

Super-Resolved Mapping of DNA Nanostar Hydrogels Reveals Ionic Modulation of Network Architecture

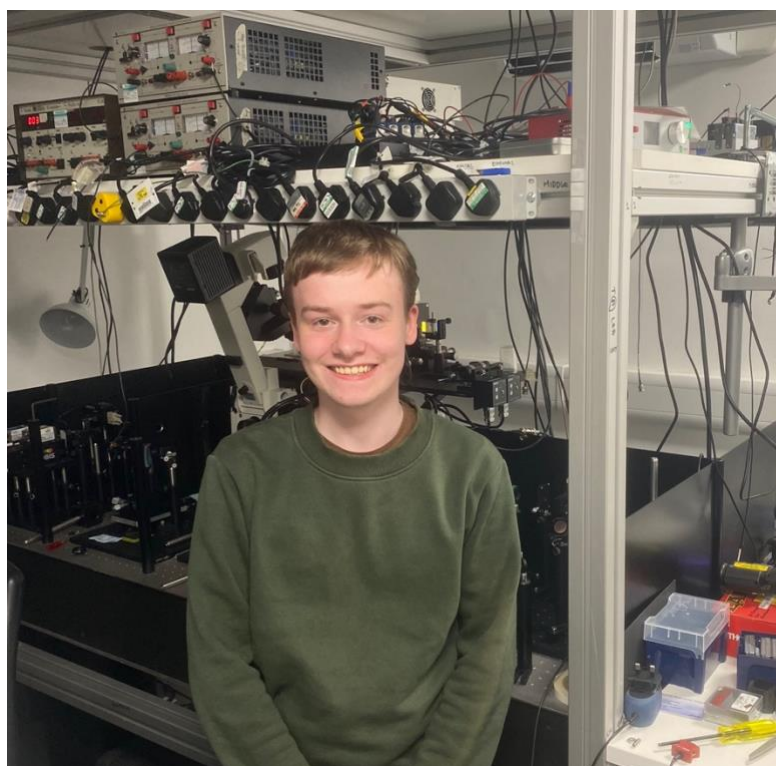
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Lay Summary

Biofilms are made up of a complex mixture of extracellular DNA, proteins and polysaccharides that surround bacterial colonies. This matrix reduces antibiotic penetration to pathogens which contributes to the rise of antibiotic resistance. Understanding the physical and biochemical properties of biofilms is therefore key to developing strategies that target both the bacteria and their protective environment. In this project, I worked with DNA hydrogels as a simplified model of biofilms. These hydrogels mimic the network structure of biofilms while allowing us to probe their physical properties under controlled laboratory conditions. Using intercalating fluorescent dyes and single-molecule localisation microscopy I investigated how changes in ionic strength perturb hydrogel organisation. To achieve this, I optimised experimental conditions for single-molecule imaging and developed an analysis pipeline using *pystachio* software. This pipeline detects dye blinking events, extracts single-spot brightness information and reconstructs super-resolved images of the network. This work demonstrates how DNA-based materials can be used to model biofilm organisation and provides a foundation for building more complex systems in the future. It also shows how environmental conditions influence the nanoscale organisation of biofilm-like networks.



Report

Aim

The aim of this project was to use DNA hydrogels as a model biofilm system and apply single-molecule localisation microscopy to investigate how ionic conditions affect network structure, while developing an analysis pipeline to reconstruct super-resolved images from dye blinking events.

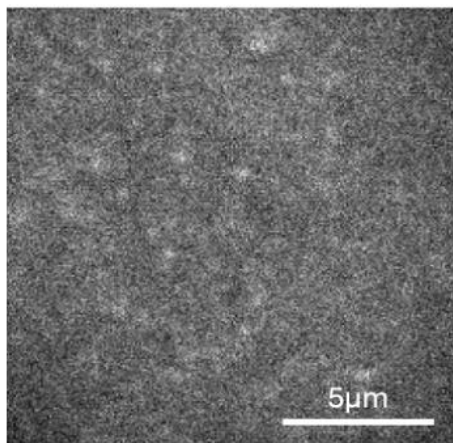
How I Addressed the Aim

To address this aim, I first prepared DNA hydrogels based on nanostar motifs which mimic the cross-linked networks found in biofilms. These hydrogels were stained with the intercalating fluorescent dye YOYO-1 that undergoes stochastic blinking under laser excitation which enables single-molecule localisation microscopy. Imaging was performed under total internal reflection fluorescence (TIRF) conditions, providing the sensitivity needed to capture individual dye molecules. I optimised imaging conditions to reliably detect dye blinking across different salt concentrations which perturb hydrogel organisation. Raw microscopy images were processed using the *pystachio* software package. The pipeline detects single fluorescent events, fits Gaussian functions to localise centroids and applies thresholding to separate true signals from background noise. By combining thousands of localisation events I generated super-resolved reconstructions of the hydrogel networks. This combined experimental and computational approach allowed me to directly assess how ionic strength influences the nanoscale structure of DNA hydrogels while also testing and refining a workflow for super-resolution analysis that can be extended to more complex biofilm models.

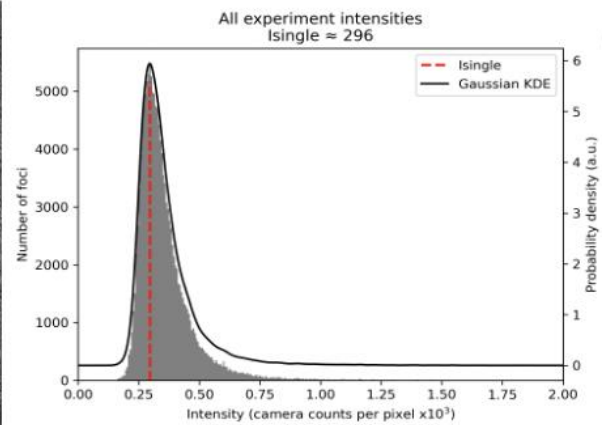
What I Found

The project successfully demonstrated that DNA nanostar hydrogels can be super-resolved using a blinking-based single-molecule microscopy approach and revealed how ionic strength affects their network organisation. In low ionic strength conditions (200 mM NaCl), reconstructions generated with a $\pm 30\%$ Gaussian intensity threshold revealed hydrogel networks that appeared diffuse. In contrast, at higher ionic strength (400 mM NaCl), the networks displayed heterogeneous organisation where regions of high density formed tightly clustered structures interspersed with areas of lower connectivity. This indicates that ionic conditions not only condense DNA nanostar assemblies but also introduce spatial variation into their organisation. Analysis was performed using the *pystachio* pipeline which fitted individual blinking events with Gaussian functions and generated point localisations for reconstruction. While the resulting images revealed clear nanoscale structure, resolution was limited by errors around centroid localisation which were not fully corrected in this first-pass analysis.

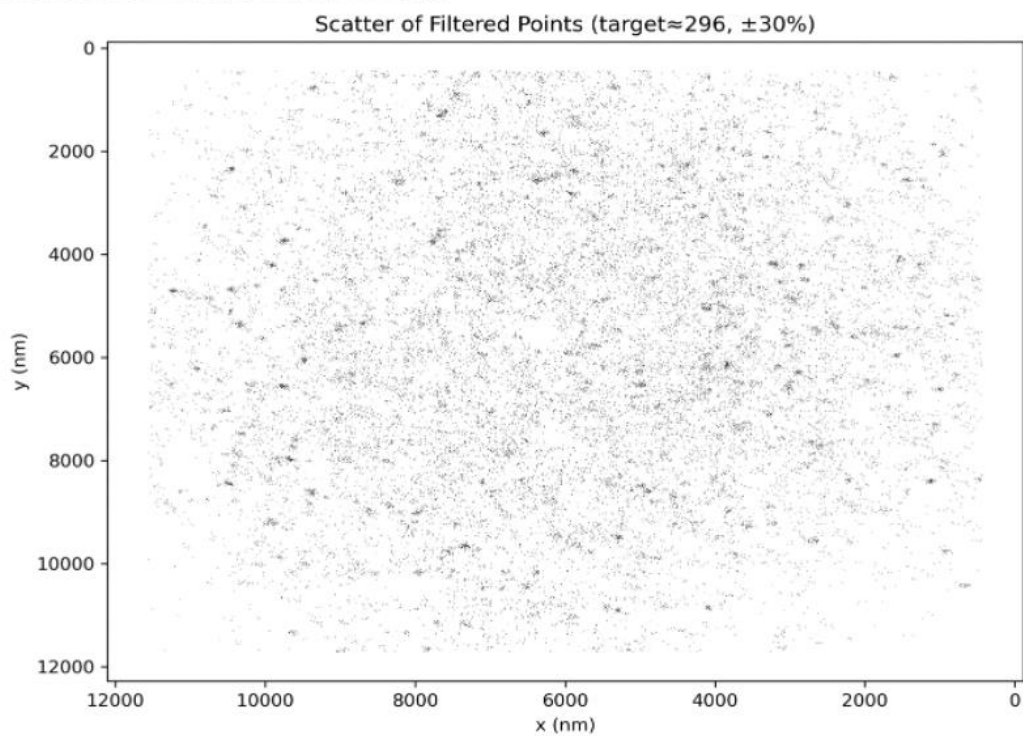
(a)



(b)



(c)



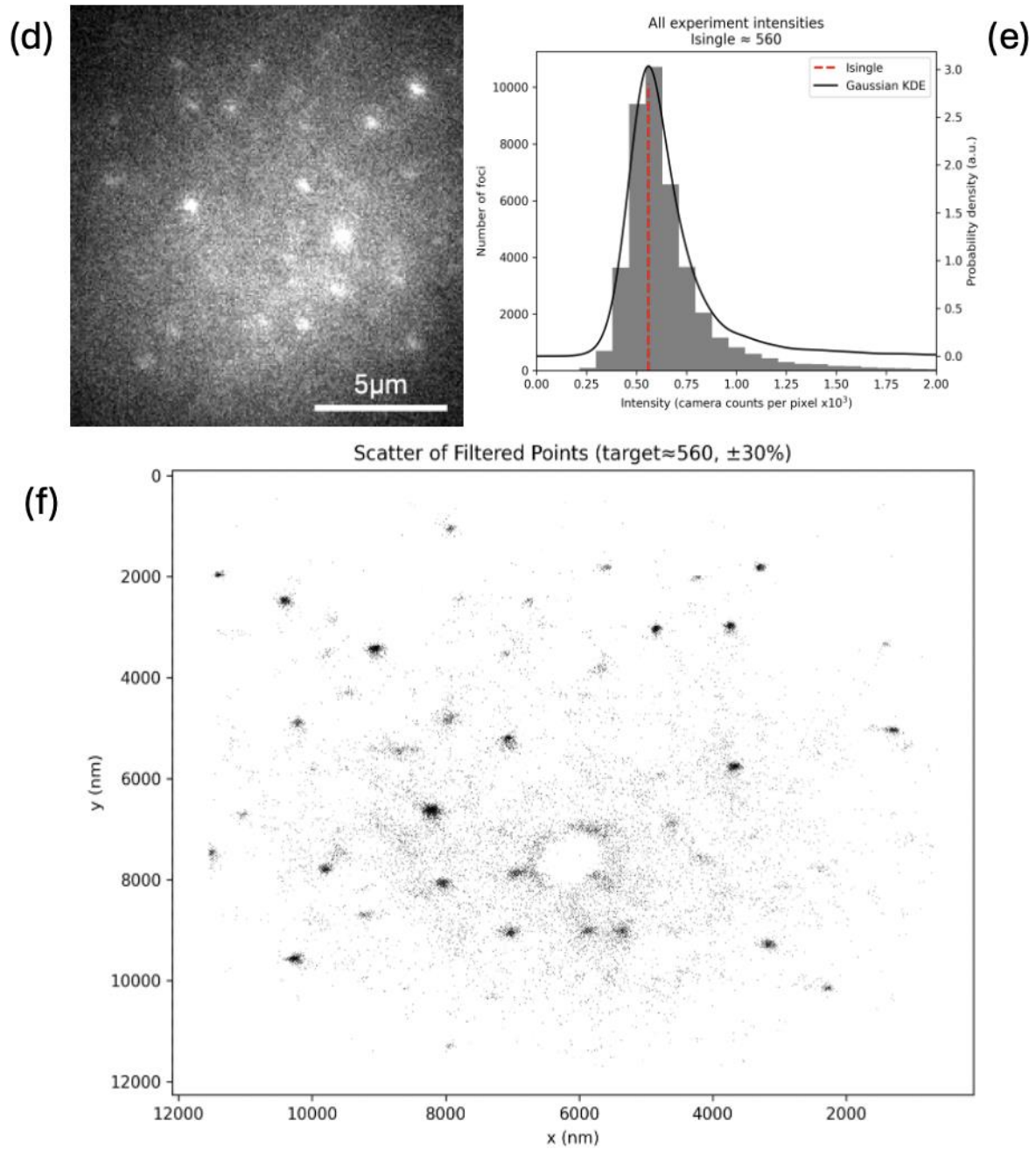


Figure 1. Super-resolution imaging and analysis of DNA nanostar hydrogels under varying ionic conditions. (a) Raw TIRF image of a DNA hydrogel stained with intercalating dye at 200 mM NaCl showing stochastic blinking events from individual dye molecules. (b) Representative Gaussian fit of fluorescence intensity profiles used to localise molecular centroids with sub-diffraction precision. (c) Scatter plot of localisations reconstructed from Gaussian fitting at 200 mM NaCl ($\pm 30\%$ intensity threshold applied) revealing a diffuse hydrogel network. (d) Raw TIRF image of a DNA hydrogel at 400 mM NaCl showing more spatially clustered blinking events. (e) Representative Gaussian fit of fluorescence intensity profiles used to localise molecular centroids with sub-diffraction precision. (f) Scatter plot of localisations reconstructed from Gaussian fitting at 400 mM NaCl ($\pm 30\%$ intensity threshold applied) revealing a heterogeneous network with tightly clustered regions interspersed with less connected areas.

Together, these findings provide proof-of-principle that stochastic blinking of intercalating dyes can be exploited to achieve super-resolution imaging of DNA nanostar hydrogels. Reconstructions revealed clear structural differences between low and high ionic strength conditions, highlighting the importance of the chemical environment in modulating hydrogel architecture at the nanoscale. These results provide a foundation for improved biofilm model systems and indicate where further refinement of the analysis pipeline could enhance localisation accuracy and resolution.

What I Learned

This project gave me hands-on experience in both experimental and computational aspects of single-molecule microscopy. I learned how to prepare and handle DNA hydrogels as a controllable model for biofilm structures and gained practical skills in operating TIRF microscopy for single-molecule imaging. On the analysis side, I developed a deeper understanding of how photo-blinking can be exploited for super-resolution and became confident in using the *pystachio* software to process raw image data. This involved working with Gaussian fitting, thresholding strategies and data reconstruction workflows. I particularly enjoyed the iterative troubleshooting process which involved optimising dye conditions, adjusting imaging parameters and refining analysis code. The project also highlighted the challenges of balancing experimental and computational approaches, teaching me to critically evaluate both data quality and computational pipelines — skills that will be invaluable in future research.

Long-Term Goals

This studentship has confirmed my enthusiasm for applying advanced microscopy to study biological systems. Moving from raw images to nanoscale reconstructions was particularly rewarding and I aim to continue developing expertise at the interface of biology, physics and computation. In the future, I hope to pursue a PhD in molecular or cellular biology with a strong imaging component, ideally focusing on how biophysical approaches can reveal the organisation of complex biological materials. This project also highlighted the importance of computational pipelines for modern microscopy, motivating me to further develop my coding and quantitative analysis skills. Overall, this experience confirmed my desire to pursue a research career and provided valuable skills that will help me contribute to imaging-based investigations in the future.