Cancer cell detection and invasion depth estimation in brightfield images

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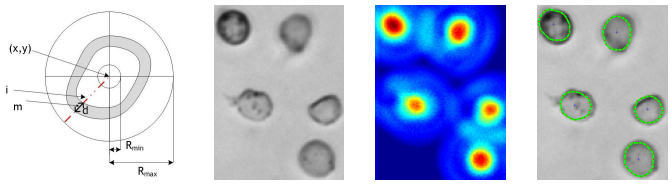
Invasion, an important phase in the development of cancer, consists on the extravasation of cells from the tissue of origin into neighbor tissues. The absence of good models to study the interactions between invasive cancer cells and the other elements of the tumor microenvironment, led to the construction of innovative 3D invasion assay [1]. However, the task of evaluating the results of such assays is performed manually by microscopic observation, which is time-consuming, fatiguing, and prone to human errors, requiring frequent repetitions towards validation [1].

We present a tool for the automatic analysis of 3D cell invasion based on multiple brightfield images taken at different depths of focus, using a new focus estimation approach. Our methodology can be divided into three steps. First, we detect all cells in each image, after 2D cell detection we search for cell detection, at adjacent planes, which are close to each other, associating them in a stack, each representing a possible cell. Finally, we estimate for each detection stack, which is the most likely image plane for the cell's location.

Most cell detection approaches are based on image segmentation. These approaches assume mostly isolated cells. However, in our case, cell's appear in groups and can appear superimposed. Our approach to cell detection is based on finding the approximated round shape characteristic of cells. To perform such detection we use a convergence index filter based on the SBF filter [3], the MSBF:

$$MSBF(x,y) = \frac{1}{N}\sum_{i=1}^{N}\left(\max_{R_{min}\leq n\leq R_{max}}\left(\frac{1}{d}\sum_{m=n-d/2}^{n+d/2}\|\cos(\theta(i,m))\|\right)\right), \quad (1)$$

where N is the number of support region lines that irradiate from (x,y), d is the band width, n is the position of the band in a line that varies from R_{min} to R_{max}, and $\theta(i,m)$ is the angle between the image gradient vector direction at location m and the direction that is currently being analyzed i. After filtering each image cells are associated with the locations of filter maxima and a minimum distance of R_{min} between maxima is enforced. Figure 1 shows the filter's response(b) and detections(c). Details on how to estimate the cell's shape can be found in the paper.



(a) (b) (c) (d)

Figure 1: SBF filter schematics (a), and examples of cell detection using the SBF filter: (b) brightfield image, (c) filter response for image (b), (d) final detection of cells.

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. For each cell detection in image the 2D distance to all other cell detections in the adjacent plane is obtained and the closest detection for the adjacent image is considered to correspond to the same cell. Cell detection correspondence is only valid if the 2D distance is lower than a threshold and if the closest detection criteria is reciprocal. The final 3D stack is composed

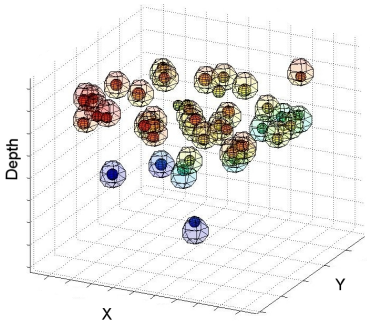


Figure 2: Final 3D detection result: smaller solid colored spheres represent detected cells, larger semi-transparent spheres represent ground truth.

of the detections, with correspondences, for each image plane in the stack. Additionally we also impose continuity of stacks and impose a minimum number of detections for a stack of cells to be valid.

One of the MSBF filter main properties is that its result is not depending on the magnitude of the image's gradient. This is particularly useful for the specific application of cell detection, allowing the detection even when image contrast is reduced. 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 [2]. The proposed focus estimation measure is:

$$FE(x,y) = \frac{1}{N}\sum_{i=1}^{N}\left(\frac{1}{d}\sum_{m=n_{max}(i)-d/2}^{n_{max}(i)+d/2} mag(grad(i,m)) * \|\cos(\theta(i,m))\|\right), \quad (2)$$

where $mag(grad(i,m))$ is the image gradient magnitude at location m in the i filter's support line and $n_{max}(i)$ is the support point for region line i.

For a given (x,y) coordinate we can now obtain a focus estimation which has a higher value if those coordinates correspond to an in-focus cell than if they correspond to an out-of-focus cell. Focus estimator value is also used to validate the cell detection stacks. If a detection stack does not have a minimum value of focus for any cell detection it is assumed that the stack contains detection of a cell that is never in focus (or noise), as such it is removed.

For each of the cells in the detection stack we can now obtain the most likely focus depth and as such detect the cell's 3D position (figure 2).

The results obtained using a database of 4 experiments, corresponding to 84 images and 320 cells, show a cell detection precision and recall of 0.896 and 0.910 respectively. Additionally we analyzed the magnitude of the cell 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.

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