

Toward closed-loop laminar fMRI: A real-time framework at 9.4T and single-block reliability of layer-resolved signals

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Introduction

Cortical computations are organized across layers, with feedforward and feedback pathways exhibiting distinct laminar activity patterns.¹ Real-time access to depth-resolved signals would enable researchers to monitor and modulate these dynamics during ongoing cognition, advancing our understanding of how cortical layers contribute to information processing and paving the way for laminar-level neurofeedback applications. However, achieving real-time laminar fMRI requires submillimeter spatial resolution, precise anatomical–functional alignment, and processing that keeps pace with the MRI repetition time, posing significant challenges at ultra-high field.² A key unresolved question is whether laminar profiles exhibit sufficient signal-to-noise ratio (SNR) and reliability to support closed-loop applications, which place particularly stringent demands on signal detectability. This study therefore pursues two complementary aims: (1) to introduce and demonstrate a real-time laminar fMRI framework at 9.4 Tesla, and (2) to characterize single-block laminar profile reliability in primary visual cortex (V1) using an offline analysis.

Methods

Participants. Three healthy adult participants were scanned at 9.4 Tesla using a GE-BOLD EPI acquisition (0.8 mm isotropic). One participant contributed to the real-time framework validation. Two additional participants completed a checkerboard paradigm with four conditions (2 Hz / 8 Hz temporal frequency $\times$ low / high contrast) in a blocked design (16 s on / 16 s off, 12 cycles per run; sub-01: 6 runs, sub-02: 4 runs) for the offline single-block reliability analysis.

Real-time framework. We developed a two-session protocol combining gold-standard ultra-high-field laminar fMRI preprocessing with a low-latency real-time pipeline. In session 1, high-resolution anatomical and functional scans were acquired to reconstruct cortical surfaces and define depth masks using established surface-based registration pipelines.³ These computationally intensive offline procedures yielded highly accurate subject-specific depth masks, while allowing for manual quality control and refinement where necessary. In session 2, these precomputed layer masks were mapped into the native acquisition space, enabling real-time extraction of depth-specific signals via a custom Pyneal-based pipeline⁵ with parallel processing architecture (Figure 1a).

Offline single-block reliability analysis. To assess whether laminar profiles are detectable at the single-block level, we designed a split-half reliability analysis. The checkerboard paradigm was chosen to emphasize feedforward-dominated responses in V1, expected to produce a characteristic middle-depth peak in the laminar profile. Cortical columns were defined using LayNii⁴ (~4,000 whole-brain) with quality criteria ensuring sufficient voxel and layer coverage. The example column shown (column 2957, left V1) contained 61 voxels spanning 9 cortical depth bins. First-half runs were used to estimate a per-column laminar reference template, with columns selected based on correlation with a Gaussian feedforward template ($r \geq 0.3$). Second-half runs were used for single-trial beta estimation via LSS GLM,⁶ and per-block percent signal change (PSC) laminar profiles were compared to the first-half template using Pearson r (Figure 1b).

Results

Real-time framework. Real-time laminar signals were successfully detected and tracked across cortical depths during online acquisition, demonstrating the feasibility of our real-time laminar fMRI pipeline. Across 168 volumes, mean processing time was 2.19 s per volume (range: 2.16–2.23 s, SD $\approx$ 0.03 s).

Although each volume exceeded the 2 s TR by ~0.19 s, the parallel processing architecture prevented cumulative latency, maintaining real-time performance throughout.

Single-block laminar reliability. Single-block laminar profiles showed substantial similarity to the independently estimated template (median Pearson $r = 0.77$) in 74 columns, with 58.1% of individual blocks achieving $r \geq 0.7$. An exemplary V1 column exhibited very high template similarity ($r \geq 0.94$), with clear feedforward-like laminar profiles peaking at middle cortical depths. These responses were strongest for high-contrast and 8-Hz checkerboard stimulation, consistent with the known ground-truth response properties of V1, and were reliably tracked at the individual block level (Figure 1b).

Conclusions

We present the first real-time laminar fMRI framework at 9.4 Tesla, combining state-of-the-art laminar precision with low-latency online processing. The offline reliability analysis demonstrates that selected V1 columns carry sufficient SNR for detection at the single-block level. Importantly, the recovered layer profiles exhibited the expected feedforward-dominated shape and stimulus-dependent modulation, with stronger and more reliable responses for high-contrast and 8-Hz stimulation, consistent with established V1 physiology. These findings suggest that meaningful laminar signals can be recovered with sufficient fidelity to support real-time applications. Key limitations include the within-session nature of the out-of-sample reliability estimates; cross-session template stability remains to be established in future work. Together, these results provide a principled foundation for closed-loop laminar fMRI experiments and future neurofeedback applications targeting layer-specific cortical dynamics.

References

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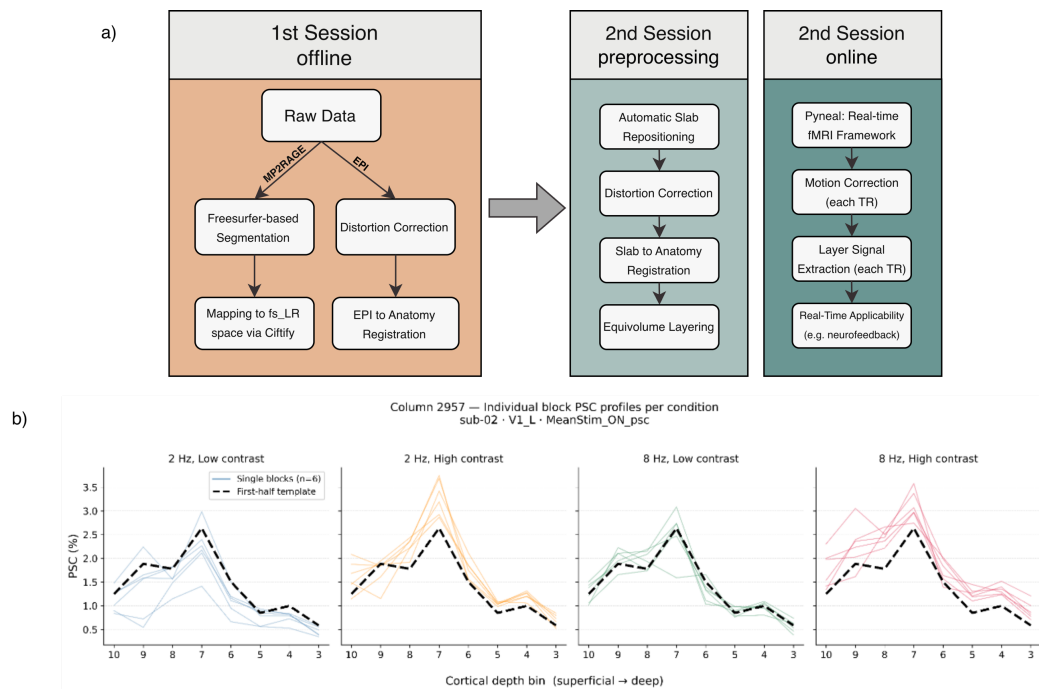


Figure 1. Real-time laminar fMRI framework and single-block reliability validation. (a) Two-session protocol: Session 1 involves offline anatomical processing and registration; Session 2 combines preprocessing with real-time layer signal extraction via Pyneal. (b) Individual block PSC profiles for an example V1 column (column 2957, sub-02; 61 voxels, 9 depth bins). Each line represents a single stimulation block; the black dashed line is the independently estimated first-half template. The consistent middle-depth peak across all four conditions reflects the expected feedforward laminar activation pattern of V1, reliably detectable at the individual block level. Response amplitude is strongest for high-contrast and 8-Hz stimulation, consistent with known V1 physiology.