Visiting Scholar Opportunity

Optical Microscopy and Analysis Laboratory

Cancer Challenge

The failure of new cancer treatments comes at an estimated average cost of $1.7 billion per drug, compounded further by an inestimable human toll caused by our inability to care effectively for many patients with cancer. All too often, initially successful treatments have proven to be temporary; recurrent cancers, resistant to the initial therapy, inevitably emerge that are frequently more aggressive than the primary tumor. The need to address this key challenge is a recognized NCI priority and is part of the core mission of the Frederick National Laboratory for Cancer Research (FNLCR).

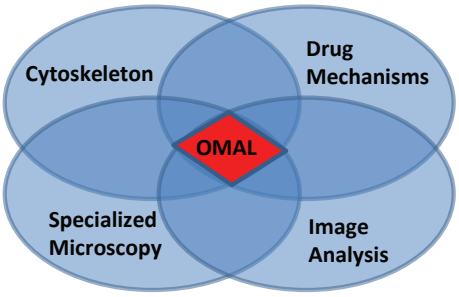
Despite the promises of "molecularly targeted therapies," only about seven percent of cancer drugs entering clinical trials actually receive regulatory approval for therapeutic applications. The majority of new cancer treatments fail due to lack of efficacy in patients, indicating that the current preclinical approaches for testing cancer drug efficacy have limited accuracy. The problem is complicated by the fact that the same drug frequently behaves differently at different locations in the cancer cell, and that cells respond differently depending on their surrounding environment in the tissue.

Consequently, standard biochemical approaches for analyzing drug mechanisms are inadequate, and there is a clear need to investigate drug mechanisms using approaches that interrogate drug action at the individual and subcellular level. There have been, and continue to be, rapid advances in optical and live cell microscopy. The chemistries for fluorescence-tagging specific proteins and the development of subdiffraction imaging technologies make it possible to investigate protein interactions in live cells,[1] and, in turn, study such interactions in response to drug exposure.

OMAL
Optical Microscopy and Analysis Laboratory

Generative Models of Cells for Understanding Drug Mechanisms

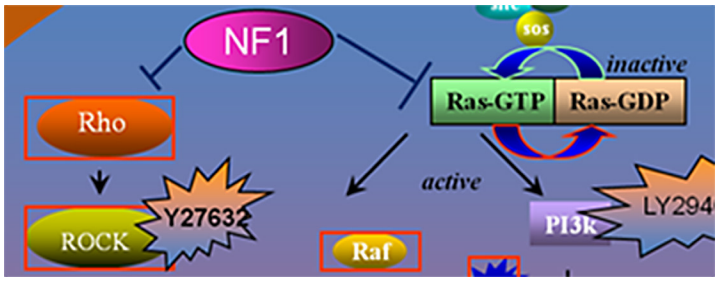
The FNLCR Optical Microscopy and Analysis Laboratory (OMAL), in collaboration with NCI's Molecular Targets Laboratory (MTL) and Mouse Cancer Genetics Program (MCGP), has an ongoing project to understand how candidate drugs alter the actin fibers (F-actin) in cells. It has long been understood that F-actin drives cell behavior and fate by controlling cell shape, the internal organization of cells, and the generation of force, and that the F-actin in cancer cells differs from that in normal cells. Furthermore, F-actin is altered by some anticancer drugs. Since more than 15 separate, spatially overlapping F-actin-based structures exist at various times in animal cells and each is assembled from a different complement of cooperating proteins,[2] it is not surprising that many drugs have some direct or indirect effect on the actin cytoskeleton.



OMAL research is multidisciplinary, ranging from the biological to the computational and from the basic to the translational.

Actin is heavily studied by others using a range of imaging technologies, including super-resolution methods to visualize actin fibers in detail[3]; live cell microscopy to track the dynamics of actin at the leading edge of cells[4]; image analysis methods to detect and track individual actin fibers[5]; and micropatterning to impose pre-defined morphology on cells[6] or for studying actin in vitro.[7]

OMAL is studying how certain natural products, specifically Schweinfurthin A (SA), exert an antiproliferative effect in tumor cells lacking the tumor-suppressor gene neurofibromin (NF1). NF1 is directly upstream of the Ras and Rho-ROCK pathways, and Rho is upstream of cytoskeletal modifications. Our results suggest that SA substitutes for NF1 when NF1 is missing in tumor cells,[8] and we are currently using quantitative microscopic imaging[9] to investigate how this natural product affects F-actin in cells.



However, it remains a major challenge to combine the disparate information from image measurements and biochemical results into a unified, deeper understanding of the mechanisms of drug action. To overcome this challenge, OMAL has begun a project to simulate the structure of F-actin in cells. The simulations are derived from known and hypothesized biophysical mechanisms, and optimized by adjusting model parameters in order to match simulated images to actual images. By studying the images of simulated F-actin in drug-treated and untreated cells, one can interpret quantitative differences in terms of the biophysical changes in both cell groups. Such biophysical changes in turn implicate not only which macromolecular alterations have occurred, but also where in the cell these alterations have occurred.

Simulation, through the technique of generative modeling, has been applied in cells over the past five years. Specifically, it was applied by Schaub et al.[10] to show that F-actin networks in keratinocyte lamellipodia can arise from a stochastic branching and capping process, confirming the molecular mechanisms proposed by Pollard et al.[11]

Working with associate professor Jason Kinser, D. Sc., at George Mason University, we have successfully used a generative model to simulate F-actin in cells. The model combines observed F-actin patterns from microscope images with the known, in vitro, biophysical properties of F-actin fibers in the cells, including the number of fibers and the nature of the bending and stretching of individual actin fibers (Figure 1A). We learned from this exercise that different models were needed to simulate the shorter, more crisscrossed F-actin fibers in the lamellipodia, and the straighter and longer F-actin fibers in the internal, lamella part of the cell (Figure 1B).

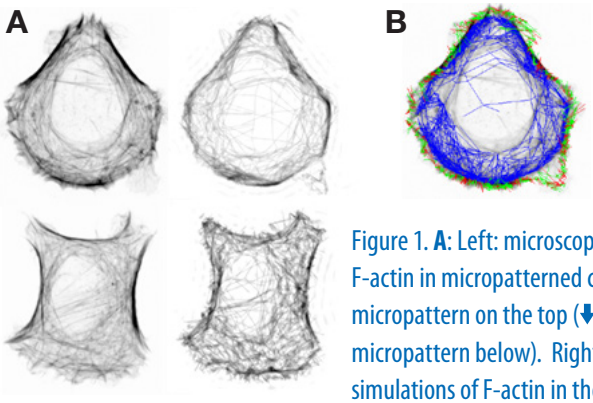


Figure 1. **A**: Left: microscope images of F-actin in micropatterned cells (Cross bow micropattern on the top (⬇), rectangular micropattern below). Right: computer simulations of F-actin in the same images. **B**: Using different generative models for lamella actin (blue) and lamellipodia actin (green and red).

Who Should Apply

We are seeking a mid-career, faculty-level visiting scholar (VS) with expertise in one or more of the following areas: (1) mathematical modeling; (2) actin dynamics and interactions; and (3) microscope-based drug screening. We foresee that the time spent by the VS at FNLCR will be part of an enduring collaboration between our laboratories. Thus, there may be accommodation for an accompanying graduate student or junior scientist should that facilitate the overall objectives.

We anticipate that the VS will be involved in one or more of the following paths of investigation: simulation of the structure of F-actin in control cells and cells treated with known actin disruptors; validation of the current model by determining where parameter re-optimization is consistent with known molecular mechanisms of action of disruptors; evaluation of the current generative model against alternative models; determination of the need to extend or replace the current model.

Once validated with known reagents, the models will be utilized to understand the mechanisms of candidate drugs. Priority will be given to candidate drugs that target key oncogenes, such as KRas. We anticipate that this visiting scholar opportunity, if successful, will result in the dissemination of the technology through software provided to NCI, a manuscript for submission to a peer-reviewed journal, and the delineation of specific aims for subsequent funding. The technology developed in this project should be applicable to the analysis of other proteins in cells, particularly other cytoskeletal proteins, or for analysis of specific organelles.

OMAL has seven full-time scientists and usually three or four personnel in training positions. OMAL activities are divided approximately equally between providing NCI at Frederick with core service, including undertaking technology developments, in the areas of optical microscopy, and performing image analysis and collaborative research that combines cytoskeletal biology, advanced microscopy, image analysis, and drug discovery to understand the molecular mechanisms of candidate pharmaceuticals.

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The Visiting Scholars Program
http://frederick.cancer.gov/Careers/VisitingScholar/Default.aspx

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