Modeling Dynamic Cellular Morphology in Images

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Abstract. This paper presents a geometric method for modeling dynamic features of cells in image sequences. The morphological changes in cellular membrane boundaries are represented as sequences of parameterized contours. These sequences are analyzed as paths on a shape space equipped with an invariant metric, and matched using dynamic time warping. Experimental results show high sensitivity of the proposed dynamic features to the morphological changes observed in lymphocytes of healthy mice after undergoing skin transplantation when compared with standard representation methods and shape features.

1 Introduction

Morphological analysis of cells features prominently in a wide range of applications including digital pathology and is essential for improving our understanding of the basic physiological processes of organisms. Although the underlying cellular structure is $3D$, a $2D$ morphological analysis can still be conclusive and effective for several applications [1]. Accordingly, advanced image processing techniques involving tasks such as cell tracking, extraction, cell shape representation and analysis [1–3] have enabled accurate analysis of $2D$ static cell images. However, these static analyses do not provide information about the dynamic cellular activity. To date, an increasing number of studies are using live-cell ($2D + t$) imaging to provide insight into the nature of cellular functions [4]. The task of cell shape analysis in image sequences ($2D + t$) is challenging due to the following reasons: i) it is difficult to compactly reduce and represent the high dimensional geometric data from information-rich image sequences for accurately capturing the biologically relevant phenomena under investigation, ii) live cells are non-rigid bodies, and thus classical methods optimized for rigid transformations are unsuitable for their analysis, and finally, iii) the geometric information from the cell shapes needs to be appropriately isolated from the pose in an invariant manner.

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In this paper we propose a dynamic framework for quantitative analysis of lymphocyte morphological changes in $2D + t$ image sequences. We represent cell shape boundaries by continuous, closed, parameterized curves, and analyze them dynamically on the shape space of such representations. Our framework conveniently lends itself to i) the interpolation of intermediate shapes in a sequence, ii) the invariant and symmetrical matching of different cell shape sequences, and iii) statistical analysis of dynamic cell morphology. We test our method on a collection of 42 lymphocyte sequences and present results on i) discrimination of normal and abnormal cellular morphology, ii) local statistical differences between abnormal and normal shape sequences, and iii) classification of shape sequences based on the dynamic cellular shape changes from one time point to another.

2 Dynamic Cellular Morphometry

2.1 Shape Analysis of Static Cellular Boundaries

While normal morphological changes in cells show subtle changes in shape, abnormal cells exhibit structural irregularities due to various disease processes or disruptions in cellular mechanisms caused by isolated cellular activity. It is critical that the shape representation be sensitive to pathological changes as well as regular normative variation. In this section, we briefly describe the representation, invariances, and matching framework for static cell shape analysis.

Shape Representation: We represent the segmented cell boundary by $2D$ closed, continuously parameterized curve [5–7] $c \subset \mathbb{R}^2$ given by $c(s) : [0, 2\pi) \rightarrow \mathbb{R}^2$. In order to analyze the geometry of them exclusively without the confounding effects of global location and scale, we represent the translation invariant geometric shape of this curve using the parameterized function given by

$$q(s) = \frac{\dot{c}(s)}{\sqrt{\|\dot{c}(s)\|}} \in \mathbb{R}^2, s \in [0, 2\pi]. \quad (1)$$

Here $\|\cdot\| \equiv \sqrt{(\cdot, \cdot)_{\mathbb{R}^2}}$, and $(\cdot, \cdot)_{\mathbb{R}^2}$ is the standard Euclidean inner product in $\mathbb{R}^2$.

Shape Invariances: The representation is automatically invariant to translation. To make that invariant to scale, we divide the q function by its magnitude $\sqrt{\int_0^{2\pi} (q(s), q(s))_{\mathbb{R}^2}}$. A rigid rotation of a curve is a shape-preserving operation and is defined as $O \cdot q(s) = Oq(s)$, where $O \in \text{SO}(2)$. Additionally the cell shape distances should also be independent of the origin of the curve. The choice of the origin is modeled as a shift operation by $r \in \mathbb{S}^1$, $r \cdot q(s) = q((s - r)_{\text{mod } 2\pi})$. Lastly, the variable speed reparameterizations of the curve are modeled as diffeomorphic group actions of $\gamma : [0, 2\pi) \rightarrow [0, 2\pi)$ on the curve and given as $q \cdot \gamma = \sqrt{\dot{\gamma}} (q \circ \gamma)$. Of these, the contribution due to the starting point, rotation, and the reparameterization are removed during the next shape matching stage.

Shape Matching: Since a cell boundary necessarily corresponds to a closed curve, we define the space of all translation and scale invariant closed curves

as $\mathcal{C} \equiv \{q|q(s) : [0, 2\pi] \rightarrow \mathbb{R}^2 | \int_0^{2\pi} (q(s), q(s))_{\mathbb{R}^2} ds = 1, \int_0^{2\pi} q(s) \|q(s)\| ds = 0\}$. Owing to the conditions of scale invariance, translation invariance, and closure, the space $\mathcal{C}$ becomes a subset of a spherical Hilbert space. For the purpose of discriminating between cell boundaries, we need a computable metric on the space of cellular shapes. We define a $\mathbb{L}^2$ inner product on the ambient Hilbert space given by $\langle u, v \rangle = \int_0^{2\pi} (u(s), v(s))_{\mathbb{R}^2} ds$, and induce it on $\mathcal{C}$. This inner product is analogous to the vector form of the Euclidean inner product and measures infinitesimal perturbations of shapes. The advantage of the ambient spherical Hilbert space is that geodesics are specified analytically. The geodesic as a function of time τ , between two cell shapes q_1 and q_2 on the sphere is given by $\chi_\tau(q_1, q_2) = \cos(\tau \cos^{-1}\langle q_1, q_2 \rangle) q_1 + \sin(\tau \cos^{-1}\langle q_1, q_2 \rangle) f$, where $f = q_2 - \langle q_1, q_2 \rangle q_1$. Then the scale and translation invariant distance between the two cell shapes is given by $d(q_1, q_2) = \int_0^{2\pi} \sqrt{\langle \dot{\chi}_\tau, \dot{\chi}_\tau \rangle} d\tau$. Thus we need to enable fully pose, and initial-point invariant, as well as elastic mappings between cell shapes. This is achieved as follows. Rotational invariance is achieved by finding the optimal distance over all rotations $O \in SO(2)$, and the invariance to starting point is obtained by searching over all starting points $r \in \mathbb{S}^1$. It is easier to implement in the discrete setting. For obtaining an elastic mapping, we optimize over all reparameterizations of the curve given by $q \cdot \gamma$. Thus the fully pose and scale invariant, elastic shape distance between two cell boundaries is given by

$$d_e(q_1, q_2) = \underset{r, O \in SO(2), \gamma}{\operatorname{argmin}} \frac{1}{2} [d(q_1, r \cdot O(q_2 \cdot \gamma)) + d(q_2, r \cdot O(q_1 \cdot \gamma^{-1}))]. \quad (2)$$

After solving Eqn. 2 using a combination of dynamic programming (for initialization) and gradient descent, we not only get a distance between the shapes q_1 and q_2 , but also get a geodesic path χ_τ between them. The distance obtained by solving Eqn. 2 is a shape distance between two static cell boundaries.

2.2 Dynamic Shape Analysis of Cell Sequences

Since our goal is to model the dynamic nature of the cell behaviors by analyzing the spatial as well as temporal changes, we now propose a general framework for representation, matching, and statistical analysis of dynamic cell shape sequences. In this paper, we assume that the cell motion for all populations is captured in a fixed time interval, $t \in [0, T]$. Fig. 1 (A) provides an overview of the acquisition of the cell shape sequences from phase contrast microscopy images as well as two representative sequences of abnormal (Fig. 1 (B)) and normal (Fig. 1 (C)) lymphocyte morphology.

We denote $X(t)$ as a time-valued sequence of cell shapes for a given observation, where $X(t) \subset \mathbb{R}^2 | X(t, s) : [0, 2\pi] \times [0, T] \rightarrow \mathbb{R}^2$. We represent the time sequence $X(t)$ as $X(t) \equiv \{q_t^X\}$, $t \in [0, T]$, where q_t^X is the observed shape in sequence X at time t . The quantity $X(t)$ can be thought of as a path of a collection of shapes exhibiting infinitesimal changes on the shape space. Now given two such shape sequences, $X(t)$, and $Y(t)$, we are interested in finding an optimal correspondence between them, that takes the temporal variation in the

Fig. 1. (A) Lymphocyte shape sequence extraction workflow. Lateral and cross-sectional views of cell sequences belonging to the (B) abnormal category and the (C) normal category.

sequences into account. Dynamic time warping (DTW) [8] has been originally used for aligning different speech time series for speech recognition, and subsequently used to compare human gait [9, 10]. Matching cellular forms is more challenging than comparing human silhouettes, since cellular shapes lack well defined structure. In this work, we extend and adapt the dynamic time warping algorithm for comparing dynamic sequences on the shape space of elastically parameterized cell boundaries.

Cell Sequence Matching. Given two shape sequences X_1 and X_2 , we want to find a distance between them that takes into account i) the changes between individual shapes along the temporal direction, and ii) the differences between individual shapes within the two sequences. Myers et al. [8] have used a square root weighting function compensated by the time rate change between two time sequences for speech recognition. Following the same principle, we define an invariant, symmetrical distance between the two shape sequences X_1 and X_2 as

$$d_s(X_1, X_2) = \min_{\psi} \int_0^T d_e \left(q_t^{X_1}, q_{\psi(t)}^{X_2} \right)^2 \{1 + \dot{\psi}(t)\} dt, \quad (3)$$

with the optimal time warp $\hat{\psi}$ given by the minimizer of Eqn. 3. This function is a weighted Euclidean distance between the two shape sequences compensated by a non-linear weighting function that adjusts the time rate change of the two sequences. Furthermore, this distance is invariant to the rate change by time t , and symmetrical with respect to the two shape sequences. For the purpose of computer implementation, Eqn. 3 is discretized by considering finite samples from the shape sequences, and solved using dynamic programming. Fig. 2 shows an example of a dynamic alignment between two lymphocyte sequences along with the optimal warping function $\hat{\psi}$ overlaid on the discrete distance matrix given by $d(X_1, X_2)$.

Statistics of Dynamic Shape Sequences. In order to find statistical differences between two populations of cell shape sequences, we establish the notion

Fig. 2. Left: Cell shape sequence (top and bottom) matching using DTW in Eqn. 3. Right: The optimal warp ψ overlaid on the distance matrix $d(X_1, X_2)$.

of an average dynamic shape sequence. The average shape sequence is the local minimizer of the variance of a collection of shape sequences. We define the dynamic shape variance as $X_\sigma^2 = \frac{1}{N-1} \sum_{i=1}^N d_s(X_i, X)^2$. Then using Eqn. 2 and 3, the dynamic shape average becomes

$$X_\mu = \operatorname{argmin}_X \frac{1}{N-1} \left[\sum_{i=1}^N \left\{ \operatorname{argmin}_{\psi} \int_0^T d_s(q_t^{X_i}, q_{\psi(t)}^X)^2 \{1 + \dot{\psi}(t)\} dt \right\}^2 \right]. \quad (4)$$

Specifically X_μ is a sequence of shapes denoted by $X_\mu \equiv \{q_t^{X_\mu}\}, t \in [0, T]$. In practice, the mean shape sequence is computed by performing the dynamic time warping of the shape sequences and then computing the Karcher mean [11] shapes of all the corresponding shapes. The Karcher mean is an intrinsic mean on the space of shapes and is computed iteratively by minimizing the sum-squared geodesic distances between all the shapes in the population.

Shape Discrimination between Cell Morphologies. Since our main goal is to differentiate the shape variation between the dynamic behavior of normal and abnormal lymphocytes, we derive a discrete shape-sequence feature vector which can be used to classify different sequences of lymphocytes. This feature vector should ideally be efficient to compute as well as capture the relevant shape dynamics along the sequence. For a given sequence $X(t)$, we first sample N shapes uniformly along the time interval $t \in [0, T]$ as $\{q_i^X\}, i = 1, \dots, N$. We then compute $N - 1$ piecewise geodesics between the adjacent shapes of the sequence and denote the respective geodesic paths by χ . We then construct a parameter vector by taking the magnitude of the velocity vector given by

$$f_i = \int_0^1 \sqrt{\langle \dot{\chi}_t(q_i^X, q_{i+1}^X), \dot{\chi}_t(q_i^X, q_{i+1}^X) \rangle} dt, i = 0, \dots, N - 2. \quad (5)$$

The adjacent velocity vectors $\dot{\chi}_\tau$ are analogous to the approximation of the difference operators in the well-known ARIMA model, although we do not impose any such parametric constraints in our work. This $N - 1$ dimensional parameter vector can now be used as the input feature for classification of dynamic lymphocyte shape changes in image sequences.

Fig. 3. Average for all the 42 lymphocyte sequences. The overlaid p-values (FDR-corrected) on the shapes denote significant differences of the invariant shape deformation fields between the abnormal and normal classes.

3 Experimental Design and Results

3.1 Data

Our data consists of 42 lymphocyte image sequences (20~30 seconds) of mice undergoing back skin transplantation (age: 6-8 weeks, weight 20-22 g) observed with phase contrast microscopy (Olympus BX51, 0.3μ resolution, 16×1000 magnification). The first group consisted of 21 healthy Balb/C mice as hosts and 21 healthy Balb/C mice as donors, whereas the second group consisted of 21 healthy Balb/C mice as hosts and 21 healthy C57BL/6 mice as donors. The lymphocytes were obtained from the blood samples of the 42 hosts collected from the tail 7 days after the skin transplant. Lymphocytes in the second group showed irregular dynamic behavior such as cell elongation from different angles and a temporary projection at the border, and were characterized as abnormal, while the lymphocytes in the first group were characterized as normal.

3.2 Dynamic Shape Differences between Lymphocytes

To find differences in changes of shape across the entire population, we computed an average shape sequence for all the 42 lymphocytes using Eqn. 4. For efficiency, we sampled each sequence into 8 shapes per sequence, aligned all the sequences to this average sequence using dynamic time warping, and measured the magnitude of the velocity vectors along the geodesics between the corresponding shapes in the sequence. Since the velocity vectors are invariant to pose, we denoted this measure as the magnitude of the element wise shape deformation between the two sequences. We then performed a t-test comparing the magnitude of the velocity vector across the abnormal and normal groups. Fig. 3 shows the mean shape sequence for all the 42 lymphocyte sequences computed using Eqn. 4 with color-code false-discovery rate (FDR) corrected p-values ($p_{FDR} < 0.0059$) denoting the differences in shape. It is observed that there are significant localized statistical differences in shape across the normal and abnormal populations.

Next, we test the discriminative properties of the shape-sequence distance by computing $42 \times \frac{41}{2} = 861$ pairwise shape distances for all the 42 sequences. The shape sequence distances were visualized by projecting the distance matrix into two dimensions using multidimensional scaling (MDS). To test the improvement due to dynamic time warping, we also computed 861 pairwise distances without DTW ($\psi(t) = t$). Fig. 4 shows a comparison of the MDS projections of the

pairwise distances between all 42 (21 abnormal (denoted by A_n), and 21 normal (denoted by N_n) shape sequences. It is observed that pairwise distances using DTW exhibit a better separation of the sequences compared to shape matching using a linear time correspondence. Additionally, Fig. 4 also shows possible outlier sequences for abnormal cases A_1, A_5, A_8 , and A_{21} .

Fig. 4. MDS projections of the pairwise distances between all 42 sequences without (left) DTW ($\psi(t) = t$), and with DTW (right) along with outlier sequences. The abnormal and normal samples are plotted in red and green respectively.

3.3 Classification of Dynamic Lymphocyte Cell Morphology

Finally we present results of the classification of lymphocyte cell sequences using the feature vector defined in Eqn. 5. We sampled $N = 7$ cell shapes from each image sequences and computed 42 6-dimensional feature vectors for the population. Learning Vector Quantization (LVQ) was then used to classify these two categories with 10-fold cross-validation. We compared our results with standard cell shape features such as area, elongation, and Fourier descriptors coefficients ($n = 360$) of cell shapes [1]. Each of these features were represented by a 6-parameter vector using the same 7 images used for the geodesic feature vectors, except computing Euclidean norms instead of geodesic distances. This ensured that the comparisons were consistent across different methodologies. All the classification experiments were randomly repeated for 100 times, trained with one prototype for each class, and the performance was evaluated over the other disjoint members of the set. Table 1 shows the comparison results of mean recognition accuracy of training (TrAc), testing (TeAc), sensitivity (TrSe) and specificity (TrSp) of training, and sensitivity (TeSe), and specificity (TeSp) of testing for these features. As seen in Table 1, our method shows a good performance in terms of recognition rate and stability. Although specificity for some features was better than ours, the balance of sensitivity and specificity for the geodesic-based features was superior. Statistically, our geodesic-based feature vector method showed significant ($p < 1e - 6$) improvement in the total recognition accuracy over the Fourier descriptor-based method.

Table 1. Classification Results of Lymphocyte Cell Sequences using LVQ

Feature	TrAc(%)	TeAc(%)	TrSe(%)	TrSp(%)	TeSe(%)	TeSp(%)
Area	86.44	68.50	86.78	86.11	56.67	80.33
Elongation	95.06	84.50	90.56	99.56	79.00	90.00
Fourier	92.58	92.33	85.17	100.00	84.67	100.00
Geodesic	95.22	94.33	95.44	95.00	92.00	96.67

4 Discussion

We have presented a morphometry method for analyzing dynamic cell boundaries, and it showed improved discriminative properties over linear time matching. The performance was visually verified by projecting the data into its MDS coordinates, as well as detailed classification comparisons using LVQ. The proposed method required minimal manual intervention, thus offering a significant advantage for analyzing large scale time-lapsed cellular imaging data.

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