NMS Innovation News

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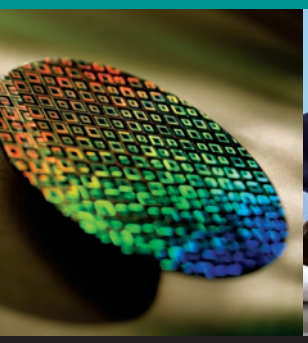



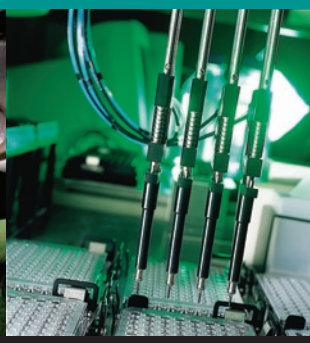

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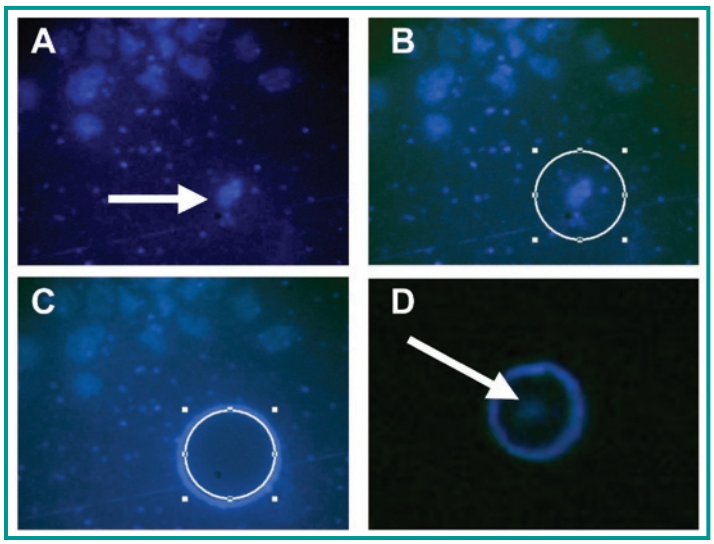
One Cell at a Time

Cell based technologies are revolutionising the healthcare industry, offering advanced tools to combat disease and aid diagnostic procedures. Such technologies offer new benefits to an NHS that spends 7.2% of UK national income on healthcare provision and is increasingly burdened by the needs of an ageing population. The UK enjoys a strong position in the development of advanced cell based therapies via a favourable regulatory framework and a strong academic knowledge base. This revolution is set to continue with the development of stem cell therapies which are adding to the regenerative medicine products that are either in the late stages of clinical trial or already competing in a marketplace estimated to be worth $10 billion by 2013.

Understanding the complex processes that drive the development of stem cell therapies is hindered, to a larger part, by the number of cells required to perform biological assays and the purity of the information that can be obtained. Cell biologists typically use thousand or millions of cells to make single measurements, generating data that is an average of the whole cell population. This type of approach, however, provides an incomplete picture due to the well established heterogeneity that exists in cell populations. This means that averaged cell responses are subject to errors in interpretation and while qualitative trends can be inferred, it is extremely difficult to generate precise quantitative measurements. One solution to this problem is the application of measurement techniques at the level of the single cell.

In recent years a number of advances in the fields of molecular, cellular, systems and computational biology have made it possible to be able to extract



Single cell isolation using laser capture microdissection. A single isolable cell is identified using the fluorescent cell marker Hoechst (arrow in A). The cell is marked using the microscope software (B) and dissected using an UV laser (C). After isolation the cell can be observed still adhered to the cell culture surface (arrow in D).

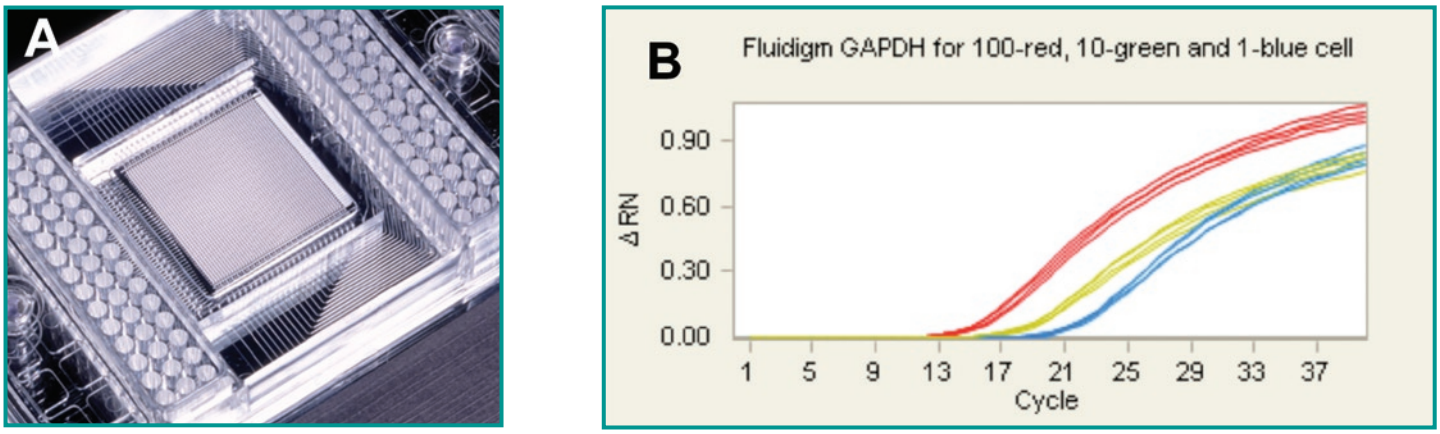
highly resolved information from isolated single cells by challenging the extreme ranges of measurement detection for cell-based systems. Using these technologies, biologists are now starting to understand the complex cascade of events that occur within individual cells and improve biological measurements by reducing the variability associated with population analysis. Translation of these information rich measurements to the single cell level offers great potential for the development of better and safer cell-based therapies for the healthcare sector, improved

nel technology for life

LGC *Setting standards in analytical science*

NPL National Physical Laboratory

Department for **Innovation, Universities** & **Skills**



Single cell quantitative PCR. The Fluidigm Biomark chip (A) allows over two thousand nanolitre PCR reactions to be performed in parallel using microfluidic loading. This system can be used to reproducibly perform dynamic quantitative PCR (B) from samples ranging from 100 cells (red lines) through to 10 cells (yellow lines) and even single cell samples (blue lines)

drug discovery platforms that exploit stem cell technology and could ultimately impact upon the wider pharmaceutical, diagnostic, environmental and forensic sectors.

At present most single cell analysis is performed in isolation using a single platform. This is providing valuable information on, for example, the gene expression profiles of individual cells but ignores the protein signalling events that ultimately govern cell fate. This lack of cross platform analysis is hindering the commercial application of single cell technologies and is slowing their widespread uptake by UK industry. The metrology challenge therefore, lies in being able to provide robust methodologies for handling, isolating and characterising single cells on multiple comparable platforms. The Innovation R&D 'Single Cell Analysis' project, which began in April 2008, seeks to answer these challenges by providing cross platform analysis of measurement techniques that are able to extract highly resolved information from isolated single cells. This will examine gene, protein and metabolite expression, enabling data to be linked and integrated to model the key performance outputs for any given cell system.

One of the first challenges for single cell analysis is sample handling. Mammalian cells are typically only 20-30µm in size and many of them require an adherent substrate on which to grow. This makes isolating single cells technically difficult, particularly as "cell stress" caused by physical isolation methods can dramatically alter gene expression profiles, reducing assay reproducibility. To overcome this, LGC has invested in a state of the art, contact-free laser microdissection microscope with optical tweezers. This new NMI capability allows adherent single cells to be imaged, prepared and isolated without physical disruption to the cell. Using this technology we have been able to isolate defined cell samples that can be used for downstream analysis to check both the limits of detection and reproducibility of a range of platform technologies.

Another major challenge associated with single cell analysis is the limited amount of material available for investigation, which again impacts all downstream analysis following cell isolation. For gene expression analysis this means that either the starting material has to be pre-amplified, which may introduce sample bias and reduce assay robustness, or only a single PCR amplification can be performed per sample, which can lead to the introduction of technical bias through a lack of replicates and controls. To address these issues, LGC is examining a range of techniques designed to enable quantitative PCR from samples as small as a single cell, while also examining the reproducibility of assays following a pre-amplification process. The recent purchase of a new breed of real-time PCR instrument is also helping to address some of the problems associated with limited amounts of sample. The BioMark system from Fluidigm, of which LGC has the first in the UK[1], uses innovative microfluidic chips to enable thousands of real-time PCR amplifications to be performed on-chip in nanolitre volumes. This technology reduces the reaction volume over a thousand fold compared with standard real-time RT-PCR and offers the ability to perform multiple analyses from a single cell sample. An additional advantage of the instrument lies in its ability to perform "digital PCR". Here, a single sample is diluted and split over thousands of individual reactions such that only a small proportion of them actually contain a target molecule. After PCR is completed, the number of wells with a positive amplification signal are counted and used to determine the amount of target DNA or RNA in the sample. This "digital" approach to PCR offers a novel system for absolute quantification of target. A further approach to be evaluated makes use of the next generation of sequencing technologies. The recent purchase by LGC of a Roche FLX sequencer[2] will enable sequencing of the single cell transcriptome to be performed, dramatically increasing the accuracy for profiling of global gene expression patterns.

Sample size is also an issue when performing protein analysis, particularly as protein samples from single cells cannot be amplified, making measurements of rare signals particularly difficult. From initial studies we have ascertained

that single cells have a total cell protein content of approximately 150pg per cell. Taking the average protein size to be 30KDa this equals to approximately 5fmol of protein per cell, which is half the normal 10fmol limit of detection for cell protein identification using conventional proteomics. This, therefore, represents a major metrology challenge, both in terms of detecting signals in a reproducible manner and in the purification of samples to isolate rare protein messages from background noise.

Going forward, LGC, in collaboration with University College London and King's College London, will optimise new methodologies for the characterisation of single cells in a clinical model for the derivation of human embryonic stem cells. The therapeutic potential for stem cells is undoubted - however the processes involved in deriving the cell lines are labour-intensive and to a certain extent, hit and miss. During the development of the inner cell mass, which is used to create stem cell lines, there is a process of zygotic genome activation when embryos switch from maternal genes and begin their own transcription. It is widely thought this switch plays a key role in determining cell fate and ultimately their potential for deriving stem cell lines. Understanding the processes which determine this fate will be central to creating the cell lines that can drive future advances in cell based therapies and can only be achieved by using the type of sophisticated single cell measurement tools that will be developed as part of this project.

For further information on this project please contact Neil Harris at LGC, email: neil.harris@lgc.co.uk

1. http://www.lgc.co.uk/news/27-aug-2008_fluidigm_ifc.aspx

2. http://www.lgc.co.uk/news/05-aug-2008_agowa_adds_roche_f.aspx

Measuring Authenticity

Researchers at NPL are currently working on a feasibility study for ways of measuring authenticity. Building on existing research into naturalness and identity, the team is trying to isolate and measure physical characteristics that will allow us to discriminate authenticity.

The purpose of the research is to pursue a measurement framework that could then be applied to validate the effectiveness of the components, devices and systems being developed to protect documents and products from counterfeiting.

NPL involvement is in response to the growing concern expressed by business, government and regulatory authorities at the economic and, more urgently, the health and safety impact of counterfeiting. The risk to public health from counterfeit pharmaceuticals has been clearly identified in the Intellectual Property Crime Report, 2007 published by the UK Intellectual Property Office (IPO) (www.ipo.gov.uk/ipcreport.pdf) which states that due to concerns of counterfeiting the Medicines and Healthcare products Regulatory Agency (MHRA) recalled five different pharmaceutical products in the UK in 2007.

The first targets to be examined are banknotes as their authenticity is validated by a set of known overt features such as holograms, watermarks, colour shifting and magnetic inks. These initial findings are likely to be of direct interest to document issuing authorities such as central banks and to security printers. The work will then be expanded to attempt to characterise authenticity in commodities such as drugs, chemicals and healthcare products that do not necessarily have overt authentication features. Prior to the start of the scientific study, a series of stakeholder consultations took place where commercial, government and regulatory representatives confirmed the need for authoritative independent science to assist in the fight against counterfeiting.

This is an excellent example of harnessing NPL capabilities to address a real UK market failure that is affecting economic development and public health.

For further information please contact Trevor Esward at NPL on 020 8943 6883 or email: trevor.esward@npl.co.uk

The NPL's Rubidium Fountain Atomic Clock Demonstrates First Results

The world's first atomic clock was operated at the National Physical Laboratory in 1955. This device used a thermal beam of caesium atoms and was based on a precise measurement of the frequency of a microwave transition in the atoms. In 1967 the SI unit of time, the second, was redefined in terms of this caesium transition frequency.

Modern primary frequency standards do not use a thermal atomic beam but instead use bunches of much slower, laser-cooled atoms. The atoms are launched upwards to the height of about 1 metre and then fall under gravity in the form of a fountain. The low velocity of the atoms in the fountain makes it possible to increase the interrogation time of the atomic transition up to half a second, and therefore to increase the accuracy of the measured clock transition frequency by several orders of magnitude.

Currently, the existing NPL primary caesium fountain frequency standard is characterized by the fractional frequency uncertainty of 2×10^{-15}, which corresponds to an accuracy of 1 second in 15 million years (or, more practically, of 1 microsecond in 15 years).

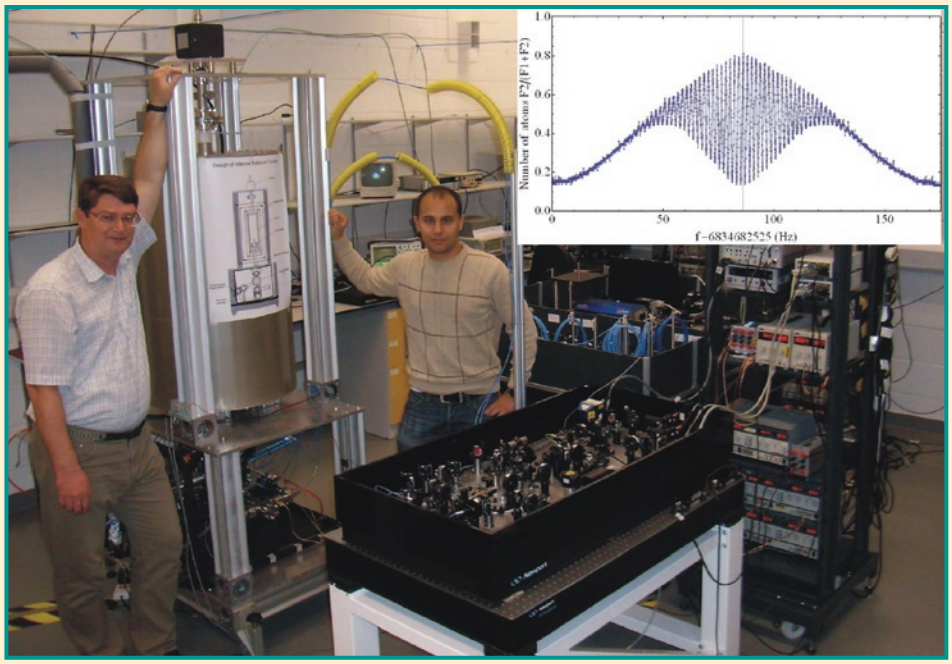
In addition to the caesium fountain standard, NPL have recently developed a new system based on rubidium rather than caesium. The rubidium fountain clock is expected to show better stability than the caesium clocks because of its smaller sensitivity to interatomic collisions. Such a rubidium fountain system recently built in the United States Naval Observatory (USNO) has demonstrated a frequency uncertainty of 5×10^{-16} in four hours.

In a fountain frequency standard, the number of atoms moved from one state of the clock transition to the other under the influence of the interrogating electromagnetic field is measured. For the atoms that interact with the electromagnetic field twice (once on the way up, and once on the way down), the corresponding dependence of the number of transferred atoms as a function of the frequency of the electromagnetic field oscillates greatly as the frequency is changed. These oscillations are referred to as Ramsey fringes.

Ramsey fringes have recently been observed in the NPL rubidium atomic fountain frequency standard for the first time (see inset figure). The maximum of the central fringe (which corresponds to the frequency of the rubidium microwave clock transition), without any averaging, already agrees at the 10^{-12} level of relative frequency uncertainty with the only other comparable measurement of the rubidium clock transition produced by the French LNE-SYRTE's dual (caesium-rubidium) fountain.

The next step will be measurement of the position of the central Ramsey fringe with better statistics to improve the knowledge of the exact frequency of the rubidium clock transition, which is already officially recognised as a secondary representation of the SI second.

For further information please contact Yuri Ovchinnikov at NPL on 020 8943 8792 or email: yuri.ovchinnikov@npl.co.uk



Researchers Yuri Ovchinnikov (NPL) and Vjacheslav Lebedev (PhD student from UCL) in front of the rubidium fountain frequency standard. The inset figure shows the first Ramsey fringes of the rubidium fountain.

One-day Workshop to Examine Metrology for Microfluidics – 28 October, Loughborough University

Whilst there are already some commercially available microfluidic devices, the number and range of microfluidic applications is set to expand rapidly. Inkjet printer heads, for example, have been commercially available for years but microfluidic devices are now being developed for applications in medicine, analytical chemistry and biology, high throughput analysis and synthesis, and process intensification.

To keep abreast with the many new designs and applications which are rapidly evolving, there is a growing requirement to develop and advance techniques in fluid modelling, measurement and control.

The design and production of microfluidic devices, physical interfacing to macro-scale systems, and the integration of the technology within business processes, all present challenges to the use of microfluidics.

To help address these challenges, a one-day workshop has been organised at Loughborough University on the 28th October 2008 to examine the metrological issues and challenges which directly affect the production and use of microfluidic devices. This free event is open to both academic and commercial organisations, and will provide developers and users a valuable opportunity to discuss measurement issues. Feedback from the workshop will identify metrology and research requirements that will be fed into the formulation of new research to support the future development and use of microfluidic technologies within the UK.

Further information can be found at www.tuvnel.com/events.aspx

Biometrology for Microfluidics: One-day Workshop – 21 November, LGC, Teddington

Microfluidic devices are now being applied in many areas of the biotechnology and life science sectors. However, in order for successful transition from an academic, research environment to a viable commercial product many technical hurdles must be overcome. Examples include the design and production of appropriate platforms, their integration with or replacement of existing test devices and procedures or the difficulties presented by the nature of the sample types analysed. Such technical hurdles present key measurement technology challenges and also provide further requirements for targeted collaborative R&D.

This FREE one day workshop will be held on 21 November 2008 at LGC, Teddington, Middlesex. It aims to identify the key trends, requirements, risks and bottlenecks affecting the production and use of microfluidic devices for biological measurement applications. Feedback from the workshop will be used to define stakeholder's current and future metrology requirements and help shape and underpin the measurement research and support required for continued development and use of microfluidic technologies within the bioscience sector in the UK.

The event is DIUS-funded as part of the Innovation R&D Programme and open to all organisations to provide developers and users the opportunity to network, share experiences and discuss their needs to help define the measurement agenda going forward.

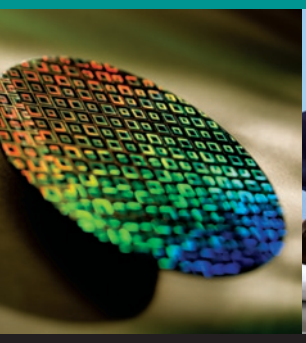
Delegate places are now available. To register your interest in participating at this Workshop, contact the Booking Hotline on +44 (0)20 8943 7423 or by email to nmshelp@lgc.co.uk.

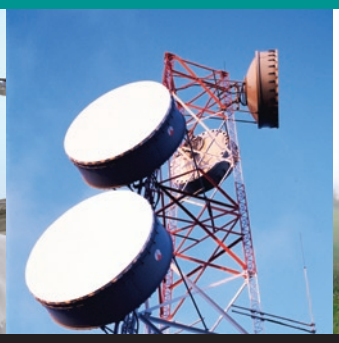
Plastic Electronics

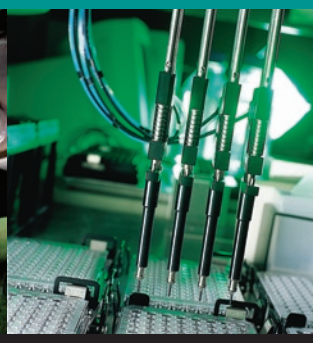
Plastic electronics is an emerging technology area with the potential to have a huge impact on many aspects of our lives. The near future is expected to see the widespread application of flexible organic electronic devices in a number of diverse areas, including displays, lighting, solar energy, smart packaging and medical diagnostics.

In order for the vision to become reality a number of challenges need to be overcome, including the determination of the chemical, optical and electrical nature of organic nanostructures, the characterisation and control of surface chemistry and surface energy for controlled patterning and printing, and the development of accurate bulk charge transport measurements and models.

Researchers at NPL are currently tackling these issues in a number of projects, in order to support UK companies in the plastic electronics arena, enabling the effective exploitation of the UK's technical lead in this critical scientific field.







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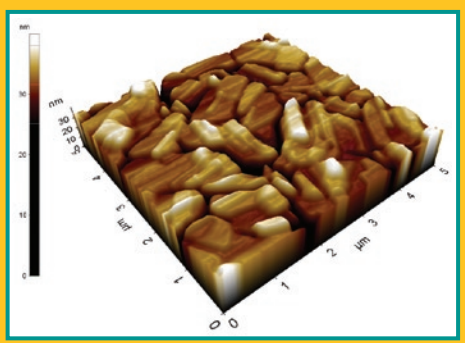
If you would like further information on any aspect of NMS Innovation R&D Programme, see www.metprog.org.uk
National Physical Laboratory | Teddington | Middlesex | United Kingdom | TW11 0LW
Helpline: 020 8943 6880 | Fax: 020 8614 0446 | E-mail: enquiry@npl.co.uk

In April 2008 the IRD Plastic Electronics project commenced. Within this programme a number of novel measurement methods are being developed, including ultra high vacuum scanning kelvin probe microscopy (UHV-SKPM), which will be used to map the spatial distribution of work function in photovoltaic organic blends and transistor semiconductors at the nanoscale, and photoconducting atomic force microscopy (PC-AFM), which will provide images of the photocurrent activity in thin-films for solar cells. Work is also ongoing to advance bulk electrical measurement techniques, such as electro-absorption and dark injection transient spectroscopy, to provide quantitative electric field and charge mobility data.

In addition to the NMS research, NPL experts in plastic electronics are engaged in two Technology Strategy Board (TSB) projects. The first, FlexDisplay, is led by NPL and is aimed at the demonstration of flexible organic transistor circuits for electronic display driving. The partners include University of East Anglia (UEA), Queen Mary College London and Qudos Technology. Measurements using advanced scanning probe microscope methods at NPL have revealed fascinating temperature-dependent behaviour of the crystalline structures in organic semiconductors synthesised by UEA.

The second TSB project, SCOPE, includes as partners Cambridge Display Technology, University of Liverpool and University of Cambridge. This project covers the challenges of ink-jet printing for manufacturing of plastic electronics, where NPL is using its world-leading expertise in surface analysis to characterise the surface chemical properties of device substrates and develop correlations with the wetting behaviour of organic inks. This work can help manufacturers to achieve increased reliability of printing processes and subsequently improved product performance.

The global market for plastic electronics is forecast to reach a value of £15B by 2015 and the research being undertaken by NPL is targeted at applying and developing metrology to understand the fundamental science of organic and flexible electronics. The results of this work will assist the UK to achieve commercial success from the existing excellent research base.



Atomic Force Microscope image of annealed organic semiconductor thin-film. 5 micron x 5 micron scan, 40nm height scale.

For further information please contact Craig Murphy at NPL on 020 8943 8703 or email: craig.murphy@npl.co.uk

Forthcoming Events

Good Lighting with Less Energy: Possibilities for the Future
9 October 2008
NPL, Teddington
www.npl.co.uk/server.php?show=nav.1078

Metrology for Microfluidics
One-day Workshop
28 October 2008
Loughborough University
www.tuvnel.com/events.aspx

Nano-Molecular Analyis for Emerging Technologies III (NMAET III) & Surface Science of Biologically Important Interfaces 10 (SSBII 10)
5 – 6 November 2008
NPL, Teddington
http://conferences.npl.co.uk/nmaet/

Developing Advanced Scientific Engineering Spreadsheet Applications
A two day training course
12 – 13 November 2008
NPL, Teddington
www.npl.co.uk/server.php?show=ConWebDoc.2032

Biometrology for Microfluidics
One-day Workshop
21 November 2008
LGC, Teddington
Contact: nmshelp@lgc.co.uk

MTDATA Introductory Course
Software for Phase Equilibria and Thermodynamic Properties
24 – 25 November 2008
NPL, Teddington
Contact: john.gisby@npl.co.uk

Electromagnetic Materials Measurement Interest Group Meeting
9 December 2008
NPL, Teddington
Contact: kevin.lees@npl.co.uk

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