IMPROVEMENT OF THE FIRST CUBAN LASER DENSITOMETER

MEJORAS AL PRIMER DENSITMETRO LÁSER CUBANO

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Received 3/6/2015; Accepted 4/8/2015

PACS: Optical absorption 42.25.Bs, measurement of density 06.30.Dr, optical instruments 07.60.-j, optical scanners 42.79.Ls

In 1988, the Cuban National Laser Commission started to collaborate with the so-called Biological Front, in order to help substituting imported equipment.

In 1990, at the former Center for Development of Scientific Instruments and Equipment (CEDEIC), the first Cuban semi-automatic Laser Densitometer (LD-01) and its first associated software version (SOFDEN) were built [1].

A densitometer is a device for measuring optical density (OD), based on the photometric principle [2], where the intensity of light after passing through a substance is compared to the incident intensity [3].

The LD-01 is used to perform qualitative and quantitative determinations of various substances (DNA, proteins, enzymes, fatty acids, dyes, etc.). It is based on optical absorption measurements of substrates that permit the transmission of luminous radiation through them. Examples of such substances, are: electrophoretic separations (agarose, starch and polyacrylamide gels, flat and tubular, cellulose acetate films), autoradiograms (X rays), photographs, slides, stains and thin layer chromatography (TLC) plates.

The instrument basically consists in a light source and optical, electronic and mechanical systems. A substrate, coupled to a scanning mechanism, is moved between the light source and the detector. The signal at its output is electronically processed. With a recording module, the density curve is plotted and the area enclosed enables sample quantification.

Sonly the Center for Genetic Engineering and Biotechnology (CIGB) got interested in the LD-01, which satisfactorily passed a battery of tests as an instrument for the evaluation of the purity of different biotechnological products [4].

The LD-01 passed another battery of tests at the Clinical Laboratory of the Abel Santamaria Hospital (Pinar del Rio), this time related to the analysis of blood serum proteins which is the most widespread application. This led to some modifications in hardware and software, achieving metrological certification in the National Institute of Metrology (INIMET).

For more than three years, the LD-01 provided services at the Clinical Laboratory of the Ameijeiras Hospital (Havana), and since 1998 it is being used at the Central Military Hospital (Havana), where it was a key element in the reopening of the electrophoresis service of serum proteins.

At the beginning of this century, with the disappearance of CEDEIC and its subsequent fusion with the Center for Technological Applications and Nuclear Development (CEADEN), the technical service to the available laser equipment has continued, including densitometers.

Between the improvements made during the last years, are:

- Decrease of the distance slit-sample, which reduces the negative effects of stray light on the photo-detector and the background noise.
- Replacement of the detector (germanium photocell) for a silicon photodiode, with greater sensitivity and linearity.
- Modernization and simplification of the electronic system of the photo-detector.
- Improvement of the correspondence between signals and actual values of absorption or OD [5].
- Increase of the scan length, from 65 to 72 mm in order to evaluate other samples, including cylindrical gels.
- Integration and optimization of subroutines, reducing processing time.
- Replacement of the ISA interface card by a suitable USB interface external module.
- Redesign of the motor control board.
- Development of new software (*CEADEN Soft Visual Densitometer*), compatible with any PC configuration and suitable to be used in virtually any other advanced densitometer [6].

Below, they are provided details of some of the most recent improvements listed above.

The obsolete ISA interface was substituted by an external USB interface [7]. The external module (see top of figure 1) has two connectors: a USB for connecting to the PC and a DB9 for connecting to the LD-01. The module was based on a single PIC18F2550 microcontroller. Through the

DB9 connector the microcontroller receives the analog signal (NA/SGN) and controls the motor rotation direction (figure 3). A small change in the densitometer hardware was made to split the motor control signal into two separate ones (BK1 and BK2).

Figure 1. LD-01 laser densitometer. Note the USB external module over the instrument.

The module gets the analog signal from the densitometer, converts it to a digital one and sends it to a PC. On the other hand, from the PC the LD-01 receives commands to stop, move or change direction of the motor.

The analog signal is proportional to the measured OD and is digitized by an internal PIC (10 bit ADC). To match the 4 VDC output signal (equivalent to 4 OD) to the ADC 2.5 VDC external reference, a resistor divider R3-R4 was used.

According to calibration, figure 2 shows the linearity of the instrument measurements vs. standard neutral density filters. The correlation coefficient of the linear fit is 0.9987.

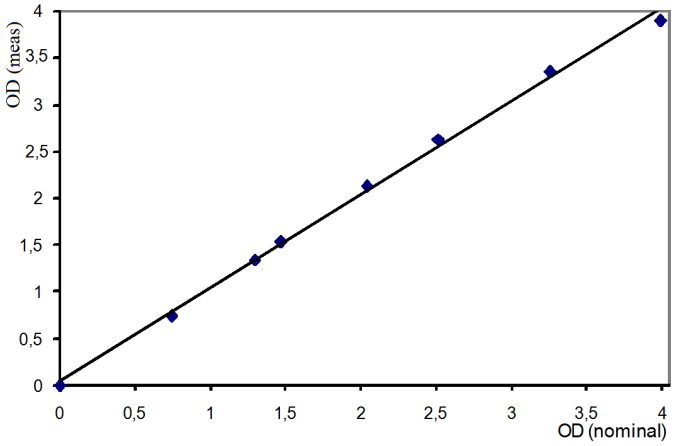

Figure 2. Linearity of response in the measurement range (0-4 OD).

The CEADEN Soft Visual Densitometer is general purpose software, made for the LD-01 densitometer (figure 4). This software offers a user interface and automation which improves the capabilities of the device, such as controlled motor movement direction with and without data acquisition, time of movement, and number of samples to read.

The LD-01 densitometer communicates with the PC trough an USB 2.0 interface with serial port virtualization. Data post-processing like curve smoothing, peak detection, calculation of areas and protein quantization, can be performed after data acquisition. All of these are tweaked by some parameters that the user can vary. The software brings a report with graph and peaks data that the user can print or save as a PDF file. In the case of standards, it also allows the obtainment of calibration curves by molecular weight or concentration very useful in several applications.

In summary, the LD-01 continues to meet the requirements of its original main application: the analysis of protein electrophoresis of serum in Clinical Laboratories. However, nowadays it has been updated to meet contemporary standards. It constitutes an excellent example of how Cuban Physics and Engineering are able to successfully insert products in the healthcare and biotechnology scenarios.

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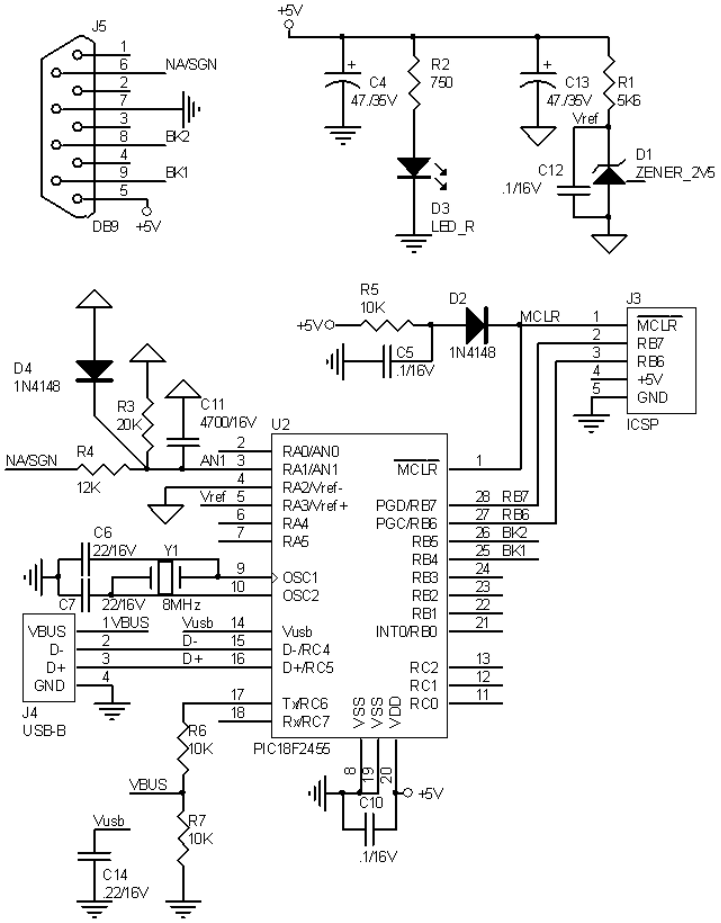

Figure 3. Electronic diagram of the USB interface external module.

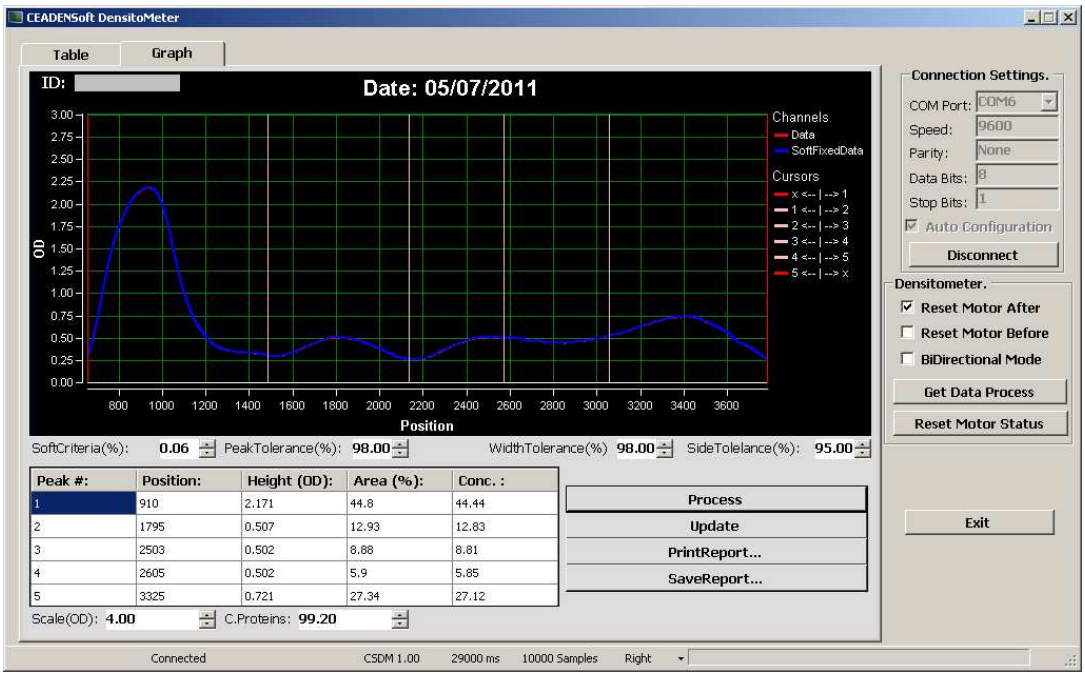

Figure 4. Viewport (Graph) displaying one typical curve and the results of densitometric analysis.