Analysis of fluorescence excitation emission matrices of endometrial tissue

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ABSTRACT.

We have analysed an endometrial tissue fluorescence excitation spectra with a purpose to determine the characteristic wavelength of excitation for multiwavelength diagnostics of tissue. Fluorescence excitation-emission matrices from 200 to 500 nm excitation and 200 to 800 nm emission were measured as a supplementary data for previous measurements to unambiguously characterize biochemicals quantitatively in endometrial tissue.

Keywords: Laser induced fluorescence (LIF), 320 -360 nm and 400 - 420 nm

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1. INTRODUCTION

Laser induced fluorescence (LIF) spectroscopy is being used in many laboratories for diagnostics of disease of human tissues. This diagnostics requires identifying of the cell biochemical components or at least the characteristic light emission bands responsible for biochemical change in tissue. Fluorescence might provide insight into the spectral features that are responsible for absence or presence of disease in tissue. Fluorescence excitation-emission matrices provide more information for tissue optical characterization.

The different systems have been designed for the fluorescence spectroscopy in vitro and in vivo [1-3] and their clinical efficiency is reported. But these systems are not well designed for a wide application therefore it is still necessary to propose more simple but the efficient systems for preliminary diagnostics. Non destructive endoscopically or sonoscopically guided optical biopsy on site in endometrial tissue could be important for fertility, uterine bleeding and cancer screening purposes [4]. It could serve as a supplement for the selective optical thermal therapy device. Chemometrics, a modern statistics techniques should also be employed to use all potential of spectroscopically obtained data [5].

This work is oriented towards more reasoned choice of the excitation wavelength that could be employed for a device design based on the light emitting diodes as an excitation source. The excitation emission matrix peculiarities are a base for this choice.

Fig.1. The average spectrum of fluorescence in "normal" (1) and hiperplasia (2) tissues and their difference (3). The components with a peak wavelength are following: 390 nm, 410 nm, 470 nm, 480 nm, 525 nm, 610 nm, 670 nm.
The endometrial tissue fluorescence excitation spectrum and light absorption spectrum were recorded by a fluorescence spectrometer (f-4500, Hitachi) furnished with a photomultiplier as a detector and a 150 W Xe lamp as an excitation source. The relative uncertainty of photoluminescence measurements was better than 7%. The measurements were performed in vitro.

Preliminary investigation of different endometrial tissues excited at 354 nm, demonstrated a possibility to perform the evaluation of components that could be with a certain uncertainty related to biochemical components [4]. Fig. 1 demonstrates these components for a hyperplasia case. Upper part of this figure shows a difference among normalized average spectra of 9 different samples, where the hyperplasia simple was identified by histology, and a normalized average spectrum of 40 "normal" endometrium tissues [6]. The lower part of figure presents both of these spectra and the average fluorescence spectrum components in hyperplasia tissues. A weakest chain of this analysis is hidden into the identification of these components. Especially it is important in the cases of different tissue metabolism processes where additional fluorescing compounds may evolve [7]. To increase reliability of component's identification an excitation spectrum of fluorescence can be used.

An excitation-emission matrix is shown in Fig. 2 and the fluorescence spectra dependence on excitation wavelength are shown in Fig. 3 and 4. By analysis of relative change of fluorescence intensity on excitation wavelength the threshold of different components excitation can be determined.

Fig. 3 data show the increase of excitation wavelength from 200 nm to 220 does not change the fluorescence spectrum, only a small decrease of fluorescence intensity is observed. During the change of excitation from 230 to 260 the fluorescence at 300-400 nm and at longer wavelength as 600 nm decrease more effectively than at 420-580 nm. The latter band intensity decreases during an increase of excitation from 260 nm to 330 nm. Further increase of the excitation wavelength to 410 nm (Fig.4) does not change its intensity. An interesting feature is observed at excitation 350-500 nm that enhances the fluorescence in the neighbouring spectral region (50 nm) that is larger than the excitation side effects could explain. Therefore we assume that in this region an overlap of the molecules excitation and fluorescence spectra exist.

A long wavelength fluorescence band at 650-750 nm is excited most efficiently if the excitation 350-370 nm is used. A spectral device diffraction artefact does not allow investigating an exact region width.

The optical density measurements showed an existence of absorption bands at 320 nm and 415 nm. The presented fluorescence spectra confirm that there are different effects of excitation in these bands on the spectral components. The existing efficient light emitting diodes today cover the spectral region from 315 to 450 nm, therefore it is possible to adjust the latter observed phenomena but the spectral component analysis would be possible after the more detailed spectral investigation.
4. CONCLUSION
The tissue fluorescence spectra decomposition into the spectral compounds in the excitation spectral regions of 320 - 360 nm and 400 - 420 nm are promising but requires more detailed investigation.

REFERENCES

Fig. 4. Fluorescence spectra excited by different wavelength. An excitation wavelength is in a range 400 – 500 nm, (The excitation wavelengths are shown in the inserts).