Two – Photon Excitation Fluorescence Cross – Correlation Spectroscopy for Ligand Binding Assays

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The in vitro assessment of large libraries of potential therapeutic agents can be achieved using high throughput screening (HTS). Conventionally, fluorescence based assays are used for HTS due to low cost output and availability of commercial detection equipment. However, fluorescence based high context screening (HCS) of live cells has been limited by intrinsic fluorescence of many cellular components. Large backgrounds arising from high levels of autofluorescence from the sample result in high instances of false positive tests. Additionally, many fluorescence assays report on secondary effects of ligand receptor interaction, such as increases in intracellular Ca^{2+} levels, rather than direct ligand binding.

Fluorescence cross-correlation spectroscopy (XCS) can be utilized to address the limitations of current fluorescence based high context screening, allowing for direct detection of ligand-receptor interactions. In XCS interacting species are labeled with fluorophores of different and nonoverlapping spectra. Two–photon excitation (TPE) enables the simultaneous excitation of both fluorophores using a single wavelength. Emission from the sample is spectrally separated and collected simultaneously in two separate detection channels. A cross–correlation signal is obtained only when the two distinctly labeled species are physically linked, and the uncorrelated signal (autofluorescence) will time average to zero. Thus, XCS is ideal for tracking the dynamics of dimerized (dually labeled) species amidst a sea monomers.

Recent developments will be described illustrating the use of TPE–XCS for monitoring binding of various ligands to a model G–protein coupled receptor (GPCR), the human μ opioid receptor (hMOR). Using this technique it is possible to simultaneously follow the kinetics of binding in addition to the changes in the structures of the bound species.