

## 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.

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### 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

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### Column definitions

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

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#### 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)
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## 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
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## 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*

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## 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