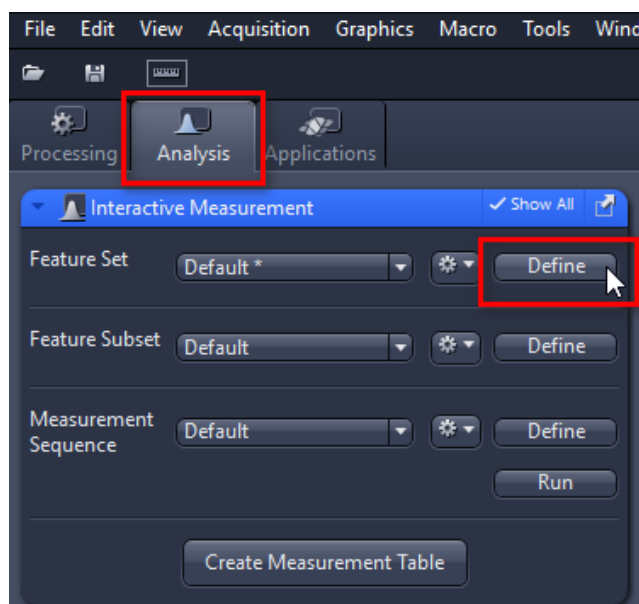
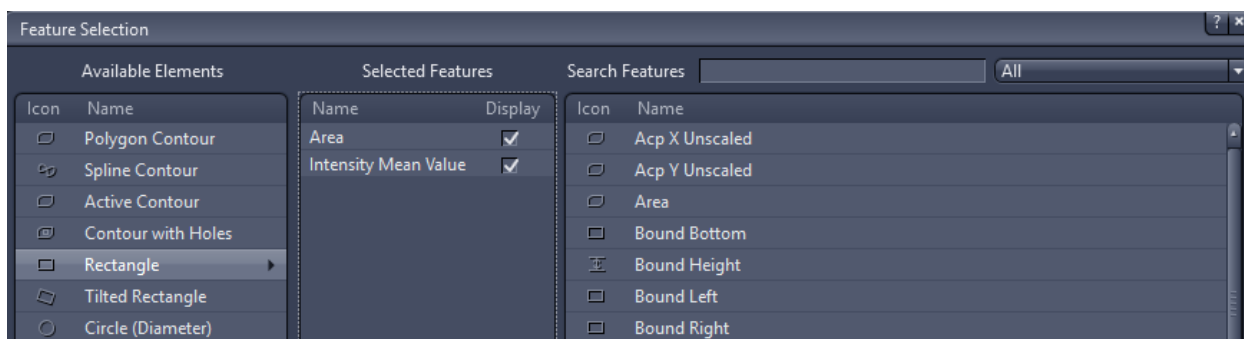


This analysis technique will allow you to acquire measurement data over time without utilizing the “MeanROI” tab. You will also be able make other measurements within a region of interest (ROI) in addition to mean intensity.

Begin by clicking on the “Analysis” tab in the top left corner of the ZEN application window. Then click on the “Define” button in the “Feature Set” subheading.

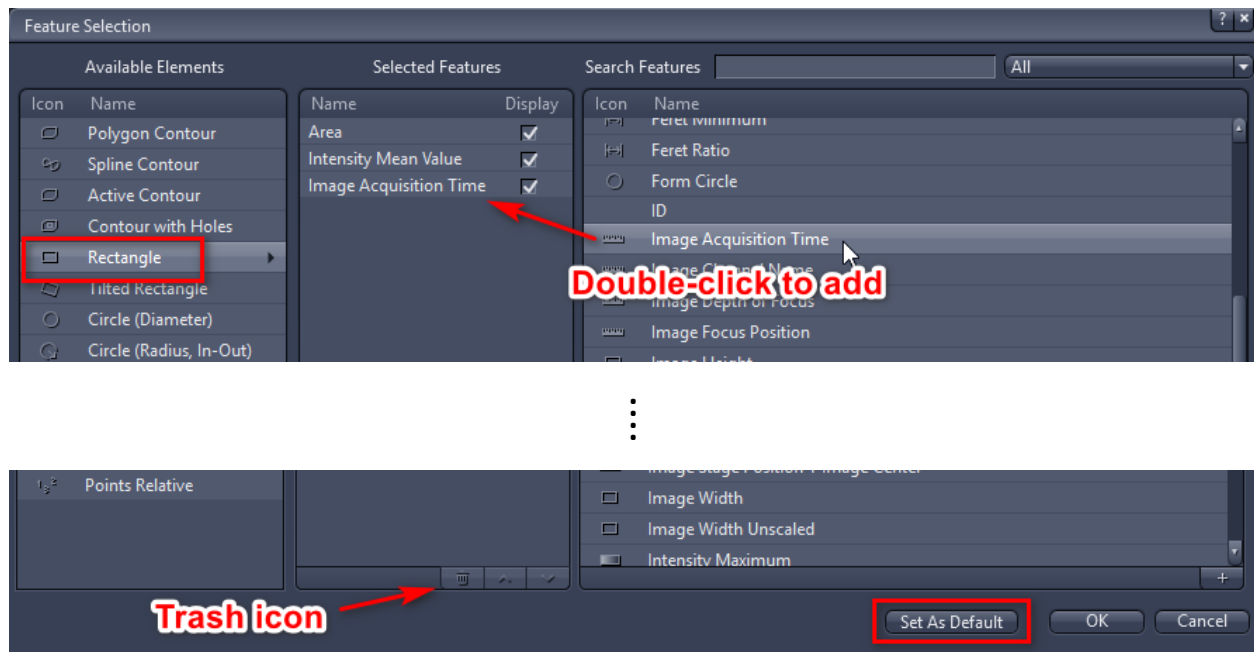


This will bring up a large dialog box allowing you to choose the measurement “features” for each ROI that is drawn on the image. The leftmost column allows you to select the “element” or ROI tool that you wish to modify. These are the ROI tools you can choose from the “Graphics” tab on the bottom of the image. Each ROI tool has its own set of measurement features that are listed in the middle column. You can add additional features from the list in the rightmost column.



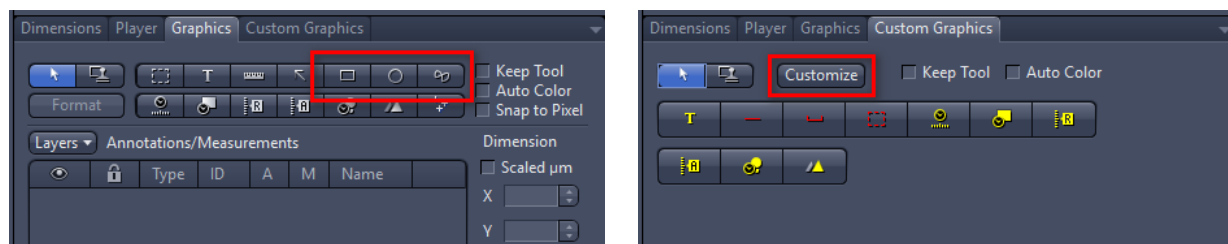
For your purposes, you want to add an “absolute time” measurement feature to the particular ROI tool you will use to draw on the dataset. First, choose the ROI tool from the leftmost column, in this example, the “Rectangle” element. Now scroll down to “Image Acquisition Time” in the rightmost column and double-click it to add it to the rectangle’s selected features list. If you want to remove a selected feature, just highlight it and press the trash icon on the bottom of the window. You can of course add other measurement features if you like.

In this example, whenever a rectangle ROI is drawn on the image, it will calculate the rectangle’s area, mean intensity in each channel and the date and time each timepoint was acquired.



If you press the “Set As Default” button, then the selected measurement features will be saved for each ROI tool so that they will be available the next time you start ZEN. Press “OK” to close the window.

Below the image, click on the “Graphics” tab. Basic ROI tools are available in this tab, such as rectangle, circle/ellipse and freehand. If you want to access other ROI tools, you can add new ones to the “Custom Graphics” tab by pressing the “Customize” button. New elements will be added to the toolbar.



Press the “Measure” tab on the lefthand side of the image. Under the “Graphics” or “Custom Graphics” tab, choose an ROI tool and draw the element on the image. In this example, I created two ROIs to simulate a background ROI and a signal ROI.

In the “Measurement” tab below the image, make sure the “Table” and “All Views” buttons are depressed in the “Data Display” subsection. “Table” will make it easier to collate the data in Excel. “All Views” will allow you to display the measurements for all timepoints, not just the current timepoint in the image display. The table next to the image will now calculate the measurement features you specified in the feature set as defined in the first part of this guide.

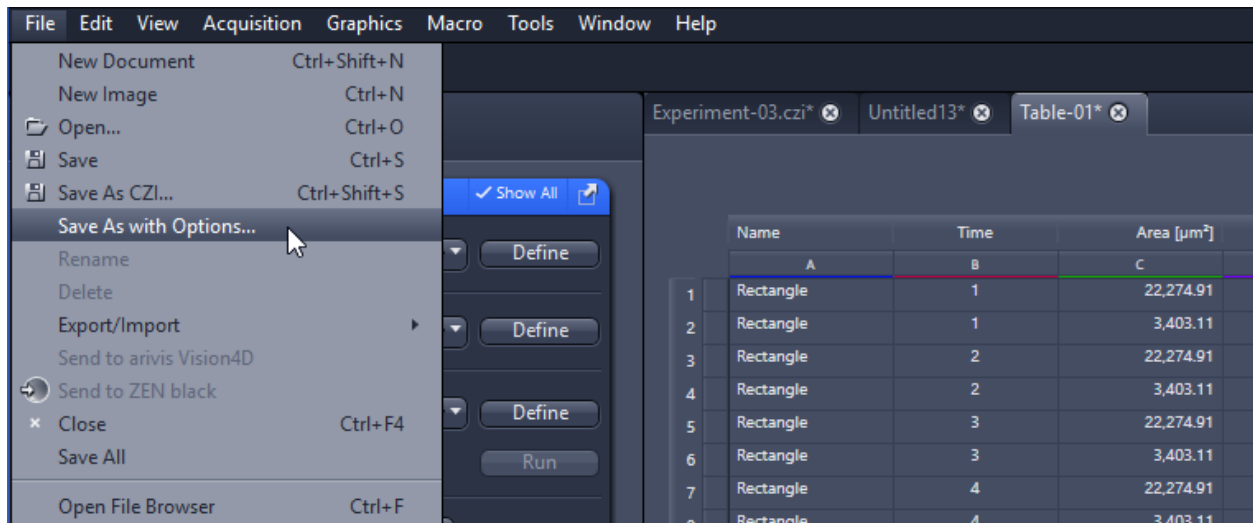
Please note that if you change an ROI’s feature set, all existing ROIs will retain the measurements before the changes were made. Only new ROIs drawn on the image will contain the changes made to the feature set.

Press the “Create Document” button to export the tabular data to another window container in ZEN.

The screenshot shows the ZEN software interface. On the left, the 'Measure' tab is selected in the sidebar. The main window displays a microscopy image with two rectangular ROIs. Below the image, the 'Measurement' tab is active, showing a table of measurements for 20 ROIs. The table has columns for Name, Time, Area [μm²], and various channel measurements. The 'Data Display' subsection is visible, with 'Table' and 'All Views' buttons selected. A red arrow points to the 'Create Document' button with the text 'Click here to export the table'.

Name	Time	Area [μm²]	Channel_1_CF...	Channel_2_YF...	Channel_1_CF...	Channel_2_YF...
A	B	C	D	E	F	G
Rectangle	1	23,723.093	2,779.639	48,986.673	8/30/2019 2:22:05...	8/30/2019 2:22:05...
Rectangle	1	23,679.577	3,291.673	59,176.824	8/30/2019 2:22:05...	8/30/2019 2:22:05...
Rectangle	2	23,723.093	2,771.280	48,869.431	8/30/2019 2:22:08...	8/30/2019 2:22:08...
Rectangle	2	23,679.577	3,280.668	59,045.051	8/30/2019 2:22:08...	8/30/2019 2:22:08...
Rectangle	3	23,723.093	2,763.757	48,760.964	8/30/2019 2:22:11...	8/30/2019 2:22:11...
Rectangle	3	23,679.577	3,272.556	58,913.826	8/30/2019 2:22:11...	8/30/2019 2:22:11...
Rectangle	4	23,723.093	2,755.756	48,675.225	8/30/2019 2:22:14...	8/30/2019 2:22:14...
Rectangle	4	23,679.577	3,263.545	58,804.618	8/30/2019 2:22:14...	8/30/2019 2:22:14...
Rectangle	5	23,723.093	2,750.429	48,588.462	8/30/2019 2:22:17...	8/30/2019 2:22:17...
Rectangle	5	23,679.577	3,255.901	58,712.144	8/30/2019 2:22:17...	8/30/2019 2:22:17...
Rectangle	6	23,723.093	2,743.901	48,518.417	8/30/2019 2:22:20...	8/30/2019 2:22:20...
Rectangle	6	23,679.577	3,249.386	58,625.327	8/30/2019 2:22:20...	8/30/2019 2:22:20...
Rectangle	7	23,723.093	2,738.458	48,449.712	8/30/2019 2:22:22...	8/30/2019 2:22:22...
Rectangle	7	23,679.577	3,243.499	58,546.644	8/30/2019 2:22:22...	8/30/2019 2:22:22...
Rectangle	8	23,723.093	2,733.196	48,374.132	8/30/2019 2:22:25...	8/30/2019 2:22:25...
Rectangle	8	23,679.577	3,237.448	58,444.238	8/30/2019 2:22:25...	8/30/2019 2:22:25...
Rectangle	9	23,723.093	2,730.201	48,315.308	8/30/2019 2:22:28...	8/30/2019 2:22:28...
Rectangle	9	23,679.577	3,233.119	58,380.158	8/30/2019 2:22:28...	8/30/2019 2:22:28...
Rectangle	10	23,723.093	2,725.051	48,281.245	8/30/2019 2:22:31...	8/30/2019 2:22:31...
Rectangle	10	23,679.577	3,227.731	58,329.538	8/30/2019 2:22:31...	8/30/2019 2:22:31...

To save the table, click on the window container with the exported data and go to “File” -> “Save As with Options...”



The “Save As...” dialog box will allow you to save the data as a .csv file for import into Excel.

