*Response to reviewers for “Functional near infrared spectroscopy of the sensory and motor brain regions with simultaneous kinematic and EMG monitoring during motor tasks”*

July 29, 2014

Dear editors and reviewers,

We thank you very much for taking the time to review and provide helpful feedback for our manuscipt. Below we have copied the text of the reviews, and included our itemized responses in italics. We hope that you find these changes satisfactory.

Kind regards,

Theresa Sukal-Moulton, Ana Carolina de Campos, Christopher Stanley, and Diane Damiano

**Editorial comments:**  
The manuscript has been modified by the Science Editor to comply with the JoVE formatting standard. Please maintain the current formatting throughout the manuscript. The updated manuscript (52391\_R0\_061214.docx) is located in your Editorial Manager account under “File Inventory." Please download the .docx file and use this updated version for any future revisions.   
  
Changes made by the Science Editor:  
1. There have been edits made to the manuscript.   
  
Changes to be made by the Author(s):

1. 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.

*The manuscript has been proofread and we believe spelling and grammar should be appropriate for publication.It was a common theme among reviewers that more details about experimental setup were expected in the Methods section, so we have moved several sentences from Representative Results into Methods to improve the flow of the manuscript.*

2. How is the room calibration done in step 1.1?

*Step 1.1 has been clarified including details of the calibration process* *, however each motion capture company has a unique calibration process.*

3. How are the rest and task periods defined?

*For all tasks except gait, visual and auditory feedback provided by cartoon animations cued participants to either relax or move according to the instructions of each task. Only auditory feedback was provided during the gait task. We clarified this point in steps 6.3 and 7.5 Additional details are provided in the Representative Results section.*

4. There is 10 page limit for the Protocol, but there is a 2.75 page limit for filmable content. 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.

*The highlights were revised to meet these requirements.*

5. Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense. Please remove the Notes from the highlighting to reduce the overall length.

*Notes were removed from highlited part of item 3.3. All the remaining highlights include actions in imperative tense.*

6. The highlighting in section 4 needs to include the placing the sensors on the skin (for continuity).

*Step 4.3 was partially highlighted for continuity.*

7. Regarding detail, please describe the basic motion capture setup used in section 1.  
 *Details were added to steps 1.1 and 1.2*

**Reviewers' comments:**  
**Reviewer #1:**   
Manuscript Summary:   
The authors outline a detailed methods description for the synchronous acquisition of functional near infrared spectroscopy (fNIRS), motion tracking and electromyography (EMG) data from subjects with cerebral palsy (CP). The aim of this multi-modal acquisition strategy is to provide auxiliary data to investigators about unintended motions that could contribute to the measured brain activation signals and thus confound results. Methods are presented for applying this multi-modal acquisition strategy to bilateral gait and cycling tasks as well as to unilateral hand squeezing and ankle flexion tasks.   
  
The methods presented are complete and well-organized. Procedures are explained clearly and their potential applicability is highlighted. The methods described are generalizable to many other motor conditions and are therefore expected to be useful to other investigators in the field of rehabilitation.   
  
Major Concerns:   
None.  
  
Minor Concerns:   
There are only a few points in the methods description that may need some more detailed explanation or clarifications:  
  
1. In the room setup procedure (step 1.1) it may be helpful to include more information about the motion tracking camera system, e.g. how many cameras were used and what heights and distances they were placed at relative to the subjects. This would be important for understanding target placement to avoid occlusions.

*Some of the parameters requested will be manufacturer-specific, and related to the exact dimensions of the room where a researcher sets up the equipment. We did add details about the size of our measurement volume, and suggested steps to take for researchers.*

2. In step 1.2 please clarify which instrument is the 'master' trigger, i.e. is data acquisition started from the instruction computer or one of the other instruments?

*Additional information has been added to this step for clarity.*  
3. In step 3.6.1 you may want to clarify that the 690 nm and 830 nm wavelengths are specific to this fNIRS instrument, but all commercial fNIRS instruments would offer two-wavelength measurements.

*This is a good suggestion and has been implemented.*  
4. In step 3.7 it may be worth commenting that the feasibility of using the motion tracking setup to determine fNIRS optode placement is specific to the camera geometry. In studies performed in smaller rooms, it is possible that target occlusions may make this task difficult and that a 3D position tracking stylus could be used instead.

*We added information about the volume of measure for our setup in step 1.1, and then commented in the first paragraph of the discussion about alternative methods including a 3D position tracking stylus.*

5. In step 3.8 it may be worth considering that, depending on where the markers need to be placed for tracking a certain task, the camera geometry could be adjusted so that it does not directly shine the tracking light onto the fNIRS optode setup on the head, which would reduce the need for optical insulation of the latter.

*Although we have been working in a relatively large space with several motion capture cameras, it has not been our experience that removing the optodes, cables or control boards from that space is helpful. For example, when turning the machine away from the cameras while still in the same room we still saw differences between covered and uncovered data collection. Although other researchers should experiment with this in their own particular setting, we prefer to leave this suggestion in the text.*

6. In step 4.3 do subjects perform a few test motions to make sure that good electrical contact has been achieved for each muscle being tracked?

*We added this validation process to 4.5.*  
7. Step 6.6 mentions that feedback is provided to participants about extraneous movements. Is the goal here to help participants consciously reduce their extraneous movements? Also, has it been checked that auditory feedback does not provide additional activation in the fNIRS field of view?

*The feedback that we give is related to movements outside of instructed ones, and could include shifting in the seat, fidgeting with another body part, tapping the hand or foot during the rest period, etc. If participants are unable to suppress the extraneous motions, such as mirroring, during the task then the movements are considered involuntary and allowed to continue.*

8. In Figure 1 caption it is mentioned that a Monte Carlo was run for the probe arrangement shown in the right side of that figure. Was this a summation of multiple runs for each one of the sources in the field of view? Were the results from all the photon runs normalized? Are there units or a color scale to describe what the plotted sensitivity color map means?

*This additional information could be useful to the reader and has been added.*  
9. It may be helpful to add some brief information on what all the other buttons in that figure panel represent. For example, what is the meaning of all those letters on the top right side, and what do the source and detector buttons represent on the bottom half of the figure?

*We had originally elected to keep much of this information out, since it is machine and manufacturer specific. However, it could be relevant to the readers’ understanding and has been added in.*

10. It would be more consistent to use the term fNIRS rather than DOT in Figure 5 caption. DOT could map something static like a tumor, but fNIRS is specific to functional imaging.

*Thank you for this suggestion, the caption has been updated.*

11. In the Materials table please provide information on the animation software and the retroreflective markers.

*This has been added.*  
  
12. In the second to last paragraph of the Discussion, would it be correct to also suggest how the multi-modal data could be used to remove bad blocks from the fNIRS data? Also a more correct literature reference for #8 would be the following:  
Nathan Hervey, Bilal Khan, Laura Shagman, et al., "Motion tracking and electromyography assist the removal of mirror hand contributions to fNIRS images acquired during a finger tapping task performed by children with cerebral palsy", Proceedings of SPIE Vol. 8565, 856563 (2013).

*The use of the signals in this way is a good suggestion and has been added. Thank you for this reference.*

Additional Comments to Authors:  
N/A  
  
  
**Reviewer #2:**   
Manuscript Summary:   
The manuscript details procedures for collecting and synchronizing fNIRS, kinematics with a 3D motion analysis capture unit, and EMG for 4 movements: treadmill walking, gross grasping, isolated ankle dorsiflexion, and a cycling motion of the lower limbs.  
  
Major Concerns:  
1. There is very little information on the instructional software/program, which guides the movements of the subject and provides a trigger for the various data collection instruments. Is this a unique program developed by your lab, and if so, what did you use to create it? Is it based on E-Prime? It would be helpful to clarify where the program came from, and whether it places a marker via a voltage change in the data for each collection instrument, or whether it initiates the start of data collection for all devices simultaneously.

*The animations were custom-made using 3DS Max software, Audacity, and Windows Movie Maker to produce a .mp4 file that included both rest and task periods. During the experiment, the video files that were created were projected onto a screen. The mouse trigger used to start the video sends a marker for both the motion capture and the NIRS systems, which were previously started. We added this information to step 6.5 and the Figure 2 caption, as well as putting the software on the materials list.*

2. In item 1.2, you indicate connecting the instructional computer to the motion capture equipment and fNIRS, but do not mention the EMG. If the EMG is automatically synched with your motion capture, please mention that here. I believe it is explained in the "representative results" section more clearly, but would be better in the beginning.

*Yes, the EMG is automatically synched with the motion capture system. This information has been included in item 1.2, and also appears in the Representative Results section.*

3. Alternative approaches to measuring movement, other than a full 3D motion analysis system, should be mentioned as an alternative, specifically for the single joint movements such as your ankle example. Depending on the research question and movement of interest, it could be mentioned that this would decrease the preparation time of the patient if the full protocol you described was not of interest.

*Thank you for the suggestion. Since a gait lab is not always available, it is relevant to note that there are alternative methods for motion analysis, for example, accelerometry. However, in this protocol we also used the motion analysis system to record optode placement; for this purpose accelerometers would not be appropriate. We addressed this point in the Discussion section.*

4. And lastly, there could be a little more explanation why you chose these particular 4 movements to pull together in your protocol.

*This protocol is part of a larger study encompassing several different task conditions in individuals with and without brain injuries. We chose this subset of tasks (gait, a bilateral multi-joint movement (cycling), one upper and one lower extremity unilateral single joint movement) to illustrate the methodological aspects and the variety of potential applications of the protocol to a range of different movements. We tried to clarify this in the Introduction section.*

Minor Concerns:  
The title, which refers to "functional movement", is a little deceptive to me. The better description would be "specific movement tasks" to be clear that the subject is not engaged in a truly functional task such as grasping a cup, walking over ground, or cycling with balance required. Thus, these movements do not accomplish an overall goal-directed function, which may be interpreted by some if you use the current title. I realize this is semantics, but clarity is important when presenting such a protocol, which others may want to use as a guideline.

*We agree with your point. “Functional movement” was replaced by “motor tasks”.*

**Reviewer #3:**   
Manuscript Summary:   
Functional near infrared spectroscopy of the sensory and motor brain regions with simultaneous kinematic and EMG monitoring during functional movement  
  
This manuscript is of large interest for the community as concurrent monitoring of blood flow changes related brain activity and motor output (movement production and muscle activity) is warranted for clinical and more fundamental research purposes. On the methods side, one major advantage for coupling all these measurements is to give highlights in the functional near-infrared spectroscopy values and its better interpretation during (loco)motor tasks. Neural control could be proposed even if the fNIRS technique is based on the neurovascular coupling (indirect approach). Interestingly, this study explored different kinds of movement with fNIRS and muscle / kinematic monitoring. Some issues for fNIRS monitoring during gait are clearly different from that during isolated hand squeezing for instance. This should be clearly stated by using some tables if any for future users.  
  
Major Concerns:  
Introduction  
In the introduction authors should reinforce the necessity / rationale to combine fNIRS and motion capture and mainly the use of electromyographic (EMG) monitoring. What these measurements are bringing us exactly? Please develop deeper this point.  
The goals are not clearly stated. Based on some points underlined in the introduction, the feasibility (level of difficulty) of such combination of techniques as well as the main (methodological) differences in the use and interpretation of fNIRS monitoring according to the motor tasks are expected. Differences in brain activation patterns with unilateral and bilateral movements are known and does not appear the focus of this study.

*We thank the reviewer for these thoughtful comments. Indeed, the results of brain activaiton patterns are not the primary focus of this article.*

Methods   
Please give information (anthropometric data, age etc..) of the sample of subjects used in the protocol.  
Optode location in 3.3 has to be specified. For instance the simple use of a 3D-digitizer-besed probabilistic registration method allows the registration of NIRS optode or channel locations onto MNI coordinate space.

*We did not include a participant table as our focus is primarily on the methods used to collect data. In the representative results figure captions, we have added participant age and head circumference as requested. Step 3.7 is our method for determining 3-D coordinate positions of each of the optodes, and is used for the registration noted by this reviewer. We have added some language to this section to be more clear about what we are using that data for.*

Physiological signals (heart rate, blood pressure) have to be collected simultaneously to cope with the large systemic influences on fNIRS signals (mainly HbO2).

*This information is not currently recorded in our laboratory, but we have added a suggestion in the disucssion to add this capability if possible to future data collection.*

EMG setup. Impedance of the skin between electrodes has to be minimised. This has to be measured accordingly after 4.2.

*The EMG electrodes that we use (Delsys Trigno) are designed to have higher signal to noise ratios, and we take steps to minimize impedence between skin and electrodes, but this is not routinely measured in our laboratory. SENIAM recommendations have been followed, and this has been added to 4.2.*

Discussion  
Authors have to discuss limitations and mention alternative approaches in case.   
The article should therefore consider some pitfalls, such as:  
Non responders: receiving a good signal can be troublesome. Moreover, it seems that not all subjects show clear hemodynamic responses. How should we deal with this problem? Please mention the problem.  
The spatial resolution of fNIRS seems to be limited and should be discussed.  
In most of the discussed studies, placement of the optodes is based on 10-20 system or by trial and error moved to the optimal position. From this point of view it is becomes difficult which cortical area is measured.  
Systemic interferences can influence the results when they are not corrected. For example when increasing the walking speed, blood pressure and heart rate increase, which influences the signals measured by fNIRS.  
While NIRS is more robust to the movement artifacts, they do exist especially during gait.

*Thank you for these suggestions. Although some points were touched upon in the introduction, we’ve substantially expanded our discussion to include the points brought up here.*

Minor Concerns:  
N/A  
  
Additional Comments to Authors:  
N/A