Intrinsic image-based projection sorting for in vivo lung and cardiac gated scans

Method note
MCT-105

1. Introduction

Normally you don’t want your sample to move during a microCT scan. It leads to movement artefacts which degrade image quality. However, imaging of breathing lungs and the beating heart in live mice under anaesthesia is a special case, where the motion cycle of breathing and the heartbeat are themselves the objective of the imaging study. The cycle should repeat the same movement and start and end in the same position.

The technique of synchronisation or “gating” allows time phase resolution of repetitive cyclical movement in a scanned object; for example, the lungs in a breathing mouse and the same animal’s beating heart. Gating can be either “prospective”, in which xray CT projection images are acquired at timepoints selected in real time corresponding to a single phase of the cycle, or “retrospective”, in which a large redundant number of images are acquired during the scan and after the scan they are sorted into different phases of the cycle.

This method note concerns the second method, retrospective listmode imaging. There are two ways in which the redundantly large number of projections acquired in a listmode scan can be sorted into different phases of the cycle. These are time based and image based sorting, and are illustrated below in figure 1.

Time-based listmode sorting depends on physiological monitoring in an *in-vivo* scanner, providing a record of time-points of the cycle of breathing or the heart beat; for instance, each inhalation, or each systole. This time-recording needs to be at the millisecond level of precision.

The image based alternative sorts projection images into their phases of the cycle based on image analysis of the projections, for instance measuring intensity (attenuation) within a selected region of interest (ROI) in the lung or heart. Image based listmode sorting does not require time points from physiological monitoring, so unlike time-based sorting, image based listmode can be done on scans without recorded physiological data.

This method note describes how to apply image-based sorting of listmode scan projection images using the Dataviewer software. It takes the example of a lung – breathing listmode scan, but the same method can equally be applied to the cardiac cycle in scans with injected blood pool contrast agent to visualise the chambers of the heart.

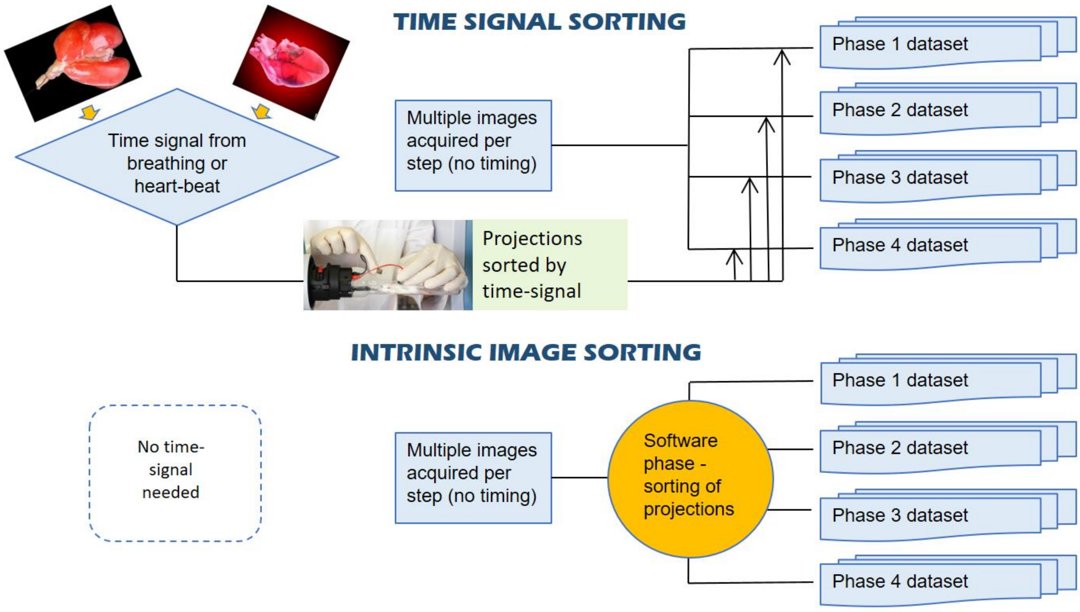
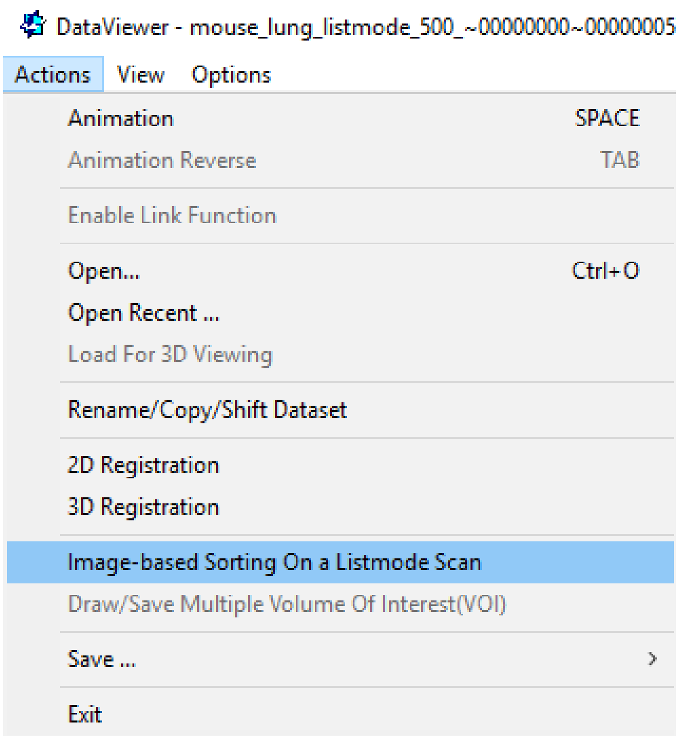


Figure 1. Schematics of the two approaches to listmode sorting of projection images in retrospective synchronisation or gating of lungs and breathing and of the heart and the cardiac cycle, from in-vivo microCT scans. Projection images can be sorted either based on physiological time signals (breathing or cardiac) acquired by monitoring of breathing and ECG heart monitoring, or by image analysis of mouse thoracic images and software-based sorting into breathing and cardiac (systole-diastole) bins. These two approaches – time based and image-based sorting, are illustrated above and below, respectively.

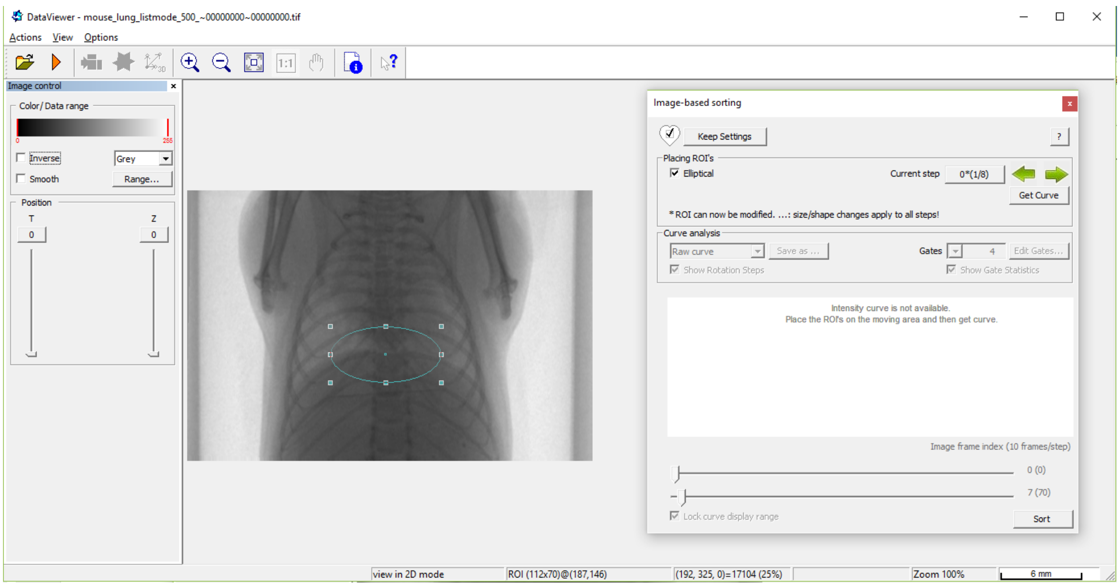
2. Method

2.1. Open Dataviewer and launch image-based sorting.

In Dataviewer version 1.5.4, image-based sorting is available in the Actions menu (see image below). Select “Image-based sorting on a listmode scan”:



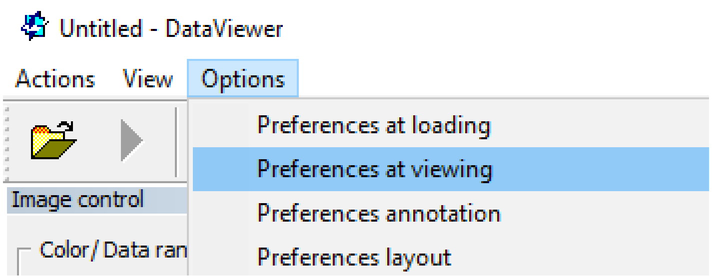
The window that opens will look like this:

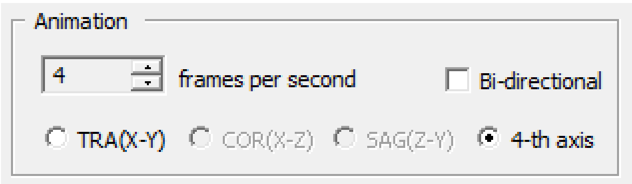


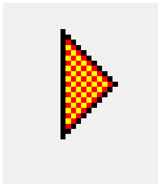
2.2. Animation viewing is important in image based sorting

During the process of setting up image based sorting, one needs to animate the displayed image – to scroll through all the different projection images at a particular rotation step to help position the region of interest (ROI).

Go to the Options menu “Preferences at Viewing” and under the “Animation” pane, set animation to the “4^{th} axis” – here this will be the time dimension in listmode sorting. Also set the animation speed in frames per second to about 4-5. Do not select “Bi-directional”.





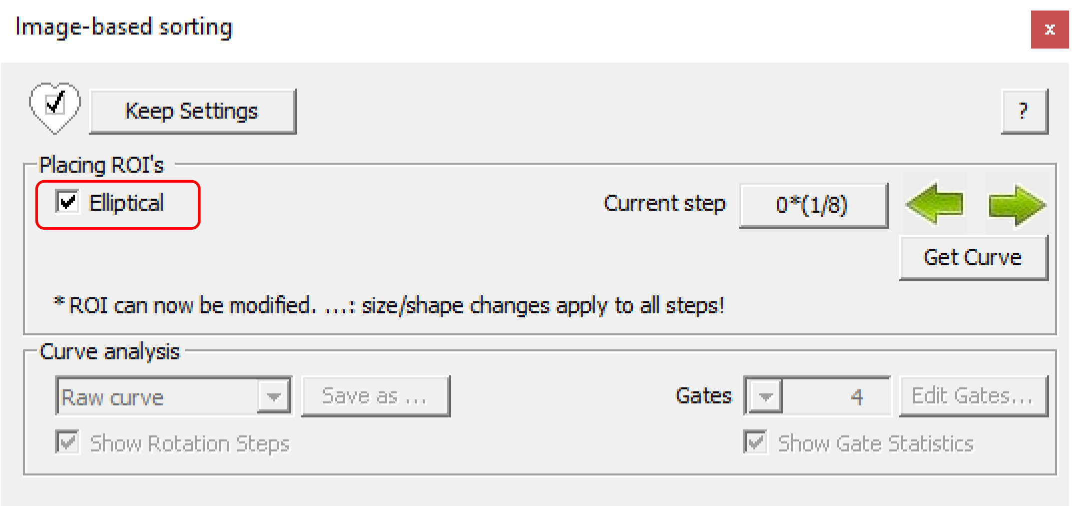


In the control window, under “ROI definition”, set to define ROI every π/4 radians, which means 45 degrees, or eight rotation positions if it is a 360-degree scan.

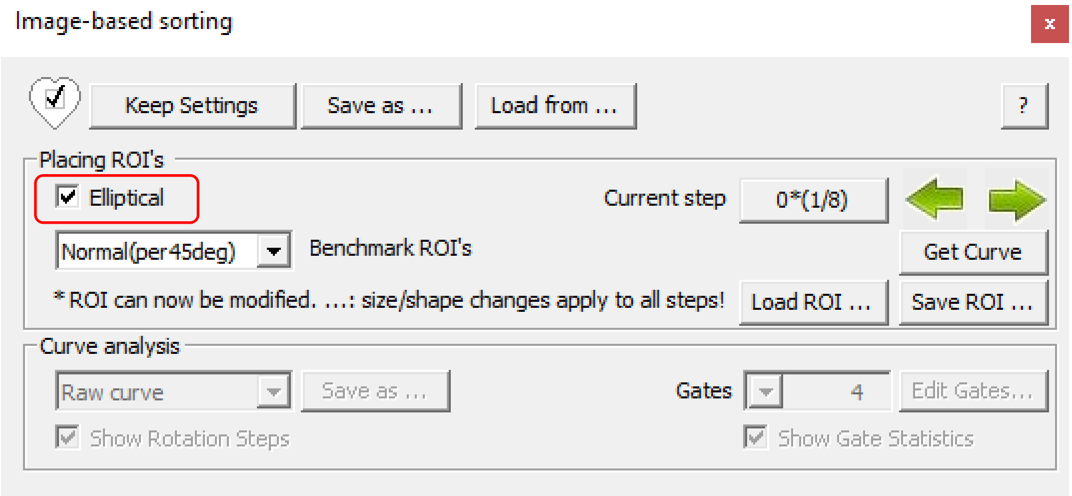
2.3. Choose how many angular views will be visited for ROI positioning

For image-based sorting to work best, a region of interest (ROI) needs to be set in each projection. It would take far too long to position an ROI in every projection. Therefore, the ROI is positioned in a small subset of projections, separated by a fixed angle. Assuming that the scan is 360 degrees for best image quality, then by default, the number of images visited for ROI positioning is 8 and the angle between them 45 degrees (or π/4 radians).

In Dataviewer 1.5.4 and above, 45 degrees (for 8 ROI positions) is the default. If you wish to change this number, go into engineering mode (Ctrl + 9). Then additional options will become active.



Engineering mode view:



The control window in Dataviewer 1.5.4 for image based sorting, without (above) and with engineering mode selected (below – Ctrl.+9). Note that the option exists for "Elliptical" shape of the ROI – as an alternative to rectangular. Elliptical is recommended.

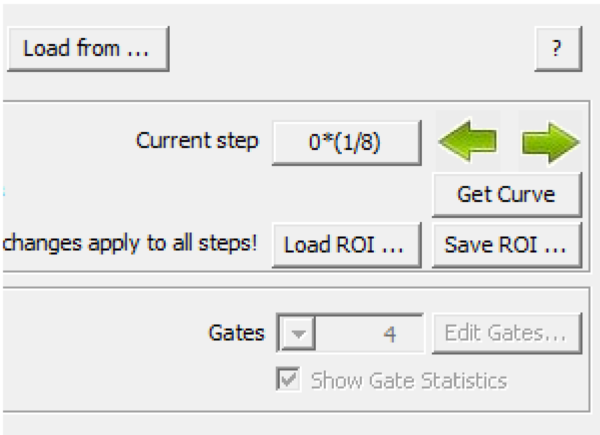
Important note: the size and shape of the ROI cannot be changed between the different viewing angles. Only the position of the ROI is changed by mouse drag-and-drop. So at the beginning, select a size and shape – for instance of ellipse – that will work for all viewing angles around the 180 or 360 degree scan. Since the projected view of the lungs varies in width, do not set the ROI too wide at first, otherwise it may be too wide for some projection angles.

In some versions of Dataviewer, with each move to the next rotation position (green arrow click) the ROI shape jumps into the bottom left corner of the displayed projection image. If this happens, then drag and drop the shape from there to the appropriate location.

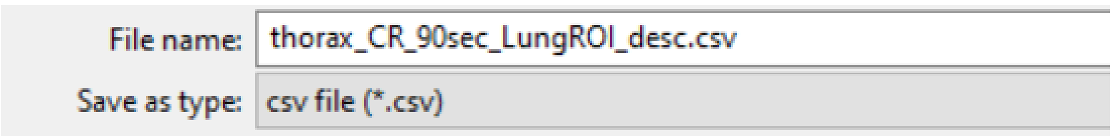
2.3.1. *Saving the ROI set*

Once the setting of the ROI position at all angle steps is complete, it is recommended to save the ROI. This will automatically be given a name depending on whether lung or heart is sorted. Remember that at the start the choice of lung or heart should be set in the menu at the top right of the sorting window.

Click on “Save ROI …”



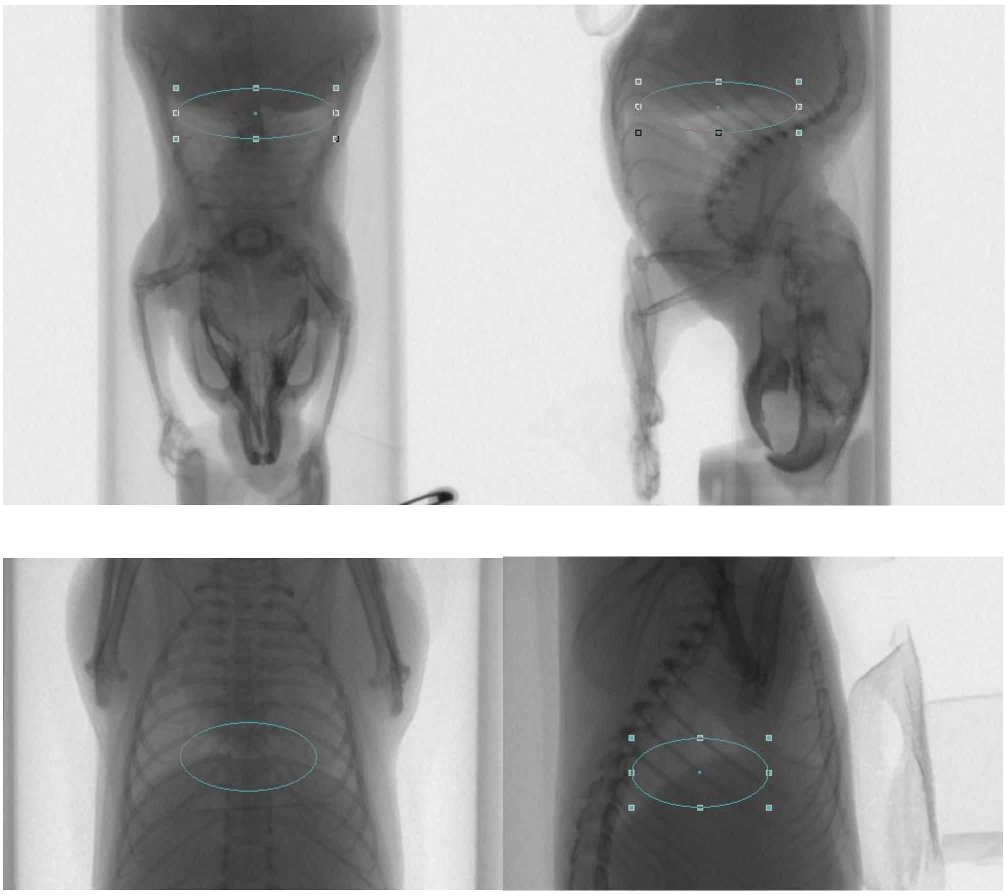
A file of this name will be saved by default in the case of lung sorting:



2.4. At each set position, place the ROI shape

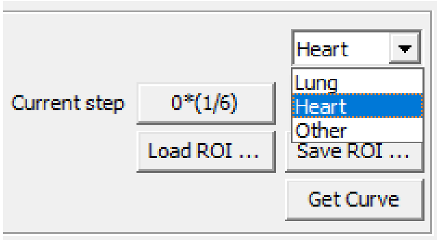
2.4.1. ROI positioning for lungs

The ROI shape that works well for mouse lungs is an ellipse. At each of those eight positions, set the ROI at the border of the posterior lung with the diaphragm as in the images below. The ellipse should be less than the diameter of the lung from any projection. You can position the ellipse with reference to the animated viewing of all the projections at the current rotation step and finding the part of the boundary between lung and diaphragm which shows the most conspicuous intensity changes associated with breathing. Including such places in your ROI will strengthen your breathing signal.

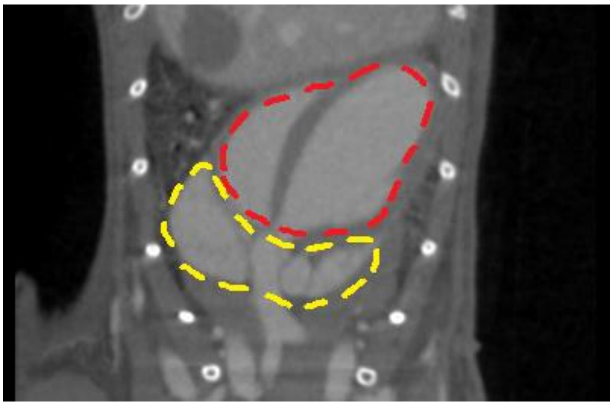


Note again: the size and shape of the ROI <u>cannot be changed</u> between the different viewing angles. So please choose a “compromise” shape that works at all angles.

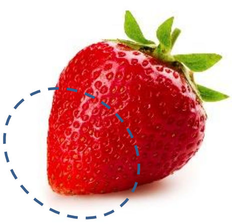
2.4.2. ROI positioning for the heart



To change the sorting from lung to heart, switch the menu selection at the top right of the image sorting window from lung to heart:

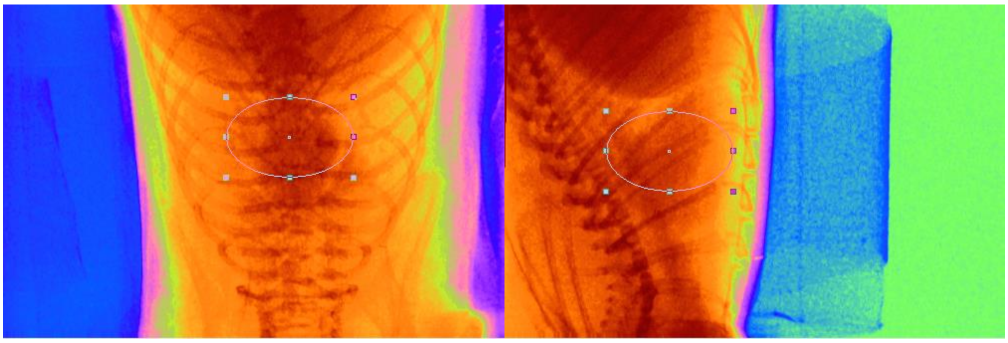


In the cardiac cycle the ventricular region (red outline) beats in different phase to the atrial region (yellow outline). The ROI ellipse used in image based listmode sorting should be placed carefully within the ventricular region for cleaner synchronisation with the main ventricular beat.



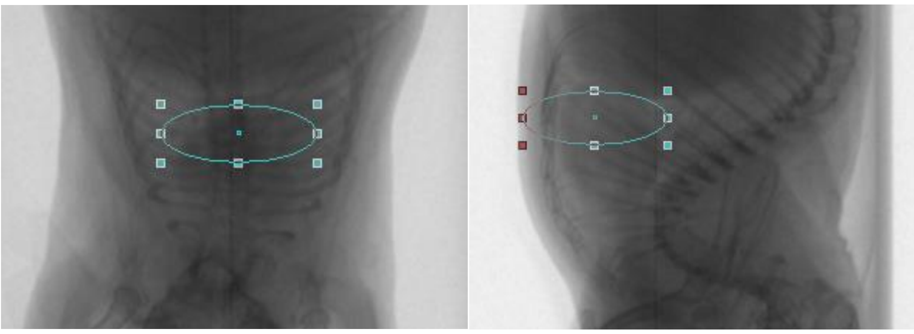
Think of the heart as having a shape of a strawberry. There is a wide base and there is a narrow tip with an overall triangular-like profile. You should position the elliptic ROI at the narrow-pointed end of the strawberry. This will select the ventricular end. You should avoid including any of the wide, “leafy” end of the strawberry in the ROI, since this would add an out-of-phase signal.

To visualise the heart in projection images, and to help accurate positioning of the ROI in the distal, ventricular end of the heart, it can sometimes help to add color-coding to the displayed projection image, using Dataviewer’s color palette.

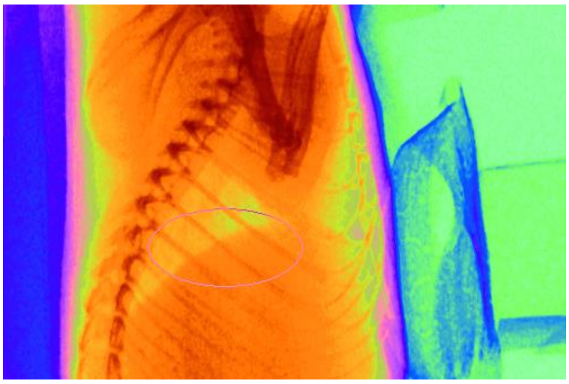


Note also – the ellipse of the heart ROI should if possible be kept away from the distal diaphragm boundary of the lungs. The diaphragm makes big movements with each inhalation, and thus a "contaminating" signal from breathing will enter the heart signal, if the cardiac ROI overlaps the diaphragm. Later in this method note, "dual gating" is described which reduces the mixing of heart and lung signal, improving cardiac image-based gating. (Dual gating applies to heart, not lung.)

Alternatively keep the images displayed in greyscale if this works just as well or better:

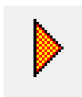


2.5. Animate through the projections at each ROI editing position, to help position the ROI



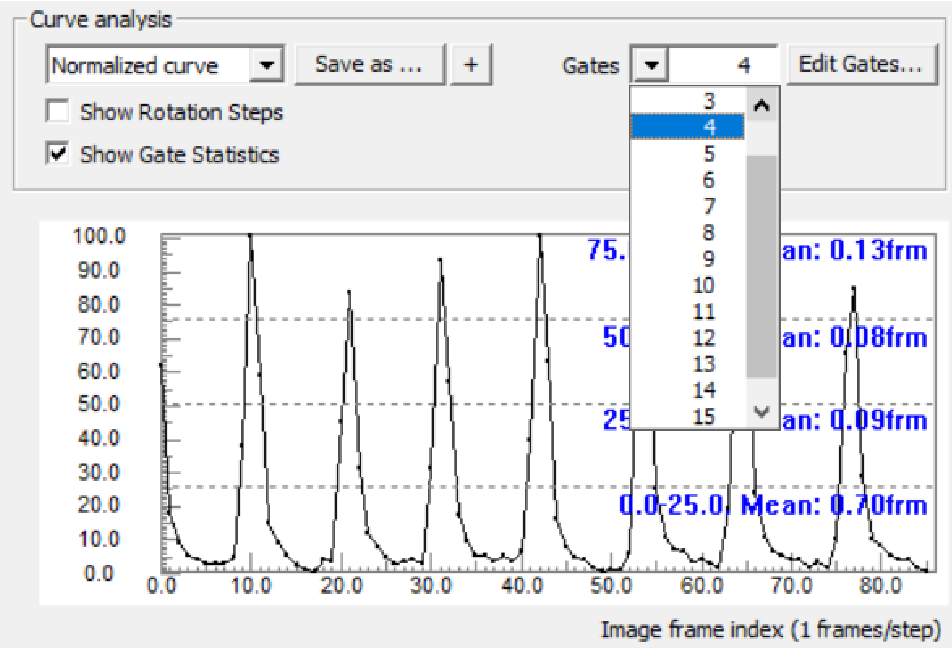
At each of the eight rotation positions, you can guide your choice of the ROI by animating through that step's multiple projections to see where the breathing movement is causing maximal intensity change, at the posterior border with the diaphragm. (Remember in "Preferences at viewing" to set animation to "4th axis".)

Sometimes color-coding the displayed projection can help to see the phase of breathing or heartbeat, which depending on the angle can be a little obscured by the ribs. Animation is started by the top play button:

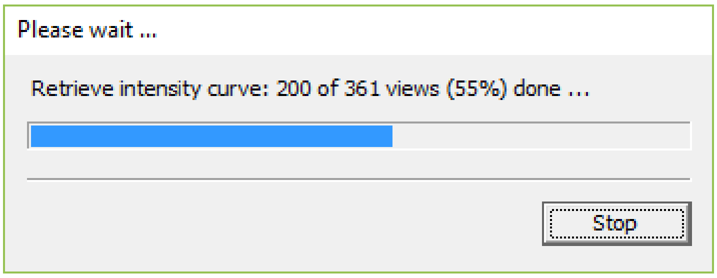


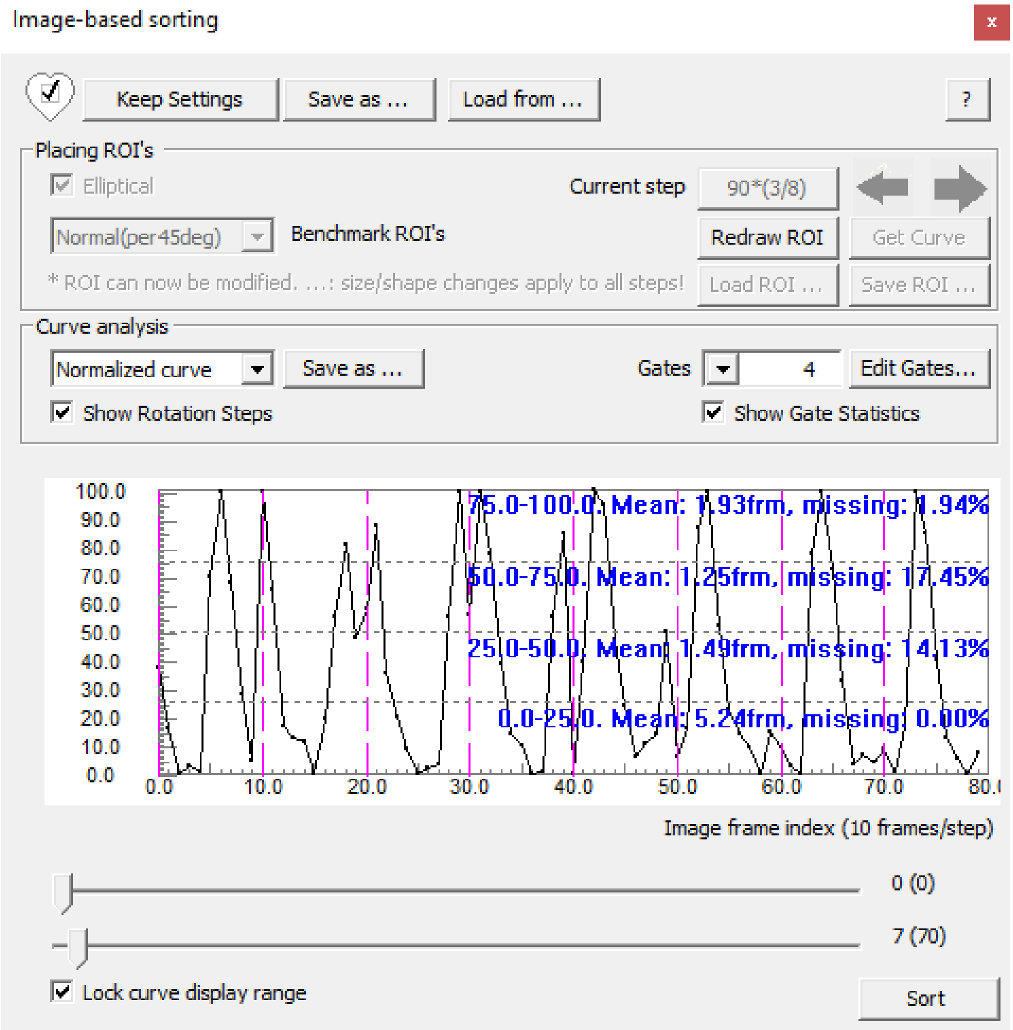
2.6. The number of gates and value range within gates can be changed

Gate number is 4 by default but can be reduced to 3 for instance, to increase signal to noise ratio or decrease the number of empty bins. Selection of the number of gates is by the drop menu after "Gates".



Note however that the window to "Edit gates", that is, change to a different number of gates, only becomes active after you have clicked on "Get curve" – see image below. Pressing "Get curve" will start the analysis of the ROI part of all projection images and the process of normalised sorting into physiological gates (breathing, cardiac). The following progress bar will denote this process:





2.6.1. *The image sorting stats: image number and missing percentage*

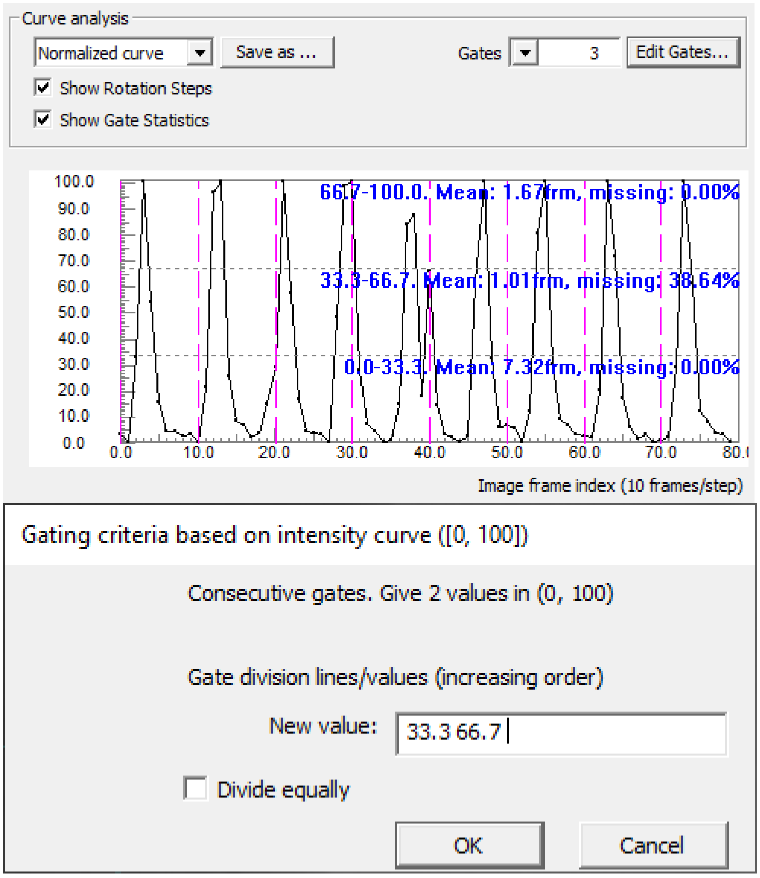
Once you've got the curve, the sorted bins are shown with the vertical (y axis) amplitude scale. Blue text on the bin dividing lines gives two values, first the average number of frames ("frm") per bin (the number each bin receives from the sorting) and second the percent of missing views. "Missing" means when a rotation step receives no projections in a given bin. Missing images are bad – this number should be small.

From the frame data it is clear the image based sorting is far from being socialistic or equitable. The lowest bin corresponding to exhalation gets the lions share – much more projections than the rest, because the exhalation phase is the longest one for a mouse under anaesthesia. This is a big difference between image based sorting and time-based sorting with T-sort, where sorting into equal time intervals assigns about the same number of projections into each sorted time-interval. Empty views should if possible be <10%. If the missing view percentage is too high you can reduce the number of gates, to 3 for instance.

2.6.2. Moving the gate intervals

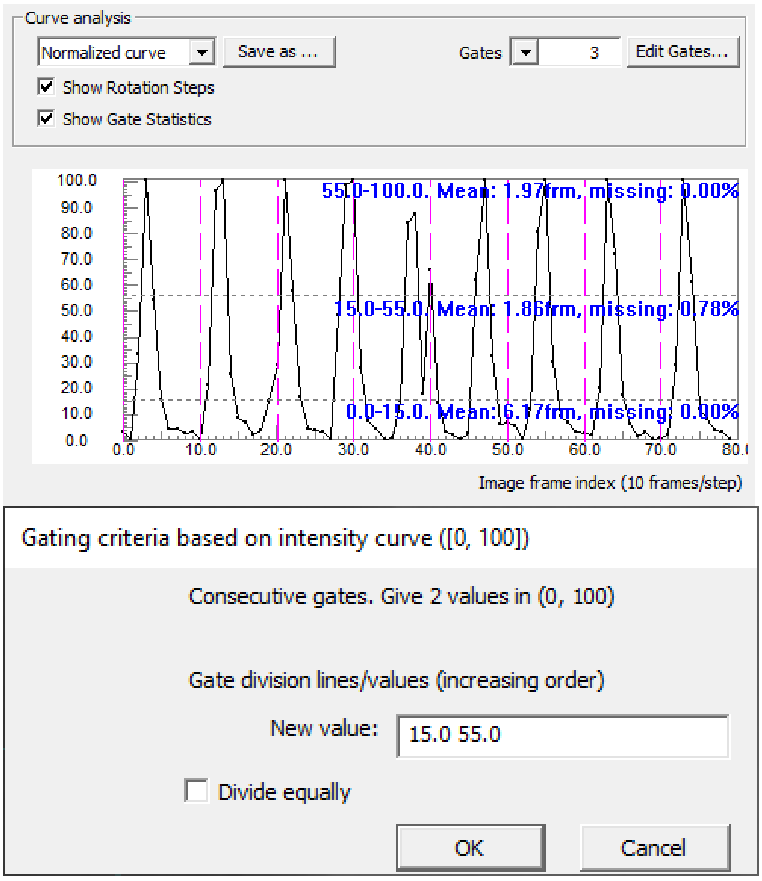
In the curve plot of image sorting, the vertical y axis is signal intensity, where higher means inhalation (more air in lungs) and lower means exhalation (less air in lungs). Along the horizontal x axis is time during the scan, with the scan start at the left.

By default, the breathing gates are divided evenly by signal intensity on the y axis. So that – in the example below – if 3 gates are chosen, the divisions between the 3 gates are at values of 33 and 67 percent.

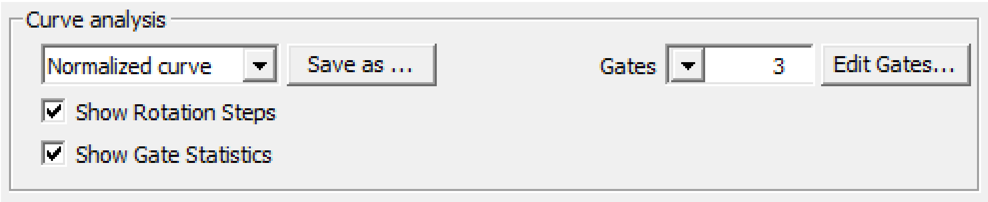


Take a look at the blue text on the curve plot. As mentioned above, in the default equal interval sorting, there are far more projections in the lowest interval (0-33% on the y axis) than in the higher bins. The lowest bin has more than 7 frames on average while the upper two bins have only 1-2 frames. The central bin has almost 40% steps that are missing any images (empty views). This is because under anaesthesia the mouse breathing cycle is not an even sine-wave but instead very asymmetrical. Inhalation gasps are short and the exhalation phase is much longer. Most of the time the lung is in a slow-moving exhalation phase, and the inhalation gasps are a much smaller percentage of the breathing cycle.

By contrast, the images below show the result of changing the boundaries in the 3 bins from 33 and 67 to 15 and 55 percent. The lowest bin becomes much narrower; even so it still has far more projections than the other intervals. However the upper bins now have more images and the problem of missing images in the central bin is solved – views with missing images are reduced to less than 1% fr all bins.

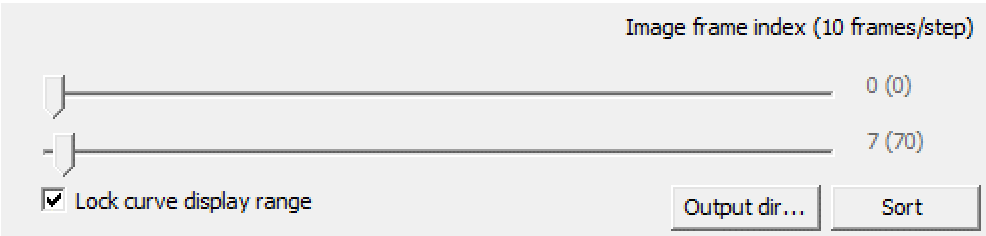


Once a normalised curve has been obtained, it can be saved, to allow it to be retreived for later analysis. This is done by clicking on “Save as” after the “Normalised curve” pane:

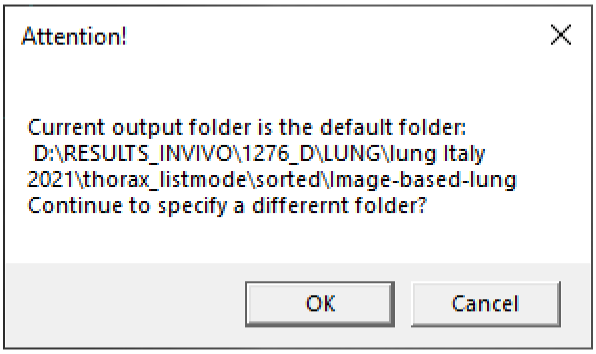


2.6.3. Changing the name of the folder for sorted projections

Finally, while there are default folder names for sorted results of lung and heart sorting, and also dual heart-lung sorting, the option exists for the user to set their own folder name for the sorting results. To do this, click on the button at the bottom for “Output dir…”.



On clicking this button the following confirmation message will appear:



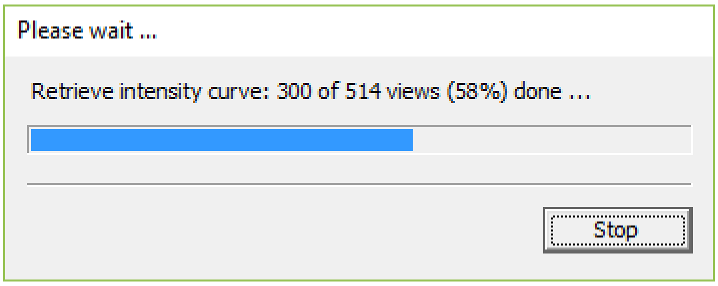
The window shows the default folder into which the sorting results will be saved. To stay with this default folder, click “Cancel”. But to change to a different folder with your choice of name, click on “OK” and proceed.

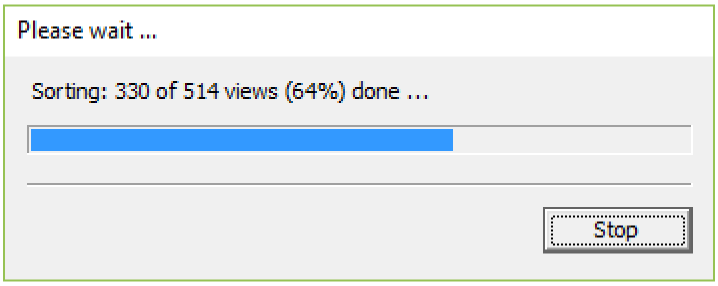
2.7. To run the sorting you can just click "Sort" or else first click "Get curve" – then sort.

If you click "Sort", it will start by getting the curve anyway – the same process that will follow if you click on "Get curve". However, clicking "Get curve" first gives you the option – if you require it – to change the number of gates, and to study the sorting results, before launching the sorting process.

Diagnostic information is shown on the curve window after the curve is obtained, showing in blue text for instance for each of the gates the number of rotation steps missing an image within that step – a parameter that in TSort is called "Empty bins". The lower this is, the better.

Using the sliders at the bottom of the window you can scroll across the whole scan, looking at the assigned gates for all images in all projections, at different zooming levels. The normalised attenuation values in the ROI are stretched between arbitrary values of 0 and 100 in the y axis of the displayed curve.





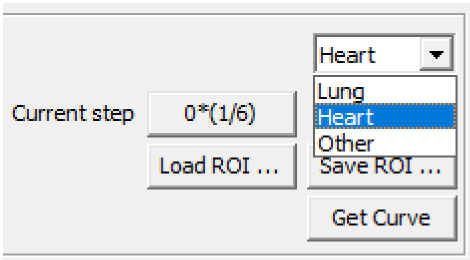
When you click "Sort"; two progress bars will run, first "Retrieve intensity curve", a detrending process, then "Sorting".

2.8. *Dual gating to both the cardiac and lung cycles*

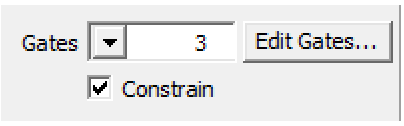
Dual cardiac gating for is also possible. This might at first seem a complicated option – however it's actually straightforward and can improve the results a lot. The purpose of dual gating for the heart is to remove the projections taken during the inhalation "gasps" of the mouse when the thorax movement is maximal. This takes advantage of the fact that inhalation gasps account for only a small percent of a mouse's breathing cycle under anaesthesia. The lung moves rapidly during inhalation and slowly during the longer exhalation phase.

For dual gating, the first step is to do the image sorting for lung, as described above. Once the lung image sorting is done, a subfolder will be created in the "Sorted" folder called "Image-based-lung".

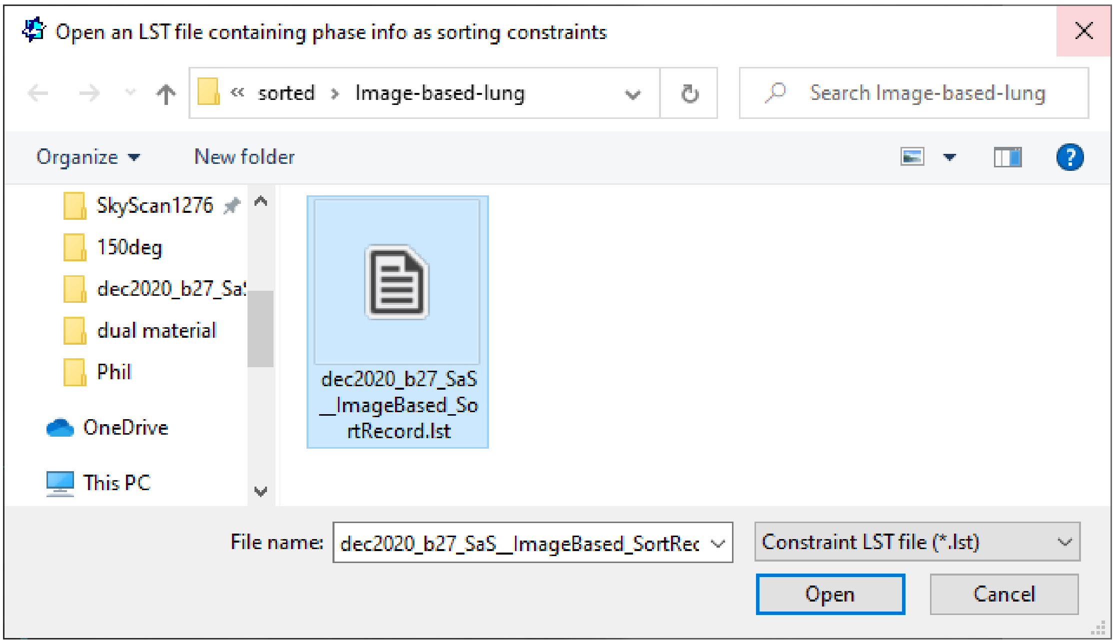
With the unsorted projection dataset still open, now change the menu selection at the top of the image sorting window from lung to heart.



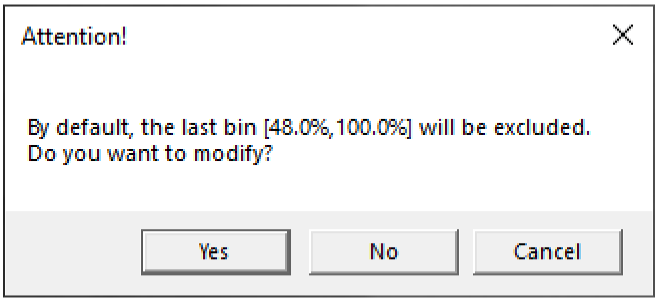
Now reduce the size of the ROI ellipse to be suitable for the distal ventricular heart region, as shown in the above section on heart sorting. Once the ROIs are selected (advancing between them with the green arrow button), click the tick box "Constrain":



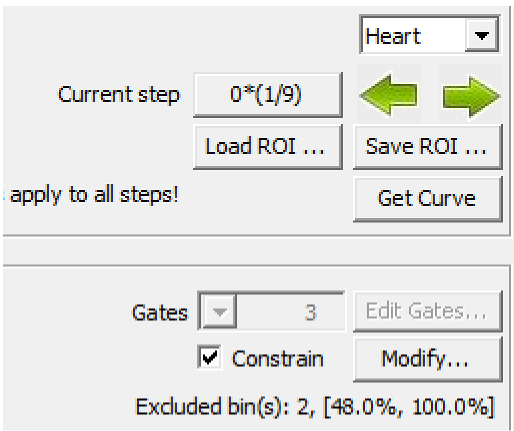
The next steps are partly automated, to avoid the need to search for the lung data to apply the lung constraint to the heart sorting. A window will open with the title "Open an LST file containing phase info as sorting constraints". However it will by default open in the “image-based-lung” folder that you have just created, and display the needed LST file from the lung sorting. Click on this file – it is the correct one:



On clicking on this lung LST file, this message will pop up: “do you want to modify?” The answer in the standard case should be “No”. We will accept the default option which is to exclude the last breathing bin which is the inhalation bin, on account of it’s having the highest air content and thus lowest mean intensity. So click “No”and move on:

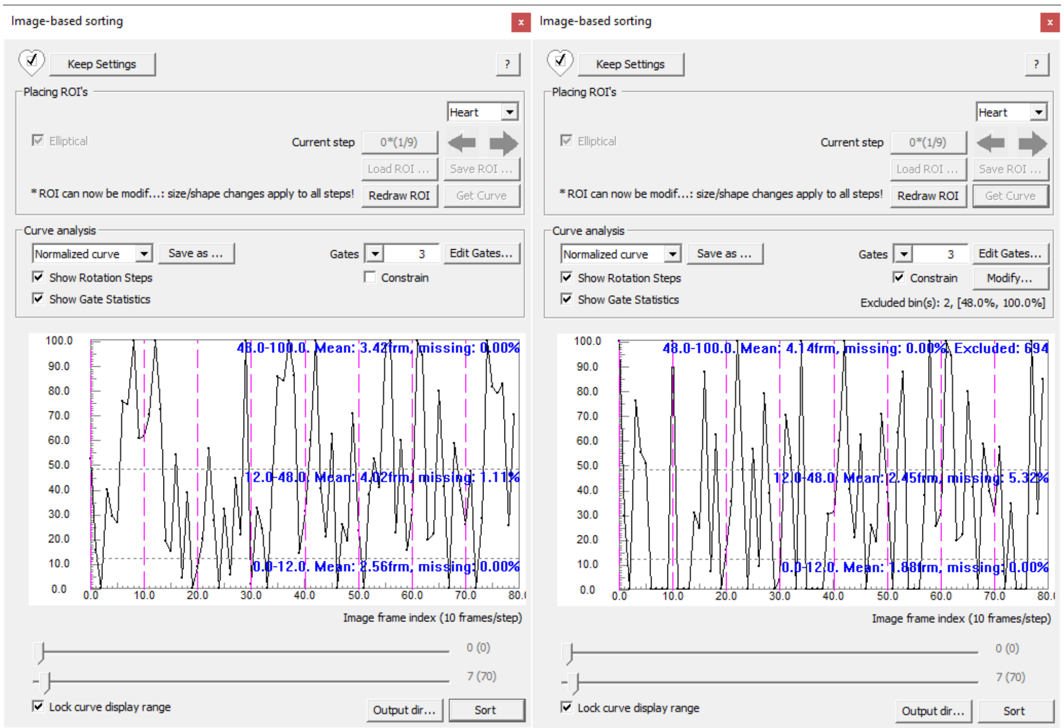


Now the details of the excluded bin will be shown below the (selected) constrain tick box.



Now we are ready again to click “Get curve”.

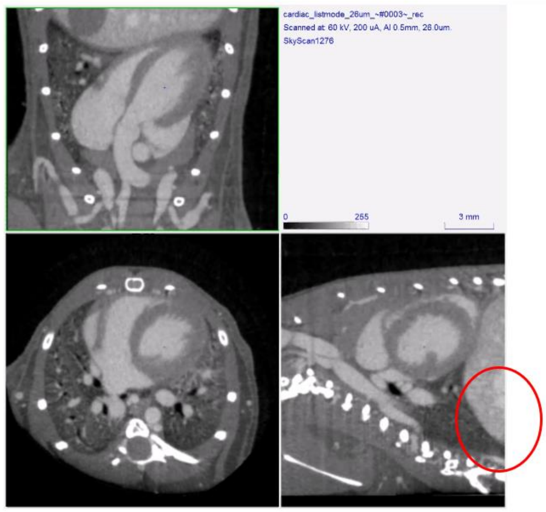
Shown below are the curves obtained by sorting for heart only (no constraint) on the left and the one obtained by dual sorting with lung constraint on the right. You can see that the event peaks are better defined and separated in the right hand window; this is the effect of removing the movement-affected inhalation bins with a resulting improvement in the accuracy of the heart phase sorting.



With the completed curve displayed, click finally on "Sort". In the latest versions of Dataviewer the dual sorted datasets (with constraint selected) will be sorted into a folder within the "Sorted" folder named "Image-sorted-dual". In earlier versions of Dataviewer this auto naming of the dual sorting results is not done so you will have to manually set a different name for the dual sorting results – unless you are happy for the dual projections to be over-written over the projections from unconstrained heart sorting.

The images below compare single sorted heart reconstructions (left) with the corresponding dual sorted reconstructions (right) with the inhalation breathing phase edited out. You can see – for instance in the red circle at the diaphragm – that the dual sorted images are a little clearer and less blurry:

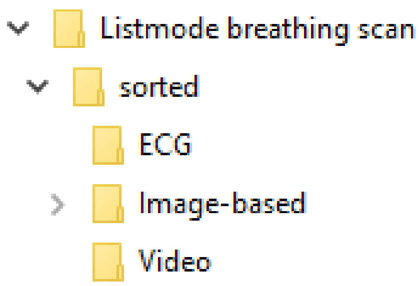
Intrinsic gated to cardiac only



Intrinsic gated to cardiac and lung

2.9. Sorted images are stored in a subfolder path Sorted\Image based

The image-sorted projections will be saved in the subfolder called “Sorted” and a further subfolder “lung-image-based” or “heart-image-based” or “Dual”, to distinguish them from sorted images from video-based time sorting or ECG based time sorting, which are in accordingly named sub folders also within the “Sorted” folder. On saving sorted projections you have the option to choose your own subfolder name – such as “Lung-15-55” (to denote gate intervals for instance). These newly saved images can now be reconstructed in Nrecon.



2.10. Filename and numbering convention of listmode scan projections

The naming convention is unchanged from the program TSort for sorting time-based gated scans (see method note 5).

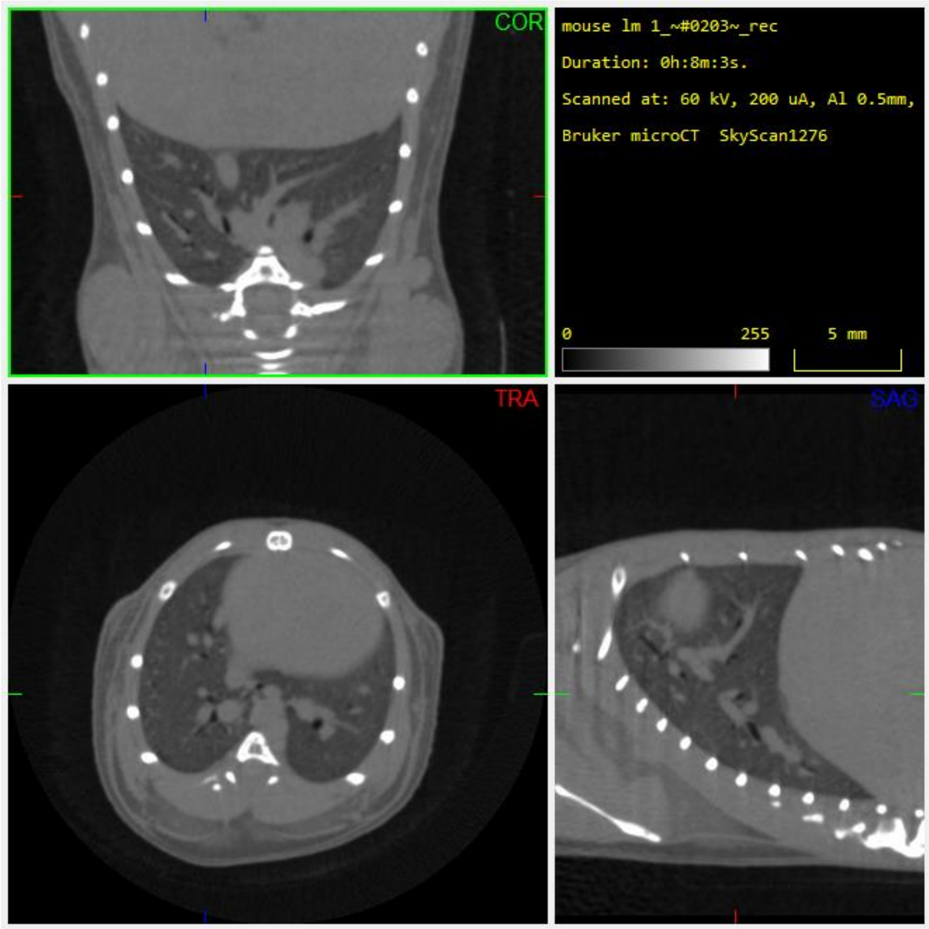
mouse_lung_listmode_500_~00000000~00000000.tif	mouse_lung_listmode_500_~#0004~00000000.tif
mouse_lung_listmode_500_~00000000~00000001.tif	mouse_lung_listmode_500_~#0004~00000001.tif
mouse_lung_listmode_500_~00000000~00000002.tif	mouse_lung_listmode_500_~#0004~00000002.tif
mouse_lung_listmode_500_~00000000~00000003.tif	mouse_lung_listmode_500_~#0004~00000003.tif
mouse_lung_listmode_500_~00000000~00000004.tif	mouse_lung_listmode_500_~#0004~00000004.tif
mouse_lung_listmode_500_~00000000~00000005.tif	mouse_lung_listmode_500_~#0004~00000005.tif
mouse_lung_listmode_500_~00000000~00000006.tif	mouse_lung_listmode_500_~#0004~00000006.tif
mouse_lung_listmode_500_~00000000~00000007.tif	mouse_lung_listmode_500_~#0004~00000007.tif
mouse_lung_listmode_500_~00000000~00000008.tif	mouse_lung_listmode_500_~#0004~00000008.tif
mouse_lung_listmode_500_~00000000~00000009.tif	mouse_lung_listmode_500_~#0004~00000009.tif
mouse_lung_listmode_500_~00000000~00000010.tif	mouse_lung_listmode_500_~#0004~00000010.tif

Left: unsorted projections. Right: sorted projections.

Images produced by sorting are distinguished from unsorted images by the # symbol, followed by four digits; the first two mean the number of the sorted phase (the first one is always 0) and the second two digits mean the number of sorted phases, e.g. 4.

2.11. Use gas anaesthesia flow rate to get mouse breathing in the range 1-1.8 breaths per second

The correct rate of isofluorane gas anaesthesia can improve listmode scan resuls for the lung (and also heart). For breathing, the best breathing rate is in the range 1-1.8 per second. The breathing rate is displayed in the physiological monitoring window in both the SkyScan 1276 and 1278.



Tri-axis view in Dataviewer of inhalation phase of mouse lungs, usin image-sorted Listmode.

Volume rendered images of listmode-sorted mouse (left) and rat (right) lungs.