Single Particle Tracking

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Introduction & Background

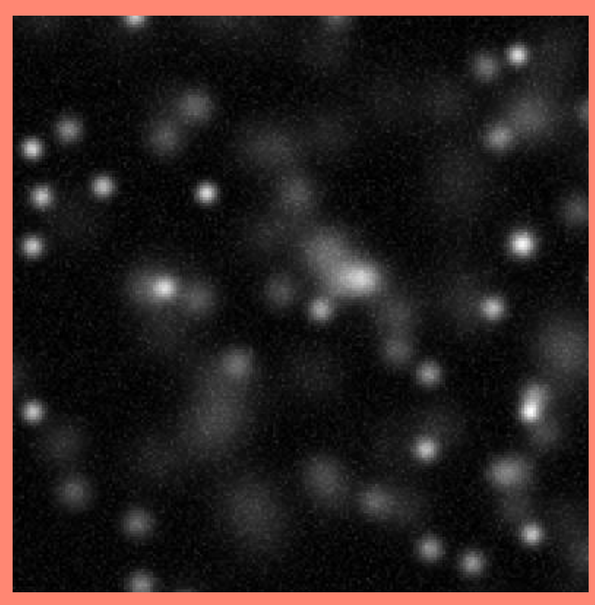
Single particle tracking (SPT) is a technique that deciphers the dynamic behaviour of individual particles by analysing raw fluorescence microscopy data. Multi-frame TIFF images capturing fluorescently labelled particles are processed using tools like Fiji's TrackMate, which leverages the point spread function for sub-pixel localization. This analysis yields trajectories from which key parameters—such as the diffusion coefficient and hydrodynamic radius—are extracted, providing insights into particle mobility and interactions at the nanoscale.

Aims and Objectives

The aim of this study is to establish an efficient workflow for quantifying particle dynamics from raw microscope data.
The objectives are to:
•Acquire high-quality multi-frame TIFF images of fluorescent particles.
•Enhance raw data quality through noise reduction and image processing.
•Use Fiji's TrackMate for precise particle detection and sub-pixel localization.
•Construct particle trajectories across frames.
•Calculate diffusion coefficients and derive hydrodynamic radii from the trajectories.

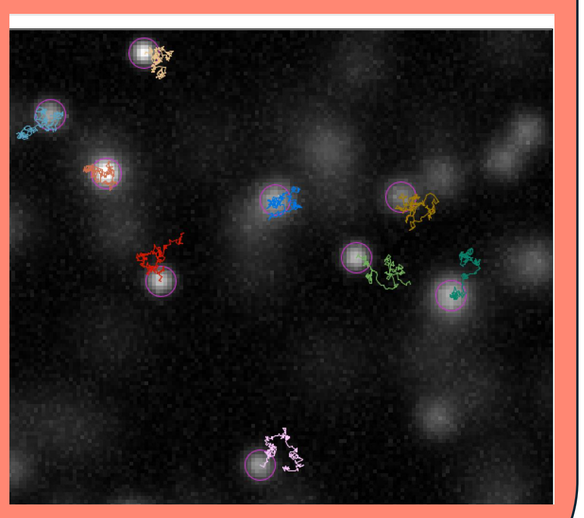
Data Collection:

Raw data is collected via fluorescence microscopy and saved as multi-frame TIFF images. Each frame captures the emission from fluorescently labelled particles under controlled illumination.



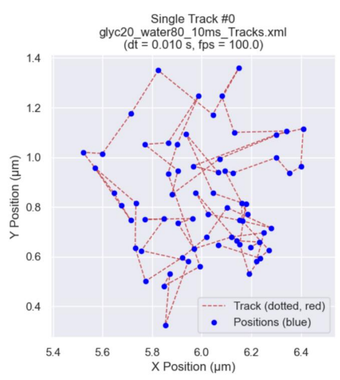
Data Conversion:

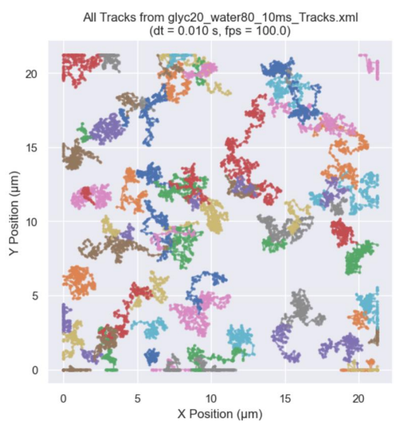
TIFF files are imported into Fiji's TrackMate, which uses the point spread function (PSF) for sub-pixel detection of fluorescent spots. After fitting intensity profiles to the PSF, TrackMate links detections across frames to form continuous trajectories, enabling robust motion quantification for further biophysical analyses

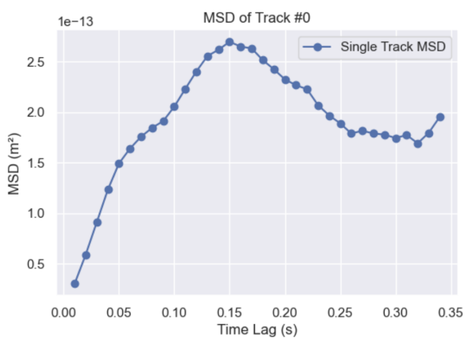


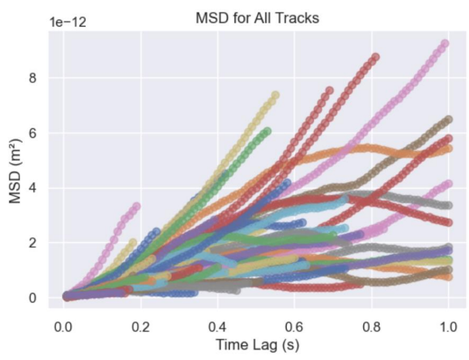
Data Analysis

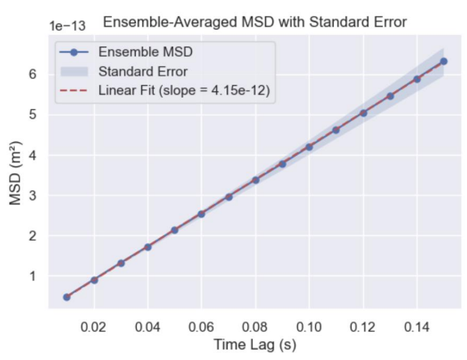
I wrote a code that accepts the continuous track field generated from TrackMate. The code extracts experimental parameters (like water percentage and frame interval) from the filenames, loads the particle tracks, and converts the positions from pixels to meters. It then computes the mean squared displacement (MSD) for each individual track and takes an ensemble average of all tracks. From the ensemble MSD, I estimate diffusion coefficients using a linear fit, and finally, I apply the Stokes–Einstein equation to calculate the hydrodynamic radius of the particles.

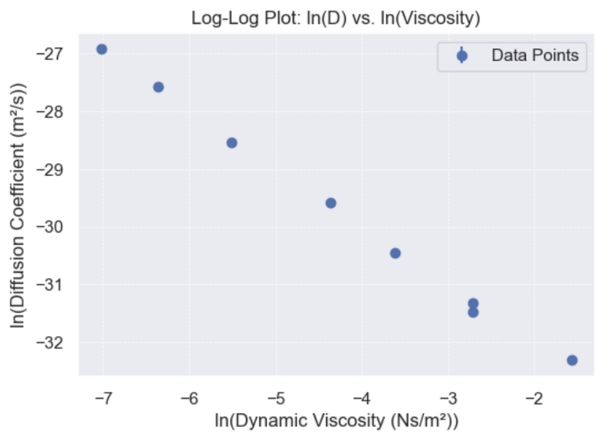


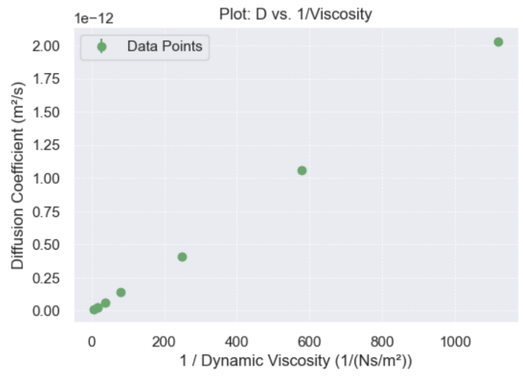












Conclusion

In conclusion, single particle tracking is a powerful technique that enables researchers to observe and quantify the behavior of individual particles in real time. This method has transformative applications across disciplines—from revealing the inner workings of cellular processes and molecular interactions in biology to informing the design of advanced materials and targeted drug delivery systems in engineering and medicine. By providing detailed insights at the nanoscale, SPT continues to drive innovation and deepen our understanding of complex systems in the real world.