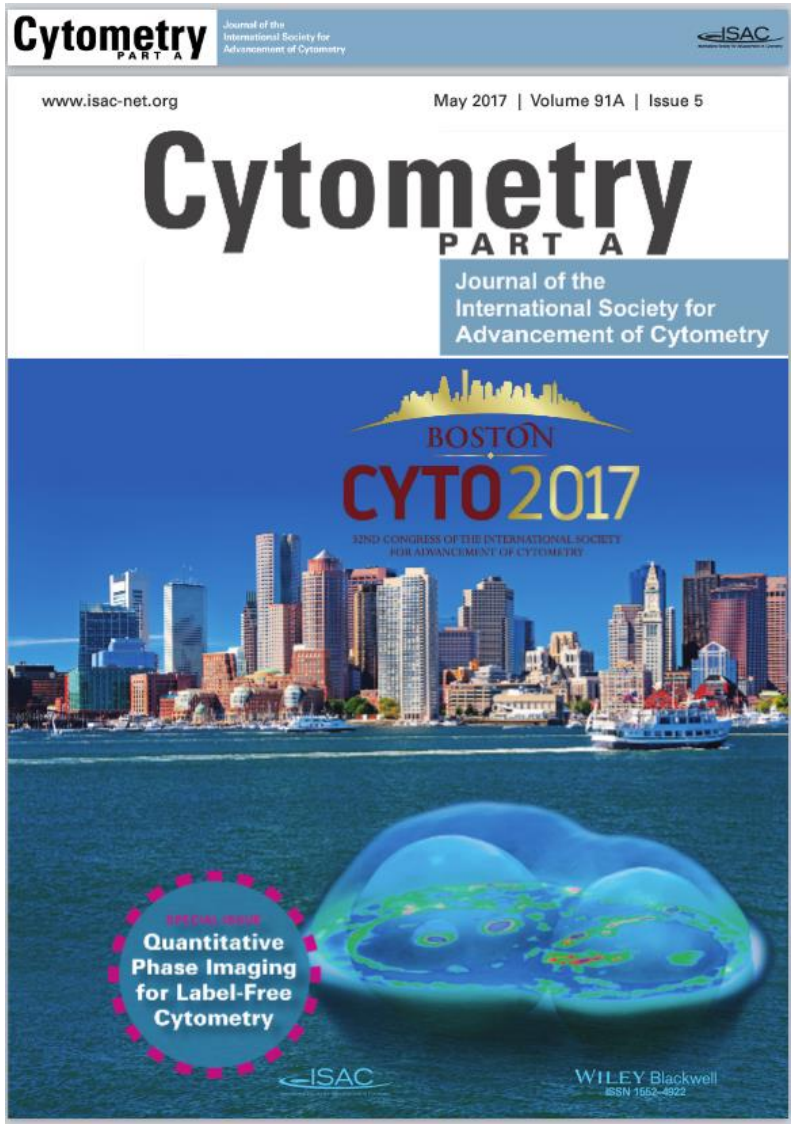
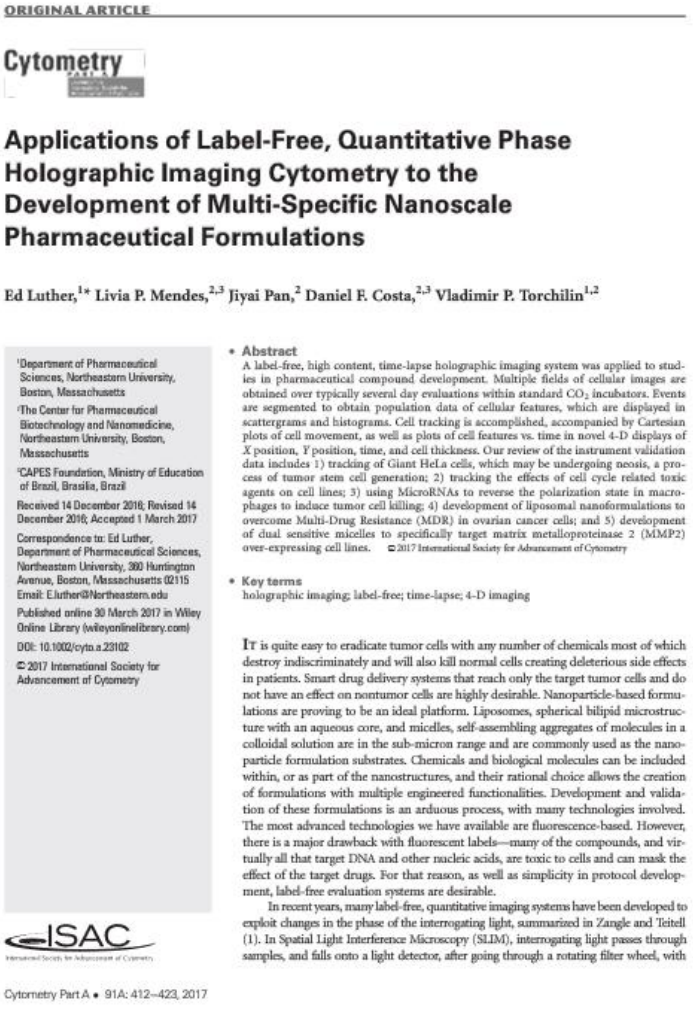
Long-Term Imaging and Tracking of In Vitro Cells and Their Environment Using Quantitative, Label-Free, High Content, Holographic Based Imaging Cytometry

Ed Luther, Livia Mendes, Jiayi Pan, Daniel Costa, Can Sarisozen and Vladimir Torchilin

- Department of Pharmaceutical Sciences
- Center for Pharmaceutical Bioscience and Nanotechnology
- NEU – PHI Program of Excellence





ORIGINAL ARTICLE

Cytometry PART A

Applications of Label-Free, Quantitative Phase Holographic Imaging Cytometry to the Development of Multi-Specific Nanoscale Pharmaceutical Formulations

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• **Abstract**
A label-free, high content, time-lapse holographic imaging system was applied to studies in pharmaceutical compound development. Multiple fields of cellular images are obtained over typically several day evaluations within standard CO_2 incubators. Events are segmented to obtain population data of cellular features, which are displayed in scattergrams and histograms. Cell tracking is accomplished, accompanied by Cartesian plots of cell movement, as well as plots of cell features vs. time in novel 4-D displays of *X* position, *Y* position, time, and cell thickness. Our review of the instrument validation data includes 1) tracking of Giant HeLa cells, which may be undergoing neosis, a process of tumor stem cell generation; 2) tracking the effects of cell cycle related toxic agents on cell lines; 3) using MicroRNAs to reverse the polarization state in macrophages to induce tumor cell killing; 4) development of liposomal nanoformulations to overcome Multi-Drug Resistance (MDR) in ovarian cancer cells; and 5) development of dual sensitive micelles to specifically target matrix metalloproteinase 2 (MMP2) over-expressing cell lines.

• **Key terms**
holographic imaging; label-free; time-lapse; 4-D imaging

IT is quite easy to eradicate tumor cells with any number of chemicals most of which destroy indiscriminately and will also kill normal cells creating deleterious side effects in patients. Smart drug delivery systems that reach only the target tumor cells and do not have an effect on nontumor cells are highly desirable. Nanoparticle-based formulations are proving to be an ideal platform. Liposomes, spherical bilipid microstructure with an aqueous core, and micelles, self-assembling aggregates of molecules in a colloidal solution are in the sub-micron range and are commonly used as the nanoparticle formulation substrates. Chemicals and biological molecules can be included within, or as part of the nanostructures, and their rational choice allows the creation of formulations with multiple engineered functionalities. Development and validation of these formulations is an arduous process, with many technologies involved. The most advanced technologies we have available are fluorescence-based. However, there is a major drawback with fluorescent labels—many of the compounds, and virtually all that target DNA and other nucleic acids, are toxic to cells and can mask the effect of the target drugs. For that reason, as well as simplicity in protocol development, label-free evaluation systems are desirable.

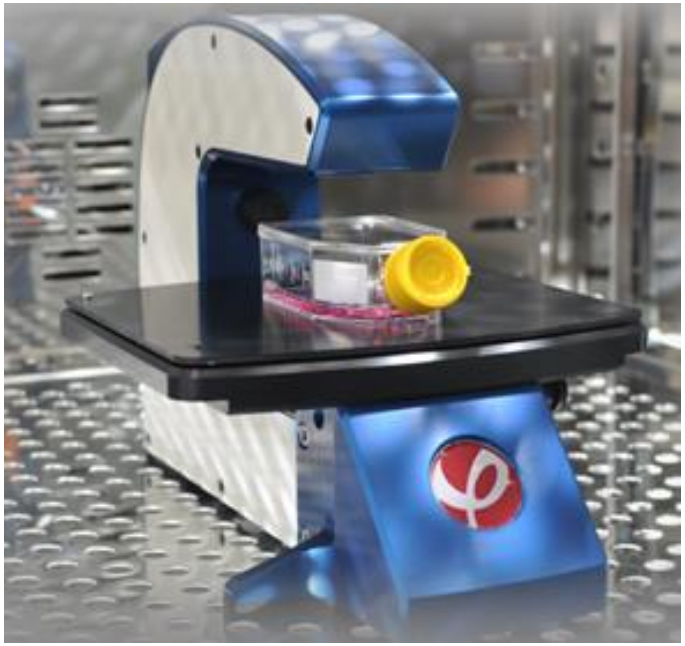
In recent years, many label-free, quantitative imaging systems have been developed to exploit changes in the phase of the interrogating light, summarized in Zangle and Teitell (1). In Spatial Light Interference Microscopy (SLIM), interrogating light passes through samples, and falls onto a light detector, after going through a rotating filter wheel, with

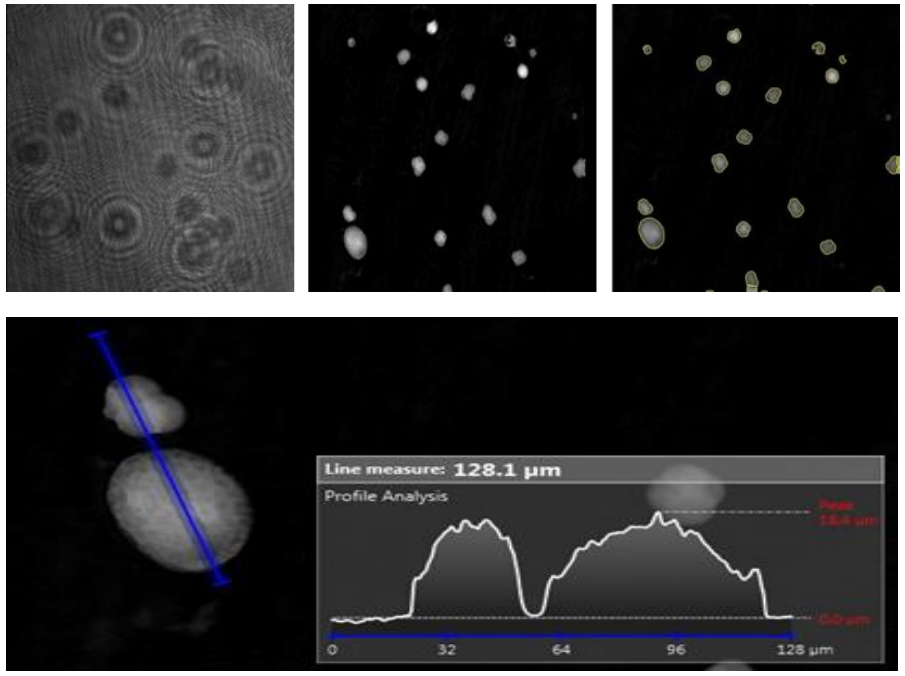
ISAC

The full description of these experiments is published in the current issue of Cytometry Part A.

DESCRIPTION OF HOLOMONITOR® TECHNOLOGY

Holographic Time Lapse Image Acquisition

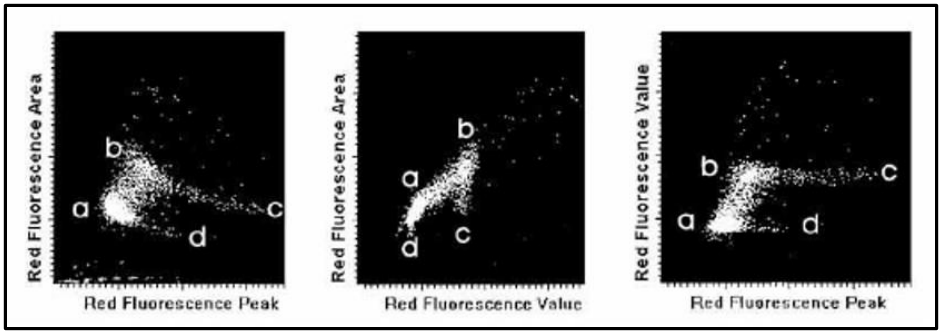




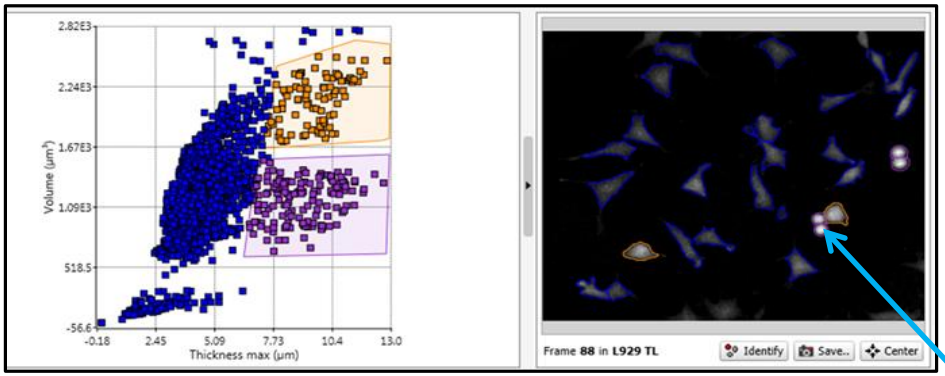
- **The HoloMonitor M4® (Phase Holographic Imaging, Lund Sweden) is an incubator adapted, label-free, quantitative time lapse imaging system.**

Dark field images of the cellular thickness are analogous to fluorescence based images. Analysis techniques that have been developed for flow cytometry and laser scanning technology are applicable.

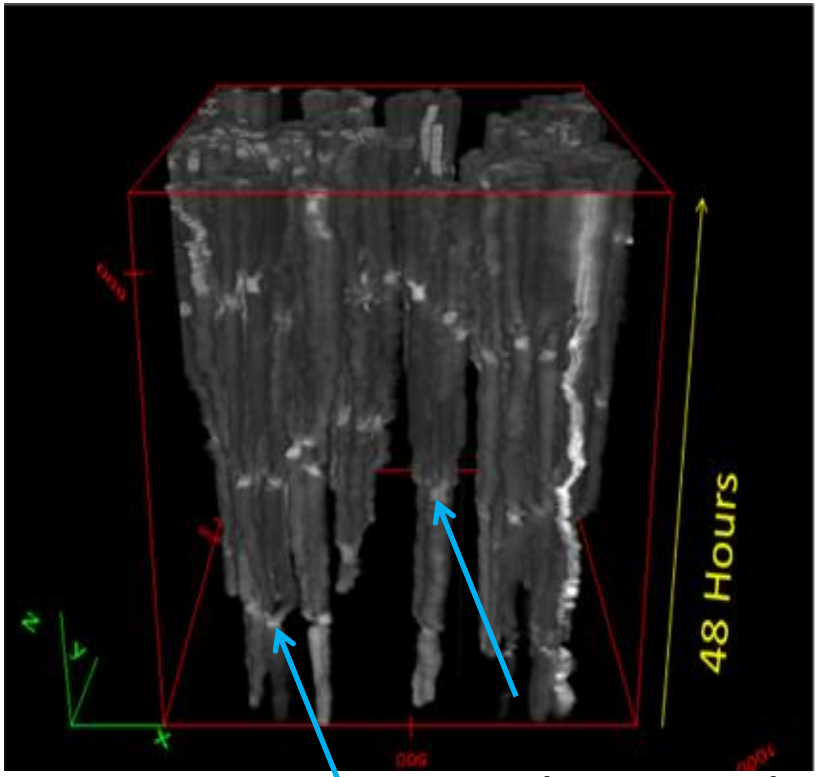
Resolution of Mitotic Cells …



- By Laser Scanning Cytometry



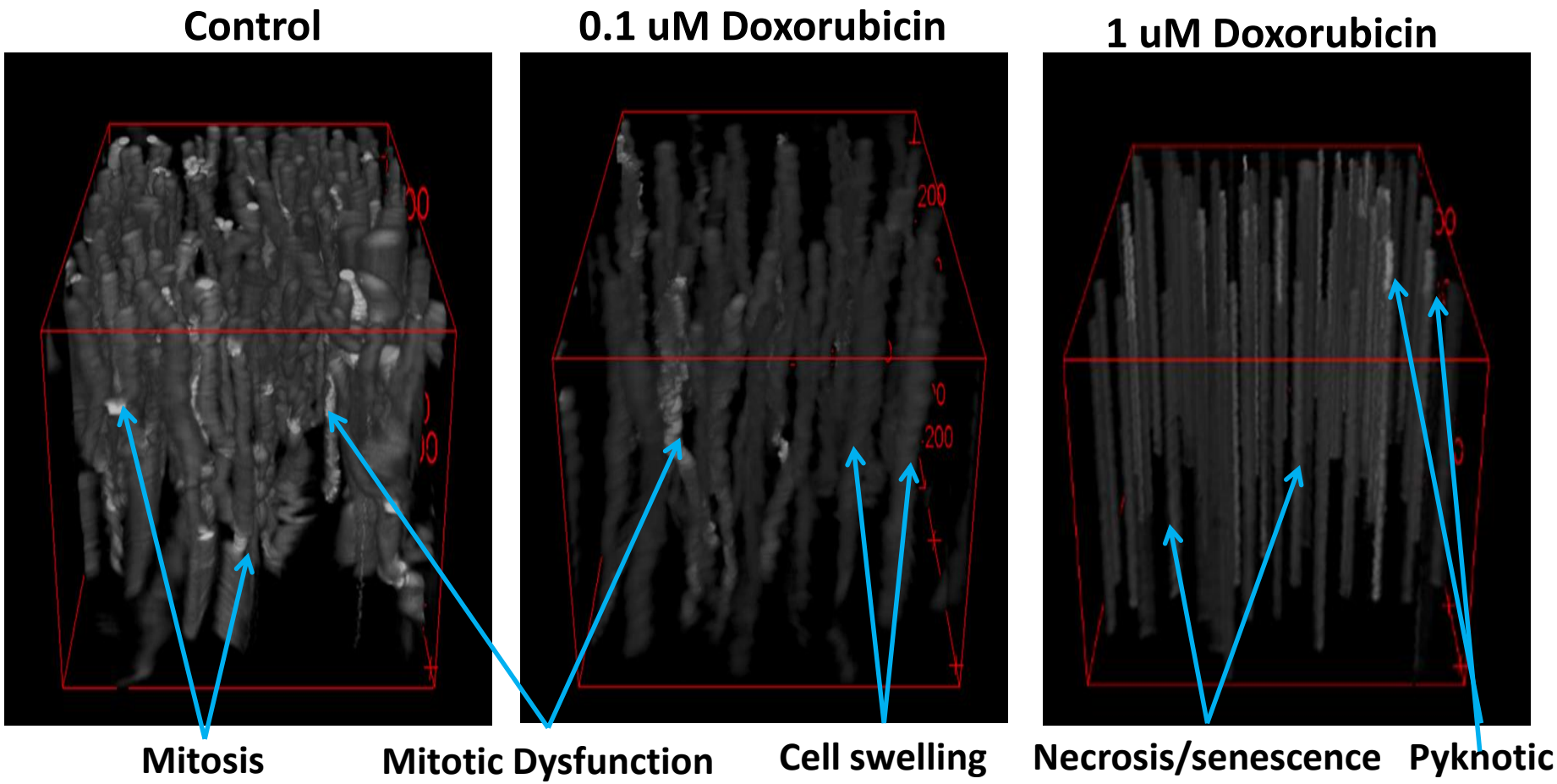
- By Quantitative Phase Holographic Imaging



- X and Y position, Time (Z-direction) and Optical Thickness (brightness)

Mitotic cells are distinguishable from interphase cells based on their increased thickness.

Four Dimensional Imaging – An Innovation Developed at NEU



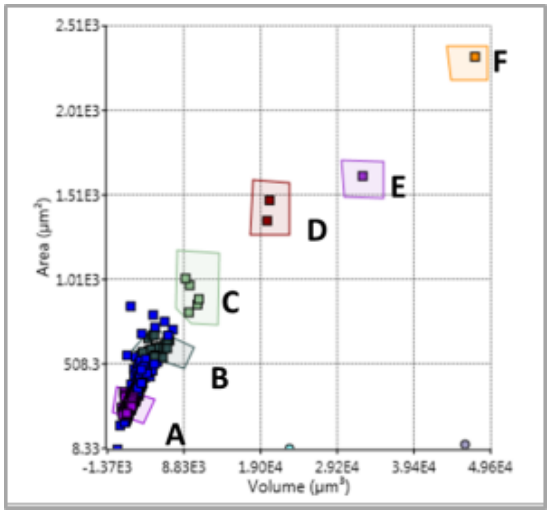
- HeLa cells treated with Doxorubicin, a potent chemotherapeutic agent.

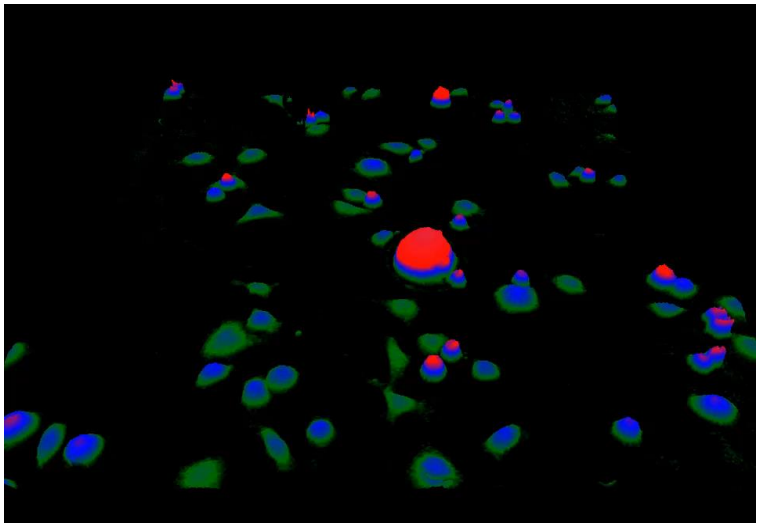
Four dimensional holographic images of in vitro cultures may have significant diagnostic potential.

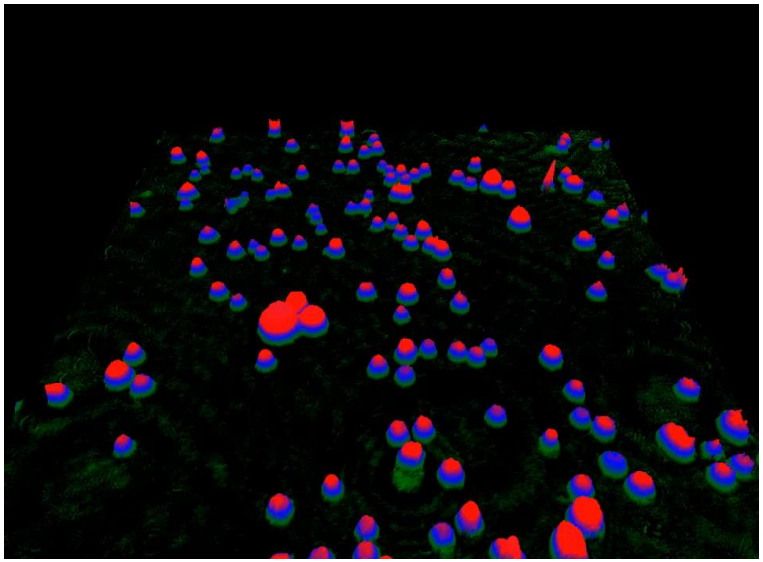
VALIDATION OF HOLOMONITOR TECHNOLOGY

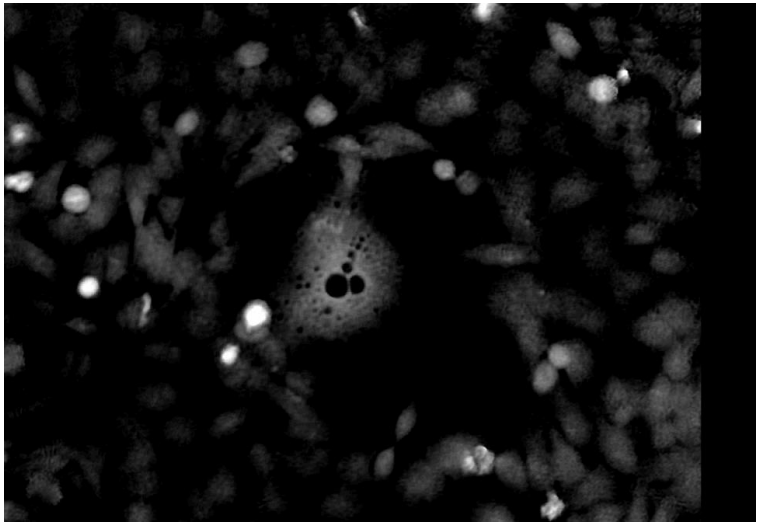
LONG-TERM CELL TRACKING

Validation of Long-Term Tracking of Selected Cells



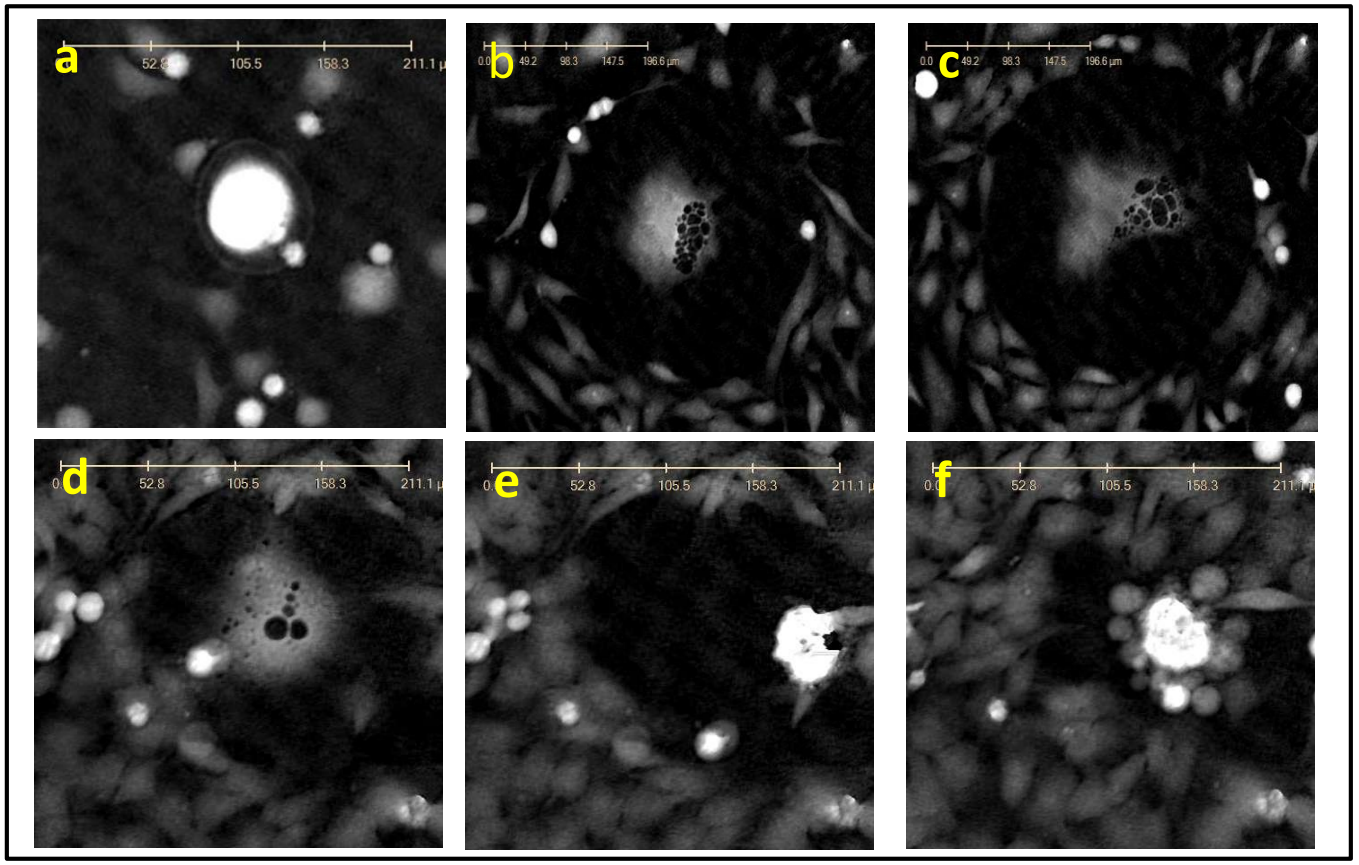






Giant HeLa cells were selected as the targets for long-term tracking.

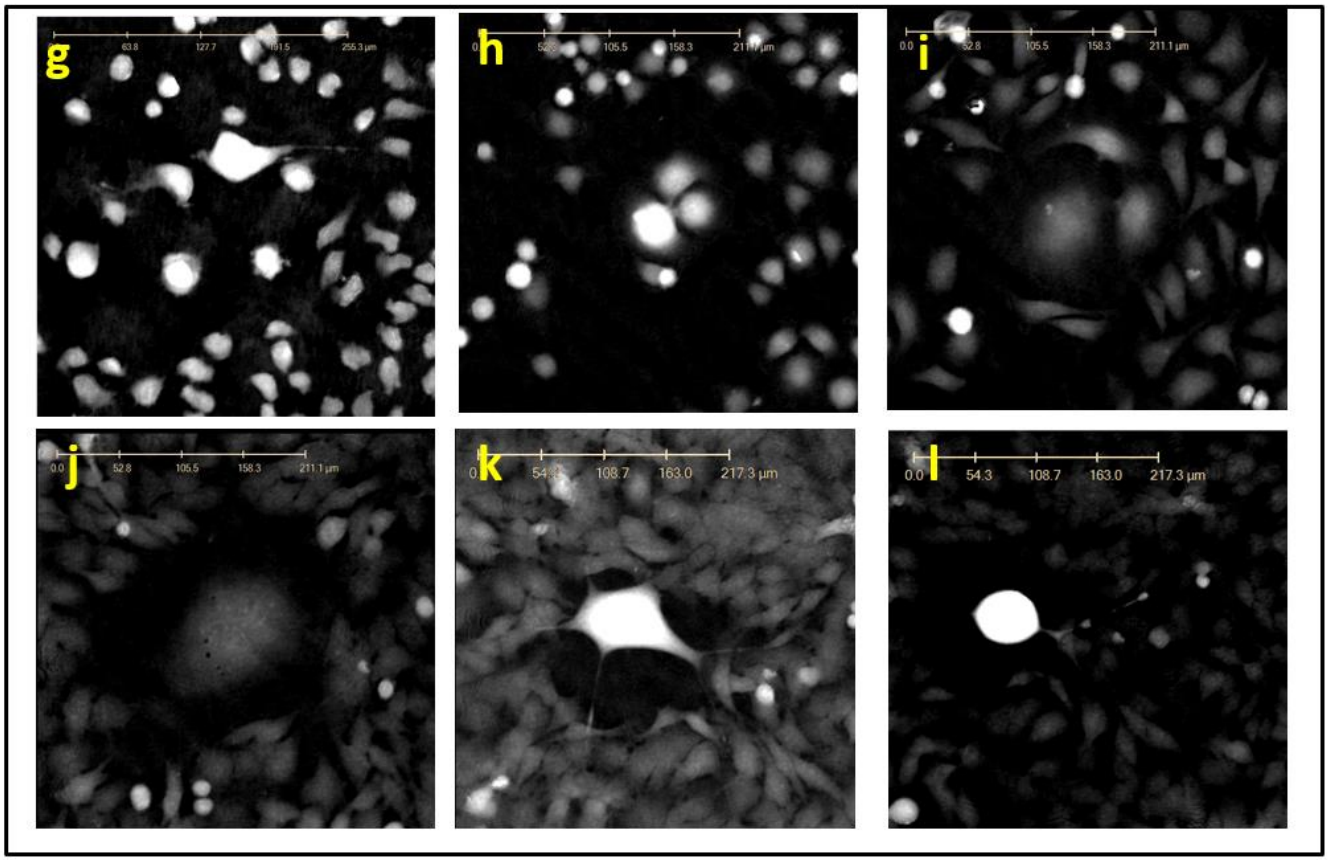
Observation of Giant HeLa Cells



- **Initial seeding, expansion of the cytoplasm forming a clear zone around the cell, autophagy of a portion of the nucleus, and the eventual release of neoplastic progeny.**

A cell with an estimated ploidy level of 32X was followed for 5 days. The processes involved are consistent with neosis, a newly discovered model of tumor cell stem cell generation.

Observation of Giant HeLa Cells



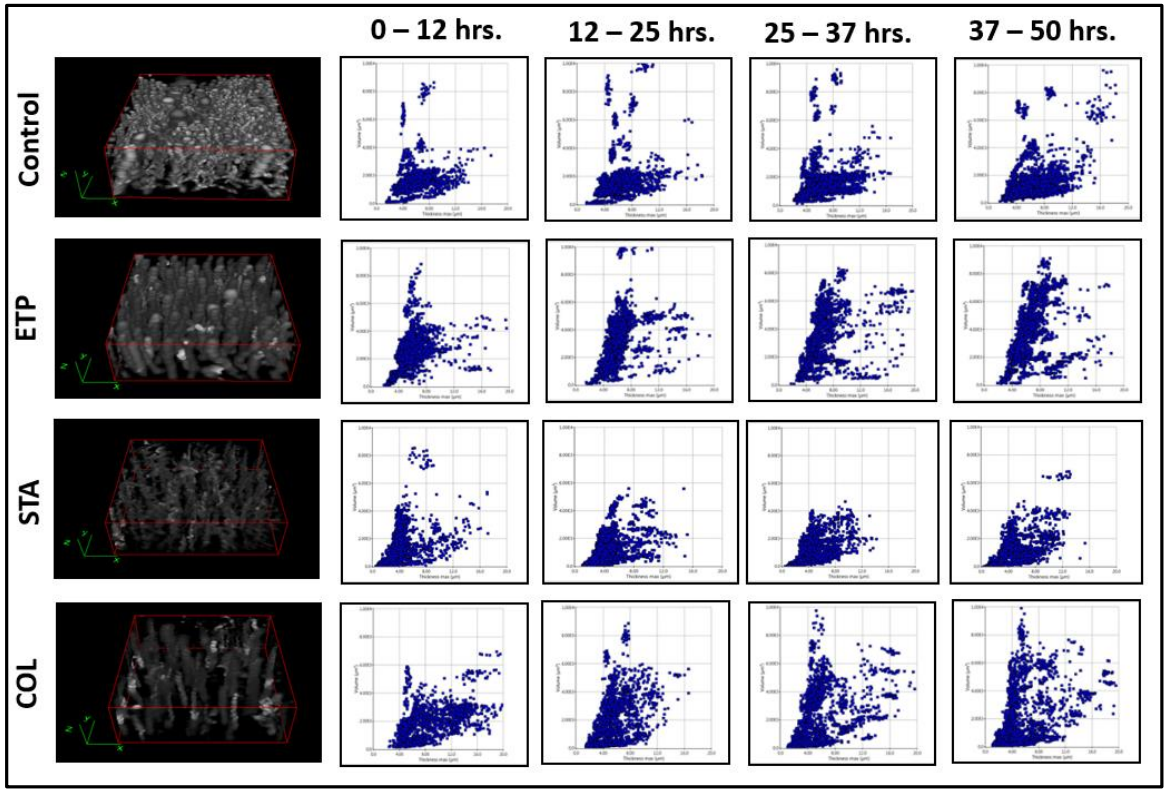
- **Trypsinization, a triad of cells after seeding, separation of the cells, reunification of the cells, and finally rounding of the cell .**

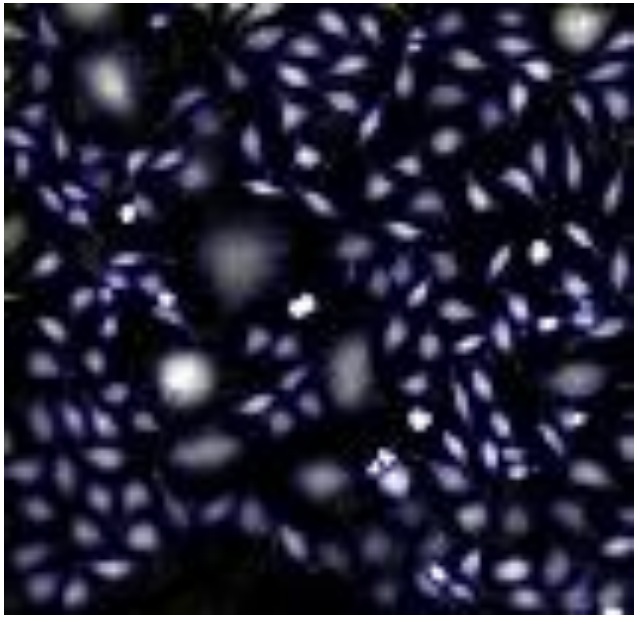
Visible connections between the cells, which cause them to form giant multinucleate cells – a suspect in tumor stem cell generation.

VALIDATION OF HOLOMONITOR TECHNOLOGY

LABEL-FREE QUANTIFICATION OF CELLULAR FEATURES

Label-free Quantification of the Effects of Cell Cycle Related Toxins

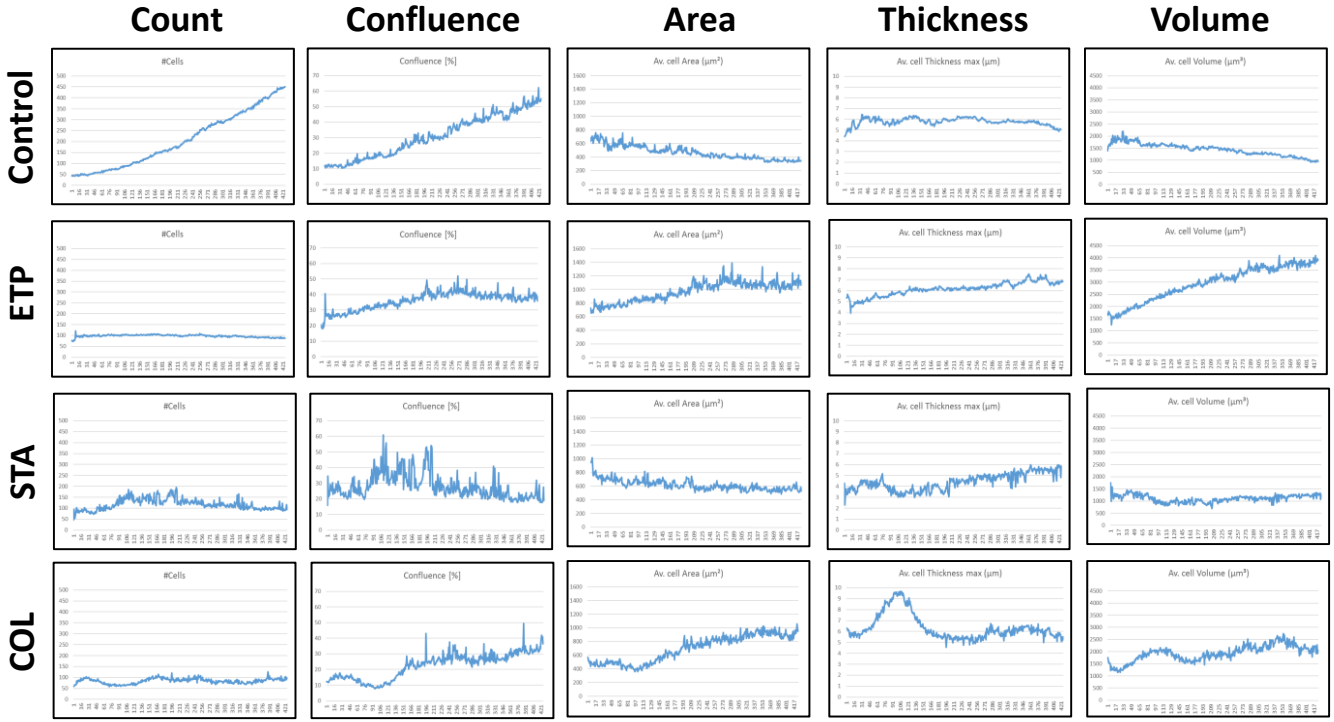


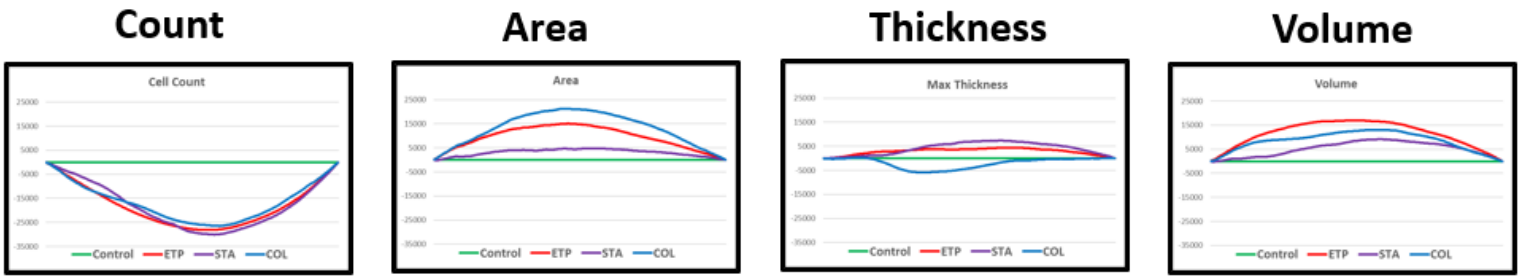


- **L929 cells treated with Etoposide, Staurosporine and Colcemide displayed in thickness vs. volume scattergrams.**

L929 cells have very strong contact inhibition. Lanks and Lehman (Can. Res, 1990) described their tendency to form giant multinucleate cells.

Label-free Quantification of the Effects of Cell Cycle Related Toxins





Time plots of feature values of the previous data set and Kolmogorov Smirnov D-value plots.

A four sample version of the Kolmogorov Test – Poster Number B56.

APPLICATIONS OF HOLOMONITOR TECHNOLOGY

Journal of Clinical & Cellular Immunology

Oka, et al., J Clin Cell Immunol 2015, 6:2
http://dx.doi.org/10.4172/2155-9899.1000326

Research Article Open Access

Macrophage Polarization and the Effect of MicroRNA-155 Administered in Water-in-Oil-in-Water Multiple Emulsion Formulations

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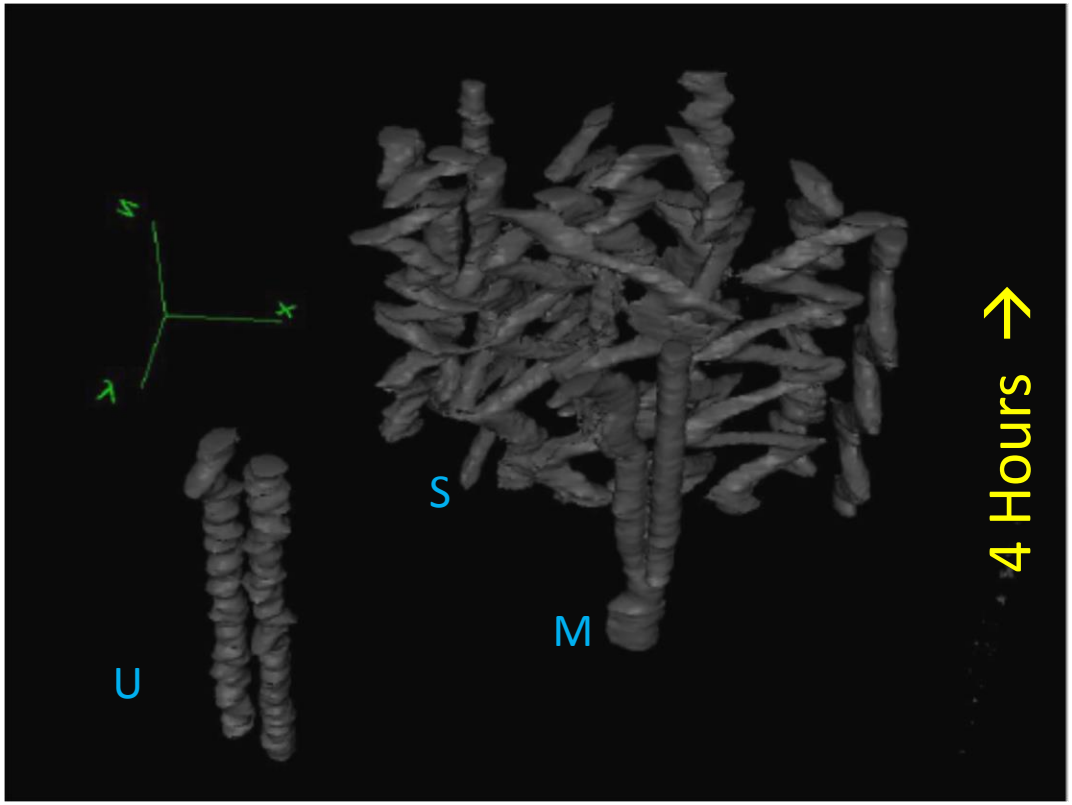
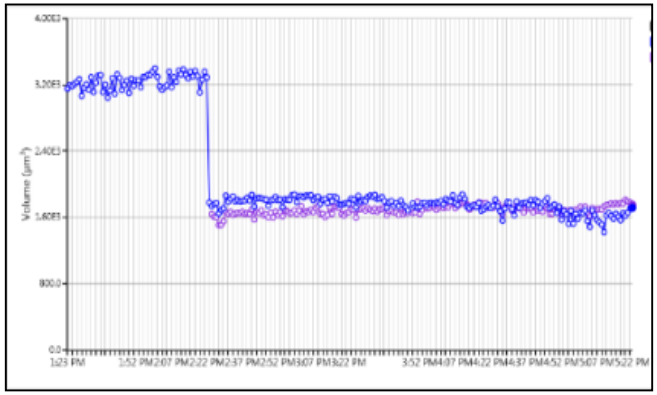
Department of Pharmaceutical Sciences, School of Pharmacy, Northeastern University, Boston, MA 02115 USA

- ***M2 macrophages are pro – tumor.***
- ***M1 macrophages are tumoricidal.***
- ***MicroRNA-155 has the property to convert M2 to M1.***

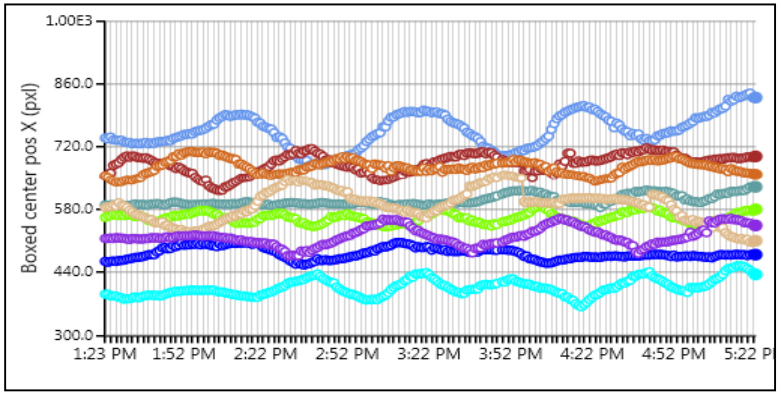


LPS Stimulated, Unreactive, and Mitotic

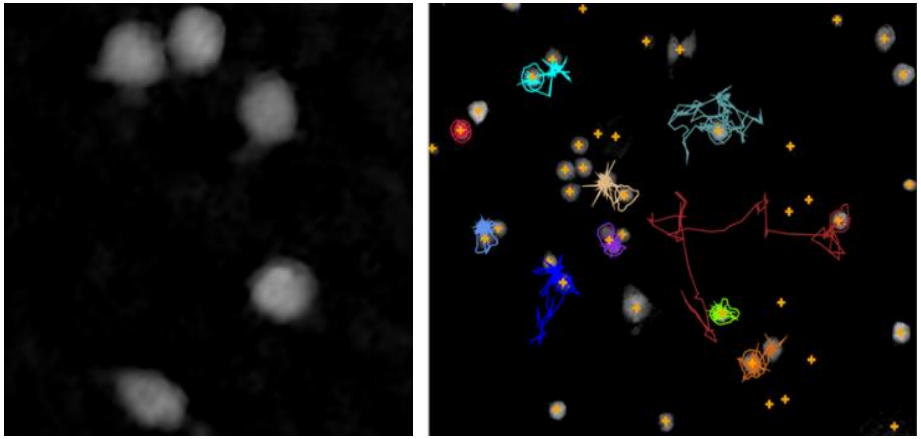
Macrophage Polarization with Chemical Stimulation



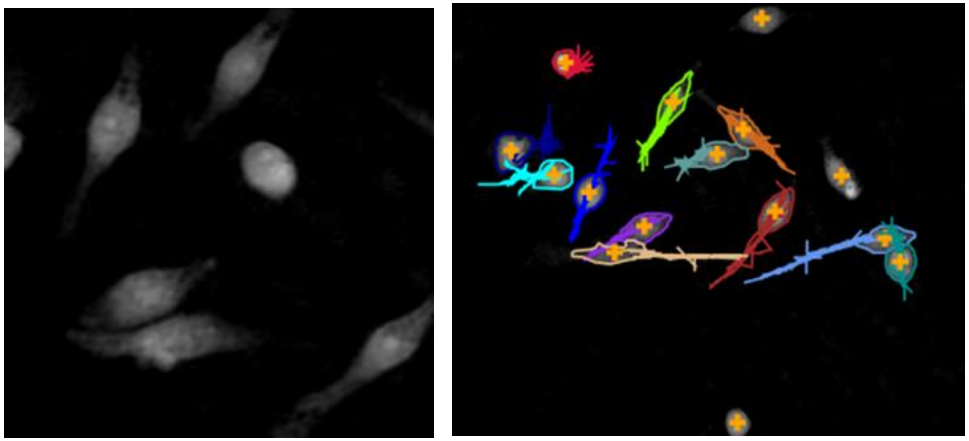
Time vs. Cell Volume plot of the previously shown mitosis.



Time vs. X Position of previously shown stimulated cells.



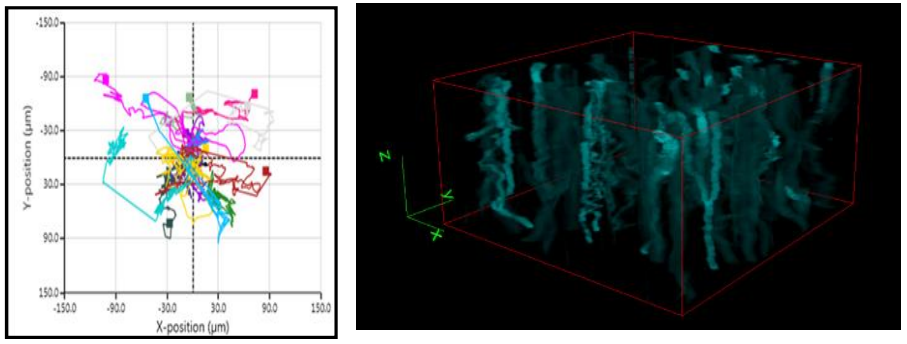
IL4 stimulated macrophages (M2).



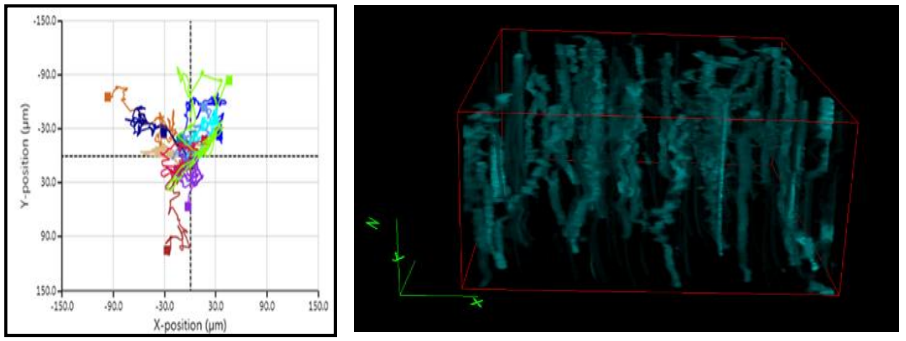
LPS stimulated macrophages. (M1)

Chemically stimulated J228 macrophages present different motility and cellular morphologies.

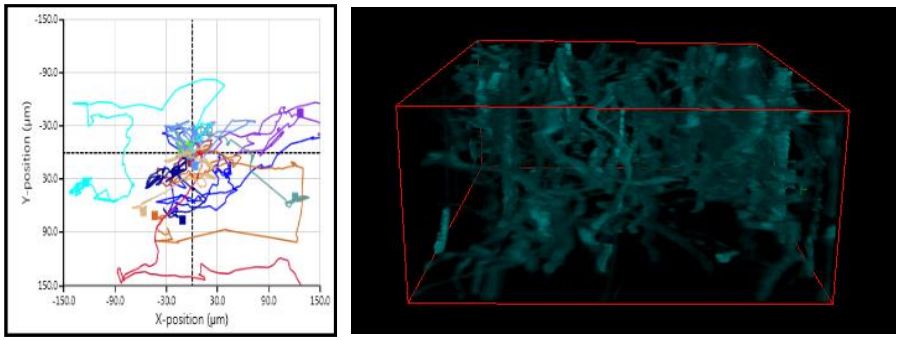
Macrophage Polarization –
Co-culture with SKOV-3 Ovarian Cancer Cells



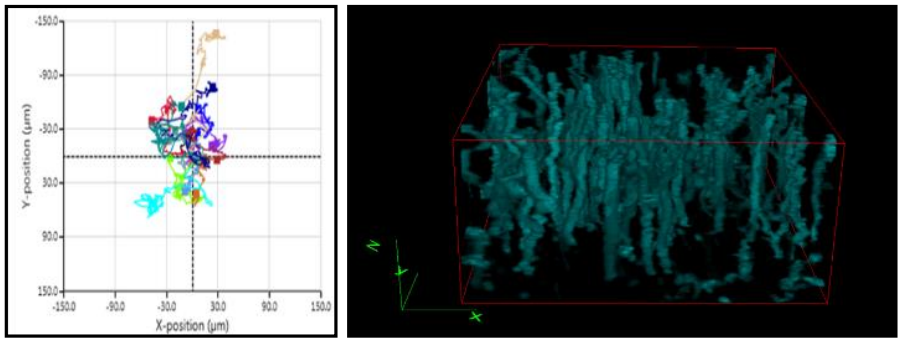
IL4 stimulated macrophages (M2)



LPS stimulated macrophages (M1)



MicroRNA Control



MicroRNA-155

The motility and patterns of the MicroRNA control emulate the IL4 treated cells while the MicroRNA-155 emulates the LPS stimulated cells.

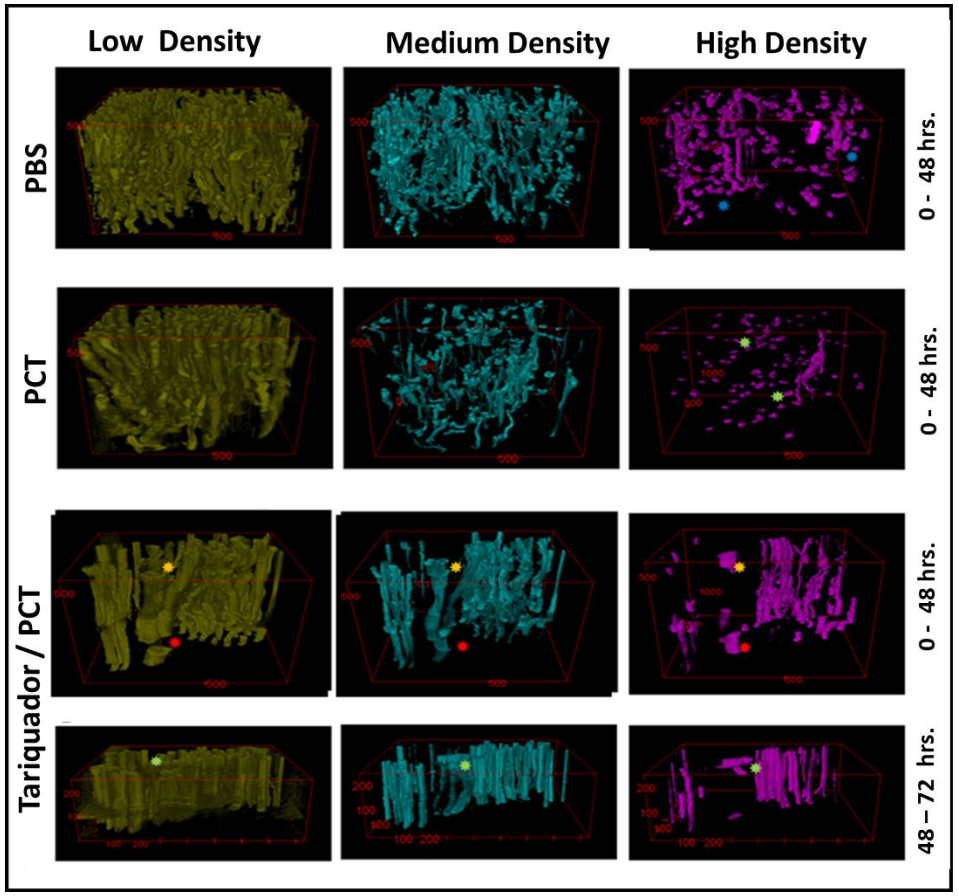
Small Molecule Therapeutics

Molecular Cancer Therapeutics

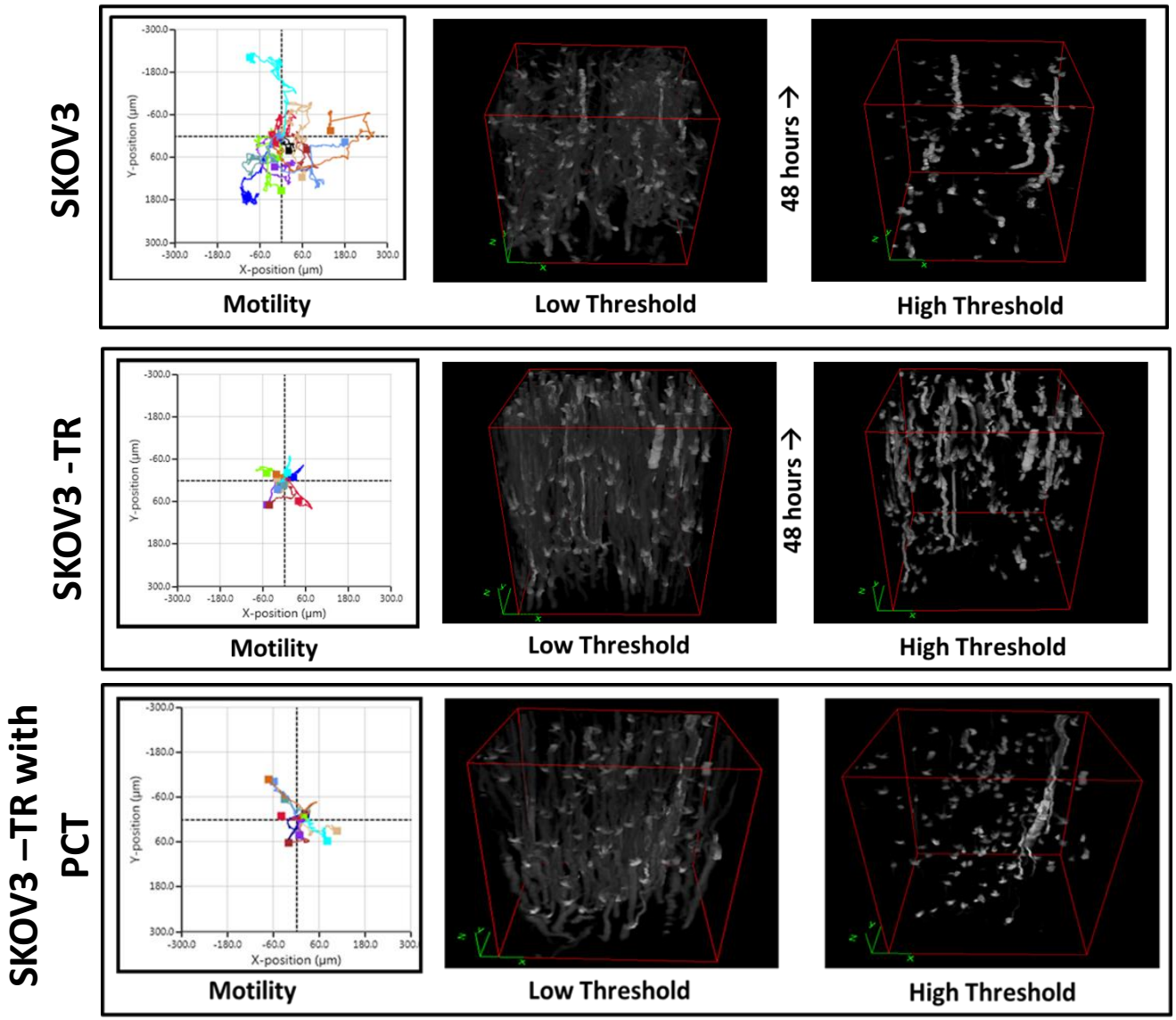
Reversal of Chemoresistance in Ovarian Cancer by Co-Delivery of a P-Glycoprotein Inhibitor and Paclitaxel in a Liposomal Platform

Yilin Zhang[1], Shravan Kumar Sriraman[2], Hilary A. Kenny[1], Ed Luther[2], Vladimir Torchilin[2], and Ernst Lengyel[1]

- *Ovarian cancer has a high mortality rate due to the development of multi-drug resistance.*
- *The strategy was to combine the toxin Paclitaxel (PCT) with a MDR inhibitor - Tariquidar- in a single liposomal delivery system.*
- *The results were evaluated in part, by monitoring SKOV3 – Taxol resistant tumor cells treated with the formulation and controls over multiple day periods.*



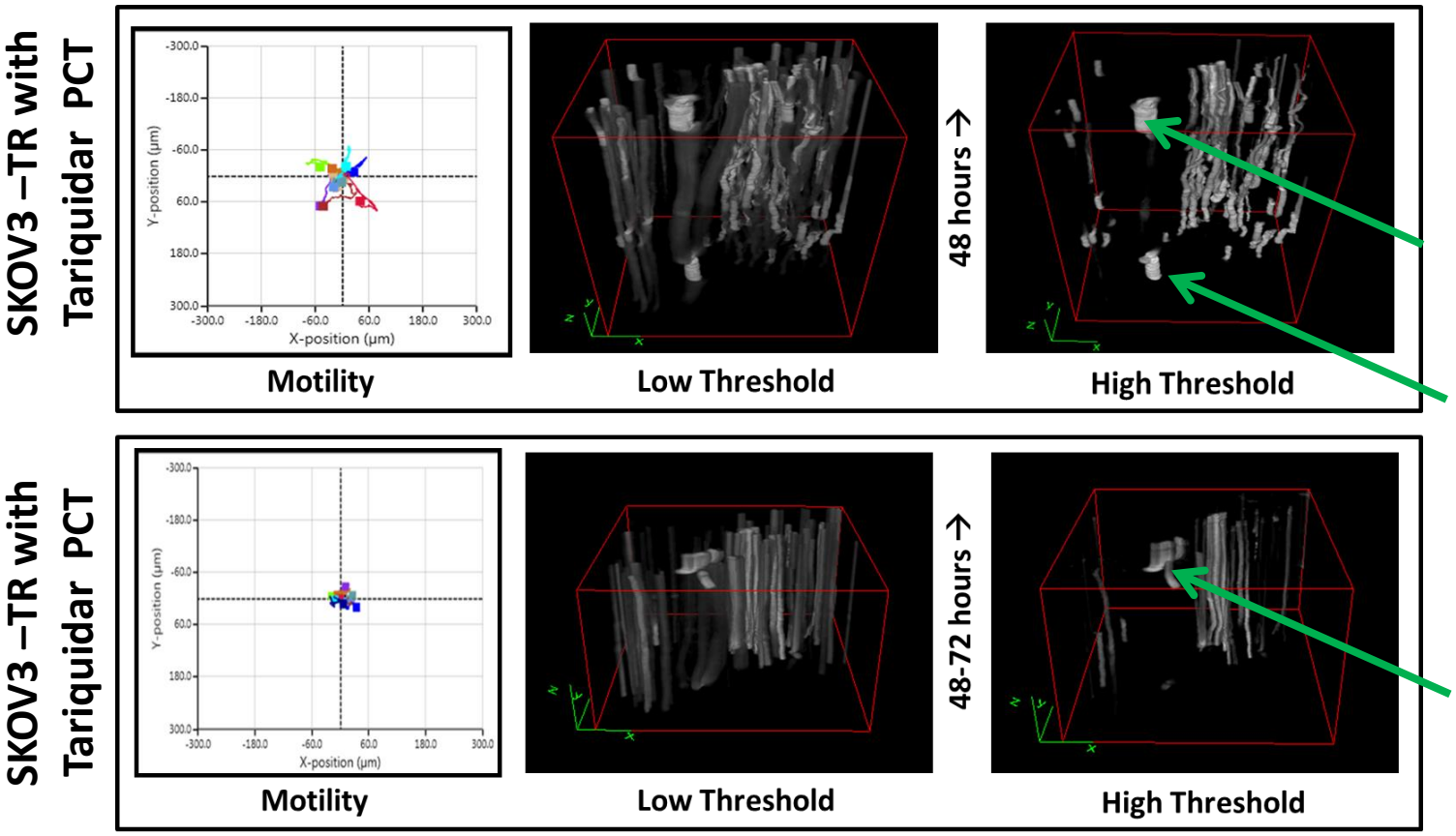
Reversal of Chemo-resistance in Ovarian Cancer



Reduced motility in the SKOV-3 TR

Increased proliferation in response to PCT

Reversal of Chemo-resistance in Ovarian Cancer



↖ a giant cell that went through three rounds of attempted mitosis

Many lines of evidence are used to validate the efficacy of the compounds. The HoloMonitor M4 data shows longer treatment time required for the giant cell.

Materials Views
www.MaterialsViews.com

small

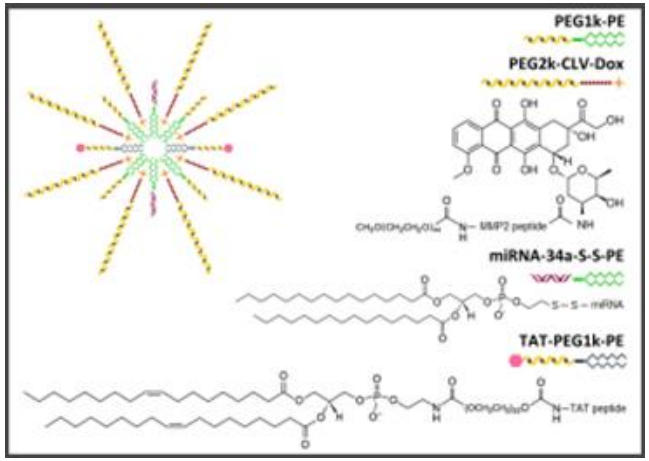
Cancer Therapy

Mixed Nanosized Polymeric Micelles as Promoter of Doxorubicin and miRNA-34a Co-Delivery Triggered by Dual Stimuli in Tumor Tissue

*Giuseppina Salzano, Daniel F. Costa, Can Sarisozen, Ed Luther, George Mattheolabakis, Pooja P. Dhargalkar, and Vladimir P. Torchilin**

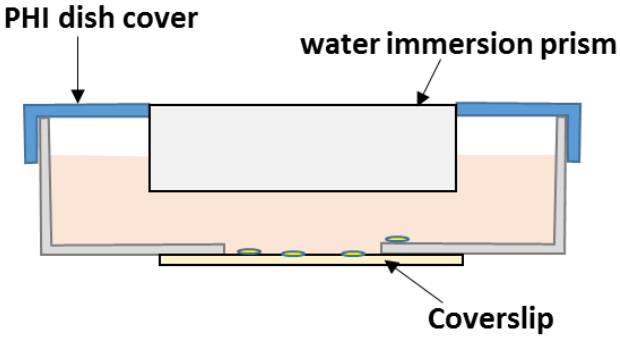
This developed formulation contains:

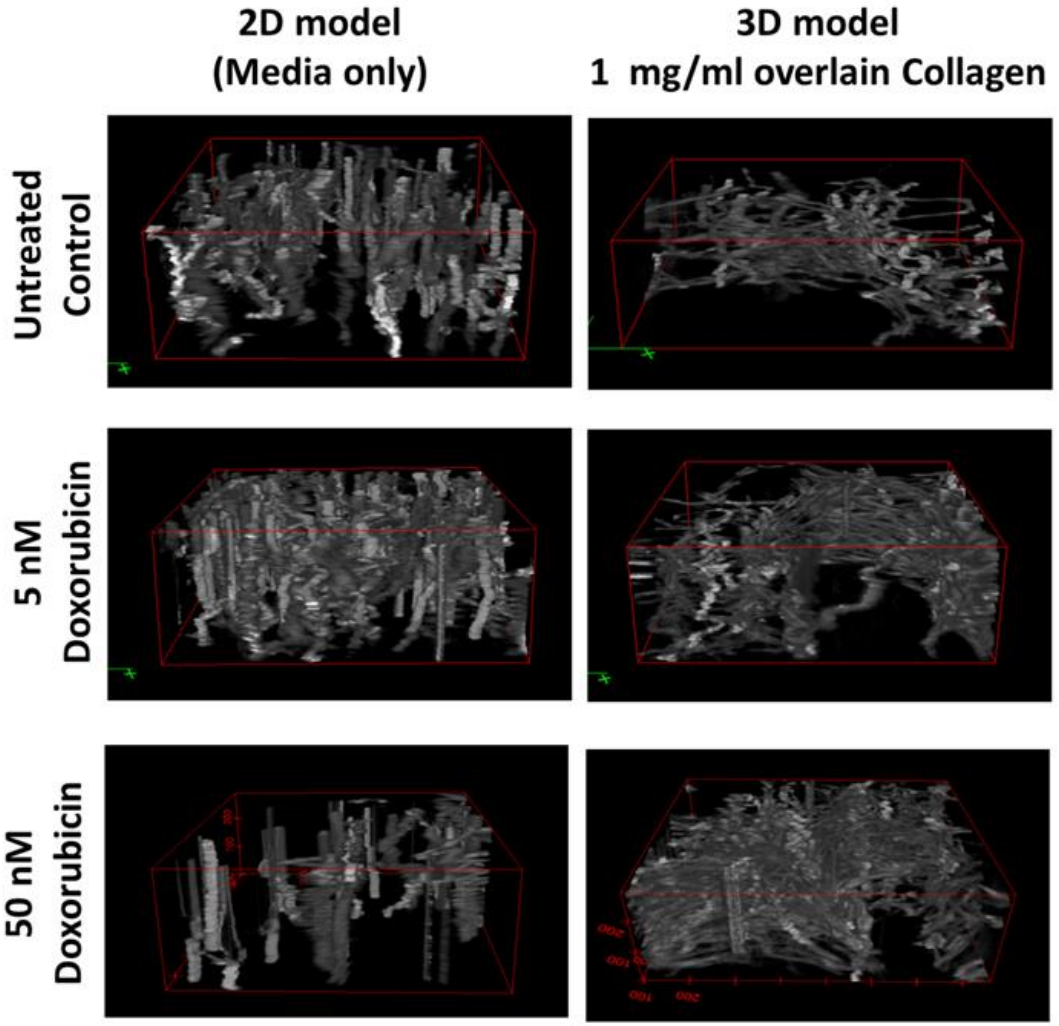
- ***CLV Dox – a prodrug that requires MMP2 to be activated.***
- ***MiRNA34a- a prodrug that requires glutathione to be activated and induce p53 down regulation.***
- ***TAT peptide to ease entry into the cells***
  The test cells are HT1080 fibroblasts MMP2 overexpressing.
- ***Exist in amoeboid and mesenchymal forms.***



Experiments were performed in 2D and 3D models .

Mixed Polymeric Micelles for Dual Stimulus

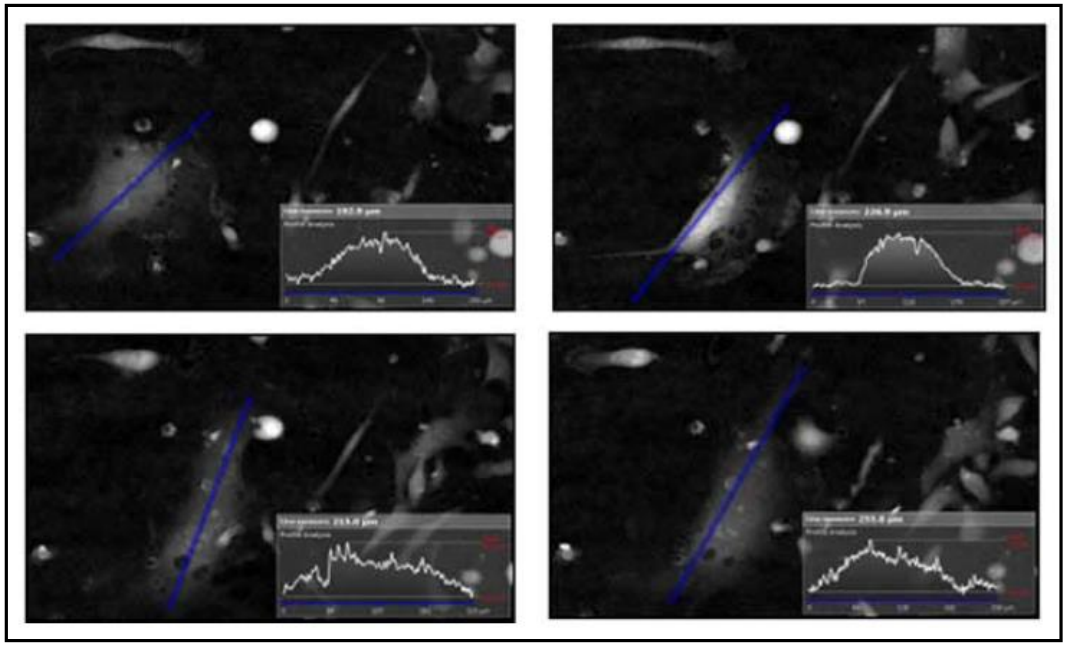


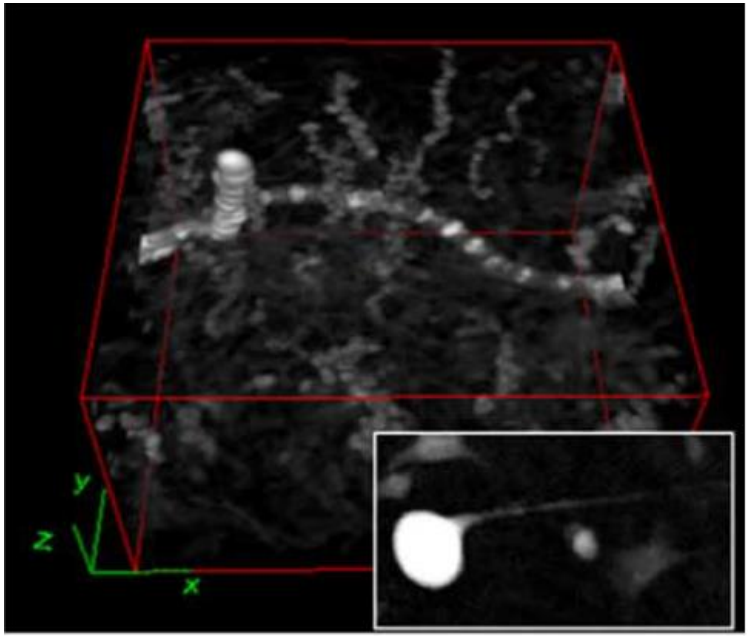


- HT-1080 cells treated with Doxorubicin in 2D and 3D models

In 3D models, HT-1080 cells have greater lateral motion and are less affected by DOX.

Mixed Polymeric Micelles for Dual Stimulus





- **Giant amoeboid and mesenchymal phenotypes found in the 3-D HT-1080 cultures**

Increasing number of publications on the subject of Giant Polyploid Cancer Cells and their role as tumor stem cells.

Incomplete chemotherapy, especially with agents like Dox, can be responsible for tumor stem line perpetuation.

Summary

- *HoloMonitor M4 adds a new dimension to our portfolio of complex imaging technologies – TIME!*
- *We developed a novel method for visualizing 4-dimensional time-lapse studies.*
- *We demonstrated that the same principles that are used in fluorescence analysis can be applied to label-free analysis.*
- *We provided examples of how phase holographic imaging is being applied in pharmaceutical nano-formulation development.*