Abstract # 202 Four dimensional quantitative label-free holographic imaging of the cell cycle in tumor cell lines

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Introduction

Label free imaging and analysis of cells using only their physical characteristics is desirable in pharmaceutical studies because it eliminates the effects of labeling compounds on the cells under study.

We used a newly developed holographic imaging system to monitor cell-cycle effects in long term cell cultures.

Materials and Methods

Instrumentation: Time-lapse holographic imaging cytometer HoloMonitor® M4 (Phase Holographic Imaging, Lund, Sweden).

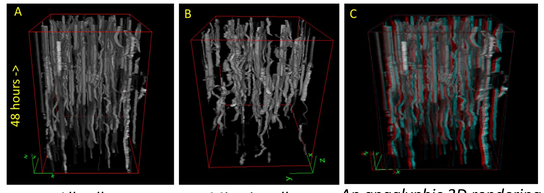
- Label-free analysis and incubator adapted.
- Holographic imaging of interference patterns from a low power 635 nm diode laser.
- Quantitative phase shift measurements are translated by software algorithms into morphological parameters – optical cell volume, thickness, etc.
- Multiple segmentation algorithms to identify objects.
- XY co-ordinates enable cell tracking over time.
- Time-lapse acquisition at selected intervals ranging from seconds to multiple days

Pharmacodynamics application: SKOV3 and taxol-resistant SKOV3 (SKOV3 TR) cells were seeded into T25 culture flasks, after treatment with various agents, and imaged for a minimum of 48 hours. Our objective was to evaluate effects on cell cycle of a newly developed liposomal formulation containing a multi-drug resistance inhibitor, tariquida, combined with the chemotherapeutic agent paclitaxol.

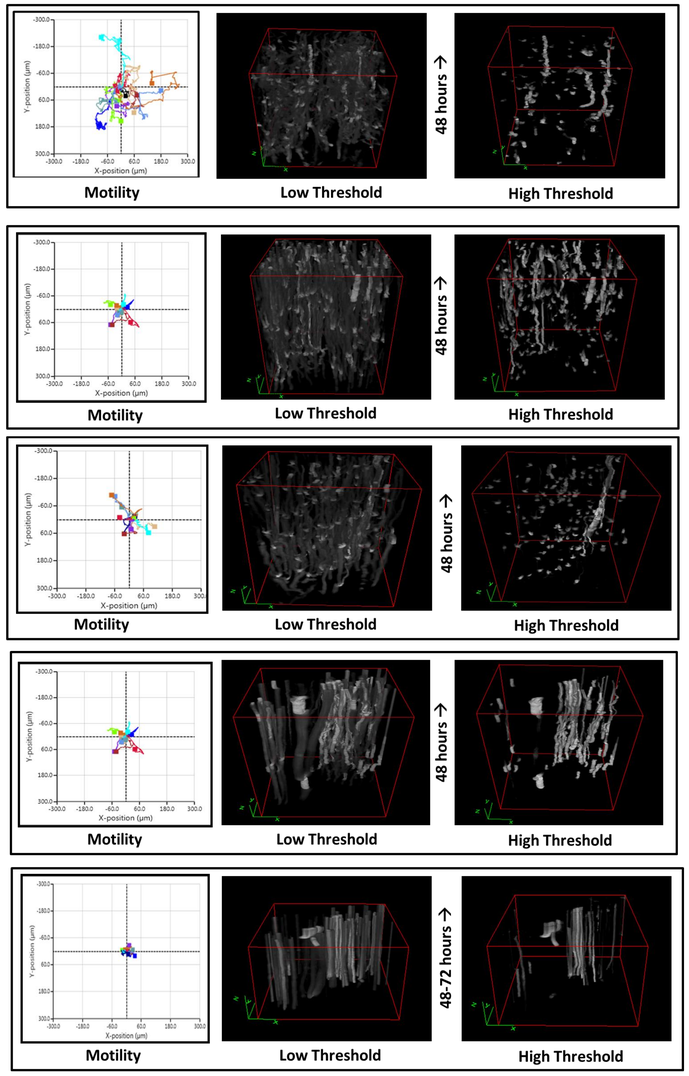
Note: Primary presentation of results in "Reversal of chemo-resistance in ovarian cancer cells by the liposomal co-delivery of MDR inhibitors and paclitaxel", Poster Section 29, Board 3, Tuesday, April 22. Shravan Sriraman et. al.

Tracking "giant" HeLa cells: HeLa cells were obtained from the ATCC and cultured under standard conditions. Representative images were acquired immediately after seeding throughout the culture vessel to obtain the cell volume distribution of the culture. Events of interest, abnormally large cells, were selected for multiple day time lapse viewing at 5 minute intervals.

4D quantitative holographic imaging: HeLa cells were treated with colchicine to block them in mitosis, and imaged for 24 hours. The brightness of an event corresponds to the spatial Z dimension, while the stack Z position corresponds to time. The passage of time is on the Y axis. Mitotic cells have a whiter color than interphase cells.



Pharmacodynamics application



Untreated

Sensitive SKOV3 cells have some degree of motility.

In the 4D plots, the short bright tracks are mitotic cells. The long bright tracks are cells stuck in mitosis.

Untreated

Taxol resistant SKOV3 cells loose most of their motility.

The number of cells blocked in mitosis appears to be increasing with time.

Taxol Only

There may be a slight effect of the taxol added to SKOV TR early in the analysis (brighter mitotic nuclei), but it diminishes over time.

Tariquidar and Taxol

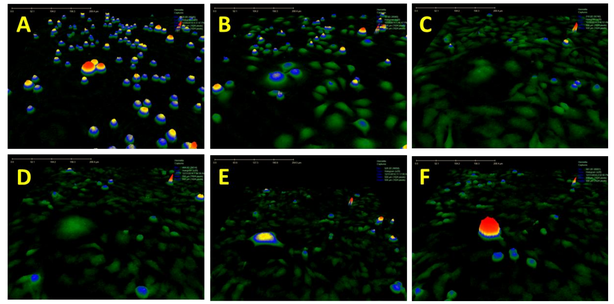
The P-gp inhibitor/taxol liposome treated cells show immediate effects. The smaller cells in the background are all in mitotic catastrophe. The large cell in the left center made it through one mitosis early, and is starting a second one.

Tariquador and Taxol

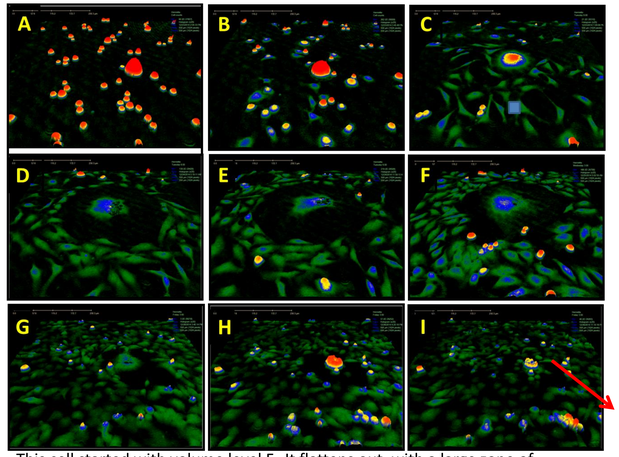
In the continued analysis of the previous sample, there is no cell motility.

The large cell goes on to attempt, but fail, a third mitosis.

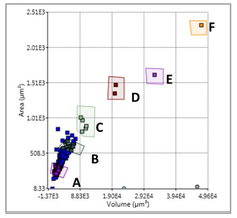
Tracking giant HeLa cells



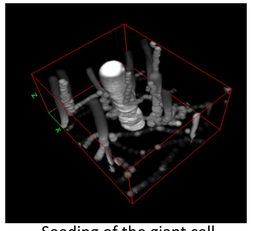
Event of interest started as a triad of giant cells (A), followed by their separation (B-C), and then reunion (D-F).



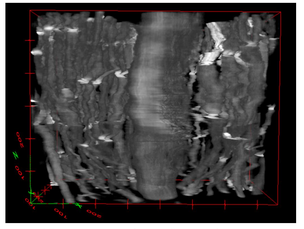
This cell started with volume level F. It flattens out, with a large zone of repugnance around it. The central part is tri-lobate, with vacuolization in one of the lobes (D-E), Eventually, goes through a mitotic crisis, and small cells emerge.



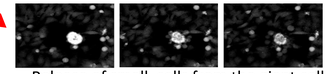
Optical Volume vs. Area of HeLa cells



Seeding of the giant cell



4D cross section of the giant cell



Release of small cells from the giant cell.

Conclusions:

1. **HoloMonitor M4 enables new non-invasive quantitative monitoring of live cells over long periods of time with high applicability in monitoring drug effects on cell cycle or tracking rare cellular events.**
2. **In the SKOV3 pharmacodynamics study, we were able to track and differentiate mitotic cells from those undergoing mitotic crisis/catastrophe.**
3. **In the giant cell tracking study, we identified cells consistent with those undergoing Neosis as originally proposed by Sundaram (2004)*.**

*Sundaram, M.,Guernsey, D.,Rajarman, M., and Rajarman, R. Neois, a novel type of cell division in cancer, Cancer biology & Therapy 3:2, February 2004