

# illuminations

Issue 5

## Farfield Achieves NPL Recognition



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Welcome to another edition of illuminations. In this issue we mainly focus on the key application areas for our **AnaLight**® product range, along with some company news.

As ever, we would welcome your feedback, comments and suggestions regarding this edition of 'illuminations'. We invite you to submit your ideas and articles for publication in future editions, and look forward to hearing from you.

Jo Maltby, Marketing  
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### *January 2006: National Physical Laboratory Verifies Farfield's Measurement Technology*

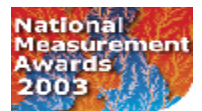
The National Physical Laboratory, Teddington, United Kingdom ([www.npl.co.uk](http://www.npl.co.uk)) has recently completed and reported the full verification of the underlying theoretical basis for Farfield's Dual Polarisation Interferometry (DPI) technology and measurements. Farfield markets first-generation DPI technology in its **AnaLight**® instrument series for quantitative measurement of dynamic structural change at the molecular level, for biophysical and nanotechnology applications.

The verification exercise was undertaken through the Department of Trade and Industry's National Measurement System Directorate Joint Industry Project (NMSD JIP) initiative and means that the basis of DPI measurements has now been confirmed by one of the world's leading independent centres of excellence in research, development and knowledge transfer in measurement and materials science. For more than a century, NPL has developed and maintained the UK's primary measurement standards and ensured accuracy, consistency and innovation in physical measurement.

Dr Neville Freeman, Development Director at Farfield Sensors commented, "Farfield is delighted to have received this influential endorsement. Combined with the host of other awards and recognition that DPI has received over the last two years, this again shows that Farfield's products are firmly rooted in the principles of quantitative, scientific measurement and analytical excellence".



INVESTOR IN PEOPLE



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## Farfield

illuminating the molecular world...

# Farfield House Opens its Doors!



January 2006

**Farfield Sensors Limited** completed the formal relocation to its new UK headquarters with the official opening ceremony for Farfield House on 18<sup>th</sup> January 2006.

Farfield House is a new, purpose built facility on Crewe Business Park, Cheshire, UK. The move to Crewe will allow Farfield to enjoy the benefits of a modern business environment whilst maintaining the company's traditionally strong commercial links with the industrial and academic research community in the North of the UK. Farfield House represents a major step forward for the company by providing increased office space, training and seminar facilities and a state-of-the-art applications laboratory.

Gerry Ronan (Farfield's CEO) and Paul Barraclough (Farfield's Chairman and Managing Director of Worknorth II) jointly hosted the ceremony, during which James Keaton, Vice President of the University of Liverpool, officially declared Farfield House open.



Attendees at the ceremony and networking event included a wide cross section of Farfield's customers, business partners and representatives from organisations who have contributed to Farfield's continued success.



The last twelve months has seen a rapid expansion for Farfield's business into a number of new export markets, and the opening ceremony marked the official completion of the company's move.

Gerry Ronan described proceedings as "a tremendously exciting and rewarding day. This expansion will underpin Farfield's growth and extend our support capability for our worldwide community of users. The expansion and relocation are a direct result of our unique positioning in the biophysics and nanotechnology marketplaces and demonstrates the pull from these markets for our unique measurement capabilities".



**Farfield**  
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# Spotlight on Applications

## Detection of Early-Stage Protein Crystallization using DPI

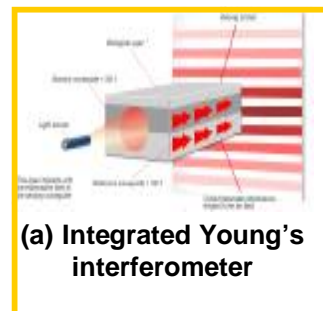
Data first presented as a poster at BioScience 2005, 17<sup>th</sup>-21<sup>st</sup> July 2005, Glasgow, UK

### Introduction

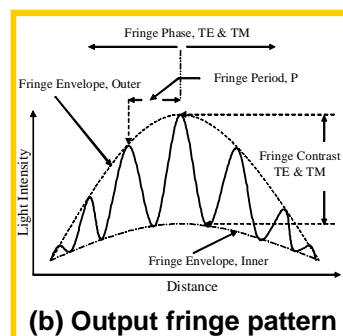
The three-dimensional structure of a protein, which is key to understanding its biological function and plays a critical role in drug design and discovery, is predominantly determined by X-ray crystallography. However, the growth of suitable protein crystals remains a major bottleneck in structural determination, as proteins are extremely hard to crystallize. Each protein has its own specific set of crystallization conditions and no generic set of crystallization rules exists. As a result, hundreds or even thousands of crystallization trials must be performed on any target protein, of which less than 1% typically yields promising results.

This application note describes the use of Dual Polarisation Interferometry (DPI) to monitor the crystallization of proteins in real time.

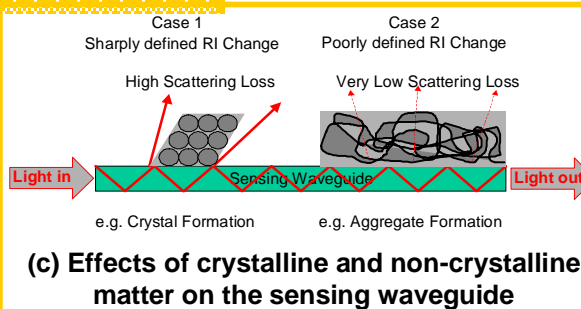
The interference fringe pattern generated by the dual slab waveguide sensor chip (**Figure 1a**) used in Farfield **AnaLight**<sup>®</sup> instruments is characterised by a number of parameters, including its period and contrast (as defined in **Figure 1b**). The contrast is a measure of the amount of light guided in both waveguides, and as such is affected by any losses such as scattering that occur on the top (active) waveguide surface. It is the contrast parameter that is used to monitor protein crystallization using DPI.



(a) Integrated Young's interferometer



(b) Output fringe pattern



(c) Effects of crystalline and non-crystalline matter on the sensing waveguide

## Experimental

The DPI experiments were performed on a Farfield **AnaLight**<sup>®</sup> instrument. The surface used was an unmodified silicon oxynitride **AnaChip**<sup>™</sup>. The temperature of the samples was controlled to 20°C throughout. Reagents were analytical grade or higher and water was high purity.

The precipitant solution was prepared a few minutes before the sample injection was made. Once the **AnaChip**<sup>™</sup> was fully covered with precipitant solution, the flow was stopped. In addition, the surface of the **AnaChip**<sup>™</sup> was monitored with a microscope operating in polarising mode mounted on top of the flow cell (above the sensor chip). Only light caused by birefringence reached the CCD camera attached to the microscope.

### Lysozyme Crystallization:

The crystallization of lysozyme was achieved by dissolving 80mg of the protein in 1.0ml of sodium acetate (aqueous, 50mM, pH 5.5) followed by addition of 1.0ml of NaCl (aqueous, 10% w/v) as a precipitant. Reducing the concentration of NaCl to 2.5% w/v inhibited the crystallization of lysozyme and resulted in a clear solution.

### β-Lactoglobulin Crystallization:

The crystallization of β-lactoglobulin was achieved by dissolving 20mg of the protein in 1.0ml of sodium acetate (aqueous, 50mM, pH 2.5). No other reagents were added. β-Lactoglobulin precipitated under these concentrations.

# Spotlight on Applications

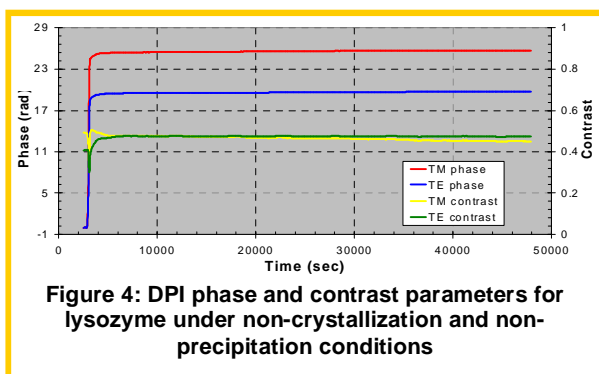
## Results and Discussion

### Lysozyme Crystallization:

**Figure 2** shows the effects of the formation of protein crystals on the DPI phase and contrast parameters. The phase increases as the protein adsorbs to the sensor surface. However, as the protein forms a crystalline structure on the surface, light from the sensing waveguide is lost through scattering (as depicted schematically in **Figure 1c**) leading to the drop in contrast. A photograph of the lysozyme crystals grown on a sensor chip is shown in **Figure 3**.

The decrease in the contrast parameter started approximately 8 min (point B in Figure 2) after the instrument flow was stopped (point A), and was complete in less than 30 min (point C). For eight experimental trials using lysozyme under crystallizing conditions, DPI consistently predicted crystallization more than an order of magnitude earlier than the birefringence technique (by computing the total intensity of the images captured by the CCD camera) could detect significant crystals.

The contrast parameter remained unchanged in the cases where no crystallization and no precipitation occurred for lysozyme (**Figure 4**). The phase increased initially due to the formation of an adsorbed protein layer, and subsequently remained constant throughout the experiment.

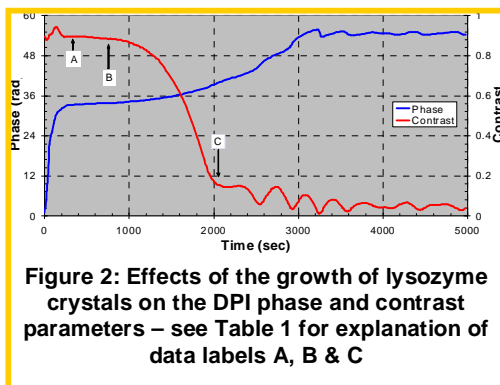


**Figure 4: DPI phase and contrast parameters for lysozyme under non-crystallization and non-precipitation conditions**

The DPI technique enables the calculation of the refractive index, thickness and coverage of the protein layer adsorbed onto the surface of the sensor chip during the first 30 min of the lysozyme crystallization experiment in **Figure 2**. The results (see **Table 1**) indicate that crystallization on the sensor proceeds *via* an adsorbed protein layer averaging 6.7nm thick and increases to 10nm immediately before crystallization.

Measurement	After Flow stopped (A)	Onset of Contrast loss (B)	Before Total Collapse (C)
Layer RI	1.452 ± 0.008	1.455 ± 0.001	1.458 ± 0.007
Thickness (nm)	6.7 ± 0.7	8.9 ± 2.2	9.9 ± 2.1
% Coverage Erect	159 ± 28	214 ± 51	246 ± 54
% Coverage Prone	238 ± 41	320 ± 76	369 ± 81

Table 1: Refractive index, thickness and coverage of the adsorbed lysozyme layer during the different stages of the crystallization experiment (see Figure 2)



**Figure 2: Effects of the growth of lysozyme crystals on the DPI phase and contrast parameters – see Table 1 for explanation of data labels A, B & C**

The results also show that the contrast loss occurred during the early stages of crystallization, and could therefore be used to monitor the process and provide an early indication of the onset of crystallization in protein crystallization trials.

### β-Lactoglobulin Crystallization:

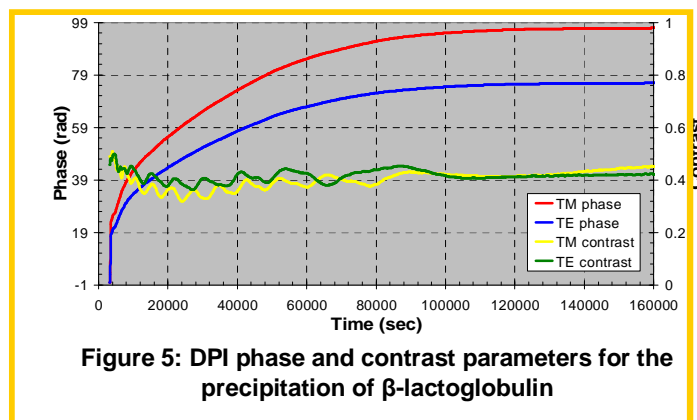
The response in the phase and contrast parameters to the precipitation of β-lactoglobulin on the surface of the sensor chip is illustrated in **Figure 5**.



**Figure 3: Photograph showing lysozyme crystals grown on an AnaChip™**

The phase increased in response to the adsorption of a thin layer of protein as the solution was flowed over the surface, and then reached a maximum. The contrast displayed some low-magnitude and low frequency oscillations but, in general, also remained unchanged.

The crystallization of a range of common proteins such as myoglobin and glucose isomerase has also been studied using **AnaLight®**, and similar results to those shown above were obtained. In addition, a MAP kinase crystallization in the presence and absence of a known specific binder has been examined. In this case, crystallization proceeds from a liquor containing precipitate. All results to date indicate that DPI can be used to differentiate between crystallization events and non-crystallization in proteins.



**Figure 5: DPI phase and contrast parameters for the precipitation of β-lactoglobulin**

# Spotlight on Applications

## Conclusions and Benefits

The **AnaLight**<sup>®</sup> instruments and their experimental protocols give the researcher a unique combination of high-resolution data in real time on the phase and contrast of the interference fringes generated by the DPI technique. DPI is an important enabling technique for protein crystallography researchers, giving them the ability to:

- Distinguish easily between protein crystallization and other macromolecular physical processes, such as precipitation and aggregation
- Detect crystallization at the early nucleation stage, the onset of the crystallization process
- Detect the onset of crystallization 10-100 times faster than with polarised microscopy.
- Enable crystallization processes to be monitored, and hence developed and optimised, in real time

Farfield gratefully acknowledges that these experiments were carried out in collaboration with

Professor Gareth R. Jones and Dr David T. Clarke from the CCLRC Daresbury Laboratory, Keckwick Lane, Warrington, WA4 4AD, UK

*Note: The use of Dual Polarisation Interferometry for the detection of early-stage protein crystallization is the subject of Patent Application PCT/GB02/02185*

## Measurement of Conformational Change in Transglutaminase on Calcium Ion Binding

### Introduction

Dual Polarisation Interferometry (DPI) is an important enabling technology for the rapid and sensitive monitoring of interactions between proteins and metal cations, and the measurement of resulting conformational changes in proteins.

**AnaLight**<sup>®</sup> DPI instruments provide density and dimensional measurements, showing mass capture events and revealing structural changes in proteins that are indicative of a response to specific binding. The sub-atomic resolution of DPI allows the detection of metal cations binding to large, immobilised proteins. This means that DPI can be used to determine whether metal cations known to be essential for protein **function** actually change the **structure** of a protein when they bind, giving a level of information beyond that provided by traditional biosensors and other kinetic techniques.

Transglutaminases (**Figure 1**) are implicated in a range of protein modifying activities including roles as diverse as fusing fibrinogen to increase its mechanical strength as part of the blood clotting cascade [\[1\]](#) to cross-linking cytoskeletal and membrane proteins and programmed cell death [\[2\]](#). Regulatory binding sites are known for Ca<sup>2+</sup> and GTP, which reciprocally modulate the cross-linking and signaling activities of transglutaminase [\[3\]](#).



Figure 1: The structure of transglutaminase

Protein structure is intimately related to function, activity and regulation of activity. Transglutaminase is presumed to undergo conformational changes on binding both Ca<sup>2+</sup> and GTP, bringing about the modulation in activity. Indirect analysis using Shallow Angle Neutron Scattering (SANS) [\[4\]](#) and Circular Dichroism Spectroscopy [\[5\]](#) has suggested that the gyration radius of transglutaminase increases by approximately 0.8nm on binding of Ca<sup>2+</sup> ions. There is no clear crystal structure data available to support this.

This application note demonstrates the use of **AnaLight**<sup>®</sup> for the analysis of the conformational changes taking place in guinea pig liver transglutaminase (tTG, 77,000 Da) when it binds calcium ions (Ca<sup>2+</sup>, 40 Da), and to validate this measurement by calculating the affinity constant K<sub>D</sub> for the interaction from the DPI data for comparison with published values for the transglutaminase-calcium interaction.

# Spotlight on Applications

## Experimental

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

**Immobilisation of Transglutaminase:** The **AnaChip**<sup>™</sup> was calibrated by injecting an 80% (w/w) ethanol/water solution into a stream of PBS running buffer (10mM, 150mM NaCl, pH7.4) at a flow rate of 50µl/min. The amine-amine linker BS<sup>3</sup> (bis [sulfosuccinimidyl]suberate, 4mg/ml in PBS) was added to both channels (experimental and reference) for 8 minutes at 10µl/min. Guinea pig liver transglutaminase solution (1mg/ml in PBS) was then added to the experimental channel only for 10 minutes at 10µl/min. Tris buffer (3M, pH8.0) was then added to both channels for 8.5 minutes at 50µl/min to block unreacted BS<sup>3</sup>.

**Protein-Metal Cation Interactions:** After stabilising the immobilised transglutaminase with sufficient rinsing with Tris running buffer (50mM, pH7.6) at 50µl/min, the protein was challenged with metal salt solutions. Stock solutions of calcium chloride (CaCl<sub>2</sub>, 30mM) and sodium chloride (NaCl, 60mM) were made up in Tris running buffer. Aliquots of each were introduced to the flow stream of Tris running buffer for 3 minutes at 50µl/min over both channels in a series of nine injections of each solution, alternating between CaCl<sub>2</sub> and NaCl. The injection sequence began with the lowest concentration of CaCl<sub>2</sub> and then the lowest concentration of NaCl and progressed through to the highest concentrations. The transglutaminase was washed with Tris running buffer for 4 minutes at 50µl/min between metal cation additions. These additions covered a CaCl<sub>2</sub> concentration range of 0.15mM to 30mM and a NaCl concentration range of 0.32mM to 60mM, designed to be equimolar with respect to chloride ion. The density, thickness and mass of the transglutaminase surface were monitored throughout.

## Results and Discussion

**Immobilisation of Transglutaminase:** Transglutaminase (tTG) has a discoid structure that is 15nm in diameter and around 5nm thick. Following immobilisation onto the amine surface using BS<sup>3</sup>, the thickness of the transglutaminase was measured at 4.6nm by DPI. This is consistent with the expected thickness value if the protein is immobilised with the disc lying parallel to the surface, which is most likely given the immobilisation strategy employed.

**Protein-Metal Cation Interactions:** A mass signature alone cannot be used as a reliable indicator to quantify protein-metal cation interactions when considering the binding of very low atomic mass metal cations, as the signal becomes diminishingly low with their interactions with much larger proteins. The capabilities of the **AnaLight**<sup>®</sup> instrument series allows for unique levels of sensitivity to metal cation and small molecule detection, by providing direct insight into protein function associated with specific binding through conformational change coupled with density variation.

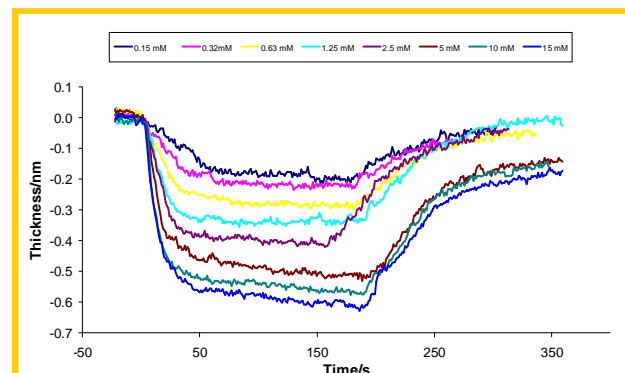


Figure 2: Thickness change in tTG as a function of Ca<sup>2+</sup> concentration showing Ca<sup>2+</sup> induced conformational changes in tTG

Whilst looking for the diminutive signals associated with metal cations binding to large proteins, the effects of changes in the buffer or bulk refractive index need to be accounted for if the measurement is to be quantified. Generally speaking, because the **AnaLight**<sup>®</sup> measures in a thin film format, it is adequate to simply subtract the reference channel signals from their counterparts on the experimental channel.

Having compensated for bulk refractive index effects from the high metal cation concentrations present, the resolved thickness data as a function of Ca<sup>2+</sup> concentration is shown in **Figure 2** and density data as a function of Ca<sup>2+</sup> concentration is shown in **Figure 3**. Clearly, each Ca<sup>2+</sup> binding event results in a concentration-dependant decrease in thickness and increase in density of the tTG, an effect not observed in the presence of the Na<sup>+</sup> ions in the control experiments.

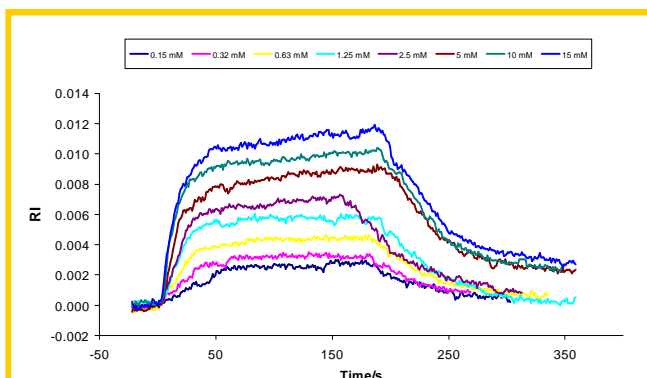
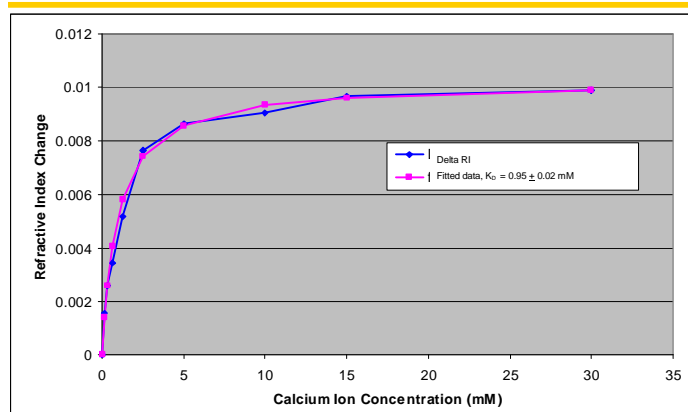


Figure 3: Density (RI) change in tTG as a function of Ca<sup>2+</sup> concentration showing Ca<sup>2+</sup> induced conformational changes in tTG

# Spotlight on Applications



**Figure 4:** Using the relative change in RI for NaCl and CaCl<sub>2</sub> at the same Cl<sup>-</sup> concentrations provides a concentration profile from which the affinity constant K<sub>D</sub> can be determined

The effects observed for the Ca<sup>2+</sup> ions indicate a **specific binding** event (density increase associated with mass capture) and a corresponding **conformational change** in transglutaminase (thickness decrease associated with structural tightening). Both of these effects are reversible with buffer washing.

The data in **Figure 2** shows clearly that Ca<sup>2+</sup> ions induce a conformational change of around 0.6 to 0.7nm. This conformational change is not seen in the presence of Na<sup>+</sup> ions in the control experiments. A conformational change of this order would be expected in tTG, as the long axis is reported to expand by approximately 3nm on Ca<sup>2+</sup> binding<sup>(4)</sup> which would induce a thickness reduction in the short axis, which is perpendicular to the surface in this immobilisation (above). It is worth noting here that the thickness measurement resolution provided by DPI (**Figure 2**) is better than +/-0.01nm.

In order to determine whether the structural changes observed in tTG (**Figures 2 & 3**) were a direct consequence of Ca<sup>2+</sup> binding, the results were verified against known parameters from the interaction. The affinity constant K<sub>D</sub> for tTG binding calcium has been reported as between 0.2 mM and 3.0 mM<sup>(6)</sup>. Curve fitting to a plot of the relative change in RI (density) of the tTG layer against Ca<sup>2+</sup> concentration (**Figure 4**) gives a K<sub>D</sub> value of 0.95 ± 0.2mM, which falls well within the range of reported values.

Farfield gratefully acknowledges that these experiments were carried out in collaboration with Kal Karim, Judith Taylor and David Cullen from the Institute of BioScience & Technology, Cranfield University, Silsoe, Bedfordshire, UK.

## Conclusions and Benefits

**AnaLight**<sup>®</sup> enables the detailed study of the intimate link between protein structure and function, activity and regulation of activity. A range of methods is used to elucidate protein structure of which x-ray crystallography provides the most detailed information. Obtaining structural information is often difficult and time consuming, and this structural information cannot always be directly related and compared to functional information obtained from other experimental techniques.

These experiments show how **AnaLight**<sup>®</sup> can be used to determine the structural changes occurring when a protein binds metal ions known to play a role in the regulation of its activity. **AnaLight**<sup>®</sup> offers a simple, convenient laboratory-based method to unambiguously measure **structural** changes and directly relate these to **functional** aspects of proteins in real time and in a single set of measurements.

The **AnaLight**<sup>®</sup> instruments and their experimental protocols give the biophysicist a unique combination of high-resolution data in real time on thickness, density (refractive index) and mass from a bench top technique. The **AnaLight**<sup>®</sup> is an important enabling tool for life science researchers, giving them the ability to:

- Rapidly and sensitively detect low atomic weight metal cations binding to large proteins
- Connect functional and structural events in biological molecules in a single set of high-content measurements, in real time
- Measure structural changes in proteins as a result of metal cation binding, moving the basis for such studies beyond simple measurement of binding affinities and revealing dynamic structural changes in proteins
- Remove the matrix effects which may dominate such measurements on biosensor technologies by using the **AnaLight**<sup>®</sup> reference channel and a little care in experimental design

- (1) G. Siefiring, A. Apostol, P. Velasco & L. Lorand. **Biochemistry** 17 (1978) 2598-2604
- (2) L. Fesus, M. Piacentini & P. Davies. **Eur. J. Cell Biol.** 56 (1991) 170-177
- (3) K. Achyuthan & C. Greenburg. **J. Biol. Chem.** 262 (1987) 1901-1906
- (4) R. Cassadio, E. Polynerini, P. Mariani, F. Spinozzi, F. Carsughi, A. Fontana, P. Polverino de Laureto, G. Matteucci & C. Bergamini. **Eur. J. Biochem.** 262 (1999) 672-679
- (5) A. Di Venere, A. Rossi, F. De Matteis, N. Rosato, A. Finazzi & G. Mei. **J. Biol. Chem.** 275 (2000) 3915-3921
- (6) J Mottahedeh & R Marsh. **J Biol Chem** 273 (1998) 29888-29895

# Spotlight on Applications

## Introduction

Dual Polarisation Interferometry (DPI) is an important and highly sensitive technique for determining interfacial properties, including those of thin spun films. Thin films are defined as being up to 200nm thick. **AnaLight**<sup>®</sup> DPI instruments can be used to provide a quantitative and sensitive measure of a range of processes such as thin film ageing, permeability, solute partitioning and changing of solvation state. In addition, **AnaLight**<sup>®</sup> can be applied to quantitative, real time measurement of the physisorption of other materials, such as proteins (bio-fouling), polymers and surfactants, onto polymer and other spin coated surfaces. Many of these processes result in only a relatively small change in the total film composition, but can dramatically affect the behaviour of the film in its intended application area.

This application note describes the determination of film properties and ageing behaviour for a thin polymer film that is applied to the **AnaChip**<sup>™</sup> surface off-instrument and removed from the **AnaChip**<sup>™</sup> surface in a solvent wash step at the end of the experiment.

## Results and Discussion

**Spun Film Properties:** Values for the thickness and refractive index of the polymer layers at the start of each experiment are shown in **Figure 1**. The calculated values for density, mass and volume fraction for the layers are also shown. The polymer volume fraction for the thinner polymer layer containing the more hydrophilic modifying agent (B) is low, indicating a structure dominated by surface roughness. The drop in fringe definition observed due to scatter of light from the surface also indicates significant levels of roughness. For the thicker polymer layer containing the hydrophobic modifying agent (A), the polymer volume fraction is significantly higher, indicating that a smaller portion of the film constitutes a rough interfacial region.

Schematic diagrams of the structures of the two PVC films are shown in **Figure 2**. The measured structures are also consistent with the method of formation of the layers, in that spinning from a relatively low boiling point solvent will produce relatively rough surfaces.

Polymer	Thickness (nm)	RI	Density (g/cm <sup>3</sup> )	Mass (ng/mm <sup>2</sup> )	Polymer Volume Fraction
(A) PVC + hydrophobic modifying agent	175	1.490	1.01	177	0.72
(B) PVC + part-hydrophilic modifying agent	56	1.444	0.69	39	0.50
Literature Values for pure PVC		1.55	1.4		1

Figure 1: Physical properties of spun PVC films measured by DPI

## High Resolution Measurements of the Properties and Ageing Behaviour of Thin Spun PVC

### Experimental

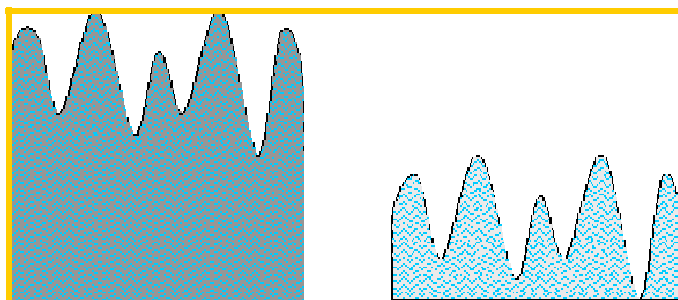
The DPI experiments were performed on a Farfield **AnaLight**<sup>®</sup> instrument. The surface used was an unmodified silicon oxynitride **AnaChip**<sup>™</sup>. The temperature of the samples was controlled to 20°C throughout. Reagents were analytical grade or higher, water was high purity and all solutions were degassed prior to use.

PVC polymer films were spin coated onto pre-calibrated **AnaChips**<sup>™</sup> from dilute THF solution. Two different polymer solutions were used in these studies. **Solution A** contained PVC plus a purely hydrophobic modifying agent and **Solution B** contained PVC plus a partially hydrophilic modifying agent.

The spun polymer coated chips were inserted into the **AnaLight**<sup>®</sup> instrument and running water was flowed over the surface at 50µl/min. The properties of the polymer films were measured by DPI. The polymer-coated chips were then subjected to a series of analyte partitioning challenges and buffer-based pH changes. At the end of the experiment, the polymer layer was removed from the chip surface by a 2 minute THF injection. The chip was then calibrated and the calibration compared against the pre-calibrated chip values to confirm that all deposited polymer had been removed from the chip surface.

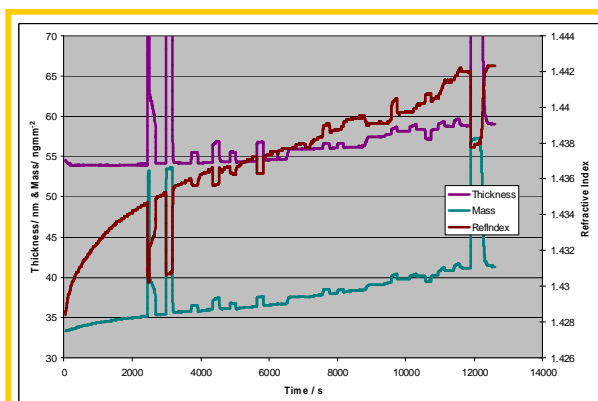
# Spotlight on Applications

**Polymer Film Ageing:** The DPI measurements of the ageing behaviour of the two PVC polymer films after 3 hours of analyte partitioning challenges and buffer-based pH changes are summarised in **Figure 3**. As an example, the real time ageing behaviour of the PVC polymer layer B (containing part hydrophilic modifier) as recorded on the **AnaLight®** instrument is shown in **Figure 4**.



**Figure 2: Schematic of PVC polymer layer (A) containing a hydrophobic modifier and PVC polymer layer (B) containing a part hydrophilic**

The PVC polymer film containing the hydrophobic modifier (**A**) shows relatively little change in properties over the 3 hours of ageing and challenges, showing a small degree of thickness increase and no change in density. By comparison, the PVC polymer film containing the part hydrophilic modifier (**B**) undergoes proportionately three times the swelling *combined with* a significant (>10%) increase in density. This indicates significant uptake of compounds from solution rather than swelling due to water (solvent) uptake. This behaviour be expected from the more hydrophilic nature of the modifier employed in this layer. Water uptake alone would lead to an increase in thickness and a decrease in density.



**Figure 4: Resolved thickness, refractive index (density) and mass for PVC polymer film B showing the ageing behaviour under a series of analyte partitioning challenges and buffer-based pH changes**

## Conclusions and Benefits

**AnaLight®** DPI instruments enable the study of the interfacial behaviour and structure of a diverse range of molecular systems. These experiments show DPI can be applied to the real time study of polymer films and their behaviour.

Importantly, the measurements provide a distinction between uptake of analytes or salts from solution and swelling due to water (solvent) inclusion. The former is observed as a mass increase, whereas the latter is observed as a thickness (or volume) increase with no mass gain. This approach also enables the differing properties of the polymer-modified surface as measured, or subsequently determined, to be related to both its structure and method of formation.

The **AnaLight®** instrument range and associated experimental protocols give the researcher a unique combination of high-resolution data in real time on thickness, refractive index (density) and surface coverage in an easy to use, bench-top technique.

Polymer	Thickness Change (nm)	% Thickness Change	RI (Density) change	% Density Change	% Mass Change
(A) PVC + hydrophobic modifying agent	+ 3	+ 1.7	0.000	0	+ 1.7
(B) PVC + part-hydrophilic modifying agent	+ 3	+ 5.4	+0.012	+ 10	+ 10.5

**Figure 3: Changes in physical properties of spun PVC films on ageing for 3 hours measured by DPI**

The **AnaLight®** is an important enabling tool for surface scientists giving them the ability to:

- Measure deposited polymer film structure and dimensions at high resolution
- Follow and understand polymer film ageing processes in real time
- Quantify the uptake of salts or analytes from solution or leaching of compounds
- Sensitive measure changes in real-time, providing information on permeability, solute partitioning and changing solvation state
- Avoid the limitations and ambiguities that are inherent in other techniques for such studies, and provide the final results and analysis rapidly

Farfield gratefully acknowledges that these experiments were carried out in collaboration with Dr. Sub Reddy, from the School of Biomedical & Molecular Sciences, University of Surrey, Guildford, UK.

# AnaLight<sup>®</sup> Product Range

## AnaLight<sup>®</sup> Flex

Automated execution platform for structure function studies

### System Features:

- Simultaneous measurements on three channels gives total confidence in data integrity
- Full automation of DPI methods giving remarkable sample throughput
- Flexible, autosampler-based fluidic design can be optimised for your application
- High sensitivity for accurate analysis
- Optimised for sub-atomic structural measurement and class-leading kinetic performance
- Wide dynamic range extends solvent and buffer handling capabilities



## AnaLight<sup>®</sup> Quantum

Uncompromising DPI performance in an entry level system

### System Features:

- Proven DPI technology in a low-cost system
- Open fluidic design can be easily optimised for your application
- Sub-atomic structural detail combined with class-leading kinetic performance
- Wide dynamic range extends solvent and buffer handling capabilities
- Flexible range of surface chemistry to suit all applications



## AnaLight<sup>®</sup> CrystaLight

New development platform for protein crystallisation studies



### System Features:

- Detection of early onset of crystallisation
- Simultaneous monitoring of crystallisation on two independent channels
- Simultaneous surface imaging on two channels
- Search of crystallisation space without the need for optically visible crystals
- Sub-atomic structural analysis
- Sample loading options
- Open fluidic design can be optimised for your needs



## AnaLight<sup>®</sup> Bio200

Established development platform for structure function studies

### System Features:

- Simultaneous measurements on three channels gives total confidence in data integrity
- Sample loading options give flexibility and precision with low sample volumes
- Open fluidic design can be optimised for your application
- Straightforward upgrade to full automation available
- Sub-atomic structural detail combined with class-leading kinetic performance
- Wide dynamic range extends solvent and buffer handling capabilities

# Distributors

With the continued success of our products worldwide, we are expanding our network of distributors to support our sales across the globe. Below we list our confirmed distributors who are fully trained to present and support our products.

*If your country of interest is not yet listed please contact us directly at [sales@farfield-sensors.com](mailto:sales@farfield-sensors.com).*



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# More Prestigious Awards and Recognition for Farfield

Farfield received another major award for the innovation behind its **AnaLight**® product range at the highly regarded Manufacturing Excellence Awards 2005 (MX2005) in London. The awards are an annual competition organised and hosted by the Institution of Mechanical Engineers (IMechE).

At the MX2005 Awards dinner at the Dorchester Hotel in London on Wednesday 29<sup>th</sup> June 2005 and hosted by William Hague MP, Farfield was singled out as 'worthy of special commendation' in the Renishaw Award for Product Innovation for its DPI technology for measuring molecular structures and nanoparticles. Mr Hague commented, "Farfield is a company that is really going places".

Farfield was selected as a MX2005 finalist after rigorous self-assessment audits, visits from independent assessors and a presentation to the MX2005 judging panel at IMechE headquarters. Farfield was one of only 17 finalists selected from 118 entrants. Other finalists included such top names as AstraZeneca, Rolls Royce and Siemens.

July 2005



IMECHE



## Farfield Awarded MNT Quality Mark

October 2005

Farfield is one of only six companies to be recognised with the award of the UK Micro and NanoTechnology Network's (MNT) highly esteemed Quality Mark. This award significantly strengthens Farfield's position as a world leader in innovation, and is a reflection of Farfield's pioneering approach to analytical measurement that meets the demands of the emerging nanotechnology market.

The MNT initiated the Quality Mark for companies involved in and supplying the nanotechnology industry. The objective of the MNT Quality Mark is to benchmark development and implementation of best practice and to set a strict minimum standard of performance and achievement.

The MNT Quality Mark is a key milestone for the UK nanotechnology industry. The MNT network was launched in 2003 and has seen the nanotechnology industry rapidly evolve and expand in the UK.

Dr Simon Carrington, Farfield's Marketing Director commented, "the global market for nanotechnology is forecast to exceed \$1,000 billion in the next decade, so we are naturally delighted to be one of the first UK companies to receive such prestigious recognition. We see the award as a clear endorsement of our position as one of the world's leading suppliers of new instrumentation" to meet the emerging and demanding measurements needs of nanotechnology.



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# Farfield

illuminating the molecular world...