

Requirements for Live-cell Imaging

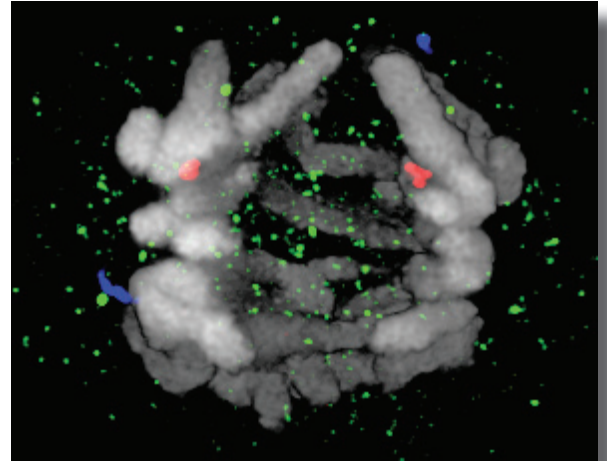
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Introduction

Cells are the basic unit of complex organisms. By studying cells, biologists are able to dissect molecular interactions essential for Discovery and Target Identification free from the costs and complexity associated with performing these same studies in intact organisms. Currently, most of the cell-based assays in these fields are fixed endpoint assays at moderate resolution. While these assays yield useable results, they suffer from lost temporal and spatial information. Recently, a number of studies utilizing living cells studied at high temporal and three-dimensional spatial resolution have produced significant results that would have been lost using fixed endpoint assays. These include mutations in yeast that are responsible for changes in kinetichore dynamics [Sorger], gene knockdown experiments that reveal proteins responsible for the regulation of centriole replication in humans [Pelletier], and the dynamics of Cajal body and RNA processing in human cells [Platani]. These studies have been enabled by digital microscopy methods that, until recently, were available only to select cell biologists. This document addresses the basic requirements for such microscopy systems and then describes a new microscopy product that delivers these capabilities to a broader range of scientists.

Requirements

Live-cell imaging requires a balance of several factors. First, cells are small relative to the wavelength of light. The organelles and structures essential to cell physiology are even smaller. Microscopy systems for studying living cells must, therefore, be capable of sampling small structures. That is, they must be highly resolving; however, the higher the resolution, the thinner the depth-of-field of the optical system. Thus the higher resolution requirements also drive the need for three-dimensional (3D) imaging or, at a minimum, extending the depth-of-field without losing lateral resolution. Secondly, cells are surprisingly dynamic and the organelles and structures that they contain are even more so. Systems designed for live-cell imaging must be able to rapidly acquire images so that these dynamic processes can be properly studied. Finally, cells are highly susceptible to photon damage. Even moderate illumination of cells can cause significant physiological changes that range from initiating checkpoints, to changing cellular kinetics, to inducing apoptosis (cell death). This last constraint on live-cell imaging systems



places serious constraints on the ability to sample at high spatial (1) and temporal (2) resolution. Many imaging methods that enable high spatial resolution such as confocal microscopes are photon inefficient and require high illumination intensities. These high intensities are not compatible with long-term live-cell imaging experiments. Described here is a microscopy system capable of imaging living cells for long periods of time (up to days or weeks) while delivering high spatial and temporal resolution.

The DeltaVision System

The DeltaVision system by Applied Precision is a fluorescence microscope that illuminates the entire field-of-view (that is, it is a wide-field microscope) yet delivers superior resolution and contrast (i.e. signal-to-noise) compared to conventional microscopy systems. From its inception, the DeltaVision system was engineered for the demands of live-cell imaging. The DeltaVision system consistently delivers image resolution that pushes the diffraction limit of about 200nm laterally and 400nm axially. The DeltaVision system is fast, capable of imaging up to 50 optical sections per second. However, the DeltaVision system is highly photon efficient, up to hundreds of times more efficient than confocal systems. It is this ability to deliver high contrast images with few photons that enables the DeltaVision system to outperform other live-cell imaging systems. It is common for DeltaVision systems installed in the laboratories around the world to deliver diffraction limited resolution of living cells over the course of days and even weeks.

One of the constraints on live-cell imaging has been throughput. Biology is rate limiting; for example, it takes time for cell division. Imaging a single cell in one field-of-view completing one round of mitosis before imaging another cell is a highly inefficient way to collect data. Some systems attempt to overcome this limitation by reducing the resolution and increasing the size of the field-of-view thereby increasing the number of cells that can be studied in a stationary view. As mentioned above, there are significant limitations to this approach in regards to small objects. Effective live-cell imaging systems must be efficient in collecting data to maximize the value of each experiment while not compromising the image quality. Applied Precision is the world leader in nano-positioning technology. By leveraging this technology into every DeltaVision system, each system is capable of multiplexing multiple fields-of-view during time-lapse experiments. The precision of the DeltaVision stage permits each field-of-view collected to be virtually stationary although many different locations may have been visited between time-points even at high resolution. By increasing the number of fields-of-view, scientists can increase their sample size and the number of experiments that can be run in parallel thus achieving high spatial resolution and large sample sizes.



It is not enough for a system to deliver images, it must also deliver data. The DeltaVision system was engineered to be a quantitative instrument. The deconvolution algorithm used in image processing on the DeltaVision has been demonstrated to be superior for live-cell imaging compared to other imaging modalities [Swedlow]. This means that, not only is the data from the DeltaVision system scientifically valid, the deconvolution software is superior to other packages. This performance is important for routine live-cell imaging and it enables advanced photokinetic experiments [FRAP, photo-activation, FRET] through the use of the optional Quantifiable Laser Module (QLM).

Live-cell imaging is data intensive and it is easy to generate 10-15 GBs of data in one experiment. Applied Precision offers solutions for data management using open standards. As a founding supporter of the Open Microscopy Foundation, creators of the Open Microscopy Environment (OME), Applied Precision has helped to create a universal, open standard to describe and define microscopy data. Applied Precision, in collaboration with Glencoe Software, has used this standard to create the Data Management Solution (DMS). DMS enables customers to manage images and the associated metadata in a database designed specifically for the demands of live-cell imaging.

By engineering a complete solution for live-cell imaging, Applied Precision has enabled seminal research in leading laboratories in the world. This performance is now available to every research laboratory in a range of products from the personalDV for the price conscious researcher to the fully enabled DeltaVision Core that has been optimized for multi-user facilities. Biologists can now have the best technology at an affordable price without sacrificing performance and data integrity.