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Cell detection and tracking in lab-on-a-chip devices by image processing

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This paper is devoted to the usage of image processing techniques in lab-on-a-chip devices for a cell detection and tracking. Advantages and disadvantages of image processing in data acquisition from lab-on-a-chip are described. A modified multiparametric object tracking method is presented. The method was tested on the lab-on-a-chip setup with living euglena and has shown plausible results for video with a low frame rate.

Keywords: image processing, lab-on-a-chip, computer vision, bioanalysis.

1. Introduction

Image processing of cells and microorganisms is an important tool in biological researches. Due to randomness and variety of the microobjects, advanced processing of the images is necessary to obtain useful and reliable data. Processing of static images may give us such parameters as: position, size, shape, opacity, deformation and inner structure of a single cell. Video processing provides a rate of change of quantities that can be obtained from static images and allows to track cell appearing, division, disappearing, movements, *etc*.

There are many methods and algorithms that enable image processing with special attention paid to cells recognition, characterization and tracking. Some of them are level adjustments, thresholding, morphological transformations, edge detection, contour search and segmentation. They can be divided into two groups. The first group (level adjustment, desaturation and thresholding) relates to the image improvement methods which adjust an image to prepare it for the further step. The second group is used to detect objects on an image.

The level adjustment is used to increase contrast and brightness of the image. Level adjustment maps the existing range of image pixel brightness values to the full possible range of the used image format. The next method is thresholding. Thresholding transforms all pixels that are brighter or darker than some specific value to white and all

other pixels to black. It allows marking objects on an image with different brightness level. It is used with color marking techniques.

The morphological transformations are some simple operations based on the image shape. It is normally performed on binary images. It needs two inputs, one is our original image, and second one is called a structuring element or kernel which decides about the nature of operation. Two basic morphological operators are erosion and dilation. Then its variant forms like opening, closing, gradient, *etc*., also come into play. In [1] thresholding morphological transformations are used to detect *E. coli* cells and measure their growth. Top hat transform is used in [2] to identify cytoplasmic regions of interest after the watershed algorithm pass.

The edge detection algorithms are used when we have to find objects with different brightness level or have uneven background on an image. The algorithms detect a rapid change of brightness value and mark it with a bright color. The simplest edge detection algorithm is the Canny algorithm. It uses Gaussian blur to remove the noise, calculates intensity gradients and detects edges by a double thresholding pass, then applies non-maximum suppression to thin the edges and eliminates all false edges by hysteresis.

Segmentation algorithms are intended to distinguish separate objects in groups. Watershed algorithm inspired by a physical principle of water basins is used to separate cells in groups, because cells are not completely connected. Feature based segmentation algorithm was used in [3] to distinguish biofilm and non-biofilm regions of an image of *V. cholerae* colony. In [4] double watershed is applied in order to detect cells on an image. It is also possible to distinguish cell phenotypes of eukaryotic cells using a feature search [4].

Contour search algorithms are used when single or a few not connected cells are presented on an image. Contours can be used to measure size, area, shape, position, circumference and momentous of a single cell. After using watershed algorithms contour search can be also used for wider range of images. Hough transform algorithm is concerned with the identification of lines, circles, ellipses or arbitrary shapes using voting procedure. Hough transform is used in [5, 6] to detect circular shapes which are assumed to be correspondingly yeast and HEK293T cells. When simple detection algorithms are not enough, machine learning techniques are used as in [7] for detection a nuclei in eukaryotic cells.

In many applications static parameters describing the cell/microorganism are used as preliminary information and then applied to start object tracking of the cell. Tracking algorithms are used to obtain movement of cells on timelapse records. In [8] authors describe traditional approaches to object tracking used in the cell biology. As described in [9] configuration, shape and quantity of bacteria can change between video frames, which is a linear assignment problem so a multiparameter model with the cost matrix is used.

Described algorithms are usually implemented in a form of an open-source or proprietary software system [9–11] or as a set of procedures in MATLAB [3, 5, 6].

T a b l e 1. Advantages and disadvantages of image processing in LOC.

Pros	Cons
1. High speed (throughput) in comparison to the manual analysis of a single image. 2. Batch processing and automation. Series of images and videos can be processed without involving human. 3. High precision and uniformity. Algorithm works in the same way for any input data. 4. Visual error detection. False positive detection can be seen by a naked eye on a resulting image.	1. Raster image contains a limited amount of information depending on its resolution and color depth. It means that low resolution images can result in a big magnitude error if processed. 2. In some cases image processing algorithms are time consuming. It is possible that high resolution images will not be processed fast enough to match the speed of real-time capturing. High speed image processing therefore requires hardware implementation (*e.g.* FPGA) that is a less universal solution. 3. Living cells are mostly semitransparent, hence need to be labeled by dye and image improvement techniques to enhance contrast. 4. Two-dimensional image of a cell is a projection of three-dimensional cells, so it has some error 5. It is hard to handle depth of field of an image captured by an optical microscope, especially when the cell is moving in 3D.

Image processing tools are also widely used with lab-on-a-chip (LOC) devices. Lab-on-a-chip devices are suitable for a sequential single cell analysis under specific conditions. There are plenty examples of usage of image processing techniques in LOC: in [6] and [11] small particles in picoliter drops can be detected; image processing is used to track yeast cells in the microfluidic matrix [12] and to measure the cytotoxicity based on fluorescence [13]. Lab-on-a-chip devices can contain complex structures (channels, mixers, chambers, valves, *etc*.) which have an influence on image background uniformity and measurement conditions. That influence can be diminished by using image processing methods. Pros and cons of the image processing for LOC are briefly presented in Table 1.

Typical cell tracking algorithms applied in LOC can fail if recognition of a cell on an image fails creating "hole" in a path. Cell tracking algorithms [9, 8, 14] are used to track cells without considering their rotation and replacement in 3D space. Microorganism can change its position and spatial configuration rapidly in a fraction of a second and can change it in the third dimension. These situations should be taken into account. What more and as it was mentioned earlier, lab-on-a-chip devices in most cases consist of a set of chambers and channels. It causes change in contrast and brightness over the image.

Therefore selection of the best detection approach to eliminate detection miss is important to decrease the probability of a "hole" appearing in a path. It is also necessary to enhance tracking algorithms to take into account cell rotation and movements in

3D space. In such cases, a shape of a cell can change, also cell image can be blurred. Also information about the shape of a cell to predict its movement direction (*e.g.*, euglena is an elliptical microorganism with a flagellum on one end therefore it can move only within the specific range of directions) should be used. Implementation of the multiparameter model for fast moving small objects should be considered. In this paper we propose the improved and optimized algorithm for cell tracking in LOC devices.

2. Methodology

Applied measurement setup is described by PODWIN *et al.* [15]. Briefly, it consists of lab-on-a-chip in a housing, light source, CCD camera (5 megapixels resolution, non-cooled) and PC with special software (Fig. 1). As a model moving microorganism, *E. gracilis* was used. The lab-on-a-chip has all glass structure with microchambers for the euglena growth (channel depth – 5 µm, channel width – 500 µm, chamber depth – 150 µm, and chamber dimensions – 4 × 2.4 mm^2).

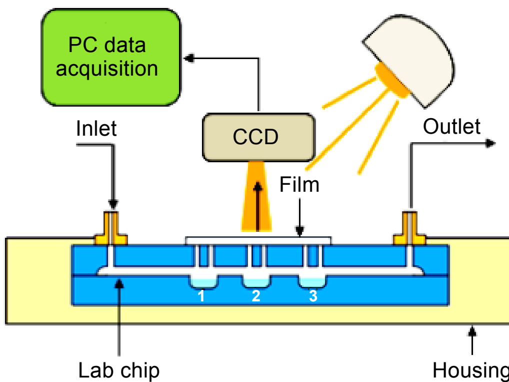

Fig. 1. Scheme of measurement setup with LOC for *E. gracilis* growth [9].

In order to use described methods in practice, there was implemented the software. The software was implemented using C++11 programming language to achieve high working speed and throughput, Qt5 Framework for GUI and OpenCV3 programming library for image processing tools. The software was tested on a computer with Intel Core i5-2450M @ 2.50 GHz processor with 8 GB of RAM.

The developed software allows the user to open and process an image or video file and save processing results in a plain text form.

3. Results

Five methods of cell detection were tested and implemented to diminish detection misses.

The simple thresholding algorithm processes an image inversely thresholding it with one threshold value. All pixels with brightness lower than threshold turn white

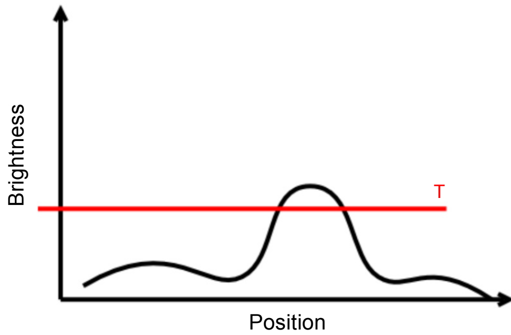

Fig. 2. Thresholding algorithm principle.

or black, otherwise accordingly to Eq. (1), and are schematically presented in Fig. 2. It is ineffective in case of brightness change over the image.

$$R(i, j) = \begin{cases} 255, & G(i, j) < t \\ 0, & \text{otherwise} \end{cases} \tag{1}$$

where: R – resulting image, G – initial image, t – threshold value, and i, j – pixel coordinates on an image.

The next method is multiple thresholding where an image is inversely thresholded few times simultaneously with decreasing or increasing a threshold value within a specific range (Fig. 3). It is effective on images with brightness change over the image.

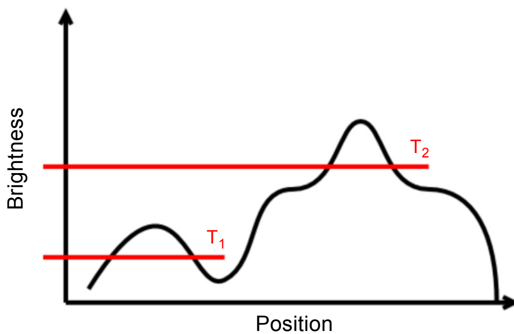

Fig. 3. Multiple thresholding algorithm principle.

Next edge detection was tested. In this method, an image is processed by Canny edge detection algorithm (Fig. 4). For this we need two threshold values, minVal and maxVal. Any edges with intensity gradient higher than maxVal are sure to be edges and those below minVal are sure to be non-edges, so discarded. Those which lie between these two thresholds are classified edges or non-edges based on their connectivity. If they are connected to "sure-edge" pixels, they are considered to be part of edges. Otherwise, they are also discarded. It is ineffective in case of image blur.

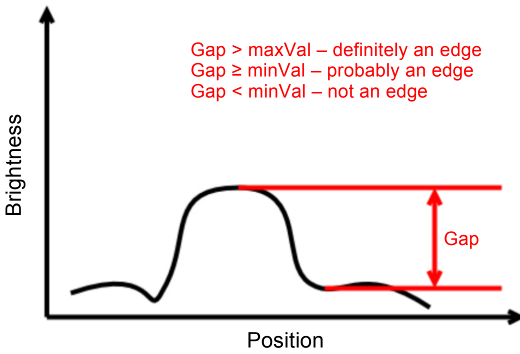

Fig. 4. Edge detection algorithm principle.

In the watershed algorithm, an image is processed to distinguish cells that are gathered in groups. Standard algorithm was extended by us with a preprocessing part. In a regular watershed algorithm the initial objects have to be marked manually. Using morphological operations we can detect objects automatically. In order to work with a group of cells with different level of "connectedness" or on a different background level, a multiple thresholding is used to detect a single cell in the group. This algorithm is relatively slow and ineffective for small particles.

The background elimination algorithm uses morphological transformations to find and remove non-uniform brightness of a background and small noise from an image leaving only features of a specific size. It is ineffective for detection of objects which are larger than approximately 20 × 20 pixels.

While processing, we were faced with overexposed images with high brightness and low contrast for which we used a minimum desaturation technique

$$\mathrm{Gr} = \min(R, \min(G, B)) \tag{2}$$

where: Gr – desaturated value, R, G, B – red, green and blue channels, respectively. It allows us to find small intensity change in cases where simple contrast-brightness adjustment is not enough (Fig. 5).

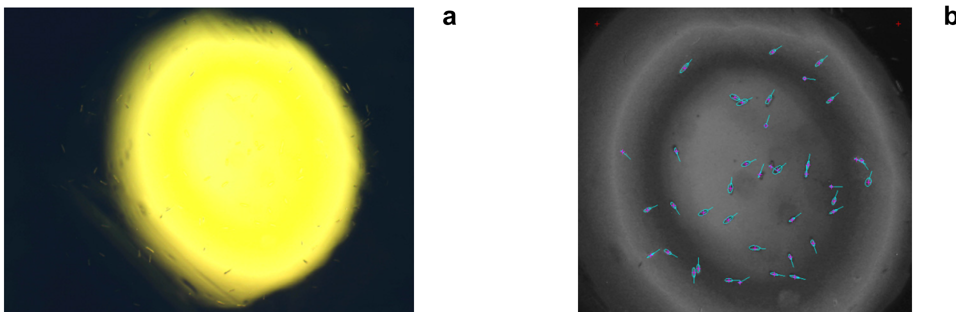

Fig. 5. Images of LOC microchamber with euglenas – overexposed (**a**) and after image processing (**b**).

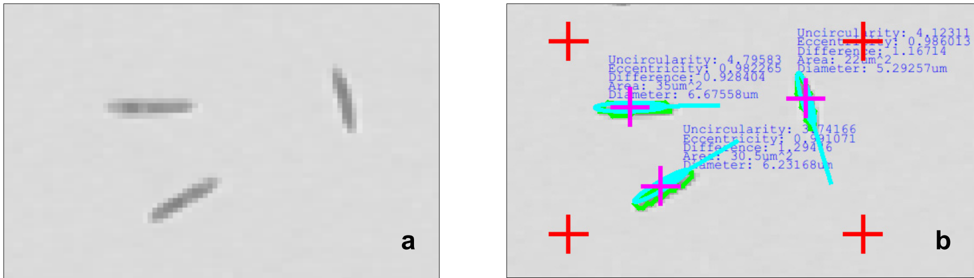

Fig. 6. Original image of euglena in LOC (**a**) and with determined parameters (**b**).

Each algorithm results in a set of contours for each detected object (Fig. 6). Various parameters of contours are calculated for further filtering of detection results and path tracking. The parameters are: uncircularity – ratio between semi-major and semi-minor axes of the ellipse, eccentricity of the ellipse, difference between areas of a contour and the ellipse, area of the contour and diameter of the contour.

The second part of tracking enhancing is a segment connection. It uses an assignment problem solving algorithm from [11] with modifications. Instead of using a sum of distance and moments as a function

$$\varphi = d + \alpha i + \beta a + \gamma s + \delta e \quad (3)$$

being minimized we are using a sum of distance and such parameters as: ellipse inclination i (an angle between long semi-major axis of the ellipse and the X axis), area a (area of the contour), size s (size of the contour calculated as a mean of semi-major and semi-minor ellipse axes), and eccentricity of the ellipse e.

Each parameter is multiplied by a coefficient (α, β, γ, δ) which represents an influence of the parameter on an overall function value. User can switch between linear and quadratic parameter values.

Other parameters such as intensity can be added further. Size, area and intensity allow us to distinguish two cells on different depth level in non-planar lab-on-a-chip devices. Inclination parameter increases the probability to distinguish two cells trajectories which are crossing within a small distance but at the significant angle.

Because of ellipse symmetry, the inclination value varies from 0 to 180 degrees. To convert it to 0–360 range, we use linear regression of a few points in a path as a vector **g** using an ordinary least squares estimator which allows retrieving a direction of a cell movement (Fig. 7). After calculating of the vector **g**, we measure angles between direct vector **d**, inverse vector **i**, regression vector **g** and reference vector **r** = (1, 0). Depending on which angle is closer to a regression vector angle, we choose between a direct and inverse vector as a vector of current cell direction.

Paths of cells are stored as arrays of points and related information. For each frame, current points are obtained by detection, and previous points are received as endings of each path. Therefore if a single path is not prolonged at the current frame, it can be done so on the next one. It requires increasing of the maximum distance in target func-

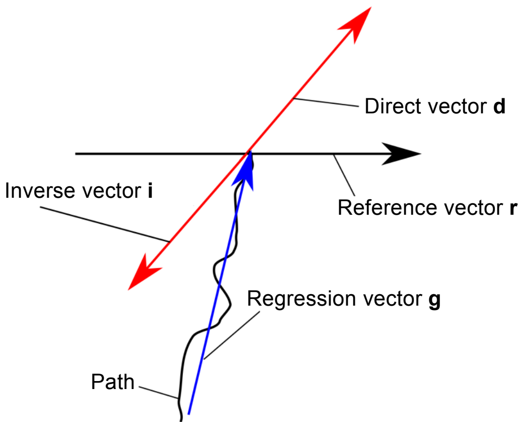

Fig. 7. Direction calculation scheme.

tion calculation which in its turn is compensated by other target function terms. Therefore "holes" in a path caused by detection miss can be omitted. Using only two consecutive frames, makes possible real-time video processing.

Relatively short displacements of a cell mass center below the user-defined value are merged together to eliminate small errors caused by frame to frame detection differences.

Table 2. Benchmark of detection methods in tracking (video: 36 seconds, 1024 × 768 pixels at 8 fps).

Algorithm	Time [s]	Maximum fps
Simple thresholding	4.6	62.6
Multiple thresholding	9.4	30.6
Edge detection	5.3	54.3
Watershed	15.93	18.07
Background elimination	7.9	36.45

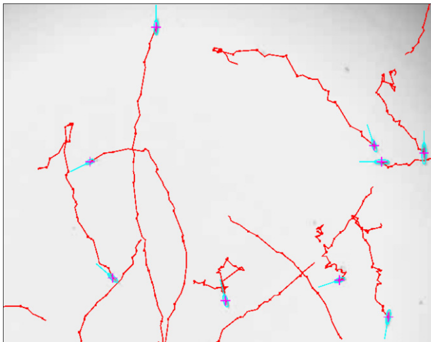

Fig. 8. Cell tracks of euglena inside a lab-on-a-chip chamber (edge detection).

The implemented algorithm was tested on a video with a frame rate equal to 8 fps (Fig. 8). At that frame rate euglena movement has a form of step sequences. Nevertheless we can obtain distinguishable tracks.

The comparative benchmark of applied algorithms was conducted (Table 2). Benchmarking results show that four of five algorithms can handle real-time processing at 24 fps frame rate. The watershed algorithm is relatively slow and need to be optimized or to be used on video streams with a lower data rate.

4. Conclusions

The paper presented the usage of image processing in lab-on-a-chip-based measurements setups for cell detection and tracking. Image processing helps to cope with the image artifacts which emerge from lab-on-a-chip features. Various detection methods were successfully tested to handle different measurement conditions caused by cells type and LOC device structure. The developed modified multiparametric object tracking method that solves a linear assignment problem is able to handle video stream processing in real-time. Therefore such method is suitable to work with long term observations without need to store video data.

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