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VISUALIZATION OF THE CONDUCTION SYSTEM OF HEART BY FLUORESCENCE SPECTROSCOPY *

E. Žurauskas [a], S. Bagdonas [b], L. Bandzaitytė [b], G. Streckytė [b], and R. Rotomskis [b]

[a] *Faculty of Medicine, Vilnius University, M.K. Čiurlionio 21, LT-03101, Vilnius, Lithuania*
[b] *Laser Research Centre, Vilnius University, Saulėtekio 9, LT-10222, Vilnius, Lithuania*

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Fluorescence measurements have been employed for the spectroscopic characterization of heart tissues. Excitation at the absorption band of the aromatic amino acids allows good differentiation between tissues of the bundle of His and those of myocardium when the ratios of the fluorescence intensities measured at 340 nm to those at 450 nm are compared. Several fluorophores with overlapping fluorescence bands are responsible for autofluorescence of heart tissues in the spectral range from 350 to 550 nm. The tissues of the bundle of His and myocardium are distinguishable by fluorescence intensity under excitation in the UVA spectral region. The best conditions are ensured by the excitation range of 360–390 nm and by recording of the fluorescence signal in the 440–480 nm spectral range.

Keywords: fluorescence, heart conductive system, visualization

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1. Introduction

Optical diagnostic techniques based on tissue autofluorescence nowadays are widely employed in many areas of medicine due to a noninvasive, nondestructive approach and instantaneous performance. Recently fluorescence spectroscopy methods were put into practice in diagnostics of multiform heart disorders. To meet the rising requirements of the heart surgery the up-to-date visualization of the heart conduction system (HCS) is needed, which could enable one to avoid incurable accidental lesions occurring during the surgical intervention.

HCS is a complex cellular structure, which is hardly distinguishable by the naked eye from cardiac muscle, myocardium (MC). HCS has faster transmission of a bioelectrical impulse than MC and is responsible for the heartbeat (depolarization) control. Precise molecular mechanism of the combined conductive performance of the HCS cells has not been identified yet. HCS consists of the sinoatrial node (SN), the atrioventricular node (AV), the bundle of His (HB), and its right and left bundle branches.

There are only few reports concerning the studies performed on HCS by using the fluorescence spectroscopy technique. The localization of the AV node was investigated in a canine heart, though no well-defined differences between the fluorescence of the AV node and that of MC tissues were observed [1]. Fluorescence intensity of auricle tissues was found being higher than that of ventricle tissues in MC samples taken from lambs [2]. The fluorescence intensity of the SN node and the AV node of the human HCS significantly differed from that of MC tissues – the measured signal in both nodes was higher than in ventricle tissues and lower than in auricle tissues [3]. However, there are no reports on autofluorescence measurements of human or animal bundle of His and its branches.

During the preliminary studies performed earlier on the specimens taken from the tissues of HB and MC of human it was found that the intensity of the absorption band with a peak at about 280 nm in the case of the HB was significantly higher than that in the spectrum of MC [4]. The observed difference in the tissues was ascribed to the different content of the aromatic amino acids – tryptophan (Trp) and tyrosine (Tyr).

In this study the fluorescence and fluorescence excitation spectra of endogenous fluorophores of HB and MC were examined.

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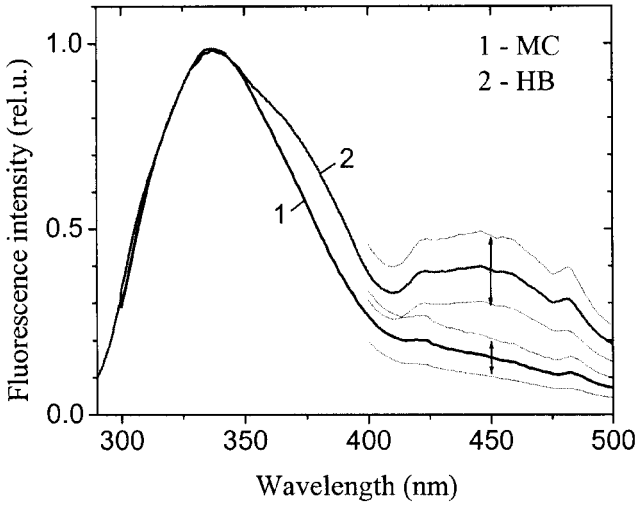

Fig. 1. Averaged fluorescence spectra of HB and MC tissues recorded at 255 nm excitation and normalized to peak intensities. Dotted curves indicate the standard deviation.

2. Materials and methods

The samples of the heart tissues were randomly chosen from 10 autopsy cases of different age disregarding pathology and prepared in the National Pathology Centre. The samples of HCS and MC were taken from tissues of HB and from tissues of ventricles, respectively. Before the measurements the specimens were kept fixed in 10% neutral solution of formaldehyde or stored at -20° C. Fluorescence and fluorescence excitation spectra were recorded with Perkin–Elmer spectrometer LS-50B. Images of heart tissues (Fig. 5) in daylight and fluorescent light were taken with a digital CCD camera ToUcam Pro.

3. Results and discussion

The fluorescence spectra of HB and MC were measured for 6 different heart samples and the averaged spectra are presented in Fig. 1.

Under excitation at the absorption region of the aromatic amino acids an intensive band of Trp fluorescence with a peak at 340 nm was observed in the fluorescence spectra of all heart tissue samples. This band seems not to be specific to a particular type of tissue and therefore it cannot be used for an unambiguous distinction between HB and MC tissues. However, within the spectral range of 400–500 nm, where no Trp fluorescence is observed, the fluorescence spectra of heart tissues significantly vary in spectral shape and relative intensity (Fig. 1) and the relative fluorescence intensity of HB is higher than that of MC. Four bands with peaks at 423,

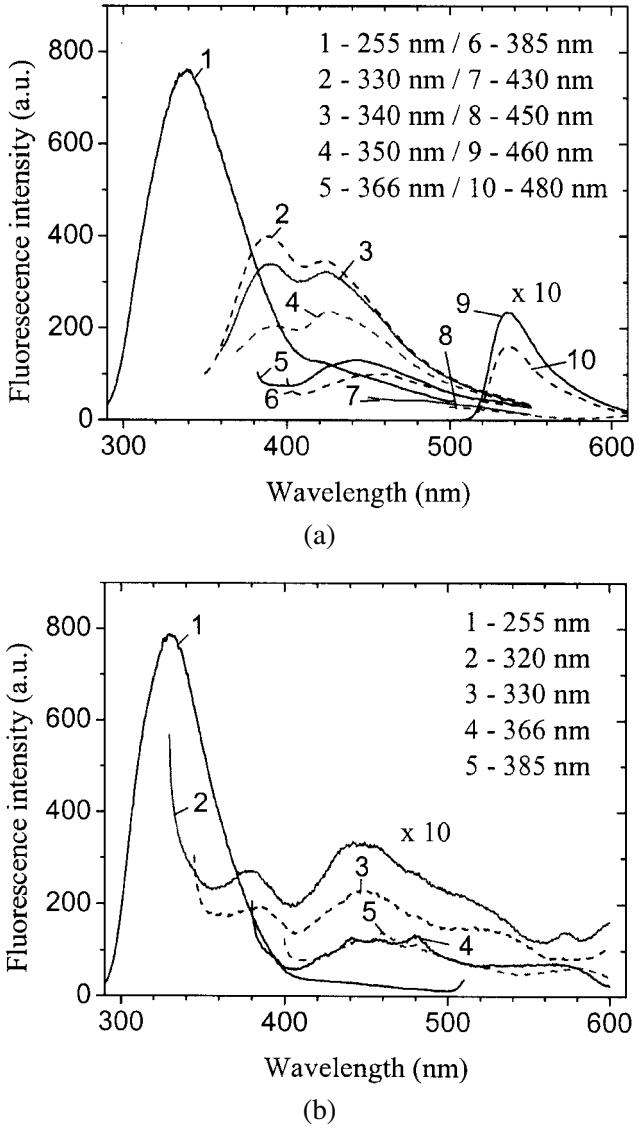

Fig. 2. Dependence of the fluorescence spectra of (a) HB and (b) MC on the excitation wavelength. Spectra *8–9* (a) and *2–5* (b) are scaled by a factor of 10.

445, 458, and 482 nm can be distinguished in the spectrum of HB, while two of them, at 423 nm and 482 nm, are present in the spectrum of MC. The ratio of the fluorescence intensity at 340 nm to that at 450 nm was calculated for both types of tissues from the averaged fluorescence spectra: the ratios determined for HB and MC were 2.5 ± 0.8 and 6.4 ± 1.8, respectively. (A standard deviation was used for error rating, the calculated reliability of differences between tissue fluorescence intensities exceeds 91%, when measured at 450 nm.)

These results show the possibility of precise differentiation between HB and MC tissues based on the ratio of autofluorescence intensities in UV and blue spectral regions. Though, due to the usage of UV radiation for the fluorescence excitation, such method of

tissue identification requires more sophisticated optical equipment and cannot be safely applied to visualize biological tissues. The spectra recorded under excitation at 255 nm (Fig. 1) implied that autofluorescence of the heart tissues in the blue spectral region can result from several fluorophores, which can probably be excited by light of the UVA and visible spectral regions. Therefore, the aim of the further studies was to determine the optimal wavelengths for the selective fluorescence excitation of heart tissues in the visible spectral region. For this reason, the fluorescence spectra of HB and MC were registered under various excitation conditions by changing excitation wavelength in small increments (Fig. 2).

It should be noted that under the excitation at longer wavelengths, where aromatic amino acids do not absorb, the intensity of HB fluorescence in the visible spectral region still remains significantly higher as compared with that of MC, what can be explained by the higher concentration of some endogenous fluorophores present in HB tissues. Two new emission bands with peaks at 390 nm and 422 nm emerge in the spectra of HB under excitation at 330 nm (Fig.2(a)). When the excitation wavelength is increased, the relative intensities of these fluorescence bands undergo redistribution – the peak at 390 nm fades out, whereas the second peak broadens and shifts towards longer wavelengths. Even under excitation in the visible blue range of the spectrum (460 nm and 480 nm) the HB fluorescence can be detected within the wavelength region of 520–650 nm.

The largest difference between fluorescence intensities of HB and MC tissues was detected under excitation in the spectral region of 330–385 nm. To reveal the spectral properties of the fluorophores, which can cause the observed differences, fluorescence excitation spectra of samples were recorded (Fig. 3).

Comparison of the fluorescence excitation spectra of HB and MC tissues shows that only the bands in the UV region attributable to Trp are similar in spectra of both types of tissues. The intensity of other excitation bands in the spectra of MC is significantly lower. The long-wavelength part of the HB excitation spectra consists of several overlapping bands, whose intensities and peak positions vary depending on the fluorescence emission wavelength (Fig. 3).

Analysis of fluorescence and fluorescence excitation spectra and comparison with literature data [5–8] allow one to point out potential endogenous fluorophores of HB and MC tissues, which are enlisted in Tables 1 and 2.

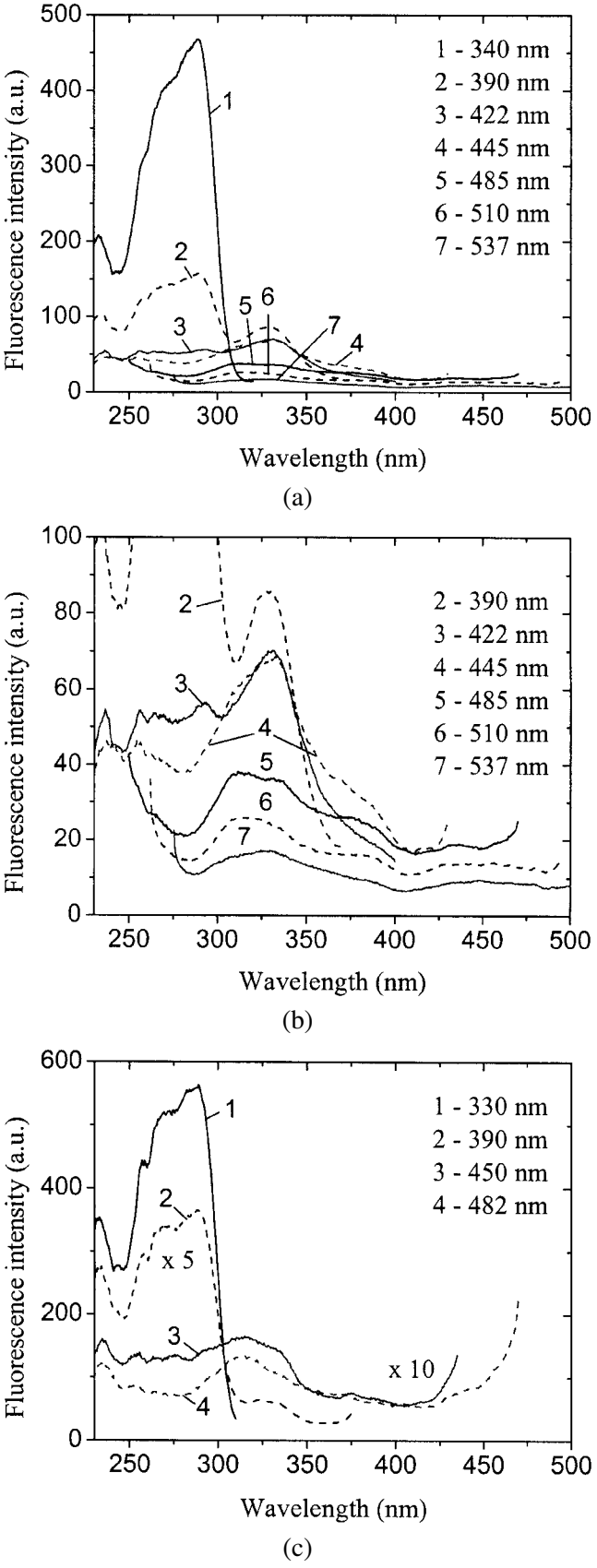

Fig. 3. Fluorescence excitation spectra of (a, b) HB and (c) MC recorded at different fluorescence wavelengths. The (b) spectra are scaled with respect to the (a) ones by a factor of 5.

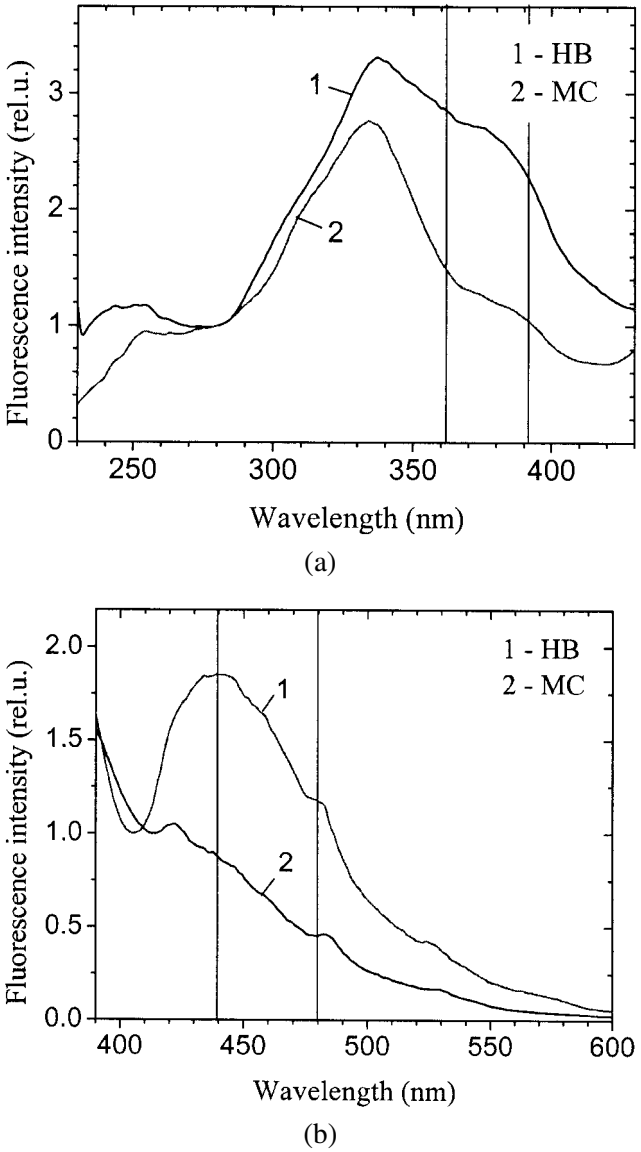

Fig. 4. (a) Fluorescence excitation spectra, $\lambda_m = 450$ nm, and (b) fluorescence spectra, $\lambda_{exc} = 360$ nm, of HB and MC normalized at 280 nm and 410 nm, respectively.

The collected spectral data reveal that the maximal difference in intensity of the fluorescence excitation spectra of heart tissues is within the spectral region of 360–390 nm, when the fluorescence is recorded at 450 nm (Fig. 4(a)). The fluorescence spectra registered under the excitation $\lambda_{exc} \in [360, 390]$ nm resulted in the largest difference between fluorescence intensities of HB and MC tissues (Fig. 4(b)).

On the basis of the collected spectral data, preliminary experiments on the visualization of HCS were performed *ex vivo* under excitation at 360 nm (Fig. 5). As a fluorescence signal was recorded within a whole range of the visible light spectrum, not only autofluorescence of HB branch was observed, but also that of other tissues. Therefore, the spectral range of the recorded signal should be narrowed to ensure the selective HCS visualization. That is envisaged to perform during the subsequent research stages.

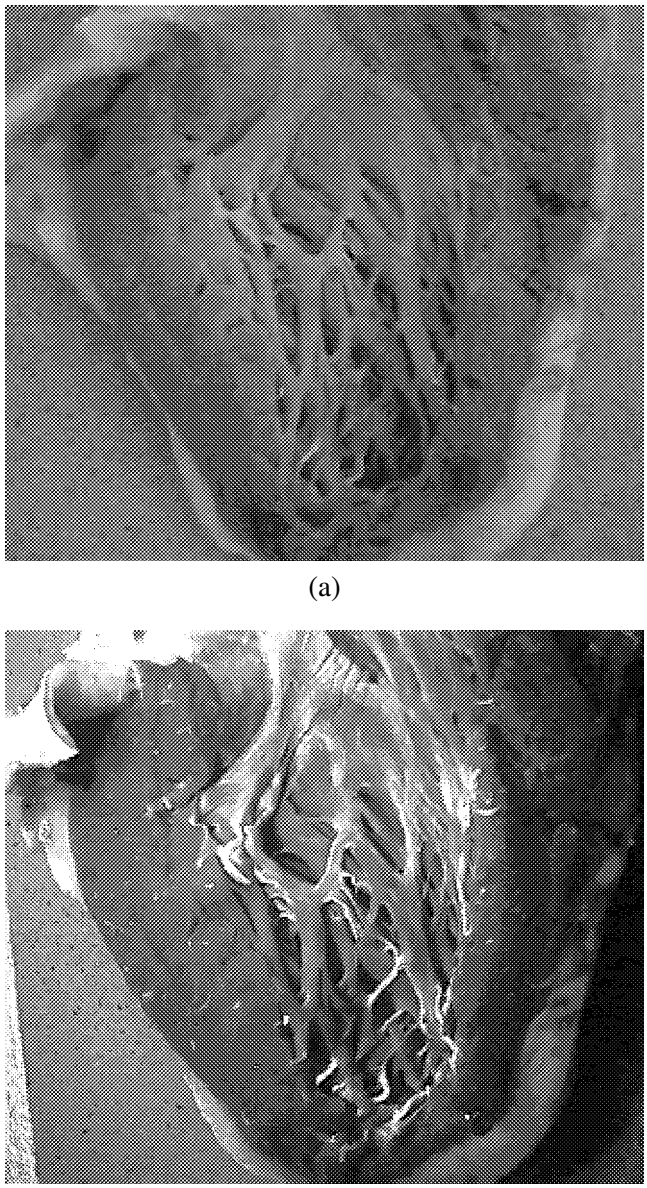

Fig. 5. Left branch of bundle of His in inter-ventricle septum (a) in the daylight and (b) under 365 nm excitation, taken in a whole range of visible spectrum.

4. Conclusions

Excitation at the absorption band of the aromatic amino acids allows good differentiation between tissues of the bundle of His and those of myocardium when the ratios of the fluorescence intensities measured at 340 nm to those at 450 nm are compared.

Table 1. Fluorescence wavelengths λ of potential fluorophores responsible for autofluorescence of HB and MC tissues.

λ_{exc}	λ (nm)		Possible
(nm)	HB	MC	fluorophores
255	340, 425	340, 425	Tryptophan, N-formylkynurenine
330	390, 425	390, 450, 530	Pyridoxine, collagen
366	450	450	Kynurenine
385	460	450	Kynurenine (?)
430	>450	>450	Lipopigments, flavins

Table 2. Fluorescence excitation wavelengths λ_{exc} of potential fluorophores responsible for autofluorescence of HB and MC tissues. Here λ_m is the emission wavelength at which the fluorescence was recorded.

λ_m	λ_{exc} (nm)		Possible
(nm)	HB	MC	fluorophores
340	285	285	Tryptophan
390	285, 330	285,330	Pyridoxine, collagen
422	330	330	Collagen, elastin
445	330, 383	330	Kynurenine
485	315, 330, 390, 430	315	Lipopigments, flavins

Several fluorophores with overlapping absorption and fluorescence bands are responsible for autofluorescence of heart tissues in the 390–550 nm spectral range. Their concentration in His bundle seems to be significantly higher than in myocardium.

The tissues of the bundle of His and myocardium are also distinguishable by fluorescence intensity under excitation in the UVA spectral region. The best conditions are ensured by excitation at $\lambda_{exc} = 360-390$ nm and by recording the fluorescence signal in the 440–480 nm spectral range.

Acknowledgements

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ŠIRDIES LAIDŽIOSIOS SISTEMOS VIZUALIZACIJA FLUORESCENCINĖS SPEKTROSKOPIJOS METODU

E. Žurauskas, S. Bagdonas, L. Bandzaitytė, G. Streckytė, R. Rotomskis

Vilniaus universitetas, Vilnius, Lietuva

Santrauka

Tirti širdies laidžiosios sistemos dalies – His'o pluošto ir širdies skilvelių vidiniame paviršiuje esančių jo šakų bei aplinkinio miokardo – bandiniai, paimti iš skirtingo amžiaus ir skirtingos patologijos širdžių preparatų, fiksuoti ir nefiksuoti buferiniame formalino tirpale, matuojant savitosios fluorescencijos bei jos žadinimo spektrus. Iš rezultatų matyti, kad šie audiniai pasižymi skirtingais fluorescencijos ir fluorescencijos žadinimo spektrais. Žadinant aromatinių aminorūgščių sugerties juostoje, galima vienareikšmiškai atskirti His'o pluošto ir miokardo audinius pagal fluorescencijos spektrų intensyvumų ties 340 nm ir 450 nm santykį, kuris His'o pluoštui yra 2,5, o miokardui – 6,4. His'o pluošto ir miokardo audinius pagal jų fluorescencijos intensyvumą galima atskirti ir žadinant ilgesnių bangų spinduliuote, kurios aromatinės aminorūgštys nesugeria. His'o pluošto mėginiuose spektriniame ruože nuo 350 nm iki 550 nm fluorescuoja keli fluoroforai, kurių fluorescencijos spektrai persikloja. Tų fluoroforų fluorescencijos intensyvumas His'o pluošte žymiai didesnis nei miokarde. His'o pluošto ir miokardo audinių fluorescencijos spektrai geriausiai atskiriami žadinant fluorescenciją 360–390 nm ruože. Audinių fluorescencijos signalo registracija 440–480 nm srityje užtikrintų optimalų širdies laidžiosios sistemos vizualizaciją.