

2015 Year In Review





The UPMC Eye Center is dedicated to patient care and research, and to seeking out new therapies and treatments that will protect, preserve, and restore vision for individuals with vision loss regardless of the causes, be they disease, trauma, or congenital malformation.

In 2015, the UPMC Eye Center was the recipient of numerous major grant awards, including several related to developing the first whole-eye transplant program in the United States. Kia Washington, MD, a plastic surgeon, clinician-scientist, and expert on complex tissue allografting, is working to develop and refine a small animal model for whole-eye transplant that is adapted from a face transplant model she has developed. Along with the work of her colleagues and collaborators, Dr. Washington is at the forefront of this exciting new research.

In July of the past year, the UPMC Eye Center welcomed Jeff Gross, PhD, to the department. Dr. Gross is a basic scientist with a research focus on retinal regeneration working in the zebrafish model, and some of his current work is profiled in this year's report. Dr. Gross also is the new director of the Louis J. Fox Center for

Vision Restoration, A collaborative effort between UPMC and the University of Pittsburgh, the Fox Center seeks to further the understanding of optic nerve regeneration, retinal regeneration, and cures for vision loss through collaborative efforts with regenerative medicine, tissue engineering, and other specialties. The work of the Fox Center is highly translational in nature and focuses on discovery and dissemination of new knowledge for clinical patient care. Notably, in late 2015, the Fox Center was the recipient of an anonymous gift of more than 2 million dollars to further the study of optic nerve regeneration.

Also during the past year, Morgan Fedorchak, PhD, joined the department as an assistant professor of ophthalmology. Dr. Fedorchak, a chemical and bioengineer with expertise in controlled drug delivery, has played an instrumental role in

developing a novel type of eye drop to treat glaucoma. She and her colleagues have invented an eye drop that turns into a solid gel and can deliver medications over a period of at least a month in small animals. This new agent and its intellectual property has been protected by the University, and work is in progress to begin clinical trials in humans and to eventually commercialize the technology. This work has profound implications for the millions who endure the burdens of this blinding disease.

Accomplishments by the clinical practitioners and researchers of the UPMC Eye Center further the understanding and treatment of eye diseases, and will continue to have an impact far into the future. We look forward to continually sharing our findings, successes, and ideas with our colleagues across the nation.

ENGINEERING

A SIMPLER GLAUCOMA EYE DROP



Researchers, clinicians, and engineers in the Departments of Ophthalmology and Chemical Engineering at the University of Pittsburgh and UPMC are on the edge of a breakthrough in reengineering how glaucoma patients will administer their medications in the future

Seeing is Believing

Perhaps the biggest obstacle to optimal outcomes for people living with glaucoma is adherence to their topical medication regimen, both in terms of the regularity of their dosages and the technique for administering the drugs. People forget. Or they are unable to administer the medication properly every time. Or they struggle to deal with the burden of multiple daily doses of eve drops for the rest of their lives. Whatever the individual reasons or difficulties, the outcome of poorly managed glaucoma is inevitable impairment or total loss of vision.

Morgan V. Fedorchak, PhD, assistant professor of ophthalmology, and head of the ophthalmic biomaterials lab, wants to simplify the way glaucoma patients take their medications.

Dr. Fedorchak received her PhD in bioengineering from the University of Pittsburgh, following chemical and biomedical engineering degrees from Carnegie Mellon University. "I always knew that I was going to be an engineer," she says, but it wasn't until after receiving a postdoctoral fellowship in the Ocular Tissue **Engineering and Regenerative** Ophthalmology (OTERO) program at the Louis J. Fox Center for Vision Restoration that she found a path into the field of ophthalmology research. The OTERO fellowship program paired Dr. Fedorchak with an ophthalmology mentor, Joel Schuman, MD, FACS, and an engineering mentor, Steven R. Little, PhD, William Kepler Whiteford Endowed Professor and Chair of the Department of Chemical Engineering.

The goal of the OTERO program is simple: to engineer a solution to a vision loss problem. In Dr. Fedorchak's case, that problem was glaucoma.



Preparing a sample of the eye drops.



"Vision loss has a profound effect on people. Their sense of vision is what they want to hold on to the most. And the current treatment options are sometimes setting people up to fail. We have drugs that work very well in treating glaucoma effectively. Our job now is to simplify the delivery mechanism to improve patient adherence," says Dr. Fedorchak.

What started in the OTERO program as a contact lens that could deliver

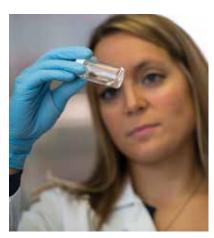
glaucoma medications in a controlled way, evolved into the study of a controlled-release glaucoma drug that would be injected monthly. Dr. Fedorchak, Ian Conner, MD, PhD, and Drs. Schuman and Little designed a study that used a single dose of brimonidine tartrate loaded in polylacticco-glycolic acid (PLGA) microspheres capable of a controlled release for 28 days after injection into the inferior fornix. At the conclusion of the study,

subjects receiving the injection saw an average decrease in intraocular pressure of 21.4 percent, compared to subjects receiving the traditional twice-daily drop who saw a 19.9 percent decrease.

The results of this study were encouraging, but a monthly injection would mean patients would have to visit a physician consistently each month to receive treatment. With the goal still to simplify the process for patients and improve adherence, the







research team began the work of engineering a topical treatment that patients could self-administer.

Elegance in Simplicity

"There is an inherent simplicity in eye drops, and most people with glaucoma are very familiar with administering their medications this way," says Dr. Fedorchak.

The problem became how to engineer an eye drop that could be selfadministered by the patient and that they would only have to do once a month, instead of the two to three times a day that most medications require.

The challenges of creating this new kind of eye drop were many. Figuring out how to get the drops to remain in the eye for an entire month without degrading or washing out, and how to get the medication to release in a controlled way, in the correct dosage, during the entire duration the drop is in the eye were paramount for success. Patient safety and comfort are additional considerations that must be addressed for long-term success.

As the base for the eye drop, Dr. Fedorchak's team is using a reversethermoresponsive hydrogel that is able to transform from a liquid at room temperature to a solid at 34 degrees Celsius — approximately human body

temperature. It gels almost immediately after contact with the eye and stays in place for the intended duration without degrading or moving around. They also have engineered the gel so that it is opaque, rather than clear. "Patients wanted to know where it would be in their eye," says Fedorchak. Removal of the drops and reapplication at the prescribed time is a fairly simple process. A patient could, essentially, push the drop out of the eye, or flush the eye with a cold saline rinse, transforming the drop back into a liquid and washing it out of the eye.

Within the hydrogel are microparticles - or microspheres - composed of PLGA, a fairly common biomaterial. The microparticles are essentially degradable polymers used to encapsulate the glaucoma medications. After the drops are inserted into the eye, the microparticles take on water which starts the process of breaking down the polymer chains over time, thereby releasing the infused medications. Formulating the microparticles so that they release the medications at the right pace over the course of 30 days was a big challenge, and an iterative process of testing and refining formulations was used to achieve the desired release. schedule of 2-3 micrograms per day.

Dr. Fedorchak and her collaborators on the project are one of the only groups in the country working on this kind of patient-administered topical delivery system for glaucoma medication. Their pre-clinical trial efficacy studies continue, and the hope is that they will have FDA approval to start clinical trials in humans in 18 months.

Looking to the Future

If Dr. Fedorchak and her collaborators work continues successfully, their new eye drop for glaucoma patients would be nothing short of revolutionary, with the potential to help millions of people. From Dr. Fedorchak's perspective, "It will mean less of a burden for patients to remember to take their medications and take them correctly. The continuous, lower-dose of drugs instead of pulses of higher concentrations in traditional drops will decrease the likelihood of adverse side effects and may also control their disease better than traditional drops by preventing extreme highs and lows in ocular pressure throughout the day." she says.

For older patients with conditions or barriers to adherence like osteoarthritis of the hands, or limited mobility and in need of assistance from a caregiver, the results would be even more profound, since administering the treatment



once a month is a much more realistic. and less burdensome scenario than multiple times every day.

"Overall, the goal is to prevent vision loss due to glaucoma by setting patients up for success with a treatment method that is much simpler and more tolerable." The delivery platform that Dr. Fedorchak and colleagues are working to develop could, in the future, be applied to treatments for other eye conditions and ocular infections.

AN AUDACIOUS GOAL:

WHOLE- EYE TRANSPLANTATION



For decades surgeons have been able to transplant organs and tissues - hearts, livers, kidneys, corneas - with increasing frequency, better and safer techniques and outcomes, saving, extending, and improving the lives of countless individuals. However, the ability to transplant an entire eye and restore sight has yet to be realized. Researchers at UPMC and the University of Pittsburgh are taking strides to make this a reality.

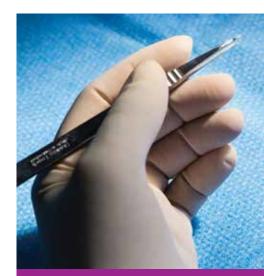
Kia M. Washington, MD, is a reconstructive surgeon and assistant professor of surgery in the Department of Plastic Surgery. Her research endeavors include being a principal investigator in the Vascularized Composite Allotransplantation (VCA) research lab focusing on improving nerve regeneration and the study of cortical reorganization after VCA.

Dr. Washington also is a principal investigator in one of several new studies being funded by the U.S. Department of Defense (DOD) Vision Research Program.

The overall goals of the projects are to establish the nation's first whole-

eye transplantation program. Dr. Washington's research is focused on establishing a baseline viability and structural integrity in an animal model, and modeling the immune system and rejection response to develop a grading system. Her team also is collaborating with Michael Steketee, PhD, assistant professor of ophthalmology who is working on developing extracellular matrix technology to help restore retinal function and decrease the likelihood of scarring after transplant. Vijay Gorantla, MD, PhD, associate professor of surgery in the Department of Plastic Surgery at the University of Pittsburgh, and administrative medical director of the Pittsburgh Reconstructive

transplant Program at UPMC also has DOD funding to explore methods of optic nerve regeneration.





The DOD funded projects, with collaborators at Harvard University and the University of California, San Diego, under the auspices of the recently formed Audacious Restorative Goals in Ocular Sciences (ARGOS) Consortium at the University of Pittsburgh, will receive additional funding from the Louis J. Fox Center for Vision Restoration of UPMC and the University of Pittsburgh to further the transplant research.

The Challenges Are Many

The scale and complexity of obstacles in successfully performing a whole-eye transplant and restoring vision in a human are daunting. "Survival time of retinal ganglion cells (RGC) in the donor eye will be one limiting factor," says Dr. Washington. RGC's do not survive very long post-harvest so understanding and mitigating ischemia times will be necessary for determining when it is appropriate to harvest the

donor eye. Developing perfusion techniques to extend the time that the donor eye remains viable for transplant also may be required.

The optic nerve does not deal well with injury, so finding ways to stimulate the regeneration process of the optic nerve from the retinal ganglion cells is crucial to success. So too is maintaining the eye vascularity, ensuring that it remains intact and functioning during and after the surgical procedures.



Restoring the function of the extraocular nerves and the controlling musculature of the eye will be necessary to obtain a normal range of movement, and preserving the upper eyelid function to ensure protection of the transplanted eye also are crucial.

"And then there is the surgery itself. Right now it would be a pretty invasive procedure requiring a trans-cranial and combined endonasal approach to expose the optic nerve and carotid artery," says Dr. Washington. Her team will devote attention to refining the

surgical procedures to make it a less invasive process.

Developing the Animal Model

Dr. Washington began working on an animal model for whole-eye transplant in 2012 while in the Vascularized Composite Allotransplantation (VCA) lab. This work ultimately led to DOD grant funding for additional research. Her team is one of the only entities in the country working on a small animal model for whole-eye transplant, furthering the progress towards what could one day

be a successful human eye transplant.

Dr. Washington's team has successfully performed an eye transplant in a small animal model, using a rat. "With rats, you can perform a lot of carefully controlled experiments to understand the basic science. You can perform a lot of transplants and refine the processes before progressing to a larger animal model," says Dr. Washington. Her team has completed more than 50 animal model transplants and is working on follow up studies on the recipients, one of which is now over 500 days posttransplant.

After the eye is transplanted, it must remain viable for long-term success. Blood flow to the eye and its structures and surrounding transplanted tissues, eye function and mobility, the integrity of the ocular fluids, and maintaining proper intra-ocular pressure are all mandatory. In the animal model, Dr. Washington's team collaborates with Kevin C. Chan, PhD, assistant professor of ophthalmology and bioengineering, and researcher in the Neurolmaging Laboratory to perform functional magnetic resonance imaging to assess the structural integrity and ocular humor dynamics posttransplant.

To assess the responsiveness of the retina and its constituent parts,

Dr. Washington's team, in collaboration with Valeria Fu, PhD, assistant professor of ophthalmology and director of the Electrophysiological Testing Service, uses retinal electrogram scans to determine whether there is any receptivity to visual stimulus in the transplanted retina. To date, the animal models have shown a lack of response. but "that's one of the next big steps we have to take. We have the basic model for a successful transplant, and while we work to refine this over time, we know ultimate success means getting our transplant subjects to respond to stimuli," says Dr. Washington.

Modeling the Immune Response and Rejection Potential

Rejection and immunosuppression are other areas Dr. Washington and her team are investigating. "One of the key parts of our grant, and something we will work extensively on, is establishing a grading system for how the immune system responds to the transplanted eye. Since we're the first ones working on this, we'll have to set the standard. Right now, we don't really know how the body will react to a transplanted eye and what will be required in the way of immunosuppression therapies to stave off rejection," says Dr. Washington. The current animal models do not experience an immune

response or rejection, as the subjects are all genetically identical. The next step in the process is to move from a syngeneic to an allotransplantation model, allowing Dr. Washington and her team to study the rejection process and immunosuppression requirements. Her team has performed a handful of these transplants so far and will begin delving further into the model in the coming year.

How rejection in the eye is eventually monitored in humans will require special attention. Multiple biopsies are not an option. A noninvasive technique, such as optical coherence tomography, will need to be constructed to safely monitor, with relative speed and ease, for rejection over the lifespan of the recipient.

It Will Be a Miracle

Dr. Washington and the other research teams and collaborators on this project understand the complexities and the potential. "This is an aggressive program with very high-risk and high-reward scenarios. We're excited to be leading the project and honored to be collaborating with global leaders in optic nerve regeneration," she says.

When asked what it would mean if the projects are successful, Dr. Washington says, "We will be achieving a miracle. The ability to give sight to someone has not been accomplished yet, and millions of people could benefit. To be on the cusp of the current research is very exciting. I want to be there when we are ultimately successful."



Detail of retinal electrogram device.

THIS SMALL FISH

COULD CHANGE HOW WE TREAT VISION LOSS

Arriving at UPMC and the University of Pittsburgh in July 2015, Jeff Gross, PhD, professor of ophthalmology, the E. Ronald Salvitti Endowed Chair in Ophthalmology Research, and the director of the Louis J. Fox Center for Vision Restoration, brings with him a passion for science and research, along with an approach grounded in mentorship and creativity.

Creativity and Collaboration

There were a number of opportunities that attracted Dr. Gross to the Department of Ophthalmology to continue his research. "The research that is being conducted to model ocular diseases fits very will with my ophthalmic research in zebrafish.

At the same time, the Louis J. Fox Center for Vision Restoration was looking for a new director to help nurture and grow the center, and expand its identity and visibility. "This was another reason I wanted to come here," explains Dr. Gross. "I'm really excited by collaborative science and working with great teams. The Fox Center's work runs the gamut from basic science to stem cell research to

whole-eye transplant to prostheses development, and I'm looking forward to expanding its reputation on a national and international level. We really have an opportunity to help physicians, researchers, and the public understand the work we are doing and its potential."

A Model Fish

The zebrafish (Danio Rerio) laboratory at the University of Pittsburgh houses 11,000 aquariums, making it the largest research colony of zebrafish in the world. For someone whose primary research inquiries use zebrafish models for understanding eye diseases, there may not be a better place to conduct research. "The zebrafish lab is tremendous," says Dr. Gross. "Just

from a practical standpoint, its scale affords so much opportunity to continue our research and expand our efforts to understand eye development



Inside the Zebrafish research facility at the University of Pittsburgh.



processes and disease, and retinal regeneration capabilities."

Dr. Gross' postdoctoral work at Harvard University was instrumental in shaping his current research and vision. During his time there, the lab where he worked was transitioning into working with zebrafish in order to understand the genetics of eye development. "My advisor, John Dowling, allowed me to do a lot of the experiments that I wanted to do. He really encouraged

the postdocs to do exciting things and branch out into new territory."

Zebrafish are ideal for studying a number of issues related to eye development and retinal regeneration properties. Their eye structure, development, and other characteristics are quite similar to that of the human eye, and their embryos are transparent and develop ex utero. Zebrafish eyes are relatively large in proportion to body size and allow researchers to use various

imaging techniques to study cellular and morphological aspects of ocular development in real time. Zebrafish also develop and breed quickly, allowing researchers to rapidly create and breed genetically modified variants to screen for developmental defects.

Optic Cup Formation and Coloboma Research

For a number of years, Dr. Gross has studied optic cup formation and pathology, with particular interest in



The Importance of Mentorship

Dr. Gross credits much of his career success and his approach to his work to his PhD advisor, Dr. David McClay. "He was really important for my career, instrumental. He had only two criteria: Your work has to be great. And it has to keep me interested," he says.

"As I run my lab and conduct research, for me, it's not so much the project but the people. I don't see myself as a boss. I'm an advisor. Training is so important. I've had really great mentors, and it's a big deal to me to continue to do this. I'm looking for creativity and fostering that kind of atmosphere, allowing our researchers to be bold and truly experimental is how we'll be successful."

coloboma pathogenesis. Colobomas — congenital defects of the eye manifest very early in both zebrafish and human embryo development. They occur when the optic or choroid fissure fails to close or only partially closes.

The affected structure of the eve, and the type and severity of the vision impairment can vary widely. Colobomas of the iris may only result in a cosmetic defect with no vision impairment, whereas a chorioretinal coloboma. where much of the retina is absent. having never enclosed within the optic cup, is quite severe. The range of affected ocular structures and types of colobomas that can develop are perplexing. "We're not sure yet if there is a heterogeneous mechanism affecting all the structures, or if there are subtle variations that cause one structure to be affected differently than another. This is one of the key elements of our research," says Dr. Gross.

A focus of Dr. Gross' research is in understanding the basic molecular and cellular processes of how the optic cup forms and the choroid fissure closes. "We're only beginning to understand these processes and which candidate genes may be responsible for various aspects of the formation and closure."

This is where zebrafish are an ideal model on which to experiment. "We can develop fish mutants and use these to understand the molecular and cell biology of the closure process of the fissure. We can isolate individual genes or proteins that may be playing key roles in the developmental process and, ultimately, screen for those," says Dr. Gross.

"The big challenge down the road, once we fully understand the biological and molecular processes at play in choroid fissure closure, will be how to develop therapies that can prevent or repair the development of colobomas," says Dr. Gross. "We also want to research how we can screen individuals for potential developmental problems in advance." Because of how early in embryonic development colobomas occur — within the first weeks in humans — screening for them will be a difficult task. This is where regenerative medicine can play an enormous role.

Retinal Regeneration Capabilities in Zebrafish

Damage to, or degradation over time, of the retina and retinal pigment epithelium (RPE) cells are at play in numerous vision-compromising human eye diseases, from age-related macular degeneration to retinitis pigmentosa and diabetic retinopathy. Dr. Gross and his molecular biology lab, with generous support provided by Charles

and Louella Snyder, are using zebrafish to model the underlying mechanisms of disease, and to understand the biology of the regenerative response, to determine what sort of signals or gene regulatory factors drive the process of regeneration.

"The zebrafish has an incredible ability to regenerate, or repair its retina and RPE if damaged or compromised by disease," says Dr. Gross. Müller glia cells, a type of support cell, in the retina of zebrafish are able to differentiate and regenerate those areas affected. They can 'sense' what cells need to be repaired, for example rod photoreceptors, and set about the task of regenerating and re-associating the necessary neuronal connections. "This is something that the human eye, and most mammalian eyes, currently cannot do, and we're trying to understand the biological processes in the zebrafish that make this work," says Dr. Gross.

Müller glia cells in the human eye will begin the process of regeneration, for example after a traumatic injury, but for some as-yet-unknown reason, the process breaks down and is unable to complete, producing scar tissue. Dr. Gross sees several possible solutions to this if we can pinpoint why the regeneration process halts itself.

Is there some sort of block or impairment in mammals that we could tweak? Can we simulate the regenerative response in some way so that it completes? Or can we just bypass the problem: create a workaround that circumvents the innate biological inability in humans for retina regeneration.

"There are so many diseases and congenital conditions of the eye and retina that in the long run we could have the ability to really help a large number of people regain vision, or partial vision, or even reverse what is now a progressive condition," says Dr. Gross.

Age-related macular degeneration (ARMD) is extremely prevalent as our population continues to age, and the number of cases may very well double and even triple as people continue to live longer. Dr. Gross says, "It's a significant problem now, but in the near future, its repercussions are going to be felt much more strongly."



Detail of gel electrophoresis device inside the Charles and Louella Snyder Laboratory for Retinal Regeneration.

FACULTY AND LOCATIONS

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Michael Steketee, PhD

Assistant Professor of Ophthalmology

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Xiangyun Wei, PhD

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Gadi Wollstein, MD

Professor of Ophthalmology Director, Ophthalmic Imaging Research Laboratories

UPMC EYE CENTER Locations

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UPMC Eye Center, Eye Care & Optical

3616 Forbes Ave. Pittsburgh, PA 15213

Director: Scott Drexler, OD

UPMC Eye Center Bethel Park

1300 Oxford Drive, Suite 1-A Bethel Park, PA 15102 Director: Ian Conner, MD, PhD

Children's Eye Center Children's Hospital of Pittsburgh of UPMC

45th St. and Penn Avenue Pittsburgh, PA 15201 Director: Ken K. Nischal, MD, **FRCOphth**

UPMC Eve Center Children's at Pine Center

11279 Perry Highway, Suite 202 Wexford, PA 15090

Director: Christin Sylvester, DO

UPMC Eye Center Mercy

1400 Locust St., Suite 3103 Pittsburgh, PA 15219 Director: Evan L. Waxman, MD. PhD

UPMC Eye Center Monroeville

125 Daugherty Drive, Suite 320 Monroeville, PA 15146

UPMC Eye Center St. Margaret

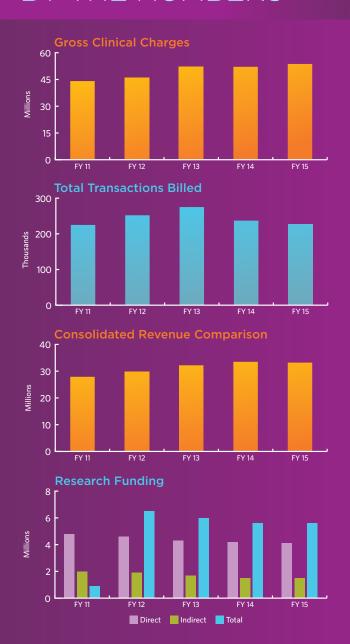
100 Delafield Road, Suite 201 Pittsburgh, PA 15215 Director: Marshall Stafford, MD

UPMC Eye Center Wexford

1603 Carmody Court, Suite 104 Sewickley, PA 15143

Director: Alexander Anetakis, MD

BY THE NUMBERS



SETTING SIGHT ON CURES

FOR VISION LOSS

The Louis J. Fox Center for Vision Restoration is a collaborative and comprehensive the McGowan Institute for Regenerative Medicine at the University of Pittsburgh. Founded in 2008, the Fox Center is focused on research and clinical programs development of novel technologies to cure vision impairment and blindness.

Research programs at the Fox Center delve into many areas and some of the most prevalent conditions associated with vision loss, including macular diseases, glaucoma, diabetic retinopathy, optic nerve disorders, ocular trauma, and corneal scarring. The researchers at the Fox Center and their collective efforts have a single, simple focus: find ways to stop or repair vision loss.

The current research endeavors at the Fox Center are quite numerous and include projects on understanding the biological causes for corneal blindness, led by principal investigator James Funderburgh, PhD, and optic nerve regeneration using extra-cellular matrix technology, under the leadership of

Michael Steketee, PhD. To learn more about the groundbreaking research in progress at the Fox Center, visit FoxCenter.Pitt.edu.

New Director of Research

In 2015, the Fox Center named Jeff Gross, PhD, as its new director, In addition to his role as director of the Fox Center, Dr. Gross is professor of ophthalmology and the E. Ronald Salvitti **Endowed Chair in Ophthalmology** Research at the University of Pittsburgh. Prior to his appointment at the University of Pittsburgh and the Fox Center, Dr. Gross was a professor in the department of molecular biosciences in the Institute for Cellular and Molecular Biology at the University of Texas at Austin.

OTERO Fellowship Program

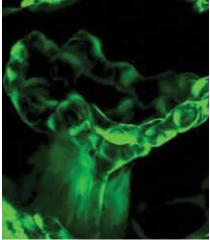
Since 2010, the Fox Center has sponsored the Ocular Tissue Engineering and Regenerative Ophthalmology (OTERO) fellowship. This postdoctoral research program pairs fellowship participants with both a UPMC ophthalmology clinical investigator and a regenerative medicine coinvestigator on research projects designed to restore, maintain, or enhance ocular function with regenerative medicine technologies. OTERO fellowships are one-year projects and funded up to \$70,000.

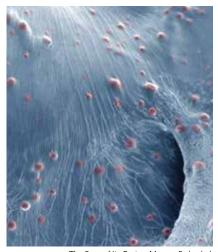
UPMC EYE CENTER

2015 Year In Review

There is a hidden beauty in the eye not apparent on its surface. These photographs, taken in the labs of our department researchers, show some of the abstract forms underlying the physical and chemical structures of the human eye. For more information about the photographs email EyeCenter@upmc.edu.







Fundus with Visible Striations - Gadi Wollstein

Hyaloid - Jeff Gross

The Sum of Its Parts - Morgan Fedorchak

