Biology

Probing metal complex dissociation in cells by fluorescence microscopy

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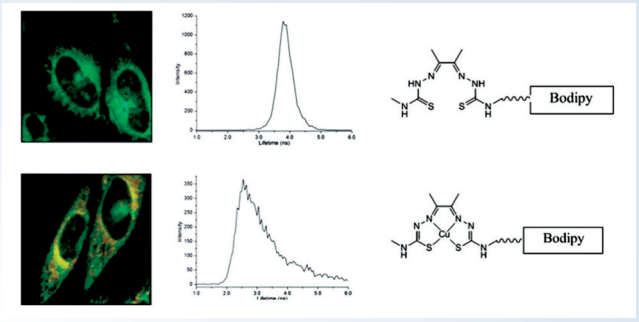
Metal complexes of thiosemicarbazone species are known to be important biological agents, and have received considerable interest in terms of radio-imaging and therapy; however, to date the precise mechanism and cellular localisation of these drugs is uncertain. Whilst PET (Positron Emission Tomography) and SPECT (Single Photon Emission Computed Tomography) imaging can provide invaluable in vivo diagnostic information at the mm level, often little is known about the fate of the metallic radionuclides of these complexes once they enter cells. Fluorescence imaging has resolution which is currently in the order of 0.1-10 μm and to this end we have attached fluorophores to the thiosemicarbazone structures to aid elucidation of the mechanism of uptake and localisation of these metal labelled conjugates. One and two photon excitation lifetime measurements and images were determined on a series of thiosemicarbazone derivatives bearing potent inherent fluorophores. Findings indicate that careful choice of fluorophore is necessary to avoid influencing the behaviour of the complexes dramatically.



Emission lifetime maps for free bodipy/ATSM ligand(1) (top) and copper complex(3) (bottom).

Biocompatible microstructured materials for tissue engineering

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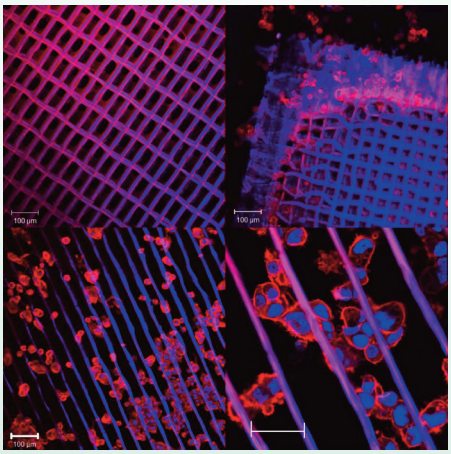
In this project microstructuring of biocompatible materials was investigated via two-photon polymerisation (2PP), a laser-based direct write technique. In 2PP a femtosecond laser is used to initiate the polymerisation via two-photon processes (2 near-IR photons are absorbed by an UV-sensitive photoinitiator). This results in a highly localised polymerisation at the focal point, and allows for 3D direct writing with excellent feature resolution (sub-micrometer). This process has been investigated for the production of biocompatible and biodegradable scaffolds for tissue engineering, using photocurable polylactide-based materials. In this project millimetre-sized patterns were written with micrometer resolution. The scaffold material showed good biocompatibility and distinct differences in neural cell growth were observed on these scaffolds depending on the scaffold geometry.



NG108-15 Cells on Microstructured Surfaces after 48h in culture. Cells have attached successfully to parallel lines of Microstructured PLA (Polylactic Acid) however they did not attach to square or rectangular structures indicating a degree of surface selectivity. Scale bars top and left, 100 μm, bottom right 10 μm.

The plant secretoryome: protein-protein interactions in the higher plant secretory pathway

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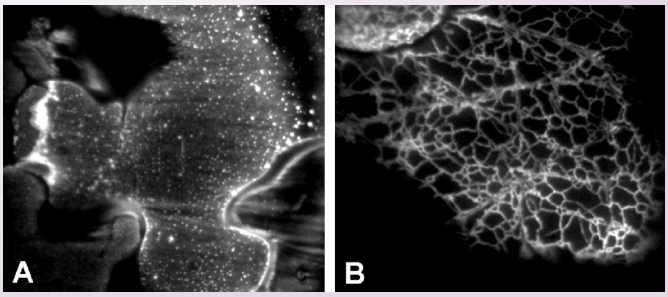
We have used FRET/FLIM to investigate protein interactions in the endomembrane system of plant leaf epidermal cells, TIRF microscopy to study the endomembrane system adjacent to the plasma membrane and are developing an optical tweezer set up on a TIRF microscope.

A family of endoplasmic reticulum proteins are involved in inducing curvature of the membranes of the plant endoplasmic reticulum (ER). As part of a project on the function of these proteins FRET/FLIM using fluorescent protein constructs in living tobacco leaf cells was used to show that in the ER membrane the reticulon proteins can interact with each other forming hetero-and/or homo-oligomers [Sparkes et al. Plant Cell (2010) 22, 1333].

FRET/FLIM has also been used to study putative interactions between Golgi matrix proteins and small regulatory GTPases. Whilst TIRF imaging has successfully imaged ER, Golgi and the distribution of plasma membrane proteins at high resolution in tobacco and arabidopsis leaf epidermal cells.



A. TIRF image of the plasma membrane of a tobacco leaf epidermal cell expressing GFP-LTI6B showing punctae and a filament associated with the PM (image courtesy of A. Martiniere).
B. TIRF image showing cortical endoplasmic reticulum in a similar cell labelled with GFP-HDEL.

Supra-molecular rules in signalling networks: A single molecule comparative study in cells and tissues

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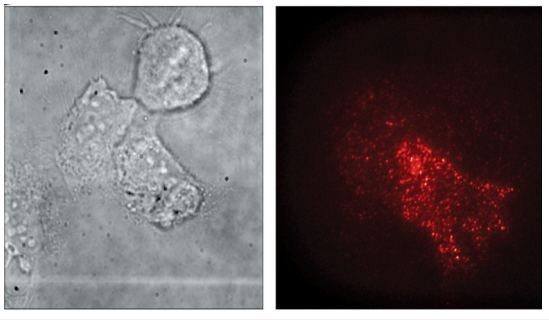
We have developed single molecule and other fluorescence techniques on the Octopus facility, combined with simulation, modelling and analysis techniques as part of a programme in the Lasers for Science Facility (LSF). Our first aim is to define patterns of conformation association, activation and signal transduction for the 4 members of the ErbB family and their correlation with plasma membrane proximal receptor traffic and signalling using cultured cells. We also aim at deriving models of ligand-induced behaviour using single molecule and ensemble FLIM and systems predictions and to establish how this is influenced by feed-back loops and perturbations. By testing these models on primary epithelial cells and tissues we will adapt the models iteratively to create a framework of response prediction for the network. Our ultimate goal is to derive probes and/or algorithms based on the models, suitable use with fixed or fresh human tissues to determine model robustness and network predictability in this real world setting.



Wide field transmission (left) and single molecule fluorescence (right) images of HeLa cells. The EGFR molecules at the cell surface were labelled with EGF tagged with the red emitting fluorophore Atto647. The spots on the figure on the right show the location of single EGF/EGFR complexes in different oligomerisation stages. The number of fluorophores is determined by the number of photobleaching steps in each spot.

The role of BER proteins in the repair of DNA damage induced following NIR multiphoton laser microbeam irradiation

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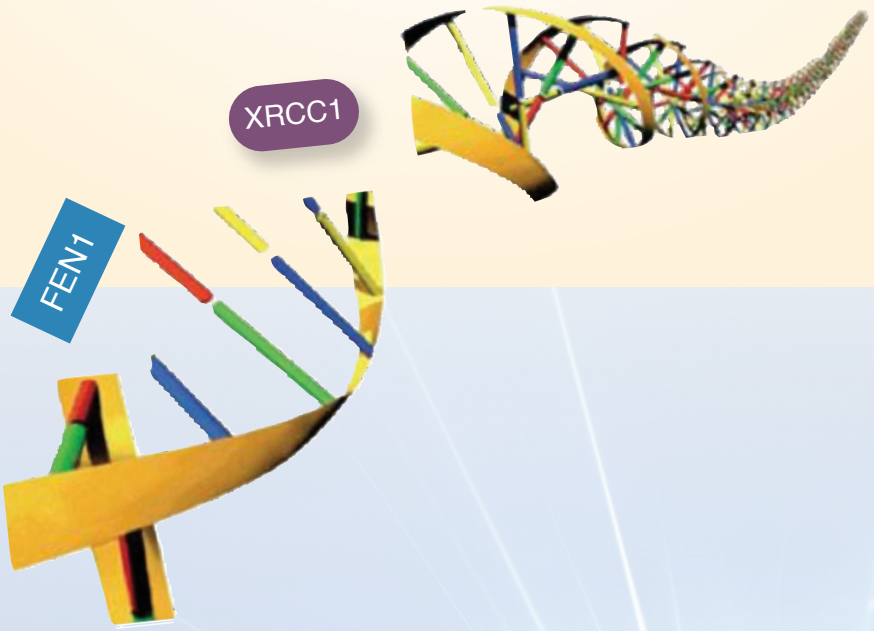
DNA damage induced by radiation has been shown to increase genome instability and mutations, which could in turn lead to the formation of cancerous cells. Ionising radiation may be considered as a two-edged sword; it may induce cancer or other adverse responses in normal tissue or alternatively it may lead to killing of tumour cells, of considerable importance in radiotherapy and cancer treatment. The repair of ionising radiation induced DNA damage has therefore been extensively studied in mammalian systems as it is essential to maintain genome stability.

The use of NIR laser microbeam has demonstrated its feasibility to study the induction of lesions in genomic DNA in living cells. Key proteins involved in a repair process required to maintain genome stability, base excision repair, are recruited and lost from sites of induced DNA damage at different speeds possibly reflecting the repair of different types of DNA damage. The identification of these types of damage which are difficult to repair may advance our understanding of cancer induction.



Chemistry

Time resolved infrared analysis of AppABLUF using isotopic labeling at mutant studies

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AppA is the first and best characterized BLUF domain photoreceptor and is found in Rhodobacter sphaeroides where it acts as an antirepressor of photosystem biosynthesis. Our studies have focused on the N-terminal domain of AppA responsible for sensing light. Previous studies determined the formation of a signaling state which is characterized by a 10 nm red shift in λmax of the flavin chromophore. The goal of the current study is to identify differences in the chemical structures of the dark and signaling state of AppA. Both AppA's dark and light excited state dynamics have been studied using time resolved infrared spectroscopy (TRIR). We have also used isotope labeling of both the flavin chromophore and of the protein in order to identify major differences in the TRIR spectra of light AppA and dark AppA. Additionally, we have studied structurally conservative AppA mutants which play a crucial role in photoactivity.

High-resolution stimulated Raman spectroscopy with photoacoustic detection (PARS) of formic acid dimer

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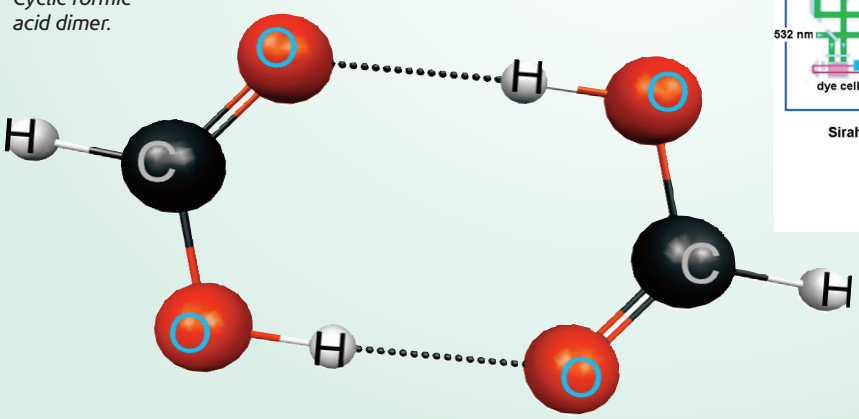
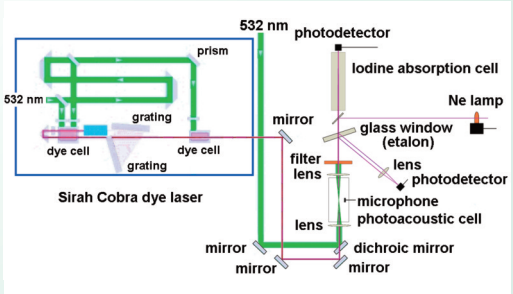
A stimulated laser Raman experiment with optophone photoacoustic detection has been set up which allows high-resolution Raman spectroscopy of gas-phase species in hostile environments, PARS. With this technique, we have observed the CH-stretching vibration of formic acid dimer which shows a distinct shift towards higher wavenumber ('blue shift'). Such shifts have in the past been attributed as a typical sign of intermolecular bonding. In the cyclic dimer, however, CH is not involved in hydrogen bonding. We propose that this is a secondary effect where increase of electron density to the C=O bond by hydrogen bonding with OH has an electron withdrawing effect. This increases the polarity and strength of the C-H bond, thus inducing a shift towards higher wavenumber.

In future work, PARS can also be used to observe the OH-stretching vibration of cyclic formic acid dimer to study the concerted double-proton transfer in the two equivalent OH...O hydrogen bonds.



Cyclic formic acid dimer.



Scheme of the PARS setup with wavelength calibration.

Transient 2D-IR spectroscopy of [FeFe]hydrogenase enzyme model compounds

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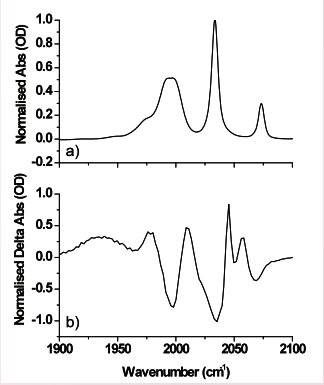
Ultrafast multidimensional infrared spectroscopy is a useful tool for probing complex reacting systems due to the ability to spread the molecular response over two frequency dimensions and thus give access to previously inaccessible information. Furthermore, the inherent ultrafast time resolution of these techniques provides the ability to observe reactions in real time. Here, transient 2D-IR (T-2D-IR) spectroscopy has been employed to determine the infrared absorption frequencies and coupling patterns of the photoproduct vibrational modes of the species formed following photolysis of $(\mu S(CH_2)_3S)Fe_2(CO)_6$, a model compound of the [FeFe]hydrogenase enzyme active site, in cyanoheptane solution. Double difference T-2D-IR analysis has revealed a number of peaks due to a single photoproduct and they show spectroscopy consistent with the formation of a pentacarbonyl solvent adduct species.



a) FTIR spectrum of ~1mM solution of 1 in cyanoheptane solution. b) TRIR spectrum of the same solution following phtoloysis at 350 nm. The data shown corresponds to a UV_{pump} -IR_{probe} delay time of 200 ps.

Ultrafast TRIR studies on tetraazidosilicon complexes

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Photoinitiated CO-release molecules

43

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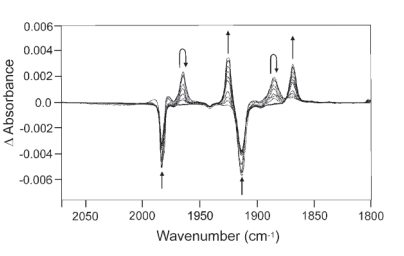
Carbon monoxide (CO) is a toxic odourless gas. Surprisingly however it is generated in living organisms during the degradation of heme by the enzyme heme oxygenase. In low concentrations carbon monoxide gas acts to dilate blood vessels in a manner similar to nitric oxide and it also has some anti-inflammatory and anti-apoptotic properties. We are currently investigating metal carbonyl complexes as possible CO Releasing Molecules (CORM) for use as therapeutic agents. Fundamental to the design of new CORM is an understanding of the photopysical processes leading to CO-loss. Two metal carbonyl systems have been chosen, $(\eta^6$-arene$)Cr(CO)_3$ and $(\mu$-alkyne$)Co_2(CO)_6$. Photoinduced CO loss from $(\eta^6$-arene$)Cr(CO)_3$ occurs from a vibrationally cold excited state while photolysis of $(\mu$-2 pyreneacetylene$)Co_2(CO)_6$ results in CO loss producing $(\mu$-2 pyreneacetylene$)Co_2(CO)_5(S)$ (S = n-heptane). These results will assist in the design of new molecular systems with specific spectroscopic and photochemical properties ideal for use as CORM agents.



The TRIR spectra obtained following 400 nm excitation of $(\eta^6$-Benzene$)Cr(CO)_3$ in n-heptane, spectra were recorded at 1, 7, 12, 21, 51, 70, 106, 160, 350, 500, 750, 1000 ps after the excitation pulse.

Excited-state dynamics of adenine thymine dinucleotides: influence of stacking

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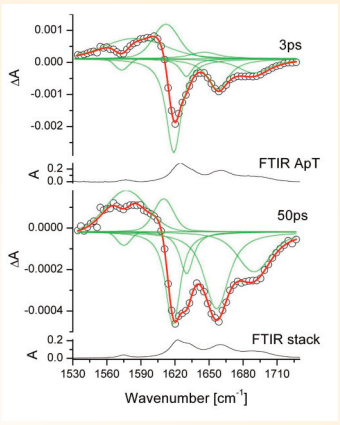
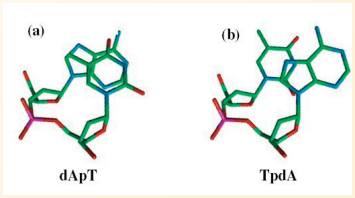
Individual bases of DNA typically decay on the ultrafast timescale however in a sequence longer-lived species are observed which are susceptible to chemical change. In DNA bases interact through stacking and hydrogen bonding and understanding their influence on the excited states is important. We studied two simple sequences, dApT and TpdA, which are both stacked and unstacked in solution (38% and 20% stacked respectively). Using picosecond time-resolved infrared (ps-TRIR) spectroscopy we observed two transient species, a short-lived species (5 ps), of the unstacked form and a longer-lived species from the stacked form. The yield and the lifetime of the longer-lived species differed significantly in dApT (75 ps) and TpdA (50 ps). Importantly, its distinctive spectroscopic signature (1500-1600 cm^{-1}) is identical to that observed for the long-lived state in poly(dA-dT). The study demonstrates the influence of stacking and confirms that the excitation in the polymer is localized on the dinucleotide unit.



ps-TRIR spectra of 10 mM dApT following UV excitation (300 fs, 267 nm) upper Voigt fitted data at 3 ps and ground state FTIR spectrum for dApT lower Voigt fitted data at 50 ps and ground state FTIR spectrum of stacked component of dApT



Overlap in the stacked form of (a) dApT and (b) 5´-TpdA-3´. Molecules were drawn using the ChemSW® package.

Antigens laid bare: the intrinsic conformation of Lewis^x

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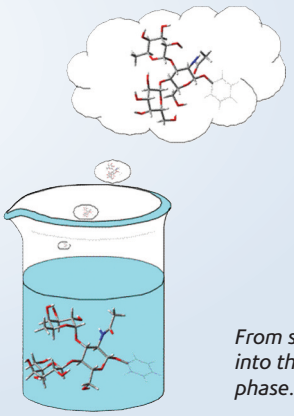
The trisaccharide, Lewis^x is expressed by many cancers of epithelial origin and is an important immunotherapeutic target. Knowing its structural preference(s), particularly in its bioactive conformation, is a key requirement in designing drugs which might inhibit its binding and activity. In aqueous or protein bound environments Lewis^x adopts relatively rigid, compact structures with its terminal units, galactose and fucose, stacked in parallel. But is this its intrinsic conformation or one controlled by the environment? A combination of laser desorption and tunable infrared laser techniques, which can interrogate individual molecular conformers isolated in the rarified environment of a cold, molecular beam, and quantum chemical computation has provided the answer.

The intrinsic structure is not stacked but hydrogen bonded and the results of these experiments suggest that the environment, particularly water, plays a key structural role in controlling the conformation of Lewis^x and in consequence, its biological function.



From solution into the gas phase.

Carbohydrate molecular recognition: probing CH-π interactions

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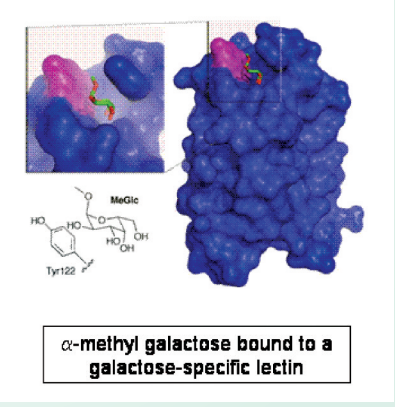
Interactions between carbohydrates and proteins are intimately involved in the biological machinery of a vast number of biological processes: mammalian infection, inflammation, fertilization and cellular recognition for example. Molecular recognition by specific carbohydrate binding modules is controlled by the disposition, conformation and configuration of the locally interacting sites. Fine controls are often provided by hydrophobic interactions between the apolar regions of the carbohydrate and aromatic residues, thought to be promoted (in aqueous environments) by the exclusion of surrounding water molecules from the apolar interface though binding to aromatic residues could also involve dispersive, so-called "CH-π" interactions. These have now been identified in model carbohydrate-arene complexes, isolated in the gas phase free from from neighbouring solvent molecules and stabilized at low temperatures in a molecular beam. The molecular complexes, probed through infrared laser spectroscopy. adopt stacked structures supported solely by dispersive "CH-π" interactions between the pyranose and aromatic rings.



Stacking in a carbohydrate-protein complex.

Polymer coil-globule transition dynamics on the nanosecond to second time scale

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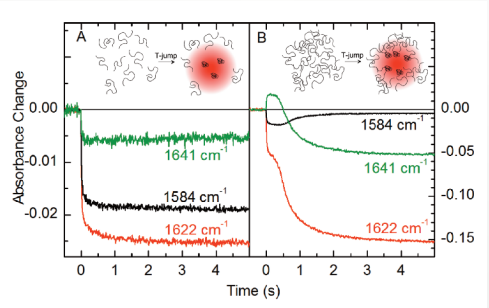
When heated over the lower critical solution temperature (LCST), poly(N-isopropylacrylamide) undergoes a change in conformation from a random coil to a collapsed structure which then leads to phase separation. This effect is investigated for a wide range of applications, including drug or DNA delivery and control of enzymatic activity. Although the transition has been studied extensively, its dynamics has received only little attention so far. We have used laser-induced temperature jumps and IR spectroscopy to follow the polymer collapse on time scales ranging from a few nanoseconds to several seconds. An increase of the temperature over the LCST leads to a local collapse of polymer sections within nanoseconds due to cooperative hydration, followed by much slower phase separation. Partial phase separation on the 100 ms time scale could be observed, as shown in the Figure, but only at concentrations resulting in significant overlap of the polymer chains.



IR absorbance changes after a temperature jump over the LCST at polymer concentrations of 10 mg/ml (A) and 45 mg/ml (B). Whereas the signal in (A) is dominated by heating of the solvent (water), (B) shows a decrease of the polymer absorbance, indicating that polymer is removed from the heated sample volume (phase separation) when polymer chains overlap.

Physics

Using tip enhanced femtosecond lasers to create graphite nanostructures on diamond

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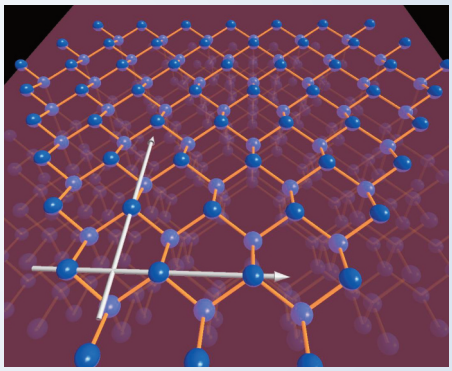
Graphene is the new wonder material; it is a zero band gap semiconductor and has electron mobilities two orders of magnitude greater than in silicon. Our interest is in thin ribbons of graphene with well defined edge states predicted to be highly magneto-resistive and potentially even half-metallic, the holy grail of spintronics research.

From our previous work we knew that a femtosecond laser will convert the (111) surface of diamond into thin layers of graphite, the interaction occurring so quickly that the bonds reform before there is any significant heating. In this work we used the laser in conjunction with an atomic force microscope to enable us to produce structures smaller than the wavelength of light.

Although we made sub-diffraction limit structures on silicon with relative ease, in these initial experiments we were unable to make structures on the non-conducting surface of the diamond.



The (111) surface of diamond showing the puckered graphite like arrangement of the atoms. The two arrows show the armchair edge direction $(2\bar{1}\bar{1})$ *or the zig-zag edge* $(0\bar{1}1)$.

Nonlinear spectroscopy of doped glass and crystal for applications in distributed fibre sensing

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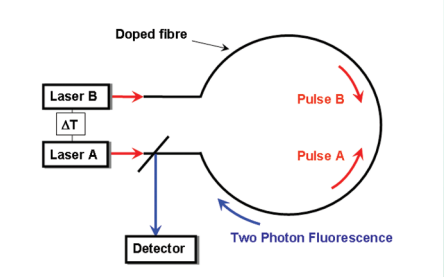
Distributed fibre sensing based on the temperature or strain dependence of the fluorescence decay time or spectrum can only be achieved using two-photon excitation. An enhanced fluorescence signal will be produced where two counter-propagating excitation pulses overlap in a doped fibre if the sum of their photon energies excites the upper level of the ions. The Laser Loan Pool NS3L optical parametric oscillator was used to investigate the nonlinear spectroscopy of glass and crystal doped with various lanthanide and transition metal ions. The two-photon absorption cross-section was obtained by comparing the two-photon excited fluorescence (TPF) yield with that for single-photon excitation for the same transition under identical conditions. Related experiments on TPF in doped glass and fibre are outlined in the accompanying synopsis, "Distributed sensing by time-correlated two-photon excited fluorescence in rare earth doped optical fibres" by I S Ruddock et al..



Schematic diagram of a distributed sensor based on time-correlated TPF in a doped optical fibre. Counter-propagating pulses (A) and (B) meet at a location depending on their relative time delay, ΔT. The TPF generated at their overlap is guided along the fibre and detected.

Distributed sensing by time-correlated two-photon excited fluorescence in rare earth doped optical fibres

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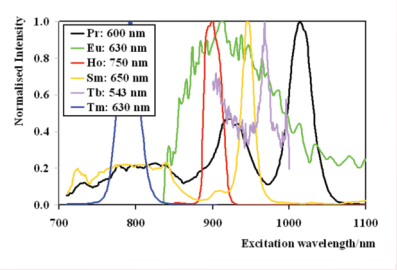
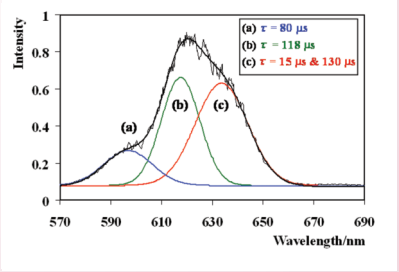
Potential dopants for distributed fibre sensing based on two-photon excited fluorescence (TPF) were identified by single- and two-photon excitation spectroscopy of bulk samples of rare earth doped glass. Using the tuneable near-infrared idler and the visible signal outputs of the Laser Loan Pool's NSL3 optical parametric oscillator, two-photon and two-step excited fluorescence was observed in praseodymium, samarium, europium, gadolinium, terbium, dysprosium, holmium and thulium. From this list, praseodymium was selected for detailed investigation in single-mode fibre geometry on account of its convenient excitation and TPF wavelengths and its potentially negligible fluorescence self-absorption due to the transitions of interest near 600 nm terminating above the ground state. An outline of fluorescence based distributed fibre sensing and related experiments on determining the two-photon absorption cross-section are given in the accompanying synopsis – "Nonlinear spectroscopy of doped glass and crystal for applications in distributed fibre sensing" by I. S. Ruddock et al..



Two-photon and two-step excited excitation spectra for a range of rare earth doped glasses of different composition. The corresponding emission wavelengths are indicated in the legend. For praseodymium (black line), note the TPF excitation peak at ~930 nm and the adjacent two-step excited fluorescence peak at ~1014 nm.



The TPF spectrum for praseodymium doped silica fibre showing the composite emission line at ~600 nm, the de-convolved components and their respective room temperature decay times, τ.