Biology

E-Combretastatins as anti-cancer prodrugs activated by photoisomerization

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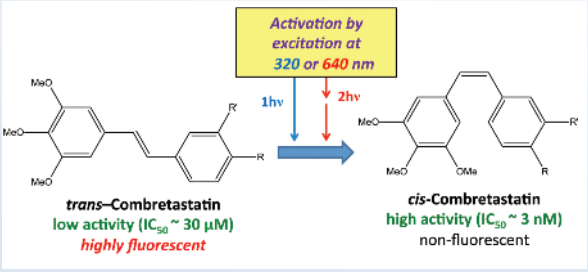
We are investigating combretastatins as anticancer drugs. Using light at wavelengths that penetrate tissues, we are exploring the potential to convert trans-isomers of combretastatins with low activity to highly active cis-isomers. This is possible through two-photon absorption at red and near-infrared wavelengths induced by intense laser pulses, even though the molecules normally only absorb in the ultraviolet region. Our objective is to design a molecule that is highly active as a drug and also efficient in the two-photon absorption process. One molecule under investigation is a 4-cyano substituted combretastatin. This has a higher two-photon absorption cross section than the parent combretastatin and undergoes two-photon excitation at longer wavelengths. These advantages are linked to intramolecular charge transfer (ICT) in the excited state. Evidence for this ICT state is obtained from steady state fluorescence spectra in a range of solvents, and from the picosecond dynamics of the infrared spectra in alcohols.



Imaging nanoparticles in fixed cells using Octopus

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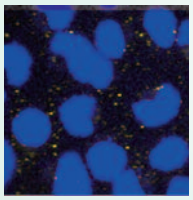
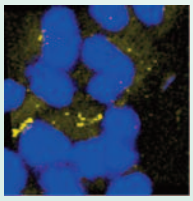
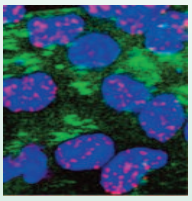
Gold nanoparticles are one of the most common cores used in developing new constructs for nanomedicine. Using the Octopus microscope cluster we have used multiphoton excitation of the surface plasmon resonance to image the gold nanoparticles within fixed cells. The size dependence of the peak wavelength of the plasmon resonance has been used to deduce information about the clumping of the nanoparticles. Furthermore, the images can be coregistered with conventional confocal microscope images so information gained using a wide range of fluorescent stains can be correlated with the nanoparticle subcellular distribution of the gold. From studies taken under a range of conditions a dynamical picture of gold nanoparticle uptake in cells is beginning to emerge. This is being used to infer the efficacy of various gold nanoparticle constructs as dose-enhancing agents in the development of a new modality of cancer therapy. It is hoped that the rapid development pipeline that has been made possible by this way of imaging the gold nanoparticles will be adapted for use in a wider range of nanomedicine projects once it is fully established.







Examples of coregistered multiphoton and confocal images taken using MDA-MB-231 breast cancer cells. In each case the cell nuclei have been stained blue. In images a) and b) the gold (shown in yellow) was allowed to accumulate in the cells for one hour and four hours respectively before fixing and imaging the cells. In image c) the gold (now shown in green) was allowed to accumulate for 24 hours and then the cells were irradiated. Resultant DNA damage is shown in magenta.

Characterization of dynamics of protein disulphide-isomerase using single-molecule FRET

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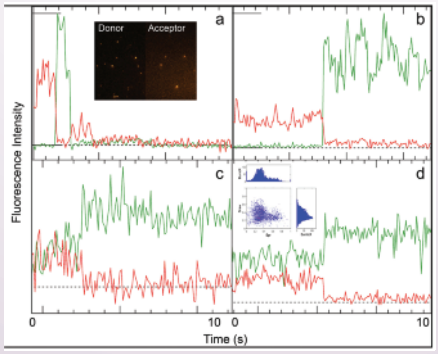
Protein disulphide-isomerase (PDI) is the key element of the machinery which ensures correct and efficient folding of proteins that pass through the 'export' compartments of the cell. These exported proteins include hormones, antibodies, growth factors, blood-clotting proteins etc. and many are now being developed as protein pharmaceuticals. Knowledge of the role and mechanism of action of PDI is crucial to attempts to generate more efficient cell factories for the production of such proteins. We have labelled key sites in the protein with fluorescent molecules, and used Forster Resonance Energy Transfer (FRET) in a number of single molecule modes to map the distances between the sites, and hence investigate the flexibility of the protein. Our first data indicate a predominantly "open" conformation accompanied by a distribution of more "closed" conformations. Work is ongoing to investigate the effect of substrates on PDI conformation and flexibility.



Single molecule TIRF fluorescence traces for immobilised PDI labelled with the FRET pair Atto 550 (green) and Atto 647N (red). Traces show single step photobleaching and correspond to high (a and b) and moderate (c and d) FRET, corresponding to distances of less than 2 nm and around 5 nm. Inset in a) shows a typical single molecule TIRF image. Inset in d) shows data from a single molecule alternating laser excitation (ALEX) measurement on immobilised PDI, indicating a predominance of "open" conformations and a distribution of shorter distances.

Protein-protein interactions in the higher plant secretory pathway

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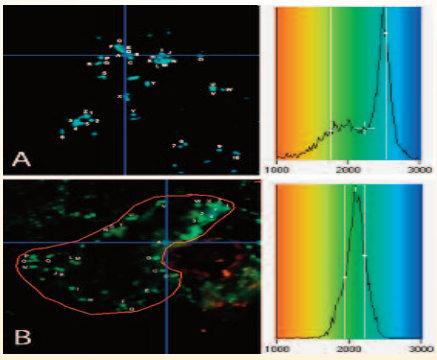
Optical trapping: With the TIRF-based laser trapping system we have demonstrated that trapping and movement of plant Golgi bodies can be used to assess properties of endoplasmic reticulum tubules. Expression of the ER membrane protein RHD3 and various mutants combined with displacement of attached Golgi bodies has revealed a potential role for the protein in regulating extensibility of ER tubules.



Life time images of A: the cis-Golgi transferase mannosidase1-GFP (MNS1-GFP) and B: the cis-Golgi transferase mannosidase 1-GFP co-expressed with the cis-transferase N-acetylglycosaminyltransferase-mRFP expressed in tobacco leaf cells. A reduced life-time of GFP suggests interactions between the enzymes when co-expressed.

Single molecule tracking: Using a photoactivatable GFP fused to a plasma membrane protein LTI6b, we have observed molecules in the PM and tracked them with time. We have obtained quantitative information about confined diffusion with the membrane. By pharmacologically modifying the PM/cell wall interface, we demonstrated that LTI6B molecule mean square displacement can be reduced and the velocity of particles decreased by 25% indicating a role for the cell wall in PM protein mobility.
Golgi transferase interactions have continued to be investigated by FRET/FLIM analysis. To date we can conclude that cis-Golgi located enzymes specifically interact with enzymes from the same or adjacent sub-compartments but it is unclear yet whether the same applies to late-Golgi enzymes.

Studying the role of protein dynamics coupled to light-activated enzyme catalysis using time-resolved infra-red spectroscopy

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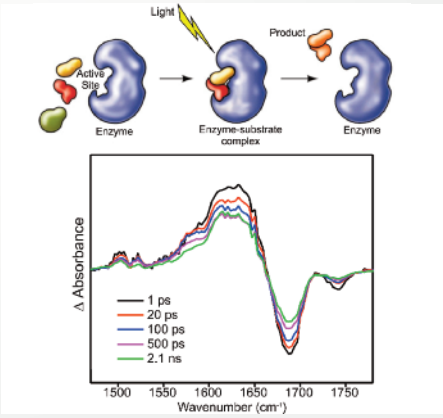
We have used time-resolved infra-red methods to attempt to understand how enzymes use dynamical processes to achieve their huge catalytic power. It is known that enzymes undergo structural fluctuations over a range of timescales but the role of protein motions/dynamics in catalysis is one of the most difficult questions to address experimentally. We have used laser-induced infra-red techniques to detect the rapid fluctuations in protein and chromophore structure that may be important for enzyme catalysis. We have shown that it is possible to observe infra-red spectral changes over a range of timescales from the fs-ms in two enzyme systems, the vitamin B12-containing ethanolamine ammonia lyase enzyme and light-activated protochlorophylllide oxidoreductase. In the future we aim to use a number of computational and experimental approaches to assign the complex spectral changes that were observed and to develop a complete and quantitative picture of catalytic processes in enzyme systems.



Human epidermal growth factor receptor (EGFR) aligned on the plasma membrane adopts key features of drosophila asymmetry

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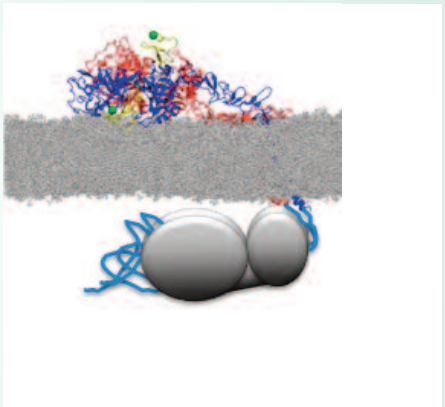
The human Epidermal Growth Factor Receptor (hEGFR, a.k.a HER1) is the founding member of the growth factor receptor tyrosine kinase family. The EGFR family has evolved from one receptor/one ligand in C. elegans, through one receptor/multiple ligands in flies, to a family comprising four receptors and 13 extracellular ligands in mammals. The function of fly EGFR is known to be modulated by the asymmetry of its extracellular domain. However, no similar asymmetry is apparent in hEGFR. To investigate this discrepancy, we developed an approach based on quantitative Förster resonance energy transfer (FRET) imaging, combined with Monte Carlo and molecular dynamics simulations, to probe receptor conformation in the membranes of cells. This revealed a new hEGFR conformation in which the receptor is aligned in contact with the membrane. Simulations suggest that the asymmetry of this new structure shares key features with fly EGFR, offering a solution to a 30-year old puzzle in EGFR research.



Cartoon of a full length HER1 holoreceptor dimer with two bound ligands based on known crystallographic structures. The extracellular domains were placed on the membrane using MD simulations and the intracellular domain disposition is informed by electron microscopy data found in the literature.

The effect of PARP inhibition on BER and B-NHEJ in the repair of simple and complex DNA damage

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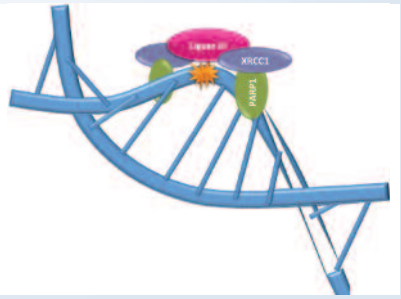
Ionising radiation induces a number of lesions within DNA and if unrepaired these lesions lead to genomic instability and cancer. The repairability of DNA lesions is subject to the complexity of damage and proficiency of repair pathways. Cellular susceptibility to genomic damage has been targeted by radiotherapy to induce tumour cell death. Extensive studies are on-going to elucidate the mechanisms of DNA repair and develop specific tumour cell targets. We have used a NIR multiphoton laser to induce DNA lesions and study kinetics of repair when repair is proficient or proteins are chemically inhibited. A protein involved in BER, XRCC1, was studied in real time following inhibition of PARP1 (recruited before XRCC1). The fast component of repair is unaffected by PARP1 inhibition although the slow component is not observed, suggesting PARP1 is involved in the repair of DNA lesions repaired slowly by XRCC1 but may not play a significant role in the repair of damage by the fast component.



Chemistry

Transient absorption spectroscopy studies of CdTe-cationic meso-tetrakis(4-N-methylpyridyl) zinc porphyrin ($ZnTMPyP_4$)

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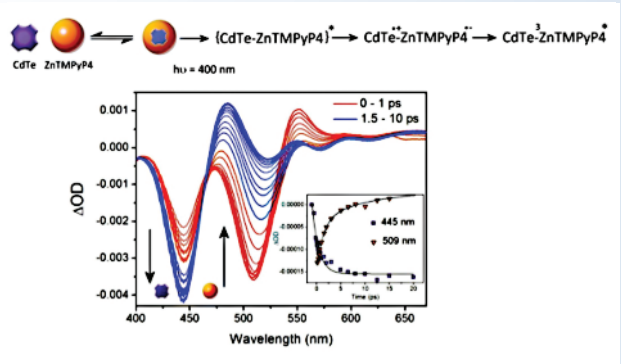
Quantum dots (QD) have attracted enormous interest due to their easily tuneable size, surface chemistry, and photophysical properties enabling a broad range of potential applications including sensing, LEDs and energy conversion. Our earlier studies of chiral CdSe QDs demonstrated the importance of working at very low energies ensuring single photon excitation. The Ultra system is ideally suited for this purpose, allowing work at pulse energies as low as 5 nJ. Ultrafast properties of a donor-acceptor system comprising a $ZnTMPyP_4$ porphyrin and CdTe QD studied using Ultra are reported. Excitation of the CdTe in the absence of porphyrin causes bleaching of the exciton band at 520 nm which recovers on a nanosecond timescale. However, this band recovers within 1.3 ps in the presence of a surface bound porphyrin, with concurrent grow in of the porphyrin bleach. This process is attributed to electron transfer and subsequent formation of the porphyrin triplet state.



A new method to probe organic films on suspended aqueous particles

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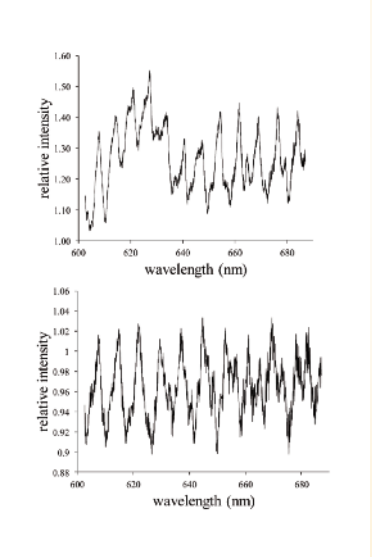

The Mie spectra for an optically trapped droplet. Top panel shows resonances for a 7.4 micron radius saltwater droplet and the lower panel the effect of coating the same droplet with a layer of oleic acid.

Particulate matter in the atmosphere (aerosol) is a critical component of the Earth's climate system and has a significant impact on clouds and rainfall. Clouds reflect light back to space and cool the planet. Pollution and chemistry in the atmosphere is changing the amount, reflectivity and drizzle potential of clouds. By trapping sea spray particles in focus of a laser beam and using Mie spectroscopy of the scattered laser light we can explore the morphology and presence of very thin films on these particles. Initial experiments have shown we can detect thin organic films. These thin films are typically organic detritus and natural reactions (atmospheric oxidation) in the atmosphere may trigger their growth to cloud condensation nuclei, and a cloud droplet, changing the reflectivity of clouds and potentially retarding rainfall.

Probing the mechanism of blue light sensing BLUF domain proteins: A study through transient infra-red spectroscopy and unnatural amino acid incorporation

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The work investigates the underlying physical processes through which plants 'see' the world around them. Plants respond to light through light sensing proteins. We have studied one example, a flavoprotein. By studying the reaction which occurs in the first few picoseconds after excitation we can see how this protein works. We have proposed a new mechanism and tested it by studying isotopically labelled proteins and proteins with artificial amino acids.

Vibrationally resolved chemical reaction dynamics in solution

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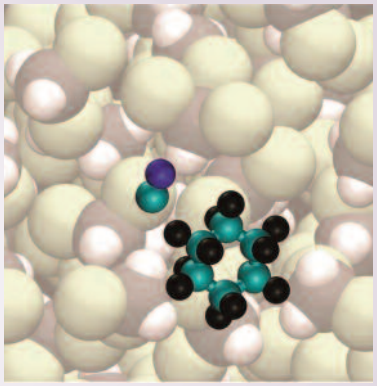
The dynamics of chemical reactions in solution in various organic solvents have been observed with vibrational quantum state resolution on picosecond timescales using transient IR absorption spectroscopy at the Ultra Facility. Exothermic reactions of CN radicals, Cl atoms and F atoms, that are known to channel their available energy into product vibrational excitation for isolated collisions in the gas phase, are shown to exhibit similar behaviour in solution. Reaction is followed by vibrational relaxation of the products on timescales as long as a few hundred picoseconds. Our experimental studies, complemented by computer simulation of the reactions, provide insights both into the ways the solvent modifies fundamental mechanisms of chemical reactions, and the interactions between solvent molecules and products in the immediate wake of the reaction. We are able to distinguish solvent caging of reactive events from reaction following diffusion into the bulk liquid medium, and to observe the energy flow between reaction products that are held in close proximity by a solvent cage.



On the photoelectron spectroscopy of gas-phase polyanions

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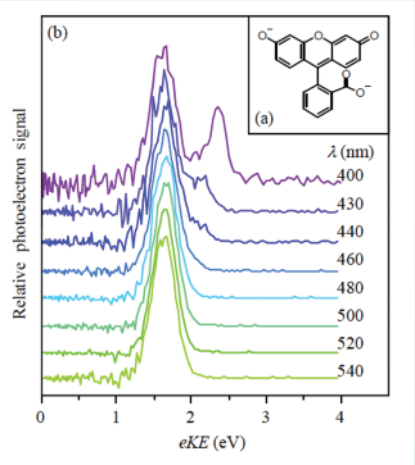
Molecular anions containing more than one excess charge are inherently unstable in the gas-phase due to the strong Coulomb repulsion between the charges. For a polyanion A^{n-}, this would lead to Coulomb explosion (fragmentation) or electron detachment, $A^{(n-1)-} + e^-$. Despite this instability, both these processes have a barrier which arises from a balance between the short range interactions and the long range Coulomb repulsion between the departing charged particle and the remaining anion. This repulsive Coulomb barrier (RCB) can result in a remarkable kinetic stability leading to the formation of exotic species such as polyanions with a negative binding energy. There has been a growing interest in the spectroscopy and dynamics of polyanions both from a fundamental perspective and because in nature, many ions are multiply charged such as in biological systems and in materials.



(a) Structure of Fl^{2-}. (b) Photoelectron spectra at various wavelengths resonant with the S1 excited state of Fl^{2-}.

Physics

Photoemission from nanoscale metal tips for time-resolved electron diffraction

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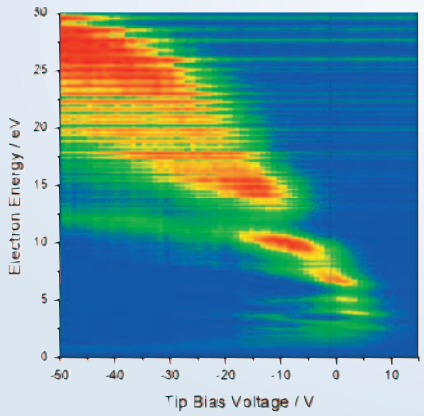
We have examined the properties of photoemission from nanoscale metal tips resulting from ultrashort laser pulses of an intensity of only 10^{10} Wcm^{-2}. Electron tunnelling into the continuum is the result of the combined ultrafast laser field and electric field enhancement by the small radius of curvature. The resultant electron pulses will be used for time-resolved ultrafast electron diffraction studies of complex biological molecules. Nanoscale tips have potential advantages over more traditional photocathodes including greater transverse coherence and the field enhancement effect allowing low energy, few-cycle near-infrared laser pulses to be used.

The number of photoelectrons and their energy spectrum has been measured using a custom magnetic bottle electron energy spectrometer. Systematic measurements have been performed for a variety of laser pulse parameters including polarisation, intensity, and relative delay of multiple pulses. We have also adjusted the field strength on the surface of the tip by altering an applied bias voltage, and report the unusual features that have been uncovered in the energy spectra resulting from these variations.



Kinetic energy spectrum of electrons ejected from a nanoscale metal tip following illumination with a femtosecond laser pulse as a function of tip bias voltage.

Feasibility study of collinear ion resonant ionization spectroscopy

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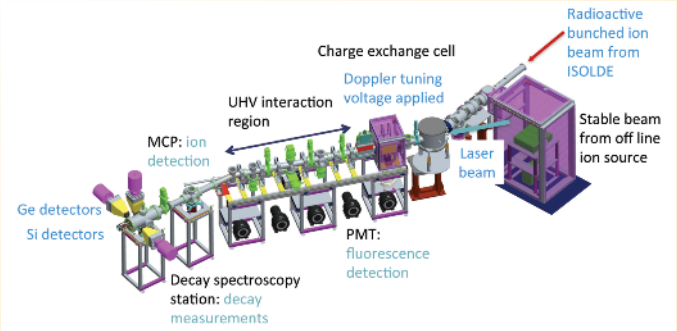
The ISOLDE facility at CERN is one of the premier nuclear physics research laboratories in the world. The facility uses protons from the CERN accelerator complex at 1.4 GeV, a sufficient energy to smash apart heavy nuclei into lighter fragments. This process produces nuclei with exotic ratios of protons and neutrons that are rarely observed in nature. Outside of the laboratory it requires the extreme environment of an exploding star or the accretion disk around a neutron star or black hole to produce these exotic nuclei. A key element to understanding these astronomical objects can only be provided by studying such nuclei in the laboratory. Even after 100 years since the discovery of the nucleus we are still far from a grand unified nuclear theory that works for all elements. Current research at ISOLDE probes nuclear matter at the extremes of existence, with the aim to further our understanding of the forces that bind protons and neutrons together.



3D drawing of the CRIS beam line and the decay spectroscopy station.

Wavelength dependence of the raman gain in synthetic diamond

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Measurements of the Raman gain in diamond were made in two ways: absolute measurements based on a pump-probe technique and relative measurements based on an assessment of the stimulated Raman scattering (SRS) threshold. Both approaches indicated that for pump wavelengths between 450 and 1450 nm, the Raman gain is inversely proportional to wavelength varying from ~40cm/GW to a few cm/GW. This is the first time to the author's knowledge that the wavelength dependence of the Raman gain in diamond has been determined experimentally. These measurements were made possible by the loan of a Continuum Panther OPO system from the EPSRC laser loan pool.

Enabling plasma medicine by unravelling the physics of plasma jets

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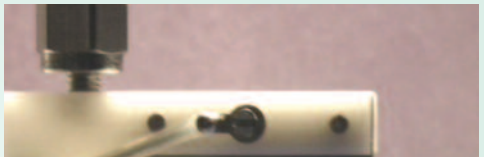
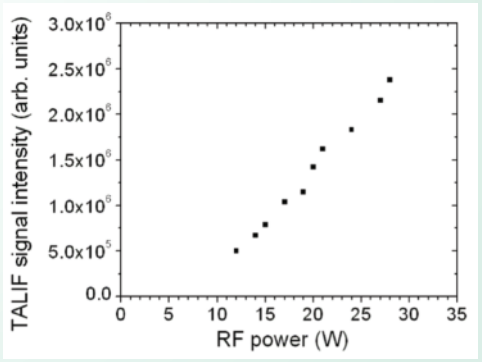
Atmospheric-pressure plasma jets (APPJ) are widely studied for multiple, novel applications in plasma medicine. To guarantee the safe and efficient use of these devices it is vital that a thorough understanding of the physics and chemistry of these plasmas is established. Reactive oxygen and nitrogen species (RONS) such as O, N, OH, NO are expected to play a crucial role in the applications of APPJs. However, so far they are only poorly understood, mainly because they are difficult to measure experimentally.

Our plasma jet is a micro-scaled APPJ device designed for optimal access for optical diagnostics. It is operated with a 1 slm helium flow with 0.2 vol% nitrogen admixture.

Using a laser from the CLF Laser Loan Pool we developed a two-photon absorption laser-induced fluorescence (TALIF) technique and performed the first direct measurements of atomic nitrogen, one of the important RONS, in APPJs.



York atmosphericpressure plasma jet.



Measured TALIF signal intensities as a function of the input rf power of the APPJ. The APPJ was operated with1 standard litre per minute helium flow with 0.2 vol% N_2 admixture. The observed signals have been corrected for collisional quenching.