

Automated Optical Density Normalization for Myelin Quantification

Cross-modal validation with 7T *ex vivo* MRI across 151 histology sections

Zahra Khodakarami, Sheina Emrani, Pulkit Khandelwal, Chinmayee Athalye, Amanda Denning, Winifred Trotman, Lisa M. Levorse, Eric Teunissen-Bermeo, Hamsanandini Radhakrishnan, Daniel Ohm, Christopher Olm, Noah Capp, Ranjit Ittyerah, Karthik Prabhakaran, John A. Detre, Sandhitsu R. Das, David A. Wolk, Corey T. McMillan, Gabor Mizsei, M. Dylan Tisdall, David J. Irwin, John L. Robinson, Edward B. Lee, Paul A. Yushkevich

University of Pennsylvania

Contact: zkm@seas.upenn.edu

Code: github.com/zkhodakarami/myelin_quantification

01 BACKGROUND

White matter hyperintensities on T2-weighted MRI are robust markers of aging and dementia, but they are **pathologically heterogeneous**, only a fraction reflect demyelination.

Luxol Fast Blue (LFB) optical density (OD) is the standard quantitative proxy for myelin, but **staining variability across slides drowns out the biological signal**. Existing color-based normalization methods don't encode what *intact myelin* looks like.

OUR REFRAME

Find intact myelin in **each slide**, then calibrate against it. We leverage a pretrained pathology-detection CLAM model, its **lowest-attention** white matter marks intact tissue, giving annotation-free reference selection.

02 METHODS

DATASET

321 LFB-CV whole-slide images from **45 hemispheres** across the AD continuum

151 sections / 31 hemispheres with co-registered 7T T2w MRI (0.3 mm³ isotropic) for cross-modal evaluation.

PIPELINE

1 White matter segmentation

nnU-Net v2 · Dice = 0.896 on held-out test

2 Blind stain deconvolution

SVD-based angular peak detection, no predefined vectors

3 CLAM-driven reference discovery

SLIC superpixels × inverted attention → lowest 2nd percentile = ref.

4 Four normalization strategies

raw / internal / calibrated (β = 1.15) / batch-normalized

VALIDATION

Expert ref.
placement n = 84

Expert grading
n = 97 ROIs

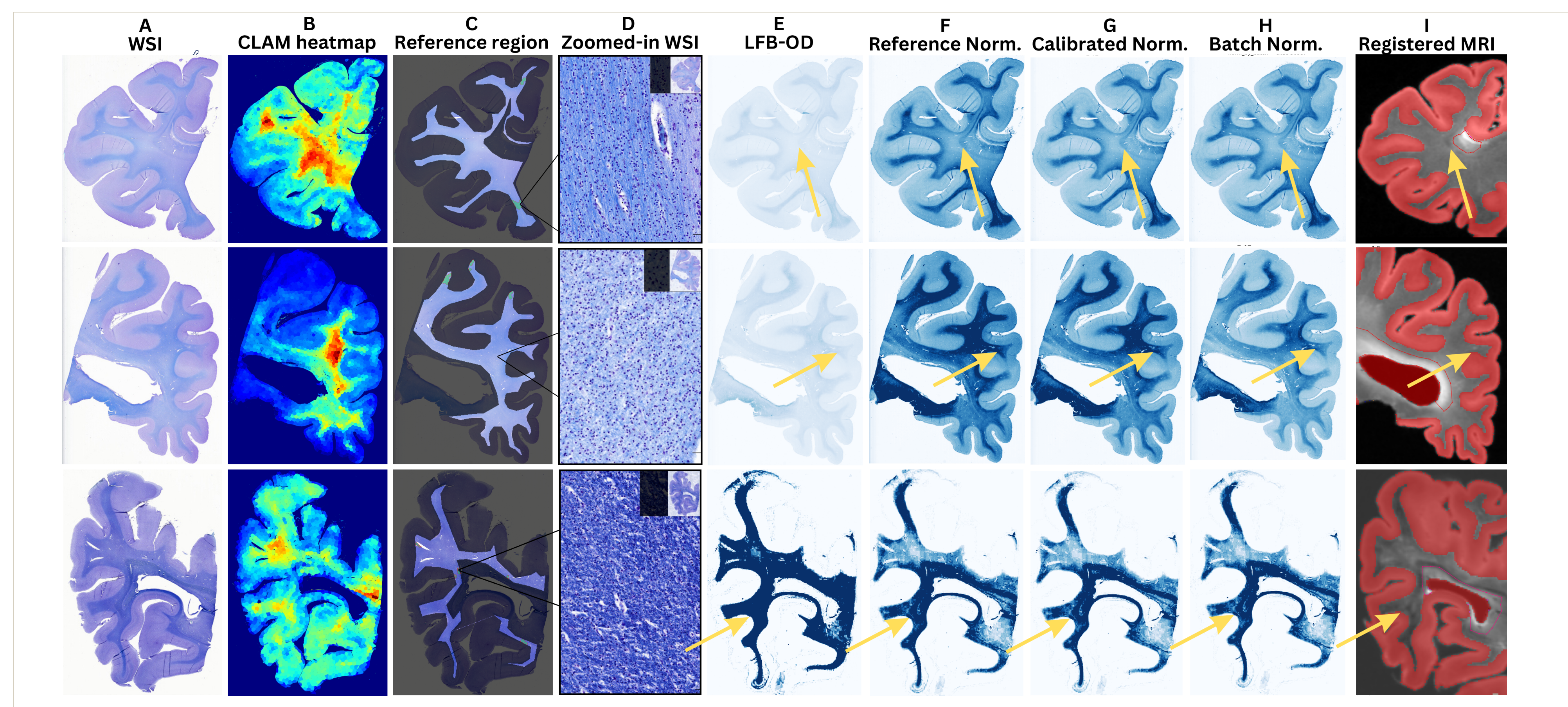
Voxel-wise vs.
MRI n = 151

07 LIMITATIONS

- Single MRI protocol.** Generalizability to other T2 sequences (FLAIR, SWI) remains to be established in follow-up work.
- Batch normalization requires multiple slides** from the same staining session; internal normalization provides a practical single-slide fallback.
- Microscopic registration error** bounds the achievable voxel-wise correlation, shift analysis shows the ceiling near $\rho \approx -0.42$.

FIG. 1 PIPELINE OVERVIEW

From whole-slide image to cross-modal MRI validation



(A) Whole-slide image → (B) **CLAM attention heatmap** → (C, D) lowest-attention superpixels selected as the **intact-myelin reference region** → (E) LFB optical density via blind stain deconvolution → (F–H) per-slide, calibrated, and batch-calibrated **normalization strategies** → (I) co-registered **7T T2w MRI** for cross-modal validation (white matter unmasked in red, WMH outlined). *Top two rows: same staining batch; bottom row: different hemisphere and batch, illustrating the cross-batch variability the pipeline corrects.*

03 RESULTS

CLAM ≈ EXPERT

r = 0.91

Annotation-free reference selection matches expert placement across 84 slides.

PATHOLOGY TRACKING

ρ = -0.64

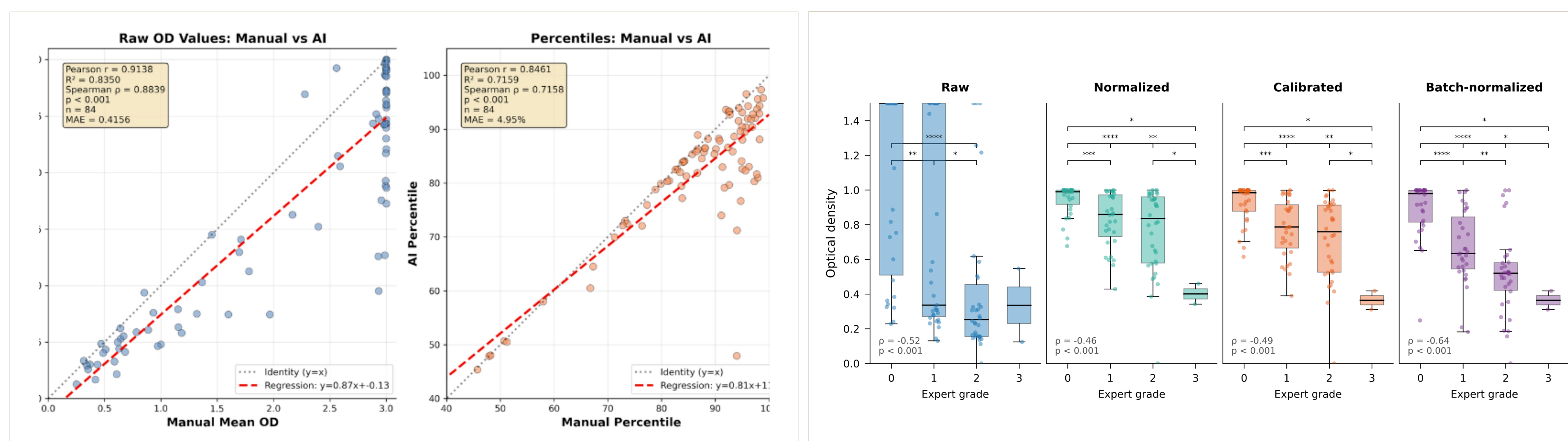
Batch-normalized OD vs. expert myelin-loss grades. AUC = 0.90 intact vs. demyelinated.

NOISE SUPPRESSED

178% → 18.5%

Within-grade coefficient of variation collapses after batch normalization.

FIG. 2, 3 CLAM vs. expert reference placement, OD by myelin-loss grade across 4 methods



n = 84 slides. Left: mean raw LFB-OD (Pearson $r = 0.91$). Right: percentile-transformed within slide ($r = 0.85$). Both confirm strong concordance with manual annotation.

97 ROIs · Deramecourt grades 0–3. Batch normalization (right) shows the steepest monotonic decrease and tightest within-grade dispersion.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

04 CROSS-MODAL EVIDENCE

TABLE 1 Voxel-wise vs. 7T T2w MRI

Metric	Raw	Intern.	Calib.	Batch
ALL WHITE MATTER · n = 151				
Spearman ρ	-23.8	-35.6	-36.6	-37.9
Best shift ρ	-27.9	-40.3	-41.3	-42.4
Pearson r	-19.6	-31.0	-32.6	-33.5
Dice	32.9	42.6	42.8	43.2
AUC-ROC	62.0	68.8	69.3	69.8
WMH ONLY · n = 125				
Spearman ρ	-23.0	-32.8	-33.1	-33.6
Pearson r	-22.8	-33.0	-33.4	-33.4

Values $\times 10^{-2}$. Best per metric in **red**.

Significant voxel-wise correlation in **148 / 151 sections (98%)** of white matter and **108 / 125 (86%)** within WMH. *Largest voxel-wise histology–MRI myelin study to date.*

05 TAKE-AWAYS

- **Annotation-free reference discovery.** A pathology-detection network, inverted, finds intact tissue. No manual labels.
- **Batch normalization wins.** Most biologically grounded OD measure across both expert grades and MRI.
- **Generalizes across cohorts.** Shared LFB chromogen → CLAM transfer works across datasets and scanners.
- **Ground truth for in vivo FLAIR.** Calibrates regional myelin-loss prediction from clinical MRI.

06 DISCUSSION & IMPACT

WHY IT WORKS

The shared LFB chromogen preserves the feature space for myelin pallor, so a CLAM model trained on a different cohort still places attention correctly. Inverting the attention map is then equivalent to detecting **intact tissue**, the network's negative-class evidence. Batch normalization then removes residual session-level intensity drift that no single-slide method can correct.

WHAT IT UNLOCKS

Calibrated, voxel-aligned myelin OD maps act as **ground truth** for training cross-modal encoders that predict regional myelin loss from **in vivo FLAIR MRI**, moving WMH analysis from coarse volumetric scoring toward pathologically specific biomarkers usable at the bedside.

08 KEY REFERENCES

- [1] Athalye C, et al. Operationalizing postmortem pathology-MRI association studies in AD. *Acta Neuropathol Commun* (2025).
- [2] McKenzie AT, et al. Interpretable deep learning of myelin histopathology. *Acta Neuropathol Commun* (2022).
- [3] Isensee F, et al. nnU-Net: A self-configuring method for biomedical segmentation. *Nat Methods* (2021).
- [4] Wardlaw JM, et al. What are White Matter Hyperintensities Made of? *J Am Heart Assoc* (2015).