PHASEQUANT (User Manual)
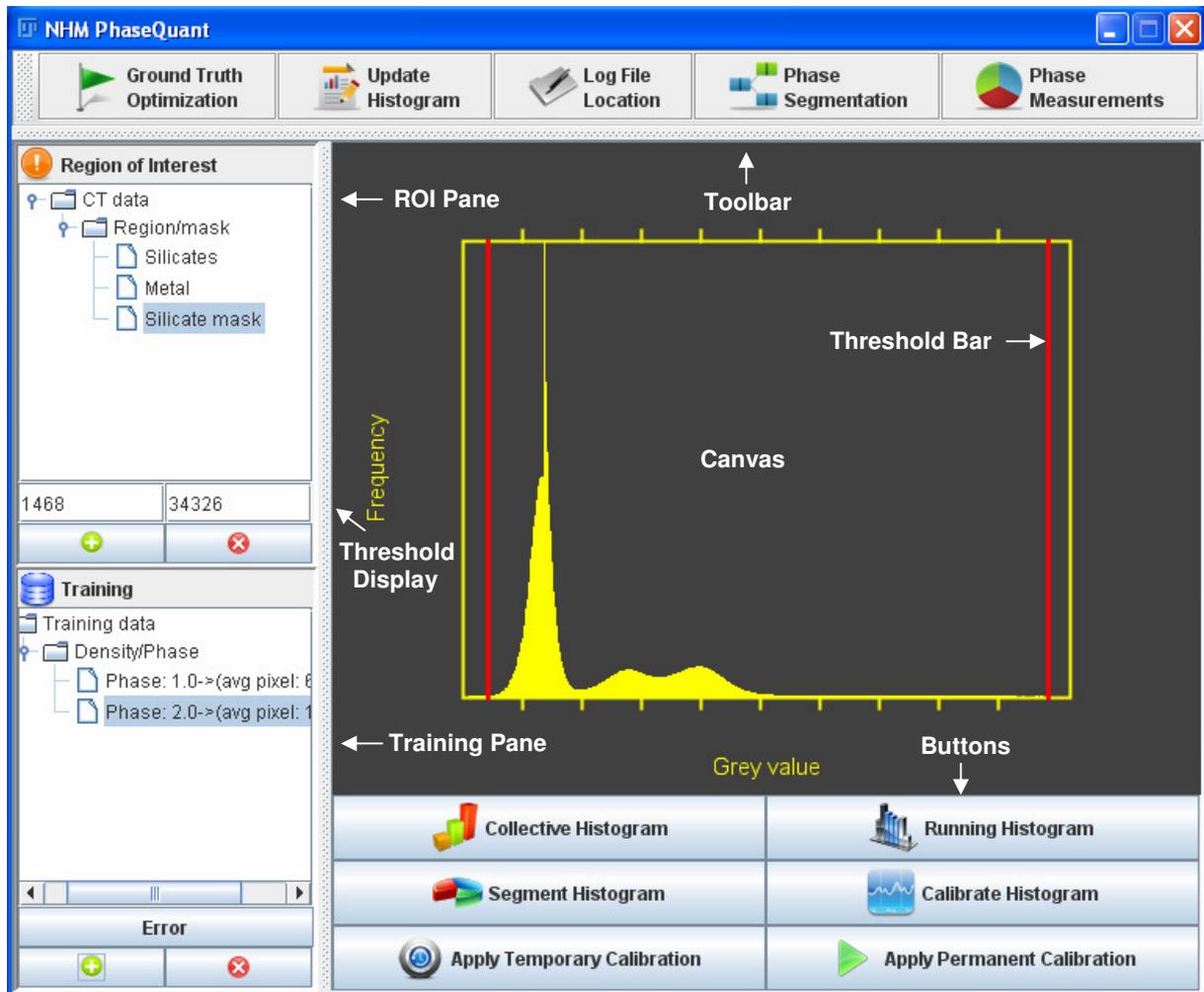

Fig 1. PhaseQuant GUI

1. INSTALLATION AND LOADING

1.1 Installing PhaseQuant

➢ Download the zip file from the following link

http://imagejdocu.tudor.lu/doku.php?id=plugin:segmentation:phasequant_:start

➢ Unzip the contents to the 'ImageJ/plugins' folder

➢ Restart ImageJ

➢ PhaseQuant can be found in NHM folder under plugins menu

**Note:** PhaseQuant will also install Object_Counter3D plug-in from http://rsb.info.nih.gov/ij/plugins/track/objects3.html.

Video Demo: http://www.youtube.com/watch?v=RF5HVUwDHaY

1.2 Loading PhaseQuant

➢ Open an image or stack

➢ Load PhaseQuant

Note: PhaseQuant will not load without an active image or stack in ImageJ

Video Demo: http://www.youtube.com/watch?v=ZdUDa1PPc0g&feature=related
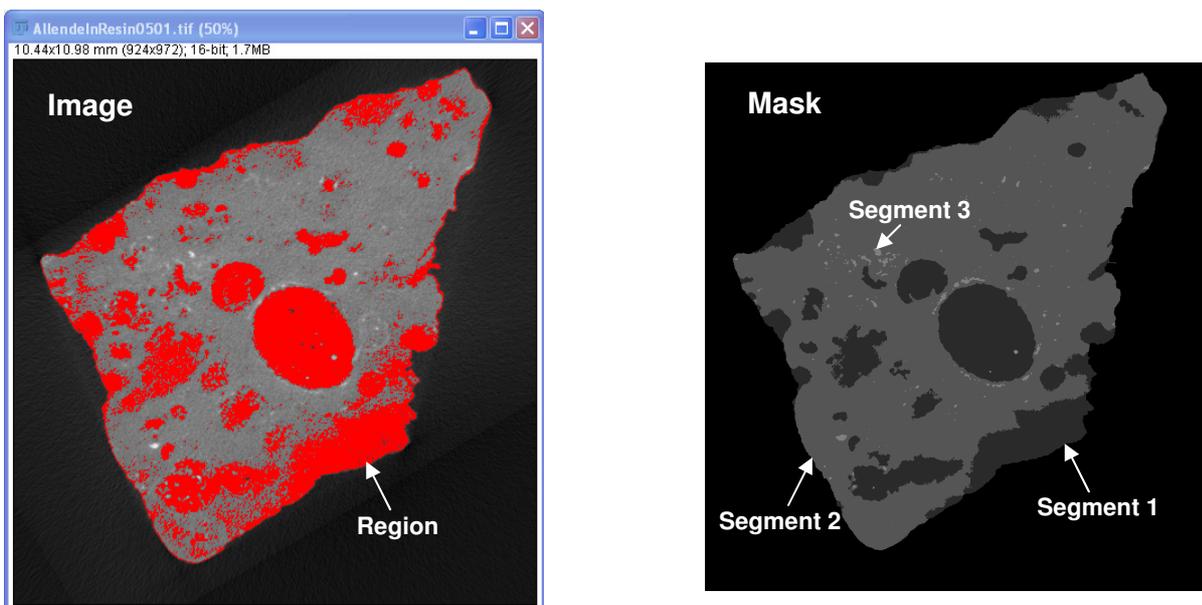
Fig 2. Regions, masks and segments

2. BASIC UTILITIES

2.1 Combined/Running histogram

➢ On clicking *Collective Histogram* button, PhaseQuant will update the canvas with histogram of the entire stack

- ➢ On clicking *Running Histogram* button, PhaseQuant will update the canvas with histogram of the current slice and will continue to refresh the canvas as the current displayed slice is changed in the stack window.

Video Demo: http://www.youtube.com/watch?v=9WDfj9EvDXM&feature=related

2.2 Update histogram

- ➢ Existing utilities in ImageJ such as smoothing and sharpening filters can be applied to the image stack while PhaseQuant is in operation

- ➢ Changes made by external utilities can be registered to PhaseQuant by clicking *Update Histogram* button in the toolbar

Video Demo: http://www.youtube.com/watch?v=dBXlBbSJrAs&feature=related

2.3 Manual segmentation

This utility is used for segmenting the stack or image into multiple regions based on thresholds.

- ➢ Click *Segment Histogram* button and subsequently, *Threshold Bars* will shown up on the histogram in the canvas

- ➢ Dragging the *Threshold Bars* will change the upper and lower thresholds and the corresponding region in the stack/image is highlighted with a unique colour map. The threshold values will be displayed in the *Threshold Display* textbox in the *Region of Interest (ROI)* pane

- ➢ A new region can be added to the PhaseQuant by pressing the *Add Region* button at the bottom of the *ROI* pane

- ➢ The new region will show up as *ROI* pane titled *New Segment* and can be renamed by clicking on the region or deleted by clicking *Delete Region* button

    Video Demo: http://www.youtube.com/watch?v=stRVcCFTDMs&feature=related

2.4 Recording training sequence

- Some segmentation methods included in PhaseQuant require training data for processing.

- To record training data, click *Add Data* ⊕ button at the bottom of the *Training* pane which will bring up a new frame

- Select the check box next to the label *phase* in the new frame and start selecting the pixel values in the image/stack window. Once completed, assign a label to the data set for identification (this must be a number)

- Click *Record* button to register the training set and the training set will show up in the *Training* pane. The training sets can be renamed or deleted as per user's convenience

- Repeat the procedure to register more training sets.

Note: On clicking *Error* button at the bottom of the *Training* pane, PhaseQuant will bring up a frame with statistical information (mean and variance) on the training datasets.

Video Demo: http://www.youtube.com/watch?v=YHbSfyZHWcc&feature=related

3. PHASE EXTRACTION MODULE

3.1 Automatic segmentation

Five segmentation methods are included in PhaseQuant, two of the methods require training sequences and others don't require training data. All the segmentation methods split the regions into two segments and create a new mask.
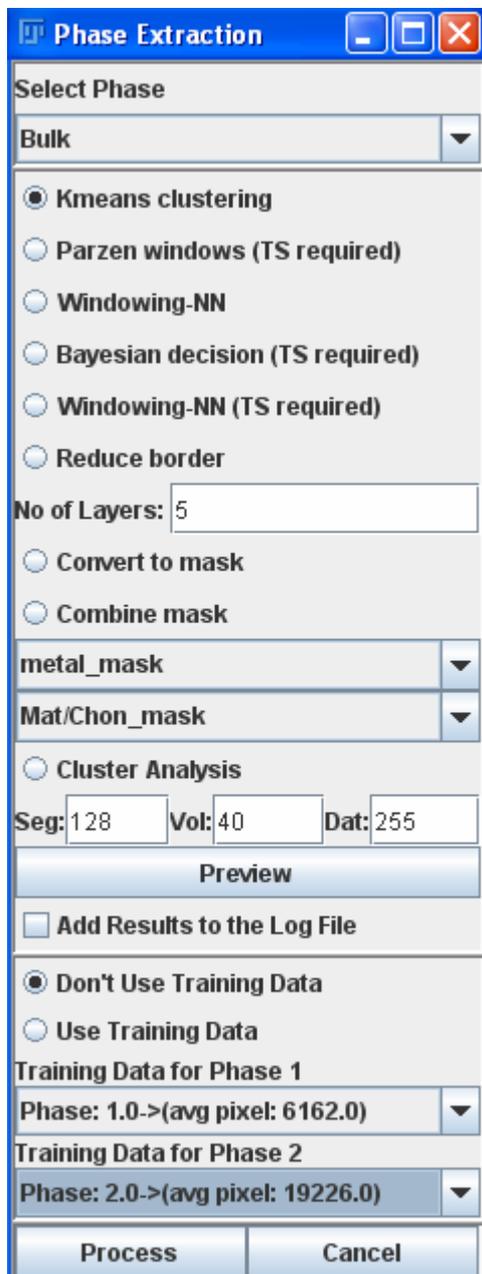
- Bring up the *Phase Extraction* frame by clicking *Phase Extraction* button in the toolbar

- Select one of the regions created by manual segmentation from the drop down box

- Choose the phase segmentation method to be used for segmentation

- If the selected segmentation method requires training sets – indicated by *(TS required)* in the phase extraction window -, then select *Use Training Data* check box at the bottom and chose the training data sets from the drop down boxes below

- Click *Process* button at the bottom of the phase segmentation frame to start segmentation

> Once segmentation is complete, a new mask titled *New Mask* is created and shows up in the *ROI* pane

> On clicking the mask, a new window opens with the segmented image.

Note: It is important not to close the windows as it might crash the program. On clicking the top level node region named *Region/mask* in the *ROI* pane, all the windows except the main window will disappear.

Video Demos: http://www.youtube.com/watch?v=nISI0JYnlAY

http://www.youtube.com/watch?v=no9yUqveeiI

3.2 Cluster analysis

Cluster analysis utility lets a user specify the minimum and maximum bounds on the cluster size of each segment in a mask

> Bring up the *Phase Extraction* frame by clicking *Phase Extraction* button in the toolbar

> Select a mask from the drop down box on which cluster analysis is to be performed

> Specify the gray value of the segment to be processed in *Seg* textbox and minimum size of the cluster in *Vol* textbox and gray value to replace with in *Dat* textbox if the cluster is smaller than the value specified in *Vol*

> Click *Preview* button to process current slice and preview the results

> Click *Process* button in the bottom of the *Phase Extraction* frame to apply cluster analysis to the entire stack permanently

Fig 3. Phase extraction module

Note: Cluster analysis can only be performed on a mask and cannot be performed on a region. After performing cluster analysis, it might be necessary to hide the mask and reopen to refresh the image data.

Video Demo: http://www.youtube.com/watch?v=nISI0JYnlAY

3.3 Convert to mask

A manually segmented region can be converted into a binary mask using this utility.

➢ Bring up the *Phase Extraction* frame by clicking *Phase Extraction* button in the toolbar

➢ Select a region from the drop down box which needs to be converted into a binary mask

➢ Select *Convert to mask* utility and click *Process* button at the bottom of the *Phase Extraction* frame to start the conversion

Video Demo: http://www.youtube.com/watch?v=no9yUqveeiI

3.4 Combing masks

The masks created by different segmentation methods can be combined together. It is often difficult to precisely segment the images manually and as a result there is an overlap between successive regions. While combining masks generated by processing the regions using the *Calculator* and *CalculatorPlus* utilities in ImageJ, it often results in an overlap as the manual segmentation may not have been precise and margins might overlap. This problem has been addressed with *Combine mask* utility.

➢ Bring up the *Phase Extraction* frame by clicking *Phase Extraction* button in the toolbar

➢ Select *Combine mask* utility and select the masks to combine in the two drop down boxes below and click *Process* button

➢ A new mask will be created in *ROI* pane with the masks combined together

➢ The mask selected at the top drop down box takes precedence over the mask selected at the bottom drop down box if there is an overlap

Note: *Calculator* and *CalculatorPlus* utilities in ImageJ can be used to combine the masks if there is no overlap between the masks, but while using those utilities the user has to make sure that the masks to be combined are visible in respective image windows and also to uncheck the *Create new window* check box

Video Demo: http://www.youtube.com/watch?v=no9yUqveeiI

3.5 Reduce border

Often it might be necessary to reduce the outer border of the specimen to remove spurious pixels that result due to partial volume averaging process.

➢ Bring up the *Phase Extraction* frame by clicking *Phase Extraction* button in the toolbar

➢ Select a mask from the drop down box which needs border reduction

➢ Select *Reduce border* utility and specify the number of pixel layers to be stripped off in *No of Layers* textbox.

➢ Click *Process* button at the bottom of the *Phase Extraction* frame to start the reduction

Note: Border reduction can only be performed on a mask and cannot be performed on a region.

Video Demo: http://www.youtube.com/watch?v=4bI11Wpso48

3.6 Log file

The results of the segmentation can be written to a log file which is a text file

➢ Create a new text file anywhere on the computer

➢ Click *Log File Location* button in the toolbar and browse to the location of the text file created in the previous step and click *open*

➢ Bring up the phase segmentation frame by clicking *Phase Extraction* button in the toolbar
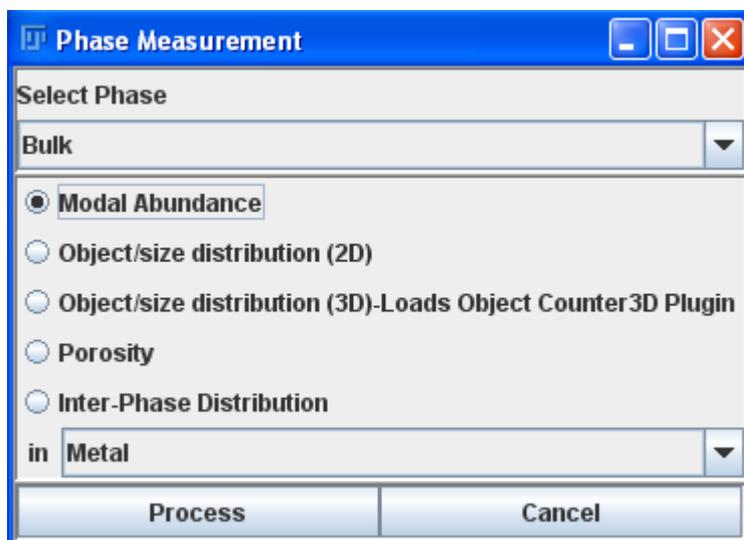
➤ Select *Add Results to the Log File* check box

➤ Now results of the segmentation will be written to the log file

Video Demo: http://www.youtube.com/watch?v=dPBAxbQeuK0

4. PHASE MEASUREMENT MODULE

4.1 Modal abundance

➤ Bring up the *Phase Measure* frame by clicking *Phase Measurement* button in the toolbar

➤ Select the region or mask from the drop down box

➤ Select *Modal abundance* option and click *Process* button at the bottom

➤ Results are displayed in a new window with volume fraction in percentage and number of pixels/voxels in square brackets

Fig 4. Phase measurement module

Video Demo: http://www.youtube.com/watch?v=no9yUqveeiI

4.2 Object/size distribution (2D & 3D)

➤ Bring up the *Phase Measurement* frame by clicking *Phase Measurement* button in the toolbar

➤ Select the mask from the drop down box

➤ Select *Object/size distribution (2D)* option and click *Process* button at the bottom

➤ After processing, PhaseQuant generates a shape mask representing each object with a unique color and also displays a result window with size distribution statistics (object identifier, phase ID, size of the object in voxels/pixels).

Note: On clicking *Object/size distribution (3D)*, PhaseQuant loads an existing plug-in named *Object Counter3D*. This plugin is well documented and further information about this plug-in can be found in the following link http://pacific.mpi-cbg.de/wiki/index.php/3D_Objects_Counter. If you are using *Object/size distribution (3D)* in PhaseQuant it is imperative to cite the reference provided in the above link. *Object/size distribution (2D)* option does not require a binary mask, whereas *Object/size distribution (3D)* requires a binary mask.

Video Demo: http://www.youtube.com/watch?v=PAYmYOYPr6c

4.3 Porosity

➢ Create a region by thresholding the bulk (everything except air and porosity) of the specimen using manual segmentation

➢ Bring up the *Phase Measurement* frame by clicking *Phase Measurement* button in the toolbar

➢ Select the bulk from the drop down box

➢ Select *Porosity* option and click *Process* button at the bottom

➢ After processing, a new mask called *Porosity* is created with segmented void spaces and shows up in the *ROI* pane

Video Demo: http://www.youtube.com/watch?v=un4LyOOOSV4

4.4 Inter-Phase Distribution

Inter-Phase distribution utility can be used for measuring distribution of a segment within other segments. This option requires two inputs one containing two segments and another containing just one segment.

➢ Bring up the *Phase Measurement* frame by clicking *Phase Measurement* button in the toolbar

➢ Select a mask containing two segments in the top drop down box and select a mask containing one segment in the bottom drop down box

➢ Select *Inter-Phase Distribution* utility and click *Process* button at the bottom

➢ After processing, a new mask called *Inter Phase* is created in the *ROI* pane. This mask will contain the segment from the second mask with the gray values replaced with the enclosing segments in the first mask.
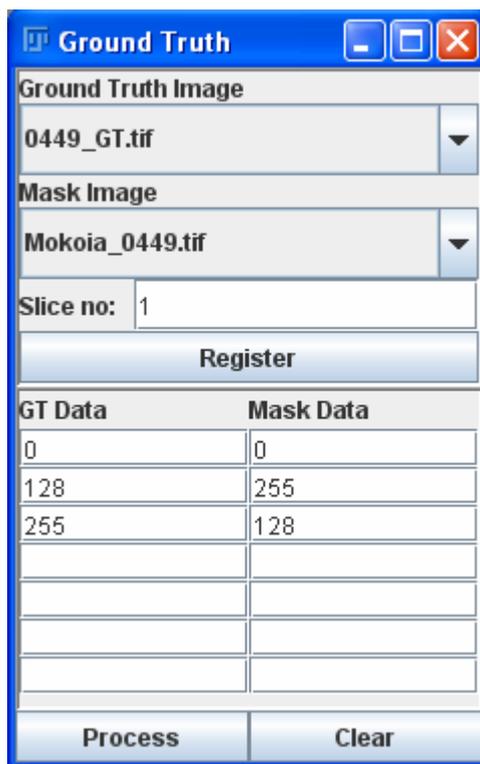
Video Demo: http://www.youtube.com/watch?v=SzcscgdAT8k

5. GROUND TRUTH OPTIMIZATION

5.1 Ground truth validation

The masks generated in PhaseQuant can be validated against Ground Truth (GT) data to measuring the margin of error.

➢ Open the ground truth image

➢ Click on the relevant mask in the *ROI* pane to make it visible

Fig 5. Ground truth optimisation module

➢ Click *Ground Truth Optimization* button from the Toolbar and this will bring up a new window named *Ground Truth*

➢ Now select the GT image from the drop down box below *Ground Truth Image* label and select the mask from the drop down box below *Mask Image* label

➢ Specify the slice number of the mask for which GT corresponds to in the textbox right next to *Slice no* label

➢ Now list all the gray values taken by segments in the mask in the textboxes below *Mask Data* label and list corresponding gray value in the GT image under *GT Data* label

➢ On clicking *Process* button at the bottom, the segments in the mask is compared against segments in the GT image and an *Error Image* visualising the discrepancy between actual and software measurements is generated along with the information on the margin of error in percentages for each segment in a separate window.

5.2 Importing training data

Training datasets can be generated automatically from the GT data using this utility

➢ Open the ground truth image

➢ Click on relevant mask in the *ROI* pane to make it visible

➢ Click *Ground Truth Optimization* button from the Toolbar and this will bring up a new window name *Ground Truth*

➢ Now select the GT image from the drop down box below *Ground Truth Image* label and select the mask from the drop down box below *Mask Image* label

➢ Specify the slice number of the mask for which GT corresponds to in the textbox right next to *Slice no* label

➢ On clicking *Register* button in the center, PhaseQuant registers the pixel values corresponding to each segment in the GT image into training data sets and lists them in *Training* pane

6. DENSITY CALIBRATION MODULE
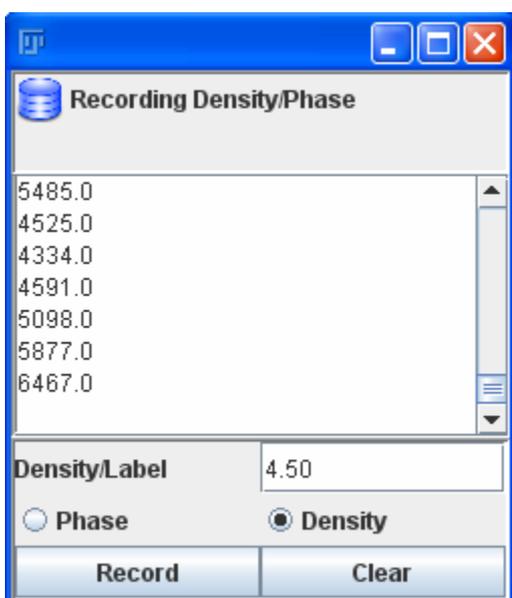
This is a module for calibrating micro-CT image stack for density so that the stack can be standardised

6.1 Recording density data

➢ To record training data, click *Add Data* button at the bottom of the *Training* pane which will bring up a new frame

➢ Select the check box next to the *Density* label in the new frame and now start selecting the pixel values in the image/stack window. Once

completed assign a density value to the data set

➢ Click *Record* button to register the density data set and this will show up in the *Training* pane.

➢ Repeat the procedure to register more density data sets.

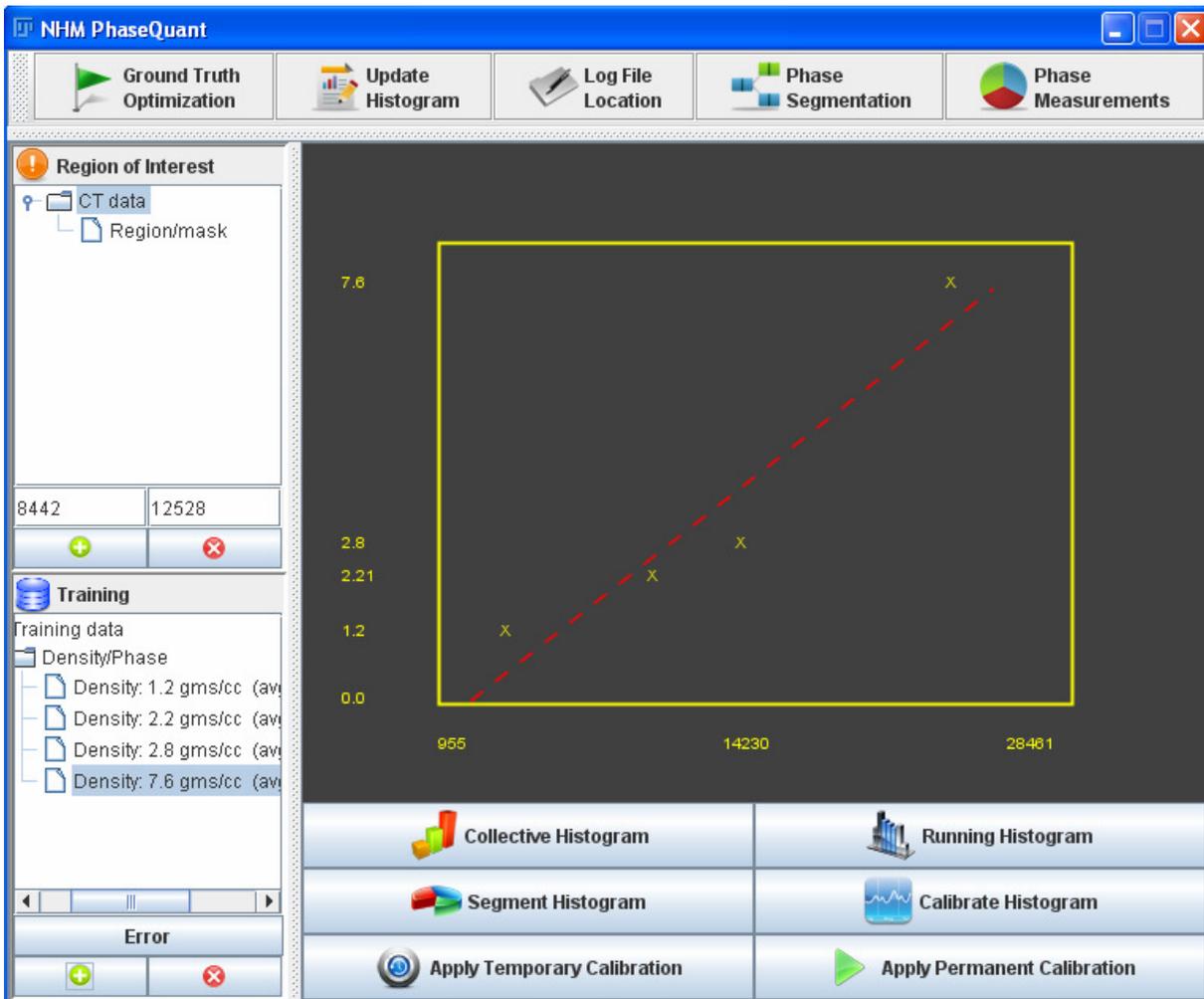*Video Demo:* http://www.youtube.com/watch?v=M_UgoS0HpF0

Fig 7. PhaseQuant GUI while density calibration

6.2 Density calibration

➢ On clicking *Calibrate Histogram* button, PhaseQuant will display density vs gray value plot in the canvas

➢ On clicking *Apply Temporary Calibration* button, PhaseQuant applies calibration to the current slice and displays the calibrated image in a separate window

➢ On clicking *Apply Permanent Calibration* button, PhaseQuant applies calibration to all the slices and updates the histogram

Video Demo: http://www.youtube.com/watch?v=M_UgoS0HpF0