# Applying Hounsfield unit density calibration in SkyScan CT-Analyser

**Method note**

**MN-39**

Hounsfield units (HU) are a standard unit of x-ray CT density, in which air and water are ascribed values of 0 and 1000 respectively. It has been found over several decades of CT imaging to be a useful general CT density calibration owing to the approximate linearity of the calibration curve of materials of different x-ray density, such that calibration with water and air data points gives a useful and highly reproducible reference curve even for materials (such as biological calcified tissue) with CT density significantly higher than that of water.

It is conventional to displace the HU value downwards by 1000 units, so that the values for water and air are 0 and -1000 respectively. SkyScan CT-analyser allows you to either adopt this convention or not, as you choose.

You may have noticed that HU calibration is possible in SkyScan scanners at the point of image reconstruction. (This involves HU calibration factors in the scanner control software.) The HU calibration done by CT-analyser is a second, alternative option, for *post-hoc* calibration of a dataset after reconstruction, by reference to a scanned phantom (tube of water). It is probably an easier, more flexible and more accurate method, and how to do it is set out in this method note.

## Before you scan the object

The ability to accurately measure density from a micro-CT scan of an object is not something that can be taken for granted. Certain artefacts of micro-CT imaging can compromise density measurement. An exact correlation between apparent grey level in the reconstructed image and object density cannot be assumed. Several key points should be considered:

- **Beam hardening**. This relates to the wide x-ray energy distribution from a micro-CT x-ray source, and selective removal of low energy photons as the x-rays pass through your object.

Uncorrected, this phenomenon gives the surface layer an exaggerated high density. If you scan an object to measure density, you should minimise beam hardening by (a) applying an aluminium filter during the scan, and (b) applying the correct beam hardening correction during reconstruction. SkyScan NRecon software includes a profile line tool useful for assessing beam hardening and optimising its correction. Also, keep the applied voltage to the minimum needed to give adequate transmission through your object.

- **Sample hydration**. If the sample is wet, such as a piece of biological tissue, it is very important to maintain a level of hydration close to natural during the scan, and prevent drying. Drying in particular will cause problems, both by causing inaccuracy in density measurement and by causing movement artefacts in the scan due to shrinkage. So wet biological samples should be maintained moist (in a physiological 0.9% saline if possible) and enclosed to prevent drying. Drying can be prevented either by wrapping in parafilm or "cling wrap" plastic (PVC-free if possible), or by scanning the object inside a narrow plastic tube of water or saline.

- **Streak artefacts, 360° scanning**. Scans over 360° will also give a slightly more uniform image density than over 180° (removing asymmetric beam hardening). In some 180 degree scan reconstructions, a high density object surrounded by lower density medium can cause dark streak artefacts around the dense object. These can be minimised by scanning over 360° rather than 180°. This will improve the accuracy of density measurement. Note however that for 360° scans it is important to check that the post-alignment correction has the right value.

- **Apply sufficient smoothing**. If an image is too noisy then the density peaks on the attenuation histogram are widely spread out,

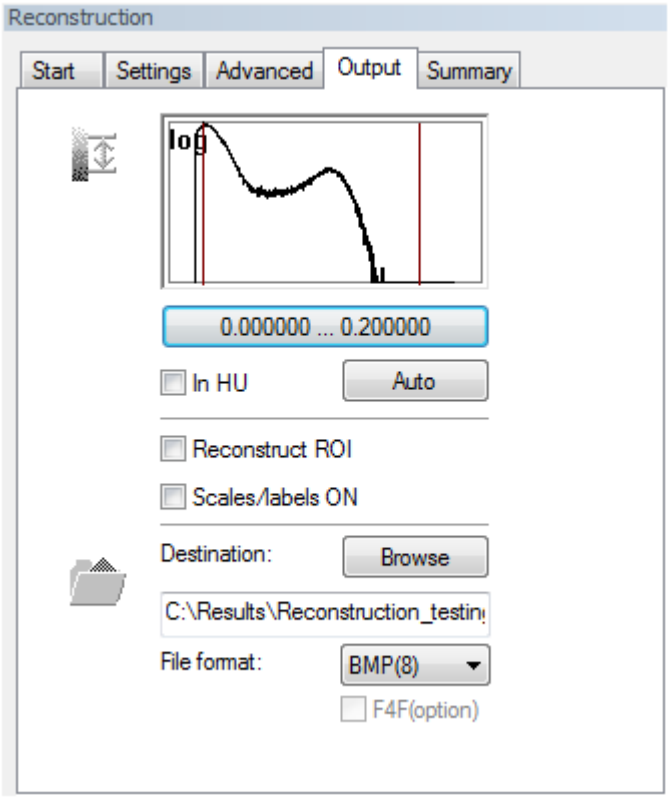
compromising the accuracy of density measurement. Smoothing should be applied in NRecon to remove excess noise from the image. For density measurement you do not really lose any image data by doing this, you can in fact improve the image quality.

## Steps to apply HU calibration

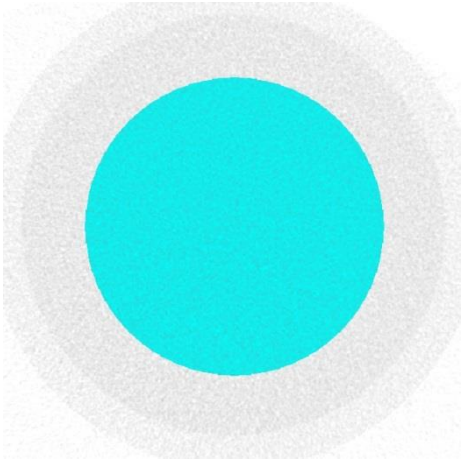
The steps to calibrating density in a sample scanned by a SkyScan micro-CT instrument are as follows:

1. Scan your sample (noting points 1-3 above for scanning for CT density). Reconstruct the dataset, taking care to minimise beam hardening, and find and apply the optimal beam hardening correction. When setting contrast limits in reconstruction, the lower limit should be zero (this simplifies density calibration). The upper limit should be above the upper end of the sample absorption histogram, so that minimal saturation occurs in the image (i.e. minimise the number of pixels reaching the maximum density 255, black "grey level"). Note that you can double-click on the density histogram in NRecon to change the y axis to a logarithmic scale.

2. Make your micro-CT HU phantom. This is a plastic tube filled with water, with a diameter about the same as the sample object(s) you are scanning. The plastic should be low density – polypropylene, acrylic, polystyrene or polycarbonate are acceptable (PVC is not).

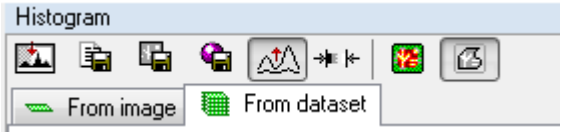



3. Scan the water phantom, and reconstruct it. All the parameters of the scan (tube settings, rotation step, etc.) and all the reconstruction settings including contrast limits, and beam hardening correction, must be identical between the scanned sample and the scanned water phantom. Thus the reconstructed water images will look quite pale if your object is more dense that water – this is OK. The one exception is that post-alignment correction is unique to each scan, and differences between scans in post-alignment do not compromise comparative measurements of density (or morphometry). This parameter is automatically calculated by NRecon and should be individually optimised for each scan.

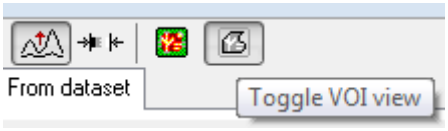


4. Open the reconstructed dataset of the water tube in CTAn. Select a circular region of interest (ROI) of water as shown (right). Make this into a volume of interest over a number of layers of water. It is best to use image levels with water only, not levels containing a denser object (e.g. bone) surrounded by water, since the dense object can slightly change the density of neighbouring water.

5. Having selected the water volume of interest (VOI), go to the next, binary page of CTAn. Above the density histogram are two tabs, "from image" and "from dataset". Click on "from dataset" to integrate the density histogram over all crossections.

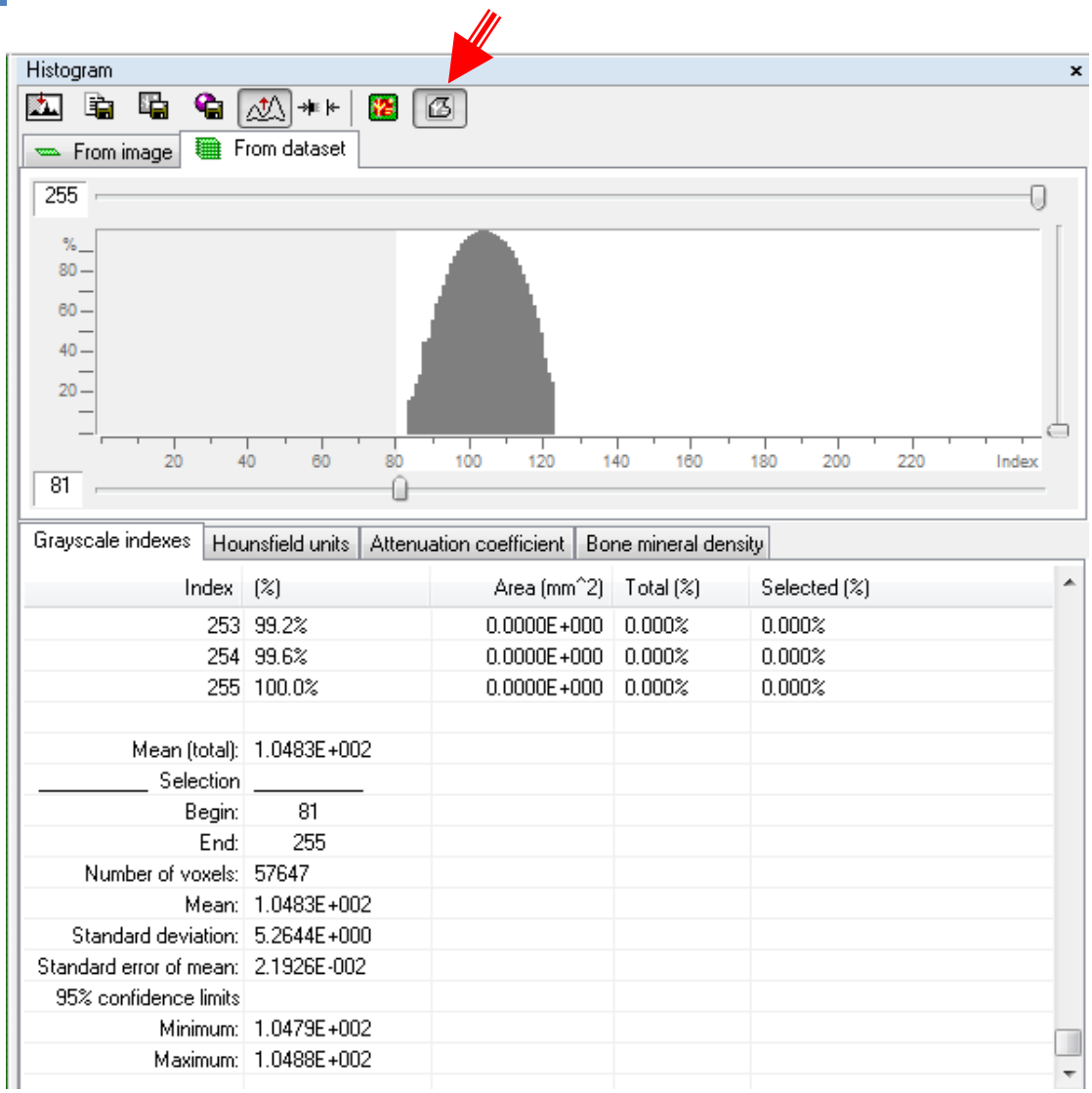



6. At the right end of the buttons under "Histogram" is the button "toggle VOI view". Please make sure that this button is pressed. This will make the density measurement correspond to the region/volume of interest. (If it is not pressed, then the output density values will be taken from the whole dataset, without reference to the VOI, resulting in a calibration error.)



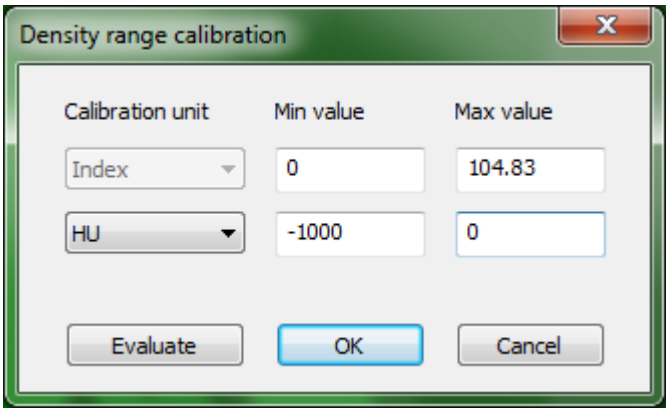

Note that clicking on the log button  above the histogram display, can make the histogram easier to see.

7. Open the HU calibration window by pressing on the button  above the density histogram – indicated by the red arrow in the image below. The HU calibration box has three columns of paired boxes, for corresponding values of grey level ("index") and

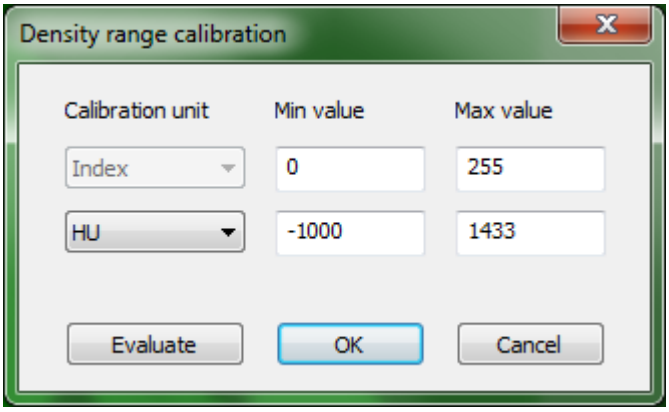



The density page in CTAn for applying HU calibration. With the VOI of water selected, and the average grey level of water displayed as "Mean (total)", the HU calibration button can be pressed (red arrow).

As indicated in the images below, enter values 0 for grey level and -1000 for HU in the first column. (Remember during NRecon reconstruction you set the contrast minimum to zero, so that zero greyscale corresponds to zero attenuation.) In the second column, enter the measured mean grey value for the water VOI – here 104.83, and the corresponding HU value for water, which is 0.




Click on OK to implement the calibration – the window will close.

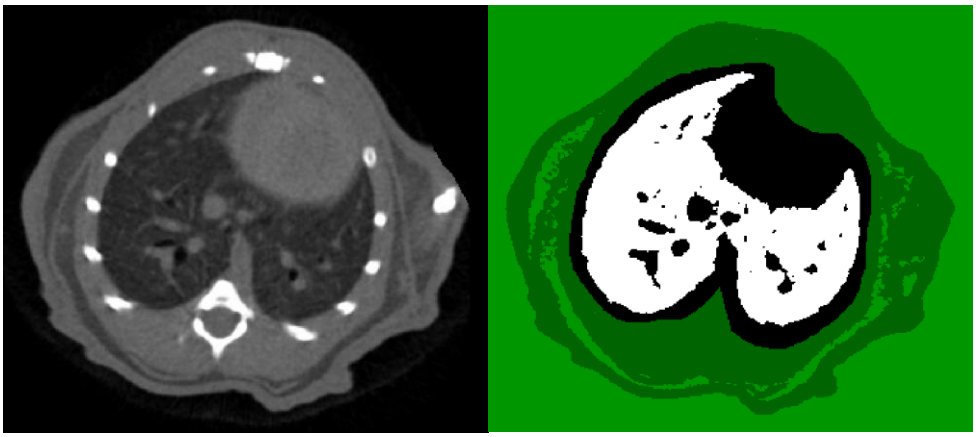



Please note that on reopening the HU calibration box, the numbers will have changed as shown in the image (left). However the calibration you entered is unchanged, but the displayed numbers have just been normalised to show the maximum density range, corresponding to the extreme grey level values of 0 and 255. If you make a note of the HU number shown above – 1433 – which corresponds to the 255 grey level, this number will allow you to implement the same HU calibration on other datasets – such as your scanned sample or animal datasets. This number, here 1433, is called the HU calibration number.

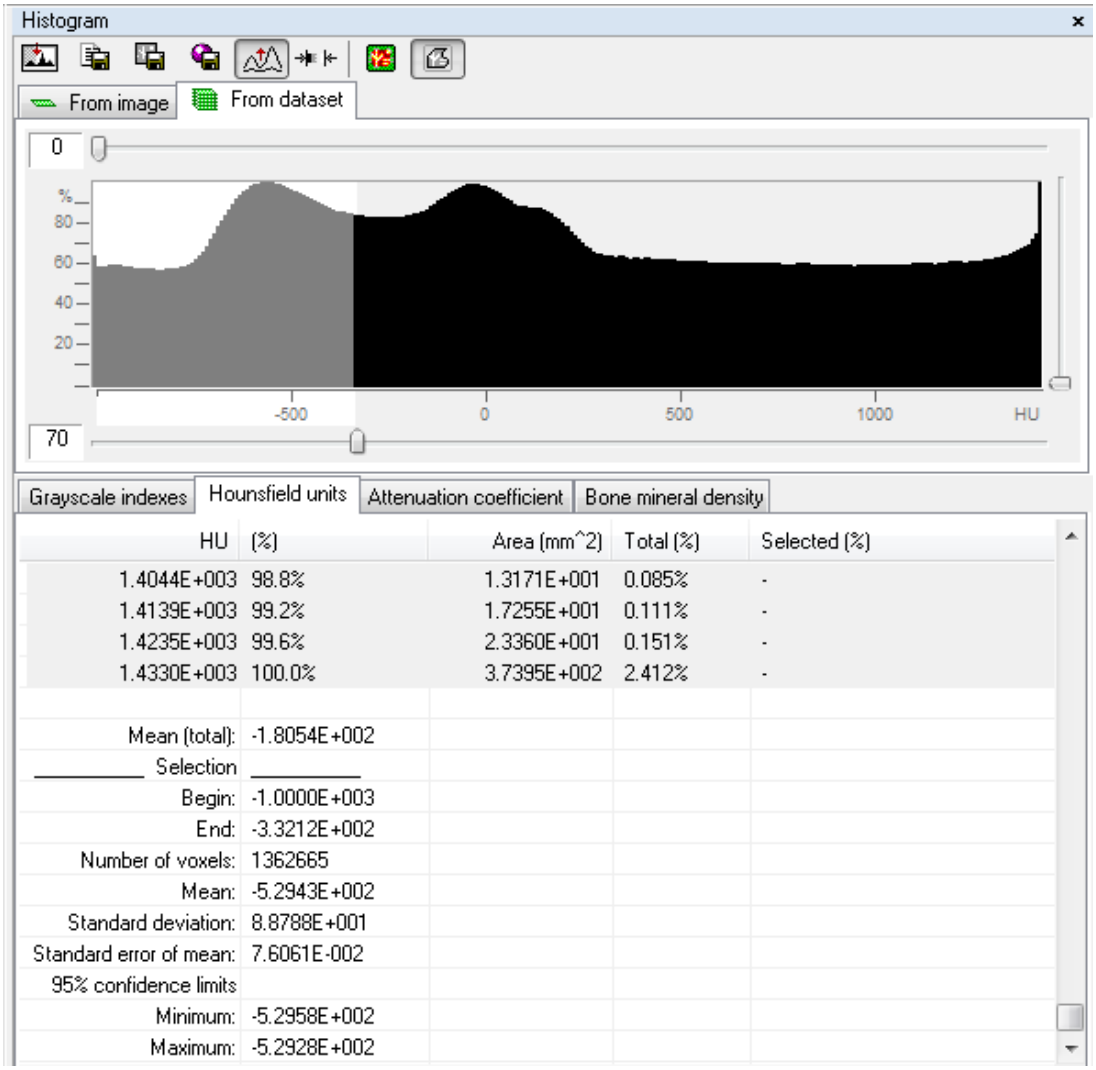
8. Apply the HU calibration from the water tube, to your measured sample. Open the sample scan dataset in CTAn, go to the binary page, and click on  which will open the "density range calibration" window, and enter the values exactly as in the second such window shown above (i.e. 255 grey = 1433 HU). Remember to set also the HU value of -1000 to correspond with the grey scale

value of 0.

9. Now the sample scan dataset is calibrated to HU. To measure HU within a volume of interest (VOI), select a VOI from your sample – the example below shows a mouse lung. Then go to the density page of CTAn and click on the "from dataset" tab to show the density histogram. Also make sure the "toggle VOI view" button is pressed.

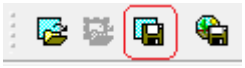


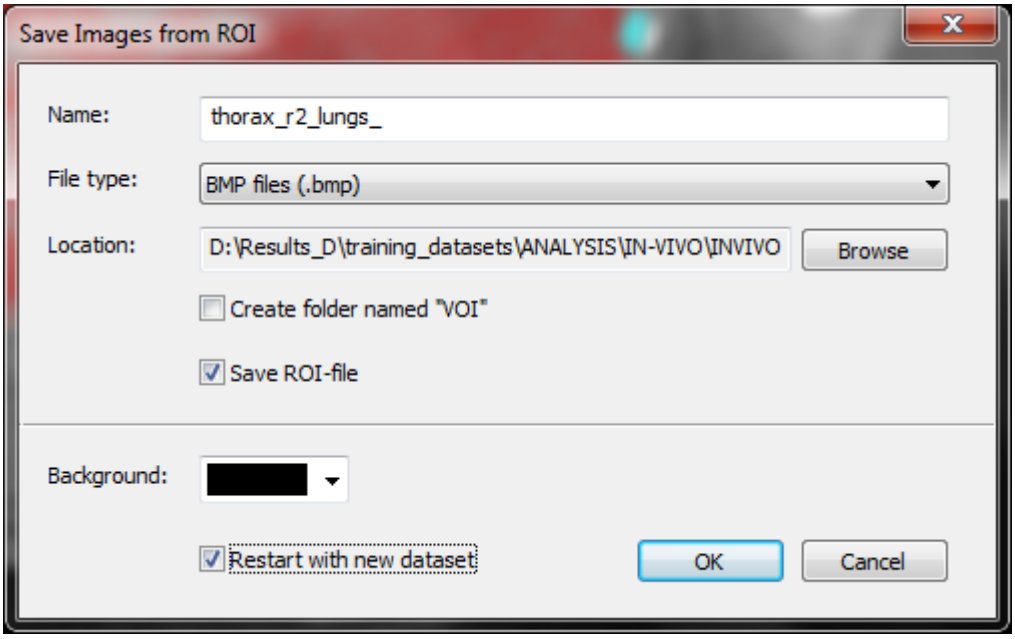
Then read the density value in calibrated HU for the manually selected lung ROI (black shape around the lung in the image above right) from the summary data. The value as shown below is -529.43 HU. Note that since we want to measure HU in the binarised white lung pixels only, then the HU value is read from the mean under the "Selection" heading, not the "mean (total)" which would output HU from the whole black area.



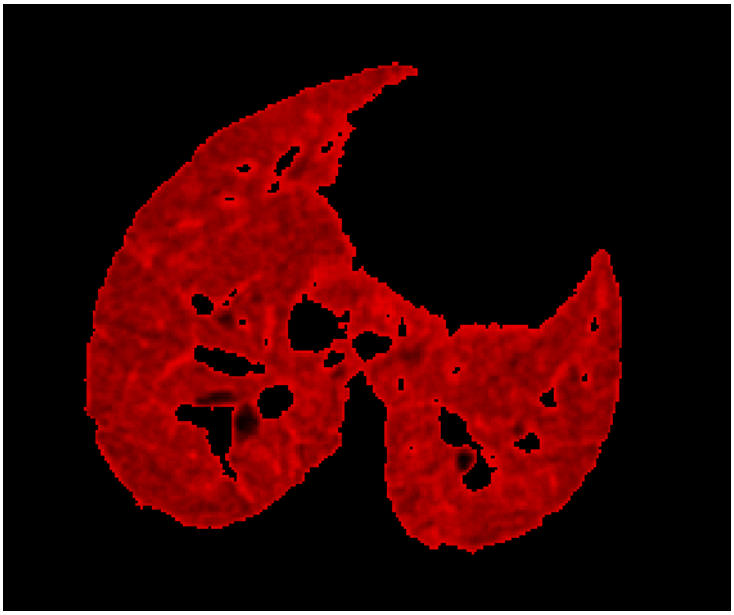

10. **Saving your calibration**. To apply the HU calibration that you have obtained from the water phantom to a sample dataset, you should record the HU number corresponding to the maximum grey level 255. In the example given above, 255 grey = 1433 HU. For example you can save it in a text file.

11. **Saving a calibrated dataset**. You can "embed" a HU calibration into a dataset in the following way. First apply the HU calibration from the button, as described above. Then go to the ROI page in CTAn, and select a volume of interest. This can either be a part of the dataset or the whole dataset (in the case that you choose no

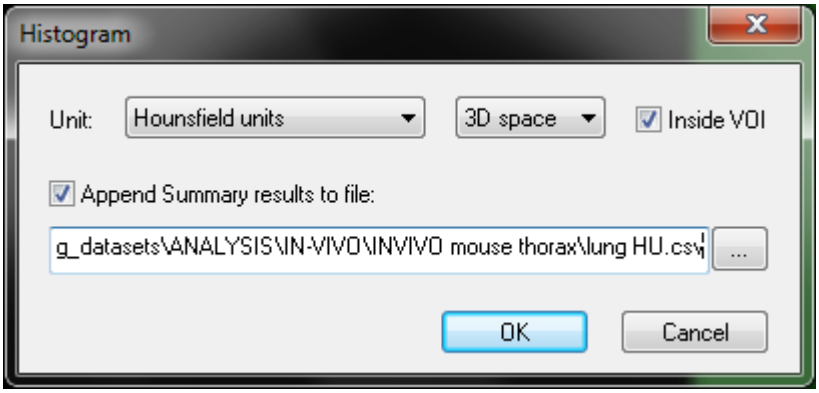
ROI). Then save a new dataset from the ROI – using the fourth of the ROI function buttons  (see the window below). This new saved dataset will have the HU calibration that you just applied embedded in it (written into the text header of each bmp file). So when you re-open this new dataset you will not need to apply the HU calibration – the correct HU calibration will already be set.




12. **Measuring HU from custom processing**. Once a HU calibration has been made for the open dataset in CTAn, density as HU can also be output from the custom processing. Here for example one can import the greyscale lung image into a binary mask of the lungs (see image below).



With the ROI set as the lung binary mask as shown in the above image, density is measured and output in custom processing using the "histogram" plugin:




The HU density data is output in the report file with the following format:

| | | | | | | |
|---|---|---|---|---|---|---|
| 2 | ---------- | | | | | |
| 3 | [ 05/26/14 11:52:22 ] Histogram (3D space) inside VOI | | | | | |
| 4 | Unit: | HU | | | | |
| 5 | Dataset name | -1.00E+03 | -9.90E+02 | -9.81E+02 | -9.71E+02 | -9.62E+02 |
| 6 | \INVIVO mouse thorax\mouse_thorax_r2\thorax_r2_cut_ | 1320 | 732 | 714 | 689 | 723 |
| 7 | Mean | Standard deviation | Standard error of mean | 95% confidence limit (minimum) | 95% confidence limit (maximum) | |
| 8 | -5.2943E+02 | 8.8788E+01 | 7.6061E-02 | -5.2958E+02 | -5.2928E+02 | |
| 9 | | | | | | |