

Real-Time DNA-PAINT Analysis: From Offline Processing to Live Microscopy Feedback

Introduction

DNA origami has emerged as a powerful platform for the construction of programmable nanoscale structures with applications in biophysics, nanotechnology, and molecular diagnostics. Combined with DNA-PAINT (DNA Points Accumulation for Imaging in Nanoscale Topography), these systems enable the observation and quantification of transient molecular binding events with single-molecule sensitivity.

Our group is currently studying the application of DNA-PAINT in the field of nanothermometry. This requires the production of large image datasets containing DNA-PAINT binding events, which are currently processed using a collection of Python scripts that extract fluorescence intensity traces, identify valid binding events, and quantify kinetic parameters. While the existing analysis pipeline produces scientifically meaningful results, it was developed primarily as a research tool and is becoming a significant bottleneck as datasets increase in size and complexity.

This project aims to redesign and modernise the analysis workflow, transforming it into a robust, scalable, and efficient software framework that will support ongoing research activities and enable future real-time data analysis during microscopy acquisition.

Goal

The proposed thesis aims at systematically analyse and redesign the existing python-based analysis workflow. The new software will then be benchmarked using existing dataset. The next step will then be to improve the traces categorization, by working on the selection parameters already included in the scripts. This will again be benchmarked against the previous software by using existing dataset. Lastly, the student will explore the integration between the new analysis pipeline and the microscopy acquisition. The objective will be to demonstrate the feasibility of generating a real time temperature readout.

Overview of the work steps

1. Optimisation and Refactoring of the Existing Analysis Pipeline

- Analyse and understand the current Python-based workflow for DNA-PAINT data processing and kinetic analysis.
- Identify computational bottlenecks through profiling and benchmarking of representative datasets.
- Rewrite the existing scripts into a modular, maintainable, and well-documented software package.

- Implement and validate performance improvements while ensuring full compatibility with the current analysis results.

2. Development of Improved Trace Selection and Quality Assessment

- Investigate the current trace evaluation strategy and identify limitations affecting robustness and reproducibility.
- Define quantitative metrics to assess trace quality and binding-event reliability.
- Develop and benchmark improved trace-selection algorithms using expert-annotated datasets.

3. Integration of Real-Time Analysis into the Microscopy Workflow

- Explore the integration of the analysis pipeline with the Micro-Manager microscopy platform through Python-based interfaces.
- Develop a proof-of-concept framework for processing images during data acquisition rather than after completion of the experiment.
- Implement live visualisation of extracted traces and analysis results alongside the ongoing measurement.
- Assess the computational requirements for real-time operation and investigate acceleration strategies, including parallel processing and optional GPU-based approaches.

Your profile

- Enrolment in a master's programme in Bioinformatics, Computer Science, Computational Biology, Data Science, Biomedical Engineering, Physics, or a closely related field
- Programming experience in Python (experience with NumPy, SciPy, pandas, scikit-image are desired, but not necessary)
- Familiarity with scientific computing concepts
- Interest in solving interdisciplinary research problems
- Ability to work independently while interacting regularly with researchers