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The Impact of Room Light and Speculae on Spectroscopic Measurements for Screening for Cervical Cancer: an Experiment Using a Simulated Gynecological Patient

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ABSTRACT

Fluorescence spectroscopy has been investigated as a means of detecting oral, lung and cervical neoplasia. However, there are many issues in developing a clinical protocol for routinely making such measurements. We conducted an experiment with a simulated gynecological patient to study the effect of room light and speculum type. The speculum is needed for the medical provider to access the cervix. We investigated whether: (1) room lights present or absent had an effect and (2) the type of speculum (no speculum, coated speculum, or metal speculum) made a difference. In recent years, simulation-based medicine has gained prominence in clinical science. A simulated gynecological model was employed as a surrogate for a human cervix, which provides a physical environment similar to the clinical setting and mitigates ethical considerations. Measurements on fluorescence standards placed inside the simulated patient were made at all combinations of lighting and speculum type. Both light and the type of speculum were significant factors, and there was a statistically significant interaction between the two factors. When the room light was absent, the measurements made with either the metal speculum and the coated speculum did not exhibit significant differences, so either type of speculum may be used when the lights are absent. When the lights are present, the coated speculum shows less room light contamination in the measurements. Because the room light can contaminate the fluorescence spectroscopic measurements, making the measurement with room lights absent is highly recommended.

Key words : Cervical cancer, Fluorescence spectroscopy, Optical medical device, Simulation medicine, Statistical interaction

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Introduction

Cervical cancer remains a major epidemic worldwide, particularly in developing countries [1]. The current standard of care for screening in developed countries - Papanicolaou smear and/or HPV test, which if positive is followed by colposcopy and possible biopsy - may be impractical in low-resource settings [1,2]. Due to these challenges, much research has been devoted to finding more cost-effective methods for cervical cancer screening and diagnosis that can be used in low resource settings.

Fluorescence spectroscopy has shown promise for real-time diagnosis of cancer and pre-cancer [3-7]. We have developed an optical device, the Fast Excitation-Emission Matrix (FastEEM) system that measures fluorescence using a point probe placed in contact with the tissue. Studies have shown that fluorescence in tissue comes from several biologically relevant fluorophores including NADH, FAD, tryptophan, and collagen [8,9]. Tissue measurements of fluorescence also show absorption, particularly from hemoglobin. We routinely measure standards (water, exalite, coumarin, rhodamine, etc.) both for quality assurance and calibration. Because of the inherent biological variability in the human measurements, it is desirable to measure standards when doing experiments [10]. All of the results reported here are based on measurements of a rhodamine standard with peak emission wavelength of 580 nm.

We have made spectroscopic measurements in clinic with the lights off because it has been suspected that room light may contaminate the measurements. In addition, a previous study determined that patients preferred the darker setting for the spectroscopic measurements [11], but it does create difficulties for the medical provider making the measurements. The current protocol is for the provider to identify the measurement location on the cervix with the lights on, place the probe at the selected location, and then turn the lights off to make the measurement. The current device has an external lamp that illuminates the cervix between the measurements. Clearly there is some inconvenience from repeatedly turning the lights off and on. The experiments reported here were made to determine if the lights off requirement is in fact necessary. In addition, it was of interest to assess whether the type of speculum used makes a difference in the measurement. Two types of speculae are commonly used in clinic: a highly reflective metal speculum or a plastic-coated speculum.

Because of inherent variability in human measurement and the issues involved with making measurements over several hours, it was desirable to conduct an experiment on a model human cervix. To this end, we obtained a simulated gynecological model (a medical simulation mannequin) in which fluorescence standards were placed and measurements made in a manner very similar to clinical practice. The use of such models has become common in simulation-based medicine and medical training.

Materials and Methods

1. Spectroscopic Measurement and Instrumentation

Fluorescence spectroscopy works by illuminating the sample with excitation light and collecting the emitted fluorescent light at longer wavelengths than the excitation light. Both the illumination and collection of the light were done with a point probe. Our devices utilized 24 excitation wavelengths ranging from 300 nanometers (nm) to 530 nm in increments of 10 nm. For this experiment, to decrease the measurement time, we chose to measure 6 excitation wavelengths (330 to 480 nm, with increments of 30 nm). We believe that this subset should capture any important effects. The wavelength of fluorescence light coming back into the probe is referred to as the emission wavelength. The range of emission wavelengths is different for each excitation wavelength. The colored areas in the plots in Fig. 3 depict these different ranges.

The FastEEM consists of a main box and a fiber optic probe attached to the box. The probe has separate fibers for illumination and collection. More complete descriptions of the device may be found in [7,12,13].

2. Description of the Simulated Gynecologic Patient

In recent years, simulation-based medicine has gained prominence in clinical fields, especially in medical education. Many procedures can be performed on simulated patients or on computerized systems to simulate real medical practice. We used a mock patient to simulate a patient measurement as closely as possible, particularly as we are interested in light that enters the region where the measurements are made. Fig. 1 shows the model and speculae employed in the experiment.

3. Description of the Experiment

We compared the measurements in six settings: (1) room light off and no speculum, (2) room light off and metal speculum, (3) room light off and coated speculum, (4) room light on and no speculum, (5) room light on and metal speculum, and

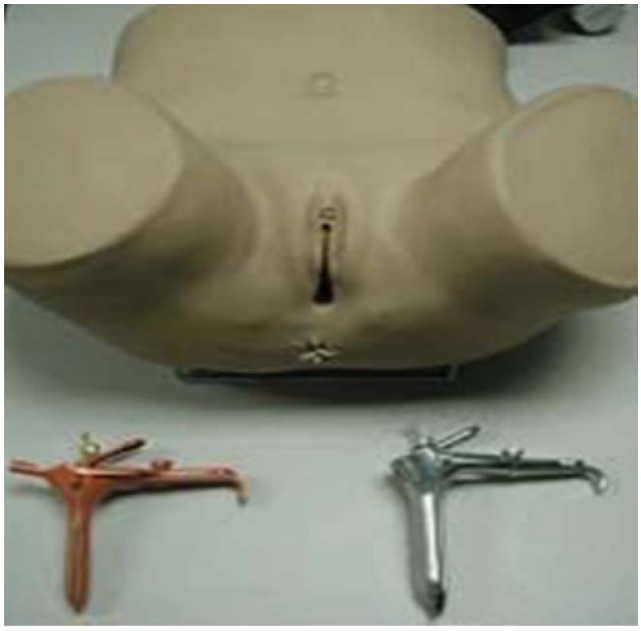

Fig. 1. The simulated gynecologic patient, shown with coated (left) and metal (right) speculum. Patient Model: S504.100 ZOE (Gaumard Scientific; Miami, FL).

(6) room light on and coated speculum. The FastEEM instrument was turned on for a one-hour warm-up. The FastEEM device was positioned next to the simulated gynecologic patient and the rhodamine standard was placed in a foam holder that was put inside the simulated patient in the place of cervix, 14 cm from the introitus. For each light-speculum combination, we inserted the probe into the simulated patient and placed it in contact with the standard (Fig. 2). We obtained five consecutive measurements, removed the probe and placed it back onto the standard and obtained five more consecutive measurements, and removed and replaced it again for a final set of five measurements. Thus, we obtained a total of 15 measurements for each of the 6 light-speculum combinations giving 90 measured EEMs. We randomized the order of the six setups to avoid selection bias. Hence, this was a statistically sound experiment with balance, replication and randomization all considered in the design. Such experimental designs simplify the statistical analysis [14] and provide more accurate results.

4. Data Processing

The raw data were processed using the following steps. A background measurement with the FastEEM device's lamp shutter closed is taken just prior to the sample measurement. The background is subtracted from the sample measurement. The wavelengths are calibrated on a daily basis using a mercury-argon (HgAr) lamp, and the values are interpolated to a 1 nm wavelength grid. The spectrum is divided by the illumination intensity measured by a power meter internal to the device. Finally, the data for each excitation wavelength are smoothed to remove additional noise.

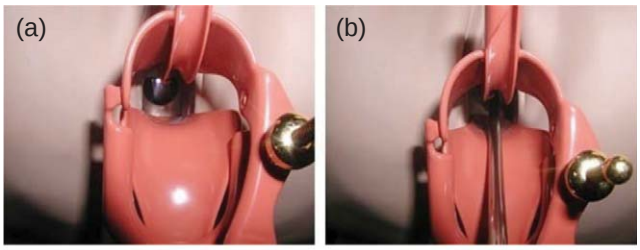

Fig. 2. Photographs of (a) the placement of the rhodamine standard (faint pink area below the bright specular reflection from the glass cuvette) inside of the simulated gynecologic patient, and (b) probe inserted into the simulated gynecologic patient in contact with the rhodamine standard.

5. Statistical Methods

Heat maps were used to visualize differences in mean intensities among the six settings (no speculum/lights off, metal speculum/lights on, etc.), as shown in Fig. 3. The color coding progresses from dark blue → light blue → green → yellow → orange → red, respectively, from low to high values. It might be appropriate to consider the entire spectra and use advanced statistical methods [10,15]. However, it can easily be seen in Fig. 3 that the differences occur primarily at peak emission wavelength, 580 nm, and the other regions of the EEM plots exhibit very similar intensity patterns. To test for the significance of light and speculum, we used multivariate analysis of variance (MANOVA) with peak intensities at the six excitation wavelengths. For the MANOVA test statistic, we chose the Wilk's lambda [16], which is widely used in practice. For the present study, there are 2 factors of interest: light and type of speculum. We tested both the main effects and the interactions for statistical significance. The normality and equal variance assumptions were satisfied for all the MANOVA models. All statistical analyses were done in the R statistical computer package (available free from http://cran.rproject.org/).

Results

Since the background measurement contains room light that is leaking into the probe, one might surmise that it is possible that the background subtraction would remove the room light

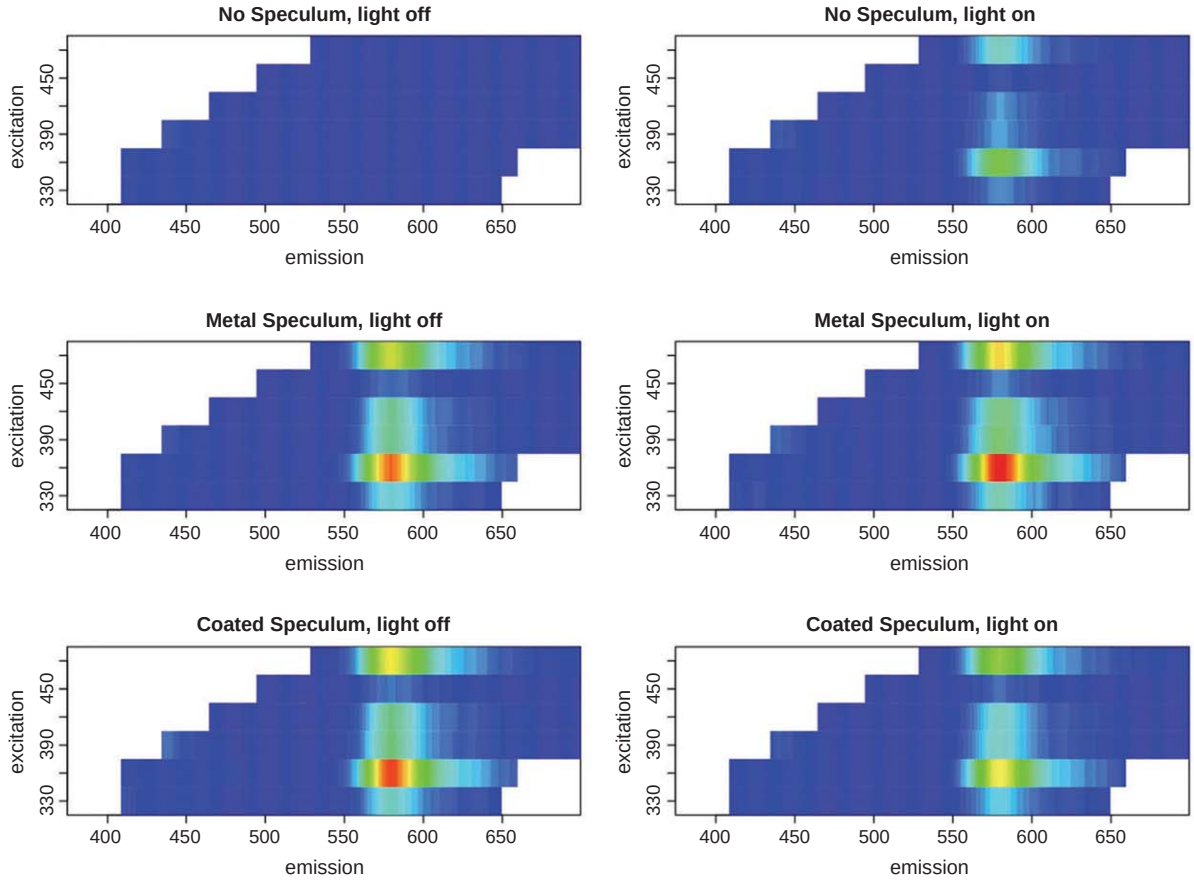

Fig. 3. The EEM plots of mean intensities. The three rows are used to compare the type of speculum, while the two columns are used to compare the lights on or off. All plots have a common scale.

effects. However, Fig. 4 shows that there is mixed success for this. The green line shows the way the background subtraction have worked in many cases, but we have also included some extreme cases such as red and blue lines where the current background subtraction have not fully calibrated the signals. Sometimes the room lights seem to cancel out, but for many measurements the background measurement of room light intensity has a different magnitude than that of the standard measurement. Since the exposure times for standards measurements are about 100 ms., which is shorter in duration than patient measurements (about 1 s.), we expect there could be more magnitude variation in the background measurement of room light of the patients. In the end, the room light leakage after the background subtraction seems to have averaged out, as seen in Fig. 3.

In Fig. 3, we observed differences between lights on and off, for any type of speculum, primarily at peak wavelength at 580 nm. At the same time, the speculum types exhibited different patterns as well. To analytically confirm these observa-

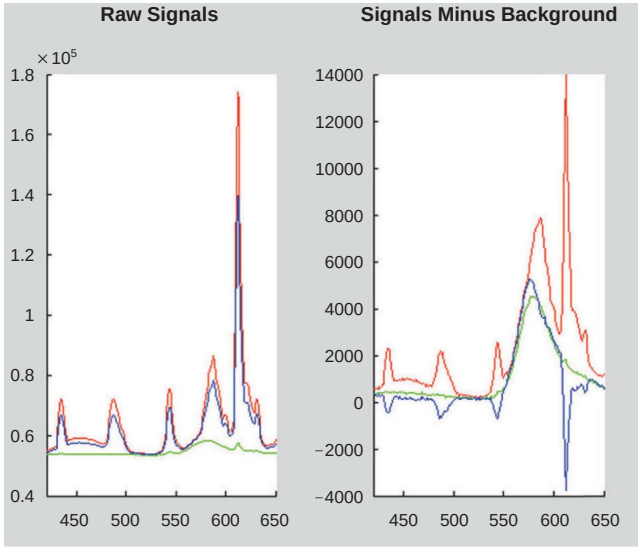

Fig. 4. Three individual measurements at excitation 330 nm, before and after background subtraction. The green line shows the way the background subtraction have worked in many cases. The red and blue lines show extreme cases where background subtraction has not the desired effect but seems to have canceled out on average.

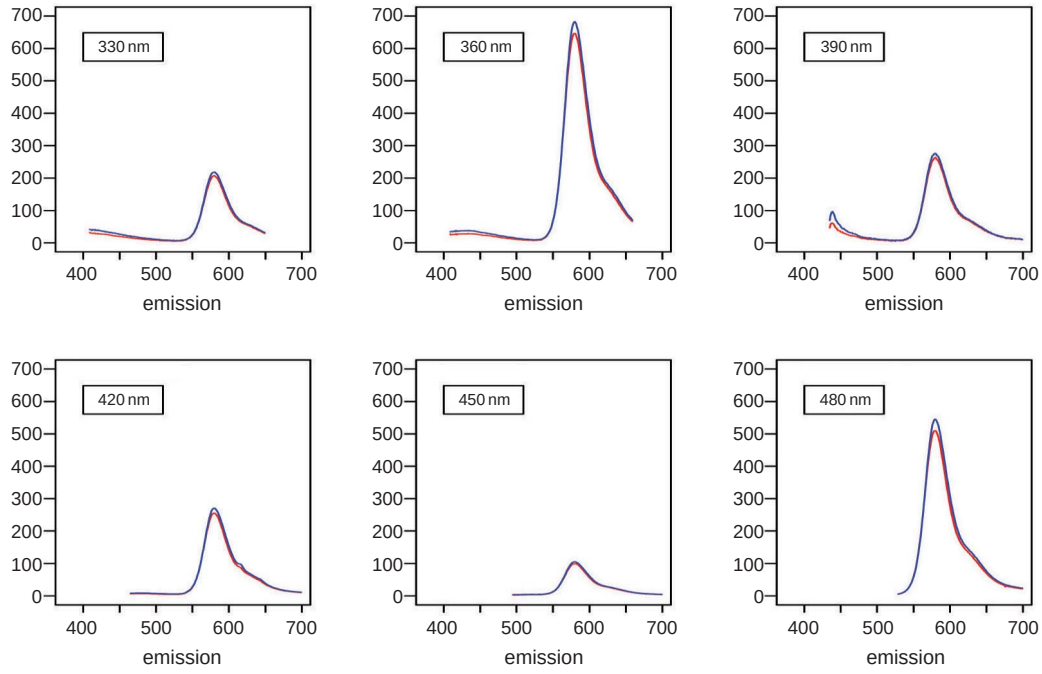

Fig. 5. The line plots comparing mean intensities of metal and coated speculae (when the light are off). The blue curves correspond to the coated speculum and the red curves to the metal. The excitation wavelengths are shown in the figure captions. The y-axis units are relative intensity.

tions, we used the MANOVA model with 2 factors - light (on or off) and speculum (none, metal, or coated), using peak emission wavelength intensities at the six excitation wavelengths as the response (six-dimensional vector). In other words, the model is given as

$$\text{Response}_{ik}=\mu+\text{Speculum}_j+\varepsilon_{ik},\ i=1,\ 2,\ k=1,\ \cdots,\ 15.$$

$$\text{Response}_{ijk}=\mu+\text{Light}_i+\text{Speculum}_j+\varepsilon_{ijk},\ i=1,\ 2,\ j=1,\ 2,\ 3,\ k=1,\ \cdots,\ 15.$$

We found that both light and speculum were significant factors ($p<0.01$). It was worth noting that, although having no speculum was not of practical concern since the actual patient measurement must be made with a speculum, it nevertheless highlighted the contrast between presence and absence of room light. We noted that when no speculum was used, having the lights off resulted in low-intensity, flat measurements, whereas having the lights on clearly showed a signal (Fig. 3).

We also considered if there was any interaction between two factors, Light and Speculum. In this setting, we have the model

$$\text{Response}_{ijk}=\mu+\text{Light}_i+\text{Speculum}_j+\text{Light}*\text{Speculum}_{ij}+\varepsilon_{ijk},\ i=1,\ 2,\ j=1,\ 2,\ 3,\ k=1,\ \cdots,\ 15.$$

Here, we saw that both factors were significant, but we also saw that their interaction was significant as well ($p<0.01$). See Table 1. In Fig. 3, it is evident that with no speculum or a metal speculum, the average intensities were higher when the lights were present than when lights were absent. However, the situation was reversed for the coated speculum, where the lights-off case showed higher average peak intensity than the lights-on.

Table 1. The MANOVA analysis results for a model that fit both light and speculum, and their interactions

Source	F statistic	Numerator degrees of freedom	Denominator degrees of freedom	p-value
Light	4.37	6	79	<0.01
Speculum	24.88	12	158	<0.01
Interaction	6.70	12	158	<0.01

Therefore, speculum type "interacted" with presence of room light, meaning that different speculum types had different mean responses than expected from adding the main effects of light on/off and speculum type. This was an interesting observation, which might warrant further investigation.

There was an important exception to the above results. Fig. 3 suggested that, when the lights were off, there might be no differences between metal and coated speculum. Fig. 5 shows line plots comparing metal speculum and coated speculum

Table 2. Comparing metal and coated speculum, when the lights are off, by MANOVA

Source	F statistic	Numerator degrees of freedom	Denominator degrees of freedom	p-value
Speculum	1.20	6	23	0.34

with lights off, which confirms the result of Fig. 3. The statistical model to be considered is MANOVA, but this time it will be one factor only (one-way MANOVA, see Table 2). The response is still the six-dimensional peak intensities as before, but we now have the subset of original data, with Lights Off and two types of Speculum (metal and coated only), so that n=30. Hence the model is now

$$\text{Response}_{ik}=\mu+\text{Speculum}_j+\varepsilon_{ik},\ i=1, 2,\ k=1, \cdots, 15.$$

Applying MANOVA to the peak intensities at 6 excitation wavelengths with lights off resulted in no significant difference between the two types of speculum (p=0.34, Table 2).

It is of interest to know the actual percentage differences between the coated and metal speculae. With the room lights on, the metal speculae ranged from 23% to 26% increased peak intensity across the six excitation wavelengths. With lights off, the increment in intensities of the metal speculae varied from −7% to a −5%, which as noted, was not statistically significant.

Conclusion and Discussion

By comparing the results for presence or absence of room light, it was determined that the room light can easily contaminate the measurement. Both presence/absence of light and the type of speculum were significant, and there was an interaction between these two factors. When the room light was absent, the measurements made with metal speculae or coated speculae did not exhibit significant differences.

Therefore, we recommend that clinical measurements of fluorescence be made with the lights absent, with either metal or coated speculum. The results in a preference study of patient satisfaction [11] indicate that patients prefer to have the lights absent as well.

One question that arises is why the room leakage does not appear on the plot of averages for each experimental condition in Fig. 3. We see in Fig. 4 that the background subtraction leaves positive and negative versions of room light spectrum. Our conjecture is that the room light leakage after the background subtraction averages out to zero, which helps to make comparison for this experiment. It should also be noted that individual spectra can show a major impact from this contamination. Nevertheless, further investigation is needed to assess how much background light can be present and still obtain measurements that provide discrimination of clinically relevant conditions. Further, it may be possible to statistically correct for known sources of contamination, like room light, provided we can accurately measure the slope of the spectra and adjust for its' magnitude.

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