RAPID RESPONSE

Methodological concerns and a note of caution on repeatability of cone density measurements with the Mona II AO-SLO system

In response to Jia et al., British Journal of Ophthalmology (doi: 10.1136/bjo-2025-329013)

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Dear Editors of BJO,

We read with interest the study by Jia and colleagues (doi: 10.1136/bjo-2025-329013) reporting excellent repeatability of cone photoreceptor measurements with the Mona II AO-SLO system and wish to raise two methodological concerns that we believe substantially limit the paper's conclusions. We close with a broader note of caution regarding the clinical interpretation of the data from this device.

The repeatability design is fundamentally flawed, and its presentation compounds this.

The study's central claim rests on a design in which three images were selected from a single imaging session at each retinal location, and ICC was computed across those three images. This is not repeatability in any clinically meaningful sense: it measures within-session frame-to-frame consistency and, in effect, tests whether the automated detection software returns consistent output when applied to the mostly identical pre-selected images. That a deterministic algorithm does so is unremarkable. True repeatability requires independent re-imaging on separate occasions, ideally by different operators, to capture the sources of variability that matter in longitudinal clinical use: day-to-day fluctuation in image quality, alignment, tear film, optical correction, and operator performance. The authors do recognize this limitation in the discussion but do not adequately address its severe consequence.

The presentation of these values compounds the problem. Table 1 reports a pooled ICC of 0.994 for cone density across the full 4.2° field as a headline result. ICC computed over a heterogeneous field spanning locations whose true cone densities differ by more than a factor of two from center to periphery will be strongly inflated by large between-subject and between-location variance, irrespective of actual measurement precision.[1] A high pooled ICC is in this context a statistical near-inevitability. By collapsing across eccentricities with very different ground-truth densities, this metric is essentially guaranteed to yield good values while masking location-specific poor performance. We note that removing this data from the paper would not resolve the underlying concerns: the location-specific ICCs in Tables 2 and 3 are subject to the same fundamental limitation; they remain within-session software consistency metrics and do also pool across a considerable density variation within a 2.4 deg field.

Absolute cone density values seem underestimated across a large part of the imaged area.

The authors acknowledge that the system cannot resolve individual cones within the central 300 µm, attributing this to "inadequate resolution." This is a known limitation of the Mona II device, and we

do not dwell on it here. It is nevertheless worth noting the internal contradiction: despite this admission, the foveal ICC of 0.992 at (0°,0°) is highlighted as the study's strongest result, and data tables as well as Fig. 2 and the abstract still present these data points. A coarse read might not catch that these quantities are completely artefactual and that the given density values do not represent the biology in any way.

More concerning is that systematic underestimation persists well beyond the foveolar center, at eccentricities where individual cones should be fully resolvable, possibly due to density pooling across large areas. At 1.5° (approximately 435 µm) eccentricity, this study reports 29,000–33,000 cells/mm² across meridians, compared with around 38,000 cells/mm² from normative research-grade AOSLO data with full cone annotation, or histology.[2] This discrepancy, and its eccentricity-dependence, must be characterized explicitly before any clinical interpretation of absolute values can be considered valid. The authors again acknowledge the missing benchmark as a limitation, but nonetheless omit to discuss it in their paper. A persistent underestimation in healthy eyes risks reduced sensitivity for detecting true photoreceptor loss in disease, and even relative comparisons between groups become difficult to interpret when the magnitude of this offset is unknown: a measurement whose relationship to the underlying structure is not characterized cannot be assumed to track change in it reliably, in either direction.

A broader note of caution

The present paper does not emerge in isolation. We have previously raised the issue of discordant absolute cone density values obtained with this device in published correspondence[3], a concern that was not adequately addressed. It is in this context that the current repeatability study presents a particular danger: without itself being benchmarked against biological ground truth, it risks serving as implicit methodological validation for the device's absolute density measurements across the broader literature, lending credibility to a body of work whose quantitative foundation has not been established.

Finally, we wish to be clear that a validated commercial AO-SLO system would be genuinely welcome: widening access to cone-resolved retinal imaging beyond specialized research laboratories would benefit both clinical practice and science enormously. The field would likely embrace such a device, provided its measurements are shown to be not only repeatable but accurate. We therefore encourage the journal to require direct benchmarking of absolute cone density values against established normative data as a standard condition, to ensure an adequate quality assessment of the data.

References

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Submitted as a Rapid Response to the British Journal of Ophthalmology (article doi: 10.1136/bjo-2025-329013), published 23. June 2026