Analysis of Inflammation in Regards to Distance of Neutrophil Migration in Histopathology Images: A Marker of Infection Severity/Duration?

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Goal

Most pathologists currently rely on a visual examination of hematoxylin and eosin (H&E) stained placenta histology slide. Such qualitative examination fails to accurately determine the extent of an infection. In this study we propose an image analytical method that could be used to provide a marker of infection severity/duration.

Summary of Methods

A qualitative examination of the placental histology slides consists in looking for inflammatory polymorphonuclear leukocytes (neutrophils). We automatize this process using key features of neutrophils, like eccentricity and size. Nevertheless, only counting the number of the neutrophils is not enough to understand the extent and duration of the infection. For a relatively fresh infection, we expect more number of neutrophils near the tissue boundary.

In this study we develop an algorithm that quantifies the extent of infection and determine the motion of the neutrophils relative to the tissue-boundary. This is achieved by plotting the histogram of the distance of the found neutrophils from the boundary.

C++ module for the identification of the neutrophils

1. To identify the neutrophils we developed a fast C++ module that allows the user to specify the parameters used to identify neutrophils in a randomly selected region of interest (ROI).

2. The parameters include area, eccentricity, and the background threshold. The user also has a control over the size of the ROIs. In figure 1, we show a snapshot of this algorithm.

3. The algorithm was made faster by coding in C++, an efficient run-length labeling algorithm given in [2] pp. 40-48.

4. The user can either accept the automatically selected ROI, or reject it depending on the location of the ROI in the slide.

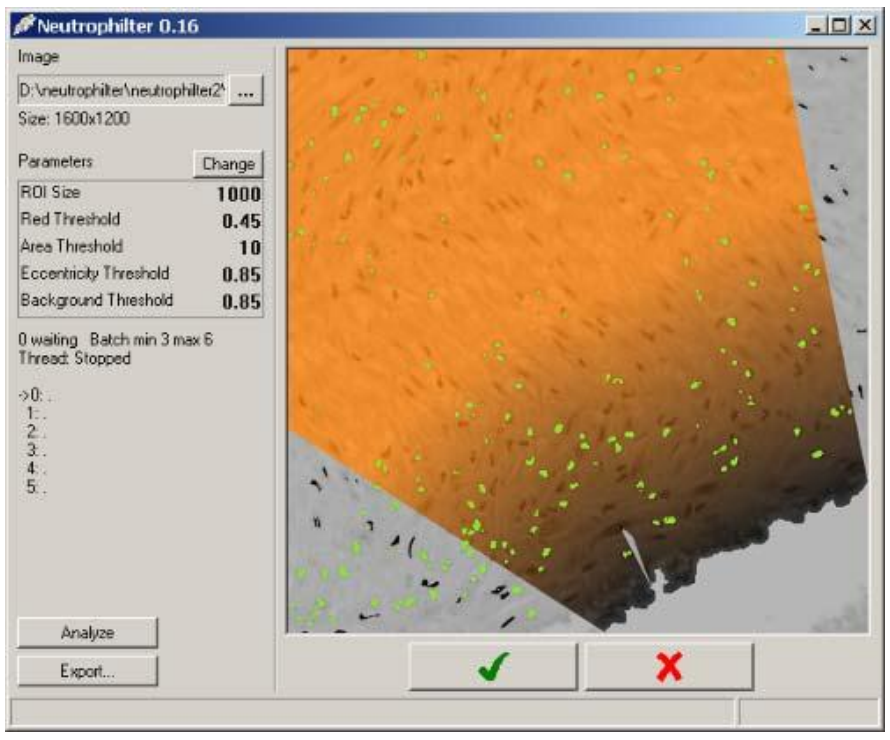

Figure 1: A snapshot of the C++ module developed for the neutrophil analysis.

Finding perpendicular distance from the boundary

The next step is to measure the *minimum* distance to the boundary from each neutrophil. This distance also needs to be a '*perpendicular*' distance from the boundary. This is essential due to occlusions in the ROI.

- ***Problem***: *Finding the minimum and perpendicular distance is difficult due to irregular shape of the boundary.*

- ***Proposed solution***: To overcome this difficulty we develop a novel algorithm, outlined below:

    1. Divide the boundary into discrete segments.
    2. Find the closest segment from the centroid of the identified neutrophil.
    3. Find the distance, d_{min}, of this centroid from the center of the closest segment.
    4. Measure the angle between this closest segment to the line joining the centroid of the neutrophil and the center of the segment.
    5. If the angle is within an accepted range from 90^o, then accept the distance d_{min} as minimum and perpendicular distance.

The advantage of using only the coarser sampling is that the angle estimates are smoother, since it does not see the finer oscillations along the boundary.

Histogram of '*neutrophil-to-boundary distance*'

In the final step, we record the neutrophils' distances from the tissue boundary. These measurements are then used to prepare histogram of the neutrophil-to-boundary distances, as shown in figure 2.

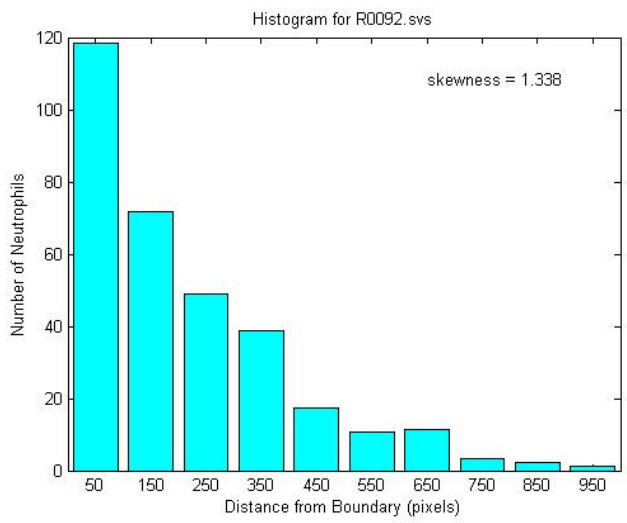

Figure 2: A histogram of the neutrophil-to-boundary distances.

Skewness and how to use it

Skewness, γ_1, measures the symmetry of a distribution and it is defined as below:

$$\gamma_1 = \mathrm{E}\left[\left(\frac{X-\mu}{\sigma}\right)^3\right]$$

Here μ is the mean and σ is the standard deviation of the data X.

- Skewness could be zero, positive or negative.
- Skewness zero for a perfectly symmetric distribution
- It is positive for a distribution with longer right tail and it is negative for a distribution of longer left tail.

A chart of ROIs of different Skewness is shown in table 1.

Roi Index	Skewness
1	0.92869
2	0.16209
3	-1.91370
4	0.38716
5	-1.63271

Table 1: Skewness of different regions of interest.

Interpretations of the histogram skewness:

1. For a histogram of '*neutrophil-to-boundary distance*', a negative skewness indicates a longer left tail. This could mean that neutrophils have traveled further away from the tissue area.

2. On the other hand, positive Skewness indicates a histogram with longer right tail. This could mean that the infection is relatively new. See the histogram in figure 2 as an example.

Conclusion

In this study we proposed a novel approach that could be used to quantitatively measure infection severity/duration, using image analytic methods.

References

[1] K. Benirschke and P. Kaufmann, *Pathology of the human placenta*, 4 ed. Springer-Verlag, 1999.
[2] R. Haralick, and L. Shapiro, *Computer and Robot Vision*, Volume I, Addison-Wesley, 1992.

** *This research was supported by Institute of Pure and Applied Mathematics, UCLA, Placental Analytics, LLC and National Science Foundation grant # DMS-0439872.*