Nikon Note 2

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Properties and Selection of Objective Lenses for Light Microscopy Applications

Abstract:

Digital imaging is a key component of modern microscope systems. New digital sensors, however, are far more sensitive than the human eye and this has required novel optical strategies including the design of new objective lenses. Key developments include the Plan Achromat-type lens, the fly-eye lens illuminator and water dipping and water immersion objectives for live cell imaging. This technical note describes the optical performance of modern objective lenses and discusses criteria for the selection of objectives for a variety of microscope applications such as fluorescence microscopy, confocal microscopy and live cell imaging.

Introduction:

Today's microscope systems can be configured to create complete imaging and analysis workstations incorporating, for example, digital cameras, laser scanning confocal systems, advanced systems for live cell fluorescence and spectral detectors. Digital imaging is a fundamental enabling technology at the heart of today's advanced imaging and analysis techniques. Compared with the human eye, however, digital sensors are far more sensitive to differences in the intensity and evenness of illumination and this has placed new demands on optical systems. The requirement for new imaging techniques such as live cell imaging, in addition, has driven the evolution of objective lens design. This technical note describes some key developments in the design of microscope optics. It also provides guidance on the selection of appropriate objective lenses for specific imaging techniques – a fundamental factor in achieving the best possible imaging results.

Technology:

In non-digital microscopy, traditional Koehler illumination provides uniform illumination of the specimen plane by spreading light from the bulb evenly over the specimen using a diffuser. This approach is adequate for observation by the human eye and for film photomicrography but new technology is required for highly sensitive digital sensors. A digital equivalent of Koehler illumination has been created through the introduction of a 'fly-eye' lens arrangement in the microscope illuminator (figure 1). This multiplies the single illuminating filament to more than 300 filaments and projects them uniformly over the field of view.

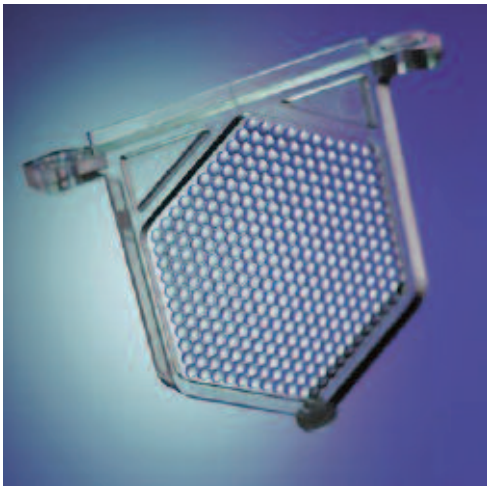

Fig 1: Fly-eye lens for uniform specimen illumination.

Further solutions for improved image quality can be found in objective lens design. The transition from the Achromat objective lens to the Plan Achromat lens is a key development. Typically, Achromat objective lenses focus blue (about 486nm, also called the f line) and red (656 nm, c line) in the same plane, providing axial chromatic aberration correction. The Plan Achromat objective lenses, additionally correct in the g line (436 nm) to provide evenly focused photographs, better resolution and flat image reproduction.

Plan Fluor objectives are available for increasingly popular fluorescence contrast techniques and Super Fluor objectives for special applications requiring ultraviolet excitation. Plan Fluor objectives are essentially Plan Achromat-style lenses with respect to correction but, because of different glass selection, show higher transmission in the infrared (IR) and blue violet (BV) parts of the spectrum. Plan Fluor lenses are also designed to correct for green (588 nm, d line). The recently developed Plan Apochromat violet-corrected (VC) series additionally cover the h line (405 nm), totally correcting axial chromatic aberration for five lines ranging from 405-656 nm (figure 2). This lens is also vignetting-free: the resolution of the objective lens is even across the entire field of view (figure 3) making it an ideal choice for digital photomicrography.

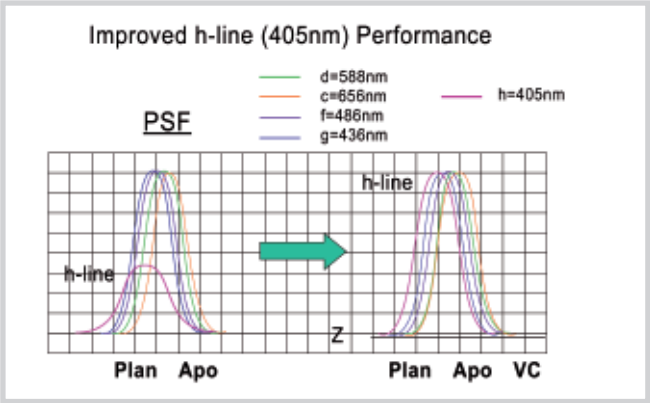

Fig 2: Chromatic aberration of objective lenses. In the CFI60 Plan Apochromat VC the h line (405 nm) is focussed in the same focal plane as the g, f, d and c lines (right) while with traditional Plan Apochromat lenses the h line is focussed in a different plane (left) CFI60 Plan Apochromat lenses are fully corrected from 405 – 656 nm.

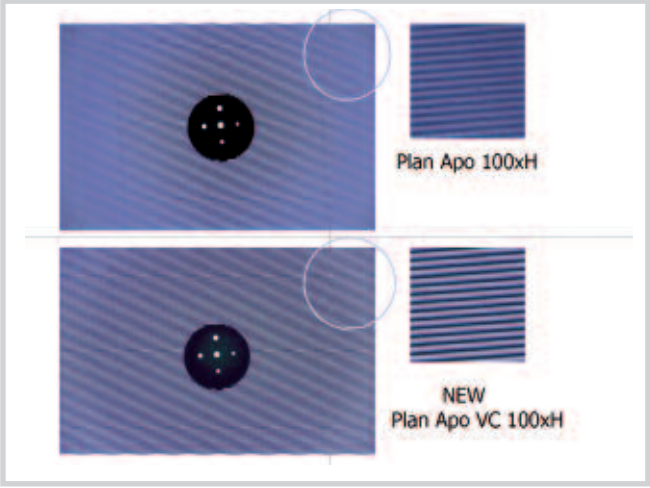

Fig 3: Comparison of normal (top) and violet corrected (bottom) Plan Apochromat oil-immersion objectives.

Live cell imaging has placed even more demands on optical systems in terms of resolution and long working distances and has resulted in the introduction of the water immersion and water dipping lenses. When cells need to be observed deep within tissues, water immersion lenses show superior performance with respect to resolution and aberration correction than oil dipping lenses. An additional benefit is that the use of water dipping lenses means that the optical path is symmetrical. Light passes from cells (watery) to glass (cover slip) to water (immersion medium) and then to glass (objective lens) again. As a rule of thumb, when observing cells up to 25 µm into the specimen, high N.A. water immersion lenses should be used as these will give both the brightest and best resolved images.

Water dipping lenses are designed to work without a cover glass and can be submerged directly in the cell culture dish. Typically, the N.A. is slightly lower than water immersion lenses (e.g. Plan Fluor 100x, N.A 1.1 compared to Plan Fluor 100x, N.A. 1.3) but the working distance is much longer – up to 2.5 mm.
The combination of water dipping and long working distance enables micromanipulation at extremely high magnifications. Because the N.A. of water dipping lenses is still relatively high, they can also be combined with digital imaging or even laser scanning confocal microscopy.

The relative intensity of the image in an optical system is given by the expression:

$$NA^4 / TM^2$$

Where N.A. is numerical aperture of the lens and TM is total magnification.

Using this formula, it can be shown that the Plan Apochromat 60x, NA 1.45 is the highest resolution lens available, while the Plan Fluor 40x, NA 1.3 is the brightest. The most flexible objective lenses currently available are the multi-immersion type (Plan 20x MI). These can be used dry or with water, glycerine or oil, and are often used as a lower magnification companion lens for a high N.A. high-resolution immersion lens – especially in confocal microscopy.

Several types of correction ring are available that allow the performance of microscope objectives to be offset against certain parameters, such as cover glass thickness, immersion medium refractive index, temperature or depth penetration into tissues, thereby achieving optimum performance.

In parallel with lens design various other developments in optical design have also taken place. It is important, for example, to match the resolving power of the objective lens (determined by its N.A.) to the digital resolution of the camera. This is achieved using a zoom system, which, when motorised, allows automatic setting of the correct position for each individual microscope objective lens. The physical properties of the lenses have also changed. The parfocal distance has grown from 33 mm to RMS 45 mm and, with Nikon, to 60mm. Similarly the objective lens diameter has grown from 20.3 mm to 25 mm – giving microscope developers more latitude in their optical designs.

Application:

The selection of appropriate lenses is critical when setting up a new imaging application, digital imaging system or confocal microscopy system. When using bright field applications in a digital microscope system, light intensity is almost never a limiting factor. Illumination evenness (fly –eye correction) and vignetting-free objectives (Plan Apochromat VC series) are desirable specifications and zoom optics are important in balancing digital and optical resolution. When using fluorescence applications, however, a choice needs to be made between maximum signal collection (plan fluor 40x, NA 1.3) in the case of photon limited applications, or Super Fluor lenses when using probes that work at UV or IR wavelengths. When the signal is not limited, Plan Apochromat lenses are preferred.

Important in live cell imaging, the green fluorescent family of proteins enable an array of techniques such as FRAP, FLIP, iFRAP, FLIM, TIRF and laser ablation techniques. Certain probes require Plan Apochromat VC optics for activation and imaging including CFP, GFP, YFP, Cherry-FP, Tomato-FP, PA-GFP, Kaede, and PS-CFP. Confocal is the technique of choice for high resolution 3-D fluorescence imaging. Most laser scanning confocal microscopes use blue (488 nm), green (543 nm) or red (633 nm) excitation. At these wavelengths correction and transmission are optimal in Plan Fluor and Plan Apochromat series lenses. When using IR excitation (as in 2-photon laser scanning microscopy) efficient IR transmission is crucial. Plan Apochromat VC lenses not only work well in the violet part of the spectrum, but also perform well in the IR enabling greater depth penetration in the specimen. This objective, capable of accommodating an excitation wavelength of 405 nm and imaging from blue to red parts of the spectrum, is also useful for quantum dot deep cell imaging techniques.

Recommended lenses for laser scanning microscopy are:

1. For most general, highest resolution, vignetting free performance: Plan Apochromat 60x VC, oil immersion, NA 1.4.
2. For live cell imaging deeper than 25 µm, vignetting free: Plan Apochromat 60x VC water immersion, NA 1.2
3. For brightest imaging in cases of photon limitation: Plan Fluor 40x, water dipping NA 1.0.

Conclusion:

With sophisticated optical design, the latest in illumination techniques and advanced detectors, modern microscopes, whether stand-alone or as part of a complete imaging and analysis workstation, are capable of delivering outstanding performance. The design and quality of the objective lens are key factors in achieving optimal results and a variety of objectives with diverse optical properties have been purpose-designed for particular applications – such as fluorescence, confocal and live cell imaging. Correct selection of the appropriate objective for each application will help ensure high quality results.

Authors Background

Peter Drent is a microscopy products manager at Nikon Instruments, Europe BV. He has a special interest in digital imaging technologies and is a frequent speaker at Nikon's Digital Imaging Seminars. Mr Drent has a degree in biology from the University of Utrecht in the Netherlands.

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References:

http://www.microscopyu.com/articles/optics/opticshome.htm

Your Nikon Imaging Centre (NIC):
www.nikonimagingcenters.com

The Nikon Instruments organisation has established a number of Nikon Imaging Centres to make available the latest microscope developments to the researchers and to create an interaction between research and the Nikon product development. Nikon Imaging centres have been established in:

- Heidelberg University, Germany
- Oxford University, United Kingdom
- Harvard Medical School, USA
- University California San Francisco, USA
- Hokkaido University, Japan
- Singapore Bioimaging Consortium, Singapore

- The fly-eye lens – the digital equivalent of Koehler illumination
- The Plan Apochromat violet-corrected (VC) series objectives lenses totally correct axial chromatic aberration for five lines from 405 – 656 nm.
- Water immersion and water dipping objective lenses – meeting the demands of live cell imaging applications

For more information on the Nikon objectives go to:

www.nikoninstruments.com/objectives

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