**AlphaTims tutorial**

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This step-by-step guide helps you to get started with our software AlphaTims.

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# Program description



AlphaTims is an open-source Python package for fast accessing of unprocessed Bruker trapped ion mobility spectrometry - time of flight (TIMS-TOF) data. It provides a very efficient indexed data structure that allows to access the five-dimensional TIMS-TOF data in the standard numerical Python (NumPy) manner. AlphaTims is a key enabling tool to deal with the large and high-dimensional TIMS-TOF data.

# Installation



Three types of installation are possible:

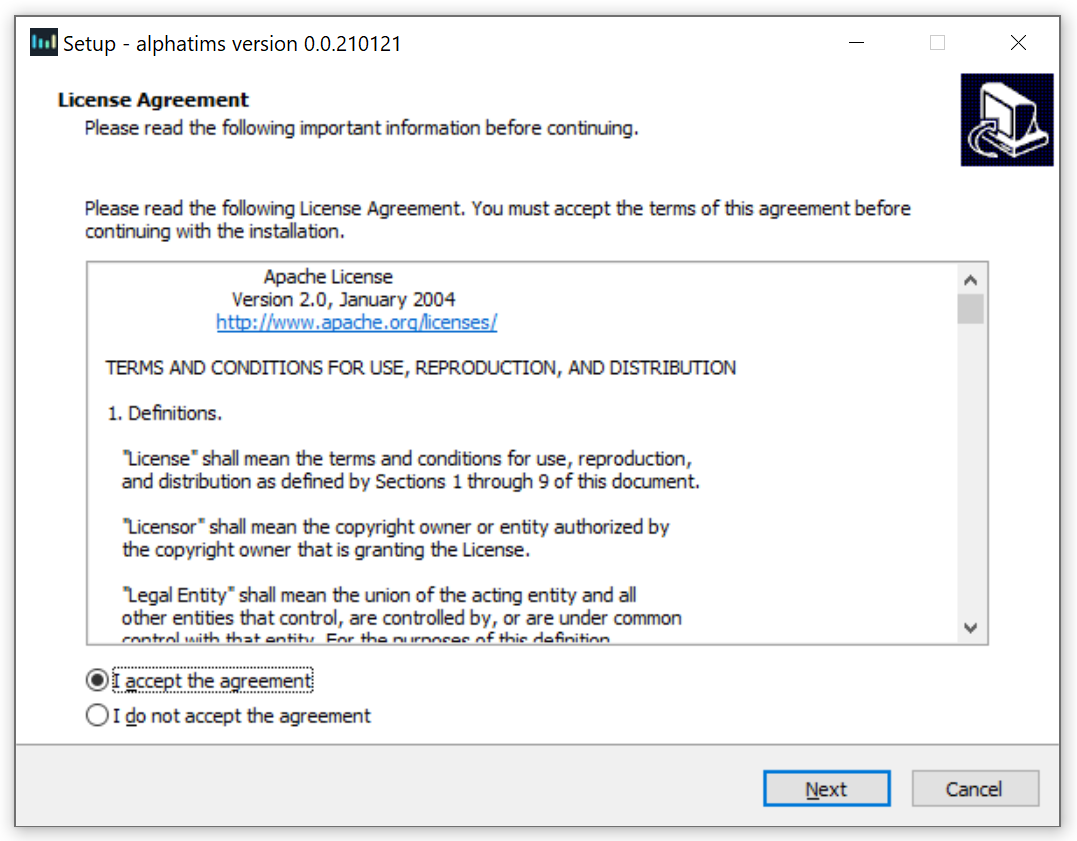
* **[One-click GUI installer:](https://github.com/MannLabs/alphatims" \l "one-click-gui)** Choose this installation if you only want the GUI and/or keep things as simple as possible.
* **[Pip installer:](https://github.com/MannLabs/alphatims" \l "pip)** Choose this installation if you only want to use AlphaTims as a Python module in an already existing Python 3.8 environment such as a Jupyter notebook.
* **[Full installer:](https://github.com/MannLabs/alphatims" \l "full)** Choose this installation if you are familiar with CLI tools, [conda](https://docs.conda.io/en/latest/) and Python. This installation allows access to all available features and modifiable AlphaTims source code. Specific extensions (GUI, CLI and notebooks) can be included in this installation as well that generally outperform the precompiled versions.

## Windows

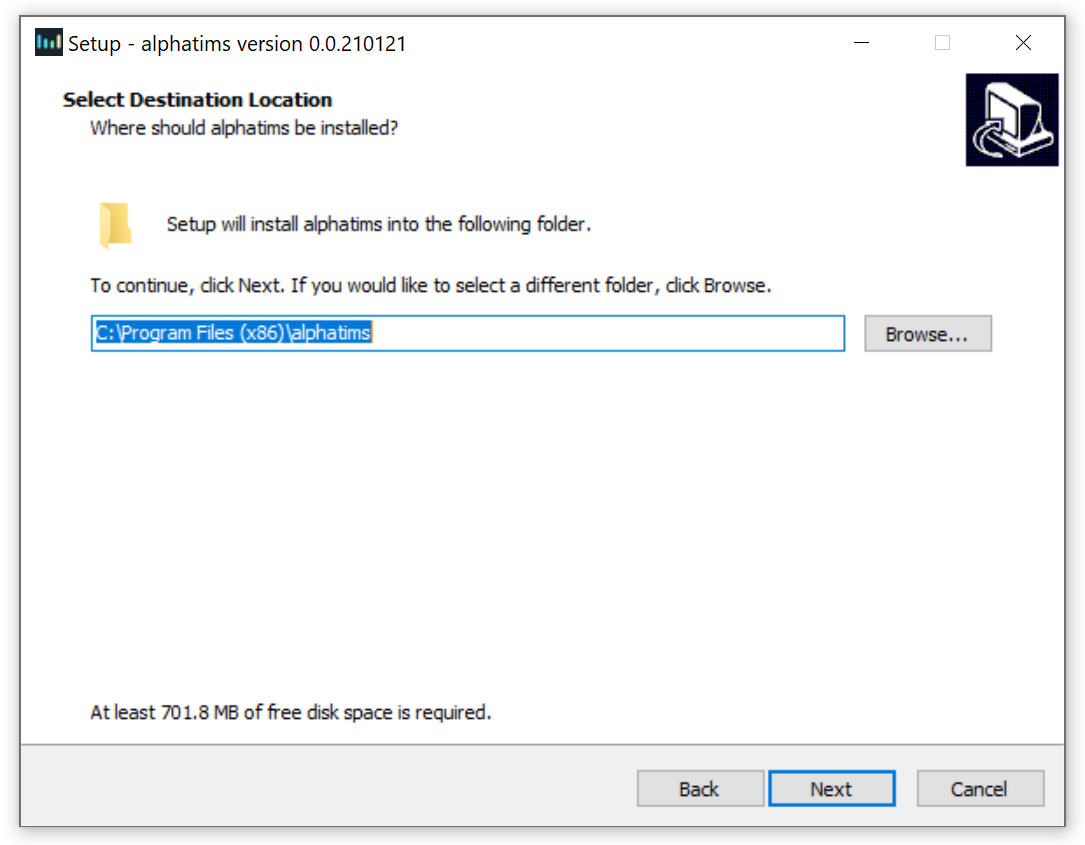
\*

If you install AlphaTims for all users, you might need admin privileges to run it (right-click on the AlphaTims logo on your desktop and select "Run as administrator").

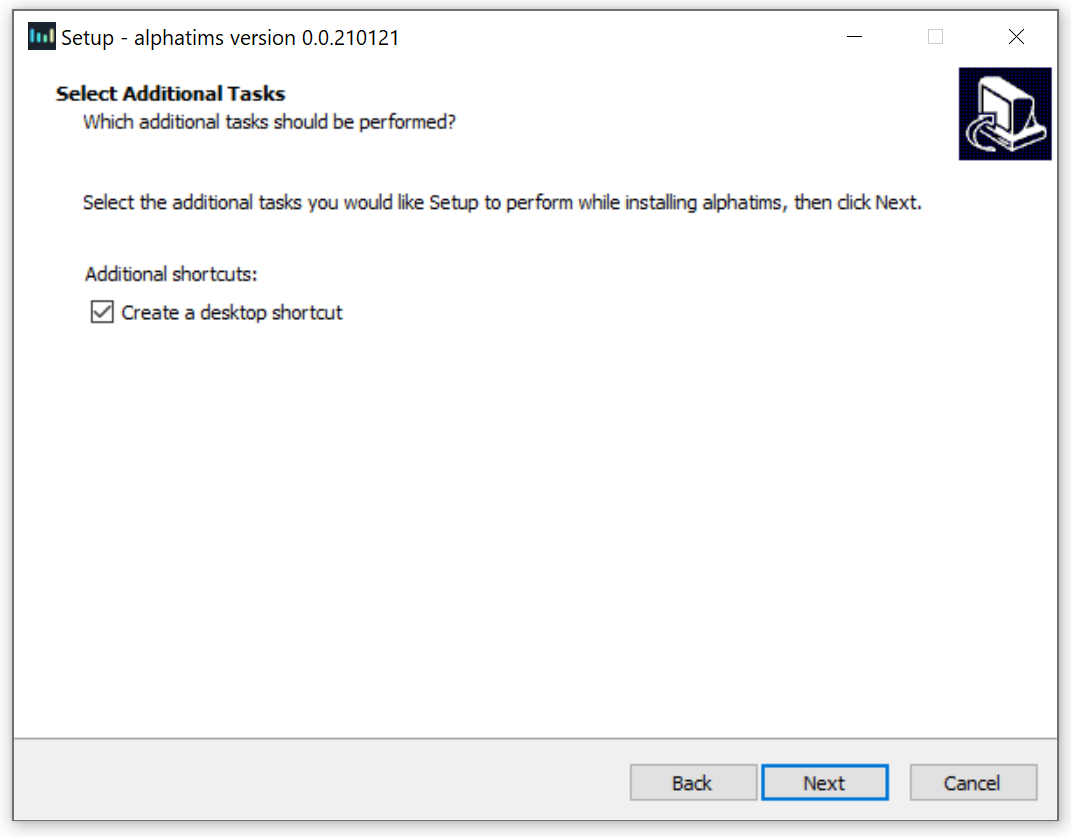
1. Download [the latest release](https://github.com/MannLabs/alphatims/releases) for Windows (alphatims\_installer\_windows.exe) from the GitHub repository and open the .exe file.
2. In the “User Account Control” dialog asking about permission for the app to make changes to your device press the “Yes” button.
3. In the appearing “Setup – AlphaTims version X.X.X” dialog window, accept the License Agreement and press the “Next” button.

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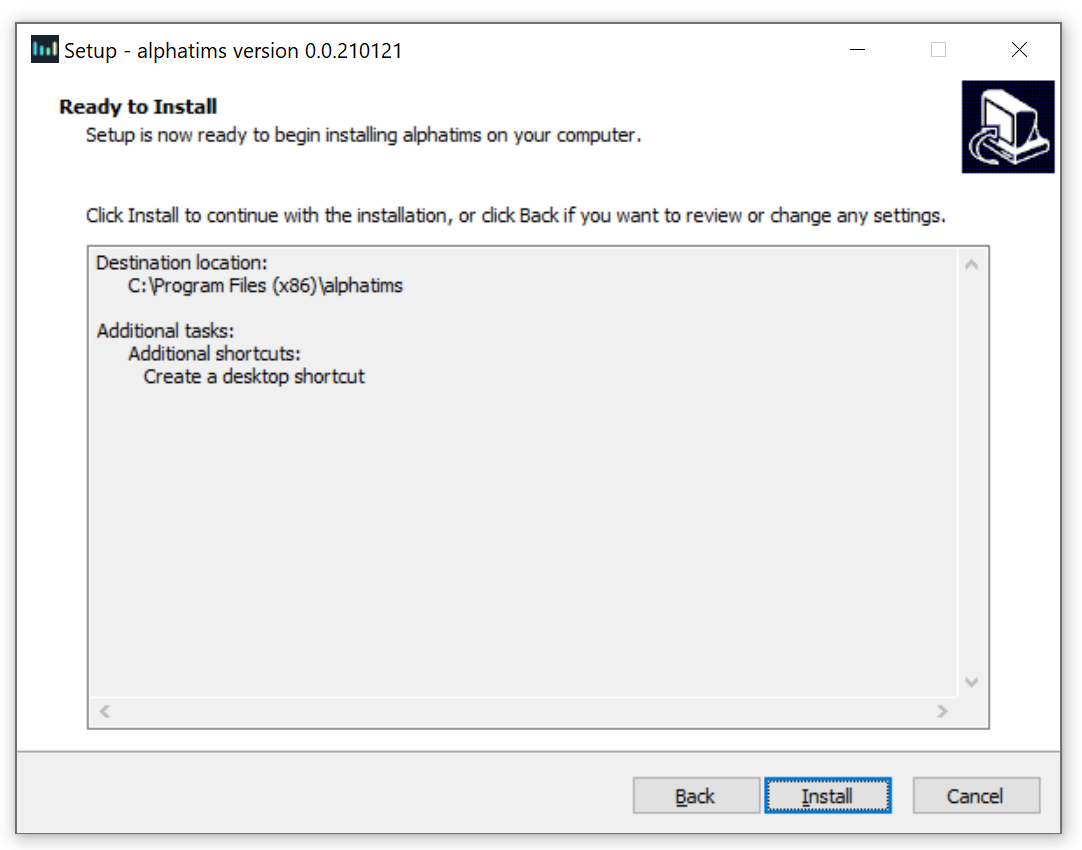
1. Select the destination location for the installation of AlphaTims software and press the “Next” button.

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1. (optional) In the next dialog window, mark the “Create a desktop shortcut” check box and press the “Next” button.

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1. Check the setting and if everything is correct, press “Install” button. You may go back to change some settings using the “Back” button or “Cancel” the installation.

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1. Wait until the installation process is finished. It is advised to directly test if the installation was successful by marking the “Launch AlphaTims” check box before pressing the “Finish” button.
2. In the appearing “Windows Security Alert” dialog window press the “Allow access” button that will prevent the Windows Defender Firewall from blocking the AlphaTims tool on your PC.
3. A new tab should have been opened in your default browser (Google Chrome or Mozilla Firefox are suggested for the fast running of the AlphaTims). You can now use AlphaTims.

## MacOS

1. Download [the latest release](https://github.com/MannLabs/alphatims/releases) for macOS (alphatims.app.zip) from the GitHub repository, unzip the file and move it to your applications folder. By doing so, you accept the terms of the AlphaTims license agreement and all third-party licenses.
2. Launch the file and take into account that the first opening of the tool on macOS takes a long time to load. The loading time will be significantly reduced upon the second launch.
3. A new tab should have been opened in your default browser (Google Chrome or Mozilla Firefox are suggested for the fast running of the AlphaTims). You can now use AlphaTims.

\* If you get a pop-up message “Do you want the application “python3.8” to accept incoming network connections?”, just click “Yes” button.

\* If nothing happens when you launch AlphaTims, you might need to grant it permissions by going to the macOS menu "System Preferences | Security & Privacy | General". If the problem still persists, it is possible that macOS already quarantined the AlphaTims app. It can be removed from quarantine by running <*xattr -dr com.apple.quarantine AlphaTims.app*> (copy everything between <>) in your terminal in the application folder where AlphaTims.app is located.

## Linux

1. Download [the latest release](https://github.com/MannLabs/alphatims/releases) for Linux (alphatims) from the GitHub repository. By doing it, you accept the terms of the AlphaTims license agreement and all third-party licenses.
2. To launch the file drag-and-drop it in the terminal and press enter to run AlphaTims.
3. A new tab should have been opened in your default browser (Google Chrome or Mozilla Firefox are suggested for the fast running of the AlphaTims). You can now use AlphaTims.

\* If nothing happens when you launch AlphaTims, you might need to grant it permissions by running <*chmod +x alphatims*> (copy everything between <>) in your terminal in the application folder where AlphaTims is located.

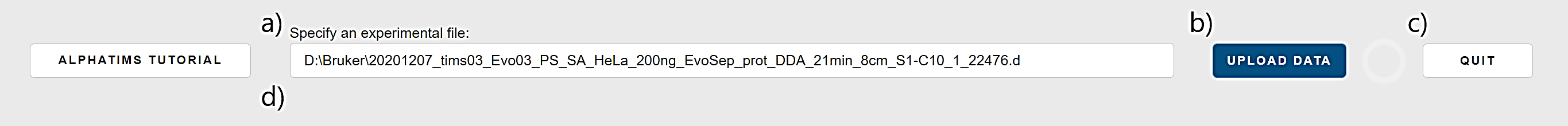
# How to use AlphaTims



1. Upload your dataset:
   1. Provide the filepath to the original raw Bruker .d folder, or a saved .hdf file, in the “Specify an experimental file:” field, e.g. “D:\Bruker\20201207\_tims03\_Evo03\_PS\_SA\_HeLa\_200ng\_EvoSep\_prot\_DDA\_21min\_8cm\_S1-C10\_1\_22476.d”.
   2. Press the "Upload Data" button. The loading process is indicated by a spinner symbol. To evaluate the required time to preload the file into memory take into account [the typical performance statistics](https://github.com/MannLabs/alphatims#performance) for the reading of raw/HDF data.
   3. Please, press the “Quit” button when your working session in AlphaTims is finished.

**IMPORTANT WARNING**! If you just close the browser tab and do not press the "Quit" button, AlphaTims will keep running in the background (possibly using a huge amount of RAM memory). This is especially important for **macOS** because of the non-showing terminal.

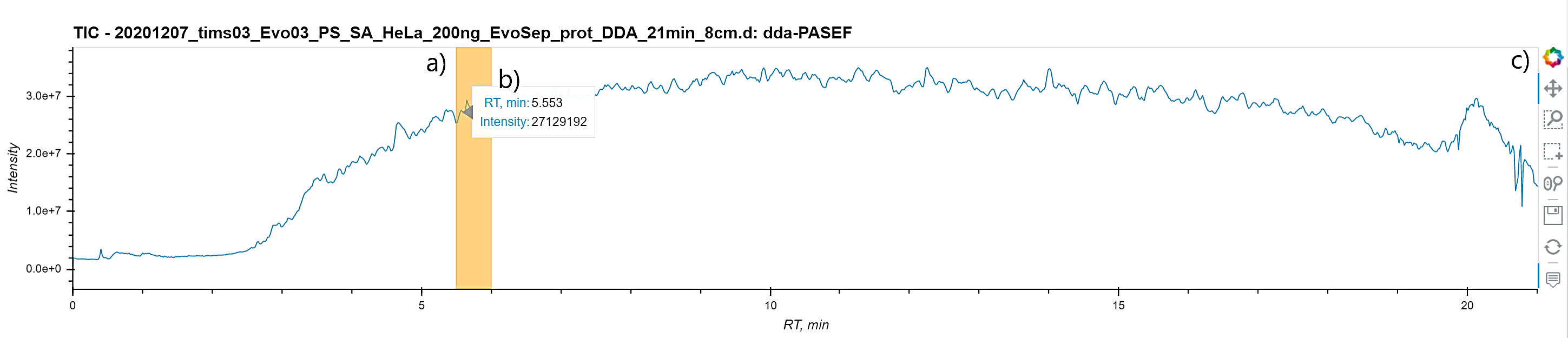
* 1. If the specified file won’t have a .d/.hdf extension or it will be impossible to upload it for exploration because of any other reasons, you will get an error message below the “Specify an experimental file:” field with the reasons what is wrong.



\* See [the example files](https://github.com/MannLabs/alphatims#test-data) for testing of the GUI in the GitHub repo.

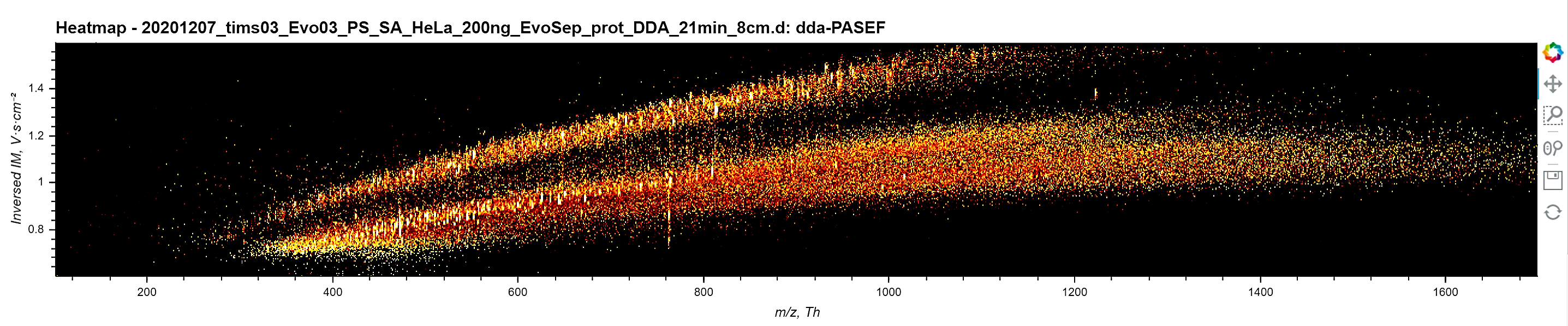
1. On the right you may see three different plots for the data exploration:

* **TIC** - a line plot that visualize the total ion chromatogram for the whole run.
  1. The sliced and currently visualized on other plots RT range is highlighted in orange.
  2. If you hover over the chromatogram, all annotation information for the position of the cursor will be displayed, such as a precise RT in minutes and an intensity value.
  3. You can use the interactive toolbar, e.g. to zoom in and out. Press “Save” button to download a .png image of the plot.

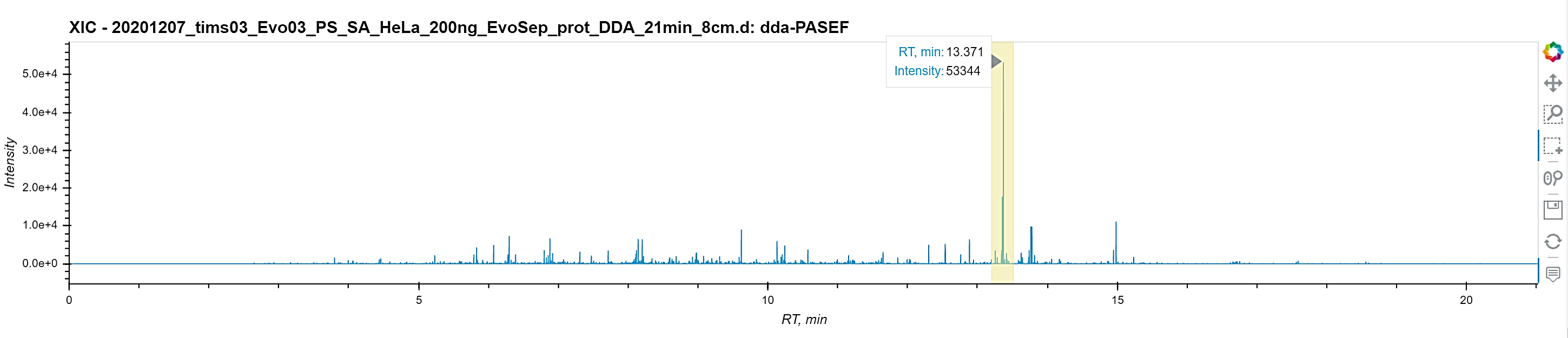


* **Heatmap** - a scatter plot for the sliced data on the requested axis (could be "m/z, Th", "RT, min" and "inversed IM, V·s·cm-2") colored by the summed intensities. This plot is generated with the use of Datashader Python library for the visualization of big data.

You can use the interactive toolbar, e.g. to zoom in and out. Press “Save” button to download a .png image of the plot. For this plot you can change the x- and y-axis in the “Select axis for plots” card in the “Parameters” section.



* **Spectrum / XIC / Mobilogram** - a line plot for the sliced data that visualize "m/z, Th", "RT, min" or "inversed IM, V·s·cm-2" against “intensity” values.
* The sliced and currently visualized on other plots RT range is highlighted in light yellow.
* If you hover over the plot, all annotation information for the position of the cursor will be displayed, e.g. a precise RT in minutes and an intensity value.
* You can use the interactive toolbar to for example zoom in and out. Press “Save” button to download a .png image of the plot. For this plot you can change the x- and y-axis in the “Select axis for plots” card in the “Parameters” section.

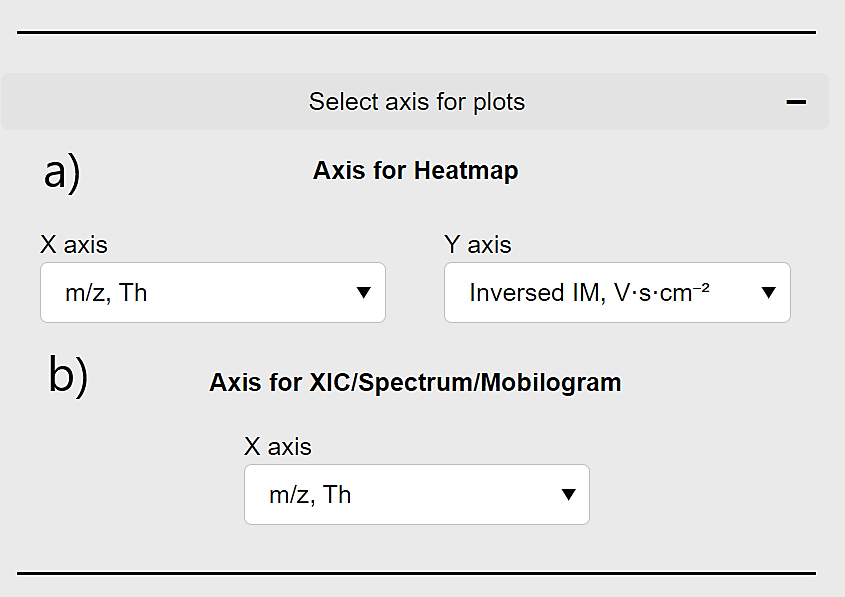


1. On the left in the “Parameters” section there are several opened/collapsed cards with options that can be specified for slicing of the original data and further visualization/exporting:

* **“Select axis for plots” card**

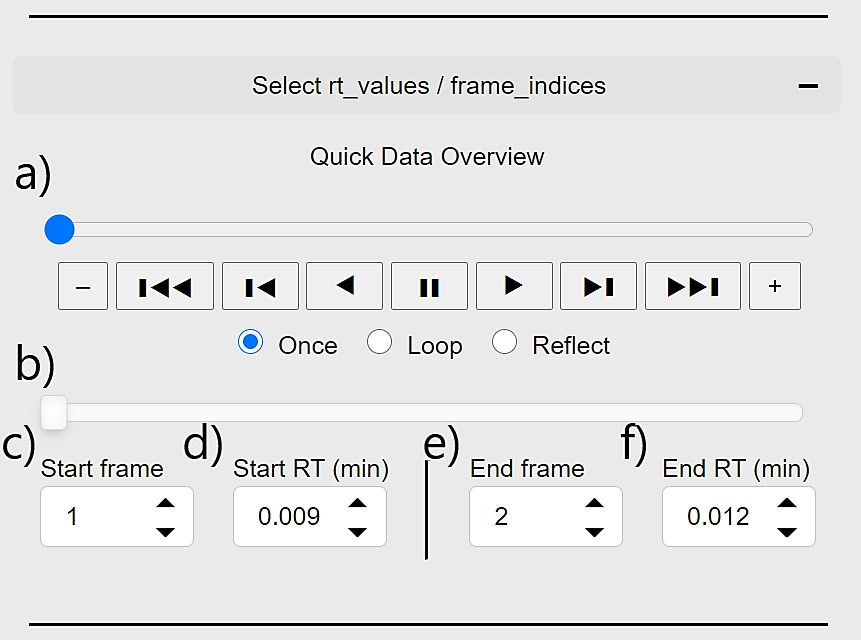
This card allows changing the axis for the two images presented in the dashboard plots. It can be done for:

* 1. The scatterplot/heatmap where you can choose between three options for x-/y-axes, such as "m/z, Th", "RT, min" and "inversed IM, V·s·cm-2".
  2. The line plot with the options to plot spectrum ("m/z, Th" vs. “intensity”), extracted ion chromatograms, or XIC ("RT, min" vs. “intensity”) or mobilogram ("inversed IM, V·s·cm-2" vs. “intensity”).



* **LC dimension: “Select rt\_values / frame\_indices” card**
  1. For a quick overview of the uploaded dataset you may use an integrated player option that allows to visualize ten MS1 frames which are distributed with the same interval through the whole gradient. The player has a number of buttons to go to the last (“ C:\Users\voytik\Downloads\next-track_icon-icons.com_70913.png ”) or the first (“ C:\Users\voytik\Downloads\next-track_icon-icons.com_70913.png ”) selected frame, step forward(“󠇯 C:\Users\voytik\Downloads\nexttrackbutton_113555.png ”) or backward (“ C:\Users\voytik\Downloads\nexttrackbutton_113555.png ”), or visualize 10 selected frames automatically one by one in a forward (“ ▶ ”) or a backward (“ ◀️ ”) order and pause the visualization (“Pause Button Transparent | PNG All”). It also provides control over the loop policy which determines whether to play the visualization 'once', 'loop' through it, or 'reflect' the process. Additionally “-” and “+” buttons slow down and speed up the player speed.
  2. Having a single frame or a range of frames for visualization, you may specify them using a frame range slider that shows the start and the end of desired frames.
  3. – f) Simplifying the work with the slider in b) you may also set manually the start – c) – and end – e) – of the frame range or the start retention time (in minutes) – d) – and the end retention time – f) – values.

\* If any specified start value is equal to or higher than the end value, the end value will be changed automatically with the value equal to “start\_value + 1”.



* **TIMS dimension: “Select mobility\_values / scan\_indices” card**

This card allows slicing mobility values and scan indices. It can be done using either a scan slider and the “Start scan”/ “End scan” input fields for manual setting of the values or the “Start IM”/ “End IM” value inputs to specify the range of inversed ion mobility values (1/Ko). For example, setting a value for the “Start scan” field will automatically update the slider start value and will change correspondingly the value of the “Start IM” field.

\* If any specified start value is equal to or higher than the end value, the end value will be changed automatically with the value equal to “start\_value + 1”.

* **QUADRUPOLE dimension: “Select quad\_values / precursor\_indices” card**

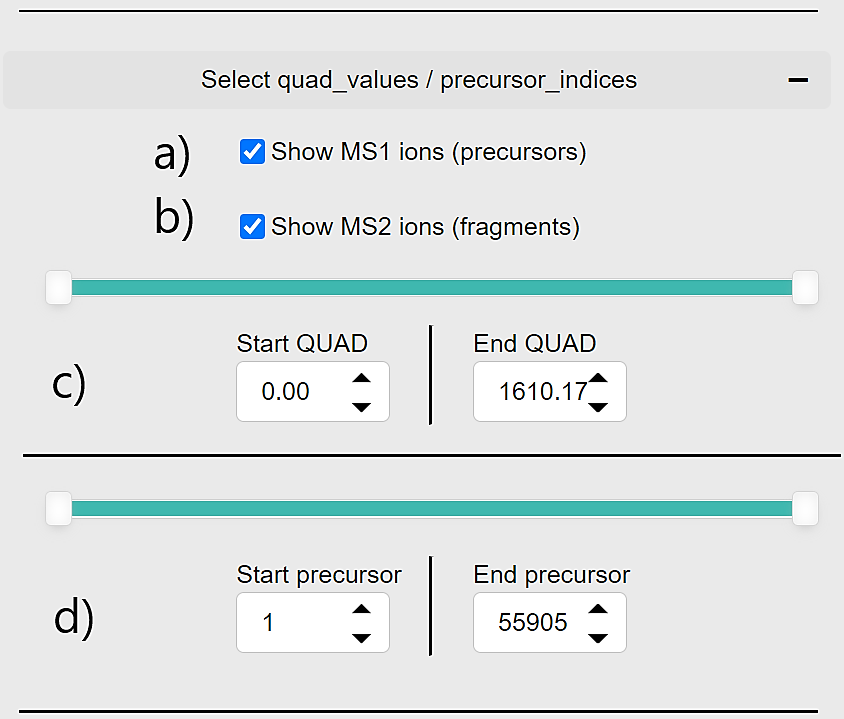
This card focusses on the quadrupole and indirectly on the collision cell. It allows to slice lower and upper quadrupole m/z values (e.g. the m/z of unfragmented ions / precursors).

1. To inactivate the quadrupole and collision cell and as a result to filter only the precursor ions, mark the “Show MS1 ions (precursors)” check box.
2. To activate both quadrupole and collision cell and as a result to slice only the fragment ions, mark the “Show MS2 ions (fragments)” check box.

\* To visualize both precursors and fragment ions just mark two checkboxes at the same time.

1. To slice quadrupole m/z values use either a quadrupole values slider or the “Start QUAD”/ “End QUAD” input fields for manual setting of the values.
2. To slice precursor indices use either a precursor indices slider or the “Start precursor”/ “End precursor” input fields for manual setting of the values.

\* If any specified start value is equal to or higher than the end value, the end value will be changed automatically with the value equal to “start\_value + 1”.



* **TOF dimension: “Select mz\_values / tof\_indices” card**

This card allows slicing TOF m/z values and indices. It can be done using either a TOF indices slider and the “Start TOF”/ “End TOF” input fields for manual setting of the TOF indices or the “Start m/z”/ “End m/z” value inputs to specify the range of fragment m/z values. For example, setting a value for the “Start TOF” field will automatically update the TOF indices slider start value and will change correspondingly the value of the “Start m/z” field.

\* If any specified start value is equal to or higher than the end value, the end value will be changed automatically with the value equal to “start\_value + 1”.

* **“Select intensity\_values” card**

This card allows filtering the data base on the intensity values of the ions. It can be done using an intensity values slider or the “Start intensity”/ “End intensity” input fields for manual setting of the intensity values.

\* If any specified start value is equal to or higher than the end value, the end value will be changed automatically with the value equal to “start\_value + 1”.

* **“Export data” card**

This card allows exporting the data into two different formats:

* HDF format

To reduce the reading time of your original file by 2-3 fold and, as even more important, to define an easily portable file which can be transferred between different OSs, it can be saved into HDF5 format that are supported for reading in step 1a.

1. Provide the path to the folder where to save a created .hdf file, e.g. “D:\Bruker\my\_file.hdf”.

\* By default, the file will be saved with the same file name to the same folder where your original .d folder exists. For example, in our case it would be: “D:\Bruker\20201207\_tims03\_Evo03\_PS\_SA\_HeLa\_200ng\_EvoSep\_prot\_DDA\_21min\_8cm\_S1-C10\_1\_22476.hdf”

1. Press the "Save to HDF" button. The loading process is indicated by a spinner symbol. In case of the successful saving of the file, you’ll get a confirmation message about it below the "Save to HDF" button.

\* a) and b) options are disabled if user originally uploads .hdf file.

* CSV format

Applying all specified slicing options, e.g. frames and scan ranges, the filtered data can be saved into .csv format with all possible information about ions:

- raw\_indices

- frame\_indices

- scan\_indices

- precursor\_indices (0 value is equal to “precursor” ion)

- tof\_indices

- rt\_values

- mobility\_values

- quad\_low\_mz\_values and quad\_high\_mz\_values (-1 value is equal to “precursor” ion)

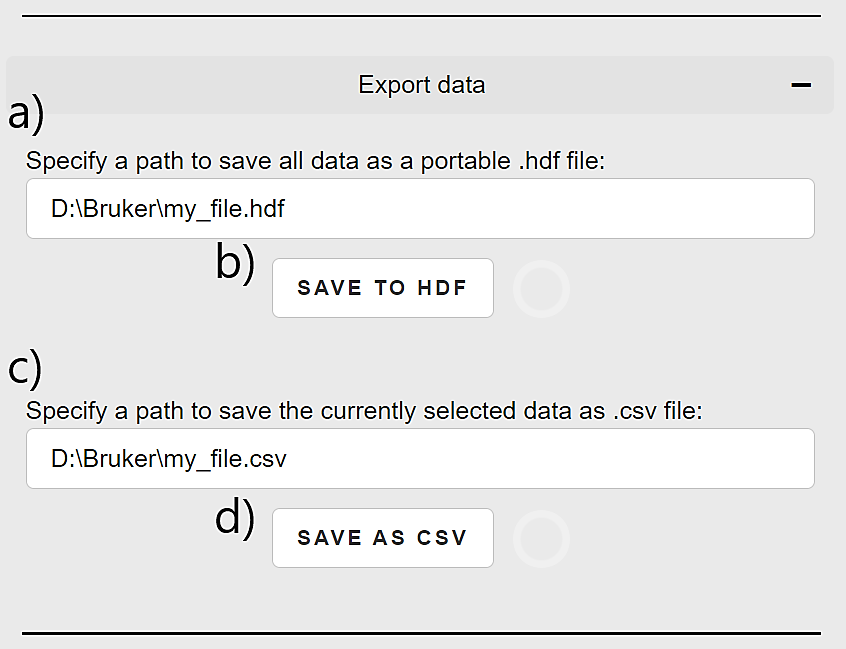
- mz\_values

- intensity\_values

1. Provide the path to the folder where to save a created .csv file, e.g. “D:\Bruker\my\_file.csv”.

\* By default, the file will be saved with the same file name to the same folder where your original .d folder exists. For example, in our case it would be: “D:\Bruker\20201207\_tims03\_Evo03\_PS\_SA\_HeLa\_200ng\_EvoSep\_prot\_DDA\_21min\_8cm\_S1-C10\_1\_22476\_data\_slice.csv”

1. Press the "Save as CSV" button. The loading process is indicated by a spinner symbol. In case of the successful saving of the file, you’ll get a confirmation message about it below the "Save as CSV " button.



* **“Undo | Redo” buttons**

To reverse your actions inside “Select …” cards where you are setting values for slicing/visualization of the data, you can use “Undo” and “Redo” buttons. They won’t have any effects on exporting, strike count estimation, image zooming or (re)loading other datasets.

You can reverse more than one action just clicking several times on the buttons. You can use “Redo” command only after “Undo” command.

\* Take into account that it could take some time to update the widgets values and the plots.

* **“Strike count” option**

This option sets a maximal limit for the number of data points that can be visualized per each plot. For example, by default it’s allowed to visualize till 10 million points and if you will try to set the RT ranges for the whole dataset, the heatmap and the line plot will be empty. This is done for time performance considerations.