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Use of Reflectance in Soft Laser Densitometry in Scanning Opaque Electrophoregrams

A High-Resolution Technique

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ABSTRACT

The present report describes a new approach in soft laser scanning densitometry performed on opaque electrophoregrams. The method employs a light reflectance system instead of absorption, which is commonly used. The data of this preliminary study were obtained by directly scanning a photograph of an electrophoregram exhibiting viral proteins separated by SDS-PAGE. Comparison of the two methods clearly demonstrate that laser reflectance offers finer resolution than laser absorbance when opaque materials are scanned.

Index Entries: Laser densitometry; scanning opaque electrophoregrams, use of soft laser densitometry in; reflectance, use of in soft laser densitometry; soft laser scanning densitometry, a new approach.

INTRODUCTION

The use of soft laser beams in scanning densitometry has been a major breakthrough in analytical protein chemistry, and since its inception in 1974 (*1*), has opened new frontiers in basic and applied medical research (*1–7*). Densitometers using the laser beam may now resolve stained protein components in an electophoregram having an interspace

of 1 μm or less. In this manner, adjacent protein bands not visually distinquished by the naked eye appear in the densitometric tracing as distinct peaks reaching the baseline, and may thereafter be easily quantitated.

However, when the scanned material is opaque, such as in thin layer chromatogrphy, the resolving capability of any densitometer operating on the principle of light absorbance decreases. This is particularly evident when the distance separating two adjacent components approaches the width of the scanning light beam. In this regard, as the light beam passes through an opaque material, it undergoes scattering and, as a result, becomes wider after transversing the medium. In this manner, a loss in the resolving capability of the densitometer is observed. Under such circumstances, scanning closely stacked bands in an electrophoregram produces tracings of poor resolution. The peaks corresponding to the scanned adjacent components fail to reach the baseline, and in many instances they may fuse, thus preventing their quantitation. To this end, the present study was undertaken to determine whether the use of laser reflectance could be an alternative method in scanning electrophoregrams in which opacity poses a problem in obtaining densitometric tracings of high fidelity.

MATERIALS AND METHODS

A picture of an electrophoregram showing viral protein bands separated by SDS-gel electrophoresis was scanned, using a soft laser densitometer, SL-TRFF (Biomed Instruments, Inc.). The scanner was interphased with an Apple IIe computer, and densitometric analysis was carried out using the "'Videophoresis II" program (Biomed Instruments, Inc.). Following several runs, the optimal scanning speed determined to produce densitometric tracings of the finest resolution was 3.5 mm/s. The laser beam was focused on the sample, using a high-resolution adapter, and changes in the intensity of the reflected light were measured by two photocells facing the surface of the sample. Hard copies of the densitometric tracings were obtained through a dot matrix printer (STAR SG-10).

RESULTS AND DISCUSSION

The laser reflectance system has now been employed in the densitometric analysis of an electrophoregram reproduced on photographic paper. This approach offers the opportunity to scan opaque material, such as thin layer chromatography, nitrocellulose, or even photographic paper, as was the case presented in this report. It has been illustrated that the use of such a system in scanning opaque material generates high resolution tracings that are superior to those obtained by laser

absorbance. A practical application of the new approach is presented in Fig. 1a, which illustrates densitometric analysis of the image of an electrophoregram in photographic paper. It can be seen that the peaks produced by laser reflectance are sharp, well-defined, and reach the baseline. Most important, the tracing reveals the presence of peaks that are not seen in the tracing produced by laser absorbance (Fig. 1a), as indicated by arrows #1, 2, and 3. The differences in the data generated in scanning by the two methods become more evident upon electronic expansion of the tracings, and Fig. 2 presents a clearer view of such discrepancies.

Equally impressive was the capability of the scanner, when laser reflectance was used, to resolve closely stacked bands not visually distinct to the naked eye. This can be better appreciated upon inspection of band "m," identified by an arrow at the right side of the electrophoregram. It is of interest that, although it is visualized as a single band, in the tracing in which laser reflectance was used, it is resolved into two components, as evidenced by the well-defined notch separating the two peaks shown in Fig. 2c.

Resolution of band "m" into two components was also observed when laser absorbance was used (Fig. 2d). However, the tracing produced was not as sharp as that obtained by reflectance.

Upon gross examination of the densitometric tracings produced by the two methods, it can be seen that the peaks in the tracing in which reflectance was used rise at a steeper slope than those observed when the conventional method was utilized. Apparently, this could be attributed to the fact that, in the latter approach, the width of the light beam is limited by the inherent physical properties of the scanned material. That is, the fine width of the laser beam can no longer be controlled after focusing on an opaque sample, since it undergoes diffusion. It becomes wider than it originally was, and, therefore, the scanning time at a given point in an electrophoregram increases accordingly. This is evident when the slopes of the peaks in the tracings illustrated in Fig. 2 are compared. It can be observed that the slopes of the peaks produced by laser absorbance are not as steep as those seen when laser reflectance is used. In further analysis, when the light beam width becomes greater than the gap between two adjacent bands, but is smaller than the space occupied by them, the peaks generated rise at an angle determined by the width of that beam. That is to say, the wider the beam width the wider the angle of deflection of the peaks. That is why, under certain conditions, tracings produced by laser absorbance are not as sharp as those observed when laser reflectance is employed.

In summary, the new approach in laser densitometry described in this communication may be engaged to prevent the loss in resolution observed when opaque electrophoregrams are scanned, and to thus increase the accuracy of the results reported.

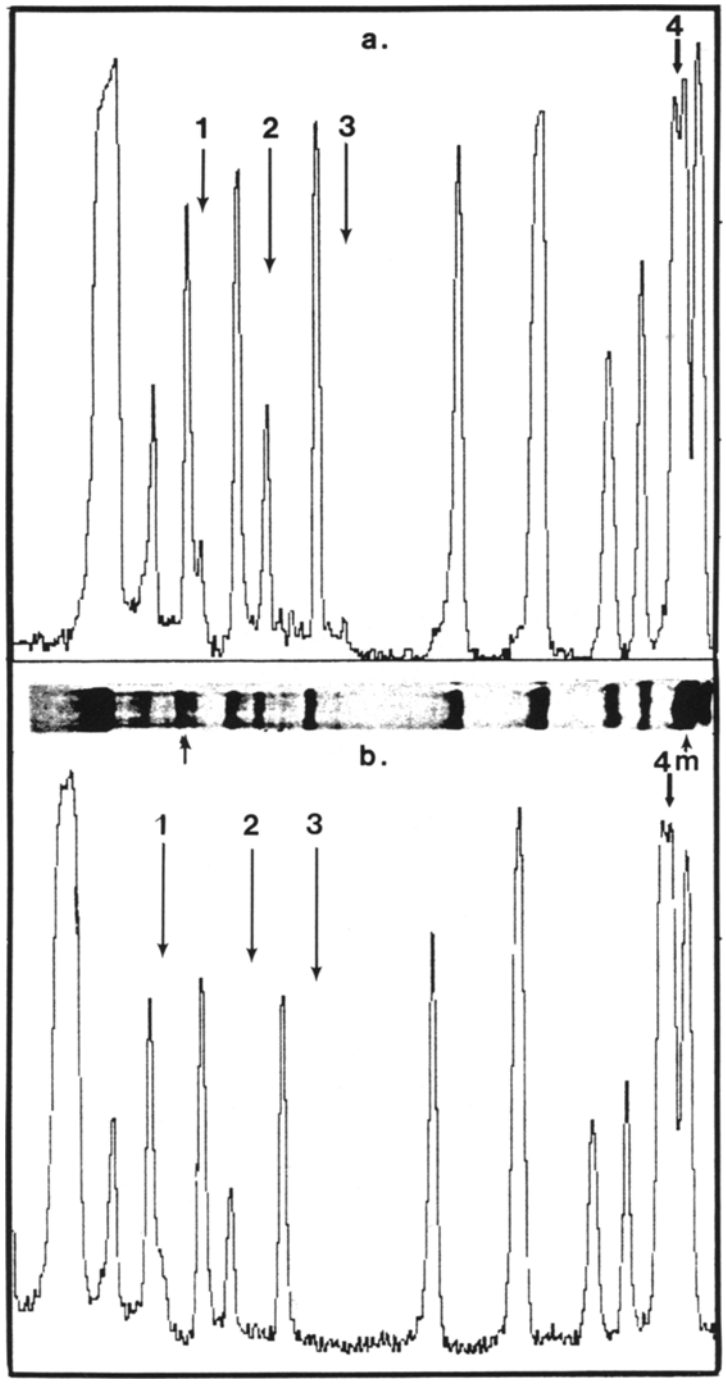

Fig. 1. Soft laser scanning densitometry of a photograph illustrating separation of viral proteins by SDS-PAGE. (a) and (b) represent tracings produced using reflectance and absorbance, respectively.

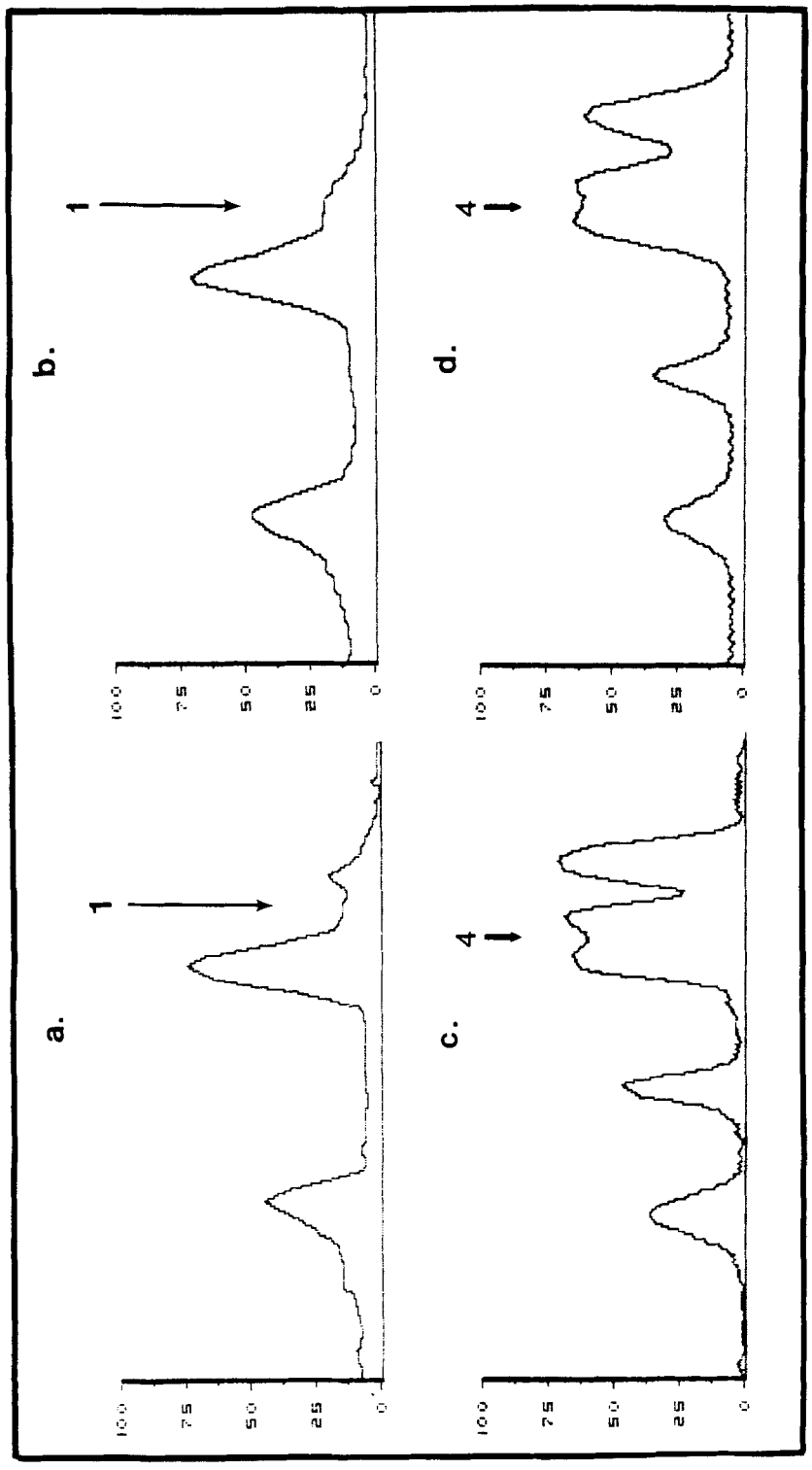

Fig. 2. Segments of tracings produced by soft laser scanning densitometry, expanded fourfold by electronic manipulations provided by a computer program (Videophoresis II). Figures (a) and (b) illustrate in greater detail peak contour of segments identified in Fig. 1a by arrows #1 and #4, respectively. Figures (c) and (d) depict details in peak contour of segments shown in Fig. 1b by arrows #1 and #4. Note the finer resolution obtained when the system of reflectance was used.

REFERENCES

1. Zeineh, R. A., and Num, W. P. (1974), *Clin. Res.* **22**:3 428A.
2. Barany, K., Barany, M., Gillis, M. J., and Kusmeric J. M. (1979), *J. Biol. Chem.* **254,** 3618.
3. Barron, J. T., Barany, M., and Barany, K. (1979), *J. Biol. Chem.* **254,** 4954.
4. Kyriakidis, G., and Zeineh, R. A., Protein Band Patterns in Isoelectric Focusing of Cerebrospinal Fluid in Multiple Sclerosis Analyzed by Soft Laser Densitometry, In preparation.
5. Zeineh, R. A., Nijm, P. W., and Al-Azzawi, F. (1975), *Am. Lab.* 7:2 51-58.
6. Zeineh, R. A. (1977), *Am. Lab.* **9**:6 71-82.
7. Zeineh, R. A. (1977). *Soft Laser Scanning Densitometry Compatible with High Resolution Obtained by Electrofocusing,* Proc. Intl. Symp., August 2–4, 1976, Hamburg, Germany, *Electrofocusing and Isotachophoresis,* pp. 147–151. Radola, B. J., and Graesslin, D., ed., Walter de Gruyter, Berlin NY.