In vivo Raman analysis of human skin lesions

Ali Tfayli, Olivier Piot, Sylvie Derancourt, Philippe Bernard, and Michel Manfait

A new Raman system, consisting of optic fiber bundles coupled to an axial Raman spectrometer has been developed for in-vivo investigations and can be used on human skin lesions.

Vibrational spectroscopy is increasingly used for the investigation of biological tissues: spectral analysis reveals hidden information at microstructural and molecular levels. Moreover, *in vitro* and *ex vivo* applications have established the potential of these techniques for detecting and diagnosing different types of lesions. Several studies show that it can successfully differentiate cancerous from benign tissues on thin sections of biopsies on colon, cervix, stomach, breast, and skin.¹⁻¹¹ For *in vivo* diagnosis, Raman spectroscopy permits a non-destructive analysis, unhindered by the aqueous nature of tissues. It does not require any previous sample preparation or labeling. In addition, it is a rapid technique enabling real-time spectral acquisition.

In 1997 M.G. Shim *et al.*¹² described a fiber-based Raman system and presented *in vivo* spectra from buccal cheek epithelium, human skin, fingernail, and tooth. Since then, several other *in vivo* studies have been done on atherosclerotic plaque,¹³ skin,¹⁴ colon¹⁵ and esophagus.¹⁵ For our work we used a new spectrometer, the unique feature of which is its size (60cm long). This allows for easy transport into operation rooms for in-situ clinical application. We used the devices to perform a study on 10 consenting patients treated for basal cell carcinomas (BCC), a very common skin cancer.

The spectrometer has an axial geometry with no moving parts. It is equipped with a laser diode emitting at 830nm, a bifurcated fiber-optic bundle (SEDI Fibers) to transport excitation and scattered light, a grism (grating and prism) to spread the light, and a charge-coupled device (CCD) detector (see Figure 1).

The size of the fiber bundle was limited to a 1.6mm diameter so it could be inserted into an endoscope. The spectral resolution is 2cm^{-1} and the output power at the central delivery fiber is 110mW. These specifications mean the system is safe for in-vivo application. However, it can acquire a Raman spectrum in just 30 seconds.

Figure 1. A Global schematic view of the setup (a), and detailed view of the axial spectrometer (b).

Figure 2. The spectra of benzene recorded with the Labram (dotted line) and the portable axial spectrometer (connected line).

The first step in the measurement process required the verification of the system efficiency, accuracy, and resolution. To this end, we collected spectra of pure products like benzene

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and naphthalene commonly used as reference products in Raman spectroscopy (see Figure 2). Then, we collected spectra from phenylalanine and tyrosine (see Figure 3), two amino acids that present highly intense bands that can easily be identified all tissue spectra. Finally we collected the signal from elastin fibers (not shown). In both Figure 2 and 3 the quality of spectra is weakly affected by the fiber signal. For elastin, the signal-to-noise ratio decreased significantly as compared to a Labram spectrometer spectra, also from the Horiba Jobin Yvon company.

In-vivo skin spectra present an intense signal below 1100cm^{-1} due to the silica in the fiber, and this needed to be corrected before any classification. The spectral windows were therefore reduced to the $1000\text{--}1800\text{cm}^{-1}$ region, where the silica fiber contribution can be mathematically eliminated via a polynomial base-

Figure 3. The spectra of phenylalanine (connected line) and tyrosine (dotted line) in the $900\text{--}1750\text{cm}^{-1}$ region recorded with the portable axial spectrometer.

Figure 4. A comparison between the mean spectrum of normal skin and the basal cell carcinoma mean spectrum.

Figure 5. A dendrogram separating into two groups the spectra of normal and cancerous skin.

line correction. All skin spectra were corrected using the same function.

Hierarchical cluster analysis (HCA) was performed to classify the spectral data. A thorough description of this statistical analysis can be found in Reference 11. The results are shown in a tree-like diagram (dendrogram), grouping spectra in clusters according to a heterogeneity scale (see Figure 5)

Comparison between normal skin and BCC spectra (see Figure 4) shows a slight frequency shift in the C-H deformation mode (around 1450cm^{-1}), a weak shoulder at 1685cm^{-1} in the protein Amide I band of carcinoma spectra, and some differences in the $1200\text{--}1250\text{cm}^{-1}$ range (Amide III and phospholipids). HCA was performed on the Amide-III band and the C-H deformation band at 1410cm^{-1} .

In spite of the poor quality of skin signal (because of the high contribution of the fused silica fiber), the combination of spectral data and HCA enables a relatively good separation.

Conclusion and perspectives

The examples presented here show that our portable Raman system could potentially be used as diagnostic tool in medical care centers. Improvements in optical instrumentation are possible by putting the appropriate filters at the tip of the bundle and by changing fiber geometry. Also, more advanced data analysis should permit improved signal quality by creating models of the fiber signal and therefore allowing for more accurate cancellation of its effect.

Further experiments will involve a larger number of patients with respect to variations in skin lesions. Sex, age, and location on the body will also be taken into account.

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Author Information

Ali Tfayli, Olivier Piot, and Michel Manfait

MéDIAN unit

UFR Pharmacy, University of Reims

Reims, France

Sylvie Derancourt and Philippe Bernard

Dermatology Service

CHU Robert Debré

Reims, France

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