

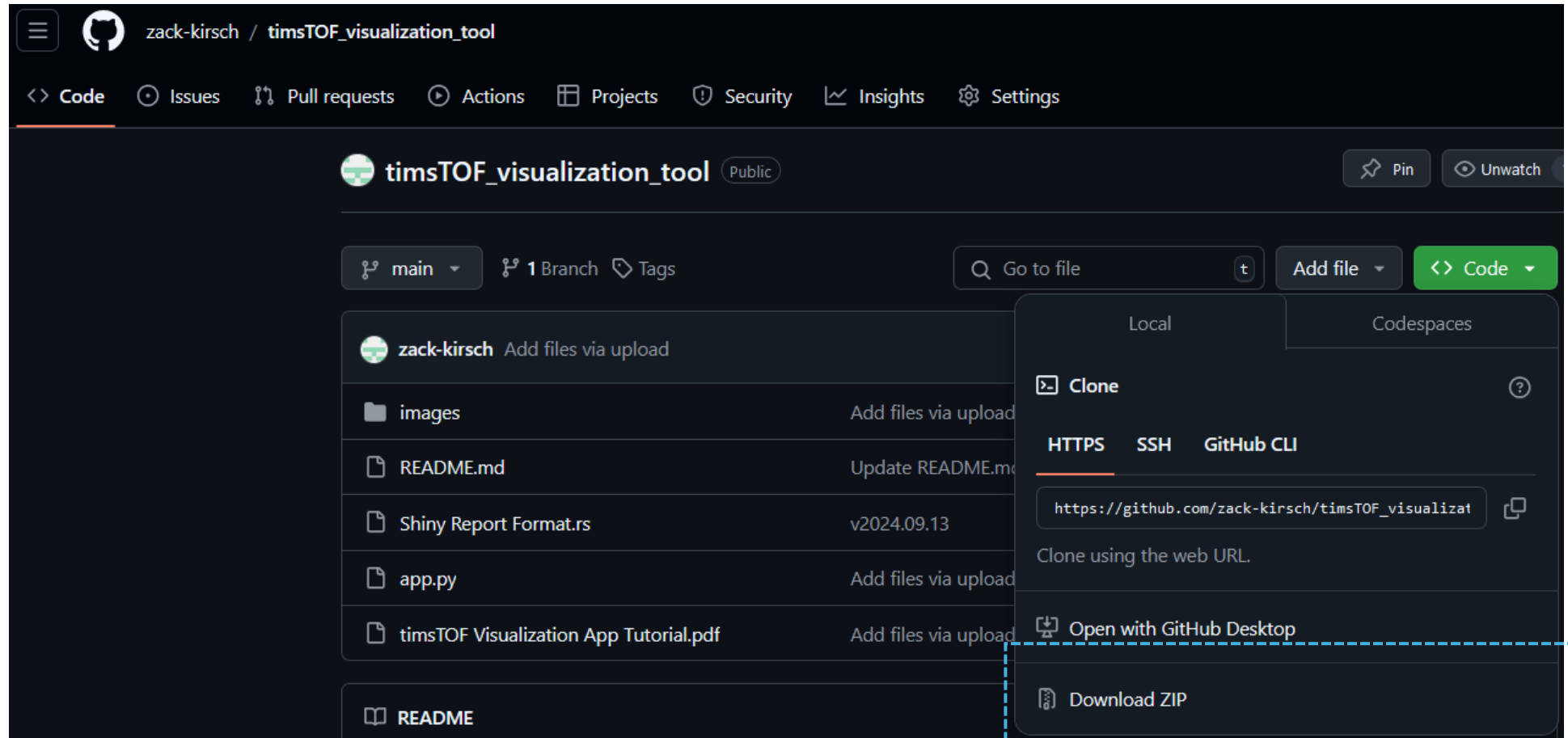
# timstTOF Visualization App Tutorial

# Software Installation

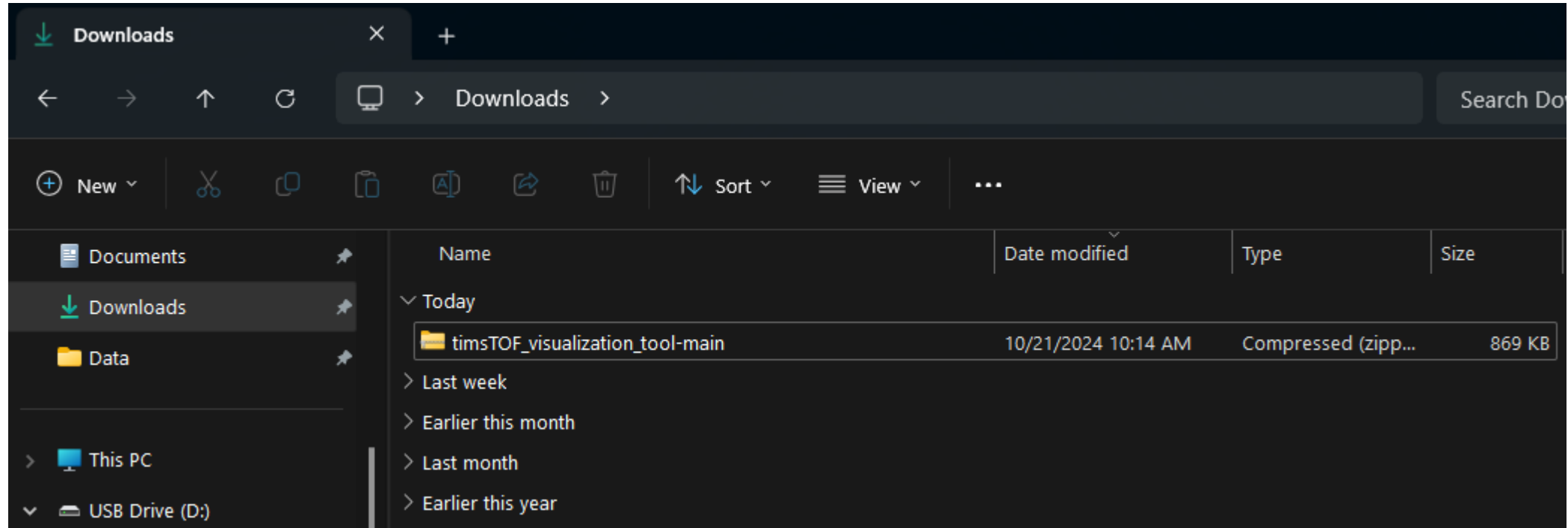
# Necessary Software

- Install Python or Anaconda
  - <https://www.python.org/downloads/>
  - <https://www.anaconda.com/download>
- Install Visual Studio Code
  - <https://code.visualstudio.com/>

# Download folder as a .zip from GitHub

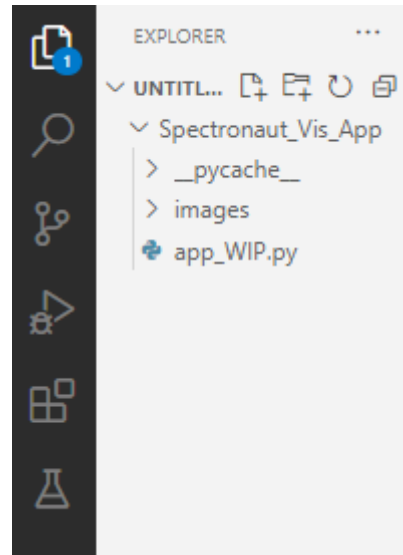
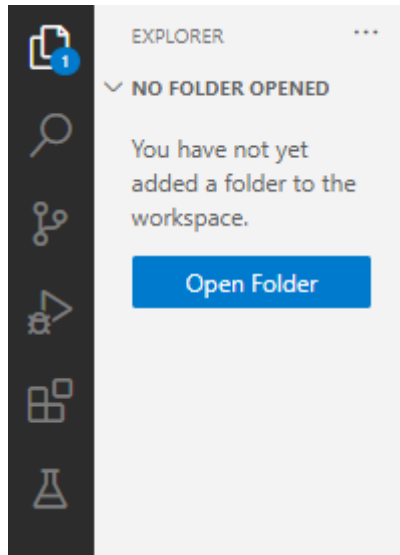


# Extract .zip file

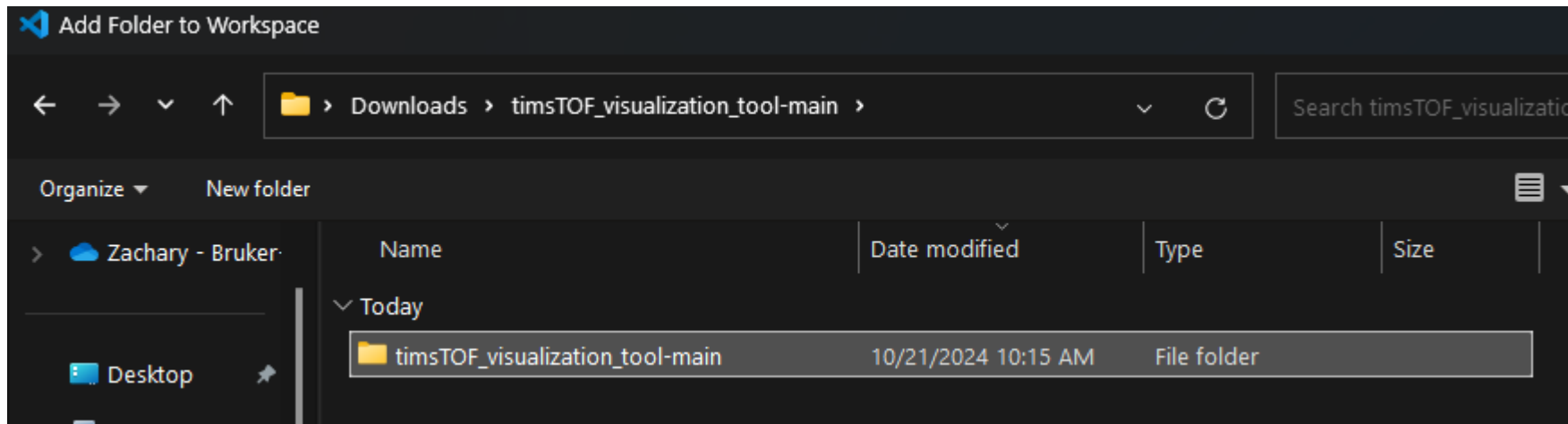


# Getting Started

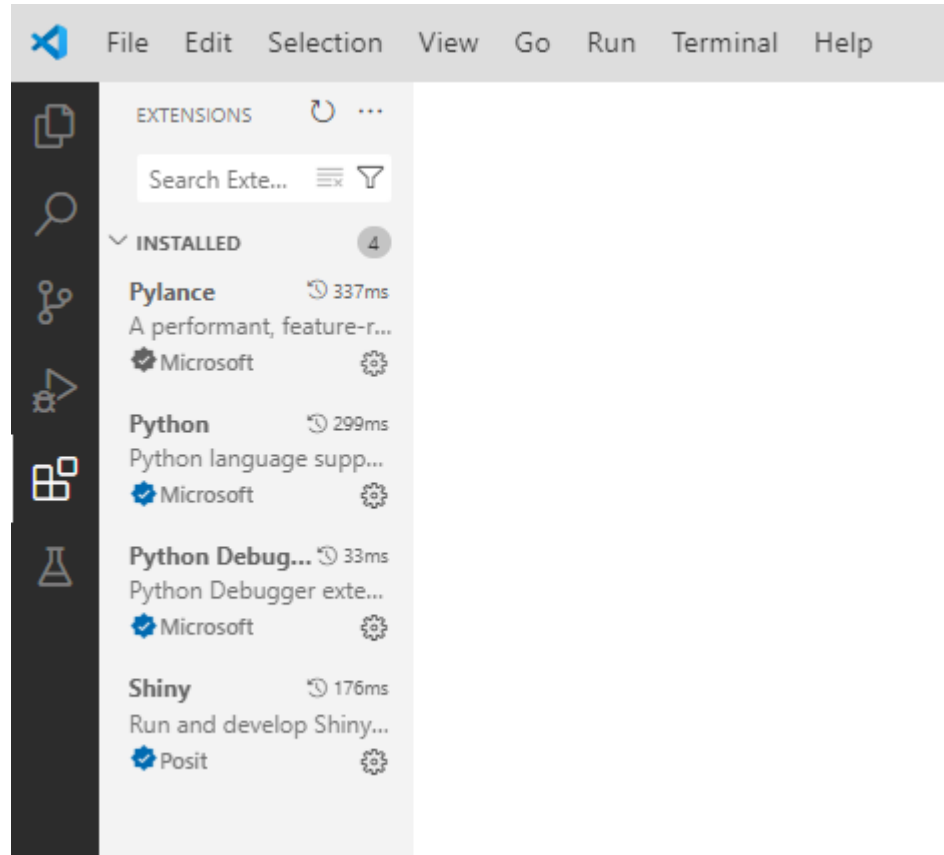
# Getting started in Visual Studio Code



- 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

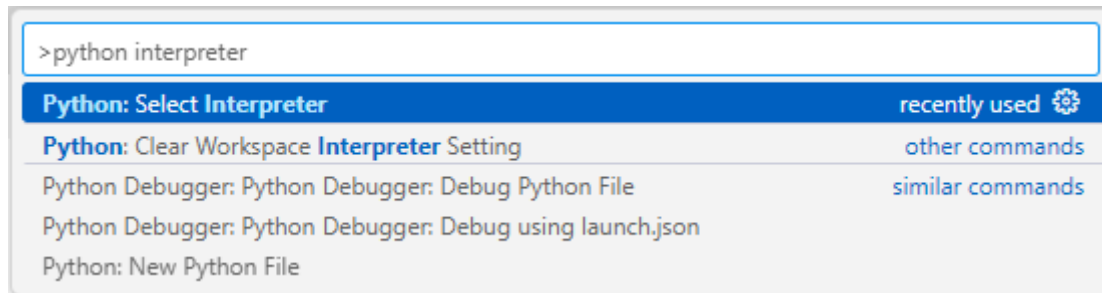


- Install Python and Shiny extensions using the Extensions:Marketplace tab

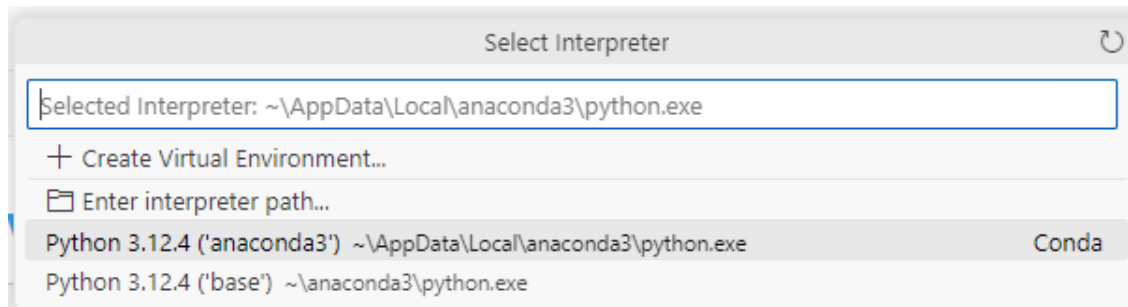


# Set Python interpreter to current installation of Python/Anaconda

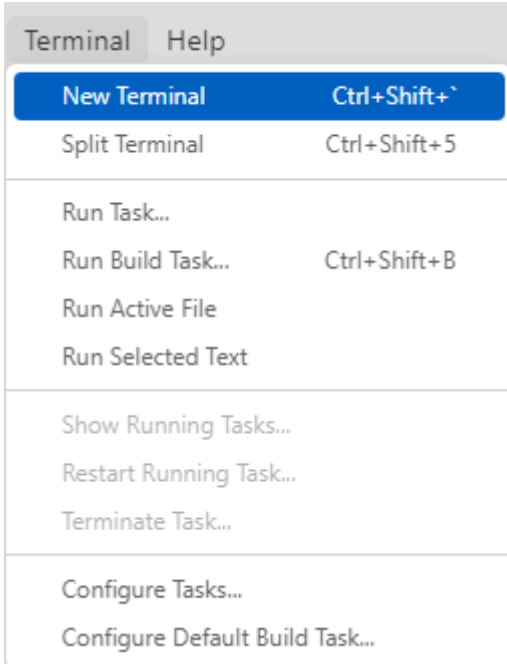
- Ctrl+Shift+P and search Python interpreter



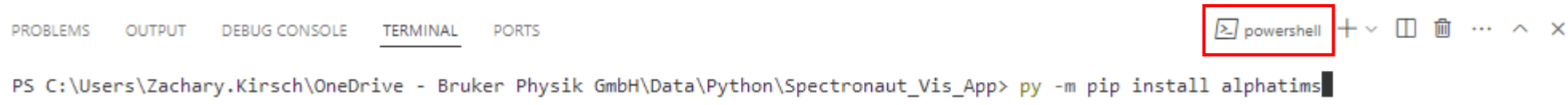
- Select current Python installation



# Installing necessary Python libraries



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



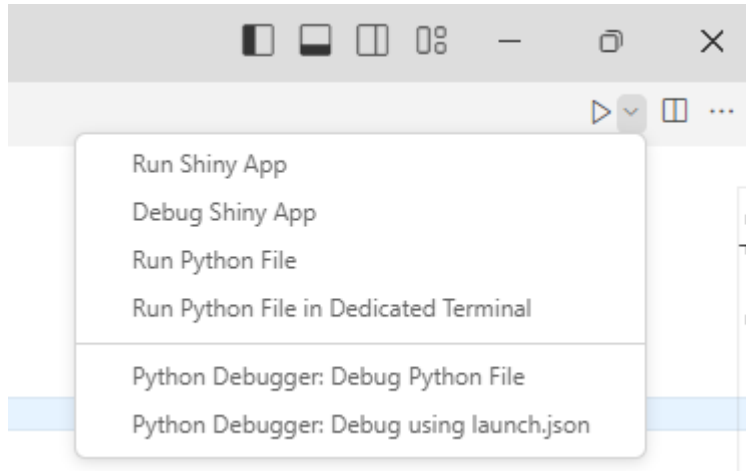
- Replace the part underlined in red with the library to install

# Installing necessary Python libraries

```
# =====  
# 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 alphasims.braker as atb  
import alphasims.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

## timsTOF Proteomics Data Visualization

File Import

Settings

ID Counts

Metrics

PTMs

PCA

Heatmaps

Mixed Proteome

PRM

Dilution Series

Raw Data

Export Tables

Upload Search Report

When uploading a new file after one has already been uploaded, click the apply changes button below

Upload search report:

Browse...

No file selected

Search software:

☒ Spectronaut

☐ DIA-NN

☐ FragPipe

☐ tims-DIANN (BPS)

☐ Spectronaut Library

Spectronaut: Make sure to use Shiny report format when exporting search results

Update from Metadata Table

☐ Update 'R.Condition' and 'R.Replicate' columns

☐ Reorder runs

☐ Remove selected runs

☐ Update 'Concentration' column

Apply changes to search report /  
reinitialize search report

Upload search report (.tsv)

Select search software

# Updating result metadata

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

☐ Reorder runs

☐ Remove selected runs

☐ Update 'Concentration' column

Apply changes to search report /  
reinitialize search report

Metadata Tables

-Double click on any cell to update its contents

-To remove runs, add an 'x' to the 'remove' column

R.FileName	R.Condition	R.Replicate	remove
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.Condition

1-1-3\_10ng

1-2-2\_10ng

order

Concentration

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

**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.

**Concentration:** (optional)  
Sets the value for each condition.

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

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

# General Navigation

## timsTOF Proteomics Data Visualization

Main navigation pane organized by the types of information to be plotted

- File Import
- Settings
- ID Counts**
- Metrics
- PTMs
- PCA
- Heatmaps
- Mixed Proteome
- PRM
- Dilution Series
- Raw Data
- Export Tables

Counts per Condition   Average Counts   CV Plots   IDs with CV Cutoff   UpSet Plot

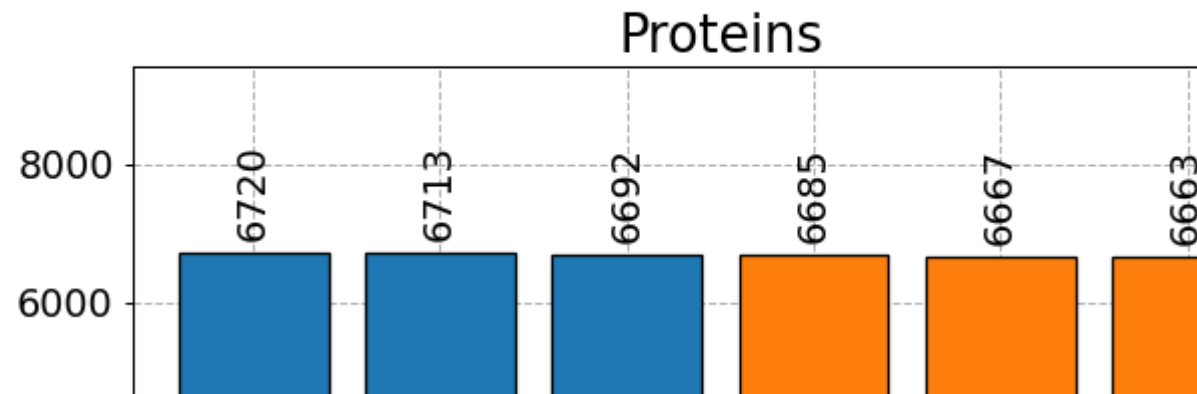
Plot width: 500 1,500 2,000

Plot height: 500 1,000 2,000

Choose what metric to plot:

all

Sub-menu navigation organized by the different available plots that can be generated





# Coloring Options

File Import

Settings

ID Counts

Metrics

PTMs

PCA

Heatmaps

Mixed Proteome

PRM

Dilution Series

Raw Data

Export Tables

Color Settings

Column Check

Control Panel

Choose coloring option for output plots:  
☐ Pick for me (rainbow)  
☒ Pick for me (matplotlib tableau)  
☐ Custom

Input color names from the tables to the right, one per line:

Note: replicates of the same condition will have the same color

Run

Color per run

Matplotlib Tableau Colors:

tab:blue

tab:orange

tab:green

tab:red

tab:purple

tab:brown

tab:pink

tab:gray

tab:olive

tab:cyan

CSS Colors:

black  
dimgray  
gray  
darkgray  
silver  
lightgray  
lightgrey  
gainsboro  
whitesmoke  
white  
snow  
rosybrown  
lightcoral  
indianred  
brown  
firebrick  
maroon  
darkred  
red  
mistyrose  
salmon  
tomato  
darksalmon  
coral  
orangered  
lightsalmon  
sienna  
seashell  
chocolate  
saddlebrown  
sandybrown  
peachpuff  
peru  
linen

bisque  
darkorange  
burlywood  
antiquewhite  
tan  
navajowhite  
blanchedalmond  
papayawhip  
moccasin  
orange  
wheat  
oldlace  
floralwhite  
darkgoldenrod  
goldenrod  
cornsilk  
gold  
lemonchiffon  
khaki  
palegoldenrod  
darkkhaki  
ivory  
beige  
lightyellow  
lightgoldenrodyellow  
olive  
yellow  
olivedrab  
yellowgreen  
darkolivegreen  
greenyellow  
chartreuse  
lawngreen  
honeydew  
darkseagreen  
palegreen  
lightgreen

forestgreen  
limegreen  
darkgreen  
green  
lime  
seagreen  
mediumseagreen  
springgreen  
mintcream  
mediumspringgreen  
mediumaquamarine  
aquamarine  
turquoise  
lightseagreen  
mediumturquoise  
azure  
lightcyan  
paleturquoise  
darkslategray  
darkslategrey  
teal  
darkcyan  
aqua  
cyan  
darkturquoise  
cadetblue  
powderblue  
lightblue  
deepskyblue  
skyblue  
lightskyblue  
steelblue  
aliceblue  
dodgerblue  
lightslategray  
lightslategrey  
slategray

slategrey  
lightsteelblue  
cornflowerblue  
royalblue  
ghostwhite  
lavender  
midnightblue  
navy  
darkblue  
mediumblue  
blue  
slateblue  
darkslateblue  
mediumslateblue  
mediumpurple  
rebeccapurple  
blueviolet  
indigo  
darkorchid  
darkviolet  
mediumorchid  
thistle  
plum  
violet  
purple  
darkmagenta  
fuchsia  
magenta  
orchid  
mediumvioletred  
deeppink  
hotpink  
lavenderblush  
palevioletred  
crimson  
pink  
lightpink

- 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



Color Settings Column Check Control Panel

Choose coloring option for output plots:

- ☐ Pick for me (rainbow)  
☒ Pick for me (matplotlib tableau)  
☐ Custom

Input color names from the tables to the right, one per line:

Note: replicates of the same condition will have the same color

Run	Color per run
1-1-3_10ng	
1-2-2_10ng	
	

Colors by condition according to the matplotlib tableau colors


Color Settings Column Check Control Panel

Choose coloring option for output plots:

- ☒ Pick for me (rainbow)  
☐ Pick for me (matplotlib tableau)  
☐ Custom

Input color names from the tables to the right, one per line:

Note: replicates of the same condition will have the same color

Run	Color per run
1-1-3_10ng	
1-2-2_10ng	
	

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

Color Settings Column Check Control Panel

Choose coloring option for output plots:

- ☐ Pick for me (rainbow)  
☐ Pick for me (matplotlib tableau)  
☒ Custom

Input color names from the tables to the right, one per line:

Note: replicates of the same condition will have the same color

Run	Color per run
1-1-3_10ng	
1-2-2_10ng	
	

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

• CSV Format:

Upload DIA windows as a .csv:

Browse...

No file selected

Choose DIA windows to overlay:

☐ Imported DIA windows


☐ Lubeck DIA


☐ Phospho DIA


☒ None


	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

- File Import
- Settings
- ID Counts
- Metrics
- PTMs
- PCA
- Heatmaps
- Mixed Proteome
- PRM
- Dilution Series
- Raw Data
- Export Tables

Multi-File Import

TIC Plot

BPC Plot

Accumulation Time

EIC Plot

EIM Plot

Paste the path for each .d file you want to upload (note: do not leave whitespace at the end):

ex - C:\Users\Data\K562\_500ng\_1\_Slot1-49\_1\_3838.d

OneDrive > ... > Data > 2024\_June-July\_ICCs > Mixtures >

Name	Status
20240612_1-1-3_K562-Yeast_EColi_500ng_1_Slot1-49_1_3838.d	✓
20240612_1-1-3_K562-Yeast_EColi_500ng_2_Slot1-49_1_3839.d	✓
20240612_1-1-3_K562-Yeast_EColi_500ng_3_Slot1-49_1_3840.d	✓
20240612_1-2-2_K562-Yeast_EColi_500ng_1_Slot1-47_1_3833.d	✓
20240612_1-2-2_K562-Yeast_EColi_500ng_2_Slot1-47_1_3834.d	✓
20240612_1-2-2_K562-Yeast_EColi_500ng_3_Slot1-47_1_3835.d	✓

- 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