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Harnessing abruptly auto-defocusing beam to enhance the Raman signal in aqueous humor: A simulation analysis

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A B S T R A C T

Raman aqueous humor detection provides a non-invasive, molecular-level approach for ingredient analysis within aqueous humor. However, current Raman aqueous humor applications are facing low signal-to-noise levels due to the trade-off between laser power and laser safety. In order to increase Raman signal while guaranteeing laser safety, in the research, we propose to use the abruptly auto-defocusing (AADF) beam as the illumination source for Raman aqueous humor spectroscopy. The ray-tracing sketch together with the propagation simulation of AADF shows its evolution within the aqueous humor. The intensity distributions are analyzed with and without the impact of corneal refraction. Results show that the efficiency of AADF is higher than the conventional focused Gaussian beam (FGB) method with center blocked. The peak value of the Raman signal intensity acquired using the AADF method is about 6 times larger than that of an FGB.

1. Introduction

The aqueous humor (AH) is a biofluid in the ocular system that provides nutrition to the anterior segment of the eye and removes metabolic wastes from the tissues herein. It is located in the anterior eye chamber, bordered by the cornea, the iris diaphragm, and the pupil, and contains mainly water and some biomolecules [1]. Imbalance of the molecular composition of these biomolecules can be indicative of systematic disease or dysfunction of the visual system. Hence, molecular profiling of the AH can reveal crucial information about these physiological and pathological changes.

There are several analytical methods to investigate the molecular composition of the AH [2]. For example, liquid chromatography (LC) can quantify AH samples with minimal sample pre-treatment. Its detection limit is able to reach the ng/mL level [3]. The mass spectroscopy shares the same advantages on the detection limit as that of LC but is easier to use and less expensive to deploy and operate than the LC [4]. The biggest shortcoming of these methods is that they need invasive biopsy sampling, and can increase the potential health risks, like inflammation and pain for patients.

As a non-invasive and non-destructive spectrometry-based technique, the applications of Raman spectroscopy in the identification and quantification of biomolecules enabled its role in various biomedical applications in ophthalmology [5]. Erckens et al. demonstrated the possibility of the Raman technique for identifying three materials of poly methylmethacrylate (PMMA), acrylic, and silicone as an intraocular lens in a patient [6]. They have also demonstrated the in-vivo evaluation of drug-induced cornea hydration changing by continuous monitoring water content in rabbit eyes [7]. Hosseini quantified the local concentration of intraocular drugs used for endophthalmitis by Raman spectroscopy [8]. Bauer et al. investigated ocular pharmacokinetics in rabbit eyes by a confocal Raman spectroscopy system [9].

Beside its applications in detecting ocular drugs and water content, Raman spectroscopy can also be employed to detect particular molecules and to discriminate between normal and diseased states. For example, Kaji et al. showed the capability of visualizing and localizing collagen fibers, proteins, lipids, and DNA/RNA in the rat cornea by coherent anti-Stokes Raman scattering [10]. Paluszkiewicz et al. reported the Raman spectrum from two human lenses after cataract surgery. They found an excess of tryptophan, tyrosine, phenylalanine, Beta-sheet, the cause being still under discussion [11]. In comparing healthy and cataractous human lenses with Raman spectroscopy, they found differences in the concentration of Tyr and Trp residues [12].

Erckens et al. reviewed Raman spectroscopy applications in ophthalmology [13] and showed that it can be used for the purpose of aqueous humor detection [14]. This idea was further substantiated by Lambert et al. [15], in which the glucose concentration in the AH is detected by Raman spectroscopy for diabetes diagnosing. Bertens and Zhang et al. explored the feasibility of Raman spectroscopy to measure ocular drug concentrations in living animals [3].

The applications of Raman spectroscopy on animal eyes are promising, but its applications on human eyes are facing a big challenge-the
trade-off between laser safety and high Raman signal. A traditional aqueous humor Raman spectroscopy setup can be regarded as a confocal system, as shown in Fig. 1(a). Laser light, which can be regarded as a collimated Gaussian beam, is focused by an objective lens into the chamber between the cornea and ocular lens. The AH herein is stimulated, producing back-scattered Raman signals to be measured by a detector. The Raman signal strength is proportional to the intensity of stimulating laser light. However, the focusing laser will pass through and be focused by the ocular lens, then further arrives at the retina, which is highly sensitive for light damage. In order to prevent the retina from being burned by laser, Zhang et al. proposed two designs with a dark-illumination approach and obtained ocular drug concentrations in animal eyes ex vivo [16,17]. An alternative solution is to place a black screen between the eye and objective lens or within the light pathway to block out part of the beam that will enter the pupil. Fig. 1(b) shows a ray-tracing sketch of this idea.

Unfortunately, such a screen will also block the back-scattered Raman signal, leading to a low signal-to-noise ratio. Hence the key issue to be addressed is how to enhance the Raman signal while preventing light damage in the retina. In other words, we need a beam that can be focused with a high-intensity spot at the focal point, with a decreased intensity thereafter. The aim of this paper is to propose an abruptly auto-defocusing beam to meet these requirements.

The abruptly auto-defocusing field (AADF) is an inverse propagating version of the abruptly auto-focusing beam (AAF) [18,19]. Fig. 1(c) shows a ray-tracing sketch using AADF for Raman spectroscopy in the AH. With the propagating of the AADF, the beams’ caustic surfaces become closer and closer and collapse at the cross-point of the caustic surfaces [19]. Optical waves interfere intensively at that point creating a high-intensity focal spot. After the focal spot, the beam’s caustic surfaces begin to separate, resulting in an abrupt decrease of intensity after the beam’s focal point and producing an intensity-empty area between the separated caustic surfaces. Considering that the caustic surfaces of an AADF beam can be pre-engineered using ray-optics [19–23], it is possible to design a special AADF beam in which the caustic surfaces have already separated enough at the pupil after it collapses at the chamber between the cornea and iris, such that no optical field will arrive at the retina.

2. Abruptly auto-defocusing field

Fig. 2 shows the Liou–Brennan eye model that we used in the following simulations [24]. It contains two spherical surfaces for the cornea, with a curvature of 7.8 mm for the outer layer and 6.4 mm for the inner layer. The center of the two spherical surfaces located on the z-axis, with a distance of 0.88 mm. The distance between the iris plane and the vertex of the inner layer is 2.6 mm.

For Raman spectroscopy, a laser beam needs to be focused inside the anterior eye chamber. Let us consider an optical beam focused on an objective lens with a given numerical aperture (NA). The distance from the geometrical focal point of the objective lens to the iris plane is . The geometrical focal point denotes a single cross point of light rays when focused by the lens, which is corresponding to the position of the focal spot of a focused Gaussian beam (FGB), as well as the auto-defocusing point of an AADF. Due to the diffraction of optical waves, the optical focal spot is located near the geometrical focal point.

According to the rotational symmetric of the eye model and both of the Gaussian beam and the AADF, we used a 3D cylindrical coordinates system to convert the 3D problem into a 2D situation, where the origin was placed at the geometrical focal point as shown in Fig. 2, . Due to the Fourier transform property of the objective lens, the output optical field, which propagates along the positive z-axis at any given observation point is given by the superposition of the
plane waves weighted by their angular spectrum [25,26]:

\[ U(r, \phi, z) = \int_{\theta_{\min}}^{\theta_{\max}} A_0(\theta) J_0(kr \sin \theta) \exp(ikz \cos \theta) \exp \left[ i\Phi(\theta) \right] \sin \theta d\theta \]  

(1)

where \( k = 2\pi / \lambda \) and \( \lambda \) is the wavelength. \( \theta \) is the angle between the direction of plane waves and the positive z-axis. \( A_0(\theta) \) is a real function that weights different components of the plane waves. \( J_0 \) is the zero-order Bessel function of the first kind. \( \Phi(\theta) \) is also a real function that denotes the phase difference of each plane wave. Different types of optical fields can be generated by adjusting \( \Phi(\theta) \). For an FGB \( \Phi(\theta) = 0 \), and for an AADF with circular caustics \( \Phi(\theta) \) is given by [27]:

\[ \Phi(\theta) = -kf \left( \theta \csc \theta_{\max} - \sin \theta \cot \theta_{\max} + \cos \theta \right) \]  

(2)

Here \( f \) is the parameter that controls the length of the position of the focal point of AADF. The half of the distance between the two caustics at the iris plane is given by [27]:

\[ R_1 = f \csc \left( \theta_{\max} \right) \cos \left\{ \arcsin \left( \frac{f - h}{f \csc \left( \theta_{\max} \right)} \right) \right\} - f \cot \left( \theta_{\max} \right) \]  

(3)

\( R_1 \) should not be smaller than the radius of the pupil. The optical aberration due to the corneal refraction is ignored since it can be compensated by adding an additional phase term to \( \Phi(\theta) \). The upper limit of the integration \( \theta_{\max} \) in Eq. (1) is given as \( \theta_{\max} = \arcsin(NA) \). The lower limit of integration, \( \theta_{\min} = 0 \) for the AADF. While for FGB, \( \theta_{\min} \) is determined by the block length and \( h \) due to the impact of the block. Let \( R_0 \) be the length of the block and \( d \) be the diameter of the pupil. We have \( R_0 > d / 2 \). \( \theta_{\min} \) can be calculated by \( \theta_{\min} = \arctan(R_0/h) \). According to Eq. (1), the NA of the objective lens must satisfy \( NA > \sin \left( \theta_{\min} \right) \) to ensure the illumination intensity is not zero for the case of FGB.

When the beam enters the anterior eye chamber, the substances at the focal spot are stimulated by the illumination field \( U(r, \phi, z) \) and will scatter Raman signal toward the objective lens. Raman scattering is an inelastic scattering and an incoherent process, in which photons are scattered in random directions [28]. The Raman detector is a barrel detector that detects the total intensity collected by the objective lens. For simplicity, we assume that the total intensity of the Raman signal collected by the objective lens from a stimulated point \((r_0, \phi_0, z_0)\) is proportional to the product of the illumination intensity \( U(r, \phi, z) \) and the objective lens. The objective lens collects all signals within the focal spot. Since the intensity of the focal spot peaks in the z-axis, we consider only the signal emitted from a single point at the peak of the intensity. The total Raman signal that is collected by the objective lens can thus be calculated using

\[ I_R \sim |U(r, \phi, z)|^2 \int_{\Omega} d\Omega \]  

\[ = 2\pi |U(r, \phi, z)|^2 \int_{\Omega} \arcsin \frac{NA}{\sin \theta} \sin \theta d\theta \]  

(4)

where \( \Omega \) is the solid angle measured from point \((r_0, \phi_0, z_0)\) to objective's aperture. Our purpose is to compare the value of \( I_R \) for both FGB and AADF. Although \( \Omega \) changes with the different positions of points on the z-axis, if we consider only the small region near the focal spot, the change of \( \Omega \) can be ignored compared with the aperture diameter of the objective lens.

3. Comparison between AADF and FGB

In the simulation part, we assume that \( h = 2300 \mu m, \lambda = 0.785 \mu m \), and the numerical aperture of objective lens \( NA = 0.58 \). The human pupil diameter is normally around 2-4 mm. However, the value of human pupil diameter in the simulation needs to be carefully chosen. On the one hand, the pupil diameter should not be very small such as 2 mm, 1 mm, or even smaller, since these cases are difficult to achieve and be stabilized in every practical experiment. On the other hand, the pupil diameter should not be very large, due to the limitation of NA of the objective lens — the smallest angle of inclination illumination component might exceed the NA. We found that \( d = 3 \text{ mm} (3000 \mu m) \) can be an appropriate situation for the diameter of the human pupil.

We ignored corneal aberrations since we assumed that these aberrations are already compensated for in the phase term in Eq. (1). The block length of FGB was taken just equal to \( d \). For AADF, we assumed that \( 2R_1 = d \). When the beam waist of the incident Gaussian beam matches the entrance pupil of the objective lens, \( A_0(\theta) = \exp(-\sin^2 \theta / NA^2) \) in Eq. (1). This value is also applied to AADF so that the total energy of FGB without block is equal to that of AADF. When the block is placed, the light energy of FGB within the range of the block is wasted.

Fig. 3(a1) shows the normalized 2D intensity distribution of an FGB at the iris plane. Due to the block, a light ring is formed which can be also used for alignment. The diameter of the center dark circle area is larger than that of the iris to prevent retinal light damage. Fig. 3(a2) shows the normalized 2D intensity distribution at the xoz-plane where \( y = 0 \). An FGB creates a focal spot at the focal point (origin).

Fig. 3(b1) shows the normalized 2D intensity distribution at the iris plane (\( z = 2300 \mu m \)) for the AADF, which is similar to Fig. 3(a1). Fig. 3(b2) shows the normalized 2D intensity distribution at the xoz plane for AADF. According to Fig. 3(b2), almost the entire surface of the cornea is illuminated by the AADF. Laser intensity is more concentrated at the vertex part of the cornea (\( x = y = 0 \)). In general, the intensity on the cornea surface is rather weaker than that of the focal spot, which is approximately located at \( z = -21.02 \mu m \). Different from Fig. 3(a2), the AADF shows an elongated focal spot in the z-direction as shown in Fig. 3(b3), resulting in a weaker peak intensity than that of FGB.

More detailed intensity distributions are shown in Fig. 4(a1) to Fig. 4(a3) for FGB. Fig. 4(a1) is the axial intensity distribution, which shows rapid focusing near the focal point. Fig. 4(a2) is the transverse distribution of the focal spot and Fig. 4(a3) the transverse distribution at the iris plane. The axial intensity distribution for AADF, Fig. 4(b1), shows an oscillating increase and reaches its peak near the focusing point (\( z = 0 \)). Hereafter the intensity rapidly decreases to zero with the beam propagating toward the positive z-axis. The transverse distribution of the focal spot is shown in Fig. 4(b2) which is similar to that in Fig. 4(a2). The transverse intensity distribution at the iris plane for AADF is shown in Fig. 4(b3), showing chirping-like oscillations, denoting the self-accelerating feature [22,29,30].

Comparing Fig. 4(a1) to Fig. 4(b1), the peak intensity of AADF is not as high as that of FGB. This can be also explained using the theory of ray optics where the light intensity is in inverse proportion to the distances of light rays [31,32]. For an FGB, light rays are tightly focused at the focal point, where the distance of all light rays is zero. For an AADF, light rays are not directly focused at the focal point, but tangent to the caustic surface. Due to the collapse of the caustic surface, the optical fields interfere at the collapse point creating the bright focal spot. At the collapse point, light rays are not as tight as that of directly focusing resulting in that the focal spot intensity of AADF is not as high as the FGB.

Although the illumination intensity for FGB is larger than that of AADF, the signal intensity of FGB is weaker than AADF as shown in Fig. 5(a) due to the presence of the block. According to Eq. (4), the signal intensity not only depends on the illumination intensity but also relies on the solid angle of \( \Omega \), within which the signal can be collected by the objective lens. For AADF, \( \Omega \approx 0.37\pi \) which can be directly calculated using the NA of the objective lens. While \( \Omega \approx 0.04\pi \) for FGB. Due to the block, a large part of Raman signals is removed and cannot be received by the objective lens. We also simulated the performance of AADF and FGB under different wavelengths and NA. Let \( I_{\text{RA}} \) and \( I_{\text{RF}} \) be the intensity of the Raman signal for AADF and FGB, respectively. With NA = 0.58 for the illumination beam, the ratio of Raman (\( I_{\text{RA}}/I_{\text{RF}} \)) signal between AADF and FGB increases with increasing wavelengths as shown in Fig. 5(b). Moreover, the signal strength of AADF is at least 5 times larger than that of FGB. This is due to the property of AADF of
Fig. 3. 2D intensity distribution for FGB and AADF. (a1) is the transverse intensity distribution of FGB at the iris plane. (a2) is the intensity distribution at the $xoz$ plane for FGB. (b1) is the transverse intensity distribution of AADF. (b2) is the intensity distribution of AADF at the $xoz$ plane. (a3) and (b3) are enlarged parts in the red box and green box in (a2) and (b2), respectively.

Fig. 4. Curves of intensity distribution for FGB and AADF. (a1) and (a2) are axial and transverse intensities for FGB near the focal spot. (a3) is the transverse intensity of FGB at the iris plane. (b1) and (b2) are axial and transverse intensities for AADF near the focal spot. The peak value of intensity for AADF is approximately located at $z = -21.02 \mu m$. (b3) is the transverse intensity of AADF at the iris plane.

which the peak intensity gets larger with the increase of wavelength as shown in Fig. 5(d).

For fixed $\lambda$, $I_{R1}/I_{R2}$ decreases with increasing NA. This can be explained as follows. First, the increase of NA will enhance the peak intensity of both AADF and FGB, however, FGB is focused more tightly than AADF, with its peak intensity also increasing faster than that of AADF as shown in Fig. 5(e), resulting in the decrease of $I_{R1}/I_{R2}$. Second, the increase of NA also enlarges the range of the solid angle that can be collected by the objective lens.

Fig. 5(f) shows the change of $I_{R1}/I_{R2}$ with respect to NA and $\lambda$. In general, the AADF will have better performance than FGB with a larger illumination wavelength and small NA. In addition, Raman signal efficiency of two alternative FGB configurations are discussed in the Appendix section.

4. Impact of the corneal refraction

In the above simulation, we ignored the impact of corneal aberration since we assumed them to be compensated in the phase term of Eq. (1). Corneal aberrations can be rather complex for individual subjects due to the difference of corneal curvature, thickness, and the mismatch of refractive index between cornea and air and cornea and anterior eye chamber. The combination of those intricate situations will make it hard to simulate the impact of corneal aberration.

However, we can simplify the analyses by considering only the case of mismatch of the refractive index, that is the impact of corneal refraction. This is a reasonable assumption due to the following reasons. First, the optical field of the incident beam at the front surface of the cornea mainly locates in a central circular area of the cornea due to the focusing properties of the incident beam. The radius of the circular is approximately equal to 1 mm. This can be also demonstrated
Fig. 5. Comparison of strength of Raman signal for AADF and FGB. (a) axial Raman signal for AADF and FGB. (b) and (c) are the ratio of Raman signal of AADF and FGB with different NA and \( \lambda \). (d) and (e) are changes of the peak intensity of AADF and FGB with different NA and \( \lambda \). (f) are \( I_{R1}/I_{R2} \) with respect to NA and \( \lambda \).

Fig. 6. Distribution of corneal aberrations. (a) aberrations for one subject. (b) and (c) are statistic distribution of corneal aberration for all 42 subjects in the red circle area through the ray-tracing method as shown in Fig. 7(a). Second, optical aberrations for the central part of the cornea can be ignored.

We verified this property by measuring corneal aberrations in 42 subjects using a Pantacam setup, a typical example of which is shown in Fig. 6(a) (512 by 512 pixels). Here the phase distribution is moded by \( 2\pi \). The Pentacam measures corneal aberrations of a circular area with a diameter of 6 mm. The red circle in Fig. 6(a) with a radius of 1.2 mm denotes the circular area of the incident beam. Fig. 6(b) shows the statistical distribution of corneal aberrations within the red circle in Fig. 6(a). Accordingly, the aberrations were mainly close to zero, meaning that the aberrations within the red circle can be regarded as uniformly distributed. The statistic distribution of corneal aberration for all 42 subjects is shown in Fig. 6(c). The result shows a similar distribution to that in Fig. 6(b).

The impact of the corneal refraction is demonstrated through ray-tracing as shown in Fig. 7(a1) for FGB and 7(b1) for AADF. Here we assumed 1.38 for the refractive index of the cornea and 1.33 for the anterior eye chamber [33]. The blocked range, \( R_0 \), for FGB and the parameter \( R_1 \) of AADF remained unchanged as in Section 4, where \( R_0 = R_1 = 1500 \, \mu \text{m} \). Further, \( h = 2300 \, \mu \text{m}, \lambda = 0.785 \, \mu \text{m} \) and NA = 0.58 for the objective lens. The direction of light rays can be determined using the geometrical relation that light rays are tangent to the caustics [31]. The direction of spatially refracted light rays can be calculated using the quaternion–rotation method [34] so that the change of optical path length can be numerically calculated according to ray-tracing, which further changes the value of the phase term \( \Phi(\theta) \) in Eq. (1).

Black dashed lines in Fig. 7(a1) denote the light rays that are blocked. Purple curves in Fig. 7(a2) are the caustics of AADF without the impact of aberrations. Due to the mismatch of the refractive index, the focal point is shifted about 321 \( \mu \text{m} \) away toward the positive \( z \)-axis. The diameter of the pupil should be further reduced to 2000 \( \mu \text{m} \) in order to prevent the retina from being damaged by light beams. The 2D intensity distributions for FGB and AADF are shown in Fig. 7(a2) and 7(b2). Fig. 7(a3) and 7(b3) show \( I_R \) along the \( z \)-axis of 7(a2) and 7(b2). The peak value of \( I_R \) for AADF (7(b3)) is about 6 times larger than that of FGB (7(a3)).

Although the position of the focal spot is shifted due to the mismatch of the refractive index, the profile of the intensity distribution hardly changes. If we ignore the change of refractive index with respect to wavelength, the relationship between peak \( I_R \), \( \lambda \), and NA is similar to the result in Section 4, i.e. the AADF has better performance than FGB with a larger illumination wavelength and small NA. Moreover, according to Fig. 7(b1) and 7(b3), the AADF still shows its abruptly defocusing property and can be used to avoid excitation light beams entering the pupil. Therefore, only Rayleigh or Mie scattered photons might reach the posterior section of the eye through the crystalline lens which is within the safe limit [35]. Compared with the strength of the Raman signal of FGB, the AADF is still able to enhance the signal strength up to 6 times.

5. Concluding remarks

In this study, we propose to use an abruptly auto-defocusing (AADF) beam to increase the Raman spectroscopy of the anterior eye chamber. Taking the advantage of the auto-defocusing feature of the AADF, a focal spot of high intensity is created inside of the anterior eye chamber. After that, the beam automatically defocuses along its caustic surface yielding an intensity-free circular area at the pupil plane, preventing the retina from being burnt by laser beams.
Fig. 7. Performance of AADF and FGB under the impact of mismatch of refractive index. (a1) and (b1) are ray tracing sketches for FGB and AADF. (a2) and (b2) are the 2D intensity distribution in $xoz$ plane. (a3) and (b3) are the detailed axial Raman signal in the $z$-axis for FGB and AADF. The black dashed lines in (a1) denote the blocked light rays. Purple curves in (b1) are the designed caustic for AADF without the impact of aberration.

The performance of the AADF is analyzed theoretically and compared with conventional focused Gaussian beam (FGB) approaches. The simulations are performed without and with the impact of the corneal refraction. The influence of wavelength and NA of the objective lens are also simulated. The results show that AADF has better performance than FGB, where the AADF produces a focal spot with about 6-fold higher intensity than that of a center-blocked FGB, which will largely increase the useful Raman signal for detection.

In a practical experiment, the AADF will not lead to additional complexity of optical aligning between the two centers of the pupil and the beam’s dark circular area than that of FGB. Both two methods face optical aligning, and unintended eye motion problems. The light rings on the pupil plane might be of help for the alignment procedure. In addition, the impact of irregular corneal shapes like keratoconus or cloudy cornea like keratitis also reduces the performance of traditional FGB and the proposed AADF methods. At the current stage, the main challenge of applying AADF to the aqueous humor Raman detection remains on how to produce such an optical field after the objective lens. Using a spatial light modulator or a phase mask could be good solutions.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix. Signal efficiency for raman system with a beam splitter or apertured mirror

In order to get rid of blocking on the detection path, we used the two optical layouts shown in Fig. A.1. In this appendix, we briefly analyze their signal efficiencies, advantages and disadvantages according to numerical simulations and experimental experiences.

In the first layout in Fig. A.1(a), a beam splitter is used to combine the detection path and illumination path into the same optical path. In this case, the beam splitter will reduce the light intensity. For example, the beam splitter has 50% penetration and 50% reflection. Thus, the intensity of the detection light is actually decreased by 75%. In this case, the signal collection angle is the same as that of AADF, but the light intensity is multiplied by 0.25. The simulation result is shown in Fig. A.1(d) in the red curve. The signal intensity is larger than that of blocked FGB but is still much weaker than that of AADF.

For the second layout shown in Fig. A.1(b), the beam splitter is replaced by an apertured mirror. This layout is better than that in Fig. A.1(a) since the intensity will not be separated. However, due to the presence of the apertured mirror, the beam width in the detection path is tailored by the mirror’s aperture. In an ideal situation, as shown in Fig. A.1(c), the beam width in the detection path is equal to the size of the pupil’s projection circle at the objective lens plane. Thus, with an upper integral limit in Eq. (4) of \( \arctan \left( d/2h \right) \) and a lower integral limit of 0, the simulation results in the blue curve in Fig. A.1(d), which is slightly larger than that of AADF.

The key issue for the apertured mirror in layout Fig. A.1(b) is the alignment of the system, as misalignment can cause the excitation laser to pass through the pupil and damage the vulnerable retina. Extra efforts need to be paid for the apertured mirror alignment in its three degrees of freedom (say, the three components of its Euler angles). In addition, since the mirror must be properly installed for both the illumination path and the detection path, the difficulty in aligning is doubled compared to the direct illumination setting.

References