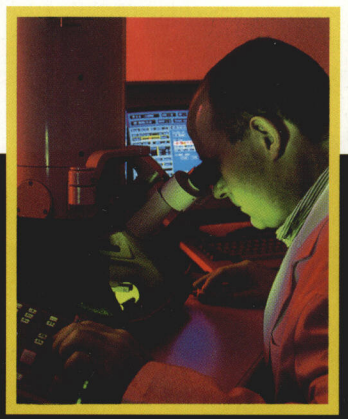
University of Pittsburgh
School of Medicine

Pitt Medicine

Medical Alumni Association Fall 1997

Quarterly

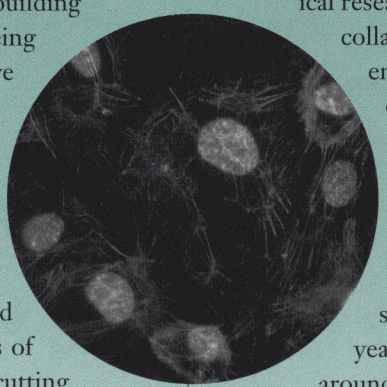
Center for Biologic Imaging provides powerful quantitative data for biomedical research



If *you* could see what *I* see:

For more than a century, the School of Medicine has probed and prodded into the frontiers of medicine where painstaking research has led to biomedical breakthroughs. Included in the discoveries are the first effective poliovirus vaccine and the first multiorgan transplant for the treatment of cancer. Today, the school continues to devote considerable prowess toward clinical and basic research, and as a measure of its stature the school ranks among the top 10 recipients of funding from the National Institutes of Health.

Many of the fresh insights offered by biomedical research are a product of the digital age, a time when the building blocks of life and the forces that destroy it are being investigated with precise pictures and quantitative data. It is a world where the School of Medicine enjoys picture-window seats courtesy of the Center for Biologic Imaging (CBI), which gets its vision from director Simon Watkins.



The CBI is on the front lines of microscopy, a graphically stunning universe that has evolved from the staid and old stain-and-count images of the 1970s to a dynamic set of tools that are at the cutting edge of research today. "We are trying to develop diagnostic methods that didn't exist," explains Simon Watkins, PhD, associate professor of cell biology and physiology at the School of Medicine. "In the past, we would biopsy and do histologic analysis. Now we can image and measure where and when molecules are expressed. We can watch molecules interact within a cell and with other cells. And we can do it in real time."

Watkins, a former research associate at the Dana-Farber Cancer Institute, Harvard Medical School, and one-time research fellow at the Pasteur Institute in Paris, came to Pitt in the fall of 1991 because the school wanted a microscopist to develop an imaging center. Today, there are only three or four comparable imaging centers in the United States.

"What's most amazing is the pace at which the CBI has grown and developed," says Raymond Frizzell, PhD, chairman of the Department of Cell Biology and Physiology. "Over about six years, starting with a collection of aging electron microscopes in the basement of Scaife Hall, Simon has assembled a centralized, state-of-the-art center for imaging biological specimens that serves the entire medical research community."

The new diagnostic tools include fast, powerful computers combined with cameras yielding more and more information from less and less light. They can record single photon events. Advances in dye technology have also played a role. Researchers can look at the molecular infrastructure of a cell. They can watch multiple molecules interact within the cell and with other cells. "Our power of magnification is like enlarging an ant to the size of the United States," says Watkins.

The imaging center's almost unimaginable power to provide quantitative data results in a matrix for much of the biomedical research conducted at the school. Imaging works collaboratively with sources, mostly at the front end of research in diagnostic biology, tumor biology, and muscle biology, where the absence, over-expression, or mutation of some cell needs to be quantified. This can be accomplished with multicolor, immunofluorescence microscopy. The center plays a role in 30 to 40 published papers a year. And the research stretches far beyond the university campus. Last year, the CBI worked with 79 different labs from around the world.

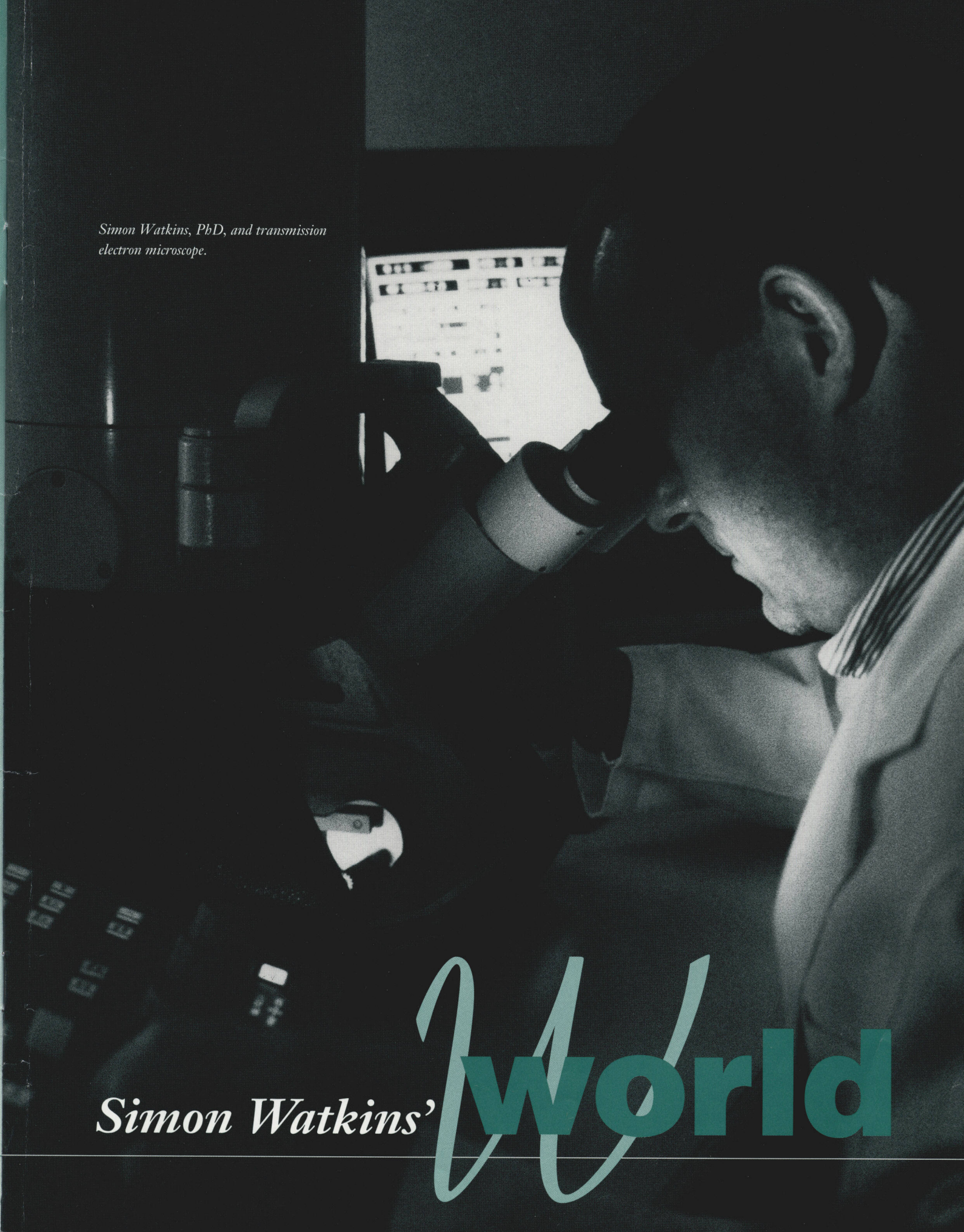
The imaging center could not have developed without the institutional support to move ahead and pursue additional grant money. Here, too, Watkins excels. He has been relentless in his pursuit of federal funds to purchase new equipment and is usually successful. For example, he recently received funding for a new state-of-the art electron microscope. His score ranked in the top 1 percent of the applicants.

"The CBI is a unique and irreplaceable resource," says Frizzell. "When you're in the computerized image analysis module of the CBI, it's like mission control and the fantastic voyage rolled into one."

Indeed, with its bank of computers and light-tight rooms housing telescope-sized electron microscopes, the CBI does seem to belong in a future world. "We can do things we dream of," says Watkins. "We can build a montage of an entire cover slip — three dimensional and spinning in space. On the horizon is more and more computing power and new multiphoton imaging."

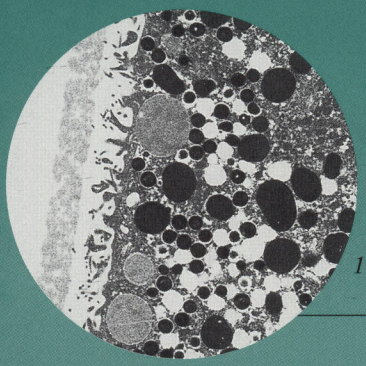
(Continued on Page 4)

Pictured in center: Endothelial cell labeled to show actin (fibrous material) and nucleus (circular organelles)



Simon Watkins, PhD, and transmission electron microscope.

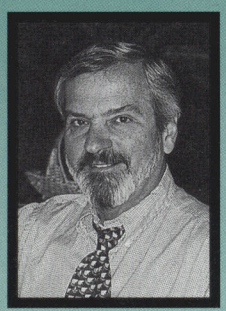
Simon Watkins' World



1

Monitoring gene therapy for *Gaucher disease*

Single-gene inherited disorders like cystic fibrosis and Gaucher disease have tantalized gene therapy specialists from the earliest beginnings of their discipline. In theory, replacing the gene might produce the first definitive cure for these as well as other deadly or debilitating disorders.



Ray Frizzell, PhD

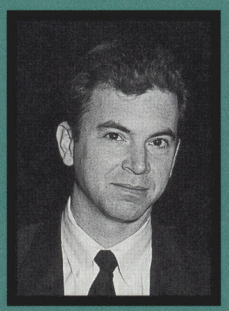
Two of the School of Medicine gene therapy studies involving Watkins and CBI include investigations of the cystic fibrosis transmembrane regulator (CFTR), in collaboration with Ray Frizzell, PhD, professor and chairman, Department of Cell Biology and Physiology, and of the molecular treatment of Gaucher disease, with John Barranger, MD, PhD, professor of human genetics, molecular genetics and biochemistry, and pediatrics.

In the Frizzell study, CBI investigators are localizing CFTR in epithelial cells using an artificial peptide tag that allows detection of genetically engineered CFTR at epithelial cell surfaces. These methods are being used to gain more information on the basic biology of CFTR and the possible role of novel pharmacologics in monitoring expression at the cell surface. Along with Barranger, Watkins and colleagues are identifying where the therapeutic glucocerebrosidase gene is expressed when myoblasts engineered with the gene are transplanted into murine muscle.

"Simon Watkins' imaging center has been instrumental in our effort to understand what determines the amount of the CFTR protein in the cell surface membrane — the site of its physiological actions," says Frizzell. "Both the microscope facilities and the sophisticated image processing capabilities of the center have made this work possible."

Imaging the many faces of *nitric oxide*

Considered a pollutant with no beneficial biological activity little more than 10 years ago, the gaseous molecule nitric oxide has moved to center stage in diseases as diverse as septicemia, cardiovascular disease, and arthritis. A number of microscopic techniques, including multicolor fluorescence analysis, confocal microscopy, and electron microscopy, have allowed Watkins and colleagues at CBI to monitor the production of NO synthase (the enzyme that produces NO), as well as the ultrastructural status of NO-producing cells.

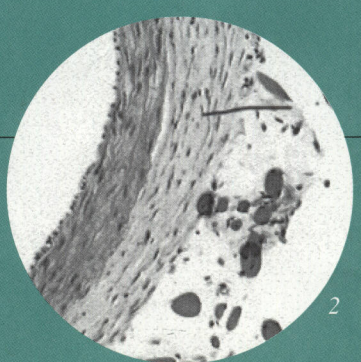


Timothy Billiar, MD

Among other studies, Watkins is collaborating with Timothy Billiar, MD, Watson Professor of Surgery, and colleagues to monitor the expression of the NO synthase gene in blood vessels following gene therapy. This gene, cloned at the University of Pittsburgh, inhibits occlusion of the blood vessel following angioplasty or during chronic rejection of a heart transplant. The group is now working to identify optimum vector and delivery systems that can prevent restenosis. This preliminary data will help develop clinical trials.

Watkins is also measuring natural NO synthase production in arthritic joints and in tissues surrounding artificial joints, helping Christopher Evans, PhD, Henry Mankin Professor of Orthopaedic Surgery, to elucidate the molecule's role in both arthritis and artificial joint loosening. And with Richard Simmons, MD, Distinguished Service Professor and chairman, Department of Surgery, and associate dean for clinical affairs, CBI is monitoring the role of NO and of inflammatory cytokines in bacterial translocation across the bowel epithelium.

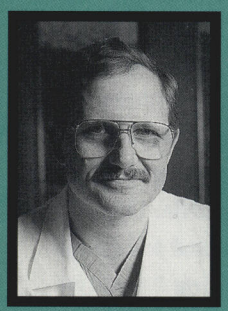
"Dr. Watkins has established a world-class tissue imaging facility here in Pittsburgh," says Billiar. "This unique resource has proved invaluable to our work."



2

Tracking the migration of tumor-antigen-bearing *dendritic cells*

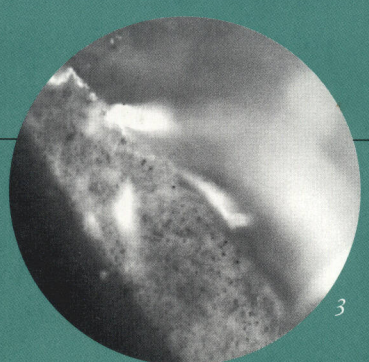
One of the most exciting frontiers in oncology today is the possibility of immunizing patients against cancer — both prophylactically and therapeutically. A School of Medicine effort involving a number of faculty members, led by Michael Lotze, MD, PhD, professor of surgery and molecular genetics and biochemistry, and co-director, University of Pittsburgh Cancer Institute (UPCI) Biological Therapeutics Program, is employing engineered dendritic cells in an attempt to present tumor antigens to the immune system.



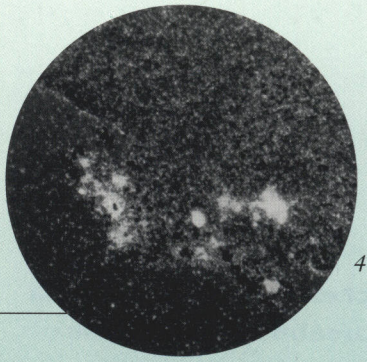
Michael Lotze, MD, PhD

Prior to clinical use, the ability of these cells to present tumor peptides to the immune system must be elucidated. Watkins has collaborated with the UPCI team, including Louis Falo, MD, PhD, assistant professor of dermatology, by examining the traffic of Langerhans cells transduced *in vivo* using the gene gun. With a combination of electron microscopy, low-light fluorescence microscopy, and two-color fluorescence techniques, Watkins and colleagues have traced the movement in mice of these transduced dendritic cells from the skin, where the gene gun is applied, to the lymph nodes. With Olivera Finn, PhD, professor of molecular genetics and biochemistry, and colleagues, Watkins has recently taken these experiments a step further, showing that labeled dendritic cells migrate to the lymph nodes in primates as well.

"Dr. Watkins' imaging capabilities have provided unique insights into the biology of this immunization strategy," says Falo. "This collaboration will continue to be essential to our ongoing efforts to understand and improve these promising immunotherapies."



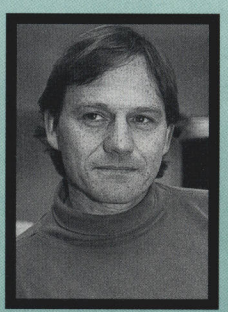
3



4

Looking for an *arthritis cure*

Recently Christopher Evans, PhD, Henry Mankin Professor of Orthopaedic Surgery, and colleagues discovered a potential role for the interleukin-1 receptor antagonist protein (IL-1Ra) in treating rheumatoid arthritis, by using it to block the inflammatory sequelae of IL-1 production. Interleukin-1 and its receptor are linchpins in the autoimmune response seen in rheumatoid arthritis, and gene therapy with its antagonist holds great promise in providing the first true medical treatment for this disease.



Christopher Evans, PhD

In collaboration with Evans and others, Watkins and CBI colleagues continue to provide an important component of the ongoing clinical trial of *ex vivo* gene therapy for rheumatoid arthritis. Their contributions have ranged from preclinical studies of the distribution of genetically engineered synovial cells when reintroduced into the rabbit knee to histological and immunocytochemical *in situ* hybridization studies of IL-1Ra and cytokine production within human gene therapy patients' joints.

1. *Electron micrograph of the plasma membrane of the* Xenopus *oocyte used to examine the cystic fibrosis transmembrane regulator.*
2. *Cross section of blood vessel treated to prevent occlusion after angioplasty.*
3. *Gold particles (dark spots) carry DNA following bio-ballistic delivery to living human skin.*
4. In situ *localization of IL-1Ra in human arthritic joint following gene therapy (light areas reflect gene expression).*