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USAGE OF VIDEO ANALYSIS FOR TRACKING OF LABORATORY ANIMALS

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Abstract: This article deals with tracking of laboratory animals (guinea pigs) in cage. Authors collective applied machine vision tools based on global automatic thresholding, adaptive thresholding, differential operators and color matching algorithms. Animal activity and trajectory is significant marker in research of selected pathologies (gastrointestinal diseases in our case). Tracking is done in the huge database of video sequences of healthy animals (reference) and animals with induced gastric pain (gastric ulcer). The solution also be used like non-expensive replacement of radio-frequency laboratory trackers.
Keywords: animal tracking, image segmentation, trajectory, thresholding, differential operators, color matching

INTRODUCTION

Laboratory animals are used in many branches of medical research (drugs testing in pharmacology, efficiency of therapy). Many times, the behavior or activity of animal is significant marker of health status. This fact is used in research of gastrointestinal diseases and influence of selected type of therapy on them (e.g. animal suffering from any kind of gastric pain is generally less vital than healthy one) [1]. Comparison of long-time (hours or days) activity between healthy and ill animals is good feedback in process of optimal therapy setting.

Therefore, it is necessary to develop suitable method for animal activity monitoring or trajectory tracking. The first possibility is to use popular RFID (radio-frequency identification) tracking systems [2] for laboratory cages. This modern solution is slightly invasive: it is necessary to implant RFID tag under the animal skin. RFID tracking system uses specified hardware (RFID pad) and software for animal position processing [3]. Their main disadvantage is high cost. Another group of tracking methods is based on visual systems. Selection of optimal and robust segmentation algorithm is crucial. From the hardware point of view, these tracking methods need "only" camera and video data storage.

In this article we bring some methods for animal tracking in video sequence based on image processing and machine vision and their verification on selected sample video sequences. The length of trajectory is equivalent to animal activity, so we also bring the proposal for trajectory analysis [4]. All algorithms were applied offline but many of them could be used in real-time tracking (e.g. using OpenCV libraries).

MATERIALS

Video sequences for offline analysis were obtained in medical environment (Jessenius Faculty of Medicine, Martin, Slovakia) during experimental phases of gastric diseases therapy. Authors collective were asked for additional animal tracking after the experiments, so offline and machine vision approach was the proper solution for this task. The database contained approximately 200 video sequences with 1 hour duration. The video sequences were captured by color industry IP camera Panasonic in Windows Media Video format.

Figure 1. Scene with animal

The scene (Figure 1) can be divided into basic parts: animal (guinea pig), bowl for food, drinking place (plastic bottle), nest (plastic tube).
On the first sight, we can find and highlight some video sequence features which make the image segmentation process easier and those characteristics which can generate false segmentation results.

Benefits

In a cage, there is always one animal during the tracking process. One animal in a cage is a condition which results from requirements for experiments from medical point of view. Only behavior of one animal is monitored – its activity with and without certain pathology, it is not influenced by any other individual. The animal's environment is natural, standard used during laboratory experiments.
The main components of scene are relatively different in color (bowl is dark brown, nest is gray, animal is always white). In all experiments, the medical specialists used white guinea pigs. Lab technicians who prepared this experiment selected only white guinea pigs. This selection was probably made in order to fulfill set genetic criteria which are connected to research of pathologies of gastrointestinal tract (for instance, features such as genealogy, weight, age, etc.). Animal selection was done before processing of obtained videos and was not connected with used visual methods for trajectory detection. This means that authors solved only the task of animals' tracking, they could not influence their appearance. The color of animal is not important in the case of differential methods of tracking, but is very important in color matching (location).

Cons

The scene is non-uniform illuminated. Cage borders generate shadows (darker places). This fact is not important for adaptive thresholding-based methods or differential methods, but important for global ones and color location.
The covering grid is not removed from the top of the cage and generates reflections.
Despite of these crucial cons, "bad" features in the image created a challenge to authors to do the robust algorithm for tracking task.

METHODS

The main idea of animal tracking is based on animal segmentation, defining the object representative point (center of mass, centroid e.g.) and building the trajectory from these points. We selected three groups of algorithms: differential methods, thresholding-based methods and color matching (location) methods.

Differential methods

Differential methods integrate basic and advanced methods for motion detection using frame subtraction or static background removal [5]. Detection of laboratory animal motion can be defined as a change in position of the animal relative to its surroundings. Area with significant motion (changes) between adjacent video frames is represented in a resulting image as an area with higher intensity levels. Such an area is then transformed to the binary object in order to calculate the centroid (representative point of animal). There is only one single animal in the cage (scene) and the background is static. This fact brings a tremendous advantage for this method, which means that the resulting differential image is composed from one huge object representing animal and isolated small particles generated by noise processes. These small particles can be removed by morphological operators.
The simplest differential method is based on subtracting of relevant video frames. In our case, we can create reference frame ("empty cage", background model) and subtract it from the actual frame or we can subtract two following frames. The mathematical formula for simple difference is as follows:

$$d(n) = |f(n) - f(n-1)| \qquad (1)$$

where f(n) is actual frame and f(n-1) is previous frame; or:

$$d(n) = |f(n) - f_{ref}| \qquad (2)$$

where f_{ref} is reference image (empty scene).
Formula (2) is applicable for short-time sequences and analysis due to possible changes of background model (changing lighting conditions).
Advanced method [6] of simple differential was used for remove ghosting effect. This method is in experimental part called *Differential method #1.* Advanced differential method operates with three adjacent video frames: current frame (CF), next frame (NF) and previous frame (PF) (Figure 2a, 2b, 2c). Differential image is then:

$$d(n) = (PF - NF) AND (CF - NF) \qquad (3)$$

After differentiation, resulting pixels are thresholded using global thresholding (Figure 2d). Finding global thresholding is crucial to remove small particles generated by noise processes.

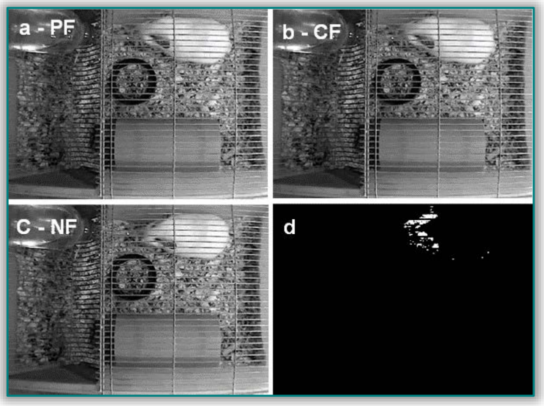

Figure 2. Advanced differential method #1: a) previous frame, b) current frame, c) next frame, d) thresholded differential image

To filter noise particles from thresholded differential image, we can compute standard deviation (StdDev) or variance of pixels and filter those with maximal value of given parameter; or we can use morphological operators for removing small particles. Resulting cluster of pixels is replaced with centroid and this is a single point of trajectory.

As *Differential Method #2* we used advanced static background removal method working in following steps:

» Creating background model from a part of video sequence or entire video sequence. The simplest way is to do the average of all frames or use advanced method [7], [8].
» Subtracting current video frame form background model and blurring the resulting image to remove small particles (Figure 3a). Blurring can be done through spatial scaling down the image and consequent resampling to original size or using blurring spatial filter (Gauss e.g.).
» Thresholding the difference image by Otsu's method [9]. After thresholding, small details are filtered out using erosion and dilation (Figure 3b). If total number of white pixels in the image is greater than MAX value or lower than MIN value, it is considered as false detection and the current frame is discarded. Position coordinates of the object in this frame is then set to predefined value or value from previous frame.

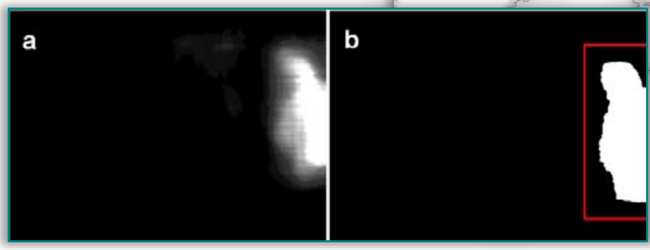

Figure 3. Advanced differential method #2: a) background removal, b) morphological filtering and construction of bounding box

Finally, in image processing lots of other combinations of subtracting two images are known. Some of the differential methods are quite simple and usually they are sensitive to the noise and ghosting [10].

Thresholding-based methods

Compared to differential methods, thresholding-based methods do not use the subtraction of neighboring frames in the video sequence but they work with frames separately. These methods suppose that the object is well separable from background (because of color or intensity). In this work we tested automatic global thresholding method based on entropy and local (adaptive) thresholding method called Background correction method.

Before using global thresholding based on entropy we converted color image from RGB space to HSI space, which is more suitable for computer vision applications. Intensity (I) layer concentrates most of image information content. Global threshold k divides image pixels into two groups: background and foreground. The entropy (information associated with black or white pixels) of background (H_b) and foreground (H_w) is then defined as [11]:

$$H_b = -\sum_{i=0}^{k} P_b(i).\log_2 P_b(i) \quad (4)$$

$$H_w = -\sum_{i=k+1}^{N-1} P_w(i).\log_2 P_w(i) \quad (5)$$

where i is gray level (0 - 255), P_b is probability (relative area) of background and P_w is probability (relative area) of foreground. Optimal threshold k maximizes following expression:

$$H_b + H_w \quad (6)$$

Additional morphology and binary filtering after global thresholding is often needed to remove small particles or filling holes in the object(s) (Figure 4c, 4d).

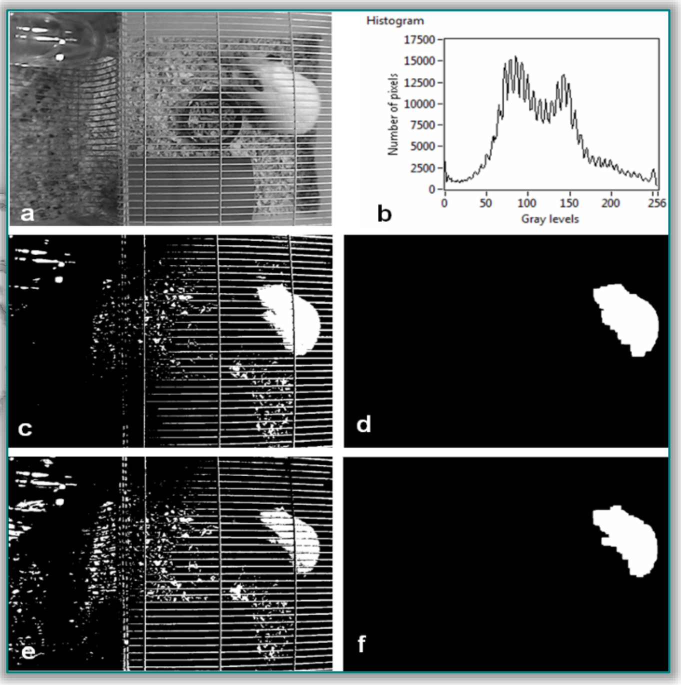

Figure 4. Global and adaptive thresholding: a) Intensity layer of actual frame, b) image histogram, c) result of thresholding using entropy, d) morphological enhancement of c), e) result of adaptive thresholding using Background correction, f) morphological enhancement of e)

Background correction algorithm combines local (adaptive) and global thresholding concepts for image segmentation [11]. In the first phase algorithm computes corrected image B(i;j):

$$B(i;j) = I(i;j) - m(i;j) \quad (7)$$

where I(i;j) is pixel value at position (i;j) in the frame and m(i;j) is average pixel value in the window centered at pixel I(i;j). Standard window size is 33x33 pixels. Corrected image is then thresholded by global technique (Otsu). Example of adaptive thresholding is in Figure 4e. Additional morphological filtering is needed (Figure 4f).

In both cases, the centroids of filtered objects are points of animal's trajectory.

Color location (Color Matching)

Color matching algorithm uses color template for which searches for in the inspected image. Algorithm determines

a level of similarity (matching score, in range 0 - 1000) between color spectrum of template and classified region in image (Figure 6). The details how to create a color spectrum of image region of interest (ROI) is in [11]. Simplified algorithm of color matching is shown in Figure 5. Key element of this method is in proper selection of template. In our case we created three variations of "animal's texture" for various lighting conditions. Algorithm tests the image ROIs for all the templates and resulting matching score is the maximum value.

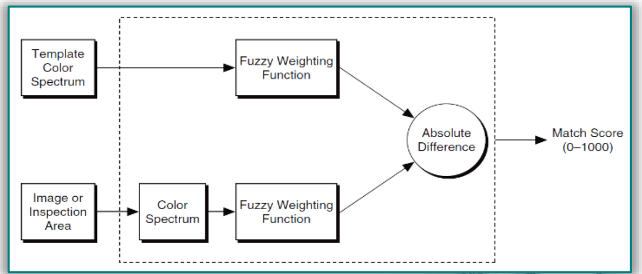

Figure 5. Block diagram of color matching algorithm

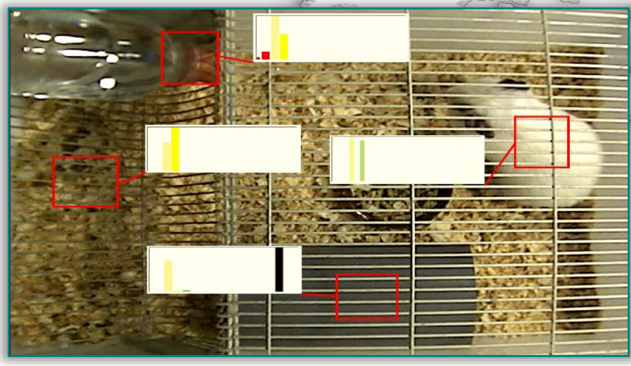

Figure 6. Examples of color spectrums in various image areas

Color matching method can work in two different ways. In the first mode, window with same size as template shifts across the image and color spectrum comparison is done at each position. If the matching score is higher than threshold value (750 e.g.), the central pixel of window is set to 1 or vice versa. The second mode searches only for 1 or some (as set) place(s) in the inspected image with the maximum matching score.

EXPERIMENTAL RESULTS

For experimental verification of laboratory animals tracking we selected 5 methods (2 differential, 2 thresholding-based and 1 color matching). Then we selected 5 representative video sequences (3 color for day mode and 2 infrared – IR - monochromatic for night mode). Color matching was tested only on color – day mode videos, because the IR videos do not contain color information. Duration of each test sequence was 1 minute. The first, we created reference trajectories. All video sequences were segmented manually by constructing bounding rectangle frame by frame around the guinea pig. Centroids for each bounding rectangle were inserted into an array for constructing the reference trajectory. After applying all 5 methods to the samples, the absolute difference between the coordinates from the reference trajectory and the coordinates generated from particular methods for adequate frames was evaluated. The example of experimental testing for each method is as follows:

1. Creating of reference trajectory (common for all methods) – Figure 7;
2. Applying the tracking method to the selected video sample (generating the new trajectory) – Figure 8;
3. Comparison of reference trajectory and the trajectory generated by the method – Figure 9;
4. Evaluation of absolute differences in pixels or relative difference in % – Tab. I.

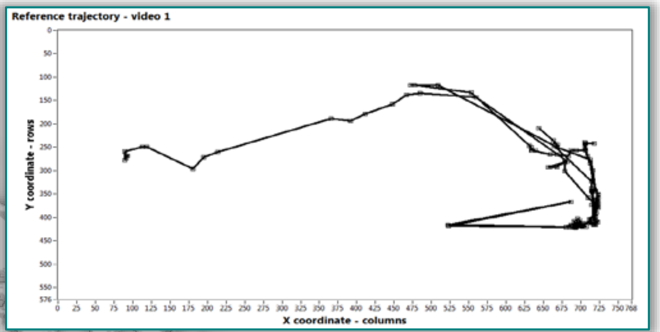

Figure 7. Reference trajectory for video sample #1

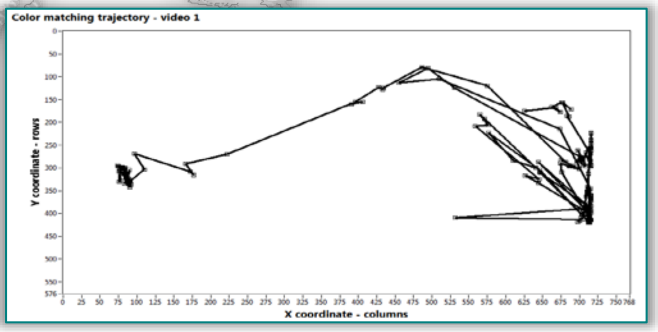

Figure 8. Trajectory for video sample #1 obtained by color matching

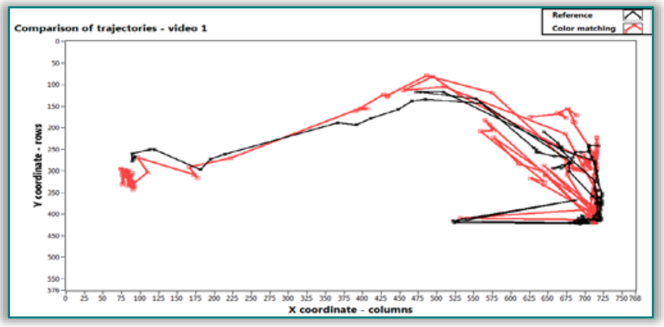

Figure 9. Comparison of reference (black) and measured (red) trajectory

According to Table 1 we can define:

- Δx [%] – average difference in x coordinate between reference and tested videos per one frame (error rate for x coordinate for each method);
- Δy [%] – average difference in y coordinate between reference and tested videos per one frame (error rate for y coordinate for each method).

Table 1. Methods Comparison

Tested video samples		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Entropy	Δx [%]	11.60	6.28	83.79	10.75	8.08
	Δy [%]	2.96	3.85	16.57	11.75	2.37
Color matching	Δx [%]	8.42	4.62	4.12		
	Δy [%]	2.93	7.91	6.75		
Background correction	Δx [%]	13.38	12.03	3.59	5.45	8.33
	Δy [%]	4.00	7.13	1.37	7.15	1.40
Differential method - 1	Δx [%]	12.17	2.57	4.13	3.88	3.35
	Δy [%]	16.26	8.47	8.90	4.07	5.08
Differential method - 2	Δx [%]	2.15	2.28	1.41	3.47	3.50
	Δy [%]	2.04	1.50	1.85	2.73	2.13

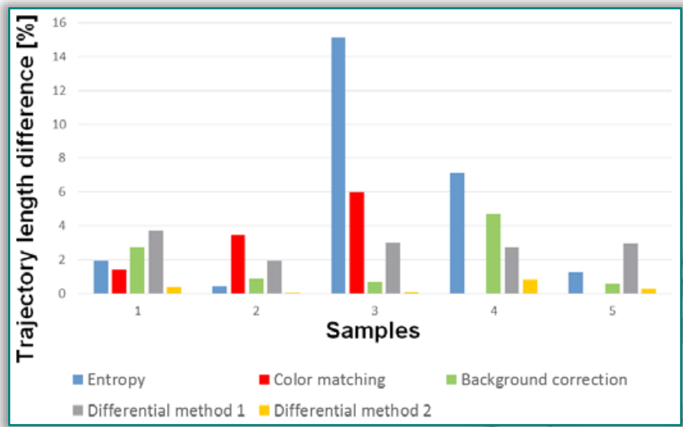

Figure 10. Comparison of methods accuracy

After trajectory points connection, we can compute absolute trajectory length. This parameter is the key element in the process of computation of single methods accuracy (Figure 10). Each trajectory length is compared with relevant reference one. In addition, if camera system is calibrated, trajectory length can be computed in real world units (cm or m e.g.).

DISCUSSION AND CONCLUSIONS

The first, we must underline that selected representative video sequences contain the natural guinea pig's scene without any preliminary modifications. The covering grid was not removed from the cage's top, the lighting was non-homogenous and so lots of shadows and reflections were presented at the scene – in order to verify the robustness of the motion detection algorithms (especially the thresholding-based methods). The entropy method was the most suitable thresholding technique for our application from the family of global thresholding techniques.

When testing the usage of global thresholding method several difficulties were undergone. In video sequences with poor lighting conditions (Sample 3), the entropy threshold value was not optimal and so that only the detection of guinea pig in certain part of its cage was possible (the well-lighted one). In order to bring the method to operate exactly, the scene in each video could be divided into two approximately homogenous parts (the well-lighted one and the one with worse lighting). Worse lighting conditions in the video sequence were obtained during the evening and were caused by combination of day light and artificial lighting (e.g. lamp). Bad accuracy of entropy technique is improved using adaptive background correction method. The accuracy of background correction is approximately at the same level for day video sequences as entropy accuracy, but much better for evening and night video sequences. It was proven that in order to bring this technique to work properly, it is necessary to consider the window size comparable with the size of sought object (guinea pig). In our application the windows size was set to 200x200 pixels for this purpose.

In moments of the video in which the guinea pig was hidden in its nest, its detected position was set into the middle of it automatically.

Detected objects whose area was less than 4000 pixels were considered as false-positive detections in moments of the video when the Guinea pig was hidden in its nest, so such small objects were excluded and the right detected position was set into the middle of the nest instead. Also the possibility of setting the ROI improved the correctness of this algorithm. Setting of the morphology operations did not change, they were the same for both entropy and background correction thresholding techniques.

Differential methods are significant with the stability of results for each sample. This is achieved by frame subtraction (Differential method #1) or static background removal (Differential method #2). In case that there was only one simple moving object in the scene, the background removal achieved the best results. Differential methods presented the best results. Due to their relative simplicity, these methods (with thresholding-based techniques) are suitable for real-time animal tracking.

Color matching operates with a chosen template containing a searched color which is normally of a small area (ca. 50 x 50 pixels), in our case it was part of guinea pig's white/bright fur. It was experimentally proven, that the method works much better if the image of the scene without the guinea pig ("empty cage") is first subtracted from each evaluated video frame. This eliminates the false-positive detection of parts of the scene, which also appear as bright ones (reflections on plastic drinking bottle). Color matching techniques are suitable for offline tracking (recording analysis) due to their computational difficulty.

All the results (trajectory lengths and deviations) are presented in pixels. Pixel units can be easily converted to real-world units (mm, cm, ...) after image calibration (with length reference) based on the fact, that camera has constant distance from the recorded scene.

Notable deviations in tracked length of animal's trajectory, is caused by varying of the centroid position. Centroid moves a little also in the situations, when guinea pig doesn‘t move (head moves, rotation by little angle etc.). This centroid varying can be successfully filtered by mean or median filtering e.g.

Generally, all methods have similar results in first and second sample. This was due to a relatively small movement of the guinea pig. In third sample, entropy thresholding and color matching failed. This was due to a greater amount of movement of guinea pig. Moreover, guinea pig moved also the nest and this was a significant source of error based on the nature of these methods. The last samples were obtained in IR mode. Especially in fourth sample each method achieved worse results. This was caused by the impacting noise. Differential methods are able to cope with this, as they compare the current image with previous. The best results for the whole sample set were achieved by differential methods, which subtract the background from the image. However, this may be caused by appropriate image post-processing (mathematical morphology and adaptive thresholding). All of the tested methods are based on simple image processing and can be easily implemented into any hardware. It makes these methods robust for other usage in animal trajectory tracking.

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Note

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References

[1] J. Halička, M. Tatár, “Upper Gastrointenstinal Visceral Pain Models In Guinea-pigs (mini-review)”, Acta Medica Martiniana, 2013, Suppl. 1, DOI: 10.2478/acm-2013-0005

[2] A. Gmiterko, M. Kelemen, I. Virgala, R. Surovec, and M. Vacková, „Modeling of a snake-like robot rectilinear motion and requirements for its actuators,“ in Proceedings of 15th International Conference on Intelligent Engineering Systems, 2011.

[3] L. Catarinucci, R. Colella, L. Mainetti, L. Patrono, S. Pieretti, I. Sergi, L. Tarricone: “Smart RFID Antenna System for Indoor Tracking and Behaviour Analysis of Small Animal in Colony Cages”. IEEE Sensors Journal, Vol. 14, No. 4, April 2014, pp. 1198-1206.

[4] Ľ. Miková, F. Trebuňa, and M. Kelemen, „Concept of locmotion mobile undercarriage structure control for the path tracking“. In Solid State Phenomena, Vol. 198, 2013, pp. 79-83.

[5] Isaac N. Bankman: Handbook of Medical Image Processing and Analysis (2nd edition). Elsevier Inc., 2009, ISBN 978-0-12-373904-9.

[6] R. T. Collins, R. Gross, and S. Jianbo, “Silhouette-based human identification from body shape and gait,” in Proceedings of 5th IEEE International Conference on Automatic Face Gesture Recognition, 2002.

[7] Sobral, Andrews. BGSLibrary: An OpenCV C++ Background Subtraction Library. IX Workshop de Visão Computacional (WVC'2013), Rio de Janeiro, Brazil, Jun. 2013. https://github.com/andrewssobral/bgslibrary.

[8] Piccardi, M. (2004, October). Background subtraction techniques: a review. In Systems, man and cybernetics, 2004 IEEE international conference on (Vol. 4, pp. 3099-3104). IEEE.

[9] Nobuyuki Otsu (1979). "A threshold selection method from gray-level histograms". IEEE Trans. Sys., Man., Cyber. 9 (1): 62–66. doi:10.1109/TSMC.1979.4310076.

[10] L. Zheng, S. Zhang, J. Zhang, Y. Zhang, „Dynamic image mosaic via SIFT and dynamic programming“, in Machine Vision and Applications, 2014, vol.25, pp. 1271-1282.

[11] National Instruments, NI Vision Concepts Help, June 2013.

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