

Practical Tips for Cellular Quantification and Imaging Using Thermo Scientific Varioskan LUX Multimode Reader and Invitrogen EVOS FL Auto Cell Imaging System

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Goal

This technical note offers practical tips for facilitating the use of **Thermo Scientific™ Varioskan™ LUX multimode reader and Invitrogen™ EVOS™ FL Auto Cell Imaging System** with the goal of achieving successful cellular quantification and imaging of mammalian cells in microplate-based assays.

Introduction

Researchers can get a significant improvement of the relevance of their microplate cell-based assays when combining imaging with quantitative data. The use of a multiplicity of parameters originating from different assays and/or endpoints has proven a valuable strategy for achieving a deeper level of understanding of complex cellular events. Particularly in drug discovery, this orthogonal approach has become a popular trend over the last years, coupled, at least in part, with a sustained re-emergence of phenotypic screening. To meet the demands of this new trend, Thermo Fisher Scientific offers a solution that combines **Varioskan LUX multimode reader with EVOS FL Auto Cell Imaging System**. Here, key practical instructions are given to aid researchers in successfully implementing this strategy. These tips are applicable to virtually any type of mammalian cellular cultures.

In this example, viable epithelial HL cells are quantified and imaged with the fluorescence-based Molecular



Probes ReadyProbes® Cell Viability Imaging kit (blue/red). The ReadyProbes Cell Viability Imaging kit (blue/red) has been formulated to be kept at room temperature, and the dyes are added to the cells followed by a short incubation. There is no need for optimizing their concentrations; the signal-to-noise ratios are excellent; and the kit is suitable for microplate readers as well as for imaging. The kit contains the NucBlue® Live ReadyProbes® reagent, which is a formulation of the classic Hoechst 33342 stain, a popular cell-permeant nuclear counterstain that emits blue fluorescence when bound to DNA ($\lambda_{\text{excitation}} = 360 \text{ nm}$; $\lambda_{\text{emission}} 460 \text{ nm}$). It also contains propidium iodide, which specifically stains the nuclei of dead cells with damaged membranes, emitting red fluorescence ($\lambda_{\text{excitation}} = 535 \text{ nm}$; $\lambda_{\text{emission}} 617 \text{ nm}$). Toxic effects caused by the exposure of HL cells to high concentrations of dimethylsulfoxide (DMSO) are quantified and imaged in this example. Cytotoxicity is shown as a decrease of the blue-to-red fluorescence ratio (B/R ratio), caused by a larger number of dead cells compared to control cells.

Practical Tips for Cell Preparation

Mammalian cells are commonly maintained in serum containing culture media at 37°C and 95% humidity in an atmosphere containing 5% CO₂. In the example here, epithelial HL cells were cultivated in RPMI 1640 medium supplemented with 7.5% of heat-inactivated fetal bovine serum, 2 mM L-glutamine and 20 µg/ml streptomycin.

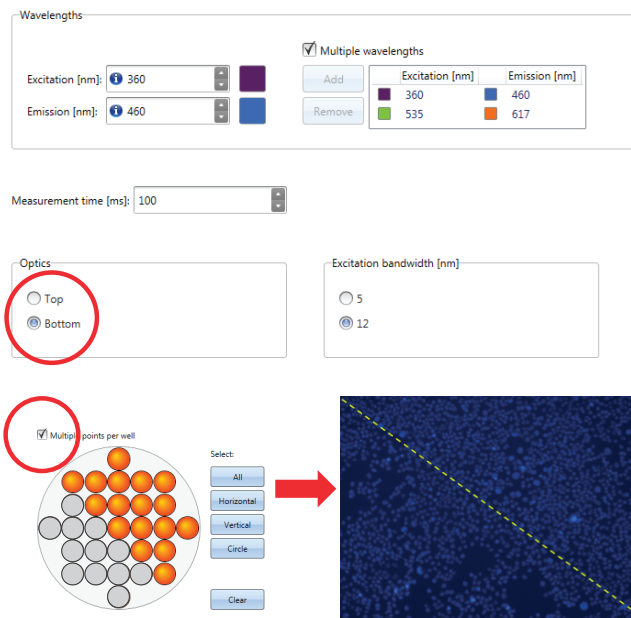
TIP 1: Cells should be **preferentially seeded in a cell-culture compatible black plate with clear bottom**, better if optimized for imaging. One example is the Nunc™ MicroWell™ 96-Well Optical-Bottom Plates with Polymer Base (catalogue number 163505). Black plates are especially optimal for fluorescence measurement in microplate readers.

TIP 2: A **high cellular density is important** for being able to perform simultaneous quantification with multimode readers and imaging. This can be easily accomplished by seeding high cellular concentrations, and incubating for at least 20-24 hours prior to measurements. In this example, a minimum of 400,000 cells/ml (80,000 cells/well) was required, and cells were incubated for 22 hours before conducting the trials.

Practical Tips for Fluorescence Quantification Using Varioskan LUX Multimode Reader

Varioskan LUX allows for accurate quantification of cellular signals with a flexible range of measurement technologies, including Fluorescence Intensity (FI) and Time-Resolved Fluorescence (TRF). FI measures can be conducted using top or bottom reading, depending on the nature of the samples. The instrument is controlled by the Thermo Scientific SkanIt software, which is particularly intuitive, and provides excellent usability and flexibility even for the most challenging microplate assays. A bottom fluorescence reading was performed here, as recommended for quantifying adherent cells.

TIP 3: A non-homogenous distribution of cells (even at high concentrations) can typically jeopardize fluorescence signal detection in multimode readers. However, Varioskan LUX offers a solution to this dilemma by allowing you to select **specific points within the well where the signal can be measured**. In the example below (scheme 1), HL cells were found to be preferentially located towards the right top region of the well, and thus multi-point measurements matching this area were chosen in the SkanIt software (bottom reading function).

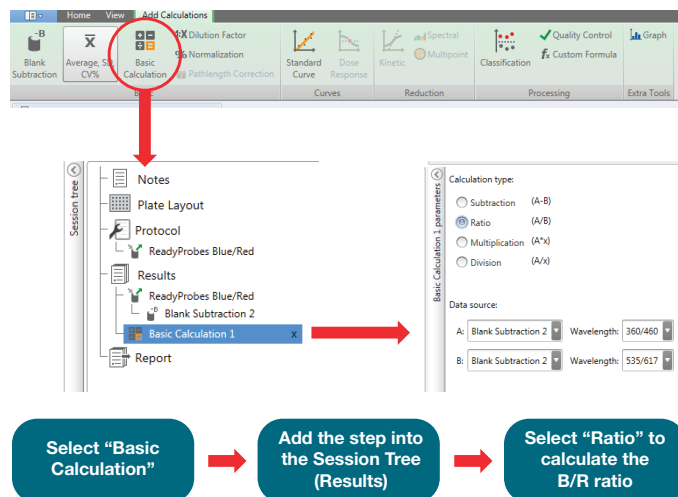


Select "Bottom reading"

Use "Ctrl" key to select the desired points where cells are located

Scheme 1: How to set up the measurement step in SkanIt using multipoint measurement with bottom reading.

TIP 4: After performing the fluorescence measurements of the two dyes, a **basic calculation** can be added to the measurement session using the SkanIt software. In this example, the direct calculation of the blue-to-red fluorescence ratio (B/R) was carried out (scheme 2).



Select "Basic Calculation"

Add the step into the Session Tree (Results)

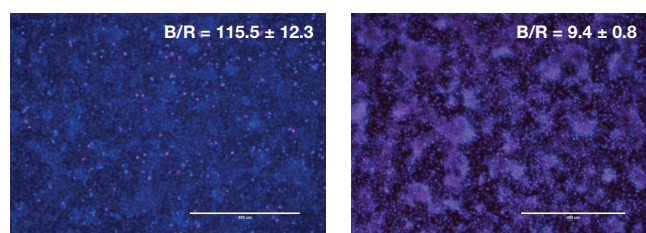
Select "Ratio" to calculate the B/R ratio

Scheme 2: How to set up a "Basic calculation" step in SkanIt to automatically determine the B/R fluorescence ratio.

Practical Tips for Fluorescence Imaging Using the EVOS FL Auto Cell Imaging System

The EVOS FL Auto Imaging System is an automated imaging platform capable of live cell imaging, area scanning, image stitching, and time-lapse imaging. The microscope supports any type of culture dish. In this example, wells were imaged in black (clear bottom) 96-well plates. Wells are automatically selected with the software; different positions within one well can also be imaged using the Medium Jog button.

TIP 6: Locate first the cells using the **Transmitted Light imaging** choice of the microscope. Once the cells are located, switch to **Fluorescence Imaging**. For the case describe here, selected DAPI and RFP light cubes, to image cells stained with the NucBlue® Live and propidium iodide (PI) dyes, respectively, were chosen. Once the separated images are captured, the software allows for further editing, for instance an “**overlay**” of the **images**. Red cells are shown as pink when overlaid with the NucBlue-stained cells. **The image to the left** shows untreated cells, with a clear prevalence of NucBlue-stained cells. By contrast, **the image to the right** shows cells exposed to DMSO 20%, with a significantly larger proportion of PI-stained cells. Cytotoxic effect of DMSO at 20% was further demonstrated by the significantly



Scheme 3: Example of combination of EVOS images with the B/R fluorescence ratios measured with Varioskan LUX (inserted values).

lower B/R fluorescence ratio quantified with Varioskan LUX. Inserted values on the images are the averages and standard deviations of the B/R ratios measured in three replicate wells.

TIP 7: Values of the blue-to-red fluorescence ratio (B/R) can be different depending on different cell types, and cellular concentrations, among others. In this example, the selected cells were easily imaged with minimal background fluorescence. **Dye quantities, as well as incubation times can be further adjusted, if needed.** In the current example, 35 µl of the dyes was added with a pipette to each well, and they were incubated with the cells for 15 minutes at room temperature. In most cases, incubations with the ReadyProbes™ Cell Viability Imaging kit (blue/red) within the range of 5 to 30 minutes are sufficient.

Conclusions

Orthogonal approaches that combine imaging with quantitative data are becoming increasingly popular. In the case illustrated here, the outcomes from the imaging and quantitative assays were merely confirmatory. However, in many other scenarios, these outcomes can provide complementary or even totally new views to the research question, thus broadening the understanding of the cellular events under study. An excellent proposition to apply these orthogonal approaches is combining **Thermo Scientific™ Varioskan™ LUX multimode reader and Invitrogen™ EVOS™ FL Auto Cell Imaging System**. The simple tips described here can help scientists answer even their most intricate research questions and advance in their cell-based discovery projects.

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