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Characterization of high-numerical-aperture lenses by spatial autocorrelation of the focal field

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We demonstrate a new method for the characterization of high-numerical-aperture lenses based on the fluorescent detection of the spatial autocorrelation of the focal field. Numerical calculations without any fitting parameters, based on scalar diffraction theory, are in excellent agreement with the experimental results. The novel method provides lens characterization parameters similar to those obtained with point-spread function measurements in real time, which may be expected to be suitable for high-numerical-aperture lens testing and alignment procedures.   © 1995 Optical Society of America

The imaging properties of a high-numerical-aperture (high-NA) lens are generally given in terms of the lens's optical transfer function or, equivalently if the imaging process is linear in the detected quantity, by the Fourier transform of the optical transfer function, the point-spread function (PSF). These functions express the ability of the lens to transfer spatial frequencies. Measurement techniques for the PSF of high-NA lenses include, among others, three-dimensional imaging— and subsequent analysis—of highly scattering, or fluorescing, spheres that are much smaller than the typical width of the PSF[1-4] either in full field or by scanning the sphere or the detector. Alternatively one can image the edge of a mirror in reflection, although some care must be taken in comparing reflection with fluorescence measurements.[4] The major drawback of these techniques is that they are slow and consequently require long-term nanometer stability of the optical setup. Also, the sample preparation imposes certain constraints on these methods. For optimization of high-NA lens systems, industrial testing of objectives, and three-dimensional image restoration techniques[5] a real-time technique would be useful for lens characterization. In this Letter we demonstrate a new technique, the point-spread autocorrelation function (PSAF) technique, which provides a straightforward, accurate, and versatile alternative for real-time characterization of high-NA lenses. First we introduce the new functional dependence in general terms, after which we demonstrate the capabilities of the technique, using theoretical modeling and experimental verification.

In the novel PSAF technique we introduce a new function, $G(\Delta\mathbf{r}, \tau)$, for the characterization of optical lens properties based on the measurement of the autocorrelation of the focal field of a (high-NA) lens:

$$G(\Delta\mathbf{r}, \tau) = \int_{-\infty}^{\infty} \mathrm{d}t \int_{-\infty}^{\infty} \mathrm{d}\mathbf{r}|u(\mathbf{r}, t) + u(\mathbf{r} + \Delta\mathbf{r}, t + \tau)|^2,$$

$$(1)$$

where $u(\mathbf{r}, t)$ denotes the electromagnetic field in space and time behind the aperture of the lens, $\Delta\mathbf{r}$ is a spatial shift, τ is a temporal delay, and $|\ |^2$ denotes the complex square. To this end one part of the focal field—denoted object in what follows—is shifted in both space and time with respect to a duplicate part, which is denoted reference. The value of $G(\Delta\mathbf{r}, \tau)$ in Eq. (1) is measured through absorption of the radiation by a fluorescing dye and the subsequent detection of the total fluorescence, integrated over space and time, emitted by the sample. Applying a periodic variation on the time delay τ of the object focal field with respect to that of the reference induces alternating constructive and destructive interferences at the absorption level and causes an oscillating (in time) fluorescence intensity to be emitted by the sample. The magnitude of these fluorescence oscillations is related to the phase relation between the two fields, whereas the frequency of the oscillations relates to the number of times per second that the phase between the interfering fields alternates. The measured PSAF signal is given by

$$I_{\mathrm{PSAF}}(\Delta\mathbf{r}) = \mathrm{Max}[G(\Delta\mathbf{r}, \tau);\ \tau \in (0, \lambda/c)]$$
$$- \mathrm{Min}[G(\Delta\mathbf{r}, \tau);\ \tau \in (0, \lambda/c)], \quad (2)$$

where Max[ ] and Min[ ] denote the maximum and the minimum, respectively, of the total space-integrated fluorescence intensity detected for temporal delays τ between the object and the reference focal field within the range of 0 to λ/c (i.e., a relative phase shift between 0 and 2π).

The novelty and also the basis of the PSAF technique presented here are the introduction of the spatial shift $\Delta\mathbf{r}$ of the object focal field with respect to that of the reference. To understand the influence of spatially shifting one of the focal fields with respect to the other one should note that high-NA lenses produce a focal intensity distribution characterized by the diffraction-determined PSF $h_{\mathrm{exc}}(\nu) = \mathrm{const.}|2J_1(\nu)/\nu|$, where $J_1(\nu)$ is the first-order Bessel function, $\nu = 2\pi r\mathrm{NA}/\lambda_{\mathrm{exc}}$, r is the lateral distance from the geometrical focal point, NA is the numerical aperture of the lens, and λ_{exc} is the wavelength of the excitation field. This distribution is characterized by a strong central peak accompanied by several sidelobes, with π phase jumps in the lateral direction at every node. Spatially shifting one of the focal fields with respect to the other thus affects their relative phase relation
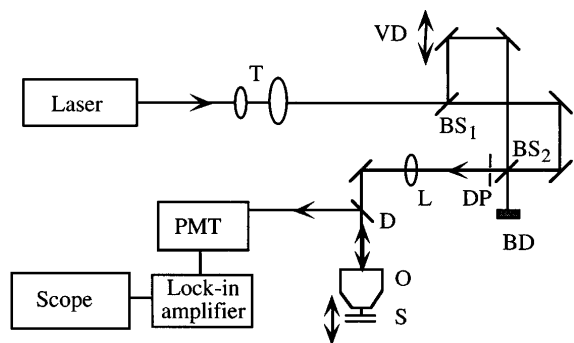
Fig. 1. Schematic of the experimental the setup for PSAF measurements: Laser, 25-mW argon-ion laser; T, telescope; VD, variable-delay line; BS_1, 50% beam splitter: BS_2, 50% beam splitter with motorized tilt control; DP, diaphragm; BD, beam dump; L, 200-mm lens; D, dichroic mirror; O, objective; S, sample on a variable-height translation stage; PMT, photomultiplier tube; Lock-in amplifier, PAR Model 121; Scope, LeCroy 9361 digital scope.

across the total distribution. By monitoring the magnitude of the fluorescence intensity oscillations, which reflect the spatial coherence of the two focal fields, as a function of the relative focal shift, a functional dependence (denoted PSAF) is measured that provides information on the characteristics of the lens in a way similar to that by the PSF.

In our experimental setup (Fig. 1), a small fraction (0.1 mW) of the output of a 25-mW argon-ion laser (Spectra-Physics) operated at 488 nm passes a telescope to enlarge the beam waist to 6 mm and make it parallel. The beam is then split by a 50% beam-splitter (BS_1). One of the beams, the object beam, passes a variable-delay line driven by a home-built shaker unit before being recombined with the other beam, the reference beam, on a second beam splitter (BS_2). We induce a lateral spatial focal shift $\Delta \mathbf{r}$ in one dimension and without additional axial shift of the object focal field with respect to the reference focal field by varying the tilt of the second beam splitter, which is controlled by a stepper motor with a range of 3.5 mrad in 500 steps, over one axis of orientation. The recombined beams are focused into the sample by the objective to be tested. A three-element compound objective (Leitz oil Plan 100, NA 1.25) was used in these demonstration experiments under appropriate tube length conditions and with the recombining beam splitter positioned telecentrically to ensure a beam-tilt-independent aperture filling. The fluorescence that results from single-photon absorption is detected by a photomultiplier tube after the scattered light is blocked with an OG530 filter. The sweep of the variable-delay line is such that the relative phase changes span a range of approximately 250π with a frequency of 19 Hz, inducing fluorescence interference oscillations at 4.8 kHz. The magnitude of these oscillations is measured with a lock-in amplifier unit. The output of the lock-in amplifier is digitized by an oscilloscope. The sample consists of a 10^{-4}-M Rhodamine 6G solution in water contained in a 0.1-mm microslide (Vitro Dynamics, Rockaway, N.J.)

In the theoretical model used to describe the PSAF technique the various focal field distributions are

calculated from the first Rayleigh–Sommerfeld integral of scalar diffraction in the Kirchhoff approximation[6] and by use of the shift-invariance approximation. Figure 2 shows the calculated PSAF and (intensity) PSF responses for various NA conditions; only the NA of the objective and the wavelength of the radiation are used as input parameters. We first note the apparent similarity of the theoretical PSAF curves with the well-known PSF responses to the nodes of the PSAF found at lateral positions close, but not necessarily identical, to those of the PSF. The background of the similarity between the PSAF and the PSF, which unfortunately cannot be understood from a simple physical picture, will be investigated further.
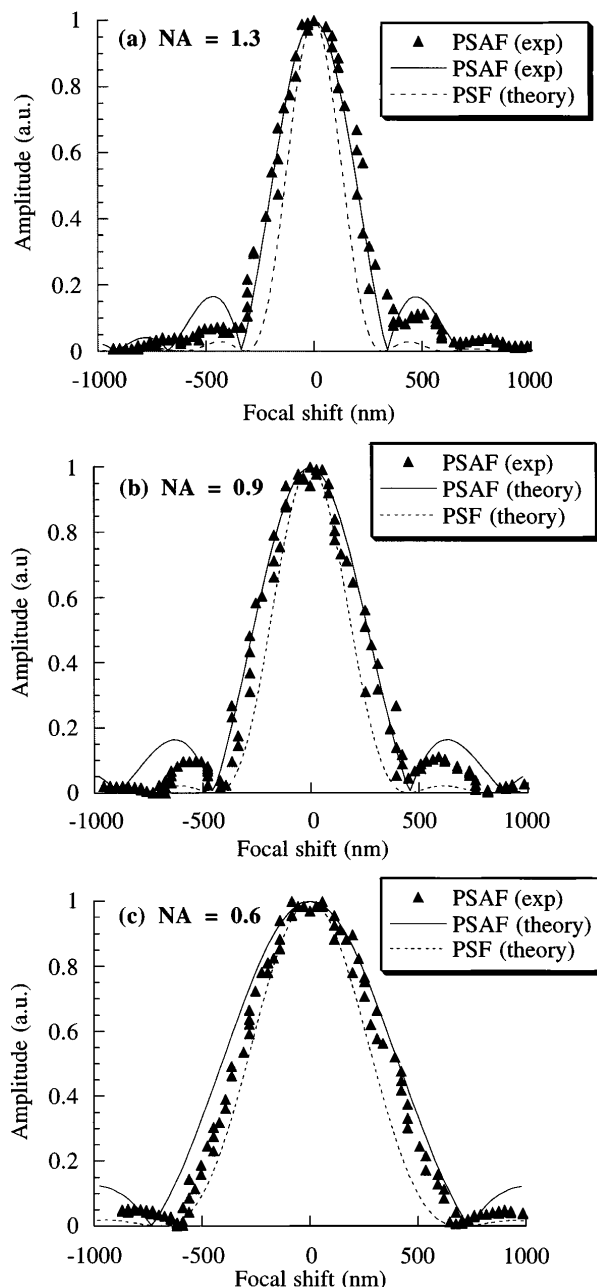

Fig. 2. PSAF for three different values of the effective NA of the objective: (a) 1.3 (± 0.04), (b) 0.9 (± 0.05), (c) 0.6 (± 0.05).

Also shown in Fig. 2 is the experimental verification of the PSAF technique. In these first experiments a separate measurement was made at each focal shift. Since then the method has been improved to provide the PSAF responses in real time. The induced focal shift is calculated from the measured tilt of the recombining beam splitter (BS_2). To obtain responses at different NA's, we changed the effective NA of the objective by adjusting the beam waist with a diaphragm placed directly behind the recombining beam splitter. Note that there is excellent agreement between the experimental data and the theoretical curves without the use of any fitting parameters. As a further indication of the potential of the PSAF technique for lens characterization we note that the width of the observed PSAF response broadens as expected when the effective NA of the objective is reduced.

The PSAF technique presented in this Letter is both simple and versatile. Because the measurement is performed in a bulk solution and with real-time speed, there are no strong requirements on either the sample preparation or the long-term optical stability of the setup, making the technique a powerful tool for the testing and alignment of microscopic objectives and other optical systems. Although this is not demonstrated experimentally in this Letter, but as follows from theoretical modeling, the technique is capable of measuring the full three-dimensional shape of the intensity distribution of a high-NA lens by a combination of a lateral and an axial focal shift. This information can be used either for system optimization or for image restoration techniques[5] and may be the basis for quantitative analysis and testing of microscope objective. The technique's sensitivity to small changes in the optical parameters indicates its great potential for comparative high-NA lens testing. One may expect also that, because the amplitude and phase distribution around the focal point of a high-NA lens is sensitive to aberrations (spherical, chromatic, coma, etc.),[7] the technique will be capable of providing data for de-tailed lens characterization. We are currently investigating these capabilities.

Although the measurements presented in this Letter refer to a high-NA system, the technique is suited principally for the characterization of any lens or combination of lenses. Also, the technique may be combined with the technique developed earlier of two-photon absorption autocorrelation[8] for the analysis of focal excitation and imaging conditions in two-photon absorption experiments employing femtosecond laser systems.

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