

Journal of Visualized Experiments

A Closed-Type Wireless Nanopore Electrode for Analyzing Single Nanoparticles --Manuscript Draft--

Article Type:	Methods Article - JoVE Produced Video
Manuscript Number:	JoVE59003R2
Full Title:	A Closed-Type Wireless Nanopore Electrode for Analyzing Single Nanoparticles
Keywords:	nanopore; Wireless Nanopore Electrode; single nanoparticles; gold nanoparticles; Single Entity; Electrochemical confined sensing
Corresponding Author:	Yi-Tao Long CHINA
Corresponding Author's Institution:	
Corresponding Author E-Mail:	ytlong@ecust.edu.cn
Order of Authors:	Rui Gao Ling-Fei Cui Lin-Qi Ruan Yi-Lun Ying Yi-Tao Long
Additional Information:	
Question	Response
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US\$2,400)
Please indicate the city, state/province, and country where this article will be filmed . Please do not use abbreviations.	Shanghai, China

Dr. Phillip Steindel
Review Editor
JoVE

Professor Yi-Tao LONG
Key Laboratory for Advanced Materials & Department of Chemistry
East China University of Science and Technology

Oct. 16, 2018

Dear Dr. Phillip Steindel,

Thank you very much for providing us this opportunity to revise our manuscript of “A Closed-Type Wireless Nanopore Electrode for Analyzing Single Nanoparticles” (EMID: bcb0938da1b6af29). According to your suggestions and comments, we have revised and improved our manuscript. Please find attached our point-by-point response letter and revised manuscript for details. All the revised parts were marked in red color in the Main Text. The essential steps for the video were in yellow. Moreover, we have certainly uploaded "Copyright" PDF which contains all the reuse permission for our figures in our first-round revision. Also, "Copyright" PDF has sent to your email em@editorialmanager.com on Oct. 11. We are confusing about your comments of “There is currently no reuse permission for your Figures uploaded to Editorial Manager” in your email on Oct. 10. If the "Copyright" PDF could not meet the requirement of *JoVE*, I am wondering if you could guide us to prepare the reuse permission.

We hope our revised manuscript can meet the requirements for *JoVE*.

Thank you very much for your consideration again.

Sincerely yours,

Yi-Tao LONG

TITLE:**A Closed-Type Wireless Nanopore Electrode for Analyzing Single Nanoparticles****AUTHORS & AFFILIATIONS:**

Rui Gao^{1*}, Ling-Fei Cui^{1*}, Lin-Qi Ruan¹, Yi-Lun Ying¹, Yi-Tao Long¹

¹Key Laboratory for Advanced Materials, School of Chemistry and Molecular Engineering, East China University of Science and Technology, Shanghai, China

*These authors contributed equally to this work.

Corresponding Authors:

Yi-Tao Long (ytlong@ecust.edu.cn)

Yi-Lun Ying (yilunying@ecust.edu.cn)

Email Addresses of Co-Authors:

Rui Gao (gaorui@mail.ecust.edu.cn)

Ling-Fei Cui (lingfeicui@mail.ecust.edu.cn)

Lin-Qi Ruan (ruanlinqi@mail.ecust.edu.cn)

KEYWORDS:

Nanopore, wireless nanopore electrode, single nanoparticles, gold nanoparticles, single entity, electrochemical confined sensing

SUMMARY:

Here, we present a protocol for fabrication of a closed-type wireless nanopore electrode and subsequent electrochemical measurement of single nanoparticle collisions.

ABSTRACT:

Measuring the intrinsic features of single nanoparticles by nanoelectrochemistry holds deep fundamental importance and has potential impacts in nanoscience. However, electrochemically analyzing single nanoparticles is challenging, as the sensing nanointerface is uncontrollable. To address this challenge, we describe here the fabrication and characterization of a closed-type wireless nanopore electrode (WNE) that exhibits a highly controllable morphology and outstanding reproducibility. The facile fabrication of WNE enables the preparation of well-defined nanoelectrodes in a general chemistry laboratory without the use of a clean room and expensive equipment. One application of a 30 nm closed-type WNE in analysis of single gold nanoparticles in the mixture is also highlighted, which shows a high current resolution of 0.6 pA and high temporal resolution of 0.01 ms. Accompanied by their excellent morphology and small diameters, more potential applications of closed-type WNEs can be expanded from nanoparticle characterization to single molecule/ion detection and single-cell probing.

INTRODUCTION:

Nanoparticles have attracted tremendous attention due to diverse features such as their catalytic ability, particular optical features, electroactivity, and high surface-to-volume ratios¹⁻⁴. Electrochemical analysis of single nanoparticles is a direct method for understanding the intrinsic chemical and electrochemical processes at the nanoscale level. To achieve highly sensitive measurements of single nanoparticles, two electrochemical approaches have been previously applied to read out nanoparticle information from current responses⁵⁻⁷. One of these approaches involves immobilizing or capturing an individual nanoparticle on the interface of the nanoelectrode for the study of electrocatalysis^{8,9}. The other strategy is driven by single nanoparticle collision with the surface of an electrode, which generates a transient current fluctuation from the dynamic redox process.

Both of these methods require a nanoscale ultrasensitive sensing interface that matches the diameter of single nanoparticles. However, traditional fabrication of nanoelectrodes has mainly incorporated the micro-electromechanical systems (MEMS) or laser pulling techniques, which are tedious and undisciplinable¹⁰⁻¹³. For example, MEMS-based fabrication of nanoelectrodes is expensive and requires the use of a clean room, restricting the massive production and popularization of nanoelectrodes. On the other hand, laser pulling fabrication of nanoelectrodes relies heavily on experience of the operators during the sealing and pulling of a metal wire inside the capillary. If the metal wire is not well-sealed in the capillary, the gap between the inner wall of the nanopipette and wire can dramatically introduce excess background current noise and enlarge the electroactive sensing area. These drawbacks largely decrease the sensitivity of the nanoelectrode. On the other hand, the existence of a gap can enlarge the electrode area and reduce sensitivity of the nanoelectrode. As a consequence, it is hard to guarantee a reproducible performance due to the uncontrollable electrode morphologies in each fabrication process^{14,15}. Therefore, a general fabrication method of nanoelectrodes with excellent reproducibility is urgently needed to facilitate electrochemical exploration of the intrinsic features of single nanoparticles.

Recently, the nanopore technique has been developed as an elegant and label-free approach for single molecule analysis¹⁶⁻²⁰. Owing to its controllable fabrication, the nanopipette provides a nanoscale confinement, with a uniform diameter ranging from 30–200 nm by a laser capillary puller²¹⁻²⁴. Moreover, this simple and reproducible fabrication procedure ensures the generalization of the nanopipette. Recently, we proposed a wireless nanopore electrode (WNE), which does not require the sealing of a metal wire inside the nanopipette. Through a facile and reproducible fabrication process, the WNE possesses a nanoscale metal deposition within the nanopipette to form an electroactive interface²⁵⁻²⁸. Since the WNE possesses a well-defined structure and uniform morphology of its confinements, it achieves high current resolution as well as low resistance-capacitance (RC) time constant for performing high temporal resolution. We previously reported two types of WNEs, open-type and closed-type, for realizing single entity analysis. The open-type WNE employs a nanometal layer deposited on the inner wall of a nanopipette, which converts the faradic current of a single entity to the ionic current response²⁶. Usually, the diameter of an open-type WNE is around 100 nm. To further decrease the diameter of WNE, we presented the closed-type WNE, in which a solid metal nanotip fully occupies the nanopipette tip through a chemical-electrochemical approach. This method can rapidly generate

a 30 nm gold nanotip inside a nanopore confinement. The well-defined interface at the tip area of a closed-type WNE ensures a high signal-to-noise ratio for electrochemical measurements of single nanoparticles. As a charged gold nanoparticle collides with the closed-type WNE, an ultrafast charging-discharging process at the tip interface induces a capacitive feedback response (CFR) in the ionic current trace. Compared to a previous single nanoparticle collision study *via* a nanoelectrode with metal wire inside²⁹, the closed-type WNE showed a higher current resolution of $0.6 \text{ pA} \pm 0.1 \text{ pA}$ (RMS) and higher temporal resolution of 0.01 ms.

Herein, we describe a detailed fabrication procedure for a closed-type WNE that has highly controlled dimensions and outstanding reproducibility. In this protocol, a simple reaction between AuCl_4^- and BH_4^- is designed to generate a gold nanotip that completely blocks the orifice of a nanopipette. Then, bipolar electrochemistry is adopted for continuous growth of a gold nanotip that reaches the length of several micrometers inside the nanopipette. This simple procedure enables the implementation of this nanoelectrode fabrication, which can be carried out in any general chemistry laboratory without a clean room and expensive equipment. To determine the size, morphology, and inner structure of a closed-type WNE, this protocol provides a detailed characterization procedure with use of a scanning electron microscope (SEM) and fluorescence spectroscopy. One recent example is highlighted, which directly measures the intrinsic and dynamic interactions of gold nanoparticles (AuNPs) colliding towards the nanointerface of a closed-type WNE. We believe that the closed-type WNE may pave a new path for future electrochemical studies of living cells, nanomaterials, and sensors at single-entity levels.

PROTOCOL:

1. Preparation of Solutions

NOTE: Pay attention to general safety precautions for all chemicals. Dispose of chemicals in a fume hood, and wear gloves, goggles, and a lab coat. Keep flammable liquids away from fire or sparks. All aqueous solutions were prepared using ultrapure water ($18.2 \text{ M}\Omega \text{ cm}$ at 25°C). The prepared solutions were filtered using a $0.22 \text{ }\mu\text{m}$ pore-size filter.

1.1. Preparation of KCl solution

1.1.1. Dissolve 0.074 g of potassium chloride in 100 mL of deionized water.

1.2. Preparation of NaBH_4 solution

1.2.1. Dissolve 0.018 g of sodium borohydride in 10 mL of ethanol.

1.3. Preparation of HAuCl_4 solution

1.3.1. Dissolve 0.010 g of potassium chloride in 1 mL of 1% chloroauric acid solution.

1.4. Preparation of silicone rubber

1.4.1. Mix silicone rubber containing Part A and Part B (see **Table of Materials**) at a ratio of 1:1 by volume.

1.4.2. Use the mixed silicone rubber to paint a reaction area at the slide immediately during the pot time of 1 min.

1.4.3. Cure the prepared silicone rubber on the slide for 5 min.

1.5. Preparation of gold nanoparticles³⁰

1.5.1. Add 4.8 mL of chloroauric acid with a mass fraction of 1% into 40 mL of deionized water with vigorous stirring.

1.5.2. Heat the solution to a boil.

1.5.3. Quickly add 10 mL of a trisodium citrate solution with a mass fraction of 1% into the solution.

1.5.4. Heat the solution for an additional 15 min until the final solution is red in color.

NOTE: In our case, the chloroauric acid solution was rapidly reduced by trisodium citrate, and it was observed that the solution rapidly changed from clear-yellow to dark-black.

2. Preparation of Experimental Setup

2.1. Preparation of current measurement system

2.1.1. Turn on the current measurement system containing the current amplifier (see **Table of Materials**) and low-noise data acquisition system (see **Table of Materials**)

2.1.2. Switch on the voltage-clamp mode.

2.1.3. Set the filter bandwidth to 10 kHz and sampling rate to 100 kHz.

2.1.4. Assemble a self-designed specific homemade copper cage to shield external noise for experimental cells and the pre-amplifier on the inverted microscope (see **Table of Materials**).

2.1.5. Ground the shell of the Faraday cage, shells of the amplifier, and inverted microscope system.

2.2. Setup of the dark-field detection system

2.2.1. Generation of the gold nanotip inside the nanopipette is monitored by the dark-field microscope.

NOTE: An inverted microscope system (see **Table of Materials**) is used to take images and scatter spectra. A true-color digital CCD camera is employed to take images of the nanopipette and nanopore electrode. A dark-field condenser [numerical aperture (NA) = 0.8–0.95] is utilized to form a dark-field illumination. 10X (NA = 0.3), 20X (NA = 0.45), and 40X (NA = 0.6) objectives are used to collect images of the closed-type WNE. Fluorescence detection is used to further verify whether there are gaps between the nanotip and inner wall of the nanopipette. This experiment is performed by another EMCCD (see **Table of Materials**) also integrated on the inverted microscope, and the excitation light is a built-in mercury lamp with a band-pass filter of 450–490 nm.

3. Fabrication of Closed-Type WNE

3.1. Fabrication of nanopipettes

3.1.1. Put the quartz capillaries (see **Table of Materials**) in a 15 mL centrifuge tube filled with acetone for 10 min of ultrasonic cleaning.

3.1.2. Pour off the acetone, then add ethanol into the same centrifuge tube.

3.1.3. Put the centrifuge tube in an ultrasonic cleaner for 10 min of cleaning.

3.1.4. Put the capillaries into another 15 mL centrifuge tube with deionized water for removal of the ethanol, with 10 min of ultrasonic cleaning.

3.1.5. Continually ultrasonic clean the capillaries three times with deionized water to remove the residual ethanol.

3.1.6. Dry the capillaries using a nitrogen gas stream.

3.1.7. Keep the capillaries in a new, clean centrifuge tube.

3.1.8. Turn on the CO₂ laser puller (see **Table of Materials**)

3.1.9. Preheat the puller for 15-20 min to ensure a steady laser power.

3.1.10. Install the cleaned capillary in the puller.

3.1.11. Set the pulling parameters of heat, filament, velocity, delay, and pulling force on the panel of the CO₂ laser puller for a specific diameter. The detailed parameter for a 30 nm diameter nanopipette in this protocol is shown in **Table 1 (Figure 1)**.

3.1.12. Fix the prepared nanopipette on a Petri dish with the reusable adhesive (see **Table of Materials**) for further characterization.

3.2. Fabrication of closed-type WNE

3.2.1. Inject 10 μL of the prepared HAuCl_4 solution into the nanopipette with a microloader.

3.2.2. Centrifuge the nanopipette for 5 min at around $1878 \times g$ for the removal of air bubbles in the nanopipette.

NOTE: For this step, we placed the nanopipette with the tip facing down into a homemade holder within a 2 mL centrifuge tube.

3.2.3. Fix the nanopipette on a coverslip with the prepared silicone rubber (see step 1.4) and define the area inside the nanopipette as the “cis” side and outside as the “trans” side.

3.2.4. Wait 5 min until the rubber is cured.

3.2.5. Put the integrated ensemble on the objective table of the inverted microscope.

3.2.6. Turn on and adjust the dark-field illumination to focus the nanopipette tip under a 10X microscope objective.

3.2.7. Change to 20X and 40X objectives for a higher spatial resolution.

3.2.8. Place one Ag/AgCl electrode inside the nanopipette.

3.2.9. Place the other grounded Ag/AgCl electrode into the trans side.

3.2.10. Connect a pair of Ag/AgCl electrodes to the pre-amplifier.

3.2.11. Turn on the current measurement system and the corresponding software (see **Table of Materials**) for ionic current recording.

3.2.12. Set the applied potential to 300 mV.

3.2.13. Slowly add 150 μL of NaBH_4 solution into the trans side to trigger the reaction between HAuCl_4 and NaBH_4 (**Figure 2**).

NOTE: The reduction of NaBH_4 in aqueous solution takes place at a violent reaction rate. Therefore, the generation of H_2 from the reduction of NaBH_4 may induce a defective structure of the nanotip by the generation of cavities during the gold nanotip growth.

3.2.14. Simultaneously, electrically and optically record the current trace and the dark-field image/scattering spectra using the current measurement and dark-field detection systems (Figure 3).

NOTE: The ethanol solution is volatile under dark-field illumination. Pay attention to the volume of ethanol during the fabrication process.

3.2.15. Turn off the applied potential after ionic current tracing back to 0 pA.

3.2.16. Wash the prepared closed-type WNE with flowing deionized water from the bottom to the tip.

3.3. Characterization of closed-type WNE

3.3.1. Characterize closed-type WNE with a scanning electron microscope (SEM), which is a general method for characterization of nanopipettes^{22,31-34}.

3.3.2. Use a calcium ion fluorescent experiment to verify the sealing condition of the gold nanotip inside the nanopipette.

3.3.2.1. Inject 10 μL of CaCl_2 solution into the cis side of the closed-type WNE and Fluo-8 solution into the trans side.

3.3.2.2. Connect the Ag/AgCl electrodes to the headstage.

3.3.3. Apply a 400 mV bias potential and use the EMCCD (see **Table of Materials**) to monitor the fluorescence response at the tip area. Use focus ions beam (FIB) to sculpt the closed-type WNE from the tip to the bottom, then determine the length of the interior metal layer or nanotip with SEM characterization.

3.4. Single nanoparticle collision with closed-type WNE

3.4.1. Change the solution in the trans and cis sides to a KCl solution after fabrication of the closed-type WNE.

3.4.2. Transfer 50 μL of 30 nm gold nanoparticle solution into the trans side. Record the current signal of single nanoparticle collision events at a potential of 300 mV (Figure 5).

3.4.3. Change the applied voltage to monitor the frequency, amplitude, and shape change of the current signal.

REPRESENTATIVE RESULTS:

We demonstrate a facile approach to fabricate a well-defined 30 nm wireless nanopore electrode based on a quartz conical nanopipette. The fabrication of a nanopipette is demonstrated in **Figure 1**, which includes three main steps. A microcapillary with an inner diameter of 0.5 mm and outer diameter of 1.0 mm is fixed in the puller, then a laser is focused on the center of the capillary to melt the quartz. By applying forces to the terminals of the capillary, it finally separates and forms two parts with nanoscale conical tips. The pulling parameters are provided in **Table 1** for fabricating 30 nm nanopipettes in our lab. It should be noted that the parameters can vary from different laser pullers. Experimenters should adjust the parameters according to the laser power, temperature, and humidity. After fabrication, SEM characterization is required to verify the true diameter of the nanopipette.

Figure 2 shows the procedure of generating a gold nanotip inside the nanopipette tip after the pulling process. First, the AuCl_4^- inside the nanopipette is consistently reduced by BH_4^- to generate a gold nanotip until the opening of nanopipettes is fully blocked. Then, the bipolar electrochemistry promotes the further growth of the gold nanotip. We used an *in situ* characterization system to monitor the fabrication process of the closed-type WNE by simultaneous recording of the current response and dark-field images (**Figure 3**). As for the SEM characterization, **Figure 4** shows top view SEM images of the bare nanopipette and closed-type WNE. After FIB splitting, a side view SEM image provides the morphology of the gold nanotip inside the closed-type WNE. In the single-nanoparticle collision experiments, the gold nanoparticles are added to the trans side of the WNE. The outstanding noise performance of this CNE uncovers the hidden signals with a high signal frequency (**Figure 5**).

FIGURE AND TABLE LEGENDS:

Figure 1: Fabrication of nanopipettes. The procedure for fabrication is as follows: step 1) install a microcapillary in a laser puller; step 2) heat the middle of the capillary with a CO_2 laser and apply force at the ends of the capillary to pull it; and step 3) the capillary tapers down and separates into two symmetric nanopipettes in several seconds.

Figure 2: Fabrication of the closed-type WNE. Step 1) HAuCl_4 and NaBH_4 solutions are added to the cis and trans sides of the nanopipette, respectively. AuCl_4^- is reduced by BH_4^- to generate gold at the nanopipette orifice. Step 2) After the orifice is blocked by generated gold, the bipolar electrochemical reaction takes place with the applied potential for further growth of the gold nanotip. Step 3) A closed-type WNE is finally fabricated with a micrometer-length gold nanotip. This figure has been modified with permission from previous work²⁵.

Figure 3: Simultaneous electrical monitoring and scattering recording during closed-type WNE fabrication. (A) After addition of NaBH_4 in the trans side of the nanopipette, the current drops immediately from 0 pA. Then, the current trace experiences a quick transition due to the generation of gold. After ~150 s, the current returns to 0 pA, demonstrating complete blockage of the nanopipette. **(B)** Dark-field images during WNE fabrication at corresponding timepoints of 0 s, 10 s, 100 s, and 150 s. This figure has been modified with permission from previous work²⁵.

Figure 4: SEM characterizations of the nanopipette and closed-type WNE. (A) Top view SEM image of a pulled nanopipette with a diameter of 30 nm. **(B)** Top view SEM image of a closed-type WNE with a diameter of 30 nm. **(C)** Side view SEM image of a closed-type WNE after FIB splitting from the tip to the back of the nanopipette.

Figure 5: Single nanoparticle collision detection with a closed-type WNE. (A) 30 nm gold nanoparticles are added to the trans side solution. A pair of Ag/AgCl electrodes are employed to apply a bias potential of 300 mV. Insert: a typical spike signal of a 30 nm gold nanoparticle collision. **(B)** A current trace without nanoparticles and after addition of 30 nm gold nanoparticles in the trans side of the closed-type WNE. This figure has been modified with permission from previous work²⁵.

DISCUSSION:

Fabrication of a well-defined nanopipette is the first step in the closed-type WNE fabrication process. By focusing a CO₂ laser onto the center of the capillary, one capillary separates into two symmetrical nanopipettes with nanoscale conical tips. The diameter is easily controlled, ranging from 30–200 nm, by adjusting the parameters of the laser puller. It is noted that the parameters for pulling can vary for different pipette pullers. The environmental temperature and humidity can also influence the final diameter of the nanopipette.

After fabrication of the nanopipette, a chemical reaction generates the solid nanotip inside the nanopipette. In this protocol, a gold nanotip is formed by the reduction of HAuCl₄; other metal nanotips can be fabricated by designing corresponding reactions. After full blockage of the nanopipette tip, the electrochemical polarization of the generated gold nanotip promotes its further growth according to bipolar electrochemistry. An *in-situ* electro-optical characterization system is then constructed to achieve simultaneous recordings of the current traces and optical information during the growth process of the gold nanotip.

For characterization, calcium ion fluorescence detection helps verify whether the gap is generated between the inner wall of the pipette and the gold nanotip. For a well-defined closed-type WNE, the tip area should be invariably dark in the fluorescence image. Also, SEM can be used to characterize both the nanopipette and closed-type WNE. FIB may be employed to sculpt along the sidewall of the closed-type WNE to expose inner metal for the subsequent SEM imaging. Therefore, the length and inside structure of close-type WNE can be determined. Following its characterization, the well-prepared closed-type WNE is capable to be used for further applications.

This protocol for closed-type WNE paves a new path for electrochemical measurements of single nanoparticles with high reproducibility. However, there are still some challenges and limitations in this fabrication process. The first limitation involves the diameter of the nanopipette tip. Theoretically, when the tip diameter decreases to a single-molecule size, the current resolution

can be dramatically enhanced. However, it is challenging to pull a nanopipette with a diameter under 30 nm with the existing pulling strategy.

The potential of this closed-type WNE protocol can be expanded to practical applications in nanosensing. By incorporating traditional nanoelectrodes with a scanning electrochemical microscope, the closed-type WNE can reveal dynamic electrochemical mapping for some special 2-D/3-D nanomaterials. Moreover, the plasmonic resonance of the gold nanotip can be used to simultaneously detect the electron transfer process by both electrical readout and optical recording. By virtue of its geometrical properties, the closed-type WNE with a conical nanotip is suited to cellular analysis with low mechanical damage.

ACKNOWLEDGEMENTS:

This research is supported by the National Natural Science Foundation of China (21327807, 21421004, and 61871183), Innovation Program of Shanghai Municipal Education Commission (2017-01-07-00-02-E00023), and the Fundamental Research Funds for the Central Universities (222201718001, 222201717003, and 222201714012).

DISCLOSURE:

The authors declare no conflicts of interests.

REFERENCES:

- 1 Vajda, S. *et al.* Subnanometre platinum clusters as highly active and selective catalysts for the oxidative dehydrogenation of propane. *Nature Materials*. **8** (3), 213-216 (2009).
- 2 Liu, G. L., Long, Y. T., Choi, Y., Kang, T., Lee, L. P. Quantized plasmon quenching dips nanospectroscopy via plasmon resonance energy transfer. *Nature Methods*. **4** (12), 1015-1017 (2007).
- 3 Hu, J. S. *et al.* Mass production and high photocatalytic activity of ZnS nanoporous nanoparticles. *Angewandte Chemie International Edition*. **44** (8), 1269-1273 (2005).
- 4 Martinez, B., Obradors, X., Balcells, L., Rouanet, A., Monty, C. Low temperature surface spin-glass transition in γ -Fe₂O₃ nanoparticles. *Physical Review Letters*. **80** (1), 181 (1998).
- 5 Fang, Y. *et al.* Plasmonic Imaging of Electrochemical Reactions of Single Nanoparticles. *Accounts of Chemical Research*. **49** (11), 2614-2624 (2016).
- 6 Mirkin, M. V., Sun, T., Yu, Y., Zhou, M. Electrochemistry at One Nanoparticle. *Accounts of Chemical Research*. **49** (10), 2328-2335 (2016).
- 7 Murray, R. W. Nanoelectrochemistry: metal nanoparticles, nanoelectrodes, and nanopores. *Chemical Reviews*. **108** (7), 2688-2720 (2008).
- 8 Anderson, T. J., Zhang, B. Single-Nanoparticle Electrochemistry through Immobilization and Collision. *Accounts of Chemical Research*. **49** (11), 2625-2631 (2016).
- 9 Fu, K., Bohn, P. W. Nanopore Electrochemistry: A Nexus for Molecular Control of Electron Transfer Reactions. *ACS Central Science*. **4** (1), 20-29 (2018).
- 10 Zaino, L. P., Ma, C., Bohn, P. W. Nanopore-enabled electrode arrays and ensembles. *Microchimica Acta*. **183** (3), 1019-1032 (2016).
- 11 Katemann, B. B., Schuhmann, W. Fabrication and Characterization of Needle-Type. *Electroanalysis*. **14** (1), 22-28 (2002).

437 12 Penner, R. M., Heben, M. J., Longin, T. L., Lewis, N. S. Fabrication and use of nanometer-
438 sized electrodes in electrochemistry. *Science*. **250** (4984), 1118-1121 (1990).

439 13 Watkins, J. J. *et al.* Zeptomole voltammetric detection and electron-transfer rate
440 measurements using platinum electrodes of nanometer dimensions. *Analytical Chemistry*.
441 **75** (16), 3962-3971 (2003).

442 14 Liu, Y., Li, M., Zhang, F., Zhu, A., Shi, G. Development of Au Disk Nanoelectrode Down to 3
443 nm in Radius for Detection of Dopamine Release from a Single Cell. *Analytical Chemistry*.
444 **87** (11), 5531-5538 (2015).

445 15 Li, Y., Bergman, D., Zhang, B. Preparation and electrochemical response of 1-3 nm Pt disk
446 electrodes. *Analytical Chemistry*. **81** (13), 5496-5502 (2009).

447 16 Kasianowicz, J. J., Brandin, E., Branton, D., Deamer, D. W. Characterization of individual
448 polynucleotide molecules using a membrane channel. *Proceedings of the National*
449 *Academy of Sciences of the United States of America*. **93** (24), 13770-13773 (1996).

450 17 Cao, C. *et al.* Discrimination of oligonucleotides of different lengths with a wild-type
451 aerolysin nanopore. *Nature Nanotechnology*. **11** (8), 713-718 (2016).

452 18 Cao, C., Long, Y.-T. Biological Nanopores: Confined Spaces for Electrochemical Single-
453 Molecule Analysis. *Accounts of Chemical Research*. **51** (2), 331-341 (2018).

454 19 Sha, J. J., Si, W., Xu, W., Zou, Y. R., Chen, Y. F. Glass capillary nanopore for single molecule
455 detection. *Science China-Technological Sciences*. **58** (5), 803-812 (2015).

456 20 Ying, Y. L., Zhang, J., Gao, R., Long, Y. T. Nanopore-based sequencing and detection of
457 nucleic acids. *Angewandte Chemie International Edition*. **52** (50), 13154-13161 (2013).

458 21 Lan, W. J., Holden, D. A., Zhang, B., White, H. S. Nanoparticle transport in conical-shaped
459 nanopores. *Analytical Chemistry*. **83** (10), 3840-3847 (2011).

460 22 Karhanek, M., Kemp, J. T., Pourmand, N., Davis, R. W., Webb, C. D. Single DNA molecule
461 detection using nanopipettes and nanoparticles. *Nano Letters*. **5** (2), 403-407 (2005).

462 23 Morris, C. A., Friedman, A. K., Baker, L. A. Applications of nanopipettes in the analytical
463 sciences. *Analyst*. **135** (9), 2190-2202 (2010).

464 24 Yu, R.-J., Ying, Y.-L., Gao, R., Long, Y.-T. Confined Nanopipette Sensing: From Single
465 Molecules, Single Nanoparticles to Single Cells. *Angewandte Chemie International Edition*.
466 doi: 10.1002/ange.201803229, (2018).

467 25 Gao, R. *et al.* A 30 nm Nanopore Electrode: Facile Fabrication and Direct Insights into the
468 Intrinsic Feature of Single Nanoparticle Collisions. *Angewandte Chemie International*
469 *Edition*. **57** (4), 1011-1015 (2018).

470 26 Ying, Y. L. *et al.* Asymmetric Nanopore Electrode-Based Amplification for Electron Transfer
471 Imaging in Live Cells. *Journal of the American Chemical Society*. **140** (16), 5385-5392 (2018).

472 27 Gao, R., Ying, Y. L., Hu, Y. X., Li, Y. J., Long, Y. T. Wireless Bipolar Nanopore Electrode for
473 Single Small Molecule Detection. *Analytical Chemistry*. **89** (14), 7382-7387 (2017).

474 28 Gao, R. *et al.* Dynamic Self-Assembly of Homogenous Microcyclic Structures Controlled by
475 a Silver-Coated Nanopore. *Small*. **13** (25), 1700234-n/a (2017).

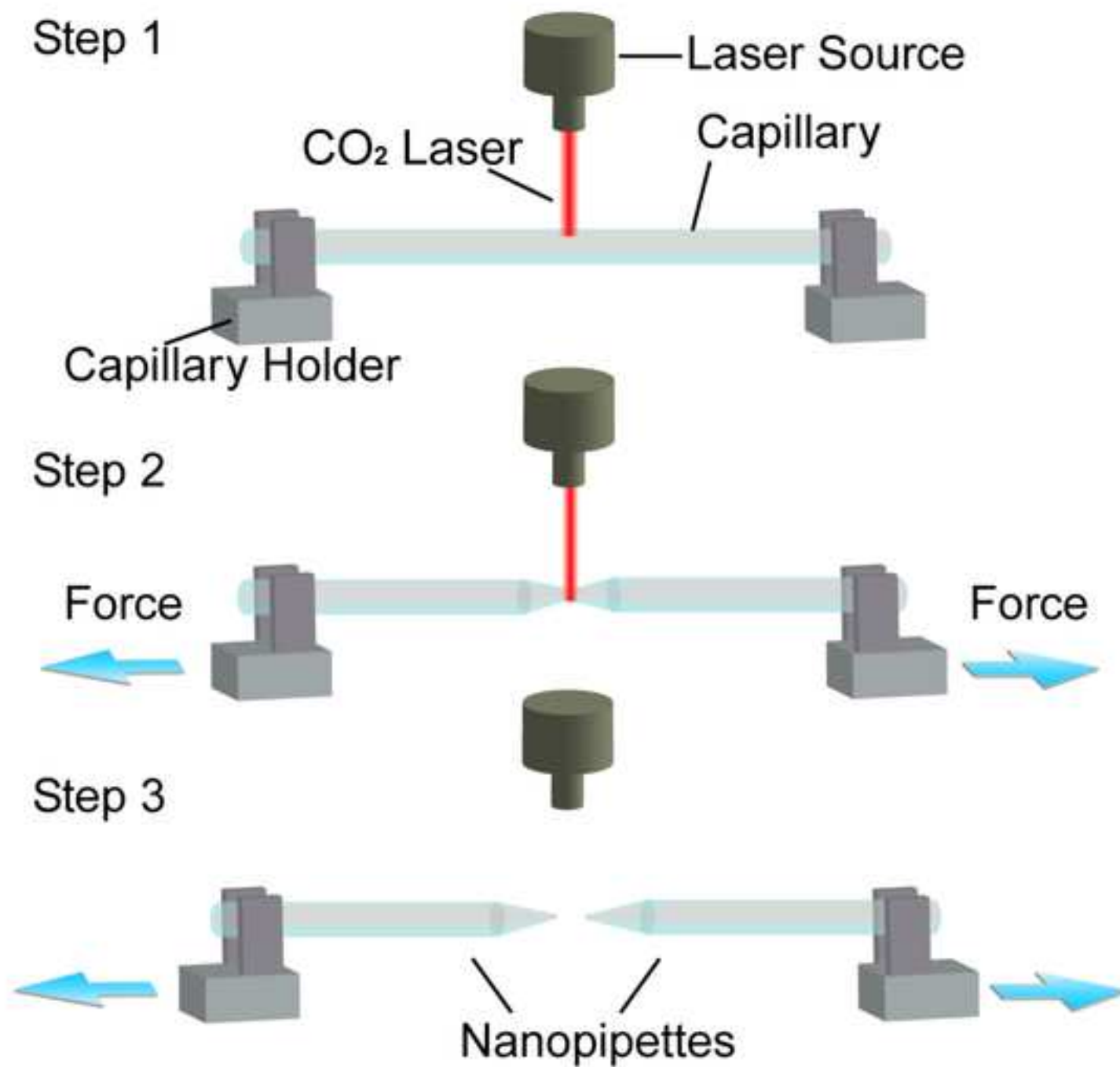
476 29 Kim, B. K., Kim, J., Bard, A. J. Electrochemistry of a single attoliter emulsion droplet in
477 collisions. *Journal of the American Chemical Society*. **137** (6), 2343-2349 (2015).

478 30 Lee, P. C., Meisel, D. Adsorption and surface-enhanced Raman of dyes on silver and gold
479 sols. *Journal of Physical Chemistry*. **86** (17), 3391-3395 (1982).

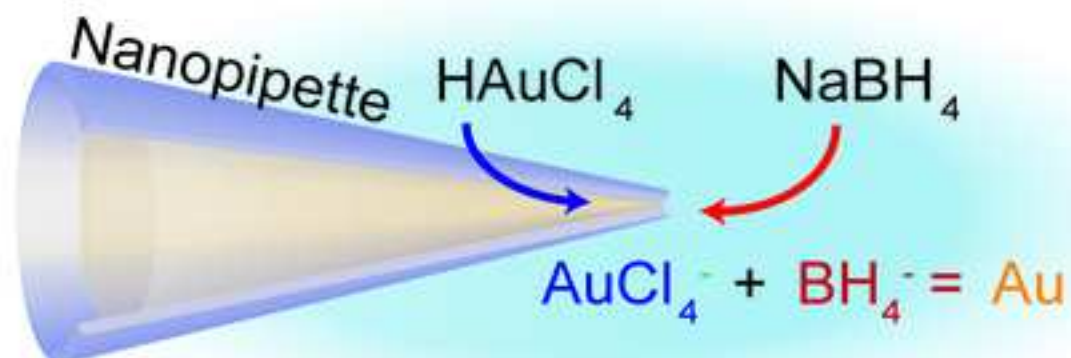
480 31 Steinbock, L. J., Otto, O., Chimere, C., Gornall, J., Keyser, U. F. Detecting DNA folding with
481 nanocapillaries. *Nano Letters*. **10** (7), 2493-2497 (2010).

482 32 Gong, X. *et al.* Label-free in-flow detection of single DNA molecules using glass
483 nanopipettes. *Analytical Chemistry*. **86** (1), 835-841 (2013).

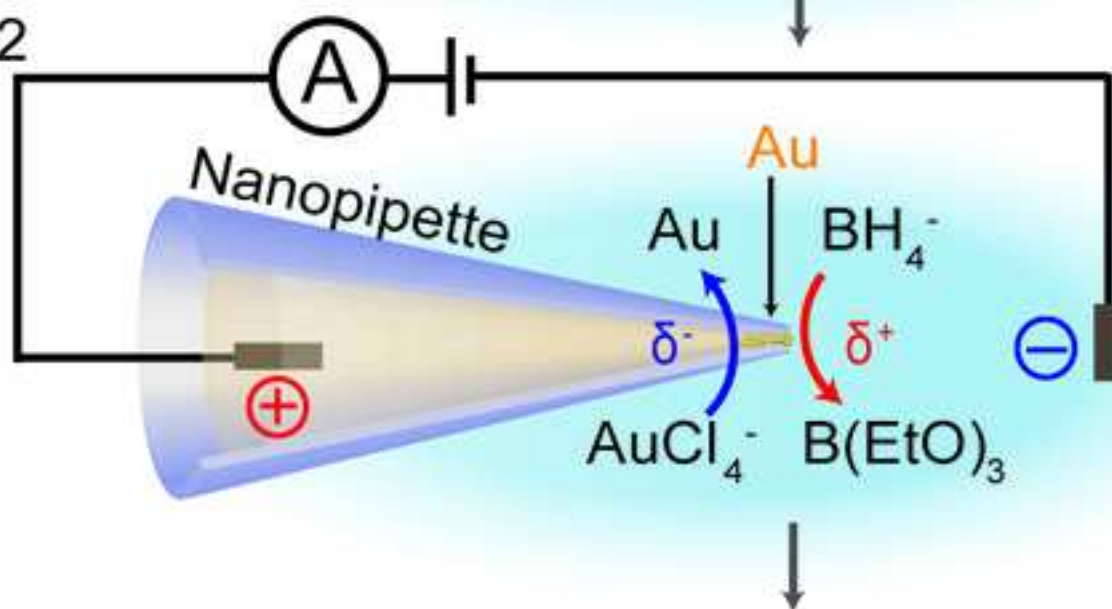
484 33 Cadinu, P. *et al.* Double barrel nanopores as a new tool for controlling single-molecule
485 34 Bell, N. A. & Keyser, U. F. Digitally encoded DNA nanostructures for multiplexed, single-
486 molecule protein sensing with nanopores. *Nature Nanotechnol.* **11** (7), 645-651 (2016).



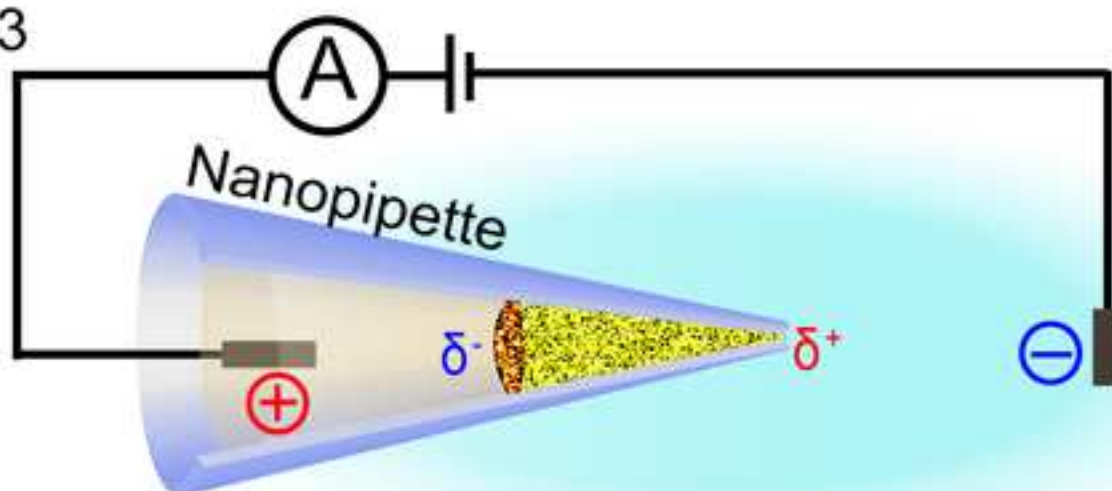
step 1



step 2



step 3



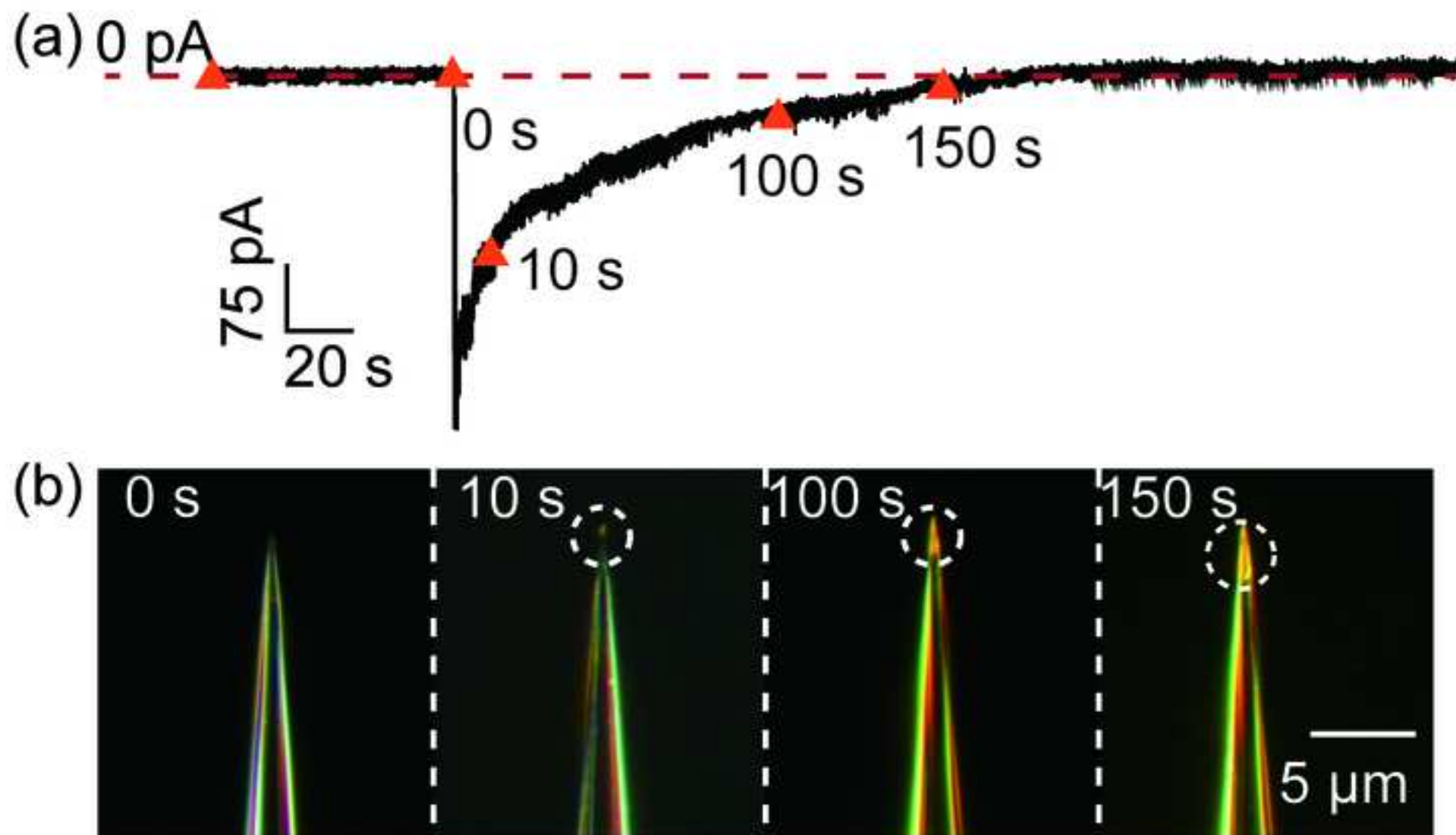
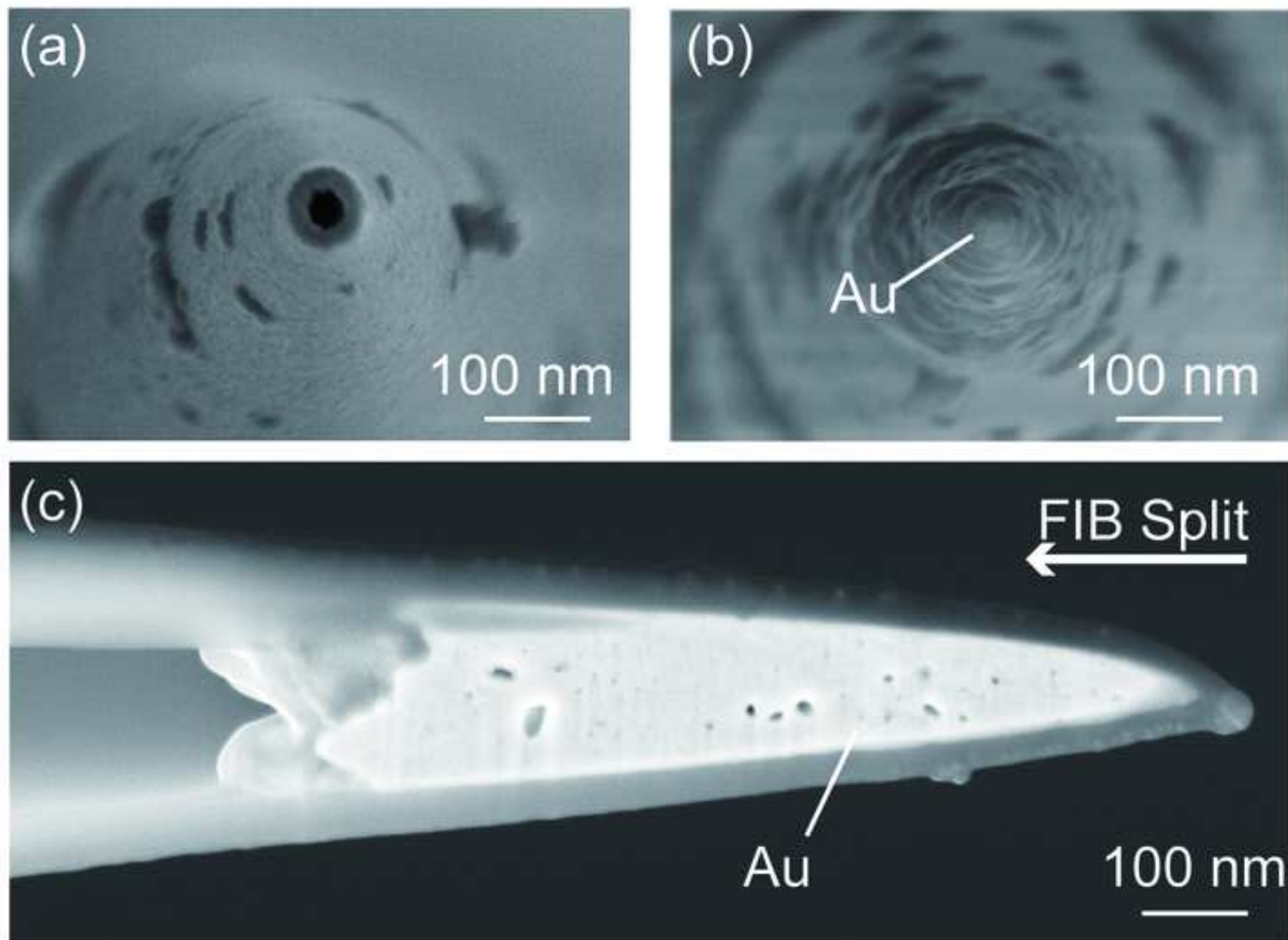
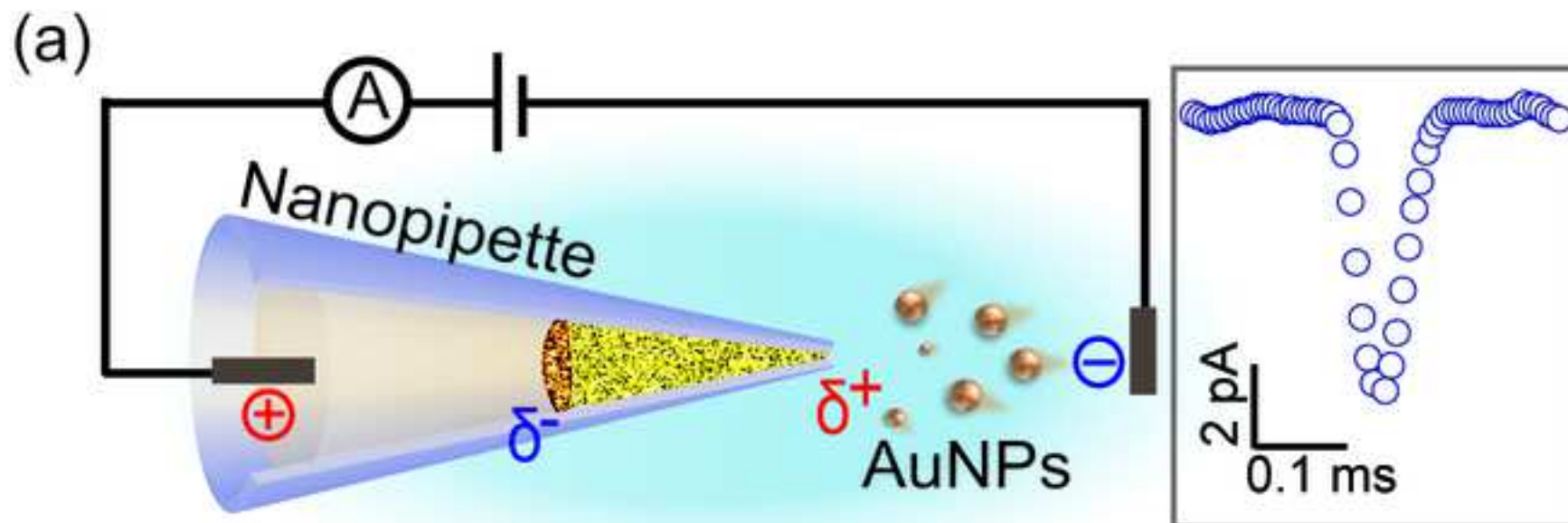
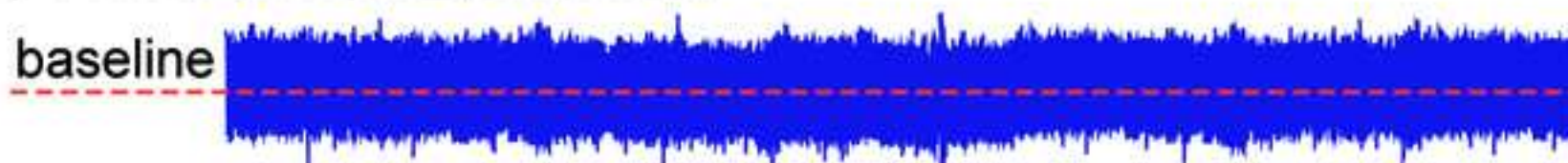


Figure 4

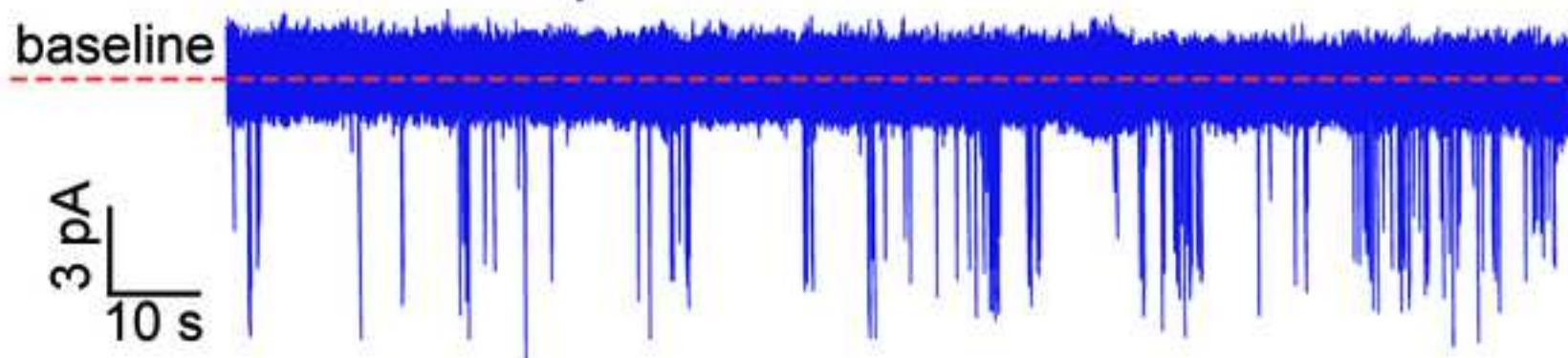




(b) Without nanoparticles



With 30 nm nanoparticles



PROJECT	HEAT	FIL	VEL	DEL	PUL
1	575	3	35	145	75
	900	2	15	128	200

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Acetone	Sigma-Aldrich	650501	Highly flammable and volatile
Analytical balance	Mettler Toledo	ME104E	
Axopatch 200B amplifier	Molecular Devices		
Blu-Tack reusable adhesive	Bostik		
Centrifuge tube	Corning Inc.		Centrifuge Tubes with CentriStar Cap, 15 ml
Chloroauric acid	Energy Chemical	E0601760010	HAuCl ₄
Clampfit 10.4 software	Molecular Devices		
Digidata 1550A digitizer	Molecular Devices		
DS Fi1c true-color CCD camera	Nikon		
Ecoflex 5 Addition cure silicone rubber	Smooth-On	17050377	
Eppendorf Reference 2 pipettes	Eppendorf	492000904	10, 100 and 1000 µL
Ethanol	Sigma-Aldrich	24102	Highly flammable and volatile
Faraday cage			Copper
iXon 888 EMCCD	Andor		
Microcentrifuge tubes	Axygen Scientific		0.6, 1.5 and 2.0 mL
Microloader	Eppendorf	5242 956.003	20 µL
Microscope Cover Glass	Fisher Scientific	LOT 16938	20 mm*60 mm-1 mm thick
Milli-Q water purifier	Millipore	SIMS00000	Denton Electron Beam Evaporator
P-2000 laser puller	Sutter Instrument		
Pipette tips	Axygen Scientific		10, 200 and 1,000 µL
Potassium chloride,+D25+A2:F2+A2:F25	Sigma Aldrich	P9333-500G	KCl
Quartz pipettes	Sutter	QF100-50-7.5	O.D.:1.0 mm, I.D.:0.5 mm, 75 mm length
Refrigerator	Siemens		
Silicone thinner	Smooth-On	1506330	
Silver wire	Alfa Aesar	11466	
Sodium borohydride,	ianlian Chem. Tech	71320	NaBH ₄
Ti-U inverted dark-field microscope	Nikon		



1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article:

A Closed-Type Wireless Nanopore Electrode for Analyzing Single Nanoparticles

Author(s):

Rui Gao[#], Ling-Fei Cui[#], Lin-Qi Ruan, Yi-Lun Ying* & Yi-Tao Long*

Item 1 (check one box): The Author elects to have the Materials be made available (as described at

<http://www.jove.com/author>) via: ☒ Standard Access ☐ Open Access

Item 2 (check one box):



The Author is NOT a United States government employee.



The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.



The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. **Defined Terms.** As used in this Article and Video License Agreement, the following terms shall have the following meanings: “**Agreement**” means this Article and Video License Agreement; “**Article**” means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; “**Author**” means the author who is a signatory to this Agreement; “**Collective Work**” means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; “**CRC License**” means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: <http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode>; “**Derivative Work**” means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; “**Institution**” means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; “**JoVE**” means MyJoVE Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments*; “**Materials**” means the Article and / or the Video; “**Parties**” means the Author and JoVE; “**Video**” means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. **Background.** The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. **Grant of Rights in Article.** In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the “Open Access” box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

ARTICLE AND VIDEO LICENSE AGREEMENT

4. Retention of Rights in Article. Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. Grant of Rights in Video – Standard Access. This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. Grant of Rights in Video – Open Access. This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. Government Employees. If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such

statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. Likeness, Privacy, Personality. The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. Author Warranties. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. JoVE Discretion. If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have

ARTICLE AND VIDEO LICENSE AGREEMENT

full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. **Indemnification.** The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in JoVE or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's


expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. **Fees.** To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US\$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. **Transfer, Governing Law.** This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to be one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

CORRESPONDING AUTHOR:

Name:	Yi-Tao Long	
Department:	Key Laboratory for Advanced Materials, School of Chemistry and Molecular Engineering	
Institution:	East China University of Science and Technology	
Article Title:	A Closed-Type Wireless Nanopore Electrode for Analyzing Single Nanoparticles	
Signature:		Date: Aug 27, 2018

Please submit a signed and dated copy of this license by one of the following three methods:

- 1) Upload a scanned copy of the document as a pdf on the JoVE submission site;
- 2) Fax the document to +1.866.381.2236;
- 3) Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02139

For questions, please email submissions@jove.com or call +1.617.945.9051

Response to the editorial comments:

1. There is currently no reuse permission for your Figures uploaded to Editorial Manager; please do so.

Response: We have uploaded "Copyright" PDF which contains all the reuse permission for our figures.

2. 3.2.2: How exactly do you centrifuge the nanopipette? E.g., what container is it in, which side is down, any special precautions, etc.?

Response: We have completed the information about how we exactly centrifuge our nanopipette as follow:

“3.2.2. Centrifuge the nanopipette for 5 minutes at a speed of around 1878 x g for the removal of the air bubbles in the nanopipette.

Note: Place the nanopipette into a homemade holder contained in a 2 mL centrifuge tube. The tip of nanopipette is placed down.”

3. 3.2.3: You earlier mention preparing silicon rubber on a slide (1.4); is this what you are referring to here?

Response: It is true that the prepared silicone rubber mentioned in (3.2.3) is actually the silicon rubber in (1.4). Revised as follow:

“3.2.3 Fix the nanopipette on a coverslip with the prepared silicone rubber (see procedure 1.4) and define the area inside the nanopipette as cis side and the outside as trans side.”

4. Results: Please define FIB.

Response: We have defined “FIB” in (3.3.3.), which refers to “Focus Ion Beam” as follow:

“Apply a 400 mV bias potential and use the EMCCD (see table of Materials) to monitor the fluorescence response at the tip area. Use focus ions beam (FIB) to sculpt the closed-type WNE from the tip to the bottom, and then determine the length of the interior metal layer or nanotip with SEM characterization.”

In compliance with data protection regulations, please contact the publication office if you would like to have your personal information removed from the database.

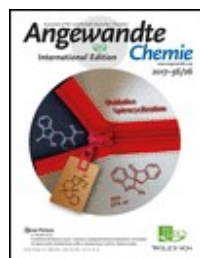


RightsLink®

Home

Account Info

Help



Title: A 30 nm Nanopore Electrode: Facile Fabrication and Direct Insights into the Intrinsic Feature of Single Nanoparticle Collisions

Author: Rui Gao, Yi-Lun Ying, Yuan-Jie Li, et al

Publication: Angewandte Chemie International Edition

Publisher: John Wiley and Sons

Date: Dec 21, 2017

Copyright © 2017, John Wiley and Sons

Logged in as:
Lingfei Cui
East China University of
Science and Technology

LOGOUT

Order Completed

Thank you for your order.

This Agreement between East China University of Science and Technology -- Lingfei Cui ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

Your confirmation email will contain your order number for future reference.

[printable details](#)

License Number	4413610850799
License date	Aug 21, 2018
Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	Angewandte Chemie International Edition
Licensed Content Title	A 30 nm Nanopore Electrode: Facile Fabrication and Direct Insights into the Intrinsic Feature of Single Nanoparticle Collisions
Licensed Content Author	Rui Gao, Yi-Lun Ying, Yuan-Jie Li, et al
Licensed Content Date	Dec 21, 2017
Licensed Content Volume	57
Licensed Content Issue	4
Licensed Content Pages	5
Type of use	Journal/Magazine
Requestor type	University/Academic
Is the reuse sponsored by or associated with a pharmaceutical or medical products company?	no
Format	Print and electronic
Portion	Figure/table
Number of figures/tables	3
Original Wiley figure/table number(s)	Figure 1 Figure 2 Figure 3

Will you be translating?	No
Circulation	5000
Title of new article	A Closed-Type Wireless Nanopore Electrode for Analyzing Single Nanoparticles
Publication the new article is in	Jove
Publisher of new article	Dr. Benjamin Werth
Author of new article	Rui Gao, Ling-Fei Cui, Lin-Qi Ruan, Yi-Lun Ying & Yi-Tao Long
Expected publication date of new article	Nov 2018
Estimated size of new article (pages)	5
Requestor Location	East China University of Science and Technology 130 Meilong Road, Xuhui District Shanghai City, China Shanghai, Shanghai 200237 China Attn: East China University of Science and Technology
Publisher Tax ID	EU826007151
Total	0.00 USD

Would you like to purchase the full text of this article? If so, please continue on to the content ordering system located here: [Purchase PDF](#)

If you click on the buttons below or close this window, you will not be able to return to the content ordering system.

ORDER MORE

CLOSE WINDOW

Copyright © 2018 [Copyright Clearance Center, Inc.](#) All Rights Reserved. [Privacy statement.](#) [Terms and Conditions.](#)
Comments? We would like to hear from you. E-mail us at customercare@copyright.com