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High-speed line-focus Raman microscopy with spectral decomposition of mouse skin☆

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ABSTRACT

Line-focus Raman microscope system was designed and constructed for high speed quantitative analysis of Raman spectral maps. Laser line generator lens was used for formation of homogeneous laser power distribution thought the line. Three-dimensional Raman maps of mouse skin were obtained using point and line illumination modes. It was shown that laser line illumination can provide Raman spectrum with signal to noise ratio comparable to point illumination mode. The speed of scanning in line-focus mode achieved is two orders of magnitude faster compared to the point illumination mode, which enables *ex vivo* measurements of large areas of skin surface during a few minutes. Non-negative least squares (NNLS) decomposition of mouse skin spectral maps was done using keratin, lipids, water, lactate, urea and natural moisture factor (NMF) components as library spectra. Fluorescence background of measured spectra was corrected using the fluorescence profile obtained from time-lapse Raman skin measurements, which was used as additional component in NNLS decomposition procedure. The lateral and depth distribution of major skin components obtained from Raman maps mainly correlates with histological information. High-quality line-focus Raman maps from large sample area expand the possibilities of studying skin chemical components distribution.

1. Introduction

Spontaneous Raman effect has relatively low efficiency ($I_{Raman} \sim 10^{-7}$–$10^{-11} \times I_{laser}$) compared to SRS and CARS processes [1]. Therefore typical pixel dwell time varies from hundreds of milliseconds to hundreds of seconds for the systems where CCD detector is used for registration of the broadband Raman spectrum. Nevertheless, due to a number of fundamental and experimental reasons spontaneous Raman technique is much more popular than coherent Raman techniques in many research fields, but it has limited use for applications, which require fast chemical mapping [1].

In order to decrease the total time needed for measuring complete Raman map of the sample three basic methods were developed: line-focus [2], line-scan [3] and direct Raman [4]. Line-focus and line-scan techniques require flat field, astigmatism free spectrograph for simultaneous measurement of spectra from many points through the line. Line-focus technique needs much less laser power per point compared to line-scan technique [3]. Therefore, line-focus method is better suited for temperature sensitive samples, where local overheating due to absorbed laser energy could be a problem. On the other hand, line-scan technique can be used with a pinhole for true confocal data registration.

Direct Raman technique is the fastest spontaneous Raman method, which makes it possible to register the 2D map at selected wavenumber simultaneously. It is possible to obtain the spectrum via acousto-optic tunable filter (AOTF) [5] or recently realized interferometric technique with Michelson interferometer and square CCD [6]. Similar to line-scan, direct Raman technique is also unfavorable for temperature sensitive samples due to high total laser power required for illumination of the large sample area.

Considering advantages and disadvantages of discussed spontaneous Raman methods we decided to realize line-focus technique in our self-made Raman microscope. Such instrument can greatly decrease the acquisition time of obtaining Raman map, which is critical for investigating biological samples. Such a map can be

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further analyzed via decomposition procedures to provide useful information about components distribution through the sample.

Raman maps obtained from large sample areas expand the possibilities of studying chemical components distribution of biological systems like skin. Line-focus microscope with line size of 400 pixels makes possible to carry measurements 400 times faster than traditional point illumination Raman microscope. Total time needed to obtain spectral map with spatial dimensions 400 × 100 × 10 μm considering 10 s exposure time and 1 μm focal spot will be 2.7 h in line-focus mode versus 46 days in point illumination mode. It is obvious that most biological samples would decay during such a long experiment, if point illumination mode were used.

The most common method used for skin investigation is staining of histological samples to study microanatomy. The main disadvantage of such a method is impossibility to observe skin tissues *in vivo*. An ultrasonography and confocal microscopy solve this issue and give opportunity to study *in vivo* skin structure but do not detect chemical components. Raman spectroscopy of skin can provide useful information about active substances, penetration of enhancers, skin water content, distribution of natural moisturizing factor (NMF) and *stratum corneum* thickness [7–9]. Monitoring of PCA and UCA volume is important to understand the behavior of the natural moisturizing factor and hydration of skin [10]. Such information can be obtained via detailed analysis of spectral peaks in mouse skin Raman spectrum. Taking into account spectral overlap between different skin components like protein, amino acids, lipids and other, intensity of the selected peak cannot be strongly correlated with concentration of the component. Concentration of species in the investigated system may be

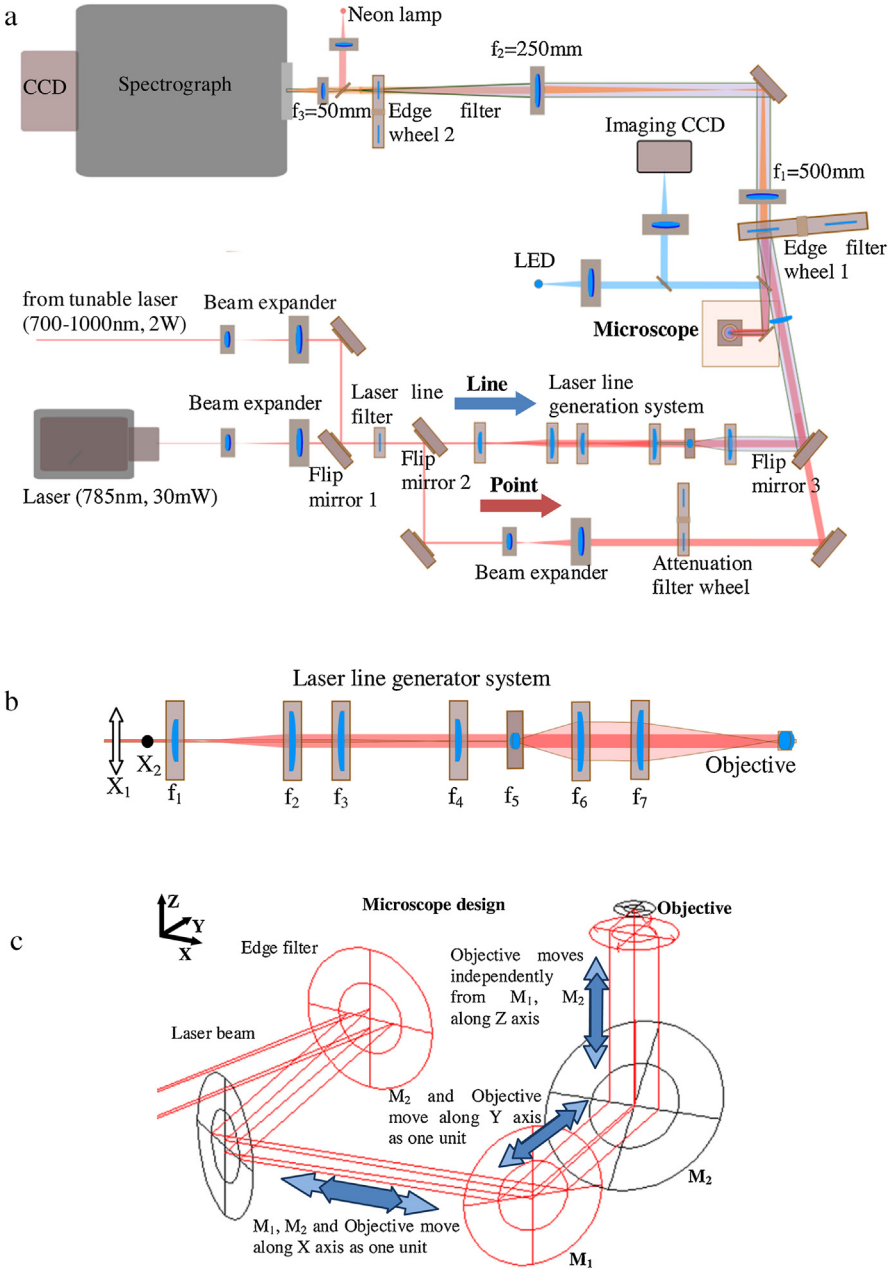

Fig. 1. Optical schemes of Raman system.
(a) Optical schemes of line-focus Raman system with homogeneous laser power distribution throughout the illumination line.
(b) Optical scheme of line generator system.
(b) Inverted microscope design.