Correcting spherical aberrations induced by an unknown medium through determination of its refractive index and thickness

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Abstract: In imaging and focusing applications, spherical aberration induces axial broadening of the point spread function (PSF). A transparent medium between lens and object of interest induces spherical aberration. We propose a method that first obtains both the physical thickness and the refractive index of the aberration inducing medium in situ by measuring the induced focal shifts for paraxial and large angle rays. Then, the fourth order angle dependence of the optical path difference inside the medium is used to correct the spherical aberration using a phase-only spatial light modulator. The obtained measurement accuracy of 3% is sufficient for a complete compensation as demonstrated in a model microscope with NA 0.3 with glass plate induced axial broadening of the PSF by a factor of 5.

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1. Introduction

Specimen induced spherical aberrations are known to severely degrade the point spread function (PSF) of high numerical aperture (NA) systems in imaging and focusing applications. A planar refractive index mismatch is introduced by a suspension medium, a glass cover slide or imaging into a thick transparent specimen, thereby inducing a focus shift and spherical aberrations. This reduces the image contrast and transversal resolution and degrades the axial resolution [1,2].

Several correction methods have been proposed to eliminate spherical aberrations. They include correcting the focus shift using an adjustable path length in optical coherence tomography [3], objective coupled illumination in microscopy [4], wave front corrections of the scanning beam in two-photon microscopy [5] or changing the microscope conditions by changing the immersion oil, tube length, objective or cover glass thickness [6,7]. For an unknown aberration inducing medium, iterative processes with adaptive feedback loops have been considered. Such adaptive aberration corrections contribute to optimizing the image quality without the a priori knowledge of the characteristics of the aberration source [8–11]. For a priori known parameters of the aberration inducing medium, i.e. the refractive index and the physical thickness, best results are obtained from geometrical calculations of the path length difference [12–14].

In order to characterize the medium, a standard measurement of the physical thickness and refractometry can be used for large, removable objects such as glass slides or fluids. Thereby unwanted alterations can occur in the sample when changing from the characterization system to the application. Furthermore, if the medium is a part of the specimen under investigation or the sample is prepared and fixed with the aberration inducing medium, these techniques do not work, and a different set of characterization methods have to be applied. Since two measurands need to be quantified, at least two measurements under different conditions or two independent measurement systems are needed. The optical thickness can be measured with interferometric methods such as digital holographic microscopy. However, the optical thickness, being the product of the physical thickness and the refractive index, cannot be separated without an additional measurement or knowledge of one of the quantities. An independent measurement of the physical thickness can be obtained, for example, with tomography [15] or with a reflective confocal measurement system [16]. By assuming a spherical shape of cell structures [17] additional information is gained by letting single cells flow through microchannels [18] or Fabry-Perot cavities [19]. To our knowledge, only a few methods yet exist, that can extract the physical thickness and the refractive index
simultaneously in a microscopic arrangement. Both values are obtained in a separate non-
microscopic system. Ellipsometry is able to analyze thin films [20], white light interferometry
obtains the group index and thickness of transparent media [21,22] and a Mach-Zehnder
multiwavelength interferometer can generate a 2D map of the refractive index distribution and
the thickness profile [23]. Further, an equivalent approach was reported whereby the
immersion medium of a digital holographic microscope was changed to obtain a set of optical
thickness maps, from which the refractive index and physical thickness was calculated [24].
The disadvantage of all these methods is that they involve an extensive additional
measurement system before the target application of focusing or imaging can be improved.

We present a new method based on a set-up that is integrated in the very same lens system
that is used in the imaging or focusing application, without the need of moving or remounting
the sample. To prove the principle, we introduce a planar refractive index mismatch into a
PSF measurement system. The first step of our method characterizes the aberration inducing
medium. Two independent measurements are taken using the non-linear dependency of
spherical aberration on the incident angle. Using geometrical optics we are able to calculate
the refractive index and physical thickness of the planar medium. The information thus
obtained is sufficient to evaluate the phase function needed for correcting spherical aberration.
In the experimental set-up, this is performed with a phase-only spatial light modulator (SLM).

We first derive the theoretical concept that relates the refractive index and the physical
thickness of an aberration-inducing medium to the focus shift. Then the PSF measurement
system based on the use of a set of apertures is presented. The experimentally obtained results
for different glass plates used to model the planar refractive index mismatch are compared to
the known values for accuracy estimations. We also show that our method is able to get good
results even if the lens system is not diffraction limited, but includes aberrations. Finally, we
use the derived equations to correct the planar refractive index mismatch with a SLM and
present results for the optimized focusing.

2. Characterization of the aberration-inducing medium based on spherical aberration

A planar refractive index mismatch is shown in Fig. 1. Depending on the propagation
direction of the light, this represents a focusing or imaging application. The microscope
objective focuses or gathers the light through a glass plate with refractive index \( n \) and
thickness \( d \). Refraction by the glass plate induces a focal shift, which is defined by
geometrical optics and Snell’s law. The maximum incident angle \( \alpha_{0\ max} \) is related to the
numerical aperture of the objective and the refractive index \( n_0 \) of the surrounding medium.

![Fig. 1. Scheme of a planar refractive index mismatch with thickness \( d \) (MO: microscope
objective; O.A.: optical axis). Parameters are explained in the text.](image-url)
In order to focus into the medium at depth \( d \), the objective must be brought closer to the interface. From geometrical optics it follows that the new distance \( z \) between the objective and the glass surface is

\[
z = \frac{\rho - h}{t_0} = \frac{\rho - d \cdot \tan \alpha}{t_0} = z_0 - d \tan \alpha
\]  

(1)

where \( \rho \) is the incident beam height in the lens plane with radial symmetry, \( h \) is the intercept height, \( t_0 \) is the tangent of the incident angle \( (t_0 = \tan \theta_0 = \rho/f) \) and \( \alpha \) is the beam angle after refraction. The \( \tan \alpha \) term can be reformulated using trigonometric relations and Snell’s law so that the axial shift \( \Delta z \) that has to be applied to the objective to focus into the glass at depth \( d \) is obtained by

\[
\Delta z = z_0 - z = \frac{n_0 \cdot d}{\sqrt{n^2 + t_0^2 \left( n^2 - n_0^2 \right)}}
\]  

(2)

The focal shift \( \Delta f \) itself is then given by

\[
\Delta f = d - \Delta z = d \left( 1 - \frac{n_r}{\sqrt{1 + t_0^2 \left( 1 - n_r^2 \right)}} \right)
\]  

(3)

where \( n_r = n_0 / n \) is the ratio of the refractive indices. We expand Eq. (3) to the second order in \( t_0^2 \) and neglect terms of the order \( t_0^6 \) and higher. The final equation for the focal shift becomes

\[
\Delta f \approx d - d n_r \left[ 1 - \frac{1}{2} t_0^2 \left( 1 - n_r^2 \right) + \frac{1}{8} t_0^4 \left( 1 - n_r^2 \right)^2 \right] =: \Delta f_{MR}
\]  

(4)

For marginal rays (MR) with large incident angles occurring in systems with high numerical apertures, the full Eq. (4) has to be considered to describe the focal shift \( \Delta f_{MR} \). In case of paraxial rays, the tangent terms can be neglected, and the focus shift becomes \( \Delta f_{PAR} = d - d n_r \).

To determine the physical thickness \( d \) and refractive index \( n \) of the medium, we measure \( \Delta f_{PAR} \) and \( \Delta f_{MR} \). We used a microscope objective with high numerical aperture in combination with a set of apertures allowing for a controllable selection of optical rays. An iris diaphragm that only transmits the paraxial rays is used to measure the focus shift \( \Delta f_{PAR} \). A dark-field diaphragm with known \( t_0 \) that blocks all inner rays and transmits only rays at large angles is used to measure the marginal ray focus shift \( \Delta f_{MR} \). By dividing the respective equations, the physical thickness \( d \) is eliminated and the resulting Eq. (5) can be solved numerically for the refractive index \( n_r \),

\[
\frac{\Delta f_{MR}}{\Delta f_{PAR}} \approx 1 - n_r \left[ 1 - \frac{1}{2} t_0^2 \left( 1 - n_r^2 \right) + \frac{1}{8} t_0^4 \left( 1 - n_r^2 \right)^2 \right]^{-1}
\]  

(5)

After solving for the refractive index \( n_r \), the physical thickness \( d \) can be evaluated using the paraxial focus shift \( \Delta f_{PAR} \).

3. PSF measurement system

The experiments are performed with a home-built PSF measurement system (Fig. 2) combined with a phase-only spatial light modulator (SLM) [25]. A green laser beam (Nd:YAG Crystalaser, wavelength 532 nm) is expanded and its polarization set along the axis of the reflective liquid crystal SLM (Pluto from Holoeye, 1980x1080 pixels). The reflected beam is passed through a 10x objective (Nikon) with NA = 0.3 and infinity correction. The distance between the SLM and the 10x objective was set to 60 mm, the shortest possible in our configuration. Its focal plane is magnified with a 100x objective (EO) with a much higher

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numerical aperture of 0.9 onto a CCD camera (Prosilica, 2 Mpix). The complete image analyzer is mounted on a translation stage with a step size of 100 nm allowing for a full measurement of the 3D PSF by scanning through the focus.

For the planar refractive index mismatch, we introduced different glass plates (Thorlabs) between the two objectives: calcium fluoride (CaF$_2$, $n = 1.43$, $d = 5.25$ mm), BK7 ($n = 1.52$, $d = 5.35$ mm), sapphire (Al$_2$O$_3$, $n_{ord} = 1.77$, $d = 5.00$ mm), and a quartz glass ($n = 1.52$, $d = 8$ mm). Their refractive indices were identified at a wavelength of 532 nm. Their thickness was measured with a calliper with a measurement accuracy of 0.05 mm, which corresponds to 1% of the thickness $d$. Each glass plate was evaluated independently to determine the measurement accuracy of our method. It is important that the focused beam travels completely through the glass to ensure the full thickness effect. This also avoids spherical aberrations or scattering at the back side, which might influence the observation by the 100x MO.

Fig. 2. Experimental setup to focus into a glass medium (BS: beam splitter, CCD: camera, SLM: spatial light modulator, MO: microscope objective).

Three different apertures were used to vary the incident angle. The aperture allowing for full field illumination (FF) comprises the full lens diameter ($\rho = 1$). Paraxial illumination (PAR) is generated using an iris aperture with normalized radius of 0.2, while marginal rays (MR) at large angles are obtained with a ring aperture with inner and outer normalized radii of 0.9 and 1, respectively. The apertures were produced by first depositing a silver film on a glass plate and then by removing it from the respective regions by UV laser ablation. With each aperture, the intensity peaks were measured through the focus and fitted by a Gaussian function to obtain the position of the maximum, followed by the determination of the axial focus shift. The actual value of the tangent $t_0$ for the MR aperture was measured in the far-field.

4. Characterization results for the glass plates

The axial intensity distribution obtained for each of the three apertures is presented in Fig. 3. The maxima of the reference peaks (Fig. 3 left), i.e. the ones obtained with the objective used in air, are given for the apertures associated with FF, MR and PAR illuminations, respectively. They are almost at the same position, indicating that the objective is well corrected for spherical aberrations. The peaks for PAR and MR illuminations are wider as compared to the one obtained for FF illumination, as the apertures reduce the effective NA. The CaF$_2$ FF measurement contains spherical aberrations, which in our case contributes to the widening of the axial peak width by a factor of 2 (Fig. 3 right). The peaks for the PAR and MR illuminations are separated, each lying at one end of the FF intensity distribution. The focal shift for the MR illumination is larger, which is consistent with Eq. (4).

The characterization results for all glass plates are summarized in Table 1. Inserting the results into Eq. (5) and solving by Matlab led to the values of the refractive indices and the physical thickness of the glass plates. From the focal shifts measured for the PAR and MR illuminations, the calculated values for the refractive index and the thickness are presented together with the percentage deviation.

The transversal FF focal spot size of the reference was measured to be 1.04 $\mu$m, while the Rayleigh resolution of a NA 0.3 system predicts it to be 0.9 $\mu$m at 532 nm wavelength. The measurement value is close to the theoretical value, indicating that the set-up is operating
close to the diffraction limit, which is also seen by the fact that the paraxial as well as the non-
paraxial rays are focused at almost the same axial position. Our measurements of the axial
position of the maximum intensity are reproducible to half a µm, which is equivalent to
introducing less than half a percent of error to the calculated values of the refractive index and
physical thickness of the glass plates. Comparing the measured results with the known values,
we obtain a measurement accuracy of better than 3%.

We have also tested the method in cases where the system is not aberration free. In this
case it is important to measure the focal shifts for each type of aperture relative to the
respective reference measurement, i.e. the focus shifts of the PAR and MR illuminations are
measured relative to the focal position of the PAR and MR values of the reference. Then, the
resulting shifts represent the additional effect of the refractive index mismatch, because the
system aberration is apparent in the shifts of the PAR and HA peaks of the reference. To
induce aberration into the system, a finite 10x objective from Nikon for 160 mm DIN tube
length was illuminated from infinity. Since the NA changed compared to the normal usage,
the tangent had to be measured beforehand in the far-field. The results using this system
yielded an accuracy of around 2-3% for the evaluation of the refractive index and the physical
thickness. It thus proves that it is possible to characterize the additional aberration source
independently from the aberration inherent in the optical system.

Fig. 3. Axial intensity measurement results for the reference (left) and for CaF$_2$ (right). (FF: full
field illumination, MR: marginal ray aperture, PAR: paraxial iris aperture)

Table 1. Results for different glass plates obtained with a 10x MO, NA 0.3 system.

|       | CaF2 | BK7 | Quartz | Sapphire |
|-------|------|-----|--------|----------|
| n (532 nm) | 1.453 | 1.519 | 1.52 | 1.770 |
| d (calliper) [mm] | 5.25 | 5.35 | 8.00 | 5.00 |
| Δf$_{PAR}$ [mm] | 1.5969 | 1.8092 | 2.7671 | 2.1757 |
| Δf$_{MR}$ [mm] | 1.6760 | 1.8944 | 2.8439 | 2.2377 |
| n (calculated) | 1.454 | 1.506 | 1.534 | 1.812 |
| % dev | +1.3% | −0.9% | +0.9% | +2.4% |
| d (calculated) [mm] | 5.12 | 5.38 | 7.95 | 4.86 |
| % dev | −2.5% | +0.7% | −0.6% | −2.9% |
5. Aberration correction with a phase SLM

After obtaining the necessary information on the medium an aberration correction is performed. The main idea is to convert the angle dependency of the focal shift to a radial dependency of the wavefront correction within the pupil plane. As the phase SLM is limited to a modulation depth of 2 pi radians, we cannot compensate for the full focal shift but only for the spherical aberration. We thus subtract the paraxial focal shift from the full focal shift for large angles (Eq. (5)) and wrap the phase function. In this case, only the angle dependent component of spherical aberration remains:

\[
\Delta f_{MR} - \Delta f_{PAR} = \frac{d}{\lambda} t_0^2 \left(1-n_r^2\right) - \frac{\rho d}{\lambda} t_0^4 \left(1-n_r^2\right)^2
\]

(6)

To obtain the corresponding phase correction, the focal shift difference has to be multiplied with the wave number \(k\) and the difference of the refractive indices; \(\phi(\rho) = k(n-n_0)(\Delta f_{MR}-\Delta f_{PAR})\). Converting the tangent of the incident angle to a radial dependency leads to the phase modulation function (Eq. (7)). Having measured the incident angle in the far-field for the three apertures relative to FF (\(\rho = 1\)), MR (\(\rho = 0.9\)) and PAR (\(\rho = 0.2\)) illumination, a linear dependency between the tangent \(t_0\) and the radius \(\rho\) in the 10x MO input plane was obtained to be \(\rho = 3.20 \times t_0\). The maximum value for the tangent is reached for the maximum value of the normalized radius, \(\rho = 1\), and becomes 0.313. This is in agreement with the specified numerical aperture of 0.3 for the MO which corresponds to \(t_0 = 0.314\).

\[
\phi(\rho) = \frac{\pi d}{\lambda} \left(1-n_r\right) \left(\frac{\rho}{3.2} \left(1-n_r^2\right) - \frac{\rho^4}{3.2^4} 4 \left(1-n_r^2\right)^2\right)
\]

(7)

Comparing the terms with Zernike’s coefficients, we note that while the square term induces a simple focus shift, the term containing the radius to the power of four represents the third order spherical aberration. We again ignore the pure focus shift and only correct the spherical aberration term with our phase SLM.

We demonstrate the correction of the spherical aberration for a 7.95 ± 0.05 mm quartz glass with refractive index of 1.534. The full 3D intensity distribution for the FF illumination is shown in Fig. 4 for three settings: focusing through air (Fig. 4a), through the glass plate (Fig. 4b), and idem with the spherical aberration corrected with the SLM (Fig. 4c). The reference PSF has a depth of field of 13 μm FWHM. After introducing the 8mm thick quartz glass, the depth of field was increased to 66 μm because of the induced aberration. By applying the aberration correction obtained from the focal shifts, we were able to compensate for the spherical aberration completely and obtain again a PSF with a depth of field of 13 μm.
Additional numerical simulations showed that a thickness and refractive index accuracy of up to 5% is good enough to compensate more than 95% of the spherical aberration. This aberration compensating setup can be combined with other aberration corrections, for example asymmetric aberrations such as coma.

6. Conclusion

The paper describes a new dual step method based, respectively, on a controllable selection of optical rays (paraxial or marginal), and a phase-only spatial light modulator for measuring and correcting spherical aberrations induced by an unknown medium between lens and object of interest, for example, in a microscope setup. The method is reliable, does not require the remounting of the sample, and the aberration inducing medium can be characterized directly in situ in various imaging application systems. Another significant advantage of the method lies in its intrinsic ability to work accurately for systems with much higher numerical apertures.

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