ABSTRACT

Monitoring of embryos is an important activity during In Vitro Fertilization (IVF) procedures. A critical stage is that of the early embryo, which is formed by a small number of cells (blastomeres): the morphology of such cells is considered to be a powerful indicator of the embryo viability. We deal with the challenging problem of automatically segmenting the different blastomeres in the early embryo while simultaneously determining their depth, by processing a Z-stack of images acquired by means of a Hoffman Modulation Contrast (HMC) microscope. We discuss experimental results on 53 embryo image stacks, and elaborate on the advantages and limitations of our approach, also briefly describing it is being integrated in the workflow of an IVF laboratory.

Index Terms— segmentation, microscopy, In Vitro Fertilization, embryo, blastomere, shape from focus

1. INTRODUCTION

We provide a technique for performing automated measurements on an early embryo, for application in In Vitro Fertilization (IVF)\(^1\). As shown in Figure 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 measures of 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 [1]. Observations are routinely performed manually, and embryos are scored by considering the number of blastomeres, their relative sizes and several other criteria; in this context, quantitative measurements can provide valuable information for decision-making[2].

Embryos are routinely imaged by means of a particular phase contrast technique called Hoffman Modulation Contrast (HMC). In the resulting images transparent embryos and their substructures have a complex, 3D-like sidellight 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 (untrained) human observers.

Due to such complexity, region-based segmentation techniques fail in this context; other techniques such as active contours [3] and level sets [4] are more suitable, but their application is not straightforward due to the large amount of clutter and artifacts in the image. For example, in [5] active contours are used for measuring the thickness of the zona pellucida in embryo images, but only after a preprocessing step aimed at removing debris and other artifacts; in [6], level sets are used...
to model the embryo, after the blastomeres segmentation is manually provided.

We model the problem in Section 2. Our technique, described in Section 3, includes a global energy minimization step for a given candidate center of the cell, and may be classified as a specialized graphcut-like [7] 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, as also described in [8]; \( c \) 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 algorithm is run with many initializations, which are shown to quickly converge to a small number of candidate blastomeres which are then either automatically filtered, or presented to the user for interactive validation. Section 4 summarizes experimental results, discussed in Section 5 which also concludes the paper and presents research directions.

2. MODEL

Our algorithm operates on a Z-stack of \( N \) HMC images (see Figure 2). 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 \( \Gamma \) is a curve in 3D space (which we assume single and 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 \( \Gamma \) in our input images \( I_{i} \), i.e. its orthogonal projection \( \gamma \) on the \((x, y)\) plane. Following the principles introduced previously, a part of \( \gamma \) is visible and well-focused in an image \( I_{i} \) if the corresponding part of \( \Gamma \) is on or near the \( z = z_{i} \) plane; in this case, such part of \( \gamma \) will exhibit large, localized gradients in image \( I_{i} \). The gradient intensity is weaker as \( \Gamma \) gets farther away from the plane \( z = z_{i} \). We account for the fact that different parts of \( \Gamma \) may lie at different depths, by detecting different parts of \( \gamma \) on different \( I_{i} \) images.

3. EMBRYO SEGMENTATION TECHNIQUE

We first introduce how a single blastomere is segmented and simultaneously its depth is determined, when a rough initialization of its center in the three dimensions is given; we also show that a moderately displaced initialization can be corrected by few iterations of the segmentation algorithm. Then, we discuss in how this algorithm is applied in parallel to several different candidate centers, and how results are filtered in order to automatically find blastomeres in an embryo.

3.1. Simultaneous Segmentation and Depth Localization of a Single Blastomere

Let us consider a candidate center \( c = (c_{x}, c_{y}, c_{z}) \). We consider a circular corona \( C \) centered on \((c_{x}, c_{y})\) with inner and outer radius respectively \( \rho' \), \( \rho'' \), which is expected to contain \( \gamma \). Also, we operate on a subset of the stack, composed by \( K \) neighboring frames \( I_{i-1} ... I_{i+K} \) centered at depth \( c_{z} \).

The circular corona in each of the \( K \) frames is converted into polar coordinates, by sampling it at \( \rho_{n} \cdot \theta_{n} \) points. Let \( J_{i-1} ... J_{i+K} \) be the resulting \( K \) images. An energy is associated to each pixel as described in [8]: such energy term leads to low energies where strong localized gradients are present, which exhibit an appearance compatible with the direction of the expected lighting.

A single directed acyclic graph is built over the stack of all \( J_{i} \) images, by instantiating a node \( n(\rho, \theta, z) \) for each pixel and for each plane (for a total of \( \rho_{n} \cdot \theta_{n} \cdot K \) nodes).

- For each node \( n(\theta, \rho, z) \), arcs are added connecting it to its three 8-neighbors at the right on the same plane: \( n(\theta + 1, \rho, z) \), \( n(\theta + 1, \rho + 1, z) \) and \( n(\theta + 1, \rho - 1, z) \);
- For each node \( n(\theta, \rho, z) \) such that \( \theta \) is a multiple of an integer \( \beta_{n} \), a set of interfocal arcs are added connecting the source node to its two neighbors on the following
The stack of $I_i$ slices (a) is transformed to $J_i$ slices in polar coordinates (b), then an energy value $E_i$ (c) is computed for each pixel and a graph is built on each (d). A single global graph is then obtained by interlinking the nodes in the individual graphs with interfocal arcs every $\beta_n$ columns.

The cost of each arc is set to the energy $E_z(\theta, \rho)$ of its source node.

A single global source node $s$ is added, with zero-cost arcs that lead to every pixel in the first column of every plane: $n(1, \rho, z) \forall \rho, z$. We also add a sink node reached by zero-cost arcs from every pixel at the last column of every plane: $n(\theta_n, \rho, z) \forall \rho, z$.

As the resulting graph is a directed acyclic graph, the minimum-cost path from the source node to the sink node can be efficiently computed in linear time. Such path passes through low-energy arcs, and is constrained by the graph topology to have a regular shape.

After removing the source and sink nodes (which have no geometric meaning), the path can be trivially brought back to cartesian coordinates: the result is a curve in the $(x, y, z)$ space which estimates the 3D contour generating curve $\Gamma$. It simultaneously represents the contour of the blastomere and identifies its main focal plane (i.e. the “equator” of the cell). Note that the obtained curve is not necessarily closed; we use this property in the following as a criterion for detecting a correct segmentation.

Larger values for ratio $\theta_n/\rho_n$, as well as a smaller $\beta_n$ parameter allow more freedom to the path built over the graph, which translates to better accommodation of an irregular cell shape or a displaced centroid $(c_x, c_y)$; at the same time, this reduces the robustness of the approach, as shape priors are less strongly enforced. Similarly, a large $\theta_n/\beta_n$ ratio allows the resulting contour generator curve to span a larger interval of focal planes; due to the quite regular shape of the blastomeres, none of our test images required a $\theta_n/\beta_n$ larger than $4^2$.

When the algorithm is initialized on a centroid significantly displaced on the $(x, y)$ plane, the resulting segmentation is often at least partially correct: the centroid of the resulting area is in most cases (see Section 4) nearer to the true centroid than the initialization. This is especially true if shape priors are loosely enforced by using larger $\theta_n/\rho_n$ values, and/or by using a wider $[\rho', \rho'']$ interval (i.e. considering a thicker circular corona). This also holds if the centroid is displaced along the $z$ direction.

3.2. Segmentation of Multiple Blastomeres

Initially, the image stack is analyzed in order to detect the approximate embryo area, by using the simple preprocessing technique in [8]. A number $B$ of candidate blastomere centers $c_i = (c_x, c_y, c_z)$ are randomly generated inside said area. Then the algorithm in Section 3.1 is iterated few times, updating the blastomere centers after each iteration.

4. EXPERIMENTAL RESULTS

We validated the approach on 53 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, using a constant $\theta_n/\rho_n = 270/70$, and a $[\rho', \rho'']$ interval starting at $[10, 160]$ and ending at $[50, 110]$, which roughly corresponds to the minimum and maximum expected radii when considering a squeezed blastomere. 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 44.1, 21.5 and 11.0 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

\[\text{Note that the obtained curve is not necessarily closed; we use this property in the following as a criterion for detecting a correct segmentation.}\]
which map to a closed curve and whose centers are spaced at least 40 μm are finally considered as the final candidates.

We manually segmented the 212 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 μm (3 slices) of the manually-determined depth, and the 2D Jaccard similarity index is higher than 0.8. The results are summarized in Figures 4 and 5.

**Fig. 4.** Left: percent of detected blastomeres as a function of the number of top candidates considered for each embryo; Right: after filtering 4 compatible candidates for each embryo, most embryos have all 4 blastomeres correctly chosen, but a significant amount has only three or less blastomeres detected.

5. **DISCUSSION, CONCLUSIONS AND FUTURE DIRECTIONS**

Experimental results show that the proposed technique is effective for segmenting blastomeres in HMC images of early embryos; in particular, a 91% of the blastomeres are detected as candidates, although in the final filtered results only 71% of the stacks have all 4 blastomeres correctly selected. Consequently, we are currently integrating the system in the workflow of an IVF laboratory, by requiring the biologists to interactively determine the correct blastomeres from the set of precomputed candidates (on average 11.0 per image); as soon as all of the blastomeres are confirmed, the system immediately outputs size and 3D morphology measurements. Integration in the current workflow is straightforward as the observation and scoring of the embryos already happen on the computer after all the embryos are imaged and placed back in the incubator; during the intervening time, the image stacks are stored on a server and our system has the opportunity to noninteractively precompute candidate blastomeres.

We are also experimenting with more sophisticated criteria to filter the final candidates; still, we believe that the reliability needed for completely automatic operation will not be reached in the near future on this delicate task; moreover, the scoring of the embryologist is also based on a number of other observations, such as presence and number of nuclei, and evaluation of the embryo fragmentation, which are unlikely to be computed automatically with the necessary reliability. On the other hand, a new set of challenges for semi-automatic processing will be evoked by the need of analyzing time-lapse imagery, which is going to be available in the near future thanks to specific hardware, allowing the observation of embryos at much shorter time intervals than is currently done, thus generating vast amounts of data.

6. **REFERENCES**


