Simplified laser fluorescence scanner for proteomics studies and early cancer diagnosis

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ABSTRACT

A compact integral system was developed capable of quantitatively measuring analytes in biological fluids. This system uses the fluorescence as a reporter molecule to provide the information about the molecular density of the target molecule in the test sample. Developed for the purpose of providing an affordable means of fast point–of-care testing of marker molecules specifically arranged in the specimen, this system proved to be able to detect the presence of the marker agent at the level of 50 *pg*/*ml* in less than 15 minutes, with the actual measurement taking less than 1.5 minutes. The design concept for this unit is presented together with the result for a few representative samples.

1. INTRODUCTION

Since the first reported observation of fluorescence by Herschel[1] in 1845, utilizing fluorescent light from a material is one of the well-accepted techniques in chemical analysis, together with absorption spectroscopy and other related techniques. The physical basis of this technique is relatively straightforward and simple[2], which makes it a reliable means of analyzing the constituents. The energy needed to excite the target molecule to an excited energy state can be of any form, yet light is a most convenient source. One might use appropriate band pass filters to take advantage of the resonance absorption, which effectively prevent the unused energy from degrading the S/N value of the detection system. The laser can be used as the exciter source should its wavelength fall within the effective absorption bandwidth of the target system. Such a technique is called the Laser Induced Fluorescence (LIF), a technique widely practiced in quantitative and qualitative analysis of chemical and organic compounds.

The success of the Human Genome Project (HGP) can be in part attributed to the mature engineering of the fluorescence handling technique. Now, with the result from the project available, one may want to further the

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understanding and to utilize the information thus made obtained. The DNA chip is a good example of this application. Here more than 10,000 gene expressions are marked on a substrate and the fluorescence expressions from these expressions are analyzed in a systematic way to convey the useful information. One of the key steps in implementing such a system is the device that reads the fluorescence from so many markers on the substrate. One area of deriving merits from such a technique is proteomics, in which researchers investigates various forms of protein. It is known that faulty DNA causes hereditary disease, and that these processes are accompanied with forewarning changes in corresponding protein. Observing the changes in one's protein might provide a warning alarm for the upcoming disease.

Proteomics and related research activities are rather costly. A typical DNA scanner that can be used in proteomics costs anywhere from around US$100,000 and up. Yet routine tasks at the clinic level do not require such a full-featured scanner to do the job. We designed and fabricated a simple and an affordable laser induced fluorescence scanner that could meet the need of this application.

2. DESIGN CONSIDERATIONS

Most DNA or protein scanners on the market have versatile modalities built-in so that the user can select or combine them to appropriate uses depending on the nature of the measurement. This versatility is one of the major factors that forces in the complexity of the system, increasing the production cost. For the system described here, we tried to eliminate all the underused features to simplify the scanner, retaining the essential functionality to meet the specific requirement for routine tasks.

2D scanners in general adopt the laser for excitation and the confocal geometry for collection of the fluorescent light. The collection efficiency *E* of a lens for a point source is limited by the numerical aperture (NA = 1/2f#) of the system, and is approximately given by

$$E = \frac{Lens Solid Angle}{4\pi} \cong \frac{1}{16\,(f/\#)^2}$$

Thus, a lens with f/# = 1.0 (an impractically fast lens) would give the efficiency of 6.25%. This low light collection efficiency has to be compensated by the increased input laser power and the costly post signal processing system. We adopted reflective collection optics to do away with the lens in order to increase this efficiency. Figure 1 is the optical schematics for the system thus conceived. The reflective elliptical mirror with diameter of 95 mm was used instead of the usual spherical mirror. The sample was located on the first foci of the ellipsoid, with a low pass spatial filter located on the other foci. The incident laser beam was focused to 75 micrometers in diameter onto the sample. The fluorescence light emanating from this spot together with the scattered incident light was to be reflected by the ellipsoidal mirror toward the second foci without any aberrations. Spherical mirrors were found unfit for this application; the spherical aberration was of the unacceptable size as one can easily predict.

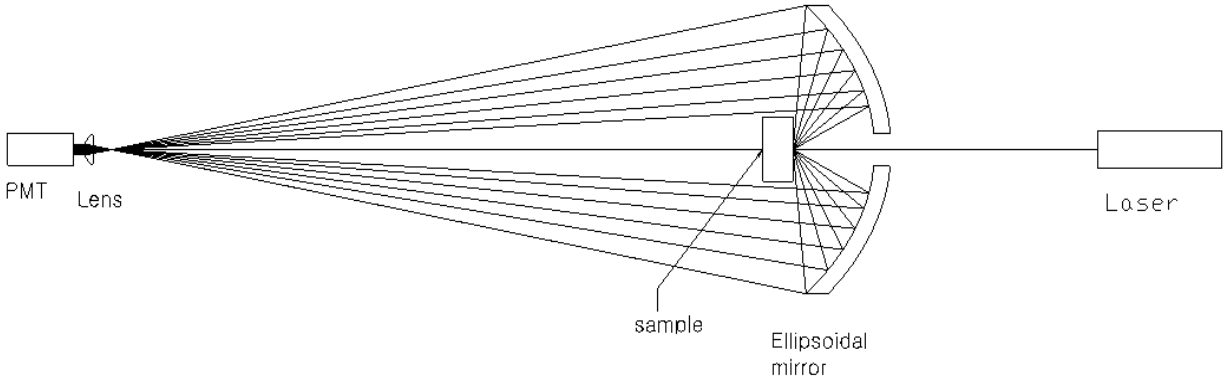


Figure 1. Optical layout of the system.

The light gathering efficiency of the system is limited only by the obscuration by the sample and the holder. In the system under consideration, the obstruction was about 20%, rendering the ideal collection efficiency of 80%. The actual value was expected to be lower due to other parameters. Most predominant of these was the imperfection of the mirror.

3. THE SYSTEM AND PERFORMANCE

We used a semiconductor laser WLM-6305NH (Lanics, with the laser diode from Samsung Electronics, Seoul, Korea) as the illumination source that emits 2.5 mW of CW radiation around 637 nm. This laser came with a focusing lens, about 4 mm in diameter, which could be used to adjust the beam divergence and the focusing properties. We used this lens to bring the laser through an excitation filter to the best focus on the sample strip. Since the strip was opaque, most of the fluorescence light as well as the diffusely scattered laser light was directed toward the ellipsoidal mirror. This mirror was positioned in such a way that the focus of the laser on the strip was poised on one of the two foci of the mirror so that any beam emanating from that focal point would eventually come to another focal point. The spatial filter was placed to remove the stray and any other unwanted light. Light coming through this filter was then directed to a collimator and the emission filter. Fluorescence energy that passed through the emission filter was eventually detected by the detector, H5784-01 (Hamamatsu Photonics KK, Hamamatsu, Japan) and was converted to a digital readout signal. The on-board computer controls each operation steps and measures signals via preprogrammed instructions to generate statistically analyzed results. Figure 2 is the system at a glance.

Upon inserting the sample, the system positions the sample in the programmed scanning position which can be selected by the user with the key pad on the front panel. For a typical run, the system scans over a span of 8.4 mm, with a sampling step of 0.1 mm and takes 100 readouts for a spot over the membrane to generate an average value for that spot. The time it takes for a typical run is 84 seconds.

Figure 2. The 1D fluorescence scanner. The width is 37cm.

The scanner was tested with a typical cartridge containing tumor or cardiac markers, the image of which is shown in figure 3. The membrane base containing these markers, exposed for the measurement, was 12mm long as manufactured by BodiTech Inc, Korea. With this cartridge, analytes were arranged in such a way that, when exposed to the blood sample, the fluorescing lines were spaced at preset locations along the length of the membrane. Figure 4 shows the response of the system with the controlled amount of antigen. The ordinate is the measure of the fluorescence level and are to be used on a relative scale. The abscissa is the distance along the sample length. The signal level was found to be not linearly proportional to the molar density of the marker. Yet with a calibration standard provided on the same membrane strip, one could estimate the quantitative value of the molar density. The lowest density thus measured was 40 pg/ml as demonstrated in the figure 4 or lower under optimal conditions. Figure 5 is a demonstration of the scanner compared with the conventional scanned image. The density of the fluorescing antigen was arranged in such a way that, as in the top image of figure 5, the 2^{nd} and the 4^{th} were of the same value. One would expect under such a circumstance identical fluorescent signal for both, which is clearly demonstrated at the scanned intensity plot of figure 5. Though not clearly discernable in figure 5, the brightness of the scanned spots is strongly correlated with the intensity plot at the bottom when viewed on a display terminal. Figure 6 is the result of a practical application. The scanned image on top of figure 6 was obtained via a commercially available scanner(GSI Lumonics). This shows the typical arrangement of the fluorescing antigen on the sample strip. Only one image is shown here. The scan plot at the bottom of figure 6 was obtained over multiple strips with differing antigen densities to reveal that there exists informative correlation between the density and the fluorescent intensities. The line at the middle of the sample was introduced as a reference source, whose fluorescent intensity was to be used as a calibration measure for other intensities. It was found that the base line of the scanned intensity could vary, and that when it happens the change in the reference intensity could provide sufficient leverage to compensate the shift of the base line.

Figure 3. The sample cassette. The strip in the middle was 12mm long.

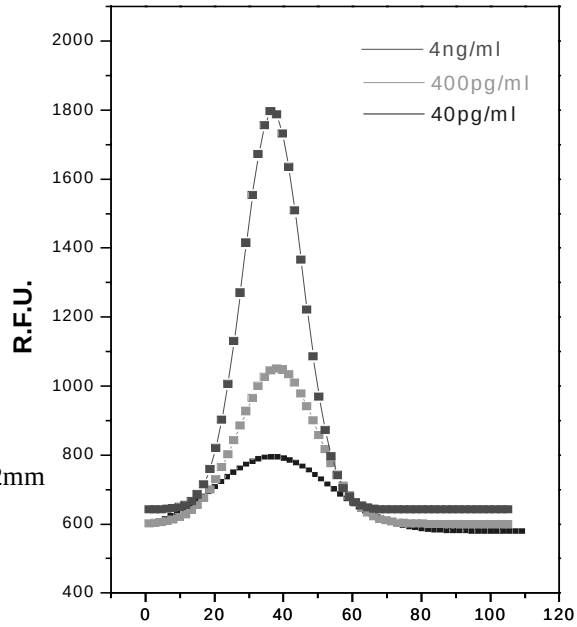
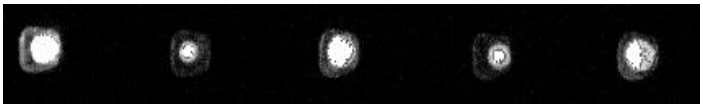


Figure 4. Comparison of signal for differing densities of the Prostate Specific Antigen (PSA). The ordinate is in arbitrary unit.



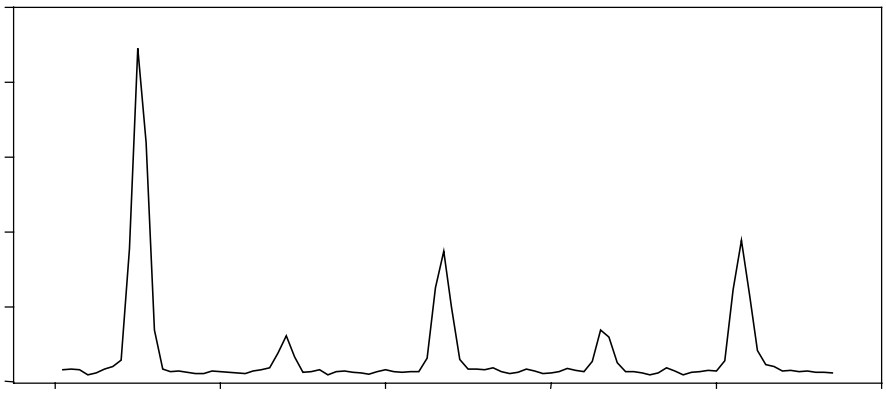


Figure 5. Comparison between the 2D image and the 1D scan.

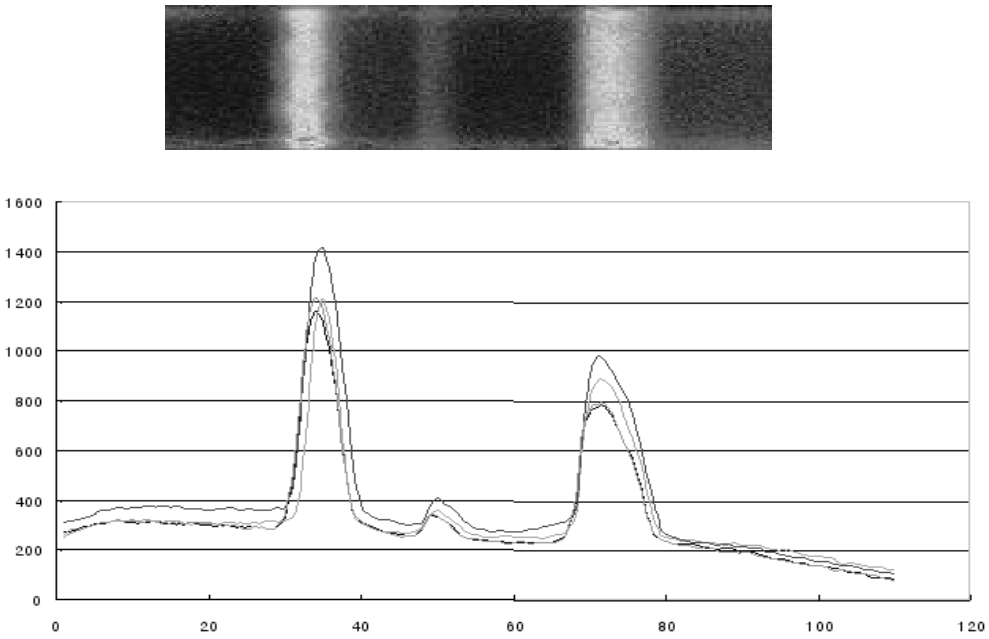


Figure 6. Comparison for Rabbit IgG

CONCLUDING REMARKS

We have demonstrated that a simple 1D fluorescence scanner is practically realizable that has the capability of detecting cancer or cardiac markers indicative of preceding conditions for the corresponding diseases. Although we designed the system for dyes CY5 or Alexa 647, one can easily generalize the system to encompass differing dyes and wavelengths at the expense of the cost of the whole system. This system is undergoing field tests and further improvements are in order to better the performance and the reliability.

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