Atomic Force Microscopy

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Biomaterials and Tissue Engineering

Introduction

Scanning probe microscopy (SPM) has revolutionised high resolution imaging of a wide range of samples. With the introduction of atomic force microscopy (AFM) high resolution images can be obtained without the need for the sample to be placed in a vacuum chamber. Furthermore, modern AFM systems are capable of producing high quality images of samples immersed in a wide range of liquids.

Technical specifications

The PSIA XE-100 is a non contact atomic force microscope.

•True non-contact mode results in improved resolution due to reduced tip and specimen damage.

•Separated X-Y & Z Scanner. No coupling between the x-y plane and the z-scanner completely removes background curvature from the fundamental level, and effectively eliminates the cross-talk and non-linearity problems that are intrinsic to conventional piezoelectric tube based AFM systems.

•Ultra High Force Z-Scanner. The key innovation that enables True Non-Contact Mode in the XE-Series. The ultra high force z-scanner allows a significantly higher resonance frequency than those of conventional piezoelectric tube scanners. This enables more than 10 times faster scan rates than is possible with a conventional tube type scanner.

•Hardware Closed-loop Feedback. Hardware, not software, feedback is used to drive all the AFM signals in order to guarantee distortion free imaging. Hardware closed-loop position control allows for the absolute scaling of AFM measurements.

•2D Guided Flexure X-Y Scanner. High resolution imaging without background curvature. This single module parallel-kinematics x-y scanner has low inertia and minimal runout, providing the best orthogonality, high responsiveness, and axis-independent performance.

The Main Unique Aspects of the Machine

•The separated and high force nature of the scanning piezos produces very high quality images without effects of tip or sample deterioration.

•The full enclosure and active anti-vibration table which support the instrument provide an optimal environment for high quality experimentation.

•The dovetail head mount, magnetic tip chip carrier and in-line optical arrangement make tip positioning and laser alignment a very simple task.

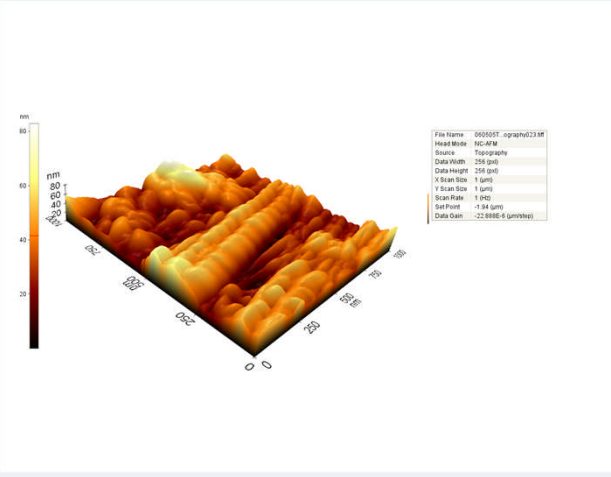
•The acquisition and analysis software is state of the art and allows detailed analysis of images and spectroscopic data.

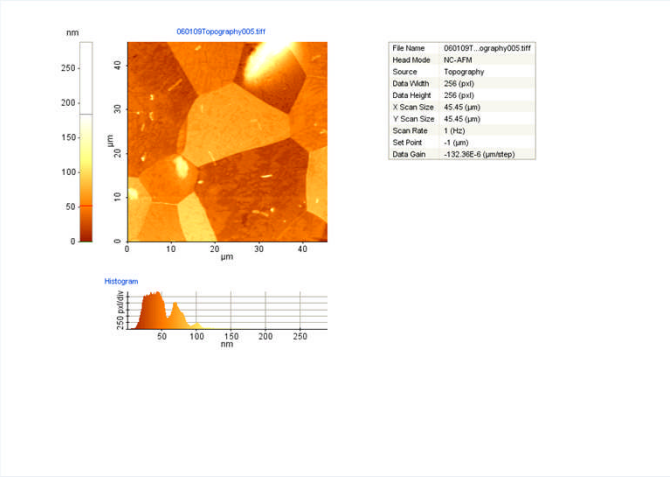
•A nanoindentor option enables surface hardness measurements to be undertaken.

Examples of Work undertaken at EDI

A wide variety of projects have utilised this technique for analysis of a range of surfaces. For example (left hand figure below) we have examined the surface of a compressed collagen scaffold for tissue engineering applications. The resolution of the microscope is such that it is possible to visualise the de-banding which occurs in collagen fibrils. This can be seen as the regular patterning which can be seen along the length of the fibrils running through the centre of the image.

The image on the right hand side shows the surface topography of an ion implanted titanium surface following argon ion etching. The combination of ion bean techniques has resulted in pronounced etching of the grain structure.





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ATR FTIR

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Introduction

ATR FTIR can provide information on the chemistry of new materials and the kinetics of chemical reactions. In FTIR spectra peaks are obtained that are associated with different chemical groups. Since the absorbance or height of the peak is proportional to the concentration of the chemical group, in a mixture changes in height with time can provide information on the relative rates of formation of different products.

Technical specifications

The Perkin Elmer series 2000 FTIR spectrometer is equipped with a temperature controlled (25-200 ºC) diamond ATR unit and both Spectrum and Timebase software that allow determination of individual spectra or spectra as a function of time respectively. These spectra can be converted to profiles of absorbance versus time at chosen wavenumbers. The instrument can also be used to generate Raman spectra.

Examples of Work undertaken at EDI

A variety of projects are currently utilising this technique for standard characterisation of new materials for example phosphate glasses and degradable polymers. The technique is also being applied to assess and quantify various possible reactions between dentine and chemicals used in dentistry. Reaction kinetic studies have included polymerisation of polymers and composites as well as setting kinetics of various cements for tooth and bone repair. In Figure 1 for example spectra of a brushite forming bone cement at different times after mixing are shown. In Figure 2 absorbance at the 980cm^{-1} peak is provided as a function of time for this cement with 2 different powder liquid ratios (PLR) and 2 temperatures. Such data can be converted into percentage conversion of a component or relative levels of reaction. For example in Figure 3 the percentage of monomer polymerised in 4 different methacrylate containing dental restorative materials determined using FTIR is given and Figure 4 the relative levels of acid reaction in four dental cements. These results can be used to explain macroscopic properties of the materials as they set.

Figure 1 ATR FTIR spectra of a brushite forming cement as a function of time after mixing

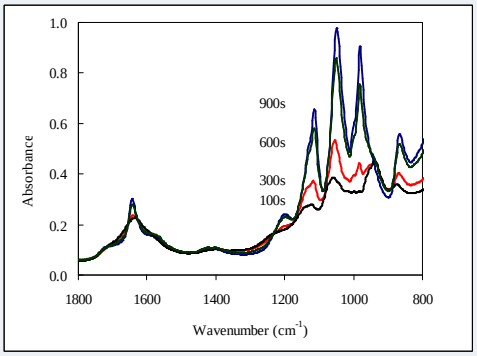


Figure 2 Absorbance profiles for a brushite cement with PLR of 3.3 and 2.0

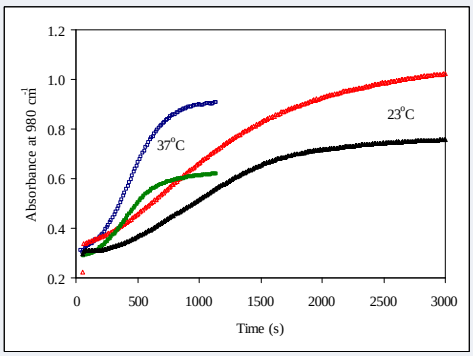


Figure 3 Percentage polymerisation of 4 hybrid dental restorative materials

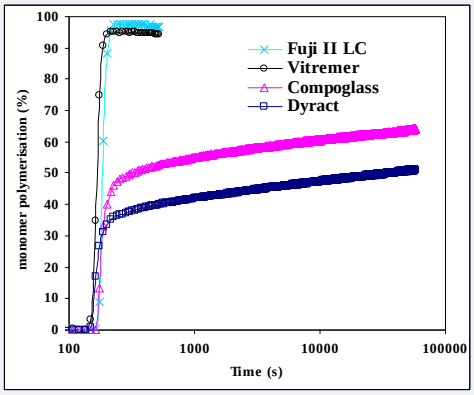
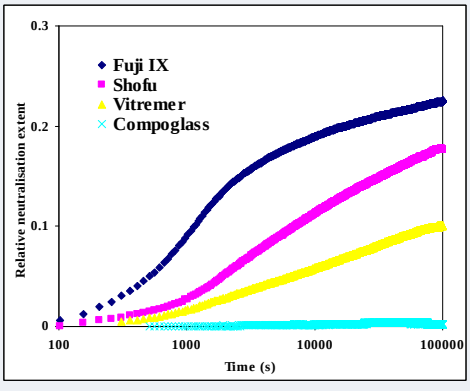


Figure 3 Relative levels of acid neutralisation in 4 dental restorative materials



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Goniometry

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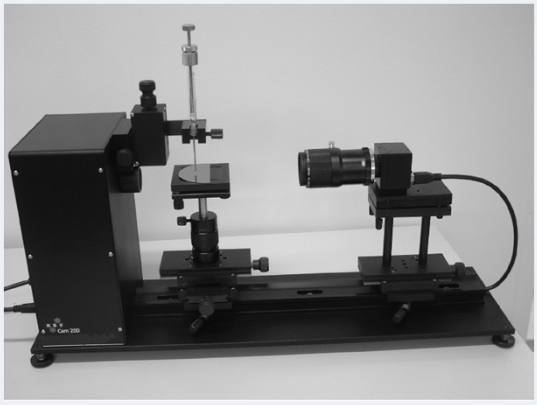
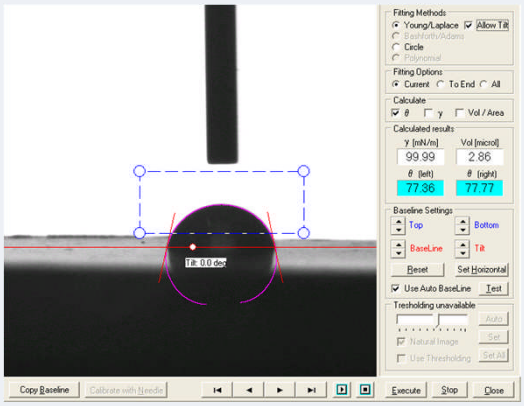
Introduction

Goniometry is a technique that is used to investigate the interactions between a solid surface and a liquid. When a small volume of liquid is placed onto a solid surface it usually forms a discrete drop on the surface. The contact angle is geometrically defined as the angle on the liquid side of the tangential line drawn through the three phase boundary where a liquid, gas and solid intersect, or two immiscible liquids and solid intersect. A high contact angle is indicative of a repellant action between the surface and liquid. Where attractive forces occur between the substrate and liquid low contact angles are observed. Water is commonly of interest when examining contact angles, since it is the most abundant liquid at room temperature. Solids which exhibit a high (>90) contact angle at a water / air interface are termed hydrophobic, while those exhibiting and angle less than 90° are known as hydrophilic.





Technical specifications

The CAM 200 consists of a camera, lens, light source and PC interface, illustrated in the top left figure. It collects images of the fluid surface interaction and stores them digitally. These images are then processed to provide the contact angle at each side of the drop, surface tension and drop volume as shown in the top right figure.

•The camera has a resolution of 512 x 480 pixels and runs at a maximum frame rate of 25 fps.

•Illumination is provided by a pulsed LED source. This provides a monochromatic light source, improving the image sharpness and decreasing sample heating.

•The lens has a zoom configuration allowing different sizes of drops to be measured while retaining optimal resolution.

•A tilt stage enables dynamic contact angle measurements to be made.

•A precision syringe allows precise control over the drop volume.

•The software enables simple, accurate calculation of the contact angle, surface tension and volume.

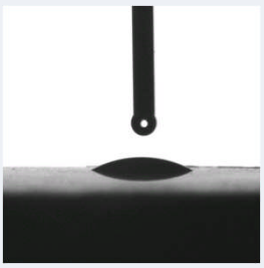
The Main Unique Aspects of the Machine

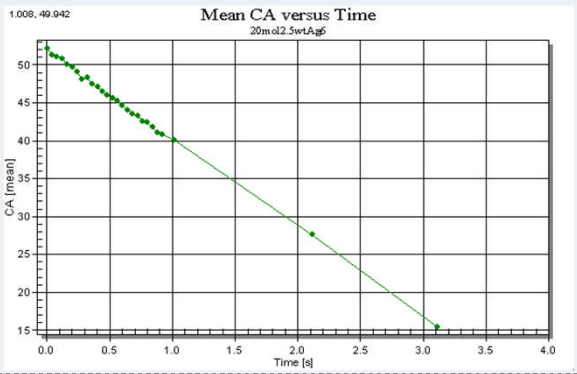
•The software is capable of capturing a large number of images at defined time periods, this is very useful for looking at the variation of contact angle with time.

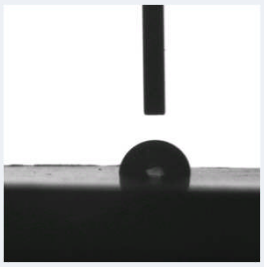
•The tilt stage enables dynamic analysis of contact angle. This give quantitative data on both advancing and receding contact angles which can determine the contact angle hysteresis. The hysteresis is related to the homogeneity of the sample surface. Roughness and surface heterogeneity contribute to the hysteresis.

Examples of Work undertaken at EDI

A number of projects have utilised this technique for assessment of a wide variety of surfaces. For example we have studied contact angles on surface modified titanium, on soluble calcium phosphate based glasses (figures) and on polymer systems degraded by a number of different biological agents. The figures below show that varying the composition of phosphate glasses can have a significant effect on the contact angle. Both the hydrophilic (figure below, left) and hydrophobic (figure below, right) surfaces are from the same system. Certain surfaces undergo changes in surface properties when exposed to water. The figure below, centre shows the rapid change in the contact angle of a phosphate glass exposed to water.







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Confocal Laser Scanning Microscopy

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Introduction

Confocal microscopy offers several advantages over conventional widefield optical microscopy, including the ability to control depth of field, elimination or reduction of background information away from the focal plane (that leads to image degradation), and the capability to collect serial optical sections from thick specimens. The basic key to the confocal approach is the use of spatial filtering techniques to eliminate out-of-focus light or glare in specimens whose thickness exceeds the immediate plane of focus. There has been a tremendous explosion in the popularity of confocal microscopy in recent years, due in part to the relative ease with which extremely high-quality images can be obtained from specimens prepared for conventional fluorescence microscopy, and the growing number of applications in cell biology that rely on imaging both fixed and living cells and tissues. In fact, confocal technology is proving to be one of the most important advances ever achieved in optical microscopy. Current instruments are highly evolved from the earliest versions, but the principle of confocal imaging is employed in all modern confocal microscopes. In a conventional widefield microscope, the entire specimen is bathed in light from a mercury or xenon source, and the image can be viewed directly by eye or projected onto an image capture device or photographic film. In contrast, the method of image formation in a confocal microscope is fundamentally different. Illumination is achieved by scanning one or more focused beams of light, usually from a laser or arc-discharge source, across the specimen. This point of illumination is brought to focus in the specimen by the objective lens, and laterally scanned using some form of scanning device under computer control. The sequences of points of light from the specimen are detected by a photomultiplier tube (PMT) through a pinhole (or in some cases, a slit), and the output from the PMT is built into an image and displayed by the computer. Although unstained specimens can be viewed using light reflected back from the specimen, they usually are labeled with one or more fluorescent probes.

Technical specifications

The Bio-Rad confocal microscope we have is fitted to an Olympus BX51 upright microscope. This allows a wide variety of specimen geometries and sizes to be imaged.

•The main confocal system comprises at its heart, two lasers, a standard HeNe laser of wavelength 543nm and a second argon laser, with lines at 457, 476, 488, 514nm.

•The lasers are mounted externally to the microscope and the light is transmitted via fibreoptics to the microscope.

•The microscope is equipped with a Solent Scientific fully enclosed incubator system to allow long term cultures and studies to be performed.

•Coupled with the incubator and to minimise and reduce the need to access the chamber, the microscope is equipped with a fully motorised ProScan II X-Y stage supplied by Prior to allow manipulation and also for producing large area composite images.

The Main Unique Aspects of the Machine

•The two laser system allows a number of different dyes to be used simultaneously for example for colocalisation experiments or live dead staining to be carried out. The image below on the right is an example of a live-dead stain of human oral fibroblasts embedded in a collagen-glass composite, with live cells stained green and dead are in red.

•The incubator and X-Y manipulation allow operation without disturbing the sample. Couple with the software, it is simple to produce time dependent studies of cells and materials.

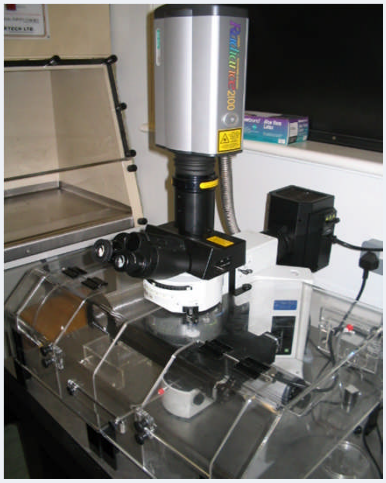
•Data interpretation is via a large suite of software, including LaserVox for 3D volume rendering. Data is also analysed using the NIH developed ImageJ software and associated plug-ins and macros.

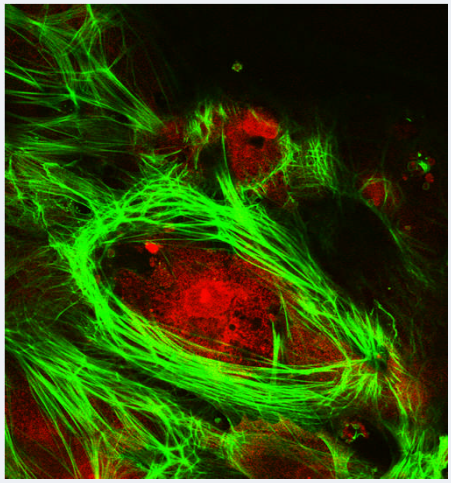
Examples of Work undertaken at EDI

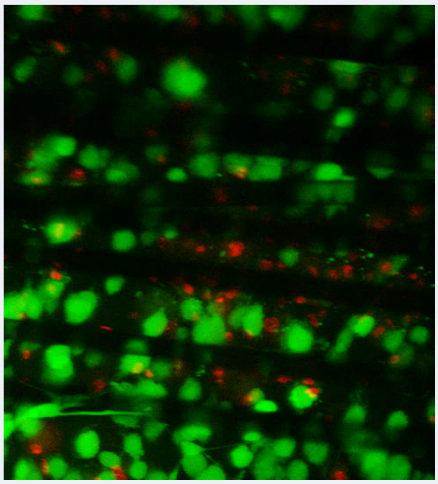
A wide variety of projects have utilised this technique for qualitative and quantitative measurement of a wide variety of parameters.

The image below in the centre is of bone marrow-derived human mesenchymal stem cells cultured upon a biomimetic calcium phosphate surface and differentiated to the osteogenic lineage and stained with phalloidin in green and a red nuclear stain

The image below on the right is an example of a live-dead stain of human oral fibroblasts embedded in a collagen-glass composite, with live cells stained green and dead cells red.







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Fatigue Testing

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Biomaterials and Tissue Engineering

Introduction

Fatigue testing is a method commonly used to characterise the survival probability of a wide variety of materials in particular ceramics, composites and those relating to dental restoratives . The machine (Dartec HC10 as shown in the image below) we have in place can be configured to apply a range of force within the constraints of the load cell suited to carry out a particular test. It comes equipped with three interchangeable loads cells each having a different limiting force (100N, 1kN and 10kN). The user can choose to set the appropriate limits within the software in order for the machine to cycle in either load or stroke (displacement) mode. The Dartec can be easily adapted to perform cyclic tests in compression, tension, bending and flexure depending on the test design. Variables such as testing under wet conditions can also be introduced to mimic the physiological environment.

Technical specifications

•The Dartec HC10 consists of a test frame fitted with a movable crosshead. The crosshead can be adjusted to a suitable height in order to accommodate a specific test design.

•The piston head is fitted with an attachment that enables the user to interchange between load cells (100N, 1kN and 10kN).

•To enable the application of cyclic loads or static loads the machine is equipped with a Dartec 9610 control unit which interfaces between the software and the actuator (piston-load cell).

•The test frame is engineered as such that the force applied by the load cell during a test is driven by hydraulic pressure supplied by a hydraulic unit.

•A servo valve fitted on to the crosshead provides a close loop flow or pressure response as a result of programmed values in the control loop application available in the software. This enables the machine to control the output pressure whilst testing.

•There is a large bolt fitting at the bottom of the test frame which runs along the axis of the actuator. A number of test designs can be manufactured and adapted to be bolted onto the bottom of the test frame. These can include models that are specifically designed to characterise the insertion and removal force of 'O' rings on dental implants over a given number of cycles.

•There is also an environment chamber available which has been manufactured to be bolted directly onto the test frame and to also accommodate testing jigs. The environment chamber can be attached to a unit comprising of a pump and a temperature controller that can apply a dynamic flow of water at regulated temperature around the environment chamber. This enables the user to characterise the behaviour of materials under wet dynamic conditions in much the same way as implant materials do under physiological conditions.

The Main Unique Aspects of the Machine

•The user is able to set up the limits and parameters of their test using the cyclic generator application under the toolkit 96 software. Upper and lower limits need to be set in either in load or stroke mode in order to carry out a cyclic test. In reality these limits would be the definitive parameters of the test, however, as the machine is subject to some given error the user can define error bands or a region of error around these limits in which the actuator is constrained to.

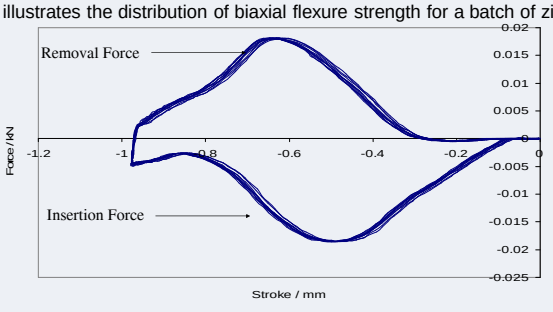
•Safety features are available in the software that can be set which allows the machine to act automatically. Such actions can involve shutting down the hydraulic pressure or subjecting the machine to operate in set up mode (idle mode) once a particular safety limit has been exceeded.

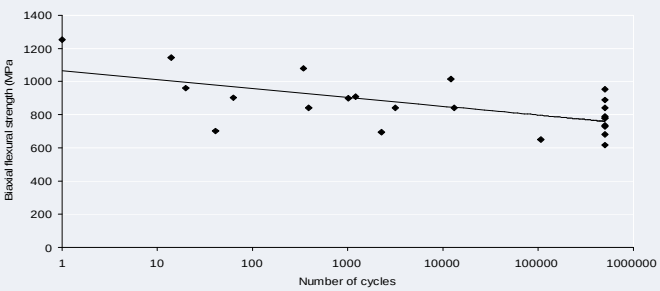
•The Toolkit 96 software has a number of data collection and analysis applications. The data acquisition tool can be set to collect data over a very short period of time i.e. over 10 or 20 seconds. It can also be set up to automatically collect a very short range of data at a given cycle number. Alternatively the user can set the software to collect data by continuous logging data points for the duration of the test.

Examples of Work undertaken at EDI

A number of projects have been carried out measuring the changes in the mechanical behaviour of materials over a given number of cycles. For example we have measured the changes in the insertion and removal forces of 'O' rings on dental implants as a function of two variables; the number of cycles and the degree of inclination for insertion and removal. The diagram below (left) illustrates some data exhibiting typical insertion and removal forces for 'O' rings on dental implants collected over 10 seconds at around 250 cycles.

We have also carried out numerous studies involving the biaxial flexure behaviour of ceramic materials such as zirconia (3M) under wet conditions. The graph below (right) illustrates the distribution of biaxial flexure strength for a batch of zirconia samples as a function of number of cycles.





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Dynamic Mechanical Analysis

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Biomaterials and Tissue Engineering

Introduction

Dynamic Mechanical Analysis (DMA) is a proven technique for the characterisation of the viscoelastic properties of materials, in particular polymers and composites, as it not only gives a quantitative assessment of materials' properties such as stiffness and damping, but also provides important structural information. The dynamic mechanical properties of materials are sensitive to all kinds of thermal transitions, relaxation processes, structural heterogeneity and morphology of multiphase systems such as crystalline polymers, polyblends and composites. DMA can also pinpoint thermal transitions e.g. typical output of tan δ versus temperature will display a peak at glass transition temperature (Tg). Above Tg, peaks correspond to the crystalline regions and eventually melting temperature (Tm). As a technique, DMA is also sensitive for the characterisation of polymers of similar chemical compositions, as well as detecting the presence of moderate quantities of additives such as plasticizers or leachable materials.

Technical specifications

• **The Perkin-Elmer DMA 7e** is used for mechanical analysis of solid and near-solid samples. It measures mechanical properties such as modulus (elasticity) and viscosity (damping) as a function of time, temperature, frequency, stress, or combinations of these parameters. The DMA 7e provides the performance and flexibility necessary for the characterization of a broad range of materials, from soft samples such as elastomers, thin films, and single filament fibers to hard samples like composites, ceramics, and some metals. The DMA 7e can also apply a constant force to perform standard thermomechanical analysis (TMA). Comprehensive calibration, verification, and validation assure the highest confidence in results reported by the DMA 7e. It has been certified to perform standard test methods defined by international organizations including ASTM and ISO.

• **Force Control-** Force motors are used to cause the sample to deflect. Precise force control is critical to obtaining accurate modulus results. The DMA 7e force motor can accurately control from very low forces for the analysis of samples in the molten or flowing state to high forces for the analysis of very high modulus solids. Forces can be calibrated and verified using traceable mass standards providing traceable modulus results.

• **Displacement Sensitivity-** Displacement sensors are used to measure sample deflections. High displacement sensitivity is important for characterizing a wide variety of sample types from polymer pellets to single filament fibers and thin films. High dynamic displacement sensitivity allows measurement of subtle mechanical transitions. Long static displacement range allows the sample to expand or contract many times its original size, up to 300%.

• **Furnace Systems-** Two furnace systems are available for use with the DMA 7e. These systems allow DMA measurements over the range of –170°C to 1000°C. Based on a resistance-heating design, these furnace systems allow continuous monitoring and temperature control, resulting in improved accuracy and reproducibility. Low-mass design permits rapid cool-down at the completion of an experiment, often requiring only a few minutes to cool back to the starting temperature. Fast-cooling experiments are best performed with the DMA 7e's unique quartz measuring systems. The result is that you can run more samples and characterize more materials. The precise temperature control features of this furnace allow heating and cooling rates from 0.1°C/min to 100°C/min.

• **Sample Types and Geometries-** The practical advantages of the DMA technique have expanded to almost every material and sample type. It is not uncommon for a single laboratory to test raw materials, intermediate products, and finished products to verify materials and end-use performance. To accommodate this broad range of sample types and test geometries, numerous measuring systems have been developed for the DMA 7e. These systems are designed to handle materials in a variety of geometries, from semisolids in the form of flat bars, pellets, cylinders, disks, films, and even fibers. Included are Extension Analysis systems for the analysis of thin films and fibers; 3-Point Bending, Dual Cantilever, and Single Cantilever systems for the analysis of a variety of thermoplastics and thermosets; and nine different Parallel Plate systems

• **Frequency Range-** With a frequency range of 0.01 to 50 Hz, the DMA 7e provides a valuable tool for the characterization or "fingerprinting" of materials. Frequency scanning provides a convenient way to observe differences between materials as a result of long and short chain branching, chain entanglements, and molecular weight distribution differences. Frequency scanning also provides a means for calculating the "zero shear viscosity" or for identifying the Newtonian region in polymers, which may then be applied to molecular weight calculations. Lower frequency data, such as one cycle per day or one cycle per week, is generated through creep recovery tests.

Typical applications for which the DMA 7e is used are

Polymers and Composites
Quality factor/tan delta
Impact performance
Effects of filler, modifiers, blending, grafting, and copolymerization
Heat set
Strength
Effect of drying
Effect of heating
Effect of chemicals, solvents, or humidity
Effect of stress or pressure
Stiffness/Modulus
Glass transition

Fiber/Film
Shrinkage
Orientation

Food/Pharmaceutical
Effect of additives

Biological/Environmental
Effect of aging, drying, or sunlight

Examples of Work undertaken at EDI

DMA has been extensively used for the characterisation of the thermal and mechanical properties of polymers and composites for biomedical applications. In particular DMA of scaffolds developed for tissue engineering have been a focus of interest. Numerous forms of scaffolds have been developed through gas foaming, particle leaching, thermally induced phase separation (TIPS), and the electrospinning of nano-fibres into porous structures. Figures 1 and 2 show the DM properties of highly porous (>90% porosity) foams of Bioactive glass incorporated PDLLA that were produced through TIPS and subsequent solvent sublimation. Figure 3 shows typical stress-strain curves obtained for highly dense native collagen scaffolds (cellular and acellular) as a function of time in culture.

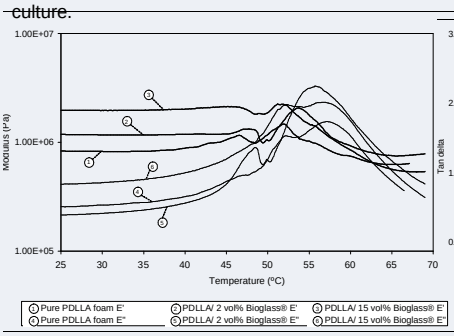

Figure 1

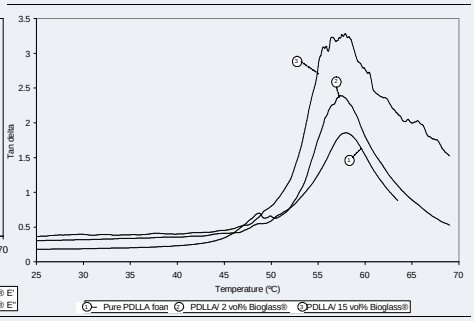

Figure 2

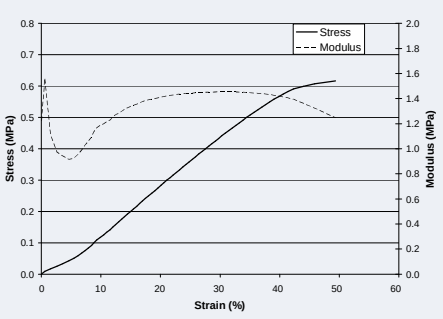

Figure 3

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Differential Scanning Calorimetry

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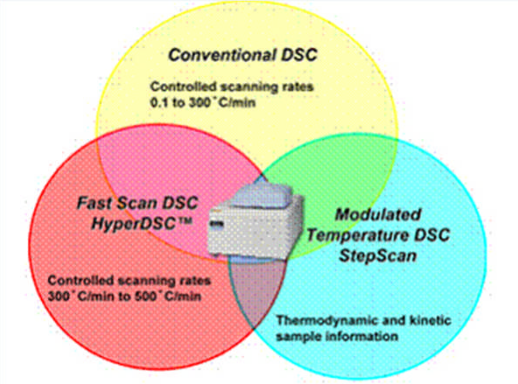
Biomaterials and Tissue Engineering

Introduction

As a technique Differential Scanning Calorimetry (DSC) has been extensively used for the characterisation of the thermal properties of all types of materials, including metals, ceramics, polymers and composites.. Typical resultant graphs of the energy flow versus temperature or time can be easily used to identify a number of endothermal or exothermal transitions occurring in materials and parameters can be identified such as glass transition temperature (Tg), crystallisation temperature (Tc), melting temperature (Tm) and the heat of cure. A number of methods could be used, under isothermal conditions, constant heating rate (typically at 10 or 20°C/min), modulated temperature (which overall is a much slower rate of heating), and recently Hyper-DSC (which operates at very high heating rates >100°C/min).



From www.Perkinelmer.com

Technical specifications

The Perkin-Elmer Diamond DSC is a unique power-compensation DSC, offering sensitivity and insights into materials processes. It is designed where the sample and reference pans are heated by two independent furnaces embedded in a temperature-controlled heat sink. This allows sophisticated analysis when performing the direct measurement of heat flow into or out of a sample The power compensation DSC design leads to sharper peaks and high sensitivity. Additionally, benefits include true isothermal operation, modulated temperature DSC (StepScan) technique and HyperDSC™ for dramatic enhancements in sensitivity, as well as greater productivity.

Features of Diamond DSC include:

- Unique power-compensation design
- Highest caloric accuracy
- Superior signal resolution and sensitivity
- Multiple cooling options for a temperature range of -170 °C to 725 °C
- HyperDSC™, the leading fast scan DSC technique
- StepScan, for Modulated Temperature DSC

Examples of Work undertaken at EDI

DSC has been extensively used for the characterisation of the thermal properties of polymers and composites for biomedical applications. DSC can also be used to assess the effect of incomplete curing of materials as shown in Figures 1 and 2 comparing the thermograms of the first heating cycles for bone cements cured at room temperature and at 37°C. As can be observed, in both the BCRT and BC37 there appears to be a low glass transition that is in the range of 40-60°C indicated by an endothermic inflection (upwards) in both thermograms. At temperatures above the transitions, both materials underwent exothermic peaks (characterised by a downward inflection in the thermograms) that reached a maximum at around 112 and 115°C for BCRT and BC37 respectively. If the curing is not complete during the initial setting of the bone cements, unreacted residual monomers trapped within the polymerised chains are released as the temperature increases above Tg, and therefore undergo further reactions. Figure 2 shows the second heating curve and, as can be seen, in all materials there was an absence of the residual exothermic peak and a clear Tg inflection showing that the heating process during the first cycle allowed the unreacted residual monomer to undergo further curing. DSC has also been used to investigate the kinetics of fast setting inorganic based calcium phosphate cements as shown in Figure 3. Three typical isothermal DSC measurements showing the normalised heat flow of the exothermic setting reaction against reaction time for a specific powder liquid ratio and three retardant concentrations

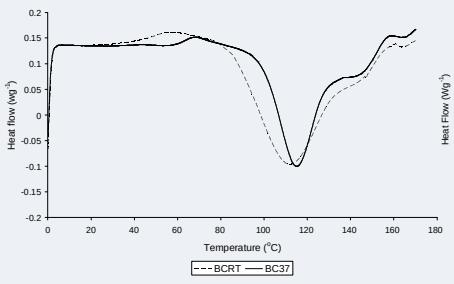


Figure 1

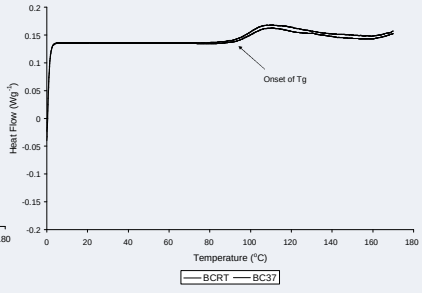


Figure 2

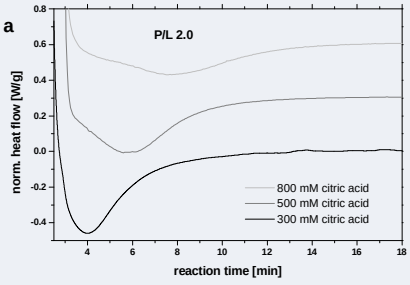


Figure 3

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Flow Cytometry

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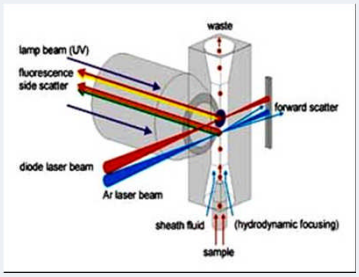
Biomaterials and Tissue Engineering

Introduction

Flow cytometry (FCM) is a technique for simultaneously measuring certain physical and chemical features of individual particles in suspension as they pass through a fixed laser beam, on the basis of angular reflection of a incident laser light. The light reflected by such particles at a low angle (< 2˚) is detected in the forward direction along the axis of the incident light and this "forward scatter" (FSC) is considered to be proportional to the relative size of the particle. Light reflected at > 2˚ is detected at 90˚ or more to the axis of the incident light and is referred to as orthogonal or "side scatter" (SSC), which is considered to be proportional to the surface and/or internal complexity of the particle. Additional information about each of the particles in the suspension can readily be obtained if they are 'tagged' with a fluorescent dye delineating a particular physical or chemical characteristic. Under these conditions, when an incident (eg, argon-ion) laser light is applied, the energy absorbed by the fluorochrome is absorbed and subsequently emitted at a higher wavelength which is specific for the fluorochrome. The intensity of the emitted fluorescent signal is proportional to the level of fluorochrome associated with the particle. Moreover, multiple parameters can be measured simultaneously using a panel of different fluorescent markers, each with a unique emission signal. A flow cytometer (eg FACScan; Becton Dickinson Ltd) is optimized for the acquisition of the data, and appropriate software is then used to analyze similarities and differences between the particles in the population.

Schematic illustration of a flow cytometer



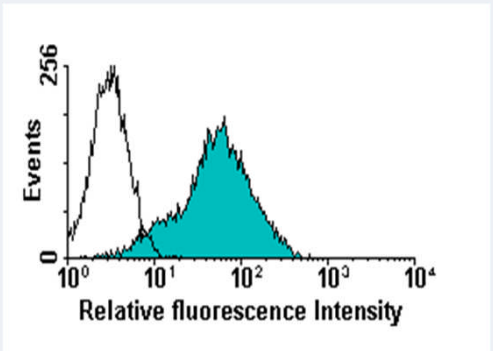
The flow cytometer uses the principle of hydrodynamic focusing for presenting particles in a fluid suspension to a laser (or other light excitation source). The sample is injected into the center of a sheath flow and as each single particle passes through the laser beam, the emitted optical signals generated by each single particle are captured and displayed for subsequent analysis.

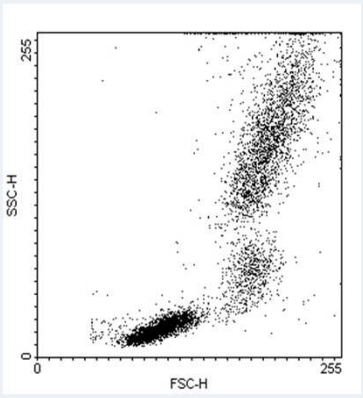
Applications of FCM

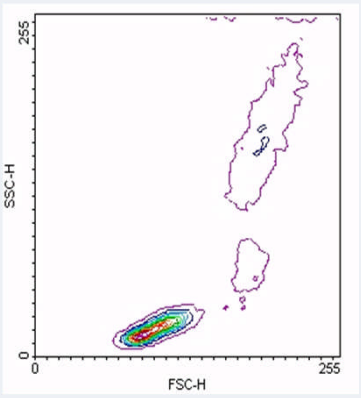
Because cells are essentially 'particles', the FCM technique has had major application in the life sciences and is now widely used in plant biotechnology, microbiology, haematology, immunology, oncology and virtually all other areas of cell and molecular biology. This has been due, in part, to the development and availability of fluorescent-labelled reagents which are specific for nucleic acids and, in particular, antibodies which identify specific cell-associated proteins. The use of monoclonal antibodies (mAb) to detect and measure many thousands of such 'antigens' has now become fundamental in assessing cell proliferation, differentiation and function, and is especially important in the analysis of suspensions containing different types of cell, including abnormal diseased cells and also putative stem cells for clinical therapy. In addition, research in the Biomaterials and Tissue Engineering Division at the Eastman has pioneered the application of the FCM technique for analysis of the precise cellular effects of conventional, modified and newly-developed materials for orthopaedic, dental and soft tissue implant surgery and for determining the potential efficacy of procedures for tissue repair and regeneration. During the past 10 years these studies, generating more than 40 publications, have had a significant impact on the criteria for evaluating cellular responses to biomaterial formulations and have now become the gold standard for determination of 'biocompatibility'.

Frequency histograms (Figure left below) display relative fluorescence or scattered light signals plotted against the number of events. The simplicity of this type of display is the main attraction. To see the relative levels of other parameters which were collected at the same time , one needs to use one of the forms of bivariate displays namely dot, density or contour plots. In these type of displays, one parameter is plotted against another in an X versus Y axis display.

The bivariate display (Figure right below) plots one dot or point on the display related to the amount of parameter x and y for each cell which passed through the instrument. Dotplots are good for detecting small numbers of events which are well separated from the main populations of the cells present but give little or no indication of the relative density of numbers of events in populations. This is particularly true for large data files. This is one reason for using a density plot.







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High performance liquid chromatography (HPLC)

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Biomaterials and Tissue Engineering

Introduction

HPLC HPLC is a chemistry based tool for quantifying and analyzing mixtures of chemical compounds. It is a technique that has been used for biotechnological, biomedical, and biochemical research as well as by the pharmaceutical industry. More recently HPLC has found a use in a variety of fields including cosmetics, food, and environmental industries. It consists of a precise gradient mixer; high pressure pumps with very constant flow; high accuracy, low dispersion, sample valves, high efficiency columns with inert packing materials, high sensitivity, low dispersion detectors, low dispersion connecting tubes for valve to column and column to detector. The columns, in general, achieve their separation by exploiting the different intermolecular forces between the solute and the stationary phase and those between the solute and the mobile phase. The column will retain those substances that interact more strongly with the stationary phase than those that interact more strongly with the mobile phase. The detector is either UV, refractive index or fluorescence.

Technical specifications

The Thermo-Separation products HPLC system is modular in style and consists of:

- Degasser that removes dissolved gas from the mobile phases so that on mixing, gas bubbles are not formed
- High performance gradient pump system consisting of two pumps working out of phase to ensure continuous flow
- Automatic sampler for unattended use
- High efficiency column
- UV detector
- PC for data acquisition

The Main Unique Aspects of the Machine

- High precision detector (5 d.p. absorption units). The limit of detection varies according to the absorbance of the species but typically this equates to a species concentration of 0.2 ppm.
- Column oven that ensures reproducibility of retention time.
- Microprocessor controlled gradient pump ensuring low pulsation and therefore an even mobile-phase flow.
- Auto sampler that allows a maximum of 120 samples to be run unattended after which a shutdown program can reduce the flow to 0.1 ml / min to reduce mobile phase loss.
- The system is interfaced with Dionex Chromeleon v6.5 for fully automated unattended measurements and full quantification capabilities. This ensures high throughput and repeatability

Examples of Work undertaken at EDI

A number of projects have used this technique for quantitative measurement.

- Measurement of lactic acid in degradation studies of poly (lactic acid)
- Drug releases from glass-ionomer cements including chlorhexidine acetate, chlorhexidine gluconate and amprolium hydrochloride.
- Release of Tetracycline hydrochloride and Vancomycin from Bio-OSS.

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Ion Chromatography

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Introduction

Ion chromatography is a versatile, sensitive and powerful technique for measuring a wide range of ions over a very broad range of concentrations. Furthermore, the analyses may be carried out over the full pH range 0-14, thus minimising sample preparation. The technique utilises functionalised polymeric separation technology for separating out the ions of interest. This can range from simple cation separation, through to more complex studies of transition metals or polyphosphates.

Technical specifications

We have three systems simultaneously available to maximise throughput.

•A Dionex ICS-1000 cation system is available (left hand picture above) for measurement of a standard wet of cations (K^+, Na^+, Li^+, Ca^{2+}, Mg^{2+} and NH_3^{2-}. The system utilises an isocratic pumping system coupled with electrolytic suppression to minimise background counts. The mobile phase is a low concentration (20-30mM) solution of methylsulphonic acid. Analysis times can be as short as 4 minutes depending on the ions of interest and a high throughput is also achieved with an AS50 autosampler equipped with 49 or 99 sample racks (9ml or 500ul vials respectively). Detection is via an electrochemical cell. Column heating is also carried out for absolute retention time stability.

•A Dionex anion system is the second system available (right hand picture above). This is routinely set up for polyphosphate separation and measurement using a AS18 column and again utilising electrolytic suppression of the background. A unique aspect to this system is the online generation of eluents via an electrolysis method. This method simplifies the production of gradient concentrations, by eliminating the need for gradient pumps. As it is electrolytic, it allows for extremely high gradients to be generated. Again an AS50 autosampler is available to ensure high throughput. Detection is eletrcochemically and column heating is standard.

•The final Dionex system is for the speciation and measurement of transition metals and utilises a PAR postcolumn colorimetric reagent to determine the ions. Detection is via a UV detector, so the system can also be configured for standard HPLC measurements. Again column heating and an AS50 autosampler are standard.

•All data collection and analysis is via the Chromeleon v6.0 suite of software which allows full control of all the systems simultaneously.

The Main Unique Aspects of the Machine

•As we have three separate systems each with an autosampler, a very high throughput can be achieved, thus allowing detailed multipoint studies to be carried out.

•The particular systems we have utilise extremely sensitive columns, allowing excellent separation of a variety of species. Also a very wide range of other columns are available for separation of a wide range of ions.

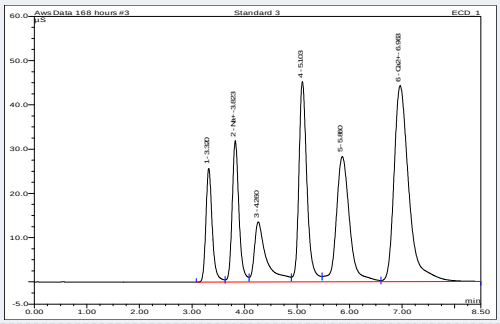
•Due to the electrolytic suppression and very sensitive detection cells, it is possible to routinely measure down to 0.1ppm. Work has been carried out on samples with concentrations as low as 0.01ppm.

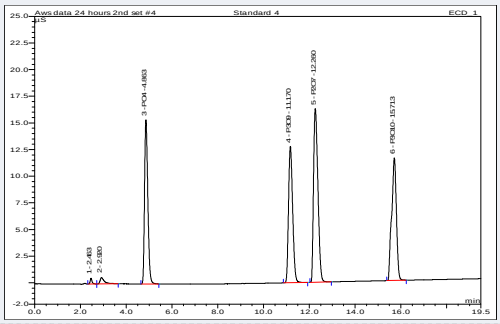
•Report generation can be automated to allow output of the data direct to Excel via a user defined report generation template.

Examples of Work undertaken at EDI

The ion chromatography systems have been responsible for a very high number of publications on a variety of ionic species in a range of different materials. The left hand chromatogram shows a separation curve for a low concentration solution of the 6 cations detectable with the ICS-100 system.

The right hand figure shows a calibration curve for measurements of a variety of phosphates. Excellent separation can be seen for each of the four phosphates for which we are calibrating (PO_4^{3-}, $P_2O_7^{4-}$, $P_3O_9^{3-}$ and $P_3O_{10}^{5-}$.





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Inductively coupled plasma mass spectrometry ICP-MS

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Introduction

The ICP-MS is an analytical technique that determines the elemental content of samples. It is accomplished by counting the number of ions at a certain mass of the element. Most samples analyzed by ICP-MS are liquid. Solid samples can be analyzed but they must be vaporized using e.g. lasers or heat cells. Gas samples can also be measured by introducing them directly into the instrument.
The ICP-MS instrument measures most of the elements in the periodic table. The elements can be analyzed with detection limits at or below the part per trillion (ppt). The ICP-MS detects only elemental ions and can determine the individual isotopes of each element

Fig. 1. Elements determined by ICP-MS and approximate detection capability (PerkinElmer

Technical specifications

An ICP-MS consists of the following components:

•sample introduction system – consist of the peristaltic pump, nebulizer, and spray chamber that introduces sample to the instrument,

•ICP torch – generates the plasma which serves as the ion source of the ICP-MS, converting the atoms to be analysed to ions,

•interface – the sample ions are extracted from the central plasma channel and separated from the bulk ions by cooled conical aperture plates with aperture openings of 1/0.8 mm in the vacuum interface (vacuum <2mbar),

•vacuum system – provides high vacuum for ion optics, quadrupole and detector,

•quadrupole – the high frequency quadrupole acts as a mass filter to sort ions by their mass-to-charge ratio (m/e). The mass resolution with constant peak widths from 0.5 to 1 amu at 10% peak height can be set in three steps,

•detector – after passing mass filter the ions are either detected through direct current measurements on the ion collector or the ions generate secondary electrons that are propagated in the multiplier. Together, both methods can cover an intensity range from a few ions/s to 10^{12} ions/s.

•data handling and system controller.

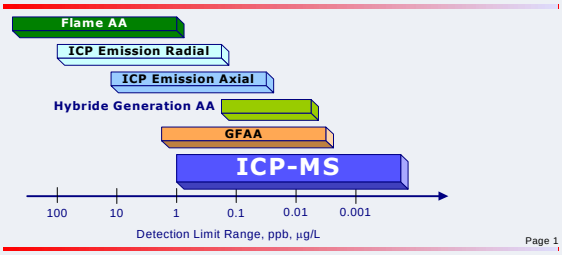

Fig. 3. Typical detection limit ranges for the major atomic spectroscopy techniques (Perkin Elmer)

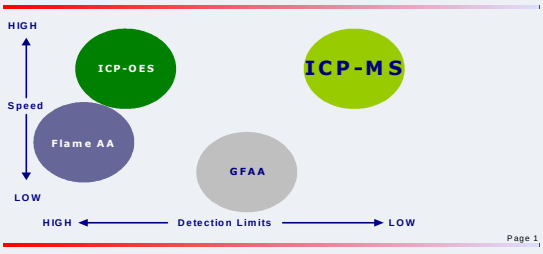

Fig. 4. General selection guide for atomic spectroscopy instrumentation based on sample throughput and concentration range (Perkin Elmer)

The Main Unique Aspects of the Machine

Speed: The quadrupole mass analyzer is able to scan the mass spectrum from 3-250amu very quickly. A mass spectrum of usable data can be acquired in just a few seconds depending on exact instrument settings.

Mass Stability: As there are no magnetic fields in the quadrupole ICP-MS, it is able to move from mass to mass with a superb degree of precision. This enables the analysis technique known as "peak hopping" in which only a single point of data is acquired at the very top of the peak at each element during an analysis.

Sensitivity: easily able to detect trace levels of many elements at levels well below a ppb (ng/g).

Cold Plasma Capability: Cold or cool plasma is a technique whereby the temperature of the plasma is reduced by lowering the RF power. This partially prevents the formation of Ar-based molecular interferences by reducing the number of Ar+ ions in the plasma. While a little awkward to use, this technique allows for the analysis of elements with large molecular interferences such as potassium and iron.

Inability to resolve target isotopes easily from molecular interferences: Commercially available quadrupole ICP-MS systems are able to resolve a mass spectrum only to unit resolution. This means that while the mass analyzer can easily tell the difference between ^{56}Fe and ^{57}Fe, they cannot resolve ^{56}Fe (mass 55.9349) from the ^{40}Ar ^{16}O molecular species (mass 55.9573), which is very easily formed in an Argon plasma. To accurately determine the concentration of some difficult elements, it is necessary to compromise sensitivity with the use of techniques such as "cold plasma."

High Background Noise: The ion optics of quadrupole mass analyzers make them susceptible to background noise on the detector, particularly when coupled to an ICP source. A few stray high-energy photons from the plasma source always seem to make it through to the detector, sending false pulses into the counting electronics. Because the ultimate limit of detection (LOD) of any system is directly proportional to variations in the background noise, higher noise levels obviously will result in compromised LODs.

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Leica DMIRB Inverted Fluorescence Microscope

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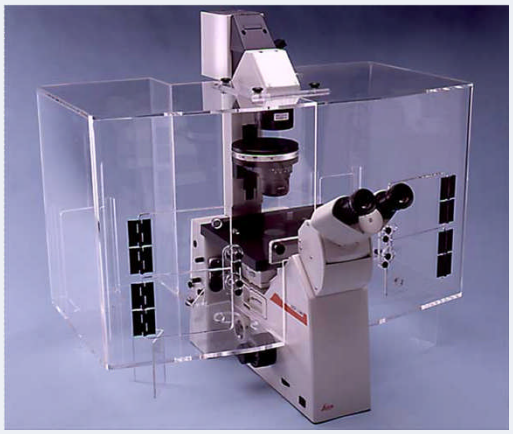
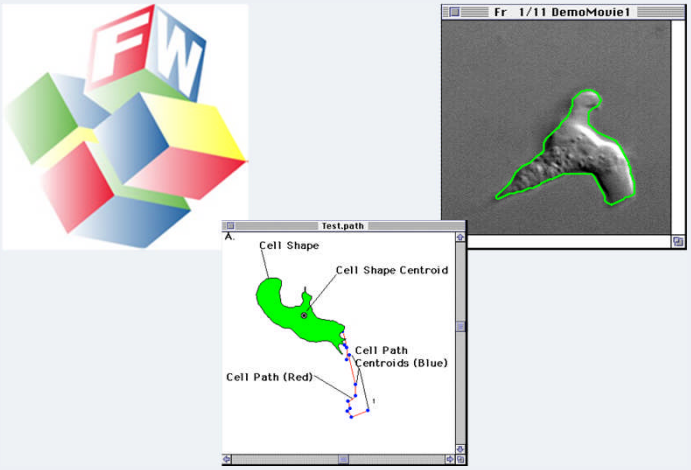
UCL

Biomaterials and Tissue Engineering

Introduction

The Leica DMIRB is a computer-controlled conventional wide-angle (non-confocal) microscope. Its inverted nature means it is uniquely designed for use with cell culture experiments. It is equipped with a stage accepting a range of plates and microscope slides and a high-resolution CCD camera. There is maximum flexibility in using the microscope for effective contrasting methods such as Brightfield, Phase Contrast, Darkfield Contrast, Polarization Contrast, Leica Modulation Contrast, DIC and Fluorescence. The microscope is fixed with a Solent Scientific transparent environmental chamber. This incubation chamber is used for prolonged studies of living cells, including time lapse image capture experiments for cell motility and cell death. The Leica DMIRB is a viable alternative to confocal microscopy that is not always the best solution for imaging needs and, in some situations, may even be counter-productive. The Leica DMIRB can produce similar results and offers as much flexibility. The dedicated FW4000TZ software handles image captures and use and offers a total fluorescence imaging solution.





Technical specifications

"Live cell" option

Microscopy – Leica Modulation Contrast optics. 10, 20 and 40x objectives.

Environment – Solent Scientific transparent environmental chamber. Warm, filtered air circulates within the acrylic chamber from a heater unit that is mechanically isolated from the environmental chamber. Two doors allow specimens to be changed and the condenser settings to be adjusted. A further two doors, below the stage, allow access to the nosepiece and objectives. Focusing and stage controls remain outside the environmental chamber. The 35mm camera, video camera, multi-viewing and fluorescence capabilities of the microscope are unaffected. The chamber can, when required, be quickly removed without the use of hand tools.

Microscope Control – FW4000TZ software (see below) allows user to perform time-lapse experiments with automated shutter control to prevent excessive exposure of specimen to transmitted light. The motorised focus allows for Z-stacking (multiple focus during experiments).

Post experiment processing – Movies generated by the FW4000 software can be converted and analysed with DIAS Software. This system represents the most advanced computer system commercially available for analyzing how cells move and change shape overtime. It contains both manual and automatic digitization modes, advanced image-processing capabilities, the ability to quantitate more than 30 parameters of motion and dynamic morphology, the capacity to generate a variety of movies demonstrating motility and dynamic morphology, and sophisticated graphing and analytical capabilities.

"Fixed cell" option

Microscopy - Brightfield, Phase Contrast, Darkfield Contrast, Polarization Contrast and Fluorescence. 20, 40 and 63x objective. Optimized Fluorescence with a 4 position filter cube turret (manual) without pixel shift, and filters for UV (dapi), FITC, cy3 and cy5

Image acquisition and processing with FW4000TZ software –

Basic Acquisition Suite
Acquisition
Basic Enhancement Tools (Contrast, brightness, gamma)
Lab Book
Basic Printing
Annotation
Annotation & Calibration
Probe meter and pseudo-colouring
Zoom and pan
Composite creation
Channel mixing
Z-stack acquisition
Time sequence builder
lapse experiments
Auto Relocate

Enhancements Module
Region of interest
No neighbours deconvolution
Sharpening/smoothing tools
Background removal
Nearest neighbours deconvolution
Maximum intensity projections

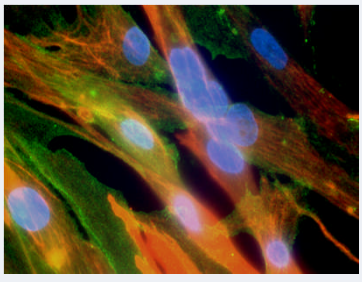
Measurement Module
Manual measurements
Automatic measurements
Object counting
Grey level profiling

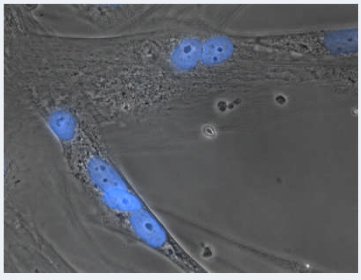
Deblur and 3D Visualize Module

Gallery Module
Interactively review experiments
Create composites on collections of image
Filter and display selected images
Select images for processing

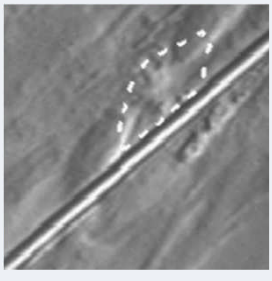
Publish Module
Compiling. avi movies
Printing reports
Print 1,2 or 4 images
Printing image montages of Z-stacks and time
Compile web documents

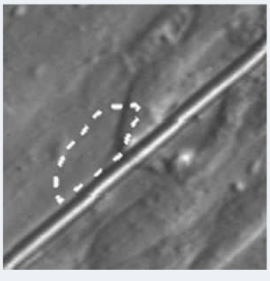
Work conducted at EDI





Examples in "fixed cell" mode
LEFT: Human jaw muscle cells fluorescently stained for cytoskeletal markers (red and green) and DNA (blue).
RIGHT: Human jaw muscle cells identified by phase contrast (greyscale) and DNA (blue).





Examples in "live cell" mode
Human jaw muscle cells were cultured on soluble glass fibres and monitored with live time lapse.

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Real time quantitative PCR and allele discrimination

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Biomaterials and Tissue Engineering

Introduction

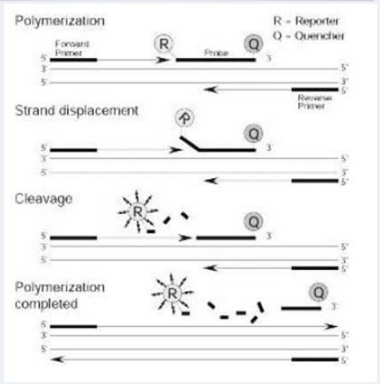
Real-time Polymerase Chain Reaction (PCR) is the ability to monitor the progress of the PCR as it occurs (i.e., in real time). Data is therefore collected throughout the PCR process, rather than at the end of the PCR. This completely revolutionizes the way one approaches PCR-based quantitation of DNA and RNA. In real-time PCR, reactions are characterized by the point in time during cycling when amplification of a target is first detected rather than the amount of target accumulated after a fixed number of cycles. The higher the starting copy number of the nucleic acid target, the sooner a significant increase in fluorescence is observed. In contrast, an endpoint assay (also called a "plate read assay") measures the amount of accumulated PCR product at the end of the PCR cycle. Applied Biosystems has developed two types of chemistries used to detect PCR products using Sequence Detection Systems (SDS) instruments, the TaqMan® chemistry (also known as "fluorogenic 5´ nuclease chemistry") and SYBR® Green I dye chemistry.

TaqMan® Chemistry
The TaqMan chemistry uses a fluorogenic probe to enable the detection of a specific PCR product as it accumulates during PCR cycles.

Assay Types that Use TaqMan Chemistry
The TaqMan chemistry can be used for the following assay types:
Quantitation, including:
- One-step RT-PCR for RNA quantitation
- Two-step RT-PCR for RNA quantitation
- DNA/cDNA quantitation

• Allelic Discrimination
• Plus/Minus using an IPC

SYBR Green I Dye Chemistry
The SYBR Green I dye chemistry uses SYBR Green I dye, a highly specific, double-stranded DNA binding dye, to detect PCR product as it accumulates during PCR cycles. The most important difference between the TaqMan and SYBR Green I dye chemistries is that the SYBR Green I dye chemistry will detect all double-stranded DNA, including non-specific reaction products. A well-optimized reaction is therefore essential for accurate results.



Assay Types that Use SYBR Green I Dye Chemistry
The SYBR Green I dye chemistry can be used for the following assay types:
- One-step RT-PCR for RNA quantitation
- Two-step RT-PCR for RNA quantitation
- DNA/cDNA quantitation

How TaqMan Sequence Detection Chemistry Works
The TaqMan chemistry uses a fluorogenic probe to enable the detection of a specific PCR product as it accumulates during PCR. Here's how it works:

Step Process
1.An oligonucleotide probe is constructed containing a reporter fluorescent dye on the 5´ end and a quencher dye on the 3´ end. While the probe is intact, the proximity of the quencher dye greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer (FRET) through space.
2. If the target sequence is present, the probe anneals downstream from one of the primer sites and is cleaved by the 5´ nuclease activity of Taq DNA polymerase as this primer is extended.
3. This cleavage of the probe:
- Separates the reporter dye from the quencher dye, increasing the reporter dye signal.
- Removes the probe from the target strand, allowing primer extension to continue to the end of the template strand.

Thus, inclusion of the probe does not inhibit the overall PCR process.

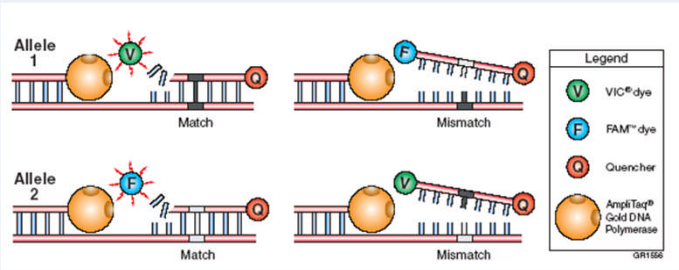
4. Additional reporter dye molecules are cleaved from their respective probes with each cycle, resulting in an increase in fluorescence intensity proportional to the amount of amplicon produced.

What Is a Quantitation Assay?
A Quantitation Assay is a real-time PCR assay. It measures (quantitates) the amount of a nucleic acid target during each amplification cycle of the PCR. The target may be DNA, cDNA, or RNA. There are three types of Quantitation Assays discussed in this chemistry guide:
- DNA/cDNA quantitation
- RNA quantitation using one-step reverse transcription polymerase chain reaction (RT-PCR)
- RNA quantitation using two-step RT-PCR

Absolute vs. Relative Quantitation
When calculating the results of your quantitation assays, you can use either absolute or relative quantitation.
What is Absolute Quantitation?
The absolute quantitation assay is used to quantitate unknown samples by interpolating their quantity from a standard curve.
What is Relative Quantitation?
A relative quantitation assay is used to analyze changes in gene expression in a given sample relative to another reference sample (such as an untreated control sample).

What is an Allelic Discrimination (AD) Assay
An allelic discrimination (AD) assay is a multiplexed (more than one primer/probe pair per reaction), end-point (data is collected at the end of the PCR process) assay that detects variants of a single nucleic acid sequence. The presence of two primer/probe pairs in each reaction allows genotyping of the two possible variants at the single-nucleic polymorphism (SNP) site in a target template sequence. The actual quantity of target sequence is not determined.

For each sample in an AD assay, a unique pair of fluorescent dye detectors is used, for example, two TaqMan® MGB probes that target an SNP site. One fluorescent dye detector is a perfect match to the wild type (allele 1) and the other fluorescent dye detector is a perfect match to the mutation (allele 2).



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Raman and FTIR mapping

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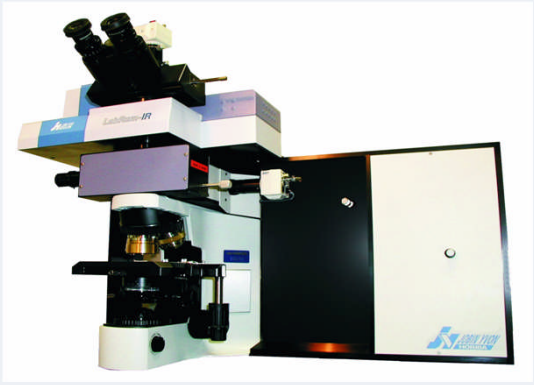
Biomaterials and Tissue Engineering

Introduction

With both Raman and FTIR spectroscopy peaks are obtained at high wavenumbers due to known specific chemical groups. In the low wavenumber 'fingerprint' region the spectra are generally unique for a given chemical. Raman and FTIR are considered to provide complimentary spectra because peaks for a specific chemical group of interest that may be weak in one spectrum is likely to be strong in the other. These techniques have been available to study the bulk properties of chemicals for many years but with the advent of Raman and FTIR microsocopy it is now possible to gain a chemical map of a surface with micron resolution.



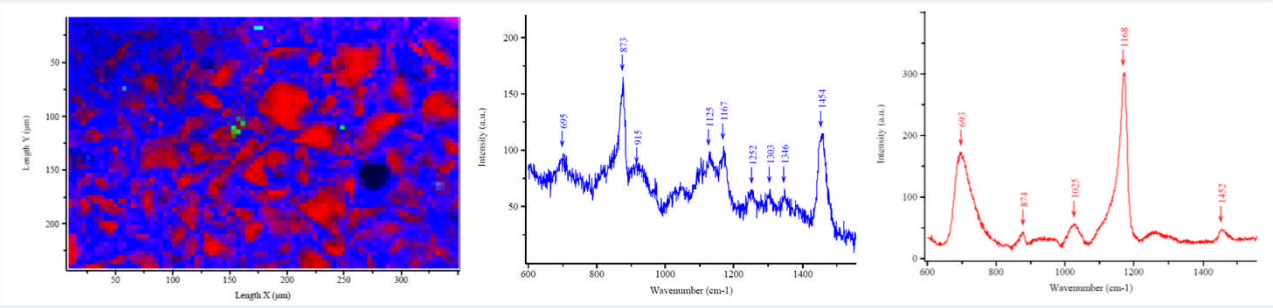
Technical specifications

The Labram 300 (Horiba Jobin Yvon) is equipped with a HeNe (633nm) laser and an xyz mapping stage. With the 1800 and 950 Raman gratings wavenumber resolutions of 1 and 2cm^{-1} respectively are obtained. Use of a video camera allows white light images of a sample on the microscope to be transferred to the computer screen. The data acquisition software then enables spectra to be obtained within selected areas in the image. Data can also be obtained as a function of time and depth. With the analysis options the sample spectra can be compared with those of known compounds. Coloured images, providing information on the relative amounts of each chemical at different points within the region mapped, are then generated.

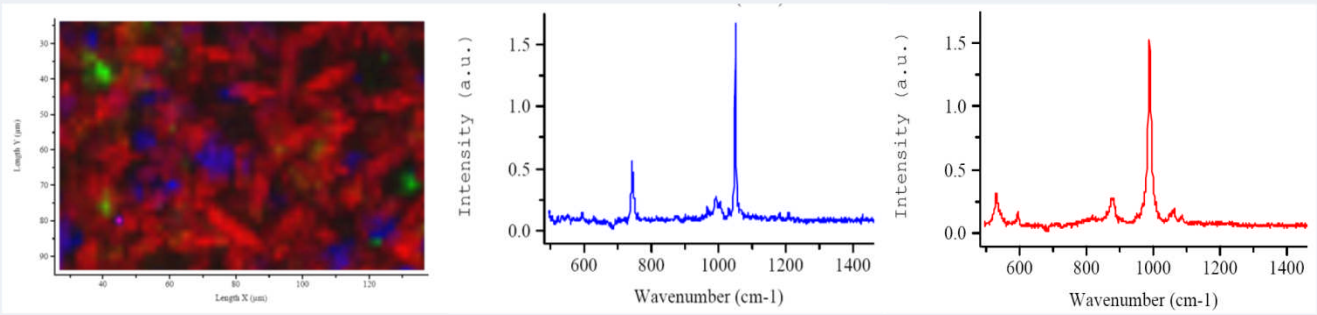
Examples of Work undertaken at EDI

A variety of projects are currently utilising this technique for qualitative and quantitative measurement of the chemistry of complex mixtures. Examples include determination of relative levels of hydroxyapatite and collagen in dentine after reaction with components used in endodontics, assessment of differences between normal and cancerous tissue, understanding of the dissolution mechanism of phosphate glass fibres, and characterisation of biomedical composites and cements. For example in Figure 1 below a Raman map of a composite is provided showing phosphate glass particles within a degradable polymer. This technique has shown that with time in water the phosphate glass dissolves preferentially. In the second example a bruhite forming bone cement image is given. This shows that in the set cement the components other than brushite are present only in low amounts.

Example 1 – Raman spectra and chemical map of a polymer (blue component) containing 20 micron size phosphate glass particles (red component)



Example 2 – Raman spectra and chemical map of a brushite (red needle – like component) forming bone cement containing low levels of other phosphates (blue component)



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Scanning Electron Microscope

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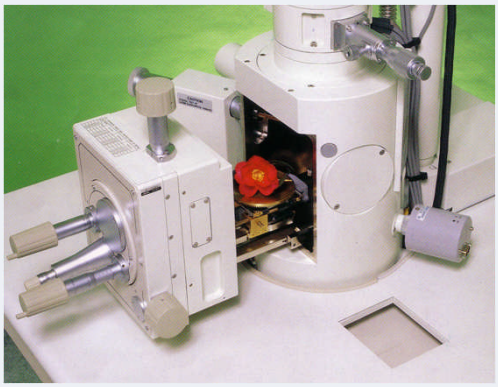
UCL

Biomaterials and Tissue Engineering

Introduction

Scanning electron microscopy is a technique that utilises a beam of electrons to image and analyse the surfaces of both biological (bacteria, cells and tissues) and material (metals, glasses and ceramics) samples and various combinations of the two. By using different detectors within the microscope chamber it is possible to obtain high resolution digital data of detailed surface topography and elemental composition at magnifications that can vary from 20x to 50,000x. Samples can be examined once fixed and dried at high vacuum or with little or no preparation using variable pressure.



Technical specifications

The JEOL JSM 5410 LVSEM is a versatile microscope with 3 attached detectors: a secondary electron detector for imaging and topography, a back-scattered electron detector giving compositional information and an X-ray detector for elemental analysis.

•The spacious specimen chamber enables examination of single large samples up to 150mm diameter with 125mm diameter viewable or several smaller samples (up to 8) in various multi-holders. The eucentric goniometer stage offers high accuracy and vibration resistance with tilt from -10 to +90˚ and continuous dynamic focus.

•In high vacuum mode the resolution is 4nm whilst in variable pressure mode the resolution remains a decent 5.5nm.

•There are several automated functions including focus, contrast and brightness settings, astigmatism correction, photo acquisition and a low vacuum mode.

•There are four sets of frame memories so that up to four separate images that can differ in magnification, detector type, accelerating voltage, etc, can be compared on one screen with a high resolution digital image capture facility which includes some measuring and image analysis functions.

The Main Unique Aspects of the Machine

•Specimen observation and analysis are possible without pre-treatment or coating. This is facilitated by operation in a low pressure environment and back-scattered electron acquisition

•The change from high vacuum to variable pressure mode is by a single button and it can be operated in an auto mode or by manual pressure setting. The low vacuum range is from 6-270Pa.

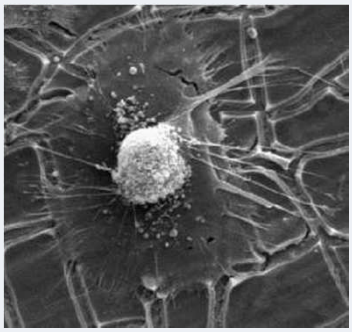
•There is a motorised stage which operates in the x, y and rotation axes and can be programmed, all movements being monitored by an infra-red chamberscope displayed on an additional TV screen.

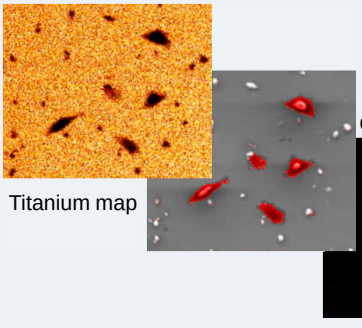
•An Oxford Instruments INCA EDX system is attached to the SEM and elemental analysis can be carried out in both high vacuum and variable pressure modes. The system allows the analysis of large or small areas, points and lines and also the localisation of individual elements can be mapped.

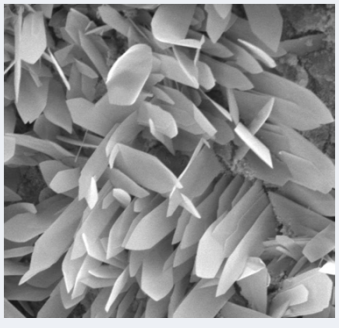
Examples of Work undertaken at EDI

A first stage in assessing the biocompatibility of many novel materials is investigating the extent to which cells will adhere to and proliferate on the biomaterial. The SEM in both high vacuum and variable pressure mode provides very detailed information about the cells (left-hand figure) and the elemental distribution (middle images). In a similar manner the initial stages of biofilm formation can be rapidly assessed by this technique, particularly relevant for the effects of antibacterial components and specialised surface coatings.

As part of the quest for information in clinical research, the SEM has been used to examine evidence of wear, fractures and composition of many dental materials, the interface between tooth and restoration (right-hand figure), biofilm attachment to failed implants







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UV-Visible Spectroscopy

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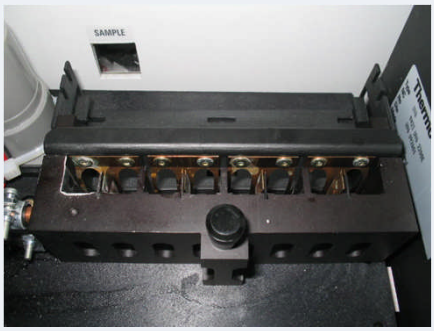
Introduction

UV-visible spectroscopy probes the electronic transitions of molecules as they absorb light in the UV and visible regions of the electromagnetic spectrum. Any species with an extended system of alternating double and single bonds will absorb UV light, and anything with colour absorbs visible light, making UV-visible spectroscopy applicable to a wide range of samples. A UV-visible spectrum usually consists of one or more broad peaks corresponding to the maximum intensity of absorbance of a particular wavelength. The technique is quantitative and is routine in biochemistry and pharmaceutical research.



Technical specifications

The Unicam UV spectrometer is a dual beam instrument with an eight position vial holder allowing seven samples and a reference to be measured in one run. It has a tungsten lamp for the visible range and deuterium lamp for the UV range. This allows it to measure from 190 nm in the UV range to 900 nm in the visible range. During a scan that traverses both the visible and UV ranges the point at which the lamps switch over can be set to between 315 nm and 340 nm to avoid interference with important peaks.

The Main Unique Aspects of the Machine

UV spectroscopy has been in use for forty years and is the simplest and most versatile of the analytical instrument techniques. The software on this machine gives the option of three detection methods:

- •Fixed, where a sample can be measured at up to ten individual wavelengths per run
- •Scan, where a sample can be measured between two wavelengths at a maximum resolution of 1nm. This is displayed as a plot of absorbance against time.
- •Quant, where a calibration plot is constructed from a range of standard solutions and the unknown samples are measured and their concentrations computed by the software.

•Examples of Work undertaken at EDI

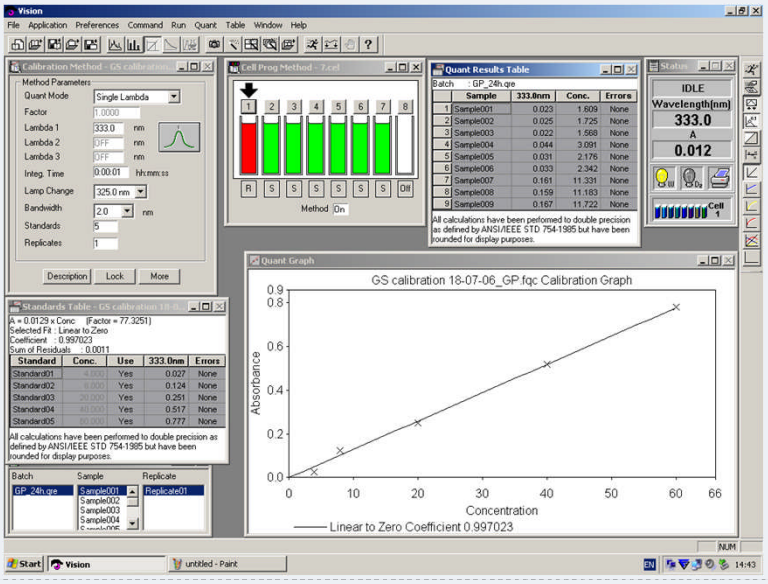
A number of projects have used this technique for quantitative measurement.

•For example (figure below) the release of Gentamicin sulphate from doped Bio-Oss was determined using the 'quant' method.

•Bio-Oss has also been doped with tetracycline and its release measured with UV spectroscopy.

•Release of tetracycline from hydroxyapatite.

•Release of chlorhexidine from hydroxyapatite.



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X-Ray Diffraction

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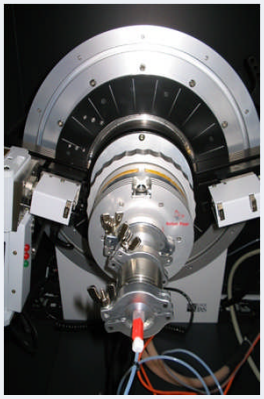
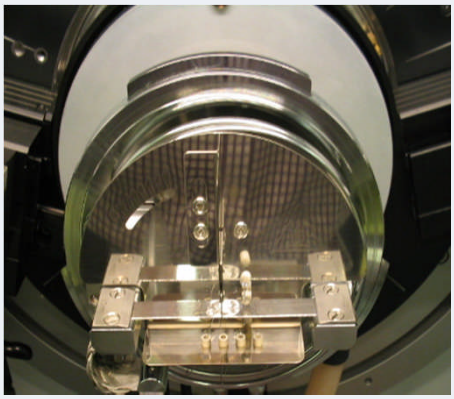
Biomaterials and Tissue Engineering

Introduction

X-ray diffraction is a technique that may be used to probe the structure of crystalline materials at a number of different levels of detail. X-ray diffraction patterns consist of a number of intense peak or 'reflections' at specific angles and contained within this pattern is information that may be as detailed as where all the atoms are within the structure. However these peak positions may be used more simply as a fingerprint, to identify the crystalline phase or phases in a sample, either qualitatively or quantitatively. The technique is non-destructive and can be applied to a wide variety of materials, such as ceramics, metals, polymers and composites in a variety of forms such as powders, solids and thin films

Technical specifications

The Brüker D8 Advance diffractometer is a vertical footprint goniometer configured in θ-θ geometry (i.e. the sample is horizontal and the X-ray source and detector move). It is configured with a CuKα X-ray source with a number of other fixtures available for different measurements

•The standard geometry θ-θ geometry utilises a high resolution scintillation counter for excellent quality datasets suitable for structure refinement.

•Long Soller slits and LiF monochromator crystal setup available for thin film measurements also known as glancing angle or grazing angle measurements). Films down to 40nm have been characterised with this attachment

•A Lynx Eye silicon strip detector is also available as an alternative to the scintillation counter. This has similar resolution, but because of the 197 strips available, can reduce the count times by a factor of almost 200. with little loss of resolution. (See Figure below right).

•A 9 position sample changer allows continuous unattended data collection, enabling large numbers of high quality data sets to be collected routinely.

•An Anton Paar HTK16 high temperature stage capable of reaching 1600 C is available and easily exchanged with any of the other optics (centre and right hand imagers above). The chamber also has gas purge facilities, for either inert or reactive atmospheres. Coupled with the Lynx Eye enables real-time measurement of phase changes and melting phenomena.

The Main Unique Aspects of the Machine

•The modular design allows simple and rapid changeover to different configurations. On most changeovers, realignment is not required.

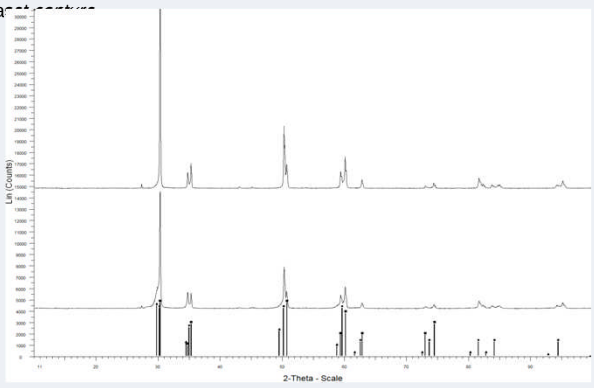
•The Lynx Eye detector allows high quality datasets to be collected in a fraction of the time for conventional scintillation detectors. This brings data refinement strategies into the realm of routine analytical methods and allows systematic studies to be performed in great detail

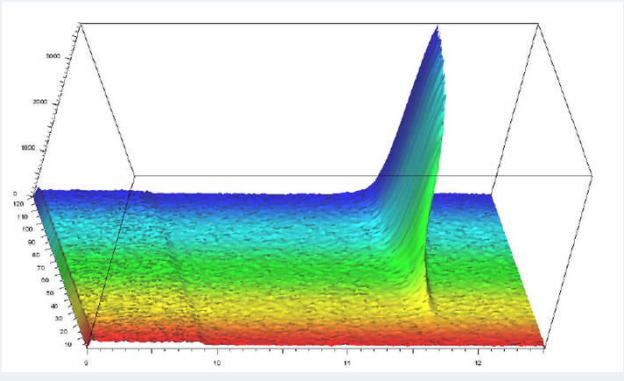
•The 9 sample auto-changer allows 24 hour unattended operation

•A significant suite of software is available for data analysis, this extends from The Brüker Eva package coupled with ICDD PDF-2 database for phase identification, through to the General Structure Analysis Software (GSAS) and also Topas for full structure determination. We also have a suite of Crystallographica Search/Match software available from Oxford Cryosystems

Examples of Work undertaken at EDI

A wide variety of projects have utilised this technique for qualitative and quantitative measurement of a wide variety of parameters. For example (left hand figure below) we have measured and quantified the presence of a strained cubic phase in zirconia following grinding (lower trace) and the disappearance of this phase following a polishing procedure.
We have also carried out studies of the crystallisation of brushite in a calcium phosphate cement. The right hand figure below shows the appearance of the main reflection for brushite as the cement reacts. There are 120 datasets and each 3.5 range took 2 seconds to collect with an 8 second delay between each dataset capture





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