1 Introduction

Colposcopy was developed in 1925 by Hinselmann to direct the cervical biopsy placement to improve the correlation between cervical cytology and histology. The transformation zone of the cervix could be colposcopically identified after 5% acetic acid was applied to the cervix. Areas of abnormality are defined colposcopically by: (i) the degree of whitening after acetic acid solution application, (ii) the roughness of the surface contour, (iii) the margins of the lesions, and (iv) the vascular pattern and intercapillary distance. The correlation between cytology and histology improved with colposcopically directed biopsies from less than 50% to almost 90% when the entire transformation zone was visible in expert hands. When the squamocolumnar junction could not be seen, the colposcopically directed biopsy was not much better than cytology alone at a 60% correlation.

There is agreement among colposcopists that, in general, expert correlation between the colposcopic image and the specimen histology ranges from 50% to 75%. This considers cross agreements between all tissue types: normal, metaplastic, all stages of cervical intraepithelial neoplasia (CIN 2/3), and cancer. Yet, in inexperienced hands, the colposcopic impression correlates to the histologic specimen less than 35% of the time, with the most difficulty encountered in differentiating benign epithelial changes from CIN 2/3.

Digital imaging in colposcopy has facilitated the development of archival records for many clinical purposes: auditing of cytology, colposcopy and histology correlations, quality assurance of care, and monitoring disease regression and progression with simple planar measurement tools. Although the advance in digital imaging has been employed mainly for image archiving of patient records, the digitized colposcopic images contain two important types of diagnostic information: tissue structure and color. Structural information appears as morphological features reflecting underlying tissue architecture, while color content, which is affected by tissue optical absorption and scattering, reflects tissue biochemistry and substructure. Although this information could be quantified and enhanced via standard image processing techniques, in conventional use these images remain unprocessed. Thus, disease diagnoses are made off-line from pathological examinations of biopsied tissue samples obtained during colposcopy. Digital imaging colposcopy now allows the colposcopist to take advantage of the mature field of image analysis and processing, where image features, color patterns, and trends can be reliably detected and quantified.

Semiautomated image processing tools can be incorporated into a computer coupled colposcope to aid the physician, once the appropriate metrics have been determined to quantify...
the important morphologic features. An example of this is the
development of neural network algorithms using intercapillary
distance, lesion area, and perimeter to aid the less ex-
perienced colposcopist in determining the most diseased cer-
vical lesion for biopsy. This type of processing may be
especially useful in problematic diagnoses, where an objective
number can be assigned to the color or structural changes seen
in the cervical tissue. While image analysis of the entire field
is possible, it is more illustrative and potentially useful to
perform analyses on regions of interest (ROI) that have been
defined within an image by the clinician. In this way, the
analysis is confined to that region as defined by the user, and
takes advantage of the knowledge of the user. If this approach
is not used, then the analysis is blind and must be able to deal
with a larger range of problems such as intensity variation
across the image or depth of focus variations. The first stage
in such a development is to determine and test the most useful
measures to analyze and quantify regions of cervical tissue,
within specified regions of interest.

In this study, three tissue types in particular have been
examined including mature squamous epithelium (MSE),
immature squamous metaplasia (ISM), and cervical intraepi-
thelial neoplasia grade 2/3 (CIN 2/3). The goal of the study was
to take a sample of representative tissue images, and deter-
mine some simple metrics which would be capable of dis-
criminating between them based upon the information from
the colposcope color image. To this end, we examined the
color information in the red, green and blue (RGB) pixel in-
tensities and intensity variations, as well as some simple tex-
ture detecting features including spatial frequency analysis,
fractal dimension, and Euler number. These analyses are the
first steps in designing a computer-aided colposcopy worksta-
tion that can serve as a useful tool and guide for the practicing
colposcopist. Additionally, the analysis of typical cervical tis-
tue features including multiple optical wavelengths and mul-
tiple spatial frequencies may potentially lead to an improved
understanding of the morphology of cervical tissue lesions.

2 Methods
2.1 Patients
Women between the ages of 18 and 65 were recruited for this
study if they had been referred to colposcopy. Referral to
colposcopy was based on abnormal cytology, [two consecu-
tive Pap tests showing atypical squamous cells of undeter-
dined significance (ASCUS), or one Pap test showing atypi-
cal glandular cells of undetermined significance (AGUS), low
grade squamous intraepithelial lesion (LSIL), high grade
squamous intraepithelial lesion (HSIL) or squamous cancer]
or an abnormal visual exam at routine screening. All women
underwent the standard colposcopic procedure. The cervix
was visualized and 5% acetic acid was applied continuously
for three to four minutes to the surface, allowing adequate
time for the most dense acetowhiteness to appear. The
squamocolumnar junction and the transformation zone were
identified; and then the abnormal lesions were analyzed.
The first image was captured at a 4× magnification giving a pan-
oramic view of the entire cervical field at maximal acetowhi-
tening. Subsequent images were taken at 10× and 16× of the
area of most concern focusing either on margins or vascula-
ture patterns that are most easily seen at higher powers. Ace-
tic acid was reapplied to the cervix when the transient ac-
etowhiteness started to fade. The images in this study were all
taken between 1 and 3 min after application of acetic acid, to
provide a uniform whitening affect across all the images.
Color filter images were taken when contrasting images were
visible, but generally the green filter darkened the digital im-
age so much that the details of the lesion were obscured.
Lugol’s solution was applied to the cervix after all acetic acid
images were taken. This iodine based solution stained the
mature cervical squamous epithelium mahogany brown, and
the immature or neoplastic epithelium a mustard yellow or
variegated pattern. Lugol’s is used clinically to distinguish the
borders of a lesion clearly from the normal mature cervical
epithelium, as it obscures whitening and vessel patterns. A
panoramic colposcopic image was taken at 4× with Lugol
staining, however these images were not used in this study as
we believed the acetowhitened images to be more useful for
the current analysis.

Images were interpreted after all patient images were taken
and biopsy confirmation had been received for suspicious
sites. For this study, nine patients with biopsy confirmed neo-
plasia were included in the study, with biopsy results included
in Table 1.

Table 1 Details of biopsy pathology results from the nine abnormal
colposcopy patients in this study. These same specimens correspond
to specimen numbers 1–9 in Figures 2–5.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Pathologic diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Focal adenocarcinoma in situ</td>
</tr>
<tr>
<td>2</td>
<td>CIN II</td>
</tr>
<tr>
<td>3</td>
<td>CIN II/III</td>
</tr>
<tr>
<td>4</td>
<td>CIN II/III</td>
</tr>
<tr>
<td>5</td>
<td>CIN II/III</td>
</tr>
<tr>
<td>6</td>
<td>CIN II/III</td>
</tr>
<tr>
<td>7</td>
<td>CIN II/III</td>
</tr>
<tr>
<td>8</td>
<td>CIN III</td>
</tr>
<tr>
<td>9</td>
<td>CIN III</td>
</tr>
</tbody>
</table>

2.2 Colposcope
The colposcope is a Carl Zeiss 1-FC system ZMS-506-II with
magnifications of 4×, 6×, 10×, 16×, and 25× illuminated by
a fiberoptic 12 V/100 W halogen light with 20× eye bin-
oculars. A DAGE-MTI DC-330 three channel charge-coupled
device color video camera, automatically white balanced, was
mounted to the colposcope allowing real-time video display
of the examination. Although this video system is not com-
monly used in Colposcopy Clinics because of its cost, the
parts to assemble it were standard. Images were captured by
an integrated video frame grabber and video display card us-
ing the RGB video signal at 24 bit resolution. ColpoShot is
the custom-designed software copyrighted by TeleComputing
Solutions, Inc. (Hanover, NH) which integrates the unique
image into the patient’s medical record. Unlimited images can be taken of any one patient. The images are stored in (JPEG) format with minimal compression.

2.3 Region of Interest Analysis

All digital images were stored as JPEG files on the computer typically at 640×480 pixel dimensions, with 16 million colors. Images were loaded into Adobe Photoshop software for separation of the RGB channels and selection of the specific regions. These regions of interest (ROI) were chosen from each image for intensity and feature analysis in order to find a metric which would allow discrimination of different tissue types. The ROI from colposcopic images with neoplasia and immature squamous metaplasia were chosen specifically to correspond to the biopsy sites, while areas of mature squamous epithelium where chosen from arbitrary representative sites of the image.

2.3.1 RGB Values

The most obvious choice of parameters to examine in a color image are the raw red, green, and blue (RGB) pixel intensity values within the regions of interest. Intergroup variability combined with the automatic gain of most imaging systems make the absolute intensity an unreliable parameter to use, so that here the relative contribution of R, B, and G are examined, which can also be called the chromaticity coordinate [i.e., R/(R+B+G)] is the relative red pixel intensity as a fraction of 1]. Images were analyzed with Adobe Photoshop software to separate RBG images of the ROI, and to count absolute RBG values along with standard deviations.

2.3.2 RGB Variations

Since variation of the intensity was observed significantly in all regions of interest, this was tracked for each site. The variation in the intensity was calculated as the standard deviation of the intensity divided by the absolute intensity of the region, for RGB values separately. This provided a relative variation measure over the region of interest. In this analysis, care was taken to make sure that the area of the region of interest was roughly the same, so that the sample size was sufficient to minimize the error due to random fluctuations to less than 1% (i.e., a minimum of 10,000 pixels in each ROI image).

2.3.3 Radial Frequency Analysis-Zeroth Frequency Amplitude

One of the easiest methods to examine patterns in an image is to use the two-dimensional (2D) fast Fourier transform (FFT) algorithm to provide quantitative numbers for the amplitude of different spatial frequencies which compose an image. For this Fourier analysis care was taken to ensure that the magnification of each image was similar (16×) to ensure that the effect of pixel size did not distort the results. The regions of interest were chosen to coincide with the biopsy results for the CIN 2/3 and the immature squamous metaplasia. The regions were cropped to 96×96 pixels to increase the allowed use of the FFT, rather than the slower full Fourier transform algorithm. All calculations were performed in MATLAB using the separated images from the red, green, and blue channels of the RGB image. The resulting transformed image data was reorganized so that the zeroth frequency was at the center of the image, and the 2D data was converted to a one-dimensional array of data by summing spatial frequencies radially around the central (zeroth) frequency. The amplitudes at each spatial frequency were then displayed as logarithmic data. In order to quantitatively compare the spatial frequency features of the images, two particular frequency regions were examined. First the zeroth spatial frequency relative to the next following frequency was calculated for all three red, green, and blue channels of each image, which is the difference in logarithmic values between the zeroth and first spatial frequency amplitudes. This measure of the difference between zeroth and first frequencies is a metric of the amount of the image which is homogeneous (i.e., no changes in intensity).

2.3.4 Radial Frequency Analysis-Slope of Logarithmic Frequency Amplitude

The second analysis was carried out by calculating the slope of the logarithm of spatial frequency amplitude as a function of spatial frequency. This slope was calculated within the frequency range between the 5th and the 25th spatial frequencies, where the slope of log amplitude versus frequency was linear in most cases. This slope is a measure of how complicated the image is, since more complex images tend to have a higher slope in this range of frequencies. In essence this number is related to the fractal dimension of the image, since it is a measure of the increasing complexity contained within the image as a function of spatial frequency. However it should be noted that the method to calculate this number is quite different than the next method (following section) so that there is no guarantee that the two numbers will agree.

2.3.5 Fractal Dimension

In an effort to describe the complexity of each image, the fractal number of each ROI was calculated. The methodology for this is described in Refs. 12 and 13 where a box counting method was detailed. The box counting method works for binary images (black and white only with no gray scale), so that the ROI in each case was converted from a blue pixel image to a binary image by automating the program to choose the threshold which produced 50% black and 50% white in the image. Initial measurements were also taken with thresholding based upon the best value to preserve the texture of the image, however the former algorithm was used to preserve objectivity in the analysis. Briefly, the box counting method uses square box regions translated around the ROI to determine the number of areas where the box is homogenous and all black. Once the number of box areas are counted, then the size of the box is increased, and the process is repeated. The algorithm used here was programmed into MATLAB and the fractal number was calculated using the equation below

\[ D_f = \lim_{d \to 0} \frac{\ln N(d)}{\ln(1/d)}, \]

where \( N(d) \) is the number of squares required to cover the details contained in the thresholded image to be analyzed, where the square has each side of length, \( d \). For simplicity, the slope of the ratio in Eq. (1) was used as the fractal dimension. If an image has no features, it has fractal dimension \( D_f = 0 \), whereas the most complicated structures have \( D_f = 3.0 \). In
practice, the value of $d$ was varied over 1.5 orders of magnitude, since fractal patterns need to be analyzed over orders of magnitude for good accuracy.

### 2.3.6 Euler Number

Another measure of the complexity of an image is to count the number of arbitrary sized objects present in the image. The MATLAB function to calculate the Euler number of the image was used for this task. The Euler number is defined as the number of objects in the image subtracted by the number of holes in these objects. While this number is a measure of the whole image, it is calculated in practice by analyzing the $2 \times 2$ pixel patterns across the image systematically for a binary image. The procedure used for analysis of ROI was to take the blue pixel map only, and convert it to a binary (black and white image only) by automatically choosing the threshold value which would convert the image to 50% black and 50% white on average. Then the MATLAB subroutine calculated the Euler number from this image using $2 \times 2$ pixels (or bit quads) for analysis. Three types of pixel patterns are counted across the image: $p1$ those with four zeros, $p2$ those with three ones and one zero, and $p3$ those with two zeros and two ones in a diagonal pattern only. From these three patterns, the Euler number is calculated by

$$E = 1/4 [n_{p1} - n_{p2} + 2n_{p3}], \quad (2)$$

where $n_{p1}$ is the number of $2 \times 2$ pixel regions with pattern $p_1$, and similarly labeled for $p_2$ and $p_3$. Interestingly, this analysis estimates the number of objects in the image minus the number of holes in the objects, but this type of analysis cannot be used to just measure the number of holes or objects alone. In the case of colposcopy images, this number is useful since many of the relevant textures are circular with intricate patterns from blood vessels and glandular regions.

### 3 Results

The ROI of CIN 2/3 in the diseased cervical images were all chosen to correspond to the biopsy site results so that pathologic confirmation of the disease state was known. The ROI of mature squamous epithelium (MSE) were chosen randomly from the images, within the homogenous tissue areas, and the ROI of immature squamous metaplasia (ISM) were chosen from the biopsy confirmed sites in each image. Table 1 shows the pathologic grade of the nine diseased cervical images used in this study. All samples with immature squamous metaplasia had similar biopsy results.

Figure 1 shows two typical colposcopic images, where the first displays a diseased region of CIN 2/3 above the squamo-columnar junction, within the transformation zone; and the second image has only ISM in the same region. While the images from all the patients varied considerably, these two images show one type of difference which is possible in the color and texture patterns of the image. The three categories of tissue chosen for this study are shown in these two images. To compare the features which can be analyzed in a typical image, a closer image of the cervix in Figure 1(a) is shown in full RGB in (a), with just the red channel in (d), just the green channel in (g), and just the blue channel in (i). The red, green, and blue fractions of the image were separated and are shown in Figures 2(d), 2(g), and 2(i), respectively. These images are all thresholded using an algorithm which was designed to produce 50% black and 50% white images (binary images). The resulting images from each channel provides unique patterns which are reflective of the tissue optical properties in that particular wavelength range. These patterned images were used for determination of the fractal dimension and the Euler number. Alternatively, the original R, G, and B images can be Fourier transformed to produce 2D plots of the spatial frequency amplitude. In Figure 2, the 3rd column (c), (f), (i), and (l) shows the result of performing a 2D FFT of the central 128×128 region of pixels from each image (a), (d), (g), and
These images are arranged so that the zeroth spatial frequency is at the center of the image, and a radial binning around this central location provides a linear array of amplitudes at increasing spatial frequency.

The ROI analysis for relative red, green, and blue values (also called the chromaticity coordinate) are shown in Figures 3(a)–3(c), respectively. The data are plotted as sample number in order to show all samples and the variance in the data points. The unpaired students T test was used to determine if there was a statistically significant separation of the data between different tissue types for all three RGB values. No comparisons between any of the tissue types for any of the three RGB values produced probability values of less than 0.1, indicating that individual colors of the tissues were not sufficient to discriminate between types.

In this case the ability to discriminate between tissue types was improved, based on the students T test. The P values for the three different tissue types and three color variations are shown in Table 2. The results indicate that the variation in the RGB values are a reliable method for separating CIN 2/3 or

<table>
<thead>
<tr>
<th>Data sets compared</th>
<th>P value (R st. dev.)</th>
<th>P value (G st. dev.)</th>
<th>P value (B st. dev.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN vs MSE</td>
<td>0.048</td>
<td>0.004*</td>
<td>0.002*</td>
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<tr>
<td>CIN vs ISM</td>
<td>0.4</td>
<td>0.44</td>
<td>0.51</td>
</tr>
<tr>
<td>ISM vs MSE</td>
<td>0.07</td>
<td>0.03*</td>
<td>0.03*</td>
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</table>
ISM from mature squamous epithelium, but that CIN 2/3 and ISM cannot be reliably separated from each other in this manner. Interestingly, the green and blue channels provide the best separation between tissues. Qualitatively the green channel appears to emphasize blood vessel and glandular locations, whereas the blue channel improves the resolution of epithelial tissue layers and patterns.

The radial spatial frequency analysis was carried out for all image ROIs for each of the R, G, and B channels separately. The typical results from a radial binning of the spatial frequencies is shown in Figure 5(a) for mature squamous epithelium, 5(b) for CIN 2/3, and 5(c) for ISM. The shapes of these curves indicate that there is a pronounced peak at the zeroth spatial frequency for all images, as expected, and that the CIN and SM data generally has a higher gradient in the amplitude with increasing spatial frequency. The data is displayed as logarithm of frequency amplitude versus spatial frequency number, in Figure 5. The peak value from these graphs at the zeroth frequency was calculated relative to the next higher frequency amplitude, providing a measure of the difference in log values between the zeroth and first spatial frequency amplitudes. This calculation was carried out for all images and for all R, G, and B channels, the results are plotted in Figure 6. An unpaired students T test was used to compare the discrimination of the calculated data, and the $P$ values from this comparison are shown in Table 3. This zeroth to first frequency amplitude difference could be used to discriminate between CIN 2/3 and MSE or between ISM and MSE using either the green or the blue images, but not the red image.

While the zeroth spatial frequency is related to the homogeneous regions of the image, the higher spatial frequencies (above zero) are related to the more complex features of the images. Based upon the data in Figure 5, it was hypothesized that the slope of the log(amplitude) versus spatial frequency image analysis for discrimination.

![Figure 5 plots](image1)

![Figure 6 calculation](image2)
would be a good indicator of the complexity of the images. The slope of the log amplitude versus spatial frequency was calculated for all of the images using red, green, and blue images for all ROI sections, using the frequency range between 5 and 25 (see Figure 5 as a reference). The data from this analysis is plotted in Figure 7, and the results from a students T test comparison is used to calculate probabilities of different distributions (Table 4). Again, the results indicate that this analysis can be used to differentiate between CIN 2/3 and MSE or between ISM and MSE, but not between CIN 2/3 and ISM, using only the blue and green channels.

The fractal dimension was calculated for each tissue type with automatic segmentation of the images. The calculation was carried out on all three R, G, and B channels using binary black and white versions of each image which were obtained by thresholding to provide 50% white and 50% black in each image. One set of typical thresholded binary images for the R, G, and B channels separately, is shown in Figure 2, in the third column. The calculated fractal dimension numbers are plotted in Figure 8. Statistical analysis indicated that the fractal number was insufficient to discriminate between any of the tissue types \( (P > 0.05) \) for all comparisons, so that a table of values is not provided here.

The Euler number was calculated for each tissue ROI with automatic segmentation of the images, and the data is plotted in Figure 9. Each of the R, G, and B channels were used again for this analysis, as in the fractal dimension, using binary images thresholded in the manner described in Figure 2. The students T-test comparison between the data sets is shown in Table 5. In this case, the Euler number cannot be used to discriminate between CIN 2/3 and MSE or between ISM and MSE. The ability to separate CIN 2/3 and ISM was significant here \( (P = 0.013) \). A further improvement in separating the data sets is possible with a different level of thresholding used in the images; however it is difficult to objectively determine which level of thresholding provides the best separation between calculated Euler number for differentiating ISM and CIN 2/3. To address this question, thresholding was done at all possible values for all blue channel images, and the Euler number was calculated as a function of the fraction of the image which appeared white (ranging between 0% and 100%). The results for this analysis for all CIN 2/3 tissue samples are shown in Figure 10(a) and for all ISM tissue samples are shown in Figure 10(b). A comparison of the data in the two graphs indicates that there is maximal deviation

![Fig. 7](https://photonicsforenergy.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics on 01 Jun 2019)

<table>
<thead>
<tr>
<th>Data sets compared</th>
<th>( P ) value (R zero freq.)</th>
<th>( P ) value (G zero freq.)</th>
<th>( P ) value (B zero freq.)</th>
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</thead>
<tbody>
<tr>
<td>CIN vs MSE</td>
<td>0.27</td>
<td>0.016*</td>
<td>0.015*</td>
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<tr>
<td>CIN vs ISM</td>
<td>0.44</td>
<td>0.87</td>
<td>0.79</td>
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<tr>
<td>ISM vs MSE</td>
<td>0.65</td>
<td>0.052*</td>
<td>0.056*</td>
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</table>

![Table 4](https://photonicsforenergy.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics on 01 Jun 2019)

<table>
<thead>
<tr>
<th>Data sets compared</th>
<th>( P ) value (R freq. slope)</th>
<th>( P ) value (G freq. slope)</th>
<th>( P ) value (B freq. slope)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN vs MSE</td>
<td>0.17</td>
<td>0.022*</td>
<td>0.009*</td>
</tr>
<tr>
<td>CIN vs ISM</td>
<td>0.91</td>
<td>0.97</td>
<td>0.40</td>
</tr>
<tr>
<td>ISM vs MSE</td>
<td>0.16</td>
<td>0.020*</td>
<td>0.004*</td>
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</table>
4 Discussion
The main goal of this study has been to determine the pertinent color features in digital colposcopy images which can be used for semiautomated discrimination of different tissue types and malignancies. While it is unlikely that feature analysis can replace expert physician inspection of images, there is a role for feature analysis in objectively quantifying the color changes and structural changes of certain regions of the image. This type of analysis can be important in several roles such as: (i) training new colposcopists, (ii) confirmation of the colposcopic impression, (iii) aiding providers in remote regions to make colposcopic impressions by telecolposcopy, (iv) providing quantitative classification of the color and fea-

between the two sets of values between threshold values of approximately 0.2 to 0.5.

![Figure 8](https://photonicsforenergy.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics/January-2000-Vol.5-No.1)

![Figure 9](https://photonicsforenergy.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics/January-2000-Vol.5-No.1)

![Table 5](https://photonicsforenergy.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics/January-2000-Vol.5-No.1)

### Table 5

<table>
<thead>
<tr>
<th>Data sets compared</th>
<th>( P ) value (( R ) Euler No.)</th>
<th>( P ) value (( G ) Euler No.)</th>
<th>( P ) value (( B ) Euler No.)</th>
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<tr>
<td>CIN vs MSE</td>
<td>0.74</td>
<td>0.32</td>
<td>0.062</td>
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<td>CIN vs ISM</td>
<td>0.17</td>
<td>0.56</td>
<td>0.013*</td>
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<tr>
<td>ISM vs MSE</td>
<td>0.15</td>
<td>0.20</td>
<td>0.99</td>
</tr>
</tbody>
</table>

**Fig. 8** Calculated fractal dimension plotted for all the tissue samples using the region of interest chosen and thresholded to provide 50% white and 50% black binary images, using the data from (a) the red channel, (b) the green channel, and (c) the blue channel. The typical appearance of the images after thresholding can be seen in the 3rd column of Figure 2. The box counting method was used to calculate fractal dimension, as described in the text.

**Fig. 9** Calculated Euler number for all of the image regions of interest using (a) the red channel, (b) the green channel, and (c) the blue channel. The Euler number was calculated from the thresholded binary images as shown in the 3rd column of Figure 2, and is defined as the number of black objects minus the number of white holes within the black objects.

**Table 5** Calculated probabilities that the data sets do not have different mean values, using an unpaired students T test to compare the data of Euler number, as plotted in Figure 8. Abbreviations are as in Table 2, and statistically significant differences are denoted with an asterisk (*)
tures associated with malignant changes in the cervix, and (v) potentially enhancement of the pertinent clinical features through online image preprocessing. The type of color analyses completed here are similar to endoscopy studies which have shown promise in diagnosing colon polyps based upon color changes in the RGB values from video images. Similarly the feature analysis here using fractal dimension is based upon other studies which showed that these can be representative of malignancy based upon the complexity of the blood vessel patterns developed. Enhancement of the pertinent features of an image has also been demonstrated in endoscopy by Nishioka and Mycek.

In epithelial tissues, many diseases are characterized by abnormalities having different blood volume content than normal mucosa. In the visible region of the electromagnetic spectrum, hemoglobin in the blood is the major chromophore in soft tissues, with two broad absorption bands in the visible spectrum roughly corresponding to the wavelengths of "blue" and "green" colors of light. Therefore, when the cervix is viewed under white light during endoscopy, the hemoglobin underlying the epithelial cell layers absorbs most of the incident blue light and green light, while scattering most of the incident red light back to the camera. For this reason, the cervix looks generally pinkish-red in color and variations in the local blood vessel density or epithelial cell layer thickness appear as changes in color relative to normal tissue. Thus, glandular epithelium, which has only one layer of columnar epithelium overlying its vasculature usually appears "redder" than normal tissue. The islands of epithelial tissue which are white upon the background of glandular and vascular tissue provide the patterns which can be interpreted with metrics such as the fractal dimension or the Euler number.

A study of the RGB color patterns in the three different tissue types studied here disappointingly indicates that discrimination of immature squamous metaplasia from CIN 2/3 is difficult, and likely impossible, simply based upon the relative RGB values or even upon the variance in these values. This finding is perhaps not surprising considering that the diagnosis of CIN 2/3 is based on a constellation of visual clues that can be extremely different from patient to patient. However, some fundamental insight about these different tissues can be gleaned from these comparisons, in that the RGB channels of the images yield distinctly different information about the tissue layers. For example, the blue light image discriminates surface cell tissue layers and also provides good contrast between epithelial tissue and blood vessels, while green light discriminates blood vessels very well from all other tissues, and red light provides much less contrast between blood and tissues. Based upon these observations, we hypothesized that the blue light channel of the images would provide the best discrimination between CIN 2/3 and immature squamous metaplasia. The ability to discriminate between features within the transition zone and the mature squamous epithelium may appear trivial to the experienced colposcopist, however achieving this in a robust automated manner with image processing is not always as trivial. Therefore the analysis in Figure 3, and Table 2 indicates that the variance in either the green or blue channels over a region can be used to segment these two different tissue types. Since this is such an easy feature to calculate, it can be a reliable and robust method for future use.

The fractal dimension is an obvious choice of a feature which is well understood in image processing, and has definitively been shown to be a reliable indicator of blood vessel pattern complexity. In this analysis, the fractal pattern was not a useful method to discriminate between tissue types, mainly due to the problems of automated thresholding of the images. The fractal analysis requires that RGB images be turned into thresholded binary images before the box counting method can be applied. In our case, to be as objective as possible, the thresholding was done with each image producing 50% black pixels and 50% white pixels automatically. In these images, variations in the light intensity produced complexity patterns even in the squamous epithelium regions of

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**Fig. 10** (a) Calculated Euler number for the immature squamous metaplasia ROI samples as a function of the level of thresholding in the image, for blue channel images only. (b) Calculated Euler number for the CIN 2/3 ROI samples as a function of threshold value, for the blue channel only. A comparison of the two graphs indicates that maximal difference between the two graphs would be between threshold values of 0.2-0.5.
the image. Thus light variations across the image can confound the feature analysis of fractal dimension, making this a less reliable metric to discriminate between tissues. This problem is clearly an artifact which can be eliminated in future work either through careful analysis of tissues which are only in a certain field of the image, or through the use of online image enhancing processors.17 One improvement to our thresholding would be to use more sophisticated methods of threshold choice, such as Max–Lloyd quantization, which is used to separate the histogram of gray-scale values into two regions, and the threshold is chosen at the minimum between these two regions. This latter method could provide a more robust method for choosing the threshold level, assuming that the images are all bilobal in histogram gray-scale values.

Fractal dimension calculated by the box counting method may also be related to the calculation of spatial frequency slope, as calculated from the Fourier transformed data. However there does not necessarily have to be a direct relation here since the former was calculated on thresholded images whereas the FFT was completed on gray-scale images. While neither of these two methods provided a clinically useful method of separating CIN 2/3 regions from squamous metaplasia, further exploration of the relative differences between these two methods may elucidate which is the more robust measure of fractal dimension calculation. It is still controversial whether angiogenesis is a good predictive indicator of cervical intraepithelial neoplasia19 so that at this time it is not obvious if the fractal dimension as calculated from the vascular pattern would be a good predictor. However in other tumor sites there is clear evidence of angiogenesis marking carcinoma, so further investigation of this fractal number seems warranted. Perhaps the most confounding problem for this analysis is that the potential fractal pattern of the vasculature is overlaid with larger regions of epithelial tissue, thus potentially obscuring the blood vessel pattern.

The feature analysis obtained with the calculation of the Euler number appears more promising for a metric which is able to discriminate between all three tissue types compared here. The separation of mature squamous epithelium from CIN 2/3 or immature squamous metaplasia could be achieved with this metric. The discrimination of CIN 2/3 from immature squamous metaplasia was not statistically significant (\(P = 0.08\)) but is potentially an indicator that this can be a first order method to categorize the complexity of the image. It is not realistic to expect that an automated feature extraction algorithm can always separate CIN 2/3 from immature squamous metaplasia, especially since this cannot always be done by the experienced colposcopist unless biopsy confirmation is used. The eventual goal of this work is to provide some objective metric which discriminates between immature squamous metaplasia, CIN 1 and CIN 2/3 with at least equal to or better specificity than standard colposcopy. This will be tested in further work involving a larger cohort of patient images, and confining the feature analyses used to those which have shown the most promise in this study.

5 Conclusions

The most important result of this study is that a feature analysis based method can distinguish between images from patients with CIN 2/3 versus images from patients with immature squamous metaplasia. In this analysis, comparisons were made with images of squamous epithelium as well simply to provide a tissue type with much simpler features. While many of the methods tested were able to distinguish between SE and SM or SE and CIN 2/3, these are not useful methods for clinical use. The calculation of Euler number was the only statistically significant way to differentiate between CIN 2/3 and SM. The CIN 2/3 images had a higher Euler number than the SM images, as measured from the blue image only. The Euler number is a measure of the number of objects in the image, minus the number of holes in those objects. Therefore the CIN 2/3 images had a higher number of surface tissue objects on a background of darker blood tissue, for the samples used here. Further study of this feature analysis is needed on a larger image data base to determine the sensitivity and specificity of this method.

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