

# Nanoparticle-Based Optical Detection of Molecular Interactions for DNA-Chip Technology

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## ABSTRACT

We adapted the nanoparticle-labeling technique from microscopical applications for DNA-chip detection. Nanoparticles can be detected by simple optical means (e.g., reflection or transmission), and exhibit a high stability. So alternative optical readout devices can be applied for the detection of specific DNA-binding on microstructured spots of complementary, surface-immobilized capture DNA. Several devices were tested, and the binding of gold-labeled DNA to complementary and non-complementary sequences was investigated using optical detection of the gold-labeled substrates before and after silver enhancement.

**Keywords:** DNA chip, nanoparticle, colloidal gold, optical detection, DNA

## 1. INTRODUCTION

Fluorescence is the standard method for detection of binding events in molecular biology. Fluorescence markers are highly sensitive, and a variety of fluorescence-conjugated biomolecules are available. However, this technique suffers from photochemical instability and environment-dependent quantum yield, and the needed readers are expensive. Metal nanoparticles promise a high stability combined with a simpler optical detection, and were therefore proposed as alternative for the detection of binding events [1-4]. The high stability of DNA-nanoparticle conjugates in solution and on solid substrates were demonstrated in various studies [5-9]. There are several possibilities for the detection of the bound nanoparticles; the simplest one is the detection of color change in solution or on a substrate with the naked eye [10]. This technique is only applicable for a greater ensemble of DNA-nanoparticle complexes. For single-molecule detection, the surface plasmon resonance of nanoparticles can be used, which can be tailor-made to enable multicolor tests [1, 2]. To meet the demand of today's molecular diagnostic, highly paralleled tests with a simple detection scheme are needed. The parallelization of nanoparticle-based tests was already demonstrated by microstructuring DNA-spots on substrates down to 50  $\mu\text{m}$  or even 4  $\mu\text{m}$  squares [4]. However, these spots were still examined by an optical microscope. Envisioning a broad application of nanoparticle-based detection, a simpler device would be helpful to minimize instrument costs and enhance the stability of measurements outside of the lab. One step in this direction was the introduction of a flatbed scanner for the study of nanoparticle-labeled DNA-binding on DNA-modified glass substrates after application of a silver amplification technique [3]. In this technique silver ions are reduced to silver metal at the surface of gold nanoparticles, which increased the scanned intensity by several orders of magnitude. We think that the step away from the use of optical microscopes is important for future applications, because the microscope cannot fulfill the requirements for a mass application of readout devices, especially outside the protected area of the lab. The needed device has to be low cost in combination with ease of use and high stability against incorrect user input. Although one can envision custom-built optical systems meeting these requirements, for now the use of already existent devices is helpful, especially for a fast spreading of the method. So we compare in this paper the application of several optical devices for the readout, with special emphasize of ease of use and the achievable resolution. The resolution is an important parameter, as pointed out by the ongoing discussion in DNA-microarray community. A pixel resolution of 3x3 for a given DNA-spot seems to be needed for a sufficient characterization of the signal.

Beside this comparison, we used a high-resolution flatbed scanner in transmissive mode for the characterization of DNA binding on microstructured spots, using gold-labeled DNA before and after silver enhancement. The high sensitivity of a flatbed scanner was demonstrated for the detection of the gold-labeled DNA on the glass substrate, and compared with the results of this test after silver enhancement.

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## 2. MATERIALS AND METHODS

### 2.1. Test substrates

To test the resolution of different optical devices, a microstructured test substrate with a pattern etched in a 100 nm gold coating (primed by 5 nm Cr) on a glass slide was used. The pattern consisted of 10x10 arrays of squares of 200  $\mu\text{m}$  each.

### 2.2. Optical devices

The following devices were used for an optical transmissive readout of the test substrate: Optical microscope (AxioTech, Carl Zeiss Jena, Germany), an USB-connected all-in-one-microscope (Intel Play QX3 Microscope, Intel), a flatbed scanner (Duoscan T2500, AGFA), and a slide scanner (Dimage Scan Dual II, Minolta). For the readout of the DNA-nanoparticle samples, the flatbed scanner was used.

### 2.3. Substrates for DNA-spots

Standard microscopy glass slides were cleaned with water, ethanol and acetone for 10 min each prior to sonication. For activation, the chips were immersed in a 1:1:1 mixture of hydrochloric acid : hydrogenperoxide : water for 10 min, washed with water and dried at 80 °C for 5 min. The chips were then given in a 1 mM octadecyltrichlorosilane solution in dry toluene for 3-4 h at 40 °C. Afterwards the substrates were covered with a photo resist, exposed to a pattern of 4 rows with 8 squares of 1x1 mm each, and developed. Then etching with oxygen plasma opened the binding areas and the resist was removed. The chips were freshly activated in the solution described above and then incubated for 6-8 h in a 10 mM solution of GOPS in dry toluene. After the reaction the chips were washed once with toluene and twice with ethyl acetate for 5 min each. The chips were used right after the washing.

The aminomodified capture DNA was solved in 0,1 M KOH to a final concentration of 50 $\mu\text{M}$ . 1 $\mu\text{l}$  droplets of that solution were given on the designated binding spots and the chips were incubated in a humidity chamber for 3-4h at 37°C. The droplets were allowed to dry. Then, the chips were washed with water until the chips were clean again.

### 2.4. DNA preparation and hybridization

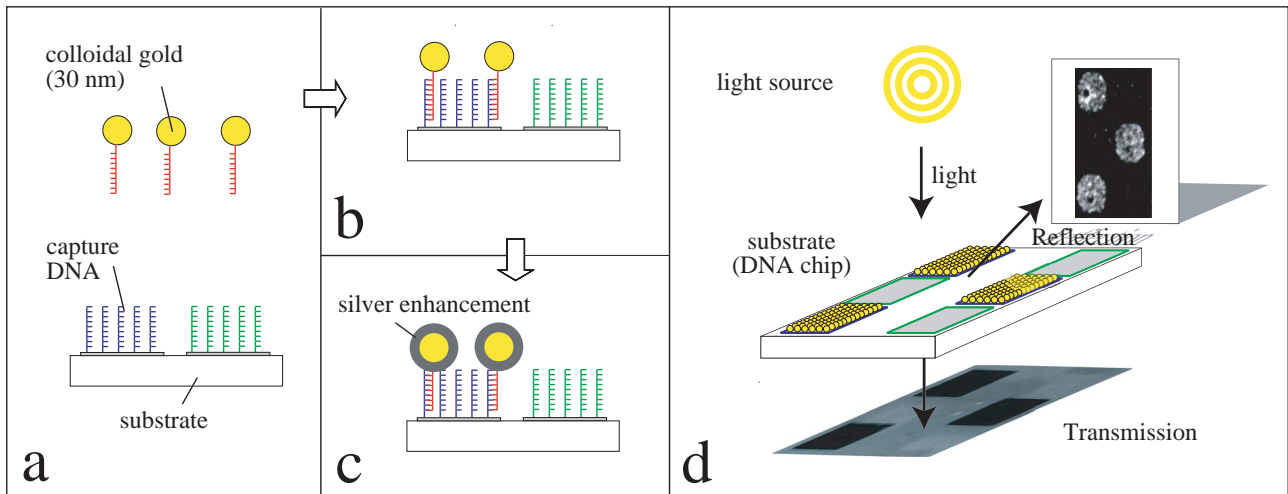
For preparation of gold-DNA complexes, 5'- and 3'-alkylthiolated oligonucleotides (BioScience, Jena, Germany) were desalted by NAP-10 Column (Pharmacia) and preincubated with gold nanoparticle solution (30 nm diameter, British BioCell) for 16 h at room temperature in a ratio of 0.33 nM gold and 200 nM DNA [10]. Another incubation was after adjusting the solution to 0.1 M NaCl/ 10 mM sodiumphosphate buffer at pH 7.0 for 48 h at room temperature. The DNA-nanoparticle complexes were repeatedly washed with buffer and redispersed in 0,1 M NaCl / 10 mM sodium phosphate buffer at pH 7.0. Solutions with higher concentrations were prepared by centrifugation and redispersion in a corresponding lower volume of buffer.

The used solution of the gold-labeled marker DNA had an optical density of 2 at 525 nm. The marker solution was applied to the chip and incubated for 30 min at 66°C in a humidity chamber and cooled slowly to 20°C. The chips were then washed again as described above.

The silver enhancement was conducted as described elsewhere [7].

### 2.5. Quantification of spot density / Image processing

The scanned images of the slide before and after silver enhancement were imported into the public domain NIH Image 1.61 program (developed at the U.S. National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/nih-image/>) as 8-bit TIFF-files. For an approximation, squared regions of interest with constant size were defined around every DNA-square (resulting in 8 measurements per row), and the mean gray-value density was determined using built-in routines. The background was determined by measuring 8 squares of the same size in the area between both double rows. The measurements for each row were averaged and plotted in Fig. 3b.



**Figure 1:** Scheme of nanoparticle-based labeling for the detection of molecular interactions. a) Molecules of interest are labeled with colloidal gold particles. Arrays of capture molecules, which are complementary to the molecule of interest, are prepared on solid substrates. b) After incubation, the molecules of interest bind to the complementary capture molecules, resulting in surface-bound colloidal particles. c) These particles can be enhanced by selective growth of silver on the nanoparticles. d) A perspective scheme shows a substrate with immobilized nanoparticles. Optical detection in reflection or transmission mode is applied for localization of the areas of nanoparticle binding.

### 3. RESULTS AND DISCUSSIONS

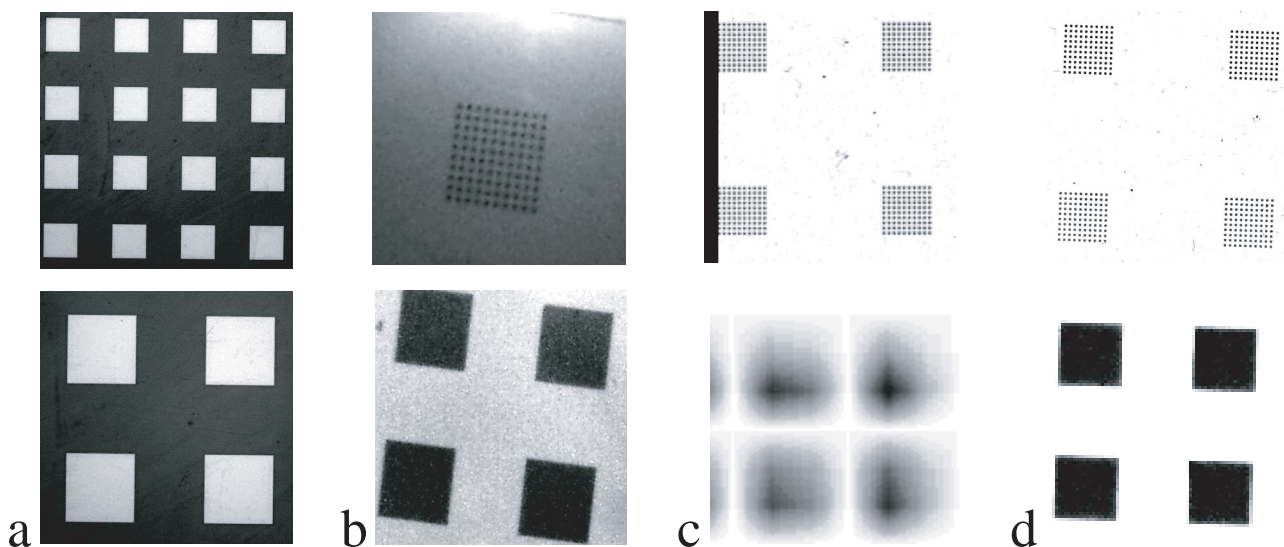
#### 3.1. Optical readout of nanoparticle labels

##### *Scheme of nanoparticle labeling*

The general setup of nanoparticle labeling for DNA-chip technology is given in Fig. 1. Immobilized capture DNA is complementary to the sample molecules of interest, so that incubation with a positive sample solution results in the binding of the sample DNA to the substrate surface (Fig. 1b). After binding, the surface-bound nanoparticle is detected by an optical readout (Fig. 1d). Based on the knowledge of the prepared pattern of capture DNA, the observed signals on the surface can be assigned to sequences. In the case of weak signals, resulting from a low density of surface-bound gold labels, an enhancement step using specific metal deposition can be applied (Fig. 1c). By using small areas (spots) with different capture molecules in a checkerboard pattern, the detection of molecular interactions can be highly paralleled, and the needed sample volume is minimized. The smaller volume results in a cost reduction in fields like high-throughput screening, and is advantageous in diagnostical applications. In the scheme in Fig. 1a, the left spot stands for capture DNA that is complementary to the gold-labeled DNA; the spot on the right exhibits a different sequence and therefore no binding (Fig. 1b). The result of a typical experiment is a substrate surface, where only a part of the squares is covered by labels (Fig. 1b-d).

##### *Optical readout*

Surfaces covered with colloidal gold particles exhibit a strong optical signal. In the case of higher particle densities and areas above the medium micrometer range they are visible by the naked eye (e.g. 50  $\mu\text{m}$  squares as demonstrated in [4]). The presence of the particles is detectable not only by their surface plasmon resonance in a dark-field microscopical setup [1], but also by simple transmission or reflection. Due to the metallic character of the particles, a significant reflection occurs already at small spots; as we could demonstrate with 4  $\mu\text{m}$  structures and a millisecond exposure time of a full-chip image [11]. These studies were made using an optical microscope, but the observed high contrast let us look for alternative low budget devices. This search was motivated by the potentially application of this detection scheme in molecular diagnostics, where a broad distribution of measurement devices would be helpful.



**Figure 2:** Comparison of different readout devices using a microstructured test sample with  $200\ \mu\text{m} \times 200\ \mu\text{m}$  squares structured in a  $100\ \text{nm}$  gold layer on a glass substrate. a) Optical microscope. b) Intel Play QX3 Microscope c) Flatbed scanner AGFA Duoscan T2500. d) Slide scanner Minolta Dimage Scan Dual II.

### 3.2. Alternative optical readout devices

#### *Requirements for readout device*

What are the requirements for such a device? If the contrast is sufficient, the next important point would be the resolution. Ongoing discussions in the microarray community point to at least 9 pixels ( $3 \times 3$ ) per spot as a minimum. Assuming a  $200\text{-}\mu\text{m}$  spot size as minimum, one pixel should image less than  $70\ \mu\text{m}$ . On the other hand, to image at least  $10 \times 10$  arrays in one exposure, the pixel number should be in the range of minimal 60 in both lateral directions. So this number could be fulfilled already by the simplest available webcam. However, one would need an optical adoption to project the chip onto the CCD at a 1:1 scale. This problem is already solved in optical scanners, which are (especially in their flatbed models) widely distributed. A resolution of  $70$  or better  $20\ \mu\text{m}$  per pixel is equivalent to 340 (1200) dpi, respectively, which is standard in today's low-cost models. Another point is the number of gray levels needed. Because the detection scheme is still in development, we are not yet at the limits of gray level resolution. For the start an 8-bit approach is probably a reasonable value, which should be checked in the course of further developments of the method.

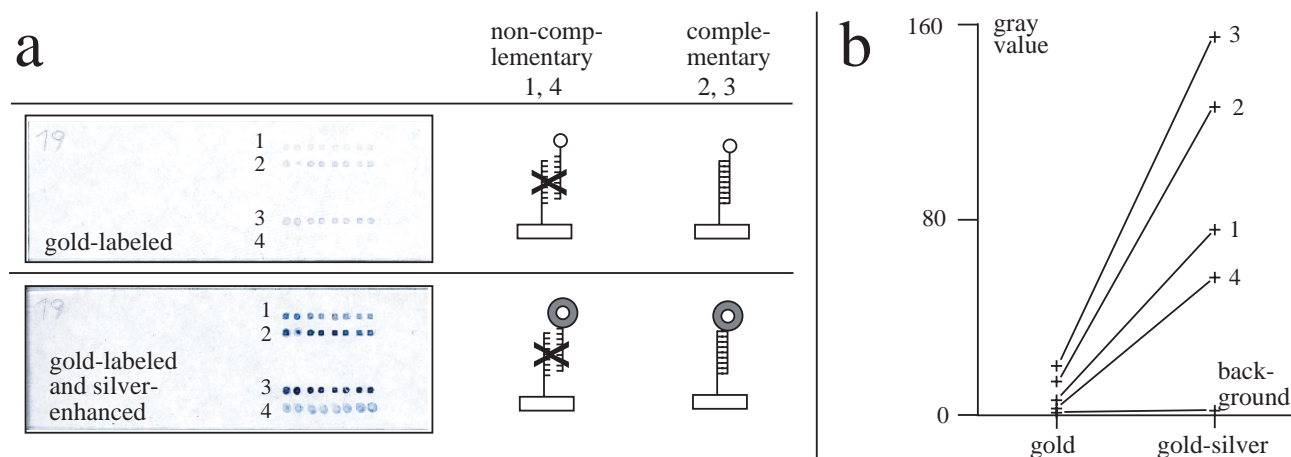
The first step toward testing of alternative devices was the preparation of a test sample, to have an identical and well-characterized test sample for comparison of different devices. Therefore, a test pattern was microstructured from sputtered gold using standard thin film technology. A checkerboard pattern of 100 squares of  $200 \times 200$  square micrometers structures was used. The size of the squares is comparable to typical sizes of DNA spots used for microarrays. This test pattern was visualized using an optical microscope in reflective mode (Fig. 2a). The overview (top) and zoom (bottom) confirmed the defined structure.

#### *Alternative devices*

The first device tested was an Intel Play QX3 Microscope, a simple microscope as USB-device. Fig. 2b shows two images of the test sample with this microscope. As evident in the overview (top), a homogeneous illumination is difficult to achieve with this device, so that an intensity gradient is observed over the imaged region. This is a knockout criterion, because the intensity values are crucial for a quantification of the spots. Another problem was related to the missing robustness of the sample holder, which made - especially at larger magnifications - focusing or controlled moving of the sample difficult. However, the resolution was sufficient. At the highest magnification of  $200 \times$  (Fig. 2b, bottom), a resolution of better than  $2\ \mu\text{m}$  per pixel was determined.

The next device tested was a flatbed scanner with transmission mode. Here the homogeneity of illumination is excellent, as demonstrated in the overview image in Fig. 2c (top). However, other problems occurred. Because the focus at scanners is often fixed, the alternative use has to ensure that the plane of interest is identical to the one at the typical scanning application. An example is given in Fig. 2c (bottom), where the individual spots are clearly out of focus. Beside this problem, the resolution of 2400 dpi is quite impressive by providing 20 pixels per 200  $\mu\text{m}$ . However, this high resolution results also in the highest price under the tested devices.

More specialized high-resolution devices are slide scanners. Here, the same resolution of 2400 dpi is standard, but they are available for a significantly lower price. The overview in Fig. 2d (top) and especially the zoom (bottom) demonstrates the excellence of the visualization. Again, 20x20 pixels are scanned per 200- $\mu\text{m}$  square, which gives a generous amount of data points for quantification. In a direct comparison with the flatbed scanner, the only disadvantage is the smaller throughput. Whereas the flatbed scanner can hold theoretically 28 full-sized slides, the slide scanner (with the filmstrip holder) keeps only 3 slides.



**Figure 3:** Slides with four rows of different capture DNA were incubated with gold-labeled DNA, which binds specifically to two rows (2, 3), but not to row 1 and 4. a) The slide imaged after gold-labeling. b) The same slide as in a) imaged after a silver enhancement step. c) Plot of the average gray values of the spots, determined before and after silver enhancement.

### 3.3. Experimental confirmation of readout scheme

#### *Optical detection of gold-labeled DNA*

A slide with four rows of immobilized capture DNA was incubated with gold-labeled DNA and imaged in the flatbed scanner (Fig. 3a, top). The four rows contain four different DNA sequences. Row 1 and 4 are non-complementary, so that no specific binding should occur. On the other side, row 2 and 3 contain complementary (with no and 3 mismatches respectively) sequences. In this case, binding of the labeled DNA is expected due to specific DNA-DNA interactions (hybridization). In the imaged slide, the rows 2 and 3 are weakly visible after gold labeling. So both rows correspond to the left spot in the scheme in Fig. 1b, and yield the expected picture (cf. scheme on the right). However, also the first row shows a barely visible signal, pointing to unspecific binding. But this signal is much lower, both in the scan and in the plot of the averaged gray values in Fig. 3b (left data points).

#### *Silver enhancement*

After applying the silver enhancement to the slide, it was scanned again (Fig. 3a, bottom). Now all four rows become visible, but in different intensities. As shown in the right scheme and also in Fig. 1c, the silver enhancement should result in a growth of the immobilized colloidal particles on the surface. Therefore, all surface-bound particles will be enhanced. This is the reason for the appearance of the fourth and the increase of the first row, where a low amount of unspecifically-bound nanoparticles became visible due to the enhancement. On the other hand, the enhancement makes the two central rows with the specific binding clearly visible. As demonstrated in the gray value plot in Fig. 3b, the

order of the four rows is kept during the enhancement, but the differences are now more pronounced. An interesting observation is the low background introduced by the silver enhancement. It is hardly visible in the scanned slide (Fig. 3a, bottom), and also barely expressed in the averaged gray value (plot in Fig. 3b). This is an important fact, because it points to a high specificity of the enhancement reaction. So the enhancement can be applied to extend the dynamic range of the gold-labeling method by visualizing levels of gold, which are not visible before amplification. Another interesting point is the geometry of the spots. By defining windows for immobilization of the capture DNA, the surface-bound DNA is concentrated in a squared area, which is helpful for evaluation of the results. So the area of specific binding is fixed, as well as the geometry. Another advantage is the prevention of cross talk based on intermixing liquid droplets from neighboring spots, which can be important in the course of further minimization of the structures.

#### 4. CONCLUSIONS

The paper describes the scheme of a simple and low-tech approach for detecting molecular interactions, especially DNA-DNA interactions, on substrate surfaces. The described and tested alternative devices demonstrate the potential of equipment not yet used for DNA-chip detection for applications in combination with the nanoparticle labels. Although the silver enhancement extends the dynamic range of the method, the used readout was able to detect even the gold-labeled DNA. The microstructuring of the spots provides optimal conditions for an optical evaluation by defining their geometry.

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