DOI 10.5162/EUROSENSORSXXXVI/PT10.227

Multifunctional polarizing microscope system- Classification silicosis tissue and collagen film

Chia-Ling Chiang[1], Lukas Jyuhn Hsiarn Lee[2,3], Chii-Wann Lin[4]

[1] Graduate Institute of Biomedical Electronics and Bioinformatics, National Taiwan University, Taipei 106319, Taiwan, R.O.C.

[2] National Institute of Environmental Health Sciences, National Health Research Institutes, Miaoli 350, Taiwan, R.O.C.

[3] Institute of Environmental and Occupational Health Sciences, National Taiwan University, Taipei 106319, Taiwan, R.O.C.

[4] Department of Biomedical Engineering, National Taiwan University, Taipei 106319, Taiwan, R.O.C.

cwlinx@ntu.edu.tw

Summary:

This study proposes a multifunctional microscope system composed of linear polarizers and CMOS polarization camera featuring different polarization directions especially. After that, we applied pathological tissue samples from patients with silicosis and collagen film as an example. According to preliminary results, it can clearly observe the arrangement of connective tissue in both tissue and crystalline particles in lymph node tissue with silicosis. Afterwards, outcomes are not only analyzed also classified by Image J and Python. The purpose is to assort different tissue in order to enhance diagnostic accuracy as a quantitative data method.

Keywords: Polarizer, CMOS, Silicosis, Lymph node, Collagen film

Background

Workers exposed to large amounts of silica particles in occupational environments for a long time are at a higher risk of developing silicosis. The main reason is that the presence of silanol (SiOH) groups on the surface of silica particles which can form hydrogen bonds with oxygen and nitrogen groups in biological cell membranes. This interaction may lead to the loss of membrane structure, lysosomal leakage, and tissue damage, resulting in inflammation of respiratory system tissues, pulmonary fibrosis, and sclerosis. In severe cases, it can lead to a decline in respiratory function, making it an irreversible lung disease and even death [1, 2]. However, despite strict regulations and management efforts, it still has 1.7 million workers approximately that is exposed to silicosis annually in the United States and other worldwide [3]. Moreover, although polarizing microscopes can display birefringent crystalline silica particles, sometimes the birefringent crystals observed within nodules are the result of inhaled silicate particles mixed with silica dust [2].

In this study, the multifunctional microscope system we proposed are not only installation of polarizers but also utilization CMOS polarizing cameras with different polarization directions on the fluorescence microscope. In addition to polarization function, each unit consists of four pixels composed of linear polarization in different four directions: 0°, 45°, 90°, and 135° for the camera in this system. Compared to traditional polarizing microscopes, the multifunctional microscope can measure physical properties which conventional imaging can not detect, such as classifying chemical isomers, analyzing stress and strain, and even enhancing contrast [4, 5]. Therefore, through measurements provided by this system, it is expected that silicosis tissue images captured at different polarization angles can distinguish from other tissues by different crystal structures and structure arrangement, aiming to quantify data and improve future diagnostic capabilities.

Material and Method

Polarized light microscope system

The original fluorescence microscope system (Olympus BX51) was modified by installing two linear polarizers, serving as the polarizer and analyzer, respectively. Due to the light source being a halogen lamp (350 to 1100 nm), polarizers with higher transmittance in the visible light spectrum were selected as the installation components. In the system configuration, it includes the following components in sequence: light source, polarizer, condenser, specimen,

objective lens, analyzer, and eyepieces. In order to observe crystal variations, a 360-degree rotating mount was installed on the polarizer to change the polarization direction additionally. Furthermore, the specimen was connected to a touch screen keypad to control movement more precisely compared to manual adjustment. It allows for more accurate positioning of samples within the field of view. Subsequently, CMOS cameras and traditional EMCCD cameras were installed above the microscope separately. Finally, captured images were processed and analyzed by Image J and python.

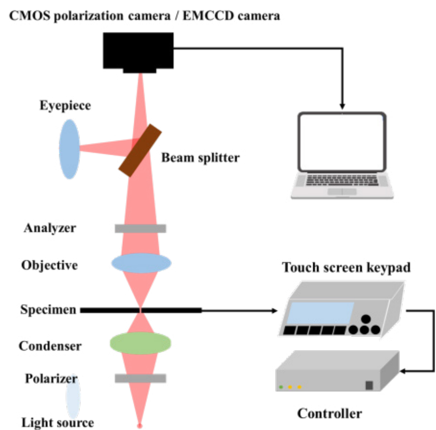

Fig. 1. Construction of polarized light microscope system.

Sample preparation

Because of oil attached on unused slides, it's difficult for the tissue samples to adhere on the surface and also to affect tissue observation. Therefore, we are able to washed it away on the surface of the glass slide with acetone first. After then, through isopropyl alcohol, it can remove the oil and the acetone remaining on the surface. Lastly, utilizing DI water is to rinse it for final cleaning. During the process, an ultrasonic vibrator is used to vibrate for 6 minutes. When replacing to the next solution, it will be rinsed with a large amount of DI water first to wash away the remaining solution on the surface of slides and then blow dry with nitrogen. After cleaning is completed, we flatted the tissue sample with DI water and covered it with a coverslip to complete the preparation.

Preliminary Results

After preparing the lymph node tissue sample from a patient with silicosis, it was placed under a polarized light microscope system for observation, as shown in the Fig. 2. Under orthogonal polarization, the arrangement in different direction of connective tissue and structure in both tissue as well as crystalline particles in Lymph node can be clearly observed through the eyepiece. Subsequently, EMCCD cameras were used to capture a series of 15 images with an exposure time of 0.15 seconds per each at 100 times magnification. Before images come out, it's significant to remove background to improve the signal-to-noise ratio for analysis of the crystalline particles. From the results depicted in the Fig 3., after setting the scale with Image J, the crystalline particles were distinguished from the background through thresholding methods. Furthermore, there are the total of 16 particles was counted as well as an average area of 9.093 nm^2 were calculated within the specified area.

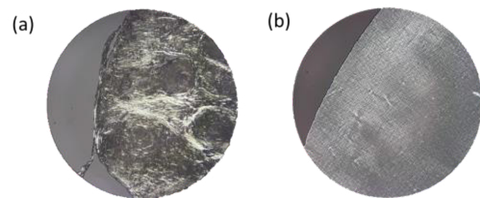

Fig. 2. The orthogonal polarized light image in a microscope: (a) Lymph node tissue (X100) (b) Collagen film (X100).

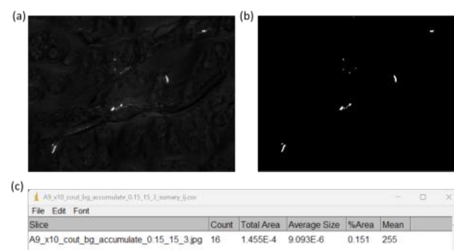

Fig. 3. The images are processed and analyzed by image J: (a) Before processing (b) After processing (C) Particle count and area summation.

References

[1] C. R. Thomas and T. R. Kelley, "A brief review of silicosis in the United States," Environmental health insights, vol. 4, p. EHI. S4628, 2010.

[2] M. I. Greenberg, J. Waksman, and J. Curtis, "Silicosis: a review," Disease-a-Month, vol. 53, no. 8, pp. 394-416, 2007.

[3] X. Liu, Q. Jiang, P. Wu, L. Han, and P. Zhou, "Global incidence, prevalence and disease burden of silicosis: 30 years' overview and forecasted trends," BMC Public Health, vol. 23, no. 1, p. 1366, 2023.

[4] X.-F. He and T. Dalsa, "Polarization-based imaging: Basics and benefits," Photon. Spectra, 2016.

[5] A. G. Andreou and Z. K. Kalayjian, "Polarization imaging: principles and integrated polarimeters," IEEE Sensors journal, vol. 2, no. 6, pp. 566-576, 2002.

[6] R. Oldenbourg, "Polarized light microscopy: principles and practice," Cold Spring Harbor Protocols, vol. 2013, no. 11, p. pdb. top078600, 2013