High Resolution Light Microscopy of Live Cells

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All living creatures, including humans, are made of cells. The majority of life forms exist as single cells that perform all functions to continue independent life. Some cell structures, cell organelles and particularly bacteria and viruses are commonly too small to be fully observed with an optical microscope. Therefore, an electron microscope is required. Since samples examined with an electron microscope are exposed to very high vacuum, it is impossible to view living cells. The sample preparation for electron microscopy requires that living cells be killed, frozen, dehydrated, and impregnated with heavy metals. These procedures, together with the electron beam, damage the sample during observations and create the possibility that some components of the cell may be lost or distorted. Furthermore, many properties can only be seen occurring in living cells. These may include such phenomena as vesicular transport, cytoplasmic streaming, Brownian motion, diffusion, phagocytosis, pinocytosis, mitosis, native fluorescence, or cellular interactions. Biomedical technologies including gene therapy, artificial insemination, new drug development, cell culturing and cloning, cell regeneration, implantation, biodetection, biotherapeutics, and others; need to visualize live cells and cellular mechanisms. While the nature of these phenomena might be inferred by examining electron micrographs before and after these processes, they can only be studied in depth while they are occurring. The only certain way to avoid these problems is to examine cells while they are alive, without fixing, freezing, or dehydrating. For these reasons, a light microscope with a special optical system that allows the observation of live cells at high resolution and contrast becomes very useful.

Figure 1. This portion of the Richardson Test Slide offers a series of parallel vertical and horizontal lines with distances between lines of 133, 126, and 120 nm. The least resolved pattern would indicate the horizontal and vertical resolution of a microscope. Olympus BX51, CytoViva150, 100X, oil, NA1.4, color digital camera, Sony DXC-S500.

The CytoViva™150 Ultra Resolution Imaging™ (URI™) system was recently introduced as an advanced optical illumination system enabling resolution well below 150 nm and detection below 60 nm to produce detailed images of living cells [1]. The enhanced spatial resolution and contrast are realized by minimizing the spot size impinging on the sample and by the reduction of stray light. Minimizing the spot size increases the irradiance (energy per unit area) of the sample and increases contrast due to increased interaction with the smallest particles of the sample, which otherwise would not produce enough light to be visible. Similar to confocal microscopy, a small focal volume reduces signal background and improves image contrast. Another benefit of a small focal volume is the capability of three-dimensional sectioning that may be used for reconstruction of three-dimensional images.

The resolution of conventional microscopes is limited by the wave nature of light and is defined by the Rayleigh criterion. For green light, with a wavelength of 546 nm, this criterion gives a resolution of about 240 nm [2]. However, unlike incandescent or halogen light sources that have a continuous optical spectra, the metal halide lamp used in the CytoViva 150 system has a few strong spectral lines that can interact with the sample to produce additional optical effects that enhance resolution and detection beyond that normally predicted by the Raleigh criterion. The geometry and high numerical aperture of the CytoViva150 also generates conditions for standing evanescent waves and thus further enhances microscope resolution [3].

Figure 2. Diatom, Pleurosigma angulatum, Olympus BX51, CytoViva150 100X, oil, 1.4 NA (Northern Biological Supplies). Left and right inserts: Pleurosigma angulatum from (Carolina Biological Supply). Olympus BX51, CytoViva150, color digital camera, Sony DXC-S500. Scale bars: central micrograph and left insert = 5 µm, right insert = 2 µm.

Figure 3. RG2 glioma, cultured rat cancer cells (brain tumor cells). Olympus BX51, CytoViva150, 40X. The single frame was captured from records by a color video camera, Sony DXC-C33. Scale bar = 40 µm. The living cells were grown and photographed in the Lab-Tek® Chamber...
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-J. Paul Robinson, PhD, Director of Cytometry Laboratory, Professor of Basic Medical Sciences and Biomedical Engineering, Purdue University

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- John A. Smith, MD, PhD, MMM, Divisional Director, Department of Pathology, University of Alabama at Birmingham

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*Samples courtesy of Scott-Ritchey Research Center, Auburn University

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The CytoViva 150 optical system is designed to provide Kohler illumination and a very high efficiency transfer of light from the light source to illuminate the sample. Thus, the system employs the only light that interacts with the sample at certain apertures thereby reducing both stray light and undesirable heating. It delivers an intense light to the sample with a low power light source, especially when compared to conventional darkfield and DIC that require high power light sources. As illumination intensity is increased, the optical difference between small light scattering particles and their background increases. At still higher illumination, smaller particles now scatter enough light to become visible. This phenomenon is an important element in allowing CytoViva 150 to achieve the detection of particles sized 60 nanometers or less. The movements of small intracellular particles and thin bacteria that are both smaller than 100 nanometers in diameter (too small to be seen in brightfield or DIC illumination) can be observed and photographed by the new microscope illumination system. A high numerical aperture condenser is an integral part of the new illumination system so that the system comprises a complete and self-contained module for high quality illumination. As a part of the illumination system, the condenser is pre-aligned and therefore additional alignment is unnecessary. Thus, CytoViva 150 does not require a highly skilled operator to operate successfully.

In order to define the useful magnification of the microscope, the resolving power of the microscope is first determined. The resolving power of a microscope may be defined as the smallest distance between two objects that a microscope will show as separate images. Having determined the physical limit of resolution of a microscope, we can estimate when the distance between two objects is visible to the eye. Hermann von Helmholtz demonstrated that the angle between two objects when they are just barely distinguishable as two to the eye is one minute of arc [4]. Accordingly, two objects visible as separate objects by the microscope must at least subtend this angle to the eye, and the magnification must be sufficient to produce this. Therefore, the useful magnification, M must be defined as M=(visual angle of image seen with microscope)/(visual angle of object seen directly); or M=(1 min. [in circular measure] × D)/R. Where D is the distance of distinct vision (250 mm), R is the limit of resolution [4]. The limits of the resolving power and the detection limit of the new microscope system have been experimentally determined with a special test slide designed to calibrate high-resolution microscopes. The Richardson Test Slide (Richardson Technologies Inc.) [5] was used in tests to confirm the high resolution and detection limit of the microscope (120 and 60 nm, respectively) (Figure 1). Using the above equation for useful magnification and the experimentally found resolving power of the microscope, we can make an estimate of the useful microscope magnification:

$$M = \left(\frac{1 \text{ min. [in circular measure]} \times D}{R}\right)$$

$$= \frac{(2.9 \times 10^{-3} \times 25 \times 10^{-3})}{120 \times 10^{-3}} = 6.41$$

A second experiment to analyze image quality and resolution was conducted with a diatom, *Pleurosigma angulatum*, a diatom, is a single cell plant that has a skeleton consisting of silica. The silica walls have a complex ultrastructure, with a regular pattern of perforations. The fine texture and regularity of this pattern make this

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