

An automated fluorescence lifetime imaging multiwell platereader for high content analysis

High content analysis (HCA) imaging assays allow many conditions to be tested in a single experiment. Fluorescence lifetime imaging (FLIM) is established in the life sciences as a method to extract quantitative information from time-resolved fluorescence images. However, FLIM has not been widely adopted in an HCA context. An automated HCA-FLIM platereader was developed to allow unsupervised FLIM experiments to be performed and is capable of recording multispectral, FLIM, and brightfield data from 600 fields of view in less than four hours.

FLIM is commonly used as a means of reading out Förster resonance energy transfer (FRET) between fluorescent fusion proteins in cells. Using the HCA-FLIM platereader has allowed statistically significant results to be obtained in FRET experiments that present changes in mean fluorescence lifetime that might otherwise be lost in biological noise. The HCA-FLIM platereader can be applied to a broad range of FRET systems in diverse biological contexts, including protein-protein interaction screens and assays to probe the spatiotemporal profile of protein activation. HCA-FLIM has potential applications in the drug discovery pipeline, illustrated by FRET experiments investigating the response of Gag protein clustering to drug candidates in an HIV-1 model. Separately, the HCA-FLIM platereader can be used to measure autofluorescence lifetime in label-free cell assays, highlighting the flexibility of the instrument in addressing a variety of biological questions.