(из интернета)

Electron Diffraction

Electron diffraction is capable of giving the highest resolution information about the structure of materials. Using aberration-corrected field-emission sources in combination with advanced computer processing, chemical bonds can now be seen in many materials. A special issue of Microscopy and Microanalysis [9(5):377-490, Nov 2003] reviews progress in methods for the quantitative electron diffraction.

Of special interest are reviews of quantitative zone-axis convergent beam electron diffraction (CBED) of elastically scattering components, low-dose, low-temperature CBED, Koehler mode selected area electron diffraction, and STEM electron nanodiffraction methods for producing the highest signal from the smallest regions.

Other papers cover: quantitative CBED measurements of low-order structure factors copper; analysis of local strain in aluminium interconnects by CBED; sufficient conditions for direct methods with swift electrons; a combination method of charge density measurement in hard materials using accurate, quantitative electron and X-ray diffraction; low-dose, low-temperature CBED and multiwavelength analysis of hydrocarbon films by ED; accurate measurements of valence electron distribution and interfacial lattice displacement using quantitative ED; the consistency of qCBED structure factor measurements for rutile; and quantitative ED evidence for one-dimensional ordering in magnetite above the Verwey transition.

High-Pressure Freezing

The holy grail of electron microscopists is to examine samples in as close to their native state as possible. For biological tissues, high-pressure freezing is probably the best currently available technique that meets this requirement. Samples are frozen in liquid nitrogen at 2000 bar which preserves them in vitreous or microcrystalline ice; they can then be processed for TEM by cryosectioning, freeze fracture or freeze substitution.

Papers from a conference on high-pressure freezing in biology and medicine held at Bad Schandau in Oct 2002 are published in the Journal of Microscopy [212(1):1-99, Oct 2003]. The 11 articles describe the preparation of tissues and cells, specimen holders, freezing apparatus, cryosectioning, freeze substitution and embedding, and many applications of the technique. This issue provides a useful and timely overview of high-pressure freezing with many detailed protocols and high resolution illustrations of a broad range of samples.

Electrical Nanobalance

Calibration of atomic force microscopes is essential for accurate measurements. Height can be calibrated with stepped-height standards, but force calibration requires knowledge of the spring constant (i.e. stiffness, or ratio of applied force to displacement) of the cantilever. A new calibration device for atomic force microscope cantilevers for the measurement of nanoNewton and picoNewton forces is described by P. Cumpson and J. Hedley [Accurate analytical measurements in the atomic force microscope: a microfabricated spring constant standard potentially traceable to the SI. Nanotechnology 14:1279-1288, 2003].

The authors made their calibration device by silicon surface micromachining. Called an electrical nanobalance, the device is essentially a capacitor with one fixed electrode and one moveable electrode suspended on a spring. First the device is itself calibrated by a combination of non-contact electrical measurements and Doppler velocimetry at a testing or metrology laboratory to give the spring constant of the moveable electrode. The device is then prepared for use by permanently connecting the two electrodes electrically. The user then calibrates their own cantilever using the moveable electrode. The authors contend the device allows accurate calibration to within 5%, is SI compatible, and allows comparison with other methods used for different instruments such as optical tweezers.

Intensifier Eyepiece

The human eye can see only light of wavelengths 400 to 700 nm. So infrared emission from fluors such as Cy5 is invisible through the eyepieces of a fluorescence microscope and an IR-sensitive CCD camera must

be used. A simple device that couples an image intensifier with a regular eyepiece to allow a user to see IR fluorescence directly has been designed by M. Siddiqi et al. [Use of a night vision intensifier for direct visualization by eye of far-red and near-infrared fluorescence through an optical microscope. Journal of Microscopy 212 (2):132-143, Nov 2003].

The authors used a GaAs photcathode-based intensifier with sensitivity in the 450-900 nm range and an output window of 25 mm diameter matching the standard eyepiece field of view. The intensifier was placed in a shortened Nikon eyepiece so that the output image is in the microscope's intermediate image plane. The output phosphor is green so IR emission is not viewed in true colour, but the sensitivity of the intensifier is much greater than that of the eye and so lower excitation intensities can be used, reducing photobleaching and phototoxicity. The authors demonstrate the use of this intensifier eyepiece on a wide range of fluors and living samples.

Advances in Light Microscopy

Nature Biotechnology [21(11):1251-1409, Nov 2003] contains commentaries on the progress and commercialization of light microscopy instrumentation and several review articles on high resolution and non-linear imaging, all under the heading Focus on Optical Imaging.

Of particular interest are the review by Stefan Hell on stimulated emission depletion microscopy, which reduces the side lobes of diffraction-limited images, so improving lateral and axial resolution, and an accompanying paper that demonstrates that a resolution of 70 nm is now possible when imaging fluorescently labelled microtubules in kidney cells.

Other papers cover second-harmonic imaging microscopy for visualizing biomolecular arrays in cells, tissues and organisms, optical coherence tomography for ultrahigh resolution *in vivo* imaging, multiphoton microscopy in the biosciences, near-field optics and FRET imaging.

Nanolithography

Various scanning probe microscopy techniques for nanofabrication using lithography of surfaces coated with organothiol self-assembled monolayers (SAM), a widely used method for surface modification and nanofabrication of metals and metal oxides, are reviewed by S. Kraemer et al. [Scanning probe lithography using self-assembled monolayers. Chemical Reviews 103(11):4367-4418, Nov 2003].

This is the most comprehensive and detailed review of the use of STM, AFM, scanning electrochemical microscopy and NSOM in nanolithography. It includes 245 references and three tables listing hundreds of applications by the type of SPM, SAM, substrate, resolution and imaging conditions.

The review covers four different techniques: elimination lithography in which material is removed from a coated substrate by the SPM tip, addition lithography which involves adding placing material onto a substrate from the tip, substitution lithography, and dip-pen nanolithography where the AFM tip is used to deliver molecules to a surface via a solvent meniscus.

The ability to arrange and organise material on a nanometre scale is a major enabling principle in nanotechnology and can only be done with advanced scanning probe microscopes so this paper is a timely review of this rapidly growing field.

Super-Resolution Light Microscopy

All far-field light microscope images are limited in resolution by diffraction. A recent issue of Micron [34 (6-7):261-344, October 2003] contains papers that describe different approaches for achieving super-resolution in a variety of microscopical methods such as conventional, confocal and 4Pi imaging. These approaches are mostly based on the use of structured illumination by the incorporation of apertures or filters in the imaging pathway; this can lead to a 55% improvement in resolution.

The contributions include: Super-resolution in computational imaging; 3D Behaviour of Frieden filters in confocal imaging; Saturated patterned excitation microscopy with two-dimensional excitation patterns; Resolution enhancement by subtraction of confocal signals taken at different pinhole sizes; Improvement of optical resolution in far-field imaging by optical multiplication; Optical sectioning by two-pinhole confocal fluorescence microscopy; Sidelobe decline in single-photon 4Pi microscopy by Toraldo rings; Hyperresolving

phase-only filters with an optically addressable liquid crystal spatial light modulator; Emulated super-resolution using quantitative phase microscopy.

Environmental Scanning Electron Microscopy

Environmental scanning electron microscopes (ESEM) operate at low vacuum - around 10 torr - and the ions generated as a result of the collisions between secondary electrons emitted from the specimen and the gaseous material, e.g. water, in the column reduce the problem of charging of uncoated specimens. This makes ESEM a powerful technique for the study of specimens in close to their natural state: anything from ice-cream to aqueous polymers can be imaged.

A new review of ESEM has been published by A. M. Donald [The use of environmental scanning electron microscopy for imaging wet and insulating materials. Nature Materials 2(8):511- 516, August 2003]. Donald's detailed review covers the instrumentational parameters and electron interactions that provide the basis for ESEM and gives examples of its use for imaging wet and uncoated insulators.

Electron Backscatter Diffraction

Electron backscatter diffraction (EBSD) Kikuchi patterns are formed by the diffraction of electrons reflected from the surface layers of crystalline samples in the scanning electron microscope. Kikuchi diffraction is now a widely used and powerful technique to deduce crystallographic, strain and orientation information of materials in a large bulk samples in the SEM. A recent review by K. Baba-Kishi [Electron backscatter Kikuchi diffraction in the scanning electron microscope for crystallographic analysis. J Materials Science 37:1715-1746, 2002] provides excellent and comprehensive coverage of the theoretical and practical aspects of EBSD for the analysis of crystals.

The author discusses the background of the technique, the geometry of backscatter Kikuchi diffraction patterns (BKDPs), gnomonic projections, and pattern centres. Experimental aspects are reviewed including: microscope operation and BKDP detection; resolution; influence of microscope parameters; specimen preparation, charging and surface contamination. A discussion of pattern contrast includes: geometrical characteristics; crystalline contrast; and reciprocity between electron channelling patterns and BKDPs. Also reviewed are symmetry determination such as crystal point and space group determination. The author covers special applications: crystal polarity; fine symmetry determination; orientation microscopy; and strain measurements. The review is copiously illustrated with many high quality images and diagrams of crytalline materials such as zinc blende, silicon, germanium, gallium arsenide, chalcopyrite, tantalum telluride, and erbium germanate. There are 146 references.

Electrostatic AFM Nanolithography

A new technique for nanolithography of polymers for the development of data storage devices and sensor arrays is reported by Sergei Lyuksyutov et al. [Electrostatic nanolithography in polymers using atomic force microscopy. Nature Materials 2(7): 468-472, July 2003].

Atomic force microscopy-assisted electrostatic lithography (AFMEN) produces features in a planar polymer film by simply biasing at 0 to 20V a highly conductive tungsten carbide tip across the film resting on a grounded conductive layer. For a wide range of process conditions, raised features such as dots and lines (1 to 50 nm) have been observed. The AFM tip locally softens the polymer by Joule heating and uses extremely non-uniform electric field gradients to rapidly polarize and manipulate the soften polymer into a pattern of lines and dots. An increase of carrier density, potentially arising from localized dielectric breakdown within the films, creates a cylinder of polymer under the AFM tip susceptible to Joule heating arising from increased current flow. The strong electric field gradient polarizes the viscoelastic polymer, drawing it towards the AFM tip. The new technique generates these features without chemical crosslinking, substantial polymer degradation or ablation.

The authors demonstrate representative structures formed by AFMEN in polymers with different physicochemical properties. The nanoscale features were patterned using constant force as well as height AFM. Dots were formed by pausing for 0.2 to 5 seconds with constant bias and lines were created at tip velocities from 0.1 to 8.0 m m s^{-1}. The optimal polymer film for patterning will provide a gradual, not catastrophic, dielectric breakdown. The authors zeroth-order modelling indicates that feature size should depend critically on the thermal characteristics of the polymer. Importantly, the resolution of their method

does not directly depend on the radius of the AFM tip, which is distinctly different from other AFM lithographic techniques.

The authors conclude that this new nanolithographic technique will, through feedback controls for current voltage position and two-dimensional tip arrays, allow rapid access to both raised and recessed structures should enable ternary data storage logic based on relative deflections (positive, zero, negative) from the common plane of the polymer.

Fluorescent Speckle Microscopy

The dynamics of subcellular cytoskeletal structures and their constituent macromolecules can be followed by labelling them either by exogenously microinjected fluorescent proteins or by endogenously generated genetic markers such as green fluorescent protein. Problems with this approach include high background fluorescence, from unincorporated markers, and the difficulties in detected changes and motion in uniformly labelled structures. These can be overcome in part with confocal microscopy and photoactivation, photobleaching and ratiometric methods.

An alternative approach to the study of the dynamics, turnover and movement of the cytoskeleton in living cells is fluorescent speckle microscopy (FSM), reviewed by G. Danuser and C. M. Waterman-Storer. [Quantitative fluorescent speckle microscopy: where it came from and where it is going. J Microscopy 211(3): 191-207, September 2003]. The authors review the development of FSM since its discovery, by accident, in 1997. It was noticed that high resolution images of cells labelled with rhodamine-tubulin showed not uniform labelling of their microtubules but a discontinuous - hence speckled - labelling. The authors discuss the resolution of the technique demonstrating that optimal contrast of obtained where speckles represent the diffraction-limited image of a single fluorophore in the cellular structure. The authors provide a useful table of key literature in this field from 1997 to 2003 which demonstrates the wide application of FSM to cytoskeletal dynamics.

The authors foresee the application of FSM in many other life and materials science processes including endocytosis, interactions of DNA and RNA, and in the mechanisms of assembly of polymeric materials.

First Atomic-Resolution Imaging of Bacterial Protein

The first atomic-resolution model of a protein derived solely from cryoelectron microscopy and image analysis has been reported by K. Yonekura et al. [Complete atomic model of the bacterial flagellar filament by electron cryomicroscopy.. Nature 424 (6949):643-650, 7 August 2003].

Using images obtained at 4K on a 300 kV JEOL JEM, the authors were able to resolve a -helices, b -sheets and side chains in the R-subunit of the bacterial flagellar protein flagellin with 0.4 nm resolution. The model was obtained by analysis of over 40,000 images of single particles. Earlier similar studies have used other information such as that provided by electron diffraction, but these authors relied solely on the information present in the protein images. Key factors in the reconstruction were the helium-cooled, highly stable specimen stage and low dose conditions which gave high quality images with minimal radiation damage, the helical reconstruction process and the high degree of structural order of flagellin. The authors predict that further single-particle image analysis of cryoelectron images will allow more protein structures to be elucidated.

Environmental Cell for TEM

A new environmental cell for transmission electron microscopy has allowed the first high resolution EM study of crystal growth at a solid-liquid interface, report M. J. Williamson, et al.[Dynamic microscopy of nanoscale cluster growth at the solid-liquid interface. Nature Materials 2:532-536, July 2003]. The study was co-authored by Frances Ross, recipient of the Burton Medal at last month's Microscopy and Microanalysis meeting in San Antonio.

The authors fabricated a special chamber made from two silicon wafer windows which surrounded a 1 m m-deep space filled with electrolyte within which the electrodeposition of copper atoms from solution onto a gold surface could be studied on the TEM stage. The dynamics of copper cluster formation by electrodeposition was examined in a TEM at 300kV; the nucleation and growth of individual nanoscale Cu clusters on polycrystalline Au was followed at video rates at a resolution of 5 nm.

Fluorescence Correlation Spectroscopy of Membrane Proteins

The movement of fluorescent markers inside cells can be followed using fluorescence correlation spectroscopy, a technique that measures changes in fluorescence intensity within very small volumes. A new study by K. Saito et al. [In situ observation of mobility and anchoring of PKCbI in plasma membrane. FEBS Letters 541:126-131, 2003] has revealed that protein kinase C (PKC), a key enzyme involved in signal transduction, undergoes a change in its localization when activated by intracellular signalling molecules.

The authors labelled human cells with PKC tagged with green fluorescent protein. Observing the cells with a confocal fluorescence microscope, the time course of fluorescence intensity distribution in the cytoplasm was followed as the cells were treated with a PKC ligand. After 10 minutes the PKC relocated from the cytoplasm to the plasma membrane by a diffusion-driven transport mechanism.

Combined AFM/FLIM for Topography and Spectroscopy

A new instrument that gives high resolution topographic information simultaneously with spectroscopic data is described by Dehong Hu et al. [Correlated topographic and spectroscopic imaging beyond diffraction limit by atomic force microscopy metallic tip-enhanced near-field fluorescence lifetime microscopy. Review of Scientific Instruments 74 (7): 3347-3355, July 2003].

The authors combined an atomic force microscope (AFM) with a fluorescence lifetime imaging microscope (FLIM). By using a globule of gold on the silicon AFM tip they generated a high local electric field which interacted with fluorescent molecules in the region of the tip; these interactions were studied with the FLIM. So the spatial resolution of the fluorescent images was as good as that of the topographic images generated by the AFM. The authors used their technique to examine POPO-stained DNA and fluorescent nanobeads.

Rapid Freezing for Cryoelectron Microscopy

Rapid freezing of viruses, cells and biological macromolecules is essential to obtain useful samples for examination by cryoelectron microscopy. In order to maintain their native state, samples must be embedded in vitreous (non-crystalline) ice; if the cooling rate is not fast enough, i.e. heat is not transferred between sample and cryogen (coolant) at a sufficiently high rate, water crystals may form, damaging the physiological state of the sample. A standard rapid freezing technique is to plunge EM grids edge-on into liquid pentane at -180^{o}C. A new study using high-speed video imaging has revealed how this process of vitrification occurs [S. Kasas et al. Vitrification of cryoelectron microscopy specimens revealed by high-speed photographic imaging. J Microscopy 211(1): 48-53, July 2003].

The authors obtained stroboscopic millisecond images of grids entering liquid pentane and found that the grid initially displaces the cryogen. This means that the grid is not immediately in full contact with the pentane so heat transfer occurs by conduction through the grid bars: this can lead to slower cooling and the formation of damaging hexagonal ice. The authors recommend that grids be plunged into the cryogen parallel to the liquid surface which both reduces evaporation from the sample during its passage towards the cryogen and results in more uniform vitrification of the sample.

Electron Energy-loss Spectroscopy

A recent issue of Micron [34(3-5):119-260, April - July 2003] contains papers based on presentations at the 2002 Microscopy and Microanalysis conference including a festschrift for Elmar Zeitler and the sessions on electron energy-loss spectroscopy (EELS).
The topics in this issue include reviews of valence excitations in electron microscopy: resolved and unresolved issues, by Archie Howie, and new techniques in electron energy-loss spectroscopy and energy-filtered imaging, by Ray Egerton.

Other papers cover thermal diffuse scattering in sub-angstrom quantitative electron microscopy, electron channeling, structure factor phases, polarity and atom site determination in crystals, forbidden and weak reflections, detectability limits for elemental mapping by EF-TEM and STEM-XEDS, optimization of EDX performance, microstructures in materials induced by electropulsing, automated crystallography for TEM, EELS performance measurements, high resolution EELS using monochromator and high performance spectrometer, inelastic electron scattering observation using EFTEM, theoretical prediction of ELNES/XANES, and simulation methods for electronic structure calculations with experimental EELS spectra.

Atomic Resolution from Electron Diffraction Imaging

To achieve the highest, i.e. atomic, resolution in transmission electron microscopy, the challenges of instrument instability, lens aberrations, specimen drift and contrast transfer function must still be overcome. Now, J. M. Zuo et al. (Atomic resolution imaging of a carbon nanotube from diffraction intensities. Science 300(5624):1419-1421, May 2003) report that electron diffraction imaging solves many of these problems and produces atomic resolution in non-periodic structures.

They measured the intensities of the diffraction patterns (DP) of a carbon nanotube obtained using a 50-nm coherent electron beam by oversampling the DP in reciprocal space. But since intensities provide only wave amplitude, and the phase of wave function is required for highest resolution, they also devised a novel phase-retrieval iterative process. In this way they were able to clearly resolve the carbon atoms with a resolution of 0.1 nm, or 1 Angstrom. The authors propose that diffraction imaging will have a wide range of application in high resolution imaging of non-periodic inorganic materials and biological macromolecules.

Fluorescent Speckle Microscopy in Living Cells

Life-science microscopists commonly use fluorescently-tagged markers to follow the fate of molecules in living cells. Fluorescent speckle microscopy (FSM) utilises low amounts of fluorescent labels, such as proteins, which incorporate randomly into cellular structures giving a distribution of fluorescence with high spatial density variation, hence the structure appears 'speckled'.

A. Ponti et al. (Computational analysis of F-actin turnover in cortical actin meshworks using fluorescent speckle microscopy. *Biophysical Journal* 84:3336-3352, May 2003) describe a new method for processing and analysing the fluctuations of hundreds of thousands of speckles in an FSM time-lapse series of living cells. They modelled the behaviour of fluorescent speckles using synthetic data and applied their model to real confocal images of fluorescently labelled actin in epithelial cells. Their software was able to remove false speckles, compensated for focus changes and photobleaching, and gave new information on the polymerisation, depolymerisation and translocation of the actin cytoskeleton.

New Instruments for Scanning Probe Microscopy

New developments in scanning probe microscopy are reported in five papers in a recent issue of Review of Scientific Instruments 74(5):2631-2941, May 2003.

A combined Kerr-effect microscope and magnetic force microscope for variable temperature ultrahigh vacuum investigations of magnetic domains in ultrathin films is reported by D. Peterka et al. (pp. 2744-2748). Improvements to a multiphoton fluorescence lifetime imaging microscopy system using a streak camera with high spatial (0.2 µm) and temporal (50 ps) resolution, described by R. V. Krishnan et al. (pp. 2714-2721), has applications in intracellular physiology and fluorescence resonance energy transfer imaging of living cells.

M. Ferrara and O. Odoardi (pp. 2735-2743) describe a highly sensitive, inexpensive and compact design for a scanning capacitance microscope based on a coaxial resonator for the study of metal-oxide semiconductors. A detailed analysis of the properties of a non-contact atomic force microscope is presented by G. Couturier et al. (pp. 2726-2734). A superconducting microwave resonator for milliKelvin magnetic resonance force microscopy is reported by H. J. Mamin et al. (pp. 2749-2753).

Electron Energy-Loss Spectroscopy

A special issue of the Journal of Microscopy 210(1): 1-118, April 2003 contains papers on TEM electron energy-loss spectroscopy (EELS) and electron energy-loss near edge structure (ELNES) studies in the life and materials sciences.

Topics covered in this issue include: the detection of single atoms of calcium and iron in biological structures; new detector and specimen-preparation technologies; subcellular localization of boron in freeze-dried cryosections; electron spectroscopy imaging to study ELNES at a nanoscale; ELNES of InGaN quantum wells; a bandstructure approach to near edge structure; analytical electron microscopy and focused ion-beam studies of copper sorption; EELS study of natural carbonaceous materials; N-K ELNES study of anisotropy effects in hexagonal AlN; electron spectroscopy imaging to study ELNES at a nanoscale;

EELS of thin films for novel gate dielectrics; EELS of nanocomposites; a spatial difference technique for EELS studies of interfaces; EELS ionization edges in intermetallic compounds; and EELS applications in tribology.

Spectrally Resolved Fluorescence-Lifetime Imaging Microscopy

The mobility of single messenger RNA molecules has been demonstrated in living cells using fluorescence-lifetime imaging microscopy (FLIM), report J-P. Knemeyer et al. (Detection and identification of single molecules in living cells using spectrally resolved fluorescence-lifetime imaging microscopy. Analytical Chemistry 75(9): 2147 -2153, May 2003).

FLIM detects the time taken for a fluorescent object to fade and hence can discriminate between short lifetime (1.3 ns) autofluorescent structures and longer lived (2.8 ns) specifically introduced fluorescent markers. In a clever adaptation of the technique, they were able, by positioning the laser beam of their microscope at the tip of the microinjection pipette, to count the number of fluorescent mRNA molecules introduced into their cells, enabling them subsequently to show that they were imaging single molecules in their cells. The authors showed that fluorescent mRMA moves in and out of the nucleus, but that 10-30% is tethered to intranuclear structures.

High Resolution Electron Microscopy

A recent issue of J Electron Microscopy 52(1):1-90, 2003 describes advances in high resolution transmission electron microscopy for the materials sciences.

The spherical aberration (C_s) of a TEM can be improved from 0.5 mm to 15 m m by adding two hexapole correctors below the objective lens report F. Hosokawa et al. (A spherical aberration-corrected 200 kV TEM. JEM 52:3-10, 2003). And in a similar study with a Cs-corrected TEM, N. Tanaka et al. (First observation of SiO_2/Si(100) interfaces by spherical aberration-corrected high-resolution transmission electron microscopy. JEM 52:69-73, 2003) were able to conventionally image oxygen atomic columns without Fresnel fringes between Si-Si bonds at a resolution of 0.13 nm.

Other papers in this issue describe: a 1 MV field-emission transmission electron microscope; electron holographic 3-D nanoanalysis of catalysts; ARHVTEM of hetero-interface chemical structure; ultrahigh-vacuum electron microscopy of junction and thinning processes; in-situ HREM study of nm-sized alloy particles; segregation and precipitation behaviour of cascade clusters under electron irradiation; creep deformation of grain boundaries; and HREM observation of nucleation and growth of nanotwins in silicon.

Anniversaries of DNA and Raman

Since the last issue we have celebrated the anniversaries of two Nobel prize-winning landmarks in microscopy: the 50th anniversary of the discovery of the structure of DNA, which catalysed the growth of modern molecular biology and genetic engineering, and the 75th anniversary of the discovery of Raman scattering, the change in the frequency of light following interaction with molecular bonds, which allowed the development of chemical analysis by Raman spectroscopy.

X-ray Crystallography of DNA

The background to the discovery of the double helical structure of DNA, published in Nature on 25 April 1953 by Crick and Watson, is recalled by Hugh Huxley (Physics World, 16(3), 29-35, March 2003). Huxley describes the development of X-ray crystallography at the Cavendish Laboratory in the early 20th century that provided a favourable environment for this momentous discovery, and recounts the challenges faced in the interpretation of X-ray diffraction patterns of DNA, which were the only structural data available at that time. Nature 421(6921), 395-453, Jan 2003 has a number of articles celebrating this anniversary including copies of the original 1953 papers. The scanning tunnelling image of the DNA double helix on page 421 is dramatic testimony to how far microscopy has progressed in the last 50 years.

Raman Spectroscopy

Sir Chandrasekhara Raman of Calcutta University, India, received the Nobel prize for in Physics 1930 for his theory of light scattering, published in Nature on 31 March 1928, which formed the foundation of Raman spectroscopy. In his Nobel acceptance speech he presciently commented: "The universality of [Raman scattering]…enables the effect to be used as an experimental aid to the solution of a wide problems in physics and chemistry."

In a short review of Raman Spectroscopy, D. Bougeard et al. [The Raman effect 75 years after. J. Raman Spectroscopy 34(2), 97-99, Feb 2003] recall the milestones in the development of the technique since 1928 and describe innovations in the detectors, lasers, spectrometers, analysis, and applications of Raman spectroscopy, incuding surface enhanced Raman spectroscopy (SERS), coherent anti-Stokes Raman spectroscopy (CARS), time-resolved Raman spectroscopy, and microspectroscopy in art, forensics and geology.

The techniques used to extract quantitative information at the ppm level from spontaneous Raman spectra are reviewed by M. J. Pelletier [Quantitative analysis using Raman spectrometry. Applied Spectroscopy 57(1), 20A-39A, Jan 2003]. He covers the effects of noise, pre-processing, and multivariate analysis of Raman spectra, and presents over 200 references to applications of the quantification of Raman data from polymers, food, biologicals, hydrocarbons, pharmaceuticals and inorganics.

Fourier-Transform Infrared Spectroscopy

Recent papers describe new developments and applications of Fourier-transform infrared (FT-IR) spectroscopy. The problem of the temporal resolution of data acquired by FT-IR is addressed by R. Bhargava and I. Levin [Time-resolved Fourier-transform infrared spectroscopic imaging. Applied Spectroscopy 57(4), 357-366, April 2003]. Currently, data sets are collected on time scales of the order of minutes. But their new method allows the detection of dynamic processes with time scales of the order of milliseconds. They illustrate this new method by imaging electric-field changes in a polymer-liquid crystal composite.

Differences between normal and malignant cancer cells can be detected by FT-IR microspectroscopy, report A. Salmana et al. [FT-IR microspectroscopy of malignant fibroblasts transformed by mouse sarcoma virus. J Biochemical and Biophysical Methods 55(2), 141-153, Feb 2003]. Significant differences in phosphate and RNA/DNA levels were noted, allowing classification of the normal and cancer cells.

In a similar study, B. Budevska et al. [Application of mulitvariate curve resolution for analysis of FT-IR microspectroscopic images of in-situ plant tissues. Applied Spectroscopy 57(2), 124-131, Feb 2003] were able to quantify starch and zein-protein levels in 8 m m sections of mature corn kernels.

The potential for fully-automated industrial polymer waste sorters for waste recycling, based on near-infrared (NIR) spectral imaging, is described by A. Kulcke et al. [On-line classification of synthetic polymers using near infrared spectral imaging. J. Near Infrared Spectroscopy 11, 71–81, 2003].

Total Internal-Reflection Fluorescence

Total internal-reflection fluorescence (TIRF) occurs when light is reflected from below a glass-water interface above the critical angle (55^{o}): an evanescent wave enters the water exciting any fluorescent molecules to a depth of only nanometres making TIRF particularly suited to the analysis of single fluorescent molecules in a bulk solution or fluorescently tagged cellular markers in the ventral regions of adherent cells.

D.-M. Yang et al. [Tracking of secretory vesicles of PC12 cells by total internal reflection fluorescence microscopy. J Microscopy 209(3), 223-227, March 2003] used TIRF to study the motion of secretory vesicles near the cell surface of living neuroendocrine cells. Good images of vesicle interactions with the plasma membrane were obtained without interference from fluorescence in other parts of the cells.

At the critical angle, the evanescent wave falls off exponentially over the first few hundred nm, but by varying the angle of incidence fluorescence can be detected deeper into the cell. Recent work by T. Nakata and colleagues is reviewed by R. Gaughan. [Critical-angle microscopy combines sensitivity and imaging depth. Biophotonics International, 18-19, March 2003]. Nakata showed that TIRF is superior to confocal microscopy for the detection of labelled proteins inside cells.

For single molecule imaging with TIRF one requires isotropic excitation in 3 dimensions; but typical TIRF set-ups are less efficient at detecting fluorescent dipoles misaligned to the plane of polarisation of the evanescent

wave. By using a special prism that that projects two orthogonal beams of similar intensity, S. Wakelin and C. Bagshaw [A prism combination for near isotropic fluorescence excitation by total internal reflection. J Microscopy 209(2), 143-148, Feb 2003] were able to study fluorescent myosin constructs in all orientations. Their new prism design will facilitate studies of the rotational motions of macromolecules and reduce flickering of fluorescence from molecular rotation..

History of Electron Microscopy

A very valuable history of the development of electron microscopy has been published by F. Haguenau, P. Hawkes, J. Hutchison, B. Satiat–Jeunemaotre, G. Simon, and D. Williams. [Key events in the history of electron microscopy. Microscopy and Microanalysis 9(2) 96-138, April 2003]. This article describes the development of electron microscopical instrumentation from the discovery of the electron by J. J. Thomson in 1897 to the commissioning of the first superSTEM in 2000. Key developments in both the materials and life sciences are also covered and the article lists many of the important papers published in the intervening years.

Low-Energy Electron Microscopy

Low-energy electron microscopy (LEEM) is a powerful technique for the study of surface structure and dynamics. The instrument has a conventional electron gun but the beam is then decelerated to only 10 eV and electrons reflected off the surface are collected to form an image or diffraction pattern with a resolution of about 5 nm. By replacing the electron beam with a light or X-ray source, the microscope can also be used for photoelectron emission microscopy which gives topographical, elemental, chemical, magnetic and orientation contrast but at lower resolution.

Papers from a workshop on low-energy electron microscopy and photo-emission electron microscopy held in Anaheim, CA, in May 2002 were published in J. Vacuum Sci. & Tech. B, 20(6), 2472-2549, Nov/Dec 2002. These 13 papers cover the instrument developments and applications of LEEM in surface science, including the nucleation, transitions and growth of thin epitaxial layers of In, Ag and Ge on silicon substrates, the growth dynamics of titanium silicide nanowires, scanning photoelectron microscopy studies of boron-doped diamond films, PEEM of ultrathin oxide covered devices, and magnetic thin-film applications.

Optical Coherence Tomography of Plants

Optical coherence microscopy uses long-wavelength coherent laser light to non-destructively image the superficial layers of biological tissues. Light that is scattered back from the sample is compared with a reference beam by interferometry to generate an intensity map of the refractive index of tissue components. OCM uses much less energy than confocal microscopy - so is less harmful - and can be used to examine tissue in situ.

OCM images of *Arabidopsis* plant leaves, trichomes and cotyledons have been obtained by A. Reeves et al. [In vivo three-dimensional imaging of plants with optical coherence microscopy. J. Microscopy, 208(3), 177-189, Dec 2002]. Their system comprised a 300 m W LED, producing 850-nm infrared light which was focused into a volume element of 5 x 10 m m which was then scanned across the specimen in a 3D array. Images of plant structures were built up by combining data from up to 10^7 volume elements using new computer methods for data processing and display. These images were obtained in about one minute while the plants were growing and, importantly, the laser had no deleterious effect on viability. Plant cell walls gave no significant OCM signal, however the authors were able to obtain images of shoot apex, leaf primordia, cotyledons, xylem channels, petioles and trichomes at a resolution of less than 1 m m.

Reviews of New Optical Techniques

A really useful overview of the most significant developments in optical sciences in 2002 is given in Optics and Photonics News, 13(12), December 2002. This overview takes the form of 48 one-page summaries of new optical devices, materials, sources, detectors and techniques as well as progress in phase optics, phase retrieval, photonic structures, quantum optics, soft X-rays, and terahertz tomography.

Of particular interest is the review on interferometric methods for analysing surface acoustic waves in solids which has applications in non-destructive testing and thin-film thickness monitoring. Another describes the

imaging of diffusion volumes in silicon using two photon optical-beam induced current to study junctions in semiconductor chips.

New techniques to probe the dynamics and structure of materials include ultrafast (femtosecond) scanning tunnelling microscopy and harmonic generation by laser beams. In the life sciences, phase resolved optical coherence tomography can now simultaneously image both tissue structure and the dynamics of blood flow in the superficial tissues of patients.

Fourier-Transform Infrared Microscopy

Two recent papers on Fourier-transform infrared (FT-IR) spectroscopy and microscopy provide a useful review of developments in this broadly applicable form of chemical analysis.

In the geological sciences, FTIR has been used to identify the various types of clay minerals [J. Madejovб. FTIR techniques in clay mineral studies. Vibrational Spectroscopy, 31(1), 1-10, Jan 2003]. The chemical composition of kaolinite, chrysotile, montmorillonite and other minerals was resolved and changes in smectites during acid modification and hydration were characterised, using both transmission and reflectance IR modes.

The conventional incident method of polarized IR illumination cannot distinguish molecular species with variable orientation in the sample. But now H. Kubota et al. [Development of a micro-FT-IR system for three-dimensional structural studies. Vibrational Spectroscopy 31(1), 11-17, Jan 2003] have designed a method for oblique transmission IR imaging that can give 3-dimensional data from a sample. The new method uses a condenser with an ellipsoidal mirror for oblique illumination of the sample and a Cassegrain mirror objective to collect the transmitted light. Their method was tested on a single crystal of stearic acid.

Fluorescence Correlation Spectroscopy

Nanomolar changes in the concentration of fluorescent molecules in living cells due to their diffusional mobility can be efficiently detected using fluorescence correlation spectroscopy (FCS) with single and multiphoton microscopy. Three recent papers in the Biophysical Journal 84 (3), March 2003, describe developments in this field.

P. Sengupta et al. describe a new algorithm for the analysis of FCS data in complex systems and have tested it on green fluorescent protein in dilute aqueous solution. The method can be used for large numbers of diffusing molecules.

Variations or errors in the fluorescence correlation spectra arising a result of the compartments in cells have been studied by C. Fradin et al. Their analysis is applicable to changes due to obstructed diffusion of molecules in the cytoplasm and to changes brought about by a dynamic cell membrane such as a vesicle.

Assistance with the planning and evaluation of FCS experiments is reported by S. Saffarian and E. Elson. Their statistics can be used over a large range of time scales.

Imaging Atomic Motion in Semiconductors

Semiconductors are enhanced by dopant atoms, but these can cluster and reduce performance. A dramatic demonstration of the power of high resolution STEM to study the migration and clustering of individual atoms is given by U. Kaiser [Direct observation of defect-mediated cluster nucleation. Nature Materials 1(2), 102-105, Oct 2002].

Using high-angle annular dark-field imaging with a 0.2 nm probe in a 200 keV FEG-STEM, Kaiser followed the motion of erbium and germanium atoms in an 8-20 nm-thick silicon carbide substrate. Columns of single atoms were imaged by atomic (Z) contrast. Erbium was found to cluster in lines, planes and 3D precipitates, whereas germanium formed compact 3D structures. The identity of the atoms was confirmed by correlated 0.3 nm-resolution electron energy-loss spectroscopy.

FFT Measurement of Resolution in the SEM

Calculating the resolution of an electron microscope by visual inspection of images is problematical. An alternative is to analyse images in the frequency domain by fast Fourier transformation (FFT). The

relationship between an object and its FFT is analogous to that of an object and its diffraction pattern in the back focal plane of a microscope - high spatial frequencies, i.e. the finest details, are located at the margins of the FFT power spectrum and give an indication of the image resolution.

A simple computer method to determine image resolution by FFT is described by D. Joy [SMART - a program to measure SEM resolution and imaging performance. J. Microscopy 208(1), 24-34, Oct 2002]. The SMART - scanning microscope assessment and resolution testing - method uses a fast Fourier transform of any selected high magnification area to generate a power spectrum of the sample; after calibration and several processing steps, the diameter of the power spectrum gives a direct value for image resolution. SMART also reports astigmatism, signal-to-noise ratios and any instrument instability. The author demonstrates the method's applicability and pros and cons for images from SEM, VP-SEM and STEM instruments. SMART is available as a macro for the free NIH Image and SCION Image programs.

Laser Tweezers for Biomanipulation

The technique of optical trapping, or laser tweezers, is described in a detailed and well illustrated and referenced review by C. Kuyper and D. Chiu [Optical trapping: A versatile technique for biomanipulation. Applied Spectroscopy 56(11), 300a, Nov 2002].

When a laser is focused by a microscope objective it creates a 3D gradient of force that can overcome Brownian motion forces and trap small particles at the centre of the beam. This allows the particle to be moved at will and isolated or experimentally manipulated. This review covers the theory, optical set-up and many applications of the technique. Optical tweezers can be used to study biological motors, to manipulate cells, particles and DNA, for microsurgery, for measuring forces, and for sorting of subcellular organelles prior to biochemical analysis.

Fluorochromes for use in Two-Photon Microscopy

As the applications of two-photon fluorescence microscopy (TPM) increase, so does the need for standardisation of staining methods with fully characterised fluorescent dyes. A wide range of popular fluorophores has been examined for their absorption and emission properties by F. Bestvater et al. [Two-photon fluorescence absorption and emission spectra of dyes relevant for cell imaging. J. Microscopy 208(2), 108-115, Nov 2002].

When a fluorophore absorbs a photon, a change in energy state results in the release of a longer wavelength photon. In TPM, two lower-energy photons do the job of the single excitational photon: this not only allows the use of less harmful infrared laser excitation but also gives the advantage that the excitation and emission wavelengths are widely separated.

The authors determined the one- and two-photon absorption and emission maxima for 28 fluors including dyes widely used for staining DNA, histones, tubulin and mitochondria, such as Alexa, Bodipy, Cy, DAPI, Hoechst, propidium iodide, FITC and rhodamine.

Most dyes behaved as predicted from two-photon excitation theory, but there was a general trend towards blue shift for absorption maxima and a red shift for emission maxima. The report included a nice demonstration of TPE of multiply labelled cells.

Magnetic Probe Microscopy of Electronic Materials

Two recent papers describe the use of probe microscopy to study magnetic fields in electronic materials at very low temperatures. C. Veauvy et al. [Scanning micro-superconduction quantum interference device force microscope. Review of Scientific Instruments, 73(11), 3825-3830, Nov 2002] present a scanning probe technique to study the mechanisms of superconductivity. Their microscope operates at 0.4K with a resolution of 200 nm. Images of magnetic vortices in semiconductors were obtained. The force microscope uses a piezoelectric quartz tuning fork as the detector and magnetic imaging is obtained by scanning μ-SQUID microscopy.

J. Gregory et al. [A scanning Hall probe microscope for large area magnetic imaging down to cryogenic temperatures. Review of Scientific Instruments 73(10), 3515-3519, Oct 2002] describe the design, construction and operation of a large-area scanning Hall probe microscope. The system has an effective lateral spatial resolution of 1.25 μm and a minimum detectable field of 0.08 μT/Hz and operates down to 35K.

The instrument was used to image local magnetic induction at the surface of various magnetic recording media such as floppy disks at room temperature as well as an array of superconducting materials at 40 K.

X-Ray Photoelectron Spectroscopy for Surface Analysis

A recent issue of Surface and Interface Analysis [33(10-11), 773-904, Oct-Nov 2002] is a festschrift celebrating Prof. Jim Castle and the surface analysis group at the University of Surrey,UK, which is a world-leading centre for the application of X-ray photoelectron spectroscopy (XPS), Auger electron spectroscopy (AES), time-of-flight secondary ion mass spectrometry (ToF-SIMS), and scanning probe microscopy (SPM) in materials science.

The papers include reviews on: Free-electron metal alloys: a study by high-energy XPS; Use and limitations of electron flood gun control of surface potential during XPS; Quantitative XPS analysis of aluminium in the presence of copper; Spatially resolved microchemical analysis of chromate-conversion-coated aluminium alloy; Investigation of the initial reactions of alloys and constituent metals with oxygen using SIMS; Passivity and its breakdown on stainless steels and alloys; Surface chemistry of cement pastes: a study by XPS; Pigment effects on ink-polymer bonding; Characterization of the interface in rubber/silica composite materials; Degradation during ARXPS measurements of polystyrene treated with an oxygen plasma; Angle-resolved XPS characterization of urea formaldehyde-epoxy systems; Nanoscale surface characterization of conducting and non-conducting materials with STM and contact SFM; Using in-situ AFM to investigate corrosion and passivation of duplex stainless steels.