Fluorescence Dashboard

Summary of the metrics you can get

The information obtained from the Fluorescence Dashboard allows you to gain in depth knowledge on changes in cell fluorescence over time at the population and single-cell level. In particular, the high-contrast label-free images produced by Livecyte facilitate robust segmentation of whole cells and quantification of individual cell fluorescence. This is a much more reliable indicator of fluorescence changes as it is independent of the number of cells in the region.

The Fluorescence Dashboard gives you a comprehensive view of fluorescence intensity where you can obtain several useful measurements including both Total Integrated Intensity and Median Integrated Intensity. This allows an opportunity to see both widespread and cell-specific changes in fluorescence.

Applications where it would be useful

Investigating both extensive and cell specific changes in fluorescence may be beneficial when assessing mechanisms of disease or toxicity in *in vitro* models. In particular when investigating the change in expression or activity using fluorescence conjugated proteins or antibodies. For example, using a fluorescence indicator allows characterisation of neoplastic changes and aberrant division in cells characteristic of a cancerous phenotype. This can further provide a robust basis to screen and understand the adverse or beneficial effects of drug compounds as well efficacy of therapeutics in ameliorating this disease phenotype. Moreover, testing within a coculture can improve physiological relevance. Using the Explore page on Livecyte's Analyse software gives you additional tools to isolate and analyse subpopulations of cells based on fluorescence expression giving you in-depth experimental data.

In addition to disease, Livecyte allows you to independently evaluate healthy pathways and may be of merit when investigating the regulation of critical biological pathways, utilising fluorescence proteins and kinase markers to validate experimental data. For instance, the use of fluorescence bioparticles or fluorescence tagged target cells in investigating macrophage immune cell responses have allowed further understanding of the mechanisms involved in the phagocytic response.

Finally, as Livecyte can intermittently image fluorescence and use the QPI images to track individual cells over time, linking up expression data, cells vulnerable to phototoxicity can be imaged for longer without perturbation leading to more physiological and reliable experimental data.

Median Integrated Intensity – The median cell fluorescence intensity over time. The high-contrast label-free images produced by Livecyte facilitate robust segmentation of cells measuring individual cell fluorescence. This is a much more reliable indicator of fluorescence changes as it is independent of the number of cells in the region. This may be especially applicable when measuring phagocytosis activity over time or quantifying specific cytoplasmic protein expression and activity.

Integrated Intensity – The fluorescence intensity for each cell on each frame, shown in a distribution plot. This may be useful in alluding to variation in fluorescence intensity between cells within the sample with dissimilarities in intensity leading to bi-modal distribution.

Total Integrated Intensity – A line graph of how total fluorescence signal across the region varies with time. This is a good preliminary snapshot of fluorescence changes over time and may be useful when assessing cell death or viability using a fluorescence indicator. However, the total intensity can be misleading when there is heterogeneity in the population or where the confluence differs from region to region.

Publication Example

Suman, R., Smith, G., Hazel, K.E., Kasprowicz, R., Coles, M., O'Toole, P. and Chawla, S., 2016. Label-free imaging to study phenotypic behavioural traits of cells in complex co-cultures. *Scientific reports*, *6*(1), pp.1-6.

Suman, R *et al* used label-free imaging alongside fluorescence to characterise microglia, apoptotic neurons and astrocytes within a coculture. By characterising individual cell fluorescence intensity, it allowed a more physiologically relevant *in vitro* model to study the effect of microglial phagocytosis in response to cyclosporine A.