Overview:

This package of Igor scripts is designed to interpret mass spectrometry data that has been saved in a .csv file format. These scripts are able to take in exact masses and names of compounds as input from the user and find and label masses corresponding to monomers and polymers of these compounds. Scripts are also available to export graphics for use in Powerpoint or other media.

These scripts were written in Igor 6 and have been updated for use with Igor 8.

Data Files:

Data files should be saved as CSV Files and be created with no header row with the m/z value first and the intensity value second. These values should be separated by either a comma or a tab. Examples in figure 1.

```
55.8,0
55.95,0
56.1,0.0070649285874105
56.25,0.0241809926909308
56.4,0.00250550044749404
56.55,0
56.7,0
56.85,0.00314644242243437
57,0.00272400339349642
57.15,0
57.45,0
57.6,0.00310274183323389
57.75,0.00364171576670645
57.9,0.00948302785650358
58.05,0.0200877041691528
58.2,0.00675902446300716
58.35,0.00591414640513127
58.5,0.0140278891333532
58.65,0.0200585704430191
58.8,0.0177424392153938
58.95,0.0105901094495824
59.1,0.0115078218227924
59.25,0.00670075701073986
59.4,0.00760390252088306
```

```
55.8    0
55.95   0
56.1    0.0070649285874105
56.25   0.0241809926909308
56.4    0.00250550044749404
56.55   0
56.7    0
56.85   0.00314644242243437
57  0.00272400339349642
57.15   0
57.45   0
57.6    0.00310274183323389
57.75   0.00364171576670645
57.9    0.00948302785650358
58.05   0.0200877041691528
58.2    0.00675902446300716
58.35   0.00591414640513127
58.5    0.0140278891333532
58.65   0.0200585704430191
58.8    0.0177424392153938
58.95   0.0105901094495824
59.1    0.0115078218227924
59.25   0.00670075701073986
59.4    0.00760390252088306
59.55   0.0142755258054893
59.7    0.0195195965095466
59.85   0.0202916402520883
60  0.0353974772523867
```

Figure 1

Loading data into Igor:

In the “Macros” menu inside of Igor select “Label_Single_Spectrum.” (Figure 2)

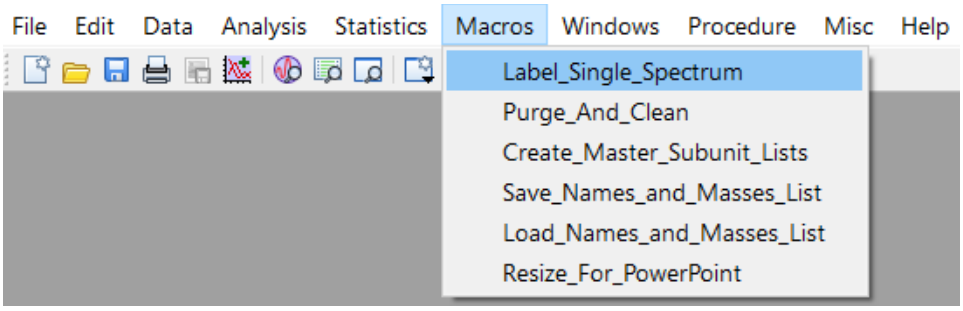

Figure 2

Browse to your .CSV, select it, and click “Open.” This will open your data into a spectrum similar to what is seen in Figure 3.

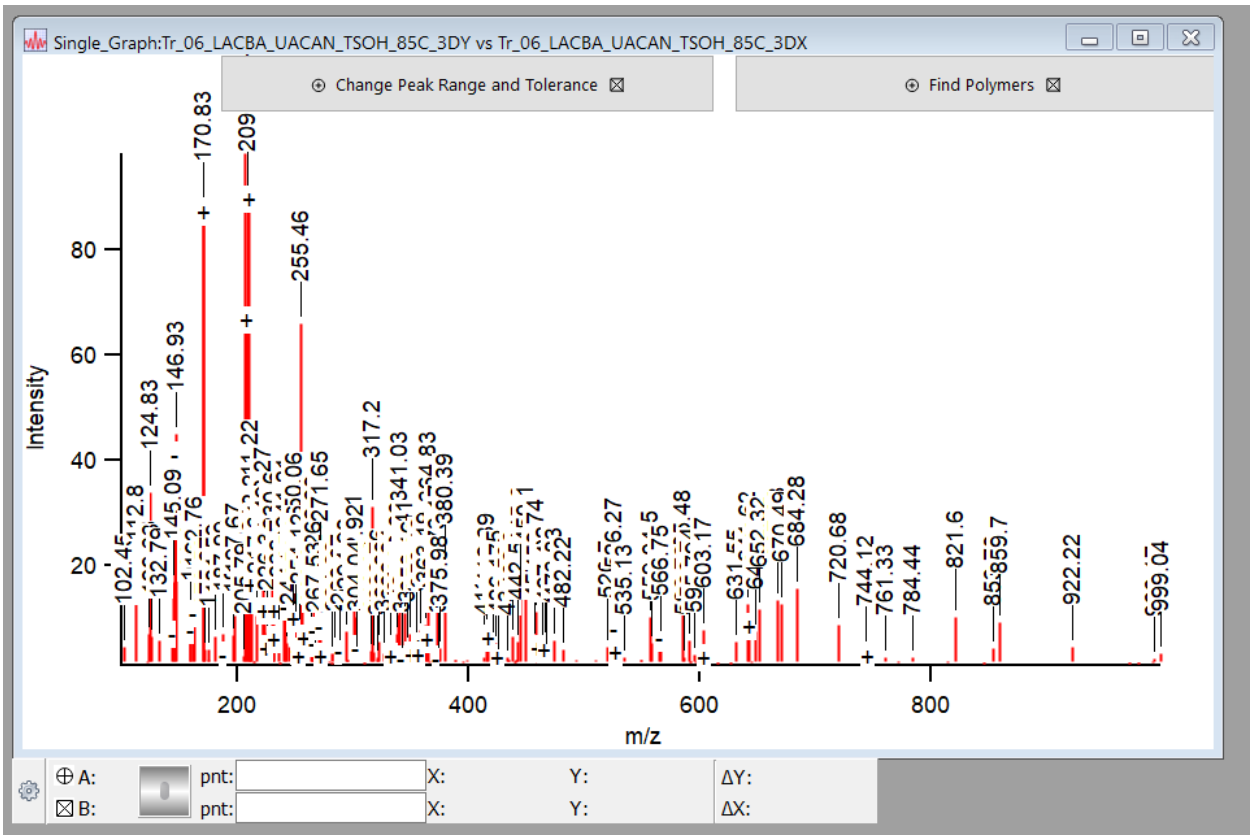

Figure 3

Processing Spectrum:

Define the processing range:

Once the spectrum is open cursors A and B must be placed on the spectrum before continuing. These cursors are available in the small rectangular window open at the bottom of the spectrum (Figure 3). Simply click and drag the symbols next to A: and B: onto the spectrum making sure that A is the left of B. These cursors define which region of the spectrum will be processed.

Exclude low intensity peaks:

In order to exclude low intensity peaks the Peak Range and Tolerance can be adjusted. Click on the "Change Peak Range and Tolerance" button located at the top of the spectrum (Figure 3). This will open a new dialog window where you can define the minimum tolerance; this value calculates a ratio between each peak's intensity and the maximum intensity in the range and excludes anything below this threshold from analysis. Press Continue when finished. If the tolerance requires further adjustment, repeat this procedure.

Find and Name Peaks:

Once the region and tolerance is sufficiently defined, click on "Find Polymers" to bring up the window where you can define what species to search for (Figure 4).

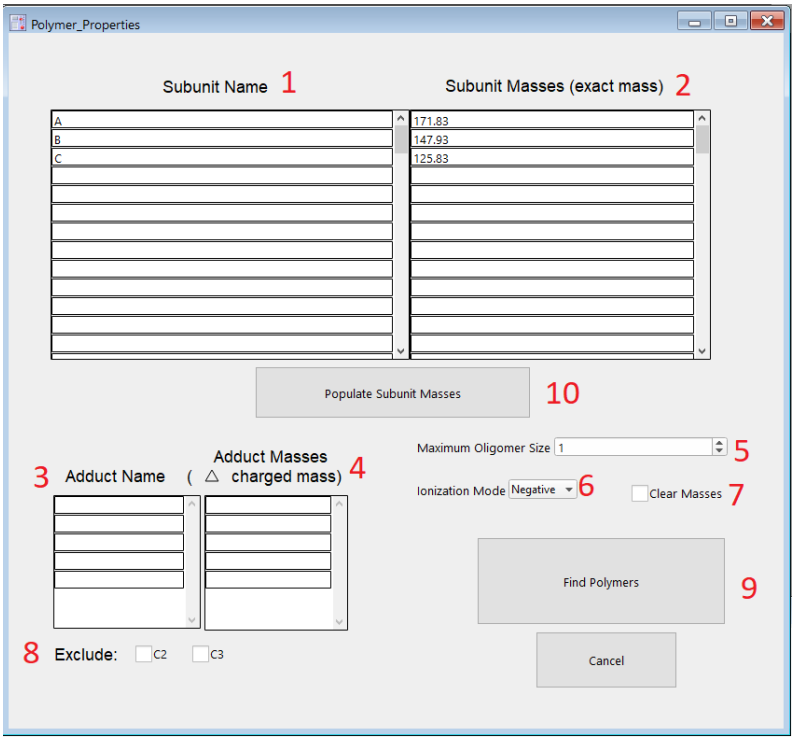

Figure 4

1 – Define the name of the species to search for.

2 – The exact mass of the species to be found from (1).

3 – If you want to search for non-covalent adducts, list the adduct to be searched for here.

4 – The exact mass difference if the non-covalent adduct is present.

5 – Maximum oligomer size to be searched for. Enter 1 for no polymers.

6 – Specify whether the spectrum is in positive or negative mode.

7 – Click this box if you want to remove the numerical masses from the spectrum and only display the species defined in (1) under Subunit Name.

8 – By default, multiply charged species of +-2 and +-3 will be searched for. Checking C2 or C3 will exclude +-2 or +-3 charged species, respectively, from the mass search.

9 – Once this window is filled out, click “Find Polymers” to proceed.

10 – If masses were previously defined and imported (as outlined later), then if the species are listed in (1) under Subunit Name then the Subunit Masses will be automatically populated in (2) by pressing this button.

Once “Find Polymers” is clicked, an annotated spectrum similar to the following (Figure 5) will be displayed:

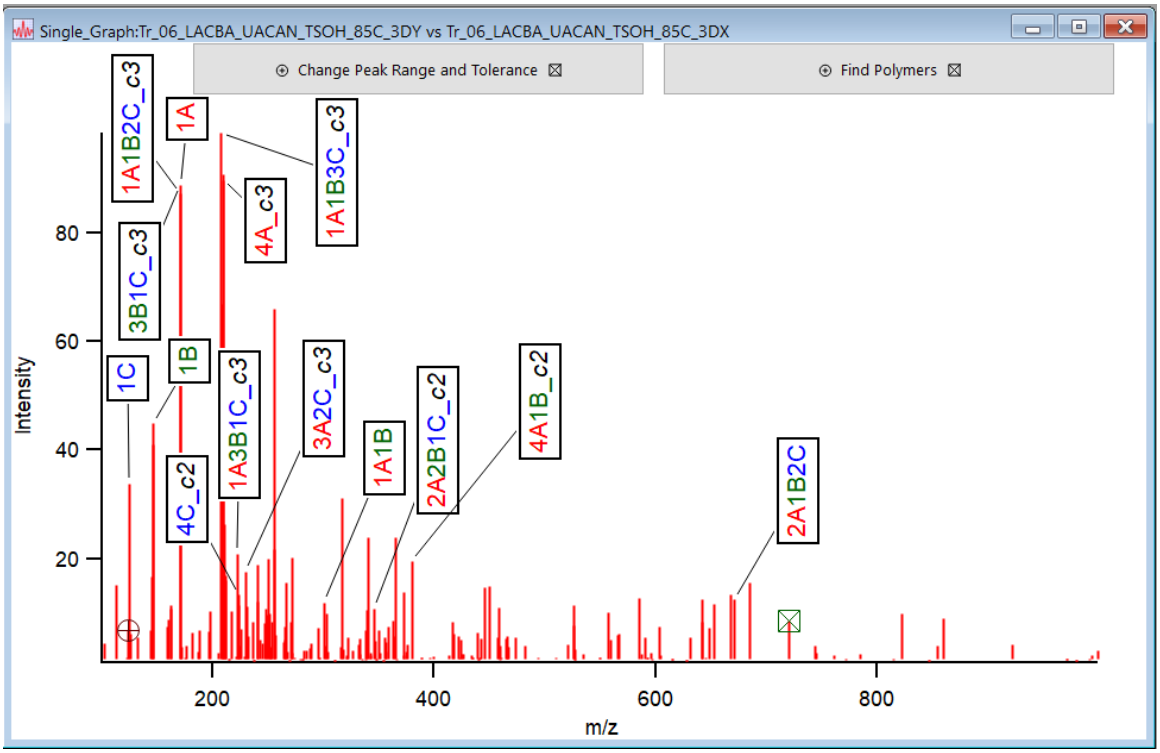

Figure 5

These annotations show which masses correspond to different combinations of the defined subunits. Some notes on the labels:

1) The labels can be freely moved around the spectrum by clicking and dragging.
2) _c2 or _c3 labels represent species with 2 or 3 charges on them.
3) _(adduct name) represents a non-covalent adduct.
4) If multiple species have the same exact mass, then each possible species will be listed. Therefore, some peaks may have multiple labels.
5) If any labels are to be removed, double-click on the label and select "Delete" from the subsequent dialog box.

Note: The "Resize_for_Powerpoint" macro can now be selected from the macro menu (Figure 2). This macro will resize the spectrum so that it can be easily imported into Powerpoint (approximately the size of half a standard slide).

Saving and Importing Mass Lists:

For Loading and Saving mass lists for future use, the "Create_Master_Subunit_Lists," "Save_Names_and_Masses_List," and "Load_Names_and_Masses_Lists" macros will be used (Figure 2).

Creating a Master Subunit List:

After a mass analysis as outlined in the previous section, if the mass list is to be saved for future use click on the "Create_Master_Subunit_Lists" command and the Subunit Names and Subunit masses previously defined will be used to generate new master lists for future use. After creating these lists, "Save_Names_and_Masses_List" must be selected. This macro will allow you to browse to a directory which you can save your current mass lists to.

Load a Master Subunit List:

Clicking the "Load_Names_and_Masses_List" macro will bring up a directory dialog. Browse to where a previous mass list was saved. Selected the files "Subunit_Masses_Master_List.ibw" and "Subunit_Names_Master_List.ibw" on at a time (in any order) and click "Load" to import them into Igor.

Once these masses are important, button (10) as outlined in "Processing Spectrum" section can be used to automatically populate subunit masses (Figure 4). In this section, populate the Subunit Names with the names of each subunit (note that this is case-sensitive) and once button (10) is pressed the corresponding masses will appear in "Subunit Masses."