OpenMIMS ImageJ Plugin Guide

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Introduction and Installation

The NRIMS Analysis Module is an ImageJ plugin to open, process and analyze images captured with NanoSIMS 50 & 50L secondary ion mass spectrometers (Cameca). The plugin has been developed at the National Resource for Imaging Mass Spectrometry (NRIMS, http://www.nrims.hms.harvard.edu), an NIH-supported National Resource developing Multi-isotope Imaging Mass Spectrometry (MIMS) for biomedical research. Images and/or stacks of images for up to 7 different isotopes can be opened, analyzed and saved. All metadata saved in the original file are preserved. Image ratios and Hue-Saturation-Intensity (HSI) maps of any combination of isotopes can be displayed and data from any number of Regions of Interest (ROIs) extracted, analyzed and tabulated for single images or entire stacks.

After downloading the Open_MIMS.zip from (http://www.nrims.hms.harvard.edu/software.php) copy all the extracted contents to the ImageJ home 'plugins' folder and restart ImageJ. Note this plugin requires a 1.43u or later version of ImageJ (http://rsb.info.nih.gov/ij/download.html) and Java 1.6.

Opening MIMS images

After launching ImageJ, place the mouse over the "Plugins" menu option and select the "Open MIMS Image" option. This action will open the OpenMIMS graphical user interface. You then open an image by clicking "File > Open MIMS Image" and navigate to the desired MIMS image file (files with a *.im*, *.nrrd* extension). Files can also be opened by dragging and dropping the file from a file browser. Only *.nrrd* files generated by the OpenMIMS plugin are guaranteed to be readable by the plugin. After the MIMS image is opened, each mass will appear in a separate window (example below) as well as the NRIMS Analysis Module window.
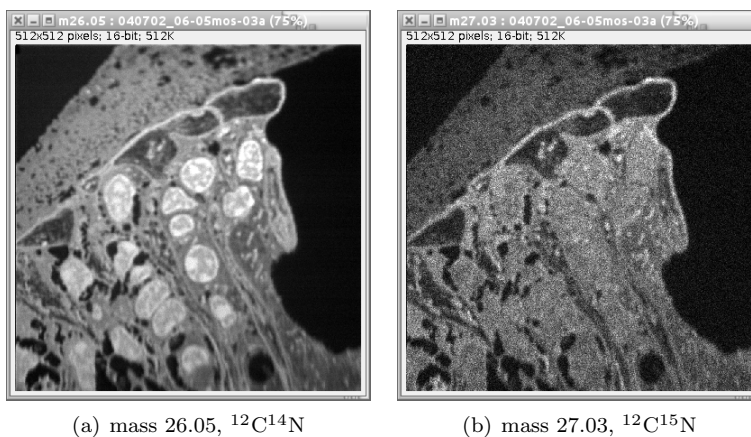

(a) mass 26.05, $^{12}C^{14}N$ (b) mass 27.03, $^{12}C^{15}N$

Figure 1: Two different mass images of the same field of view.

Graphical Interface

The NRIMS Analysis Module window contains 7 tabs:

- **MIMS Data:** Displays metadata and other information for the current file.

- **Process:** Used to generate ratio images and HSI maps.

- **Contrast:** Controls the contrast and brightness settings for mass, ratio and sum images.

- **Stack Editing:** Used for manipulating the mass images (applying x and y translations, dropping planes, etc).

- **Tomography:** Basic plotting of statistics for whole images or ROIs.

- **Segmentation:** Perform an algorithmic segmentation of an image.

- **MIMS Log:** Displays more header metadata and a record of the user's actions and debug output.
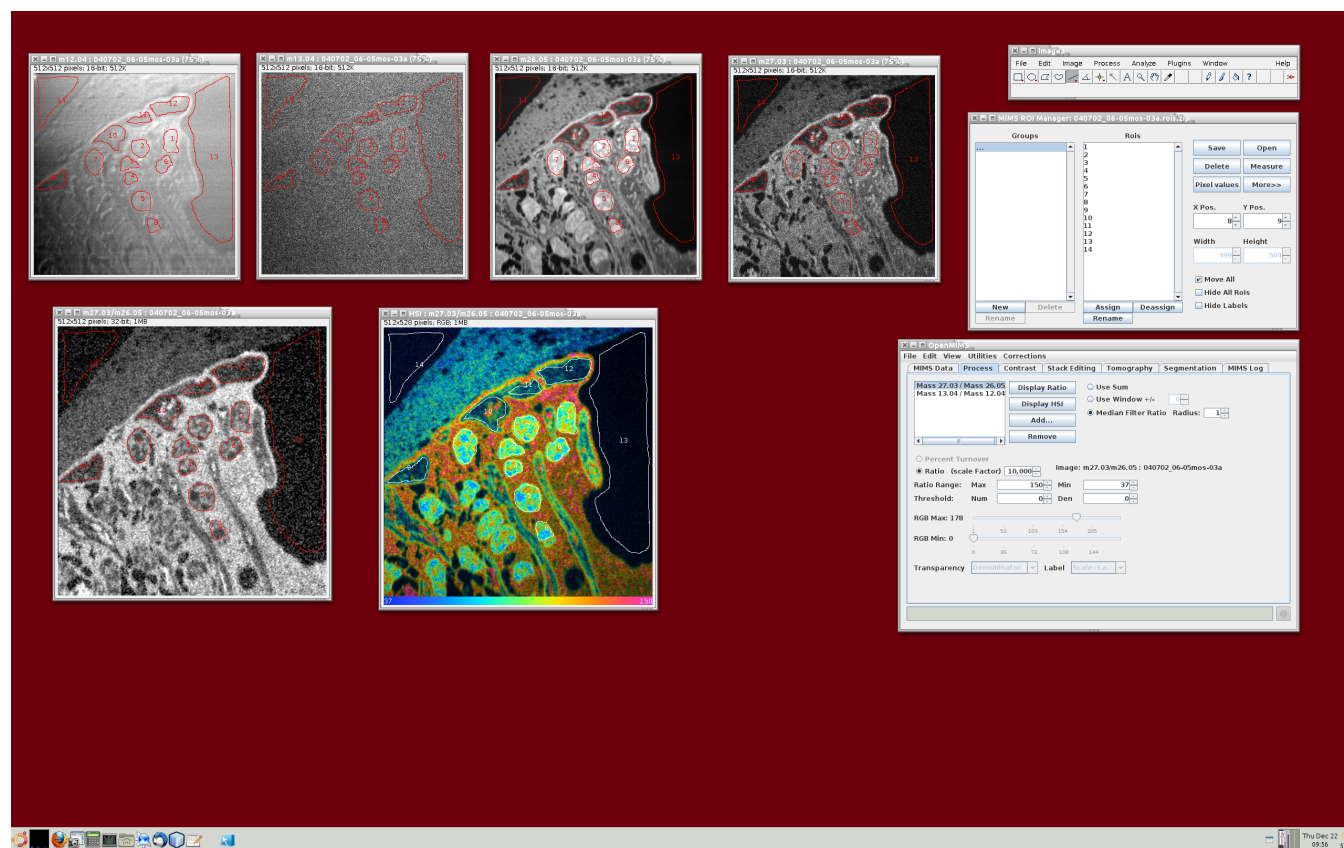

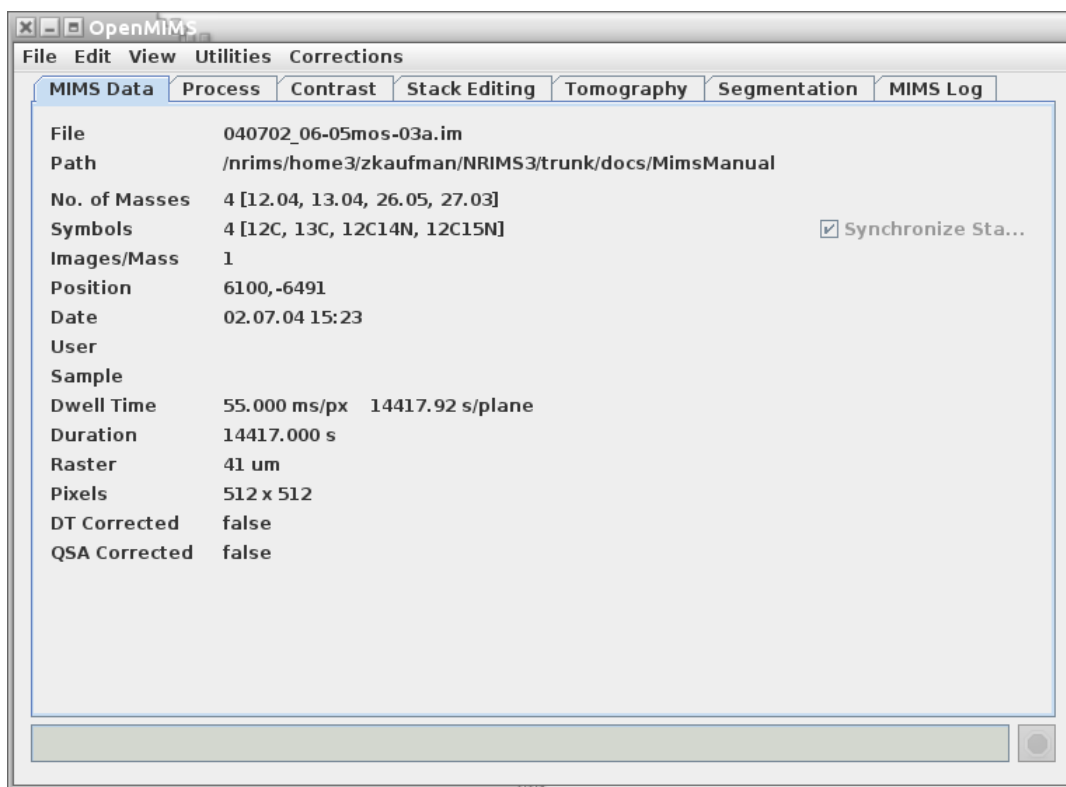Figure 2: A desktop screenshot of the OpenMIMS application.

MIMS Data:

Figure 3: The **MIMS Data** tab displays file information and meta data.

The **MIMS Data** tab displays a subset of the image's meta-data including the absolute path of the current image, the number of masses and their AMU values, number of planes in the stack, position, date of image, user name, dwell time, duration, raster size and image size. The **Synchronize Stacks** check box enables updating of all masses simultaneously while scrolling through an image stack. Adjusting the slider (plane selector) at the bottom of any mass automatically selects the same plane for all masses.
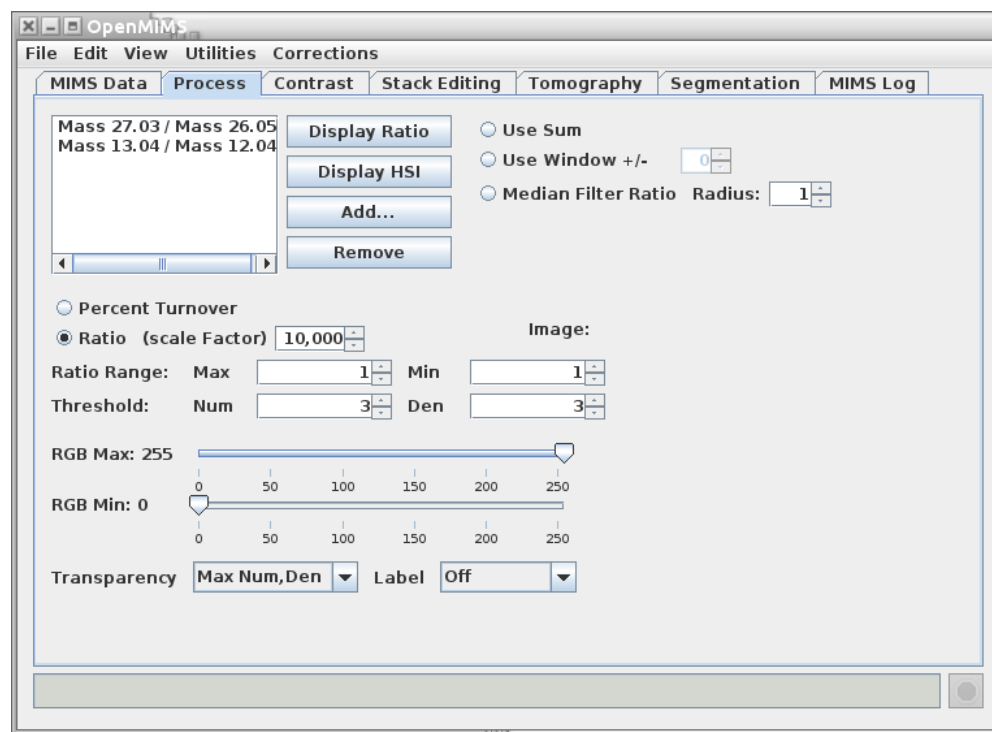
Process:

Figure 4: The **Process** tab is used to generate ratio and HSI images.

The **Process** tab allows the user to generate ratio images and HSI maps. Ratio and HSI images are the result of dividing one mass image by another. Masses that have similar values will automatically show up in list form in the **Process** tab. Others that do not show up by default can be entered manually using the **Add...** button. A ratio image will appear when the user selects one (or more) in the list and clicks **Display Ratio**. When moving the mouse pointer over the ratio image, the status box along the lower border of the NRIMS Analysis Module window displays the raw numerator and denominator counts as well as the ratio value.

HSI images are similar to ratio images but are different in that they use a combination of the the ratio value of a pixel, the counts of one of the masses for the intensity, and a constant saturation value, to generate pixels in the RGB color space. Clicking the **Display HSI** will make the selected HSI image appear. An example HSI image is provided in **APPENDIX A**. When displaying HSI images, the user has the option of displaying the actual ratio values (multiplied by the **Ratio Scale Factor**) or displaying percent turnover by selecting the **Percent Turnover** radio button. Percent turnover is determined by the naturally occurring ratio of that specific isotopic pair along with the maximum achievable ratio based on the experimental protocol.

The **Threshold** option sets the minimum number of counts in the numerator and/or denominator. Any pixels below that threshold will be ignored and appear black. The **Ratio Range Min** and **Max** values determine the range of the colormap used to display the image. The **RGB Min** and **RGB Max** values determine the intensity scale used in the image. The **Transparency** selects the method for computing the intensity component of the HSI image, and the **Label** option enables a color scale bar to be become visible. Selecting the **Use Sum** radio button will generate a single HSI (or ratio) image representative of the entire stack. Selecting the **Use Window** radio button will use the sum of both masses over a sliding window with the specified window size. This can be very useful for finding small features in large image stacks that have low counts. Also, a median filter can be applied to a ratio or HSI image by selecting the **Median Filter Ratio** radio button.
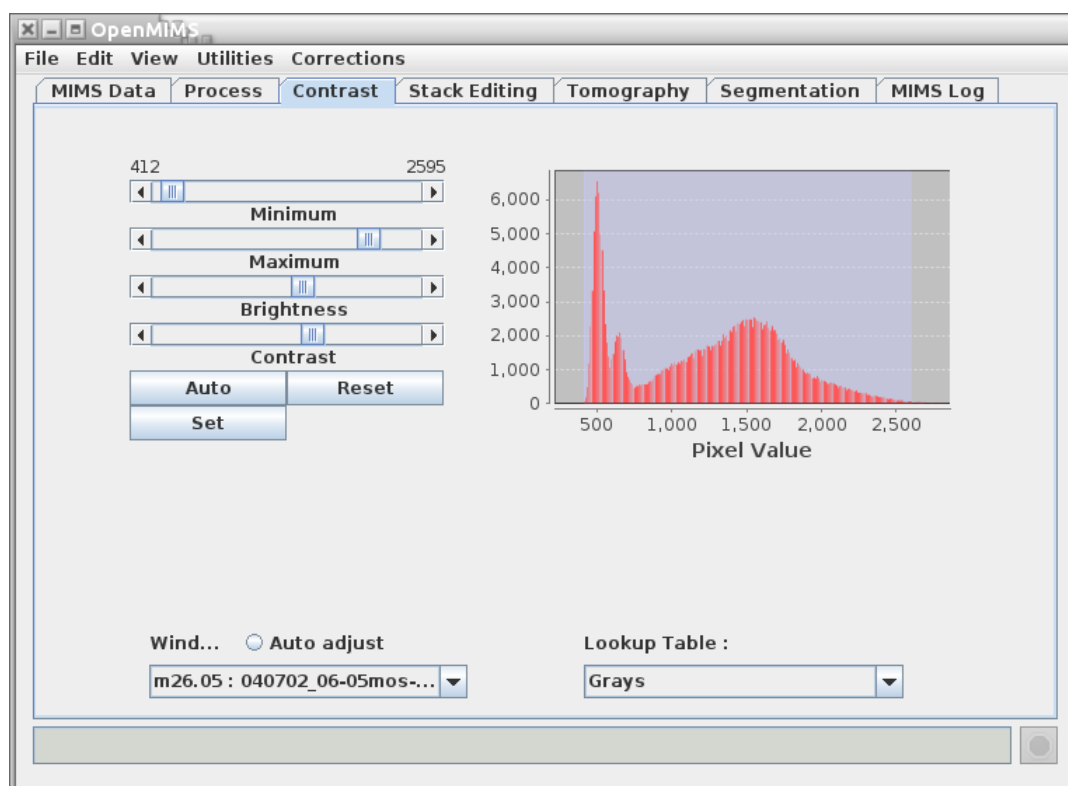
Contrast:

Figure 5: The **Contrast** tab controls and displays contrast settings for mass, ratio and sum images.

The **Contrast** tab allows the user to control the contrast settings, by adjusting the sliders, for any given mass, ratio or sum image (HSI images do not have contrast settings, instead their display parameters are controlled using the **Process** tab). It also displays a histogram of intensity values for any of these types of images. Clicking on an image brings it into focus and the histogram and contrast settings will update to reflect the values of the current image in focus. The histogram and contrast settings will also update by selecting the window in the combobox located at the bottom of the **Contrast** tab. All mass, ratio and sum images should appear in the combobox.

If changes to the default contrast settings are made, clicking the **Reset** button will bring those settings back to their default values. Clicking **Auto** will iterate through a set (usually 5 or 6) of contrast settings, eventually returning back to default values. The **Set** button allows the user to input values for min and max that are outside the range of those provided by the **Minimum** and **Maximum** sliders.

Mass, ratio and sum images can also be set to **Auto Adjust** meaning that each time a new slice is selected in the stack, new contrast settings are calculated. If **Auto Adjust** is not selected, the contrast settings for the first image in the stack are used throughout the stack.

The user has the additional capability of displaying the image using **Lookup Tables** other than the gray table used by default. There are several options for displaying the data and each image can be set independently.
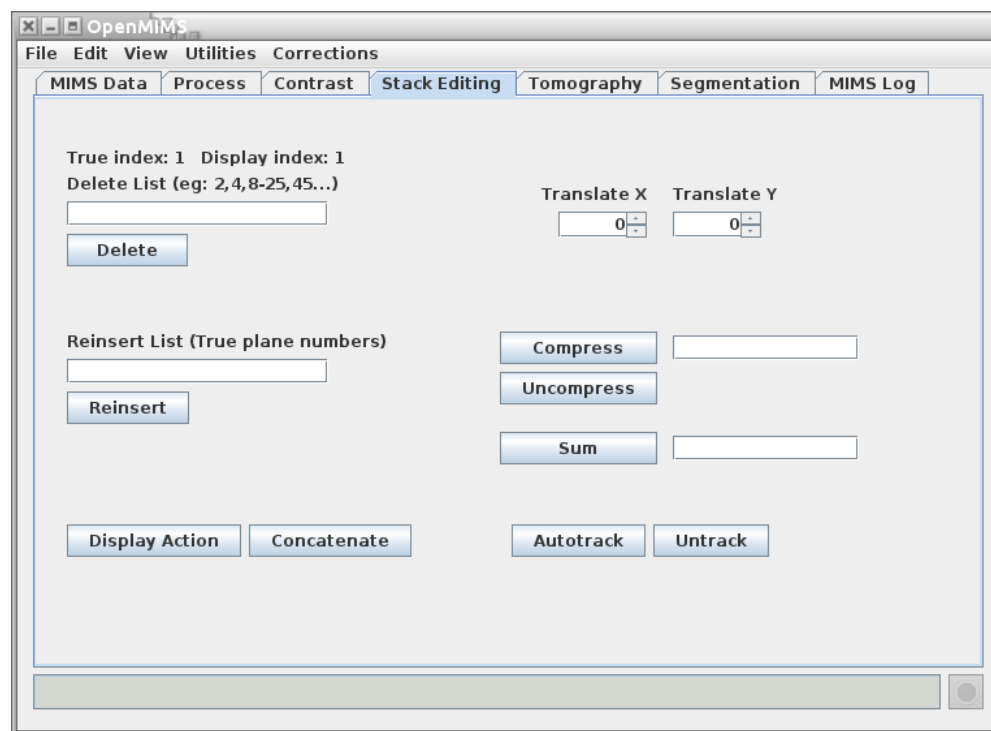
Stack Editing:

Figure 6: The **Stack Editing** tab is used for image manipulation.

The **Stack Editing** tab is reserved for functions that relate to the editing and manipulating of mass images. This includes deleting and reinserting planes, applying translation, compressing the image and generating sum images. One important thing to note is that there are two indices for an image, both of which are displayed: **True index** and **Display index**. The True index of a plane in an image never changes, but the Display index depends on what planes have been deleted. For example: entering 1-5 and clicking the **Delete**, then entering 6-10, and clicking Delete will -not- delete the first 10 planes of an image. Doing that is equivalent to entering 1-5, 11-15 and clicking **Delete**. The **Reinsert** button uses the indices of the original data, so entering entering 1-5, 11-15 would reinsert the previously deleted planes.

A plane that is currently displayed can be translated using the **Translate X** or **Translate Y** spinners, or by entering a value those text fields. The image can also be registered automatically by clicking on a specific mass to use and then clicking the **Autotrack** button. This will call the autotrack algorithm which will automatically attempt to determine the best per image translations for a best fit alignment throughout the stack. Clicking **Untrack** will reset all translations to zero.

The **Concatenate** button allows another image (or image stack) to be prepended or appended to the current image set. The **Sum** button will generate a sum image from whichever mass image or ratio image was most recently clicked. If the field is blank the entire image is summed, or a range of planes can be entered in the textfield (e.g. 1-20). The **Compress** button compresses the images into blocks of the size entered in the text field. Entering a value of 4 in the field will sum the pixel values in blocks of 4 planes, resulting in a stack of images 1/4 the original size. Any remaining planes at the end of the stack are summed together into a single block. The **Uncompress** button undoes the compression and restores the image to its original state - minus any translations applied and planes deleted.
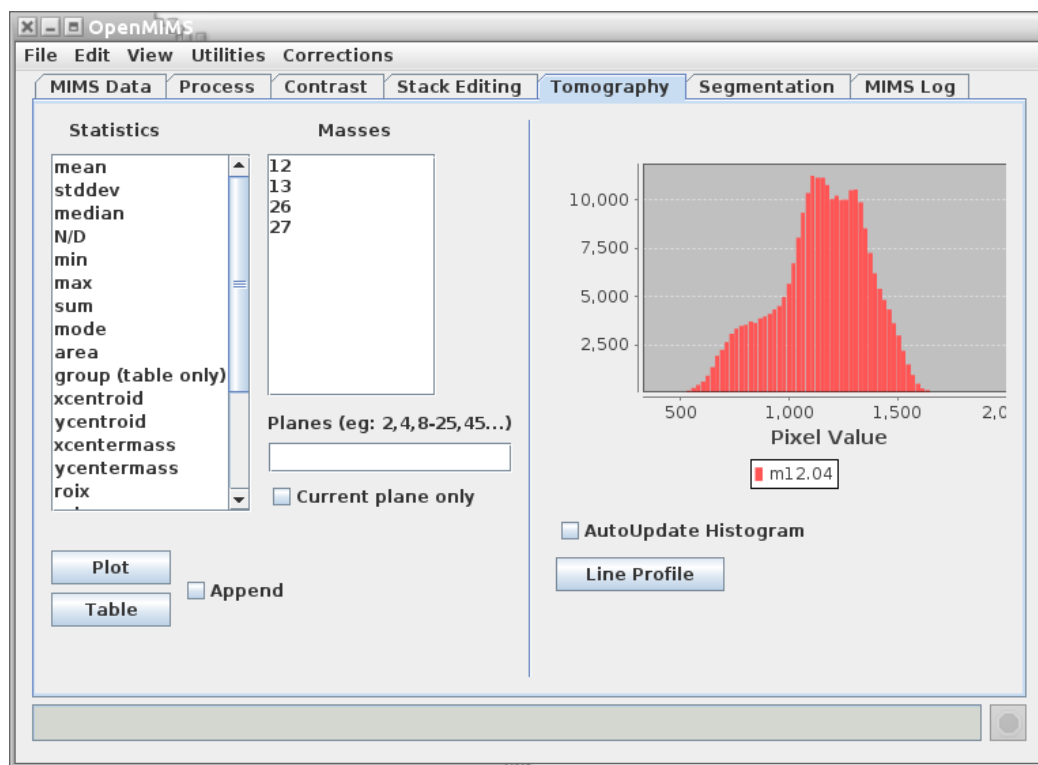
Tomography:

Figure 7: The **Tomography** tab is used to generate plots and tables of ROI statistics.

The **Tomography** tab allows the user to generate simple line plots or tables of ROI statistics through the stack. Simply select a set of ROIs from the **ROI Manager**, the desired statistics, and the mass, ratio and/or sum images and click **Plot** or **Table**. The **Planes** field allows the user to enter which planes are included in the plot (or table). If this field is left blank, all planes will be included. Checking the **Append** box will append the data to an existing plot or table if one exists, otherwise a new one will be created. An example of the type of plot produced can be seen in Figure 8.
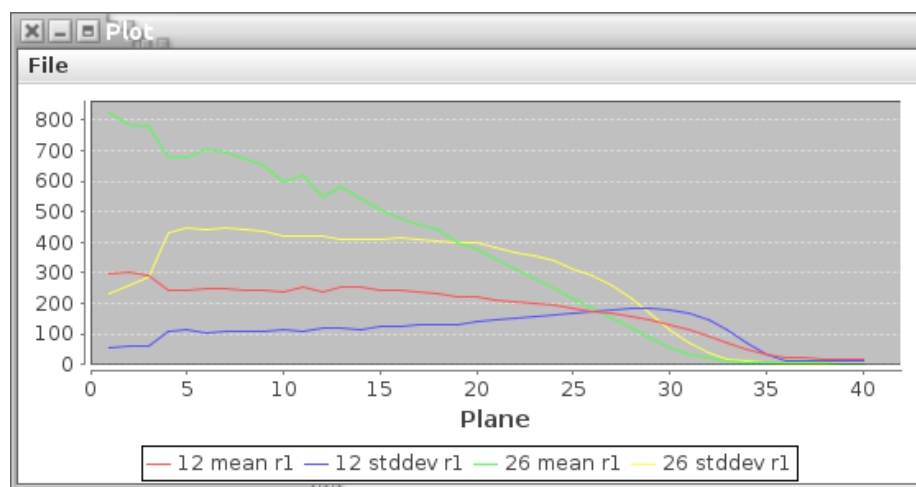

Figure 8: An example plot of the *mean* and *stdev* statistics for two different masses.

The table output will vary depending on the type of images for which statistics are being generating. Mass and ratio images that are more than one plane will output one row of data per plane. In the case of sum images and other images that are only one plane, one row of data will be produced per ROI. See **APPENDIX B** for a sample data output table.

The right side of the tab includes a histogram that displays the intensity values for the pixels within a given ROI. To set which ROI values are displayed by the histogram, the user only needs to scroll the mouse over the desired ROI. After a ROI has been drawn, it can be moved anywhere within the image by either dragging it with the mouse, or using the position spinners in the **ROI Manager**. If the **Autoupdate Histogram** radio button is selected, the histogram will update as the ROI is being moved by the mouse. Otherwise, it updates when the move is complete.

A line ROI represents a special case because it has no enclosed area, so its values will not be represented in the histogram. Instead, the user can select the **Dynamic Profile** button to generate a profile plot of the pixels which the line intersects. An example of a mass image with a line ROI and its profile plot is provided below.
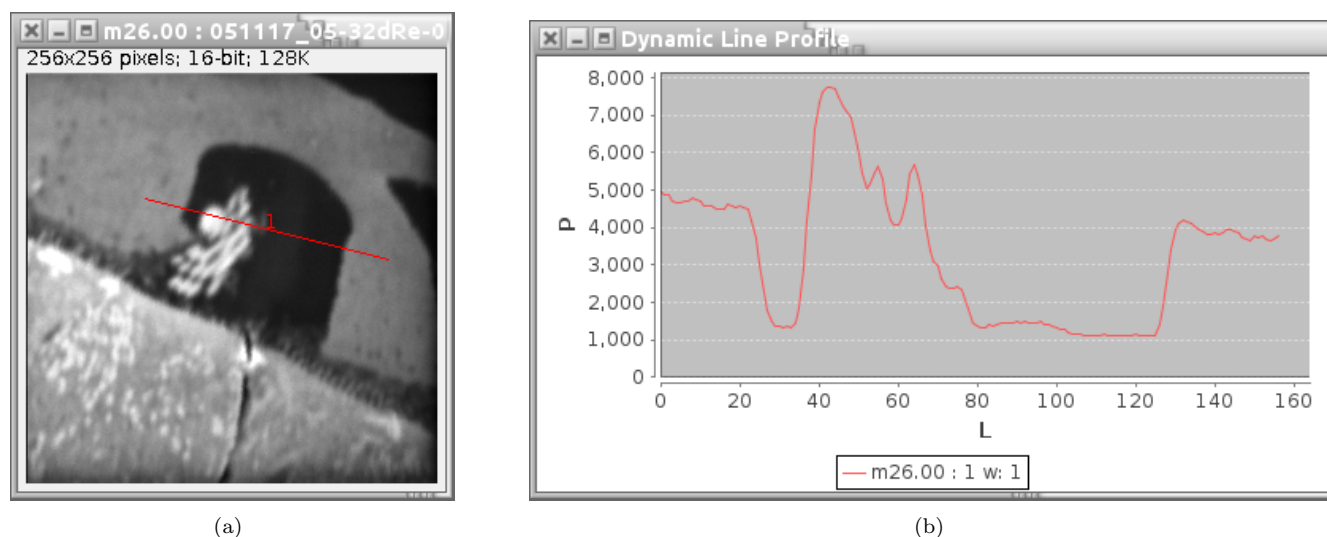

(a)   (b)

Figure 9: A mass image with a line ROI (a) and its corresponding Profile Plot (b).
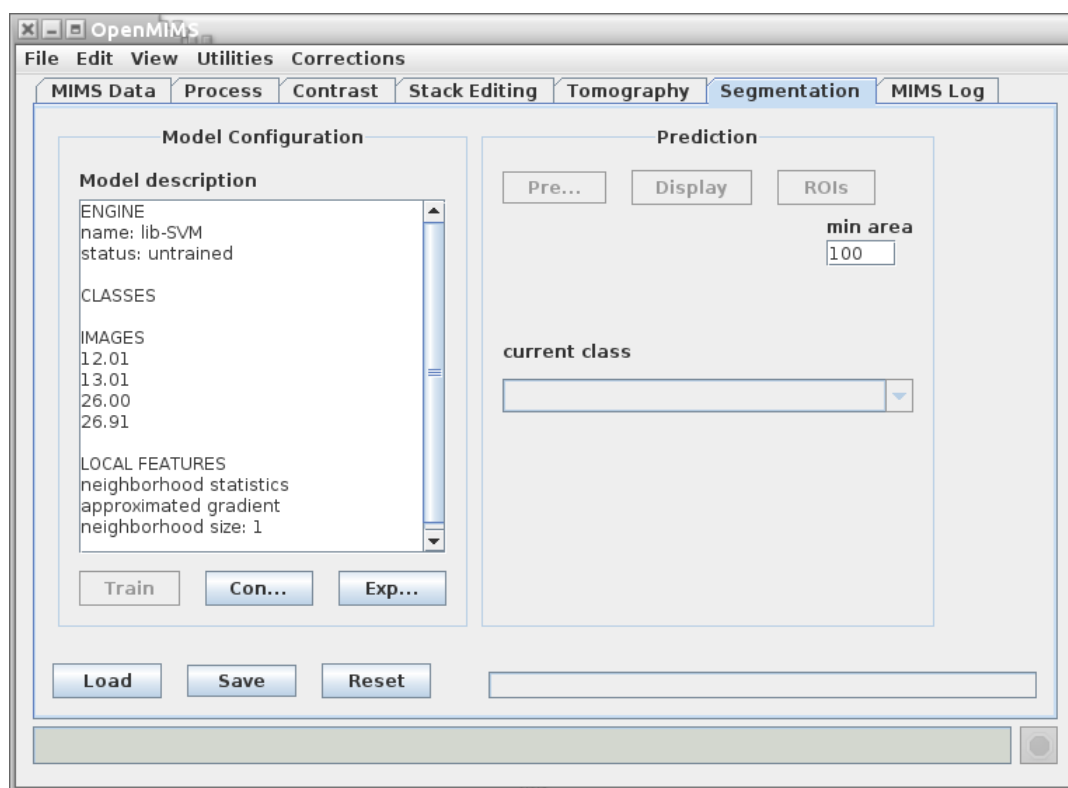
Segementation:

Figure 10: The **Segementation** tab.

The **Segmentation** tab allows the user to perform an algorithmic segmentation of a MIMS image to automatically generate ROIs for a given image plane. The algorithm used is a support vector machine (SVM) based segmentation that classifies pixels in the image using methods similar to *Fuller et. al.* [1]. A full description of SVMs is outside the scope of this document but will be detailed in a forthcoming paper. The algorithm can use many values or features to classify a pixel: the value of that pixel in a set of mass or ratio images, the mean and standard deviation in a neighborhood around that pixel, and the gradient around that pixel. Note that the ability to use other features may be added later. To perform a segmentation the user clicks on the **Config** button and is presented with a window as shown in Figure 10. The **Export** button will export the SVM and data if the SVM has been trained using the libSVM format.

Here the user may select which mass and ratio images to use for the segmentation (segmentation can not be performed on Sum images). One can choose whether or not to use the **Neighborhood** parameters (mean and standard deviation) and the **Gradient** as features, as well as the size of the neighborhood radius. Some parameters used by the SVM library (in our case libSVM [2]) by clicking the **Setup** button, specifically the type of kernel to use and the level of cross validation. We recommend using the radial basis function (RBF) as the kernel. The level of cross validation may be reduced to increase speed at the possible cost of accuracy.

To train the SVM the user needs to choose at least several ROIs that represent a given class of pixels. First a class is added to the **Class Manager** by clicking the **Add** button. Then the representative ROIs are drawn. Then the **Sync** button is clicked to sync the ROIs in the **ROI Manger** to the class in the **Class Manager**. Finally the users clicks the **Ok** button to complete the SVM configuration.
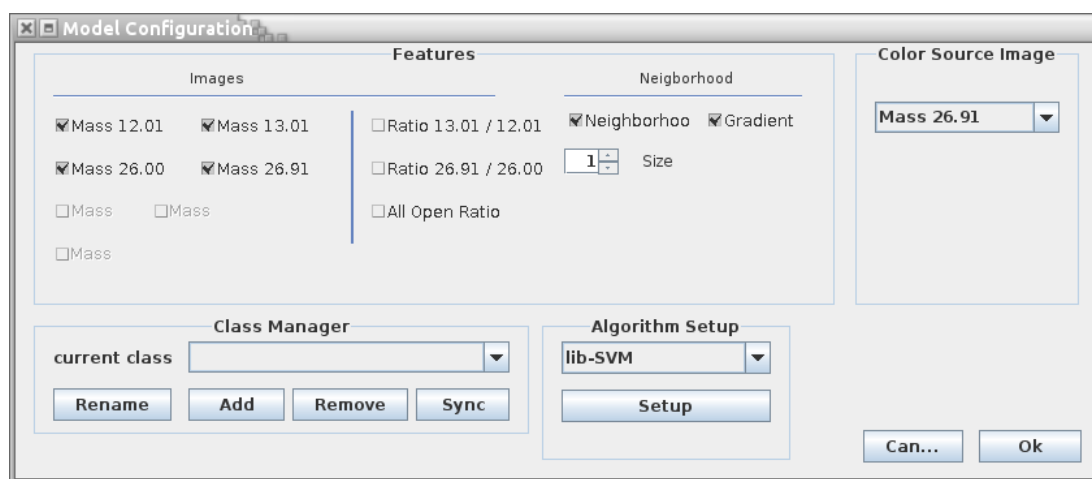
Figure 11: SVM configuration GUI.

The SVM must be trained by clicking the **Train** button, located below **Model description** in Figure 11. It should be noted that the SVM can be saved either before training (saving all parameter settings and training ROIs) or after training (also saving the trained SVM). The segmentation of the entire image can be performed by clicking **Predict** and the result of this prediction can be shown by clicking **Display**. Currently the SVM only segments 2D images. Generating ROIs from the prediction can be done by clicking **ROIs** which will generate a set of ROIs for each class ignoring groups of pixels of the same class that are below the value in the **min area** field. An example of a segmentation can be seen in **APPENDIX C**.
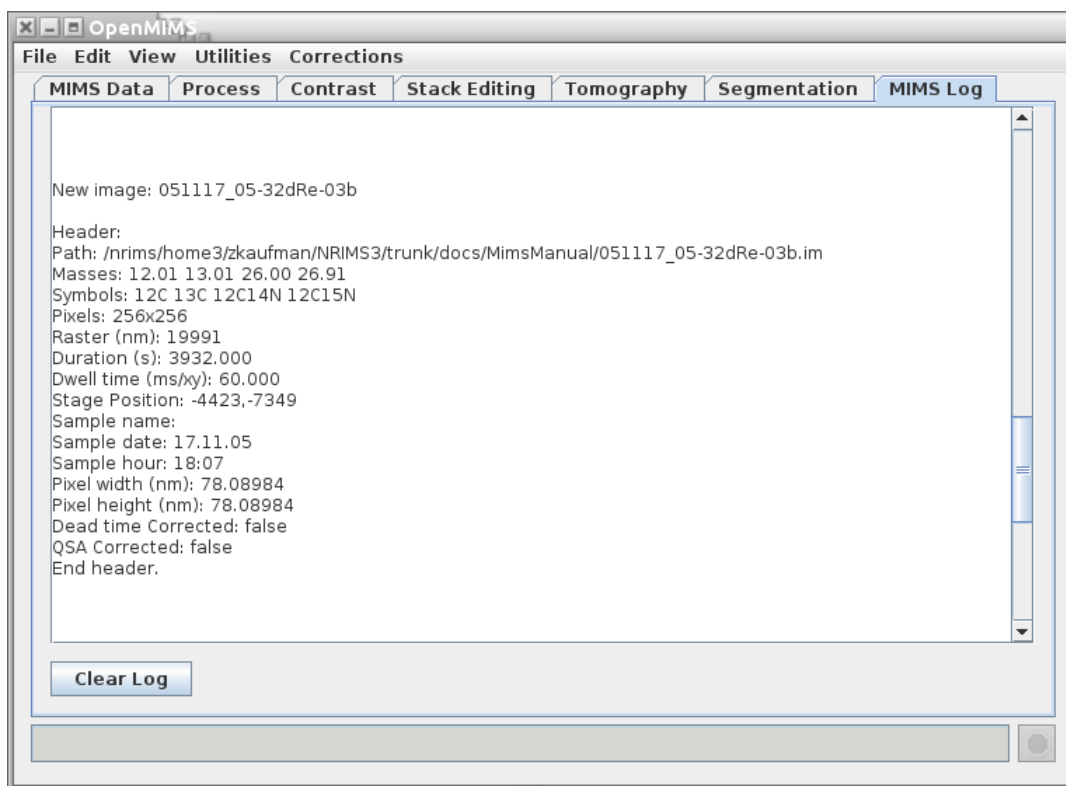
Mims Log:

Figure 12: The **MIMS Log** tab contains metadata and debug information.

The **MIMS Log** tab keeps a record of what the user has done: e.g. deleting planes, translating planes, autotracking, etc. Various bits of degub data are also displayed on this tab. This information is not saved and is most likely of little use to the most users.
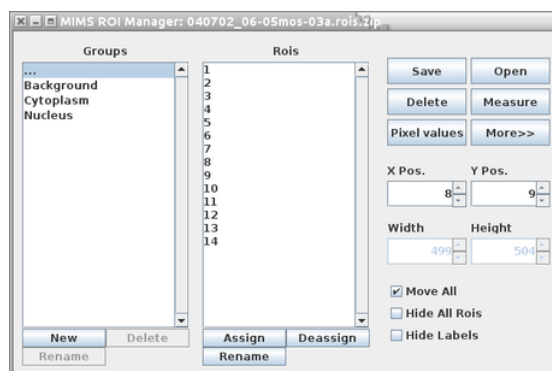
ROI Manager:

Creating Regions of Interest (ROIs)

The ROI Manager (see Figure 13) gives the user functionality relating the ROIs in a given image. It is comprised of two lists, one labeled **Groups** and one labeled **Rois**, as well as a panel on the right hand side containing buttons, spinners and checkboxes.

To create a ROI, first select the type of ROI to draw by clicking one of the corresponding toolbar buttons in ImageJ's main window (any of the five buttons on the left, i.e Rectangle, Circular, Polygon.. etc, see the top right window in Figure 2). Then place the mouse over the area and drag the mouse while holding the left mouse button down. When the left mouse button is released, the corresponding ROI will appear in all image windows as well as in the list of ROIs in the ROI manager. Clicking on a previously drawn ROI in **ROI Manager** list will automatically highlight the selected ROI in all mass, ratio, sum and HSI images.
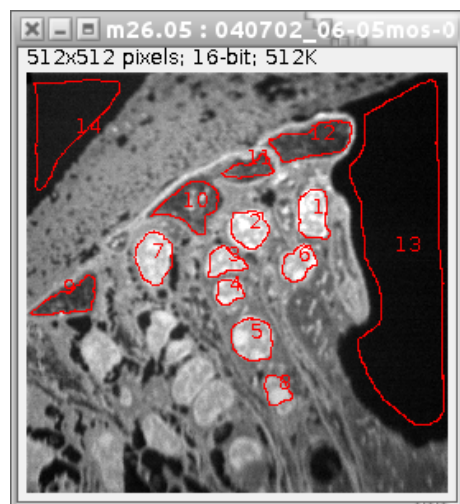
(a) Mass image with ROIs, $^{12}C^{14}N$

(b) The ROI Manager

Figure 13: A mass image with serveral ROIs (a) and the corresponding ROI Manager (b).

The **Groups** list allows the user to organize ROIs into groups. The user can create new groups, delete existing ones, and assign ROIs into groups. An ROI can only belong to a single group. Clicking on a group will reduce the **Rois** list to only those ROIs belonging to that group. Clicking on the elipses group (**...**) will always show all ROIs.

Delete will delete the selected ROIs in the ROI list. If none are selected then it will delete them all, after prompting the user. **Rename** allows the user to rename the ROI from its default name. **Open** will open an ROI file that had been saved from a previous session and overwrites the current list. **Save** will save a single ROI to a file or a group of them to a *.zip* file. **Measure** will open a statistics box that will display statistics for all ROIs for the current image. **Deselect** will deselect any ROIs that have been highlighted. The **More>>** button offers the user some more complicated features relating to the combining and splitting ROIs. ROIs can be moved on a pixel by pixel basis using the **X Pos.** and **Y Pos.** spinners. ROIs can also be moved by the user by dragging them across the image with the mouse cursor. Adjusting the **Width** and **Height** spinners will adjust those values but only for rectangular and circular ROIs.

If the user moves an ROI, it will move for all images in the stack unless the **Move All** checkbox is unchecked, in which it will only be moved for the current plane. The user can choose to hide all ROIs by checking the **Hide All Rois** checkbox or just hide the labels by checking the **Hide Labels** checkbox.

Menu Items

This section provides a brief description of features provided in the menu items of the MIMS application

File

Open MIMS Image: This menu item will bring up a file chooser that allows the user to select which file should be opened. The plugin can read *.im* files and *.nrrd* files [3] (It can only open the *.nrrd* files that were generated by the plugin). Additionally it can open any *.ratio*, *.hsi*, and *.sum* file that was generated by the plugin when saving from a previous session.

Save Image: This menu item will save whatever modifications have been made to the original *.im* file (translations, dropped planes, etc.) into a new binary file with a *.nrrd* file extension. The OpenMIMS plugin is capable of reading the both *.im* files and the *.nrrd* files that it generates.

Save Session: This menu item is similar to the **Save Image** menu item however in this case additional files will be created for each ratio, hsi and sum image that is open at the time of saving. These files can be opened individually at a later date so long as the *.nrrd* file that was created with them is stored in the same directory.

About OpenMIMS: Displays version and other information related to the OpenMIMS application.

Exit: Exits the application. ImageJ stays open as well as all opened images.

Edit

Preferences: Opens a dialog box which allows the user to set customized preferences.

Restore Mims: This menu item will reset the current image to its original state, all translations will be set back to zero and all dropped images will be reinserted. Functionally it is the same as reopening the current image.

View

Tile Windows: This menu item will take all of the currently open *image* windows and rearrange them in a grid on the desktop.

ROI Manager: This menu item open the ROI Manager. The ROI manager will also open whenever a ROI is drawn.

Additional menu items are located here allowing the user to view or hide individual mass images.

Utilities

Image Notes: Opens up a text area that allows user to enter notes regarding an image. These notes will be stored with other metadata when saving the file.

Generate Report: Opens a dialog box which captures the current image and has a text area that allows the users to enter notes. When the user clicks OK a .rtf formated report is generated. Subsequent images and notes can be appended to the report. This functionality is useful for recording important information while analyzing images.

Sum All Open: Generates a sum image for all open mass and ratio images. It will not generate a sum image for open HSI images. See the **Process** section to see how to generate sum images for HSI images.

Import .im List: The OpenMIMS application has the ability to read a text file with a list of file names and it will open those image files, appending them to one another. *NOTE* *All image files must exist in the same directory as the text file that references them.* An example image list file is provided in **APPENDIX D**.

Capture Current Image: Selecting this menu item will produce a screen capture of the the last image clicked by the user (whichever image has the current focus). The new image is an RGB image of **exactly** what is displayed on the screen, including things like ROI outlines.

Batch convert to nrrd: Allows the user to batch convert a set of .im files into .nrrd files. Can also perform tracking of the image while converting.

Export...> Export All Derived (png): Exports all derived images (ratio, HSI and sum images) as .png files.

Close...> Close All Ratio Images: Closes all currently open ratio images.

Close...> Close All HSI Images: Closes all currently open HSI images.

Close...> Close All Sum Images: Closes all currently open sum images.

Generate Stack: Generates a new ratio or HSI image with independent scrollbars rather than the single plane ratio or HSI images that are generated by default.

Composite: Selecting this menu item will bring up a dialog which allows the user to quickly create a composite image with up to 4 channels. Any mass, sum, or ratio image can be used for the Red, Green, Blue, or Grey channels or a channel may be left blank. Each channel uses a simple color LUT. The min/max values for each LUT are taken from the underlying images. For example if the m26 mass image is used for the Red channel that channel will have the same min/max values. Changing the min/max values (or brightness/contrast) on the Contrast tab for the m26 image will automatically update the Red channel in the composite.

Corrections

Apply dead time correction: Applies a dead time correction to the data. A 44 nanosecond correction is applied to the data.

Apply QSA correction: Applies a QSA correction to the data. Applying the QSA correction automatically applies a dead correction.

[1] Fuller et. al. *Segmentation of Three-dimensional Retinal Image Data.* IEEE Trans. Vis. Comput. Graph. 16(6):1719-1726, 2007.

[2] libSVM *http://www.csie.ntu.edu.tw/ cjlin/libsvm/*
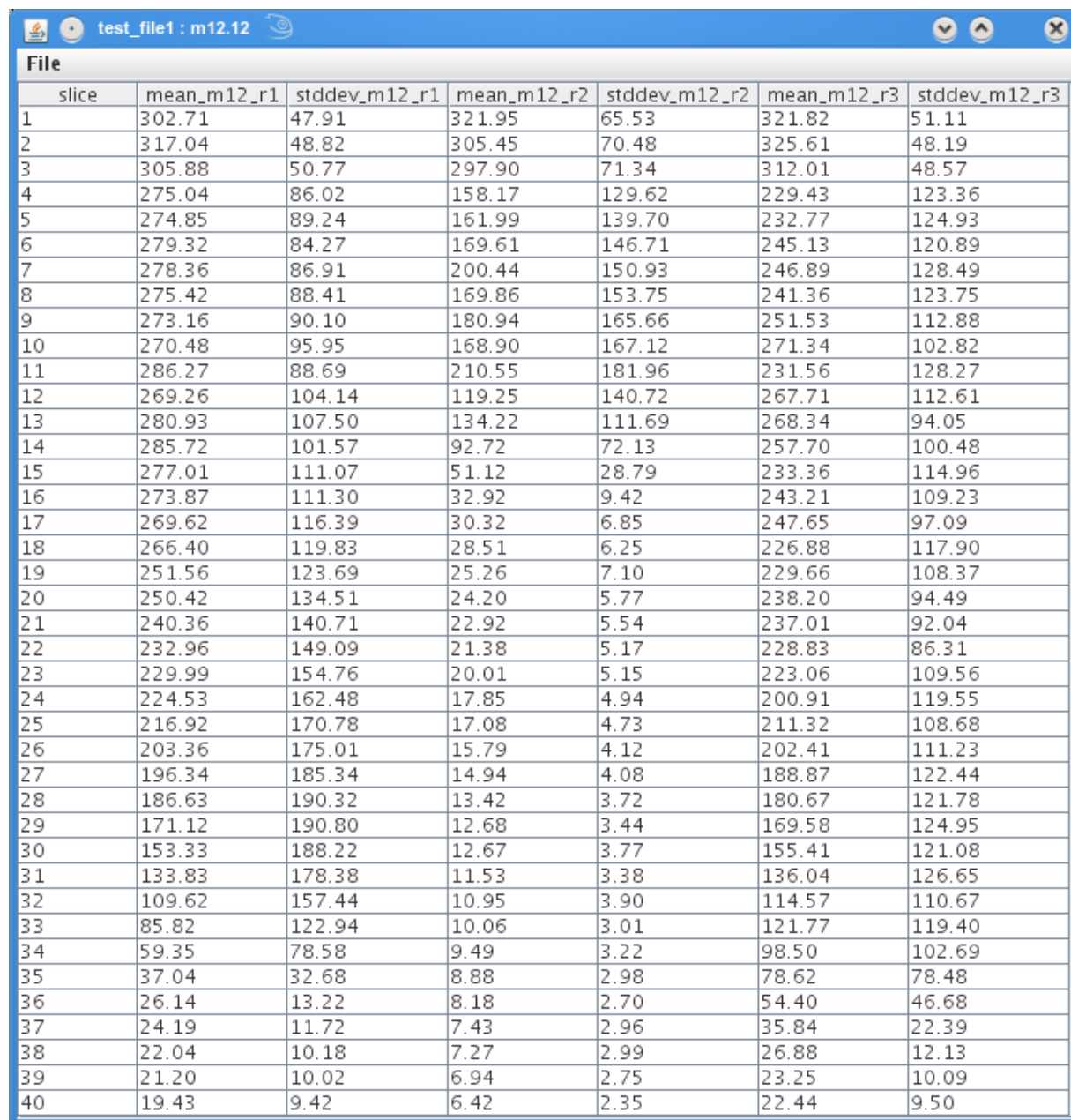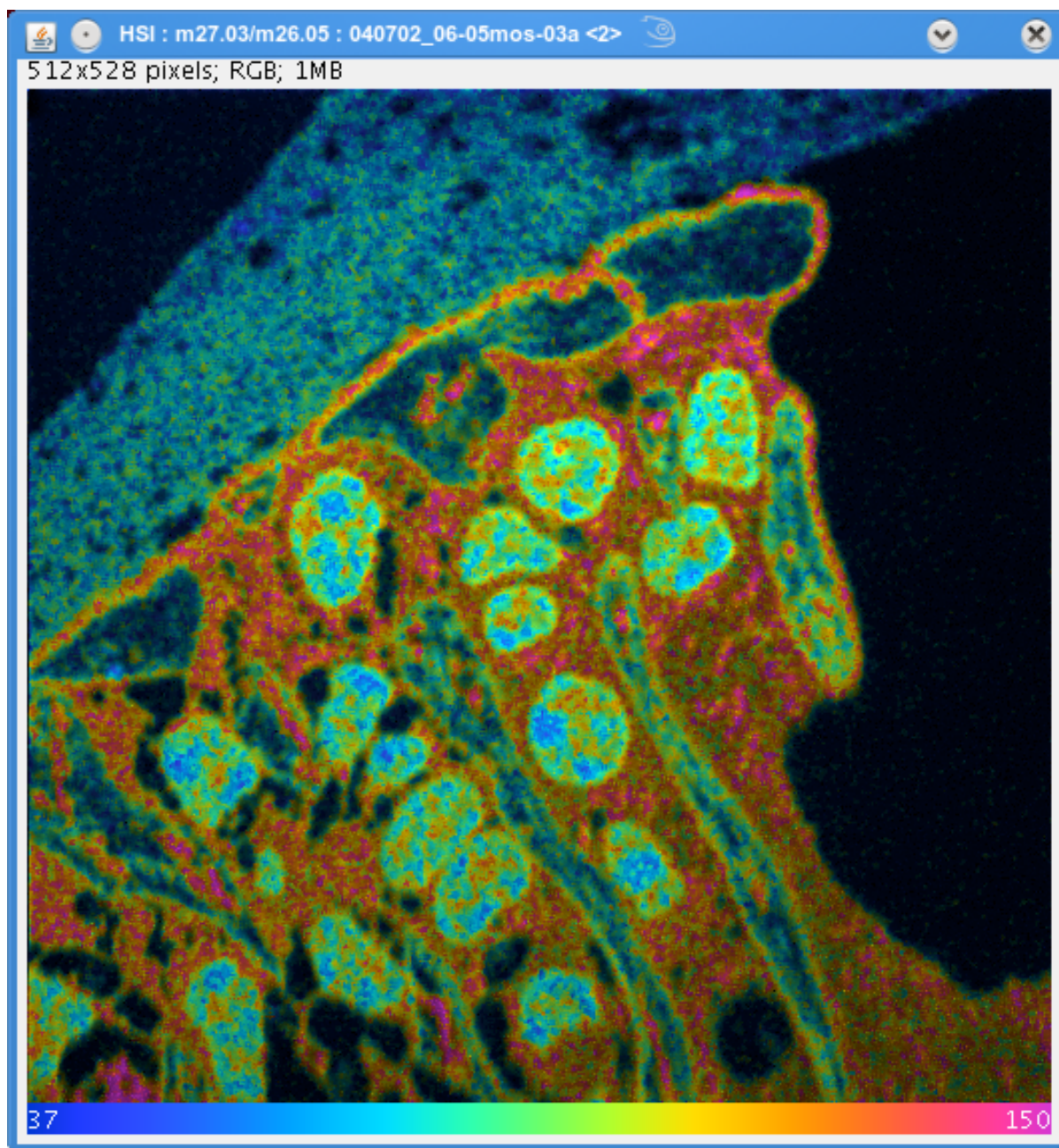
[3] http://teem.sourceforge.net/nrrd/

APPENDIX A

Figure 14: An example of an HSI image.

APPENDIX B

slice	mean_m12_r1	stddev_m12_r1	mean_m12_r2	stddev_m12_r2	mean_m12_r3	stddev_m12_r3
1	302.71	47.91	321.95	65.53	321.82	51.11
2	317.04	48.82	305.45	70.48	325.61	48.19
3	305.88	50.77	297.90	71.34	312.01	48.57
4	275.04	86.02	158.17	129.62	229.43	123.36
5	274.85	89.24	161.99	139.70	232.77	124.93
6	279.32	84.27	169.61	146.71	245.13	120.89
7	278.36	86.91	200.44	150.93	246.89	128.49
8	275.42	88.41	169.86	153.75	241.36	123.75
9	273.16	90.10	180.94	165.66	251.53	112.88
10	270.48	95.95	168.90	167.12	271.34	102.82
11	286.27	88.69	210.55	181.96	231.56	128.27
12	269.26	104.14	119.25	140.72	267.71	112.61
13	280.93	107.50	134.22	111.69	268.34	94.05
14	285.72	101.57	92.72	72.13	257.70	100.48
15	277.01	111.07	51.12	28.79	233.36	114.96
16	273.87	111.30	32.92	9.42	243.21	109.23
17	269.62	116.39	30.32	6.85	247.65	97.09
18	266.40	119.83	28.51	6.25	226.88	117.90
19	251.56	123.69	25.26	7.10	229.66	108.37
20	250.42	134.51	24.20	5.77	238.20	94.49
21	240.36	140.71	22.92	5.54	237.01	92.04
22	232.96	149.09	21.38	5.17	228.83	86.31
23	229.99	154.76	20.01	5.15	223.06	109.56
24	224.53	162.48	17.85	4.94	200.91	119.55
25	216.92	170.78	17.08	4.73	211.32	108.68
26	203.36	175.01	15.79	4.12	202.41	111.23
27	196.34	185.34	14.94	4.08	188.87	122.44
28	186.63	190.32	13.42	3.72	180.67	121.78
29	171.12	190.80	12.68	3.44	169.58	124.95
30	153.33	188.22	12.67	3.77	155.41	121.08
31	133.83	178.38	11.53	3.38	136.04	126.65
32	109.62	157.44	10.95	3.90	114.57	110.67
33	85.82	122.94	10.06	3.01	121.77	119.40
34	59.35	78.58	9.49	3.22	98.50	102.69
35	37.04	32.68	8.88	2.98	78.62	78.48
36	26.14	13.22	8.18	2.70	54.40	46.68
37	24.19	11.72	7.43	2.96	35.84	22.39
38	22.04	10.18	7.27	2.99	26.88	12.13
39	21.20	10.02	6.94	2.75	23.25	10.09
40	19.43	9.42	6.42	2.35	22.44	9.50

Figure 15: Sample output table showing mean and standard deviation for a 40 plane image file with three ROIs
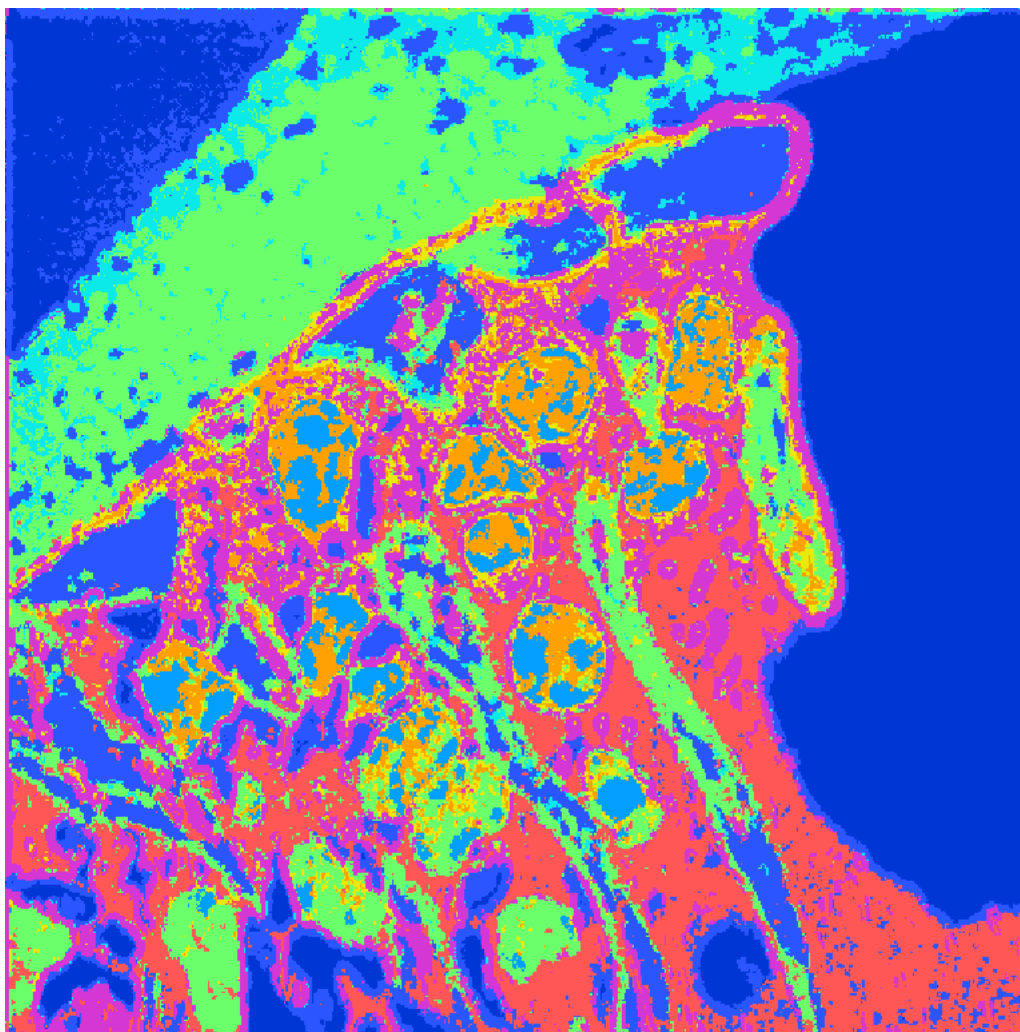
APPENDIX C

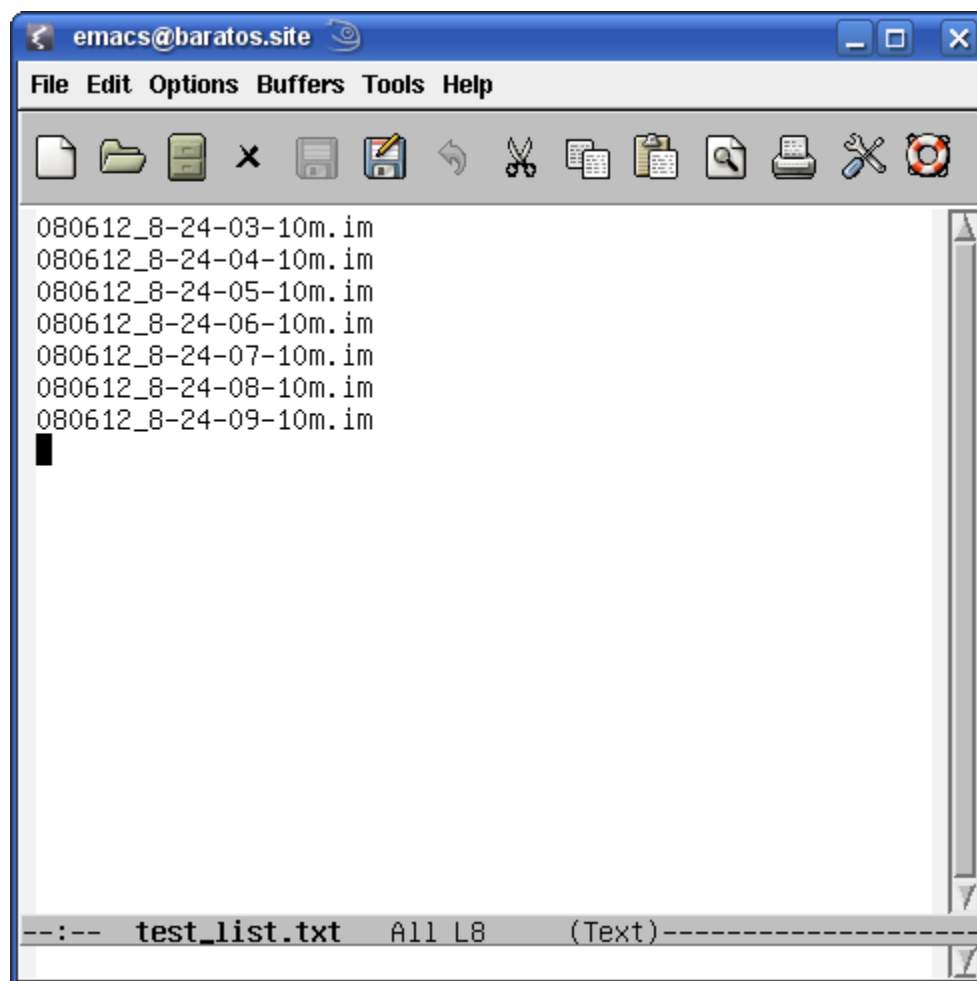Figure 16: A segmented image with 9 classes and no post processing.

APPENDIX D

Figure 17: A sample image list file.