Local Morphologic Scale: Application to Segmenting Tumor Infiltrating Lymphocytes in Ovarian Cancer TMAs

Second Progress Report

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by

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under the guidance of

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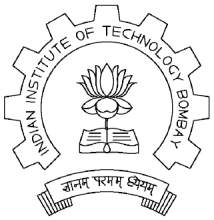

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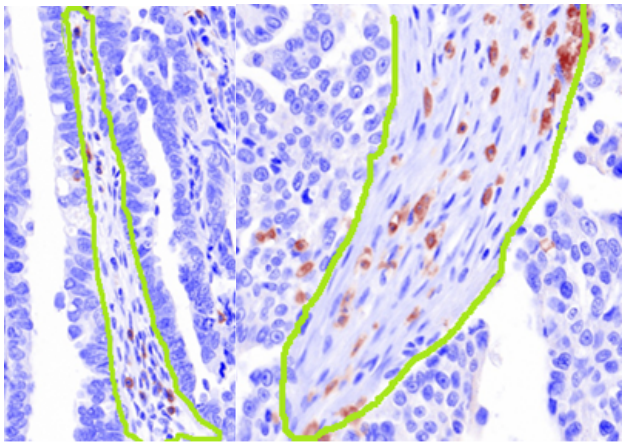
1 Abstract

The idea of a locally adaptive scale (e.g., generalized, tensor, and ball scale) has been previously introduced for facilitating a large number of image processing operations including filtering, intensity standardization, bias field correction, image registration, and segmentation. These scale notions are centered on the idea of associating a local descriptor with every spatial location. For instance, generalized scale represented the largest connected set associated with every image location, with all elements of the set satisfying some pre-defined homogeneity criterion. In this progress report we present a framework for a novel local morphological scale (LMS) notion which associates with every spatial location in the image, a descriptor capturing the shape of the largest unimpeded neighborhood. This is a departure from previous scale formulations that were defined based on a locally connected set of pixels, all of which satisfied some pre-defined homogeneity criterion, or were constrained by some shape criterion. LMS yields a unique signature of local structure at every spatial location. LMS scenes thus constructed could be applied to a number of image processing applications, including image registration and segmentation. By characterizing the physical concept of fluid flow associated with particles emanating from a point of interest, we are able to define a local region corresponding to the catchment area created by the flow of these particles. Shape descriptors quantifying the morphology of this local catchment region are used to define LMS. These shape descriptors can be used in conjunction with supervised classifiers for scene segmentation and classification. By using a monte-carlo sampling technique, LMS is efficiently determined through parallelized computations. As mentioned in the previous progress report, we apply LMS to the specific problem of classifying regions of interest in Ovarian Cancer (OCa) histology images as either tumor or stroma. This approach is used to classify lymphocytes as either tumor infiltrating lymphocytes (TILs) or non-TILs; the presence of TILs serving as an important prognostic indicator. We present preliminary results on the tumor/stroma classification of 8,000 randomly selected locations of interest, across 11 images obtained from 6 patient studies. Our supervised learner, after generating features at 38 milliseconds a sample, was able to reach an Area Under the Curve (AUC) of 0.808.

2 Introduction

It is estimated that 21,650 women will be diagnosed with and 15,520 women will die of cancer of the ovary (OCa) in 2008. The 5-year survival rates of these women are highly correlated to accurately determining a course of treatment based on the inferred prognosis. In order for doctors to determine prognosis they analyze various factors, of which new ones are being discovered. Recent work has suggested that a valuable indicator is based on the extent to which the patient's own immune response had attacked the cancer. This response can be characterized by the behavior of lymphocytes. A lymphocyte is a type of white blood cell that is sent to the



(a) (b)

Figure 1: Figure 1 Stroma region circled in green. Lymphocytes stained in red. Notice the stark difference in sizes between the two green regions in (a) and (b), selecting an appropriate window size for the texture features would be difficult.

proximity of objects which the body considers foreign. In this case, the object of interest is a tumor leading to a classification of the lymphocyte into two categories. A tumor infiltrating lymphocyte (TIL) and a non-TIL. To summarize, it is believed that the more TILs that are present in a tumor, the more severe reaction the body's immune system has (i.e. the ability to detect that the cancerous tumor should not be there), and thus leads to a better prognosis for the patient.

Lymphocytes of both classes are easily detected using a combination of targeted staining and advanced image analysis techniques such as HNCut. Given the location of the lymphocytes, the problem then becomes the classification of the cell into the aforementioned two groups, TILs and non-TILs. As expected, it is impossible to determine the class of a lymphocyte by merely examining it by itself. A more global approach is needed in order to accurately place them. This placement falls into two parts, either the lymphocyte is in the tumor or the lymphocyte is in the stroma. The stroma of the ovary is a soft tissue, abundantly supplied with blood vessels, consisting for the most part of spindle-shaped cells with a small amount of ordinary connective tissue. This creates for us the dual problem of detecting stroma versus tumor regions.

Traditional approaches to this problem would involve texture features, and some detection of the spindle like cells located in the stroma and or the derivation of domain specific features such as cell density (counting). It is well known that the computation of textural features requires a large amount of computation time, and that cell counting is another non-trivial task. Regardless of the drawbacks of other approaches, the main issue remains in the selection of an appropriate window size for these standard algorithms. Not only is this difficult but in some cases it is impossible. The stroma region is often nestled between areas of tumor, making not only its boundaries not clearly defined but the size of the associated region difficult to pre-determine.

We can see from Figure 1 that the pre-selection of a texture window would be challenging because of the varying sizes and shapes of such a window (a normal rectangle would not work). Additionally, we can see that the segmentation of individual cells would be difficult since they clump together as a result of a 3 dimensional tissue sample being scanned in two dimensions.

To circumvent these problems, we present in this progress report a paradigm shifting framework for the generation of features for tissue classification. This volcano gradient based technique, attempts to compute global features of the local region using a monte-carlo sampling technique leading to a definition of Local morphological scale. We show that the new approach is extremely efficient as it can be pushed to parallel machines such as GPUs. Additionally we hope to motivate the creation of an easily extendible framework which opens the door to a large amount of future work. We compare the computation time, precision and accuracy with an industry standard texture approach.

2.1 Previous Work

The concept of scale was introduced to characterize varying levels of image detail so that localized image processing tasks could be performed that would yield an optimal result globally. Early research indicated that having a locally adaptive definition of scale was necessary even for moderately complex images [1]. In order to take local structure into account, the notion of ball-scale[2] was introduced by Saha and Udupa. Ball-scale assigned to every spatial location in the image, a value corresponding to the radius of a circle encompassing all neighboring locations satisfying some pre-defined homogeneity criterion. In [3], Saha extended the ball-scale idea to a tensor-scale (*t*-scale), such that every spatial location fit an ellipse instead and used its major and minor axis along with the dominant orientation to characterize each spatial location. Generalized scale (*g*-scale) was introduced by Madabushi and Udupa in [4] an attempt to free the scale definition from shape constraints. *G*-scale was defined as the largest connected set associated with every spatial location, such that all elements of which satisfied a pre-defined homogeneity criterion.

2.2 Local Morphlogic Scale

In this progress report we present the concept of Local Morphologic Scale (LMS) which attempts to define a local morphometric signature for every spatial location. The spirit behind LMS is that every spatial location is associated with an identical potential energy. This energy allows particles from the spatial location under consideration to move with an initial velocity in different trajectories. If the particle moves along an unimpeded trajectory (i.e. it does not encounter an obstruction in the form of a location with a very different intensity than that of its current location) it gains momentum. Obstructions reduce the particle's momentum and cause changes in the trajectory. When the particle's momentum goes to zero it stops moving. The catchment region $\mathcal{R}(c)$ is defined by the locus of the trajectories of all particles, after they have come to a halt, at any spatial

location c. Morphometric signatures (area, perimeter, etc) derived from $\mathcal{R}(c)$ may be used to define the LMS, specifically $\mathcal{L}(c)$ at c. Unlike ball-scale and *t*-scale, LMS does not impose any shape constraint on the scale definition. Additionally, unlike previous scale definitions, LMS is focused on capturing local spatial morphology and not primarily driven by homogeneity considerations.

The implications of LMS are extensive in that the local morphometric signatures associated with every spatial location could have applications in the context of registration, segmentation, and classification. An LMS based representation of an image might allow for better image alignment/registration and region classification since it captures local structural details.

In this work we demonstrate an application of LMS for the problem of classification of regions as either stroma or endothelial in Ovarian Cancer (OCa) histology slides. It is believed that [5] lymphocytes that are tumor infiltrating (TILs) can serve as a prognostic indicator. Since lymphocytes have a homogeneous appearance, identifying TIL's requires identifying the kind of tissue that the lymphocyte is embedded in. In conjunction with powerful supervised classification techniques, we show that the LMS descriptors can serve as robust tissue classifiers by exploiting local morphological differences.

3 Methods

3.1 Theory

An image is defined as $\mathcal{C} = (C, f_q)$ where C is a 2D grid representing N pixels $c \in C$, with $c = (x, y)$ representing the Cartesian coordinates of a pixel and f_q is a function that assigns an quantized integer intensity value to c and is defined as $f_q(c) : \mathbb{R}^2 \rightarrow \{0, \ldots, q\}$. Additionally, to all $c \in C$, we assign a set of particles $p_i(c), i \in \{1, \ldots, m\}$, which are associated with an initial velocity $u_0(p_i)$.

The initial velocity $u_0(p_i)$ provides energy so that the particle p_i can migrate from c along a certain trajectory $T(p_i)$, where $T(p_i) = \{d^{(1)}, d^{(2)}, \ldots, d^{(k)}\}$ with $d^{(t)} \in C$. Here t is a sequentially increasing time variable starting at 1. As such, at the time step $t+1$, $u_{t+1} = u_t - f_q(d^{(t)}) - f_q(d^{(t+1)})$ and $d^{(t+1)} = \underset{\delta}{\operatorname{argmin}} f_q(d^{(t)}) - f_q(\delta)$, where δ is the set of adjacent pixels in front of and to the left and right of $d^{(t)}$. Intuitively, this can be described as the path of least resistance going away from c.

At some point, the particle comes to a stop when $u_{j+1} \leq 0$, on account of the particle's trajectory encountering multiple obstructions (i.e. locations with significantly different intensity as compared to those previously encountered), or the end of the specified simulation period. The locus obtained by connecting all $d^{(k)} \forall p_i(c), i \in \{1, \ldots, m\}$ yields the LMS, $\mathcal{L}(c)$.

We present the output of the algorithm in Figure 3.1 for two different classes of tissue. It is interesting to note that the emergent behavior of the particles, as seen in (d) and (h), creates a local morphological scale that has many quantifiable features which can help to distinguish between the two tissue classes. The features we are

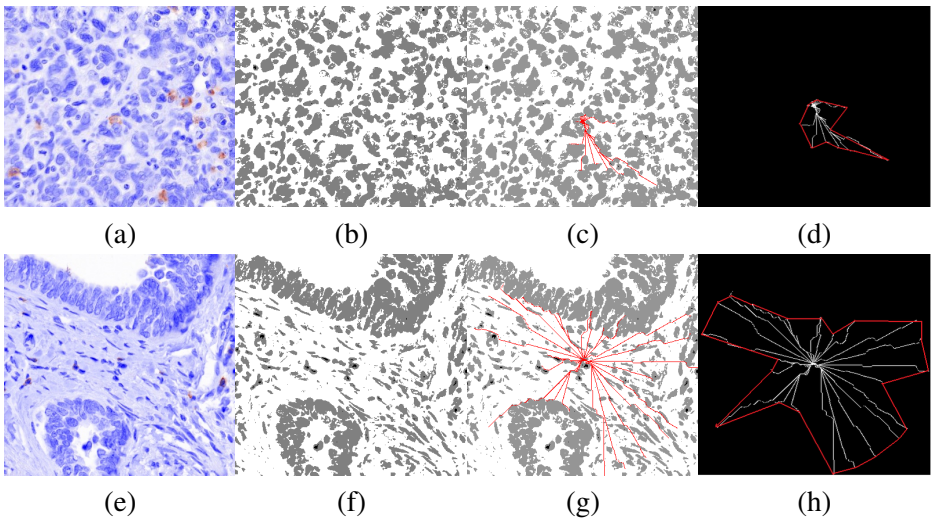


Figure 2: Two original images (a) non-stroma and (e) stroma , their quantized input ((b) and (f)), and the paths followed by the particles in red ((c) and (g)). We can see that the two patterns of the paths are drastically different, which allows us to create features based on the paths and use them in a supervised classifier to distinguish different regions within the scene. In (d) and (h) we take the end points and connect them to allow for a unique morphologic finger print, this finger print defines our local morphological scale which we can then associate with the center of the region of interest.

currently using include: a probability density functions derived from the number of obstacles hit by particles, angles between neighbors, average length of path, number of particles that reach completion, average number of unobstructed pixels on both sides of the particle during its path. Additionally, to add robustness, we also calculate those same distributions using the top 15% of the values and again with the bottom 15%. Higher order sophisticated features include area of the region creating by the endpoints and the distance between neighboring particles ending locations.

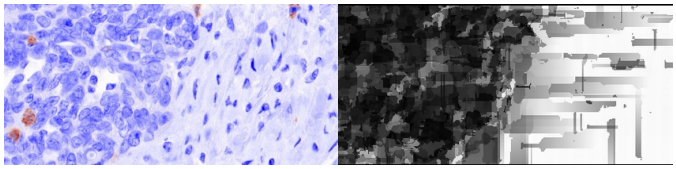
4 Results

4.1 Distinguishing Stroma and Endothelium on Ovarian Cancer TMAs

We evaluate our algorithm qualitatively and quantitatively by testing its ability to differentiate between stroma and tumor classes of tissue. To specifically illustrate the difference for the reader, in reference to Figures 3.1 (a,e), the stroma region is smoother and less dense as compared to the more complex tumor region. The classification of these regions allows for the identification of a lymphocyte as either a TIL or non-TIL. The presence of TILs is considered to be an important prognostic marker in ovarian cancer. To generate qualitative results we used LMS with only 4 particles at 90 degree intervals to save on computation time and applied it to every spatial location. We set the LMS-scale value to the area of the convex hull created by the morphometric signature, and displayed the results as a gray scale image where darker shades are associated with smaller

values. The quantitative results were computed using a supervised classifier, Probabilistic Boosting Tree (PBT) [6], on the computed features mentioned above. For this purpose, we use 11 different Ovarian Cancer histology images across 6 different patient studies for which we have manually annotated ground truths demarking the two tissues classes. We randomly selected 500 different tissue locations from each of the 1400 x 1400 pixel images, creating 8,000 individual locations. The training set consisted of 80% of the locations, and the test set the remaining 20%. All results presented are averaged across 5 runs. The timings were recording with an equipment setup consisting of an 8 core processor at 2.66Ghz with 72 gigabytes of RAM.

4.2 Quantitative and Qualitative Results



(a) (b)

Figure 3: We display the original sample in (a) and show a LMS-scale feature computed for each spatial location in (b). The gray scale value in (b) is proportional to the size of the convex hull created by the LMS at centered at the respective point. We can see that it is easy to discern between the left and right sides of the image, which in reality belong to the tumor and stroma class respectively.

Qualitatively, from Figure 3, we can see that our approach, using only a single feature, creates a very easily discernible class difference from the left tumor and right stroma regions. Additionally, we can see from Figures 4 and 4 that our preliminary quantitative results are very encouraging. At this time, we have yet to test varying levels of quantization (the above results were generated with q=2) and more sophisticated shape metrics, yet our area under the curve measure of .808 indicates that we are indeed on the right track. Additionally, we would like to note that the computation time per 500 regions was proportionally adaptable with respect to the number of particles. Due to the straightforward definition of a particle life, we have evidence suggesting that the process could be pushed to a GPU, resulting in an immense jump in resolution with a significant drop in computation time.

5 Novel Contributions

The innovations of this years work include:

1. Novel local morphometric scale, differentiated from *t*- and *b*- scales in that it does not assume or require an explicit shape *a priori*.

2. An intuitive concept of particle life motivated by physics. Catchment regions associated with particle trajectories are used to extract morphological attributes, defining a local signature for every spatial location.

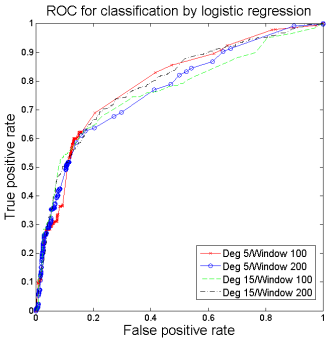


Figure 4: A Receiver operating characteristic curve (ROC) displaying the average result of the supervised classifier across 5 cross-sampled runs for the 4 different configurations specified to the right in Figure 4.

Degree Interval	Window Size	Seconds Per 500 Samples	Area Under Curve
5	100 x 100	190	.808
5	200 x 200	450	.7941
15	100 x 100	86	.801
15	200 x 200	187	.779

Figure 5: Area under the curve and computation time results for 4 different configurations. The first two used a higher sampling rate by placing a particle at 5 degree intervals, while the last two used a lower sampling rate by placing a particle at every 15 degrees. The window size considered for the first and third configurations were 100 x 100, while the second and fourth were set at 200 x 200.

3. An elegant LMS implementation that can take advantage of user selectable signature resolution and parallel processing resources.

6 Conclusion / Discussion

We have presented a novel framework for the definition of local morphological scale. Our sampling technique has been demonstrated to not only be efficient but powerful in its ability define a catchment region associated with the locus of the trajectories. The quanitzation and extraction of morphologic features associated with the catchment region yields a unique morphologic signature. When applying our algorithm to tissue classification, we have seen qualitatively how even with using only a single feature we are able to visually discern the two regions. Future work will involve expanding the concept, more fully developing LMS properties, and lastly applying the method in the context of image registration.

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