Re: Using functional Near Infrared Spectroscopy (fNIRS) Neuroimaging to Study Global Child Development: Imaging Field Methods in Rural sub-Saharan Africa

Kaja K. Jasinska\* and Sosthène Guei

\*Corresponding Author

Dear Dr. Vineeta Bajaj:

We sincerely thank you for considering our manuscript for publication in *Journal of Visualized Experiments* and especially for the opportunity to improve this

manuscript as per the specific suggestions provided by the reviewers. We greatly

appreciate the time and effort that went into their thoughtful reviews. We are delighted to report that the manuscript is now improved following from their helpful suggestions.

Every suggestion identified by the reviewers is now addressed. Please see our

detailed replies to each reviewer’s critiques below. The overall expansion of our

descriptions reflect our deep gratitude and extraordinary respect for the reviewers’

invaluable input. We thus sincerely hope you will now find our manuscript suitable for

publication in your esteemed *Journal of Visualized Experiments*.

Sincerely,

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**Editorial comments**

Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version. Response: All corrections have been made.

1.    Please abbreviate all journal titles. Response: All corrections have been made.

2.    Please define all abbreviations before use. Response: All corrections have been made.

3.    Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file. Response: A new table has been included.

4.    Please add commas to separate affiliation numbers for the authors. Response: All corrections have been made.

5.    Please provide an email address for each author.  Response: All corrections have been made.

6.    Please revise the title to better reflect the technique shown.

Response: Our title has been revised to: “Neuroimaging field methods using portable functional Near Infrared Spectroscopy (fNIRS) Neuroimaging to Study Global Child Development: Rural sub-Saharan Africa.”

7.    Please rephrase the Short Abstract to clearly describe the protocol and its applications in complete sentences between 10-50 words: “Here, we present a protocol to …”. The current short abstract is above 50 words.

Response: Our abstract has been revised as follows: “Portable neuroimaging approaches (functional Near Infrared Spectroscopy) provide advances to the study of the brain in previously inaccessible regions; here, rural Côte d’Ivoire. Innovation in methods and development of culturally-appropriate neuroimaging protocols permits novel study of the brain’s development and children’s learning outcomes in environments with significant poverty and adversity.”

8.    Unfortunately, there are a few sections in the manuscript that show overlap with previously published work. Though there may be a limited number of ways to describe a technique, please use original language throughout the manuscript. Please see lines: 82-86

Response: We have corrected this text.

Please see lines 81-94: “fNIRS neuroimaging is well-suited for field research. Similar to functional magnetic resonance imaging (fMRI), fNIRS measures the brain’s hemodynamic response6. However, fNIRS uses a series of light emitting optodes and light detectors rather than generate electromagnetic fields. There are no restrictions on metal in or near the testing area, and no electric shielding is necessary, as in the case for electroencephalography (EEG). A key advantage of fNIRS is its portability (i.e. some systems may fit in a suitcase) and ease of use. fNIRS is also easy to use with children; the child is comfortably seated in a chair during the experiment and the fNIRS system tolerates movement well compared to fMRI. Compared with fMRI, fNIRS also provides separate measures of deoxygenated and oxygenated hemoglobin during recording, compared to fMRI which yields a combined blood oxygen level density (BOLD) measure. fNIRS has superior temporal resolution to fMRI: sampling rates can vary between ~7-15 Hz. fNIRS has good spatial resolution: fNIRS’ depth of recording in the human cortex is less than fMRI, measuring about 3 to 4 cm in depth, this is well-suited for studying cortical functions, especially with infants and children who have thinner skulls than adults 3,7-10.“

9.    JoVE cannot publish manuscripts containing commercial language. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.

For example: Shimadzu LightNIRS, etc.

Response: All references to Shimadzu LightNIRS have been removed.

10. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.). Response: All corrections have been made.

11. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly. Please include all safety procedures, etc. Response: All corrections have been made.

12. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Response: We have adjusted the protocol and added explanatory “notes” to clarify specific protocol steps. We have also moved text that corresponds to discussion into the Discussion section.

13. The Protocol should contain only action items that direct the reader to do something. Please move the discussion about the protocol to the Discussion. For e.g. 2.1.1, 2.2, 3.2.1, 4.2.2, 5.1.1, 7.1, etc.  Response: See note above.

14. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Response: We have added additional details to the protocol section.

Please add section 3.5 along with the ethics statement. Response: We have added details of the IRB procedure in country and the ethics statement in section 3.4.

4.2.1: Please provide the specific scalp locations so this manuscript can stand alone. Response: Scalp locations are noted in the international 10-20 scalp location standard.

4.3.2: Please explain how to arrange the 3D digitizer in detail. Where is the second sensor placed? Please be more specific.

Please see revision 4.3.2: “Arrange 3D digitizer equipment. One sensor should be placed on the participant’s head at Cz and affixed securely (i.e. using elastic or hair accessory), and the second block sensor should be placed behind the participant. For example, the participant can be seated in a chair with their back to a table. The second sensor can be placed on the table directly behind the participant’s head. Neither sensor can move during the course of obtaining the 3D digitizer measurement.“

4.4.3: Please explain how to select the probe arrangement. What is the basis of the experimental design?

Please see revision 4.4.2: “Using the fNIRS built-in software, select the probe arrangement that corresponds to the experiment design. fNIRS probes can be placed to cover the entire head (i.e. full head coverage), or alternatively, an array can be placed over general regions of interest. For example, this protocol used a 10 x 3 probe array (30 probes arranged in 3 rows of 10 probes each). This probe arrangement was placed to maximally overlay left hemisphere language areas and their right hemisphere homologues, as well as the frontal lobe. See Figure 2.”

4.4.4: Please describe the optode map. Where does it come from? Please see revision on 4.4.4: “Using the optode map in the fNIRS inbuilt software as a guide, place each optode in the appropriate optode opening on the cap. The optode map indicates the location of each optode in the array (e.g. 10 x 3)”

5.3: How are the experimental tasks presented? Is there a rest period? How long is each task presented?

How are the speech and non-speech sounds presented? How are the rhyming and non-rhyming words selected?

How are the images presented on the screen and how are they selected?

5.8: Is there a specific reason to measure the given tasks? Are there any other tasks which can be measured?

Response (to above comments re: task): We have revised the session describing the task. In fact, any researcher following this protocol will customize the experimental task to suit their research aims. Our tasks are only an example. In the first version of the manuscript, our protocol gave the incorrect sense that these are required steps. We have noted our error and as such, we have limited our description and clearly state that

Please explain how to analyze the data in more details. Response: We’ve added additional details as per the reviewers’ (particularly reviewer 1) incredibly helpful and insightful comments.

7.4.1: Corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s). Response: This is now corrected.

7.5.1: Citation? Response: This is now corrected.

16. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. The highlighted steps should form a cohesive narrative with a logical flow from one highlighted step to the next. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

17. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense. Response: Relevant sections are highlighted.

18. Please note that only steps 4, 5, and 6 can be filmed. The other steps are not appropriate for the video. Response: This is noted. We do also have a lot of footage we accumulated in Cote d’Ivoire that we would be happy to potentially share with the production team if deemed relevant.

19. Please explain the figures in more detail in the Representative Results. Response: We have added additional details to our figures, particularly following Reviewer 1’s suggestions as well.

20. Please do not have one sentence paragraphs. Response: This is now corrected.

21. Please revise discussion to also include the significance with respect to existing methods and any future applications of the technique. Response: We have added a section into our discussion for existing methods and future applications.

**Reviewers' comments:**

**Reviewer #1:**

Manuscript Summary:

This article demonstrated a protocol of using a portable neuroimaging technique fNIRS to study child labor on brain development in rural Africa area. The practical protocol including fNIRS equipment shipping and set up, fNIRS data analysis, and finding local research team, can be very useful to researchers in the field.

Response: We sincerely appreciate the reviewer’s careful reading of our manuscript. Particularly the reviewer’s detailed attention to analysis issues that were lacking or unclear in our previous version of the manuscript, including nirs preprocessing steps. This is a very critical point in the fNIRS field right now, particularly because robust analysis methods are not always utilized. As such, we are very grateful for the opportunity to make the important improvement to our manuscript, which now expresses a higher standard for nirs data preprocessing and statistical inference.

Major Concerns:

1. It is suggested to provide a picture of the NIRS device set up as well as the mobile lab, so that readers can see the size of the NIRS system and the mobile lab.

Response: We thank the reviewer for this wonderful suggestion. We have added a new figure with a schematic of our laboratory set up and photos. See Figure 1. We have also accumulated video footage of laboratory setup, in addition to photos, to share with JoVE team for potential production.

2. Is there any data backup plan, since online data storage service is usually not available on site?

Response: This is a very important concern, and in fact, in the field, we did not have any access to online data storage. As such, data was transferred to multiple to external portable hard drives during field data collection dates.  We note this data backup plan in section 7. See Line 340:

“7.Backup data

7.1. Ensure that data is exported and backed up to multiple portable hard drives, as internet access for online data storage is unlikely to be available. Data should be transferred to online data storage as sufficient internet connectivity is available.”

Minor Concerns:

3. protocol 1.2.1, more details about how temperature affect fNIRS functionality are expected. e.g. what temperature range does fNIRS equipment require, and within the range, will the signal quality change along the temperature variation.

Response: Electronics operating in high humidity conditions, generally above 60%, are more susceptible to corrosion as excessive moisture can settle on parts and react with metal. Humidity levels in an indoor lab (e.g. inside a university building) are generally between 30-50%. Humidity in southern Côte d’Ivoire ranges between 80 and 90%. We have revised the text, see section 1.2.2.: “1.2.1. Prepare for climate conditions in the field. Note: Temperature and humidity conditions in the field can vary significantly from laboratory setting and may affect equipment function and longevity, as well as participant comfort during experimentation. Electronics operating in high humidity conditions, generally above 60%, are more susceptible to corrosion as excessive moisture can settle on parts and react with metal components. Humidity levels in an indoor lab (e.g. inside a university building) are generally between 30-50%. Humidity in southern Côte d’Ivoire can be 80-95%. Set up a portable air conditioning unit with low wattage demands.”

4. protocol 4.1.2, the authors should describe how to use the measured head size information, e.g. how to use the information when placing the cap on participants, the description in 4.2.1 is vague.

Response: We appreciate the reviewer bringing this important detail to our attention. We have revised the text to improve clarity. Please see section 4.2.1.: “4.2.1. Place the fNIRS optode holder cap onto the participant’s head, aligning the cap to the international 10-20 system for scalp locations16. Cap position should be identical for all participants. Align points on the cap (e.g. probe holder) with scalp positions. For example, center the front of the cap on the head to frontopolar (FP) position. This position corresponds to 10% of the nasion-inion over top distance dorsal to nasion position.”

5. protocol 4.3.2, if a sensor of the 3D digitizer is placed at Cz, it can be very easily moved during the localization data collection, please provide details of how the sensor is secured.

Response: Please see revision in 4.3.2.: “Arrange 3D digitizer equipment. One sensor should be placed on the participant’s head at Cz and affixed securely (i.e. using elastic or hair accessory), and the second block sensor should be placed behind the participant. Neither sensor can move during the course of obtaining the 3D digitizer measurement.”

6. Protocol 7.5.1, please provide more details about the time derivatives, the authors should describe the reason of including the time derivatives in the analysis model.

Response: We are grateful to the reviewer for raising this important analysis issue, which indeed should be further clarified. Please see our revisions in section 8.5.1.:

“The hemodynamic response function can have greater variability in higher cortical regions and across participants. These types of variability can be accommodated in analysis models by expanding the HRF to include temporal derivatives. A temporal derivative can model differences in the time to peak hemodynamic response (Friston et al., 1998).”

7. Since 7.4.1 and 7.4.2 was optional preprocessing steps, please discuss the performance of the low pass filter and Wavelet artifact correction method. It is also helpful to introduce more methods for data preprocessing so that readers can make their selection.

Response: Indeed, many rigorous analysis approaches exist, including outlined by Huppert et al (2009). We now stress in the manuscript that fNIRS researchers have to make a selection that should be guided by careful investigation of methods that are best suited to their research projects, and we indicate other approaches to consider.

Please see revisions in section 8.5: “Data preprocessing. Multiple options exist for preprocessing fNIRS data. For example, Huppert et al. (2009) propose very rigorous methods for different sources of noise20. These include eigenvector-based reduction of motion artifacts, bandpass filtering techniques and eigenvector-based reduction of spatial covariance for physiological interference in data (e.g. respiration, blood pressure, heart rate). Huppert also shares a thorough commentary on sources of noise in fNIRS research and implications for statistical analysis 21. The fNIRS researcher must investigate preprocessing applications that are most appropriate for a given study. Below, an analysis approach modeled after Worsely and Friston (1995)24 and Jang et al. (2009)23 is presented.”

8. Figure 1 panel C, why not also provide a coronal view of the digitization results?

Response: We have now added a coronal view.

9. Figure 4, the axis labels need revision, is the signal normalized?

Response: Thank you for bringing this to our attention. We have revised the figure and figure caption. A - raw data, not normalized, B - filtered data after wavelet detrending. Please see Figure 4.

10. Figure 5 and Figure 6, the authors should provide statistical results, e.g. labelling the color bar as t-value, also please provide the p-value associated with the results, especially for Figure 5 panel b.

Response: We have updated our figures. Figure 5 panel b presents a visualization of oxy and deoxy responses with standard error (shaded areas). This visualization is obtained from Cui Xi’s plotAverage.m code (http://www.alivelearn.net/?p=1191)

**Reviewer #2:**

Manuscript Summary:

This is an excellent manuscript that addresses and situates the scientific and social benefits of an inclusive, collaborative, and culturally appropriate research protocol for rural contexts. As the authors write, "Methods that advance our field's capacity to study development in a fuller range of human experience can dramatically advance our understanding of the complex relation between brain development and the life experiences that shape it." This manuscript lays out the practical means to achieve this important objective for our field.

The scientific and social benefits (as well as the risks of not following this paradigm) are made clear as they relate to multiple parties (the researcher, the participants' community, and the research scientists based in that region.) This manuscript is also timely given the increasing interest in fNIRS systems and will serve well both new and established fNIRS users that may be seeking to extend their research into studying populations that have been historically underrepresented in research studies.

The fNIRS protocols for setting up the equipment for participants, spatial registration, data collection, and data analyses are all appropriate and are within published cognitive neuroscience standards.

Response: We are very grateful for the reviewer’s positive comments about our manuscript -- particularly noting the importance of “scientific and social benefits of an inclusive, collaborative, and culturally appropriate research protocol for rural contexts”. We have addressed all the very valuable issues the reviewer raised with respect to culturally-appropriate engagement. We are deeply appreciative of the reviewer raising important issues related to culture and science capacity building that pushed us to improve this new version of the manuscript.

Major Concerns:

§6.4: Given the emphasis provided on culturally appropriate engagement with rural communities and the importance of establishing appropriate channels for approval, the manuscript abruptly ceases to consider this after the cap is removed from the participant's head. This important consideration extends beyond obtaining consent to collect data. At the very minimum, section 6.4 should at least provide some discussion about how the participant is released from the experiment. Do they accept payment for time/participation? What is the appropriate method of acknowledging and thanking the participant? It would be more ideal if there was some mention of efforts to return the knowledge back to the community. This is partially alluded to in earlier sections that discuss the importance of building research capacity in rural regions. The manuscript would benefit well from a more explicit discussion on this matter.

Response: We thank the reviewer for this pertinent observation. We have addressed the question related to the benefits and compensation for participants and other stakeholders in the Discussion Section.

Please see revisions in Discussion:

Local Science Engagement and Opportunities for Capacity Building  
One of the main challenges that local, particularly junior, researchers in Côte d’Ivoire are faced with when they complete their studies is the lack of opportunity for hands-on research experience with experienced researcher mentors and/or international collaborators.  For this purpose, researcher should make all efforts to establish robust collaboration with local researchers from relevant disciplinary backgrounds, and include trainees at all levels (undergraduate, graduate, postdoctoral). Trainees can leverage the insight gained from this experience to work independently and further research. This experience can also be a stepping stone to build their capacities as researchers and develop their competitiveness at the international level in writing research proposals, papers and applying for grants. A research team excluding local researchers may have a reduced chance of success as local researchers will best know the local social and cultural values and systems, the local languages spoken in addition to the geographic knowledge of the area. Their contribution is therefore extremely important in understanding the local realities and designing culturally-appropriate protocols for the successful research projects.  
  
Culturally-Appropriate Research Methods  
The development of informed consent protocols to conduct research in rural settings specifically in Cote d’Ivoire is critical and failure to adopt the appropriate approach can inhibit the successful achievement of the research even though well-intentioned and scientifically robust 28-32. Generally, in rural settings in Côte d’Ivoire, asking a villager to read a consent form and sign it can break any trust building between the researcher and the participant. In fact, the perceived formality of this procedure may create a psychological distance and a feeling of insecurity in the participant’s mind. This may result in a clear or unexpressed unwillingness to collaborate. This attitude can be explained by many factors including a long history of oral tradition whereby communication is more oral than written and high rates of illiteracy that may be found in target communities. Communities in rural settings trust their chief and rely on his decision-making power. Therefore, the protocol presented incorporates the consent of the chief of the village at the community level. This is arguably more culturally important than individual consent. Additionally, participants and community members may have had limited or no exposure to neuroimaging technology or computers before. Therefore, researchers need to take into consideration that the informed consent procedure, and instructions, may be misunderstood. The function of the fNIRS system should be communicated in lay terms and appropriate language easily understandable by child participants and community members who may have had very limited exposure to technology. These considerations can strongly influence the comfort and confidence of all community members involved in a field neuroimaging research project.  
  
The protocol presented here also highlighted the importance of sharing research findings with community members and government partners. Partnerships built on continued dialogue aid in the eventual translation of research findings into policy. It is imperative to arrange post data collection field visits to disseminate research findings and deliver reports and, possibly, share any tools that resulted from the study (e.g. assessments in local languages). Participating communities in rural settings may never otherwise receive information about study completion and findings given lack of internet service and/or computers. Likewise, researchers in country may have limited access to academic journal subscriptions and poor internet connectivity at regional universities. Published results should be shared in a regional forum, and made available in an accessible language.

Minor Concerns:

Line 81 on page 1: "the fNIRS system tolerates movement well compared to both fMRI and EEG." NIRS systems are not any more tolerant of motion than EEG systems, but they are certainly much more tolerant of motion than fMRI systems (and even PET or MEG systems, which can tolerate small minor movement.) This sentence should be corrected.

Response: We have made this correction, thank you for bringing this important detail to our attention.

§7.1: It is correct that NIRS-SPM & Homer2 are widely used, but the fNIRS Toolbox by Huppert is quickly gaining considerable attention and use. To reflect current trends, this toolbox should also be cited.

Response: We are grateful for this important note. Indeed, we are excited about the development of the fNIRS toolbox and plan to incorporate the advance into our future research program. We did note that on the fNIRS Toolbox website (<https://bitbucket.org/huppertt/nirs-toolbox/wiki/Home>) there is currently an ommission for the direct citation of the toolbox.

Please see our revision: “Multiple data analysis packages exist for fNIRS18. Statistical Parametric Mapping for Near-Infrared Spectroscopy (NIRS-SPM)19 , Homer220 (widely used), and the fNIRS toolbox 21,22 (new and gaining popularity) are used for fNIRS data analysis. This protocol reviews data analysis methods using NIRS-SPM, but it is to the discretion of the researcher to select preferred method of analysis.

Typos:

§7.4.1: Citation for Jang is not formatted correctly.

Figure 3: 2 instances of "Trail" as opposed to "trial"

Response: We have made these corrections. Thank you for noting this.

**Reviewer #3:**

Manuscript Summary:

The protocol described using portable functional near infrared spectroscopy (fNIRS) in studying children's reading development and ability in rural sub-Saharan Africa, especially in regions with a high risk of illiteracy. The study is performed in Côte d'Ivoire where an estimated of 1.3 millions of child labor in the coca agricultural sector. It is an important topic because little has been studied about the impact of child labor on brain development and learning. The manuscript is well-written and easy to understand. The protocol can help researcher understand fNIRS is portable, low-cost and easy to operate in field research, especially in the pediatric populations. Although similar setup of fNIRS field studies in low-resource countries have been published (Begus et al, 2016, PMID: 26782222; Papademetriou et al, 2014), the protocol provides useful information, including preparations for traveling with laboratory devices in low-resource contexts and set up, to researcher who interested in setting up field fNIRS studies.

Response: We would like to thank the reviewer for their thoughtful comments on our manuscript. Particularly referencing the growing group of fNIRS research in low-resource contexts. We have added an additional section to our discussion to review some of the existing fNIRS neuroimaging studies in similarly low-resourced contexts. We agree with the reviewer that is in important to note the extent to which portable NIRS technology is already revolutionizing cognitive neuroscience.

Major Concerns:

Are the tasks used in the fNIRS measurements culturally appropriate or adapted to children who can not read or/and write? Please address the considerations of the potential impact on the test results.

Response: The fNIRS tasks and measurements are adapted to children in a village setting who have very low/beginning reading ability. Indeed, most of the participants and community members have not have exposure to neuroimaging technology or computers before, but efforts are made by the experimenters to explain the instructions and the function of the fNIRS system in lay terms and appropriate language easily understandable by child participants. Experimenters also ensure that the participants feel confident and relaxed all along the experiment. From our experience, children are keen to participate in the experiment whatever their literacy level as they consider the research activities like a game. All these factors strongly influence their comfort with participating and have a positive impact on the quality of data collected.

Please see 5.4.2.1.: “Note: Tasks should be created in a manner suitable for the participant. In the study reference here, researchers were investigating language, cognitive, and reading development in children who were very poor readers. The reading neuroimaging task developed with words that would be appropriate for a child with minimal literacy skills. As well, children were selected for the neuroimaging paradigm based on scores obtained on a reading assessment.”

Minor Concerns:

The author stated the protocol has been approved by the Institutional Review Board of the University of Delaware. Please confirm the study is also approved by local IRB in Côte d'Ivoire. If not, please explain why.

Response: We sincerely appreciate the reviewer raising this issue. IRB approval was obtained from the University of Delaware (and now noted in the manuscript). At the time of writing this manuscript, Cote d’Ivoire did not have any formal IRB procedures, albeit there has been mention that a branch of government was working on developing a comprehensive policy and procedure. Date of completion and policy implementation are not known. In lieu of this, we obtained approval directly from the Ministry of Education.

Please see revision in 3.5: “All research activities need to be approved by local authorities, for example, the Ivorian Ministry of Education or the Ministry of Higher Education and Research. The country the project will take place in may not have a formal procedure for ethical approval through an institutional review board (IRB). Check regulation to ensure you are following the correct protocols for obtaining ethical approval for research. Note: at time of obtaining approval, Cote d’Ivoire did not have a formal IRB review process. In lieu of this, the research team proceeded by preparing documentation akin to a IRB application to submit to the Ministry of Education. Multiple meetings were arranged with Ministry of Education and Ministry of Higher Education and Research officials. At these meetings, the research team presented the proposed research plan to all officials followed by group discussion and question and answer sessions. Ethical approval was obtained directly from the Ministry of Education in the form of a signed document granting authorization to conduct research with children at specific schools.