顕微鏡対物レンズ CFI Plan Apochromat λD シリーズの開発

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Development of the CFI Plan Apochromat Lambda D Series Objectives for Biological Microscopes

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プランアポクロマート λD シリーズを2021年12月に発売した．世界最高レベルの NA と WD を有し，視野全域における優れた光学性能を誇り，広波長帯域の色収差補正がなされたプランアポクロマート対物レンズシリーズである．ニコンの顕微鏡対物レンズの最高峰に位置するプランアポクロマート対物レンズシリーズには，これまで λ シリーズと VC シリーズがあった．本論文では λ シリーズと VC シリーズの長所を継承した λD シリーズの光学設計技術と製造技術について紹介する．

顕微鏡システムにおいて最も重要な心臓部と言われる顕微鏡対物レンズの重要事項の 1 つに光学硝材選択がある．特に，広波長帯域の色収差補正を実現するには，最適な光学硝材を選択する必要がある．ここでは 2 枚の薄肉単レンズの色消しから出発し，3 枚の薄肉単レンズの色消しについて説明する．更に共焦点レーザー走査型蛍光顕微鏡における色収差の影響を解説し，観察画像を比較する．

ニコンの誇る波面収差計測技術と最適化技術から生まれた波面収差最適化システムについて説明する．この波面収差最適化システムのシミュレーション結果を報告する．

In December 2021, we launched seven models of CFI Plan Apochromat Lambda D Series objectives for biological microscopes. The Plan Apochromat objective lens series provides the world's highest levels of Numerical Aperture and Working Distance, excellent optical performance over the entire field of view, and chromatic aberration correction across a wide wavelength range.

To date, the Plan Apochromat objective lens series, which is the highest spec of Nikon's microscope objective lenses, comprised the λ and VC series. Herein, we introduce the optical design and manufacturing technologies of the λD series, which has the advantages of both λ and VC series.

We begin with the achromatism of two thin lenses, explain that of three thin lenses and the influences of chromatic aberration in a confocal laser scanning fluorescence microscope, and finally show the actual images.

Furthermore, we explain the wavefront aberration optimization system developed using Nikon's wavefront aberration measurement and optimization technologies. Finally, we report the simulation results of this wavefront aberration optimization system.

Key words 対物レンズ，解像限界，顕微鏡
objective lens, resolution limit, microscopy

1 Introduction

In recent years, remarkable progress has been made in optical microscopy systems. However, from the time of E. Abbe to the present day, the objective microscope lens remains the heart of the optical microscope and determines its resolution.

This paper introduces the CFI plan apochromat λD series launched in December 2021 (Fig. 1).

2 Development Background

Thus far, among the pinnacle of Nikon's plan apochromatic objective lens series were the λ and VC series.

The λ series has high aberration performance with wide and peripheral fields of view, which are the most prominent features of Nikon microscope systems, and high transmittance from the visible to the near-infrared region using Nano Crystal Coat. On the other hand, the VC series features an axial chromatic aberration performance in the visible range from 405 nm, which the λ series does not have, and was

highly regarded in the market, especially concerning confocal laser scanning fluorescence microscopy.

In recent years, with the development of digital imaging technology and fluorescent dye technology in the biological microscopy market, there has been a desire for a microscope objective lens that enables observation in a wide field of view and a wide wavelength band from bright-field observation to confocal laser scanning fluorescence microscopy. Indeed, the microscope objective lens, which inherits the advantages of the λ series and VC series, was what was needed.

Fig. 1 Images of the present objective lens series

3 Specifications

The CFI plan apochromat λD series is a series of plan apochromatic objective lenses with the world's highest numerical aperture (NA) and working distance (WD) standards, excellent optical performance at the field of view periphery, and chromatic aberration correction over a wide wavelength band. It supports all Nikon microscopes and systems. In developing this new series, the power layout was reviewed from the ground up, and everything from design to manufacturing and inspection was completely redesigned.

The specifications of the objective lens series in this report are shown in Table 1.

Table 1 Specifications of CFI plan apochromat λD series

	60x	100x	40x	20x
NA	1.42	1.45	0.95	0.80
WD [mm]	0.15	0.13	0.21*	0.80
Chromatic correction	Apo	Apo	Apo	Apo
Flatness	Plan	Plan	Plan	Plan
Immersion medium	Oil	Oil	Dry	Dry

	10x	4 x	2 x
NA	0.45	0.20	0.10
WD [mm]	4.00	20.0	8.50
Chromatic correction	Apo	Apo	Apo
Flatness	Plan	Plan	Plan
Immersion medium	Dry	Dry	Dry

* with correction ring

4 Chromatic Aberration Correction in the Wide Wavelength Range

One of the essential factors in the optical design of microscope objectives is the selection of optical glass material. Here, we introduce the axial chromatic aberration based on three thin lenses necessary to realize the correction of axial chromatic aberration in a wide wavelength range and explain the basic concept of lateral chromatic aberration correction.

First, the first-order and second-order color cancellation conditions for two thin lenses, a and b, are confirmed, and then the conditions are extended to three thin lenses. First-order chromatic aberration means that the focal points of two wavelengths overlap, and second-order chromatic aberration means that the focal points of three wavelengths overlap. Second-order chromatic aberration correction is essential for correcting chromatic aberration in a wide wavelength band. The composite focal length and conditions for achromatization for two thin lenses can be written as follows:

$$\Phi_a + \Phi_b = \Phi \tag{1}$$

$$\frac{\Phi_a}{\nu_a} + \frac{\Phi_b}{\nu_b} = 0 \tag{2}$$

$$P_a \frac{\Phi_a}{\nu_a} + P_b \frac{\Phi_b}{\nu_b} = 0 \tag{3}$$

Here, νa, Pa , Φa, represent the Abbe number, partial dispersion ratio, and power of the thin lens, respectively. The power is the reciprocal of the focal length. The optical glass material that satisfies the condition in Eq. (4) is required to achieve first-order and second-order color cancellation with two thin lenses. To achieve the above Conditions for achromatization as close as possible, the dispersion properties of glasses of the optical glass of thin lenses a and b must have a larger Abbe number difference and a smaller partial dispersion ratio difference.

$$\frac{(P_a - P_b)}{(\nu_a - \nu_b)} \Phi \cong 0 \tag{4}$$

However, optical glass materials are discrete and limited

even when fluorite or other materials are used, making it impossible to achieve axial chromatic aberration correction over a wide wavelength band.

Therefore, we extend the idea of two thin lenses to three thin lenses, *a*, *b*, and *c*.

$$\Phi_a + \Phi_b + \Phi_c = \Phi \tag{5}$$

$$\frac{\Phi_a}{\nu_a} + \frac{\Phi_b}{\nu_b} + \frac{\Phi_c}{\nu_c} = 0 \tag{6}$$

$$P_a\frac{\Phi_a}{\nu_a} + P_b\frac{\Phi_b}{\nu_b} + P_c\frac{\Phi_c}{\nu_c} = 0 \tag{7}$$

These equations can be expressed as follows: [1]–[3].

$$\Phi_b = \frac{P_c - P_a}{P_b - P_a}\frac{\nu_b}{T}\Phi \tag{8}$$

$$T = \frac{\nu_a(P_b - P_c) + \nu_b(P_c - P_a)}{P_b - P_a} - \nu_c \tag{9}$$

Fig. 2 shows the Glass map of partial dispersion ratio and Abbe number. Here, T is the line length extending from point c to the line connecting points **a** and **b** parallel to the horizontal axis in axis in the Glass map of partial dispersion ratio and Abbe number. It is difficult to satisfy the achromatic conditions for two thin lenses, by using two optical glass materials, **a** and **b**, a virtual optical glass material d' is created. Especially, the sign of Φ_b is noteworthy: Φ_b has a positive power when glass materials with dispersion properties of glasses are used, as shown in Fig. 2. This convention shows that axial chromatic aberration can be reduced by adding flint-type optical glass with a positive partial dispersion ratio, in addition to fluorite and crown-type optical glass.

In existing optical systems, in practice, a more complex design is required. More than a dozen pieces of optical glass

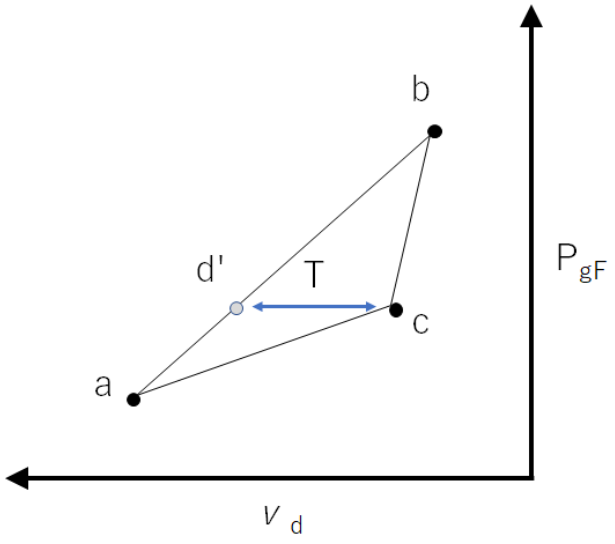

Fig. 2 Glass map of partial dispersion ratio and Abbe number

are combined to correct axial chromatic aberration over a wide wavelength band.

Before explaining the correction of lateral chromatic aberration, I would like to mention the history of the development of microscope objective lenses. The compensating method compensates for lateral chromatic aberration using an eyepiece and objective lens combination. Hence, lateral chromatic aberration occurred when observing a camera without an eyepiece. Therefore, around 1976, Nikon adopted the chromatic aberration Free (CF) method and developed a microscope system in which the objective and eyepiece lenses are each individually corrected for aberration [4]. Furthermore, in confocal laser scanning fluorescence microscopy, which is widely used as a general observation method, the shading in the periphery of the field of view due to the magnification chromatic aberration, which has not been a problem until now, has become an issue.

This paper introduces technology that can correct lateral chromatic aberration, especially in high-magnification objective lenses.

As shown in Fig. 3, the main power configuration of a high magnification objective lens is arranged in the order of the front group with positive power and the rear group with negative power, starting from the object (sample) side. For simplicity, the figure shows the d-line ray (green) as the primary ray and only the direction of the F-line ray (purple) to show the chromatic aberration at short wavelengths. The front group corrects image curvature, axial chromatic, and spherical aberrations. On the other hand, the rear group is at a greater distance from the optical axis that the rays pass through. As shown in Fig. 3, both the front group with positive power and the rear group with negative power produce positive lateral chromatic aberration on the image plane. As shown in Fig. 3, the flint-type optical glass has higher dispersion than the crown-type optical glass, so its F-line can be corrected more efficiently.

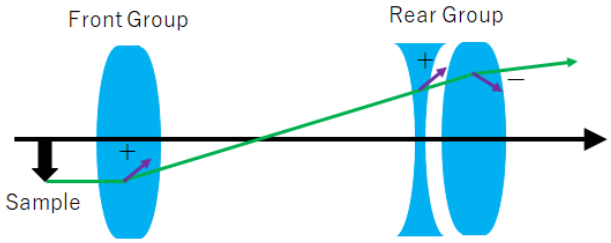

Fig. 3 Power configuration of high-magnification objective lenses

In particular, the immersion medium of oil-immersion objective lenses has higher dispersion than water or silicone immersion lenses. Since the chromatic aberration caused by

the oil is large, the chromatic aberration that must be corrected with the oil immersion objective lens is also large. High-dispersion optical glass may be used for the flint-type optical glass with the positive power described above. In this case, the second-order spectrum of lateral chromatic aberration is generated as the residue after the first-order spectrum of lateral chromatic aberration is corrected. For this reason, it is desirable to use optical glass with a lower partial dispersion ratio for the positive lens in the rear group and optical glass with a higher partial dispersion ratio for the negative lens. Nikon has a high degree of freedom in selecting glass materials because the development of the production process of optical glass materials is performed within the Nikon Group, enabling optical design that corrects for the axial chromatic aberration and second-order spectrum of lateral chromatic aberration. The effect of this lateral chromatic aberration on image quality and the effect of the designs are described in the next section.

5 Confocal Laser Scanning Fluorescence Microscope

This section discusses the effect of the second-order spectrum of lateral chromatic aberration on confocal laser scanning fluorescence microscopy. The confocal laser scanning fluorescence microscope is configured as follows (Fig. 4).

Excitation light from a laser light source passes through the objective lens and irradiates a fluorescent sample, and the fluorescence emitted is detected as a signal by the objective lens. At that moment, the wavelength of the excitation light and the fluorescence wavelength differs by several 10 to several 100 nm due to the Stokes shift. Here, we consider how the slight chromatic aberration in this wavelength difference affects image quality.

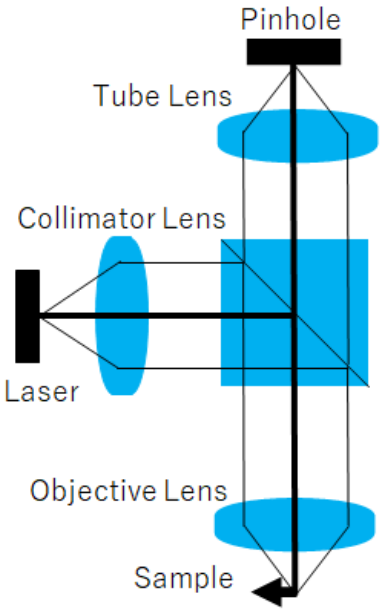

Fig. 4 Schematic of the confocal laser scanning microscope

$PSF_{ex}(x)$ is the Point Spread Function of the excitation optics of the confocal laser scanning fluorescence microscope, and $PSF_{em}(x)$ is Point Spread Function of the fluorescence optics. $PH(x)$ is the transmittance distribution representing the pinhole shape, and the Point Spread Function I obtained by laser scanning is multiplied by

$$I = o(x) * [PSF_{ex}(x) \cdot (PSF_{em}(x) * PH(x))] \quad (10)$$

where $o(x)$ is the density distribution of the fluorescent sample. $*$ is the convolution integral. The effective Point Spread Function $PSF_{eff}(x)$ is then defined as follows [5]:

$$I = o(x) * PSF_{eff}(x) \quad (11)$$

$$PSF_{eff}(x) = [PSF_{ex}(x) \cdot (PSF_{em}(x) * PH(x))] \quad (12)$$

Fig. 5 shows the Point Spread Function $PSF_{ex}(x)$ of the excitation light, $PSF_{em}(x)$ of the fluorescence, transmittance distribution (PH) of the pinhole, effective Point Spread Function $PSF_{eff}(x)$, Point Spread Function of excitation light $PSF_{ex}(x)$, Point Spread Function of fluorescence $PSF_{em}(x)$, and the effective Point Spread Function of the pinhole $PSF_{eff}(x)$. Here, $PSF_{ex}(x)$, $PSF_{em}(x)$, and $PH(x)$ are normalized

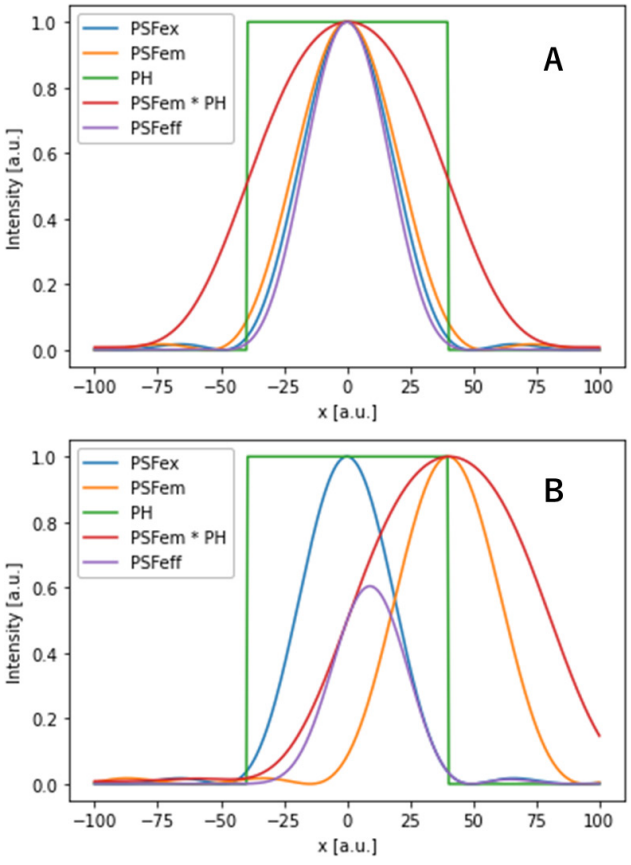

Fig. 5 Effective Point Spread Function of confocal laser scanning microscope PSF_{eff}

to be at most 1.

On the other hand, Fig. 5B shows the calculation results when lateral chromatic aberration occurs. The intensity distribution of the excitation light $PSF_{ex}(x)$ and the fluorescence $PSF_{em}(x)$ are shifted horizontally, and the intensity of the effective Point Spread Function $PSF_{eff}(x)$ is reduced. As a result, the confocal laser scanning fluorescence microscope image appears as shading.

More intuitively, the fluorescent sample excited by the laser passes through the detection optics, and the signal is detected through the pinhole, and if the detection optics has chromatic aberration, the signal intensity is reduced. If the detection optics has chromatic aberration, the signal intensity is reduced. For axial chromatic aberration, similar behavior is observed in the optical axis direction.

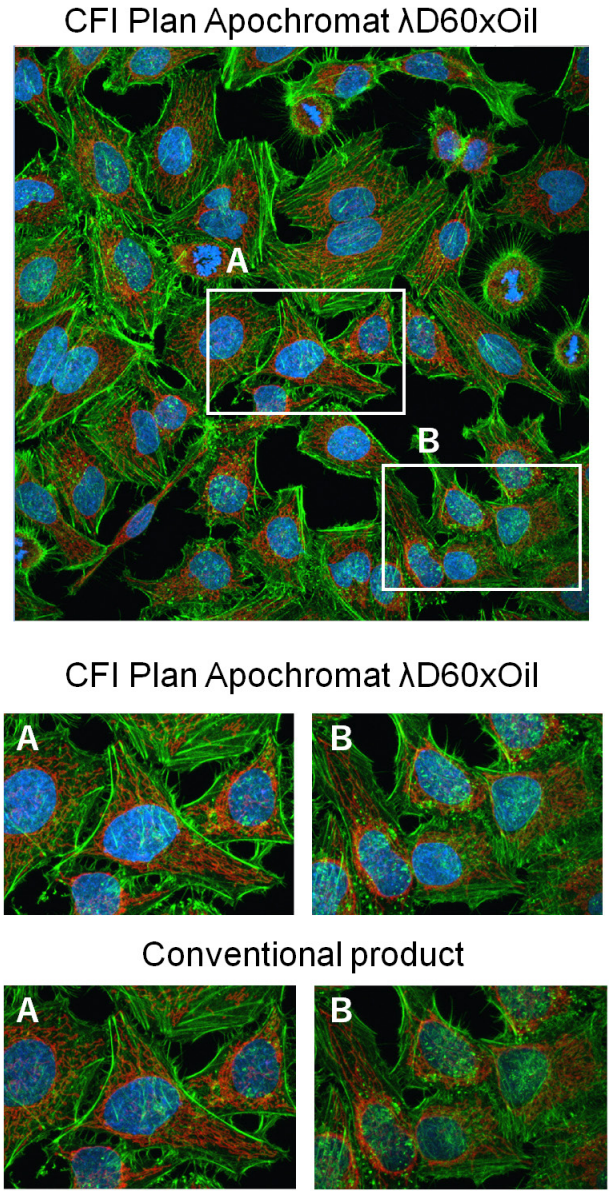

Fig. 6 Confocal laser scanning microscope FOV25 images of CFI plan apochromat λD60xOil and conventional objective lens
Hela cells: DAPI, 488-Actin, 568-Mitochondria

In the calculations shown in Fig. 5, the effect of lateral chromatic aberration was not included in the discussion of the effect of lateral chromatic aberration. However, when off-axis coma aberration and off-axis asymmetry occur, the Effective Point Spread Function $PSF_{eff}(x)$ decreases, and the shape of $PSF_{eff}(x)$ collapses, reducing resolving power. In the newly developed series of objective lenses, coma and asymmetric aberrations have been thoroughly eliminated to obtain sharp images with a little shading to the periphery of the field of view.

To demonstrate the effect of the developed objective lens, we compared the developed objective lens with a conventional objective lens in wide-field confocal laser scanning fluorescence microscopy with an FOV25. All observation conditions other than the objective lens were the same. In the conventional product, DAPI-stained cell nuclei are dark even at the center of the field of view due to axial chromatic aberration and extremely dark on the periphery of the field of view due to lateral chromatic aberration.

This shading change from the field of view center to the periphery does not change even when the laser power is increased. On the other hand, the CFI plan apochromat λD 60x, in which axial and lateral chromatic aberrations are corrected, produces sharp and uniform image qualities from the center of the field of view to the periphery.

6 Wavefront Aberration Optimization System

This section introduces the manufacturing technology of microscope objective lenses. Although microscope objective lenses are designed to be nearly aberration-free, aberrations occur due to manufacturing errors. Therefore, it is vital to have a wavefront aberration measurement device that accurately measures the wavefront aberration amount of the objective lens and an optimization technique that brings it as close to the designed value as possible.

A microscope observes a collection of point light sources emitted from a sample as an object. The light emitted from an ideal point source is converted to a parallel plane wave through the microscope objective. If the microscope objective lens has an aberration, it has an error from the ideal plane wave. This small error is called wavefront aberration and is used to evaluate the imaging performance of the optical system. In particular, the Zernike polynomial is an orthogonal polynomial defined on the unit circle, which is a good match for optical aberration. Nikon's wavefront mea-

surement technology is used in microscope objectives but also in semiconductor lithography equipment and interchangeable camera lenses, making it one of the most important technologies supporting imaging technology.

Even if each lens is manufactured with manufacturing tolerances of a few microns, the generated wavefront aberration cannot be ignored because the wavelength of light is several 100 nm. Therefore, the assembly must be optimized to minimize wavefront aberrations, equivalent to solving the following problem. For example, it can be expressed as in Eq. (13). In Eq. (13), the measured wavefront aberration expressed by the Zernike polynomial is denoted by z, and the variable x represents the state of the compensator. The change matrix for the sensitivities is denoted by $\boldsymbol{H}$. We must find the optimal solution x of the compensator so that each Zernike component is zero. Such a calculation can often be performed using the least-squares method to obtain the optimal solution [6].

$$min_x \left\| \boldsymbol{H}x - z \right\|^2 \tag{13}$$

Microscope objectives require high aberration performance over the entire field of view. More than ten lenses are used in the same parfocal distance of only 60 mm to correct axial chromatic aberration over a wide wavelength band. Therefore, the components of the compensator's variation matrix H, which indicates the sensitivity to aberration components, are hardly independent. For example, if the components of this variation matrix are not independent, compensating for the aberration component z1 using one compensator x1 increases another aberration component z2. In reality, the compensator has a finite range of motion, and the compensator has errors. Hence, it cannot be expressed in a simple expression like Eq. (13) and becomes a more complex constrained optimization problem. Alternatively, the complex constrained optimization problem must be solved and optimized for each objective lens measured by wavefront aberration. Before the advent of wavefront aberration optimization systems, microscope lens operators had to be adjusted by hand. In particular, high magnification, high-NA objectives have extremely high sensitivity, and spherical and eccentricity-coma aberrations are required to the resolution limit. These requirements make the manufacture of these objectives extremely difficult.

Even before recent AI technology became widely adopted, Nikon had developed its own optical design software and continued research and development over many years in the optimization field along with evaluation calculations for optical systems.

We applied our proprietary optimization engine to solve the complex constrained optimization problem during the assembly and adjustment of microscope objectives. As the simulation results of the optimization system, Fig. 7A and Fig. 7B show the wavefront aberration of the objective lens before adjustment and after optimization using the wavefront aberration optimization system, respectively. The wavefront aberration of the ideal optical system has a flatter shape. In other words, the optical system is approaching aberration-free image formation (Fig. 7B).

These wavefront aberration measurement and optimization technologies bring each microscope's objective lens as close as possible to the optical design value. This manufacturing system is the ideal system for manufacturing objective lenses.

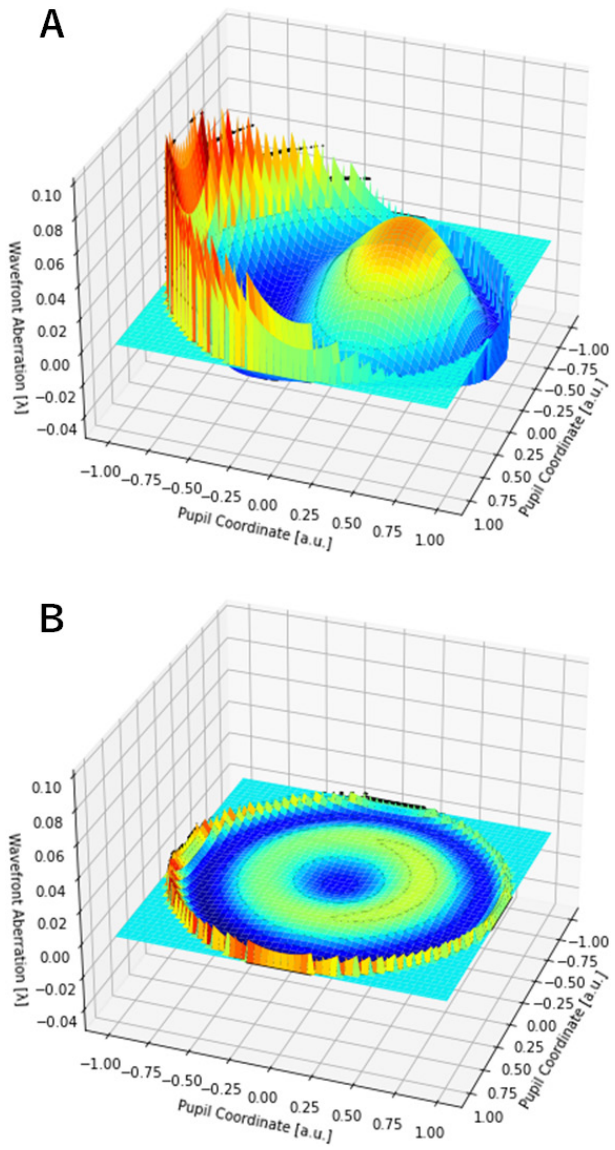

Fig. 7 Simulation of the wavefront aberration optimization system

7 Conclusion

The CFI plan apochromat λD series is a microscope objective lens series that integrates the λ series and VC series to achieve NA, WD, flatness, and field of view. This objective lens series is suitable for the heart of Nikon microscope systems with high aberration performance in the wide and peripheral fields of view, which are the essential features of the Nikon microscope systems, and with chromatic aberration correction over a wide wavelength band.

Nikon's optical design and manufacturing technologies are combined to make this objective lens series significantly contribute to developing scientific and industrial technologies, including bioimaging.

Though I have taken the lead in writing this article, it represents the work of many dedicated individuals who have contributed to this development. I want to take this opportunity to express my deepest gratitude to them.

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