

Title: CMAP Scan MUNE (MScan) - A Novel Motor Unit Number Estimation (MUNE) Method

Authors and Affiliations:

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1. Introduction (Experimental Goal and Author Interviews) – As the beginning of your video, the introduction should clearly present the goal of your method to the viewer and its significance. Other information can be provided according to the various statements below, but the total introduction should not exceed 150 words.

A. Experimental Goal: (read by voice talent at JoVE)

The overall goal of this procedure is to describe MScan, a new method used to estimate the number of functioning motor units in a muscle.

B. Required Interview Statements: (Said by you on camera. Don't forget to smile!)

- 1.1. Hatice Tankisi: MScan is a noninvasive method used to estimate the number of functioning motor units in a muscle. MScan works by fitting a model to a detailed stimulus-response curve, or CMAP scan [1-MED].
 - 1.1.1. Hatice speaks to camera, looking slightly off center. Interview style.
- 1.2. Anna Bystrup Jakobsen: This technique has several advantages. It can be completed quickly, it is easy to perform and analyze, and it has excellent reproducibility [1-MED].
 - 1.2.1. Anna speaks to camera, looking slightly off center. Interview style.

E. Ethics title card: (for human subjects or animal work, does not count toward word length total)

- 1.9. All subjects must give their written consent prior to examination, and the recording protocol must be approved by the appropriate local ethical review board(s). All methods described here were approved by the Regional Scientific Ethical Committee and the Danish Data Protection Agency.

Protocol: (read by voice talent at JoVE)

2. Subject Preparation

- 2.1. To begin, request written consent from the subject. All subjects must give their written consent prior to the examination [1-WIDE]. Then, explain that the subject will experience a tickling sensation in their hand and wrist and that the examination can be halted at any time, should they experience too much discomfort [2-MED].

2.1.1. Talent enters shot and shakes hands with subject. Talent is shown speaking with subject, using body language cues that indicate that the talent is explaining the procedure to the subject. Talent then hands a written consent form to the subject. The subject signs the form.

2.1.2. Talent and subject are sitting down and speaking. Talent is shown indicating by pointing where the subject may feel a tickling sensation. Show talent nodding their head, to indicate that the subject understands this information.

2.2. First, use skin prep gel and alcohol to clean the subject's hand and forearm **[1-MED-over the shoulder]**. Position the active recording electrode over the abductor pollicis brevis muscle and then place the reference electrode on the metacarpo-phalangeal joint of the thumb **[2-CU]**. Place a ground electrode on the dorsum of the subject's hand and connect the electrodes to the pre-amplifier **[3-CU]**.

2.2.1. Talent cleans subject's hand and forearm as written.

2.2.2. Close up shot of the subject's hand as the talent positions the electrodes as written.

2.2.3. Close up shot of the subject's hand as the talent gently turns it over and places the ground electrode on the back of the subject's hand.

2.3. After positioning and connecting the electrodes to the pre-amplifier **[1-MED-over the shoulder]...**, tape the subject's fingers together to reduce noise and artifacts caused by voluntary movement **[2-CU]**.

2.3.1. Talent connects the electrodes to the preamplifier.

2.3.2. Talent gently tapes the subject's fingers together as written.

3. Recording the CMAP Scan

3.1. To start the semi-automated computerized system, use the standard protocol for motor nerve excitability tests. From the main menu, select 'MScan R' recording protocol, and accept default settings for the pre-amplifier and the stimulator. Then, accept the default parameters for scan step, interstimulus interval, and stimulus width **[1-SCREEN-TEXT]**.

3.1.1. SCREEN: To be provided by authors. **TEXT:** Stimulus width = 0.2 ms, Scan step = 0.2%; interstimulus level = 0.5 s

3.2. In the 'Select recording parameters' form, enter a 2-3 letter operator prefix in the 'Output file' box. Then, click 'O.K.' to continue. When the screen displays the raw EMG input, accept the default parameters and click 'O.K.' **[1-SCREEN-TXT]**.

3.2.1. SCREEN: To be provided by authors. **TEXT:** 0.2ms stimulus width; 0.2% scan step; 0.5s scan step

3.3. To find the site of lowest threshold at the median nerve of the wrist position a repositionable stimulating electrode **[1-CU]**... and click 'O.K.' to begin stimulation. The next display will show the stimulus and the EMG response. Continue to adjust the position of the electrode until the largest EMG response has been achieved. Once the site of lowest threshold has been located, click 'O.K.' to continue **[1-SCREEN]**.

3.3.1. Talent positions the electrode on the subject's wrist as written

3.3.2. SCREEN: To be provided by authors. **Authors:** You will need to take a screen capture video for all steps after the repositionable stimulating electrode is placed.

3.4. Next, the 'Modified' response will be displayed with the 'window', where the response is measured. The magenta line indicates the response, measured in the window, while the short green line immediately before the window indicates the baseline [1-SCREEN].

3.4.1. **SCREEN:** To be provided by authors

3.5. Replace the repositionable electrode with a non-polarizable adhesive stimulating cathode electrode [1-CU]... and place an anode 2 centimeters proximally along the median nerve [2-CU]. Encourage the subject to find the most relaxed position for their hand to minimize spontaneous activity [3-MED]. Click the 'OK' button to continue [1-SCREEN].

3.5.1. Talent replaces the old electrode on the subject's wrist with the new one.

3.5.2. Talent uses index finger to indicate the proper placement of the anode and then places the anode as written.

3.5.3. Talent is shown speaking with subject, using body language to indicate that they are giving directions. Subject nods and then places their hand in a comfortable position.

3.5.4. **SCREEN:** To be provided by authors. Authors: You will be getting screen capture video of clicking the O.K. button here.

3.6. Manually increase stimulus intensity by steps of 3% by pressing the "Insert" key. Click the 'OK' button to continue. Make fine, 1% stimulus adjustments until the stimulus current is above the level for the maximal amplitude of the CMAP. Then, check window position for the maximal response before clicking the 'OK' button to start the CMAP scan. [1-SCREEN]. After the responses to 20 supramaximal pre-scans are recorded, the stimulus intensity will automatically decrease with the frequency and percent steps selected previously.

3.6.1. **SCREEN:** To be provided by authors

3.7. Once there is no longer a motor response from the subject, click the 'OK' button to finish the recording with a series of 20 CMAP post-scans [1-SCREEN-TEXT].

3.7.1. **SCREEN:** To be provided by authors. **TEXT:** To check whether the subject was relaxed during the exam, refer to the text protocol.

3.8. Finally, click the 'OK' button in the lower right corner to finish the recording and save the data [1-SCREEN].

3.8.1. **SCREEN:** To be provided by authors

4. Fitting the Model to the CMAP Scan

4.1. To do analyses offline, start the analysis program and select the MScan recording to be analyzed. Select 'Fit MScan on a QZD file' from the menu [1-SCREEN].

4.1.1. **SCREEN:** To be provided by authors

4.2. Then, the program will automatically generate a preliminary model and optimize the fit. The program will continue to run without user intervention until the multi-colored progress bar in the 'Optimization' box is complete and the 'Stop' button is grey [1-SCREEN].

4.2.1. **SCREEN:** To be provided by authors

4.3. The original scan, in black, can be plotted next to the scan generated by the model, in magenta. Make serial adjustment to minimize the difference between the recorded and simulated scans and optimize the model. [1-SCREEN].

4.3.1. SCREEN: To be provided by authors

4.4. In the 'Plot type' box, different plotting options may be used to track the optimization process. A multi-colored progress bar will also be displayed in the optimization panel **[1-SCREEN-TXT]**.

4.4.1. SCREEN: To be provided by authors. **TEXT:** Options include: contour plots, error v N units, model units, and cumulative amplitude. Video editor: show this text overlay when VO says "different plotting options may be used to track the optimization process."

4.5. Use a contour plot to assess the accuracy of the model by selecting the 'Diff' and 'Contour map' displays. These displays show the differences between the recorded and modeled CMAP scans as a contour plot. As the optimization process runs, these differences will be minimized **[1-SCREEN]**.

4.5.1. SCREEN: To be provided by authors.

4.6. When the optimization process is complete, view the analysis results on the 'MScanFit text display'. Finally, click on the 'O.K. button to save the model in a MEM (pronounce "mem" as is memory) file **[1-SCREEN]**.

4.6.1. SCREEN: To be provided by authors

5. Results: "CMAP Scan MUNE Differentiates ALS Patients from Healthy Controls"

(Authors: Please note that the directions listed in blue are intended for our video editors.)

5.1. In this study, MScan MUNE is used to derive an estimation of the number and sizes of functional motor units by fitting a model to CMAP Scan **[1-LM]**. This technique is tested for sensitivity and specificity in both healthy controls and ALS patients **[2-LM]**. As expected, the median number of motor units in patients with ALS is significantly lower than in healthy controls **[3-LM]**.

5.1.1. 56806_Tankisi_Fig_2: Video Editor: When VO is saying "In this study, MScan MUNE is used to derive an estimation of the number and sizes of functional motor units by fitting a model to a CMAP Scan." show Figure 2 in its entirety.

5.1.2. 56806_Tankisi_Fig_2: Video Editor: When VO says "in both healthy controls" in the second line, zoom onto section A of Figure 2.

5.1.3. 56806_Tankisi_Fig_2: Video Editor: When VO says "and ALS patients" zoom into sections B, C, and D simultaneously. Show the sections of the figure in a horizontal row in that order (B, C, then D). Because the smaller graphs within figure 2 are not labeled, please label as follows: label B with "ALS patients with normal MScan", label C with "ALS patient with moderate motor unit loss", and label D with "ALS patient with sever motor unit loss". Please make these labels look like they are part of the original figures.

5.2. As shown by the ROC curve, the ability of MScan MUNE to distinguish the healthy controls from ALS patients is compared to that of CMAP **[1LM]**.

5.2.1. 56806_Tankisi_Fig_3: Video Editor: When VO says "MScan MUNE" highlight the red curve on Figure 3. When VO says "CMAP" highlight the green curve.

5.3. Area under the curve or AUC, is used to measure the ability of both techniques to distinguish between ALS patients and healthy controls. The AUC of MScan MUNE is significantly higher than that of CMAP **[1LM]**.

5.3.1. 56806_Tankisi_Fig_3: Video Editor: When VO says " The AUC of MScan MUNE" highlight the AUC value written in red inside of the results box on Figure 3. When VO says "than that of CMAP" highlight the AUC value written in green inside of the results box on Figure 3.

6. Conclusion (said by authors on camera)

- 6.1. Anna Bystrup Jakobsen: Once mastered, this technique can be completed in 6 minutes, if it is performed properly. The analysis process is also fast and should take less than 5 minutes.
- 6.2. Anna Bystrup Jakobsen: When attempting this procedure, it's important to remember to achieve adequate relaxation of the subject.
- 6.3. Hatice Tankisi: MScan is a method that may have the potential to be implemented in a clinical setting for diagnosis and monitoring of neuromuscular disorders, such as ALS.
- 6.4. Hatice Tankisi: Further studies with other neuromuscular disorders and larger study groups, are warranted. Studies on the application of MScan in different muscles should also be conducted.
- 6.5. Anna Bystrup Jakobsen: After watching this video, you should have a good understanding of how to perform and analyze MScan examinations.