

# Fast Track

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## Short Communication

### Low cost, portable detection of gold nanoparticle-labeled microfluidic immunoassay with camera cell phone

This paper describes the use of camera cell phone to detect the gold nanoparticle-labeled immunoassay results on microfluidic chip. On-chip heterogeneous immunoassay between anti-human IgG and human IgG (hIgG) was performed and the results were amplified by gold nanoparticle-enhanced silver staining to transform the nano-scale probe into detectable silver precipitation film. The detection results obtained by camera phone showed good correlation with the concentration of hIgG. This detection scheme is simple, low cost and easy to use and has significant promise for portable diagnosis and point of care testing on microfluidic platform.

#### Keywords:

Camera phone / Detection / Gold nanoparticle / Immunoassay / Microfluidics

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Immunoassay, which takes advantages of the specific reaction between antibody and antigen, has become a powerful tool to detect trace compounds in many fields such as clinical diagnosis, food inspection and environmental analysis [1]. However, the conventional immunoassay formats such as micro-titer plate ELISA suffer from the long incubation time and much reagents and sample consumption, which are caused by the relatively large dimensions of the reactors (millimeter scale) [2, 3]. Microfluidics platform, also known as “lab-on-a-chip”, has the benefit of scaling dimension (micrometer scale) that enables rapid diffusion and reaction and is attracting more and more attention in the biological and medical sciences in recent years [4–8]. Its ease of integration makes it have great potential for parallel processing and high-throughput analysis [9]. A wide range of work has been demonstrated for microfluidic immunoassay [9–18]. Several different detection methods have been used to monitor the immunoassay results on the microfluidic platform, such as fluorescence microscopy [9, 10, 15, 18], thermal lensing [11], electrochemical detection [12, 13] and surface plasmon resonance [14]. However, most of the previously

reported detection equipment suffered from high cost, difficult to use and large size, which are not compatible with the small size and the portability of microfluidic chip. These shortcomings hinder maximum utilization of the advantages of microfluidic immunoassay [19]. As there is great potential for microfluidic immunoassay to become important tools for point-of-care applications, a cheap and easy-to-use detector is highly desirable to make it acceptable for practical use.

Here we used a cell phone embedded with camera as a low cost, portable and easy-to-use detector to monitor the gold nanoparticle-labeled immunoassay results on the microfluidic platform. Using gold nanoparticle as a biocatalytic probe, on-chip heterogeneous immunoassay between anti-human IgG and human IgG (hIgG) was performed. With gold nanoparticle-enhanced silver staining as the amplifying method [20, 21], the signal of the immunoassay area on the microfluidic chip was imaged by a camera cell phone. The images could be analyzed in a personal computer with image processing and analysis software by converting each image to an 8-bit grayscale mode and measuring the average grayscale values within the reaction zones. The detection scheme is shown in Fig. 1A. This detection method has the advantages of ease of use, low cost, transmissibility, high portability and mobility. We believe that it will be very useful for microfluidic immunoassays and has a significant promise for low-cost

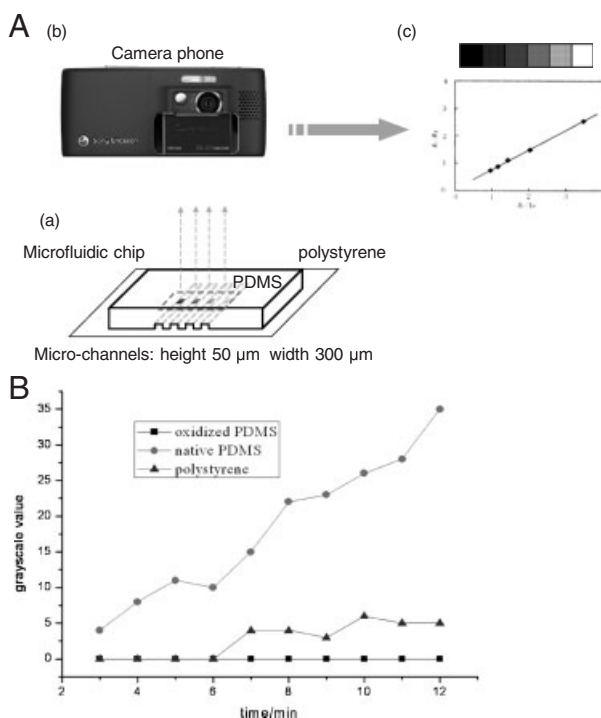
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**Abbreviations:** g-hIgG, goat anti-human IgG; hIgG, human IgG

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**Figure 1.** (A) Schematic illustration of camera phone detection for gold nanoparticle-labeled microfluidic immunoassay (a) the hybrid microfluidic chip made of PDMS with microstructures and a flat polystyrene with immobilized antibody strip; (b) the camera phone used in the experiment for image catching with following parameters: camera 3.2 megapixel, digital zoom:  $16\times$ ; auto focus; (c) the immunoassay signal is obtained with image analysis software by converting each image into the grayscale mode and measuring the average grayscale values within the immunoassay area. (B) Comparison of the autocatalytic reduction/precipitation background of the silver staining solution on different substrates including native PDMS, polystyrene and PDMS oxidized in the plasma cleaner. The substrates were sealed with an oxidized PDMS layer, where microwells with 3 mm diameter were punched out. The silver enhancer solution was pipetted into the microwells and the substrates were incubated with it. At the indicated times, dry the solutions in microwells and wash them with PBST buffer. The resulting silver films on the substrates were measured by a flatbed scanner and the average grayscale values were obtained with the imagepro software (the minus results between the grayscale values in the microwells and the background).

monitoring of health in remote regions and application in future telemedicine.

The layout of the microfluidic chip used in our experiment is shown in Fig. 1A(a), which is composed of two layers. The upper layer is a PDMS chip, which has straight micro-channels (height:  $50\ \mu\text{m}$ , width:  $300\ \mu\text{m}$ , length: 3 cm) with separate inlet holes and outlet holes for fluid handling; the bottom layer is a flat polystyrene substrate (Greiner, Germany). Antibody strip (1 mm width) was immobilized on the polystyrene surface beforehand. Here we used the polystyrene instead of PDMS to pattern antibody strips onto its surface for the following two reasons: (i) we found that the silver staining background was much

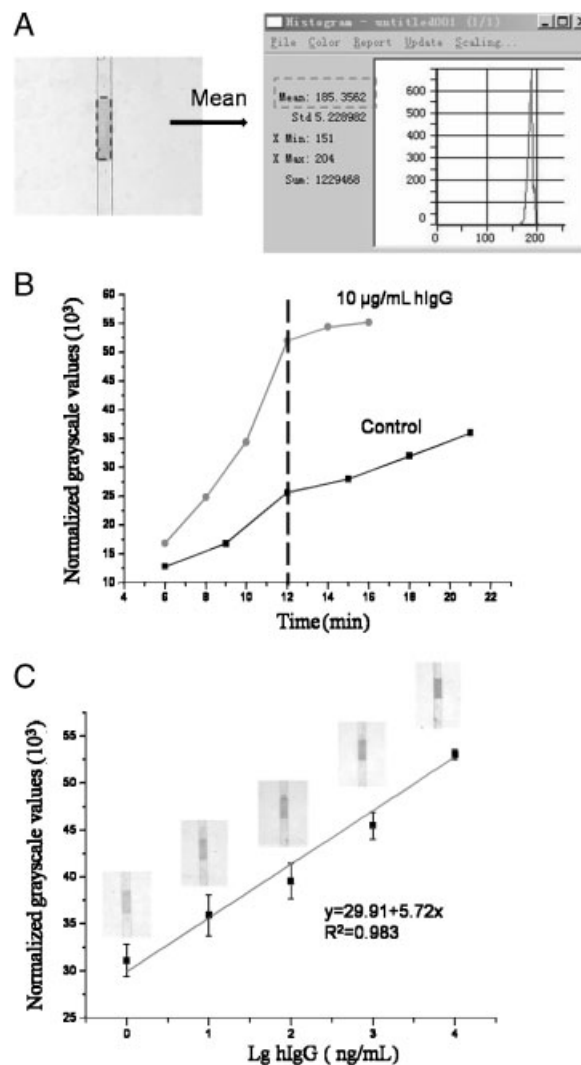
higher on native PDMS than on polystyrene (Fig. 1B), which is probably because the PDMS is more hydrophobic and tends to adsorb more silver onto its surface, and (ii) the protein adsorbed onto the polystyrene surface can maintain its activity much longer time than on PDMS, and this feature is quite important for long-term use (It is reported that the immobilized protein on PDMS surface can remain stable for less than 4 wk [22].)

The PDMS master with positive relief was fabricated with rapid prototyping technique as described elsewhere [23] ([http://www.microchem.com/products/su\\_eight.htm](http://www.microchem.com/products/su_eight.htm)). It included the following procedures: (i) SU-8-2025 photoresist (Microchem, Newton, CA) was spin-coated onto silicon wafers at 1500 rpm for 30 s. (The wafers were pre-cleaned with Piranha solution (3:1 v/v 96%  $\text{H}_2\text{SO}_4$ : $\text{H}_2\text{O}_2$ ).) (ii) Pre-exposure soft baking was finished at  $65^\circ\text{C}$  for 15 min and  $95^\circ\text{C}$  for 15 min on a hotplate. (iii) Expose SU-8 photoresist to ultraviolet light with the mask to get the microchannels pattern on the photoresist. (iv) The post exposure baking was done following the pre-exposure soft baking protocol. (v) The photoresist was developed in SU-8 developer at room temperature. Following the developing, the photoresist was hard-baked at  $150^\circ\text{C}$  for 1 h. After that, masters were silanized by exposure to tridecafluoro-1,1,2,2-tetrahydrooctyl trichlorosilane vapor (Sigma Chemical, St. Louis, MO, USA) and positioned in Petri dishes for replica molding. PDMS prepolymer (PDMS, Sylgard 184, Dow Corning, Midland, MI) was mixed thoroughly at 10:1 v/v ratio. Then it was poured onto the master and cured in the oven at  $80^\circ\text{C}$  for 4 h. After cooling, the cured PDMS slab was peeled off from the master, the PDMS slab was cut to the desired size and the inlet and outlet holes were punched out (2 mm diameter). Then the PDMS layer was oxidized in the plasma cleaner (Harrick, USA) to tailor the PDMS surface hydrophilic to resist protein adsorption and facilitate the fluid handling (The silver staining background on the oxidized PDMS is very light; hence it will not bring much background signals.) After plasma oxidation, it was sealed onto the polystyrene substrate and the microchannels were placed orthogonally to the immobilized goat anti-human IgG (g-hIgG) strip on the polystyrene surface.

After the PDMS and polystyrene layers were assembled, there were three steps to complete the immunoassay procedures on the microfluidic chip. First, 2.5% BSA (Roche, Switzerland) in PBST buffer (PBS+0.05% Tween 20) was introduced into the microchannels to block the unpatterned surface for 30 min, which is quite important to reduce the background signals. Second, hIgG sample with varied concentration were added to react with the patterned antibody. Third, 1:20 dilution of gold nanoparticle-labeled g-hIgG (Boaosen Biotech., P. R. China) was introduced to form the immuno-complex on the polystyrene surface. The incubation time for each reaction is set at 10 min and the microchannels were washed with PBST buffer three times before each new reagent was introduced. After a thorough washing of the microchannels with double distilled water to remove unbonded gold nanoparticle-labeled protein and

avoid the interference of ions, a freshly prepared silver enhancer solution (mix at 1:1 v/v ratio) (Silver Enhancer Kit, SE-100, Sigma) was added to start the staining process. Silver ions were reduced by hydroquinone to silver metal at the surfaces of the gold nanoparticles and immunoassay signals were greatly amplified [21]. Silver-staining-based amplifying method is quite sensitive to a great deal of factors such light, temperature and contamination, which will lead to nonspecific silver aggradations. Hence, the handling of the immunoassay and staining processes should be as careful and clean as possible. The resulting silver film's opacity on the polystyrene surface was proportional to the concentration of the analyte hIgG, transforming the nano-scale tracers to detectable signals.

After completing the staining process and washing the microchannels with PBST to remove the unbonded silver, the microfluidic chip was ready for detection. We imaged the reaction results by a camera cell phone (SonyEricsson K790C, camera 3.2 megapixel, digital zoom: 16 $\times$ ; auto focus). The detection process was quite simple with camera directly placed vertically on top of each detection area to ensure its lens parallel to the surface of the microfluidic chip. After half pressing the camera button to let the camera focus on the immunoassay area automatically, press the button and capture the image. To enhance the resolving power of the camera phone, a magnifying glass (3 $\times$ ) was fixed in front of the camera *via* glue to strengthen its micro-shot ability. With the rapid advances in digital technology, more and more camera phones will combine the optical zoom ability to realize ever-increasing high resolution and capability. In our experiment, the camera can focus in about 1.5 cm above the microchannels with the aid of the magnifying glass. Turning camera close to the detection area can reduce the background light variedness. Meanwhile, an LED lamp base was placed under the microfluidic chip to ensure the equal distribution of the background light intensity. After image capturing, all these pictures can be uploaded into the personal computer through USB or infrared connection, or it can be transmitted faraway through internet to be evaluated. The images could be analyzed using image processing and analysis software (Image-Pro, Media Cybernetics, USA) by converting each image to an 8-bit grayscale mode and measuring the mean pixel values within the immunoassay zones, which is shown in Fig. 2A. The signal was obtained by normalizing the grayscale values by  $A = (I_0 - I)/t$ , where  $A$  denotes the normalized grayscale intensity of the immunoassay area;  $I_0$  and  $I$  are the averaged grayscale values of the background and the reaction area, respectively; and  $t$  is the exposure time of the image captured by the camera phone. We characterized the variation of the images caused by the dithering of the image-catcher. Six pictures were taken sequentially on the same immunoassay area and analyzed on the image-pro software; the standard deviation of the grayscale values obtained was about 1.37%, which indicated that the results obtained by the camera phone were quite reproducible. Through the established detection strategy, calibration curve for hIgG



**Figure 2.** (A) The signal analysis with the image-pro software to get the average grayscale values within the immunoassay zones; (B) the time profile of silver deposition in immunoassay, the highest signal to background ratio were obtained after 12 min of silver staining; (C) the calibration curve of hIgG with the optimized immunoassay conditions as follows: 500 µg/mL of g-hIgG in 50 mM NaCO<sub>3</sub> buffer (pH 9.6) for antibody immobilization on the polystyrene surface, 1:20 dilution of gold nanoparticle-labeled g-hIgG, 12 min for silver staining (the micropatches shown in the figure are 1 mm  $\times$  0.3 mm).

was conducted with the optimized conditions (Fig. 2C). The linear regression showed a good correlation between the signal and the concentration of hIgG.

Simple and effective detection methods are critically important for realizing rapid on-site analysis and point of care testing. The immunoassay signals amplified by silver staining are visible to eyes. However, eye inspection can generate only qualitative information, which is not adequate to differentiate and judge the results in some cases. When the quantitative or sub-quantitative results are needed, some kinds of detection equipment are needed. The camera mobile phone, which is accessible to more and more people,

provides the feasibility and a simple way to detect the immunoassay results. With the aid of professional image analysis software, these images can be turned into specific values and evaluated to obtain the diagnostic results. Meanwhile these images can also be transmitted faraway to be judged by an off-site expert to realize on-site detection and off-site diagnostics. The camera phone detection coupled with gold nanoparticle label and silver staining offers several advantages: (i) low cost: the camera cell phone we used costs only about 150 US dollars, which is quite cheap compared with professional detection equipment such as surface plasmon resonance, thermal lensing or fluorescence microscopy; (ii) portable and mobile: the weight of the cell phone we used is only 115 g and the size is 105 mm × 47 mm × 22 mm; thus it is easy for people to take it around; (iii) user friendly: the cell phone is now widely used and taking pictures is also easy for people; hence, this detection scheme can be easily adopted by non-experts; (iv) transmit the results directly and quickly: we can turn to professional to get advices and feedback quickly, which is quite helpful in remote areas. Advanced camera phones will combine personal digital assistant together and install the image analysis software directly in the cell phone to make it more powerful; (v) battery powered: this feature is quite useful for the cases when the detection must be done in the field or remote regions where the power source may be a big problem.

In conclusion, we utilized the widely used camera cell phone as the detector to monitor the gold nanoparticle-labeled immunoassay results on the microfluidic platform. This detection strategy is small, low cost, portable, easy to use and carry. It is not only suitable for immunoassay on microfluidic platform but it can also be implemented on other bioassay platforms such as DNA microarray, the strip assays such as nitrocellulose lateral flow strip including women fertility testing, cardiac markers, drugs of abuse, infectious diseases and many others. We have great confidence that this kind of detection method can be implemented for on-site analysis in remote areas in the near future [24].

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