Fluorescence detection improves malignant melanoma diagnosis

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Fluorescence spectroscopy combined with new diagnostic algorithms can be used to differentiate between early-stage benign and malignant skin lesions.

In clinical practice, the early diagnosis of tumors is still based on biopsy, a procedure that involves the removal of tissue samples for microscopic examination. But the technique has many disadvantages. In some patients, it may lead to bleeding and complications. The histology examination is also very time-consuming. Last, but not least, the medical personnel performing the procedure must be highly qualified. Other imaging diagnostic tests are also available, such as x-ray radiography, magnetic resonance imaging, ultrasound, and positron emission tomography. But these techniques only reveal abnormalities and can neither confirm nor detect the early stages of a tumor. Recent advances have shown, however, that optical investigations—especially fluorescence, one of the most sensitive spectroscopic techniques—can be used for early tumor detection and to supplement other diagnostic tools. An additional advantage of optical techniques is that they can overcome the problem of practitioner subjectivity since they provide objective and reproducible qualitative measurements.

The medical applications of fluorescence spectroscopy now include the early detection of malignant tumors, investigations of blood vessel atherosclerotic transformations, and skin or mucosa pathologies. Although the ‘best’ method for detecting and differentiating cutaneous malignant lesions is still much debated, there is overwhelming agreement that early detection of malignant neoplasia is critical in improving patient survival rates and prognosis.

To differentiate between normal and neoplastic tissue, a fluorescence excitation wavelength around 340nm has been shown to be most appropriate. Accordingly, for our studies, we selected a compact nitrogen laser emitting at 337nm (ILGI-503, Russia) and a highly sensitive fiber-optic spectrometer (Ocean Optics, USA). Clinical investigations began with the lesions of interest visually classified by an experienced dermatologist using digital epiluminescence microscopy (MoleMax II, DERMA Instruments, USA). In the final analysis, control histological examinations were performed to ascertain lesion type and verify our results.

We obtained spectra of the native endogenous fluorescence of benign compound nevi, dysplastic nevi, and malignant melanoma. Algorithms based on the fluorescence spectral features were developed and introduced at the oncological center to discriminate between skin lesions with high sensitivity and specificity.

Skin tissue contains many types of chromophores with different absorption spectra and different quantum efficiencies, which still remain to be accurately identified. However, the major components are known: melanin, hemoglobin, and water. Other fluoresce...
The fluorescence emission of human skin is therefore a complex signal involving several fluorophores and absorbers. Our results, shown in Figure 1, nonetheless report significant differences in the intensity of the fluorescence signals of normal, benign, and malignant tumor tissues. The fluorescence intensities (maxima at 480–490nm) of benign and dysplastic nevi were similar, but could be easily distinguished from those of the melanoma lesions.

The hypervascularization of malignant neoplasia has now become a diagnostic criterion, and this abnormal formation of blood vessels should result in an increased hemoglobin spectral signature. Even in the weak fluorescence of malignant melanoma lesions we were able to observe the presence of hemoglobin: a faint minimum in the 540–580nm spectral region. In all cases, the effect of native absorbers in the tissue was included in the evaluation of the spectra. This is because the distortion of spectral shapes due to the absorption of native skin chromophores can lead to the appearance of false maxima, which can be misrepresented as new fluorophores in the lesion area.

The most significant changes in the fluorescence spectra of melanin-pigmented lesions were observed in the intensity values shown in Figure 2. We accordingly developed an algorithm based solely on the intensity values of the main fluorescence maximum. Our results provide improved statistical values (see Table 1) when compared with data acquired by surface microscopy or epiluminescence microscopy, two methods currently used as noninvasive diagnostic techniques in dermatology. Our results are also comparable—if not better—to those obtained by experienced personnel, since optical detection does not depend on clinical staff expertise.

Table 1. Diagnostic statistical criteria in the differentiation of benign and malignant melanin-pigmented cutaneous lesions using laser-induced autofluorescence spectroscopy. SN: Sensitivity. SP: Specificity. DA: Diagnostic accuracy.

<table>
<thead>
<tr>
<th>N</th>
<th>Comparison</th>
<th>SN%</th>
<th>SP%</th>
<th>DA%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal skin/Compound nevus</td>
<td>91.2</td>
<td>100</td>
<td>91.2</td>
</tr>
<tr>
<td>2</td>
<td>Compound nevus/Dysplastic nevus</td>
<td>82.9</td>
<td>86.8</td>
<td>69.6</td>
</tr>
<tr>
<td>3</td>
<td>Dysplastic nevus/Malignant melanoma</td>
<td>89.6</td>
<td>78.7</td>
<td>74.1</td>
</tr>
</tbody>
</table>

The most important feature of the algorithm we developed is that it can indeed differentiate between malignant and nonmalignant lesions. Were the algorithm absolutely accurate, 100% of lesion types could be predicted. But every diagnostic test has its own limitations: one procedure may miss several lesions and return a few false diagnoses, while another may miss only a few lesions, but with a higher number of false diagnoses. Overall, the fluorescence detection of skin benign and malignant pigmented lesions yields very good diagnostic performance for the identification of malignant melanoma lesions in vivo. Our next step will be to extend the technique to the diagnosis of other skin lesions and the development of improved algorithms and equipment for early noninvasive skin cancer detection.

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Ekaterina Borisova started to work in the field of biomedical photonics in 1999 at the Institute of Electronics, BAS. Her research covers the optical spectroscopy of hard and soft human skin. Continued on next page
tissues and their pathological changes, as well as the problems associated with photodynamic therapy applications. She has been a secretary and member of the organizing committees of two issues of the International School on Quantum Electronics, and has written numerous papers for this and other SPIE events related to biomedical optics.

References