

iTheraMedical

Listening to Molecules

View MSOT

inSight 64

inVision 128

inVision 256-TF



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1 OVERVIEW

This manual provides an overview of how to use the ViewMSOT™ software. ViewMSOT™ can be operated on the MSOT inSight 64, inVision 128 and inVision 256-TF systems to acquire, process, visualize and analyze Multispectral Optoacoustic Tomography (MSOT) data. For desktop systems, a version of ViewMSOT™ without data acquisition module is available.

Chapter 2 illustrates how to start the software and set up imaging studies. ViewMSOT™ allows to select between already established Study Presets for several distinct applications and contrast agents. It is also possible to individually generate Study Presets.

Learn how to acquire optoacoustic raw data with the MSOT system in chapter 3.

Chapter 4 shows how to convert the raw data into anatomical optoacoustic tomographic images by reconstruction. Furthermore Multispectral Processing (MSP) can be performed on those images to unmix the provided information according to given spectra.

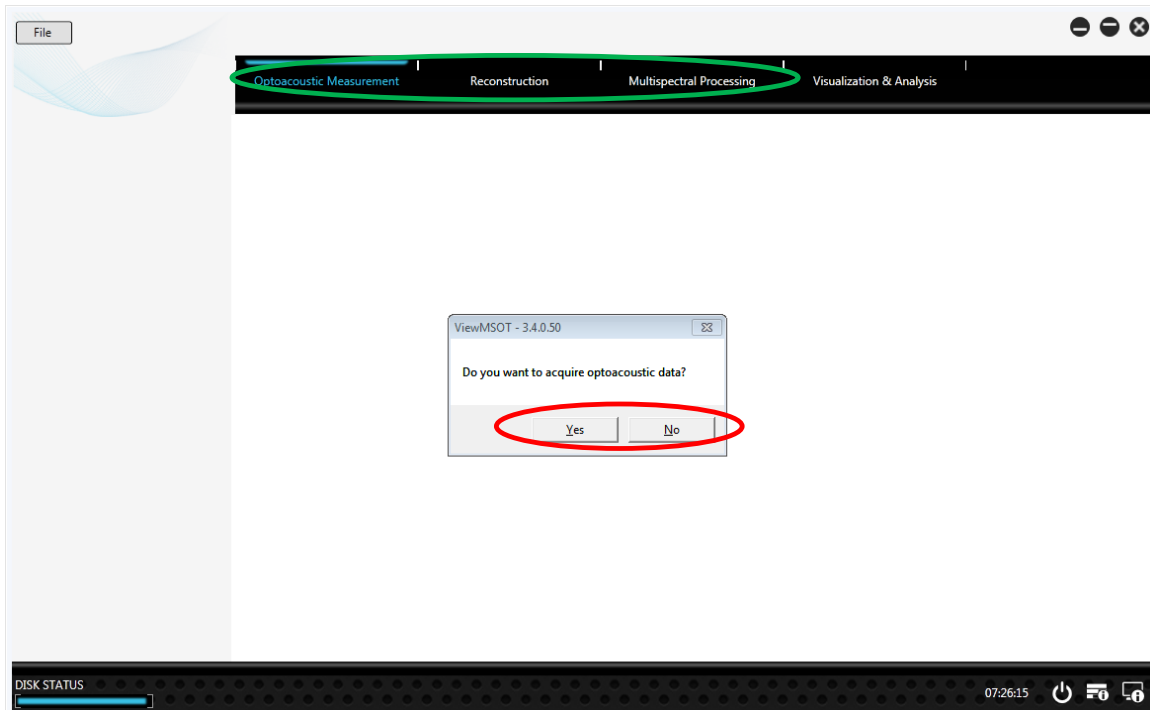
In chapter 5, it is shown how to use the Visualization functionality in order to inspect, analyze and improve the images. This includes image post-processing tools like thresholding and filtering as well as displaying region of interest (ROI) graphs from the image data. The images as well as the content of the ROI graphs can be exported.

The last chapter (chapter 6) is dedicated to Maintenance operations needed to manually control hardware, as well as access hardware and error logging.

For questions and comments please contact your local distributor or iThera Medical at support@ithera-medical.com.

2 STARTING ViewMSOT™ AND SETTING UP A STUDY

After starting the software the main screen of the Graphical User Interface (GUI) opens and a dialog box appears asking if acquisition of optoacoustic data follows. When clicking Yes the hardware components – laser, chamber and data acquisition unit – are prepared for data acquisition. If you do not want to acquire data, e.g. in order to export or process the data click No.



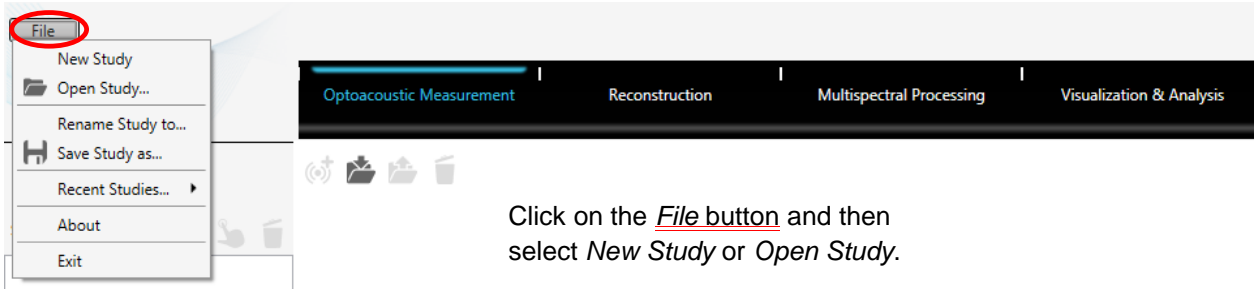
The four main functional areas of the software are reflected by four tabs on the screen.

- 1) **Optoacoustic Measurement** (OAM): acquiring data on an MSOT system
- 2) **Reconstruction**: reconstructing images from the raw data
- 3) **Multispectral Processing**: multi-spectrally processing those images to extract spectrum dependent component images
- 4) **Visualization and Analysis**: displaying and post processing of the images as well as extracting statistical measures from the data

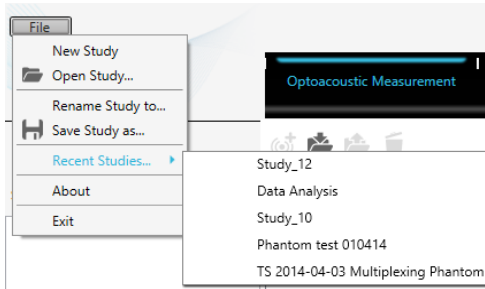
ViewMSOT™ uses a study-centered concept to store the data. A study may contain a multitude of different scans, which will be visible in a list in the main window of the software. Different columns of this list make it easy to identify the particular scan.

NOTE: The system's hardware can also be initialized later as explained in 3 IMAGE ACQUISITION. However, initializing the hardware environment before choosing the study and/or selecting the Study Preset (see 2.1 SELECTING A STUDY PRESET) is advised to save time.

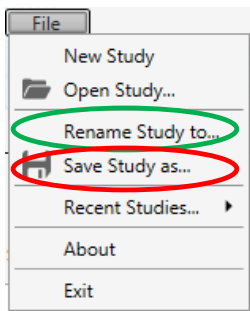
Prior to the actual experiment an existing study needs to be opened or a new one has to be set up.



Click on the File button and then select *New Study* or *Open Study*.

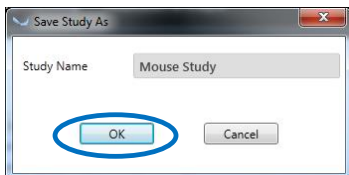


Or choose one of the *Recent Studies*.



To change the name of the current study select Rename Study to...

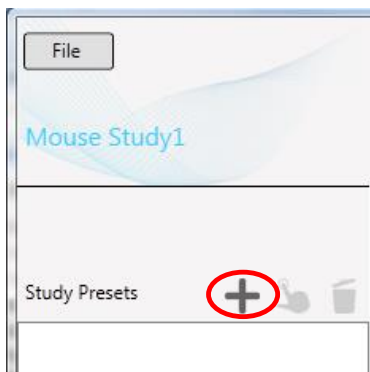
Select Save Study as... to save a copy of the current study.



Enter a *Study Name* and press OK to save it.

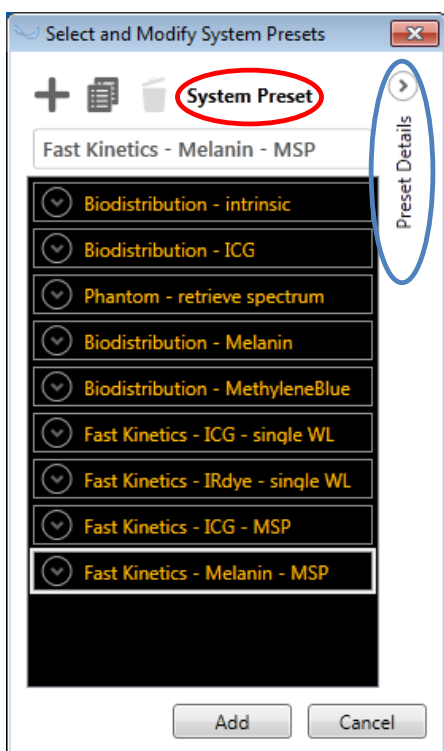
2.1 SELECTING A STUDY PRESET

For different types of studies *System Study Presets* that include presets for data acquisition as well as image reconstruction and multispectral processing are available. Study Presets allow to have one or more fixed set(s) of parameters for a whole study that can be applied to scans without being required to re-enter all parameters for each scan separately.



To select a Study Preset press the “+” button.

The *Select and Modify System Presets* dialog opens.



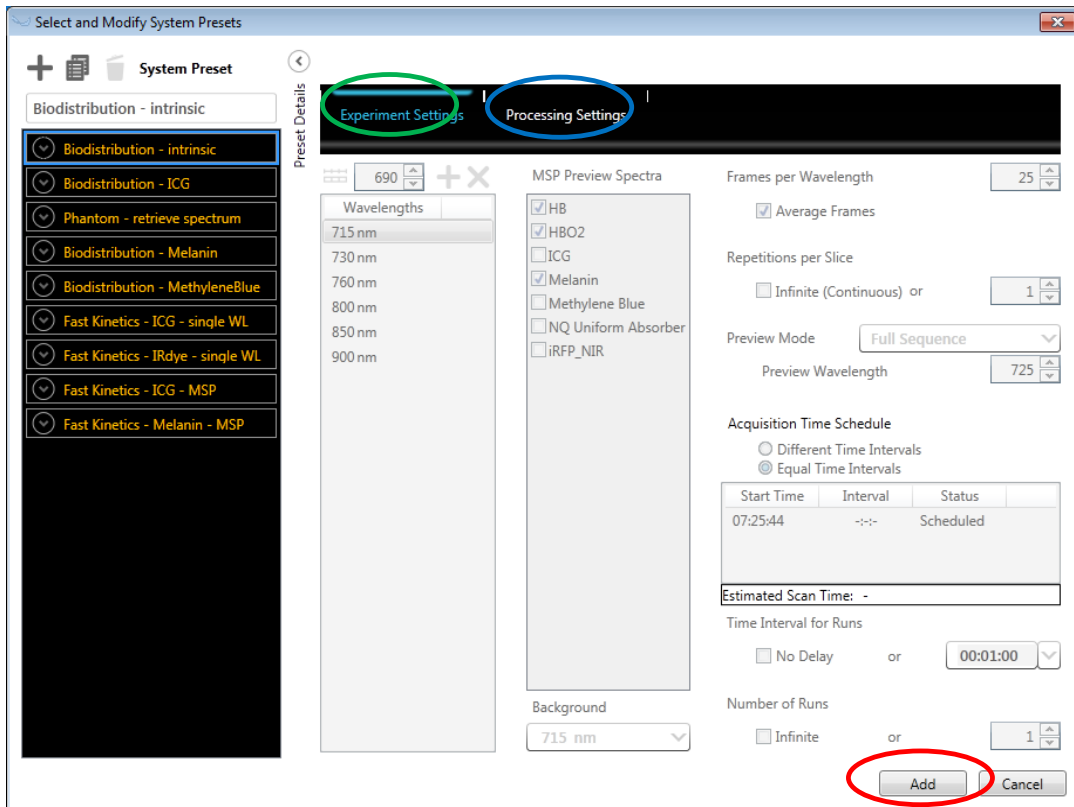
The software contains different System Study Presets for particular applications, e.g.

- Determination of blood oxygenation
- Measurement of lymph nodes
- Whole-body anatomical mouse scan
- Etc.

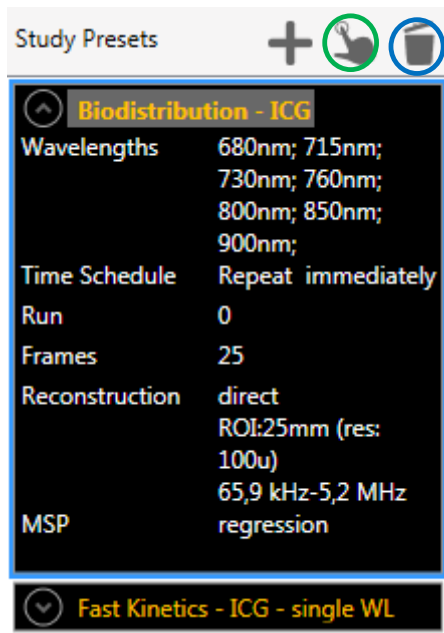
To review the existing System Study Presets click Preset Details.

NOTE: The user cannot modify or delete these optimized System (inherent) Study Preset, but can generate new Study Presets or duplicate the existing presets and modify them (see section 2.2 CREATING STUDY PRESETS).

The *Study Presets* contain Experiment Settings defining the relevant parameters for data acquisition and Processing Settings containing the parameters for reconstruction and multispectral processing. The content of the Experiment Settings and the Processing Settings tab will be explained in detail in 2.2 CREATING STUDY PRESETS.



Press Add to add the selected Study Preset from the list to the current study and close the dialogue.



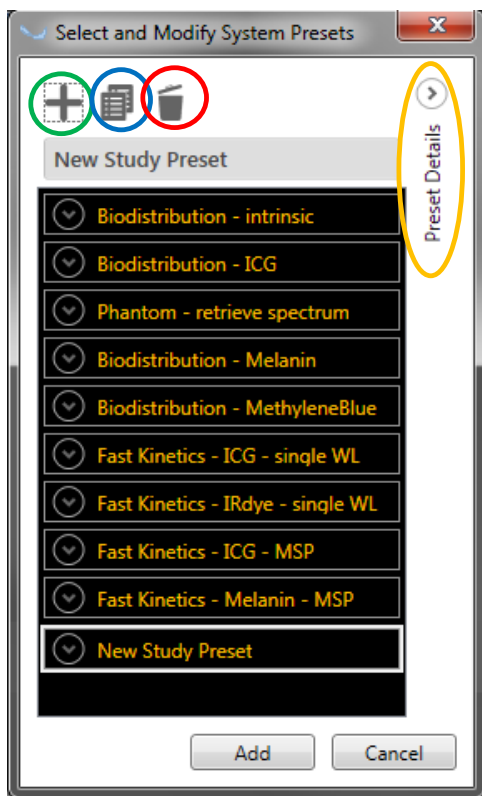
Click on one of your Study Presets (selection is indicated by a blue frame) to extend the information.

Click the Activate icon to select the preset for the scans to record during the subsequent acquisitions. The currently active Study Preset is highlighted by a grey background behind the study name.

Press the Trash Bin icon to delete the selected Study Preset from your Study

NOTE: Once a Study Preset is removed from the Study the user needs to add that Study Preset to the Study again as described above. There is no undo functionality. **Make sure to check which list you are deleting from:** While using the Trash Bin icon on the main screen will only delete a preset from the current study, the one in the Select and Modify System Presets list will delete that preset from the system permanently.

2.2 CREATING STUDY PRESETS



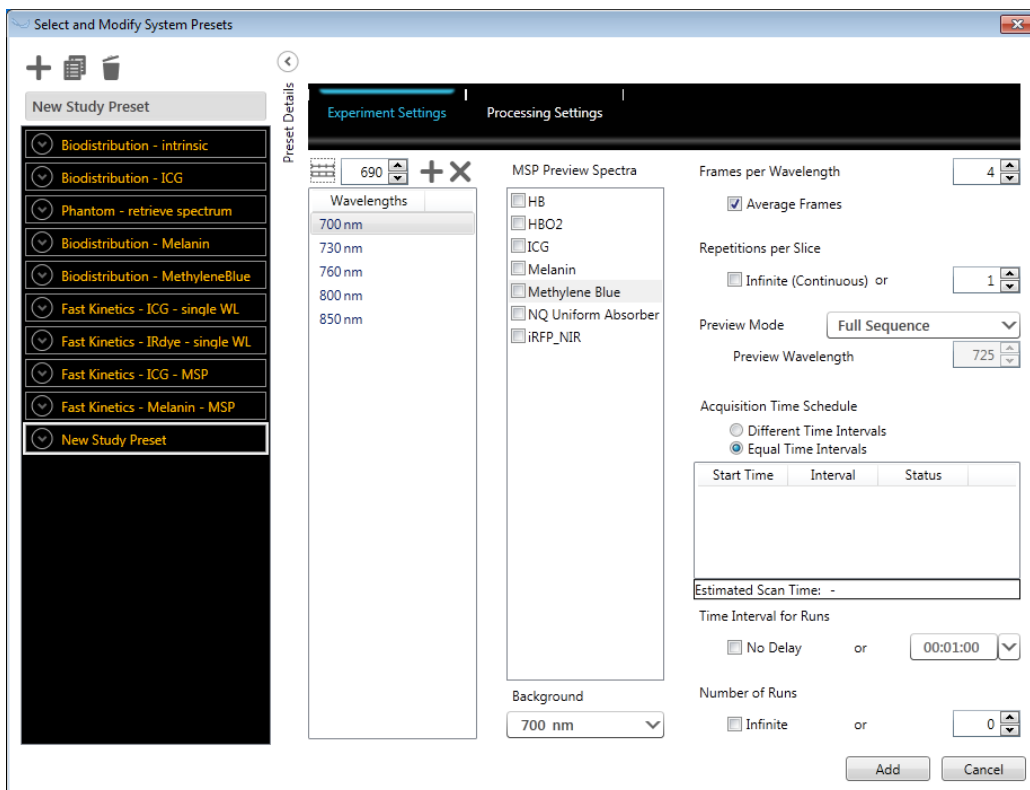
Open the *Select and Modify System Presets* dialogue as described in 2.1 *SELECTING A STUDY PRESET* and click "+" to generate your own Study Preset or duplicate one of the existing Study Presets to modify it according to your needs.

Delete one of the Study Presets.

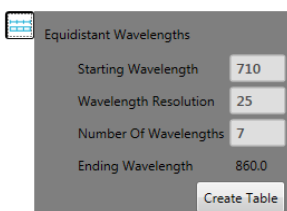
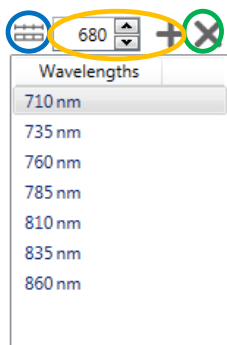
NOTE: There is no possibility to delete System Study Preset. Only presets generated by the user can be deleted.

NOTE: Deleting a Study Preset will delete that preset from the system permanently. There is no undo functionality.

Click *Preset Details* to open an area where you can modify the chosen Study Preset.



2.2.1 Experiment Settings

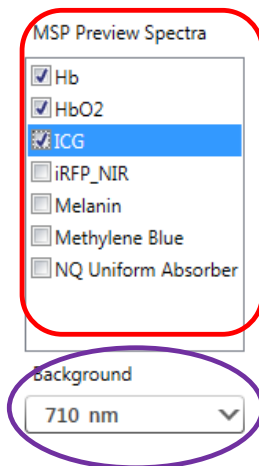


When creating Study Preset a default choice of wavelength for multispectral acquisition is presented in the Wavelengths list. Adding wavelengths to this list is possible in two ways:

- 1) Different wavelengths can be selected and added to the active list manually. In this way it is possible to select any pattern of wavelength.
- 2) If a set of equally distributed wavelengths is needed there is a faster alternative method: Define the *Starting Wavelength*, the *Wavelength Resolution* as well as the *Number of Wavelengths* in this case.

After clicking the *Create Table* button the wavelengths will be added to the active list.

Any entry in the wavelengths list can be deleted by clicking "X".



Frames per Wavelength
 Average Frames

4

To display a live multispectrally decomposed image during live preview and data acquisition for certain absorption molecules like Indocyanine Green (ICG) or melanin check the boxes for the spectra of interest in the MSP Preview Spectra box.

For the decomposition of the multispectral image (layers) the linear regression algorithm, a fitting procedure using known spectra that allows for easily reproducible results across studies, is used (see section 2.2.2 Processing Settings).

Select one of the single wavelength images as a Background anatomical image for the MSP preview. The multi-spectral components will then be overlaid with semi-transparency on top of that background image.

Define the number of *Frames per Wavelength* to be acquired. The raw data for each wavelength from multiple consecutive pulses is averaged to create a single image. This can be useful to increase the ratio of signal to noise or to account for motion.

NOTE: The ideal number of averages should be determined empirically depending on the use case (temporal profile of the experiment, motion from heart beat or breathing, etc.). As images are generated at 10Hz, there is a tradeoff between temporal resolution and the number of averages used, especially considering that multiple wavelengths are used to generate a multispectral analysis.

Furthermore, multispectral analysis compares the signal intensity at different wavelengths on a pixel by pixel basis; therefore, the user must consider whether motion or a change in concentration in an observed probe in the region of interest could affect the measurement in the time it takes to acquire a single multispectral data set. In addition, averaging the data to account for motion also reduces the apparent resolution.

Repetitions per Slice
 Infinite (Continuous) or

1

Repetitions per Slice defines the number of repetitions that the software acquires at all defined wavelengths with the defined number of frames at the same position before moving to the next position.

Repetitions per Slice
 Infinite (Continuous) or

1

Preview Mode Full Sequence

Preview Wavelength 725

Acquisition Time Schedule
 Different Time Intervals
 Equal Time Intervals

| Start Time | Interval | Status |
|------------|----------|--------|
| | | |

Estimated Scan Time: -

Time Interval for Runs

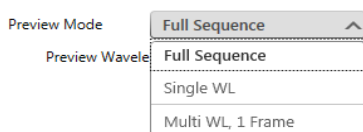
No Delay or 00:01:00

Number of Runs

Infinite or 0

Check *Infinite (Continuous)* to continuously acquire the defined set of wavelengths with the defined number of frames at one tomographic slice (position). When you use *Infinite (Continuous)* repetitions setting up imaging regions (see 3.2.5 Setting up Scan Region(s)) as well as setting up an Acquisition Time Schedule (see below) becomes obsolete. It is grayed out in the Experiment Settings screen.

Choose the Preview Mode of the live Image Preview (see 3 IMAGE ACQUISITION). Live preview is used to explore the imaging object and to set up the imaging regions before the actual acquisition with the defined settings is started:

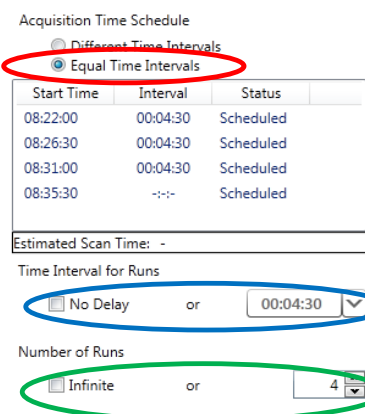


- 1) *Full Sequence*: Preview is done with the defined number of frames at the defined wavelength of the acquisition presets (what you see is what you get).
- 2) *Single WL*: Define a single wavelength for preview (if you are only interested in the anatomy of the imaging object).
- 3) *Multi WL, 1 Frame*: Perform live preview with the defined wavelength of the acquisition presets but with one frame per wavelength (the fastest method to preview multispectral data).

NOTE: The selection that is chosen in the Preview Mode is for image preview only. The acquisition always uses the defined presets.

NOTE: If *Infinite (Continuous)* is checked the *Preview Mode* is always *Full Sequence*.

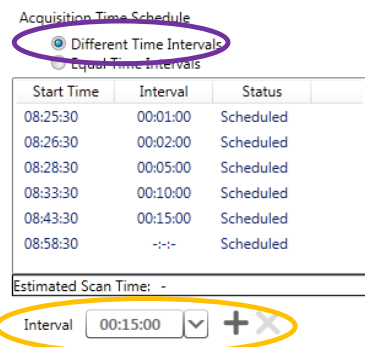
The acquisition of the same scan regions repeatedly within one scan can be done with the use of multiple Runs in the *Acquisition Time Schedule*. Choose between *Different Time Intervals* (meaning freely chosen points in time) and *Equal Time Intervals*.



For *Equal Time Intervals* it is possible to choose a defined time Interval between subsequent runs, or to proceed immediately to the next Run by clicking *No Delay*.

If the time interval chosen is too short and conflicts with the estimated scan time, this conflict will be indicated by a red box around the *Estimated Scan Time*.

The selection of the *Number of Runs* can be either *Infinite* where the system acquires images in an open loop manner. Alternatively, a fixed *Number of Runs* can be defined.



Another option is to choose *Different Time Intervals* between subsequent Runs.

Different intervals between the different Runs can be added and removed.

Section 3.3 STARTING THE ACQUISITION explains how to stop acquiring data after a run is finished or after a full set of wavelengths has been acquired.

00:04:00

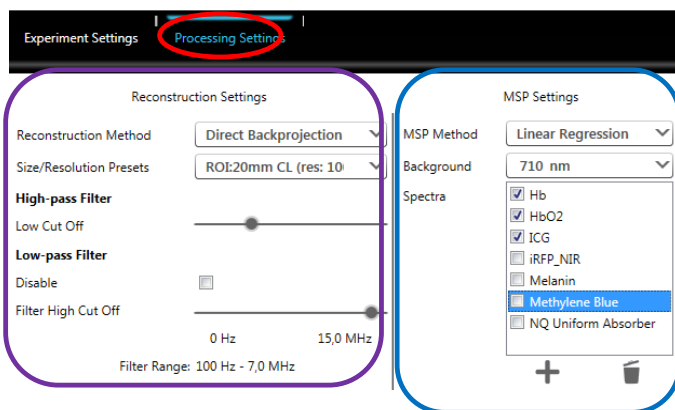
00:04:02

NOTE: Click into the text box next to the dropdown menu to modify the time interval if you want to apply a time interval different from the choices in the dropdown menu.

NOTE: Once a measurement has started the time schedule cannot be changed (see section 3.1 MSOT ACQUISITION).

Note: The *Start Time* in the Time Schedule is referring to the current time. This *Start Time* is updated when entering the acquisition and starting the measurement.

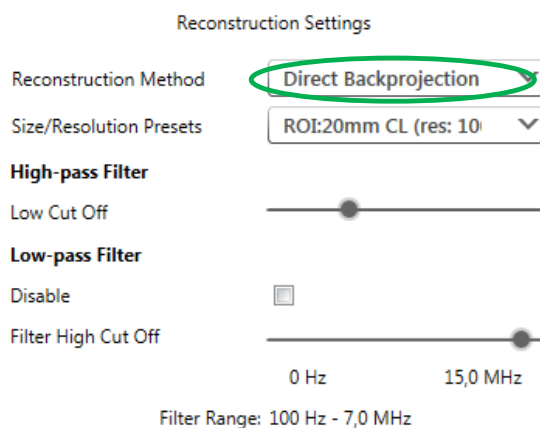
2.2.2 Processing Settings



The *Processing Settings* contain parameters for image reconstruction and multispectral processing.

NOTE: *Processing Settings* do not affect the acquisition mode but will be used during reconstruction and multispectral processing. All parameters that affect the acquisition and acquisition preview are located in the *Experiment Settings* tab of the screen.

For image reconstruction the following parameters need to be set up:

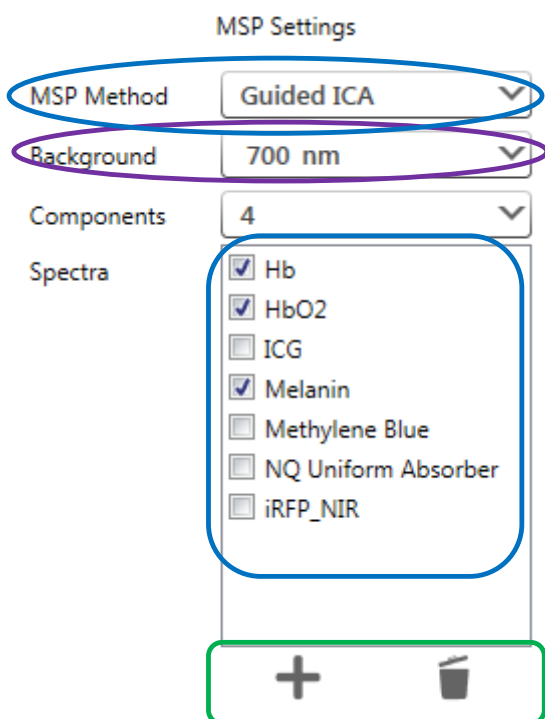


NOTE: Increasing the resolution leads to higher processing time effort.

- 1) *Reconstruction Method:*
 - a) *Back Projection* reconstruction is a very fast and semi-quantitative reconstruction algorithm that allows efficient generation of anatomical images. The back projection algorithm is also used for the live preview.
 - b) *Model Based* reconstruction is a more precise way of reconstructing that is computationally more demanding and thus takes significantly more time to process, but incorporates a detailed model of the detection geometry that allows for more quantifiable images
- 2) *Size and Resolution Presets* are adjustable for different imaging objects.
- 3) *Bandpass Filter:* With *Direct Backprojection* the *Low* and the *High Filter Cut Off* frequencies can be modified. Otherwise the system will use the recommended presets of 50kHz to 7MHz

Under *MSP Settings* the multispectral processing method is chosen:

- 1) *Difference*: Subtracts an image acquired at one wavelength from an image acquired at another wavelength. Subtract a wavelength reflecting maximum absorption from the absorber of interest from a wavelength of low absorption. Use of this algorithm is limited to cases where the change in pixel intensity is only defined by the change in concentration of one absorber.
- 2) *Ratio*: Similar to the subtraction algorithm, this method divides the signal acquired at one wavelength from the signal acquired at another, according to the formula $[(a-b)/a]$. The same conditions and limitations described above for the subtraction algorithm apply to this algorithm.



- 3) *ICA*: Independent Component Analysis (ICA) is a blind method that does not require prior spectral knowledge and iteratively searches for relevant absorbers. The number of components equals to the number of input wavelengths.
- 4) *Linear Regression*: Fitting procedure using known spectra that allows for easily reproducible results across studies.
- 5) *PCA/ICA*: Blind method that does not require prior spectral knowledge. It can retrieve spectra based on the statistical properties of the dataset. Principle component analysis (PCA) is performed initially, which identifies the most dominant absorber in the image. This dominant absorber is the basis for the subsequent ICA algorithm. Components are then randomized during the ICA procedure.
- 6) *guidedICA*: An adaptive algorithm that uses a-priori knowledge of the absorption spectra in the sample in order to best adapt to the individual situation. It makes use of the statistical properties of the dataset, and thus does not allow comparisons between different MSP data sets.


Choose a *Background* single wavelength image. On top of this the multispectral unmixed components are overlaid with semi-transparency.

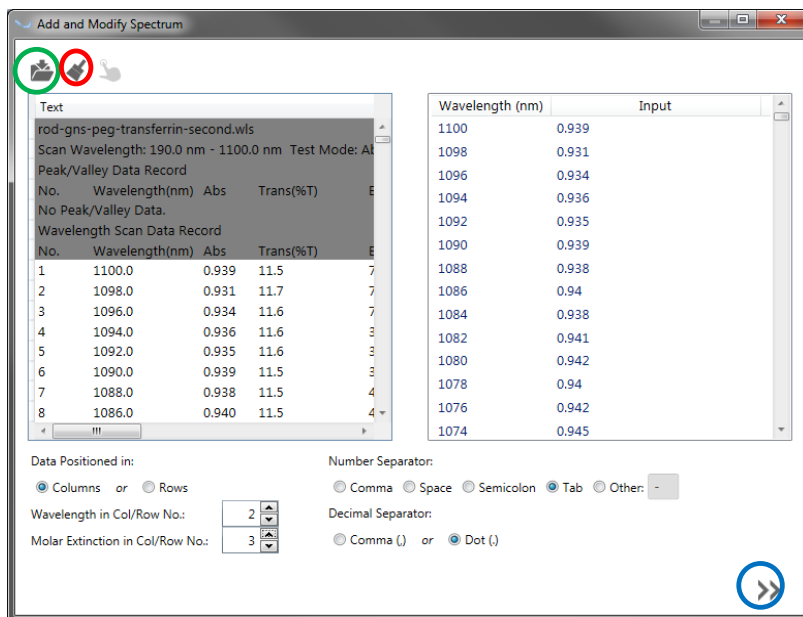
Press “+” to add a new spectrum or the Trash Bin icon to remove an existing one.

2.2.3 Interactive Spectrum Import Wizard

Open the spectrum import wizard by pressing + under the MSP Settings. There are two different ways to add a spectrum:

2.2.3.1 Import Values for Wavelength and Absorption

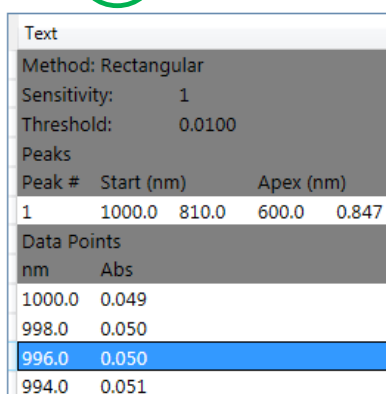
- 1) Click the Import Spectrum File icon to open a .txt or a .csv-file containing the spectrum data.
- 2) Import a spectrum by copy & paste: Copy the spectrum (wavelength and absorption) from Excel or from a text document and paste it into the import wizard by pressing .



The software expects the wavelength to be the first row and the absorption data to be the second row. It automatically tries to detect the:

- File header: e.g. information from the spectrometer the data was acquired with which is not part of the spectrum data. The part of the text data being recognized as the header is grayed out in the left sided text window
- Number Separator and
- Decimal Separator

A preview of the import data - wavelength and input absorption - is shown on the right. Check if the software detected the data you wanted and press the arrow button at the bottom right to continue to the next screen. If auto-detect did not work correctly please modify the import settings as explained below.



1) **Remove Information Header** from the input file:

In the example on the left the auto-detect has missed to omit 1 1000.0 810.0 600.0 0.847.

Click the lines you want to omit and press the *Select/Deselect Comments* icon.

To select/deselect multiple lines keep the left mouse button pressed when you move over the comment lines.

According to the header selection the preview on the right is updated.

| Text | |
|-------|-------|
| 980.0 | 0.060 |
| 978.0 | 0.061 |
| 976.0 | 0.062 |
| 974.0 | 0.064 |
| 972.0 | 0.065 |

Text
0,966187810548308;1;0,96122616850974;0,796648
680;700;720;740;760;780;800;820;840;860;880;900;

Data Positioned in:

Columns or Rows

| No. | Wavelength(nm) | Abs | Trans(%T) |
|-----|----------------|-------|-----------|
| 1 | 1100.0 | 0.939 | 11.5 |
| 2 | 1098.0 | 0.931 | 11.7 |
| 3 | 1096.0 | 0.934 | 11.6 |
| 4 | 1094.0 | 0.936 | 11.6 |
| 5 | 1092.0 | 0.935 | 11.6 |

Wavelength in Col/Row No.:
Molar Extinction in Col/Row No.:

| Wavelength (nm) | Input |
|-----------------|-------|
| 1100 | 0.939 |
| 1098 | 0.931 |
| 1096 | 0.934 |
| 1094 | 0.936 |

Number Separator:

Comma Space Semicolon Tab Other:

Decimal Separator:

Comma (,) or Dot (.)

Press the arrow button **>>** at the bottom right to continue to the next screen.

- 2) **Change the reading orientation** for the input data text:

As default the wizard expects the wavelength data as well as the absorption value of the import file to be arranged in columns.

If the data is oriented horizontally – the wavelengths and the absorption values being arranged in rows – instead, please change the orientation under *Data Positioned in*.

- 3) **Modify the selection of the columns/rows** for wavelengths and the absorption values:

As default the wizard expects the wavelength values in the first column/row and the absorption values in the second column/row.

Please modify this selection if necessary by changing the column/row numbers for wavelength and/or absorption.

- 4) **Modify the number separator and decimal separator:**

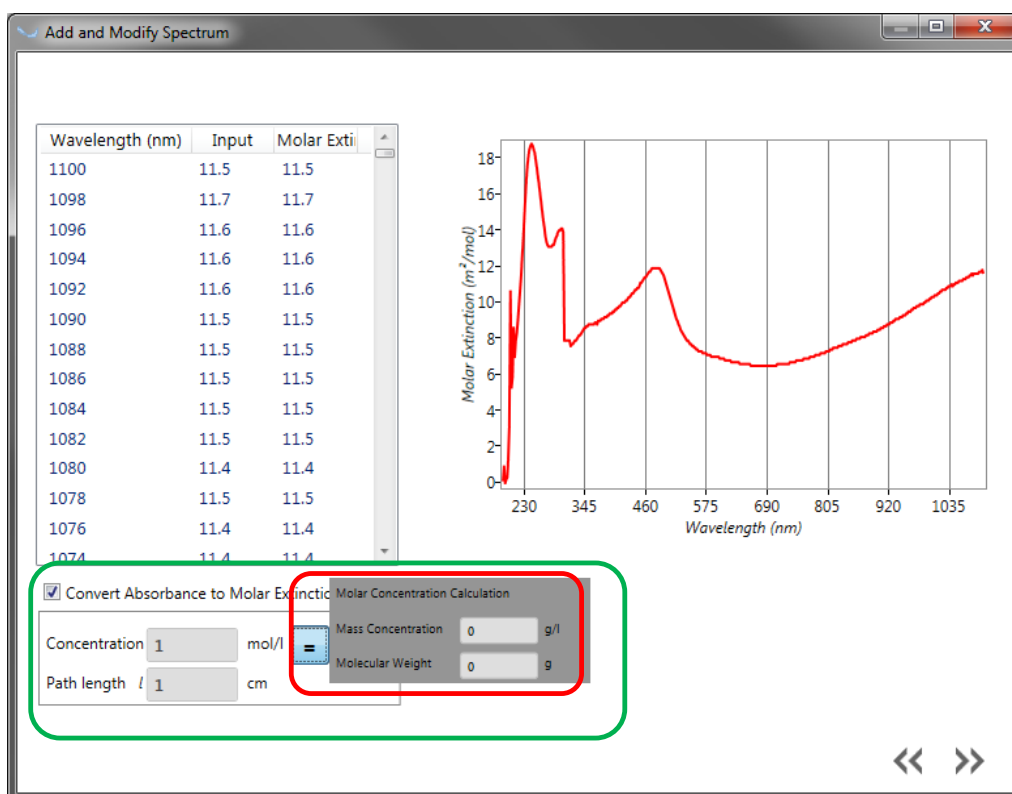
If the wizard does not auto-detect the wavelength and the spectrum input correctly, review the *Number Separator* and *Decimal Separator*.

2.2.3.2 Review Spectrum

Review the spectrum curve you want to import on the second screen of the import spectrum wizard. [Click the check mark to convert the absorption values \(Input column\) into Molar Extinction](#). The Molar Extinction $\epsilon(\lambda)$ is calculated by the equation:

$$\epsilon(\lambda) = \frac{A(\lambda)}{c \cdot l}$$

Where $A(\lambda)$ is the wavelength dependant absorption – that is the absorption value which is delivered by the spectrometer – c is the molar concentration in moles/liter and l is the path length.



Calculate c by entering the Mass Concentration ρ and the Molecular Weight M in the popup according to the equation

$$c = \frac{\rho}{M}$$

Press the arrow button \gg at the bottom right to continue to the next screen.

2.2.3.3 Modifying the Spectrum and Import

The tab on the right of the third spectrum import screen shows the values for wavelength and spectrum (either absorption or molar extinction).

Choose any line of the table and press “x” to delete it from the data to import, e.g. to remove an outlier or add an absorption/molar extinction value at a certain wavelength.

| Wavelength (nm) | Molar Extinction |
|-----------------|------------------|
| 932 | 8.9 |
| 934 | 9 |
| 936 | 9 |
| 938 | 9 |
| 940 | 9 |
| 942 | 9.1 |
| 944 | 9.1 |
| 946 | 9.1 |
| 948 | 9.2 |
| 950 | 9.2 |
| 952 | 9.2 |
| 954 | 9.3 |
| 956 | 9.3 |
| 958 | 9.4 |
| 960 | 9.4 |

Added values show up in the table ordered by wavelength.

Both the values from the tab on the left (blue dots) and an interpolated curve (red line) are shown in the graph on the right.

The order of the spline interpolation can be changed if necessary.

Entering a name and press the import icon to save the final interpolated curve.

NOTE: If you delete a spectrum it will not only be deleted from the current Study Preset but from the system the software is installed on. That way it will not be possible to use the deleted spectrum for further studies/ Study Presets. Old studies containing those spectra will not be affected because they keep the chosen spectra in the study data.

3 IMAGE ACQUISITION

After creating a study and having chosen the Study Presets new scans can be added to that study. If multiple Study Presets exist the user needs to activate the desired one for the current measurements (see 2.1 SELECTING A STUDY PRESET).



Before acquiring a scan, the hardware components – laser system, acquisition system etc. – have to be initialized by pressing the Power On/Off icon in the task bar, if this has not been done when starting the software (see 2 STARTING ViewMSOT™ AND SETTING UP A STUDY).

The Power On/Off icon turns from white to green as soon as the hardware is initialized.



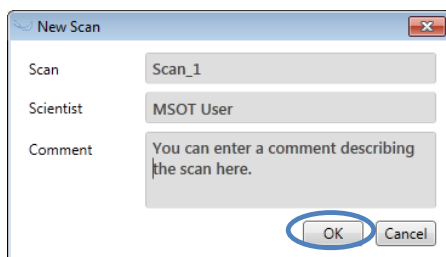
The current Temperature of the water in the imaging tank is shown in the task bar

NOTE: Only insert the animal when the intended temperature of 34°C (changeable on request) is reached.

NOTE: It normally takes some minutes until the animal's core body temperature has acclimated to the water temperature. Experimental results can vary as a function of animal body temperature.



As soon as the system is ready the Add Optoacoustic Scan icon button becomes active. Pressing it will bring up the New Scan window.



The subsequent scan can be named and annotated in this window. Those entries can also be changed later during or after leaving the acquisition.

Pressing OK closes the New Scan window and enters the MSOT acquisition window.

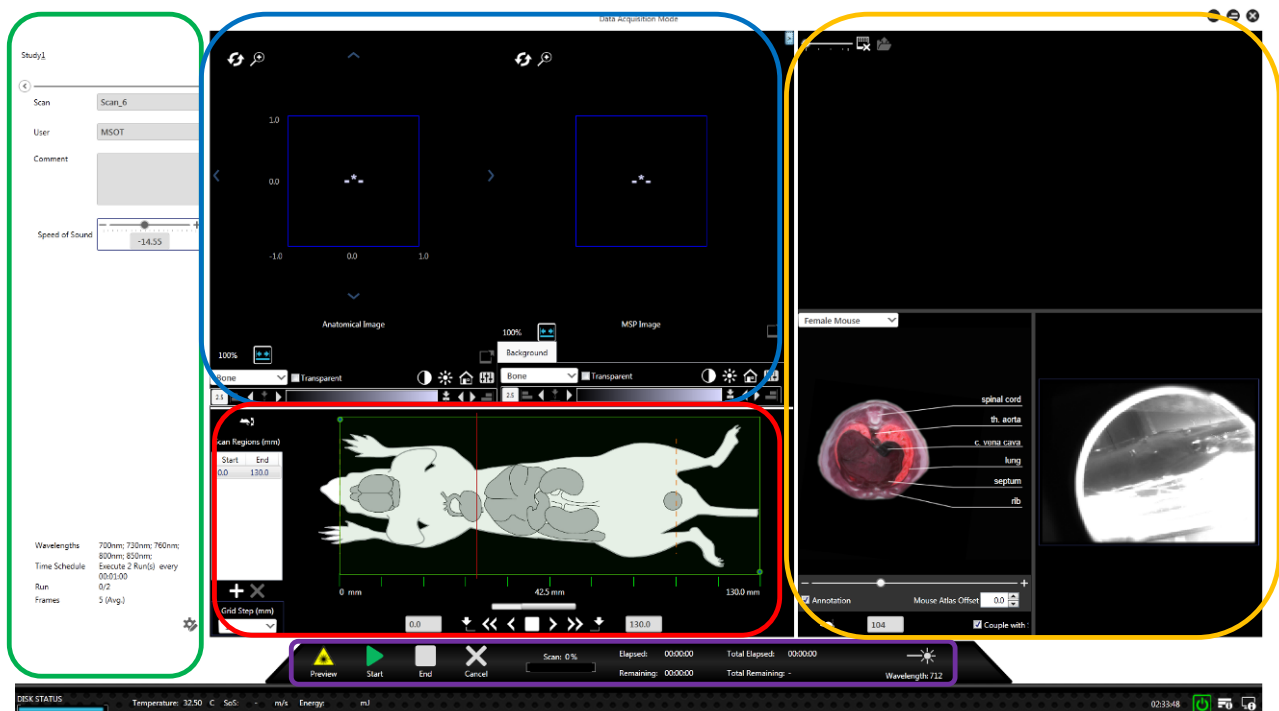
NOTE: The Add Optoacoustic Scan icon is only active if the hardware is initialized.

3.1 MSOT ACQUISITION

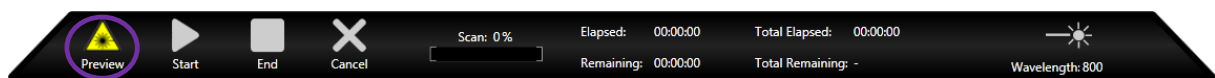
In the MSOT Acquisition window the user is able to monitor the scanning procedure in live mode. The screen is set up in five sections:

- 1) Scan Information extender: Shows all relevant scan information such as name, user, comment, speed of sound trimming, as well as the acquisition parameters.

- 2) Live Image Preview and Image Control: Monitoring of the live image – single wavelength and multispectral image - during acquisition. Via Image Control preview image properties and scaling can be modified on the fly.
- 3) Positioning Information and Setup panel: Moves the imaging plane in relation to the imaging object and allows exploring and defining scan regions of interest for the subsequent acquisitions.
- 4) Control Panel: Start acquisition and view acquisition progress, as well as interrupt an acquisition.
- 5) Dashboard: Consists of windows for CryMOUSE™ atlas, live CCD camera chamber view (if installed) and a Snapshot Window where snapshots from the live image preview - single wavelength or MSP - can be kept.



By clicking the Preview button in the *Control Panel* the system fires the laser and starts showing a live tomographic image. Clicking the button a second time turns off the preview.

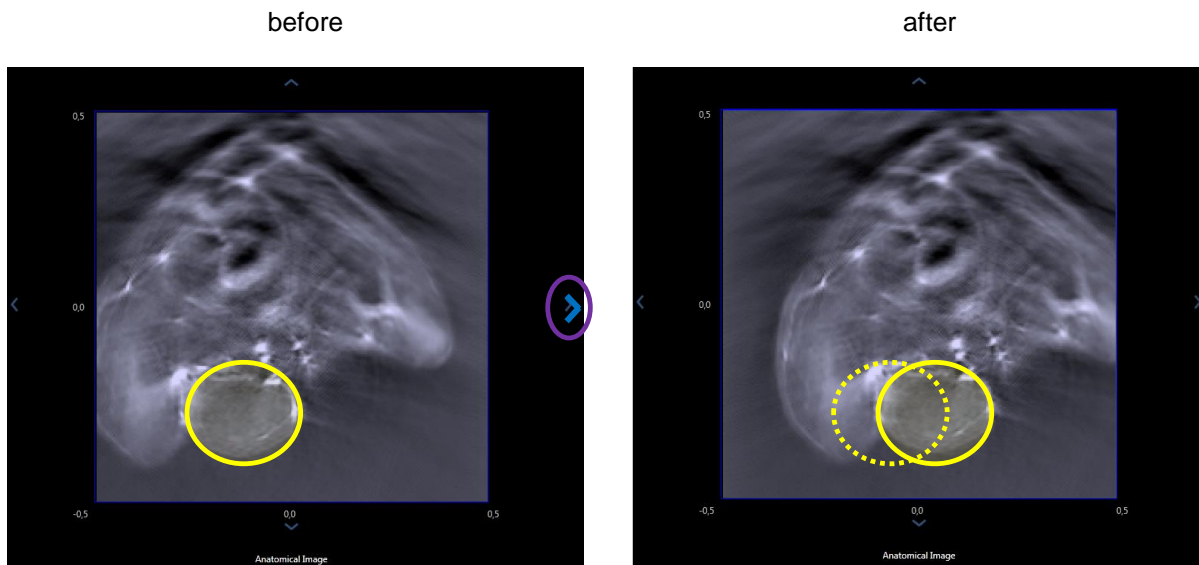


NOTE: To start the data acquisition the desired Scan Region(s) need to be defined.

3.2 EXPLORING AND SETTING UP SCAN REGION(S)

3.2.1 Positioning the Imaging Object in the Imaging Plane

Using the blue arrow buttons to the left, right, top and bottom of the Live Image Preview the imaging object can be moved to center it in the middle of the screen. Note that the arrows move the object and not the view window.



In the example the right arrow is pressed to move the mouse to the right.

3.2.2 Setting the Orientation of the Mouse

As can be seen in the schematic drawing in the Positioning Setup and Information panel the animal needs to be inserted into the device with the head facing to the left side. This is required to maintain that the tomographic image, the Positioning Information and Setup panel, the mouse atlas and later during image analysis the ROI analysis graph are all synchronized.

It is possible to insert the animal with the belly facing down or with the belly facing up. Click the *Mouse Orientation* icon in the right top of the Positioning Information and Setup panel to change from belly down to belly up orientation:



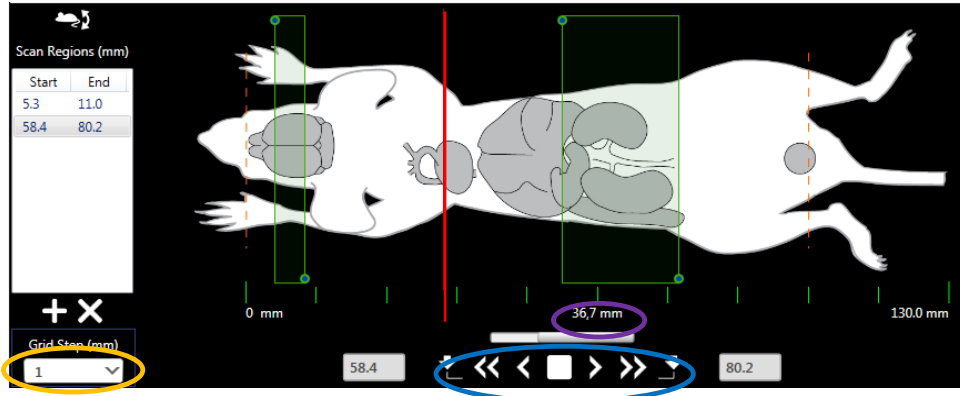
The tomographic single wavelength and the MSP live image are modified to have exactly the same orientation as the images in the CryoMOUSE™ atlas. No matter if the mouse is positioned on its belly or its back, the image in the preview has the same orientation.

NOTE: This orientation correction is internally kept as information and applied also during Visualization.

3.2.3 Exploring the Imaging Object

By clicking the arrow buttons inside the Positioning Setup and Information panel the user moves the imaging plane indicated by the red line in relation to the imaging object and explores it in the Image Live Preview.

- “<” and “>”: Click these buttons to make the imaging plane move one step with the step size defined in Grid Step.
- “<<” and “>>”: The double arrows make the imaging plane move continuously. Pressing those arrows more than 2 seconds makes the imaging plane move until stopping it.



- “□”: to stop the movement of the imaging plane.
- **Double click on a position** in the positioning window, e.g. the kidneys, moves the imaging plane to the location of interest.

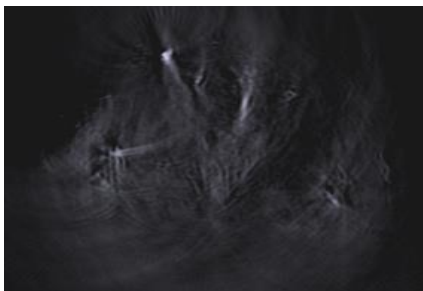
The position information of the imaging plane is displayed below the scale under the mouse.

If a continuous scan is acquired the user does not need to set up a *Scan Region*. By pressing the Start icon the acquisition begins (see Section 3.3 STARTING THE ACQUISITION).

NOTE: It is possible to freely move the imaging plane during the acquisition when using continuous mode. This is not the case for automatic scan.

3.2.4 Synchronize the current Imaging Plane with the Mouse Atlas

It is possible to synchronize the imaging plane visible in the live image preview with the corresponding image of the CryMOUSE™ atlas and with the red line showing the current position in the Positioning Setup and Information panel.



Move to the tail region of the mouse right where the tail and the legs still can be seen as one connected region.

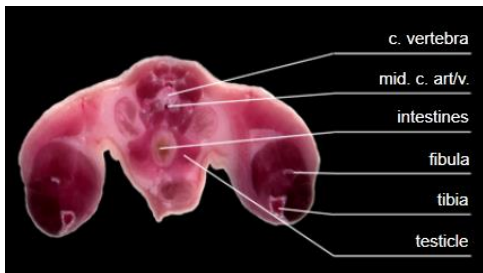
Press the Mark Mouse Tail icon underneath the mouse atlas.

The current imaging plane position of the live image preview is set as tail position.

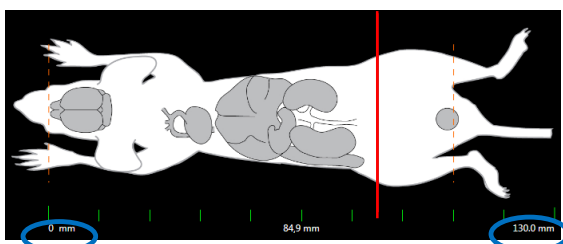


Alternatively the length of the mouse (in mm) can be entered for synchronization of mouse atlas and image preview.

The mouse atlas is adjusted and jumps to the corresponding tail slice.



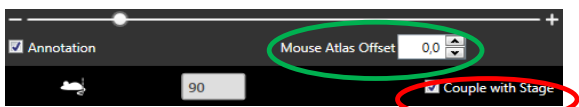
The Positioning Setup and Information panel is also adjusted accordingly. The orange dashed line in the left holder which corresponds to the first slices of the mouse atlas.



The dashed line on the right represents the region where the mouse tail is marked.

The length of the scale underneath the mouse schematics is automatically adjusted to the size of the mouse.

The synchronization can also be switched off for free browsing through the mouse atlas by unchecking *Couple with Stage*.

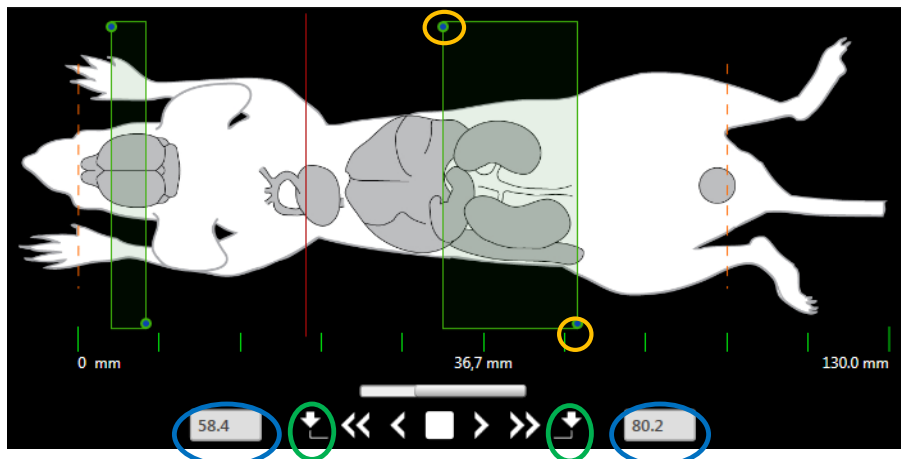


If the anatomy of the mouse in the mouse atlas is slightly shifted in comparison with the live view a Mouse Atlas Offset (in mm) can be used to compensate.

Further functionality of the CryMOUSE™ atlas that is not related to the synchronization of the imaging object's position is explained in 3.6.1 CryMOUSE™ Atlas in detail.

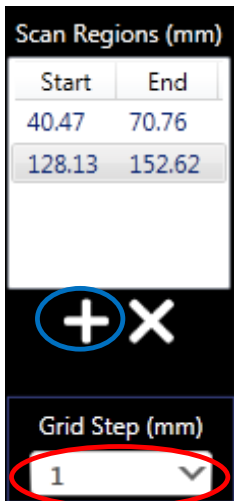
3.2.5 Setting up Scan Region(s)

If the Study Preset is set to Automatic scan one or more scan region(s) or position(s) need to be set up.



The start and end position for the scan can be defined in three different ways:

- 1) Move the linear stage and select the start and end using the Mark Current Position as Scan Start/End icon.
- 2) Click and drag the handles above the stage display (green-blue dots) to the desired positions.
- 3) Enter the desired values. After pressing the Enter key the handles move to the indicated position.

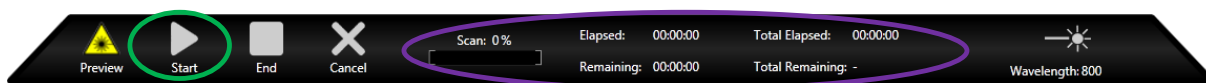


The defined scan region shows up in the *Scan Regions* list. By pressing '+' multiple scan regions can be added, which will be scanned sequentially.

Define the Grid Step size to determine the distance between two imaging planes (or slices) within a *Scan Region*.

3.3 STARTING THE ACQUISITION

Clicking the Start button in the Control Panel starts the data acquisition. Additional acquisition status information such as remaining time, elapsed time, current wavelength, etc. is shown in the status bar. For multiple *Run* acquisitions the *Remaining* time for the current run and the *Total Remaining* time until the last acquisition run ends are displayed.



The acquisition can be interrupted by pressing *Cancel*. When pressing *Cancel* all wavelengths of the current scan position will be finished before the scan is aborted. If the user is running a measurement including multiple *Runs* pressing *End* will finish the scan after the current *Run* is complete, i.e. the last position of the current run is acquired.

3.4 SCAN INFORMATION EXTENDER/EDIT SCAN PRESET

Mouse Study1

←

Scan **Before injection**

User **Peter Mustermann**

Comment **Multispectral measurement of the kidney region of a balb/c**

Speed of Sound **4.31**

Wavelengths 690nm; 715nm; 730nm; 760nm; 800nm; 850nm; 900nm;

Time Schedule Execute 3 Run(s) every 00:01:00

Run 0/3

Frames 25

The *Scan Information* extender contains all relevant scan information. The user can enter or modify *Scan Name*, *User Name*, and *Scan Comment*.

If the live image is distorted, the *Speed of Sound* trim should be changed before starting the scan. The speed of sound is dependent on the water temperature and the material of the sample that is imaged. The MSOT scanners automatically adjust the speed of sound to the water temperature, however different imaging objects – or even different areas inside the imaging objects – require slight changes of the speed of sound.

During an acquisition this slider is locked and the entered value is kept as preset for further reconstructions of this scan.

Also shown are the acquisition presets that were pre-defined for the active study.

Clicking the *Scan Settings* icon opens a window containing all acquisition-relevant presets which were defined at the Experiment Settings of the Study Presets (see chapter 2.2



CREATING STUDY PRESETS).

Inside *Scan Settings* it is possible to change those settings for the subsequent scan.

Pressing **OK** stores the modified settings for the subsequent scans.

NOTE: By changing the Scan Settings the Study Preset is not modified. When closing the acquisition screen and reentering it from the main screen the Scan Settings will automatically be taken from the active Study Preset.

NOTE: The description of the parameters of this menu are explained in section 2.2 CREATING STUDY PRESETS).

Scan Settings

Wavelengths 690

Wavelengths 680 nm, 690 nm, 693 nm, 715 nm, 730 nm, 760 nm, 800 nm, 850 nm, 900 nm

MSP Preview Spectra: HB, HBO2, ICG, Melanin, Methylene Blue, NQ Uniform Absorber, iRFP_NIR

Background 680 nm

Frames per Wavelength 25

Average Frames

Repetitions per Slice 1

Preview Mode Full Sequence

Preview Wavelength 725

Acquisition Time Schedule: Different Time Intervals, Equal Time Intervals

| Start Time | Interval | Status |
|------------|----------|-----------|
| 09:16:58 | 00:02:00 | Scheduled |
| 09:18:58 | 00:02:00 | Scheduled |
| 09:20:58 | 00:02:00 | Scheduled |
| 09:22:58 | 00:02:00 | Scheduled |
| 09:24:58 | -:-- | Scheduled |

Estimated Scan Time: 00:01:59

Time Interval for Runs: No Delay or 00:02:00

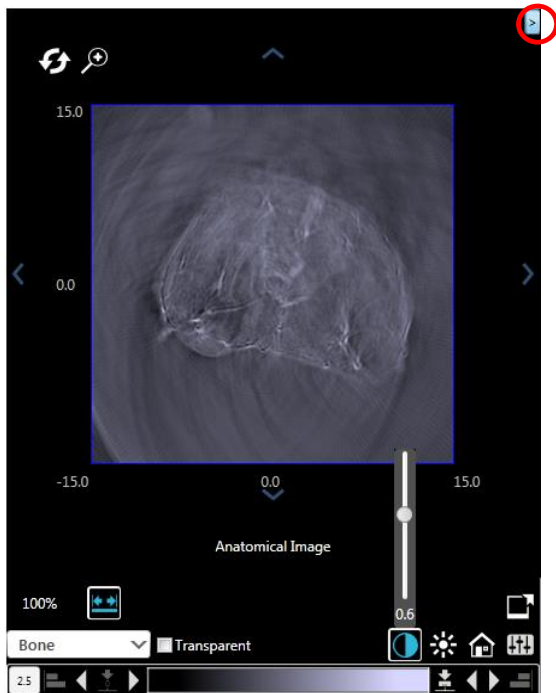
Number of Runs: Infinite or 5

OK Cancel

3.5 IMAGE PREVIEW WINDOW & IMAGE CONTROLS

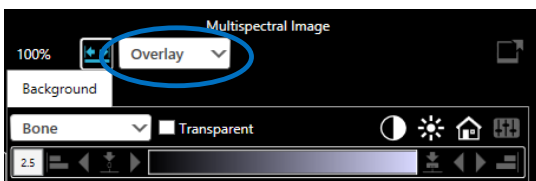
While using the MSOT Acquisition window the live image can be seen in the Image Preview window. Different controls and tools are provided to manipulate the way the scanned images are displayed in the live view. Additional information tools will help to interpret the image. Note that changes to the image at this stage are only affecting the preview image and are not stored with the acquired data.

The following image control tools are also available in the Visualization window. They are explained in detail in section 5.6 IMAGE CONTROLS TO MODIFY THE IMAGES.



- Color bar of the displayed image
- Auto-Scaling of the color bar
- Changing the color map of the image
- Arrow buttons to adjust the color bar in the current image
- Changing Contrast and Brightness
- Changing alpha (transparency) value for the color map

Click the Open MSP Window extender to enable a second tomographic image displaying the live MSP Preview.



Toggle between the two imaging modes Overlay and Color Mixing. They are explained in section 5.6.1 Overlaying and Enabling Difference Layer(s) and MSP-Layers in detail.

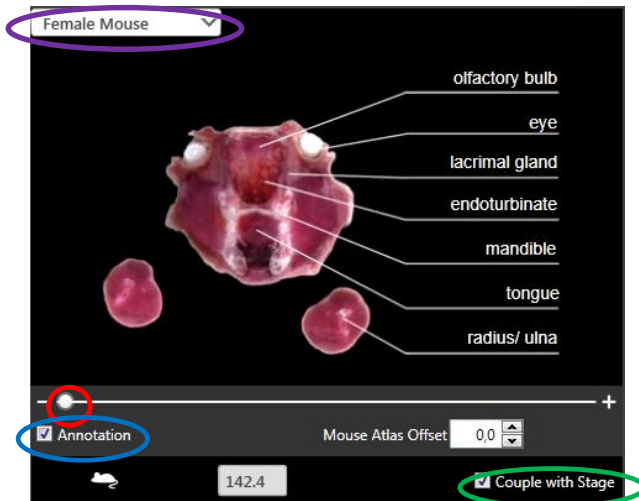
NOTE: At least two spectra need to be chosen for a meaningful MSP Preview.

3.6 ACQUISITION SCREEN DASHBOARD

The Dashboard in the MSOT acquisition window is split into three regions. It contains the CryoMOUSE™ atlas for anatomical reference as well as a live camera view of the tank interior for monitoring the object (if installed) and the live image dashboard for snapshots of the current reconstruction and MSP live preview.

3.6.1 CryoMOUSE™ Atlas

The mouse atlas contains annotated images of an adult male and female CD1 mouse. CryoMOUSE™ is an optional software component and not installed on all systems.



A dropdown allows toggling between female and male mouse atlas, as well as other if the imaging object is not a mouse.

The slider can be moved to different body positions of the mouse.

Annotations of the main anatomical features of the mouse can be displayed or hidden.

3.2.4 Synchronize the current Imaging Plane with the Mouse Atlas
Synchronize the current Imaging Plane with the Mouse Atlas explains in detail how the mouse atlas can be synchronized with the current tomographic slice of the live image preview.

This synchronization is switched off for the mouse atlas to freely browse through the atlas by unchecking Couple with Stage.

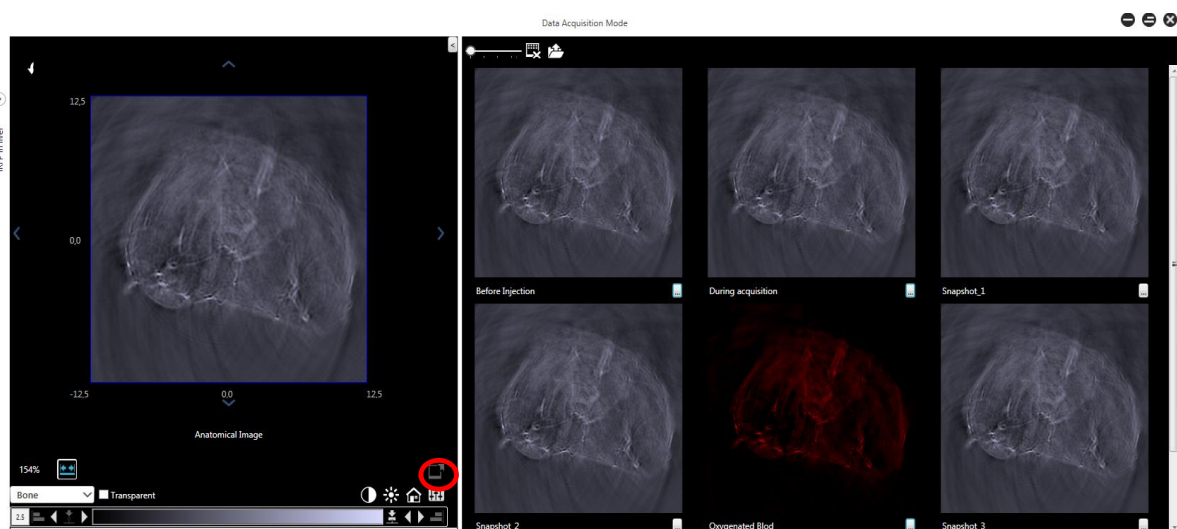
NOTE: The controls in the mouse atlas window that are not mentioned in this section are explained in 3.2.4 Synchronize the current Imaging Plane with the Mouse Atlas .

3.6.2 Camera Preview

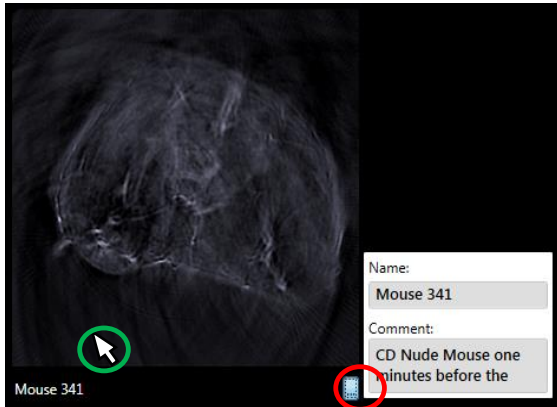
A live camera view displaying the inside of the imaging chamber is displayed in the bottom right part of the acquisition screen Dashboard and used for monitoring the animal during acquisition. The live camera is an optional hardware component and not installed on all systems.

3.6.3 Snapshot Window

A snapshot image of the current anatomical or MSP Image can be added to the Snapshot window by pressing the respective icon in the Image Control panel.



Images are arranged in the Dashboard according to the order in which they have been added.



Mouse 341
CD Nude Mouse one minutes before the injection of ICG.

Name: iRFP in liver
Scientist: User Name
Creation Time: 01.07.2013 15:44

Image Information:
Time: 01.07.2013 15:56
Run: -
Position: 250,0 mm
Repetition: -
Wavelength: 725,0 nm
Average Frames: -
Laser repetition rate: -
Attenuation/Laser Energy: -/-

Reconstruction Parameters:
Speed of Sound: 1518,35
Method: Direct
Resolution: 250
Roi High: 0,053
Roi Low: 0,028
Filter: 50,0 kHz - 7,0 MHz
Chebychev I - 4th Order, zero phase

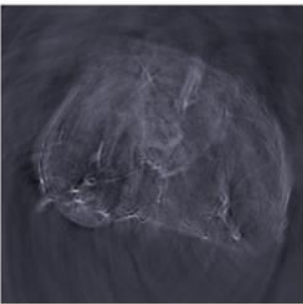


Name: Before Injection
Comment:

Name: iRFP in liver
Scientist: User Name
Creation Time: 28.06.2013 14:07

Image Information:
Time: 28.06.2013 14:10
Run: -
Position: 119,1 mm
Repetition: -
Wavelength: 725,0 nm
Average Frames: -
Laser repetition rate: -
Attenuation/Laser Energy: -/-

Reconstruction Parameters:
Speed of Sound: 1515,63
Method: Direct
Resolution: 250
Roi High: 0,0535
Roi Low: 0,0285
Filter: 50,0 kHz - 7,0 MHz
Chebychev I - 4th Order, zero phase



To change the name of the snapshot click the "..."-
icon under the reconstruction/MSP snapshot.

It is also possible to add a comment in the popup.

Move the mouse pointer over a snapshot to get
further information on the image:

- Name and Comment
- Study information
- Acquisition information
- Reconstruction and MSP(if the snapshot was made from an MSP) parameters

The image size can be adjusted in the snapshot
window by moving the slider.

The icon next to the slider clears all images from
the snapshot window.

Click the Export icon and choose a folder to save
all snapshots from the dashboard.


The reconstruction and/or MSP snapshots are
saved as .png-files along with the information that
is also shown in the mouse over and described
above.

NOTE: The snapshot images along with the additional information are also stored within the study. They can be accessed from the OAM tab of the main screen (see 3.8.4 Reviewing Snapshots).

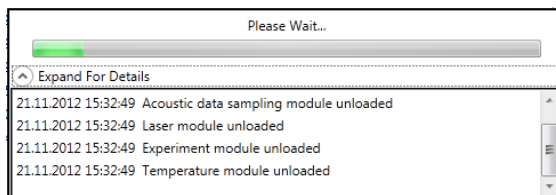
NOTE: All images will be removed when clearing the Snapshot window. It is not possible to clear individual images.

3.7 EXITING ACQUISITION MODE & TURNING HARDWARE OFF

After having finished a scan another scan can be added to the current study or the acquisition window can be closed.

After closing the MSOT acquisition window the hardware of the system stays initialized, i.e. by returning to the acquisition window (pressing  in the Optoacoustic Measurement tab of the main window) another scan can be acquired without reloading the hardware components.

After finishing all scans the system hardware needs to be unloaded by pressing the *Power On/Off icon* in the status bar of the main screen before shutting down the system.



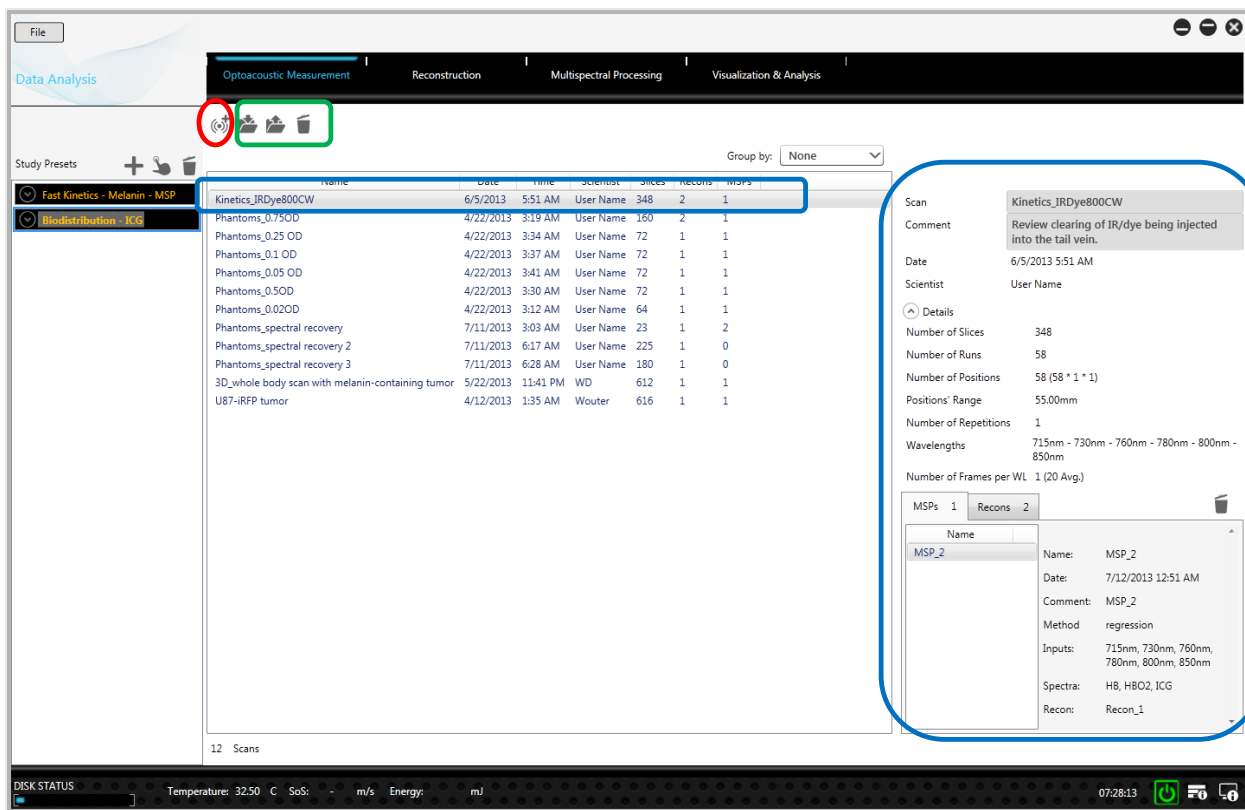
After clicking Power On/Off, an information window displays the progress of the hardware unload (turning off) procedure.

NOTE: During unloading the water is not drained to save time in case the user wants to re-enter acquisition mode shortly. Only when exiting the application the water is drained from the chamber. It is possible to drain out or pump in the water from the Maintenance (see section 6.1.16.1 MAINTENANCE) window manually.

NOTE: Only drain out the water manually if you unload the hardware immediately afterwards. Staying in acquisition mode longer than two minutes after the chamber was emptied will heat up the chamber. For safety reasons the system will be set to standby state. The user needs to quit acquisition mode and re-enter it (after the chamber has cooled down).

3.8 THE OPTOACOUSTIC MEASUREMENT TAB

The **Optoacoustic Measurement** tab serves to start a measurement as explained in 3.1 MSOT ACQUISITION. This tab also allows browsing among the scans and snapshots that were already acquired and review information about these. Furthermore scans can be imported, exported and deleted.



The table shows all the scans and snapshots that were acquired inside the current study. To the right, additional information is shown, such as the name of the scan/snapshot, date and time of its acquisition, as well as the scientist name, number of slices that were acquired and number of reconstructions or MSPs (see 4 IMAGE PROCESSING).

3.8.1 Exporting, Importing and Deleting Scans

A snapshot or a scan including its reconstructions and MSPs can be deleted by clicking the *Trash Bin* icon at the top.

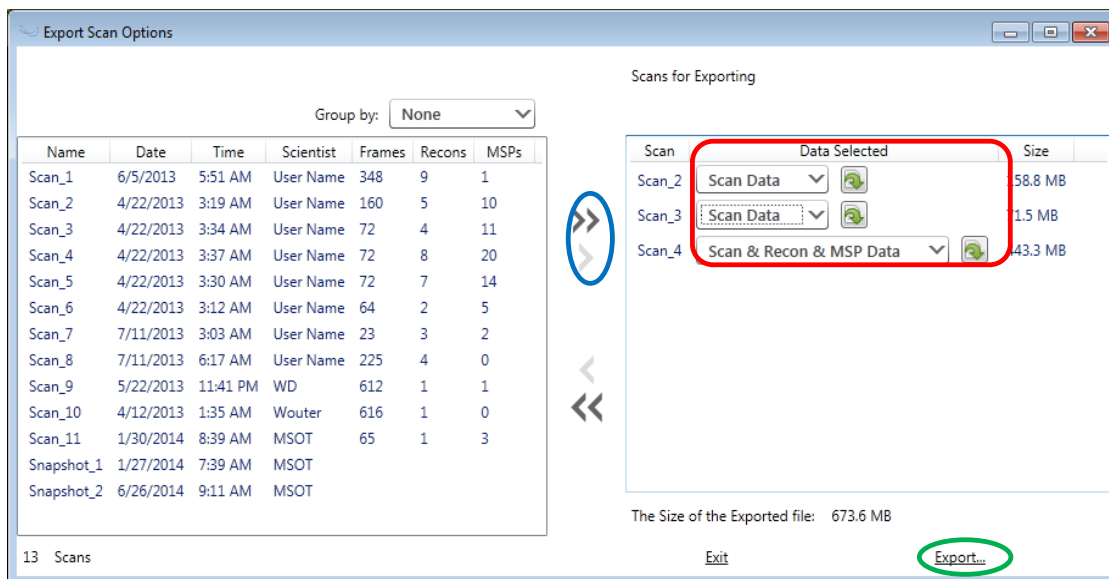


NOTE: There is no possibility to restore the snapshot or scan or any of its Reconstructions and MSPs which are deleted along with the scan.

NOTE: The MSPs and Reconstructions that are deleted along with the scan will be removed from all Views in which they appear (see 5.1 VISUALIZATION AND ANALYSIS TAB). The views itself will not be deleted.



The *Export* icon opens a dialog in which scans of the current study can be added to the export. The dropdown boxes allow to export only the scan data or additionally the reconstruction and MSP data.



After clicking Export the software asks for a name and location to store the set of scans.

NOTE: The scans are automatically zipped into one file. **Do NOT try to unzip this file**, but use it as it is for the import to prevent errors during import.



The *Import* icon can be used to import a .zip-file containing one or more scans that was exported with the functionality described above into the current study.

NOTE: Exporting and importing one or multiple scans from the acquisition system to a desktop system or a reconstruction workstation does not automatically carry the Study Presets with it that have been set up before acquisition. The proposed approach is to copy the whole study folder (located in the MSOT data root folder) from one system to the other when transferring MSOT data for that study for the first time. For following scans the export /import functionality – described above - has to be used. When further Study Presets are added on the acquisition system after the user has worked on the data of that study on a reconstruction system he needs to add these presets also in his system (see chapter 2 STARTING ViewMSOT™ AND SETTING UP A STUDY).

NOTE: Copying scans of a study manually through the browser and adding them to another study will destroy that study which internally keeps an XML file containing the information about the content of the study additionally to the scan folders.

NOTE: When automatically writing the data to a network folder and accessing it from multiple systems (i.e. acquisition system and reconstruction PC) it is required that only one system accesses a particular study at the time. There is no merging functionality so working on the same study from two systems at the same time (i.e. reconstructing the existing scans on the reconstruction workstation and at the same time modifying visualizations of that same study on a different PC) will destroy that study.

3.8.2 Rearranging and Grouping Scans and Snapshots

Click on one of the titles in the table, to sort the table (e.g. in the example the data is sorted in the order of the acquisition date).

The screenshot shows the software interface with a table of scans and snapshots. The 'Date' column header is circled in red. A 'group by:' dropdown menu is highlighted with a green box, showing options: None, Session, Scan, and Date. The table lists scans and snapshots for two sessions: one on 28.06.2013 and another on 01.07.2013. The 'Mouse 341' scan is selected, and its details are shown on the right, including the comment 'CD nude mouse before injection of ICG' and a corresponding image.

| Name | Date | Time | Scientist | S |
|-----------------------------------------------|------------|-------|-----------|-----------|
| Under No Session - 6 Scans/Annotations | | | | |
| - 8 Scans/Annotations | | | | |
| Before Injection | 28.06.2013 | 14:10 | 14:10 | User Name |
| During acquisition | 28.06.2013 | 14:10 | 14:10 | User Name |
| Snapshot_1 | 28.06.2013 | 14:11 | 14:11 | User Name |
| Snapshot_2 | 28.06.2013 | 14:11 | 14:11 | User Name |
| Oxygenated Blod | 28.06.2013 | 14:11 | 14:11 | User Name |
| Mouse 341 | 01.07.2013 | 15:45 | 15:45 | User Name |
| Snapshot_4 | 01.07.2013 | 15:53 | 15:53 | User Name |
| Snapshot_5 | 01.07.2013 | 15:56 | 15:56 | User Name |

14 Scans

Group the scans and snapshots of a study by:

- 1) **Date:** scans and snapshots are found under their day of acquisition by clicking the *Extender* icon
- 2) **Scan:** by clicking the *Extender* icon of a Scan the user can access that scan as well as all snapshots that have been acquired during that particular scan. Snapshots that have been acquired before or after a scan are stored *Under No Scan*.
- 3) **Session:** Each time the user enters the OAM screen from the OAM tab to collect new data a Session is started. All scans and snapshots that are acquired until the OAM screen is left are arranged under one Session

3.8.3 Reviewing Scans

| | |
|-----------|--------------------------------------------------------------|
| Scan | Kinetics_IRDye800CW |
| Comment | Review clearing of IR/dye being injected into the tail vein. |
| Date | 6/5/2013 5:51 AM |
| Scientist | User Name |

| | |
|-------------------------|-----------------------------------------------|
| Details | |
| Number of Slices | 348 |
| Number of Runs | 58 |
| Number of Positions | 58 (58 * 1 * 1) |
| Positions' Range | 55.00mm |
| Number of Repetitions | 1 |
| Wavelengths | 715nm - 730nm - 760nm - 780nm - 800nm - 850nm |
| Number of Frames per WL | 1 (20 Avg.) |

| MSPs 1 | Recons 2 | | | | |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|--|-------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <table border="1"> <thead> <tr> <th>Name</th> <th></th> </tr> </thead> <tbody> <tr> <td>MSP_2</td> <td> Name: MSP_2 Date: 7/12/2013 12:51 AM Comment: MSP_2 Method: regression Inputs: 715nm, 730nm, 760nm, 780nm, 800nm, 850nm Spectra: HB, HBO2, ICG Recon: Recon_1 </td> </tr> </tbody> </table> | | Name | | MSP_2 | Name: MSP_2 Date: 7/12/2013 12:51 AM Comment: MSP_2 Method: regression Inputs: 715nm, 730nm, 760nm, 780nm, 800nm, 850nm Spectra: HB, HBO2, ICG Recon: Recon_1 |
| Name | | | | | |
| MSP_2 | Name: MSP_2 Date: 7/12/2013 12:51 AM Comment: MSP_2 Method: regression Inputs: 715nm, 730nm, 760nm, 780nm, 800nm, 850nm Spectra: HB, HBO2, ICG Recon: Recon_1 | | | | |

Clicking on a particular scan in the scan table of the Optoacoustic Measurement tab shows additional information on the scan and its reconstructions/MSPs.

The Scan name and scan Comment – that were given to the scan during acquisition – can be modified.

The Details section shows important acquisition parameters of the scan.

The tab below lists all Reconstructions and MSPs that have been processed for the current scan.

Clicking on a specific reconstruction or MSP brings up the reconstruction/MSP parameters like resolution, method etc.

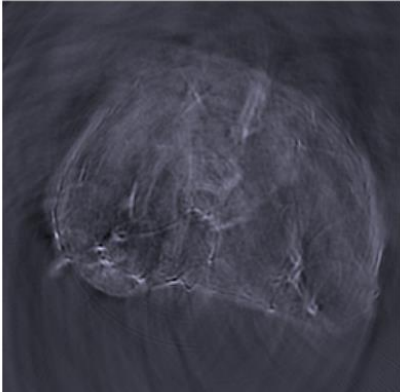
A reconstruction or MSP can be deleted by selecting it and clicking the Trash Bin icon.

3.8.4 Reviewing Snapshots

| | |
|-----------|------------------|
| Name | Before Injection |
| Comment | |
| Date | 28.06.2013 14:10 |
| Scientist | User Name |

| | |
|--------------------------|------------------|
| Image Information | |
| Time | 28.06.2013 14:10 |
| Wavelength | 725,0 nm |
| Run | - |
| Average Frames | - |
| Position | 119,1 mm |
| Laser repetition rate | - |
| Repetition | - |
| Attenuation/Laser Energy | -/- |

| | |
|---------------------------|-------------------------|
| Reconstruction Parameters | |
| Speed of Sound | 1515,63 |
| Method | Direct |
| Resolution | 250 |
| Roi High | 0,0535 |
| Roi Low | 0,0285 |
| Filter | 50,0 kHz - 7, Chebychev |



Clicking on a snapshot in the scan table of the Optoacoustic Measurement tab shows additional information for that snapshot.

The name and comment – that were given to the snapshot during preview or acquisition – can be modified.

The section below shows important acquisition parameters of the snapshot as well as the parameters of the reconstruction (and MSP) which were used.

Click the Export icon to export the current snapshot including the information to a .png-file.

4 IMAGE PROCESSING

Image processing is done in two steps. First the raw data is reconstructed, i.e. tomographic slices are processed from the acquired frames. Those tomographic images are recorded at certain wavelengths, positions and time points. In a second step multispectral processing is performed. The images acquired at different wavelengths at the same time point and position are combined and result in component images, each linked to a different spectrum.

A list of multiple processing Jobs containing reconstructions and MSPs from the scan data can be set up.

4.1 SETTING UP A RECONSTRUCTION JOB

Switch from the **Optoacoustic Measurement** tab to the **Reconstruction** tab to set up a list of scans for the next *Reconstruction Job*.

| Name | Date | Time | Scientist | Slices | Recons | MSPs |
|--------------------------------------------------|-----------|----------|-----------|--------|--------|------|
| Kinetics_IRDye800CW | 6/5/2013 | 5:51 AM | User Name | 348 | 2 | 1 |
| Phantoms_0.75OD | 4/22/2013 | 3:19 AM | User Name | 160 | 2 | 1 |
| Phantoms_0.25 OD | 4/22/2013 | 3:34 AM | User Name | 72 | 1 | 1 |
| Phantoms_0.1 OD | 4/22/2013 | 3:37 AM | User Name | 72 | 1 | 1 |
| Phantoms_0.05 OD | 4/22/2013 | 3:41 AM | User Name | 72 | 1 | 1 |
| Phantoms_0.5OD | 4/22/2013 | 3:30 AM | User Name | 72 | 1 | 1 |
| Phantoms_0.02OD | 4/22/2013 | 3:12 AM | User Name | 64 | 1 | 1 |
| Phantoms_spectral recovery | 7/11/2013 | 3:03 AM | User Name | 23 | 1 | 2 |
| Phantoms_spectral recovery 2 | 7/11/2013 | 6:17 AM | User Name | 225 | 1 | 0 |
| Phantoms_spectral recovery 3 | 7/11/2013 | 6:28 AM | User Name | 180 | 1 | 0 |
| 3D_whole body scan with melanin-containing tumor | 5/22/2013 | 11:41 PM | WD | 612 | 1 | 1 |
| U87-iRFP tumor | 4/12/2013 | 1:35 AM | Wouter | 616 | 1 | 1 |
| Mouse 341 | 1/27/2014 | 7:39 AM | MSOT | | | |

| Scan | Slices |
|--------------------------------------------------|--------|
| U87-iRFP tumor | 616 |
| 3D_whole body scan with melanin-containing tumor | 612 |
| Phantoms_spectral recovery 2 | 225 |

The list view in the center of the window shows all acquired scans of one study. The columns Recons and MSPs show the number of reconstructions and multispectrally processed scans that already exist.

Press the double arrow icon to add all scans that have not yet been reconstructed. Alternatively, select scans to reconstruct on the left and press the single arrow icon to add them to the processing list on the right.

Group by: None

| Name | Date | Time | Scientist | Slices | Recons | MSPs |
|----------------------------|--------------|-------|----------------|--------|--------|------|
| GastricEmptying1 | 28.06.2012 1 | 14:18 | Scientist Name | 11 | 2 | 0 |
| GastricEmptying2 | 28.06.2012 1 | 14:42 | Scientist Name | 3 | 1 | 2 |
| GastricEmptying3 | 28.06.2012 1 | 14:47 | Scientist Name | 255 | 2 | 0 |
| pharmacokinetics with NP | 16.05.2012 1 | 18:16 | Scientist Name | 4 | 3 | 6 |
| pharmacokinetics with NP | 16.05.2012 1 | 18:18 | Scientist Name | 711 | 1 | 0 |
| pharmacokinetics with NP | 16.05.2012 1 | 18:20 | Scientist Name | 30 | 2 | 4 |
| pharmacokinetics with NP | 16.05.2012 1 | 18:36 | Scientist Name | 28 | 3 | 3 |
| PK with negative liposome | 29.06.2012 1 | 15:45 | Scientist Name | 1 | 1 | 0 |
| PK with negative liposome | 29.06.2012 1 | 15:49 | Scientist Name | 212 | 1 | 0 |
| PK with negative liposome | 29.06.2012 1 | 16:16 | Scientist Name | 1 | 12 | 14 |
| PK with PEG lipo 062912_1 | 29.06.2012 1 | 17:27 | Scientist Name | 1 | 1 | 0 |
| PK with PEG lipo 062912_2 | 29.06.2012 1 | 17:02 | Scientist Name | 202 | 1 | 0 |
| PK with PEG lipo 062912_3 | 29.06.2012 1 | 17:27 | Scientist Name | 1 | 1 | 2 |
| PK with positive liposome: | 29.06.2012 1 | 14:43 | Scientist Name | 1 | 1 | 1 |
| PK with positive liposome: | 29.06.2012 1 | 14:17 | Scientist Name | 307 | 1 | 0 |
| PK with positive liposome: | 29.06.2012 1 | 14:15 | Scientist Name | 1 | 1 | 0 |
| multiple slice multiple WL | 25.05.2012 1 | 10:17 | Scientist Name | 28 | 2 | 9 |
| multiple run kinetics | 10.07.2012 1 | 15:41 | Scientist Name | 164 | 2 | 1 |
| m1_post | 14.05.2012 1 | 10:47 | Scientist Name | 41 | 1 | 5 |
| Scan_19 | 05.12.2012 1 | 11:06 | User Name | 47 | 0 | 0 |
| Scan_20 | 05.12.2012 1 | 11:19 | User Name | 2 | 0 | 0 |

24 Scans

Scans for next Reconstruction Job

| Scan | Slices |
|-------------------------------------|--------|
| pharmacokinetics with NPs 2 | 711 |
| PK with positive liposomes 062912_2 | 1 |
| PK with positive liposomes 062912_1 | 1 |

Press the single left arrow icon to remove a selection of scans from the reconstruction job list or the double left arrow icon to clear the reconstruction job list completely.

By clicking the Add to Processing icon the current *Reconstruction Job* is added to the processing queue. The status of running reconstructions and MSP jobs can be displayed by clicking the Processing Status icon in the system information bar.



The *Processing Jobs* window shows all *Reconstruction* and *MSP Jobs* including their processing status (*Complete*, *Processing*, *Pending* or *Canceled*) and the time/date when they were finished.

Processing Jobs

| Job Type | Items | State | Abort | Finished |
|--------------------|-------|------------|-------|------------------|
| Reconstruction Job | 3 | Complete | ✓ | 23.04.2014 10:41 |
| MSP Job | 25 | Complete | ✓ | 23.04.2014 10:42 |
| Reconstruction Job | 1 | Canceled | ✓ | 23.04.2014 10:42 |
| Reconstruction Job | 3 | Processing | ✗ | - |
| MSP Job | 4 | Canceled | ✓ | 23.04.2014 10:42 |

Recon Job Details:

| Scan | Frames | State | Processing | Abort |
|------------------------------|--------|------------|------------|-------|
| Phantoms_spectral recovery | 23 | Complete | ██████████ | ✓ |
| Phantoms_spectral recovery 2 | 225 | Complete | ██████████ | ✓ |
| Phantoms_spectral recovery 3 | 180 | Processing | ██████████ | ✗ |

Stop Resume

By clicking on a *Reconstruction/MSP Job* all scans/reconstructions added to that job including their progress can be displayed.

A scan or a whole *Reconstruction/MSP Job* can be canceled by clicking "x". Processing can be stopped and resumed with the buttons at the bottom right.

4.2 ADVANCED RECONSTRUCTION

Scans for next Reconstruction Job

| Scan | Slices |
|-------------------------------------|--------|
| pharmacokinetics with NPs 2 | 711 |
| PK with positive liposomes 062912_2 | 1 |
| PK with positive liposomes 062912_1 | 1 |



Click the *Advanced* icon at the bottom of the main screen to modify the Reconstruction Settings of the Reconstruction Job list.

The *Set up Reconstruction Job* window has the following four areas:

- 1) Current job list: the job list contains all the scans that were chosen for reconstruction in the *Reconstruction* tab. The *Trim Speed* (third column of the job list) represents the speed of sound presets that were chosen for each scan during acquisition. If necessary, press the autofocus icon to automatically find the optimal *Trim Speed* or modify it manually. The effects are visible in the Image Preview window.
- 2) Image Preview window: the window shows a Back Projection reconstruction image. Choose any slice from the scan and get a fast Back Projection reconstruction preview. Additionally, the effects of filtering modifications and speed of sound changes can be previewed.

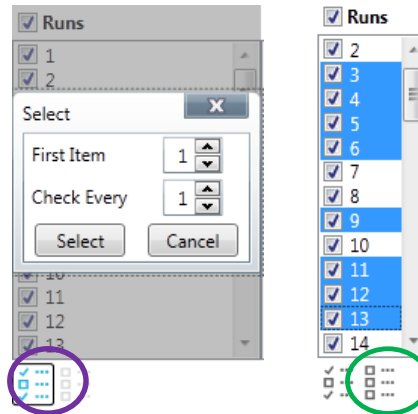
- 3) Reconstruction Settings: The default settings for the reconstruction are defined in the Study Presets, but can be changed here. The Reconstruction Settings are explained in section 2.2.2 Processing Settings).

NOTE: Changed Reconstruction Settings are applied to ALL scans in the current Reconstruction Job.

- 4) Reconstruction data selection: Used to select *Runs*, *Slices* and *Wavelengths* to be reconstructed.

The Pattern Selection icon button opens a dialog box facilitating the selection. Enter a *First Item* and then *Check Every* as a step size for selecting the items.

Uncheck a selection of checked items by clicking the "x" icon.

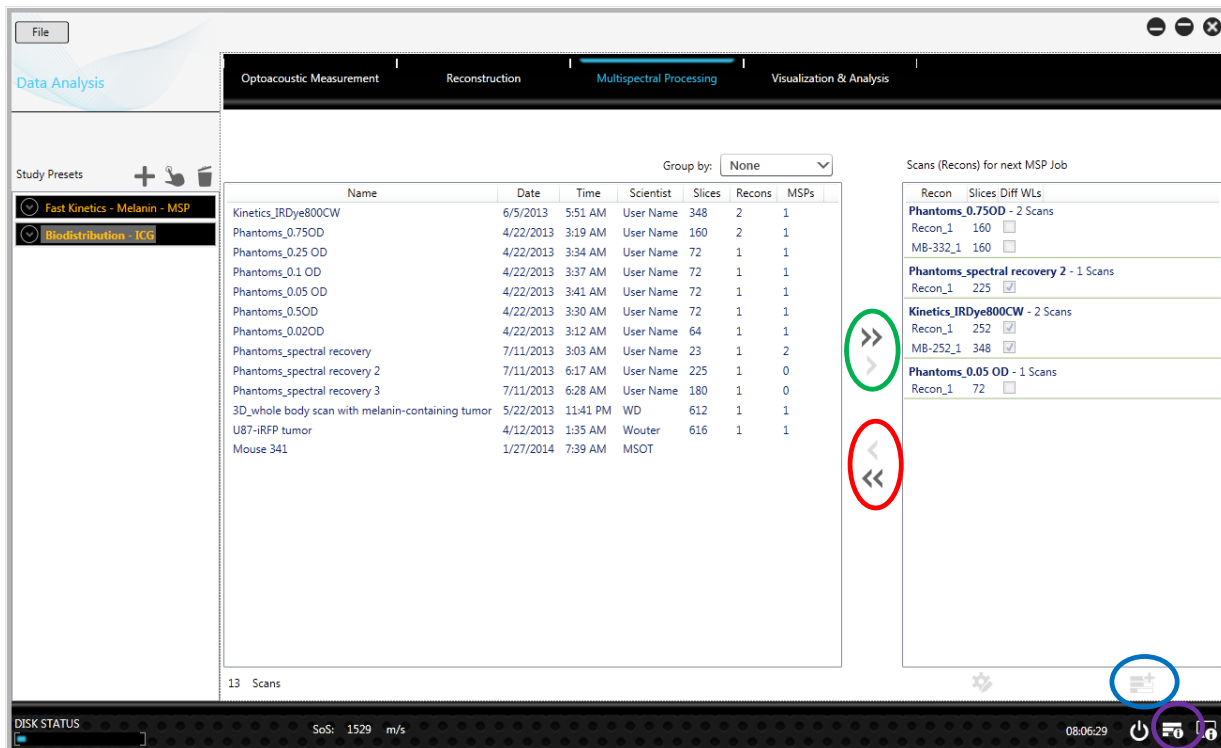


4.3 SETTING UP A MULTISPECTRAL PROCESSING BATCH

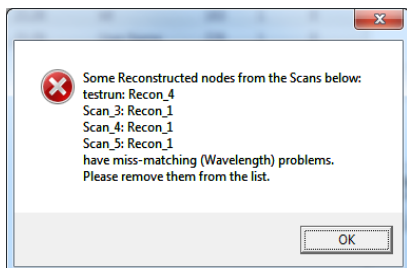
To multispectrally process the reconstructed image data switch to the **Multispectral Processing** tab. The functionality of the tab is very similar to the Reconstruction tab described in 4.14.1 SETTING UP A RECONSTRUCTION.

NOTE: To multi-spectrally process a scan, the scan needs to include at least one reconstruction, because the multispectral processing is performed on the reconstructed images and not on the raw data.

Add all scans which have no MSP(s) yet or select the scans that should be processed and add them to the *MSP Job*. Multispectral processing is only possible on scans that have already been reconstructed. If a scan has multiple reconstructions, all of them will be added to the *MSP Job* and MSP will be performed on each reconstruction.



Selected reconstructions can be removed from the MSP Job. Clicking the Add to Processing icon sends the MSP job list to processing. The processing list is explained in 4.14.1 SETTING UP A RECONSTRUCTION.



If a Reconstruction that does not contain the wavelengths needed for the chosen MSP is added to an MSP Job list a warning pops up.

| Recon | Slices | Diff WLS |
|------------------------------------------------------|--------|-------------------------------------|
| GastricEmptying1 - 2 Scans | | |
| Recon_2 | 11 | <input type="checkbox"/> |
| Recon_3 | 1 | <input checked="" type="checkbox"/> |
| GastricEmptying3 - 2 Scans | | |
| Recon_1 | 3 | <input checked="" type="checkbox"/> |
| Recon_2 | 3 | <input checked="" type="checkbox"/> |
| PK with PEG lipo 062912_1 - 1 Scans | | |
| Recon_1 | 1 | <input type="checkbox"/> |
| PK with positive liposomes 062912_1 - 1 Scans | | |
| Recon_1 | 1 | <input type="checkbox"/> |

In the MSP Job list those reconstructions are marked in the *Diff WLS* column with a checked box.

These reconstructions need to be removed from the MSP Job before proceeding.

NOTE: Multiple reconstructions of one scan having different reconstruction settings need to be set up sequentially for MSP using the Advanced icon.

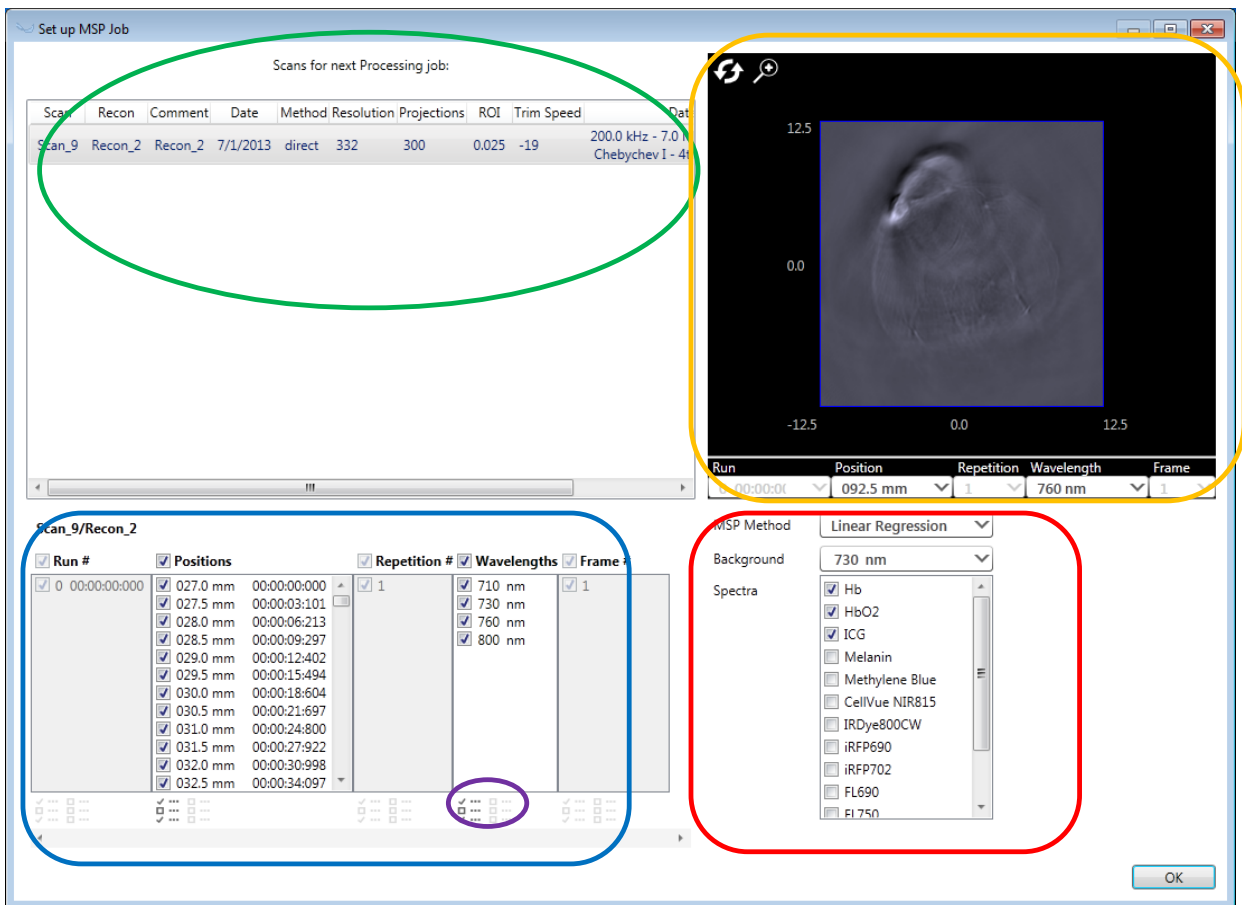


Pressing the Advanced icon opens the advanced MSP job popup where the MSP Settings can be modified.

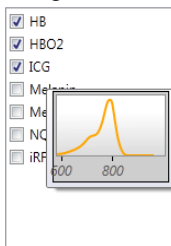
4.4 ADVANCED MSP

By clicking the Advanced icon the MSP parameters of the MSP Job list can be changed. The advanced MSP popup window is very similar to the Setup Reconstruction Job window explained in 4.2 ADVANCED RECONSTRUCTION and has the following four areas:

- 1) Current job list: the job list contains all reconstructions that were chosen for MSP.
- 2) Image Preview window: any slice from the reconstructed images can be displayed in the preview.



- 3) MSP Settings: The default settings for the MSP are defined in the Study Presets but they can be changed here. The MSP settings are explained in section 2.2 CREATING STUDY PRESETS.



Move the mouse over one of the spectra to see the graph of the spectrum.

- 4) Slice Selection: Can be used to choose a selection of *Runs*, *Slices* and *Wavelengths* to be multispectrally processed. With the help of the pattern selection a subset of images can be selected automatically, e.g. every 5th slice.

5 VISUALIZATION AND ANALYSIS

The 4th tab of the ViewMSOT™ main screen – **Visualization and Analysis** – is used to create new and open existing Views in a Visualization screen. A View serves to display, post-process and analyze reconstructed anatomical images as well as multispectrally processed MSP component images.

Open one or multiple reconstructions or MSPs, inside a View, select images to be displayed and determine how they should be arranged on the screen. Image manipulation tools like thresholds, different color maps etc. can be applied to all visible images. It is also possible to draw regions of interest (ROI) and show graphs of these ROIs.

The Visualization screen includes exporting capabilities for images (and image stacks), videos, spectrum and ROI analysis data.

5.1 VISUALIZATION AND ANALYSIS TAB

The *Visualization and Analysis* tab contains a list of all Views that are saved in the current study. This list includes the name of the View and additional information such as the number of Sessions displayed inside the View.

Views can be created or existing Views deleted. To open a View in the Visualization screen press the Load View icon or double click the View. Several Views can be opened at the same time.

| Name | Sessions | Creation Time | Modified Time |
|---------|----------|---------------|--------------------|
| View_1 | 1 | - | - |
| View_2 | 1 | - | 6/18/2014 10:36 AM |
| View_3 | 2 | - | 6/24/2014 7:54 AM |
| View_4 | 2 | - | 5/28/2014 12:07 PM |
| View_5 | 1 | - | 6/23/2014 7:49 AM |
| View_6 | 1 | - | - |
| View_7 | 6 | - | - |
| View_8 | 7 | - | 6/25/2014 11:17 AM |
| View_8 | 1 | - | - |
| View_9 | 1 | - | - |
| View_10 | 1 | - | - |

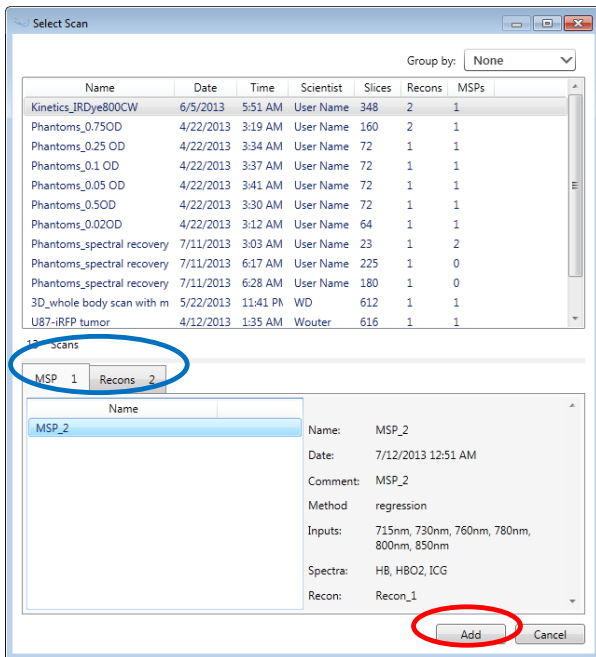
The name of a View can be changed by selecting it and entering the name into the text field on the right of the screen. Add a comment if necessary.


| Name | Sessions | Creation Time | Modified Time |
|--------|----------|------------------|------------------|
| View_1 | 1 | 28.05.2014 20:16 | 28.05.2014 20:16 |
| View_2 | 1 | 28.05.2014 20:16 | 28.05.2014 20:16 |
| View_3 | 2 | 28.05.2014 20:17 | 28.05.2014 20:17 |
| View_4 | 2 | 28.05.2014 20:17 | 28.05.2014 20:17 |
| View_5 | 1 | 28.05.2014 20:17 | 28.05.2014 20:17 |

Click on the column titles to sort the list according to the respective column.

Click the title a second time to invert the order.

5.2 ADDING A RECONSTRUCTION/MSP TO THE CURRENT VIEW



Create a new View by pressing the  -icon.

In the *Select Scan* window choose a scan from the list showing all scans belonging to the current study.

The MSP and the Recons tab list all MSPs and reconstructions processed from the chosen scan.

After selecting a reconstruction or MSP additional information is shown on the right side of the window.

Pressing Add includes the selected data set in the current View.

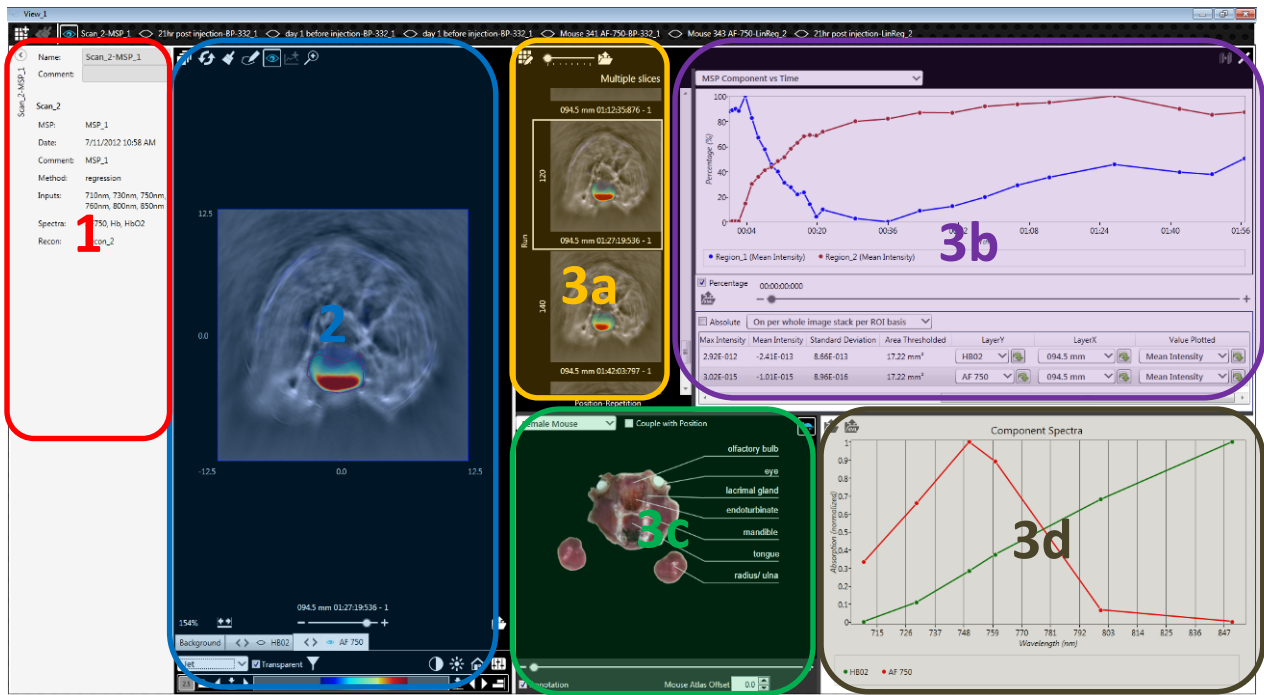


Click the Add Scan to View icon to add another reconstruction or MSP to the current View.

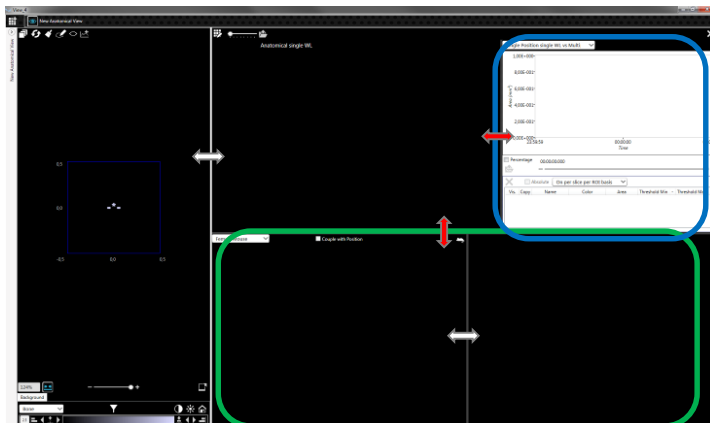
5.3 OVERVIEW OF THE MSOT VISUALIZATION WINDOW

The MSOT Visualization screen consists of the following areas which will be more extensively described in the subsequent chapter:

- 1) Information extender
- 2) Image Master View including Image Control Panel
- 3) Dashboard including windows for
 - a. Image Window
 - b. ROI graph
 - c. CryoMOUSE™ Atlas
 - d. Component Spectra graph



The windows of the Visualization Screen can be dragged with the mouse pointer to adjust their size.

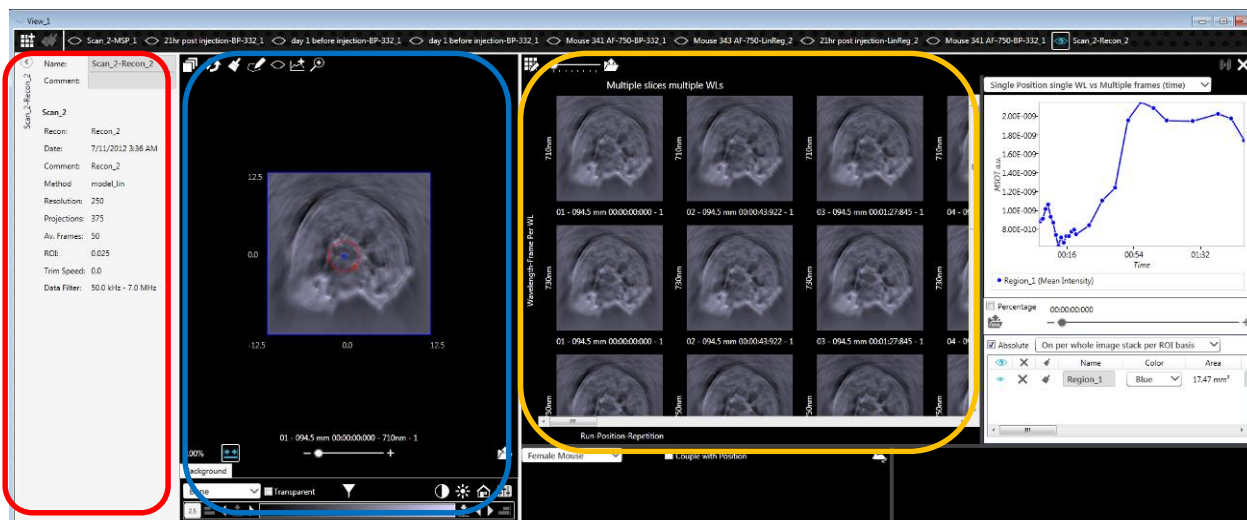


Double click the line separating the Image Window and the ROI graph (red double arrows) to shrink the ROI graph to the left. The ROI graph can be extended by again double clicking the line, or dragging the line from the right back into the middle of the Dashboard.

The lower part of the Dashboard containing the CryMOUSE™ Atlas and the Component spectra graph shrinks to the bottom if the line separating it from the upper part of the Dashboard is double clicked.

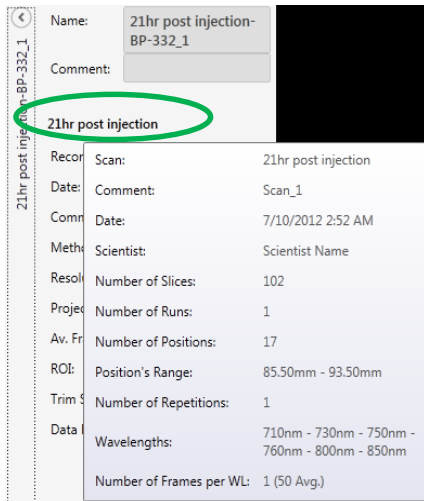
5.4 DISPLAYING IMAGES

ViewMSOT™ uses a master-detail concept for the visualization of data sets, i.e. there is one Master Image window and a Detail Images window which is part of the Dashboard. The Detail Images window displays all images that are chosen for the current View.



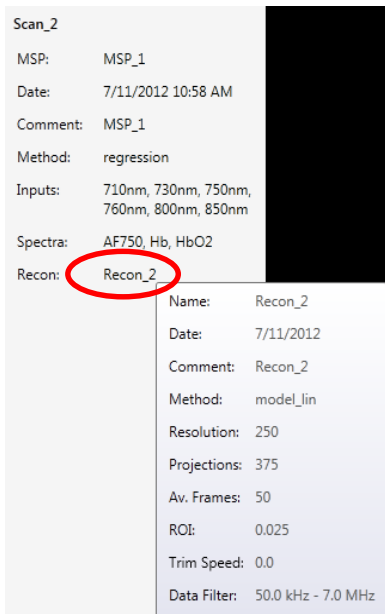
The Master Image window displays one of the images shown in the Detail Images window. On this Master Image the user can apply modifications – e.g. thresholding – or draw ROIs, which will then be automatically applied to all images in the Detail Images window.

A default selection of images is presented in the Detail Images window when adding a reconstruction or MSP to the View.



Information on the chosen Reconstruction/MSP can be reviewed by opening the [*Information Extender*](#).

Move the mouse pointer over the scan name from which the current reconstruction or MSP view has been processed, to review the acquisition information of that scan.



For MSPs move the mouse over the reconstruction name to gain additional information about the reconstruction underlying the MSP.

Controls related to the Detail Images window are located in the top left of the Dashboard. Functionality of the icons from left to right:

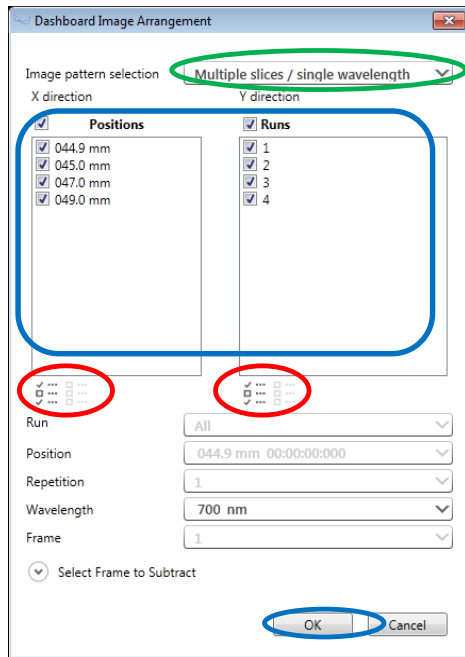


- 1) Select Images to show in Dashboard (see 5.4.1 Modifying Selection of Recon Images in the Detail Images Window) and select Difference/Gradient Slice (see 5.6.2 Choosing the Difference Image)
- 2) Slider to change the size of the images in the Detail Images window
- 3) Export Images (see 5.7 EXPORTING IMAGES))

5.4.1 Modifying Selection of Recon Images in the Detail Images Window



Press the Select Image icon to open the Dashboard Image Arrangement window and to modify the selection of the displayed images and options for image arrangement.



First decide – depending on the type of scan – what information should be displayed. For reconstructions there are different options:

- 1) *Multiple slices / single wavelength*: showing images of the same wavelengths at different (anatomical) positions (and for different Run times)
- 2) *Multiple slices / multiple wavelengths*: show images at different positions and different wavelengths
- 3) *Single slice / multiple wavelengths*: show multiple wavelengths at one position (for multiple runs)

Depending on what is chosen, the options for horizontal (x-axis) and vertical (y-axis) direction are shown. The axes refer to horizontal and vertical directions in the image dashboard, i.e. different wavelengths could be shown in horizontal direction and positions vertically.

In the example above *Multiple slices / single wavelength* was chosen, so the wavelength is fixed while position (in x-direction) and Runs (in y-direction) are variable.

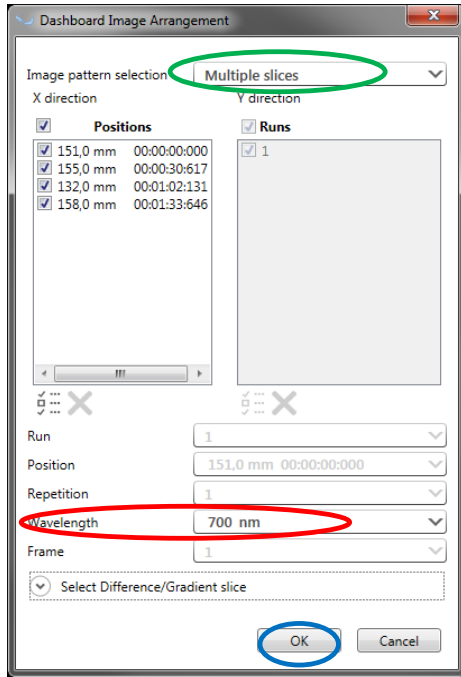
By marking the checkboxes choose a selection for the View. The Pattern Selection icon button opens a dialog box facilitating the selection. Uncheck a selection by clicking the “x”-icon.

In the bottom part of the Dashboard Image Arrangement window, the fixed parameters can be chosen. In the current example where positions and runs are variable the wavelength value is fixed.

By pressing OK the images are arranged in the Detail Images window of the Visualization Screen.

5.4.2 Modifying Selection of MSP Images in the Detail Images Window

Modifying the selection of MSP images in the Detail Images window works similarly to the one for reconstruction images.

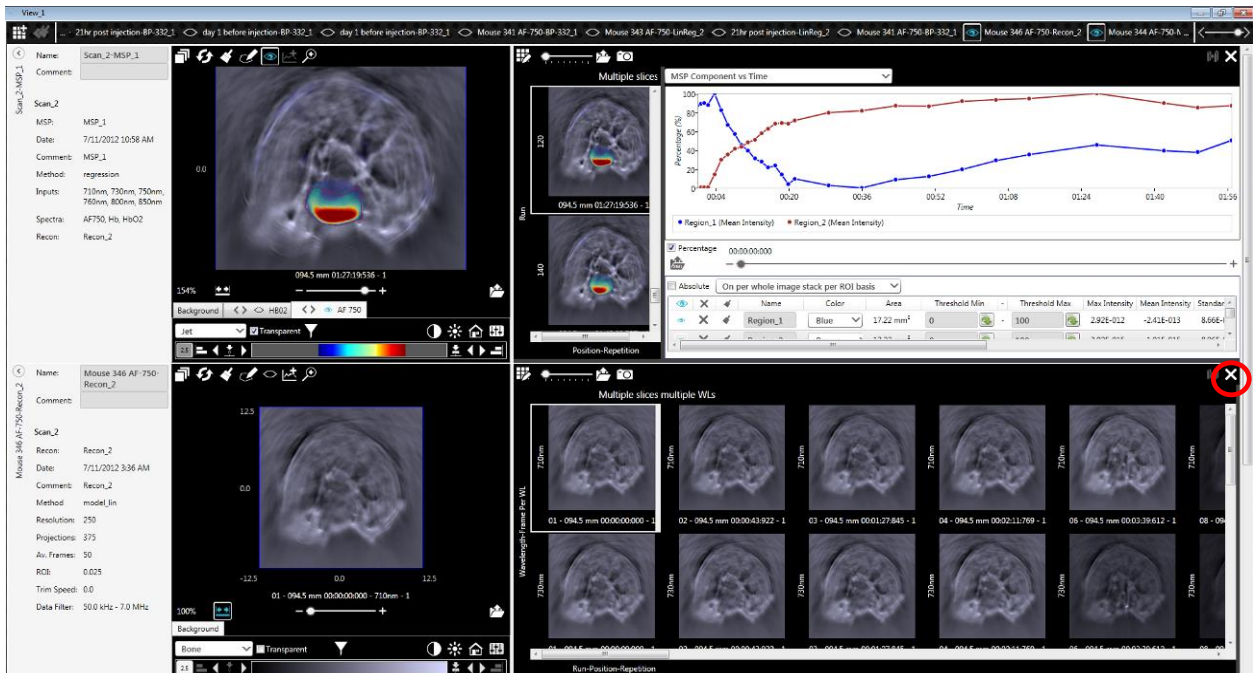


Select the MSP images to be shown for different (anatomical) positions and for different Runs (and/or Repetitions). The Wavelength selection in this case is used for the background single wavelength image. The MSP images can be overlaid with semi-transparency which is explained in 5.6.15.6.1 Overlaying and Enabling Difference Layer(s) and MSP-Layers. Pressing OK loads the images to the *Details Images* window.

NOTE: Depending on whether a reconstruction or an MSP was chosen for the View, the *Dashboard Image Arrangement* window is adjusted according to which images can be selected and how these are arranged in the *Detail Images* window.

5.5 OPENING MULTIPLE RECONSTRUCTIONS/MSPS IN ONE VISUALIZATION

Perform the steps explained in 5.2 ADDING A RECONSTRUCTION/MSP TO THE CURRENT VIEW to add additional reconstructions or MSPs to the current View.



To remove an image data set from the View click the x-icon.

Click the Eye icon of the associated image data set name in the Visualization Bar on the top to visualize or hide the particular reconstruction or MSP from the View.

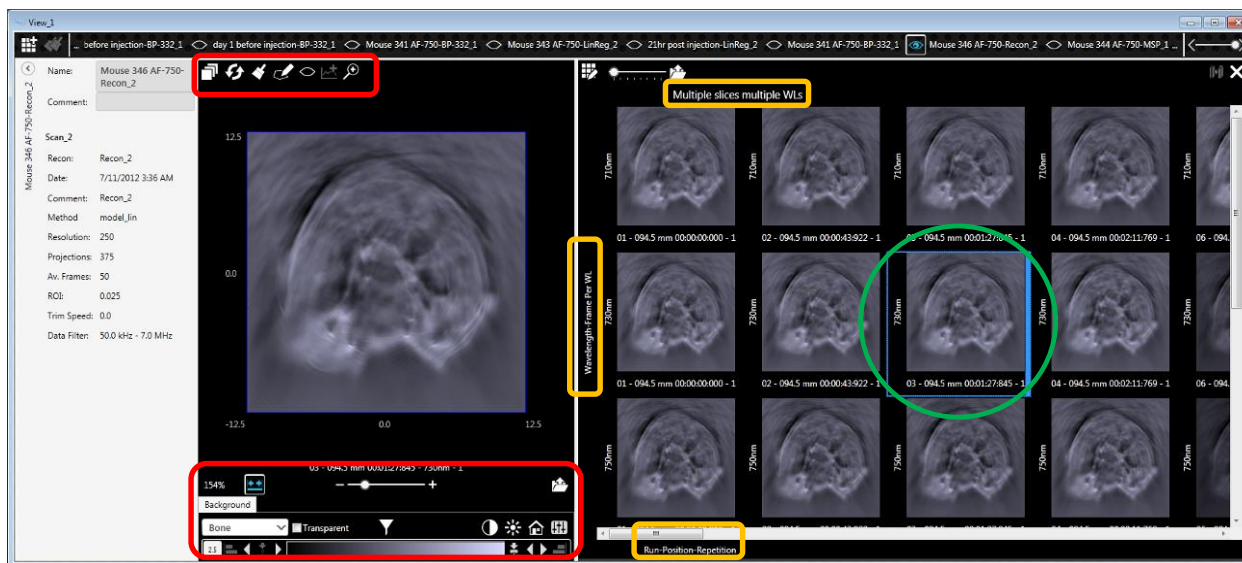


If multiple Views are added to the Visualization stretching out over the length of the views bar, use the left/right arrows or the scrollbar.

NOTE: The image data sets are added from top to bottom in the Visualization screen and from left to right in the Visualization Bar.

5.6 IMAGE CONTROLS TO MODIFY THE IMAGES

Select the image to be shown in the Master Image window by clicking on one of the images in the Detail Images window. A blue (white, if inactive) frame surrounding the image in the Detail Images window indicates the choice. The title and axis description show the selections explained in section 5.4 DISPLAYING IMAGES.



A multitude of different image controls are available to modify the way the images are presented. There are two types of images:

- 1) Single layer images: Single layer images result from reconstructed single wavelength images for a certain run, position and wavelength.
- 2) Multi layer images: For the MSP (containing information of different spectral components) or difference images it makes sense to overlay multiple component layers (with semi-transparency) on top of a background single wavelength image for a certain run and position.

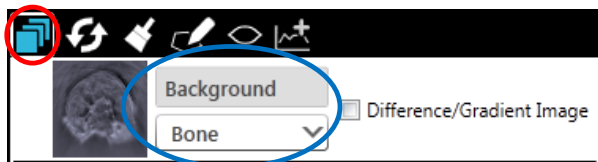
There are some common controls always affecting the whole image including possible overlays (e.g., rotation) and other image controls which can be modified individually for each image layer (e.g., choosing the color map).

5.6.1 Overlaying and Enabling Difference Layer(s) and MSP-Layers

Reconstruction (Single Wavelength) Image(s)

Having added a reconstruction to the View it is possible to overlay a semi-transparent *Difference Image* on top of the single wavelength image. This *Difference Image* is calculated by subtracting one of the single wavelength images from the other images. As default the first slice is selected for subtraction. The selection of the slice to be subtracted is explained in 5.6.2 Choosing the Difference Image.

Click the Select Layers icon at the top of the Master Image window to open the Select Layer dialog.

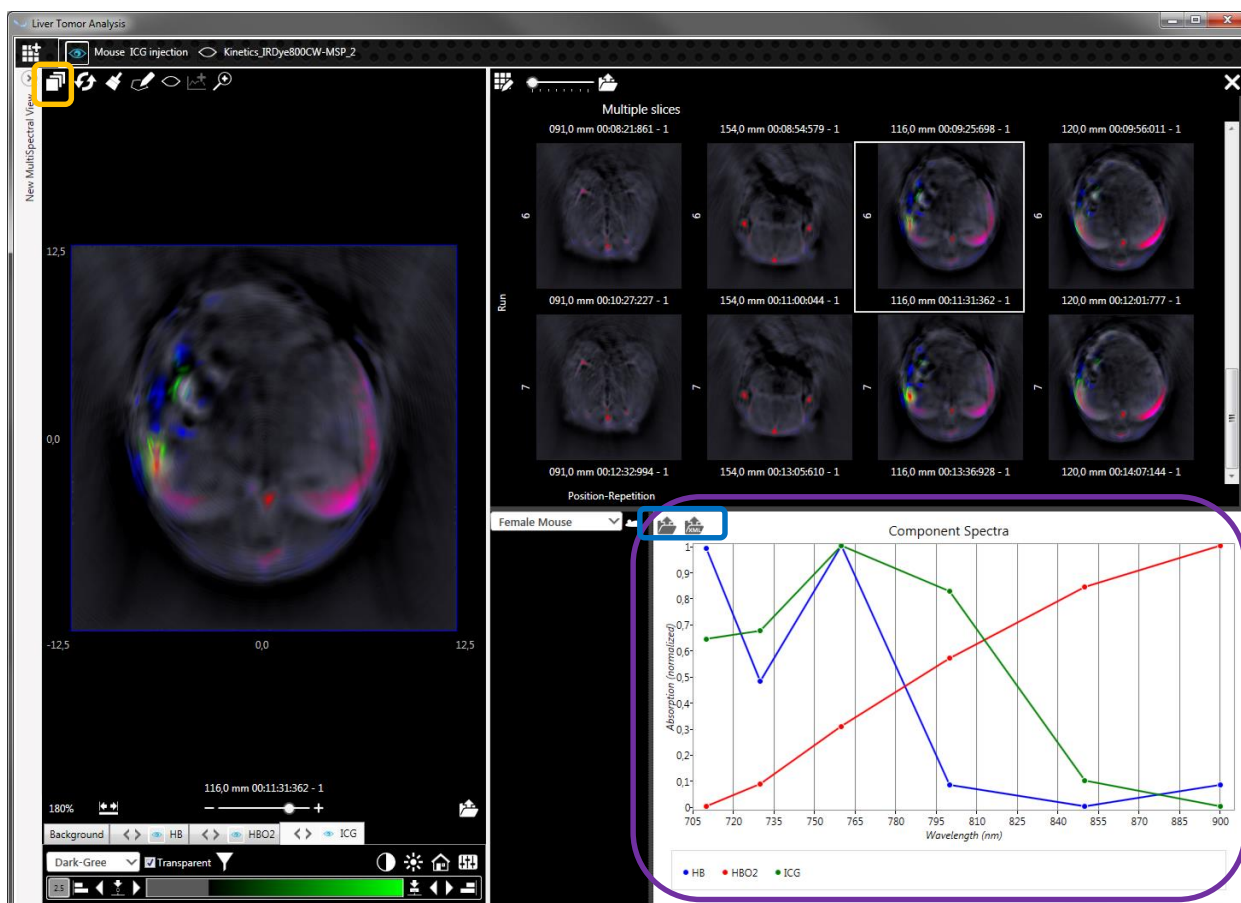


The name of the layer can be modified and the color map can be changed.

Check *Difference/Gradient Image* to enable the semi-transparent overlay of the Difference Image.

MSP Image(s)

When adding an MSP to a View all the MSOT layers are overlaid with semi-transparency on top of the single wavelength background image by default. The spectra associated with the layers are shown in the Component Spectra graph.



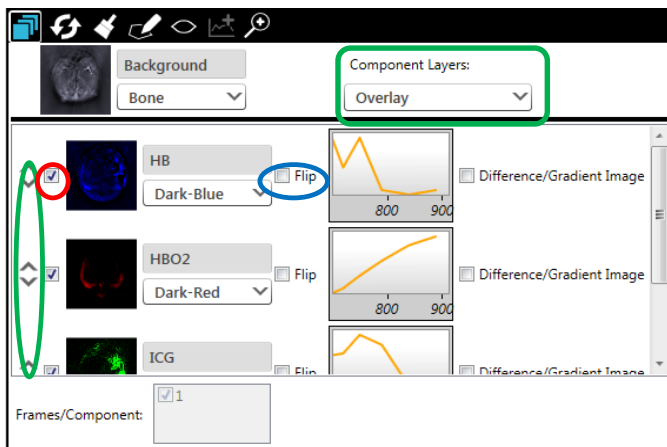
The Component Spectra graph can be exported:

- 1) As a .tiff or jpg image
- 2) As an xml file containing the spectra values. The xml-file can be opened in excel.

Two different methods to generate the components overlay are available in ViewMSOT™:

- 1) **Overlay** (default when opening an MSP in the Visualization): On top of the non-transparent single wavelength background image, the images of the available spectral components are overlaid in a semi-transparent way. The order in which the component images are stacked effects the resulting image (components layered on top may overlay the lower layered components).
- 2) **Color Mixing** (this method is only applicable for three different components – red, green, blue): Three spectral component images – one red, one green, one blue – are mixed. The resulting RGB image is semi-transparently overlaid on top of the non-transparent single wavelength background image.

Click the Select Layers icon to:



- 1) Uncheck any layer to remove it from the *Image Control Panel* and the *Component Spectrum graph*
- 2) Toggle between Overlay and Color Mixing. For Overlay use the arrow up/down buttons to change the order.
- 3) Flip image
- 4) Preview Spectrum belonging to the layer
- 5) Enable/Disable *Difference Image* for the particular layer
- 6) Change name and color map of single wavelength background image as well as MSP overlays.

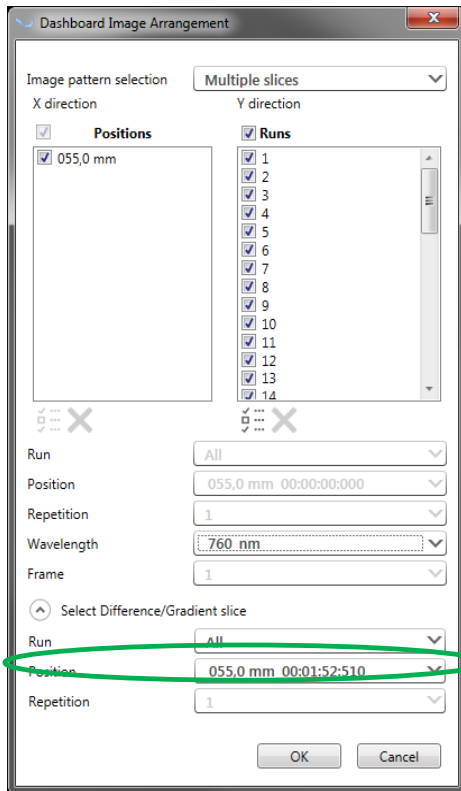
NOTE: Enable/Disable *Difference Image* is only available for 2D Views.

NOTE: The layers can be made visible and invisible also from the image controls explained in 5.6.4 Individual Layer Controls. However, only in the Select Layer dialog they will also be removed from the Component Spectra graph. The color maps of the layers can also be modified from the image controls.

NOTE: For color mixing only the three base colors red, green and blue can be chosen.

5.6.2 Choosing the Difference Image

In a reconstruction or MSP session one of the slices can be subtracted from all others and the resulting image can be overlaid on top of the background image (see 5.6.1 Overlaying and Enabling Difference Layer(s) and MSP-Layers).



By default, the first slice from the image data set is used for subtraction.

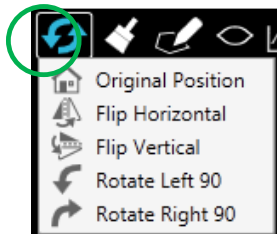
Click the Select Images icon to open the *Dashboard Image Arrangement* window.

Use the Position (Time) dropdown to select the slice that should be subtracted from the image data set.

NOTE: On the particular slice that is used for subtraction the Difference Image is empty/"0".

NOTE: When applying Difference to single wavelength images the difference is always calculated for each wavelength separately on the desired set of positions and time points.

5.6.3 Common Image Controls



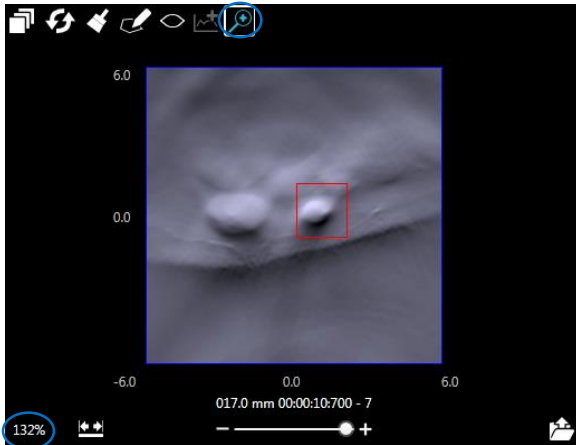
Click the Rotate icon to choose several rotation and flip options applied to the image including all layers and also overlaid regions of interest (ROI):

- 1) *Original Position* reverts everything to the initial state
- 2) *Flip Horizontal* and *Flip Vertical*
- 3) Rotate 90° to Left or Right



The Format Painter icon is explained in chapter 5.6.5 Applying Image Manipulation from one Reconstruction/MSP to another. The icons on the right side serve for the analysis of regions of interest (ROIs) and are explained in 5.9 REGION OF INTEREST (ROI) ANALYSIS.

The mouse scroll wheel as well as the F2/F3 buttons can be used to zoom in and out of the image in the Master Image window. The zoom factor is shown at the bottom left.

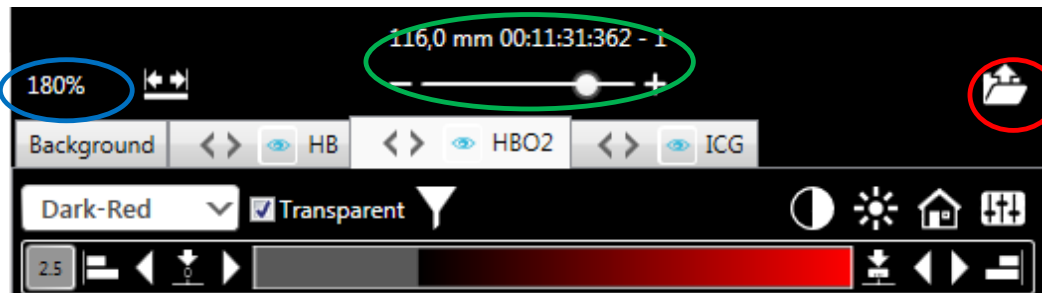


Alternatively, click the [zoom icon](#) and draw a (red) rectangle around the region to be magnified. Click the first time to start drawing, click a second time to apply. The region of interest will be zoomed to the full size of the Master Image window.

The [zoom factor](#) is shown at the bottom left of the Master Image Window together with other image controls that contain individual layer-dependent controls as well as some controls that affect the whole image. The layer-dependent controls are explained in section 5.6.4 Individual Layer Controls.

With the [slider below the image the user can scroll through the stack of images](#). Information on position and acquisition time is displayed. The blue movable frame – mentioned in section 5.6

IMAGE CONTROLS TO MODIFY THE IMAGES– surrounding the current image in the Detail Images window stays in sync with the slider position.

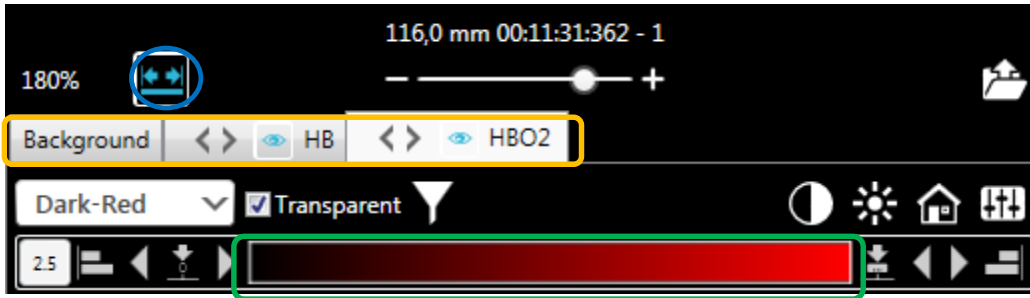


Click the [Export Visible Frame as an Image icon](#) to export the image currently shown in the master view. The Export Image Stack dialogue opens which is explained in detail in Chapter 5.8 EXPORTING IMAGES.

5.6.4 Individual Layer Controls

The MSP-layers that were added to the image – see section 5.6.1 Overlaying and Enabling Difference Layer(s) and MSP-Layers – are [visible as tabs in the image controls](#). Clicking on the *Eye* icon hides or shows the layers on the images. Clicking on the tabs allows access to the individual image controls of each layer. Click on the arrow buttons inside the tab titles to change the order of the component overlay (if overlay and not color mixing is applied).

5.6.4.1 Autoscaling and Thresholding of the color bar



If the Auto-Scale icon is pressed all images - in the Master Image window as well as in the *Detail Images* window – and all image layers are auto scaled, i.e. the range of the color map of each layer is adjusted to each layer's maximum and minimum pixel value.

Unchecking *Auto-Scale* will apply the current scaling – minimum and maximum of each layer of the current image in the Master Image window to the full range of the individual component color maps - to all other images in the Detail Images window, i.e. all images will have the same scaling for the same component making them visually comparable.



Clicking the arrow buttons to the right or left of the color bar modifies the range of the color bar in the current Master Image window (these changes will also be applied to all images in the Details Image window).



In the example the left side of the color bar is in saturation, i.e. all pixels below a certain threshold are black in the resulting image. To the right side the range of the color bar is not fully used. The white line represents maximum pixel intensity in that image.

Modify the stepsize for the arrow buttons.

NOTE: For multiple images scaled to the same range it is clear that both, the saturation as well as the partial use of the range, is varying from image to image because each image has a different range but the assignment of color shades and MSOT values stays the same.



Clicking on the icons next to the arrow buttons adjusts the color bar to the minimum (on the left) or to the maximum (as demonstrated in the example below) of the pixel value of the current Master Image.



Set the beginning of the color bar to zero or manually type in the value for the upper and lower limit of the color bar, e.g. in the color map below the image pixels with data values that are below the lower limit are black, the image pixels with data values that are above the upper limit are red.



5.6.4.2 Changing the Color Map

The following color maps are available:



- Bone ➤ Gray ➤ Hot ➤ Dark-Red ➤ Light-Red
- Dark-Green ➤ Light-Green ➤ Dark-Blue ➤ Light-Blue ➤ Cyan
- Magenta ➤ Yellow ➤ HSB-HSL ➤ Jet ➤ Union Jack

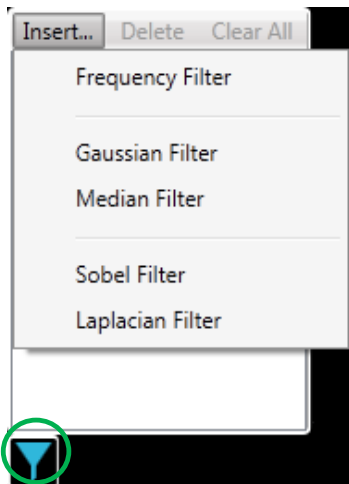
5.6.4.3 Modify Contrast and Brightness

Modify Contrast and Brightness of the images as well as set them back to default by pressing the Home icon.



5.6.4.4 Applying Image Filters

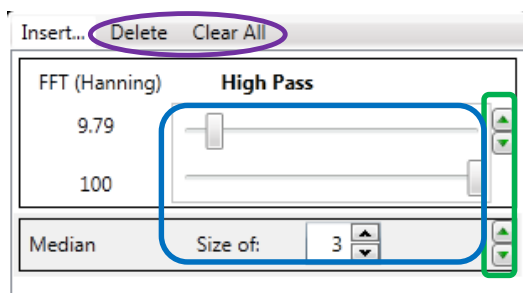
The image filters are available for post-processed 2D data in 2D Visualizations:



Click the [Filter icon](#) and *Insert...* to apply one or several of the following image filters to the current image layer:

- *Frequency Filter*: Hanning filtering of the image frequencies
- *Gaussian* and *Median Filter* to smoothen the image
- *Sobel* and *Laplacian Filter* for image edge detection

When adding more than one filter they will be applied on top of each other.

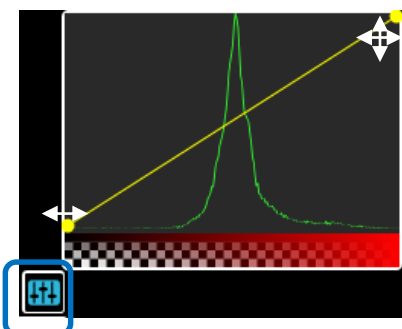


In addition:

- [Modify the filter parameters](#),
- [Change the order](#) in which the filters are applied to the image,
- [Delete the selected filter](#),
- [Delete all filters](#).

5.6.4.5 Apply Transparency and Transparency Modification Tool

[Check or uncheck Transparency to enable/disable the semi-transparent behavior of the current layer](#). If *Transparent* is checked the *Transparency Tool* can be applied to the current layer. This tool is available for all modes except single Wavelength during acquisition.



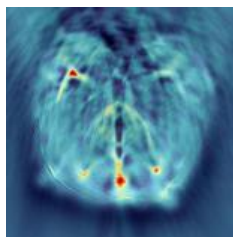
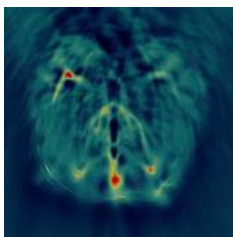
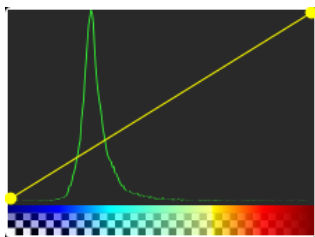
With the help of the [Transparency Tool](#) the user can change the transparency/opacity – which by default is linear to the color bar color intensities – with respect to the color bar and independent from the relation between color bar and image values (which is modified by the arrows next to the color bar as explained above).

Transparency Tool

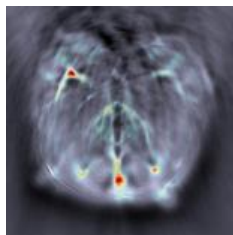
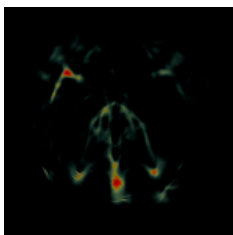
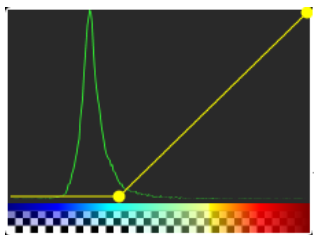
**Image
(no background)**

**Image
(with background)**

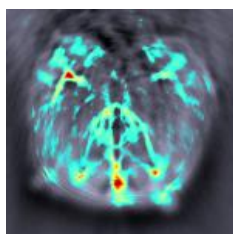
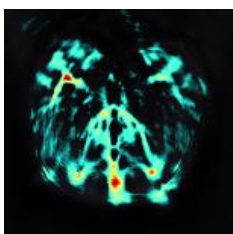
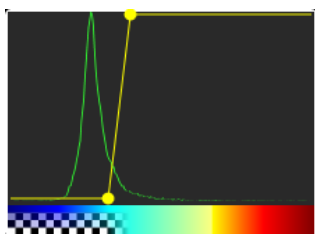
Description:



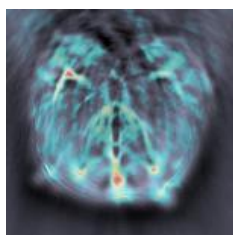
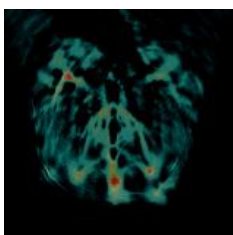
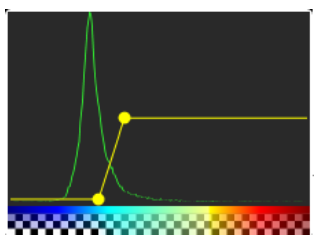
Default setting: Transparency is linear to the color bar. Low color bar values (blue) are fully transparent. High values are fully opaque.



Increase the maximum color bar value for full transparency makes more parts of the image invisible. Full opacity is still only given for the highest values.



Decrease the minimum for opacity. Above that value the color bar is fully opaque.



Decrease the overall maximum level of opacity/transparency (in this example to 50%)

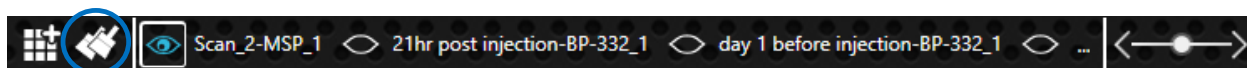
NOTE: If the *Component Layers* method is set to *Overlay*, disabling *Transparent* makes all layers below the current one invisible. If the *Component Layers* method is set to *Color Mixing*, disabling *Transparent* makes the whole overlay opaque.

5.6.5 Applying Image Manipulation from one Reconstruction/MSP to another







Click the Copy Image Settings icon on a reconstruction or MSP to apply the image post processing settings from this data set to another, by clicking onto the Master Image window.


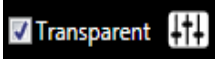
The Copy Image Settings functionality can also be applied between different Views that are opened at the same time. Apply the Image Settings to all image data sets of a View by clicking the Paste to All icon in the Visualization Bar.



The following image post processing tools can be applied from one reconstruction/MSP to another:

- Orientation/rotation of the image 
- Auto-Scaling, color maps and scaling of the color maps 
- Image Filters 
- Contrast/Brightness 

Additionally, for MSP image data sets:

- Visibility of a component in the tab 
- Transparency and transparency thresholds (low/high) 

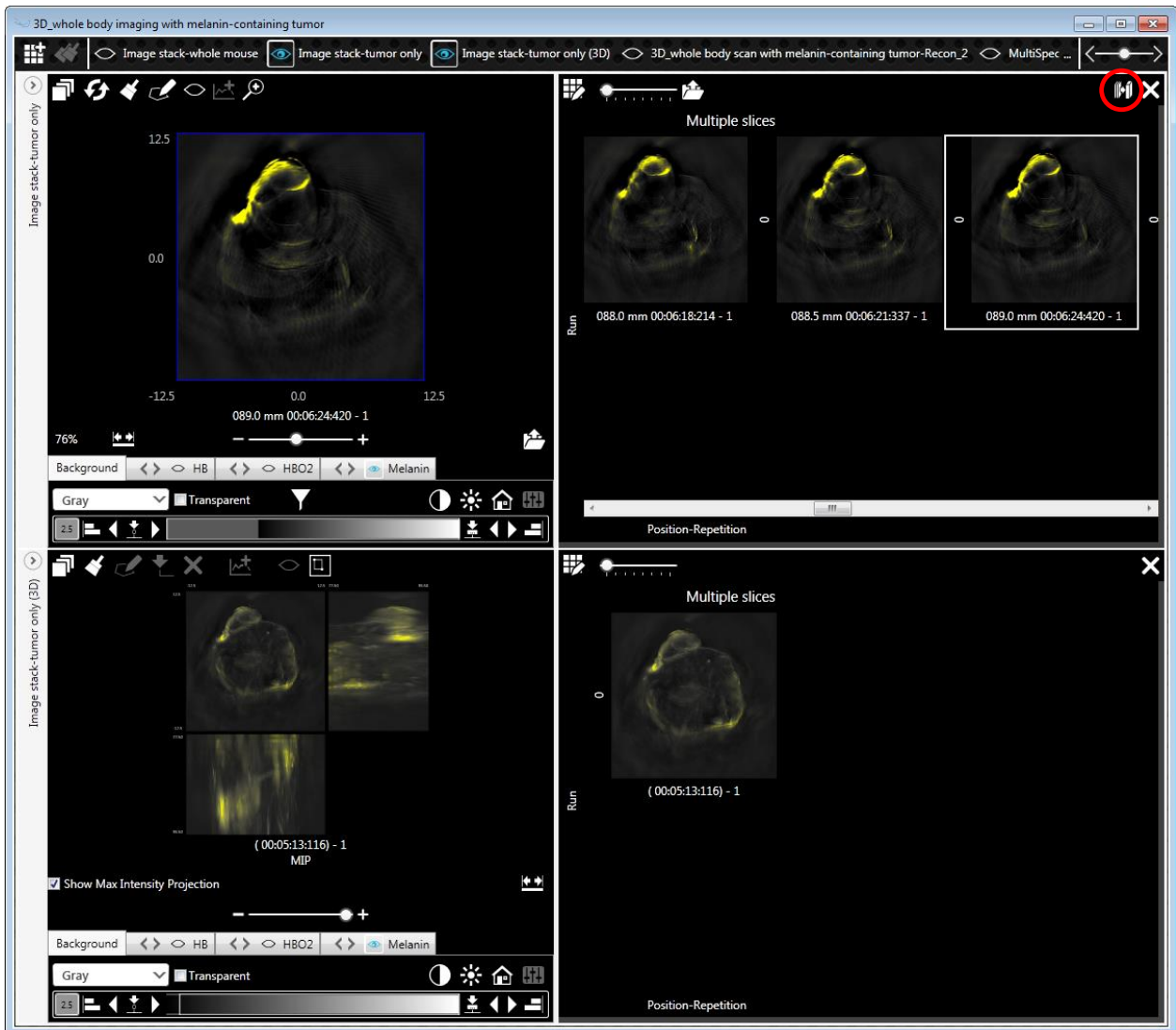
NOTE: It is not possible to adopt regions of interest (ROI), layer selections, layer flipping, and enable/disable Difference Images from one image to another.

NOTE: Copy and paste of parameters is only performed between layers with exactly the same name. The order of the overlaid layers is not changed when applying parameters from one data set to another.

NOTE: When applying the Copy Image Settings from an MSP to a reconstruction or vice versa only the single wavelength background image of the MSP is taken into account.

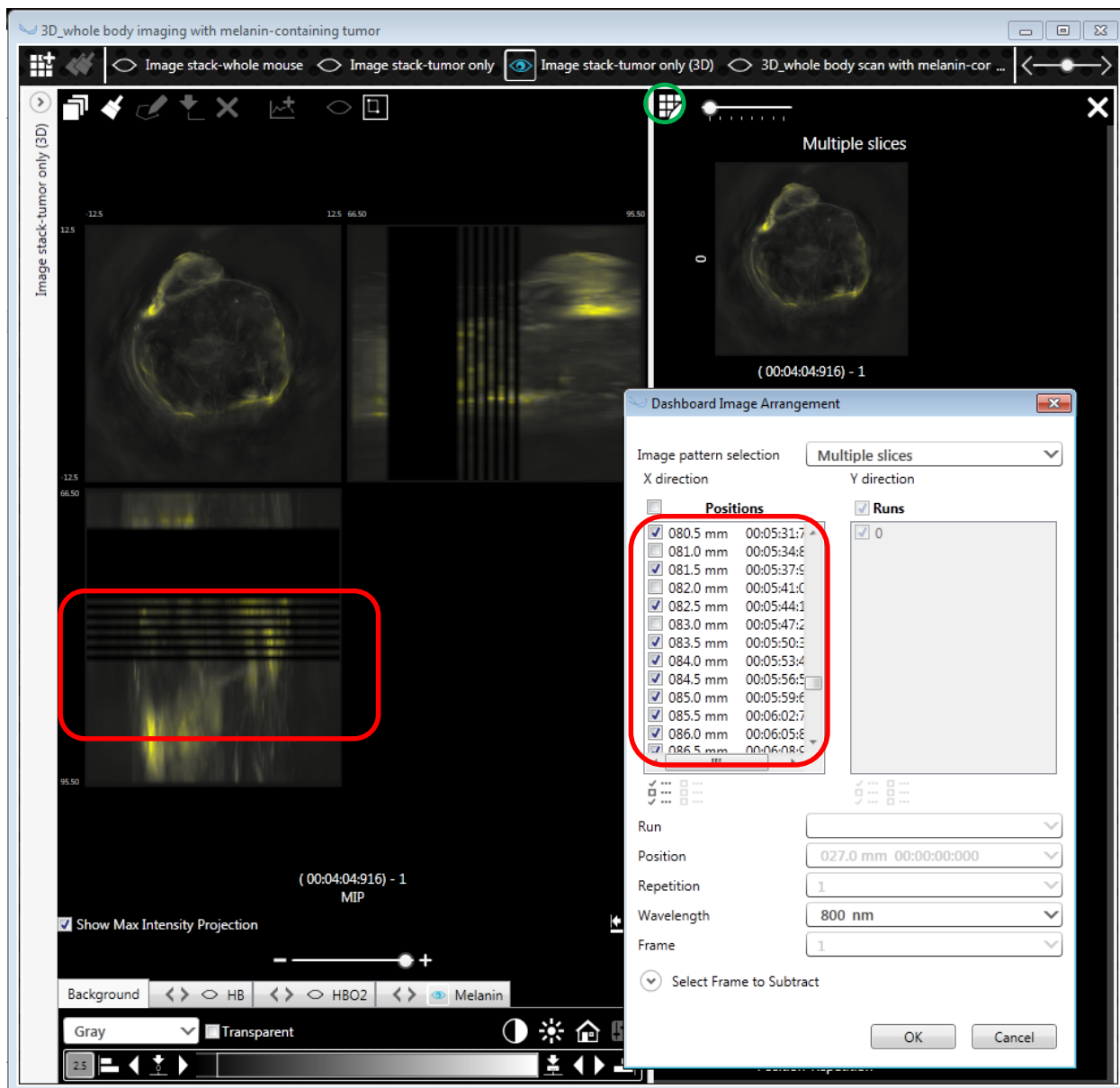
5.7 GENERATE 3D VOLUME OUT OF MULTI POSITION 2D DATA

Click the 3D View icon to stack multi-position 2D data. Below the original View – reconstruction or MSP – a 3D view is added, showing the maximum intensity projection (MIP) of the 3 planes (x-y, x-z and y-z) of the composed cuboid.



5.7.1 Selecting Slices

Initially the 2D positions selection of the reconstructed (or multispectrally processed) image data is converted to and shown in the 3D view. The user can select or unselect parts of the z-axis image data, by opening the Dashboard Image Arrangement popup and modifying the selection of the positions in the Positions table as explained in 5.4.1 5.4.1 Modifying Selection of Recon Images in the Detail Images Window and 5.4.2 Modifying Selection of MSP Images in the Detail Images Window.



ViewMSOT recognizes the positioning grid step size that has been used during acquisition and approximates the x-z and the y-z image plane for this grid step. If the users chooses a preset of the positions and the unselected positions are in the beginning or in the end of the positions range, the x-z and the y-z image plane start with the first and end with the last selected position. If the unselected positions are located inside the displayed x-z and y-z plane, this will lead to gaps in those planes.

NOTE: These gaps can also happen when choosing only a subset of positions during advanced reconstruction and MSP (see 4.2 ADVANCED RECONSTRUCTION and 4.4 ADVANCED MSP) for processing or when the slices are located more than 3mm apart from each other during acquisition. In these cases the gaps occur, even when all positions are chosen to be displayed in the Visualization.

5.7.2 3D Image Controls

Most of the image controls of the 3D Views coincide with the image controls for the 2D views: Layer selection, ROI drawing, color bar (color selection, thresholds, etc.), brightness/contrast and transparency. These controls are explained in detail in 5.6 IMAGE CONTROLS TO MODIFY THE IMAGES.

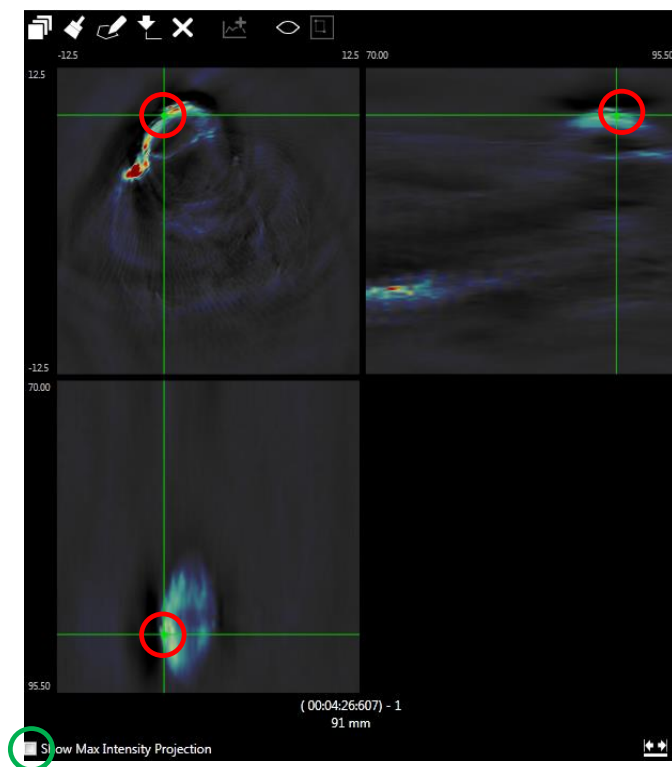
The user can view the 3D data in two different ways:

- 1) Orthogonal Planes view
- 2) Maximum Intensity Projection view

The views and the associated controls are explained below.

NOTE: There is no Difference/Gradient Image processing (see 5.6.2 Choosing the Difference Image) available for the 3D views.

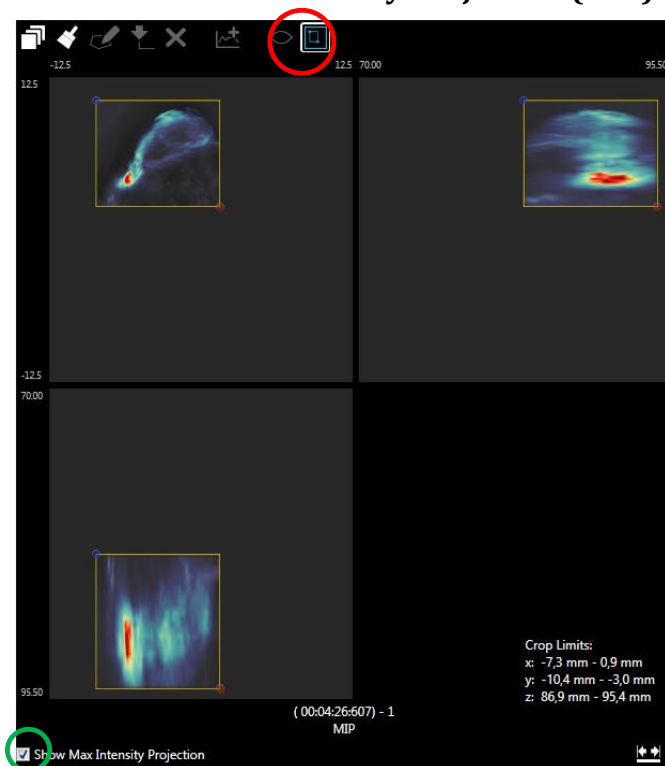
5.7.2.1 Orthogonal Planes View



Uncheck Show Max Intensity Projection to use the orthogonal planes view.

Drag the green cross hair to navigate to the plane(s) of interest.

5.7.2.2 Maximum Intensity Projection (MIP) View



Check [Show Max Intensity \(MIP\) Projection](#) to use maximum intensity projection view.

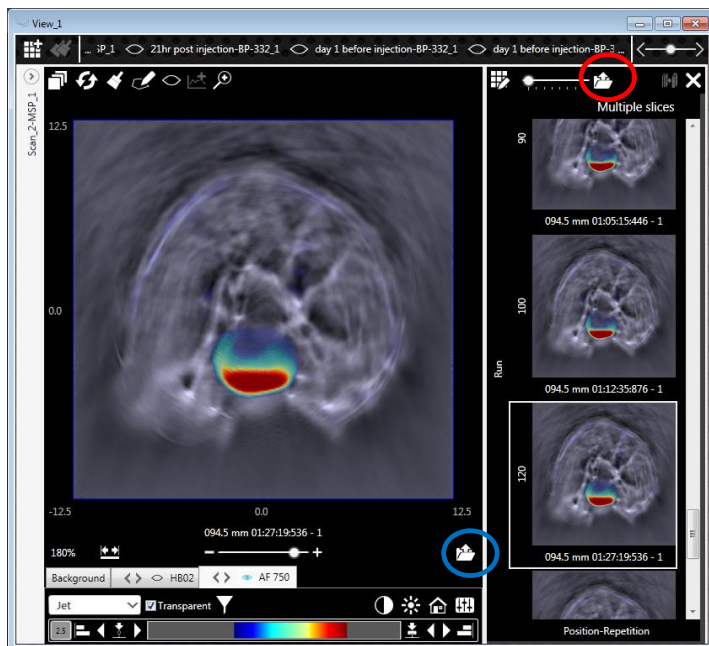
Starting with the image cuboid a MIP for each of the three planes – x-y, x-z and y-z - is processed by finding the maximum voxel intensity being projected onto the plane.

Use the [MIP crop tool](#) to define a sub-volume of the cuboid for the MIP. Only the voxels being selected with the MIP crop tool are scanned for the maximum voxels when generating the MIP.

The *Crop Limits* information at the bottom right displays the coordinates of the MIP cuboid.

5.8 EXPORTING IMAGES

5.8.1 General Image Export



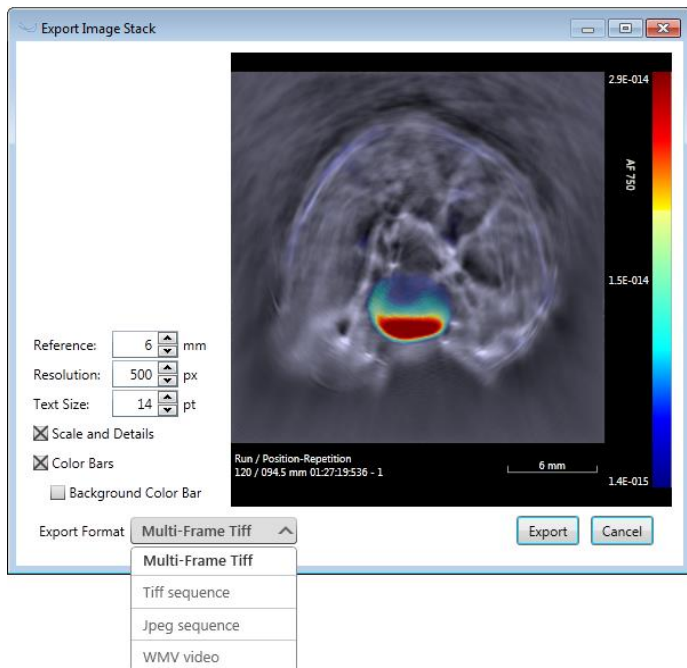
By clicking the [Export icon](#) in the *Detail Images* window all images that are present in the *Detail Images* window can be exported.

When clicking the [Export icon](#) below the *Master Image* only the currently selected image is exported.

In the *Export Image Stack* window the following parameters can be chosen for image export:

- Length of the reference scale bar
- Image resolution
- Size of the text in the image
- Availability of scale bar, information text and color bars

Select one of the following export formats:



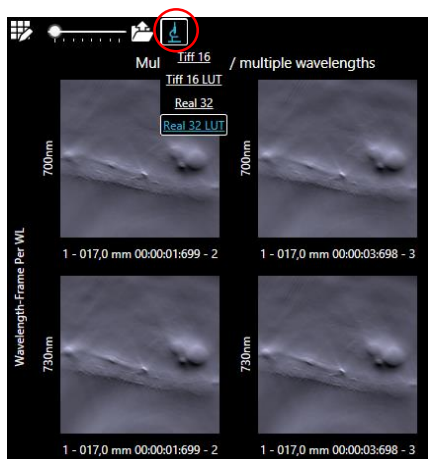
- 1) *Multi-Frame Tiff*: one single Tiff-file containing all images in the *Detail Images* window. The order of the images is from left to right and row by row.
- 2) *Tiff and Jpeg sequence*: separate image files with automatic naming convention:

```
<Scan name>
<Run> <Position>
<hours_minutes_seconds_hundredth>
.<format>
```
- 3) *Mpeg video*: frames are converted into a video. The order of the images is from left to right and row by row.

NOTE: Tiff and Jpeg sequence are saved in an automatically generated folder named according to the Scan name.

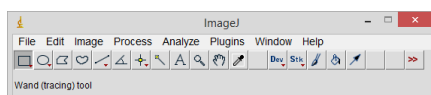
5.8.2 Direct Import into ImageJ

Click the *ImageJ* icon to open the current dashboard image selection as a hyperstack in ImageJ. Choose between the following types of ImageJ import:



- *Tiff 16*: import dashboard selection as 16 bit gray scale tiff images
- *Tiff 16 LUT*: import dashboard selection as 16 bit colored tiff images. Colors will be transferred to ImageJ by generating a look up table (LUT).
- *Real 32*: import dashboard selection as 32-bit images to ImageJ. Each pixel prevents the original (real) reconstruction/MSP value (after thresholding).
- *Real 32 LUT*: same as Real 32 but the colors will be transferred to ImageJ.

The following user adjustments from the Master Image are applied to the ImageJ import:

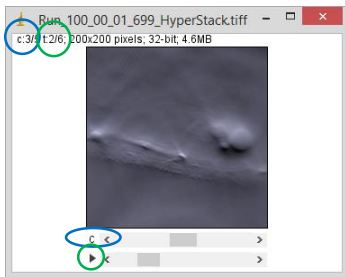


- Rotation of the image
- Filtering of the image layers
- Color bar thresholding/autoscaling

For Tiff 16 LUT and Real 32 LUT also:

- Contrast/Brightness
- Color maps

The following parameters are NOT applied:



- Image overlay options including transparency and transparency map
- ROIs/VOIs

The hyper stack c-dimension keeps the different wavelength for a reconstruction image data set. For MSP visualizations the c-dimension keeps the single wavelength background image and those MSP layers that are visible in the Master Image. The t-dimension keeps the time dimension (Repetitions) of the scan.

NOTE: documentation of the ImageJ software is available under <http://imagej.nih.gov/ij/docs/>

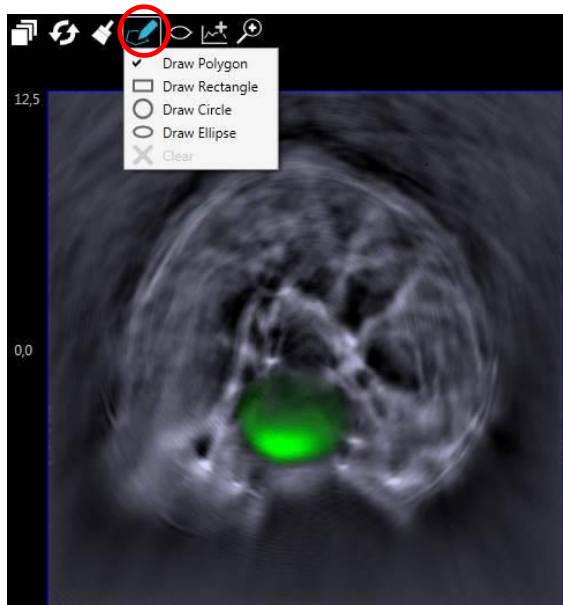
5.9 REGION OF INTEREST (ROI) ANALYSIS

ViewMSOT™ contains region of interest (ROI) analysis tools. ROIs can be drawn onto the Master Image and statistical measures can be extracted, e.g., mean value or maximum pixel value. A graph showing the statistical measure versus one of the variables of the images presented in the Detail Images window can be displayed in the dashboard.

The values of the ROI-measures can be exported to an XML-file.

In the following the ROI analysis is shown for 2D views first, in 5.9.2 Creating VOIs (for 3D Views) VOI analysis on 3D views is explained.

5.9.1 Creating ROIs (for 2D Views)

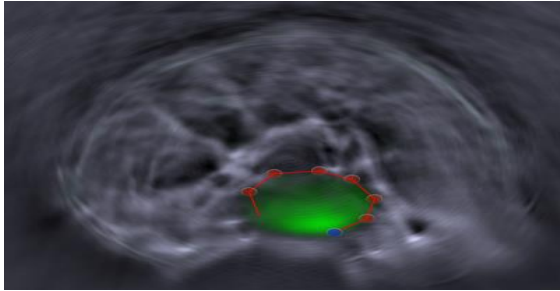


By clicking on the *Select Region* icon different shapes can be selected:

A polygon, rectangular, circle or ellipse shaped ROI can be drawn onto the Master Image Window.

When choosing *Draw Polygon* draw the first point by left clicking. This first point is shown in blue.

Moving the mouse and performing further left clicks continues to draw the polygon.



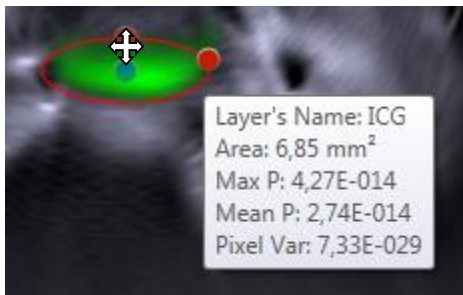
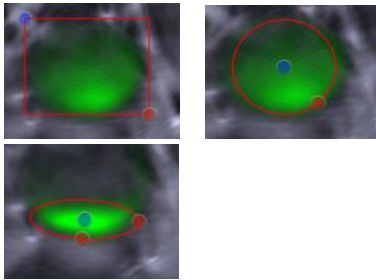
By right clicking, pressing Esc or by unchecking the check mark in the selection dropdown menu the polygon is closed.

Draw Rectangle: on the first left click the blue starting point is set. Moving the mouse pointer the size of the rectangle is chosen. Left clicking again finishes the shape.

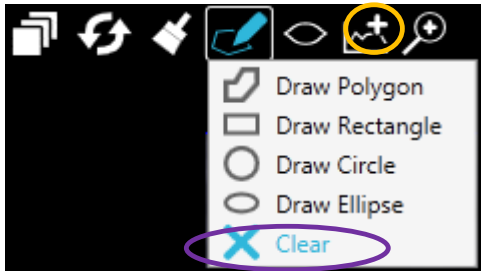
Draw Circle: on the first left click the center is defined (blue dot). With the second left click (red dot) the diameter is chosen.

Draw Ellipse: on the first left click the center is defined (blue dot). With two further left clicks (red dot) the diameters are chosen.

NOTE: Move a shape by dragging the blue dot. Dragging the red dot(s) change(s) the shape.



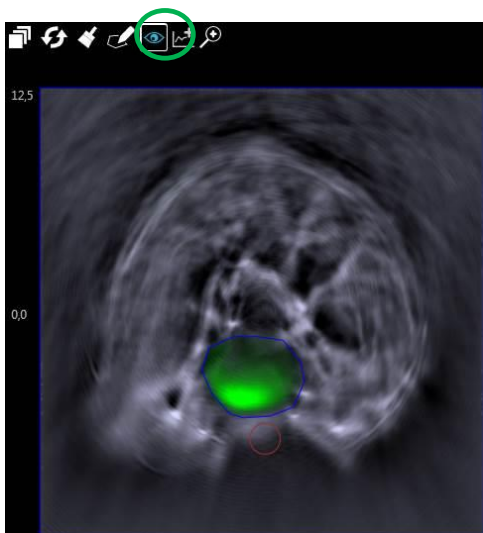
Moving the mouse pointer over one of the red or blue dots surrounding the ROI displays different statistical measures of that ROI, referring to the active layer chosen in the Image Control (see section 5.6.4 Individual Layer Controls).



Click the Import Region icon to add the current ROI to the graph view of the Dashboard explained in section 5.9.3 ROI/VOI Graph.

Selecting Clear removes the current ROI from the *Master Image* window.

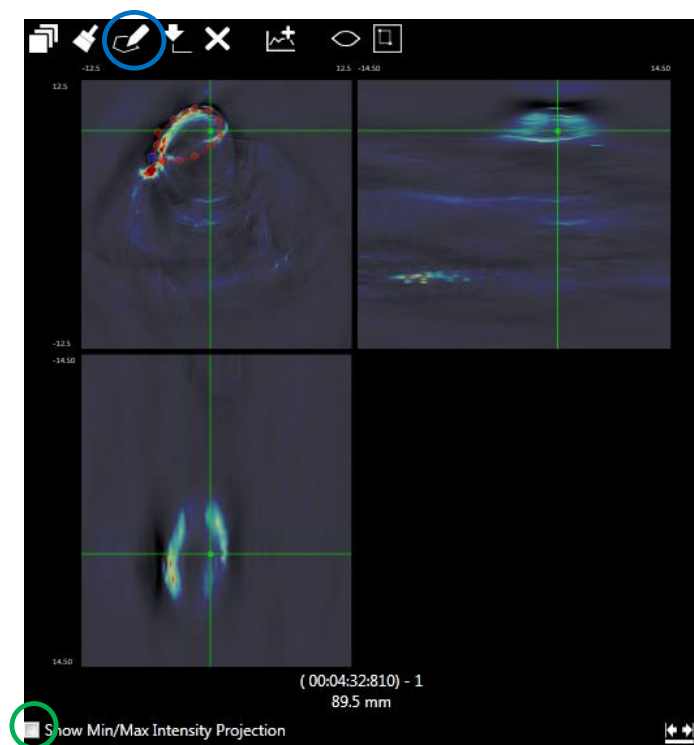
NOTE: This does not remove the ROI in the ROI graph.



Click the Show/Hide Regions icon to overlay the ROIs onto the *Master Image* window or to hide the ROIs.

5.9.2 Creating VOIs (for 3D Views)

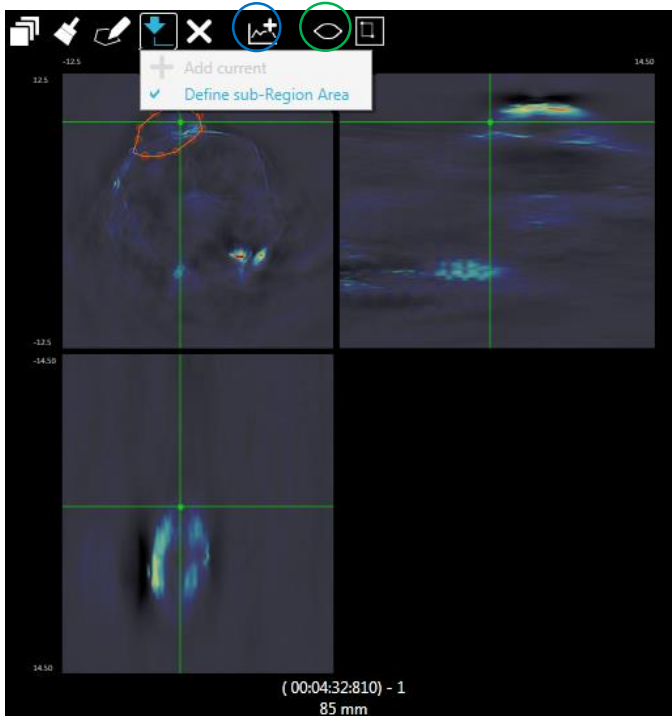
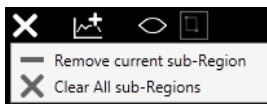
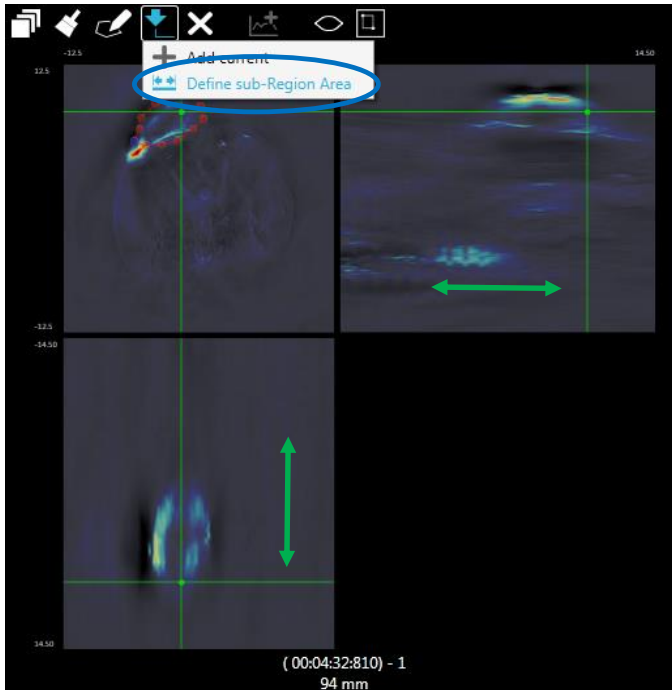
Similarly to a 2D ROI analysis the user can perform a 3D VOI analysis. VOIs can only be drawn on the orthogonal planes view (see 5.7.2.1 Orthogonal Planes View) and not in the MIP view.



In the Orthogonal Planes View inspect the object by browsing through the image cuboid using the cross hairs.

Click the Draw Shapes for Region of Interest icon to draw the profile for the volume of interest (VOI) in the x-y-plane using the different shapes explained in 5.9.1 Creating ROIs (for 2D Views).

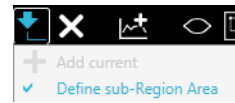
NOTE: The resulting shape will always be symmetrical in the z-dimension, because the ROI which is drawn in the x-y plane stays constant for all z-positions.



Move the cross hair along the z-axis in the x-z or the y-z plane to find the start point, the first slice/position of the VOI.

Mark this z-axis position of the VOI by choosing Define sub-Region Area.

Move the cross hair along the z-axis, find the end of the object and mark the end position of the VOI by unchecking Define sub-Region Area.



The ROIs on the x-y- plane of the image constituting the VOI are visible in orange color, when moving through the z-axis.

Remove single sub-ROI from the volume of interest or clear the whole VOI that has been generated.

Import the VOI to the ROI/VOI graph and table for analysis (see 5.9.3 ROI/VOI Graph).

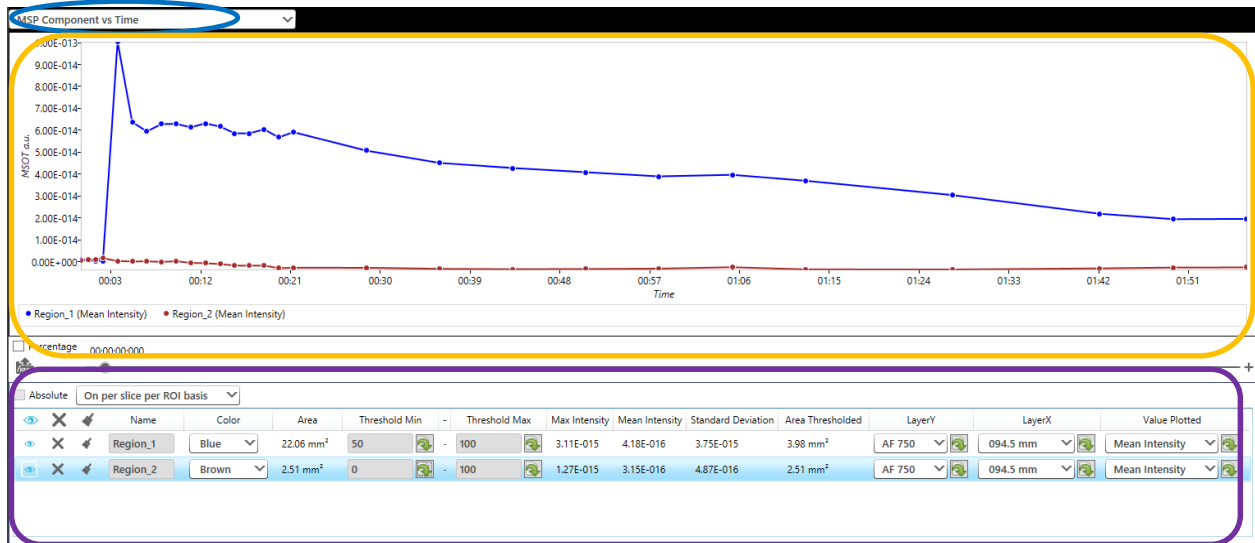
NOTE: The VOI is only visible - after clicking the eye icon - as ROI in the x-y image plane for the defined z-position range. The VOI is not visible in the x-z and in the y-z plane.

5.9.3 ROI/VOI Graph

The top right of the Dashboard contains the ROI/VOI graph window. The ROI graph window includes:

- 1) Dropdown to select the type of graph

- 2) Graph showing the ROI curve depending on the selections
- 3) Region of interest table containing all ROIs that are imported



5.9.3.1 Selecting the Type of ROI/VOI graph

Depending on the type of the currently loaded image data – from reconstruction or MSP – and also depending on the type of scan – e.g. single position or multiple positions – there are different possibilities for the ROI graph type.

For a **reconstruction image data** select:



- 1) *Single Position single WL vs Multiple frames (time)*: observe how a region/volume of interest behaves over time for a certain wavelength.
- 2) *(Average) single WL frame vs Positions*: ROI at one wavelength over different positions (anatomical)
- 3) *(Average) single Position frame vs WL*: spectrum of the ROI at a defined slice

For an **MSP image data** select:



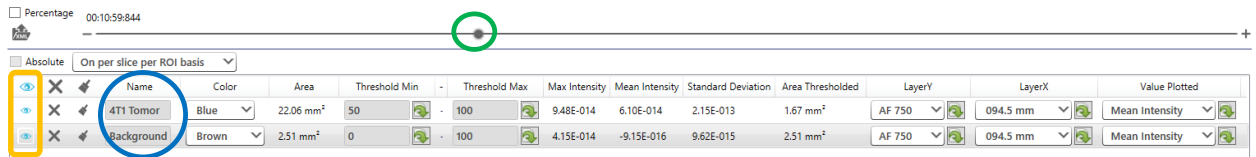
- 1) *MSP Component vs Time*: observe how a slice of interest behaves over time for an MSP component
- 2) *MSP Component vs Position*: ROI of an MSP layer over different positions (anatomical)

5.9.3.2 Content and Functionality of the Interactive ROI Table

All ROIs that are imported from the Master Image are listed in a table. Click the Eye icon to show/hide a ROI table entry – or all - from the graph as well as the ROI(s) on the Master Image. Further entries and selections possible in the table are:

- 1) Give a specific Name to each ROI.

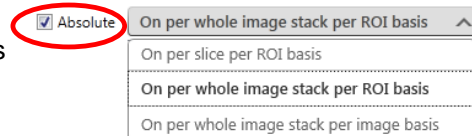
- 2) **Color:** selection of the color of the curve and ROI in the ROI-image.
- 3) **Area:** surface area of the ROI after thresholding in mm squared.



- 4) **Threshold Min and Max:** an upper and lower threshold in percent or absolute MSOT a.u. taken into account for the calculation of the statistical measures (see 5) and for the graph. Different possibilities how the thresholds are applied to the data are explained below.
- 5) **Max Value, Mean Intensity and Pixel Variance:** statistical measures calculated from the ROI applying the thresholds explained in 3. The values refer to the position on the graph's x-axis indicated by a grey line in the ROI graph and by the movable slider below the graph.
- 6) **LayerY/LayerX:** selection for the fixed values. In the example, MSP component versus time is chosen, so the position (LayerX) and the Component (LayerY) need to be fixed for the graph. The ROI graph shows the curves extracted from the ROIs depending on the selection of the graph type and the definition of LayerY and LayerX in the ROI table.
- 7) **Value Plotted:** select the measure plotted in the graph from the available statistical measures of the table.

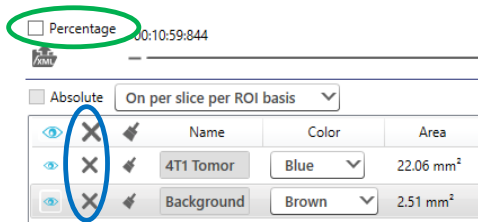
5.9.3.3 ROI Thresholding

Apply a Threshold Min and/or a Threshold Max to limit the image data pixels for the calculation of the statistical measures and to find out how the Area that is defined by the number of pixels within the thresholds behaves over time or position. The thresholds can be applied in four different ways:



- 1) **On a per slice per ROI basis:** apply thresholds on a per image and per ROI basis, e.g. 75% to 100% means for each image/layer only the highest 25% of the pixel values of each ROI separately are used to calculate the statistical measures (in the graph and in the table) of that particular ROI.
- 2) **On a per whole image stack per ROI basis:** apply threshold on each ROI separately but for the complete image stack (depending on what was chosen to be shown on the x-axis of the graph).
- 3) **On a per whole image stack per image basis:** apply the threshold again to the whole stack that is chosen for the ROI analysis but for the whole image (all the pixels not the ROIs separately or only the pixels that belong to the ROIs)
- 4) **Absolute:** enter the Threshold Min and Threshold Max by typing the MSOT a.u. in scientific notation: e.g. 0.000135 is written as 1.34E-4

NOTE: Absolute is only enabled for options 2 and 3.

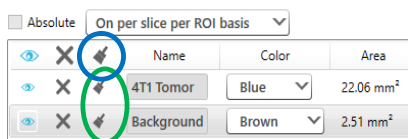


Check Percentage and each ROI curve in the ROI graph will be stretched to the 0% to 100% range with 0% being the minimum value of each curve and 100% being the maximum value.

Selecting an entry of the ROI table and clicking the “x” deletes the ROI. Click the delete icon in the title bar to delete all the ROIs from the current view.

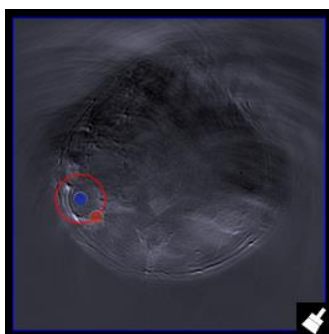
NOTE: Selections for the ROI graph are only applied to images shown in the Detail Images window selected with the Select Images dialog (see section 5.4 DISPLAYING IMAGES).

5.9.3.4 Copy the Shape of a ROI to another Image Data Set



To compare multiple different image data sets - inside the same View or across different Views – ViewMSOT™ allows to apply ROIs from one data set to another.


Click the brush icon in the title bar of the ROI table to generate a ROI graph and table with the same ROIs on another data set. The mouse pointer becomes a brush. Click either on the Master Image of another data set to apply the ROIs to that data set or click the ‘Apply to All’ button in the upper task bar to generate ROI graphs and tables for all data sets inside the visualization.



Click the Brush icon in the ROI table to copy the shape and location of that particular ROI. Now click on that Master Image Window of the image data set where you want to apply the ROI.

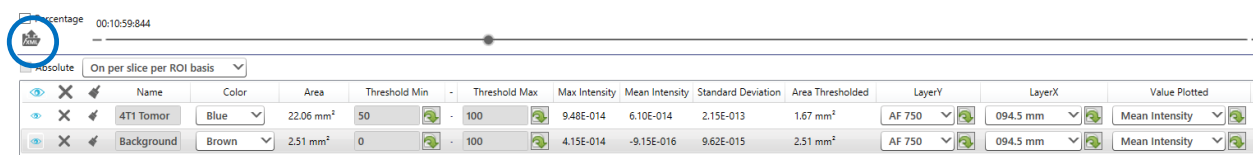
NOTE: It is also possible to copy ROIs between different MSP component layers or in between reconstructions and MSPs.

NOTE: The image resolutions of the image data sets need to be the same. Otherwise ViewMSOT™ will report a resolution mismatch.

NOTE: The brush icon in the title bar and the brush icon for particular ROIs/VOIs work different: Clicking the ROI brush to copy a single ROI only the ROI shape will be copied. Click  to import the ROI/VOI and set up the ROI/VOI graph as explained in 5.9.3 ROI/VOI Graph. Clicking the ROI/VOI brush in the ROI/VOI table title line to copy all ROIs/VOIs automatically generates a ROI/VOI graph and table with the same parameters.

5.9.4 ROI-XML export

Pressing the Export to XML icon exports the content of the table for all ROIs and for all x-axis positions of the graph to an XML file.

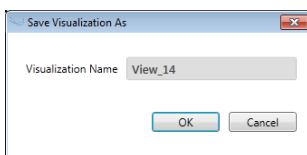
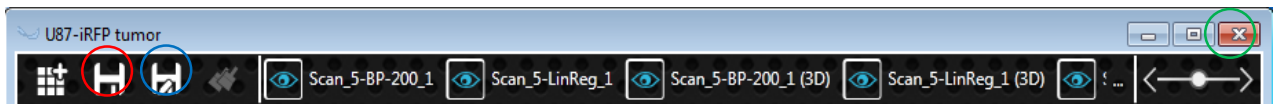


Opening the exported file in a spreadsheet program allows the user to process the ROI data.

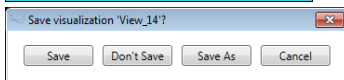
| Name | Calculation Method | Method | Region's Name | Area (mm ²) | Minimum Threshold (%) | Maximum Threshold (%) | Position (mm) | Run | Repetition | Component's Name |
|-------|----------------------------|-----------------------|---------------|-------------------------|-----------------------|-----------------------|---------------|-----|------------|------------------|
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 1 | 1 | 1 HB |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 1 | 1 | 1 HBO2 |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 1 | 1 | 1 ICG |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 2 | 2 | 1 HB |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 2 | 2 | 1 HBO2 |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 2 | 2 | 1 ICG |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 3 | 3 | 1 HB |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 3 | 3 | 1 HBO2 |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 3 | 3 | 1 ICG |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 4 | 4 | 1 HB |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 4 | 4 | 1 HBO2 |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 4 | 4 | 1 ICG |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 6 | 6 | 1 HB |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 6 | 6 | 1 HBO2 |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 6 | 6 | 1 ICG |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 8 | 8 | 1 HB |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 8 | 8 | 1 HBO2 |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 8 | 8 | 1 ICG |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 10 | 10 | 1 HB |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 10 | 10 | 1 HBO2 |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 10 | 10 | 1 ICG |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 12 | 12 | 1 HB |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 12 | 12 | 1 HBO2 |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 12 | 12 | 1 ICG |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 14 | 14 | 1 HB |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 14 | 14 | 1 HBO2 |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 14 | 14 | 1 ICG |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 16 | 16 | 1 HB |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 16 | 16 | 1 HBO2 |
| MSP_1 | On per slice per ROI basis | MSP Component vs Time | 4T1 Tumor | 20,97 | 80 | 100 | 94,5 | 16 | 16 | 1 ICG |

5.10 SAVE VIZUALIZATION AND EXIT VIZUALIZATION

Click the save icon to save the current status of the Visualization. If the Visualization has not been named yet you will be asked for a name.



Save the current Visualization under a new name keeping the last saved status of the Visualization under its previous name.



Click the 'x' button to close the current Visualization. If modifications have been done on the Visualization that have not yet been saved a respective Save dialog will be shown.

6 APPENDIX

6.1 MAINTENANCE

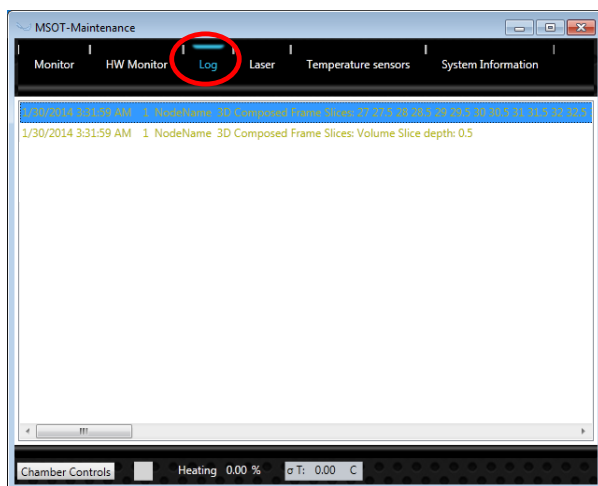
Press the *Maintenance* icon - bottom right button in the main window – to access the MSOT Maintenance. Usually this module is only used by the iThera Service team.



However, in case of system failures the user can check the error log where all system actions are displayed in a temporal order.

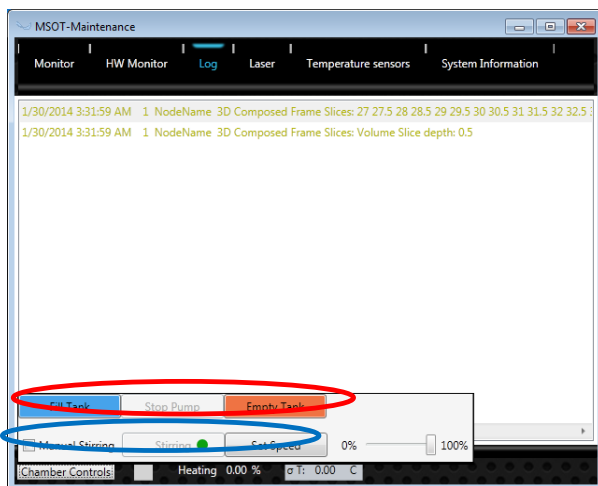
It is also possible to manually operate the water supply pump and the stirring pump with the help of the Maintenance window.

6.1.1 Data Log



In case an error message pops up review the system log. In order to see the log call up the MSOT Maintenance window. The *Log* tab shows a list of all log messages including date and time.

6.1.2 Manual Control of the Pumps



To change the water level in the imaging chamber click on either *Fill Tank* or *Empty Tank*. *Stop Pump* will interrupt the filling or emptying procedure.

Manual Stirring enables access to the stirring pump controls. It is possible to disable/enable the stirring by pressing *Stirring*. To control the flow rate of the pump use the slider and press *Set Speed*.

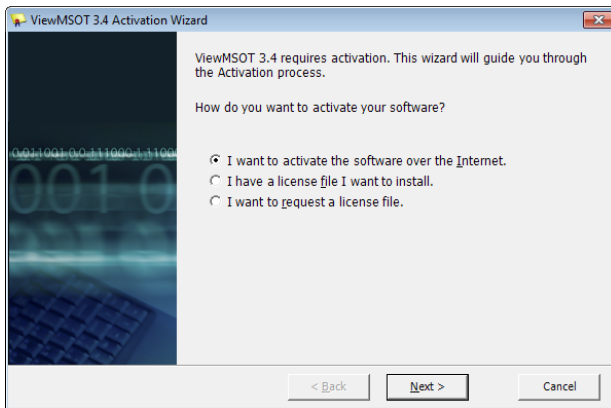
Note: Stirring is fully automatic. Normally there is no need for user intervention.

Note: After pressing the control buttons there might be a delay of approximately 2 seconds.

6.2 ACTIVATION OF ViewMSOT LICENSE

Your license of ViewMSOT can be activated through the internet with the help of a product key. If no internet is available on the machine an additional license file needs to be transferred.

6.2.1 Activate ViewMSOT from PC being connected to the Internet



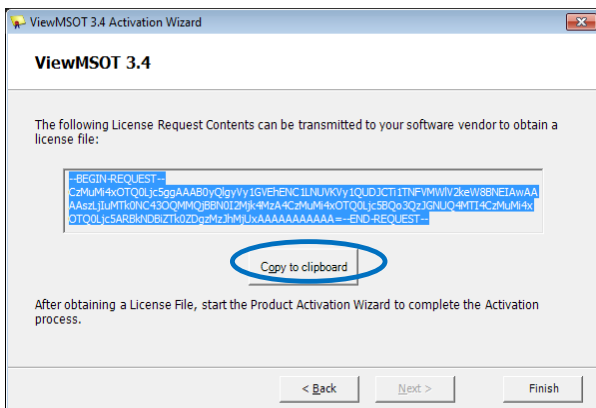
When starting ViewMSOT on a system where no ViewMSOT license has been activated yet, the license activation window appears.

Click the first option *I want to activate the software over the Internet* and then *Next* if your PC is connected to the internet.

Please enter your product activation key when asked and click *Next*.

Your activation key is now validated via the internet.

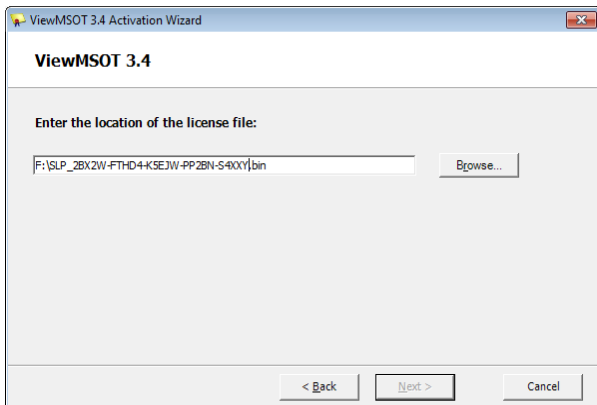
6.2.2 Activate ViewMSOT from PC that is not connected to the Internet



Click the third option *I want to request a license file* and then *Next*, if your system as no connection to the internet.

Please enter your product activation key when asked and click *Next*.

Copy the content of the request and transfer it to iThera e.g. via Email and close ViewMSOT.



As soon as you receive the license file, start ViewMSOT and click the second option *I have a license file I want to install* in the license activation popup.

Please browse for the license file key when asked and click *Next*.

Your license file is loaded and ViewMSOT is activated.

NOTE: If you face any problems during the activation process please contact support@ithera-medical.com.