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Filling nanopipettes with apertures smaller than 50nm: dynamic micro-distillation

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Abstract

Using nanopipettes with very small apertures ($<10\text{nm}$) is a good way to improve spatial resolution in scanning conductance experiments, to monitor single molecule delivery, to strain long molecule stretching in translocation, etc. However, such nanopipettes can be difficult to fill. Here we describe a dynamic micro-distillation technique that successfully fills all nanopipettes, whatever their shape or tip radius. Even elongated or bent nanopipettes with a small angle tip are completely filled using this new technique. The nanopipettes are first filled with pure water, which is later replaced with the desired electrolyte via electro-migration. Electrical measurements are used to check that filling is complete.

Keywords

Nanopipette; filling; distillation; current rectification

Introduction

Nanopipettes and nanocapillaries are often used as a single-molecule tool in liquid. A wide range of studies use capillaries with nanometric-size apertures [1,2,3,4,5,6]. For example, simulated translocation with nanopipettes is currently being explored worldwide [7]. A large community of researchers is currently working on characterizing nanopipette geometry, using different methods to address aperture size [8,9,10,11,12,13]. While nanopipettes are known to be simple and easy to fabricate, batch-to-batch reproducibility is poor, particularly for the difficult-to-obtain small-hole nanopipettes. Electrical characterizations depend on the complete filling of nanopipettes, which means no gas bubbles inside, to ensure good conductivity. The capacity to fill all types of nanopipettes, including small-hole nanopipettes ($<10\text{nm}$), bent nanopipettes, with both small and large angles, would therefore be highly desirable. It would help improve spatial resolution in scanning conductance experiments, optimize the signal-to-noise ratio during single molecule delivery, strain long molecule stretching in translocation, etc.

Small nanopipette apertures make backfilling unfeasible. There are many other ways to fill nanopipettes, but none is very efficient. One way is to apply high pressure

behind the fluid placed in the large part of the capillary. Conversely, the so-called desiccator method consists in applying a depression of about 1mbar in the external environment. A third solution is to use a nanopipette with a glass filament inside. The filament is melted during tape manufacturing, which can lead to glass impurities in the capillary and geometrical irregularities at the very end of the nanopipette. In some cases, the intended use of the nanopipette, especially translocation of objects, can introduce undesired microfluidic in-homogeneities, even contributing to aperture clogging. The fourth method, plasma treatment, involves improving wettability by hydrophilizing the glass surface in an O₂ plasma [8,9]. Zweifel et al. [8] observed that electrical (I-V) curves were strongly modified, possibly due to surface chemistry modifications like reactive H activation. Regardless of whether this is intentional or not, and considered an advantage or a drawback, the initial surface chemistry seems to be changed by the treatment and probably also the surface roughness. Moreover, the very small aperture range, our particular focus, was not explored in this work.

When high back-pressure is applied to the filling fluid, we showed in a previous article that the fluid meniscus follows an air-flow Poiseuille law [14] enabling the size of the hole to be determined. However, given the filling velocities, filling capillaries this way is very time-consuming. Our previous study found that for elongated nanopipettes with a tip longer than 10 mm and high back-pressure of about 2.5 bars applied, meniscus velocity is about 0.02 μm/sec for a nanopipette with a diameter of 40nm. Filling can thus take several days (about 6 days in the present example), even though velocity increases with decreasing diameter.

In a recent article, Dr. S. Hennig [15] even wrote that *“it can be very difficult, if not impossible, to fill a nanopipette with a small diameter of 10 to 20 nm and an extended taper length of 4 to 5 mm, even if glass capillaries with filament are used”*.

Here, we first show that traditional methods are ineffective in filling certain kinds of nanopipettes, especially elongated and small-hole nanopipettes.

Second, we demonstrate that the new filling method, dynamic micro-distillation, is capable of filling any capillary. Filling time, complete filling and electrical equilibrium were assessed on “basic nanopipettes”, without modification of their internal geometry or chemistry. The nanopipettes used have an inner diameter as low as 7 nm, without filaments. They were filled, without plasma treatment and using a simple method. This nanopipette filling process is usable for all types of nanopipettes. Details of some are provided in this paper, together with the method and reproducible electrical measurements to prove complete filling.

Results and discussion

Quartz capillaries, being pure silica, have certain intrinsic properties (lowest dielectric constant, lowest loss factor, highest volume resistivity, strength and chemical purity)

that make them appropriate for the reproducible electrical measurements described below. Notably, they eliminate ion leakage, and offer the lowest possible background noise levels in electrical current recording. The capillaries, puller and set of parameters used to obtain nanopipettes are described below, under Method.

The general shape of nanopipettes can vary, but tip length and hole diameter are usually in the expected range for a given set of parameters. Due to variations in builder calibration, this set of parameters can change from one puller to another. The very end of each tip is always different and has to be characterized. Here, we determined tip shape and aperture *via* different methods: first using the velocity of the water meniscus and an optical microscopy view of the tip [14]; second, using scanning electron microscopy (SEM) after capillary use (this method is destructive); third, via electrical characterization of the nanopipette.

Figure 1 shows the SEM view of two typical nanopipettes. The nanopipette in Figure 1a was filled by applying high back-pressure. The nanopipette in Figure 1b, which was impossible to fill by the usual methods, was filled using the technique proposed here. Probably due to a parameter set which favors glass sinking flow before pulling, it is bent at its tip, with an 11nm hole. This can be viewed as an artefact, but it illustrates the strength of the method. Klenerman et al. [16], at least in their schematic setup, found this design useful. The last angle is invisible in optical microscopy. The nanopipettes that proved impossible to fill here by the high back-pressure method showed these types of defects, which are common. Nanopipettes are still open but the tip shape can be highly irregular. Irregular tip shapes obviously make filling these nanopipettes harder.

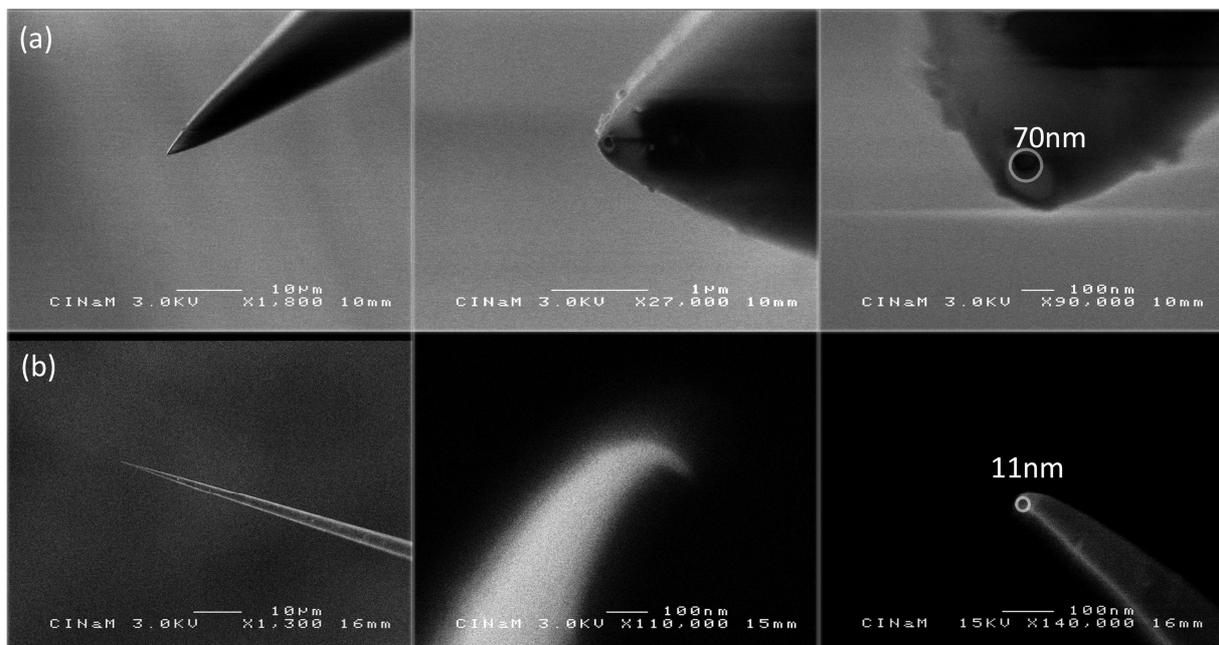


Figure 1: Upper line (a): Three SEM images of the same nanopipette filled by applying high back-pressure; Lower line (b): Three SEM views of a capillary

impossible to fill by applying high back-pressure (see fabrication program 2, Method). It is very thin and elongated, bent at its tip with a $d_{tip} = 11\text{nm}$ hole.

Our success in using the high back-pressure method to fill this kind of nanopipette, thin and elongated with a small-angle end tip, was random (roughly 20%), with a high probability of breaking the tip. Actually, what we observed was that filling became very difficult when apertures were smaller than 50nm . Often, the very end of the capillary quickly became plugged by water condensation or air pollution, which completely and irreversibly stopped the filling. For shorter nanopipettes, the high back-pressure method is efficient but still takes time, several hours or even 2 or 3 days. The surrounding atmosphere needs to be particularly clean.

Our tests applying high back-pressure applied to filament capillaries were not very successful: filling remained impossible with elongated nanopipettes.

Thus, current nanopipette filling methods appear inefficient, time-consuming and wasteful (up to 80% waste). Hereunder (Method), we propose a new technique that fills 100% of nanopipettes. This dynamic micro-distillation process can be applied to fill any nanopipette, whatever its shape or tip hole. The process is as follow. Due to the heating involved, a quartz nanopipette is recommended. A wall of heat is moved along the nanopipette. Deionized water boils inside the nanopipette and re-condenses to the tip in the cold part. The tip water plug grows with the motion of the heat-wall, water being chosen because it is the solvent of potassium chloride, the salt used for the electrical measurements. Only the very end of the tip is filled with pure water, the micrometric part of the capillary being replaced by the saline solution desired. Although ion diffusion at the very end of the tip only concerns a negligible volume, in such a confined situation it can take a long time, from hours to days, to reach electrical equilibrium by diffusion alone. Nanopipettes are filled faster than with high back-pressure, in roughly one hour. The technique works with all nanopipettes, filling even closed nanopipettes. Results are tested directly by electrical conductivity to check that filling is complete, that the nanopipette is open and that electrical equilibrium is reached. The electrical measurement setup is described under Method. Figure 2a corresponds to the typical recording made with an elongated, small-angle and small-hole nanopipette: $r_{tip} = 13\text{nm}$, $\theta = 9^\circ$ and $l_{tip} = 15\text{mm}$. Voltage supply is increased from 0 to $\pm 500\text{mV}$; the intensity is recorded at the same time. The first measurement is made directly after the first electrolyte loading at a concentration $[KCl] = 10^{-4}\text{mol/L}$.

This first electrical measurement confirms that the nanopipette is filled: the current flows; ion exchange is ensured between the Ag/AgCl electrodes. However the tip of the nanopipette is still filled with pure deionized water. The intensity level does not correspond to the stationary regime: it changes slowly with diffusion.

To ensure that electrolyte concentration reaches its nominal value throughout the nanopipette, electro-migration is performed (Figure 2b): ion exchanges are produced via voltage applied for a given time.

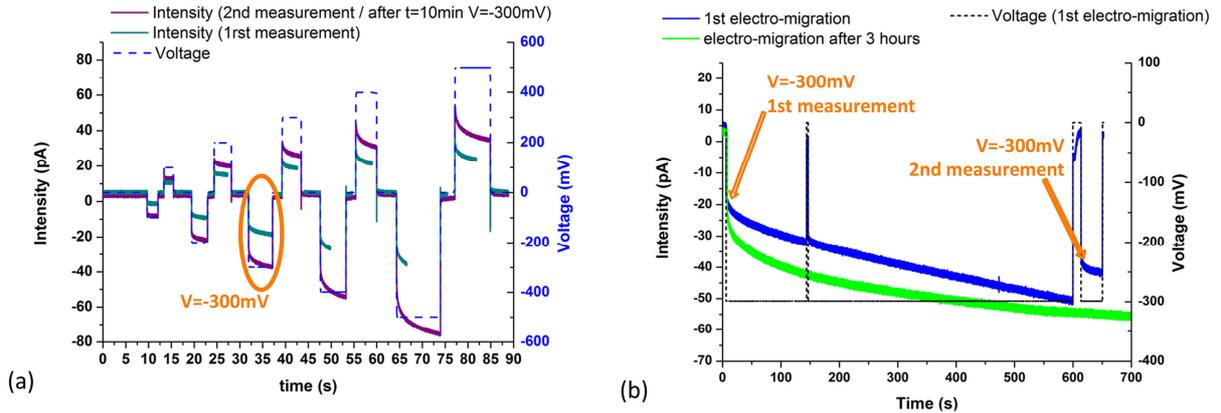


Figure 2: Typical $I(V)$ measurements with pClamp: I and V are recorded as a function of time. $[KCl] = 10^{-4} mol.L^{-1}$ and the geometrical nanopipette characteristics are: $r_{tip} = 13nm$, $\theta = 9^\circ$ and $l_{tip} = 15mm$; (a) The first measurement (cyan) directly after the KCl filling shows the electrical conductivity of the system: the nanopipette is open and filled. The second measurement (purple) after the electro-migration shown in (b) shows the nominal intensity values with voltage: electrical characteristics are reproducible; (b) Electro-migration directly after filling and three hours later: $V = -300mV$ during $t = 10min$.

Here, electro-migration is performed by maintaining the power supply at $V = -300mV$ during $t = 10min$. During these 10min, the intensity level increases from $I_{-300mV} = -30pA$ to an upper limit of about $I_{-300mV} = -55pA$. A second intensity versus voltage measurement is then performed (in purple in Figure 2) and is higher than the previous one. To check that the stationary regime has been achieved, after three hours and a new electro-migration process ($V=-300mV$ during 10min), the intensity versus voltage measurement is performed again. These new electrical current values exactly match the second measurement (in purple, Figure 2). The repeatability of measurements after several hours confirms that electro-migration leads to homogenized ion distribution more efficiently and faster than diffusion alone. All the nanopipettes filled with this technique were electrically tested. Some of « intensity pairings » as a function of estimated hole-size are given in Table 1. An intensity pair represents pre- and post-diffusion intensity measurements for an applied voltage of about $V = -500mV$ during $t = 10min$ with the given concentration. Tip-hole size uncertainties depend on the measurement techniques used and any overlap among measurements.

Tip hole size (nm)	Concentration $[KCl](mol.L^{-1})$	Intensities ($I \pm 5$)pA before / after electro-migration
$(109 \pm 6)nm$	$10^{-4} mol.L^{-1}$	-111 / -256pA
$(81 \pm 5)nm$	$10^{-4} mol.L^{-1}$	-56 / -135pA
$(20 \pm 5)nm$	$10^{-2} mol.L^{-1}$	-261 / -418pA

$(15 \pm 5)nm$	$10^{-2} mol.L^{-1}$	-60 / -135pA
$(7 \pm 1)nm$	$10^{-2} mol.L^{-1}$	-34 / -75pA

Table 1: Intensity measurements made at $V = -500mV$ and at the given concentration before and after electro-migration during $t = 10min$ for different tip sizes.

All these nanopipettes, more than 20, were filled, whatever their shape or tip hole. All showed a signature related to their shape. For example, in Figure 3, the intensity versus voltage (I-V) is found with results from Figure 2. I-V exhibits a rectification effect already observed for this kind of nanopipette [1,10,12,13]: both intensity values and ICR (intensity current rectification) ratio at $[KCl] = 10^{-4}mol.L^{-1}$ about $ICR_{500} = \frac{I_{-0.5V}}{I_{+0.5V}} = 1.53$ are comparable with values previously measured. This confirms that the proposed filling technique is reliable.

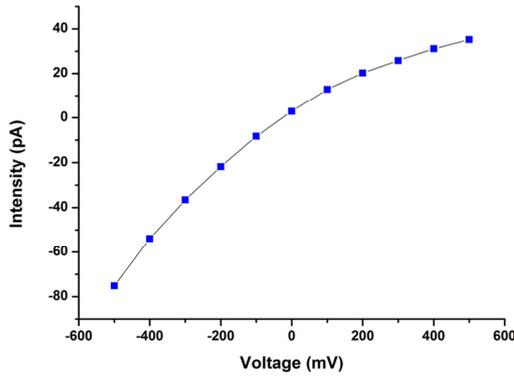


Figure 3: $I(V)$ at $[KCl]=10^{-4}mol.L^{-1}$ for a capillary with $r_{tip}=13nm$, $\theta=9^\circ$ and $l_{tip}=15mm$. The intensity versus voltage found with results from Figure 2 exhibits a rectification effect: $ICR_{500} = \frac{I_{-0.5V}}{I_{+0.5V}} = 1.53$ at $[KCl] = 10^{-4}mol.L^{-1}$.

Conclusion

100% of nanopipettes were filled here using the dynamic micro-distillation method. The process involves growing a deionized water plug at the tip of the nanopipette by moving the water-filled capillary into the vicinity of a heated filament. The water is brought to boil inside the capillary and re-condensed. This re-condensed water is deposited on the plug, which grows until it reaches the micrometric part of the capillary.

After complete filling, the initial water in the large part of the capillary is replaced by the desired electrolyte. Electro-migration yields the electrical stationary regime after some minutes. Here we present an electrical setup used in our experiment, but the stationary regime could be obtained using a cheaper power supply.

This nanopipette filling process is usable for all types of nanopipettes, including small-hole nanopipettes (<10nm), bent nanopipettes, with a small or large angle,

even closed. All the filled nanopipettes were open and exhibited electrical characteristics related to their shape. The process works despite surrounding atmospheric pollution and humidity, which usually impede complete filling. Using a programmable piezo-electric actuator, the process could be automated and certainly optimized with a smooth and regular flow.

The overall experimental technique leads to a workable system within a reasonable time. Pulling the nanopipette, determining its size by the Poiseuille technique, filling it and carrying out the electrical measurement takes only a few hours, making the nanopipette reliably usable in any experimental application.

Method

Nanopipette fabrication. The capillaries used are pure silica tubes (called quartz tubes) from Sutter (HERAEUS HSQ300) with a 1mm outer diameter and 300, 500 or 700 μ m inner diameters. Due to their high melting point, they are pulled with a P-2000 Laser Based Micropipette Puller from Sutter Instruments. Table 2 shows typical programs used to pull nanopipettes and likely to produce a small hole ($\Phi < 100nm$).

Quartz tube (ϕ_{in} μ m) Sutter ref.	Heat	Filament	Velocity	Delay	Pull	General shape
300 Q100-30-7.5	750	5	65	126	185	$l_{tip} > 10mm$
700 Q100-70-10	750	5	65	126	185	$l_{tip} > 10mm$
700 Q100-70-10	700	4	60	145	175	$l_{tip} < 10mm$
500 Q100-50-10	750	4	53	135	100	$l_{tip} < 10mm$

Table 2: Puller programs with the general shapes obtained with hole diameters $d_{tip} < 100nm$.

Dynamic micro-distillation. The new technique uses dynamic micro-distillation, as shown in Figure 4. The nanopipette is first filled with deionized water up to the micrometric accessible part of the capillary. A 200nm syringe filter is used to keep the capillary free of dust. The capillary is held by a (x,y,z) micromanipulator and the water meniscus is positioned in a loop of a tantalum filament under a large field optical microscope. The filament of 250 μ m tantalum wire is 5mm long and its loop diameter is about 5mm. Tantalum was chosen because of its high melting point and its resistance to oxidation. The filament is heated by Joule effect, with a power supply of $U=3V$, which means at roughly $I=3.5A$ intensity. The filament becomes white without burning at atmospheric pressure. When the filament is white, the water inside the capillary is brought to a boil. The water vapor re-condenses on the large part of the capillary (left of the tantalum loops), but only slightly, due to the heat. However these

water vapor molecules are not visible since they do not greatly affect the large meniscus. Usually, most of the vaporized water heads for the narrow part of the nanopipette (right side on the pictures) and re-condenses as a string of droplets as soon as it encounters a significantly colder region. Moving the nanopipette slowly and regularly in the loops away from the meniscus (velocity of about 1mm/s) either pushes the water vapor with a “moving wall of heat” or re-vaporizes the condensed string of droplets, which re-condenses further. The droplets in the string become smaller and smaller as the tip narrows. The process transports the water up to the very end of the nanopipette by “distillation”. The transported water becomes visible under the microscope because a small meniscus signaling the border between the vapor phase and the liquid region appears and moves gradually toward the large part of the nanopipette (due to the back-and-forth motion of the nanopipette). Paying much attention, the reader can see the thin lines on Figure4(5) delimiting the droplets and the coming meniscus in picture (we did not enlarge line thickness on purpose to maintain a global coherent scale). The process is repeated so that the tip water plug grows until the micrometric part of the capillary becomes accessible with the micropipette syringe. Then the water in the large part is removed and replaced with the desired electrolyte solution without bubbles.

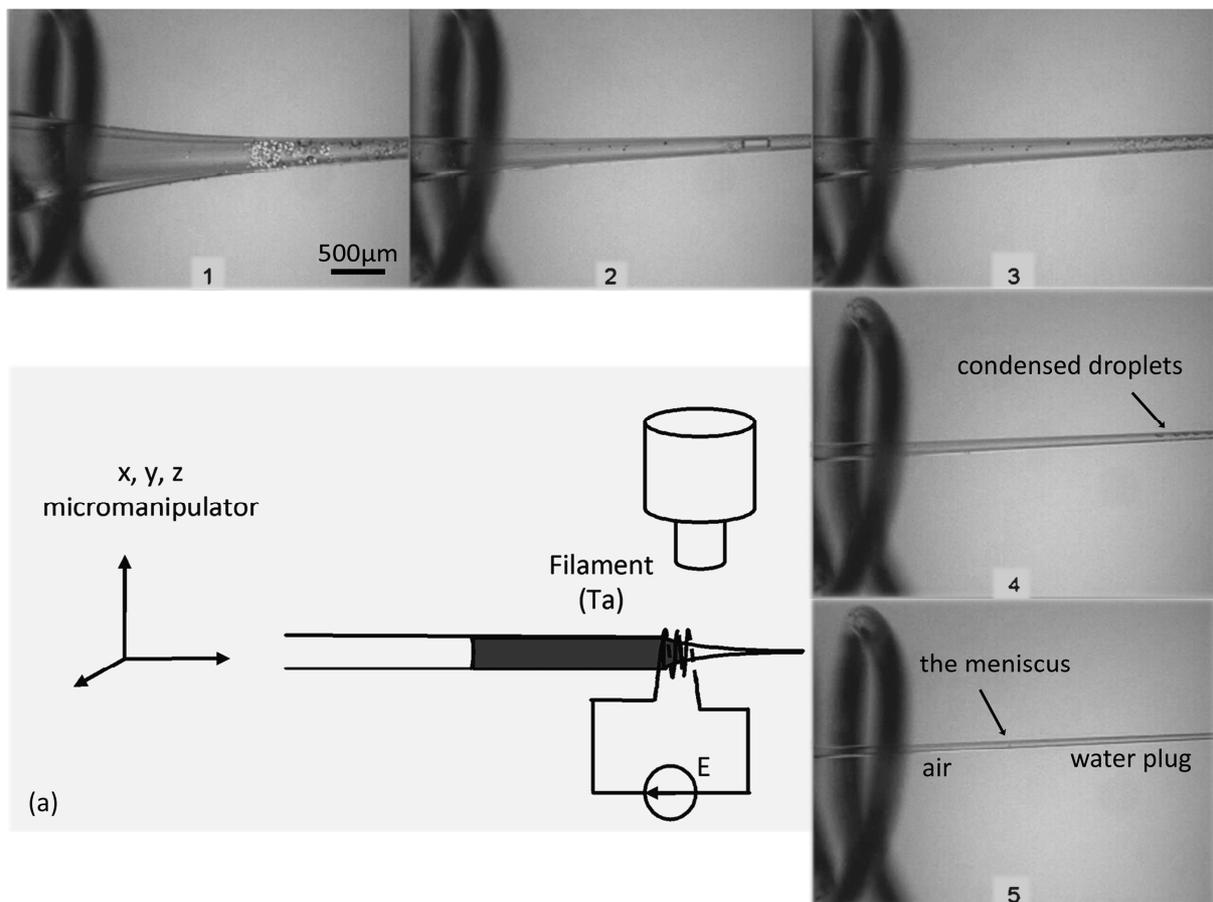


Figure 4: (a) New filling technique experimental setup – The nanopipette is inserted into a tantalum loop (wire diameter: 250µm, filament size: diameter and length =5mm) powered electrically by a stabilized generator ($U=3V$, $I= 3.5A$). The

nanopipette can be moved with a (x, y, z) piezoelectric manipulator and is loaded with water up to its millimeter-to-micrometer region using a micro-syringe. The loop of the filament is placed under an optical microscope. Images 1 to 5 illustrate the process. (1) The heated filament brings the water to a boil inside the capillary; water re-condenses further on the cold wall of the nanopipette. (2) The capillary is moved to heat the part where the water has re-condensed as droplets. (3) The droplets are boiled again and water is re-condensed further on the next cold part of the nanopipette. (4) The process is repeated. (5) The water is re-condensed at the very end of the tip; the meniscus formed by the water plug is visible under the optical microscope.

Electrical measurements. To ensure that electrolyte concentration reaches its nominal value throughout the nanopipette, electro-migration is performed in an electrical measurement setup described in Figure 5. When the electrolyte is in the large part of the capillary, the nanopipette is plunged into a tank full of the same electrolyte. One Ag/AgCl electrode is inserted into the capillary and another into the tank. The nanopipette electrode is connected to the pre-amplifier of a pClamp (Axopatch 200B) and the entire electrical measurement setup is placed under a Faraday cage to prevent electrical noise.

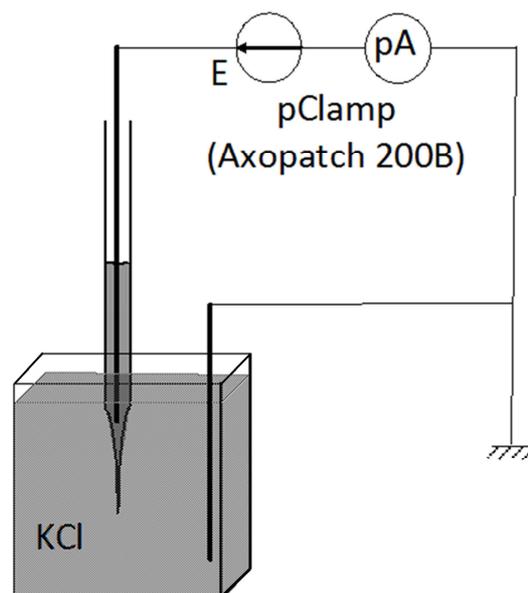


Figure 5: Electrical measurement: one Ag/AgCl electrode is introduced into the large part of the capillary loaded with KCl electrolyte and connected to the head-stage of the Axopatch 200B, the tip of the capillary is plunged into the same concentration KCl bath, a second Ag/AgCl electrode is plunged into this bath and directly connected to the ground. The entire electrical measurement setup is placed under a Faraday cage.

Additional information

The dynamic micro-distillation technique was patented in 2013: B. Tinland, E. Guirleou (Salançon), patent WO 2013079874 A1, "Method and device for filling nanopipettes via dynamic micro-distillation".

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