A Nano-Toolbox for Biomolecular Fluorescence Imaging

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Abstract

Diagnosis and monitoring in biochemistry is presently based on measuring average concentration, molecular form and bioactivity of large ensembles of proteins and other macromolecules, thereby losing sensitivity and the ability to discriminate amongst the range and pattern of biomolecular heterogenity in pathology.

This project seeks to overcomes this limitation by combining the confinement and control of single molecules trapped in nanopores with high-resolution fluorescence imaging techniques.

The industrial collaborators of this project are a Glasgow-based fluorescence division of HORIBA Scientific, which is one of the largest scientific instrument manufacturers globally and the National Physics Laboratory Biotechnology Division.

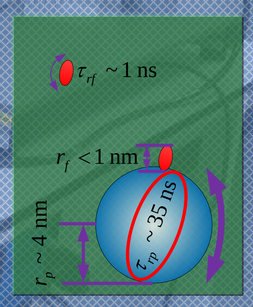
Decay Measurements & Nanometrology

Fluorescence decay measurements are performed via the Time Correlated Single Photon Counting (TCSPC) method using a HORIBA Scientific FluoroCube® combined with the state of the art electronics of the Deltahub® and high repetition rate of the DeltaDiode® pulsed light sources. For nanometrology of silica nanoparticles, dyes were electrostatically or covalently attached to the nanoparticles. Fluorescence lifetime curves were recorded with polarisers set at V and H orientations to construct an anisotropy decay curve.

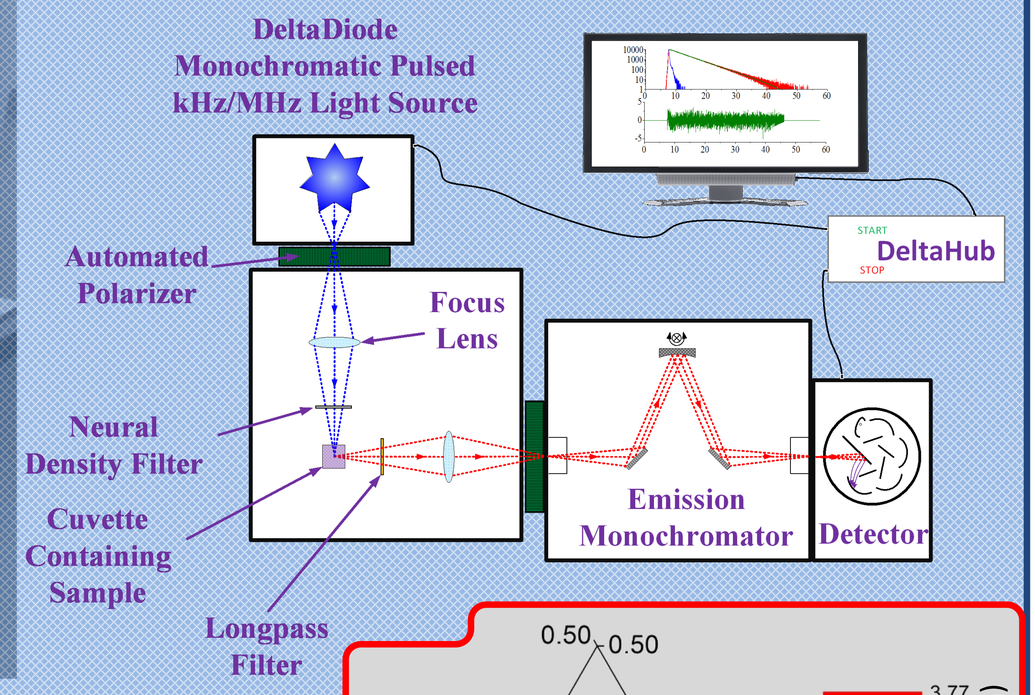
$$R(t) = \frac{\overbrace{I_{VV}(t) - GI_{VH}(t)}^{\text{Difference}}}{\underbrace{I_{VV}(t) + 2GI_{VH}(t)}_{\text{Sum}}}$$

$$\tau_{rp} = \frac{\eta V_h}{kT} \Rightarrow R = \sqrt[3]{\frac{3\phi_c kT}{4\pi\eta}}$$

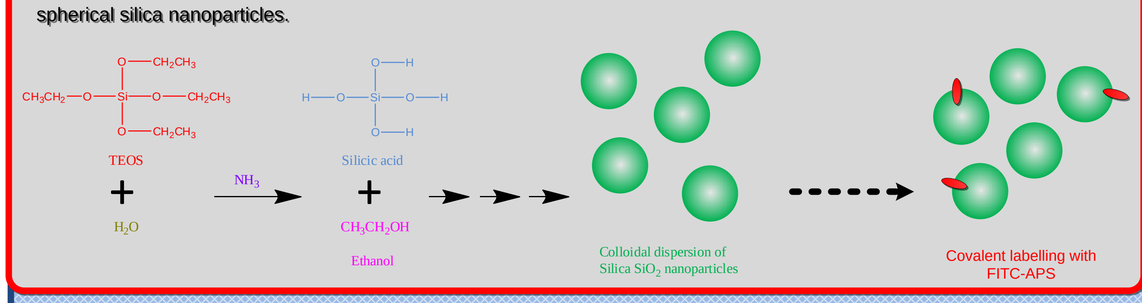
$$G = I_{HV} / I_{HH}$$

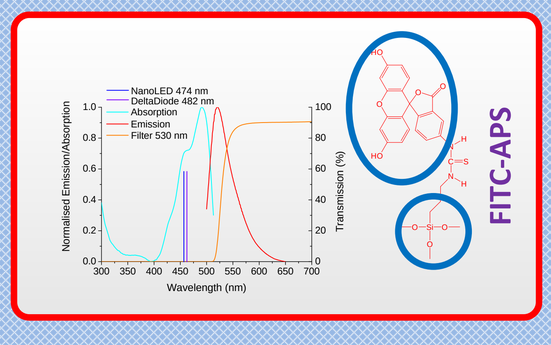


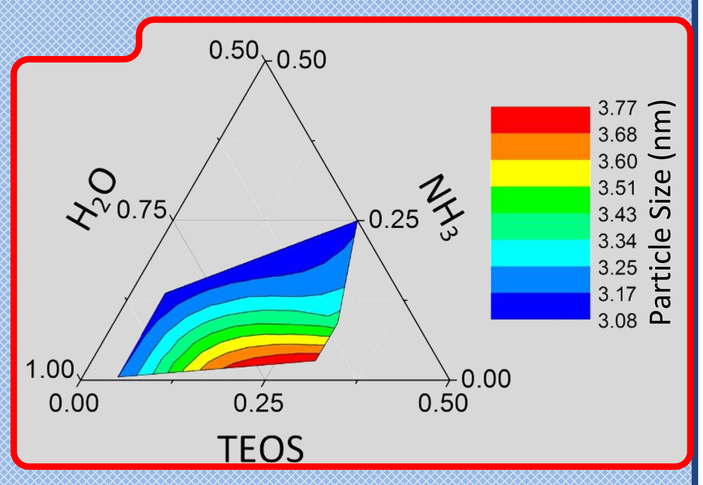
By fitting exponentials to the sum and difference one may find the rotational correlation time, which is the time it takes a nanoparticle to rotate by one radian. From this time, one can calculate the hydrodynamic volume and hence radius.



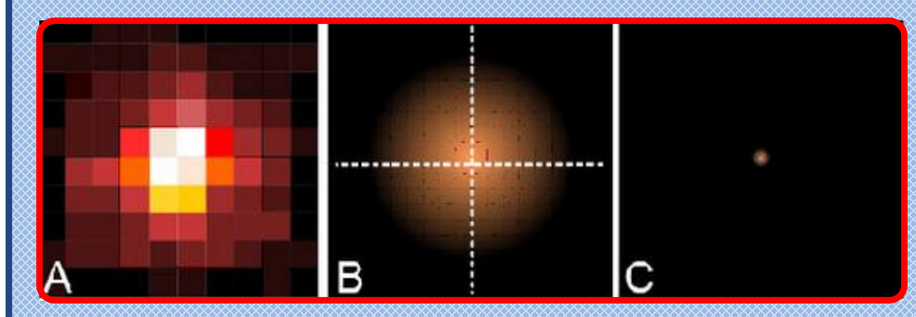
Silica nanoparticles were created via Stöber synthesis, the ammonia-catalysed reaction of an orthosilicate e.g. tetraethylorthosilicate (TEOS: $Si(OR)_4$; RC_2H_5) with water in low-molecular-weight alcohols to produce monodispersed, spherical silica nanoparticles.



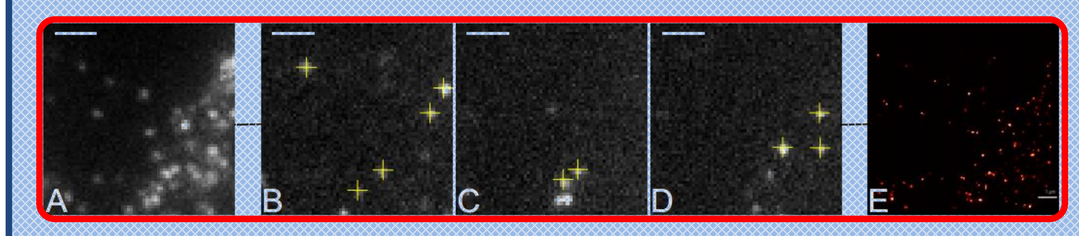




Super-resolution Microscopy dStorm



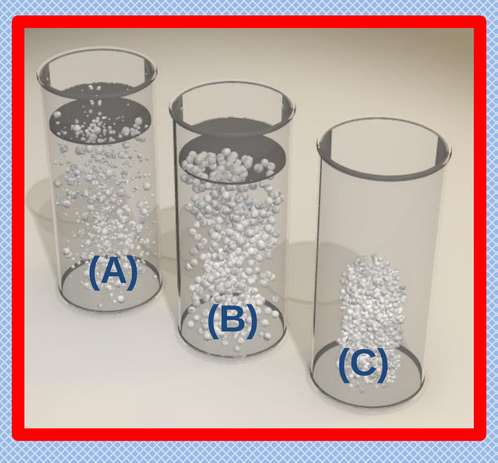
One main challenge in light microscopy is the ability to distinguish features that are close together; the best optical resolution is ~250 nm due to the diffraction limit. This is too large to observe the interplay of molecules and structures within cells. The image of a single molecule (A) can be analysed and fitted with a 2D gaussian function (B) to determine the actual localisation with nanometer precision (C). This is the principle behind the localisation-based super-resolution approaches, such as dSTORM (direct stochastic optical reconstruction microscopy) and PALM (photoactivated localisation microscopy).



(A) A large number of molecules closely-spaced within the diffraction limit . In order to localise them, dSTORM uses blinking fluorescent dyes. (B-D) Multiple images are recorded ~10,000. In each image a random subset of dye is fluorescing. Localisation of each blink (yellow crosses) is carried out and processed to create (E) a final super-resolution image. Resolution can be resolved to ~20 nm. Scale bars are 2 µm.

Future Work

Further work will be carried out with Hydrogels; another type of silica sol-gel created by the controlled mixing of Sodium Silicate, Water and Sulphuric Acid. The physical properties of Hydrogels are highly influenced by variables such as pH and temperature. The particle size with respect to time will be investigated by use of fluorescence anisotropy and the DeltaHub electronics so that a robust protocol can be established. As the sol-gel is hydrated and biocompatible, the aim is to encapsulate single biomolecules/systems within the gel. This project will also incorporate other work being carried out with within the group – gold & silver surface plasmon signal enhancement with the aim being to control, preserve and study single biomolecules/systems.



Sol to gel transition: (A) A nanometre scale particles composed of clusters of silica form. (B) Nanoparticles merge and form a growing network. This spans the containing vessel at a time t_g. (C) The network shrinks forming pores.

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