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On-chip Electrophoresis Devices: Do's, Don't's and Dooms.

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Background

On-chip electrokinetic injections and on-chip electrophoresis are well-established techniques, and the field is about 15 years old [1].The techniques for on-chip sample loading, voltage control, injection, separation, visualization, and electropherogram detection have been described in numerous publications [2, 3]; a few of these are summarized by Sharp et al. [4].

Below we present a few informal "tips" listed under various categories that we hope may be useful to new users of this technology. These instructions are in no way comprehensive, are not even quantitative, but we hope they will save someone somewhere some time.

Chip Maintenance

- Do properly pre-treat your channel surfaces. First time you use a glass chip, treat the surface with, say, NaOH to establish a repeatable, high surface charge density. A recommended cleaning and surface treatment is rinse channels with 100 mM NaOH for 15 minutes, then 15 minutes with filtered deionized water (DI). After this, you can introduce your buffer of choice, flush with it for another 15 min, wait some time, and then flush again with buffer. We have seen measured zeta potentials take more than 2 hrs after introduction of a new buffer to equilibrate.

- Do rinse/clean your chips often. If you are re-using your chips for multiple experiments (as most of us do), be sure to carefully clean and rinse your chip before and after each experiment (e.g., you might occasionally repeat the "break in" procedure above). Use DI, buffer, and/or a strong base solution. Remember that surface charge and resultant zeta potential can vary depending on the history of your chip. You can use a vacuum pump to repeatedly flush/refill wells. As a check, you might run electroosmotic flow with a field of, say, 500 V/cm until the measured current stabilizes (this is very important for very small channels of order 1 um depth or less).

- Don't quickly give up on a clogged chip. Try mating the barbed fitting of an empty (air filled) syringe to an Upchurch fitting (see below). You can use high pressure to try and push the liquid in the reservoir to clear the clog.

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- Don't store glass chips dry. Instead, store them with channels filled with DI and at the bottom of a small container partially filled with filtered DI. We have found that this is the best way to keep them clean; it prevents electrolytes from crystallizing in the channels.

- Doom: Cleaning/wiping a chip with ordinary tissue paper can scratch a glass microchip, this can reduce the quality of your optical access. Wipe your chip with lens paper.

Experimental considerations

- Do filter ALL solutions immediately before putting them in chip reservoirs. Syringe filters work great.

- Do check the mercury bulb alignment on an epifluorescent microscope before starting a serious experiment.

- Doom: Differing heights of the liquid columns in chip reservoirs can cause significant pressure driven flows. These can lead to high dispersion and low resolution. You can check for these by, say, starting an electrokinetic focusing/injection and then suddenly deactivating the applied potentials. Once the field is off, pressure gradients will show themselves as drifts in the (e.g., fluorescent stream) material lines.

- Do save a data set whenever possible (as in all experiments). For quantitative imaging, also remember to take a flatfield image often (in fact, whenever it is convenient and perhaps more often). An accidental bump of the microscope stage can void hours of quantitative imaging data (since your background illumination pattern is now different).

- Don't assume that the dye fluorescence remains constant in your reservoirs over time when running long experiments with large applied potentials (in the hundreds of volts and higher). Most fluorescent dyes are broken down electrochemically; see for example [5].

- Do experiment with and optimize your electrokinetic sample injection schemes. A good injection can significantly increase the resolution of the electrophoretic injection, and properly balance the demands of signal to noise ratio and resolution [6]. As a first step, instead of simply injecting sample from one channel to another, pinch the injection by introducing "focusing streams" on both sides of the injected stream [7]. See Fig. 1a. This also applies to injections in "double T" channels. Note that the method of gated injection can also give clean injections [8], especially if you are hungry for signal and want a relatively large sample plug. If you choose the pinching method, it is possible to insert a step of flow reversal before the injection in the main channel for further optimization, by reversing the flow of analyte for a very short time (Fig. 1b) [6].

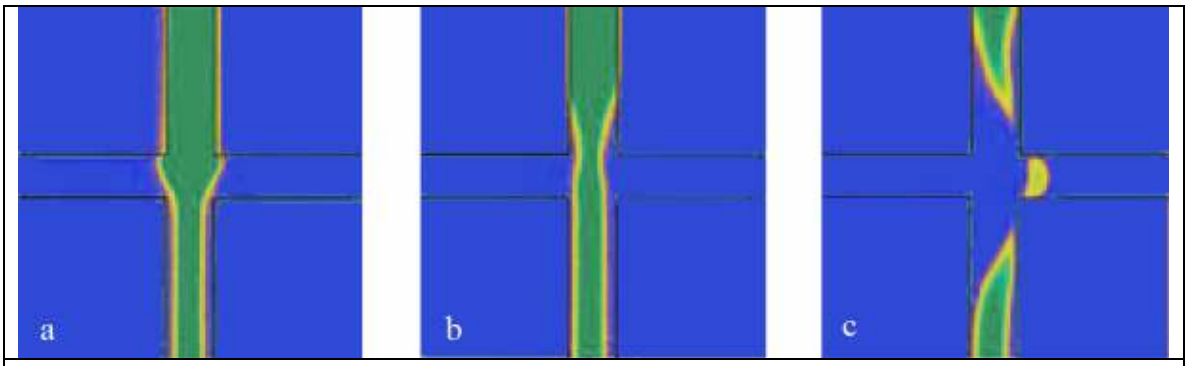

Figure 1: Optimization of On-Chip electrophoretic injection [6]. (a) is the pinching step, (b) the flow reversal step, and (c) the dispensing in the separation channel. The same procedure is applicable to a double T channel geometry.

To conclude this section, we provide a step-by-step description of a typical On-Chip electrophoretic injection experiment:

- Make sure your setup is protected from external light to limit noise.
- Rinse the channels with buffer for >15 minutes. If you want to inject sample from the North channel (as shown on Fig. 1), fill the West, South and East wells and apply vacuum at the North well.
- Remove the vacuum from the North well and rinse the well (not the channel) with DI water. Empty it before filling it with your sample. (This rinsing is critical if the background buffer of the sample is to be significantly different than that in the other wells).
- Place platinum electrodes (e.g., wires) in the wells. Make sure they don't touch each other or another surrounding metallic part like the stage (to avoid short circuits). Ideally, place the wire in the well but well away from the channel inlet. (This keeps the region where electrolysis occurs away from the channel inlet.) Also, make sure there are no unwanted "liquid bridges" connecting two wells above or below the chip.
- Take a background image. Taking a background image at this point will capture any stray fluorescence that may be visible from the well, although the channel should still be empty of sample.
- Inject liquid from North to South before starting any injection. After starting this flow, you can start your flows from East and West to the South (as per your pinching scheme).
- Initiate the rest of your electric field scheme. A simple scheme is suggested in Table 1, but you can customize it depending on your setup and interests.
- After optimizing your scheme, re-focus your microscope and camera on the region of interest within the chip and take another background image prior to injection (this background will be locally free of sample). This image will help you determine background signal due to autofluorescence of solution, channel wall, etc.
- Inject and image away!
- After acquiring your data, don't move the chip or the stage. Instead, first rinse all of your channels with DI and then fill all channels with your fluorescent sample solution, focus your microscope and camera on the

region of interest of the channel, and take a "flat field"/background image. A "flat field" image is one where the entire region of interest is filled with fluorescent dye. This image will help you correct for non-uniformities in illumination, non-uniform channel depths, etc. This step can also be performed before actually taking data.

- Rinse the channels with DI water and go back to step 1 or store properly.

Table 1: Electric field scheme for optimized sample injection, assuming all streams have the same conductivity [6]. E_i is the value of the electric field in the channel i.

Injection step	Duration (s)	E_n/ E_s	E_e/ E_s	E_w/ E_s
Pinching step	20	0.42	0.29	0.29
Flow reversal step	0.2	2.32	0.66	0.66
Dispensing step	100	1.00	0.43	2.43

Hardware

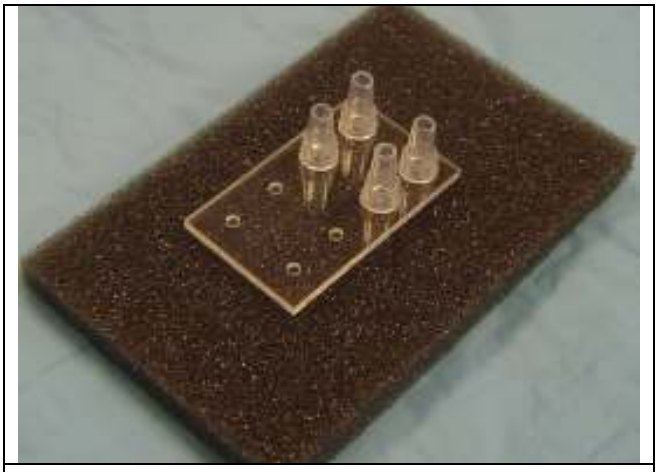

Figure 2: 200 μL pipette tip wells attached to a glass microchip using 30 min epoxy. Epoxy was allowed to set for 24 hours before filling the wells.

- Don't use 5 minute epoxy for attaching wells to quartz or glass substrates. 2-Ton 30 min epoxy (ITW Devcon, Danvers, MA) holds well and is still removable after curing. Cut the narrow end off of a pipette tip, invert it (wide side down), and epoxy over a microchip reservoir for a quick custom fitting as shown on Fig. 2. Table 2 shows a panel of recommended well size depending on the experimental conditions. To remove the well and epoxy, use a razor blade held at a shallow angle to the chip surface.

- Don't rely too much on PDMS for high quality electrophoretic chip separations. This material notoriously adsorbs and absorbs molecules [9, 10], conducts ionic current (through the substrate), and is highly hydrophobic (favoring bubbles) [11]. In our experience, published surface treatments (e.g., oxygen plasma) wear off rapidly (within minutes if dry; within hours if wet). PDMS also has very low thermal diffusivity and conductivity (compared to glass), and so it favors Joule heating at relatively low electric fields. The latter limits achievable resolution and separation speed.

Table 2: Recommended well size for given buffer concentration, allowable $|\Delta pH|$ and nominal current/experiment duration

$\|\Delta pH\|$	0.2	1	0.2	1	0.2	1	0.2	1	0.2	1
Current and duration / Buffer concentration	100 pA for 10 min		500 pA for 20 min		1 μA for 60 min		10 μA for 60 min		100 μA for 60 min	
100 μM	10 μL	700 nL	100 μL	7 μL	600 μL	40 μL	6 mL	400 μL	60 mL	4 mL
1 mM	1 μL	70 nL	10 μL	700 nL	60 μL	4 μL	600 μL	40 μL	6 mL	400 μL
10 mM	100 nL	7 nL	1 μL	70 nL	6 μL	400 nL	60 μL	4 μL	600 μL	40 μL
100 mM	10 nL	700 pL	100 nL	7 nL	600 nL	40 nL	6 μL	400 nL	60 μL	4 μL

- Do consider the buffering capacity of your wells. Table 2 uses the simple Henderson-Hasselbach equation [12] for a (typical) buffer which uses a very strong acid (or strong base) and which has one dominant pK_a value. The columns depict the desirable tolerance for changes in pH and the applied charge. The rows depict various buffer molarities, starting from a (typically unacceptable) value of 100 μM to a relatively strong buffer of 100 mM.

- Do consider mechanically secured (i.e., versus glued) fittings and reservoirs for chip-to-world interfaces when using glass microchips (as shown on Fig. 3). Each chip is worth a hundred dollars or so and it may be easier to get the chip thoroughly cleaned if the reservoirs are not permanently attached. Threaded Upchurch fittings, tapped plastic pipette tips, and red (not the black) silicone O-rings work very well. Silicone (red) o-rings works well because they don't seem to shed microparticles that can jam up your chip (Buna-N o-rings are especially bad).

- Do consider Upchruch "Nanoports" to interface a chip with tubing in your Lab-on-a-Chip design (as shown on Fig. 4). They bond permanently to the chip, but are handy and come in different types and sizes depending on the

application. They also serve as reservoirs. A large number of fittings are adaptable to Nanoports [13].

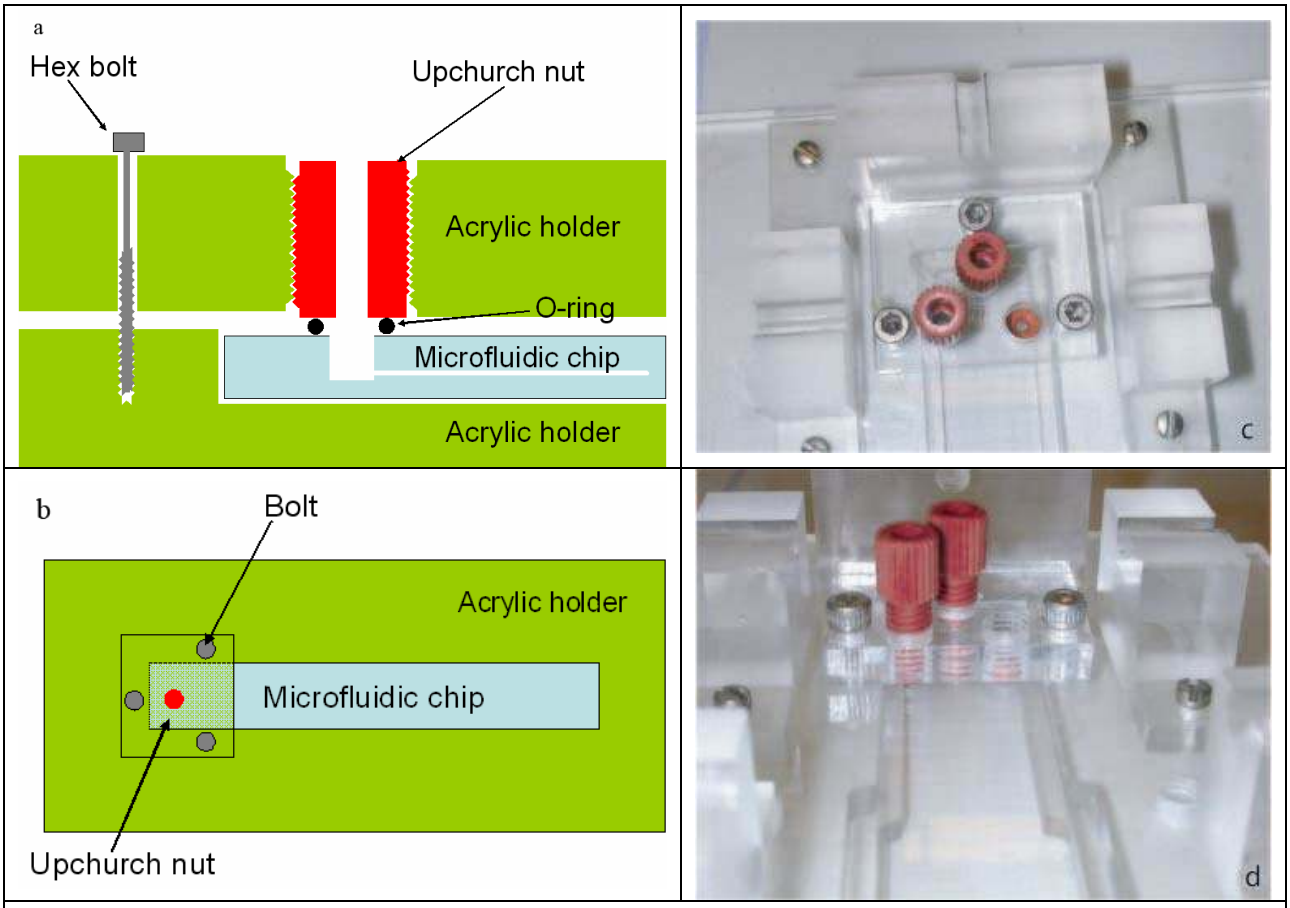

Figure 3: Chip-to-world interface for glass microchip. Schematic of chip holder, side (a) and top view (b), and example of acrylic holder with nuts and O-rings (c,d).

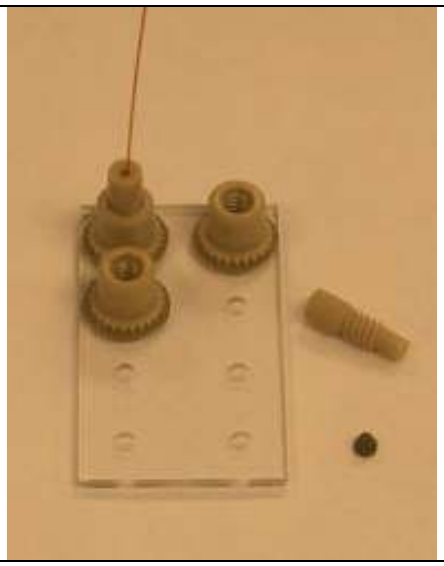

Figure 4: Upchurch Nanoports bonded to glass chip, and interfaced to a capillary with nut and ferrule (black).

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