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LIGHT SCATTERING SPECTROPHOTOMETRIC DEVICE FOR CELLULAR STRUCTURE CHARACTERIZATION

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Light scattering spectrophotometry is a new optical-probe technique suited for accurately measuring of *in situ* cellular structure features. This paper presents an original experimental optical device (design and construction) witch is based on the Mie light scattering theory and microscopically control of the investigated field.

Key words: Light scattering spectra; Mie theory; Microphotography; Cell cultures

1. INTRODUCTION

Characterization of cellular structures in cell cultures is an important problem in biomedical sciences. The most optical methods used to characterize cell structures are based especially on image analysis and flow cytometry. Recently a new spectrophotometer method has been developed using the Mie theory, started from the necessity of cellular structures analysis, with a view to establish the amount of cellular nuclei dimension distribution. The Mie theory provides an adequate method for extracting information about cell nucleus [1].

Because cellular nuclei have diameters between 5-10 μm for normal cellular and respectively up to 20 μm for transformed cells, the Mie theory corresponds to particles larger than the wavelength of incidence light [2].

The general rigorous Mie theory is based on a complete mathematical-physical theory of electromagnetic radiation scattering by non-absorbing isotropic spherical particles [3].

Particles here means an aggregation of material that constitutes a region with refractive index n that differs from the refractive index of its surroundings medium n_0. The dipole backward radiation pattern from oscillating electrons in the molecules of such particles superimposes to yield a strong net source of scattered radiation. Also, the backward radiation patterns from all the dipoles do not cancel in all but the forward direction of the incident light as it is true for a homogeneous medium, but rather interfere both constructively and destructively in a radiation pattern. Hence, particles scatter light in various directions with varying efficiency.

Mie's classical solution is described in terms of two parameters, m and x. The magnitude of refractive index mismatch between particle and medium expressed as the ratio of the n for particle and medium

$$m = \frac{n}{n_0} \tag{1}$$

The size of the surface of refractive index mismatch which is the "antenna" for backward radiation of electromagnetic energy, expressed as a size parameter x which is the ratio of the meridional circumference of the sphere ($2\pi r$, where radius = r) to the wavelength λ of light in the medium, and the wavelength λ_0 of light in the vacuum.

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$$x = \frac{2\pi r}{\lambda} = \frac{2\pi r n_0}{\lambda_0} \tag{2}$$

For a dielectric non-absorbing particle and an unpolarized primary radiation of unit intensity, the relative scattered intensity depends on the angle θ and distance r from the centre of the sphere appears as:

$$I_\theta = \frac{\lambda^2}{8\pi^2 r^2}\left[i_1(\theta) + i_2(\theta)\right], \tag{3}$$

where i_1 and i_2 are the intensity functions.

A Mie theory calculation will yield the efficiency of scattering Q_s which relates the cross-sectional area of scattering, σ_s [cm^2], to the true geometrical cross-sectional area of the particle, $A = \pi r^2$ [cm^2]:

$$\sigma_s = Q_s A, \tag{4}$$

Finally, the scattering coefficient μ_s [cm^{-1}] describes a medium containing many scattering particles at a concentration described as a volume density, ρ_s [cm^{-3}]. The scattering coefficient is essentially the cross-sectional area per unit volume of medium.

$$\mu_s = \rho_s \sigma_s \tag{5}$$

Before using Mie theory to approximate the scattering behaviour of biological tissues, let's briefly examine the Mie calculation.

Spectrophotometer devices presented in literature, were aimed to evidence malign transformation of epithelial tissue by observing the fine periodical structure of tissue reflectance spectra, with a view to quantify cellular nuclei size distribution *in situ*. Next this technique was extended to diagnose epithelial mucosa dysplasia and carcinoma from oral cavity, oesophagus and colon epithelium [4-6].

Because of bigger diameter of the nuclei in dysplasic cells compared with normal cells, beside the dimension distribution evaluation, spectrodiffusiometry provides a non-invasive and fast tool to diagnose cellular dysplasia in a more synthetic manner than microscopically images and particularly flow cytometry could do. As we can notice, the advantages of using these techniques are obvious considering the efficiency of the method and also the information provided.

Also, the retro diffusion results lead to distributions characterized by statistical assessments, like standard deviation and average value, or to deconvolutions obtained using Fourier transform, witch can be graphically represented [7-9].

The device presented in the present paper was designed, starting from a previous version [10] to simultaneously allow the obtaining of the microscopic image of the investigated area and the characterization of structure distributions, more synthetically, using fractal analysis; witch could be a helpful tool to differentiate normal from pathological shapes and, for the last one, to estimate evolutionary stages.

2. MATERIAL AND METHODS

The spectrodiffusiometric device represents a system which allow the recording of the backward diffusion spectrum recording the light reflectance gathered from a sample mounted onto the microscope platinum to be analyzed online using software tools with a view to obtain data about cellular structures observed in the optical field of the object lens.

The experimental arrangement of the device is presented in Fig. 1.

The basic components of experimental device there are:

- collimated light source with a NARVA 6V-20W halogen lamp;
- optical neutral filters;
- optical microscope ML-4M IOR;
- digital Creative PC-CAM 300 camera;
- MCS 420 Zeiss diode array multichannel UV-VIS spectrophotometer;

- Pentium III computer with interface for MCS 420 Zeiss multichannel spectrophotometer.

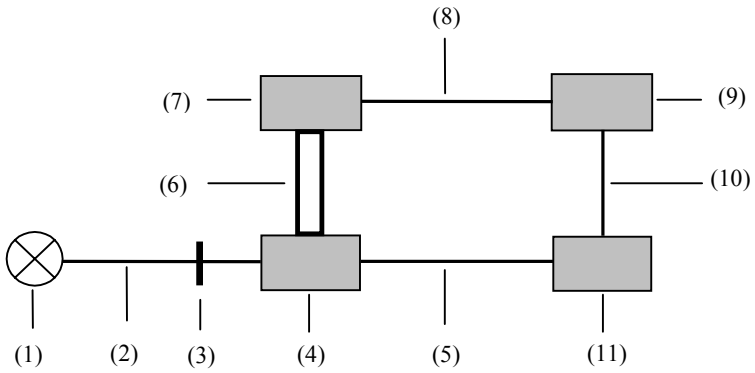

Fig. 1 Block diagram of the spectrophotometer device

(1) – Light source; (2) – Optical fibre guide; (3) – Neutral filters; (4) – Optical connector; (5) – Optical fibre guide; (6) – Microscope; (7) – Digital camera; (8) – USB cable; (9) – Computer; (10) – Computer spectrophotometer interface; (11) – Spectrophotometer.

Also we must specify that the spectrophotometer MCS 420 Zeiss uses the ASPECT V2.02 software for data acquisitioning and processing.

The component parts 1, 5, 6, 7, 8, 9, 10 (Fig. 1) remained fixed during the process, the component parts 2, 3, 4 are mobile.

The following types of optical fibre guides presented in table 1 were tested.

Table1 Optical fibres guide tested

System	*Type*	*Model*
standard	quartz	Fibroflex UV-VIS, ϕ = 1mm l = 2 m with terminal parts CZ # 772610-9018 mm
	glass	Fibrox ϕ = 1 mm, l = 0,55 m with terminal parts
	plastic	VIVANCO V3123 TOS/TOS with l = 1,5 m VIVANCO V3126 TOS/3.5 with l = 1,5 m
non-standard	glass	Fibroflex RANK ϕ = 0,97 mm with l = 1,5 m Fibroflex RANK ϕ = 1,94 mm with l = 1,5 m
	plastic	Optical fibre ϕ = 1,5 mm with l = 2 m

Among types and models tested as optical fibre guides the quartz standard ones brings together al the conditions imposed.

Regarded the optical connector (4) two methods were tested:

a) optical fibre guide (2) brings light to probe and the second one (4) gathers backward diffusion reflected light under a sharp angle;
b) optical connector with two guiding channels, using fibres guided through a common channel to probe. Optical fibre guide (2) brings light to probe through channel one and the diffuse reflected light is taken through channel two by optical fibre guide (4) (Fig. 2).

Optical connector with two guiding channels proved to be the best alternative having the advantage of removing non-alignment error by fixing joining extremities using a mechanical guide, and satisfying the low attenuation conditions.

Optimal working conditions in case of light intensity measurements were: a higher light intensity for data gathering and a lower light intensity for photography processing.

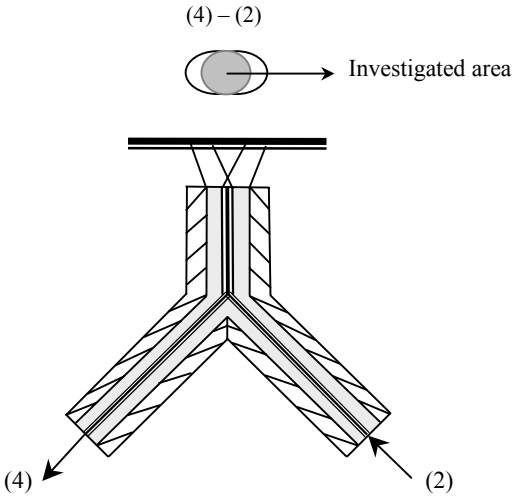

Fig. 2 Optical connector with two guiding channels

For these reasons, to microphotograph the investigated area is necessary to use neutral or polarisation filters.

The culture conditions have been described elsewhere [11]. Briefly, HeLa cells (human cervix epitheloid carcinoma cell line) were grown in DNEM (Sigma) supplemented with 10% fetal calf serum (Sigma) at 37^0C, 5% CO_2, in a humidified atmosphere.

Exactly $5x10^5$ cells were seeded onto 35 mm Petri dishes. After 24h, cells grown on Petri dishes were fixed in methanol for 5 min, and subsequently stained with May-Grünwald-Giemsa.

As cell cultures are developed on transparent surfaces, a part $I_0(\lambda)$ of the incident light intensity spectrum is reflected geometrically, and the scattering contribution $I_d(\lambda)$ results as a difference between the total recorded spectrum $I_s(\lambda)$ and $I_0(\lambda)$.

$$I_d(\lambda) = I_s(\lambda) - I_0(\lambda) \tag{6}$$

With the view to verify the performances of the system the on Petri dishes developed, unstained and stained same cell cultures were used.

3. RESULTS AND DISSCUSIONS

In figure 3 are represented the results obtained using the described optical system, recording the 200x amplified microscopic image of the field subjected to Mie scattering measurements done on the stained HeLa cells investigated, while de curve corresponds to the unstained ones.

The microscopic image in the above figure represents the stained analogous cell culture, in which nuclei and other structural components of the cells are obvious.

The Mie scattering spectrum data between 350 and 619 nm in $I_s(\lambda)$ energy units, representing the automatic calculated means of successive three light intensity values at 0.8 nm intervals, and represented with a 1 nm resolution, as given by the ASPECT V2.02 firm software, was recorded for three times on the HeLa cell culture field, and the average curve was used for the further calculations. The corresponding $I_0(\lambda)$ mean spectrum was recorded for a cell empty part of the Petri dish, in which the data are used as reference ones.

The curve presented in figure 3 represents the difference $I_d(\lambda)$ between each value $I_s(\lambda)$ and $I_0(\lambda)$ for the same wavelength value, representing a difference Mie scattering spectral curve, and noted I(M) versus wavelength in nm.

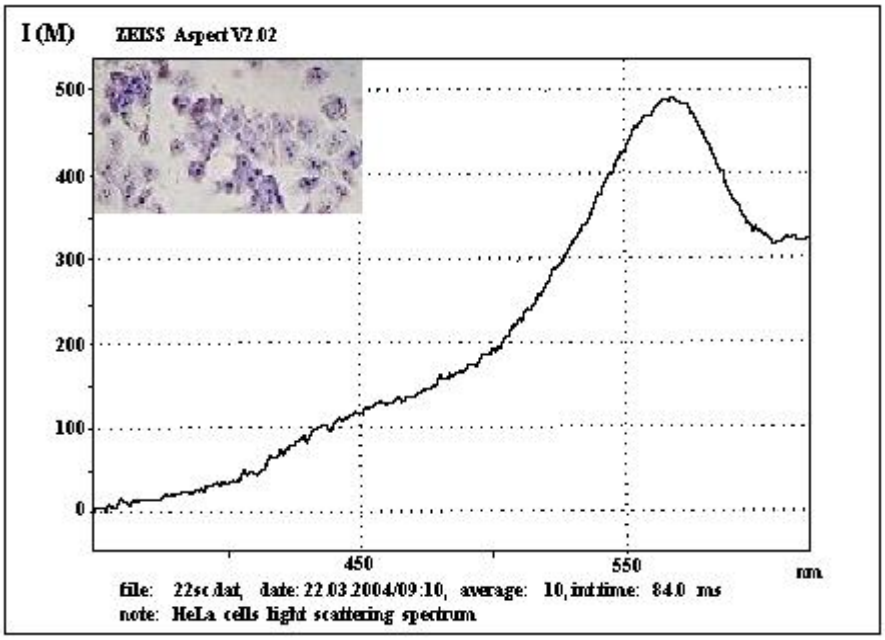

Fig. 3 HeLa cells light scattering spectrum I(M) and microphotography

The I(M) curve in figure 3 shows a specific Mie scattering pattern at least for two diameter distributions of the scattering centres, one in the 350-500 nm range, an the other, much intensive maximum, in the 500-600 nm range, which could be attributed to the nuclei dimension ranges of the transformed HeLa cells investigated.

The Rayleigh scattering curve would decrease monotonously and without intensity maximum values, so that this effect does not influence significantly the phenomenon used for cell nuclei distribution characterisation.

4. CONCLUSION

The device presented is consistent with the initially imposed conditions for the practical simultaneous record of both the microscopic image of the investigated cell culture area and the Mie scattering measurements.

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