

# Fabrication of microsensors using unmodified office inkjet printers

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## Abstract

In this paper, a simple microfabrication technique for delivering biological macromolecules and patterning microelectrode arrays using desktop inkjet printers is described. In particular, we have demonstrated that: (1) phospholipid films can be patterned into pre-designed features (dots, lines and grids) with a resolution down to 50  $\mu\text{m}$ ; (2) miniaturized electrode sets can be prepared via “printing” an alkanethiolate self-assembled monolayer on a gold CD-R and subsequent wet chemical etching; (3) multiplex chemical sensors (e.g., for iodine vapor) can be fabricated using a multi-step inkjet printing, selective etching and polymer deposition protocol. It is remarkable that the entire fabrication process has been carried out in a regular wet laboratory setting, for which no hardware or software modifications to the commercial printer are required and regular CD-Rs/glass slides are used as substrate materials. This technique outlines a generic, “amateur”-friendly approach for the rapid and economical construction of disposable multiplex microsensors and portable electroanalytical units.

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## 1. Introduction

Abundance in electronic and computer hardware is fueling interdisciplinary research nowadays. Particularly, research in chemical and biomedical sensor development toward health science and disease diagnostics is effectively exploiting these opportunities. One example of such novel research reported in the literature is the utilization of one of the most popular media for storing and retrieving digital information, recordable compact discs (CD-Rs) for modern materials and analytical chemistry [1,2]. CD-Rs with the reflective layer made of pure gold are known for their exceptional environmental stability and long storage life expectancy. It has been discovered that the same sputtered gold layer can serve as an inexpensive and convenient substrate for the fabrication of self-assembled monolayers (SAMs) [3]. Angnes et al. pioneered the application of gold electrodes prepared from CD-Rs (so-called “CDtrodes”), and compared their performance with that of commercial ones [4]. They also used them to construct flow cells for electrochemical quantification of mercury in natural waters [4,5]. Similar

devices were developed from these planar CD-R gold electrodes by Westbroek et al. for the amperometric determination of Ce(IV) [6]. In addition, CD-Rs have been used as pre-patterned substrates for the preparation of micro/nanostructures [7–9]. For example, zirconia thin films with tunable topography can be deposited electrochemically on gold CD-R surfaces [7]; the polycarbonate CD-R base can be taken as polymeric stamps to fabricate metal nanostructures [8], or molded to produce microfluidic devices [9]. Based on the CD-R reading mechanism, La Clair and Burkart reported that the binding of proteins to different ligands attached to a CD surface may be detected by a conventional optical drive [10]. A surface plasmon resonance sensor array based upon the grating substrate (made from gold CD-Rs) has been recently developed by Singh and Hillier for the detection of biomolecular interactions [11].

Several methods (either lithographic or non-lithographic) have been reported in the past decade for patterning gold films supported on solid substrates, with potential applications including fabrication of electrode sets or microelectrode arrays [12]. Notably, one of these techniques uses micro-contact printing ( $\mu\text{CP}$ ), a stamping technique that takes solutions of alkanethiols as “ink” to form SAMs on gold with designed features [13–18]. These closely packed organic monolayers then act as etching resist (mask) to protect the gold film against aqueous etchants

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while unprotected gold regions are dissolved to produce the desired microstructures [18]. Elastometric stamps can be produced via standard lithographical protocols, after initial preparation of a master template or mold [13]. Once made, the latter is difficult to modify. Recently, Daniel and Gutz have produced sub-millimetric tailored gold electrode sets via laser printing the electrode design on wax paper (then heat-transferring it onto a gold CD-R) and subsequent wet chemical etching [19,20]. Gold microelectrodes (in single or twin sets) for amperometric analysis of a mixture of iodide, ascorbic acid and uric acid in capillary electrophoresis (CE) have also been constructed by this method [21]. This technique is much simpler than the traditional protocols to prepare elastometric stamps.

Inkjet printing and drop-on-demand (DOD) technology are now popular not only for printing graphics and photographs, but also for other applications that require deposition of micro/nanoliter volumes of functional materials into desired locations. Inkjet printing technology has been effectively employed in building microstructures, such as microlens and ceramic pillar arrays [22,23], manufacturing of polymer/organic light-emitting diodes (PLEDs and OLEDs) [24], multicolor flat-panel displays [25], flexible displays [26] and flexible thin-film transistors (TFTs) [27,28]. Inkjet printing technology has been also explored for delivering materials with specific chemical and biological functionalities. Kido et al. have proposed to construct circular indexed protein microarrays with an inkjet printer on polycarbonate CDs in 2000, however, experimental exploration has not yet followed [29]. Bietsch et al. have deposited alkanethiolate monolayers using an inkjet dispenser with a three-axis micropositioning system on gold surfaces and compared their resist qualities with those of  $\mu$ CP SAMs after selective wet-etching [30]. Pardo et al. have also created alkanethiolate SAMs and protein arrays on gold substrates with specially designed inkjet printers [31]. Recent work by Watanabe demonstrated the fabrication of microfluidic channels using an inkjet printer [32].

In this paper, we describe a rapid and reliable method to deliver nanoliter volumes of biomaterials and to pattern microstructures by direct inkjet printing with a mass-produced commercial desktop printer without any modification to either the hardware or software driver. To illustrate the practical applications of this novel technique, a disposable, multiplex iodine vapor sensor based on the conductivity changes of phospholipid films printed on top of gold microelectrode arrays (prepared from gold CD-Rs) was tested. Stemming from our continued interest in the development of CD-based biosensing technology [1,2], this work explores an inkjet method for rapidly prototyping complex structures and devices, which does not require access to conventional clean-room and photolithographic facilities.

## 2. Experimental

### 2.1. Reagents

1-Octadecanethiol (98%), potassium thiosulfate ( $K_2S_2O_3$ ), potassium ferricyanide ( $K_3Fe(CN)_6$ ), potassium ferrocyanide

(II) ( $K_4Fe(CN)_6$ ), diethylene glycol, poly(3,4-ethylenedioxythiophene) (PEDOT) and poly(styrenesulfonate) (PSS) were purchased from Aldrich Chemical Co. (Milwaukee, WI); 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) from Avanti Polar Lipids (Alabaster, AL). Deionized water ( $>18.3 M\Omega\text{ cm}$ ) was from a Barnstead EasyPure UV/UF compact water system (Dubuque, IA). All other chemicals are of ACS reagent-grade or higher quality and used without further purification.

### 2.2. Instrumentation and procedures

Mitsui 700MB Gold CD-Rs (Q-MAM-CD700LB) were obtained in bundles locally. To prepare gold substrates from CD-Rs for printing and patterning, first the protective film must be removed. This is accomplished by immersing the entire CD-R (or pieces that were cut out with a pair of scissors) in concentrated  $HNO_3$  [3]. After approximately 2 min of immersion, the protective film swells. It can be cleanly removed by dipping the disc in and out of  $HNO_3$  repeatedly. This way the separated protective film gently slides away from the gold surface without other mechanical assistance leaving the gold surface clean and scratch-free. The CD-R is then rinsed with anhydrous ethanol and dried with  $N_2$ . Fig. 1(A) shows a gold CD-R after removal of the protective polymer film.

An Epson Stylus Photo R200 Inkjet Printer with refillable inkjet printer cartridges (CIS Print For Less, Cumberland, MD)

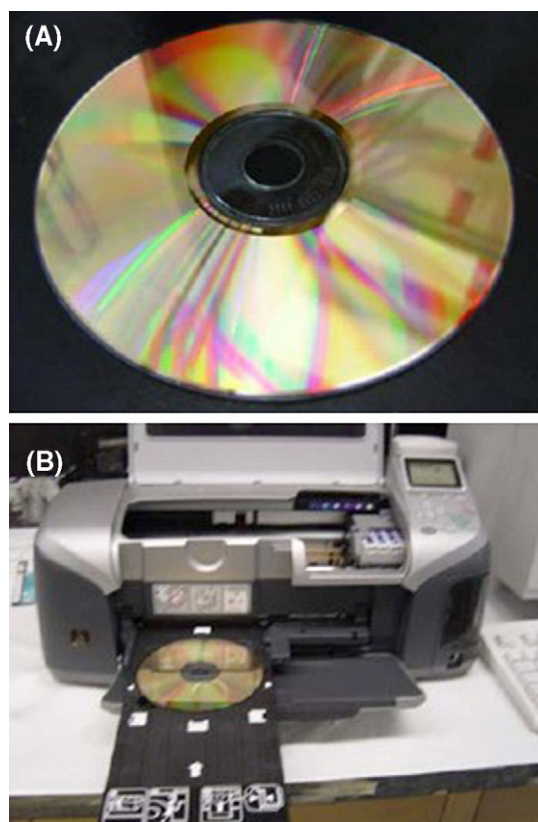


Fig. 1. Optical images of gold CD-Rs and the inkjet-printing process. (A) Gold CD-R after removing the protective polymer film. (B) Epson Stylus Photo R200 inkjet printer with a gold CD-R on the front-loading tray.

was used for printing the phospholipid films and alkanethiol solutions. After removing the polymer film, a gold CD was loaded into the front-loading tray for printing (Fig. 1(B)). We then used a regular PC to control the printing of specially prepared “inks” on the CD-R surface. After each printing step, the pattern was inspected through an optical microscope with an 80× objective lens for magnification. Phospholipids and 1-octadecanethiol solutions were made at different concentrations in 2-propanol. These “ink” solutions were pumped into the refillable inkjet cartridges with a gas-tight syringe. Excess ink was wiped off using an absorbent cloth before the cartridge was installed into the printer.

Once the pattern of 1-octadecanethiol solutions was printed on the gold CD-R surface with desired features, the CD-R was allowed to dry for at least 20 min. To etch the unprotected gold, the printed CD-R was gently immersed in an aqueous 0.1 M  $K_2S_2O_8$ /1 M KOH/0.01 M  $K_3Fe(CN)_6$ /0.001 M  $K_4Fe(CN)_6$  solution for 10–15 min [17]. After the etching step, the alkanethiolate monolayer may be removed by UV exposure (254 nm UV radiation for 60 min). The substrate with patterned gold was subsequently washed with ethanol and dried under  $N_2$  before the electrical measurements or the device fabrication.

We constructed a prototype chemical vapor sensor by printing a phospholipid film on the CD-R with patterned gold electrodes (see Section 3 for design details). After soldering electrical wires to the connection pads on the CD-R substrate, the entire unit was placed inside an enclosable Petri dish with a port-hole through which gas/vapor can be injected. The electrical wire connections were led out and the edges of the dish were sealed with a silicone sealant. Electrical measurements were performed with an Autolab electrochemical analyzer (Model: PGSTAT30, Eco Chemie B.V., Utrecht, The Netherlands) or a HP 3478A digital multimeter in a range from  $-5.0$  to  $+5.0$  V at a scan rate of 0.1 V/s.

### 3. Results and discussion

#### 3.1. Choice of printer and preparation of “inks”

Inkjet printing has been explored extensively [22–31] as a versatile research tool in materials and bioanalytical chemistry. However, such experiments usually require either specialized inkjet dispensing devices (which are often very expensive) or substantial modifications of commercial printers (which are tedious and challenging). We chose Epson Stylus Photo R200 (Fig. 1(B)), an inexpensive and mass produced office printer, based on its following special features [33]: (1) the attached CD-print tray and labeling software make the creation of desired complex features directly on the surface of CDs and DVDs quick and easy; no modifications to either the printer hardware or the software driver were needed. As shown in Fig. 1(B), a CD-R can be positioned accurately for printing, enabling repeated delivery of different solutions at the same position ( $\pm 0.5$  mm). We prepared blank CDs with cutouts (3 in.  $\times$  1 in.) that hold a standard microscope glass slide, which permit the printing on glass or other solid substrates. (2) This model can be operated with

commercially available refillable ink cartridges. Their simple internal structures make them easy to clean and reuse, which are very suitable for multiplex, multistep printing experiments with different types of solutions.

The piezoelectric print head of Epson printers provides excellent inkjet delivery with very precise volume dispensation and high-resolution print quality. However, it is made of plastic and therefore easily damaged by organic solvents. We prepared most of our ink solutions in water or alcohols, mixed on rare occasions (e.g., to dissolve water-insoluble polymers) with THF or chloroform. 2-Propanol was chosen as solvent for 1-octadecanethiol and phospholipids (DPPC) because its high viscosity closely matches that of normal inkjet printer inks, thereby avoiding potential clog of the print heads.

It should be noted that the Epson Stylus Photo R200 is not the only workable printer, as the later, upgraded versions (R220, R300 and R800) indeed offer equivalent or better printing results when they were tested. Other manufactures (Canon and Primera, for example) also carry similar products, i.e., inexpensive desktop printers with a CD-print tray. The advances in the printer manufacture industry guarantees that the technology described herein is not short-lived.

#### 3.2. Printing of phospholipid films

Phospholipids are the main components of cellular membranes (glyceryl esters) and consist of hydrophilic polar phosphate head groups and two hydrophobic carboxy-terminated alkyl chains. Because of their ability to form bilayers, micelles and liposomes in aqueous solution, they often serve as models for the study of membrane interactions [34,35] and as drug carriers [36,37]. Herein, we use them to demonstrate that microstructures of biological macromolecules (lipids, proteins and DNA), which are of technological importance in the fabrication of microarray devices, can be prepared with standard inkjet printers.

In order to examine the resolution and uniformity of inkjet-printed phospholipid films, we have designed different types of structures including dots, lines and grids with dimensions from 5 to 200  $\mu\text{m}$ ; the phospholipid concentrations were examined from 1.0 to 5.0 mM. Pictures of the designs were first drawn in image authoring software and then imported into the CD labeling program. The clear borders and the contrast between the film-covered and bare gold areas (Fig. 2) indicate that patterns with dimensions of 100  $\mu\text{m}$  are quite satisfactory. There was no clear dependence on the concentration; however, we noticed that when the dimension is below 50  $\mu\text{m}$  (higher resolution), the patterns were uneven and considerably deformed (particularly for dots). Such a resolution (50  $\mu\text{m}$ ) is lower than the optimized resolution claimed by the manufacturer (5760 dpi  $\times$  1440 dpi corresponding to 17.6  $\mu\text{m}$  laterally). We believe that this is due to spreading of the phospholipid solutions on the hydrophilic gold CD-R surface. More importantly, we found that the printing carried out with this printer is not single-drop (3 pL) at a time, rather multiple drops are “fired” even when a single pixel dot is specified. A close look at the printed phospholipid films (Fig. 2(D)) shows vesicle-like features, which may be the result of discrete individual drops. The resolution and

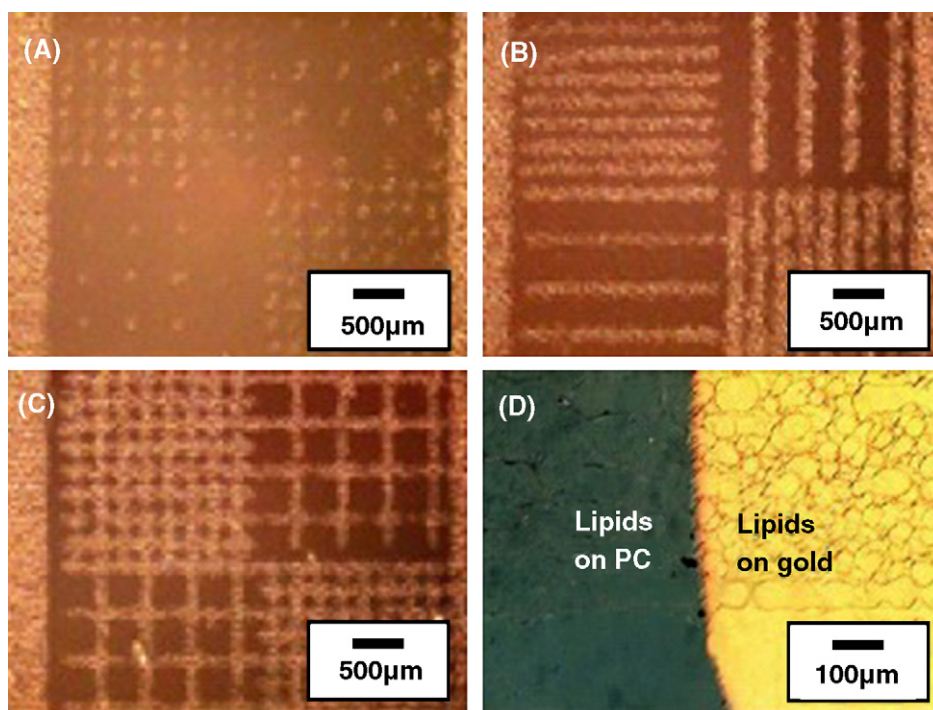


Fig. 2. Inkjet printing of phospholipids on a CD-R. (A)  $100\ \mu\text{m} \times 100\ \mu\text{m}$  dots, (B)  $100\text{-}\mu\text{m}$  wide lines, (C) grids made from  $100\text{-}\mu\text{m}$  wide lines on gold. (D) Morphology of phospholipid film printed on gold and polycarbonate (PC) surfaces.

quality of the printed microstructures could be improved by fine-tuning the technology and upgrading to state-of-the-art printers.

### 3.3. Patterning microstructures of gold and electrical testing

As mentioned above, fabrication of gold microstructures is the essential goal of preparing gold microelectrode arrays for potential applications in electroanalytical chemistry. This can be easily achieved by inkjet printing alkanethiol solutions on gold CD-Rs to form a resist layer and subsequent etching in aqueous gold etchant. To demonstrate the capability of inkjet patterning, we fabricated centimeter-long gold wires that are  $100\ \mu\text{m}$  wide and loop every  $8\ \text{mm}$ , with  $300\ \mu\text{m}$  spacing between adjacent parallel sections (Fig. 3(A)). Other electrode array geometries can be easily designed with graphics software. Besides the much simpler preparation protocol than previously reported methods [12–21], the resolution achievable with inkjet patterning is considerably better than that obtained by heat transfer of laser-printed toner masks [19–21].

We also carefully studied concentration effects of alkanethiol solutions ( $100\ \mu\text{M}$  to  $2\ \text{mM}$ ) on the formation of gold patterns and found  $0.5\ \text{mM}$  1-octadecanethiol in 2-propanol to be most suitable. Higher concentrations resulted in bulky patterns with poor edge resolution, lower concentrations in non-uniform and low-quality monolayers that were insufficient to protect the gold film effectively during the etching step. Ten to 15 min etching time was sufficient to achieve the ideal pattern resolution; how-

ever, this will vary with the concentration of the etchant as well as the thickness of the gold film on the substrate.

Since the patterned gold electrodes are eventually to be used for conductometric sensor applications or electroanalytical chemistry, it is imperative that they exhibit appropriate electrical characteristics. We recorded the resistance of each serpentine gold wire pattern as a function of wire length with a pair of microelectronic probe tips (inset of Fig. 3(A)). The linear response (Fig. 3(B)) shows that the patterned gold wires are purely ohmic and ideally suited for conduction measurements. The slopes of the three wires of different widths vary as expected; using the literature value ( $2.2 \pm 0.2 \times 10^{-8}\ \Omega\ \text{m}$ ) for gold electrical resistivity [38], the calculated thickness of the gold film is  $10.8 \pm 1.5\ \text{nm}$ . This is of the same order of magnitude, but much thinner than that generally reported ( $50\ \text{nm}$ ) [1–3]. The difference is assumed to be due to the chemical etching and possibly also to temperature/dimension effects to the gold conductivity. The suitability of the CD-R gold substrates (without patterning) for preparation of high-quality SAMs, electrochemical characterization and surface spectroscopic studies has been reported previously [3,7].

### 3.4. Construction and testing of prototype iodine vapor sensor

Recent studies have shown that phospholipids can be employed as sensing entities for detecting molecules, such as glucose [39,40], xanthine [41], water [42] and various ionic compounds [43,44]. In particular, several groups reported a dramatic increase of the conductivity of phospholipid films upon exposure

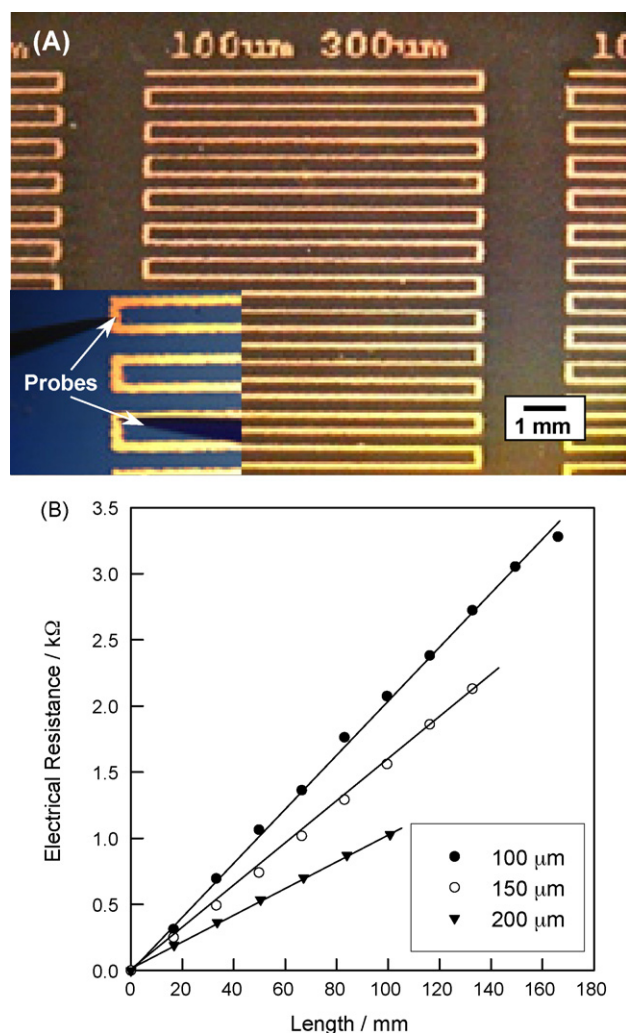


Fig. 3. (A) Serpentine gold wires fabricated by inkjet printing alkanethiolate monolayers on a gold CD-R and subsequent chemical etching. The wires are 100  $\mu\text{m}$  wide, and the loops (8 mm long per turn) are 300  $\mu\text{m}$  apart. The inset shows two probe electrodes. (B) Plot of electrical resistance vs. wire length and width.

to iodine vapor [45–47]. To demonstrate the potential applications of inkjet patterning with an office printer, we therefore decided to build a CD-R based, prototype iodine sensor by the following three steps: design and patterning of gold electrode arrays; printing phospholipid films across the microelectrodes; sealing and testing the device in a closed chamber.

Starting with a simple four-probe arrangement of the gold electrodes, the results were not satisfactory because of the low conductivity of phospholipid films [45], particularly when only thin films were inkjet printed. Using free software called GIMP (<http://www.gimp.org>) we designed a new pattern, covering the entire surface of a gold CD-R with 84 sets of interdigital gold microelectrodes configured in parallel (Fig. 4(A)). Each set consists of four pairs of 200- $\mu\text{m}$  wide gold finger electrodes insulated from the connect pads at both ends. This configuration prevents potential breakdowns when any two electrodes are cross-linked due to unsatisfactory etching. The electrical connections are made by depositing conducting polymer on the connection pad (blue-colored area in the inset of Fig. 4(A)), and

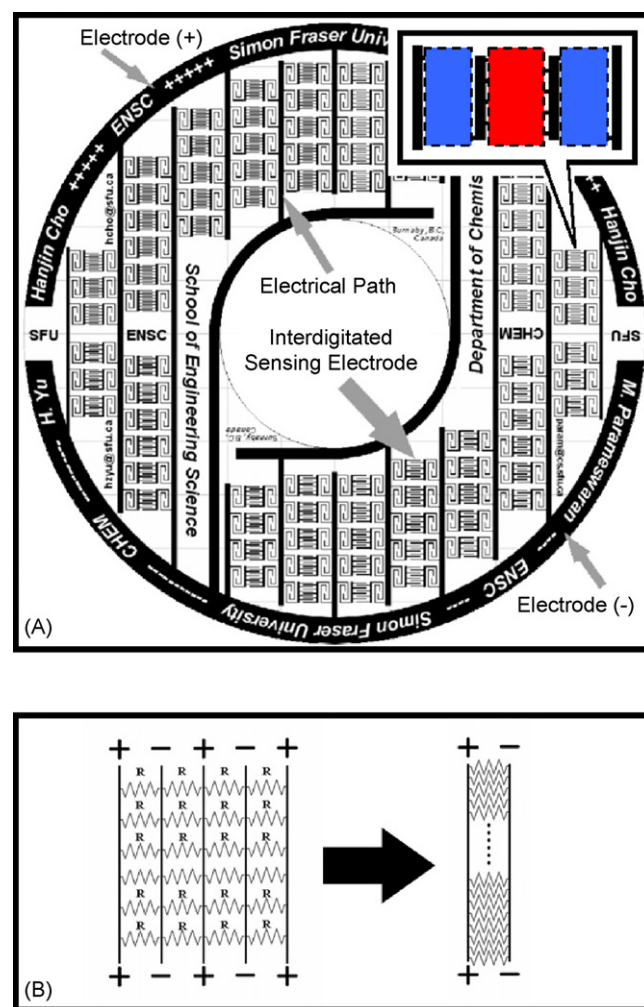


Fig. 4. (A) Design of a multiplex electrical sensor consisting of 84 sets of interdigital microelectrodes on a gold CD-R. The inset shows an enlarged view of a set of microelectrodes (pink area will be coated with lipid film, blue areas with conducting polymer). (B) Equivalent electrical circuit (schematic) of the sensor design shown in (A). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

a thin film of phospholipids is printed on top of the interdigitated electrodes to serve as the sensing entity.

This arrangement (Fig. 4(A)) results in a higher signal-to-noise ratio of the final sensing unit [48]. As all 84 sensing units were parallel connected (Fig. 4(B)), the resistance  $R_{\text{lipid}}$  of the phospholipid film covering each pair of the interdigital electrodes can be calculated from the general formulas [49]:

$$\frac{1}{R_{\text{total}}} = \frac{1}{R_1} + \frac{1}{R_2} + \dots + \frac{1}{R_{84}} = \frac{n}{R_{\text{lipid}}} \quad (1)$$

This model assumes negligible capacitance and inductance, which is generally acceptable for DC measurements [48].

It is remarkable that the design shown in Fig. 4(A) can be successfully patterned onto a gold CD-R, i.e., via inkjet printing of an alkanethiolate monolayer and subsequent chemical etching. Fig. 5(A) shows thus prepared multiplex sensor electrode arrays on an entire CD-R. Before printing, the phospholipid and depositing conducting polymer films, we tested the insulating

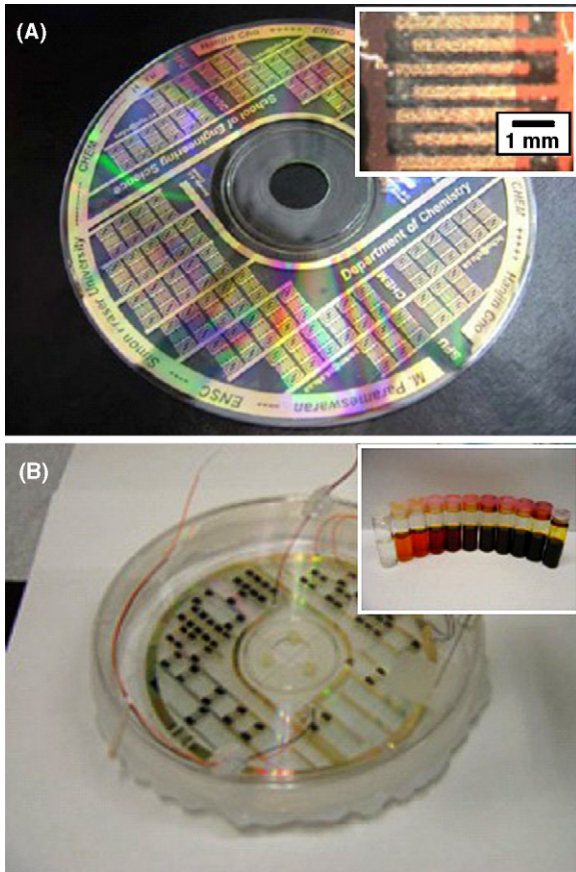


Fig. 5. (A) Multiplex electrical sensor (for the detection of iodine vapor) constructed on a gold CD-R. The inset shows a patch of phospholipids (yellow) inkjet-printed on one set of the interdigitated sensing electrodes. (B) Sealed chamber (Petri dish) enclosing the sensor into which the iodine solution is injected. The “black dots” on the CD are the conducting polymer films deposited at both ends of the interdigitated electrodes for making the electrical connections. The inset shows iodine solutions at 10 different concentration levels. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

properties of each set of the interdigital electrodes by monitoring the electrical resistance between the two ends and found it to be much greater than  $30\text{ M}\Omega$  in most cases. Ideally, it should be infinity: some electrically conducting impurities in the polycarbonate substrate and/or imperfect patterning may be responsible for the observed residual conduction. A few sets exhibited a lower resistance and were discarded.

After obtaining the electrode structure and confirming the electrical characteristics and integrity of the electrodes, phospholipid films were printed on top of the interdigitated area by the same inkjet printing technique (inset of Fig. 5(A)). Conducting polymer films were deposited using a micropipette onto the contact pads at both ends of the interdigital electrodes; this also can be done by repeated printing of the polymer solution. Fig. 5(B) shows the prototype multiplex sensor for  $\text{I}_2$  vapor that was carefully sealed in a Petri dish. The inset shows testing solutions of  $\text{I}_2$  at 10 different concentrations; the electrical tests were performed after a small amount of each solution ( $5\ \mu\text{L}$ ) was injected into the device through the port-hole. The iodine solutions were prepared in diethylene glycol ( $\text{C}_4\text{H}_{10}\text{O}_3$ ) as this

solvent is inert to phospholipid films and does not produce an electrical response in the sensor unit. The iodine absorbed from the vapor phase into the phospholipid film will, however, produce an enhanced electrical response [46].

Fig. 6 shows the sensor’s current–voltage response to different concentrations of iodine vapor. The curves are generally linear in the voltage range  $-2.0$  to  $+2.0\text{ V}$ , indicating ohmic conduction behavior in phospholipid films (Fig. 6(A)). This supports the assumption (Eq. (1)) that only resistance needs to be considered. Measurement of the slopes of the current–voltage curves enabled us to evaluate the correlation between the conductance of the device and the iodine concentration. The conductivity change of this sensor device is significant (Fig. 6(B)) when the

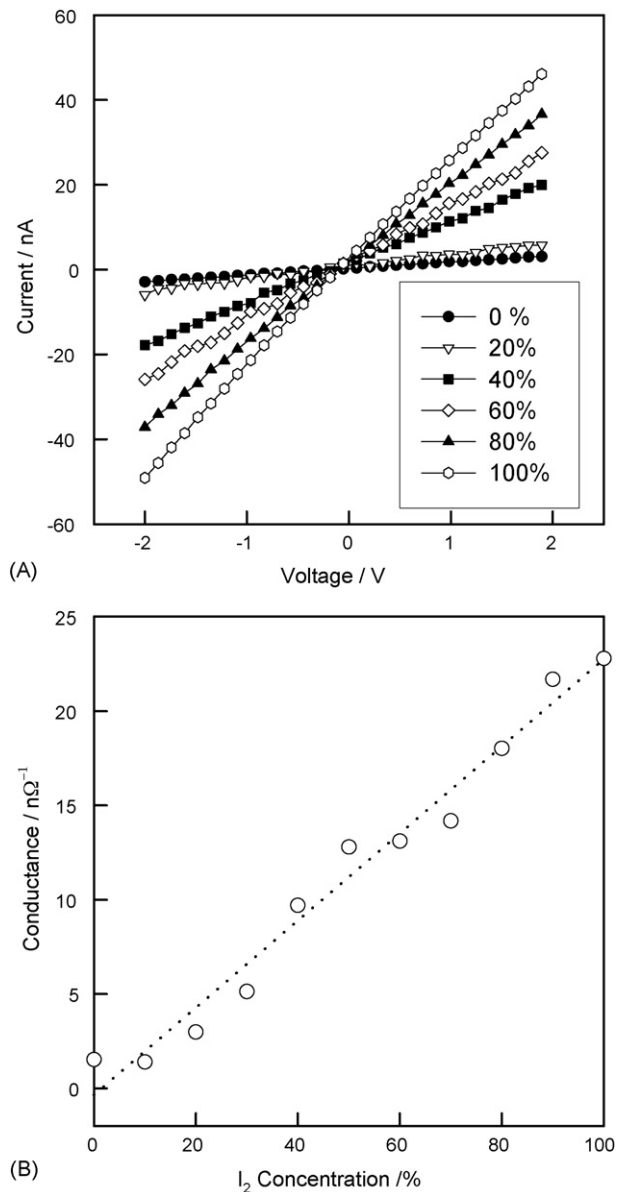


Fig. 6. (A) Current–voltage curves of the multiplex sensing CD when iodine solutions of different concentrations are added. (B) Plot of electrical conductance vs. iodine concentration (100% = saturated solution in diethylene glycol, for which the corresponding iodine vapor pressure in the detection chamber is  $10.1\text{ Pa}$ ).

concentration of  $I_2$  increases from infinite dilution to saturation (this, in turn, corresponds to an increase in the vapor pressure of  $I_2$  in the chamber). The simplest explanation of the conductivity change in phospholipid films is that  $I_2$  or  $I_3^-$  accumulates in the head-head group region of the lipid bilayer [45–47]. This interpretation deserves further investigation, as it is critical for optimizing the sensitivity of the prototype  $I_2$  sensor.

#### 4. Conclusion

We have demonstrated a simple and quick technique for patterning biomaterials and microelectrode arrays by an office inkjet printer without any modification to either the hardware or software. Phospholipids can be deposited into different features (locations) on a gold CD-R with a lateral resolution of 50  $\mu\text{m}$  and with unprecedented flexibility. The gold film on a commercially available CD-R can be patterned into multiplex electrode sets or microarrays by printing alkanethiol solutions (to form SAMs as etching-resist layer) followed with a wet chemical etching protocol. We have also demonstrated the fabrication and testing of a CD-based prototype iodine vapor sensor by using phospholipid films on top of interdigitated electrodes as sensing entities. The inkjet printing technique along with the gold CD-R patterning technique lends itself to rapid fabrication of sensing devices for research, and it can be effectively used for commercial production of low-cost biosensors and electroanalytical units. In contrast to photolithographic techniques for microfabrication, this inkjet printing protocol does not require any expensive instrumentation and/or clean rooms.

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