

Fluorescence cell-based high throughput assays for drug discovery

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In drug discovery, fluorescence assays predominate in primary screening of large chemical libraries. For example, within the group of ion channel drug targets, 74% libraries are screened by fluorescence assay based either on membrane potential (40%) or ion flux (34%) [Comley 2005]. Fluorescence High Throughput Screening (HTS) is used for a wide spectrum of targets, biological responses and experimental systems. Experimental set-ups may be based on detection of a signal from single molecules, complex proteins, cell organelles, whole cells, even a whole organism as complex as a fish (small enough to fit in the well...). Biological processes such as cell proliferation, cellular toxicity, receptor modulation, protein signaling and gene expression can be measured using fluorescence signal alteration due to the changes in membrane potential, pH, Ca concentration, molecule abundance, structural conformation, co-localization, binding and migration.

The majority of current HTS campaigns are cell-based and use culture plates with 96, 384 or 1536 wells. The inherent variability of the “biological” component of these assays together with the “miniaturization” and “robotization” artifact creates a major hurdle for an effective HTS and requires an often tedious process of assay optimization.

The talk will outline ways of automation in cell culture, liquid handling and fluorescence imaging, and explain approaches used in image analysis, assay quality assessment, screen quality assurance, data analysis and “hit” selection. The fluorescence based primary screen process will be illustrated with an example of a GABA_A receptor modulation assay aimed to find candidates for a non-sedative anti-epileptic drug development.

Reference

Comley J. 2005. Ion Channel Trends 2005: Current Practices & Technology Preferences. SBS News 19, 5-6.