

## Real Time Measurement of Actin Polymerisation by Dual Polarisation Interferometry

### Introduction

This application presents Dual Polarisation Interferometry (DPI) as an important tool for investigating and understanding how structural proteins function. Actin is a structural protein that forms microfilaments *via* the polymerisation of actin monomers. These actin microfilaments are important for cell locomotion, cell structure, secretion and cell division. It is believed that actin polymerisation is nucleated by the actin binding protein gelsolin.

We were interested in using DPI to measure the time-dependent structural changes that occur when actin monomers were added to a gelsolin surface, and comparing these structural changes, expressed in terms of layer thickness, density and mass, to those occurring on a surface without gelsolin present in a parallel experiment. Such information cannot often be achieved from the measurement of mass change alone

### Experimental

The DPI experiments were performed on a Farfield dual-channel **AnaLight<sup>®</sup> Bio200** instrument. The surface used in all studies was an amine-derivatised **AnaChip<sup>™</sup>**. The temperature of the samples was controlled throughout to 20°C. Water used in buffer preparation was deionised and free of organic impurities. All buffers and reagents were analytical grade or higher, and solutions were degassed prior to use.

#### Preparation of Gelsolin Surface

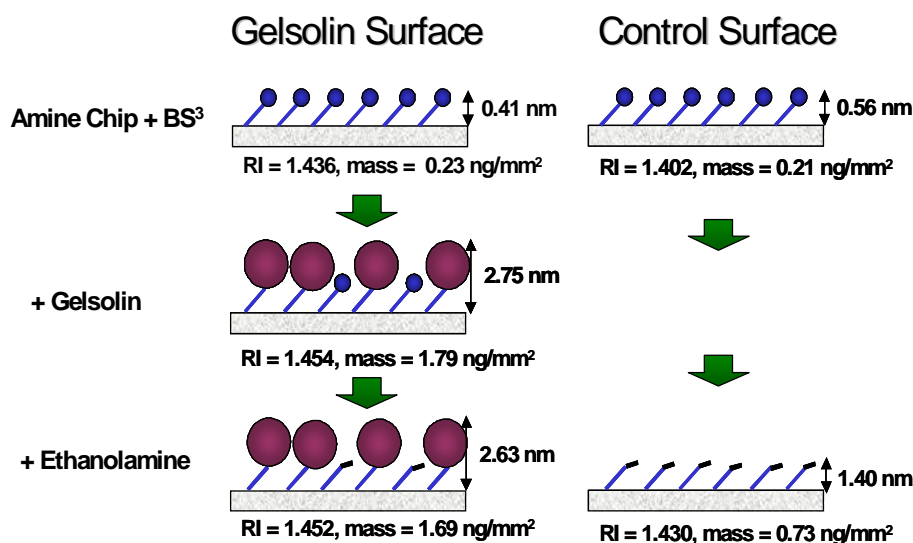
Phosphate running buffer (PBS, aqueous, pH8.0) was flowed over both channels of the **AnaChip<sup>™</sup>** surface at 100µl/min until equilibration. The amine-amine linker BS<sup>3</sup> (bis[sulfosuccinimidyl]suberate, 2 mg/ml in PBS) was added to both channels (experimental and reference) for 3 minutes at 100µl/min. Gelsolin solution (17.3µM in PBS) was then added for 6 minutes at 50µl/min onto channel 1 (experimental channel) alone. Ethanolamine (pH 8.0, 0.1M in PBS) was then added for 2 minutes at 100µl/min to both channels to block any unreacted BS<sup>3</sup>.

#### Polymerisation of Actin Monomers

Once the deposited gelsolin layer was stable in the running buffer, the buffer system was switched to buffer G+KM (pH8.0), a specialised tris-based buffer that contains calcium ions and supports actin polymerisation. Actin monomer solution (11µM in buffer G+KM) was then added at 100µl/min for 2.5 minutes to both channels on the **AnaChip<sup>™</sup>**. The flow was then stopped, and both channels were incubated under no flow for 80 minutes.

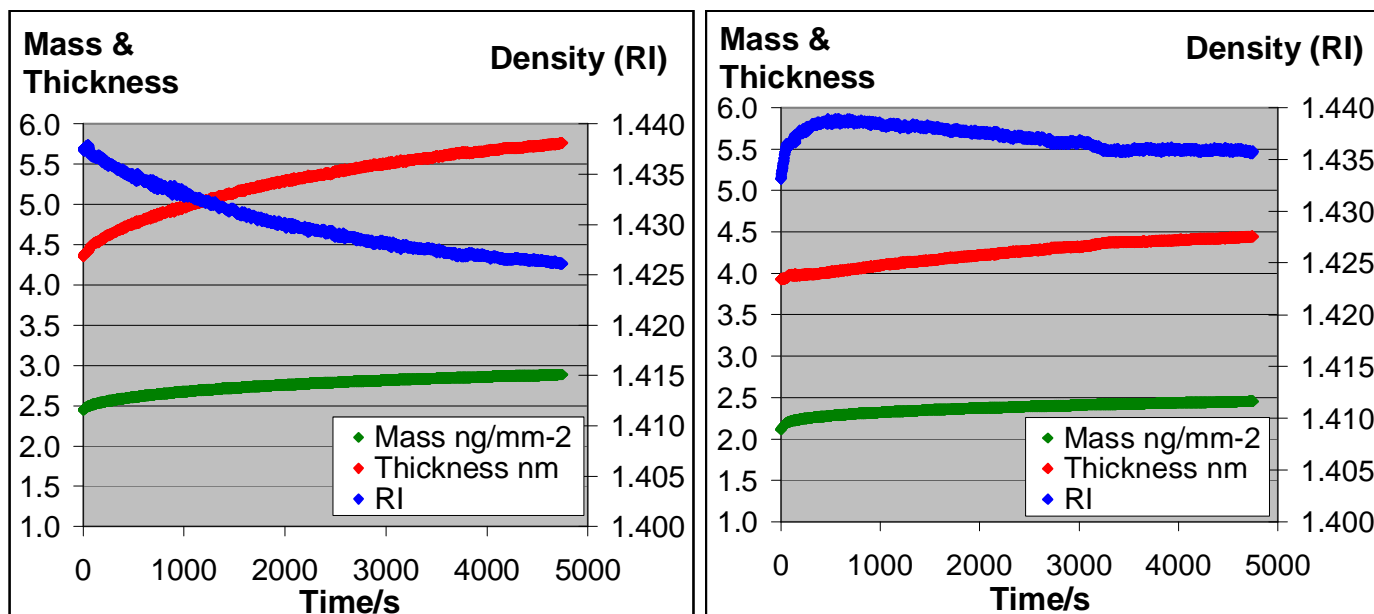
### Results and Discussion

**Figure 1** shows a visual representation of the surface substructures for each channel on the **AnaChip<sup>™</sup>**.



**Figure 1: Visual representation of formation of gelsolin surface (channel 1) and control surface (channel 2)**

**Figure 2** shows the results from the subsequent addition of actin monomers to the two measurement channels on the **AnaChip™**. A gradual increase in mass with time (*green lines*) can clearly be seen on both the gelsolin surface and the “control” surface during the incubation period with actin monomers. This confirms the accumulation of actin on both surfaces but, as the rates of mass change are similar for both channels, it is difficult to deduce any further information from these mass profiles alone.



**Figure 2: Real time mass, density (RI) and thickness values for the actin structural changes during incubation over the gelsolin surface (left) and “control” surface (right)**

If we consider the thickness (*red lines*) and density (refractive index, *blue lines*) profiles for the two channels we can gain a different level of understanding about the two different processes taking place. On the gelsolin surface, a gradual increase in thickness accompanied by a gradual decrease in density is observed during the incubation with actin monomers. This is a classic signature of filamental growth at the surface and firmly indicates polymerisation of actin. The thickness and density profiles during actin incubation over the “control” surface are consistent with the simple physisorption of actin molecules onto the surface. A dense actin layer forms in the initial stages of incubation followed further actin molecules gradually adsorbing into a second, less dense layer for the remainder of the incubation.

## Conclusions and Benefits

DPI provides measurements and mechanistic information on structural changes taking place in proteins. This experiment shows the time dependent changes that occur when actin polymerises on a gelsolin surface, and distinguishes the polymerisation process from that occurring on a control surface. The **AnaLight®** instruments and their experimental protocols give the researcher a unique combination of high-resolution data in real time on thickness, refractive index (density) and surface coverage from a bench-top technique. The **AnaLight®** is an important enabling tool for protein structural studies, giving the researcher:

- Structural information not available from the mass measurements provided by traditional biosensors
- Clearer understanding of the molecular mechanisms involved in protein polymerisation
- High resolution, real time measurements of protein structural change from a bench-top instrument
- The capability to avoid the limitations and ambiguities that are inherent in other techniques for such studies

**Farfield gratefully acknowledges that these experiments were carried out according to a protocol and using samples provided by Dr Sutherland Maciver from the Division of Biomedical Sciences at the University of Edinburgh, United Kingdom.**

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