
CLIN. CHEM. 34/11, 2367–2370 (1988)

A Reflectance Photometer with a Square Photodiode Array Detector for Use on Multilayer Dry-Film Slides

William E. Nessley

This semiautomated prototype reflectance photometer measures reflected light from multilayer dry-film slides. The instrument makes use of a square photodiode array detector, a Hewlett-Packard desktop computer, and a modified mechanical transport mechanism from an Ektachem DT60 analyzer. When 2 μL of serum is placed on a dry-film slide, a colored spot is formed. The slide is automatically transported to an incubation area and then to the photometer area. There the spot is illuminated with dual tungsten lamps, and the reflected light passes through an interference filter, where it is focused on a square photodiode array containing 10,000 individual detectors. The analog signal from each detector is digitized and transmitted to a computer for calculation of the percentage of reflectance. I used a series of algorithms to locate the spot, estimate spot area, correct for minor variations in sample volume, and compute the average reflectance from a central spot area. To evaluate the instrument’s performance, I ran parallel glucose determinations in the Beckman Astra; results correlated well. The small sample size along with no dead sample volume makes the system useful for small sample volumes.

Additional Keyphrases: prototype instrument · pediatric chemistry

For the past 15 years several companies have produced dry-reagent systems for measuring common analytes in serum and urine (1). Their technique involves flooding the slide or strip with sample, illuminating a small portion of the slide with a small beam of light, and then measuring the amount of reflected light. More recent advances have resulted in the development of layered-film technology that permits more control over the reactions and the use of much smaller sample volumes (2, 3). We have described two earlier systems in which 1 to 2 μL of serum is placed on a multilayer dry-film slide, a colored spot developed, and a linear photodiode array used to measure the amount of reflected light across the middle of the spot (4, 5). Both systems greatly depend on positioning the film element so that the reflectance measurement is taken across the middle of both spots. In both cases the film element was removed from its plastic support.

In the current design, I use a square photodiode array and an automated slide-transport mechanism with an incubator. Software algorithms are used to locate the spot, which greatly relaxes the requirements for exact sample positioning. I compute each sample volume based on spot area and provide a computed correction to compensate for small variations in sample size. By using a 37°C incubator I have been able to extend the time between calibrations.

Materials and Methods

Instrument. A photograph of the instrument is shown in Figure 1. I used a slide-transport assembly and incubator from an Ektachem DT60 analyzer (Eastman Kodak Co., Rochester, NY). Two additional optical limit switches were added to aid in monitoring the position of the slide transport mechanism. The stepping motor for the slide transport mechanism was controlled by the computer via a 16-bit I/O

---

Division of Laboratory Medicine, Department of Pathology, University of California School of Medicine; and Veterans Administration Medical Center, San Diego, CA 92161.

Received July 14, 1988; accepted August 26, 1988.

---

Fig. 1. Photograph of the instrument, with partial removal of panels to show internal components
interface. A modified Ektachem DT digital pipet was used, so that a range of sample volumes from 1 to 10 μL could be digitally selected.

I used a matrix camera containing a square photodiode array with 10 000 photodiodes arranged in a 100 × 100 matrix, having a center-to-center spacing of 60 μm (Model 521), a 55-mm camera lens, and a matrix camera controller (Model RSS21; all from E.G.&G. Reticon, Sunnyvale, CA). The sensing area for each photodiode is ~2100 μm². The charge-transfer photodiode array couples the advantages of diffuse photodiodes for a broad spectral response with analog registers for low noise output (6). I designed and constructed the interface circuits between the matrix camera controller and our computer system. The clock speed of the camera was adjusted so that the 10 000 photodiodes could be scanned in ~100 ms. The video output signal from the sample-and-hold circuitry was amplified by a differential operational amplifier to reduce common mode noise, and to amplify and buffer the signal. The signal was digitized by a 12-bit analog-to-digital converter (Model ADC 1102; Analog Devices, Norwood, MA). The digitized signals were transmitted to a computer (Model 9846B; Hewlett-Packard, Palo Alto, CA) via a 16-bit I/O interface. I used direct memory access to facilitate the high-speed data transmission. All programs were written in Hewlett-Packard BASIC language.

Figure 2 is a diagrammatic representation of the reflectance photometer design. Light was provided by two 10-W, 6-V DC halogen lamps positioned on opposite sides of the multilayer slide, to provide a 45° angle illumination with respect to the planer slide surface. The light duration was controlled by the computer interface circuitry; the lights were illuminated for only 2 s while the reflectance measurement was made. Dual lighting ensures uniform sample illumination, and little heat is generated during the short illumination time. I used three cavity interference filters with a 10-nm bandpass (Ditric Optics, Hudson, MA).

Before any reflectance measurements were taken, a blank slide was used to obtain a 100% reflectance reading. To accommodate variations in the light source between different slides, reference reflectance bars were used as shown in Figure 2. I constructed a thin metal mask with a central hole. Along two opposite sides and adjacent to the hole, a reference reflectance bar consisting of a narrow strip of white photographic film was attached to the metal mask. The slide mechanism automatically moves each slide so that it rests on the top of the mask. The area viewed by the photodiode array included the colored spot, the surrounding area, and the two reference bars.

Reagents. Ektachem calibrators (no. KP7435J) and multilayer film slides (Ektachem Clinical Chemistry Slides, Eastman Kodak Co.) for glucose were used.

Procedure. A multilayer dry slide was placed in the transport assembly. Then the slide was advanced manually to the sample application area, where 2 μL of sample, control or reference was applied with the Ektachem Digital Pipet. The slide advanced automatically from the sample area to an incubation area for 5 m, and then to the photometer area, where the reflectance of the slide is measured.

Calibration. The linear transformations were based on the work of Williams and Clapper (7) as modified by Curme et al. (2) and later by Neeley (5). Linearization of the calibration curve is computed by converting reflectance to reflectance density, Dᵣ, and then to transformed reflectance density, Dᵣ:

\[ Dᵣ = -0.194 + 0.469Dᵣ + 0.422[1 + 1.179e(aDᵣ)] \]

a is determined by iteration and best fit. The instrument was calibrated every three days by assaying, in duplicate, reference sera containing glucose in concentrations ranging from 400 to 5000 mg/L.

Results

Data processing. Four dark-signal measurements for each photodiode were taken and averaged before each measurement. The average was stored as a digital matrix:

\[
\begin{align*}
    d(1,1), & \quad d(2,1), \quad \ldots \quad d(100,1) \\
    \vdots & \quad \vdots \\
    d(1,100), & \quad d(2,100), \quad \ldots \quad d(100,100)
\end{align*}
\]

At the beginning of each run a blank slide was measured to obtain a 100% reflectance signal for each photodiode. Two blank-signal measurements were taken, averaged, and stored in a digital matrix:

\[
\begin{align*}
    b(1,1), & \quad b(2,1), \quad \ldots \quad b(100,1) \\
    \vdots & \quad \vdots \\
    b(1,100), & \quad b(2,100), \quad \ldots \quad b(100,100)
\end{align*}
\]

For each sample two measurements for each photodiode were taken, averaged, and stored in a matrix:

\[
\begin{align*}
    (s(1,1), & \quad s(2,1), \quad \ldots \quad s(100,1)) \\
    \vdots & \quad \vdots \\
    s(1,100), & \quad s(2,100), \quad \ldots \quad s(100,100)
\end{align*}
\]

The dark signal for each photodiode was subtracted from both the blank and sample readings. I corrected for minor differences in light output between the blank and the sample readings by adjusting the digitized sample voltages proportionally. I based this correction on changes observed in the amount of light reflected from the reference bars, as measured by the reference photodiodes located along two opposite edges for each row. The correction is applied to each separate row as follows:

\[
    r(i,j) = s(i,j) \cdot \left[ \frac{b(1,j)}{s(1,j)} + \left( \frac{b(100,j)}{s(100,j)} - \frac{b(1,j)}{s(1,j)} \right) \cdot (i - 1)/99 \right] 
\]
For a given value of \( j \) between 1 to 100, \( i \) is varied from 1 to 99. Percent reflectance is computed by the following:

\[
R(i,j) = \frac{r(i,j)/b(i,j)}{100}
\]

for \( i = 1 \) to 100; \( j = 1 \) to 100.

**Determining spot location and measuring reflectance.** The advantage of using a square photodiode array detector is that the spot can be located anywhere within the detector viewing area. I developed software algorithms to identify the exact location of the spot from the scan data. Once the spot was located, I computed the \( x \) and \( y \) coordinates for the center and then the spot diameter in both the \( x \) and \( y \) directions. The total reflectance for each spot was determined by computing the average reflectance of all photodiodes located within a central circular area having a radius 0.8 that of the spot radius.

**Estimation of sample volume.** The area of the spot produced by a serum sample is proportional to the original sample volume. To confirm this relationship, I applied different volumes of serum to the slides with a graduated syringe, allowed the color to develop, and then measured the reflectance. The image of the colored spot and the surrounding area was focused upon the square photodiode array. I estimated sample volume by summing the number of photodiodes covered by light reflected from the spot. For a 2-\( \mu \)L sample, the area encompasses \( \sim 1200 \) photodiodes.

**Corrections for variations in sample volume.** The reflectance for a given concentration of analyte is dependent, to a small degree, on sample volume as demonstrated in Figure 3. I compensated for minor variations in reflectance with respect to sample volume by developing a series of curves based on different reflectances, sample volumes, and concentrations. I used these data to create a series of algorithms to normalize all reflectance measurements to a 2-\( \mu \)L sample volume. In most instances, the correction was very small because the pipetting error was minimal. For example, for a single serum sample with different sample volumes of 1, 2, and 3 \( \mu \)L, the reflectance for each sample was 69.7\%, 59.4\%, and 57.5\%, respectively; the glucose values, without correction for sample volume, computed from a normal calibration curve, were 2260, 2430, and 2670 mg/L, respectively. This example represents an extreme case where the sample volume varied from 50% to 150% of the 2-\( \mu \)L target value. By applying the correction algorithm for sample volume, the effects of minor variations in sample volume are minimized and precision is increased.

**Precision.** Instrument precision was assessed by making repeated scans of reflectance standards having reflectance values from 100\% to 0.6\%. If the slides were allowed to remain stationary, precision, expressed in terms of the coefficient of variation (CV), was 0.051\% at 100\% reflectance, 0.070\% at 12\% reflectance, and 0.197\% at 5\% reflectance at 540 nm. Similar overall precision was found at wavelengths from 450 nm to 700 nm. If the slides were automatically transported into the area where the reflectance measurements are taken, the corresponding values were 0.32\% at 100\% reflectance, 0.45\% at 12\%, and 0.78\% at 5\%.

Twentyfold analysis of two serum samples for glucose produced the following results: mean = 630 mg/L, SD = 15 mg/L, CV = 2.4\%; and, for the second sample, mean = 2050 mg/L, SD = 58 mg/L, CV = 2.8\%. Day-to-day precision was assessed over a period of 30 days. The respective results for controls 1 and 2 were: \( n = 30 \), mean = 870 mg/L, SD = 34 mg/L, CV = 3.9\%; and \( n = 30 \), mean = 2960 mg/L, SD = 136 mg/L, CV = 4.6\%.

**Correlation with the Beckman Astra.** Serum samples were split into two portions and analyzed concurrently by both the Astra (Beckman Instruments, Brea, CA) method and the present method. Three different lots of slides were used. The results by this method (\( y \)) demonstrated acceptable agreement with those by the Astra method (\( x \)); \( y = 0.988x + 25.4 \) mg/L (\( n = 112 \), \( r = 0.992 \), \( S_{xy} = 81.7 \) mg/L).

**Discussion**

In an earlier design we attained excellent photometric precision with the use of a xenon flashlamp and a linear photodiode array. At the beginning of this project I attempted to use dual xenon flashlamps as the light source because of their high intensity over a broad wavelength range and low heat production. Xenon flashlamps could not be used with the square photodiode array because of excessive variability in light distribution over the entire array from one flash to the next. I found that dual tungsten lamps provide a marked improvement in illumination, and I was able to compensate easily for variations in lamp intensity. The lamps were illuminated only for a brief period while the reflectance measurements were being taken.

I adapted the slide-transport mechanism from an Ektachem DT60 to substantially improve automation. The main advantages of the adapted transport method include a pipette support for sample application, slide incubation at 37 °C, processing of multiple slides at the same time, and an automatic slide transportation mechanism to move slides from the incubator to the reading station and then to a waste container. These features represent major improvements over our earlier designs, by eliminating the need to remove the slide elements from their plastic support holders and the task of placing the slide elements in the reflectance photometer manually for measurement.

The photometric precision of the square photodiode array, light source, and supporting circuitry was excellent. A combination of factors caused photometric errors, as evidenced by the increase in imprecision that occurred when the slides were moved as compared with being measured in a stationary position. These factors include slight variations in the slide position in the area where the reflectance measurements are taken, variation in the flatness of the film element within the plastic support, and the lack of parallel light illuminating the slide from a 45° angle. The complete slide consists of a thin, multilayer film element mounted in a plastic support. Ideally, the film element...
should have a flat surface parallel to the plane of the slide support. In reality, there is slight variation in the surface of the film element from one slide to the next. The surface varies from slightly convex to concave. When the entire slide is illuminated, the problems are magnified. The surface variation together with the non-parallel illumination results in excessive front-surface reflection and loss in precision from slide to slide. Eastman Kodak has minimized similar errors in its DT60 design by forcing the slide element to be in contact with a single bundle of light pipes transmitting light to and from the slide. Currently, we are working on newer designs, both to improve our light supply and reduce the front-surface reflection.

This work was supported in part by grants from Eastman Kodak Company.

References

Corrections
Vol 34:
  p 813: In the fifth full paragraph in column 2, the second sentence should read "In 1934, von Meduna, a Hungarian psychiatrist . . . " (instead of 1983), and in the third sentence "1983" should read "1938." In the acknowledgements section to this paper, "MH-4115" should read "MH-4115."
  p 1018: Add Richard A. Patrick to this list of authors’ names.
  p 1113: second column, 14th line from bottom, "32.1%" should read "10.9%" and, in the next line, "consistent" should read "inconsistent." Two lines further down, "60.7%" should read "18.5%" (see Letter by M. R. Hammer, in press).
  p 1493: It is stated (column 2, third paragraph) that a DB-1701 column does not contain a nitrogen atom in its stationary phase. This is incorrect. The phase is a 14% cyanopropylphenylsiloxane polymer.
  p 1541: In the first full sentence in the right-hand column, "similarly higher" should read "similar."
  p 1629: In the right-hand column, the second-to-last paragraph, the ninth line from the bottom should read " . . . 0.5 mL of a reagent solution containing, per liter, 10 g of dextran sulfate (50 000 Da) and 0.5 mol of MgCl2."
  p 1945: In line 6, column three, "0.65" should "0.965."
  p 2148: Add I. Morales to this list of authors’ names.