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MULTIDIMENSIONAL FLUORESCENCE IMAGING AND METROLOGY USING TUNABLE SUPERCONTINUUM SOURCES

The research of Paul French and his group, concerns the application of photonics technology to the study of disease and the development of diagnostic tools and therapies. This is achieved utilising fluorescence readouts to provide molecular contrast with a particular focus on fluorescence lifetime.

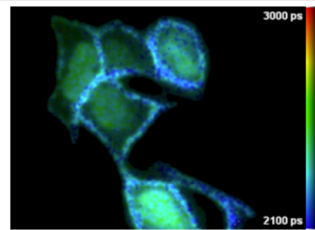
Using **Supercontinuum lasers** from NKT Photonics to provide tunable ultrashort pulse excitation, they apply this approach across the scales from cuvette-based studies of molecular interactions [1] through rapid fluorescence lifetime imaging (FLIM) of fixed and live cells, e.g. for FRET readouts using fluorescent proteins [2,3], to preclinical molecular imaging using optical tomography techniques [4,5].

Current projects exploiting supercontinuum sources include automated medium throughput FLIM implemented in multiwell plate readers to screen protein-protein interactions for drug discovery [6,7,8] and optical projection tomography (OPT), including FLIM OPT [9], of disease models such as zebrafish in which they can directly visualise key processes underlying disease and inflammation including tumour growth, angiogenesis, and cell migration.

FEATURES AND BENEFITS

For the FLIM-based research the team require tunable ultrashort excitation pulses. Unfortunately high peak power optical radiation is associated with photobleaching and phototoxicity. Picosecond pulses are therefore preferred to femtosecond pulses, making the supercontinuum source attractive for FLIM.

A single SC-400-6 unit can provide excitation of the most important fluorophores for the groups work including CFP, YFP, GFP, mCherry and further red fluorescent proteins as well as most dye-based fluorophores and some endogenous fluorophores including flavoproteins and porphyrins. This makes it an unrivalled cost-effective source for their research and fluorescence microscopy in general, particularly FLIM. The team also value the compact nature and portability of these sources, which enables them to be rapidly deployed across a range of experiments.



Fluorescence lifetime image of live Cos 7 cells presenting FRET between Raf RBD-EGFP and Ras-mRFP observed at cell membrane following EGF stimulation (from ref [2])

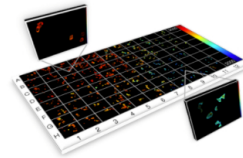
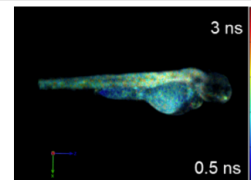


Illustration of automated multiwell plate FLIM FRET of HIV Gag protein aggregation in HeLa cells (from ref [6])

FLIM OPT image of live zebrafish embryo expressing CFP



"We use fibre-laser-pumped supercontinuum sources from NKT Photonics across our research programs and we simply would not have been able to undertake most of this research without them.

For us, the flexibility conferred by having ultrashort pulses on tap, tunable across the visible and NIR spectrum, is incredibly empowering and has enabled us to explore many different kinds of fluorescence imaging modality and many potential applications of our technology with a wide variety of samples and labels.

While such radiation could have been obtained from other laser sources, it would have been prohibitively expensive to match the capabilities of a single supercontinuum source and unthinkable to replicate this capability in the number of instruments we have developed."

Professor Paul French, Faculty of Natural Science, Imperial College London

Website: <http://www.imperial.ac.uk/natural-sciences/>

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