# SFC-MS User Guide

Columbia University
Department of Chemistry

## Contents



## 1. Introduction

Welcome to the SFC-MS instrument in 544 Havemeyer. In order to obtain swipe access to 544 Havemeyer and to get an account on the instrument, you must be a trained user. To sign up for training or if you encounter any problems with the instrument that are not addressed in these instructions, please contact Brandon Fowler (brandon.fowler@columbia.edu).

*What is SFC-MS?* SFC-MS analysis combines analytical chromatography with mass spectrometry to provide information about sample purity and identity. Supercritical fluid chromatography (SFC) uses supercritical CO2 (>31 °C at >1070 psi) as the primary eluent. The eluent strength of the supercritical CO2 depends on its temperature and pressure as well as any co-eluents. Close to its critical point, supercritical CO2 is similar to hexanes in its elution strength, and most SFC methods include a co-eluent such as methanol, isopropanol, or acetonitrile. There is a wide variety of column chemistries available for SFC, and a list of the columns available on this instrument is provided in section IV. There are currently seven different columns loaded on the instrument. Compounds that elute from the columns are monitored by both a UV-vis detector (210–800 nm) and a single-quadrupole mass analyzer (up to 3000 m/z). The mass spectrometer can generate and analyze both positive and negative ions. The ionization methods are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). The instrument can utilize both methods and both polarities simultaneously in a single run.

*What does SFC-MS tell me?* Much like an automated flash chromatography instrument or HPLC instrument, you will get a UV-vis chromatogram at the end of your run showing peaks at times when any absorbent compounds eluted. Furthermore, within each time-point, the instrument collects a full UV-vis spectrum over the range 210–800 nm. Likewise, the SFC-MS instrument produces an ion chromatogram at the end of your run showing peaks at times when there was an increase in the number of ions being detected. There is an ion chromatogram for each type of ionization: ESI+, ESI–, APCI+, and APCI–. Within each time-point in each ion chromatogram, the instrument collects a full mass spectrum over a specified range. Therefore, for every time-point, the instrument will provide a UV-vis spectrum and four mass spectra.

*Why is the SFC-MS instrument useful?* The most common uses of this instrument will be reaction monitoring and identification of fractions from preparative chromatography. As a normal phase chromatography instrument, it is intended for small organic molecules (100–2500 Da). The instrument is also equipped with chiral columns and can be used for rapid analysis of enantiomeric ratios. The mass spec data produced on this instrument are considered low-resolution (within 0.5 Da) and are good for qualitative analysis of a sample; whereas, high-resolution data are required in most publications for proof of molecular formula. Email Brandon Fowler if you require high-resolution MS analysis.

## 2. Sample Preparation

Sample preparation is extremely important for obtaining consistent, high quality results. If your sample is not prepared correctly, you can contaminate the columns and/or instrument, which may require instrument down-time and expensive cleanings or replacements. Materials for sample preparation are not provided, and you should prepare your samples before bringing them to the instrument. In order to prepare your samples, you will need the following materials:

HPLC vials (12 mm outer diameter x 32 mm height, 9 mm opening, 2 mL)
Vial caps **with pre-slit septa** (9 mm open top, 6 mm hole, pre-slit septum)
Syringe filter (unless your sample has already been purified by chromatography or distillation)
0.2–1.0 mg of sample
An appropriate sample solvent (see below)

HPLC vials and caps (**with pre-slit septa**) can be purchased from VWR (97055-986 or 89239-028), Fisher Scientific (03-246-327 or 03-410-738), or Sigma-Aldrich (29652-U). Syringe filters with pore sizes 0.45 µm and smaller are acceptable. Be sure your syringe filter is compatible with (i.e., doesn't dissolve in) the solvent you are using. HPLC and LC-MS grade solvents are recommended for the highest quality results.

**Recommended sample solvents include:** isopropanol, methanol, acetonitrile

**Other permitted sample solvents include:** hexanes, heptane, methylene chloride, chloroform, ethyl acetate, tetrahydrofuran, toluene

The instrument runs normal phase chromatography; aqueous samples are prohibited. Extremely volatile solvents such as diethyl ether and pentane and very low volatility solvents such as DMF and DMSO are not recommended unless diluted with one of the solvents above. If you have any questions about a solvent, contact Brandon Fowler.

Dissolve your sample (0.5–1.0 mg/mL) in one of the solvents above and push the solution through a syringe filter into an HPLC vial. You will need at least 500 µL of sample solution. There must be **no particulates** and your solution must be **completely transparent**. If you have a colored sample and cannot see completely through the vial, dilute your sample until you can.

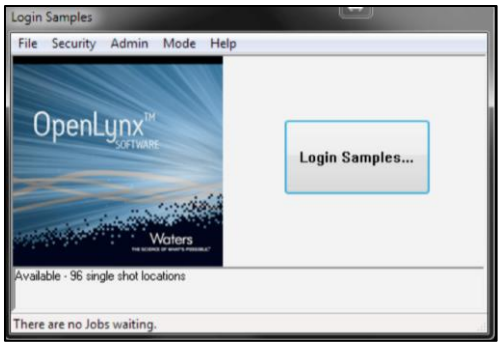
Samples with RADIOACTIVE ISOTOPES ARE STRICTLY FORBIDDEN on the SFC-MS instrument.

****Nota bene**** If you make a SFC-MS sample from a NMR sample, be aware of exchangeable protons. If your compound of interest has an exchangeable proton and your NMR solvent has an exchangeable deuteron, you may find your expected mass is off by 1 Da or more (depending on the number of exchangeable protons).

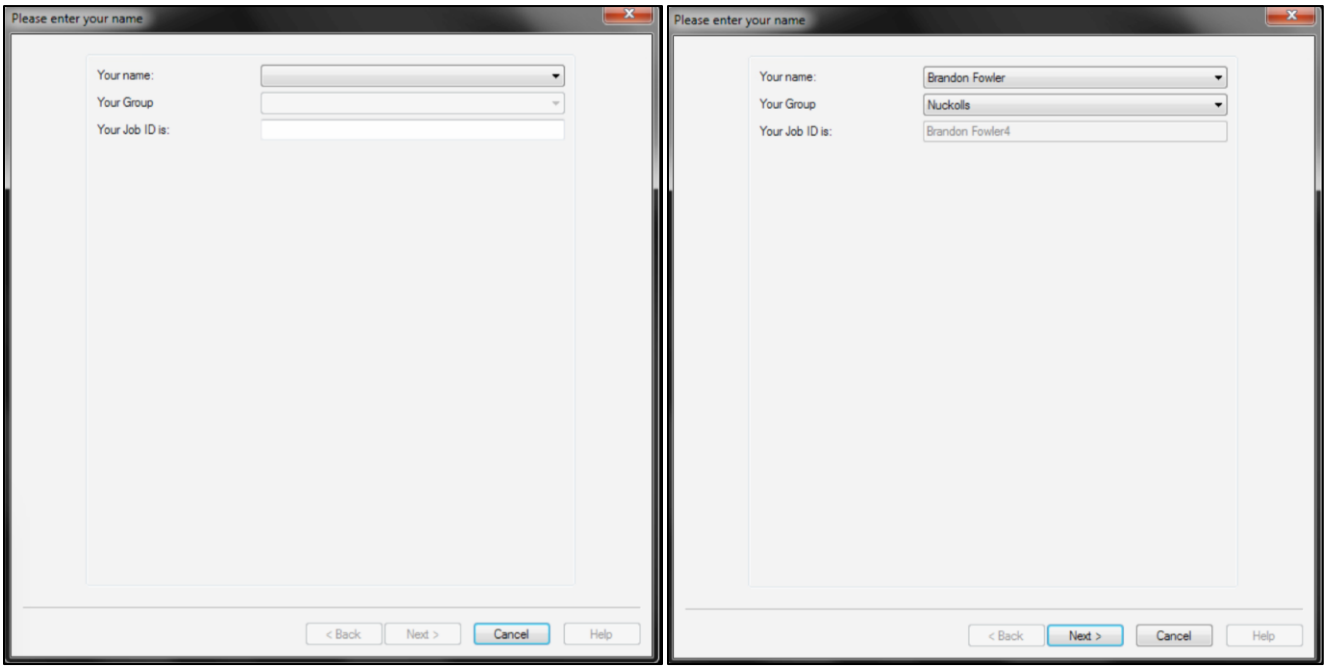
## 3. Logging in Samples

After you prepare your sample, you can log it into the queue for the SFC-MS instrument.

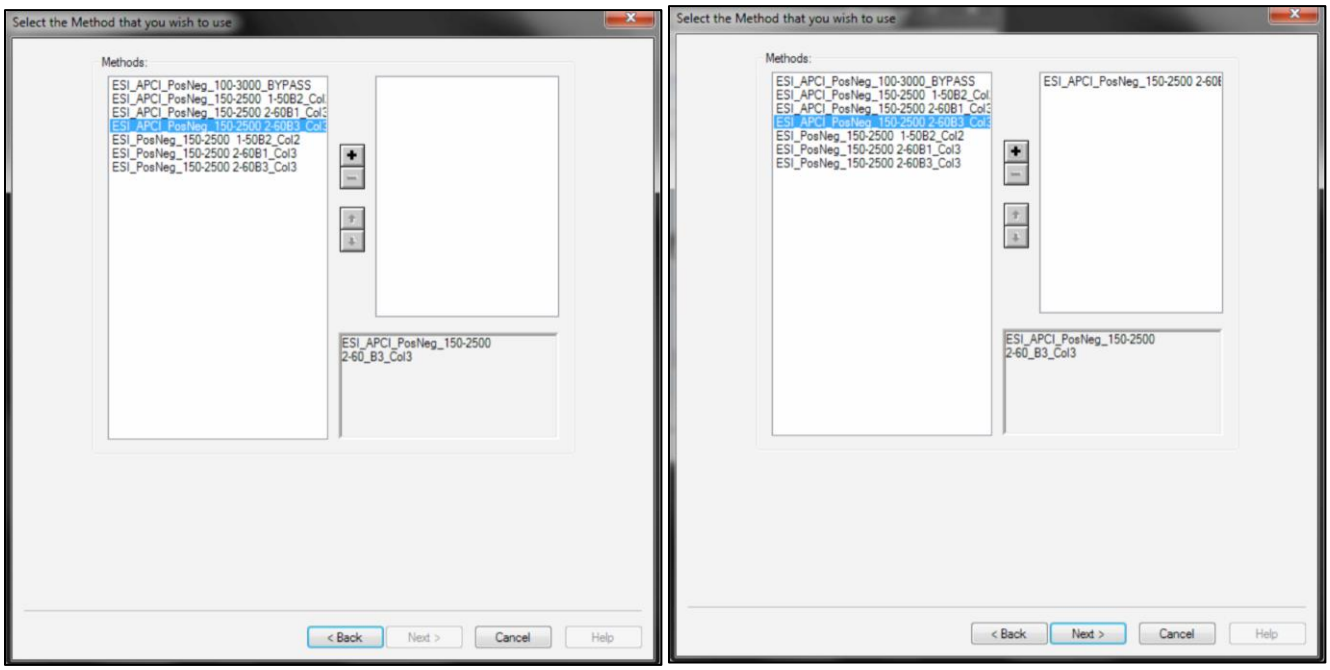
**Step 1.** At this point, you should see the small OpenLynx window shown below. Click on the button that reads "Login Samples…"



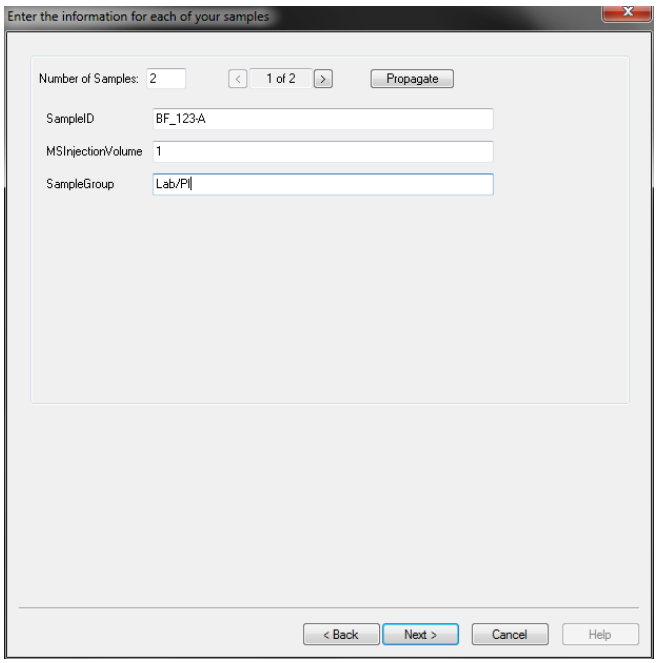

**Step 2.** Choose your name from the menu, at which point your Group and Job ID will be populated automatically. Click "Next".



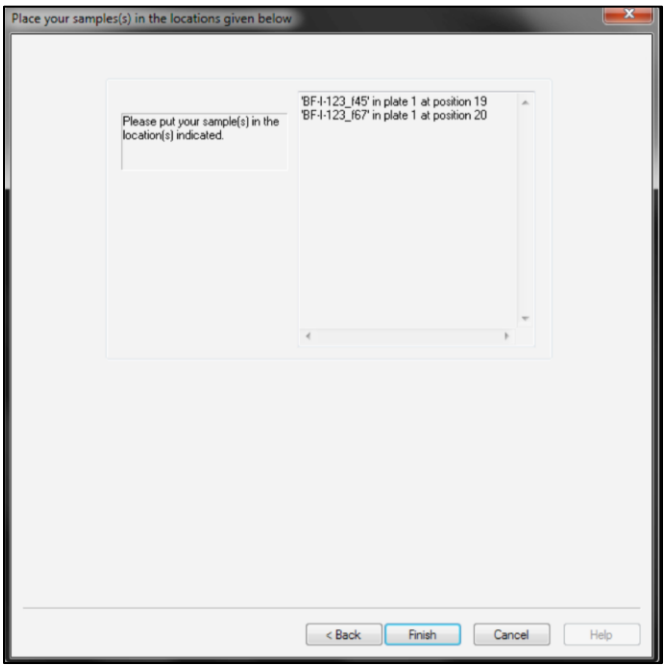
**Step 3.** Select a method from the list on the left. A description of each method including typical analytes, column, solvent, ionization methods, mass ranges, and run time can be found in the bottom right panel. Once you've found the method you want, click the "**+**" button to add it to the list of methods you'll be using for this session. If you are screening multiple methods for the same sample, you can choose multiple methods here (in this case, you may only submit one sample at a time in Step 4). Click "Next".




**Step 4.** Enter the number of samples you would like to submit. Enter the Sample ID for the first sample. **Sample IDs may contain only letters, numbers, dashes (-), and underscores(_)**. Any other characters might make the resulting data file unusable. Enter an Injection Volume – this is in microliters, and typically an injection volume of 1-2 uL is appropriate. In the SampleGroup field, enter the name of your Lab/PI. If you are submitting more than one sample, you can toggle between samples at the top of the window. When you have entered all the information for your samples, click "Next".



**Step 5.** Place your sample(s) in the position(s) indicated, then click "Finish". When you open the autosampler door, it will present Plate 1 in front. If the autosampler beeps at you when you open the door, it is in the process of moving a vial or drawing a sample. Close the door, wait 10 seconds, and try again. If there is a vial already in the position where you are placing your vial, place the old vial in the blue bin labeled "Discarded SFC-MS Samples". When estimating run times, keep in mind that each run will have 2-3 mins of equilibration before injecting. The data from your run can be accessed remotely as described in the next section. Alternatively, data can be processed in MassLynx on the instrument computer (ask Brandon Fowler for help with this).



## 4. Column Chemistries

| Position | Column Chemistry* | Uses/Notes |
|---|---|---|
| **1** | BEH Phenyl, 1.7 um, 2.1 x 100 mm | General small molecules (traditionally reversed phase column) |
| **2** | Torus 1-AA, 1.7 um, 2.1 x 100 mm | Steroids, fat-soluble vitamins, and other lipids |
| **3** | Torus 2-Pic, 1.7 um, 2.1 x 100 mm | General small molecules (best starting place) |
| **4** | BEH, 1.7 um, 2.1 x 100 mm | General small molecules (traditionally reversed phase column) |
| **5** | Torus Diol, 1.7 um, 2.1 x 100 mm | Most similar to silica gel |
| **6** | Trefoil AMY1, 2.5 um, 2.1 x 150 mm (Chiral) | Separation of enantiomers (like AD columns) |
| **7** | Trefoil CEL1, 2.5 um, 2.1 x 150 mm (Chiral) | Separation of enantiomers (like OD columns) |
| **8** | Bypass (No Column) | Direct injection to MS |

* Additional column chemistries available including Torus DEA, Trefoil CEL2, and Viridis BEH 2-EP.

## 5. Accessing Data

SFC-MS data are remotely accessible from a shared Google Drive link. Contact Brandon Fowler for the link.

In the shared data folder, find your data files. To download, select the ".raw" file with your sample name, right-click on it, and choose Download from the menu. This will create a .zip file that will be downloaded to your computer (this may take a few minutes, pay attention to where the file gets saved on your computer). Once the download is complete, navigate to the folder that contains the .zip file and right-click on the file. Choose "Extract All…" from the menu and choose a folder where you would like to keep your data. When extracting, you may need to add ".raw" to the end of the folder name. At this point, the file can be viewed in MestreNova, as described in section VI.

## 6. Processing Data

SFC-MS data can be processed on the instrument computer using MassLynx (ask Brandon Fowler for help with this). Alternatively, data can be processed on a remote computer using MestreNova. We have a campus license (up to 100 users) for MestreNova MS processing software, which runs on Mac, Windows, and Linux.

1. If you don't already have the MestreNova software for NMR, you can download it here:
   Download MestreNova
   The software downloaded here has both NMR and MS processing capabilities, but requires a different license for each plug-in.

2. Download the three license files listed below (or only the third license file if you already use MestreNova for NMR), which are available on the NMR data server, nmr22.chem.columbia.edu, in the root (/) directory. This directory can be accessed using WinSCP or Cyberduck. The first two license files are for the NMR software and last license file is for the MS plugin.

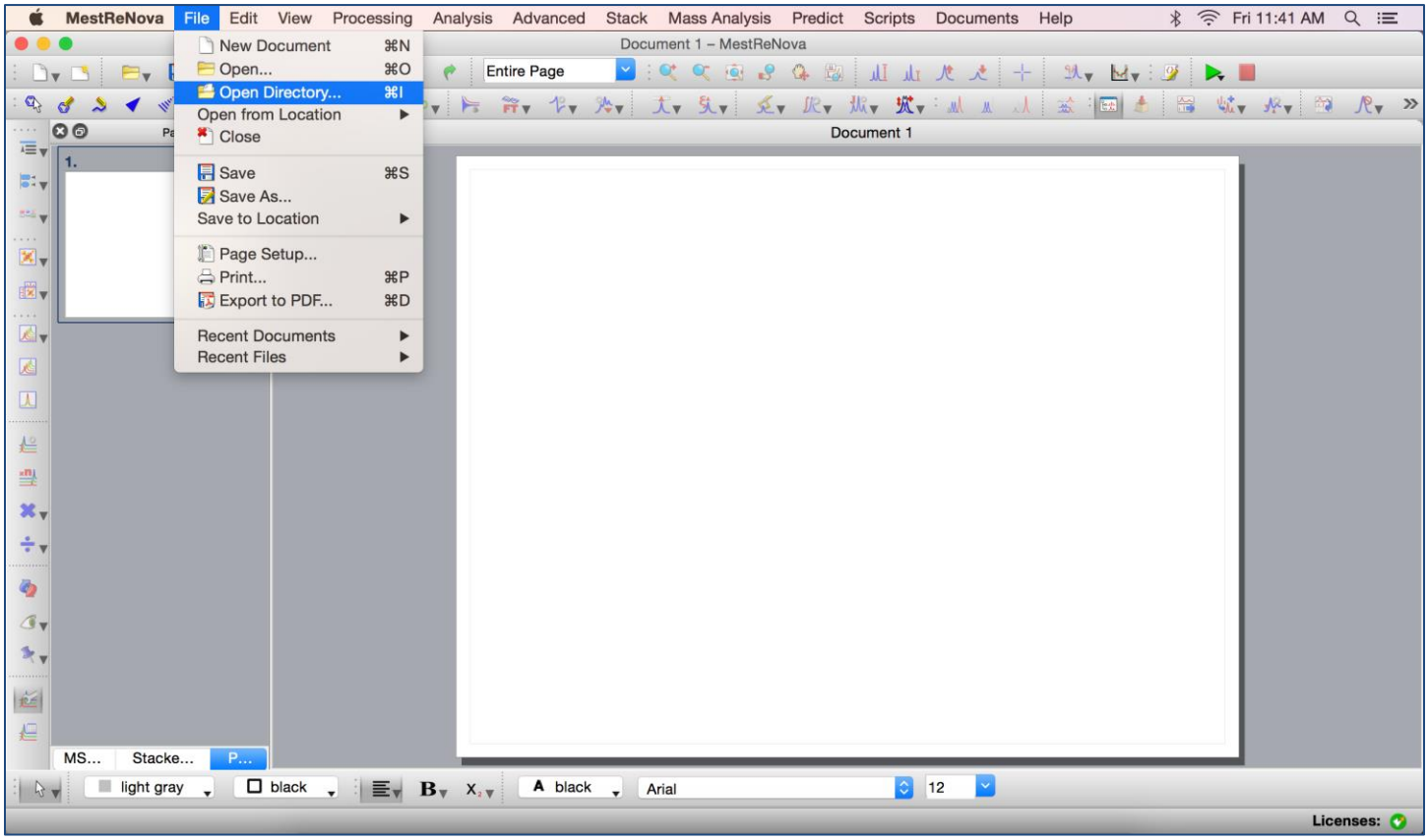
   **NMR License1:** ColumbiaUniversity125Extension.lic
   **NMR License2:** Columbia University_Conc1_USunltd_NPL.lic
   **MS License:** Columbia University Campus100 USUNLTD Mass.lic

3. To install the software and activate the license, follow the procedure in this guide:
   MestreNova License Activation Instructions
   The steps are summarized as follows. Within MestreNova, under Help, choose "Evaluate/Buy", click "Yes", click "Activate…", then find the license file(s) you downloaded above and choose "Open". Exit and restart MestreNova to start processing data. As part of this site license, up to 100 users for MS (200 for NMR), can obtain a license to MestreNova (based on each user's computer Host ID). If a user doesn't open the program and connect to the license server for three months, that user is dropped from the server list and that license becomes available again. If you experience an error connecting to the server, you may be behind a firewall.

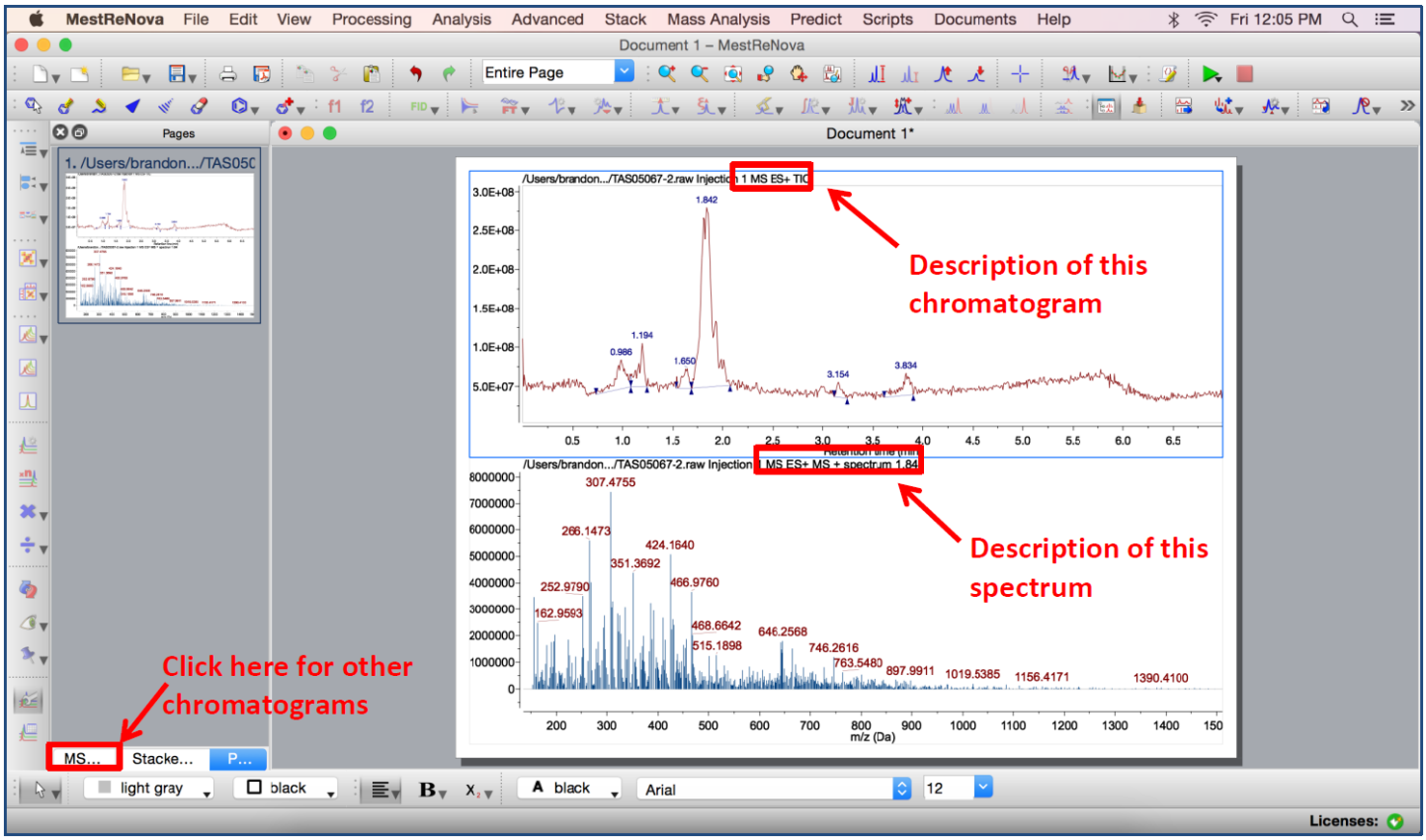
***Note bene*** By default you must be connected to the Columbia network to validate your license.

The following steps provide a basic tutorial for working up your SFC-MS data. For a more detailed tutorial, please follow this link: MestreNova MS Tutorials.

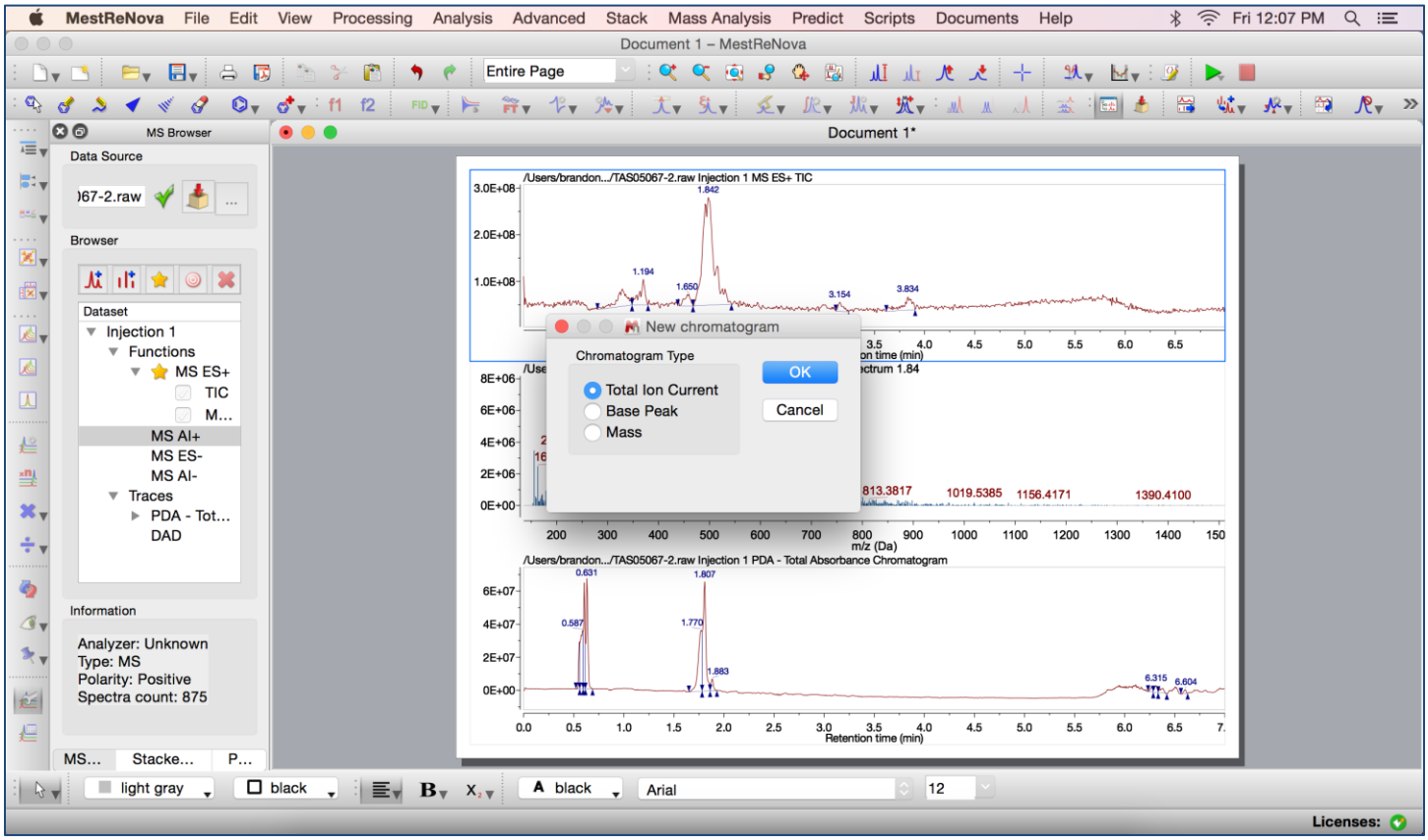
**Step 1.** Open MestreNova and click "File" at the top of the screen. Choose "Open Directory…". This will bring up a new window. Find the folder for the sample of interest (it should end with ".raw") and click "Open" in the bottom right of the window.



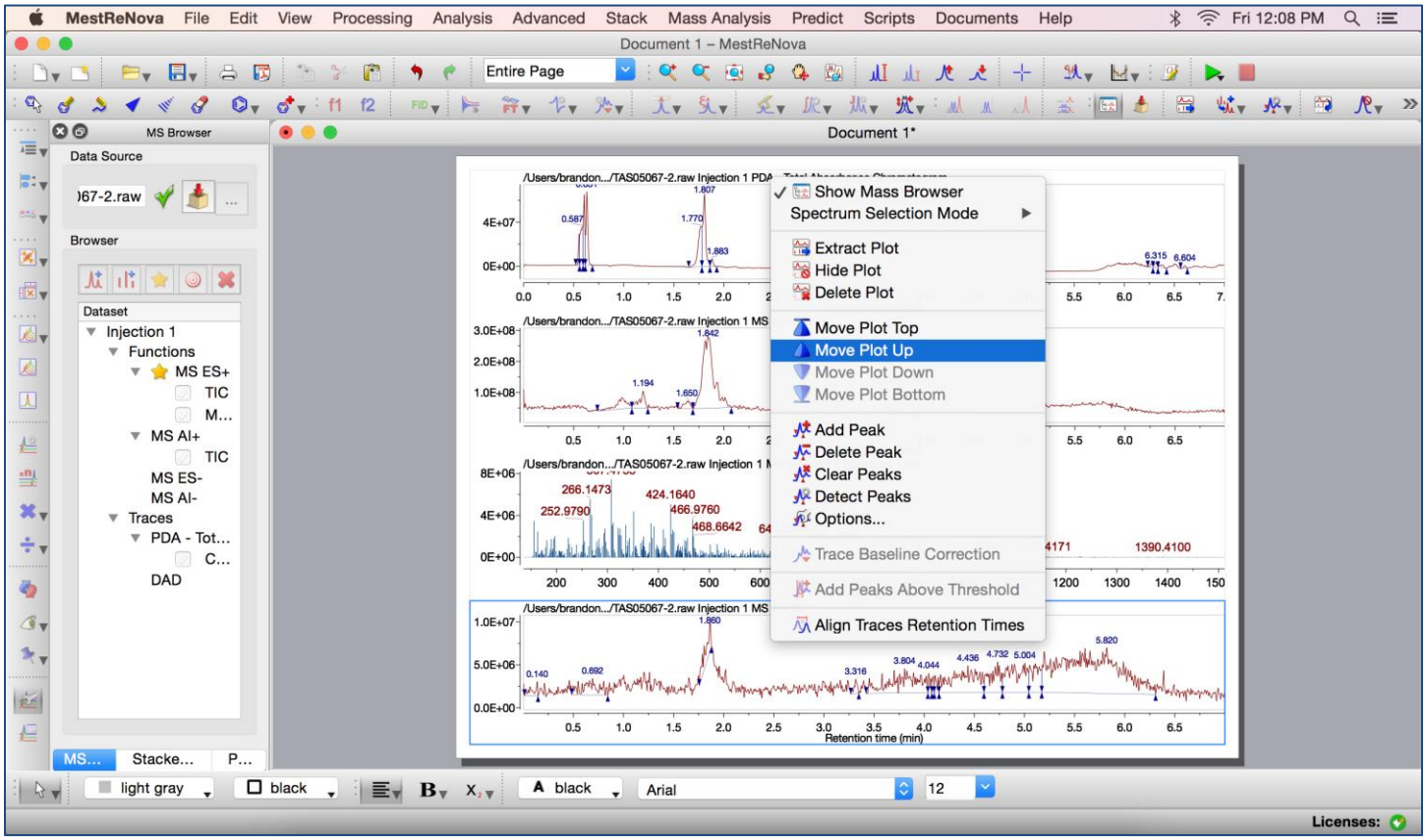
**Step 2.** The file will open with the mass chromatogram that has the most intense peak and will show the mass spectrum for that peak. In the example below, the ES+ total ion chromatogram (TIC) is shown on top and the ES+ mass spectrum for the time-point 1.84 min is shown on bottom. In the bottom left of the window, click on the "MS Browser" to access the other chromatograms from the run.



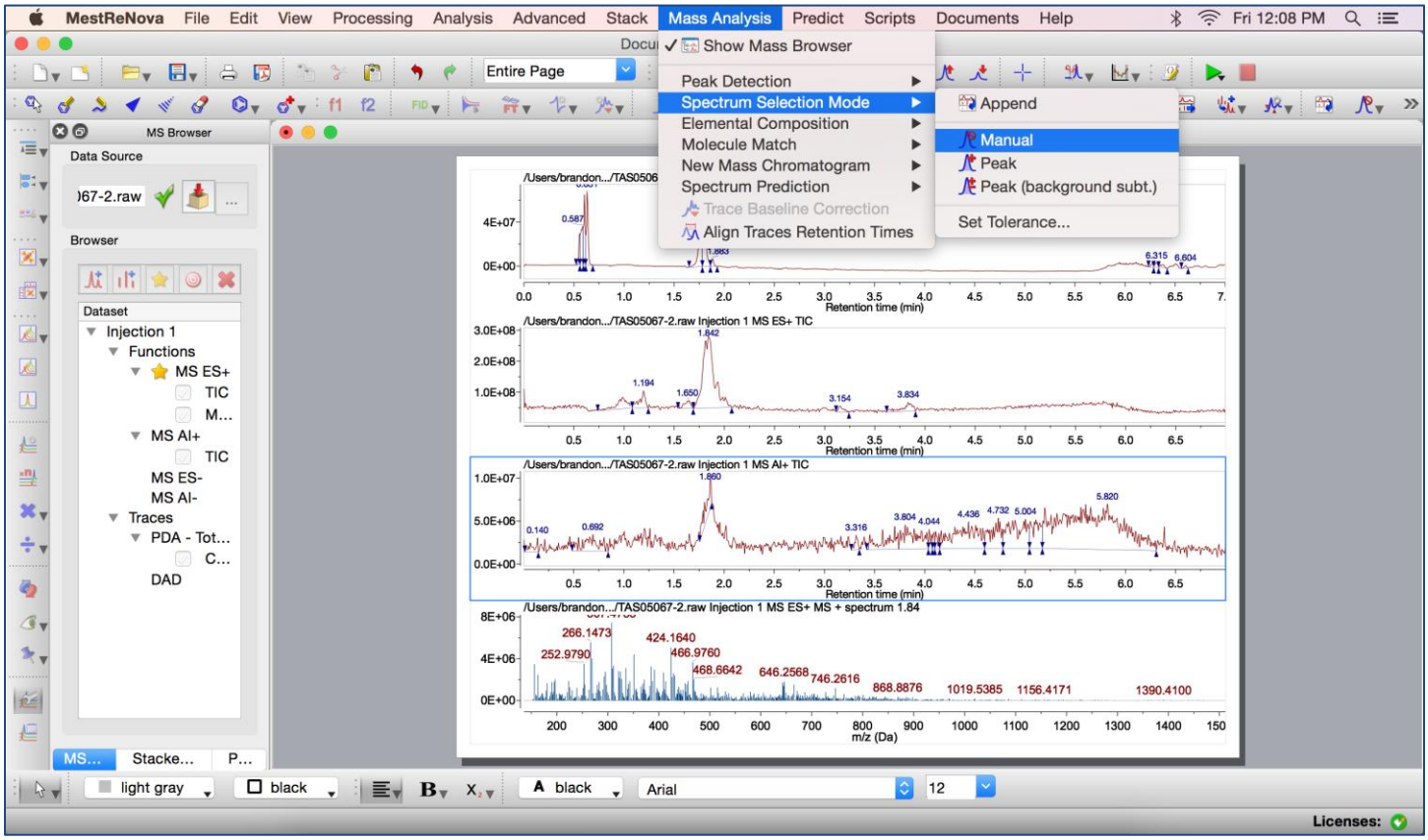
**Step 3a.** In the MS Browser, double click on "PDA" under "Traces" to see your UV-vis chromatogram. You can also choose any mass chromatogram you would like to see and double click on it. A small window will pop up, choose "Total Ion Current" and click "OK". (For an alternative way to find a mass of interest, see Steps 3b and 4b after Step 8).



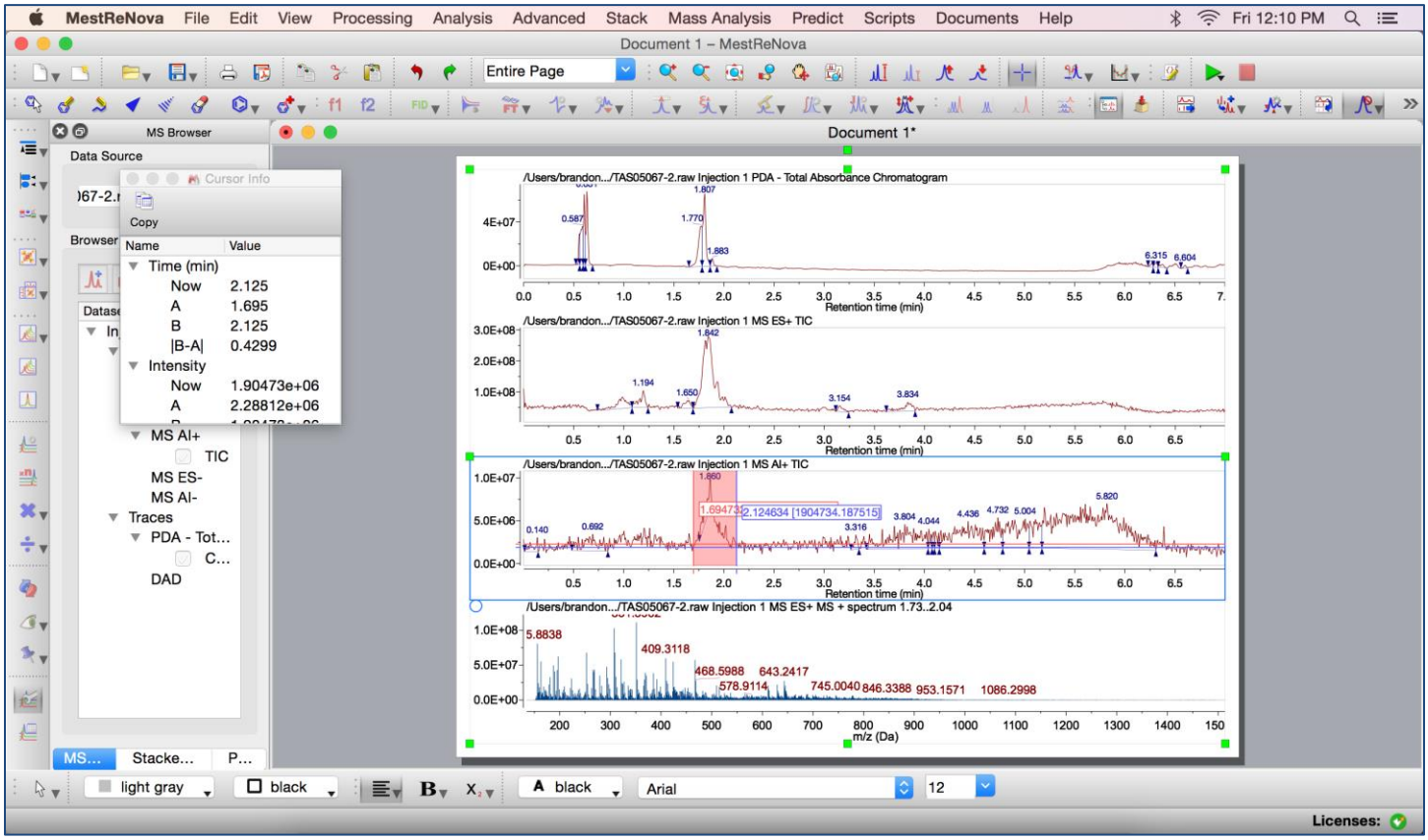
**Step 4a.** Rearrange your plots by right-clicking (or control+click) on a plot to choose where to move that plot.



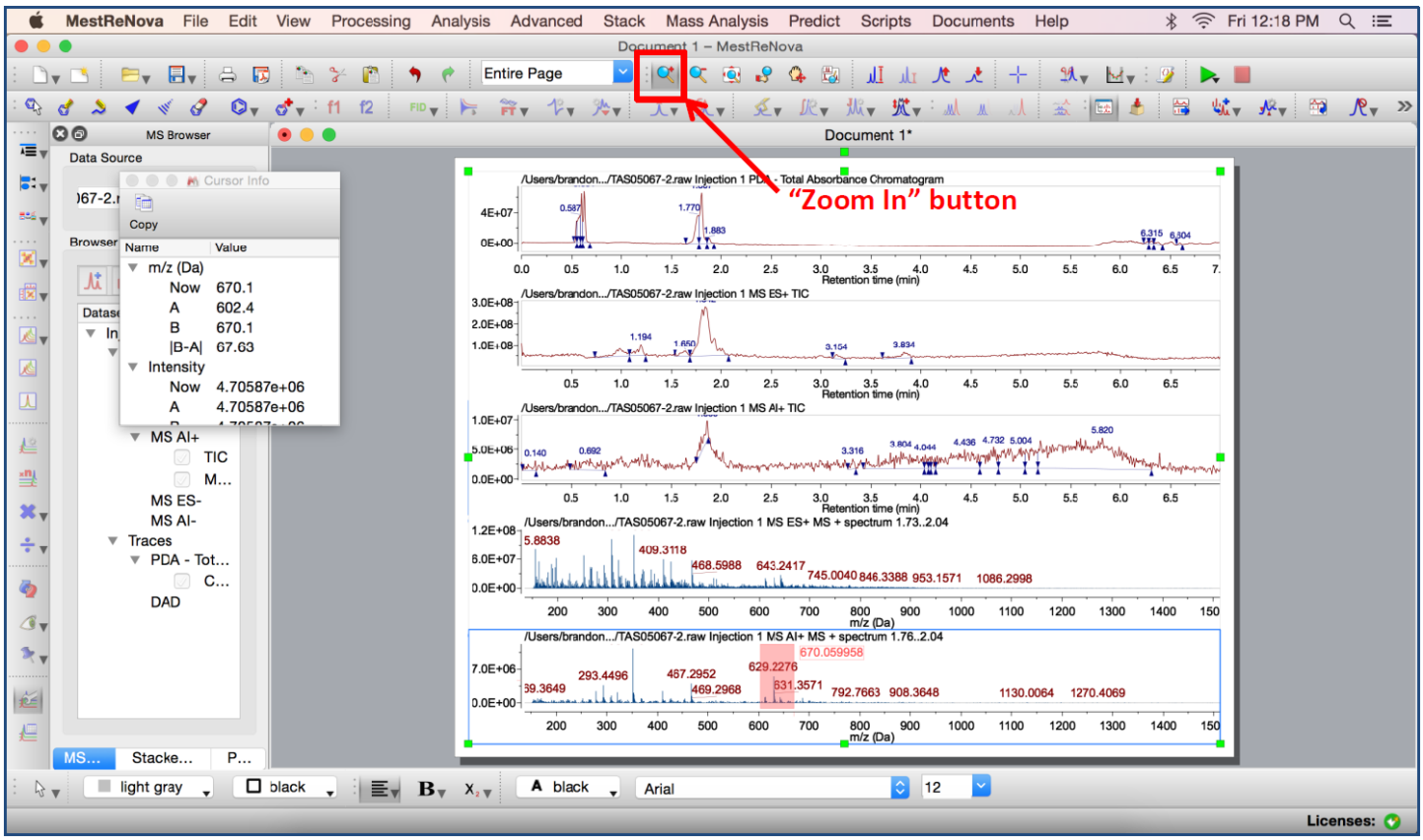
**Step 5.** To select a peak of interest from one of the mass chromatograms, go to the “Mass Analysis” menu at the top of the screen, choose “Spectrum Selection Mode”, and choose “Manual”.



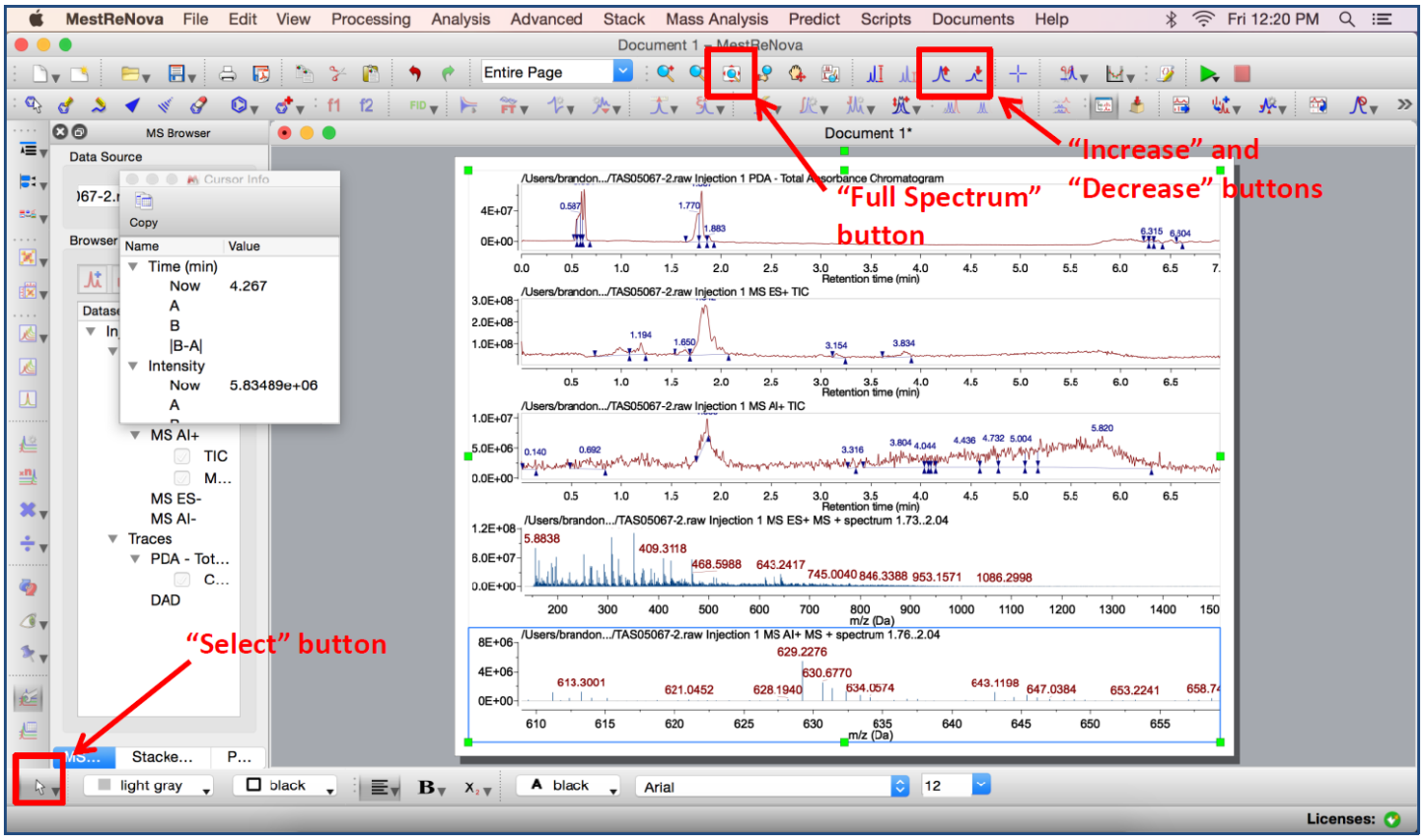
**Step 6.** Manual selection mode will change your cursor from an arrow to cross-hairs and a new window "Cursor Info" will pop up. Place the cross-hairs at the start of a peak of interest, click and drag horizontally to the end of the peak of interest. If you perform this peak selection on a chromatogram for which there is already a spectrum open (in this case, ES+), it will adjust the already-open mass spectrum to match your selection. If you perform this peak selection on a chromatogram for which there is no spectrum open (in this case, AI+), it will insert a new mass spectrum to match your selection (shown in the image for Step 7).



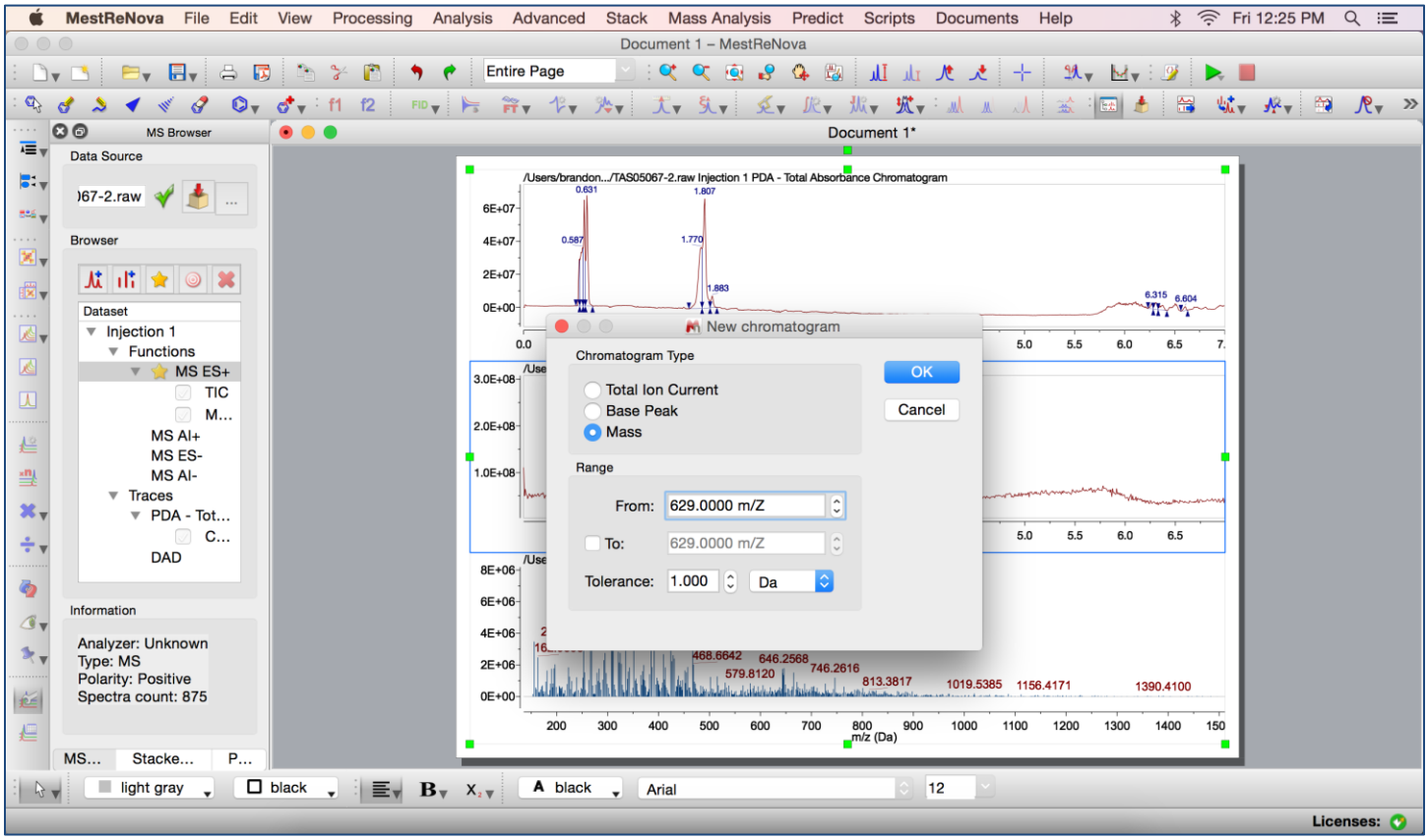
**Step 7.** Once you have the mass spectrum for your peak of interest, you can zoom in on the mass of interest by clicking on the "Zoom In" button at the top of the window. Your cursor will change to a magnifying glass, and you can click and drag across the mass range of interest.



**Step 8.** If a peak is too tall in a plot, you may not be able to see the peak label. Select the plot you want to adjust by clicking on the "Select" (white arrow) button at the bottom left of the window, then click on the plot to select it. Adjust the peak height by clicking on the "Increase" or "Decrease" buttons at the top of the window. If you want to return to the full spectrum view, click on the "Full Spectrum" button at the top of the window.



**Step 3b.** As an alternative way to find a mass of interest (especially useful if you have many peaks in the chromatogram), double-click on the chromatogram you want to view and choose "Mass" in the "New Chromatogram" window that pops up. You may then enter your mass of interest (or a range if you click the checkbox). A tolerance value of 1 Da is good for low-resolution mass data. Click "OK".



**Step 4b.** The new chromatogram that was inserted will show peaks where your mass of interest (± the tolerance value) is significantly more intense than noise. You can use the manual spectrum selection mode to get the mass spectrum of any peaks in your new mass chromatogram (see Steps 5 and 6, above).