Neurosphere fate prediction: an analysis-synthesis approach for feature extraction

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Abstract—The study of stem cells is one of the current most important biomedical research field. Understanding their development could allow multiple applications in regenerative medicine. For this purpose, we need automated methods for the segmentation and the modeling of neural stem cell development process into a neurosphere colony from phase contrast microscopy. We use such methods to extract relevant structural and textural features like cell division dynamism and cell behavior patterns for biological interpretation. The combination of phase contrast imaging, high fragility and complex evolution of neural stem cells pose many challenges in image processing and image analysis. This study introduces an on-line analysis method for the modeling of neurosphere evolution during the first three days of their development. From the corresponding time-lapse sequences, we extract information from the neurosphere using a combination of fast level set and curve detection for segmenting the cells. Then, based on prior biological knowledge, we generate possible and optimal 3-dimensional configuration using registration and evolutionary optimisation algorithm.

I. INTRODUCTION

The study of stem cells development plays a key role in modern biomedical research. From the different types of stem cells studied worldwide, neural stem cells (NSCs) and progenitors (NPs) are by far the most unknown. But they are also the most promising for a better understanding of the brain, the cure of neurodegenerative diseases (e.g. Parkinson, Alzheimer, Brain cancer, …) and the improvement of regenerative medicine. The lack of knowledge concerning these particular cells comes from the fact that there is no biomarker or other process that enables their identification. Beside, their high fragility to external aggressions (such as high intensity light) and their particular need of freely developing environment make their study very difficult to initialise. A growing protocol was developed for NSCs called neurosphere formation assay (NFA) [1], in which a set of neural cells composed of a majority of NPs and a small number of NSCs, extracted from an embryonic neural tissue, are dispatched in suspension into a developing factor solution. NSCs contained in the set of cells divide over time and proliferate until they form neural cell colonies called neurospheres, which is a 3-dimensional spherical aggregate of neural cells. Understanding how NSCs develop and give rise to those types of structures is a fundamental question in stem cell study. Monitoring and analysing this process is the key aspect for extracting possible patterns of development and identifying particular behaviours. Several research works have been done in the domain of living cell imaging and analysis, going from tracking to lineage construction and fate prediction [2]–[4]. Most of the analysis are done on two dimensions cell culture which could possibly affect general cell’s behaviour. We are developing a monitoring platform for neurospheres observation under phase-contrast time-lapse sequences at high magnification. The goal of this platform is to extract relevant biological features and analyse cell’s behaviour in three dimensions culture, which should give a better simulation of in vivo environment. As the NFA is a 3-dimensional process where cells evolve freely in suspension into a solution, we include a structural 3-dimensional modeling aspect in the monitoring to enhance the visualisation of neurospheres cells configurations over time. For this, we propose an analysis and synthesis approach by statistically determining the most probable 3-dimensional configuration, based on our observation of the image and prior biological knowledge. This model would provide topological information on cells and would allow studying the 3-dimensional structural aspect of the neurosphere formation.

The rest of the paper is organised as follows: section 2 details related work on living cells tracking and analysis. Section 3 explains the overall method we propose and section 4 details its implementation. Experiments and results are exposed in section 5 and a conclusion is given in section 6.

II. RELATED WORK

The study of living cells in phase-contrast time-lapse sequences is used for high-throughput imaging analysis of cell behaviour such as migration (movement), mitosis (division), apoptosis (death), lineage relation and fate prediction, generating important information for genomic research, tissue engineering, and stem cell biology. Most experiment are usually monitoring cells evolving in two dimensions and the process is commonly done manually,
off-line, using software tools to annotate cells, frame per frame, like in Eilkin et al. work [5]. This represents an important time consuming labour for the biologist, especially in the case of important amount of data or cells.

In order to improve cell analysis, several automated on-line algorithms for living cells study have been developed so far: one of the most know works about cells tracking and tracing has been done by Al-Kofahi et al. [2], who developed a lineage construction method for phase contrast observation and analysis of murine neural progenitor cells. The segmentation and tracking is based on intensity segmentation and the bright halo, specific to phase contrast images. Cells are then associated between frames using a likelihood score determined by extracted feature vectors during the segmentation. Based on those features, a detection of cell division and death is performed. This method is efficient but can fail on close contact cells and cells aggregate, which have high chance to be detected as one single cell.

Bao et al. [6] and Chen et al. [7] proposed similar processes for 3-dimensional fluorescence images cells, using confocal or phase contrast modality. The cell tracking was done by associating cells between frames using nearest neighbours, which can lead to errors in large multi-target tracking. Li et al. [3], [8] describe a sophisticated method based on level set segmentation and stochastic motion filter for cell tracking and tracing, and incorporate a backward tracing correction for possible gap or errors in the process. The method gives overall good results, especially in high-density cell images. More recently, Cohen et al. [4] developed a fate prediction algorithm for retinal progenitor cells called AITPD using intensity and pixel clustering segmentation and provide a prediction on the type of division based on cells features such as movement, size, orientation, etc. Based on Cohen’s work, Winter et al. [9], proposed a lineage tracing protocol for fixed NSCs including the AITPD tracking and fate prediction and Al-Kofahi’s lineage tracing construction into a open source program called LEVER.

While those work is oriented on observation and analysis of cell evolving in 2D, different works have been done on similar phase-contrast data, but allowing cell to freely evolve and proliferate in three dimensions, requiring different process and analysis. Such as Xiong et al. [10] on segmenting neurosphere at low magnification for drug testing, and Rigaud et al. [11] on neurosphere robust tracking to illumination change and dust presence in phase-contrast time lapse sequences using movement detection.

III. METHOD

The goal of our system is to extract relevant information from the neurosphere formation sequences and then, estimate a relevant 3D configuration of the neurosphere. Our system integrates three modules (Fig. 1), including: (1) an observation module, which detects, segments and analyses the microscopic images; (2) a modeling module based on rules, that generates a diversity of possible models of the observed cells called population; and (3) a convergence module, which will determine the best models and make them converge to a better optimum solution.

The system starts by taking as input the images sequentially generated by the microscope. The image is processed by the observation module that will segment each cell present in the neurosphere. It provides information on the neurosphere shape, the number of detected cells, and some other features present in the image. Based on theses outputs, the model module generates a set of models that could represent the current neurosphere and the cells configuration. Because multiple model can correspond to the observation, each model is scored according to its likelihood with the observation (Fig. 2a). The likelihood is determined by comparing different parameters between the observation and the different models. The best-generated models are selected according to their score and modified iteratively in order to make them converge towards an optimal representation of the current observation done by the first module. In the model construction, we aim to incorporate several rules and features extracted from the observation of NSCs, in order to improve the generated models and the model ranking.

Rules, that are mainly provided by the biologists, using prior biological and physical knowledge about the cells, must be adapted or translated into a mathematical concept in order to be used. Table I lists the relevant points in three categories: appearance, configuration and proliferation; and their interpretation for integration into the model generation module. Most of the current proposed rules concern: cells rendering under phase-contrast microscopy, which provide information on the type of material encountered by the phase light and can possibly provide information about overlapping objects; cell life cycle, providing information on division frequency and mechanism; and neurospheres prior configuration know-
TABLE I
BIOLOGICAL KNOWLEDGE AND INTERPRETATION

<table>
<thead>
<tr>
<th>Biological knowledge</th>
<th>Visual interpretation</th>
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<tbody>
<tr>
<td>Appearance</td>
<td>The PhC modality render cells with a visible grey level variation. High intensity on the cell membrane and low intensity in the centre of the cell. Interpret those intensity effect as a texture specific to each cells, modeled by a mixture of inverted Gaussian function.</td>
</tr>
<tr>
<td>Mitotic process interfere with the cell’s organelles density and disposition, in particular DNA, and should impact on the PhC observation. The global cell intensity increase before drastically decrease once the cell has divide.</td>
<td></td>
</tr>
<tr>
<td>Configuration</td>
<td>Cell division form two new cells from a unique parent cell, therefore, those two new cells will obligatory be neighbour and touch each other. All new cells will necessary appear in contact with already existing cell, and all neighbour of a cell shall be a close parent to this one.</td>
</tr>
<tr>
<td>Cell membrane is composed of extrinsic protein in charge of the cell interaction with neighbour cells and create links between cells membranes. Once in contact cells will stay attached to each other. This behaviour disappear with is the cell dies. All living cells will stay attach to each other and form colony.</td>
<td></td>
</tr>
<tr>
<td>Proliferation</td>
<td>A cell can either deform and move, divide into two new cells, or die. If no division or death, then modification of the cell population size is due to cells entering or leaving the field of view. A division and death probability can be determine by a cycle timer specific to each cells.</td>
</tr>
<tr>
<td>A cell reach its maturity and will divide after a 20-30 hours cycle or die.</td>
<td></td>
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</table>

ledge based on observation. Using the knowledge list and its interpretation, the likelihood score between an observation and a model is determined by three characteristics: the shape, the texture and the visibility. The shape gives information on the cell configuration but is not enough as multiple model solutions can give similar shapes. The texture information is based on the halo on the edge of cells characteristic of the phase-contrast modality. A simulated texture of the halo is applied to the model and provides additional information on the cell configuration and appearance. Finally the topological information is the visible topological aspect of the neurosphere. It provides information on the proliferation aspect of the neurosphere and the number of cells that are not visible. Those three characteristics provide a global score of the model solution (Fig. 2b).

IV. IMPLEMENTATION

A. Segmentation and cell detection

Based on automatic intensity threshold [12] and prior knowledge of the experiment which states that the cell will be kept in the center of the microscope field of view, a region of interest (ROI) is determined in order to lower the processing time by avoiding empty area of the image and limiting possible noise and presence of dust (Fig. 3b). Phase contrast modality can be either dark or bright contrast; we stand in the dark case where the background appears darker than the cells. Despite the high contrast between NSCs and the background, classic methods for segmentation such as edge detection using gradient norm, like Canny-Deriche edge detection, does not provide satisfactory results due to the non uniformity of the intensity of the cell membrane, the unclear delimitation of the cells, and illumination variation in the image sequence. Those variations are often present in phase contrast modality. For a more automatic and robust segmentation, we first apply an anisotropic diffusion function [13] that will remove important noise present in the image but preserve and sharpen edges. Then a level set method [14] to segment the neurosphere is applied. The method is initialized with the ROI detection, and the curve will converge and stop at the edge of the neurosphere (Fig. 3c). Segmenting each NSCs inside the neurosphere is not trivial, even for the human eye, because they are closely packed and attached to each other. A heat map of the centroids and radius of each visible NSCs is determined by detecting partial curve in the neurosphere, using Hough transform [15].

As the detected partial curve from the NSCs membrane is not
complete and, most of the time, does not describe a perfect partial circle, an approximation of the centroids is made, based on local maxima of the heat map (Fig. 3d, 3e). At the end of the process, a detection of the visible cells is obtained (Fig. 3f).

B. Evolutionary algorithm

EAs are applied as a problem-independent optimization algorithm, but many different EA and EA’s instance exist, depending on qualitative parameter, such as the individual representation, the mutation operator chosen, or the quantitative parameter like the starting population size or the mutation probability [16, 17]. In this optic, we will detail the individual representation, fitness function and evolutionary rules chosen for our problematic.

1) Representation model: The goal is to determine the configuration of cells that would fit the current observation. Therefore, the EA representation chosen for each individual is a configuration of cells in a $\mathbb{R}^3$ space. Each cells is simulated as a sphere determined by a center $c = \{c_x, c_y, c_z\}$ and a radius $r$, and the global topology of all the spheres shall represent a possible configuration of the neurosphere, which will give an individual. The configuration must respect biological and physical rules in order to avoid impossible configuration and to simulate as much as possible the neurosphere development. In this purpose, the individual generation is an iterative process that add a new sphere, according to the size of the individual required, at a random position that validate the spatial constrain, as described in the table 1 - configuration. Each added sphere should be located at coordinate $p$ complying with the constraint that it exists at least another sphere at coordinate $q$ in the space for which

$$d(p,q) \in [r, 2r]$$

where $d$ is the Euclidean distance between the two sphere centers $p$ and $q$. This will prevent spheres to fully overlap but force them to stay in contact with at least one sphere. This constrain comes from physical and biological statement that two NSCs can not occupy the same place and that NSCs posses membrane’s proteins that link them to each other in order to form colonies of cells. Each individual modeled has a projection in $\mathbb{R}^2$ using an orthogonal projection on an arbitrary axe, here the $z$-axe. The projection results of the individual will then be compared with the observed neurosphere in order to determine how well the model corresponds, or not, to the neurosphere. This comparison will serve as a fitness function to determine the importance of the individual. As multiple configurations are possible, we generate an initial population big enough to cover the possible space solution.

2) Fitness function: As a first implementation, the registration is based on shape comparison between a projected individual and the segmented neurosphere of the microscope image. Using a gradient descent, this registration approximates, the best transform parameter $\{\theta, c, \tau, \sigma\}$, where $\theta$ and $c$ are respectively the angle and the centre coordinate of the rotation, $\tau$ the translation vector and $\sigma$ the scale, that register the two images. We calculate a metric value between the two images given by a mean-square error function $f$, such as

$$f(M, m) = \frac{1}{N} \sum_{i=0}^{N} (M_i - T(m_i))^2$$

with $N$ the number of pixel, $M$ and $m$ are respectively the fixed image and the moving image, and $T$ the transform function. The metric function gives us a rating value of the current transformation parameters. A gradient descent is used to optimize the metric function until a stopping criterion is reached, such as a maximum iteration or a minimum variation in the gradient descent step. Applied on binary images, this registration is used to determine the transformation between the shape of a 2D projected model and the shape of the segmented neurosphere of the image. The texture information, based on the phase-contrast halo, is extracted by applying a simulated texture on each cell of the model (Fig 4). Using the same transform used for the shape registration, a comparison of the observed and the simulated texture is made. The metric value is saved as a rating score. This score, which is actually an error that will have to be minimised, will be used to determine the best model, as a fitness function, for the evolutionary algorithm.

3) Evolutionary rules: A stochastic search of the different solution is then done using an EA optimisation. The algorithm
1: $p_m \leftarrow$ mutation probability
2: $f \leftarrow$ fitness function
3: $t \leftarrow 0$
4: while until stopping criterion is reached do
5: for each individual $x \in X$ do
6: $y \leftarrow$ mutation($x, p_m$)
7: if $f(y) > f(x)$ then
8: $x \leftarrow y$
9: end if
10: $t \leftarrow t + 1$
11: end for
12: end while

Fig. 5. Dynamic ($\mu + \lambda$) Evolutionary Algorithm

(Fig. 5) is based on the dynamic ($\mu + \lambda$) EA [18], [19], usually applied on genetic bit string, that introduces a heuristic randomized search. This model only uses a mutation operator to generate new individual. In the same way, for all models, we apply a mutation, corresponding to a random translation of one sphere that composed an individual by a normalized vector. If the applied mutation lowers the registration error, we keep the modified model as it brings the algorithm closer to the correct solution, else we keep the parent in order to apply different mutations.

Each cell of the model has uniform probability to mutate of $1/N$, where $N$ is the number of spheres present in the individual. If a mutation occurs, a translation transform $\tau$ is applied to the cell’s centre coordinates, where $\tau$ is a random translation with $\tau_x, \tau_y, \tau_z = [0, 1]$. The goal of the evolutionary algorithm is to discard unwanted models and to improve the best models by stochastically testing variation of those models. The translation vector $\tau$ is equivalent to the iteration step size of the search.

V. EXPERIMENT

A. Data

The system was tested on an experimental set of data composed of twenty sequences of phase contrast images over time, at $40 \times$ magnification and a $3 \times 3$ binning, using a microscope. All the sequences start with a single cell in culture and were monitored over two days, with a frame rate of 20 min, for a total of 135 images per sequence. Each sequence was made using an on-line tracking algorithm [20] that adapts the microscope $x, y$ position and its $z$ focus to the cells movement in order to maintain each observed neurosphere in the centre of the field of view and at a correct focus. Without this pre-processing, at this magnification, there is a high risk that neurosphere leaves the field of observation of the microscope, hindering their observation. During the data acquisition, possible interferences could lower the quality of the output. For example, presence of dust near the observed cell.

<table>
<thead>
<tr>
<th>Population size</th>
<th>10</th>
<th>100</th>
<th>1000</th>
<th>10000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time process (sec)</td>
<td>23.00</td>
<td>186.21</td>
<td>1747.60</td>
<td>10787.50</td>
</tr>
<tr>
<td>Mean Score (%)</td>
<td>21.20</td>
<td>9.90</td>
<td>4.30</td>
<td>4.00</td>
</tr>
</tbody>
</table>

B. Results

Several experiments were made to test the different parts of the process and their parameters. From the cell segmentation process to the quantitative parameters of the EA such as the starting population size, the mutation probability or the criterion stop, we observe their influence on the result and the global process.

On the observation module, the cell detection was parametrised on 3 time lapse sequences and tested independently on the 17 remaining sequences. It reaches an overall mean performance of 85.5% good cell detection. Fig. 6 is the Precision-Recall graphic, with correct cell detection consider as true positive, failure in detecting a cell as false negative and any incorrect detection, such as dust detection, as a false positive. The 14.5% of error in the detection is due to particular cases of the image acquisition modality. Acquisition occurring during a cell division or a cell movement will produce a deformation of the cell physiognomy, make it lose its circular form, and will also produce a blur on the observed neurosphere (Fig. 7). Other interferences already explained in the section 5.A may also lead to detection errors. On those particular images, the detection process usually return an over-detection of cell.

On the modeling, registration and optimisation modules, we tested various initial population sizes for the model generation module. A population size $=100$ provides an acceptable cover of the solution space and a good initial diversity of individuals (Table. II). The same experiment was performed again to observe the impact of the optimisation step, for a fixed population. We can clearly observe an improvement of the global best score (Table. III) at a cost of an increase in the time processing. Based on the previous observation, we applied our method, with a population size of 100 and a 5 step optimisation, on the entire data set containing various complex neurosphere. Result example can be observed in the figure 8, where we were able to obtain good cell detection and modelisation of simple but also more complex neurosphere. The method was developed using C++ language, the ITK$^1$ API and the VTK$^2$ API which are libraries oriented for image processing, analysis and visualization. The different tests were made on an Intel core i5 at 2.53GHz and 4G of memory.

VI. CONCLUSION

We proposed an observation method for the monitoring of the neurosphere development process. It allow the 3D visualisation of cells configuration, based on texture analysis

$^1$http://www.itk.org/
$^2$http://www.vtk.org/
and shape registration. In this method, we combined image processing with evolutionary methodology and optimization into a analysis-synthesis approach. It operates in an acceptable speed while providing cell detection and neurosphere modeling with a low percentage of errors. Future works will continue by integrating the topological aspect of the registration for more accurate results. We also set the base for a future work on neural stem cells data mining and neurosphere fate prediction.
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REFERENCES


5http://imaging-dev.imb.a-star.edu.sg/