Computer-aided screening system for cervical precancerous cells based on field emission scanning electron microscopy and energy dispersive x-ray images and spectra

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Abstract. The capability of field emission scanning electron microscopy and energy dispersive x-ray spectroscopy (FE-SEM/EDX) to scan material structures at the microlevel and characterize the material with its elemental properties has inspired this research, which has developed an FE-SEM/EDX-based cervical cancer screening system. The developed computer-aided screening system consisted of two parts, which were the automatic features of extraction and classification. For the automatic features extraction algorithm, the image and spectra of cervical cells features extraction algorithm for extracting the discriminant features of FE-SEM/EDX data was introduced. The system automatically extracted two types of features based on FE-SEM/EDX images and FE-SEM/EDX spectra. Textural features were extracted from the FE-SEM/EDX image using a gray level co-occurrence matrix technique, while the FE-SEM/EDX spectra features were calculated based on peak heights and corrected area under the peaks using an algorithm. A discriminant analysis technique was employed to predict the cervical precancerous stage into three classes: normal, low-grade intraepithelial squamous lesion (LSIL), and high-grade intraepithelial squamous lesion (HSIL). The capability of the developed screening system was tested using 700 FE-SEM/EDX spectra (300 normal, 200 LSIL, and 200 HSIL cases). The accuracy, sensitivity, and specificity performances were 98.2%, 99.0%, and 98.0%, respectively. © 2016 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.OE.55.10.103110]

Keywords: cells; imaging technique; signal processing; feature extraction; computer vision; intelligent screening.

1 Introduction

Cervical cancer is the third most commonly diagnosed cancer and the fourth leading cause of cancer death in females worldwide, which contributed 12% (528,000) of the total new cancer cases and 7.5% (266,000) of the total cancer deaths among females in 2012.1 Cervical cancer develops over a period of two to three decades, providing sufficient time for a precursor screening.2 Therefore, early detection and routine screening can reduce the incidence and mortality rates related to this disease. A multidisciplinary guideline panel and systematic reviews of randomized controlled trials and observational studies were conducted to minimize the incident and mortality rates.3 Several recommendations have been introduced for screen-and-treat strategies to prevent cervical cancer, including human papillomavirus (HPV) testing, cytology testing, and visual inspection with acetic acid.4

Computer-aided screening techniques have been developed for this purpose to help cytotechnologists and pathologists in cervical cancer diagnosis.5 Due to the recent advancement of imaging technology, much progress has been made in computer-aided screening systems based on ThinPrep,6 colposcopy,7 cervicography,8 fluorescent in situ hybridization (FISH),9 and Fourier transform infrared,10 which can reduce human error and processing time.

Computer-aided cervical cancer screening systems based on cellular level data have achieved better results compared to cervicography and colposcopy, which are based on extracted tissues.11 Table 1 shows the comparisons among data acquisition techniques of cervical cancer computer-aided screening systems. The ThinPrep and FISH techniques only provide qualitative images to show the nucleus and cytoplasm of the cells. Both techniques require expert input to diagnose the abnormality of the cells based on the morphological characteristics such as the ratio of cytoplasm to the nuclear size and the concentrations of cell nuclei in the images. Although the characteristics can be computerized and developed into an automated system, there are limitations, such as the need for complicated algorithms due to the poor images and complications in classification.12,13 The developed computerized systems have produced significant results; however, accuracies of the systems have yet to be improved.14

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Meanwhile, the spectroscopy techniques provide more objective results and are independently operated without expert input. The techniques measure the chemical compounds in the sample specimen. Although they have achieved good accuracy with simple algorithms, their classification processes are based on the evaluation of the tissues rather than individual cervical cells. Evaluation on each cervical cancer cell is essential for cancer screening and important to determine the state of the cancer.

FE-SEM/EDX is an electron microscopy technique that produces high-resolution images; it captures and scans the structural surface of a material at the micro- and/or nanoscale level for organic (such as polymers, enzymes, cells, and membranes) or inorganic (such as ceramics, pigments, minerals, and composite materials) materials. It can be used as an analytical technique to investigate molecular surface structures for material characterization, understanding its mechanism and mode of formation, as well as explaining/predicting its properties. Recently, some researchers have applied the FE-SEM/EDX to fungal cells, human carotid plaques, yeast cells, stem cells, and human brain tumors.

FE-SEM/EDX produces both qualitative and quantitative data concurrently. Higher resolution images and elemental distributions of the prospective cells in the images are used for classification purposes. These representative data are believed to provide more accurate results with minimum computational expenses and rapid processing time, as specific parts in the images can provide objective results (elemental distribution) without an expert consideration. Therefore, cervical precancerous cell features can be extracted using FE-SEM/EDX to observe the morphology and elemental composition features.

The morphological features from images are presented by intensities, topology structures, colors, and texture as presented in Ref. 24. The texture of the image is one of the significant features to be analyzed in image processing for various applications including automated inspection. Currently, a gray level co-occurrence matrix (GLCM) technique has been applied to characterize the most malignant tumors, script identification, facial expression recognition, mammography, brain morphology, and so on. Findings indicated that feature extraction based on the GLCM techniques have achieved better classification results than other methods. Therefore, based on the capability of the FE-SEM/EDX as a data acquisition and image processing technique, this study proposed an automatic system for cervical precancerous screening based on FE-SEM/EDX and GLCM.

## 2 Materials and Methods

The medical ethics committee of the University of Malaya Medical Centre has approved the collection of the liquid-based cytology specimens (i.e., ThinPrep vial) from the pathology department. These specimens underwent cervical screening for cervical precancerous cells and were diagnosed by the pathologist. In this research, the collected samples were cervical cells in the vials from which the rest of the samples were used for screening by the experts. For the next process, these samples were sent to various laboratories at the University of Malaya, Kuala Lumpur, for sample preparation and capture of images using FE-SEM/EDX.

### 2.1 Sample Preparation

In the first step of sample preparation, the specimens were fixed in a McDowell-Trump fixative prepared in 0.1-M phosphate buffer or cacodylate buffer (pH 7.2) at 4°C for 2 h and then washed twice with 0.1% in phosphate-buffered saline for 10 min each time. For the dehydration process, a series of ethanol dilution dehydrations was implemented at 50%, 75%, and twice at 95% for 15 min each time, and three times at 100% with an equilibration step of 20 min each time. In the drying process, the dehydrated specimens were immersed in 1 to 2 mL of HMDS for 10 min, and the HMDS was then decanted into the specimen vials, put into the desiccator, and left to air dry at room temperature. The next process was to mount the dried specimens on circular stainless steel molds, coated with 10 nm of pure gold in a vacuum sputter coater and kept in a desiccator or under vacuum at all times before they were viewed under FE-SEM/EDX.

### 2.2 Field Emission Scanning Electron Microscopy and Energy Dispersive X-Ray Spectroscopy System

This research used FE-SEM with field emission gun 250 SEM. The capturing of the FE-SEM image was implemented at 10 mm working distance and operated at low voltage (10 kV). Elevation, tilt, and azimuth degrees in the data

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### Table 1: Characteristics of data acquisition techniques of computer-aided screening system for cervical precancerous cells.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Thin prep</th>
<th>FISH</th>
<th>Optical spectroscopy</th>
<th>FE-SEM/EDX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Property</td>
<td>Light microscopy</td>
<td>Fluorences microscopy</td>
<td>Spectroscopy</td>
<td>Electron microscopy and spectroscopy</td>
</tr>
<tr>
<td>Resolution</td>
<td>Medium</td>
<td>High</td>
<td>—</td>
<td>Very high</td>
</tr>
<tr>
<td>Data</td>
<td>Qualitative image</td>
<td>Qualitative image</td>
<td>Quantitative spectrum</td>
<td>Qualitative image and quantitative spectrum</td>
</tr>
<tr>
<td>View cell ability</td>
<td>Cell (nucleus and cytoplasm)</td>
<td>Cell (nucleus and cytoplasm)</td>
<td>—</td>
<td>Cell (surface)</td>
</tr>
<tr>
<td>Spectral absorption types</td>
<td>—</td>
<td>—</td>
<td>Sample chemical compounds</td>
<td>Cell elemental distributions</td>
</tr>
<tr>
<td>More fields</td>
<td>Limited</td>
<td>Limited</td>
<td>Not limited</td>
<td>Not limited</td>
</tr>
</tbody>
</table>

Optical Engineering 103110-2 October 2016 • Vol. 55(10)
acquisition were set up at 35 deg, 0.01 deg to 0.5 deg, and 0 deg, respectively. Both inlens and Everhart–Thornley detectors were used to create the images. The FE-SEM/EDX imaging system for data acquisition was presented in detail in our previous paper. A total of 82 ThinPrep vials were used for the samples in this study. Based on the samples, 150 FE-SEM images were captured from the samples and the elemental distributions of certain areas in the images were measured by using the FE-SEM/EDX technique. There were a total of up to 20 cervical cells in each captured FE-SEM image. Thus, the elemental distributions were measured in up to 20 scanned areas.

3 Proposed Automatic Screening System

An automated screening system for cervical cancer was proposed. The proposed system was developed to improve our previous work. A flow chart of the proposed system is shown in Fig. 1. The data input for the system had two types of data: FE-SEM/EDX images and FE-SEM/EDX spectra. The system differentiated three classes of the cervical cells to be normal, low-grade intraepithelial squamous lesion (LSIL), and high-grade intraepithelial squamous lesion (HSIL). In this section, images and spectra processing algorithms for feature extraction were applied on the input FE-SEM/EDX data. Details of the automated feature extraction technique proposed are presented below.

3.1 Features Extraction Based Field Emission Scanning Electron Microscopy Images

For the image processing technique, a magnification of 5000 for the FE-SEM/EDX images of the cervical cells was used as the region of interest (ROI) for feature extraction purposes. A large ROI that did not interfere with the image scale was needed to observe the texture and pattern at specific intervals and directions. Cervical cancer is caused by HPV infection. In the used ROI for the feature extraction, the existence of the virus in the abnormal cells (LSIL and HSIL) images can be viewed as referred by the arrow head in Figs. 2(b) and 2(c). Figure 2 shows the textural differences among three cervical cells images of the normal, LSIL, and HSIL classes. The textural differences were presented due to the existence of the virus in abnormal (LSIL and HSIL) cervical cells.

However, the captured images mostly had nonuniform illumination. Basically, in order to obtain uniform illumination, enhancement was needed to improve the visibility of the features for better extraction and classification. Therefore, based on the image condition, an image processing algorithm was needed to improve and clarify the textural features, which will be extracted from the enhanced images.

In this study, two preprocessing steps were applied to the original FE-SEM/EDX images. In the first step, contrast stretching was applied to the original images for growth intensity distribution to enhance its quality. The detail on contrast stretching is widely available in image processing textbooks. Next, a morphological opening operation was applied to the enhanced contrast image as explained previously. The goal of the morphological opening operation in this preprocessing step was to increase the detectability of the cell features and decrease undesired features within the image.

Then, the textural features were extracted based on the resulting morphological opening images. In this study, we used a statistical approach, which extracted the second-order statistical information based on the GLCM to compute the properties of the texture. As presented in Ref. GLCM has four parameters for consideration: region size, quantization levels, displacement, and orientation value. The current technique for feature calculation is based on the rotation variant, which is calculated for each angle and then averaged across all angles.

The properties of GLCM parameter used in this study were a 924 × 540 region size, nine quantization levels,
$d = 1$ and $2$ pixels of displacement and $\theta = 0$ deg, $45$ deg, $90$ deg, $135$ deg orientation. The region size was an optimal view of the textural contexts in the image to be differentiated among the cervical cell classes as presented in Fig. 2. Several quantization levels (i.e., $5, 9, 15,$ and $32$ quantization levels) were tested to obtain the optimum quantization levels for the images, since the gray-level condition in our images (after preprocessing) were minimally scattered as presented in the results section. Furthermore, a large displacement value will result in the inability of the co-occurrence matrix to capture the textural information in the image. Thus, displacements of $1$ and $2$ ($d = 1$ and $2$) pixels were selected to ensure that the textural and pattern information can be extracted for classification purposes.

For the FE-SEM images of cervical cells, there was no systematic pattern based on orientation. The cell images and the existence of the virus in the abnormal cervical cell images rotated and positioned themselves in all possible orientations. Therefore, we used $\theta = 0$ deg, $45$ deg, $90$ deg, and $135$ deg angles to efficiently cover all directions, as shown in Sec. 3.2.

The joint probability of two pixels with specific intensity levels displaced a distance $d$ and calculated along a specified direction, $h$. The spatial dependence matrix was defined as

$$P_{d,h}(i,j) = \sum_{x=1}^{N-d} \sum_{y=1}^{M-d} \left\{ \begin{array}{ll} 1, & \text{if } I(x,y) = i \text{ and } I(x + dx, y + dy) = j \\ 0, & \text{otherwise} \end{array} \right.$$

(1)

where $I$ is the input image, $N$ and $M$ are the height and width of $I$, respectively, $i$ and $j$ are the two specific intensity levels, and $d$ denotes the offset between the two pixels $(x,y)$ and $(x + dx, y + dy)$.

Four directions of $\theta$, $0$ deg, $45$ deg, $90$ deg, and $135$ deg, were considered in the distance, $d$, which varied from $1$ to $2$ pixels. The image texture analysis was executed in a slice-by-slice basis. For each direction resulting from a certain neighborhood type and a given distance, $d$, we computed four features among those most frequently used in the literature: contrast, correlation, energy, and homogeneity. The four features were calculated using the following equations: based on the co-occurrence matrixes as presented in Fig. 4, the features were calculated as follows:

1. Contrast, $f_1 = \frac{1}{n^2} \sum_{i=1}^{N} \sum_{j=1}^{N} p(i,j) |i-j| = n$.
2. Correlation, $f_2 = \frac{1}{\sigma_i \sigma_j} \sum_{i} \sum_{j} [p(i,j) - \mu_i \mu_j]$.
3. Energy, $f_3 = \sum_{i} \sum_{j} p(i,j)$.
4. Homogeneity, $f_4 = \sum_{i} \sum_{j} \frac{p(i,j)}{1 + |i-j|^2}$.

where $\mu_i$, $\mu_j$, $\sigma_i$, and $\sigma_j$ are the means and the standard deviations of the corresponding distributions. $p$ is the joint probability of two pixels with specific intensity levels as described in Eq. (1). $N_g$ is the number of gray levels in the image. $i$ and $j$ are the two specific intensity levels.

### 3.2 Feature Extraction-Based Energy Dispersive X-Ray Spectroscopy Spectra

In addition to the FE-SEM/EDX images, the scanning of the cervical cells specimen area using point EDX and/or line scanning EDX can produce a spectrum of the area. The scanning time was determined for the same duration for each produced spectrum. In this study, as presented in Sec. 2, the elemental distributions of cells were scanned in a certain area of the FE-SEM images. Figure 3 describes the visible spectral differences among normal, LSIL, and HSIL cells. In the normal cells, the carbon (C), nitrogen (N), oxygen (O), and sodium (Na) elemental peaks were detected strongly by the FE-SEM/EDX system. LSIL cells consistently had C and O elements, and they sometimes had Na elements. Meanwhile, C and O elements were higher in the HSIL cells while the N and Na elements were not present. Therefore, C, N, O, and Na elements can be used as features to differentiate among the normal, LSIL, and HSIL cells.

The elemental distribution in the cervical cells, as tabulated in Table 2, had standard peak positions in terms of keV based on the periodic table of elements. By using C, N, O, and Na elements as the discriminative features, a signal...
Fig. 3 Description of the visible spectral difference among normal, LSIL, and HSIL cells images. *Noted: (A1) normal cells image with five EDX scanning area; (A2)-(A6) EDX spectra of the scanning area; (B1) LSIL cells image with five EDX scanning area; (B2)-(B6) EDX spectra of the scanning area; (C1) HSIL cells image with five EDX scanning area; (C2)-(C6) EDX spectra of the scanning area.
processing algorithm was utilized in this study. The height of the peak elements, the corrected area under the peaks, the ratio of peak elements, and the ratio of corrected area under the peaks were calculated from the FE-SEM/EDX spectra. The maximum peak and base points algorithm were applied to the spectra to automatically detect the peaks and base points, as shown in Fig. 4 (used in calculating the corrected area), in the range of the 0 to 10 keV region. After detecting the peaks and the base points, calculation of the height of the peaks and the corrected area under the peaks were performed and the ratio of the peaks and corrected area were calculated.

An automatic features extraction algorithm, the image and spectra of cervical cells features extraction (ISCFE) algorithm for extracting the discriminant features in FESEM/EDX data, was introduced in this section. Based on the previous section shown in Fig. 1, the ISCFE algorithm is a combination of image and spectra processing techniques for FE-SEM/EDX data as follows:

- Implemented two preprocessing algorithms to the original FE-SEM/EDX images of the cervical cells: contrast stretching and morphological opening algorithms.
- Set four parameters of the GLCM technique: the region size, quantization levels, displacement, and orientation value.
- Applied the parameters and calculated the co-occurrence matrix using Eq. (1).
- Applied the GLCM technique to extract contrast, correlation, energy, and homogeneity features set using Eqs. (2)–(5), respectively, then arranged in dataset $A$.
- Detected the important peaks in the FE-SEM/EDX spectra of the cervical cells as tabulated in Table 2 using the maximum height value between $a$ and $b$ for the important peaks, where $a$ and $b$ were $-3$ and $+3$ of the location of the important peaks heights.
- Found the peak height of the important peaks.

### Table 2 Elements in cervical cell samples and their peak positions by using FE-SEM/EDX spectra.

<table>
<thead>
<tr>
<th>Features based on elemental distribution</th>
<th>Peak positions (keV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon (C)</td>
<td>0.27</td>
</tr>
<tr>
<td>Nitrogen (N)</td>
<td>0.39</td>
</tr>
<tr>
<td>Oxygen (O)</td>
<td>0.53</td>
</tr>
<tr>
<td>Sodium (Na)</td>
<td>1.04</td>
</tr>
</tbody>
</table>

### Table 3 The features of cervical cells based on FE-SEM/EDX spectra.

<table>
<thead>
<tr>
<th>Features</th>
<th>Detail peaks and corrected area ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Features 1</td>
<td>$p_1/p_2$</td>
</tr>
<tr>
<td>Features 2</td>
<td>$p_1/p_3$</td>
</tr>
<tr>
<td>Features 3</td>
<td>$p_1/p_4$</td>
</tr>
<tr>
<td>Features 4</td>
<td>$p_2/p_3$</td>
</tr>
<tr>
<td>Features 5</td>
<td>$p_2/p_4$</td>
</tr>
<tr>
<td>Features 6</td>
<td>$p_3/p_4$</td>
</tr>
<tr>
<td>Features 7</td>
<td>$ca_1/ca_2$</td>
</tr>
<tr>
<td>Features 8</td>
<td>$ca_1/ca_3$</td>
</tr>
<tr>
<td>Features 9</td>
<td>$ca_1/ca_4$</td>
</tr>
<tr>
<td>Features 10</td>
<td>$ca_2/ca_3$</td>
</tr>
<tr>
<td>Features 11</td>
<td>$ca_2/ca_4$</td>
</tr>
<tr>
<td>Features 12</td>
<td>$ca_3/ca_4$</td>
</tr>
</tbody>
</table>

![Fig. 4 Scanning EDX area in the FE-SEM image and FE-SEM/EDX spectrum with its peak and base points.](image-url)
Calculated the ratio of the specific peaks (i.e., \( p_1, p_2, \ldots \)) as determined in Table 3 where, \( p_1, p_2, p_3, \) and \( p_4 \) were peaks of C, N, O, and Na, respectively, and arranged in dataset B.

- Determined the prominent base points of the important peaks, as presented in Fig. 4, to be used in features calculation.

- Calculated the corrected area (\( Ca \)) under the peaks (i.e., \( ca_1, ca_2, \ldots \)) based on the detected base points according to Eq. (6)

\[
Ca = \frac{\int_{x_1}^{x_2} s(x) \, dx - [s(x_1) + s(x_2)] \times (x_2 - x_1)}{2},
\]

where \( d(x) \) is the height of the spectrum, \( x_1 \) and \( x_2 \) are the base points for specific important peaks, and \( s(x_1) \) and \( s(x_2) \) are the height of the base points.

- Calculated the ratio of the area under the peaks as tabulated in Table 3, where \( ca_1, ca_2, ca_3, \) and \( ca_4 \) were the corrected area under the peaks of C, N, O, and Na, respectively, and arranged in dataset C.

- Combined datasets A, B, and C as a matrix input data for classification screening part.

Based on the FE-SEM/EDX images, there were four main features based on intensity variation (contrast and homogeneity), intensity distribution (correlation), and intensity power difference (energy). For the contrast feature, the value of contrast was high if the variation in image intensity was high. On the contrary, the homogeneity feature was the opposite of the contrast value, where a high-contrast image indicated a nonhomogeneous region. Meanwhile, for the correlation feature, the value of the correlation was high if the image had strong gray level linear dependence between the pixels at the specified positions relative to each other. Then, the energy value was high if the pixel intensities were concentrated in certain coordinates for the energy feature. For example, for normal, LSIL, and HSIL in Fig. 5, the contrast feature in the normal image was lower than the LSIL and HSIL images, and the homogeneity of the normal image was higher. Meanwhile, the correlation for LSIL and

<table>
<thead>
<tr>
<th>Original images</th>
<th>Output of contrast stretching</th>
<th>Output of morphological opening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSIL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSIL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 5 Presentation of normal, LSIL, and HSIL images and their contrast stretching and morphological opening output images.
HSIL images was higher than normal images. In LSIL and HSIL images, the energy value was lower than the normal image due to the existence of the virus in the LSIL and HSIL images, as the pixel intensities were not concentrated in certain coordinates.

Based on the selected parameters for the GLCM technique, eight co-occurrences matrices were created for the four features calculation. Thus, eight values for each feature (i.e., contrast, correlation, energy, and homogeneity) were obtained depending on the displacement $d = 1, 2,$ and the orientation $\theta = 0$ deg, 45 deg, 90 deg, and 135 deg. Based on the rotational invariance of the technique, for each distance, a one value for each feature was eventually obtained. The total number of features taken into account of the two displacements could be eight features on the FE-SEM/EDX images.

Based on the previous explanation, the features of cervical cells based on the FE-SEM/EDX spectra consisted of peak height and corrected area under the peaks. The ratio of the peak height and the corrected area was used as a feature based on spectra in this study. The total features based on the ratios of the peak height and corrected area under the peaks (i.e., C, N, O, and Na element peaks) was 12, as tabulated in Table 3. A total of 20 features were extracted from the FE-SEM/EDX images and spectra and fed as the input to the automated screening system.

### 3.3 Classifications

Data from four quantization levels (i.e., 5, 9, 15, and 32) of the GLCM technique for images were compared to obtain the optimal results. The quantization levels were used for the ISCFE algorithm. The capability of the ISCFE algorithm for the features extraction techniques was tested using a 10-fold cross-validation and applied for data distribution. In the classification stage, a discriminant analysis (DA) technique was applied. This screening system used validity measures of normal and abnormal (i.e., LSIL and HSIL) classes. Confusion matrices were used for evaluation purposes. Comparison of performance between this study and related published work was performed by calculating the accuracy, sensitivity, and specificity of the screening results. The cervical cell data from the systems were compared with those of cytology (gold standard).

### 4 Results and Discussions

Based on the previous explanation, the ISCFE algorithm extracted features from FESEM/EDX data (i.e., images and spectra). FE-SEM/EDX images provided statistical feature information based on pixel intensities, specifically, contrast, correlation, energy, and homogeneity. Furthermore, the FE-SEM/EDX spectra can provide information on the ratio of the peaks and the ratio of the corrected area under the peaks.

Figure 3 shows the FE-SEM/EDX data consisting of images and spectra of the cells on the FE-SEM images. From 150 FE-SEM images, we have obtained 700 spectra of the cells on the images of 300 normal, 200 LSIL, and 200 HSIL spectra. The features from the FE-SEM image were considered as an individual prospective cell for classification purposes.

Based on the DA technique, four confusion matrixes of 10 times 10 cross-validations for each tested quantization level and in the GLCM technique in the ISCFE algorithm were used for evaluation. The comparison results are presented in Table 4. Based on Table 4, the screening performances of cervical precancerous cells achieve more than 97% of accuracy, sensitivity, and specificity when the FE-SEM/EDX data were used as input for the automated system. The results of the automated system were achieved when the ISCFE algorithm with nine quantization levels were 98.2%, 99.0%, and 98.0% of accuracy, sensitivity, and specificity, respectively. Meanwhile, with 15 quantization levels, 98.0% accuracy, 99.0% sensitivity, and 97.2% specificity were obtained. When the ISCFE algorithm with 32 quantization levels was operated, the system achieved 98.0%, 99.0%, and 97.2% of accuracy, sensitivity, and specificity, respectively. Among the results, the ISCFE algorithm with nine quantization levels was chosen for an optimal result of the system. The results achieved were very stable. By using nine quantization levels in the GLCM, it still represented well the information of the images, and it did not incur as much computational load as other higher quantization levels (i.e., 15 and 32 quantization level). Meanwhile, if we chose a low enough number of quantization levels, we would not be able to preserve enough information in the images.

Based on the screening system results in this study, the FE-SEM/EDX image and spectra features, ISCFE algorithm, and DA classifier were used for a screening system as a second opinion for human experts to classify cervical precancerous cells. To show the capability of our proposed system, the performance results were compared with other published cervical precancerous screening systems. The comparison of performance of the cervical screening system and several previously mentioned approaches are tabulated in Table 5 in terms of accuracy, sensitivity, and specificity.

Five systems, systems A,13 B,8 C,36 D,14 and E (proposed system) were included for comparison. In the automated system were achieved when the ISCFE algorithm with 32 quantization levels was operated, the system achieved 98.0%, 99.0%, and 97.2% of accuracy, sensitivity, and specificity, respectively. Among the results, the ISCFE algorithm with nine quantization levels was chosen for an optimal result of the system. The results achieved were very stable. By using nine quantization levels in the GLCM, it still represented well the information of the images, and it did not incur as much computational load as other higher quantization levels (i.e., 15 and 32 quantization level). Meanwhile, if we chose a low enough number of quantization levels, we would not be able to preserve enough information in the images.

### Table 4 Comparison of DA classification result with several quantization levels of GLCM technique for both images and FESEM/EDX spectra features.

<table>
<thead>
<tr>
<th>System performances (%)</th>
<th>5</th>
<th>9</th>
<th>15</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>97.1</td>
<td>98.2</td>
<td>98.0</td>
<td>98.0</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>97.0</td>
<td>99.0</td>
<td>99.0</td>
<td>99.0</td>
</tr>
<tr>
<td>Specificity</td>
<td>98.0</td>
<td>98.0</td>
<td>97.2</td>
<td>97.2</td>
</tr>
</tbody>
</table>

### Table 5 Comparison between the published systems and the proposed system results. Note: system A13, B8, C36, D14, and E (proposed system) were included for comparison.

<table>
<thead>
<tr>
<th>Performances</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>93.5</td>
<td>83.5</td>
<td>75.0</td>
<td>85.4</td>
<td><strong>98.2</strong></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>93.2</td>
<td>73.0</td>
<td>70.0</td>
<td>84.5</td>
<td><strong>99.0</strong></td>
</tr>
<tr>
<td>Specificity</td>
<td>94.1</td>
<td>94.0</td>
<td>80.0</td>
<td>86.4</td>
<td><strong>98.0</strong></td>
</tr>
</tbody>
</table>
systems, the first three groups used images\textsuperscript{8,13,36} while the rest used spectra.\textsuperscript{14} Among the systems based on the images, FISH\textsuperscript{13} presented a better classification performance with 93.5\%, 93.2\%, and 94.1\%, of accuracy, sensitivity, and specificity, respectively. Meanwhile, as shown in Table 5, system B, which used cervicography, had better results than system C, which used colposcopy. Meanwhile, the system using the Raman spectra\textsuperscript{14} applied preprocessing using a Savitzky–Golay smoothing filter, principal component analysis, and linear discriminant analysis-based multivariate analysis for classification purposes. The system achieved 85.4\%, 84.5\%, and 86.4\% of accuracy, sensitivity, and specificity, respectively.

Therefore, based on the aforementioned explanation, our system can better differentiate the cervical cells as it applied the combination of image and signal data. By using both image and spectra data, there was a significant increase in information, so the tendency to produce accurate results was higher. Our proposed screening system had better classification performances where it achieved 98.2\%, 99.0\%, and 98.0\% of accuracy, sensitivity, and specificity, respectively. In addition, our system ran the 10 times 10 cross-validation technique to determine its classification accuracy in an unbiased manner. Therefore, based on the results, our system is better than other published systems, as presented in Table 5.

5 Conclusions

In this paper, the capability of FE-SEM/EDX to scan material structures at the microlevel and characterize the material with its elemental properties inspired the development of an FE-SEM/EDX-based cervical cancer screening system. The developed computer-aided screening system consisted of two parts: automatic features extraction and classification. The automatic feature extraction applied an ISCFE algorithm, which extracted significant information from both FE-SEM/EDX image and spectra data. Classification-based DA was employed. The performance of this system is better than other current published systems. Better results had been achieved due to more information based on images and spectra compared to other systems. Therefore, a cervical precancerous screening system based on FE-SEM/EDX can provide accurate results as indicated by the high accuracy, sensitivity, and specificity values.

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