

FLUORESCENCE ANISOTROPY AND FLIM APPROACHES TO FUNCTIONAL IMAGING IN HIGH CONTENT SCREENING

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When genome- or proteome-wide high content/throughput screening of drug compounds is considered within a physiological context, the library under examination is of a size which is inaccessible to standard "single cell" assays. The main and very important distinction between the work-flows for high content screening and single-cell assays is that the majority of the steps are done in an automated fashion with minimal user intervention.

Functional imaging can provide a level of quantification that is not possible in what might be termed traditional high content screening. This is due to the fact that the current state-of-the-art high content screening systems take the approach of scaling-up single cell assays, and are therefore based on essentially pictorial measures as assay indicators. Such phenotypic analyses have of course become extremely sophisticated [1], advancing screening enormously, but this approach can still be somewhat subjective. Here we describe the development, and validation, of a prototype high content screening platform that combines steady-state fluorescence anisotropy imaging [2],[3] with fluorescence lifetime microscopy (FLIM). This functional approach allows objective, quantitative screening of small molecule libraries and siRNAs in protein-protein interaction assays. We discuss the development of the instrumentation, the process by which information on fluorescence resonance energy transfer (FRET) [4] can be extracted from wide-field, acceptor anisotropy imaging with cross-checking and quantification of this modality using FLIM by time-correlated single photon counting. We have validated this approach using a small-scale inhibitor screen of the Cdc42 variant of the so-called Raichu biosensor probe [5]. This has been expressed in an endothelial cancer cell line (A431) and was prepared in a 96 well-plate format. The assay was able to demonstrate all the capabilities of the instrument, image processing and analytical techniques that have been developed. We are able to demonstrate that acceptor anisotropy imaging and donor FLIM are highly correlated in FRET assays, providing an opportunity to screen proteins, interacting on the nano-meter scale, using wide-field imaging.

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