

## Proliferation Dashboard

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### Summary of the metrics you can get

The information obtained from the Proliferation Dashboard allows you to gain in depth knowledge on the division and growth of cells over time, at a population level. In addition to standard confluence values, you can compare different treatment proliferation rates, through analysing cell count and cell doubling times. Furthermore, you can quantify cellular biomass accumulation using our dry mass values, giving an indication of the growth of cells independent from proliferation.

### Applications where it would be useful

These metrics may be useful in understanding the adverse or beneficial effects of drug compounds as part of a cytotoxicity assay, or effects of genetic manipulation on cell division and growth. As cells can be monitored over long periods of time, Livecyte allows you to control and optimise the quality of your cell culture growth conditions and give you consistency in your assay. This may be particularly pertinent in experiments using a specific growth factor cocktail for stem cell differentiation or investigating viability during transfection. The merit of exploring cell growth and proliferation independently is valuable where there is an uncoupling of these two pathways. For example, in *in vitro* models of disease such as cancer, where there is hyperproliferation, or senescence where cells become enlarged and are unable to divide.

In addition, these metrics can be used in conjunction with other assays, for instance in immune cell assays to investigate how proliferation may be affected in response to immune cell interactions and activation.

**Confluence** – A graph showing the surface area of the region of interest taken up by cells over time. This is a measure of cell density and gives a preliminary indicator of the health and viability of a population of cells. However, it is affected by both proliferation and growth of cells as well as any changes in cell morphology.

**Cell count** – A graph of the number of cells in the imaging region over time. This is a measure of the rate of proliferation of cells and again gives a preliminary indicator of the health and viability of the population. As this is a direct measurement of the number of cells it is a more accurate and reliable measure of the rate of proliferation than confluence alone.

**Cell doubling time** – This is the time in hours it takes for the number of cells within the region of interest to double and is calculated from the total cell count quantified in the 'cell count' graph. An increase in cell doubling time may suggest cells are dividing at a slower rate – this is independent of the growth of cells.

**Dry mass** – A quantitative measure of the biomass within cells. This graph shows the *total dry mass of all cells* within the region of interest over time, in picograms. The rate of dry mass accumulation is indicative of cell growth in the population of cells, independent from proliferation.

**Dry mass doubling time** - The time it takes for total dry mass of all cells identified to double throughout the experiment and is calculated from the total dry mass quantified in the 'dry mass' graph. Dry mass doubling is influenced by the growth of cells over time and is independent of the proliferation of cells.

**Starting dry mass** – The total dry mass measured in picograms within the region of interest in the first frame of the experiment. This graph explicitly highlights the starting conditions for each variable giving you a better understanding of your initial experimental setup and allowing you to compare between treatments with more certainty.

### Publication Example

Ottina, E., Panova, V., Doglio, L., Kazachenka, A., Cornish, G., Kirkpatrick, J., Attig, J., Young, G.R., Litchfield, K., Lesluyes, T. and Van Loo, P., 2021. E3 ubiquitin ligase HECTD2 mediates melanoma progression and immune evasion. *Oncogene*, pp.1-12.

Ottina et al studied the role of the ubiquitin ligase HECTD2 on tumor progression of melanoma cells. They observed an increase in cell count and dry mass as well as a decrease in the cell and dry mass doubling times in cells with HECTD2 over expression, indicative of an increase in cell growth and proliferation. Furthermore, inhibition of HECTD2 led to a reversal in both these metrics suggesting a key role for HECTD2 in tumorigenicity of melanoma cells.