

X Line Objectives Offer Revolutionary Optical Performance Thanks to Advanced Manufacturing Technology

Researchers use microscopes as an essential tool for advancing their science, and objective lenses are crucial components of the system. Many applications benefit from high-quality images with a large field of view, but there is usually a tradeoff where improvements in one area of imaging, such as flatness, lead to a decrease in another area. Thanks to novel manufacturing technology, Olympus X Line high-performance objectives offer improved optical performance in three critical areas – a larger numerical aperture (NA), better image flatness, and a wider range of chromatic correction. These advances enable high-quality, large field of view (FOV) imaging for versatility in numerous applications.

Background

Conventional objective lens manufacturing technology forced a tradeoff between numerical aperture, image flatness, and chromatic correction, making it difficult to improve all three in one objective. In the past, our engineers concentrated on making objectives that were exceptional in one of the three areas discussed above so that users could choose the objective that best suited their application. These objectives were engineered to acquire high-resolution images in a relatively narrow area (smaller FOV). Sometimes, these images were problematic when using image analysis software since the processing algorithms normally assume that the images have no optical aberration or peripheral darkening. It is difficult to obtain reliable, accurate processing data unless the objectives deliver high-resolution, high-quality images over a large FOV. The advanced manufacturing technology behind X Line objectives solves these challenges.

At a Glance

- X Line exploits novel, proprietary lens manufacturing technology
- New objectives deliver an improved numerical aperture, image flatness, and chromatic correction
- Improved optical performance enables more accurate image acquisition and consistent analysis data



X Line Objectives Offer Revolutionary Optical Performance

X Line Objectives Deliver an Improved Numerical Aperture, Image Flatness, and Chromatic Correction

The UPLSAPO60XO objective with an NA of 1.35 and the PLAPON60XO objective with an NA of 1.42 are both manufactured using conventional technology. To achieve the high NA, we reduced the image flatness and reduced the chromatic correction from 435–1000 nm to 435–656 nm. However, the X Line UPLXAPO60XO objective is engineered with an NA of 1.42 while improving image flatness and increasing the chromatic correction range to 400–1000 nm. This is just one example of the improved chromatic accuracy, brightness, resolution, and image flatness that X Line objectives deliver.

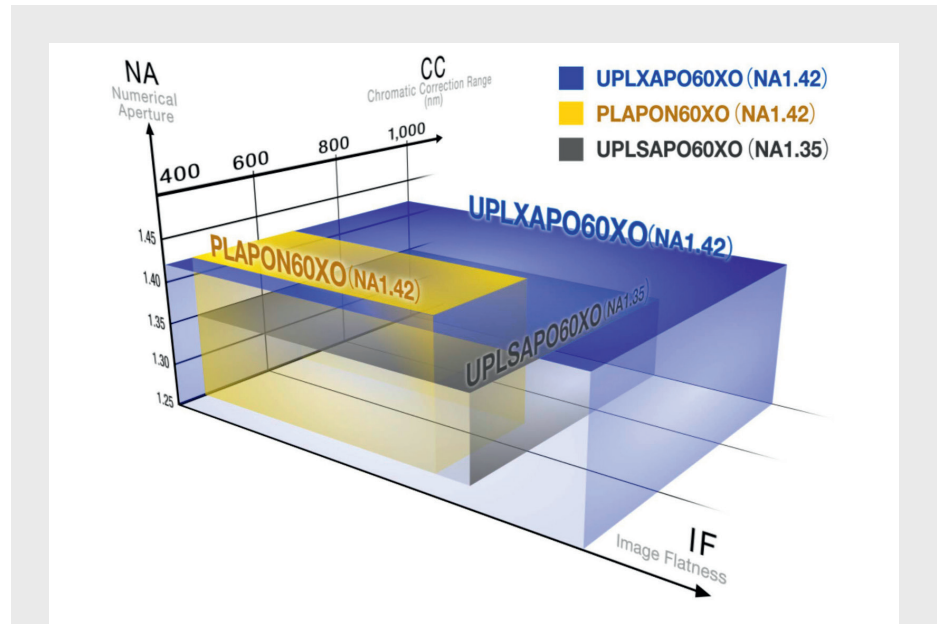


Figure 1

A comparison showing how advanced manufacturing technology improves the NA, image flatness, and chromatic correction of X Line objectives

Key Factors for High-Resolution Imaging Over a Large Field of View

Numerical Aperture

An objective's NA is a measure of its ability to gather light. It is an important factor in determining the resolution, depth of focus, and the brightness of images. Objectives with a larger NA gather a wider range of light, resulting in brighter, higher resolution images. NA is important when observing extremely fine structures or detecting dim signals during fluorescence observation. In X Line objectives, such as the UPLXAPO40XO, an NA of 1.40 is maintained without deteriorating other aspects of the optical performance (Figure 2).

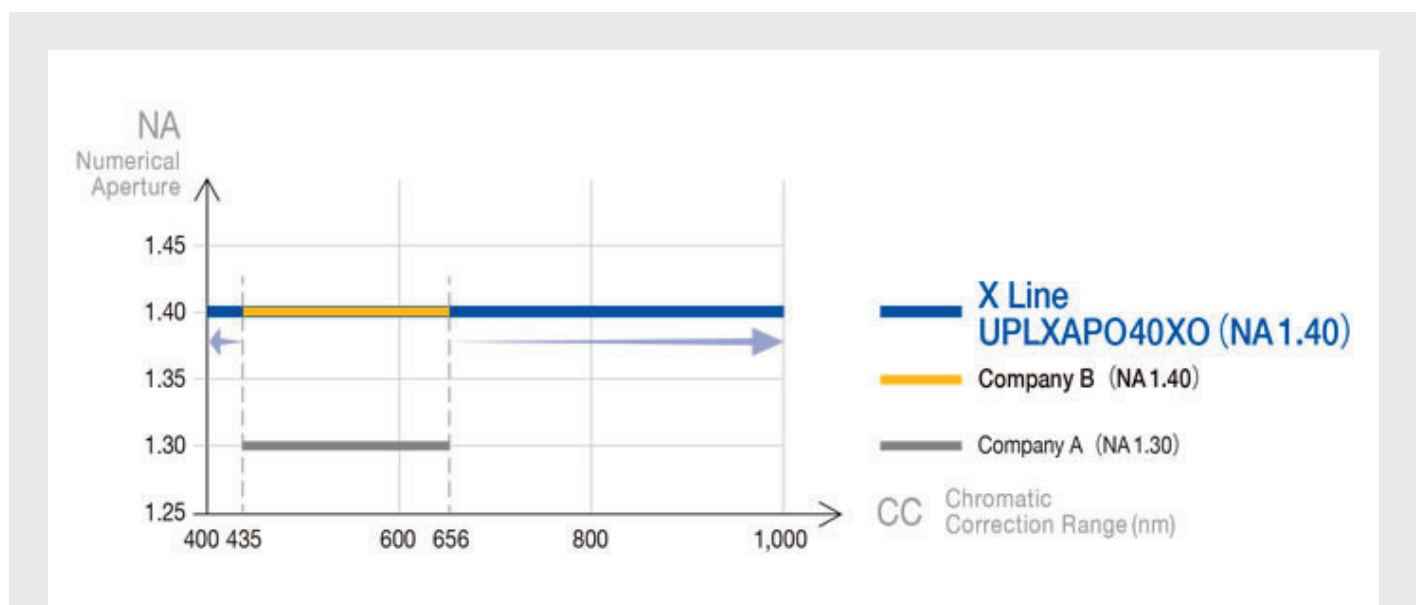


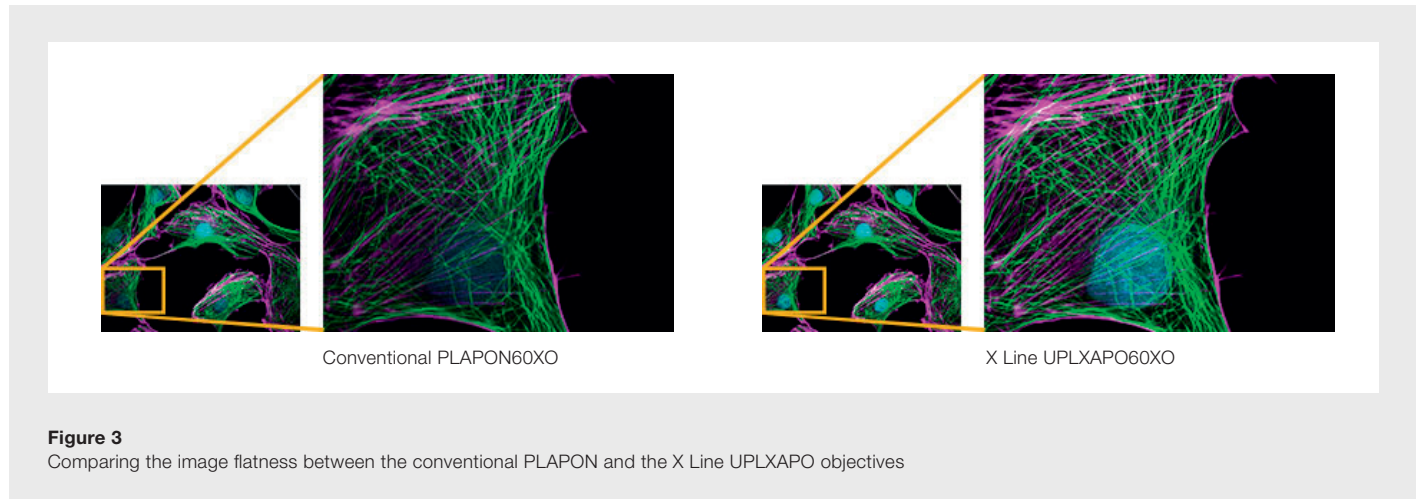
Figure 2

Improved NA with no deterioration in the chromatic correction range

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Image flatness

An objective's image flatness is limited by a lack of sharpness caused by spherical and coma aberration, astigmatic aberration, and the effect of field curvature and peripheral darkening. Image flatness is an important indicator of the reliability of quantitative analytical data from observations that require a high resolution over a large FOV. Our X Line objectives provide outstanding image flatness from the center to the periphery without sacrificing NA or chromatic correction, even in large FOV observations.

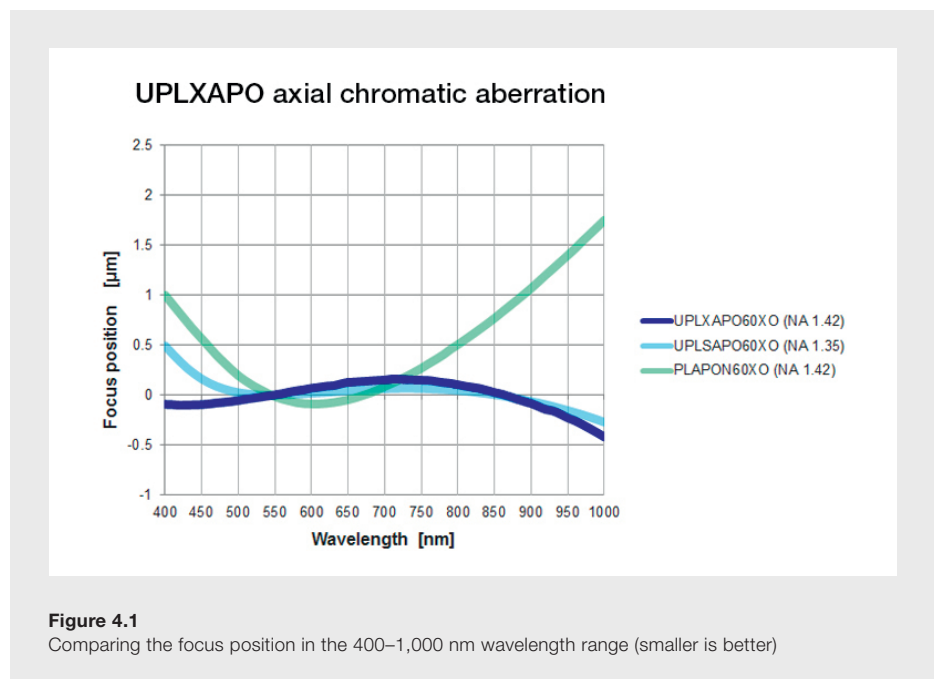


Chromatic correction

Lenses are made of glass, and glass has a different refractive index for each wavelength of light.

The lens' inability to focus different wavelengths of light in one spot is called chromatic aberration. During microscope observation, correcting for chromatic aberration is important for acquiring quality images. With lenses manufactured using conventional technology, such as the UPLSAPO60XO and the PLAPON60XO, the chromatic correction ranges are 435–1000 nm and 435–656 nm, respectively. Because of these ranges, the focus position is not colocalized for short wavelengths (< 435 nm) and long wavelengths (> 656 nm) for the UPLSAPO60XO.

By contrast, the UPLXAPO60XO X Line objective has a chromatic correction range of 400–1000 nm while maintaining an NA of 1.42 (Figures 3 and 4).



X Line Advanced Manufacturing Technology

Olympus has been designing and manufacturing objectives for 100 years. With extensive knowledge and expertise over the years we have developed a novel proprietary lens polishing technology. This technology enables us to manufacture convex lenses with ultra-thin edges as well as ultra-thin concave lenses. The lens' shape enables capturing of light at a wider angle than conventional lenses. Because they are so thin, more lenses can be packaged in each objective housing, enabling us to increase the NA, image flatness, and chromatic correction range.

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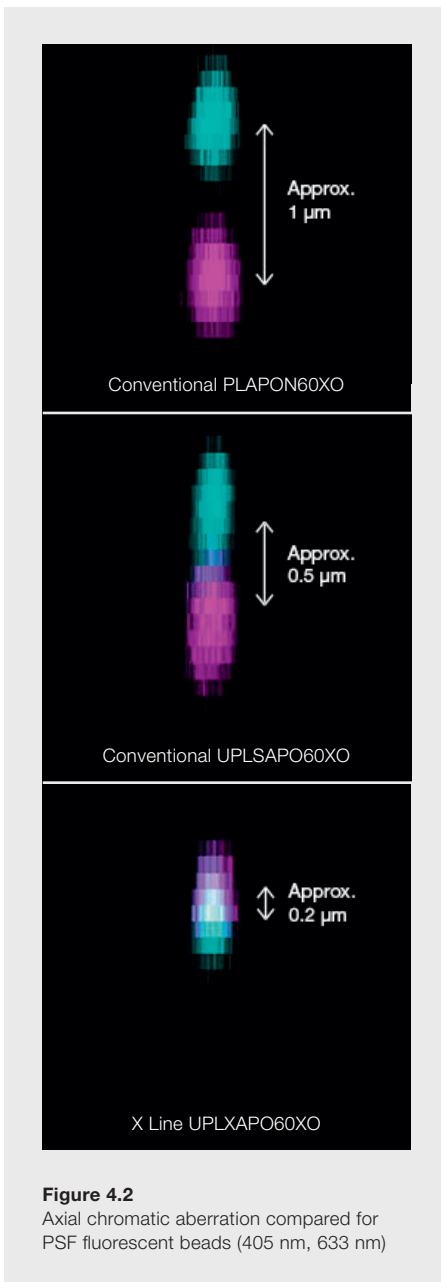


Figure 4.2
Axial chromatic aberration compared for PSF fluorescent beads (405 nm, 633 nm)

The microscope objective is composed of several convex and concave lenses with different refraction indices to correct various optical aberrations. These lenses are combined in 45mm length, the international standard. A simple model of a convex-concave-convex three lens system can be used to understand the improvements made on the X Line objectives. These improvements consist of increase in numerical aperture, image flatness and wide range chromatic correction (Figure 5.1). Composite focal length of 3 lenses (f) is defined by the equation below. Composite focal length is a constant value which determines magnification of objective.

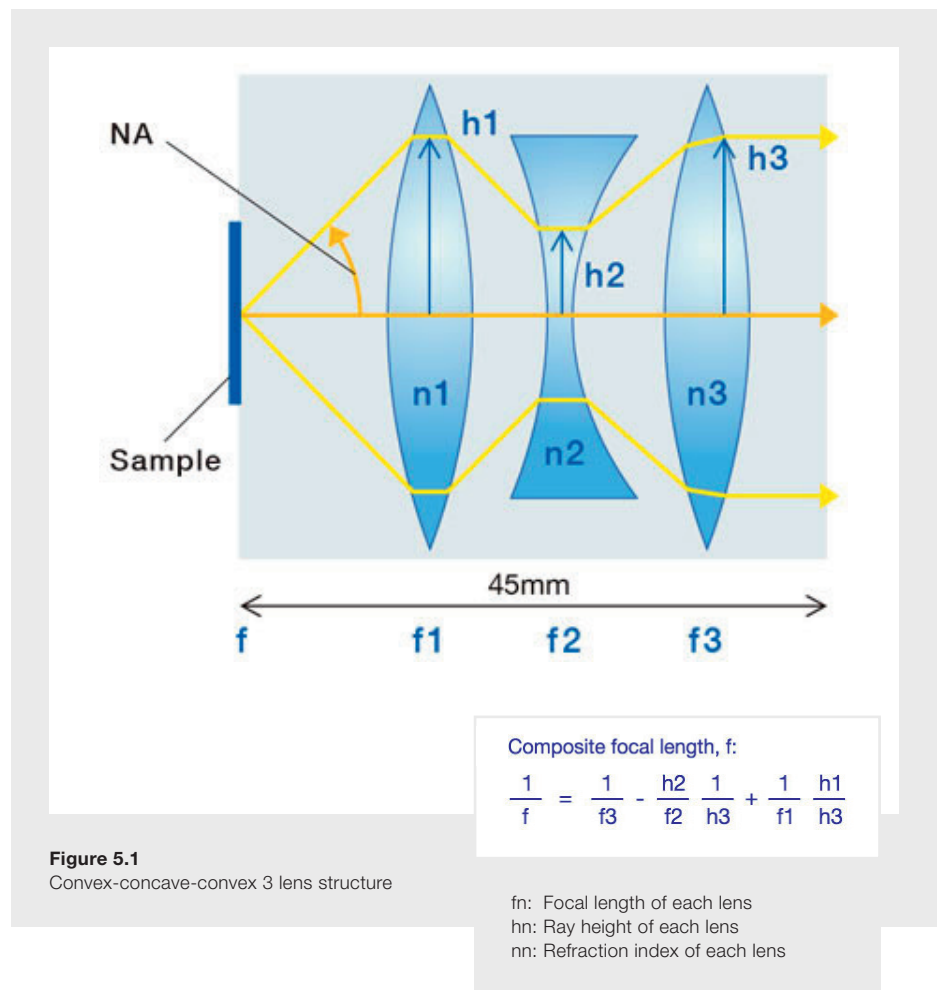


Figure 5.1
Convex-concave-convex 3 lens structure

Expansion of NA: for high resolution and high S/N

To design objectives with high numerical aperture (NA), h1 should be higher because the wider angle for collecting ray is required. Furthermore, high NA is associated with high h3 (pupil radius) coefficient through the equation of $h_3 = NA \times f$. In order to obtain high h1 and h3, effective diameter of lens should be increased. In order to improve the NA while maintaining the total length (45 mm) of the objective lens, it is necessary to make the rim of the convex lens (the thickness of the lens outer peripheral part) t (Figure 5.2 (a)) extremely thin. (Figure 5.2(b))

Improving Image Flatness: Acquisition and observation of uniform and clear image around the field of view

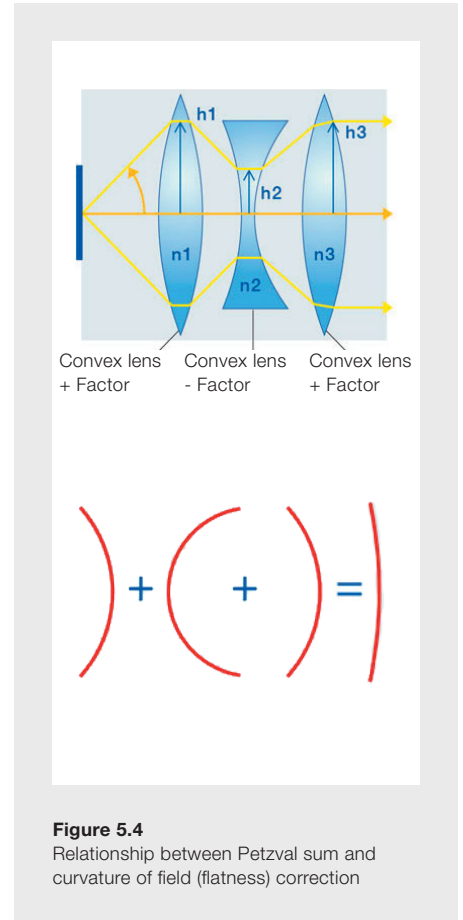
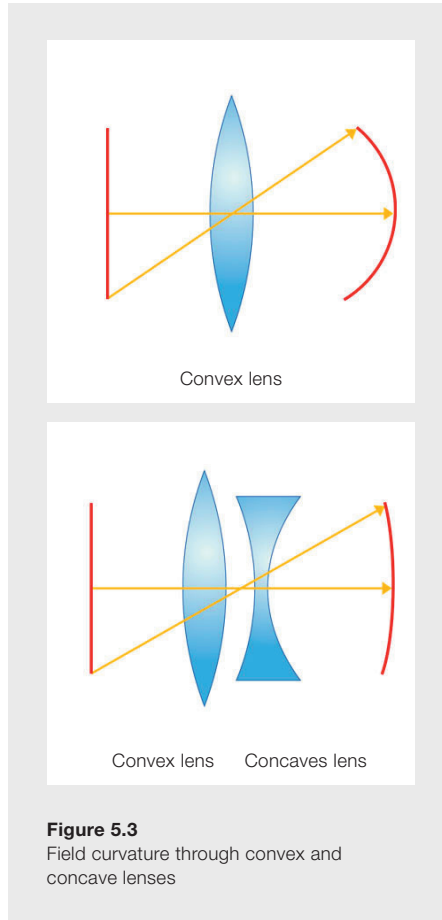
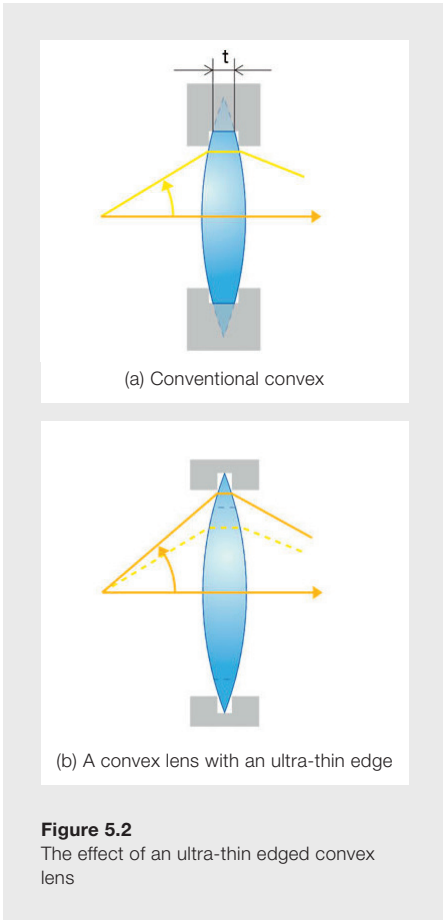
To design the objectives with improved image flatness (IF), small Petzval sum (P) is required. Petzval sum (P) is defined as an equation at the right.

Petzval sum

$$P = \frac{1}{n_1 \cdot f_1} - \frac{1}{n_2 \cdot f_2} + \frac{1}{n_3 \cdot f_3}$$

Convex lens + Factor
Concave lens - Factor
Convex lens + Factor

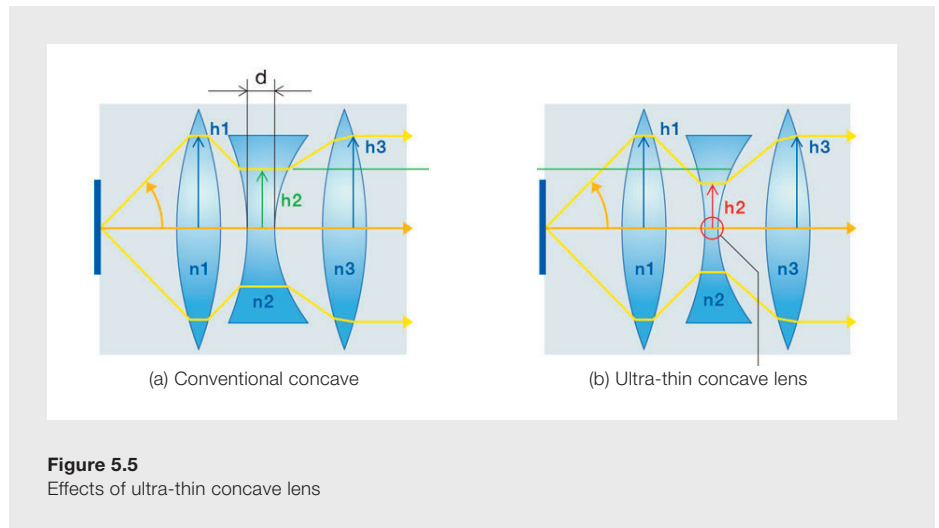
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Positive (+) factor of convex lens indicates that convex lens inflects the image so that the peripheral part of image is closer to sample, as shown in figure below. Concave lens inflects the image in an inverse direction compared to convex lens, serving as a negative (-) factor.

Since the objective of microscope serves as a convex lens, positive (+) factor in (P) is larger than negative (-) factor. Therefore, large negative (-) factor is required for improved IF with small absolute value of P. (Figure 5.4)

To create a small value of P, both f_2 and h_2 coefficients should be lowered, since h_3 becomes constant to control the magnification of the objective (composite focal length, f) and NA. The coefficient h_2 can be lowered by increasing the

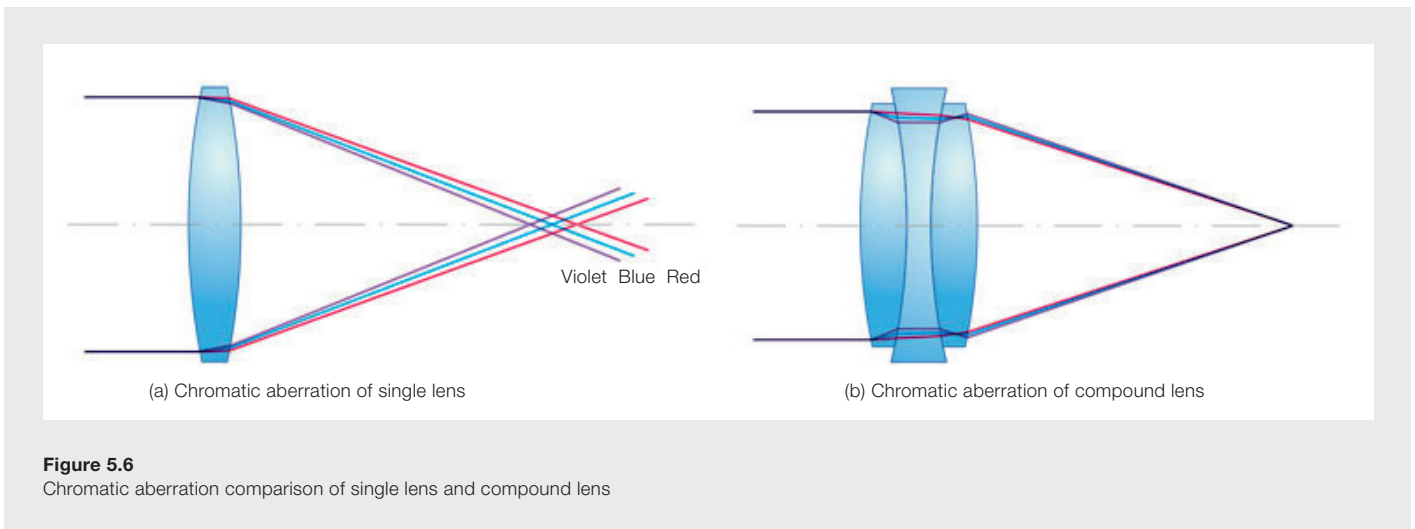


distance between convex and concave lenses, however in the limited space, thin center concave lens with small (d) is required. (Figure 5.5)

Expansion of chromatic correction wavelength range: Clearer and more accurate information acquisition

Chromatic aberration is caused by glass that has different refraction index for each wavelength. Generally, refraction index of glass is higher for shorter wavelength.

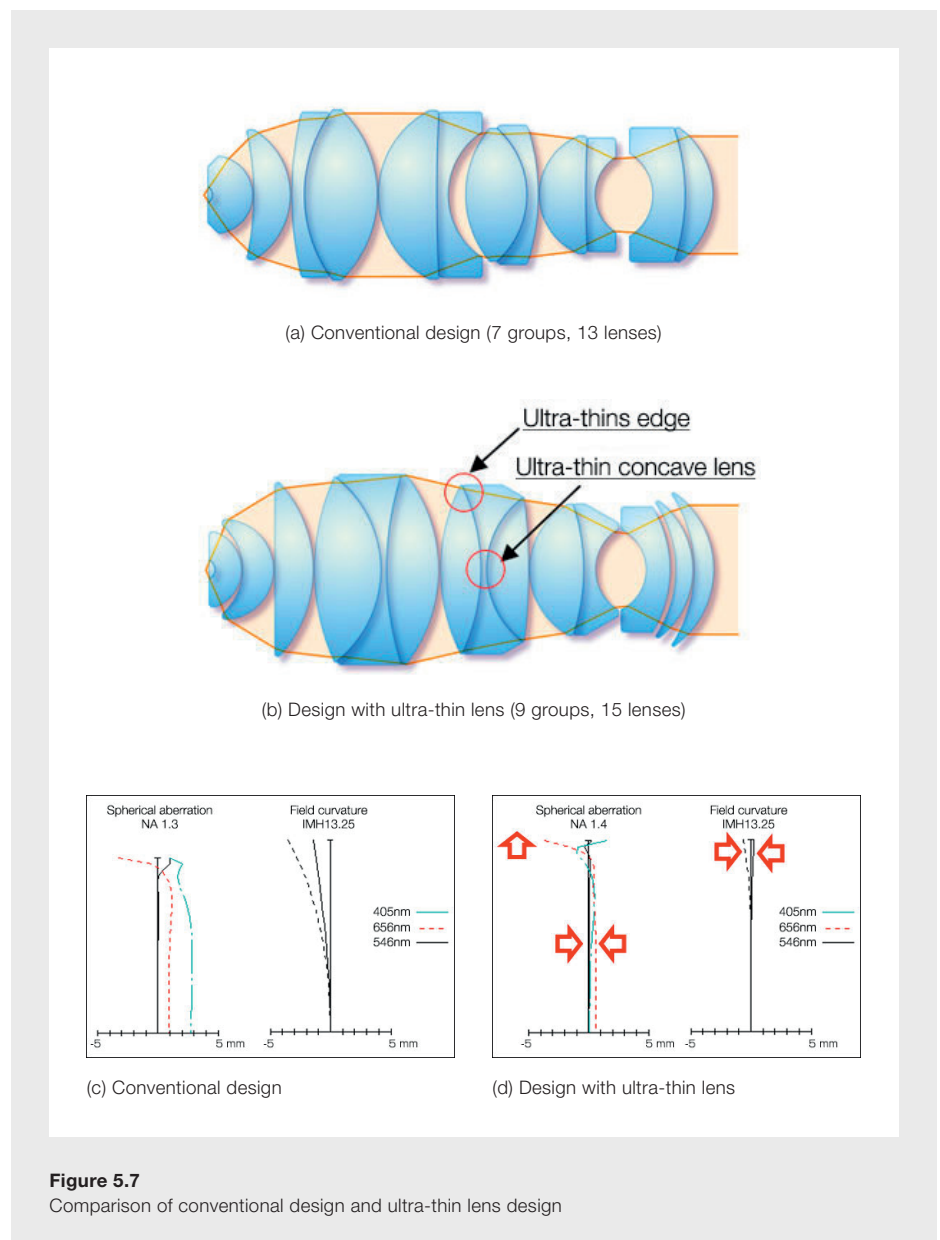
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Accordingly, in convex lens, short wavelength light focuses on closer position to lens, and long wavelength light focuses at a distal position from lens. Concave lens diffuses shorter wavelength light. Therefore, chromatic correction (CC) is performed by combining convex and concave lenses so that red and blue light focus on the same position (Figure 5.6).

For wide range correction of chromatic aberration, several cemented lenses composed of 2 or 3 lenses are required. Furthermore, thin lenses are a crucial component in a limited space. In the conventional design with 13 lenses in 7 groups, the performance was as shown in the left figure (c). Design using ultra-thin lens with 15 lenses in 9 groups can greatly improve the performance as shown in the right figure (d). (Figure 5.7).

As described above, the X Line objectives are designed by using ultra-thin lenses while maintaining the overall length of the objective lens at the international standard of 45 mm, which is compatibility with existing microscopes. The improvements in design and manufacturing have led to creating the X Line objectives, with high NA, improved IF and wide wavelength range CC.



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Stable quality provided by wavefront aberration control

The manufacturing process for X Line high-performance objectives is sensitive to fluctuations in the production line, so advanced wavefront aberration control is applied during the assembly process to assure consistency from one objective to the next. (Figure 6a and 6b).

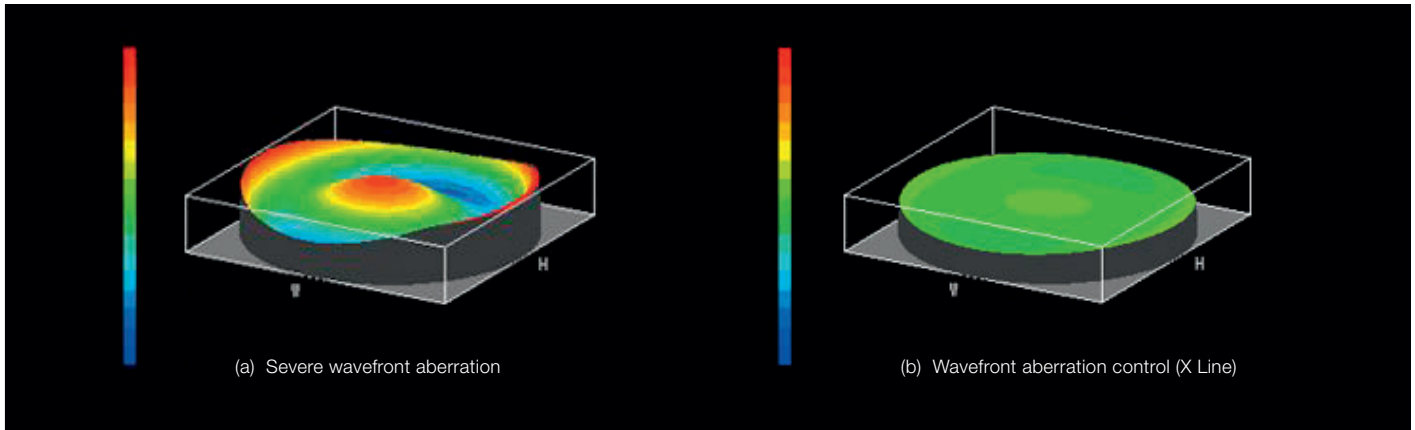


Figure 6
Wavefront aberration control

Innovative Imaging

Better image flatness improves whole slide imaging (WSI)

The X Line objectives have an improved image flatness compared to conventional objectives, which is beneficial for whole slide imaging applications. One of the X Line objectives, the UPLXAPO20X, has a numerical aperture of 0.80, significantly improved image flatness in the entire FOV (Figure 7b), and improved resolution when compared to a conventional objective (Figure 7a). These improvements yield better image quality in each image as well as more efficient acquisition of tiled images.

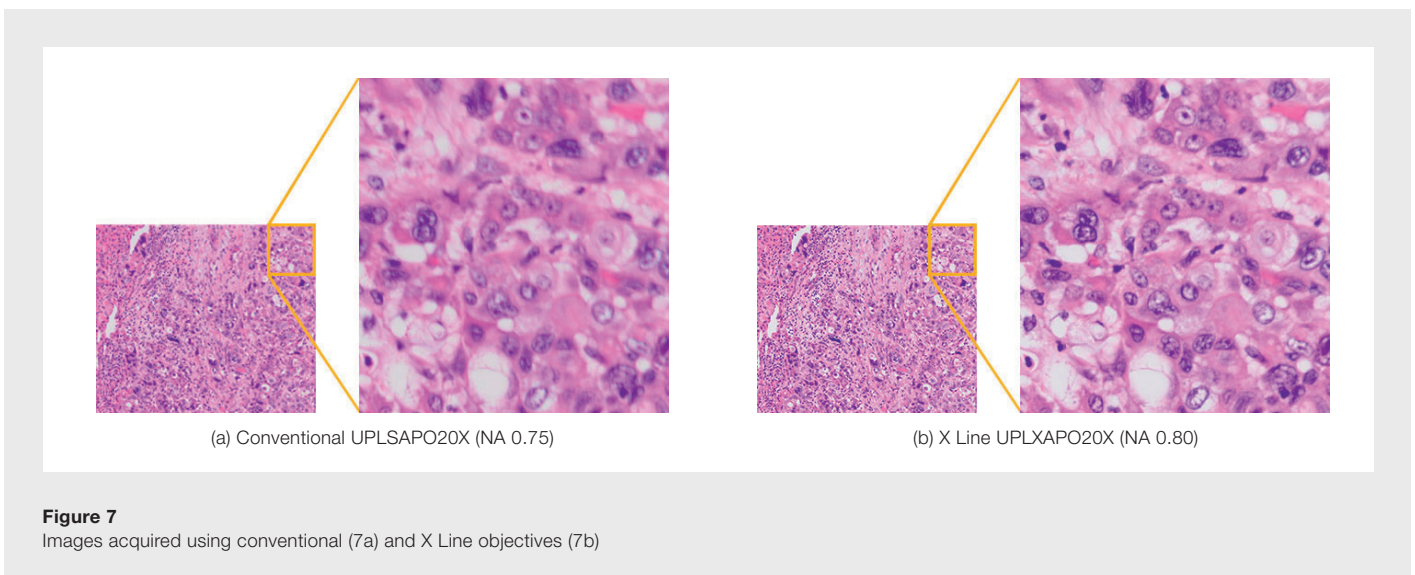


Figure 7
Images acquired using conventional (7a) and X Line objectives (7b)

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The NA improvements of the X Line objectives also help with fluorescence imaging. An increase in NA correlates to less photons needed to illuminate the sample reducing phototoxicity and photobleaching during fluorescence observation. The images are consistently bright over the entire FOV, which leads to more accurate analysis.

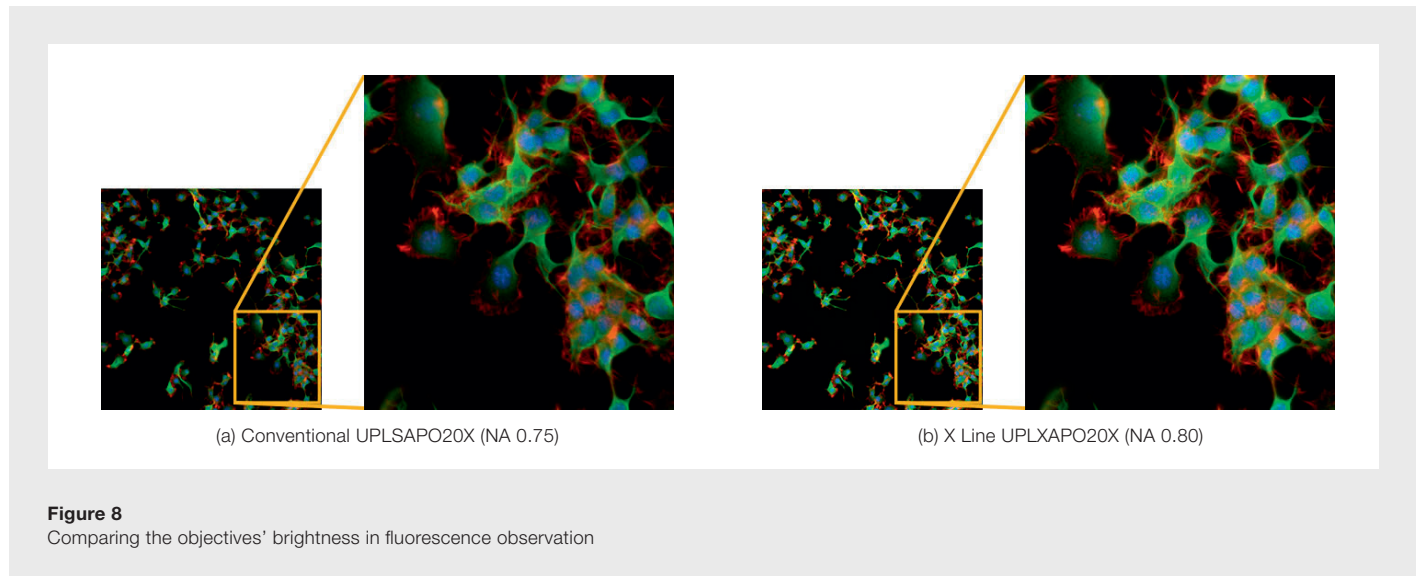


Figure 8
Comparing the objectives' brightness in fluorescence observation

Acquiring reliable multicolor fluorescence images with a high NA

X Line objectives with improved chromatic correction in the range of 400–1000 nm provides accurate data of multicolor fluorescence observation. HeLa cell labeled by FISH technique CEP17 (Spectrum Green), CEP18 (Spectrum Orange), Nuclear (DAPI). When observing with conventional objectives, signals located at the bottom of cell nuclear appears outside the nuclear.

Accurate quantitative results with chromatic correction in a wide range of wavelengths

Qualitative analysis and imaging with a large FOV are common thanks to advances in digital technologies. For your analysis to be reliable, high-quality raw data are essential. Figure 10 shows fluorescence images of nuclei stained with DAPI (405 nm excitation wavelength) acquired with different objectives and analyzed to automatically recognize nuclei using analysis software.

Peripheral nuclei are not accurately recognized in the image acquired with a conventional objective because the 405 nm excitation wavelength is outside the chromatic correction range, and the image flatness is poor (Figure 10a). However, using the X Line objective (UPLXAPO60XO; NA 1.42), even peripheral nuclei are accurately recognized because of the improved chromatic correction of X Line objectives at 405 nm (Figure 10b).

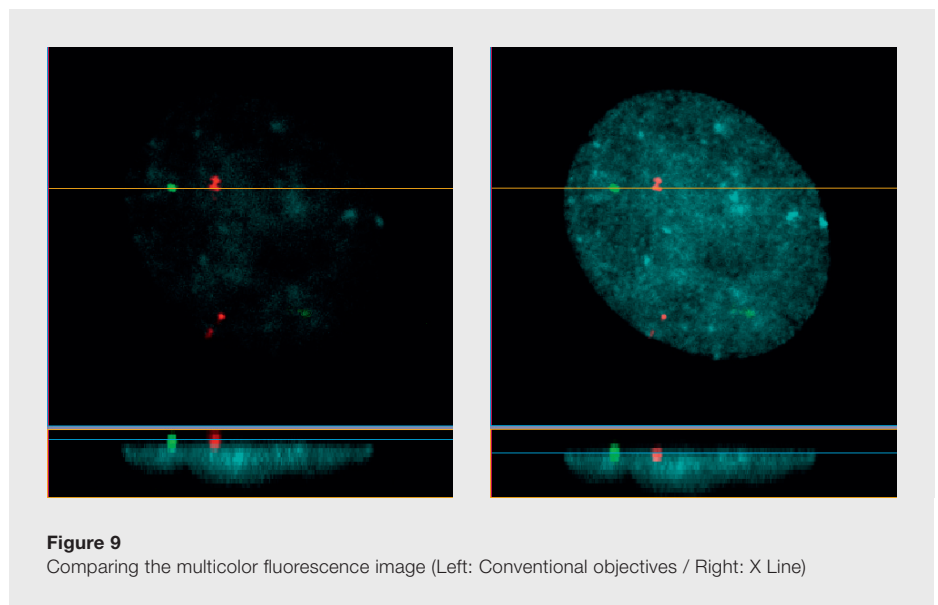


Figure 9
Comparing the multicolor fluorescence image (Left: Conventional objectives / Right: X Line)

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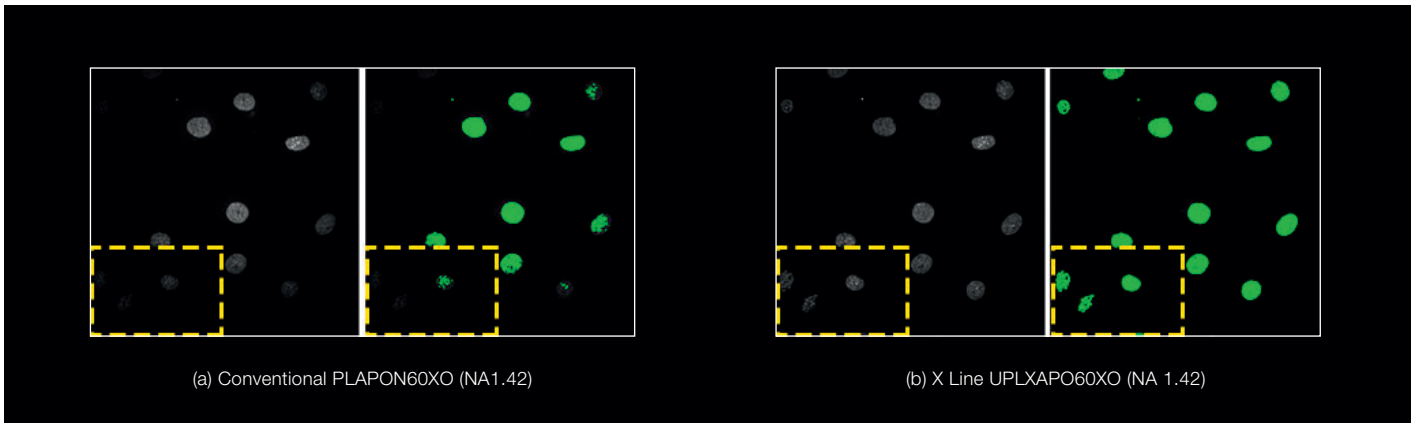


Figure 10
Comparing images at 405 nm (Left: Raw image, right: analysis results)

High usability of the X Line UPLXAPO40XO objective with an NA of 1.4 enables efficient experiments

Conventional 40X objectives have a relatively large FOV and are suitable for observing multiple cells. Consequently, users often employ 40X objectives to search for a region of interest and then switch to higher NA objectives, such as a 60X or 100X objective, to acquire high-resolution images.

The UPLXAPO40XO X Line objective has an NA of 1.40, which is equivalent to that of conventional 60X or 100X objectives. This enables the user to acquire images with adequate resolution over a relatively large FOV using just the 40X objective as shown in Figure 11. There is no need to change objectives or add oil, and the risk of losing the sample's position is minimized.

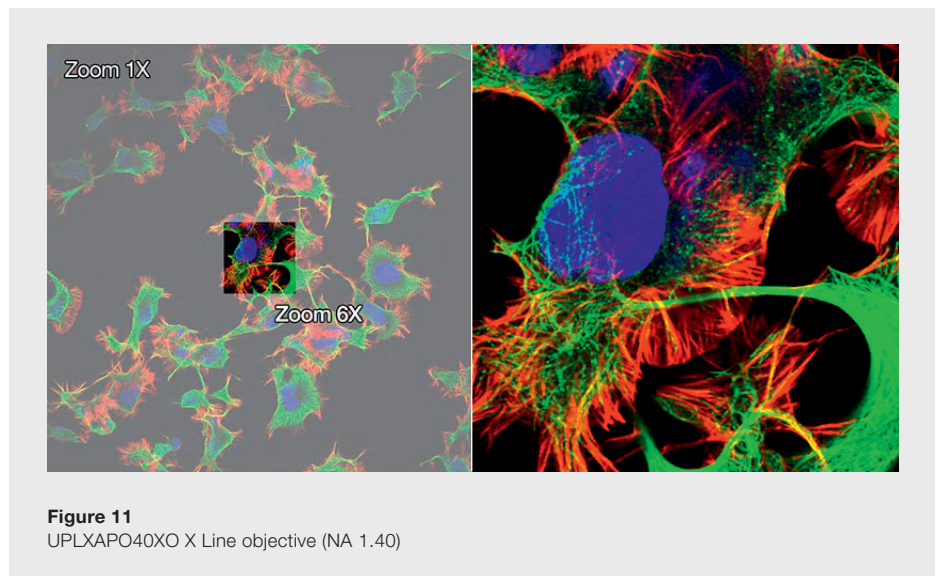


Figure 11
UPLXAPO40XO X Line objective (NA 1.40)

High-resolution multicolor tiled images, including 405 nm excitation

To get a complete image of a large specimen, such as a tissue section, a number of small images are usually acquired and then tiled together to form one large image. The quality of the tiled image is dependent on the image flatness. The better the image flatness, the more seamless the tiled image. The UPLXAPO60XO X Line objective offers high image flatness and wide chromatic

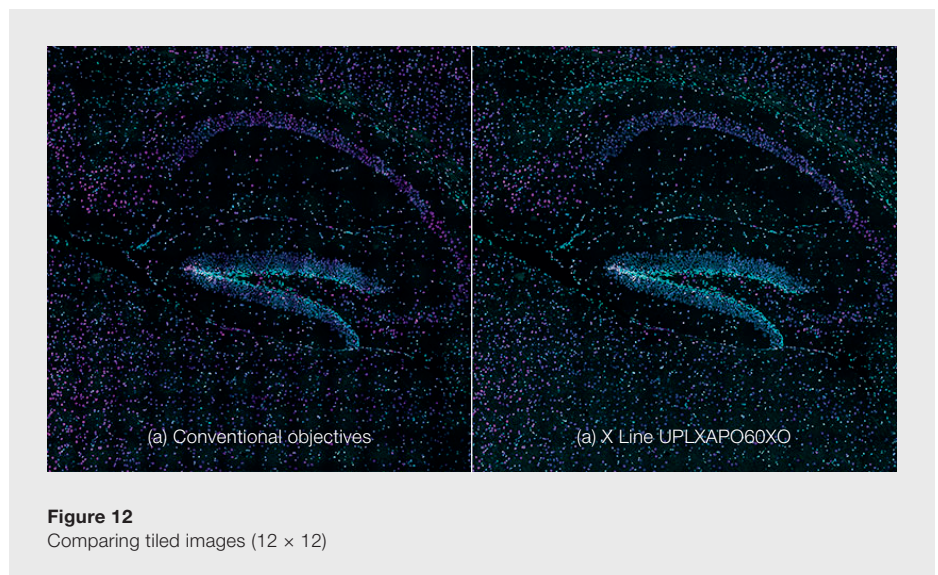


Figure 12
Comparing tiled images (12 × 12)

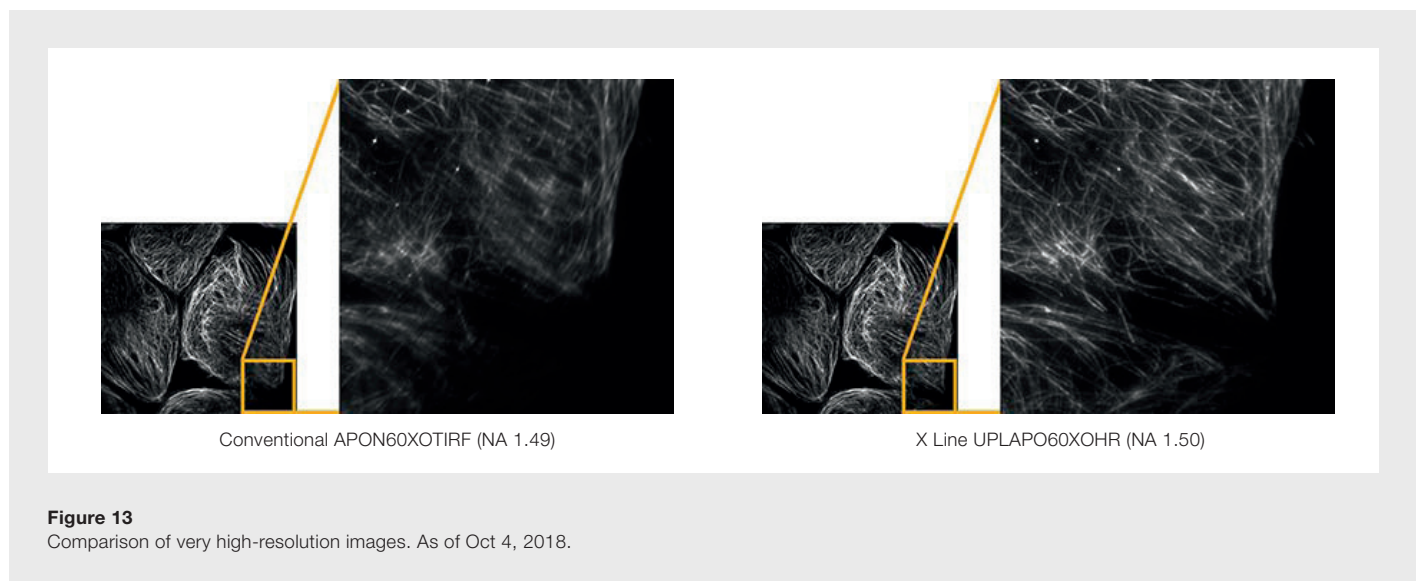
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correction which helps create high-quality tiled images, even at an excitation of 405 nm (Figure 12: Brain section of Fucci2 Tg mouse; Stitched image of 12x12 images acquired by FV3000 using 60x oil immersion objective (NA1.42)

cyan: DAPI (405nm) magenta: mCherry (561nm). Image data courtesy of Laboratory for Cell Function Dynamics, RIKEN Center for Brain Science Takako Kogure, Atsushi Miyawaki).

The world's first*¹ NA 1.50, Plan-corrected Apochromat objective

The technological advancements introduced by our advanced lens polishing technology enabled us to realize the world's first Plan-Apochromat objective with an NA of 1.50. These high-resolution objectives work well for super resolution or TIRF imaging.



*¹ According to Olympus research of objective lens using common immersion oil (refractive index $n_e = 1.518$).

X Line and High-Resolution Objectives

Series	Objective name	NA	Working Distance [mm]	Chromatic correction range
X Line	UPLXAPO4X	0.16	13	400–1000 nm
	UPLXAPO10X	0.40	3.1	400–1000 nm
	UPLXAPO20X	0.80	0.6	400–1000 nm
	UPLXAPO40X	0.95	0.18	400–1000 nm
	UPLXAPO40XO	1.40	0.13	400–1000 nm
	UPLXAPO60XO	1.42	0.15	400–1000 nm
	UPLXAPO100XO	1.45	0.13	400–1000 nm
	UPLXAPO60XOPH	1.42	0.15	400–1000 nm
	UPLXAPO100XOPH	1.45	0.13	400–1000 nm
HR (High-resolution objectives)	UPLAPO60XOHR	1.50	0.11	435–656 nm
	UPLAPO100XOHR	1.50	0.12	435–656 nm

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Summary

Combining high-quality objectives with digital technology (for both computation and device sides) is increasingly important. Olympus' X Line high-performance objectives offer improved numerical aperture, image flatness, and chromatic correction thanks to our revolutionizing lens polish technology, helping create reliable quantitative and qualitative imaging results.

Author

Masahiro Sakakura

R&D Group

Optical System Development Div.

Olympus Corporation

 www.olympus-lifescience.com

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OLYMPUS CORPORATION
Shinjuku Monolith, 2-3-1 Nishi-Shinjuku,
Shinjuku-ku, Tokyo 163-0914, Japan