Scienze della Vita

Dipartimento di Biologia e Biotecnologie Charles Darwin
Sapienza Università di Roma
IBPM CNR

The informational content of cellular imaging in studies of dynamic biological processes

Guarguaglini G.[1,2], Asteriti L.[1,2], Paiardini A.[3], Degrassi F.[1,2], Valente D.[1,2], Cirigliano P.[4], Giannini G.[5], Rosa A.[6,7], Silvestri R. [8], La Regina G. [8], Schininà M.E. [3], Lavia P. [1,2]

1. IBPM Institute of Molecular Biology and Pathology- CNR Consiglio Nazionale delle Ricerche, c/o Sapienza University of Rome, IT; 2. Nikon Reference Center for Central-Southern Italy at IBPM; 3. Department of Biochemical Sciences, Sapienza University of Rome, IT; 4. Nikon Instruments S.p.A., Campi Bisenzio, IT; 5. Department of Molecular Medicine, Sapienza University of Rome, IT; 6. Center for Life Nano Science, Istituto Italiano di Tecnologia, Rome, IT; 7. Department of Biology and Biotechnology Charles Darwin, Sapienza University *of Rome, IT; 8. Department of Drug Chemistry and Technologies, Sapienza University of Rome, IT.*

email: patrizia.lavia@uniroma1.it

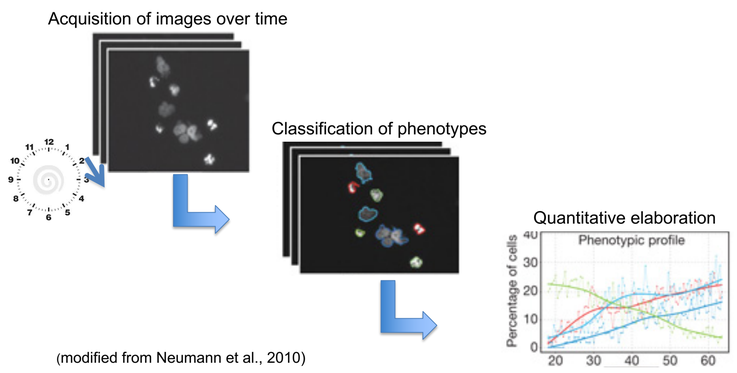
Strumentazioni e Tecniche

INTRODUCTION

High content imaging of dynamic biological processes

Image-based assays in the biomedical field are rapidly evolving from qualitative to more quantitative approaches ("high content") that provide high resolution spatio-temporal information.

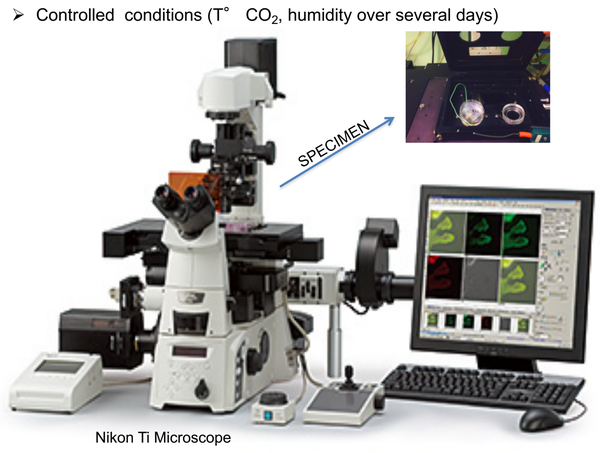
Time-lapse video recording of living cells depicts dynamic information that would remain otherwise unnoticed with analyses of fixed samples and that are fundamental to understand complex biological processes (e.g., cell division, cell differentiation, senescence, intra- and inter-cellular signalling, cellular migration, infection, host/pathogen interactions, response to drug treatments, induction of cell death).



(modified from Neumann et al., 2010)

THE IBPM-CNR MICROSCOPY PLATFORM - NIKON REFERENCE CENTER

- The platform hosts microscopes for high resolution imaging of **fixed** samples and **living** cells.
- Full automation
- Controlled conditions (T° CO_2, humidity over several days)

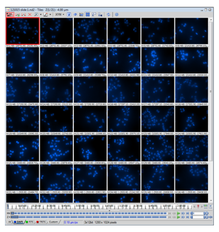


Nikon Ti Microscope

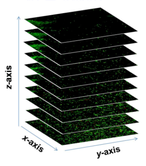
- Providing high content information and supporting simultaneous analysis of cells under several conditions

- Multidimensional acquisition:

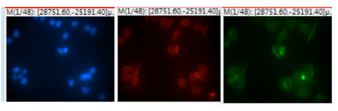
a. xy multipoint



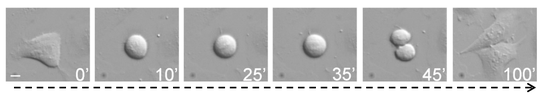
b. Z-stack



c. multichannels (λ, fluorescence)



d. time



http://bbcd.bio.uniroma1.it/bbcd/archivionotizie/cnr-microscopy-platform-nikon-reference-center-ibpm

CELLULAR IMAGING: FIELDS OF APPLICATION

The IBPM microscopy platform supports versatile applications, in which dynamic studies (time-lapse recording) are coupled with high resolution qualitative and quantitative image analysis of cells and cellular structures

Applications:

Real-time visualization of dynamic processes (cellular signalling, intracellular transport, organization of organelles and subcellular structures)

Single-cell analysis, to visualise cell heterogeneity and rare behaviours within a cell population

Recording of cellular morphological changes or cell death in response to particular stimuli (physical / chemical damage, stress conditions)

Measurements of cell migration

High definition analysis of subcellular structures (5 fluorescence excitation channels, simultaneous visualization of 4 stainings, image deconvolution, 3D reconstruction)

Proximity ligation assays for *in situ* protein interactions and *in situ* post-translational modifications.

Biological processes

Studies of cell division and checkpoints

Tumour cell growth and inhibitory drugs / molecules /modulating genes

Differentiation of stem and progenitor cells

Assay / design of innovative therapeutic strategies

Cell response to parasites, bacteria and viral infectious agents

Novel biocompatible matrices to support cell growth in tissue regeneration

The uptake of nanoparticles and functionalised nanomaterials within cells

CREATION OF CELL MODELS AND SET-UP OF *AD-HOC* WORKFLOWS FOR HIGH THROUGHPUT (HT) AND HIGH CONTENT (HC) AUTOMATED IMAGE ANALYSIS

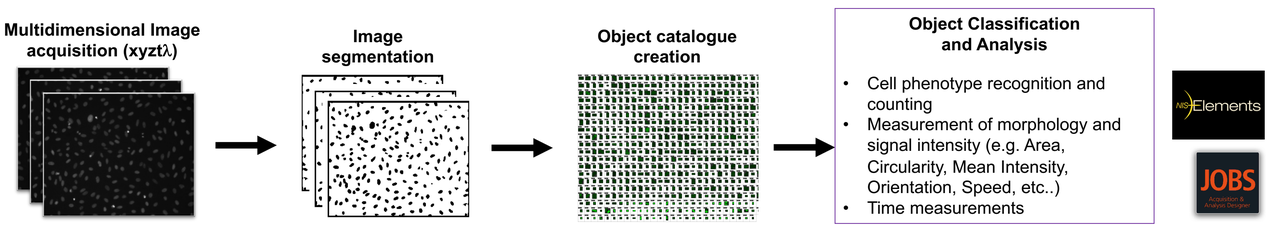
Creation and validation of informative cell models useful for automated analysis

Cell lines can be engineered to visualize cellular components using fluorescent proteins or fluorescent dyes suitable for live imaging

Set-up of specific protocols for live cell imaging (will take into account: phototoxicity assessment, definition of exposure time and intervals; concentration of staining dye etc.)

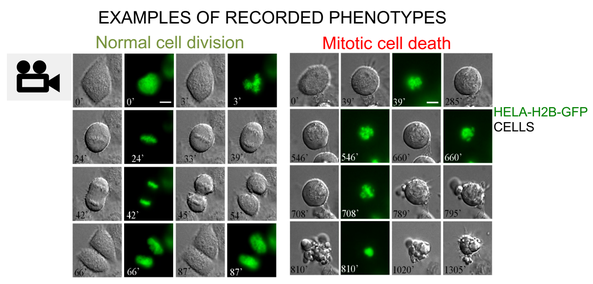
Automation of image acquisition and data analysis

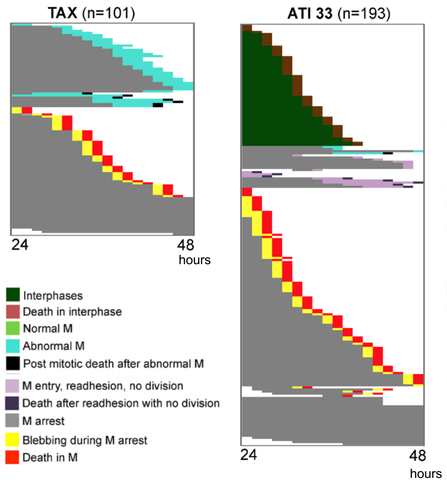
1. Creation of *ad-hoc* acquisition workflow to maximize the amount and the quality of imaging data (JOBS module of Nikon proprietary Nis Elements software)
2. Classification of phenotypes of interest using the machine learning-based Nis Elements classifier
3. Automated analysis of phenotypes of interest



EXEMPLIFYING RESULTS

1. Single-cell recording depicts stochastic phenomena in cell biology: heterogeneous responses to novel mitotic inhibitors, including rare yet biologically significant behaviours





Different cell fates were visualized in cell cultures (HeLa) treated with novel antimicrotubule compounds (ATI).
Cells were videorecorded 24 to 48 h after treatment.

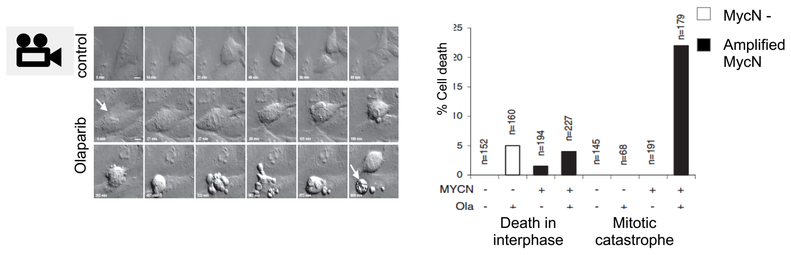
Each horizontal bar in the graph represents the fate of a single cell.

Treated cell populations show a heterogeneous profile of responses to the treatment

Video-recording is essential to evaluate the frequency of "escaper" cells in response to new anti-cancer treatments.

(Di Cesare et al., 2017)

2. Time-lapse recording shows that the outcome of therapeutic treatments is modulated by different genetic backgrounds (precision medicine)

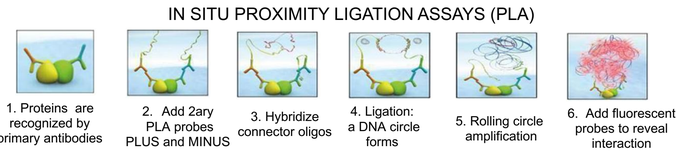


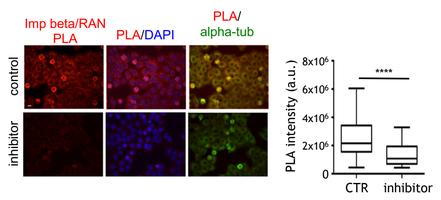
Time lapse video-recording of neuroblastoma cells:
shows that treatment with PARP inhibitors induces differential fates, depending of the status of MYCN amplification (a driver of aggressiveness in neuroblastoma);
the fraction of cells that undergo a specific cell fate can be quantified.

(Colicchia et al., 2017)

3. Automated detection and analysis of protein-protein interactions in fixed cells

IN SITU PROXIMITY LIGATION ASSAYS (PLA)

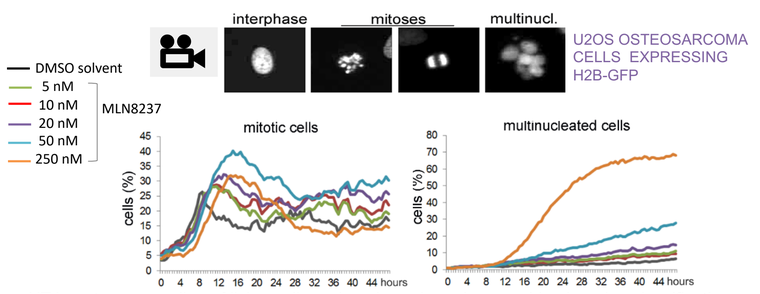




We developed a workflow for the automated detection of PLA signals.
This yields rapid and accurate information on i) genuine validation, and ii) subcellular localization of Importin beta interactors selected in proteome-wide screening.

(Di Francesco et al., 2018)

4. A high-throughput video-recording approach and automated analysis to follow the fate of cells over time



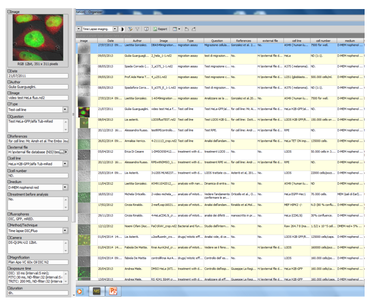
HT long-term recording of living cells and analyses based on machine learning classification:
Data can be collected from large samples, thus facilitating the detection of rare phenotypes and increasing the statistical power of the experiments.

(Asteriti et al., 2014)

EURO-BIOIMAGING EMBL Advanced Light Microscopy Facility

Our time-lapse imaging database

We created a "time-lapse imaging" database, including both "manual" and automated annotations with optimized parameters for each experiment to help reproducibility, comparison to standards and data sharing.



Experimental categories

- Test cell line
- Treatment with drugs
- Mitotic effects
- Bacterial and or nanoparticles uptake
- Migration assay (single cell or wound healing)
- Cell differentiation

General information: Date, Author, Image, Type, Question, External file, Comments, References.
Experimental conditions: Cell line, Cell number, Supports, Treatment before analysis.
Protocol: Medium, Anti-evaporation oil.
Microscope settings: Duration, Method/Technique, Magnification, Fluorophores, Exposure, XY points, Z slices

We acknowledge support from Regione Lazio - "Progetti di Gruppi di Ricerca LR13/2008 - Progetto n. 85-2017-15348"

- Di Francesco L, Verrico A, Asteriti IA, Rovella P, Cirigliano P, Guarguaglini G, Schininà ME, Lavia P. 2018. Visualization of human karyopherin beta-1/importin beta-1 interactions with protein partners in mitotic cells by co-immunoprecipitation and proximity ligation assays. Sci Rep. 8:1850. doi: 10.1038/s41598-018-19351-9.
- Ferrara M., Sessa G., Fiore M., Bernard F., Asteriti I.A., Cundari E., Colotti G., Ferla S., Desideri M., Buglioni S., Trisciuoglio D., Del Bufalo D., Brancale A., Degrassi F. 2018. Small molecules targeted to the microtubule-Hec1 interaction inhibit cancer cell growth through microtubule stabilization. Oncogene. 37:231-240. doi: 10.1038/onc.2017.320.
- Colicchia V., Petroni M, Guarguaglini G, Sardina F, Roncero MS, Carbonari M, Ricci B, Heil C, Capalbo C, Belardinilli F, Coppa A, Peruzzi G, Screpanti I, Lavia P, Gulino A and Giuseppe G. 2017. PARP inhibitors enhance replication stress and cause mitotic catastrophe in MYCN-dependent neuroblastoma" Oncogene. 36:4682-4691. doi: 10.1038/onc.2017.40.
- Di Cesare E, Verrico A, Miele A, Giubettini M, Rovella P, Coluccia A, Famiglini V, La Regina G, Cundari E, Silvestri R, Lavia P. 2017 Mitotic cell death induction by targeting the mitotic spindle with tubulin-inhibitory indole derivative molecules. *Oncotarget* Feb 1. doi: 10.18632/oncotarget.14980
- Asteriti I.A., Di Cesare E., De Mattia F., Hilsenstein V., Neumann B., Cundari E., Lavia P.· Guarguaglini G. 2014.The Aurora-A inhibitor MLN8237 affects multiple mitotic processes and induces dose-dependent mitotic abnormalities and aneuploidy. Oncotarget 5:6229-42