NOVEL REFLECTED LIGHT MICROSCOPY ASSAY FOR RAPID, LABEL FREE ASSESSMENT OF CELLULAR PROCESSES AND CELLULAR STATUS AT SINGLE CELL LEVEL

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Background: Whole cell-based biosensing systems are uniquely capable of providing functional information on the impact of a sample on cell physiology including related toxicity or pharmacology effects. As a result cell-based sensing grounded on dynamic, real-time, label-free, and non-invasive analysis of cellular events, (including cell adhesion, cell viability, cell morphology, and cell motility) is aimed for as a significant enabling technology for biological research, environment assessment and pharmaceutical industry.
Aim: Develop a rapid, label free and sensitive assay to investigate cell viability and cytotoxicity at single cell level compatible with cellular platforms format and useful in: cell-cell, pathogen-host interactions evaluation.
Method: Endothelial *bEnd-3* cells under normal and (chemical, microbiological) stress conditions, were monitored, in real time, using reflected light dark field microscopy configuration. The MTT (methyl-thiazolyl-tetrazolium) assay, a gold standard in viability evaluation, is deployed in single cell analysis format to enable method calibration: high resolution quantitation of cytological mechanisms of MTT uptake, reduction to formazan nanocrystals, formazan compartmentalization and its extrusion is quantitatively assessed in conjunction with the type and extent of stressors.
Results and discussion: The (scattered) reflected light intensity and cell localization changes reveal dynamic differences between cells cultured in normal and stress conditions enabling label free assessment of cellular processes and cellular status (viability, membrane integrity, organelle/vesicles dynamics, cell surface contacts) at single cell level. The progress of individual steps in the known MTT cytological mechanisms of uptake, reduction to formazan nanocrystals, formazan compartmentalization and its extrusion is innovatively used to demonstrate the dynamic, quantitative access to phenotypic and functional cell analysis.
Conclusion: This method assures results in a shorter time, compared to gold standard MTT and allows real-time single-cell analysis and an accurate quantification of formazan crystal products, in a dynamic manner.

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