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# *Microscopical Society of Ireland*

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## ORAL PRESENTATION ABSTRACTS

### **THE MICROSCOPE - A PAST, PRESENT AND FUTURE FORENSIC TOOL.**

**[Invited, Roche Lecture]**

P. Hamer, Forensic Alliance, UK

Forensic microscopes have evolved from the simple monocular microscope to hybrid analytical instruments. This review will touch on the evolution but concentrate on current use and future potential with a focus on particulate trace evidence. There is often a significant delay in the introduction of new techniques from the academic environment into forensic work. Some of the reasons for this will be explored using as examples FTIR/ microscope systems and Raman instruments.

### **THREE DIMENSIONAL IMAGING OF DAMAGE IN STRUCTURAL MATERIALS UNDER LOAD USING HIGH RESOLUTION X RAY MICRO-TOMOGRAPHY.**

**[Invited]**

J.Y. Buffière

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X-ray micro-tomography is a very attractive technique which enables the visualisation of internal features in a sample. Being a non destructive method of observation, it also enables, in principle, in situ visualisation of damage during loading and provides therefore the chronology of damage initiation and growth. In spite of all these advantages, this technique has been rarely used in materials science mainly because its resolution (typically around 100  $\mu\text{m}$  for classical medical applications) was of limited efficiency in terms of damage characterisation. In the last ten years, however, significant progress has been made in terms of resolution with both the availability of new third generation synchrotron X-ray sources as well as new detectors. Spatial resolution close to that of an optical microscope can now be achieved which opens (or re-opens) wide areas of research.

In this presentation, recent experimental results obtained at the European Synchrotron Radiation Facility (ESRF) are shown. They illustrate the potential of high resolution X-ray micro-tomography for the characterisation of damage development in structural materials. Fatigue crack initiation and growth has been investigated in metallic alloys submitted to various type of loading conditions. Quantitative data can be extracted from the three dimensional images and used to test/validate damage models. The limitations of the technique, in its current state of development, are also shown, giving indications for future developments.

### **A TEM ANALYSIS OF MESOPOROUS SILICATE STRUCTURE**

S. Hudson, D.A. Tanner\*, W. Redington, E. Magner, K. Hodnett, and S. Nakahara  
Materials & Surface Science Institute, University of Limerick, Limerick.

Mesoporous silicate materials contain a unique architecture of ordered pore structure with a narrow pore size distribution. This class of mesoporous materials has indeed attracted considerable interest in recent years, offering an opportunity for possible applications in the area of catalysis and sensors. A powder form of mesoporous silicates is commonly produced by means of a cooperative assembly process, whereby the inorganic moiety (e.g. amorphous silica) is condensed between ordered surfactant micelles. The shape of each powder particle thus obtained is ellipsoidal and the particle generally exhibits a mesoporous structure, in

which ordered pores are organised in the form of a hexagonal (honey-comb-like) cell on the circular cross-section. Their pore structure has been characterized using a transmission electron microscope (TEM). In the TEM, finger-print-like parallel lines running along the long axis are often observed in addition to the honey-comb structure seen on the circular face. Although this contrast is known to vary sensitively with defocusing, a detail of the contrast behaviour has not been discussed clearly to relate to the pore geometry. Motivated by the appearance of these complex contrast features, we conducted a transmission electron microscope study on mesoporous silicate materials. The TEM results were then compared with pore information (the shape/size of the pore and the wall thickness of silicate) obtained by complimentary techniques such as gas adsorption and x-ray diffraction methods. The origin of the contrast and its relationship to the pore geometry will be discussed.

## **SELF-STRATIFYING COATINGS**

N. Stobie, M. Wyer, and J. Colreavy

CREST, Dublin Institute of Technology, Kevin St, Dublin 8

A self-stratifying coating comprises a multi-functional coating in one application. Conventional multi-coat systems consist of two or more discrete layers applied to a substrate. Multi-coat systems can have the disadvantage of poor interfacial adhesion which may lead to inter-coat boundary problems. Self-stratifying coatings improve the surface and adhesive properties when only one single coating system is applied. One of the main driving forces behind self-stratification is the difference between the surface energies of the two resins with the migration of the low surface moiety to the surface. Self-stratifying blends for wood and steel were examined in this study. Standard wood systems consist of a metal primer (Al), an alkyd undercoat (fatty acid ester) for hiding and surfacing properties, and for exterior use a urethane modified alkyd topcoat for UV resistance, gloss and colour. Alkyds due to their oxidative curing mechanism progressively cross-link and crack over time, thus requiring the need for additional coatings. In this study, a waterborne system consisting of an alkyd and a low surface energy resin was examined for stratification.

A typical coating for steel consists of an epoxy based primer containing an inhibiting anti-corrosive pigment and excellent adhesive properties from the hydroxyl functionality of the epoxy. A top coat for steel would comprise a urethane modified acrylic with good UV resistance and colour. A blend of an epoxy with a fluoropolymer was examined for evidence of stratification on steel.

FT-IR was used to detect whether stratification had occurred for the epoxy/fluoropolymer blend. The top and bottom layers of the coating were analysed to chemically distinguish between the two layers. The spectra of the top and bottom layers were compared to the parent compounds and showed that stratification had occurred. SEM-EDX was used to examine the films on both wood and steel systems in cross-section for evidence of stratification. Due to the low atomic number of the fluorine atom, it was difficult to detect with EDX. Elemental dot-mapping for chlorine, which was also present in the epoxy/fluoropolymer blend, indicated that the fluoropolymer stratified towards the air interface. Pigmentation of the alkyd resin with the titanium oxide, prior to mixing with the low surface energy resin showed that the titanium oxide was most concentrated at the wood/coating interface. Surface energy data from video microscopy revealed that the overall energy was low. Optical microscopy revealed the appearance of stratified multi-layer systems. While stratification has been previously reported with solvent borne systems, this represents the first report of a waterborne self-stratifying coating.

## **CONSERVATIVE AND DISSIPATIVE FORCE IMAGING OF SWITCHABLE ROTAXANES WITH FREQUENCY MODULATION ATOMIC FORCE MICROSCOPY**

A.A. Farrell,<sup>1</sup> T. Fukuma,<sup>2</sup> T. Uchihashi,<sup>1</sup> E.R. Kay,<sup>3</sup> G. Bottari,<sup>3</sup> D.A. Leigh,<sup>3</sup> H. Yamada,<sup>2</sup> and S.P. Jarvis<sup>1</sup>

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We compare constant amplitude frequency modulation atomic force microscopy (FM-AFM) in ambient conditions to ultra high vacuum (UHV) experiments by analysis of thin films of rotaxane molecules. Working in ambient conditions is important for the development of real-world molecular devices. We show that the FM-AFM technique allows quantitative measurement of conservative and dissipative forces without instabilities caused by any native water layer. Molecular resolution is achieved despite the low Q-factor in air. Furthermore, contrast in the energy dissipation is observed even at the molecular level. This should allow investigations into stimuli-induced sub-molecular motion of organic films.

## **CHARACTERISATION OF STRAINED Si ON VIRTUAL SiGe SUBSTRATES USING RAMAN SPECTROSCOPY**

A. Waldron<sup>1</sup>, T. Perova<sup>1</sup>, R.A. Moore<sup>1</sup>, K. Lyutovich<sup>2</sup>, E. Kasper<sup>2</sup>, M. Oehme<sup>2</sup>, J. Werner<sup>2</sup>

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Micro Raman spectroscopy has been employed to investigate the degree of strain in Si layers on SiGe virtual substrates grown by molecular beam epitaxy (MBE). An initial growth stage during MBE at a temperature-ramp down to below 200°C causes misfit-dislocation generation by nucleation of point defects and provides early relaxation in the SiGe buffers. A 20 nm thick strained Si layer is deposited at the end of the process at temperature of 5000°C. Micro-Raman spectroscopy in backscattering geometry with 514.5 nm excitation of Ar<sup>+</sup> laser has been used for the analysis of stress and Ge content in the layers. Investigation of Raman spectra in the Si-Si mode region allows data on stress in strained Si layers on virtual SiGe substrates to be obtained. Firstly, investigation of the strained Si Raman peak allows the value of stress and strain in the layer to be found as well as the lattice constant of the strained Si. Secondly, analysis of Si-Si, Si-Ge and Ge-Ge Raman peaks from the SiGe virtual substrate allows the degree of relaxation to be found as well as the Ge content of this layer. Whether the strain obtained in the overlying Si layer is maximal for a particular fabricated structure can also be established. The lattice constant of the SiGe structure is also found, taking into account the degree of relaxation in the layer. Strain values obtained from Raman results associated only with the strained Si layer are in good agreement with those obtained from Raman results for the SiGe layer.

## **PHOTOINDUCED SURFACE RELIEF STUDIES IN ACRYLAMIDE-BASED PHOTOPOLYMER**

K. Pavani, I. Naydenova, S. Martin, V. Toal

Centre for Industrial and Engineering Optics, School of Physics, Dublin Institute of Technology, Kevin Street, Dublin 8

Surface relief gratings have been optically recorded in dry, self-developing acrylamide based photopolymer. An investigation of the dependence of photo induced surface relief amplitude and profile on recording intensity, UV post exposure, thickness of the sample, composition of the photopolymer and temperature at constant spatial frequency was carried out using white light interferometry. Unusual surface relief grating profiles which depend on sample thickness were observed at low spatial frequency.

The surface relief effect is intended to be applied to the alignment of liquid crystals for different applications such as voltage controllable diffraction gratings, lenses, polarizing components and switches.

## **CILIA: STRUCTURE, IMMOTILITY AND DISEASE. A DIAGNOSTIC DILEMMA.**

**[Invited]**

D.C. Cottell

The Electron Microscopy Laboratory, Core Technology Conway Institute of Biomolecular and Biomedical Research. UCD Dublin

Primary cilia dyskinesia (PCD) is a genetically determined disease with pathological significance in all the histological systems in which cilia are found. Thus, the clinical features of PCD may include chronic recurrent infection of the upper and lower respiratory tract, bronchiectasis, sinusitis, rhinitis, retinitis pigmentosa, otitis media and infertility with a 50% incidence of situs inversus. By definition PCD includes also the triad of Kartagener's syndrome i.e. chronic bronchitis, sinusitis and situs inversus. It has long been established that all of the above lesions are a consequence of structural and functional defects in the cilia of the affected epithelium.

After clinical suspicion of the disease a diagnosis of PCD can be made using several methodologies e.g. measurement of mucociliary clearance in the respiratory tract, determination of beat frequency of cilia from a brush biopsy and ultrastructural studies. However, the former two procedures are difficult to perform and are often erroneous leaving the diagnosis to rest on the results of ultrastructural analysis. Unfortunately, many of the structural defects seen in genuine PCD are also found in acquired cilia dyskinesia hence the diagnostic dilemma, is it PCD or is it not? It is important to make the diagnosis because in PCD all the cilia in the body are affected and will forever be so whereas in the acquired condition effects may be localised and will only ever be temporary.

This presentation will focus on the structure and function of cilia, the significance of structural anomalies and the exploration of diagnostic difficulties.

## **A COMBINATION OF LIVE CELL MICROSCOPY AND COMPUTATIONAL MODELLING IDENTIFIES KEY PLAYERS OF APOPTOTIC SIGNALLING – A SYSTEMS BIOLOGY APPROACH**

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Apoptosis, or programmed cell death, removes superfluous or damaged cells from the body of multi cellular organisms. Enhanced or repressed apoptosis has been shown to contribute to developmental defects, autoimmune diseases, cancer, and neurodegenerative disorders.

Intracellular apoptotic signalling is accompanied by the release of mitochondrial proteins into the cytosol, mitochondrial depolarization, and subsequent activation of proteases of the effector caspase family. In previous studies we established multi-parameter imaging routines to analyse these signalling events spatiotemporally in single living cells using epifluorescence and confocal microscopy: Mitochondrial depolarization was observed by the membrane potential sensitive dye tetramethylrhodamine methylester (TMRM), the release of mitochondrial proteins was detected in cells expressing the fusion proteins cytochrome c-GFP and Smac/DIABLO-YFP. Subsequent caspase activation was detected by an effector caspase specific fluorescence resonance energy transfer (FRET) probe (CFP-DEVD-YFP).

The processes taking place between the mitochondrial release events and the subsequent caspase activation are complex. As naturally experimental set ups are confined to monitor only a limited number of parameters per experiment, quantitatively and temporally little is known about the interactions of the many other proteins within the apoptotic signalling network under physiological conditions. We therefore combined our live cell microscopy experiments with a comprehensive computer model based on the biochemical properties of the proteins involved.

With this systems biology approach of live cell imaging and mathematical modelling we were able to characterize the temporal profiles of a multitude of different protein fractions and identified XIAP (X-linked inhibitor of apoptosis protein) as a possible key regulatory protein in the signalling network. Model-generated hypotheses on the progression of apoptotic signalling upon overexpressing XIAP were subsequently tested by live cell microscopy. We indeed identified a sharp XIAP dependent threshold deciding on the efficient activation of effector caspases at an intracellular concentration range closely resembling the computer model prediction.

## **RAPID CELLULAR SIGNALLING IN MESENCHYMAL STEM CELLS AFTER APPLICATION OF A MECHANICAL FORCE USING ATOMIC FORCE MICROSCOPY.**

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Mesenchymal stem cells (MSC's) are bone marrow stromal stem cells which exist postnatally and occur in low incidence with extensive renewal potential. If stimulated MSC's have the ability to initiate a cascade of events leading to the formation of bone, cartilage, muscle, tendons, ligaments and fat. How initiation of this cascade occurs has not been fully elucidated nor have the signal transduction events that occur downstream. Differentiation of bone precursor cells toward bone formation have been observed to be responsive to mechanical force, with the magnitude of the force combined with its frequency being vital for transduction of the signal. The aim of this work was to investigate signal transduction in

MSC's after application of a mechanical force on a single MSC. Using atomic force microscopy (AFM) indentation a controlled mechanical force was applied to a single MSC. Combined fluorescent microscopy and AFM enables the study of intracellular and mechanical cell signalling. Signalling events were investigated locally (around the area of indentation) and globally (across the whole cell) in order to enhance understanding of the signal. In this study, MSC were transfected with a pAcGFP1-Actin vector in order to study actin distribution in real time after indentation. Increase's in intracellular calcium was monitored using Calcium Green in real time after indentation. Using the fluorescent technique "spectral unmixing", both actin distribution and intracellular calcium was monitored. This study has shown that mechanical stimulation can induce rapid signalling events which lead to the differentiation of MSC's along the pathway to bone.

## **VIBRATIONAL SPECTROSCOPY AS A NOVEL TOOL FOR CHARACTERISATION OF CELLULAR FUNCTION POST-IONISING RADIATION EXPOSURE**

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The biological applications of vibrational spectroscopy have received very considerable attention and development over the past decade. As a result techniques involving the use of confocal Raman spectroscopy have seen it employed as a tool for cell phenotyping (Notingher et al, 2004), tissue screening (Ó Faolain et al, ) and recently in the characterisation of cellular function (Eliasson et al, 2005; Nithipatikom et al, 2003) as an application of SERS and SERRS. The latter approach involves the introduction of fluorescent material and nanoparticles into the cell whose own spectra, or that produced when they form complexes with molecular species in the cell, allow the multiplexed assay of many cellular functions or morphologies in parallel. The present study demonstrates the application of Raman spectroscopy as a tool for the characterisation of cellular function post-ionising radiation exposure in human skin cells, with reference to established biochemical assays of cell function, and without the external introduction of complexes into the cell, and thus modification of cell function. Results indicate that many cellular functions modified by ionising radiation exposure (metabolism, liposome activity, ROS concentration, mitochondrial potential, and cell wall permeability), among others, may be assayed from the Raman spectrum alone.

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## **FASCIOLA HEPATICA: TEGUMENTAL CHANGES FOLLOWING IN VITRO TREATMENT OF AN IMMATURE TRICLABENDAZOLE-RESISTANT ISOLATE WITH THE EXPERIMENTAL FASCIOLICIDE, COMPOUND ALPHA.**

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Estimated to inflict an annual loss in ruminant livestock productivity totalling \$3 billion (US), infection with the liver fluke, *Fasciola hepatica* is of major concern throughout the farming community. It is the immature stage of the fluke's life cycle that is responsible for the pathological aspect of the disease as the parasite migrates through the host liver. Triclabendazole (TCBZ) is the current drug of choice for the treatment of liver fluke infection. Escalating incidence of resistance to this drug, however, has intensified the need to explore alternative means of treatment. A TCBZ derivative, compound alpha, has recently been developed and has been shown to be active against a field isolate of immature *F. hepatica* in vitro and in vivo. In this study, TCBZ-resistant immature *F. hepatica* were treated in vitro with the compound alpha sulphoxide metabolite for 6 h and 18 h at a concentration of 10µg/ml. Scanning and transmission electron microscopy (SEM; TEM) were employed to examine the fluke tegument. Changes in tubulin distribution within the tegument were determined with immunocytochemistry, using an anti-tubulin polyclonal antibody. The surface of the tegument displayed extensive swelling and blebbing which became progressively more severe toward the posterior end of the fluke. At the TEM level, the basal infolds were seen to be swollen; also, disorganisation and swelling of the mitochondria within the tegument syncytium were typical features at both time periods. Following treatment, the distribution of tubulin immunoreactivity in the tegumental syncytium was altered following treatment. These findings indicate the potential of compound alpha as an alternative to TCBZ and a means of treating TCBZ-resistant isolates of *F. hepatica*.

## **BIOLOGICAL APPLICATIONS OF ATOMIC FORCE MICROSCOPY IN LIQUID**

### **[Invited]**

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Atomic force microscopy (AFM) utilizes a microfabricated cantilever with a sharp tip mounted at its end for measuring a wide range of interaction forces acting on the tip. The obtained force signal is used for making an image of the scanned surface or for performing 'force spectroscopy' where the force is characterised as a function of tip displacement relative to the sample surface. The applicability of AFM to liquid environment and its high-spatial resolution makes it a promising technique for molecular-scale investigations of biological materials in their physiological environments. In our group, we have been developing and applying a variety of novel AFM techniques to the study of biological materials.

The high force sensitivity of AFM makes it possible to measure interaction forces of the order of picoNewtons. The range of applications and versatility of the AFM technique when specifically applied to biology will be highlighted, for example: the method has proved sensitive enough to quantitatively measure solvation forces on a variety of surfaces including biological materials such as lipid bilayers immersed in water. Using the same technique we have also managed to mechanically isolate single protein molecules in the extracellular matrix of living systems and measure their unfolding forces. By functionalising the AFM probe tip it

has been possible to measure single receptor-ligand interactions. Finally by using the AFM to indent localised regions within a single cell and combining the AFM with fluorescence microscopy, we have been able to monitor expression of individual cells in response to nanoNewton applied forces.

#### **Mo6S4.5I4.5 NANOWIRES: STRUCTURE STUDIES BY HRTEM AND ABERRATION CORRECTED STEM**

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Recently one-dimensional inorganic nanowires have been receiving growing attention as a viable alternative to carbon nanotubes and nanostructured, tube-like materials in general. The most promising of these are the Mo6S9-xIx family. Easy fabrication, easy dispersability and processability, and uniformity in terms of metallic character as well as diameter, makes Mo6S9-xIx nanowires one of the most promising one-dimensional materials. However little is still known about these materials.

Here we present the study of the solubility of Mo6S4.5I4.5 nanowire soot in a range of common solvents by performing sedimentation studies, spectroscopic and microscopic characterization. A sedimentation theory has also been derived, showing that the concentration of any insoluble dispersed component decrease exponentially with the settling time. We find that in all solvents, Mo6S4.5I4.5 nanowire soot contains three different phases, two insoluble ones and a soluble one. The first soluble phase consist of spherical impurities and sediments rapidly out of solution, resulting in purification of the system. The second phase consists of insoluble nanowires and sediments more slowly than the first phase, leaving behind a stable dispersion of soluble nanowire bundles. Soluble nanowires tend to have a diameter smaller that their insoluble counterparts. Uv-Vis spectroscopy, electron microscopy and x-ray photoelectron spectroscopy characterizations of the three phases reveal their intrinsic diversity.

Here we also present a combined Atomic Resolution Transmission Electron Microscopy and Aberration Corrected Scanning Transmission Electron Microscopy study of Mo6S4.5I4.5 soluble nanowires. Electron microscopies, crystallographic and electron microscopy-simulated studies allow us to determine both the unit cell structure and the packing structure of the wires in the bundles.

#### **ATOMIC RESOLUTION IMAGING AND ANALYSIS IN ABERRATION CORRECTED SCANNING TRANSMISSION ELECTRON MICROSCOPY**

P.D. Nellist

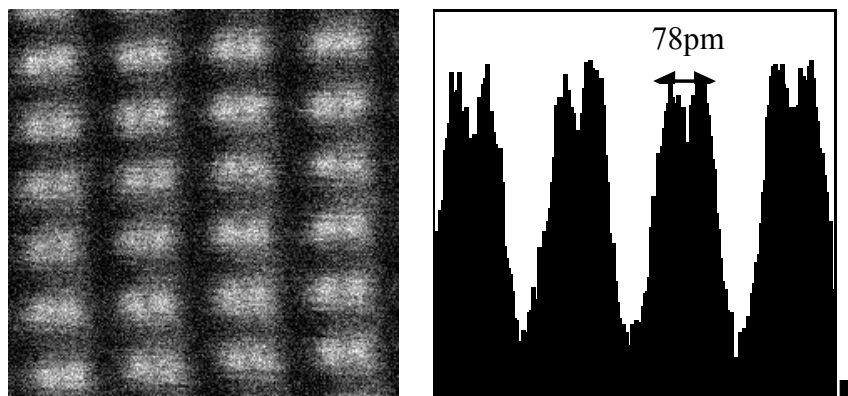
Department of Physics, University of Dublin, Trinity College, Dublin 2.

In recent years, correction of aberrations in the electron microscope has changed from being an interesting laboratory project to being a commercially available reality. The improvement in microscope performance has been particularly dramatic in the scanning transmission electron microscope (STEM), and recent data showing a spatial resolution significantly better than 1 angstrom (0.1 nm) will be presented (see Figure 1). The technological advances necessary to achieve this dramatic improvement in performance will be discussed.

Not surprisingly with such remarkable capabilities, aberration-corrected machines are revealing details on the atomic configuration of materials that, in many cases, were

unsuspected. We will present recent results on the application of aberration-corrected STEM to nanoscale systems that illustrate the new capabilities.

Aberration correction is also enabling new experiments to be performed that have hitherto not been possible. In particular, there are increased opportunities for performing experiments in-situ in the electron microscope, and it is also possible to retrieve information in three dimensions using the reduced depth of focus of aberration corrected optics to provide optical depth slicing.



*Figure 1. (a) A STEM image of a Si crystal oriented along the  $\langle 112 \rangle$  direction. The atoms project into columns and appear bright in the image. The closest spacing of atomic columns in this projection is 78 pm, and can be seen to be resolved in the image. (b) A line profile through the image as indicated, summing over a line 10 pixels wide.*

## **A STUDY EXAMINING THE EFFECTS OF TISSUE PROCESSING ON HUMAN TISSUE SECTIONS USING VIBRATIONAL SPECTROSCOPY**

E. Ó Faoláin<sup>1</sup>, M. Hunter<sup>2</sup>, P. Kelehan<sup>2</sup>, H. Lambkin<sup>3</sup>, H.J. Byrne<sup>4</sup> and F.M. Lyng<sup>4</sup>

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The use of vibrational spectroscopy in the detection of cancer is a newly emerging diagnostic field, which has shown great potential to date. The majority of investigations have been carried out on snap frozen tissue samples, which by their very nature are hard to obtain. However, histology departments have archives of thousands of tissue samples, preserved and mounted in wax blocks. If this archival material can be shown to yield good Raman and IR spectra capable of differentiating between normal and cancerous tissue, it would broaden the diagnostic capabilities of spectroscopy even further. Due to the prevalence of these formalin-fixed paraffin processed (FFPP) tissue sections, a better understanding of the effects of processing could unlock the potential diagnostic capabilities of FFPP sections. This study investigated the effect of snap freezing, formalin fixation, wax embedding and dewaxing using both IR and Raman spectroscopy. Spectra were recorded from parallel tissue sections to examine biochemical changes before, during and after processing with both Raman and IR spectroscopy. The efficacy of current dewaxing methods (xylene, Histoclear, and Trilogy) was also investigated. FFPP sections were shown to provide good quality Raman and IR spectra, and spectral differences between normal and abnormal cervical tissue were found. Results relating to the effects of tissue processing are presented and discussed.

## **DIGITAL SPECKLE INTERFEROMETRY ON A MICROSCOPIC SCALE**

E. Mihaylova

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Digital speckle interferometry records the speckle interference using a charged-coupled device (CCD) camera. The interferograms before and after the loading of the object are compared and the results are displayed directly on the computer monitor. Therefore film material, involving wet-chemical or other processing, and holographic reconstruction are not required. Digital speckle interferometry is applied to measure shape and surface deformation, and refractive index variation.

In this review article digital speckle interferometry of small objects is discussed. Attention is given to the general laws governing digital interferometric recording of the speckle field under high magnification. As magnification increases the speckle field decorrelates due to the object displacement. The main decorrelation is produced by the in-plane displacement. A method of reducing the decorrelation effects of in-plane displacement is presented.

A recent application of digital speckle interferometry is digital holographic microscopy (DHM). It offers high resolution and real-time observation capabilities. It has applications in metrology and biological cell imaging. DHM is a unique tool to evaluate shape and deformation at the nano-level.

## **POSTER PRESENTATION ABSTRACTS**

### **FOCUS ON APOPTOTIC SIGNALLING IN LIVING CELLS**

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Apoptosis is a highly controlled cell death mechanism that is crucial for eliminating damaged or superfluous cells in multi cellular organisms. After the induction of apoptosis cells can die within hours or days depending on the stimulus used or type of cell. With the help of state-of-the-art live cell microscopy we now can dissect the signalling steps leading to apoptosis. Mitochondria are key organelles as they integrate death signals and determine the cells fate after outer mitochondrial membrane permeabilisation, while most of the mitochondrial signalling occurs in a limited time frame of less than 30 min. To resolve the sequence of signalling events we use GFP fusion proteins. Thereby, we can confirm the function of the involved proteins spatiotemporally under in vivo conditions. Exploiting live cell imaging we focus mainly on signalling processes involving mitochondria and the post mitochondrial execution phase of apoptosis:

- The outer mitochondria membrane is permeabilised by pro apoptotic Bcl 2 protein family members like Bax, which translocates to mitochondria after it's dimerisation. We detect this in cells expressing Bax CFP and Bax YFP fusion proteins when FRET occurs between the fluorescent proteins.
- Mitochondrial inter membrane proteins like cytochrome c are released after the outer mitochondrial membrane permeabilisation to initiate the apoptotic execution. We use cytochrome c GFP or SMAC YFP fusion proteins to observe this event [1, 2]
- After this release the inner mitochondrial membrane potential depolarises. We measure the mitochondrial membrane potential via the intensity of TMRM, that distributes across polarised membranes according to the Nernstian Equation [1, 2].

Subsequently, proteases of the caspase family get activated to execute the apoptotic cell death. This can be detected with FRET applying a CFP-DEVD-YFP fusion protein; while the DEVD sequence is a specific executioner caspase cleavage site [3]. Currently we use the mitochondrial membrane potential depolarisation as the temporal reference point for all other signalling events. Developing further experimental protocols including new fusion proteins especially for the detection of their intracellular interaction via FRET will allow us to extend the number of observable apoptotic signalling events, to detect them with higher precision, and to confirm signalling protein interactions in single cells.

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**COMBINATION OF LIVE CELL MICROSCOPY AND COMPUTATIONAL MODELLING IDENTIFIES KEY PLAYERS OF APOPTOTIC SIGNALLING – A SYSTEMS BIOLOGY APPROACH**

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Apoptosis, or programmed cell death, removes superfluous or damaged cells from the body of multi cellular organisms. Enhanced or repressed apoptosis has been shown to contribute to developmental defects, autoimmune diseases, cancer, and neurodegenerative disorders.

Intracellular apoptotic signalling is accompanied by the release of mitochondrial proteins into the cytosol, mitochondrial depolarization, and subsequent activation of proteases of the effector caspase family. In previous studies we established multi-parameter imaging routines to analyse these signalling events spatiotemporally in single living cells using epifluorescence and confocal microscopy: Mitochondrial depolarization was observed by the membrane potential sensitive dye tetramethylrhodamine methylester (TMRM), the release of mitochondrial proteins was detected in cells expressing the fusion proteins cytochrome c-GFP and Smac/DIABLO-YFP. Subsequent caspase activation was detected by an effector caspase specific fluorescence resonance energy transfer (FRET) probe (CFP-DEVD-YFP).

The processes taking place between the mitochondrial release events and the subsequent caspase activation are complex. As naturally experimental set ups are confined to monitor only a limited number of parameters per experiment, quantitatively and temporally little is known about the interactions of the many other proteins within the apoptotic signalling network under physiological conditions. We therefore combined our live cell microscopy experiments with a comprehensive computer model based on the biochemical properties of the proteins involved.

With this systems biology approach of live cell imaging and mathematical modelling we were able to characterize the temporal profiles of a multitude of different protein fractions and identified XIAP (X-linked inhibitor of apoptosis protein) as a possible key regulatory protein in the signalling network. Model-generated hypotheses on the progression of apoptotic

signalling upon overexpressing XIAP were subsequently tested by live cell microscopy. We indeed identified a sharp XIAP dependent threshold deciding on the efficient activation of effector caspases at an intracellular concentration range closely resembling the computer model prediction.

### **XIAP INFLUENCES THE ONSET OF EFFECTOR CASPASE ACTIVATION: A SINGLE CELL ANALYSIS**

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Apoptosis, also known as Programmed Cell Death, plays a vital role in the removal of superfluous or damaged cells from the bodies of multicellular organisms. This highly regulated process is dependant on a group of proteases known as caspases. Activated caspases are inhibited by the Inhibitor of Apoptosis family of proteins (IAPs). The most potent and best characterised IAP is the X-linked Inhibitor of Apoptosis Protein (XIAP). A direct association between overexpression of XIAP and tumour malignancy has been reported in several studies. Furthermore, high levels of XIAP have been shown to enhance cellular survival in response to chemotherapeutic agents.

This project aims to analyse how exactly the effects of XIAP manifest spatiotemporally on the molecular level in single living cells under physiological conditions. It is believed that apoptosis can only proceed efficiently when effector caspases cleave XIAP and that after XIAP cleavage positive feed back signalling results in a sharp increase of effector caspase activity.

Previously we have established a microscopic monitoring system to detect effector caspase activity at the single cell level using epifluorescence and confocal microscopy. Caspase activation was detected by a caspase specific FRET substrate comprised of enhanced cyan fluorescent protein (CFP), a linker containing a caspase 3 specific cleavage site (DEVD), and enhanced yellow fluorescent protein (YFP). We are now using an improved FRET substrate which reaches a ten fold higher sensitivity, enabling us to sense even a slight effector caspase activity during the early stages of caspase activation.

Using XIAP knock out, parental and XIAP overexpressing cancer cell lines we analyse how different XIAP levels manifest in the temporal profile of caspase activation, reaching a time resolution of seconds. Mathematical analysis of the caspase dependent FRET disruption kinetic then directly reveals how XIAP can influence the onset of caspase activation.

### **THE CORRELATION OF INTRINSIC RADIOSENSITIVITY WITH BYSTANDER FACTOR PRODUCTION IN INDIVIDUALS**

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Certain individuals cannot tolerate 'conventional' doses of radiation therapy. In vitro testing of various human cell lines and tissue show a wide range of radiosensitivity and radioresistance, and the intrinsic radiosensitivity of a tumour is an important determinant of a patient's response to radiotherapy. The G2 chromosomal radiosensitivity assay is an in vitro cytogenetic based assay, employed to examine individual radiosensitivity through assessing radiation-induced chromatid damage microscopically. Other radiobiological in vitro studies have described radiation induced bystander effects which have been shown to occur when an irradiated cell communicates with non-irradiated cells via secreted factors and / or gap

junctional intercellular communication, and the non-irradiated cells exhibit responses that are normally characteristic of irradiated cells. The aim of this study was to correlate intrinsic radiosensitivity with bystander factor production in individuals and assess their clinical significance. Whole blood was taken from normal human donors and colorectal cancer patients and replicate lymphocyte cultures (1 x control and 1 x irradiated) were set up for the G2 assay and the bystander assay. At 72 h of incubation, one of each set of the replicate cultures was irradiated with 0.5Gy of cobalt 60 gamma rays (IR) while the other culture was sham irradiated. For the G2 assay, colcemid was added to the cultures 30 minutes post IR for 1 hour to arrest the cells in metaphase. The chromosomes were then harvested and fixed onto slides for microscopical analysis at x100 magnification. Structural and numerical aberrations were scored, and the radiation-induced G2 chromatid score was calculated from the structural chromatid damage. For the bystander assay, the cultures were re-incubated for 1.5 h and the medium was harvested. Recipient HPV-G reporter cells were treated with this medium for 24 h and cell viability was assessed with the alamar blue assay. The initiation of apoptosis was also assessed in the HPV-G reporter cells exposed to the bystander medium by measuring calcium flux levels with fluo-3 intracellular calcium indicator on a confocal microscope. The preliminary data showed variability in individual response (for both the normal and cancer population) to IR observed by G2 chromosomal radiosensitivity and bystander factor production.

#### **QUANTIFYING THE INNATE IMMUNE RESPONSE IN PRIMARY EPIDERMAL CULTURES FROM ONCORHYNCHUS MYKISS FOLLOWING EXPOSURE TO IRISH ESTUARINE SEDIMENT ELUTRIATES.**

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The role of mucosal surfaces such as the epidermis in fish innate immunity, has frequently been overlooked in ecotoxicology. Toxicants may have complex effects on skin mucus including alterations in its' amount, release, physical state and, possibly, composition. This in turn may alter the immuno-susceptibility of the fish. In vitro primary cell cultures derived from fish are particularly suited to the study of toxic mechanisms as they provide an economical and ethical alternative to whole fish tests, while retaining the specialised functions of the tissue of origin. The purpose of this study was to evaluate the immunotoxicity of elutriates extracted from three Irish estuarine sediments using rainbow trout cultures as a model system. We have developed a method which allows the exposure of a dilution range including whole elutriate sample (100%) to the cultures. Studies were initially performed with zinc chloride in order to establish this metal salt as a reference chemical and to assess the reproducibility of the test method. The cultures were exposed to the toxicant (metal or elutriate) for a period of 48-h, fixed and stained accordingly in order to (1) measure goblet cell area (2) quantify goblet cell numbers and (3) investigate whether changes occur in the proportions of mucin type. Results obtained to date show a dose dependent reduction in goblet cell area (due to secretion of mucus onto the surface of the cultures) following exposure to zinc chloride. A similar effect is also observed with cultures exposed to sediment elutriate samples. Based on these results the applicability of this culture model to assess the innate immune response following exposure to environmental contaminants will be discussed.

## **DEVELOPMENT OF A MULTI-TROPHIC BATTERY OF BIOASSAYS FOR THE ECOTOXICOLOGICAL EVALUATION OF IRISH MARINE SEDIMENTS.**

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Sediments have been recognised as both a source and a sink for persistent contaminants in the aquatic environment. Contaminants accumulated in the sediment have the potential to cause adverse effects to indigenous biota and aquatic organisms, should they become bioavailable or remobilised following chemical, physical or biological processes. The monitoring of contaminants in sediments should therefore form an integral part of any water quality management plan. At present in Ireland, such monitoring is reliant on chemical identification and quantification, with little direct assessment of the associated toxicity, bioavailability or synergistic effects. A three-year collaborative project between the RESC and the Marine Institute of Ireland, entitled 'Integrated Approach to the Toxicity Evaluation of Irish Marine Sediments' commenced in June 2004, with the aim of correlating results from biological tests with chemical analysis. The role of the RESC in the project is to address the need for suitable biological assessment tools for determining the severity of sediment contamination. A comprehensive assessment of potential sediment toxicity requires the consideration of multiple exposure phases. In addition to the evaluation of multi-exposure phases the use of a battery of multi-trophic test species has been advocated by a number of researchers as testing of single or few organisms may not detect toxicants with a specific mode of action. The Microtox® solid phase test and the 10-d acute amphipod test with *Corophium volutator* were used to assess whole sediment toxicity. Porewater and elutriates were assessed with the Microtox® acute test, the marine prasinophyte *Tetraselmis suecica*, the marine macroalgae *Ceramium tenuicorne* and the marine copepod *Tisbe battagliai*. This paper will describe the ecotoxicological evaluation of estuarine/marine sediments of varying contaminant loads from around the Irish coast using the described battery. The optimisation, sensitivity, and applicability of all tests will be discussed.

## **ULTRASTRUCTURAL AND IMMUNOLOGICAL ANALYSIS OF THE GASTROINTESTINAL TRACT OF THE SHEEP SCAB MITE, PSOROPTES OVIS**

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The mite *Psoroptes ovis* is an important obligate ectoparasite of sheep and is responsible for causing sheep scab disease in flocks throughout the UK and Ireland. Although control of the mite is normally through dipping or endectocide treatment, there are serious concerns over the use of these chemicals with respect to parasite resistance as well as environmental and welfare issues. One possible strategy for control is to harness the natural immune response of the host animal and produce a vaccine against the mite. Electron microscopic analyses of the alimentary canal was performed with the aim of identifying and localising cells in the gut that may be responsible for the production of antigens/allergens that elicit the immune response within the host animal. Immunocytochemistry (ICC) in conjunction with electron immunocytochemistry (EICC) was employed to identify and localise specific allergen producing sites within the mite alimentary canal.

## **LIGHT AND ELECTRON MICROSCOPY STUDY ON THE BIOADHESIVE SECRETIONS AND ASSOCIATED STRUCTURES IN BRAVOHOLLISIA SPP. OF MONOGENEA**

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Bravohollisia species are ectoparasites of the pomadasyid fish, Pomadasys hasta. They produce bioadhesive secretions from anterior adhesive organs and haptoral reservoirs to assist in their attachment to the host gill. This study examines the structures associated with bioadhesive secretions of Bravohollisia species using light and electron microscopy. Results indicate that three pairs of head organs are located in the antero-lateral region of the worm and the gland cells responsible for the secretions are positioned lateral to the pharynx. At the ultrastructural level, two types of membrane-bound secretory bodies occur in the anterior adhesive organs: rod-like bodies and oval bodies. Single ciliated structures, probably sensory in function, are found near each opening of the head organ. The haptoral reservoirs contain 3 types of inclusions and are extruded externally along grooves in the haptoral anchors to form net-like structures at the tip of the anchors. Peptidergic and serotonergic nerves observed in the muscle adjacent to the head organ and haptoral reservoir may regulate secretory activity.

## **THE STRUCTURE AND INNERVATION OF THE OVIJECTOR OF THE PARASITIC NEMATODE ASCARIS SUUM**

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Transmission electron microscopy (TEM) and confocal scanning laser microscopy (CSLM) interfaced with immunocytochemistry were used to study the neuromuscular structure of the ovijector of *Ascaris suum*. Neuronal interconnections observed between the ventral nerve cord and vagina vera innervation identified this as the centre of control of ovijector activity. The organization and arrangement of supporting musculature and points of innervation have also been identified and localized. An extensive nerve plexus containing both FaRPergic and non-FaRPergic components extend over the outer surface of the ovijector. The non-FaRPergic component is derived from nerve branches of the ventral nerve cord, whereas the FaRPergic component emanates from two large FMRFamide-immunoreactive neurons. Using TEM, the fine structure of the body wall and ovijector were described. The vagina vera contains numerous myofibrils which are circular in orientation. Many of these myofibrils divide and extend over a short distance in longitudinal and diagonal directions; their myofilaments are also orientated in a variety of directions. Parallel nerve fibres run in tracts along the length of the vagina vera with branches that penetrate the muscle layers. Immunogold labeling has identified and localized FaRPs associated with innervation of the ovijector.

## **THE EFFECT OF P-GLYCOPROTEIN INHIBITORS ON THE ACTION OF TRICLABENDAZOLE AGAINST A RESISTANT ISOLATE OF THE LIVER FLUKE FASCIOLA HEPATICA.**

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The increasing number of reports of resistance to triclabendazole by the liver fluke, *Fasciola hepatica* is a growing concern. Although triclabendazole is a benzimidazole drug, resistant isolates do not appear to show the  $\beta$ -tubulin changes associated with drug resistance in nematodes. Alternative mechanisms of resistance include changes in the uptake, metabolism or excretion of the anthelmintic. Specific multidrug resistant (MDR) reversal agents such as Verapamil were used in this study to inhibit the action of P-glycoprotein-linked drug efflux pumps. Adult liver flukes from the resistant Sligo isolate treated in-vitro with TCBZ.SO in the presence of Verapamil. Changes to the tegument were assessed by means of scanning and transmission electron microscopy. The flukes showed increased disruption in comparison with that caused to the fluke by TCBZ.SO alone

## **DEVELOPMENT OF A WIDE FIELD TIME RESOLVED FLUORESCENCE LIFETIME IMAGING MICROSCOPE USING A FAST GATED ICCD CAMERA**

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A fluorescence lifetime imaging microscope system is being developed to investigate the initial growth of biofilms. As the biofilm grows and develops, lifetime images will be taken and the results analysed. Lifetime imaging offers advantages over normal fluorescence imaging since lifetimes vary relative to pH levels and other factors. This should allow greater understanding of biofilm growth.

In comparison with confocal fluorescence microscopy, the ICCD camera will allow the imaging of a larger area. Confocal microscope systems scan the area to be imaged pixel by pixel, a process that can be very time consuming. A wide field fluorescence lifetime system can reduce the time spent looking for a region of interest in a sample.

The ICCD camera (Stanford Computer optics Inc.) offers a minimum exposure time of 200ps and a spectral range from 380nm to 820nm. The camera is mounted onto an Olympus bx60 microscope. Excitation is provided by either of two diode lasers (405nm or 635nm), input through the transmitted light port of the microscope. A dichroic mirror reflects the excitation light down through the microscope objective and allows the transmission of the emitted fluorescence. The emitted fluorescence of the sample is then filtered to remove stray light using a long pass filter, and is finally detected by the ICCD camera. The ICCD camera allows images to be acquired in rapid succession. It is hoped that in-house software can be developed to allow lifetime calculations to be performed automatically following image acquisition.

It is planned to extend the system to allow single photon counting measurements to be made at the same time as lifetime imaging is being performed. Single photon counting will be performed using a SPC-730 photon counting card from Becker & Hickl, and will be fibre coupled to the microscope.

## **INVESTIGATION OF PHOTOETCHING PROCESSES IN CDTE QUANTUM DOTS**

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Fluorescent semiconductor nanocrystals (quantum dots) have recently attracted the focus of attention of scientists working in biological imaging and photonics worldwide. The properties of quantum dots such as wide spectrum of emission wavelengths tunable by particle size and the possibility of custom modifications of surface characteristics make them prospectively very powerful and versatile tools for biomedical research, clinical applications and light emitting devices. We have synthesized water-soluble CdTe QDs capped by thioglycolic acid (TGA) and investigated the effect of etching in the presence of water and different buffers (such as RPMI 3+ (fetal calf serum (FCS) present) and RPMI(-) (no FCS present) . The aqueous synthesis of TGA stabilised quantum dots was refined through a systematic study of the various reaction conditions and their influence on the quantum yield of the sample. The quantum efficiencies of TGA stabilised QDs were further enhanced by photoetching using 500W Hg lamp and the effect on the Photoluminescence lifetime after etching was investigated using a MicroTime200 Time Resolved Luminescence Spectrometer. It was found that CdTe QDs photoetched in water show an increase in Photoluminescence lifetime with increasing quantum efficiency. However, the QDs irradiated in buffer gave different results. Those irradiated in RPMI(-) (no FCS present) buffer shows a decrease in luminescence lifetime with a decreasing quantum efficiency, however those irradiated with FCS present show a decrease in quantum efficiency but an increase in the fluorescence lifetime. These results demonstrate a direct correlation between the fluorescence of the QDs and their lifetimes, Further research on detailed investigation of QDs behaviour for potential biological imaging applications is currently under way.

## **FABRICATION OF SUB-MICRON TRENCHES BY E-BEAM LITHOGRAPHY AND DEEP REACTIVE ION ETCHING**

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This paper describes the fabrication of sub-micron trenches in silicon using a combination of electron beam lithography and deep reactive ion etching. These trenches have applications in photonic and nano-electronic devices. In this work, the trenches are etched into the silicon substrate with resist or silicon dioxide masking. PMMA positive type resist and ma-N type negative resists were used.

The trench patterns are exposed in the e-beam resist using a LEO Supra-25 SEM with an integrated Raith Elphy Plus lithography system. Resolution of exposed patterns is dependent on resist thickness and write-field area. In order to achieve a good 100nm pattern resolution, resist thickness of 200nm was chosen with a 50 $\mu$ m square write-field area. After the exposure and developing process, the trench patterns are etched in an Alcatel AMS-100 deep reactive ion etching system using alternating SF<sub>6</sub> and C<sub>4</sub>F<sub>8</sub> plasmas, where SF<sub>6</sub> is an etch gas and C<sub>4</sub>F<sub>8</sub> as a passivation gas. It is desirable that at least 50nm of the mask layer remains after etching to inhibit roughening of the silicon surface.

Results achieved show good etch selectivity between silicon and resist with etch ratio values of 19.5 for silicon to PMMA and 24 for silicon to ma-N resist. When using a silicon dioxide mask, etch selectivity as high as 70 was achieved. Trench widths down to 150nm with 100nm pillars were successfully etched to a depth of 4.4 $\mu$ m. There was still a significant amount of resist or oxide remaining after etching, with SEM images showing a smooth top pillar surface.

The etch recipe used was also able to produce smooth trench sidewalls, with sidewall scalloping of approximately 25nm.

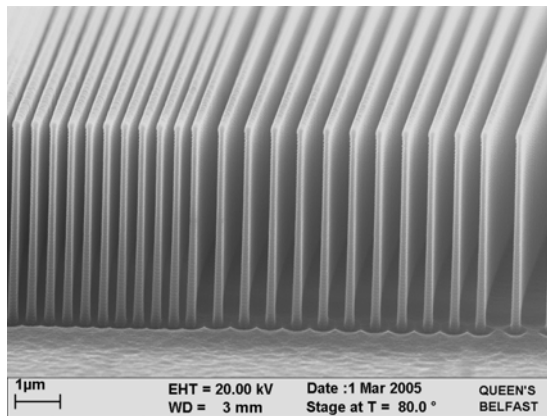


Fig 1. Silicon trenches etched with SiO<sub>2</sub> mask.

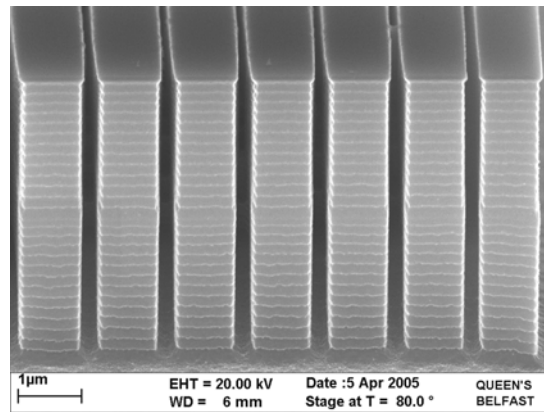


Fig 2. Silicon trenches etched with resist mask.

## CHARACTERISATION AND CONFORMAL COATING OF CHEMICAL VAPOUR DEPOSITION IRON

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The growth and analysis of iron thin films by chemical vapour deposition (CVD) from a liquid source precursor iron pentacarbonyl (Fe(CO)<sub>5</sub>) is reported. The thin iron layers were deposited on 100mm oxidized silicon substrates in a low-pressure process chamber using a radiantly heated graphite susceptor. Hydrogen at 500ml/min was used as diluent for the removal of the reaction by-products, and could be used as the carrier gas for the precursor to the chamber. To produce the CVD iron films, the precursor was chilled at -50°C and supplied to the chamber by direct draw through a narrow restrictor. Iron films were deposited at 200-400°C and 0.75 mbar with a deposition rate at 6.5nm/min. Layer thicknesses between 120nm and 245nm were achieved. Experiments were carried out to study the layer properties such as resistivity, film purity (x-ray diffraction) and grain structure. For layer deposited at 250°C and below, SEM micrographs showed the layer to consist of large cubic grains, made up of smaller cubes. With layers deposited at 350°C, the grain structure lose their cubic nature and are much more continuous. One advantage of CVD, namely conformal coating, is illustrated by depositing CVD iron on the 4µm x 4µm silicon trench. The aspect ratio was found to be 0.886 for a 240nm layer. Layer resistivity, 20µ-Ω-cm, was found to be 2 times larger than that of bulk values for a 120nm layer. α-Fe 110 & 200 body-centered-cubic (bcc) peaks were found from X-ray diffraction (XRD) analysis before rapid thermal annealing.

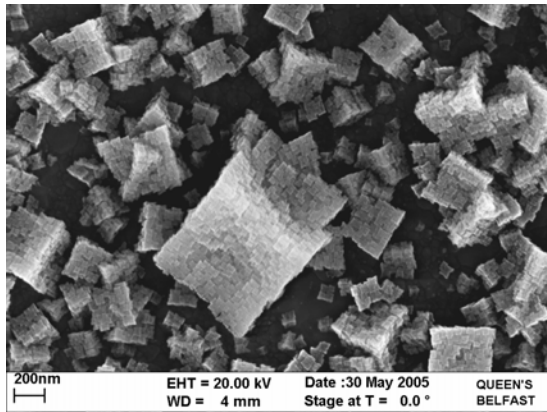


Figure 1 CVD Iron deposited at 200<sup>0</sup>C

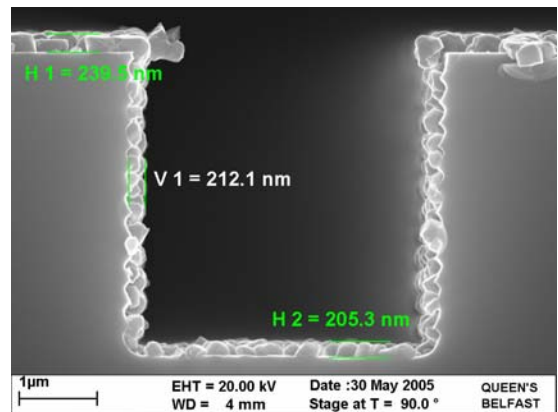


Figure 2 Conformal coating of CVD Iron

## ADJUSTMENT OF THE PHOTONIC BANDGAP OF SILICON BASED 1D PHOTONIC CRYSTALS INFILTRATED WITH NEMATIC LIQUID CRYSTAL E7

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Photonic crystals (PCs) are structures with a high-contrast periodic modulation of their complex refractive index, which leads to the appearance of a photonic band gap (PBG). The presence of these PBGs gives PCs the ability to control and manipulate the propagation of electromagnetic waves within a given frequency range. This makes these structures very attractive for the fabrication of all-optical integrated circuits. Si-based PC structures have received much attention in the research community as fabrication of the structures on a silicon substrate provides the advantage of easier integration with current semiconductor processing technology.

Our research concentrates on Si based 1D PCs. One of the main advantages of these PCs is that the position and width of the PBG can be adjusted in a wide spectral range by varying the crystal lattice parameters. These structures can be infiltrated with different compounds such as liquid crystals (LCs) hence changing the optical properties of the structure leading to the adjustment of the PBG. PBG tuning in real time is a challenging task. However, the refractive index of LC can be changed under external forces such as an electric field or a change in temperature, hence, by infiltrating 1D PCs with LCs, PBG tuning is attainable.

In the design, fabrication and investigation of 1D PCs, it was necessary devise a theoretical model to calculate the parameters required to fabricate structures to obtain specific PBGs. A program was developed to construct gap maps in order to analyse the effect of lattice parameters and refractive indices on PBGs.

A number of structures with different lattice parameters were fabricated on Si-on-Insulator wafers and these structures were infiltrated with nematic LC E7. The refractive index of this material changes under the influence of an electric field or under thermal influence. The variation of the PBG depending on these effects was investigated.

## **INFLUENCE OF SI-MFI NANOPARTICLES ON THE HOLOGRAPHIC PROPERTIES OF ACRYLAMIDE-BASED PHOTOPOLYMER**

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The influence of porous Si-MFI nanosize crystals on acrylamide-based photopolymer holographic properties has been studied. The photopolymer sensitivity, dynamics of recording, postrecording stability and shrinkage have been characterised and compared to the holographic characteristics of non-doped photopolymer. Nanoparticles with controlled morphology having diameters smaller than 100 nm have been used as additives in this study. The dynamics of recording have been studied by real time monitoring of the build up of holographic diffraction gratings with spatial frequencies from 200 to 2000 1/mm. The photopolymer shrinkage after holographic recording has been characterised by recording slanted transmission gratings and observation of the change in the reconstructing beam incidence angle.

The surface of the modified photopolymer has been characterised by utilising White Light Interferometer.

## **PREPARATION AND CHARACTERISATION OF FIRST MAGHEMITE-SILICA NANOTUBES**

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Research on the preparation of nanowires and nanotubes has received considerable attention recently. These new nanostructures possess novel properties unlike those of corresponding bulk materials and have important potential applications in microelectronics and catalysis. To date, research into the synthesis of iron oxide nanotubes has been very limited. Only  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> nanowires of 20-40nm diameters grown from the surface of an iron substrate has recently been reported. The investigation of iron oxide nanowire and nanotube formation is an important and rapidly growing area of research as these structures could provide novel materials for use as MRI contrast agents, where iron oxide nanoparticles are already being utilised. Here we report preparation and characterisation of magnetic g-Fe<sub>2</sub>O<sub>3</sub> – silica nanotubes prepared by a sol-gel technique. An organometallic precursor was used for the preparation of this new nanocomposite material. These novel nanocomposites have been studied via X-ray powder diffraction (XRD), transmission (TEM) and scanning electron microscopy (SEM), IR and Mössbauer spectroscopy and magnetization measurements. Properties and morphology of the g-Fe<sub>2</sub>O<sub>3</sub> – silica nanotubes will be discussed. This novel magnetic material might have a number of interesting potential applications in information and biotechnology.

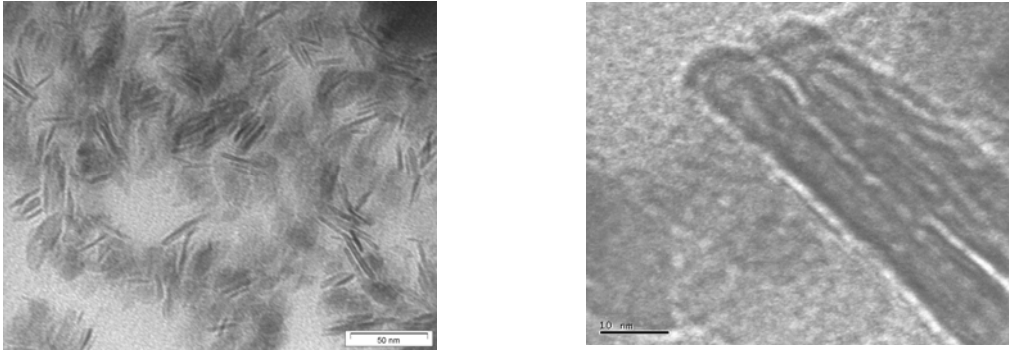


Figure 1: TEM (a) and (b) images of the iron-oxide silica nanocomposites

## **EFFECT OF VARIATION OF PROCESSING PARAMETERS ON THE FORMATION OF ZINC OXIDE PARTICLES SYNTHESISED THROUGH A SOL-GEL ROUTE.**

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Zinc oxide is one of the materials that has been proposed as a possible dilute magnetic semiconductor (DMS). If doped with the correct amounts of certain transition metal ions, zinc oxide are predicted to exhibit the characteristics of both magnetic and semiconducting materials. Sol-gel preparation is a simple and cost-effective route to synthesize homogeneously doped zinc oxide, and as such may used to produce DMSs. The processing parameters required to produce nanoscale zinc oxide through existing sol-gel routes have been examined. Solution techniques involving inexpensive zinc salts, and the effect of changes in processing steps on the purity and morphology of zinc oxide formed, have been investigated. Initial results using powder x-ray diffraction (XRD) and transmission electron microscopy, (TEM), indicate that for zinc oxide formed from zinc oxalate, there exists a relationship between the reaction time over which the zinc oxalate is formed, and the temperature at which this material decomposes to form zinc oxide. The possibility of zinc hydroxide being formed from a sol, and the conditions under which this occur have also been studied. The presence and extent of several chelating agents, mostly amine groups such as diethanolamine, required to produce ZnO powders has been explored, and the characteristics of these powders examined. Phase analysis and investigation of the morphological properties of these materials has been undertaken using TEM, XRD and DSC.

## **PHOTOLUMINESCENCE SPECTRUM OF LEVITATED MICROSPHERES**

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A laser beam tightly focused on a small particle can be applied as an optical tweezers to move or lift the particle by radiation pressure without any physical contact. The two forces exerted on the sphere are the radiation pressure force pointed in the laser beam direction due to the light scattered from the sphere surface and the gradient radiation force which is pointed towards the intensity gradient of the laserbeam and therefore perpendicular to the beam direction. The magnitude of the two forces vary depending on the focus position of the beam

resulting in either a downward pressure or an upward pressure applied to the microsphere. We carried out experiments with a Renishaw micro-Raman spectrometer equipped with both HeNe and Ar-ion lasers as excitation sources. The setup allows us to tightly focus the laser which causes a strong radiation force affecting the sphere. Our samples consist of melamine-formaldehyde latex spheres with a diameter of 3 microns. They are coated with colloidal CdTe nanocrystals applied on the sphere surface. To analyse the radiation pressure force we used a theoretical model based on the generalised Lorenz-Mie theory (GLMT). The incident Gaussian shaped laser beam was modeled with the localised approximation method to compute the beam-shape coefficients of the GLMT. The calculations for a 3  $\mu\text{m}$  sphere predict a reversed radiation pressure strong enough to overcome the weight of the sphere resulting in a upward force which lifts the sphere from the substrate. The photoluminescence (PL) spectrum of a single sphere was taken under changing radiation pressure conditions depending on the laser focus position and the beam power. The effect of the radiation pressure on the resonant modes (whispering gallery modes) in the PL spectrum of the microsphere was analysed and the results will be presented.

## **VAPOUR DEPOSITION GROWN CARBON NANOTUBES FOR INTERCONNECT TECHNOLOGY**

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Multiwall carbon nanotubes have been grown by catalytic chemical vapour deposition using iron catalyst particles drop cast onto etched silicon wafers. The catalyst used was poly(styrene-vinylferrocene) in toluene solution which has an iron content of 2.1%. The etched silicon wafers have trench regions of varying widths, ranging from 0.32 to 1  $\mu\text{m}$ . For trench widths below 0.5  $\mu\text{m}$  the number of “interconnecting” tubes growing from one side of the trench to the other increases sharply. A significant proportion of these “interconnects” are found to be Y-junction and multiple junction MWNTs. A systematic study of the effects of each of the growth conditions (temperature, run time, gas flow, catalyst concentration and trench width) versus interconnect yield was carried out. Densities of  $\sim 1.6$  interconnects per micron of trench are obtained, with junction structures accounting for 38% of these interconnects. Densities can be controlled through modification of chemical vapour deposition conditions.

## **THE ANALYSIS OF THE INTERACTION OF SWNT WITH TERPHENYL**

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This paper shows the solubilisation and de-bundling of SWNT with an aim to obtaining individual SWNT. By achieving this goal their theoretically proposed properties can be verified and their industrial potential realised. It is well documented that Single Wall Carbon Nanotubes (SWNTs) show varying degrees of solubility in a number of organic solvents. Solubility of SWNTs in toluene was found to be negligible. With toluene having such a poor affinity for SWNT it is clear that the solvent interaction with the SWNT is negligible. Therefore toluene is an ideal candidate for monitoring the improvements in the solubility of the SWNTs as a result of interaction with dye molecules such as terphenyl. The suspensions formed are stable for periods greater than thirty-six months. Spectroscopic analysis clearly shows interaction and de-bundling of SWNT is also evident. The fluorescence of terphenyl is quenched on interaction with SWNTs and the spectrum is red shifted which gives further support to the notion of interaction. With the quenching in fluorescence of the dye molecules

signifying interaction, a large range of concentrations were studied in order to quantify the degree of interaction between the SWNT and dye molecules. It was found at high concentrations such as  $1 \times 10^{-3}$  M, that both the dye molecules and SWNT formed aggregates. At lower concentrations such as  $1 \times 10^{-9}$  M for terphenyl and it was found that free dye and individual SWNT were interacting. Raman spectroscopy of the composites formed on interaction show vibrational modes that are not present in either the SWNTs or dye powder. It was found that the dye and SWNTs had Infra Red (IR) active vibrational modes at the positions at which these new or unique Raman modes occur in the composite spectra. It is therefore thought that the new Raman modes in the composite samples are related to the IR modes. The Raman Radial Breathing Modes (RBMs) give detail as to how diameter selective the dye samples are when compared to the pristine SWNT modes. Red shifting of the RBMs for the composite spectra was observed. It is believed that such a result is due to the de-bundling of the SWNT on interaction with the dye molecules.

### **CORRELATION OF RAMAN INTENSITY IN CONJUGATED ORGANIC SYSTEMS**

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A series of  $\pi$  conjugated oligomers and polymers were studied by Raman spectroscopy. It has been shown that both the electronic transition energies and the Stokes shift exhibit a well-defined relationship with increasing conjugation length, implying a correlation between the electron-vibrational coupling and chain length. This correlation is further examined using both modeled IR and Raman spectroscopy, whereby the integrated Raman scattering is seen to behave superlinearly with chain length. There is a clear indication that the vibrational activity and thus nonradiative decay processes are controllable through molecular structure. The correlations between the Stokes energies and the vibrational structure are also observed in a selection of PPV based polymers and a clear trend of increasing luminescence efficiency with decreasing vibrational activity and Stokes shift is observable. The implications of such structure property relationships in terms of materials design are discussed.

### **STUDY OF PROTEIN ADSORPTION ON SILICA GLASS SURFACE BY CONFOCAL FLUORESCENCE MICROSCOPY.**

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The interaction of proteins with solid surfaces is of major concern in a wide spectrum of fields such as biology, medicine, nanotechnology, biomaterials and biotechnological processes. Several studies show that the biological performance in vitro depends on how proteins adsorb on the surface of biomaterials.<sup>1</sup> Knowledge about the driving-forces and the structural conformation of the adsorbed protein as well as its activity are crucial to understanding the performance of biomaterials and biological systems. Innovations in instrumental techniques and molecular biology have enabled a significant increase in the ability to characterize most current biological reactions on biomaterial surfaces by revealing the adsorption behaviour of proteins in detail. In order to obtain more accurate and new physical insights into the protein adsorption process on surfaces, a confocal fluorescence microscopy study has been done with Bovine Serum Albumin labelled with tetramethylrhodamine (BSA-TRh).<sup>2</sup> The isotherms curves in time domain of BSA-TRh adsorption on silica surface were obtained from confocal fluorescence images on  $100\mu\text{m} \times 100\mu\text{m}$  scanned area at 532nm laser excitation. The effect of

protein concentration on the rate constant of adsorption process is investigated. The data are discussed taking into consideration the difference of protein size and shape that depend on the conformational states of the protein.

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### **RF MEMS CAPACITIVE SILICON SWITCHES FOR WIRELESS APPLICATIONS**

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Micro-Electro-Mechanical Systems (MEMS) is the integration of mechanical elements and electronics on a common silicon substrate. MEMS have the potential to enable greater integration of RF systems with the possibility of combining the devices such as variable capacitors, switches and phase shifters, together with the electronics within a single silicon chip. MEMS devices can also offer performance advantages; switches for example offer, low insertion loss, high isolation and virtually no power consumption making them ideally suited for use in modern telecommunications and wireless devices. RF MEMS capacitive switches utilize electrostatic force to produce mechanical movement to achieve a short or an open circuit in an RF transmission line.

Our current research has involved the design, fabrication, and characterisation of RF MEMS capacitive switches. Bridge type switch structures have been produced using polycrystalline silicon as the structural material with silicon dioxide as the sacrificial layer. The bridge passes over an actuation line. When the structure is released and dc voltage exceeds an actuation value. A layer of silicon nitride on the actuation line prevents ohmic contact and short-circuit of the dc supply. Capacitance- voltage characteristics are measured to confirm the switch operation and determine the actuation voltage. SEM examination of the fabricated switches is used to verify several aspects of the fabrication process and measure critical dimensions. Residual stress in the bridge material would cause deflection of the bridge on release and consequently affect the actuation voltage of the switches. SEM images reveal that the polysilicon bridges remain fairly flat after release and therefore have low residual stress.

### **ANALYSIS OF 3D X-RAY DIFFRACTION DATA FROM A SAMPLE OF 316LVM STAINLESS STEEL**

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The clinical demands for smaller, flexible stents have created the need for more detailed models of the materials used in their construction. As the dimensions of implants approach those of the materials microstructure, previous bulk material theories fail to accurately predict the material behaviour under stress. New X-ray diffraction techniques enable analysis of materials to provide data which enable reconstruction of the microstructure of the material in three dimensions. This will aid in understanding the materials behaviour more accurately.

This project used data obtained during experiment ME-869, carried out at the European Synchrotron Radiation Facility (ESRF) in February 2005. During this experiment, a sample of large grained 316LVM stainless steel was scanned to obtain diffraction information from the microstructure. Grain indexing software (Image\_D11) developed by Dr. Jonathan Wright (ESRF) was used to identify all grains present within the sample. Further searching of the X-ray diffraction results provided information on the location and orientation of each grain. By examining plots of diffracted intensity of a peak taken from each grain vs. its location in the sample, the grain centre and general shape was deduced.

To support grain identification, a model of the surface grain boundaries was also developed. Co-ordinates of the boundaries were taken from images of the sample, taken using a scanning electron microscope. A scaled model of the sample was created and the grain boundaries were wrapped to its surface.

The analysis has provided a methodology for analysing 3D X-ray diffraction data for large-grained samples. The results give a detailed list of each grain orientation matrix, location of grain centre and approximate shape. These data will be useful in developing accurate models of the materials microstructure in future work. For example, the model along with a model of the deformed sample can be used to compare with predictions using theories such as crystal plasticity theory.

## **PREPARATION AND CHARACTERISATION OF LUMINESCENT/MAGNETIC NANOCOMPOSITES**

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Magnetic and fluorescent nanomaterials are of immense importance in the field of biomedicine. Currently, fluorescent semiconducting nanoparticles (quantum dots) have been shown to be promising materials for bio-labelling, noninvasive biological imaging, and highly specific detectors in biological assays. The combination of magnetic nanoparticles with the properties of quantum dots is therefore an attractive prospect, enabling the engineering of a targeted-nanoscale device which can be manipulated within the body via an external magnetic field.

In our work the magnetic core was created using magnetite nanoparticles ( $\text{Fe}_3\text{O}_4$ ), which were prepared by co-precipitation of  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  with  $\text{NH}_4\text{OH}$  in an aqueous solution. The particles were then coated in silica, using TEOS and sodium silicate. The magnetic nanoparticles were then further functionalised using either aminopropyl- or mercaptopropyl-triethoxysilane derivatives. Transmission electron microscopy (TEM), X-ray powder diffraction (XRD) and IR spectroscopy were used to characterise the new core-shell nanoparticles. Then the luminescent “shell” was created using two separate routes depending on how the magnetite particles were functionalised. First mercaptoacetic acid stabilized CdS nanocrystals were prepared by co-precipitation of  $\text{CdCl}_2$  and  $\text{Na}_2\text{S}$  in water at the presence of stabiliser. Then the aminopropyl- functionalised magnetite nanoparticles and the luminescent CdS nanoparticles were linked together by traditional peptide coupling reactions of carboxyl- and the amino- groups. When using the mercaptopropyl- functionalised magnetite the CdS was simply co-precipitated around the magnetite “core”, using the mercapto's groups as linkers. The new light emitting magnetic nanocomposites were characterised by IR, UV-Vis and photoluminescence spectroscopy, TEM and XRD.

## **INFLUENCE OF PROCESSING PARAMETERS ON THE TEXTURE AND MICROSTRUCTURE OF IMITATION CHEESE**

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The impact of processing parameters on the functionality of imitation cheese (IC) has not been extensively investigated. In this study, light microscopy (LM) was used in correlation with scanning electron microscopy (SEM) to study the effects of cooker design and blade/auger speed on the texture and rheology of IC. Cheeses with similar compositions were manufactured (using water, rennet casein, vegetable oil and emulsifying salts) in two different cookers i) a single-blade cooker operated at an auger speed of 750, 1125 or 1500rpm (maximum setting) and ii) a twin-auger cooker operated at speeds of 100 or 200rpm (maximum setting). The basic constituents of IC were identified by LM of sections using histochemical staining, Fast Green for protein and Sudan III for fat. Increasing the agitation speed of the single-blade cooker reduced the mean fat particle size from 3-0.1 $\mu$ m; decreased the meltability of IC from 112-66mm and increased cheese hardness from 392-533N. Increasing the auger speed of the twin-auger cooker from 100 to 200rpm reduced the fat particle size from 10-3 $\mu$ m but had only a minor affect on the hardness and meltability values of cheeses. The results suggest that processing conditions may be varied to modify the texture and meltability of IC and that some of the ensuing changes are attributable to alterations in the microstructure. The high magnification of SEM images was useful in elucidating features of the IC microstructure e.g. fat particle size and characteristics of the protein matrix, which helped to explain some of the observed differences in physical properties. However the small sample size of samples used in SEM reduces the sampling accuracy of the original subject. Broad sampling techniques such as LM used in conjunction with SEM increased the sampling accuracy and full advantage of high resolution and high magnification was obtained.

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