

Semi-Automated Tracking of Vibrissal Movements in Free-Moving Rodents Captured by High-Speed Videos

Hyun June Kim, Tailong Shi, Seden Akdagli, Sam Most, Yuling Yan

Abstract—Quantitative analyses of whisker movements provide a means to study functional recovery and regeneration of mouse facial nerve after an injury. However, accurate tracking of the mouse whisker movement is challenging. Most methods for whisker tracking require manual intervention, e.g. fixing the head of the mouse during a study. Here we describe a semi-automated image processing method, which is applied to high-speed video recordings of free-moving mice to track the whisker movements. We first track the head movement of a mouse by delineating the lower head contour frame-by-frame that allows for detection of the location and orientation of the head. Then, a region of interest is identified for each frame; the subsequent application of a mask and the Hough transform detects the selected whiskers on each side of the head. Our approach is used to examine the functional recovery of damaged facial nerves in mice over a course of 21 days.

Keywords—Mystacial macrovibrissae, whisker tracking, head tracking, facial nerve recovery.

I. INTRODUCTION

RODENTS such as rats and mice move their mystacial macrovibrissae (whiskers) to explore the environment [1], [2]. By moving their whiskers back and forth, they acquire information about their surroundings such as textures and object features they encounter [3]-[5]. These whisker movements, also known as ‘whisking’, have been used as a tool to study facial nerve injury and recovery in rodents; the functional recovery of the whisker movement has been measured to indirectly measure the recovery of the facial nerve function. It has been used to study the effect of various parameters such as overexpression of *bcl2*, the antiapoptotic gene, the corticosteroid treatment, and administration of nimodipine, a calcium channel blocker, in functional recovery of injured facial nerve [6]-[8]. However, it is challenging to track the whisker movements, especially in freely-moving rodents, as whiskers are thin and move rapidly, and their movement patterns can be complex [1], [9]-[11]. As a result, most studies either rely on visual observation or employ some type of manual interventions [6]-[8], [12]-[16]. Yet, visual observation, which generally uses a subjective scoring system for assessing the functional recovery of whisker

movement rather than quantifying the recovery of whisker movements, may result in subjective and less reliable results, and whisker tracking methods that require manual interventions such as fixing the head of the mouse through surgical interventions or tagging a whisker with a small marker are time consuming and may require complex work, although they provide more objective and reliable evaluations [6]-[9], [12]-[17]. To address these limitations, methods to track whisker movements in unrestrained rodents have been developed [10], [18]-[21]. For example, infrared LED spotlights have been employed to create eye reflections in both eyes that can be used as a cue to track the head movement, and method to track head using the location of the tip snout and center of intersection of the whiskers, which requires a user to mark the location of the tip of the snout and the center of intersection of the whiskers in the first frame, has been developed to track the mean angular position of all the detected whiskers on each side [18], [19]. In this paper, we propose a new method to track whisker movements in freely moving rodents from high-speed videos without the requirement of manual intervention in tracking the head movement. Moreover, minimal manual input is required for tracking a single whisker, that is, selection of the whisker and the size of the mask allowing for accurate tracking of the selected whisker. Our method involves two steps: 1) tracking of the head movement and 2) tracking of the selected whisker. First, the location and the orientation of the head of the rodents are detected for each frame to track the head movement. Then movement of the selected whisker, determined by the change in the whisker angle during the whisking behavior, is tracked by detecting the selected whisker frame-by-frame using the Hough transform.

II. METHOD

A. Experimental Set-Up

Adult transgenic mice were filmed using a high-speed video camera (MS40k, Mega speed corporation, Minnedosa, MB, Canada) over a course of 21 days of facial nerve recovery following surgery. During video recording, the mice were awake and unrestrained, and the whisker movements were captured with 1280x1020 resolution at 500 frames/s for a duration of 2~6 seconds. Each unrestrained mouse was first positioned in a way that its snout pointed downwards and head portion of the mouse was filmed with overhead camera locked at a fixed position. At least three whisking cycles were analyzed, representing a range of 200 to 300 image frames (0.4 ~ 0.6 sec) of the video data.

H. Kim is with the Department of Bioengineering, Santa Clara University 500 El Camino Real, Santa Clara, CA 95053 USA (e-mail: hkim7@scu.edu).

T. Shi was with the Department of Bioengineering, Santa Clara University 500 El Camino Real, Santa Clara, CA 95053 USA (e-mail: tshi@scu.edu).

S. Akdagli, MD and S. Most, MD are with the department of Otolaryngology, Stanford University, 801 Welch Road, Stanford, CA, 94305 (e-mail: sakdagli@ohns.stanford.edu, Smost@ohns.stanford.edu).

Y. Yan is with the Department of Bioengineering, Santa Clara University 500 El Camino Real, Santa Clara, CA 95053 USA (corresponding author to provide phone: 408-554-4485; fax: 408-554-4874; e-mail: yyan1@scu.edu).

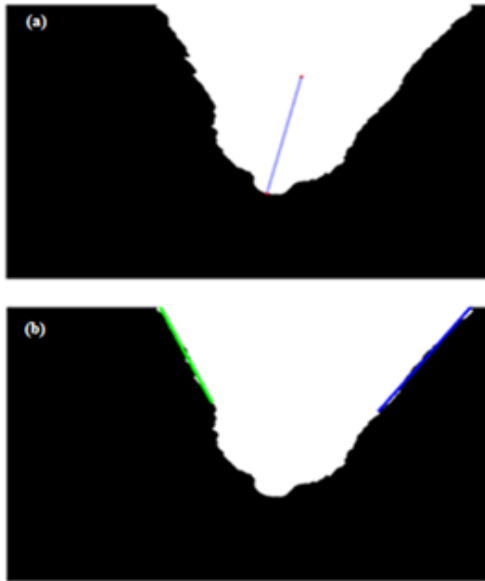


Fig. 1 Determination of the head orientation for each frame: (a) Estimation of the tip of the snout, the centroid of the segmented head; (b) Performing linear regression on each side of the snout

B. Design and Implementation

To track the whisker movements in unrestrained mice, we developed a two-step algorithm that enables us to track the movement of the selected whisker by tracking both head movements and whisker movements. By removing the effect of head movements, we can compute the true movements of the selected whisker. The proposed method was implemented in MATLAB language, and is described in more detail in the following sections.

C. Tracking of the Mouse Head

The head location and orientation were determined by tracing of the head contour frame-by-frame and subsequent linear regression operation on each side of the head. Following steps were designed to track head movements.

- 1) Segmentation of head image: To detect the orientation and the location of the head for each frame, we first segment the head image from the background. As there is need for rapid process of a large number of frames, we choose Otsu's unsupervised thresholding method to segment the head image [22]. Regions with stationary features present in the video are removed using motion cue before we perform segmentation [23]. Erosion followed by dilation is performed to the segmented image to remove any isolated objects and the whiskers to only leave the head image.
- 2) Tracing the boundary of head: After the successful segmentation of head image, we delineate the lower contour of the mouse head by tracing the boundary of the segmented image, which is the head of the mouse. Then, we estimate the location of the tip of the snout by finding the point farthest from the centroid of the segmented head. Using this point, the traced boundary is divided into two sides, representing left and right side of the face.
- 3) Performing linear regression: For each side of the traced

boundary, linear regression is performed to calculate the slope of each side of the face. To make sure that we are tracking the actual head movement, and avoid effects of movements caused by facial muscles leading the whisker movements, the lower half of the contour of the face is excluded when calculating the linear regression and its slope. The calculated slopes for each side of the face are then used to find the angles between the x-axis and each side of the face. Using these angles, we calculate the average angle, which estimates the angle between the midline of the face and the x-axis. The calculated angle in each frame represents the orientation of the face, and is recorded to track the head movement. Fig. 1 illustrates the results obtained from the two-step process: detection of the tip of the snout, and linear regression performed on each side of the face.

D. Tracking of the Selected Whisker

The Hough transform is an efficient feature extraction method that is commonly used to detect lines and has been used to detect whiskers in rats to track mean angular position of the whiskers [19], [24]. Here, we treat each whisker as a line, and use the Hough transform to detect the selected whisker and determine its orientation frame-by-frame. The steps designed for tracking the selected whisker are summarized below.

- 1) Identifying the Region of Interest (ROI): In the first frame we select the whisker to be tracked through manual input. After the whisker is selected, a bounding rectangle, or our ROI is created. The location of the ROI is recorded with respect to the estimated tip position of the snout, and is updated for each frame so that the ROI moves along with the estimated snout position in each frame.
- 2) Detecting a whisker in the first frame: To create a binary image, the Sobel edge enhancement operator can be applied before applying the Hough Transform for the detection of whiskers [19]. Within the bounding rectangle ROI, we apply the Sobel operator followed by the Hough transform to detect the designated whisker.
- 3) Creating a mask: After the line is detected, the orientation and the midpoint of the detected line are recorded. Because rodents generally have multiple whiskers on each side, and there may be several whiskers present within the ROI, we create an elliptical mask within the rectangular ROI that only bounds the selected whisker to avoid the inclusion of other whiskers that are present near the one to be tracked. The size of the ellipse is determined by the user, depending on the whisking amplitude and how closely the whiskers are located. The elliptical mask has its center at the midpoint of the detected line, which is recorded with respect to the position of the snout, and is rotated accordingly through:

$$\begin{aligned} &((x-h)\cos\alpha+(y-k)\sin\alpha)^2/a^2 \\ &+((x-h)\sin\alpha+(y-k)\cos\alpha)^2/b^2=1, \end{aligned} \quad (1)$$

where α is the angle between the detected line and the x-axis. The elliptical mask, which is updated every frame according to

the orientation and midline of the detected line, is applied to the subsequent frame and the Hough transform is only applied to the area bound by the elliptical mask. Fig. 2 shows the illustrative results of the steps described above. Since the acquisition rate of the video is high (500 frames/sec) and thus the movement of the whisker between the two consecutive frames is small, the selected whisker is still within the elliptical mask and can be detected.

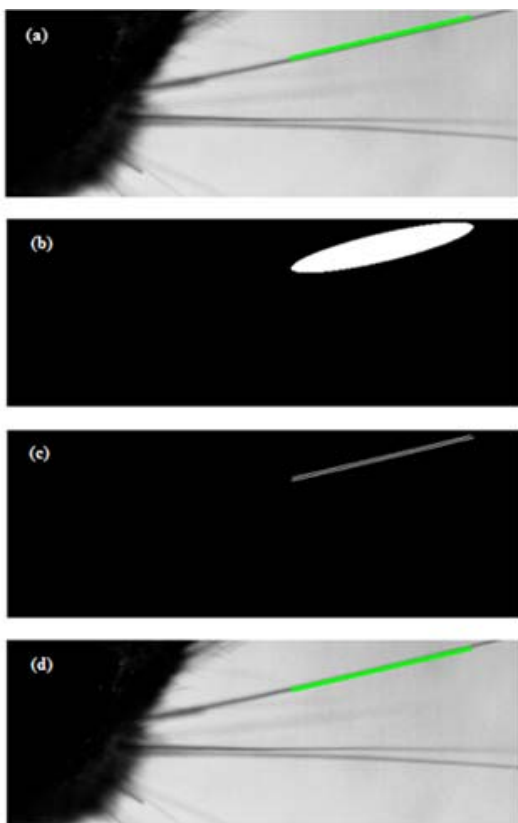


Fig. 2 Tracking of the selected whisker: (a) Detection of a specific whisker using the Sobel operator and the Hough transform within ROI; (b) Creation of elliptical mask according to the orientation and location of the whisker; (c) Application of the mask to the next frame after applying the Sobel operator; (d) Detection of the selected whisker

TABLE I
STD VALUES OF THE WHISKER ANGULAR POSITIONS

	Day7		Day13		Day21	
	Right	Left	Right	Left	Right	Left
#1	5.7062	2.399	8.6453	10.153	9.2716	6.067
#2	14.155	6.138	5.041	7.018	4.9791	8.752
#3	5.9135	2.59	7.5835	5.6581	8.5262	6.341
#4	7.267	5.608	6.2089	5.9653	3.5238	3.498
#5	2.4105	0.818	12.989	13.518	6.2452	5.512
#6	8.9227	4.189	6.4532	9.9095	6.7199	8.557
#7	11.048	4.963	8.1463	8.7148	7.4107	7.641

Std values of the whisker movements (over at least 3 cycles of whisking) detected on Day7, Day13, and Day 21 respectively after the facial nerve surgery. Whisker movement in the right side (unaffected by surgery) is compared with that in the left side.

4) Removing the effect of head movement: After the angle

between the whisker and the absolute x-axis is determined and recorded for every frame, and the orientation of head for each frame incorporated, we can track the actual movement of the selected whisker. The orientation of the whisker is calculated as the angle between the whisker and the rotated axis, where the whisker orientation on the left side is recorded with the clockwise angle between line and the negative x-axis and that on the right side is recorded with the counter-clockwise angle between line and the positive x-axis. When the method fails to detect a line due to any sudden blurriness for 1-2 frames, the average of angular positions of the whisker recorded from previous and subsequent frames of the frame(s) without the detected line (whisker) is computed and recorded as the orientation of the whisker.

III. RESULTS AND DISCUSSION

The proposed method was used to tracking whisker movements in unrestrained transgenic mice over a period of 21 days after facial nerve surgery being operated on their left side. Whisker movements, reflected by changes in whisker angular positions during the whisking behavior, were studied to observe functional recovery of the damaged facial nerve. To assess the functional level of the left whisker movements, the left whisker movements (side affected by the surgery) were compared with the right whisker movements (unaffected side). Fig. 3 shows an example of the results, obtained from the proposed tracking methods for both the head and the whisker movements.

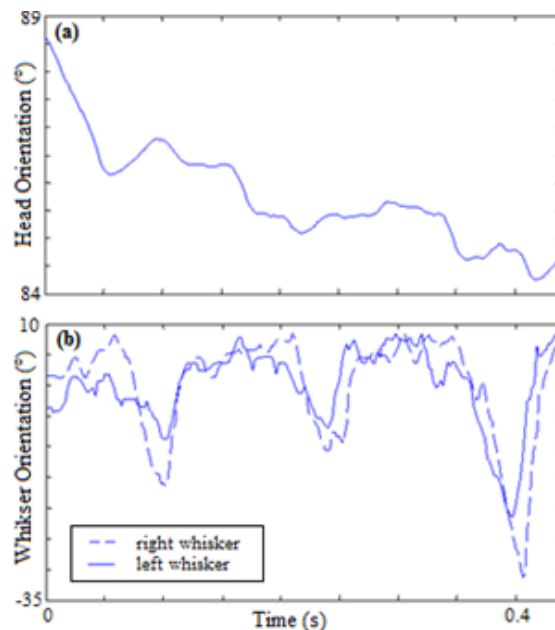


Fig. 3 Results of head tracking and whisker tracking: (a) Result of head tracking (head orientation in degree). Low pass filter was applied so any high frequency movements not caused by the actual head movements were excluded; (b) An example of whisker movements of a transgenic mouse 21 days after the facial nerve surgery. Dashed line represents the movement of the right whisker and solid line represents the movement of the left whisker

As damaged facial nerve on the left side begins to regenerate, whisking function of the left side starts to recover and an increase in the left-side whisking amplitude can be seen. Fig. 4 shows an example of changes in the movements of a left-side whisker compared to that of a right-side whisker observed over the period of 21 days after facial nerve injury. The left-side whisker, which undergoes almost no movement compared to the whisker on the right side on day 1, shows a significant increase in whisking movements on day 21, indicating a recovery of the left-side whisking function driven by the regeneration of damaged facial nerve. Furthermore, the fact that the left-side whisker is unable to move as much as the right-side whisker suggests that the injured facial nerve has not fully recovered.

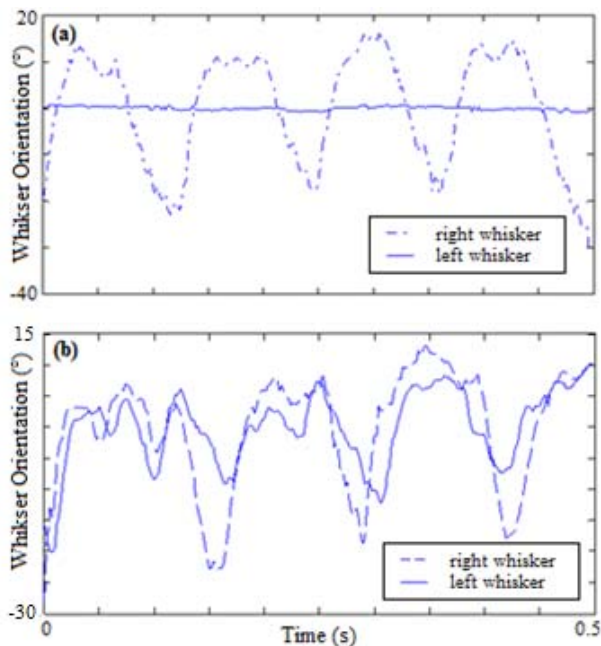


Fig. 4 Comparison of whisker movements after the left facial nerve injury. Dashed line represents the movement of the right whisker and solid line represents the movement of the left whisker: (a) Whisker movements from day 1; (b) Whisker movements from day 21

IV. CONCLUSION

Analyzing the whisker movement in rodents after facial nerve injury can provide useful information for studying the regeneration and functional recovery of injured facial nerve. In this paper, we have proposed a semi-automatic whisker-tracking algorithm that enables tracking of a selected whisker in unrestrained rodents, where cumbersome manual intervention such as fixing the head of the mouse is not required. We first track the movement of the head by performing linear regression on each side of the snout and estimate the orientation of the head in each frame. Then, we track the selected whisker by identifying a region of interest, which gets updated every frame, using both the location of the snout tip and the orientation and location of the selected whisker. Using this method, we were able to successfully track

the whisker movements in adult transgenic mice over the period of 21 days of recovery following the facial nerve injury.

However, there are a few limitations in the proposed method that requires future work. One of the biggest limitations in the proposed method is that user intervention is required in cases where the selected whisker is occluded by nearby whisker or when they are too close each other. Also, since accurate delineation of the head contour is a crucial step in head tracking, only frames with significant portion of the unobstructed head present can be processed for accurate and reliable tracking results. In the future, further improvement is desired for the detection of head image and the tracking of the selected whisker.

ACKNOWLEDGMENT

This work is supported by a research grant awarded to Y. Yan and H. Kim from the School of Engineering at Santa Clara University.

REFERENCES

- [1] S. B. Mehta, D. Whitmer, R. Figueroa, B. A. Williams, and D. Kleinfeld, "Active spatial perception in the vibrissa scanning sensorimotor system," *PLoS biology*, vol. 5, no. 2, e15, Jan. 2007.
- [2] B. Mitchinson, *et al.*, "Active vibrissal sensing in rodents and marsupials," *Philosophical Transactions of the Royal Society B: Biological Sciences* vol. 366, no. 1581, pp. 3037-3048, Oct. 2011.
- [3] M. Brecht, B. Preilowski, and M. M. Merzenich, "Functional architecture of the mystacial vibrissae," *Behav. Brain Res.* Vol. 84, no. 1-2, pp. 81-97, March 1997.
- [4] G. E. Carvell, D. J. Simons, "Biometric analyses of vibrissal tactile discrimination in the rat," *J Neurosci*, vol. 10, no. 8, pp. 2638-2648, Aug. 1990.
- [5] T. Prigg, D. Goldreich, G. E. Carvell, and D. J. Simons, "Texture discrimination and unit recordings in the rat whisker/barrel system," *Physiol. Behav.*, vol. 77, no. 4-5, pp. 671-675, Dec. 2002.
- [6] S. P. Most, "Facial Nerve Recovery in bcl2 Overexpression Mice After Crush Injury," *Arch Facial Plast Surg.*, vol. 6, no. 2, pp. 82-87, March 2004.
- [7] D. M. Lieberman, T. A. Jan, S. O. Ahmad, S. P. Most, "Effects of Corticosteroids on Functional Recovery and Neuron Survival After Facial Nerve Injury in Mice," *Arch Facial Plast Surg.*, vol. 13, no. 2, pp. 117-124, March 2011.
- [8] R. W. Lindsay, J. T. Heaton, C. Edwards, C. Smitson, T. A. Hadlock, "Nimodipine Accelerates Functional Recovery of the Facial Nerve after Crush Injury," *Arch Facial Plast Surg.*, vol. 12, no. 1, pp. 49-52, Jan/Feb. 2010.
- [9] N. G. Clack, *et al.*, "Automated tracking of whiskers in videos of head fixed rodents," *PLoS computational biology*, vol.8, no. 7, e1002591, July 2012.
- [10] J. T. Ritt, M. L. Andermann, and C. I. Moore, "Embodied information processing: Vibrissa mechanics and texture features shape micromotions in actively sensing rats," *Neuron*, vol. 57, no. 4, pp.599-613, Feb. 2008.
- [11] D. H. O'Connor, *et al.*, "Vibrissa-based object localization in head-fixed mice," *J Neurosci.*, vol. 30, no. 5, pp. 1947-1967, Feb. 2010.
- [12] T. Hadlock, J. Kowaleski, S. Mackinnon, J.T. Heaton, "A novel method of head fixation for the study of rodent facial function," *Exp Neurol.*, vol. 205, no. 1, pp. 279-282, May 2007.
- [13] T. Hadlock, *et al.*, "Functional Assessments of the Rodent Facial Nerve: A Synkinesis Model," *The Laryngoscope*, vol. 118, no. 10, pp. 1744-1749, Oct. 2008.
- [14] T. Hadlock, *et al.*, "The Effect of Electrical and Mechanical stimulation on the Regenerating Rodent Facial Nerve," *The Laryngoscope*, vol. 120, no. 6, pp. 1094-1102, June 2010.
- [15] J. T. Heaton, *et al.*, "A system for studying facial nerve function in rats through simultaneous bilateral monitoring of eyelid and whisker movements," *Journal of neuroscience methods*, vol. 171, no. 2, pp. 197-206, June 2008.

- [16] S. Venkatraman, K. Elkabany, J. D. Long, Y. Yao, J. M. Carmena, "A system for Neural Recording and Closed-Loop Intracortical Microstimulation in Awake Rodents," *IEEE Transactions on Biomedical Engineering*, vol. 56, no. 1, pp. 15-22, Jan. 2009
- [17] S. Roy, J. L. Bryant, Y. Cao, D. H. Heck, "High-precision, three dimensional tracking of mouse whisker movements with optical motion capture technology," *Front Behav. Neurosci.*, vol. 5, no. 27, pp. 1-6, June 2011.
- [18] P. M. Knutsen, D. Derdikman, and E. Ahissar, "Tracking whisker and head movements in unrestrained behaving rodents," *Journal of neurophysiology*, vol. 93, no. 4, pp. 2294-2301, April 2005.
- [19] G. Gyory, *et al.*, "An algorithm for automatic tracking of rat whiskers (Published Conference Proceedings style)," in *Proc. International Workshop on Visual Observation and Analysis of Animal and Insect Behavior (VAIB), in conjunction with ICPR, Istanbul, 2010*, pp.1-4.
- [20] I. Perkson, A. Kosir, P. M. Itskov, J. Tasic, and M. E. Diamondg, "Unsupervised quantification of whisking and head movement in freely moving rodents," *Journal of Neurophysiology*, vol. 105, no. 4, pp.1950-1962, April 2011.
- [21] J. Voigts, B. Sakmann, T. Celikel, "Unsupervised Whisker Tracking in Unrestrained Behaving Animals," *Journal of Neurophysiology*, vol. 100, no. 1, pp.504-515, July 2008.
- [22] N. Otsu, "A threshold selection method from gray-level histograms," *IEEE Transactions on Systems, Man, and Cybernetics*, vol. 9, no. 1, pp. 62-66, Jan. 1975.
- [23] T. Shi, G. Ling, and Y. Yan, "Tracing Vocal-fold Vibrations Using Level-set Segmentation Method. (Published Conference Proceedings style)," in *Proc. 3rd International Conference on Computational and Mathematical Biomedical Engineering, Hong Kong, 2013*, pp. 63-66.
- [24] R. O. Duda, and P. E. Hart, "Use of the Hough transformation to detect lines and curves in pictures." *Communications of the ACM*, vol. 15, no. 1 pp. 11-15, Jan. 1972.