

Evaluating the Effect of Optical Restoration on Retinal Imaging Quality in Fixed Post-Mortem Human Eyes

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Introduction

- The retina is an extension of the central nervous system providing a non-invasive window into neural and vascular brain health
- Clinical eye imaging modalities, including confocal laser scanning ophthalmology (cSLO) and optical coherence tomography (OCT), enable *in vivo* assessment of retinal vasculature



- Post-mortem human eye specimens provide a unique opportunity for improved clinicopathological correlation
- However, post-mortem changes reduce optical clarity and image quality, limiting visualization of retinal structures
- Optical clearing techniques may improve transparency and restore visualization of retinal vasculature, increasing utility for biomarker discovery and clinicopathological correlation

Purpose: To evaluate whether optical and corneal restoration can improve imaging quality and visualization of retinal structures in fixed post-mortem human eyes

Methods

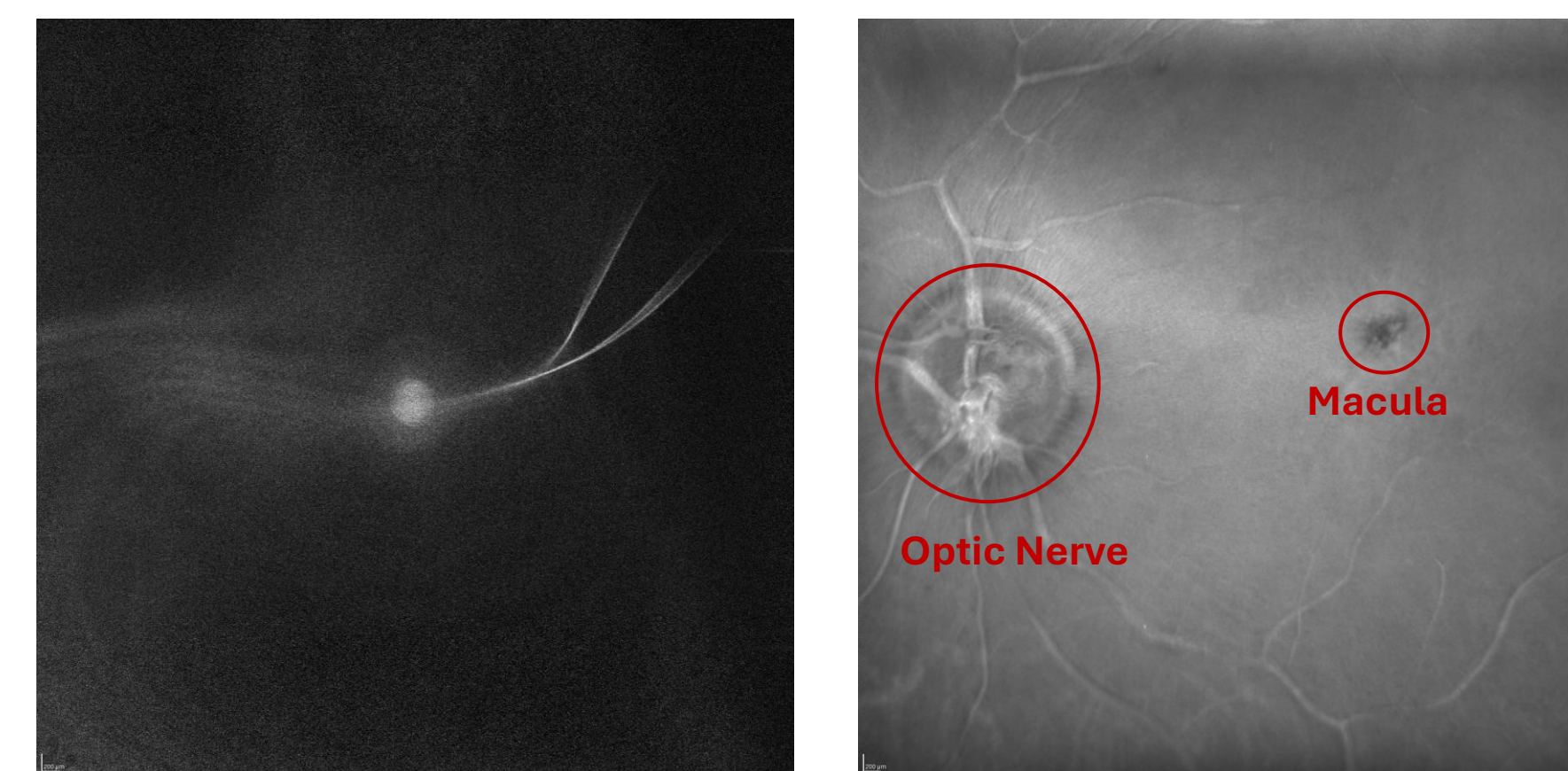
- Paired pre-post intervention pilot study (n = 10 post-mortem human eyes)
 - Approved by institutional Research Ethics Board
- Intervention of 1-hour incubation in optical clearing reagent
- Retinal imaging was performed before and after intervention with Heidelberg Spectralis device (Spectralis, Heidelberg Engineering)

Image Analysis:

- Masked images were processed and analyzed using ImageJ (ImageJ, NIH)
- cSLO images assessed for vascular visualization
 - Quantified with integrated density; evaluating vessel brightness and extent of visible vasculature
- OCT images assessed for deeper layer signal intensity
 - Quantified with mean gray value; evaluating average signal intensity within a defined region of interest underlying the retina
- Pre- and post-intervention measurements were compared using the Wilcoxon signed-rank test

Results

Paired Pre-Post cSLO Image (Male)

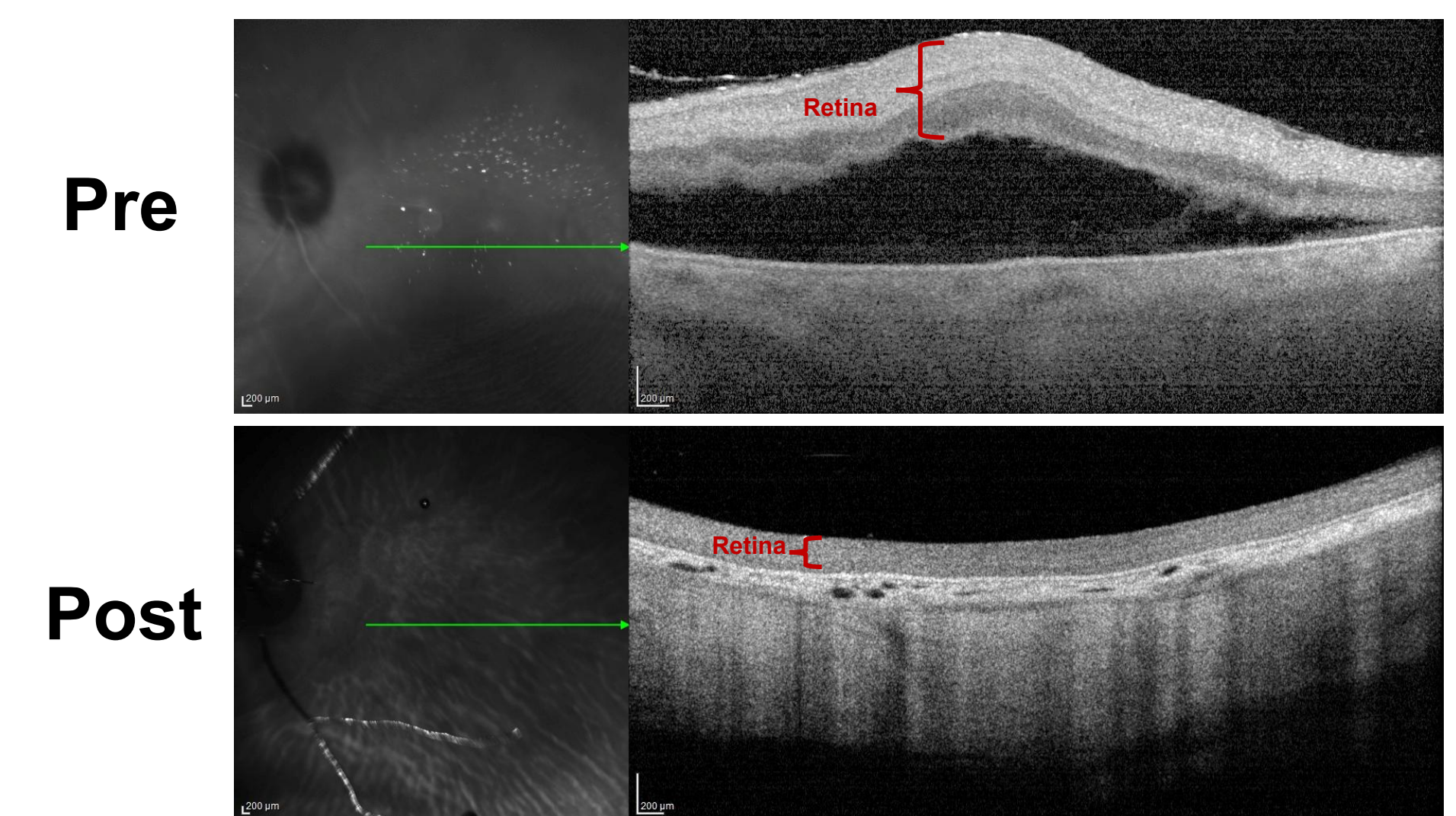


Pre

Post

Pre- and post-intervention cSLO images of a male post-mortem left eye. The optic nerve and macula are identified.

Paired Pre-Post cSLO and OCT Images (Female)

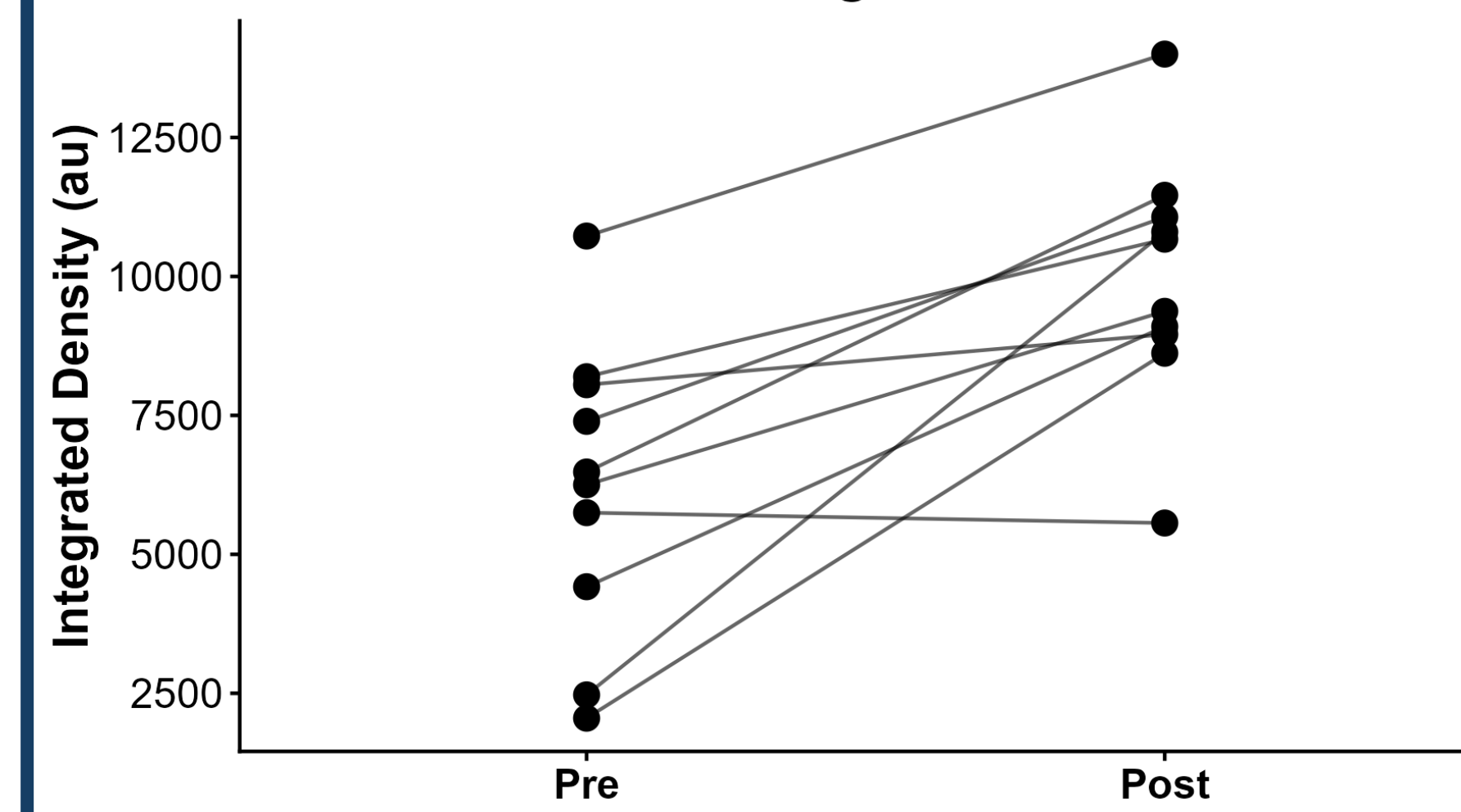


Pre

Post

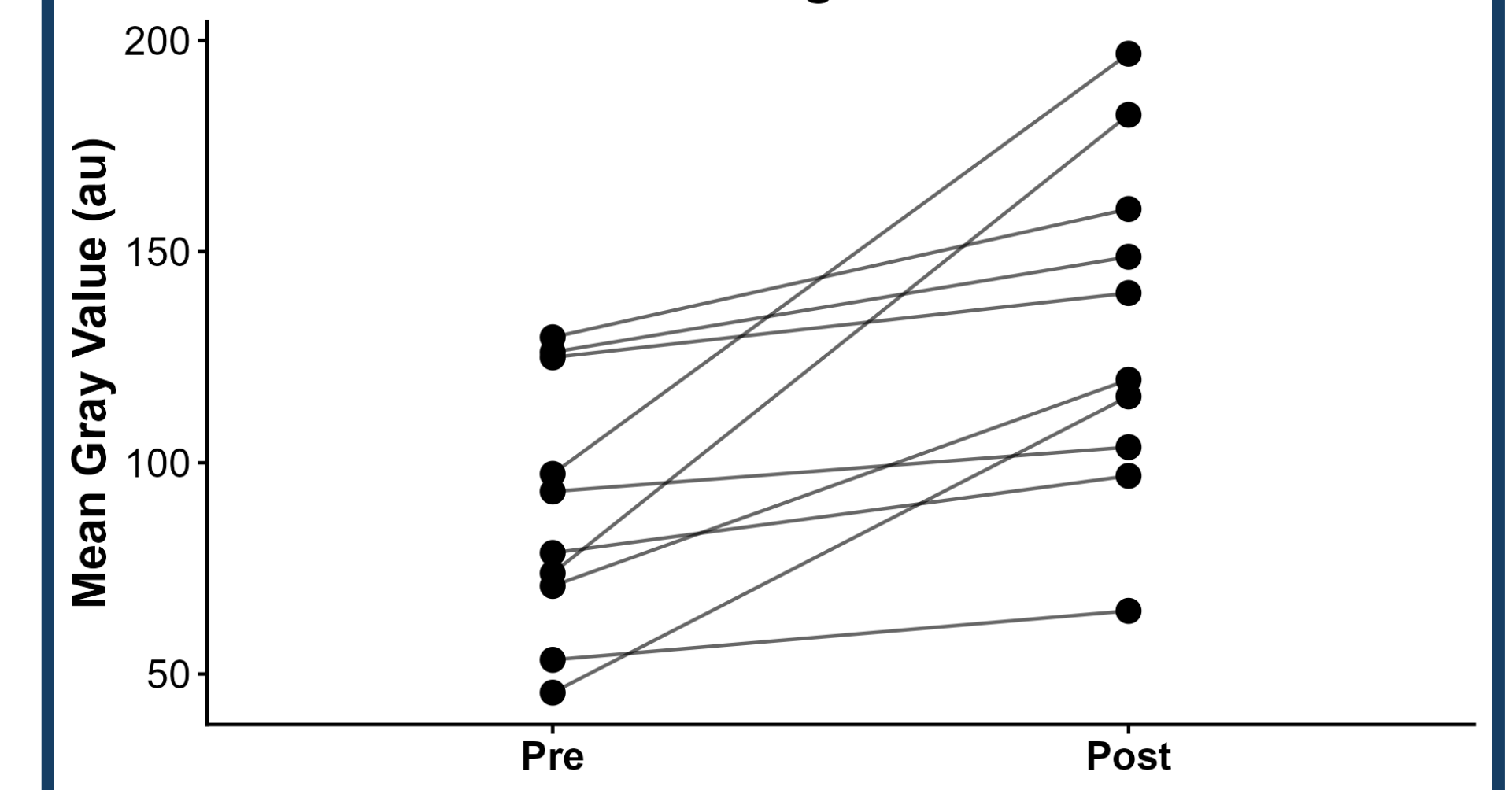
Pre- and post-intervention cSLO (left) and OCT (right) images of a female post-mortem left eye. The green horizontal line indicates the level of OCT scan. The retina is identified.

Increase in cSLO Integrated Density Following Intervention



Paired pre- and post-intervention cSLO integrated density measurements (n = 10).
The mean increase was 3782.09 ± 2519.17 au ($p = 0.004$) (Wilcoxon signed-rank test).

Increase in OCT Mean Gray Value Following Intervention



Paired pre- and post-intervention OCT mean gray value measurements (n = 10).
The mean increase was 43.51 ± 36.82 au ($p = 0.002$) (Wilcoxon signed-rank test).

Conclusion

- Optical clearing techniques significantly improved retinal image quality in post-mortem human eyes
- Enhanced retinal vascular visualization on cSLO and increased deeper signal intensity on OCT seen following intervention, supporting the feasibility of optical restoration of post-mortem eyes
- Further studies with larger sample sizes are warranted to validate these findings and assess potential applications in ocular biomarker research

References

Kam PK, Abdulla WH. Advancing Ocular Imaging: A Hybrid Attention Mechanism-Based U-Net Model for Precise Segmentation of Sub-Retinal Layers in OCT Images. *Bioengineering* 2024;11:240–240. doi:https://doi.org/10.3390/bioengineering11030240.

Nioi M, Napoli PE, Demontis R, et al. Postmortem Ocular Findings in the Optical Coherence Tomography Era: A Proof of Concept Study Based on Six Forensic Cases. *Diagnostics* 2021;11:413. doi:https://doi.org/10.3390/diagnostics11030413.

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