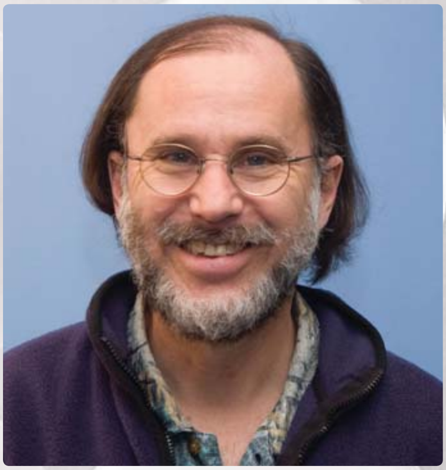
Scintillating **science**

Harnessing the technology of large ground-based telescopes, scientists led by **Dr Joel Kubby** are working to develop microscope technology that reveals the workings of stem cells in real time



Could you begin with a brief overview of the AO Wide-Field Microscope project and its relationship to stem cell research?

The adaptive optical (AO) microscopy project began in 2006 with a multidisciplinary group of biologists, astronomers and optical engineers who had been involved with the Center for Adaptive Optics at the University of California in Santa Cruz. We were inspired by the technology of the twin 10 m telescopes at the W M Keck Observatory.

It is important to obtain diffraction limited optical images of living stem cells in order to study phenomena such as stem cell differentiation in the niche. Our overall objective was to enable deep imaging into living cells and tissues, in order to study the moment when life begins with the replication of the cell nucleus and how neurons evolve as we learn.

What prompted you to look to the world of astronomy as inspiration for the development of the wide-field microscope?

Both fields require imaging through intervening layers that cause image degradation, although on greatly different scales. In astronomy, AO systems correct wavefront aberration – the blurring of telescope images caused by light from a star travelling through turbulence in the Earth's atmosphere. In microscopy, blurring is caused by light travelling through the cytoplasm and the internal structures of living cells.

How recently was this technology developed? To what extent is your work pioneering?

The first adaptive optical system for astronomy was proposed in the 1950s and implemented in the 1970s when the requisite technology became available. Adaptive optics has been used in microscopy since 2000, initially based on maximising image brightness or contrast. Our project is the first to apply the 'guide-star' approach of astronomy to microscopy. Our unique angle has been to develop a biological guide-star as a reference beacon so that we can measure the blurring effect as they do so successfully in astronomy.

What are the main drivers of the project?

We wish to advance understanding of the basic mechanisms behind stem cell structure and behaviour in Drosophila and mouse model systems. Currently, adaptive optics are used clinically in the scanning laser ophthalmoscope, enabling resolution of the mosaic of individual rods and cones in the retina, for application in diseases such as macular degeneration and retinitis pigmentosa. I believe that AO microscopy will eventually enable early detection and treatment of cancer.

How readily will your results translate to humans and have you a timescale for this?

While it is hard to forecast the time required to translate basic research done on animal models to humans, we note that the stem cell treatment for spinal cord injury that was approved for human trials in 2009 was initiated in animal trials in 1999. While that trial was discontinued, it nonetheless demonstrates that basic stem cell research can be translated to human trials within a decade.

How important has collaboration been to the project?

Collaboration between biologists, astronomers, and optical engineers has been crucial to the success of our programme.

Biology plays a critical role in determining the blurring effects that obscure images. In essence, when imaging through thick tissues, the inhomogeneous biological sample becomes a part of the optical path of the microscope. Also, we have used genetic modification to incorporate fluorescent proteins into different cellular structures, providing the reference beacons that we use to measure aberration. We need the help of the biologists to incorporate these fluorescent protein guide-stars into biological specimens.

We have substantial astronomical expertise. We have reapplied many of the same algorithms developed for measuring and correcting the blurring effect of the earth's atmosphere in telescopes to measure and correct the blurring effect of biological tissues.

Lastly, optical engineers are crucial in translating telescope AO systems to the new field of microscopy.

Is there is a single challenge that you hope to surmount in your career – or one in which there is exciting potential?

Throughout my career, imaging has been important for gaining an understanding of various scientific phenomena.

I believe that there is exciting potential in AO microscopy to image through tissues to enable earlier detection of tumours, at the micron level: three orders of magnitude smaller than current techniques.

We would be very interested to hear of new challenges where adaptive optics could provide an advantage for diffraction limited imaging through intervening tissue.

Guiding **light**

A project at the **W M Keck Center for Adaptive Optical Microscopy** at the University of California is creating technology to enable clear observation of the cellular processes of stem cell life cycles in real time

THE W M KECK OBSERVATORY in Hawaii hosts two giant telescopes, the largest in the world, each 10 m in diameter and made from 270 tonnes of steel. The telescopes enable observation and tracking of objects in deep space and embody state-of-the-art technology for correction of image variations caused by the telescopes' own characteristics and movements and interference from the Earth's atmosphere. The telescopes produce high definition images easily comparable with those obtained by the Hubble space telescope.

Professor Claire Max, Director of the Center for Adaptive Optics at the University of California in Santa Cruz (UCSC), was instrumental in the development of much of the state-of-the-art adaptive optics (AO) technology that is used in the W M Keck telescopes' imaging system. Recently, she has lent her support to the adaptation of this technology to collecting images of much, much smaller targets: the workings of the stem cells of fruit flies and mice.

THE SANTA CRUZ AO MICROSCOPY CENTRE

Most of the AO research at UCSC was focused on astronomy requirements until a group of scientists and engineers joined forces to apply AO in microscopes. The principal investigator, Joel Kubby, is an Associate Professor of Electrical Engineering at Baskin School of Engineering at UCSC. His co-investigators in the group include Professor William Sullivan and Assistant Professor Yi Zuo of Molecular, Cell and Developmental Biology at UCSC. Co-investigators Donald Gavel, Director of the UCSC Laboratory for Adaptive Optics, and Scot Olivier, the Associate Physics Division Leader for Applied Physics at Lawrence Livermore National Laboratory (LLNL), who both helped Max to develop the W M Keck AO system, provide their expertise to the group. For Kubby, his work with AO microscopy is a natural progression: "When I was at the AT&T Bell Laboratories, we developed one of the early scanning tunnelling microscopes that was used to image semiconductor surfaces. We developed a new technique for looking at the layers below the surface that helped to understand the growth of semiconductor crystals," he reflects.

The collaboration of biology, AO engineering and astronomy attracted interest first from the California Institute for Regenerative Medicine (CIRM) and then from the W M Keck Foundation, which recently provided US $1 million to fund the set up of the W M Keck Center for Adaptive Optical Microscopy at UCSC.

THE PRINCIPLES OF ADAPTIVE OPTICS

To obtain clear images of objects and events in space, AO systems in telescopes correct the image distortion from intervening atmospheric turbulence, such as winds and temperature changes, through computer-controlled adjustment of a deformable mirror. As Kubby points out, the resulting images are much sharper, greatly enhancing the possibilities for research:

Real-time image analysis of living stem cells will deliver greater understanding of their cellular mechanisms

"They allow astronomers to see out into our universe to study the origins of time and space".

The underlying principle of AO is that obtaining such precise images needs a firm point of reference: "AO systems for telescopes use a point source of light – either a bright star or an artificial guide-star created by a laser – as a reference beacon for measuring atmospheric blurring," explains Kubby. "Having measured the degree of blurring," he continues, "while maintaining reference to the beacon the system then calculates the corrections needed to counteract the distortion of the image, applies the correction by bouncing the incoming light off a deformable mirror configured with the opposite shape to the distortion, and repeats the whole process hundreds of times per second."

AO AND STEM CELL RESEARCH

The notion of applying AO to microscope imaging of stem cells arose from Sullivan's perception of the limitations of current microscope technology in his biological research. Current live imaging techniques are ineffective at depths greater than 30 microns, principally due to changes in refractive index caused by tissue and other molecules in the cell: "An important bottleneck for developing stem cell therapies is the inability to follow live injected stem cells and monitor their mechanisms and fates below the surface of tissue," Kubby observes.

It became apparent to Kubby's group that there were indeed parallels with the problem of imaging for astronomy: "They both involve blurring as light passes through intervening layers between the objects – stars or internal cellular structures – and the imaging optics -telescopes or microscopes". The scientists were confident that with improved imaging technology, the fundamental stem cell mechanisms such as cell renewal, differentiation and asymmetric division, and the role of the niche in stem cell division, might be revealed.

AO WIDE FIELD MICROSCOPES AND BIOLOGICAL GUIDE-STARS

In March 2009 the researchers set about developing an improved wide field microscope for imaging deep within the dense cell layers containing stem cells. They developed a technique to measure the magnitude of image aberrations as a function of tissue depth and prepared the design for a microscope with sufficient wavefront correction for the aberrations. In 2010 they then built the first prototype AO wide field microscope, with the support of the CIRM.

"In astronomy, a 'guide-star' is used as a reference beacon, but there are no similar guide-stars in biological specimens," explains Kubby. Thus, using stem cells in fruit fly embryos and in mouse models, which are typically used to model stem cells prior to human clinical trials, the team are now developing genetically engineered fluorescent cellular proteins, using jellyfish genes, for use as their 'biological guide-stars'. Different colours can be used in tagging to signal different proteins or parts of the cell, and ultimately, different tissue and cell types.

They have now developed AO systems for both a wide field and a confocal microscope, and plan to enhance the confocal microscope design to create a much more powerful two photon AO microscopy system. This will enable better correction of aberrations, improve image clarity and limit the need to move either sample or microscope when tracking changes in real time.

THE FUTURE OF AO MICROSCOPY

Looking ahead, the collaborators envisage that the AO microscope will become the norm for stem cell biologists and are factoring into their design means of reducing the eventual cost of the systems, by including mass-produced components and tried and tested technology where applicable.

Kubby is keen that once they have a viable prototype, AO microscope technology will be commercially developed in California, though most interest is being expressed by major microscope vendors at present. He is also keen to find new applications for the technology in the future.

Results to date have validated Kubby's confidence that real-time image analysis of living stem cells will deliver greater understanding of their cellular mechanisms, providing invaluable information about the complex events that take place during cell formation and development, including the processes that govern the evolution of undifferentiated stem cells into specific cell types. Such information will provide clues to the origins of disease, such as why and how tumour cells and other abnormalities develop. It will also support the development of cell replacement therapies and enable better targeting for drug testing. Kubby is hopeful that the group's work will help in treating diseases such as cancer: "Eventually, we believe that AO microscopy could be used in clinical applications," he reflects. "For example, by enabling earlier detection, it may be possible to minimise the suffering that cancer patients must go through in treatments such as radiation and chemotherapies."

INTELLIGENCE

AO FIELD-WIDE MICROSCOPE

OBJECTIVES

The objectives of this project are to develop enabling technologies and critical procedures to overcome longstanding barriers and vastly improve deep tissue biological imaging. The approach is inspired by the highly successful use of adaptive optics in the W M Keck Telescopes, which allows astronomers to see much more clearly and deeply into space.

The results may enable dramatic improvements in imaging of the cellular universe, enabling biologists to examine crucial living processes deep within tissues and organs at a scale previously impossible, and could lead to new biomedical interventions, such as stem cell therapies.

KEY COLLABORATORS

Professors William Sullivan and **Yi Zuo,** Molecular, Cell and Developmental Biology at UC Santa Cruz

Dr Donald Gavel, Director of the Laboratory for Adaptive Optics at UC Santa Cruz

Dr Scot Olivier, Associate Physics Division Leader for Applied Physics and **Dr Diana Chen**, Optical Scientist, Lawrence Livermore National Laboratory (LLNL)

Drs Oscar Azucena and **Xiaodong Tao**, Department of Electrical Engineering at UC Santa Cruz

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DR JOEL KUBBY is an Associate Professor of Electrical Engineering in the Baskin School of Engineering at UC Santa Cruz. Prior to joining UCSC in 2005, he was at the Xerox Wilson Centre for Research and Technology (1987-2005) in Webster, New York, working in the area of micro-electro-mechanical systems. Prior to Xerox he was at the Bell Telephone Laboratories in Murray Hill New Jersey, working in the area of scanning tunnelling microscopy.