Characterization of macrophage behavior by 4-dimensional label free, quantitative holographic imaging

Abstract # 2341

Ed Luther, Meghna Talekar, Adwait Oka, Qijun Ouyang, Mansoor Amiji. Northeastern University, Boston, MA

E.luther@neu.edu

Macrophages in the Tumor Microenvironment

The tumor microenvironment consists of several cell types including cancer cells and stromal cells (fibroblasts and inflammatory cells) embedded in extracellular matrix. Among the inflammatory cells, macrophages are critical mediators of tumorigenesis. Their behavior and activation is controlled by a large number of chemo-stimulants, and are broadly classified into two polarization groups, M1 (classically activated macrophages), that enable the release of cytokines and chemokines that induce tumor suppression and M2 (alternatively activated macrophages), that induce tissue repair and angiogenesis. In the tumor microenvironment this translates into tumor promotion. We employed a newly developed label free holographic time lapse imaging system to characterize the morphology and motility of macrophages in response to stimulants and tumor cells in 4-dimensional models of the tumor microenvironment.

Materials and Methods

Pharmaceutical

J774A.1 murine macrophage cell line and SKOV3 human ovarian cancer cell lines were purchased from American Type Culture Collection (ATCC; Manassas, VA), and cultured by standard tissue culture techniques.

In some experiments, cells were stimulated with lipopolysaccharide (LPS) to induce M1 polarization or IL4 to induce M2 polarization.

In other experiments, the cultures were treated with an emulsion system encapsulating miR-155 encoding plasmids, to attempt to switch the polarity of M1 polarized macrophages to the M2 polarization state.

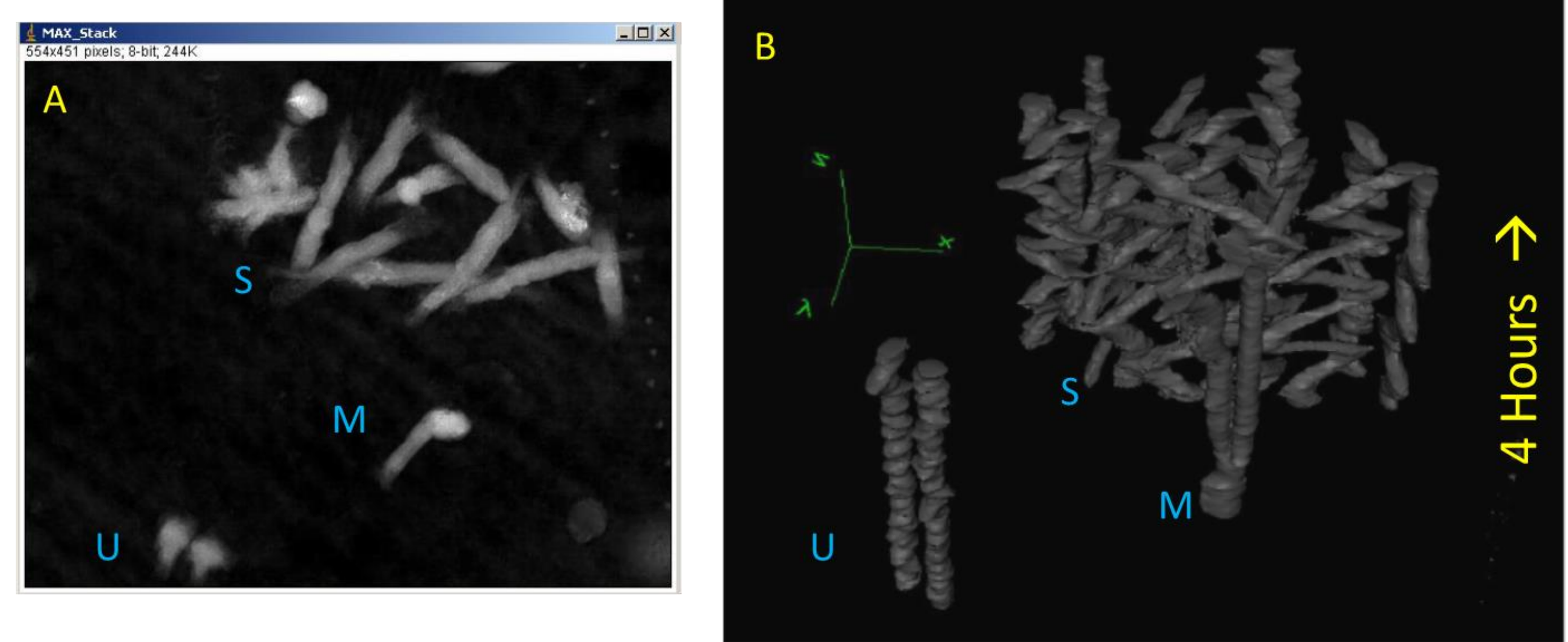
On analysis day, the macrophage populations were first imaged for a period of 5 hours at 5 minute intervals to characterize cellular motility and morphology. Vessels containing SKOV3 TR were prepared and imaged for 2 hours at 5 minute intervals to allow the cells to adhere, and then obtain base line tumor cell measurements. Thereafter, treated cultures to set up a co-culture system. Imaging was performed using HoloMonitor M4 on this co-culture system for 16 hours at 5 minute time intervals .

HoloMonitor® M4 manufactured by Phase Holographic Imaging, Lund, Sweden

- Incubator adapted.
- Digital imaging of interference patterns from a low power 635 nm diode laser.
- Software "unwraps" the interference patterns to obtain 2D or 3D images of the optical thickness of the field of view.
- Label-free analysis.
- Time-lapse acquisition at selected intervals ranging from seconds to multiple days
- Multiple segmentation algorithms to identify objects.
- Calculates quantitative features for each event.
- Stores XY co-ordinate information for cell tracking
- Time-lapse analyses are performed using the HoloStudio software (PHI).
- The series of individual image frames are exported as two dimensional images, preferably using a continuous grey scale.
- The images are imported into Image J software and converted to stacks
- Stacks can be viewed as time lapse videos; Z-projections of maximum intensity can be obtained, as well as fully rotatable 3D images using the 3D viewer plugin
- Stereoscopic renderings are produced in Stereophotomaker software.

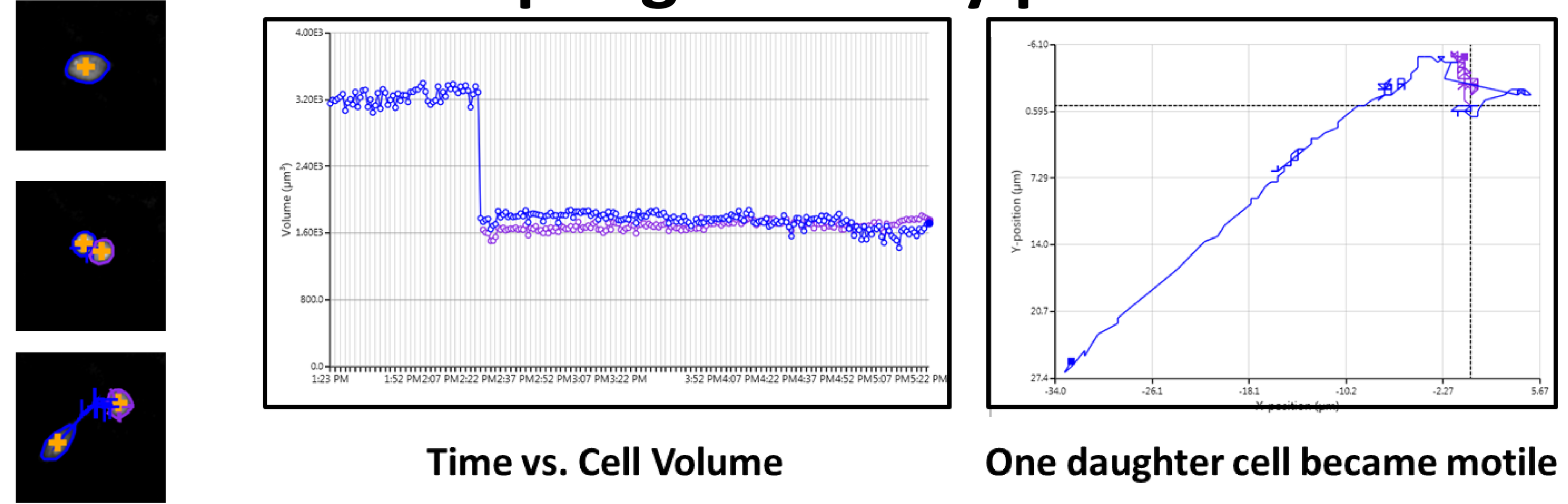
4-dimensional , quantitative holographic Imaging

Holomonitor M4 images precisely quantify the optical thickness, or cell thickness. This is particularly relevant to cell cycle studies, where mitotic cells have a greater thickness than interphase cells. Macrophages, in general, have a greater thickness than tumor cells. We take advantage of the correlation between the cell thickness and the brightness of the pixels in the gray scale to allow us to obtain 4-dimensional images of the cells in the viewing area.

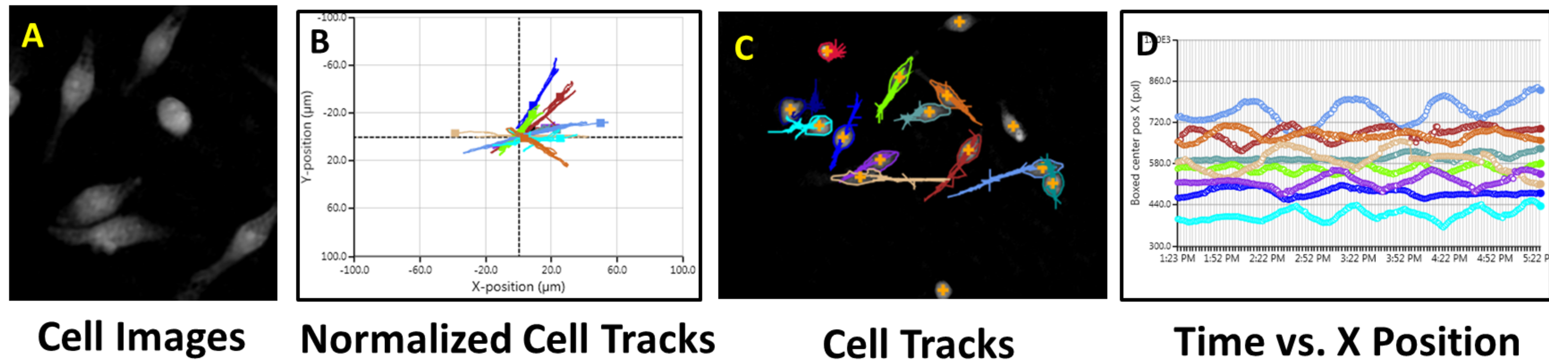


4D renditions of an analysis of LPS stimulated macrophages. **A** shows a maximum pixel Z projection of the 4D stack. The "objects" are the area covered by the cells over time. **B** shows an axiometric 3D projection. Letter **M** refers to a mitotic event, **S** refers to shuttling behavior, and **U** refers unstimulated cells.

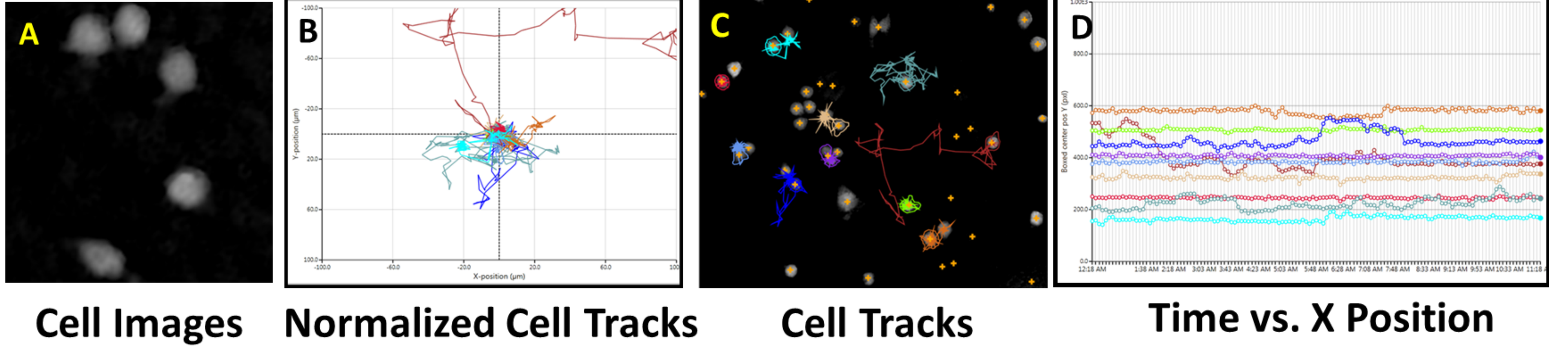
Macrophage motility patterns



One type of cellular motility is mitosis related, when cells pull apart from each other during the cytokinesis. Note the accuracy in the cell volume measurements. Interestingly, only one of the daughter cells becomes motile. This is the M event from the 3D rendition.



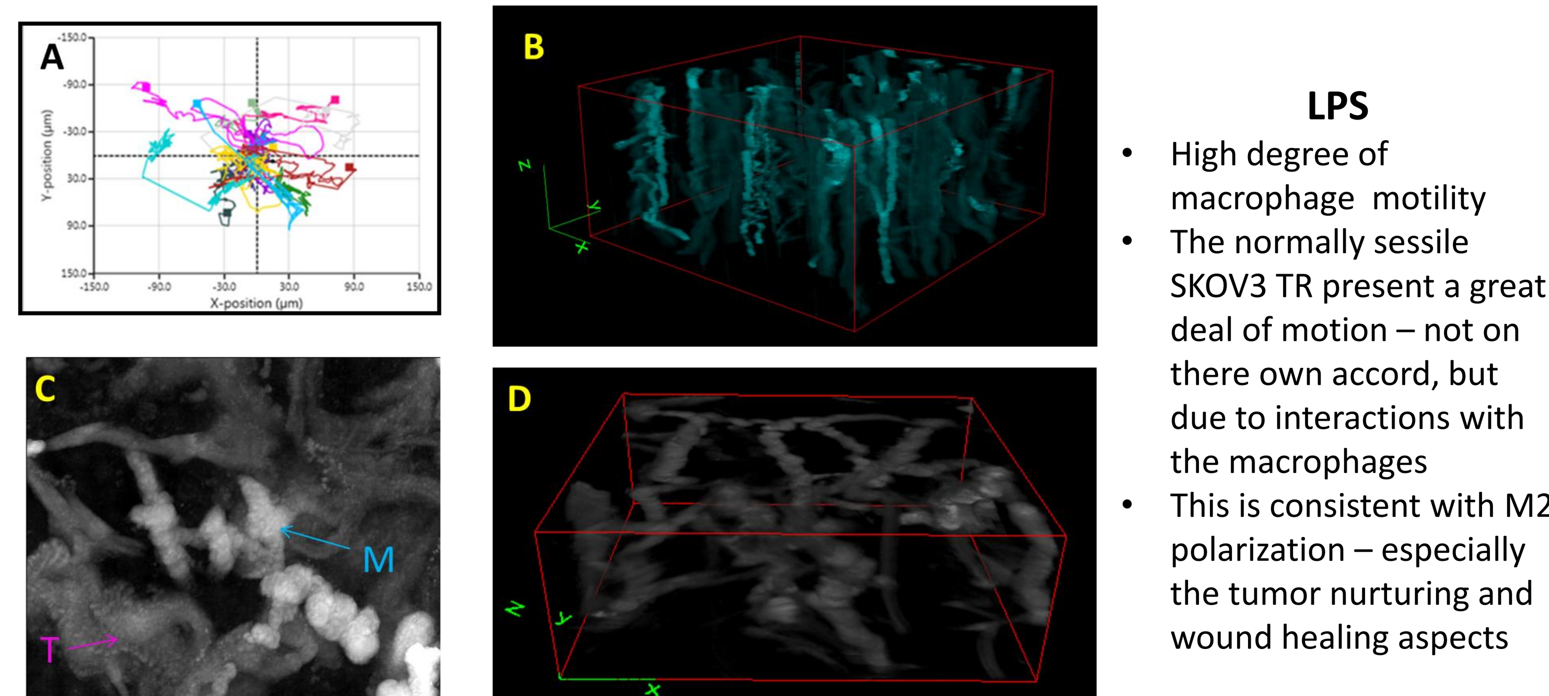
This is the S event, a cluster of cells that are responding to LPS stimulation. Their morphology changes to bilaterally symmetrical, with prominent alternating filipods. In our system, unlike standard Chemotaxis assays, there is no directionality to the signal. They are ready to go, but they do not know where. We believe their bi-lateral symmetry is a hallmark of M2 polarization.



The morphology of IL4 stimulated macrophages remains radially symmetrical, with small projections appearing randomly. As before, there is no directionality to the signal, so instead of forming linear tracks, they form starbursts. When they detect a directional chemo-attractant signal, they track as "open starbursts" – periods of directed motion interrupted by random changes in direction – a.k.a. The Drunkard's Walk. We interpret this as a hallmark of M1 polarization.

Macrophage tumor cell interactions

In this section, data from overnight (16 hour) co-incubations of treated macrophages with SKOV3 TR macrophages is presented. Each set consists of A - tracking of randomly selected macrophages, B - Low magnification 4D plot of the entire field of view, C - high resolution z-stack projection, and D – high resolution 4D plot. Remember, macrophages have a whiter color than the tumor cells.

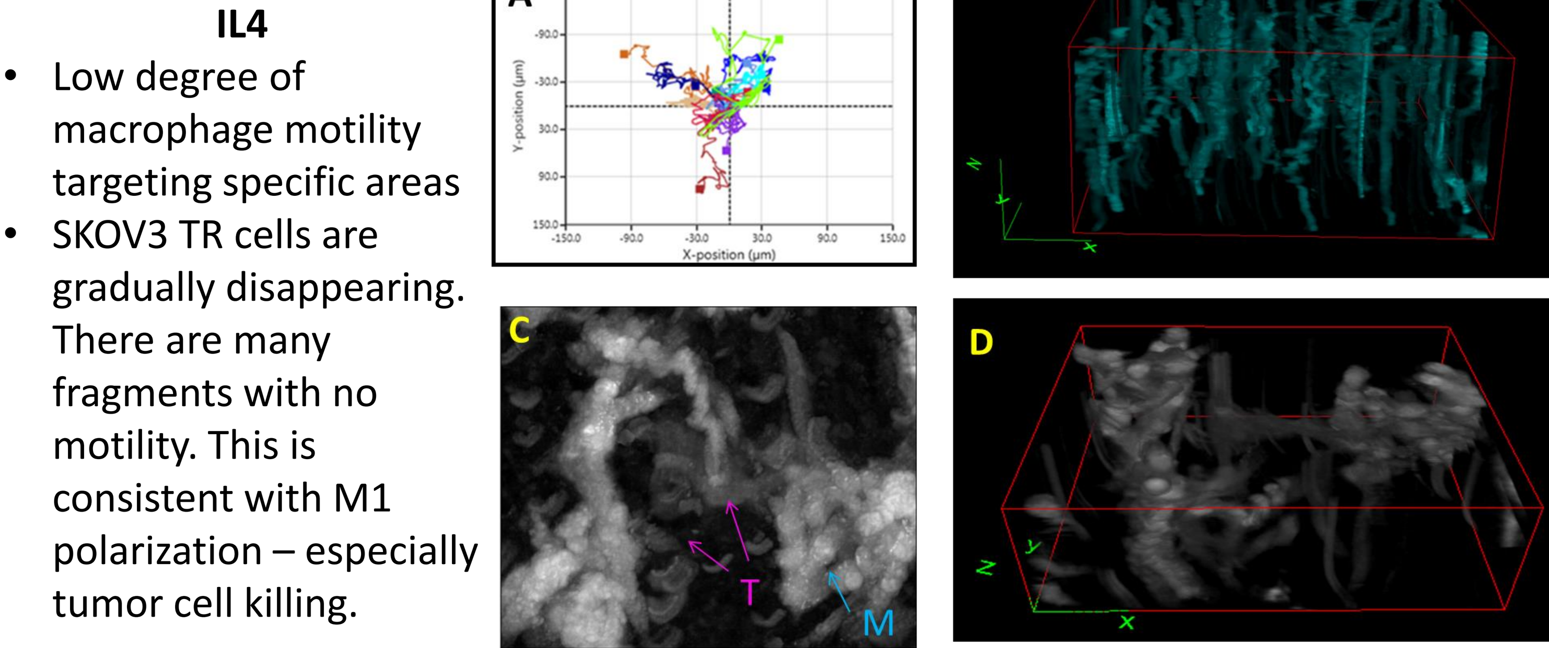


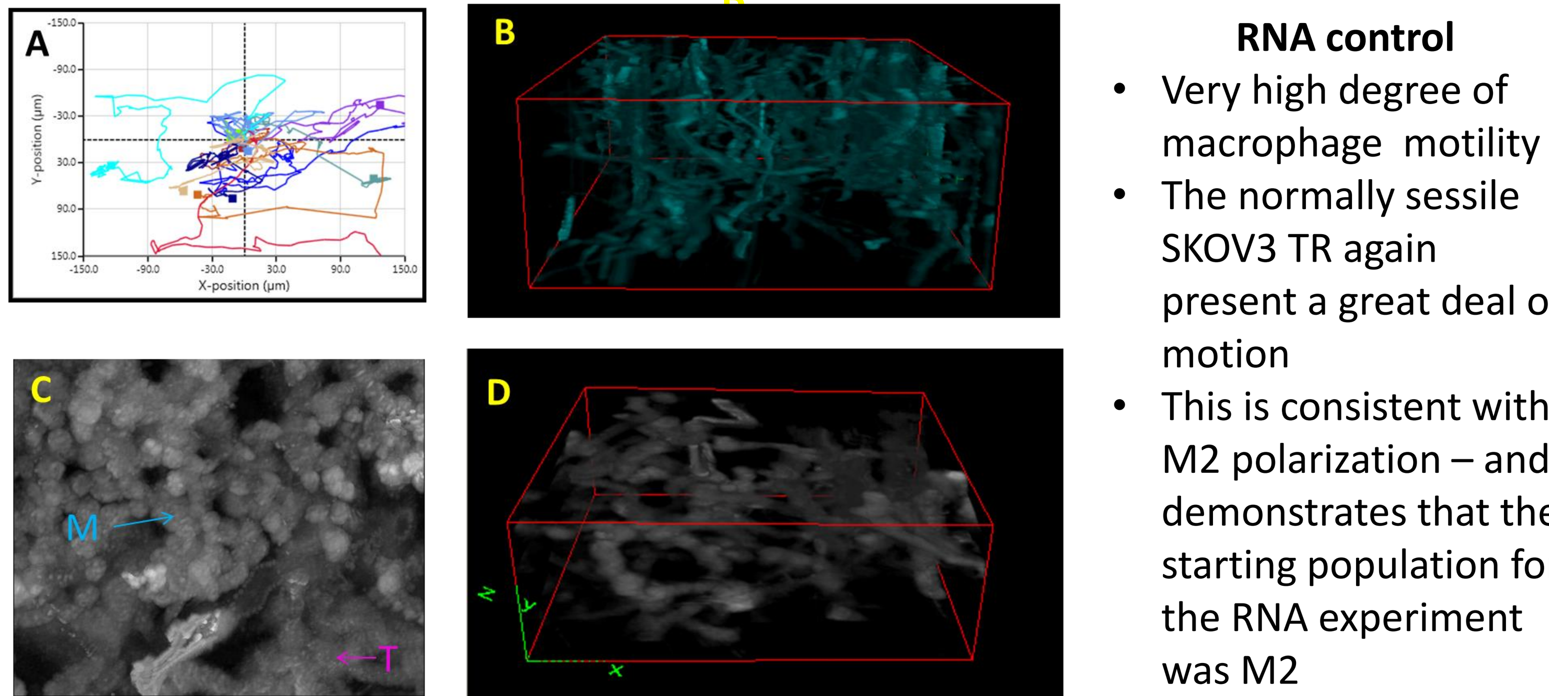
LPS

- High degree of macrophage motility
- The normally sessile SKOV3 TR present a great deal of motion – not on there own accord, but due to interactions with the macrophages
- This is consistent with M2 polarization – especially the tumor nurturing and wound healing aspects

IL4

- Low degree of macrophage motility targeting specific areas
- SKOV3 TR cells are gradually disappearing. There are many fragments with no motility. This is consistent with M1 polarization – especially tumor cell killing.





RNA control

- Very high degree of macrophage motility
- The normally sessile SKOV3 TR again present a great deal of motion
- This is consistent with M2 polarization – and demonstrates that the starting population for the RNA experiment was M2

Mir 155

- Low degree of macrophage motility targeting specific areas.
- SKOV3 TR cells are disappearing and are difficult to identify.
- This is consistent with M1 polarization. In this case via a reprograming of the M2 state via the micro RNA

Summary

We used a newly developed holographic imaging system, to track the motion and behavior of microphages and their interactions with tumor cells within the tumor microenvironment over time. Our results are completely consistent with those obtained using standard pharmaceutical evaluation techniques applied to the same samples.

This technique offers a new tool for the study of the tumor microenvironment.