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Article

Variation of the Performance of Machine-Learning Based Image Classifier in Automated Detection of Itch-Induced Scratch

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Abstract: A 'little brother' of pain, itch is an unpleasant sensation that creates a specific urge to scratch. To date, various machine-learning based image classifiers (MBICs) have been proposed for quantitative analysis of itch-induced scratch behaviour of laboratory animals in an automated, non-invasive, inexpensive and real-time manner. In spite of MBICs' advantages, the overall performances (accuracy, sensitivity and specificity) of current MBIC approaches remains inconsistent, with their values varying from ∼50% to ∼99%, for which the reasons underlying have yet to be investigated further, both computationally and experimentally. To look into the variation of the performance of MBICs in automated detection of itch-induced scratch, this article focuses on the experimental data recording step, and reports here for the first time that MBICs' overall performance is inextricably linked to the sharpness of experimentally recorded video of laboratory animal scratch behaviour. This article furthermore demonstrates for the first time that a linearly correlated relationship exists between video sharpness and overall performance (accuracy and specificity, but not sensitivity) of MBICs, and highlight the primary role of experimental data recording in rapid, accurate and consistent quantitative assessment of laboratory animal itch.

Keywords: Itch; Scratch; Automated real-time detection; Machine-learning based image classifier; Image sharpness

1. Introduction

Itch is a sensation that causes the desire to scratch [1–3]. In laboratory animal studies, scratch has been used as an objective correlate for quantitative assessment of itch [4–7]. Usually, scratch is counted by human annotators by playing the video back and forth, which is a time-consuming and laborious process with inherent subjectivity in quantitative assessment of laboratory animal itch. To this end, several machine-learning base experimental approaches have been developed to address this issue [8–10]. Very recently, Park and his colleagues [11] reported an automated real-time approach using machine learning-based image classifier (MBIC), which can be of practical use for the quantitative analysis of mouse scratching recorded with commercially available video cameras. This MBIC approach is non-invasive, inexpensive and suitable for objective counting of scratching with

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overall accuracy similar to or better than existing automated counting methods [11–13]. Moreover, it requires neither an attachment nor a surgical implantation of a device to mouse [11].

Nonetheless, MBICs' overall performances to date still remains inconsistent, including accuracy, sensitivity and specificity [11]. On average, the two-step decision tree (2DT) MBIC approach [11] performs with a sensitivity and specificity of 95.19% and 92.96%, respectively. While its sensitivity ranges from 94.8% to 99.1%, its specificity possesses a wider range from 68.6% to 85.1%, as reported by Park and his colleagues [11]. As for other MBIC approaches, such as support vector machine (SVM), k-nearest neighbour (kNN), convolutional neural network (CNN), recurrent neural network (RNN) and long short-term memory (LSTM), their sensitivities and specificities possess a even wider range from 55% to 97% [11]. In addition to the inconsistency of MBICs' performances, the reasons underlying have yet to be investigated further, both computationally and experimentally. Since various computational MBIC approaches have been reported already [8], and essentially all machine learning methods possesses an inherent trade-off between specificity and sensitivity that is controlled through the classification threshold, this article therefore chooses to focus on the experimental data recording step, i.e., the primary wet-lab step before any step(s) computational, and aims to investigate the relationship(s) between the MBICs' overall performances and the sharpness of the laboratory animal scratch video (LASV).

2. Materials and Methods

2.1. Chemicals and laboratory animals

Compound 48/80 (C48/80, Sigma, USA) is a polymer that provokes itch histamine-dependently in both human and mice [14]. Prior to use, Compound 48/80 was dissolved in 150 mM saline (Sichuan Kelun Pharmaceutical Co., Ltd, Sichuan Province, China) and stored at 4 °.

All animal-related experimental procedures were approved by Animal Ethical Committee of Nantong University (Jiangsu Province, China). Adult male health C57BL/6 mouse (weight: 24 ± 2 g) were purchased from Laboratory Animal Center of Nantong University, Jiangsu Province, China. All mice were housed singly and were maintained on a 12/12 day/night cycle at 22-25 ° with free access to rodent food and water in environmentally controlled specific pathogen-free (SPF) conditions.

2.2. Video recording, processing and preliminary analysis

Before LASV recording, a C57BL/6 mouse was placed into a box (dimensions 16 cm x 14 cm x 15 cm, Figure 1) for 15 minutes to ensure their familiarity with the box environment. Afterwards, the box (Figure 1) was cleaned with 70% ethanol and distilled deionized water. Of the LASV recording process, the general experimental setting is illustrated in Figure 1 briefly as below.

1. First, C57BL/6 mice were anesthetized and placed in the box (Figure 1) before an neck-subcutaneous injection of 50 μl C48/80 at 2 mg/ml.
2. A digital video camera recorder Sony™ HDR-CX405 (Sony Corporation, Japan) was employed to record a set of LASVs (time duration: 40 to 60 minutes) at 25 frames per second (fps) with a 1280 x 720 pixel resolution, assuming that the camera is fast enough to capture all scratching behaviors of C57BL/6 mouse [4,15]. By default, the focus is adjusted automatically by the Sony™ HDR-CX405 camera.
3. Each C57BL/6 mouse scratch was counted first by human annotators by playing LASVs back and forth, also recorded manually were the time labels (i.e., the beginning point and the ending point) of each set of scratch movements of the C57BL/6 mouse.
4. All LASVs were splitted into a series of image sequences with the *FFmpeg* command at 10 fps on an in-house Ubuntu machine.
5. With a set of in-house python scripts, all LASV images were linked to their respective time labels and manual annotations, i.e., *scratch* or *non-scratch*, for the subsequent training step of the machine-learning image classification processes.

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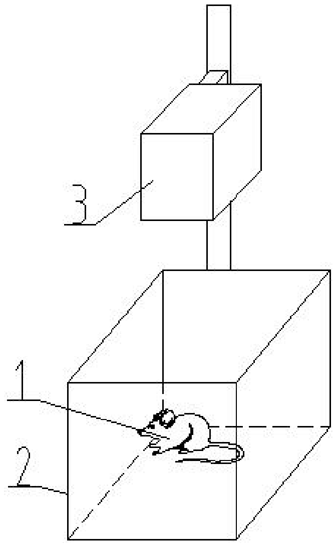

Figure 1. A minimalist sketch of the experimental setting for LASV recording. In this figure, labels 1, 2 and 3 represent the C57BL/6 mouse, the box and the position of the video-recording camera, respectively.

6. The sharpness of every LASV image was quantified as the variance of its Laplacian (supplementary file *detect_blur.py*).

Given that image sharpness is the focus here, it is necessary to ensure a reasonable scale coverage of the LASV sharpness, representing at least LASVs with high, medium and low degrees of sharpness. Therefore, we manually selected five from all experimentally recorded LASVs, labelled them according to their original file names, namely 305 (high sharpness), 306 (median sharpness), 309 (low sharpness), 310 (low sharpness) and 311 (high sharpness), respectively (Table 1).

2.3. Keras MBIC image classification

In total, 135769 LASV images were extracted from the manually selected five videos (Table 1). First, the 135769 images were pooled into one directory, and subsequently grouped into two sub-directories according to human annotations (i.e., *scratch* or *non-scratch*), with one sub-directory named *scratch* and another named *nonscratch*, respectively. For the MBIC's training step, 13650 LASV images (representing *scratch*) were imported into the open source MBIC software package (implemented in Python with Keras [11]), along with 13650 LASV images (representing *non-scratch*) which were randomly selected from the rest of all LASV images. The whole Keras MBIC software package is submitted as a supplementary file, along with its two main python scripts for MBIC image classification (Table 2), for which further details of the training and testing steps are included in Tables 2 and 1.

Of all 135769 computationally classified LASV images (Table 2), each and every image was subsequently linked to its time label, human annotation (*scratch* or *non-scratch*) and automated MBIC annotation (*scratch* or *non-scratch*), too. With both manual and computational annotations in place, a set of in-house python scripts were employed to statistically analyze the overall performances of the Keras MBIC approach and their potential relationships with the sharpness of experimentally recorded LASVs.

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Video number	305	306	309	310	311	Total
Total number of images (X1)	25542	27659	27020	28268	27280	135769
Trained Scratch (X2)	330	2350	3860	5820	1290	13650
Trained Non Scratch (X3)	2786	2834	2644	2526	2860	13650
Trained Total (X4=X2+X3)	3116	5184	6504	8346	4150	27300
Trained Scratch Ratio (X2/X1)	0.0129	0.0849	0.1428	0.2058	0.0472	0.1005
Trained Non-Scratch Ratio (X3/X1)	0.1091	0.1027	0.0978	0.0893	0.1048	0.1005
Trained Total Ratio (X4/X1)	0.1220	0.1876	0.2406	0.2951	0.1520	0.2010
C48/80 injection	No	Yes	Yes	Yes	Yes	Yes

Table 1. Statistics of the LASV images used for the training step of machine-learning based image classification of the five LASVs. For video 305, 150 mM saline was injected subcutaneously to the neck of C57BL/6.

Step	Input	Python script	Output
Training	27300 images	train_network.py	scratch_not_scratch.model
Testing	all 135769 images	test_network.py	*scratch* or *non-scratch*

Table 2. Training and testing of the machine-learning based image classification. For the training step, half of the 27300 images were with the manual annotation *scratch*, the other half *non-scratch*. In this table, all python scripts are submitted as supplementary files.

3. Results

3.1. Quantification of the sharpness of LASVs

With a preliminary visual inspection of the five LASVs, their sharpness (or blurrinesses) did vary in a manner of both inter- and intra-video, which is briefly illustrated in Figure 2 with two LASV images.

Figure 2. One LASV image with high degree (51.22, left) of sharpness (file name is 311out4381.png) and one LASV image with low degree (6.23, right) of sharpness (file name is 306out11856.png). Here, supplementary file *detect_blur.py* was used to quantify the sharpness of the two LASV images. The original versions of the two cropped and edited LASV images are submitted in supplementary file *supplementary.pdf*.

In addition to Figure 2, Table 3 presents a quantitative statistical analysis of the sharpness of the five LASVs, a necessary step for further investigation into its relationship(s) with the Keras MBIC's overall performances, which are to be described and discussed below in details.

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Video name	Average	Standard deviation	Maximum	Minimum	Sharpness
305	44.38	3.56	67.27	5.27	High
306	34.10	3.13	53.01	6.23	Median
309	29.91	2.67	37.66	6.95	Low
310	28.26	3.08	37.04	8.11	Low
311	41.47	2.16	58.91	12.28	High

Table 3. A statistical analysis of the sharpness of the five LASVs. In this table, the sharpness of each video was quantified as the average and the standard deviation of the sharpness of all 135769 images extracted from the five LASVs, the sharpness of each image was quantified as the variance of its Laplacian.

3.2. Overall performance of the Keras MBIC approach

Among all 135769 computationally classified LASV images (Table 2), there were a total of 108146 (96100 + 12046, Tables 4) images where the computational annotation is the same as its manual counterpart, with an overall accuracy at 79.65%. With a further video-specific analysis (Tables 4), it turned out that

1. The Keras MBIC approach here performed with accuracies ranging from 57.55% to 97.87%.
2. The Keras MBIC approach here performed with sensitivities ranging from 61.52% to 95.58%.
3. The Keras MBIC approach here performed with specificities ranging from 48.44% to 98.35%.

Overall, these findings align well with the performances of the previously reported MBIC approaches [11], where a range of 55% - 97% was reported for their specificities and the sensitivities of the Keras MBIC approach here.

Video number	305	306	309	310	311	Total/Average
Scratch Test True (X5)	203	2100	3587	5394	762	12046
Scratch Test False (X6)	127	250	273	426	528	1604
Scratch Test Total (X7=X5+X6)	330	2350	3860	5820	1290	13650
Non-Scratch Test True (X8)	24796	21465	13653	10874	25312	96100
Non-Scratch Test False (X9)	416	3844	9507	11574	678	26019
Non-Scratch Test Total (X10=X8+X9)	25212	25309	23160	22448	25990	122119
Sensitivity (X5/X7)	0.6152	0.8936	0.9293	0.9268	0.9558	0.8824
Specificity (X8/X10)	0.9835	0.8481	0.5895	0.4844	0.9739	0.7869
Accuracy ((X5+X8)/X1)	0.9787	0.8520	0.638	0.5755	0.9558	0.7965

Table 4. Overall performance of the Keras MBIC approach. In this table, X1 is the same as in Tables 4. To sum up, this table is combined with Table 4 as Table 1 in supplementary material *supplementary.pdf*.

With these quantitative video-specific analysis of the Keras MBIC approach's performances in place, it is now possible to further investigate their potential relationships with LASV sharpness. Here, with a set of in-house python scripts, the overall accuracy, the overall sensitivity and the overall specificity of the five LASVs were plotted against their respective sharpness, as shown in Figures 3, 4 and 5.

Specifically, Figures 3 and 5 demonstrate that the LASV sharpness is a linear correlate of both accuracy and specificity of the Keras MBIC approach here, with two Pearson's correlation coefficients 95.54% and 94.38%, respectively. On the other hand, no linear correlation was detected between LASV sharpness and the sensitivity of the Keras MBIC approach here, as shown in Figure 4.

To test the importance of specificity in the overall performance of the Keras MBIC approach here, we plotted its accuracy against its specificity and found a strong linear correlation between them with a Pearson's correlation coefficient of 99.84%, as shown in Figure 6.

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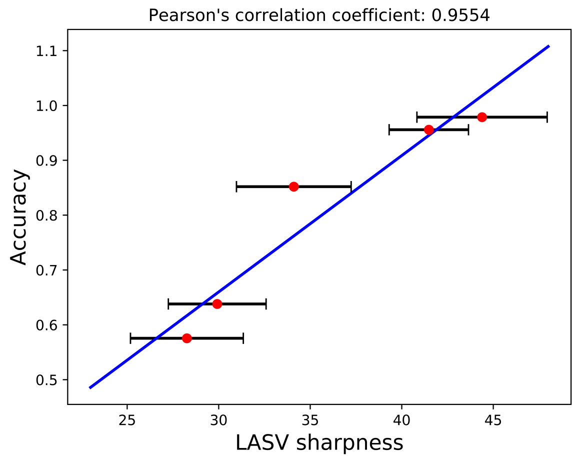

Figure 3. LASV sharpness influences the accuracy of the Keras MBIC approach in a linearly correlated manner.

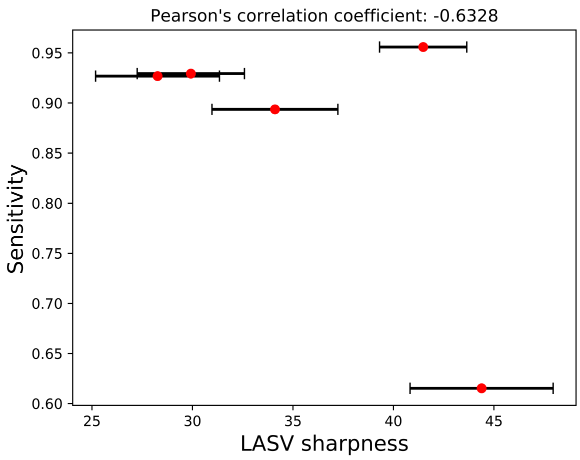

Figure 4. LASV sharpness does not influence the sensitivity of the Keras MBIC approach.

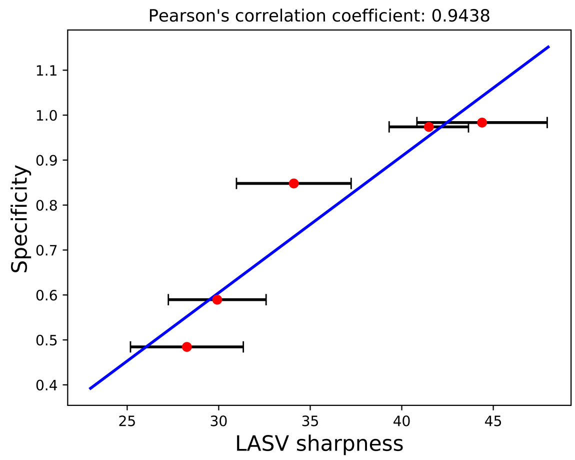

Figure 5. LASV sharpness influences the specificity of the Keras MBIC approach in a linearly correlated manner.

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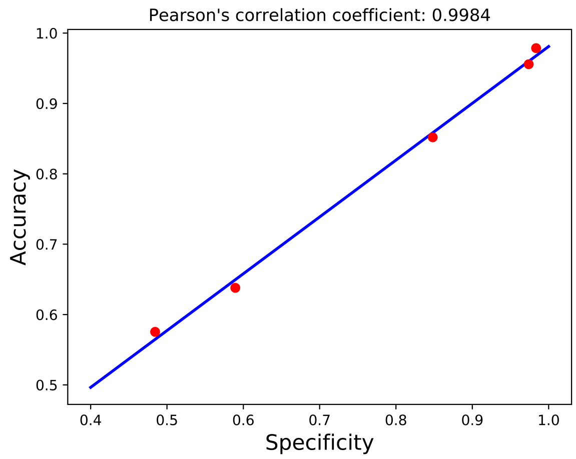

Figure 6. A linear correlation of the specificity and the accuracy of the Keras MBIC approach.

4. Variation of the performance of MBICs in automated detection of itch-induced scratch: an experimental perspective

While it has been already previously demonstrated that MBIC is a useful tool for automated quantitative assessment of laboratory animal itch [11], and on the fundamental and practical points of view, it also appears true that MBIC accuracy comes with LASV sharpness, no direct experimental study was reported yet as of January 17, 2020 to investigate the relationship between the two factors (i.e., accuracy and LASV sharpness) in a quantitative manner for the use of MBIC in automated detection of itch-induced scratch. Of the Keras MBIC approach here, this article puts forward a set of experimental evidences and reports for the first time that

1. LASV sharpness is a linear correlate of its accuracy.
2. LASV sharpness is not a linear correlate of its sensitivity.
3. LASV sharpness is a linear correlate of its specificity.
4. A linear correlation was observed between its specificity and accuracy.
5. No linear correlation was observed between its sensitivity and accuracy.

In addition to accuracy, sensitivity and specificity of the overall performance of MBICs, this article also highlights the importance of reproducibility and consistency of the overall performance of MBICs, which are just as important in rapid and accurate analysis of scratching for preclinical studies and high throughput drug screening for diseases like atopic dermatitis [16,17].

5. LASV blurriness: why and what to do next?

As discussed above, this article focuses on the primary wet-lab step, for which the experimental recording of LASVs is the weakest link in the loop. With a close naked-eye inspection of the five LASVs, we ask this questions: what makes the sharpness of all 135769 LASV images different from one another, both intra- and inter-video, as shown in Figure 2 and Table 3? To answer itr, we eyed on the experimental data recording step again, and boiled it down to two things: initial experimental setting and auto-focus of the video recording camera, for the accuracy and the precision of the focus of the video camera on C57BL/6 mouse, respectively.

1. The initial experimental setting (Figure 1) for LASV recording is fundamentally involved in the image quality of LASVs,
    (a) because the video camera must be told accurately where to focus first before LASV recording, otherwise it will be difficult for the camera to achieve satisfactory focus on the subject (i.e., C57BL/6 mouse), and the camera therefore might give up on the automated adjustment

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of the focus, resulting in failed auto-focus [18]. That is, the initial experimental setting (Figure 1) needs to be as accurate as possible, as it is the first step of LASV recording, hence the primary determinant for LASV sharpness.

(b) Therefore, with the statistical analysis and the discussion above, we call for the establishment of a universal standard for the initial experimental setting, including all necessary parameters, such as the size and the color of the box (Figure 1), the position of the camera (Figure 1), the manufacturer of the camera, LASV recording frequency, LASV image resolution/size, etc. With this universal standard in place, it becomes possible to compare the results obtained with video sequences of varying (but constant) qualities to clearly determine the minimum level of LASV sharpness to apply MBIC to detect itch-induced scratch.

(c) In the long run furthermore, with such a universal standard, the MBIC training step will produce a universally applicable *scratch_not_scratch.model* (Table 2),too, as an input for the MBIC testing step directly, and thereby strengthening the efficiency and the consistency and the reproducibility of MBIC-based automated detection of itch-induced scratch [19,20].

2. The focus of Sony^{TM} HDR-CX405 on C57BL/6 mouse is also inextricably linked to the image quality of LASVs, as by default the focus is adjusted automatically by the Sony^{TM} HDR-CX405 video camera. In general, there are four factors affecting the auto-focus performance of a camera: light level (level of illumination), subject contrast, camera motion and subject motion. Moreover, these factors are not independent; in other words, one may be able to achieve autofocus even for a dimly lit subject if that same subject also has extreme contrast, or vice versa [18].

   (a) For LASV recording, we suggest an anti-vibration [7] disturbance-free laboratory area with appropriate light level, where no laboratory member walks by the box and the camera (Figure 1) every now and then, to avoid light level variation and enhance the autofocus performance of the video camera. Furthermore, we also suggest the use of appropriate LASV recorders and video acquisition settings in the application of MBIC in automated detection of itch-induced scratch.

   (b) Supplementary file **animate.gif** included a truncated LASV built with three consecutive frames extracted from video 311, namely 311out85.png, 311out86.png and 311out87.png, representing the image of laboratory animal at 8.5, 8.6 and 8.8 seconds, respectively. The 0.3-second-long **animate.gif** is a clear representation that when a mouse scratches, it stands on one of its hind paws and uses another one of its hind paws to scratch its neck where C48/80 was injected, and that the subject contrast is strong enough for both naked-eye and MBIC to tell whether the mouse is scratching its neck or not at certain time point.

   (c) As shown in Figure 1, the position of the camera is set from the beginning, camera motion is therefore not an issue for the Keras MBIC approach here.

   (d) Mouse scratching can happen at ten times per second or more [3,6], which is a rather fast movement compared to the time (usually a fraction of a second [18]) necessary for one automated adjustment of the focus by the video camera. While most commonly available 30 fps video cameras are equipped with image sensors fast enough to clearly record these scratch behaviour [15,18], chances are that those recorded LASV images blur because the mouse scratches its neck too fast for the video camera to achieve a satisfactory focus on the subject and to clearly record each and every scratch movement of the mouse. To address this subject motion issue, further hardware improvements might be necessary for the autofocus sensor of LASV recording cameras.

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