

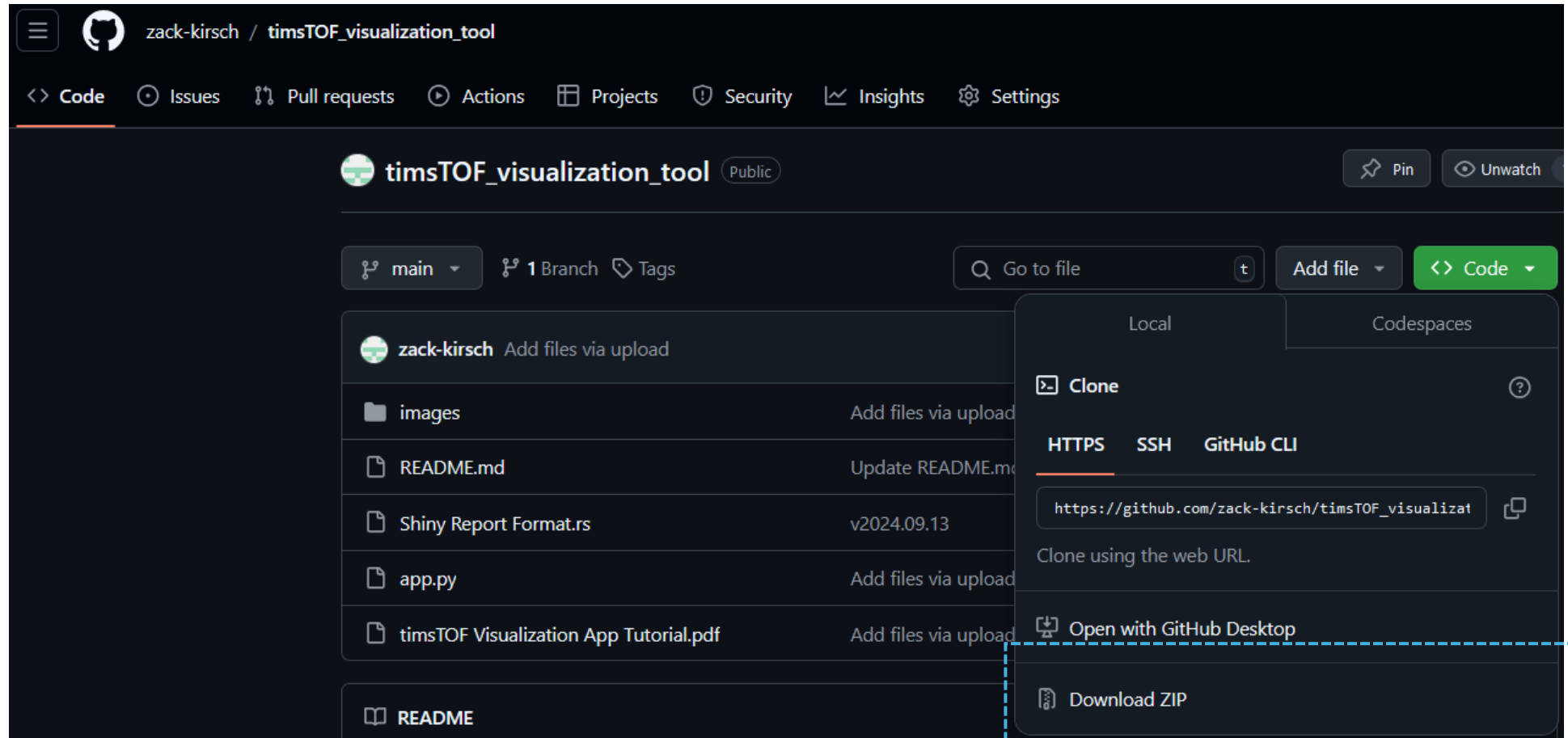
timeTOF Visualization App Tutorial

Software Installation

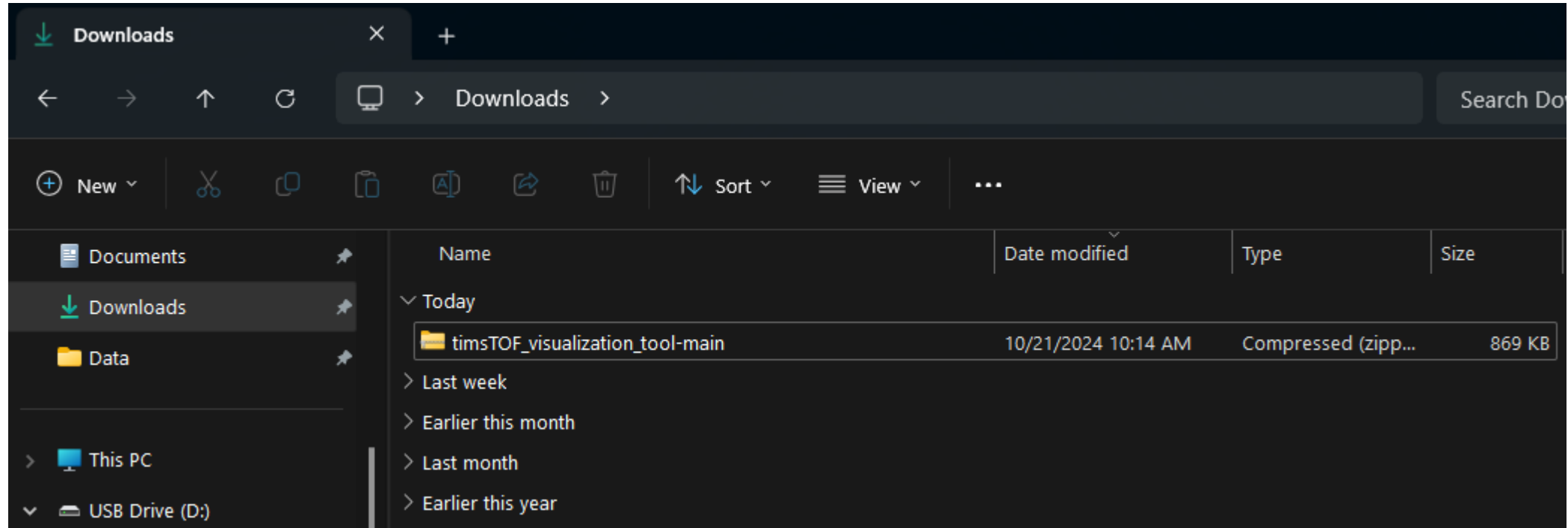
Necessary Software

- Install Python or Anaconda
 - <https://www.python.org/downloads/release/python-3128/>
 - *NOTE* Alphasims is only supported on Python versions prior to 3.13
- Install Visual Studio Code
 - <https://code.visualstudio.com/>

Download folder as a .zip from GitHub

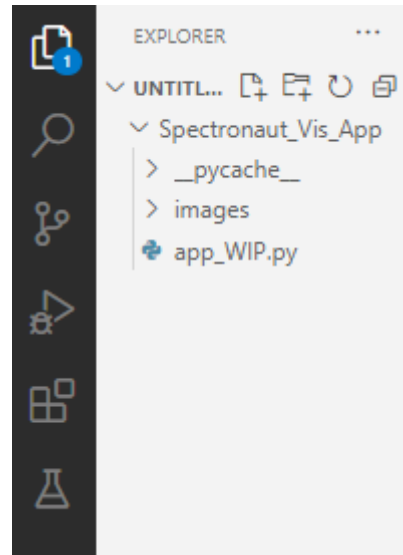
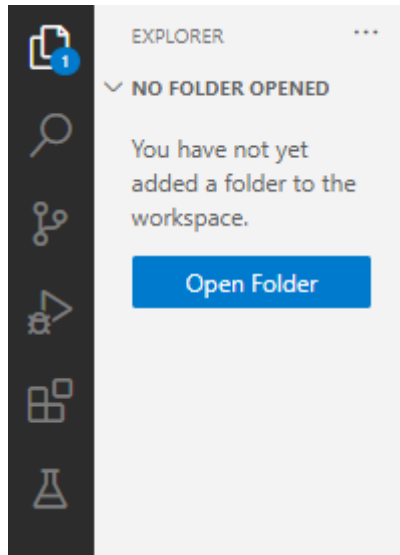


Extract .zip file

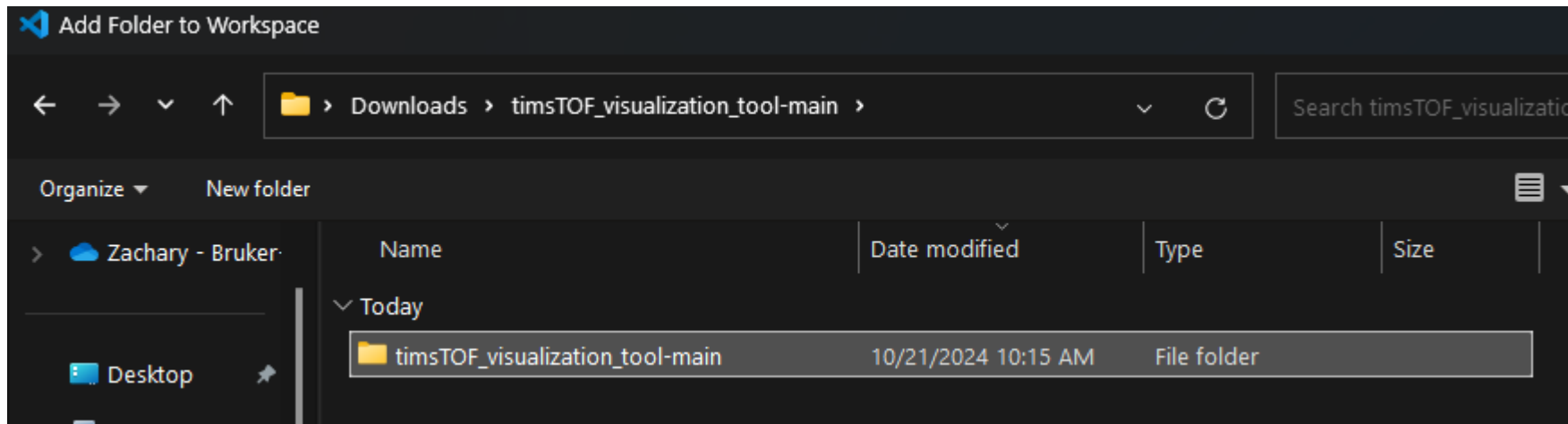


Getting Started

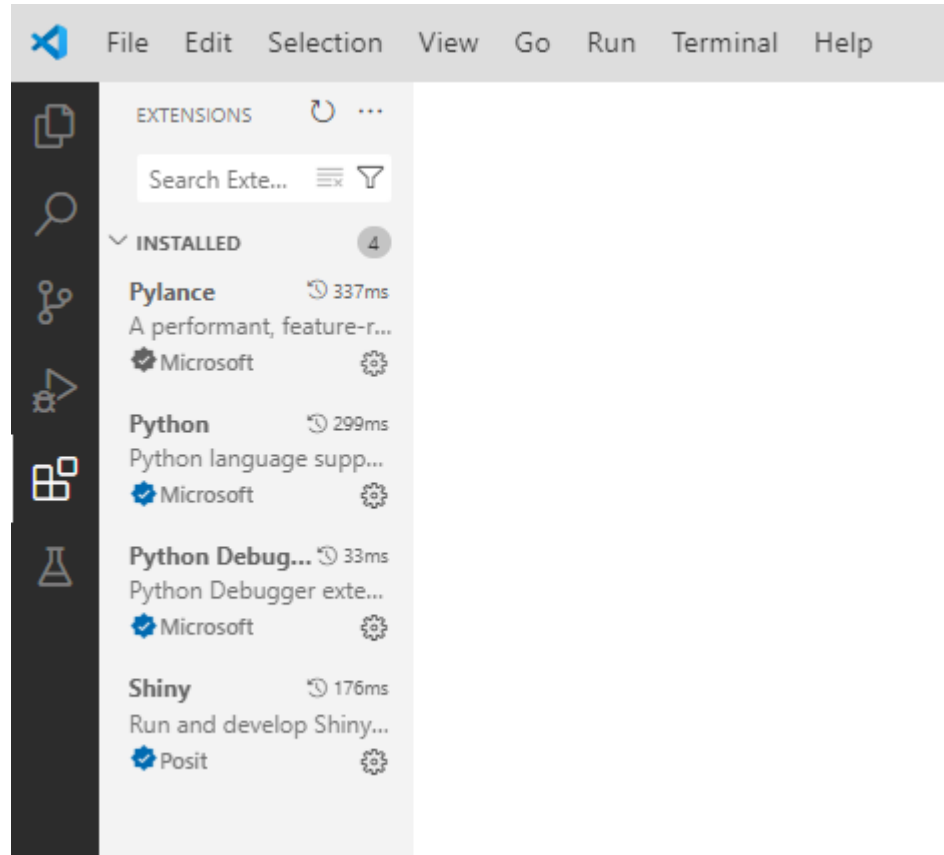
Getting started in Visual Studio Code



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



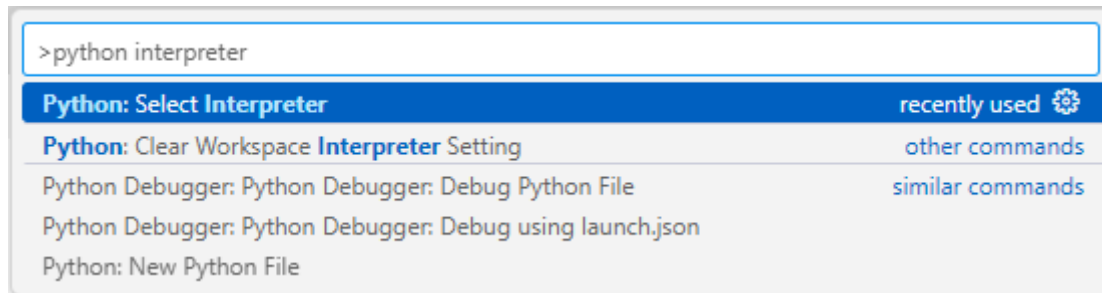
Install necessary extensions



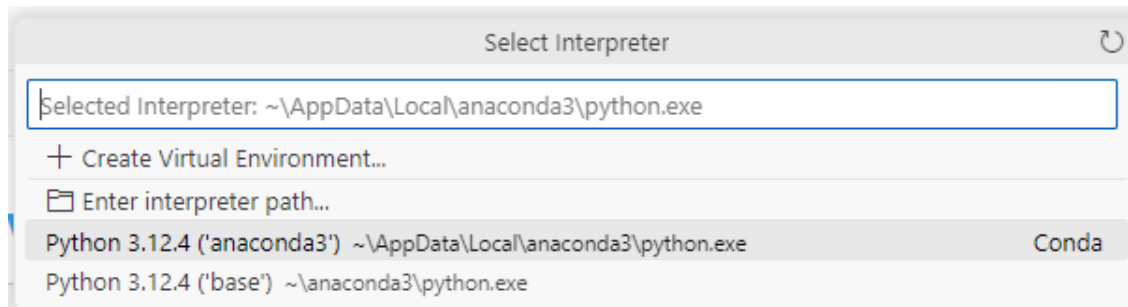
- Install Python and Shiny extensions using the Extensions:Marketplace tab

Set Python interpreter to current installation of Python/Anaconda

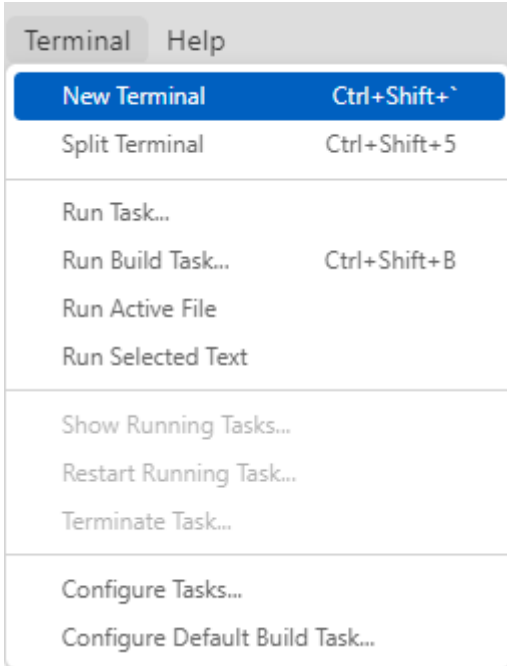
- Ctrl+Shift+P and search Python interpreter



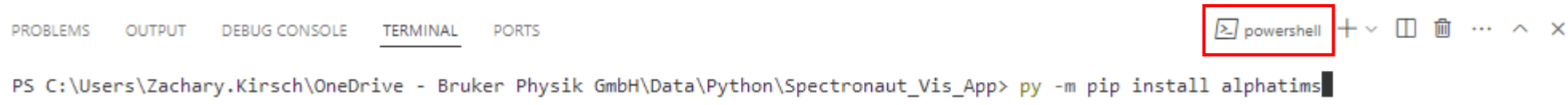
- Select current Python installation



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

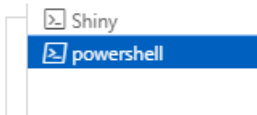
This path will just be the file path to the app folder

Installing necessary Python libraries (Alternative)

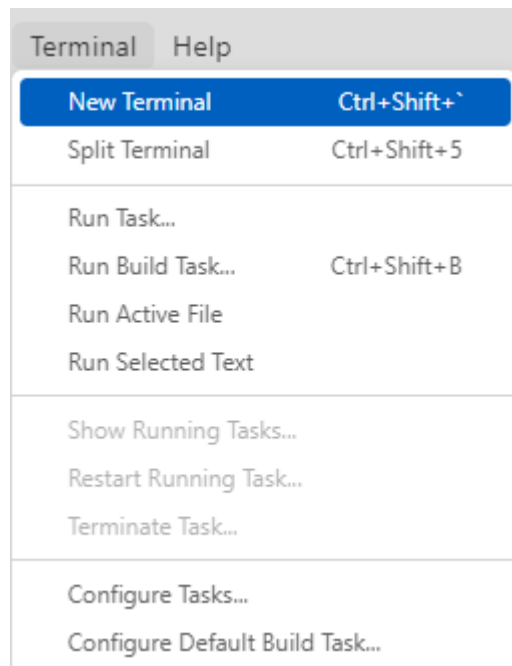
- Open a new powershell terminal and use pip to install

PROBLEMS OUTPUT DEBUG CONSOLE TERMINAL PORTS

PS C:\Users\Zachary.Kirsch\OneDrive - Bruker Physik GmbH\Data\Python\Spectronaut_Vis_App> py -m pip install -r requirements.txt



- Make sure the requirements.txt file is located in the same folder as the app.py file
 - The path shown in the figure here (underlined in blue) will vary to wherever you moved the downloaded folder from GitHub

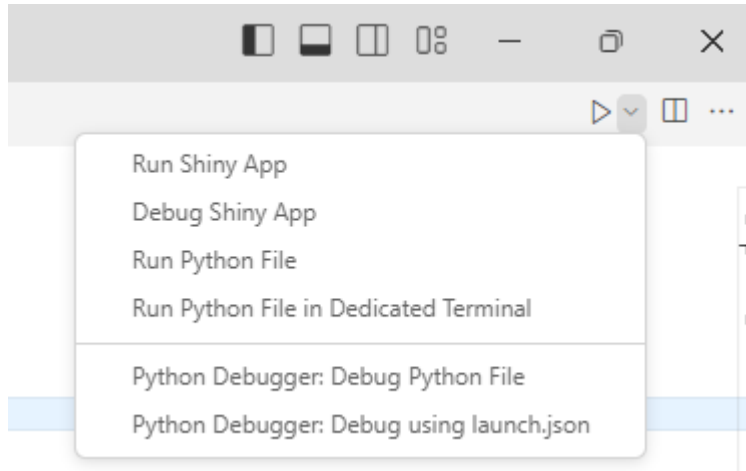


Installing necessary Python libraries

```
# =====  
# 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

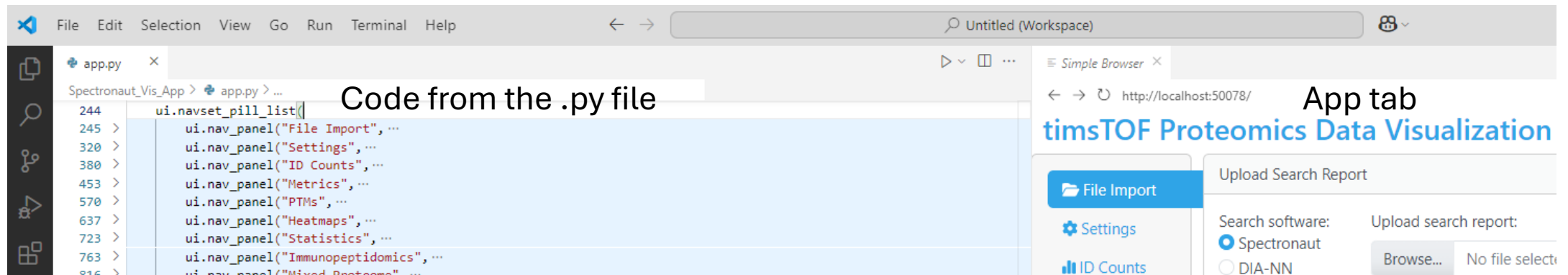
Starting the Shiny app



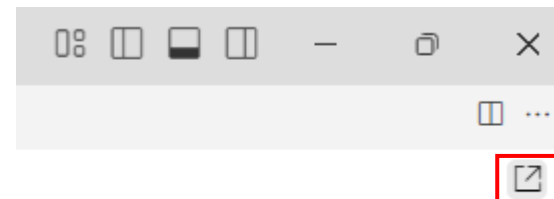
- With the extensions and libraries installed, you should be able to run the app with Visual Studio Code recognizing it as a Shiny app

Starting the Shiny app

- The app interface will launch in a tab within VSCode, which can be maximized to fill the screen by dragging the tab out of VSCode



- It can also be opened in a browser window by clicking this icon in the top right of the app window



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

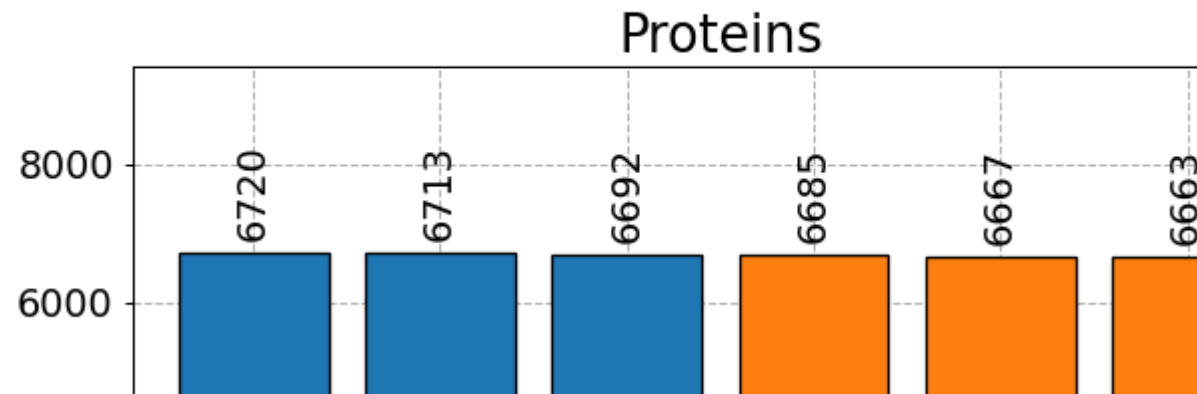
Plot width: 500 1,500 2,000

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	
	

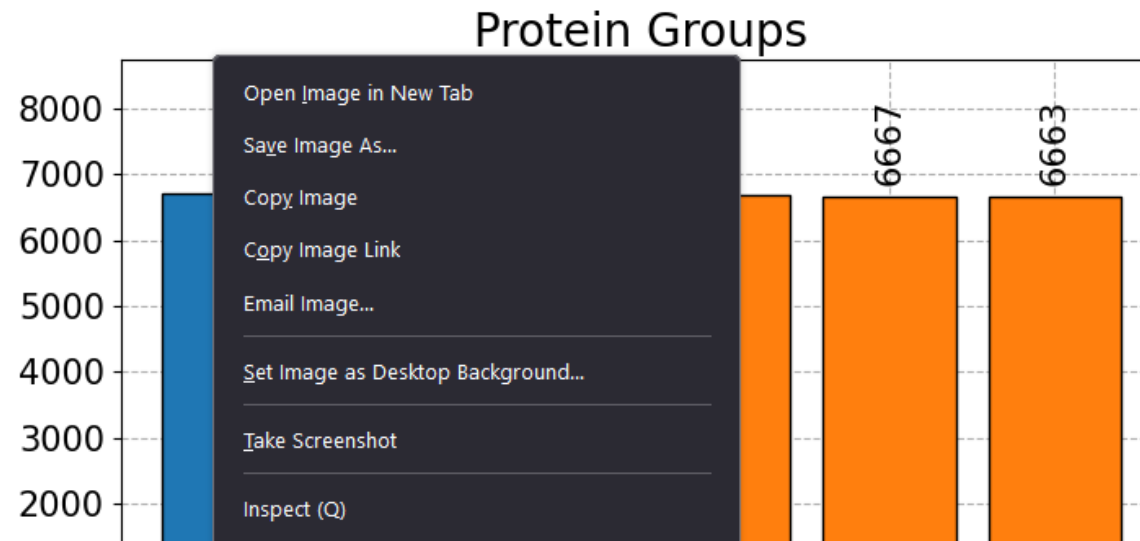
Colors by condition according to specified colors in the text area

Saving rendered plots

- Saving images is easiest when the app is being run in an external browser
- Once a plot is generated and the desired dimensions are set, you can right-click on the plot and save it to a .png file

Choose what metric to plot:

All



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 (individual files)

The screenshot displays a software interface for importing raw data files. On the left is a sidebar with navigation options: File Import, Settings, ID Counts, Metrics, PTMs, Heatmaps, Statistics, Immunopectidomics, Mixed Proteome, PRM, Dilution Series, Glycoproteomics, Raw Data (highlighted), and Export Tables. The top bar contains tabs: Multi-File Import (active), TIC Plot, BPC Plot, Accumulation Time, EIC Plot, and EIM Plot. The main area under 'Multi-File Import' shows 'Load raw data from:' with 'Individual Files' selected and 'Directory' unselected. Below this is a text input field with the instruction: 'Paste the path for each .d file you want to upload (note: do not leave whitespace at the end):' and an example path: 'ex - C:\Users\Data\K562_500ng_1_Slot1-49_1_3838.d'. An overlaid file explorer window shows the path 'OneDrive > ... Data > 2024_June-July_ICCs > Mixtures >' highlighted in red. Below this, a table lists files with their names and status (green checkmarks).

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

Referencing raw data files (directory)

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

Multi-File Import

TIC Plot

BPC Plot

Accumulation Time

EIC Plot

EIM Plot

Load raw data from:

☐ Individual Files

☒ Directory

Paste the path for the directory containing the raw files to upload (note: do not leave whitespace at the end):

ex - C:\Users\Data

- Paste the path to the directory containing raw data files
- The app will search for any .d files in the directory and import them