FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 68350
Scriptwriter Name: Debopriya Sadhukhan
Project Page Link: https://review.jove.com/account/file-uploader?src=20846798

Title: PIPEMAT-RS: Development and Validation of a Standardized MATLAB Pipeline for Resting-State EEG Preprocessing

Authors and Affiliations:

Lucas Murrins Marques[1*], Kevin Pacheco-Barrios[2,3*], Lucas Camargo[2], Melina Houlis[1], Jordan Vieira[1], Ana Castellani[4], Sara P. Barbosa[4], Marcel Simis[2], Felipe Fregni[2], Linamara R. Battistella[4,5]

[1]Mental Health Department, Santa Casa de São Paulo School of Medical Sciences
[2]Neuromodulation Center and Center for Clinical Research Learning, Spaulding Rehabilitation Hospital and Massachusetts General Hospital, Harvard Medical School
[3]Vicerrectorado de Investigación, Unidad de Investigación para la Generación y Síntesis de Evidencias en Salud, Universidad San Ignacio de Loyola
[4]Instituto de Medicina Física e Reabilitação, Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo
[5]Faculdade de Medicina, Universidade de São Paulo

[*]These authors contributed equally

Corresponding Authors:

Lucas Murrins Marques (lucas.murrins@fcmsantacasasp.edu.br)

Email Addresses for All Authors:

Lucas Murrins Marques	(lucas.murrins@fcmsantacasasp.edu.br)
Kevin Pacheco-Barrios	(kevin.pacheco.barrios@ppcr.org)
Lucas Camargo	(lcamargo@mgh.harvard.edu)
Melina Houlis	(melihouliss@gmail.com)
Jordan Vieira	(jordan.vieira@aluno.fcmsantacasasp.edu.br)
Ana Castellani	(anarcastellani@gmail.com)
Sara P. Barbosa	(sara.barbosa@ppcr.org)
Marcel Simis	(marcelsimis@gmail.com)
Felipe Fregni	(ffregni@post.harvard.edu)
Linamara R. Battistella	(linamara.battistella@hc.fm.usp.br)

Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

3. Filming location: Will the filming need to take place in multiple locations? **NO**

Current Protocol Length

Number of Steps: 12
Number of Shots: 30

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

Videographer's NOTE: It was a bit difficult to find a location for the testimonies, since it was a public hospital. We filmed in the most suitable place I could find.

As for the photos of the authors, only Lucas was present at the hospital, so I couldn't take any photos of anyone else.

REQUIRED:

1.1. **Lucas Murrins Marques:** We developed a standardized EEG preprocessing pipeline to improve signal quality, reduce noise, and support consistent neurophysiological research across diverse clinical and experimental datasets.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1, 3.2.2.*

What are the most recent developments in your field of research?

1.2. **Lucas Murrins Marques:** Recent pipelines like RELAX and Automagic offer automated EEG cleaning but often lack flexibility or clear documentation for researchers and students who need transparency and consistency of results.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What technologies are currently used to advance research in your field?

1.3. **Lucas Murrins Marques:** We use EEG, MATLAB, EEGLAB, and ICA-based artifact rejection combined with automated classifiers like ICLabel to enhance data quality and streamline preprocessing workflows.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.1, 4.2.2., 4.2.3.*

What significant findings have you established in your field?

1.4. **Lucas Murrins Marques:** It has been found that PIPEMAT-RS improves EEG signal quality, increases SNR, and supports robust biomarkers in stroke, fibromyalgia, and chronic pain studies.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 2B, 2C.*

What research gap are you addressing with your protocol?

1.5. **Lucas Murrins Marques:** This protocol addresses the lack of an editable, well-documented, and standardized pipeline for resting-state EEG that is both consistent and accessible to researchers worldwide.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.6.1, 3.6.2, 3.6.3.*

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions:

Videographer's NOTE: It was a very short script, so there are few files. I took the opportunity to take a wide shot and a close shot for all the testimonies.

It was a bit difficult to find a location for the testimonies, since it was a public hospital. We filmed in the most suitable place I could find.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Lucas Murrins Marques: Instructor Professor in Mental Health, Santa Casa de São Paulo School of Medical Sciences:** Publishing with JoVE will greatly expand the reach of our pipeline by making it visually accessible and easy to understand, especially for international labs and early-career researchers seeking clear, step-by-step EEG preprocessing guidance.

1.6.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE?

1.7. **Lucas Murrins Marques: Instructor Professor in Mental Health, Santa Casa de São Paulo School of Medical Sciences**: We expect JoVE to streamline onboarding in our lab by reducing training time. The video format will also facilitate the adoption of PIPEMAT-RS in partner institutions and support collaborations in multicenter EEG studies.

1.7.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

This research has been approved by the Ethics Committee of the Clinics Hospital, University of São Paulo Medical School

Protocol

2. File Format Conversion Procedure

Demonstrator: Lucas Murrins Marques

Videographer: Please record the screen for all the SCREEN shots as a backup.

NOTE: The author wants to use still images only and to make it dynamic add emphasis, zoom in, zoom out as needed.

2.1. To begin, open MATLAB *(Mat-lab)* and navigate to the directory containing the raw electroencephalogram data files **[1]**. Define the directory path to the raw data files **[2]** and create a list containing the subjects **[3-TXT]**.

2.1.1. WIDE: Talent seated at a workstation opening MATLAB and browsing to the specified directory.

2.1.2. SCREEN: Screen_212.tif *Video Editor: Emphasize or highlight the selected part in the image.*

2.1.3. SCREEN: Screen_212.tif **TXT: Skip this step if the dataset is already in .mat format** *Video Editor: Emphasize or highlight the selected part in the image including the text written in green above this (% List of raw EEG....).*

2.2. Load the raw data file using the **load** function for .mat *(dot-Mat)* files **[1]** and save each file in .mat format, ensuring data integrity and retaining all relevant metadata **[2]**.

2.2.1. SCREEN: Screen_221.tif *Video Editor: Only show the MATLAB window.*

2.2.2. SCREEN: Screen_222.tif

3. EEG Montage, Downsampling and Filtering, and Artifact Rejection

3.1. Open MATLAB and navigate to the directory containing the MATLAB files **[1]**.

3.1.1. SCREEN: Screen_3.1.1.tif

3.2. Use the **pop_loadset** *(Pop-Loadset)* function from the **EEGLAB** *(E-E-G-Lab)* toolbox to load each subject's EEG *(E-E-G)* dataset **[1]**. Ensure the appropriate electrode location file corresponding to the EEG cap configuration is available in the working directory **[2]**.

3.2.1. SCREEN: Screen_3.2.1.tif *Video Editor: Only show the MATLAB window and emphasize or highlight the selected part in the image.*

3.2.2. SCREEN: Screen_3.2.2.tif *Video Editor: Emphasize or highlight the selected part in the image.*

3.3. Use the **pop_chanedit** *(pop-underline-chanedit)* function to apply the electrode locations **[1]**. Visually inspect the channel layout using the EEGLAB's GUI *(G-U-I)* to confirm the correct positions of all electrodes **[2]**. Save the updated EEG dataset using the **pop_saveset** *(pop-underline-saveset)* function **[3]**.

3.3.1. SCREEN: Screen_3.3.1.tif

3.3.2. SCREEN: Screen_3.3.2.tif

3.3.3. SCREEN: Screen_3.3.3.tif

3.4. If required, reduce the sampling rate of the EEG data to 250 hertz using the **pop_resample** *(Pop-re-sample)* function **[1]**. Apply a band-pass filter from 1 hertz to 50 hertz to channels 1 to 128 using the **pop_basicfilter** *(Pop Basic Filter)* function with a Butterworth design and filter order of 2. Use zero-phase forward and reverse filtering to prevent phase distortion **[2]**.

3.4.1. SCREEN: Screen_3.4.1.tif *Video Editor: Emphasize or highlight the selected part in the image*

3.4.2. SCREEN: Screen_3.4.2.tif *Video Editor: Only show the MATLAB window and emphasize or highlight the selected part in the image.*

3.5. Apply a notch filter at 50 or 60 hertz to channels 1 through 64 using **pop_basicfilter** with the **PMnotch** *(P-M-Notch)* design and a filter order of 180 **[1]**. For the artifact rejection, use the **clean_rawdata** *(Clean Raw Data)* function with the given parameters to automatically detect and remove flatline channels, noisy segments, and low-frequency drifts **[2,3]**.

3.5.1. SCREEN: Screen_3.5.1.tif

3.5.2. SCREEN: Screen_3.5.2.tif *Video Editor: Only show the MATLAB window.*

AND,

3.5.3. TEXT on PLAIN BACKGROUND:

FlatlineCriterion (5): Removes channels flat for more than 5 s.

ChannelCriterion ([-1]): Disables channel correlation-based rejection.

LineNoiseCriterion (0.7): Removes channels with excessive line noise above this threshold.

Highpass (−1): Disables additional high-pass filtering during cleaning.

BurstCriterion (8): Removes data bursts that exceed 8 standard deviations.

WindowCriterion (0.85): Requires 85% of data in a window to be clean to retain it.

Video Editor: If possible, show 3.5.3 as an inset or something similar during 3.5.2.

3.6. Visually inspect the EEG data using **eegplot** *(E-E-G-Plot)* from **EEGLAB** to identify any residual artifacts missed by the automated method **[1]**. Manually mark and remove the remaining artifacts to ensure high data quality **[2]**. Use **pop_reref** *(pop-underline-reref)* to rereference the EEG signals to the average of all electrodes, ensuring the original reference electrode is retained **[3]**.

3.6.1. SCREEN: Screen_3.6.1.tif

3.6.2. SCREEN: Screen_3.6.2.tif *Video Editor: Highlight the green part in the plot.*

3.6.3. SCREEN: Screen_3.6.3.tif

4. Independent Component Analysis and ICLabel Classification Procedure

4.1. Load the file with the suffix **_loc_filt_cleanraw_reref.set** *(Loc-underline-filt-underline-cleanraw-underline-reref-dot-set)* using **pop_loadset [1]**. Run Independent Component Analysis or ICA *(I-C-A)* using the **pop_runica** *(pop-runica)* function with the **runica** algorithm to decompose the EEG data into independent components **[2-TXT]**. Save the new dataset with the suffix **_loc_filt_cleanraw_reref_ICA** using **pop_saveset** to indicate ICA completion **[3]**.

4.1.1. SCREEN: Screen_4.1.1.tif

4.1.2. SCREEN: Screen_4.1.2.tif **TXT: The 'runica' algorithm maximizes the statistical independence of the components** *Video Editor: Only show the MATLAB window and emphasize or highlight the selected part in the image.*

4.1.3. SCREEN: Screen_4.1.3.tif

4.2. Import the ICA dataset using **pop_loadset [1]**. Run the **pop_iclabel** *(Pop-I-C-Label)* function with the **default** model to classify independent components into brain and artifact categories **[2]**. Identify all components with brain activity probability above 0.7 **[3]**.

4.2.1. SCREEN: Screen_4.2.1.tif

4.2.2. SCREEN: Screen_4.2.2.tif

4.2.3. SCREEN: Screen_4.2.3.tif

4.3. Use the **pop_subcomp** *(pop-subcomp)* function to remove all components with ICLabel *(I-C-Label)* brain probability below 0.7 **[1]**. Retain only components with a brain activity probability above 0.7 to preserve genuine signals while ensuring the effective removal of artifacts **[2]**. Save the cleaned dataset with the suffix **_loc_filt_cleanraw_reref_ICA_Cleaned** *(Loc-underline-filt-underline-cleanraw-underline-reref-underline-I-C-A-underline-Cleaned)* using **pop_saveset [3]**.

4.3.1. SCREEN: Screen_4.3.1.tif *Video Editor: Only show the MATLAB window and emphasize or highlight the selected part in the image.*

4.3.2. SCREEN: Screen_4.3.2.tif

4.3.3. SCREEN: Screen_4.3.3.tif

4.4. Finally, import the dataset with artifact-free ICA components using the **pop_loadset** function **[1]** and save the fully preprocessed and normalized dataset with the suffix **_loc_filt_cleanraw_reref_ICA_Normalized** using **pop_saveset [2]**.

4.4.1. SCREEN: Screen_4.4.1.tif *Video Editor: Only show the MATLAB window and emphasize or highlight the selected part in the image.*

4.4.2. SCREEN: Screen_4.4.2.tif

Results

5. Results

5.1. The initial state of the EEG dataset prior to any preprocessing, including both **[1]** the raw signal traces **[2]** and their corresponding spectral characteristics **[3]**, is illustrated in this figure **[4]**.

5.1.1. LAB MEDIA: Figure 2A.
5.1.2. LAB MEDIA: Figure 2A. *Video Editor: Highlight the left image (Scroll Plot).*
5.1.3. LAB MEDIA: Figure 2A. *Video Editor: Highlight the right image (Spectral Plot).*
5.1.4. LAB MEDIA: Figure 2A.

5.2. The raw EEG data exhibited high levels of noise and irregular signal traces across multiple channels, compromising clarity and interpretability **[1]**.

5.2.1. LAB MEDIA: Figure 2A.

5.3. Manual cleaning of the EEG data reduced signal artifacts **[1]** and improved waveform uniformity **[2]**, but some residual noise and inconsistencies remained visible **[3]**.

5.3.1. LAB MEDIA: Figure 2A, 2B. *Video Editor: Highlight the left image in 2B (Scroll Plot).*
5.3.2. LAB MEDIA: Figure 2A, 2B. *Video Editor: Highlight the right image in 2B (Spectral Plot).*
5.3.3. LAB MEDIA: Figure 2B.

5.4. Data processed with PIPEMAT-RS *(Pipe-Mat-R-S)* displayed the cleanest EEG waveforms with consistent signal structure and minimal visible artifacts across channels **[1]**.

5.4.1. LAB MEDIA: Figure 2B, 2C. *Video Editor: Highlight 2C.*

Pronunciation Guides:

montage
Pronunciation link:
https://www.merriam-webster.com/dictionary/montage
IPA: /ˈmɑnˌtɑʒ/
Phonetic Spelling: mon-tahzh

 downsampling
Pronunciation link:
https://www.collinsdictionary.com/us/dictionary/english/downsampling
IPA: /ˈdaʊnˌsæmpəlɪŋ/
Phonetic Spelling: down-SAMP-ling
 artifact
Pronunciation link:
https://www.merriam-webster.com/dictionary/artifact
IPA: /ˈɑrtəˌfækt/
Phonetic Spelling: AR-tuh-fakt
 notch
Pronunciation link:
https://www.merriam-webster.com/dictionary/notch
IPA: /nɑtʃ/
Phonetic Spelling: notch
 resample
Pronunciation link:
https://www.merriam-webster.com/dictionary/resample
IPA: /riˈsæmpəl/
Phonetic Spelling: ree-SAMP-uhl
 independent
Pronunciation link:
https://www.merriam-webster.com/dictionary/independent
IPA: /ˌɪndəˈpɛndənt/
Phonetic Spelling: in-duh-PEN-dent