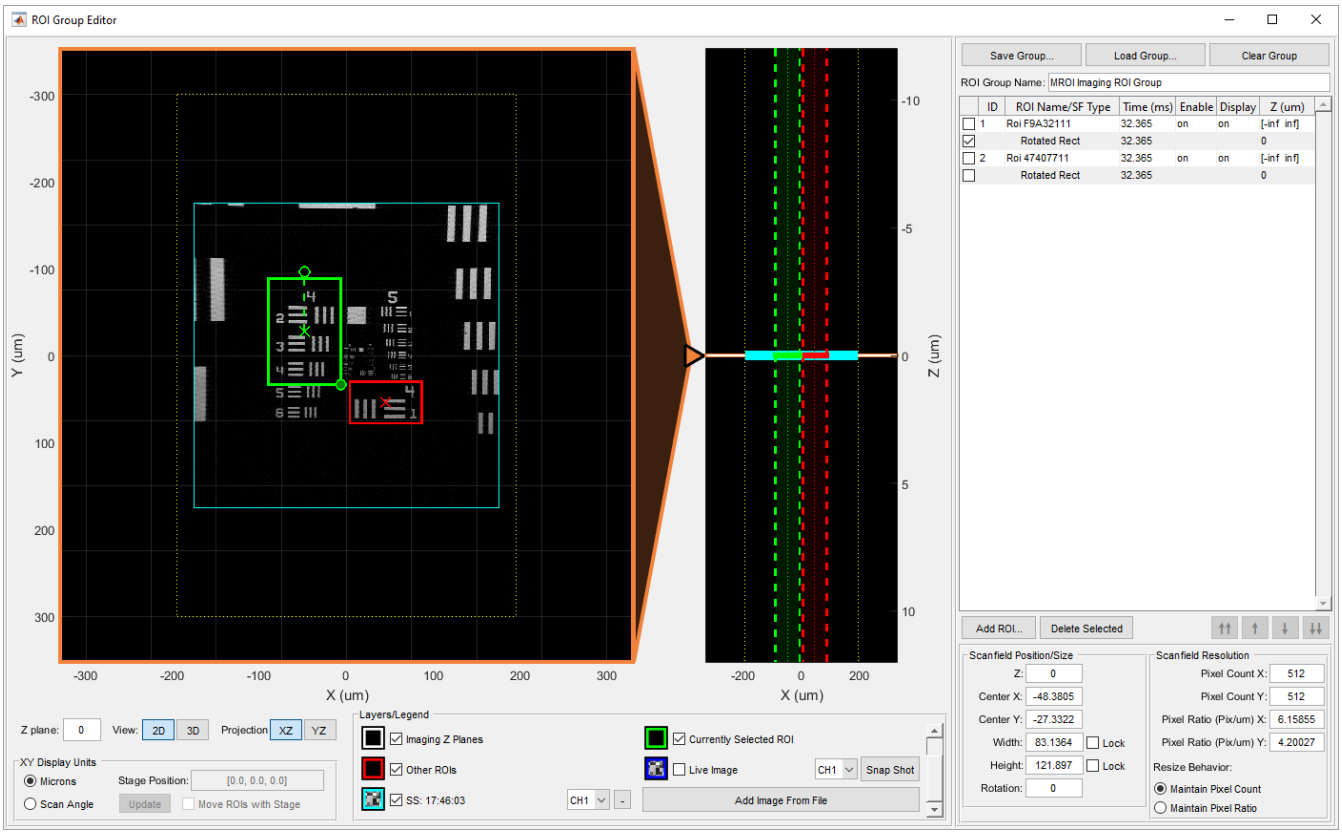
ROI/Stimulus Group Editor



Regions of Interest/Stimulus Editor Panel

Overview

The **ROI/Stimulus Group Editor** is used for defining ROI's for imaging, photostimulation, and image analysis. The interface facilitates designing 3D regions and scan paths using collected images as a reference.

Imaging ROI groups consist of one or more 3D ROIs, which are defined by 2D scanfields. You can find a complete description of multiple region of interest imaging in the following concepts guide articles:

- Multiple Region of Interest (MROI) Imaging
- Scanfields, ROIs, ROI Groups

Stimulus groups are special cases of ROI groups where scanfields are parametric 2D stimulation paths instead of fields to raster scan. More details about photostimulation and stimulus groups can be found in the following articles:

- Photostimulation
- Creating Stimulus Groups

ROI Display

The main display contains a number of different elements identified by the legend at the bottom. The display can be switched between 2D

and 3D viewing modes. In 2D mode the main display shows data at the current Z position with an accompanying side projection display at the right. The side projection can be switched between XZ and YZ modes with the controls below. The pointer between the main view and the projection view can be used to move the z plane which the main view is currently showing. The main view and side projection view both support mouse click and drag to pan and mouse wheel zoom. If you hold shift while using the mouse wheel (or if the mouse is hovering over the z control slider between the main and projection view) it will move the current Z position. The ROI display includes the following elements, all of which can be shown/hidden using the check boxes in the legend.

Scanner Field of View	This shows a representation of the XY area that the scan mirrors are capable of addressing. All ROIs must stay within these boundaries
Editor Z Plane	In the side projection view, this shows an indication of what point in Z the main view is currently displaying
Imaging Z Planes	In the side projection view, these lines indicate where images will be taken using the current stack/volume acquisition settings
Currently Selected ROI	The ROI/scanfield currently selected for editing ⓘ In the main view, you can click and drag the selected scanfield to move it in XY, resize it using the handle in the bottom right corner, and rotate it using the handle on the top. In the projection view you can click and drag to move the selected scanfield in Z. ⓘ For imaging and analysis ROI groups, the ROI at a given plane can either be defined or interpolated between two defined scanfields. If there is a scanfield defined at the current Z for the selected ROI, it will show in the main view with a solid border. If the scanfield at the current Z has been interpolated, it will show with a dashed border. For stimulation patterns, normal stimulus functions will show with a solid line. Transition and park functions will show with a dashed line.
Other ROIs	Other non-selected ROIs in the current ROI group ⓘ For imaging and analysis ROI groups, the ROI at a given plane can either be defined or interpolated between two defined scanfields. If there is a scanfield defined at the current Z for the selected ROI, it will show in the main view with a solid border. If the scanfield at the current Z has been interpolated, it will show with a dashed border. For stimulation patterns, normal stimulus functions will show with a solid line. Transition and park functions will show with a dashed line.
Live Image	A context image that updates in real time from the currently active video stream. The drop down allows selecting which channel to display and the **Snap Shot** button allows capturing the current context image
Additional Context Images	Additional context images that have been added to the view either by taking a snap shot of the live image or by loading a context image from a file

In addition to the legend, the bottom panel contains additional view controls:

Z plane	Indicates what point in Z the main view is currently displaying
View (2D/3D)	Switches the main view between 2D and 3D modes
Projection (XZ/YZ)	Select the XY dimension used for the side projection view
XY Display Units (Microns, Scan Angle)	Select the units used for XY coordinates throughout the interface ⓘ The base coordinate system ScanImage uses is in angular units (degrees). This translates directly to the output that will be sent to the scan mirrors. When units are displayed in microns, the objective resolution from the first section of the machine data file is used as a conversion from angle to position.

ROI Group Table/ROI Group Controls

The right hand side of the interface displays information about the ROI group currently being edited. The controls at the top operate on the entire ROI group:

Save Group	Save the current ROI group to a *.roi file ROIs are stored in the configuration file along with the rest of the ScanImage configuration. This option allows you to store ROI groups separately in order to easily access them without loading an entire configuration.
Load Group	Clears the contents of the ROI group that is currently being edited and replaces them with the ROIs from the selected file.
Clear Group	Clears the contents of the ROI group that is currently being edited
ROI Group Name	Specifies a name for the current ROI group For stimulus patterns, this is the name that will show up in the list in the PHOTOSTIMULATION CONTROLS interface

The ROI table displays information about all the ROIs in the ROI group and all the scanfields within each ROI. This table displays different information depending on whether you are currently editing an imaging ROI group, stimulation pattern, or analysis ROI group.

The checkbox on the leftmost column in the table indicates which item in the table is currently selected

Imaging ROI Table
For each ROI in the group and each scanfield in each ROI, the following information is listed in the table

ID	Numeric identifier of the ROI
ROI Name/Scanfield Type	For ROIs, the name. If a name has not been specified, a randomly generated unique identifier is used. For scanfields, type type of field
Time	For ROIs, the time required to scan the largest scanfield in the ROI. For scanfields, the time required to image the field. This value is displayed in milliseconds
Enable	For ROIs, indicates if the ROI is enabled for imaging. When disabled the ROI will not be scanned
Display	For ROIs, indicates if the ROI will be shown in the display window. When disabled, the ROI will still be scanned but will not be displayed in the live image
Z	For ROIs, indicates the range over Z that the ROI spans. For scanfields, indicates the Z position of the scanfield.

Stimulation Pattern Table
For each stimulus function in the pattern, the following information is listed in the table

ID	Numeric identifier of the stimulus pattern
Stimulus Function	Selected stimulus function
Time	The duration specified to output the stimulus function
Repetitions	The number of times to repeat the stimulus function
Power	The laser power speficied for the stimulation function
Z	The Z position of the stimulation function

Analysis ROI Table
For each ROI in the group and each scanfield in each ROI, the following information is listed in the table

ID	Numeric identifier of the ROI
Analysis Type	The type of analysis to be performed Currently only integration is supported
Channel	The channel on which to perform the analysis
Threshold	The threshold to compare the integration with for output of a logic state. Only evaluated when Processor is set to FPGA
Processor	The processor used to perform the analysis Currently only CPU is supported
Z	For ROIs, indicates the range over Z that the ROI spans. For scanfields, indicates the Z position of the scanfield.

ROI Table Controls
Below the ROI table, there are controls for adding, removing, and reordering ROIs

Add ROI	Opens the **New ROI** Panel detailed in the next section
Delete Selected	Deletes the selected item in the table
Move to Top	Moves the selected ROI to the top of the list
Move Up	Moves the selected ROI up in the list
Move Down	Moves the selected ROI down in the list
Move to Bottom	Moves the selected ROI to the bottom of the list

New ROI Panel

To access the new ROI panel, click the **Add ROI** button or use the hotkeys detailed in a later section. With the **New ROI** panel open there are multiple ways to create a new ROI. You can click and draw a ROI in the main view, create using position and size entered in the text fields, or use the cell picker.

Drawing ROIs in the Main View
To draw an ROI in the main view, select **Top Left Rectangle** or **Center Point Rectangle** depending on how you want to draw the ROI. Click and drag in the main view to draw the ROI in the desired location. If you click and release without dragging, an ROI will be created at the position you clicked using the default size entered in the new ROI panel. If the lock checkbox is selected for either width or height, the corresponding dimension will be constrained to the entered value while dragging. After drawing the ROI, if the **Draw Multiple** checkbox is off, the new ROI will be selected and the **New ROI** panel will close to be replaced by the **Selected ROI** panel showing propertied of the ROI that was just created. If the **Draw Multiple** checkbox is on, the **New ROI** panel will stay open allowing you to quickly draw multiple ROIs.

Create ROI Using the Entered Position
To create an ROI by entering the position, **Top Left Rectangle** or **Center Point Rectangle** must be selected. You can then manually enter values for center position, width, height, and rotation. After entering the desired position, click the **Create Using Defaults** button to create the ROI. After creating the ROI, if the **Draw Multiple** checkbox is off, the new ROI will be selected and the **New ROI** panel will close to be replaced by the **Selected ROI** panel showing propertied of the ROI that was just created. If the **Draw Multiple** checkbox is on, the **New ROI** p anel will stay open allowing you to quickly add multiple ROIs.

Create ROI using the Cell Picker
Using the cell picker is detailed in a later section.

Additional Parameters
In addition to the position and size, additional parameters for the new ROI can be specified on the right hand side of the **New ROI** panel. The available parameters depend on what kind of ROI group is being edited.

Imaging ROI Parameters
For imaging ROIs, the pixel resolution can be specified. This can be done in terms of pixel count or pixel ratio. If pixel ratio is used, the pixel

count will be set by the size of the new ROI.

Stimulus Function Parameters
For stimulus groups, the following parameters can be specified:

Function	The parametric function that defines the scan path for this stimulus function
Function Arguments	Custom arguments to use as parameters for the parametric function
Duration	Duration of the function
Repetitions	Number of times to repeat the function
Beam Power	Laser power to use while outputting the function

Analysis ROI Parameters
For analysis ROIs, the following parameters can be specified:

Channel	The channel to perform the analysis
Threshold	The threshold to compare the integration with for output of a logic state
Processor	The processor used to perform the analysis Currently only CPU is supported

Selected ROI/Scanfield Properties Panel

If there is an item selected in the ROI table, the bottom right panel will show properties of the selected item. The following properties will be available for the indicated type of selected item:

Imaging ROI

ROI Name	Display name for the ROI
Unique ID	Unique identifier for the ROI assigned at creation
Enabled	Indicates if the ROI is enabled for imaging. When disabled the ROI will not be scanned
Show in Image Display	Indicates if the ROI will be shown in the display window. When disabled, the ROI will still be scanned but will not be displayed in the live image
Discrete Plane Mode	Indicates if the ROI should be a discrete plane ROI
Number of Scanfield Control Points	Indicates number of scanfields in the ROI
ROI Minimum Z	Indicates the minimum Z position contained by the ROI
ROI Maximum Z	Indicates the maximum Z position contained by the ROI
Edit/Add Scanfield At Current Z	If there is a scanfield defined for this ROI at the current editor Z plane, this button will select it to show/edit its properties. If there is not a scanfield at the current Z plane, this button will add one to the ROI and select it for editing
ROI Specific Power Controls	These parameters allow specifically controlling the power of an ROI. If any of the values are unset, the parameters from the POWER CONTROLS interface are used like normal
Powers	Laser power. If there are multiple pockels cells, a value for each should be entered separated by commas
Enable P/z Adjust	Enter 1 or 0 (to indicate on or off) to control whether power/depth adjustment is enabled for this ROI
Length Constants	Length constant for each pockels cell for power/depth adjustment

Imaging Scanfield

Z	Z position of the scanfield
Center X/Y	The position of the center of the scanfield
Width/Height	The size of the scanfield
Rotation	The rotation angle of the scanfield
Pixel Count (X/Y)	Pixel count for the scanfield
Pixel Ratio (X/Y)	Ratio of pixels to scanfield size
Resize Behavior	Indicates whether pixel count or pixel ratio should be held constant as ROI is resized

Stimulus Function

Z	Z position of the scanfield
Center X/Y	The position of the center of the scanfield
Width/Height	The size of the scanfield
Rotation	The rotation angle of the scanfield
Function	The parametric function that defines the scan path for this stimulus function
Function Arguments	Custom arguments to use as parameters for the parametric function
Duration	Duration of the function
Repetitions	Number of times to repeat the function
Beam Power	Laser power to use while outputting the function

Analysis ROI

ROI Name	Display name for the ROI
Unique ID	Unique identifier for the ROI assigned at creation
Enabled	Indicates if the ROI is enabled for analysis. When disabled the ROI will not be processed
Show in Integration Display	Indicates if the ROI will be shown in the integration display window. When disabled, the ROI will still be processed but will not be displayed in the live integration trace
Discrete Plane Mode	Indicates if the ROI should be a discrete plane ROI
Channel	The channel on which to perform the analysis
Threshold	The threshold to compare the integration with for output of a logic state
Processor	The processor used to perform the analysis Currently only CPU is supported
Number of Scanfield Control Points	Indicates number of scanfields in the ROI
ROI Minimum Z	Indicates the minimum Z position contained by the ROI
ROI Maximum Z	Indicates the maximum Z position contained by the ROI
Edit/Add Scanfield At Current Z	If there is a scanfield defined for this ROI at the current editor Z plane, this button will select it to show/edit its properties. If there is not a scanfield at the current Z plane, this button will add one to the ROI and select it for editing

Stimulation Quick Add Panel

When editing stimulation patterns, it is important to put pauses between stimulation functions to allow the scanner time to transition from one ROI to another. The quick add panel provides the means to do so quickly.

Add Pause	Adds a pause function to the end of the stimulus pattern using the specified duration
Add Park	Adds a park function to the end of the stimulus pattern using the specified duration
Duration	Specifies the duration to use when adding a pause or park using the **Quick Add** panel

Using the Cell Picker

The cell picker allows you to select ROIs from a context image using image segmentation to identify features in the image. The cell picker is available for creating imaging ROIs, stimulus patterns, and analysis ROIs. In order to use the cell picker, you must have a context image currently showing. You can obtain a context image by showing the live context image, taking a snapshot, or loading an image from a file. The cell picker can be accessed from the **New ROI** panel by selecting **Cell Picker**. Once in cell picker mode, You can choose a selection mode. The selection mode selects which segmentation routine is used to identify cells in the image. The built in modes are Disk, Annular, and Manual (manual mode does not use segmentation; it will just use a default cell size around where the mouse is clicked). You can also define your own custom segmentation routine. Once the desired selection mode is chosen, click anywhere within a context image in the main view to find a cell at the clicked point. If the segmentation routine finds a cell, it will be indicated with a green highlight. At this point, you can continue clicking to identify more cells. The currently selected cell can be increased in size using the **Dilate** button or shrunk using the **Erode** button. Once all the desired cells are selected, click the **Add Selected Cells** button to create the ROIs.

Depending on the type of ROI group currently being edited, additional options are available for how the ROIs are created:

Imaging ROI Cell Picker Options

ROI Margin	Indicates how much larger than the cell to create the ROI
Create as Discrete Plane ROI	Indicates that the created ROIs should be discrete plane ROIs

Stimulation Function Cell Picker Options

Pause Between Stims	Indicates duration of a pause function to add between stimulation functions for selected cells

Imaging ROI Cell Picker Options

ROI Margin	Indicates how much larger than the cell to create the ROI
Create as Discrete Plane ROI	Indicates that the created ROIs should be discrete plane ROIs
Create with Mask	Indicates if the ROIs should be created with masks so that only the pixels identified from the segmentation routine will be included in the analysis

Hotkeys

The following hotkeys are available in the ROI editor interfaace:

"a" / "insert"	Open the **New ROI** panel
"d" / "delete"	Delete the selected item in the ROI table
"c"	Enter cell picker mode
"p"	If currently editing a stimulation pattern, adds a pause function using the quick add panel
"k"	If currently editing a stimulation pattern, adds a park function using the quick add panel
"escape"	Deselects any currently selected item and exits the **New ROI** and cell picker modes

The following hotkeys are available when the **New ROI** panel is open

"r"	Toggle between **Top Left Rectangle** and **Center Point Rectangle** modes
"c"	Enter cell picker mode
"m"	Toggle **Draw Multiple** option

The following hotkeys are available when the **New ROI** panel is open in cell picker mode

"a" / "insert"	End the cell pick session and create ROIs for the selected cells (same as clickind the **Add Selected Cells** button)
"d"	Dilate selected cell
"e"	Erode selected cell
"delete"	Delete selected cell
"m"	Cycle through cell picker selection modes