Visible wavelength multiphoton excitation for UVA-C combined with Fluorescence Lifetime Imaging to study medicinal drug pharmacodynamics

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Despite its current success, Fluorescence Lifetime Imaging Microscopy (FLIM) has not been utilised extensively in medicinal research. This is because a large number of drugs mainly absorb and emit fluorescence in the UV region where almost all standard research microscopes do not transmit. Using two-photon excitation in the visible region (400- 650 nm) it is possible to excite and image the uptake and localisation of previously inaccessible drugs. Imaging studies related to the processes of synthesis and drug sequestering/release from cell secretory vesicles are essential for the understanding of their mechanisms. The tunability of modern ring-cavity optical parametric oscillators and highly efficient second harmonic generation from widely tunable femtosecond sources means sub-picosecond high repetition rate lasers in operating the visible are now widely available for multiphoton processes to access UV absorbing molecules.

We will discuss our advances using visible two-photon FLIM technique on serotonin, (neuro-transmitters with Abs < 315 nm) propranonol (289 nm), Ink128 and AZD2014 (pan-mTOR cancer drugs with Abs 280 and 370 nm). We have investigated the real-time uptake, subcellular localisation and their correlation with efficacy. We found that serotonin and propranolol accumulate up to 50 mM (revealed by FLIM) in mammalian subcellular organelles. Serotonin is not released when the drug is withdrawn. Whilst propranonol was uptaken (half-life of several minutes) into the cytosol of cells but released instantaneously with a half-life of less than 1 min. However INK128 and AZD 2014 accumulates up to 0.25mM in as yet unknown cell organelles with a half-life of ~ 1 min and are not released upon withdrawal of the drugs. Excited state lifetime (FLIM) is used to accounts for concentration errors due to self-quenching that reduces actual cellular concentration in all the studies.

Furthermore, by carefully selecting the multiphoton excitation wavelength together with the application of lifetime, drugs can be easily identified from interfering background, which is not the case when using one-photon excitation and emission wavelength alone. For example INK218, serotonin and propranolol all have similar excitation and emission to tryptophan. However, the use of lifetime and visible multiphoton excitation means the drugs have a 100x detectability over tryptophan auto fluorescence background in cells. The lifetimes are INK128- 4.7$\pm$ 1.3 ns, AZD2014- 6.6 $\pm$ 1.6 ns, propranolol ~10 ns, serotonin 3.8$\pm$ 0.2 ns and tryptophan 2.8 ns. Such detectability difference between the drugs and tryptophan is negligible when one-photon UV excitation is applied due to the high extinction coefficient of tryptophan over the other drugs.

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