A Brief History of Time-Lapse

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at timed intervals using a camera attached to the microscope. The resulting sequences of images were then assembled into a montage that portrayed the events in question. These early methods of recording living cells and tissues failed to convey the exquisite beauty of the simultaneous movements of cells during development and the plethora of motions of organelles within single cells.

INTRODUCTION

Time-lapse is used for imaging the dynamics of living cells and tissues with the light microscope. The technique involves recording a single microscope field at intervals of lapsed time, along with keeping the specimen alive and in good health on the microscope stage throughout the experiment. When the full sequence of images is subsequently played back as a movie, the event is speeded up in proportion to the interval between frames. A structure that appears unremarkable in a still image can take on a new identity when a sequence of the images is played back as a time-lapse movie. For example, when cells are imaged using fluorescent speckle microscopy (25), they appear as a series of bright dots (speckles) in still images. The speckles take on a new identity when viewed as a time-lapse movie; for example, the speckles may line up in the case of fluorescently labeled microtubules (25), or they may move together in the case of fluorescently labeled actin (24).

THE EARLY ANALOG YEARS

The first time-lapse studies were performed by simply observing a specimen (e.g., a developing embryo) by eye in the light microscope and drawing a picture of it at regular time intervals, often with the aid of a microscope drawing tube, which facilitated the drawing process by projecting the image onto a sheet of paper where it could be traced by hand. Even though these early methods were subject to investigator bias and some artistic license, a wealth of detailed information about cellular architecture and dynamics was documented in this way (8,9).

Later, photomicrographs were collected onto 35-mm film

TIME-LAPSE CINEMATOGRAPHY

The first time-lapse movies of cells growing in tissue or organ culture were collected onto 16-mm film. Single frames were collected at preset intervals using a cine camera that was controlled by an intervalometer. This device opened the camera shutter at regular time intervals, held it open for a specified time to optimally expose the film, and then advanced the film by a single frame. More sophisticated instrumentation also controlled a shutter between the light source of the microscope and the specimen. This was an advantage because the specimen was only illuminated during exposure of the film and photodamage to the cells was much reduced.

Processing of the 16-mm film often took several days because they were usually sent to an outside photo laboratory, and the quality of the movies tended to be variable. The results were excellent when the fine focus of the microscope did not drift and the film was optimally exposed. Care had to be taken to eliminate vibrations of the specimen and focus drift from differential heating of various metallic microscope components. Extreme measures were often adopted to prevent focal drift; for example, the microscope was placed in a hot room at the temperature required for optimal cell growth to equilibrate all of the different components of the microscope with the specimen chamber.

In the early days of time-lapse, contrast in the image was provided from structures in the specimen itself using bright-field optics. Later, optical means were used to generate contrast using dark-field, phase-contrast, differential interference contrast (DIC), polarization, or interference reflection microscopy (Figure 1). The images were viewed as a movie using a cine projector, and they could be analyzed on a frame-by-

Figure 1. Different modes of light microscopy for imaging living cells. (a) Dark-field image of a chick heart fibroblast. (b) DIC image of 3T3 cells from the side. The cells were plated onto fine glass fibers. (c) Interference reflection imaging of the leading lamellum of a chick heart fibroblast using reflected light confocal microscopy. The black streaks in the image are the cell substratum adhesions (3).

frame basis, usually by tracing specific features onto paper by hand. Images were published as a montage of still photographs that were selected and printed from the hundreds of images in the movie sequence (Figure 2). Observations made using 16-mm film laid the foundations for the field of cell behavior (3).

An alternative to time-lapse imaging developed around this time was the “phagokinetic tracks” method, which obviated the use of expensive time-lapse imaging equipment (1). Cells were plated onto a coverslip coated with a layer of colloidal gold particles. As the cells moved around on it, they phagocytosed the gold particles and left tracks in the gold be-

Figure 2. Montage of phase contrast images extracted from a time-lapse video sequence (time between each frame is 5 min). The sequence shows the collision behavior between a mouse fibrosarcoma cell (top) and a chick heart fibroblast—an example of nonreciprocal contact inhibition of locomotion (5).

hind them. The tracks appeared as areas of black against a bright background of gold particles when viewed using dark-field microscopy or by oblique illumination in a stereomicroscope. The shapes of the tracks revealed the behavior patterns of entire populations of cells on coverslips.

VIDEO MICROSCOPY

The introduction of time-lapse video recorders revolutionized the time-lapse imaging of living cells (12). Even though the resolution of video was less than that of 16-mm film, it had several attractive advantages over film. The use of video eliminated the exposure problems associated with 16-mm movie film, and the video sequences could be viewed immediately or, at least, at the end of each recording session. This meant that a repeat experiment could more easily be performed using a sample from the same specimen pool. Many more recordings could be made using video than using film because video was much cheaper and the tapes could be reused.

A further development was the addition of real-time image processors between the video camera and the video recorder. A method of subtracting a previously collected image of the background from each of the “in-focus” images and digitally

enhancing the contrast in real time was developed, and it became known as the video-enhanced contrast-differential interference contrast (AVEC-DIC) method (2). Using this method, structures that were previously not visible in the light microscope, either by eye or with a video camera alone, appeared on the screen of the video monitor (11); for example, preparations of isolated microtubules were made visible in the light microscope for the first time. This technical advancement proved to be vital in the development of microtubule-based motility assays and the subsequent discovery of motor proteins such as kinesin (17).

The optical memory disc recorder (OMDR) was a further improvement to time-lapse video recording (12). The OMDR proved to be more flexible for recording and playing back images than a time-lapse video recorder, especially when a computer was used to control it. Single frames from a long time-lapse sequence could be retrieved by the computer and arranged into a montage for publication. The videodiscs were more expensive than videotape and, unlike videotape, they were write once only.

DIGITAL IMAGING

Time-lapse imaging methods now use sensitive digital cameras (21) or photomultiplier tubes, large and fast computers and sophisticated yet user-friendly image acquisition software. Time-lapse sequences are captured as digital images directly onto the computer hard disc. Representative sequences can be easily extracted from the series of images and printed using a digital printer or placed onto the Web. Programs have been developed for analyzing the wealth of information in the digital movies (20,27).

Improvements in the sensitivity of detectors [e.g., charge-coupled device (CCD) cameras] have made fluorescence imaging of living cells a more practical option (26). Novel probes have been developed for lighting up specific cellular components; for example, the green fluorescent protein (GFP) is currently a popular reporter for gene expression in living cells (6,10). GFP is a small, nontoxic protein that naturally fluoresces in blue light and is resistant to bleaching. GFP, along with calmodulin, has also been incorporated into the cameleons, a new class of fluorescent protein indicators for calcium (7,14).

With these improvements in both instrumentation and fluorescent probes for living cells, more images can be collected from a living sample, and time-lapse imaging methods are no longer restricted to two dimensions over time. Microscopes are now commercially available that collect 3-D data sets over time of living tissues using a computer-controlled stepper motor to control the fine focus. These systems include wide field (18), deconvolution (16), confocal (19), and multiple photon (15) microscopes.

The collection of optical series over time is called 4-D imaging (23), in which individual focal planes or optical sections (X and Y dimensions) are collected at different depths in the specimen (Z dimension) at different times (the fourth dimension of time) (i.e., one time and three space dimensions) (Figure 3). The dimensional notation has now been

Figure 3. Early 4-D imaging—optical series of a sea urchin embryo injected with 3,3'-dihexyloxcarbocyanine iodide (DiOC6) and imaged using a laser scanning confocal microscope (22). (A and B) Confocal Z-series at two time points. (C–K) 3-D reconstructions of the Z-series—each time point was rendered separately using the same parameters (specimen courtesy of Steve Stricker).

Table 1. Web Sites

http://rsb.info.nih.gov/nih-image/
http://www.basic.nwu.edu/g-buehler/cellint.htm
http://sdb.bio.purdue.edu/dbcinema/
http://util.ucsf.edu/sedat/marsh/opticell.html
http://pantheon.cis.yale.edu/~wfm5/gfp_gateway.html
http://www.welc.cam.ac.uk/~brandlab/
http://www.theimagingsource.com/
http://listserv.acsu.buffalo.edu/cgi-bin/wa?S1=confocal
http://www.videomicroscopy.com

stretched even further to six dimensions imaging in which the fifth and the sixth dimensions represent multiple wavelengths and different positions in the specimen, respectively. Multiple dimensional images can be presented in several different ways (4,13)—as a simple montage of the images, as stereo pairs, or as 3-D reconstructions of each time point projected as a digital movie (Figure 3).

CONCLUSIONS

Time-lapse imaging continues to be used for the study of cellular dynamics using the range of magnifications and the different modes available in the light microscope, from cell movements in tissues and embryos to the dynamics of macromolecules within single cells, and from phase contrast to fluorescent speckle microscopy. Time-lapse imaging has progressed a long way from its analog beginnings and has now greatly benefited from the introduction of digital imaging techniques. Much of the process can now be automated and the final digital movies are easily transferred and displayed using the Web (Table 1).

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Suggestions for contributions to the BioImaging feature are welcomed by its editor, Dr. Steve Paddock (paddock@facstaff.wisc.edu)