https://msda.unistra.fr
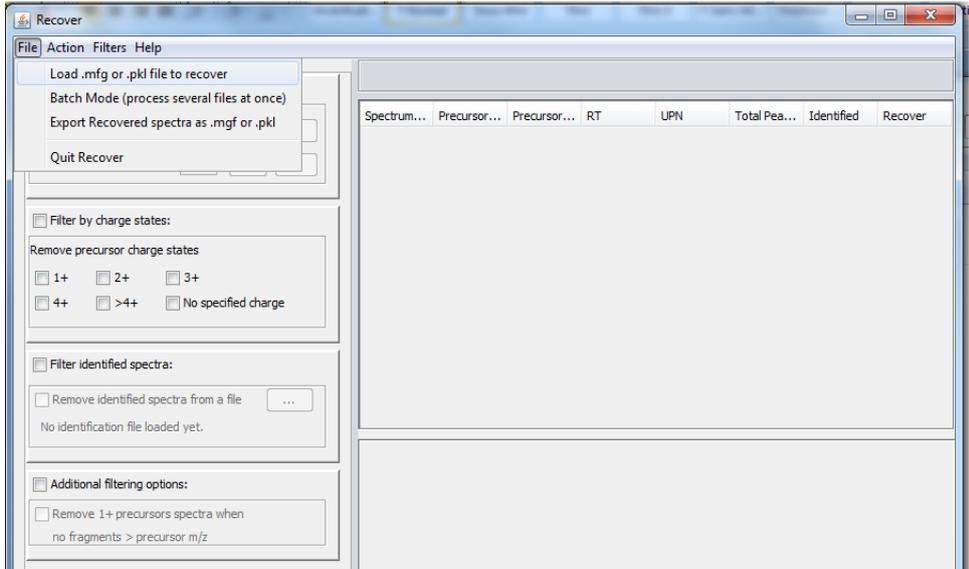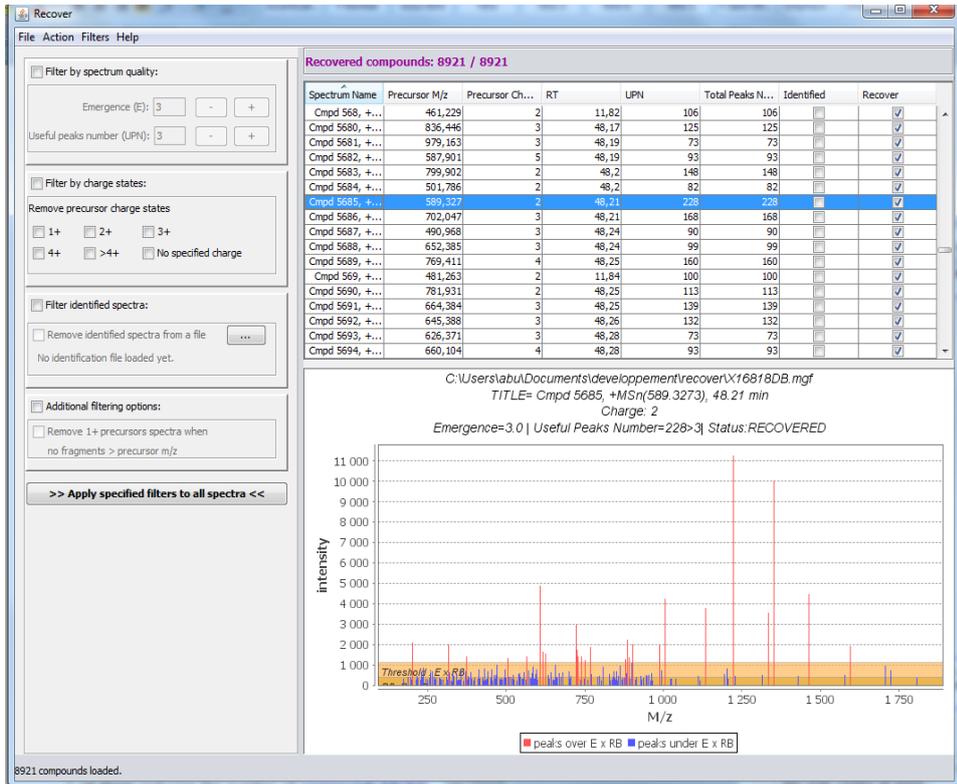
MSDA tutorial: How to use RECOVER

Open a peaklist file (.mgf or .pkl) in order to display its MS/MS spectra

Click on the "File menu" and then "**Load .mgf or .pkl file to recover**"

You can now see the list of compounds in the upper right table. You can then select a line and see its spectrum displayed in the bottom right panel.

Filter options

You can select and adjust several types of filters, each of them reducing the number of MS/MS spectra to keep for recovery. When you set a new value for a filter, you have to click on the button "Apply specified filters to all spectra" to re-compute the table. You can also individually check and uncheck compounds from the table and play with the action menu which allows global check, uncheck of all or validated compounds. **Note that those actions reset the Recover check**.

You can follow the statistics of recovery after application of a given filter in the upper left corner of the table.

Filter by spectrum quality

You can set the Emergence (E) and Useful Peaks Number (UPN) values in order to remove low quality spectra from your peaklist. The emergence is the multiplication factor applied to the noise level (computed with an appropriate algorithm for each spectrum) allowing to define "Useful Peaks" with intensities higher than a threshold=E x noise level. The Useful Peaks Number (UPN) is the actual number of peaks above this threshold. If the actual Useful Peaks Number is higher than the desired UPN parameter, the spectrum is considered high quality and thus gets the Recover tag.
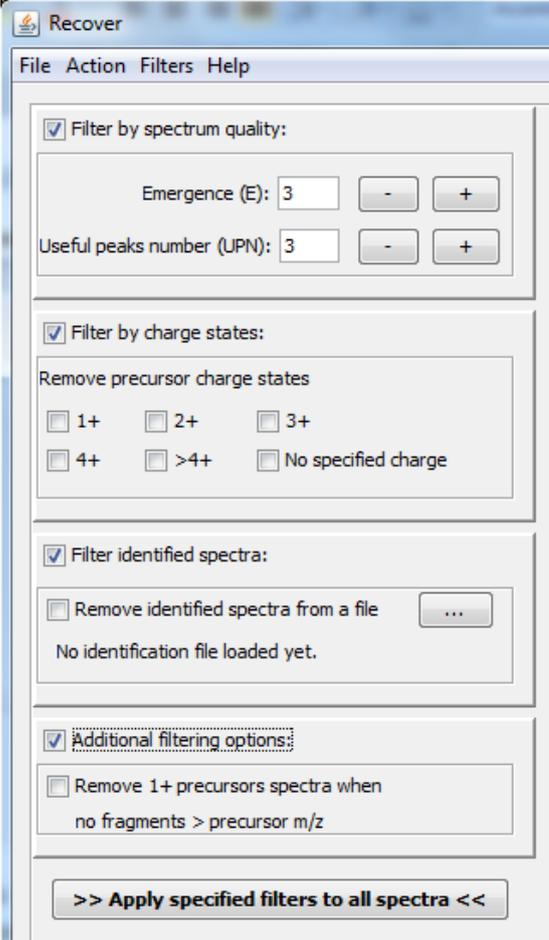
Filter by charge states

The charge state filter allows removing precursors with given charge states. For instance, checking +1 will remove all precursors annotated as singly charged in the peaklist from recovery. Multiple charge states can be simultaneously selected.

Filter identified spectra

An identification results file can be loaded in order to specifically mark spectra that have been previously identified using database searches for instance. Identification results have to be loaded from a Microsoft Excel 2007 (or higher) file. When loading the Excel file, indicate the cell containing the first compound of the identified compounds column.

Spectra for which identification results are found will be marked as identified in the compound table. You can then choose to exclude identified spectra from recovery.

Additional filtering options

Additional filters are available such as removing spectra with no fragment ions higher than the precursor m/z (this allows removing singly charged precursor ions fragmentation spectra, not annotated as singly charged in the peaklist).

Apply specified filters to all spectra

Once the filters are properly adjusted, clicking on the "Apply specified filters to all spectra" button applies the filters to the complete peaklist and displays a refreshed list of recovered spectra in the table.

Filters menu

The filters menu allows enabling or disabling each filter category. Selecting "Show only recovered spectra" will hide all spectra checked as recovered (by filters or manually).
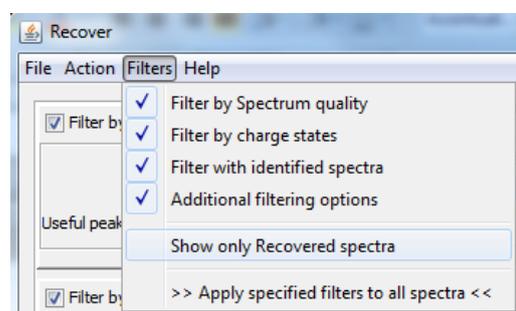
Action menu

This menu contains some generic functions like "Check 'Recover' for all spectra". The purpose of this menu is to help the user to select a wide range of spectra in just a few clicks.
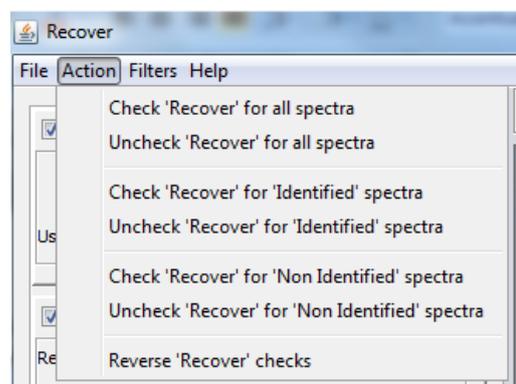
Export recovered spectra

When you are done with setting your filters, you can export the recovered spectra in a new peaklist file. This new file will be a subset of the original file, containing only the spectra with the Recover tag.

Run recover in batch mode

You can also use these filters in **Batch Mode**. You only have to select the files you want to process, and indicate the output folder.