Microfluidics with the LabSmith LabPackage: Making a Microfluidic Injection on a Chip

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LabSmith's LabPackage (Figure 1) makes it easy to build, control and monitor simple and complex 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 test equipment, chips and reagents to ensure that they are working properly. Though the experiment can be attempted with other equipment, the settings and consumables listed here are specifically for use with LabSmith equipment.

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.

Figure 1. Components of LabSmith's LabPackage.

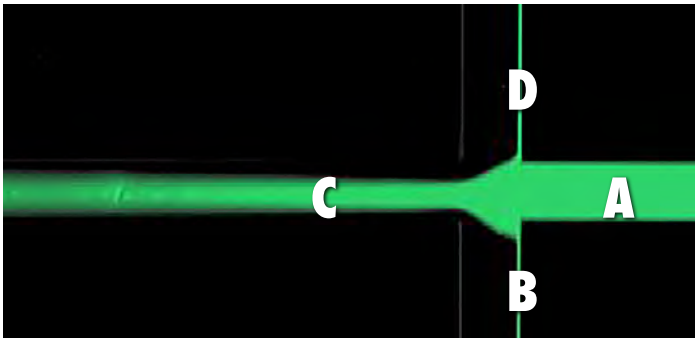

Figure 2. Load step using pinching voltage. Oregon Green dye in Caliper NS 12A Chip.

PERFORMING A PINCHED INJECTION

Preparing the Chip

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

Figure 3 shows a diagram of a generic microfluidic chip. The steps below require a chip with capillary/tubing connections already in place (information on applying CapTite bonded port connectors is included on Page 3 if needed).

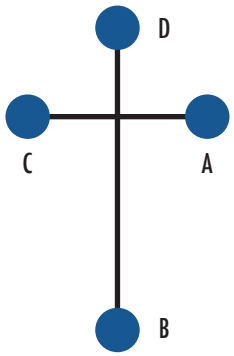

Figure 3. Diagram of basic cross microfluidic chip. Reservoir A=Sample; B=Buffer waste; C= Sample waste; D=Buffer.

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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, using a syringe with a one-piece fitting connected to capillary or PEEK tubing, fill from bonded port connector B.

3. Place the chip on the LabSmith SVM340 Synchronized Video Microscope using either the LabSmith integrated Bread Board (iBB) or the stage plate to secure the chip.

4. Launch LabSmith's uScope™ software.

5. In uScope, examine the channel for bubbles or debris. Continue to flush the channel with solution if bubbles are observed, to ensure correct electrokinetic flow.

6. Once the channel is successfully filled, twist CapTite™ reservoirs into the bonded port connectors until finger-tight.

7. 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).

8. Insert a piece of capillary into the reservoirs to mechanically dislodge bubbles trapped in the cone of the reservoir fitting.

Placing Electrodes and Connecting to HVS448 Voltage Sequencer

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.

1. Insert platinum wire in the LabSmith microclip connectors or labeled white wire from the appropriate LabSmith HVC High Voltage Cable. Be sure to match cable labels (**A, B, C, D**) with the terminals into which they are connected.

2. Place the electrodes in the CapTite™ reservoirs and connect them to the terminals of the HVS448. Make sure that **Terminal A** goes to sample, **B** to buffer waste, **C** to sample waste, and **D** to buffer (Figure 4).

Verify Electrical Connections

1. Turn on the HVS448 and open the Sequence™ software.

2. Click the **Online** button in the Sequence toolbar.

3. Click the **Enable High Voltages** button.

4. Select **High Voltage Power Supply/Monitor** in 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.

CAUTION: The wires are now live and exposed, at high voltage. If at any time you need to touch the electrodes or reservoirs (for example, to refresh the solutions), click the Disable button (Figure 5).

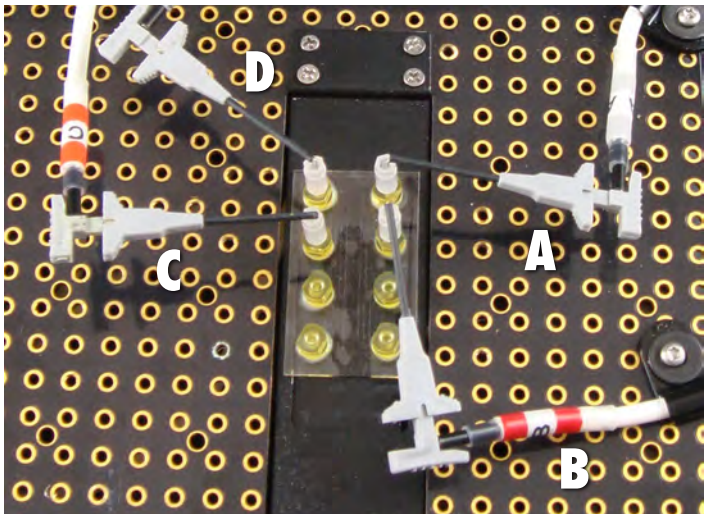

Figure 4. Correctly matched HVC cables with reservoirs and corresponding channel labels for the HVS448 Sequence program.

Writing the Voltage Sequence Program

1. If necessary, open the Sequence™ software.

2. In Sequence choose **Tools > Simple Sequence Wizard**.

3. On the **Step A** tab change the Step name to "Load."

4. 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 reservior names correspond to HVS channel labels.

5. Select **Step B** in the Sequence Wizard.

6. Name the step "Inject."

7. For the standard Caliper NS12A chip , enter the voltages from the "Inject" column in Table 1 for Reservoirs A-D.

8. Press **Apply**.

9. Choose **File > Save As** and name the file.

10. 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).

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 1-8 using these values rather than the Table 1 values.

TABLE 1. HVS448 Sequence Voltages for Caliper NS12A Chip

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

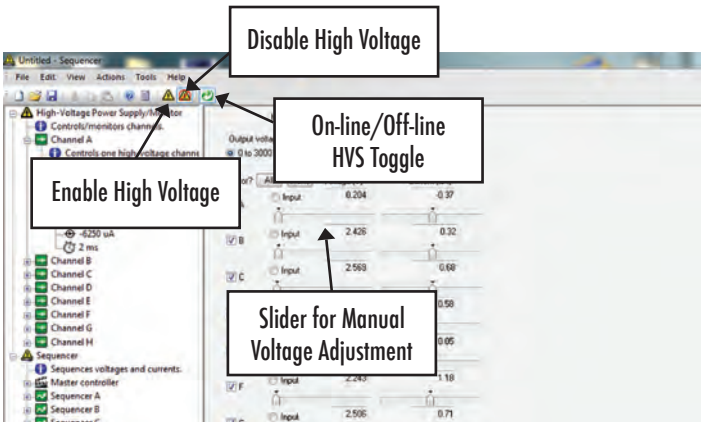

Figure 5. Sequence Software screen showing Enable, Disable and Manual Mode.

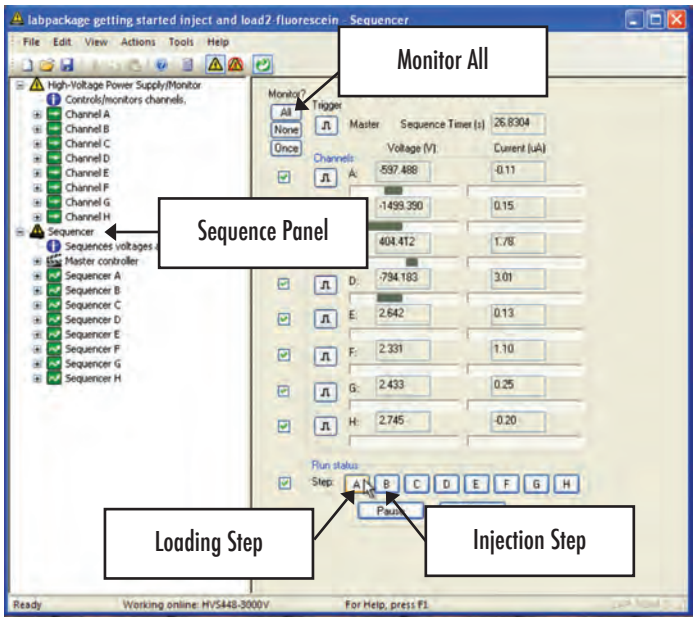

Figure 6. Picture of Sequence Software screen when running Load and Inject sequences.

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 CAPTITE™ 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 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.

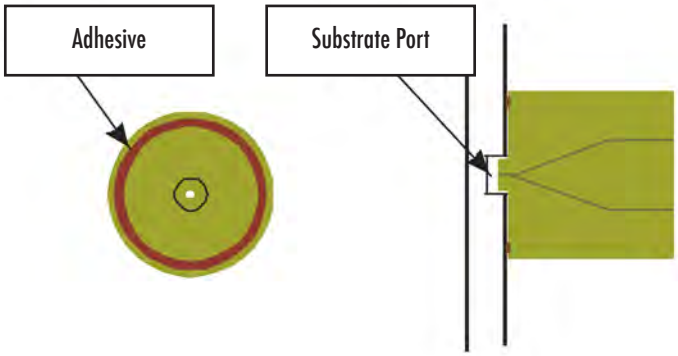

Figure 7. Bonded port glue application and alignment.

SUPPLIES

Table 2 and Table 3 on the following page list the reagents, materials, and LabSmith equipment required for a microfluidic pinched injection.

ACKNOWLEDGEMENTS & REFERENCES

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

Jacobson, S. C.; Hergenroder, R.; Koutny, L. B.; Warmack, R. J.; Ramsey, J.M. Anal. Chem. **1994**, 66, 1107-1113.

TABLE 2. Reagents and Materials

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

TABLE 3. LabSmith Equipment

Component	Part Number	Quantity
High Voltage Control (HVS448-LP)		
Eight-channel high-voltage sequencer with 3000 V maximum differential voltage	Assumes HVS448-3000D	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 Wire- 3 cm	A-HVPT8-STD	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
Epi-fluorescent Camera for Oregon Green	EPI-Blue	1
Stainless steel sample stage for SVM340 with two rectangular openings: 20 x 32 mm and 22 x 66 mm	A-SVM-Stage	1
Integrated Bread Board	iBB	1
SVM light shield, sits on top of SVM to block ambient light	A-SHIELD	1
Fluid Control and Connectors – specify kit for 360µm capillary		
One-Piece Fittings	C360-100	8
One-Piece Plugs	C360-101	4
Bonded Port Connectors*	C360-400*	4
Luer-Lock Adapters	C360-300	4
Reservoirs	C360-RES	1
Hex wrench	LS-HEX	1
Torx wrench	LS-TORX	1
Fused silica capillary cutting stone	LS-CUTTER	1
Epoxy Adhesive*	LS-EPOXY*	1*
150µm ID fused silica capillary	1m	1

* Only included in 360µm and 1/32” kits.

Prices will vary depending on tubing size and/or substitutions.

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