Multi Time Series Rev. 4.0.12

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 location where the autofocus finds the reference feature in focus (location of maximum intensity - e.g. location of the cover slip reflection in the reflected light configuration) to the location, 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** (group) **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)., otherwise the "**Bleach After Number of Scans**" and "**Bleach Repea**t" parameters in the "**Edit Bleach**" are ignored and **Bleach** is done first followed by the delay and the block scan.

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 then the image acquisition. The multi-track 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 Experiment 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 Experiment 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 Experiment 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 *.lsm 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".lsm.

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 Experiment 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 Experiment 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 4.0.12 Beta Version

Single Location	Multiple Locations
View 1	1: X=-93.50 Y=-96.00 Z=-76.80

List of Blocks: 5

BlkNo	Groups	G.Rep.	BkIntv	ZStack	AutoF	Bleach	Scans	Intvl
1	1	5	0	No	No	Yes	3	0
2	1	5	0	No	Yes	Yes	3	0
3	2	3	0	No	No	Yes	3	0
4	2	3	0	No	Yes	Yes	3	0
5	3	5	0	No	No	Yes	3	0

Add Block	Insert Block	Remove Blck	Start Group	End Group	Store/Apply

? | L-GR-G | GR-L-G | GR-G-L

Experiment Repetitions 2

Group Repetitionss:

Views: 1   5

Block 5 Parameters; Single Location

☐ All Loc's
☐ Autofocus (XY)
☑ Bleach (XY)
☑ Trigger In: Start/Pause (XY)

Wait Interval: (ms) [XY when All Loc] 0   ms

Define Autofocus	Define Bleach	Define Configuration

Configuration, Time Interval and Number of Scans

Multi Track (XY/View)   Multi1

☐ FindBC XY
☐ Pause XY
☐ ZStackXY
☐ ZHyper XY

?   Load Conf.   Save Conf.

No of Scans (XY/View):   3

Interval(XY/View):(ms)   0   ms

Trigger In to Start Block (XY)   None
Trigger Out at Block Start (XY)   None
Trigger In to Pause Block (XY)   None
Trigger Out at Block End (XY)   None

Ready

Z=-76.80   Current Pos: X=0.00 Y=0.00

Base File Name   New   ☐ Single File Output   ?

Select Image DB   C:\a_shared\LsmImages\MultiTime.mdb\MultiTime.mdb

Open Image DB   OpenTemp DB   At Location 1   Tile Mode

Close
Start Time
STOP Stop
Finish
End Block
Pause
Autofocus
Update
Center
Edit Locations
Move To Loc.
Next Loc.
Replace XYZ
Replace Z
Help
Options
Tile Utilities

Multi Time Series 4.0.12 Beta Version ✕

Single Location	Multiple Locations

| View 1 ▾ | 1: X=-93.50 Y=-96.00 Z=-76.80 ▾ |

List of Blocks: 5

BlkNo	Groups	G.Rep.	BkIntv	ZStack	AutoF	Bleach	Scans	Intvl
1	1	5	0	No	No	Yes	3	0
2	1	5	0	No	Yes	Yes	3	0
3	2	3	0	No	No	Yes	3	0
4	2	3	0	No	Yes	Yes	3	0
5	3	5	0	No	No	Yes	3	0

Add Block	Insert Block	Remove Blck	Start Group	End Group	Store/Apply

?	L-GR-G	GR-L-G	GR-G-L

Experiment Repetitions: 2 ◄ ─────── ►

Group Repetitionss:

Views: 1 ◄ ─────── ► 5 ◄ ─────── ►

Block 5 Parameters; Single Location

☐ All Loc's
☐ Autofocus (XY)
☑ Bleach (XY)
☑ Trigger In: Start/Pause (XY)

Wait Interval: (ms) [XY when All Loc] 0 ◄ ─────── ► ms ▾

Define Autofocus	Define Bleach	Define Configuration

Bleach

Config. (XY) [MultiBleach1 ▾] ☑ Spot XY

? Wait Delay(XY/View):(ms) 0 ◄ ─────── ► ms ▾

ROI (XY/View): [BleachFRET ▾] Get Spot

Ready

Z=-76.80	Current Pos: X=0.00 Y=0.00

Base File Name [New] ☐ Single File Output ?

Select Image DB   C:\a_shared\LsmImages\MultiTime.mdb\MultiTime.mdb

Open Image DB	OpenTemp DB	At Location 1 ▲▼	Tile Mode

Close
Start Time
STOP Stop
Finish
End Block
Pause
Autofocus
Update
Center
Edit Locations
Move To Loc.
Next Loc.
Replace XYZ
Replace Z
Help
Options
Tile Utilities

Multi Time Series 4.0.12 Beta Version

Single Location	Multiple Locations
View 1	1: X=-93.50 Y=-96.00 Z=-76.80

List of Blocks: 5

BlkNo	Groups	G.Rep.	BkIntv	ZStack	AutoF	Bleach	Scans	Intvl
1	1	5	0	No	No	Yes	3	0
2	1	5	0	No	Yes	Yes	3	0
3	2	3	0	No	No	Yes	3	0
4	2	3	0	No	Yes	Yes	3	0
5	3	5	0	No	No	Yes	3	0

Add Block | Insert Block | Remove Blck | Start Group | End Group | Store/Apply

? | L-GR-G | GR-L-G | GR-G-L

Experiment Repetitions: 2

Group Repetitionss:

Views: 1 | 5

Block 5 Parameters; Single Location

☐ All Loc's
☐ Autofocus (XY)
☑ Bleach (XY)
☑ Trigger In: Start/Pause (XY)

Wait Interval: (ms) (XY when All Loc) 0   ms

Define Autofocus | Define Bleach | Define Configuration

Autofocus

? | ☑ Use Cnf (XY) | Autofocus 633 nm 10x | (XY)

☐ Correct All Loc. | Load Conf. | Save Conf.

Fast Z Scan

Z Offset (XY): -20   µm

Find Z Offset

8

Z Range (XY): 100   µm

0

Scan Speed ☐ Use Threshold

☐ ZHyper XY

Return to Z before Autofocus

Ready

Z=-76.80 | Current Pos: X=0.00 Y=0.00

Base File Name | New | ☐ Single File Output | ?

Select Image DB | C:\a_shared\LsmImages\MultiTime.mdb\MultiTime.mdb

Open Image DB | OpenTemp DB | At Location 1 | Tile Mode

Close
Start Time
STOP Stop
Finish
End Block
Pause
Autofocus
Update
Center
Edit Locations
Move To Loc.
Next Loc.
Replace XYZ
Replace Z
Help
Options
Tile Utilities

Yellow Section indicates parameters which may be edited individually for each block and each location/view. Selecting block in the block list and location or view from the location or view combo boxes displays block and location specific parameters in the Yellow Area. These parameters can be edited individually for each block and location or view.

To copy the same parameters to ALL locations, simply switch to the **Single Location** mode and then select back **Multiple Locations** mode.

The same applies to the multiple views, switching from the single view (**Views** number set to 1) to multiple views (**Views** greater then one) copy first view parameters to all other views. New blocks created by **Add Block** have all the parameters copied from the previous block for all locations or views.

The Tabs: Define Autofocus, Define Bleach, Define Configuration switch between windows defining corresponding parameters.

Close: Ends the program.

Start Time: Starts the 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 location). (The very first block is executed immediately, without wait). Pressing **End Block** button terminates the wait period.

In **Multiple Locations** case one has an option to execute the **Block Wait** at each location, after stage moves to the next location (**All Loc's checkbox** checked), or only at the first location (**All Loc's checkbox** unchecked).

Autofocus (if selected) is performed next.

The **Block Configuration** specified in the **Define Configuration** window is loaded and time series of specified **Number of Scans** with specified **Interval** or **Delay** is executed. In particular, when Number of Scans is set to "1" the **Interval** or **Delay** is ignored. One can set the **Number of Scans** in the **Block** to "0" to use the block for **Autofocus** or **Bleach** only purpose, without the scanned image(s). The **Block Configuration** has to be compatible with the mode selected in the **Configuration Control** dialog.

All **Block Configurations** have to be of the same type: **Channel Mode**, **Lambda Mode** or **Online Fingerprinting** mode. The **Block Configurations** of the different mode then the one selected in the **Configuration Control** will not be loaded.

When optional **Bleach checkbox** is checked, the scans in the block are executed with the **Bleach** function according to the settings specified in the **Bleach Configuration** and in the region specified by the selected **ROI**.

In case of **Time Delay after Bleach** set to zero, bleach is executed after number of scans defined in the **Bleach Configuration** (Bleach After Number of Scans). Make sure that the Number of Scans in the Block is greater then Bleach After Number of Scans.

In case of **Time Delay after Bleach** greater then zero, the **bleach** is executed before the scan, and there is the delay of **Time Delay after Bleach** between bleach and scan. The value of the **Bleach After Number of Scans in the Bleach Configuration** is ignored.

Scan Mode is defined in the **Scan Control Mode** dialog: **Spot**, **Line** or **Plane** should be set in the **Scan Control** and not changed during experiment. **Multi Track** configurations store and reuse remaining **Configuration Control** and **Scan Control** parameters including number of slices in the **Z Stack** or **Z Scan** (check "**Options**"->"**Settings**"->"**Recording/Reuse**" parameters). **Single Track** configurations store only **Configuration Control** parameters. All other parameters are defined in the **Scan Control** window.

When **Z Stack** is checked, Z Sectioning or Z Scan is executed; otherwise only single slice at the selected X,Y,Z location is scanned. **Z Stack** check box is location dependent. The number of the z slices is stored in **Multi Track** configuration, or for **Single Track** configurations, is specified in the Scan Control. 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 **Pause** checkbox, when checked, pauses before the scan of each block. Press **Resume** button to continue

The block data is saved in the selected Temporary 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".lsm.

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

There is a possibility to create tiles of the same or different number of tiles at the different stage locations. Multiple tiles can be programmed by "**Add Tile Locations**" function in the **Edit Locations** -> **Tile** section of the program.

At the end of the data collection, one Time Series file with the images from all locations will be generated, and, in addition, separate stitched tile images. These separate stitched tile images might be 2, 3 or 4 dimensional, depending on the experiment design.

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: During block scan, Interrupts execution of the block saves the partial data and continues with the acquisition of the next block. During block wait/delay, terminates the wait/delay.

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.**

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.

Update: 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 locations are marked when Multi Time is running) one has to click **Update** button.

Help: Displays this document.

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

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

Next Loc: Moves the stage and the focus motor to the next location in the Multi Location list.

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

Replace Z: Replaces the focus motor coordinates of the selected location in the Multi Location list. Leaves the X and Y coordinates unchanged

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 locations in the Stage and Focus Control (different XY and Z locations), by creating grid, tile or Z Stack locations 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 Loc** button.

The Location List can be changed in **Edit Location menu,** by applying previously created Recipe or by marking locations in Stage and Focus Control and clicking the "**Update**" 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	300	No	No	Yes	3	0
2	1	5	1200	No	Yes	Yes	3	0
3	2	3	300	No	No	Yes	3	0
4	2	3	1200	No	Yes	Yes	3	0
5	3	5	300	No	No	Yes	3	0
Add Block	Insert Block	Remove Blck	Start Group	End Group	Store/Apply			

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

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.

Insert Block: Inserts new block before the selected one with the parameters equal to the parameters of the selected 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

Add Block | Insert Block | Remove Blck | Start Group | End Group | Store/Apply

? | L-GR-G | GR-L-G | GR-G-L

Experiment Repetitions 2

Views: 1

Group Repetitionss: 5

L-GR-G:
Loop Number of Experiment 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 Experiment 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 Experiment 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

Experiment 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 grater then 1, drop down list of Views becomes active and one can specify block parameters for each view separately.
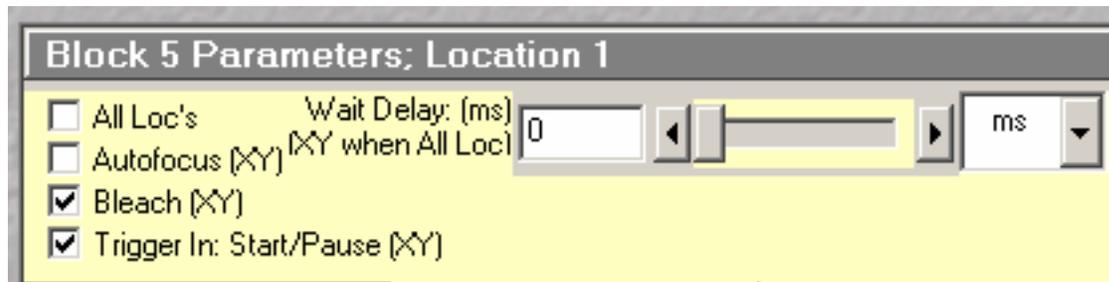
Wait Interval/Delay: The time the program will wait idle before starting the current block. The wait time is counted from the beginning of the previous block to the beginning of the current block (**Time Interval**) or from the end of the previous block top the beginning of the current block (**Time Delay**).
During that time user has full control of the microscope, can relocate the sample in XY and Z etc. During wait time, **Status Bar** displays remaining idle time and the **End Block** button is active.
Clicking on **End Block** 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.
When **All Loc's** is checked, one can program different time delay/interval at each location.

Autofocus: When checked, **Block** scan will be preceded with **Autofocus** function defined in **Define Autofocus** dialog.

Bleach: When checked, **bleach** function defined in the **Define Bleach** dialog is executed during **block** scan.

Trigger In: Start/Pause when checked **block** execution waits for the trigger signal specified in the **Define Configuration** dialog. If the **Trigger In to Start Block** is set to "None" **block** executes normally.

Block 5 Parameters; Location 1

☐ All Loc's
☐ Autofocus (XY)
☑ Bleach (XY)
☑ Trigger In: Start/Pause (XY)

Wait Delay: (ms)
(XY when All Loc) `0` ◄ ═══════ ► ms ▼

| Define Autofocus | Define Bleach | Define Configuration |

Autofocus

? | ☑ Use Cnf (XY) | Autofocus 633 nm 10x ▼ | (XY)
☐ Correct All Loc. | Load Conf. | Save Conf.

Fast Z Scan

Z Offset (XY): `-20` ◄ ═══════ ► µm
Find Z Offset

Z Range (XY): `100` ◄ ═══════ ► µm

8 ▼

Scan Speed ☐ Use Threshold `0` ◄ ═══════ ►

☐ ZHyper XY

Return to Z before Autofocus

Autofocus (XY) 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).

Correct All Loc. Check box: When checked, **Autofocus** z correction (the difference between z value from the location list and the z position found by the Autofocus) is applied to all locations – the z values of all locations are updated. This mode is designed to correct the focus drift for all locations by executing autofocus function on first location only.
When this checkbox is unchecked the z correction is applied only to the current location. This mode can be applied to dynamically find the correct scanning locations.

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

Load Conf. Loads the selected Autofocus configuration

Save Conf. Saves the selected Autofocus configuration. The program prompts if it is OK to overwrite.

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.

Find Z Offset: Automatically finds the z distance from the present z location to the reference plane and enters this value to the **ZOffset(XY)** field. The XZ scan is executed (like in **Autofocus** function) and additional image with two horizontal lines, one (green) at present z location and the other (yellow) at the reference plane is created. In addition, there is an intensity profile line (red) which is used to determine the reference plane (at the maximum or at the threshold).

Z Hyper: When checked, Piezo Objective Positioner (I present) or hyperfine z drive is used for Autofocus scan Piezo Objective Positioner has to be in the light path for the checkbox to be enabled.
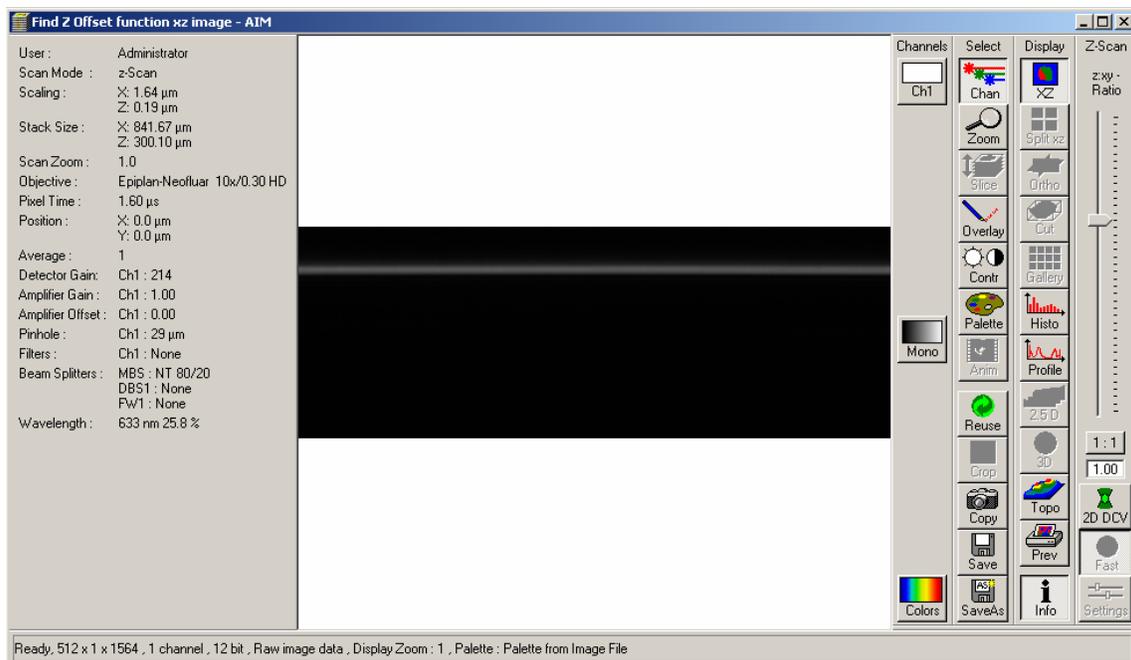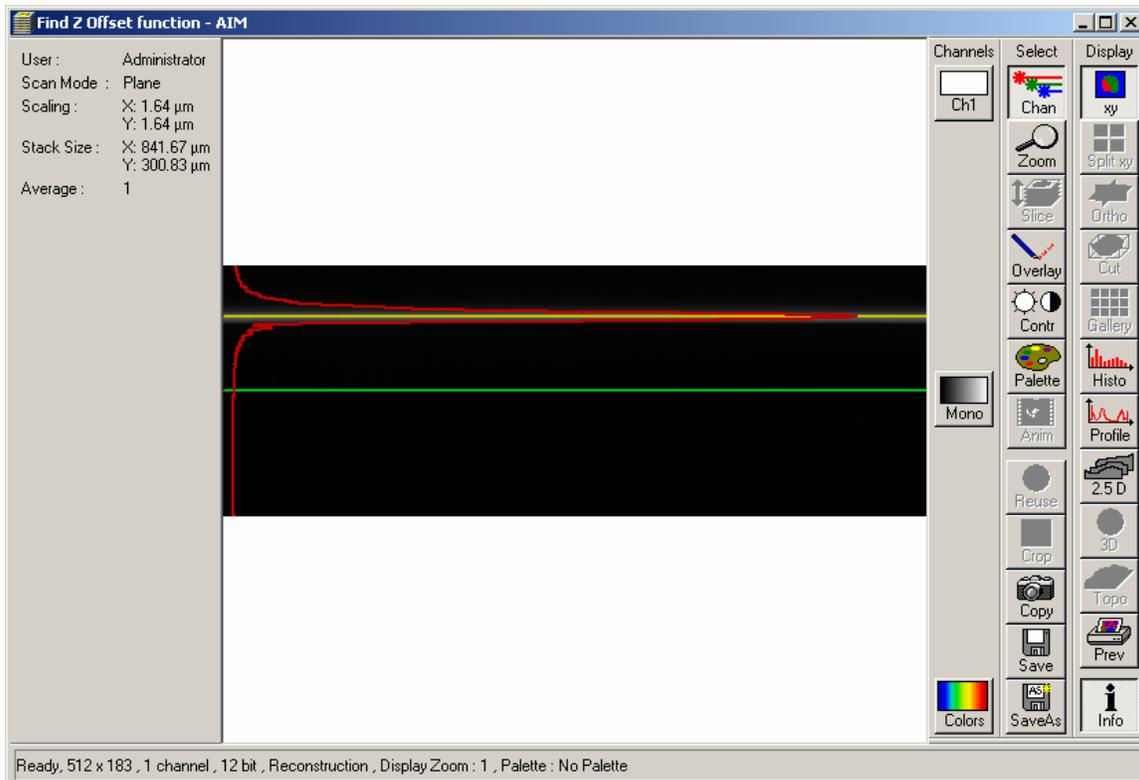
The **Z Range(XY)** should be greater then 2 times the expected value of the **Z Offset(XY)** if the starting value of the **Z Offset(XY)** is set to "0". Also, only in that case, both lines will be visible on the cross section image. **Find Z Offset** function will work with the smaller ranges of the **Z Range(XY)**, providing that the starting value of the **Z Offset(XY)** is close to the real value (differs from the extimate less than the half of the **Z Range(XY)** value). Afterwards, the **Z Range(XY)** can be narrowed to twice of the expected range of the focus drift. Execution of the **Autofocus** function afterwards moves the desired imaging plane into focus. Possible systematic error can be corrected by the **Autofocus Correction** value set in the **Options.**

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 then 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.
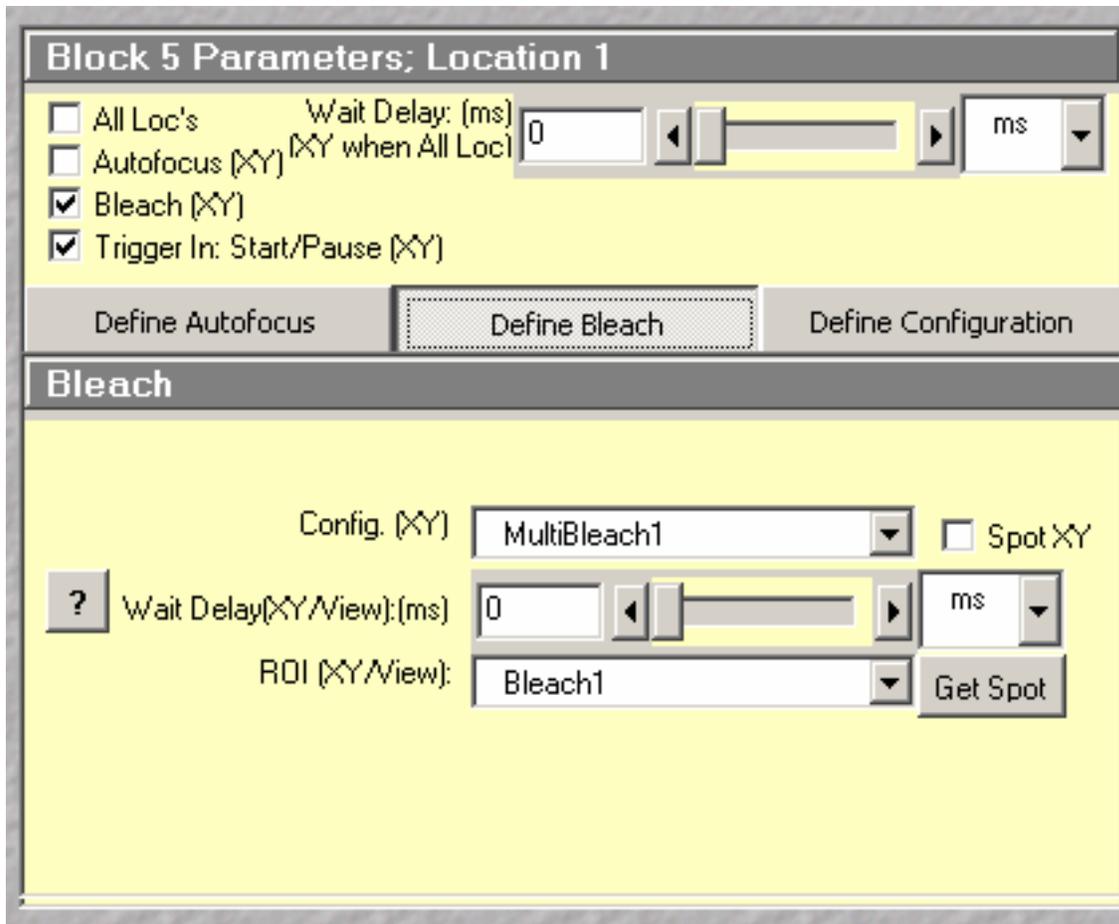
Bleach checkbox: When checked, bleach is executed in the given block, after autofocus (and possible wait) and before the data acquisition. **Bleach checkbox** can be checked only when **Bleach Configuration** and **Bleach ROI** are defined.

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.
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)., otherwise the "**Bleach After Number of Scans**" and "**Bleach Repea**t" parameters in the "**Edit Bleach**" are ignored and **Bleach** is done first, followed by the delay and the block scan.

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 then the image acquisition. The multi-track configuration specified in the **block**

configuration window is used during bleaching (defines scan speed, zoom, scan rotation and offset).The **bleach** track and bleach ROI defines laser attenuation and the ROI for bleach.
This option is activated when the number of scans in the block is set to 0.

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 Select. function in Scan Control Window to select the Spot location)

Single Track/ Multi Track: Configuration for data acquisition in the selected block. These configurations are location 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).

Load Conf. Loads the selected Multi or Single Track configuration

Save Conf. Saves the selected Multi or Single Track configuration. The program prompts if it is OK to overwrite.

Time Interval: Time interval between consecutive images or z-stack of images within the block. It is applied only when the **Number of Scans** in the block is greater then 1.

The selection between the **Time Interval** and **Time Delay** within block is done in "Options"-> "Settings"->"Time Series" settings.

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

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

Z Hyper checkbox: Is active only when Objective Piezo Positioner is in the light path. When checked and when Z Stack checkbox is checked, Z Sectioning is done with the Objective Piezo Positioner instead of microscope focus drive. In that case "Middle of the Z Stack" option is automatically selected (see **Options** section of this document).

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

Trigger In to Start Block: When **Trigger In Start/Pause** checkbox is checked, and **Trigger In to Start Block** is different from "**None**" **block** scan waits for the TTL signal on the specified trigger line.

Trigger In to Pause Block: when the selected trigger signal is received, block scanning is paused. The image scanned (Line, Frame or Stack) when trigger signal is received will be finished. The scan will be resumed when **Trigger In to Start Block** is received.

Trigger Out at Block Start: Selected trigger signal is sent at the beginning of the block. This signal can be used to synchronize external equipment

Trigger Out at Block End: Selected trigger signal is sent at the end of the block. This signal can be used to synchronize external equipment
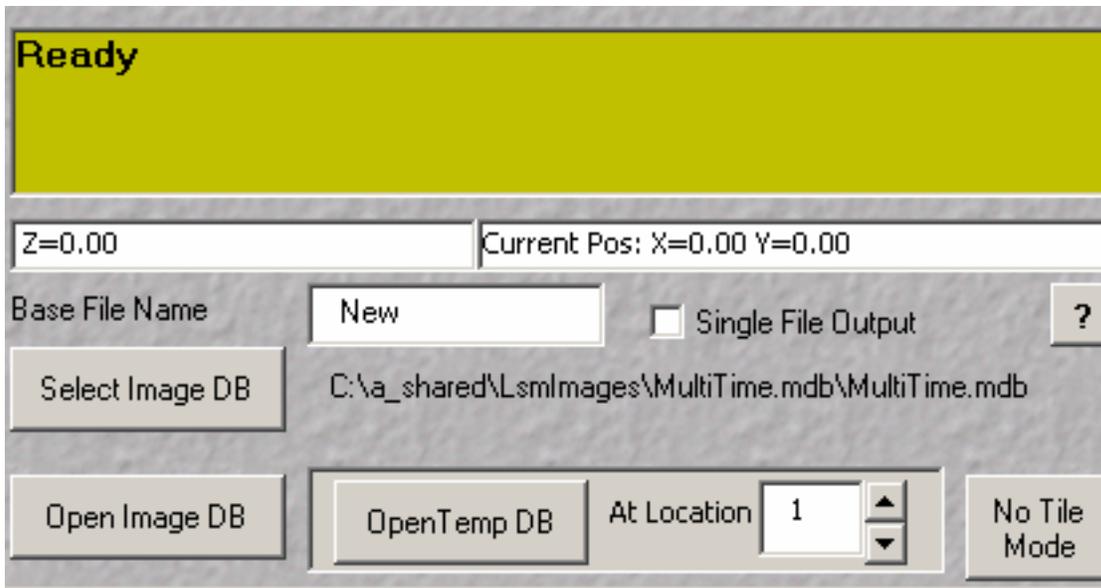
Status bar: Displays the current state of the system.

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".lsm.

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. This option is necessary in the Tile Mode. In that mode, in addition to the Single File Time Series, the tiled images are generated
        Time series for individual locations can easily be extracted from the single file using "Subset" function.

Open Image DB: Opens selected image database with final result images.

Open Temp DB: Opens the temporary image database from the selected location. Use **Location** edit field to select the location.
Note! Use **Options** to **Select Folder for Temporary Image Databases**

In **Options** one can select **Single Temporary Image Database** option.

Select Image DB: Selects the image database where the final images are stored:
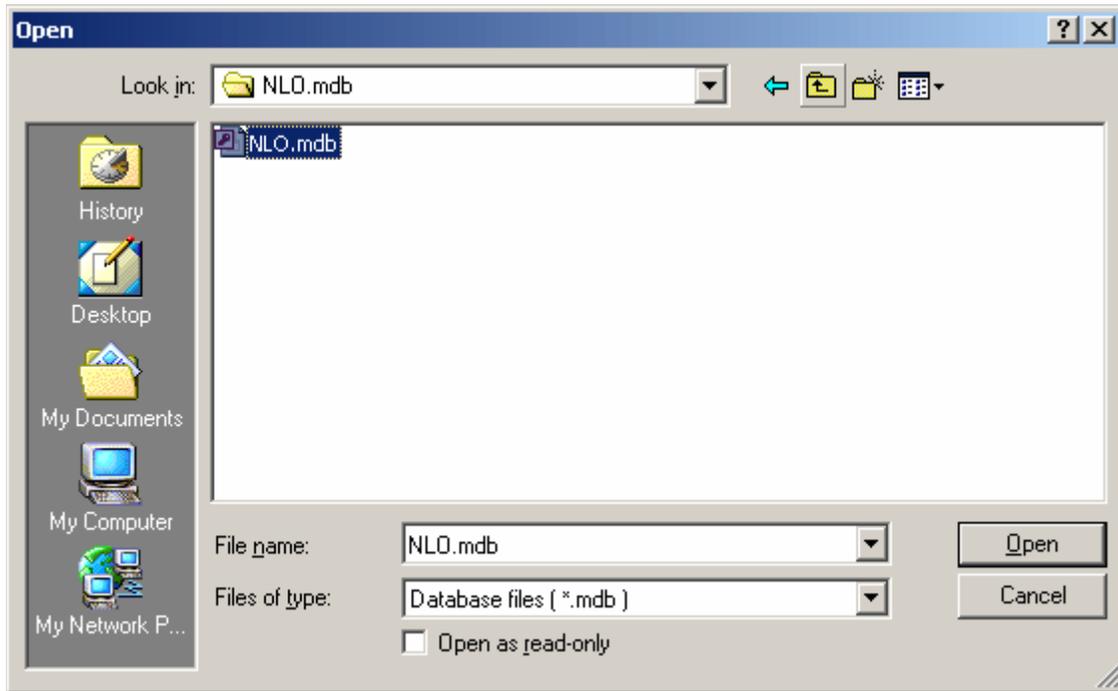
Open DB and Open TempDB opens specified Image Databases.

Options: Opens Options dialog box
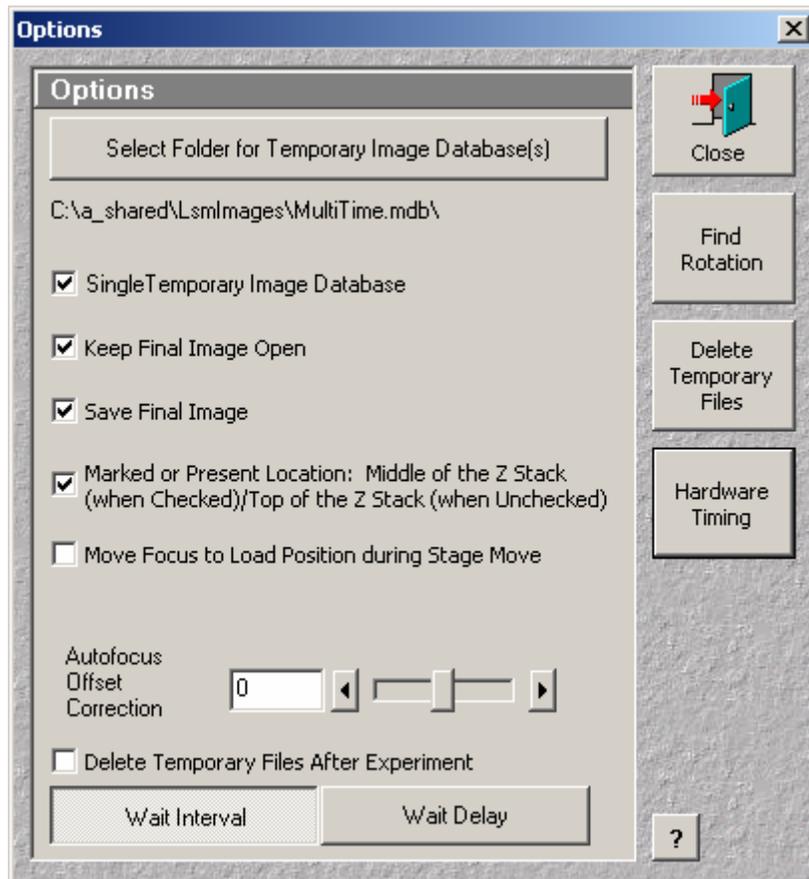
Select Folder for Temporary Image database(s): Selects the folder where intermediate image database(s) are stored: This function selects only the folder and the user do not have to provide any (valid) filename in the File name field. Files from each location/view are stored in separate Temporary Image 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.
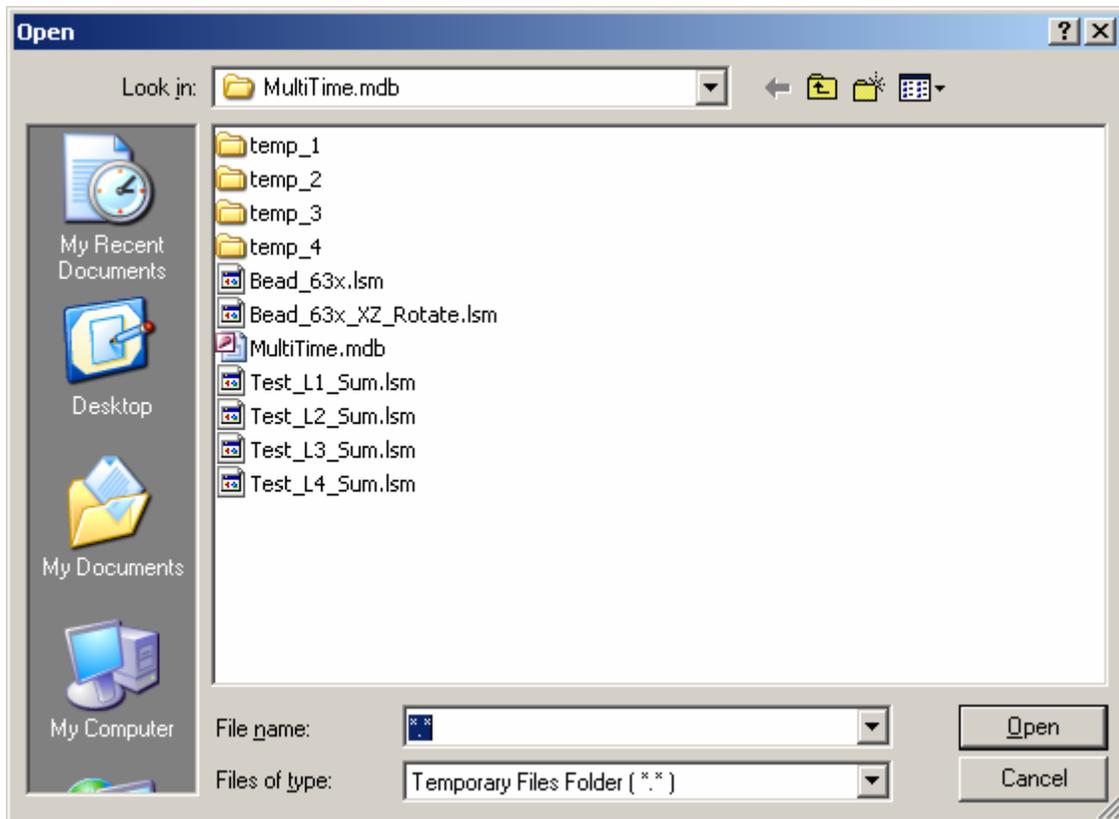
Single Temporary Image Database: When checked, ALL images from ALL locations are stored in the single Temporary Image Database: temp_1.mdb. This selection is convenient when creating tile images.

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

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

Marked or Present Location: Middle of the Z Stack (when Checked)/Top of the Z Stack (when Unchecked) - The current location (or each location 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 location. The z location is adjusted to the z location listed for the new location and corrected by the autofocus function.
Autofocus executed at one location corrects the z locations for ALL locations.

Autofocus Offset Correction value can be used to correct systematic error in **Find Z Offset** function. Otherwise it's value should be set to "0".

Wait Interval; Wait Delay: Toggle buttons which selects **Wait Interval** (time counted from the beginning of one block to the beginning of the next block); **Wait Delay** (time from the end of the previous block to the beginning of the next block).

Delete Temporary Files After Experiment – when checked, after the complete experiment user is prompted to delete ALL **Temporary Files** and **Folders.**

Delete Temporary Files– when clicked, user is prompted to delete ALL **Temporary Files** and **Folders.**

? Help

Hardware Timing opens dialog:
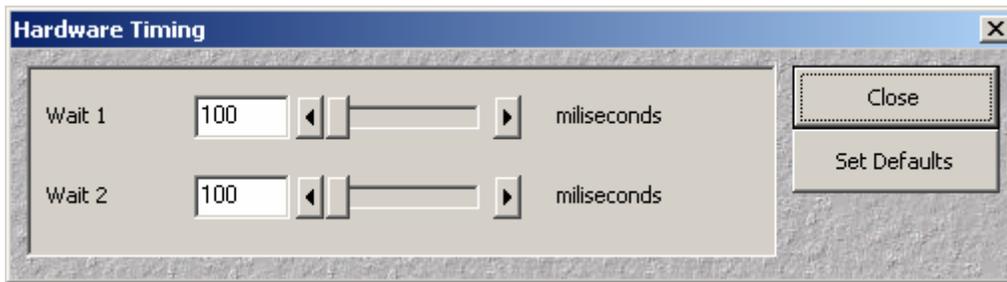
Set Defaults set default timing values (100 milliseconds for Wait1 and Wait2) Increase wait values if you experience timing problems

Edit Locations:
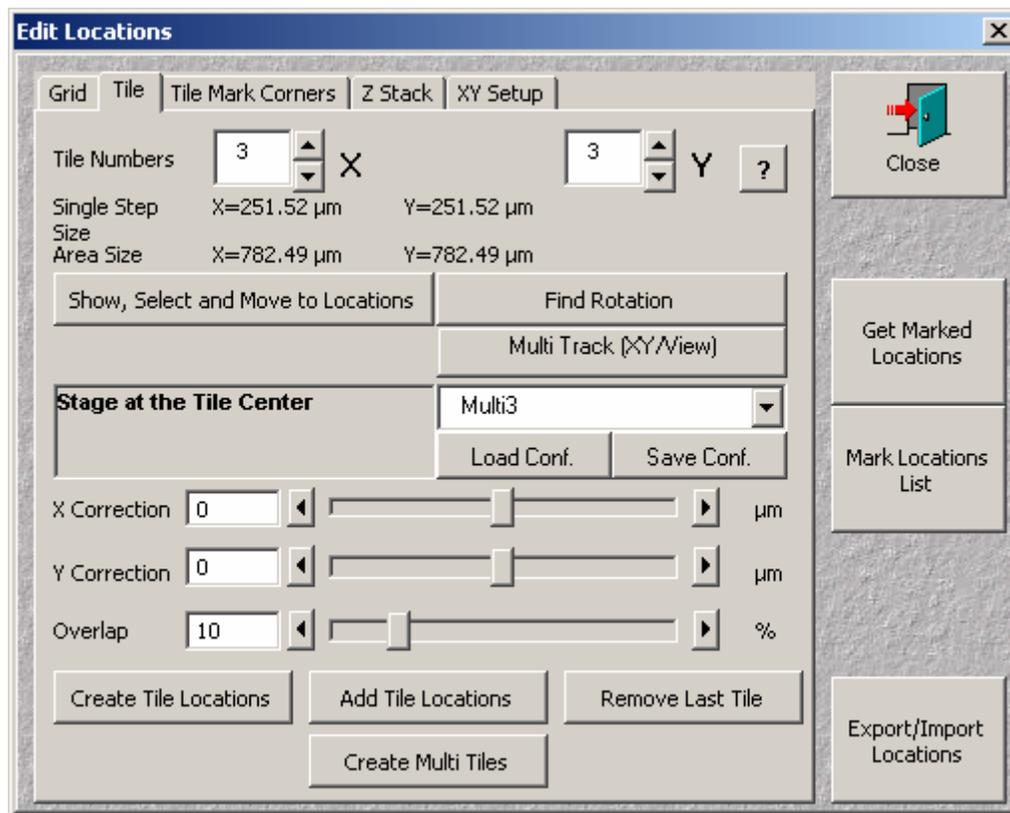
Warning! Edit Locations Loads Scanning Configuration Defined at the Selected Block and Selected Location!

Create Tile Locations: 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 scanning configuration used for the tile locations can be selected from the configuration list (either Single Track or Multi Track), available in the **Edit Locations** menu. The selected configuration is automatically loaded and the **Single Step Size** and the **Area Size** automatically adjusted to the zoom factor of the configuration.

The configuration can be modified and saved by pressing **Save Conf.** button. New zoom factor will be applied to the tile parameters.

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 **Experiment 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.

Add Tile Locations: Appends additional grid of locations of adjacent scan fields (of actual scan settings) to the existing list of tile locations. The current stage location is in the center of the new tile group.
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 **Experiment Repetitions** which will result in time series of tiles.
Start Time will create the **Single File Output** time series file and the multiple stitched tile files, one for each tile.

Remove Last Tile removes all locations added by the last **Add Tile Location**. One can repeated this procedure by removing coordinates of the last of the remaining tiles.

Create Multi Tiles: Automatically creates multiple tiles around locations which are originally in the location list. The original location list is overwritten with consecutive locations from consecutive tiles. Each original location is the center of the corresponding tile.

One possible scenario is to create the grid of locations for the multi-well plate and then to execute **Create Multi Tiles** with, say 3x3 tiles. This will create the appropriate list of locations.
The result of the experiment will be the set of 3x3 tiles (2 or 3 dimensional) from all well locations, each being the time series of the number of **Experiment Repetitions** time points.

Show, Select and Move to Location opens the graphical representation of the intended tile scan, with each tile represented by the square in the image.
Right mouse click on any of those squares moves the stage to that location.
One can visually (eyepieces) or by scanning inspect the intended scan area and enable or disable given tile. Disable tiles will not be included in the location list and will not be scanned. That will speed up the collection of images , by not scanning areas where there are no specimen. In the stitched tile image this areas will remain black.
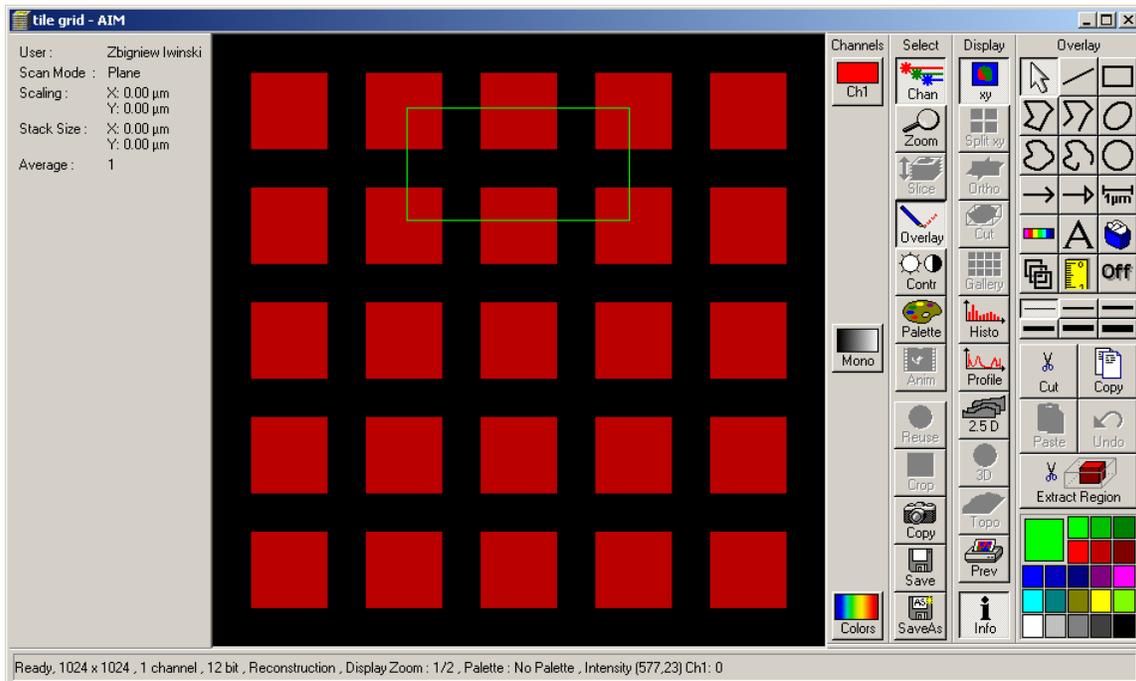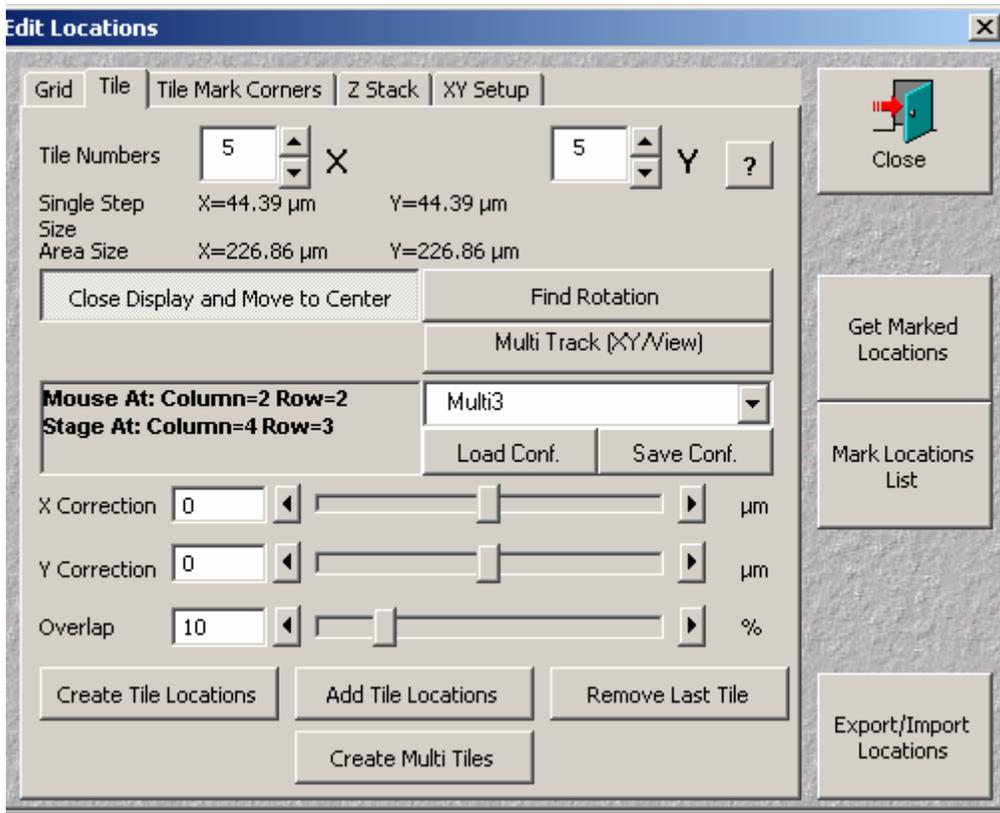
Moving the mouse with the left mouse button pressed, draws the rectangle on the image, which can be used for selecting or deselecting locations.
After releasing the left mouse button one is prompt:
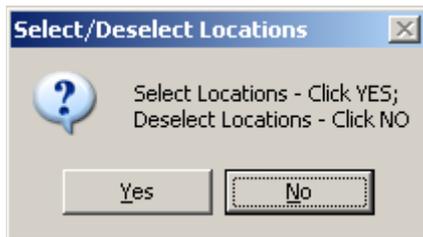
Replying NO results in deselecting corresponding tiles – these locations will not be scanned. This procedure can be repeated arbitrary number of times.
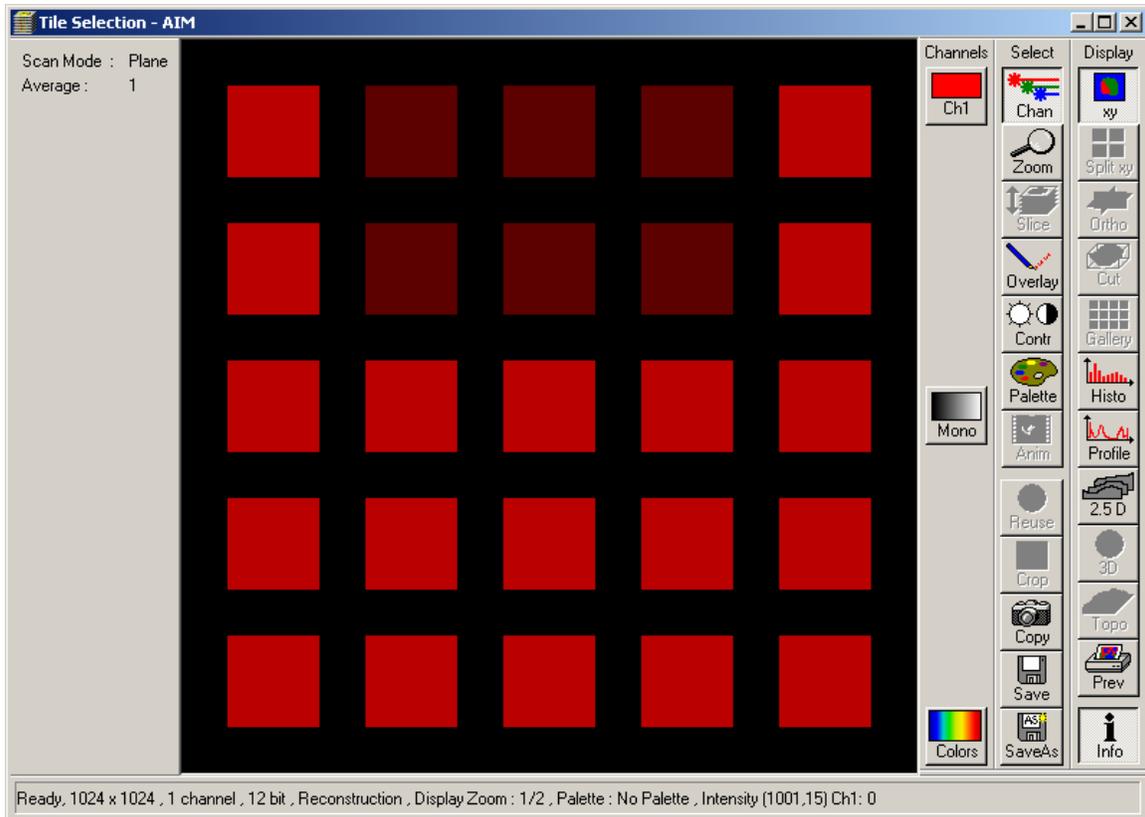
There is the **Status Window** in the **Edit Locations** menu informing about the stage location (due to the stage moves generated by the right mouse click on the corresponding square) and the mouse position:

Edit Locations

Grid | **Tile** | Tile Mark Corners | Z Stack | XY Setup

Tile Numbers: `5` ▲▼ X   `5` ▲▼ Y   ?

Single Step Size: X=44.39 µm   Y=44.39 µm
Area Size: X=226.86 µm   Y=226.86 µm

Close Display and Move to Center	Find Rotation
	Multi Track (XY/View)

Mouse At: Column=2 Row=2
Stage At: Column=4 Row=3   Multi3 ▼

Load Conf.   Save Conf.

X Correction `0` ◄ [─────────●─────────] ► µm

Y Correction `0` ◄ [─────────●─────────] ► µm

Overlap `10` ◄ [──●──────────────] ► %

Create Tile Locations	Add Tile Locations	Remove Last Tile
	Create Multi Tiles	

Close | Get Marked Locations | Mark Locations List | Export/Import Locations

Find Rotation corrects the possible rotation of scanners versus stage axis. This rotation is smaller then 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).

Find Rotation function automatically finds an angle between the scanner axis and the stage axis.
This parameter is also used in the TILE SCAN.

Tile Scan Rotation

Rotation [°] `-0.01` ◄ [─────────●─────────] ►   Calibrate   Close

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

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 correlation of 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 Locations replaces the current list of locations with the list of marked locations created in the **Stage and Focus Control** menu.

Mark Locations List replaces the list of marked locations in the **Stage and Focus Control** with the list of **Multi Time** locations

Export/Import Locations opens the following dialog box:
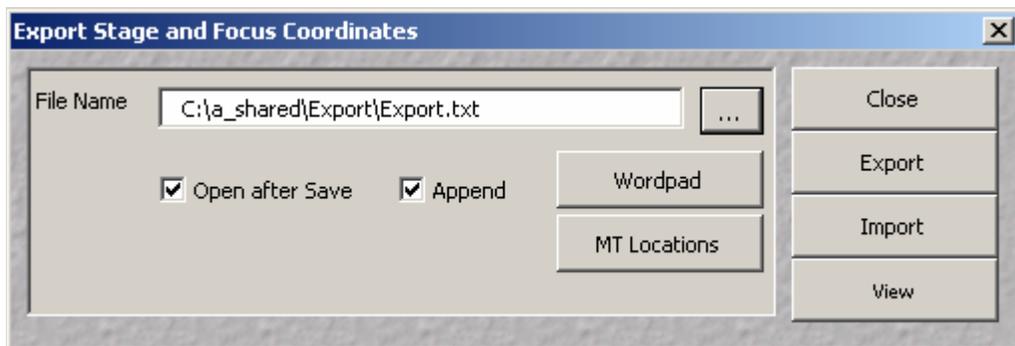
Export exports the list of coordinates from Stage **Marked Locations** or from **Multiple Locations** list into the selected text file:

Import imports the list of X, Y, Z coordinates from the Coordinates text File into the **Multiple Locations** list and, simultaneously, into the Stage **Marked Locations** if the **Marked Locations** option is selected.

The coordinate file has to have following format:

```
$Coordinates
File Name=   C:\a_shared\Export\Coordinates-new.txt
Number of Locations =     4
1      0.00   0.00   0.00
2      -200.00        0.00   20.00
3      -200.00        -400.00        -25.00
4      -600.00        -400.00        -50.00
```
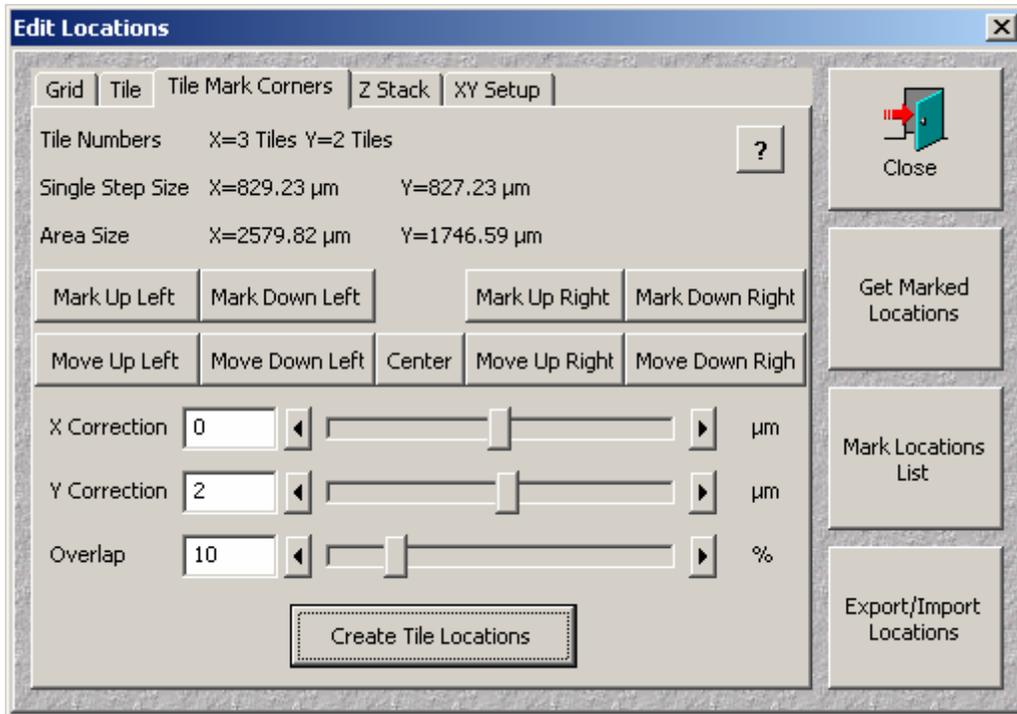
View displays the selected file, either in **Notepad** or in **WordPad** application.

Close closes the dialog box

Open after Save opens automatically exported file.

Append appends the existing text file with exported coordinates

? Help

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 location as the new Upper Left Corner of the Tile region. The Tile region is re-calculated and the number of tiles adjusted.
Mark Down Left: Selects the current stage location as the new Lower Left Corner of the Tile region. The Tile region is re-calculated and the number of tiles adjusted.
Mark Up Right: Selects the current stage location as the new Upper Right Corner of the Tile region. The Tile region is re-calculated and the number of tiles adjusted..
**Mark Down Right:** Selects the current stage location as the new Lower Right Corner of the Tile region. The Tile region is re-calculated and the number of tiles adjusted.

? Help
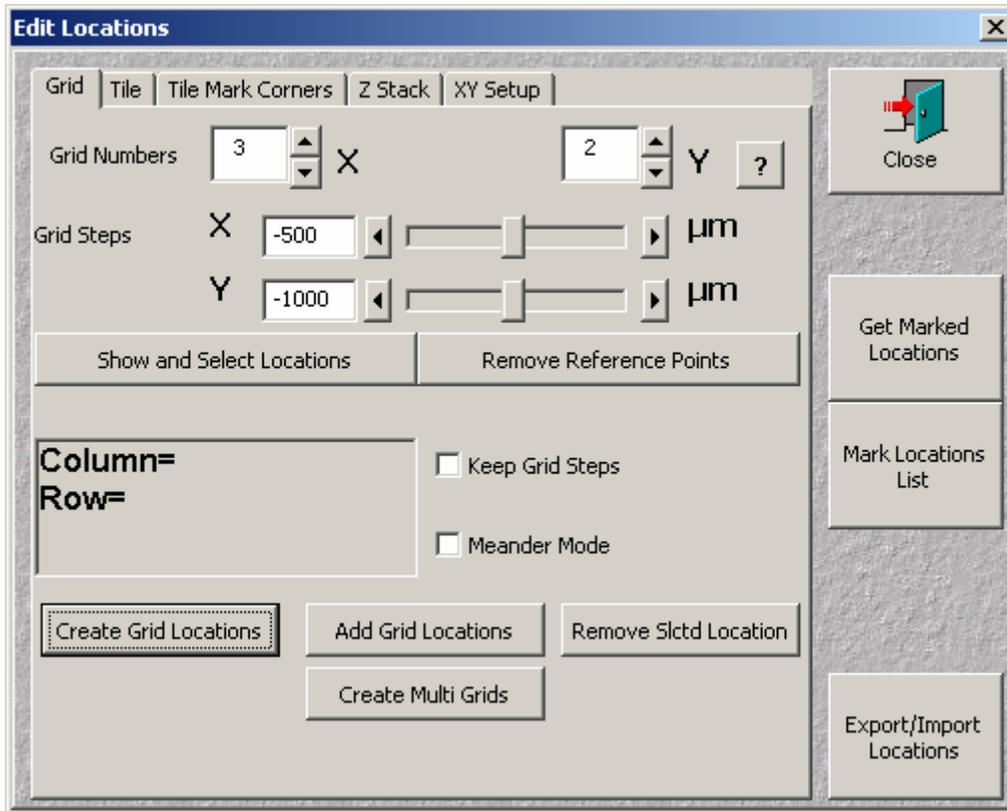
Create Grid Locations: Creates grid of locations with user defined steps in X and Y directions. The first location of the grid is at the present location, when no reference points are defined. User can interactively define the reference points, by positioning stage at the specific grid position and by providing information about the grid position at which the stage is located.

Show and Select Locations: Opens the window with the graphical representation of the grid:
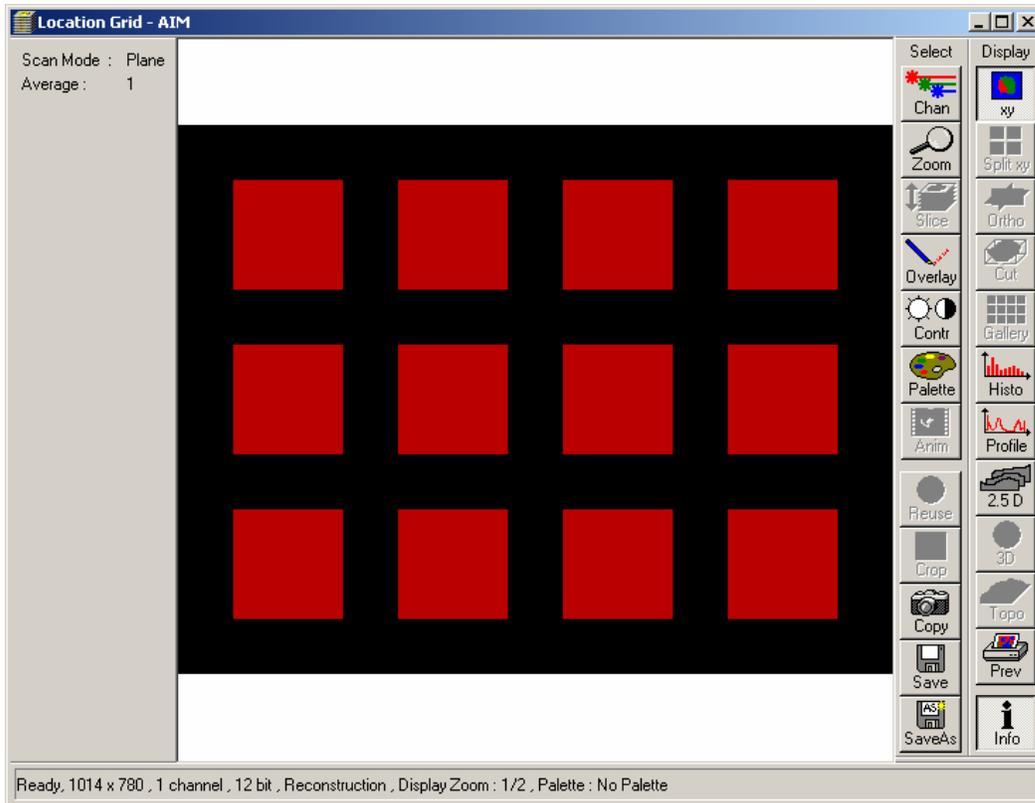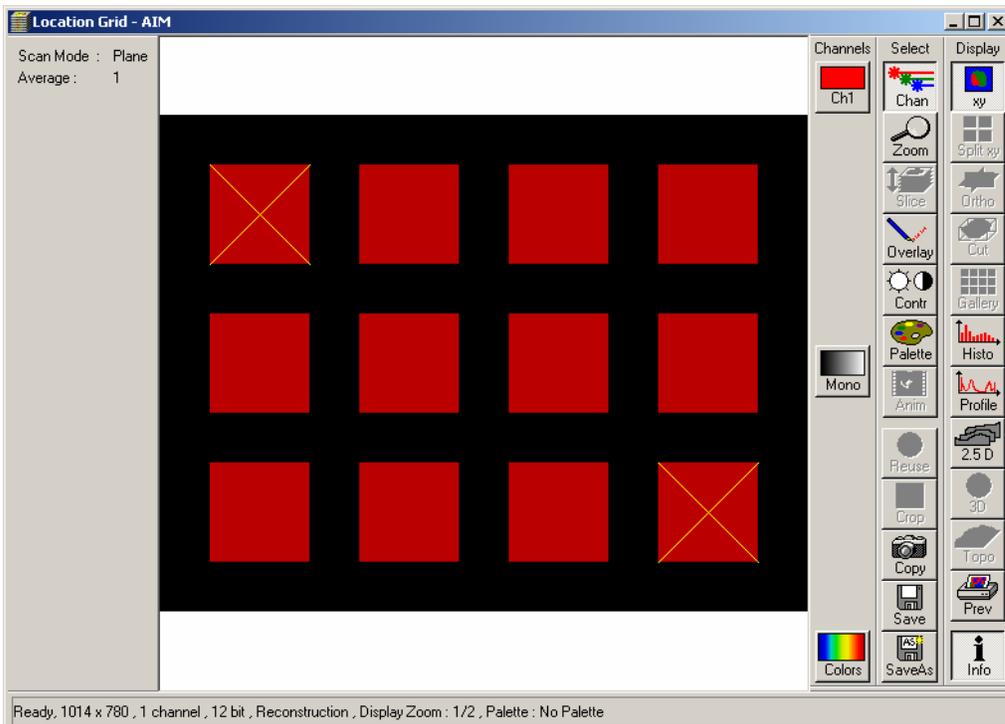
Right mouse click on the grid selects the reference point:
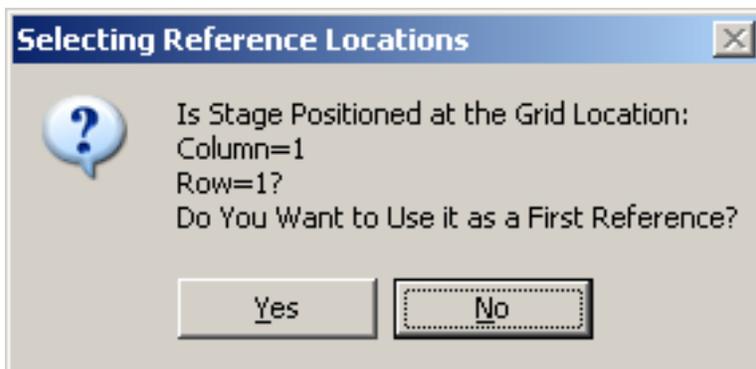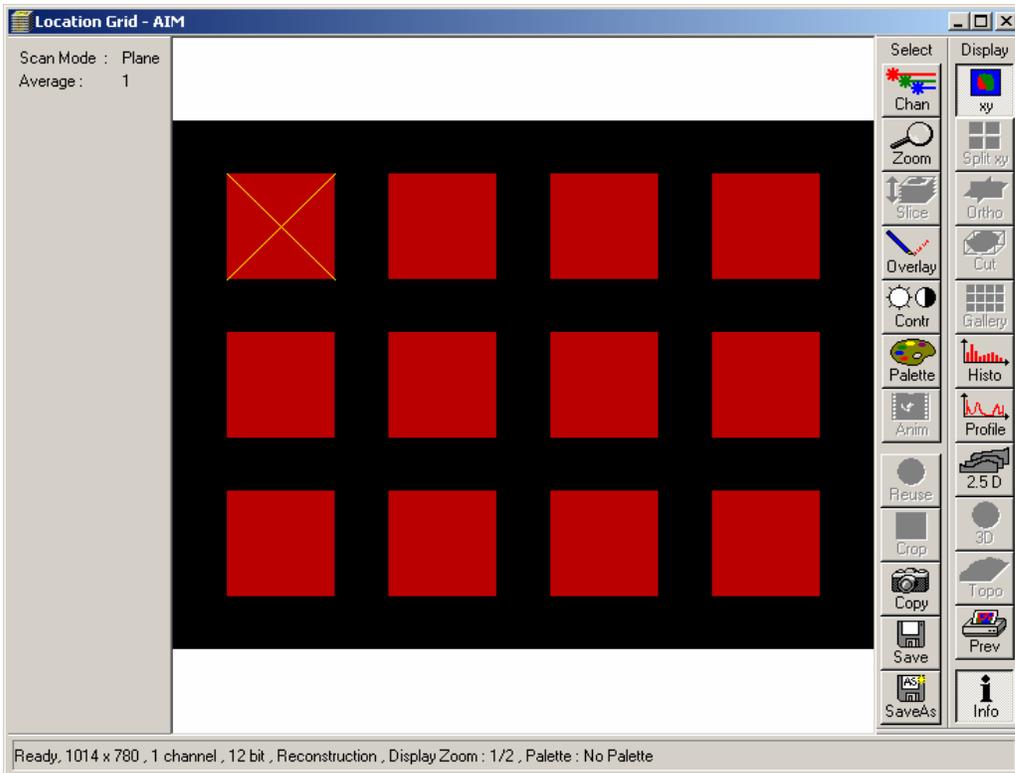
After selecting the second reference point program will automatically calculate the grid step in X and Y direction and the proper stage movement direction (positive or negative grid steps)

Alternatively, when the grid steps are known (from the multi well manufacturer), one can check **Keep Grid Steps** checkbox, and then two reference points are used to adjust stage positions on the reference points.

Show and Select Locations: One can select and deselect grid points for scanning by drawing the interactive rectangle with the left mouse button:
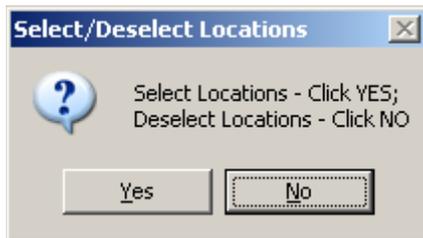
Deselected locations will be displayed with darker colors and will not be included in the location list.
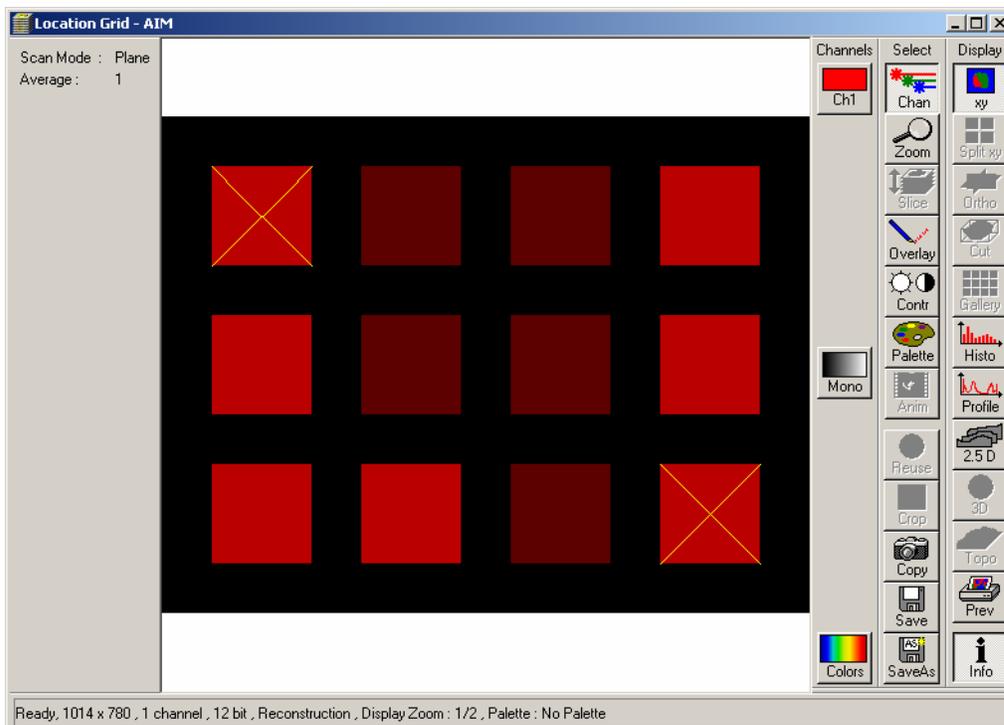
Remove Reference Points: Removes selected reference points

Create Grid Locations Creates the list of coordinates in comb (when **Meander Mode** checkbox is unchecked) or meander mode (when **Meander Mode** checkbox is checked). When no reference points are selected the current stage location is considered the grid location of the first row and the first column.

Otherwise the reference points define the grid.

Positioning mouse pointer over the active grid location displays the grid position and the location list order.

Create Grid Locations may also be used, when one wants to mark several locations, not arranged in any particular pattern.
First, one has to generate the appropriate number of locations, by creating, say, grid of 5x1 when 5 locations are needed.

Then one can navigate manually to the desired locations (either scanning or by visual inspection) and use **Replace XYZ** button to replace the placeholder location with the actual stage coordinates.

Remove Selected Location removes location selected in the **Multiple Locations** list.

Add Grid Locations appends the list of locations with the additional grid, specified by the new Grid Numbers and the Grid Steps. One can also deselect some of the grid locations by using **Show and Select Locations** tool.
In particular one can add single location at the present stage position by adding grid of 1x1.

Create Multi Grids: Automatically creates multiple grids around locations which are originally in the location list. The original location list is overwritten with consecutive locations from consecutive grids. Each original location is the first location of the corresponding grid.

One possible scenario is to create the grid of locations for the multi-well plate and then to execute **Create Multi Grids** with, say 3x3 sites and small X and Y steps to fit all 9 locations within each well. This will create the appropriate list of locations.
The resulting experiment will scan (2 or 3 dimensional images) consecutively at 9 locations arranged in the grid pattern in each of the wells.
When the number of **Experiment Repetitions** is greater then 1, one will result with the time series at each location.
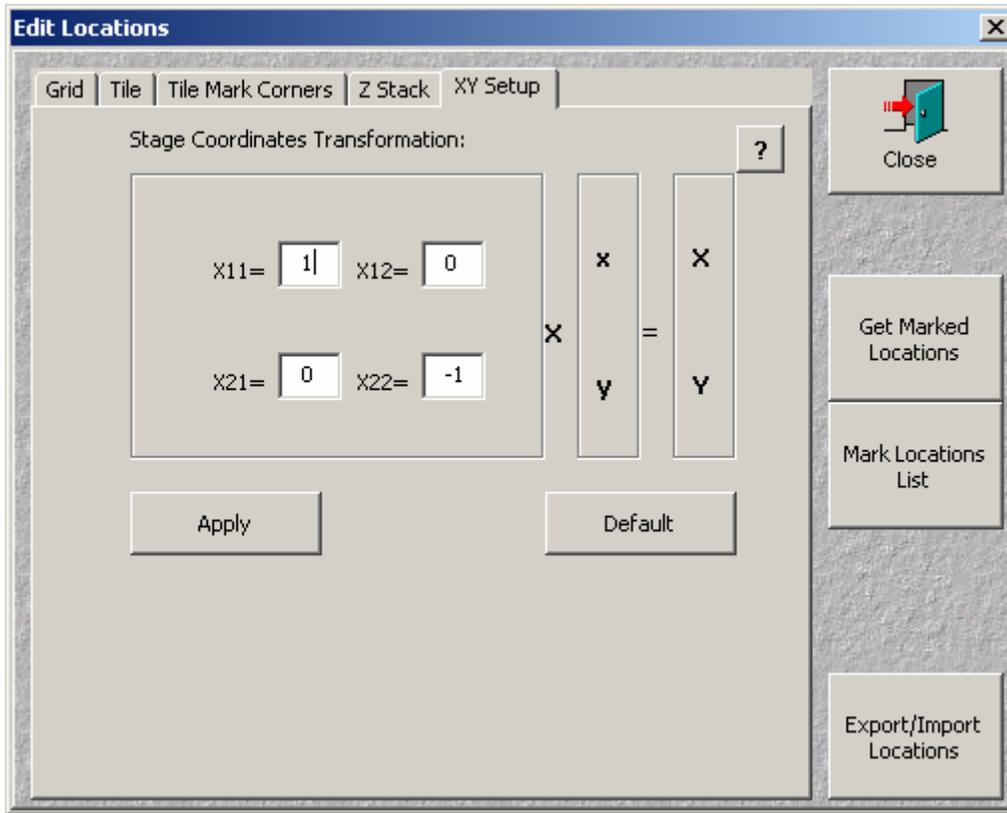
? Help
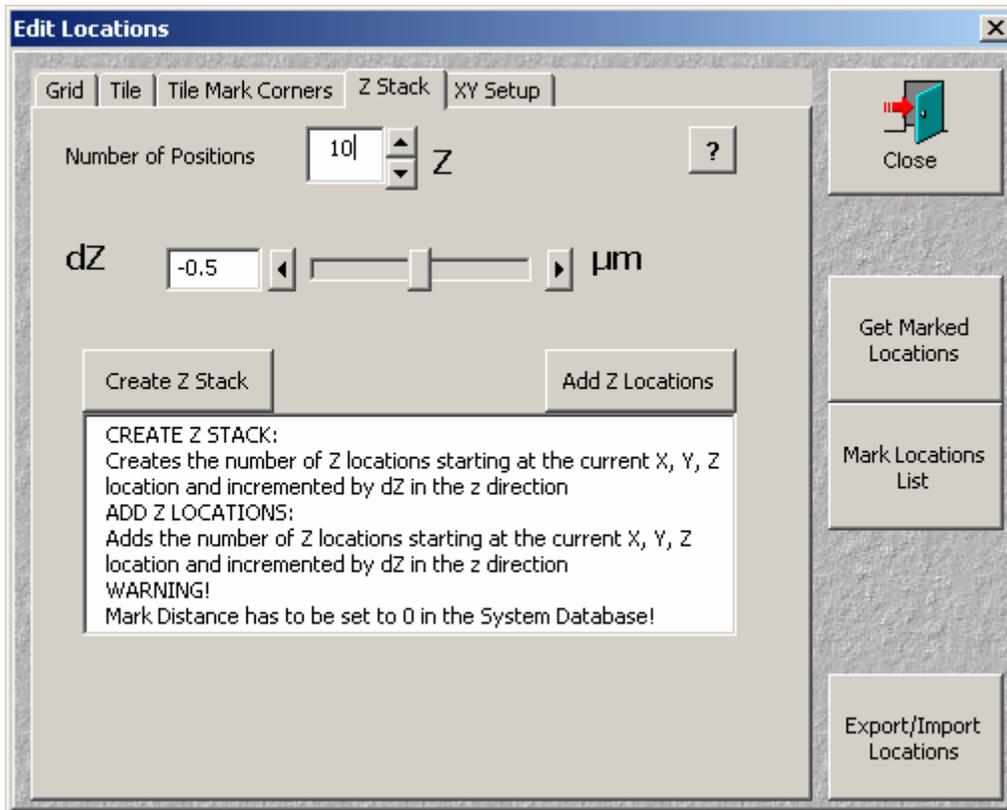
Create Z Stack: Creates the stack of z locations starting with the current x, y, z location, with user defined step **dZ** in Z direction.
Add Z Locations: Adds the stack of z locations starting with the current x, y, z location, with user defined step **dZ** in Z direction.
**?** Help

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.

? Help

Tile Utilities: Opens the following dialog:
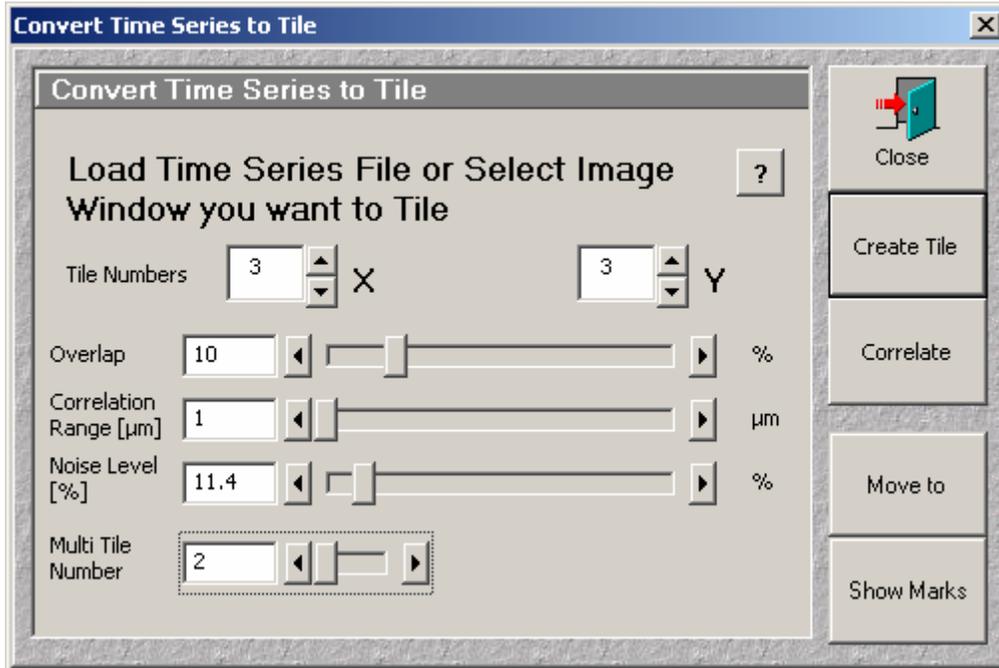
Create Tile converts selected Time Series File into a gallery of stitched images. Images from consecutive time slots are stitched together into (TileNumberX) x(TileNumberY) image.

In case when the **Time Series File** was created during the **Tile Scan** with the **Overlap** value greater or equal to 10%, one can use the **Correlate** button to create the **Tile** image with stitching improved by correlation of the adjacent tiles over the overlap region.

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 correlation calculations.

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

When the tiles were created in the Multi Tile experiment (more then one tile file were created in the time series experiment) the parameters of the individual tiles can be accessed by the **Multi Tile Number** slider.

The **Create Tile** and **Correlate** buttons stitch corresponding time series into tiles

according to the tile recipes in the **Multi Tile** experiment.

When the tiles were created with some disabled locations the stitching will be done accordingly.

The **Correlate** function **does not work** on the tiles with disabled locations (tiles which have empty spaces).

It is possible to stitch any time series file into tile, with the arbitrary arrangement of tiles in X and Y directions. To avoid stitching with empty spaces (in case when previous tile scan was with disabled location), switch off the previous tile mode by pressing **Tile Mode/No Tile Mode** toggle button to exit from the tile mode.

Then the stitching will place consecutive images from the time series into the consecutive rows and columns of the tiled image.

The original **tile** / **multi tile** experiment can be stored as a **Multi Time** recipe and later recalled

Move To activates the stage navigation with the use of the Tile Image. In case when Tile Image was created during the Tile Scan, by **Create Tile** or **Correlate** function, one can navigate to any stage location by clicking the corresponding pixel in the Tile Image. The proper navigation is ensured only when the Time Series Image was created in the **Tile Mode** and the stage coordinates are the same as during image acquisition.

? Help

Show Marks displays the **Marked Positions** from Stage Positions Mark List on the Tile Image
One can add Marked Positions by clicking the left mouse button on the desired pixel in the Tile Image.

Show Marks and **Move To** functions are similar to the functions in the Stage control.

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.
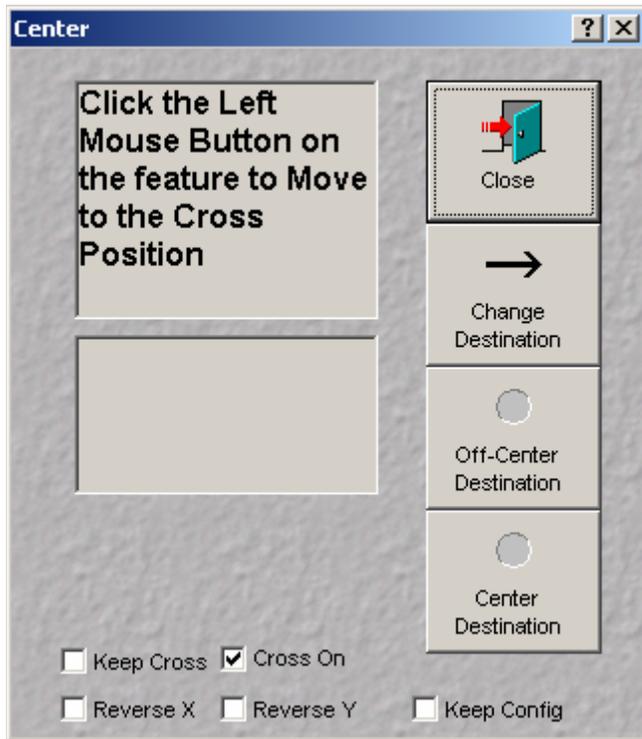
Modifications History:

New features in version 4.0.12

Hardware Timing option. Shorter time delays to speed up **Autofocus** function.
Click **Set Default** button for optimal hardware timing.
Corrected **Store/Apply** function

New features in version 4.0.11

Insert Block function.
Number of Locations increased to 900
X and Y correction parameter range in the TILE function increased to the range:
-200 microns to 200 microns (from -50 to +50)
After **Find Offset** function, MTS displays the parameters for the selected location
(in the previous versions the display returns to the first location)
Block Delay/Interval is location dependent when **All Loc's** checkbox is checked
Change of temporary files naming convention:
      When Multi Location or Multi View:
            When **L-GR-G** button pressed
                "BaseFile Name"_R"Repetition Number"_L"Location
                Number"_GR"GroupRepetitionNumber"_B"Block
                Number".lsm.
            When **GR-L-G** button pressed
                "BaseFileName"_R"RepetitionNumber"_
                GR"GroupRepetitionNumber"_L"Location Number"_
                B"Block Number".lsm.
            When **GR-G-L** button pressed
                "BaseFileName"_R"RepetitionNumber"_
                GR"GroupRepetitionNumber"_
                B"Block Number"_L"Location Number".lsm.
      When Single Location
                "BaseFileName"_R"RepetitionNumber"_
                GR"GroupRepetitionNumber"_
                B"Block Number".lsm.
New Grid and Tile functionality:
      Selecting and deselecting Grid or Tile locations
      Adding tiles and/or grids.
      Deleting Tiles and Locations.
      Creating Multiple Grids
      Creating Multiple Tiles
      Interactive tool for creating Grid and Tile locations.

New features in version 4.0.10

Block parameter definitions split into three tabs:
Define Configuration
Define Bleach
Define Autofocus
Each block (at each location) can be optionally started and/or paused on trigger,

and one can program trigger out signals independently for each block.
Autofocus can optionally work with Hyperfine Z drive.
Z correction determined by **Autofocus** can be applied to all locations, or only to the location where autofocus is performed.
New, interactive **Grid** design features

New features in version 4.0.9

Help Files are in the PDF format (Please install Acrobat Reader)

Add Tile Locations: Appends additional grid of locations of adjacent scan fields (of actual scan settings) to the existing list of tile locations. 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 **Experiment Repetitions** which will result in time series of tiles.
Start Time will create the **Single File Output** time series file and the multiple stitched tile files one for each tile.

Add Tile Locations provides the method of scanning multiple tiles of images at different locations once or over time.

The images scanned with the Multi Time have the stage and the focus coordinates stored in the file in the "Tags" section of the file header (see file format document: LsmFileFormats40.pdf

The temporary files from the scan of each block have the following tags:
Tag Name   Value

Tag Name	Value
Z_Coordinate	**0**
X_Coordinate	**94.5030953586075**
Y_Coordinate	**-93.5030953586075**

Concatenated file of two time points have the following tags:

Tag Name	Value
X_Coordinate_At_T_Stamp_1	**510.993809282785**
Y_Coordinate_At_T_Stamp_1	**-93.5030953586075**
Z_Coordinate_At_T_Stamp_1	**0**
T_Stamp_1(s):	**0**
X_Coordinate_At_T_Stamp_2	**700**
Y_Coordinate_At_T_Stamp_2	**-93.5030953586075**
Z_Coordinate_At_T_Stamp_2	**0**
T_Stamp_2(s)	**2.17198107580771**

X_Coordinate_Tile   **700**
Y_Coordinate_Tile   **0**

Tiled Image has the following tags:

X_Coordinate_Tile   **700**
Y_Coordinate_Tile   **0**

New features in version 4.0.8
Area of Block specific parameters is displayed in light yellow color.
Buttons with question marks provide the function specific help.
Buttons operating on motorized stage have dark gray color.
Selection of the "Temporary Image Folder" and the selection of "Single File Output" moved to the "Options".
"Find BC'" and "Pause" checkboxes are Location/View dependent.
"Z Hyper XY" checkbox activates Hyperfine "Z Scan" or "Z Sectioning".
This checkbox is only active when Piezo Objective Positioner is present in the system and the Positioner is in the light path.
This location dependent checkbox works only in combination with "Z Stack XY"
"End Block" button, when pressed during "Block Delay", terminates the delay and starts immediate scan of the block.
"Pause" button pauses the execution of Multi Time Script (the current single scan – line, plane or stack will be finished). If the script is paused in the middle of the block (block with more then single scan) the block parameters and the focus position cannot be changed.
However when the "Pause" is pressed during execution of the block with the single scan the block is finished, focus and stage are moved to the next location and new configuration is loaded.
User can modify parameters of the block, which is about to scan, during block wait time or when Pause is pressed.

All temporary image files and the final concatenated time series files have X,Y and Z (stage and focus) coordinates stored in the file tag section (see "LsmFileFormats40.pdf" document).

New features in version 4.0.7
Bug fixing:
      Blocks with 0 scans
      "Pause" checkbox
      Unexpected switches to the "Line" scan mode

New features in version 4.0.6
Minimum Autofocus step in the "Step" mode set to 0.1 microns

Help file in PDF format (requires Acrobat 7, otherwise RTF help file)

New features in version 4.0.5
Find Offset function displays the intensity profile used by Autofocus to determine reference plane.
Correction to Autofocus - drift problem
Corrected Autofocus on LIVE
Systems with tunable lasers (e.g. Chameleon or Mai Tai):
        Blocks with the different Multi-track configurations with the different laser wavelengths of the tunable laser can be used to dynamically tune the laser and scan with the different wavelengths.
        To ensure that the laser is tuned and mode-locked before the scan is done, use the block delay/interval of minimum of 10 seconds.

New features in version 4.0.4
Navigation on Tile Image created by **Create Tile** or **Correlate**
Bug fixing of version 4.03

New features in version 4.0.3
Correction of the initialization errors in Multi Views.
Correction to the XYZ navigation with the focus move to the Load Position at each stage move.
Prompt to load scanning configuration, when selecting "**Edit Locations**" dialog box.

New features in version 4.0.2
The same functionality as 3.5.4

New features in version 3.5.4
The same functionality as 3.2.Z5
Functional Mapping is available as a separate program

New features in version 32Z5
Find Z Offset function in **Autofocus**. Automatically finds the z distance (**Z Offset**) from the desired scan focal plane to the reference plane
Delete Temporary Files button in **Options**. Delets **Temporary Files** and **Folders**.
Delete Temporary Files After Experiment checkbox in **Options**. Automatically delets (after prompt) **Temporary Files** and **Folders** at the end of the experiment.
Bug Fixing in **Tile Utilities**

New features in version 32Z4
Pause checkbox, pauses before the scan of the block. Press **Resume** to

continue.

New features in version 32Z3

Import Stage and Focus Coordinates from the text Coordinate File

New features in version 32Z2
1 Fast Functional Mapping
2 Import and Image Display of Electrophysiology data
3 Trigger Out before and after each uncaging event
4 Correction of the **Tile Utilities**

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.
Export Coordinates List
Navigation and marking on the Tile Image.
New functionality in the Functional Mapping.
Autofocus on the LIVE systems.

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, Aufofocus 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 prevous 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 >= 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.