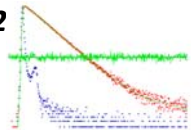


# Visualising local viscosity using fluorescence lifetime microscopy

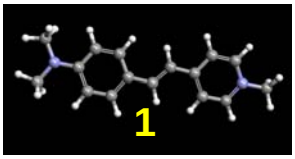
***The use of fluorescent molecules, known as molecular rotors, is advantageous in estimating the local (nanoscale) viscosity in microheterogeneous systems, since it just requires the measurement of their fluorescence lifetime. This measurement is simpler to perform using microscope systems as corrections for photobleaching do not need to be made, as may be needed if a fluorescence anisotropy measurement was performed. The usage of molecular rotors is demonstrated using the HORIBA Scientific DynaMyc to monitor the gelation of a polysaccharide film.***

## Polysaccharide films made from Gellan Gum

Polysaccharides are biologically important polymeric molecules, in which repeat units of monomers are linked via glycosidic bonds. These carbohydrates play an important role in animal and plant nutrition and structure. Gellan gum consists of a linear tetrasaccharide repeating unit and this versatile polysaccharide has found many applications, ranging from the food industry to drug delivery and tissue engineering applications. Upon heating the helical polysaccharide backbone undergoes a helix to coil transition accompanied by a large change in viscosity. Subsequent cooling produces a *sol* to *gel* transition at 33 to 34°C, which is close that that of biological interest. There have been many reports evaluating its viscosity behaviour over a range of concentrations and upon addition of metal ions, as cations help to crosslink gellan's negatively charged polysaccharide helices, forming helix-helix aggregates. Usually studies on its viscosity measure the bulk property, but as interest in its biological application has increased, including its ability to produce biocompatible films, this has lead to a need to examine changes on the microscope scale.

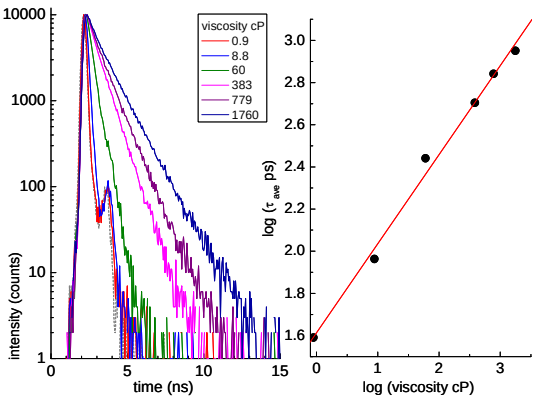
## Molecular rotors

A well known feature of fluorescence is the sensitivity of a fluorophore to its microenvironment (ie viscosity change). Normally a fluorescence anisotropy measurement, where rotational molecular diffusion is monitored, maybe considered, but there are fluorophores that undergo a viscosity dependent intermolecular rearrangement that effects their fluorescent lifetime. This makes the decay acquisition faster by reducing the number of measurements and in the case of microscopy, avoids problems associated with photobleaching. An example of such a molecule, made use of in this work, is shown below.



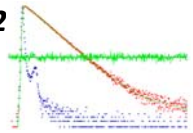

*The stilbenoid dye DASPMI*

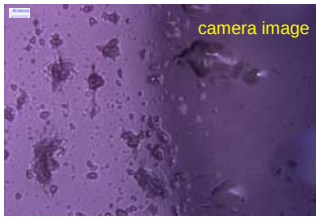
In order to make use of this phenomenon a "calibration" is made by measuring their fluorescence lifetimes in solutions of known viscosity (this should produce a linear calibration on a log-log plot of lifetime vs viscosity). An example of the fluorescence decays, measured on a HORIBA Scientific *TemPro*, and a plot for the average lifetime of DASPMI at different viscosities is shown in the figure below.



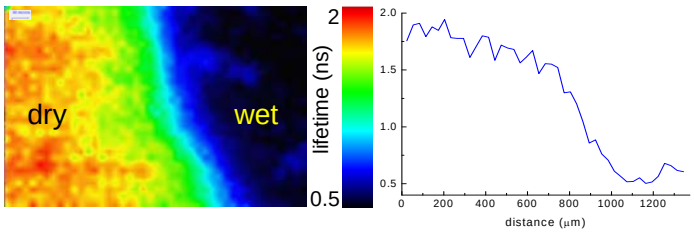

## Monitoring the viscosity of gellan gum

The measurements were carried out using a *DynaMyc* (shown above) with FLIM (fluorescence lifetime imaging) performed making use of a 470nm emitting *DeltaDiode* laser running at 25MHz. A drop of dye containing liquid gellan gum was placed on a microscope slide and allowed to dry at ambient conditions. A drying sample is shown below in a recessed (long side approx 1cm) well on a slide.



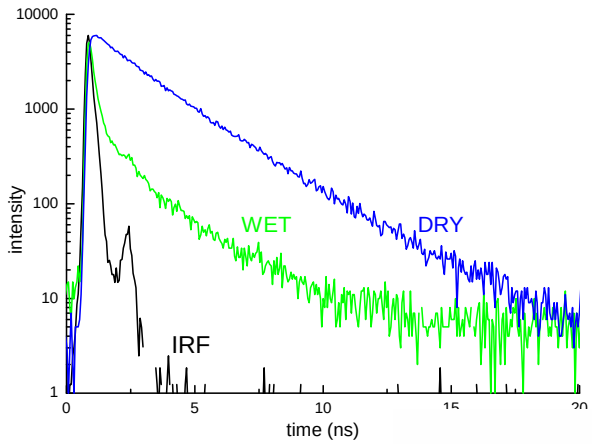




An area across the drying front was chosen for imaging (see above for approx. selection area and CCD camera image of the area viewed through a beam splitter using the microscope illuminator). The corresponding FLIM measurement is shown below,




This shows a decrease in the average lifetime, illustrated using a rainbow scale, on going from the drier region to the wetter one (left to right, with example cross-section). This corresponds to a decrease in viscosity. Making use of the lifetime "calibration" allows the estimation that the viscosity increased ~600 times from the wet to dry region.

It is also possible, using the microscope, to monitor the fluorescence lifetime of DASPMI at a single point, as the gellan gum film dries. Examples of the fluorescence decays obtained as given below,




This shows decays from which the average lifetime can be obtained using DAS6, after reconvolution with the instrumental response (IRF), which was obtained via scattering light from the microscope slide.

**Summary**

The time-resolved measurements made on the compact *DynaMyc* show that using fluorescence lifetime measurements it is possible to spatially monitor changes in viscosity associated with a gelation and drying process.

The results shown in this note are based on the following the paper,

G. Hungerford, M. Toury, D. McLoskey, N. Donaldson, A.S. Holmes-Smith, 2012. *In-situ formation of silver nanostructures within a polysaccharide film and its application as a potential biocompatible fluorescence sensing medium*. Soft Matter, 8, 653-659.