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An Analysis-Synthesis Approach for Neurosphere Modelisation Under Phase-Contrast Microscopy

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Abstract— **The study of stem cells is one of the most important biomedical research. Understanding their development could allow multiple applications in regenerative medicine. For this purpose, automated solutions for the observation of stem cell development process are needed. This study introduces an on-line analysis method for the modelling of neurosphere evolution during the early time of their development under phase contrast microscopy. From the corresponding phase contrast time-lapse sequences, we extract information from the neurosphere using a combination of phase contrast physics deconvolution and curve detection for locate the cells inside the neurosphere. Then, based on *prior* biological knowledge, we generate possible and optimal 3-dimensional configuration using 2D to 3D registration methods 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 and progenitors 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, *etc.*) and the improvement of regenerative medicine.

One of the main tools for cell study is based on high-throughput screening. It consists mainly to observe living cell and their behaviour, such as mitosis (division), apoptosis (death), cells movement, lineage relation and fate prediction. This information is used in several research field such as genomic research, tissue engineering, and stem cell biology. Most of these experiments are usually monitoring cells evolving in two dimensions.

Several methods propose to detect and segment the cells and to automatically track and trace their lineage over time. These methods use either frame-frame segmentation and association [1], or motion filter tracking [2]. More recently, methods were proposed for predicting the fate of the observed cells [3], [4]. Such methods use features extraction and classification methods to predict the fate of a cell, like death, division, or dividing into a specialised cell.

However, the most interesting part of the neural stem cells comes from the expression of their characteristics (self-renewal population, multi-potency, *etc.*). Such characteristic cannot be fully observed in standard culture. A protocol, called neurosphere formation assay [6], was developed to force neural stem cells to express these characteristics by making the cells grow into 3-dimension cell structure. A stem cell is isolated and put into suspension. This cell is going to divide and create a population of cells that will compose a spherical structure called neurosphere. Until recently, this protocol was not monitored, but more and more research are done to extract information for the development of neurosphere. Some work have been done to track the neurosphere over time [7], or to segment them at low magnification of drug tests [5].

In this study, we improve and facilitate the monitoring of the neurosphere growth in 3-dimension for biologist. More precisely, we propose a monitoring platform for neurosphere observation under phase-contrast time-lapse sequences at high magnification. As the neurosphere formation assay is a 3-dimensional process where cells evolve freely in suspension into a solution, we include a structural 3-dimensional modelling aspect in the monitoring to enhance the visualisation of neurosphere cells configurations over time. Thus, 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.

The paper is organised as follows: section 2 explains the overall proposed method and section 3 details its implementation. Experiments and results are in section 4.

II. METHOD

The goal of our system is to extract 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 *modelling 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. Every image is processed by the observation module, which will segments the cell(s) present in the neurosphere. This module provides information

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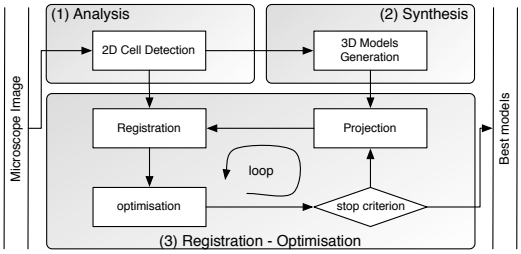

Fig. 1: System workflow. The image is, first, passed to the observation module (1), the modelling module (2) uses data extracted from the observation module to generate a set of possible models, and the convergence module (3) makes the best model converging to the observation.

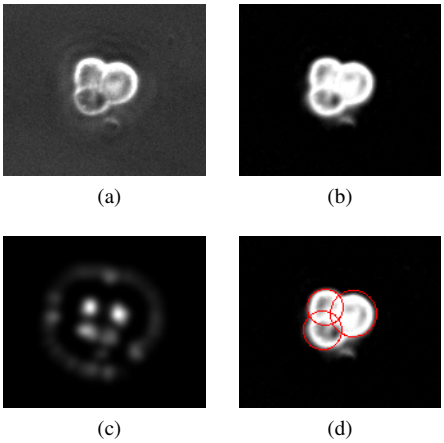

Fig. 2: Observation module process. (a) The raw image from the microscope of a 3-cell size neurosphere. (b) The restored phase-contrast image. (c) The cell centroids heat map determined from the Hough space. (d) Detected cells result.

on the neurosphere shape, the number of detected cells, and some other features present in the image. Based on these outputs, the *model module* generates a set of models that could represent the current neurosphere and the cells component configuration. Because multiple models can correspond to the observation, each model is scored according to its likelihood with the observation. The likelihood is determined by comparing different parameters, such as shape and texture, between the observation and the different models proposed. The best-generated models are selected according to their scores and modified iteratively in order to converge towards an optimal representation of the current observation done by the first module.

III. Implementation

A. Phase-contrast cell segmentation

Despite the high contrast between neural stem cell and the background, classic segmentation methods such as edge detection using gradient norm (*i.e.* Canny-Deriche filter) does not provide satisfactory results due to the phase-contrast artefacts such as non uniformity of cell membrane intensity,

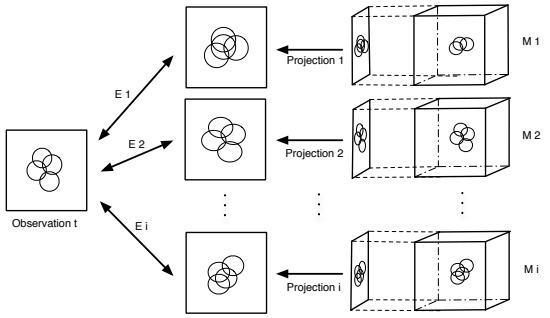

Fig. 3: Ranking of model by projection and registration.

unclear delimitation of the cells, and illumination variation in the image sequence. These variations are often present in phase contrast modality. In our previous work [9], a classic approach with level set method [8] to segment the neurosphere was used. However such method was still affected by the different artefacts of the phase-contrast and was not providing a robust result on all our data.

In this paper, we use instead, the phase-contrast restoration algorithm proposed by Yin *et. al.* [10] to remove these artefacts and calculate an artefact-free image. The restoration algorithm is using an approximation model of the phase-contrast physics, such as

$$g(x) \propto (\delta(r) - airy(r)) * f(x) + C \quad (1)$$

where g is the observed image, f the restored image to be determined, C a constant corresponding to the background, δ the Dirac function and $airy$ an obscure Airy pattern. The reconstruction of f from g is defined as the following minimisation problem

$$O(f) = ||g - Hf||_2^2 + \gamma smooth(f) + \beta sparsity(f) \quad (2)$$

where H is a sparse matrix, $||.||_2$ a $\mathcal{L}_2$-norm, and γ,β are weights respectively to the smoothness and sparcity term of the optimisation. Further details on the method can be found in [10]. An example of the reconstruction of our data can be observe in fig. 2b.

Once the restored image is done, a heat map of the centroids and radius of each visible cell is determined by detecting partial curve in the image, using Hough transform. As the detected partial curve from the cell 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. 2c). At the end of the process, a detection of the visible cells is obtained (Fig. 2d).

B. 2D-3D registration

Our approach relies on the principle of 2D-3D registration methods. More precisely, we try to find a corresponding 3D model to a 2D microscope image. In order to find the transformation T that link the data, they have to be brought to the same dimension. Based on the review work of Markelj *et. al.* [11], we use a *projection* strategy that consist in projecting the 3D data into the $\mathcal{R}^2$ space of the 2D data and then search

for the transformation T by registering the 2D data and the projected data (Fig. 3). Such as

$$T : \mathcal{P}(T(X_A^{3D})) = X_A^{2D} \Longleftrightarrow X_B^{2D} \quad (3)$$

Where $\mathcal{P}$ is the projection, T the transformation and X_A and X_B the two dataset.

A random population of models is generated based on *prior* knowledge on neurosphere configuration. Each model is represented by a configuration of spheres that represent the cells in $\mathcal{R}^3$. A particular configuration is given by a set of spheres that respect configuration criteria: cell-cell overlapping, cell-cell contacts and cell proliferation rates.

Once a population of model is generated, we can start the registration process. Each model is projected into the 2D space of our observation. An intensity based registration focusing on two criteria functions (CF): a shape comparison and a texture comparison. The shape comparison is made between the shape of the projected individual and the segmented shape of the neurosphere of the microscope image. The texture comparison is based on the phase-contrast halo. The cells usually appear with a dark interior and a bright boundary, which is a common aspect under phase-contrast microscopy. Using a gradient descent optimisation and a mean square error, this registration approximates the best rigid transform parameter $\{\theta, c, \tau, \sigma\}$ that register the two images, where θ and c are respectively the angle and the centre coordinates of the rotation, τ the translation vector and σ the scale.

The metric value is calculated using the different criteria function, shape score and texture score, are summed and saved as a score for the corresponding model, such as:

$$T = \arg\max_{T} \sum_{j=1}^{N} CF_j(\mathcal{P}(A^T(X_A^{3D})),\ B(X_B^{2D})) \quad (4)$$

where $N = \{1, 2\}$ is the total number of criterion function and CF the a criterion function. This score, which is actually an error, provides us a ranking of the couple $\{model; transform\}$ so generated.

C. Model optimisation

Once a set of model has been selected according to its registration score, we search to optimise the different model to improve their score. Thus, we propose a stochastic search of the different solution using an evolutionary optimisation. The algorithm is based on the dynamic $(\mu + \lambda)$ evolution algorithm [12], 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. Once modified, we apply the model corresponding transform and recalculate its score. If the model is improved, we keep the modified model otherwise we discard it.

TABLE I: 2-dimension cell detection results

	Precision	Recall	F-Score
Previous method [9]	0.870	0.838	0.854
Current method	0.884	0.906	0.895

TABLE II: Parameters effects on global process time (sec.)

	0 iteration	25 iteration	50 iteration	100 iteration
Pop. 10	104.01	117.35	129.07	158.51
Pop. 25	120.48	152.46	185.24	250.18
Pop. 50	146.37	209.55	284.20	414.17

IV. Experiments and Results

A. Data

The system was tested on an experimental set of data composed of twenty sequences of phase contrast images over time, at 40 magnification and a 3×3 binning. All the sequences start with a single cell in culture and monitored over two days, with a frame rate of 20 min per image, for a total of 135 images per sequence. Each sequence was made using an on-line microscope tracking algorithm [13] 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.

B. Results

We calculated the precision and recall score (Tab. I) of the cell detection process presented at the beginning of the section 3. Such as $precision = tp/(tp + fp)$, $recall = tp/(fp + fn)$ and $f - score = 2 * (precision * recall)/(precision + recall)$. We define the *true positive* (tp) as a cell correctly detected, the *false positive* (fp) a detection that do not correspond to a cell, and the *false negative* (fn) a cell that was not detected by the process. We have obtained the precision and recall score respectively 0.884 and 0.906. Based on our previous test [9], we have globally improved the cell detection f-score, from 0.854 to 0.895.

We compared the registration results by observing the variation of the score of the best model for multiple runs with a population size of 100 (Fig. 4). We can see an improvement of the score compared to previous results. However, we can observe that the process does not always

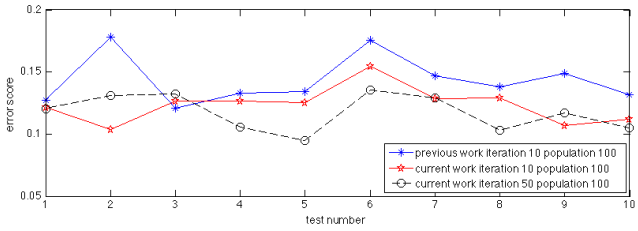

Fig. 4: Best score over 10 simulations on an neurosphere image.

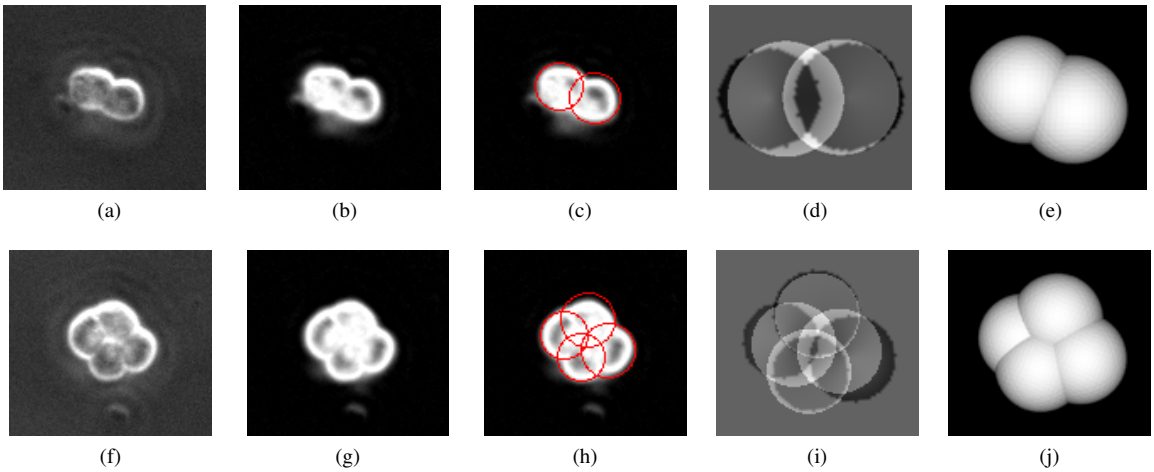

Fig. 5: Examples of results on different time-lapse sequences and at various times. First column represent the raw images of the cells. The second and third column is the restoration result of phase-contrast and the cell detection results. The fourth column the 2D-3D registration temporary results. The last column is the 3-dimensional model representation of the cells observed.

converge to the best solution. This is due to the fact that 2D-3D registration does not have a unique solution. The bad convergence of the model can be corrected by using more parameters into the criteria function during the registration process, but it will affect the global cost of the process.

We have tested our modelling methods on different sequences, for each image containing various complex neurosphere at early stage of development (Fig. 5), where we were able to obtain good cell detection and modelling of simple but also more complex neurosphere. The time process depend of the parameters used (Tab. II) and can be important. However, it remains relatively fast compare to the time resolution of the image sequence we are working on, and the cell cycle of division (~ 24h).

The method was developed using C++ language, the Insigth Toolkit (ITK) API and the Visual Tookit (VTK) API. The different tests were made on an Intel core i5 at 2.53GHz, 4G of memory.

V. CONCLUSIONS

We proposed a modified method for the monitoring of the neurosphere development process. It uses phase-contrast reconstruction method to produce a new observation image that is used to detect cells present in neurosphere and allow a 3D visualisation of cells configuration in neurosphere, based on texture analysis and shape registration. This new method achieved an improvement of 5% on the cell detection and a global improvement of 5% to 9% of the model generation.

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