

# Multimodal multispectral imaging of the cervix *in vivo* for the detection of neoplasia

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## ABSTRACT

Optical spectroscopy has been shown to be an effective method for detecting neoplasia of epithelial tissues. Most studies to date in this realm have applied fluorescence or reflectance spectroscopy alone as a preferred method of disease detection. We have been developing instrumentation which can acquire both reflectance and fluorescence images of the human cervix *in vivo*, with the goal of combining multispectral information from the two spectroscopic modalities. This instrumentation has been tested on a group of patients in a clinical setting. We have applied spectral and spatial analysis techniques to the acquired images to assess the capabilities of this technology to discriminate neoplastic from normal cervical tissue.

Keywords: cervix, neoplasia, fluorescence, reflectance, spectral imaging

## 1. INTRODUCTION

Cervical cancer is one of the most common malignancies in women, represented annually as at least 370,000 new cases globally<sup>1</sup> and as 12,900 new cases in the US alone<sup>2</sup>. The implementation of annual screening using the Papanicolaou (Pap) smear has significantly reduced cervical cancer mortality rates in the US, primarily by identifying women with precancerous changes, also known as cervical intraepithelial neoplasia (CIN) or, more concisely, cervical dysplasia. Despite the success of the Pap smear in reducing mortality rates, the test has demonstrated high false positive rates, which causes women with benign conditions to unnecessarily proceed to more expensive and invasive procedures. In a meta-analysis conducted by Fahey et al<sup>3</sup>, they reported an average sensitivity of 58% (95% CI 49-67%) and average specificity of 69% (95% CI 62-77%) of the Pap smear for detecting dysplasia in a screening setting. A more accurate, point-of-care test for cervical dysplasia has the potential to not only improve cervical cancer prevention but also reduce costs of health care.

Various forms of steady-state optical spectroscopy have been shown to be efficacious in the detection of CIN, as well as similar abnormalities of other epithelial tissues<sup>4-14</sup>. Fluorescence-based methods<sup>4-11</sup> and diffuse reflectance (or elastic scattering)-based methods<sup>12-14</sup> are among the most common spectroscopic techniques in this area. Nearly all studies have exploited either fluorescence or reflectance alone to provide information about the tissue relevant to the presence of disease. Measuring fluorescence and reflectance concurrently could offer a more complete picture of the biochemical and morphologic conditions of the interrogated tissue.

We have developed instrumentation to acquire both fluorescence and reflectance images of the cervix *in vivo* at a number of different ultraviolet (UV) and visible wavelengths. This multimodal multispectral imaging (MMI) system has been used in a clinical setting to acquire images from a cohort of patients with a broad distribution of cervical abnormalities, including those with a normal cervix and those with various grades of CIN. We have analyzed the images from these patients and present results of the analysis which indicate the ability of our system to discriminate normal from dysplastic tissue.

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## 2. MATERIALS AND METHODS

### 2.1 Instrumentation

Wavelength bands for fluorescence imaging were chosen based on the excitation and emission wavelengths of endogenous fluorophores present in cervical epithelium. Fluorescent species include tryptophan, collagen, elastin, reduced nicotinamide adenine dinucleotide (NADH), oxidized flavin adenine dinucleotide (FAD) and porphyrins. A number of studies have shown that the measured fluorescence intensity from these fluorophores may be significantly modulated by the onset and progression of dysplasia<sup>4,7,8,15,16</sup>.

The diffuse reflectance properties of the cervix, as with most other human tissues, are dominated by hemoglobin absorption. Because increased vascularity has been associated with dysplastic processes, we considered it essential to acquire reflectance images at one or more visible wavelength bands corresponding to the absorption peaks of hemoglobin. We also wanted to acquire reflectance images at other wavelength bands across the UV-visible spectrum, especially those not as strongly affected by hemoglobin, wherein effects such as epithelial scattering might indicate the presence of dysplasia.

We designed and constructed an imaging system capable of providing illumination and detection at a variety of UV-visible wavelength bands and suitable for use in a clinical setting. A layout of the optical system is shown in Figure 1. The illumination path consists of a xenon flashlamp as a broadband illumination source followed by a collimating mirror, optical filter, and finally a beamsplitter which directs light to the tissue. Fluorescent or reflected light passes back through the beamsplitter, followed by an optical filter and then an imaging lens system which places an image onto a CCD camera (512 x 512 pixels). Motorized filter wheels were used to independently select both illumination and collection filters. A tube was placed in front of the foreoptic to shield the exposed light path from ambient light.

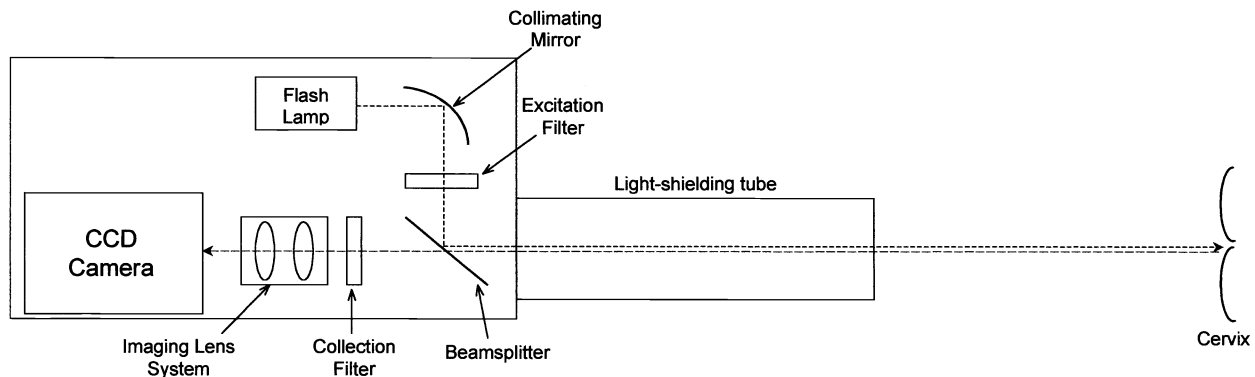


Figure 1. Optical system layout.

All the major electro-optical components of the MMI system were mounted in a contained unit on top of a vertical pole stand with wheels so it could be positioned in front of the patient like a standard colposcope used to examine the cervix. The imaging system could be manipulated via height and tilt adjustment. The stand was tethered to a base unit which contained power supplies, control circuitry, and a computer. Custom software was written to provide a simple user interface for system control and data acquisition. Ten fluorescence images and seven reflectance images were acquired per patient, with a total image acquisition time of approximately 1.5 minutes.

Because our system uses UV excitation wavelengths, we conducted an analysis to ensure patients would not be exposed to unsafe levels of UV light during the course of measurements. For the benefit of researchers developing electro-optical systems for detecting CIN, the Food and Drug Administration (FDA) has published a draft guidance document<sup>17</sup> which recommends a UV exposure limit for cervical tissue. This limit is equivalent to a threshold limit value set by the American Conference of Governmental Industrial Hygienists (ACGIH) for UV light incident on the eye and skin by non-laser light sources. The FDA draft guidance document states specifically that the device emission spectrum weighted with the action spectrum published by the ACGIH should be less than 3 mJ/cm<sup>2</sup>. Upon measuring the output of the MMI system at the UV

excitation wavelengths, we determined that the effective radiant exposure of our system was approximately  $0.5 \text{ mJ/cm}^2$ , well below the threshold limit value.

## 2.2. Clinical data collection

After obtaining institutional review board-approved informed consent, non-pregnant women 18 years of age or older with an intact cervix scheduled for colposcopy were enrolled in our study. Colposcopy involves examination of the cervix *in situ* with low power magnification (3X-15X), typically after applying acetic acid to the cervix. Acetic acid acts as a contrast agent by temporarily whitening (acetowhitening) areas which tend to be suspicious of CIN. The colposcopic examination included digital color image capture with a video colposcope for documentation and data analysis purposes. When clinically indicated, either a biopsy was performed if CIN was suspected, or an excision treatment was performed to remove already-confirmed dysplastic tissue. Some patients were also scheduled for hysterectomy, usually for reasons unrelated to dysplasia or cancer. All biopsies and excised specimens underwent histologic analysis by pathologists at the clinical site.

Image acquisition with the MMI system took place before colposcopy. After the vaginal speculum was inserted, but prior to image acquisition, a mark was placed on the cervix to ensure all the images could be properly registered. The MMI system was then positioned so that the front of the tube was just inside the opening of the speculum and a test image of the cervix appeared in focus. Figure 2 shows some typical images acquired with the MMI system. Because we acquired images before colposcopy, they were without the contrast enhancement offered by acetic acid. For the purposes of this study, we wanted to determine if the native, unbiased properties of cervical tissue could provide adequate information to permit detection of dysplasia. In addition, since acetowhitening is a transient phenomenon typically lasting only several minutes, there was concern that there would be variable amount of acetowhitening influencing each image in an acquisition sequence.

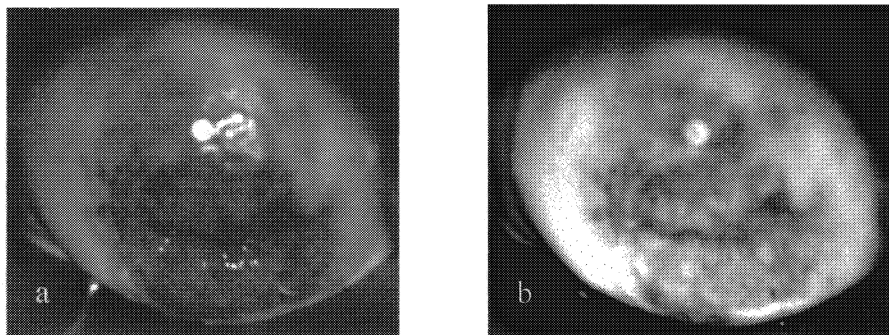


Figure 2. MMI system images of cervix *in vivo*: (a) reflectance image, (b) fluorescence image.

## 2.3. Data analysis

Images from 34 patients have been analyzed for this study. This group consists of 18 with a normal cervix, eight with only non-dysplastic lesions (metaplasia, inflammation), and eight with dysplasia. Of the dysplastic cases, one had only mild dysplasia (CIN 1, low grade) and seven had moderate to severe dysplasia (CIN 2+, high grade). Two of the CIN 2+ cases also had CIN 1 lesions, and another two of the CIN 2+ cases also had non-dysplastic lesions. The presence of dysplasia was always confirmed by histopathology; however, histologic specimens were typically not available to confirm non-dysplastic or normal conditions. Normal epithelium and non-dysplastic lesions were identified by colposcopy.

For each patient, the colposcopist drew a detailed colposcopic impression directly on the captured digital color image. The boundaries of any dysplastic or non-dysplastic lesions, as well as the boundary between the two normal cervical epithelial types (squamous and columnar) were recorded. This information was then transferred to the MMI system image set to delineate image regions and classify each region into one of five categories: normal squamous, normal columnar, non-dysplastic lesion, CIN 1 lesion, CIN 2+ lesion. It should be noted nearly every cervix contained multiple regions, and many times more than one category was present. During this classification process, image areas were also excluded from subsequent analysis for any of the following reasons: non-cervical tissue, registration mark, excessive blood or mucus, specular reflection artifact (CCD saturation), unknown/uncertain classification.

Prior to applying quantitative analysis techniques, images were first corrected by subtracting the offset introduced by the CCD. In addition, 5 x 5 pixel binning was applied to all images for data smoothing and data reduction purposes. Any mentions of the term “pixel” in the remainder of this paper are referring to the average of a 5 x 5 pixel block. Table 1 lists the number of patients, regions, and pixels in each category that were analyzed in this study.

	<b># Patients</b>	<b># Regions</b>	<b># Pixels</b>
<b>Normal squamous</b>	34	44	24372
<b>Normal columnar</b>	9	11	551
<b>Non-dysplastic</b>	10	16	386
<b>CIN 1</b>	3	5	254
<b>CIN 2-3</b>	7	8	836

Table 1. Sample sizes for this study.

An expected source of intensity variability in the images was that arising from intrinsic differences in fluorescence yield between patients. This variability is unrelated to and can be larger than the fluorescence intensity differences between dysplastic and normal tissue. To investigate the possibility of reducing the effects of this variability, we generated a complementary set of images in which each pixel was normalized by the mean intensity of all pixels located on normal squamous epithelium from the same image. Normal squamous epithelium was selected as the normalization category because every cervix in the study population, and practically every cervix in the general population, contains some amount of this native epithelium.

Because the cervix has an irregular surface and its anatomical orientation varies significantly between patients, we expected and observed spatial intensity variations unassociated with system variability or relevant biological factors. In other words, there was intra- and inter-patient variability of excitation-collection geometry in every image. Therefore, we believed our best opportunity to extract useful quantitative information from the images was by analyzing ratio images, i.e. one image divided by another, pixel-by-pixel.

For both the original and squamous normalized image sets, all possible ratio images (excluding inverses) were generated from the ten fluorescence and seven reflectance image types, giving rise to 272 total ratio images per patient. For each ratio image type, all pixels from all patients together were then grouped by category. After separating the categories into two groups (i.e. normal squamous vs. all others, non-dysplasia vs. dysplasia), we applied the Mann-Whitney test, a non-parametric analog to the Student’s *t*-test. The p-value from this test provided a metric which indicated how separate the two groups were for each ratio image type. The ratio image types were then ranked by p-value in order to select one or more types for basic discrimination algorithms.

### 3. RESULTS

We first wanted to determine an algorithm using the original images to discriminate normal squamous epithelium from all other tissue types. This was expected to be the simplest of our tasks at hand, because normal squamous can be visually identified on the cervix by even a minimally trained eye. Combining two ratio image types provided improved discrimination most critically between normal squamous and dysplasia, and also separated normal columnar and non-dysplastic lesions from normal squamous as well. Figure 3 plots all the pixels from the two ratio image types in feature space. With the decision lines shown, the discrimination performance of non-squamous normal (positive) from squamous normal (negative) was 87% sensitivity and 88% specificity.

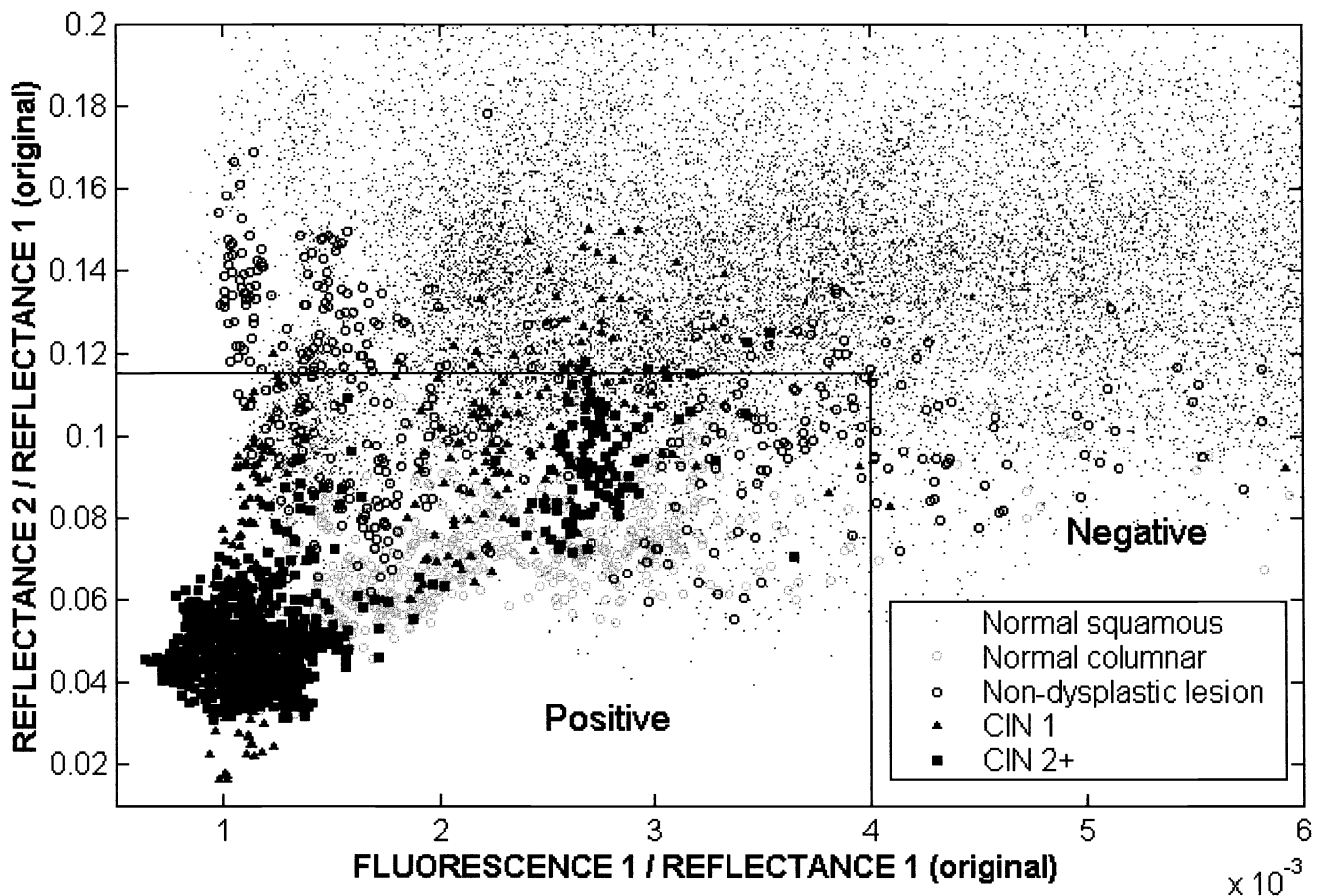


Figure 3. First algorithm using two ratio images to discriminate squamous normal tissue from all other tissue types.

Having completed our simpler task, our second task was to discriminate the remaining non-dysplastic pixels from the dysplastic pixels. Two different ratio image types were found, one from the original image set and the other from the squamous normalized image set, which again provided improved discrimination when combined. Figure 4 plots all the pixels from the two ratio images, again in feature space. Note that only pixels called positive by the first algorithm are plotted here, including normal squamous false positives. Similarly, pixels called negative by the first algorithm are excluded here, including dysplastic false negatives. For the decision line shown, the performance of this algorithm is 86% sensitivity and 86% specificity. The combined performance of these two algorithms to discriminate dysplasia from non-dysplasia is 83% sensitivity and 98% specificity.

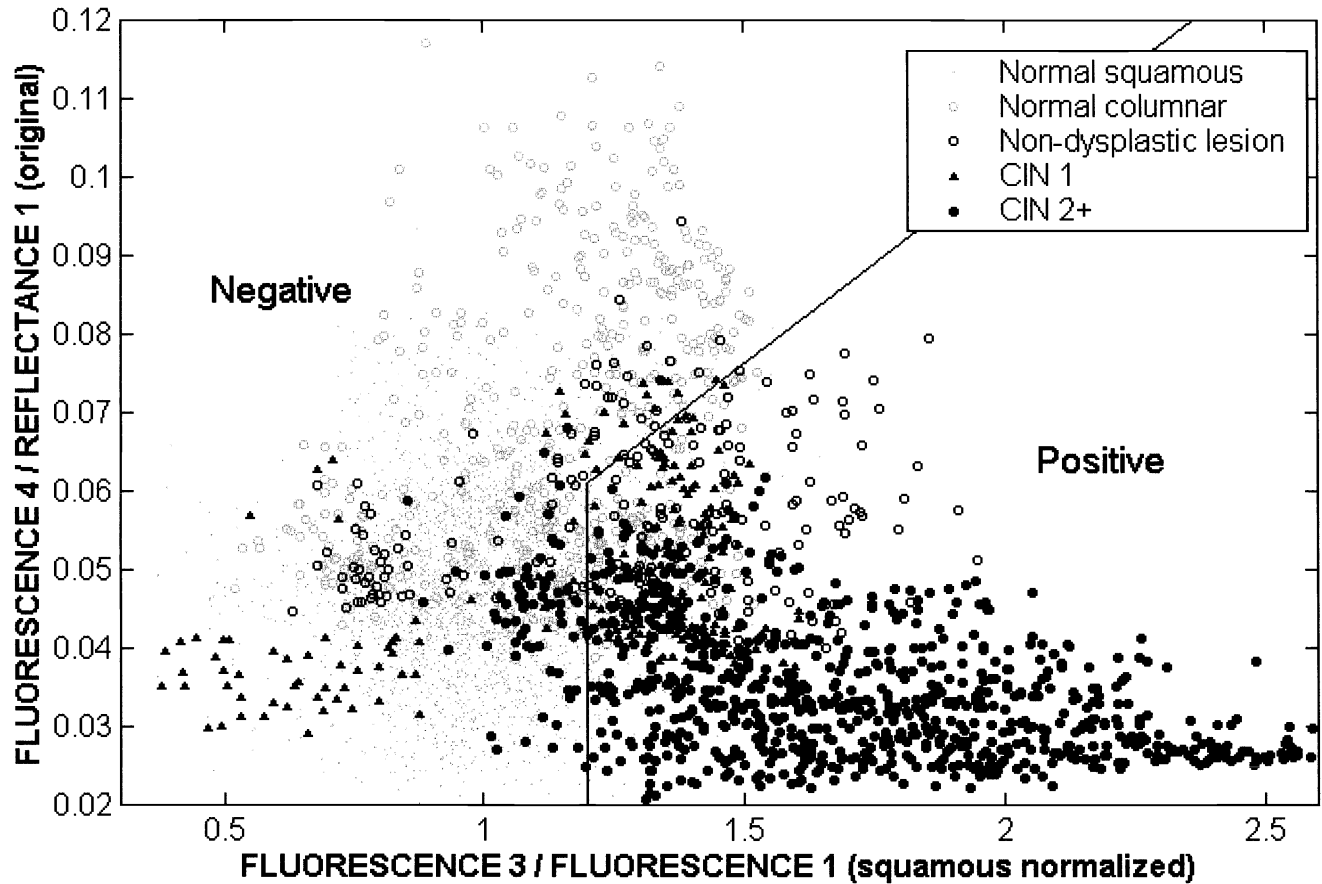


Figure 4. Second algorithm using two ratio images to discriminate dysplasia from non-dysplasia.

#### 4. CONCLUSIONS

With basic analysis approaches, we were able to develop simple algorithms to demonstrate feasibility of discrimination between non-dysplastic and dysplastic tissue in this data set. The algorithms incorporated both fluorescence and reflectance information, thereby exploiting the multimodal capability of the MMI system. It is important to note that this level of discrimination was achieved even without the use of acetic acid, which is known to be essential for visual assessment of the cervix at colposcopy and has been shown benefit spectroscopic techniques<sup>18</sup>. The sensitivity and specificity as demonstrated in this study can potentially reduce the number of false positive cases and improve the detection rate of cervical dysplasia.

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