

PEAKS Data Analysis Pipeline

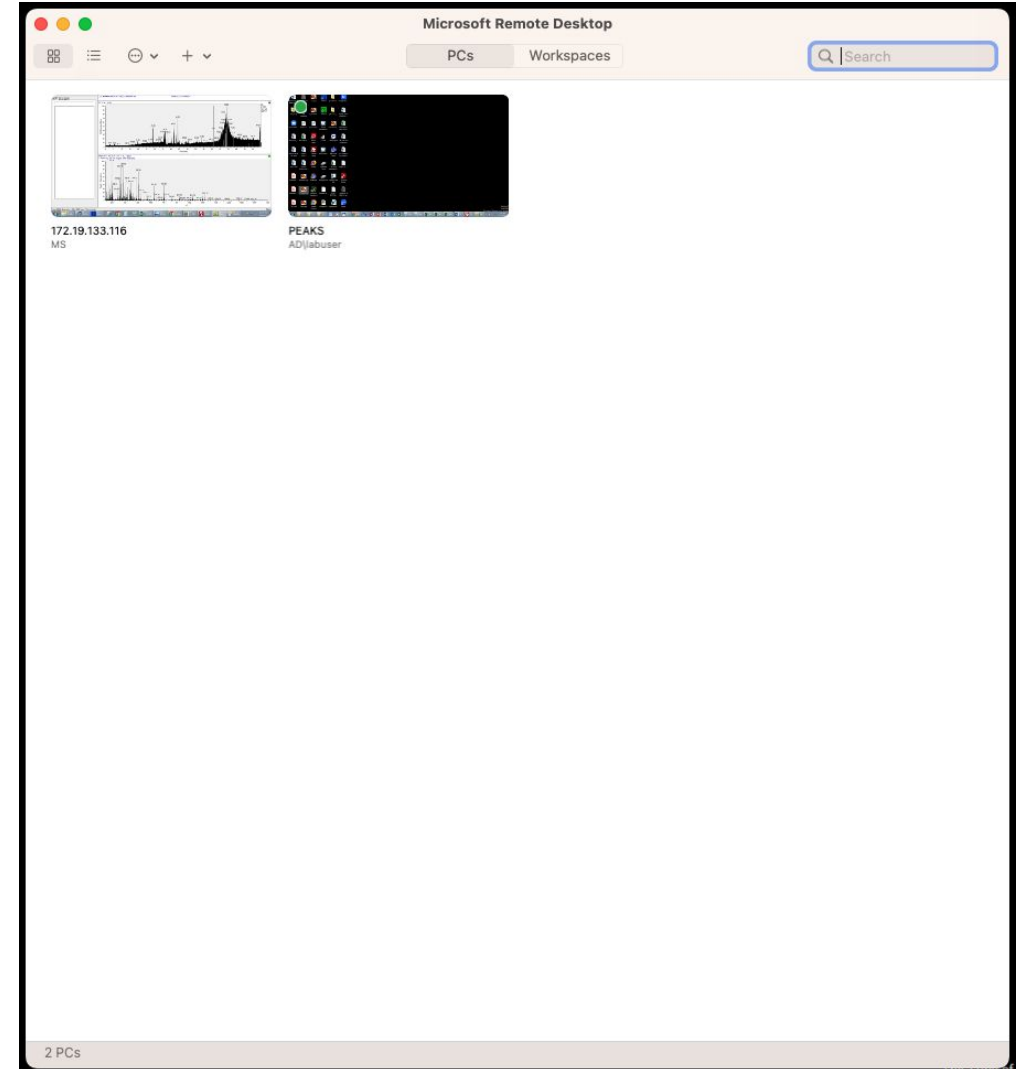
Anthony O'Donoghue, Lawrence Liu, Brianna Hurysz, Diego Trujillo

1. Go to Microsoft team viewer; Select the Peaks computer

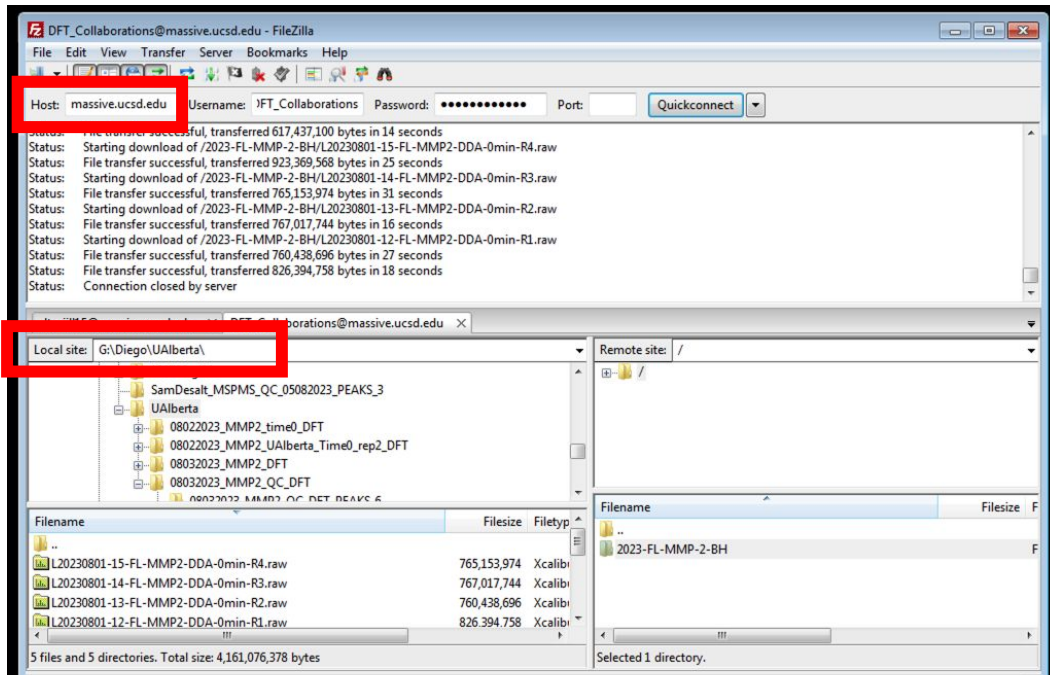


To remote in to the MS computer and PEAKS computer (3rd floor, Hook Lab), use UCSD Wifi or UCSD VPN with Cisco AnnyConnect (Icon above)

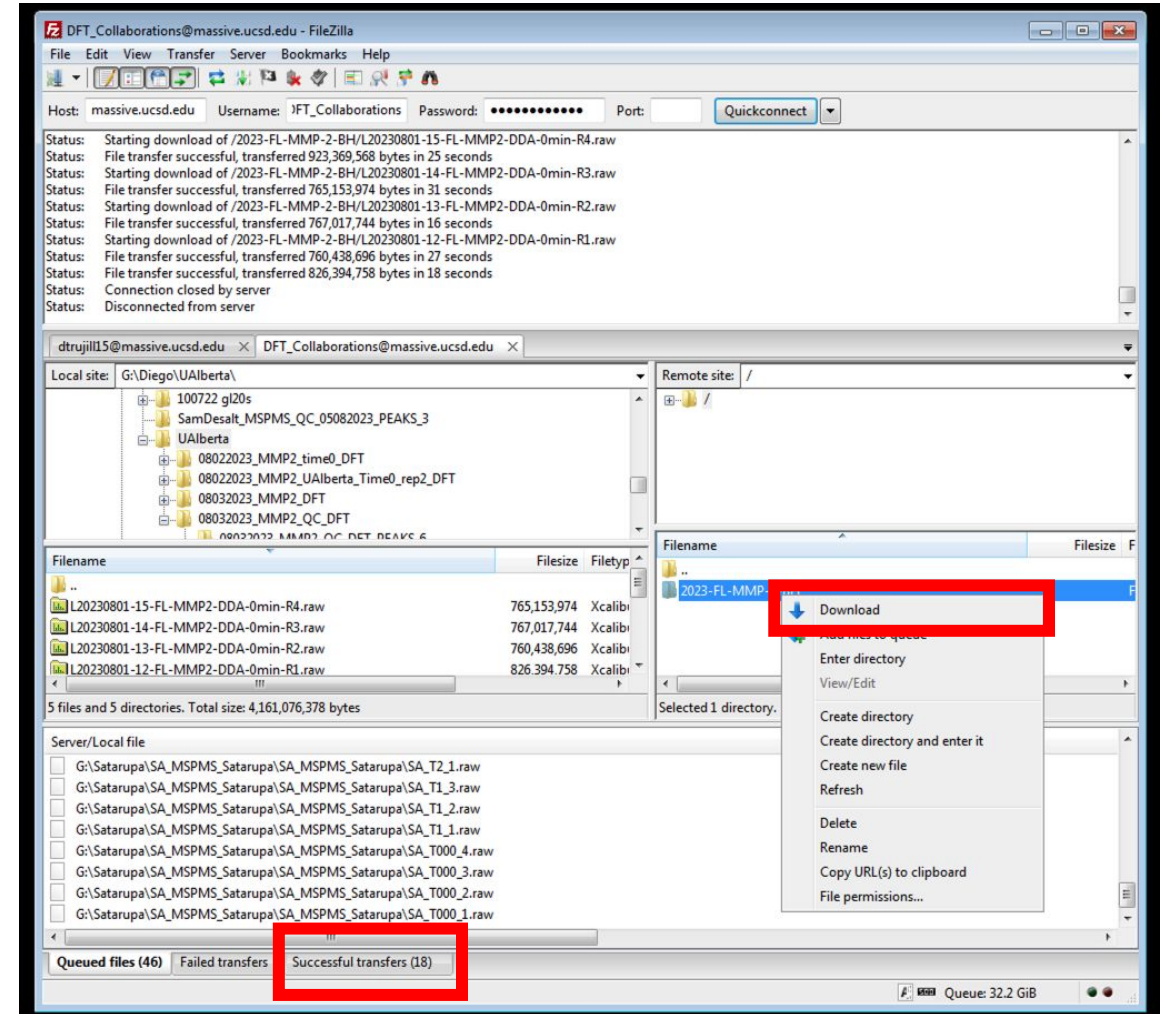
pwd for MS comptuer = Cathep\$inV



2. Use Massive/Filezilla to transfer MS/PEAKS data to each other and to home CPU

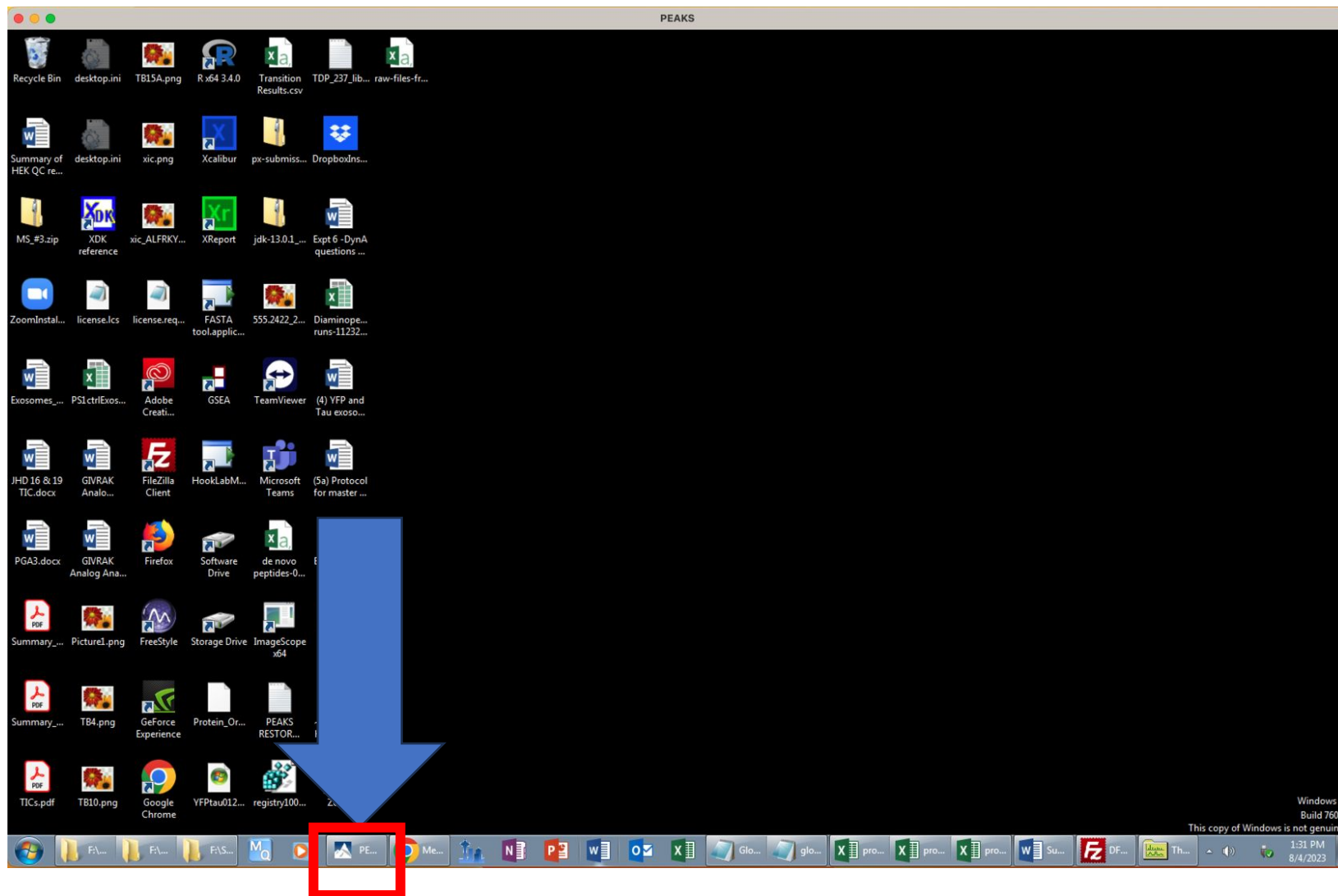


- Sign in using the host “massive.ucsd.edu” (shown above) and with personal massive credentials
 - For this step, we will want the data to be on our remote site already; this means it should have been placed on massive/filezilla from the MS computer prior to this protocol
- Find local directory where you want your data on the left side; this is usually in the G drive (easystore)

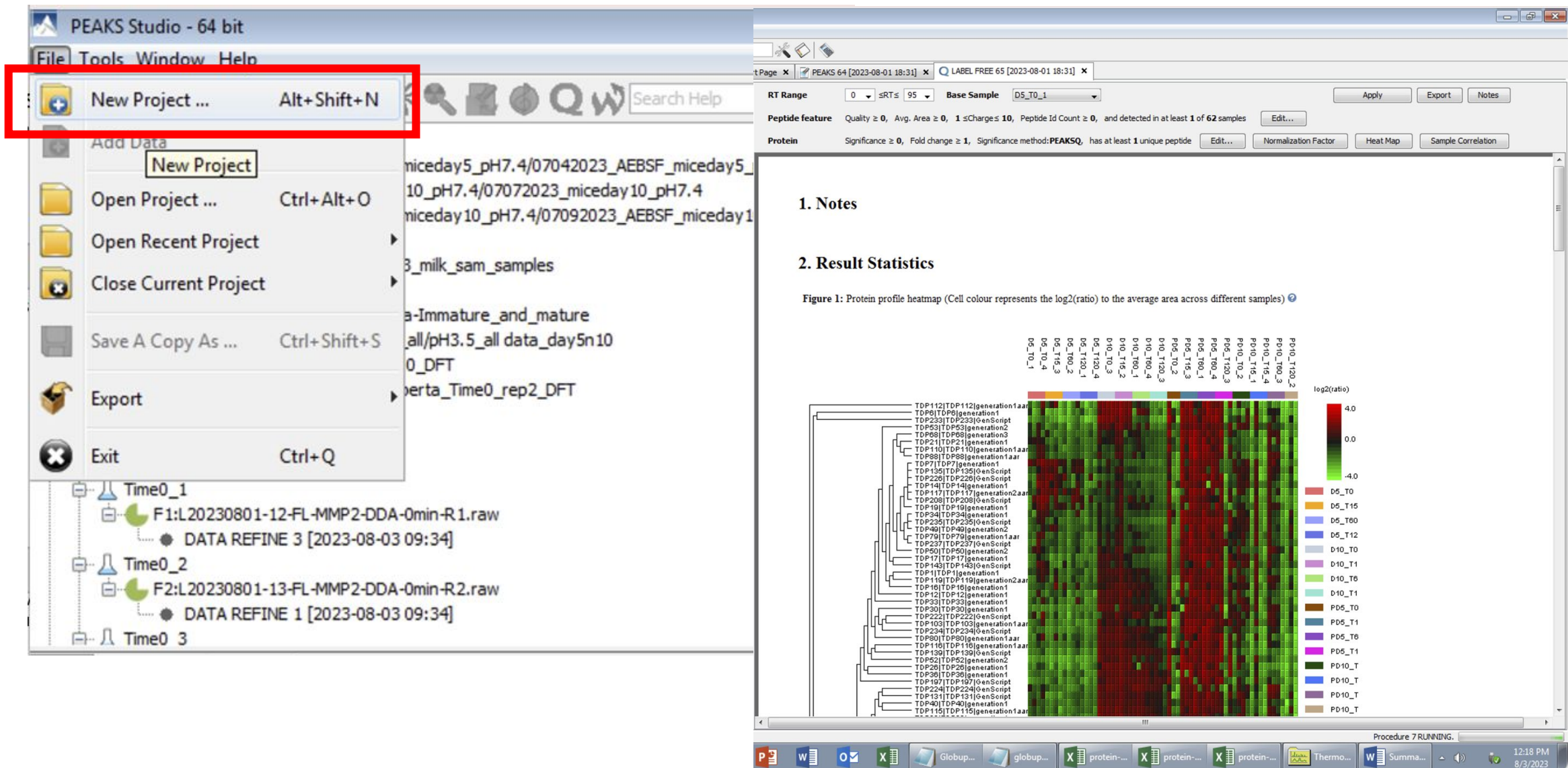


- Double/Right click to get a menu selection; Download from the remote site to the local site on the PEAKS computer
- Find local directory to ensure transfers were made successfully. Can also check the tab at the bottom in peaks

3. Open Up PEAKS Software

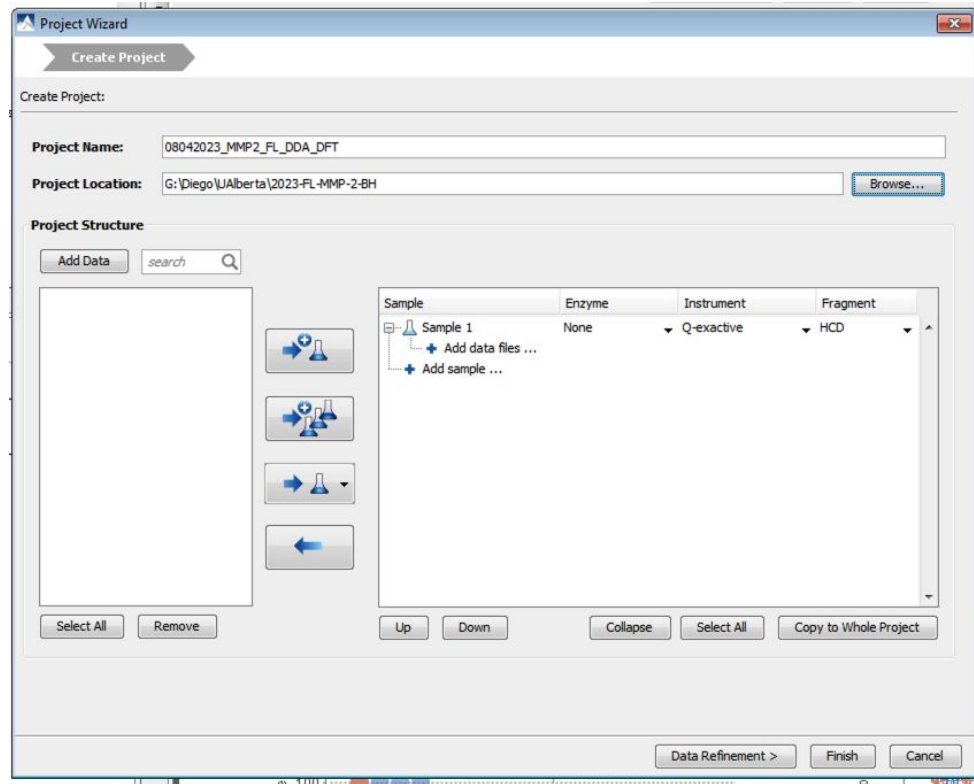


4. Create a New Project in PEAKS



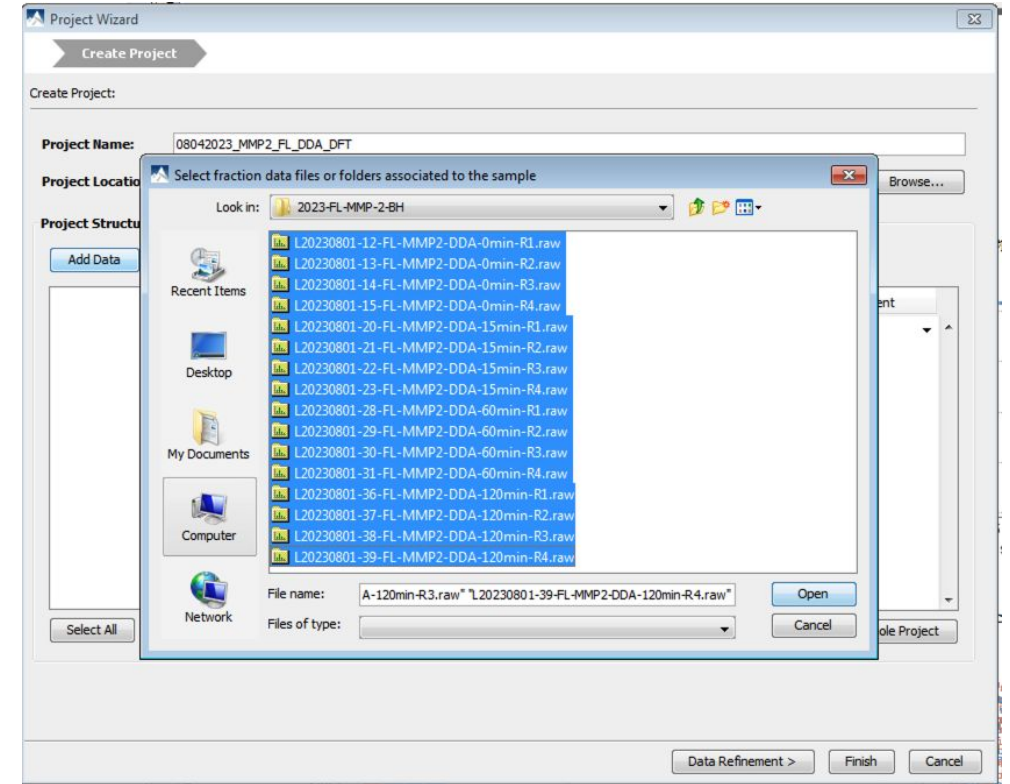
5. Select data to add to PEAKS project

A.



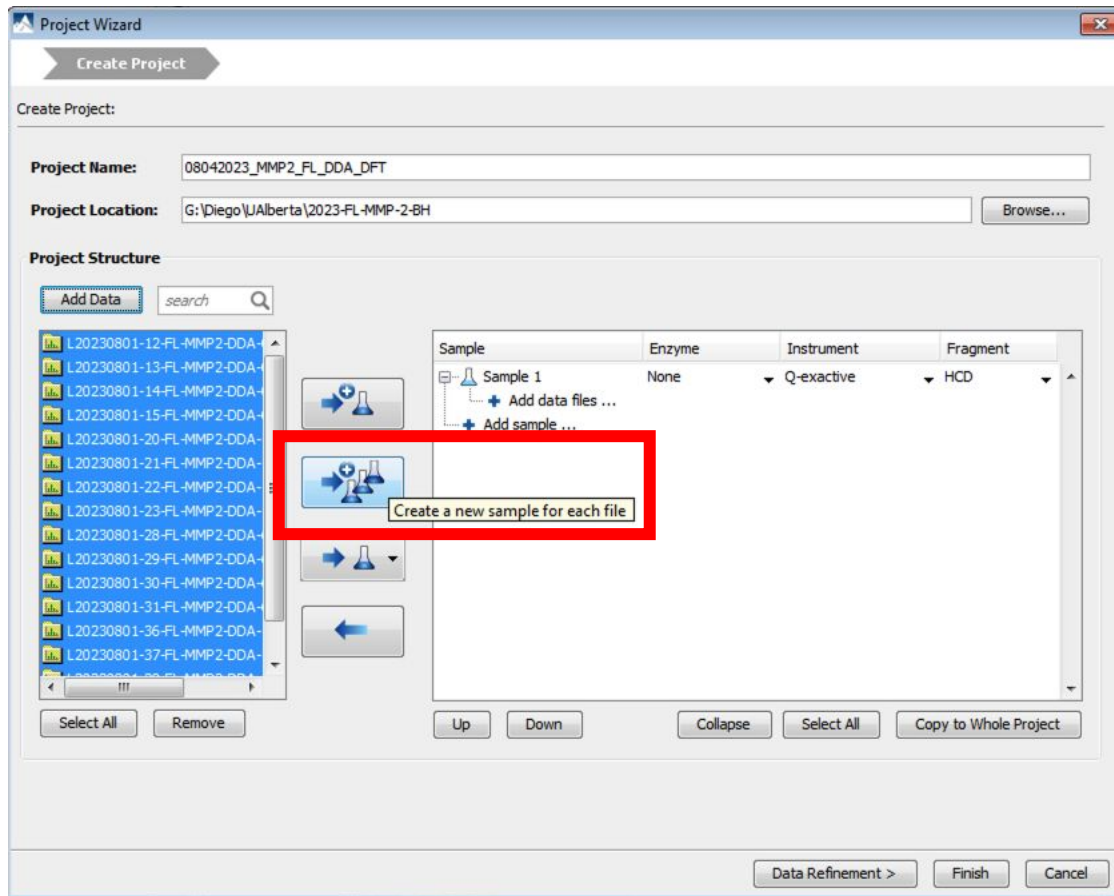
- First, create a project name, and select a project location
 - i.e. Easy Store; G:\Diego

B.

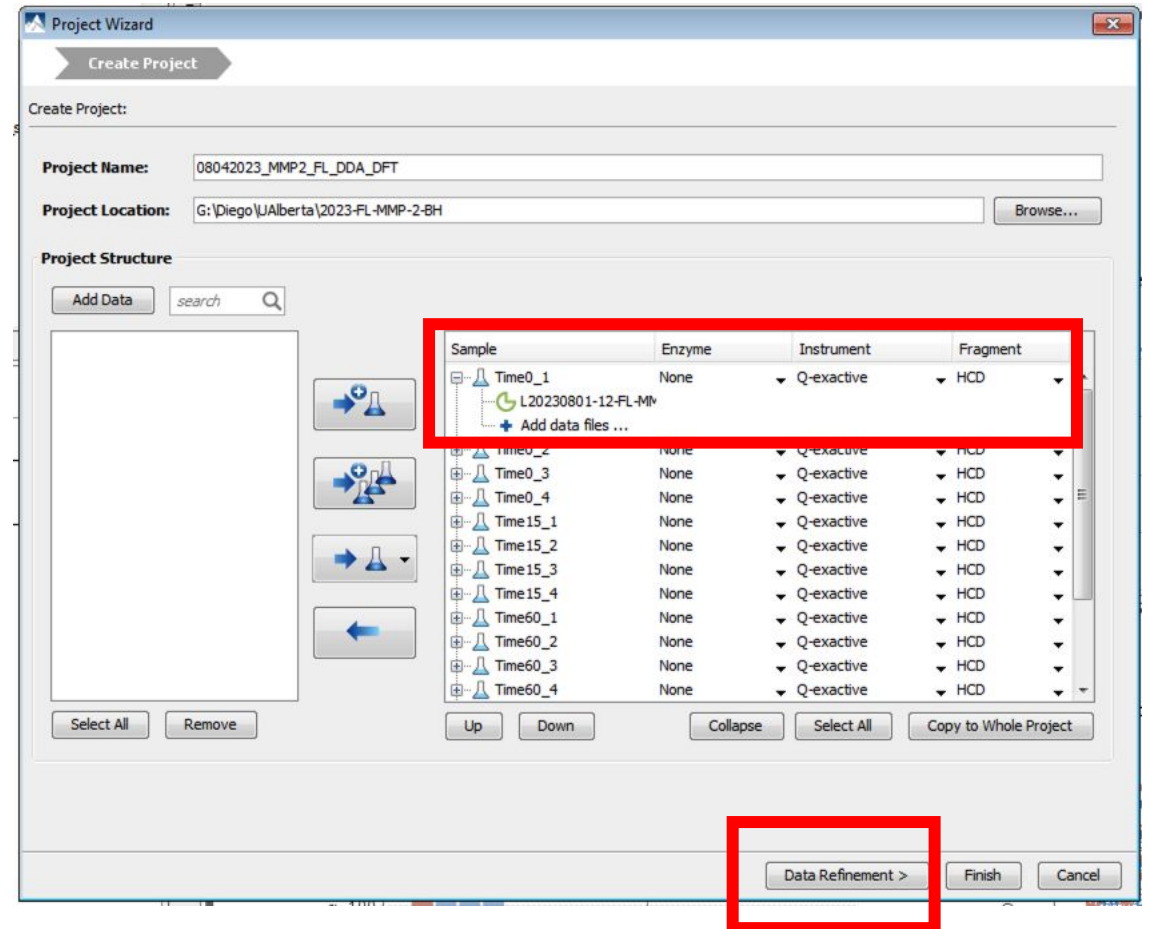


- Select the files you would like to analyze

6. Add data, rename samples, set correct parameters



- Highlight all the added data.
- Select Create new sample for each file



- Select appropriate instrument and fragmentation method
- Set Enzyme to "None"
- Rename samples according to enzymes, time points, and replicates
 - Can also ensure appropriate data is selected for these
- Select "Data Refinement" to continue

7. Data Refinement

Project Wizard

Create Project Data Refinement

Data Refinement

☒ **Merge Scans**

Retention time window (for raw files only): 0.8 min

Precursor m/z error tolerance: 10.0 Da ☐ ppm ☒

☐ Merge CID and HCD scans together

☒ **Correct Precursor**

☒ Mass only (recommended)

☐ Mass and Charge states

Min charge: 1 Max charge: 3

☒ **Filter Scans**

Only keep scans satisfying:

☐ Precursor mass between and Da

☒ Retention time between 0.0 and 95.0 min

☐ Quality value greater than (suggest 0.65)

Predefined parameters MSP-MS

< Back Identification > Finish Cancel

- On the top right, select MSP-MS as the predefined parameters
- Parameters shown are what should be used for MSP-MS data analysis
- Select “Identification” to continue

8. Peptide Identification

A.

Project Wizard

Create Project Data Refinement Identification

PEAKS Search Predefined parameters MSP-MS

Error Tolerance
Precursor mass: 20.0 ppm using monoisotopic mass Fragment ion: 0.01 Da

Enzyme
None View

Allow non-specific cleavage at both ends of the peptide.
In None Enzyme mode, PEAKS Search will process all peptides with length up to 65.

PTM
Carbamidomethylation Set PTM
Acetylation (N-term) Remove
Oxidation (M) Switch type

Maximum allowed variable PTM per peptide 3

Database
Select database Database: TPD_237 View
Paste sequence Taxa: all species Set/View taxa...
Contaminant database clos17 View

General Options
☒ Estimate FDR with decoy-fusion.
☐ Find unspecified PTMs and common mutations with PEAKS PTM Advanced Settings
☐ Find more mutations with SPIDER

Skip Identification < Back Quantification > Finish Cancel

- On the top right, select MSP-MS as the predefined parameters
- Parameters shown are what should be used for MSP-MS data analysis

B.

Project Wizard

Create Project Data Refinement Identification

PEAKS Search Predefined parameters MSP-MS

Error Tolerance
Precursor mass: 20.0 ppm using monoisotopic mass Fragment ion: 0.01 Da

Enzyme
None View

Allow non-specific cleavage at both ends of the peptide.
In None Enzyme mode, PEAKS Search will process all peptides with length up to 65.

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General Options
☒ Estimate FDR with decoy-fusion.
☐ Find unspecified PTMs and common mutations with PEAKS PTM Advanced Settings
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Skip Identification < Back Quantification > Finish Cancel

- Identification -> Select Database -> TPD_237

C.

Project Wizard

Create Project Data Refinement Identification

PEAKS Search Predefined parameters MSP-MS

Error Tolerance
Precursor mass: 20.0 ppm using monoisotopic mass Fragment ion: 0.01 Da

Enzyme
None View

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PTM
Set PTM
Remove
Switch type

Maximum allowed variable PTM per peptide 3

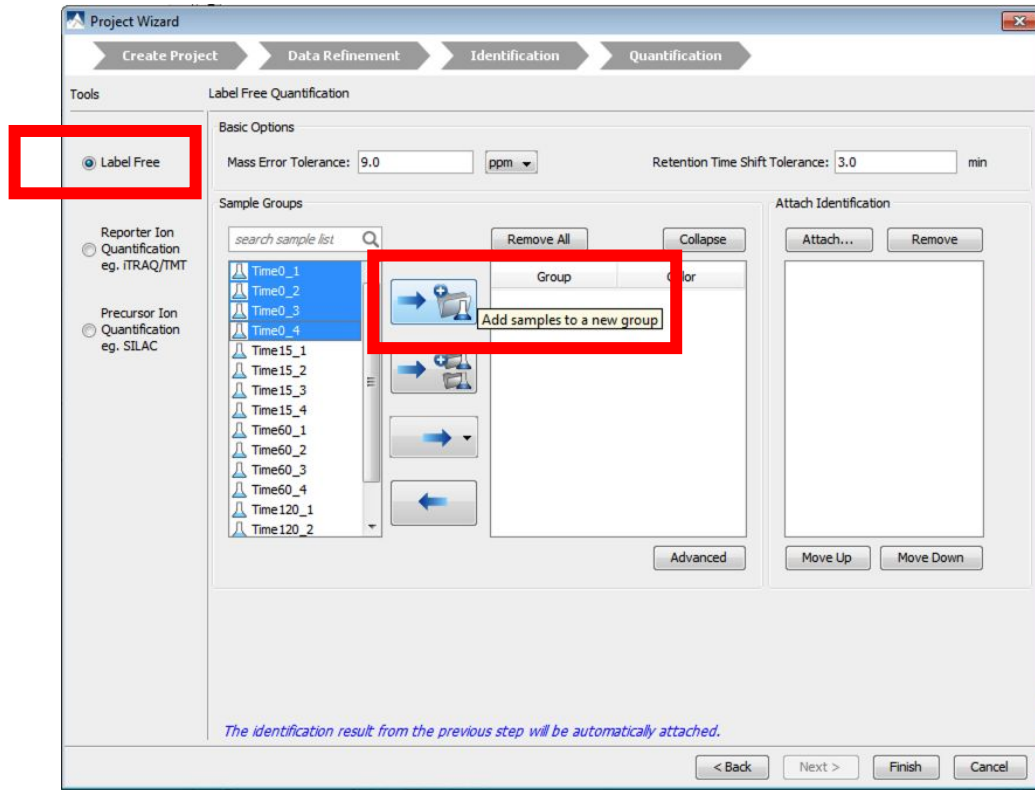
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General Options
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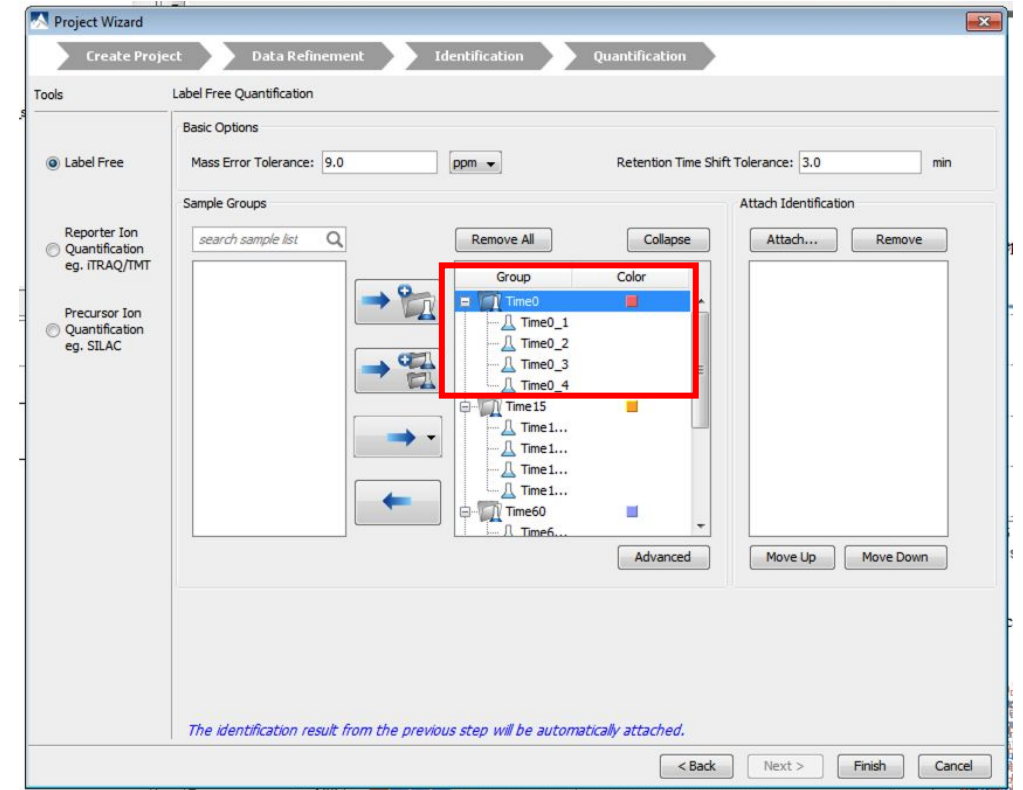
Skip Identification < Back Quantification > Finish Cancel

- Identification -> no PTMs
 - Can remove the PTMs by highlighting them and selecting Remove

9. Label Free Quantification



- Make sure Label Free is selected
- Group samples -> add quadruplicates of samples to new group
 - Note: I never really play with the Basic option on the top, I just leave these as is



- Rename samples according to time points
- Select Finish. Let PEAKS finish running the analysis
 - Usually takes at least 4 hours for MSP-MS data
- Once complete, use R scripts to further process data
 - See "MSP-MS Data Analysis" protocol for R scripts