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 and share 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 possibilities of line-scanning (p16). This figure shows raw time courses for a superficial and deep voxel (Figure 6A), and the position estimates (Figure 6B), response magnitude (Figure 6CD), and variance explained (Figure 6E) as a function of depth:*

“For one of these subjects, we show a more detailed profile of depth-dependent measures by modeling all voxels covering the ribbon independently (Figure 6). For this subject, the time courses of superficial and deep layers exhibited the classical pattern (Figure 6A), 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 6B), 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 6CD) and variance explained (Figure 6E) scaled with cortical depth, an effect often reported in fMRI literature (Koopmans et al., 2011; Lawrence et al., 2019; Polimeni et al., 2010; Self et al., 2019; Siero et al., 2011; 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. that curvature majorly impacts the efficacy of line-scanning. Their approach consisted of anatomical screening of subjects. Though such a method is desired to avoid voxel loss due to curvature, it imposes additional criteria on the subject pool and ignores the functional aspect of the target area. Our approach integrates functional and structural properties that allows for optimization of experimental setup for the target area.*

*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:*

“In the current 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](#Figure 3. Assessment of line-placement accuracy using anatomical measures. The registration cascade from target vertex (A) to line-scanning acquisition (B; outer right panel) is known after registering the anatomical image from session 1 (high-res) to the)). 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 manifested as loss of effective resolution; signals coming from non-gray matter were mixed into the line.

*The discussion has been extended to include discussion regarding the effects of curvature:*

*[add paragraph here]*

*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. (2021a/b). We have added this reference to 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).”

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 denoising version we used can be found in* (Raimondo et al., 2023)*.  
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.*

*We have added the following to highlight the specific usage of NORDIC (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. 2023a).”

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 (p16). This figure shows raw time courses for a superficial and deep voxel (Figure 6A), and the position estimates (Figure 6B), response magnitude (Figure 6CD), and variance explained (Figure 6E) as a function of depth:*

“For one of these subjects, we show a more detailed profile of depth-dependent measures by modeling all voxels covering the ribbon *independently* ([Figure 6](#Figure 6. Single subject depth-dependent outcomes. (A) Raw time courses (thin lines) and model predictions (thick lines) for a superficial (red) and deep (blue) voxel. (B) Position estimates from voxels covering the cortical ribbon in color that scales fr)). For this subject, the time courses of superficial and deep layers exhibited the classical pattern ([Figure 6A](#Figure 6. Single subject depth-dependent outcomes. (A) Raw time courses (thin lines) and model predictions (thick lines) for a superficial (red) and deep (blue) voxel. (B) Position estimates from voxels covering the cortical ribbon in color that scales fr)), 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 6B](#Figure 6. Single subject depth-dependent outcomes. (A) Raw time courses (thin lines) and model predictions (thick lines) for a superficial (red) and deep (blue) voxel. (B) Position estimates from voxels covering the cortical ribbon in color that scales fr)), 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 6CD](#Figure 6. Single subject depth-dependent outcomes. (A) Raw time courses (thin lines) and model predictions (thick lines) for a superficial (red) and deep (blue) voxel. (B) Position estimates from voxels covering the cortical ribbon in color that scales fr)) and variance explained ([Figure 6E](#Figure 6. Single subject depth-dependent outcomes. (A) Raw time courses (thin lines) and model predictions (thick lines) for a superficial (red) and deep (blue) voxel. (B) Position estimates from voxels covering the cortical ribbon in color that scales fr)) scaled with cortical depth, an effect often reported in fMRI literature due to ascending draining veins (Koopmans et al., 2011; Lawrence et al., 2019; Polimeni et al., 2010; Self et al., 2019; Siero et al., 2011; 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 (p18) highlighting different issues that affect effective spatiotemporal resolution, including subject motion, physiological noise, T2\*-blurring due to multi-echo acquisition schemes, and contamination within and from outside the line:*

[add paragraph from manuscript here once approved]

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:*

“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 to be inflated further by imperfect OVS bands.”

*The discussion has been extended to include discussion regarding the effects of curvature:*

[add paragraph from manuscript here once approved]

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?

*We have extended the discussion on 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 significant 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. Approaches such as DeepRF (Thielen et al., 2019) or fast, real-time pRF mapping (Bhat et al., 2021) can reconstruct pRFs based on minimal data. These approaches demand significant resources from the software as well as the experimenter. Currently, the most robust implementation requires two sessions.”

*Theoretically, it is possible. However, some stages of the process take significant 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.*

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 site 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 results 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).*

[add paragraph from manuscript here once approved]

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.

*Considering the reviewers’ remarks, we have added an additional figure showing laminar results for the best performing subject to highlight the possibilities of this method. The scope of the paper, however, remains the experimental setup.*

References

[add refs]