Part 1a: Data Processing for NIST MSMS Search Using Thermo Freestyle Software

(*Favorite Approach* Part 1 ***plus*** *"*Nearby Precursors" Enhancement)

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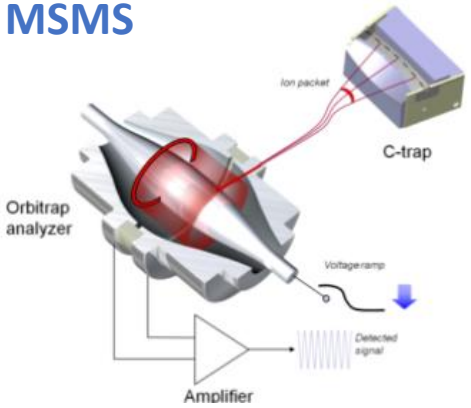
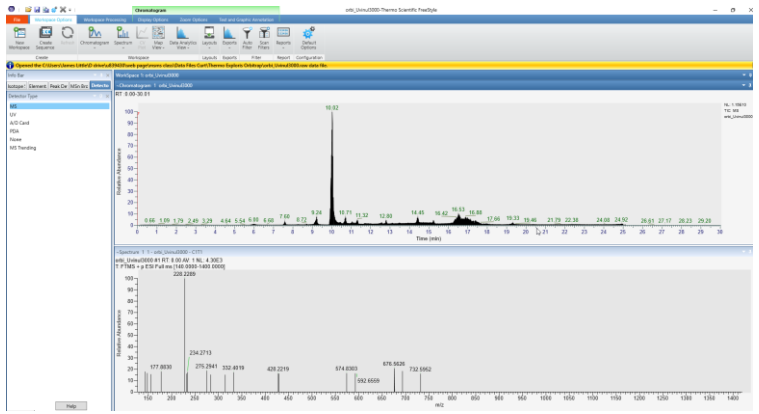
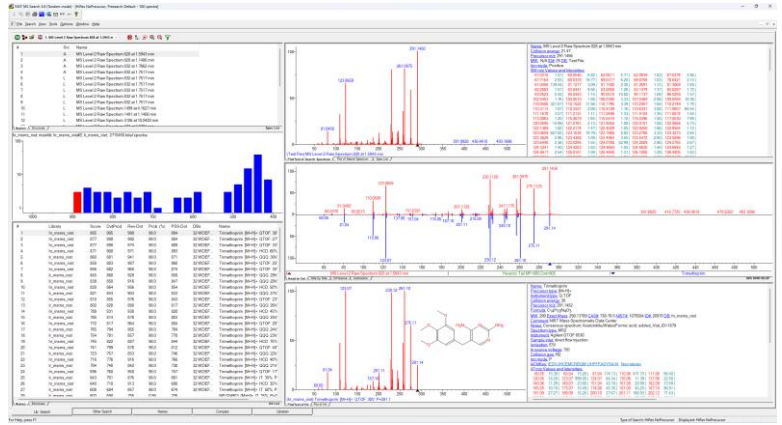
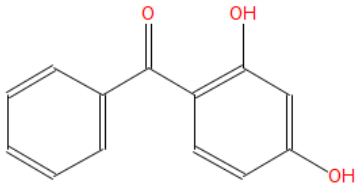


Thermo Freestyle



NIST MSMS Search



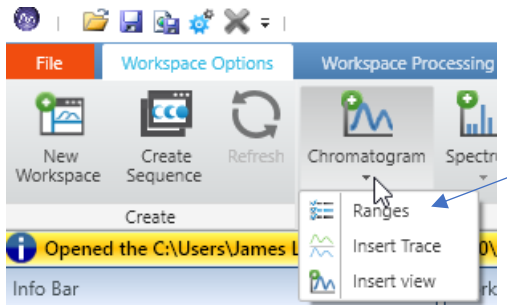


Uvinul 3000
MW 214
LC-MS and MSMS Analyses (Positive and Negative Mode)
with
Photo Array Detector Array (PDA) and UV-VIS Analyses

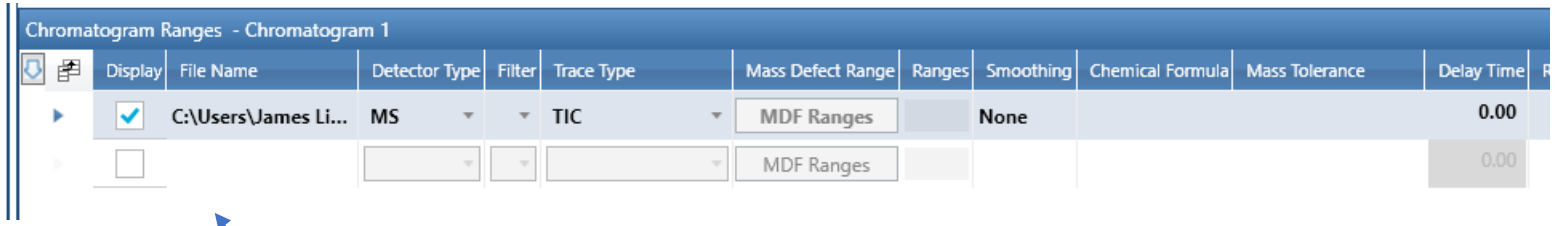
In My Opinion, Preferred Way of Processing Data

(Do the Same Steps as in the Preferred Approach in Part 1)

- 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!



Select ranges



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

(Do the Same Steps as in the Preferred Approach in Part 1)

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!

(Do the Same Steps as in the Preferred Approach in Part 1)

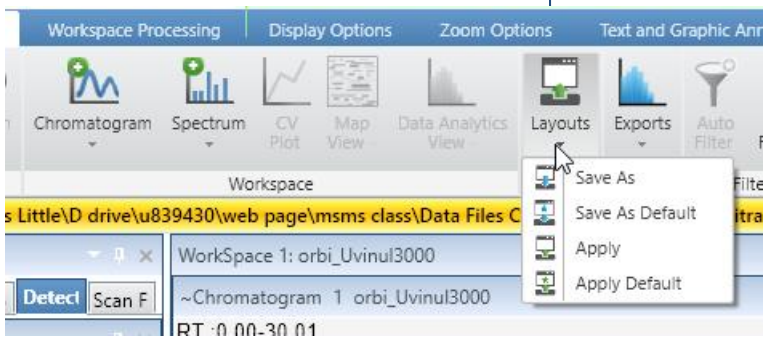
Positive MS1

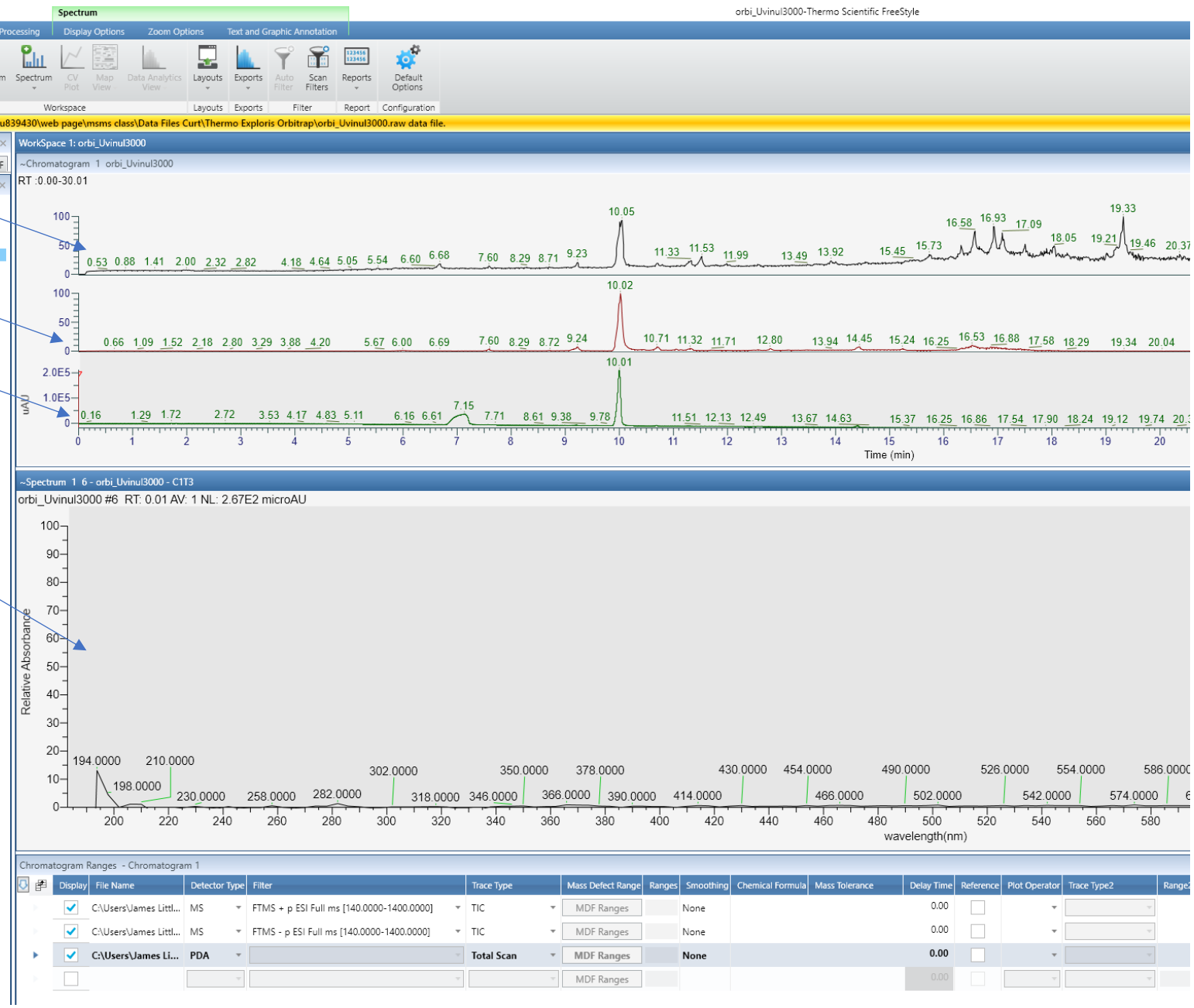
Negative MS1

PDA

Spectrum

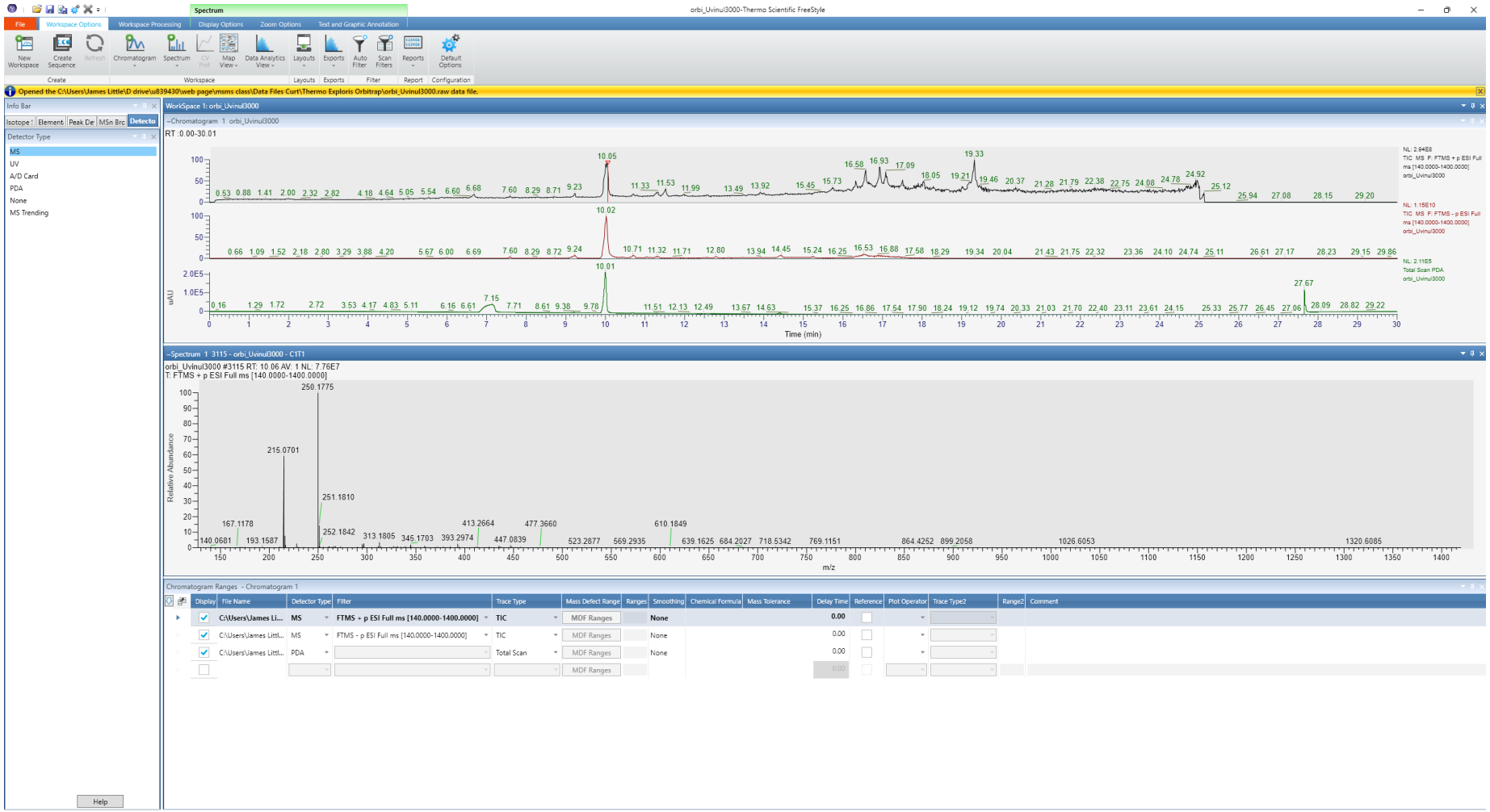
Note, Now be sure to save layout so when opening a new file, it will be automatically applied





Then Use Nearby Precursors Option

- Left click on any peak in either the positive or negative chromatogram
- Below, I clicked on the positive ion for chromatogram to get peak at 10.05 minutes
- That will give the spectrum for peak in second window from top
- Double left click on the purple circle, and the MS2 spectrum will appear as ***shown in next slide***
- ***NOTE: "Nearby Precursors" CANNOT be used with an averaged MS Spectrum!***

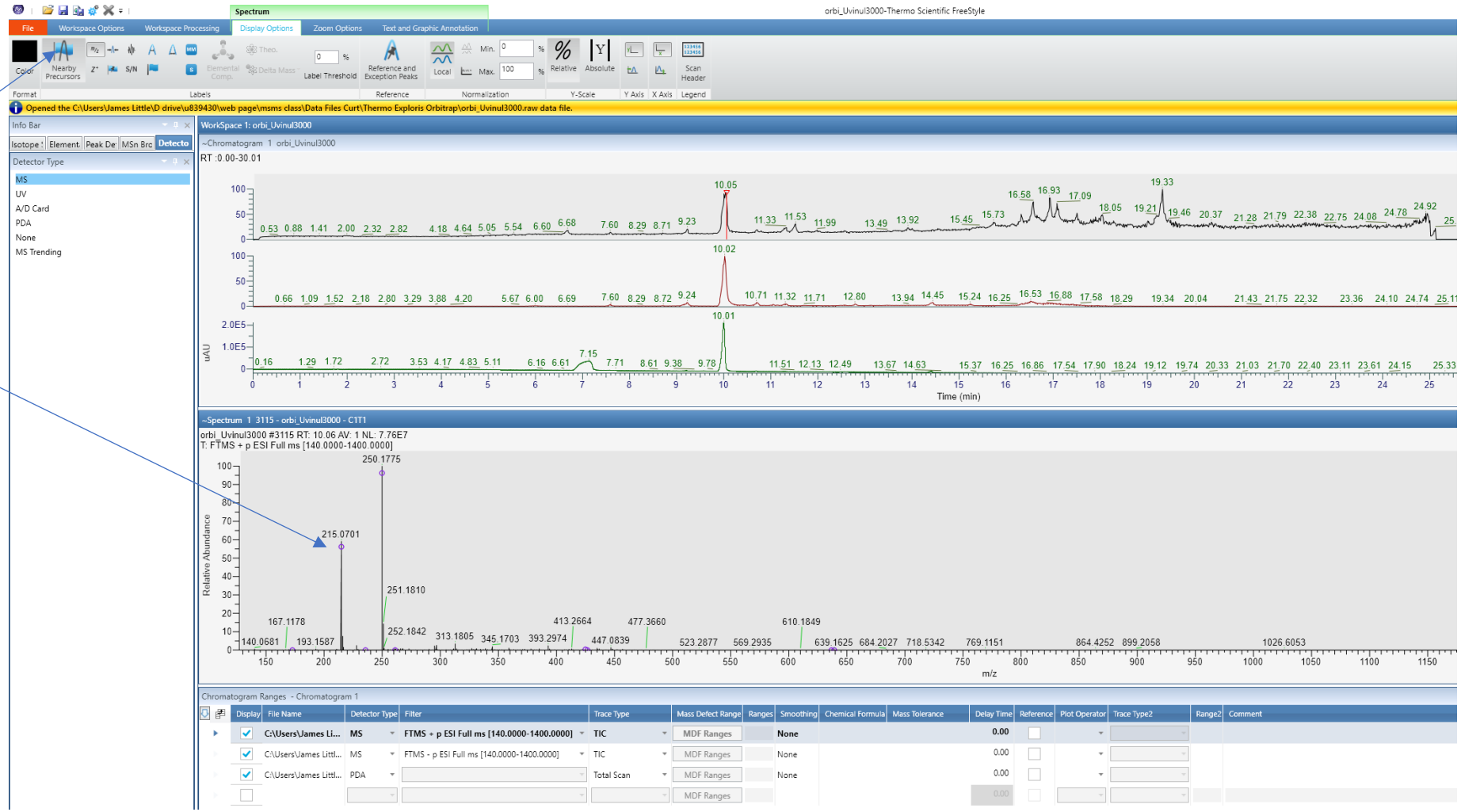


Then Use Nearby Precursors Option

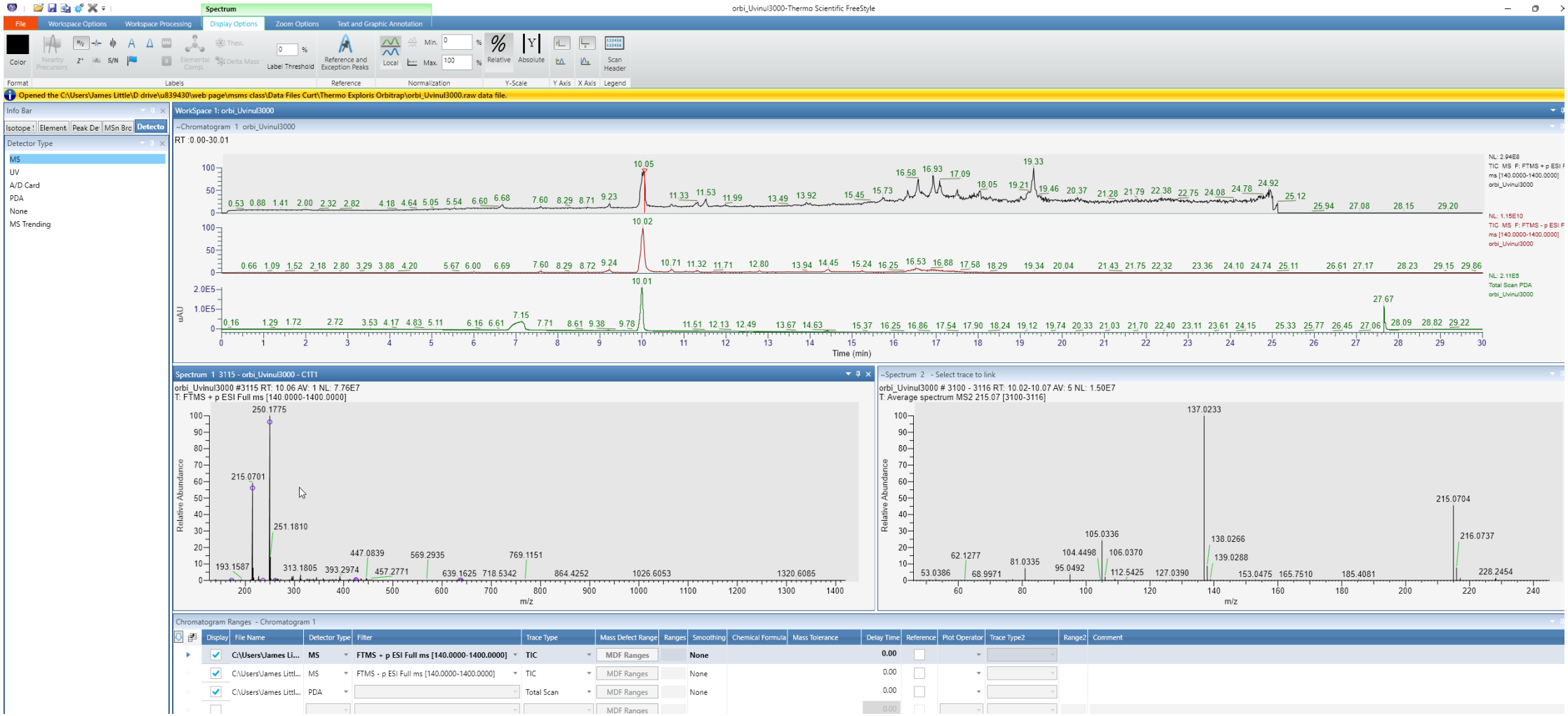
- All peaks with a purples circle have an associated MS2 spectrum
- Double left click on the purple circle, and the MS2 spectrum will appear as ***shown in next slide***
- ***NOTE: "Nearby Precursors" CANNOT be used with an averaged MS Spectrum!***

Nearby Precursors

Double Left Click on Purple Circle to get MSMS Spectrum associated with ion



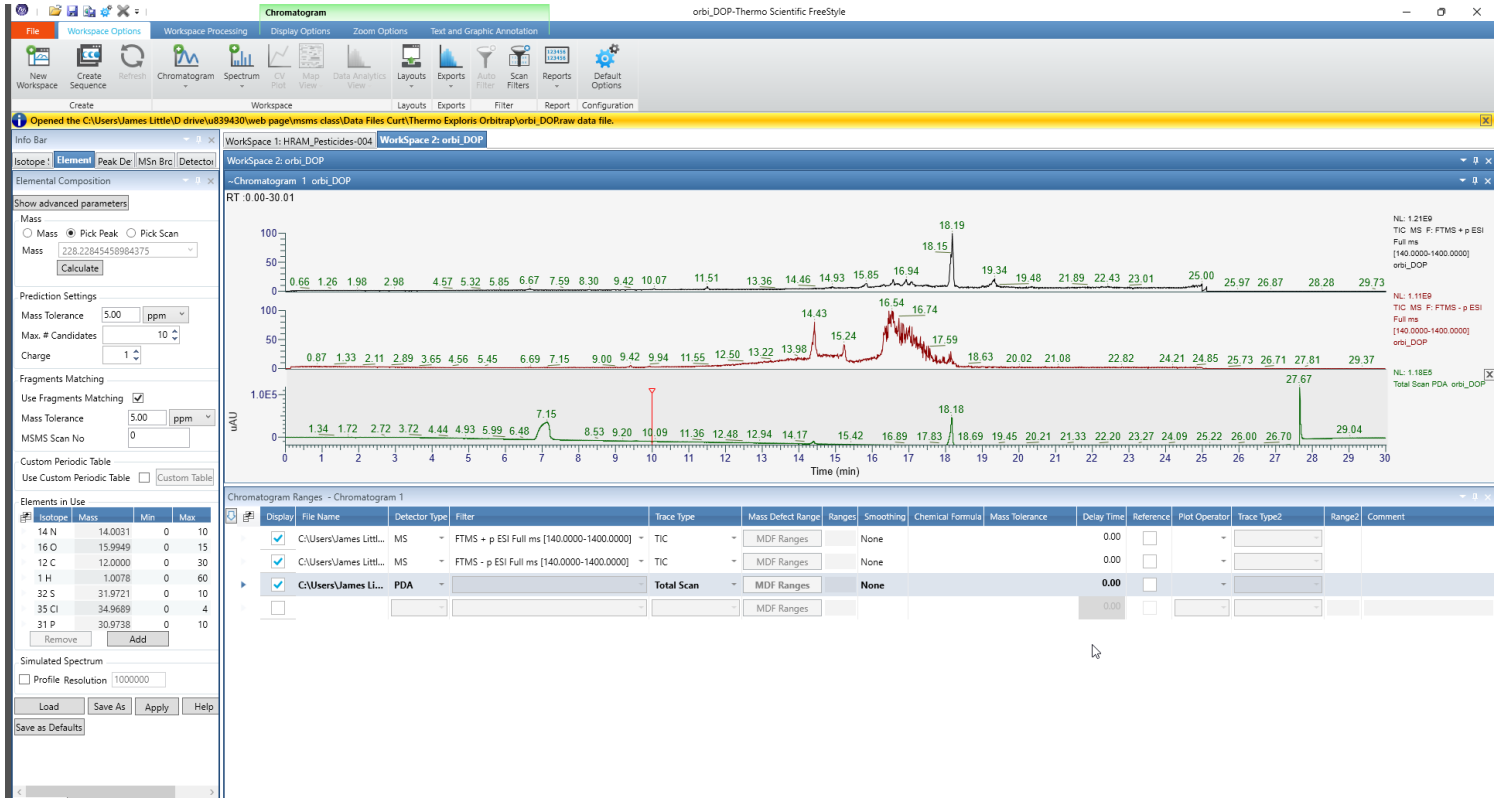
- After *double left clicking* on purples circle for ion at 215.o701, the MS2 spectrum for that peak is displayed
- Can then select bar above spectrum with left click and send to NIST search as before as detailed in Part 1 of this series
- Jut click on the x in box to remove the MS2 spectrum and get ready to select another one
- If you inadvertently remove all the spectra, go to main toolbar Workspace Options/Spectrum/Insert View



If You Delete All the MS and MS2 Windows by Mistake

- If you remove all the spectra windows by mistake (1)
- Go to Workspace Options/Spectrum/Insert View (2)

(1)



(2)