Automatic Segmentation of Zona Pellucida in HMC Images of Human Embryos

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Abstract

An important prognostic parameter for assessing the success of an in vitro fertilization treatment is the variation in thickness of the zona pellucida. Zona pellucida, the envelope of the human embryo, is usually visualized using Hoffman modulation contrast microscopy (HMC). The paper addresses the problem of segmenting the zona pellucida in HMC images of embryos. We propose a variational method based on an image model for the zona which takes advantage of the characteristic appearance of HMC images. The simple topology of the embryo allows us to focus on parametric models. Our approach is partly inspired by the works of Chan and Vese, to which it has some similarities.

1. Introduction

Success during in vitro fertilization treatment (IVF), defined as clinical implantation and ensuing pregnancy, culminating in live birth of healthy offspring, depends on a complicated interplay between several variables. Among these are, for example, the patient’s age, aetiology of infertility and embryo quality. Recent studies have suggested that the zona pellucida thickness variation (ZPTV) is an important prognostic parameter for assessing the success of an IVF treatment [4]. Figure 1 shows an image of a human embryo. The image is taken using a video camera mounted on an HMC microscope.

The HMC technique is a light microscopy contrast technique which generates contrast from phase gradients, originating from differences in the refractive index of the specimen [5]. Since this technique requires no staining it is well suited for studies of living specimen. The HMC microscope uses a modulator with a given orientation. This results in phase differences being most clearly visible in one direction and invisible in the orthogonal direction. Parts of the image pertaining to the same structure may vary in intensity from dark to light, as the zona does in Figure 1.

Figure 1. An HMC image of a human embryo. The zona pellucida is the non cellular layer enveloping the embryo.

There are commercially available tools for assisting laboratory technicians in measuring embryo parameters, such as the ZPTV [6]. To our knowledge these systems are either manually operated or semiautomatic. An automated system would be time-saving, and the measurements obtained would be independent from the system operator. Today it is hard to achieve reproducible measurements among different technicians, because the zona is rather diffuse.

Olsen et. al. have also studied HMC images of embryos [9], but they do not consider automatic segmentation.

This paper considers the problem of automatically segmenting the zona in HMC images, which must be considered a crucial step in an automated procedure for ZPTV measurements. We assume that a rough estimate of the background can be found using for instance mathematical morphology. This estimate is used to normalize the images such that the intensity of the background becomes essentially constant and equal to zero.

An appealing method for segmentation problems is to
use some sort of active contour model [7, 10]. However, most active contour models are based on the use of edge maps, and it is difficult to develop any good edge maps in our case. Instead, we consider a model-based segmentation method, similar to what has been proposed by Chan and Vese [1], which is independent of edges in the image.

The segmentation of the zona can be separated into two problems; (a) the segmentation of the embryo from the background, i.e. finding the outer circumference of the zona, and (b) the segmentation of the zona from the rest of the embryo, i.e. finding the inner circumference of the zona. In both cases we use a variational formulation of the problem.

2. Variational Segmentation

The image is considered as a real valued function $I : R \rightarrow [-1, 1]$ defined on some open rectangular subset $R \subset R^2$. Points in the image domain $R$ are denoted $x = (x, y)$. The object is to determine the subset $\Omega^*$ of $R$ which corresponds to the embryo. The latter looks more or less like a distorted disc, so we assume that $\Omega^*$ belongs to the class $D$ of subsets $\Omega \subset R$ which are open and simply connected. Thus $\Gamma^* = \partial \Omega^*$ is a simple closed curve which corresponds to the outer circumference of the embryo, and the complement $\Omega^c$ of $\Omega^*$ (with respect to $R$) is the background. The segmentation $\Omega^*$ is defined as a minimizer of the energy functional:

$$J(\Gamma, I_0, I_1) = \int_{\Omega^c} |I - I_0|^2 \, dx + \int_{\Omega} |I - I_1|^2 \, dx,$$

where $I_0, I_1 : R \rightarrow [-1, 1]$ are image models for the background and the embryo, respectively, and $\Gamma = \partial \Omega$.

If $I_0$ and $I_1$ are chosen to be real constants, then (1) coincides with the minimal partitioning problem of Mumford and Shah [8], studied in [1, 2, 3] by Chan and Vese. However, apart from the constant intensity model for the background:

$$I_0(x) = c_0,$$

for some $c_0 \in R$, ($c_0 = 0$ if the image is normalized), we use an intensity model for the embryo which depends on a vector $w \in R^2$, whose direction is a characteristic of the HMC image:

$$I_1(x) = c_1 + w^T N(x),$$

where $N(x) = (x - x_0)/|x - x_0|$. Here the point $x_0 \in R$ is uniquely defined by the condition:

$$\int_{\Omega} N(x) \, dx = 0.$$

For symmetric domains, for instance ellipses, $x_0$ equals the centroid of $\Omega$.

When $x_0$ is close to the centre of the embryo, $I_1$ will be a good approximation of $I$ in the zona. The model is unlikely to be correct in the interior of the embryo, but if the latter is always contained in $\Omega$, then the error will be approximately constant, and will not affect the segmentation result.

If $J = J(\Gamma, c_0, c_1, w)$ denotes the functional (1) with $I_0$ and $I_1$ given by (2) and (3), respectively, then the problem is to find $\Omega^* \in D$, $c_0^*, c_1^*, w^* \in R$ and $w^* \in R^2$ such that

$$J(\Gamma^*, c_0^*, c_1^*, w^*) = \min_{\Omega, c_0, c_1, w} J(\Gamma, c_0, c_1, w).$$

Note that along with $\Omega^*$ we also determine the characteristic vector of the HMC image, $w^*$.

As in [1] and [8] we use that

$$\min_{\Omega, c_0, c_1, w} J(\Gamma, c_0, c_1, w) = \min_{c_0, c_1, w} \min_{\Omega} J(\Gamma, c_0, c_1, w),$$

which is advantageous because the parenthesis can be computed explicitly. In fact

$$\min_{c_0, c_1, w} J = \min_{c_0} \int_{\Omega^e} |I - c_0|^2 + \min_{c_1, w} \int_{\Omega} |I - I_1|^2$$

is a quadratic optimization problem. The minimizer of the first term is

$$c_0^* = c_0^* (\Omega) = \frac{1}{|\Omega^e|} \int_{\Omega^e} I \, dx,$$

where $|\Omega^e|$ is the area of $\Omega^e$. The optimum for the second term is easily seen to be

$$c_1^* = c_1^* (\Omega) = \frac{1}{|\Omega|} \int_{\Omega} I \, dx,$$

and

$$w^* = w^* (\Omega) = \left( \int_{\Omega} NN^T \, dx \right)^{-1} \int_{\Omega} I N \, dx.$$

For each choice of $\Omega$ the model parameters $c_0, c_1$ and $w$ are uniquely determined, so with $J(\Gamma) = J(\Gamma, c_0^*, c_1^*, w^*)$ the segmentation problem finally becomes

$$\Omega^* \in D : \quad J(\Gamma^*) = \min_{\Omega \in D} J(\Gamma).$$

To compute the Gâteaux derivative of $J$, consider an arbitrary vector field $\delta \Gamma : I \rightarrow R^2$ defined on $\Gamma$. If $t \in R$ is close to zero, then the curve $\Gamma + t \delta \Gamma$ corresponds to a domain $D$, and

$$\delta J(\Gamma; \delta \Gamma) = \frac{d}{dt} J(\Gamma + t \delta \Gamma)|_{t=0} = \int_{\Gamma} V(x) \delta \Gamma N \, d\sigma$$

where $V(x) = |I - c_1^* - NN^T w^*|^2 - |I - c_0|^2$, $N$ is the outward unit normal to $\Gamma$, and $d\sigma$ is the Euclidean curve element on $\Gamma$. Observe that the dependence $x_0 = x_0(\Omega)$ has been deliberately ignored in the calculation of (8). The strategy is to approximate the optimum $\Omega^*$ by solving the Euler-Lagrange equation: $\delta J(\Gamma^*; \delta \Gamma) = 0$, for all displacements $\delta \Gamma$. 

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3. Finding the Outer Circumference

Rather than attempting to solve (7) in its full generality, we try to find a good approximation to $\Omega^*$ by restricting the admissible domains to be in the class $D_E$ of ellipses. Thus, we seek the solution of the finite dimensional optimization problem:

$$
\Omega^* \in D_E : \quad \hat{J}(\Omega^*) = \min_{\Omega \in D_E} \hat{J}(\Omega). 
$$

(9)

Let $D = \{\mathbf{u} = (u, v) : u^2 + v^2 < 1\}$ denote the unit disc in $\mathbb{R}^2$. Then an element $\Omega \in D_E$ may be parametrized by an affine mapping:

$$
\Omega = \Omega(\mathbf{a}, \mathbf{A}) : \quad \mathbf{x} = \mathbf{a} + \mathbf{A}\mathbf{u}, \quad \mathbf{u} \in D,
$$

(10)

where $\mathbf{a} \in \mathbb{R}^2$ and $\mathbf{A}$ is a 2-by-2 matrix. We may assume, without loss of generality, that $\det(\mathbf{A}) > 0, \text{tr}(\mathbf{A}) > 0$ and $\mathbf{A}^T = \mathbf{A}$. Notice that if $\mathbf{u} \in \partial D$ then $\mathbf{u} = (\cos \theta, \sin \theta), 0 \leq \theta < 2\pi$, thus $\Gamma$ is represented by a trigonometric sum.

We are now going to use the formula (8) to find expressions for the gradient $\nabla \hat{J} = (\nabla_\mathbf{a} \hat{J}, \nabla_\mathbf{A} \hat{J})$ of $\hat{J}$ with respect to the parameters $\mathbf{a}$ and $\mathbf{A}$.

Suppose $\mathbf{A}$ is kept fixed and $\mathbf{a} \rightarrow \mathbf{a} + \delta \mathbf{a}$, where $\delta \mathbf{a} \in \mathbb{R}^2$. Then it is easy to see that $\delta \Gamma = \delta \mathbf{a}$, and so it follows from (8) that

$$
\delta \hat{J}(\Gamma; \delta \Gamma) = \delta \mathbf{a}^T \int_\Gamma V(\mathbf{x}) \mathbf{n} d\sigma.
$$

This shows that

$$
\nabla_\mathbf{a} \hat{J}(\Gamma) = \int_\Gamma V(\mathbf{x}) \mathbf{n} d\sigma.
$$

Now, if $\mathbf{a}$ is fixed and $\mathbf{A} \rightarrow \mathbf{A} + \delta \mathbf{A}$, $\delta \mathbf{A}$ symmetric, then $\delta \Gamma = \delta \mathbf{A} \mathbf{u} = \delta \mathbf{A} \mathbf{A}^{-1}(\mathbf{x} - \mathbf{a})$, so by (8),

$$
\delta \hat{J}(\Gamma; \delta \Gamma) = \int_\Gamma V(\mathbf{x}) \mathbf{n}^T \delta \mathbf{A} \mathbf{A}^{-1}(\mathbf{x} - \mathbf{a}) d\sigma.
$$

If we substitute the orthogonal basis elements $\left[\begin{smallarray}{c} 0 \\ 1 \end{smallarray}\right]$, $\left[\begin{smallarray}{c} 1 \\ 0 \end{smallarray}\right]$ and $\left[\begin{smallarray}{c} 1 \\ 1 \end{smallarray}\right]$ for $\delta \mathbf{A}$ and set

$$
\mathbf{B}(\Gamma) = \mathbf{A}^{-1} \int_\Gamma V(\mathbf{x})(\mathbf{x} - \mathbf{a}) \mathbf{n}^T d\sigma,
$$

then we get

$$
\nabla_\mathbf{A} \hat{J} = (\mathbf{B}(\Gamma) + \mathbf{B}(\Gamma)^T)/2.
$$

(Without the symmetry requirement: $\nabla_\mathbf{A} \hat{J} = \mathbf{B}(\Gamma)$.)

To find a solution to the Euler-Lagrange equation we use steepest descent: Start with an ellipse $\Omega_0 = \Omega(\mathbf{a}_0, \mathbf{A}_0) \in D_E$, and solve the initial value problem:

$$
\left\{ \begin{array}{l}
\dot{\mathbf{a}} = -\nabla_\mathbf{a} \hat{J}(\Gamma), \\
\dot{\mathbf{A}} = -\nabla_\mathbf{A} \hat{J}(\Gamma),
\end{array} \right. \quad \mathbf{a}(0) = \mathbf{a}_0, \quad \mathbf{A}(0) = \mathbf{A}_0,
$$

where $\Gamma = \Gamma(\mathbf{a}, \mathbf{A})$.

4. Finding the Inner Circumference

Constructing an image model for the interior of the embryo is difficult, so the earlier approach, with two image models, cannot be applied in finding the inner circumference. Instead we use a method based on a single model, namely $I_1$ defined in (3), which is a good approximation to the zona. Starting from a set $A_0$, a prediction for zona–we seek to expand this set without increasing the standard deviation of the model error more than twofold.

To find the initial set $A_0$ we use the segmentation of the embryo $\Omega^* = \Omega(\mathbf{a}^*, \mathbf{A}^*)$. Assuming that the zona has some minimal thickness, a number $s \in (0, 1)$ can be a priori determined such that the annular region $A_0 = \Omega^* \setminus \Omega(\mathbf{a}^*, s\mathbf{A}^*)$ is contained in the zona. With $\mathbf{x}_0 = \mathbf{a}$ and $\Omega = \Omega_0$ equation (4) holds. Thus $c_1(A_0)$ and $w(A_0)$ can be measured using (5) and (6). These parameters, and hence $I_1$, are from here on fixed.

The standard deviation of the model error over a set $A$ is

$$
\sigma(A) = \left[ \frac{1}{|A|} \int_A |I - I_1|^2 \, d\mathbf{x} \right]^{1/2}.
$$

We define the segmentation $A^*$ of the zona as a minimizer of the energy functional

$$
J_Z(A) = |A| \left( \sigma^2(A) - 4\sigma_0^2 \right),
$$

(11)

where $\sigma_0 = \sigma(A_0)$. Notice that if a point $\mathbf{x}$, for which it holds that $|I(\mathbf{x}) - I_1(\mathbf{x})| < 2\sigma_0$, is included into $A$, then the value of $J_Z$ is decreased. So without any priors on $A^*$ we would end up with a thresholding procedure. However, we restrict the problem to the class $A$ of annular domains, that is, to sets of the form $A = \Omega^* \setminus \Omega$, where $\Omega$ is an ellipse contained in $\Omega^*$, and seek a solution of the optimization problem:

$$
A^* \in A : \quad J_Z(A^*) = \min_{A \in A} J_Z(A).
$$

(12)

The Gâteaux derivative of the functional (11) is given by (8) with $V(\mathbf{x}) = |I - c_1 - N^T \mathbf{w}|^2 - 4\sigma_0^2$. Now (12) can be solved using the same framework as in Section 3.

5. Experiments

Initially the images are normalized: A linear intensity model is adapted to the image in a manually specified, rough segmentation of the background. The determined model is then subtracted from the entire image. This implies that the mean intensity in the background is zero, in particular we may use $c_0^* = 0$ in all computations. The model $I_1$ for the embryo is, as mentioned earlier, in good agreement with the zona, but quite inadequate for the interior of the embryo. This results in the annoying fact that
the method, for finding the outer circumference, works best if we simply set \( c^*_1 = 0 \) instead of using (5). Fortunately, the orthogonality relation (4) implies that \( w^* \) can be determined independently of \( c^*_1 \). For finding the inner circumference, (5) and (6) are used unchanged.

Figure 2a shows the result of the proposed segmentation method on the same embryo as shown in Figure 1. The method has been tested on several images, and works well for images of good quality. Unfortunately, not all images are of such good quality. Since living embryos are studied, it is essential to keep photographic exposure time to a minimum. It may therefore be impossible to produce images without disturbances. Figure 2b shows an example of an image with two dark artifacts, and as can be seen, the segmentation scheme fails miserably for this image.

To cope with such disturbances, a simple modification of the proposed scheme is made: We apply masks to cover the artifacts, and perform the usual calculations outside the masks. The resulting segmentation can be seen in Figure 2c, which also shows the masks superimposed in white.

Conclusions and Further Work

We have presented an area based method for segmentation of zona pellucida in HMC images of human embryos. The method is robust and does not depend on edges in the images. Instead an image model is used, which takes advantage of the characteristic appearance of HMC images. The results look very promising, but a more thorough evaluation should be performed.

The considered shapes have been restricted to annuli with elliptic boundaries, which seems an adequate approximation of the zona. However, it is straightforward to generalize the method to more complex shapes by including more terms in the trigonometric sums for the boundaries of the object.

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