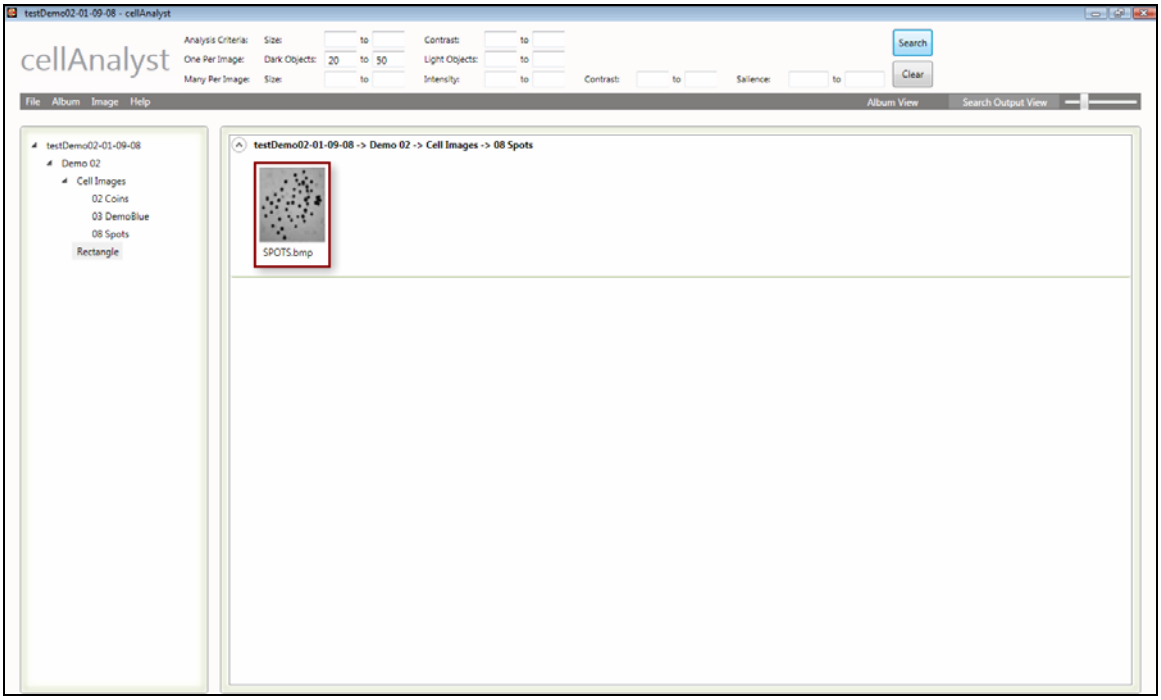
Before executing the next query, select the "Clear" button which will insert null values in all the search criteria and remove all the entries from the "Search Output View."

Query 2

Retrieve all images that contain between 20 and 50 dark objects.

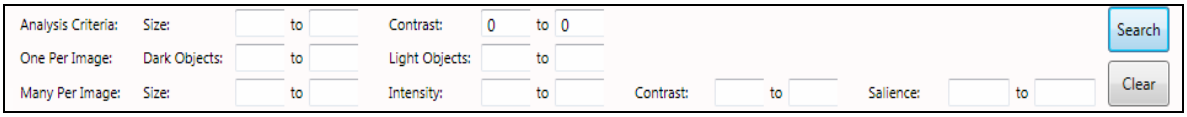


Enter the values 20 and 50 in the "One Per Image/Dark Objects" boxes and push the "Search" button. The program will display Image 3 in the "Search Output View."

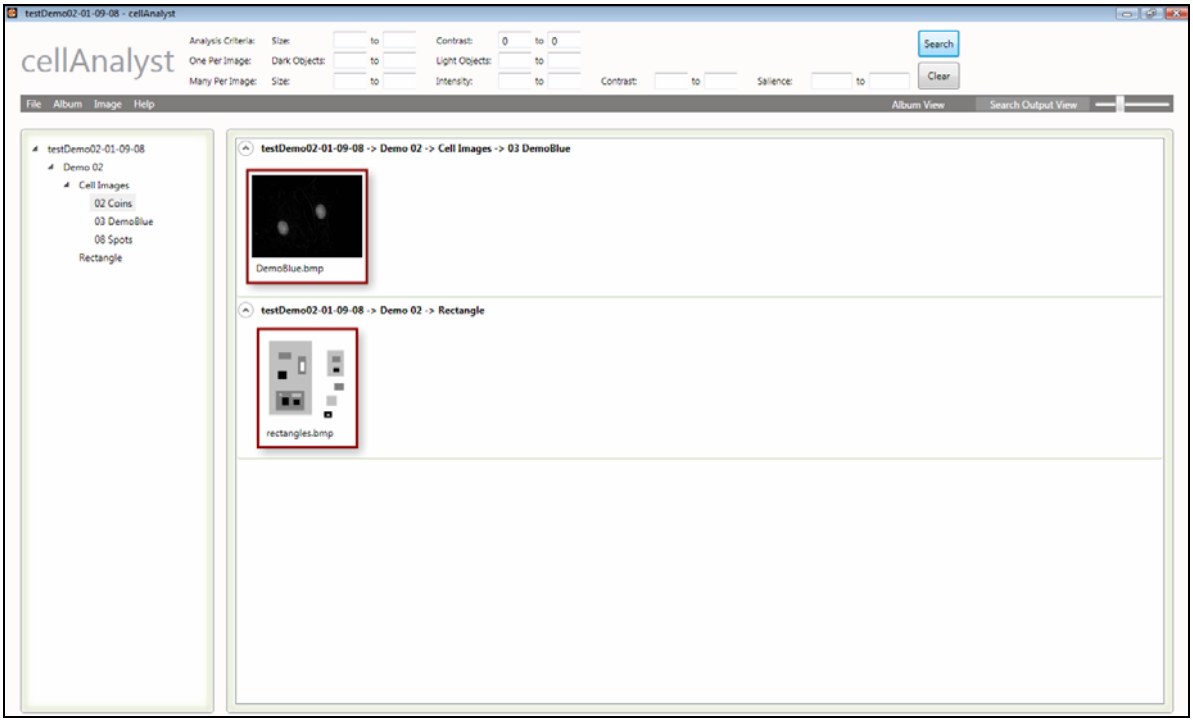


Query 3

Retrieve all images that have been analyzed with contrast setting of 0.

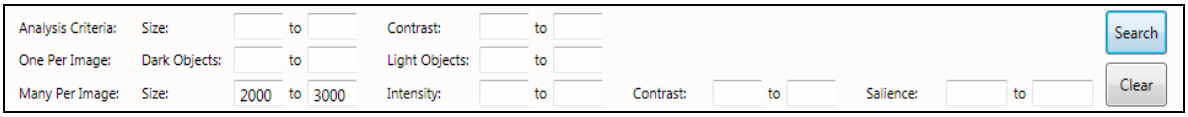


Enter the values 0 and 0 in the "Analysis Criteria/Contrast" boxes and select the "Search" button. The program will display Images 2 and 4 in the "Search Output View."

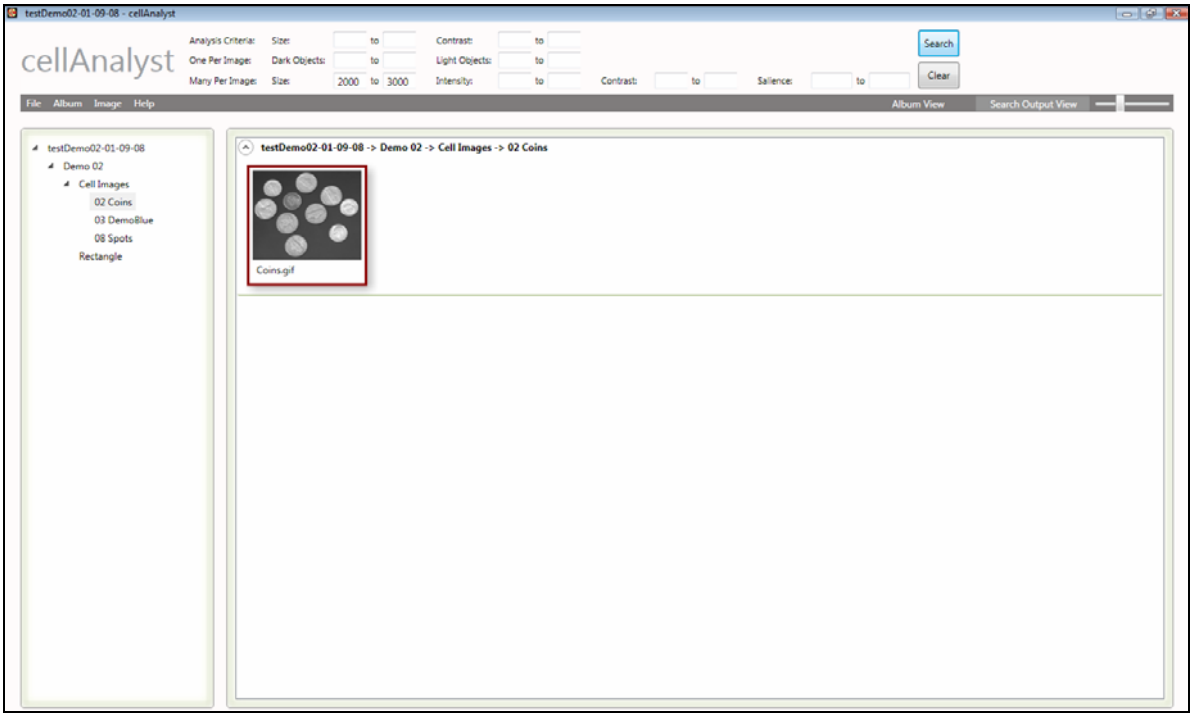


Query 4

Retrieve all images that have objects of size between 2000 and 3000 pixels.



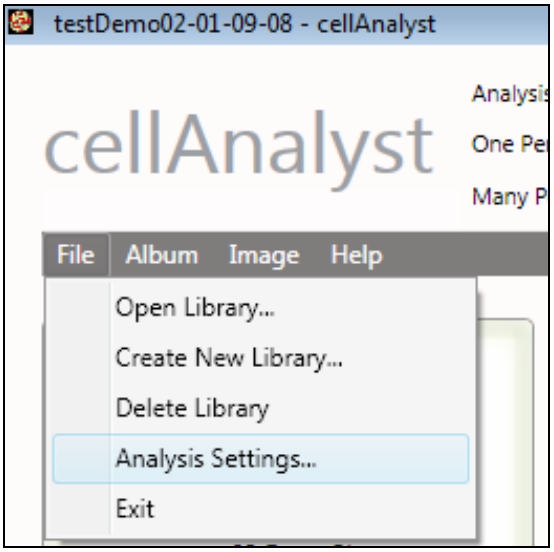
Enter the values 2000 and 3000 in the "Many Per Image/Size" boxes and push the "Search" button. The system will display Image 1 in the "Search Output View."

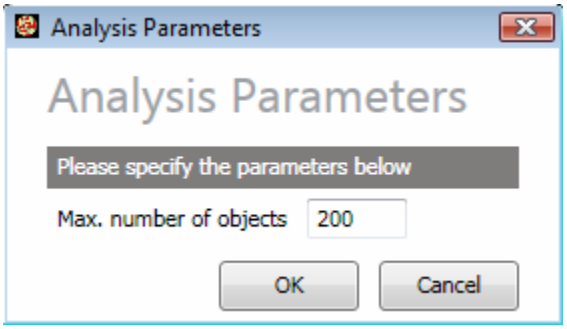


Analysis Settings

cellAnalyst is designed to display the first 200 objects found in an image. This limit is reasonable because images commonly have less than 200 cells. This limit is also necessary because having more than 200 objects in a single image would be difficult to comprehend, manage and store.

If user needs to increase this limit, user can select the menu command “File/Analysis Settings” and increase this limit to any desired number.





Product Evolution

AssaySoft will continue to evolve and refine *cellAnalyst*. User feedback provides valuable insight into user results and expectations. We invite your comments and suggestions for individual as well as community use. Feel free to use the "Contact Us" page of our web site for this purpose.

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