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Electrical detection of single pollen allergen particles using electrode-embedded microchannels

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Abstract

We report on the development of a microfluidic system for the electrical detection of single pollen allergen particles. Our device consists of 500 nm electrode gaps fabricated in an 800 nm wide fluidic channel. We flowed pollen allergen particles of average size 330 nm along the channel via fluid pumping and simultaneously monitored temporal change in dc current flowing through the sensing electrodes. Current spikes were detected, which can be attributed to a capacitance discharging upon trapping/detrapping of single allergens in the electrode gap. This sensing mechanism may open new avenues for a highly sensitive pollen allergen sensor.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

There has been increasing interest in utilizing nanostructures for better functionality in biomolecule analyses such as molecular sieving, high throughput screening, and DNA sequencing [1–5]. Nanopores and nanochannels are considered as among the promising biosensing platforms for counting and sizing biomolecules on the single-molecule level [3–9]. This emerging technology is essentially similar to the Coulter counter principle [10] making use of an ionic current blockade effect via volume exclusion of ions by an individual molecule or particle translocating through a pore [7, 8].

Recently, much effort has been devoted to adding additional sensing electrodes to nanopores and nanochannels [10–17]. The device concept is based on sorting single molecules by size with the lithographically defined fluid channel while identifying the molecular signatures from a temporal change in the electrical current flowing through the embedded electrodes that occurs as individual molecules pass by the electrode gap [3, 4, 16–21]. We herein examine the potential of this approach at the sub-micron

scale by fabricating an electrode-embedded microchannel architecture and demonstrating electrical detection of single pollen allergen particles with simultaneous measurements of ionic current flowing through the fluidic channel and the embedded sensing electrodes (figure 1). The results presented here can be used for development of a highly sensitive pollen allergen sensor, which is of practical importance considering that hay fever is one of the most common seasonal allergic respiratory diseases worldwide.

2. Experimental details

2.1. Fabrications of electrode-embedded microfluidic channels

The procedure of fabrication of electrode-embedded microfluidic channels is summarized in figure 2. Briefly, microelectrode patterns were delineated by a photolithography processes using a photoresist AZ5206E on a 30 mm square piece of a (100)-oriented silicon wafer coated with a 300 nm SiO₂ layer. Subsequently, Cr (2 nm thick), Au (30 nm thick), and Pt (2 nm thick) were deposited by a

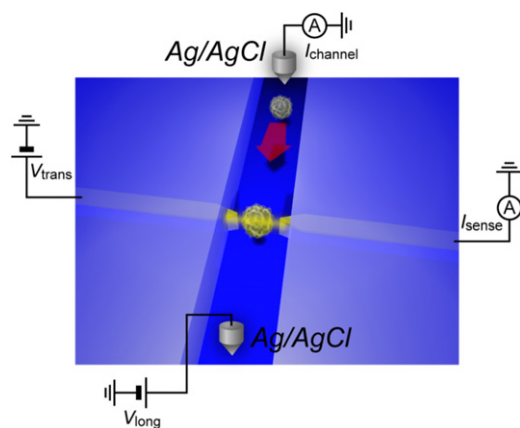


Figure 1. Conceptual diagram showing a device structure of an electrode-embedded microchannel and its use for the detection of single allergen particles. Allergen particles are electrophoretically injected into the channel with a dc bias voltage of V_{long} . At the same time, the ionic currents flowing across the channel I_{channel} and the sensing electrode gap I_{sens} are measured simultaneously.

radio-frequency (RF) magnetron sputtering method. The Cr layer was used for better adhesion of the microelectrodes on the SiO_2 substrate, while the Pt layer coated on the Au surface served as an adhesion layer for the SiO_2 overlayer deposited in the later processes. The sample was then immersed in *N,N*-dimethylformamide for half a day and sonicated for 10 min to peel off the photoresist. As a result, we obtained Cr/Au/Pt microelectrodes. Following this, two nanoelectrodes were patterned by an electron beam (EB) lithography technique using the resist ZEP-520A. Then, a Pt (2 nm thick)/Au(30 nm thick)/Pt(2 nm thick) layer was deposited by the RF magnetron sputtering method. Peeling off the EB resist by means of sonication provided Pt/Au/Pt nanogap electrodes whose gap size and thickness were about 500 nm and 30 nm, respectively. After fabricating these sensing electrodes, we formed a microfluidic channel by first depositing an SiO_2 (300 nm)/Cr (50 nm) layer by RF magnetron sputtering on the sample substrate. Subsequently, a fluidic channel was patterned by the EB lithography process using a resist ZEP-520A. Two L-shaped markers formed by the photolithography process at both sides of the sensing electrodes were utilized to align the channel with the electrode gap (figure 3(a)). Arrays of square patterns were also drawn in the upstream and the downstream regions of the microchannel. The exposed Cr layer was then removed by wet etching in a diluted hydrochloric acid solution of ceric ammonium nitrate (15 wt%) for about 10 s. In the next step, the sample was subjected to CF_4/O_2 isotropic reactive ion etching to partially etch away the SiO_2 film. As a result, a straight fluidic channel (length 6 μm , width 0.75 μm , and height 550 nm) and micropillars (cross-section 2 $\mu\text{m} \times 2 \mu\text{m}$, height 550 nm, and pillar-to-pillar spacing 2 μm) were created. Finally, the residual Cr layer was removed by the aforementioned wet etching process.

Scanning electron micrographs of the electrode-embedded microchannel are displayed in figures 3(a)–(b). It is comprised of sensing electrodes embedded in a channel and micropillar

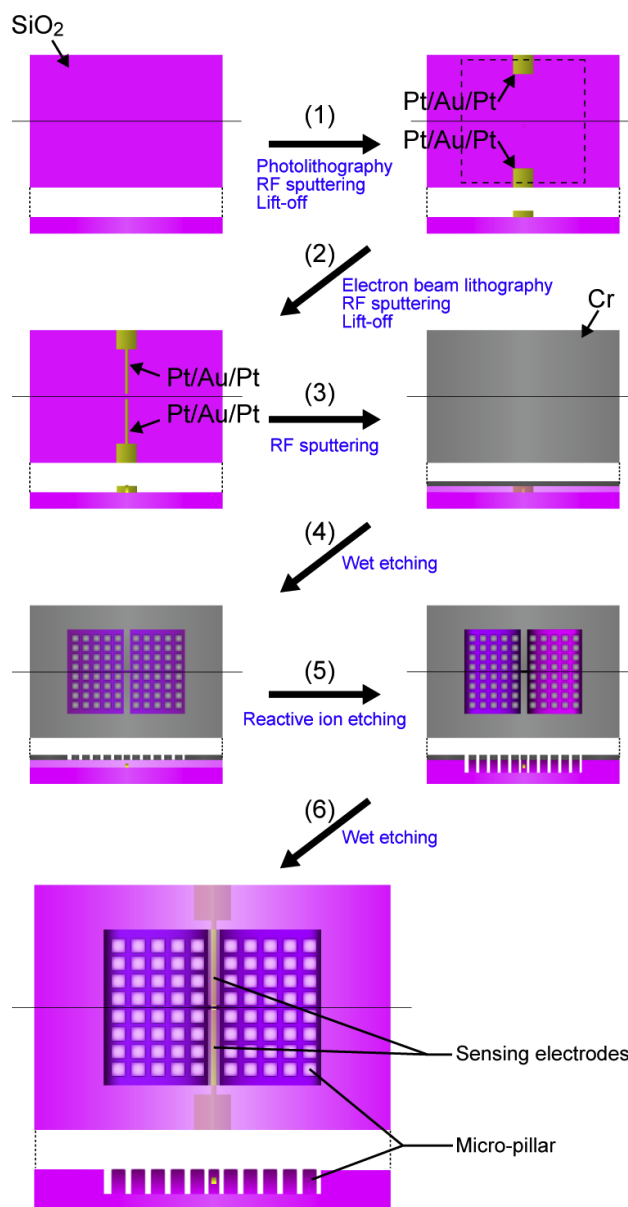


Figure 2. Fabrication scheme for electrode-embedded microchannels. (1) Microelectrodes are fabricated on a SiO_2/Si substrate using a photolithography method followed by radio-frequency (RF) magnetron sputtering and lift-off processes. The top and bottom illustrations describe plan and side views of the sample respectively. The solid black line at the center of the plan view indicates the position where the side view is taken. SiO_2 is colored pink. (2) Sensing nanoelectrodes are then formed using electron beam lithography, RF sputtering, and lift-off processes. (3) After that, a micrometer-scale fluidic channel is fabricated by first depositing SiO_2/Cr layer by the RF sputtering method. Cr regions are colored gray. (4) A Cr mask was then patterned by EB lithography and subsequent wet etching processes. (5) Using the Cr mask, a fluidic channel was formed by reactive ion etching of the SiO_2 layer with a CF_4/O_2 gas. (6) Finally, the residual Cr layer on the top surface is removed by the wet etching procedure. The device yield using these processes is about 75%.

arrays in the downstream and upstream regions. These pillars serve as spacers for preventing roof collapse of PDMS lid bonded on the substrate to seal the fluidic channel.

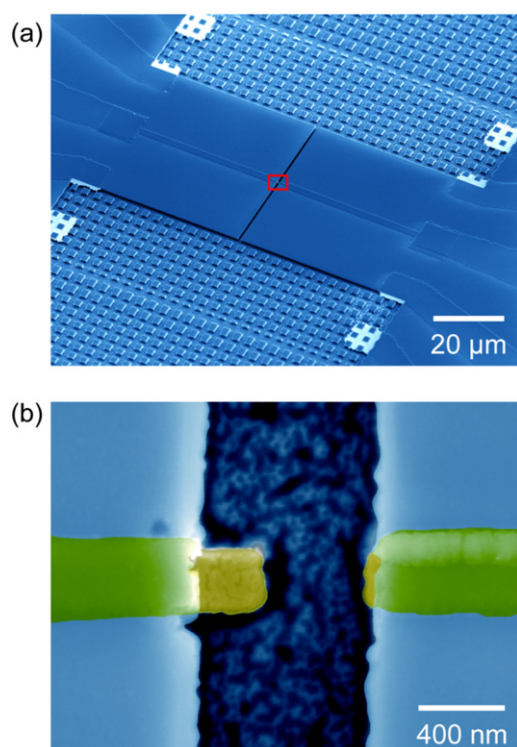


Figure 3. Scanning electron micrographs of an electrode-embedded microchannel. (a) The device structure consists of a sensing electrode-embedded microchannel and micropillar arrays in the upstream and downstream regions. (b) A magnified view of the red square region in (a). Two nanoelectrodes are embedded in the microchannel.

2.2. Sealing of electrode-embedded microchannels

The microchannel sealing was implemented by putting a piece of polydimethyl siloxane (PDMS) on the sample substrate. The PDMS lid had two fluidic channels of width 0.4 mm and height 0.2 mm with a gap of 0.2 mm at the center on one side of the surface for injecting the particle solution into the microchannel/sensing electrodes. This PDMS channel was prepared by first fabricating a polymer mold of SU-8 photoresist on a silicon wafer. In short, SU-8 was spin coated on a square piece of silicon substrate. The resist was then baked at 95 °C for 40 min. This formed an SU-8 film about 0.2 mm thick. After slowly cooling the substrate to room temperature, we contacted a photomask to the resist-coated substrate and exposed it to UV light. Finally, an SU-8 mold was obtained by immersing the substrate in a developer solution and then rinsing with 2-propanol.

A PDMS block was prepared by first mixing a curing agent and a prepolymer in a 1:10 weight ratio (SYLGARD 184 silicone elastomer kit, Dow Corning Co.). The PDMS mixture was poured into the SU-8 mold and baked at 70 °C for 1 h. After cooling to room temperature, a 20 mm × 20 mm piece was cut out. Four holes were punched in the block: two for inserting Ag/AgCl electrodes and the other two for the inlet and outlet for a particle solution. The conventional oxygen plasma treatment was applied to ensure sealing of PDMS on the SiO₂ microchannel [22]. Specifically,

we exposed the PDMS surface and the sample substrate to O₂ plasma at 25 W for 25 s and 50 W for 50 s, respectively. Subsequently, the PDMS was immediately attached to the microchannel for permanent bonding. All these processes were conducted in a clean room.

2.3. Ionic current measurements

The electrode-embedded microchannel device was mounted on a stage of a home-built probe system. A dilute solution of target allergen particles (1 mM tris-HCl) was introduced through Teflon tubes connected to the inlet and outlet holes of the PDMS lid. Two cables were connected to the Pt wires coated with Ag/AgCl paste (BAS Co.) immersed on both sides of the channel for driving electrophoretic flow and monitoring ionic current through the microchannel (figure 1). Two additional probes were contacted to the microelectrode pads for measuring the transverse current flowing through the sensing electrodes (figure 1). Each measurement circuit had a dc voltage source. The current was amplified by a homemade current amplifier. The output voltage was measured using a digitizer PXI-5922 (National Instruments Co.) at a sampling rate of 10 kHz and stored on a RAID drive HDD-8264 (National Instruments Co.).

3. Results

3.1. Pollen grains and orbicles

Figure 4 shows scanning electron micrographs of cypress pollen particles. The spherical grains have a diameter of 30 μm (figure 4(a)), which tends to collapse when they are put in water. As a result, the allergenic protein residing inside tends to spread out. A magnified view of the pollen surface reveals numerous orbicles that contain allergen Cha o 1 (figure 4(b)) [23]. These allergen particles have a sub-micrometer-scale burr-like morphology. It is known that in addition to the large pollen particles themselves, orbicles released and suspended in the atmosphere are also a serious allergic factor. Although pollen grains can be observed by conventional optical methods, the spatial resolution is not high enough for detecting sub-micrometer size orbicles. In the present study, therefore, we attempted to detect single orbicles by two-probe current measurement using an electrode-embedded microchannel structure. Particle size analyses of an orbicle solution (buffered with tris-HCl 1 mM and filtered with 800 nm filters) using a Zetasizer reveal a Gaussian distribution of the particle size centered at 330 nm (figure 4(c)). Furthermore, the zeta potential ζ was −36.5 mV with a full width at half-maximum of 13 mV (figure 4(d)). The fact that $|\zeta| > 30$ mV ensures monodispersity of orbicles in the solution. These results indicate that the diameter of single cypress orbicle is 330 nm on average.

3.2. Ionic current blockading by single allergen particles

We introduced an orbicle solution (prepared at concentration of 1 μg ml^{−1} in tris-HCl 1 mM) into an electrode-embedded

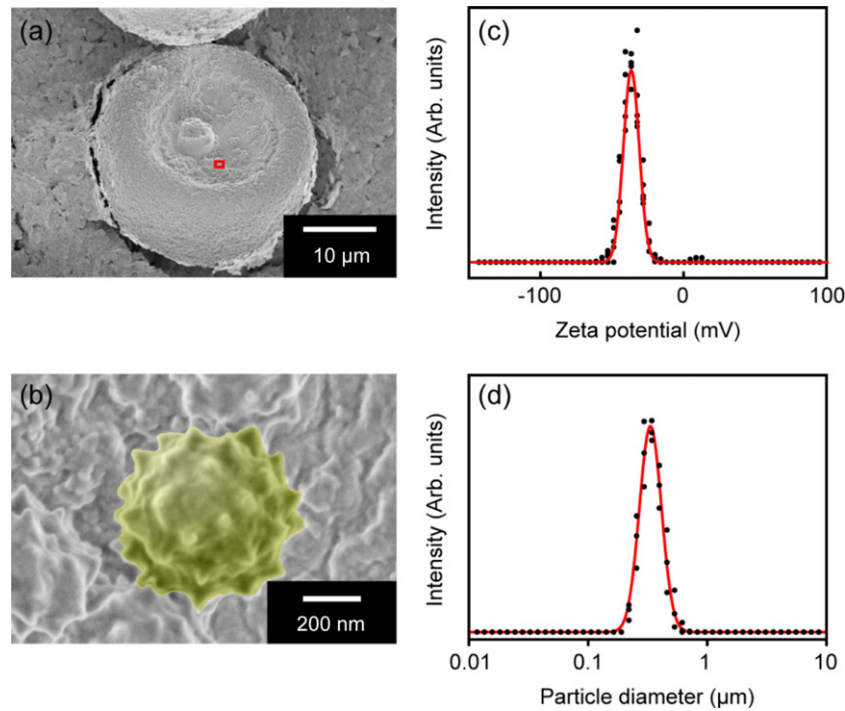


Figure 4. Scanning electron microscopy images and size distributions of cypress pollen allergen particles. (a) Cypress pollen has a spherical shape of size 10–30 μm . (b) A closer look at the pollen surface showing a false colored single orbicle that contains allergenic proteins. (c) The zeta potential and (d) particle size distributions of orbicles in a dilute solution of tris-HCl.

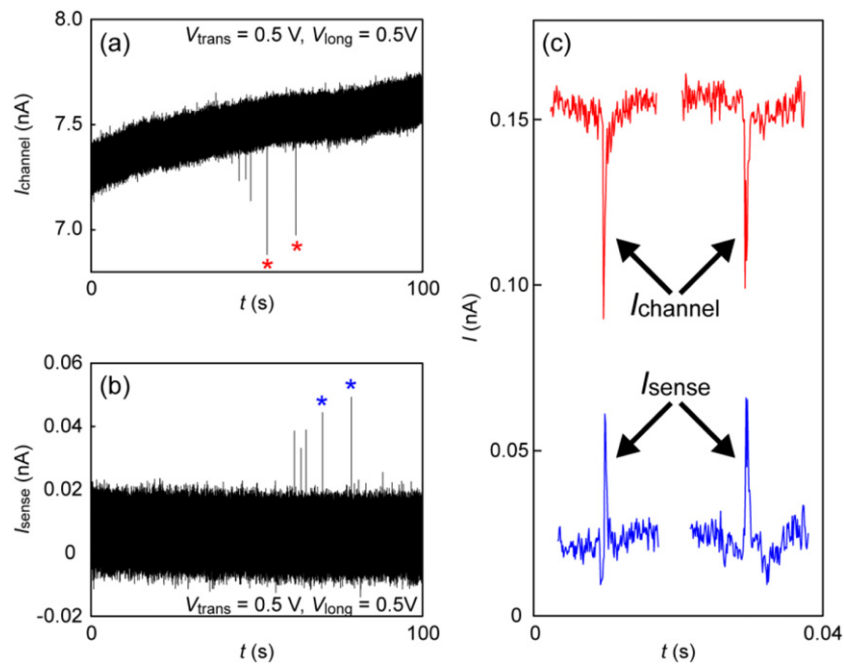


Figure 5. Typical ionic current traces measured in a dilute solution of cypress orbicles. (a) Temporal change in the ionic current flowing through a microchannel I_{channel} and (b) sensing electrodes I_{sense} . (c) A magnified view of the four signals marked by asterisks in (a) and (b). The curves are shifted vertically for clear comparison. The color coding follows those of (a) and (b).

microchannel through a PDMS inlet port and measured the ionic current flowing across the channel I_{channel} and the sensing electrodes I_{sense} under dc voltages of $V_{\text{long}} = 0.5 \text{ V}$ and $V_{\text{trans}} = 0.5 \text{ V}$, respectively (figure 5). We find very different background current for the ionic current

flowing through the channel and the sensing electrodes: $I_{\text{channel}} \sim 7 \text{ nA}$ and $I_{\text{sense}} \sim 0.003 \text{ nA}$ (figures 5(a) and (b)). The background current being more than three orders of magnitude lower in the $I_{\text{sense}}-t$ curve is attributable to the relatively small surface area of the sensing electrodes exposed

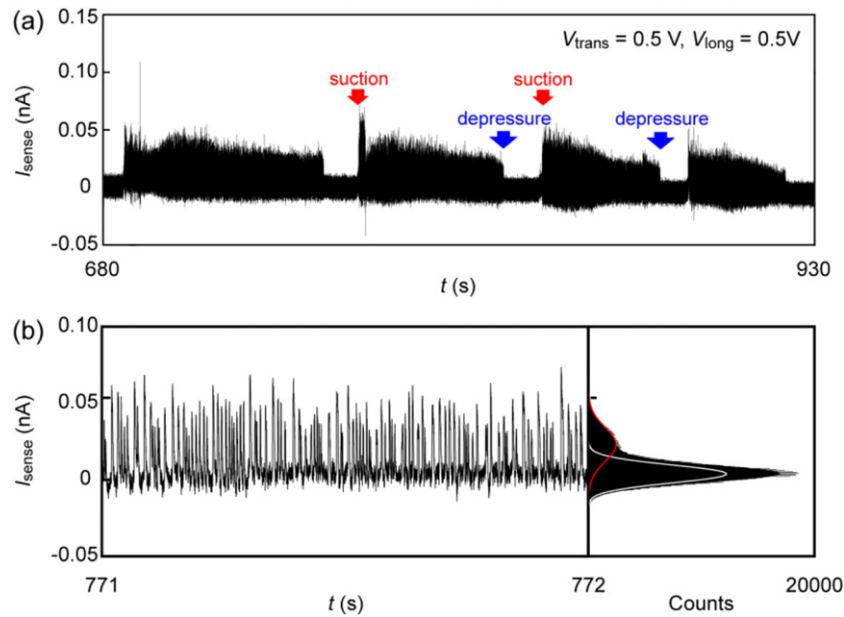


Figure 6. Orbicle translocation through a microchannel induced by external liquid pressure. (a) Characteristic current spikes signifying translocation of single orbicles through a microchannel were observed when a pressure was added using a syringe pump. (b) A closer look at the I_{sense} trace in (a) reveals many current spikes. The right panel displays an I_{sense} histogram constructed with the $I_{\text{sense}}-t$ curve in (a) that contains about 20 000 spike-like signals. White and red curves are Gaussian fits to the current distribution, corresponding to those of the background current and the orbicle-related I_{sense} signals, respectively.

to the ionic solution. Most of the sensing electrodes was covered with SiO_2 layer and the exposed apex had an area of only about $0.1 \mu\text{m}^2$, which is much smaller than that for the Ag/AgCl electrodes (about 0.8 mm^2) used to measure I_{channel} . The offset current tends to increase with increasing bias voltage and/or ion concentration of the solution.

The $I_{\text{channel}}-t$ curves obtained for the allergen particle solution occasionally exhibited a transient decrease in I_{channel} (figures 5(a) and (c)). This can be interpreted as a negative contribution from the temporal volume exclusion of ions by a single allergen particle passing through the microchannel for the ionic conductance across the channel [24–26]. The rate of emergence of these current spikes was typically within a range of 0.1–0.01 Hz. We also performed control experiments in a buffer solution containing no allergen particles. These measurements gave only featureless I_{channel} traces, which further suggest that the characteristic I_{channel} spikes signify translocations of single allergen particles through the microchannel.

3.3. Electrical detection of single allergen particles using sensing electrodes

It is noticeable that the I_{sense} trace also shows distinct transient signals concomitant with the emergence of the ionic current blockade derived I_{channel} spikes (figures 5(b) and (c)); a constant time gap of about 16.7 s is an external artifact stemming merely from a difference in the time of starting the two independent current measurement systems for recording I_{sense} and I_{channel} . By analogy, the spike-like I_{sense} signals is also ascribable to a response of the current flowing through the embedded sensing electrodes associated with single orbicles

passing through the electrode gap. However, while I_{channel} demonstrated ionic current decrease via the ionic current blockade mechanism, the corresponding I_{sense} exhibited a sharp current rise. This can be explained as a result of sudden change in the stray capacitance of the nanogap electrodes during single-orbicle translocation that causes a capacitor discharging induced current pulse. Nevertheless, it is also possible that cross-talk between I_{sense} and I_{channel} may cause I_{sense} signals observed here as predicted theoretically very recently [27]. Further effort is required to elucidate the physical origin of the I_{sense} signals.

3.4. Pressure-driven flow of allergen particles

Having demonstrated that the electrode-embedded microchannel can be used for electrical detection of single orbicles, it is important to further examine what information we can possibly acquire from I_{sense} measurements. For this, we added an external pressure to facilitate the particle translocation through the microchannel so as to obtain enough I_{sense} signals for conducting statistical analysis. We observed characteristic I_{sense} spikes when liquid pressure was exerted by a syringe connected to the outlet hole of the PDMS lid (figure 6(a)). These signals disappeared when the external pressure was removed. The rate of single-particle detection increased to about 100 Hz (figure 6(b)). A current histogram constructed with a consecutive $I_{\text{sense}}-t$ curve containing about 20 000 signals shows a shoulder peak centered at 16 pA in addition to the large background current peak offset to 0 A (figure 6(c)). Although clear understanding of the physical origin of the I_{sense} signals is still lacking, this result suggests a potential use of the I_{sense} distribution for identification of pollen allergen particles of different types.

4. Summary

We have fabricated an electrode-embedded microchannel and applied it for the detection of single allergen particles. Ionic current flowing through the microchannel was found to decrease during electrophoretic single-orbicle translocation, which could be explained by the ionic current blockade mechanism. In contrast, the transverse ionic current flowing through the sensing electrode gap showed a sharp rise suggestive of capacitor discharging via a temporal change of the stray capacitance. Statistical analysis of the I_{sense} distributions indicated a possible use of the transverse current method for single-particle identification of pollen allergens.

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