A Nano-Toolbox for Biomolecular Fluorescence Imaging

Philip Yip
Email: ph.yip@googlemail.com
Supervisor: David Birch (Strathclyde). Yip Chen (Strathclyde), Alex Knight (NPL), Daniel MacColl (NPL), Graham Hungerford (HORIBA)
University of Strathclyde Photophysics Website: http://www.photophysics.strath.ac.uk

Absorbed Scientific Fluorescence Spectroscopy Website: http://www.horiba.com/scientific/products/fluoresent-spectroscopy/

Departmental Address: Photophysics Research, Department of Physics, John Anderson Building, University of Strathclyde, 217 Cathedral Street, Glasgow G4 0NG, United Kingdom

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 heterogeneity in pathology. This project seeks to overcome this limitation by combining the confinement and control of single molecules trapped in nanoparticles 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.

\[
R(t) = \frac{I_V(t) - G I_H(t)}{I_V(t) + 2 G I_H(t)} \\
G = \frac{4 \pi n^2}{3} \frac{kT}{n^2} \frac{n}{4 \pi n^2}
\]

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.

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 (phototivated localisation microscopy).

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 within the group – gold & silver surface plasmon signal enhancement with the aim being to control, preserve and study single biomolecules/systems.

References

HORIBA Scientific Website: http://www.horiba.com


Publications


A Nano-Toolbox for Biomolecular Fluorescence Imaging

Philip Yip
Email: ph.yip@googlemail.com
Supervisor: David Birch (Strathclyde). Yip Chen (Strathclyde), Alex Knight (NPL), Daniel MacColl (NPL), Graham Hungerford (HORIBA)
University of Strathclyde Photophysics Website: http://www.photophysics.strath.ac.uk

Absorbed Scientific Fluorescence Spectroscopy Website: http://www.horiba.com/scientific/products/fluoresent-spectroscopy/

Departmental Address: Photophysics Research, Department of Physics, John Anderson Building, University of Strathclyde, 217 Cathedral Street, Glasgow G4 0NG, United Kingdom

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 heterogeneity in pathology. This project seeks to overcome this limitation by combining the confinement and control of single molecules trapped in nanoparticles 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.

\[
R(t) = \frac{I_V(t) - G I_H(t)}{I_V(t) + 2 G I_H(t)} \\
G = \frac{4 \pi n^2}{3} \frac{kT}{n^2} \frac{n}{4 \pi n^2}
\]

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.

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 (phototivated localisation microscopy).

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 within the group – gold & silver surface plasmon signal enhancement with the aim being to control, preserve and study single biomolecules/systems.

References

HORIBA Scientific Website: http://www.horiba.com


Publications