

SFC/MS User Guide

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Department of Chemistry

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I. Introduction

Welcome to the SFC/MS instrument in the Shared Materials Characterization Lab in 544 Havemeyer. In order to obtain swipe access to 544 Havemeyer and to get an account on the instrument, you must be trained by a super-user. To sign up for training, place your name and contact information on the sign-up sheet outside of 544 Havemeyer. If you encounter any problems with the instrument that are not addressed in these instructions, please contact a super-user:

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What is SFC/MS? SFC/MS analysis combines analytical chromatography with mass spectrometry to determine 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. Neat 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 posted next to the monitor. 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 quadrupole mass detector (150–2500 Da). The mass spectrometer can ionize samples by two methods in both positive and negative polarities. 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 between 210 and 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 flowing into the mass detector. 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.

What is the SFC/MS instrument good for? 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 (150–2500 Da). If you would like to inject anything other than small organic molecules, please talk to a super-user first. The instrument is also equipped with chiral columns and can be used for rapid analysis of enantiomeric ratios (talk to a super-user if this is your planned use). 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.

II. 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 downtime and expensive cleanings or replacements. If you repeatedly submit samples that do not fit the criteria below, **YOU WILL BE BANNED FROM THE INSTRUMENT**. Materials for sample preparation will not be 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:

1. HPLC vials (12 mm outer diameter x 32 mm height, 9 mm opening, 2 mL)
2. Vial caps **WITH PRE-SLIT SEPTA** (9 mm open top, 6 mm hole, pre-slit septum)
3. Syringe filter (unless your sample has already been purified by chromatography or distillation)
4. 0.2–1.0 mg of sample
5. A recommended or permitted 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

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 are prohibited. Very low volatility solvents such as DMF and DMSO are prohibited. If you have any questions about a solvent, contact a super-user.

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.

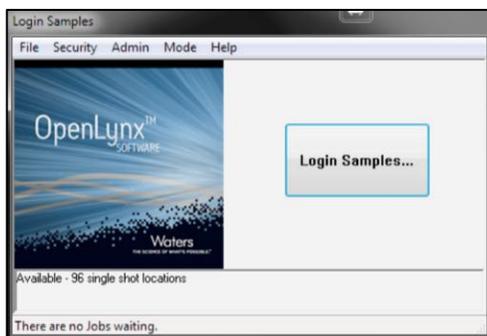
Samples with **RADIOACTIVE ISOTOPES ARE STRICTLY FORBIDDEN** on the SFC/MS instrument.

****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).

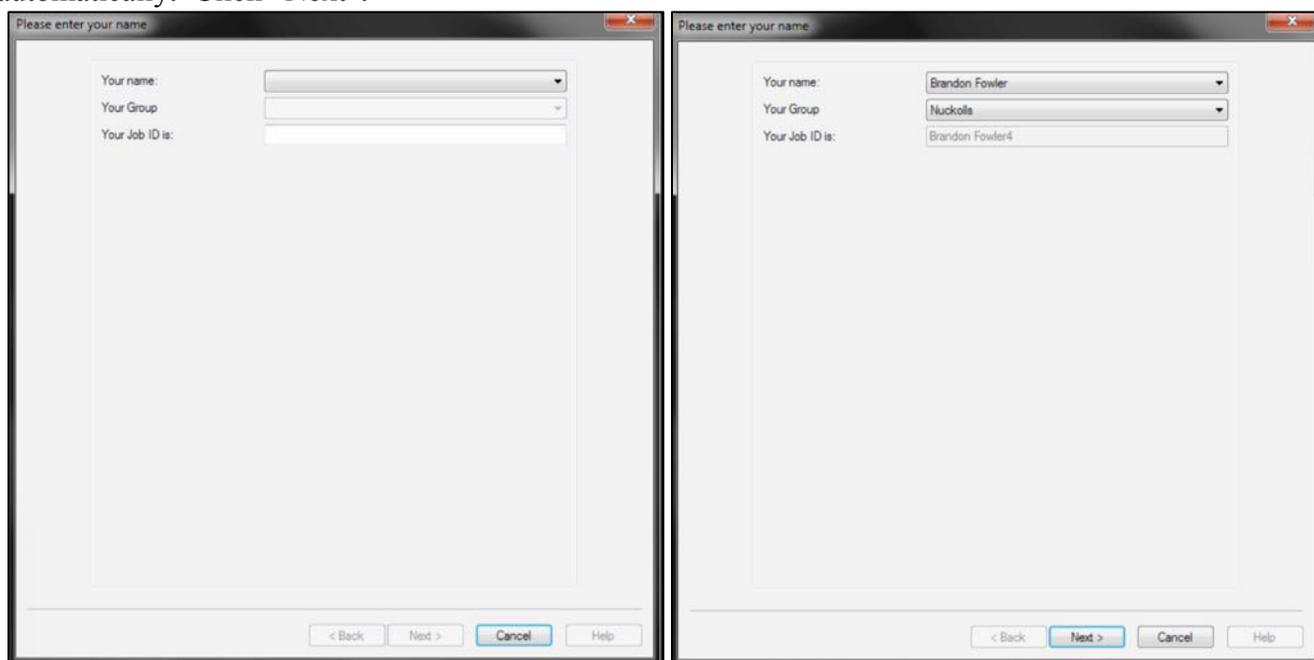
III. Logging in Samples

After you prepare your sample, you can log it into the queue for the SFC/MS instrument. When you approach the computer, it may be locked. If so, press Ctrl+Alt+Delete and enter the password “waters”.

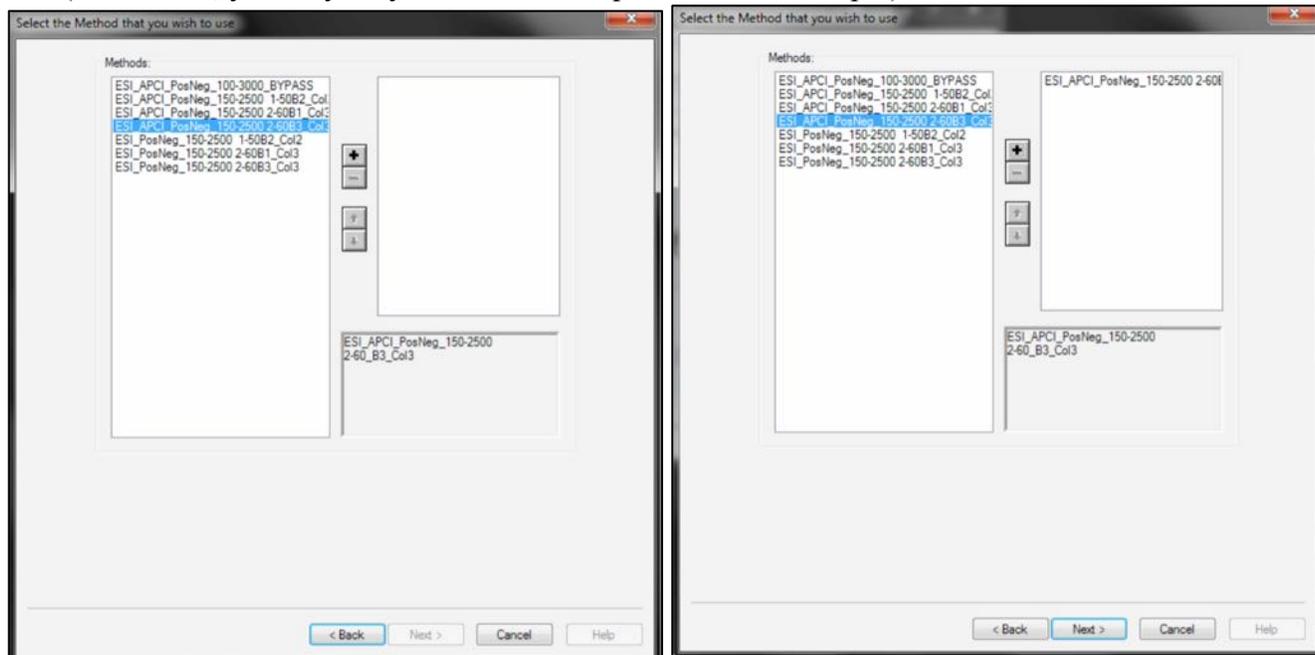
Step 1. At this point, you should see the small OpenLynx window shown below. Click on the button that reads “Login Samples...”



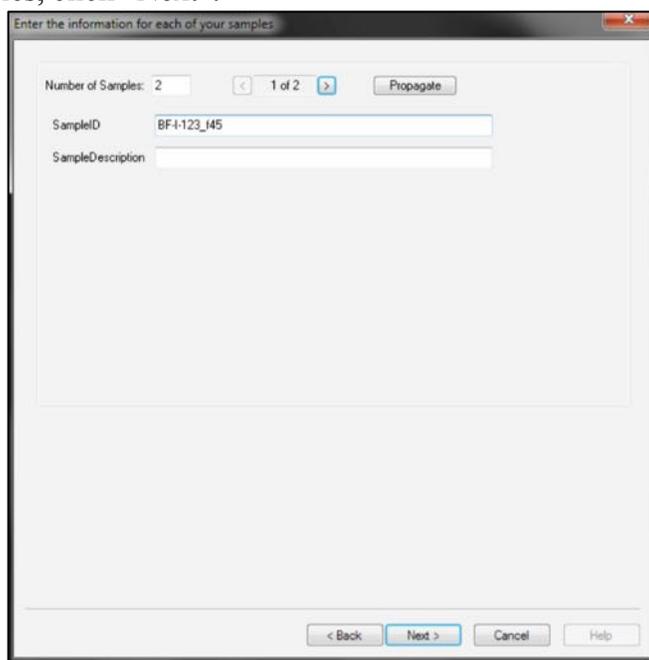
Step 2. Choose your name from the menu, at which point your Group and Job ID will be populated automatically. Click “Next”.



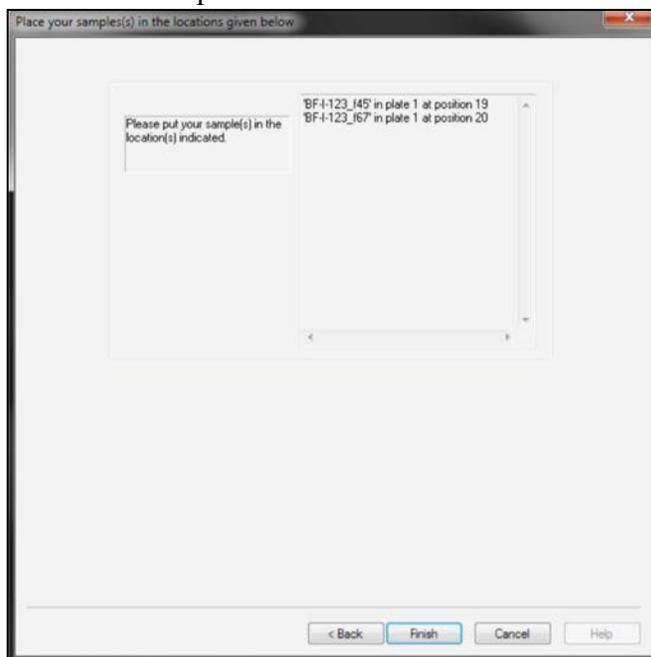
Step 3. Select a method from the list on the left. A description of each method including column, solvent, ionization methods, and mass ranges can be found in the bottom right panel. Once you've found the method you want, click the "+" button to add it to the list of methods you'll be using for this 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 (up to 2 at a time) you would like to submit. Enter the Sample ID for the first sample (and a description if you want). **SAMPLE IDs MAY CONTAIN ONLY LETTERS, NUMBERS, DASHES (-), and UNDERSCORES(_)**. Any other characters might crash the software. You may enter any characters in the Sample Description. 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”. Most runs will take approximately 8-9 minutes to finish. The data from your run can be accessed remotely as described in the next section. There will be no data processing on the instrument computer.



IV. Accessing Data

Thanks to the work of John Decatur, you can access your SFC/MS data remotely, much like your NMR data. The SFC/MS data server updates every 5 minutes for quick access to your results. Your SFC/MS data can be processed on the same MestreNova software as NMR data; however, you will need to download the license to access the SFC/MS processing capabilities. For more information about processing your data, see Section V.

To download your data (and MestreNova license) from the MS server onto your Mac or PC, you need a client ftp program. On Windows, the free ftp client program is WinSCP. On the Mac, it is Cyberduck or Fetch. I recommend Cyberduck.

Windows. Download WinSCP from <http://winscp.net/eng/index/php>. Choose the latest version and the “installation package” type of download. During the install, select all components and the Commander type interface. Launch WinSCP and enter the following information:

Hostname: msdata1.chem.columbia.edu (or 128.59.112.131), port is 21

Username: msuser

Password: _____ (get from a super-user at the end of training)

File protocol: ftp

Encryption: TLS/SSL explicit

Click login. Once the program logs in, your windows directories will be on the left. On the right is the data server. On the data server, navigate to the following directory:

Path: /Public/NetBakData/Administrator@WATERS-S4AKWLB/Disk C/MassLynx/current.pro/data

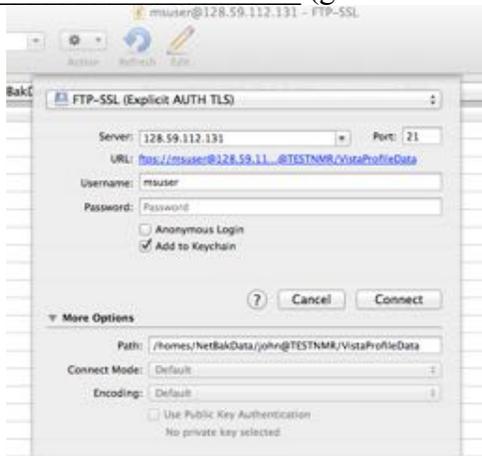
To download, select the folder with your sample name and press F5 (or right-click the mouse and select copy). You will need the entire folder to open your data in MestreNova.

Mac. Download and install Cyberduck from <http://cyberduck.ch/>. After it downloads, click on cyberduck.zip to decompress it and then move it to your application folder. After Cyberduck is launched, choose connect using FTP-SSL and enter the following information (see figure below):

Hostname: msdata1.chem.columbia.edu (or 128.59.112.131), port is 21

Username: msuser

Password: _____ (get from a super-user at the end of training)



You will get a Verify Certificate message. Please ignore and click “Continue”. On the data server, navigate to the following directory:

Path: /Public/NetBakData/Administrator@WATERS-S4AKWLB/Disk C/MassLynx/current.pro/data

To download, select the folder with your sample name and right-click (or control+click) and select “Download”. You will need the entire folder to open your data in MestreNova.

V. Processing Data

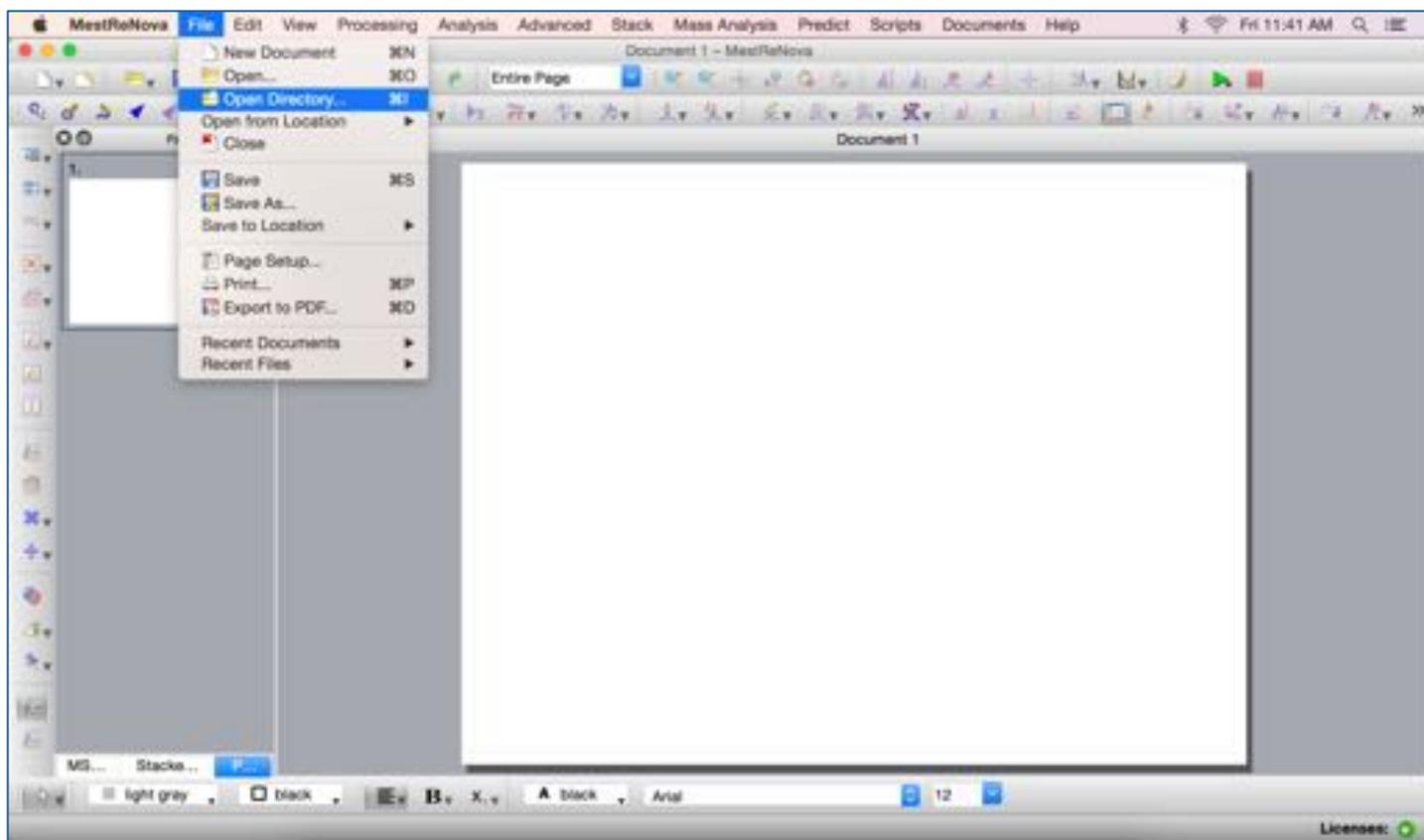
Unlike the NMR labs, there are no workstations to process your data. The only way for users to process data from the SFC/MS is on a remote computer using MestreNova. We now have a campus license (up to 100 users) for MestreNova MS processing software, which runs on Mac, Windows, and Linux.

1. If you don't already have the MestreNova software for NMR, you can download it here:
<http://www.mestrelab.com/Products/Mnova/Product-download.html>
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 the WinSCP or Cyberduck programs mentioned in Section IV. 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:
<http://mestrelab.com/resources/mnova-suite/installing/how-to-install-mnova>
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.

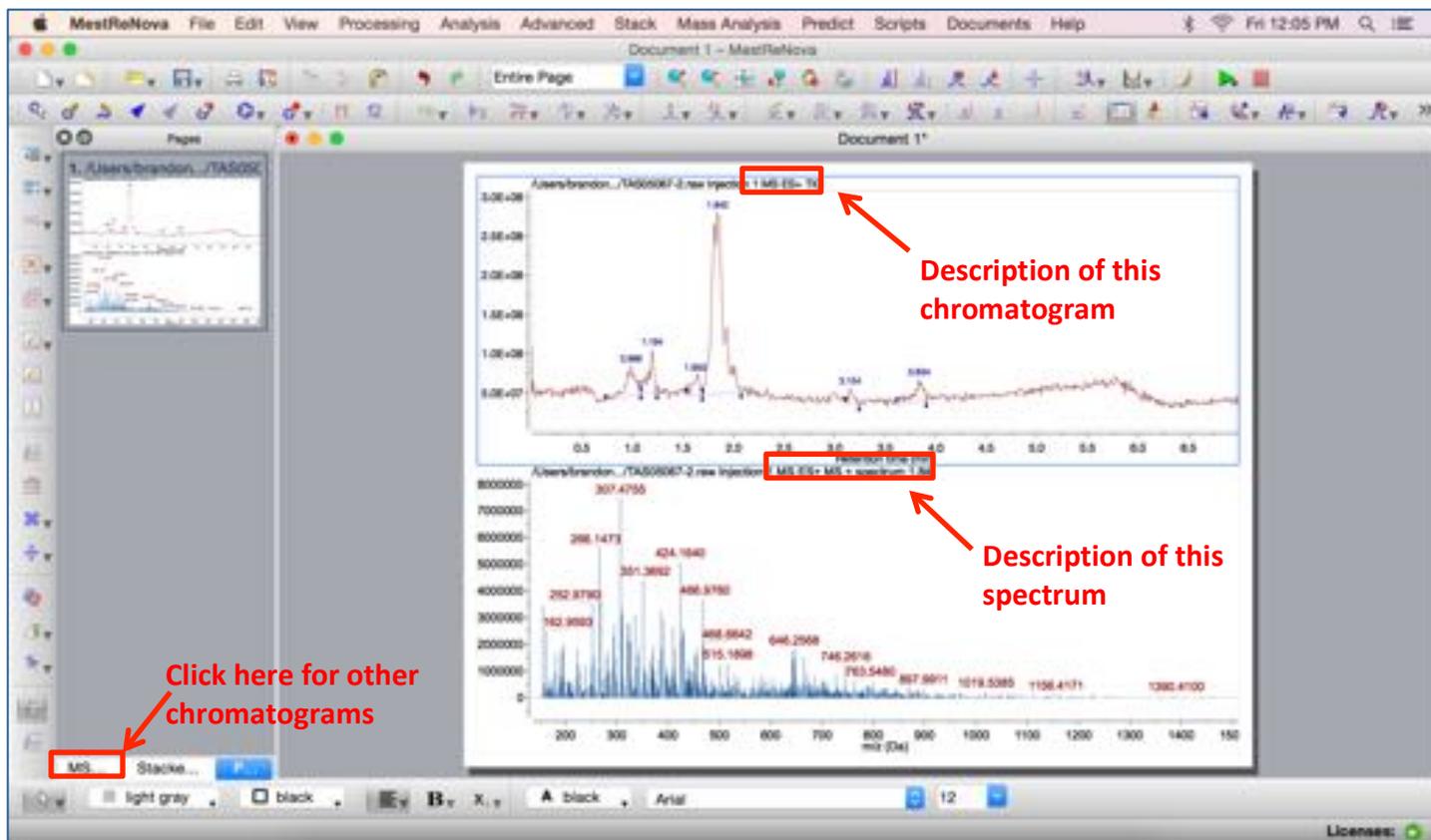
****Note bene**** By default you must be connected to the Columbia network to validate your license.

The following steps provide a basic tutorial for working up your SFC/MS data. For a more detailed tutorial, please follow this link: <http://mestrelab.com/pdf/mnova-ms-tutorial.pdf>.

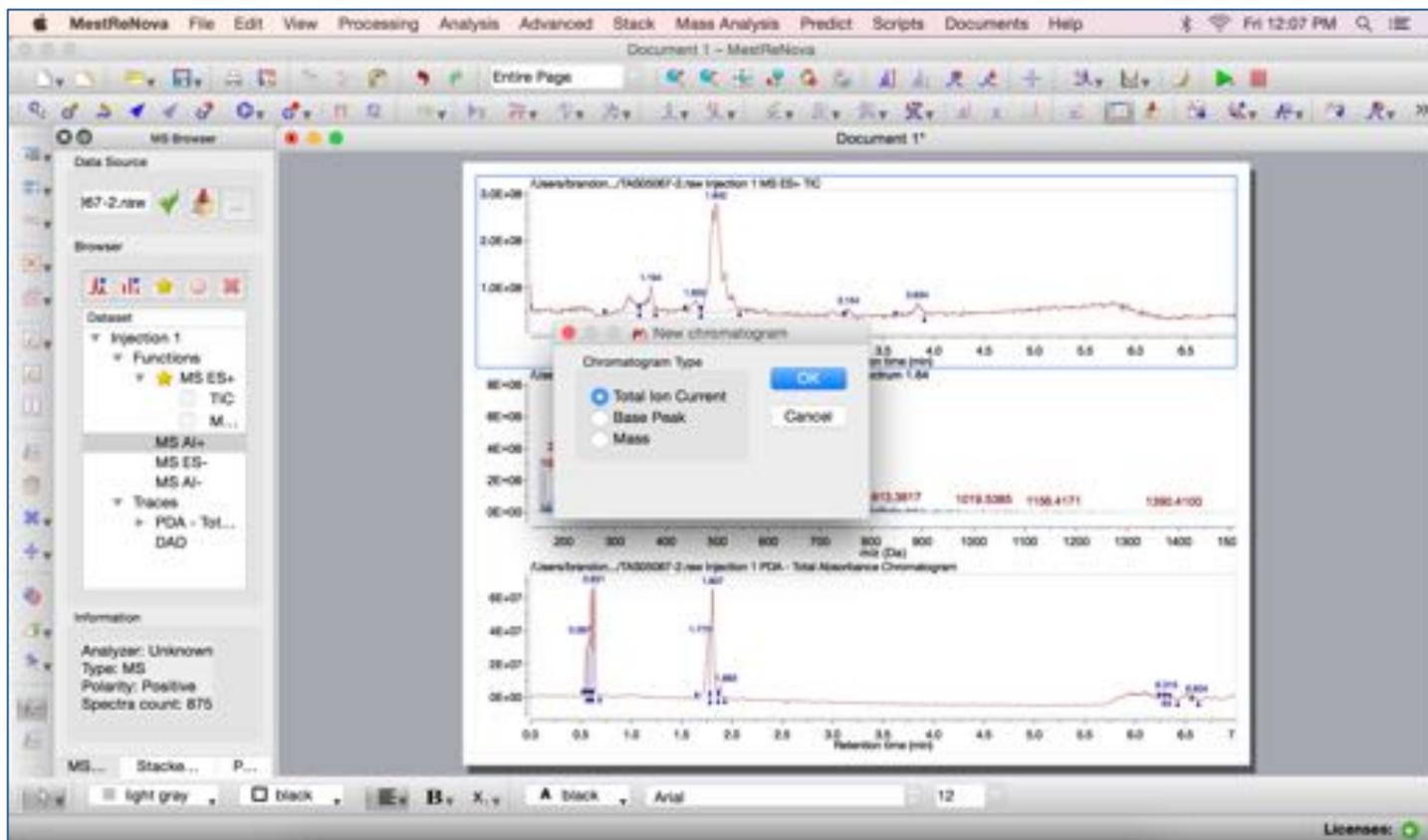
Step 1. Open MestreNova and click “File” at the top of the screen. Choose “Open Directory...”. This will bring up a new window. Find the folder for the sample of interest (it should end with “.raw”) and click “Open” in the bottom right of the window.



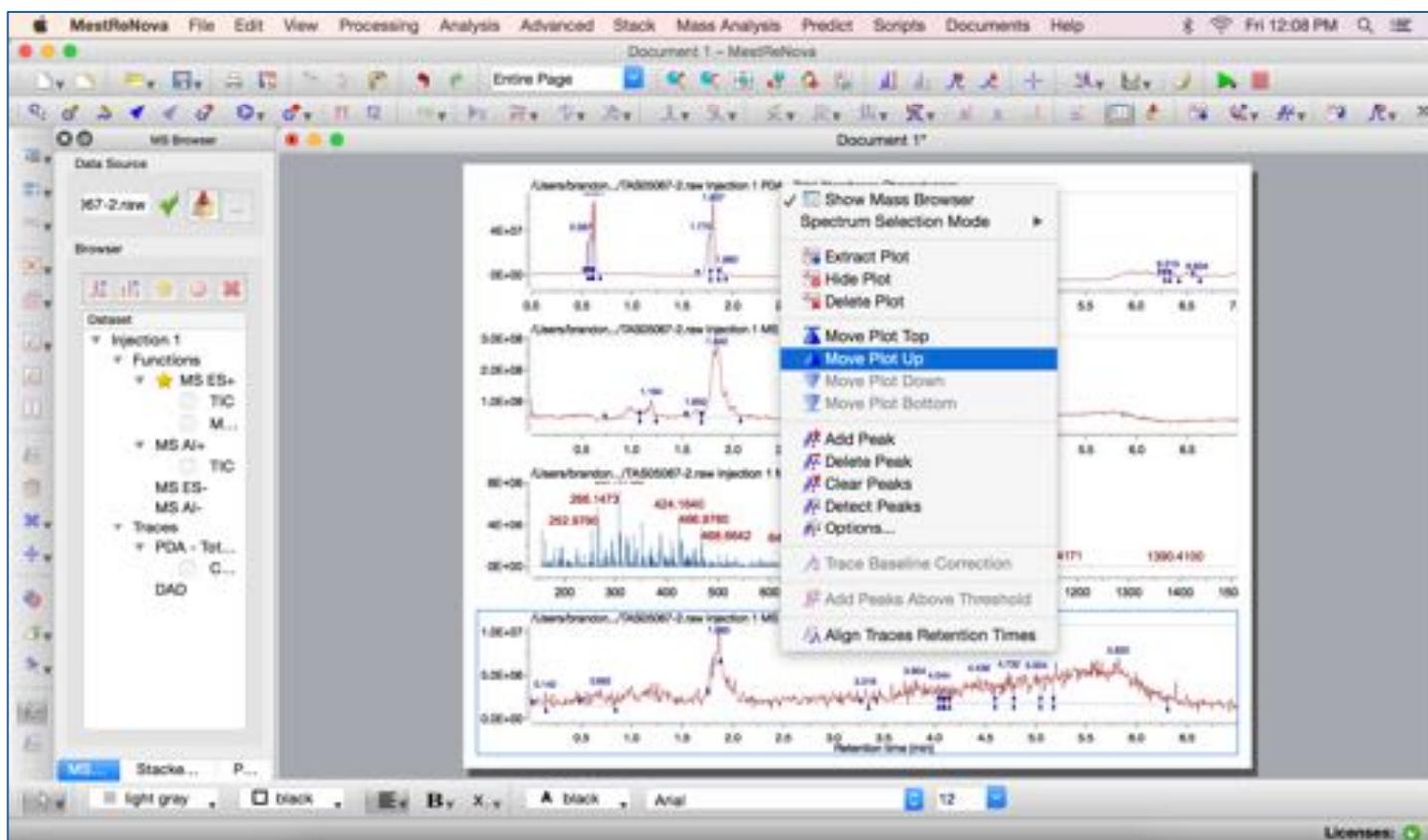
Step 2. The file will open with the mass chromatogram that has the most intense peak and will show the mass spectrum for that peak. In the example below, the ES+ total ion chromatogram (TIC) is shown on top and the ES+ mass spectrum for the time-point 1.84 min is shown on bottom. In the bottom left of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



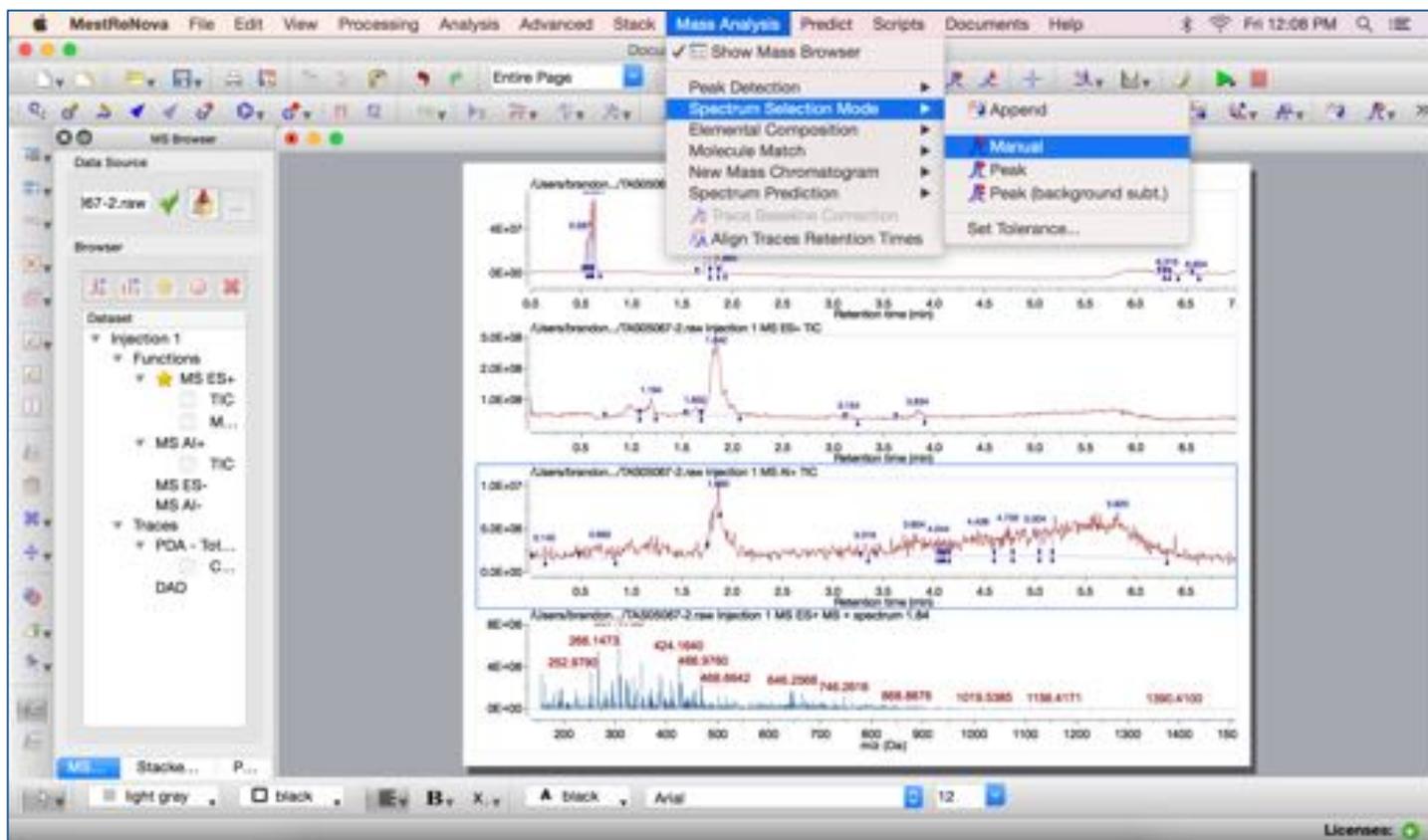
Step 3a. In the MS Browser, double click on “PDA” under “Traces” to see your UV-vis chromatogram. You can also choose any mass chromatogram you would like to see and double click on it. A small window will pop up, choose “Total Ion Current” and click “OK”. (For an alternative way to find a mass of interest,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



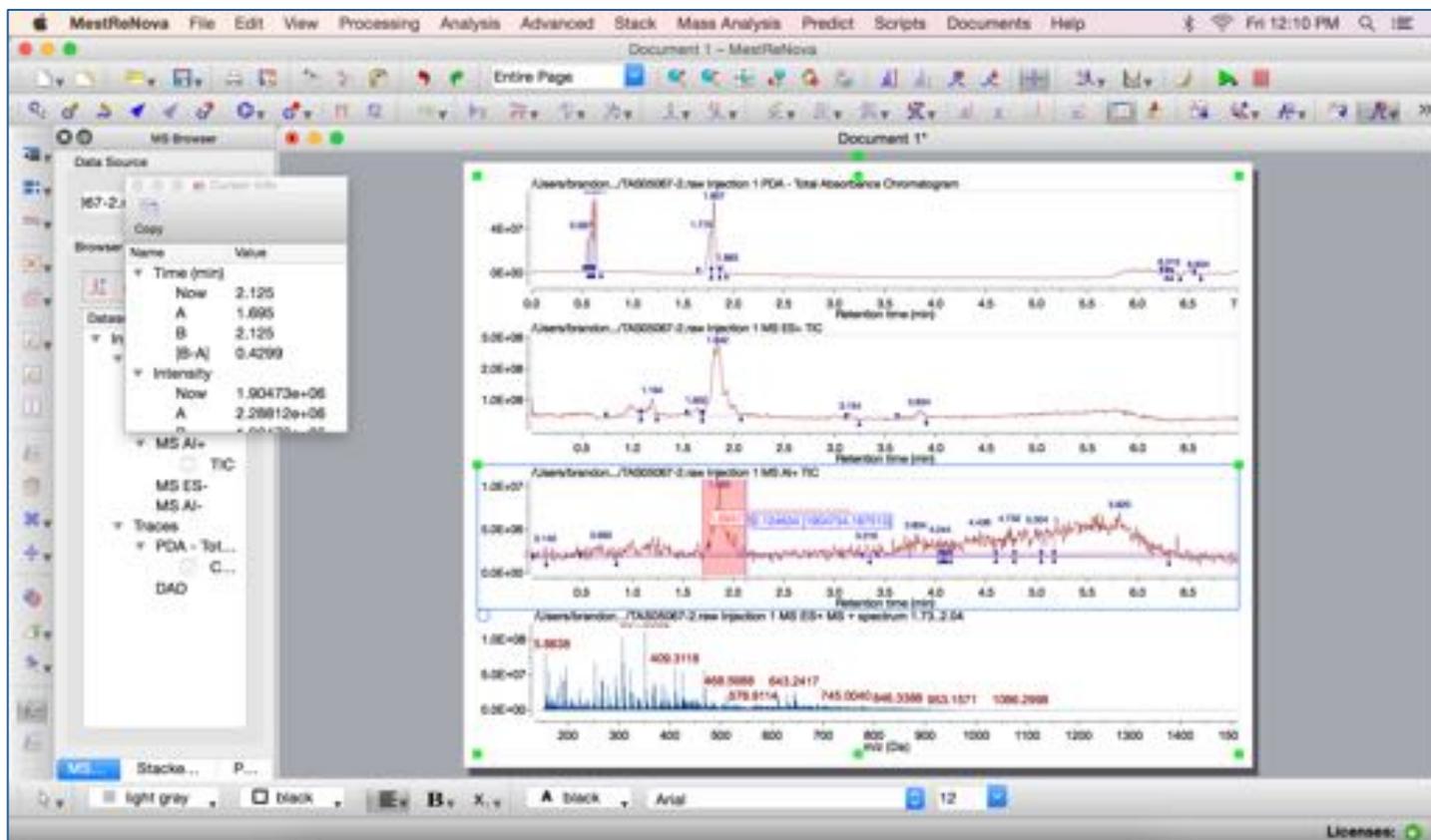
Step 4a. Rearrange your plots by right-clicking (or control+click) on a plot to choose where to move that plot.



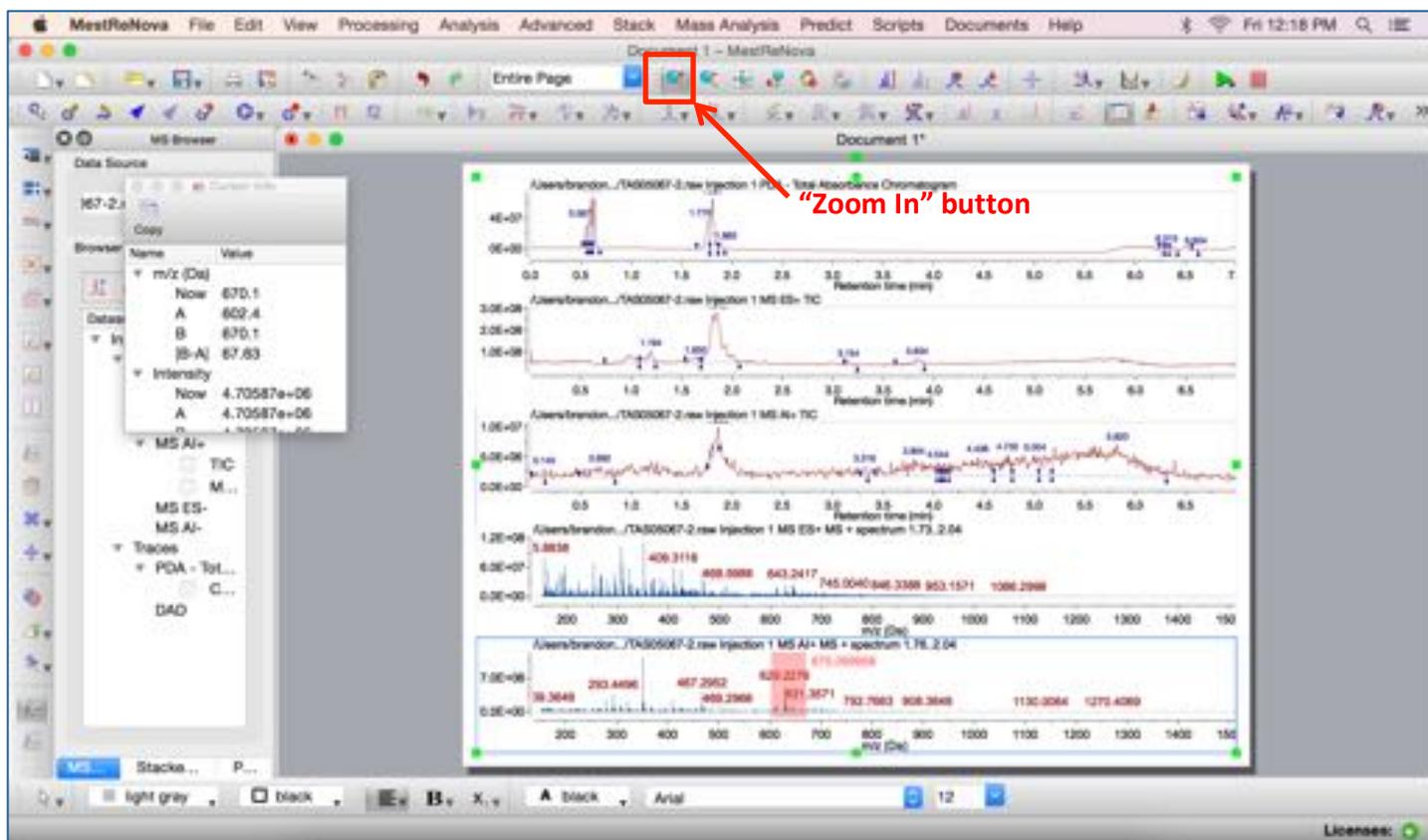
Step 5. To select a peak of interest from one of the mass chromatograms, go to the “Mass Analysis” menu at the top of the screen, choose “Spectrum Selection Mode”, and choose “Manual”.



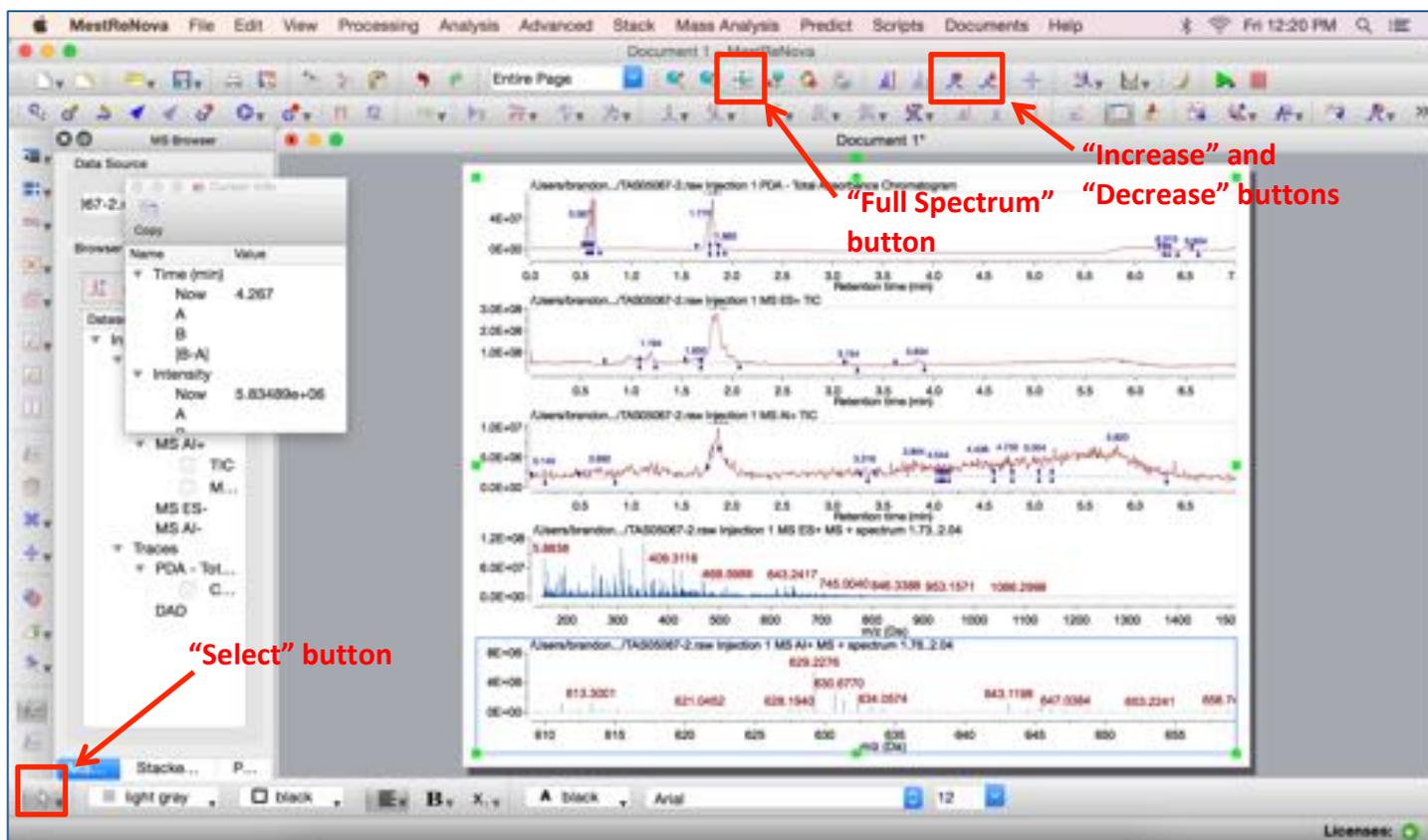
Step 6. Manual selection mode will change your cursor from an arrow to cross-hairs and a new window “Cursor Info” will pop up. Place the cross-hairs at the start of a peak of interest, click and drag horizontally to the end of the peak of interest. If you perform this peak selection on a chromatogram 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).



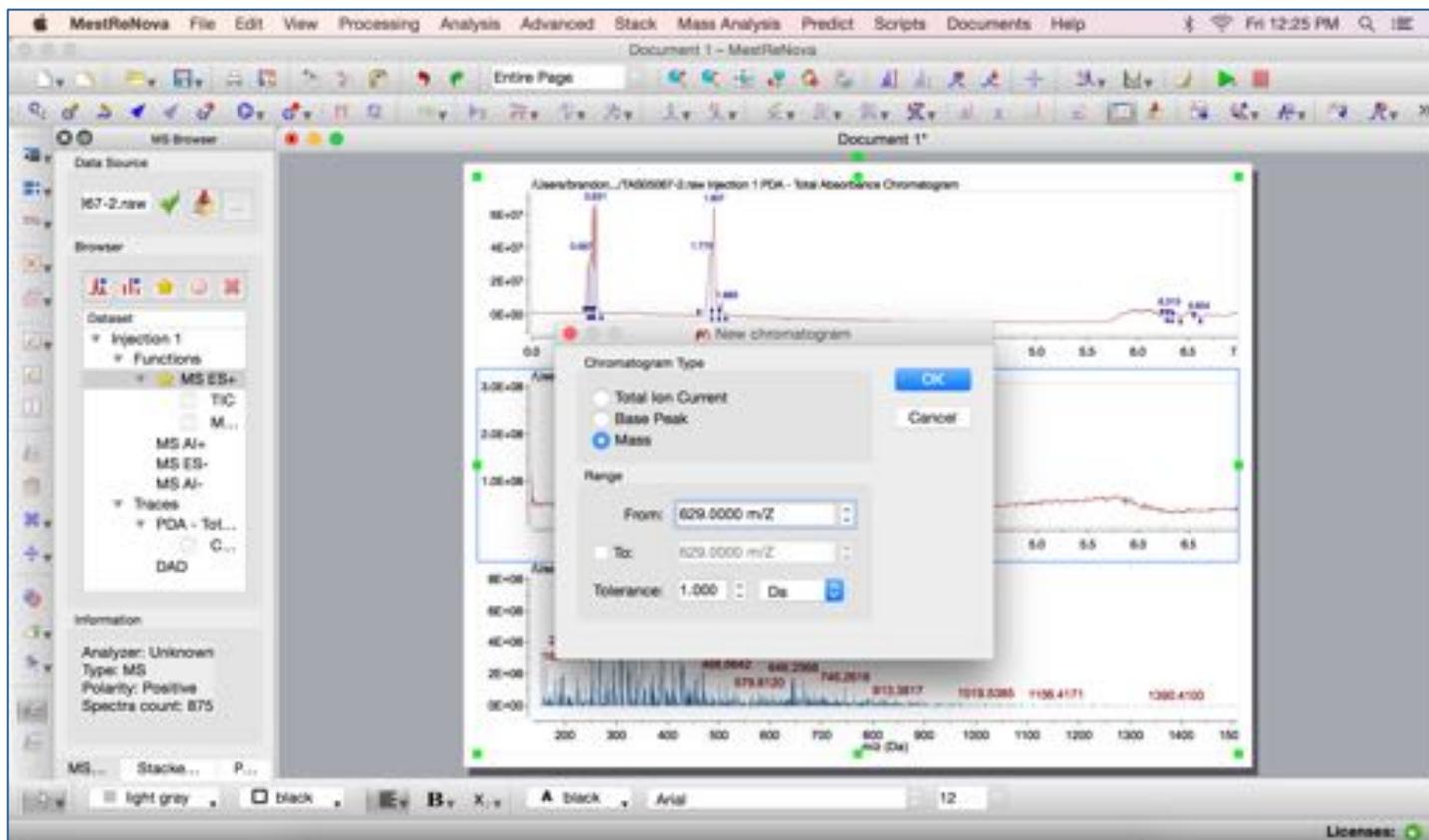
Step 7. Once you have the mass spectrum for your peak of interest, you can zoom in on the mass of interest by clicking on the “Zoom In” button at the top of the window. Your cursor will change to a magnifying glass, and you can click and drag across the mass range of interest.



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 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 (\pm 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).

