Digital histology of gastric tissue biopsies with liquid crystal-based Mueller microscope and machine learning approach

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ABSTRACT

We investigated gastric tissue biopsies using a liquid crystal-based Mueller microscope and a machine-learning approach to examine the degree of inflammation. Machine learning and statistical analysis were performed with the multi-dimensional dataset including the polarimetric properties (linear retardance and dichroism, and circular depolarization) and total transmitted intensity images of the unstained thin sections of gastric tissue to identify and quantify the microstructural differences between healthy control, chronic gastritis, and gastric cancer.

Keywords: Mueller microscopy, Optical anisotropy, Statistical image analysis, Gastric cancer

1. INTRODUCTION

Chronic gastritis is a known precancerous condition of stomach that has the potential to evolve into gastric cancer [1] if left without the appropriate treatment. Therefore, precise detection of the degree of gastric inflammation based on quantitative analysis of microstructural heterogeneity for healthy and inflammatory tissue of gastric biopsies is essential during medical diagnosis. The gold standard diagnosis is based on the conventional tissue histology analysis of thin tissue sections of biopsies taken from a patient during the endoscopy test. However, it requires time-consuming sample preparation (tissue fixation, paraffin embedding, sectioning, and staining) and is operator-dependent. Therefore, quantitative metrics to examine unstained gastric tissue are remarkably important in examining the degree of gastric inflammation from the gastric tissue biopsies. In this study we used liquid crystal-based Mueller microscopy [2]–[4] combined with a machine learning approach to provide the quantitative metric of the degree of inflammation to support pathologists during medical diagnosis.

2. METHODS

2.1 Sample preparation

Gastric tissue biopsies were randomly taken from three cohorts of patients with different pathological conditions (healthy control, chronic gastritis, and gastric cancer) during the endoscopy test. The samples were prepared using the conventional histological analysis protocol: tissue fixation, paraffin embedding, sectioning, and dewaxing. Then unstained thin tissue sections were measured with a custom-built transmission Mueller microscope. As a cross-validation, the adjacent thin tissue sections were stained and annotated by a pathologist for the gold standard diagnostics.

2.2 Transmission Mueller microscope

The interaction between polarized light and sample changes the polarization state of light depending on the sample's optical properties. Based on these changes of polarization of probing light beam, we estimate the polarization properties of a sample and characterize its microstructure. For the polarimetric characterization of thin sections of gastric tissue biopsies we used a custom-built transmission Mueller microscope. This instrument makes use of the electrically driven ferroelectric

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liquid crystals (FLCs) for the polarization modulation and analysis. The microscope operates in a visible wavelength range. The beam passes through a polarization state generator (PSG) composed of linear polarizer, and fixed half wave plate sandwiched between two FLCs and shines on a sample. The polarization state of light transmitted by a sample is analyzed by polarization state analyzer (PSA), composed of the same optical components, but assembled in a reverse order. White LED (Stemmer Imaging, Germany) is used as a light source, light passes through a bandpass filter (spectral bandwidth of 20 nm, central wavelength 533 nm) before being modulated by PSG. A CCD camera (AV Stingray F-080B, Allied Vision, Germany, image resolution 600 × 800 pixels) is used as a detector. In order to measure 16 intensity images of a sample four different polarization states are produced by PSG and each is projected into 4 different polarization states of PSA after being transmitted through the tissue section. Based on these 16 raw intensity measurements, we obtain a 4 × 4 Mueller matrix using the eigenvalue calibration method [5].

2.3 Polarimetric data post-processing

Since physical interpretation of Mueller matrix elements is not always straightforward, we applied the logarithmic decomposition of Mueller matrices (LMMD) [6] for the extraction of polarimetric properties of gastric tissue biopsies. This decomposition is particularly suitable for the analysis of a few micrometers thick biological samples measured in transmission, because it does not assume the sequential appearance of polarimetric effects along the optical path of a probing beam. Both linear and circular retardance and dichroism, as well as linear and circular depolarization were extracted by using the LMMD method. Next we applied a polynomial regression model to the multi-dimensional dataset that contained total transmitted intensity, linear retardance, linear dichroism and circular depolarization images, in order to develop a quantitative metrics for the diagnosis of the degree of inflammation of gastric tissue. We calculated the regression model $\hat{\beta}$ from the feature space Z, which is consisted of multi-dimensional polarimetric images from healthy control gastric tissue group and target image $\vec{Y}$, which highlights inflammatory cells and gastric tissue gland walls within the polarimetric images of healthy control tissue. Feature space Z consisted of polynomial basis functions of the second degree. Both feature space Z and target image $\vec{Y}$ were created based on the vectorized and standardized dataset images. We defined the regression model by minimizing mean squared error (MSE) between target value y and predicted value $\hat{y}$ (see Eqs 1-3) [7]. The obtained regression model $\hat{\beta}$ was applied to the images of healthy control, chronic gastritis, and gastric cancer tissue biopsies to examine the degree of inflammation.

$$\hat{y} = Z\hat{\beta} \tag{1}$$

$$MSE(\hat{\beta}) = \frac{1}{N}\sum_{i=1}^{N}(y_i - \hat{y_i})^2 \tag{2}$$

$$\hat{\beta} = (Z^T Z)^{-1} Z^T \vec{Y} \tag{3}$$

3. RESULTS AND DISCUSSION

Using standard histology diagnosis as a ground truth, we observed the structural changes of gastric glands (appeared at healthy control and chronic gastritis tissue biopsies) and gastric epithelium infiltration by the inflammatory cells. Similarly, polarimetric images of linear retardance from healthy control and chronic gastritis tissue exhibit high contrast for the connective tissue of gastric gland walls that forms a 'honey-comb' structure, also visible in the transmitted intensity images. In contrast, such 'honey-comb' structures were not observed in the corresponding images of gastric cancer tissue, which reflects the re-arrangement of gland wall fibers by malignancy. Apart from the structural changes we observe several spots with relatively high linear retardance, dichroism and circular depolarization values that are not visible in the intensity images. This phenomenon most likely is related to the presence of intra-nuclear birefringent inclusions (IBI) due to the insufficient dewaxing [8]. Since these spots are located along the gastric gland walls, we expected them to represent the inflammatory cells [9]. Therefore, to obtain the quantitative metrics for monitoring the degree of inflammation, we focus our analysis on the presence of inflammatory cells and structural changes of gastric gland wall compared to the healthy control tissue using the multi-dimensional dataset of polarimetric images. To enhance the contrast of the gastric gland walls and inflammatory cells, we defined the target image with thresholding of transmitted intensity and linear retardance values (Label 1: transmitted intensity ≤ 0.65; Label 2: transmitted intensity > 0.65; Label 3: linear retardance > 0.1). Then, to extend and include the various polarimetric properties of inflammatory cells and gastric gland wall, we included transmitted intensity, linear retardance, linear dichroism, and circular depolarization to the feature space. We calculated a regression model based on the training set of healthy control tissue, and then this trained regression model was applied to the images of tissue from all three classes to show the enhanced contrast of inflammatory cells and gastric gland wall in the predicted images (see Fig.1). For the predicted images for healthy control, chronic gastritis, and gastric cancer tissues

we performed two-Gaussian fit of the distributions of the predicted values obtained from the regression model, in order to quantify the degree of gastric tissue inflammation. First Gaussian peak did not show a significant variation with the degree of inflammation. On the contrary, the second Gaussian peak shows a strong variation with the pathology evolution. The predicted value of peak position and its full width at half maximum (FWHM) stay constant. However, we observed that the height of second peak increases drastically: healthy control - 80; chronic gastritis - 150; gastric cancer - 200. Based on these results, we suggest to use this parameter as a quantitative metrics for the precise and accurate diagnosis of gastric inflammation state [10].

4. CONCLUSIONS

Our studies propose a quantitative approach to grading gastric inflammation state based on multi-dimensional polarimetric dataset, machine learning and statistical analysis. Measuring unstained tissue with a custom-built transmission Mueller microscope will accelerate preparation of tissue sections compared to the conventional histology protocol. Further studies are needed to test the suggested metrics under blind conditions with respect to the pathological status of studied biopsies.

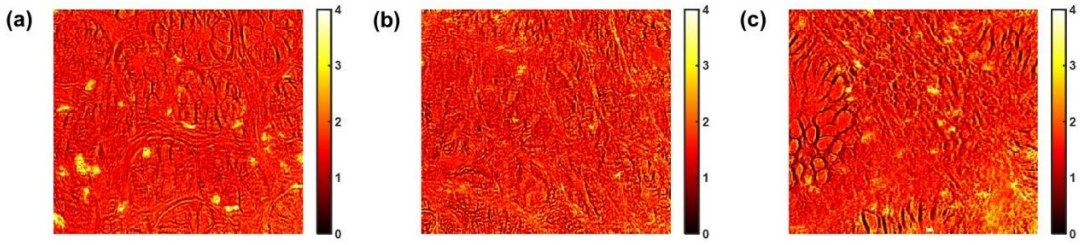

Figure 1. (a-c) Predicted images obtained from trained regression model on three different types of gastric tissues (healthy control, chronic gastritis, and gastric cancer). Adapted from [10].

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REFERENCES

[1] R. M. Genta and M. Rugge, “Assessing risks for gastric cancer: New tools for pathologists,” *World J. Gastroenterol.*, vol. 12, no. 35, pp. 5622–5627, 2006, doi: 10.3748/wjg.v12.i35.5622.

[2] P. Li *et al.*, “Analysis of tissue microstructure with Mueller microscopy: logarithmic decomposition and Monte Carlo modeling,” *J. Biomed. Opt.*, vol. 25, no. 01, p. 1, 2020, doi: 10.1117/1.jbo.25.1.015002.

[3] H. R. Lee *et al.*, “Digital histology with Mueller microscopy: how to mitigate an impact of tissue cut thickness fluctuations,” *J. Biomed. Opt.*, vol. 24, no. 07, p. 1, 2019, doi: 10.1117/1.jbo.24.7.076004.

[4] H. R. Lee, I. Saytashev, V. N. Du Le, M. Mahendroo, J. Ramella-Roman, and T. Novikova, “Mueller matrix imaging for collagen scoring in mice model of pregnancy,” *Sci. Rep.*, vol. 11, no. 1, pp. 1–12, 2021.

[5] E. Compain, S. Poirier, and B. Drevillon, “General and self-consistent method for the calibration of polarization modulators, polarimeters, and Mueller-matrix ellipsometers,” 1999.

[6] O. R. Gil-Perez J.J., *Polarized Light and the Mueller Matrix Approach*. CRC Press, 2016.

[7] F. Akdeniz and H. Erol, “Mean Squared Error Matrix Comparisons of Some Biased Estimators in Linear Regression,” *Commun. Stat. - Theory Methods*, vol. 32, no. 12, pp. 2389–2413, 2003, doi: 10.1081/STA-120025385.

[8] A. Vitkunaite *et al.*, “Intranuclear birefringent inclusions in paraffin sections by polychromatic polarization microscopy,” *Sci. Rep.*, vol. 11, no. 1, pp. 1–7, 2021, doi: 10.1038/s41598-021-85667-8.

[9] G. Pennelli *et al.*, “Gastritis: Update on etiological features and histological practical approach,” *Pathologica*, vol. 112, no. 3, pp. 153–165, 2020, doi: 10.32074/1591-951X-163.

[10] M. Kim, H. R. Lee, R. Ossikovski, A. Malfait-Jobart, D. Lamarque, T. Novikova, “Optical diagnosis of gastric tissue biopsies with Mueller microscopy and statistical analysis”, J. Europ. Opt. Soc. Rapid Publ., vol 18, no. 2, pp. 10, 2022