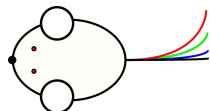# User guide v.1.0 by Nikita A. Ivanov

Welcome to Topino!

Whether it's your first time analyzing Molecular Stream Separations (MMS) or something that you do regularly, please take time to read this small user guide to familiarise yourself with *Topino*, a handy software specifically designed for MSS. Topino is a simple and intuitive software that will help you to analyze MSS pictures, extract raw data, and generate manuscript-grade figures.
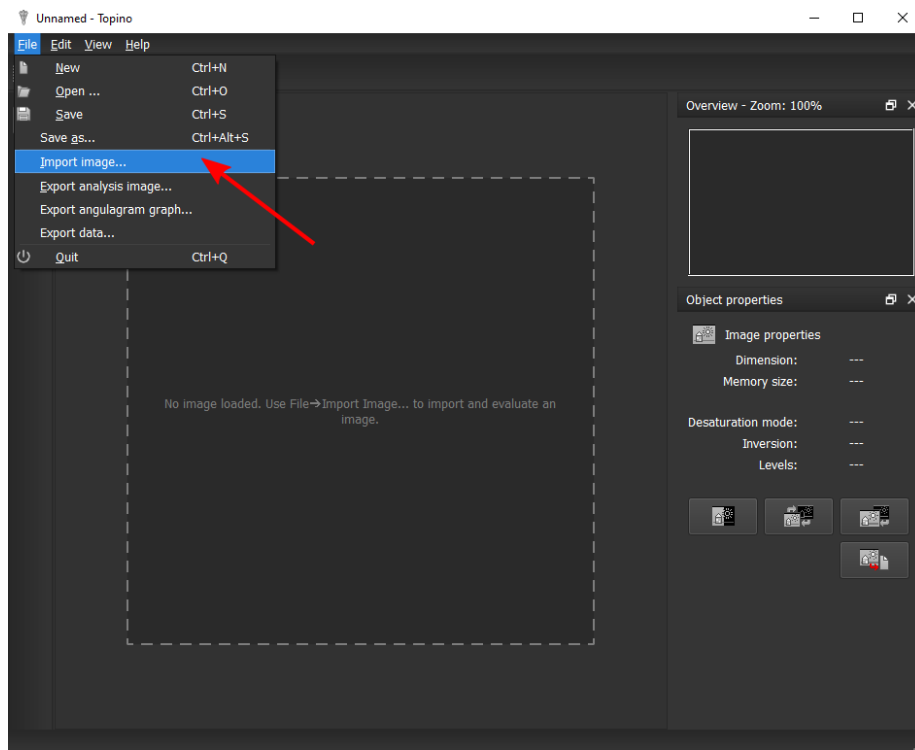
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Image importing and preprocessing

Image file import

In order to begin your journey with *Topino*, click on the *File* in the the top left and select the *Import image...* option from the drop-down menu.

Topino accepts most popular image files formats such as PNG (default) and JPEG. You can attempt to import a non-supported format by select the *All Files* option from the drop-down menu in the *Open*-dialog.
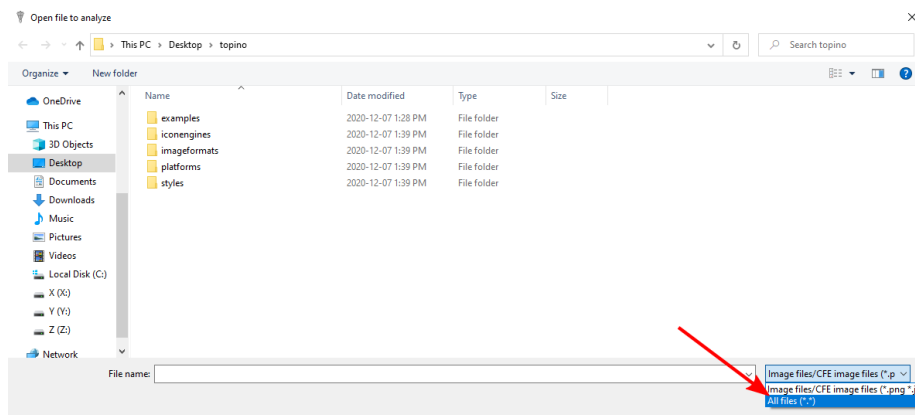
It is not guaranteed that the import will be successful. In principle, *Topino* supports all [formats that are supported by the Qt framework]. However, the import might produce artifacts or the like, *e.g.* with RAW files. Future version of *Topino* might support more image formats.

Inlet tool

On the left side of the *Topino* interface you can find the toolbar. It contains the *Inlet tool* required to specify the position of your inlet as well as the *Ruler tool*. The latter is not required for the basic operation of *Topino* and, therefore, discussed in Additional features.
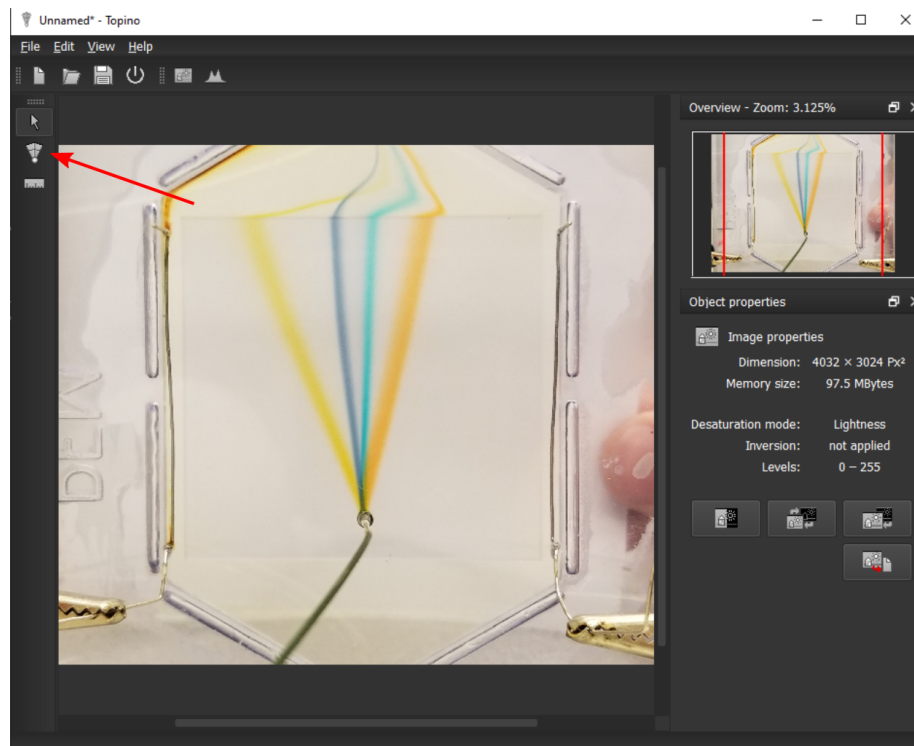
The first step of preprocessing your image is to tell *Topino* where your inlet is located. The *Inlet tool* generates an adjustable annular grid with a small red circle of variable size at the center. Your objective is to cover the inlet area with the small red circle and rotate the grid in the direction of the flow. By default, the range is set from -30° to +30° and can be adjusted either by dragging on the boundaries of the grid with the mouse cursor or by changing values in the *Object Properties* discussed in the next section. Only the area covered by the grid is used for further processing. The small red circle is used to exclude the inlet area as it often contains non-useful information.
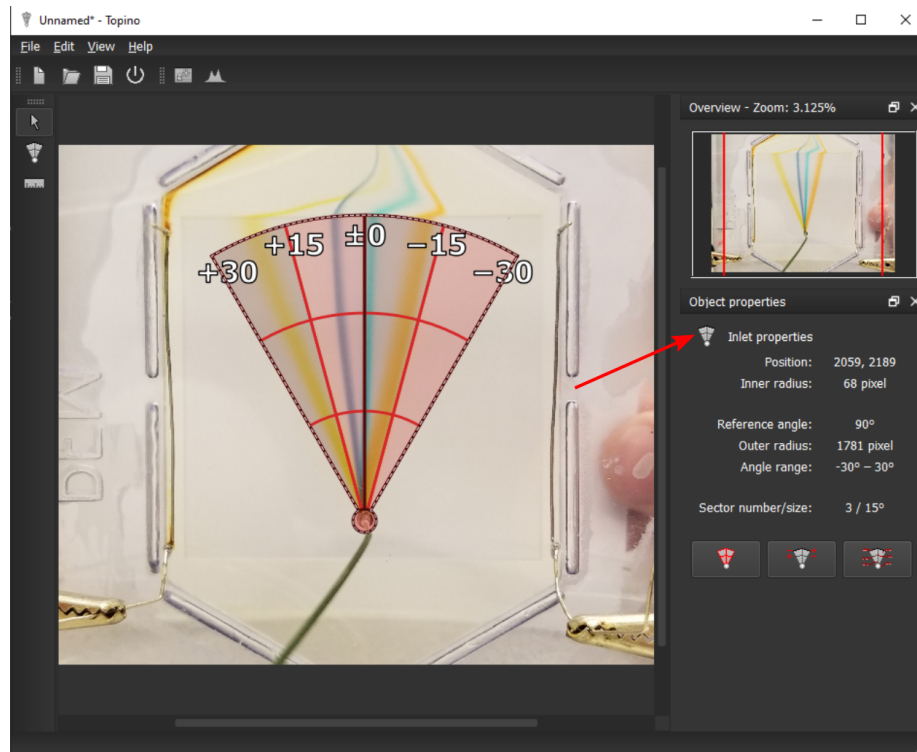
Select the *Inlet Tool* and position your mouse cursor at the center of your inlet. Click and hold the left mouse button (LMB) dragging it towards the edges of your inlet. When the entire inlet zone is covered by the small red circle, release LMB. This action should generate a visible annular grid similar to the one in the following picture that you can rotate, expand, and move around.
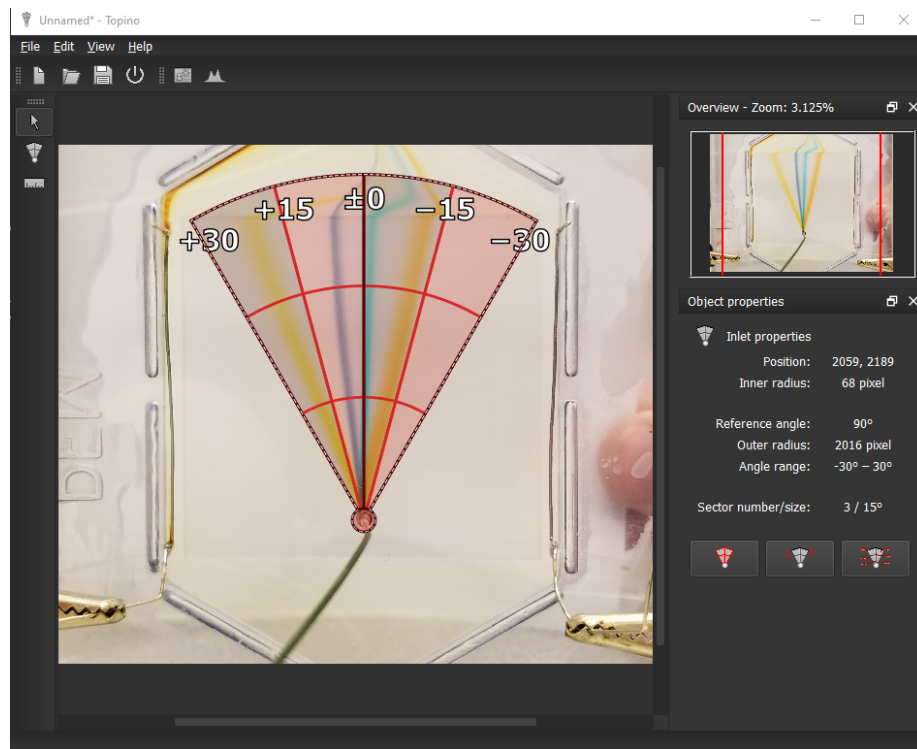
Object properties bar

On the right side of the *Topino* interface, you can find the *Object Properties*. It can display either object properties of the imported image (if nothing is selected) or properties of objects on the image such as the inlet depending on what is currently selected.

Let us explore *Inlet properties* first. For this, make sure that the inlet you created in the previous step is selected:

Button	Function
	Sets your currently selected inlet as the *Main Inlet* if multiple sample inlets are specified. Only one *Main inlet* can be designated at a time because the polar coordinate system can have only one origin.
	Reverses positive and negative annular values on the displayed grid.
	Shows a dialog that allows modification of the parameters of the annular grid. This dialog has the advantages of holding additional parameters, as well as their exact numerical values, not accessible through direct cursor interaction with the grid. One can use it to fine-tune a quickly placed annular grid.

The *Image properties* can be viewed when nothing is selected (just click on any empty space, the image, or press *Esc*). It contains several important functions and useful image information such as resolution, selected desaturation mode, and adjusted histogram levels. There are 4 buttons visible:

Let us explore *Image properties*. For this, make sure that nothing is selected by simply pressing *Esc*:

| Button	Function
	Shows the *Image preprocessing* dialog. Proper image preprocessing is crucial for angulagram analysis. Select a desaturation mode and carefully tweak the histogram. The objective is to maximize the contrast between image background and molecular streams of interest. The selection of the right desaturation mode will depend on the colours of the background, streams, and image noise and is left at your discretion. A preview on the left of the dialog is included for your convenience. For images with a bright background and dark streams it is recommended to invert the preprocessed image to generate positive peaks in the angulagram. When preprocessing is complete you should see a high-contrast image with the previously placed and adjusted overlay grid.
	Swaps between preprocessed and the original imported image
	Resets the image to its original state.
	Exports the preprocessed image as PNG file.

Analyzing the angulagram

Understanding your preprocessed data

This chapter begins with the preprocessed image overlayed with the annular grid. By clicking on *Show and edit the angulagram* the second part of the analysis is initiated.
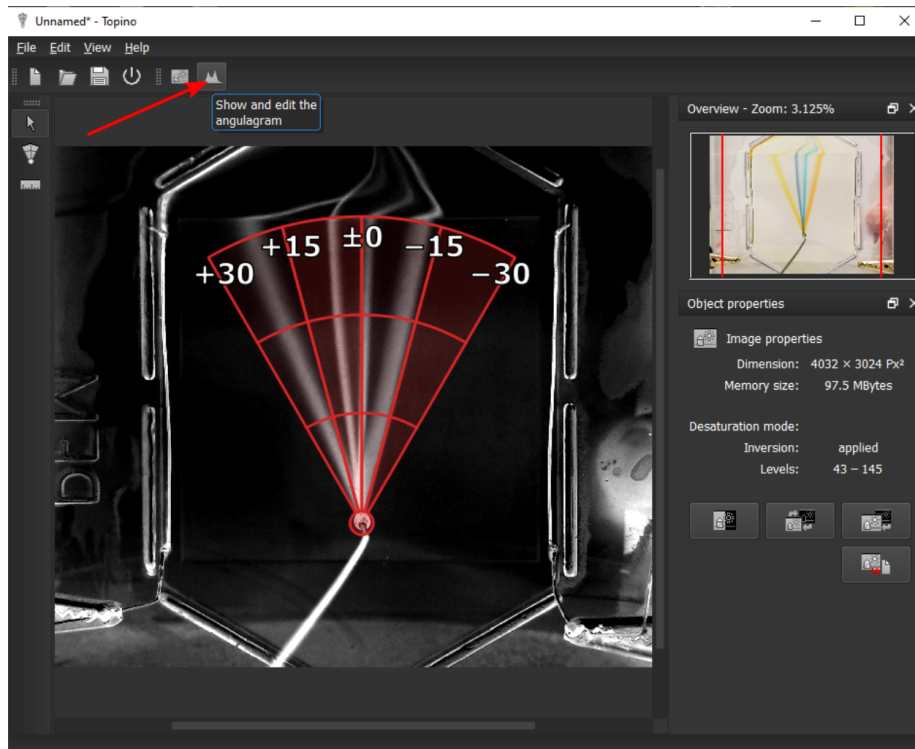
Topino will convert the imported image from Cartesian to Polar coordinate system with the previously specified main inlet at the origin. The resulting *Polar image* serves as the template for the angulagram generation. To view the converted *Polar image*, press the respective button on the object properties bar.

The polar image is then integrated over the radius for each angle and the preliminary angulagram is presented in the main window. If good contrast was achieved during the preprocessing step all streams should appear as well-spaced distinct peaks.
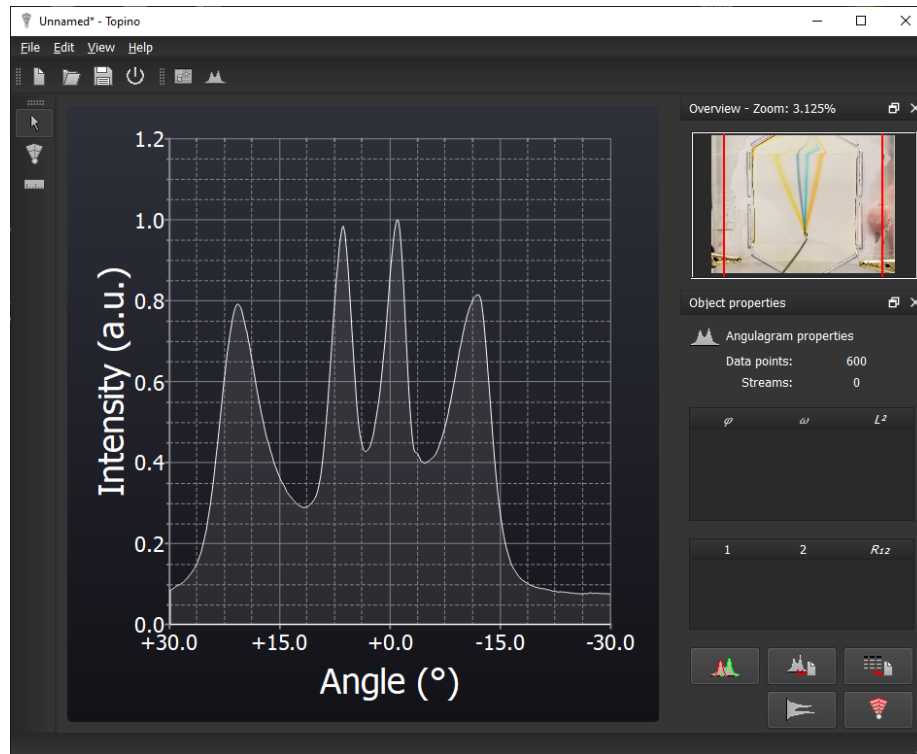
Analyzing the angulagram by fitting peaks

Click on the *Evaluate angulagram* button on the right side of *Topino* to start the fitting process. Fitting is done mostly automatically in three steps: First, data is smoothed by a Gaussian kernel. Second, *Topino* detects maxmima and minima to split the angulagram into sections (ideally: one peak per section). Third, each section is fitted to a Lorentzian function by the Levenberg-Marquardt algorithm.

In the preview dialog, you can adjust some of the fitting parameters such as the Gaussian noise and threshold. For instance, adjusting the threshold will allow to remove the noise on the left and right side of the angulagram, which is often incorrectly identified as peaks. Adjusting the smoothing parameters will remove false-positive peaks (small minima/maxima). If you are happy with the fit preview, simply press *Ok* to accept these parameters and fitting.

After fitting your peaks, *Topino* will automatically calculate all stream parameters (deflection, width, linearity, resolution) and display it in the table on the right side of the main window.
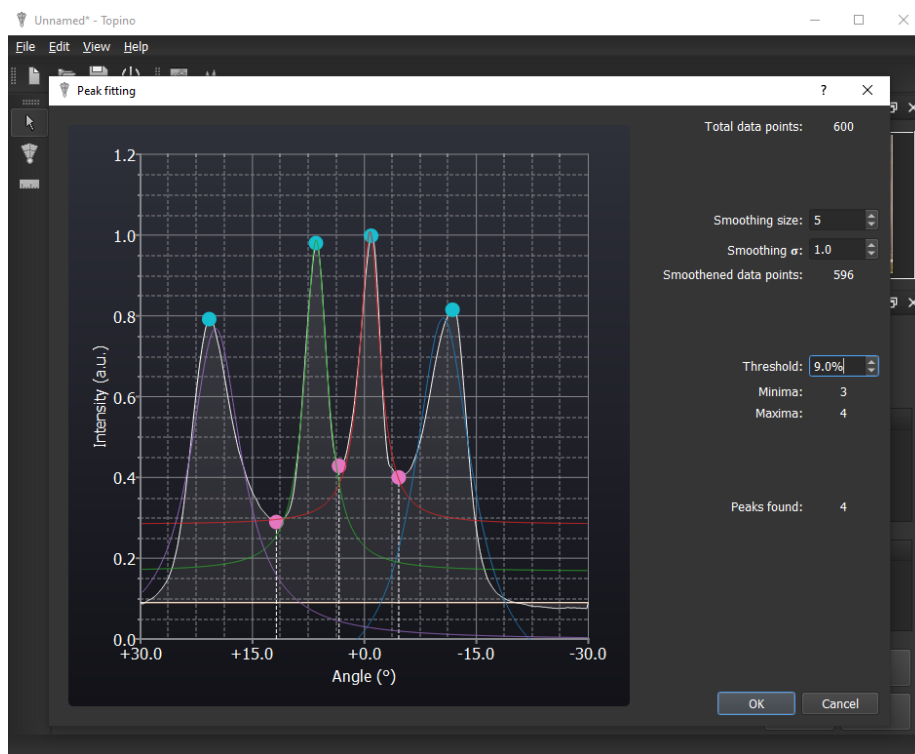
Note: If small manual modifications to the fitting parameters cannot give you a good fit, please return to the preprocessing step and modifiy its histogram values and/or the desaturation method (or capture a better image).

Angulagram properties

Let us explore *Angulagram properties*:

Button	Function
	Evaluates the angulagram by fitting peaks.
	Exports the angulagram as raster image (PNG) or vector image (SVG).
	Exports the raw data as text file suitable for import into any spreadsheet application (Excel, Origin, etc.).

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Button	Function
	Show the polar image generated. It can be used to verify the fidelity of the previously placed inlet and its size. Ideally, all streams in the polar image should look like cones that get progressively thinner as they approach the right boundary. You can also export the polar image as PNG here.
	Generate and show the radialgram. This is an experimental feature not required for standard processing.

Additional and experimental features

Topino has two advanced and experimental features, *i.e.* the *ruler tool* and the *radialgram.* All these advanced functions are not needed for every day assessment of MSS but are meant as power features for advanced users that need an extra bit more control and precision for difficult-to-assess border cases.

The *ruler tool* can be used to quickly align and expand the annular grid to any image feature (such as corners and the like) used as reference. This tool can be employed to offset rotated images and fine adjust angles, etc.

The *radialgram* integrates the polar image over the angle instead of the radius, which can be used to follow the progression and dispersion of streams through the separation zone. A typical radialgram is shaped as a half-peak that follows progressive loss of signal intensity due to molecular diffusion of streams. Any deviations from this expected shape can point to debris found on the MSS device (sudden narrow drops), non-uniformity in lightning or exposure (overall curve shape change), or errors in pre-processing of the image (random peaks or drops).