Multi-Modal Registration of Embryonic Images for In Vitro Fertilization

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Abstract

This paper presents a robust and fully automated registration algorithm for alignment of embryonic images taken by Optical Quadrature Microscopy (OQM) and Differential Interference Contrast (DIC) Microscopy. Registration of such images allows recognition of distinguishing features of viable embryos, such as growth rate, to reduce multiple births from In Vitro Fertilization. OQM is an optical interferometer whereas the DIC images gradient of the phase. Due to the very different nature of the image formation, the interior of the embryo appears substantially different if not totally uncorrelated between different modalities. We present an algorithm which extracts and aligns the silhouettes of the embryo—the most invariant features between different modalities. The algorithm is tested with 28 image pairs taken from different embryos at various development stages. The success rate is 85.7\% if only affine transformation is adopted, and 96\% if similarity transformation is allowed for embryos without sufficient outline complexity.

1. Introduction

Infertility now affects over 15\% of the reproductive age population with over 25\% of couples unable to conceive in the worst-affected areas [13]. A common method of assisted reproduction is In Vitro Fertilization (IVF) where the egg is fertilized in vitro and the resulting embryo is transferred to the mother to develop naturally [11]. Due to the low success rate of IVF, multiple embryos are transferred in each cycle to increase the likelihood of success, which often results in multiple births and a multitude of problems for both the mother and the child, including preterm delivery, low birth weight, congenital malformations, and infant death [6], [10]. To strive for a higher rate of live births and to reduce the rate of multiple births, non-invasive methods of embryo evaluation have been adopted to assess the quality of the embryo during the preimplantation period. Many parameters influence the prediction of embryo viability, among which the rate of embryo growth (number of cells) has been considered the parameter of the highest predictive value in many studies [1], [3].

Warger et. al. [14], [15] developed the non-toxic phase-subtraction cell-counting method that combines the images of optical quadrature microscopy (OQM) and differential interference contrast (DIC) to facilitate cell counting in developing mouse embryos past the eight-cell stage limit provided by the use of DIC imaging alone. OQM is an interferometric imaging modality that measures the amplitude and phase of the light that travels through the embryo. The phase of the light is related to refractive index mismatch between the embryo and the culture medium and is generally converted to an image of optical path difference (OPD). DIC images the gradient of the phase, and provides distinct cell boundaries for cells within the focal plane. Figure 1 shows an example of an OQM image with the corresponding DIC image. When well aligned, the boundary of a single cell from the DIC image and the maximum OPD of the cell from the OQM image can create an ellipsoidal model cell of OPD. Subtracting the model cell in the OQM image reveals the OPD of the culture media or another cell underneath. The technique subtracts all of the visible cells in the DIC image and then analyzes the remaining OPD to subtract the remaining cells. The cell count is complete when no more cells can be subtracted.

A key component of the phase-subtraction cell-counting method is accurate registration between the OQM and DIC images. When registration is performed manually, points where cells intersect along the boundary are potential landmarks for alignment. However, it is not always easy to manually identify enough corresponding landmarks with sufficient accuracy to constrain the transformation, as demonstrated in Figure 1. With an automatic approach, a sufficient number of landmarks can be generated to mitigate the error induced by few landmarks with high uncertainty. There is a rich body of literature on inter-modality image registration, especially in the domain of medical imaging [5], [9], [8], [12], [16]. In this paper, we present a correspondence-based method which registers images of DIC and OQM without any human intervention. Due to the fundamental differences in contrast that form each image, the most invariant feature shared between the images is the silhouette of the embryo. Our method adopts the correspondence-based approach to automatically extract the edge points from the silhouettes that are consistent between the two modalities as the correspondences and perform the registration by matching the entire silhouette in each image to properly constrain an affine transformation.
2. Methodology

The silhouette we are interested in for registration is the outline of the cell cluster (blastomeres), since inclusion of the surrounding structure—zona pellucida—makes the shape of the embryo less distinctive. To extract the silhouette, we have adopted the Random Walk algorithm proposed by Grady [4], which is an interactive segmentation algorithm that requires manual placement of the seed points to initiate the process. To remove the need for user input, we have developed a method to automatically determine the potential foreground and background seed points for our application, so that extraction of silhouette is fully automated.

Edge points of each image are identified using Canny edge detection [2] to identify points in the embryo as the foreground and the points outside as the background. The algorithm performs non-maximum suppression on the gradient magnitudes to identify potential edge points, and hysteresis with two thresholds to obtain the final set. Figure 2(b) is the result of the operation on a DIC image. Since the culture medium is relatively textureless, Canny edges are mostly found along the boundaries of the cells and the zona pellucida. To identify only points in the embryo, edge points are assigned to clusters, each having the minimum distance between intra-cluster points below a threshold of 25 in our experiment. The cluster with the maximum number of points represents the embryo and the points are treated as the foreground seed points. Outmost foreground seed points are identified by taking the left- and right-most points of each row and the top- and bottom-most points of each column. Each outmost foreground seed point defines a background seed point by moving 3 pixels away from the center of the cluster in the radial direction. Figure 2(c) shows the foreground points in blue and background points in red.

The random walk algorithm proposed by Grady [4] performs K-way image segmentation given a small number of pre-defined seed points with region labels. For our application, K = 2 and the pre-defined seed points are generated from Canny edges. For each unlabeled pixel, the algorithm analytically determines a K-tuple vector, with each element storing the probability that a random walk starting at this pixel will first reach one of the corresponding pre-labeled seed locations. The segmentation is achieved by assigning each pixel to the label with the greatest probability. The result of the random walk is a mask image with the region of the embryo (excluding the zona pellucida) in white and the background in black. Figure 2(d) and (f) are the results of random walk for a pair of DIC and OQM images, respectively.

With the silhouettes of the embryo available from both
modalities, we perform shape matching that allows rotation, scaling, and translation between the two images. We have adopted the Generalized Dual-Bootstrap Iterative-Closest-Point (GDB-ICP) method by Yang et al. [16] to align the silhouettes. The primary choice of the transformation model is affine to accommodate possible aberrations that are not consistent in both imaging modalities and deformation due to movement of the embryo. GDB-ICP is a feature-based method that uses Lowe’s keypoints [7] to initialize a transformation, and corner and edge points for refinement to achieve sub-pixel accuracy, as shown in figure 3(a). Figure 3(b) is the checkerboard mosaic of the registered images of Figure 1 with an accuracy of 0.4 pixels. If two images are well aligned, lines are well connected at the boundaries of adjacent blocks coming from different images, as observed in Figure 3(b).

3. Experimental Analysis

The test suite consisted of 28 DIC-OQM image pairs without pre-screening on the quality of images. If the transformation model is restricted to affine, there were 4 failures resulting in a success rate of 85.7% and an average alignment accuracy of 0.46 pixels. The algorithm is robust even for image pairs involving OQM images with unwrap failure (see Figure 2(e)). 3 of the 4 failed image pairs can be registered if the similarity transformation is chosen as the final model. An explanation for the success with a lower order model is exclusion of the mis-matched regions as outliers, while a better fit using a higher order model would include such regions for the computation of accuracy, resulting in a higher alignment error.

The accuracy measure provided by GDB-ICP indicates the fitness of the two silhouettes computed by the segmentation algorithm. However, it does not necessarily reflect the correctness of the alignment of the raw images since there are errors in segmentation. To validate the correctness of the system, we generated the ground truth transformation for each image pair by manually selecting the landmark points at the locations where the cells intersect along the outer perimeter using the registration toolbox in Matlab. The DIC image was transformed to the image space of the OQM image using both the ground truth transformation and the transformation generated by our algorithm. The GDB-ICP algorithm was performed to align both transformed images and the accuracy measure indicates the correctness of the transformation generated by our algorithm. The average error was 3.8 pixels for 27 image pairs which can be registered using either the affine or similarity model. Figure 4 shows the case with the lowest alignment error of 1.6 pixels. To accentuate the difference between the two transformations, we placed the ground-truth-transformed DIC image in red and our transformed DIC image in green. When the two images are well-aligned, the image should be in yellow, as shown in Figure 4(b).

The average validation error is higher than expected. It can be partially explained by the difficulty in manually selecting corresponding points with high certainty. For example, when there is a low number of cells, such as the two-cell embryo, it is not always possible to manually gather enough reliable correspondences to sufficiently constrain an affine transformation. As shown in figure 5, our transformation provides a more superior checkerboard mosaic than the ground truth, with an alignment error of 7.14 pixels between the two transformed DIC images.
4. Discussion and Conclusion

We have presented a robust algorithm to automatically align images of optical quadrature microscopy and differential interference contrast for study of embryo viability for *In Vitro* Fertilization. The algorithm consists of the improved random walk segmentation algorithm to automatically delineate the embryo and the Generalized Dual-Bootstrap Iterative-Closest-Point algorithm to align the segmented images. With a suite of 28 image pairs, the method achieved a 85.7% success rate with an affine transformation model, and 96% when a similarity transformation was accepted for embryos without sufficient outline complexity. The method has outperformed the manual registration in terms of accuracy when the image pairs are lacking salient landmarks as seen in Figure 5. The methodology can be widely applicable to multi-modal image registration problems outside the domain of embryonic study if the images contain objects for alignment with perceptible boundary definition and the background is relatively textureless.

References


