Array Analyze Software

ACTIVE MOTIF®

Format: PC compatible

Applications: For use with the MODified™ Histone Peptide Array (Catalog Nos. 13001 & 13005)

Description: The Array Analyze software program is designed for use with the MODified™ Histone Peptide Arrays (Cat. Nos. 13001 & 13005). The software program will analyze the intensity of spot interactions from the MODified Histone peptide array and generate a graphical analysis of the histone peptide modification interactions. The Excel file containing the list of histone modifications and their associated locations has been incorporated into the software to help automate the identification process for the arrays. With the Array Analyze Software, information about spot intensity, averages and errors can be saved in Excel-compatible files to allow for individual analysis. For added convenience, up to three individual histone modifications can be displayed in superposition to the experimental data.

Installation: The software is designed for use on PCs only. Download the software file to your computer desktop, and unzip the file. Open the unzipped folder and begin installation by running the setup.exe file. If you get an error during installation or if the software does not run after installation, you should first run VisualBasicPowerPacksSetup.exe & MSChart.exe before running setup.exe.

A shortcut for the Array Analyze program should automatically appear on your desktop.

Usage Instructions:

1. Follow the MODified™ Histone Peptide Array manual to perform experiments.
2. Capture an image of the processed array. Crop the image to the size of the slide using normal exposure settings. Sometimes it helps to over-expose the image to see the edges of the slide. Make sure to return the exposure settings back to normal before capturing the image.

    Note: If there are only a few spots visible, this is a good time to over-expose the slide and identify them. Even spots that do not react with the antibody should be faintly visible on the array. Once all the spots are visible, identify the spots that did react and make a note of their position for use later with the spot assignment in the array software.

3. Save the cropped image as a .tif file.
4. Start the Array Analyze program by clicking the shortcut on your desktop.
5. Select Open Slide…
6. Upload the .tif file.
7. Optionally crop the image if the array spots do not fill the display window.
    a. Use the mouse to click-and-drag a rectangle around the border of the array image, making sure to leave a small amount of space outside of the spots.
    b. Click the Crop button. (The original image file will not be modified).
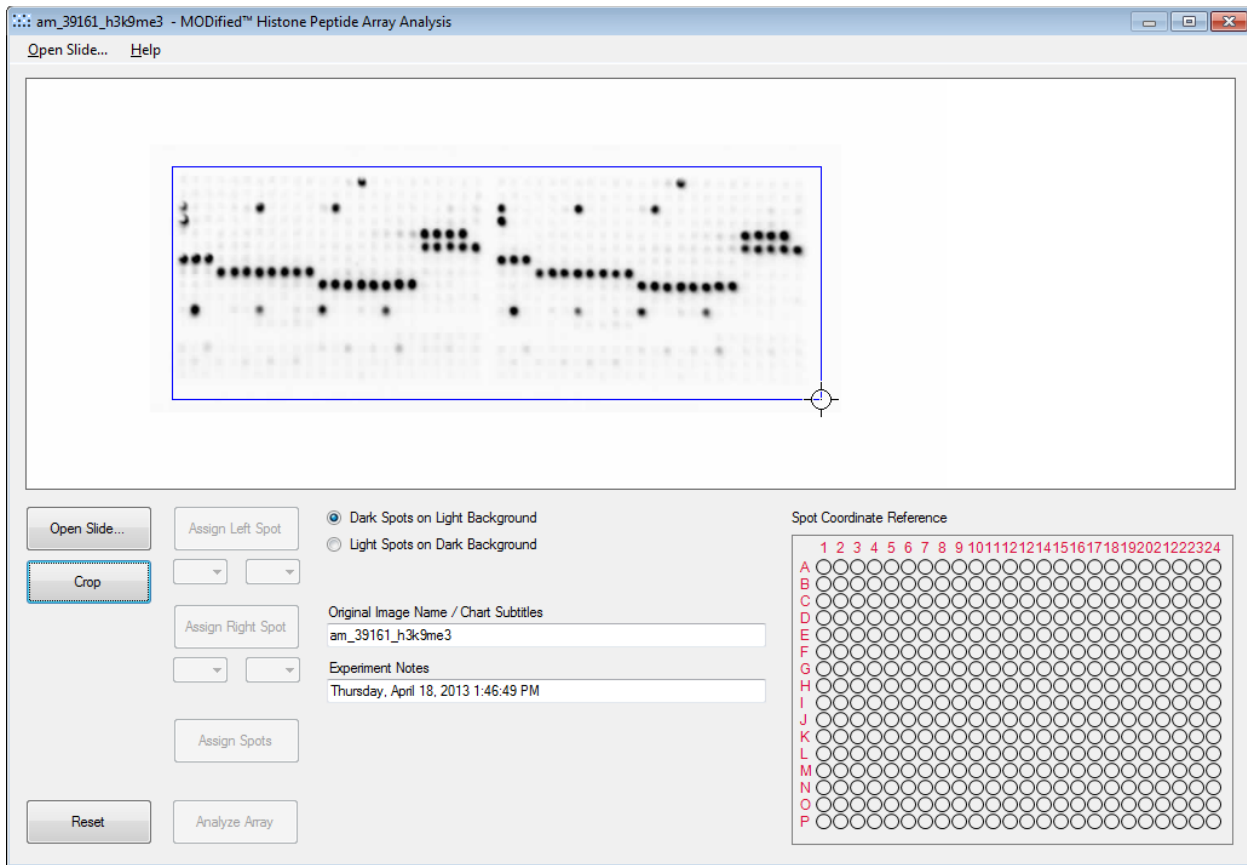
8. Select the option "Dark Spots on Light Background" or "Light Spots on Dark Background" which corresponds to your image type.

9. In order to align the array reference grid with the slide you will need to identify two spots. It is best if spot 1 is in the upper left
corner of the left half of the array and spot 2 is in the lower right corner of the right half of the array. However, in the case that only a few spots show up, take care to correctly identify their location and use those spots as the reference spots.

   a. Align the cursor with the center of a spot on the upper left side of the left array.

   b. Click on the center of this spot.

   c. Use the drop down menu to select the coordinates of the selected spot (Row A to P, Column 1 to 24).

   d. Click the Assign Left Spot button.

   e. Repeat for the lower right side of the right array and click the Assign Right Spot button.

10. Once the left and right spots have been assigned, press the Assign Spots button.

11. The grid image will be overlaid onto the array image. Verify the grid is in alignment with the array spots. If not, click the Reset
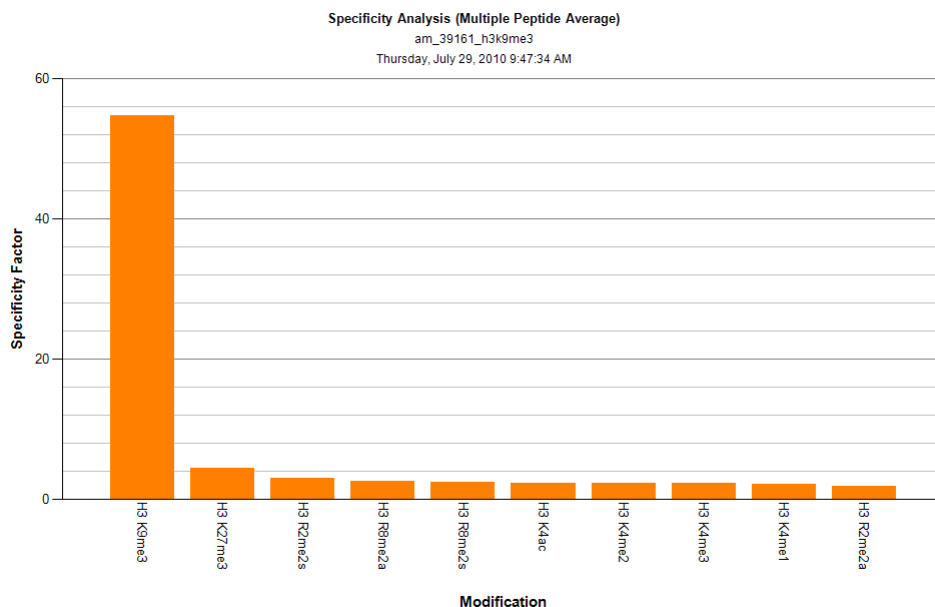button and repeat spot alignment. See the example image below.
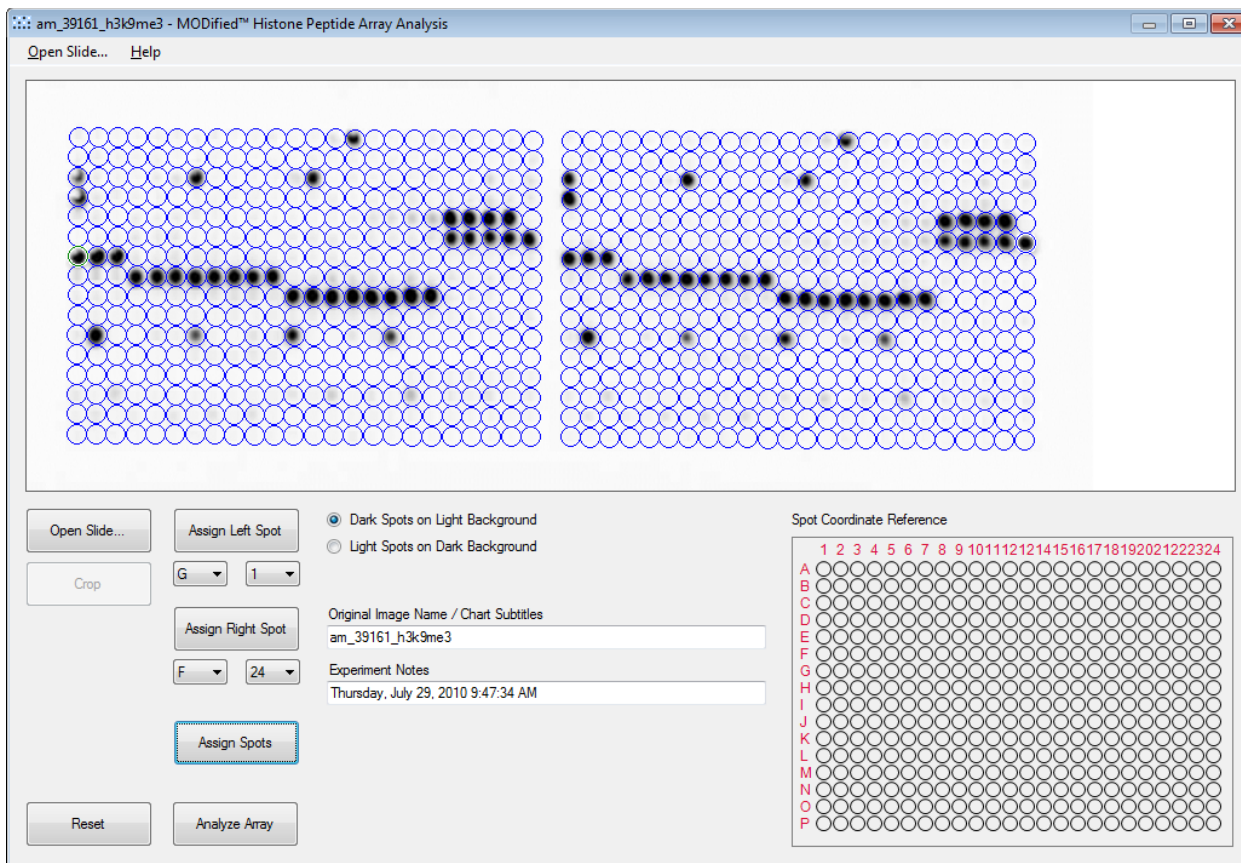
12. Optionally edit the fields for Chart Subtitles and Experiment Notes. By default these contain the original image name and the date that the original image was saved. These fields will appear as annotations on results graphs.

13. Click the Analyze Array button. A new tabbed Window will open containing the analysis results.

14. To Save any of the results, select one of the Save options from the File menu. Save All saves results from all tabs, or you may Save the output displayed on each tab individually. By default, the results will be saved in the same directory as the original image file. The location and names of the files can be changed if desired by editing the information in the Save dialog. Note that tabs which have multiple display options will only save the selected option when Save All is used. To save multiple graphs from a single tab, use the Save As option under the File menu.

15. The Spot Alignment tab contains an image of the grid overlay. If saved, this image will be called "name_slide.png".

16. The Spot Statistics tab gives a comparison of the intensities of each spot in the left and right array (left image). The data points ideally should be on a straight line connecting 0,0 and 1,1 which indicates perfect duplicates. Outliers (or poor replicates) will not fall along the linear line. If saved, this figure will be called "name_figXY.png". The distribution errors (right image) displays the errors of the intensities between the right and left spot, normalized to the maximum intensity. It is ideal to have most of the peptides contain an error range of 0-5% (which means very little variation between the left and right sides). If saved, this figure will be called "name_fig1.png".

17. The Background Setting tab allows you to define the background for the calculation of the Specificity, Reactivity, and Modification Analysis profiles. Click on the graph to manually change the background level (orange line on graph), or select the Use Automatic Background Level button for the default background values. Lowering the background level will decrease the specificity of the analysis allowing weakly associated spots to be included, while raising the background level will increase the specificity of the analysis so only spots with higher signal levels will be
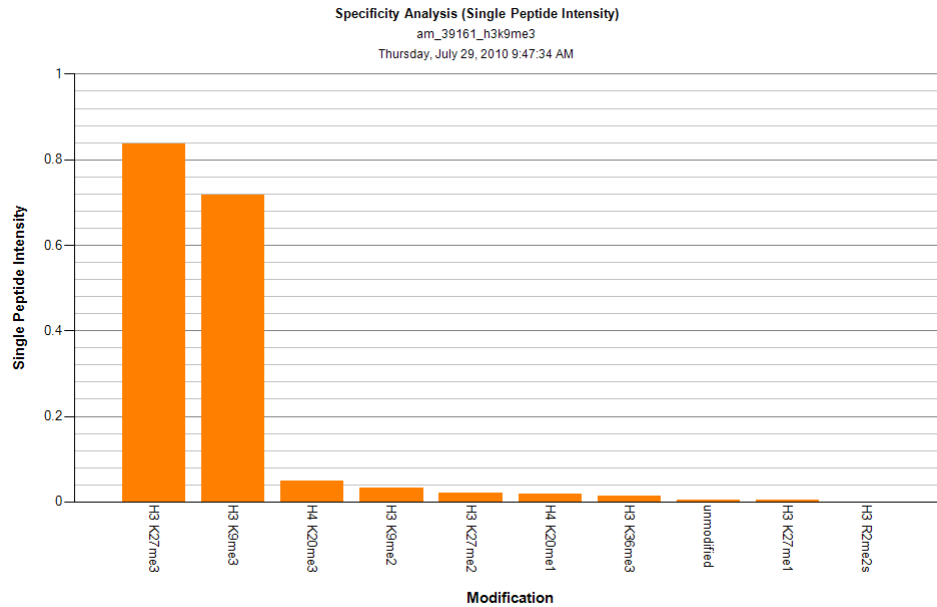
considered positive. If saved, this figure will be called "name_fig2.png".

18. The Specificity tab displays a background subtracted graph of the 10 modifications with the greatest specificity factors. While all modifications have been accounted for in determining the specificity factor, only the 10 modifications with the highest specificity factor values are graphed. The larger the difference in specificity factor values for the mark of interest versus other marks, the more specific the interaction. The Multiple Peptide Average option calculates the ratio of the average intensity of all spots containing the mark divided by the average intensity of all spots not containing the mark. Non-specific signals will decrease the specificity factor values. These non-specific signals can be caused by improper antibody dilutions, over development, and non-specific binding (false positives). Note that the data used to generate this graph can be viewed and saved in the Raw Data tab (see below). The Single Peptide Intensity option displays only single modified peptides without averaging all spots containing the mark. If saved, the graph will be called "name_fig3.png". See the example graphs below.

Note: When using the Multiple Peptide Average view to interpret the specificity of your binding event, it is important to understand that this information is provided as a general overview of specificity, but the graph should not be considered all inclusive of the information needed to assess complete specificity. The presence of "false positives" and "false negatives" will alter the average intensity values and can change the specificity factor value for a particular modification. False positives indicate non-specific binding of a modification that was not anticipated. The intensity of the false positive spots will be averaged into the denominator of the ratio, resulting in a reduced specificity factor value. Alternatively, false negatives can result from the inhibition of binding due to the presence of a neighboring modification. In this case a peptide that contains the modification of interest will have a lower than expected signal. This reduced intensity value is averaged into the numerator of the ratio generating a reduced specificity factor value. The result is that a modification with very specific binding may display a lower specificity factor value than a less specific binding event due to the presence of neighboring modifications.

To address these influences, utilize the Reactivity tab (see below) to look at individual modifications that show binding. Then use the Modification Analysis tab (see below) and select the modifications identified by the Reactivity tab to evaluate the effects of neighboring modifications on binding specificity.

Specificity Analysis (Multiple Peptide Average)
am_39161_h3k9me3
Thursday, July 29, 2010 9:47:34 AM

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Specificity Analysis (Single Peptide Intensity)
am_39161_h3k9me3
Thursday, July 29, 2010 9:47:34 AM

19. The Reactivity tab displays the background subtracted intensity values for all single modified peptides, as well as for control spots. Selecting a modification from the pull down menu scales the intensity of the selected modification to 1.0. Select "none" to display unscaled values. The impact of neighboring modifications on the single modified peptides can be further investigated using the Modification Analysis tab and selecting for the modification of interest (see below). If saved, the graph will be called "name_fig6.png". See the example graph below.

Single Modification Peptide Reactivity (Scaled)
am_39161_h3k9me3
Thursday, July 29, 2010 9:47:34 AM
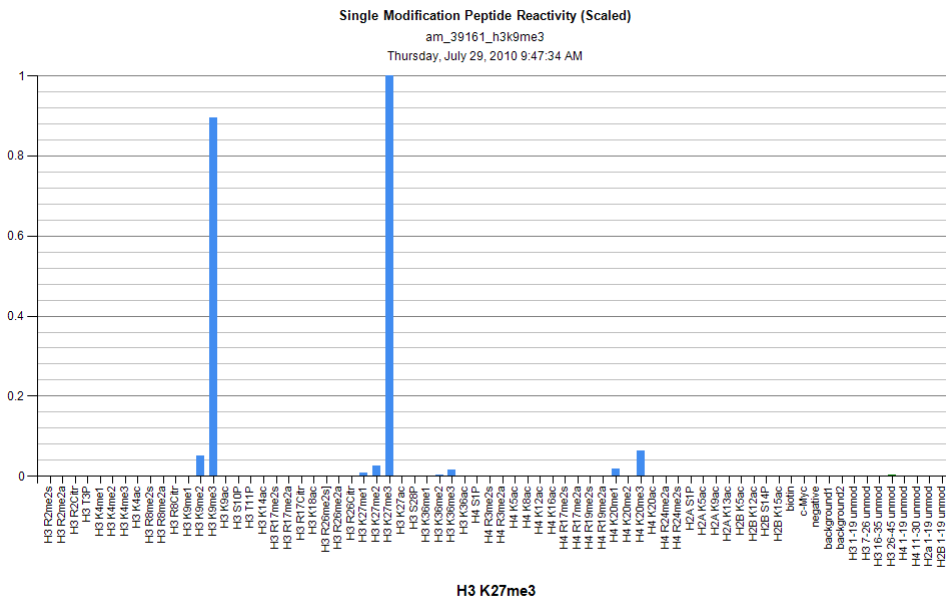
20. The Modification Analysis tab displays background subtracted intensity values for all spots containing the selected modification. If saved, the graph will be called "name_fig5.png". See the example graph below.

Modification Analysis
am_39161_h3k9me3
Thursday, July 29, 2010 9:47:34 AM

H3 K9me3
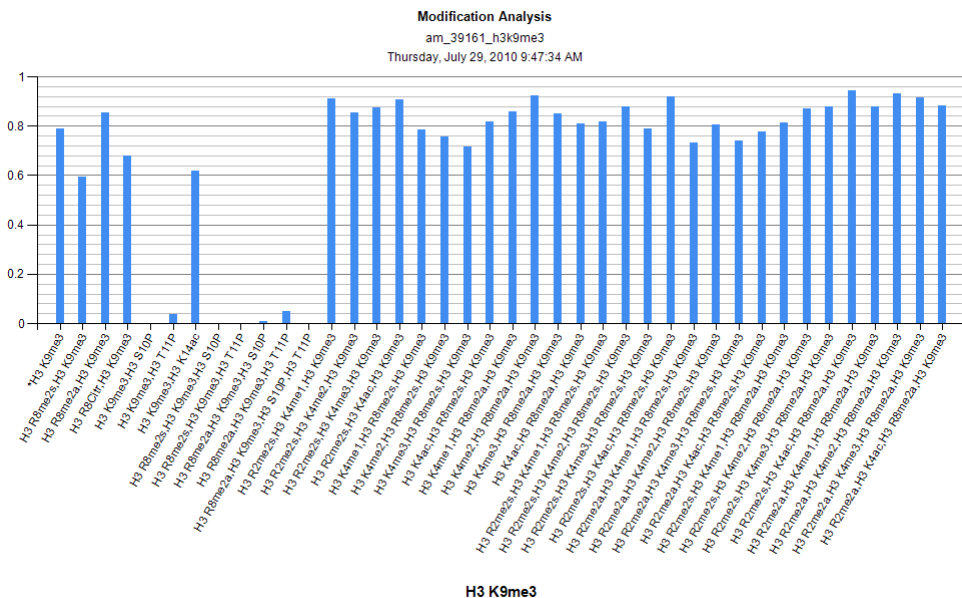
21. The Annotated Array tab allows you to select up to three modifications to be displayed in superposition to the experimental data. Select these modifications from the drop down menus. Press the Select Most Specific button to automatically display the top 3 modifications from the Specificity chart. Check the boxes in order to see the array grid overlay or to identify the modifications on both sides of the array. Use the color coding to identify the modifications. If saved, this graph will be called "name_fig4.png". See the example chart below.

Annotated Array
am_39161_h3k9me3
Thursday, July 29, 2010 9:47:34 AM

H3 K27me3	H3 K27me3, H3 K9me3
H3 K9me3	H3 K27me3, H4 K20me3
H4 K20me3	H3 K9me3, H4 K20me3
H3 K27me3, H3 K9me3, H4 K20me3	
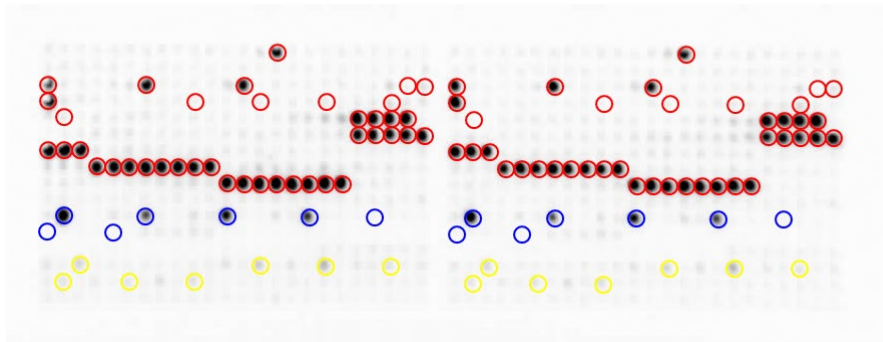
22. The Raw Data tab displays the comma separated value (CSV) data for the peptide intensities for all spots, the ranked intensities for all spots, and the ranked modifications. This CSV data can be saved in Excel compatible files to allow for individual analysis. If saved, these files will be called "name_data.csv", "name_ranked_list.csv", and "name_ranked_modifications.csv", respectively.

The data in the "name_data.csv" file is the raw data for the array. The fields are:

   a. Peptide No.   the peptide number (1 through 384)

   b. Location   the grid coordinates of the spot on the array (A1 through P24)

   c. Name   the peptide name

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d. Mod 1   the first modification, if present

   e. Mod 2   the second modification, if present

   f. Mod 3   the third modification, if present

   g. Mod 4   the fourth modification, if present

   h. Intensity left   the intensity value for the spot on the left side of the array

   i. Intensity right   the intensity value for the spot on the right side of the array

   j. Intensity av.   the average between the duplicate (left and right) spots

   k. Error   the error between the duplicates (the lower the value, the better the
       duplicate readings. This is
                          visually depicted as figXY)

The data in the "name_ranked_list.csv" file is ranked according to intensity values from highest to lowest. The control and background spots on the array should be found ranked towards the bottom if there was low background and good specificity. The fields are:

   a. Rank   the peptide rank (1 through 384)

   b. Pept. No.   the peptide number (1 through 384)

   c. activity   the average intensity value from the name_data.csv file

   d. norm. act.   the normalized activity. This is calculated by dividing the activity by the
       activity of the number one
                          ranked peptide.

   e. The remainder of the fields is the data for each modification, with a value of 1 indicating the
       modification is present in the peptide, and a value of zero indicating that the modification
       was not present.

The data in the "name_ranked_modifications.csv" file list each modification ranked by specificity factor, from highest to lowest. This is the same data used to generate the Multiple Peptide Average chart. The fields are:

   a. Rank   the rank of the modification

   b. Modification   the name of the modification

   c. No pos   number of peptides on the array that contain the modification

   d. No neg   number of peptides on the array that do not contain the modification

   e. Av Int pos   average intensity of all spots containing the modification

   f. Av Int neg   average intensity of all spots that do not contain the modification

   g. Spec   Specificity factor (Av Int Pos divided by Av Int Neg)

23. The Protocol Log tab displays a log of steps used to perform the analysis. If saved, this log will be called "name_protocol.txt".

24. To start a new analysis or edit the analysis of the current image, select Open Image… from the File menu.

25. Close out of the program by selecting Exit from the File menu, or by using the X in the upper right corner of the program.