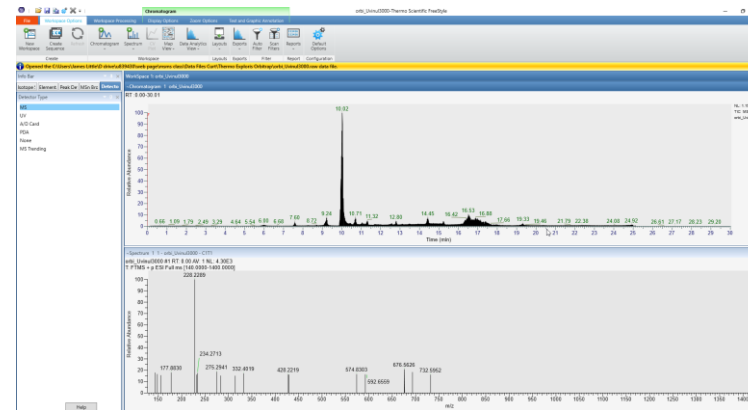
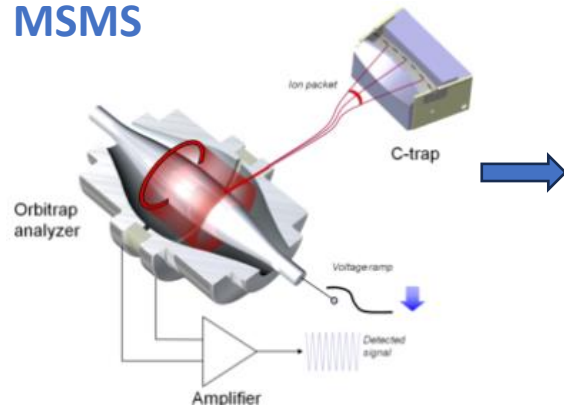


Part 1: Data Processing for NIST MSMS Search Using Thermo Freestyle Software (*My Favorite Approach*) (*shared with me by colleague*)

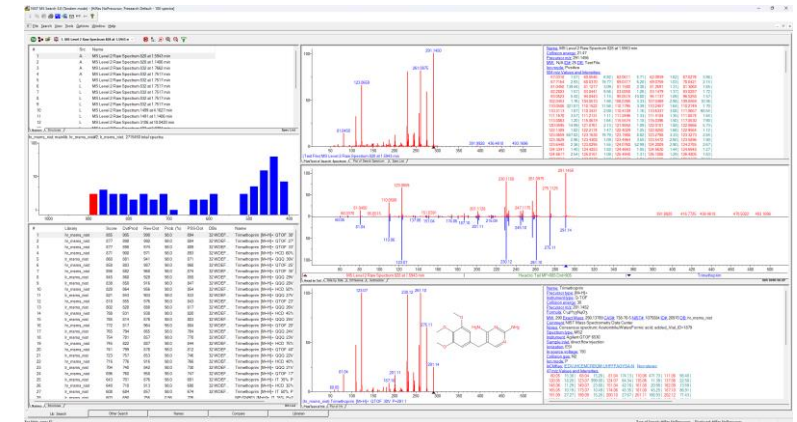
James Little
Mass Spec Interpretation Services
<https://littlesandsailing.com/>
11/06/2025

Thermo Freestyle

Thermo Orbitrap MSMS

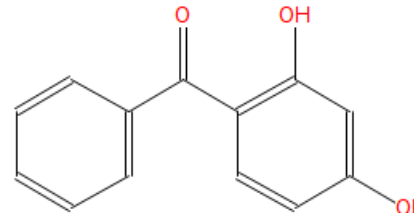


NIST MSMS Search

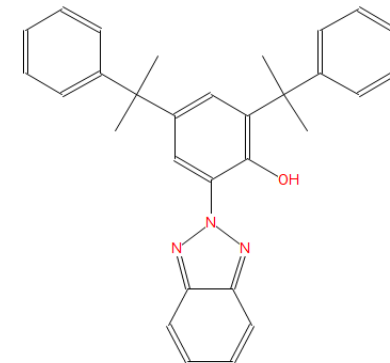


Orbitrap Data One Acquisition:

- LC-MS and MSMS Analyses
- Non-targeted acquisition of MSMS
- Positive and Negative Mode
- Photo Detector Array (PDA) and UV-VIS Analyses



Uvinul 3000
MW 214



Tinuvin 234
MW 447

NIST 2023 Tandem Library *Great* Resource for Unknown Identifications

2023 Release

51,501 Compounds, **60%** More than 2020
400 K Precursor Ions – 2.4 M Spectra

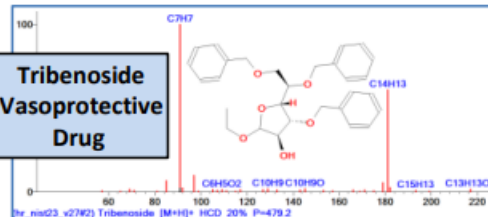
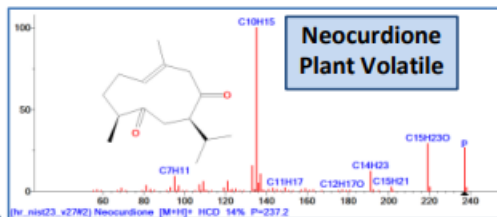
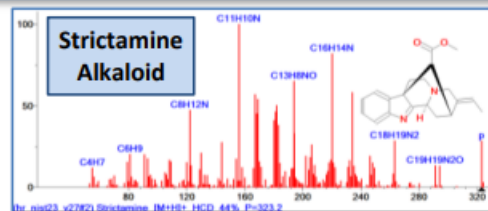
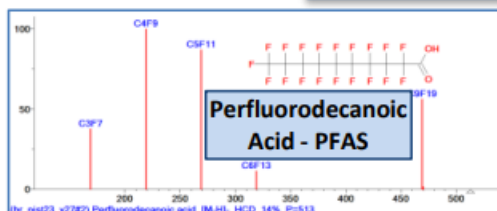
Fragmentation Methods

49,590 HRAM (High Res Accurate Mass) Compounds
50,071 QTOF, HCD, IT-HRAM, QqQ Compounds
49,561 Ion Trap Compounds (Low Res., up to MS⁴)
561 APCI HRAM Compounds

Precursor Ion Types

44,191 Protonated
19,620 Deprotonated
14,318 Water/Ammonia Loss
44,547 Other In-Source Generated

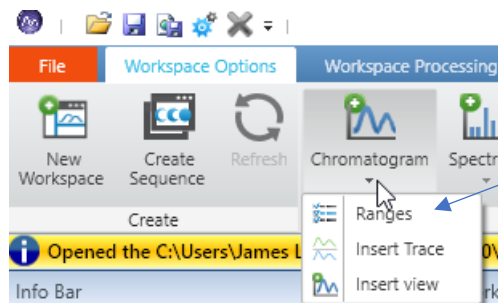
**ALL COMPOUNDS SELECTED FOR RELEVANCE
MEASURED AT NIST, THOROUGHLY EVALUATED**



Citation	New	Total
Wikipedia	1618	6424
EPA Tox	3181	8146
Food DB	602	4491
EU Contaminants	6553	15818
Protein Data Bank	1246	4945
Human Metabolite DB	2071	9686
PFAS	90	116

My Favorite Way of Processing Data (shared with me by colleague)

- Open three windows in the bottom of Freestyle
- Top one is positive ion MS1
- Second one in negative MS2
- Bottom is either UV or PDA trace
- After setting up, be sure to save in Workspace Options/Layouts
- Then when new file opened, and layout is loaded, data is parsed into these three window automatically!
- **NOTE**, will add **Nearby Precursors** to **simplify** selection of MSMS in an **accompanying video(1A)**
- **Also NOTE**, “Nearby Precursors” option **DOES NOT work with averaged MS spectra!**



Select ranges

Chromatogram Ranges - Chromatogram 1											
Display	File Name	Detector Type	Filter	Trace Type	Mass Defect Range	Ranges	Smoothing	Chemical Formula	Mass Tolerance	Delay Time	
☒	C:\Users\James Li...	MS		TIC	MDF Ranges		None			0.00	
☐					MDF Ranges					0.00	

Bottom window will appear, keep adding windows to get three total

Three working
boxes for
processing

Spectrum window

Boxes for selecting
functions

Select filter to change top one to
positive ion and second one to negative
ion, Select Detector type to get PDA or
UV



Note, positive and negative options are last two items in the filter list when opened!

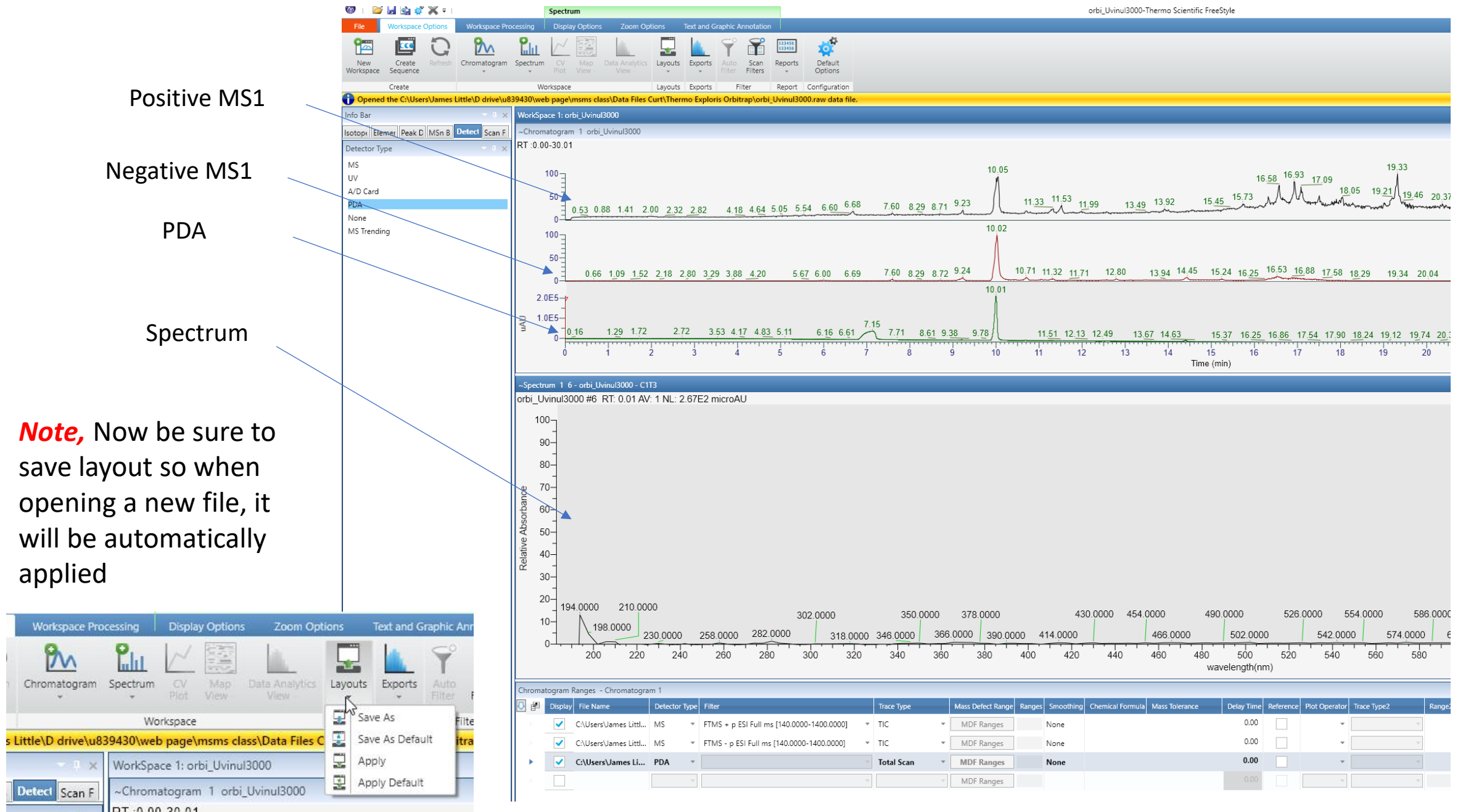
Positive MS1

Negative MS1

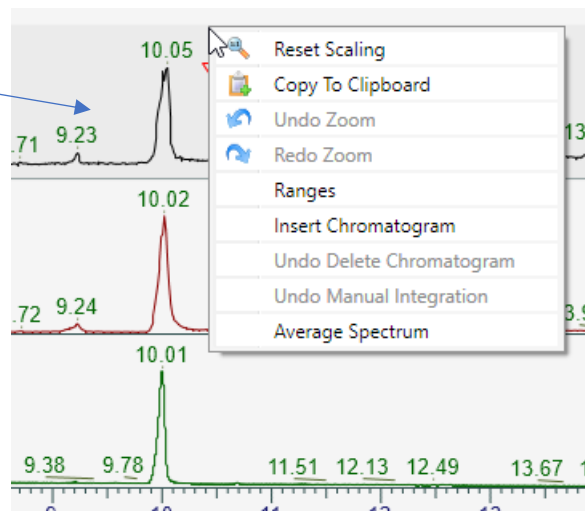
PDA

Spectrum

Note, Now be sure to save layout so when opening a new file, it will be automatically applied

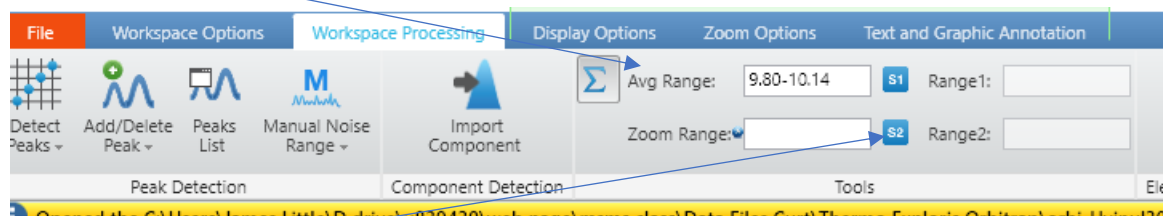


Left click in top box for positive ion chromatogram, then right click in order to average spectrum in Spectrum box



Can subtract background, not after averaging the Avg Range box is populated

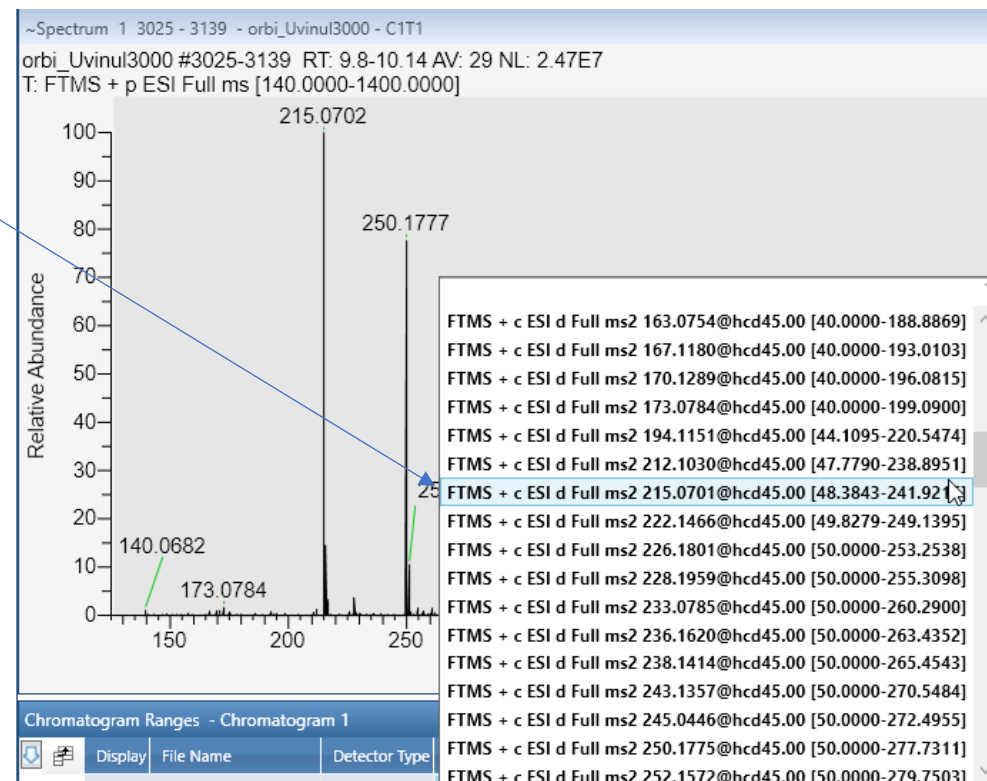
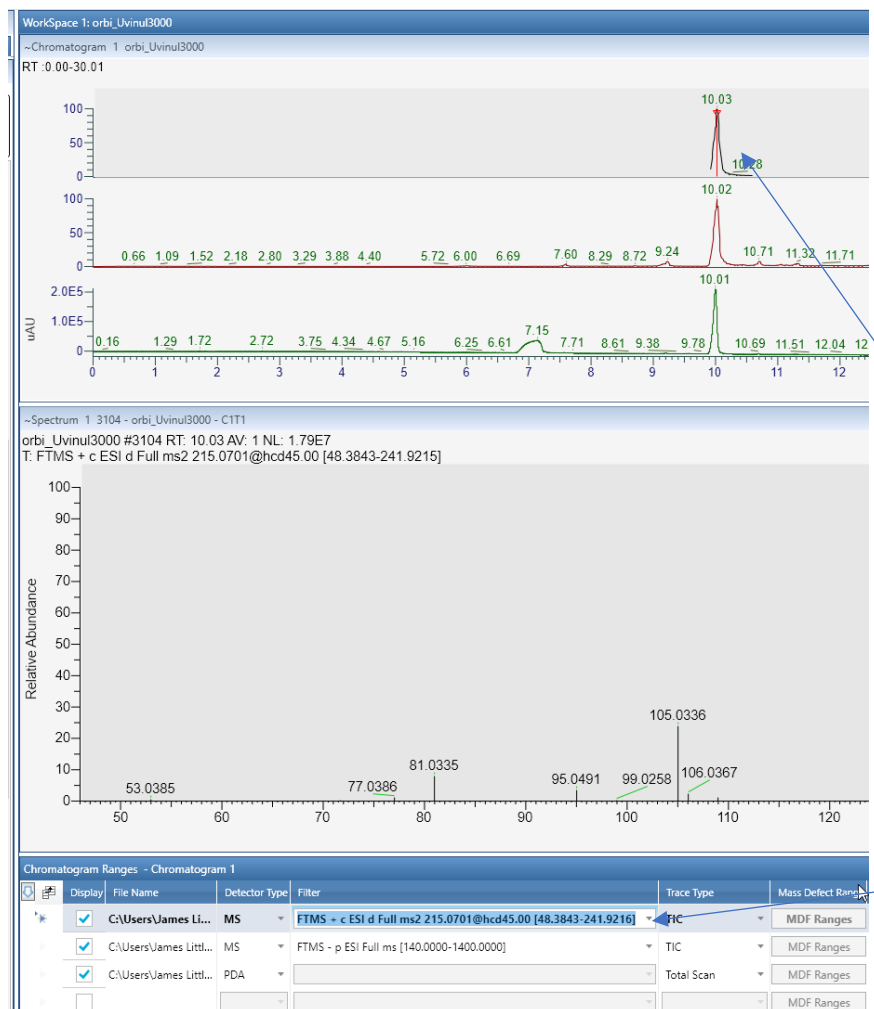
Then click on S1 and make sure you click on Range 1 box before going to Positive ion chromatogram to specify range



Note, If you use average range in a MS2 chromatogram, the accurate mass data will **only be nominal** when sent to NIST search!

Thus, for **obtaining MS2** spectrum for NIST search, **just single left click** on the peak of interest!

After deciding you are interested with m/z 215.0702, go to filter in Chromatogram range for positive ion and find that ion in the list, it(MS2) will be sent to the top chromatogram box and replace the MS1 chromatogram



MS2 is now in the top chromatogram and you can see that is shown in the bottom box as the filtered ion,

You can now click on the peak of interest to get the MS2 spectrum, again **remember**, average MS2 spectra are converted to nominal mass when imported into NIST search, so **just single click** to get the MS2 for sending to NIST search

- The positive ion MS2 (MSMS) spectrum is now displayed in the Spectrum display box, can repeat steps to get the Negative Ion MS2 (MSMS) spectrum
- The spectra can be sent to the NIST MS search for processing, shown in in another part of the course

