Schedule of Events

The Boots Science Building

Morning Session

10.00 Biomedical Array Imaging: Life Changing Measurements
Dr Andrew M. Shaw

10.15 Inductive Science and the Demands on Analytical Technologies of a More Quantitative Biology
Professor Simon Gaskell

Coffee served in the Atrium

11.45 2D Attogram Surface Plasmon Imaging
Dr Andrew M. Shaw

12.00 Differential Surface Plasmon Imaging
Professor J. Roy Sambles

12.30 Custom CMOS cameras for modulated light detection
Dr Mark C. Pitter

Lunch served in the Atrium

Afternoon Session

14.00 COPD: Problems and Prospects of Early Detection
Dr Lucy Fairclough

14.30 Making use of gold - first steps in array reading
Professor Bill Barnes

Tea served in the Atrium

15.30 Kinetic Analysis of Array Assays: Biomarker Expression Profile
Dr Andrew M. Shaw

16.00 The Biological Data Problem; collection, analysis and interpretation
Professor Paul O'Shea

Transfer to the University Staff Club via Hopper or a short walk up the hill.

Staff Club

Poster Session

Please join us for poster presentations of the various elements of the work of the project and enjoy a glass of wine.

Dinner will be in the Hemsley Restaurant at the University Staff Club

Introduction

2D-Attogram Surface Plasmon Imaging

A Collaboration between the Universities of Exeter and Nottingham

The 2D Attogram Surface Plasmon Imaging project is a joint project between the Universities of Exeter and Nottingham funded as a Basic Technology Grant (£3M) from Research Councils UK. We are developing a new label-free high-throughput screening technology based on the photonic properties of gold plasmons as a detection technology. We are also developing a novel CMOS camera to image the 500 ´ 500 array printed onto a gold surface or gold nanoparticle surface that is sensitive to surface binding. The gold photonic surface is functionalised with antibodies for the target species and the antibody-antigen binding event is imaged using the change in the optical properties of the gold plasmon. The biomedical target for the project is to screen cytokine biomarkers in whole blood and biopsy samples from patients that may be suffering from the lung condition Chronic Obstructive Pulmonary Disease (COPD) to derive expression profile in the cytokines as a predictor for COPD.

Our project is multidisciplinary involving physicists, biologists, electrical engineers, immunologists and physical chemists. The project members are:

Dr Andrew Shaw, PI University of Exeter
Prof Roy Sambles, CI University of Exeter
Prof Bill Barnes, CI University of Exeter
Prof Paul O'Shea, CI University of Nottingham
Prof Mike Somekh, CI University of Nottingham
Dr Mark Pitter, University of Nottingham
Dr Lucy Fairclough, University of Nottingham

Dr Ian Hooper, University of Exeter
Dr Andrew Murray, University of Exeter
Dr Rouslan Olkhov, University of Exeter
Baptiste Auguié, University of Exeter
Artem Jerdev, University of Exeter
Ciaran Stewart, University of Exeter

Bo Fu, University of Nottingham
Dr Nicholas Johnston University of Nottingham
Dr Elizabeth Lunt, University of Nottingham
Rebecca Metcalf , University of Nottingham
Dr Joanna Richens, University of Nottingham

Christi Hetzler, Project PA, University of Exeter

"Biomedical Array Imaging: Life Changing Measurements" is our mid term conference two years into the project and will report progress in all areas of the project towards the development of our Basic Technology.

Dr Andrew M. Shaw, Principal Investigator

Biomedical Array Imaging: Life Changing Measurements

Dr Andrew M. Shaw, School of Biosciences, University of Exeter

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Sequencing of the Human Genome revealed perhaps 30,000 genes but the body is considerably more complex than this small number of genes would suggest; the proteome is approximately 1 million and the metabolome is approximately 2500. The resulting complex system must be profiled if we are to understand the health of an individual at the level of phenotype and realise the vision of personalised medicine. Large numbers of molecules will have to be screened from which their relative concentrations will then be determined. The blood itself contains over 280 proteins with concentrations varying from 100 mg ml^{-1} to pg ml^{-1}: 11 orders of magnitude. The Biomaker Hypothesis suggests that a protein profile of the blood will enable differential diagnosis, pre-symptomatic diagnosis of disease states and prediction of the onset of disease. Screening target groups of the population may enable significant improvements in healthcare, personalised treatment regimens and a better understanding of the susceptibility to disease; what is required is an enabling technology.

Current high-throughput screening technologies allow massively large numbers of molecules to be screened but the screen yields relatively little information. The molecules are labelled with fluorophores and the arrays are then screened to provide simple yes/no binding information. The large size of the proteome or metabolome suggests that the chemistry for labelling molecules will produce a large preparation overhead for large-scale screening and that a label-free approach would be better. Further, having decided to print a large scale array, with all of the preparation overhead costs, more information is required than the current yes/no results.

A surface-plasmon-based technology removes the need to label molecules producing a label free-technology with capacity to screen a 500 x 500 array (250,000 assay array elements) from which we can derive analyte concentrations and binding kinetics producing a highly sensitive (attogram $pixel^{-1}$) accurate measure of biomarker concentrations. We are targeting our biomedical validation of the technology on blood and biopsy cytokine levels as predictors of COPD. Early diagnosis of this chronic condition will extend the treatment window for many patients and increase the quality of life and management of a disease which is predicted to be the third biggest killer in the UK by 2012.

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IS GRATEFULLY ACKNOWLEDGED

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The RSC recognises that the partnership of chemistry and biology will continue to drive forward the creation of new products and services in the bioscience related industries. With this in mind, the RSC Chemistry Biology Interface Forum is pushing ahead with an agenda that focuses on meeting the needs of industry and academia working at the interface between the chemical sciences and the life sciences. The current programme includes conferences and workshops, as well as working in partnership with other professional societies, funding agencies and industry on key policy issues.

www.rsc.org/chemicalbiology

Invited Speaker

Inductive Science and the Demands on Analytical Technologies of a More Quantitative Biology

Professor Simon Gaskell

Michael Barber Centre for Mass Spectrometry, School of Chemistry and Manchester Interdisciplinary Biocentre, University of Manchester

The increasing appreciation of the importance of inductive reasoning in experimental biology need not diminish the significance of hypothesis-driven research, but does introduce new approaches to hypothesis generation. Furthermore, a new emphasis on the importance data generation, particularly in the context of 'omics studies, has increased demands on analytical technologies and raises questions about the nature of the information produced. This presentation addresses such issues, exemplified by proteome analyses.

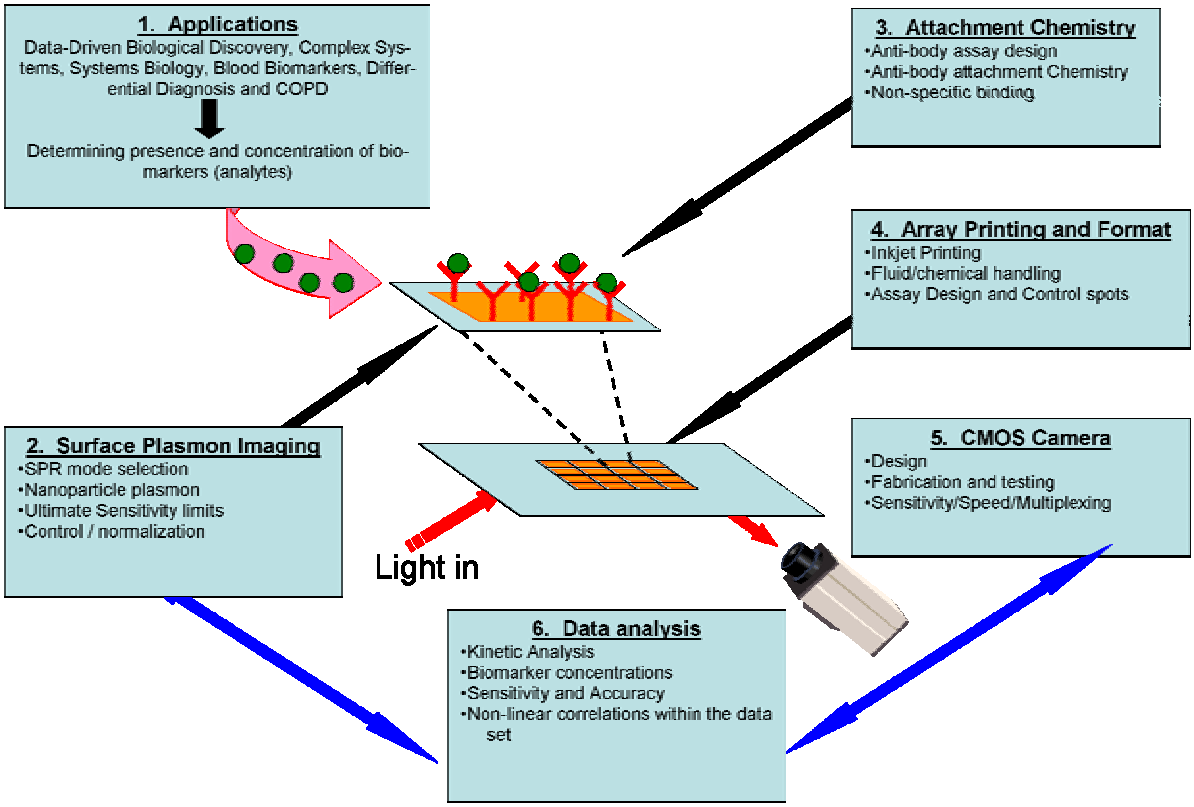
Key questions concern the nature of the information required for full definition of the proteome. The majority of the literature relates to qualitative analyses, with recent attention to relative quantification (e.g. the change in a component's concentration following a change in growth conditions). The common assertion of protein "identification" is rarely correct, however; strictly speaking, data are generated consistent with the expression of a particular gene, and analyses may be blind to protein structural changes following translation, such as N- or C-terminal processing, phosphorylation, etc. Moreover, the cataloguing of expression products is unlikely to reflect the full dynamic range of components, an issue of critical importance in biomarker identification. (The dynamic range of protein constituents in blood exceeds 11 orders of magnitude, emphasising the challenge of biomarker identification in this medium.) In the context of systems biology, it is increasingly important that quantitative data should take the form of *absolute* estimates (i.e. copy number per cell), that the stoichiometry of post-translational modification should be defined, and that consideration should also be given to the dynamics of the proteome – the rates of synthesis and degradation.

The recognition that characterisation of the proteome (and equivalently, other analytical challenges) is a multi-dimensional problem raises questions about the appropriate analytical approaches to be taken. High-throughput approaches are clearly required to generate the high volumes of data required to enable the application of inductive reasoning, but can we introduce sufficient sophistication into such methods to address the multi-dimensionality of the information available?

2D- Attogram Surface Plasmon Imaging

Dr Andrew M. Shaw, School of Biosciences, University of Exeter

The 2D-Attogram Surface Plasmon Imaging project (Attogram Project) is developing a new basic technology to enable label-free high-throughput screening of a large 500 x 500 array using two plasmon-based techniques; differential phase surface plasmon resonance and localised particle plasmon resonances. The project falls broadly into 6 areas of activity outlined below and will be discussed in detail during the day:



The project mid-term achievements in the project are:

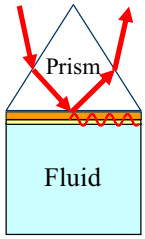
- Demonstration of differential phase surface plasmon resonance technique with sensitivity of 2 x 10^{-7}. This precision can be maintained over an extended dynamic range of 10^{-7} to 10^{-2}.
- The differential technique has been developed into a differential phase imaging technique with a over a 5 mm^2 area with a refractive index sensitivity of 5 x 10^{-5}.
- A novel differential phase imaging CMOS camera has been constructed with 128 x 128 pixel currently sampling data at 200 kHz with a 12-bit vertical resolution.
- A novel hyrazide coupling technology has been developed to tether primary amine groups to the array assay spots and is being compared with the conventional techniques.
- A small-form array reader has been developed using a nanofabricated array surface that has a bulk refractive index sensitivity of 5 x 10^{-5} corresponding to a µg ml^{-1} biomarker concentration.

Differential Surface Plasmon Imaging

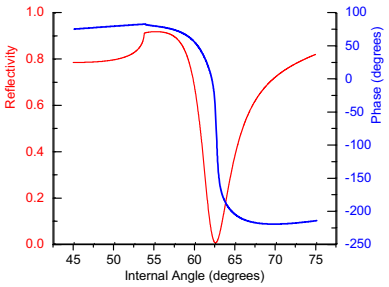
Exeter: Ian Hooper, Ciaran Stewart and Prof J. Roy Sambles
Nottingham: Mark Pitter, Nick Johnston, Li Chunhua (Dennis) and Mike Somekh

Surface plasmons, which are electron density oscillations coupled with highly localised electromagnetic waves propagating along the interface between a metal and a dielectric may be used to optically sense changes at the interface. In the visible region of the spectrum the use of gold and silver as the metal of choice is commonplace, with gold being often selected because of its chemical inertness.

In order to probe the changes of the surface plasmon associated with changes in an overlayer on the gold substrate, forming thereby a biosensor, two essential conditions have to be met. Firstly one needs to have a simple and repeatable way of optically probing (exciting) the surface plasmon and secondly one needs a method for measuring as accurately as possible any changes. This is readily done using what is known as the prism coupling technique with a thin gold film and p-polarised (Transverse Magnetic) incident light tunnelling through the film to excite the surface plasmon on the exposed gold surface. Examining the reflected signal as a function of wavelength or angle of incidence is often then undertaken to characterise the surface plasmon resonance. The changes due to absorption of material on to the gold will result in changes in the resonance position either in angle or in wavelength. This is all relatively straightforward. The challenge is to make this into an imaging technique where one can visualise very small changes in the surface plasmon over a significant area. This means combining a sensitive differential technique for detecting changes with some form of smart pixilation with differential signal recovery on the camera.



The sensitivity required may be achieved using some form of differential technique. In the present case we chose to use optical phase (essentially established here by monitoring the optical polarisation) which changes very rapidly through the surface plasmon resonance. Then using a small optical polarisation dither imposed on the input laser beam we detect at the dither frequency very small changes in the output polarisation state of the light. This gives a sensitivity of ~10^{-5} of a monolayer (~80 Attograms for a 100 micron diameter pixel) or better, While this is relatively easy to achieve with a single pixel and a photodiode detector the implementation of an imaging version is much harder. What is then required is a pixelated camera as the detector in which each pixel is itself a differential detector. This is the essence of the camera designed and fabricated for this project at Nottingham. This has provided a general differential imaging camera with enormous potential, used here with the optical phase dither technique to visualise over a substantial area very small changes in the surface plasmon.



Custom CMOS cameras for modulated light detection

Mark C Pitter, Nicholas Johnston, Roger A Light and Mike G Somekh

In order to exploit fully the highly sensitive imaging techniques for surface binding that have been developed by the Attogram grant, we need a new form of array camera that can demodulate AC signals. Existing camera technology suffers from insufficient bandwidth (we wish to demodulate a 50 kHz AC signal, which requires that we obtain sample measurements at 200 kHz) and/or inadequate dynamic range (the AC modulation is often at a very low level compared to the DC background).

Conventionally, signals of this nature would be demodulated using a photodiode and a lock-in amplifier. However, this only allows one channel of measurement at any one time. One custom camera described in this talk consists of an array of 64 simple lock-in amplifiers which can be connected to any row of a 64 x 64 photodiode array. This camera is currently installed in d-SPR system at Exeter, and allows for very sensitive demodulation, but obviously the image has to be acquired one row at a time.

Another approach under investigation is to modify the standard CMOS active pixel to our purposes. The active pixel is an integrating sensor analogous to a CCD camera. We have increased the dynamic range of the standard active pixel by adding addition high density capacitors and enable high bandwidth acquisition by building four analogue memories into each pixel. With this architecture, we acquire four sequential images at 200 kHz. These are then downloaded and processed to obtain the AC signal we require. This camera is capable of very fast acquisition as it is fully parallel, but does not allow for on-chip averaging, meaning that several frames must be acquire to obtain the necessary signal to noise ratio.

A third architecture will also be introduced that attempts to combine the best features of the initial prototypes to allow for a high frame rate with on chip averaging. This involves various trade-offs, and the collaborative work with the d-SPR team at Exeter is working towards establishing the most suitable final architecture.

COPD: Problems & Prospects of Early Detection

Dr Lucy Fairclough[1], Dr Jo Richens[2], Rebecca Metcalf[1,2], J. Corne[3] and Prof Paul O'Shea[2]

Chronic obstructive pulmonary disease (COPD) is a treatable and preventable disease state, characterised by progressive airflow limitation that is not fully reversible. It is a current and growing cause of mortality and morbidity worldwide, with the World Health Organization (WHO) projecting that total deaths attributed to COPD will increase by more than 30% in the next 10 years. The pathological hallmarks of COPD are destruction of the lung parenchyma (pulmonary emphysema), inflammation of the central airways (chronic bronchitis) and inflammation of the peripheral airways (respiratory bronchiolitis). Although COPD is primarily a disease of the lungs there is now an appreciation that many of the manifestations of disease are outside the lung, leading to the concept that COPD is a systemic disease.

Currently, diagnosis of COPD relies on largely descriptive measures to enable classification, such as symptoms and lung function. The inaccuracies in measure of lung function can however lead to difficulties in the detection of COPD in the early stages of disease, often delaying the onset of treatment when it would be most effective. It is therefore important to identify peripheral markers of both early disease and disease activity, which may have implications for diagnosis, treatment, prognosis and measurement of drug efficacy.

We are using existing technologies, such as flow cytometry and RT-PCR, to identify a panel of proteins and genes that will enable early identification of disease, as well as for slide validation purposes. However, current strategies used thus far are not without problems of their own. Once documented, genes and proteins identified will be used by the novel strategy being designed by the scientists collaborating in the RCUK BT grant. It will develop an easy-to-use label-free system enabling the technology to be utilised by all health care professionals. It will also be cost effective, enabling all individuals to be tested and thus enhance early detection of COPD.

[1]Department of Immunology, University Hospital, University of Nottingham [2]Cell Biophysics Research Group, University Park, University of Nottingham [3] Division of Respiratory Medicine, University Hospital, Nottingham

Making use of gold - first steps in array reading

Baptiste Auguié, Andrew Murray, Rouslan Olkhov, Andrew M. Shaw, Professor Bill Barnes
School of Physics, University of Exeter

Whilst the development of super-sensitive surface-plasmon based sensing methods and new camera technology are key components of the Attogram project, so too is developing the array format technology. We needed to be able to develop the some aspects of the array technology without waiting for the super-sensitive sensing developments to be completed. We also wanted to move as quickly as possible with a technique that would allow us to gain biologically/clinically relevant data, as early as possible. To fulfil both of these demands we developed a simple 'low tech' array format technique. Whilst not having the sensitivity that we are developing elsewhere in the project, this work allows us to establish a number of other techniques that we need in the project and, as we have found, is likely to provide a useful technology in its own right.

The concept is relatively simple. We have printed arrays of spots ~ 100 μm in size with seed particles that nucleate the growth of gold nanoparticles. These seed particles are developed chemically, functionalized, and then interrogated optically to detect binding of target analyte molecules. In doing this we are able to test and gain experience with a number of key project techniques, array printing, image processing, functionalization, data analysis and interpretation. Our 'low tech' approach, though simple, already shows signs of promise[1].

In the talk we will look at the science underlying the operation of this array reader, identify some of the project-generic issues, such quality of the printed spots, and discuss some early test results. So far we have been able to produce and analyze arrays having 96 spots.

The value of this particle array approach would be significantly improved if we could increase the sensitivity of the technique. Ultimately the approach described here will be overtaken in sensitivity by the differential surface plasmon approach, but nonethe-less, improving the sensitivity our 'low tech' array reader would give it greater scope. To that end we have been exploring the use of different types of metallic nanoparticles. Results will be presented that show how careful design of such particles might lead to the increased sensitivity we desire.

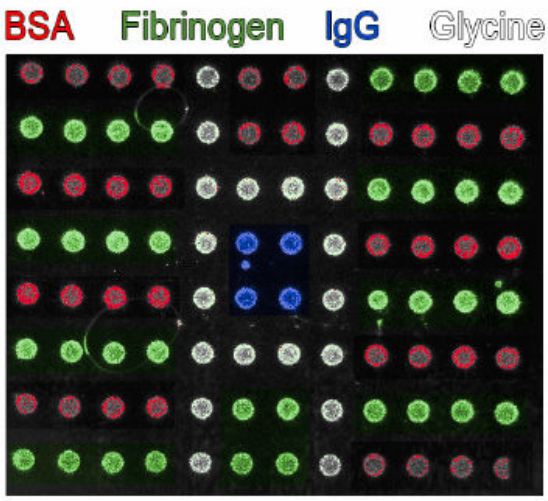


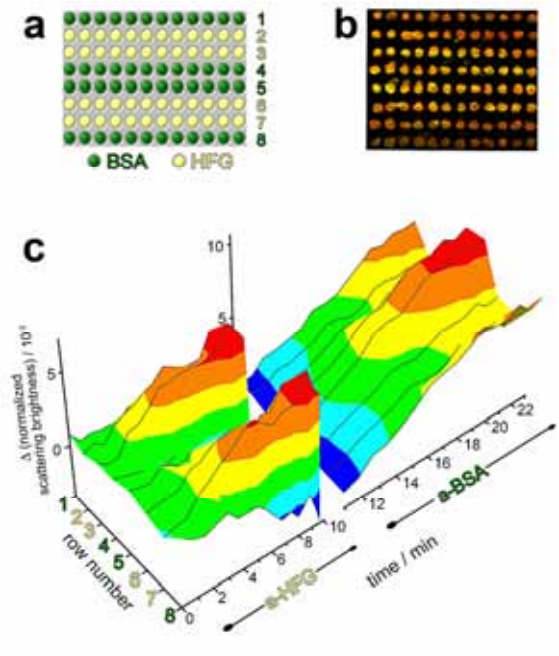
Figure caption.
A 96 spot array. Four types of protein have been printed on the spots. In the picture they are coded by false colour. The picture simply demonstrates our ability to print different biochemistries onto a suitable array format.

1. R. V. Olkhov and A. M. Shaw, Biosensors and Bioelectronics **In Press, Corrected Proof** (2008).

Kinetic Analysis of Array Assays: Biomarker Expression Profile

Rouslan V. Olkhov and Andrew M. Shaw, School of Biosciences, University of Exeter

The expression profile of biomarkers requires the accurate determination of the concentration of the target species in the hostile environment of the blood plasma. The label-free technique we employ is to monitor the binding kinetics of the target antigen to its monoclonal antibody. The first demonstration of label-free plasmon-based arrays has screened antibodies from whole antiserum for the target proteins Human fibrinogen (HFG) and Bovine Serum Albumen (BSA). Seed gold nanoparticles 4 nm in diameter are printed into the array configuration using an inkjet printer, and developed on the surface to form larger particles of different shapes. The target proteins are then printed on the array chip which is then placed in a small-form cost effective array reader. The array is illuminated by two lasers of different wavelength in near field configuration and the scattered light imaged using a conventional video camera. The current refractive index sensitivity of the array reader is 5×10^{-4}, for a bulk refractive index change. BSA and HFG antisera are passed the array with specific binding kinetics on the antibodies on their respective epitopes. The association rate constant for specific binding is 10^6 M s^{-1} whilst the non-specific association rate constant is 10^3 M s^{-1}. The current sensitivity for screening BSA and HFG in whole anti-serum is µg ml^{-1}. The multi-spot, multi-assay array format allows repetition of the assay many times to improve the measurement precision and may be compared to a number of control and reference spots which act as internal standards to ensure the precision of the biomarker concentration expression profile. The limitations of the kinetic analysis for specific binding discrimination will be explored and the design of control and reference spots and the array format will also be discussed.



Epitope screening on a nanofabricated array in whole serum of Bovine Serum Albumin (BSA) and Human Fibrinogen (HFG): (a) the array key; (b) scattered radiation image of the array; and (c) epitope screening kinetics for fibrinogen and BSA

The Biological Data Problem; collection, analysis & interpretation

Professor Paul O'Shea and the 2D Attogram team, Cell Biophysics Group, School of Biology, University of Nottingham

This presentation will reiterate the vision and goals of the 2D Attogram project. Some of the problems encountered with the overall goal will be discussed in light of the technologies that are being developed as part of the programme of work. Emphasis will be placed on the biomedical exemplar of the project, namely the exquisite detection of patterns of molecular markers in a complex biological fluid ie blood. This strategy will be developed to show that there are numerous additional potential applications. Some of which may potentially provide a road-map to accommodate the enormous demands from the biological and biomedical sciences for multi-parametric data at unprecedented levels of sensitivity. Finally, these applications may not just be confined to life science problems and some discussion will be directed towards applications of the technology to detection solutions for environmental and even national security issues.

Designing Plasmonic Nanoparticles for Sensing

Baptiste Auguié, Andrew Murray, Bill Barnes, School of Physics, University of Exeter

Plasmonic materials such as arrays of metallic nanoparticles have been recently considered in the design of highly sensitive and miniaturized optical sensors. Their very specific optical properties arise from the fact that they support localized surface plasmon resonances (LSPR). These resonances comprise electromagnetic fields that are bound to the particles through the interaction of the electromagnetic field with the free electrons in the surface of the metal. This interaction is resonant at a frequency (wavelength) that depends strongly on the particle's size, shape, composition and, crucially, environment. At resonance the fields associated with these modes are very significantly enhanced, thus when white light is incident on such a particle only wavelengths that match the resonance are strongly scattered. Further, the enhanced fields are concentrated near the surface of the metal so that the resonant response is easily perturbed by changes to the surface of the particle [1], e.g. when an anti-body is bound to it. Monitoring changes in the scattered spectrum forms the basis of this sensing technique [2].

The performance of such sensors depends on several parameters, most notably the spectral width and intensity of the plasmon resonances. In this regard we study the influence of the particle shape (aspect ratio) and volume, and compare the results for single particles and both ordered and disordered particle arrays.

Using e-beam lithography (EBL) we explore the precise effect of small modifications of single particles on the LSPR they support by conducting optical measurements using dark field spectroscopy. The same EBL technique allows us to fabricate arbitrary arrangements of nanoparticles. Of particular interest is the response of regular arrays with a spacing of the order of the wavelength of the LSP resonance. It has been shown in the literature [3] that radiative coupling between the nanoparticles can lead to a drastic sharpening of the resonant feature. In fact, the spectral lineshape is mostly dictated by geometrical factors and is found to be much sharper than the single particle resonance. This is a clear advantage over disordered arrays which exhibit strong inhomogeneous broadening. We demonstrate this effect experimentally, and study the influence of the particle size, shape, separation, and environment on this novel feature.

We compare our results with alternate techniques for mass production of large areas of nanoparticles, such as nanosphere lithography, and evaporated thin films that form a discontinuous arrangement of gold nano-islands. Basic refractive index sensitivity measurements are carried out on each type of sample, by varying the bulk environment.

References

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2. Haes, A.J., S.L. Zou, G.C. Schatz, and R.P. Van Duyne, *Nanoscale optical biosensor: Short range distance dependence of the localized surface plasmon resonance of noble metal nanoparticles*. Journal of Physical Chemistry B, 2004.
3. E. M. Hicks, S. Zou, G. C. Schatz, K. G. Spears, R. P. V. Duyne, L. Gunnarsson, T. Rindzevicius, B. Kasemo, and M. Kall. *Controlling plasmon line shapes through diffractive coupling in linear arrays of cylindrical nanoparticles fabricated by electron beam lithography*. Nano Lett, 5(6):1065–70, Jun 2005.

Polarisation Modulated Surface Plasmon Sensing

Ian Hooper, Ciaran Stewart and Roy Sambles, Thin Film Photonics, School of Physics, University of Exeter

The interest in optical biological and chemical sensing technologies has never been higher than in recent years. The demand for increased sensitivity and parallelism has arisen not only from areas of pure research, such as the burgeoning field of proteomics, but also from the pharmaceutical industries due to its utilisation in drug discovery processes. The Surface Plasmon (SP)[1] resonance sensor is one such optical method and works by allowing very small changes in the local refractive index of the medium bounding the SP active surface to be determined by monitoring the excitation condition of the resonance. By functionalising the SP active surface specific binding of analytes can be monitored in real time. There are many techniques allowing small changes in the resonance condition to be determined, usually based on angle or wavelength interrogation[2]. Here we present a new method based upon monitoring the polarisation state of light reflected from a Kretschmann-Raether SP system[3-5]. The sensitivity to bulk refractive index changes in both gases and liquids is shown to be of the order 10^{-7}RIU, which is comparable to the very best currently available commercial systems. Also presented are data from a dual channel system allowing referenced sensing, and initial bio-chemistry sensing experiments. The method is ideally suited to array sensing [5].

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3. Hooper I R and Sambles J R 2004 Sensing using differential surface plasmon ellipsometry *J. Appl. Phys*. **96** 3004-3011
4. Stewart C E, Hooper I R and Sambles J R, Surface Plasmon Differential Ellipsometry of Aqueous Solutions for Biochemical Sensing (accepted by J.Phys.A)
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Custom CMOS Cameras for Biological Imaging Using the Detection of Surface Plasmon Resonance

Nicholas Johnston, Mark C Pitter and Mike G Somekh
School of Electrical and Electronic Engineering, University of Nottingham

At the University of Nottingham, we are trying to create a portfolio of custom CMOS cameras that utilise a variety of different detection techniques and electronic processing of the signals obtained. In this poster we describe two of these systems, namely continuous time and integrating pixel arrays, that while specifically targeted at differential Surface Plasmon Resonance (d-SPR) sensing, can be applied to many generic scientific imaging problems where array based demodulation of an AC signal is required.

The light signal resulting from the d-SPR setup comprises DC, 50 KHz and 100 KHz components. However the information about the plasmon is contained only on the 50 KHz signal which therefore must be isolated. Two different architectures and pixel types have been fabricated, with a third currently being fabricated.

The architectures being investigated are a column based design which implements signal processing analogous to a linear array of lock-in amplifiers and a fully parallel phase stepping array based on modified integrating pixels. Both designs have been prototyped as 64 x 64 pixel arrays and are fully scalable. The column based approach allows for more on-chip processing power, whereas the fully parallel design lends itself to higher frame rates. An investigation into the trade-offs between logarithmic continuous time pixels and linear integrating pixels is also underway. The results from these two prototype arrays will lead to an optimised detector combining the best features of both of the arrays.

Development of intelligent biomedical diagnostic materials; surface and chemical studies into rapid label-free multi-parametric detection microarrays

E A M Lunt[a], J L Richens[a], J Zhang[b], X Chen[c], M C Pitter[b], M G Somekh[b], and P O'Shea[a]

The development of novel materials for next-generation microarray platforms will allow the possibility of ultra high-throughput and very rapid screening of thousands of biological interactions simultaneously. The technology, using a spatially resolved, at-togram sensitive surface plasmon resonance technique (SPR), will provide kinetic data of binding events for a variety of different biological molecules on one chip. Particular focus on the advantages available with SPR over current fluorescence techniques is emphasized. The results of pilot studies towards the optimisation of protein microarray and interro-gation techniques (SPR and AFM) are shown. Chemistries studied have been chosen to enable comparison between different levels molecular orientation. The most suitable have been taken forward for further studies of relative reactivity and surface density.

[a] Cell Biophysics Group, School of Biology, University of Nottingham, University Park, Nottingham
[b] Applied Optics Group, Department of Electrical Engineering, University of Nottingham, University Park, Nottingham
[c] School of Pharmacy, University of Nottingham, University Park, Nottingham

Studying protein interactions with gold nanospheres; enabling studies of reaction kinetics and the effects of surfaces on reactivity and structure

E A M Lunt[a], M C Pitter[b], M G Somekh[b], and P O'Shea[a]

The study of protein interactions is an area of much interest, particularly towards obtaining more detailed information about biological processes. Here, we show how information about the binding of proteins to conjugated gold nanospheres can be obtained using straightforward experimental techniques. Firstly, a Perkin Elmer LS 55 luminescence spectrometer was used to observe the changes in light scattering caused by the binding of complementary proteins to conjugated 40 nm nanoparticles, measured by the intensity change over time. Further kinetic studies have been carried out at 530 nm to obtain more detailed information about the processes involved in the binding reaction. Secondly, UV-Vis spectrometry has been used to study the interactions of cytochrome C and its derivatives with 100 nm gold nanospheres. The results of this study will be used as a platform for further work on SPR techniques within the Attogram collaborative.

[a] Cell Biophysics Group, School of Biology, University of Nottingham, University Park, Nottingham
[b] Applied Optics Group, Department of Electrical Engineering, University of Nottingham, Nottingham

The Cell-Cell Interactions In Chronic Obstructive Pulmonary Disease

B. Metcalf[1,2], P. O'Shea[1] and L. Fairclough[2]

Chronic obstructive pulmonary disease (COPD) is defined by the presence of emphysema, chronic bronchitis and respiratory bronchiolitis. At present the condition is the fourth commonest cause of death worldwide and is closely associated with cigarette smoking. COPD results in irreversible airflow limitation with small airway and lung parenchyma inflammation. During the inflammatory response, the influx of cells such as macrophages and cytotoxic T lymphocytes (CTLs) has been observed. In addition resident cells of the lung have been shown to be involved in the pathophysiology of COPD, such as airway smooth muscle cells, alveolar macrophages and alveolar epithelial cells. The interactions of the immune cells with these lung cells have not yet been characterised. In order to study these cellular interactions, we isolated CTLs from the peripheral blood of COPD patients, non-COPD smokers and non-smokers and co-cultured them with an alveolar epithelial cell line. We have measured the production of various cytokines from each cell type over a time course using flow cytometry. The information obtained will be used to identify markers of disease susceptibility and will provide a biological model for the 2D Attogram technology.

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Lung Cytokine Production in Chronic Obstructive Pulmonary Disease

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Chronic obstructive pulmonary disease (COPD) is a condition that consists of three main pathologies, namely emphysema, respiratory bronchiolitis and chronic bronchitis. Emphysema is characterised by enlargement of the alveolar air spaces, loss of elasticity in the lung, and lung parenchyma destruction. The combination of the three diseases results in irreversible airflow obstruction and deterioration in lung function. The main risk factor for developing COPD is cigarette smoke, however it is estimated that only 15-20% of the smoking population go on to develop COPD.

In order to identify possible biomolecules that can be used by the attogram technology to characterise disease susceptibility and severity, we have used lung tissue obtained from smokers with COPD and smokers without COPD. Using standard histological techniques, we have characterised the severity of emphysema in each patient by looking at the morphology of the alveoli. We then analysed the production of a variety of cytokines from the lung cells using the luminex kits. The aim is to see if there is any correlation between disease severity and the pattern of cytokines being produced within the lungs.

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CMOS integrating camera for modulated light and arrays of single photon avalanche diode arrays

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At the University of Nottingham, we are trying to create a portfolio of custom CMOS cameras that utilise a variety of different detection techniques and electronic processing of the signals obtained. This drive has produced a number of cameras that are useful in different types of optical experiments. In this poster we describe two of these systems, namely integrating pixel linear array detectors and single photon avalanche diode arrays (SPADs).

A common feature of the optical experiments is the use of modulated light, where the modulation either occurs as an integral part of the experiment or is used as an input to the experiment to tag the signal of interest. In this scheme, the signal usually consists of a very small modulation on top of a large background, as is the case in Surface Plasmon Resonance imaging. A camera chip denoted 'atto2' has been developed to measure these modulated signals. The camera is a linear array of 64 integrating pixels, each with four large storage capacitors that provide an improved signal-to-noise ratio and allow the four phase operation required to detect modulation.

Other types of experiment require different detectors to function well. In fluorescence imaging the detected signal consists of a low signal of interest on a low background. An exciting solution for this type of imaging is to use SPAD arrays. SPADs operate in a fundamentally different way to the integrating pixels used in 'atto2' and are able to detect single photon events and provide information on when the event occurred and this makes them suitable for making measurements in very low light situations. A chip for characterising SPADs has already been designed and manufactured and is showing promising results in terms of very low dark counts and high quantum efficiency. The design of an array camera chip using SPADs is currently under way.

Optical properties of single gold and silver nanoparticles

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Gold and silver nanoparticles have been studied extensively during the past decade owing to their interesting optical properties. By fabricating nanoparticles with a variety of sizes and shapes it is possible to dramatically change how light that is directed towards the nanoparticle is scattered and absorbed. This is an affect particularly observed for Au and Ag nanoparticles since there are electrons in the metal that are free to move under the influence of an applied electromagnetic field. Light is composed of just such a field that acts to induce a driven oscillation of the electrons resulting in resonant scattering and absorption (collectively referred to as extinction) within the visible and near infra-red regions of the electromagnetic spectrum. These resonant oscillations of the electrons are referred to as localized surface plasmon resonances (LSPR) [1] leading to an enhanced electromagnetic field relative to the incident field at, and very close to, the surface of the nanoparticle. The position (wavelength at which maximum extinction occurs) and width of the LSPR depend upon a number of factors including the size, shape and composition of the particle [2]. However, the properties of the LSPR also depend sensitively on the composition of the embedding medium thus enabling their use as biosensors.

Here results are presented that are obtained by monitoring the optical response of single Au and Ag nanoparticles. Two fabrication techniques are employed for realizing nanoparticles with a variety of sizes and shapes, (1) electron beam lithography (EBL) and (2) chemical synthesis. Dark-field microscopy combined with spectroscopy is then used to collect and analyze the light scattered by individual nanoparticles. By incorporating samples into a custom-built flow cell and cycling through solvents with different refractive indices the sensitivity of the LSPR to a bulk change in the medium surrounding the nanoparticle is investigated. Furthermore, the LSPR is monitored during and after deposition of thin layers of material onto the nanoparticle thus allowing inference of the sensitivity of the LSPR to molecular adsorption.

Experiments are also described that attempt to extend the single particle approach by observing the effect of introducing a second nanoparticle in close proximity to the first. By post-processing chemically synthesized nano-rods using a focused ion beam it is possible to produce separations of ~10 nm. It is suspected that small gaps between nanoparticles leads to a larger enhancement of the electromagnetic field associated with the LSPR and accordingly a higher sensitivity to a change in the medium surrounding the nanoparticle.

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Problems for detecting low abundance cytokines in plasma samples

JL Richens[1], EAM Lunt[1], L Fairclough[2] and P O'Shea[1]

COPD is a mainly considered a disease of the respiratory system but evidence suggests that it also has large systemic consequences. The relative non-invasiveness of blood collection makes analysis of plasma components for disease diagnostics preferable over other more invasive procedures such as induced sputum, bronchoalveolar lavage and tissue biopsies that are also appropriate for respiratory diseases. When being used in disease diagnostics, it is vital that the results obtained from samples are accurate and reproducible. However, detection of low level molecules, particularly cytokines, is particularly difficult in samples containing albumin. Albumin is found in many clinical samples, but especially in plasma where it is present at a concentration of 35-50mg/ml. This equates to at least 10^7 orders of magnitude higher than the concentration of cytokines such as IL-6 and IL-8. We have undertaken studies to investigate the effect of albumin on the observed levels of cytokines in chip-based protocols. Here we demonstrate that the presence of albumin has an effect on detection levels of IL-8 and other potential markers. We have also identified potential reaction conditions that may improve detection levels of low abundance proteins in plasma.

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Application of commercially available multiparametric technologies for target molecule identification and chip validation

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Living organisms are complex entities resulting from interactions between multiple cellular, genetic and molecular components. Disruption at any level can alter the biomolecular profile of a patient, triggering disease onset and providing potential indicators of disease status. There are a multitude of technologies available that allow detection and identification of such biomolecules at both the protein and molecular level. Well established techniques such as ELISA, flow cytometry and PCR have been modified to provide opportunities for multiparametric analysis. These adaptations include microarrays, bead-based fluorescent ELISA systems and real-time PCR systems. Such technologies enable identification of target biomolecules for disease diagnosis and classification. They also serve as validation tools for the development of our disease-specific microarray chip. Consequently, it is vital that intra- and inter-kit variability is low and that values obtained for sample analyte concentrations are reproducible between assays. Here, we present the relative advantages and disadvantages of current techniques and demonstrate their applicability to patient samples.

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Label-Free Antibody-Antigen Binding Detection by Optical Sensing Array Reader Based on Surface-Synthesized Gold Nanoparticles

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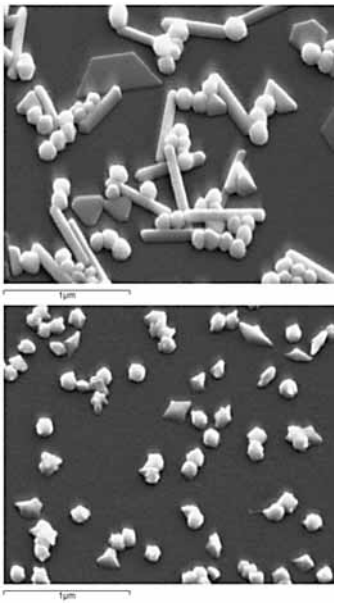
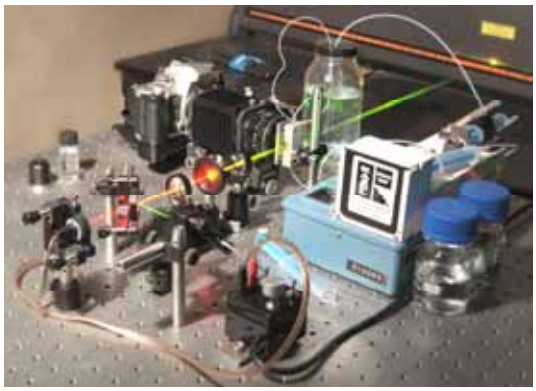


SEM images of surface particle geometries

Gold nanoparticles are grown *in situ* from printed seed particles (4 nm in diameter) on a glass substrate and have been fabricated into a biosensor array. Each spot contains different shaped particles; rods, triangular prisms and truncated prisms. The light scattering properties of the resulting nanoparticle surfaces, illuminated in near field, show sensitivity to changes in the local refractive index with an optimum sensitivity of 1×10^{-4} RIU for bulk refractive index changes. Each array spot, containing gold nanoparticles, is functionalised with a protein of choice. The scattered radiation intensity is used to monitor the refractive index change on label-free specific bingeing of the antibodies to their antigens from whole blood antisera. The simple optical configuration produces a small form low-cost array reader with potential point-of-care applications. We have demonstrated antibody screening from whole anti-sera for Bovine Serum Albumin and Human Fibrinogen with antibody concentrations of order 10 nM, or 1 μg ml^{-1}. Non-specific binding does not interfere with specific binding at these concentration levels and a clear screening of each antiserum is possible. The application of the array reader technology to biomedical array imaging and high-throughput screening is discussed.



The array reader

Surface Plasmon Differential Imaging for Sensing Applications

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The Surface Plasmon Resonance (SPR) has been utilized in various forms in sensors for many years [1]; usually based on angle or wavelength interrogation. Recently a new SPR sensor has been developed [2] that utilises ellipsometric interrogation of the SPR. It is a technique that exploits the change in phase of Transverse Magnetic (TM) light as the SPR is traversed when compared to Transverse Electric (TE) light (which does not excite SPR on a flat surface).

Linearly polarised light (containing both TM and TE components) from a high intensity LED is passed through a polarisation modulator, resulting in a small amplitude 47 kHz modulation in the polarisation angle. This light is incident on a gold film ~50 nm thick evaporated onto the base of a SF2 prism in the Kretschmann-Raether configuration [3]. The coupling of the TM component of the polarised light to the SPR is heavily dependant on the properties of the dielectric medium adjacent to the gold film. The resonance shifts when this sensed medium undergoes a change in refractive index. This in turn causes a change in the reflected polarisation state. The resultant reflected signal is interrogated through the use of a camera (64 by 64 pixels) with the equivalent of a lock in amplifier on each of the 4096 pixels. Information can then be extracted about the changing polarisation state. By imaging in this way it is possible to produce a multi channel sensor capable of analysing changes within a large bio-addressable array.

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