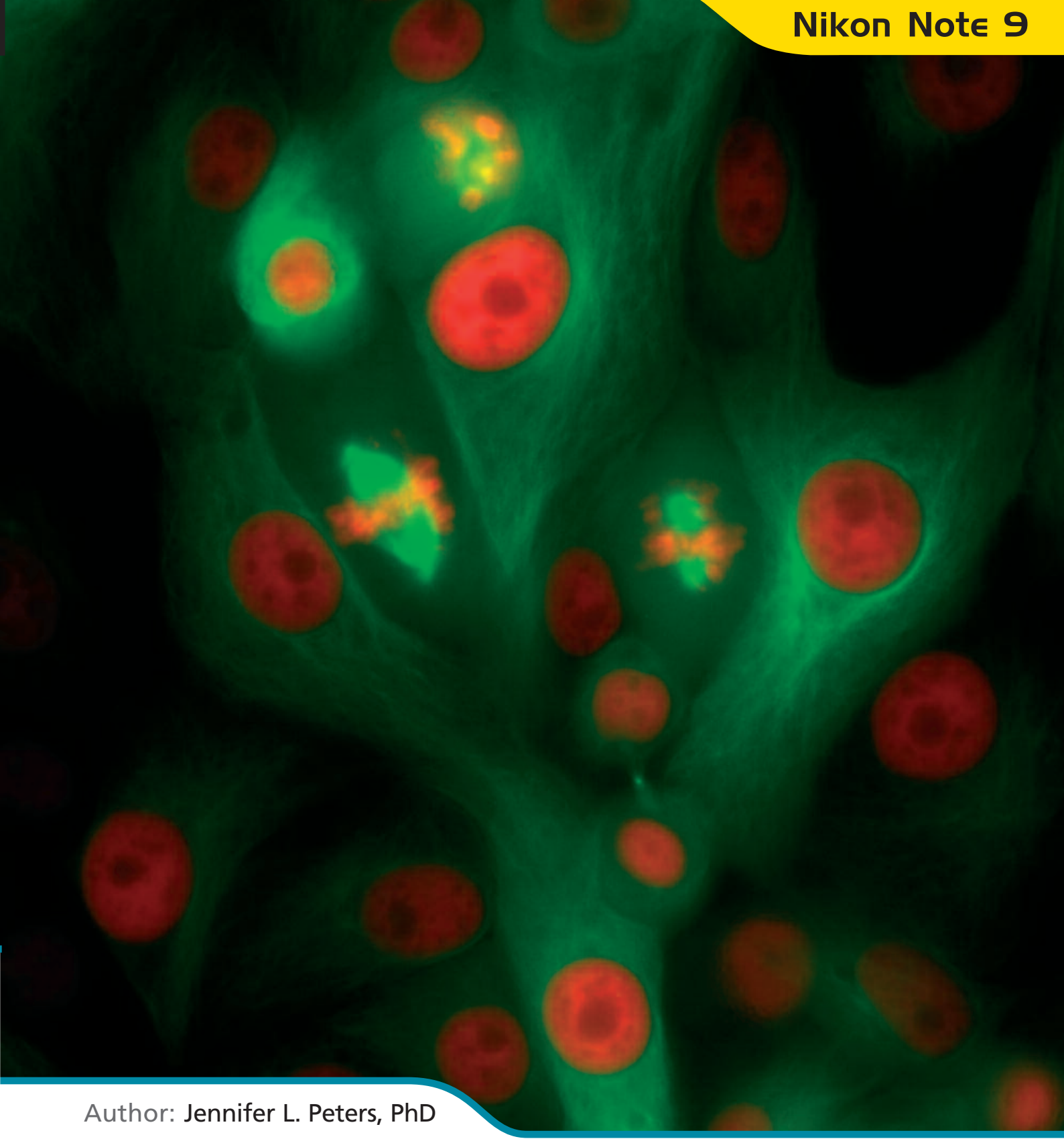


Author: Jennifer L. Peters, PhD

Environmental Considerations for Live Cell Imaging

Abstract:

Long term timelapse imaging of living cells is becoming an increasingly important technique. However, living cells have specific environmental requirements in terms of temperature, CO_2 concentration, pH and osmolarity that must be met to ensure that cells remain healthy throughout imaging and that the resulting experimental data is reliable. Various options are available for maintaining living cells on the microscope, including stagetop incubators, full microscope incubator enclosures, and the BioStation IM. If appropriate steps are taken to ensure that cells remain alive and healthy throughout an experiment, phenomena such as cell growth, division, and protein expression can be imaged over several hours and even days.

Introduction:

Many of biology's most interesting questions, including those governing the growth, division and apoptosis of living cells, can be answered by microscopic observation of cells using long term timelapse imaging. Live cell imaging experiments pose many challenges, however, not the least of which is keeping cells alive and healthy throughout the experiment. Not only are cells vulnerable to photodamage from the light needed to image them, but maintaining environmental conditions necessary to keep cells alive on the microscope stage for hours, days or even weeks is far from trivial. These environmental considerations are discussed here together with technologies (including the BioStation IM) for maintaining living, healthy cells during long term timelapse imaging.

Critical Environmental Factors for Live Cell Imaging Experiments

Most cell lines require a maintenance temperature of ~37°C to achieve normal cell growth and good overall health of the culture. Also important (and closely linked) are pH and CO_2 concentration. Most cells require bicarbonate buffering, and for the pH to be buffered at the required pH of ~7.4, the CO_2 concentration in the environment surrounding the cell culture vessel must be maintained at ~5% (considerably higher than the ambient CO_2 concentration in air). While other buffering systems not requiring CO_2 (such as HEPES) can sometimes be substituted, these must be evaluated carefully as they can result in reduced growth rates and other non-optimal behavior, especially when used long term. Finally, cells are sensitive to changes in osmolarity, commonly caused by evaporation of the cell culture media during the experiment - a particular problem when working at 37°C. Steps must be taken to reduce evaporation by humidifying the environment surrounding the culture vessel, or by using a vessel that is sealed or covered in a thin layer of mineral oil.

Failure to control environmental factors during long term timelapse imaging typically results in unhealthy cells that fail to divide, round up, detach, or exhibit other abnormal morphologies as shown in Figure 1.

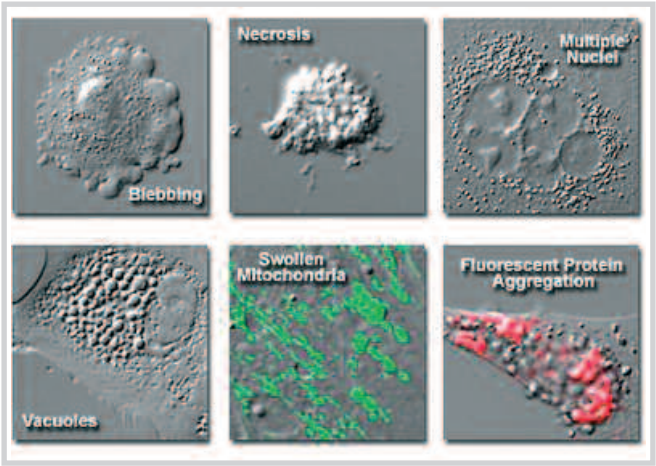


Figure 1: Examples of unhealthy morphologies in live cell imaging experiments. Image courtesy of Michael Davidson, Florida State University.

Technology for Maintaining the Live Cell Environment During Long Term Timelapse Experiments

The imaging chamber must control environmental factors accurately, while also being robust, easy to use and cost effective. Traditionally, two main strategies have been used for maintaining cells on the microscope stage during long term imaging: stagetop incubators and full incubator enclosures that encompass the entire microscope.

Stagetop incubators are small incubator enclosures that fit over the culture vessel on the microscope stage. Such enclosures must allow access by the microscope objective and often permit introduction of reagents and fresh media during the experiment. While these systems are easy to use and relatively inexpensive, the sample and its immediate environment are at a different temperature from the rest of the microscope and the laboratory environment, making fluctuations in temperature difficult to avoid. Not only are temperature changes (even of just a few degrees) detrimental to cells and resulting data, they usually cause focus drift during the course of the experiment. One method of mitigating this effect is to use an objective heater, thereby eliminating it as a heat sink.

Full incubator enclosures (see Figure 2) are another option for maintaining the live cell environment. These are most often constructed of Plexiglas and enclose most of the microscope with access doors for positioning and changing the sample. Temperature is maintained with an external heating unit (often forced air) and CO_2 is controlled by means of a CO_2 sensor and a regulated tank of pure CO_2 gas. Typically, humidity is saturated inside the incubation chamber to prevent evaporation of the culture medium. While this type of incubator has the advantage

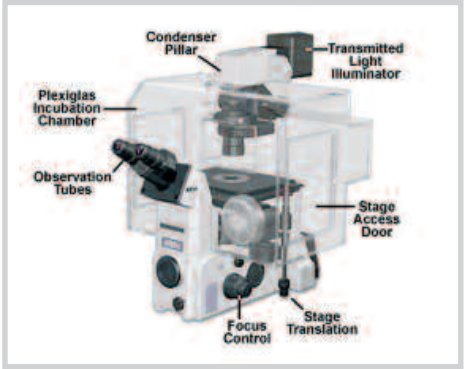


Figure 2: An inverted microscope equipped with a full incubator enclosure. Image courtesy of Michael Davidson, Florida State University.

that it keeps the majority of the instrument at constant temperature and lessens focus drift, it can be cumbersome, difficult to use and expensive. Exposing microscope components to saturated humidity, in addition, can be damaging to optics as well as metal surfaces.

Combining the two incubation methods described above is becoming increasingly popular. A small stagetop enclosure is used to supply humidified 5% CO_2 to the sample, while a larger enclosure surrounds the whole microscope and is used to maintain the temperature at 37°C. Humidity is kept away from most of the microscope optics, while the entire microscope is kept at constant temperature to prevent focus drift.

Recently, Nikon Instruments introduced the BioStation family of products, which provide additional options for monitoring living cells in a cell-friendly environment. BioStation CT combines a complete tissue culture incubator with an inverted microscope, allowing cell cultures to be observed and monitored without removing cells from the incubator. BioStation IM (Figure 3) provides a new option for long term live cell timelapse imaging. This is an all-in-one, live cell incubation and monitoring system combining all components of a live cell imaging system in one instrument. This includes illumination, microscope and optics, high sensitivity camera, acquisition and control software, and live cell incubation chamber controlling temperature, humidity and CO_2. BioStation IM includes two channel fluorescence imaging as well as phase contrast; magnifications of 20X - 80X (or 10X to 40X, depending on the version) and motorised X, Y and Z for multipoint imaging. All aspects of the BioStation IM's design reflect the needs of live cell imaging including the use of cell friendly red light for the diascopic illumination and movement of the objective in x and y planes (rather than the stage) in order to avoid disturbing the cell culture.

Figure 3: BioStation IM

Most significantly, the temperature, CO_2 and humidity control are fully integrated and designed to perfectly regulate the live cell environment and eliminate focus drift. By means of a heater, circulating fan and multiple temperature sensors, the temperature is maintained at precisely 37°C in virtually the entire instrument, keeping the cells healthy and the focus stable, even during timelapse experiments of several days duration. CO_2 concentration and humidity are controlled in a small, specially designed sealed chamber in the top of the instrument, which not only regulates pH and reduces evaporation, but protects the optics from humidity. As a result, cell survival within the incubation chamber is comparable with a standard cell culture incubator, facilitating long term timelapse imaging.

Live Cell Imaging Applications

The emerging array of live cell imaging applications requiring long term monitoring and, consequently, reliable environmental control is almost limitless. One area of interest is imaging cells as they grow and divide, often through several mitotic cycles over several days. Figure 4 shows an example of this type of experiment. Here, LLC-PK1 cells (a pig kidney cell line that remains relatively flat during mitosis) were transfected with eGFP tubulin and mCherry H2B. BioStation IM was use to take dual channel fluorescence images every 10 min for 15 hours; 8 images, 1 per hour for the first 8 hours, are shown. Cells undergoing mitosis can be observed in several images. Evidence of mitosis is important regardless of the phenomenon being studied, as it indicates that cells are healthy and functionally normal. These data demonstrate that BioStation IM keeps cells alive and in focus throughout long term timelapse imaging experiments (this particular cell culture was kept alive and observed for over 96 hrs with no adverse effects).

Conclusion:

Researchers must consider many factors when selecting the imaging and incubation components for long term, live cell timelapse imaging. Importantly, the environmental needs of the sample must be met and constantly maintained over the entire timelapse study. Environmental factors (temperature, pH, CO_2 concentration and osmolarity) are critically important in promoting growth and normal function of living cells and in avoiding potential artifacts in the interpretation of experimental results. BioStation IM offers excellent imaging capabilities and trouble-free integrated incubation while offering the flexibility to configure a system to suit researcher's individual needs.

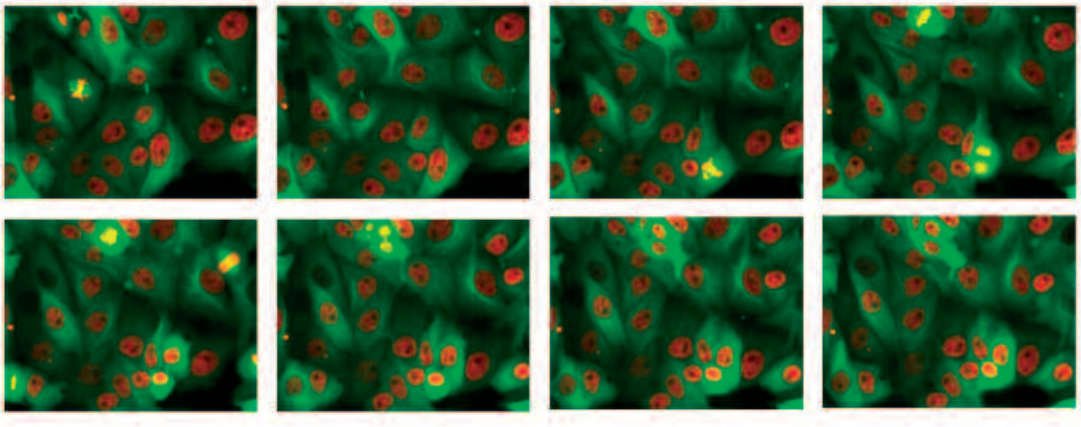
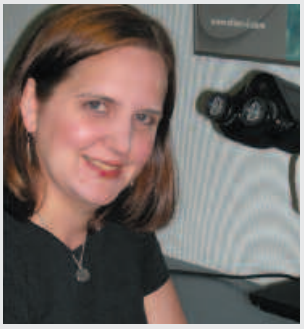


Figure 4: Timelapse imaging of cells undergoing mitosis.



Dr Jennifer Peters

Authors Background

Dr. Jennifer Peters is the Advanced BioSystems Applications Scientist for Nikon Instruments Inc. Jennifer earned her PhD in analytical chemistry in 2001 from the University of Pittsburgh. She then went on to do postdoctoral work in imaging at the University of Michigan, before starting her career at Nikon in 2005.

Acknowledgement:

We would like to thank Michael W. Davidson, Director of the Optical Microscopy Section at the National High Magnetic Field Laboratory at the University of Florida, for his assistance with this article.

References:

http://www.microscopyu.com/articles/livecellimaging/index.html

Product Links
BioStation IM and BioStation CT:
http://www.nikoninstruments.eu/biostation/
Ti: http://www.nikoninstruments.eu/ti/
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Environmental factors including temperature, pH, CO_2 concentration and osmolarity are critically important in maintaining healthy live cells and in ensuring the veracity of the data obtained during long term timelapse experiments.

Various systems, including stagetop incubators and full microscope incubator enclosures, can be used to maintain the live cell imaging environment on the microscope stage

BioStation IM is an additional option, providing incubation, microscopy and imaging integrated into a single, easy to use instrument

For more information on Nikon Biostation systems go to:

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