

A universal framework for microscope sensorless adaptive optics: Generalized aberration representations ^{EP}

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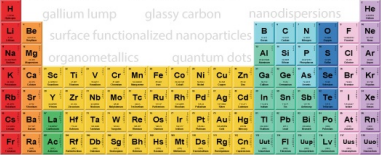
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ABSTRACT

Adaptive optics (AO) methods are widely used in microscopes to improve image quality through correction of phase aberrations. A range of wavefront-sensorless AO schemes exist, such as modal, pupil segmentation zonal, and pixelated piston-based methods. Each of these has a different physical implementation that makes direct comparisons difficult. Here, we propose a framework that fits in all sensorless AO methods and facilitates systematic comparisons among them. We introduce a general model for the aberration representation that encompasses many existing methods. Through modeling and experimental verification in a two-photon microscope, we compared sensorless AO schemes with a range of aberration representations to correct both simulated and sample induced aberrations. The results show that different representations can provide a better basis for correction in different experimental scenarios, which can inform the choice of sensorless AO schemes for a particular application.

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I. INTRODUCTION

Adaptive optics (AO) is often used in microscopes to compensate phase aberrations introduced by optical inhomogeneities in specimens and to restore near-diffraction-limited operation.^{1–4} This is achieved by estimating aberrations through the use of a wavefront sensor or through indirect optimization approaches. Instead of measuring phase aberrations directly, wavefront-sensorless aberration correction methods (known concisely as “sensorless AO”) infer aberration correction from a set of image measurements that are obtained when intensity and/or phase perturbations are introduced into the system. Sensorless AO methods are commonly used because they require no extra hardware for direct wavefront sensing and hence allow simpler optical designs and avoid non-common path sensing errors. There are also cases where the signal level is too low for sensor-based methods to be feasible, leaving sensorless methods the only viable option.

Many sensorless AO approaches have proved to be effective on various types of microscopes. These include the modal method,^{5–13} pupil segmentation zonal methods,^{14–17} and pixelated piston-based methods.^{18–20} Unfortunately, no study has systematically compared, discussed, and summarized the trade-offs of these methods since different approaches were all developed separately for different applications, meaning a fair comparison among the methods was difficult to conduct. Furthermore, the apparent conceptual separation between these correction schemes meant that there were no practical guidelines to advise AO users about choosing the more effective method for a certain application.

In this paper, we introduced a universal framework in which all of these sensorless AO methods could be interpreted. The framework requires the definition of three components: an aberration representation, an optimization metric, and an estimation algorithm. We show that one of the main differences between a range of common sensorless AO methods (such as the three approaches we

mentioned above) lies in the representation basis used for aberration correction. We hence used this framework to conduct a fair, side-by-side comparison between the various bases. We further showed that the aberration representations used in the common sensorless AO approaches were in fact special cases from a broader spectrum of using a hybrid zonal/modal representation. We explored the effectiveness of these different bases when correcting aberrations of different frequencies and amplitudes under different signal to noise ratios (SNRs). By systematically investigating this broad spectrum of aberration representations, we not only proposed guidelines for the selection of sensorless AO schemes but also provided possibilities for other sensorless correction schemes that could be tailored to particular applications.

II. A UNIVERSAL SENSORLESS FRAMEWORK

We present in this section a generalized framework that is broad enough to encompass all published image-based sensorless AO systems. The only measurement available to a sensorless AO scheme is the image, which consists of an ordered collection of intensity measurements from the imaging plane. Using fluorescence microscopy as an example, the image output of the microscope is a fluorescence intensity distribution, which has a mathematical expression of a convolution between the point spread function (PSF) of the system and the fluorophore density of the sample. Since the effects of phase aberration are confined to the PSF²¹ and normally sample structures are unknown, more than one measurement is commonly required to deduce the PSF unambiguously. The typical SNR in microscopes makes the retrieval of phase even more challenging. Therefore, all existing variations of the sensorless AO methods require in practice multiple exposures. Each of these exposures is obtained with a different perturbation to the system, which is typically in the form of a pre-determined bias phase aberration introduced by AO devices. This set of measurements is then used to optimize a metric that quantifies the quality of each recorded image²² (Fig. 1). Mathematically, this process can be expressed as an optimization problem in the

form

$$\operatorname{argmax}_{\mathbf{a}} \{f[\mathbf{O}, \Psi(\mathbf{a})]\}. \quad (1)$$

Here, f is chosen to reflect the image quality and ideally should have a clearly defined global optimum that corresponds to diffraction limited operation. This expression can be interpreted as finding an optimized value of the parameter vector $\mathbf{a}$, representing the aberrations, which maximizes f . The metric f defines the quality of the output images, and in many cases, f could be expressed explicitly as a function of the object structure, $\mathbf{O}$, and the microscope PSF, which in turn is a function of the total aberration $\Psi(\mathbf{a})$.²¹ Ψ is a function of $\mathbf{a}$, which can be expressed as

$$\Psi(\mathbf{a}) = \Phi + \sum_i^N a_i \varphi_i = \Phi + \mathbf{a} \cdot \boldsymbol{\varphi}, \quad (2)$$

where Φ is the combination of system and sample induced aberrations that need to be corrected. The summation term represents the aberration correction imparted by a wavefront shaping device: φ_i is the i th mode of a set of aberration representation modes, a_i is the coefficient of the mode φ_i , and N is the total number of modes used in the correction. $\sum_i^N a_i \varphi_i$ is also written as the scalar product of $\mathbf{a} \cdot \boldsymbol{\varphi}$ where the vectors $\mathbf{a}$ and $\boldsymbol{\varphi}$ contain all a_i and φ_i components up to order N , respectively, such that the i th entry of $\mathbf{a}$ is a_i and the i th entry of $\boldsymbol{\varphi}$ is φ_i . Note that Φ , Ψ , and $\boldsymbol{\varphi}$ are all phase functions defined over the pupil of the system.

Adaptive aberration correction is often considered as a process that minimizes the variance of the wavefront error in the pupil. In practice, however, the system would usually minimize the variance of the wavefront error modulo 2π radians. The optimization process in Eq. (1) should be an alternative way to achieve the same effect. Considering the nature of the wavefront error provides a way to understand the limitations of correction schemes.

If Φ contains only the first N aberration representation modes $\boldsymbol{\varphi}$, it is fully correctable by the adaptive element. However, in general, Φ will include modes that are outside of the set $\boldsymbol{\varphi}$ and so cannot be corrected fully. In many practical situations, it is assumed that we can find an optimal solution for Φ such that $\Phi + \mathbf{a}_0 \cdot \boldsymbol{\varphi} = \epsilon$, where $\mathbf{a}_0$ is a set of optimized coefficients of modes $\boldsymbol{\varphi}$ and ϵ is the remaining non-correctable aberration that is outside and orthogonal to the set $\boldsymbol{\varphi}$ and should be small enough to be neglected. Therefore, it is important to minimize the size of ϵ , which can be done not only by determining the optimal coefficients in $\mathbf{a}_0$ but also by the optimal choice of the aberration representation modes $\boldsymbol{\varphi}$.

The above formulation provides the basis for the universal framework. All approaches contain three major components—aberration representation modes ($\boldsymbol{\varphi}$), an optimization metric (f), and an algorithm for the optimization process ($\operatorname{argmax}_{\mathbf{a}} \{f(\mathbf{a})\}$). The fundamental differences between all sensorless AO methods consist of variations of one or more of these three components, and each of these components needs to be considered when defining an effective sensorless scheme.

III. A SYSTEMATIC COMPARISON OF DIFFERENT ABERRATION REPRESENTATIONS

We concentrate in this paper on the use of a generalized aberration representation to allow a fair comparison between various

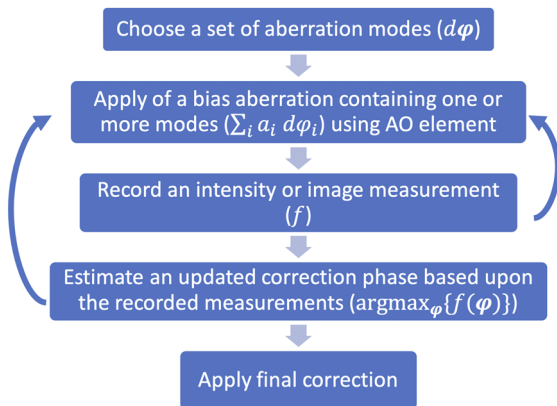


FIG. 1. Flowchart of the generalized sensorless AO aberration correction process.

sensorless AO methods within this framework. A systematic comparison can be conducted to search for a more effective choice of representation for certain imaging applications. Before explaining the general representation, we provide a brief review of existing methods for the necessary context.

In the pixelated piston-based methods, the pupil is divided into a large number of separate pixels within which the phase is modeled as a constant piston value.^{18–20} This constitutes a piecewise constant model for phase aberration. The applied bias aberration (ϕ) consists of a single pixel, whose phase is changed between measurements [Fig. 2(a)]. The phase of a portion of the pixels each varies at a different frequency, and the changing fluorescence intensity is recorded into a video. The video is Fourier transformed with respect to time to determine its phase that corresponds to the phase of each pixel.

In “pupil segmentation” zonal methods, the pupil is separated into a number of zones within which the phase is defined by three parameters corresponding to the piston, tip, and tilt components. This constitutes a piecewise linear model of the aberration. There are two implementations of the method—single zone illumination^{14–16} and full pupil illumination.¹⁷ For both implementations, a series of different piston, tip, and tilt components (ϕ) were applied in different pupil zones to modulate the pupil wavefront [Fig. 2(b)]. Optimization was performed either through the estimation of the lateral location of the object^{14–16} or through the maximization of the fluorescence intensity depending on the implementation.¹⁷

In modal sensorless AO methods, the aberrations are represented by a series of modes defined over the whole pupil. Common choices of bias aberrations ϕ used in modal methods include the Zernike polynomials^{5–10} [Fig. 2(c)], deformable mirror deformation modes,¹¹ or (polar) Walsh modes.^{12,13} In these methods, a mode is chosen and a short sequence of different bias amplitudes are applied in order to provide an estimate correction for that mode through the chosen optimization algorithm to maximize an image-based quality metric; alternatively, a sequence of bias aberration modes are applied before optimization in order to estimate several modes simultaneously.^{13,23,24}

These methods, as originally introduced, were conceived as different sensorless AO concepts. However, by placing them into the proposed framework, we can see that the primary difference between them is contained within the aberration representation. In the first case, the aberration is represented by a large number of separate zones with only piston applied within each zone [Fig. 2(a)]; in the second case, there are a number of zones with three modes applied within each zone [Fig. 2(b)]; and in the final case, only one zone and multiple modes are applied within the zone [Fig. 2(c)]. These

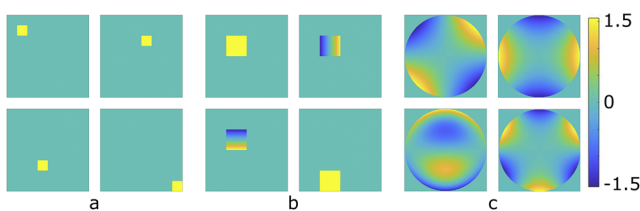


FIG. 2. Four examples of modes used in (a) the pixelated piston-based method, (b) the “pupil segmented” zonal method, and (c) the Zernike based method.

three seemingly different methods can now be considered as special cases of a more general scheme in which aberrations are defined both in terms of zones within the pupil and modes within each of the zones. A wider range of hybrid zonal/modal representations (ϕ) can be defined, which form a spectrum ranging from purely zonal through hybrids to purely modal methods, while maintaining the same degrees of freedom in each case.

In order to ascertain whether, for a particular task, a more suitable basis set exists in the whole spectrum of aberration representations, we chose to explore this broader range of hybrid representations rather than just the specific cases that have been presented previously in the literature.

Dependent upon the imaging application, including the nature of the specimen and microscope design, the nature of aberrations can vary: they can have smooth low-order shapes (due to the refractive index mismatch and system misalignments) or complex high-order variations (such as when imaging deep inside a scattering tissue), and the amplitude of aberration can be small or large. Moreover, there can be significant variations in the signal to noise ratio (SNR): the signal can be bright or dim, dependent on numerous factors such as fluorophore density and imaging efficiency. Thus, we narrow this wide parameter space by choosing representative scenarios for modeling that encompass different combinations of simple vs complex aberration shapes, small vs large aberration amplitudes, and high vs low SNRs.

A. Choice of microscope

For the purpose of comparing different aberration representations, we chose to simulate aberration correction processes and perform experiments on a two-photon (2-P) excitation fluorescence microscope (Fig. 3); see the [supplementary material](#), I: Fig. 1 for a full diagram. The 2-P microscope has been the subject of most adaptive optics developments including the various methods we mentioned in Sec. II. The 2-P microscope has often been used for deep tissue imaging²⁵ where sample induced aberrations can be significant.

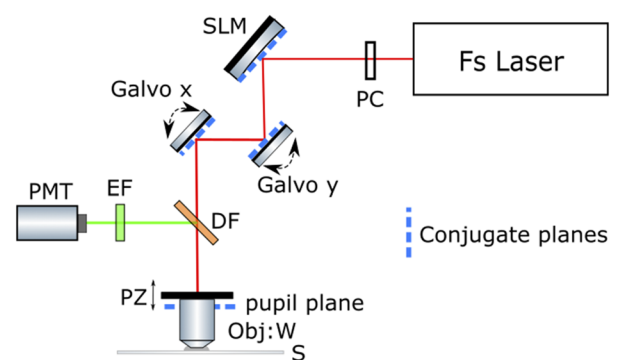


FIG. 3. A simplified diagram including the main features of the 2-P microscope: Ti:sapphire femtosecond laser, polarization control (PC), spatial light modulator (SLM), X and Y galvanometer scanners (Galvo x and Galvo y), water immersion objective lens (Obj:W) mounted on a Z-piezo translation stage (PZ), and dichroic filter (DF) allowing fluorescence to be reflected through an emission filter (EF) into a photo-multiplier tube (PMT). Various beam conditioning and relay optics are omitted for clarity.

The spatial light modulator (SLM) is used to introduce both the input and correction aberrations (which will be explained later in Secs. III B and III C) for the AO scheme.

While the image formation processes vary between different microscope modalities, we expect the general trends revealed through studying the 2-P microscope will also be observed in other microscope systems. This approach is justified as many of the approaches already used in sensorless adaptive optics have been shown to function in a similar manner across different types of microscopes.^{12,26,27}

B. Choice of aberration representations

In order to perform a fair comparison between the different approaches, we need to define a spectrum of aberration representations that permit variations of the aberration expansion while maintaining constant the total degrees of freedom. To enable a rigorous comparison at the modeling stage, we initially chose a 2-P system with a square pupil, which can be subdivided into equal sized square zones; different layouts allow us to assess aberration representations with sub-zones of different sizes; and different sized sub-zones support a different number of modes within each zone. This arrangement facilitates comparisons across different zonal/modal ratios in a way that would be difficult to formulate using circular pupils. While this square pupil is not normally used in practical microscopes, the difference should not affect the overall conclusions drawn from the results concerning the relative merits of the different zonal/modal aberration representations. Circular pupils are addressed in later experiments. In all of these cases, we consider only full pupil illumination rather than illumination of individual sub-apertures, which has been used in some previous demonstrations.¹⁴

We defined a particular set of modes to have Z zones, each of the zones supported M modes, such that the total degrees of freedom were given by the product $N = ZM$. To facilitate comparisons between different sets of representations, we selected the number of degrees of freedom N to be constant. To maintain square symmetry, we set $Z = 4^\zeta$ and $M = 4^\mu$, where ζ and μ are non-negative integers such that $\zeta + \mu = \text{constant}$.

Given the square symmetry of the zones, we chose to use a 2D Legendre expansion within each zone. The 2D polynomials are defined as $P_j^{2D}(x, y) = P_m(x)P_l(y)$, where $P_m(x)$ is a Legendre polynomial of order m , and there are restrictions on the values of indices j , m , and l (see the [supplementary material](#), II).²⁸ These Legendre modes demonstrate over a square region of support the same orthogonality as do Zernike polynomials over a circular region. For the case where $Z = 1$, the set of modes across the full pupil is defined by the first $M = N$ 2D Legendre polynomials. For cases where $Z \neq 1$, each of the full pupil modes is defined such that $Z - 1$ of the zones has zero value, and the remaining zone contains one of the first M 2D Legendre modes, where $M = N/Z$. In all of these cases, there are N modes in total defined across the full pupil.

In numerical modeling, the total degrees of freedom N was chosen to be 64. We, therefore, included four sets of representations into this analysis: Z64M1, Z16M4, Z4M16, and Z1M64, where $ZpMq$ is the notation used for the set with p number of zones in the pupil and q number of modes in each zone (Fig. 4). For a complete list of all the modes in the four aberration representation sets, please see the video in the [supplementary material](#).

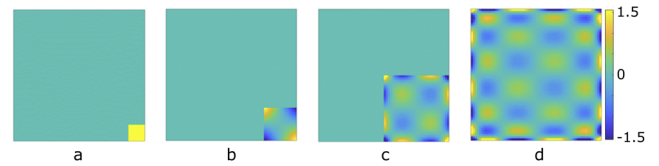


FIG. 4. An example of one mode from each aberration representation set: (a) Z64M1, (b) Z16M4, (c) Z4M16, and (d) Z1M64, respectively.

C. Choice of simulated input aberrations for correction

It is important to prevent bias when comparing different aberration representations. For example, it should be expected that input aberrations comprising a sum of Legendre modes would be corrected most precisely by an aberration representation set defined using the same modes. However, in most practical situations, the aberrations experienced in the microscope will contain components that cannot be compensated perfectly by the adaptive element. For this reason, we chose to model the input aberration using the Fourier components, which is a different basis to that used for correction. This ensures that the residual aberration $\epsilon \neq 0$ for all demonstrations.

An input aberration $\Phi(\mathbf{x})$ can be generated as a linear sum of several chosen unit Fourier components (Φ_w) at frequency w and each component with a chosen root mean square (rms) amplitude A_w ,

$$\Phi(\mathbf{x}) = \sum_w A_w \Phi_w(\mathbf{x}). \quad (3)$$

The definition of the Fourier components Φ_w is explained in detail in the [supplementary material](#), III.

In this model, the low frequency Fourier components are analogous to the low-order aberrations, such as imaging through a medium with a refractive index mismatch, encountered in microscopes [Fig. 5(a)]. Aberrations induced from highly disordered media, such as when imaging deep through thick tissues, contain high frequency components and can be simulated by high order Fourier components [Fig. 5(b)].

For the discussion of this paper, we use a Fourier series with order below 8π in one dimension ($w \leq 8\pi$), i.e., a maximum of 16 cycles across the x - y pupil plane.

D. Choice of optimization metric and algorithm

In this paper, we chose the two-photon fluorescence intensity to quantify the output image quality. It is a common choice

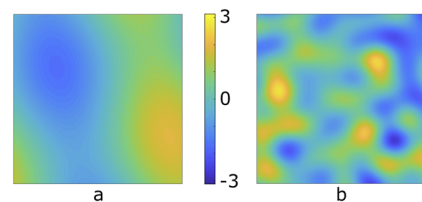


FIG. 5. An example of (a) a randomly generated low order ($w = 1.6\pi$) Fourier based aberration and (b) a random combination of Fourier components containing high orders. The maximum order (w) appearing in this pupil is 7.6π .

for many sensorless AO applications in two-photon fluorescence microscopy.^{6,17,18} It was calculated using the common approximation of the focal field through a scaled Fourier transform of the pupil field.^{21,26} The intensity distribution was then found as the square of the modulus of the focal field. The mathematical definition of this metric is presented in the [supplementary material](#), IV.

Noise was simulated to follow a Poisson distribution with a mean and variance (σ) proportional to the square root of the photon count.²⁹ The simulation results presented in this paper are acquired when no noise is added to the output metric f (high SNR case) and when Poisson noise with $\sigma = \sqrt{500}$ was added to f (low SNR case).

To optimize the quality metric, we chose a three-point quadratic optimization algorithm (3N algorithm) for the convergence of each aberration representation mode.²⁴ (For the details of this 3N algorithm, please see the [supplementary material](#), V.) This algorithm permits precise correction of aberrations if the signal follows closely a quadratic approximation as a function of the input mode amplitude. Such an approximation is appropriate if the sampling spacing around the peak of the function is even over a suitable range. Optimization schemes employing more measurements can be useful to cover a larger range of aberration amplitudes, although this comes at the expense of requiring more specimen exposure and time.²⁴

In initial modeling, we limited our discussion to only one iteration of optimization for each mode. The effects of further iterations are considered in the [supplementary material](#), VI. Though some schemes perform better with more iterations, the additional specimen exposure and time required generally have a detrimental effect on the imaging task.³⁰

E. Simulation and experimental results comparing the four aberration representations

As we mentioned in Secs. III B and III C, we investigated the effectiveness of four aberration representations (Z64M1, Z16M4, Z4M16, and Z1M64) for correction when input aberrations have varying frequencies ($0.2\pi \leq w \leq 8\pi$) and amplitudes ($0.1\pi \leq A_w \leq \pi$), where w and A_w were defined in Sec. III C. For each selection of w and A_w , 20 aberrations were generated by randomly choosing the amplitude ratio A , the orientation R , and the spatial offset τ (see the [supplementary material](#), III for the definitions of A , R , and τ). The statistics was then derived from this ensemble of results by averaging them for each frequency w and amplitude A_w setting. The four correction models were simulated to correct the aberrations and results presented below. We also studied two scenarios for high and low SNRs [Figs. 6(a) and 6(b), respectively] to observe which correction model was more robust toward noise.

Figures 6(a) and 6(b) show simulations comparing four different aberration representations—Z64M1, Z16M4, Z4M16, and Z1M64—used to correct the same set of input aberrations. From the noise-free results [Fig. 6(a)], we saw that the single zone set of modes (Z1M64 in red) gave the best correction for most of the simulated aberrations. Figure 6(a1) shows the profiles of the mean corrected intensities of correcting 20 randomly generated aberrations for each set of representations along the line labeled “a1” in Fig. 6(a) where frequency $w = 1.4\pi$. The black hexagons indicate the average initial intensity before correction. We note that the input aberration could contain significant tip, tilt, and defocus components,

particularly at lower values of w , which did not affect the intensity since we assumed a uniform volume specimen. The colored markers indicate the mean corrected intensity for different aberration representation sets. In all cases, correction succeeded, although the benefit was the greatest for Z1M64 (red stars). Figure 6(a2) shows the equivalent profiles along line “a2” in Fig. 6(a), which correspond to more complex input aberrations. In this case, the average corrected intensity was much lower than that shown in a1. At higher amplitudes, above $A_w = 1$ rad, there was little benefit in correction from any of the four methods. However, at lower amplitudes, it could be seen that all methods provided some level of correction with Z64M1 (blue crosses) performing marginally better than the others.

As many practical imaging scenarios worked at low SNR, we also explored the case when Poisson noise was introduced to the system in our simulation. Comparing Fig. 6(b) to Fig. 6(a), it was observed that the small sized zone set (Z64M1 in blue) was more susceptible to the effects of noise and the medium sized zone sets Z4M16 and Z16M4 performed better for more complex, low amplitude aberrations. Furthermore, all sets failed when correcting wavefronts with a large A_w and w at low SNR.

The trends observed in the simulations were confirmed through experiments on fluorescence bead specimens. Two- μm fluorescence beads were diluted and dried on a coverslip before sealing to a sample slide. The spherical aberration introduced by the coverslip was compensated mostly by the correction collar of the objective lens; any residual system aberration was compensated by the SLM using the Zernike polynomials over the whole circular pupil before experiments being carried out. During the experiment, only light from the square pupil enclosed by the full circular objective pupil was allowed to enter the objective lens, and light outside of the square pupil was deflected away by a grating pattern displayed on the SLM. When an aberration with $A_w = 2.5$ and $w = 1.4\pi$ was introduced into the system [Fig. 6(c)] for correction, the images were best after Z1M64 correction, and the correction process plot [Fig. 6(c1)] also suggested that Z1M64 was the fastest as the fluorescence intensity was close to the optimized value after correcting the first 32 modes, while the other three methods only reached the maximum after correcting all the 64 modes; thus, the single zone method (Z1M64 in red) was shown to be the most suitable model for correcting simple shaped wavefronts with small frequency components w . Figure 6(d) shows the results with input aberration $A_w = 1.0$ and $w = 6.8\pi$, and the medium sized zone sets (Z4M16 in purple and Z16M4 in green) gave better corrections than the other two; the correction using Z16M4 was also observed to be the fastest as we can see in Fig. 6(d1), and after correcting the first 45 modes, the fluorescence intensity of Z16M4 was much higher than the other three. Figure 6(e) presents results with input aberration $A_w = 1.5$ and $w = 7.2\pi$; the shapes of the beads were revealed after small zone correction (Z64M1 in blue), but remained blurred after using the others; Z64M1 also gave the fastest correction process among the four as the slope of the relative fluorescence intensity growth is the steepest [Fig. 6(f1)]. Figure 6(f) shows the same scenario as Fig. 6(e) but with lower SNR, which was affected by reducing the excitation laser power; no representation set provided effective correction, and only the single zone set (Z1M64 in red) gave marginal improvement of the image brightness after correction; it was similarly shown in Fig. 6(f1) where no improvement in fluorescence intensity was observed throughout the correction process.

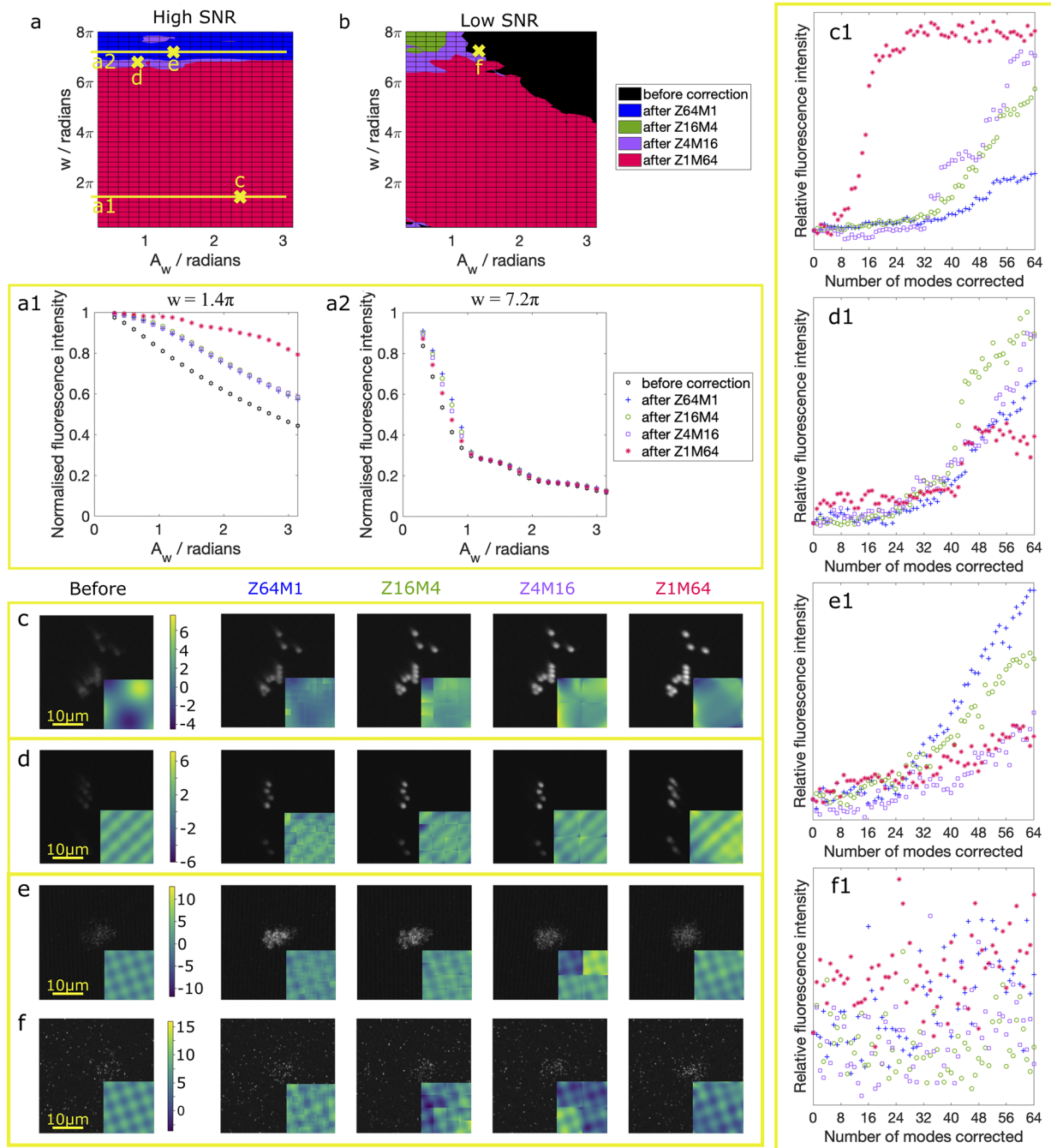


FIG. 6. [(a) and (b)] Simulation results comparing the correction using the four different aberration representations at high and low SNRs, respectively. The color indicates the method that provided the greatest mean signal (of 20 simulated experiments) after one cycle of correction, as a function of rms aberration amplitude A_w and complexity, defined as the frequency component w . The region that is colored black indicates that no correction using the four aberration representations was effective. The labels "c," "d," "e," and "f" show the properties of the input aberration used in the corresponding sections of this figure. (a1) and (a2) represent the sections through the plot, as shown in (a). They show the mean (of 20 simulated experiments) signal level after correction for each of the representations. [(c)–(f)] Experimental results imaging $2\ \mu\text{m}$ fluorescence bead clusters when correcting the Fourier based aberration using the four aberration representations, Z64M1, Z16M4, Z4M16, and Z1M64. Aberrations were both introduced and corrected using the SLM. The first column from left shows the images with the input aberration; the second to fifth columns show the images after correction using the four aberration representations. The inset in the bottom right of each image shows the wavefront before (the first column) and after (the second to fifth column) correction. The colorbar shows the wavefront phase in radians for each row. The four plots (c1), (d1), (e1), and (f1) show the relative fluorescence intensity variations when each mode of the four methods was corrected during the experiments; they displayed the correction process when correcting the four aberrations from (c)–(f), respectively.

Note that the noise conditions present when obtaining the results of Fig. 6(f) are not directly comparable to those used in the simulation of Fig. 6(b). In a real system, many factors, such as the laser power, fluorophore density, aberration amplitude, scanning speed, quantum efficiency of the photodetector, and background noise,²⁹ can all affect the SNR. However, the results are representative of the trends to be expected in a noisy system.

IV. EXPERIMENTAL COMPARISON OF ABERRATION REPRESENTATIONS OVER A CIRCULAR PUPIL

The results in Sec. III E provided mathematically complete and systematic like-for-like comparisons of the effectiveness of different aberration representations for correction while maintaining fixed degrees of freedom. However, the square pupils used in the analysis are not commonly used in practice for microscopy. We therefore performed further comparisons among the most common three aberration representation sets over circular (or near-circular) pupils, using a similar conceptual approach. In these cases, the approach necessitated slight variations in the pupil shape and degrees of freedom. For these experimental demonstrations, we used thin fluorescently labeled biological specimens and simulated aberrations through the introduction of controlled phase patterns using the SLM.

A. Circular pupil aberration representations

Similar to the approach in Sec. III B, we defined a spectrum containing different aberration representation sets over a circular pupil that permitted variations of the aberration expansion while maintaining the total degrees of freedom N . In this case, we aimed for N to be as close to 36 as possible. For convenience in the zonal/modal expansion, 36 is chosen. We also aimed to create aberration representation sets to cover a plane as close to a circular pupil as possible. For the small sized zone set, we had a total of 37 square zones ($Z = 37$) mapped to a circular pupil with piston ($M = 1$) applied to each zone [Fig. 7(a)]. For the medium sized zone set, we had 12 square zones ($Z = 12$) mapped to a circular pupil with piston, tip, and tilt ($M = 3$) applied to each zone [Fig. 7(b)]. For the large sized single zone set, we chose to use the Zernike polynomials up to mode $M = 36$ (Noll index³¹) over the whole circular pupil [Fig. 7(c)]. Note that the small sized zone Z37M1 set corresponds to the representation used in the pixelated method¹⁸ and the medium sized zone Z12M3 set closely modeled the representation used in the “pupil segmentation” zonal method¹⁴ mentioned in Sec. III. We note that for the sets Z37M1 and Z12M3, some areas of the circular pupil are missing.

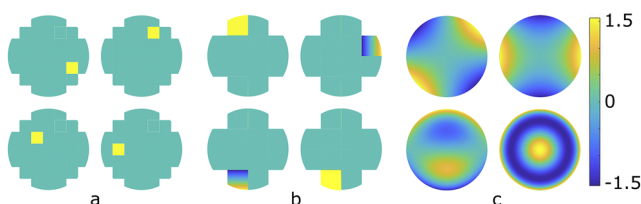


FIG. 7. [(a)–(c)] Four examples of each of the aberration representation sets Z37M1, Z12M3, and Z1M36, respectively.

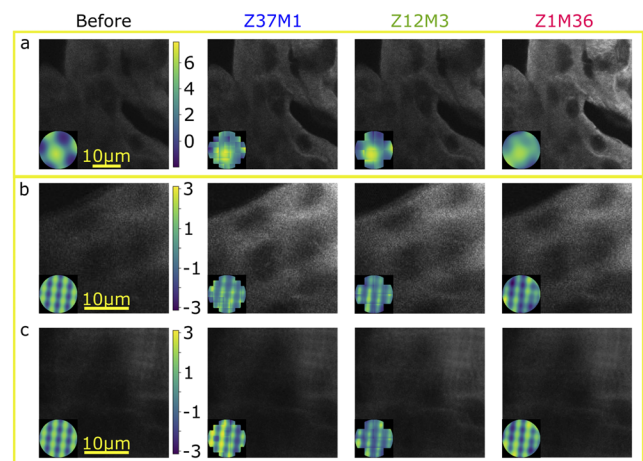


FIG. 8. Correction of aberrations over a circular pupil using a FluoCells prepared slide containing a 16 μm cryostat section of mouse kidney stained with Alexa Fluor 488 wheat germ agglutinin (W-11261), Alexa Fluor 568 phalloidin (A-12380), and DAPI (D-1306). These demonstrations show the relative performance of the different aberration representation sets for variations in input aberration: (a) a low frequency input aberration and [(b) and (c)] a high frequency input aberration. In (b), imaging was performed with high SNR, and in (c), imaging was performed with low SNR. The inset in the bottom left of each image shows the wavefront before (the first column) and after (the second to fourth column) correction. The colorbar shows the wavefront phase in radians for each row.

However, as they cover about 94% and 91%, respectively, of the full pupil area, this would not have a significant effect on our comparison.

B. Experimental results using tissue specimen and SLM-induced aberrations

For these experiments, the input aberration was defined as in Sec. III C using a Fourier basis and applied using the SLM. The slide for imaging was a thin (16 μm) mouse kidney specimen. Two particular demonstrations were performed using two different types of input aberration: one with low spatial frequency components [Fig. 8(a)] and the other with high frequency components [Figs. 8(b) and 8(c)]. In the latter case, the laser power was adjusted to obtain images with large [Fig. 8(b)] and small [Fig. 8(c)] SNRs.

The experimental results agreed with our previous conclusions that the large sized zone representation (Z1M36) corrected the best when aberration had simple shapes (small w) [Fig. 8(a)]. The small sized zone set (Z37M1) performed slightly better when the input aberration had complex shapes (large w) but was more susceptible toward noise [Fig. 8(b)]. For low SNRs, no set gave effective correction [Fig. 8(c)].

V. COMPARISON OF CIRCULAR REPRESENTATION SETS CORRECTING SPECIMEN-INDUCED ABERRATION

To further validate the above observations, we extended the experimental investigation to cover the correction of specimen-induced aberrations in thick samples. We tested the three aberration

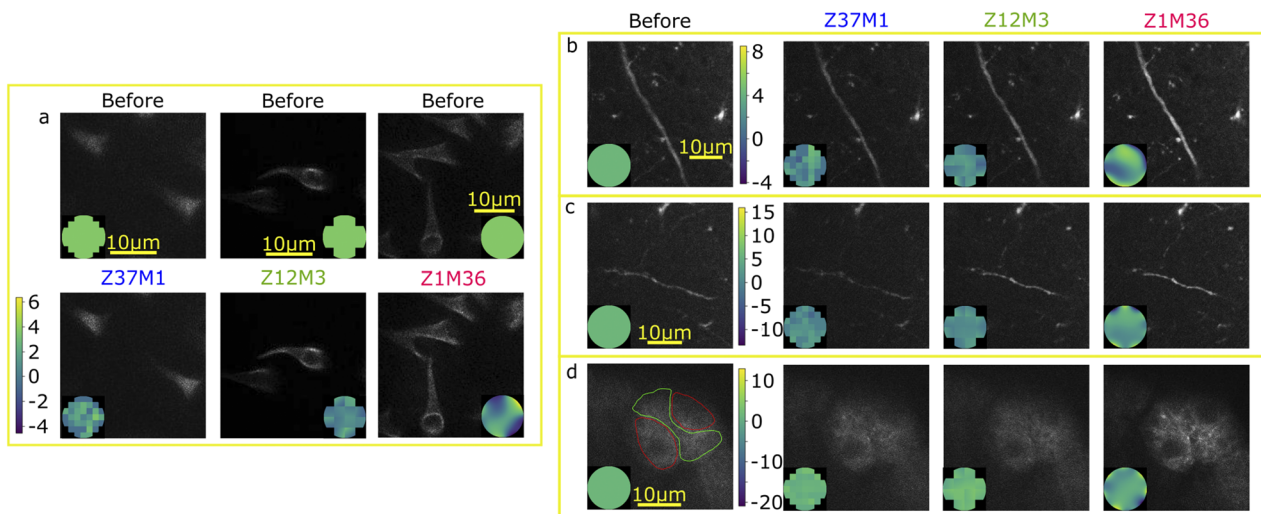


FIG. 9. (a) Denticle cells imaged at $100\ \mu\text{m}$ deep inside a *Drosophila* larval fillet sample. The first row shows the images before aberration correction and the second row includes the images after correcting using representation sets Z1M36, Z12M3, and Z37M1. Wavefronts before and after correction are shown in the insets. The colorbar shows the wavefront phase in radians. [(b) and (c)] Neuronal processes at $183\ \mu\text{m}$ and $225\ \mu\text{m}$, respectively, in the fixed, extracted brain of a Thy1-YFP mouse in a region where a dendrite was axially contained within the field of view. The first column shows the images before aberration correction, and the second to fourth columns include the images after correcting using representation sets Z1M36, Z12M3, and Z37M1. (d) *Drosophila* larval brain neuroblast (enclosed by red line) and progeny (enclosed by green lines).

representation sets described in Sec. IV A in three different biological samples, where aberrations had been caused by focusing through the tissue at different depths. (For details of all specimens, see Methods section, the [supplementary material](#), VII.)

Figure 9(a) shows images of denticle cells at $100\ \mu\text{m}$ deep inside a *Drosophila melanogaster* larva. Specimen-induced aberrations were corrected using the three aberration representations at three different sites to obviate the effects of photo bleaching. As these sites were adjacent, the sample induced aberrations were expected to be similar in terms of shapes and amplitudes. All three sets improved the image quality, as seen in the images after correction, which were brighter and showed sharper features. The large sized zone Z1M36 set gave the more effective correction, as seen in the image improvement.

Figure 9(b) showed neuronal processes at $180\ \mu\text{m}$ depth in a fixed Thy1-GFP mouse brain. In this case, the large sized zone Z1M36 set provided the best aberration correction. After Z1M36 correction, more specimen details were revealed, and the brightness of the neuronal process was clearly increased. For the other correction schemes, the improvement was less obvious.

Figure 9(c) shows the correction for imaging of neuronal processes at a depth of $225\ \mu\text{m}$ in the same fixed Thy1-GFP mouse brain. At this location, the wavefront correction appeared to contain higher order components with large amplitudes and the initial SNR reduced significantly. In this case, the single zone Z1M36 set gave the best correction compared to the other two methods, possibly, because it is more robust in noisy scenarios, although Z12M3 also generated an improvement.

In Figure 9(d), we imaged neuroblasts at about $100\ \mu\text{m}$ deep inside a freshly extracted, non-fixed *Drosophila* larval brain. After Z1M36 correction, the definition of membranes (marked by

mCD8-GFP) was greatly improved with the outlines of the small progeny cells and subcellular structures visible. The Z12M3 correction made some progeny outlines visible, whilst the Z37M1 correction achieved sharper membrane and subcellular structures relative to pre-correction. Though all methods provided some level of correction, Z1M36 performed the best for this specimen.

It is interesting to observe that in Figs. 9(c) and 9(d), the wavefronts after correction using the single zone scheme Z1M36 can contain large amounts of some high order modes, despite successful correction overall. This is manifest in the “wrinkles” that can be observed around the edge of the pupil, which are due to the presence of high azimuthal order Zernike polynomials in the correction aberration. These high order modes are likely to be artifacts due to incorrect optimization as it is improbable that these modes are actually introduced by the specimen. The real specimen induced aberration is likely to be smaller in amplitude than the correction aberration shown. It is probable that the method has incorrectly introduced these modes in an attempt to correct high order spherical-like aberrations, which are likely present. In advanced schemes, prior information on the nature of the specimen-induced aberrations could be used to regularize such unwanted phenomena.

VI. DISCUSSION

Overall, the modeling and experimental results point to the conclusion that large sized zone sets are better at correcting aberrations with simpler shapes and they are also less sensitive to noise; small sized zone sets work slightly better when aberrations have more complex shapes, while they are more susceptible to noise.

The results can be explained because the spatial correlation of the aberration representation sets goes down as the number of zones

goes up; with a large number of small sized zones, the set is potentially better at correcting aberrations with low spatial correlation (with large frequency components w). Furthermore, large sized zone sets are expected to be more robust against the effect of noise as they create a greater change in the fluorescence signal when phase modulation is applied to the pupil plane.

Results following the correction of specimen-induced aberrations suggest that [Figs. 9(a)–9(d)] the specimen-induced aberration can be better corrected by the large sized zone Z1M36 set. It is possibly because of limitations of the other representations in low SNR scenarios.

It is known that specimen-induced aberrations can take many forms, including smooth forms, high spatial frequency components, and discontinuities.²⁷ A Fourier representation for modeling input aberrations was chosen in order to cover a range of possibilities while not being biased toward any choice of correction sets. All sets have limitations in the form of aberrations that they can adequately correct as they are limited by the representation used. For example, in all of the zone-based sets, there exist discontinuities at the zone boundaries. This might be alleviated by inter-zone phasing if assumptions can be made about phase continuity. However, in other situations, sudden steps in phase may be required for optimal aberration correction.²⁷

The systematic analysis in this paper provides fair comparisons across the spectrum of different aberration representation bases to illustrate their relative capabilities for realistic scenarios. The detailed analysis has concentrated on a single cycle of correction to provide a like-for-like comparison that seeks to minimize measurements and hence specimen exposure. Several demonstrations in the literature have used multiple iterative correction cycles. Through modeling of these scenarios, we found (see the [supplementary material](#), VI) that multiple cycles increased the effectiveness of all sized zone sets, although at the cost of higher specimen exposure.

The conclusions drawn here could also be readily extended to other aberration representation bases, such as deformable mirror deformation modes,¹¹ Walsh modes,^{12,13} or other arrangements of pupil zones, since in this paper, we demonstrated phenomena using in both Legendre and Zernike based modes in both square and circular pupils, respectively, to correct both Fourier based introduced aberration and sample-generated aberration.

VII. CONCLUSION

We have brought together a range of image-based wavefront-sensorless AO methods into the same framework. This has permitted for the first time a systematic fair comparison of different aberration representations for sensorless AO schemes. This was illustrated through the simulation and experimental demonstration of their operation in a two-photon microscope. Furthermore, we have shown that these representations are all special cases of a broader spectrum of zonal/modal hybrid methods. This opens up the possibility of other aberration representations beyond those already presented in previous research.

Overall, the results suggest that the large zone schemes generally provide better correction in a single cycle and are more robust against noise, and the small zone schemes exhibit better performance when the input aberration has complex high order shapes, but they are more susceptible to error in low SNR imaging.

The results have been presented in a manner that decoupled the essential phenomena from the chosen aberration representations. We therefore expect that the major conclusions will be broadly applicable and are not limited to the particular aberration representations employed here. Moreover, we expect that many of the phenomena observed here through demonstration on a two-photon microscope will also be common to other microscope modalities. Hence, the results will be useful in the design of future sensorless AO schemes for a wide range of applications.

SUPPLEMENTARY MATERIAL

See the [supplementary material](#) for the supplementary document and video.

AUTHORS' CONTRIBUTIONS

Q.H. and M.J.B. contributed to concept and theoretical development; Q.H., J.W., J.A., and M.W. contributed to experimental system implementation; Q.H., M.H., R.T., and D.G. contributed to imaging experiments; Q.H. and M.J.B. helped in writing the paper. All authors edited the paper and approved the final version.

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The authors declare no conflicts of interest.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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