

Meeting the Challenges of Long-term Time-lapse Imaging

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Recent technological developments in the sequence capture of cellular events through a light microscope can be combined to image multiple independent experiments automatically, with many advantages.

Capturing sequences of microscope images has become an invaluable tool for studying mechanisms underlying cell signaling, growth, and differentiation. Recent technological developments can be combined to image multiple independent experiments automatically, with the requisite combination of multiparametric read-out, high resolution optics, high precision sample repositioning, and long-term maintenance of optimal cell growth conditions.

History and applications

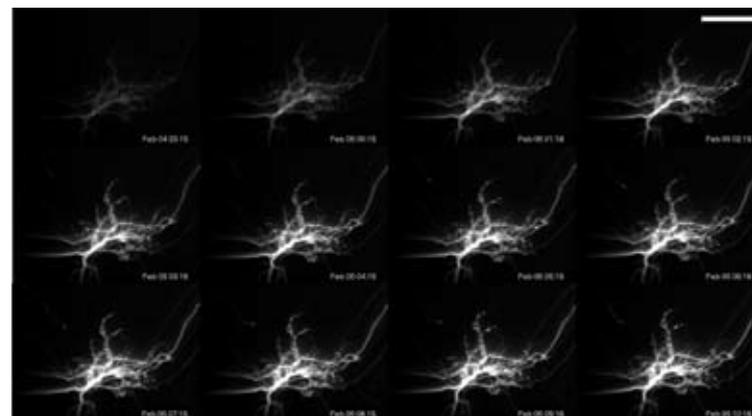
Prior to the discovery and development of green fluorescent protein (GFP), live cell imaging was confined to structure and a few fluorescent labels that were able to penetrate live cells. Antibodies labeled specific molecules, an approach that often required fixation and permeabilization, allowing only frozen “moments in time” from

which the dynamics of a cellular process could be deduced. Since the advent of fluorescent proteins, live cell imaging has increased dramatically, driving the emergence of imaging solutions with varying degrees of hardware and software integration. Customized systems with requirements for sophisticated microscopy and programming expertise are now joined by commercial platforms that focus more on ease of use.

The ability to track multiple fluorescent probes simultaneously over time in a single experiment correlates with an increase in the need for time-lapse imaging experiments. Recent developments in digital

image capture, with improved signal-to-noise ratios, higher quantum efficiencies, and better usability, along with a parallel revolution in the availability of new fluorescent probes for long-term use with living specimens, have expanded the field. Finally, developments in creating stable, controlled environments for living cells have evolved significantly toward making live cell imaging possible over time.

Although the use of live cell fluorescence imaging has been growing, long-term time-lapse observation has been limited by technical issues. First is the difficulty of controlling the environment sufficiently for cells



Left, Figure 1. Olympus VivaView FL Incubator Fluorescence Microscope, an ultra-long-term live cell incubation and imaging system that can handle observation of multiple locations in up to eight dishes simultaneously and handle high-resolution photomicrography. (Source: Olympus America Inc.)

Above, Figure 2. Representative fluorescent images from a 16-hour time-lapse experiment, acquired automatically with no user intervention. Expression of chimeric mTomato fluorescent protein after transfection into primary neuron cultures. Figures 2, 3, 4, and 5 were captured using the Olympus VivaView FL microscope system. (Source: Dr. Thomas Blanpied, University of Maryland School of Medicine, Department of Physiology)

to maintain normal function throughout an experiment lasting hours, days, or even weeks. Some cell types are exquisitely sensitive to environmental perturbation even in imaging experiments lasting just seconds. Cells may be cultured in a controlled environment and then transferred to a microscope but, even when heaters and other devices regulating temperature, gas, humidity, and pH are used on the microscope, the transfer itself can contribute to cell demise. Recently, custom and commercial systems are beginning to combine inverted microscopes, incubation chambers and cameras, adding features to compensate for thermal and mechanical drift caused by environmental differentials.

Another concern is the lack of full-featured, integrated hardware and software. Researchers require repeatable positioning for multi-field time-lapse imaging, along with notification if the temperature changes or CO₂ runs out. In addition, commercial software has not fully integrated functions such as autofocus, time-lapse acquisition, storage, image naming, and post-capture analysis. Most available systems lack the ability to image multiple samples on the same microscope, making it difficult to analyze results with a variety of analytes or conditions, or to run an exact simultaneous control. Furthermore, the volume of

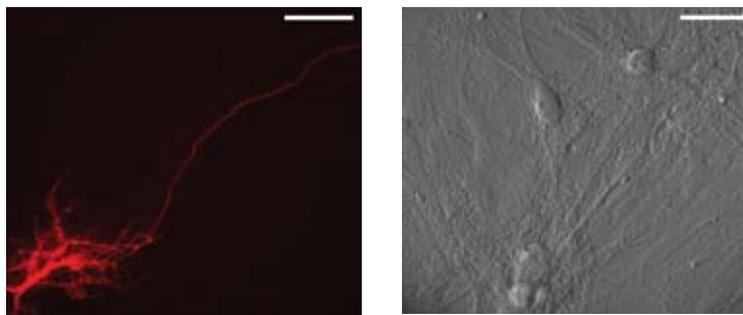


Figure 3. Fluorescence (left) and differential interference contrast (DIC) (right) images of mTomato fluorescent protein expression after transfection into primary neuron culture. (Source: Dr. Thomas Blanpied, University of Maryland School of Medicine, Department of Physiology)

data in many of today's experimental protocols is enormous, and most commercial systems have not been ready to take on such immense data management tasks. For example, a recent publication in *Science* described a home-built automated microscope that imaged for three days, capturing 216 multicolor images per protein every 20 minutes ("Dynamic proteomics of individual cancer cells in response to a drug." Cohen AA, *et al. Science*. 2008 Dec 5; 322(5907):1511-6).

The third key issue has been financial. By the time a researcher purchases the microscope, imaging system, software, focus drift system, incubator, and other components necessary for the experiment, the cost swells well into six figures.

The final concern is the ability to do multi-parameter fluorescence imaging at subcellular resolution. Although several

commercial live cell imaging systems are available, most are limited to relatively low magnification, and compromises in imaging quality and optical capability have been made to satisfy throughput demands. Furthermore, transmitted light imaging has been restricted to phase contrast microscopy, lacking the structural detail afforded by differential interference contrast (DIC) imaging.

Enhancing time-lapse micrography

Scientists have taken a number of steps to address these issues, building custom systems or adapting their experimental protocols so that there are limited and rapid transitions between microscope and incubator.

Commercial systems also are coming to market. For instance, **Olympus** introduced a new type of high-resolution time-lapse imaging system,

the VivaView FL Incubator Fluorescence Microscope, a live cell incubation and imaging system that can handle observation of multiple locations in up to eight dishes simultaneously for hours, days, or weeks at a time. The closed-box system incorporates an incubator, fully motorized inverted microscope, high-performance non-drift optics, cooled CCD camera, and computer. Intuitive, seamless operation is provided by MetaMorph for Olympus software, which has been specifically tailored to provide a system for managing complex experiments. VivaView is a compact solution that offers flexibility, ease of use, and optical performance (Figure 1, page 24) enabling researchers to better image and analyze the growth, kinetics, morphology and protein expression in the most sensitive cell types.

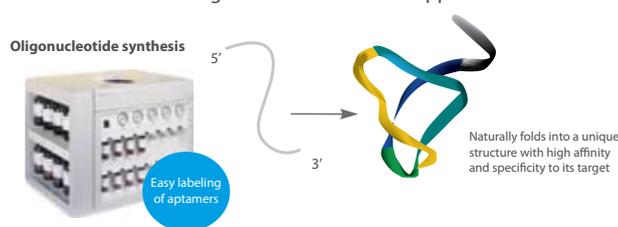
Multi-channel fluorescence and DIC images from 20× to 80× magnification can be acquired using a 40×, N.A. 0.95 UPLSAPO objective. The high numerical aperture dry objective avoids thermal instabilities that can be created by the use of oil immersion objectives, and is maintained at 37 °C. Scientists can change media in mid-experiment, and can add compounds, growth factors, agonists or inhibitors with minimal disruption to the environment. Protocols can involve

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References:
Troita, P.P. et al. (1995) *Medicinal Research Reviews* 15(4), 277-298
McGown, L.B., et al. (1995) *Analytical Chemistry*, 663A-668A
Nielson, P. (1991) *Bioconjugate Chemistry* 2(1)

multiple observations in each of eight separate dishes, at multiple focal points, along with Z-stacking and deconvolution. The software also allows intermittent review of results and images, even while the experiment is underway.

At Japan's **Mitsubishi Kagaku Institute of Life Sciences**, the VivaView has been used extensively to study specific amino acid transport systems involved in the metabolism of cultured adipocytes. Fluorescence and DIC time-lapse images obtained using the VivaView visualized the intracellular trafficking of the GFP-labeled transporter protein ATA2 in submicron sized vesicles moving from a perinuclear location to the plasma membrane in response to insulin. ("Amino acid transporter ATA2 is stored at the trans-Golgi net-

work and released by insulin stimulus in adipocytes." Hatanaka T, *et al. J Biol Chem.* 2006 Dec 22; 281(51):39273-84.)

Researchers at the **University of Maryland** used the instrument to study the expression of mTomato fluorescent protein after transfection of primary neuron cultures. Expression was observed after four hours, peaked at nine hours and continued at this level for the remainder of the study some 17 hours after transfection, with images automatically acquired every 15 minutes. A montage of images commencing 5.5 hours post-transfection (Figure 2, page 24)

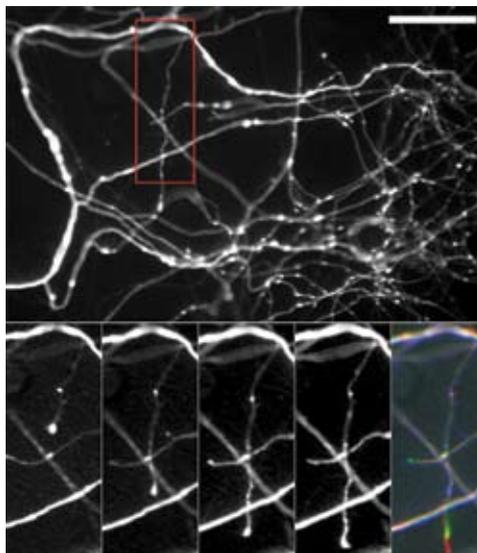


Figure 4. Top: Fluorescence image of mTomato expression at end of experiment, 17 hours after transfection. Red box indicates region of interest shown in successive one-hour intervals (9, 10, 11, and 12 hours into the experiment) below. Bottom right image shows the latter three timepoints overlaid and pseudocolored blue for the earliest, with subsequent timepoints pseudocolored green and red respectively, showing the growth of the neuronal process. (Source: Dr. Thomas Blanpied, University of Maryland School of Medicine, Department of Physiology)

illustrates the continued viability of the cells, maintained expression of the transfected construct, and the accurate focus and repositioning of the region of interest throughout the experiment. Fluorescence images are registered with DIC images to reveal transfected neurons among other cells in the culture (Figure 3, page 25) as well as the spatial relationship of neuronal outgrowth (Figures 4 and 5).

The benefits of long-term time-lapse imaging of living cells has similarly been proven in disciplines as diverse as cell biology, neuroscience, phar-

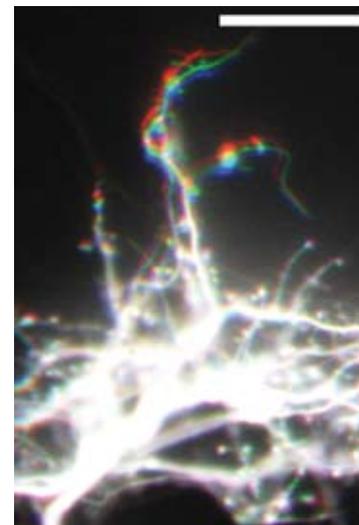


Figure 5. Pseudocolored images showing changes in the position of neuronal outgrowth over time. The earliest image is pseudocolored blue; subsequent images at 30 and 60 minutes later are green and red respectively. The majority of the neuronal processes remain in the same position, shown by the white color due to the exact overlay of red, green and blue pseudocolored images from successive timepoints. However, morphological changes in the outgrowth are clearly indicated by distinct red, green and blue images of the neuronal processes. (Source: Dr. Thomas Blanpied, University of Maryland School of Medicine, Department of Physiology)

maceutical research, and *in vitro* fertilization. Key applications include protein folding, membrane and lipid dynamics, tumor spheroid or organ culture, neural cell reaggregation, chemotaxis, stress response, gene regulation, cell-matrix interaction, and many more.

Recent advances in software, hardware, environmental control, and ease of use will contribute to the growth and importance of long-term time-lapse imaging as a technique for studying both qualitative and quantitative changes in biological systems over time. ■

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