NEWS FROM THE ADVANCED TECHNOLOGY PROGRAM

ATP Update

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Director's Point of View

New Imaging Capabilities Developed at ATP

Tim Harris, Ph.D., Director, ATP

One aspect of the Advanced Technology Program (ATP) that I have been learning about since arriving here as the Director, and with which I was a whole lot less familiar than I am with genetics and genomics or proteins and proteomics, is imaging and nanoparticle characterization. The Image Analysis Laboratory, run by Stephen Lockett, Ph.D., is part of the Imaging and Nanocharacterization Group and is, as its name suggests, focused on finding and applying novel ways to analyze images collected by confocal microscopy or by electron microscopy (EM).

As you will read in this issue of the newsletter, Dr. Lockett and Jack Collins, Ph.D., of the Advanced Biomedical Computing Center, have been deriving new algorithms for interpreting cellular images. This is of great interest to our NCI customers because it enables more in-depth analysis of cell–cell communications that drive normal tissue development, tissue homeostasis, and tumorigenesis. We are also working with Sriram Subramanian, Ph.D., of NCI, to investigate ways of correlating optical images with those collected by EM.

One of the most exciting parts of working at the ATP is the breadth of technologies at our disposal. We are rapidly moving towards the time when we will be able to correlate cellular and subcellular images with those collected by molecular techniques, such as top-down proteomics or transcriptional profiling of the same cells. These new developments are all part of the plan to provide NCI with an integrated set of technologies to help understand tumor biology and drug response.

Inside This Issue

Analysis of Individual Cells in Tissue and Cell Culture

By Stephen Lockett, Ph.D.

The Image Analysis Laboratory (IAL) has developed several algorithms that facilitate the quantification of fluorescence-labeled macro-molecules in the individual cells of intact tissue and cell culture samples. These algorithms are being made available to the scientific community in collaboration with the Advanced Biomedical Computing Center (ABCC) (see "New Imaging Group Formed at the ABCC" on page 2).

Quantification at the Individual Cell Level in Tissue

By analyzing specific molecules in the individual cells while the cells remain in their tissue context, we gain an understanding of the mechanistic basis of cell–cell communications that in large part drive normal tissue development, tissue homeostasis, and tumorigenesis.

While fluorescence labeling combined with high-power confocal microscopy enables the visual localization of macro-molecules in living or fixed tissues, much more in-depth information is derived from quantification, the process of precisely measuring the amount and distribution of fluorescence labels within each cell, the shape of each cell, and the organization of the cells in the tissue. This process necessitates computational analysis that consists of first segmenting (delineating) individual cells; however, this capability is not readily available to the cancer biologist because

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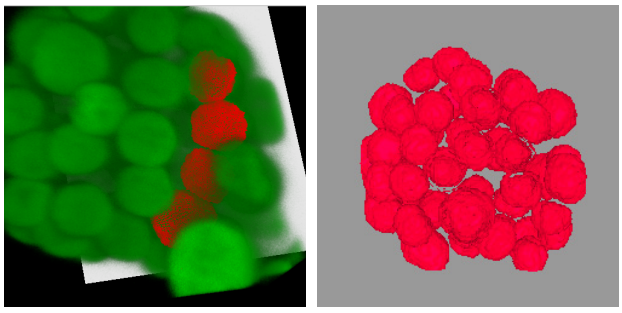
Analysis *continued*

computational methods are still very much under development.

Computational Segmentation Method Developed

Recently we developed a computational segmentation method based on dynamic programming (DP) that is highly reliable and accurate across a wide variety of microscopically imaged 2D and 3D samples encountered in cancer biology. It requires cell surfaces to be fluorescence labeled, or the nuclei to be labeled when segmenting cell nuclei. The method is practical to use, requiring the user to make three mouse clicks per cell, plus additional clicks to correct any visually observed segmentation errors. It is also highly reliable when applied to very noisy images that arise from using low levels of fluorescent dyes and excitation light intensity in order to minimize damage to living cells. The software, developed by Dr. Dean McCullough,[1] is available at: http://www-fbsc.ncifcrf.gov/tab_sw_resources.shtml. A new graphical user interface was written by Dr. Jusub Kim while a Ph.D. student at the University of Maryland, and will be available for download shortly at the same URL.



Left: Volume rendering (green) on fluorescence-labeled cell nuclei in a 3D tissue-like structure (acinus) formed from MCF-10A cells. The red surface rendering shows 4 nuclei that have been segmented (delineated) by the algorithm. Right: Surface rendering of all the nuclei in the acinus. (Microscope images kindly provided by Dr. K. Meaburn and Dr. T. Misteli, Cell Biology of Genomes Group, National Cancer Institute, Bethesda, MD.)

Adept Algorithm Adapts for High-throughput Applications

The key advantage of the segmentation method described above is the guarantee of correct segmentation of each cell based on visual assessment. However, the requirement of user interaction limits utility for high-throughput applications. Consequently, Dr. Prabhakar Gudla and Kaustav Nandy, both of the IAL, have investigated fully automatic methods for segmenting cell nuclei in 2D cell culture samples. They demonstrated for the first time that DP can achieve highly accurate delineation of nuclei, with 97% correct detection.[2] Then, by adopting a different approach, based on multi-scale enhancement and on-the-fly self-tuning to the characteristics of the imaged nuclei in different datasets, performance further improved. Self-tuning enables the algorithm to judge for itself whether a nucleus is correctly segmented or not. Out of a test population of over 4,000 nuclei, 84% were considered correctly segmented by the algorithm. Out of these 84%, all except 8 were visually confirmed as correct, resulting in a specificity of 99.8%.[3]

[1]D.P. McCullough, P.R. Gudla, B.S. Harris, J.A. Collins, K.J. Meaburn, M. Nakaya, T.P. Yamaguchi, T. Misteli, and S.J. Lockett. Segmentation of whole cells and cell nuclei from 3D optical microscope images using dynamic programming. IEEE Trans Med Imaging, Published online, 2007.

[2]K. Nandy, P.R. Gudla, and S.J. Lockett. Automatic segmentation of cell nuclei in 2D using dynamic programming. Proc. MIAAB, 2007. Published online at: http://www.miaab.org/miaab-2007-papers.html

[3]P.R. Gudla, J. Collins, K. Nandy, K.J. Meaburn, T. Misteli, and S.J. Lockett. A high-throughput system for segmenting nuclei using multi-scale techniques. Cytometry 73A(5): 451–466, 2008.

New Imaging Group Formed at the ABCC

By Jack Collins, Ph.D.

In recognition of the growing demand for medical imaging and microscopy resources needed to diagnose, treat, and understand cancer, NCI has established funding for an image analysis and visualization group within the Advanced Technology Program. Combining the expertise of the Image Analysis Laboratory, Small Animal Imaging Facility, and the Advanced Biomedical Computing Center (ABCC), this funding will maximize the insight that imaging can bring to our research efforts by supporting new personnel dedicated to imaging. Research efforts will include developing new capabilities

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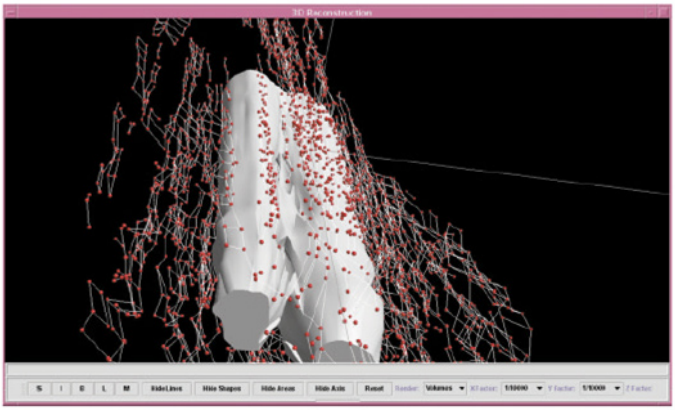
New *continued*

such as image analysis software, and algorithmic and mathematical modeling for in vivo small animal imaging studies as well as detailed tumor reconstruction. Training classes will also be developed to aid researchers in analyzing their images.

Small animal biomedical imaging technologies are still in the early stages of their development and have the potential to make a much greater impact on cancer research by integrating several modalities into a unified view. We anticipate that the new imaging group will focus on automating and accelerating the process of tumor detection and accurate quantitative analysis of tumor growth and location. In addition, this group will enhance the impact of experimental studies by improving our ability to access, integrate, and evaluate genomic and proteomic data and information within the context of cancer biology.

The new group is expected to build technology for computational 3D reconstruction of excised, serially sectioned animal tumors imaged at high resolution using optical microscopy following live animal imaging. These efforts will enable multiple, detailed, and quantitative structural and molecular analyses of tumors and neighboring normal tissue.



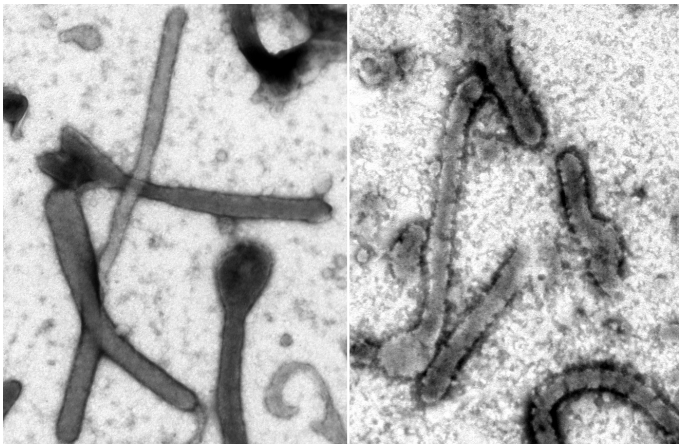
Example of tissue reconstruction of a mouse lymph node. 3D reconstruction of the H&E stained, manually annotated mouse case used to develop experimental versions of the algorithms. Lymph node volumes have been surface-rendered to show their three-dimensional shapes. Ducts are identified by spheres and connected by lines within and between sections.

Originally published in Fernandez-Gonzalez R, Jones A, Garcia-Rodriguez E, Chen PY, Idica A, Lockett SJ, Barcellos-Hoff MH, Ortiz-De-Solorzano C. System for combined three-dimensional morphological and molecular analysis of thick tissue specimens. Microscopy Research and Technique 59:522–530, 2002.

We envision that these new developments in whole animal imaging and 3D microscopic tumor reconstruction are components of a broader, multi-scale imaging effort that also includes microscopic analysis of cancer protein dynamics and interactions in living cells, and 3D whole cell electron microscopy (EM). We believe the full benefits of in vivo medical imaging in cancer research and treatment will be realized through the research and development efforts of this new group.

PEL Supports Ebola, Marburg Research

By James Hartley, Ph.D.,



Electron micrograph of Ebola virus (left panel) and Ebola VLPs (right panel). Image courtesy of Dr. Kelly Warfield, USAMRIID.

Events of recent years have heightened the need for improved vaccines against exotic and deadly diseases. Vaccine experts such as Dr. Kelly Warfield of the U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID) have seized upon three biological phenomena to fashion new tools to protect people from these threats. First, the human immune system has evolved to efficiently recognize viruses. Second, incomplete viruses, called virus-like particles (VLPs), can be prepared using small and safe pieces of viral DNA. Third, VLPs can incorporate heterologous antigens into their structures. Thus, artificially engineered VLPs represent a new, fast, and effective alternative to producing vaccines.

For the past three years, the Protein Expression Laboratory (PEL) of the ATP has been making VLPs for Dr. Warfield and her colleagues at USAMRIID, using both

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PEL *continued*

mammalian and insect cells. In particular, VLPs produced in insect cells have been shown to protect non-human primates against both Ebola and Marburg viruses (reported at the American Society for Microbiology meeting on Biodefense and Emerging Diseases, February 26, 2008). The insect cell production system is especially amenable to fast and cost-efficient scale-up, which makes it an attractive alternative to mammalian cell culture.

Supporting Dr. Warfield and her associates have been PEL staff members Dominic Esposito, Leslie Garvey, Jen Mehalko, Butch Hopkins, Veronica Roberts, and Cammi Bittner. The PEL team has supplied clone advice and construction and has produced large quantities of VLPs specific for Ebola and Marburg viruses on a challenging schedule. A press release from the ASM describing these findings can be viewed at http://www.asm.org/Media/index.asp?bid=56621.

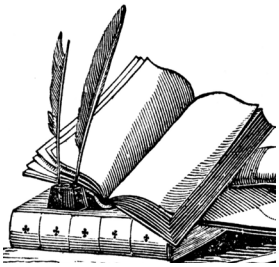
On Effective Communication

What's the Story?

By Ken Michaels

Matt Rix, one of the speakers at a meeting I recently attended, talked about the pernicious problem of misinterpretation and how we frequently cause ourselves trouble by jumping to erroneous assumptions and conclusions. He illustrated his point by recalling an experience he had while waiting for a flight in a very small airport somewhere in Nebraska. A lilting female voice came over the public address system with the request, "Will the man who dropped his pants at the ticket counter a few minutes ago please return to the counter?" The airport was small enough that reactions from all over—gasps and snickers—were clearly audible. About a minute later, the same voice, more cross than lilting this time, announced, "The pants were on a hanger!"

Matt's presentation was titled "Facts Tell, But Stories Sell" and emphasized the power of stories to paint memorable visual pictures.



Storytelling is as old as language itself as a means of education and entertainment in virtually all cultures. Historical icons such as Mark Twain taught, and amused, by effectively using stories to illustrate the points he wanted to make.

Many of the best public speakers are those who recognize the value of really ramming a point home with a story that will stick with the listener, and breathe some life into the facts of the matter. Following are Matt's eight elements of an effective story:

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Bob Fisher, Director of the Protein and Proteomics Supergroup, convened the group for its first off-site retreat at the Beaver Creek Country Club in April 2008.

What's *continued*

1. Premise: What's going on?
2. Problem or conflict: Difficulties that beg to be resolved are intrinsically captivating.
3. Payoff: The resolution can be funny, enlightening, or both.

The first three elements are simple and are present in virtually every story of any interest. It's the final five elements that transform a good story into a great one.

4. People: Good storytellers talk about real people. They say "Angela" rather than "a friend."
5. Places: Real places help the listener form a picture. "I was headed south on I-270" creates a more vivid mental image than "I was out on the highway."
6. Dialog: First person accounts with real dialogue are highly effective.
7. Educational: A really good story has a message that teaches something.
8. Entertaining: Things that give us a chuckle tend to be more memorable than things that don't.

Storytelling is not joke-telling. A great story doesn't have to be funny, and in fact some of the greatest stories of all aren't the least bit funny, but they're memorable all the same.

A phrase often heard in an oral presentation is "If you remember only one thing from this presentation, remember that …" Try this: The next time you give an oral presentation, identify that "most important" item and see if you can't come up with a story that illustrates it and makes it really memorable. It may be the key to a highly effective conclusion, and your audience will appreciate, and remember, it.

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