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. However, we have added the raw time course to Figure 4A in light black, overlayed with the original low-pass filtered time course. Additionally, we have added Figure 6 to highlight the possibilities of line-scanning. This figure shows time courses of a pial (red) and white-matter (blue) voxel, and population receptive field location in visual space and variance explained profiles across depth obtained by fitting voxels across depth individually.*

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 is 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 an 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 included a section in the results section delineating the curvature measures in the lines and commented on subsequent loss of curvature.*

*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:*

*“*These signals are predominantly transmitted at timescales on the order of milliseconds (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?

*Because of the low voxel contents, line-scanning is vulnerable to thermal noise. Therefore, a thermal denoising step was applied. In collaboration with the original authors of NORDIC, a custom implementation was written to accommodate the line-scanning data described in Raimondo, et al. (2023a). Briefly, denoising was applied in k-space data before the coil and echo combination. A singular value decomposition (SVD) of the data was then submitted to “hard thresholding” that eliminates all the components indistinguishable from zero-mean Gaussian noise. This is different to the original implementation in that … .*

***Luisa..* add code to repo**

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.

***Can we show SOMETHING..?***

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.

*In previous work, we have shown the effect of prospective motion correction (PMC) using different flavors of navigators (Raimondo, 2023a). This work highlighted 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 design are desired to evaluate response shapes to different stimuli (see also discussion for potential experiments highlighting this). In such scenarios, it will be extremely challenging to separate task-related signals from navigator-induced signals. Another possibility for PMC involves the use of optimal instruments, which have a reported accuracy of 0.1mm and ~0.1°.*

*Another improvement would be to abandon the idea of “a single vertex” that is target. In the current study, we selected a the vertex with the lowest curvature among all vertices that survived the initial criteria. However, it could be the case that there are patches of vertices with similar functional properties. It could then be beneficial to select the center of that patch at the cost of the curvature metric. This would increase the number of functionally relevant vertices being projected into the line.*

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.

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 separate the answer in two scenarios. One in which the experiment is fully tailored to the target area (e.g,. in visual experiments) and experiments that do not require this manipulation.*

*In the first scenario, where we would like to manipulate properties of the experiment, surface-based approaches would be extremely challenging. The fastest surface reconstruction de software currently available is FastSurfer (*[*https://deep-mi.org/research/fastsurfer/*](https://deep-mi.org/research/fastsurfer/)*). This is a FreeSurfer alternative rooted in deep-learning. Despite its near instantaneous volumetric segmentation (~1 min), its surface reconstruction still takes about 1hr. This is not feasible to fit within an MRI-session. Alternatively, one could turn to deep-learning based pRF fitting software. Alternatively, we could imagine an MRI session consisting of 1-2 retinotopic runs, which are then exported and fitted using pRF estimation software based on deep-learning. One such software is DeepRF (*[*https://www.biorxiv.org/content/10.1101/732990v1*](https://www.biorxiv.org/content/10.1101/732990v1)*), which is able to produce pRF estimates within seconds. A vertex solely based on functional measures could then be selected.*

*Scenarios where the experiment itself is not manipulated could involve somatosensory and/or motor experiments. In such scenarios, one could create regions-of-interest (ROIs) based on atlases beforehand and obtain the coordinate-of-interest. After a quick anatomical scan the transformation between this atlas and the anatomical scan could be applied to the target vertex.*

2.  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?

*These processes primarily depend on bash, python, and ANTs. Given the widespread availability of these tools at other research sites, this pipeline could be implemented everywhere. Successful implementation depends on the ability to export data to a location where these tools are available. Additionally, for correct calculation of translation and orientation parameters, one would need to know the coordinate system of the MRI itself. While for some scanners more posterior locations are denoted by negative values, some coordinate systems do the opposite. This framework is currently compatible with Phillips, and it should be feasible to translate it to Siemens or GE systems.*

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

*In setups where the experiment is manipulated in order to match the functional properties of the target location – e.g., pRFs –, essential. For larger visual field stimuli, or sensorimotor experiments, it is possible to find a functionally relevant area. However, manual estimation of local curvature and perpendicularity is significantly more challenging.*

4.  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.

*In light of the reviewers request, 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.*