FINAL REPORT FOR AWARD # 9612240

Richard C Haskell ; *Harvey Mudd College*

Optical Coherence Microscopy in Developmental Biology

Participant Individuals:

CoPrincipal Investigator(s) : Scott E Fraser; Daniel C Petersen; Mary E Williams; Ruye Wang

Senior personnel(s) : Barbara M Hoeling

Undergraduate student(s) : Tiffany C Arnal; Francis R Carr; Michael T Cope; Andrew D Fernandez; Jason A Fredrickson; Jody L Hamilton; Sarah A Jacobson; Steven L Johnson; Mark Reyes; Sharon E Ungersma; Peter Boothe; Jason Brudvik; Mathias Gehl; David Herman; Jeremy Liu; James Minnis; Sarah Reisinger; Whittier R Myers; Aaron Boyer; Eric Clement; Jonathan Daehnke; Andrew Harrington; Seth Heidkamp

Graduate student(s) : James Hettinger

Undergraduate student(s) : Eric Huang; Matthew Mattozzi; Andrew Schile; Phu Tran

Participants' Detail

Partner Organizations:

California Institute of Technology: Facilities; Collaborative Research

Dr. Scott E. Fraser, a Co-PI on this grant, is Professor of Biology and Director of the Biological Imaging Center at Caltech. He and his staff (lab technicians, Caltech undergraduates, graduate students, and postdocs) have supplied expertise and frog embryos for imaging. Dr. Fraser has been generous with his time, energy, and lab facilities. This collaboration has not only focused on developmental biology, but has also moved into the area of 3-D visualization with participation from members of Caltech's Applied Mathematics Department.

Colorado State University: Facilities; Collaborative Research; Personnel Exchanges

Dr. June I. Medford, Assistant Professor of Biology at CSU, has collaborated with us since September, 1997. Dr. Medford visited our lab at Harvey Mudd and collected images of developing plants on 6 or 7 occasions, each visit lasting typically a week. During the summer of 1999, a graduate student in Dr. Medford's lab, James Hettinger, spent 10 weeks at Harvey Mudd collecting and analyzing OCM images of plants. A postdoc, Dr. Ronald Parsons, and another graduate student, Aaron Reeves, from Dr. Medford's lab also worked with us on occasional visits.

Dr. Medford has contributed her expertise in plant development, and her lab facilities for histology and electron microscopy have been crucial in comparative studies of the OCM technology. Our 6 month extension of the grant was primarily aimed at supporting our collaboration, culminating in a paper published in Plant Physiology. While Dr. Medford did not draw a salary from our grant, we did support the work of James Hettinger during the summer of 1999 at Harvey Mudd,

and we provided partial support for his subsequent OCM work in the lab of Dr. Medford.

Beckman Laser Institute and Medical Clin: Collaborative Research

For the past decade, Harvey Mudd College has enjoyed a close working relationship with BLIMC on the campus of UC Irvine. In particular, there are several scientists at BLIMC who actively work in OCM and who have collaborated with us during visits to our campus, especially during end-of-the-summer student presentations. Dr. Zhongping Chen and Dr. Johannes F. de Boer have visited several times and have employed Harvey Mudd student researchers at BLIMC for 10 weeks during the summer. While the BLIMC work has focused on Doppler OCT and polarization-sensitive OCT, we have found much common ground in our research programs. In addition, Dr. Thomas Milner, now at U. of Texas, Austin, was another helpful collaborator when he was at BLIMC during the early years of the grant.

Activities and findings:

Research and Education Activities:

Please see the Activities PDF file.

Findings:

Please see the Findings PDF file.

Training and Development:

This research project in optical coherence microscopy has been highly interdisciplinary and has involved students and faculty in nearly all of the traditional academic disciplines. The 28 student researchers listed in the 'Participants' section of this report, plus roughly 22 more students performing research for course credit during the academic year, were drawn from all of the major programs at Harvey Mudd College -- biology, chemistry, computer science, engineering, mathematics, and physics. They worked side-by-side with peers and faculty with very different backgrounds and formal trainings, and attacked a challenging research problem with significant scientific and medical goals. It is clear to us that this research experience has kindled in nearly every participant a scientific curiosity and enthusiasm that has generated a professional identity that fulfills a large part of the College's mission. The process of learning to work on a multidisciplinary team, benefitting from and respecting the expertise of others, will serve these students well as they embark on graduate programs or jobs in science and engineering.

It is important to mention that the faculty involved in this project

have also benefitted enormously from the exposure to different perspectives and tools common in other disciplines. The words 'synergy' and 'diversity' have taken on much richer meanings as a result of this research effort. We are now much more likely to pursue significant research problems that stretch across disciplinary lines -- and those problems are becoming increasingly a larger percentage of the outstanding problems in science and technology.

As mentioned in the 'Activities' section of this report, this research project in optical coherence microscopy and developmental biology has generated new courses and course material in physics, engineering, and computer science at Harvey Mudd College. But perhaps more importantly, the research activities supported by this grant have themselves served as the most effective pedagogical tool in undergraduate education -- original research on significant and challenging problems in laboratories equipped with instruments and technology reflective of the current state-of-the-art, and with faculty mentors who are actively and passionately engaged in that work. These research activities have served as the major focus for at least 5 senior theses at Harvey Mudd and will serve as a major component of 2 Ph.D. theses at Colorado State University.

Outreach Activities:

None applicable.

Journal Publications:

Barbara M.Hoeling, Andrew D. Fernandez, Richard C. Haskell, Eric Huang, Whittier R. Myers, Daniel C. Petersen, Sharon E. Ungersma, Ruye Wang, Mary E. Williams, and Scott E. Fraser, "An Optical Coherence Microscope for 3-Dimensional Imaging in Developmental Biology", *Optics Express (http://www.opticsexpress.org/oearchive/source/19250.htm)*, vol. 6, (2000), p. 136. Published

James W. Hettinger, Matthew de la Pena Mattozzi, Whittier R. Myers, Mary E. Williams, Aaron Reeves, Ronald L. Parsons, Richard C. Haskell, Daniel C. Petersen, Ruye Wang, and June I. Medford, "Optical Coherence Microscopy. A Technology for Rapid, in Vivo, Non-Destructive Visualization of Plants and Plant Cells", *Plant Physiology (http://www.plantphysiol.org)*, vol. 123, (2000), p. 3. Published

Barbara M. Hoeling, Andrew D. Fernandez, Richard C. Haskell, and Daniel C. Petersen, "Phase Modulation at 125 kHz in a Michelson Interferometer Using an Inexpensive Piezoelectric Stack Driven at Resonance", *Review of Scientific Instruments*, vol. , (), p. . Accepted

Book(s) of other one-time publications(s):

Other Specific Products:

Internet Dissemination:

http://www.physics.hmc.edu/research/ocm.html

This website provides a brief description of the OCM research project and the NSF award that supports it. OCM images described in the Activities and Findings sections are served by linked web pages. Our OCM publications to date are also accessible as pdf files on linked web pages.

Contributions:

Contributions within Discipline:

It is clear that our optical coherence microscope (OCM) is capable of imaging the dynamic processes that occur in the early development of plants and animals. OCM imaging is non-invasive and non-destructive, so the complete development of an individual organism can be followed. OCM images can be obtained at depths up to 1 mm in tissue where conventional light microscopy fails because of the high light scattering properties of most embryonic tissue. OCM is emerging as a very useful tool in the field of developmental biology.

Contributions to Other Disciplines:

Our 3-dimensional approach to optical coherence microscopy (3-D OCM) can contribute significantly to clinical applications of OCM in dentistry, dermatology, ophthalmology, and endoscopic medicine. Often in clinical diagnoses, the 3-dimensional spatial relationships of cells and groups of cells are critical to a full understanding of the state of the biological tissue. In these situations, the longer acquisition time of 3-D datasets may be offset by the more complete information obtained. Our techniques and software for 3-D visualization of OCM images will also contribute to the larger field (and growing exponentially) of medical imaging.

Contributions to Education and Human Resources:

This research project in optical coherence microscopy has been highly interdisciplinary and has involved students and faculty in nearly all of the traditional academic disciplines. The 28 student researchers listed in the 'Participants' section of this report, plus roughly 22 more students performing research for course credit during the

academic year, were drawn from all of the major programs at Harvey Mudd College -- biology, chemistry, computer science, engineering, mathematics, and physics. They worked side-by-side with peers and faculty with very different backgrounds and formal trainings, and attacked a challenging research problem with significant scientific and medical goals. It is clear to us that this research experience has kindled in nearly every participant a scientific curiosity and enthusiasm that has generated a professional identity that fulfills a large part of the College's mission. The process of learning to work on a multidisciplinary team, benefitting from and respecting the expertise of others, will serve these students well as they embark on graduate programs or jobs in science and engineering.

It is important to mention that the faculty involved in this project have also benefitted enormously from the exposure to different perspectives and tools common in other disciplines. The words 'synergy' and 'diversity' have taken on much richer meanings as a result of this research effort. We are now much more likely to pursue significant research problems that stretch across disciplinary lines -- and those problems are becoming increasingly a larger percentage of the outstanding problems in science and technology.

Contributions to Resources for Science and Technology:

As mentioned in the 'Activities' section of this report, this research project in optical coherence microscopy and developmental biology has generated new courses and course material in physics, engineering, and computer science at Harvey Mudd College. But perhaps more importantly, the research activities supported by this grant have themselves served as the most effective pedagogical tool in undergraduate education -- original research on significant and challenging problems in laboratories equipped with instruments and technology reflective of the current state-of-the-art, and with faculty mentors who are actively and passionately engaged in that work.

While this research project is therefore a 'consumable resource' for research and education, the skills and methodology for designing, funding, and executing these sorts of programs can be documented and disseminated as a rich 'resource for research and education.' Harvey Mudd College has recently received an AIRE award (Award for the Integration of Research and Education), and nearly all of the faculty participating in this OCM research project have participated in AIRE activities on the HMC campus. It is clear that workshops associated with this AIRE grant, presentations to trustees, prospective trustees, and faculty visiting from other institutions, and discussions with educators who are members of visiting accrediting teams all constitute methods of dissemination of our experience in integrating research in education.

Contributions Beyond Science and Engineering:

Our use of optical coherence microscopy (OCM) to image developing plants has led to the realization that OCM has potential importance in

the agricultural industry. OCM can provide a rapid, non-invasive assay of the progression of disease and of the mechanism of pest attack in food crops, as well as a high-throughput assay of the effects of gene mutations in the search for genetically superior strains of crops. We are now negotiating with several major agricultural corporations to collaborate on the use of OCM in their research and development activities. Our patent application concerning the use of OCM in imaging plants is part of a coordinated program to commercialize our OCM technology. We hope that ultimately our progress in OCM technology will benefit the US and world populations by providing plentiful and healthful food crops.

Categories for which nothing is reported:

Participants: Other Collaborators
Products: Book or other one-time publication
Products: Other Specific Product

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Activities

The goal of this research has been to design and construct an optical coherence microscope (OCM) to study fundamental problems in developmental biology. As initially proposed, we have used our OCM to explore two model systems, the frog *Xenopus laevis* and the plant *Arabidopsis thaliana*. The OCM instrument we have constructed performs as well as we had hoped—with a lateral resolution of 5 μm, a depth resolution of 10 μm, and a signal-to-noise ratio (SNR) limited only by fundamental photon noise. The acquisition time for a 3-D image containing one million voxels is 6 minutes, and there is potential for reducing that to about 1 minute in the next few months.

A detailed description of our OCM instrument and its performance can be found in two papers: "An Optical Coherence Microscope for 3-Dimensional Imaging in Developmental Biology," which was published in March 2000 in the web-based electronic journal *Optics Express*, and "Phase Modulation at 125 kHz in a Michelson Interferometer Using an Inexpensive Piezoelectric Stack Driven at Resonance," which has been accepted for publication in the *Review of Scientific Instruments.* The salient points of those papers as well as a few instrument details that have not yet been published are summarized in the Findings section of this report.

Using our OCM we have non-invasively and non-destructively imaged events in the early development of both animals and plants that occur 400 to 500 μm below the tissue surface. The large amount of light scattering in these tissues prevents the successful application of other optical imaging techniques at this depth. As a check and calibration of OCM, we have imaged a developing *Arabidopsis* plant with OCM and then immediately fixed the plant for examination by conventional (but destructive) techniques, i.e., scanning electron microscopy and histological sectioning for light microscopy. Comparison of the images obtained with these different techniques supports a straight-forward interpretation of the OCM images. As we report in the Findings section, the ability of OCM to follow *in vivo* the developmental process in a single animal or plant does indeed prove to be a powerful tool for revealing dynamic processes.

Our studies of the plant *Arabidopsis thaliana* have been reported in a paper: "Optical Coherence Microscopy: A Technology for Rapid, *in Vivo*, Non-Destructive Visualization of Plants and Plant Cells," which was published in the new "Breakthrough Technologies" section of the May 2000 issue of *Plant Physiology*. With the images of *Arabidopsis* that we collected, we have made QuickTime movies and placed them on our website (http://www.physics.hmc.edu/research/ocm.html). These movies are rotating 3-D images of developing plants.

We also used our OCM to image developing frog embryos. We had some success in stages 10 through 20 (gastrulation through neurulation), and acquired some remarkable images of frogs in the later stages 40 through 45. Our *Optics Express* article includes a QuickTime movie that consists of a rotating 3-D image of a stage 41 *Xenopus laevis*. We

have assembled OCM images of gastrulation and neurulation into time lapse movies. These unpublished movies are available on our website.

Another key objective of this project has been to engage undergraduates in a significant research project to help prepare and motivate them to pursue careers in multidisciplinary activities in science and technology. By any set of criteria, the educational component of this project has been a notable success. Roughly 50 student researchers participated in the project over the three years of the grant, either during a 10-week period in the summer or for academic credit during the school year. Oral presentations by student researchers were the culmination of the summer activities, and oral presentations and written theses were a required part of the research program during the academic year. Students were drawn from all of the six major programs at Harvey Mudd—biology, chemistry, computer science, engineering, mathematics, and physics.

The seven faculty participants in the OCM project came from three institutions and three traditional academic disciplines. A new biophysics half-course was conceived and offered, and course material from it migrated to an introductory course in biomedical engineering. Our visualization software package served as a project-oriented assignment in a computer science course. Activities associated with this research project made a significant impact on the educational programs of the College and on the students and faculty who participate in those programs.

In addition to the three scientific journal articles mentioned above, we gave oral presentations at two professional meetings. The first was a contributed talk at the SPIE meeting in San Jose in January 1999, and the second was an invited talk at the Annual Meeting of the Optical Society of America in September 1999. Professor Haskell described our OCM research at a physics colloquium at Cal State Long Beach in October 1998. Our collaborator, Professor June Medford in the Biology Department at Colorado State University, has given numerous talks on our joint OCM work with plants. Perhaps the most notable were the talks at the Plant Signaling meeting in Coeur d'Alene, ID, in February 1999 and at the Eleventh International Meeting on Arabidopsis Research in Madison, WI, in June 2000.

The design of our OCM and especially our application to plants and agriculture are sufficiently unique that we have applied for a patent. Harvey Mudd College has generously supported this patent venture. The six inventors include our collaborator, Professor Medford, who has given three talks to interested corporate researchers, Novartis (NADI – Novartis Agricultural Discovery Institute) in San Diego in February 2000, the Dupont Plant Biotechnology Group in Wilmington, DE, in May 2000, and Paradigm Genetics in the Research Triangle Park, NC, in September 2000.

Findings

We have divided our major findings into three areas: (1) the design and performance of our optical coherence microscope (OCM), (2) insights gained from our OCM images of developing plants (*Arabidopsis thaliana*), and (3) conclusions drawn from our OCM images of developing frogs (*Xenopus laevis*).

Instrument Performance

Our final OCM instrument provides the resolution and speed of image acquisition necessary to follow *in vivo* and in real time the development of an individual plant or frog. The design principles of our OCM and most of the instrumental details are described in our *Optics Express* article (http://www.opticsexpress.org/oearchive/source/19250.htm) or in our manuscript accepted for publication in the *Review of Scientific Instruments* (see the pdf file on our website http://www.physics.hmc.edu/research/ocm.html). We summarize the most critical performance specifications.

Brief description of instrument (See Figure 1.) The depth resolution of our OCM is 10 μm (full-width-at-half-maximum (FWHM) in tissue with n = 1.4) and is determined by the coherence length of the 850 nm superluminescent diode used as a light source.

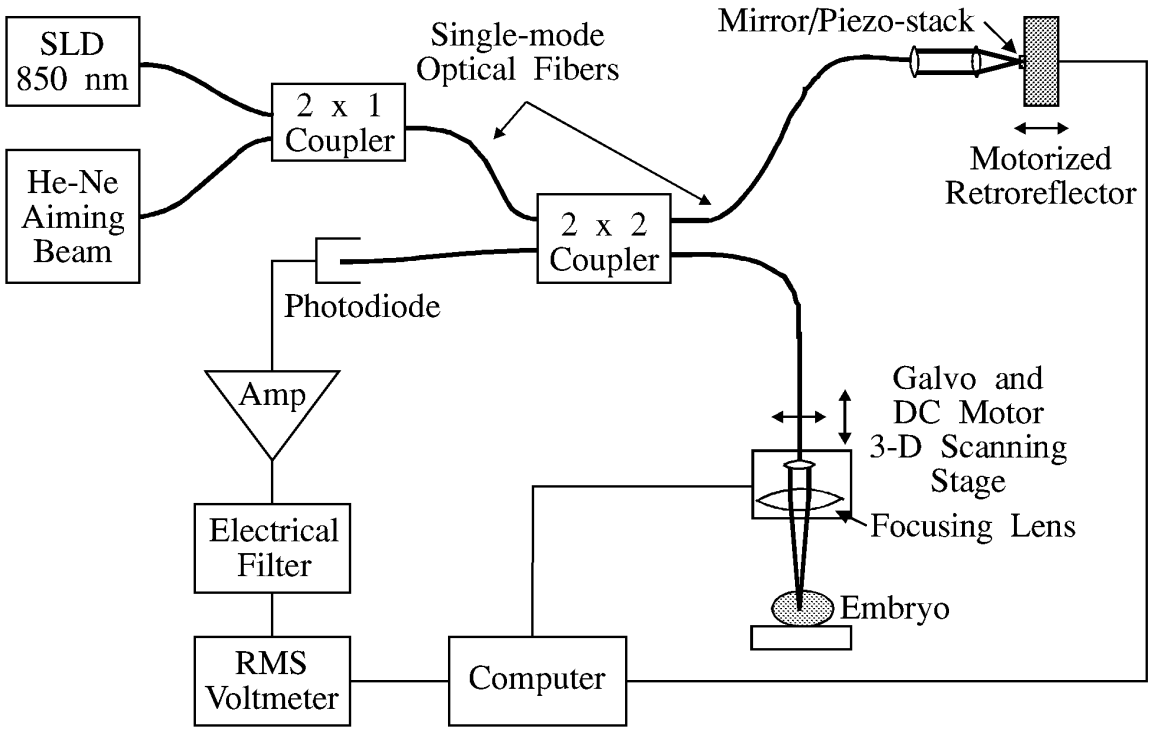

Fig. 1. Optical schematic of the instrument.

The lateral resolution is 5 μm (FWHM) and corresponds to the waist diameter of the focused beam. A pair of galvoscanning mirrors moves the beam waist quickly over the x-y plane, then the focusing lens is stepped down along the z-axis (depth) and a slightly deeper x-y plane is scanned. At the same time the focusing lens is moved, the reference mirror is translated to keep the equal path length position of the OCM interferometer coincident with the focused beam waist. This latter procedure maintains the 5 μm lateral resolution throughout the depth of the sample.

Fringes are produced at the output of the OCM interferometer by oscillating the reference mirror at 125 kHz. The amplitude of these fringes is ultimately the output signal of the OCM. A tiny reference mirror (1.5 mm × 1.5 mm × 0.1 mm) is glued to a small piezoelectric stack positioned at the rear of a cat's-eye retroreflector (see Fig. 2). The reference beam is focused onto the mirror by the retroreflector lens, and the piezo stack is driven at a resonance frequency of 125 kHz to produce a phase modulation amplitude of roughly one fringe at the OCM interferometer output. This method of phase modulation is much less expensive than a technique employing an electro-optic device. The problems of phase wander and piezo wobble were circumvented as described in the *Optics Express* article and in the *Review of Scientific Instruments* manuscript.

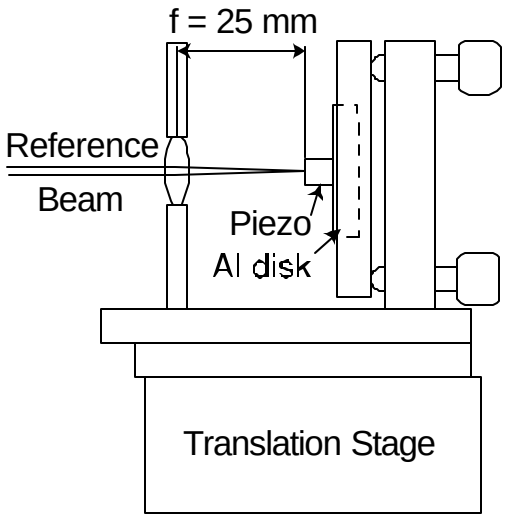

Fig. 2. Sketch of the cat's-eye retroreflector with reference mirror mounted on the piezoelectric stack.

The short lengths of optical fiber (~ 1 meter) comprising the sample and reference arms of the instrument exhibit no fiber birefringence drift. This is a delightful improvement over our preliminary OCM instrument that utilized 10 meters of fiber in each arm. Despite the lack of polarization-preserving fiber, our heterodyne efficiency can be kept easily above 90%. We simply twist the sample and reference fibers occasionally (once every few months) to compensate for relative polarization rotation in the two arms. However, we are not able to detect and record any sample birefringence. We want to redesign the instrument in the future to achieve polarization sensitivity.

The performance of our OCM is limited by fundamental photon noise. We discovered that this noise is dominated by Bose-Einstein photon bunching, so we attenuated the reference beam by a factor of four to optimize the signal-to-noise ratio. Our use of an unbalanced OCM interferometer configuration was guided by convenience. Some researchers claim an improvement with a balanced configuration, albeit at the expense of instrument complexity. We are seriously considering a balanced configuration that would allow greater power in the reference beam but would circumvent photon bunching by using balanced detectors.

Finally, we are in the process of replacing the electrical filter and RMS voltmeter in the detection electronics with a digital signal processing unit (DSP). This will provide flexibility in the trade-off between image acquisition speed and image quality. In addition, the DSP will improve the linearity of the instrument and increase the dynamic range. Because of sub-optimal choices for bandwidths in the present electrical filter, the DSP will decrease the image acquisition time for a million voxel image from the current 6 minutes to roughly 1 minute. The DSP will also pave the way for future acquisition of the entire fringe waveform, potentially yielding valuable phase information.

The entire process of image acquisition is directed by computer programs written in the software environment of LabView (National Instruments, Austin, TX). This graphical programming language runs on a Pentium III computer with a Windows NT 4.0 operating system. This is the same hardware/software platform used by our visualization software, making it possible in the future to acquire and visualize an OCM image on a single, relatively inexpensive computer.

Visualization software We are currently using Visualization Express 5.0 (available from Advanced Visualization Systems, Inc., Waltham, MA) on a Pentium III computer running Windows NT 4.0. Visualization Express allows us to "volume-render" our 3-D data sets onto a 2-D viewing (computer) screen. In this process, the contents of volume elements (voxels) are "blended" with the contents of voxels directly behind them to yield the final number assigned to a pixel on the viewing screen. An "opacity" parameter in this blending algorithm may be reduced to achieve a translucent appearance in which structures deep within the sample can be seen, or it can be increased to yield a superficial rendering of the sample. We find that rotating a translucent, volume-rendered 3-D data set is remarkably effective in producing a 3-D perspective.

We can also rotate, slice, or crop the 3-D data set, yielding 2-D images that can be analyzed using standard methods. Often a 3-D perspective is used to determine the optimal 2-D slice to use in quantitative analyses. Section 4 of the *Optics Express* paper describes in more detail the operation of Visualization Express.

While Visualization Express has proved effective, we are in the process of writing our own visualization software package in order to simplify the user-interface and provide open access to the source code. We are using a combination of Microsoft Visual C++, OpenGL, and Microsoft Foundation Class (MFC). The data handling program is written

in C++ and includes calls to OpenGL for graphic display of the data. Access to the operating system and hence to the graphics board and computer monitor is provided by MFC. This software package has been the focus of a project-oriented activity for a team of students in an HMC computer science course, and we hope to have a new working visualization package within a year.

Plant Developmental Biology

Our OCM work with the plant *Arabidopsis thaliana* is described in a paper published in *Plant Physiology* (123: 3-15 (May 2000)). We focused on the shoot apex of the plant where a series of leaves are formed in a characteristic spiral pattern. Considerable effort in the plant biology community is being devoted to the study of the formation of this leaf pattern (phyllotaxy). Attention is paid particularly to the relation between gene expression and the series of morphological changes in which leaf primordia emerge from the shoot apical meristem, a group of undifferentiated cells lying below the early leaves and embryonic cotyledons. In an intact plant the meristem and leaf primordia are shielded from the view of conventional light microscopes, so we used the OCM to monitor the appearance and growth of successive leaves during the development of an individual plant. The *Arabidopsis* plants used were growing in soil, and they showed no indication of damage or abnormal development despite many days of OCM imaging.

We found that *Arabidopsis* tissue is highly scattering. We measured an attenuation coefficient at 850 nm of roughly 15 /mm or equivalently an attenuation length of 70 μm. We imaged typically 300 to 400 μm deep into tissue. For comparison, confocal fluorescence microscopy is limited to depths less than 100 μm. In addition, our early imaging attempts were frustrated by the strong light scattering of trichomes, hairlike structures on leaf surfaces. This problem was circumvented by using the *glabrous* (*gl-1*) mutant of *Arabidopsis* which lacks trichomes. As a result, the OCM was able to follow readily the appearance and growth of successive leaves in the characteristic spiral pattern during development of a single plant. Images taken 10 minutes apart were virtually identical, but images taken an hour or two apart showed definite signs of growth. We have not yet, however, imaged unambiguously the emergence of leaf primordia from the meristem. The spatial resolution of our current OCM may or may not be adequate for this latter task—we are currently working to resolve this question.

We also tried to image the plant seeds intact in their silique. Pattern formation (bipolar root-shoot organization) and organogenesis (formation of roots and cotyledons) occur in the very early embryo. These events are obscured from view by the silique and ovule walls, so we hoped that OCM would allow us to follow these processes in real time in a single plant, presumably for the first time. Unfortunately, we found the attenuation at 850 nm was simply too great. We are anxious to see if the seed embryo in a silique can be visualized with an OCM employing a wavelength of 1300 nm where light scattering is presumably reduced.

In our *Plant Physiology* paper we present OCM images beside or superposed upon electron micrographs or histological sections of the same plant. It is clear from these comparisons that OCM images are reliable, though they typically have poorer resolution than the scanning electron micrographs and histological sections. On the other hand, producing the electron micrographs and sections destroyed the plant, preventing observation of subsequent development. While the full-width-at-half-maximum (FWHM) resolution of the OCM is a cylinder with diameter 5 μm and depth 10 μm, we routinely see image detail on the scale of 2 to 3 μm. This finer detail is achieved with OCM sampling intervals of 2 μm.

We also used *Arabidopsis* mutants to test our OCM instrument. Using the *shoot-meristemless* mutant, both OCM images and electron micrographs of the same plant show a void where the meristem should be.

The origin of the OCM light-scattering signal was investigated. *Arabidopsis* aneuploid cells grown in suspension are roughly 20 to 30 μm in size and can be resolved easily in OCM images. The nuclei and cytoplasm are bright in the OCM images, while the vacuoles are very dark regions. As other researchers have reported, nuclei are large (Mie) scatterers and backscatter significantly, giving rise in our case to an OCM signal. They scatter enormously more in the forward directions and hence contribute strongly to the average attenuation coefficient that reduces the OCM signal with depth into the sample. Small (Rayleigh) scatterers in the cytoplasm scatter isotropically—enough in the backward direction to yield a detectable OCM signal when their concentration is sufficiently high, but not enough in all directions to contribute significantly to the overall attenuation coefficient of the cells. It is interesting to note that we can readily obtain OCM images of individual polystyrene latex spheres with diameter 523 nm executing Brownian motion in water. These spheres are not so large as to contribute strongly to the average attenuation coefficient of the sample, and we are considering them as a contrast agent or marker for OCM imaging.

Our collaborators at Colorado State University have used OCM images of *Arabidopsis* to generate movies that illustrate the capability of our OCM instrument and visualization software. Images are cropped, sliced, rotated and viewed as semi-transparent 3-D images to visualize the internal structure of plant tissue. These movies have been placed on our website and are a part of our *Plant Physiology* publication.

Frog Developmental Biology

Our early OCM studies of frog development were performed on stage 41 frogs (approximately 3-day-old tadpoles) that had been lightly fixed (2% paraformaldehyde overnight at 4º C). From the many OCM images that we collected of these fixed frogs, we created a QuickTime movie that we submitted as part of the *Optics Express* paper (also available on our website). The movie illustrates the structure that is evident and the 3-D perspective that can be achieved by rotating a volume-rendered image. The highly scattering spinal cord is clearly visible, and the waning notochord appears as a low-

scattering cylinder. There is a small range of viewing angles during which one can see directly down the notochord axis. A dotting of melanophores (pigment cells) throws shadows onto deeper tissue. Developing somites form diagonal stripes (chevrons) in a side-on view. The movie also illustrates the effect of altering the opacity value from a low, translucent setting to a high, superficial-view value.

We measured an average attenuation coefficient at 850 nm for these later stage frogs to be roughly 10 /mm, yielding an attenuation length of 100 µm. We often imaged 400 µm deep into the frog tissue.

OCM studies during the summers of 1999 and 2000 focused on the development of live frogs during gastrulation (stages 10 to 13, 10 to 15 hours post fertilization) and neurulation (stages 14 to 20, 16 to 22 hours post fertilization). Attenuation at 850 nm is very high in these early stages of development, roughly 25 /mm. Nevertheless, we were able to produce a time-lapse movie of the movement of mesodermal cells underneath and across the ectodermal layer during gastrulation. Another time-lapse movie follows the formation of the neural fold during neurulation. These unpublished movies are available on our website.

The high attenuation at 850 nm makes it difficult to image deep into the embryo at these early stages of development. We are anxious to repeat these image sequences at a wavelength of 1200 or 1300 nm where we expect scattering to be less.

Directions of Future Work

There are many new lines of investigation that seem important to pursue. We mention here just a few of the central topics for future work.

We would like to improve and extend the capabilities of our current OCM instrument. To achieve greater speed and better depth resolution, we would like to develop a higher power (tens of milliwatts) light source with a broader spectrum and hence shorter coherence length. We would also like to explore image quality and depth penetration at a wavelength of 1200 or 1300 nm. We expect scattering at these wavelengths to be less and hence depth penetration to be greater. However, it remains to be seen if spatial variations of scattering in tissue will be large enough at these longer wavelengths to provide good contrast in OCM images. A titanium-sapphire or chromium-forsterite laser combines these source characteristics, and we are seriously considering constructing one of these sources. In the meantime we will try to obtain exploratory images using a longer wavelength OCM at another research laboratory.

We would also like to modify our OCM to be able to detect birefringence in a biological sample. At the moment, birefringent structures may give rise to misleading features in our OCM images. It also seems wise to experiment with a balanced interferometer configuration including balanced detectors to remove intensity noise at the higher power levels.

In terms of *Arabidopsis* studies, we plan to focus on making time-lapse movies of dynamic processes, like phyllotaxis. We have not yet successfully imaged the initiation of leaf primordia at the shoot apical meristem, and we would like to do so. It will be important to correlate the changing morphology with gene expression, so the development of reporter genes whose products are visible in the OCM will be an essential area of work. It is also important to try to image the embryo intact in the silique using a 1200 or 1300 nm light source.

Our *Xenopus* studies have reached the point where we can begin to ask serious questions about events during gastrulation and neurulation. Greater depth penetration in these early stages would be helpful, so we plan to experiment with a wavelength of 1200 to 1300 nm. It is clear that the quality of our images at stages 40 to 45 are sufficiently good to focus on specific dynamic events during this time period. As with *Arabidopsis*, it will be important to design reporter genes that can correlate the changes in morphology with gene expression. In addition it would be useful to develop OCM contrast agents that will allow us to construct fate maps for cells in the early stages of *Xenopus* development.

Other Project Participants

Fraser E Scott : CoPrincipal Investigator
Has worked for more than 160 hours : Yes
Contribution to project : Dr. Fraser served as leader and advisor of the frog developmental biology group. He and his lab supplied the live or fixed frog embryos, and provided the expertise in care, handling, and imaging of living embryos. He also served as advisor on OCM instrument development.

Petersen C Daniel : CoPrincipal Investigator
Has worked for more than 160 hours : Yes
Contribution to project : Dr. Petersen shared the leadership with Dr. Haskell of the instrument development group. Like Dr. Haskell, he also played an active role in the developmental biology groups and in the image visualization work.

Williams E Mary : CoPrincipal Investigator
Has worked for more than 160 hours : Yes
Contribution to project : Dr. Williams served as the leader of the plant developmental biology group, and as the local coordinator of frog developmental biology activities. She also played an advising role in the image visualization work.

Wang Ruye : CoPrincipal Investigator
Has worked for more than 160 hours : Yes
Contribution to project : Dr. Wang served as the leader of the image analysis and visualization group. A considerable amount of his time was spent in development of the AVS visualization software.

Hoeling M Barbara : Senior personnel
Has worked for more than 160 hours : Yes
Contribution to project : Dr. Hoeling worked full-time with our OCM research group during the summers of 1997, 1998, and 1999. While we were able to offer Dr. Hoeling only a small stipend for the summer of 1997, we received Research Opportunity Awards for her support during the summers of 1998 and 1999. Dr. Hoeling also worked part time on the OCM research project during the academic years 1996-97, 1997-98, 1998-99, and 1999-2000, without compensation. She contributed valuable expertise in optical physics and quantum optics in the instrument development group.

Hettinger James : Graduate student
Has worked for more than 160 hours : Yes
Contribution to project : James is a graduate student in the Biology Dept. at Colorado State University. He was a graduate researcher in the HMC OCM lab during the summer of 1999. James is a Ph.D. candidate in the lab of our collaborator, Professor June Medford at CSU.

Arnal C Tiffany : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Tiffany was an undergraduate researcher during the summer of 1997. She contributed to the plant developmental biology efforts. Tiffany graduated from Harvey Mudd in 2000 as a physics major.

Carr R Francis : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Francis was an undergraduate researcher during the summer of 1997. He contributed to the instrument development efforts and to the Monte Carlo simulations. He graduated from Harvey Mudd in 1998 as an engineering major.

Cope T Michael : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Michael was an undergraduate researcher during the summers of 1997 and 1998. He contributed to the instrument development efforts. Michael graduated in 2000 as an engineering major.

Fernandez D Andrew : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Andrew was an undergraduate researcher during the summer of 1997. He contributed to the instrument development efforts. He graduated from Harvey Mudd in 1999 as an engineering major.

Fredrickson A Jason : Undergraduate student
Has worked for more than 160 hours : Yes

contributed to Monte Carlo simulations of the instrument. Jason graduated from Harvey Mudd in 1999 as a physics major.

Hamilton L Jody : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Jody was an undergraduate researcher during the summer of 1997. She contributed to the frog developmental biology work, including OCM imaging. Jody graduated from Harvey Mudd in 2000 as a chemistry major.

Jacobson A Sarah : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Sarah was an undergraduate researcher during the summer of 1997. She contributed to the instrument development efforts. Sarah graduated in 1998 as an engineering major.

Johnson L Steven : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Steven was an undergraduate researcher during the summer of 1997. He contributed to the instrument development efforts. Steven graduated in 1997 as a physics major.

Reyes Mark : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Mark was an undergraduate researcher during the summer of 1997. He contributed to the image visualization efforts. Mark graduated from Harvey Mudd in 1998 as a computer science major.

Ungersma E Sharon : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Sharon was an undergraduate researcher during the summers of 1997 and 1998. She contributed both to instrument development and to developmental biology activities. Sharon graduated from Harvey Mudd in 1998 as a physics major.

Boothe Peter : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Peter was an undergraduate researcher during the summer of 1998. He contributed to the image visualization efforts. Peter graduated from Harvey Mudd in 2000 as a computer science major.

Brudvik Jason : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Jason was an undergraduate researcher during the summer of 1998. He

physics major.

Gehl Mathias : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Mathias was an undergraduate researcher during the summer of 1998. He contributed to the plant developmental biology efforts including OCM imaging. Mathias graduated from Harvey Mudd in 1999 as a biology major.

Herman David : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : David was an undergraduate researcher during the summer of 1998. He contributed to the image visualization efforts. David anticipates graduating from Harvey Mudd in 2001 as a computer science major.

Liu Jeremy : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Jeremy was an undergraduate researcher during the summer of 1998. He contributed to the image visualization efforts. Jeremy anticipates graduating from Harvey Mudd in 2001 as a physics major.

Minnis James : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : James was an undergraduate researcher during the summers of 1998 and 1999. He contributed to the Monte Carlo simulations of the OCM instrument. James graduated from Harvey Mudd in 1999 as a physics major.

Reisinger Sarah : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Sarah was an undergraduate researcher during the summer of 1998. She contributed to the developmental biology efforts. Sarah graduated from Harvey Mudd in 1999 as a biology major.

Myers R Whittier : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Whittier was an undergraduate researcher during the summers of 1998 and 1999. He contributed to the instrument development efforts. Whittier graduated from Harvey Mudd in 1999 as a physics major.

Boyer Aaron : Undergraduate student
Has worked for more than 160 hours : Yes

Aaron contributed to the instrument development efforts. Aaron anticipates graduating from Pitzer College in 2002 in computer science.

Clement Eric : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Eric was an undergraduate researcher during the summer of 1999. He contributed to the instrument development efforts. Eric anticipated graduating from Harvey Mudd in 2002 as an engineering major.

Daehnke Jonathan : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Jonathan was an undergraduate researcher during the summer of 1999. He contributed to the instrument development efforts. Jonathan anticipates graduating from Harvey Mudd in 2001 as a physics major.

Harrington Andrew : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Andrew was an undergraduate researcher during the summer of 1999. He contributed to the instrument development efforts. Andrew graduated from Harvey Mudd in 1999 as an engineering major.

Heidkamp Seth : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Seth was an undergraduate researcher during the summer of 1999. He contributed to the image visualization efforts. Seth graduated from Harvey Mudd in 2000 as a computer science major.

Huang Eric : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Eric was an undergraduate researcher during the summer of 1999. He contributed to the image visualization efforts. Eric anticipates graduating from Harvey Mudd in 2002 as a computer science major.

Mattozzi Matthe w : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Matthew was an undergraduate researcher during the summer of 1999. He contributed to the plant developmental biology efforts including OCM imaging. Matthew anticipates graduating from Harvey Mudd in 2001 as a chemistry major.

Schile Andrew : Undergraduate student

Contribution to project : Andrew was an undergraduate researcher during the summer of 1999. He contributed to the frog developmental biology efforts. Andrew anticipates graduating from Harvey Mudd in 2001 as a biology major.

Tran Phu : Undergraduate student
Has worked for more than 160 hours : Yes
Contribution to project : Phu was an undergraduate researcher during the summer of 1999. He contributed to the image visualization efforts and to the instrument development activities. Phu graduated from California State Polytechnic University in Pomona in 1999 as a physics major.

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We welcome comments on this system