**Table S1. A complete list of all 39 trajectory features calculated by the diff-classifier Python package.** Included for each feature is a brief description and how it is determined. Additional documentation can be found in the TraJ GitHub repository (<https://github.com/thorstenwagner/TraJ.git>).

| **Feature** | **Model Abbreviation** | **Description** | **How it is determined** |
| --- | --- | --- | --- |
| Alpha () | alpha | Exponent of the anomalous diffusion equation. | Non-linear least squares is used to fit raw MSD vs lag time () data to the anomalous diffusion equation: |
| Effective diffusion coefficient () | D\_fit | Coefficient of the anomalous diffusion equation | Non-linear least squares is used to fit raw MSD vs lag time () data to the anomalous diffusion equation: |
| Kurtosis () | kurtosis | The fourth moment of the projected positions on the dominant eigenvector of the radius gyration tensor (T). |  |
| Asymmetry1 () | asymmetry1 | Characterizes the asymmetry of the trajectory. Asymmetry1 equals 0 for circularly symmetric trajectories and 1 for linear trajectories. | where and are the eigenvalues of radius of gyration tensor T: |
| Asymmetry2 () | asymmetry2 | The ratio of the smaller to larger principal radius of gyration. |  |
| Asymmetry3 () | asymmetry3 | An asymmetry feature that accounts for non-cylindrically symmetric point distributions. |  |
| Aspect ratio (*AR*) | AR | The ratio of the long and short side of the trajectory's minimum bounding rectangle. Perfectly symmetric trajectories have an aspect ratio of 1, and aspect ratio increases as trajectories become more elongated. |  |
| Elongation | elongation | An estimation of amount of extension of the trajectory from its centroid. |  |
| Boundedness (*B*) | boundedness | Boundedness quantifies how much a particle with diffusion coefficient *Deff* is restricted by a circular confinement of radius *r* when diffusing for a period of time . |  |
| Fractal Dimension (*Df*) | fractal\_dim | Fractal dimension is a measure of how "complicated" a self similar figure is. |  |
| Trappedness () | trappedness | The probability () that a particle with duffusion coefficient *Deff* is trapped in a region (*r0*) for a period of time. |  |
| Efficiency (*E*) | efficiency | The ratio of the squared net displacement to the sum of step lengths. |  |
| Straightness (*S*) | straightness | The ratio of the net displacement to the sum of step lengths. |  |
| MSD Ratio () | MSD\_ratio | MSD ratio characterizes the shape of the MSD curve. For Brownian motion, it is 0; For restricted motion it is < 0; For directed motion it is > 0. |  |
| Frames | frames | The total number of frames the trajectory spans. | Frames = *N* |
| Mean Turning Angle ) | angle\_mean | The trajectory mean of the turning angle which is the counterclockwise angle from one point to another |  |
| Mean Turning Angle Magnitude () | angle\_mag\_mean | The trajectory mean of the magnitude turning angle which is the counterclockwise angle from one point to another |  |
| Turning Angle Variance (*)* | angle\_var | The trajectory variance of the turning angle which is the counterclockwise angle from one point to another |  |
| Total Distance (dtotal) | dist\_tot | Total distance particle travels throughout trajectory |  |
| Net Distance (dnet) | dist\_net | Net distance traveled throughout trajectory |  |
| Progression | progression | The ratio of the net distance traveled, and the total distance traveled |  |
| Effective Diffusion Coefficient 1 | Deff1 | Effective diffusion coefficient at 0.33 s. |  |
| Effective Diffusion Coefficient 2 | Deff2 | Effective diffusion coefficient at 3.3 s. |  |
| Mean values were calculated based on surrounding datapoints for alpha, D\_fit, asymmetry1, asymmetry2, asymmetry3, AR, elongation, boundedness, fractal\_dim, trappedness, efficiency, straightness, MSD\_ratio, Deff1, and Deff2 | | | |
| ***MSD*: mean squared displacement τ: lag time**  ***N*: number of frames *xp*: projected 2D position**  ***σ xp*: standard deviation of the projected 2D positions *T*: gyration tensor**  **λ1, λ2: eigenvalyes of radius of gyration tensor : average x and y location**  ***Deff*: effective diffusion coefficient : inverse of frame rate**  ***r*: radius of circular confinement *d*: largest distance between any two positions**  ***n*: frame number *L*: total length (sum over all steplengths) of trajectory**  ***r0*: radius of trapped region σ*I*: standard deviation of pixel intensities**  ***IBack*: average background pixel intensity** | | | |