

Multi Time Series Rev. 35

The Multi Time Series program is designed to provide flexible programming of automated time dependent experiments.

The basic programming unit is a single Time Series Block in line, frame or Z-stack mode. In each block user can define configuration for the data acquisition (single or multi-track), the number of images and the time interval/delay between images. User can activate an optional autofocus function before each block, pre-program time interval before each block (from the beginning of the previous block to the beginning of the current block) or time delay (from the end of the previous block to the beginning of the current block), and/or execute bleach track with arbitrarily specified bleach ROI's, laser line(s) and power.

The autofocus function can be executed using specified multi-track configuration, or with the configuration used for imaging (using the first channel of the first track). One can define the z-offset, the distance in the z direction from the position where the autofocus finds the reference feature in focus (position of maximum intensity - e.g. position of the cover slip reflection in the reflected light configuration) to the position, which is moved into focus plane when the image acquisition begins (e.g. into the tissue). The image acquisition is done with the configuration specified for the given block. Autofocus search parameters: Z-offset as well as the Z-range (the distance in the z direction over which the autofocus procedure searches for the plane of maximum intensity) can be set independently for different stage locations (on the systems with the motorized stage). Alternatively, instead of searching for the plane of maximum intensity, autofocus can be programmed to search for the plane of the first occurrence of the threshold intensity in the z direction (Use Threshold checked and the threshold value specified).

User can pre-program time intervals between blocks - counted from the beginning of the previous block (start of its autofocus function, when activated) to the beginning of the current block or time delays between blocks - counted from the end of the previous block to the beginning of the current block. When the block time interval is set to zero or if is shorter then the time required for execution of the previous block, the current block is executed immediately after the end of the previous block.

The time interval for the first block does not have any effect at the start of the program - it is only used when number of block repetitions is greater then 1.

During time delay before the start of the image acquisition user has full interactive control of the microscope. The status bar in the Multi Time Series informs about the time remaining before the start of the next block (or that the

system is waiting for the trigger).

Time interval between blocks can be executed before the block scan at each location (All Loc's check box checked), or only at the first location (All Loc's checkbox unchecked).

The image acquisition starts with the (optional) autofocus function followed by the (optional) bleach function followed by the image acquisition.

User can program the time delay between bleach function and the first scan. When the bleach time delay is set to zero the bleach function is executed according to the bleach track protocol (specified in "Edit Bleach" function).

It is possible to define block with bleach function only, without consecutive image acquisition (and with or without autofocus). This option is useful when bleach has to be performed with much slower scan speed than the image acquisition. The multitrack configuration specified in block configuration window is used during bleaching (defines scan speed, zoom, scan rotation and offset). The bleach track and bleach ROI define laser attenuation and ROI for bleach.

This option is activated when the number of scans in the block is set to 0.

On the systems equipped with motorized stages one can mark multiple stage locations (XYZ coordinates) and activate Multiple Locations options in the program. Each block will then be executed at consecutive stage and focus locations. All parameters marked (XY/View) can be independently set for each stage location (or view). In particular, the bleach ROI's can be independently specified for each stage location (and for each block).

New in release 3.2 p and later is that Autofocus, Autofocus Configuration, Bleach, Bleach Configuration can be set independently for each stage location (or view).

In addition, number of scans within each block and the time interval (delay) between them can be set independently for each stage location (or view)

Alternatively, one can specify multiple views - different zoom, offset and rotation settings within single field of view, with different excitation parameters (all scanning parameters stored in Multi Track configuration and specified in "Options"->"Settings"->"Recording/Reuse").

Each block will be executed at consecutive views, recalled from stored Multi Track configurations. The bleach ROI's can be independently specified within each view.

Only Multi Track configurations can be used in multiple views option - only Multi Track configurations modify scan zoom, offset, rotation and speed. Single Track configurations do not change these parameters. However, one can create Multi Track configuration with single track only.

Blocks can be combined in groups, and the whole group of blocks will be executed at the same location or at the same view, before moving to

the next location or view. The whole group of blocks can be repeated by the Group Repetition number.

The order in which program executes group of blocks, their repetitions and movement to the consecutive locations/views is specified by the one of the three selections:

L-GR-G:

Loop Number of Repetitions
Step through ALL Groups
Within Each Group:
Loop Number of Locations/Views
At Each Location/View:
Loop Group Repetitions
Step through ALL Blocks in the
Group
Next Group Repetition
Next Location/View
Next Group
Next Repetition

GR-L-G:

Loop Number of Repetitions
Step through ALL Groups
Within Each Group:
Loop Group Repetitions
Loop Number of Locations/Views
At Each Location/View:
Step through ALL Blocks in the
Group
Next Location/View
Next Group Repetition
Next Group
Next Repetition

GR-G-L:

Loop Number of Repetitions
Step through ALL Groups
Within Each Group:
Loop Group Repetitions
Step through ALL Blocks in the Group
At Each Block
Loop Number of Locations/Views
Next Location/View
Next Block
Next Group Repetition
Next Group
Next Repetition

To specify group of blocks, select the first block of the group and click "Start Group" button. Then select the last block of the group and click "End Group" button. The remaining blocks (blocks after the End Group) will form separate groups and can be combined afterwards).

Each block of data is saved automatically as *.ism file in the image database in the user specified Temporary Image Folder. The program automatically creates sub-folder(s) **temp_1.mdb, temp_2.mdb, etc...** and the image database(s) **temp_1.mdb, temp_2.mdb, etc...** . The file name is created in the following way:

"BaseFile Name"_L"Location Number"_B"Block Number"_GR"GroupRepetitionNumber"_R"Repetition Number".ism.

The temporary images during scans with Multiple Locations or Multiple Views (see below) are stored in separate image databases, in separate subdirectories: temp_1.mdb, temp_2.mdb, temp_3.mdb etc. in folders temp_1, temp_2, temp_3 etc... respectively.

One can link arbitrary number of blocks with independently specified parameters.

The entire list of blocks can be repeat by specified number of times (Number of Repetitions).

The independently saved time series files are combined into single time series file(s) (separate file(s) for each location or view), containing all the proper time stamps, at the end of the program.

The experiment can mix z-stacks with single plane images, providing that the first block of data is marked as the z-stack. The z-stack parameters are set in Z-Stack Scan Control (for "Single Track" configurations) or specified in "Multi Track" configuration.

When using "Single Track" configuration z-stack has to be active in Scan Control in order to use Z Stack option.

Program will create separate time series files, independently for each location (or view) at the end of the program run. These files are automatically saved in the Image Data Base specified in the macro. The Most Recently Used Image Database is the default destination for the storage of the final time series image.

There is an option of "Single File Output". When this option is checked, single time series file is created, collecting images from all locations (or views). This option is useful for an easy overview of all

collected data.

Time series for individual locations can easily be extracted from the single file using "Subset" function.

Optionally, acquisition of each block can be triggered by Trigger1 line and paused by Trigger2 line. In this mode program waits for the trigger at the beginning of each block.

During execution of each block user can use interactively all the controls provided in the standard Time Series menu - set markers, change time interval, pause and resume.

The Multi Time experiment design is automatically saved on exit from macro (in System registry) and automatically re-loaded when macro starts. Different experiment design can be saved, recalled and deleted. The list of multiple locations for the given experiment is saved and may be recalled (an option "Recall Location List"). The list of stage locations is generated in the Stage Menu.

There is an additional "Edit Locations" button, where user can generate grid of locations, specifying number of locations in X and Y direction and the X and Y distance between locations. This provides easy programming of automatic data collection from specimens arranged in the grid patterns.

The second option in the "Edit Locations" is the creation of the tile grid - grid of locations of adjacent scan fields. User specifies the number of tiles in X and Y direction and program calculates the step size in X and Y directions based on the scan field size.

The third option in the "Edit Locations" is the creation of the stack of z locations for non-equidistant z sampling of the specimen or for the special experiments, where each z slice has to be controlled by the external trigger signal.

WARNING: Mark Distance has to be set to 0 in the System Database.

This option can be used on the systems without the scanning stage, providing that the "dummy" stage is activated in the System Database.

Ask LSM specialist for details.

Program automatically generates the time series of Tile Images (in addition to the Time Series file), providing that there is only single scan specified at single block (repeated by Number of Repetitions) and the tile grid of locations was created in "Edit Locations". "Single File Output" has to be checked. Both files are automatically saved (if the "Save Final Image" option is checked) to the Image Data Base. Time Series file has the name with the "Raw_..." prefix and Tile file has the name with "Tile_..." prefix.

Maximum size of the tile cannot exceed 16384x16384 pixels in X and Y directions.

In particular one can design time experiment of tiled z stacks with or without autofocus function at each location (to correct for potential drift in the z direction)

The main dialog box:

Multi Time Series Version 3.5.1

Single Location Multiple Locations

View 1 1: X=0.00 Y=0.00 Z=0.00

List of Blocks: 5

BlkNo	Groups	G.Rep.	BkDelay	ZStack	AutoF	Bleach	Scans	Intvl
1	1	2	60	No	Yes	No	1	0
2	1	2	180	No	No	No	1	0
3	2	3	60	No	No	No	1	0
4	2	3	120	No	No	No	1	0
5	3	5	60	No	No	No	1	0

Add Block Remove Block Start Group End Group Store/Apply

Repetitions: 1 L-GR-G GR-L-G GR-G-L
 Views: 1 Group Repetitions: 5

Block 5 Parameters

All Loc's Wait Delay: 1 min

Autofocus

Autof (XY) Use Cnf (XY) Autofocus633 (XY)
 Fast Z Scan Z Offset (XY): 0 μm
 Z Range (XY): 80 μm
 Scan Speed 9 Use Threshold 50

Bleach

Bleach (XY) Config. (XY) bleach1 Spot XY
 Wait Delay (XY/View): (ms) 2 ms
 Mapping ROI (XY/View): Get Spot

Configuration, Time Interval and Number of Scans

Single Track (XY) Multi Track (XY/View) Find BC
 Multi1 Multi1
 Wait Interval (XY/View): (s) 0 s
 Number of Scans (XY/View): 1 ZStack XY

Ready

Z=0.00 Current Pos: X=0.00 Y=0.00

Trigger Start/Pause/Resume Trigger1/Trigger2 Single File Output

Base File Name New Single Temp DB

Image Data Base C:\a_shared\LsmlImages\MultiTime.mdb\MultiTime.mdb

Tmp Image Folder C:\a_shared\LsmlImages\Export\

Open DB OpenTemp DB Location 1

Close

Start Time

Stop

Finish

End Block

Pause

Autofocus

Refresh

Center

Edit Locations

Move To Pos.

ReplacePos

Replace Z

Load Conf.

Save Conf.

Help

Options

Tile Time Series

Close: Ends the program.

Start Time: Starts data acquisition. Each block is executed according to the recipe. , First, there is an optional block wait (interval or delay), during which one has the full control of the microscope (e.g. to check and correct focus position). (The very first block is executed immediately, without wait). Pressing Resume button terminates the wait period. Autofocus (if selected) is performed next In **Multiple Locations** case one has an option to execute the block wait at each location, after stage moves to the next position (**All Loc's** checked), or only at the first location (**All Loc's** unchecked). If there is a group of blocks executed at each location, the wait period of the first block after stage moves to the new position is skipped. The remaining blocks in the group are executed with the specified block wait.

Optional **Bleach** is executed next, followed by the specified number of scans. In case of **Time Delay After Bleach** set to zero, bleach is executed according to the settings in the **Edit Bleach** dialog.

Scanning is done using configuration specified in the block definition, with all the parameters set in the Scan Control menu including Z Stack settings. When **Z Stack** is checked, multiple slices at selected z position are scanned; otherwise only single slice at the selected z position is scanned. **Z Stack** check box is location dependent. The number of the z slices is stored in **Multi Track** configuration, or is specified in the Scan Control for **Single Track** configurations. The z step size is specified in the Scan Control.

When the **Find BC** checkbox is checked, the **FIND** function adjusts detector gain and offset before the scan of the block.

The block data are saved in the selected Image Database in the file with the name created according the following rule:

"BaseFile Name"_L"Location Number"_B"Block Number"_GR"Group Repetition"_R"Repetition Number".ism.

In **Multiple Locations** case the stage moves to the next location and the same block (or group of blocks) is executed according the same recipe. Block parameters marked (**XY**) are location specific, for example **Z Offset (XY)**, **Z Range (XY)** and bleach **ROI (XY)**.

When the **Number of Group Repetitions** is greater then one, the whole cycle (group of blocks at all locations) repeats that number of times The next group of blocks from the list is executed, and so on...

After the last block is scanned all the partial time series files from each location are concatenated into single time image for each location. These images are saved automatically, when **Save Final Image** option is checked in the Options menu.

Alternatively, when **Single File Output** is checked, single time image is created from all time stamps from all locations. Subset function can be used to extract time series image for each location separately.

Additional **Tile** image is created in case when Multiple Locations are generated

by the **Tile** function (Tile function requires **ONE BLOCK ONLY** with **Number of Scans** in this block set to one). This block can be repeated arbitrary number of times.

At the end of the entire cycle, stage and focus return to the location at which **Start Time** was activated.

Stop: Stops the execution of the Time Series. Intermediate images are saved but not concatenated.

End Block: Interrupts execution of the block saves the partial data and continues with the acquisition of the next block.

Pause/Resume: During data acquisition user can **Pause** the scanning. The **Pause** button changes to **Resume** button. **Resume** continues data acquisition. The button changes to **Pause**.

Re-Initialize: The list of **Single** and **Multiple** Configurations, ROI's, Bleach configurations as well as the list of stage locations is filled when the program starts. In order to update those lists (when new configurations are created or new stage positions are marked when Multi Time is running) one has to click **Re-Initialize** button.

Autofocus: Executes the Autofocus function with the parameters specified in the selected block. The focus correction is applied to the ALL locations (for systems with the motorized stages), therefore it is sufficient to execute the Autofocus at one location only.

Help: Displays this document.

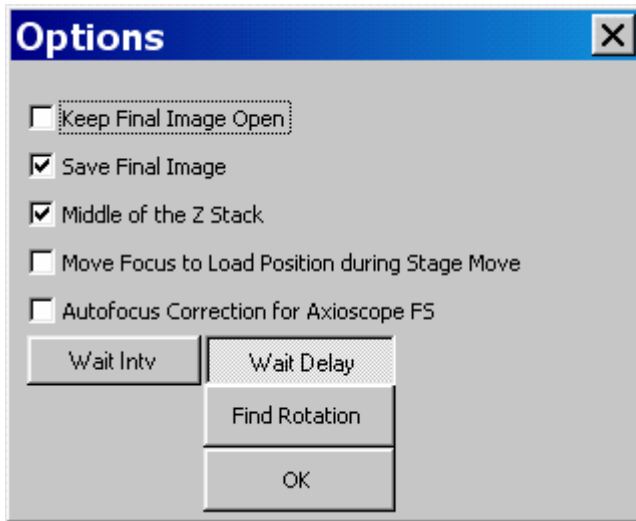
Load Conf: Loads current configuration

Save Conf: Saves current configuration (overwrites, after prompt, selected configuration for current block and location).

Move To Pos: Moves the stage and the focus motor to the selected position in the Multi Location list.

Replace Pos: Replaces the stage and the focus motor coordinates of the selected position in the Multi Location list.

Options: Opens Options dialog box



Keep Final Image Open - Concatenated result(s) remains displayed in the Window

Save Final Image - Concatenated result(s) are saved in the Image Database

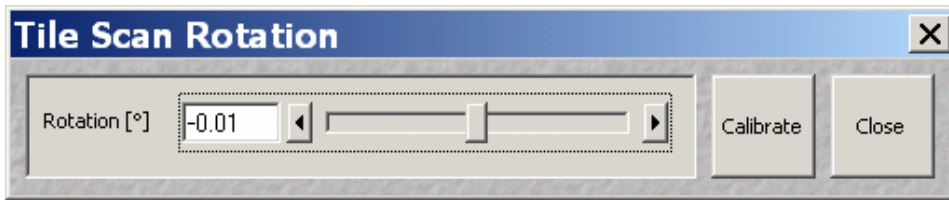
Middle of the Z Stack - The current location (as well as the destination locations in Multiple Locations mode) will be in the center of the z stacks. Otherwise, the marked locations are at the top of the z stacks

Move Focus to Load Position during Stage Move - When checked, focus drops to the Load Position before stage movement to the next location. Otherwise, stage moves to the next location keeping the current z position. The z position is adjusted to the z position listed for the new position and corrected by the autofocus function. Autofocus executed at one location corrects the z positions for ALL locations.

Autofocus Correction for Axioscope FS - When checked, Autofocus in **Fast Z Scan** mode on Axioscope FS systems executes special correction procedure to compensate for incorrect **Fast Z Line** scans on some systems.

Find Rotation - Systems with motorized stage only, with software version greater or equal 3.0!

Find Rotation function automatically finds an angle between the scanners axis and the stage axis. Used in the TILE SCAN.



Calibrate - Automatically finds the rotation angle between the scanners and the stage axis.

Single Location/Multiple Location: Multiple Locations option is available on systems with motorized stage only. On those systems, when Multiple Locations Option is selected, the Locations Combo Box displays all locations at which time series will be executed.

This location list can be created by marking stage positions in the Stage and Focus Control (different XY and Z positions), by creating grid, tile or Z Stack positions in **Edit Location** menu or by recalling previously created **Recipe**. Most parameters in the Multi Time Dialog are location specific. Those parameters (e.g. autofocus Z-Offset, Z-Range, Bleach ROI, etc...) are marked with (XY) identifier. One can view and set these values by selecting appropriate location in the Locations Combo Box. The stage and focus motor do not move to the selected locations. One can move to any of the marked locations using **Move to Pos** button.

The Location List can be changed in **Edit Location menu**, by applying previously created Recipe or by marking positions in Stage and Focus Control and clicking the **“Re-Initialize”** button.

Single Location		Multiple Locations							
View 1		1: X=0.00 Y=0.00 Z=0.00							
List of Blocks: 5									
BlkNo	Groups	G.Rep.	BkDelay	ZStack	AutoF	Bleach	Scans	Intvl	
1	1	5	600	Yes	No	Yes	1	0	
2	1	5	600	Yes	No	No	1	0	
3	1	5	600	Yes	No	No	1	0	
4	2	5	600	Yes	Yes	No	1	0	
5	2	5	600	Yes	Yes	Yes	1	0	
Add Block		Remove Block		Start Group		End Group		Store/Apply	

The Location List can be saved and recalled using Store/Apply function.

List of Blocks: 5								
BlkNo	Groups	G.Rep.	BkDelay	ZStack	AutoF	Bleach	Scans	Intvl
1	1	5	600	Yes	No	Yes	1	0
2	1	5	600	Yes	No	No	1	0
3	1	5	600	Yes	No	No	1	0
4	2	5	600	Yes	Yes	No	1	0
5	2	5	600	Yes	Yes	Yes	1	0

The list of blocks displays the general settings for all blocks and enables selection of individual blocks. Multi Time menu displays and allows editing of parameters of the selected block.

Add Block: Creates new block with parameters equal to the parameters of the previous block. Parameters dependent on the stage location are also copied.

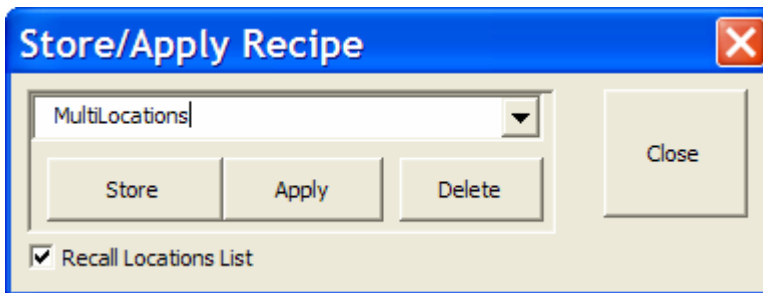
Remove Block: Removes the selected block from the list.

Start Group: To specify group of blocks, select the first block of the group and click "**Start Group**" button. Then select the last block of the group and click "**End Group**" button. The remaining blocks (blocks after the **End Group**) will form separate groups and can be combined afterwards).

End Group: The selected block becomes the last in the group of blocks, providing that the block selected by the **Start Group** is listed above the **End Group** block in the list of blocks.

All blocks below the **End Group** block become consecutive groups of single blocks.

Store/Apply: Opens Store/Apply dialog box.



Store: Stores the Multi Time Recipe (all settings for all blocks and locations) in the system registry with the user provided name.

Apply: Applies the settings stored in selected recipe. When "**Recall Location List**" is checked the list of XYZ locations is loaded into Stage and Focus Control list of locations. (as well as into the list of Multiple Locations). This list marks the

locations with respect to the origin - point (0,0,0). It is important to set the same physical point of the sample as the (0,0,0) point at the time when the location list is created and at the time when the location list is recalled.

Delete: Deletes selected recipe.

Close: Closes **Store/Apply** dialog box and returns to the **Multi Time** menu



L-GR-G:

Loop Number of Repetitions

Step through ALL Groups

Within Each Group:

Loop Number of Locations/Views

At Each Location/View:

Loop Group Repetitions

Step through ALL Blocks in the

Group

Next Group Repetition

Next Location/View

Next Group

Next Repetition

GR-L-G:

Loop Number of Repetitions

Step through ALL Groups
Within Each Group:
Loop Group Repetitions
Loop Number of Locations/Views
At Each Location/View:
Step through ALL Blocks in the
Group
Next Location/View
Next Group Repetition
Next Group
Next Repetition

GR-G-L:
Loop Number of Repetitions
Step through ALL Groups
Within Each Group:
Loop Group Repetitions
Step through ALL Blocks in the Group
At Each Block
Loop Number of Locations/Views
Next Location/View
Next Block
Next Group Repetition
Next Group
Next Repetition

Repetitions: Number of repeats of the entire list of blocks.

Number of Group Repetitions: Number of repeats of each group.

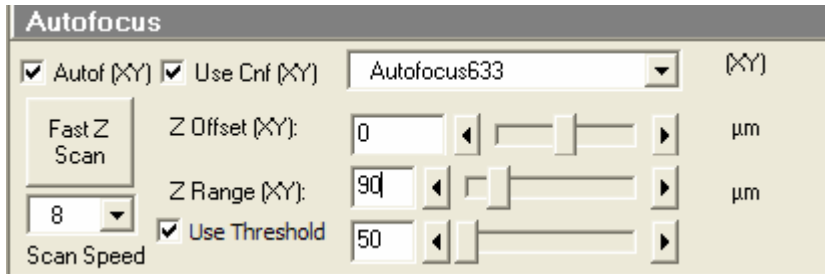
Views: Active only in **Single Location** mode. When greater than 1, drop down list of Views becomes active and one can specify block parameters for each view separately.



Wait Before Block Scan: The time the program will wait idle before starting the current block. The wait time is counted from the beginning of the previous block to the beginning of the current block (**Time Interval**) or from the end of the previous block to the beginning of the current block (**Time Delay**). During that time user has full control of the microscope, can reposition the sample in XY and Z etc. During wait time **Status Bar** displays remaining idle time and the Resume button is active.

Clicking on **Resume** button can start the data acquisition.

All Loc's: When checked, the program will wait specified time before the start of data acquisition at each stage location at the beginning of each block (active only in "Multiple Locations" case). When unchecked, program waits only at the first location at the beginning of each block.



Autofocus check box: When checked **Autofocus** is executed at the beginning of the block.

Use Configuration Check box: When checked **Autofocus** is executed using configuration specified in the **Configuration Combo Box**. Otherwise, the actual configuration is used (first channel of the first track).

Configuration Combo Box: Displays the Single Track configuration, which is used in Autofocus function when **Use Configuration Check box** is checked.

Z Offset (XY): Autofocus function places the plane distanced by **Z Offset** from the plane of maximum intensity in the focal plane of the microscope. Different **Z Offset** values can be set for different locations.

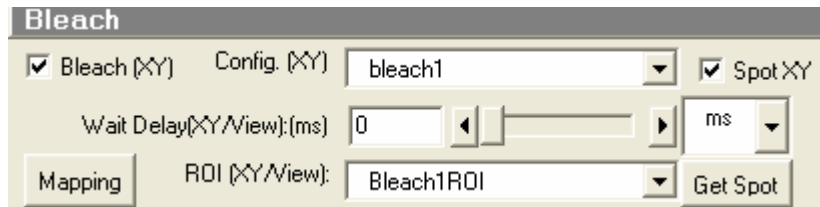
Z Range (XY): Distance in the Z direction over which Autofocus searches for the plane of maximum intensity. Autofocus function moves first the specimen by (**-Z Offset**) then searches one half of the **Z Range** below and half of the **Z Range** above that plane.

Step/Fast Z Line button: Toggles between two z-line modes, **Step** - slow but more accurate and **Fast Z Line** - much faster but less accurate.

Scan Speed: One can set the laser scanning speed for the Autofocus function.

Use Threshold: In the semi-transparent samples one can specify reference plane on the sample surface rather than on the brightest plane. The reference plane is defined when the average intensity exceeds the threshold value. In software release 3.0 and higher the autofocus z-scan stops when "Use Threshold" is checked and mean intensity exceeds the threshold value.

All Autofocus parameters are stored in the Multi Time Recipe.



Bleach checkbox: When checked, bleach is executed in the given block, after autofocus (and possible wait) and before the data acquisition.

Configuration List Box: Bleach configuration (edited in Edit Bleach function) used to bleach sample in the selected block.

Time Delay After Bleach: Time between Bleach function and the data acquisition. When the **Time Delay After Bleach** is set to zero, data acquisition starts immediately after bleach.

ROI (XY): ROI used for bleach. ROI is defined separately for each location and for each block.

Spot: Use the Spot Bleach

Get Spot: Stores the selected spot coordinates (Use the Spot Sel. function in Scan Control Window to select the Spot position)

Mapping: Activates/Uncage all points in the image specified by the multitrack scan configuration. Activation of each point is done by the spot scan of the duration of the pixel dwell time multiplied by 1000 and multiplied by the number of bleach repetitions (defined in the **Bleach Control**). There is an approximately 1 second delay between consecutive activations. user specifies the number of activation events in each line. The **Activation/Uncaging** dialog displays the distance between adjacent activation points, total number of uncaging events and the activation time of each point.

The activation follows the following pattern:

First pixel, first row
Middle pixel, first row
First pixel, middle row
Middle pixel, middle row
Second pixel, first row
Middle pixel+1, first row
Second pixel, middle row

Middlepixel+1, middle row

.....

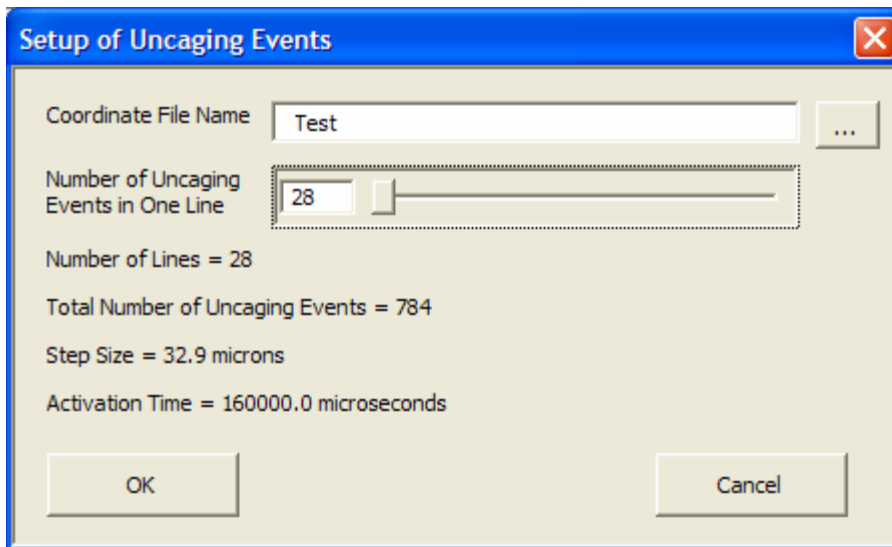
First pixel, second row

Middle pixel, second row

First pixel, middle row+1

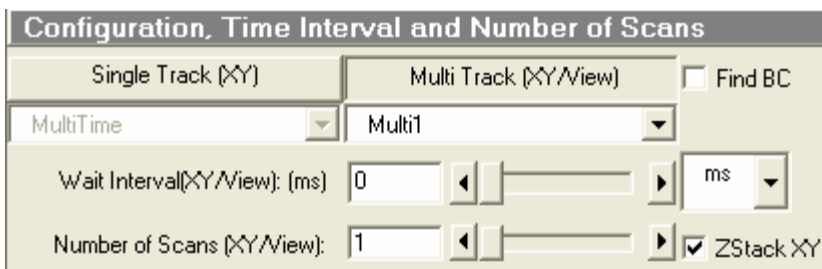
Middle pixel, middle row+1

.....



OK: Starts the **Functional Mapping**. **Stop** button stops the activation.

Cancel: Returns to the **Multi Time** main dialog



Single Track/ Multi Track: Configuration for data acquisition in the selected block. These configurations are position dependent. Different configurations can be specified for different locations

Each block can have different configuration and the images from the different blocks are concatenated together, based on the channel names.

Only channels with identical names from different configurations will be concatenated together.

The efficient way to deal with different configurations for different blocks is

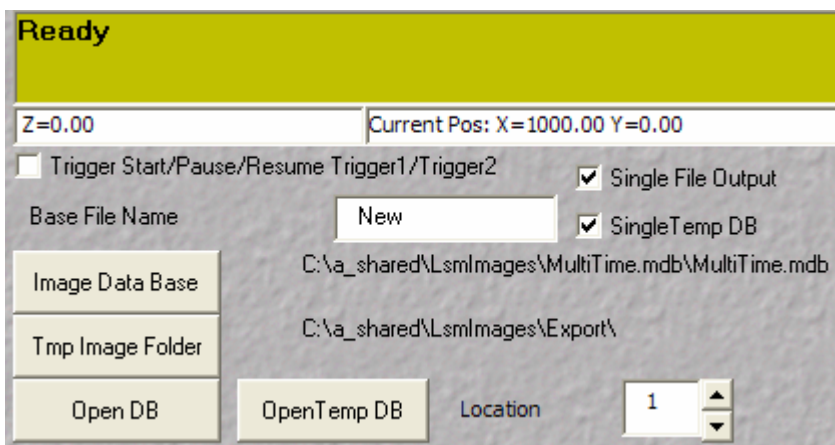
to create the list of dedicated configurations for Multi Time (e.g. Block1View1, Block1View2, etc.). These configurations can be overwritten for each new sample. The selections in the Multi Time dialog box for Multi Track (or Single Track) remain unchanged and only the actual configurations (Block1View1...) are changed (Config -> Store/Apply).

Time Interval: Time interval between consecutive images or z-stack of images.

Number of Scans: Number images (stacks) in selected block

Z Stack checkbox: When checked z-stacks are acquired, otherwise single plane images. In order to mix z-stack and single plane images the first block has to be a z-stack.

Find BC checkbox when is checked, the **FIND** function adjusts detector gain and offset before the scan of the block

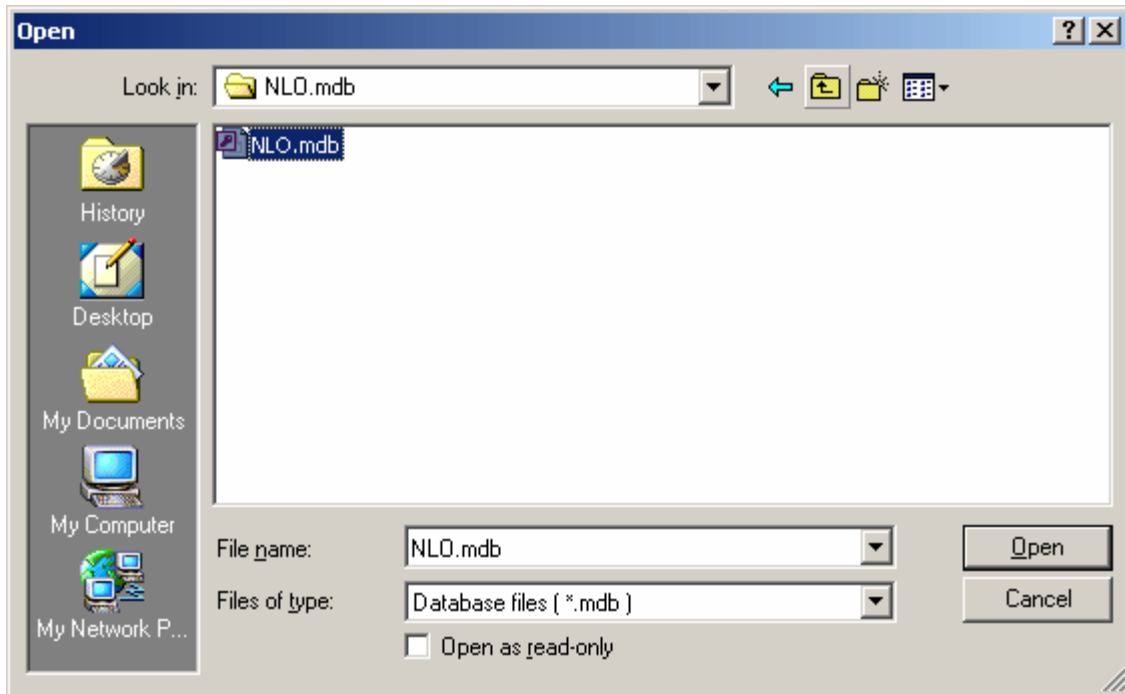


Status bar: Displays the current state of the system.

Trigger Start/Pause/Resume Trigger1/Trigger2: When checked, each block of the data starts when Trigger 1 is sent. Trigger 2 pauses the data acquisition and Trigger 1 resumes the data acquisition.

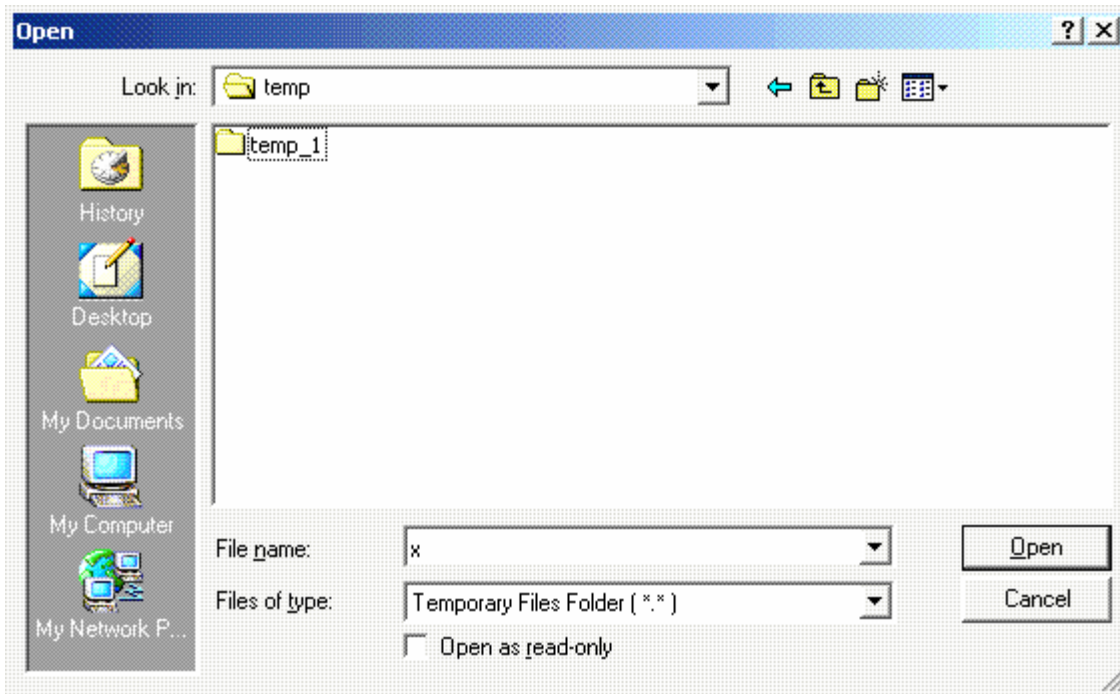
Base File Name: Images from each block are automatically saved in the specified Temporary Image Database with the file names following the convention:
"BaseFile Name"_L"Location Number"_B"Block Number"_R"Repetition Number".ism.

Image Data Base: Selects the image database where the final images are stored:



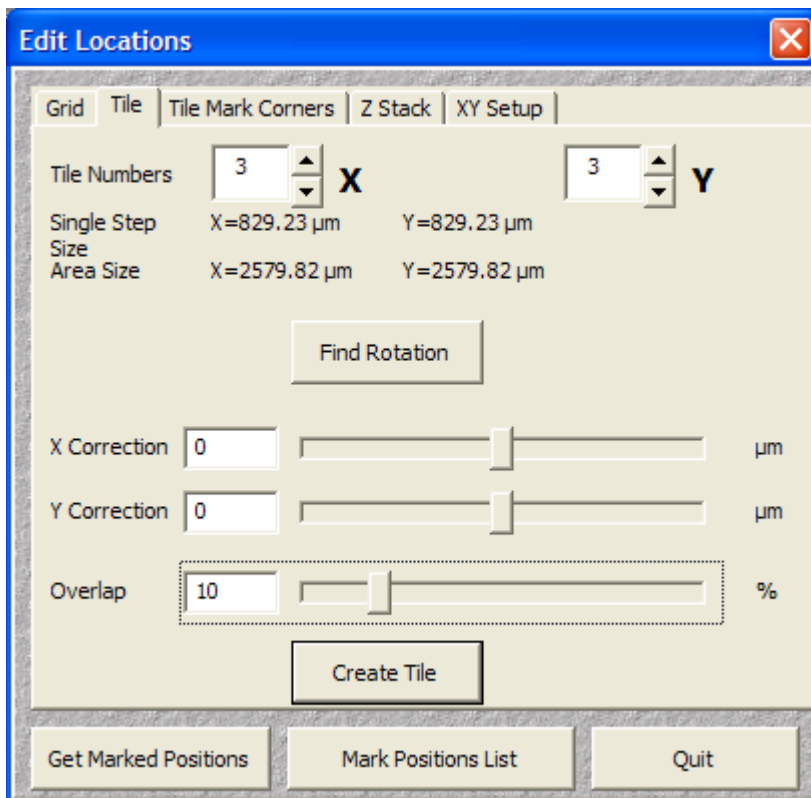
Temporary Image: Selects the folder where intermediate image database(s) are stored: This function selects only the folder and the user do not have to provide any (valid) filename in the File name field. Files from each location/view are stored in separate Temporary Image Database. These Temporary Image Databases (temp_1.mdb, temp_2.mdb,) are automatically created for each location/view in subfolders named temp_1, temp_2, respectively.

Single Temp DB: When checked, ALL images from ALL locations are stored in the single Temporary Image Database: temp_1.mdb. This selection is convenient when creating tile images.



Open DB and Open TempDB opens specified Image Databases.

Edit Locations:



Create Tile: Creates grid of locations of adjacent scan fields (of actual scan settings). The current stage location is in the center of the entire area. The settings in the Multi Time menu will be automatically set to the "Multiple Locations" scan of the single block with single scan (plane or **Z Stack**) One can set arbitrary number of **Repetitions** which will result in time series of tiles.

Start Time will create the **Single File Output** time series file and the stitched tile file.

Rotation Correction corrects the possible rotation of scanners versus stage axis. This rotation is smaller than 1 degree and has to be empirically adjusted (the rotation of 1 degree would result of the shift of 9 pixels in the Y direction over the field of 512 pixels). The automatic **Rotation Correction** is provided in **Options** -> **Find Rotation** menu.

One can also specify **X Correction** and **Y Correction** (in microns) to correct for the stitching imperfections, which cannot be corrected by magnification adjustment.

In addition one can specify the **Overlap** region (0-50 %) of consecutive tiles in X and Y directions. The non-zero **Overlap** may result in more accurate stitching.

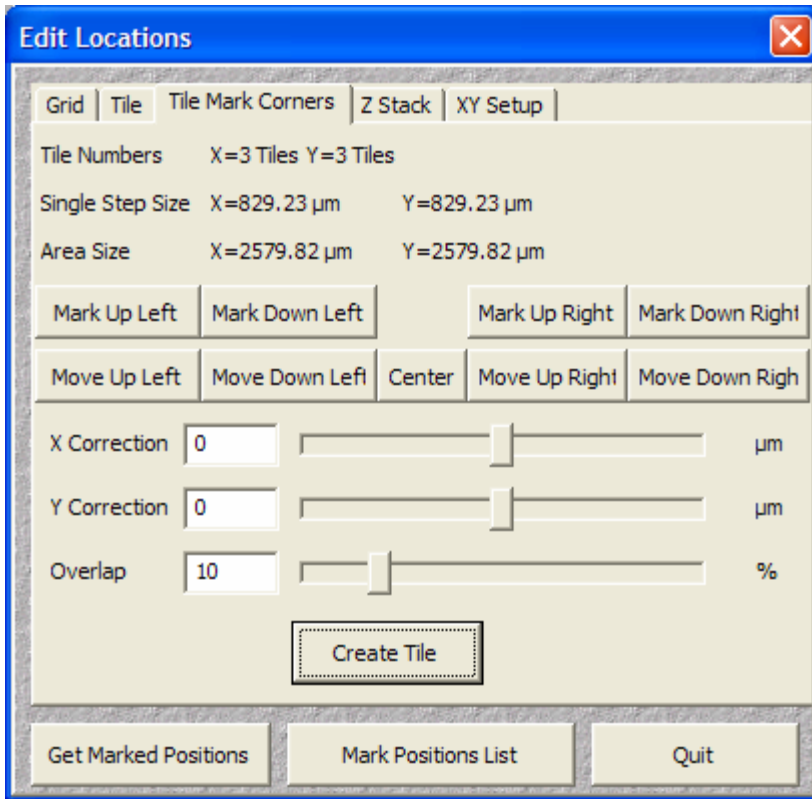
The **Overlap** of 10% and more allows to correlate images within the overlap regions to improve the stitching of the tiles.

The **Correlate** function is available in **Tile Time Series** menu. The **Correlate** function creates the tiled image from the **Single File Output** file.

Single File Output file is created together with the tiled image during the tile scan and automatically saved when "**Save Final Image**" option is checked.

Get Marked Positions replaces the list of locations with the list of marked positions created in the **Stage and Focus Control**

Mark Positions List replaces the list of marked positions in the **Stage and Focus Control** with the list of locations



Tile Mark Corners defines the **Tile** region by selecting corners of the region.

Move Up Left: Moves the stage to the Upper Left corner of the Tile region

Move Down Left: Moves the stage to the Lower Left corner of the Tile region

Center: Moves the stage to the Center of the Tile region

Move Up Right: Moves the stage to the Upper Right corner of the Tile region

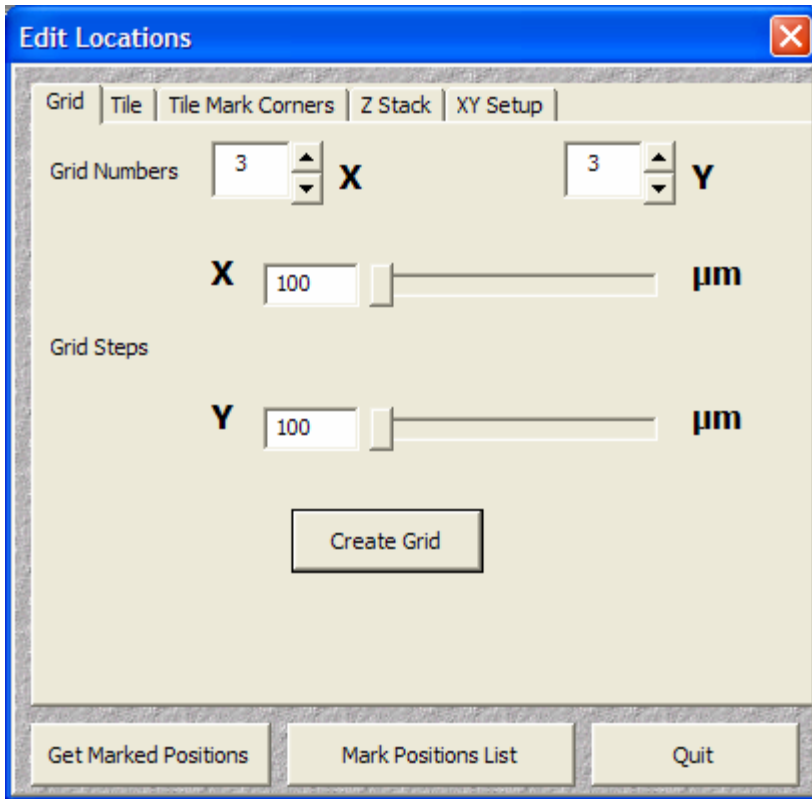
Move Down Right: Moves the stage to the Lower Right corner of the Tile region

Mark Up Left: Selects the current stage position as the new Upper Left Corner of the Tile region. Recalculates the Tile region, number of tiles and the three other corners of the Tile region.

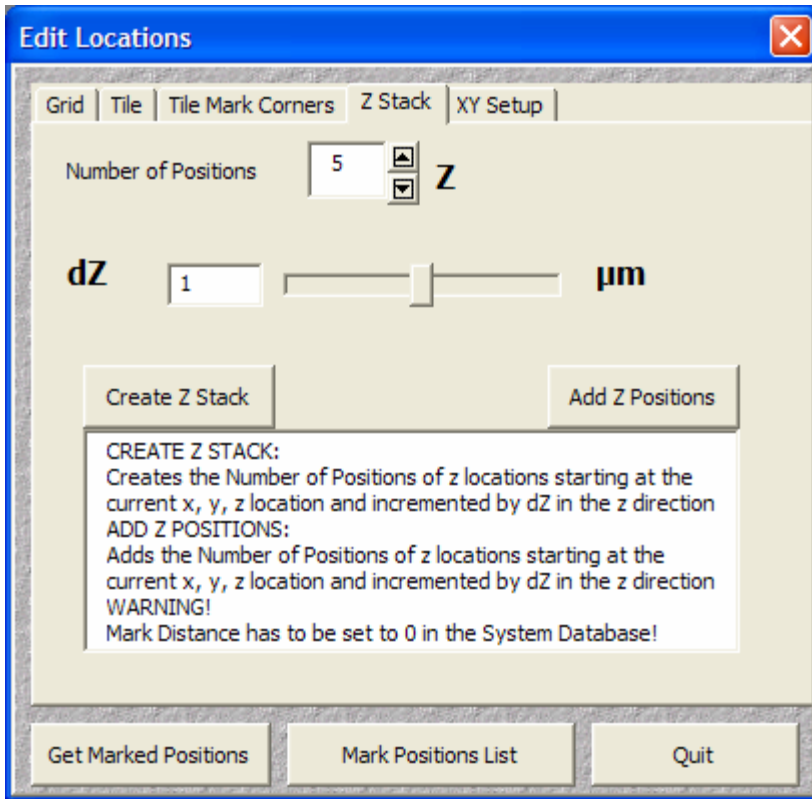
Mark Down Left: Selects the current stage position as the new Lower Left Corner of the Tile region. Recalculates the Tile region, number of tiles and the three other corners of the Tile region.

Mark Up Right: Selects the current stage position as the new Upper Right Corner of the Tile region. Recalculates the Tile region, number of tiles and the three other corners of the Tile region.

Mark Down Right: Selects the current stage position as the new Lower Right Corner of the Tile region. Recalculates the Tile region, number of tiles and the three other corners of the Tile region.

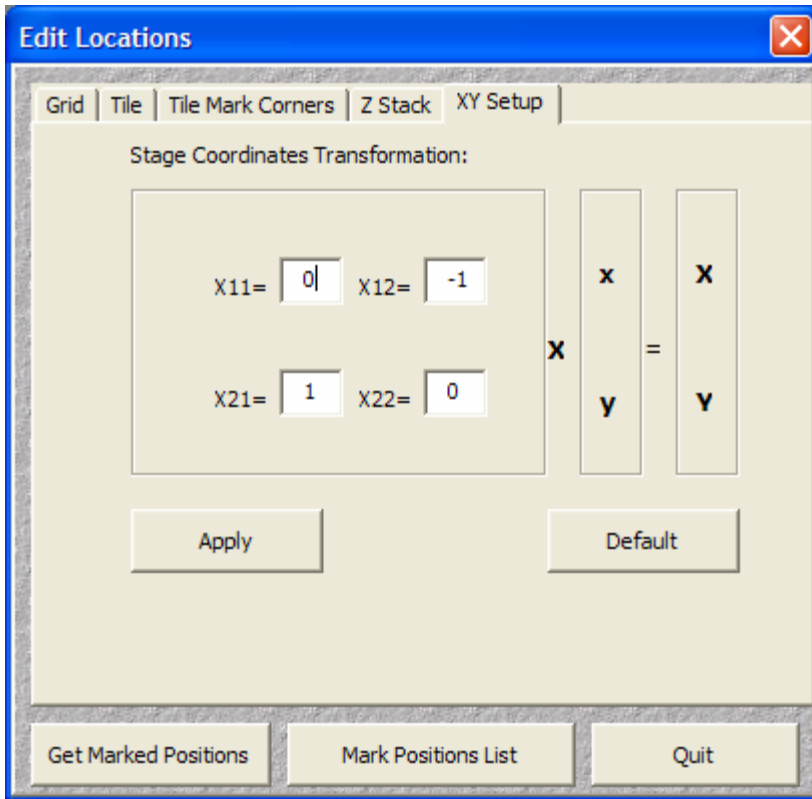


Create Grid: Creates grid of locations with user defined steps in X and Y directions. The first location of the grid is at the present location



Create Z Stack: Creates the stack of z locations starting with the current x, y, z location, with user defined step **dZ** in Z direction.

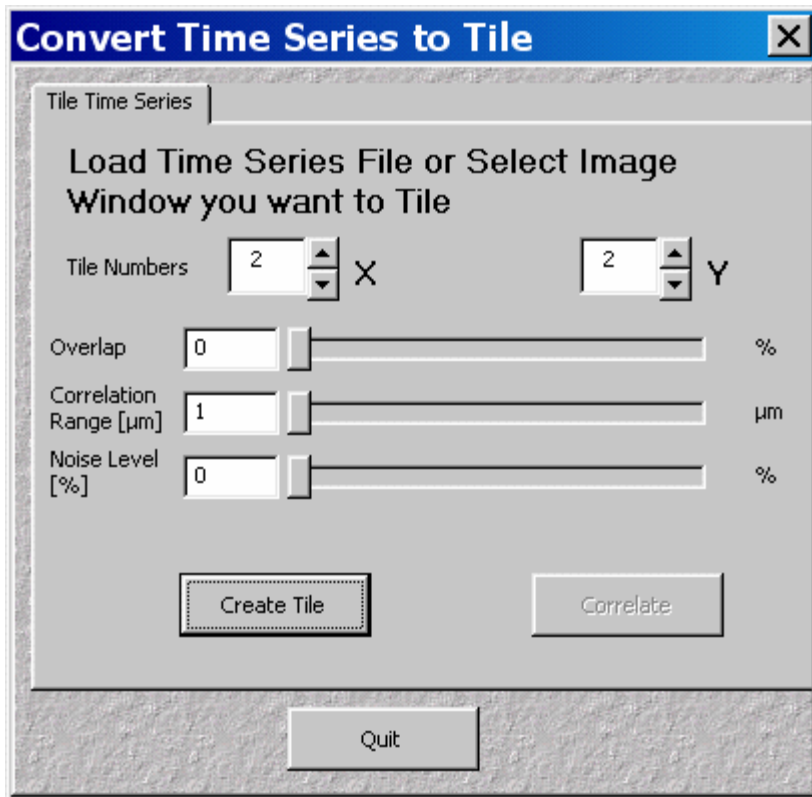
Add Z Positions: Adds the stack of z locations starting with the current x, y, z location, with user defined step **dZ** in Z direction.



XY Setup: LSM program requires stage coordinates transformation dependent on the type of microscope and the scan head position. In case when the default transformation results in incorrect generation of grid or tile coordinates, user can correct transformation parameters and press **Apply** button

Default button returns the default values of parameters.

Tile Time Series: Opens the following dialog:



Converts selected Time Series File into a gallery of stitched images. Images from consecutive time slots are stitched together into TileNumberX x TileNumberY image.

In case when the Time Series File was created during the Tile Scan with overlap settings greater or equal to 10%, one can use **Correlate** button to create the Tile image.

Correlate requires **Overlap**, **Correlation Range** and **Noise Level** parameters to create tiling.

Overlap determines the region which is scanned in consecutive tiles and used for the correlation procedure.

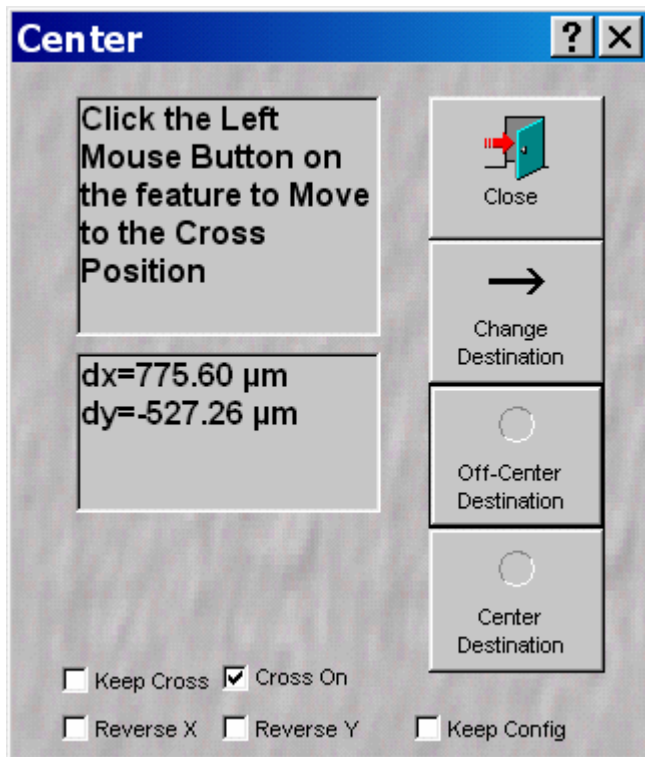
Pixels outside the **Correlation Range** and with intensities below the **Noise Level** do not contribute to the coreelation calculations.

The **Correlate** button stitches the tiles and uses the overlap regions to improve the stitching accuracy.

Center function (on systems with the motorized stage) opens the following dialog box and moves the clicked feature in the sample to the Cross Position.

User can switched between two destination positions:

One in the center in the field of view and the second, arbitrarily selected.



Modifications History:

New features in version 35

There are no additional new features in version 35.
This version was modified to run in the LSM software release 3.5
Functionality in this version is identical to version 32Z1

New features in version 32Z1

Modified Trigger In function
Single Temporary Data Base Option
Find BC works in 512x512 resolution mode regardless on the Scan Configuration.

New features in version 32Z

- 1 Corrected **Wait Delay/Wait Interval** display.
- 2 **Functional Mapping**
- 3 Corrected **Spot Scan**

New features in version 32Y

- 1 Corrected **Wait Delay/Wait Interval** display.

- 2 **Find Rotation** uses macro from Macros directory.
- 3 Improved stage navigation
- 4 **Tile Mark Corners** method of defining the tiled region

New features in version 32X

- 1 **Replace Position** replaces selected X,Y and Z coordinates in the **Multiple Location** list with the current location.
- 2 **Z Stack** checkbox is location dependent
- 3 **Find BC** checkbox when is checked, the **FIND** function adjusts detector gain and offset before the scan of the block
- 4 **Save Conf:** Saves current configuration (overwrites, after prompt, selected configuration for current block and location).

New features in version 32W

Autofocus Correction for Axioscope FS - When checked, Autofocus in **Fast Z Scan** mode on Axioscope FS systems executes special correction procedure to compensate for incorrect **Fast Z Line** scans on some systems.

New features in version 32U

- 1 **Correction in the Z navigation**
- 2 **Correction to the Center function**

New features in version 32T

- 1 **Z positions in the Multiple Location List are updated to the new values, after executing of the Autofocus function.**
- 2 **Center function, stores and toggles between two destination positions - one in the center of the field of view and the second arbitrarily selected.**
- 3 **Load Configuration corrected.**

New features in version 32R

- 1 **Location List is independent of the Marked Position List in Stage and Focus Control (those lists were linked together in the previous MT releases) This results in the more efficient execution of the multilocation scans.**
- 2 **Location List can be created in Edit Locations (Grid, Tile or Z Stack) can be red from the list of Marked Positions in Stage and Focus Control (Get Marked Positions or Refresh buttons) or can be red from the previously stored Recipe.**
- 3 **Location List can be used to create Marked Positions List in Stage**

and Focus Control (Mark Positions List button).

4 Create Tile has an additional parameter, Overlap (0-5 %)

5 Tile Time Series has the "Correlate" button. Correlate button may improve stitching of the tiles acquired with the overlap region $\geq 10\%$.

Correlation requires three parameters: Overlap (10-50%), Correlation Range (1-50 microns) and the Noise Level (0-100%).

Pixels outside the Correlation Range and with intensities below the Noise Level do not contribute to the correlation calculations.

6 Autofocus focus correction is applied to ALL locations (systems with motorized stage), therefore it is sufficient to do the autofocus function at one (usually the first) location.

7. New Option: "Move Focus to Load Position during Stage Move" drops the focus to the Load Position before moving to the next location. (This parameter is set in the "Options" dialog box).

8. Automatic saving (when "Save Final Image" option is checked) of the Tiled Image (during Tile Scan) and the Single Output File of time series of images from all locations.

9. Tile function works in the camera mode.

New features in version 28-32q

1 Edit Locations

Tile

X Overlap and Y Overlap can have negative values.

New features in version 28-32p

1 The new location/view depended block parameters are:

Autofocus Use

Autofocus Configuration

Bleach Use

Bleach Configuration

Number of Scans in the Block

Time interval for those scans

2 Group Repetitions

3 Three modes (L-GR-G, GR-L-G and GR-G-L) for the sequencing of the program execution.

4 Use of ROI data acquisition mode compatible with the use of Autofocus function.

5 Option of block delay or block interval

New features in version 28-32m

1 Temporary Image Folder can be selected without specifying file name in the "Open File" Dialog Box.

New features in version 28-32 j

- 1 Tile of Time Series - Time Series from multiple locations (or single location) can be converted into time series of mosaics of NxM tiles.
- 2 Markers in the file indicating change of the time interval

New features in version 28-32 i

- 1 **Edit Locations:**
Create Z Stack: - Creates the Number of Positions of z locations starting at the current x, y, and z location and incremented by dZ in the z direction.
Add Z Positions: - Adds the Number of Positions of z locations starting at the current x, y, and z location and incremented by dZ in the z direction.
WARNING: Mark Distance has to be set to 0 in the System Database.
This option can be used on systems without the scanning stage, providing that the "dummy" stage is activated in the System Database.
Ask LSM specialist for details.

New features in version 28-32 c (from version 28-32c)

- 1 Autofocus configuration is changed from Single Track to Multi Track type. This change provides greater flexibility in use of Autofocus function. Use of Multi Track configuration instead of Single Track allows to specify zoom, offset and rotation of autofocus scan (these parameters are stored in Multi Track configurations, and not in Single Track configurations). The existing Autofocus Single Track configuration can be easily converted into Multi Track configuration in the Control Panel. To accomplish that, load your existing Single Track Autofocus configuration, then switch to Multi Track tab and save this new Multi Track configuration (containing only single track). **NOTE:** Image from the first channel in the first track is used to determine focus position).
- 2 Corrections in the macro initialization procedure.
- 3 Improvements in the overall functionality.

New features in version 28-32 c (from version 28-32a and b)

- 1 Corrections in Single Track handling (Multiple Locations, Tile, Grid)
- 2 Tile of Time Series - Time Series from multiple locations (or single location) can be converted into a mosaic of NxM tiles.

New features in version 28-32 a (from version 28-30)

- 1 Works in Vers 2.8, 3.0 and 3.2 of LSM program

- 2 The ability to group blocks, which are executed at the given location or view before switching to the next location or view.
- 3 Threshold mode in "Autofocus" function.
- 4 "LoadConf" - Load Configuration button
- 5 Move To Pos - Move to Position
- 6 "Options" button with following options:
 - Keep Final Image Open - Concatenated result(s) remains displayed in the Window
 - Save Final Image - Concatenated result(s) are saved in the Image Database
 - Middle of the Z Stack - The current location (as well as the destination locations in Multiple Locations mode) will be in the center of the z stacks. Otherwise, the marked locations are at the top of the z stacks
- 1 Multi Time has the option to bleach in the spot mode and then collect images in the frame, z stack or line mode
- 2 Multi Time can collect stacks of fluorescent images and single camera images using two views mode.

This program works in versions 2.8, 3.0 and 3.2 of LSM 510.

If you were using previous versions of Multi Time you have to re-do the stored recipes.

The systems with motorized stage: Select "Edit Locations"-> "XY Setup" -> "Default"-> "Apply" to update the coordinate transformation matrix.

