

Lab-on-a-Chip Application: Making a Pinched Injection on a Chip

LabSmith's LabPackage (Figure 1) makes it easy to build, control and monitor microfluidic manipulations. This experiment was designed to help you train your laboratory team and test competency on microfluidic techniques. It can also be used to ensure that equipment, chips and reagents are working properly. Though the experiment can be attempted with other equipment, the settings and consumables listed are specifically for use with LabSmith equipment.



Figure 1. Components of LabSmith's LabPackage.

Introduction

One of the most important advantages of microfluidic channels on planar substrates is the ability to use electric fields to confine volumes without creating dead volumes or carry over. Described here are the steps and requirements to perform a so-called "pinched injection"¹. In a pinched injection the load step uses applied voltages at all four reservoirs to define a time-independent sample plug, between the sample and sample waste reservoirs. An inject step applies a different voltage sequence to pull back the sample and sample waste fluid while sweeping the defined sample plug from buffer to buffer waste.

Figure 2 shows an image of the load step of the pinched injection captured using the LabSmith SVM340 synchronized video microscope with uScope™ software.

Performing a Pinched Injection

Preparing the Chip

LabSmith's CapTite™ bonded port connectors and microfluidic fittings provide a simple method for connecting capillaries, tubing, or fluid reservoirs to chips.

Figure 3 shows a diagram of a generic microfluidic chip. The steps below assume your chip has CapTite bonded port connectors installed. (information on applying CapTite bonded port connectors is included on Page 3).

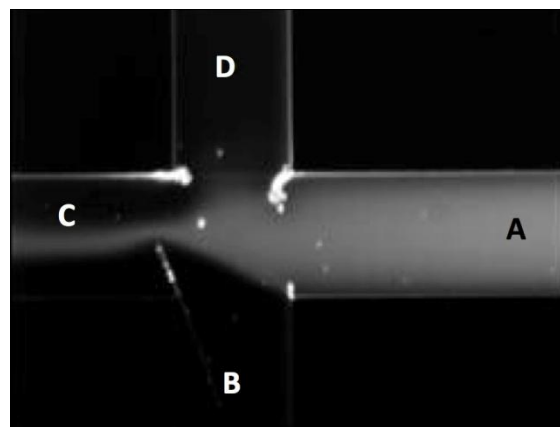


Figure 2. Load step using pinching voltage. Oregon Green dye in Caliper NS 12A Chip.

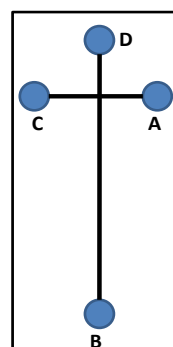
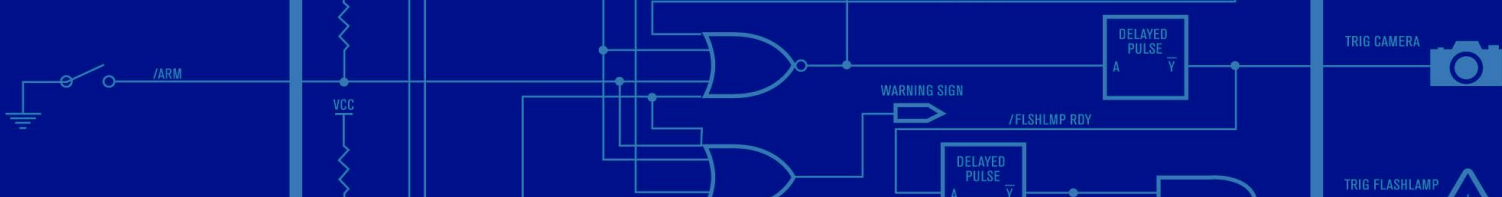


Figure 3. Diagram of basic cross microfluidic chip. Reservoir A=Sample; B=Buffer waste; C= Sample waste; D=Buffer.

Filling the Channel

1. Filter and degas your solution (water or buffer). Sonication for 5 to 10 minutes is suitable for degassing up to 10 mL volumes.
2. Referring to Figure 3, connect a ~10 cm piece of capillary or tubing to port B using a CapTite one-piece fitting. Connect the other end of the capillary to a manual syringe and fill the channels of the chip with your solution via the channel B.



- Place the chip on the LabSmith SVM340 Synchronized Video Microscope stage or to the LabSmith integrated Bread Board (iBB) and secure.
- Launch LabSmith's uScope™ software.
- In uScope™, examine the channels for bubbles or debris. Continue to flush the channels with solution until no bubbles are observed, to ensure correct electrokinetic flow.
- Once the channel is successfully filled, remove the capillary and one-piece fitting from channel B and install CapTite™ reservoirs on all four the bonded port connectors until finger-tight.
- Using an insulin syringe or syringe with needle, fill reservoirs B, C and D with buffer; fill reservoir A with the sample (in this case, Oregon Green™ or fluorescein dye).
- Insert a piece of capillary into the reservoirs to mechanically dislodge bubbles trapped in the cone of the reservoir fitting.

- Install the interlock (50 Ω terminator) in the back of the HVS448.
- Turn on the HVS448 and open the Sequence™ software.
- Click the **Online** button in the Sequence toolbar.
- Click the **Enable High Voltages** button.
- Select **High Voltage Power Supply/Monitor** in the left pane.

In the right pane, select **Monitor All** to monitor all the voltages and currents of the electrodes. Test the connections by using the voltage sliders to adjust voltages in the four channels you will use. Note typical currents.

WARNING: Ensure high voltage is disabled before continuing.

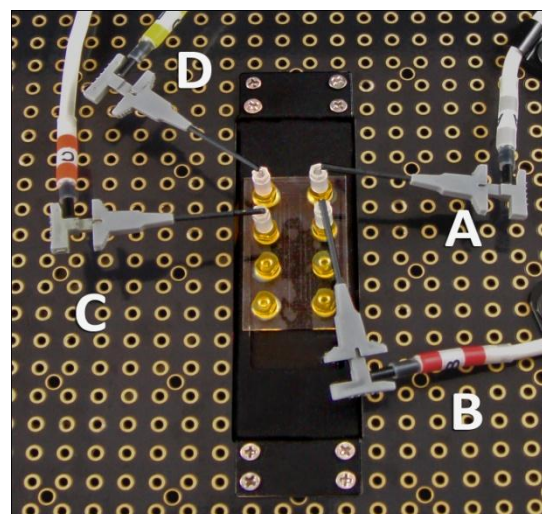


Figure 4. Correctly matched HVC cables with reservoirs and corresponding channel labels for the HVS448 Sequence file.

Placing Electrodes and Connecting to HVS448

The HVS448 high voltage sequencer will control the electric fields. You will use four of the eight channels on the HVS448 to perform this experiment. To connect the high-voltage cables to the HVS448:

- Turn off the HVS448 and remove the interlock (50 Ω terminator) to disable the high voltage.
- Plug high voltage cables A-D in channels HVA-HVD on the back of the HVS448.
- Install a microclip and platinum electrode on the labeled end of each cable. Alternatively, press the electrode directly into the end of the cable.
- Follow the instructions in the **Grounding the HVS448 Channels** section of the Sequence™ User's Manual (pps. 11-12) to ground the unit.
- Place the electrodes in the CapTite™ reservoirs, making sure that cable **A** goes to sample, **B** to buffer waste, **C** to sample waste, and **D** to buffer (Figure 4).

Writing the Voltage Sequence Program

- If necessary, open the Sequence™ software.
- In Sequence™ choose **Tools > Simple Sequence Wizard**.
- On the **Step A** tab change the Step name to "Load."
- If you are using a standard Caliper NS12A chip with pH 7 buffer, refer to Table 1, enter the voltages from the "Load" column for Reservoirs A-D. The reservoir names correspond to HVS channel labels.
- Select **Step B** in the Sequence Wizard and name the step "Inject".
- For the standard Caliper NS12A chip, enter the voltages from the "Inject" column in Table 1 for Reservoirs A-D and press **Apply**.
- Choose **File > Save As** and name the file.
- Now it's time to run the sequence. Press **A** to run the **Load** voltage sequence. Press **B** to run the **Inject** voltage sequence (Figure 6).

Verify Electrical Connections

WARNING: You are about to apply high voltage. Read and follow safety precautions outline in the HVS448 User's Manual (Cautions and Warnings and Grounding the HVS448 Channels sections of user's manual pp. 9-12) before proceeding. Click the Disable button (Figure 5) if you need to touch the microclips, electrodes or reservoirs.

Table 1. Voltages for Caliper NS12A Chip

	Reservoir	Load (V)	Inject (V)
Sample	A	-600	61
Buffer Waste	B	-1500	-100
Sample Waste	C	402	-11
Buffer	D	-792	-1500

If you are using a different chip or buffer, use Sequence's Manual Mode (Figure 5) to adjust the voltages until you observe the desired conditions. Note the voltages used and follow Steps 1-6 using these values rather than the Table 1 values.

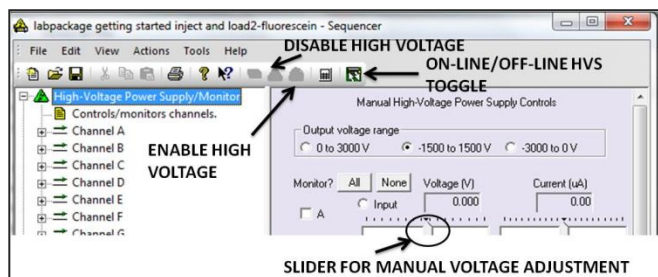


Figure 5. Sequence Software screen showing Enable, Disable, and Manual Mode.

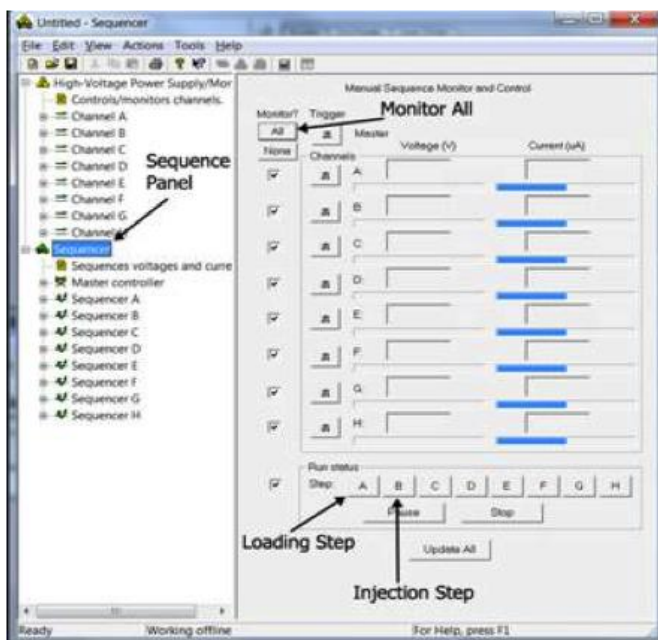


Figure 6. Picture of Sequence™ Software screen when running Load and Inject sequences.

Channel Cleaning and Storage

For **Polymer Chips**, flush with water then running buffer. For **Glass Chips**, flush with 10 mM HCl, water, 10 mM NaOH,

water, then running buffer. Store cleaned chips with sterile deionized water in channels, or air dry the channels and store them dry.

Mounting Bonded Port Connectors

1. Using the 3M DP420 two-part epoxy supplied with the LabPackage, mix two parts white base with one part amber accelerator.
2. Using a small wire or piece of fused silica capillary apply the epoxy mixture to the outer edge of the bottom of the CapTite™ bonded port connector (Figure 7).
3. Align centering nub with center of via hole in chip (Figure 7).
4. Press firmly. Repeat for all wells.
5. Cure hours at 60°C.

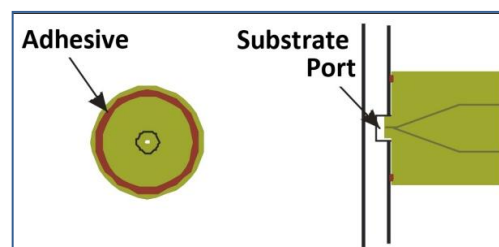


Figure 7. Bonded port glue application and alignment.

Supplies

Table 2 lists the materials, and Table 3 lists the LabPackage components used for this experiment.

Table 2. Reagents and Materials

Reagent/Supply	Source
Glass Chips	Caliper
Polymer Chips	microfluidic ChipShop
Oregon Green	Life Technologies
Buffer, HCl, NaOH	ThermoFisher Scientific

Acknowledgements

LabSmith gratefully acknowledges Professor Sumita Pennathur of University of California Santa Barbara for the teaching laboratory procedure on which these instructions are based.

Reference

- (1). Jacobson, S. C.; Hergenroder, R.; Koutny, L. B.; Warmack, R. J.; Ramsey, J.M. *Anal. Chem.* **1994**, *66*, 1107.

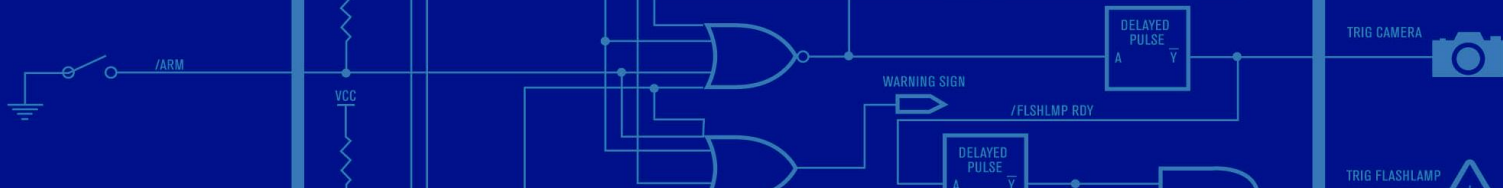


Table 3. LabSmith LabPackage Components *Indicates Equipment Used in This Experiment

COMPONENT	PART NUMBER		QUANTITY
High Voltage Control (HVS448-LP)			
* Eight-channel high-voltage sequencer with 3000 V maximum differential voltage	A - Base price assumes HVS448-3000D or HVS448-3000D-LC B - HVS448-6000D or HVS448-6000D-LC		1
* High-voltage cable kit	A-HVC8-STD		1
* Micro-clip set for use with high-voltage cable kit (includes 8 clips)	A-MC8-01		1
* Platinum electrode wire, 0.584 mm dia (23 gauge), 10 cm length	A-PT-ELECTRODE		1
Visualization (SVM-LP)			
* Synchronous video microscope. Includes control and acquisition software, RS-170-BW camera module, LED-B illuminator module, 10X objective, and motorized X-Y focus traverse stage	SVM340		1
* Schott filter for use in camera module. Wavelengths available: 495, 515, 530, 550, 570, 590, 610, 630, 645, 665 nm [†]	A-FILTER-[WL]		1
* Stainless steel sample stage for SVM340 with two rectangular openings: 20 x 32 mm and 22 x 66 mm	A-Plate		1
* SVM light shield, sits on top of SVM to block ambient light	A-SHIELD		1
Fluid Control (uProcess-LP) – Syringes and Valves for use with uProcess Software			
Syringe Pump Bodies	SPS01		2
Syringe glass and plunger – specify size:	SG-XXX-C360, SG-080-T116		4
Tools for changing syringe glass and plunger set	SPS-TOOLS		1
Two-Position Automated Valve (choose from 360 μm o.d. and 1/16” o.d compatible)	AV201-360	AV201-T116	2
4 Valve Electronic Manifold	4VM01		1
Electronic Interface Board	EIB-100		1
*Integrated breadboard with 8 device connections and 1”x3” cutout for chip viewing via SVM340. Includes mounting hardware for attachment to SVM340.	iBB		1
Connectors (CAPTITE-LP) - specify kit for 360 μm capillary, 1/32” tubing or 1/16” tubing			
One-Piece Fittings	xxxx-100		75
One-Piece Plugs	xxxx-101		10
Tee	xxxx-203		5
Cross	xxxx-204		5
* Bonded Port Connectors (only included in 360μm and 1/32” kits)	xxxx-400		25
Luer-Lock™ Adapters	xxxx-300		10
*Breadboard mounting hardware and tools. Includes TORX wrench, hex wrench, and screws for mounting 5 valves, 10 interconnects to breadboard plus 4 chip clips and 8 electrode clips for mounting chips and electrodes to the iBB.	iBB-TOOLS		1
Breadboard Reservoirs – 1.1 mL reservoirs with pressurizable caps and 3 connections	C360-BBRES	T116-BBRES	2
Epoxy Adhesive (only included in 360μm and 1/32” kits)	LS-EPOXY		1*
microfluidic ChipShop chip – cross, 100 μm x 100 μm , 87 mm PMMA or TOPAS	LS-CROSSCHIP-PMMA	LS-CROSSCHIP-TOPAS	2
*360μm kit: 150μm ID fused silica capillary with cutting stone; 1/32” kit: 250μm ID PEEK tubing; 1/16” kit: 0.03” ID PEEK tubing			1m

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