iMSD toolbox

`iMSD_2026.m` manual for iMSD analysis of fluorescence image stacks

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ABSTRACT

The given toolbox provides a comprehensive collection of scripts for performing image-derived Mean Square Displacement (iMSD) analysis, a fluctuation-based approach for quantitatively characterizing the average dynamics of subcellular structures -such as proteins, organelles, and nanoparticles- in living cells. The method relies on the computation of spatiotemporal correlation functions from time-resolved fluorescence microscopy data, followed by Gaussian fitting of the correlation peak variance to extract the iMSD curve and the associated dynamic parameters. The provided scripts are designed to facilitate the quantitative assessment of different modes of intracellular motion, including diffusive, confined, and directed transport regimes, enabling the investigation of complex biological processes at the subcellular scale. The intent is to simplify iMSD data analysis and support the interpretation of fluctuation-derived measurements, thereby promoting reproducible and accessible studies of intracellular dynamics in live-cell imaging experiments.

Getting started

The toolbox requires MATLAB (MathWorks) version R2022a or later. Prior to analysis, the `.tif` image stack to be analyzed should be placed in the same directory as `iMSD_2026.m` and all other associated scripts, i.e.

- `iMSD_01_import.m`,
- `iMSD_02_spatial.m`,
- `iMSD_03_spatiotemporal.m`,
- `iMSD_04_output.m`, `interactiveImagStack.m`,
- `spatialCorrelationViewer.m`,
- `spatiotemporalCorrelationViewer.m`.

Then, edit `iMSD_2026.m` to input the specific instrumental and processing parameters for the experiment. In detail,

- `filename`: name of the image stack .tif file;
- `px_size`: pixel size in µm (e.g., 0.05).
- `frame_time`: time-lapse between successive frames in seconds (e.g., 0.4).
- `SpatialLimit`: linear extent of the spatial domain for the computation of the spatial and spatiotemporal correlation functions, in pixel (e.g. 32).
- `TauLimit`: upper limit of the temporal domain for iMSD analysis, expressed as the number of frames. The default is 20% of the total number of frames.

Run the script

Image stack import and visualization

The script imports the selected image stack and automatically determines its key properties, including the total number of frames and the lateral dimensions of each image. An interactive "Image Stack Viewer" window will then appear (as shown in Fig. 1), enabling direct frame-by-frame visualization of the dataset. The viewer also displays the corresponding intensity distribution for each frame, as well as the temporal evolution of the average, minimum, and maximum intensity values.

Preliminary spatial correlation analysis

The spatial correlation function is first computed for each frame. This initial calculation provides the starting parameters required for subsequent analyses and enables a direct assessment of whether the chosen spatial domain extent is appropriate. Indeed, an interactive "viewer of Spatial Correlation Function" will appear. As reported in Fig. 2, this window shows for each frame the computed spatial correlation function, its corresponding Gaussian approximation, along with the residual heatmap, and the sections at $\xi = 0$, and $\eta = 0$. Furthermore, relevant fitting parameters are reported, including the Gaussian amplitude, baseline, width, and R2 coefficient.

Spatiotemporal correlation analysis

The script computes the spatiotemporal correlation function. This is the core of the analysis and is the most computationally intensive part. The spatiotemporal correlation function is calculated in the Fourier domain. Specifically, the Fourier transform of each image is multiplied by its complex conjugate to obtain the power spectral density, and the inverse Fourier transform of this product yielded the real-space autocorrelation. The resulting correlation map is then centered and normalized by the square of the mean intensity and by the number of pixels. At the end of the computation an interactive "viewer of Spatiotemporal Correlation Function" will appear. As reported in Fig. 3, this window shows for each time-lag the computed spatiotemporal correlation function, its corresponding Gaussian approximation, along with the residual heatmap, and the sections at $\xi = 0$, and $\eta = 0$. Furthermore, relevant fitting parameters are reported, including the Gaussian amplitude, baseline, width, and R2 coefficient. The script extracts the Gaussian variance $\sigma^2(\tau)$ for each time lag τ. This variance is the iMSD.

Inspect the result

The last step of the computational analysis focuses on the processed Gaussian function that best approximates the experimental spatiotemporal correlation function. The trends of the Gaussian amplitude, Gaussian peak location and Gaussian variance are shown in a dedicated "iMSD output" window, are reported in Fig. 4. Importantly, the variance $\sigma^2(\tau)$ represents the iMSD curve. It is first fitted to a power-law function for a first motion characterization, i.e. sub-diffusive, Brownian, or super-diffusive, according to Eq. 6 (Box 3). Then, on the basis of the obtained outcome, it is further fitted to a confined-motion equation (Eq. 8 for sub-diffusive behaviors, Box 3), or to an active-transport equation (Eq.9 for super-diffusive behaviors, Box 3). All the relevant fitting parameters are reported in the MATLAB command window, in the MATLAB Workspace, and can be exported into a .xlsx file (if `export_value` is set to 1).

Interactive Graphical User Interface

Overall, the provided scripts generate four GUI windows for quality control. They can be summarized as follows:

1. **Image Stack Viewer** displays the image stack, and its intensity distributions, frame by frame (Fig. 1).

2. **Spatial Correlation Viewer** shows the spatial correlation function and the extracted fitting function, along with the obtained fitting parameters (Fig. 2).

3. **Spatiotemporal Correlation Viewer** shows the spatiotemporal correlation function and the extracted fitting function, along with the obtained fitting parameters (Fig. 3).

4. **iMSD output** displays the trends of Gaussian amplitude, peak's location and variance as a function of the time-lag τ (Fig. 4). As $\sigma^2(\tau)$ represents the iMSD curve, it is employed for motion characterization and evaluation of all the relevant dynamic parameters, e.g. diffusion coefficients at short and long timescales, active transport speed, if any, average spot size.

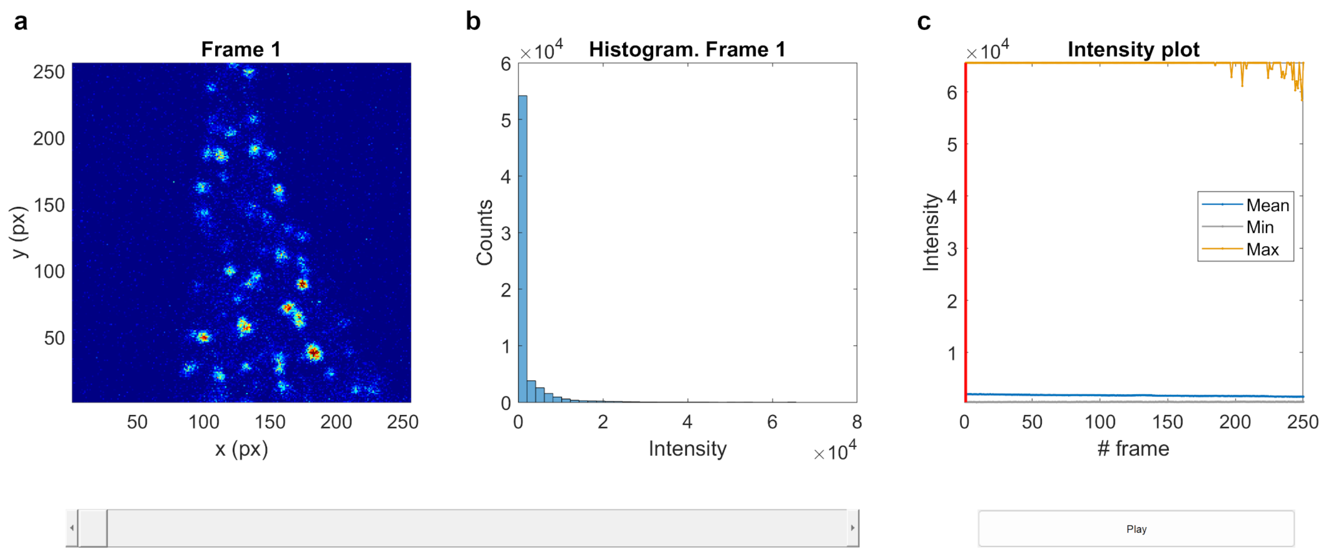

Figure 1. Image-stack viewer. The image stack imported by `iMSD_2026.m` can be explored through (a) individual frame displays, (b) intensity histograms, and (c) plots of average, minimum, and maximum intensity across frames. During the analysis, "play" button and slider bar allow users to move through the image frame by frame.

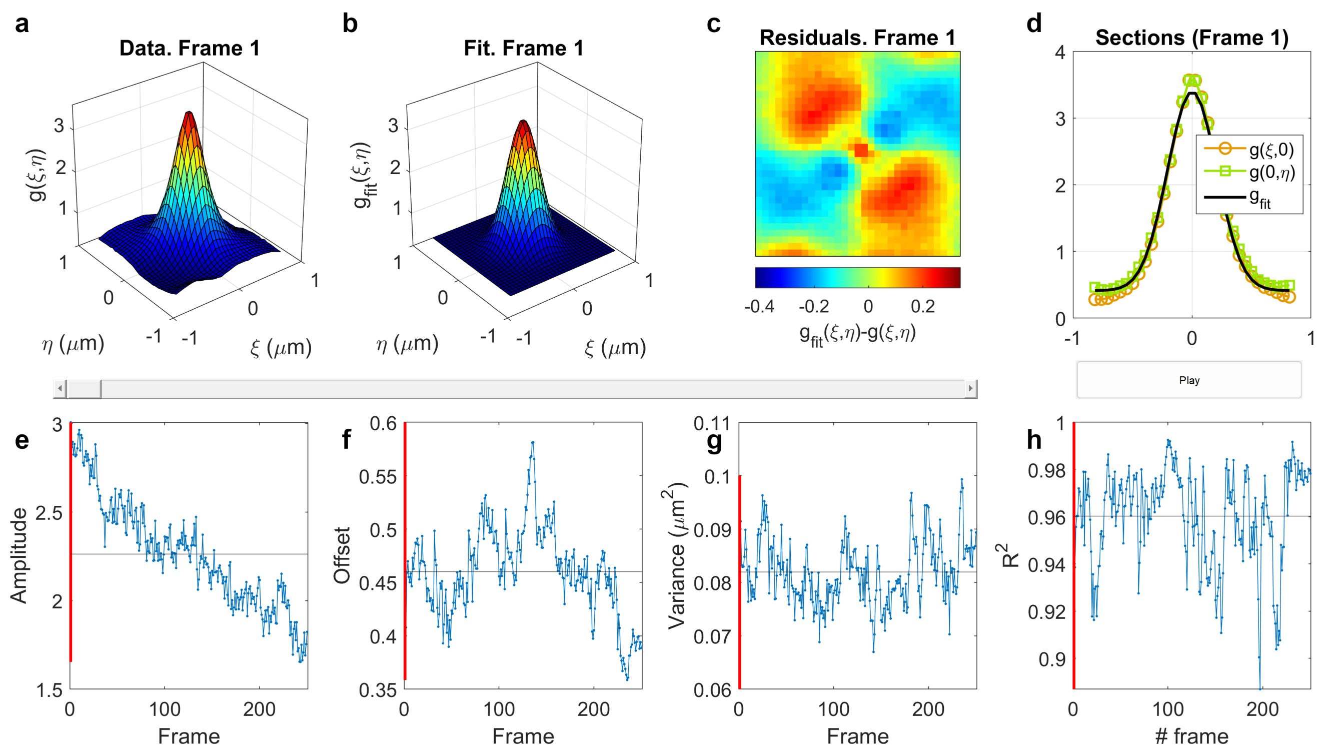

Figure 2. Spatial correlation viewer. (a) The spatial correlation function computed by iMSD_2026.m is reported for each frame, along with (b) the corresponding Gaussian fitting function, (c) the residuals plot, and (d) the sections at $\xi = 0$, and $\eta = 0$. Computed (e) Amplitude, (f) offset, (g) variance, and (h) R^2coefficient are shown. During the analysis, "play" button and slider bar allow users to move through the plots frame by frame.

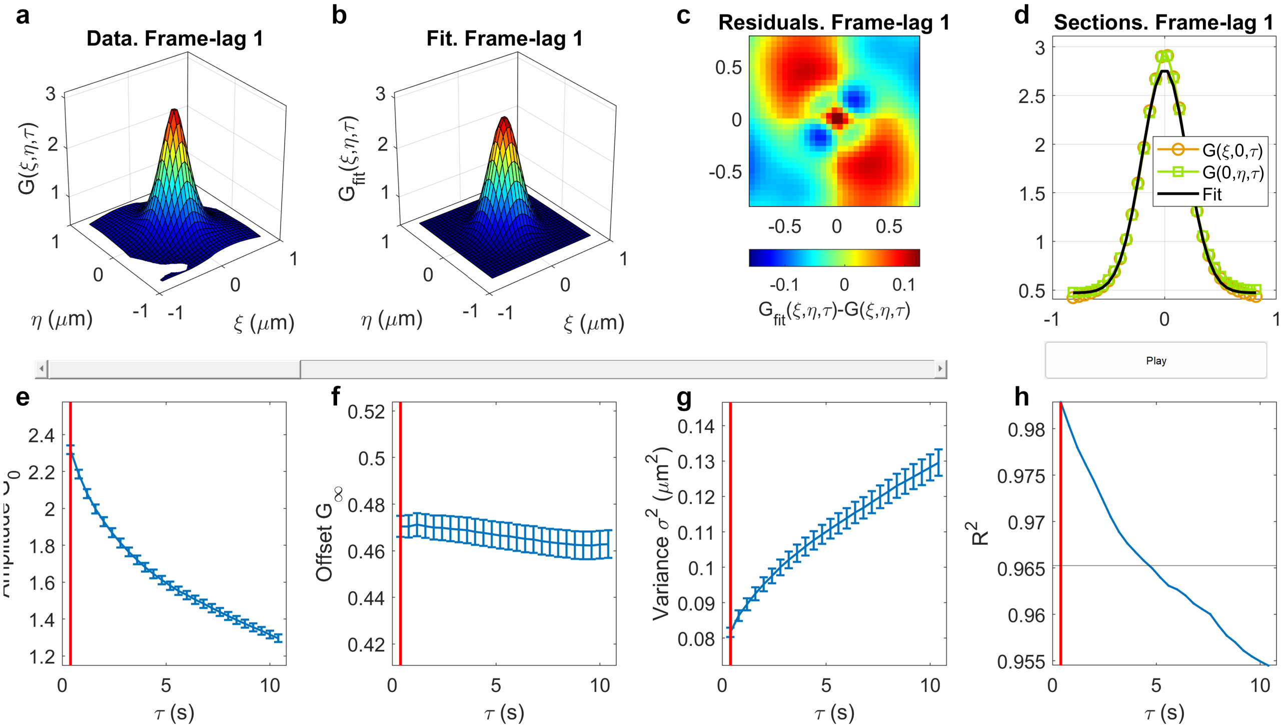

Figure 3. Spatiotemporal correlation viewer. (a) The spatiotemporal correlation function computed by iMSD_2026.m is reported for each time-lag τ, along with (b) the corresponding Gaussian fitting function, (c) the residuals plot, and (d) the sections at $\xi = 0$, and $\eta = 0$. Computed (e) Amplitude, (f) offset, (g) variance σ^2, and (h) R^2 coefficient are reported as functions of time-lag. During the analysis, "play" button and slider bar allow users to navigate through the plots at different time-lag points.

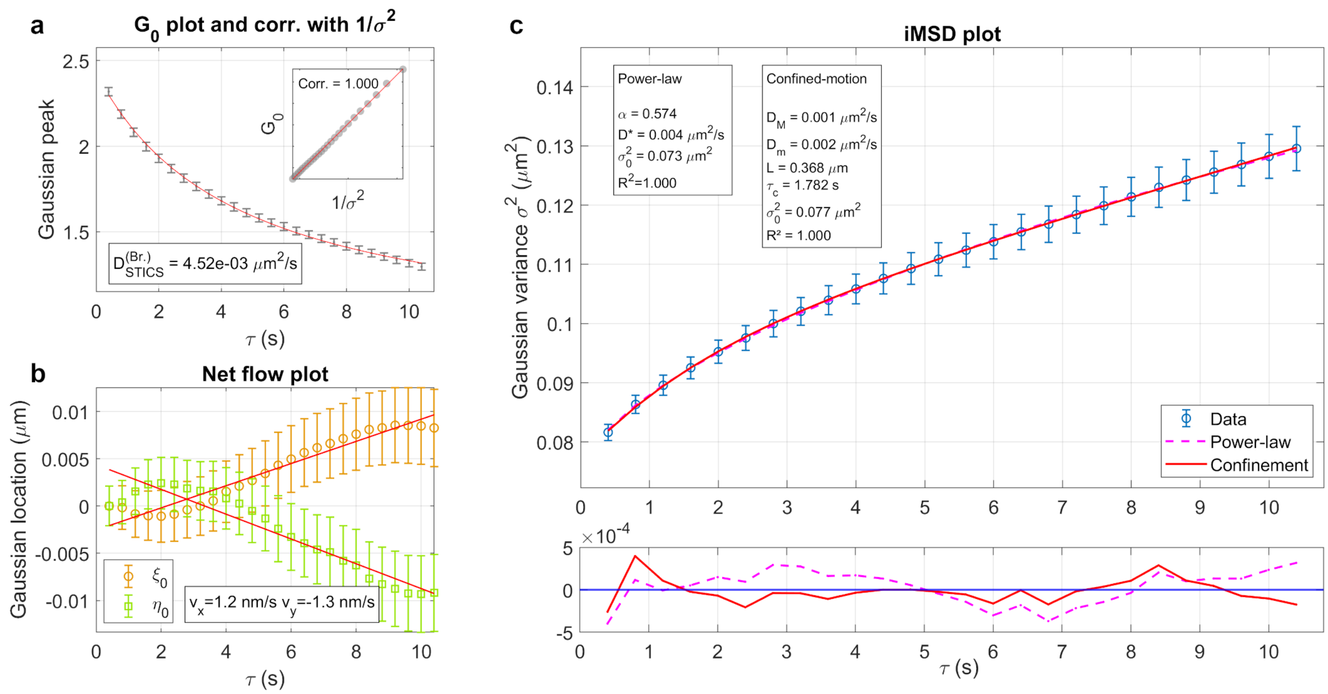

Figure 4. iMSD output viewer. (a) Amplitude G_0, (b) peak's location, and (c) variance σ^2 of the Gaussian function approximating the spatiotemporal correlation function. Correlation between G_0 and $1/\sigma^2$, computed net flow, and relevant dynamic parameters are reported in the insets.