Analyst

A Troubleshooting Guide for Laser Pulling Platinum Nanoelectrodes

Journal:	*Analyst*
Manuscript ID	AN-ART-02-2023-000268.R1
Article Type:	Paper
Date Submitted by the Author:	12-Apr-2023
Complete List of Authors:	Lim, Koun; University of Utah, Department of Chemistry Goines, Sondrica; The University of North Carolina at Chapel Hill, Chemistry Deng, Mingchu; The University of North Carolina at Chapel Hill, Chemistry McCormick, Hadley; The University of North Carolina at Chapel Hill, Chemistry Kauffmann, Philip; Purdue University, Chemistry Dick, Jeffrey; Purdue University, Chemistry; The University of North Carolina at Chapel Hill, Chemistry

A Troubleshooting Guide for Laser Pulling Platinum Nanoelectrodes

Koun Lim[a, §], Sondrica Goines[a, §], Mingchu Deng[a], Hadley McCormick[a], Philip J. Kauffmann[c], Jeffrey E. Dick*[a, b,c,d]

[a]Department of Chemistry, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

[b]Lineberger Comprehensive Cancer Center, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

[c]Department of Chemistry, Purdue University, West Lafayette, IN 47906, USA

[d]Elmore Family School of Electrical and Computer Engineering, Purdue University, West Lafayette, IN 47906, USA

*To whom correspondence should be addressed: jedick@email.unc.edu

[§]These authors contributed equally to this work.

ABSTRACT

While there are numerous publications on laser-assisted fabrication and characterization of Pt nanoelectrodes, the exact replication of those procedures is not as straightforward as following a single recipe across laboratories. Often, the working procedures vary by day, by laser puller, or by person. Only a handful of nanoelectrode fabrication papers record their parameters, and even fewer offer troubleshooting advice. Here, we provide a step-by-step guide for laser-assisted Pt nanoelectrode fabrication using low-cost equipment including a laser-puller, voltammetry, and simple microscope images captured via cell phone. We also offer solutions for common failures experienced throughout the process to guide beginners as they troubleshoot their own fabrication procedures.

KEYWORDS

Research, Analytical Chemistry, Testing, Lasers, Nanotechnology

With heightened interest in higher sensitivity measurement science, electrochemists have geared up to discover new truths of nature within complex systems via nanoelectrochemistry. Characterized by increased

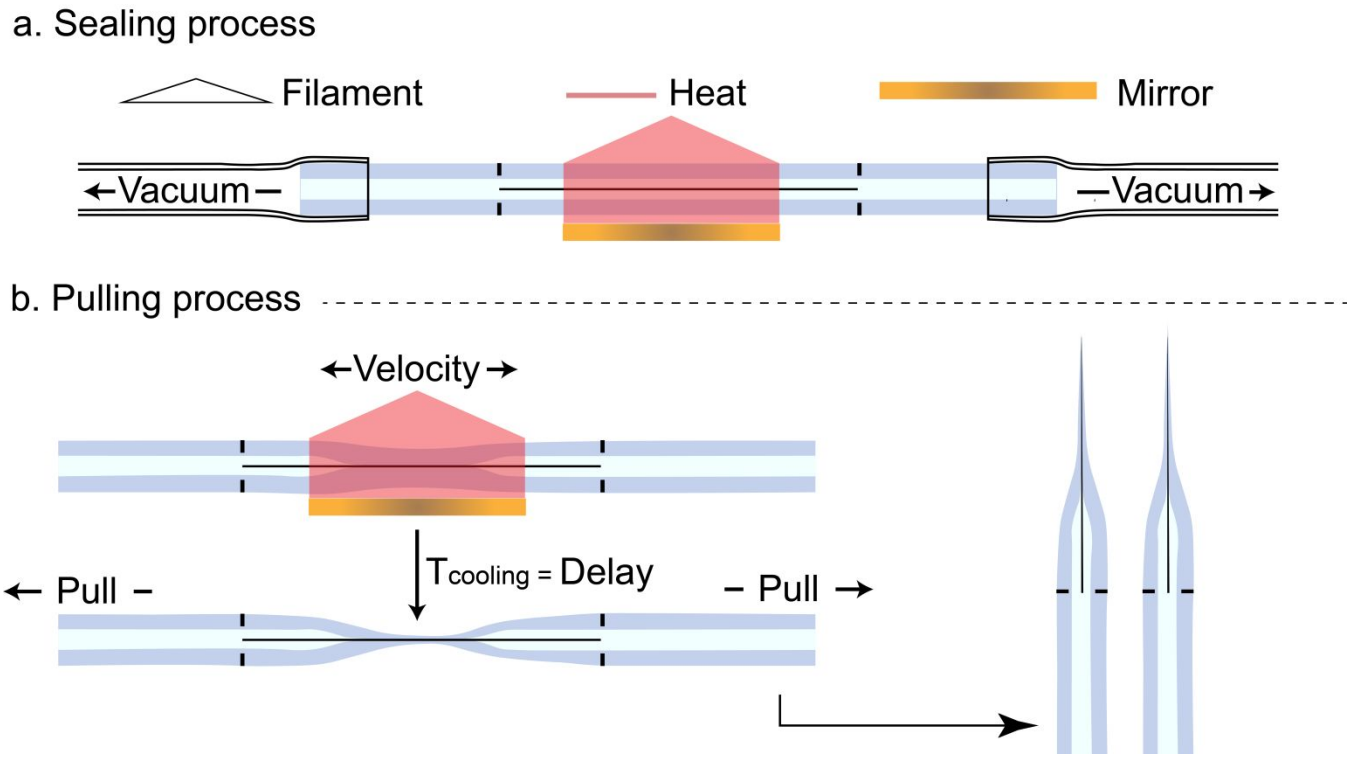
signal-to-noise and decreased double-layer capacitance[1], nanoelectrodes are designed to probe the limits of electron-transfer kinetics[2, 3] and study the redox chemistry of single entities. In particular, nanoelectrodes have been used to study single metal crystals[4], single nanoparticles[5], and single molecules (*e.g.,* single enzymes[6]) as well as to elucidate dynamic changes in mass transport at the nanoscale level within materials chemistry and biochemistry[7]. Additionally, nanoelectrodes are invaluable tools for studying intracellular redox chemistry, especially those that have been functionalized to be metabolite specific[8,9]. Numerous procedures detail the fabrication of submicron electrodes via lithography[10], electrochemical deposition[11], electrochemical etching[12-13], laser pullers[14-16], focused ion beam (FIB) milling[17], and chemical vapor deposition[18]. Some fabrication methods even use a combination of techniques[19, 20] or unique techniques, such as interfacial reactions[21], to obtain the desired electrode material and size.

Laser-assisted fabrication using laser-based micropipette pullers (*i.e.,* laser pullers) is very popular due to its ease and safety.[2, 14-16, 22-25] However, fabrication procedures using laser pullers initially included little to no information on the specific pulling parameters.[22, 23] Since then, more publications have offered insight into laser-assisted nanoelectrode fabrication. Katemann *et al*. were among the first to report their laser puller parameters, while noting the challenge of reproducibly sealing and pulling electrodes in this manner.[14] Some reports have modified the fabrication process in an effort to reproducibly create submicron electrodes.[15, 16] However, none of the referenced publications provide an in-depth look at laser puller parameters, parameter effects on the outcome of electrode fabrication, or how to adjust these parameters based on electrode outcomes. Thus, despite the number of publications that used laser-assisted fabrication procedures, specific fabrication guides are difficult to find, which in turn make the reproduction of such protocols challenging. This is not only because these electrodes have fragile tips leading to significant random errors and various defects[24] when used, but also because of systematic differences between instrumentation.

Here, we carefully varied each parameter involved in laser-assisted submicron and nanoelectrode fabrication to guide a user in finding a parameter setting specific to the laser puller available to them. Our goal is to outline troubleshooting guidelines in each step of the laser pulling process without the need for less-accessible equipment such as TEM imaging or ion beam polishing. We note that we focus on the fabrication of nanoelectrodes and not

nanoelectrode polishing, a complex step that is necessary to reproducibly fabricate useful nanosensors. In addition, we found that the specific parameters used to create working nanoelectrodes varied from one instrument to the other, and so in some cases specific values are not provided as they may not work with a different puller. We provide our values as a general starting point, but each user may need to adjust depending on the issues encountered during or after the completion of the fabrication process.

Typically, nanoelectrode fabrication using a laser puller has two parts: 1) the sealing process where a quartz capillary is heated under vacuum to encase a Pt wire (**Scheme 1a**), and 2) the pulling process where the Pt-sealed quartz capillary is pulled into two separate pieces with a fine tip (**Scheme 1b**). Ultimately, there are six parameters involved in the fabrication process: 1) vacuum, 2) heat, 3) filament, 4) velocity, 5) delay and 6) pull. Here, we varied each parameter to show the optimization of a laser-assisted fabrication procedure. The procedure outlined specifically works with 0.025 mm Pt wires and quartz capillaries (ID:0.3 mm, OD: 1 mm). In all experiments, a Sutter P2000 laser puller was used.



Scheme 1. A schematic illustration of submicron electrode fabrication using a Sutter P2000 laser puller. The first step in the fabrication is to seal a Pt wire in a quartz capillary (a) using a program involving filament, heat and vacuum and the second step in the fabrication is to pull the Pt-sealed quartz capillary (b) using a program involving heat, filament, velocity, delay and pull.

MATERIALS AND METHODS

Laser-based micropipette puller systems (Model P-2000) and quartz capillaries (ID:0.30 mm, OD: 1.0 mm, Item#: Q100-30-15) were purchased from Sutter Instrument Company and Pt wire with a diameter of 0.025 mm (Purity: 99.99%, PT005114) was purchased from Goodfellow. BV-10 microelectrode beveler and its corresponding diamond abrasive plates (*i.e.,* 104C – coarse, 104D – fine, 104E – very fine, and 104F – extra fine) were purchased from Sutter Instrument Company. Nichrome wire (0.25 mm, product# 13082) was purchased from Ted Pella and tungsten wires (W559504) were purchased from Advent. Tinned copper wire (30 AWG) was purchased from Treedix. A rotary vacuum pump (RZ 6) was purchased from Vacuubrand. Vacuum tubes were purchased from Fisher Scientifics (60985-540, 14-469-1A) and New Age Industries (1400154). All other chemicals were purchased from Sigma-Aldrich.

From the vacuum pump to the laser puller, a series of vacuum tubes were connected. Lastly, a Y-adaptor was used to part a single vacuum line into two using smaller vacuum tubes that tightly wrap around each end of the quartz capillary. A small hole was drilled on each side of the laser puller cover to bring the vacuum tubes in (**Figure S1**). The quartz capillaries were always handled with gloves to avoid smudging the glass. When necessary, the outer glass was cleaned with acetone or isopropyl alcohol prior to loading with Pt wire. A single Pt wire (approximately 4 cm long) was loaded into a clean quartz capillary, then pushed into the center of the capillary with a nichrome wire (Ted Pella, 0.25 mm, product #13082). The location of the ends of the Pt wire was marked on the quartz capillary using a Sharpie marker to indicate the location of the Pt wire. Marking the capillaries in this way allowed us to better visualize if the Pt wire was centered within the capillary. At least five quartz capillaries were loaded with Pt wire before the sealing process. We found it useful to have multiple capillaries ready for fabrication, as events that render electrodes useless (melting the wire, chipping the capillary, etc.) can be frequent as one learns how to pull nanoelectrodes. Before placing the Pt wire-loaded capillary into the laser puller, the Sharpie marks were removed with acetone or isopropyl alcohol to prevent debris from coating the

gold plated retro mirror of the puller during heating. Next, the puller bars of the instrument were held in place with horizontal metal bars (2'' × 1'') placed between the center bearings and the puller bars (**Figure S2a**). The Pt wire-loaded capillary was centered in the laser puller using the clamping knobs (**Figure S2b**). Once the Pt wire-loaded quartz capillary was centered and secured in the laser puller, a vacuum line was connected on each end of the quartz capillary (**Figure S2c**). The vacuum was turned on for at least 2 minutes prior to the sealing process and stayed on until the end of the sealing process. The placement of each clamp within the laser puller was marked on the quartz capillary using a marker to approximate the placement of the quartz capillary within the puller throughout the entire process. A bench-top upright microscope was used to examine the sealing process between cycles to determine the status. All images used in this publication were taken with a personal smartphone, Galaxy S9+, by aligning the phone with an eyepiece of the microscope.

One cycle of the sealing process means turning the laser on and off once. For a complete seal, the laser was turned on for 30 seconds and off for 30 seconds. Generally, this cycle was repeated 4 times. While the on and off time for the laser can be arbitrarily picked, it is advised for the laser safety to turn off the laser as long as the laser was turned on. It is also possible to reduce the number of cycles and increase the laser exposure time to complete the seal. Experimentally, the laser puller was automatically turned off after approximately a minute of constant exposure. Therefore, it is possible to heat the Pt wire-loaded capillary for longer but, in order to keep the laser safe and constant control over the variables within the fabrication procedure, the laser was turned on for 30-second intervals.

Here, two Sutter P2000 laser pullers were used to prepare submicron electrodes. Therefore, parameter values featured in figure captions may vary based on the puller in use, however the troubleshooting steps outlined here may be used consistently to optimize fabrication procedures across laboratories. For an optimal seal using laser puller #1 (used in **Figures 1-5** & **7**), the puller was programmed: Heat: 840, Filament: 5, Velocity: 120, Delay: 129, Pull: 0. However, the only important parameters were heat, filament, and pull settings. The other parameters (velocity and delay) could be arbitrarily set. For the subsequent pulling process, the puller was programmed: Heat: 817, Filament: 2, Velocity: 120, Delay: 128, Pull: 250. **Table 1** summarizes the suggested parameters for puller #1, as well as the effect each parameter has on the outcome of the electrode fabrication procedure. The

voltammograms featured in **Figures 6** & **8** were prepared using a second Sutter P2000 laser puller. To seal the Pt wire featured in **Figure 6**, puller #2 was programmed: Heat: 700, Filament: 4, Velocity: 60, Delay: 140, Pull: 1. To pull the final electrode, the puller was programmed: Heat: 695, Filament: 2, Velocity: 60, Delay: 100, Pull: 200. To seal the Pt wire featured in **Figure 8**, puller #2 was programmed: Heat: 775, Filament: 5, Velocity: 120, Delay: 129, Pull: 1. For the subsequent pulling process, the puller was programmed: Heat: 800, Filament: 3, Velocity: 120, Delay: 128, Pull: 200. It is important to note, the pullers are labeled #1 and #2 based on the order in which they were purchased. Furthermore, the laser pullers differ significantly in the parameters used to seal and pull electrodes, not only because of inherent differences between instruments, but also because puller #1 has been in use for a longer period of time than puller #2. It was apparent that the laser may become weaker overtime based on how much higher the heat setting must be for puller #1 compared to puller #2 to seal an electrode. Thus, the associated parameter values are provided as an initial reference and may be subject to optimization using the steps outlined in this manuscript.

Table 1. Optimal Parameter Settings & Summarized Parameter Effects

Parameter	Vacuum	Heat	Filament	Velocity	Delay	Pull
Increasing Values	Displaced wire	Melted wire	Longer seal area (seal); larger radii (pull)	Thinner outer glass	Higher noise	Smaller electrode radii
Decreasing Values	Unsealed wire	Unsealed wire	Shorter seal area (seal); smaller radii (pull)	Thicker outer glass	Smaller radii	Larger electrode radii
Starting Seal Settings	Strong	840	5	120	129	0
Starting Pull Settings	N/A	817	2	120	128	250

THE SEALING PROCESS

Sealing a Pt wire in a quartz capillary requires reforming the quartz capillary to encase the Pt wire. A complete seal shows a smooth and tight encasing of the quartz around the Pt wire without degradation (**Figure 1a**). A simple microscopic examination of the seal is important in ensuring a complete seal from all angles and higher magnifications (**Figure 1b**). The final check of a good seal is to perform cyclic voltammetry to see if there is capacitive current introduced from a poor seal. A successful sealing process is governed by 3 parameters: 1) vacuum, 2) heat, and 3) filament. Specifically, finding a combination of heat and filament under a sufficient vacuum is key to the sealing process.

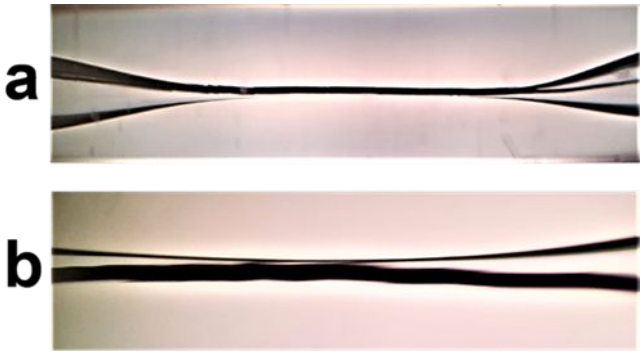


Figure 1. Representative microscopic images of a complete seal (a) using 10x objective and an incomplete seal (b) observed using a 20x objective of a bench-top upright microscope. All images were taken using a cellphone by aligning it with the eyepiece of the microscope.

Vacuum and Heat

For vacuum, it is either strong enough or not. Typically, if the vacuum strength was not sufficient, the seal didn't occur or didn't complete despite the number of sealing cycles. As a result of increasing the sealing cycles, the capillaries show uneven and elongated deformation of capillaries without a complete seal (**Figure S3**). A sudden inability to seal is typically related to a loss of vacuum strength due to clogged vacuum tubes, cracked quartz capillaries, or defective machines. On the other hand, a sufficient or strong vacuum may result in drawing the Pt wire out of the quartz capillary. If this occurs, the glass capillary can be pre-thinned before introducing Pt wire into the capillary by running one or two heat cycles and then inserting the Pt wire and finishing the sealing process. Careful consideration must be taken to not thin the glass such that the Pt wire cannot be inserted into the capillary. Once Pt wire is inserted into the capillary, both ends of the quartz capillary should be connected to the vacuum lines before turning on the vacuum so that an uneven vacuum pull does not dislodge the Pt wire in one direction.

Similar to vacuum, the heat parameter is either high enough or not. Usually, the heat was applied at 30-second intervals (30 seconds on/30 seconds off) over the course of 3-4 cycles to ensure that the outer and inner diameters would shrink to make a proper seal. The cycle number was often adjusted to complete a seal if a seal was almost complete (**Figure 1b**). Otherwise, after finding an optimal heat parameter, a constant 4 cycles with 30-second intervals were used as the standard procedure. Moreover, even with the appropriate heat setting, there were occasions where the loss of the Pt wire integrity was observed when the wire itself was damaged prior to the sealing process (**Figure S4**). Thus, in order to find the heat setting most suitable for the sealing process, it was crucial to always insert clean and unused Pt wires with care to ensure the conservation of Pt wire integrity.

To elucidate the correlation between the heat value and the seal status, the protocol was set to vary heat but kept other parameters constant: Filament:5, Velocity: 120, Delay: 128, Pull: 0, 30-second interval, 4 cycles. If the heat setting was too high, the inner diameter started to completely seal, resulting in a complete blockage with a line of melted Pt wires tracing where the inner capillary was (**Figure 2a**). The inner quartz reached the melting temperature of the Pt wire and the Pt wire started to melt forming spheres prior to the seal completion. As the heat started to approach the proper heat value, the Pt wire was partially sealed but still disconnected in the middle

where the heat was applied (**Figure 2b**). When the vacuum and heat were sufficient, a complete seal was achieved (**Figure 2c**). On the contrary, if the heat was insufficient, the seal wasn't complete (**Figure 2d**), compromising the Pt wire integrity at times (**Figure 2d**) even with a clean and uncompromised wire in the beginning. We must emphasize that the ideal heat setting may vary when using a different puller, or if alternative glass and wire material are used. Thus, we recommend a starting value of 840 for our successful sealing of platinum wire in a quartz capillary (**Figure 2**), but the value may need to be adjusted. The heat values were typically adjusted in units of 10, 5, or 1 depending on the seal status. However, when changing the heat parameter by a unit of 1 either resulted in melting of the Pt wire or an incomplete seal (**Figure 2d**), the filament was often changed as an easier and faster option for obtaining a complete seal.

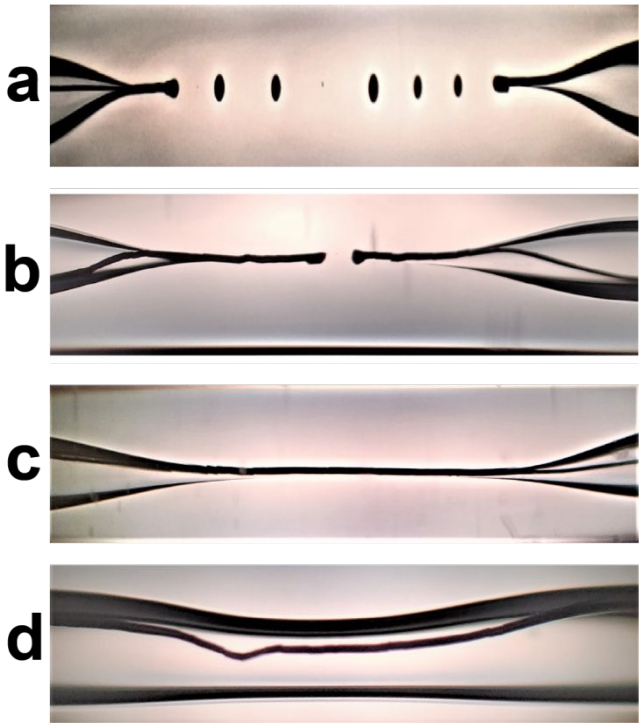


Figure 2. The effect of heat on the sealing process while keeping the other parameters constant (Filament: 5, Velocity: 120, Delay: 128, 30 seconds on/off, 4 cycles). A melted and discontinued Pt wire (a) was due to the heat setting too high (Heat: 880). A melted and discontinued Pt wire with a seal (b) was due to the heat setting approaching the appropriate heat value (Heat: 865). (c) A smooth and complete seal was observed (Heat: 840). Lastly, an incomplete seal was observed when the heat setting was too low (Heat:800). When the heat is too low, more heating cycles are needed, and so at times the integrity of the Pt wire was compromised (d). All images were taken using a cellphone by aligning them with the eyepiece of the microscope at 10x objectives.

Filament

The filament is defined as a distribution of heat (**Scheme 1a**). Higher values of filament indicate a wider distribution of heat. Here, it is essential to know that there is a total of 16 values (0-15) available for the filament parameter, but the P-2000 laser-based micropipette puller only supports values up to 5. As the manual states, any value above 5 repeats the previous heat distribution pattern (**Figure S5**); thus, 1, 6, and 11 are supposed to be the same value, as are 2, 7, 12, etc. The experimentally varied filaments confirmed a distribution pattern (**Figure 3** and **S6**). However, laser puller #1 showed a change of heat distribution among filaments from 0-6 (**Figure 3**), not just 0-5 as suggested by the manual. Therefore, we highly recommend testing this systematic difference between each laser puller on the filament distribution while holding the heat value constant. Once the first distribution pattern is observed on the glass capillaries (i.e. the heat distribution for filament settings 0-5), we advise not using the "repetitive" filament values (such as 6-10 or 11-15).

A higher heat value is used with a higher filament resulting in a wider seal. Increasing the heat with the filament value is necessary to apply similar heat across a wider section of the capillary. Increasing the filament without also increasing the heat will result in an insufficient seal. For our puller, we generally found that when the filament was increased by 1, the heat value needed to also be increased by approximately 60-100 for a complete sealing process (**Figure S7**). Filament values lower than 3 harshly degraded Pt wires before a complete seal was obtained, and filament values above 5 were avoided due to the repeating heat distribution. Thus, the filament value was varied from 3 to 5 to elucidate the effect of filament on the sealing process.

Comparing 3 completely sealed quartz capillaries under three different filaments, a filament of 3 completed a seal with the shortest seal area (**Figure 4a**). Next, a higher heat setting was used for a filament of 4 to complete a seal (**Figure 4b**). Despite the higher heat setting, the integrity of the Pt wire was maintained. Lastly, a filament of 5 needed the highest heat setting to seal the Pt wire without damage (**Figure 4c**), and the seal area was the longest among the varied filament values. Therefore, when it came to sealing a Pt wire in a quartz capillary, using a higher filament such as 4 or 5 was more forgiving in terms of the fluctuation of heat values or the duration of heat application.

In summary, for a complete and efficient sealing process, ensuring that the vacuum is sufficient, try a starting heat value of 680-740 for a filament of 4 or a starting heat value of 750-860 for a filament of 5. However, it is important to note that these values of heat and filament are dependent on the current status of the laser puller. Therefore, it is advised to compare the filament effects first and then, set the heat. Other researchers have reported the pre-thinning of the glass before threading a Pt wire into the quartz capillary[15]. While this is feasible, the heat set for the seal after the pre-thinning would be different, leading to another optimization step. Therefore, in this paper, the pre-thinning of the quartz capillary is not discussed.

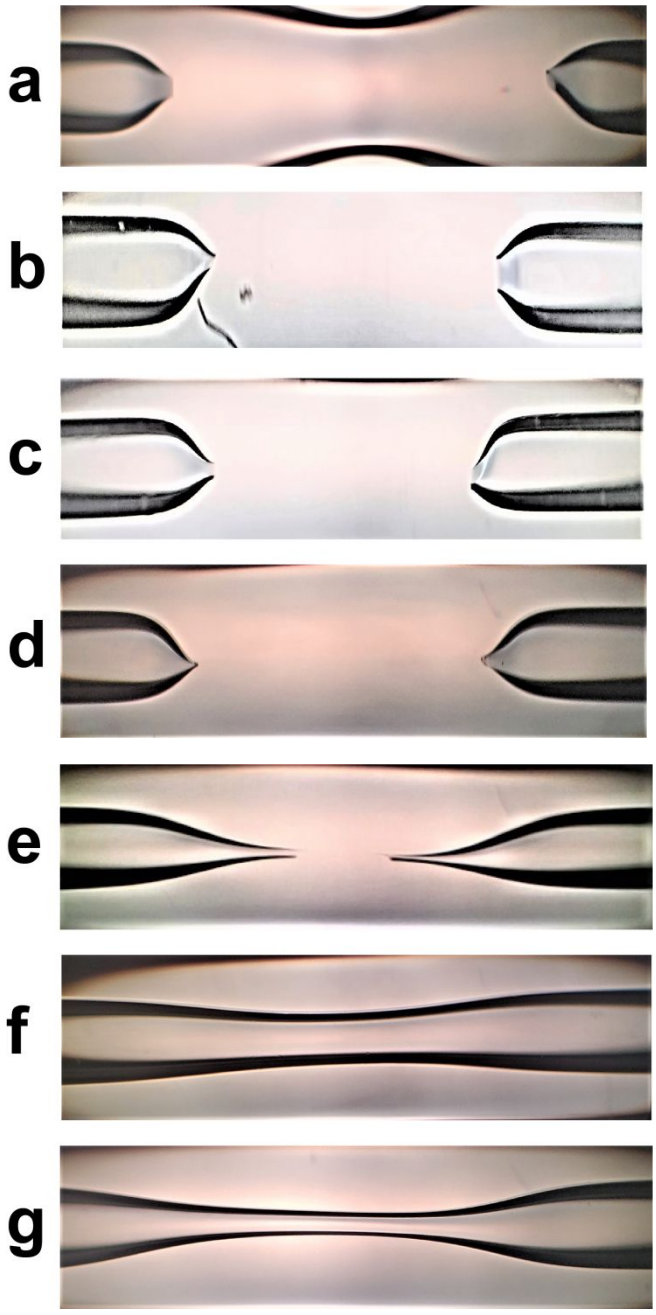


Figure 3. The difference in a heat distribution length according to a filament value. Using a constant temperature of 740, a filament range from 0 (a) to 6 (g) were tested, revealing the first set of distribution pattern. All images were taken using a cellphone by aligning it with the eyepiece of microscopes at 10x objectives.

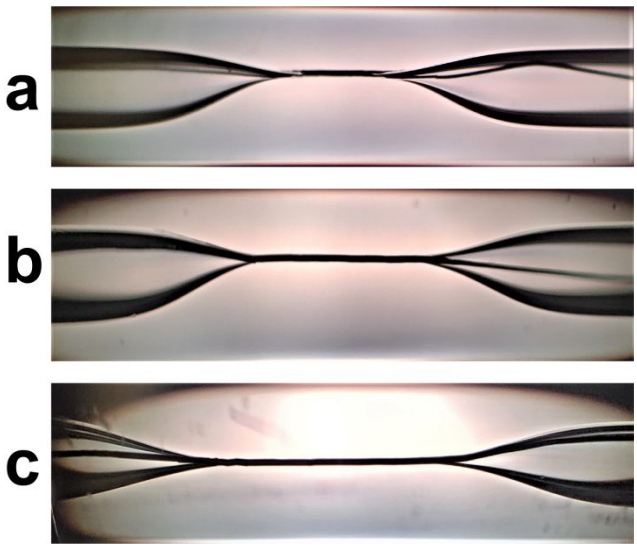


Figure 4. The effect of filament values on the sealing process. While all filament values from 3(a) to 5(c) made a complete and smooth seal, a higher filament value produced a longer seal area. All images were taken using a cellphone by aligning it with the eyepiece of microscopes at 10x objectives.

THE PULLING PROCESS

Under the assumption that the seal was complete and smooth, pulling submicron or nanoelectrodes is governed by 5 parameters: 1) heat, 2) filament, 3) velocity, 4) delay and 5) pull. According to the laser puller manual, the resulting electrode radius is smaller when all the parameters except delay are higher. Thus, the pull value was typically set at a value ≥200 to give a smaller radius. For nanoelectrode fabrication (*i.e.*, radii < 500 nm), we suggest a pull setting of 250, the highest number possible for the pull value. The other parameters were varied systematically to elucidate their effects on laser-assisted fabrication.

Filament

When deciding a filament value, the easy rule is to use a higher filament value for sturdy and longer tips with a bigger radius and a smaller value for flexible and shorter tips with a smaller radius. When a filament of 5 or 4 was used, the resulting electrodes were longer and physically harder at the tip with more quartz surrounding the Pt wire (**Figure 5a**). On the other hand, when a filament of 2 or 3 was used, the resulting electrodes were shorter and more flexible (**Figure 5b**). Thus, the filament can be varied depending on the experimenter's goals. We noticed a trend that when a filament of 4 or 5 was used, a larger capacitive current was observed. We also noticed that the Pt wire of these electrodes were often pinched (**Figure S8**), although why this may contribute is not clearly understand. Most likely, the capacitance is due to a poor seal, in which case the electrode should be examined

under the microscope to see if any defects in the seal can be observed. If it is an issue with the sealing, the following steps may need to be taken on the next electrode: ensure that the vacuum is strong and not obstructed by anything; increase the heat setting for the seal process; or add one or two extra heat cycles with the previous heat setting. Our highest success rate was found using a filament of 2 or 3, and so we recommend this as a starting point for nanoelectrode fabrication.

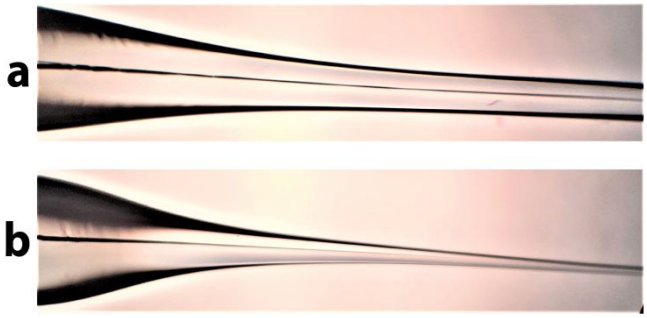


Figure 5. The different outer diameters of quartz rod depending on the filament setting during the pull setting. Using a higher filament such as 5 (a), the electrodes are pulled with more quartz rod expanded, resulting in a physically sturdy electrode with a bigger radius. Using a lower filament such as 2 or 3 (b), the electrodes are pulled with a finer tip, resulting in the more fragile electrode with a smaller radius. All images were taken using a cellphone by aligning it with the eyepiece of microscopes at 10x objectives.

Delay, Velocity, and Heat

The delay value indicates the cooling time between the heat off and the hard pull setting. Any value above 128 means there is a (delay – 128) ms between the laser off and the start of the hard pull. A delay of 128 means the hard pull follows immediately after the laser is turned off. Lastly, any delay value below 128 means the hard pull is activated while the laser is on, and then, the laser is turned off after a (128 – delay) ms. Thus, if the delay was set for 130, it resulted in a 2 ms delay between the laser off and the hard pull. If the delay was set for 126, it resulted in a hard pull, and then the laser was turned off after a 2 ms delay. As previously mentioned, smaller delay values result in a smaller radius (**Figure 6** vs **Figure 8**), as the wire has less time to cool before being pulled, which in turn allows for the softened wire to be stretched further. However, when the delay parameter is set below 128, the resulting electrodes sometimes show electrical noise (**Figure S9**). This noise could be electromagnetic noise which was not eliminated despite the use of a Faraday cage and proper grounding of the

electrochemical setup. Another reason could be that when the delay is < 128, the pull occurs before the laser is turned off. This exposes the fragile electrode tip to the laser, likely inducing defects that result in noise. Thus, while a nanoelectrode can be pulled with a delay below 128, we had the most success with a delay of 128.

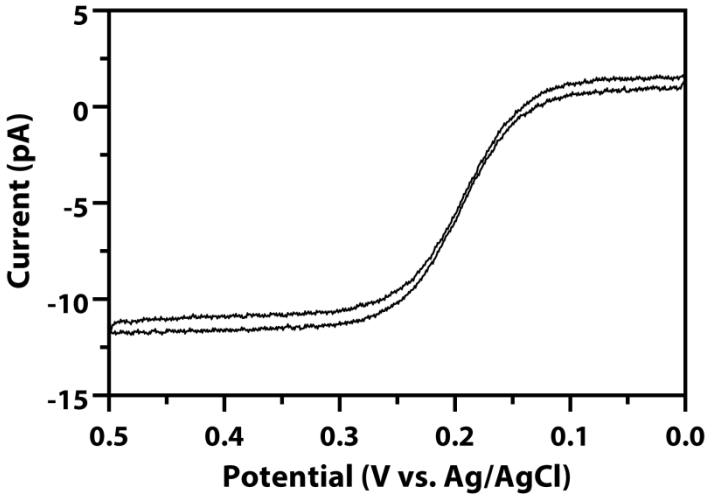


Figure 6. Representative cyclic voltammogram of nanoelectrode fabricated with a delay of 100. Nanoelectrode (r = 88 nm) fabricated with laser puller #2 [Seal (30 s on/30 s off, 4×) – Heat: 700, Filament: 4, Velocity: 60, Delay: 140, Pull: 1; Pull – Heat: 695, Filament: 2, Velocity: 60, Delay: 100, Pull: 200]. Voltammogram captured in 0.5 mM ferrocenemethanol in Dulbecco's Phosphate Buffered Saline (1X) vs. Ag/AgCl (1 M KCl), using a CHI potentiostat, plotted in the US convention.

Next, velocity indicates the speed that the soft pull force must be moving in order to execute the hard pull. There is a small, constant force (soft pull) from the pull bars tugging the glass capillary in opposite directions. When no heat is applied, the rigid glass will be unmoved. As heat is applied at the center, the two ends of the capillary begin to pick up speed in opposite directions. When this speed reaches the program velocity value, the delay parameter takes effect. Thus, the velocity values are indirectly connected to the temperature of the glass. Lower velocities will be reached with less heat, while higher velocity values require the glass to be hotter before the hard pull is initiated. Previously, it has been reported that nanoelectrodes with exceptionally small radii (*i.e.,* radii ≤ 50 nm) can be pulled when the ratio of the diameter of the Pt wire to the outer diameter of the quartz capillary is between 0.2 to 0.4[23]. Thus, if Pt wires of 25 μm diameter were used, the outer diameter should be between 62.5 and 125 μm. However, the exact variance of the outer diameter as the capillary softens is a

challenge to know specifically. Intuitively, a higher velocity will result in softer glass when the hard pull is initiated, and so should result in a thinner layer of outer glass. We fabricated working electrodes with a velocity of 120 for puller #1, and a velocity of 60 for puller #2. Thus, depending on equipment used and the user's desired application, an appropriate velocity can be determined for a thinner or thicker outer layer of glass.

Interconnected to velocity is the heat value. A higher heat value can accommodate a higher velocity with even taper lengths. Technically, the heat value can be varied, but the electrodes should hard pull within 4-8 seconds for the best reproducibility (this value is displayed once the program completes). We used a higher heat value that pulled around 4 seconds because the higher heat typically results in a smaller radius. As the user begins to familiarize themselves with the parameters and behavior of the instrument, blank quartz capillaries can be used to discern if a given heat pull program (heat, filament, velocity, delay, and pull) will reproducibly hard-pull within the 4-8 second window. Overall, under a given filament of 2 or 3 and a delay setting of 128, the starting values of heat and velocity were determined first using blank quartz capillaries.

How to Interpret Failure

Largely, there were two different types of failures with nanoelectrode fabrication: 1) melted Pt wires (**Figure 7a**) and 2) small discontinuities throughout pulled Pt wires (**Figure 7b**). Typically, the melted Pt wire shown in **Figure 7a** indicated that the hard pull occurred when the Pt wire was too hot; whereas the discontinuity in the Pt wire shown in **Figure 7b** could be the result of the use of a bent Pt wire, insufficient vacuum during the sealing procedure, or pulling under insufficient heat. While troubleshooting, it is important to note the strong interconnection between heat, velocity, and delay. Changing one parameter influences the other parameters and certain steps need to be retaken. As a general rule, it is preferred to decrease delay to a minimum of 128 and to increase heat and velocity when it is feasible to ensure the resulting electrode has as small a radius as possible with a laser puller setting.

When melted Pt wires are observed (**Figure 7a**), the first effort can be made with increasing delay by a factor of 3 or less to cool down the heated Pt wire. If the delay doesn't help, then, the second effort should involve decreasing the heat by a factor of 1. When discontinuity of the Pt wire is observed (**Figure 7b**) as the first failure or the sequential failure from a melted Pt wire, the first effort was to ensure the vacuum seal was sufficient to

reduce the possibility of air bubbles. The next step was to decrease the delay until it reaches 128 to address the issue of discontinuities in the Pt wire. If these changes did not correct the issue, the heat was increased by an increment of 1. If none of those efforts fixed the discontinuity of the Pt wire, then, the velocity was decreased by an increment of 1. The heat was increased before decreasing the velocity to avoid losing a smaller electrode radius. Through the navigations of failures, it is also important to understand that temporary malfunctions of laser pullers can change parameters within minutes or between each pull attempt. Thus, it is important to understand the status of each laser-based micropipette puller in use and interpret each failure encountered, instead of relying on one absolute procedure to pull. Parameter values may be adjusted according to results, variation in heat and filament, and systematic differences between the puller and the researcher.

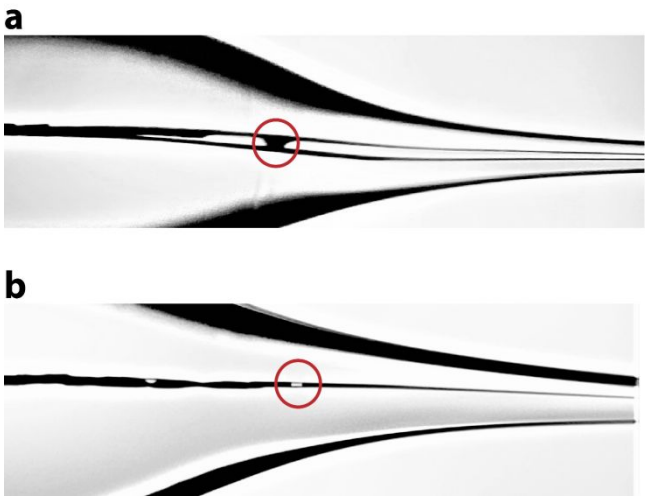


Figure 7. Representative microscopic images of nanoelectrode fabrication failures. The Pt wire showed a characteristic deformation of the Pt wire (a) as if the Pt wire had melted as shown with a red circle, and the rest of the Pt wire were pulled out of the seal. The pulled Pt wire under a longer delay (b) showed cleaner cuts at the disconnected sites as shown with a red circle, and the rest of the Pt wires typically stayed within the glass seal. All images were taken using a cellphone by aligning it with the eyepiece of microscopes at 10x objectives.

MAKING CONNECTIONS AND MAINTENANCE

When handling the Pt-sealed quartz capillaries, it is advised to ensure the handler is grounded to avoid unnecessary displacement of static charge to the electrode surface. Once the Pt-sealed quartz capillary was pulled, a conductive wire was dipped into liquid gallium and inserted into the backend of the capillary to make electrical contact with the sealed Pt wire. Electrical contact can be made with various conductive wires (*e.g.,* tungsten,

copper wire, tinned-copper wire, etc.), paints (*e.g.,* silver paint), or powders (*e.g.,* graphite powder), but it is advised to use the least resistive materials available. Typically, a thin layer of glass will encase the Pt wire tip. Thus, careful polishing of the electrode tip with a beveling pad will be needed to remove the glass and expose the inlaid Pt disk, as discussed below. Once the inlaid Pt disk is exposed, the radius of the working electrode may be calculated via cyclic voltammetry using the following equation (**Equation 1**),

$$i_{limiting} = 4nFDC^{*}r \qquad \textbf{Equation 1}$$

where $i_{limiting}$ is the limiting current value indicated with the red box (**Figure 8**), n is the number of electrons involved in the redox reaction, F is Faraday's constant, 96485 C mol^{-1}, D is the diffusion coefficient of the redox species used in the system, C* is the bulk concentration of the redox species, and r is the radius of the electrode (*i.e.,* the unknown to solve for). **Figure 8** shows a voltammogram of a laser pulled and beveled nanoelectrode. The calculated radius of the electrode from the limiting current is 250 nm.

At times, cleaning of the nanoelectrode surface is necessary to remove blockage. Nanoelectrodes should not be polished on a polishing pad with alumina. We would like to make the important point that polishing a nanoelectrode with alumina or a polishing pad with ~ 1 μm grit size is like polishing a macroelectrode with bowling balls. We suggest chemical or electrochemical methods to polish. Dipping the nanoelectrode in piranha solution for 10 – 20 seconds is one method to rid the surface of organics. Cyclic voltammetry cycling can also be used in strong acid but we would like to alert the reader to emerging investigations indicating platinum dissolution under such conditions.[30-31] Another common problem is electronic noise observed from sensitive nanoelectrodes, which is generally a result of having a poor Faraday cage or poor electrical connection to the potentiostat in use. Thus, always maintaining a clean and stable working environment is key in nanoelectrode characterization.

If no faradaic current was observed, it typically means the metallic Pt electrode surface is still covered by sealed quartz glass despite proper electrical connection within the barrel of the pulled electrode (**Figure S10**). In this case, beveling the electrode tip with a special micropipette beveler (**Figure S11**, as opposed to the previously discouraged alumina pads) was required to expose the conductive Pt surface. The act of beveling was also beneficial to reduce the recessed electrode area (*i.e.,* seemingly small radii and large capacitive current), dirty electrode surfaces (*i.e.,* resistive cyclic voltammograms), and exposed Pt wire (*i.e.,* seeming large radii) to make

reliable nanoelectrodes. However, beveling is a double-edged sword. When beveling, special considerations must be made. First, using a microscope will allow better visualization of when the nanometer sized tip is in contact with the beveler. Second, more beveling will lead to larger electrode radii. This is because the cylindrical platinum wire, once pulled, becomes more conical in shape. The thin tip can rapidly enlarge as it is polished down. Thus, the minimum amount of beveling required to expose the Pt surface should be performed. This will ensure the smallest Pt radii possible. Finally, the improper cleaning of the beveling pads between each use could result in a pile-up of small broken shreds from previous bevels or a complete mask of the electrode surface with fine dust (**Figure S12**). While a thorough water wash of electrodes could significantly remove the pile-ups, it cannot thoroughly rinse the masked electrode surface. Therefore, those masked electrodes must be beveled again using a clean beveling pad or scored to reveal the electrode surface once more. Because of the skill required to properly bevel a nanoelectrode, it is difficult (but not impossible) to attain sub-100nm nanoelectrodes. One of the best ways to polish nanoelectrodes is by focused ion beam.[28-29] However, we recognized that not all laboratories have access to such equipment, and so beveling can accomplish the necessary purpose with enough care and skill.

Additionally, proper care and maintenance of laser pullers as instructed in the manual is required to obtain reproducible results between sealing and pulling procedures. If an incomplete seal is obtained using the tested optimal parameters for vacuum, heat, and filament, we suggest cleaning the gold-plated retro mirror prior to adjusting parameters. This mirror may become covered in debris and materials discharged from the glass surface during previous heating steps. To minimize transfer, glass should be cleaned with acetone or isopropyl alcohol prior to loading into the instrument; in addition, glass should always be handled with clean gloves. To clean this mirror, the shroud covering the laser beam path must be removed, then the mirror can be wiped with a Kimwipe dampened with acetone or isopropyl alcohol. If a seal is obtained, but it is an uneven seal under sufficient vacuum, the scanning mirror tilt may be misaligned. First, check the scanning mirror tilt micrometer towards the back of the puller. If the puller is no longer in the manufacturer default setting listed on the label in the back of the instrument, the tilt may be inspected using thermal paper. Specifically, the shroud must be removed, an empty capillary must be inserted between puller bars, and the thermal paper must be inserted between the capillary and the retro mirror (shiny side toward the glass). Next, the following pull program should be used: Heat: 200,

Filament: 5, Velocity: 0, Delay: 40, Pull: 0. If the resulting marks are asymmetric, the micrometer behind the should be adjusted as directed in the manual. In addition, if electrode tips are uneven after the pulling process, the puller bars should be dusted with a clean dry Kimwipe (or dry cotton swab as instructed in the manual). Specifically, the top edges of the puller bars and puller bar grooves should be dusted to maintain a reproducible pulling process. If the pulley system is a concern, contact the instrument company prior to adjusting. Lastly, the exterior should be dusted periodically with a dry cloth or Kimwipe.

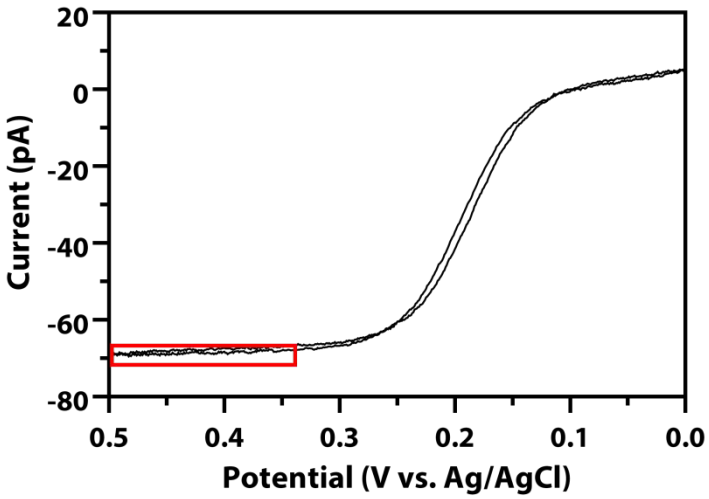


Figure 8. Representative cyclic voltammogram of nanoelectrode characterization using cyclic voltammetry with a CHI potentiostat, plotted in US convention. Voltammogram captured in 1.02 mM ferrocenemethanol in 250 mM KCl vs. Ag/AgCl (1 M KCl). The region marked with red box is where the limiting current is observed indicating r = 250 nm.

CONCLUSION

In this paper, 6 parameters are discussed to succeed in the nanoelectrode fabrication: 1) vacuum, 2) heat, 3) filament, 4) velocity, 5) delay and 6) pull. The first part of the fabrication is to seal the Pt wire without any damages. Here, it is important to adjust three parameters: vacuum, heat, and filament. Once the vacuum and heat are sufficient (*i.e.,* the quartz capillary encases an intact Pt-wire), the filament may be adjusted to optimize the width of the seal. Single-digit filament values should be used to establish the correlation between the numeric value of the filament and the microscopic images of the effect on the quartz capillary to minimize the systematic variation between laser-based micropipette pullers. The second part of the fabrication is to pull the Pt-sealed

quartz capillary into two separate electrode tips. As there is no one-size-fits-all procedure for submicron electrode fabrication, this paper provides examples on how to interpret each failure and vary each parameter to troubleshoot through each individual laboratory's fabrication procedure.

AUTHOR INFORMATION

Corresponding Author
*E-mail: jedick@email.unc.edu

ACKNOWLEDGMENTS

We acknowledge the generous support from the National Institutes of Health under grant number 1-R35-GM138133-01. Additional graduate student support was provided by the National Science Foundation Graduate Research Fellowship under Grant No. DGE-1650116.

REFERENCES

(1) Jena, B. K.; Percival, S. J.; Zhang, B. Au disk nanoelectrode by electrochemical deposition in a nanopore. *Anal Chem* **2010**, *82* (15), 6737-6743. DOI: 10.1021/ac101261m.
(2) Velmurugan, J.; Sun, P.; Mirkin, M. V. Scanning Electrochemical Microscopy with Gold Nanotips: The Effect of Electrode Material on Electron Transfer Rates. *J Phys Chem C* **2009**, *113* (1), 459-464. DOI: 10.1021/jp808632w.
(3) Yu, Y.; Sun, T.; Mirkin, M. V. Toward More Reliable Measurements of Electron-Transfer Kinetics at Nanoelectrodes: Next Approximation. *Anal Chem* **2016**, *88* (23), 11758-11766. DOI: 10.1021/acs.analchem.6b03392.
(4) Velmurugan, J.; Noel, J. M.; Nogala, W.; Mirkin, M. V. Nucleation and growth of metal on nanoelectrodes. *Chemical Science* **2012**, *3* (11), 3307-3314, 10.1039/C2SC21005C. DOI: 10.1039/c2sc21005c.
(5) Li, Y.; Cox, J. T.; Zhang, B. Electrochemical responses and electrocatalysis at single au nanoparticles. *J Am Chem Soc* **2010**, *132* (9), 3047-3054. DOI: 10.1021/ja909408q.
(6) Bard, A. J. Toward single enzyme molecule electrochemistry. *ACS Nano* **2008**, *2* (12), 2437-2440. DOI: 10.1021/nn800801z PubMed.
(7) Liu, Y.; Li, M.; Zhang, F.; Zhu, A.; Shi, G. Development of Au Disk Nanoelectrode Down to 3 nm in Radius for Detection of Dopamine Release from a Single Cell. *Anal Chem* **2015**, *87* (11), 5531-5538. DOI: 10.1021/ac5042999.
(8) McCormick, H. K.; Dick, J. E. Nanoelectrochemical quantification of single-cell metabolism. *Anal Bioanal Chem* **2021**, *413* (1), 17-24. DOI: 10.1007/s00216-020-02899-9.
(9) Sun, P.; Laforge, F. O.; Abeyweera, T. P.; Rotenberg, S. A.; Carpino, J.; Mirkin, M. V. Nanoelectrochemistry of mammalian cells. *Proc Natl Acad Sci U S A* **2008**, *105* (2), 443-448. DOI: 10.1073/pnas.0711075105.
(10) Wang, C.; Hu, X. Fabrication of nanometre-sized platinum electrodes by controllable electrochemical deposition. *Talanta* **2006**, *68* (4), 1322-1328. DOI: 10.1016/j.talanta.2005.07.048.
(11) Chen, S. L.; Kucernak, A. Electrodeposition of platinum on nanometer-sized carbon electrodes. *Journal of Physical Chemistry B* **2003**, *107* (33), 8392-8402. DOI: 10.1021/jp0348934.
(12) Penner, R. M.; Heben, M. J.; Longin, T. L.; Lewis, N. S. FABRICATION AND USE OF NANOMETER-SIZED ELECTRODES IN ELECTROCHEMISTRY. *Science* **1990**, *250* (4984), 1118-1121. DOI: 10.1126/science.250.4984.1118.

(13) Sun, P.; Zhang, Z. Q.; Guo, J. D.; Shao, Y. H. Fabrication of nanometer-sized electrodes and tips for scanning electrochemical microscopy. *Analytical Chemistry* **2001**, *73* (21), 5346-5351. DOI: 10.1021/ac010474w.
(14) Katemann, B. B.; Schuhmann, W. Fabrication and characterization of needle-type Pt-disk nanoelectrodes. *Electroanalysis* **2002**, *14* (1), 22-28. DOI: 10.1002/1521-4109(200201)14:1<22::aid-elan22>3.0.co;2-f.
(15) Mezour, M. A.; Morin, M.; Mauzeroll, J. Fabrication and Characterization of Laser Pulled Platinum Microelectrodes with Controlled Geometry. *Analytical Chemistry* **2011**, *83* (6), 2378-2382. DOI: 10.1021/ac102482f.
(16) Li, Y. X.; Bergman, D.; Zhang, B. Preparation and Electrochemical Response of 1-3 nm Pt Disk Electrodes. *Analytical Chemistry* **2009**, *81* (13), 5496-5502. DOI: 10.1021/ac900777n.
(17) Lanyon, Y. H.; De Marzi, G.; Watson, Y. E.; Quinn, A. J.; Gleeson, J. P.; Redmond, G.; Arrigan, D. W. M. Fabrication of nanopore array electrodes by focused ion beam milling. *Analytical Chemistry* **2007**, *79* (8), 3048-3055. DOI: 10.1021/ac061878x.
(18) Guo, D. J.; Wu, X. Y.; Lei, J. G.; Xu, B.; Kometani, R.; Luo, F. Fabrication of micro/nanoelectrode using focused-ion-beam chemical vapor deposition, and its application to micro-ECDM. *18th Cirp Conference on Electro Physical and Chemical Machining (Isem Xviii)* **2016**, *42*, 733-736. DOI: 10.1016/j.procir.2016.02.310.
(19) Zhang, B.; Galusha, J.; Shiozawa, P. G.; Wang, G. L.; Bergren, A. J.; Jones, R. M.; White, R. J.; Ervin, E. N.; Cauley, C. C.; White, H. S. Bench-top method for fabricating glass-sealed nanodisk electrodes, glass nanopore electrodes, and glass nanopore membranes of controlled size. *Analytical Chemistry* **2007**, *79* (13), 4778-4787. DOI: 10.1021/ac070609j.
(20) Arrigan, D. W. M. Nanoelectrodes, nanoelectrode arrays and their applications. *Analyst* **2004**, *129* (12), 1157-1165. DOI: 10.1039/b415395m.
(21) Zhu, X. Y.; Qiao, Y. H.; Zhang, X.; Zhang, S. S.; Yin, X. H.; Gu, J.; Chen, Y.; Zhu, Z. W.; Li, M. X.; Shao, Y. H. Fabrication of Metal Nanoelectrodes by Interfacial Reactions. *Analytical Chemistry* **2014**, *86* (14), 7001-7008. DOI: 10.1021/ac501119z.
(22) Pendley, B. D.; Abruna, H. D. Construction of submicrometer voltammetric electrodes. *Analytical Chemistry* **1990**, *62* (7), 782-784. DOI: 10.1021/ac00206a029. Shao, Y.; Mirkin, M. V.; Fish, G.; Kokotov, S.; Palanker, D.; Lewis, A. Nanometer-Sized Electrochemical Sensors. *Analytical Chemistry* **1997**, *69* (8), 1627-1634. DOI: 10.1021/ac960887a.
(23) Fish, G.; Bouevitch, O.; Kokotov, S.; Lieberman, K.; Palanker, D.; Turovets, I.; Lewis, A. Ultrafast Response Micropipette-Based Submicrometer Thermocouple. *Review of Scientific Instruments* **1995**, *66* (5), 3300-3306. DOI: Doi 10.1063/1.1145498 (acccessed 2021/08/23).
(24) Nioradze, N.; Chen, R.; Kim, J.; Shen, M.; Santhosh, P.; Amemiya, S. Origins of Nanoscale Damage to Glass-Sealed Platinum Electrodes with Submicrometer and Nanometer Size. *Analytical Chemistry* **2013**, *85* (13), 6198-6202. DOI: 10.1021/ac401316n.
(25) Katemann, B. B.; Schulte, A.; Schuhmann, W. Constant-Distance Mode Scanning Electrochemical Microscopy. Part II: High-Resolution SECM Imaging Employing Pt Nanoelectrodes as Miniaturized Scanning Probes. *Electroanalysis* **2004**, *16* (1-2), 60-65, https://doi.org/10.1002/elan.200302918. DOI: https://doi.org/10.1002/elan.200302918 (acccessed 2022/09/08).
(26) Percival, S. J.; Dick, J. E.; Bard, A. J. Cathodically Dissolved Platinum Resulting from the O2 and H2O2 Reduction Reactions on Platinum Ultramicroelectrodes. *Anal. Chem.* **2017**, *89* (5) 3087-3092.
(27) Deng, X.; Galli, F.; Koper, M. T. M. In Situ Electrochemical AFM Imaging of a Pt Electrode in Sulfuric Acid under Potential Cycling Conditions. *J. Am. Chem. Soc.* **2018**, *140* (41), 13285-13291
(28) Chen, R.; Hu, K.; Yu, Y.; Mirkin, M. V.; Amemiya, S. Focused-Ion-Beam-Milled Carbon Nanoelectrodes for Scanning Electrochemical Microscopy. *J Electrochem Soc.* **2016**, *163* (4):H3032-H3037.
(29) Kai, T.; Zoski, C. G.; Bard, A. J. Scanning Electrochemical Microscopy at the Nanometer Level. *Chem. Commun.* **2018**, *54*, 1934-1947.