Segmenting Embryo Blastomeres in 3D from Hoffman Modulation Contrast Image Stacks

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Abstract

We describe a technique for segmenting embryo blastomeres while simultaneously determining their 3D positions, by processing a Z-stack of images acquired by means of an Hoffman Modulation Contrast (HMC) microscope; in particular, the depth at which each blastomere lies is identified by localizing the focal planes where its contour appears sharp. The problem is particularly challenging because of the complex image appearance due to HMC, and because images of different blastomeres at different depths often project to overlapping regions. We discuss experimental results and detail the system implementation.

1 Introduction

We provide a segmentation technique for performing automated measurements on an human embryo, for application in In Vitro Fertilization (IVF)[1]. The embryo is a 3D structure with a roughly spherical shape, which contains a variable number of cells (blastomeres); in this work, we are mainly dealing with 4-cell embryos, which is the most common configuration at day 2 after fertilization. Our technique segments the blastomeres from a set of images taken at different focus levels (Z-stack), while simultaneously estimating their depth: we can therefore provide quantitative data on their apparent sizes, shapes, and 3D spatial relationships (see Figure 1).

Observation of embryos plays an important role during IVF procedures, as embryologists closely follow the embryos' development in order to determine their viability [5]; such evaluation provides fundamental data for performing critical decisions, such as determining which and how many embryos should be trasferred to the woman. Observations are routinely performed manually, and embryos are scored by considering the number of blastomeres, their

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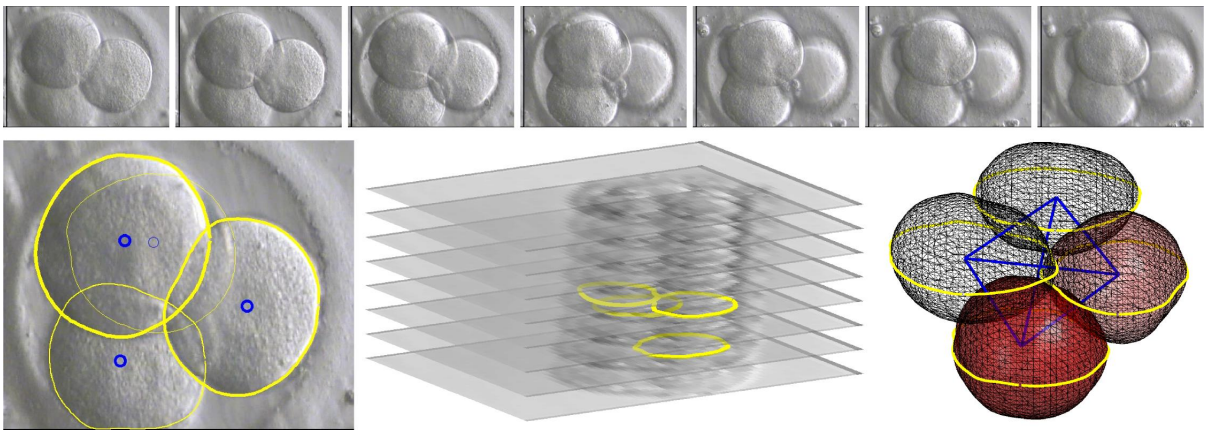

Figure 1: Top row: original Z-stack: the 4 embryo cells are visible at a different focus planes. Bottom row: segmentation of the blastomeres from different focus planes; note that there are two cells at the top left, at different depths. From the 3D contours, we extrapolate the cell shapes and their 3D relationships.

relative sizes and several other criteria; in this context, quantitative and objective measurements can provide valuable information for decision-making [4].

Embryos are routinely observed by means of a particular phase contrast microscopy technique called Hoffman Modulation Contrast (HMC). In the resulting images, the embryos and their substructures (which are transparent and would not be visible with brightfield imaging) gain a complex, 3D-like sidelit appearance which eases interpretation by human observers, but is often considered an hindrance for automated processing; moreover, blastomeres are grouped in a thick 3D topology: therefore, their images overlap while being affected by varying defocus, and are often difficult to identify even by human observers.

Due to such complexity, region-based segmentation techniques fail in this context; other techniques such as active contours [8] and level sets [7] are more suitable, but their application is not straightforward due to the large amount of clutter and artifacts in the image stacks; for example, in [6] level sets are used to model the embryo, after the blastomeres segmentation is manually provided. A more technical description of the technique is available in [3].

2 Model

Our algorithm operates on a Z-stack of N HMC images. We denote the input images as $I_1, I_2, ..., I_N$, and their respective focal planes $z = z_1, z_2..z_N$. Such focal planes can be considered horizontal slices at different depths of a 3D space whose cartesian axes are (x, y, z).

The underlying HMC imaging model is extremely complex, especially if the effect of out-of-focus features is taken into account. Still, several intuitive principles hold, on which we base our approach: *a)* structures which lie on or near the current focal plane z_i appear sharp and exhibit strong localized gradients in the image intensity I_i; *b)* as the focal plane depth moves farther from the structure's depth, the structure image becomes blurred. Consequently, the gradients of the structure's image lose locality and strength, although the *global* contrast and visibility of the feature may not be affected, or may even be emphasized in some situations.

Let S be the surface of the blastomere, which we assume to be smooth, in the 3D space (x, y, z). The contour generator curve Γ is a curve in 3D space (which we assume single and

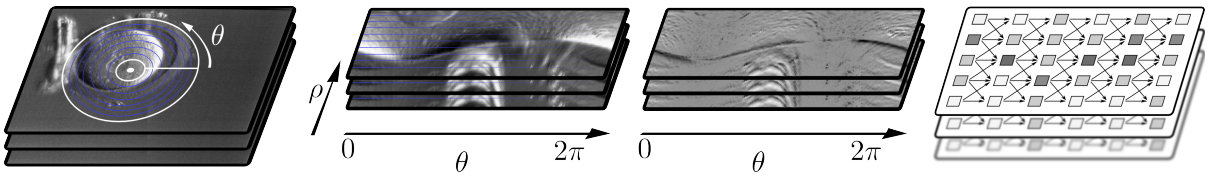

Figure 2: The stack of I_i slices (left) is transformed to J_i slices in polar coordinates around the candidate center c_i, then an energy value E_i (third illustration) is computed for each pixel and a graph is built for each focal plane (right). A single global graph is then obtained by linking a single source and sink node to the left and, respectively, right pixels of each focal plane. The minimum-cost path on such graph symultaneously summarizes contour information, and identifies the depth of the structure.

closed due to the regularity of the cell shape), identified by the locus of points P on S such that the tangent plane to S in P contains the z direction.

We are interested in detecting the image of Γ in our input images I_i, i.e. its orthogonal projection γ on the (x,y) plane. Following the principles introduced previously, a part of γ is visible and well-focused in an image I_i if the corresponding part of Γ is on or near the $z = z_i$ plane; in this case, such part of γ will exhibit large, localized gradients in image I_i. The gradient intensity is weaker as Γ gets farther away from the plane $z = z_i$. We account for the fact that different parts of Γ may lie at different depths, by detecting different parts of γ on different I_i images.

3 Embryo Segmentation Technique

Initially, the image stack is analyzed in order to detect the approximate embryo area, by using a simple preprocessing technique [1, 2]. A number B of candidate blastomere centers $c_i = (c_x, c_y, c_z)$ are randomly generated inside said area, from all focal planes.

Given a candidate center, segmentation is performed by using the graph-based approach described in [2] for segmenting ovocytes, which handles the peculiar HMC lighting and enforces shape priors – conditions which also hold for blastomeres. Such approach may be classified as a specialized livewire-like approach, where: *a)* priors on the blastomere shape are accounted for by operating on a spatially-transformed image and searching for a minimum-cost path on a directed acyclic graph; *b)* priors on the contour appearance due to HMC lighting are directly integrated in the energy terms; *c)* information at different focal planes is represented in a single large graph, which allows us to simultaneously detect the shape and depth of the cell (the two problems are strictly related). The approach is summarized in Figure 2.

Experimental results show a significant robustness to a displaced candidate center initialization c_i when iterating the algorithm few times, re-initalizing the centroid of the new iteration to the centroid of the recovered mask in the previous iteration. Therefore, the algorithm is run in parallel on all B candidate blastomere centers, then iterated after redundant candidates (i.e. candidates with a significant overlap with others) are discarded.

As we show in the following Section, this procedure quickly converges to a small number of candidate blastomeres which are then either automatically filtered, or presented to the user for interactive validation.

4 Experimental Results

We validated the approach on 71 Z-stacks of 4-cell embryos. The Z-stacks are acquired during the routine activity of an IVF lab, by means of an Olympus IX51 Microscope equipped with HMC 40x optics, and a 720x576 video camera attached through a 0.5x video adapter to the microscope video port. Each stack is composed by $N = 24$ slices, spaced approximately $5\mu m$. In the resulting images, the embryos have an apparent diameter of about 300 pixels, whereas the blastomeres have an average apparent diameter of roughly 140 pixels.

The images are processed without any user supervision, and each image stack is segmented in less than a minute. The preprocessing step aimed at detecting the embryo position in the image returns acceptable results in all of our images, which is expected as the problem is trivial due to the background uniformity. The segmentation was performed by considering $B = 100$ initializations, randomly distributed in the cylinder defined by the embryo circle and all of the available slices. We perform 3 iterations of the segmentation algorithm in [2]. After each iteration, we remove all candidates which are nearer than $15\mu m$ in the 3D space to a candidate whose segmentation has a lower energy; this boosts performance and leaves on average 43, 19.9 and 11.9 candidates after the first, second and final iterations, respectively. This also demonstrates the property of the candidates to converge to the same solutions. The 4 lowest-energy candidates which map to a closed curve and whose centers are spaced at least $40\mu m$ are finally considered as the final candidates.

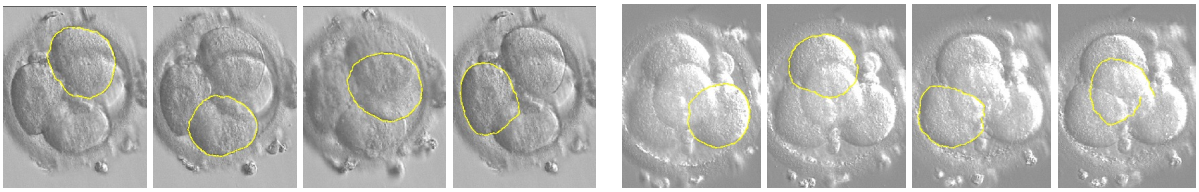

Figure 3: Examples of successful segmentations (note that each contour is detected at a different depth).

We manually segmented the 276 blastomeres in all of the input stacks, in order to derive quantitative results; we consider a candidate as a correct segmentation of a blastomere if its average depth is within $15\mu m$ (3 slices) of the manually-determined depth, and the 2D Jaccard similarity index is higher than 0.8. Eventually, 90% of the blastomeres are among the candidates detected after the last iteration. After automatically filtering the 4 best candidates in each stack, 71% of the stacks have all 4 blastomeres correctly detected; in other cases, the algorithm detects one or two blastomeres in the wrong position, mostly in instances with high fragmentation, which causes strong spurious edges.

5 Discussion, Conclusions and Future Directions

Experimental results show that the proposed technique is effective for segmenting and localizing blastomeres in HMC images of early embryos; however, automatic detection of the actual number of blastomeres, as well as automatic filtering of the correct candidates, would not provide the required robustness for practical application.

Therefore, the technique is deployed as follows:

- image stacks are acquired by users during routine observations, using a dedicated software connected to the microscope and controlled through a keypad placed near the

microscope focus knob; this optimizes a time-critical operation, as embryos must be placed back into the incubator as soon as possible;
- while other embryos are acquired, our system noninteractively computes candidate blastomeres for each acquired stack;
- later, users review embryo image stacks on the computer and take the necessary time for evaluation; in this phase, our system allows them to interactively determine the correct blastomeres from the set of precomputed candidates; as soon as all of the blastomeres are confirmed, the system immediately outputs size and 3D morphology measurements. As this is a supervised process and users themselves confirm the segmentation results, the resulting measurements can be trusted.

We are currently experimenting with more sophisticated criteria to filter the final candidates, while also improving the user interface presented to the user for selecting the blastomere contours among the set of candidates; this is a nontrivial Human-Computer Interaction problem, as it requires an useable visualization of 3D features on image stacks.

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