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Nanoparticle-based photoacoustic analysis for highly sensitive lateral flow assays

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This paper demonstrates a photoacoustics-based lateral flow test that takes advantage of the strong interaction of light and gold nanoparticles to quantitatively detect a disease biomarker. For a commercially available lateral flow test strip, the photoacoustic analysis improved the detection limit by two orders of magnitude compared to colorimetric measurements.

Lateral flow immunoassays (LFAs) enable the simple and rapid analysis of biomolecules in complex biological samples, such as human body fluids.¹ LFA tests require minimal effort for sample preparation and are thus desirable for point-of-care testing at home and in clinics.²⁻⁴ Robust, inexpensive, and portable LFA tests have been successfully used for food safety monitoring, disease diagnostics, and drug tests.^{5,6} Most LFA sensor strips consist of a nitrocellulose substrate that contains a series of functional areas, each of which stores a specific chemical reagent. Driven by capillary force, liquid samples transport along the LFA strip and react with the reagents. The presence of the target analyte causes a change in the output signal, whose intensity is measured for qualitative or semiquantitative analysis. Colorimetric sensing is the most often used signal readout method for LFA tests.⁷ The colorimetric analysis allows a read-out with the naked eye but has the drawback of poor sensitivity compared with laboratory-based molecular techniques. On the other hand, high detection sensitivity is critical for some disease-relevant analyses, particularly in cases where the analyte concentration is extremely low. For example, a protein biomarker expressed by cancer cells may be present at concentrations below 100 pg mL⁻¹ in a blood sample.8

To improve the sensitivity of LFA tests, new technologies have been investigated, including the methods for concentrating analytes on paper strips,^{9,10} quantitative readout systems,¹¹⁻¹⁴ and new detection mechanisms.¹⁵⁻²⁸ For instance, hand-held LFA readers with integrated sensing elements or smartphone-based readers that utilize the built-in hardware of the phones,^{12,13} have been developed to readout LFA signals. These readers can detect and quantify signals better than naked eyes and thus improve the sensitivity of LFAs. New detection mechanisms, including fluorescence,¹⁵ chemiluminescence,¹⁶ surface enhanced Raman scattering (SERS),¹⁷ and electrochemical detection,¹⁸ have also been demonstrated in conjunction with sensitive detection instruments. Moreover, as highlighted in the recent review,¹⁹ nanomaterial labels, including metal nanoparticles,²⁰⁻²² quantum dots,²³⁻²⁵ magnetic nanoparticles,²⁶⁻²⁸ are capable of boosting the performance of LFA tests by generating strong and stable signals. Each of these technologies offers clear advantages but with some constrains. For example, the methods that concentrate analytes can increase the analyte concentration above the detection limit before the measurement takes place. However, these methods require large sample volume and increase the overall assay time. Compact LFA readers are compatible with most commercial LFA sensor strips but still lack sensitivity for the detection of low concentration analytes. In contrast, the new detection mechanisms offer high sensitivities at the expense of making major changes to the existing LFAs.

Another direction for improving the LFA is to explore the localized surface plasmon resonance (LSPR) of the gold nanoparticles (AuNPs), which have been adopted in many commercial LFA tests. For example, Bischof's group demonstrated a way to improve LFA sensitivity by using the photothermal signals generated by AuNPs.²⁹ The photothermal detection utilizes the strong light absorption of AuNPs owing to LSPR mode, which represents the oscillation of free electrons in metal nanoparticles.³⁰ Based on the LSPR effect, the metal nanoparticles have been exploited for many chemical and biomolecule sensing applications.^{31–36} In this paper, we exploited AuNPs for the development of a photoacoustics (PA)-based

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detection mechanism to achieve a highly sensitive and quantitative LFA test. PA detections relying on the light-induced acoustic signals have been widely used in chemical analysis and *in vivo* disease diagnostics.^{37–47} For example, PA-Fourier transform infrared spectroscopy is a well-established analytic method.^{37,38} The PA spectroscopy can also detect chemicals using thin-layer chromatography plates.^{39,40} PA tomography and imaging techniques have been recently developed for *in vivo* disease diagnostics using high-power pulsed lasers as the excitation source.^{41–47}

The PA method benefits LFA tests in two aspects: (i) the reduced system noise owing to the input and output signals residing in two different energy domains, and (ii) the strong and reliable PA signal generated by the AuNPs. PA measurements are performed without experiencing any difficulties with the collection and detection of photons that are common to reflection/transmission, fluorescence, and Raman spectroscopies; thus, expensive photodetectors and optical filters are not needed.^{48,49} When illuminated by a laser at the LSPR wavelength, AuNPs can efficiently heat the surrounding medium and generate strong PA signals.^{50–54} Our group recently demonstrated that PA detection was capable of sensing less than 10 AuNPs within an area of 100 μ m² using an inexpensive instrument.⁵⁵

As shown in Fig. 1a, to start a LFA test, an aqueous sample is pipetted onto a paper strip that contains antibody-conjugated AuNPs and other reagents. On the conjugation pad, the analyte binds to the AuNPs. The resulting analyte-AuNP complex wicks up the paper strip under the action of a capillary force and is captured at the test line, where the capture antibodies reside. The density of AuNPs at the test line reflects the concentration of the analyte. Meanwhile, a control line develops which indicates the proper flow of the sample. To quantitatively measure the AuNPs at the test line, we adopted the PA-based sensing approach. The PA technique generates quantitative signals based on a 3-step process including the light absorption by AuNPs, the conversion of the absorbed energy into heat, and the subsequent heat-induced thermal expansion of the adjacent air that generates pressure oscillations. In this work, the setup uses a continuous wave laser source and a commercial PA detector. The LFA sample is enclosed in a small-volume and air-tight chamber. The acoustic signal, in the form of a pressure oscillation, is produced in the small-volume chamber by heat transfer from the sample to the air in its vicinity, resulting in thermal expansion and pressurization of the air with little contribution from thermal expansion within the sample itself. The frequency of acoustic signals in this work is determined by the modulation frequency of the laser beam, which is in the infrasound frequency range (<20 Hz). Two customized PA detection schemes were implemented for the LFA analysis.

The first scheme, so-called the "chop mode", employed an optical chopper to modulate the intensity of the excitation laser as shown in Fig. 1b. The resulting PA signals appeared as triangular waveforms, and the peak-to-peak value was used as the PA signal amplitude (Fig. 2a). The amplitude of the PA signal reflects the concentration of AuNPs at the test line, which is proportional to the concentration of the analyte. The drawback of the chop mode is that the AuNPs and the paper strip both generate PA signals. The paper strip absorption contributes to the background signal (blue trace of Fig. 2a), and since this background varied from strip to strip, it becomes a problem when comparing data from one strip to another. To overcome the background signal problem, we devised a new measurement scheme, the "scan mode", in which an unchopped continuous wave (CW) laser beam was scanned across the test line where the AuNPs resided (Fig. 1c). In contrast to the chop mode, the scan mode of PA signal generation from the AuNPs employed spatial modulation by the pattern of the test line, resulting in a strong PA signal as shown in Fig. 2b. In the scan mode, the paper strip absorbs a constant amount of the excitation light at different locations as the laser beam scans across the strip. Without a change of the heat generation, the sample temperature maintains at a constant value, and the system is in a thermal equilibrium state.



Fig. 1 Schematics of the LFA paper strip and two PA detection setups. (a) Shows an LFA paper strip illuminated by a laser beam to generate PA signals. (b) and (c) Illustrate the PA detection systems for the chop mode and the scan mode, respectively. (Not to scale).



Fig. 2 Measured PA waveforms for AuNPs absorbed in a porous substrate. The PA waveforms shown in (a) and (b) were measured using the chop mode and the scan mode, respectively. The chopping frequency was 13 Hz and the scanning speed was 3 mm s⁻¹. For both schemes, the peak-to-peak values were calculated as the PA signals.

Thus, the background PA signal (blue trace of Fig. 2b) is nearly zero. The result suggests that the scan mode is capable of generating a AuNP-specific signal when the laser beam is scanned across the test line with a significantly reduced background signal from the paper strip.

To demonstrate that the PA measurement is suitable for a highly sensitive analysis, we first measured the AuNPs absorbed into a polyvinylidene difluoride (PVDF) membrane. The waveforms of PA signals for three different concentrations (1011 nanoparticles NPs mL⁻¹, 3.3×1010 NPs mL⁻¹, and 0 NPs mL⁻¹) are compared in Fig. 2. A CW green laser (P_{max} = 50 mW, beam size = 0.5 mm, and λ = 532 nm) and a commercial PA chamber (PAC-200, MTEC Photoacoustics Inc.) were used for both modes of PA detections. The microphone (B&K 4176, Brüel & Kjær) installed in the small PA chamber (volume < 1 cubic centimeter) measured PA signals in the form of pressure oscillations. In the chop mode, a modulation frequency of 13 Hz was chosen, and the PA output was a triangular wave (Fig. 2a). For the scan mode, the scanning speed of the laser beam was fixed at 3 mm s^{-1} and the PA output was an under-damped pulse, as shown in Fig. 2b. Additional details regarding the testing parameters can be found in the Experimental section.

We also compared the detection sensitivity of the PA methods with that of the conventional colorimetric method. AuNPs samples were prepared on PVDF membranes, with AuNP concentrations ranging from 0 NPs mL⁻¹ to 1012 NPs mL⁻¹. The colorimetric signals were obtained by analyzing pictures (Fig. 3a) of the samples using an image processing software (ImageJ). Fig. 3b shows the dose-response curves for the PA-based measurements and the colorimetric method. The colorimetric data points exhibited significant fluctuations when the AuNP concentration was below 3.3×1010 NPs mL⁻¹.



Fig. 3 Comparison between PA-based detection methods and colorimetric analysis for AuNPs on porous substrates. (a) Optical images of the AuNP coated PVDF porous membranes with AuNPs in a two-fold dilution series. (b) Dose response curves of AuNPs on the PVDF membrane, obtained using three different detection methods. Both the signal response and the AuNP concentration are plotted on a logarithmic scale. Within the proper sensitivity ranges, the data points were fitted using a linear function for all three data sets.

For the PA-based measurements, the chop mode improves the sensitivity to below 1010 NPs mL⁻¹. In contrast, the scan mode was capable of detecting AuNPs at the concentration as low as 10^9 NPs mL⁻¹ and exhibited the largest dynamic range: 10^9 to 10^{12} NPs mL⁻¹ because the background signal caused by the optical absorption of the PVDF membrane had been effectively reduced.

Assured by the feasibility study, the PA-based LFA tests were carried out by using cryptococcal antigen (CrAg) as an example. For patients infected by human immunodeficiency virus, cryptococcosis is a fatal fungal disease. The detection of CrAg in a human body fluid sample can enable a definitive diagnosis of cryptococcosis.⁵⁶ Here, the PA-based detections were applied to quantify CrAg using a Food and Drug Administration (FDA)-approved LFA kit (IMMY Inc.). Before the experiment, the standard CrAg sample contained in the kit was diluted to prepare the CrAg titer with two-fold dilutions. CrAg samples (200 μ L) were pipetted onto the sample pad of the LFA paper strips. For each concentration, we prepared three replicates by applying the sample to three different LFA substrates. The detailed assay protocol is described in the Experimental section. Fig. 4a shows the photos of four LFA



Fig. 4 Results of the CrAg titer on the LFA paper strips. (a) Photographs of the LFA paper strips for three CrAg samples with CrAg concentrations of 100, 10, and 1 ng mL⁻¹, as well as a negative control sample. The test and control lines on the paper strips are labeled. At the lowest concentration (1 ng mL⁻¹), the test line cannot be distinguished from the background with naked eye. Using the 100 ng mL⁻¹ sample, we imaged a region inside the test line by SEM, in which the AuNPs appeared as the bright spots. (b) Comparison between dose–response curves of the colorimetric, chop mode and scan mode PA measurements. The measurement results are fitted using the five-parameter logistic curves provided in the Experimental section. The LODs for the colorimetric readout, the chop mode and the scan mode PA measurement, were calculated as 1.10, 0.57 and 0.01 ng mL⁻¹, respectively.

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paper strips after the control and test lines were fully developed. The colloidal AuNPs absorbed on the nitrocellulose fiber at the test line were illustrated by the scanning electron microscopy (SEM) image in Fig. 4a. For colorimetric analysis, the pictures of the paper strip for each CrAg sample concentration were taken and analyzed using ImageJ. The PA signals from the test line on each paper strip were measured using the previously described system. The dose-response curves of the colorimetric analysis, chop mode and scan mode PA detections are compared in Fig. 4b. To quantitatively evaluate the performance of each detection method, the measured data points were fitted using the non-linear curve fitting shown in the Experimental section. The limits of detection (LODs), at which the signal intensity was equal to the control signal plus three times the standard deviation of its replicates, were identified using the fitting curves. For each of the three detection methods, i.e. colorimetric, chop-mode and scan-mode analysis, three negative control samples were measured. The average values of the negative controls and their standard deviations (σ) for the colorimetric method, PA chop mode, and scan mode are: 7.5 cts. (σ = 3.78 cts.), 0.37 V (σ = 0.053 V), and 0.27 V ($\sigma = 0.054$ V), respectively. The colorimetric method, chop mode, and scan mode PA detection exhibited the LODs of 1.1 ng mL⁻¹, 0.57 ng mL⁻¹, and 0.010 ng mL⁻¹, respectively. The results clearly showed that both the PA-based methods provided a more sensitive and quantitative analysis than the conventional approach. In particular, the LOD of the scan mode PA measurement was more than 100 times lower than that of the colorimetric method.

Two additional experiments were performed to study how the power and the wavelength of the excitation light affected the PA signals. Using the scan mode PA approach, the samples for the CrAg titer were measured again under a different excitation condition. First, the output power of the 532 nm NdYAG laser was reduced to 5 mW using a neutral density filter. Fig. 5a compares the dose–response curves for the CrAg titer measured using 50 and 5 mW outputs. The PA signals decreased approximately 10-fold when the excitation intensity was reduced by a factor of 10. As we expect, the PA signal is



Fig. 5 Dose response curves of the same CrAg dilution series measured under different excitation conditions. All the results were obtained using the scan mode PA approach. (a) Dose–response curves obtained using the green laser with 50 and 5 mW outputs. (b) Dose–response curves measured using the green laser and red laser, respectively.

proportional to the intensity of the excitation light. When the excitation power was 5 mW, the average PA signal of the negative control samples was 0.021 V ($\sigma = 0.004$ V), which was approximately 10 times lower than the value measured using the 50 mW excitation. The LODs were calculated to be 0.012 ng mL⁻¹ and 0.010 ng mL⁻¹ for the excitation power of 5 mW and 50 mW, respectively. Since the PA signal noise caused by the paper strip has been minimized in the scan mode, the result suggests that the background noise is caused by the non-specific binding of the AuNPs at the test line.

The LSPR mode of AuNPs varies with the surrounding medium and distribution of AuNPs. Therefore, the absorption capability of AuNPs is wavelength dependent. The effect of the laser wavelength was investigated on the measurement of AuNPs embedded in a porous paper substrate. The same dilution series of LFA strips were measured using the green laser and a HeNe laser at 632.8 nm; both lasers had an output power of 5 mW. The PA signals were measured in the scan mode. As shown in Fig. 5b, the PA signals slightly changed with respect to the excitation wavelength. There were subtle differences between the results for the two lasers. At concentrations below 1 ng mL⁻¹, the PA signals excited by the green laser were stronger, as the LSPR mode of well-dispersed AuNPs had a resonance wavelength near 532 nm. Using the 632 as the excitation, the average PA signal of the negative control samples was 0.021 V (σ = 0.004 V) and the LOD was calculated to be LOD of 0.013 ng mL⁻¹, close to the 0.012 ng mL⁻¹ LOD of the 532 nm excitation. On the other hand, the PA signals for the samples with concentrations over 1 ng mL⁻¹ were stronger when the red laser was used. This phenomenon was mainly caused by an increase in contribution from the inter-particle resonance coupling because the average distance between AuNPs could be smaller at higher concentrations and hence the inter-particle resonance was stronger.

Conclusions

To summarize, a new PA detection method was demonstrated to improve the sensitivity of LFA tests. In contrast to the semiquantitative colorimetric analysis, the PA method enabled the quantitative and sensitive detection of a low concentration analyte using LFAs, owing to the strong LSPR of AuNPs and the effective elimination of a PA background signal associated with ambient light noise. Two types of PA measurement schemes were implemented, which modulated the laser light absorption in different ways. With scan mode PA detection, the light absorption was modulated spatially and was specifically designed to enhance the PA signal from the test line on standard LFA strips. This customized PA detection scheme effectively minimized the background signal arising from the paper substrate. The PA-based LFA was applied for the detection of CrAg using an FDA-approved LFA kit. The LODs of the analyte for colorimetric measurements, chop mode PA detection, and scan mode PA detection were 1.1 ng ml⁻¹, 0.57 ng ml⁻¹, and 0.010 ng ml⁻¹, respectively. The PA method was capable of decreasing the detection limit of the CrAg detection by a factor over 100. It is worth noting that this factor was characterized using standard CrAg samples. Our follow-up research will apply the PA-based LFA test to measure patient samples with the potential of reducing the LOD of paper-based sensors to the physiological concentration range of cytokine biomarkers in human serum.57 The simple and low-cost PAbased LFA will be valuable for in vitro diagnosis of early stage diseases using a drop of blood collected by a finger prick. Such a capability currently requires a laboratory-based approach, such as an enzyme-linked immunosorbent assay. Here, the PA apparatuses were developed using the off-the-shelf components. There is plenty of room to miniaturize the size and reduce the cost of the detection system for the application in resource-limited settings. For example, the laser source can be replaced using a LED that emits in the absorption band of the AuNP and the light source, microphone, and readout circuit can be fully integrated with the detection chamber into a palm-size box.

Furthermore, the tremendous progress in paper-based microfluidics enables the detection of multiple analytes.^{58,59} A panel of biomarkers associated with a complex disease, such as cancer, can be simultaneously measured using one LFA strip, by creating multiple lanes separated by hydrophobic barriers. In addition, recent developments in paper-based synthetic biology provide an alternative method to the antibody-antigen binding to deal with a wide variety of chemical and biological substances.⁶⁰ Together with these new advances, PA-based LFA will enrich the applications of LFAs in the field of point-of-care disease diagnostics, food safety, and environmental monitoring.

Experimental section

Preparation of bare AuNP on porous substrates

For the feasibility test, bare AuNPs suspension in water (Nanopartz) was diluted to the following concentrations (in NPs mL⁻¹): 1×1012 , 3×10^{11} , 1×10^{11} , 3×10^{10} , 1×10^{10} , 3×10^{9} , and 1×10^{9} . The size of the colloidal AuNPs is 40 nm with the size distribution of CV < 10%. In our work, 20 µL of each sample was pipetted onto a PVDF membrane and dried in air for 15 minutes. For each concentration, three replicates were prepared. After being dried, the samples were ready for further measurements. In the PVDF substrate, the AuNPs exhibited a strong absorption in the wavelength range of 500 nm-600 nm.

Lateral flow assay

The lateral flow device used in this work composes of a dipstick-shaped membrane with the five consecutive zones: the sample filter pad, conjugation pad with AuNP-conjugated anticryptococcal and goat IgG (control) antibodies, test line with immobilized anti-cryptococcal monoclonal antibodies, control line with immobilized bovine anti-goat IgG antibodies deposited near the other end of the membrane, and absorbing pad. The tests were performed by placing LFA dipsticks in centrifuge vials containing liquid samples. The sample pad was submerged in the specimen that allowed the wicking up of the specimen together with the AuNP-conjugated antibodies. The complex continued to wick up the membrane until it reached the test line where the AuNP-conjugated cryptococcal antibody-antigen complex was immobilized at the test line by the anti-cryptococcal antibodies. The accumulation of AuNPs developed as a visible line at the test and control lines.

The CrAg samples were prepared in a dilution series that contained the following concentrations (in ng mL⁻¹): 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781, 0.391, 0.195, 0.098, 0.049, 0.024, 0.012, and 0.006. During a test, the LFA paper strip was dipped into a vial containing the samples and was incubated for 10 minutes at room temperature. For each concentration, three duplicated strips were prepared. Then the strips were taken out of the vials and dried at room temperature for 15 minutes. After drying the samples were ready to be measured.

Visual contrast measurement

Visual images of the AuNPs on PVDF membranes and the LFA test strips were taken by a digital camera without flash light. The images were analyzed using ImageJ (an open source image processing program). The captured 24-bit RGB images were split into red, green and blue channels. Since the scattering of the AuNPs is strong in the green wavelength range, we chose the green channel to generate the visual contrast value. A number from 0 to 255 represented the brightness of each pixel. To analyze a sample, an area of interest was selected and the averaged brightness was calculated for the area with and without the AuNPs. The visual contrast of the sample was calculated as the difference between the averaged brightness of the blank substrate and AuNPs coated area. We repeated the above steps for all the samples to obtain the contrast values for each sample.

Photoacoustic measurement

For both chop and scan modes, we measured the samples using the MTEC PAC-200 PA detector. An oscilloscope (TDS 2014, Tektronix) was connected to the PA detector to measure the PA waveforms. Since the AuNPs used in our experiments exhibited the LSPR resonance in the wavelength range of 500 nm–600 nm, a CW, frequency-doubled, Nd:YAG laser (λ = 532 nm, P_{max} = 50 mW, PN: G15012, Mingnuo Optoelectronics Inc.) was used as the excitation source. For the chop mode, the intensity of the laser beam was modulated by an optical chopper (MC2000B, Thorlabs Inc.) at a modulation frequency of 13 Hz. The optical chopper generated a square-wave modulated excitation. The resulting PA waveform was a triangular wave at the same frequency. The rising edge and falling edge corresponded to the on time and off time of the square-wave excitation, respectively. In the scan mode, the laser intensity was kept constant and the laser beam was scanned across the sample at a speed of 3 mm s^{-1} . Since the PA signal increases with the decrease of the modulation frequency/scanning speed, a low frequency/speed was chosen to enhance the

signal-to-noise ratio of the measurements. For both modes, the peak-to-peak amplitude value (Fig. 2) was defined as the PA signal. A neutral density filter was used to adjust the power of the excitation. For the study of wavelength effect, the green laser was replaced by a He–Ne laser ($\lambda = 532$ nm, $P_{\text{max}} = 5$ mW, Melles Griot).

Analysis of dose response curve

The measured dose response results were fitted using the 5 Parameter Logistic (5-PL) fitting formula:

$$y = A_{\min} + \frac{A_{\max} - A_{\min}}{\left(1 + \left(\frac{x}{x_0}\right)^{-h}\right)^s},$$

where A_{\min} is lower asymptote, A_{\max} is upper asymptote, x_0 is the dose where the response is halfway between bottom and top, h is the hillslope, and s is a control factor. The fitting was performed using OriginLab 9.0. To calculate the LOD, we calculated the signal of the respective control (the sample without analyte) for each titer, added three times of its standard deviation, and then identified the corresponding concentration of this value through the use of the aforementioned 5-PL fitting curve. The found concentration of analyte was defined as the LOD value. The standard deviation was calculated using three replicated LFA strips for each analyte concentration.

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References

- 1 G. A. Posthuma-Trumpie, J. Korf and A. Van Amerongen, *Anal. Bioanal. Chem.*, 2009, **393**, 569–582.
- 2 A. K. Yetisen, M. S. Akram and C. R. Lowe, *Lab Chip*, 2013, 13, 2210–2251.
- 3 M. Sajid, A.-N. Kawde and M. Daud, *J. Saudi Chem. Soc.*, 2015, **19**, 689–705.
- 4 V. Gubala, L. F. Harris, A. J. Ricco, M. X. Tan and D. E. Williams, *Anal. Chem.*, 2012, **84**, 487–515.
- 5 C. Parolo and A. Merkoci, *Chem. Soc. Rev.*, 2013, **42**, 450–457.
- 6 L. Anfossi, G. D'Arco, M. Calderara, C. Baggiani, C. Giovannoli and G. Giraudi, *Food Addit. Contam., Part A*, 2011, 28, 226–234.
- 7 S. Rong-Hwa, T. Shiao-Shek, C. Der-Jiang and H. Yao-Wen, *Food Chem.*, 2010, **118**, 462–466.
- 8 C. S. Huang, S. George, M. Lu, V. Chaudhery, R. Tan, R. C. Zangar and B. T. Cunningham, *Anal. Chem.*, 2011, 83, 1425–1430.

- 9 R. Tang, H. Yang, J. R. Choi, Y. Gong, J. Hu, S. Feng,
 B. Pingguan-Murphy, Q. Mei and F. Xu, *Talanta*, 2016, 152, 269–276.
- 10 B. Y. Moghadam, K. T. Connelly and J. D. Posner, Anal. Chem., 2014, 86(12), 5829–5837.
- 11 D. V. Borich, M. McAleer, A. Milder, D. Mitchell and S. Savoy, US Pat, 7803322, 2010.
- 12 D. J. You, T. S. Park and J. Y. Yoon, *Biosens. Bioelectron.*, 2013, 40, 180–185.
- M. Zangheri, L. Cevenini, L. Anfossi, C. Baggiani, P. Simoni, F. Di Nardo and A. Roda, *Biosens. Bioelectron.*, 2015, 64, 63–68.
- 14 K. May, M. E. Prior and I. Richards, US Pat, 6187598, 2001.
- 15 J. A. Ho and R. D. Wauchope, *Anal. Chem.*, 2002, 74(7), 1493–1496.
- 16 M. Mirasoli, A. Buragina, L. S. Dolci, M. Guardigli, P. Simoni, A. Montoya, E. Maiolini, S. Girotti and A. Roda, *Anal. Chim. Acta*, 2012, 721, 167–172.
- 17 X. Fu, Z. Cheng, J. Yu, P. Choo, L. Chen and J. Choo, *Biosens. Bioelectron.*, 2016, 78, 530–537.
- 18 D. Du, J. Wang, L. Wang, D. Lu and Y. Lin, Anal. Chem., 2012, 84(3), 1380–1385.
- 19 D. Quesada-González and A. Merkoçi, *Biosens. Bioelectron.*, 2015, 73, 47-63.
- 20 R. H. Shyu, H. F. Shyu, H. W. Liu and S. S. Tang, *Toxicon*, 2002, 40(3), 255–258.
- 21 J. S. Oh, G. W. Ha, Y. S. Cho, M. J. Kim, D. J. An, K. K. Hwang, Y. K. Lim, B. K. Park, B. K. Kang and D. S. Song, *Clin. Vaccine Immunol.*, 2006, 13(4), 520–524.
- 22 G. Liu, X. Mao, J. A. Phillips, H. Xu, W. Tan and L. Zeng, *Anal. Chem.*, 2009, 81, 10013–10018.
- 23 Z. Li, Y. Wang, J. Wang, Z. Tang, J. G. Pounds and Y. Lin, *Anal. Chem.*, 2010, 82(16), 7008–7014.
- 24 A. N. Berlina, N. A. Taranova, A. V. Zherdev, Y. Y. Vengerov and B. B. Dzantiev, *Anal. Bioanal. Chem.*, 2013, 405(14), 4997–5000.
- 25 X. Zhang, D. Li, C. Wang, X. Zhi, C. Zhang, K. Wang and D. Cui, *J. Biomed. Nanotechnol.*, 2012, 8(3), 372–379.
- 26 J. M. Barnett, P. Wraith, J. Kiely, R. Persad, K. Hurley, P. Hawkins and R. Luxton, *Biosensors*, 2014, 4(3), 204–220.
- 27 X. Zhang, L. Jiang, C. Zhang, D. Li, C. Wang, F. Gao and D. Cui, *J. Biomed. Nanotechnol.*, 2011, 7(6), 776–781.
- 28 D. Tang, J. C. Sauceda, Z. Lin, S. Ott, E. Basova, I. Goryacheva, S. Biselli, J. Lin, R. Niessner and D. Knopp, *Biosens. Bioelectron.*, 2009, 25(2), 514–518.
- 29 Z. P. Qin, W. C. W. Chan, D. R. Boulware, T. Akkin, E. K. Butler and J. C. Bischof, *Angew. Chem., Int. Ed.*, 2012, 51, 4358–4361.
- 30 K. A. Willets and R. P. Van Duyne, *Annu. Rev. Anal. Chem.*, 2007, **58**, 267–297.
- 31 J. N. Anker, W. P. Hall, O. Lyandres, N. C. Shah, J. Zhao and R. P. Van Duyne, *Nat. Mater.*, 2008, 7, 442–453.
- 32 Y. Xianyu, Y. Xie, N. Wang, Z. Wang and X. Jiang, *Small*, 2015, **11**(41), 5510–5514.
- 33 Y. Guo, Y. Zhang, H. Shao, Z. Wang, X. Wang and X. Jiang, Anal. Chem., 2014, 86(17), 8530–8534.

- 34 K. Zhu, Y. Zhang, S. He, W. Chen, J. Shen, Z. Wang and X. Jiang, Anal. Chem., 2012, 84(10), 4267–4270.
- 35 T. A. Taton, C. A. Mirkin and R. L. Letsinger, *Science*, 2000, 289, 1757–1760.
- 36 Y. Zhao, M. Cao, J. F. McClelland, Z. Shao and M. Lu, *Biosens. Bioelectron.*, 2016, **85**, 261–266.
- 37 A. Rosenewaig, *Photoacoustics and Photoacoustic Spectroscopy*, Wiley, New York, 1980.
- 38 D. W. Ball, Spectroscopy, 2006, 21, 14–16.
- 39 S. L. Castleden, C. M. Elliott, G. F. Kirkbright and D. E. M. Spillane, Anal. Chem., 1979, 51(13), 2152–2153.
- 40 R. L. White, Anal. Chem., 1985, 57(9), 1819–1822.
- 41 X. Wang, Y. Pang, G. Ku, X. Xie, G. Stoica and L. V. Wang, *Nat. Biotechnol.*, 2003, 21(7), 803–806.
- 42 L. V. Wang and S. Hu, Science, 2012, 335(6075), 1458-1462.
- 43 L. V. Wang, Nat. Photonics, 2009, 3(9), 503-509.
- 44 J. Laufer, C. Elwell, D. Delpy and P. Beard, *Phys. Med. Biol.*, 2005, **50**(18), 4409.
- 45 G. B. Christison and H. A. MacKenzie, *Med. Biol. Eng. Comput.*, 1993, 31(3), 284–290.
- 46 H. A. MacKenzie, H. S. Ashton, S. Spiers, Y. Shen, S. S. Freeborn, J. Hannigan, J. Lindberg and P. Rae, *Clin. Chem.*, 1999, 45(9), 1587–1595.
- 47 A. C. Tam, Rev. Mod. Phys., 1986, 58, 381-431.
- 48 J. F. Mcclelland, IEEE Trans. Sonics Ultrason., 1981, 28, 362-362.
- 49 J. F. Mcclelland and R. N. Kniseley, Appl. Opt., 1976, 15, 2658–2663.

- 50 S. Mallidi, T. Larson, J. Tam, P. P. Joshi, A. Karpiouk, K. Sokolov and S. Emelianov, *Nano Lett.*, 2009, 9, 2825– 2831.
- 51 X. M. Yang, E. W. Stein, S. Ashkenazi and L. H. V. Wang, Wires Nanomed. Nanobi., 2009, 1, 360–368.
- 52 Q. Zhang, N. Iwakuma, P. Sharma, B. M. Moudgil, C. Wu, J. McNeill, H. Jiang and S. R. Grobmyer, *Nanotechnology*, 2009, 20.
- 53 P. Manivasagan, S. Bharathiraja, N. Q. Bui, I. G. Lim and J. Oh, *Int. J. Pharm.*, 2016, **511**(1), 367–379.
- 54 G. Ku, M. Zhou, S. Song, Q. Huang, J. Hazle and C. Li, *ACS Nano*, 2012, **6**(8), 7489–7496.
- 55 Y. F. Zhao, K. Y. Liu, J. McClelland and M. Lu, *Appl. Phys. Lett.*, 2014, 104.
- 56 J. N. Jarvis, A. Percival, S. Bauman, J. Pelfrey, G. Meintjes, G. N. Williams, N. Longley, T. S. Harrison and T. R. Kozel, *Clin. Infect. Dis*, 2011, 53, 1019–1023.
- 57 P. C. Mathias, N. Ganesh and B. T. Cunningham, *Anal. Chem.*, 2008, **80**, 9013–9020.
- 58 A. W. Martinez, S. T. Phillips, G. M. Whitesides and E. Carrilho, Anal. Chem., 2010, 82, 3–10.
- 59 C. Z. Li, K. Vandenberg, S. Prabhulkar, X. N. Zhu, L. Schneper, K. Methee, C. J. Rosser and E. Almeide, *Biosens. Bioelectron.*, 2011, 26, 4342–4348.
- 60 K. Pardee, A. A. Green, T. Ferrante, D. E. Cameron, A. DaleyKeyser, P. Yin and J. J. Collins, *Cell*, 2014, 159, 940–954.