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Laser-based double beam absorption detection for aggregation immunoassays using gold nanoparticles

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Abstract A laser-based double beam absorption detection system for aggregation immunoassays has been developed. The assay was based on the aggregation of gold nanoparticles that are coated with protein antigens in the presence of their corresponding antibodies. The aggregation of the gold nanoparticles results in an absorption change that is monitored at 635 nm using the double beam spectrometer. The noise level of the spectrometer is 1×10^{-6} arbitrary units. This corresponds to a tenfold improvement in comparison to commercial absorption detectors and is comparable with previously reported more complicated laser-based absorption spectrometers. The dye Nile-Blue-A was used to test the analytical performance of the system. A limit of detection of 3×10^{-8} M Nile-Blue-A was observed. The relative standard deviation between consecutive measurements was lower than 1.5%. The system is suitable for field applications of aggregation-based immunoassays.

Keywords Diode laser · Double beam absorption spectroscopy · Gold nanoparticles · Aggregation immunoassay

Introduction

Recently we have developed an aggregation-based immunoassay using protein-coated gold nanoparticles [1]. The capabilities of the assay were demonstrated by detecting anti-protein-A using protein-A-coated gold nanoparticles. The aggregation of gold nanoparticles during the assay led to the formation of a new absorption band at longer wavelengths due to electric dipole-dipole interaction and coupling between the plasmons of neighbouring particles in the aggregates formed. We could quantify the amount of anti-protein-A by measuring the absorption change of the gold colloid suspension at 635 nm. A state of the art UV-VIS-NIR spectrometer was used for these measurements. This work focuses on the replacement of the full frame absorption spectrometer with a simpler and less expensive diode laser-based double beam absorption spectrometer, which would be more suitable for field applications.

Commercial absorption spectrometers in which conventional lamps are used as light sources offer typical detectability of 1×10^{-4}absorbance units (AU). In state of the art systems the minimum noise level is 2×10^{-5} AU using a single wavelengths [2, 3]. Due to the relatively low intensity of lamps at single wavelength their signal to noise ratio (S/N) is often shot noise limited. There have been several attempts to construct dual beam spectrometers using pulsed high intensity LEDs [4] and vertical-cavity surface-emitting lasers [5] in UV-VIS and atomic absorption spectrometry [6, 7]. The use of a laser beam enhances the optical properties of a conventional lamp-based light source such as collimation and focusing power. It could potentially eliminate noise contributions originating from long term drifts and systematic spikes. However, utilizing a laser beam as a light source for absorption detection does not improve the noise level compared to conventional lamps, contrary to shot-noise considerations. This is because the intensity stability of a typical continuous wave (CW) laser is barely above 0.1% [2]. Theoretically, CW lasers could be used to enhance absorption signals by inducing a thermal lens effect [8]. However, while the thermal lens effect increases the observed absorption signal it does not reduce the noise. In fact, due to the instability of lasers the noise level actually increases. Therefore, the noise of these lasers must be reduced in order to significantly improve the detection performance of laser-based spectrometers. Double beam laser-based absorption spectrometers have been developed and applied in liquid chromatography [2, 3, 9] and capillary electrophoresis [10, 11, 12, 13, 14]. In these spectrometers an electronic noise canceller developed earlier by Hobbs et al. [15, 16] was used to eliminate the noise contributions associated with

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the instability of laser beams in order to acquire shot-noise limited absorption signals. These double beam devices provided inexpensive and rather sensitive alternative spectrometers suitable for field applications. In this study we used a home-built diode laser-based double beam spectrometer to monitor the immuno aggregation process of gold nanoparticles coated with protein A in the presence of anti protein A. The method could be adopted to a large number of immunoassays and to other assays that are based on aggregation of the analyte solution as a basis for analyte quantification.

Materials and methods

Laser-based dual beam absorption spectrometer. The electronic circuit of the double beam detector was constructed following Campbell et al.[4] with slight modifications. Briefly, the detector circuit consists of signal and reference photodiodes. Each photodiode is connected to the inverting input of a low noise operational amplifier. The amplifier is configured as a current-to-voltage converter with a feedback resistor giving a sensitivity of 1 mV/μA. A high sensitivity is not needed for this system because of the 3 mW laser source and the high sensitivity of the photodiode (0.5 A/W). The output from the amplifier is scaled from 0 to 1 V to match the input range of the 22-bit analog to digital (A/D) converter used for data acquisition. This configuration gives a highly linear output voltage proportional to the intensity of the incident light with a noise level of about 20 μV. The output current of each photodiode is converted to voltage by the operational amplifier to produce voltage outputs as transmittances. Campbell et al. [4] obtained the ratio of the photodiode signals from an AD633JN 4-quadrant multiplier device, which operates in a divider mode. In our experiments we used Excel software to eliminate direct current (DC) noise contributions and to correct for intensity differences between the signal and reference channels. The system was tested with Nile Blue A before being applied to monitor the immuno aggregation of protein A-coated gold nanoparticles in the presence of anti protein A. The schematic diagram of the laser-based aggregation spectrometer is shown in Fig. 1. A 3-mW LAS200–635–3 diode laser (Lasermax, Rochester, N.Y., USA) at 635 nm powered by a 9-V DC power supply (GP-4303D, EZ Digital, Korea) was used as a light source. A black shield with a 10 mm aperture was used to block the Fabry-Perot interference fringes generated by the laser [17]. The laser beam was split using a 25 mm 50R/50T Tech Spec beam splitter (L45–852, Edmund Industrial Optics, Barrington, N.J., USA). The two well-correlated laser beams were used as signal and reference beams, respectively. The beams passed through a sample and reference quartz cuvettes before hitting the corresponding photodiodes (1336–8BK Si, Hamamatsu, Santa Clara, Calif., USA), which were connected to the inverting input of a low noise operational amplifier (OP 284, Analogue Devices, Norwood, Mass., USA). Quartz cuvettes (Starna Cells, Calif., USA) with a 2 mm internal width and non-transmitting self-masking sidewalls were used to minimize the sample consumption and eliminate stray light noise contributions. The experiments were performed in a dark box to prevent noise contributions due to exposure to room light. Signal and reference data were acquired at 0.5 Hz by the two channels of a standard 22-bit A/D interface data acquisition module Personal Daq (Iotech, Cleveland, Ohio, USA). The data were acquired and converted to absorbance readings using a PC-compatible personal computer equipped with Excel data analysis software.

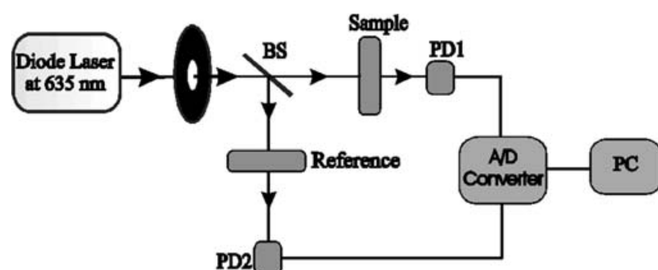

Fig. 1 A schematic diagram of the laser based double beam spectrometer

Absorption spectroscopy. A UV-VIS-NIR absorption spectrophotometer (Varian, Cary 500), which is run by Varian's Cary Win UV software version 2.0, was used to characterize the spectral properties of Nile Blue A and the gold nanoparticle suspensions. A Genios absorption microplate reader (Tecan U.S., USA) was used as a standard technique to monitor the aggregation rate of the protein A-coated nanoparticles in the presence of anti protein A. The same experiments were carried out using our laser-based double beam absorption spectrometer.

Materials and reagents. Nile Blue A, polyclonal antibodies to protein A, 10 nm colloidal gold nanoparticles labelled with protein A, bovine serum, and phosphate buffered saline at pH 7.4 were purchased from Sigma (USA). Anti protein A was reconstituted with 2 ml of deionized water. After reconstitution, the solution was stored frozen in working aliquots. Gold nanoparticles suspensions were kept under light-tight conditions to prevent aggregation and oxidation when exposed to room light. All reagents were used as received without further purification.

Results and discussion

Analytical performance of the laser-based double beam spectrometer

As previously mentioned the noise cancellation was performed by data processing using the commonly used software Excel, instead of a hardware configuration used by various researchers in the early 1990s [2, 3, 9, 10, 11, 12, 14]. The simplification of hardware-based devices by using software to perform functions previously controlled by hardware is a common trend in computer electronics as computing power and data storage capabilities increase rapidly. It may eventually lead to the elimination of most hardware from data acquisition systems. The acquisition and treatment of the spectral data are summarized in Fig. 2. The output signals of the sample (S_{sample}) and reference channel (S_{ref}) are shown in Fig. 2a and b. As expected, the noise traces co-track since they follow the noise level of the same laser. However, the signal of the reference channel is slightly lower than that of the sample channel since the beam was not split equally between the signal and reference channels by the beam splitter. The data of the reference channel was treated with a multiplying factor to correct for an intensity difference of the signal between the sample and reference channels. The absorbance of the sample was calculated using the following equation:

$$A = \log\left(S_{sample}/S_{ref*}\right) \quad (1)$$

where A is the absorbance and S_{sample} and S_{ref*} the voltage outputs of the sample and corrected reference beams respectively. Using this data treatment technique, the effect

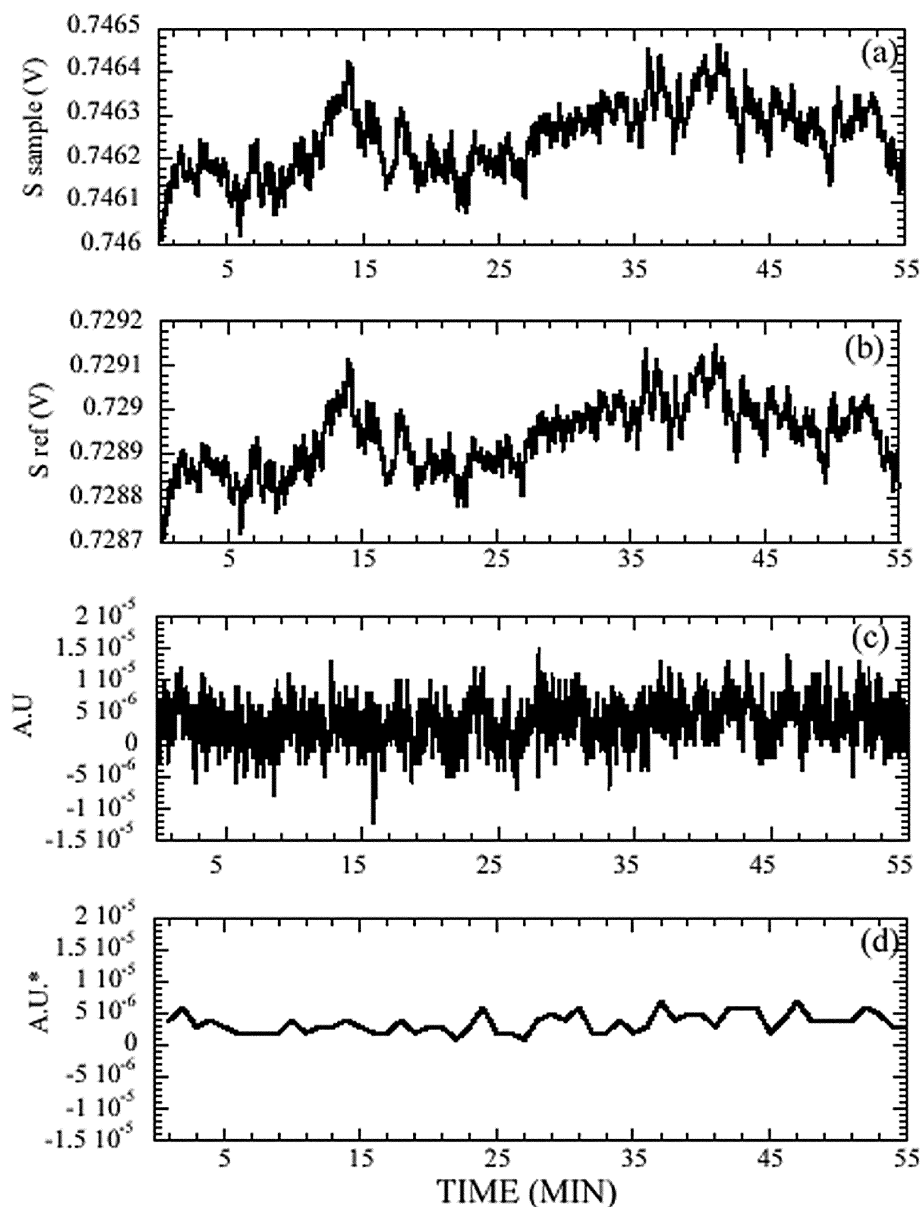

Fig. 2a–d The noise cancellation process. **a** The output voltage of the sample channel. **b** The output voltage of the reference channel. **c** The log output of (S_{sample}/S_{ref*}). **d** Average log output of every 30 data points

of laser noise and background fluctuations on the analyte signal was cancelled out (Fig. 2c). The data was collected at a frequency of 0.5 Hz. The noise level was further reduced by a boxcar averaging treatment. The effect of averaging every 30 data points to a single point is shown in Fig. 2d. The noise level was calculated to be around 1×10^{-6} AU.

The system was then calibrated using Nile Blue A, which has an absorption peak maximum at 635 nm. A calibration curve describing the concentration dependence of the absorption intensity of Nile Blue A at 635 nm is shown in Fig. 3. The dynamic range of the spectrometer was between 3×10^{-8} and 1×10^{-4} M of Nile Blue A (only the lower end of the calibration curve is shown). The limit of detection of the double beam spectrometer system was 3×10^{-8} M Nile Blue A, which is 5-fold lower than that obtained using a UV-VIS-NIR commercial spectrometer and 20-fold lower than the limit of detection obtained using an absorption plate reader. The correlation between the absorbance of Nile Blue A measured using our home-built double beam laser-based spectrometer and the absorption of Nile Blue A measured using a UV-VIS-NIR absorption spectrometer previously used in our aggregation assays [1] is shown in Fig. 4. The data points are distributed symmetrically around the linear line, $y=x$, which represents maximum correlation. The two systems are well correlated, with a correlation coefficient of 0.9998.

Aggregation based immunoassay using protein A-coated gold nanoparticles

The aggregation of protein A-coated gold nanoparticles in the presence of anti protein A takes several hours to com-

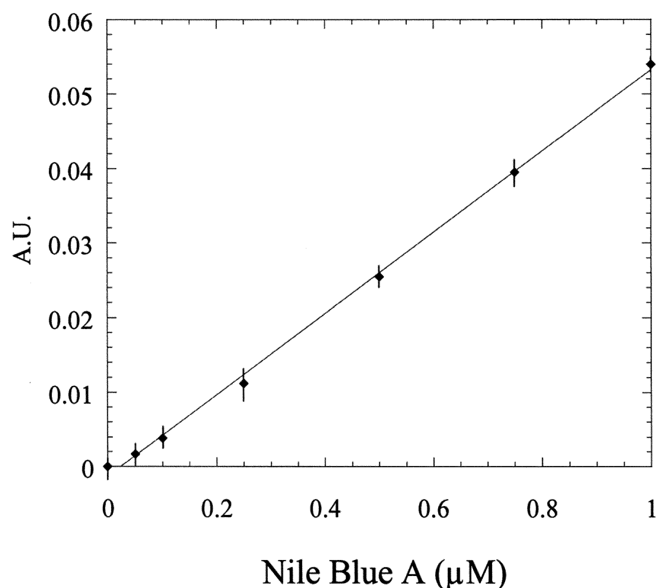

Fig. 3 A calibration curve describing the concentration dependence of the absorption intensity of Nile Blue A constructed using the laser based double beam absorption spectrometer. Each data point is an average of quadruple measurements. The relative standard deviation is lower than 1.5%

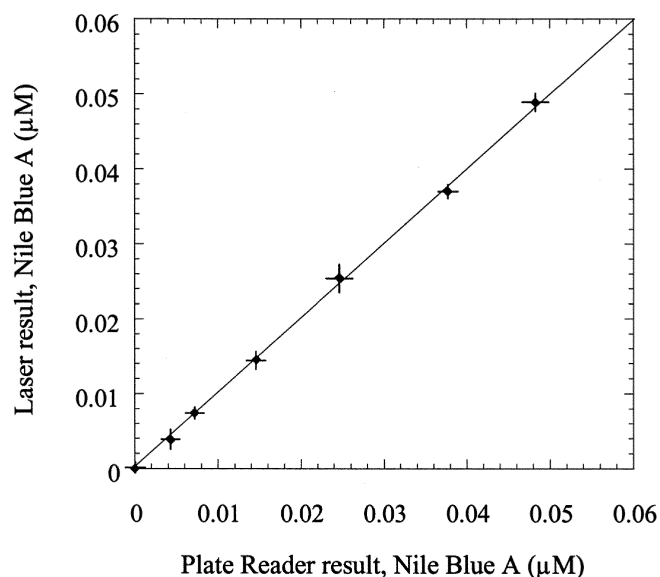

Fig. 4 The absorbance of Nile Blue A at 635 nm measured using the laser-based spectrometer as a function of the absorbance of Nile Blue A measured with a commercial UV-VIS-NIR absorption instrument

plete [1]. In our measurements we limited the assay time to 90 min. At longer times a precipitate of large aggregates and a clear supernatant are formed and the absorption intensity is no longer concentration dependent. The UV-VIS absorption spectra of a solution containing protein A-coated gold nanoparticles in the absence and presence of anti-protein A are shown in Fig. 5. Spectrum a, measured at t=0, is a typical spectrum of a 7.8×10^{11} gold

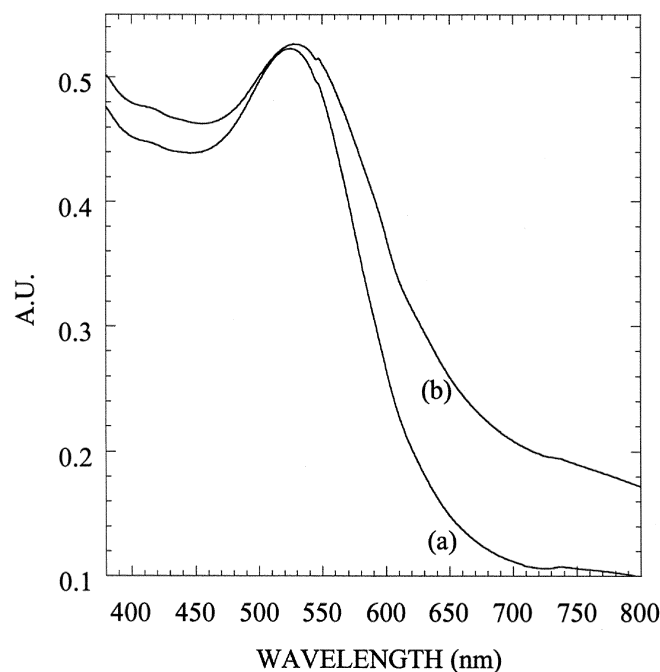

Fig. 5 UV-VIS Spectra of gold nanoparticles solution during the aggregation process at t=0 min (*a*) and t=60 min (*b*). The solution contains 7.8×10^{11} gold nanoparticles/ml and 30 μg/ml anti-protein A in phosphate buffered solution at pH 7.0

nanoparticles/ml solution containing 30 μg/ml anti protein A. A typical plasmon resonance peak at 520 nm is observed. The aggregation of gold nanoparticles leads to an increase in absorbance at longer wavelengths (600–800 nm) as seen in spectrum b (t=90 min). In this study we measured the absorbance changes at 635 nm. A diode laser at this wavelength is commercially available. This wavelength was particularly suitable for our measurements since it enables monitoring the aggregation rate while minimizing the effect of light scattering by the gold nanoparticles on the absorption measurements.

In Fig. 6, the absorption intensity of the gold nanoparticles is plotted versus time during the aggregation assay at different concentrations of anti protein A. Curves a, b, c and d show the aggregation of 7.8×10^{11} protein A-coated gold nanoparticles/ml in the presence of 10% bovine serum and 2, 5, 10 and 50 μg/ml anti protein A respectively. It can be seen that the absorption at 635 nm increased with time due to the formation of aggregates. As expected, the rate of aggregation increased with the concentration of anti protein A. The aggregation rate was comparable with that monitored by the UV-NIR-IR absorption spectrometer [1]. This proves the reliability of our new instrument. An absorption change at 635 nm versus concentration calibration curve for anti-protein A in 10% bovine serum is shown in Fig. 7. The anti-protein A solution concentrations ranged between 0 and 30 μg/ml. Each data point represents the average of three measurements. The standard deviation between the measurements is less than 3% as evidenced by the small error bars in Fig. 7. The polynomial fit that connects the data points, is for guiding

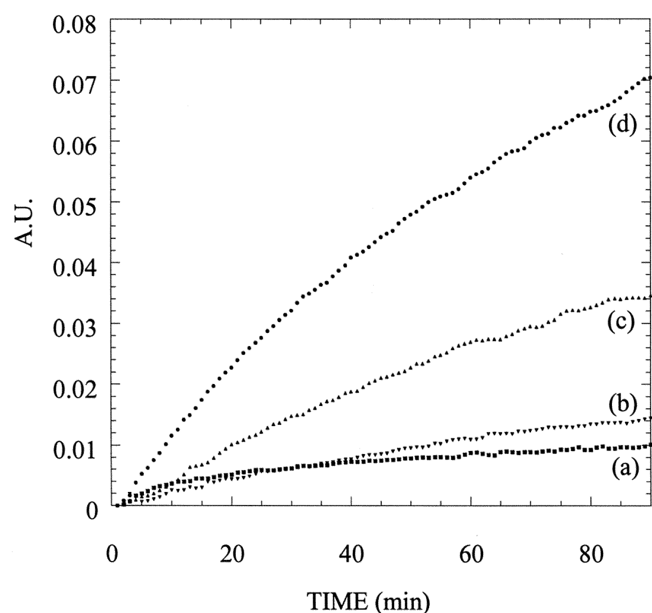

Fig. 6 The dose dependence of the immunoaggregation process. The assays are carried out in 10% bovine serum and phosphate buffered solutions at pH 7.0 and monitored for 90 min. The absorption change at 635 nm is measured at: 7.8×10^{11} protein A-coated gold nanoparticles/ml and 2 μg/ml anti protein A (*a*); 7.8×10^{11} protein A-coated gold nanoparticles/ml and 5 μg/ml anti protein A (*b*); 7.8×10^{11} protein A-coated gold nanoparticles/ml and 10 μg/ml anti protein A (*c*); and 7.8×10^{11} protein A-coated gold nanoparticles/ml and 50 μg/ml anti protein A (*d*)

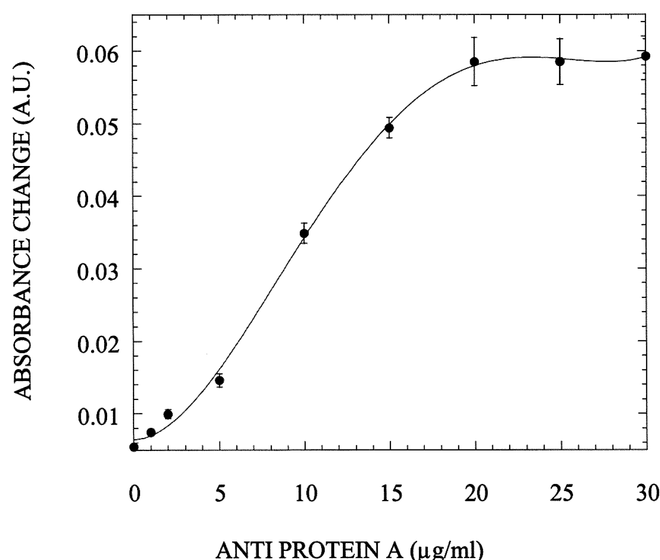

Fig. 7 A calibration curve describing the absorption change at 635 nm against anti-protein A concentration in a 10% bovine serum solution. A limit of detection of 1 μg/ml anti-protein A is observed.

purposes only, and further studies are needed to explain the concentration dependence of the aggregation process. The limit of detection of the assay in serum samples was 1 μg/ml anti-protein A, which is comparable with that measured with a commercial spectrometer [1]. This result implies that the absorption spectrometer is not the limiting factor in the assay properties. Furthermore, using a simple but robust laser-based spectrometer rather than a full frame expensive spectrometer does not compromise the analytical properties of the gold aggregation immunoassay.

In summary, a diode-laser-based double beam absorption detection system for immunoaggregation assays using protein coated gold nanoparticles was developed. The background noise due to the light source was reduced substantially. While the system is simple, robust and inexpensive, it offers high sensitivity for many biological assays. The limit of detection of 1 μg/ml anti protein A is well within the range of the physiological levels of anti protein A of around 10 μg/ml. With the availability of various diode laser sources at different wavelengths, the system can be used broadly for different applications. The new system is compact, inexpensive to build and very versatile, which makes it very suitable for field applications. The assay time of 90 min is shorter than most immunoassays, which may take up to 24 h to complete. However, with the ever-increasing need for rapid assays it is important to further develop the technique in order to realize even faster assays. Currently we are investigating whether it would be feasible to replace the gold nanoparticles with nanocomposite particles of gold and iron oxide. This may facilitate the shortening of assay times by applying an external magnetic field to induce faster aggregation.

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References

1. Thanh NTK, Rosenzweig Z (2002) Anal Chem 74:1624–1628
2. Rosenzweig Z, Yeung ES (1993) Appl Spectrosc 47:1175–1179
3. Rosenzweig Z, Yeung ES (1993) Appl Spectrosc 47:2017–2021
4. Campbell M, Lynch F, Brown G (1994) Int J Electron 76: 153–161
5. Vogel P, Ebert V (2001) Appl Phys B 72:127–135
6. Liger VV, Kuritsyn YA, Krivtsun VM, Snegirev EP, Kononov AN (1997) Quantum Electron 27:360–365
7. Liger V, Zybin A, Kuritsyn Y, Niemax K (1997) Spectrochim Acta A 52:1125–1138
8. Buffet CE, Morris MD (1982) Anal Chem 54:1824–1829
9. Rosenzweig Z, Yeung ES (1993) J Chromatogr 645:201–207
10. Xue Y, Yeung ES (1993) Anal Chem 65:1988–1993
11. Xue Y, Yeung ES (1993) Anal Chem 65:2923–2927
12. Xue Y, Yeung ES (1994) Appl Spectrosc 48:502–506
13. Culbertson CT, Jorgenson JW (1999) J Microcolumn Sep 11: 652–662
14. Tong W, Yeung ES (1995) J Chromatogr A 718:177–185
15. Hobbs PCD (1991) SPIE Proc 1376:216
16. Hobbs PCD (1991) Opt Photon NewsApril:17
17. Liger VV (1999) Spectrochim Acta A 55:2021–2026