**Wavefront sensing for ophthalmic adaptive optics**

Most AO ophthalmoscopes use *Shack-Hartmann wavefront sensors* to measure the monochromatic aberrations of the eye and drive wavefront correction. The only alternatives demonstrated to date are (sensor-less) image sharpness or intensity metrics, and computational wavefront sensing, in which one or more wavefronts across the field of view are derived from optical coherence tomography (OCT) data.

The pyramid wavefront sensor (<https://doi.org/10.1080/09500349608232742>) is likely to succeed the SHWS in ground-based astronomical telescopes, due to its tunable sensitivity and can operate with very few photons. This wavefront sensor exhibits a non-linear response that might be problematic for large amplitude aberrations, although this might not be a problem when operating in closed loop. Despite an initial demonstration in the eye (<https://doi.org/10.1364/OE.10.000419>), its value for retinal imaging has not been thoroughly tested yet. One of the questions that must be explored is, how this sensor would be affected by reflections from various retinal layers and/or intraocular scattering.

Using the SHWS in rodents is challenging, apparently due to strong back-scattering from multiple layers (<https://doi.org/10.1364/BOE.2.000717>, <https://doi.org/10.1364/BOE.3.000715>). Should other wavefront sensors be considered? Currently only SHWS (<https://doi.org/10.1167/iovs.13-12581>, <https://doi.org/10.1364/BOE.6.002106>) and sensor-less (<https://doi.org/10.1364/BOE.5.000547>, <https://doi.org/10.1364/BOE.7.000001>, <https://doi.org/10.1364/BOE.6.002191>, <https://doi.org/10.1364/BOE.6.000580>) AO ophthalmoscopes are in use.

South et al. recently demonstrated the benefit of combining AO correction with a deformable mirror and computational wavefront correction (<https://doi.org/10.1364/BOE.9.002562>).

Wavefront RMS estimated from the SHWS data is the preferred (11 labs, 1 company) metric to display *AO performance* in real time, although AO control parameters are tuned to minimize pixel displacement across all lenslets (e.g., Euclidean or L2 norm). Although total wavefront RMS informs on the quality of the correction that is achieved, it should be the RMS of the projection onto the wavefront corrector(s) space that should be used to tune the AO control parameter or test AO control algorithms. Strehl ratio is monitored by a small number of labs, mostly by calculating it from the wavefront reconstructed from a SHWS signals, and with only the Doble Lab using a point spread function camera.

Current SHWS for AO retinal imaging use almost two order of magnitudes more *photons per lenslet* than astronomical SHWSs. More photons result in lower photon/shot noise, but it remains to be explored what is a minimum acceptable number of photons to use for retinal imaging. Although not critical, we should strive as a community to minimize retinal light exposure.

Our survey indicates that three types of *centroid algorithms* are used, with the most popular being iterative centroiding with shifting and shrinking search box (9 labs, 1 company), followed by non-iterative centroiding (3 labs), a two-step iterative centroid in which the second iteration uses the location of the maximum pixel value on the first iteration as the center of the second iteration search box (1 lab) and a single iteration using the maximum pixel value in lieu of the centroid (1 lab). In all cases, but one, search box boundaries are defined in integer pixel coordinates, even though the use of “fractional” pixels provides lower centroiding errors (Akondi and Dubra manuscript in preparation).

SHWS software typically assume that the beacon light originates from a single plane. Is that a valid approximation? If not, how is wavefront estimation affected and what can be done to mitigate any potential error/bias? Miller and others pointed out that there is no convincing data on the strength of the back-scattering of each retinal-layer, and no experiments have been proposed to address this knowledge gap. Any study to address this would be further complicated by the fact that the relative backscattering intensity of each retinal layer varies with retina pigmentation, retinal location, wavelength and imaging modality. Miller noted that optical coherence tomography, whether polarization-sensitive or not, only informs about light that remains polarized, providing little to no information about multiply-scattered light. Two-layer retinal modelling by Akondi and Dubra shows that when/if aberrations do not to change with retinal depth, then adequately larger search boxes in SHWS centroiding algorithms almost completely eliminate error/bias due to the presence of multiple-layer beacons (submitted for publication).

**Ophthalmic WS and AO design and assembly**

It has been over two decades since ophthalmic wavefront sensing and AO were first demonstrated, and technical questions are still asked every time a new instrument is designed, with current literature not offering clear succinct answers. The notes below review some currently used criteria and suggest best practices for the specification, design and assembly of point-scanning AO ophthalmoscopes. Some of these are also applicable to flood illumination and line scanning instruments.

The first step in the design of an AO ophthalmoscope, is to assemble a list of desired or necessary specifications, each with a justification. These justifications are important, as they will guide any technical compromises that have to be made during the design process. A non-exhaustive example of such a list is provided next in Table 1, with the reasoning and some thoughts in the text that follows (edited from a manuscript in preparation by Samuel Steven).

1. Sample AO ophthalmoscope specifications for an adaptive optics scanning light ophthalmoscope (Dubra lab).

|  |  |
| --- | --- |
| **Specifications** | **Target** |
| Optical elements with power | Reflective |
| Light collection transmission | > 50% |
| Optical element type | Spherical mirrors |
| Wavelength range | 450 – 1300 nm |
| Source & detector vergence range | ± 1.5 D |
| Spectacle prescription vergence range | -11 to +9 D |
| Maximum field of view (FOV) | > 1.5° square |
| Full field of view with steering | > 4.0° square |
| Clearance between eye and system | > 150 mm |
| Pupil diameter at the eye | 7.5 – 8 mm |
| Pupil RMS spot size @ 450 nm (at the eye and the DM) | < 0.5 DAiry over all vergences and FOV |
| Pupil distortion @ deformable mirror relative to zero source vergence | < 50 (< 25) μm for all FOVs |
| Retina RMS wavefront @ 450 nm | < λ/14 all vergences over the maximum FOV |

There is a portion of the optical setup that is common to both the illumination, imaging and wavefront sensing. For this common optics, *reflective or refractive optical elements* with power could be used. The choice between these two types (or a hybrid approach) could be driven by minimization of wavefront aberrations, cost/availability of components, ease of alignment, desired instrument size, or light throughput (which is related to light safety).

It could be argued that in research settings, minimization of wavefront aberrations and light safety should be the most important factors, as the goal is not to mass-produce instruments at low cost, but rather develop instruments that could be easily modified to advance the technology. The consideration of light safety is of particular importance given recently reported retinal changes in response to light exposures below the maximum permissible exposures in light safety standards. It is also important to keep in mind that there is no data on the susceptibility of diseased or injured retinas to light damage, and thus, we should strive to minimize retinal exposure to light and not just aim to comply with light safety standards.

When using refractive optics, crossed-polarizers often have to be used to mitigate undesired back-reflections on the lens surfaces in non-interferometric single-scattering imaging, which could substantially reduce the detected signal, in a manner that is eye- and retinal location-dependent. These back-reflections can be particularly problematic in interferometric imaging, even if they do not directly affect the final images, by reducing the effective dynamic range of the detector (e.g., use a substantial pixel well depth portion in spectrometer-based optical coherence tomographers). Back-reflection from optical and ocular surfaces are absent in fluorescence imaging.

Most current AO Various refraction studies indicate that strong myopes are substantially more common than strong hyperopes. This indicates that when limited by the stroke of the wavefront corrector, *biasing the focus range* could help increase the fraction of the population that can be imaged.

*Optical element coatings* determine the throughput, and thus the safety, of the instrument. When using reflective optics, metallic coatings such as protected silver and protected gold are preferred due to their high reflectivity over a broad spectrum band. Dielectric coatings should be avoided when using short-pulsed lasers (e.g., for two photon microscopy) or optical coherence tomography due to their dispersion. In an average AOSLO light undergoes 14 reflections off of spherical and flat mirrors, which if using protected silver coatings with ~96% reflectivity, will result in a 55% transmission. That is, 45% of the light coming back from the eye will be lost to these mirrors alone.

The wavelength or combination of wavelengths to be used in an AO ophthalmoscope affects the optical performance in terms of wavefront aberrations. In particular, it should be considered whether light will propagate through the optical system not only at the various scanning angles necessary to capture the desired images, but also at various *vergences* due to spectacle prescription and relative foci between wavelengths. If multiple wavelengths will be used simultaneously, then the variability of longitudinal chromatic aberration across the population and the retinal thickness in units of diopters must be considered, noting that the latter can change substantially across species.

The *field of view* of AO ophthalmoscopes should ideally be the largest over which diffraction-limited wavefront correction can be achieved, that is the isoplanatic patch, which varies with wavelength, pupil diameter, across retinal locations and eyes. Existing experimental estimations of the *isoplanatic patch* of the human eye were captured with modest pupil sampling, retinal sampling, with a single wavelength and a single pupil diameter (~6 mm), not allowing extrapolation to other experimental conditions. Therefore, the choice of field of view is currently arbitrary. In addition to diffraction-limited imaging, AO ophthalmoscopes might have larger field of views as a form of viewfinder to quickly identify retinal locations of interest, or image large structures that can be studied with lower resolution.

Most current AO ophthalmoscopes use one or more wavefront correctors in pupil conjugate planes, producing isoplanatic patches estimated to be ~1° of visual angle. Increasing the isoplanatic patch further requires correction in additional conjugates, hence the term multi-conjugate AO.

The isoplanatic patch size is linked to the pupil diameter, and because of it, the choice of field of view affects transverse resolution and vice versa. If for example, resolving the cone and rod photoreceptor mosaic requires the largest possible pupil (> 7 mm) and small fields of view (~1°), while imaging the retinal capillaries for the purpose of studying perfusion can be achieved with smaller pupils (~5 mm) and larger fields of view (> 2°).

If *field of view steering* is desired for real-time eye movement compensation, then the optical design should account for this, aiming to minimize not only wavefront aberrations across the field of view, but also image distortion. To date, most AO ophthalmoscopes implement this steering through pupil conjugate optical scanners, achieving retinal stabilization but no pupil stabilization. The latter, which has often been ignored, results in poor wavefront sensing and correction. Thus, optical steering in planes conjugate to the center of rotation of the eye should be considered.

Burns has built and used scanning AO ophthalmoscopes in which the field of view can be steered over large retinal areas. This approach replicated at the U. of Rochester and U. of Pittsburgh, is now commercialized by Boston Micromachines Corporation and deployed at various institutions. So, why has this approach not been more widely used? This approach has the potential benefit of not having to move the subject to recenter the pupil every time a new retinal location is selected, as opposed to systems in which the field of view is steered by moving a fixation target. A disadvantage is that the field of view rotates and distorts across the steering range, potentially requiring complex distortion calibration and eventual removal during retinal image post-processing.

An issue of practical importance when designing an AO ophthalmoscope is the *working distance*, that is the distance between the eye and the first optical element with power. Often, an internal fixation target is provided by inserting a beam splitter (e.g., 90T:10R or dichroic) between the eye and the final element of the imaging path with power, requiring a longer working distance.

It is generally agreed that it is desirable for AO ophthalmoscopes to achieve diffraction-limited *retinal imaging performance*, that is, wavefront RMS below 1/14th of the wavelength of interest across all points in the field of view in the imaging channel. The challenge is achieving this across the entire spectacle prescription range and field of view steering range. Often optical designs are only evaluated using ray tracing software ignoring manufacturing and assembling imperfections. If possible, the “as built” performance, meaning a system built using components with known manufacturing tolerances (provided by the manufacturer) and assembly tolerances should be considered, and a process called “desensitization” to these tolerances must be performed.

Currently, there is no agreement on what the *pupil imaging performance* should be between pupil planes. “Pupil wandering,” meaning the translation of the pupil outline as the imaging beam is scanned across the field of view has been identified as a potential source of AO performance degradation, as this results in the wavefront sensor and wavefront correctors seeing blurred versions of the ocular aberrations. When using a SHWS, it would be reasonable to keep this pupil wandering and the point-spread function width substantially below the size of a (SHWS) lenslet.

At a minimum, the optical designer of AO ophthalmoscopes should aim to minimize aberrations between: a) the wavefront sensor and wavefront corrector planes, b) the wavefront corrector plane(s) and the eye, and c) the illumination/detector and the retina planes (not the intermediate retina conjugate planes) for the entire vergence and spectacle prescription ranges. Monitoring and/or optimizing performance at additional planes to avoid vignetting or aberrations might be desirable too.

AO ophthalmoscopes can be thought of as a number of pupil conjugate elements (e.g., scanners, and wavefront correctors) and a number of optical element sets, each of which relays one pupil plane element onto the next one. There is no rule of thumb about how to *order optical elements in pupil planes*, but there is an overall sense that one should minimize the propagation of rays at large angles through the optical system, starting at the eye. This thinking has been incorporated in the latest generations of AO ophthalmoscopes: a) modest combined steering plus field of view place the wavefront corrector with the largest stroke in the first pupil plane after the eye, because spectacle prescription is the dominant reason for large ray angles of incidence in optical elements, while b) large steering (e.g., ±15°) plus field of view AO ophthalmoscopes put the steering scanners in the first pupil plane.

What figures of merit are used to evaluate optical performance? Wavefront RMS for retinal and pupil planes, spot size for pupil planes and beam wander at the pupil planes. It was suggested that maybe MTF-derived metrics such as average power over pre-determined bands could be used during AO operation for tuning control algorithm parameters and/or quantify performance.

The performance of instruments after assembly can be estimated by placing point sources in the desired planes and either wavefront sensors in Fourier transform planes, or point-spread function (PSF) cameras and phase retrieval algorithms.

Are there special specifications for AO-OCT? Yes. Achromatizing lenses are needed for broadband sources. Also, and as in non-AO OCTs, similar optical elements should be considered for both the sample and reference arms.

**Topics to continue discussion:**

* What camera specifications should be prioritized for SH wavefront sensing for ophthalmic AO?
* How to choose a lenslet array for SHWSs to be used in an AO ophthalmoscope? How many lenslets across the pupil? How many pixels? How do we balance dynamic range and sensitivity? Are we matching the wavefront corrector(s)? Cross-talk? Magnification between eye and array and between array and camera sensor?

**AO calibration**

The two essential steps in the calibration of an AO system are the presentation of a known “reference” wavefront and the measurement of the wavefront corrector(s) response(s). The order in which these steps are performed is not critical, although for convenience, the reference wavefront is often captured first.

The reference wavefront is necessary because the AO control algorithm needs to know what the desired wavefront is. Ideally, one would use a flat or spherical wavefront with minimal to no aberrations, and then calculate other desired wavefronts. These desired wavefronts could be the reference wavefront plus known amount of defocus, in order to change the focus of the AO ophthalmoscope imaging channels. In vision science experiments, these desired wavefronts could be known combinations of Zernike polynomials (e.g., adding spherical aberration to induce increase depth of focus at the expense of some transverse resolution in the focal plane).

Our survey shows that two methods are used for generating the reference wavefronts, with both of them using the output of a single-mode optical fiber as a light point source. In the first method, which we term the “free-space” method, the fiber is positioned a known distance (2-4 m) from a wavefront sensor pupil conjugate, and the response that would be seen by a flat wavefront is then calculated based on the known distance. This approach requires a long setup, that might be susceptible to air turbulence (that could be mitigated by averaging multiple measurements) and that requires one or more fold mirrors, each of which could introduce aberrations. In the second method, the light from the fiber is collimated by lens, typically an achromatic doublet with focal length greater than 250 mm. This results in a more compact setup at the expense of including the aberrations of the lens into the reference wavefront. The choice of pupil plane, which is not affected by the choice of the previous methods, might not be obvious. If the wavefront is delivered at the pupil plane where the eye is placed, then by the time it reaches the wavefront sensor, the wavefront will have been distorted by the aberrations of the entire optical setup, and that would result in the AO only correcting the aberrations of the eye, but not those of the optical system. If at the other extreme, we choose the pupil plane of the wavefront sensor itself, there might be optical elements that are not common to the imaging channels that are missed, and thus the AO would end up with larger non-common path aberrations. Thus, ideally, one should choose the pupil plane that both maximizes the measurement of the aberrations of the optical path common to the illumination, imaging and wavefront sensor, while also minimizing the non-common path aberrations.

Current wavefront corrector response calibration methods assume that the relationship between the wavefront corrector control signals and the wavefront sensor response is linear (even though the actual signals send to a physical device such as an electrostatic deformable mirror might be quadratic). The most widely used wavefront corrector basis if that formed by the individual actuators. The calibration itself consists in applying a set of wavefront corrector basis functions, one at a time first with a positive amplitude and then with a negative amplitude (not necessarily the same as the positive amplitude and potentially different across actuators). Then the difference between the positive and negative wavefront sensor responses are calculated and divided by the corresponding amplitude differences. The response matrix, also referred to a “poke” or “forward” matrix, is then assembled by placing the resulting vectors as columns in a matrix, with each column corresponding to a wavefront corrector basis element.

Unlike for the reference wavefront calibration, the choice of pupil plane for measuring the response matrix is not critical, but the choice of whether the calibration beam passes once (single-pass) or twice (double-pass) through the wavefront corrector. The single-pass illumination requires additional optics and turning the optical scanners used to create the imaging raster off. In this configuration, the wavefront sensor “sees” (i.e., measures) the tip and tilt induced by the wavefront corrector(s), and thus it is the preferable approach. The double-pass usually is achieved by using the AO ophthalmoscope beacon and a model eye at the end of the ophthalmoscope with a scattering retina (e.g., a piece of paper) with the optical scanners used to create the imaging raster turned on. Here, it is assumed that the wavefront induced in the first pass through the wavefront corrector is erased, and that the wavefront sensor only sees the aberrations induced by the wavefront corrector in the second pass, other than for tip and tilt (if the beam is focused on the model retina). This approach is less desirable because it requires removing tip and tilt from the AO control loop signals, which can only be done assuming a “calculated,” as opposed to an experimental tilt. This will result in poorer wavefront correction and sometimes in unstable AO correction.

The response matrix is then “inverted” to calculate the AO control matrix. An exact inverse matrix rarely exists because the linear system that links the wavefront sensor and corrector is overdetermined and affected by noise. Thus, a least-squares approximation, the Moore-Penrose pseudoinverse, is used instead with some manipulation to remove some system modes, which in the singular value decomposition of the response matrix have low values. The idea here is that if the system has modes to which is not very sensitive (meaning that a large wavefront corrector signal would result in a small change of the wavefront sensor output) could result in large erroneous wavefront control signals in the presence of wavefront sensor noise. Practically, this often manifests as either the AO closed-loop becoming unstable and/or the wavefront corrector saturating in the absence of any noticeable aberration. Typically, the method to remove the offending system modes is to use the singular value decomposition to calculate the pseudoinverse of the response matrix, replacing the corresponding inverse of the singular values with zeros. An alternative method, is the Tikhonov regularization, in which small values are added to some/all the singular values before inversion (Burns), effectively reducing the inverse of the singular values, and thus having a similar (but milder) effect to the other method.

Another AO calibration step, that is currently not widely adopted is the measurement of the aberrations in the non-common optical path between the wavefront sensing and illumination and/or imaging channels in the AO ophthalmoscope. This measurement is easiest in channels in which the illumination and imaging share optics, such as most AO-OCT ophthalmoscopes, with the approach driven by the exploration of the wavefront corrector space to maximize an image sharpness or intensity metric. When the illumination and imaging portions of an imaging channel have non-overlapping optics, as it is the case in most AOSLOs, then a choice has to be made about whether to correct the aberrations of the illumination or the imaging path. Because the confocal detection aperture is almost always comparable to or larger than that of the core of the single-mode fiber used for illumination (relative to the Airy disk), then the correction of the aberrations in the illumination path should be prioritized. The calibration of aberrations on this path should be pursued by exploring the wavefront corrector space while seeking to maximize an image sharpness and using the largest possible confocal aperture (or no confocal aperture at all).

**SHWS calibration**

An additional calibration step is necessary to perform wavefront reconstruction in terms of Zernike polynomials or other basis (other than that of the wavefront corrector(s)). In a SHWS, this step is the measurement of the distance between the lenslet array and the sensor, scaled by any magnifying optics to the pupil plane of interest. This can be achieved by illuminating the wavefront sensor with a point source at a known distance and capturing various measurements (a minimum of two) while translating the source by known distances. If the translation is within a plane perpendicular to the optical axis of the SHWS and the source light is collimated by a lens, then the measurement will yield tilt, while if it is along the optical axis it will yield defocus. Although both types of translation are acceptable, most users prefer to measure tilt.

**AO control and operation**

AO control algorithms in AO ophthalmoscopes are rarely described in the literature. Our survey indicates that they have two modes of operation, one which we term asynchronous, in which the SWHS camera operates in free-run mode (i.e., capturing one frame after another as soon as possible) and the wavefront corrector is updated whenever new control signals are available, irrespective of where in the capture cycle the SHWS camera is in. In this mode, if the control algorithm is an integrator, the gain has to be low (e.g., 0.3) and is related to the latency between the capture of a SHWS frame and the update of the wavefront corrector. If the gain is high, then the AO correction becomes unstable.

The alternative mode is “synchronous,” (this is not the best terms, as explained below) with the SHWS image capture happening after the wavefront corrector was updated and had time to settle the control signals that resulted from the previous SHWS measurement. In this approach, the frame rate is lower, the retinal light exposure is proportionally lower and identical correction bandwidth is achieved because higher gains (up to 1.0) are possible. Hence, this operation mode is preferred.

In scanning AO ophthalmoscopes, the camera exposure should either matching the inverse of the ophthalmoscope frame rate (e.g., 60 ms for a 16.67 Hz frame rate), or be synchronous with it. In the latter case, for example, the active portion of the scanning raster (i.e., the time during which the image is captured) is shorter (e.g., 40 instead of 60 ms). Either solution ensures that the aberrations are measured (and averaged) across the field of view. In AOSLOs, due to the resonant scanner slowing down towards the edges of the field of view, this gives slightly higher weight to the aberrations on the left and right edges of the field of view.

Some AO control integrators allow the possibility of “bleeding” the wavefront, that is the removal of a small fraction of the previous wavefront correction signals to avoid the creeping up of unsensed modes. This capability is currently implemented but no longer used, as it is problematic in subjects with large spectacle prescription, as even a small bleed (e.g., 2%) could have a dramatic effect in image sharpness.

Currently, no wavefront sensing prediction is being explored or implemented.

Often, the pupil of the subject is smaller than that of the SHWS and/or the tear film breaks, leading to some SHWS lenslet images being diffuse or dark. How this is handled varies across teams. A popular approach is to force the spot displacement to be zero for these lenslets that have a low signal-to-noise ratio (SNR) and keep using the same control matrix. An alternative approach, and one that should be preferred, when computational resources allow doing so in real time, is to re-calculate the control matrix, starting by removing the rows of the response matrix that correspond to the SHWS lenslets with low SNR.