

Introduction

Welcome to **SimplePCI**. You have purchased a high-performance imaging application designed to meet the need for high-speed image analysis and processing with ease of use and flexibility.

At the core of **SimplePCI** are the Image Document and Data Documents, allowing powerful image analysis to be applied to images using hand-drawn regions of interest (ROI), or objects identified by intensity and color threshold. Over 150 measurements plus custom measurements are available and include object count, size, shape, position, color and intensity for object and field analysis.

SimplePCI enables complex imaging problems to be solved easily and efficiently by providing intuitive solutions for most of your common imaging needs.

Expand the functionality of **SimplePCI** by adding the following optional modules:

- **AIC**, automated control and image acquisition
- **DIA**, dynamically measures intensity changes on- or off-line
- **IPA**, develops icon-driven workfiles for automatic image analysis and processing
- **IPA-MTA**, tracks and analyzes moving objects
- **QFA-FRET**, provides accurate FRET measurements and cross talk correction
- **VIS**, provides rapid 3D visualization of multi-dimensional data sets.
- **DNN**, removes out-of-focus haze from optical images using fast algorithms
- **DNN-2D**, a Point Spread Function is derived and used in restoration

Getting Started

Quick Start contains examples of how to utilize **SimplePCI**. For further assistance, refer to the online help, manual, or visit support at <http://www.cimaging.net> for access to the latest **How to's** and frequently asked questions. Additional support is available via e-mail: support@cimaging.net or Tel: 412-741-7920.

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Load an Image Document

Images captured through the **Automated Image Capture (AIC-CD, AIC-SD, AIC-AD, or AIC-EP)** or through TWAIN can be loaded into an image document. The image document toolbar provides a variety of controls for image enhancement and image analysis. The controls in the image document toolbar allow the user to analyze, zoom, add annotation, apply contrast, merge images, scroll, print, copy to clipboard, and export to disk in a wide variety of formats.

To load an image into the image document, proceed with the following steps:

1. Click on the **Open** folder icon, or go to the File Menu and select **Open**.
2. Select **Image Document (fig.1)** from the Open File window and click **OK**.
3. Locate and preview the image to be opened (**fig.2**) and click **Open**.
4. The Image Document (**fig.3**) is open and ready to be used.

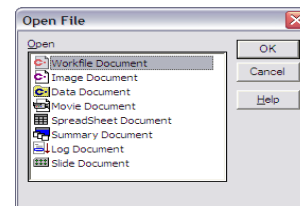


fig.1

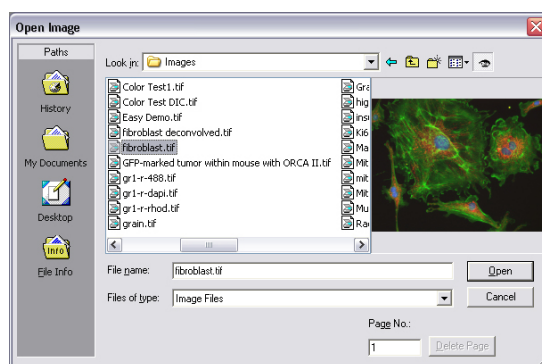


fig.2

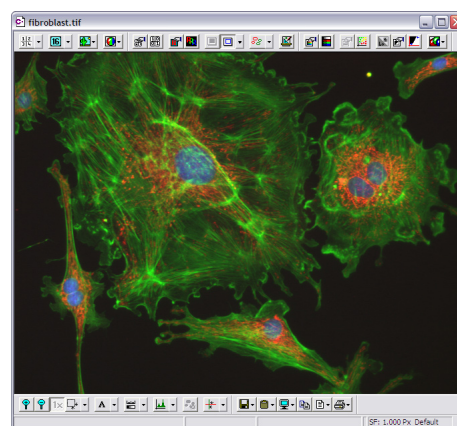


fig.3

Open a Data Document

The Data Document is a sequence of images captured through the **Automated Image Capture (AIC)** module and saved in a ***.cxd** format. The Data Document contains a variety of data types, including images, objects, field measurements and statistics. Data stored in the Data Document can be enhanced, analyzed and exported as a sequence of tiff images, multipage tiffs, a spreadsheet or an AVI file.

To open a data document proceed with the following steps:

1. Click on the **Open** folder icon, or go to the File Menu and select **Open**.
2. Select **Data Document** from the Open File window and click **OK**.
3. Locate the Data Document to be opened (**fig.1**) and click **Open**.
4. The Data Document (**fig.2**) is open and ready to be used.

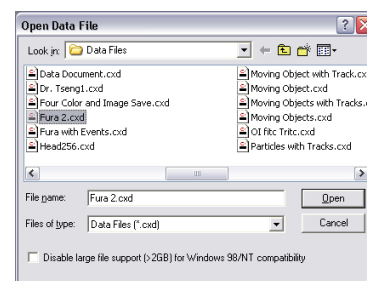


fig.1

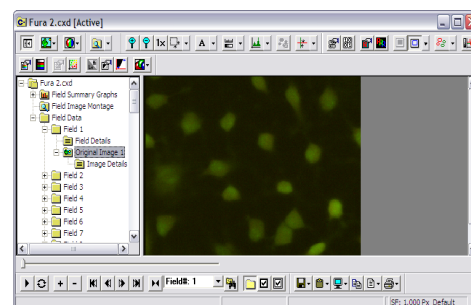


fig.2

Import Images into Data Document

1. Go to the File Menu and select **Import Images**.
2. Click **Source** on the dialog window (**fig.1.A**). Select the first image from the series and click **Open**. If the images are not in a series select the first image, hold the shift key and click the last image of the sequence. Click **Open**. Note: For best result, make sure the image are named sequentially. For example, image001.tif., image002.tif, etc.
3. Click **Dest.** (**fig.1.B**), select a file path and name. Click **Save**.
4. Check **Add Field Details** (**fig.1.C**) and enter the time interval between images in the **Incr.** field. Time is in the hh:mm:ss format.
5. Click **OK**, a cxd is created with all the images.

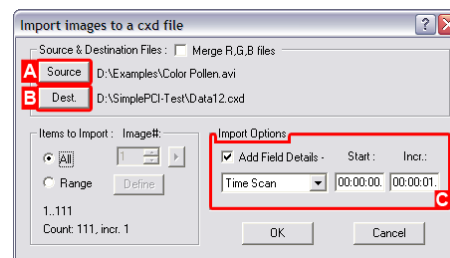


fig.1

Calibrate an Image from Pixels to Microns

1. Open an image with some known distance, for example a reticle or micrometer.
2. Press and hold the Scalebar down-down menu (**fig.1.A**) and select **Calibration**; the Calibration Window appears (**fig.2**).
3. Enter a Title for the calibration file, e.g., 10x. Select the Units and symbol from the drop-down menu.
4. Click **Calibrate** in the Calibration window (**fig.2**). Move the Calibration window to the side so you can see the image. Move the cursor to the start of a known distance; click and drag a line to span the distance you wish to measure, as shown in **fig.1.C**.
5. Enter the known distance of the line you have drawn (**fig.3**) and click **OK**. The Calibration Factor in the Spatial Calibration Menu will be updated.
6. Click **OK** to close and save the calibration window. The Spatial Calibration is displayed in the lower right-hand corner of image and data documents (**fig.1.B**).

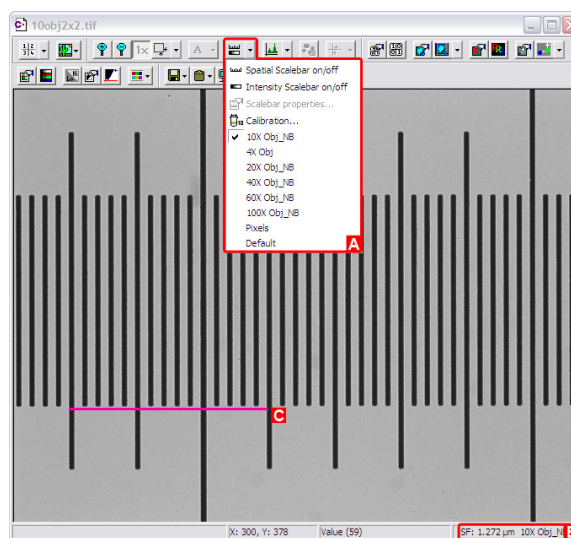


fig.1

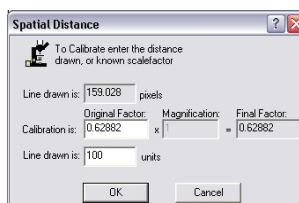


fig.3

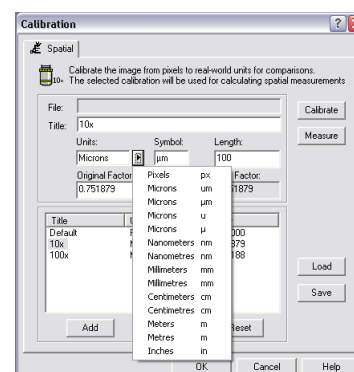


fig.2

Tint a Monochrome Image

1. Load an Image or Data Document and click on the **Quick Tint** icon (fig.1). Select a wavelength color from the Tint List.
2. To save the Display Image, click on the **Save** icon and select **Display Image** (fig.2)

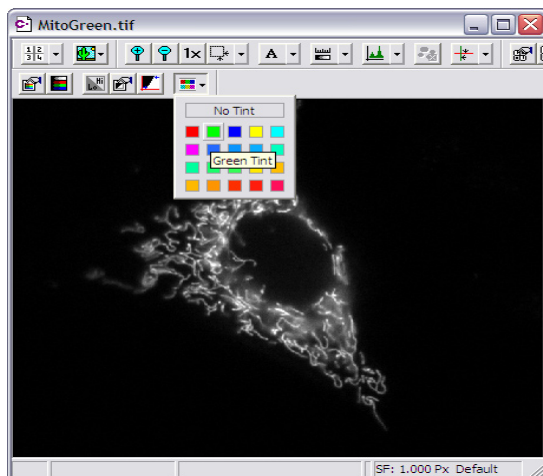


fig.1

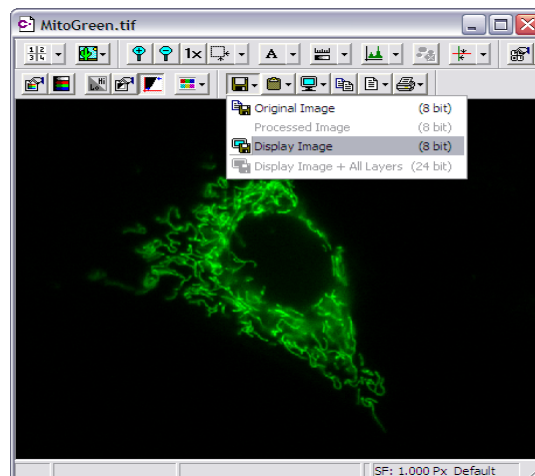




fig.2

Enhance Contrast of an Image

1. Load an Image or Data Document and then click on the **Auto Contrast** icon  to apply contrast automatically (fig.1.A).
2. To adjust contrast manually, click on the **Contrast Properties** icon  (fig.1.B); the **Display Contrast** window appears (fig.2).
3. Move the top and bottom slider to the right or left until the desired contrast is achieved. To apply contrast on each channel separately, toggle **Locked** to **Unlocked** (fig.2.A) and click on the tab for each of the color channels, then change the contrast. To apply the contrast simultaneously to all the channels, check the **Locked** icon. Also, for other contrast effects, select from the different transforms available from the drop-down list (fig.2.B).
4. Once you are happy with the contrast close the **Display Contrast** window to view changes (fig.3).

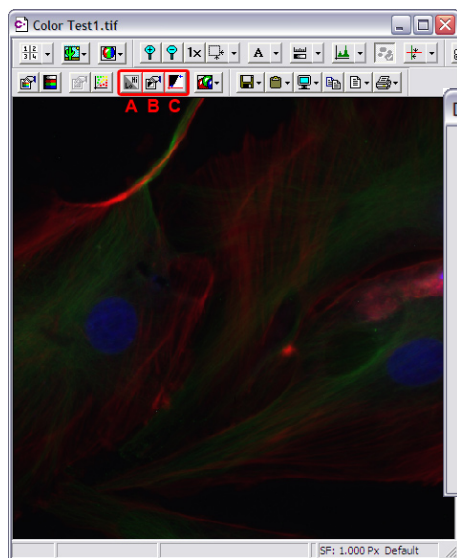


fig.1

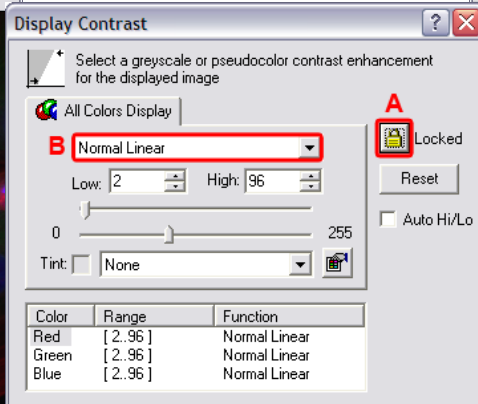


fig.2

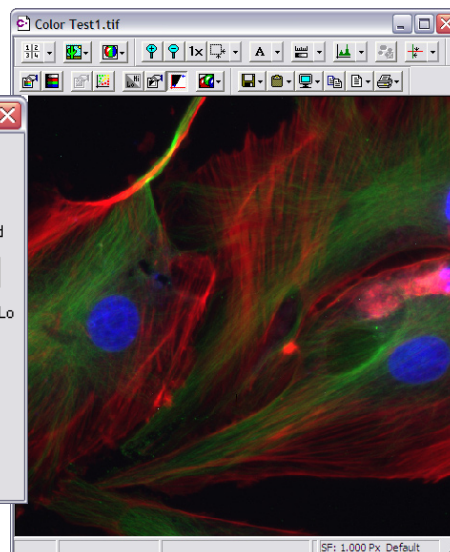




fig.3

How To Blend Several Monochrome Images

1. Go to the File Menu, select **Open**, select **Image Document**, and select all images you wish to blend from the **Open Image** dialog. Click **Open All** to open each image separately (**fig.1**).
2. Click on the **Merged Display Properties** icon  (**fig.1.A**); and the **Merged Display** window appears (**fig.2**). Select **Blend** from the drop down list underneath **Apply** (**fig.2.A**).
3. With the current image highlighted (i.e. Cy3-Elastin.tif), click the **Tint** icon, and choose from the list of available tints (**fig.2.B**). Click **Apply** to preview the tinted image.
4. Add additional images from the list of open images (**fig.3**) by selecting the next line.
5. **Tint** each image as appropriate and if necessary, you may adjust the contrast of each image individually using the **Contrast** icon (**fig.2.C**).
6. Click **Apply** to view the results of the blend (**fig.4**).
7. Toggle the blended images on and off by clicking on the **Apply Merged Display** icon  (**fig.4.A**).

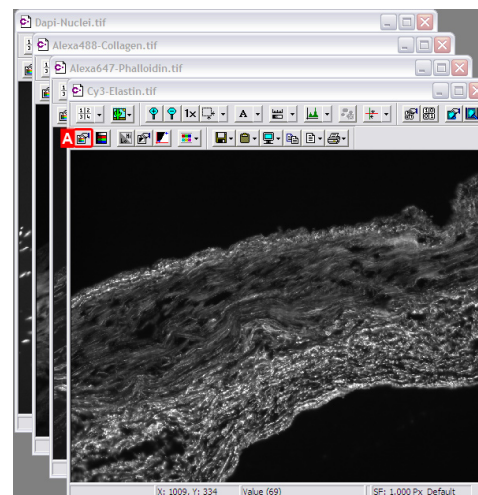


fig.1

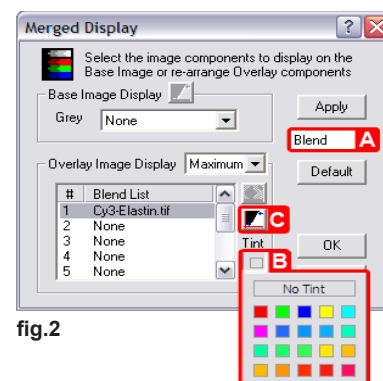


fig.2

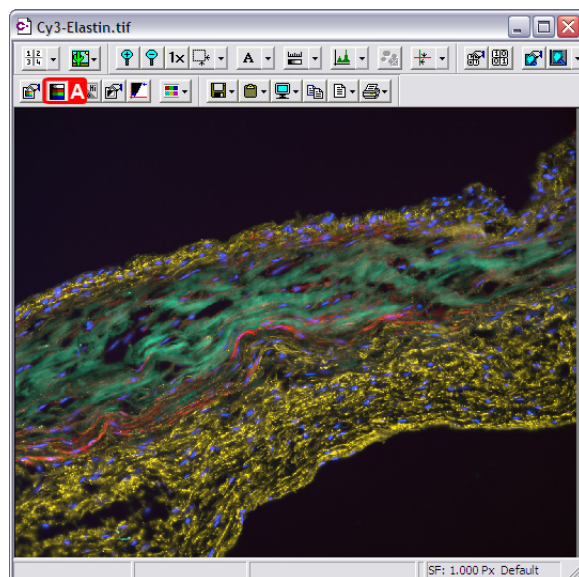


fig.4

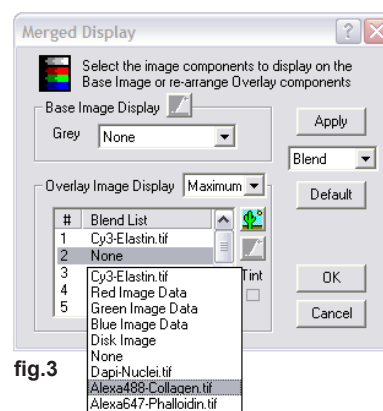


fig.3

Create a Montage From a Data File

1. Open a Data Document and click on the **Montage** drop-down menu in the main toolbar (fig.1.A).
2. Select **Best-Fit Square** from the **Montage** drop-down menu (fig.1.B). If you would like to customize or edit the montage image, go to step 3, otherwise go to step 5.
3. Click on the **Montage Properties** drop-down menu and the Montage Properties Window Opens (fig.2).
4. Select **Custom Grid** from the **Display Montage** drop-down menu and enter the width and height of the frames to be displayed. Check **Frame Separation** and enter the width for the **Horizontal** and **Vertical** grid to separate the images. Select a color for the grid and background. Click **OK**.
5. Save, Print and Copy the image montage by right-clicking on the image and selecting **Save**, **Print** or **Copy** from the menu.

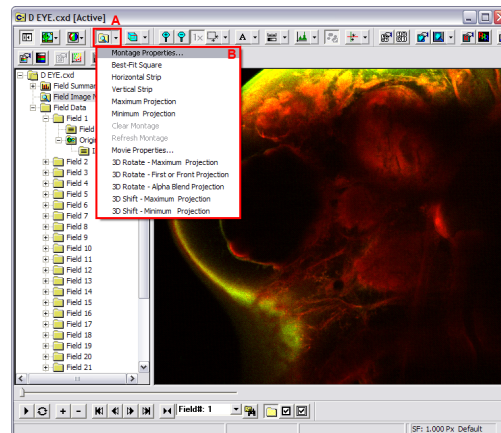


fig.1

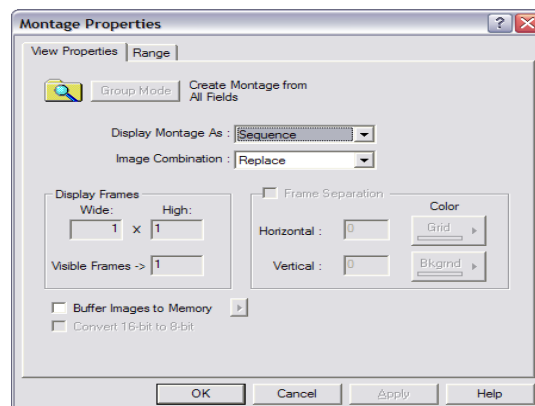
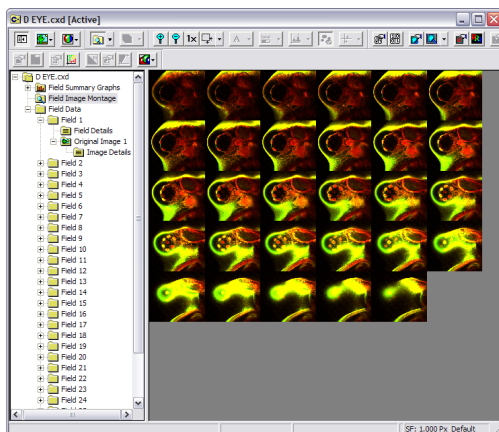



fig.2

How to Split an Image into RGB Components and Create a Montage

1. Once you open an image file, click the **Split Window for Quadrant View** icon .
2. The image will be split into four components, Original Image, Red, Green and Blue Components (fig.1).
3. Click on the **Split Window** down-arrow and select **Copy to Montage** from the Split Window sub-menu.
4. Add Annotation, Text, and Calibration Bars to the montage by selecting the respective tools from the Annotation toolbar. Save the montage by clicking on the **Save** icon.

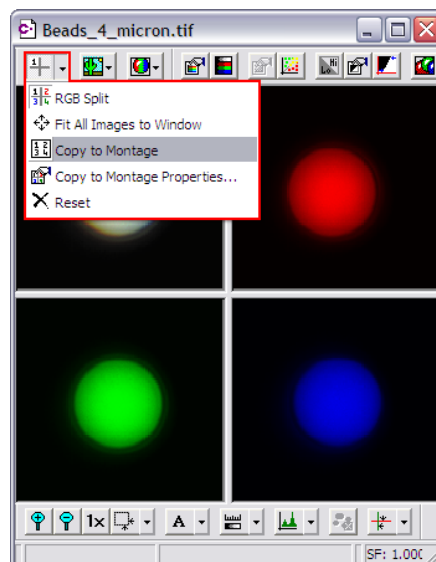



fig.1

How to Create a 3D Movie from a Z Series

1. Load a Data Document. Click on the **Montage**  drop-down menu. Select **Movie Properties** from the drop-down list (fig.1). The **3D Movie Properties** window appears (fig.2).

Note: If you want to use the default setting of the movie properties, select **3D Rotate** from the **Montage** sub-menu and skip Steps 2 and 3.

2. Select the type of image projection from the **Image Projection** drop-down list (fig.2.A). In the **3D Movie Rotation Setting** box enter the **Start Angle**, **End Angle**, **Increment**. Select **X-axis** or **Y-axis** rotation in the **Rotate About** check-box. The **Custom Threshold** box is only activated if Alpha Blend and First or Front projection are selected. Enter the **X,Y,Z** step size in the **Image Scanning Step Size**. You can retrieve these values from the data document by clicking on the **X,Y** and **Z** buttons. Select the type of interpolation from the **Z Interpolation** drop-down list (fig.2.B).
3. Click **OK**, assign a file name and file path to your data file and click **Save**. **SimplePCI** will start creating the 3D movie; a preview of the image as it is being rendered appears.
4. Play the rendered movie by clicking the **Play** button (fig.1). If the movie is playing too fast, click the "-" icon to decrease the playback speed.

Note: If the 3D movie appears too flat, ensure you are entering the correct **Z** step size, and you have enough slices. The total number of slices required should be equal to the thickness of the scan.

5. To create a movie file of the movie, right-click on the image and select **All Components** from the **Export Image Sequence** sub-menu, the **Export Images** window appears (fig.3).
6. Select **Movie Files (*.avi)** from the **Type** drop-down list (fig.3.A). Click on **Browse**, assign a file name and path. Click **Save** and **OK**. The movie will open in a new window (fig.4).

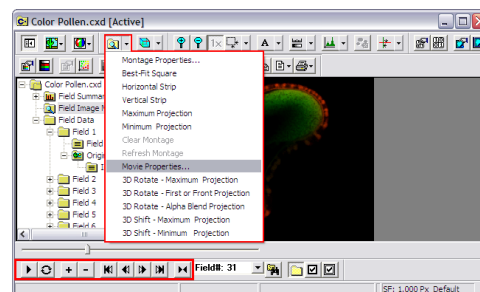


fig.1

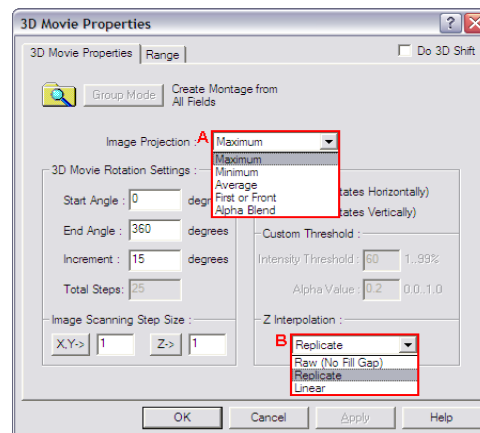


fig.2

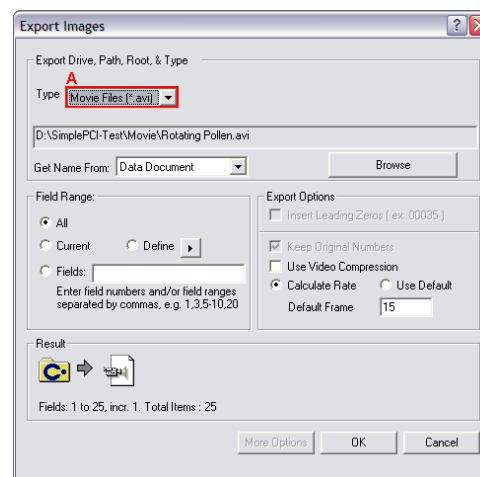


fig.3

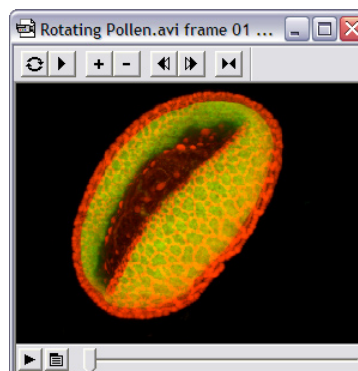



fig.4

Background Correction

1. Open an Image document, click on the **Enhance** icon  in the main toolbar. The **Image Enhancement** window opens (fig.1).
2. Select **Process** (fig.1.A), and the **Select Operations** window (fig.2) will open. For this example, we will select **Ratio Shade Correction**. Click **OK**, then select a previously saved background image when prompted.
 - Use **Shade Correction** and **Ratio Shade Correction** for bright field images. The process corrects the source image using an image of the background. **Hint:** Shade Correction is less destructive to image intensity
 - **Background Subtraction** is suited to fluorescence images and works by subtracting an image of background from the source image. **Hint:** make sure exposure and gain settings are the same for source and background images
3. You may toggle the Image Enhancement icon on or off to view the difference. Figure 3 shows the image before and figure 4 shows the image after Image Enhancement..

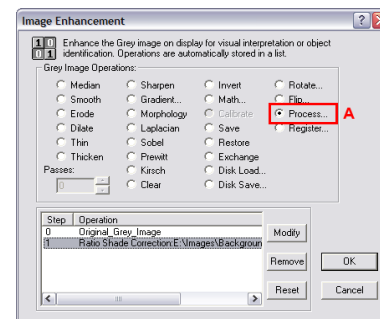


fig.1

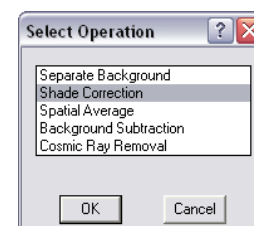


fig.2

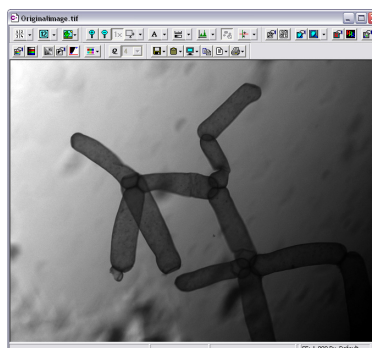


fig.3

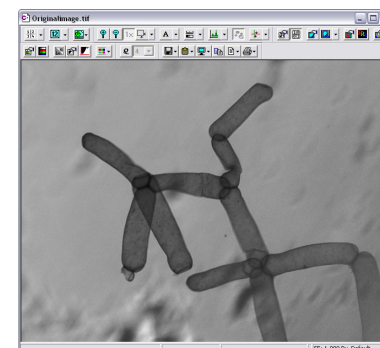


fig.4

Add Annotation To Data Document

1. Open a Data Document and Click on the **Annotation** icon (fig.1.A) to activate the annotation toolbar (fig.1.B).
2. Use the annotation tools (fig.1.B) to add text, arrows, time stamp and ROIs to the image.
3. To export the image sequence with Annotations, go to the **File** menu and select **Display Image + All Layers** from the **Export Image Sequence** sub-menu. You may export to AVI, Image Files, or a new Data document.

Hint: Use the # button on the Annotations toolbar to place **Active Text** in the displayed image. **Active Text** includes: Field Number, Time From Start, Time From Last, Image Width, Image Height, Image Depth, Group Number, Group Index, and Group Size.

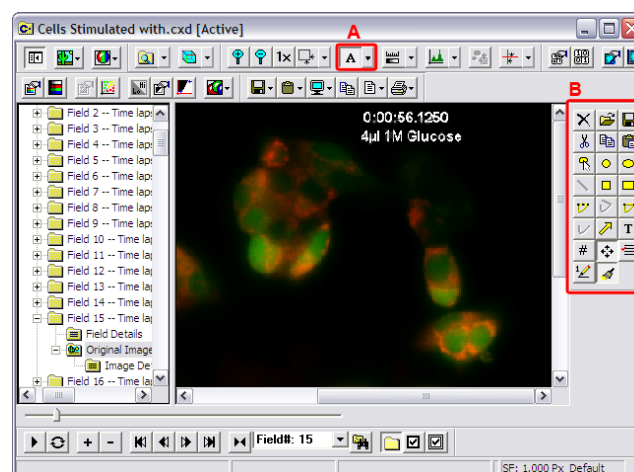





fig.1

Analyze an Image by Intensity Threshold

1. Open an image document. Click the **ROI Shape** icon  (fig.1.A), the Shape toolbar will appear (fig.1.B).
2. Press the **Identify** icon  (fig.1.C), and the Identify Objects window appears (fig. 2). Set the intensity threshold by adjusting the Min and Max slider until the objects of interest are highlighted by a green binary overlay (fig.2). Click **OK**.
3. The **Use Advance Detection** window appears. Click **Yes** if you want to apply more advanced binary operations or reject unwanted objects, otherwise click **No** and go to step 7.
4. The **Binary Modification Window** appears. Select any binary operation you need to modify the binary image with a binary filter, such as Erode, Dilate, Close, Open, etc; click **OK**.
5. The **Qualify Object** window appears (fig.3.A). Reject unwanted objects by area from the binary overlay by adjusting the Min and Max slider. Objects will appear red or blue to highlight the under-size and oversize range, while objects to be measured will be highlighted in Green. Additional qualifying parameters can be added using Grey level, Length, Roundness and other parameters by clicking on the **Add** button. To reject an object touching the border of the image, click the **ROI Edge Objects** icon (fig.3.B) and select **Delete All Edge Objects**. Click **OK** when finished qualifying the objects.
6. Click the **Measure** icon  in the image toolbar (fig.3.C).
7. Check the type of measurement you want from the Measurements window. You can customize the measurements by clicking on **Define** and creating customized measurements.
8. Once you have selected the measurements click **Measure to Data Doc**.
9. Enter a name and path for your data file and click **Save**.

10. Review the measurements (fig.4). Click on a data value to identify the object that corresponds to the data value and vice-versa. The corresponded object is highlighted in red as shown (fig.4). This interaction between objects and data is maintained even after closing the document.

The data document will display object summary statistics, object summary graphs, field summary statistics, field summary graphs, field image montages and field data. Data can be printed or exported to a spreadsheet document by selecting **Copy to Spreadsheet** under the **Edit** menu.

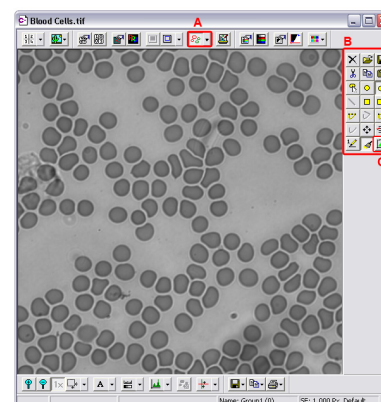


fig.1

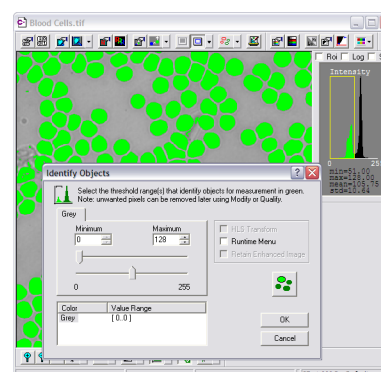


fig.2

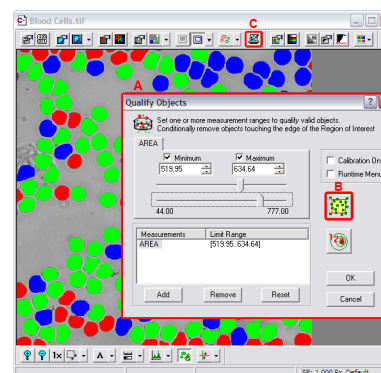


fig.3

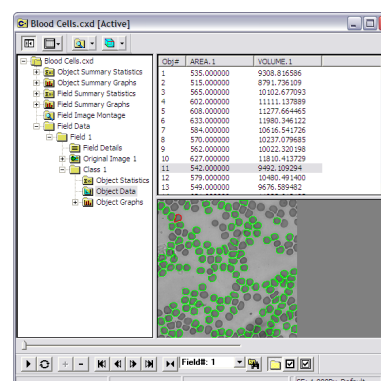


fig.4