Published in final edited form as:

Proc IEEE Workshop Appl Comput Vis. 2015 January ; 2015: 436–441. doi:10.1109/WACV.2015.64.

Classification of 3D Multicellular Organization in Phase Microscopy for High Throughput Screening of Therapeutic Targets*

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Abstract

The current trend in high throughput screening is the utilization of more complex model systems that mimic both structural and functional properties of cellular processes in vivo. In this context, 3D cell culture models have emerged as effective systems to study tumor initiation and cancer behavior, where colony organization represents distinct phenotypic signatures that enable differentiation of cancer cells in culture using phase imaging and in the absence of clinical markers. If the colony organization can be classified into different phenotypes, it will enable rapid drug screening using phase microscopy. In this paper, we propose a novel method based on locality-constrained dictionary learning for the discrimination of aberrant colony organization in phase images, which encodes original SIFT (Scale-Invariant Feature Transform) features into high dimensional sparse codes with locality-preserving landmark points on the nonlinear manifold, and summarizes the sparse features at various locations and scales through spatial pyramid matching for robust representation. Experimental results demonstrate the significant improvement of performance, compared to the state-of-art in the field.

1. Introduction

The current trend in high throughput screening is the utilization of more complex model systems that mimic both structural and functional properties of cells in vivo. In this context, 3D cell culture models have emerged as effective systems to study tumor initiation and cancer behavior.

3D cell culture models offer certain advantages over the 2D models. In the former, each cell forms an ellipsoid with dimensions of 10–30 μm [1], and the entire cell surface is exposed to other cells or the Extracellular Matrix (ECM). However, in the latter, each cell remains flat with a thickness of a few micrometers, and only a very small percentage of the cell surface area is exposed to the other cells. These differences in shape and surface are especially significant given that approximately 30% of the genome is thought to encode membrane-bound macromolecules [2]. Studies have shown that these simple morphological

*This work was supported by NIH grant R01 CA140663 carried out at Lawrence Berkeley National Laboratory under Contract No. DE-AC02-05CH11231 with the University of California.

organizations have a profound impact on cellular functions such as morphogenesis [3] and response to treatment [4–7]. *3D cell culture models can be imaged using confocal microscopy in 3D or bright field microscopy in 2D. The latter enables high throughput imaging, captures gross colony organizational features, and provides a simple screening mechanism for a range of aberrant phenotypes effectively. Because of these properties, the focus of this paper is on colonies that are imaged using bright field microscopy.*

Presently, breast cancer has one of the better model systems for studying biological processes with more than 60 established cell lines. These lines recapitulate several subtypes of breast cancer that include (i) Luminal A, which is estrogen and progesterone positive (e.g., ER+, PR+) and ERBB2 negative; (ii) ERBB2 positive; and (iii) Triple Negative (e.g., ER–, PR–, and ERBB2–). Luminal A is a model system for ductal in situ carcinoma (DCIS). When these cell lines are cultured in 3D, they form distinct phenotypic signatures as a result of aberrant organization. Colony organization represents distinct phenotypic signatures that enable differentiation of cancer cells in culture using phase imaging and in the absence of clinical markers. In this context, colony formation for normal cell is round and circular, while colony formation for cancer lines is typically more invasive [8].

Colony organization provides an endpoint for classifying therapeutic responses since research has demonstrated that targeted inhibitors can reverse aberrant disorganization [9]. Therefore, robust methodology is needed to discriminate aberrant organization for rapid drug screening. Since colony organization is a major indicator for a more aggressive phenotype and phase contrast microscopy provides fast image acquisition, classification of the phenotypic signature can provide the necessary endpoint for evaluating therapeutic targets. Although low resolution phase contrast microscopy cannot distinguish DCIS from normal colony organization, it can classify reversion of Triple Negative and ERBB2+ breast cancer colony formation to a more indolent and normal-like state. Differentiating DCIS model from normal colony formation will more likely need fluorescence microscopy, which is not the focus of this paper.

Having established the motivation and requirements of robust classification and the trend in using extensible techniques for classification, we formulate the classification problem as the locality-constrained dictionary learning based on the extracted SIFT [10] features.

2. Related Work

From our perspective, the main trends in phase contrast microscopy image analysis are (i) cell segmentation and tracking [11,12]; and (ii) cellular event (e.g., mitosis, apoptosis, and cell division) detection [13–15]. However, to the best of our knowledge, the classification of 3D multicellular organization using phase imaging has not been explored, which can actually play an important role for high throughput screening of therapeutic targets.

In the context of computer vision research on image categorization, spatial pyramid matching (SPM) [16] has clearly become the major component of the state-of-art systems [17] for its effectiveness in practice. Meanwhile, sparsity/locality-constrained feature encoders, through dictionary learning, have also been widely studied, and improvement of performance has been confirmed in various applications [18–20].

Most recently, Locality Constrained Dictionary Learning (LCDL) [21] has been proposed for nonlinear dimensionality reduction, which greatly improves the embedding quality and reduces the complexity compared to the state-of-the-art dimensionality reduction algorithms, such as K-SVD [22], LCC [23] and LLC [19]. However, the power of LCDL, as a locality-constrained feature encoder, for the task of image classification has not been explored.

Due to the effectiveness of LCDL and SPM in feature encoding and feature summarization, respectively, we propose to combine them into the same framework (LCDLSPM) for image classification, which leads to a system that outperforms the state-of-the-arts for the classification of 3D multicellular organization in phase microscopy.

3. Approach

The proposed approach is shown in Figure 1, where the traditional SIFT is replaced with sparse code, generated through LCDL, within the SPM framework. It consists of the following steps:

1. Extract SIFT features from images.
2. Sparse Coding based on LCDL. Let $\mathbf{Y} = [\mathbf{y}_1, \dots, \mathbf{y}_N]$ be a set of SIFT features, $\mathbf{X} = [\mathbf{x}_1, \dots, \mathbf{x}_N]$ be the corresponding sparse codes, and $\mathbf{B} = [\mathbf{b}_1, \dots, \mathbf{b}_K]$ be the dictionary to be learned, the LCDL optimization problem is then formulated as:

$$\begin{aligned} \min_{\mathbf{B}, \mathbf{X}} \quad & \|\mathbf{Y} - \mathbf{B}\mathbf{X}\|_F^2 + \lambda \sum_{i=1}^N \sum_{j=1}^K [x_{ji}^2 \|\mathbf{y}_i - \mathbf{b}_j\|_2^2] + \mu \|\mathbf{X}\|_F^2 \\ \text{s.t.} \quad & \begin{cases} \mathbf{1}^T \mathbf{x}_i = 1 & \forall i & (*) \\ x_{ji} = 0 & \text{if } \mathbf{b}_j \notin \Omega_\tau(\mathbf{y}_i) & \forall i, j & (**) \end{cases} \end{aligned} \quad (1)$$

where $\Omega_\tau(\mathbf{y}_i)$ is defined as the τ -neighborhood containing τ nearest neighbors of $\mathbf{y}_i$, and λ, μ are positive regularization constants. $\mu \|\mathbf{X}\|_F^2$ is included for numerical stability of the least-squares solution. The sum-to-one constraint (*) follows from the symmetry requirement, while the locality constraint (**) ensures that $\mathbf{y}_i$ is reconstructed by atoms belonging to its τ -neighborhood, allowing $\mathbf{x}_i$ to characterize the intrinsic local geometry. During training, Equation 1 is optimized with respect to both $\mathbf{B}$ and $\mathbf{X}$; In the coding phase, for a new set of $\mathbf{Y}$, the learned $\mathbf{B}$ is applied, and Equation 1 is optimized with respect to $\mathbf{X}$ only.

3. Construct spatial pyramid representation for the linear SPM [18]. Let $\mathbf{X}$ be the sparse codes calculated through Equation 1 for a descriptor set $\mathbf{Y}$. Based on pre-learned and fixed dictionary $\mathbf{B}$, the image descriptor is computed based on a pre-determined pooling function as follows,

$$\mathbf{f} = \mathbb{P}(\mathbf{X}) \quad (2)$$

In our implementation, $\mathbb{P}$ is selected to be the max pooling function on the absolute sparse codes

$$f_j = \max\{|x_{j1}|, |x_{j2}|, \dots, |x_{jN}|\} \quad (3)$$

where f_j is the j -th element of $\mathbf{f}$, x_{ij} is the matrix element at i -th row and j -th column of $\mathbf{X}$, and N is the number of features extracted in the region. The choice of max pooling procedure is justified by biophysical evidence in the visual cortex [24], algorithms in image categorization [18], and our experimental comparison with other common pooling strategies (see Table 3). Similar to the construction of SPM, the pooled features from various locations and scales are then concatenated to form a spatial pyramid representation of the image, and a linear SPM kernel is applied as follows,

$$\kappa(\mathbf{f}_i, \mathbf{f}_j) = \mathbf{f}_i^\top \mathbf{f}_j = \sum_{l=0}^2 \sum_{s=1}^{2^l} \sum_{t=1}^{2^l} \langle \mathbf{f}_i^l(s, t), \mathbf{f}_j^l(s, t) \rangle \quad (4)$$

where $\mathbf{f}_i$ and $\mathbf{f}_j$ are spatial pyramid representations for image I_i and I_j , respectively, $\langle \mathbf{f}_i, \mathbf{f}_j \rangle = \mathbf{f}_i^\top \mathbf{f}_j$, and $\mathbf{f}_i^l(s, t)$ and $\mathbf{f}_j^l(s, t)$ are the max pooling statistics of the sparse codes in the (s, t) -th segment of image I_i and I_j in the scale level l , respectively.

4. Construct multi-class linear SVM for classification. In our implementation, the classifier is trained using the LIBLINEAR [25] package.

4. Experiments And Discussion

We have evaluated five classification methods on the dataset captured through phase contrast microscopy. The five methods are:

1. LCDLSPM: the linear SPM that uses linear kernel on spatial-pyramid pooling of SIFT sparse codes generated through Locality-Constrained Dictionary Learning (LCDL);
2. PSDSPM [20]: the nonlinear kernel SPM that uses spatial-pyramid histograms of image-patch sparse codes generated through Predictive Sparse Decomposition (PSD);
3. ScSPM [18]: the linear SPM that uses linear kernel on spatial-pyramid pooling of SIFT sparse codes generated through sparse coding;
4. LLCSPM [19]: the linear SPM that uses linear kernel on spatial-pyramid pooling of SIFT sparse codes generated through Locality-Constrained Linear Coding (LLC);
5. KSPM [16]: the nonlinear kernel SPM that uses spatial-pyramid histograms of SIFT features and χ^2 kernels;

In our experiments, the configurations were fixed as follows empirically, to achieve the best performance,

1. For LCDLSPM, LLCSPM, ScSPM and KSPM, the dense SIFT features were extracted on 16×16 patches sampled from each image on a grid with step-size 8 pixels;

2. For LCDLSPM, the regularization parameters λ and μ were fixed to be 0.3 and 0.001, respectively, and the neighborhood size τ was fixed to be 5;
3. For PSDSPM, the sparse constraint parameter λ was fixed to be 0.3, image patch size was fixed to be 20×20 , and the number of basis functions was fixed to be 1024 (Examples of computed basis functions are shown in Figure 3);
4. For LLCSPM, the regularization parameters λ and σ were fixed to be 500 and 100, respectively;
5. For ScSPM, the sparse constraint parameter λ was fixed to be 0.15;
6. For both PSDSPM and KSPM, standard K-means clustering was used for the construction of dictionary, where the elements was randomly initialized and iteratively refined in the Euclidean space.
7. Additionally, for all the approaches above, the level of pyramid was fixed to be 3, and linear SVM was adopted for classification.

The dataset for evaluation contains 3 classes: DCIS model, ERBB2+, and Triple Negative, which were collected from 22 breast cancer cell line, and scanned with a 10X objective. Examples can be found in Figure 2. The number of images per category are 36, 40 and 40, respectively. Most images are 1024×768 pixels. In this experiment, we trained on 9 and 18 images per category and tested on the rest, with three different dictionary sizes: 256, 512 and 1024. All experimental processes were repeated 10 times with randomly selected training and testing images. The final results, as shown in Table 1, were reported as the mean and standard deviation of the classification rates, which was defined as the average classification accuracy among different classes.

To further examine the performances of different approaches, we also applied pair-wised tailed t-test (with significant level α set to be 0.05) on the best performances achieved with 18 training images per category, and reported the test results in Table 2.

The experiments, conducted above, indicate that,

1. Locality constrained sparse coding improves the performance for classification of 3D multicellular organization using phase imaging. As shown in Tables 1 and 2, both LCDLSPM and LLCSPM consistently outperform ScSPM, PSDSPM and KSPM, which demonstrates the effectiveness of locality constrained sparse coding for classification of 3D multicellular organization using phase imaging. One possible explanation is that, locality is more essential than sparsity, as locality must lead to sparsity but not necessary vice versa [26]. Furthermore, our proposed approach (LCDLSPM) significantly outperforms LLCSPM due to the effectiveness of LCDL for learning locality-preserving landmark points on nonlinear manifolds [21].
2. Image-patch-based unsupervised feature learning performs equally well compared to SIFT sparse codes without locality-constraint for classification of 3D multicellular organization using phase imaging. As suggested by Table 2, there is no significant difference between the performances of PSDSPM and Sc-SPM,

which indicates that there is no preference between image-patch sparse code and SIFT sparse code for classification of 3D multicellular organization using phase imaging.

3. Raw SIFT feature is not preferred for classification of 3D multicellular organization using phase imaging. As shown in Table 1, KSPM performs the worst among all the approaches in comparison. One possible explanation is that, the sparsity/locality-constrained feature encoder adds one more layer of feature extraction to the system, which is believed to benefit the performance [27].

To study the impact of pooling strategies on the LCDLSPM method, we also provide an experimental comparison among max pooling and two other common pooling methods (i.e., square root pooling and absolute value pooling), which are defined as follows,

$$\begin{aligned} Sqrt &: f_j = \sqrt{\frac{1}{N} \sum_{i=1}^N x_{ij}^2} \\ Abs &: f_j = \frac{1}{N} \sum_{i=1}^N |x_{ij}| \end{aligned} \quad (5)$$

where the meaning of the notations are the same as in Equation 3. As shown in Table 3, the *max* pooling strategy slightly outperforms the other two; however, the pair-wised tailed t-test (with significant level $\alpha = 0.5$), as shown in Table 4, indicates that the differences of performance among these pooling strategies are not statistically significant.

5. Conclusion and Future Work

In this paper, we proposed a SPM approach based on SIFT sparse codes obtained from locality-constrained dictionary learning (LCDL) for classification of 3D multicellular organization in phase contrast microscopy for high throughput screening of therapeutic targets. Due to the effectiveness of LCDL algorithm and the SPM framework, our method significantly outperforms traditional ones (i.e., LLCSPM, ScSPM and PSDSPM), and provides a robust and effective way for the discrimination of aberrant organization for rapid drug screening. Future work will be focused on (i) validating our proposed method independent datasets; and (ii) applying our proposed method for the task of rapid drug screening.

Acknowledgments

We would like to thank Yin Zhou for sharing his implementation of the LCDL algorithm.

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Figure 1.
Computational steps of our proposed approach (LCDLSPM).

Figure 2.
Examples: First row: DCIS model; Second row: ERBB2+; Third row: Triple Negative.

Figure 3.
Computed basis functions through PSD.

Table 1

Performance of different methods.

	Method	Dictionary Size=256	Dictionary Size=512	Dictionary Size=1024
18 training	LCDLSPM	79.43 $\pm$ 4.53	93.09 $\pm$ 2.98	93.54 $\pm$ 2.54
	LLCSPM [19]	89.94 $\pm$ 3.28	90.94 $\pm$ 4.05	91.60 $\pm$ 4.37
	ScSPM [18]	83.90 $\pm$ 4.19	86.44 $\pm$ 4.55	88.52 $\pm$ 4.02
	KSPM [16]	81.99 $\pm$ 4.40	83.77 $\pm$ 4.47	84.29 $\pm$ 4.35
	PSDSPM [20]	87.15 $\pm$ 4.32	86.28 $\pm$ 2.20	85.39 $\pm$ 4.03
9 training	LCDLSPM	75.59 $\pm$ 4.83	89.03 $\pm$ 3.91	89.46 $\pm$ 4.45
	LLCSPM [19]	84.63 $\pm$ 4.87	86.80 $\pm$ 5.04	85.60 $\pm$ 5.56
	ScSPM [18]	79.57 $\pm$ 4.15	81.16 $\pm$ 5.26	83.30 $\pm$ 5.18
	KSPM [16]	78.54 $\pm$ 5.22	79.83 $\pm$ 5.77	80.65 $\pm$ 6.17
	PSDSPM [20]	79.03 $\pm$ 7.75	77.63 $\pm$ 8.67	76.60 $\pm$ 8.29

Table 2

Pair-wised tailed t-test of the best performances among different approaches with 18 training images per category.

H0	H1	H	P-Value
LCDLSPM = LLCSPM	LCDLSPM > LLCSPM	1	0.0047
LLCSPM = ScSPM	LLCSPM > ScSPM	1	1.9958e-07
ScSPM = PSDSPM	ScSPM > PSDSPM	0	0.2851
PSDSPM = KSPM	PSDSPM > KSPM	1	0.0403

Table 3

Comparison of performance for LCDLSPM using different pooling strategies with fixed dictionary size (1024) and 18 training images per category.

	Sqrt	Abs	Max
Dictionary Size=1024	93.20 $\pm$ 3.12	93.02 $\pm$ 3.18	93.54 $\pm$ 2.54

Table 4

Pair-wised tailed t-test of the performances of LCDLSPM (with fixed dictionary size = 1024, and 18 training images per category) among different pooling strategies.

H0	H1	H	P-Value
<i>max</i> pooling = <i>Abs</i> pooling	<i>max</i> pooling > <i>Abs</i> pooling	0	0.3037
<i>max</i> pooling = <i>Sqrt</i> pooling	<i>max</i> pooling > <i>Sqrt</i> pooling	0	0.3361
<i>Sqrt</i> pooling = <i>Abs</i> pooling	<i>Sqrt</i> pooling > <i>Abs</i> pooling	0	0.4819