Variability of spectra of laser-induced fluorescence of colonic mucosa: Its significance for fluorescence detection of colonic neoplasia

Barbara W. Chwirot, Małgorzata Kowalska, Natalia Plóciennik, Mariusz Pwiński*, Zbigniew Michniewicz & Stanisław Chwirot

Interdisciplinary Group of Optical Methods of Early Detection of Cancer, Institute of General and Molecular Biology, Nicholas Copernicus University, ul. Gagarina 9, PL 87-100 Toruń, Poland
*Institute of Physics, Nicholas Copernicus University, ul. Grudziadzka 5, PL 87-100 Toruń, Poland

To determine the extent of a natural variability of the spectra of the autofluorescence and its significance for a reproducibility of different approaches typically used in studies on fluorescence detection of colonic lesions. Two independent series of experiments have been conducted during three years in the same laboratory. Macroscopic tissue specimens obtained during operations of patients with colonic cancers were studied in vitro. The tissues were excited using UV lines of c.w. He-Cd laser and pulsed nitrogen laser and the autofluorescence spectra were recorded for areas visually diagnosed as normal or pathologically changed mucosa. Natural variability of the autofluorescence spectra of colonic tissues seems to be most important factor limiting sensitivity and specificity of the diagnostic algorithms. The mean fluorescence spectra obtained for normal mucosa and its neoplastic lesions differ significantly but the differences are difficult to observe because of the high natural variability among the individual spectra. Further studies of biological basis of the colonic autofluorescence are necessary for a progress in the field of fluorescence detection of colonic neoplastic lesions.

Keywords: Autofluorescence, Colonic neoplasma, Colorectal cancer, Fluorescence detection, Laser-induced fluorescence, Multivariate analysis, Ultraviolet excitation, Variability of spectra.

Colorectal cancers are major health problem in the global scale\(^1\). It is now generally accepted that in almost all cases the disease starts from known neoplastic lesions and develops for a relatively long time of the order of several years. At the same time the cancers are often detected only in late stages with resulting low success rate of therapeutic treatments. Screening for the colorectal cancers relies mostly on testing for occult blood and endoscopic examinations. The occult blood tests are neither very sensitive nor specific. The endoscopic examinations are expensive and their result depends to a large extent on experience of the endoscopist. Blind biopsies which are often a screening method of choice allow in typical conditions for examination of less than 0.05% of the colonic area of interest\(^2\).

Since the beginning of 1990\(^{'}\) several groups reported on studies on a new fluorescence technique of detecting premalignant and malignant lesions in human colon\(^{2,10}\). The general idea of all such methods is based on a simple assumption that metabolic and structural changes resulting from neoplastic transformation are reflected in changes in intensity and emission spectra of a native fluorescence (autofluorescence) of the tissues of interest. These changes may result both from variations in a content of endogenous fluorophores and from differences in architecture of tissues and cells. The latter may influence a propagation of the exciting and fluorescence light. However, with a complexity and natural variability of living systems it is very difficult to differentiate between the changes related to occurrence of a pathologic condition and those resulting from the natural variations of metabolic and structural features of tissues of interest. During the last decade several groups described diagnostic algorithms for analysis of the autofluorescence spectra allowing for a sensitive and specific detection of premalignant and malignant colonic lesions. On the other hand none of those method has found a way to clinical applications. It seems that the main problem is just the natural variability of the spectra of the autofluorescence of both the regular mucosa and its neoplastic lesions. The present study has been aimed at investigating the range of such natural spectral variations and also of a sensitivity of the fluorescence methods to the experimental conditions. The main
goal of this study is to assess the significance of both the above mentioned factors for a reproducibility of diagnostic parameters calculated from the ratios of the intensity of the autofluorescence emitted in different spectral bands.

**Materials and Methods**

The materials for study were macroscopic samples of human colons obtained during surgical operations of patients with colonic tumours. The study was approved by Commission of Ethics of Scientific Research at Medical School, Bydgoszcz, Poland.

Spectral measurements were carried out in two series: one in 1997-1998 and the second in 1998-1999 (from hereafter respectively referred to as A and B). During the first series of measurements the autofluorescence spectra were obtained for 296 cases of regular mucosa, mucosal lesions of non-malignant character, adenomatous polyps, colonic cancers, cancerous infiltrations under the mucosa and border regions separating tumours and normal mucosa, all present in the material obtained from 47 patients. In the second series 211 spectra were obtained for areas of histologic characteristics similar as in the series A located in the samples obtained from 28 patients. In both the series the same procedures and the same equipment were used. However, the measurements as such were carried out in the series A and B by different persons who had in each experiment to align the optics for coupling the laser beams into the fiber and to position the probe with respect to a sample surface in a position suitable for exciting and detecting the autofluorescence.

Immediately after the resection large samples of colonic tissues were placed in cold saline and transferred to laboratory. Then the specimen was placed on a cold table (+4°C). The areas of interest were localised visually and marked with pins of different colours. The autofluorescence of the selected areas was excited using either with 325 nm line of a He-Cd c.w. laser or with pulses of 337 nm radiation of a nitrogen laser. At the end of the measurements the tissue material was fixed in buffered formaline and subject to histologic examinations. Detailed histopathologic descriptions of the places subject to fluorescence investigation were then used for differential analysis of the autofluorescence spectra.

Figure 1 shows a schematic representation of the set-up used. The exciting light was delivered to the sampled surface via 6 quartz fibres of a bifurcated cable located symmetrically around the central quartz fibre (0.2 mm diameter) which collected the light of the autofluorescence (Ocean Optics). During the measurements the fibre optic probe was held at a right angle at a distance of about 1 mm from the surface of the sample. At such conditions the exciting light illuminated an area of about 1-2 mm². The intensity of the exciting light on the sample surface was of the order of 2 mW/mm² in 325 nm line of c.w. He-Cd laser (Omnichrome 2056) and about 1 MW/mm² for the 100 ps pulses of 337 nm radiation of nitrogen laser (Laser Photonics). Typical repetition rate of the nitrogen laser pulses was 10 Hz and thus the mean power delivered to tissue surface was 1 mW/mm².

The autofluorescence spectra were obtained using S1000 spectrometer (Ocean Optics) with a resolution of about 10 nm. A multilayer cut-off filter placed at the input of the spectrometer was used to eliminate the UV light reflected from the tissue surface. A small amount of the 337 nm light was transmitted by the filter and produced a second order maximum at 674 nm. The 325 nm line of the He-Cd laser was separated from the 442 nm line using a quartz prism. There are, however, indications that in some cases the diffused 442 nm light may have contaminated the spectra and modified a narrow part of the wide main autofluorescence maximum. To avoid possible systematic errors the spectral band of 420-460 nm was not taken into account in the analyses concerning a variability of the autofluorescence spectra and in calculations of the diagnostic coefficients. The spectra were corrected for spectral sensitivity of the linear CCD matrix of the spectrometer and normalised to the intensity of the autofluorescence emitted in 507-540 nm band. Both
the normalised intensities and the spectral characteristic of the autofluorescence emitted in that band were similar for a regular mucosa and neoplastic lesions of interest.

Results

The autofluorescence spectra were recorded for central and border regions of the colonic lesions and for sites within areas visually diagnosed as normal mucosa.

However, histologic examinations showed that on many occasions the visually normal mucosa had been in fact changed pathologically: in the material investigated in the A series the visual diagnosis was confirmed by histopathologists in 61 cases but in 111 samples of supposedly normal mucosa several pathologic states were detected in microscopic examinations and among them 4 adenocarcinomas within the mucosa, 2 cases of submucous spread of adenocarcinoma and 1 case of anaplastic carcinoma beneath the mucosa. In the series B the visual macroscopic diagnosis of a normal mucosa was confirmed for 34 cases and contradicted for 86 cases. The latter included 7 cases of adenocarcinoma within the mucosa. The other pathologic conditions detected microscopically within the macroscopically normal mucosa comprised most often inflammatory reaction, ulceration, lymphoid hyperplasia, lymphonoduloplastia, oedema, hyperemia etc. Such results clearly indicate a need for developing new auxiliary tools for endoscopic examinations of colonic mucosa.

The mean spectra of the autofluorescence obtained in the series A and B for the normal mucosa and for the pathologic lesions using the 325 nm line excitation are shown in Figs 2-8 (A and B symbols label accordingly the data obtained in the two experimental series).

Similar series of the autofluorescence spectra is presented in Figs 9-15 for the excitation with 337 nm pulses of nitrogen laser. Slightly different wavelength and a very high transient power of the 100 ps pulses of the nitrogen laser result in different excitation conditions compared to the excitation with c.w. line of He-Cd laser. Thus, one could expect differences in absorption probabilities, kinetics of the excitation and emission of the autofluorescence and in effective penetration depth of the exciting light.

---

Fig. 2 — Average normalised spectra of the autofluorescence of regular mucosa excited with 325 nm line of He-Cd laser
(A: n = 48; B: n = 36)

Fig. 3 — Average normalised spectra of the autofluorescence of malignant colonic tumours excited with 325 nm line of He-Cd laser
(A: n = 55; B: n = 46)
All the spectra shown in Figs 2-15 for the groups of the tissues of similar histologic characteristics demonstrate a relatively large scatter of the results well illustrated by magnitudes of the standard deviations. The extent of the differences was similar for the spectra collected from different locations in the material obtained from given patients and for the spectra obtained for similar places in samples from different patients. The variability of all the spectra is highest in the short wavelength part and this can probably be explained in terms of differences in a composition and architecture of tissue layers and of a different blood content of the investigated tissue samples. The autofluorescence in the 390 nm band is usually related to collagen fibres while the minimum around 425 nm is thought to be caused by reabsorption of the fluorescence light by hemoglobin. It seems significant that the variability of the autofluorescence spectra of the
normal mucosa was very similar to that of the spectra of all other types of the lesions. Such an observation suggests strongly that the observed variations of the spectral patterns are due at least to a similar extent both to a natural variability of the complex biological systems and to a presence of pathologic conditions and host responses to the disease, especially to neoplasia.

The mean autofluorescence spectra of the normal mucosa and of the non-tumorous lesions are very similar for the He-Cd laser excitation and almost the same for tissues excited with the nitrogen laser. At the same time there are clear differences between the mean autofluorescence spectra obtained for normal mucosa and for the neoplastic lesions of different types. The spectra obtained for the normal mucosa and for the neoplastic lesions differ most significantly in the short wavelength region i.e. for wavelengths below ca. 475 nm. The regular tissues are clearly characterised by a more pronounced peak between 375 and 400 nm while the mean spectra of the lesions typically show slightly higher fluorescence intensities.

![Fig. 7](image7.png)

*Fig. 7 — Average normalised spectra of the autofluorescence of hyperplastic polyps excited with 325 nm line of He-Cd laser (A: n = 3; B: n = 2)*

![Fig. 8](image8.png)

*Fig. 8 — Average normalised spectra of autofluorescence of non-tumorous lesions excited with 325 nm line of He-Cd laser (A: n = 90; B: n = 90)*

![Fig. 9](image9.png)

*Fig. 9 — Average normalised spectra of the autofluorescence of regular mucosa excited with 337 nm line of nitrogen laser (A: n = 51; B: n = 37)*
for longer wavelengths above 550 - 600 nm. It also seems interesting that the spectra obtained using the He-Cd and nitrogen laser excitations are very similar in the 350 - 650 nm band investigated in the present work.

A comparison of the data obtained in the two independent measurement series A and B provides an interesting insight into a problem of reproducibility of the results and the sensitivity of the autofluorescence spectra to the experimental conditions. The spectra of the autofluorescence excited using 325 nm He-Cd laser line in the series B show a clear indication of the emission peaks around 440 nm. A closer analysis reveals the existence of similarly located but much smaller peaks also in the spectra obtained in the series A. Initially those peaks were thought to be a charac-

Fig. 10 — Average normalised spectra of the autofluorescence of malignant colonic tumours excited with 337 nm line of nitrogen laser (A: n = 64; B: n = 49)

Fig. 11 — Average normalised spectra of the autofluorescence of border regions of malignant colonic tumours excited with 337 nm line of nitrogen laser (A: n = 17; B: n = 10)

Fig. 12 — Average normalised spectra of the autofluorescence of regions of cancer spread beneath the regular mucosa excited with 337 nm line of nitrogen laser (A: n = 14; B: n = 10)
teristic feature of the autofluorescence of the tissues under investigation but after carrying out the suitable tests it was discovered that they were related to a diffused 442 nm component of the laser beam (extremely weak but strong enough to contaminate the real data). Perhaps similar effects may be responsible for differences in the autofluorescence spectra reported for colonic mucosa by different groups using similar excitation wavelengths.

First studies on a fluorescence detection of neoplastic lesions suggested that a transformation towards malignancy was associated with a significant qualitative change of the autofluorescence spectra. It was suggested that differences in the autofluorescence of normal and neoplastic tissues may result from occurrence of a new fluorescence compound or from considerable changes in a pool of existing endogenous fluorophores. However, the first very promising re-

![Fig. 13 — Average normalized spectra of autofluorescence of adenomatous polyps excited with 337 nm line of nitrogen laser (A: n = 12; B: n = 8)](image)

![Fig. 14 — Average normalized spectra of autofluorescence of hyperplastic polyps excited with 337 nm line of nitrogen laser (A: n = 3; B: n = 1)](image)

![Fig. 15 — Average normalized spectra of the autofluorescence of non-tumorous lesions excited with 337 nm line of nitrogen laser (A: n = 100; B: n = 74)](image)
sults\textsuperscript{11,12} were not confirmed by further studies conducted by other authors. It was soon clear that the differences in the spectra were more subtle and had to be detected against a background of variations due to a complexity and a variability of the tissues of interest.

Generally two classes of diagnostic algorithms have been developed and used by different groups. Some authors use a stepwise multivariate linear regression analysis (MVLR) to analyse the whole spectra and/or to localize several spectral bands relevant for detection of neoplasia. The others, prefer a more simple approach based on ratioing the intensities of the autofluorescence emitted in two spectral bands. The latter technique brought the first clinically tested and commercially available apparatus for the fluorescence detection of premalignant and malignant lesions within bronchial tree\textsuperscript{13} and a patented method for detecting several cancers in microscopic samples\textsuperscript{14}. More recently Cothren \textit{et al.} reported very good results of clinical tests of a fluorescence technique for detecting neoplastic lesions in human colon also based on ratioing the intensities of the autofluorescence emitted in different parts of the visible spectrum. Also the groups using the MLRY algorithms reported very good results of feasibility studies\textsuperscript{3,15} but up to now the practical significance of those data has not been confirmed in full clinical trials. It is possible that the multiparameter methods may be too susceptible to multiple local changes in the spectral patterns i.e. the optimum values of the diagnostic parameters may change from one calibrating set of tissues to another.

The ratios of the intensities of the autofluorescence emitted in the spectral bands which seemed to show most significant changes related to different pathologic conditions were calculated and used to compare the autofluorescence spectra obtained in the present study in the two independent experimental series. From hereafter these intensity ratios will be referred to as the diagnostic coefficients (D\textsubscript{i}) calculated according to following definitions:

\text{series A:}\n\begin{align*}
D_1 &= \frac{I_{385.395}}{I_{410.470}}, \quad D_2 = \frac{I_{385.475}}{I_{575.650}}, \\
D_3 &= \frac{I_{385.420} + I_{400.470}}{I_{575.650}}.
\end{align*}

\text{series B:}\n\begin{align*}
D_1 &= \frac{I_{385.395}}{I_{410.470}}, \quad D_2 = \frac{I_{385.475}}{I_{575.650}}, \\
D_3 &= \frac{I_{385.420} + I_{400.470}}{I_{575.650}}.
\end{align*}

The results presented in Tables 1-3 confirm the existence of considerable differences between the spectra of the autofluorescence emitted by histologically similar tissue structures. The minimum and maximum values of the D\textsubscript{i} parameters differed in some cases even by one order of magnitude for the areas of the mucosa histologically classified in the same group. At the same time the mean values of the parameter D\textsubscript{i} determined in the two experimental series A and B are very similar as they agree within the limits of one standard deviation. Such a result supports a suggestion that the main source of the scatter of the autofluorescence spectra recorded for the tissues of the same type is rather the characteristics of the biological material than the changing experimental conditions.

Another interesting finding is the similarity of the D\textsubscript{i} parameters calculated from the spectra of the autofluorescence excited with the ultraviolet lines of the He- Cd and nitrogen lasers. The slight difference (12 nm) of the excitation wavelength should not significantly influence the excitation of the endogenous fluorophores characterised \textit{in situ} by wide emission and excitation maxima (see for instance the excitation-emission matrices in Richards-Kortum \textit{et al.}\textsuperscript{2,3}). However, both a kinetics of the excitation and the effective penetration depth of the beams of c.w. He-Cd laser and pulsed nitrogen laser are significantly different. The similarity of the spectra of the autofluorescence excited by the two lasers can be clearly seen in Figs 2-15 while the data presented in Tables 1-3 show it in a more quantitative manner. A good reproducibility found for the mean values of the D\textsubscript{i} parameters in independent measurements together with a similarity of the D\textsubscript{i} values obtained at different excitation conditions indicate that the fluorescence diagnostic algorithms based on ratios of spectrally resolved autofluorescence intensities may be transportable i.e. they may produce similar results while used in clinical conditions in different laboratories.

The data shown in Tables 1-3 illustrate a potential of the autofluorescence spectral analyses for a detection of pathologic conditions in human colon and for differentiating between lesions of different types. On the basis of the values of the parameter D\textsubscript{i} one can detect with a high probability a presence of adenomatous polyps. Assuming that D\textsubscript{i} < 1.0 indicates a presence of such a polyp it is possible at the 325 nm excitation to detect colonic adenomas present in the present material with a sensitivity C = 100% with a positive predictive value PPV = 48%. The relatively low value of PPV is due to false positive results obtained for about 25% cases of normal mucosa. The malignant tumours could be detected with C = 63.1% and differentiated from regular mucosa with PPV = 57% while similar parameters for non-tumorous lesions were C = 60.2% and PPV = 59.6%. Similar approach applied to the spectra of the autofluorescence excited
Table 1 — Values of the $D_1$ parameter obtained in two experimental series for the normal mucosa and pathologic lesions

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Series A</th>
<th>Series B</th>
</tr>
</thead>
<tbody>
<tr>
<td>m.t.</td>
<td>Min. 0.46</td>
<td>Max. 2.12</td>
</tr>
<tr>
<td>e.m.t.</td>
<td>Min. 0.85</td>
<td>Max. 1.65</td>
</tr>
<tr>
<td>t.i.m.</td>
<td>Min. 0.10</td>
<td>Max. 1.74</td>
</tr>
<tr>
<td>a.p.</td>
<td>Min. 0.37</td>
<td>Max. 0.89</td>
</tr>
<tr>
<td>n.t.</td>
<td>Min. 0.52</td>
<td>Max. 2.78</td>
</tr>
<tr>
<td>reg.</td>
<td>Min. 0.71</td>
<td>Max. 1.92</td>
</tr>
</tbody>
</table>

Table 2 — Values of the parameters $D_0$ and $D_1$ obtained using 325 nm excitation for the normal mucosa and pathologic lesions

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>$D_0$ Series A</th>
<th>$D_1$ Series B</th>
</tr>
</thead>
<tbody>
<tr>
<td>m.t.</td>
<td>Min. 0.38</td>
<td>Max. 2.43</td>
</tr>
<tr>
<td>e.m.t.</td>
<td>Min. 0.60</td>
<td>Max. 1.40</td>
</tr>
<tr>
<td>t.i.m.</td>
<td>Min. 0.60</td>
<td>Max. 1.66</td>
</tr>
<tr>
<td>a.p.</td>
<td>Min. 0.46</td>
<td>Max. 1.04</td>
</tr>
<tr>
<td>n.t.</td>
<td>Min. 0.62</td>
<td>Max. 2.78</td>
</tr>
<tr>
<td>reg.</td>
<td>Min. 0.60</td>
<td>Max. 2.18</td>
</tr>
</tbody>
</table>

Table 3 — Values of the parameters $D_0$ and $D_1$ obtained using 337 nm excitation for the normal mucosa and pathologic lesions

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>$D_0$ Series A</th>
<th>$D_1$ Series B</th>
</tr>
</thead>
<tbody>
<tr>
<td>m.t.</td>
<td>Min. 0.65</td>
<td>Max. 10.02</td>
</tr>
<tr>
<td>e.m.t.</td>
<td>Min. 1.30</td>
<td>Max. 7.60</td>
</tr>
<tr>
<td>t.i.m.</td>
<td>Min. 2.09</td>
<td>Max. 6.53</td>
</tr>
<tr>
<td>a.p.</td>
<td>Min. 2.36</td>
<td>Max. 12.26</td>
</tr>
<tr>
<td>n.t.</td>
<td>Min. 0.52</td>
<td>Max. 12.95</td>
</tr>
<tr>
<td>reg.</td>
<td>Min. 1.66</td>
<td>Max. 10.95</td>
</tr>
</tbody>
</table>

by pulses of the 337 nm radiation yielded for the adenomatous polyps $C = 91.6\%$ with $PPV = 42.3\%$, for the malignant tumours $C = 57.6\%$ and $PPV = 58.2\%$ and for the non-tumorous lesions $C = 51.9\%$ and $PPV = 57.7\%$. Thus, if similar results can be obtained during in vivo studies a simple fluorescence algorithm based on a determination of the $D_1$ value can be a useful auxiliary technique for a more efficient endoscopic detection of adenomatous polyps.

On the other hand, the malignant tumours can be efficiently detected using another diagnostic parameter $D_2$. The distribution of the values of that parame-

...er is highly asymmetric and sensitive and quite specific a detection of the malignant lesions can be achieved with the threshold value much higher than the mean value of the parameter. Assuming that the malignant lesions were identified as those characterised by $D_1 < 6.5$ it was possible for the 325 nm excitation to detect correctly 89.3% of tumours in the material under study with PPV = 69.4%. The algorithm worked even better for the 337 nm excitation and yielded.

$C = 92.9\%$ with PPV = 70.2%.

The $D_1$ parameter did not allow for a good detection of any of the lesions investigated in the study.

It should be noted that the diagnostic parameters discussed above should be considered examples illustrating the potential of the method. Further analysis of the spectra is under way now and we have found recently that the adenomatous polyps can be detected with a better efficiency using a new parameter $D_2 = 1_{353.859} / 1_{348.475}$. For the He-Cd laser excitation with a condition that $D_1 < 0.25$ we obtained for the polyps $C = 88.2\%$ and PPV = 83.3% while a condition of a $D_1 < 0.3$ resulted in $C = 94.1\%$ and PPV = 72.7%.

The same approach used for the analysis of the data from the material excited with the nitrogen laser yielded respectively $C = 94.1\%$, PPV = 84.2% and $C = 100\%$, PPV = 68%.

Discussion

The present results demonstrate clearly that the variability of the spectra of the autofluorescence of both regular colonic mucosa and of its pathologic lesions is to a large extent a result of the natural variability of the tissues and cells which is typical for biological systems. It is also possible that on several occasions the spectra obtained for areas histologically classified as neoplastic may in fact have been obtained for tissues of different character located in their vicinity. Such situations may happen because the dysplastic and malignant lesions may at initial stages be restricted to small areas of the mucosa and of the adenomatous polyps which may not be included within the small region illuminated by the exciting light. However, the mean autofluorescence spectra obtained in the two independent series of measurements conducted over a period of three years are for all the tissue types very similar. The variability of the spectra measured by magnitudes of the deviations is very similar too.

It is also interesting that the spectra of the autofluorescence excited using ultraviolet lines of the He-Cd and nitrogen lasers do not show significant differences in the 350 nm - 650 nm band investigated in this work. The observed differences are of the same order (or even smaller) as the differences between the spectra reported for the colonic tissues by different groups using the same excitations wavelengths (see for instance Schomacker et al. and Eker et al.) and significant differences can be seen while comparing the spectra obtained by the groups using the nitrogen laser (Schomacker et al., Eker et al.) and He-Cd laser excitation (Kapadia et al.). All such differences point to the importance of all steps of experimental protocols for a comparability of the data and of the diagnostic algorithms based on the fluorescence measurements. Several authors indicated the dependence of the spectra on factors like tissue handling, geometry of the excitation and detection of the autofluorescence and the intensity of the exciting light.

The relatively high variability of the autofluorescence spectra found in the present work and also indicated by the discrepancies between the earlier results reported by different groups may explain the difficulties with clinical implementation of the diagnostic algorithms based on the MLR approach. Such algorithms typically involve simultaneous analysis of the intensities of the autofluorescence emitted in many narrow spectral bands and values of relevant coefficients are calculated from the results obtained for a so-called training set of the tissue samples of known histologic characteristics. As such the MLR algorithms are sensitive to changes of the relative intensities of the autofluorescence that may occur locally in the spectra. Such local changes are less significant for the coefficients calculated as ratios of the intensities emitted in broad spectral bands or for the methods based on comparing the intensities of the autofluorescence emitted in selected wide spectral bands by different regions of the mucosa and measured simultaneously in digital images of the autofluorescence.

In conclusion, at the present stage of development of the fluorescence detection of colonic neoplastic lesions it is the natural variability of the spectra and of the intensity of the autofluorescence that is the most important factor limiting the sensitivity and the specificity of diagnostic algorithms. Better understanding of a biological basis of the autofluorescence, especially of the origin of differences between the mean fluorescence spectra of the normal colonic mucosa and of its pathologic lesions seems to be a necessary condition for achieving a real progress in the field. Only then it will be possible to select optimum excita-
tion and detection conditions allowing for a sensitive and specific observation of the spectral features characteristic for the lesions of interest despite the background of natural and experimental variability of the measured spectra.

Acknowledgement

This work was supported by a grant from the State Committee for Scientific Research (KBN no. 4P05B 055 12). The authors thank colleagues in Toruń and the staff of histopathological units at those hospitals for providing histological evaluations of lesions subjected to fluorescence studies.

References


