THE SIGNAL TO NOISE RATIO IN MICROSPECTROPHOTOMETRY OF LIGHT SCATTERING SAMPLES

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Summary

The paper describes the results of investigations into the signal to noise ratio of microspectrophotometry of light scattering samples. It is demonstrated that in microspectrophotometry of light scattering systems the noise characterizing the sample has to be taken into account. The effects of various methods for improving signal to noise ratio are demonstrated. ·

The objects of our surroundings are, from the optical point of view, light scattering systems. During criminal activity, as a result of mechanical interaction, large numbers of microscopic, light scattering samples are produced, their analytical investigation being one of the most frequent problems in forensic chemistry. The aim of the present paper is to show the results of the investigations into the signal to noise ratio of microspectrophotometry of light scattering samples.

Light scattering samples are characterized by 1–100 µm inhomogeneities. During their interaction with light, if we disregard luminescent phenomena, the elementary processes of scattering and absorption of light occur [1]. During the interaction of the investigating light beam and the sample these elementary processes are summed and combined in large numbers. As a result the optical properties of the system change fundamentally, they are no longer additive. The optical properties of the light scattering system are determined, beyond physical constants by the structure of the system. The characteristic remission and transmission spectrum of the light scattering system being formed as the result of the combination of the effects [2]. An additional source of noise, as compared to non-scattering systems, is the statistically non equalized character of the various inhomogeneities. The defined measurement conditions in microspectrophotometry of light scattering systems can be determined taking into account the noise characterizing the sample.

The measurements were performed with the microspectrophotometer developed jointly with the Institute of Physics of the Technical University of Budapest, on cca. 100 sq. µm part of the samples prepared from Neolux paints (Budalakk Company, Budapest, Hungary). Further experimental details are described elsewhere [3].

Results and Discussion

Microspectrophotometric spectra recorded with natural (unpolarized) light and Köhler illumination at different positions of the sample exhibit different characters, as shown by two typical spectra of a sample recorded at different positions (Fig. 1). For scattering samples this illumination does not give reproducible results. This can be explained by the statistically non-equalized nature of the signal. In the case of macroscopic measurements very large numbers of phases with dimensions of a few micrometers are present. The number of particles in the sample decrease with the third power of the linear dimensions of the sample. In the case of microscopic dimensions, where the geometrical measurements of the sample and the particles do not differ by orders of magnitude, the statistical equalization cannot be insured by the sample. The measurement technique must be selected, so as to insure the large number of interactions between the phases and the photons, or to exclude from the measurement the photons, which did not participate in sufficient number of interactions, to achieve statistical equalization of the interactions.

To achieve the above aims, the sample was measured with a numerical aperture larger than necessary for the useful enlargement [4] and was placed
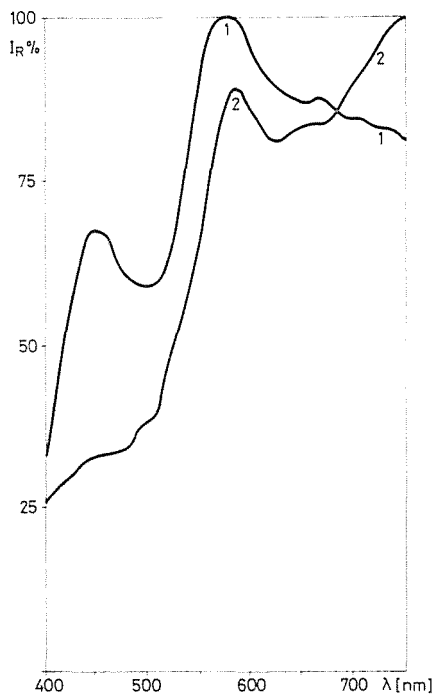

Fig. 1. Spectra from two different parts of the sample, recorded with an objective with a numerical aperture of 0.1, illuminated with natural (unpolarized) light

Table 1

The effect of different signal to noise ratio increasing methods on the standard deviation of the signal samples

No.	Characteristic data of the method combination			D	D^*	$\dfrac{S_i}{S_1}$
	smoothed	NA	average			
1.	no	0.1	1	1362		1
2.	no	0.5	1	157		0.115
3.	yes	0.5	1		25.9	0.019
4.	no	0.5	81	14.9		0.011
5.	yes	0.5	81		2.45	

where NA —numerical aperture
D —the standard deviation of the signal samples by approximate difference test (in detail see (6))
D^* —the standard deviation of the signal samples by the efficiency of the smoothing (in detail see (7))
S_i —the standard deviation characterizing the noise in the case of the i-the method combination.
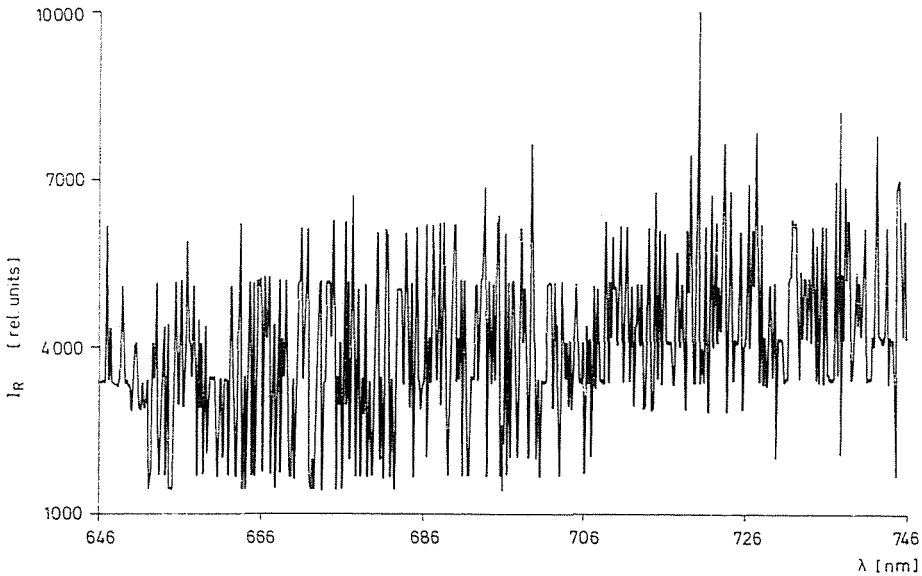

Fig. 2. Part of a spectrum measured according to row 1 of Table 1

between crossed polarizators [5]. The amount of light reaching the detector carrying the information coded in the intensity change as a function of the wavelength was increased by enlarging the numerical aperture. The polarization state of the light also changes on interaction with light scattering samples, and this also contains information about the sample. By the use of crossed polarizators this information, coded in the polarization state of the light, was used to increase the signal to noise ratio.

The effect of the various signal to noise ratio increasing methods is summed in Table 1. Increasing the signal to noise ratio could only be quantified after the first step, detailed above, as measured without crossed polarizers quantifiable data could not be produced (as a good approximation it can be stated that the signal to noise ratio is infinitely small). By the crossed polarizers
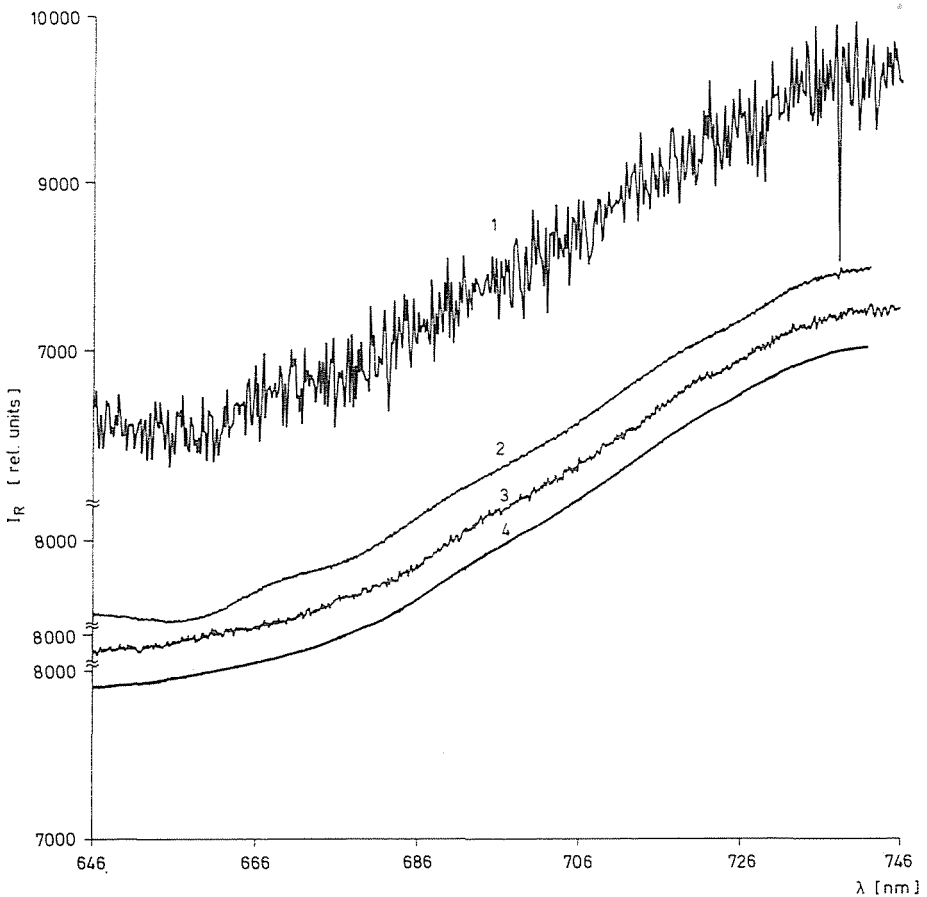

Fig. 3. The effect of different signal to noise ratio increasing methods on the part of the spectrum shown in Figure 2.
1. measured according to the 2nd row of Table 1; 2. measured according to the 3rd row of Table 1; 3. measured according to the 4th row of Table 1; 4. measured according to the 5th row of Table 1

the information carrying part of the light was selected from the noise, but this increased the role of the noise of electronic origin. By enlarging the numerical aperture the number of those photons was increased which had repeatedly interacted with the particles of the sample, thereby increasing the useful signal. The signal to noise ratio was further improved by time averaging and smoothing with a moving, triangularly weighted, average, giving the spectrum, characterizing the scattering sample.

To demonstrate the phenomenon, we show the spectrum range of 646–746 nm in different phases of increasing the signal to noise ratio in Figs 2 and 3. The spectrum recorded with the parameters of the first row of Table 1 can be seen in Fig. 2, from which information, other than the electronic noise, cannot be gathered. Curves 1, 2, 3 and 4 in Fig. 3 show the spectra recorded with the parameters of the 2, 3, 4 and 5 row of Table 1. The decrease of both high and low frequency noise is evident. Furthermore, the increase of spectral information can also be observed.

Figure 4 shows the averages of the remission spectra recorded at different positions on the same sample. The measurements were made with the parameters listed in the 5th row of Table 1. The values of the signal to noise ratio are also shown according to the definition below:
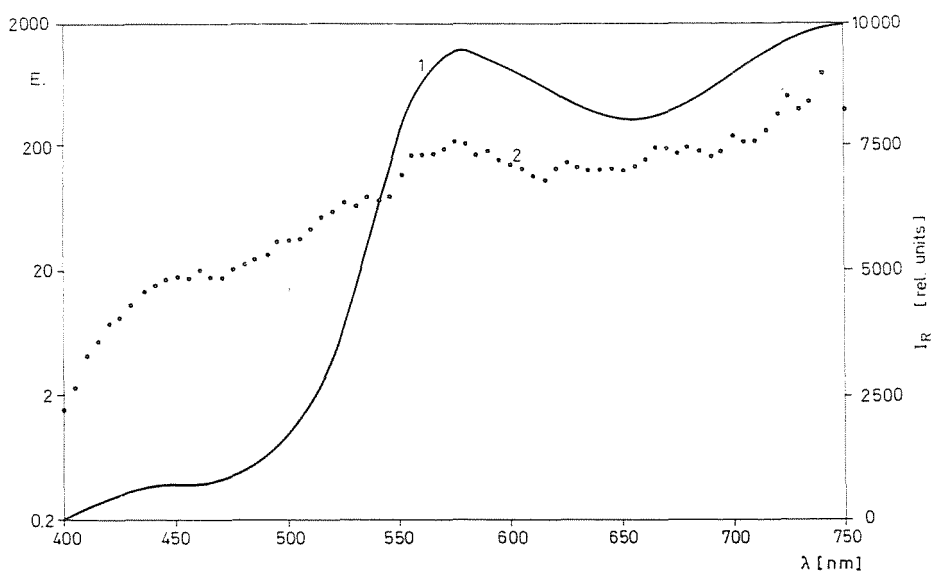
$$E = \frac{I_{R,\lambda}}{S_\lambda}$$

Fig. 4. Spectrum averaged over ten points on the surface of the sample (continuous line) and the signal to noise ratio (broken line)

where $I_{R,\lambda}$ is the remission at wavelength, S_λ is the estimated standard deviation based on ten parallels. It can be seen, that the signal to noise ratio is a few hundreds, with the exception of the high absorption part of the spectrum. The quality of the spectra can be further improved, if the number of time averages is changed with the wavelength, for recording the whole spectrum at the same signal to noise ratio.

References

1. HUSZÁR J.: The efficiency of light absorption in light scattering systems. (A fényelnyelés hatékonysága diffúzszóró rendszerekben.) Dissertation of CSc. degree, Budapest, Hungary, 1969.
2. LÁNG L.: The significance of the relative light absorption method in absorption spectrophotometry. (A relatív fényelnyelés módszerének jelentősége az abszorpciós spektroszkópiában.) Dissertation. of DSc. degree, Budapest, Hungary, 1971.
3. LEISZTNER L.: Computerized microanalytical system in forensic science. (Számítógépes mikroanalitikai rendszer a kriminalisztikában.) Dissertation of DSc. degree, Budapest, Hungary, 1983.
4. LAING, D. K., DUDLEY, R. J., ISAACS, M. D. J.: Forensic Science International, *16*, 159 (1980)
5. UMOW, N.: Physikalische Zeitschrift, *6*, 674 (1905)
6. LEISZTNER L., BARNA P.: Journal of Automatic Chemistry, *5*, 155 (1983)
7. ENKE, C. G., NIEMAN, T: A.: Analytical Chemistry, *48*, 705A (1976)

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