iThera Medical

Listening to Molecules

ViewMSOT

MSOT inSight 64
MSOT inVision 128
MSOT inVision 256-TF
MSOT inVision 512-echo

CONTENTS

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, inVision 256-TF and inVision 512-TF and -echo 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.

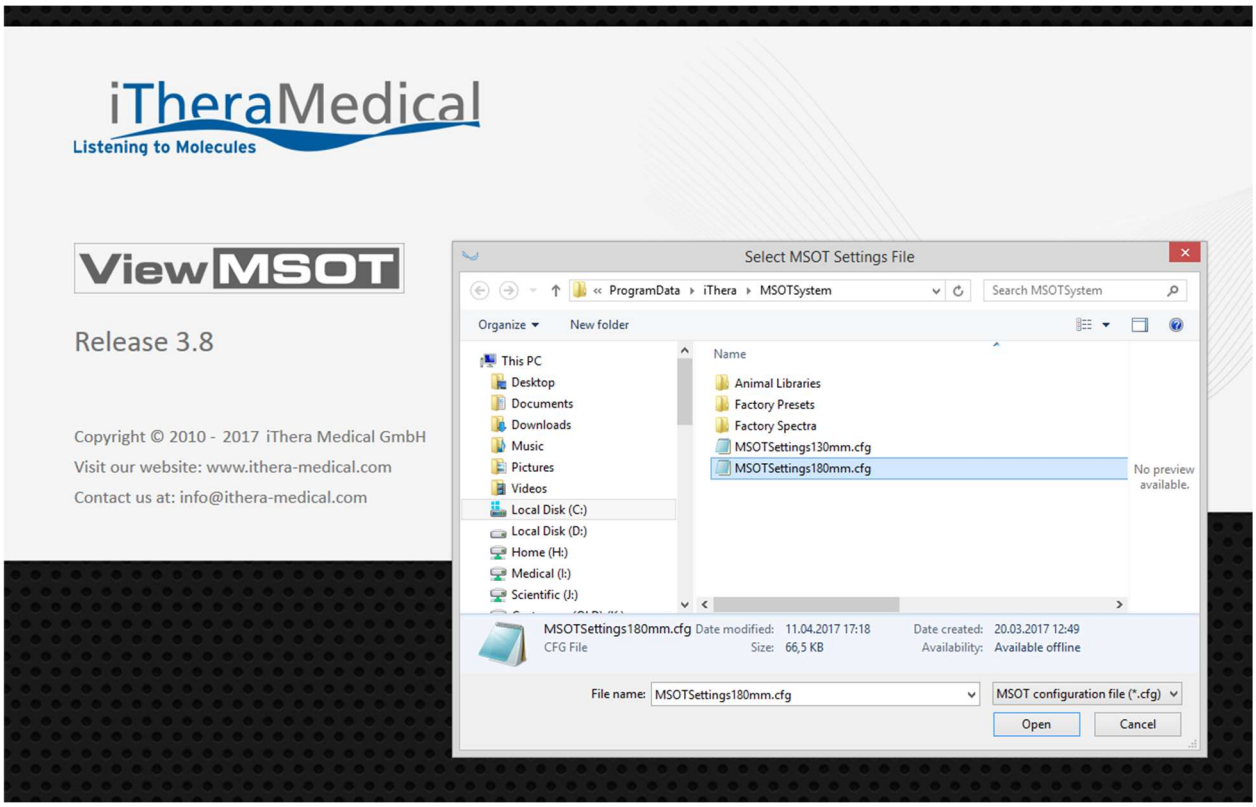
In chapter 5, it is shown how to use the Visualization functionality in order to inspect, analyze and improve the images. This includes image post-processing tools like thresholding and filtering as well as displaying region of interest (ROI) graphs from the image data. The images as well as the content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

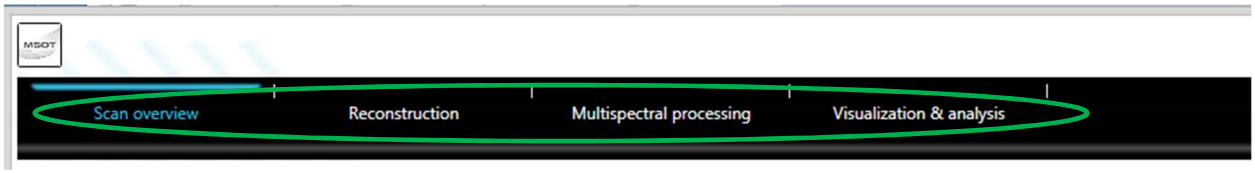
For questions and comments please contact your local distributor or iThera Medical at support@ithera-medical.com.

2 STARTING ViewMSOT™ AND SETTING UP A STUDY

Note: if animal holders of different lengths are available you will see a dialog letting you choose the MSOT Settings suitable for the animal holder you want to image with. Select the correct Settings file and click *Open*.



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.

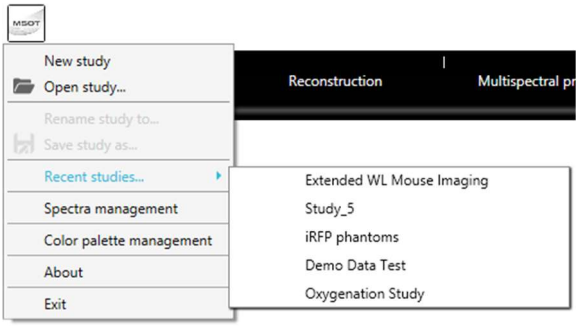
1) ***Optoacoustic Measurement*** (OAM): acquiring data on an MSOT system
2) ***Reconstruction***: reconstructing images from the raw data
3) ***Multispectral Processing***: multi-spectrally processing those images to extract spectrum dependent component images
4) ***Visualization and Analysis***: displaying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

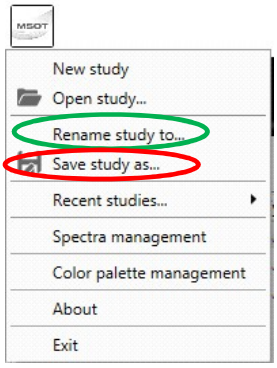
NOTE: The system's hardware can also be initialized later as explained in 3 IMAGE ACQUISITION. However, initializing the hardware environment before choosing the study and/or selecting the Study preset (see 2.1 SELECTING A STUDY PRESET) is advised to save time.

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

Click the Home button and then select *New study* or *Open study*.

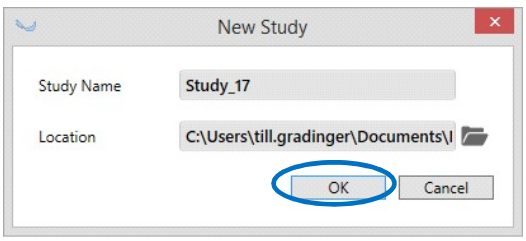


Or choose one of the *Recent studies*.



To change the name of the current study select *Rename study to…*.

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



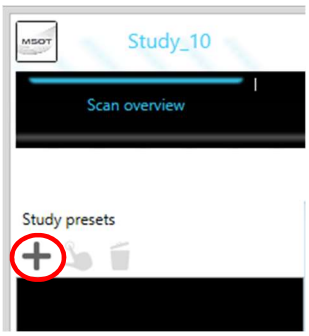
Enter a *Study Name* and click *OK* to save it.

NOTE: In the desktop version of ViewMSOT™ it is also possible to choose the location of the Study.

2.1 SELECTING A STUDY PRESET

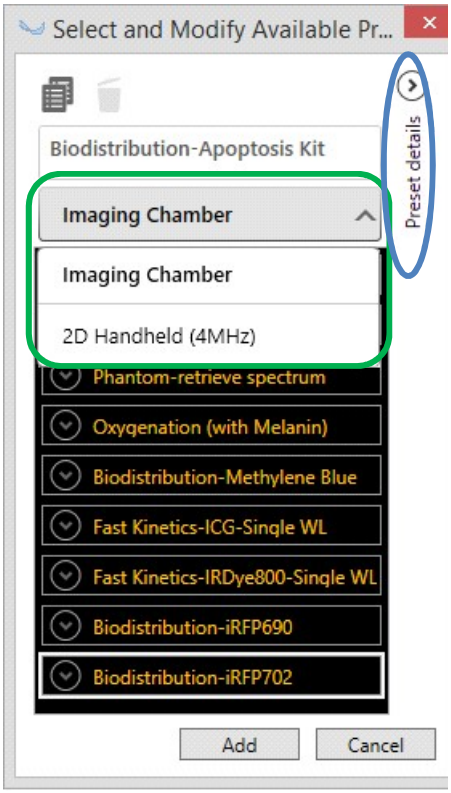
For different types of studies factory *Study presets* that include parameters for data acquisition as well as image reconstruction and multispectral processing are available. Study presets allow to have one or more fixed set(s) of parameters for a whole study that can be applied to scans without being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

NOTE: Adding, creating, removing and modifying study presets is not available in the Desktop version of the software.



To select a Study preset click the "+" button.

The *Select and Modify System Presets* dialog opens.



The software contains different factory Study presets for particular applications, e.g.

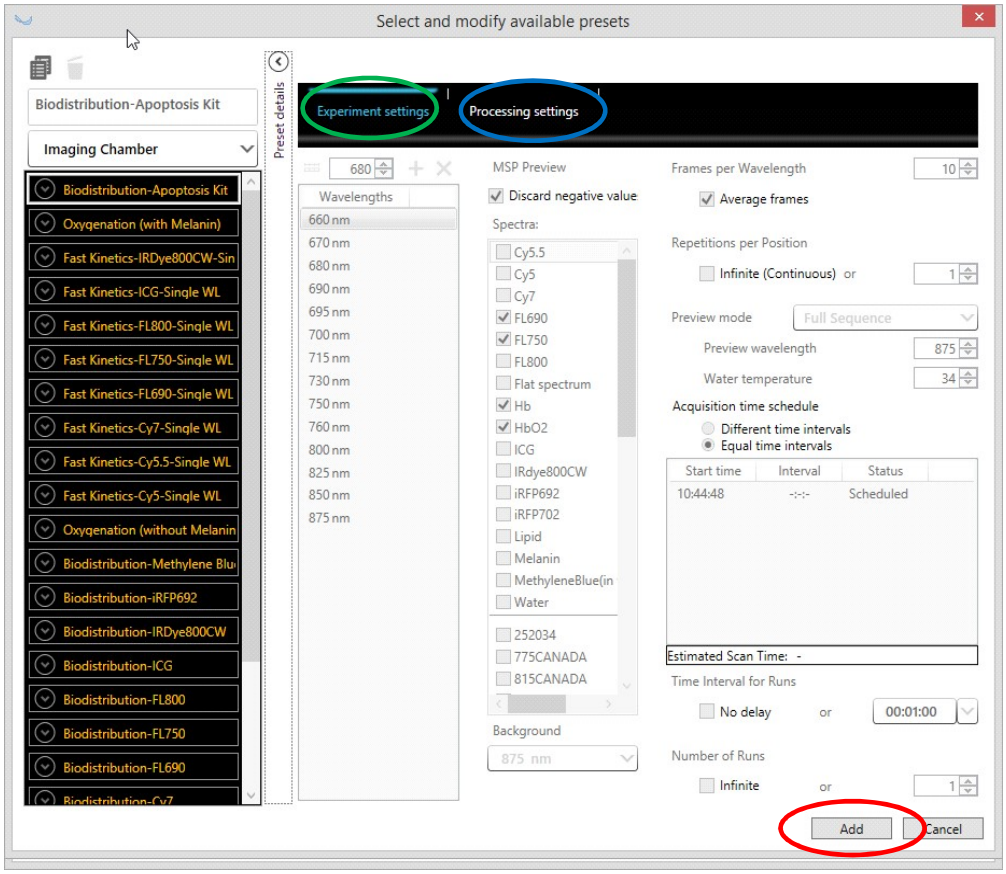
- Determination of blood oxygenation
- Detection of dyes (e.g. Methylene blue, ICG, etc.)
- Whole-body anatomical mouse scan
- Etc.

Access the Study presets available for different ultrasound probes, if your system contains more than one probe, e.g. by a handheld extension.

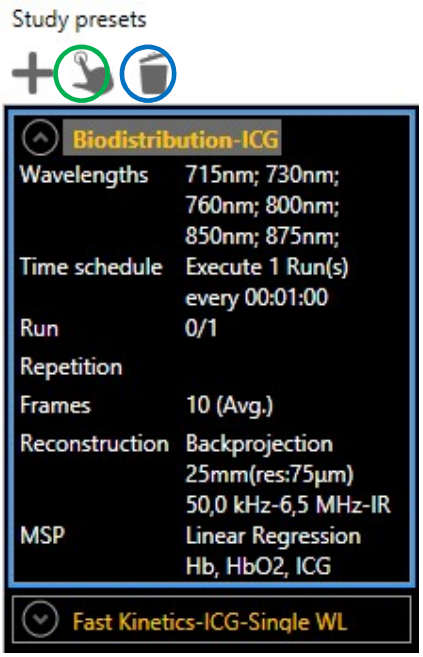
To review the existing System Study Presets click *Preset Details*.

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

The *Study Preset*s 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.



Click *Add* to add the selected Study preset from the list to the current study and close the dialogue.



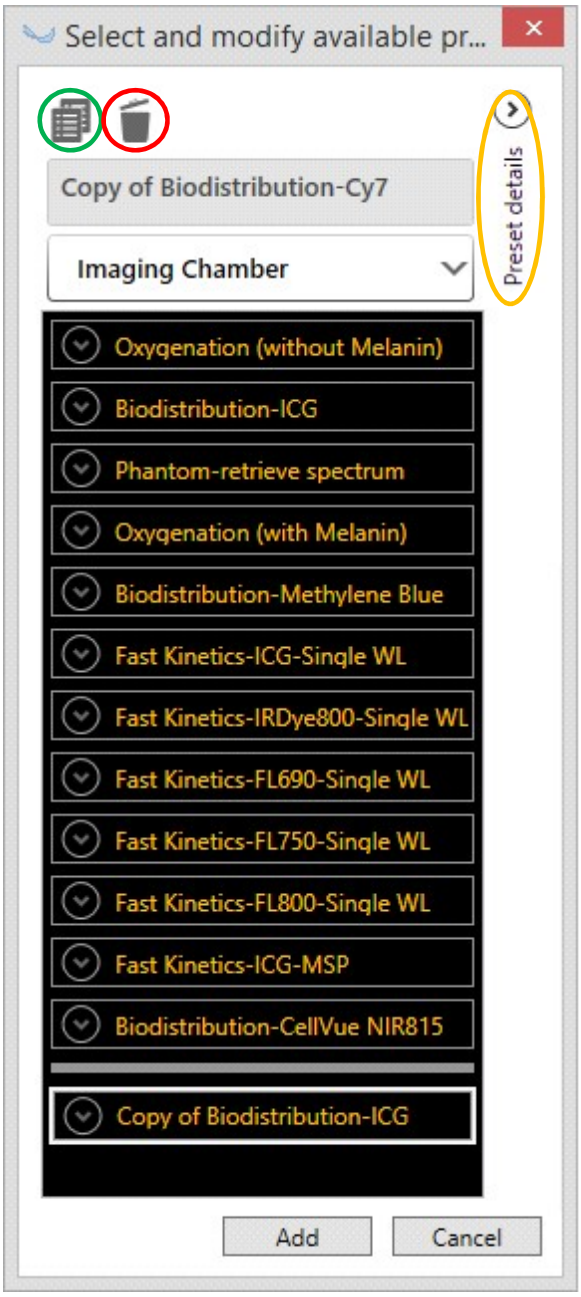
Click one of your Study presets (selection is indicated by a blue frame) to extend the information.

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.

Click 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



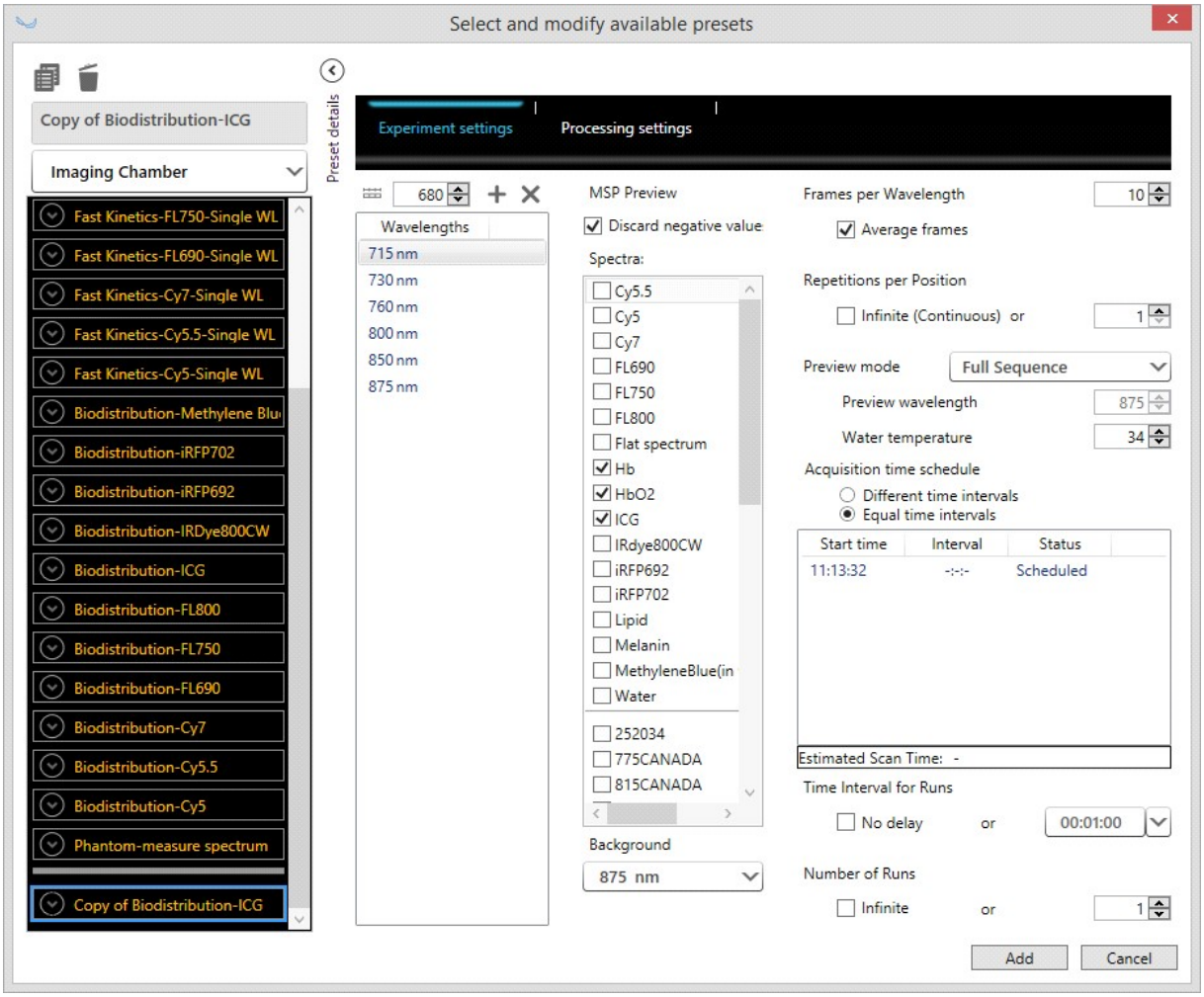
Open the *Select and modify available presets* dialog as described in 2.1 SELECTING A STUDY PRESET and duplicate one of the existing presets to modify it according to your needs.

Delete one of the presets.

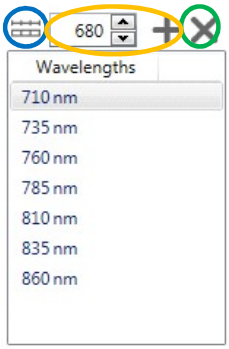
NOTE: There is no possibility to delete factory Study preset. Only presets generated by the user can be deleted.

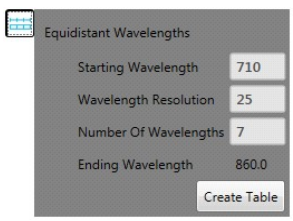
NOTE: Deleting a Study preset will delete that preset from the system permanently. There is no undo functionality. It will, however, remain part of any existing studies.

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



2.2.1 Experiment settings





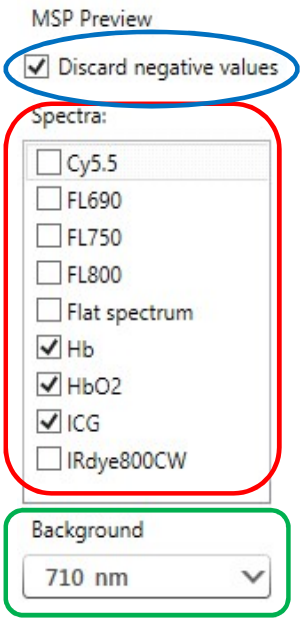
When creating Study preset a default choice of wavelengths for multispectral acquisition is presented in the Wavelengths list. Adding wavelengths to this list is possible in two ways:

1) Different wavelengths can be selected and added to the active list manually. In this way it is possible to select any pattern of wavelengths.

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*".



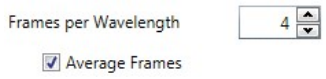
To display a live multispectrally decomposed image during live preview and data acquisition for certain absorption molecules like Indocyanine Green (ICG) or melanin check the boxes for the spectra of interest in the *Spectra* box. See 0 SPECTRA MANAGEMENT to import your own spectra.

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).

Check *Discard negative values* to exclude pixels in which a number of wavelengths (>25%) show negative signal in order to avoid reconstruction artifacts that affect multispectral processing.

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.

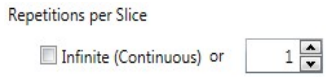
For in vivo imaging usually longer wavelengths are a better choice for better penetration depth. 800nm is a common choice because of the isosbestic point of hemoglobin. (the background wavelength can also be changed later, see 5.2.2 Selecting MSP images to be shown in visualization).



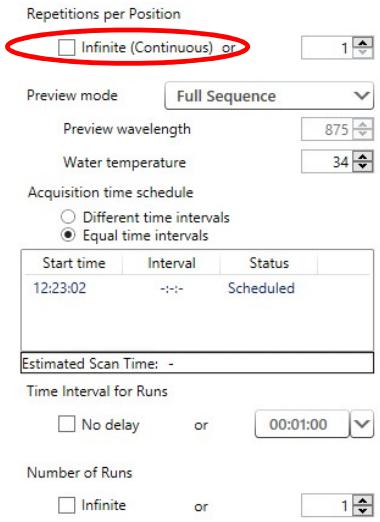
Define the number of *Frames per Wavelength* to be acquired. The raw data for each wavelength from multiple consecutive pulses is averaged to create a single image. This can be useful to increase the signal to noise ratio or to account for motion while reducing the amount of stored data.

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.

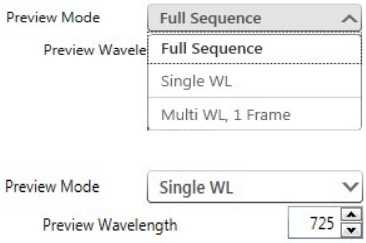


Repetitions per Slice defines the number of repetitions that the software acquires at all defined wavelengths with the defined number of frames at the same position before moving to the next position.



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

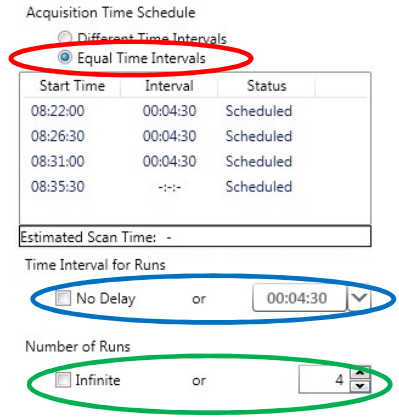
NOTE: The selection that is chosen in the Preview Mode is for image preview only. The acquisition always uses the defined presets.

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

Choose the desired water temperature in the range of 25°C to 37°C.

NOTE: 34°C is the suggested temperature for mouse imaging.

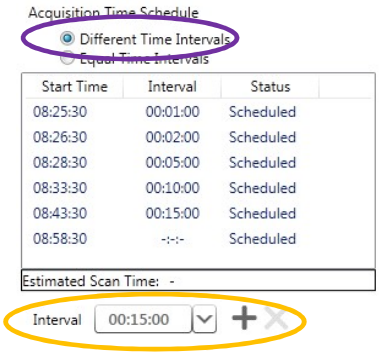
The acquisition of the same scan regions repeatedly within one scan can be done with the use of multiple Runs in the *Acquisition Time Schedule*. Choose between Different Time Intervals (meaning freely chosen points in time) and Equal Time Intervals.



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*.

The time interval refers to the time between the beginning of two consecutive Runs. If it is chosen too short and conflicts with the estimated scan time, this conflict will be indicated by a red box around the *Estimated Scan Time*. If the time interval is not adjusted accordingly, the next Run will begin immediately on completion of the current Run.

The selection of the *Number of Runs* can be either *Infinite* where the system acquires images in an open loop manner until a manual end command is issued. 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.

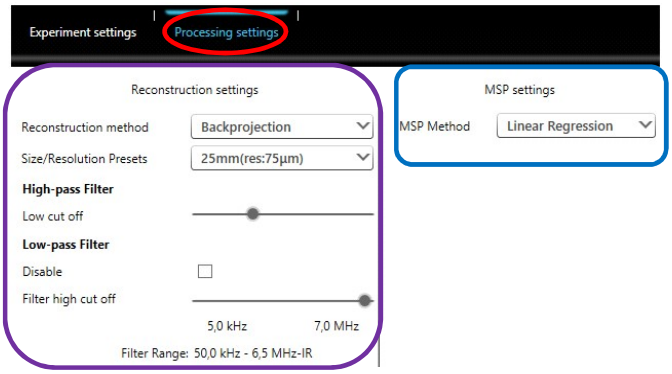
Section 3.3 STARTING THE ACQUISITION explains how to stop acquiring data after a run is finished or after a full set of wavelengths has been acquired.

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 **Error! Reference source not found. Error! Reference source not found.**).

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

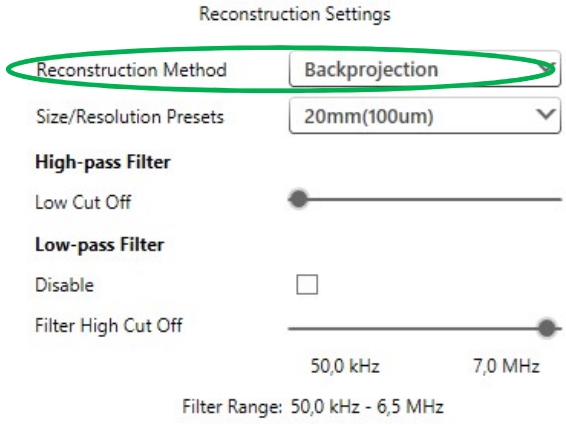


The *Processing Settings* contain parameters for image reconstruction and multispectral processing.

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.

2.2.2.1 Image reconstruction settings

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



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

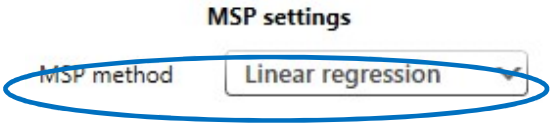
1) *Reconstruction Method*:
   a) *Backprojection* reconstruction is a very fast and semi-quantitative reconstruction algorithm that allows efficient generation of anatomical images (for details see [1]). The back projection algorithm is also used for the live preview.
   b) *Model-based* reconstruction 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 (for details see [2]).
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

NOTE: A description of the backprojection and model-based reconstruction algorithms can be found in [1] and [2].

2.2.2.2 Multispectral processing(MSP) settings

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

1) Linear Regression
2) guidedICA
3) PCA/ICA
4) AMF
5) Difference

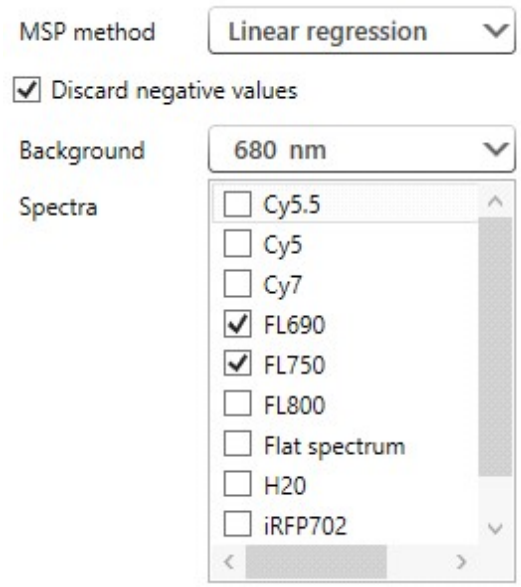


Some methods are able to incorporate a-priori knowledge of spectral absorbers present in the imaging object.

The same selection of spectra which is set up for acquisition (see 2.2.1 Experiment settings) is applied. This is also true for the selection of the background wavelength and the one for Discard negative values.

NOTE: Algorithmic details and formulas to some of the implemented methods can be found in [3].

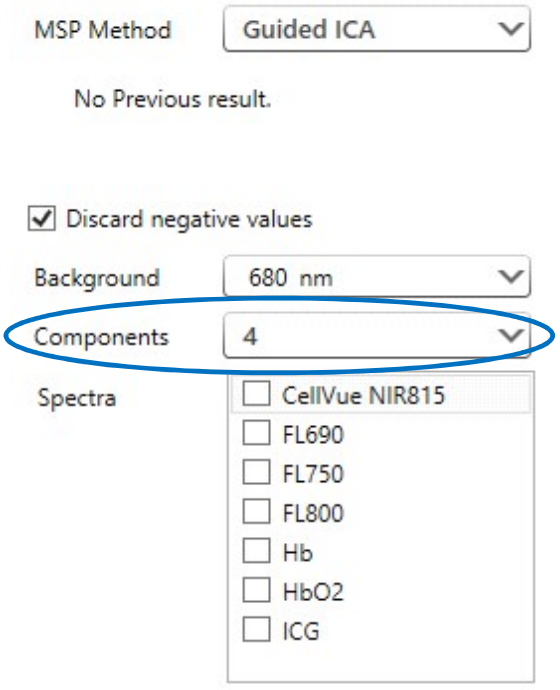
Linear regression



Linear Regression is a fitting procedure using known spectra. It allows for easily reproducible results across studies.

NOTE: Be sure to select all significant spectra to avoid misleading results. The imaging data is fitted only to the chosen spectra (that means: if a spectrum that is prominent in the imaging data is not selected its image content will appear in one or multiple of the other spectra's MSP results).

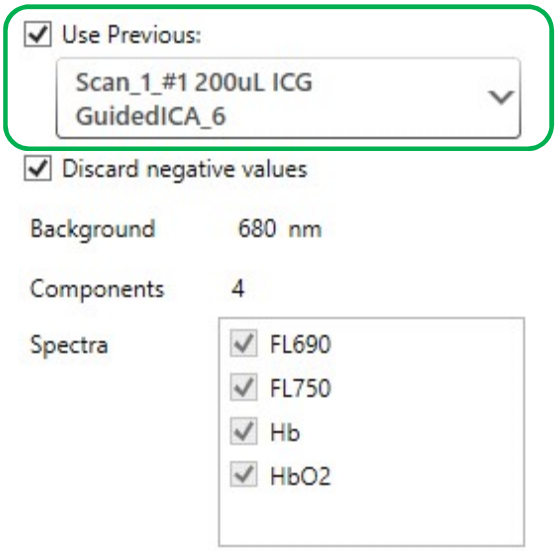
Guided ICA



Guided ICA is an adaptive algorithm that uses a-priori knowledge of the absorption spectra in the sample in order to best adapt to the individual situation. While in general it is very similar to purely algebraic unmixing (such as Linear Regression), it needs to operate in mean-free space to make use of the statistical properties of the dataset to optimize the unmixing matrix. Hence, comparisons between datasets with very different characteristics (e.g. brain vs. liver, healthy vs. immunocompromised) can lead to misleading results (use the approach suggested below instead).

Choose the number of *Components* to limit the MSP layers to a meaningful number, ideally choose the same number of components as input spectra.

NOTE: The number of components needs to be greater than or equal to the number of Spectra that are used for the Guided ICA.

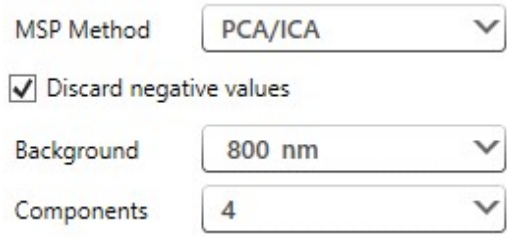


Enable *Use Previous* to re-use a previously generated unmixing matrix to maintain comparability of the results of different scans inside one Study. Unmix a **true positive** dataset to achieve an ideal unmixing matrix, then apply this to the remaining datasets in the study to obtain comparable numbers. Selecting a previously processed Guided ICA result will execute the same unmixing again without the optimization step, so no other options are available.

NOTE: Selecting a previous unmixing matrix will automatically disable the possibility to modify the wavelength selection and apply the wavelengths used for the selected previous unmixing.

PCA/ICA

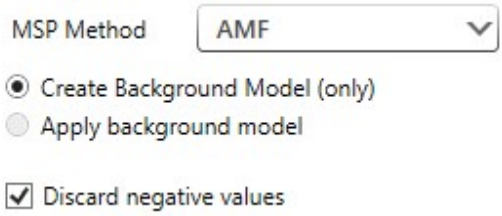
PCA/ICA is a blind method that does not require prior spectral knowledge. It can retrieve spectra based on the statistical properties of the dataset. Principle component analysis (PCA) is performed initially to reduce the dimensionality of the dataset by discarding noise and unimportant variations. The remaining data is the basis for the subsequent ICA algorithm which tries to separate remaining components using statistical independence. Components are randomized in the process of the ICA procedure, where a second round of analysis will produce a different order of components.



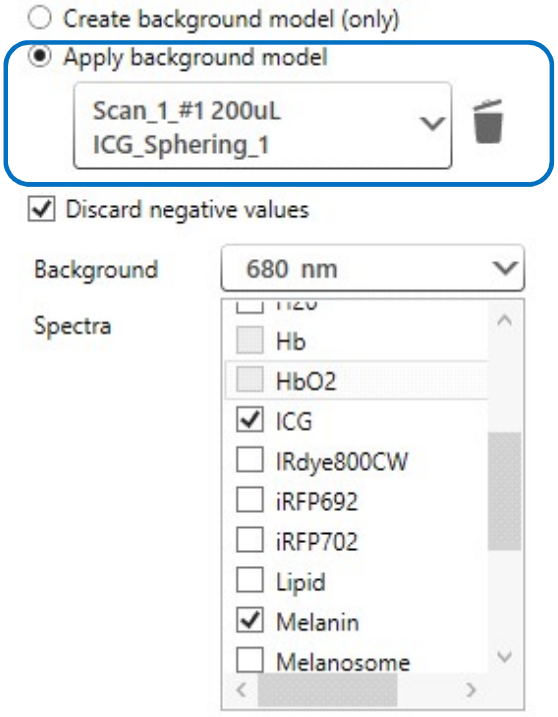
Choose the number of components retained after the PCA step, which is equivalent to the number of resulting components. Given the dominant contribution of hemoglobin (Hb, HbO_2) plus a potential contrast agent, retaining 3 or 4 components is recommended (for algorithmic details see [3]).

AMF

Adaptive Matched Filters (AMF) use signal theory to separate absorbers from background. AMF requires the use of a background model in absence of the chromophore of interest – or where the chromophore of interest has little influence. Hence it cannot be used to determine Hb and HbO2 separation in *in vivo* datasets because they are part of the background, but is used mainly to analyze biodistribution of injected agents (for details see [3]).



Create a background model first on a **true negative** dataset (e.g. acquired before injection of an agent or in a naïve mouse of the same strain, phantom filled with control absorber).

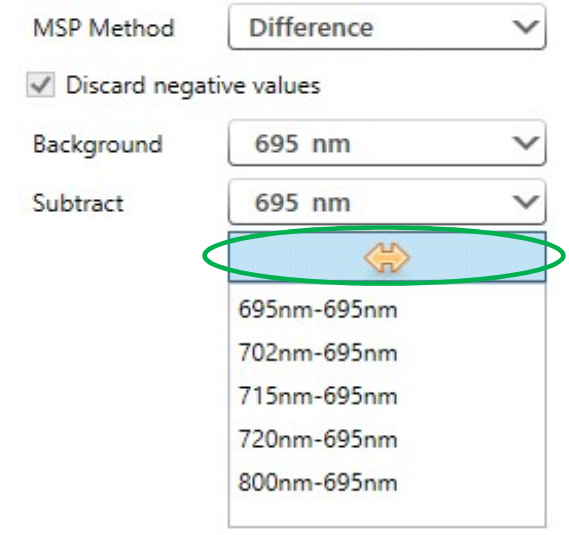


This allows the characterization of the background in absence of the desired chromophore, and hence will **not** produce a MSP result that can be used in visualization.

Then use the background model to obtain the distribution of the chromophore of interest as selected in the list.

NOTE: When using Apply background model the selection of the wavelengths is disabled and the wavelength used to generate the background model are automatically applied.

Difference

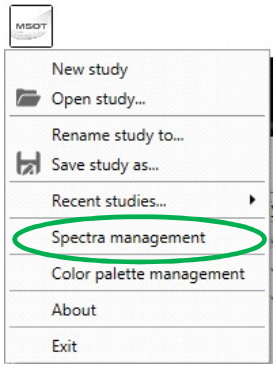


Difference subtracts an image acquired at one wavelength from an image acquired at another wavelength. Subtract a wavelength of low absorbance of the chromophore of interest from its peak wavelength in order to separate it from background. Use of this algorithm is limited to cases where the change in pixel intensity is only defined by the change in concentration of one absorber.

Click the double arrow icon to exchange minuend and subtrahend.

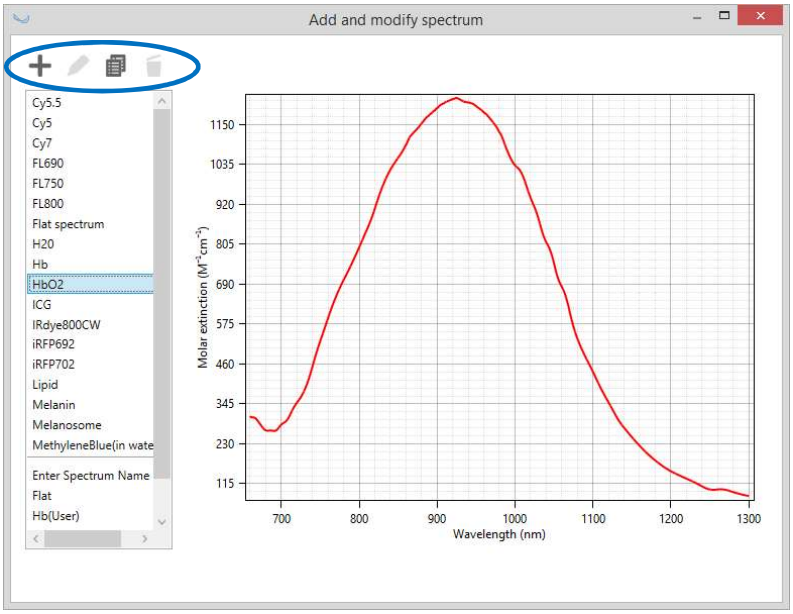
2.3 SPECTRA MANAGEMENT

Click the *Home* button and then select *Spectra management* to open the *Add and modify spectrum* dialog.



Select the spectra in the list on the left to preview the spectra. Factory spectra are listed in the upper part user defined spectra below the separator.

Add, Modify, duplicate or delete spectra by clicking on the respective button.



NOTE: Factory spectra cannot be modified or deleted by the user.

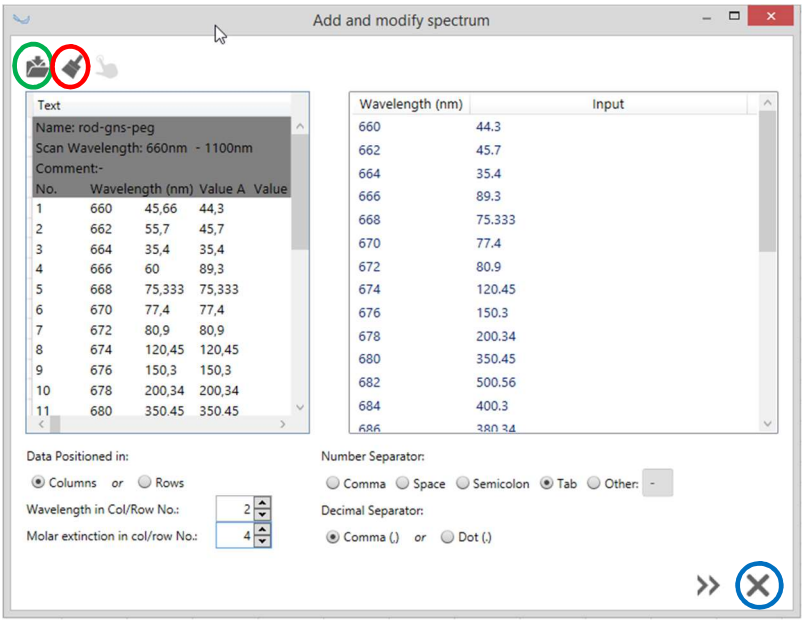
NOTE: If you delete a spectrum it will not only be deleted from the current Study but from the system the software is installed on. That way it will not be possible to use the deleted spectrum for further studies/ Study presets.

2.3.1 Add spectrum - Import values for wavelength and absorption

Open the spectrum import wizard by clicking ➕. There are two different ways to add a spectrum:

1) Click the Import Spectrum File icon to open a .txt or a .csv-file containing the spectrum data.

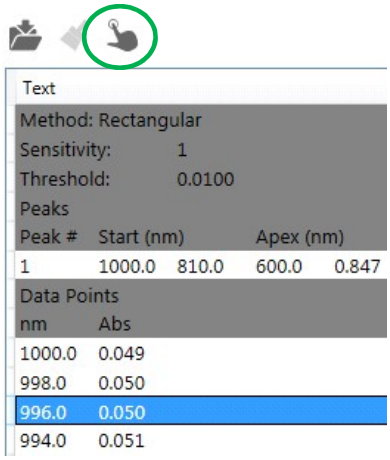
2) Import a spectrum by copy & paste: Copy the spectrum (wavelength and absorption) from Excel or from a text document and paste it into the import wizard by clicking 🧹. The spectrum can also be retrieved from the ROI graph (see 5.9.3.6 Copy ROI graph content to spectrum manager)



The software expects the wavelength to be the first column and the absorption data to be the second column. 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 click 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.

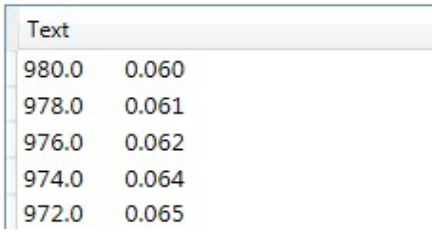


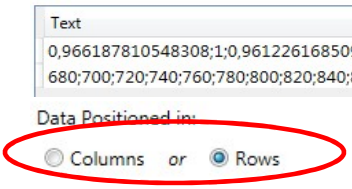
1) **Remove Information Header** from the input file:

   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 click the *Select/Deselect Comments* icon.

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

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

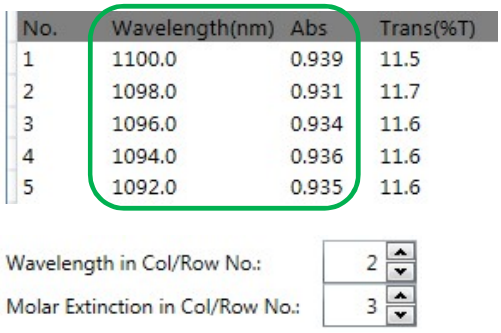




2) **Change the reading orientation** for the input data text:

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

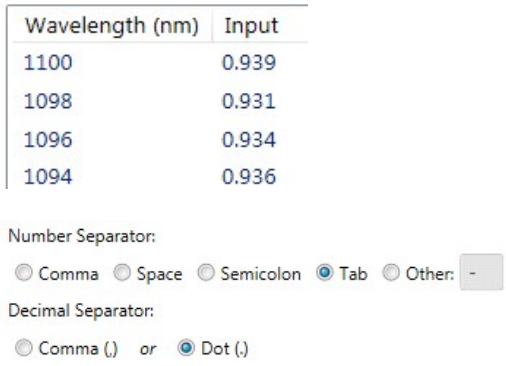
If the data is oriented horizontally – the wavelengths and the absorption values being arranged in rows – instead, please change the orientation under *Data Positioned in*.



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*.

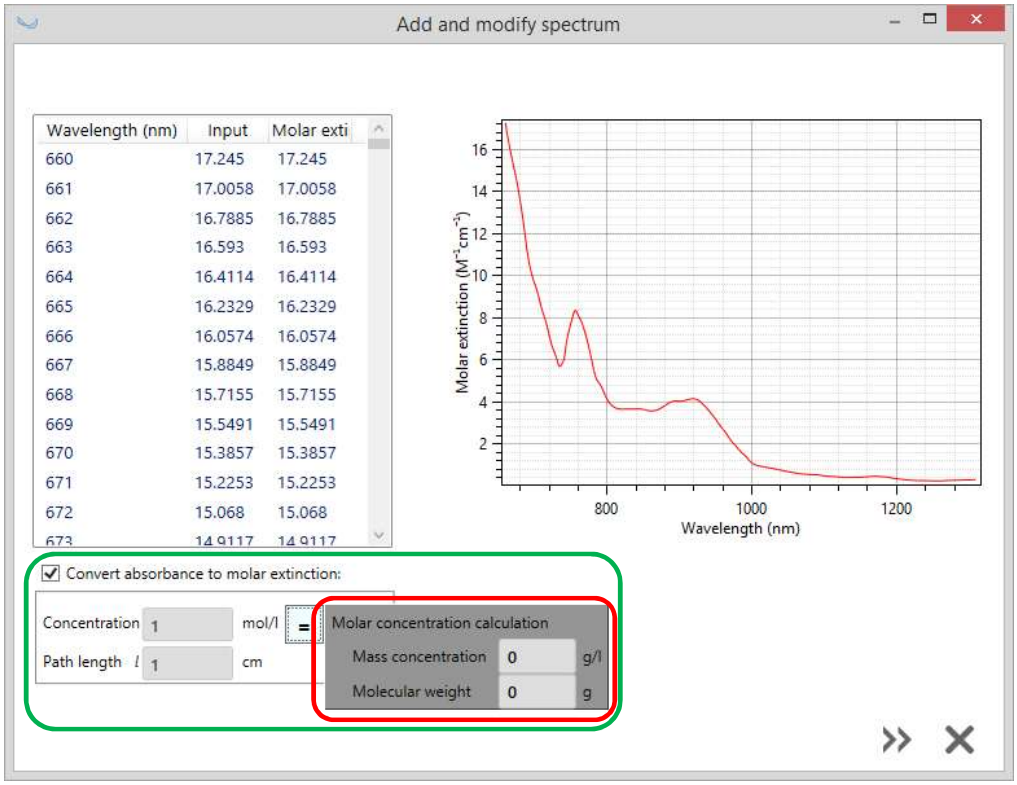
Click the arrow button ›› at the bottom right to continue to the next screen.

2.3.1.1 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* ε(λ) is calculated by the equation:

$$\varepsilon(\lambda) = \frac{A(\lambda)}{c \cdot \ell}$$

Where A(λ) is the wavelength dependent 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 p* and the *Molecular weight M* in the popup according to the equation

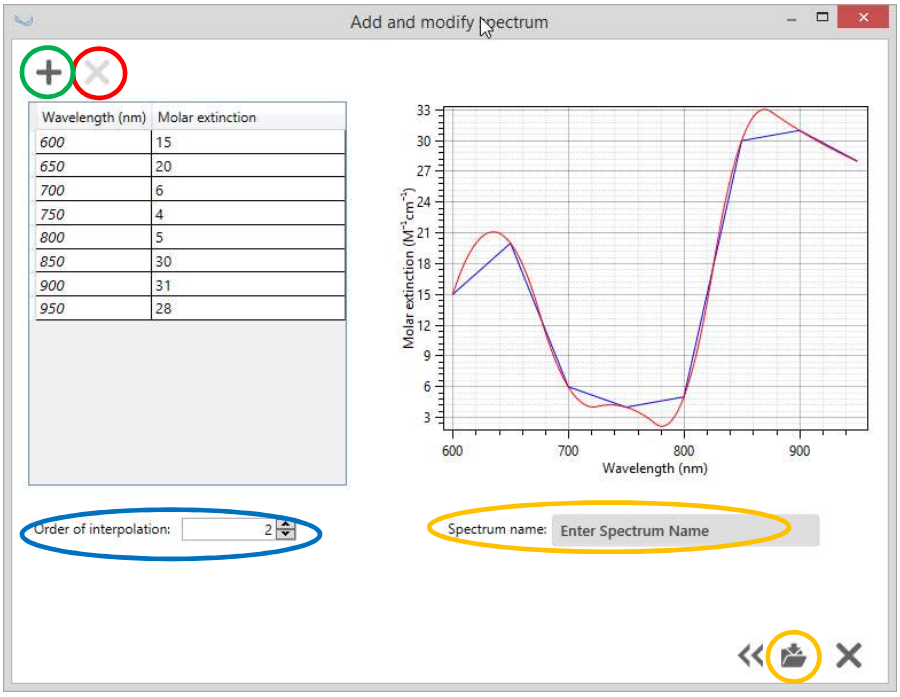
$$c = \frac{\rho}{M}.$$

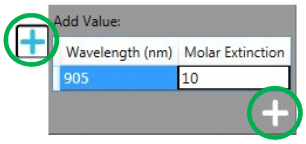
Click the arrow button ⟩⟩ at the bottom right to continue to the next screen.

2.3.1.2 Modify and import the spectrum

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 click “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.





Added values show up in the table ordered by wavelength.

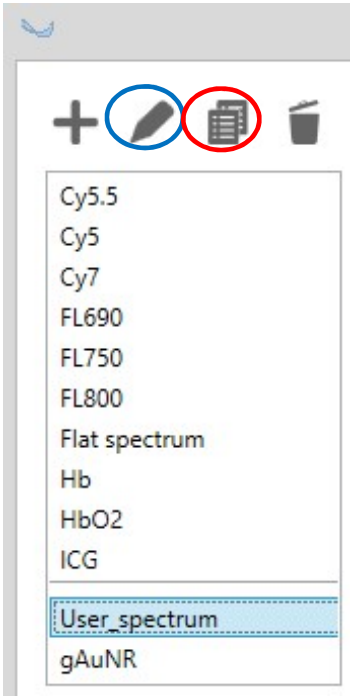
Both the values from the tab on the left (blue dots) and an interpolated curve (red line) are shown in the graph on the right.

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

Enter a name and click the import icon to save the final interpolated curve.

Click the "x" button in the lower right of the dialog to close the spectrum import wizard and return to the *Add and modify spectrum* dialog showing the spectra overview.

2.3.2 Modify or duplicate an existing spectrum

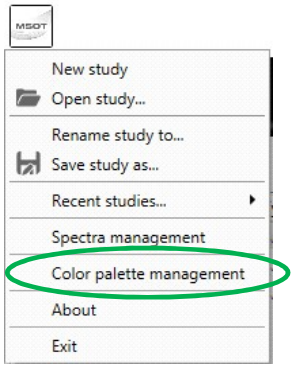


Select a spectrum and modify or duplicate it. Review, modify and import the spectrum curve using the spectrum import wizard explained above in detail (see 2.3.1.1 Review spectrum).

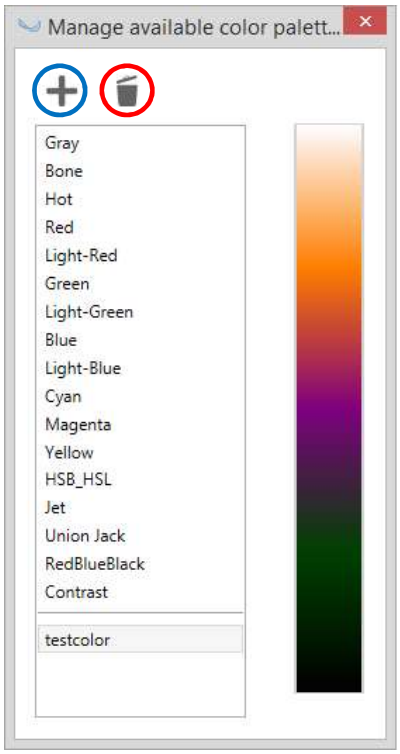
NOTE: Factory spectra cannot be modified. To be able to modify first duplicate it.

2.4 COLOR PALETTE IMPORT

Click the *Home* button and then select *Color palette management* to open the *Manage available color palettes* dialog.



Add a new color palette (.lut file from ImageJ). You can also select an existing user defined color palette and delete it.



NOTE: It is not possible to delete factory color palettes.

NOTE: The import works specifically for .lut files which can be generated in the ImageJ software.

NOTE: When deleting a color palette which is in use inside a Visualization from the *Manage available color palettes* dialog, it is still part of the particular data set.

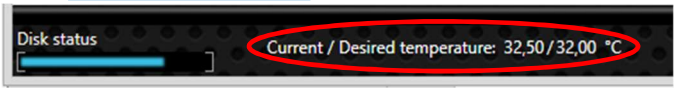
3 IMAGE ACQUISITION

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 clicking the *Power On/Off* icon in the task bar, if this was not 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.

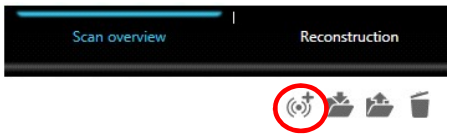


...

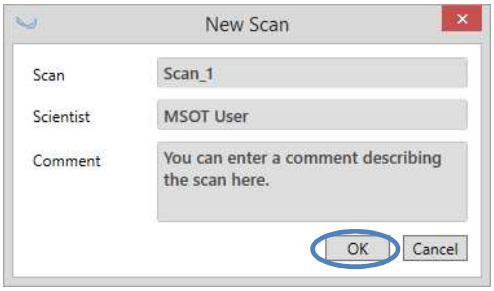
The *Current* and the *Desired Temperature* of the water in the imaging tank is shown in the task bar.

NOTE: Only insert the animal when the intended temperature (generally around 34°C) is reached. The desired temperature can be modified when setting up a Study preset (see 2.2.1 Experiment settings).

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



As soon as the system is ready the *Acquire new scan* button becomes active. Clicking 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.

Click *OK* to close the *New Scan* window and enter 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:

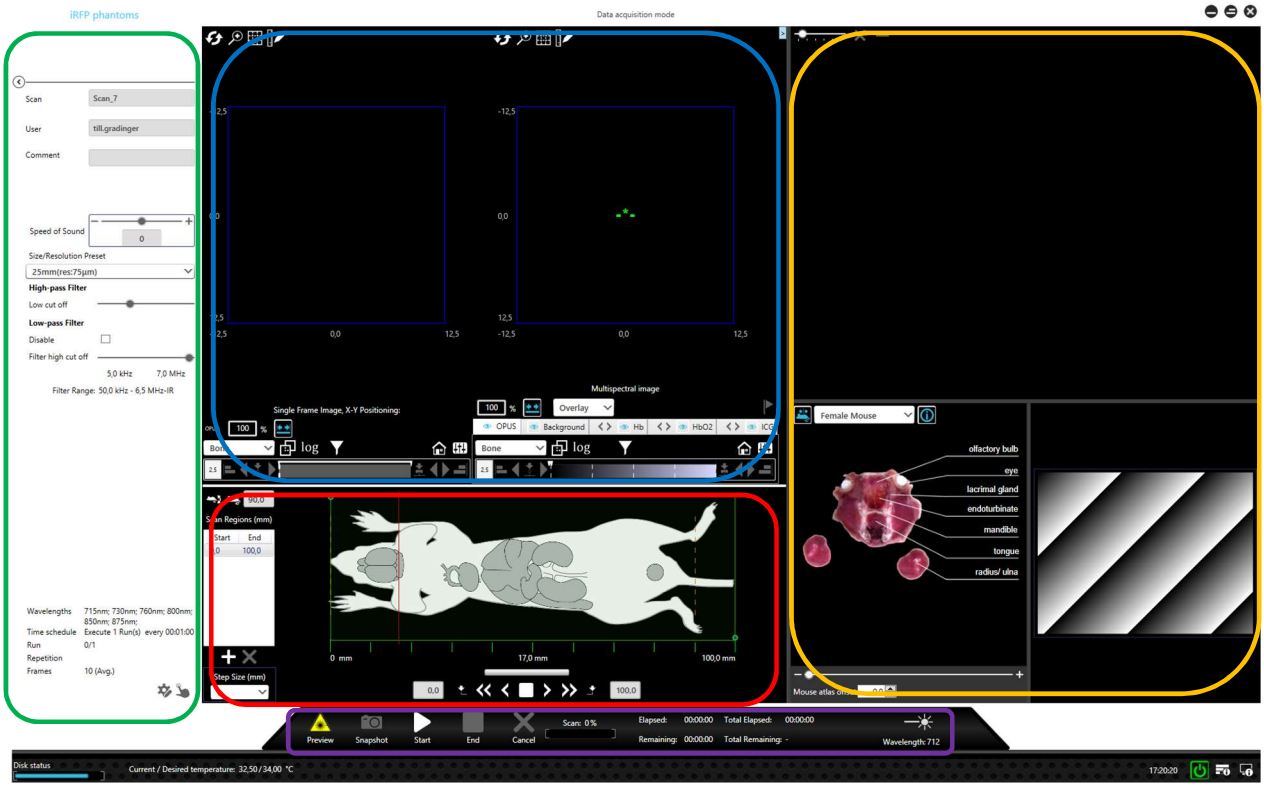
1) *Scan Information* extender: Shows all relevant scan information such as name, user, comment, speed of sound trimming, as well as the acquisition parameters.

2) *Live Image Preview and Image* Control: Monitoring of the live image – single wavelength, ultrasound 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 CryoMOUSE™ atlas, live CCD camera chamber view (if installed) and a Snapshot Window where snapshots and tagged images from the live MSP image preview can be kept.



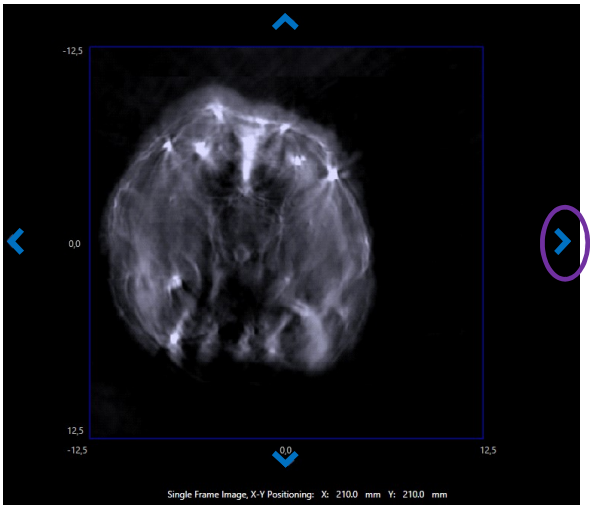
By clicking the *Preview* button in the *Control Panel* the system fires the laser and starts showing a live tomographic image. Clicking the button a second time turns off the preview.

3.2 EXPLORING AND SETTING UP SCAN REGION(S)

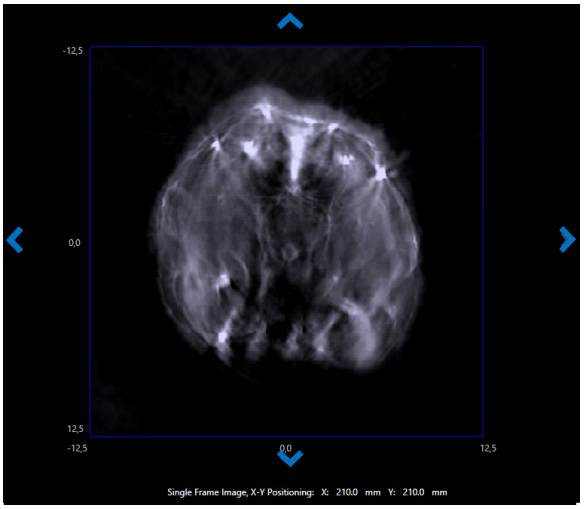
3.2.1 Positioning the imaging object in the imaging plane

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.

before



after



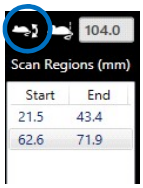
In the example the right arrow was clicked to move the mouse to the right.

3.2.2 Setting the orientation of the mouse

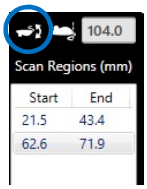
As can be seen in the schematic drawing in the Positioning Setup and Information panel the animal needs to be inserted into the device with the head facing to the left side. This is required to synchronize the tomographic image, the Positioning Information and Setup panel, the mouse atlas and later during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

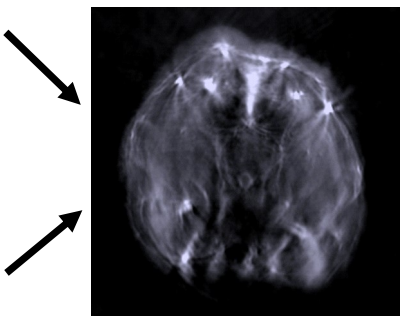
It is possible to insert the animal with the belly facing down (prone) or with the belly facing up (supine). Click the *Mouse Orientation* icon in the top left of the Positioning Information and Setup panel to change from belly down to belly up orientation:

Belly down positioning



Belly up positioning





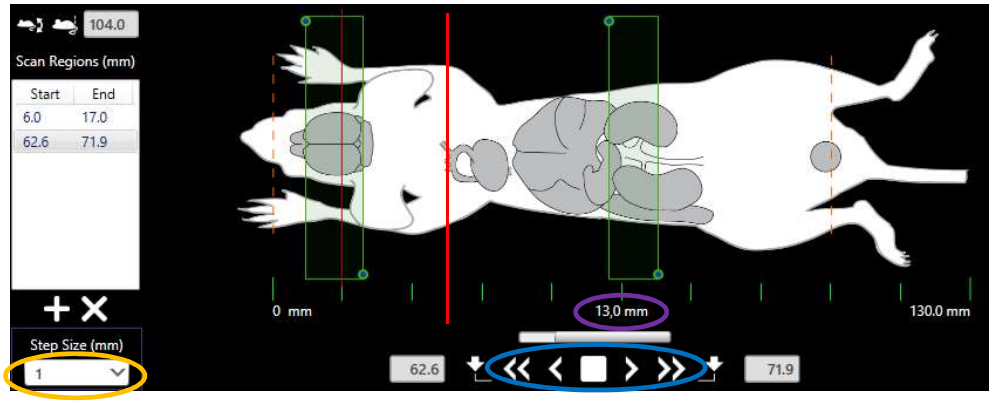
The tomographic single wavelength and the MSP live image are modified to have exactly the same orientation as the images in the CryoMOUSE™ atlas. No matter if the mouse is positioned on its belly or its back, the image in the preview has the same orientation.

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. Clicking those arrows more than 2 seconds makes the imaging plane move until stopping it.



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

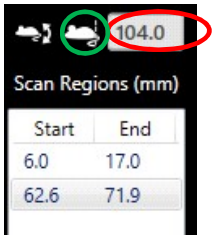
The position information of the imaging plane is displayed below the scale under the mouse.

If a continuous scan is acquired the user does not need to set up a *Scan Region.* By clicking 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 CryoMOUSE™ atlas and with the red line showing the current position in the Positioning Setup and Information panel.



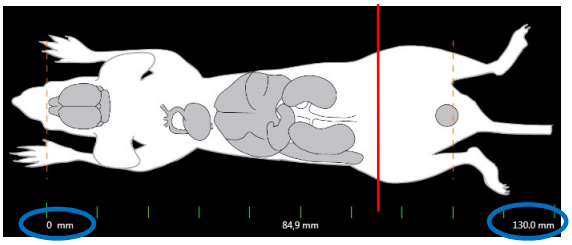
Move to the tail region of the mouse right where the tail and the legs still can be seen as one connected region.

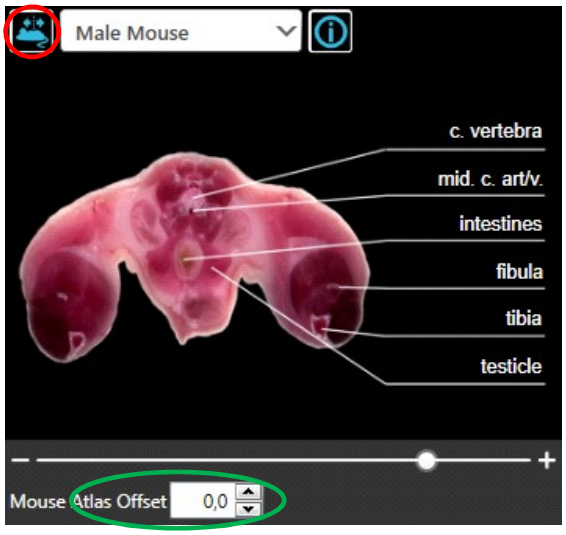
Click the *Mark Mouse Tail* icon underneath the mouse atlas.

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 manually for synchronization of Positioning Setup and Information panel, mouse atlas and image preview.

The Positioning Setup and Information panel is adjusted: The orange dashed line on the left represents the position of the tooth clamp in the mouse holder which corresponds to the first slice 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 mouse atlas is adjusted accordingly and jumps to the corresponding tail slice.

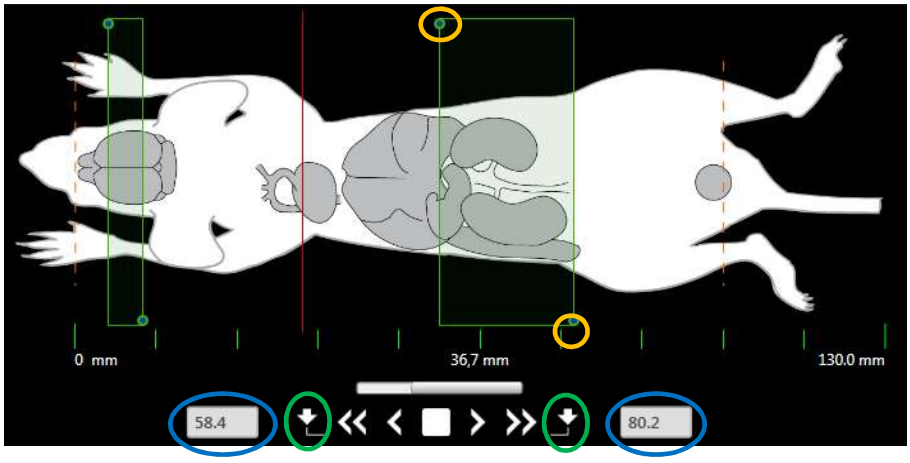
The synchronization of the mouse atlas can also be switched off for free browsing through the atlas by unchecking the *Couple Atlas Slice with Stage Position* icon.

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 CryoMOUSE™ atlas that is not related to the synchronization of the imaging object's position is explained in 3.6.1 CryoMOUSE™ 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.

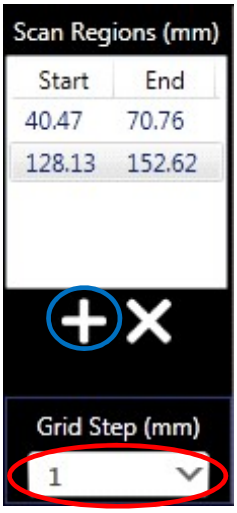


The start and end position for the scan can be defined in three different ways:

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 clicking '+' 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*.

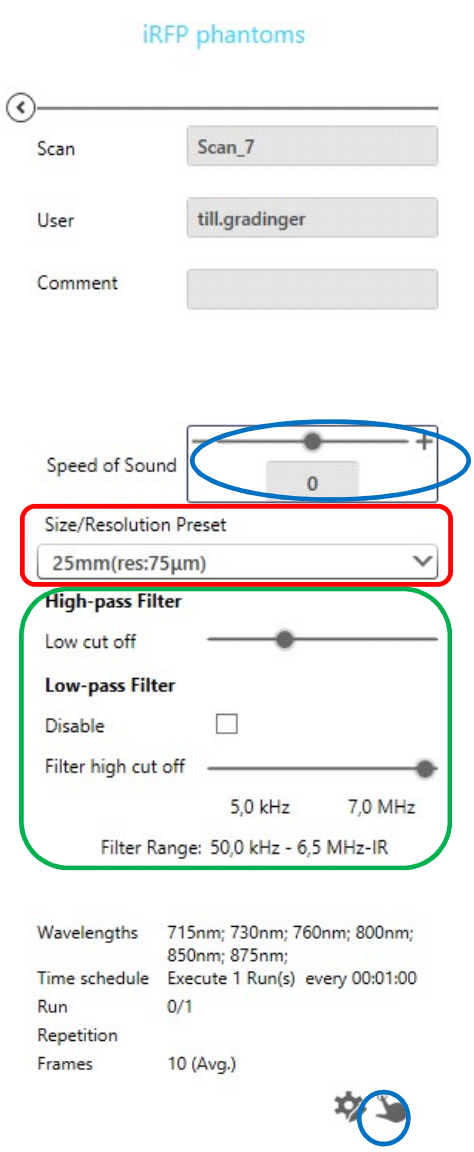
3.3 STARTING THE ACQUISITION

Click *Snapshot* to save the current MSP image. The MSP image will be displayed in the Snapshot window (see 3.6.4 Snapshot window) as well as saved as scan data.

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 clicking *Cancel*. If the user is running a measurement including multiple Runs clicking *End* will finish the scan after the current Run is complete, i.e. after the last position of the current run is acquired.

3.4 SCAN INFORMATION EXTENDER/EDIT SCAN PRESET



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.

Modify the Size/Resolution Preset to adjust the field of view according to the size of the imaging object.

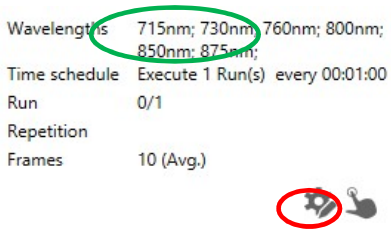
Change the filter Cut Off frequencies to modify the bandpass filter.

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

Open the list of Study presets which were added to the current Study.

Select a Study preset from the list and click *Select*.

NOTE: Open the *Select and Modify System Presets* dialog (see 2.1 SELECTING A STUDY PRESET) from the Scan overview screen to add other Study presets to the list.



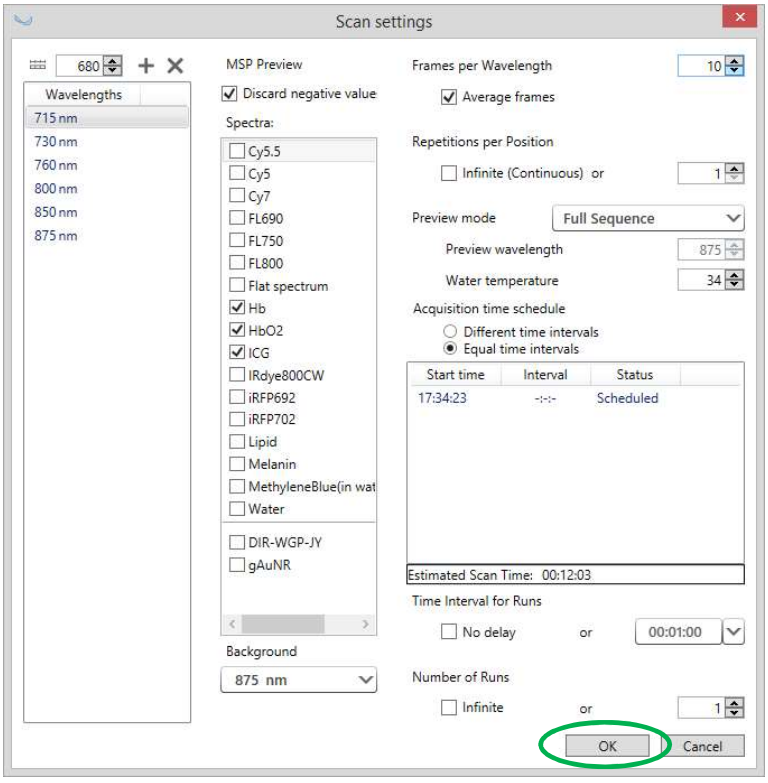
Click the *Scan Settings* icon to open a dialog containing all acquisition-relevant presets which were defined at the Experiment Settings of the Study presets (see 2.2.1 Experiment settings)

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

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

NOTE: By changing the Scan settings the initial Study preset is not modified.

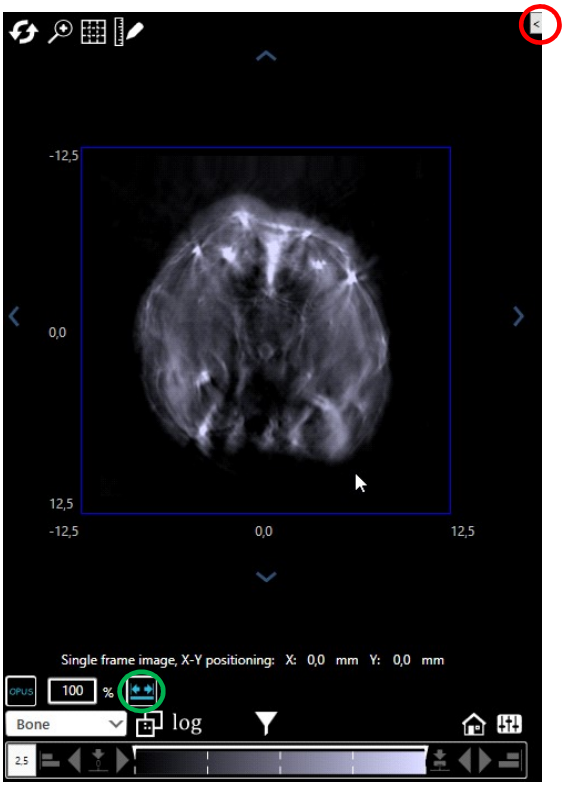
NOTE: The description of the parameters of this menu are explained in section 2.2.1 Experiment settings.



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.

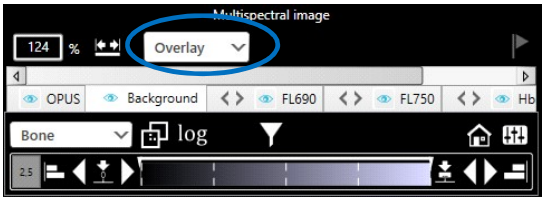


- Color bar of the displayed image
- Auto-Scaling of the color bar
- Changing the color map of the image
- Arrow buttons to adjust the color bar in the current image
- Changing alpha (transparency) value for the color map
- Etc.

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

By default the range of the color map of each layer is adjusted from “0” to the maximum value of each layer’s maximum and minimum pixel value.

Unchecking *Auto-Scale* will apply the maximum of each layer of the current image to all other images, i.e. all images will have the same scaling for the same component making them visually comparable. See 5.6.5.1 Thresholding of the color bar for more details.

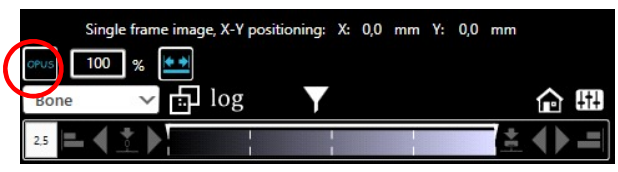


Toggle between the two imaging modes Overlay and Color Mixing. They are explained in section 5.6.1 Overlaying and enabling TLP-layer and MSP-layers in detail.

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

3.5.1 Ultrasound images - OPUS

MSOT inVision 512-echo systems include ultrasound acquisition. Toggle between the single wavelength preview and the ultrasound (OPUS) preview.



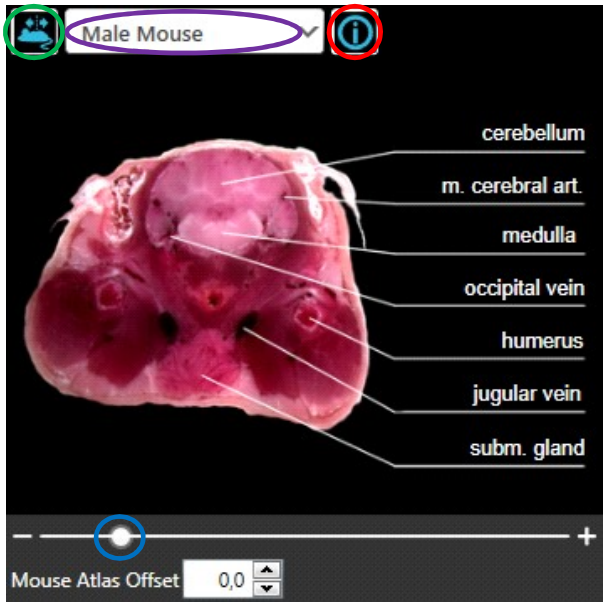
The multispectral image preview shows the ultrasound image as an additional layer below the MSOT background layer. In sections 5.6.1 Overlaying and enabling TLP-layer and MSP-layers and 5.6.5 Individual layer controls modifying the image layers is explained in detail.

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 *Phantom* (see 3.6.2 Phantom atlas) and *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.

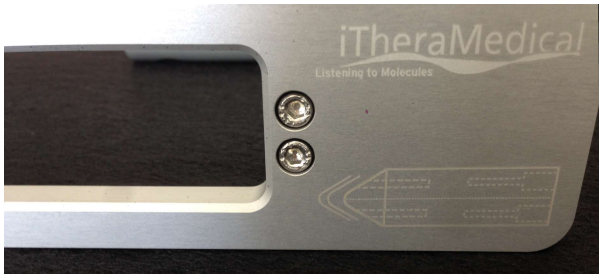
3.2.4 Synchronize the current imaging plane with the mouse atlas explains in detail how the mouse atlas can be synchronized with the current tomographic slice of the live image preview.

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 .

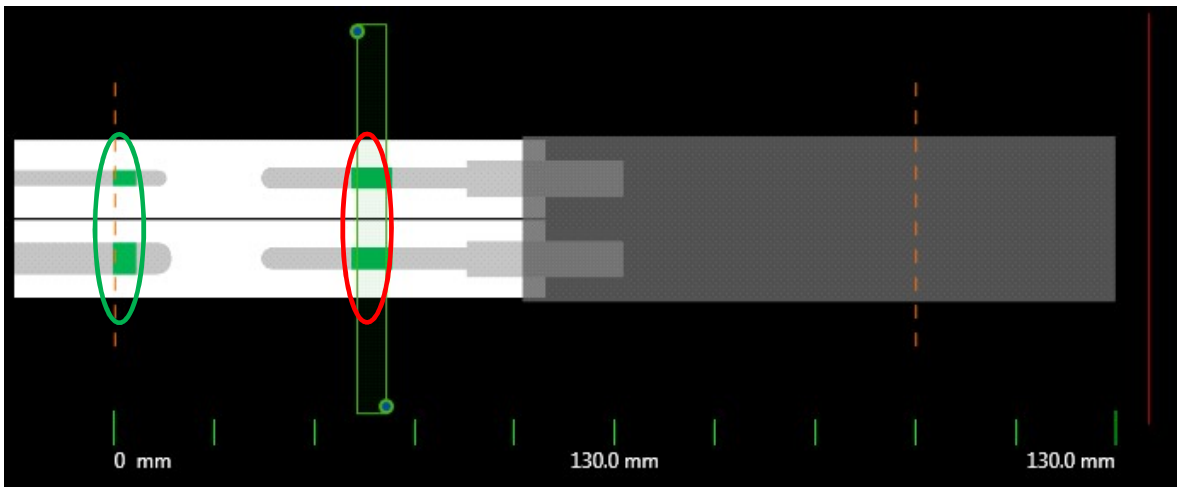
3.6.2 Phantom atlas

When a durable measurement phantom (iThera Medical) is placed into the phantom holder (left image), the flat end of the phantom containing the samples of interest should be placed towards the retractable brace. The holder should then be placed into the imaging chamber so that the 'iThera Medical' label is on the right (right image).



In this configuration, accessing the Phantom Atlas allows synchronization between phantom position and the interactive stage control.

The interactive stage control can be used to image different parts of the phantom. The similarly-sized wells allow simultaneous imaging of two different samples, while two reference inclusions which contain 0.5 OD ink of two different reference sizes (see image below) can provide a standard sample to image over time.



For home-made agar phantoms, 'Other' can be chosen in the dropdown menu above to remove the display of the phantom or male/female mouse, and the interactive stage control can still be used.

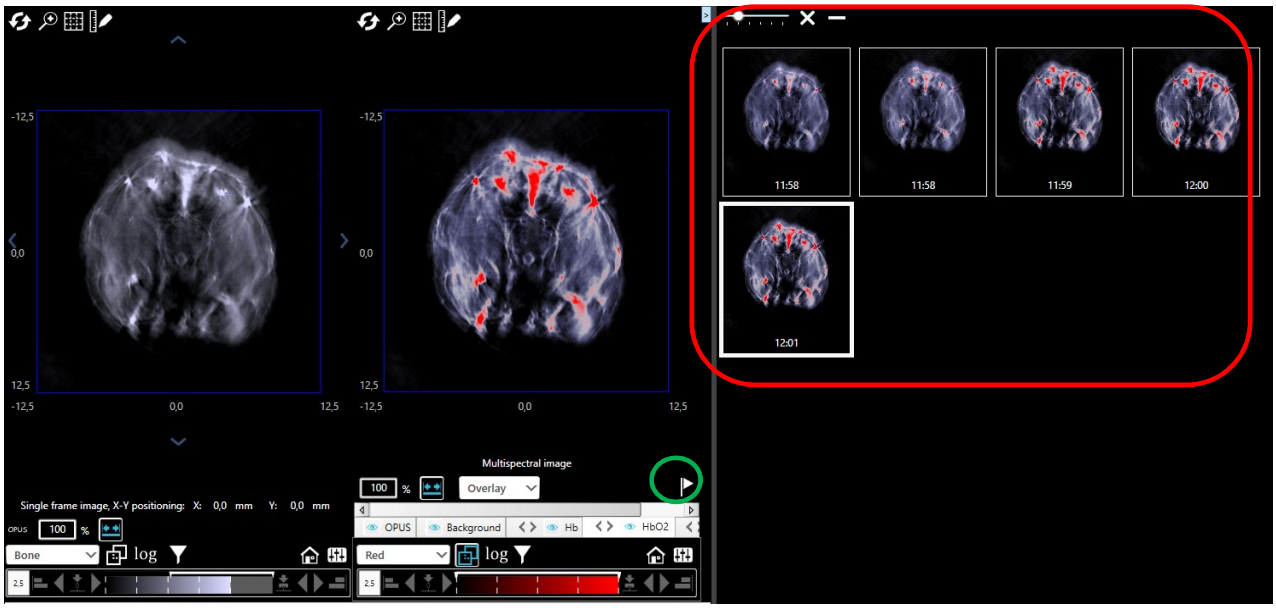
3.6.3 Camera preview

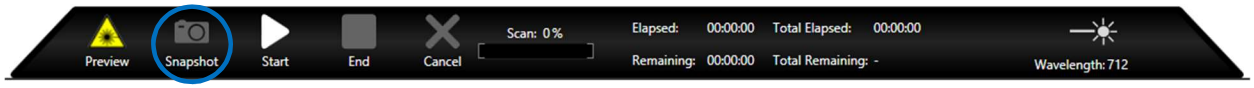
A live camera view displaying the inside of the imaging chamber is displayed in the bottom right part of the acquisition screen Dashboard and used for monitoring the animal during acquisition. The live camera is an optional hardware component and not installed on all systems.

3.6.4 Snapshot window

A snapshot image of the current MSP image can be added to the Snapshot window of the acquisition screen by either

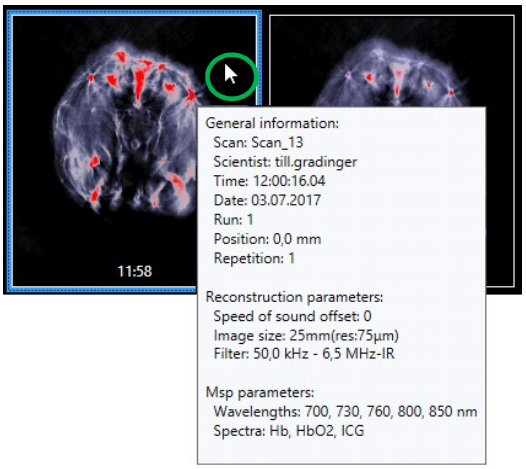
1. Clicking *Snapshot* during image preview or by
2. Clicking the Tag icon during an ongoing acquisition.





NOTE: Clicking *Snapshot* during preview saves a separate data set of the current MSP frame, while clicking the tag icon during an ongoing acquisition, tags the current MSP frame inside the acquired scan. In the Visualization the tagged frames will be highlighted by a red checkmark. See chapter … on tagging and changing tags in the Visualization.

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

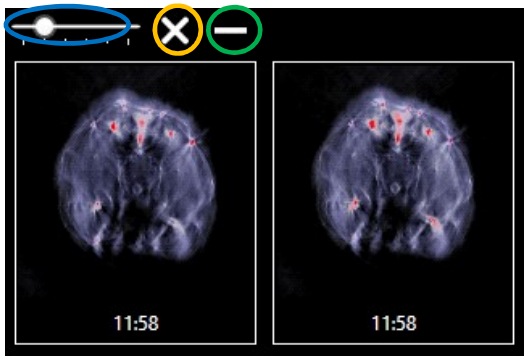


Move the mouse pointer over a snapshot to get further information on the image:

- *General information* (Scan name, Scientist name, date & time, frame information)
- *Reconstruction parameters* (Speed of sound, Image size, filter)
- *MSP parameters* (Wavelength, spectra)

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.



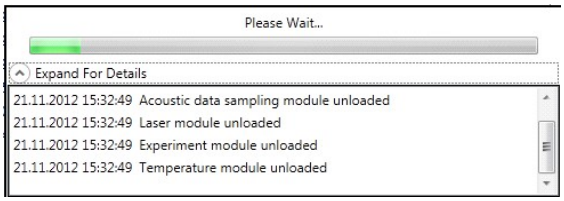
Select a snapshot and click *Remove selected thumbnail* to remove one snapshot from the dashboard.

3.7 EXITING ACQUISITION MODE & TURNING HARDWARE OFF

After finishing 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 (clicking in the Optoacoustic Measurement tab of the main window) another scan can be acquired without reloading the hardware components.

Click the *Power On/Off* icon in the status bar of the main screen to unload the system hardware after finishing the acquisition of scans, if further post processing needs to be performed on the system. Click "x" in the top right of the main screen to shut down the software including unloading the system hardware.



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

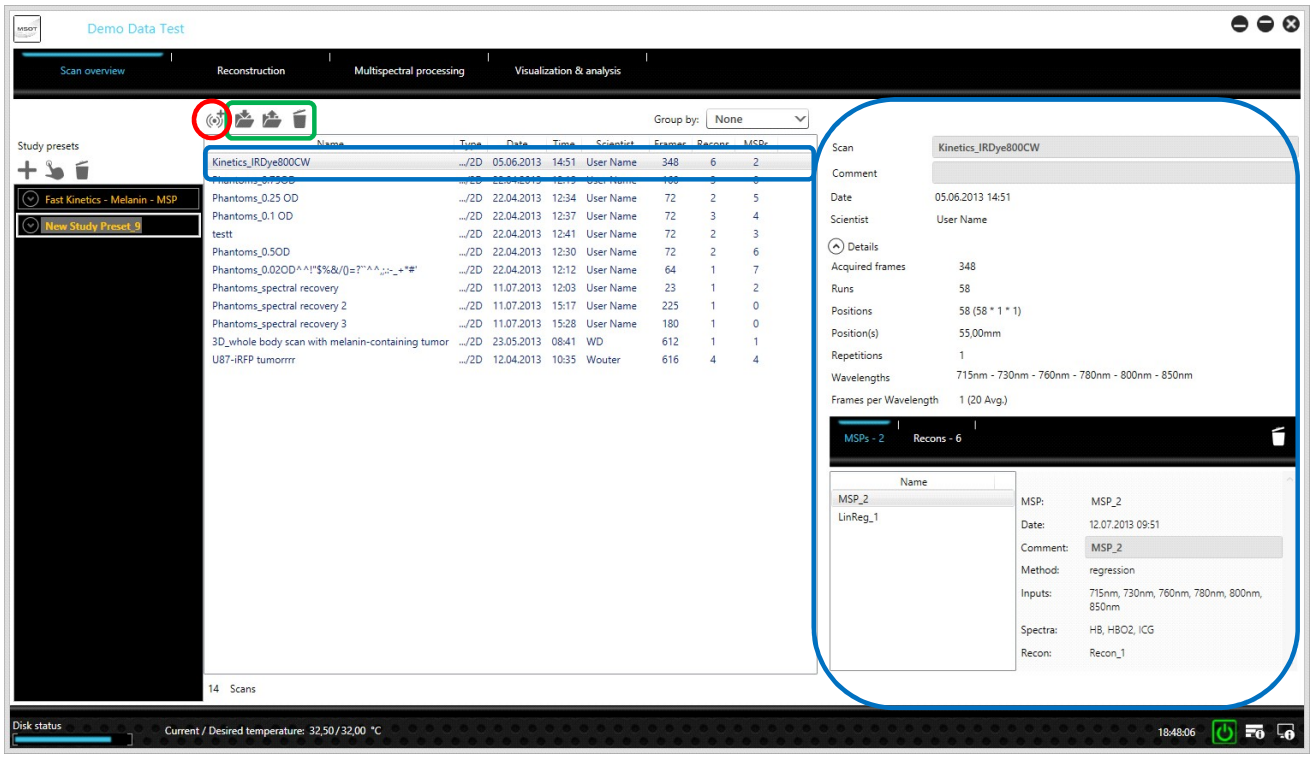
NOTE: During unloading the water is not drained to save time in case the user wants to re-enter acquisition mode shortly. Only when exiting the application the water is drained from the chamber. It is possible to drain out or pump in the water from the Maintenance (see section 6.1.16.1 MAINTENANCE) window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

acquired and review information about these items. 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).

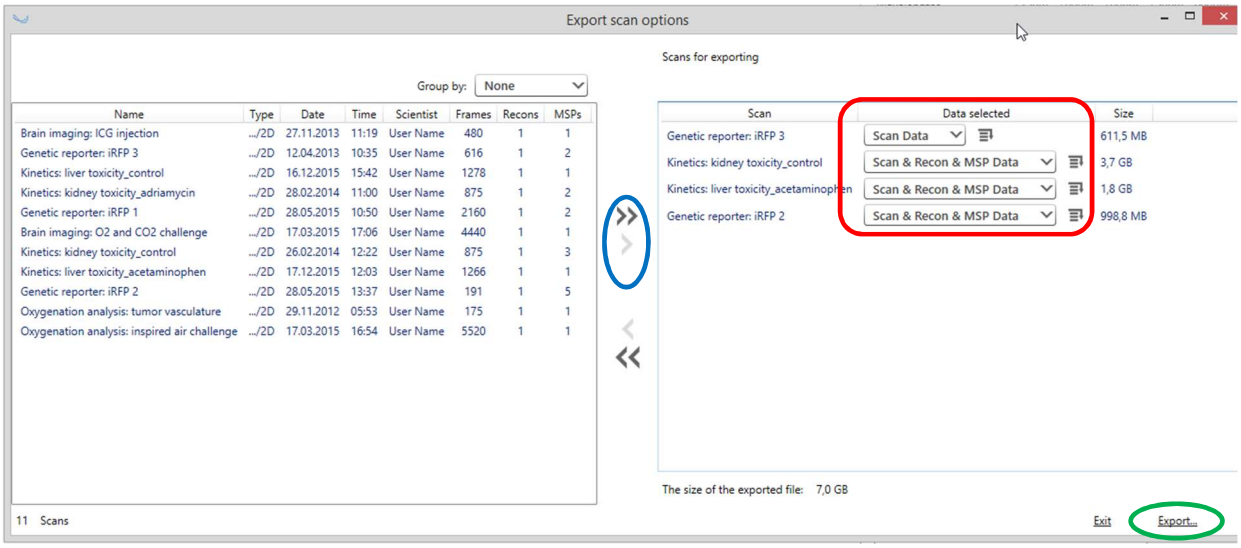
3.8.1 Exporting, importing and deleting scans

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.

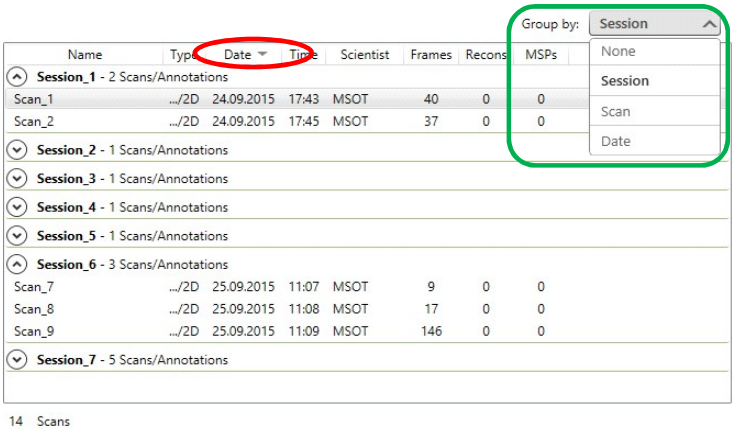
The *Import* icon can be used to import a .zip-file containing one or more scans that was exported with the functionality described above into the current study.

NOTE: 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 accessible to multiple systems, only one system at a time is allowed to access a Study. For other users opening a Study in use is prevented.

3.8.2 Rearranging and grouping scans

Select one of the titles in the table, to sort the table accordingly (e.g. in the example below the data is sorted based on the acquisition date). Click the title a second time to invert the order.



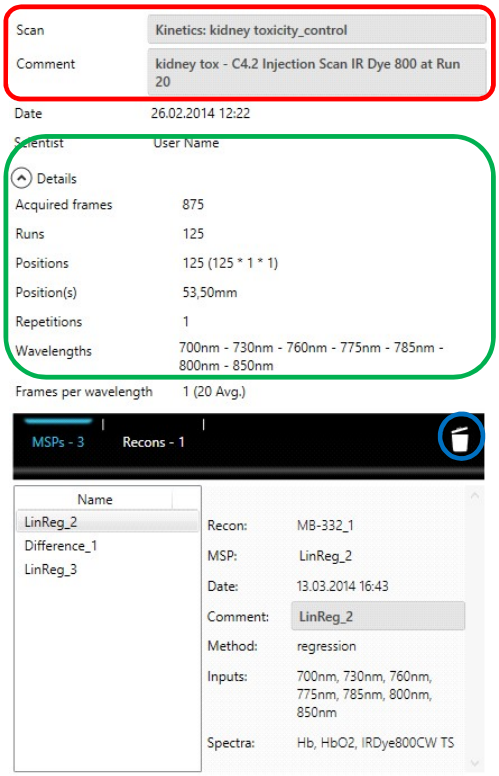
Group the scans of a study by:

1) *Date*: scans are grouped 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.

3) *Session*: Each time the user enters the OAM screen from the OAM tab to collect new data a Session is started. All scans that are acquired until the OAM screen is left are arranged under one Session

3.8.3 Reviewing scans



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 displays 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.

NOTE: There is no possibility to restore Reconstructions and MSPs after they are deleted.

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

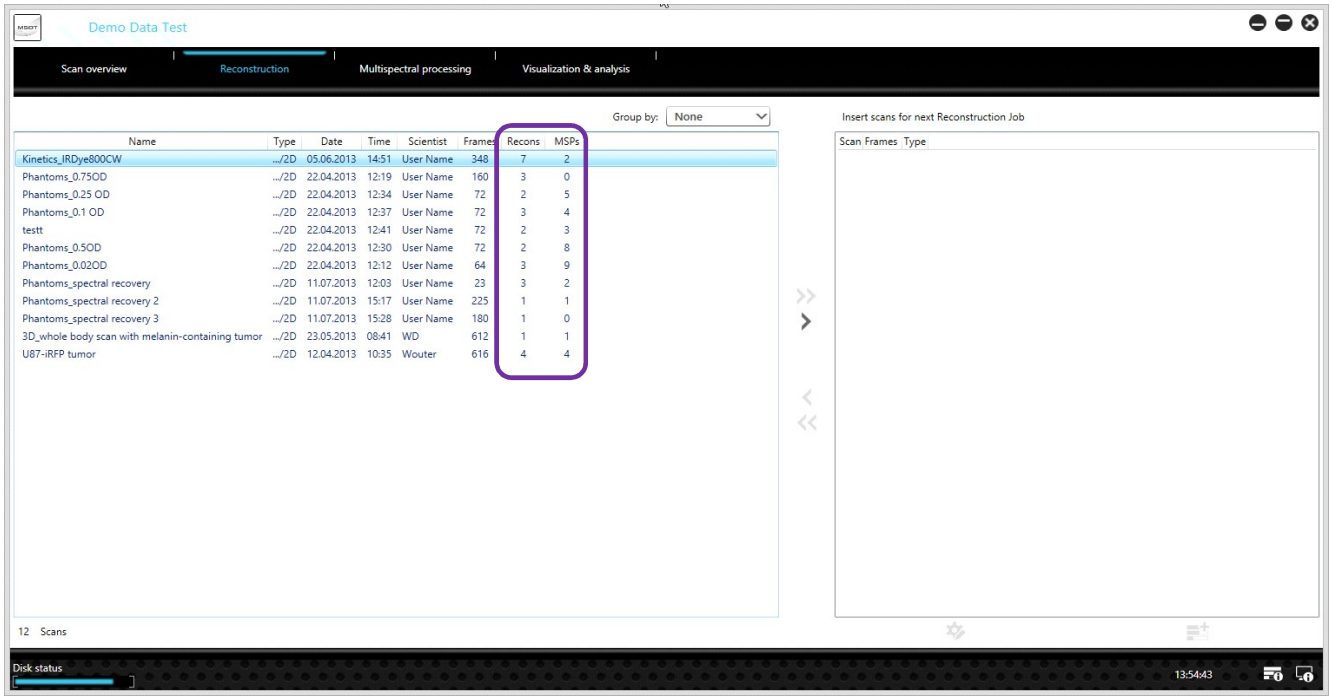
4 IMAGE PROCESSING

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 defined wavelengths, positions and time points. In a second step multispectral processing is performed. The images acquired at different wavelengths at the same 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.

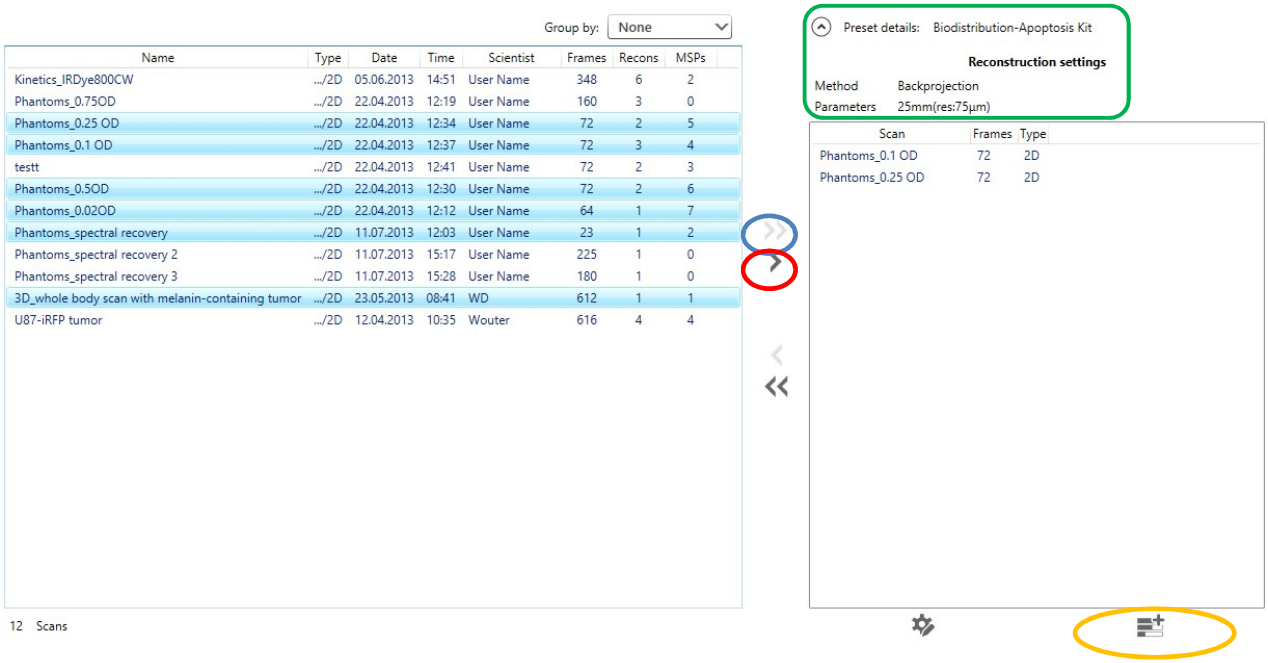
4.1 SETTING UP A RECONSTRUCTION JOB

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



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

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



The Reconstruction settings can be checked by clicking on the respective extender.

Click 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.

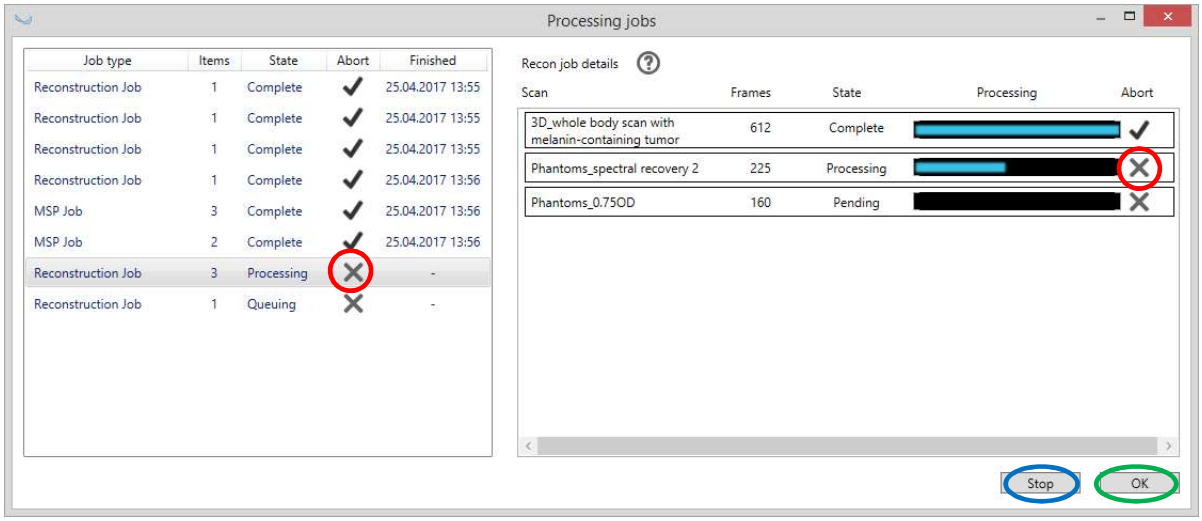
By clicking the *Add to Processing* icon the current *Reconstruction Job* is added to the processing queue. The status of running *Reconstruction* and *MSP Jobs* is shown by the *Processing Status* icon in the system information bar.

Icon is white: No *Reconstruction/MSP Job* currently active. Either no reconstruction/MSP was started or all Reconstruction and MSP Jobs have been completed.

Icon is green: Reconstruction/MSP currently ongoing.

Icon is yellow: *Reconstruction/MSP Jobs* were interrupted/stopped by the user.

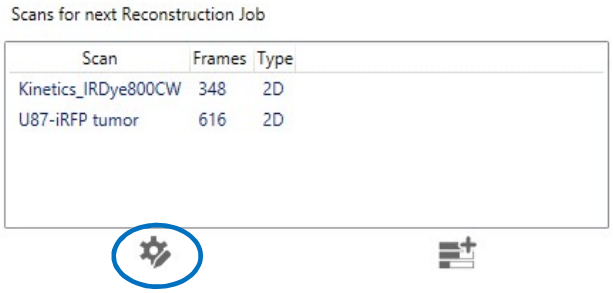
Click the *Processing Status* icon to open the *Processing Jobs* window that shows all *Reconstruction* and *MSP Jobs* including their processing status (*Complete*, *Processing*, *Pending* or *Canceled*) and the time/date when they were finished.



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



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, click the autofocus icon to automatically find the optimal *Trim Speed* or modify it manually. The effects are visible in the Image Preview window. Click Apply to all, if all scans of the reconstruction job list shall be reconstructed using the same Trim Speed.

NOTE: The autofocus functionality does not work on all data sets. Depending on what is imaged the user may need to manually change the speed of sound to a meaningful value.

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 image preview. Additionally, the effects of filtering modifications and speed of sound changes can be previewed.

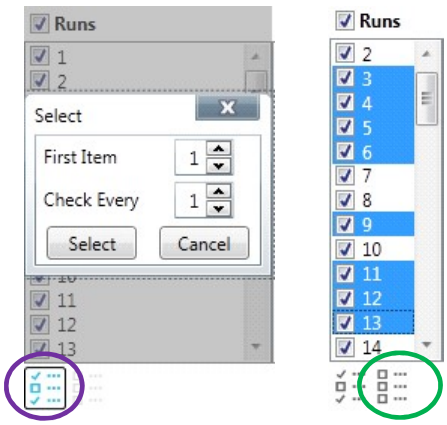
3) *Reconstruction Settings*: The default settings for the reconstruction are defined in the Study presets, but can be changed here. The Reconstruction Settings are explained in section 2.2.2 Processing settings).

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.



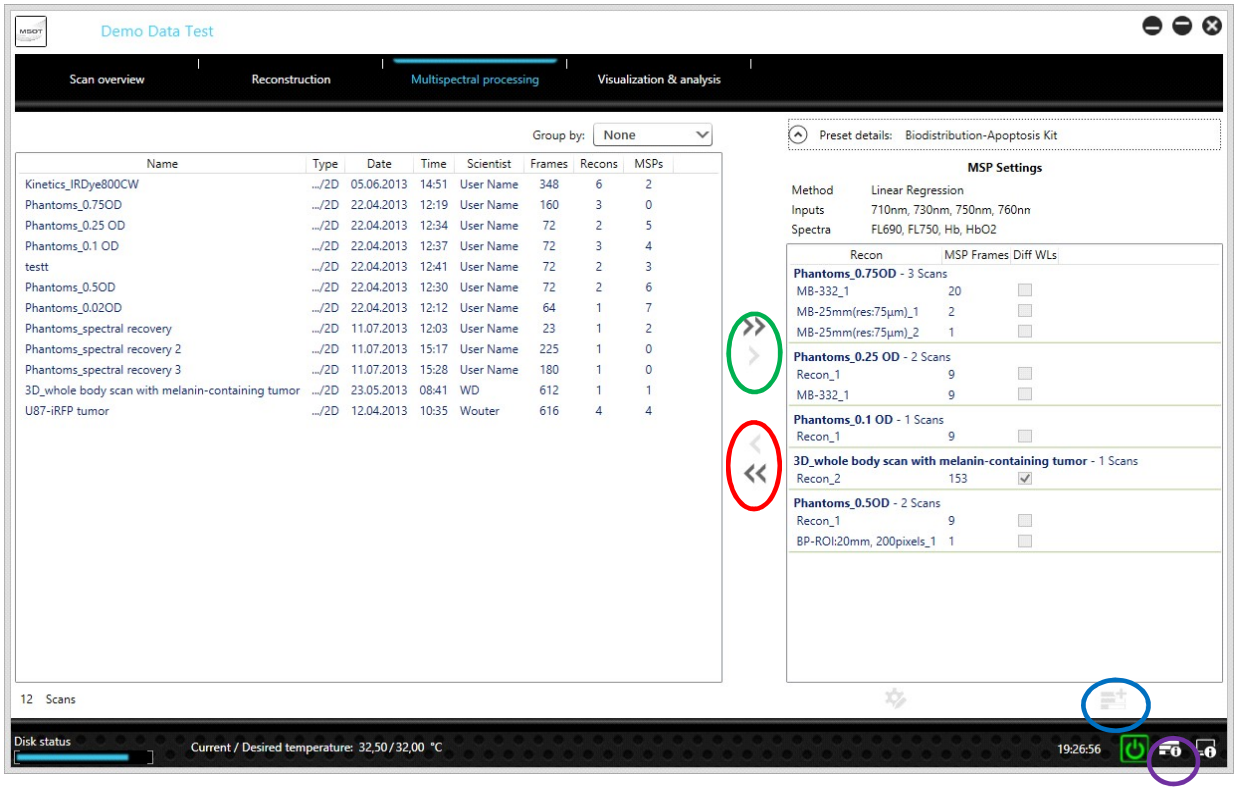
NOTE: Ultrasound frames cannot be reconstructed with different reconstruction parameters, because only the reconstructed frames are saved.

4.3 SETTING UP A MULTISPECTRAL PROCESSING BATCH

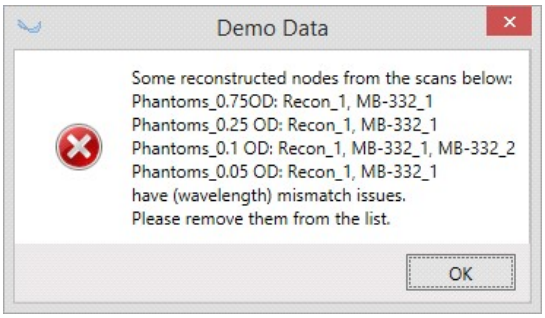
To multispectrally process the reconstructed image data switch to the **Multispectral Processing** tab. The functionality of the tab is very similar to the Reconstruction tab described in 4.14.1 SETTING UP A RECONSTRUCTION.

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.

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: Different reconstructions of one scan having different reconstruction parameters need to be set up sequentially.

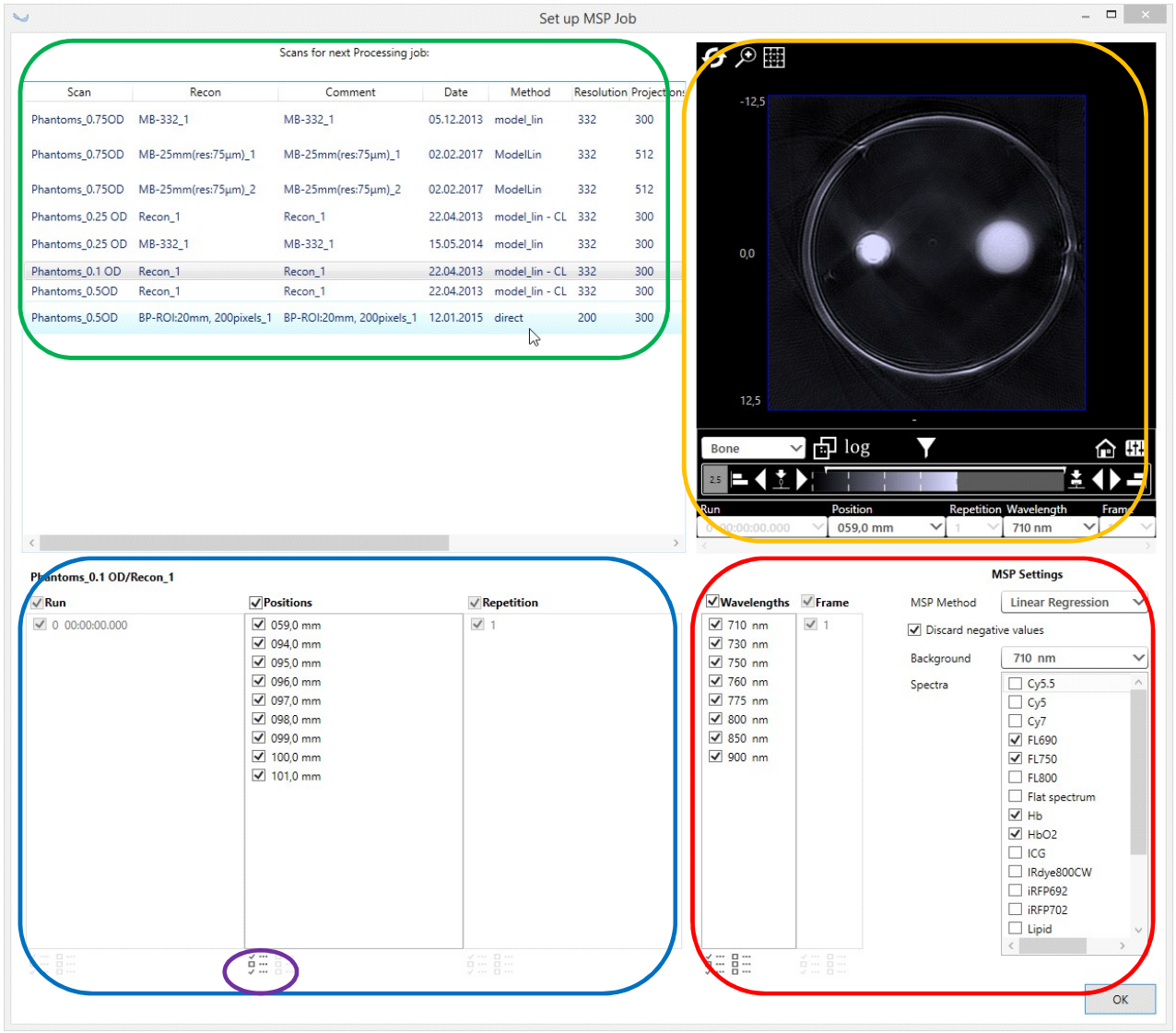
Click the *Advanced* icon to open the *Set up MSP job* dialog where the MSP Settings can be modified.

4.4 ADVANCED MSP

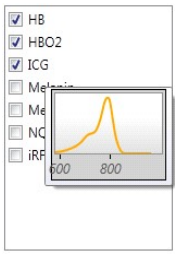
By clicking the Advanced icon the MSP parameters of the MSP Job list can be changed. The advanced MSP popup window is very similar to the Setup Reconstruction Job window explained in 4.2 ADVANCED RECONSTRUCTION and has the following four areas:

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*, *Positions* and *Repetitions* to be multispectrally processed. With the help of the pattern selection a subset of images can be selected automatically, e.g. every 5th slice.

NOTE: The wavelength selection is part of the MSP Settings to avoid processing a set of reconstructions each with varying wavelengths leading to non-comparable results.

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.

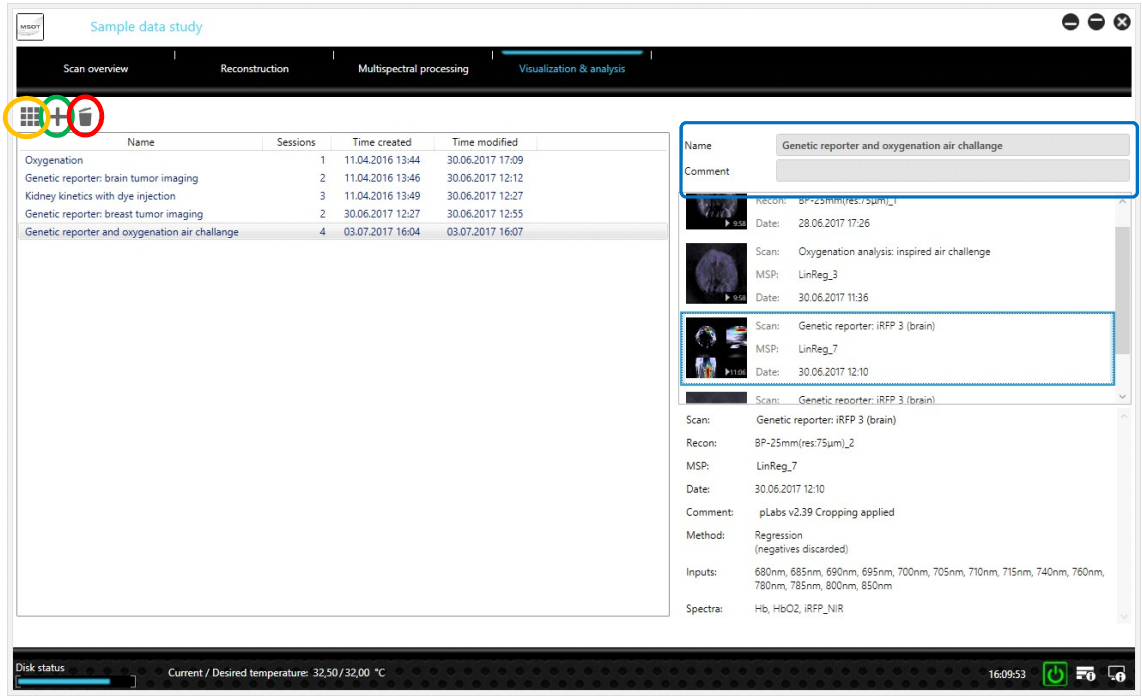
Open one or multiple reconstructions or MSPs, inside a View, select images to be displayed and determine how they should be arranged on the screen. Image manipulation tools like thresholds, different color maps etc. can be applied to all visible images. It is also possible to draw regions of interest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

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

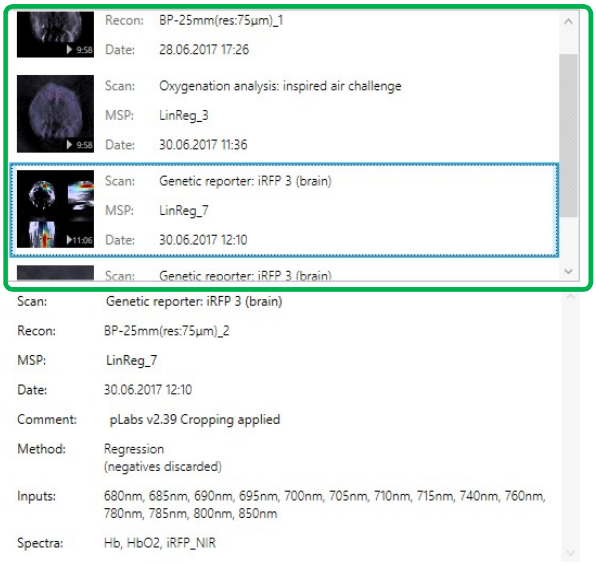
5.1 VISUALIZATION AND ANALYSIS TAB

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 data sets displayed inside the View.

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

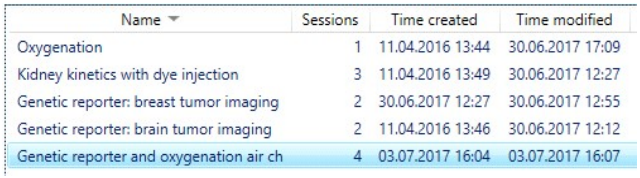


The name of a View can be changed by selecting it and editing the name in the text field on the right of the screen. Add a comment if necessary.



Below the content of the currently selected View is shown. Each data set – reconstruction or MSP – is represented by a thumbnail.

Select one of the reconstructions or MSPs to check the dataset's details.

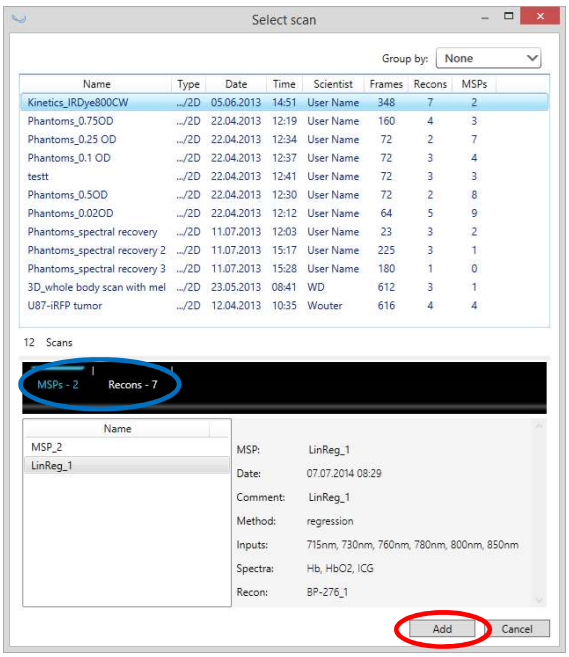


Name	Sessions	Time created	Time modified
Oxygenation	1	11.04.2016 13:44	30.06.2017 17:09
Kidney kinetics with dye injection	3	11.04.2016 13:49	30.06.2017 12:27
Genetic reporter: breast tumor imaging	2	30.06.2017 12:27	30.06.2017 12:55
Genetic reporter: brain tumor imaging	2	11.04.2016 13:46	30.06.2017 12:12
Genetic reporter and oxygenation air ch	4	03.07.2017 16:04	03.07.2017 16:07

Click one of the column titles to sort the list accordingly.

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 clicking the ➕-icon.

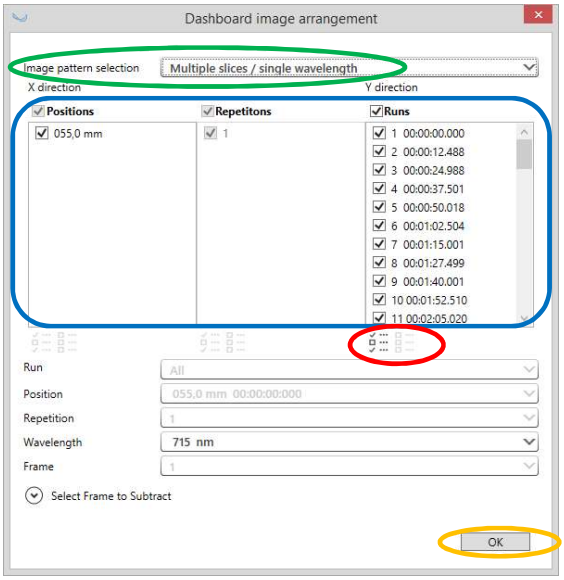
In the *Select Scan* window choose a scan from the list showing all scans belonging to the current study.

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.

Click *Add* to proceed to the *Dashboard Image Arrangement* dialog.

5.2.1 Selecting recon images to be shown in visualization



Decide – depending on the type of scan – which image frames should be displayed. For reconstructions there are the following options:

1) *Multiple slices / single wavelength*: showing images of the same wavelengths at different (anatomical) positions (and for different run and repetition times)

2) *Multiple slices / multiple wavelengths*: show images at different positions and different wavelengths for different run and repetition times

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 positioning of the images in the Visualization, i.e. different wavelengths could be shown in horizontal direction and positions vertically.

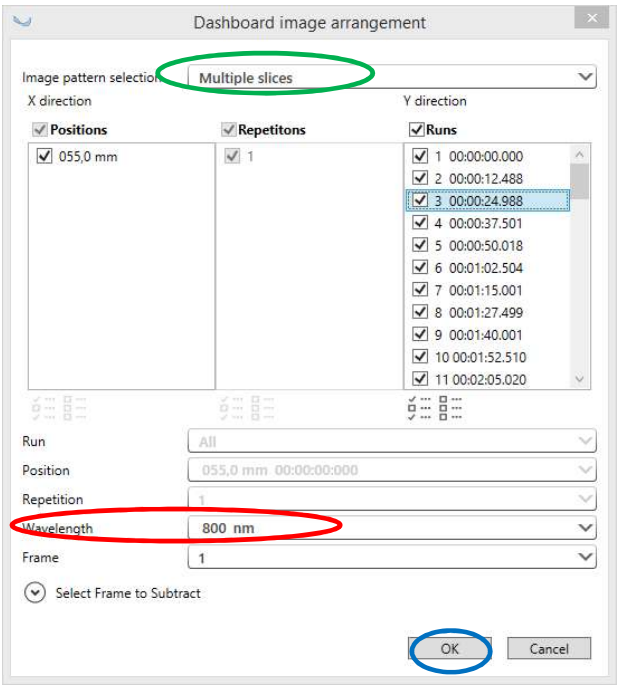
By marking the checkboxes choose a selection for the View. The *Pattern Selection* icon button opens a dialog box facilitating the selection. Uncheck a selection by clicking the “x”-icon.

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.

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 clicking *OK* the Visualization screen opens and the images of the chosen data set are added to the View.

5.2.2 Selecting MSP images to be shown in visualization



Select the MSP images to be shown for different (anatomical) positions, repetitions and for different runs. 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 TLP-layer and MSP-layers.

By clicking *OK* the Visualization screen opens and the images of the chosen data set are added to the View.

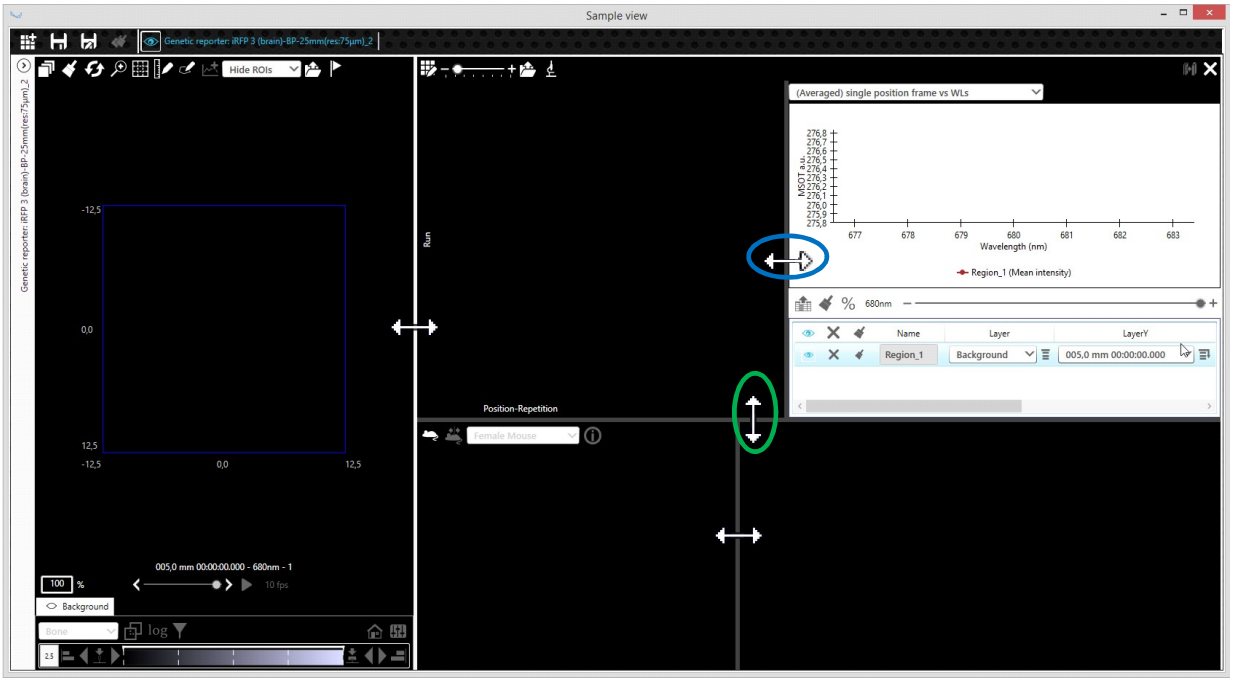
NOTE: Depending on whether a reconstruction or an MSP was chosen for the *View*, the *Dashboard Image Arrangement* window is adjusted according to which images can be selected and how these are arranged in the *Detail Images* window.

5.3 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

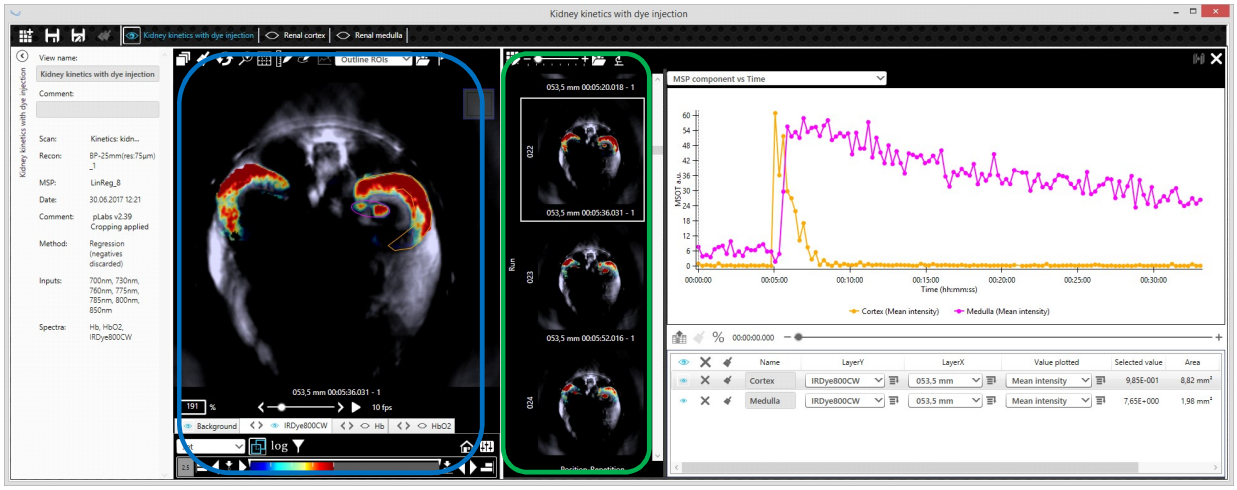
The windows of the Visualization screen can be dragged with the mouse pointer to adjust their size. Double click the line separating the Image Window and the ROI graph to shrink the ROI graph to the left. The ROI graph can be extended by again double clicking the line, or dragging the line from the right back into the middle of the Dashboard.



The lower part of the Dashboard containing the CryoMOUSE™ 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.

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

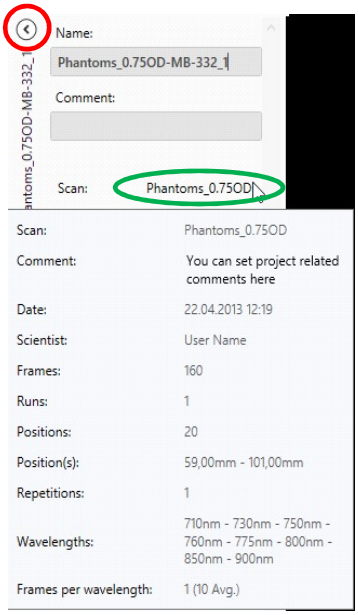
1) Select Images to show in Dashboard and select Difference/Gradient Slice (see 5.6.2 Choosing the difference image) Slider to change the size of the images in the Detail Images window

2) Export Images (see 5.7 EXPORTING IMAGES))

3) Import images from Dashboard directly to ImageJ (see 5.8.2 Direct import into ImageJ).

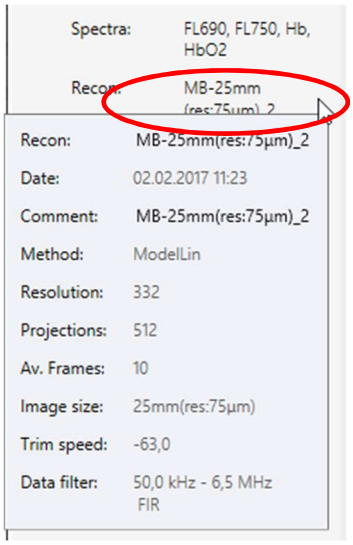
Click the Select Image icon in the top left of the dashboard to open the Dashboard Image Arrangement window and to modify the selection of the displayed images and options for image arrangement.

The Dashboard Image Arrangement window is explained in detail under 5.2.1 Selecting recon images to be shown in visualization and 5.2.2 Selecting MSP images to be shown in visualization.



Information on the chosen Reconstruction/MSP can be reviewed by opening the *Information Extender* to the left of the main image.

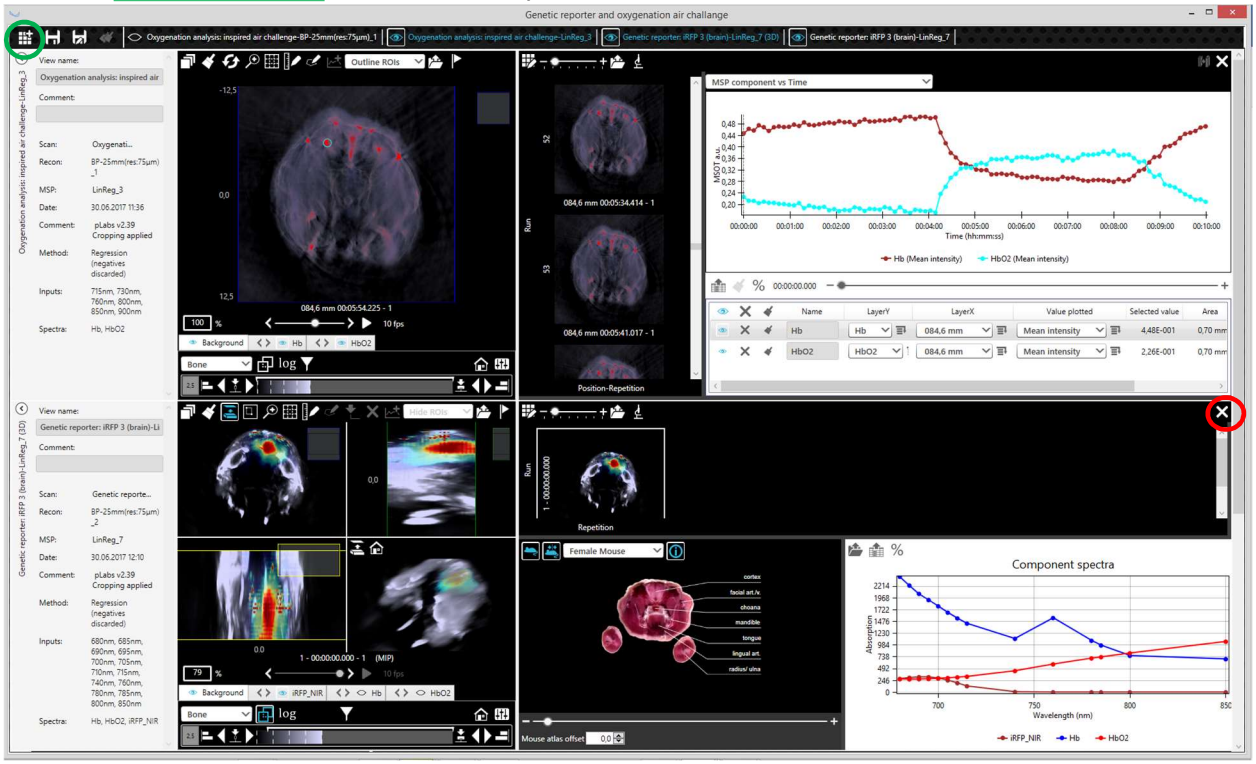
Move the mouse pointer over the scan name from which the current reconstruction or MSP view has been processed, to review the acquisition information of that scan.



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

5.5 OPENING MULTIPLE RECONSTRUCTIONS/MSPS IN ONE VIEW

Click the *Add Scan to View* icon to add multiple reconstructions or MSPs to the current View.



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

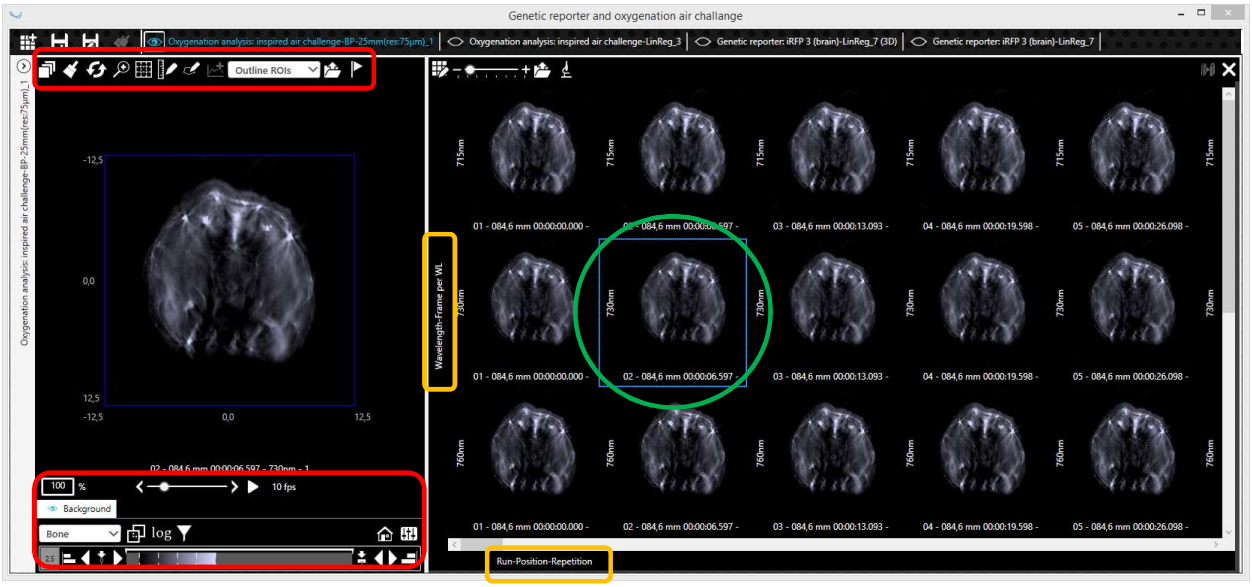
Click the Eye icon of the associated image data set name in the Visualization Bar on the top to visualize or hide the particular reconstruction or MSP from the *View*.

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 axis description shows 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 images (containing information of different spectral components), the data sets containing MSOT and ultrasound and the difference images it makes sense to overlay multiple component layers (with semi-transparency) on top of a background single wavelength or an ultrasound image.

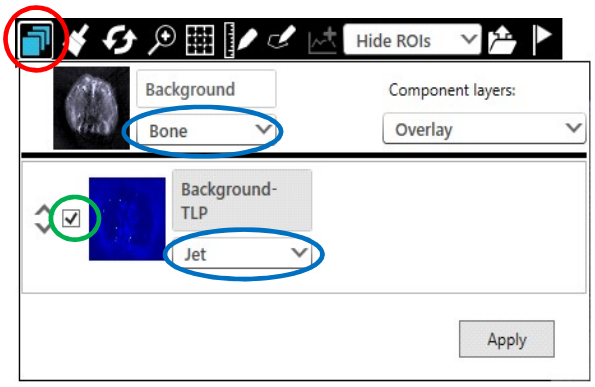
There are some common controls always affecting the whole image including possible overlays (e.g., rotation) and other image controls which can be modified individually for each image layer (e.g., choosing the color map).

5.6.1 Overlaying and enabling TLP-layer and MSP-layers

Reconstruction (single wavelength) image(s)

Having added a reconstruction to the View it is possible to overlay a semi-transparent time-lapse processing (TLP) layer on top of the single wavelength image. This TLP layer 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 to:



1) Change the color maps of the layers.

2) Click checkbox to enable the semi-transparent overlay of the TLP Image.

3) Change the name of the TLP layer.

Click outside the dialogue or on the *Apply* button to apply the modifications.

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. 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 reduce the pixel intensity of 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) Preview Spectrum belonging to the layer

4) Flip image

5) Enable/Disable *Difference Image* (TLP) for the particular layer

6) Change the color map of single wavelength background image as well as MSP overlays.

7) Change the name of the MSP overlays

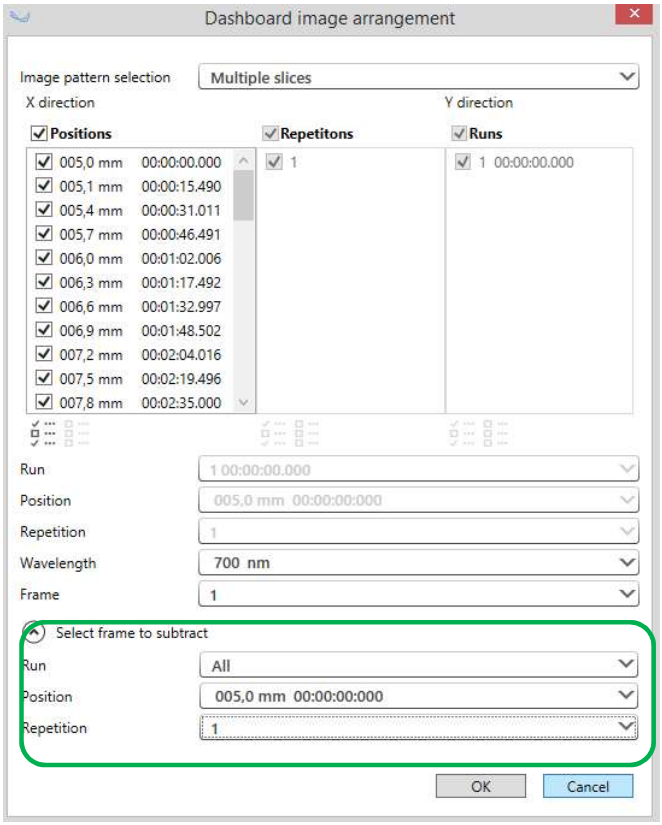
NOTE: Enable/Disable Difference Image is only available for 2D Views.

NOTE: The layers can be made visible and invisible also from the image controls explained in 5.6.5 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 TLP-layer 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.

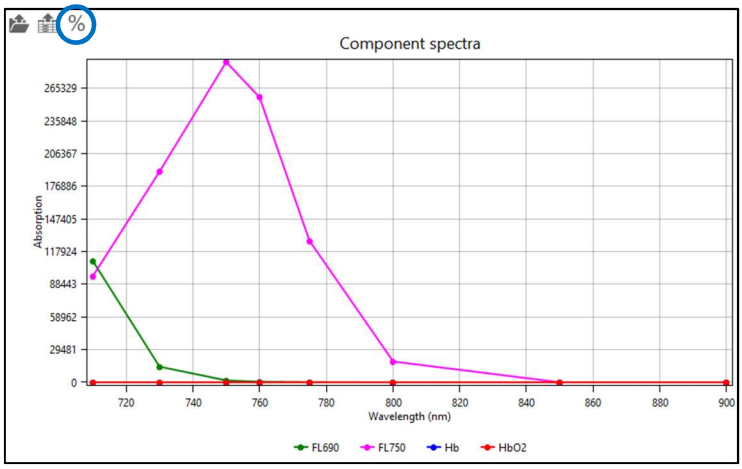
Extend *Select frame to subtract* and use the Run, Position and Repetition dropdowns to select the slice that should be subtracted from the image data set.

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

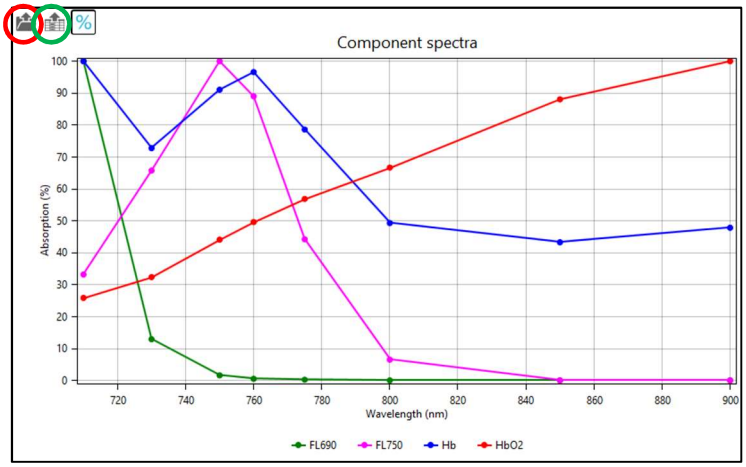
NOTE: When applying Difference to single wavelength images the difference is always calculated for each wavelength separately on the desired set of positions and time points.

5.6.3 Component spectra graph

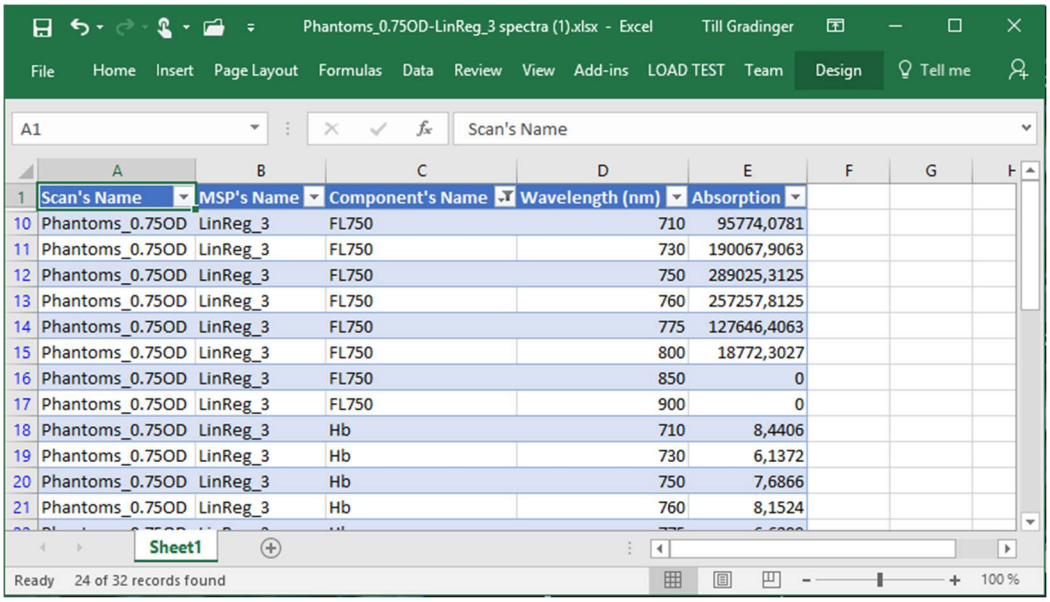
The spectra associated with the MSP layers are shown in the *Component Spectra* graph.



Toggle the Percentage (Y axis) button to normalize the spectra to their absorption maxima, with 100% being the maximum absorption value of each spectrum.



Export the *Component Spectra* graph as a .tiff or .jpg image or open the spectra in Microsoft Excel.



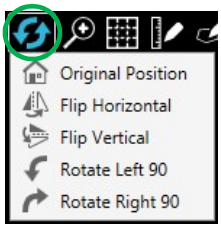
	A	B	C	D	E
1	Scan's Name	MSP's Name	Component's Name	Wavelength (nm)	Absorption
10	Phantoms_0.75OD	LinReg_3	FL750	710	95774,0781
11	Phantoms_0.75OD	LinReg_3	FL750	730	190067,9063
12	Phantoms_0.75OD	LinReg_3	FL750	750	289025,3125
13	Phantoms_0.75OD	LinReg_3	FL750	760	257257,8125
14	Phantoms_0.75OD	LinReg_3	FL750	775	127646,4063
15	Phantoms_0.75OD	LinReg_3	FL750	800	18772,3027
16	Phantoms_0.75OD	LinReg_3	FL750	850	0
17	Phantoms_0.75OD	LinReg_3	FL750	900	0
18	Phantoms_0.75OD	LinReg_3	Hb	710	8,4406
19	Phantoms_0.75OD	LinReg_3	Hb	730	6,1372
20	Phantoms_0.75OD	LinReg_3	Hb	750	7,6866
21	Phantoms_0.75OD	LinReg_3	Hb	760	8,1524

NOTE: Only the absolute values and not the normalized ones are sent to Excel.

NOTE: If Microsoft Excel is not installed on the device, the spectra are exported as an .xml-file.

5.6.4 Common image controls

The *Format Painter* icon serves to apply modifications from one image data set to the other and is explained in chapter 5.6.6 Applying image parameters from one reconstruction/MSP to another.



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

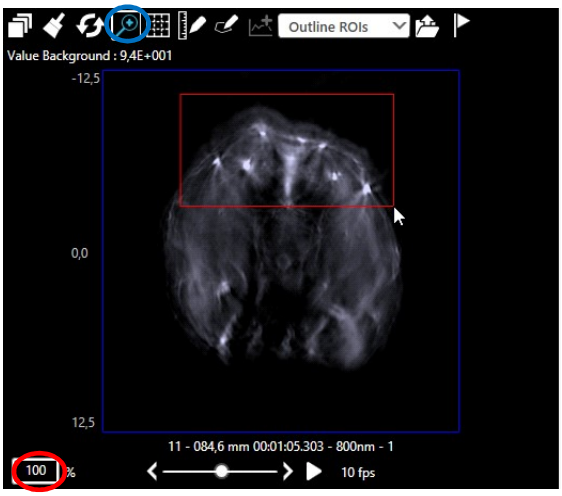
1) *Original Position* reverts everything to the initial state

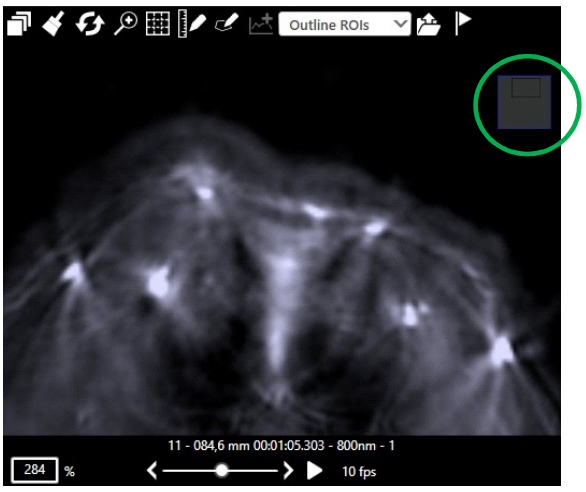
2) *Flip Horizontal* and *Flip Vertical*

Rotate 90° to Left or Right

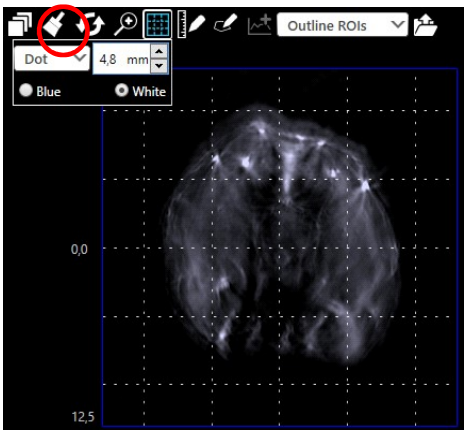
The mouse scroll wheel as well as the F2/F3 buttons can be used to zoom in and out of the image in the Master Image window. The zoom factor is shown at the bottom left of the Master Image window and can also be entered there manually.

A square shaped indicator at the top right of the Master Image window shows the location of the zoomed image sector in relation to the whole field of view.





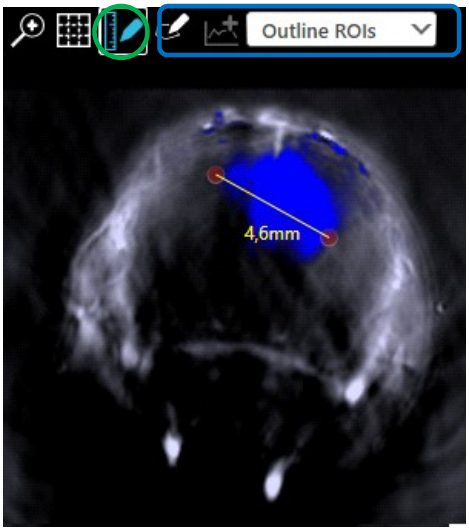
Alternatively, click the zoom icon and draw a (red) rectangle around the region to be magnified. Click the first time to start drawing, click a second time to apply. The region of interest will be zoomed to the full size of the Master Image window.



Click the *Select Grid Options* icon to overlay a grid of a certain size on top of the image.

Modify the following options:

- Grid type
- Grid size
- Grid color



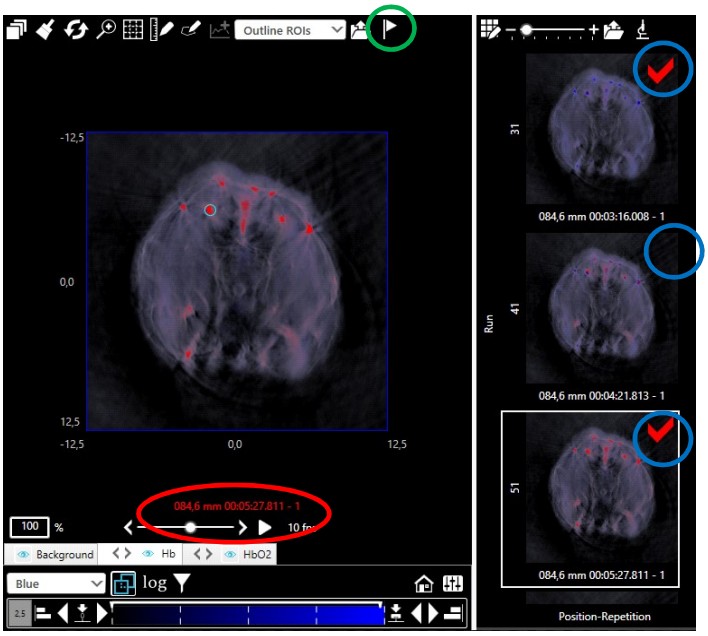
Click the Draw Ruler icon to measure a distance in the current image.

To remove the ruler from the image, click the ruler or the text indicating the length and click the delete or the backspace key.

The icons used for measuring and the analysis of regions of interest (ROIs) are explained in 5.9 REGION OF INTEREST (ROI) ANALYSIS.

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.

Tag or un-tag the image currently visible in the Master Image window by clicking on the Tag icon. Alternatively click in the upper right corner of a dashboard image to tag and un-tag it. Tagged frames are identified by the red check mark on the dashboard image and by the slice information in the Master Image window being shown in red.



Tagging can already be applied during the acquisition of a scan (see 3.6.4 Snapshot window).

NOTE: Tagging is always applied to an MSP frame. When displaying multiple wavelength in a Visualization of a reconstruction, tagging and un-tagging always applies to the full set of wavelengths and not to the individual image.

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 Preview Images icon to play the images shown in the Detail Images window.

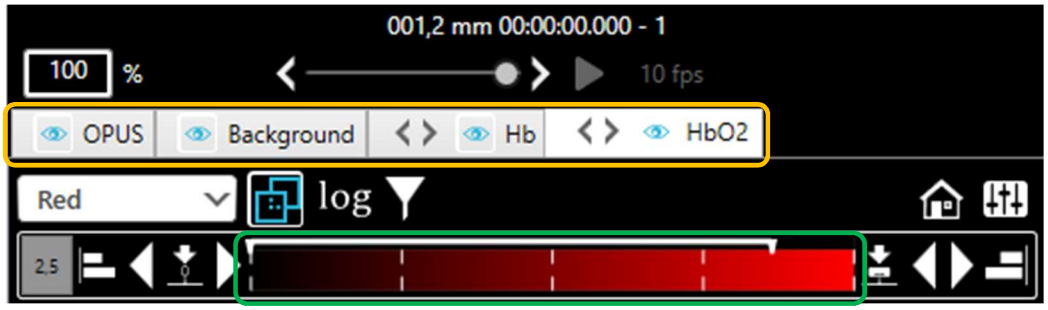
Click Frame Rate to modify the frame rate in frames per second.

5.6.5 Individual layer controls

The MSP-layers that were added to the image – see section 5.6.1 Overlaying and enabling TLP-layer 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 the arrow buttons inside the tab titles to change the order of the component overlay (if overlay and not color mixing is applied).

5.6.5.1 Thresholding of the color bar

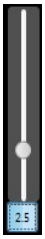
All images have the same scaling for the same component making them visually comparable. The white ticks inside the color bar represent the minimum, ¼, ½, ¾ and the maximum of the color bar.



As default the images - in the Master Image window as well as in the *Detail Images* window – are scaled from “0” to the maximum data value of the images in the Detail Images window (all selected images) for each layer separately. The white data range indicators on top of the color bar show how the image data range is related to the color bar range in the currently selected layer in the Master Image:

- Left sided data range indicator, “0”-indicator: data value “0”
- Right sided data range indicator “max”-indicator: maximum image data value of the selected layer in the current Master Image

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.



In the example above, on the left side an image data value greater than “0” is assigned to the minimum of the colorbar, i.e. all pixels below a certain threshold greater than “0” are black in the resulting image. To the right side the range of the color bar is not fully used. The right data range indicator marks the maximum pixel intensity in that image.

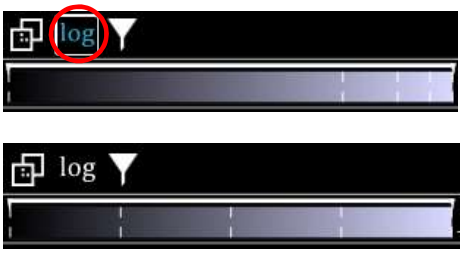
Modify the step size for the arrow buttons.

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

NOTE: On the left side of the color bar the position of the “0”-indicator in relation to the color bar minimum will vary from image data set to image data set, because it is dependent on the image data range of the data set.

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 “0” are black, the pixel with the highest data value is at around ¾ of the color bar maximum.

5.6.5.2 Logarithmic color bar



Click the Log button to apply a logarithmic color map to the current image layer.

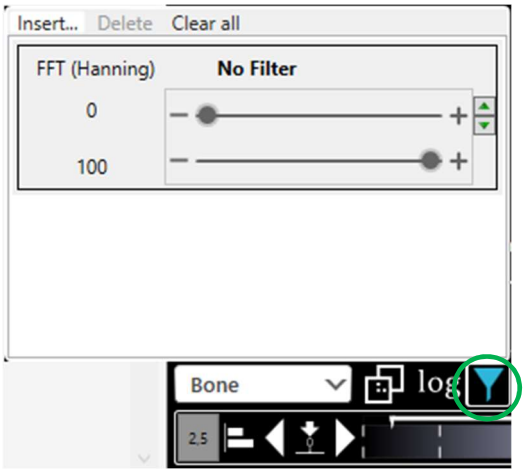
As for the linear color map the white ticks inside the color bar represent the minimum, ¼, ½, ¾ and the maximum of the color bar.

5.6.5.3 Changing the color map

The following color maps are available:

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

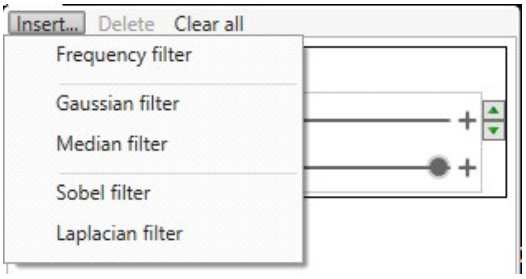
5.6.5.4 Applying image filters



Click the Filters icon to access the filter menu.

By default an FFT (Hanning) filter of the image frequencies is shown.

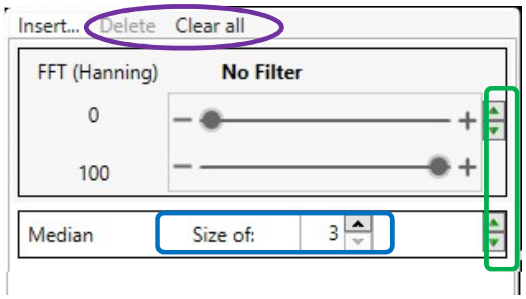
Move the sliders to apply a lower (high pass) or upper filter threshold (low pass) or both (band pass/stop).



Click *Insert…* to add more image filters to the current image layer:

- *Frequency Filter:* see FFT filter above.
- *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:

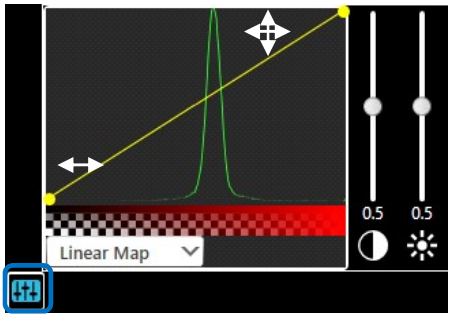
- Modify the filter parameters,
- Change the order in which the filters are applied to the image,
- *Delete* the selected filter,
- Delete all filters.

NOTE: For 3D data only the Frequency and Sobel filters are available.

5.6.5.5 Transparency

Toggle the Transparency button to enable/disable the semi-transparent behavior of the current layer. If Transparent is checked the Transparency Tool inside of the Advanced Image Controls (see 5.6.5.6 Advanced image controls) can be applied to the current layer.

5.6.5.6 Advanced image controls

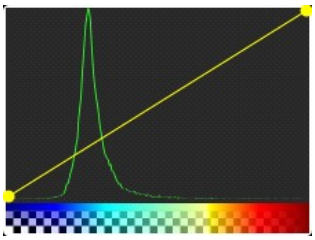
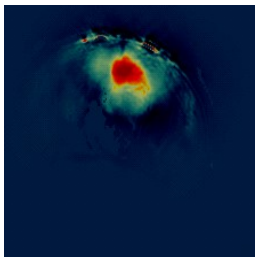
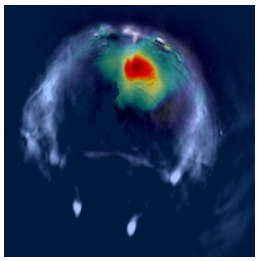
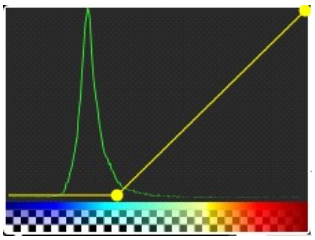
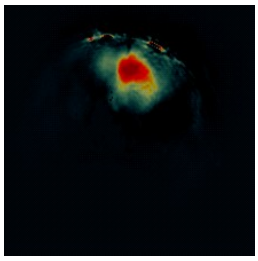
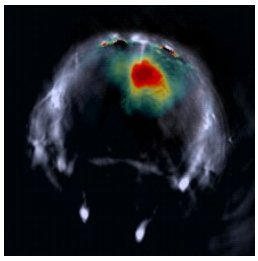
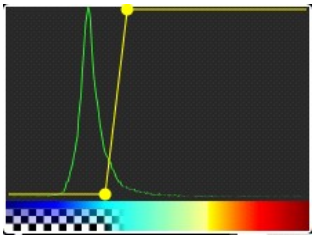
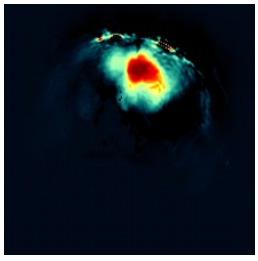
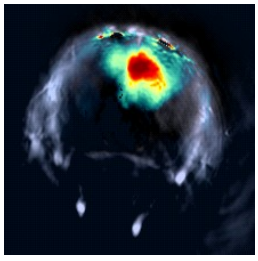
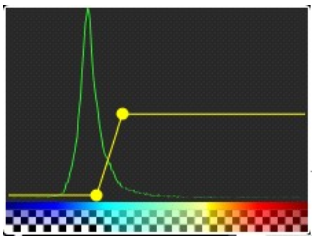
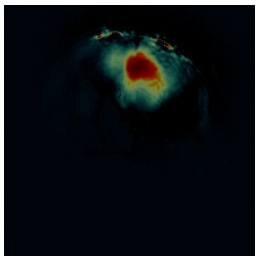
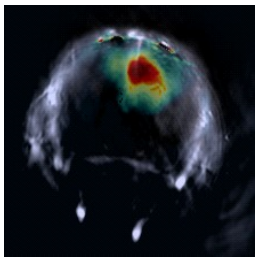


The Advanced Image Controls dialog consists of:

- Color bar histogram: histogram showing the number of pixels over the color bar intensity
- Transparency Tool: modify the transparency/opacity of imaging layers
- Controls for contrast and brightness

Transparency tool

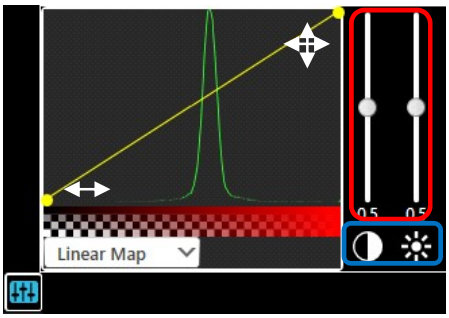
With the Transparency Tool the user can change the transparency/opacity – which by default is linear to the color bar color intensities – with respect to the color bar and independent from the relation between color bar and image values (which is modified by the arrows next to the color bar as explained 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: The Transparency Tool is only enabled when the Transparency button is clicked.

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.

Modifying contrast and brightness



Modify *Contrast* and *Brightness* of the images as well as set them back to default by clicking the icons below the sliders.

5.6.6 Applying image parameters 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 
- Image Filters 
- Contrast/Brightness 
- Visibility of a component in the tab 

- Transparency and transparency thresholds (low/high) 
- Logarithmic color bar 

NOTE: It is not possible to adopt layer selections, layer flipping, and enable/disable Difference Images from one image to another.

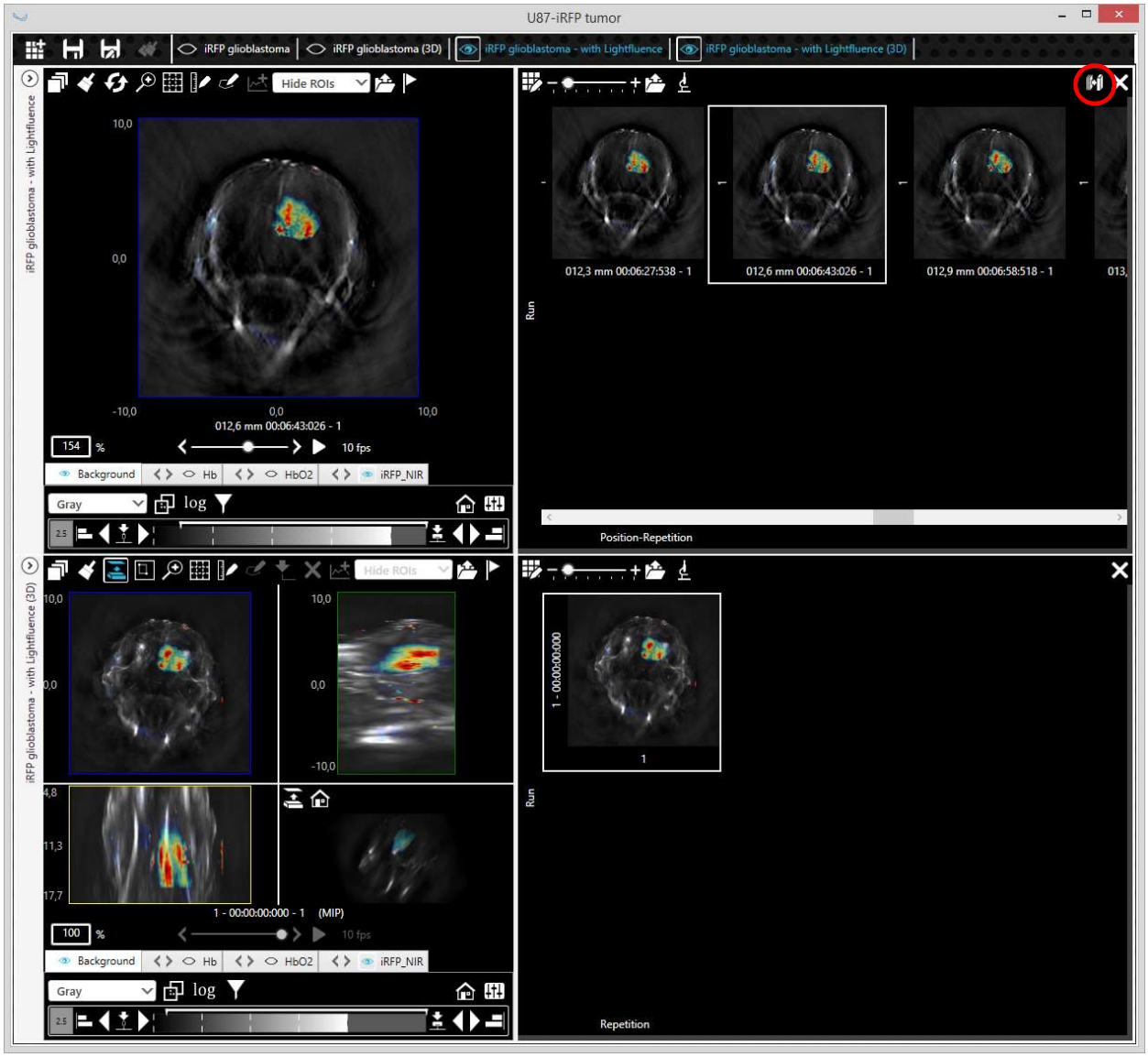
NOTE: Copy and paste of parameters is only performed between layers with exactly the same name.

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.

NOTE: It is not possible to copy image settings between two different studies.

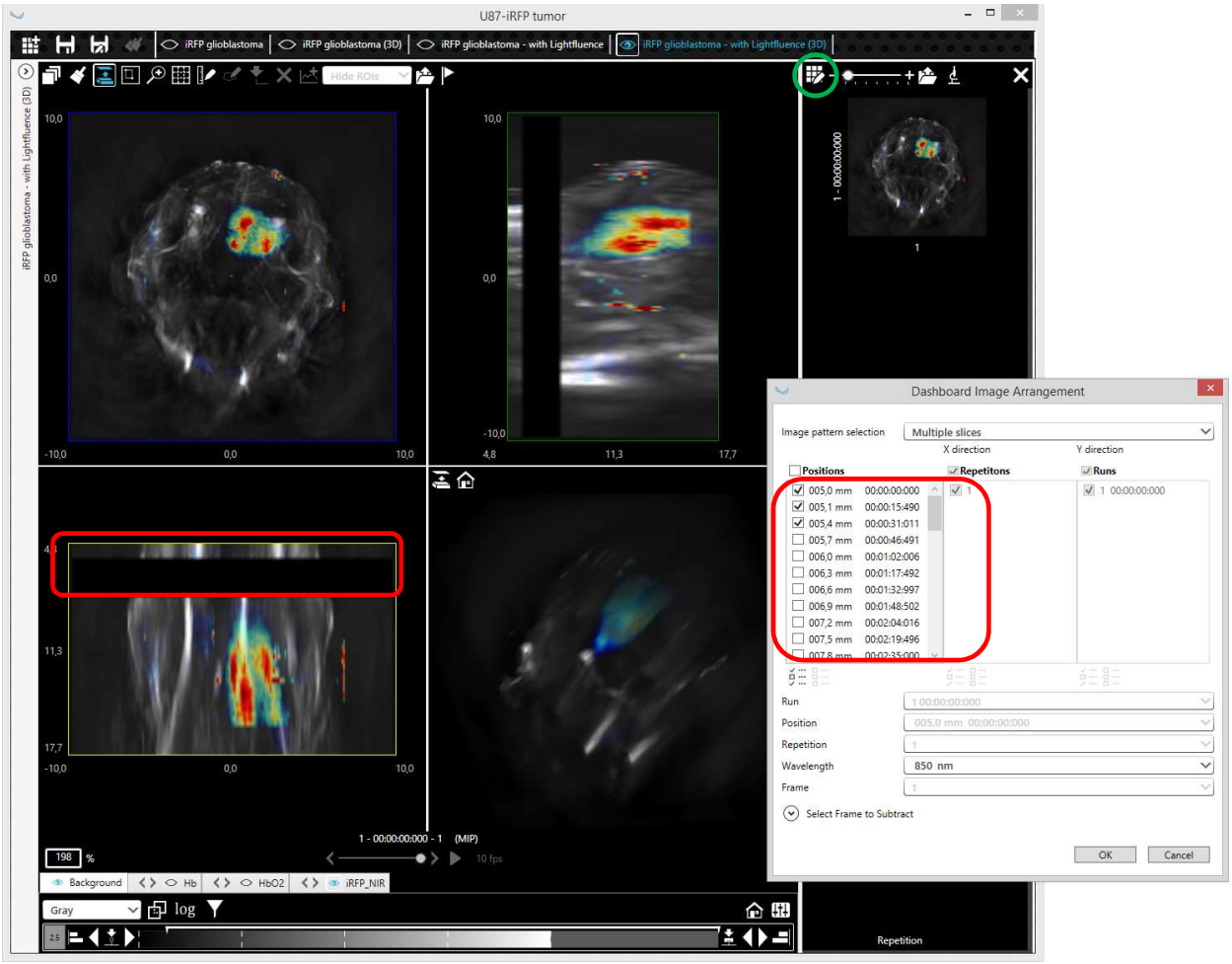
5.7 GENERATE 3D VOLUME OUT OF MULTI-POSITION 2D DATA

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) as well as the volume of the composed cuboid (in the 4th quadrant of the Master Image).



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 DISPLAYING IMAGES.



ViewMSOT recognizes the positioning grid step size that has been used during acquisition and approximates the x-z and the y-z image plane to this grid step. If the user has chosen a set of positions and the unselected positions are at the beginning or at the end of the position's 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) Maximum Intensity Projection / Volume view

2) Orthogonal Planes / 3D Orthogonal Planes view

The views and the associated controls are explained below.

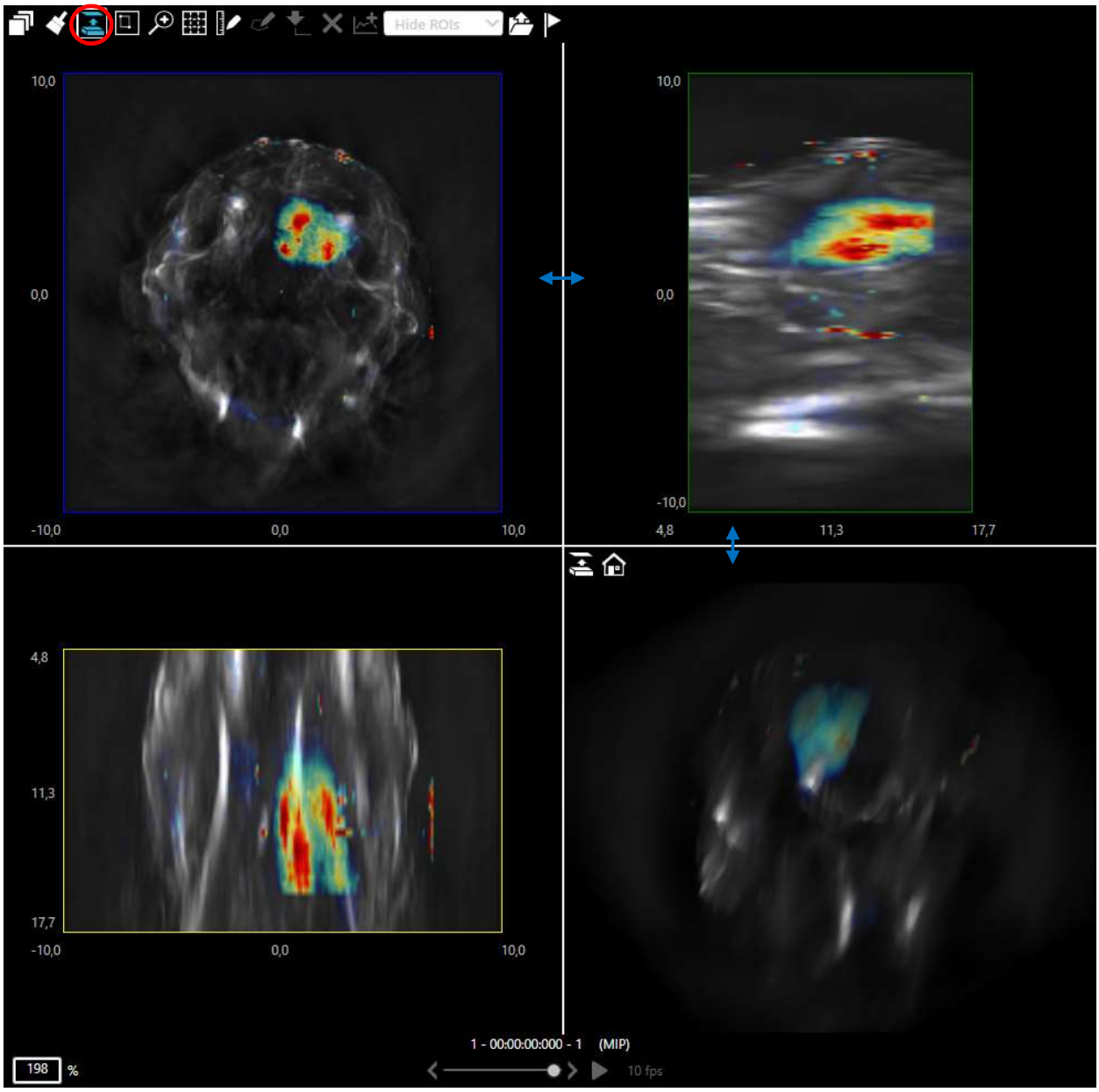
NOTE: There is no Difference/Gradient Image processing (see 5.6.2 Choosing the difference image) available for the 3D views.

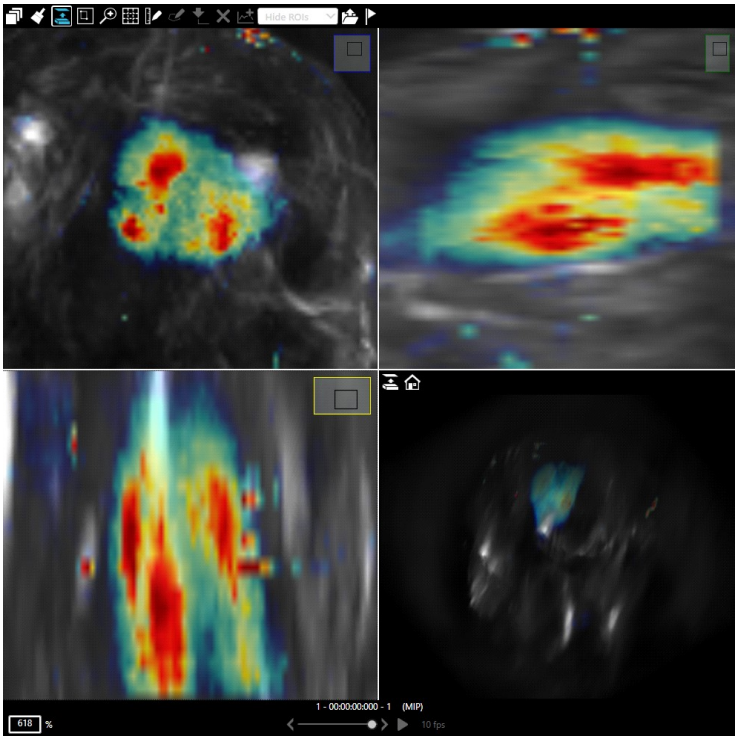
5.7.2.1 Maximum intensity projection (MIP) / volume view

Converting a data set to a 3D view is presented to the user as a maximum intensity projection by finding the maximum voxel intensity being projected onto each of the three (x-y, x-z and y-z) planes.

The four quadrants of the Master Image are resizable and contain:

- Top left: x-y MIP plane.
- Top right: y-z MIP plane
- Bottom left: x-z MIP plane
- Bottom right: rotatable volume



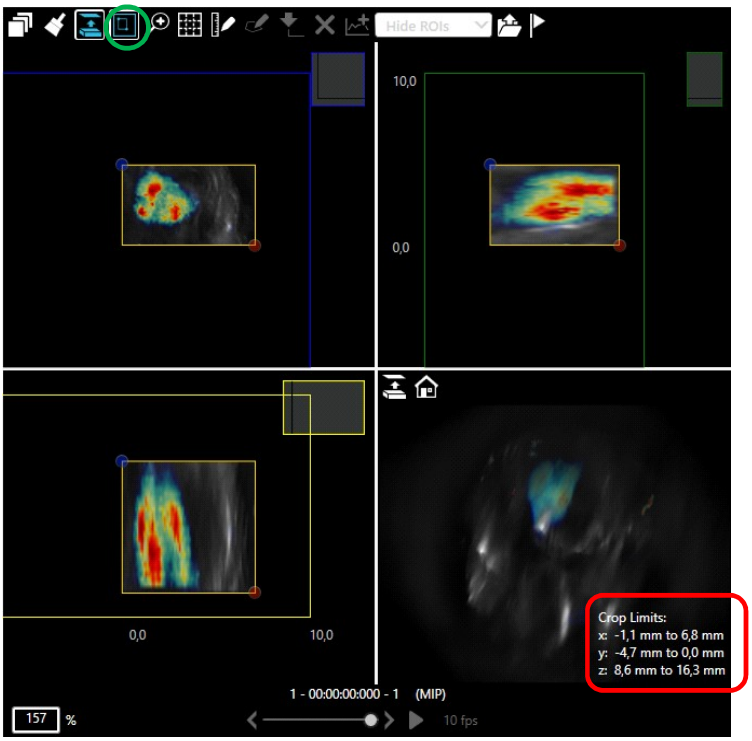


Zoom into one of the MIP planes and the other two are updated accordingly.

A small rectangular shaped indicator in the right top of each of the 3 planes show the location of the zoomed image sector in relation to the whole field of view.

The zooming functionality is explained in 5.6.4 Common image controls in detail.

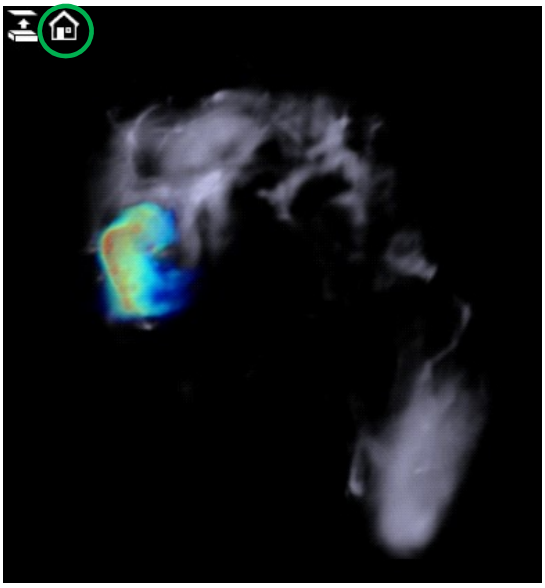
MIP cropping tool



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 included for the maximum voxels when generating the MIP.

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

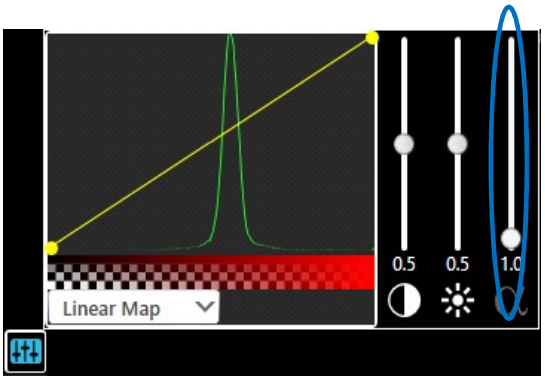
Rotatable volume



The 4th quadrant of the Master Image containing the rotatable volume is independent from the 3 planes for the following functionalities:

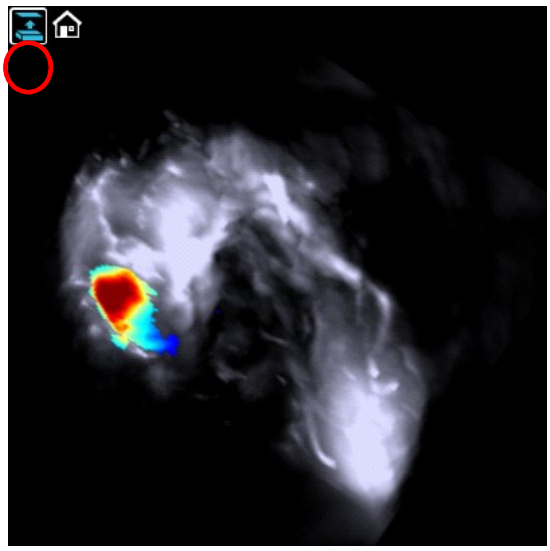
- Zooming: use the mouse scroll wheel to zoom into the volume
- Rotate volume: click inside in the volume and drag the intended rotation.

Click the *Home* icon to undo all rotations and zooming. The volume will be shown in its initial size and angle, x-y plane being orthogonally centered.



Modify transparency (explained in 5.6.5.6 Advanced image controls) and color bar thresholds (explained in 5.6.5.1 Thresholding of the color bar) to remove background noise.

Modify the *Rendering Weight for Component* to give priority to the component currently selected (in the tab) in relation to the other image components.

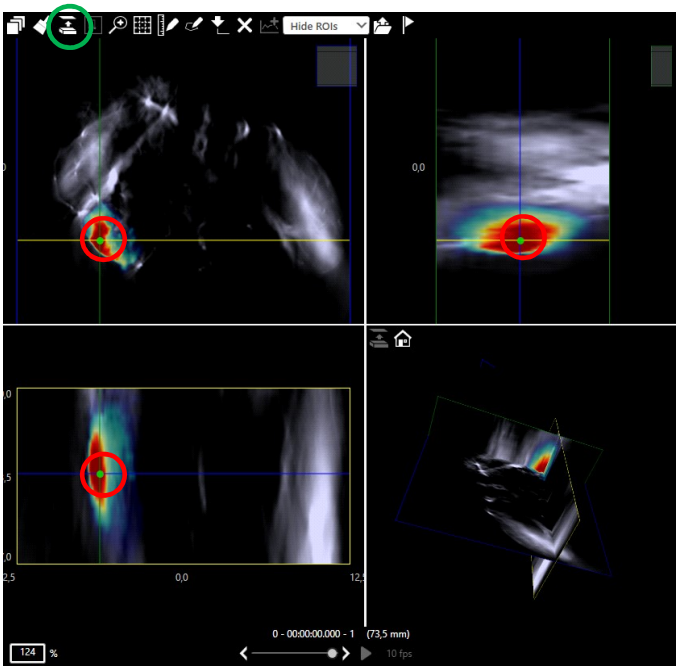


Click the MIP Volume Rendering icon to show the rotational volume as a maximum intensity projection.

NOTE: When MIP Volume Rendering is applied, the transparency settings are disregarded.

NOTE: If the Color Mixing is chosen as overlay method (see 5.6.1 Overlaying and enabling TLP-layer and MSP-layers) also the rotational volume will look different, because another method supporting color mixing will be used to generate the volume. New adjustments of the color bar thresholds and the transparency need to be performed.

5.7.2.2 Orthogonal planes view



Deselect the Show Max Intensity Projection button to use the orthogonal planes view.

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

In the 4th quadrant of the Master Image the three orthogonal planes are shown in 3D.

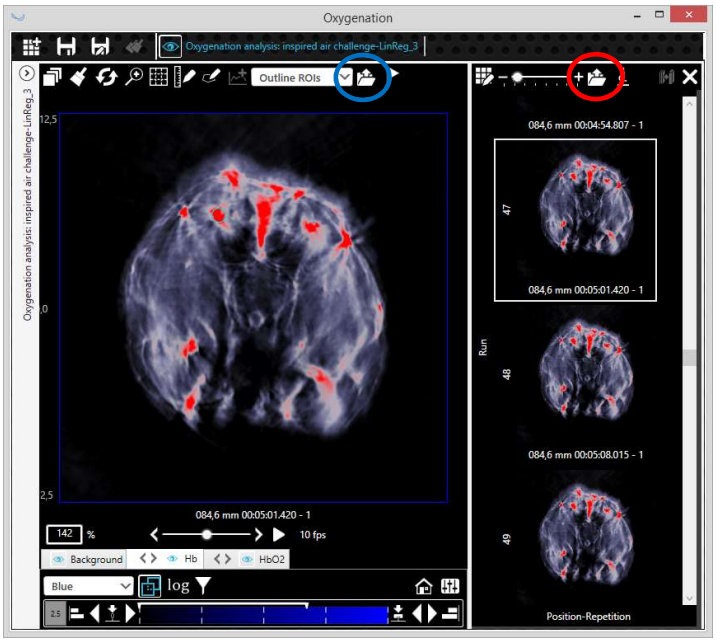
Rotate the 3D orthogonal planes by clicking inside in the volume and dragging the volume.

The three orthogonal imaging planes can be re-identified in the different window quadrants (frame of the planes) and the crosshair lines by their color:

- x-y plane: blue
- y-z plane: green
  x-z plane: yellow

5.8 EXPORTING IMAGES

5.8.1 General image export



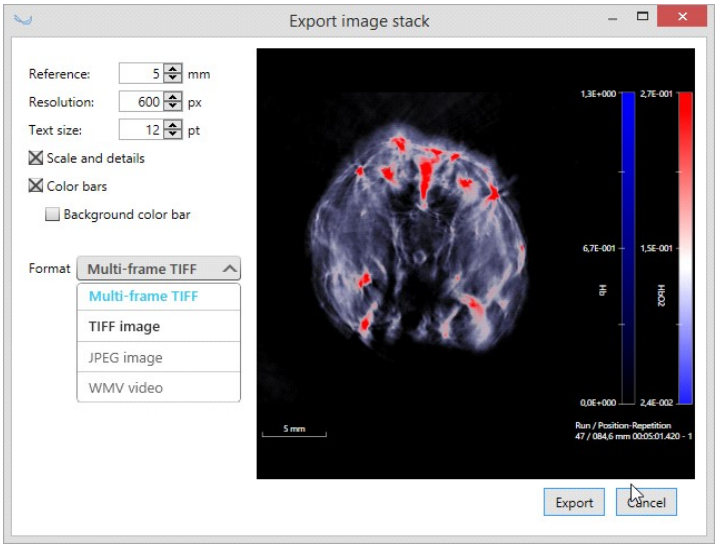
By clicking the *Export* icon in the *Detail Images* window all images that are present in the *Detail Images* window can be exported.

When clicking the *Export* icon in 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*
- *Text size* in the image
- Availability of scale bar, information text and color bars

Select one of the following export formats:

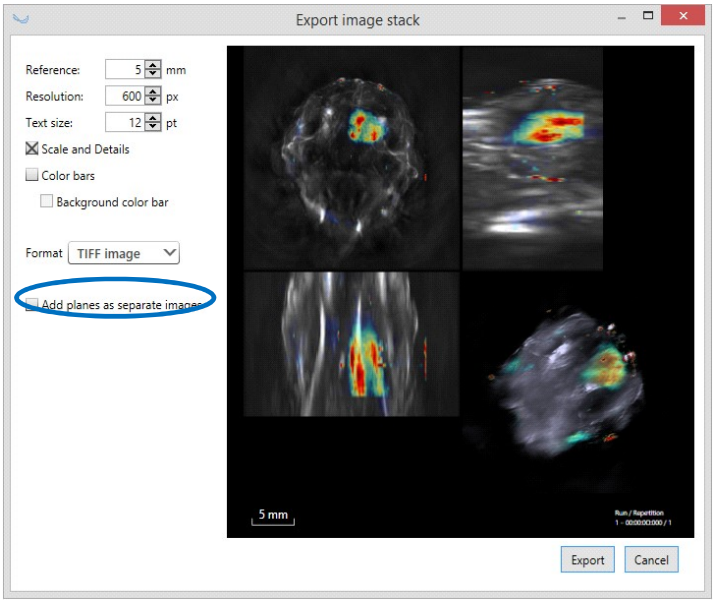


1) *Multi-Frame Tiff*: one single Tiff-file containing all images in the *Detail Images* window. The order of the images is from left to right and row by row.

2) *Tiff* and *Jpeg sequence*: separate image files with automatic naming convention:

   <Scan name>
   <Run> <Position>
   <hours_minutes_seconds_hundredths> .<format>

3) *Mpeg video*: frames are converted into a video. The order of the images is from left to right and row by row.



Depending on which visualization method – orthogonal planes view or MIP view – was chosen in the Master Image window, the 3D images will be exported accordingly. Additionally, the following choices will be applied from the Master Image window ("what you see is what you get") to the export:

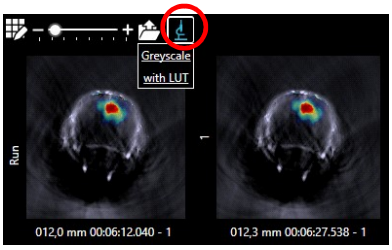
- MIP cropping regions for MIP
- Planes selection for orthogonal planes view
- ROIs in x-y plane, resulting from 3D ROI analysis

Check *Add planes as separate images* to additionally export each plane into a separate image file (without scale bar, information text or color bars).

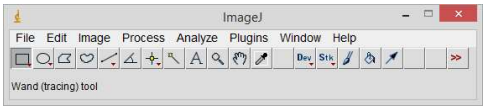
NOTE: Tiff and Jpeg sequence are saved in an automatically generated folder named according to the view name.

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:



- *Greyscale*: import dashboard selection as 32-bit greyscale images to ImageJ. Each pixel retains the original (real) reconstruction/MSP value (after thresholding).
- *with LUT*: same as Greyscale but the colors will be transferred to ImageJ by generating a look up table (LUT).



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 *with LUT* also:

- Contrast/Brightness
- Color maps

The following parameters are NOT applied:

- Image overlay options including transparency and transparency map
- ROIs

The hyper stack c-dimension keeps the different wavelengths 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/

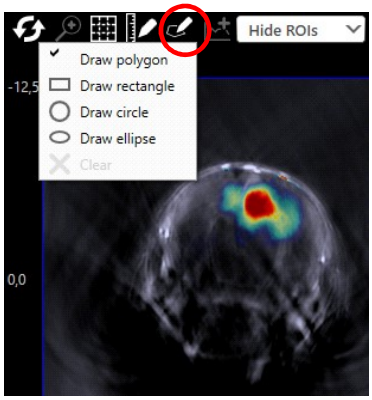
5.9 REGION OF INTEREST (ROI) ANALYSIS

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 explained for 2D views first, in 5.9.2 Creating 3D ROIs (for 3D views) ROI analysis on 3D views is explained.

5.9.1 Creating ROIs (for 2D views)

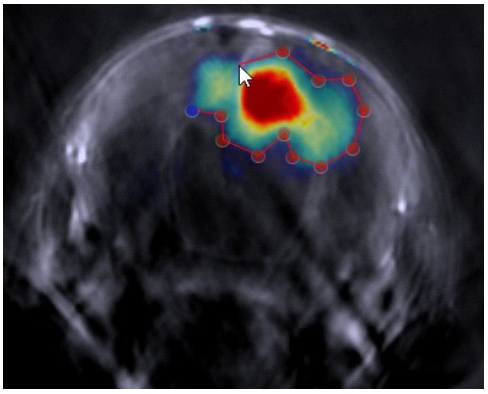


By clicking on the *Select Region* icon different shapes can be selected:

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.

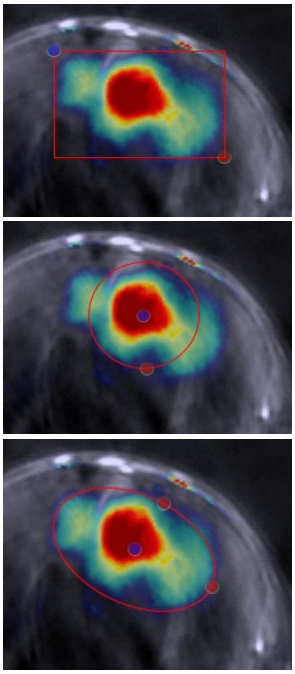
Moving the mouse and performing further left clicks continues to draw the polygon.



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

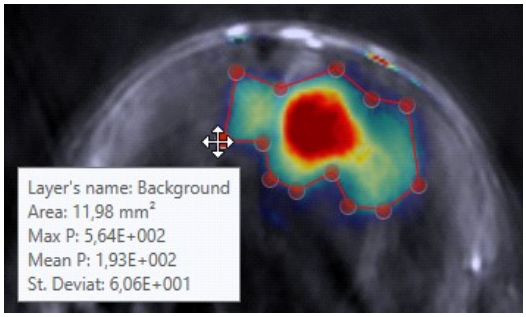
Draw Rectangle: on the first left click the blue starting point is set. Moving the mouse pointer the size of the rectangle is chosen. Left clicking again finishes the shape.

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

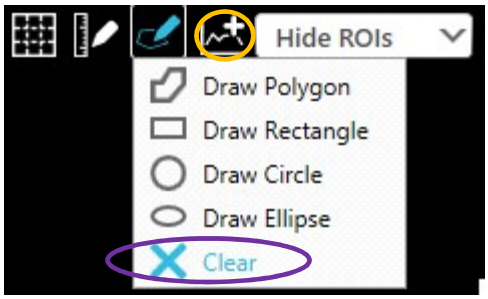


Draw Ellipse: on the first left click the center is defined (blue dot). With two further left clicks (red dot) the diameters are chosen.

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 5.6.5 Individual layer controls).



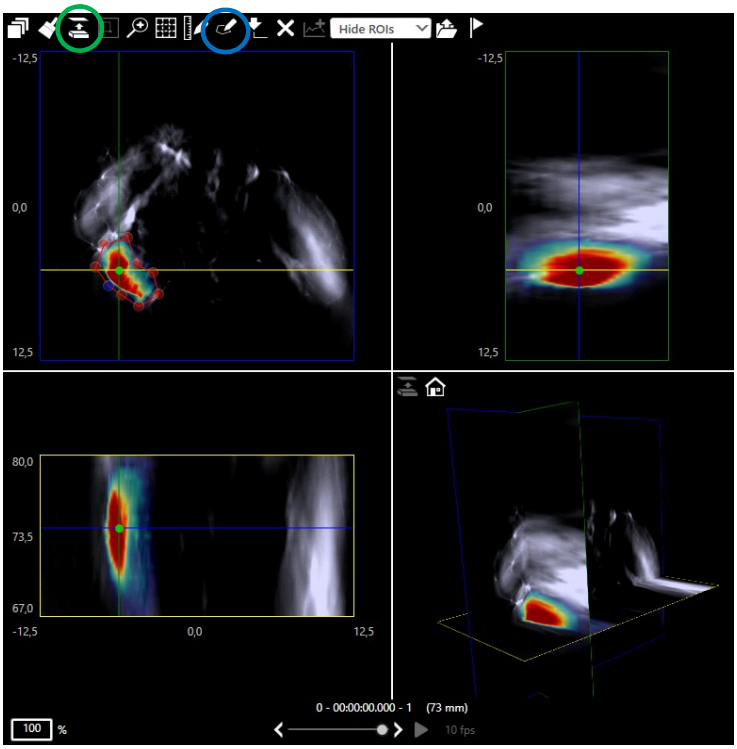
Selecting *Clear* removes the current ROI from the *Master Image* window.

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

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

5.9.2 Creating 3D ROIs (for 3D views)

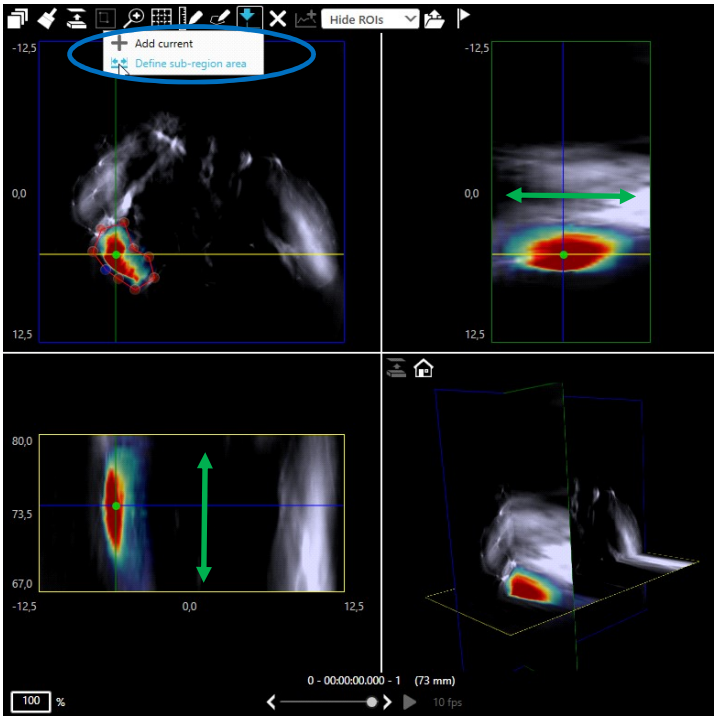
Similarly to a 2D ROI analysis the user can perform a 3D ROI analysis. 3D ROIs can only be drawn on the orthogonal planes view (see 5.7.2.2 Orthogonal planes view) and not in the MIP view.



In the Orthogonal Planes View (the MIP button needs to be disabled) 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 3D ROI 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 3D ROI.

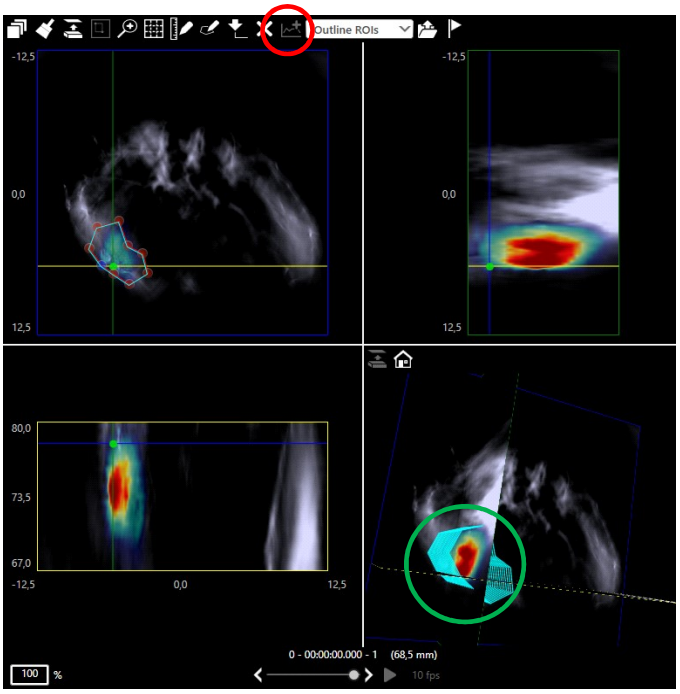
Mark this z-axis position of the 3D ROI by choosing Define sub-Region Area.

Move the cross hair along the z-axis, find the end of the object and mark the end position of the 3D ROI by unchecking Define sub-Region Area.

The 2D ROIs on the x-y- plane of the image constituting the 3D ROI are visible in orange color, when moving through the z-axis.

Add as many sub-regions as required to your 3D ROI

Remove single sub-ROI from the volume of interest or clear the whole 3D ROI that has been generated.



Import the 3D ROI to the ROI graph and table for analysis (see 5.9.3 ROI graph).

The 3D ROI is also visible in the 3D orthogonal planes view in the 4th quadrant.

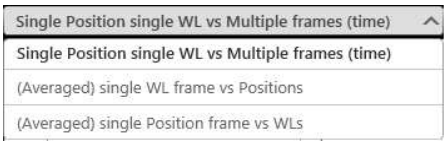
5.9.3 ROI graph

The top right of the Dashboard contains the ROI 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 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:

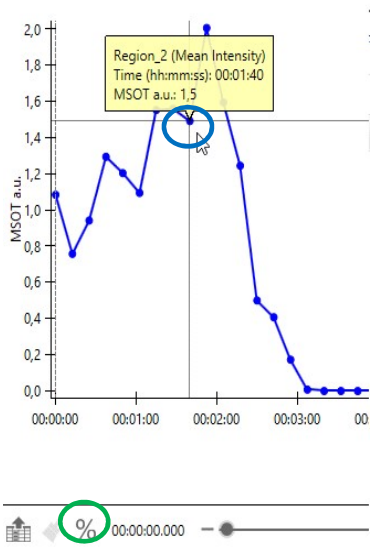
1) *Single Position single WL vs Multiple frames* (time): observe how a region/volume of interest behaves over time for a certain wavelength.
2) *(Average) single WL frame vs Positions*: ROI at one wavelength over different positions (anatomical)
3) *(Average) single Position frame vs WL*: spectrum of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

All ROIs that are imported from the Master Image are shown in the graph in the respective color.



Click a curve in the graph area to display

- ROI name
- Type of statistical measure applied to the ROI
- Y-axis value, e.g. time, wavelength
- X-axis value

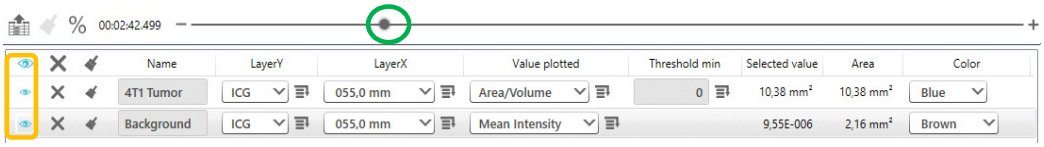
in a menu.

Click the *Percentage (y axis)* button to normalize the ROI graph curves to their absorption maxima, with 100% being the maximum value of each curve.

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).

5.9.3.3 Content and functionality of the interactive ROI table

The ROI graph curves are listed in a table below the graph. The table consist of the following columns:



1) Visibility: 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.

2) Delete: Click "**x**" to delete a ROI from the Master image and the ROI table. Click the delete icon in the title bar to delete all the ROIs from the current view.

3) Copy and paste: Apply ROI(s) from current data set to another (see 5.9.3.5 Copy the shape of an ROI to another image data set)

4) *Name*: Give a specific name to each ROI.

5) *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. Click *Apply to all regions* next to the dropdown menu, to apply the selection to all ROIs.

6) *Value Plotted*: select the measure plotted in the graph from the available statistical measures of the table. The statistical measures which can be applied are explained in detail below (see 5.9.3.4 Statistical measures applied for the ROI-value plotted)

7) *Threshold Min*: a lower threshold in absolute MSOT a.u. taken into account for the calculation of the statistical measure: Area/Volume (see 5.9.3.4 Statistical measures applied for the ROI-value plotted).

8) *Selected value:* statistical measure calculated from the ROI referring 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.

9) *Area*: surface area of the ROI in mm^2 (for 2D ROI) or in mm^3 (for 3D ROI)

10) *Color*: selection of the color of the ROI graph curve and the ROI in the Master Image.

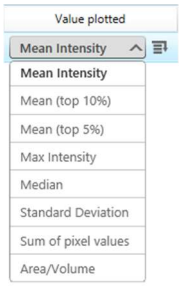
5.9.3.4 Statistical measures applied for the ROI-value plotted

Select the statistical measure to be applied to a ROI graph in the column *Value plotted*.

1) *Mean Intensity*: mean value over all pixel values inside the ROI.

2) *Mean (top 10%)*: For each frame, for each ROI identify the pixel values from 90% to 100% with 0% being the minimum and 100% being the maximum pixel value inside the ROI of the frame.

3) *Mean (top 5%)*: same as Mean (top 10%) just the top 5% are applied (95% to 100%).

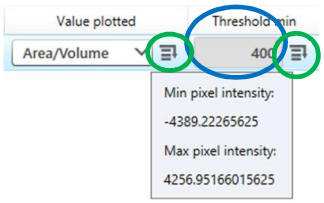


4) *Max Intensity*: highest pixel value in the ROI

5) *Median*

6) *Standard Deviation*

7) *Sum of pixel values*



8) *Area/Volume*: Set *Threshold min* to determine the surface area/volume of the ROI which is equal or above the applied MSOT a.u. value.

   Hover over the text box to display the minimum and the maximum MSOT a.u. value of the ROI for all frames.

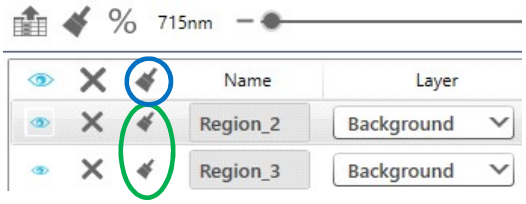
Click the *Apply to all regions* button next to the control, to apply the type of statistical measure to all ROIs.

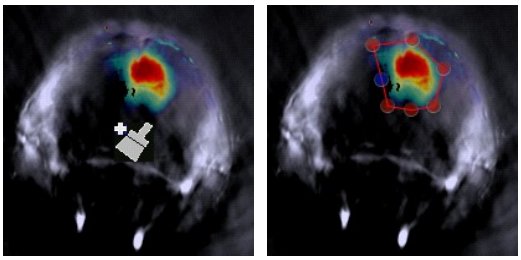
NOTE: *Threshold min* is only applied for the *Area/Volume* measure.

NOTE: Negative pixel values are set to "0" for the calculation of the statistical measures except:

- *Area/Volume*.
- Difference method (see 2.2.2.2 Multispectral processing(MSP) settings) was used to calculate the component layers. In that particular case, negative pixels are counted

5.9.3.5 Copy the shape of an 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 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, but not between different studies.

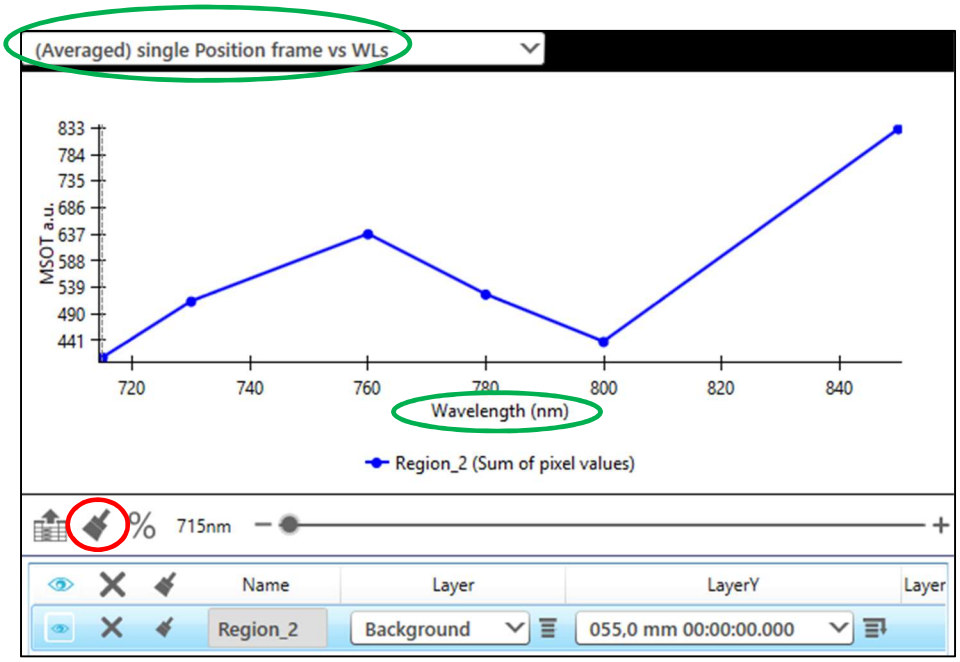
NOTE: The image resolutions of the image data sets need to be the same. Otherwise ViewMSOT™ will report a resolution mismatch.

NOTE: The brush icon in the title bar and the brush icon for particular ROIs work different: Clicking the ROI brush to copy a single ROI only the ROI shape will be copied. Click  to import the 2D/3D ROI and set up the ROI graph as explained in 5.9.3 ROI graph. Clicking the ROI brush in the ROI table title line to copy all ROIs automatically generates a ROI graph and table with the same parameters.

5.9.3.6 Copy ROI graph content to spectrum manager

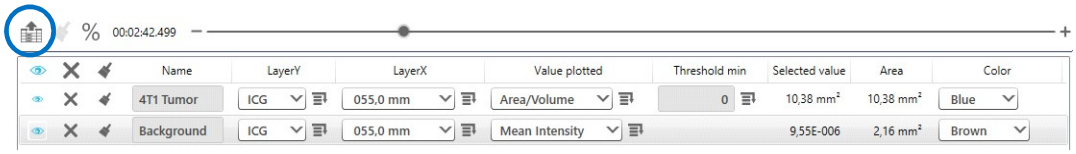
The results of ROI graph showing a spectrum can be copied and reused for MSP by adding it to the list of spectra by pasting it inside the Spectrum manager. Select *(Average) single Position frame vs WL* to display the spectrum of the ROI at a defined slice.

Select a ROI by clicking on the ROI table entry and click *Copy spectral info of Region into clipboard*. Open the Spectrum management dialog and past the selected curve (see 2.3.1 Add spectrum - Import values for wavelength and absorption).



5.9.4 ROI-XML export

Clicking 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.



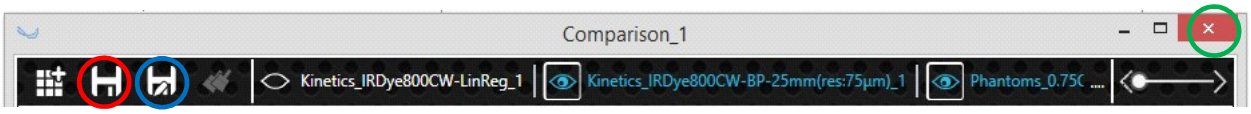
Opening the exported file in a spreadsheet program allows the user to process the ROI data.

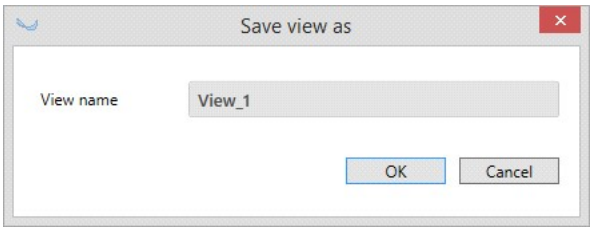
Name	Calculation Method	Method	Region's Name	Area (mm²)	Minimum Threshold (%)	Maximum Threshold (%)	Position (mm)	Run	Repetition	Component's Name
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	1	1	HB
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	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	ICG
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	2	1	HB
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	2	1	HBO2
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	2	1	ICG
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	3	1	HB
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	3	1	HBO2
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	3	1	ICG
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	4	1	HB
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	4	1	HBO2
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	4	1	ICG
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	6	1	HB
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	6	1	HBO2
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	6	1	ICG
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	8	1	HB
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	8	1	HBO2
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	8	1	ICG
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	10	1	HB
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	10	1	HBO2
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	10	1	ICG
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	12	1	HB
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	12	1	HBO2
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	12	1	ICG
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	14	1	HB
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	14	1	HBO2
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	14	1	ICG
MSP_1	On per slice per ROI basis	MSP Component vs Time	4T1 Tumor	20,97	80	100	94,5	16	1	HB

NOTE: If Microsoft Excel is not installed on the device, the ROIs are exported as .xml-file.

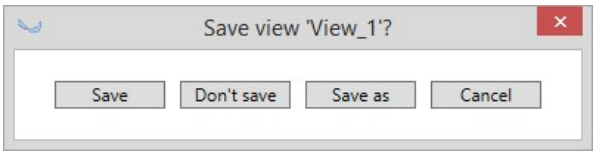
5.10 SAVE VIEW AND EXIT VISUALIZATION

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.





Click Save as icon to 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.

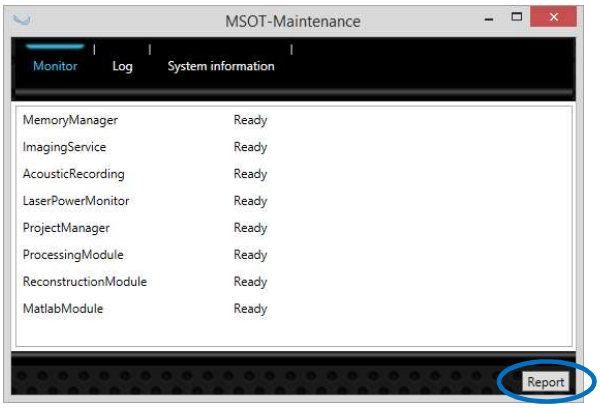
6 APPENDIX

6.1 MAINTENANCE

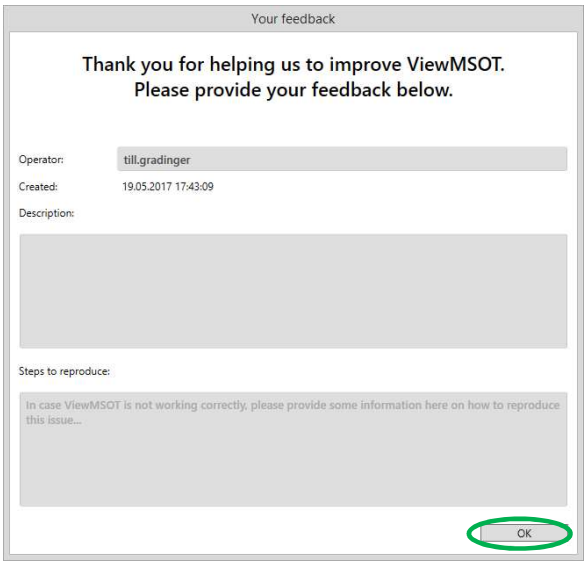
Click the *Maintenance* button – in the task bar at the bottom of the main screen – to access the *MSOT Maintenance* dialog. The maintenance dialog mainly contains service functionalities and system status information.

However, it can be used to enter a feedback report or to re-execute the laser self-test. It is also possible to manually operate the water supply pump and the stirring pump.

6.1.1 Feedback report



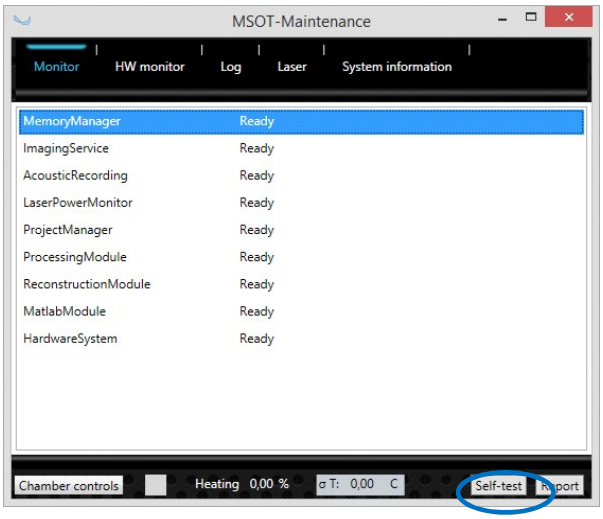
Click *Report* to enter your feedback.



Enter your name and your comments.

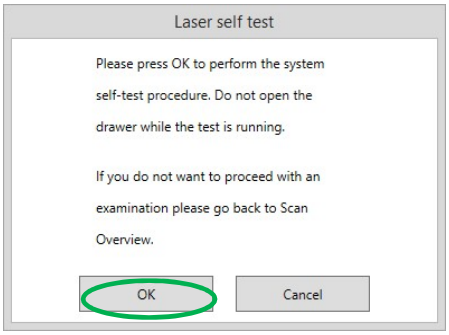
Click *OK* to save the feedback report.

6.1.2 Laser self-test

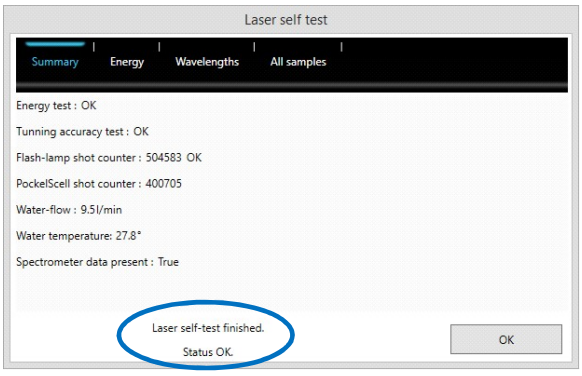


Leave the acquisition screen and go to the Scan overview screen. Be sure that the drawer is closed.

Click Self-test.



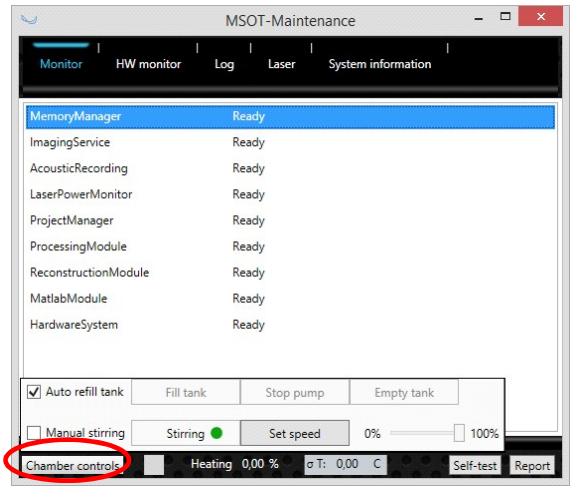
Click OK to execute the laser self-test procedure.



Do not open the drawer during the laser self-test.

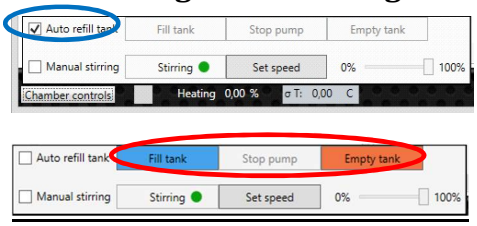
Once the laser self-test has finished a dialog provides the result. If the *Status* is not OK, please contact support@ithera-medical.com.

6.1.3 Manual control of the pumps



Click *Chamber controls* to change the water level in the imaging chamber or to manually control the water stirring pump.

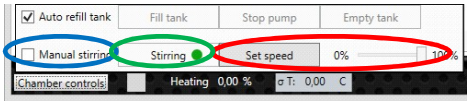
6.1.3.1 Filling in or draining water



To change the water level in the imaging chamber disable *Auto refill tank* and then click either *Fill tank* or *Empty tank*.

Stop pump will interrupt the filling or emptying procedure.

6.1.3.2 Water stirring pump



Manual Stirring enables access to the stirring pump controls.

Disable or enable the stirring by clicking *Stirring*.

To control the flow rate of the pump use the slider and click *Set speed*.

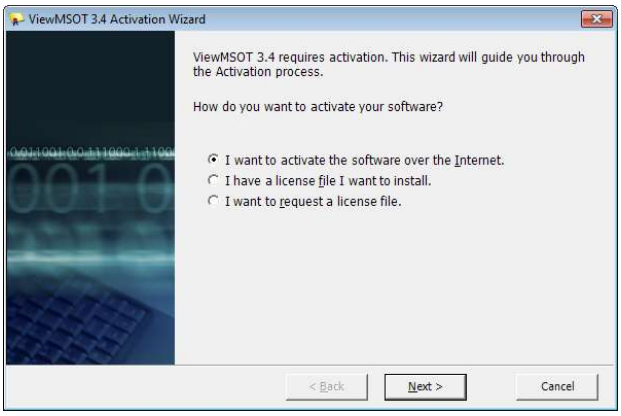
Note: After clicking the control buttons there might be a delay of approximately 2 seconds.

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

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 with internet access



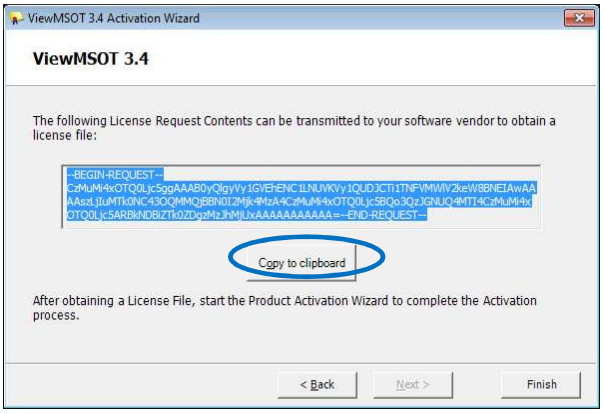
When starting ViewMSOT on a system where no ViewMSOT license has been activated yet, the license activation window appears.

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.

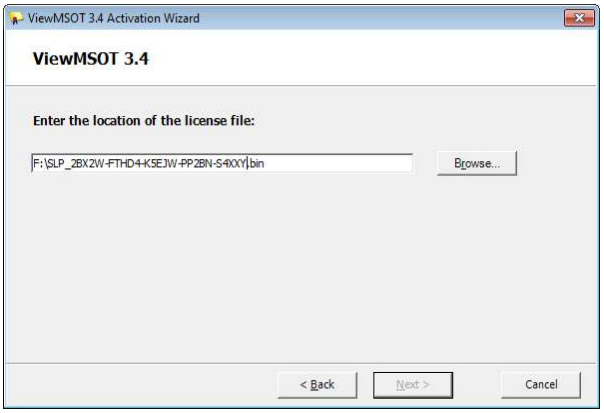
6.2.2 Activate ViewMSOT from PC without internet access



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.

7 REFERENCES

[1] *Xu M, Wang LV.Universal back-projection algorithm for photoacoustic computed tomography. Phys Rev E Stat Nonlin Soft Matter Phys. 2005 Jan;71(1 Pt 2):016706. Epub 2005 Jan 19. Erratum in: Phys Rev E Stat Nonlin Soft Matter Phys. 2007 May;75(5 Pt 2):059903.*

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