Macro solutions:

The LSM 5 lets you implement added LSM software functionality and completely automated problem solutions controlled by macros including everything from LSM setting control to image processing and documentation. Different LSM software versions require specific macro versions, which are indicated by the release number.
This appendix describes a collection of macros available for LSM software release 3.2, 3.5 and 4.0 (some additionally for releases 2.8 and 3.0).
Some macros have been developed for specific hardware configurations.

Table of content:

How to assign macro buttons and start macros:

- Click **Macro** button on **LSM – Expert Mode**

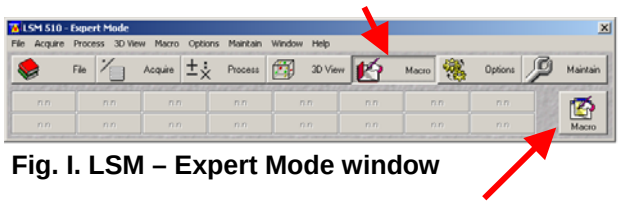


Fig. I. LSM – Expert Mode window

- Click **Macro** icon to open **Macro Control**
- Click **Assign Macro to Button** (Fig. II).
- Choose button number for macro.
- Click " **…**" button, to open the macro file dialog

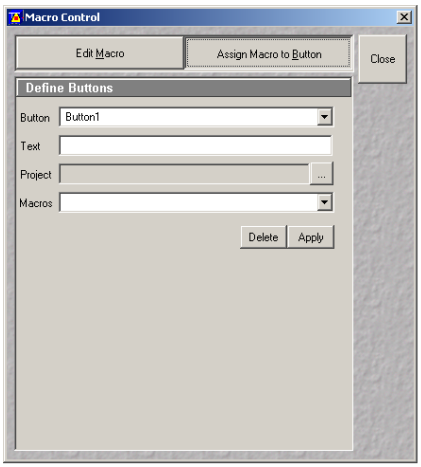


Fig. II. Macro Control dialog

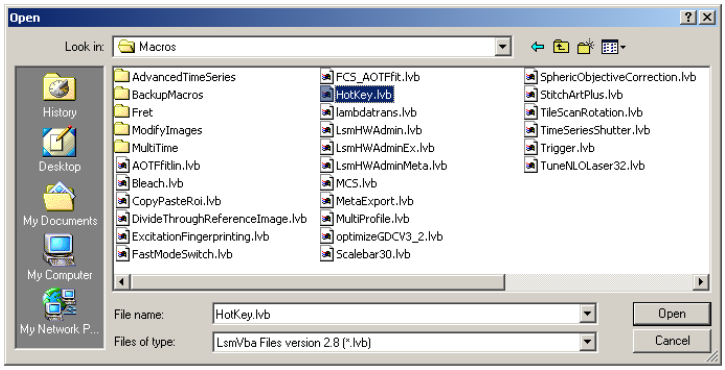


Fig. III. Macro file dialog (default path: C:/AIM/Macros)

- Open macro file. Linked path will be shown in **Project** field (**Macro Control** window).
  Type name of macro button in **Text** window (e.g. Hot KEY).

- Click **Apply** button to assign macro button.

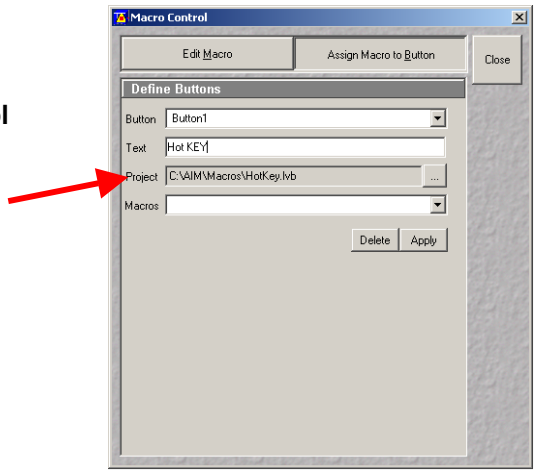


Fig. IV. Macro Control with filled windows

- To open the macro click on assigned macro button.

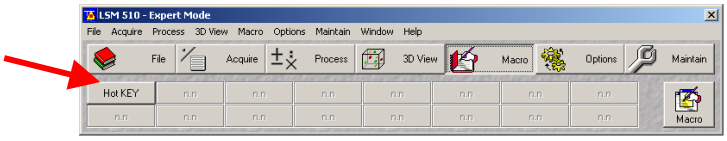


Fig. V. LSM – Expert Mode window with assigned macro button

- For further macros assign button 2 to button 16.

1. Macro name: **Copy Paste Overlay**

Filename: CopyPasteOverlays28-35.lvb
CopyPasteOverlays40.lvb

System requirements: LSM 5 system, software release 2.8 to 4.0

Short description: Copies drawing element of overlay into clipboard and pastes it into other selected windows

Description:
Copy Paste Overlay macro is used to transfer drawn overlays from one image to another.

Copy an overlay and paste it to an other image:

- Draw overlay in one image.
- Click **Copy** button in **Copy Paste Overlay** macro (then **Paste**, **Replace** buttons become active). If one specific overlay element is selected this element will be copied, otherwise all overlay elements of the active window will be copied.
- Select another image, time series or z- stack and click **Paste** button. The copied overlays will appear in the new image, series or stack.

Note: The existing overlays in the destination image will remain unchanged. **Replace** button replaces existing overlays in the destination image with the overlays from the source image. (Version 3.2: Overlays can not be copied out of lambda stack images and or be pasted into them.)

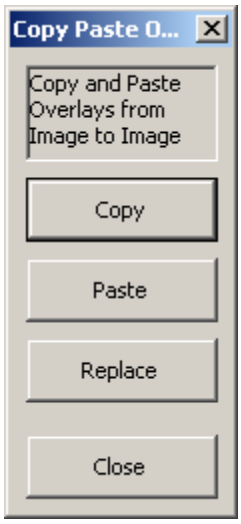


Fig. 1.1 Copy Paste Overlay macro window

The buttons of Copy Paste Overlay are:

Copy button: Copies the active overlay into the clipboard. If no specific overlay is active, all overlays in the active window are copied.

Paste button: Pastes the clipboard to the active image window.

Replace button: Replaces the activated overlay by the clipboard. If no specific overlay is active, all overlays in the active window are replaced.

Close button: Closes the **Copy Paste Overlay** macro.

2. Macro name: **Copy ROI to Overlay**

Filename: CopyRoisToOverlay28-35.lvb
CopyRoisToOverlay40.lvb

System requirements: LSM 5 system, software release 2.8 to 4.0

Short description: **Copy ROI to Overlay** macro transfers ROI drawings to the overlay interface.

Description:
The **Copy ROI to Overlay** macro converts ROI (Region of interest) drawings to overlay elements.

Convert ROI to overlay:

- Draw ROI or activate ROI from ROI List in **Edit ROI** menu
- Open **Copy ROIs to Overlay** macro
- Click **Copy Scan ROIs** button on the **Copy ROI to Overlay** menu. The ROI will be converted to an overlay.

Note: Copy Scan ROIs button converts an active ROI to an overlay and copies this overlay to the clipboard. The **Add Scan ROIs** button pastes the clipboard to the active window. This macro can be used for showing ROIs and overlays at the same time in one window or attaching an outline of a scan ROI to an image for annotation and export.

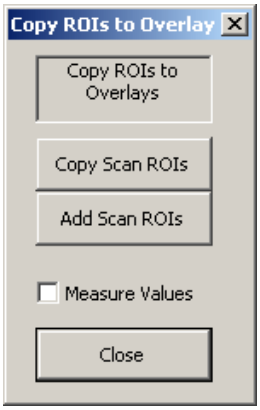


Fig. 2.1 Copy ROIs to Overlay macro window

Copy Scan ROIs button: Copies scan ROIs to the clipboard

Add Scan ROIs: Pastes the previously copied scan ROIs to the active image window as an overlay item.

Measure Values check box: If checked, area A and circumference I of ROIs are shown.

Close button: Closes this macro.

3. Macro name: **Divide through reference image**

Filename: DivideThroughReferenceImage-32.lvb
DivideThroughReferenceImage-35.lvb
DivideThroughReferenceImage40.lvb

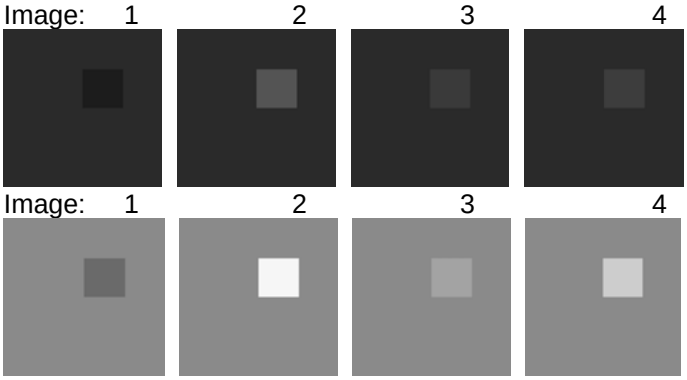
System requirements: LSM 5 system, software release 3.2 to 4.0

Short description: Divide complete time series through a single image/part of the series
Duplicates a single image or part of a time series.

Description:
This macro extends the possibilities of image processing (add, subtract, ratio, copy, dup….). **Divide through reference image** macro divides the images of the time series through the selected reference image in that time series and multiplies it by a selected factor. The macro assigns the areas of the image, which do not change over time a constant intensity value (equal to the "Factor" value) and visualizes the areas which are changing over time.

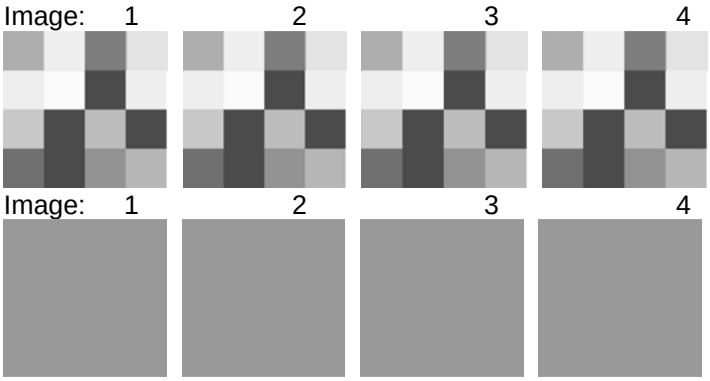
Following equation is used for the calculation: Q = new intensity value, D(x) = intensity value of pixel x, F = factor chosen by the user, S(x) = intensity value of pixel (x) in reference image
→ Q = D(x) * F / S(x).

Example: Image 3 in a series of 4 images is chosen to be the reference image. The intensity of pixel x in the reference image is 10 (grayscale value between 0 and 255). This pixel has a value of 5 in image 1, value 20 in image 2 and value 15 in image 4. After calculation with the **Factor** 100 the intensity value of pixel x in image 1 is 50, in image 2 it is 200, in the reference image the intensity is equal to the factor 100 and in image 4 the intensity has a value of 150.



→ The over all intensity level is raised and the differences of the intensity values are greater.

Looking at different pixels in which the intensity value stays constant in the series, no matter which intensity value the pixel has, the pixel will have the same intensity value as the chosen factor in the resulting series. For example: pixel *a* has the intensity value 40 in all images of the series, pixel *b* has in all images the value 200, these pixel will show the intensity value of the factor (e.g.100) in the calculated image series.



Duplicate button in this macro duplicates a selected reference image into anew series with the same number of images like the original series and can be used for subtraction.
Example: A series of 4 images is selected and one of the images represents background information. Choose this image as a reference image. The **Duplicate** button creates a new series of 4 images. All 4 images are identical to the reference image itself. Subtracting this new series from the original series will result in a new series of images with excluded background information.

Divide complete time series through a single image:

- Open image series (gallery mode shows image row to pick reference image).
- Define factor and reference image for division.
- Click **Divide** button, a new image series window will open, showing the result.

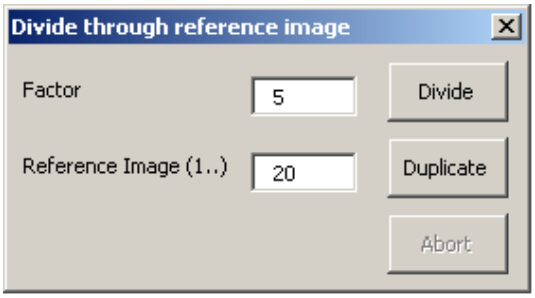


Fig. 3.1 Divide through reference image window

Factor field: Choose intensity factor for division formula.

Reference Image (1…) field: Choose reference image (type image number according to image gallery)

Divide button: Starts the division process and opens a new image series, showing the result**.**

Duplicate button: Duplicates the reference image and opens a new image series with duplicated image(s).

Abort: Aborts calculations and duplication actions.

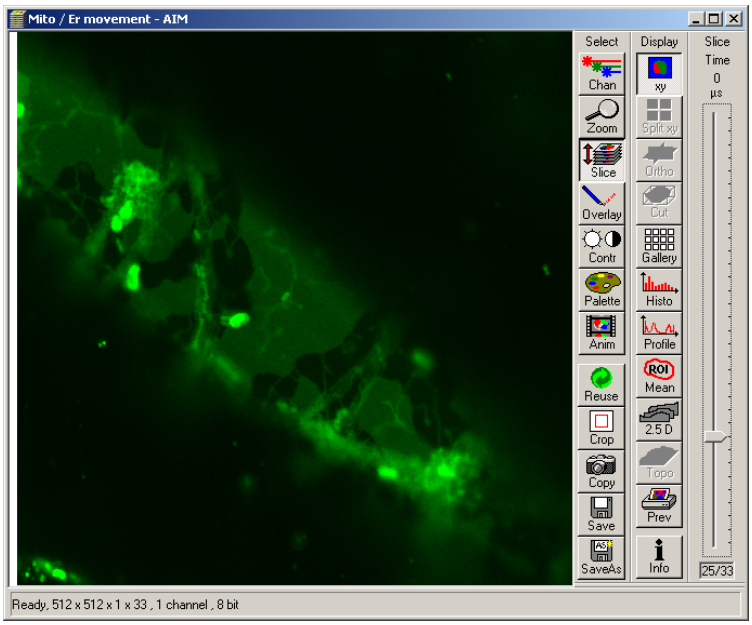


Fig. 3.2 Example of time series image

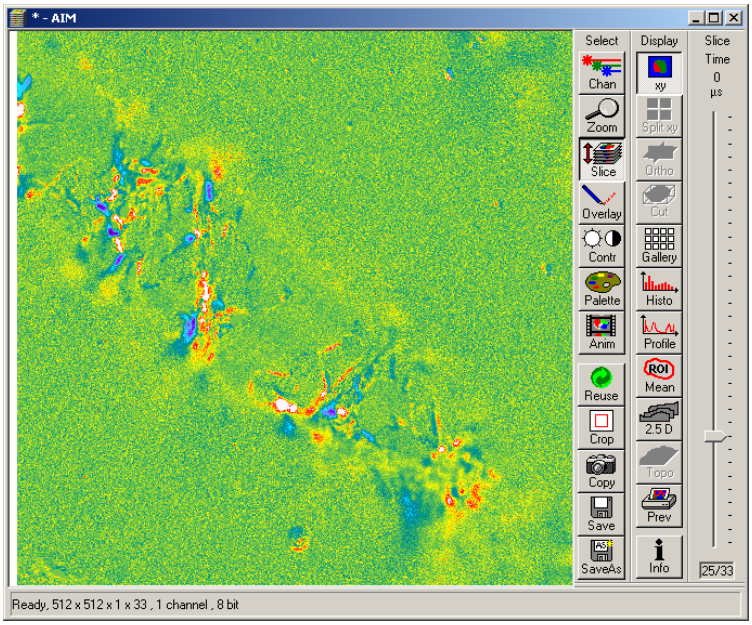


Fig. 3.3 Example of time series image after division process factor 125 (rainbow palette).

4. Macro name: **Excitation Fingerprinting**

Filename: ExcitationFingerprinting-32.lvb
ExcitationFingerprinting-35.lvb
ExcitationFingerprinting40.lvb

System requirements: LSM 510 NLO or LSM 510 Meta NLO system in combination with a software tunable ultra fast laser, software release 3.2 to 4.0
Laser power meter

Short description: Automated acquisition of excitation lambda stacks for generating excitation spectra which can be used for linear unmixing to separate spectrally overlapping dyes. Any detector including internal PMTs as well as external NDDs can be used.

Description:
With the LSM 510 NLO and LSM 510 META NLO in combination with a software tunable ultra fast laser (multiphoton lasers such as Coherent Chameleon or Spectra Physics Mai Tai) one can acquire excitation spectra and use these spectra to perform linear unmixing. This process is called Excitation Fingerprinting. Additionally to this description take a look at the guided tour on the installation CD-ROM or DVD.

- For Excitation Fingerprinting, a macro is provided to perform the necessary steps for the calibration of the system and the image acquisition.
- The calibration of the system ensures a constant laser power in the specimen plane by adjusting the AOM transmission in coordination with the applied wavelength.

(1) Configuration for calibration and image acquisition:

Calibration requires a suitable laser power meter. Calibration is not absolutely necessary to conduct Excitation Fingerprinting but recommended for realistic spectra. Each combination of Main Dichroic and objective requires a specific calibration, which is stored in individual calibration files.

- Use the KP 650 as main dichroic and the KP 685 as emission filter e.g.
- Select excitation laser line and set transmission values mode.
- To perform the AOM calibration place the power meter into the specimen plane using the objective and main dichroic you will use for image acquisition later on.
- Select Frame as the scanning mode.
- Use the bidirectional scanning mode.
- Adjust the zoom to the maximal value (40) by moving the slider to the far right position.
- Set the scan speed to Max
- Start the Macro

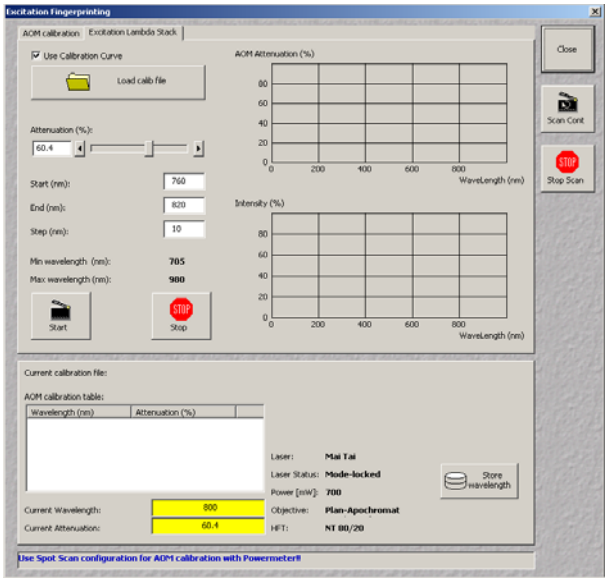


Fig. 4.1 Excitation Fingerprinting macro window

- Start scanning with **Scan Cont**.
- Change view to **AOM calibration**
- Chose lowest wavelength available.
- Set AOM Transmission to a value around 5 or 10.
- Activate the **Auto+** option checkbox (**Auto-** is chosen when starting with the highest wavelength available).
- By pressing **Add and + 10 steps** the AOM Value will be added to the list and the laser is tuned to the next wavelength: 10 nm further.
- Adjust the AOM Transmission to keep the power indicated at the power meter constant, then press **Add and + 10 steps** again.
- Repeat this procedure until you have acquired the AOM settings for all wavelengths; 10 nm steps are fully sufficient.

Note: The step size for tuning can be edited. Choosing **Manual** (checkbox) requires to perform the tuning using the slider or typing in a value. Single values or the whole list can be deleted. **Normalize list** will set the highest value to 100 and adjust the others accordingly. Mean intensity (%) refers to the overall image intensity value; The value is displayed as % of the maximum intensity value at chosen data depth (8 or 12 bit). Calibration files are stored as *.aom files in a folder of choice. Scanning can be started and stopped using the buttons **Scan Cont** and **Stop Scan** on the right side of the Macro Window.

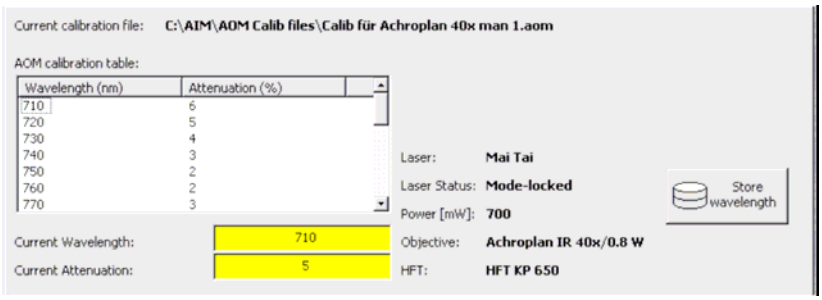


Fig. 4.2 Example using a Mai Tai Laser

(2) Using a Mai Tai Laser:

In the lower part of the window the calibration table is shown together with the file currently used, the laser status, objective and main dichroic; the latter will influence the laser power in the sample -> use calibration curves acquired with the same settings as image acquisition is performed.
The store wavelength button will update the laser wavelength in the main SW.

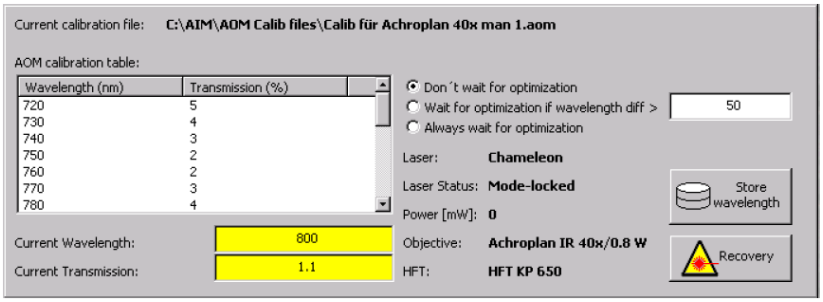


Fig. 4.3 Example using a Chameleon Laser

(3) Using a Chameleon Laser:

In the lower part of the window the calibration table is shown together with the file currently used, the laser status, objective and main dichroic; the latter will influence the laser power at the sample -> use calibration curves acquired with the same settings as image acquisition is performed. When tuning at whatever process you are performing the option **Wait for optimization** can be set as desired.

Important Note: Do not tune to a new wavelength if the laser status is indicated as busy! If the laser does not mode lock after several minutes press Recovery and wait for mode locking (takes three to five minutes).

Note: For calibration of the AOM a power meter is needed. It is recommended to use a power meter with less then 1mW resolution.

Excitation Fingerprinting can be performed using predefined Excitation Spectra or using ACE (Automatic Component Extraction). The most convenient and best working method varies depending on the labeling of the specimen. If the different fluorophores show no or only moderate spatial overlap in the specimen, ACE will work well. If the fluorochromes are strongly co-localized, it is recommended to use predefined Excitation Spectra of the individual fluorochromes as reference spectra for linear unmixing.

(4) Acquisition of an Excitation Lambda Stack to generate Excitation Spectra

- After focusing the specimen and setting the AOM transmission such that a good image is generated at the chosen wavelength switch to Excitation Lambda Stack
- Load the calibration file for the AOM required for the configuration of Main Dichroic and Objective used
- Indicate the wavelength range to be used and the step size (this does no have to correspond with the range or step size of the calibration curve)
- Start the image acquisition; A lambda stack will be acquired. The **Data** button (Gallery Mode) displays the wavelengths used for excitation of each image.

(5) Linear Unmixing

- Use the **Mean** button to display the dialog needed to perform Excitation Fingerprinting.The Spectra can be defined manually using the drawing tools to define the appropriate region in the image or by applying ACE (Automatic component extraction).
- Click on the 

icon. The system performs the unmixing algorithm and displays the unmixed images.

Note: The parameters for the unmixing procedure can be set in the main menu under **Process** and **Unmix**.

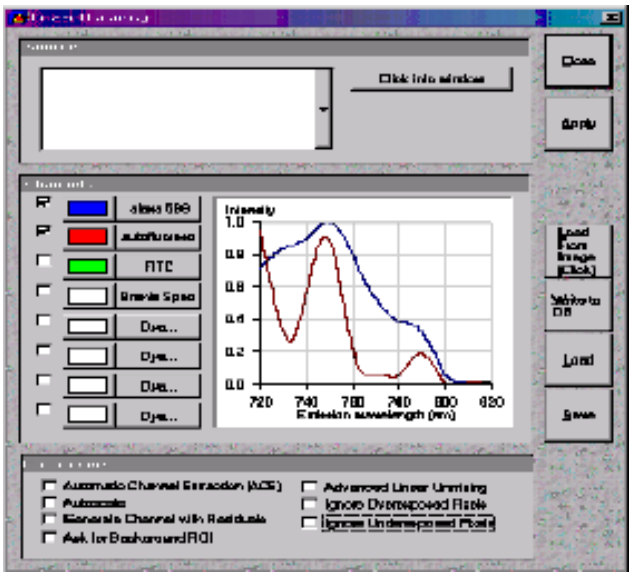


Fig. 4.4 Linear Unmixing window

Autoscale: Balances the brightness of the unmixed images.

Advanced Linear Unmixing: The algorithm used for Linear Unmixing calculates the minimal difference between the unknown spectra and the reference spectra for all possible mixtures of the reference spectra (lowest square fit of acquired spectrum). This can produce negative values for the contribution of individual spectra. Advanced Linear Unmixing ignores the negative values and starts a new calculation based on the remaining spectra. (Without performing Advanced Linear Unmixing, these values will be set to zero).

Ask for Background ROI: Subtracts a spectrum defined by a ROI in the acquired Lambda Stack before performing the Linear Unmixing. This parameter is suitable to remove homogenous background signals.

Ignore overexposed and underexposed pixels: Saturated pixel or pixel with the intensity value will not be included in calculation.

Generate Channel with Residuals: Generates an additional „Channel with Residuals“ which is a pixel-by-pixel display of the difference between fit and original data (for the channel of the Lambda Stack that shows the greatest deviation) Perfect fit for a given pixel is indicated by a pixel with intensity 0 in the corresponding channel displaying the residuals.

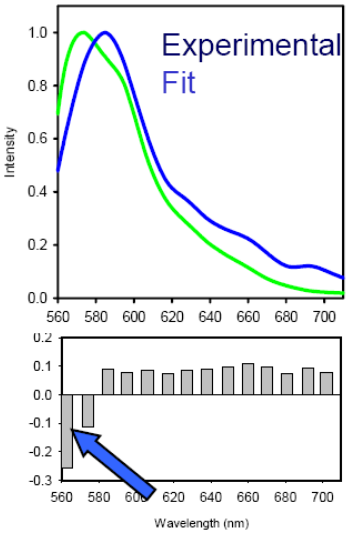


Fig. 4.5 Channel with Residuals

5. Macro name: **Fast Mode Switch**

Filename: FastModeSwitch-32.lvb
FastModeSwitch-35.lvb
FastModeSwitch40.lvb

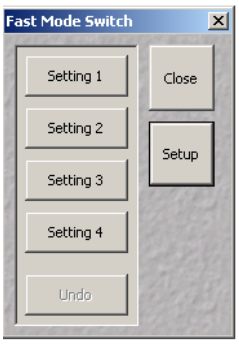
System requirements: LSM 5 system, software release 3.2 to 4.0

Short description: Store settings from **Scan-Control** and reuse.

Description:
This macro enables the user to switch quickly between different scan control settings (one button click) after assigning the settings to a button.

(1) Using Fast Mode Switch

- Open **Fast Mode Switch** macro.
- Click **Setting 1** button, saved scan control settings 1 will be set.



Setting (1,2,3,4) buttons: Change settings according to the assigned scan control settings.

Close button: Closes this macro.

Setup button: Opens the setup menu (see below)

Fig. 5.1 Fast Mode Switch macro window

(2) Saving the scan control setup for Setting 1 - 4 button:

- Adjust the settings in the **Scan Control** menu (Acquire mode),
- open **Fast Mode Switch** macro, **Setup** menu and
- click the **Store** button at the bottom of the setting 1 setup row.
- For assigning **Setting** button 2, 3 and 4, proceed accordingly.

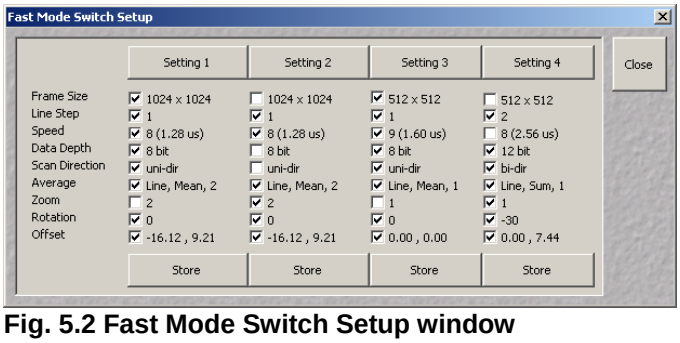


Fig. 5.2 Fast Mode Switch Setup window

The checkboxes: Activate the assigned setting value.

Setting buttons: LSM settings can be switched quickly.

Close button: **Fast Mode Switch Setup** window closes. The setup menu and the settings can still be switched with the **Fast mode switch**.

6. Macro name: **Hot key**

Filename: HotKey-32.lvb
HotKey-35.lvb
HotKey40.lvb

System requirements: LSM 5 system with transmitted light PMT, software release 3.2 to 4.0

Short description: Control of the **Start Scan**, **Stop Scan** and **focus control** with the computer keyboard

Description:
The **Hot key** macro activates or disables the following **Hot key** functions: Start scan, stop scan, focus up, focus down.

Start the Hot key function:

- Open **Hot key** macro
- Click **Start** button, **Hot keys** are activated.

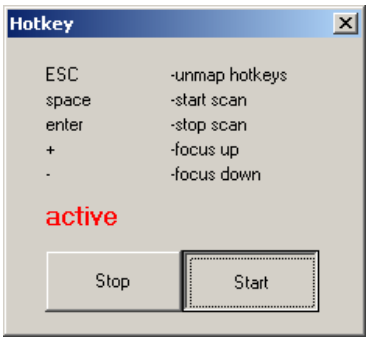


Fig. 6.1 Hot key macro window

ESC key: Disables the **Hot key** function

Space key: starts a scan

Enter key: stops the scan

+ key: focus up

- key: focus down

Stop button: Deactivates **Hot key** functions.

Start button: Activates **Hot key** function.

Note: The step size of the focus can be modified with the focus step slider in the stage and focus control (Acquire Mode).Closing the hot key macro window does not deactivate the hot key functions.

7. Macro name: **Lambda mode plus TPMT image**

Filename: lambdatrans-32.lvb
lambdatrans-35.lvb
lambdatrans40.lvb

System requirements: LSM 510 Meta system, software release 3.2 to 4.0

Short description: Creates a time series alternating between lambda mode and transmission PMT mode.

Description:
This **Lambda mode plus TPMT image** program adds a new time series functionality. It is designed to provide the possibility alternate between lambda stack and transmission PMT acquisition. (E.g. it can be used to measure spectral characteristics of auto fluorescence in combination with observing movement of an organism using DIC imaging).

(1) Configurations for lambda transmission and TPMT image time series:

(a). Choosing the lasers and there power settings:

Lambda stack Laser settings (Lambda mode) are adopted (Fig. 7.1).
Transmission PMT image: The program is designed to use the laser of the first track from Multi Channel mode (will automatically be activated by the macro, Fig. 7.2) or all lasers activated in Single Mode (Figure 7.3).

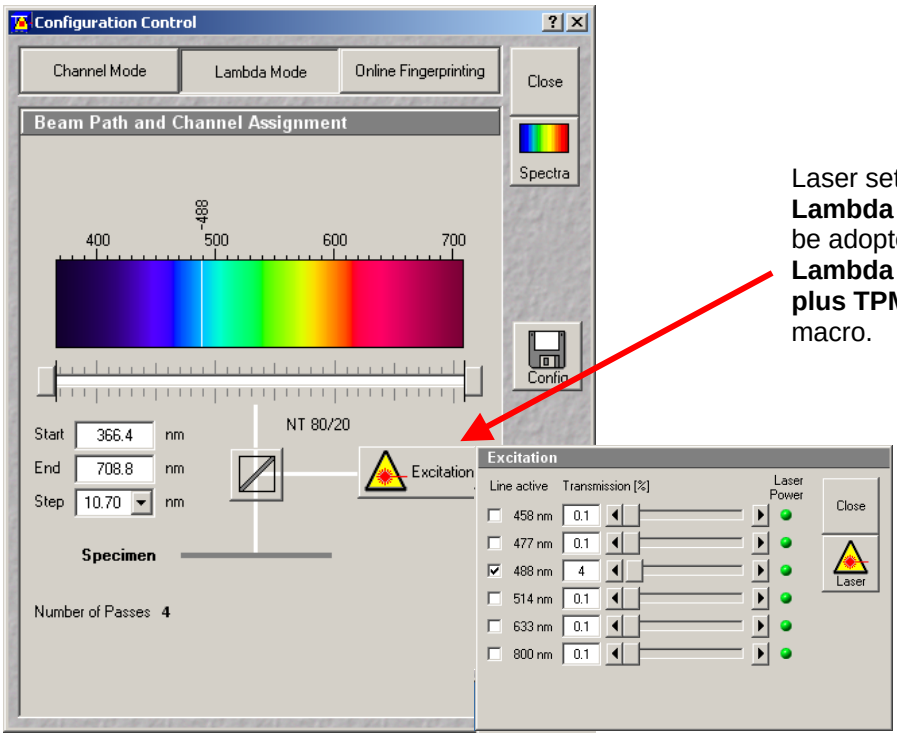
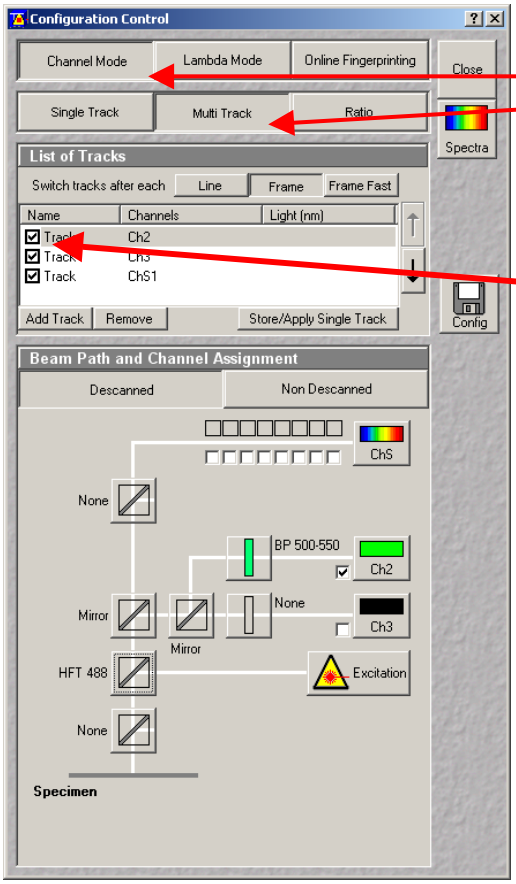


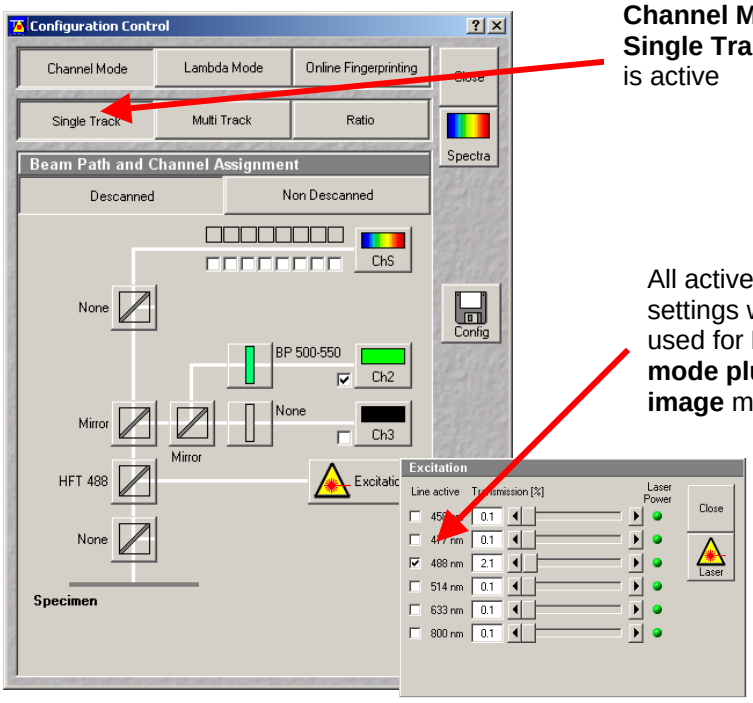
Fig. 7.1 Configuration Control window and Lambda Mode and Excitation window



Channel Mode, **Multi Track** mode is active:

First Track Laser settings will be used for TPMT image.

Fig. 7.2 Configuration Control window Channel Mode Multi Track view



Channel Mode, **Single Track** mode is active

All active Laser settings will be used for **Lambda mode plus TPMT image** macro.

Fig. 7.3 Configuration Control window Channel Mode Single Track view

(2) Handling lambda transmission and TPMT image time series:

- Focus specimen and set configurations for good image acquisition.
- Choose laser and power settings for lambda stack and ChD image (as described above)
- Open **Lambda mode plus TPMT image** macro.
- Adjust detection gain by clicking **Scan Cont** and defining the detection gain with the **Detector Gain ChD** slider.
- Stop continuous scan by clicking **Stop** button.
- Define the number of lambda stacks and TPMT images in the series by typing the count in the **Number Images in 1 Timeserie** box.
- Define the time interval (seconds) of time series acquisition in **Time** box.
- Start time series.
- Two new series windows open and show acquisition.

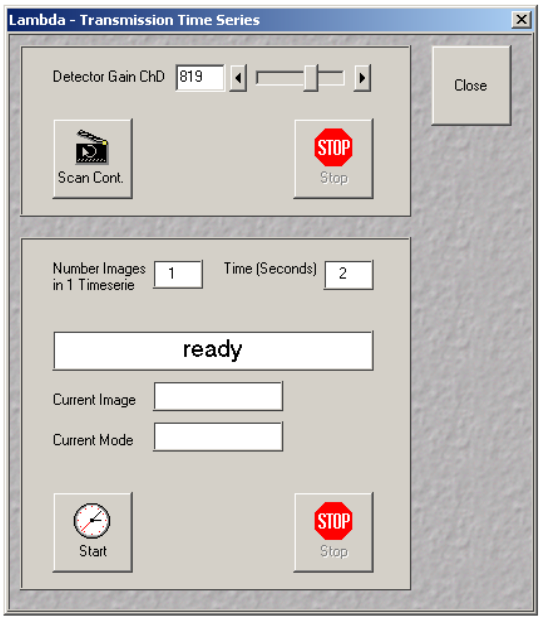


Fig. 7.4 Lambda – Transmission Time Series window

Detector Gain ChD slider: Defines the detection gain of the TPMT

Scan Cont. button: Starts continuous scan of ChD for adjusting the detection gain.

Stop button: Stops continuous scan of ChD .

Number Images in 1 Timeserie box: Defines the number of lambda stack and TPMT images in the series.

Time (Seconds) box: Defines the time interval of time series image acquisition.

Start button: Starts time series with alternating lambda mode and TPMT channel.

Stop button: Stops time series

8. Macro name: **LSM Configurations Export**

Filename: LSM-ConfigurationExport-with-Form-30-35.lvb

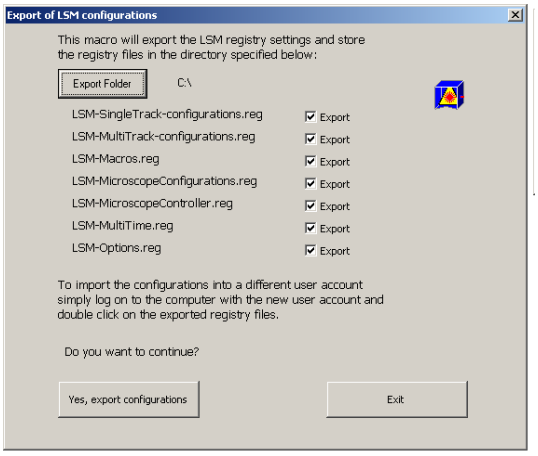
System requirements: Windows 2000, Windows XP, LSM 5 system, software release 3.0 to 4.0

Short description: Export configurations (single-track, multi-track, microscope settings, macro buttons, software option settings, etc.) into individual *.reg files for importing into new user configurations.

Description:
This macro allows the user to save various configuration settings (as listed in the macro window) as *.reg (registry) file to create backups or to transfer configurations from one user account to another.

Saving microscope configurations as *.reg files:

- Choose folder for exporting *.reg files. Click **Export Folder** button.
- Enable the files for export by activating the tick.
- Click **Yes, export configurations** button.
- All exported files will be confirmed in a pop up window. Click on each exported file pop up window and the overview confirmation window **OK**.



Export Folder button: Opens the file dialog, user chooses target folder for *.reg files.

Checkboxes: Ticks mark on/off enables or disables specific file export.

Yes, export configurations button: Copies the tagged configurations to the export folder.

Exit button: Closes the Export of LSM configuration macro.

Fig. 8.1 Export of LSM configurations window

LSM-Single-Track-configurations.reg file:	contains all single track configurations of the current user
LSM-Multi-Track-configurations.reg file:	contains all multi track and lambda mode configurations of current user
LSM-Macros.reg file:	contains all macro button configurations of the current user
LSM-MicroscopeConfigurations.reg file:	contains microscope configurations of the current user
LSM-MicroscopeController.reg file:	contains microscope controller configurations
LSM-MultiTime.reg file:	contains configurations of the LSM Multi Time macro
LSM-Options.reg file:	contains configurations of the LSM options

Note: To open the *.reg files in a different account, change registry to the new account and open the exported *.reg file by double click.

9. Macro name: **Meta Export**

Filename: MetaExport-32l.vb
MetaExport-35.lvb
MetaExport40.lvb

System requirements: LSM 510 Meta system, software release 3.2 to 4.0

Short description: Export all images of a lambda stack as a series of images in different formats (tif, bmp, jpg, etc.)

Description:
This program exports all images of a lambda stack as a series of single images with wavelength indication in different formats.

Export lambda stack images:

- Start **Meta Export** macro (Fig. 9.1) and load or activate lambda stack image window (Fig.9.2).
- Define the format of the images for export from the **File Type** drop down list (Fig. 9.1).
- Click **Export** button.
- Choose destination and base filename of export files.
- Click **Save** button in file dialog to confirm the destination. The program exports all images of a lambda stack as a series of images and appends an underscore followed by the lambda wavelength to each image filename.

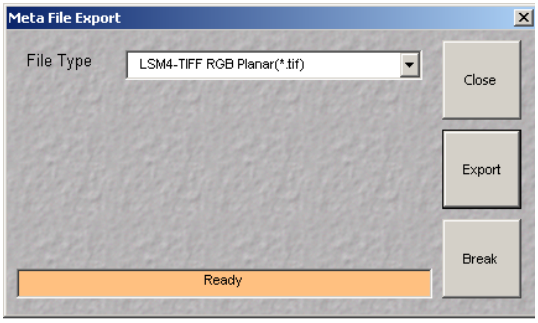


Fig. 9.1 Meta File Export macro window

File Type drop down list: Defines the format of export file

Export button: Opens file dialog for export files and exports lambda stack images.

Break button: Discontinues file export

Close button: Closes “Meta File Export” macro

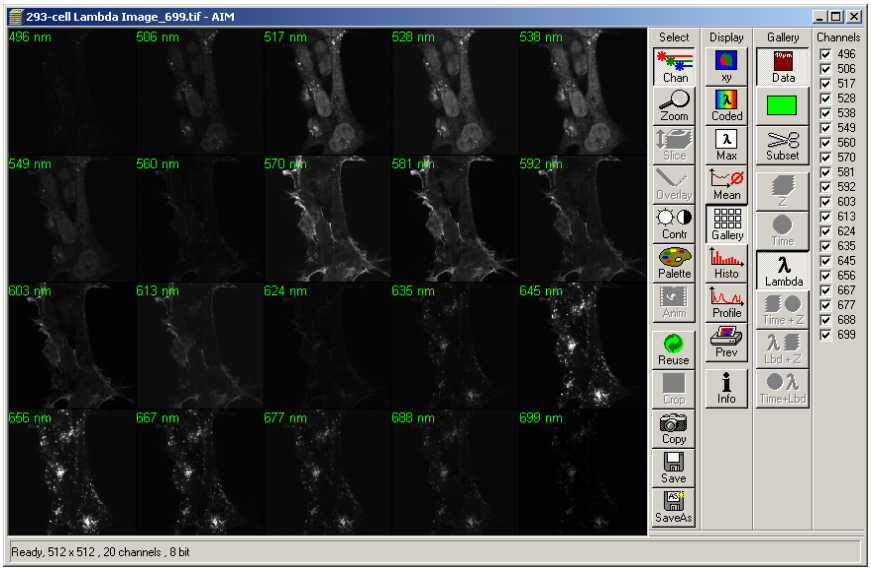


Fig. 9.2 Gallery of lambda stack images (each WL indicated in the upper right image corner)

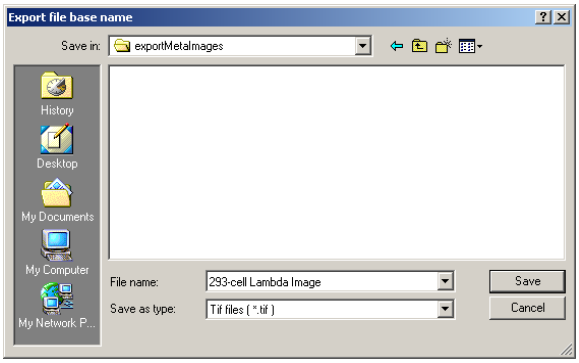


Fig. 9.3 File dialog

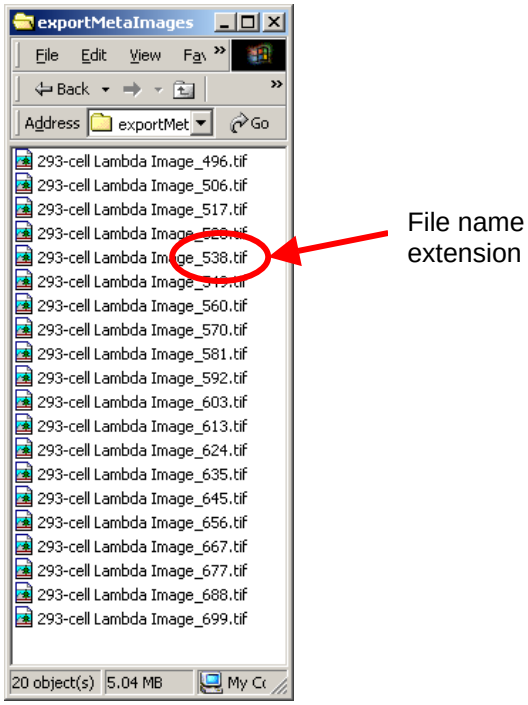


Fig. 9.4 Example list of exported lambda images with wavelength added to the filenames

10. Macro name: **Scale Bar**

Filename: Scalebar30-32.lvb
Scalebar-35.lvb
Scalebar40.lvb

System requirements: LSM 5 system, software release 3.0 to 4.0

Short description: Indication of self defined intensity levels assigned to a ROI as scale bar in the image; also attaches tick marks and concentration values to the grayscale/color wedge.

Description:
This program attaches tick marks and concentration values to the grayscale/color wedge.

(1) Attaching tick marks and concentration values to a grayscale wedge

- Add grayscale wedge to image (**Overlay** mode)
- Open **extended scalebar** macro
- Click **Edit** and define concentration values according to the intensity values of image in **Edit concentration** window. Table of concentration and intensity values can be saved and applied with the **Store/Apply** button in **Edit concentration** window.
- Check use table checkbox
- Click **Attach** button, tick marks with concentration values appear.

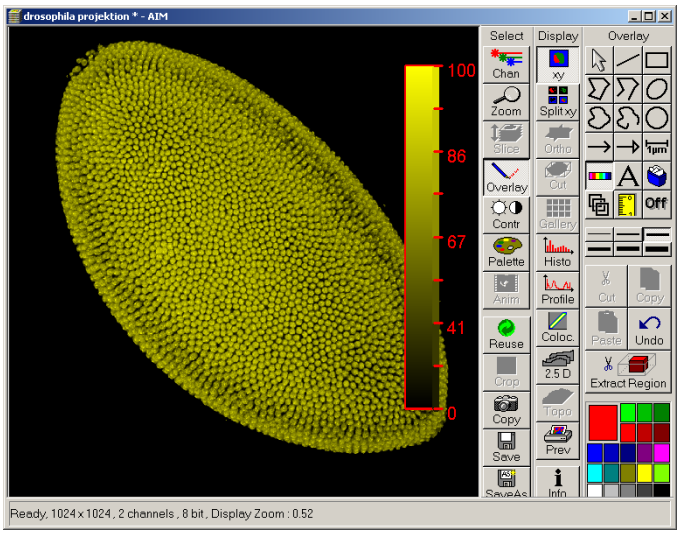


Fig. 10.1 Image with scalebar

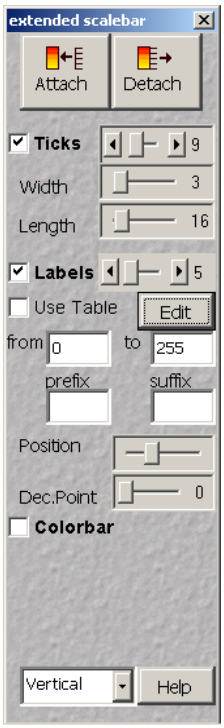


Fig. 10.2 Extended scalebar macro window

Main dialog of Extended Scalebar:

Attach button: attaches tick marks and/or labels (concentration values) to the gray/color wedge. The wedge has to be drawn into the image window using the **Overlay** toolbar before executing the **Extended Scalebar** window. The wedge can be resized afterwards, and the labels and tick marks will remain attached to it. Depending on the initial aspect ratio of the wedge the tick marks/labels will be attached vertically or horizontally.

Detach button: removes the tick marks and labels from the image.

Ticks checkbox: Tick marks on/off

Ticks scrollbar: Specifies number of attached tick marks

Width scrollbar**:** Modifies ticks width

Length scrollbar: Modifies ticks length

Labels checkbox: Ticks on/off

Labels scrollbar: Positions labels relative to the tick marks

Use Table checkbox: When checked, concentration values are displayed, otherwise linear scale of intensity values from Min to Max.

Edit button: Opens **Edit Concentration** table dialog box. See below.

Intensity Range: Editing minimum and maximum values of intensity range

Prefix: Any text displayed in front of the intensity/concentration value

Suffix: Any text displayed after the intensity/concentration value

Position scrollbar: Shift of position of the labels with respect of the tick marks

Dec. Point scrollbar: specification of the number of decimal digitals (0-3) of the displayed intensity/concentration values

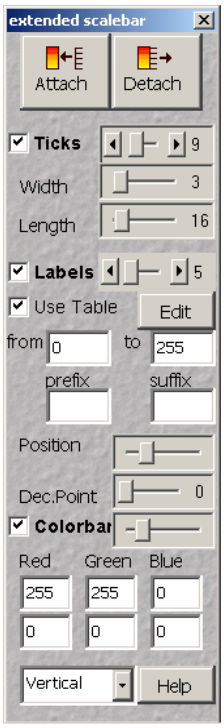


Fig. 10.3 Extended scalebar macro with selected options

Color bar checkbox: when checked, color bar is displayed alongside of the wedge. Activates the input fields for the minimum and maximum values of the Red, Green and Blue components of the color bar.

Color bar width scrollbar: width of the color bar.

Red: Min and Max values of the Red component of the color bar

Green: Min and Max values of the Green component of the color bar

Blue: Min and Max values of the Blue component of the color bar

Vertical/Horizontal combo box: specifies where the labels/labels are attached vertically or horizontally to the wedge.

Help button: opens help document.

Edit Concentration:
Defines the concentration as the function of the intensity by editing the arbitrary number of intensity - concentration pairs. The concentration values for intensity values not defined in the table are calculated by linear interpolation of two adjacent intensity-concentration points

Intensity: enter intensity value for the new data point

Concentration: enter concentration value for the new data point

Add Point: Inserts new intensity - concentration point. If the intensity value already exists in the table the new one replaces the old pair

Delete Point: Deletes the selected pair from the list

Return: returns back to the extended scrollbar dialog.

Store/Apply: Opens Store/Apply Table menu

Fig. 10.4 Edit Concentration window

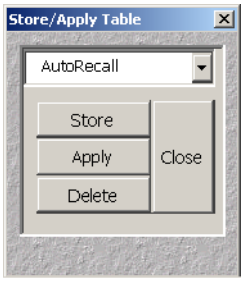


Fig. 10.5 Store/Apply Table window

Combo box: lists previously stored concentration tables. **AutoRecall** table contains the last used concentration table.

Store: stores new table

Apply: applies selected table

Delete: deletes selected table

Close: returns to **Edit Concentration** menu

11. Macro name: **Modify Images**

Filename: ModifyImages-32.lvb
ModifyImages-35.lvb
ModifySeries40.lvb

System requirements: LSM 5 system, software release 3.2 to 4.0

Short description: Modifies z-stacks and time stacks like rotation or mirroring, conversion of time stacks into z-stacks and vice versa; conversion of multi channel images into lambda stack

Description:
The macro enables the user to edit single images time and Z-stack Series. New functions have been added to the **ModifyImages-35.lvb** version.

(1) Single image:
Following actions are possible:
- Concatenation for time series or z-stack production
- Time stamp manipulation (new function 3.5 version)
- Rotation and horizontal flipping.
- Conversion of multi channel images to a lambda stack, wavelength stamp can be manually defined (new function 3.5 version).
- Time series generation from single image (new function 3.5 version)

(2) Time series:
Following actions are possible:
- Rotation, flipping horizontal
- Concatenation of different time series (preserves original times and events)
- Conversion of time series to z- stack.

(3) Z- stacks:
Following actions are possible:
- Rotation, flipping horizontal
- Conversion of z- stacks to time series
- Reversion of z-stacks
- Concatenation of different z-stacks
- Creation of new z-stacks with consecutive slices equal to XZ cuts with interpolation of the original stack
- Creation of new z-stacks with consecutive slices equal to YZ cuts with interpolation of the original stack

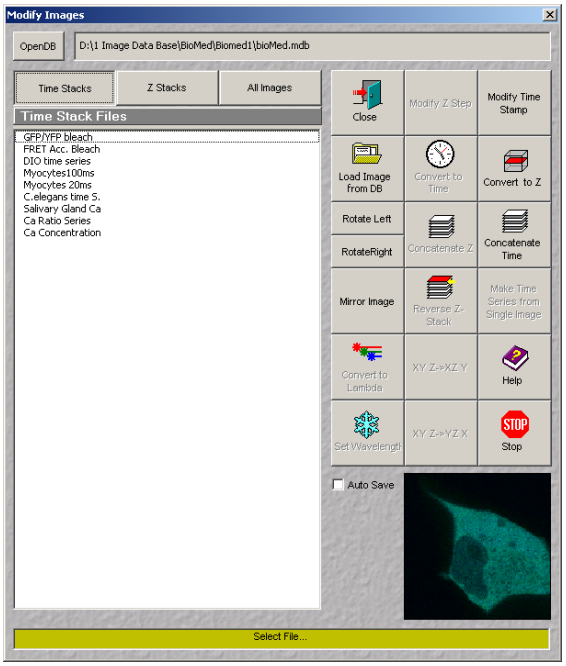


Fig. 11.1 Modify Images macro window - Time Stacks view

Open DB button: Opens the image database. Select images to process in the file list box (Ctrl-Left Mouse Button or Shift-Left Mouse Button)

Time Stacks button: When selected, only time sequence images in the DB are listed in the box. **Concatenate Time** button, **Convert to Z** button is active (Fig. 11.1).

Z Stacks button: When selected, only z-stacks in the database are listed in the list box. **Modify Z Stack** button, **Convert to Time** button, **Concatenate Z** button, **Reverse Z Stack** button, **XYZ→XZY** button, **XYZ→YZX** button become active (Fig. 11.3).

All Images button: When selected, single images, time sequence, z-stacks and lambda stacks in the DB are listed in the box. **Make Time Series from Single Image** button becomes active (Fig. 11.2).

Load Image from DB button: Loads selected image from the open image database.

Rotate: Rotates the stack or images by 90 degrees in XY plane, **Left** counter clockwise or **Right** clockwise.

Mirror Image: Creates mirror image of the stack along YZ plane.

Convert to Lambda: This function converts images with more than one channel to a lambda stack. Each channel represents one wavelength.

Set Wavelength: Opens **Set Wavelength** window (Fig. 11.6)

Modify Z Step: Opens Enter Z Step window (Fig. 11.4)

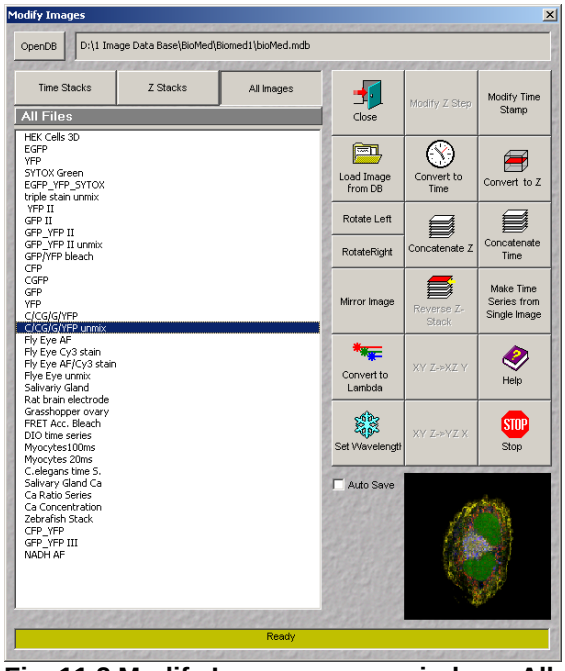


Fig. 11.2 Modify Images macro window - All Images view

Convert to Time: Active only for Z-stacks. Creates new time series image out of the selected Z-stack Time interval of the new sequence is equal to the z interval of the Z-stack image. New image can be analyzed using Mean of ROI's function, which effectively provides profiles through the stack of images.

Concatenate Z stacks: Combines selected z stacks into single z stack in order of appearance in file list. All images will be shown with the same z-stack interval. The first stack determines the Z- step distance. (This function together with **Reverse Z-stack** and **Mirror Image** provides a method to combine two z-stacks taken from the top and the bottom of the thick sample. E.g. stack A is acquired from the top of the sample, after turning the sample around stack B is acquired from the bottom of the sample. To concatenate stack A to B in a way that the middle optical slices of the sample will be fitted to the middle slices of the stack, mirror and reverse stack B before concatenation)

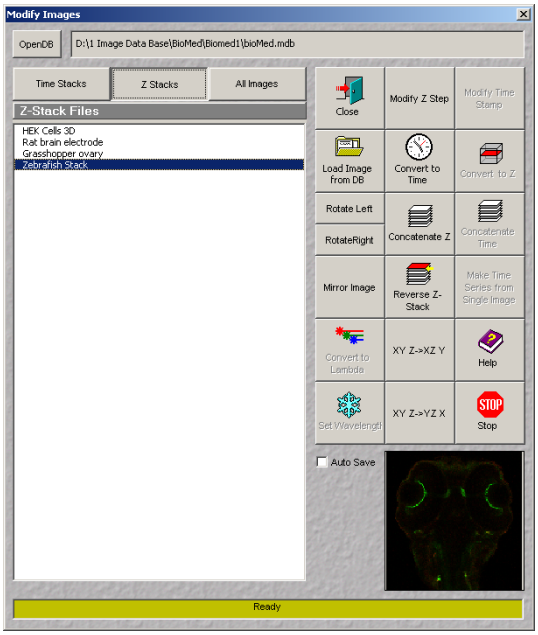


Fig. 11.3 Modify Images macro window - Z Stacks view

Creates new z-stack with slices in reverse order. Reverses bottom and top of the stack.

XY Z->XZ Y: Active only for Z-stacks. Creates new image stack with consecutive slices equal to XZ cuts with interpolation of the original stack.

XY Z->YZ X: Active only for Z-stacks. Creates new image stack with consecutive slices equal to YZ cuts with interpolation of the original stack.

Modify Time Stamp: Opens **Modify Time Stamp** window (Fig. 11.7)

Convert to Z: Active only for time stacks with single plane images for each time (not applicable for 4D). Creates new z-stack with consecutive z slices equal to consecutive time slices. Z interval is set to X pixel dimension (can be modified by **Modify Z Step** function).

Reverse Z-stack: Active only for z-stacks.
Concatenate Time series: Combines selected time series into single time series. (Adds duration of series regardless of time stamp)

Make Time series from single Image: Active for **All Images**, Opens **Enter Number Of Time Images** window.

Help: Opens help file.

Stop: Stops processing images.

Close button: Closes **Modify Series/Images** program.

Concatenate Time Stacks: Creates single time sequence out of the selected files. Preserves correct times and events.

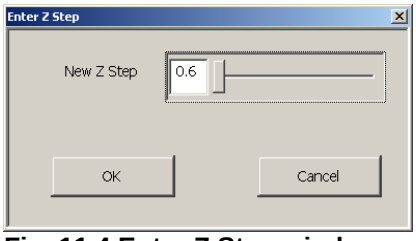


Fig. 11.4 Enter Z Step window

Modify Z Step: Active only for z-stacks. Creates new z-stack with z interval equal to the selected value. (E.g. for correction of refractive index (mismatch between specimen and immersion medium))

Make Time series from single Image: Active for **All Images**, Opens **Enter Number Of Time Images** window.

Number of Times slide: Defines number of image copies

Time Interval slide: Defines indicated time interval in time series

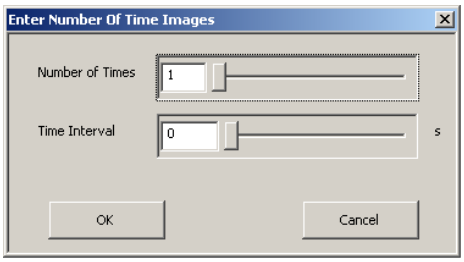


Fig. 11.5 Enter Number Of Time Images window

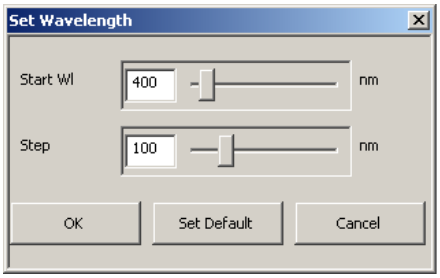


Fig. 11.6 Set Wavelength window

Start WI slider: Defines indicated wavelength for converting to Lambda stack action. Default setting is 400nm

Step slider: Defines indicated Lambda wavelength steps for converting to lambda stack action. Default setting is 100nm

OK -button: Confirms defined values above and closes **Set Wavelength** window

Cancel -button: Closes **Set Wavelength** window.

Set Default -button**:** Returns settings to default values. (Start WL 400nm Step 100nm)

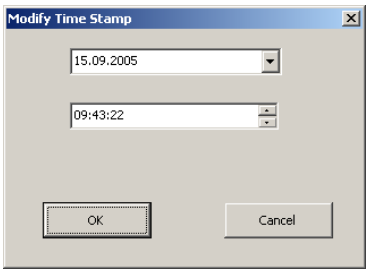


Fig. 11.7 Modify Time Stamp window

Date drop down window: Calendar opens for definition of time stamp date

Time box: Defines time of time stamp.

12. Macro name: **Batch File Export**

Filename: FileExport30-32.lvb
FileExport-35.lvb
FileExport40.lvb

System requirements: LSM 5 system, software release 3.0 to 4.0

Short description: Exports one or more selected images according to the selected file format; Exports image intensity values in ASCI format

Description:
The "Batch File Export" macro exports images in different file formats.

(1) Example: Export to *.jpg

- Open database file by clicking **Open** button.
- Shift-click to select file(s) for export.
- Click **Export File Name, Save in** file dialog will open.
  (a). **Save in** selects destination folder. If this button is not used and no path for image saving is selected, files will automatically be stored in the current database folder.
  (b). For **File name**, give a prefix for file names you are exporting. Exported file name will be "prefix_databasefilename000", "prefix_databasefilename001", etc.
- Keep **long file names** checked
- Image saved:
  (a). For single section - use **Content of image window** or **Single Image with raw data** Note: for single section of dual channel scan, you can check **Split screen** which will save an image like the **Split XY** option on the image display window (ch1, ch2, merged). **Split screen** works only with **Content of image window** mode.
  (b). For series - use **Series with raw data** or **Content of image window**
- Choose file type as ***.jpg**
- Click **Start Batch Export**

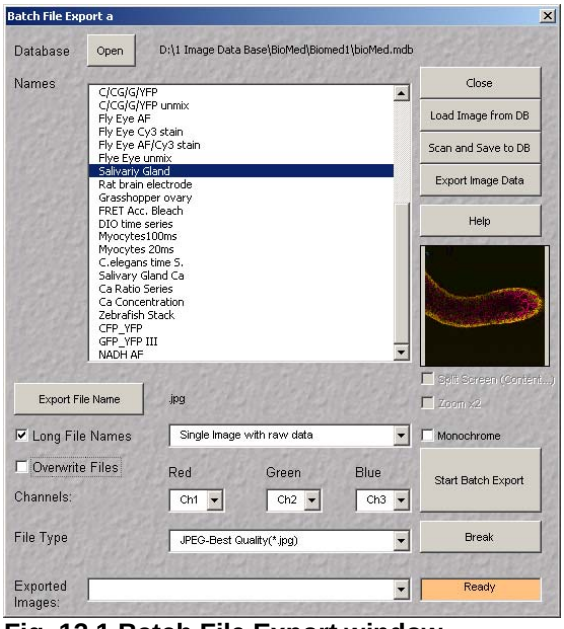


Fig. 13.1 Batch File Export window

Open button: Open the Image Database. Select images to export in the file list box. (Ctrl-Left Mouse Button or Shift-Left Mouse Button)

Close button: Close **Batch File Export** program.

Load Image from DB: Loads the image from DB

Scan and Save To DB: Scans and Saves new image to DB (according to Auto save settings).

Export Image Data: Exports Image intensity values into ASCII file. Selected Image is loaded and the following dialog box displayed: Fig. 13.2

Help button: Opens help file.

Export File Name button: File dialog will open to selects folder and the base file name for the exported images.

Start Batch Export: Start exporting images

Break button: Stops exporting images

Channels and **Export File Type** selection is the same as in the LSM program.

Exported Images : List of successfully exported images (corresponding to selected criteria).

Long File Names **ON**
Exported file name is generated from the base file name appended with "_" and the file name in the image database. Series images are appended with "000", "001" etc.
OFF
Exported file name is the first character of the base file name, appended with the image number in the list and the letter "E". Series images are appended with "000", "001" etc.

Overwrite files **ON**
Exported files are overwritten without prompt.

Split Screen **ON** (active only in the Content of the Image Window)
Exports split screen images. (Images acquired with more than one channel can be shown in a split screen window, each channel separately plus one image showing all channels. This split screen window can be exported when **Split Screen** is **ON**)

Zoom x2 **ON**
Exports **Content of the Image Window** zoomed twice

Monochrome **ON**
Exports single channel in monochrome format.

Export button: Starts export of Image intensity values into ASCII file.

Reduction: None: All pixels exported or 2, 4, 8, 16: Every second, forth, eighth, sixteenth pixel exported.

View button: Displays exported file.

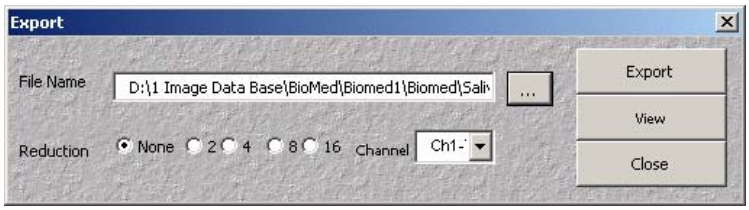


Fig. 13.2 Export

13. Macro name: **Concatenate Images**

Filename: ConcatenateImages-35.lvb
ConcatenateImages40.lvb

System requirements: LSM 5 system, software release 3.5 and 4.0

Short description: Enables the combination of different images regardless of their size and data depth into a single time series. Type of concatenation (fitted to the smallest frame or ROI, fixed size or fitted to the largest frame) can be defined by the user.

Description:
This program compounds open image files (single images, z-stacks, time series) to a single file series.

(1) Example: Concatenate two images:

- Open **Concatenate Images** macro
- Click **Add** button followed by a click in the open image window. An image link will appear in the row of icons below (time line).
- Click **Add** button followed by a click in the next open image window. A second image link will appear in the time line.
- Click **Apply** button, images or series will be concatenated in a new image window.

The main menu of Concatenate Images is:

Add button: Click **Add** button followed by a click in the open image window. An image link will appear in the row of icons below (time line). The palette and the channel settings will be transferred. Overlays will only appear in concatenated images in RGB-mode.

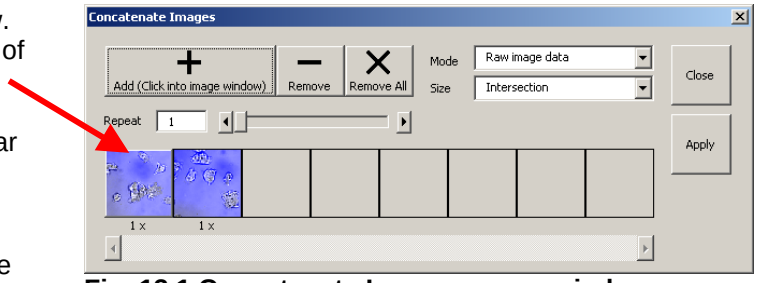


Fig. 13.1 Concatenate Images macro window

Remove button: Removes the active image link out of the concatenate image row.

Remove All button: Clears the concatenate image row.

Repeat slide: Adjusts the number of image copies to be used in the concatenated time series. Repeated image copies can e.g. be used for adjustment of playback frame rate when creating a movie. (If an animation is to be played back with a frame rate of 15 images/sec and if a single image is supposed to be displayed for 2 seconds the repeat slider needs to be set at 30 image copies.)

Mode selection: Selects the options of data storage.
Two options are possible: RGB e.g. can be used for exporting data and making movies. Raw image data e.g. can be used for further image processing and concatenating time data in image series.

(a). Concatenating images in RGB mode:
All Images from time series, stacks and single images are concatenated in one series regardless of data depth (Channels, Z-depth, slice number).

(b). Concatenating images in Raw image data mode:
Intersection mode: The number of channels in the concatenated image series depends on the image with the fewest channels. The color of the channel is adjusted depending on data of the first concatenated image in the row. XYZC data is treated similar.

Size selection: Selects the image size adjustment by concatenation of image data.

(a). Intersection:
Adjusts the image size to a common form (In RGB mode e.g. concatenate frame size 256x 256 with 512x 512, frame size 256x 256 will be adopted to 512x 512 pixel by stretching)

(b). Union:
Adopts the established image sizes (in this case 256x 256 frame size being concatenated with a frame size of 512x 512, both image sizes will be shown in the series).

(c). Fixed size:
Width and height of the frame size can be set. Square images will be modified according to the smallest side of the frame (e.g. width is set to 300pixel and height is set to 250pixel, a square image will be shown in a frame size of 250x 250pixel).

Apply button: Linked images in the icon row will be concatenated to a time series image stack.

Close button: Closes the **Concatenate Images** macro window.

(2) Creating a time series:

Concatenate time series images, single images or z-stacks using raw image data mode. Icons in the time line need to be in an ascending time order (check time stamp). Negative time succession results in a false depictive representation. Time data is shown in the galley view. It is calculated regarding the time stamp connected to each image data.

(3) Creating a time series by concatenating z-stacks:

The slice distance (z-depth) is preserved from the first stack in the concatenated row. The final number of slices is determined by the smallest number of slices in the concatenated z- stack row (when. Concatenating a z-stack of 20 images with a z-stack of 15 images, the concatenated series will consist of two stacks of 15 images). Time and z-stack data can be shown in the gallery view.

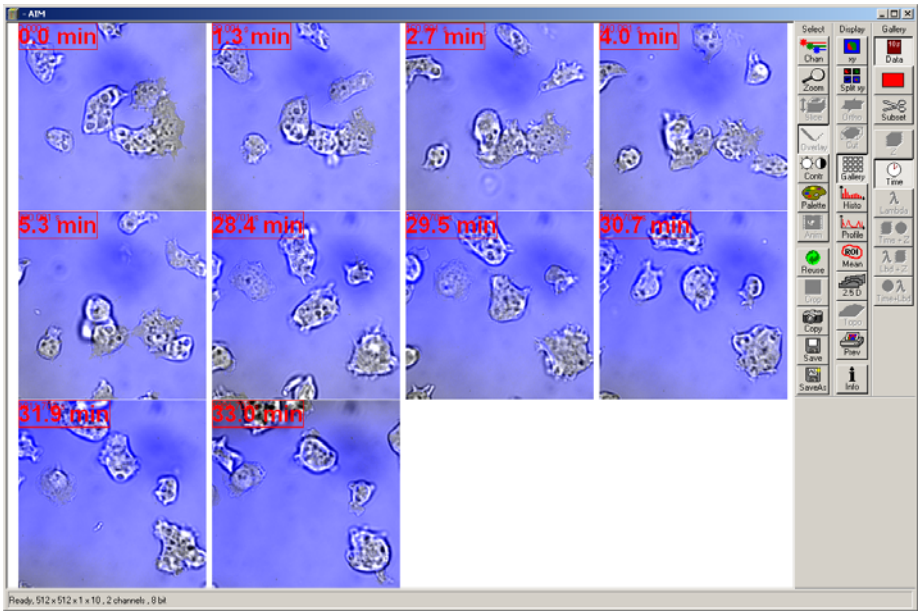


Fig. 13.2 Concatenated time series example (Gallery mode).
The time stamp is automatically updated according to the recorded time information of each series. (E.g. first 5,3min time lapse is recorded at 1.00pm, a second autonomous 4,6min (4min 36sec) time series was recorded at 1.28.24pm. The concatenated time stack results in 33 minutes because of the temporal shift.)

Note: The **Modify Time Stamp** function does not modify the time information of the image, which the concatenate program relates to. The **Modify Image** macro changes the time information shown in the image information only.