Cancer cell detection and invasion depth estimation in brightfield images

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Abstract

The study of cancer cell invasion under the effect of different conditions is fundamental for the understanding of the cancer invasion mechanism and to test possible therapies for its regulation. To simulate invasion across tissue basement membrane, biologists established in vitro assays with cancer cells invading extracellular matrix components. However, analysis of such assays is manual, being time-consuming and error-prone, which motivates an objective and automated analysis tool.

Towards automating such analysis we present a methodology to detect cells in 3D matrix cell assays and correctly estimate their invasion, measured by the depth of the penetration in the gel. Detection is based on the sliding band filter, by evaluating the gradient convergence and not intensity. As such it can detect low contrast cells which otherwise would be lost. For cell depth estimation we present a focus estimator based on the convergence gradient's magnitude. The final cell detection's precision and recall are of 0.896 and 0.910 respectively, and the average error in the cell's position estimate is of $0.41\mu m$, $0.37\mu m$ *and* $3.7\mu m$ *in the x, y and z directions, respectively.*

1. Introduction

Invasion, an important step in cancer development, consists on the extravasation of cells from the tissue of origin into neighbor tissues. The absence of good models to study interactions between invasive cancer cells and other elements of the tumor microenvironment, led to an innovative 3D invasion assay [1]. However, the assay's evaluation is performed manually, which is time-consuming, fatiguing, and prone to errors. These limitations constitute a clear motivation for analysis automation.

We present a tool to evaluate 3D cell invasion based on the analysis of multiple brightfield images taken at different depths of focus, using a new focus estimation approach. Cell depth is in this case characterized by the best focal plane and is based on the variation of depth of focus towards the surface focus.

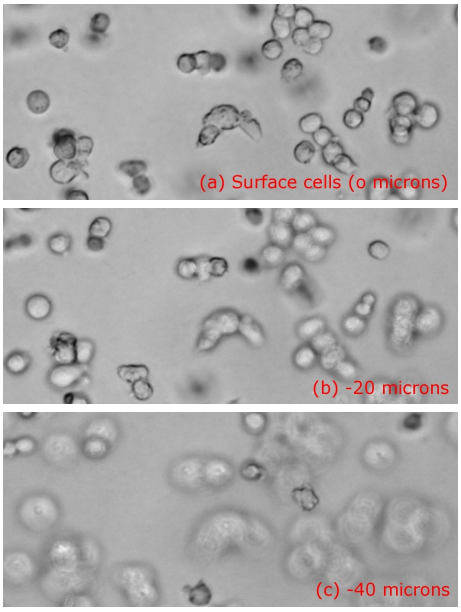

Figure 1. Cancer cell invasion assay images at different depths.

To obtain a successful detection of clustered cells even at low contrast we investigate the use of a particular convergence filter, the Modified Sliding Band Filter (MSBF) [3]. However, due to the cell's transparency, cells appear at several focal planes giving rise to multiple detections. To solve this problem we propose the stacking of multiple detections and filtering of false detections. From these 3D stacks of cell detection we apply a focus measure to estimate the degree of focus of each detection, determining in this way the depth and the full location of each cell.

Experimental Setup and Data Collection In their general formulation cancer cell invasion assays consist of gels of extracellular matrix components (collagen type I or Matrigel, for instance), on top of which isolated cancer cells, treated or not with specific drugs. After 24 hours of in-

cubation at 37°C and 5% CO_2 atmosphere, the system is visualized. A stack of images is collected varying the depth of focus, in $5\mu m$ steps (Figure 1).

To obtain the image data which will enable us to estimate cell invasion, a stack of images is collected varying the depth of focus. The images are collected from a depth above the surface until past the depth of the most invasive of all cells within the field of view, with focus being varied in $5\mu m$ steps. The joint focal length and camera CCD resolution give a spatial scale of $0.256\mu m$ per pixel, each image size being 1388×1040 pixels.

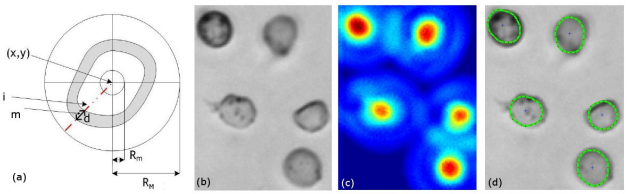

Figure 2. MSBF based cell detection:(a) MSBF filter schematics, (b) brightfield image, (c) filter response, (d) final detection.

2 Methodology

Our methodology is divided into three steps: Cell detection in each image leading to in-focus and out-of-focus detections. After 2D detection we search for cell detections, at adjacent planes, which are close to each other, associating them in a stack, each representing a possible cell at a determined (x, y) location. However, the z for each cell is still unknown. Finally, for each detection stack, we estimate the most likely image plane for each cell. This enables the determination the 3D position for each cell.

2D Cell Detection Our approach to cell detection is based on finding the cell's characteristic convex shape. To perform such detection we use a convergence filter, the modified sliding band filter [3], based on the SBF filter [2]. This filter defines a support region formed by a band of fixed width, whose position is changed in each direction to allow the maximization of the sum of the gradient convergence at each point. The set of band positions that maximizes the convergence index at each point will be called as band support points. The MSBF is defined by:

$$MSBF = \frac{1}{Nd}\sum_{i=1}^{N}\left(\max_{R_m \le n \le R_M}\left(\sum_{m=n-d/2}^{n+d/2} \|\cos(\theta_{i,m})\|\right)\right),$$

where N is the number of support lines, d is the band width, n is the band position in a line that varies from R_m to R_M, and $\theta_{i,m}$ is the angle between the image gradient at location m and the i line direction (Figure 2(a)).

After the application of the MSBF filter, cells are associated with the filter's maxima response. We can now estimate cells' shapes by investigating, for each filter maximum, the positions of the sliding band that contributed to that particular maximum. These are the band support points $SP = \{(x_{SP}(i), y_{SP}(i)), i = 1 \dots N\}$ defined as:

$$\begin{aligned} x_{SP}(i) &= x + n_o(i)\cos\left(\tfrac{2\pi}{N}(i-1)\right) \\ y_{SP}(i) &= y + n_o(i)\sin\left(\tfrac{2\pi}{N}(i-1)\right) \\ n_o(i) &= \arg\max_{R_m<n<R_M}\left(\tfrac{1}{d}\textstyle\sum_{m=n-d/2}^{n+d/2}\cos(\theta_{i,m})\right) \end{aligned}$$

where $n_o(i)$ corresponds to the radius of the support point for line i. The final border for the cell detection is the line connecting the band support points.

Cell Detection Stacking Given all the cells detected in each individual 2D image plane of the 3D stack of images we must now relate each cell in a 2D plane with all possible corresponding cells. This is performed based on a 2D distance between cells in adjacent planes, with the requirement of reciprocity. The final 3D stack is composed of the detection indices for each image plane in the stack, or zero in the case where the stack has no detections.

Additionally we also impose continuity of stacks (splitting stacks if there is no correspondence) and impose a minimum number of detections for a stack of cells to be valid. Using these constraints reduces the probability that erroneous detections may be stacked as it is unlikely for noise to be spatially consistent in z. More details on the specific values used for this implementation are presented in the result section.

Depth Estimation One of the MSBF filter main properties is that its result is not depending on the magnitude of the image's gradient. We propose a focus estimator based on the magnitude of the convergence at the band support points. It is a know property from depth from focus methods that an object is more focused if its borders are sharper [4]. As such we define the focus estimation measure as:

$$FE = \frac{1}{Nd}\sum_{i=1}^{N}\left(\sum_{m=n_o(i)-d/2}^{n_o(i)+d/2} \|grad(i,m)\| * \|\cos(\theta_{i,m})\|\right),$$

where $\|grad(i,m)\|$ is the gradient magnitude of the image at location m in the i filter's support line and $n_o(i)$ is the support point for line i.

We can now obtain a focus estimation for each detection. By evaluating the focus for all detections in each stack we find the better focused detection obtaining in this way the depth for that detection and the full 3D location.

Table 1. Cell detection results:precision, recall and error values for each experiment and the overall average.

	FP	FN	Pre.	Rec.	n. of cells
experiment 1	11	14	0.885	0.859	96
experiment 2	4	6	0.939	0.910	67
experiment 3	12	6	0.876	0.934	91
experiment 4	8	4	0.886	0.939	66
Overall (average)	8.75	7.5	0.896	0.910	80

Table 2. Cell's position estimation average error in the (x,y,z) cell's position (in μm).

	x error	y error	z error
experiment 1	0.44	0.41	4.8
experiment 2	0.40	0.32	3.5
experiment 3	0.35	0.36	3.2
experiment 4	0.45	0.40	3.5
Overall	0.41	0.37	3.7

3 Results and Discussion

To evaluate our methodology we use a dataset of 4 independent invasion assays comprising a total of 84 image planes (every $5\mu m$ in depth) and 320 cells. For parameter setting we used an auxiliary dataset with 25 images (70 cells). The dataset was annotated, by a researcher under supervision of the project's leading biologist, by selecting the best (x, y, z) coordinate for each cell. We applied the MSBF filter to all images and obtained detections for each plane, we then stacked and searched for the in-focus detection. We evaluated the correspondences between detections and annotation (Table 1). In addition to evaluating cell detection we evaluate the positional errors in the final cell's coordinates (Table 2).

4 Conclusion

We evaluated a new focus estimator for cell invasion depth estimation in brightfield images, based on of the Sliding Band Filter for cell detection. The results obtained using a database of 4 experiments, corresponding to 84 images and 320 cells, show a good cell detection precision (0.89) and recall (0.91). Additionally we evaluated the detection's positional errors and found that in average the x, y and z errors were of $0.41\mu m$, $0.37\mu m$ and $3.7\mu m$ respectively (Table 2). Overall the results were both numerically and visually promising towards full automation of cell invasion quantification (Figure 3).

The next steps in the search for an objective invasion assay evaluation are the determination of the surface z location and the computation of the percentage of cells invading. However, determining the surface is both obscured by the noise in depth detection and complicated since cells stack and move the gel matrix causing an undetermined depth variation.

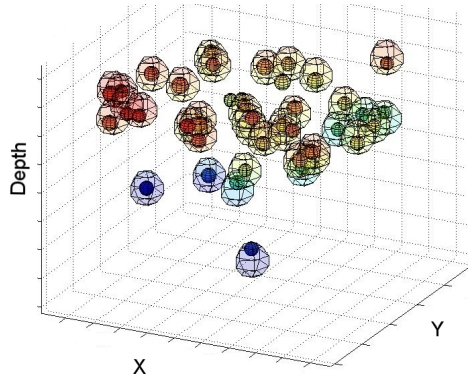

Figure 3. Final 3D detection example. Blue cells are deeper than red cells, transparent cells are annotation locations.

Acknowledgements

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