Automated Histological Analysis System for Quantifying Microstructural Damage Accumulation to the Annulus Fibrosus

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Abstract

In this paper, we proposed an automated histological analysis system for quantifying microstructural damage accumulation to the annulus fibrosus. This system takes in a digital histology image and uses Gaussian mixture model based segmentation, followed by connected components analysis to extract and label possible clefts. The image is then refined through spatial and size constraints. Finally, the required statistics for quantifying microstructural damage are calculated.

1 Introduction

Structural damage to the annulus fibrosus is commonly observed in degenerated intervertebral discs (IVD) [1]. Internal disc disruptions, characterized by damage to the internal structure of the IVD, account for approximately 40% of low back pain. These disruptions are also the precursor for herniation [2]. Studying the magnitude of microstructural damage helps in understanding damage progression mechanisms. This study can be achieved by observing microstructural damage accumulation over a protocol known to induce herniation through histological analysis [3].

Histological analysis is performed by examining a thin slice of tissue, prepared with a specific sequence of technical procedures, under a microscope [4]. Digital image processing techniques for histological analysis have attracted more and more attention in recent years; being more reliable, reproducible, and faster than traditional manual assessment as well as independent of operator experience. In this study, we propose an automated system that characterizes accumulation of clefts in order to quantify microstructural damages to the annulus fibrosus at various time points during a herniation protocol.

Methodology

In this section, we will outline the methods used in our proposed system to calculate the necessary statistics from the histology color image.

Figure 1 shows a high level view of the proposed system. A histology colour image is first loaded. Then the initial segmentation is done using Gaussian Mixture Models. A connected components algorithm is then used to extract and label possible cleft regions. The segmented image is then refined by applying spatial and size constraints. Finally, the required statistics for quantifying microsturctural damage accumulation are calculated. Initial segmentation will now be discussed.

Initial Segmentation

Initial segmentation was achieved using Gaussian Mixture Models with Expectation Maximization optimization (GMM-EM). A color histology image is composed of a finite number, g, of segments (or 'populations') of interest: $G_1, ..., G_g$. Under the finite mixture model [5], every colour pixel x in a histology image is assumed to be part of a super population G, which is a mixture of a finite number of populations $G_1, ..., G_g$ in some proportions $\pi_1, ..., \pi_g$ that represent the different segments of the image such that:

$$\sum_{i=1}^{g} \pi_i = 1, \text{ with } \pi_i \geq 0 \tag{1}$$

In the finite mixture form, the probability density function (pdf) of an observation x is given by:

$$p(x, \phi) = \sum_{i=1}^{g} \pi_i \cdot p(x|i, \theta) \tag{2}$$

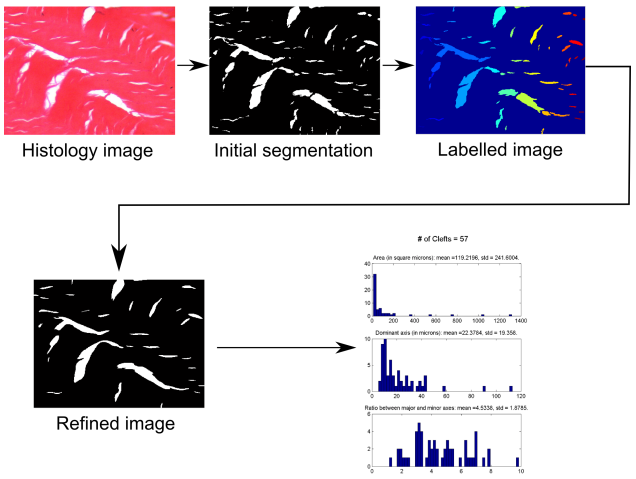

Fig. 1: High level view of the proposed system.

where $p(x, \theta)$ is the pdf corresponding to G_i:

$$p(x|i, \theta) = \frac{1}{(2\pi)^{d/2}|\sum_i|^{1/2}} \exp -\frac{1}{2}(x - \mu_i)^T (\sum_i)^{-1}(x - \mu_i) \tag{3}$$

with θ consisting of the elements of the mean vectors μ_i and the distinct elements of the covariance matrices $\sum_i$ for the g component densities, and ϕ:

$$\phi = (\pi^T, \theta^T)^T \tag{4}$$

is a vector of all unknown parameters belonging to some parameter space Ω, and is estimated with the expectation maximization (EM) algorithm.

The mixture density for the case of Gaussian components contain three adjustable components: π_i μ_i and $\sum_i$. The negative log-likelihood for the data set is given by:

$$E = -\ln L = \sum_{j=1}^{n} \sum_{i=1}^{g} \pi_i p(x_j|i) \tag{5}$$

which can be regarded as the error function [5]. Minimizing E is equivalent to maximizing the likelihood L.

The EM algorithm starts with an initial guess for the parameters of the Gaussian mixture model, these parameters are then re-evaluated using a set of equations that aim to lower the value of the error function. The change in error function can be expressed as:

$$\Delta^{t+1} = E^{t+1} - E^T = -\sum_{j}^{n} \ln \frac{p^{t+1}(x_j)}{p^t(x_j)} \tag{6}$$

where $p^{t+1}(x)$ is the evaluated pdf using the updated parametrs. The update equations for the parameters can be obtained by setting the derivatives of Δ^{t+1} to zero [5].

Once initial segmentation is complete, the input histology image will be split into two segments: possible clefts and non cleft regions. Post processing will now follow.

Post Processing

Given the segmented image, a connected components algorithm is used to extract and label the possible cleft regions. The basic steps in finding the connected components are:

1. Search for the next unlabeled pixel, p.

2. Use a flood-fill algorithm to label all the pixels in the connected component containing p.

3. Repeat steps 1 and 2 until all the pixels are labeled

Once all possible cleft regions are labeled, the image is refined by applying size and spatial constraints.

The size constraint is used to ensure small non-cleft artifacts are removed. The spatial constraint ensures that disconnected clefts that are part of the same cleft are reconnected (Figure 2). This is done through anisotropic diffusion. The coherence filtering scheme developed by Kroon [6] was used, which ensures diffusion is acting along the edges, preserving cleft shape. The result is the refined segmented image.

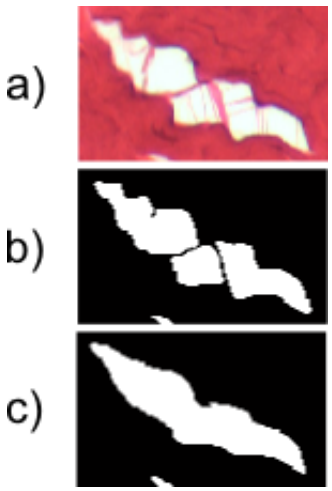

Fig. 2: Using coherence filter to enhance image. a) shows a difficult case for GMM segmentation, where it should be one continuous cleft, b) shows initial GMM segmentation, where teh cleft is divided into three regions. c) shows the result after using the coherence filter.

With the refined image containing the desired labelled clefts, statistical analysis is performed to extract information on the segmented clefts, including: area, dominant axis length, ratio between major and minor axis for all clefts, as well as the overall number of clefts. These statistics will help quantify microstructural damage to the annulus fibrosus.

Results

To evaluate the performance of our proposed system, the calculated area for three clefts from a segmented image (shown in Figure 3) from the proposed system were compared against ground truth area measurements made via manual segmentation. The results are summarized in Table 1.

Table 1: **Comparison between calculated area of proposed method vs. ground truth area measurments for the segmented areas of the three clefts from Figure 3. The areas shown are in μm^2.**

Cleft #	Proposed System	Ground Truth	error
1	1.1610	1.1521	7.70%
2	0.5363	0.5059	6.01%
3	0.0678	0.0648	4.63%

These results show that our proposed method is capable of producing numbers that are close to the ground truth values, with an average error of 6.11%. This low average error was found to be sufficient for for quantifying microstructural damage accumulation to the annulus fibrosus[3].

Discussion

In this research, we propose an automated histologcical analysis system for quantifying microstructural damage to the annulus fibrosus. This system takes in a digital histology image and segments it into possible cleft regions using GMM. Using connected components, the possible cleft regions are extracted and labeled. The labelled image is refined through spatial and size constraints as well as a coherence filter. The required statistics for quantifying the microstructural damages are then extracted.

Future work include a more comprehensive evaluation involving calculated statistics from experienced operators on different types of histology slices.

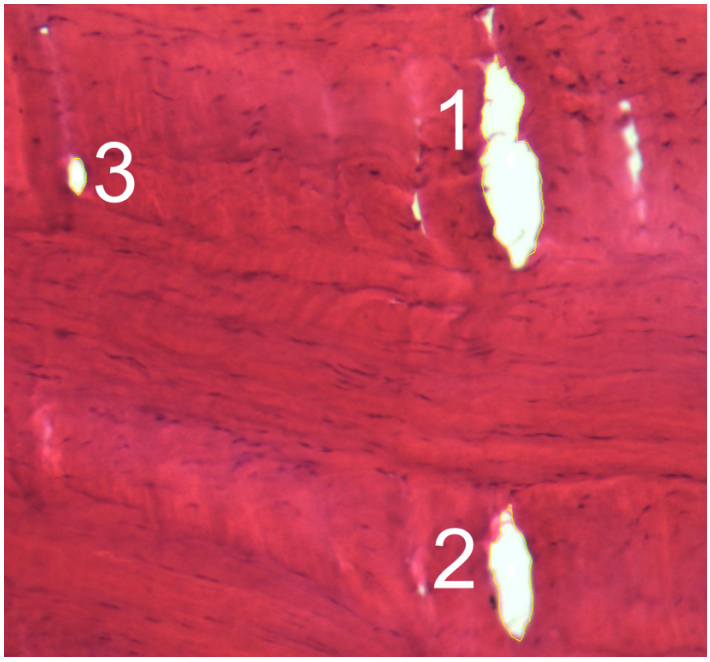

Fig. 3: The three clefts used for comparison

Acknowledgments

This research was funded by the Natural Sciences and Engineering Research Council of Canada, the Canada Research Chairs Program, and the Ontario Ministry of Research and Innovation.

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