Insight: Measurement Tool

User Guide

Open Microscopy Environment: http://www.openmicroscopy.org

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1   Introduction to the Measurement Tool

This document complements the **OMERO Client User Guide** by focusing on the Measurement Tool – a new feature to the OMERO.Insight Beta 2.2 release, which significantly extends the image measurement and analysis functionalities.

These include:

- Defining Regions of Interest (ROIs) on the image – and on each z-section or timepoint of multi-dimensional images;

- Annotating ROIs with textual annotations;

- Assigning stroke and fill colours to shapes;

- Getting measurements of the ROI shapes, including area, length and angle;

- Plotting intensity values;

- Loading existing ROIs and saving ROIs to file; and

- Managing ROIs – editing shapes, viewing, deleting and adding.
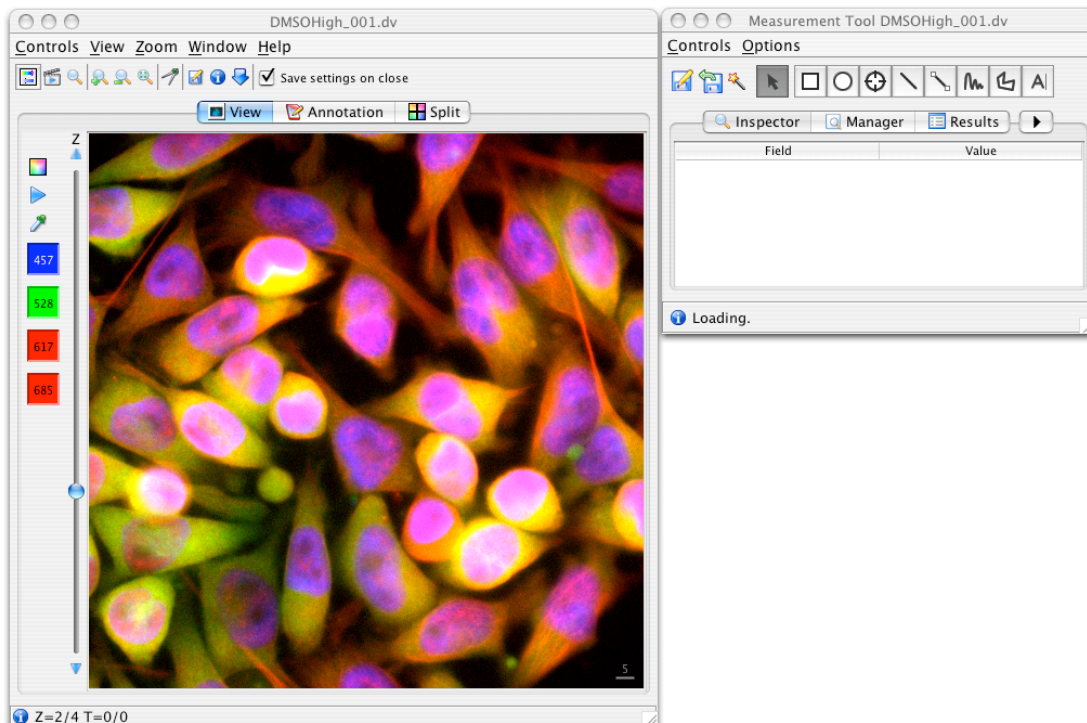

Figure 1: Image Viewer with Measurement Tool

1.1 Accessing the Measurement Tool

Assuming the image on which you wish to perform measurement is currently open in the Image Viewer, you can open the Measurement Tool by one of two ways:

- Click on the measurement tool icon ⟋ in the main Image Viewer toolbar above the image; or

- Open the **Controls** menu, select **Measurement**.

This will launch the Measurement Tool (see **Figure 1**).

1.2 The Measurement Tool interface

It is worth familiarising yourself with the Measurement Tool user interface, shown in **Figure 1**. It consists of two key areas – a series of buttons identified by icons, and below that a series of tabbed navigation options. Additionally, there are menu options available at the top of the application window, and in specific situations, available by right-clicking the mouse.

The Measurement Tool has a toolbar giving access to the key features of the tool, shown in **Figure 2**:

Figure 2: Measurement Tool toolbar

Below this are a series of tabs giving access to key views of data relating to ROIs present in the selected image (see **Figure 3**).
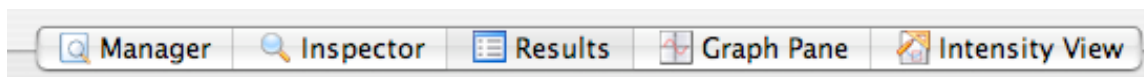

Figure 3: Measurement Tool's tab navigation

In turn, these tabs give access to the following features of the Measurement Tool:

- **Manager** shows a hierarchical view of all ROIs and constituent shapes present in the image. This allows you to select an individual ROI shape or ROI (a collection of shapes) for analysis or editing.

- **Inspector** allows you to edit the appearance of an individual ROI shape, including colour of line, fill and any text associated with the image.

- **Results** view shows a summary of key measurement data for all ROI shapes present. It allows you to select an individual ROI shape for further detailed analysis.

- **Graph Pane** generates graphical representations – histogram and, for line ROI shapes, line plots of intensity values, by channel, for the selected ROI shape. These can be saved as images.

- **Intensity View** provides numerical data of individual pixel intensity values plus values such as mean, maximum and minimum values, plus an option to export this data for viewing in spreadsheet applications, such as Excel, as a CSV file.

2 Creating ROIs

You can create Regions of Interest (ROIs) of varying types on a single plane, which can then be expanded to become multi-dimensional.

2.1 What is an ROI?

In OMERO, we use the following definitions:

- ROI – a multi-dimensional object, consisting of one or more ROI shapes, indicated by the icon: .

- ROI shape – a two dimensional object that appears on one plane only, indicated by the icon: .

An ROI shape also automatically exists as an ROI. An ROI across multiple dimensions (z-sections or timepoints) will be created when an individual ROI shape is selected and propagated.

Propagating ROI shapes to create multi-dimensional ROIs is discussed more in **Section 2.4**. Viewing and managing ROIs and ROI shapes is discussed more in **Sections 4** and **5**.

2.2 Creating a single shape

To create a single ROI shape on a single plane (z-section or timepoint) on an image, select the appropriate toolbar shape option and draw the shape.

To draw a **rectangle**:

1. Select the Rectangle toolbar button
2. Hold down the mouse button on one corner of the area you wish to form the ROI, and drag to the opposite corner.
3. Release the mouse button; the rectangle is now created.

To draw an **ellipse**:

1. Select the Ellipse toolbar button:
2. Click on the edge of the area you wish to form the ROI, and drag the mouse pointer until the circular shape approximates the ROI you wish to produce.
3. Release the mouse button; the elliptic ROI is now created.

To draw a **circular point** (for example for counting spots):

1. Select the Point toolbar button:
2. Click on the area of the image you wish to mark.
3. The point is now created (see **Figure 4** for an example of multiple points drawn on an image).

To draw a **straight line**:

1. Select the Line toolbar button
2. Move the mouse pointer to the area of the image at which you want to start drawing the line.
3. Hold down the mouse button and drag the mouse pointer to the point you wish to finish the line.

4. Release the mouse button; the line is now created. The length of the line will be shown, along with the angle of the line to the horizontal (see **Figure 5**).

To draw a **connecting line between two ROI shapes**:

1. Select the Line Connection toolbar button

2. Click inside or on the first ROI shape you wish to connect.

3. Click inside or on the second ROI shape you wish to connect.

4. A connecting line will be drawn between the two ROI shapes (see **Figure 6**).

To draw a **freehand line (scribble)**:

1. Select the Scribble toolbar button

2. Move the mouse pointer to the area of the image at which you want to start drawing the line.

3. Hold down the mouse button and drag the mouse pointer to form the line.

4. Release the mouse button; the line is now created.

To draw a **polygon**:

1. Select the Polygon toolbar button

2. Move the mouse pointer to a point on the image at which you want to start drawing the polygon.

3. Hold down the mouse button and drag the mouse pointer to create the polygon shape.

4. Release the mouse button; the polygon is now created.
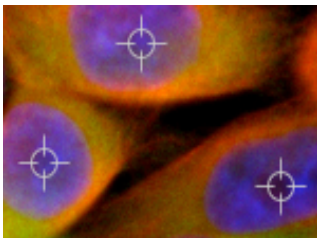
Figure 4: point ROI shapes
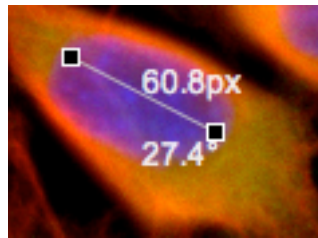
Figure 5: line ROI shape showing length and angle detail
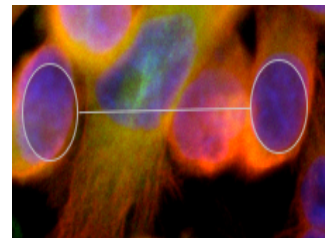
Figure 6: elliptical ROI shapes and connector line

2.3 Adding multiple ROI shapes on one plane

There is an option to allow to quickly add many ROI shapes of the same kind on the same image plane:

- To move to multiple addition mode, open the *Options* menu, select *ROI Creation*, and then select *Create Multiple ROIs* which allows you to add many ROI shapes.

- When you have finished adding shapes, click the **Selection** button to select an ROI shape.

- To return to single addition mode, open the **Options** menu, select **ROI Creation**, and then select **Create Single ROI**.

Alternatively, you can toggle between single and multiple addition mode by right clicking on the ROI shape format button before adding a shape of that format. This will allow you to add multiple ROI shapes of the selected format. To move from multiple addition mode to Selection mode you will have to click the **Selection** button as above.

2.4 Creating an ROI across z-sections and timepoints

ROI shapes, once drawn on a single plane, can be propagated (copied) across multiple z-sections or timepoints to create a multi-dimensional ROI.

To propagate an ROI shape:

1. Select the ROI shape to be propagated.

2. Open the ROI Assistant by selecting the ROI Assistant button

3. The ROI Assistant shows the timepoints and z-sections in which the selected ROI shape currently appears with the symbol of the ROI shape's type (see **Figure 7**, which shows an elliptical ROI shape)

4. Click on ROI shape symbol, and drag it across the z-sections or timepoints into which you wish the shape to be copied.

5. Selected timepoints or z-sections will be highlighted (see **Figure 8**).

6. Make sure the **Add ROI** radio button is selected.

7. Select the **Close** button.

8. The ROI shape, and any accompanying information, including annotations (see **Section 3**), will now be propagated across the selected z-sections or timepoints.

The ROI Assistant can also be used to remove ROI shapes from selected z-sections or timepoints, by following the instructions above, but selecting the **Remove ROI** radio button.
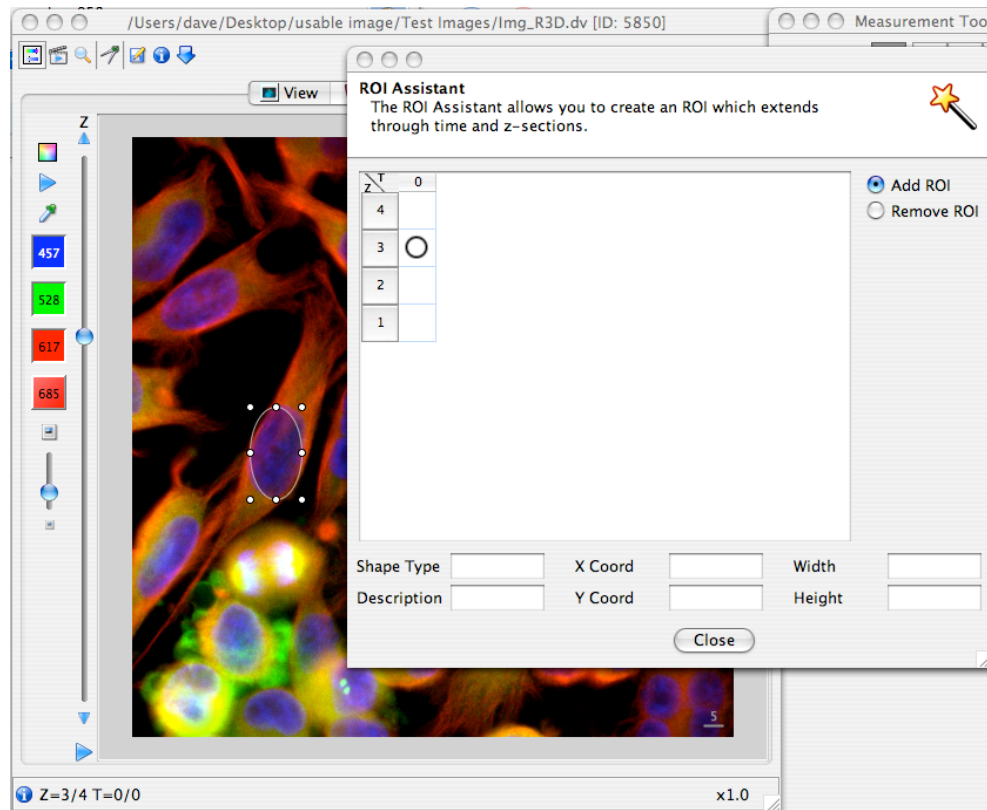
Figure 7: Propagating ROI shapes (e.g., an elliptical ROI shape) with the ROI Assistant
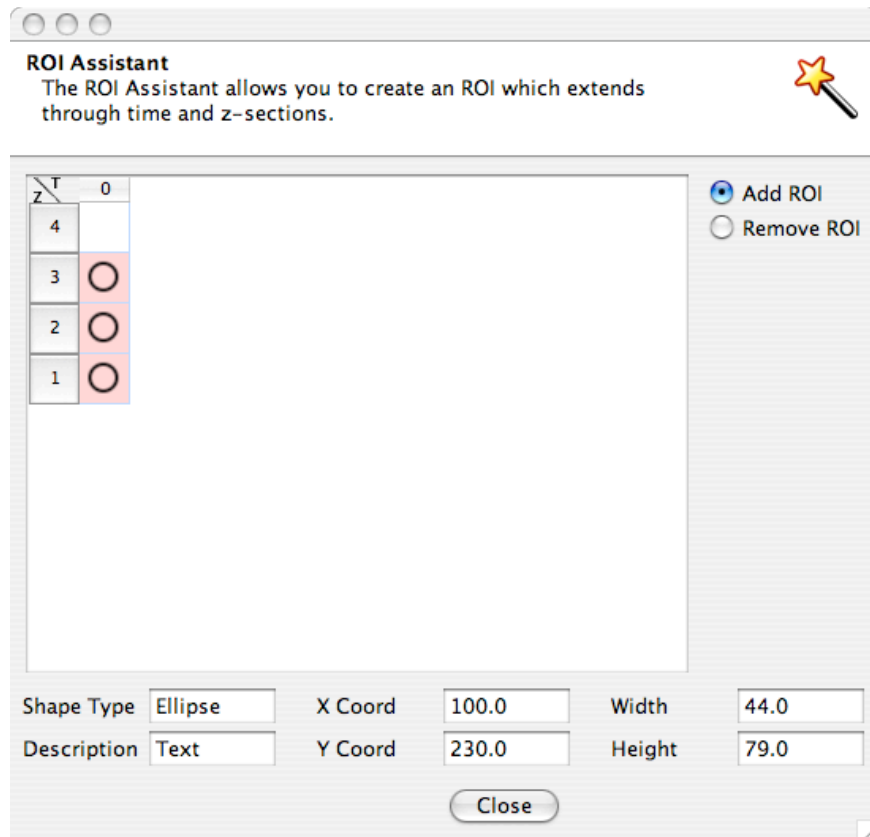
Figure 8: Propagating ROI shapes - selecting z-sections as propagation destinations

3   Saving and loading ROIs

ROI data need to be saved manually in order to be able to recall it in future. This is because ROIs and associated date are saved separately to the image itself, and ROI information is currently not saved automatically in OMERO.

3.1 Saving ROIs

To save all ROI information for an image:

1. Select the Save button

2. Choose the location for the saved ROI file using the file browser window that appears.

3. Select *Save*.

NB If you try to close the Measurement Tool or Image Viewer with unsaved ROI information, a prompt will ask you whether or not you wish to save this information.

3.2 Loading ROIs

To view ROIs associated with an image:

• Open the image in Insight's Image Viewer.

- ROIs associated with the image will be automatically loaded, so long as they were created on the same computer used to view them.

4 Editing ROIs

4.1 Selecting ROIs

There are a number of ways to select one or more ROI shapes for editing.

Using the Image Viewer window:

- To select a single ROI shape on a single plane, click on the ROI shape in the Image Viewer window.

- To select multiple ROI shapes on a single plane, right click on one ROI shapes, and select **Select All** from the menu that appears.

- To select only ROI shapes of the same type, right click on one ROI shapes, and select **Select Same** from the menu that appears.

Using the ROI Manager:

- Open the Manager view (see **Figure 9**), and select an ROI shape by clicking on it.

- To select multiple ROI shapes, hold down the Shift key while clicking.

Figure 9: ROI Manager window

For more on editing ROIs (groups of ROI shapes), see **Section 4.6**.

4.2 Annotation and labelling

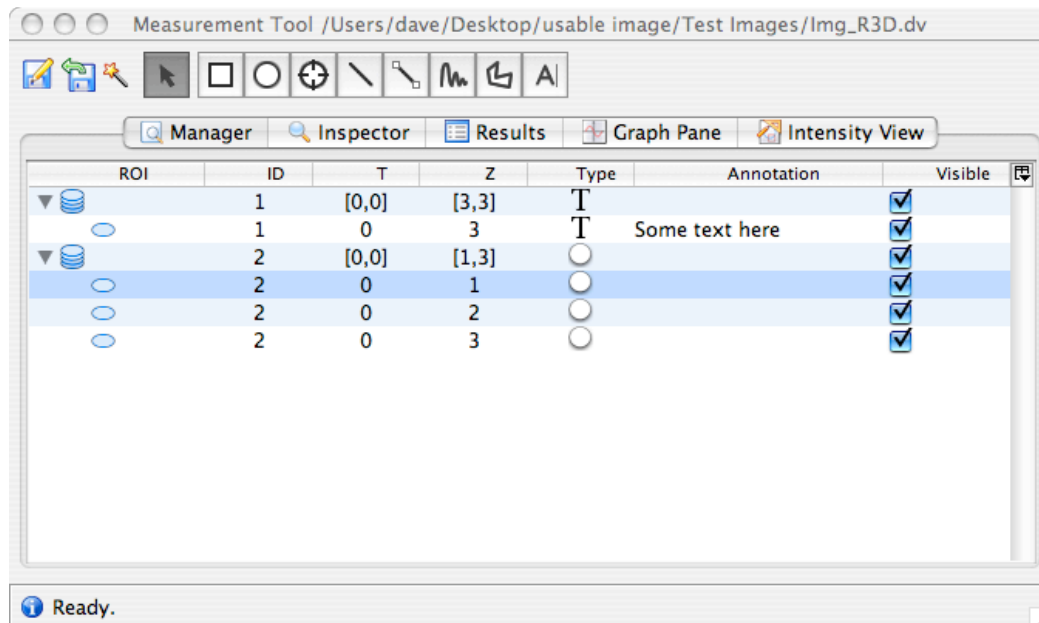You can add a text annotation to an existing ROI shape; or label an area of the image directly by adding a text ROI.

4.2.1 Annotating an existing ROI shape

Separate to text ROIs, an existing ROI shape can be annotated in one of three ways:

1. Double-click on the shape; you can either edit the current annotation text or add new ones;

2. Use the ROI Manager:

    i.   Open the ROI Manager.

    ii. Select the shape to be annotated: the selected ROI shape will be highlighted.

    iii. Type the annotation; the annotation will appear as you type in the Annotation column.

    iv. The annotation will appear within the ROI shape.

3. Use the ROI Inspector:

    i.   Select the shape to be annotated - the selected ROI shape will be highlighted.

    ii. Open the ROI Inspector.

    iii. Double click on the Annotation field.

    iv. Edit the existing text, or add new texts.

See details of adjustments that can be made to the appearance of the annotation text in **Section 4.4.1**.

4.2.2 Labelling Images

To add a text label ROI shape:

1. Select the Text toolbar button

2. Click on the area of the image in which the label is to be added.

3. A text box with the word 'text' will appear.

4. Double click on the text box to add the desired label text.

5. The label is now shown – resizing the area of the text label will adjust the size of the text.

Note that the text label will exist as an ROI with a single shape, and appear as such in the ROI Manager, Inspector and Results views. Double-clicking on the text ROI in the ROI Manager and Inspector allows you to edit the text.

4.3 Moving or resizing a single ROI

Once an ROI shape has been created, it is possible to move or resize it as required.

4.3.1 Moving a single ROI shape

To adjust the position of an ROI shape:

1. Select the Selection toolbar button:

2. In the Image Viewer window, move the mouse pointer to a point *inside* the ROI shape you wish to move.

3. Hold down the mouse button – the mouse pointer will change to the drag cursor.

4. Drag the shape to the desired location.

5. Release the mouse button.

4.3.2 Resizing a single ROI shape

To adjust the shape of an ROI shape:

1. Select the Selection toolbar button

2. In the Image Viewer window, move the mouse pointer to the border of the ROI shape you wish to move.

3. Hold down the mouse button – the mouse pointer will change to the resize cursor.

4. Adjust the ROI shape to the desired appearance.

5. Release the mouse button.

NB For ROI shapes propagated across multiple z-sections or timepoints, resizing an ROI shape will only change the shape on the one plane, **not** all z-sections or timepoints.

4.4 Changing the appearance of an ROI shape

Various changes to the visual display of a selected ROI shape can be made through the ROI Shape Inspector (see **Figure 10**).
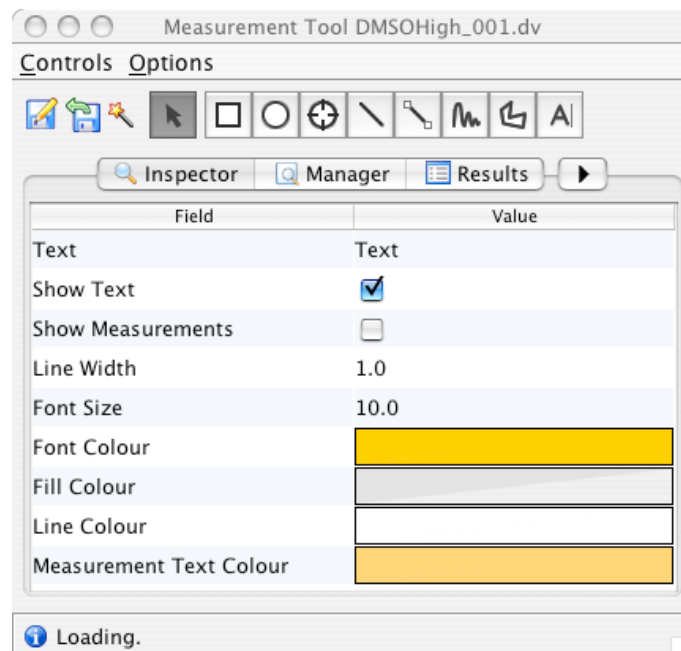
Figure 10: ROI shape Inspector view

To bring up the Inspector view adjust the appearance of an ROI shape:

1. Select the ROI shape, either by clicking on it in the Image Viewer window, or by selecting it in the ROI Manager or ROI Results view.

2. Select the Inspector tab.

3. Current display settings for the ROI shape will be displayed.

4.4.1 Adjusting display of annotation text

The following adjustments can be made to the ROI shape's annotation text:

- Click the *Show Text* checkbox to turn on/off annotation text.

- Click the *Show Measurements* checkbox to turn on/off measurement text.

- Change the size of the annotation text by selecting the *Font Size* menu and selecting the appropriate value.

4.4.2 Adjusting display of the ROI shape

The following adjustments can be made to the appearance of the ROI shape itself:

- Change the width of the ROI shape's line by selecting the *Line Width* menu and selecting the appropriate value.

- Change the colour of:
  - o the ROI shape's text.
  - o the ROI shape's fill (for polygonal ROI shapes)
  - o the ROI shape's boundary line.
  - o the ROI shape's measurement text.

To change a colour:

1. Double-click the appropriate coloured area in the Inspector window.

2. In the colour picker window that appears, select the appropriate colour from the options displayed.

3. Adjustments can be made to the Alpha value by using the slider.

4. Select *Accept* to make the change.

Annotation text is also shown here, but cannot be edited.

4.4.3 Hiding ROI shapes

To hide selected ROI shapes from display:

1. Open the ROI Manager (see **Figure 9**).

2. For the ROI shapes to be hidden, uncheck the *Visible* checkbox.

Repeating the above actions will make currently hidden ROI shapes visible again.

4.5 Deleting a single ROI shape

To delete an individual ROI shape:

1. Select the shape to be deleted by clicking on the shape in the Image Viewer window (for rectangles, ellipses and polygons, clicking inside the shape will select it).

2. Then

- Either press the Delete key on your keyboard to delete the selected shape;

- Or open the ROI Manager, right click on the selected ROI shape, select *ROI Management Options* and then *Delete*.

4.6 Editing multiple ROI shapes and ROIs

You can select whole ROIs, including all constituent ROI shapes, or selected ROI shapes for editing:

1.   Right-click on the ROI Manager.

2.   Select *ROI Management Options*.

This gives access a menu that allows you a number of options:

- *Duplicate* the selected ROI or ROI shape. A copy of the selected ROI or ROI shape will appear in the ROI Manager as a new ROI.

- *Merge* two selected ROIs (with constituent ROI shapes) into a single ROI

- *Split* – removing selected ROI shapes from their parent ROI and putting them into a new ROI.

- *Propagate* – propagating the selected ROI or ROI shape as described in

- *Delete* the selected ROI or ROI shapes.

5 Viewing ROI information

A range of information relating to the ROI shapes present in the selected image can be viewed.

5.1 Viewing summary ROI information

A summary information for all ROIs of an image can be revealed by clicking on the *Manager* tab.

All ROIs and constituent ROI shapes are listed (see **Figure 9**).

indicates an ROI

indicates an ROI shape.

For each ROI/ROI shape, the following essential information is shown:

- For ROIs, all constituent ROI shapes (this will be the case where an ROI shape has been propagated across z and/or t to create a multi-dimensional ROI).

- The unique ID number of the ROI/ROI shape.

- For ROIs, the timepoints and/or z-sections across which it exists.

- The type of ROI shape (one of Rectangle, Ellipse, Point, Line, Connector, Polygon or Text) is indicated by the appropriate symbol.

- Annotation text.

- Whether or not the ROI/ROI shape is currently visible.

To adjust the amount of data shown in the ROI Manager, the display can be adjusted in the following ways:

- ROIs can be expanded to show constituent ROI shapes by clicking on the grey 'Expand' arrow to the left of the ROI: ▶

- To collapse an expanded ROI, select the grey 'Collapse' arrow: ▼

5.2 Measurement results

The ROI Results View shows summary measurement results for all ROIs and ROI shapes (see **Figure 11**). It also allows you to configure which data should be included in any results data file to be saved for exporting to other applications.
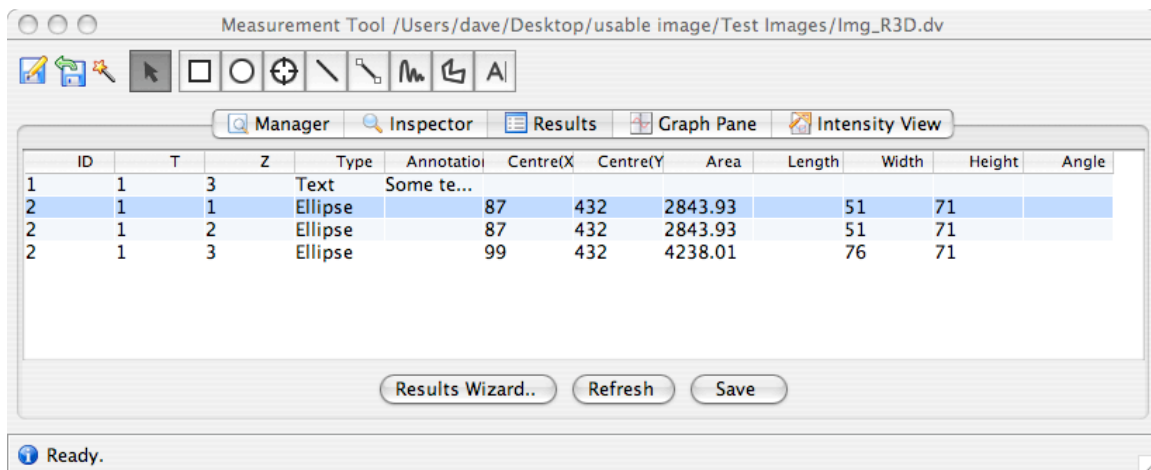
Figure 11: Results view

5.2.1 Showing summary results

The data displayed by default in the Results view includes:

- Unique ID of the ROI shape;

- The T-section in which the ROI appears;

- The z-section in which the ROI appears;

- Type of ROI shape;

- Annotation text;

- x and y co-ordinates of the centre of the ROI;

- The area of the ROI (if a rectangle, ellipse or polygon) in square microns or pixels (depending on the units specified in the **Options** menu);

- The length of the ROI (if a line);

- The width and height of the ROI (if a rectangle, ellipse or polygon);
- The angle of the ROI from the horizontal (if a line).

In addition, it is possible to view:

- The length of the perimeter of the ROI (if a rectangle, ellipse or polygon);
- x,y values for the start co-ordinates of the ROI;
- x,y values for the end co-ordinates of the ROI;
- x,y values for all constituent co-ordinates of the ROI.

The specific data values displayed in the Results view can be configured by selecting the Results Wizard button (see **Figure 12**).

It may be necessary to use the scrollbar to reveal all columns of data; alternatively the window can be resized to show all data.
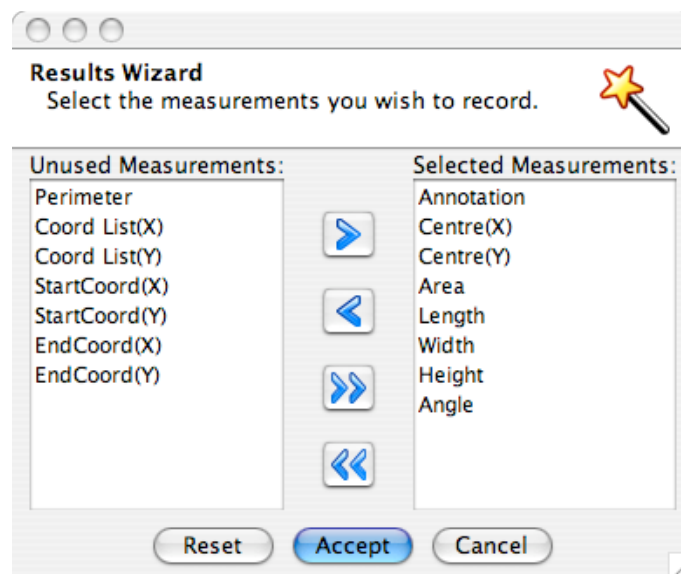

Figure 12: The Results Wizard

5.2.2 Refreshing summary data

If any changes are made to the image's ROIs – for example by resizing them, adding new ROI shapes or deleting existing ROI shapes, the data shown in the Results view will not be displayed unless being refreshed through the *Refresh* button.

5.2.3 Saving summary data

To save the currently displayed data:

- Select the *Save* button.
- Specify the location of the file to be saved.
- Choose the format for the saved data. At present this is limited to Comma Separated Variable (CSV) format.
- Select *Save*.

5.3 Viewing pixel intensity values

Pixel intensity values for an individual ROI shape can be viewed graphically or as figures in tabular format.

5.3.1 Graphical view

Selecting the Graph Pane tab shows intensity values as a histogram. Additionally, a line profile will be shown for line ROI shapes (see **Figure 13**).
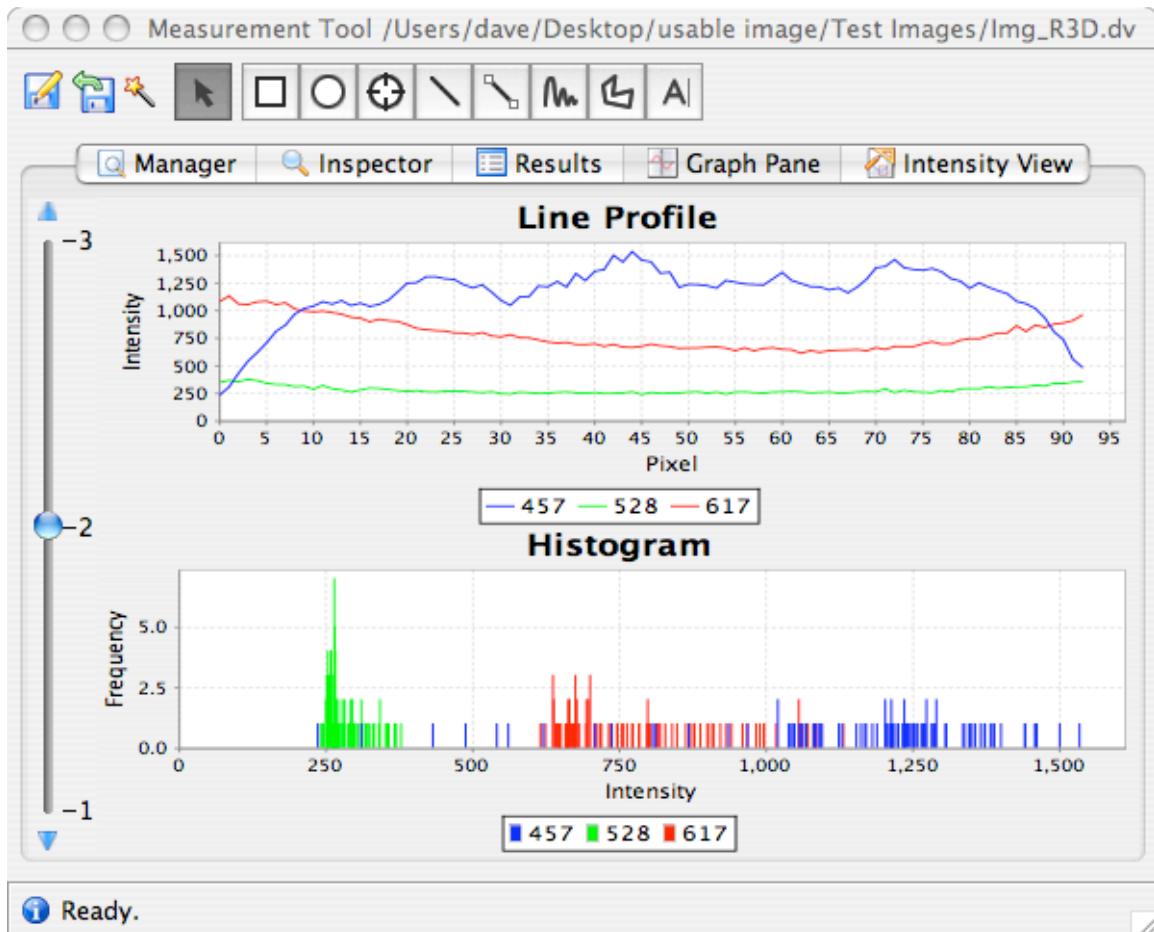

Figure 13: Graph pane view of pixel intensities

If the ROI shape is moved or resized, or another ROI shape is chosen, the histogram and line profile (if shown) will be automatically updated to reflect these changes.

5.3.1.1 Configuring the Graph view

To change the magnification of the Graph view, right click on the histogram or line profile. This brings up a menu that allows you to:

- Apply a 'zoom in' or 'zoom out' effect to the data by changing the visible values of the x and/or y axes, increasing or reducing the range of values shown in the graph.

- Apply an automatic scaling effect so that the ranges of the x and y axes accommodate exactly the maximum and minimum values of the intensity data of the ROI shape in question.

If the selected ROI shape is part of a multidimensional ROI, slider bars will be provided to allow you to move through each timepoint and/or z-section, in a similar way to the Image View window. In the example shown in **Figure 13**, the vertical slider bar to the left of the figure allows you to change the z-section for which the intensity data should be shown.

5.3.1.2 Saving graphical views

The graphs shown can be saved as images:

- Right click on the graph to be saved
- Select the *Save as...* option in the menu that appears.
- Choose the location of the image file to be saved
- Select the *Save* button.

5.3.2 Intensity View

Selecting the Intensity View tab shows intensity data for the selected ROI shape as a table of values (**Figure 14**).
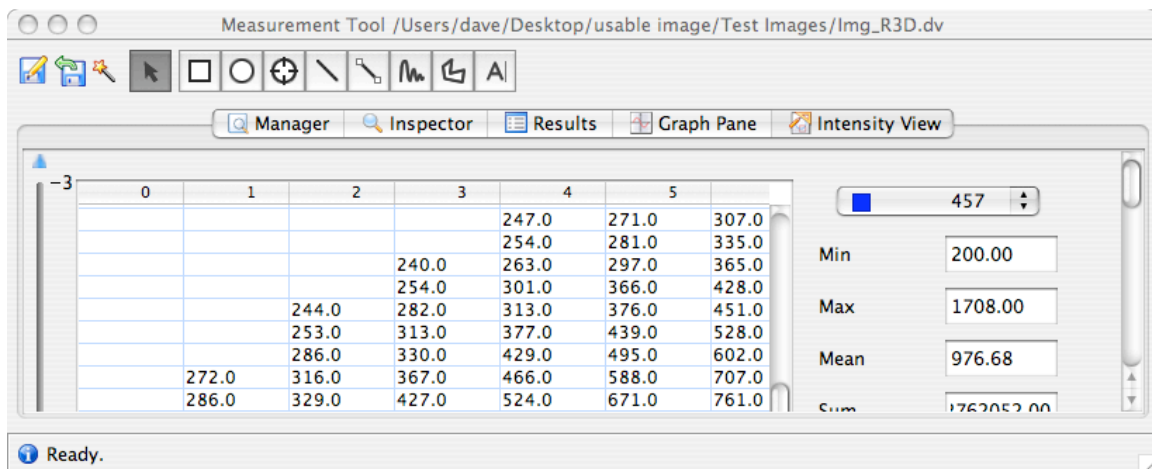

Figure 14: Intensity view of pixel intensities

The Intensity View window shows intensity values for a selected channel. The selected channel can be changed using the menu towards the top right of the window.

It also shows summary intensity data, to the right of the tabular display of intensities, including:

- Minimum intensity value;
- Maximum intensity value;
- Mean intensity value;
- Sum of all intensity values;
- Standard deviation;

- X and Y co-ordinates of the ROI shape;
- Width and height of the ROI; and
- X and Y co-ordinates of the centre of the ROI shape.

If the ROI shape is moved or resized, or another ROI shape is chosen, the values will be automatically updated in the Intensity View window.

If the selected ROI shape is part of a multidimensional ROI, menus will be provided to allow you to move through each timepoint and/or z-section. In the example shown in **Figure 14,** the menu to the left of the figure allows you to change the z-section for which the intensity data should be shown.

Results shown in the Intensity View can be saved in a similar way to the Results view, by selecting the *Save* button at the bottom right of the Intensity View window.

6 Future additions

A number of additional features are planned to be added to the Measurement Tool. These include the following:

- The ability to treat ROIs as volumes for measurement;
- Enhanced management of annotations; multiple text annotation for ROI shapes;
- The ability to assign relationships between ROIs;
- The ability to assign categories to ROIs (other than text annotation);
- Automatic creation of ROIs around cells and objects;
- Lineage management; and
- Enhanced measurement.