NIS-Elements: Using Regions of Interest (ROIs) & ROI Statistics

Various ROI tools and functions

NIS-Elements has several ROI functions designed for data analysis and image quantification. ROIs are a core feature in AR and BR packages and work and link with several dialogs within NIS-Elements: ROI Statistics, Object Count, Automated Measurement Results, Time Measurement, Colocalization, FRET calibration, and Volume Measurement.

> Note number: 0001
> Date: 4/2008
> Date Modified: 8/2008
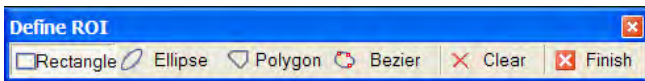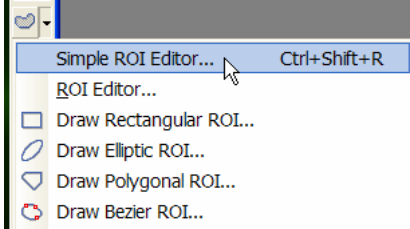> Software Version: 3.0
> Package: AR / BR

Access to ROIs:

To create ROIs on an image, click the small black arrow next to the ROI icon 🖾 on the right border of the image window.
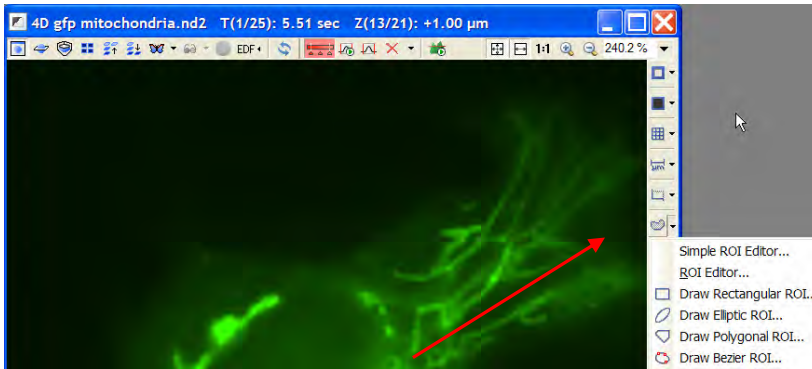
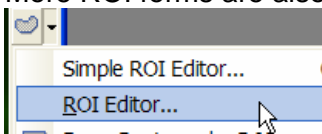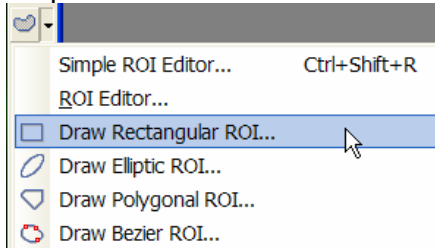**Options for creating ROIs:**

- ROIs can be drawn using the Simple ROI Editor:

- The Simple ROI Editor gives the choice of common ROI forms: Rectangle, Ellipse, Polygon, and Bezier.

- More ROI forms are also available in ROI editor for additional options.

- Rectangle, Ellipse, Polygon, and Bezier are also directly available from the ROI menu drop down.

Simple ROI Editor...	Ctrl+Shift+R
ROI Editor...	
☐ Draw Rectangular ROI...	
⬭ Draw Elliptic ROI...	
▽ Draw Polygonal ROI...	
⟳ Draw Bezier ROI...	

Drawing the ROIs on the image:

- Select the ROI form (For this example, click on 'Polygon.')
- Draw around selected area.
- When complete, either right click or double click to create the ROI. Depending on settings, a number and transparent layer will appear on the ROI.
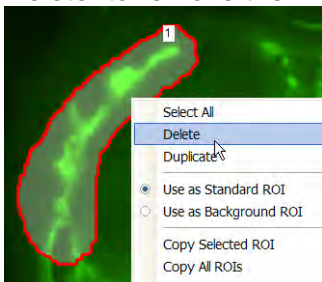- If desired, continue to add additional ROIs on image.
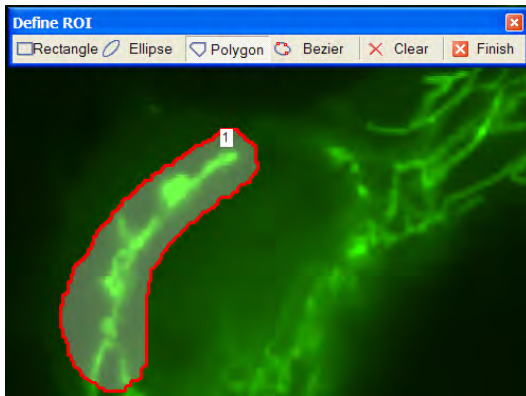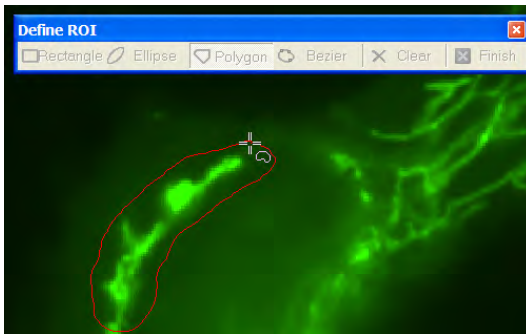
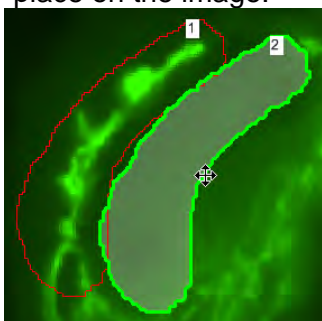- To clear ROIs, select 'Clear' from the Simple ROI Editor.
- Click Finish.

When the Simple ROI Editor is closed, additional options are available by right clicking on the ROI itself:
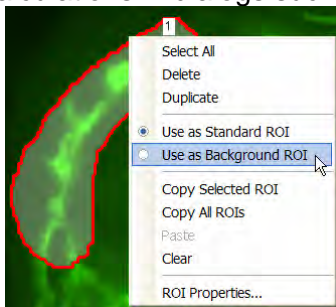
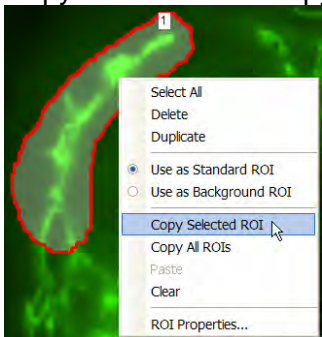- Delete: to remove the ROI

- Duplicate: to create an exact copy of size and shape of ROI to place on the image.
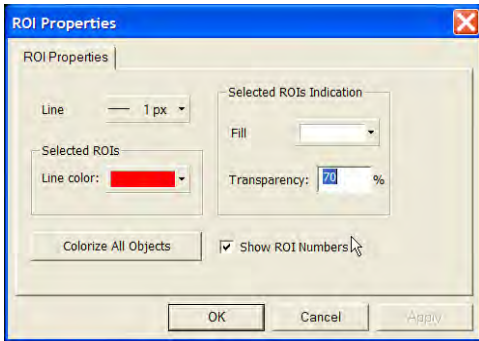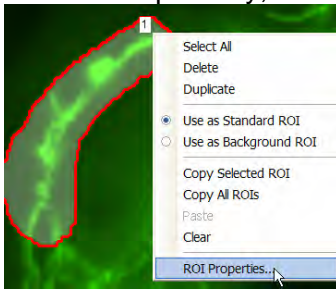

- Use as Background ROI: to designate that the ROI should be used for background calculations in dialogs such as Time Measurement.

- Copy Selected ROI: copy the ROI to transfer (copy/ paste) to another image or dataset.
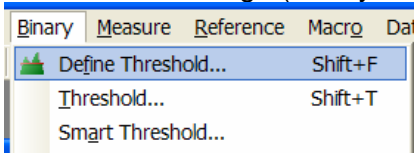
• ROI Properties…: additional options for labeling and appearance of the ROI such as transparency, color, and number labels.


Converting Binary to ROIs:

The thresholded area(s) of the image can be converted to an ROI(s). This is a useful and automated method of creating ROIs.

• Threshold the image (Binary Menu → Define Threshold)

• Select the click the small black arrow next to the background ROI icon to view a drop down of converting options. In this example, select 'Copy Binary to ROI.' The thresholded area(s) will be copied to an ROI(s).
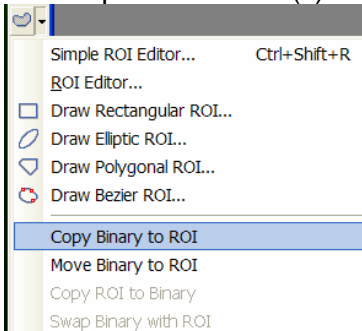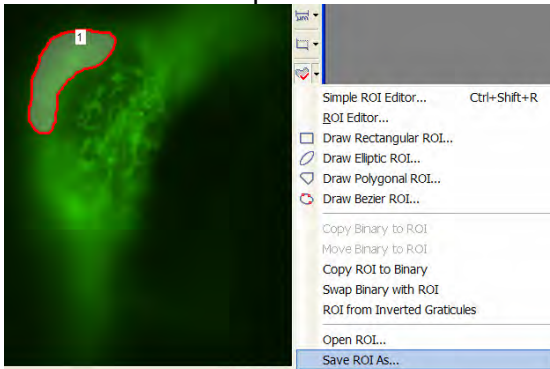

Note: The move and copy work similarly to how Microsoft 'Copy' and 'Move" function. 'Move Binary to ROI' will move the binary out of the stored binary layer to the ROI. The binary layer will be cleared.
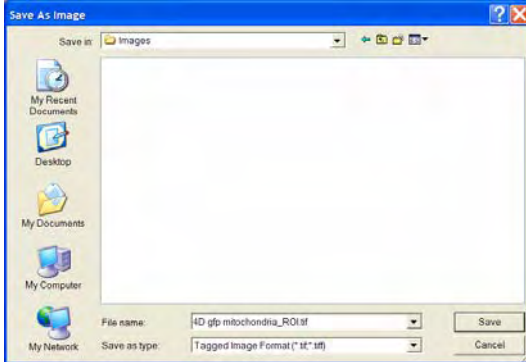

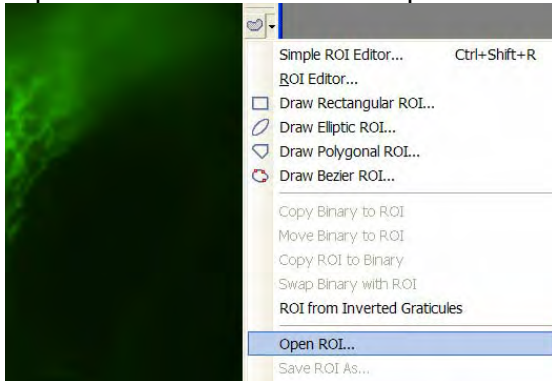**Saving and Loading/Opening ROIs on the image:**

- Draw the ROI(s) on the image.
- Select from the drop down list 'Save ROI As…'

- Select 'Save ROI As…' from the ROI drop down list
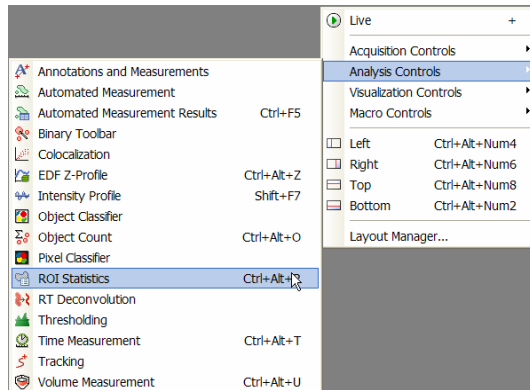- Type name of ROI file. *Recommended file type is 'Tagged Image Format (.tif, .tiff)'*

- To load/ open/ apply previously saved ROIs to an image or dataset, select 'Open ROI…' from the ROI drop list.

Access to ROI Statistics:

Right click on the NIS-Elements desktop and select 'Analysis Controls," then select 'ROI Statistics.'


Using ROI Statistics Dialog:

- Make an ROI active by clicking on the ROI in the image.
- ROI statistics will report Area, Mean, Min, Max, Sum StDev Intensity and the ratio of Signal/ Background.
- Note: If a binary level (or threshold has been set) is active with the image, ROI information can be viewed side by side. The area fraction will represent the percentage of the binary portion of the image that is also an area within an ROI(s).
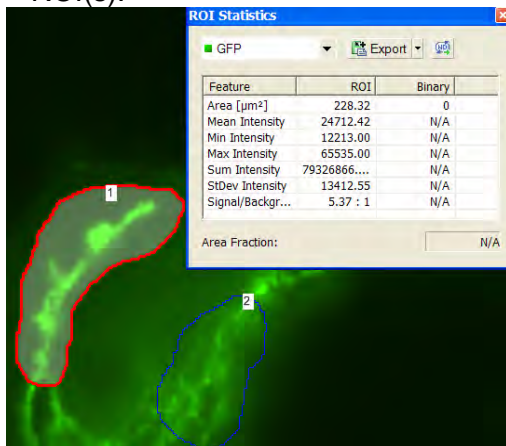
• ROI Statistics will reflect the information of the selected ROI. To view more than one ROI, hold down the keyboard Ctrl key + click on ROI(s). The ROI Statistics will update accordingly.

Ctrl + Click on ROI to select
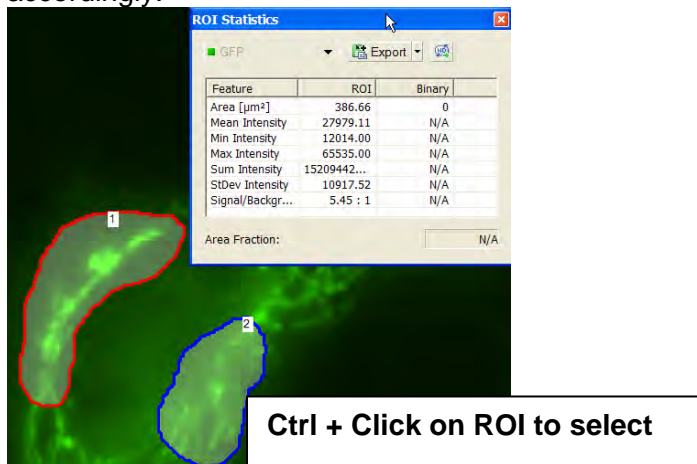
Exporting Data from ROI Statistics:

To export ROI data over a ND2 dataset:[1]
• Select the small black arrow to view a drop down of possible export locations.
• Select the desired export location.
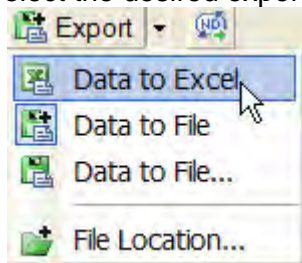
• Click on the 'Export' button to export the data to the selected location.

• Exporting across an ND2 dataset is also available. Click the ND export icon .

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[1] Exporting Across an nD2 file is possible in NIS-Elements AR and limited in Br.
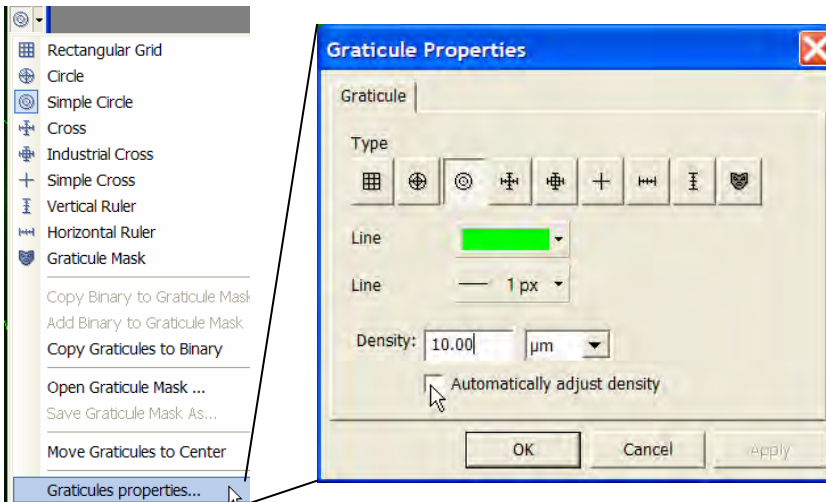
Using Graticules to Create ROIs:

- An additional method for ROI creation is using the graticule overlay.

- Select the click the small black arrow next to the graticule icon to view a drop down of graticule options. For this example, 'Simple Circle' has been selected.
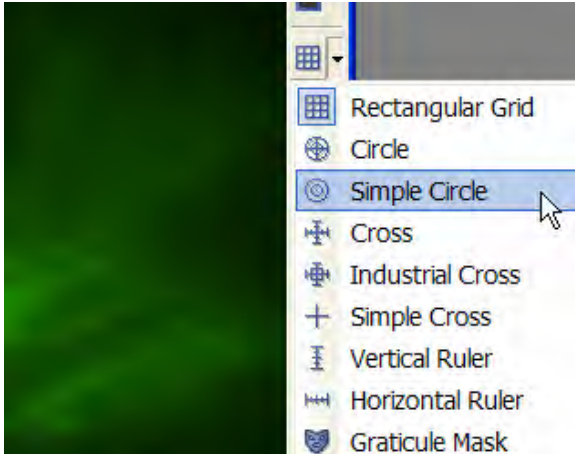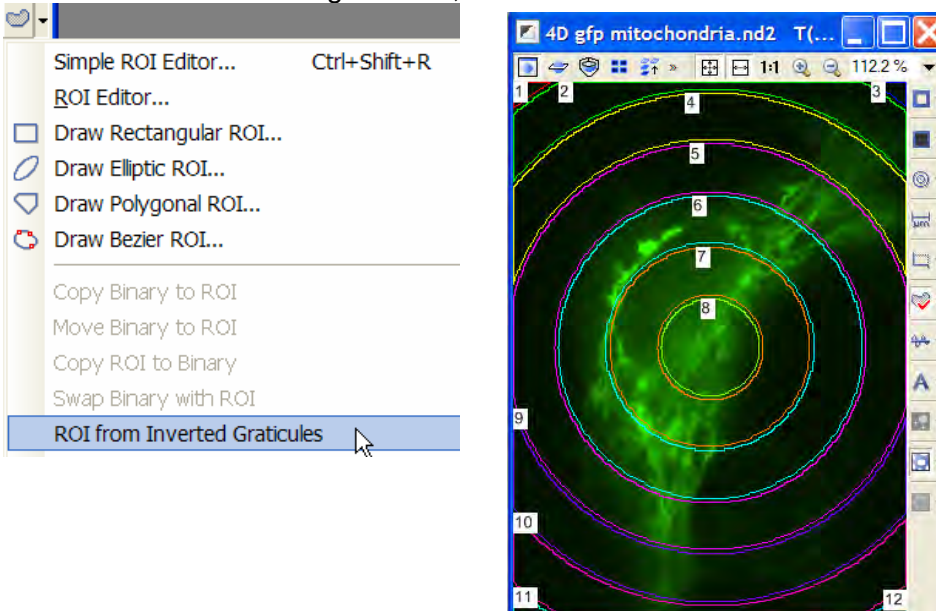

- To modify the density of the graticules, select 'Graticules properties.'

- To create ROIs from the graticules, select 'ROI from Inverted Graticules.'

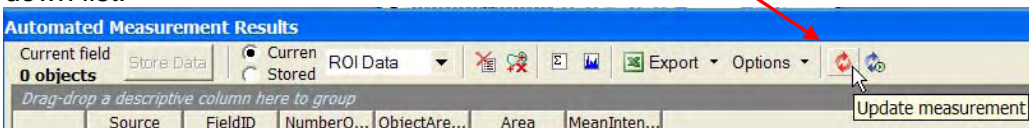
Using Automated Measurement Results + ROI data:

- Once ROIs are on the image or dataset, many other NIS-Elements can link to the ROIs.
- In this example, Automated Measurement Results will display and export ROI data as well.
- To access Automated Measurement Results, right click on the NIS-Elements desktop and select 'Analysis Controls," then select 'Automated Measurement Results.'
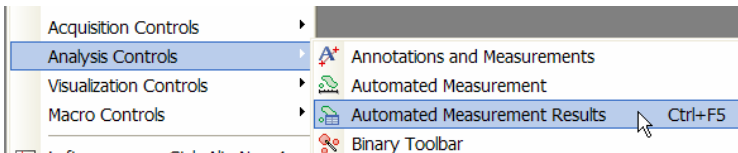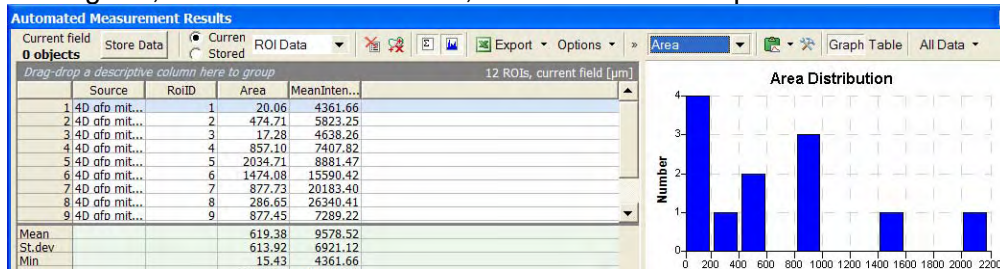
- Within the Automated Measurement Results dialog, select ROI data from the drop down list.
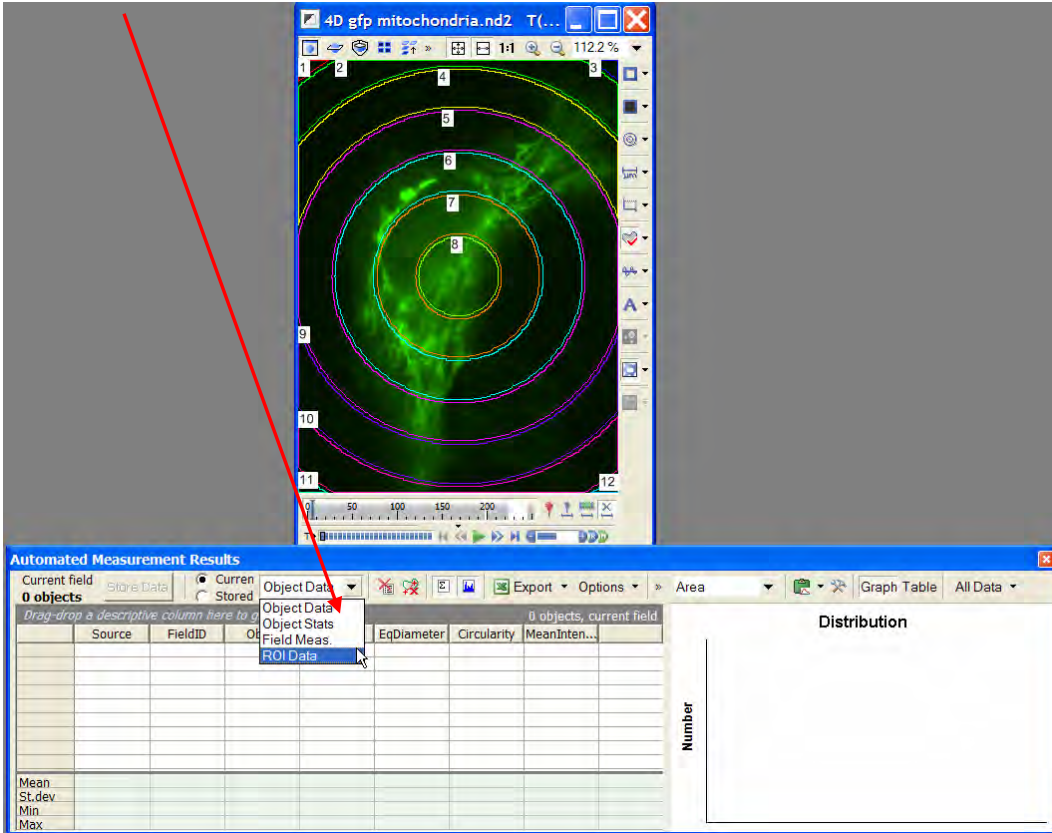
- To update the data within the Automated Measurement Results dialog, select 'Update down list.

- The Automated Measurement Results dialog will report the ROI data, summary statistics, histogram, and a table. In addition, ROI data can be exported.
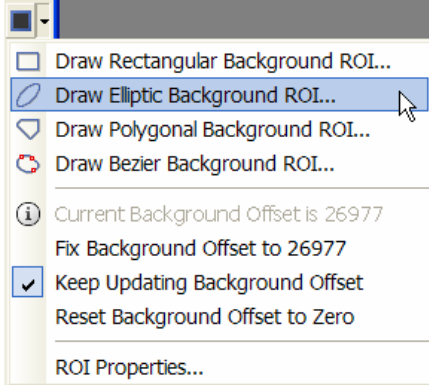

- More on this dialog will be described in a future Technical Note.

Using an ROI for background subtraction:

- Select the click the small black arrow next to the background ROI icon ■ to view a drop down of background ROI option. For this example, 'Elliptic' has been selected.
- Create the ROI on the image to be used for background subtraction.

- Open the Image Menu, select 'Background' and select 'Subtract Background Using Background ROI…"
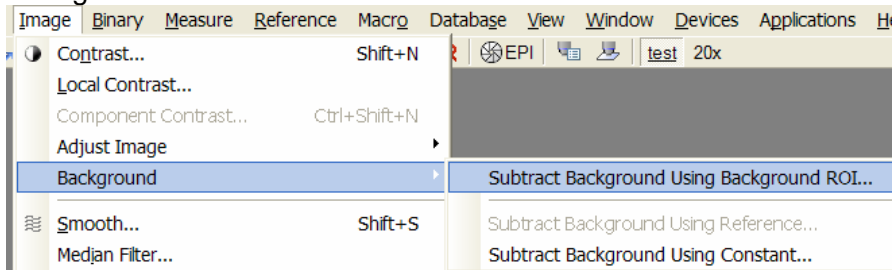
- The following dialog will open for further options for applying the substraction on the image or full dataset.