Biomolecular deposition on multiple field-emitter tips

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A deposition fixture is described for placing proteins and nucleic acids onto field-emitter tips from aqueous solution. The fixture allows the coverage of these species on each of four tips to be independently selected by an appropriate choice of deposition parameters. Common rinsing volumes can be used without cross contamination of the tips. Four tips can be dosed in 20 min, the time previously taken to cover one tip with biological molecules. The fixture makes molecular deposition from aqueous solution both simple and routine.

INTRODUCTION

In a recent paper we described a procedure for placing proteins and nucleic acids on a single field-emitter tip by depositing them from aqueous solution. This study was motivated by our interest in the binding properties of biomolecules on metal and semiconductor substrates, and by our desire to image these species with field-ion techniques. In our procedure, a single tip was sequentially placed into deposition, fixing, and rinsing volumes. If more than one tip was being prepared, the entire deposition process had to be repeated. Under optimum conditions, three tips could be dosed in 1 h.

This note describes a deposition fixture that provides the capability of independently dosing each of four tips with biological molecules at the same time. Four different molecules, at different concentrations in solution, can be deposited simply and efficiently in tandem. The fixture is designed to be used with disposable plastic beakers that contain the required rinsing solutions. Tip placement within the rinsing solution is predetermined by the fixture which is supported by the beaker's rim. The fixture makes molecular deposition from aqueous solution both simple and routine.

I. APPARATUS

Figure 1 is a schematic drawing of the deposition fixture. A clear plastic cup was fabricated from Plexiglas and threaded to accept four (#10-32) stainless-steel screws. The end of each screw was drilled and then slotted in order to hold a single field-emitter tip. The screw assembly is used to position a tip at the center of a small coil of tungsten wire. Each coil retains a 10-μl droplet of liquid throughout the deposition procedure. This ensures that the apex of a tip, centered within a coil, will always be immersed in liquid as the fixture is transferred from one rinsing solution to another. The tip apex must remain wet until the entire deposition protocol has been completed if deposition artifacts are to be minimized. Figure 2(a) shows a screw assembly being inserted into the deposition fixture.

II. DEPOSITION PROTOCOL

The protocol developed for placing proteins and nucleic acids onto field-emitter tips has been described previously. The main features of the protein deposition protocol (modified for multiple tip deposition) are as follows:

1. Molecules are deposited on each tip from a 10-μl droplet of buffer that contains the molecule of interest.
The tips are rinsed for 5 min in 10 ml of buffer.

Molecules that remain on each tip are fixed by placing the tips in gluteraldehyde (0.6% in buffer) for 5 min.

The tips are rinsed for 1 min in 10 ml of fresh, double (glass) distilled water.

The tips are rinsed for 1 min in 10 ml of a 20% mixture of double (glass) distilled ethanol in double distilled water.

The tips are rinsed for 1 min in 10 ml of a 50% mixture of double distilled ethanol in double distilled water.

The tips are rinsed for 1 min in 10 ml of a 70% mixture of double distilled ethanol in double distilled water.

The tips are rinsed for 1 min in 10 ml of 100% double distilled ethanol, removed into air, and dried.

III. DEPOSITION PROCEDURE

Four, 10-μl droplets of buffer containing the molecule of interest are placed on a sheet of Parafilm. The four droplets are spaced on 1.1-mm centers that coincide with the positions of the four tips retained in the deposition fixture. To begin the deposition, the plastic cup is positioned above the four droplets of solution as shown in Fig. 2(b). As the cup is lowered and placed on the Parafilm, the wire loops that surround the tips will touch the droplets (but not the Parafilm). Surface tension forces will draw the droplets onto the loops, enclosing each tip within a well-defined deposition volume. After the droplets are captivated on the wire loops, the cup can be removed from the Parafilm as shown in Fig. 2(c), and placed on an empty plastic beaker until the 2-min deposition period has ended. The beaker prevents any particulate matter in the air from reaching the droplets.

Deposition is stopped by rinsing the tips in buffer. This is accomplished by placing the cup on the rim of a beaker filled with 10 ml of buffer as shown in Fig. 2(d). As the cup is placed on the beaker, the wire loops enter the buffer, and the deposition solution that they contain is quickly dispersed throughout the buffer volume. Equilibrium is rapidly achieved (typically taking 30–60 s) as visually judged from a control experiment in which four, 10-μl droplets of dye replaced the usual droplets of deposition solution. Deposition stops because the concentration of molecules in the vicinity of a tip is reduced by a factor of about 1000, the ratio of the volume captured by a wire loop (10 μl) to the volume of buffer in the beaker (10 000 μl). If the concentration of molecules in the original droplet is several milligrams per milliliter, two rinsing volumes are used. The object is to quickly reduce the concentration of molecules in solution to less than 1 μg/ml so that cross contamination of the tips will not occur during the final rinse.

Fixing (step 3 of the deposition protocol) is essentially a repeat of the initial deposition procedure. Four, 10-μl droplets of a 1.2% solution of gluteraldehyde in buffer are placed on a clean piece of Parafilm. As the cup is lowered onto the parafilm, the 10-μl droplet of buffer that remains on each wire loop from step 2 of the protocol will combine with each 10-μl droplet of gluteraldehyde to form a 20-μl droplet at the required fixing concentration of 0.6%. The cup is usually left in position, on the Parafilm sheet, during fixing.

In order to facilitate rinsing we designed a covered plastic turntable to hold the rinsing volumes. Figure 3 shows the

Fig. 3. A plastic turntable (with clear plastic dust cover) used to facilitate tip rinsing. A deposition fixture is shown in place on a plastic beaker that contains a rinsing volume. The timer placed on the dust cover records the rinsing time.

Fig. 4. TEM images of four tips dosed in tandem using the deposition fixture and the protocol described in the text. (a) 28 μg/ml of hemocyanin (from busycan canalicularum for 2 min). (b) 55 μg/ml of the same protein for 2 min. (c) 100 μg/ml of (6 x crystallized) horse spleen ferritin for 2 min. (d) Control tip. Deposition from pure buffer for 2 min.
deposition fixture placed on one of the plastic beakers that contain a rinsing volume. At the end of each step in the rinsing protocol the fixture is lifted through the slotted dust cover, a new rinsing volume is rotated into position, and the fixture is lowered onto the new plastic beaker. The timer shown in Fig. 3 on the plastic dust cover is used to time each step in the rinsing protocol.

After rinsing, the tips are removed from the deposition fixture and dried in air. If the tips are to be imaged in the transmission electron microscope (TEM), they are first rotary shadowed in high vacuum with a thin layer of tungsten in order to improve image contrast. 8

Figure 4 shows the result of using the deposition protocol and the deposition fixture described in the text. Four tungsten tips were dosed simultaneously. Two tips [shown in Figs. 4(a) and 4(b)] were dosed using different concentrations of hemocyanin, a respiratory protein found in the hemolymph of certain molluses and arthropods. 9 One tip [shown in Fig. 4(c)] was dosed with a saturation coverage of horse spleen ferritin, a respiratory protein found in the liver and spleen of many mammals, including man. 10 The remaining tip [shown in Fig. 4(d)] was dosed in pure buffer and acted as a control. The tips were rotary shadowed with tungsten prior to TEM imaging.

The clean surface of the control tip, and the appearance of the other TEM images indicate that cross contamination of the tips during tandem rinsing is negligible. The large feature near the center of Fig. 4(c) is probably a cluster of ferritin molecules, deposited from solution during the first step of deposition protocol. 8 The amount of ferritin seen in Fig. 4(c) is characteristic of a saturation coverage of ferritin on tungsten. The coverage is in qualitative agreement with the coverage observed in the TEM for a ferritin monolayer on Lexan. 11

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5 A typical buffer for protein deposition consists of 50 mM HEPES, 150 mM NaCl, pH 7.5 [see Ref. 1]. The coverage of molecules on the tip surface is determined by their concentration in solution and the deposition time. We find it convenient to adjust the concentration and keep the deposition time fixed at 2 min.

6 This step of the protocol is eliminated if the molecules are fixed in solution prior to step 1 of the protocol.

7 The American Can Company (Dixie/Marathon), Greenwich, CT.


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