CAPABILITY OF LOW VOLTAGE SCANNING ELECTRON MICROSCOPE TO DIFFERENTIATE CERVICAL CELL CLASSES

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Abstract- Field Emission Scanning Electron Microscopy (FE-SEM) has become a popular imaging tool to elucidate the structure of a material at the micro-and/or the nano-scale level. Due to the great capability of the FE-SEM in characterizing the biological material, this study has investigated the capability of the FE-SEM to differentiate the cervical cell to be three classes. The classes are normal, low-grade squamous intraepithelial lesion (LSIL), and carcinoma. The cervical cells are scanned using FE-SEM including Energy Dispersive X-ray (EDX) and line scanning process. The results of the scanning are presented in electron images as qualitative results, and containing elements as quantitative results in this paper. Based on the quantitative results, FE-SEM has shown a great capability to differentiate the cervical cell classes.

Keywords- Biomaterial, Cervical Cell, Classification, Electron Image, Line Scanning

I. INTRODUCTION

Cervical cancer is a disease that attacks and causes mortality among women after breast cancer worldwide. In statistics, the disease suffered by those who are in developing countries. In developed countries, the number of patients with this disease statistically shows good progress. Besides awareness for screening, the experts and the technology used are better in the developed countries.

Pap smear and liquid based Pap cytology (LBC) are screening method that has been applied and gives good progress in the world of pathology. Through the screening methods, the potential cervix cells will be detected based on the morphology of the cells, so that the disease can be prevented in advance to get worse. The potential cervix cells take over a period of two to three decades to develop into malignant cells, providing sufficient time for the screening for precursors (Cronjé, 2005). Therefore, this screening method is important and obviously needs to be upgraded.

Recently, potential of FE-SEM is hotly discussed in science and engineering fields (Pawley, 1997), (Dalgleish, Spagnuolo, & Douglas Goff, 2004), (Felisari et al., 2011), (Moran & Coats, 2012). It is being able to elucidate the structure of a material at the micro-and/or the nano-scale level. It is indeed crucial to characterizing the material, understanding its mechanism and mode of formation, and explaining/predicting its properties and performance under a given set of environmental or load conditions. Secondary electron imaging from FE-SEM is commonly used to reveal surface topography, grain morphology and size, phase composition, and fracture profile. In such cases highest resolution and contrast are needed to illustrate the finest structural features. Imaging of organic and biological materials is strongly facilitated and represents a real challenge to investigators (Dalgleish et al., 2004), (Moran & Coats, 2012). Nowadays, there are limited published papers of FE-SEM for the cervical cells. Especially, paper presented cervical cell FE-SEM images for classifications. Therefore, based on the good capability of FE-SEM technique, the capability of FE-SEM to differentiate the cervical cells is also investigated in this study.

II. MATERIALS AND METHODS

A. Cervical cell sample

In this preliminary study, cervical cell samples in Surepath (one of the LBC methods) are obtained from Gribbles pathology laboratory, Petaling Jaya, Malaysia. These cervix cells are the routine screening on February 2014. Three samples are used to investigate the capability of FE-SEM in cervical cell screening. The samples are normal, LSIL, and carcinoma cases.

B. Sample preparation

Sample preparation for FE-SEM occupies an important role. The clarity of the resulted image on the view in FE-SEM is the role of sample preparation. For this preliminary study, the manual technique is used for sample preparation. Cervical cells are taken slightly and smeared into the microscope slides then dried using a hair dryer to make sure the sample does not contain water. After the cell is dried, the cell is inserted into FE-SEM equipment for scanning process.

C. FE-SEM Scanning

FE-SEM imaging captures specimens in their natural hydrated state. As stated in previous subsection, the manual technique is used for this paper. FE-SEM is performed on an INCA brand of FE-SEM with low voltage vacuum method. In this work, there are three

types of data acquisitions done. First, FE-SEM imaging captures the slide to show the cell presentation image. Second, EDX technique scans the slide generally to check the chemical compound of the cell. Third, the line scanning technique is also run to obtain detail composition of the chemical compound in the cell.

III. RESULTS AND DISCUSSIONS

There are qualitative and quantitative results for this investigation. For qualitative results, the electron images of cervical cells are presented in Figure 1. Figure 1(a-f) are normal, LSIL, and carcinoma images for 4000 and 2000 of magnifications, respectively. The quantitative results are performed using line scanning and EDX methods. The results of line scanning and EDX are presented in Figure 2 and Figure 3, respectively.

A. Qualitative

As morphology, carcinoma, normal, and LSIL samples are presented different in clear images. However, LSIL image is presented not clear due to water from sample suspension in LSIL slide is still exist. Sample preparation is a critical step in scanning electron microscopy imaging. This is especially true for biological samples because of charge build-up and sensitivity to vacuum and electron beam damage. In terms of ultrastructure imaging, a variety of advancements in detectors and approaches have improved biological imaging such that fewer steps are required for sample preparation.

As presented in Figure 1(a) and (b), the normal cells images are shown clear and no contained water in them. The cells are look like oval ball (in the right top of images). The normal cells are healthy. In the LSIL cells images as presented in Figure 1(c) and (d) are not shown clear. It is due to far distance and these images still contain much water. So these images are not preventative images. The images are not representative due to the cells are not viewed clearly. Then, Figure 1(e) and (f) are the carcinoma cells images. Based on the images, the cells are presented in far distances. However, the quality of images are good, no contained water. The images show many tissues of the carcinoma cervical cells.

B. Quantitative

Figure 2 and Figure 3 show quantitative results in term of line scanning and EDX results. Line scanning presents the chemical compound in a sample in certain position. In figure 3, the chemical compositions of the cells are presented clearly. Each component is presented and calculated in precisely percentage.

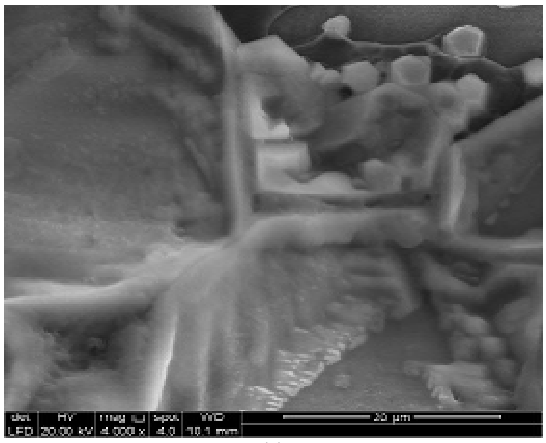
1) Line Scanning results

Based on Figure 2, the graph of chemical compounds of the cells is presented different among the cells. In the LSIL cell, the chlorine component is higher than silicon component but they still exist in high composition. In contrast, the silicon component is high when the chlorine is low composition for the normal cell, and the chlorine component is high when silicon is low composition for carcinoma.

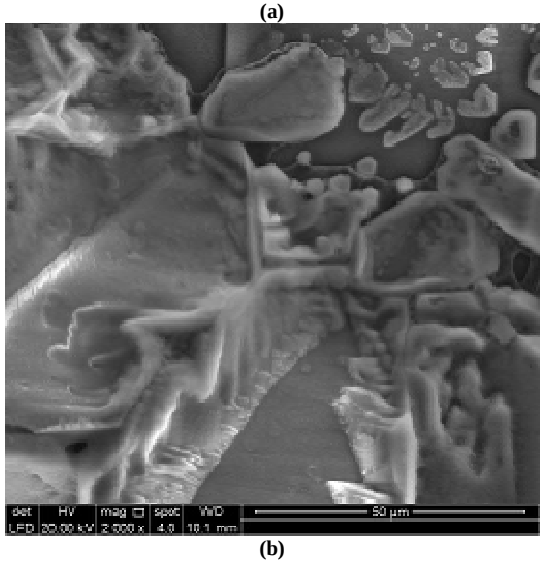
2) EDX results

Based on Figure 3, there are five components to be presented significantly different for each cases (i.e. normal, LSIL, carcinoma). The components are carbon, oxygen, natrium, silicon, and chlorine. The oxygen in cells is highest among other components and the oxygen in the normal cell is higher than in LSIL and carcinoma cells. Silicon component contained in normal cell is higher than silicon in other cells. However, carbon and chlorine component contained in normal cell is lower than carbon and chlorine in the other cells.

Based on the results, the investigation of FE-SEM for cervical cell screening can be developed. The different characteristics for cervical cells (i.e. normal, LSIL, and carcinoma) can be detected significantly. The capability of the FE-SEM for developed intelligent screening system for cervical cell precancerous is investigated in this study.



(a)



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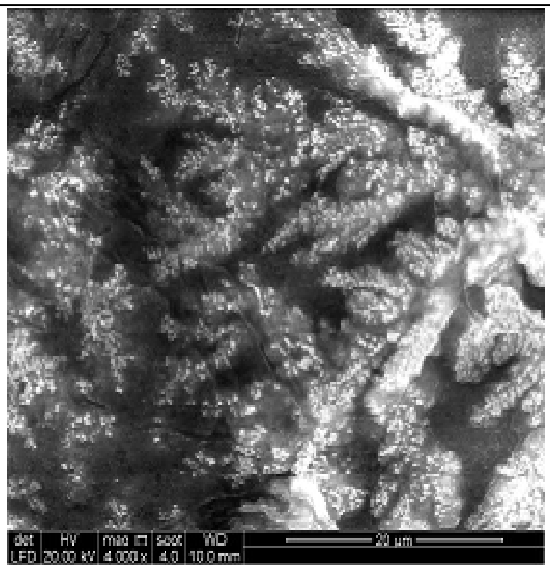

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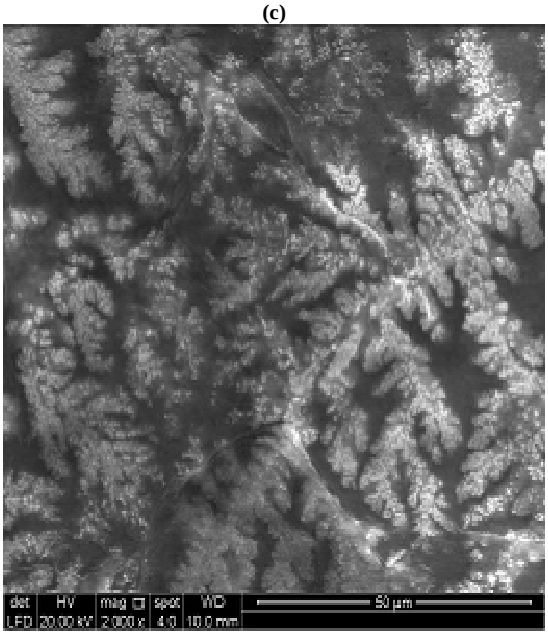

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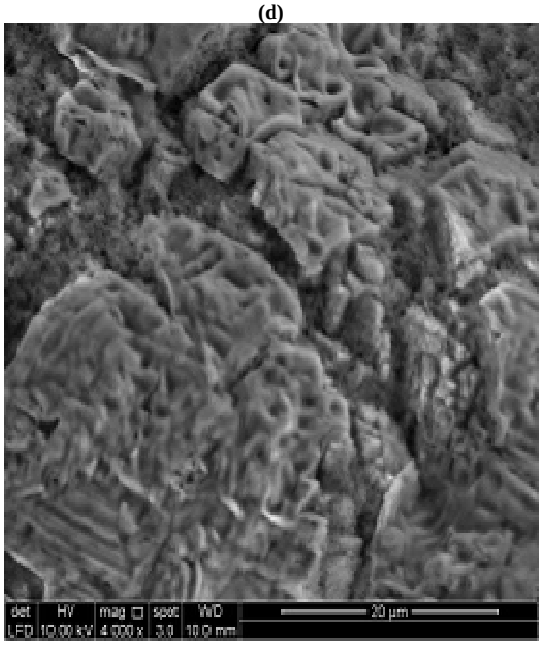

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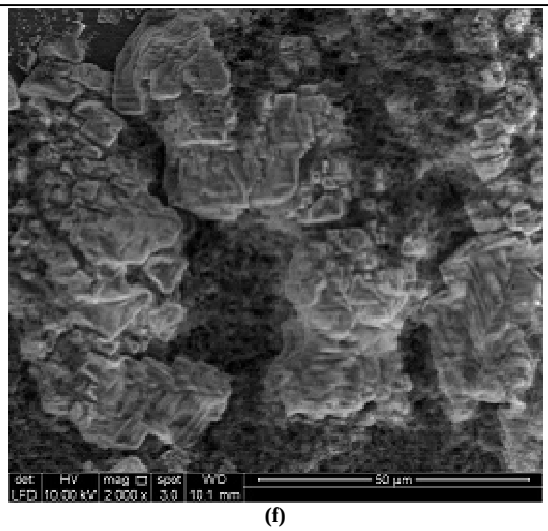
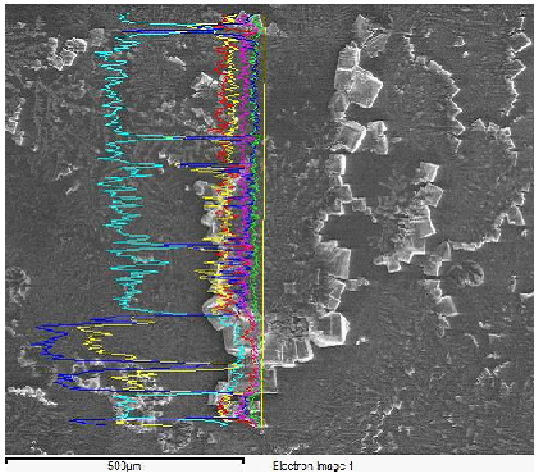


(f)

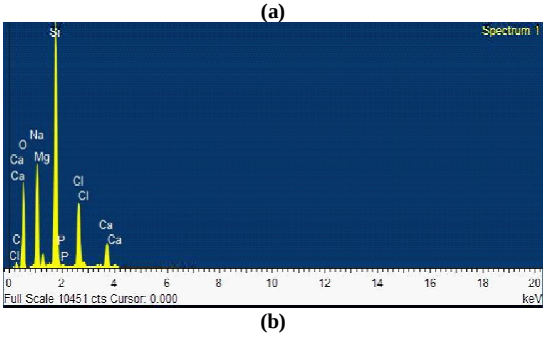
Figure 1. Cervical Cell images, (a), (b) Normal cells 4000 and 2000 magnification; (c), (d) LSIL cells 4000and 2000 magnification; (e), (f) Carcinoma cells with 4000 and 2000 magnification.

CONCLUSION

FE-SEM presented good results as potential tool to differentiate cervical cells to be three classes in this study. The quantitative results present good results for chemical component existing in the cells. The composition of the chemical component shows the significant classification of the cervical cells. Due to the good preliminary results of this study, FE-SEM is used for acquisition technique in the future study for classification system for cervical precancerous with new sample preparation technique.



(a)



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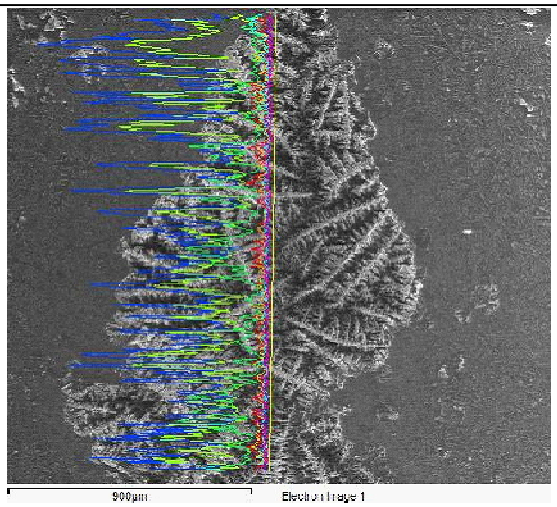

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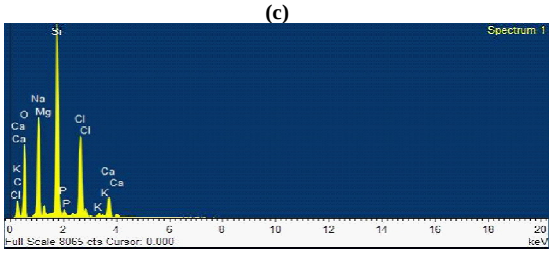

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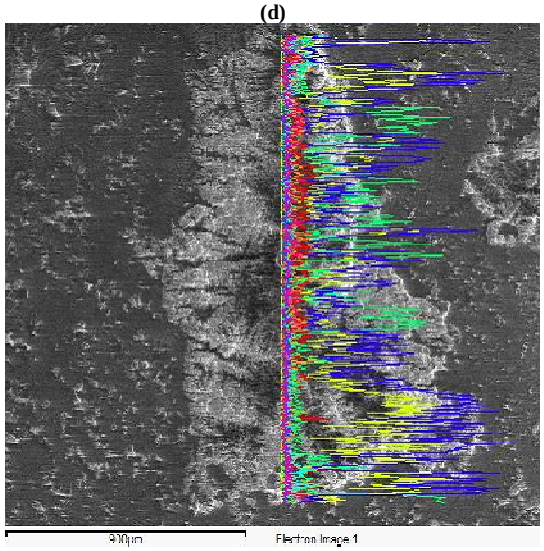

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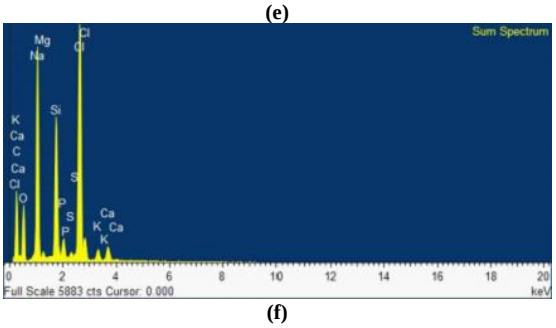
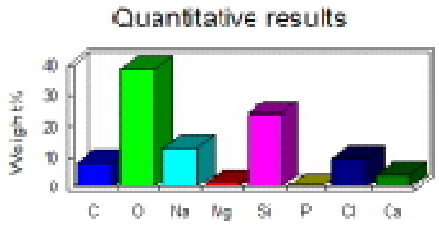
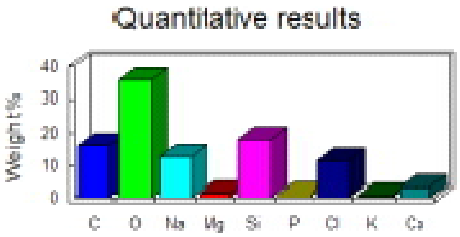


(f)

Figure 2. Line scanning results, (a) Position of line scanning in Normal FE-SEM image; (b) Sum of the spectra elements; (c) Position of line scanning in LSIL FE-SEM image; (d) Sum of the spectra elements; (e) Position of line scanning in Carcinoma FE-SEM image; (f) Sum of the spectra elements.



(a)



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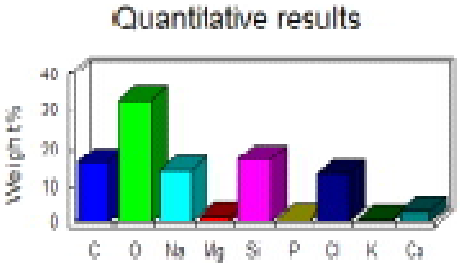

(c)

Figure 3. EDX results, (a) Normal; (b) LSIL; (c) Carcinoma.

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