Reviewer comments

Dear Editor,

We have prepared a revision of our manuscript titled: “*A selection and targeting framework of cortical locations for line-scanning fMRI*” for publication in Human Brain Mapping. We have edited the manuscripts according to the reviewer’s and associate editor’s points. We would like to thank the Editor and the Reviewers for their useful comments, which we feel have improved the manuscript significantly.

On behalf of the co-authors,

Sincerely,

Jurjen Heij

*We would like to thank both reviewers for their time and careful reading of the manuscript. A point-by-point list of the amendments we have made is provided below. All changes in the manuscript are highlighted in the annotated version.*

Reviewer 1

The manuscript entitled “A selection and targeting framework of cortical location for line-scanning fMRI” describes the workflow of how to use previously described line-scanning sequences and embed them in future experimental setups of neuroscientific application studies.

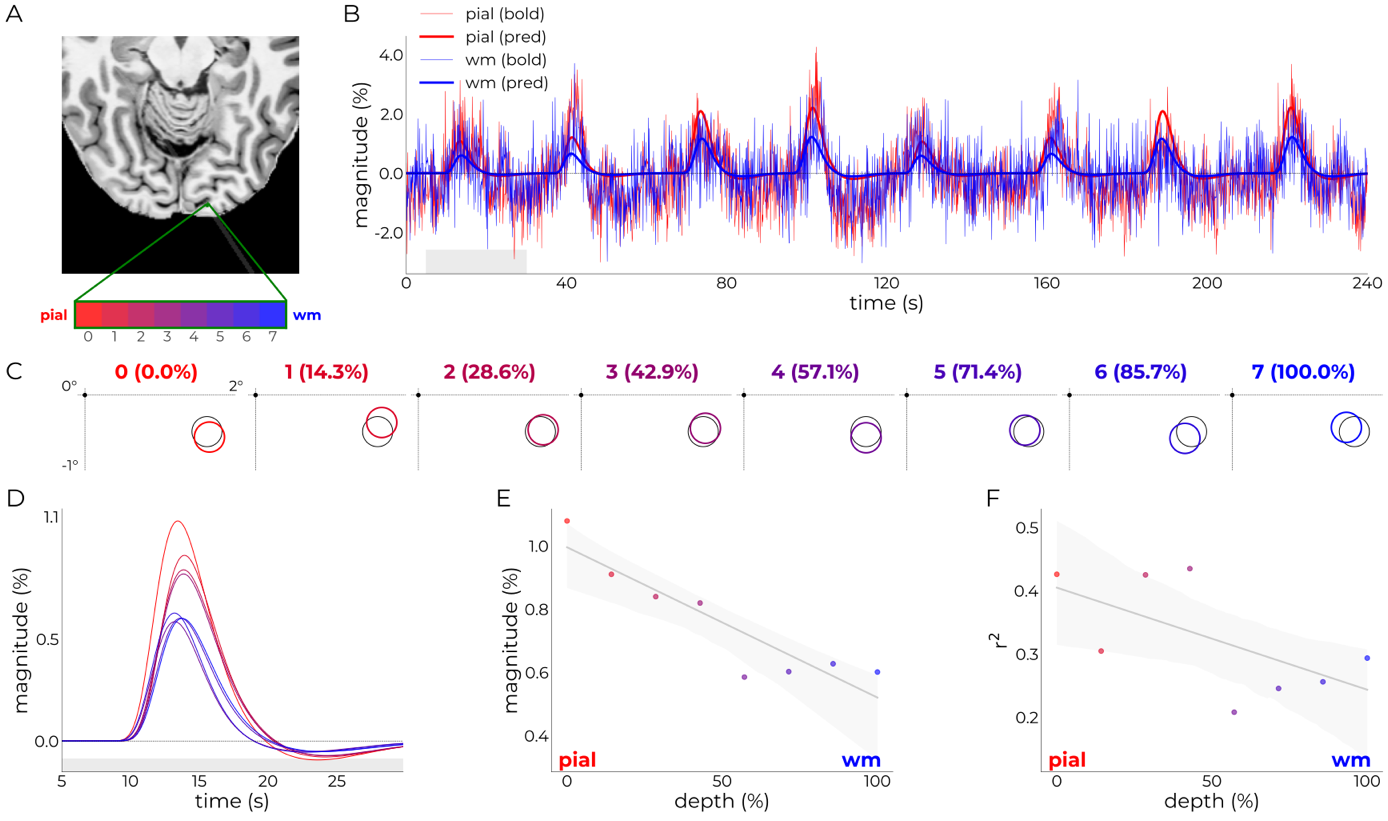
Line-scanning fMRI in humans is an emerging method in the field of layer-fMRI imaging. There are a handful of labs that are actively pursuing it. This method promises higher sampling rates across time and layers. It remains to be seen however, if the higher sampling rates translate to a detectability of more fine-scale signatures of neural activity. The sluggish HRF and the large draining veins in GE-BOLD might blur the spatio-temporal activity beyond the dense data sampling in line-scanning. This study brings the field a bit closer to finding out.

The study represents a substantial technical achievement to make line-scanning more useful and bring it closer to neuroscientific applications and I believe it will be of significant interest to the technically focused readership of HBM. I was particularly impressed by the vertex gymnastics described here to target specific lines in the right coordinate system of the scanner in the second session.

*We thank the reviewer for their kind comments on the manuscript.*

After reading the introduction on the great potential of line-scanning to bridge the gap towards electrophysiology with their spatial and temporal resolutions, I was a bit disappointed by the results. This manuscript does not show any high-resolution data, whatsoever.

While the layer-sampling density is 0.25mm, no layer profiles are presented. While the temporal sampling is 105ms, the only shown time course (Fig. 4) refers to temporally low-pass filtered smooth data. I am aware however, that the focus of this manuscript is chosen to be on the experimental framework. The authors do not make any claim that line-scanning can reveal fine scale features that are not visible with conventional mesoscale imaging. Thus, my disappointment should not be misunderstood as a low reviewer score.



*We understand the feelings expressed by the reviewer. Indeed, the main focus of the manuscript was to highlight the experimental setup. To address the reviewer’s remark, we have added a new figure (Figure 6) to showcase the spatial and temporal resolution of line-scanning (p17). This figure shows an axial slice from T1-image with the imaged line in white shading and the relevant voxels highlighted in green (Figure 6A), raw time courses for a superficial and deep voxel (Figure 6B), and the position estimates (Figure 6C), response magnitude (Figure 6DE), and variance explained (Figure 6F) of all voxels within the cortical ribbon for a single subject, highlighting the fine spatial detail of these data:*

“For one of these subjects, we show a more detailed profile of depth-dependent measures by modeling all voxels covering the ribbon *independently* (Figure 6A). For this subject, the time courses of superficial and deep layers exhibited the classical pattern (Figure 6B), where the magnitude of the superficial time courses was almost double the magnitude of the deeper time course. The position estimates were stable across cortical depth (Figure 6C), with a slight non-systematic scatter around the average – similar to early electrophysiological (Hubel and Wiesel, 1974) and fMRI (Fracasso et al., 2018) results. The response magnitude (Figure 6DE) and variance explained (Figure 6F) scaled with cortical depth, an effect often reported in fMRI literature due to ascending draining veins (de Hollander et al., 2021; Fracasso et al., 2018; Koopmans et al., 2011; Lawrence et al., 2019; Polimeni et al., 2010; Self et al., 2019; Siero et al., 2011; van der Zwaag et al., 2018; van Dijk et al., 2020).”

If the authors are interested, they could include a few additional minor revisions:

1.) One of the previous line-scanning studies from the Glasgow group, also looked at Population-receptive fields (Morgan et al., <https://doi.org/10.1101/2020.06.30.179762)>. In their study, they made a big deal about the curvature within the line and to target lines solely from flat patches of the cortex.

Here the line is 4mm wide (nominal), which makes it also susceptible to cortical curvature. The manuscript states that the authors picked the patch with the least curvature. However, I think it would be nice to give a quantitative statement about this. How big was the curvature radius at the lines? What is the corresponding resolution loss of the 0.25mm ‘pancakes’ within the layers? Maybe the authors can also comment on the spatial selectivity of the OVS bands and how much signal might be coming from areas outside the 4mm line?

*We agree with Morgan et al., (2020) that curvature impacts the specificity of the voxels in line-scanning. Their approach consisted of anatomical pre-screening of subjects. Though such a method is effective in avoiding voxel loss due to curvature, it imposes additional criteria on the subject pool and ignores the functional properties of the target area. Our approach integrates functional and structural properties that allows for optimization of experimental setup for the target area in each individual subject or patient (see new paragraph in discussion copied below).*

*We have now added an extra panel in Figure 3 (F; p13), showing the distribution of curvature values within the patch of interest. Subsequently, we included a section in the results section delineating the curvature measures in the lines:*

“In the current line-planning implementation, the target coordinate was selected by minimizing the curvature in a patch of cortex that survived the initial pRF-criteria. For each subject, we estimated the distribution of curvature values present in the line (Figure 3F). This showed that on average, the curvature was predominantly flat (0.04±0.003 1/mm). For some subjects, the initial criteria (e.g., variance explained and/or visual field position) moved the area within which to optimize for curvature towards areas with more curvature. This effected in a loss of spatial specificity within the line; signals coming from non-gray matter were mixed into the line. From manual segmentations, we estimated that the average line consisted of 88.34%±1.07% gray matter, 8.22%±2.77% white matter, and 3.44%±3.18% cerebrospinal fluid (CSF) (Figure S5). These estimations are likely conservative given the imperfect OVS bands.”

*The discussion has been extended to include a paragraph on the effects of curvature (p19):*

“Though we scanned at a spatiotemporal resolution of 100ms and 250µm, the effective resolution will be lower due to several factors impacting spatial and temporal resolution (Chen et al., 2019). First, the amount of curvature in the line affects the effective spatial resolution (Leprince et al., 2015; Shamir et al., 2019; Trampel et al., 2019). Our approach optimizes for minimal curvature after including structural and functional properties. The corollary of such an approach is that the cortical patch is not always exactly flat, causing signal coming from the white matter and CSF to be mixed into the deeper and superficial layers, respectively. In this dataset, this was approximately 11%. This comes, however, with the advantage that the method can be applied to any subject, including patients.”

*Regarding spatial selectivity OVS bands and signal from outside the line, we refer to our first implementation of line-scanning described in Raimondo, et al. (2023a; 2021). We have expanded the sentences including this reference in the section describing the OVS bands (p9, first line of section 2.2.3.1. Line-scanning fMRI acquisition), which now reads:*

“The line-scanning functional acquisition used a modified multi-echo 2D gradient-echo sequence where the phase-encoding gradients are removed and two OVS bands are used to suppress signals outside the line (Raimondo, et al 2023a; 2021a). With this sequence, 94.3±1.3% of undesired signal outside the region of interest is suppressed (Raimondo, et al 2023a; 2021).”

2.) The second sentence in the introduction sounds like the neural activity is in the millisecond regime. Native readers might understand this as saying that there is no neural activity at slower time scales. Maybe the authors can rephrase this statement.

*We have changed the sentence to highlight that these signals are not exclusively transmitted at millisecond time scales. It now reads (p4):*

*“*These signals are transmitted at time spans down to millisecond range (Moro et al., 2010; Schroeder et al., 1998; Self et al., 2013)”

3) I am a bit puzzled by the application of NORDIC: a patch-wise low-rank denoising method. Since the data here are solely one-dimensional, it is unclear how NORDIC was applied. In this case, it sounds to me like a conventional removal of principal components that look like gaussian noise? Since there is no parallel imaging used (GRAPPA/SENSE), the other features of NORDIC also do not make much sense to me in this context?!? Which version of the NORDIC-implantation was used? Maybe it makes sense to mention the version number and/or GitHub hash?

*We used an adaptation of NORDIC for one-dimensional data, where the singular value decomposition was performed on the k-space data of every channel and every echo separately. The thresholding of the diagonal matrix with the eigenvalues was evaluated at the elbow of the scree plot of the eigenvalues versus the components. The denoised k-space data were then combined through weighted channel combination and Sum of Squares echo combination to obtain final magnitude data. A description of the NORDIC-inspired denoising version we used can be found in* (Raimondo et al., 2023b)*. Since line-scanning is a small FOV one-dimensional recoding we decided not to use the patch separation for the NORDIC denoising approach. Moreover, the fact that no acceleration was applied, simply means that it is not necessary to normalize the time series through the geometry factor (g-factor =1) before either. The source code for reconstruction and denoising has been added to the* [*linescanning repository*](https://github.com/gjheij/linescanning/tree/main/recon)*.*

*We have added the following to highlight the specific usage of the NORDIC-inspired denoising step (p11):*

“Briefly, raw k-space data of each channel and echo was subjected to singular value decomposition separately. The thresholding of the diagonal matrix with the eigenvalues was evaluated at the elbow of the scree plot of the eigenvalues versus components (Raimondo et al., 2023b)”

Reviewer: 2

Comments to the Author

There is growing interest in getting high temporal and/or high spatial resolution fMRI data to assess neural information that may be obtained such as laminar organization of functional responses or ordering the onset of functional responses.  Often these approaches sacrifice resolution in two dimensions to gain spatial (and temporal) resolution in one dimension. One such approach is line-scanning where-by a region is defined in two dimension using outer volume suppression and high spatial information is obtained along the third dimension. For fMRI this required pre-determining the area that is activated and within that area defining a region that is appropriate for low resolution in two dimensions and high resolution in a third dimension.  The authors describe a process that lets them determine good regions for line scanning fMRI from anatomical and fMRI images obtained prior to the line-scanning.  The method was applied to defining areas n visual cortex of most interest after receptive field mapping although should be general to any area of the brain.  The process they used is well described and data is given to show the accuracy and potential issues associated with area selection, motion, partial volume etc.....  The paper would be improved if the following issues were addressed:

1) While fMRI data from the line scanning is compared to whole brain responses from the area selected from whole brain fMRI to determine how good the agreement, it would have been interesting to show some of the depth resolved data that was obtained with some discussion of the results with respect to other line scanning results or other laminar specific fMRI results.

*We have added Figure 6 to showcase the possibilities of line-scanning (p17). This figure shows an axial slice from T1-image with the imaged line in white shading and the relevant voxels highlighted in green (Figure 6A), raw time courses for a superficial and deep voxel (Figure 6B), and the position estimates (Figure 6C), response magnitude (Figure 6DE), and variance explained (Figure 6F) of all voxels within the cortical ribbon for a single subject, highlighting the fine spatial detail of these data:*

“For one of these subjects, we show a more detailed profile of depth-dependent measures by modeling all voxels covering the ribbon *independently* (Figure 6A). For this subject, the time courses of superficial and deep layers exhibited the classical pattern (Figure 6B), where the magnitude of the superficial time courses was almost double the magnitude of the deeper time course. The position estimates were stable across cortical depth (Figure 6C), with a slight non-systematic scatter around the average – similar to early electrophysiological (Hubel and Wiesel, 1974) and fMRI (Fracasso et al., 2018) results. The response magnitude (Figure 6DE) and variance explained (Figure 6F) scaled with cortical depth, an effect often reported in fMRI literature due to ascending draining veins (de Hollander et al., 2021; Fracasso et al., 2018; Koopmans et al., 2011; Lawrence et al., 2019; Polimeni et al., 2010; Self et al., 2019; Siero et al., 2011; van der Zwaag et al., 2018; van Dijk et al., 2020).”

2)  An advantage of the line scan technique is that both high spatial and temporal resolution can be obtained. The authors high pass filter to lose information of the higher temporal resolution data they obtained.  This was important for comparing to the whole brain fMRI data.  It is not clear how higher temporal resolutions will be obtained in the face of fluctuations (motion, physiological, etc...) and the authors make passing reference to this issue without any discussion of how, at such high spatial resolution they will overcome these issues.  A further discussion of how best to achieve the full potential of line scanning fMRI in the face of the errors they estimated so well in the present work would improve the discussion.

*We have added a paragraph in the discussion (p19) highlighting different issues that affect effective spatiotemporal resolution, including subject motion, physiological noise, and contamination within and from outside the line:*

“Though we scanned at a spatiotemporal resolution of 100ms and 250µm, the effective resolution will be lower due to several factors impacting spatial and temporal resolution (Chen et al., 2019). First, the amount of curvature in the line affects the effective spatial resolution (Leprince et al., 2015; Shamir et al., 2019; Trampel et al., 2019). Our approach optimizes for minimal curvature after including structural and functional properties. The corollary of such an approach is that curvature is not always exactly flat, causing signal coming from the white matter and CSF being mixed into the deeper and superficial layers, respectively. In this dataset, this was approximately 11%. This comes, however, with the advantage that the method can be applied to any subject, including patients. As previously described, subject motion also poses challenges to line-scanning in both the spatial and temporal domain (Godenschweger et al., 2016; Zaitsev et al., 2015). In previous work, we have shown the effect of prospective motion correction (PMC) using different flavors of navigators (Raimondo et al., 2023b), highlighting the use of PMC in MRI-naïve subjects using *block* paradigms. In such paradigms, the T1­-decay induced by the navigators can be clearly observed and separated from task-signals. The full potential of line-scanning lies in its ability to sample extremely fast. Therefore, event-related designs are desired to evaluate response shapes to different stimuli (Dale, 1999; Mumford et al., 2015). In such scenarios, it will be challenging to separate task-related signals from navigator-induced signals. Another possibility for PMC involves the use of external hardware (Godenschweger et al., 2016; Maclaren et al., 2012; Schulz et al., 2012; Stucht et al., 2015). Finally, because of the high sampling rate (~0.1Hz), cardiac and respiratory frequencies are resolved (Chen et al., 2019). This allows us to accurately characterize physiological noise due to cardiac and breathing fluctuations and target these frequencies for removal using aCompCor (Behzadi et al., 2007). In our experience, this method is more effective (and stable) than using regressors from pulse-oximeter/respiration belt recordings for line-scanning data.”

3) From the anatomical images the authors should be able to estimate how much CSF and white matter is in the cortical line. Considering the low resolution in two dimensions it would be useful to estimate this.  The calculation of where to best do the line scan finds the best area but an estimate of how good this area is in terms of SSF and white matter contamination would be of interest.

*We have now added an extra panel in Figure 3 (panel F; p13), showing the distribution of curvature values within the patch of interest. Subsequently, we included a section in the results section delineating the curvature measures in the lines and the resulting CSF and white matter contaminations:*

“From manual segmentations, we estimated that the average line consisted of 88.34±1.07% gray matter, 8.22±2.77% white matter, and 3.44±3.18% cerebrospinal fluid (CSF) (Figure S5). These estimations are likely conservative given the imperfect OVS bands.”

*The discussion has been extended (p19) to include discussion regarding the effects of curvature (see also paragraph appended to question 2):*

“First, the amount of curvature in the line affects the effective spatial resolution (Leprince et al., 2015; Shamir et al., 2019; Trampel et al., 2019). Our approach optimizes for minimal curvature after including structural and functional properties. The corollary of such an approach is that curvature is not always exactly flat, causing signal coming from the white matter and CSF being mixed into the deeper and superficial layers, respectively. In this dataset, this was approximately 11%. This comes, however, with the advantage that the method can be applied to any subject, including patients.”

Associate Editor

Comments to the Author:

The following should also be addressed:

1. The current approach relies on two sessions, but the authors should note the feasibility of this approach in a single session. Is it possible to conduct such experiments within a single session?

*Theoretically, a single session is possible. However, some stages of the process take a significant amount of time. There are packages that can aid in this situation, but this will typically come at the cost of accuracy. We believe that a two-session approach is currently the most robust.*

*We have extended the discussion (p19) to include considerations for a single-session approach:*

“In all, we have demonstrated the ability to target a specific location in cortex allowing the functional properties of this location to be probed. The implementation described in this work included two sessions. Theoretically, it is possible to conduct such experiments within a single session. However, certain steps along the pipeline – including surface reconstruction and population receptive field (pRF) modeling – take a significant amount of time. Several concessions could be made, typically at the cost of accuracy. For instance, several software packages such as BrainVoyager (Goebel et al., 2006), CAT12 (<https://neuro-jena.github.io/cat/>), or FastSurfer (Henschel et al., 2020), are able to create surfaces quickly (15-60 mins). These packages might encode coordinates differently, so the exercise becomes translating those coordinate systems to the coordinate system of the scanner. Likewise, approaches such as DeepRF (Thielen et al., 2019) or fast, real-time pRF mapping (Bhat et al., 2021) can reconstruct pRFs based near real-time. These approaches demand significant resources from the software as well as skills from the experimenter. Currently, the most robust implementation requires two sessions.”

1. Given the complexity of the process, what is the feasibility of this approach at other sites? How specialized are these pipelines in terms of implementation?

*We believe that the approach can easily be translated to other sites due to the widespread availability of the tools it relies on. We have therefore added the following in the “Data and code availability” section (p21):*

“The described pipeline primarily depends on bash, python, and ANTs. Given the widespread availability of these tools, this pipeline could be implemented at other sites. Successful implementation requires data export to a location where these tools are available. For correct calculation of translation and orientation parameters, one needs to translate the target coordinates back to the coordinate system of the MRI*.*”

1. How important is this process in terms of getting the right line? How much more accurate is this approach than standard line placing?

*Thank you for the question. Given that this is of crucial importance, we have rephrased the introduction to highlight the importance of accurate line-planning (p5).*

“Line placement is critical for line-scanning. First, the line must be placed perpendicular to the cortex to avoid loss of spatial resolution in the cortical depth dimension. This ensures that only signals from a particular layer are sampled by a given voxel in the line (Balasubramanian et al., 2022, 2021). The first implementation of line-scanning involved a rodent study (Yu et al., 2014). Due to the nature of the mouse cortex, planning the line perpendicular is a rather straightforward task. The cortex of humans has a much more intricate folding architecture (Van Essen et al., 2019), complicating (automatic) planning procedures. In earlier work (Raimondo et al., 2023a, 2023b, 2021), this was done by manually placing the line as perpendicular as possible to the cortical surface while maintaining a coronal slice orientation. As the procedure is based purely on anatomy, it remains unclear whether the functional task will activate the imaged area and how the signal is sampled across depth. Second, line placement is critical to target the exact functional region the experimenters want to probe, for example, primary visual cortex (V1) or a specific region of V1 such as the cortical representation of the blind spot (Tong and Engel, 2001). Ultimately, line-scanning can also be extended to clinical research, such as the lesion projection zone in V1 of macular degeneration patients where the balance between ascending or descending signals is disrupted (Baker et al., 2005; Masuda et al., 2008). The well-known functional specialization of the brain and the variation of functional specialization domains between subjects, which can be in the order of ±1cm relative to anatomical landmarks (Dumoulin et al., 2000), also demand accurate line placement on a per-subject basis.”

1. The authors should potentially rephrase the introduction of their manuscript to bring it in line with what will be presented given that no high-resolution data is shown.

*Following the reviewers’ remarks, we have added an additional figure (Figure 6, see above, p17) showing results of a single subject to highlight the spatial and temporal resolutions of the line-scanning method. The main objective of this paper, however, remains the experimental setup for line-planning.*

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