Kymolyzer

Results table legend

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The *Results table*

The *Kymolyzer* macro outputs a single comma-separated values (CSV) table containing data from all analyzed cells across all experiments. The table begins with a header followed by rows of quantitative results for each individual cell (ROI), organized into clearly labeled columns. Each column is explained below.

Table header

Each line of the header starts with a pound sign (`#`) and is automatically ignored by *R* scripts (use of our R scripts is recommended).

- **Macro name** – name of the macro used
- **Macro version** – version of *Kymolyzer* used (indicated in the macro filename and internally as the `version` variable)
- **Date and time** – when the macro was executed (run), in the YYYY-MM-DD HH:MM:SS format; the timestamp in the output filename corresponds to the end of the macro run
- **Image type** – indicates whether quantification was performed on transversal (equatorial) or tangential images
- **Channel** – fluorescence channel used for quantification (also noted in the file name)
- **Abbreviations** – defines commonly used short forms in column headers:
  - *fwd* – forward
  - *bwd* – backward
  - *stat* – static
  - T – lifetime
  - v – speed

Column definitions

Each row corresponds to a single analyzed cell (ROI) and contains the following parameters, grouped by origin:

From folder structure

- **exp_code** – experiment identifier (accession code), extracted from the folder three levels above the data folder

- **BR_date** - date of biological replicate; extracted from the name of the folder 2 levels above the data folder (first 6 characters)

for details on data structure consult Fig. 6 in Zahumensky & Malinsky, 2024

From file name

These labels are defined by the user via the *Naming scheme* input in the *Kymolyzer* dialog, in the *Analyze kymograms* option. The number of comma-separated fields must match the structure of the filenames.

- **strain** – yeast strain
- **medium** – cultivation medium
- **time** – cultivation time
- **condition** – treatment condition (e.g., control, heat shock, drug)
- **frame** – frame identifier (e.g., different images from the same sample)

From image analysis

- **mean_background** – average background intensity; subtracted from all intensity measurements
- **frame_interval** – time between consecutive frames (in seconds); extracted from metadata or entered manually if missing — *ensure accuracy for correct speed/lifetime quantification!*
- **cell_no** – ROI identifier, matching the number shown in Fiji's ROI Manager

Quantified dynamics (by direction)

- **traces_fwd/bwd/stat** – number of traces in the forward (right), backward (left), or static direction
- **v_fwd/bwd/stat [nm/s]** – average speed of traces in each direction
- **T_fwd/bwd/stat [s]** – average lifetime of traces in each direction (i.e., duration between appearance and disappearance of a focus)
- **mean_v [nm/s]** – weighted average of absolute speeds across all directions
- **mean_T [s]** – weighted average of lifetimes across all directions
- **mobile_fraction [%]** – estimated fraction of mobile signal relative to total signal

note that only traces that both start and end within the kymogram, or span it entirely, are taken into account during quantification

Coupled signal analysis (based on colocalization with second channel)

- **coupled_v_fwd/bwd/stat [nm/s]** – average speed of coupled (colocalized) foci in each direction
- **coupled_T_fwd/bwd/stat [s]** – average lifetime of coupled foci in each direction
- **coupled_fwd/bwd/stat_fraction [%]** – proportion of traces in each direction that colocalize with traces in the second channel
- **coupled_mean_v [nm/s]** – weighted average speed (absolute) of all coupled foci
- **coupled_mean_T [s]** – weighted average lifetime of all coupled foci