timsTOF Visualization App Tutorial
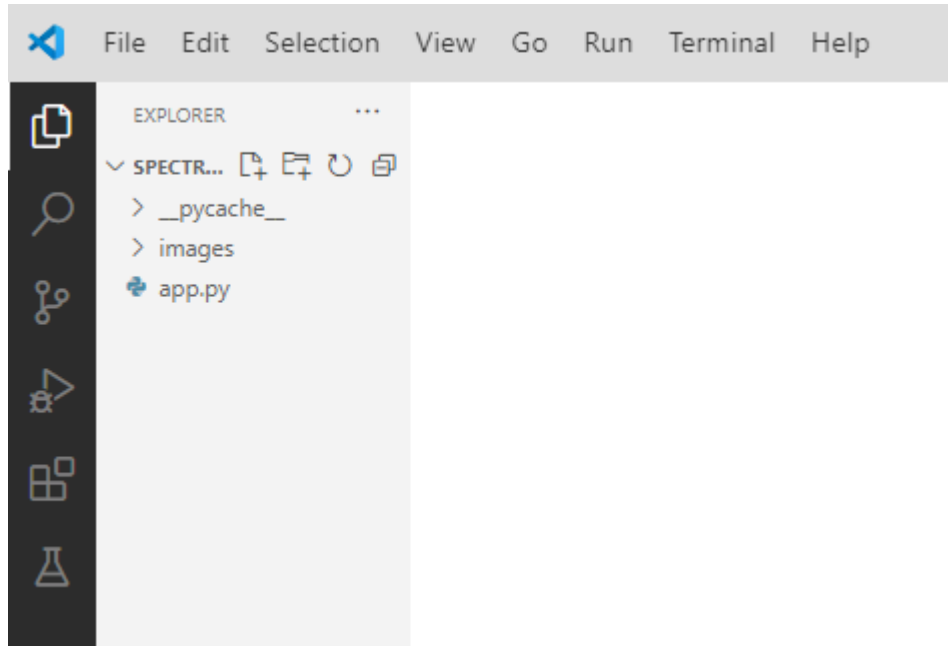
Software Installation

Necessary Software

- Install Python or Anaconda
    - https://www.python.org/downloads/
    - https://www.anaconda.com/download

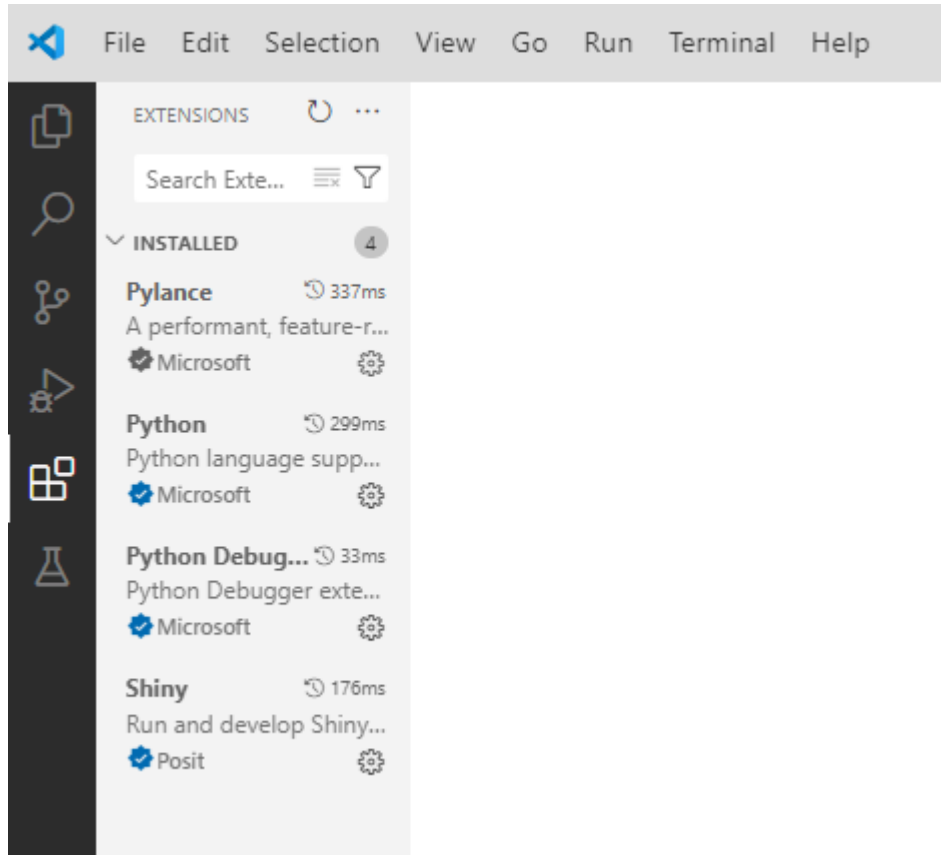- Install Visual Studio Code
    - https://code.visualstudio.com/

Getting Started

Getting started in Visual Studio Code

- Set the directory containing the app.py file as the working directory
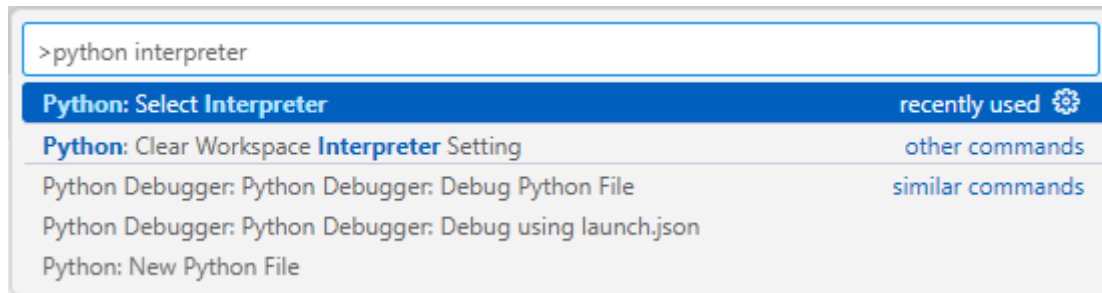- Open the app.py file by double-clicking on the file
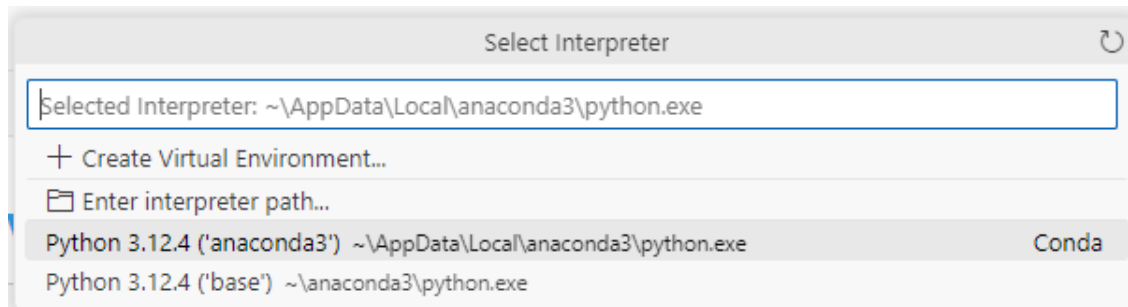
Install necessary extensions

- Install Python and Shiny extensions using the Extensions:Marketplace tab

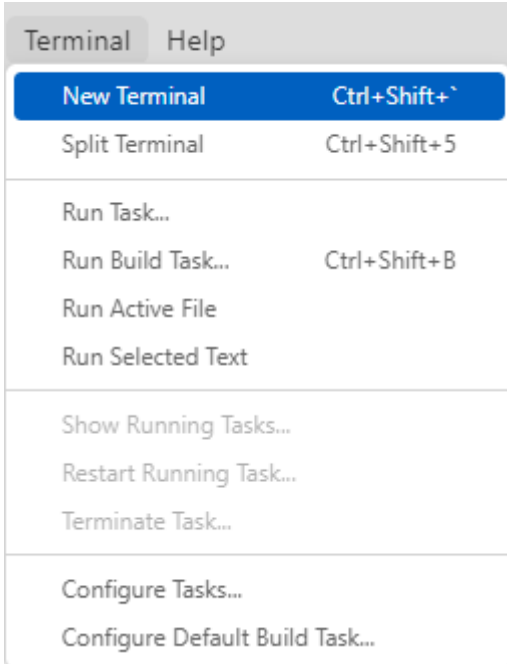# Set Python interpreter to current installation of Python/Anaconda
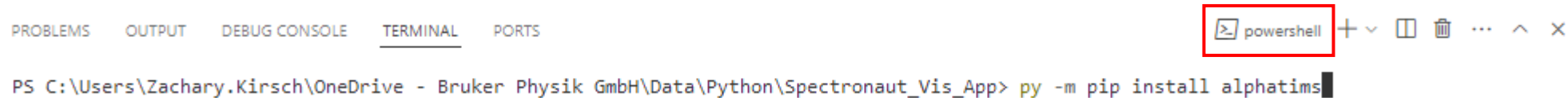
- Ctrl+Shift+P and search Python interpreter

- Select current Python installation

Installing necessary Python libraries

- Open a new powershell terminal and use pip to install
  - alphatims, colorcet, faicons, hvplot, matplotlib-venn, scikit-learn, shiny, shinyswatch, and upsetplot

- Replace the part underlined in red with the library to install

Installing necessary Python libraries

```python
# ====================================================
# Library Imports
# ====================================================
#region
from shiny import App, Inputs, Outputs, Session, reactive, render, ui, module
from shinyswatch import theme
#https://rstudio.github.io/shinythemes/
from shiny.types import ImgData
import alphatims.bruker as atb
import alphatims.plotting as atp
from collections import OrderedDict
from datetime import date
from faicons import icon_svg
#https://fontawesome.com/search?o=r&m=free
import io
import itertools
from itertools import groupby
import math
import matplotlib
import matplotlib.pyplot as plt
from matplotlib.pyplot import cm
import matplotlib.colors as mcolors
from matplotlib.patches import Rectangle
from matplotlib_venn import venn2,venn2_circles,venn3,venn3_circles
import numpy as np
import os
import pandas as pd
import pathlib
import re
from scipy.stats import norm
import seaborn as sns
from sklearn.decomposition import PCA
from sklearn.pipeline import Pipeline
from sklearn.preprocessing import StandardScaler
from tkinter import *
from upsetplot import *
```

- If any libraries are underlined in yellow, it means they aren't recognized by the IDE

- If you've already installed a library and it's not being recognized, either restart Visual Studio Code or make sure the correct Python interpreter is selected

Starting the Shiny app
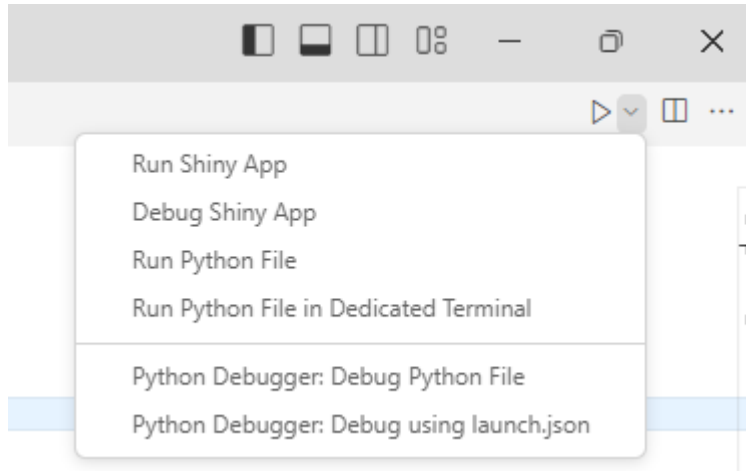
- With the extensions and libraries installed, you should be able to run the app with Visual Studio Code recognizing it as a Shiny app

Using the App

File import

Updating result metadata

This is necessary when condition names are not native to the search report or if changes are needed for the order, presence, or names of samples

If changes are made to the metadata table, use these switches to specify the change and then click the "apply changes" button

Update from Metadata Table

- ○ Update 'R.Condition' and 'R.Replicate' columns
- ○ Update 'Concentration' column
- ○ Remove/resort samples

[Apply changes to search report / reinitialize search report]

Metadata Table

-Double click on any cell to update its contents

-To remove samples, add an 'x' to the 'remove' column. To reorder samples, number them in the order you want them to appear in the 'order' column

R.FileName	R.Condition	R.Replicate	order	remove	Concentration
20240613_1-1-3_K562-Yeast_EColi_10ng_OFF_1_Slot1-50_1_3848	1-1-3_10ng	1			
20240613_1-1-3_K562-Yeast_EColi_10ng_OFF_2_Slot1-50_1_3849	1-1-3_10ng	2			
20240613_1-1-3_K562-Yeast_EColi_10ng_OFF_3_Slot1-50_1_3850	1-1-3_10ng	3			
20240613_1-2-2_K562-Yeast_EColi_10ng_OFF_1_Slot1-48_1_3844	1-2-2_10ng	1			
20240613_1-2-2_K562-Yeast_EColi_10ng_OFF_2_Slot1-48_1_3845	1-2-2_10ng	2			
20240613_1-2-2_K562-Yeast_EColi_10ng_OFF_3_Slot1-48_1_3846	1-2-2_10ng	3			

R.FileName: (required, auto-filled)
Will be pre-filled from the report file.

R.Condition: (required)
Experimental condition names. If Condition was specified in Spectronaut, it will be added here. Otherwise, it needs to be filled out.

R.Replicate: (required)
Replicate numbers. Should be unique in each condition. . If Replicate was specified in Spectronaut, it will be added here. Otherwise, it needs to be filled out.

Order: (optional)
Specify the numerical order you want the runs shown in.

Remove: (optional)
Adding an 'x' means that you want to remove the run from the subsequent figures.

Concentration: (optional)
Should be the same value for each condition.

General Navigation

Coloring Options

- Multiple color setting options for colors to be used in plotting
- Can mix and match from those listed when using custom colors
- If there's only one sample condition, the "Pick for me" sections pick a color at random

Coloring Options

Colors by condition according to the matplotlib tableau colors

Colors by condition according to a rainbow color series split based on the number of conditions

Colors by condition according to specified colors in the text area

File Import Formats

Uploading DIA windows to overlay

File Import

Settings

ID Counts

Metrics

PTMs

PCA

Heatmaps

RT, m/z, IM Heatmaps | **Charge/PTM Precursor Heatmap**

Upload DIA windows as a .csv:

Browse... | No file selected

Choose DIA windows to overlay:
- ○ Imported DIA windows
- ○ Lubeck DIA
- ○ Phospho DIA
- ● None

- CSV Format:

	A	B	C	D	E	F	G
1	#MS Type	Cycle Id	Start IM [1/K0]	End IM [1/K0]	Start Mass [m/z]	End Mass [m/z]	CE [eV]
2	MS1	0	-	-	-	-	-
3	PASEF	1	0.965	1.12	725.13	736.24	-
4	PASEF	1	0.805	0.965	559.8	567.5	-
5	PASEF	1	0.6	0.805	350.68	382.04	-
6	PASEF	2	0.977	1.15	746.34	758.38	-
7	PASEF	2	0.87	0.977	574.21	582.01	-
8	PASEF	2	0.6	0.87	412.39	425.4	-
9	PASEF	3	0.986	1.19	769.41	782.64	-
10	PASEF	3	0.89	0.986	588.81	596.81	-
11	PASEF	3	0.6	0.89	437.42	447.23	-
12	PASEF	4	0.995	1.23	794.87	808.65	-
13	PASEF	4	0.9	0.995	603.8	612.07	-
14	PASEF	4	0.6	0.9	456.05	464.9	-

Uploading peptide list to track

File Import

Settings

ID Counts

Metrics

PTMs

PCA

Heatmaps

Mixed Proteome

PRM

Dilution Series
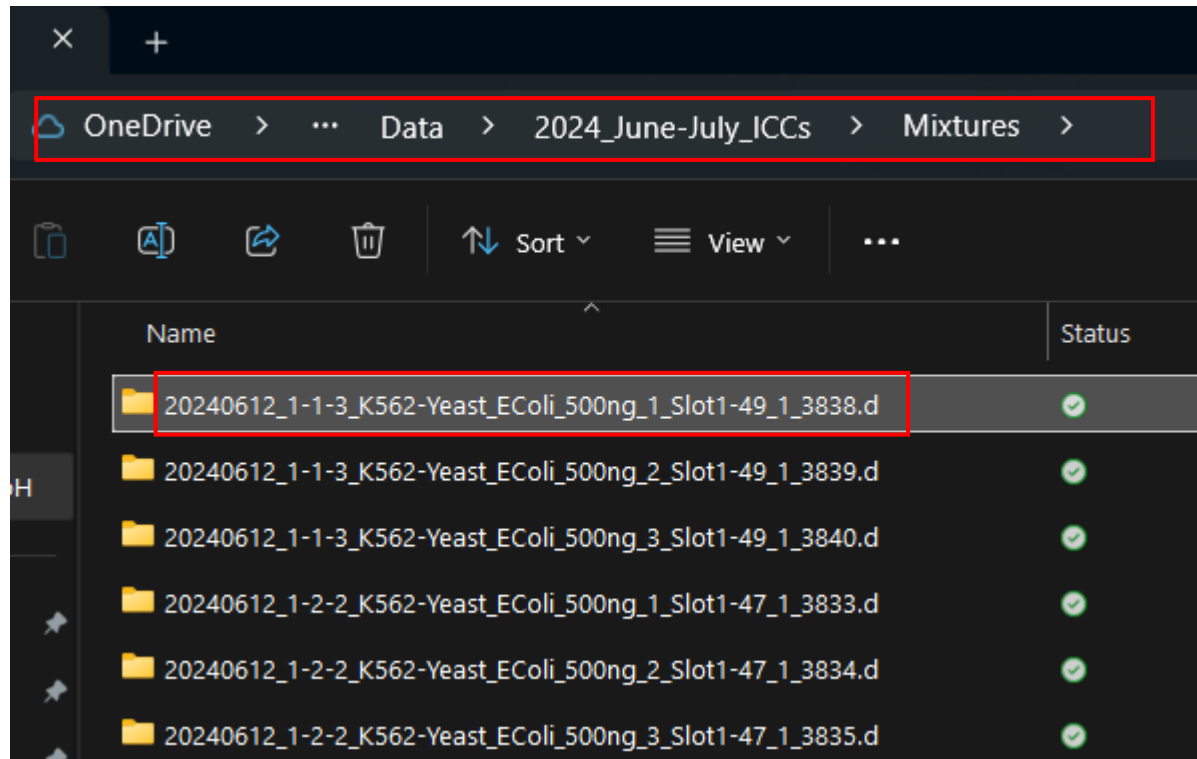
PRM List   PRM Table   PRM Peptide

Upload Peptide List:

Browse...   No file selected

- CSV Format:

	A	B
1	PG.ProteinGroups	EG.ModifiedPeptide
2	P00359	_TASGNIIPSSTGAAK_
3	P0A853	_KYDIPVVMDSAR_
4	P00330	_[Acetyl (Protein N-term)]SIPETQK_
5	P68104;Q5VTE0	_IGGIGTVPVGR_
6	P00924	_SIVPSGASTGVHEALEM[Oxidation (M)]R_
7	P00549	_GVFPFVFEK_
8	P00924	_IEEELGDNAVFAGENFHHGDK_

Referencing raw data files

- Paste the full path of the .d folder for each file you want to plot into the text area

- Should look like the example text shown

- Avoid spaces, use a new line for each file