In-Vitro/FSCV Microelectrode Flow Cell User Guide

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1. Preface

1.1 Scope

This User Guide describes the Pine Research In-Vitro/FSCV Microelectrode Electrochemical Flow Cell system. This guide is written for the professional scientist or engineer (or student of science and engineering) and assumes a basic knowledge of scientific measurement and data presentation. Portions of this manual devoted to electrochemical concepts assume some familiarity with the subject of electrochemistry. Additional documentation may be found at Pine Research Instrumentation's online site at the following URL:

https://www.pineresearch.com/shop/product-category/neuroelectrochemistry/fscv-flow-cells/

1.2 Copyright

1.3 Trademarks

1.4 Use Limitation

This instrumentation is not designed for use in experiments involving human subjects and/or the use of electrodes inside or on the surface of the human body.

Any use of this instrument other than its intended purpose is prohibited.

1.5 Service and Warranty Information

For questions about proper operation of the system or other technical issues, please use the contact information below to contact Pine Research directly.

TECHNICAL SERVICE CONTACT
Pine Research Instrumentation, Inc. **http://www.pineresearch.com** **Phone: +1 (919) 782-8320** **Fax: +1 (919) 782-8323**

If the system or one of its components or accessories must be returned to the factory for service, please contact Technical Service (see above) to obtain a Return Material Authorization (RMA) form. Include a copy of this RMA form in each shipping carton and ship the cartons to the Factory Return Service Address (below).

FACTORY RETURN SERVICE ADDRESS
Pine Instrument Company **ATTN: RMA # <RMA number>** **104 Industrial Drive** **Grove City, PA 16127** **USA**

RETURN MATERIAL AUTHORIZATION REQUIRED!

Do not ship equipment to the factory without first obtaining a Return Material Authorization (RMA) from Pine Research Instrumentation.

LIMITED WARRANTY

The Pine Research In-Vitro/FSCV Microelectrode Flow Cell System (hereafter referred to as the "Flow Cell") offered by Pine Research Instrumentation (hereafter referred to as "PINE") is warranted to be free from defects in material and workmanship for a one (1) year period from the date of shipment to the original purchaser (hereafter referred to as the "CUSTOMER") and used under normal conditions. The obligation under this warranty is limited to replacing or repairing parts which shall upon examination by PINE personnel disclose to PINE's satisfaction to have been defective. The customer may be obligated to assist PINE personnel in servicing the Flow Cell. PINE will provide telephone support to guide the CUSTOMER to diagnose and effect any needed repairs. In the event that telephone support is unsuccessful in resolving the defect, PINE may recommend that the Flow Cell be returned to PINE for repair. This warranty being expressly in lieu of all other warranties, expressed or implied and all other liabilities. All specifications are subject to change without notice.

The CUSTOMER is responsible for charges associated with non-warranted repairs. This obligation includes but is not limited to travel expenses, labor, parts and freight charges.

1.6 Icons

Special icons are used to call attention to safety warnings and other useful information found in this document (see: Table 1-1).

	**STOP:** **For a procedure involving user action or activity, this icon indicates a point in the procedure where the user must stop the procedure.**
	**NOTE:** **Important or supplemental information.**
	**TIP:** **Useful hint or advice.**
	**CAUTION:** **Indicates information needed to prevent damage to equipment.**
	**WARNING:** **Indicates information needed to prevent injury or death to a person or to prevent damage to equipment.**

Table 1-1. Special Icons used in this Document.

2. Product Specifications

2.1 Flow Cell Description

The In-Vitro/FSCV Microelectrode Flow Cell provides for the rapid measurement of transient solution changes at microelectrodes. Incorporation of flow cells enable facile quantitative and qualitative analytical measurements of solutions in a background electrolyte. Scientists often use flow cells to validate and calibrate electrochemical sensors. When this sensor is a microelectrode, scientists often employ a common electrochemical measurement technique called fast-scan cyclic voltammetry (FSCV). FSCV is a background subtracted (*i.e.*, differential) technique; FSCV measures changes in analyte concentration as a function of time.

In most common setups, FSCV uses working electrodes whose active areas are on the microscale or nanoscale. When evaluating new electrodes or developing new protocols, it is desirable to work within a controlled environment where known analyte concentration changes over time. Flow Cells for these types of calibration and validation experiments have not been commercially available, which was the motivation for Pine Research to bring this product to market.

2.2 Physical Dimensions

The base of the assembled flow cell is $10 \times 9 \times 0.5\ in.\ (25.4 \times 22.9 \times 1.3\ cm)$. The height of the flow cell, as measured from the base, is $12.4\ in.\ (31.5\ cm)$.

2.3 Gravity Feed Flow System

The supplied fluidic pathway uses a gravity feed system, which provides cost-effective performance. Gravity-fed systems use gravity to force and maintain solution flow in the fluidic system, from the reservoir to the flow cell to the waste vessel. Based on a four-way cross adapter, the flow cell fluidic pathway includes run buffer, analyte deliver, and priming lines. The instructions provided in this guide illustrate the most common and typical fluidic pathway setup, but users can make adjustments as needed to suit their research needs. The gravity feed flow cell uses compression fittings, designed for use with liquid chromatography; therefore, users can easily customize the fluidic pathway with fittings available from many commercial sources.

Users can easily upgrade their flow cell by adding a syringe pump to the run buffer supply line (not available from Pine Research). Changing the buffer supply line mechanism from the as supplied gravity feed to a syringe pump enables a known volume flow rate to be continuously supplied to the flow cell outlet/microelectrode. This alteration will be discussed briefly in Section 5.1. Regardless, this manual will focus on the as supplied gravity feed set-up and its utilization for flow cell experiments.

The supplied fluidic parts include syringes, tubing, connectors and adapters (see: Section 2.4.1). In this guide, we use the following terms:

- the *run buffer* is the continuous stream of background solution (*i.e.*, electrolyte, buffer, etc.) flowing through the system
- the *sample* is a solution containing the analyte of interest, typically injected into the run buffer stream

In the gravity feed setup, the following syringes have the described function:

- $60\ mL$ syringe serves as the run buffer reservoir
- $1\ mL$ syringe serves as the sample reservoir and injector
- $5\ mL$ syringe aids in fluidic system priming

In a gravity feed fluidic path, the resistance of the tubing and the height of the solution above the flow cell outlet govern solution flow rate. These facts have two practical implications as follows:

- Flow rates are non-linear. As the run buffer volume in the supply reservoir changes, the flow rate also changes. The greater the difference in height between the flow cell outlet and the run buffer solution level, the higher the flow rate.
- Flow rates change as a function of tubing length and/or inner diameter used in the fluidic pathway system. Throughout this guide, instructions and data are based on a fluidic pathway that uses a $14\ in.$ long run buffer supply line and $10\ in.$ long flow cell delivery line.

Solution flow rates for the system described here have been characterized (see: Figure 2-1). As shown, flow rates vary from about $2\ mL/min$ when the run buffer reservoir is full to about $0.5\ mL/min$ at the lower volume limit of the reservoir. Across all flow rates and volumes, a full run buffer reservoir will flow for around 50 minutes. Users may wish to occasionally add more run buffer to the reservoir to keep the flow rate variability smaller and extend the time of use.

TIP:

Avoid flow rates $< 0.5\ mL/min$. Use of such flow rates can result in turbulent mixing of sample and run buffer, which is undesirable for analytical measurements.

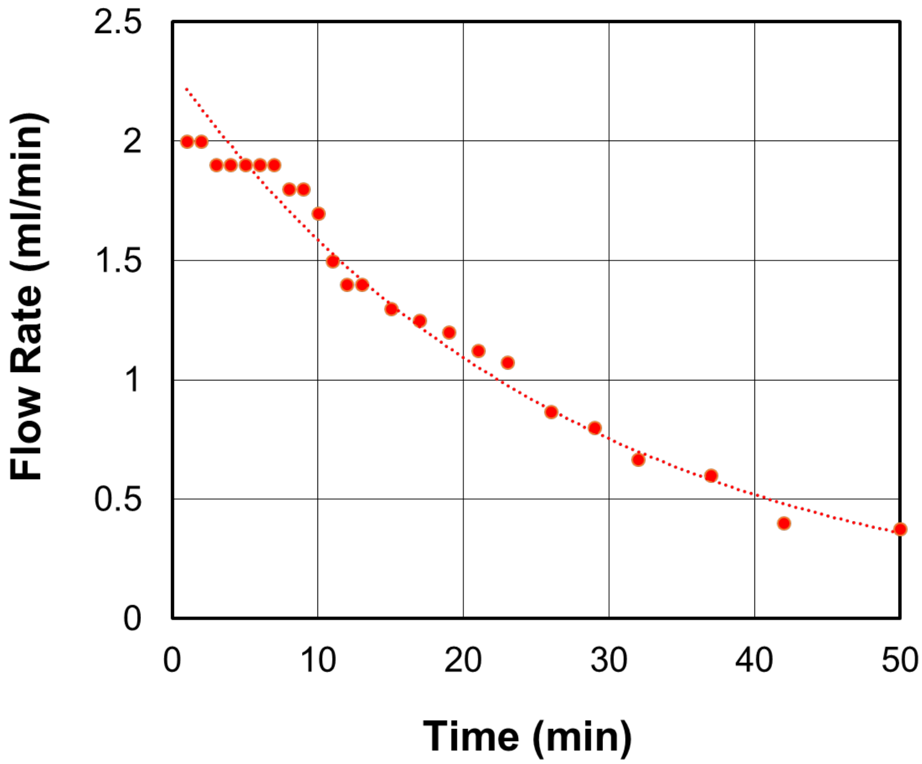


Figure 2-1. Gravity Feed Flow Rate Performance (dashed line running average)

2.4 System Components

The In-Vitro/FSCV Microelectrode Flow Cell system ships partially assembled from the production facility. The basic system does not include the micromanipulator (xyz translational stage) while the complete system does include it. Pine Research expects nearly all users will require the micromanipulator; however, labs that already have the component may wish to purchase the flow cell basic system (see: Table 2-1). Detailed descriptions of each part are in Section 2.4.2.

2.4.1 Fluidic Parts Kit

All of the threaded fittings used in this system and on the mechanical flow cell assembly are tapped with $1/4" - 28$ threads for compression fittings, selected for use with the included $1/16"$ OD $\times$ $0.040"$ ID tubing. All parts are available in a replacement kit (see: Table 2-2).

1

2

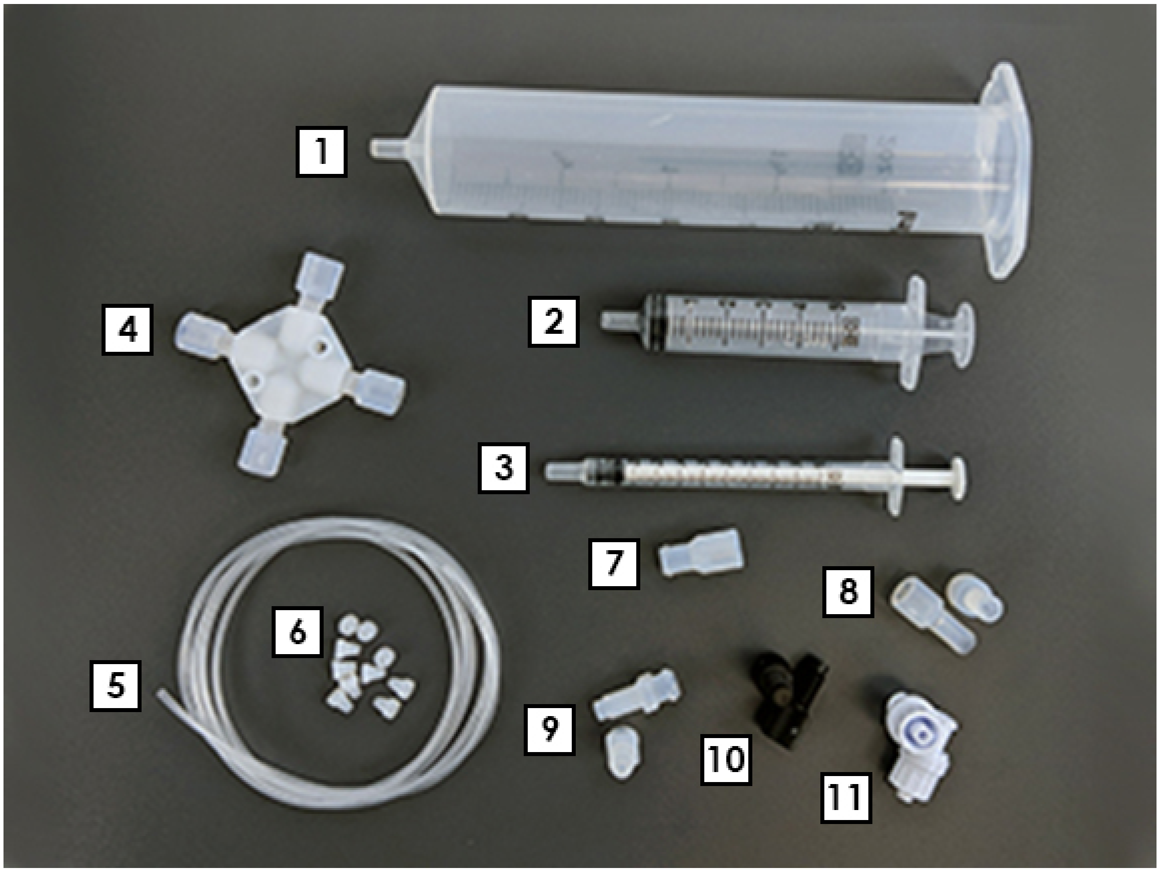
3

4

5

1	**Fluidic Parts Kit**	Box of fluidic parts, including fittings, tubing, connectors, and syringes. Specific parts included in this kit are detailed in Table 2-1
2	**Flow Cell Body**	Machined flow cell body, attaches to structural assembly, polyamide
3	**Flow Cell Structural Assembly**	Base and arm, anodized aluminum
4	**Waste Reservoir**	Glass waste container, for collecting spent buffer and analyte
5	**Buffer Reservoir Loop**	Hook and Loop type fabric strap for holding $60\ mL$ syringe
6	**Micromanipulator**	Optional xyz translational stage, connects to structural assembly to hold and position microelectrodes

Table 2-1. Primary Components Included with the Flow Cell System



	Description	Part #	Qty.
1	60 mL syringe with Luer lock	RRFC11	3
2	5 mL syringe with slip tip	RRFC10	5
3	1 mL syringe with slip tip	RRFC09	5
4	Cross assembly, EFTE polymer, 1/16" OD × 0.020 through hole	RRFC02	1
5	Tubing, EFTE polymer, 1/16" OD × 0.040 ID, 5′	RRFC03	1
6	Flangeless ferrule, EFTE polymer, 1/4" – 28 flat bottom, for 1/16" OD	RRFC08	10
7	Compression fitting, PFA polymer, female to 1/4" – 28 male	RRFC05	2
8	Compression nut, PFA polymer, 1/4" – 28 flat bottom, for 1/16" OD	RRFC06	2
9	Luer Adapter, female to Luer union, female	RRFC07	1
10	Threaded plug, 1/4" – 28, black	RRFC04	2
11	Luer-to-Luer check valve	RRFC12	2

Table 2-2. Components Included in Fluidic Parts Kit

2.4.2 Mechanical Parts

Flow Cell Base and Arm

The base and arm of the flow cell feature a rigid, anodized aluminum construction. The base ships with rubber feet installed ~~onto the base~~. Prior to shipment, Pine Research assembles the base, arm and feet (see: Figure 2-2). The arm includes machined groves and holes for the installation of the flow cell, the buffer reservoir syringe strap, and the micromanipulator.

Figure 2-2. Flow Cell Base and Arm as Assembled prior to Shipment

Flow Cell Body

The flow cell body is machined from solid polyamide. It is a lightweight material that is resistant to corrosion, which can arise from long term exposure to buffer/electrolyte. As shipped, the flow cell has several threaded bores in place for making appropriate fluidic line and drain connections. The top of the flow cell features a unique design for delivery of buffer and analyte, with a sloped drain to minimize injection errors and draining issues (see: Figure 2-3).

The flow cell body features different machined elements on the top and bottom sides of the basin (see: Table 2-3). There are three 1/4" – 28 threaded holes on the bottom of the flow cell, which are used for fluidic line delivery, reference electrode reservoir, and for solution draining (see: Table 2-3). There are additional bores in the flow cell for undefined uses, as dictated by the user as necessary.

Figure 2-3. Flow Cell Body

The flow cell includes four black thumb screws, ideal for securing external leads into the flow cell, such as a reference electrode. Common external reference electrodes are an Ag/AgCl element connected to a lead wire. To hold the reference electrode in position in the system, thread a thumb screw (see: Figure 2-4) into a bore near the reference electrode reservoir side of the flow cell body. Then use this thumb screw to hold down the lead wire of the reference electrode (see: Figure 2-5). A black plug (Part number 10 on Table 2-2) should be threaded on the bottom of the flow cell body to form a reference electrode reservoir (threaded hole#1 in Table 2-3). This maintains a well of buffer that improves contact between the solution and electrodes.

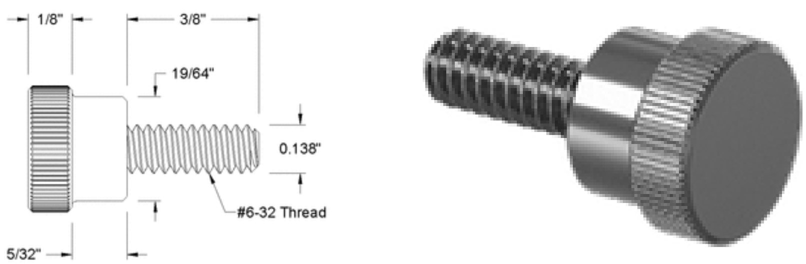


Figure 2-4. Black Plastic External Lead Thumb Screw

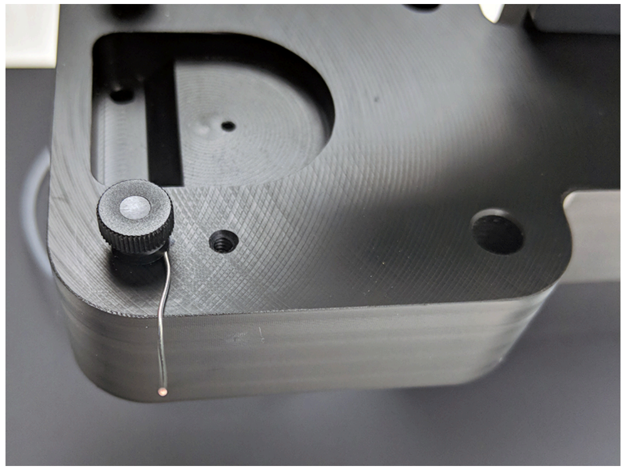
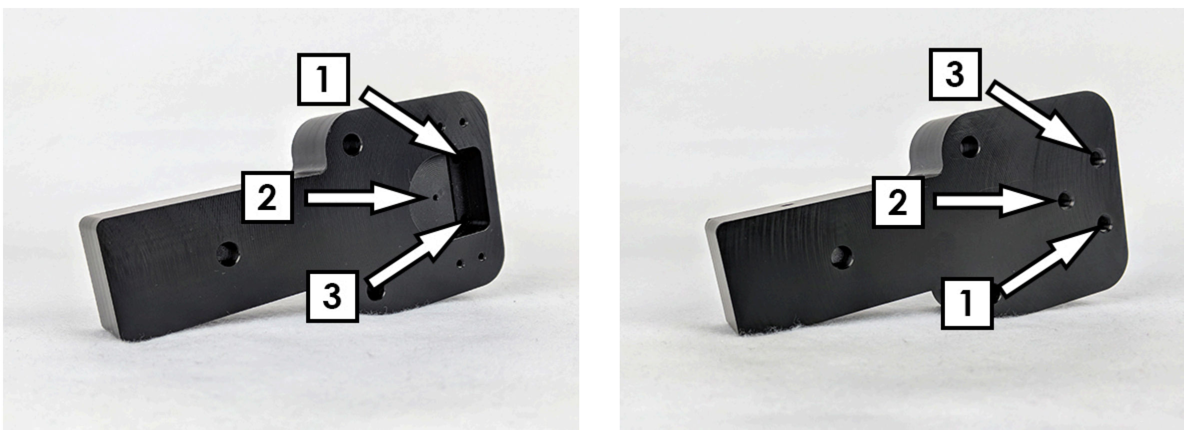


Figure 2-5. External Lead Thumb Screw Securing Reference Electrode Lead



	Description
1	1/4" – 28 bore reference electrode recess (plug with number 10 from Table 2-2)
2	Solution delivery hole (connect to fluidic line delivery)
3	1/4" – 28 bore drain (leave open)

Table 2-3. Flow Cell Body Hole Definitions

Buffer Reservoir Strap

The buffer reservoir is a $60\,mL$ syringe with plunger removed (see: Table 2-2). The syringe must be suspended above the flow cell to ensure proper gravity feed. The flow cell includes a simple hook-and-loop strap. Two slots on the top of the flow cell arm secure the strap in place, which fits around the syringe to keep it suspended and in place (see: Figure 2-6). Installation instructions for the buffer reservoir strap appear in Section 3.2.2.

Figure 2-6. Buffer Reservoir Strap Installed on Arm (Left) Holding Syringe (Right)

Waste Reservoir

During use, buffer and analyte solutions will flow out from the four-way cross assembly to the flow cell. As the volume delivered increases, solution will drop across the sloped edge of the flow cell and exit though an unplugged hole in the drain basin (see: Figure 2-3). The simple glass waste reservoir sits beneath the cell to collect waste solution. The waste reservoir holds approximately $180\ mL$.

Micromanipulator (xyz Translational Stage)

In a complete flow cell, the most expensive component is typically the micromanipulator. This mechanical device allows fine control of the x, y, and z plane of an installed electrode. Each direction has an independent fine control knob. These knobs are useful in positioning the microelectrode directly above the flow cell solution delivery hole.

Such tools like the micromanipulator are often already in laboratories. Pine Research integrated their flow cell for use with a third-party micromanipulator, manufactured by World Precision Instruments (model: M3301R, see: Figure 2-7). The flow cell arm has pre-drilled threaded holes for mounting this micromanipulator. The micromanipulator includes an acrylic electrode holder. Optional, modified Mali Adapters are available from Pine for holding microelectrodes, if needed, see Pine's website for details.

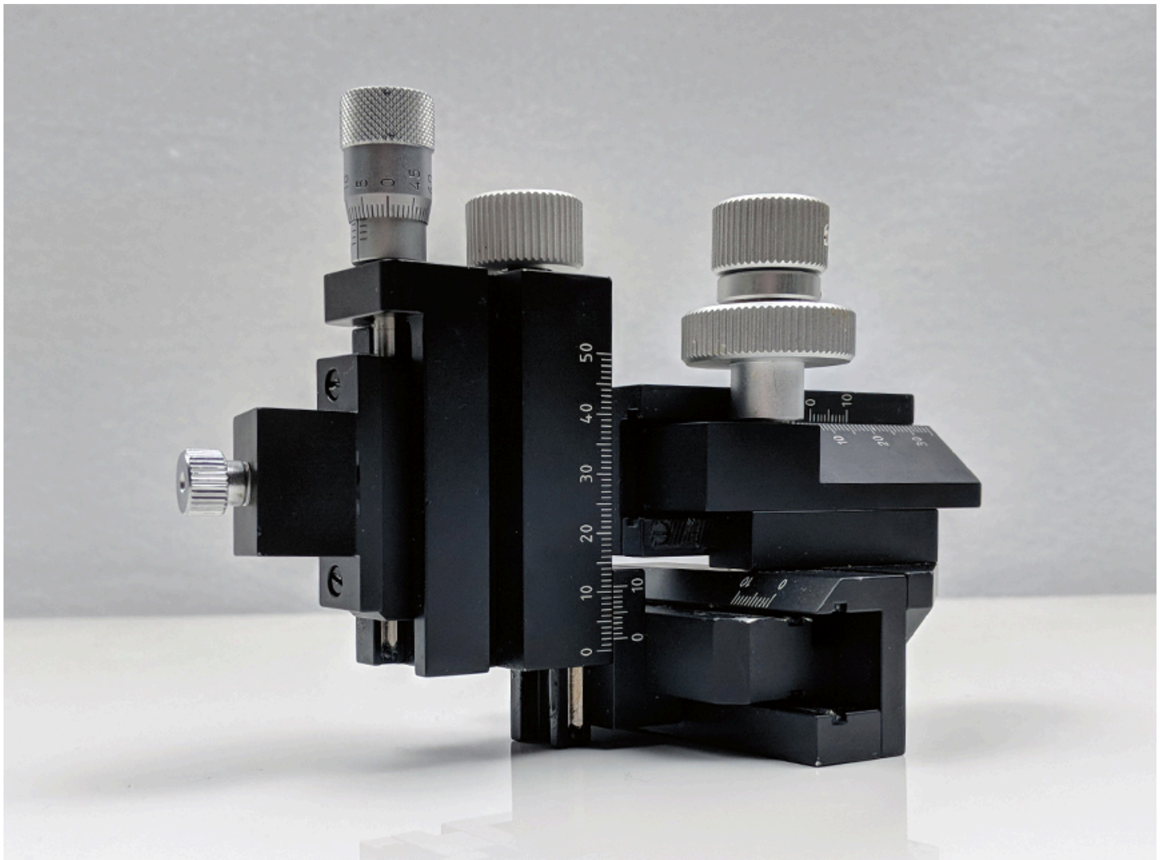


Figure 2-7. Optionally Available Micromanipulator (xyz Translational Stage)

3. System Assembly and Installation

The shipping box contains the structural portion of the flow cell mechanical assembly, flow cell body, a box containing parts for the fluidic path, buffer reservoir strap, waste reservoir, and if purchased, a micromanipulator (see: Table 2-1). All parts should be removed from the box and placed on a sturdy table/bench for assembly. Physical installation includes mechanical assembly and fluidic line assembly.

3.1 Physical Installation

The flow cell should be setup on a sturdy, stable location. In general, the system is used for electrochemical experiments where small (nanoampere or less) currents are measured; thus, the system is most commonly installed inside a Faraday cage to minimize noise.

3.2 Flow Cell Assembly

Prior to shipment, the production facility partially assembled the flow cell by connecting the base to the arm and installing the rubber feet. The first step in assembly involves configuring the fluidic lines and fittings. In the second step, users must assemble/attach the buffer reservoir strap, flow cell body, and optional micromanipulator.

3.2.1 Fluidic Line and Fittings Assembly

The supplied fluidic path is based on a four-way cross assembly for the introduction of solutions to the flow cell. The four-way cross has four inputs with each input having a compression fitting installed (see: Figure 3-1). The four inputs are used for sample injection, priming of the fluidic lines, supply of running buffer, and output flow to flow cell body.

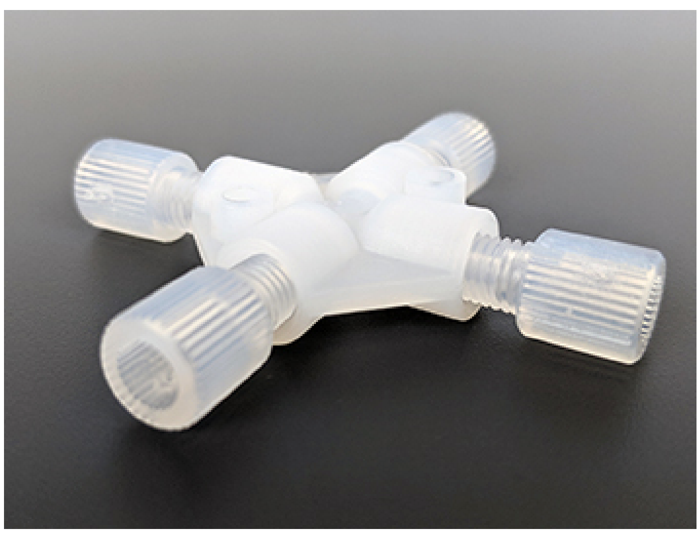


Figure 3-1. Four Way Cross Assembly

Configure Four-Way Cross Assembly

On the four-way cross assembly, remove two perpendicularly placed compression fittings and replace them with female Luer to 1/4" – 28 threaded male adapters (number 7 in Table 2-2) (see: Figure 3-2).

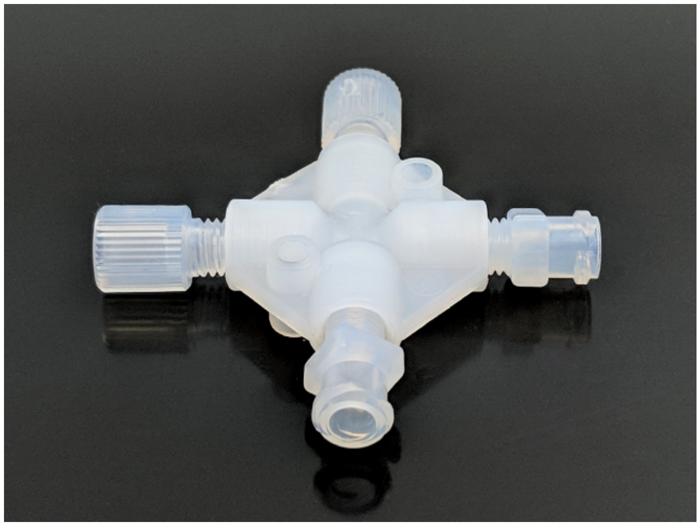


Figure 3-2. Four-Way Cross Assembly with Luer to Threaded Adapters

Onto the threaded adapters, install one Luer-to-Luer check valve (number 11 in Table 2-2) (see: Figure 3-3). The check valve allows solution to flow in only one direction, into the four-way cross. With a check valve installed solution will not be lost when the syringe is removed. The port with the check valve will be the sample injection port. The port with the threaded Luer connector is the priming port, used to prime the fluid path (using a 5 mL syringe) at the beginning of an experiment.

Figure 3-3. Installation of Check Valve on Cross Assembly

Configure Flow Cell Delivery Tubing and Fittings

The remaining two compression fittings on the four-way cross will be connected to tubing. They will be used to bring run buffer from the reservoir to the four-way cross and then from the four-way cross to the flow cell body.

Remove the remaining two compression fittings from the four-way cross. Cut a 10" length of tubing, which will be fitted to connect the flow cell body to the four-way cross. Place one of the compression fittings (removed from the four-way cross) onto the tubing and then install a flangeless ferrule (number 6 in Table 2-2) onto the tubing. The flat side of the ferrule should be facing away from the compression nut toward the end of the tubing and the flat side of the ferrule should be flush with the tubing (see: Figure 3-4). Repeat the installation of a ferrule and compression nut on the other end of the tubing (see: Figure 3-5).

Figure 3-4. Installation of Ferrule and Compression Nut onto Tubing

Figure 3-5. Ferrule and Compression Nut Installation on both Tubing Ends

Move onto configuring the buffer supply tubing and fittings, after which instructions will be provided for installation into the four-way cross adapter.

Configure Buffer Supply Tubing and Fittings

The fluidics line preparation for the buffer supply connection is analogous to the preparation of the flow cell tubing. Cut a 14" length of tubing, which will be fitted to connect the supply reservoir to the four-way cross. Place one of the compression fittings (removed from the four-way cross) onto the tubing and then install a flangeless ferrule (number 6 in Table 2-2) onto the tubing. The flat side of the ferrule should be facing away from the compression nut toward the end of the tubing and the flat side of the ferrule should be flush with the tubing (see: Figure 3-4). Repeat the installation of a ferrule and compression nut on the other end of the tubing (see: Figure 3-5).

To one end of the tubing fitted with a ferrule and compression nut, connect a compression fitting to threaded Luer adapter (number 7 in Table 2-2), then connect a Luer-to-Luer check valve (number 11 in Table 2-2) onto the free end (see: Figure 3-6).

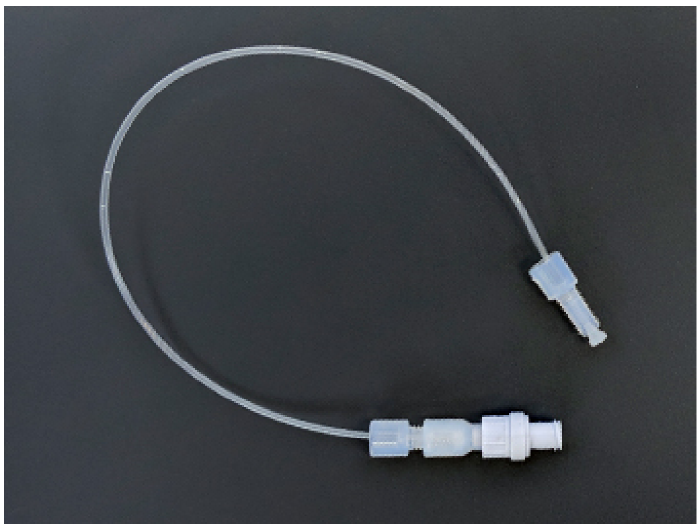


Figure 3-6. Buffer Supply Line Complete with Fittings

Connect All Fluidic Components Together

After preparing the four-way cross adapter, flow cell delivery tubing, and buffer supply tubing, all parts can be connected to form the complete fluidics component of the flow cell.

Install one end of the 10" (flow cell) tubing with compression nut into the four-way cross adapter. If the ferrule is not flush with the tubing or the assembly does not easily connect into the four-way cross adapter, remove and attempt to install again. Increase the torque applied to the compression nut. Check the connection again to ensure the ferrule is properly sealed to the tubing.

Install the compression nut end of the 14" (buffer supply) tubing into the four-way cross, perpendicular to the flow cell tubing. If the ferrule is not flush with the tubing or the assembly does not easily connect into the four-way cross adapter, remove and attempt to install again. Increase the torque applied to the compression nut. Check the connection again to ensure the ferrule is properly sealed to the tubing.

At this assembly stage, the fluidic system will be complete and ready to connect to the buffer reservoir and flow cell body (see: Figure 3-7). This guide will move to the mechanical installation steps next, followed by connection of the fluidics lines to the fully assembled flow cell.

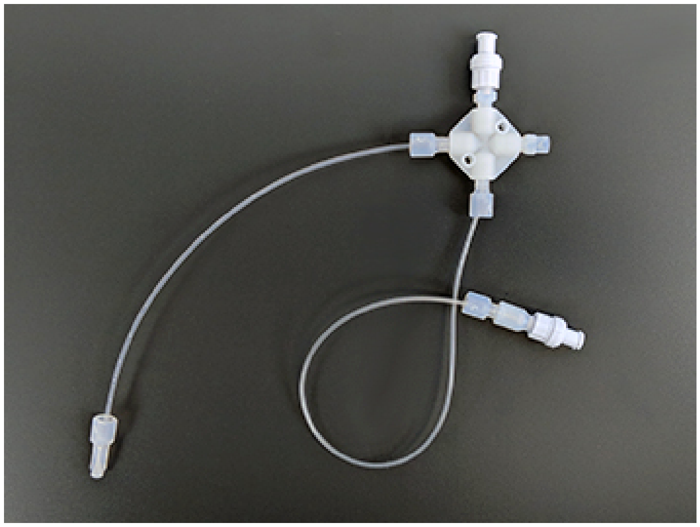


Figure 3-7. Fully Assembled Fluidics System

3.2.2 Mechanical Assembly

Attach Buffer Reservoir Strap

There are two long, parallel machined holes at the top of the flow cell arm (see: Figure 3-8). Thread the buffer reservoir strap through these holes as shown.

Start by inserting the strap into the arm on the side with flow cell body and micromanipulator cuts (see: Figure 3-9).

Figure 3-8. Flow Cell and Micromanipulator Installation Side of Arm

Follow through the installation images provided (see: Figure 3-9). The "push tab" of the syringe rests on the top of the arm, to ensure that the syringe is securely in place during use.

Figure 3-9. Attachment of Buffer Reservoir Strap (left to right, top to bottom)

INFO:

If the flow cell will be used with a syringe pump (not gravity feed), the buffer reservoir strap installation can be skipped.

Attach Flow Cell Body

Before attaching the flow cell body to the flow cell arm, connect the flow cell fluid delivery line to the bottom of the flow cell (this makes it easier to install). The flow cell delivery line terminates with a ferrule and compression nut. Thread the compression nut into the solution delivery hole in the bottom of the flow cell body (see: Table 2-3 and Figure 3-10). Before proceeding to the next step, we recommend also installing a black threaded plug on the bottom of the flow cell in one of the drain positions, to create the reference electrode reservoir (see: Table 2-3 and Figure 3-10).

Next proceed with attaching the body to the arm. One side of the flow cell arm has a notch to receive the flow cell body (see: Figure 3-8). The flow cell body slides into the lower notch and is secured to the arm with three socket head screws on the opposite side (see: Figure 3-11).

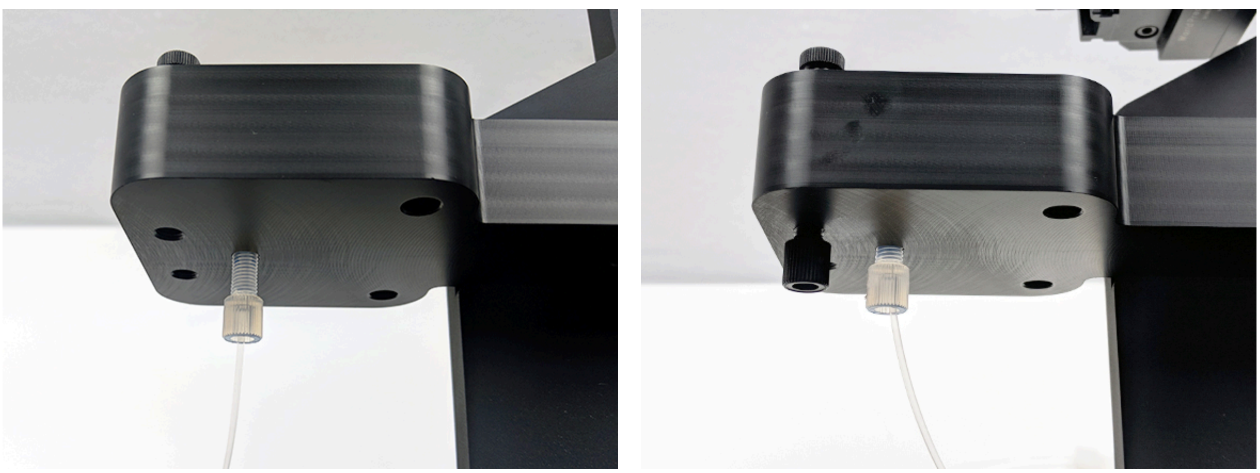


Figure 3-10. Flow Cell Delivery Line Connection to Flow Cell Body

The assembly hardware required (socket head screws and washers) have been provided with the flow cell (see: Table 3-1).

CAUTION:

There are two types of socket-head screws included with the flow cell. Be sure to use the longer of the two screws to install the flow cell body. The shorter of the two screws are for use with the micromanipulator.

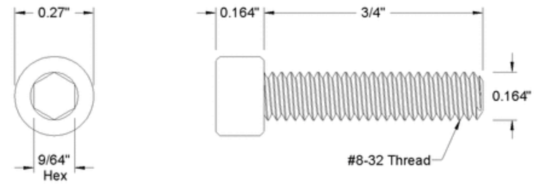
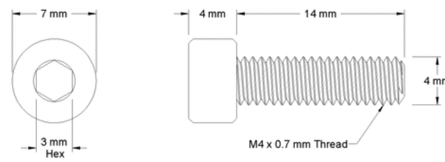
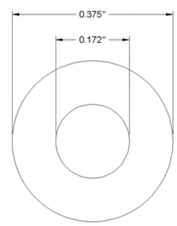
Qty.	Part Number	Description	Image
3	KSM0832S12HS	SCREW, 8-32 X 3/4 L SOC SS	
3	KSMM4S14HS	SCREW, M4X.7 14MM L SOC SS	
6	KWP008S	Washer, Flat #8 .031 THK SS	

Table 3-1. Mechanical Fasteners Required for Flow Cell and Micromanipulator Assembly

Slide the flow cell body into the lower notch on the flow cell arm (see: Figure 3-8 and Figure 3-11). Use 3x KSM0832S12HS screws, longer screws, and 3x KWP008S washers (see: Table 3-1) to connect the flow cell body to the arm. Slide a washer onto the screw first, then insert through the arm hole (side opposite the cut). Use a 9/64" hex wrench/driver to tighten the three screws. Screws should be finger tight. Do not over tighten, or this may damage the inner threads of the flow cell body.

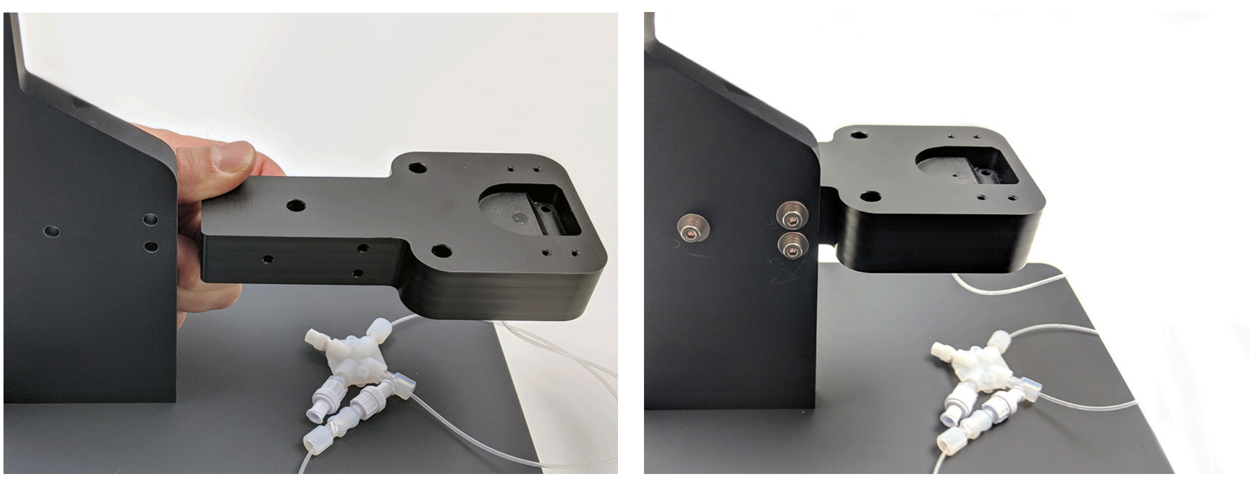


Figure 3-11. Attachment of Flow Cell Body to Arm

3.2.3 Micromanipulator Installation (if purchased)

As shipped, the micromanipulator includes a bracket that must be removed prior to installation onto the flow cell arm.

Use a $3\,mm$ hex driver/wrench to remove two socket head screws (see: Figure 3-12). The removed bracket is not used with the flow cell.

Figure 3-12. Removal of Micromanipulator Bracket

One side of the flow cell arm has a notch to receive the micromanipulator (see: Figure 3-8). The micromanipulator slides into the upper notch and is secured to the arm with three socket head screws on the opposite side (see: Figure 3-11).

Slide the micromanipulator into the upper notch on the flow cell arm (see: Figure 3-8 and Figure 3-11). Use 3x KSMM4S14HS screws, shorter screws, and 3x KWP008S washers (see: Table 3-1) to connect the micromanipulator to the arm. Slide a washer onto the screw first, then insert through the arm hole (side opposite the cut). Use a $3\,mm$ hex wrench/driver to tighten the three screws. Screws should be finger tight. Do not over tighten, or this may damage the inner threads of the micromanipulator ~~flow cell body~~.

CAUTION:

There are two types of socket-head screws included with the flow cell. Be sure to use the longer of the two screws to install the flow cell body. The shorter of the two screws are for use with the micromanipulator.

Figure 3-13. Attachment of the Micromanipulator to Arm

3.3 Final Assembly

From the four-way cross assembly on the fluidic line, connect the tubing with check valve installed onto a $60\ mL$ syringe, whose inner plunger has been removed. Attach the syringe to the arm and secure in place with the buffer reservoir strap (see: Figure 3-14). The $1\ mL$ syringe connects to the four-way cross assembly on the check valve and the $5\ mL$ syringe connects to the open Luer lock on the four-way cross adapter (see: Figure 3-14). Finally, place the buffer waste reservoir beneath the flow cell body.

Figure 3-14. Completed Fluidic and Mechanical Assembly of Flow Cell

4. System Testing and Evaluation

~~Place the buffer waste reservoir beneath the flow cell body.~~ This section describes how to initiate gravity feed flow in the fluidic system, connect working electrodes to the micromanipulator, connect the reference electrode, and perform sample injection.

4.1 Fluidic Line Preparation

Before testing the system make sure to place the buffer waste reservoir beneath the flow cell body. Next ensure that solution flows through the fluidic lines and there are no leaks. Priming is the process of filling the fluidic lines with solution and is necessary when relying on a gravity feed system. During priming air is replaced by solution in the fluidics lines and adapters. The pressure difference between the buffer reservoir and the solution delivery hole drives fluid flow.

Users should refer to the fluidic line parts list (see: Table 2-2) and assembly instructions (see: Section 3.2.1) to gain familiarity with the component names.

TIP:

Always use solutions that is at room temperature or slightly above. Cold solutions will generate bubbles as they warm-up. These bubbles will stop flow and are a common issue for gravity feed systems. During priming watch bubbles in the fluid line to monitor, if the solution is flowing. Ensure that the sample syringe is installed while priming the fluidic lines.

4.1.1 Priming Instructions

1. Remove the $5\,mL$ (priming) syringe from the four-way cross adapter. Fill the priming syringe with $\sim 5\,mL$ run buffer (the same solution in the buffer reservoir). Tap the syringe to remove any trapped air. Connect the filled syringe onto the four-way cross adapter Luer lock.
2. Fill the $60\,mL$ reservoir (buffer reservoir) with run buffer. Observe the solution delivery hole (number 2 in Table 2-3) and the fluidic tubing pathways. It is possible that solution will begin to flow from the buffer reservoir, to the four-way cross adapter, and out through the delivery hole in the flow cell.
3. If the flow does not start immediately, slowly depress the priming syringe. The solution from the syringe will fill the fluidic lines. It is possible this may cause solution to move through the system. Observe the delivery hole for solution flow.
4. If flow still does not start, remove the priming syringe from the four-way cross adapter. This should cause buffer to fill the four-way cross adapter. While the priming syringe is removed, refill it with buffer, then connect it to the four-way cross adapter. Observe the solution delivery hole in the flow cell body. If flow does not start, slowly inject solution from the priming syringe again. Flow should begin.
5. If flow still does not start, repeat step 4 until solution flows.

To determine flow rate, record the volume change in the buffer reservoir as a function of time. The volume markings on the $60\,mL$ syringe do not have good precision, so realize the rate you determine will be an estimate. Flow rates between $3\,mL/min$ to $0.5\,mL/min$ are commonly used for microelectrode flow cell experiments.

TIP:

If the lines are not priming, removing the priming syringe from the four-way cross. Removing this syringe will allow solution to flow from the reservoir to priming outlet of the four-way cross. By changing the elevation of the four-way cross the flow of solution can be controlled. When solution is visible at the priming inlet on the four-way cross, reinsert a priming syringe full of solution and inject a volume of solution to start the fluid flow.

TIP:

Avoid flow rates $< 0.5\, mL/min$. Use of such flow rates can result in turbulent mixing of sample and run buffer, which is undesirable for analytical measurements.

4.2 Reference Electrodes

Commonly used reference electrodes in FSCV research are Ag/AgCl. Each lab has a preference, but in general, these Ag/AgCl reference electrodes are based on a wire lead with the Ag/AgCl element at one end. The Ag/AgCl element must remain immersed in run buffer, which maintains electrical contact with the working electrode.

The flow cell body features two holes in the drain basin (see: Table 2-3). Plugging one of these holes creates a small reservoir that fills with electrolyte during a flow cell experiment (see: Figure 3-10). This reservoir is an ideal location to secure the reference electrode. Four holes in the side of the flow cell body fit the black plastic thumb screw, which can be used to secure the reference electrode lead (see: Figure 2-4 and Figure 2-5). Bend the wire and ensure the Ag/AgCl element is positioned into the hole as shown (see: Figure 4-1). The wire extending from the thumbscrew is easily connected to the reference electrode line of the FSCV potentiostat. With the WaveNeuro, the white connector slide of the electrode connection leads onto the end of the reference electrode wire with ease (see: Figure 4-1).

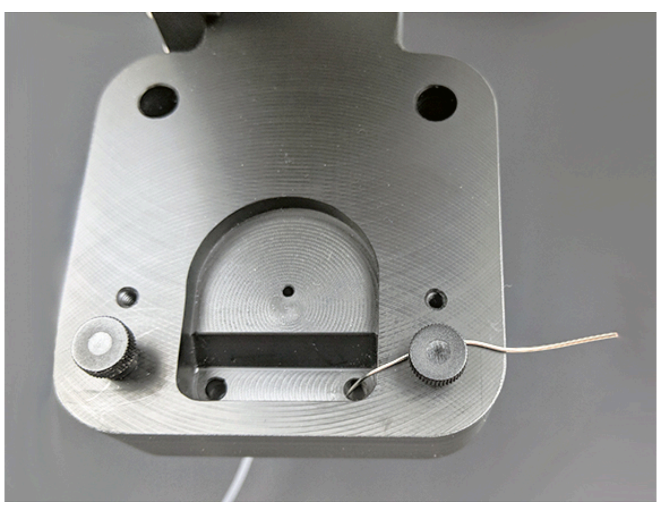


Figure 4-1. Reference Electrode Connection/Installation in Flow Cell

4.3 Working Electrodes

Typical FSCV microelectrode experiments use two electrodes: reference and working electrodes. The working electrode is typically a carbon fiber microelectrode and the reference electrode is typically a Ag/AgCl reference electrode. Due to the measurements of very small current, a counter electrode is not required as with other cyclic voltammetry experiments. With a system like the WaveNeuro FSCV Potentiostat system, the counter electrode circuit connects to the reference electrode circuit.

The Pine Research Flow Cell provides two methods for addressing, connecting, and manipulating carbon fiber microelectrodes (CFME). Both require the micromanipulator. One method uses the acrylic micromanipulator electrode holder (included with micromanipulator purchase) to mount a CFME, while the other method utilized electrophysiology-style electrode holders, which require a special adapter (Malli Adapter for Flow Cell) to connect to the potentiostat, like the WaveNeuro.

4.3.1 Installation into Micromanipulator Holder

Each lab has their preferred method for constructing (or using purchased) CFMEs. Some labs backfill the electrode with salt solution for making electrical connection. Others backfill the electrode with conductive epoxy and insert a lead wire. Some lead wires have a pin connector soldered to the end, and some of the pins are different sizes. The instructions the follow are general enough to accommodate each lab preference. Connection to the potentiostat may require special connectors or adapters, if not already compatible with the Pine Research WaveNeuro FSCV Potentiostat system.

Install the microelectrode the electrode holder by loosening the screw, placing the glass body of the CFME into the groove beneath the acrylic washer, and carefully tightening the screw until the washer applies sufficient force against the glass capillary (see: Figure 4-2).

CAUTION:

Tighten the microelectrode holder screw very gently and slowly. The screw and washer must only be tightened to hold the electrode in place. Over tightening will damage and likely destroy the microelectrode.

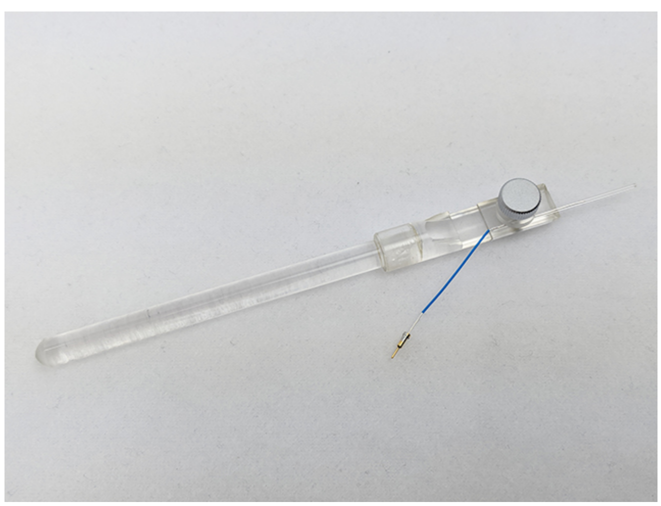


Figure 4-2. Installation of Microelectrode into Micromanipulator Electrode Holder

After the CFME is installed into the holder, carefully install the holder into the micromanipulator, as follows:

1. Raise the z-axis (up and down) of the micromanipulator as high as it will go by turning the z-axis knob.
2. Install the electrode holder by loosening the spring-loaded screw holding the black L-bracket, on the front of the micromanipulator.
3. Slide the electrode holder arm (at its smaller diameter) into the clip and tighten the screw to secure the holder in place (see: Figure 4-3).
4. Use the x-axis (left and right) and y-axis (into and out of the page) to center the CFME above the solution delivery hole. Some labs use flashlights to aid in this process (see: Figure 4-3).
5. Connect the working (and reference) electrode to your potentiostat. This will vary by lab and vary by potentiostat. For the Pine Research WaveNeuro, connect the yellow lead to the working electrode and the white lead to the reference electrode (see: Figure 4-4).
6. Slowly lower the electrode by turning the z-axis knob until the electrode is just above the solution delivery hole. At this point, the CFME should be centered above the solution delivery hole and a short distance ($< 3mm$) from making contact with the flowing run buffer.

TIP:

When adjusting the x, y, and z axes, use a flashlight and move to several angles to successfully guide the CFME into the solution delivery hole.

A convenient method to determine when the CFME has reached buffer is to observe the live electrochemical response as you are lowering the CFME toward solution. When lowering the CFME, observe the *Scope* tab in HDCV. This view shows the Waveform as one trace and another trace for voltage sensed at the working electrode. As the electrode element (carbon fiber) makes contact with electrolyte, the waveform will apply between reference and working electrodes, giving rise to a unique "shape" of the of plot on the *Scope* tab.

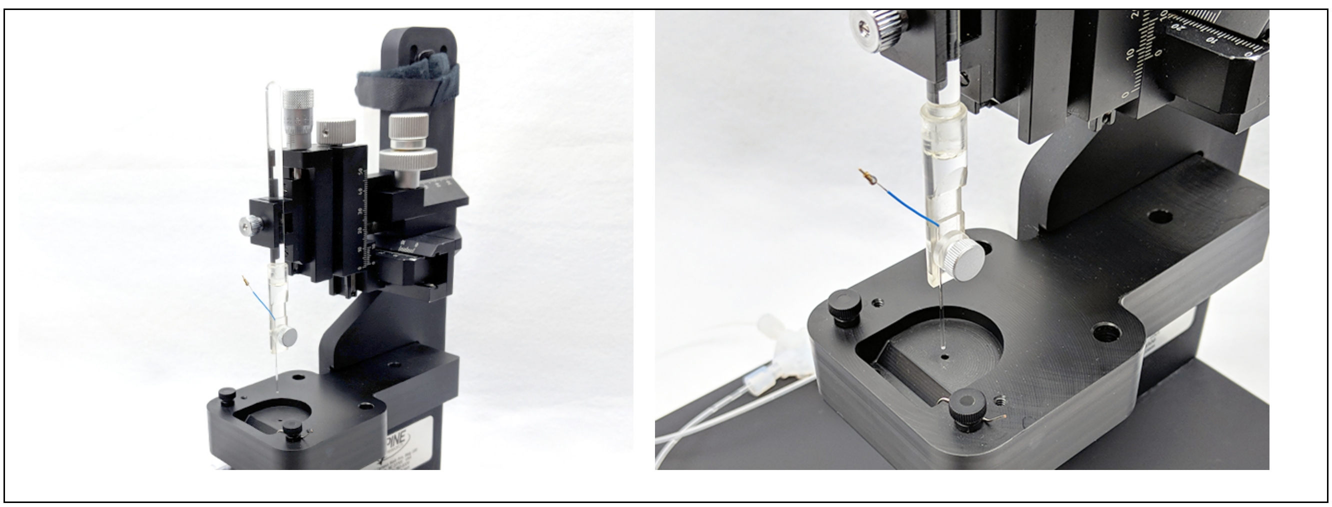


Figure 4-3. Installation of Microelectrode Holder into Micromanipulator

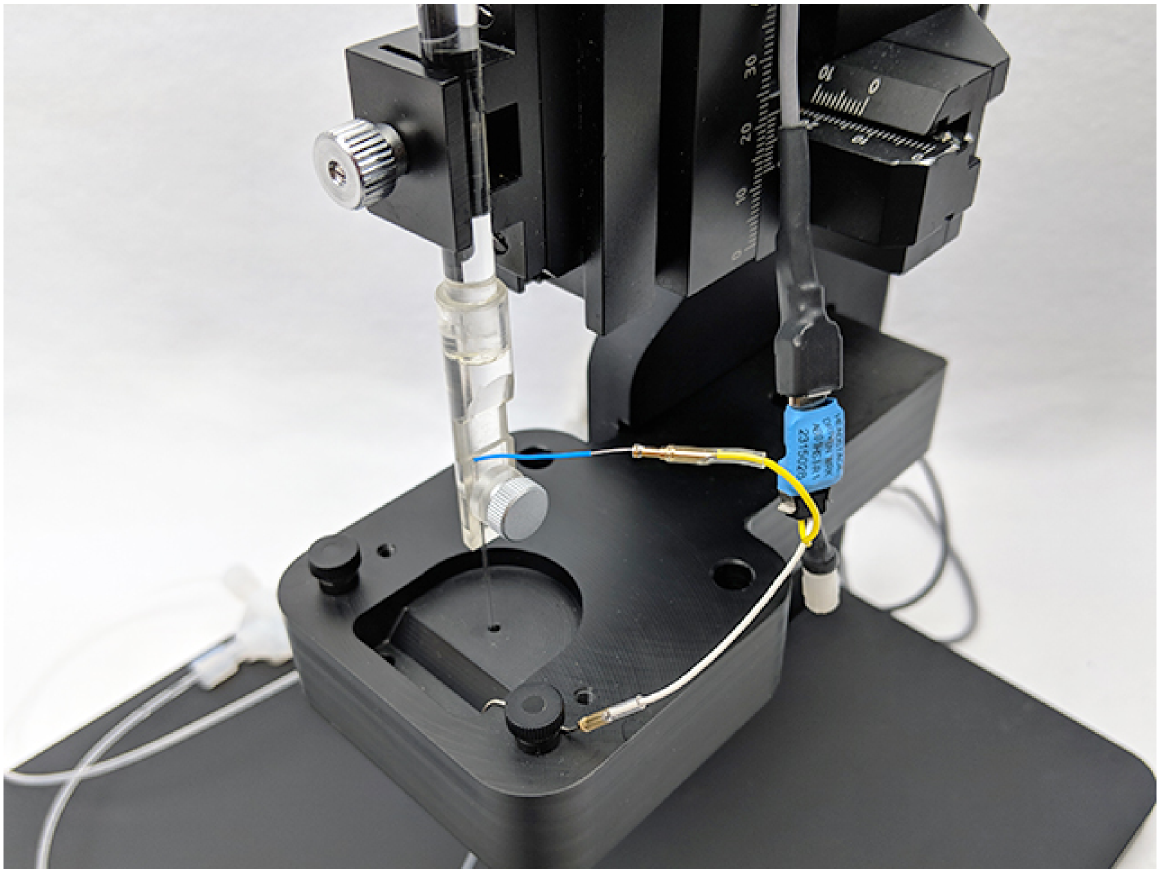


Figure 4-4. Complete Electrode Holder Style Setup

4.3.2 Installation into Malli Adapter for Flow Cell

Some labs use electrophysiology style electrode holders to hold and connect their CFME (see: Figure 4-5), which are available from many neuroscience-type companies. These holders have a thin silver wire that is addressed to a male BNC pin at the opposite end. The thin silver sire is typically inserted into the open end of a CFME that has been back-filled with KCl, for electrical conductivity. An O-ring holds onto the glass CFME. The entire assembly is then connected to a Malli Adapter, which maps the working electrode to amplifier (headstage) correctly.

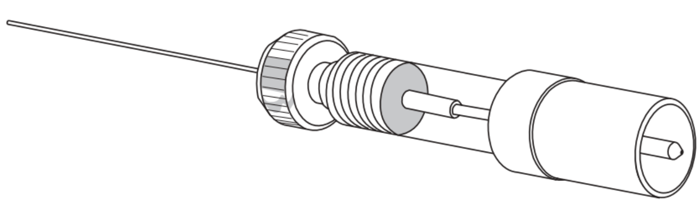


Figure 4-5. Warner Instruments Q Series Holder with BNC Connector.

Pine Research offers a Malli Adapter for Flow Cell, which is a slightly modified version of the Malli Adapter for non-flow cell applications/use. As shown, the electrode holder connects to the Malli Adapter by sliding the BNC connector on the electrode holder into the Malli Adapter BNC (see: Figure 4-6).

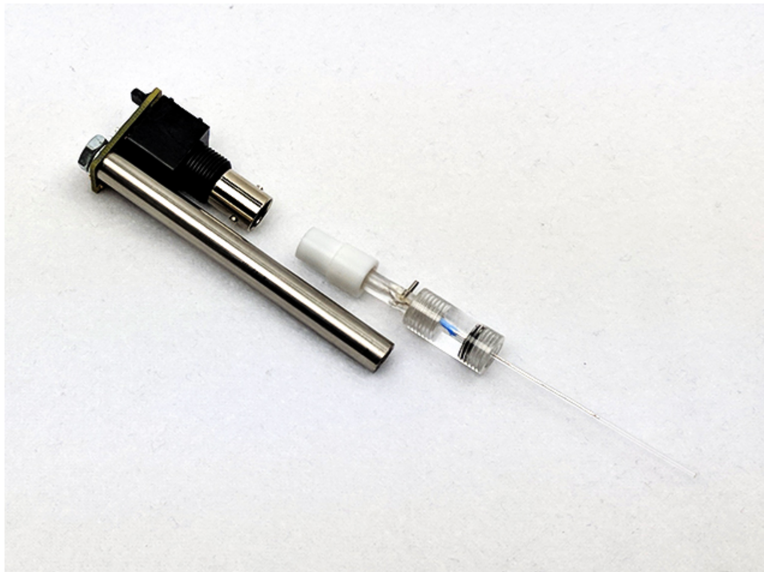


Figure 4-6. E-Phys Style Electrode Holder with Malli Adapter for Flow Cell

After the CFME is installed in the holder and the holder is installed into the Malli Adapter for Flow Cell, carefully install the Malli Adapter into the micromanipulator, as follows:

1. Raise the z-axis (up and down) of the micromanipulator as high as it will go by turning the z-axis knob.
2. Install the electrode holder by loosening the spring-loaded screw holding the black L-bracket, on the front of the micromanipulator.
3. Slide the electrode holder arm (at its smaller diameter) into the clip and tighten the screw to secure the holder in place (see: Figure 4-8).
4. Use the x-axis (left and right) and y-axis (into and out of the page) to center the CFME above the solution delivery hole. Some labs use flashlights to aid in this process.
5. The arm on the Malli Adapter for Flow Cell is not centered on the circuit board. Therefore, it may be necessary to swivel the adapter once installed into the micromanipulator. If it appears as though there is not sufficient y-axis adjustment distance, swivel the entire adapter accordingly to regain sufficient y-axis movement space about the solution delivery hole (see: Figure 4-7).
6. Install the headstage amplifier onto the Malli Adapter for Flow Cell. The WaveNeuro FSCV Potentiostat cell cable will connect to the headstage amplifier (see: Figure 4-8).
7. Connect the external reference electrode to the Malli Adapter for Flow Cell, using either a 2 mm banana cable and alligator clip (see: Figure 4-8) or via a $0.037''$ – $0.043''$ pin mounted on the adapter.
8. Slowly lower the electrode by turning the z-axis knob until the electrode is just above the solution delivery hole. At this point, the CFME should be centered above the solution delivery hole and a short distance ($< 3mm$) from making contact with the flowing run buffer (see: Figure 4-9).

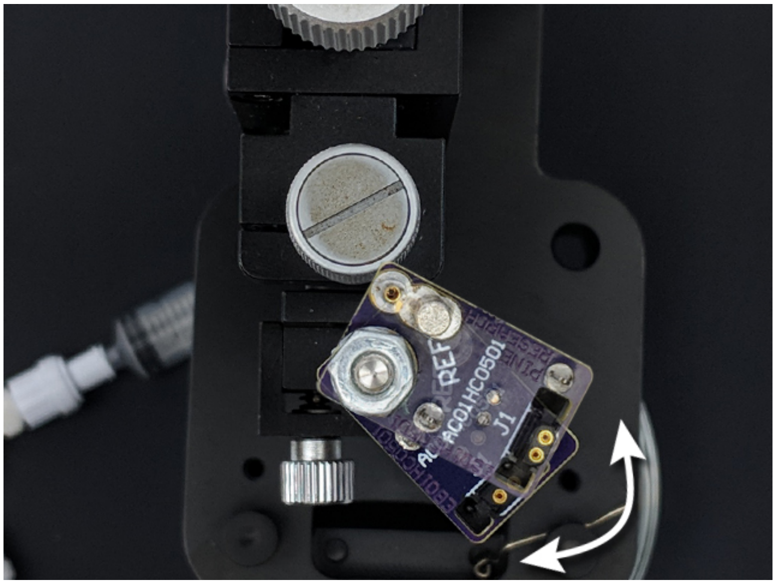


Figure 4-7. Malli Adapter for Flow Cell Variable Position

TIP:

When adjusting the x, y, and z axes, use a flashlight and move to several angles to successfully guide the CFME into the solution delivery hole

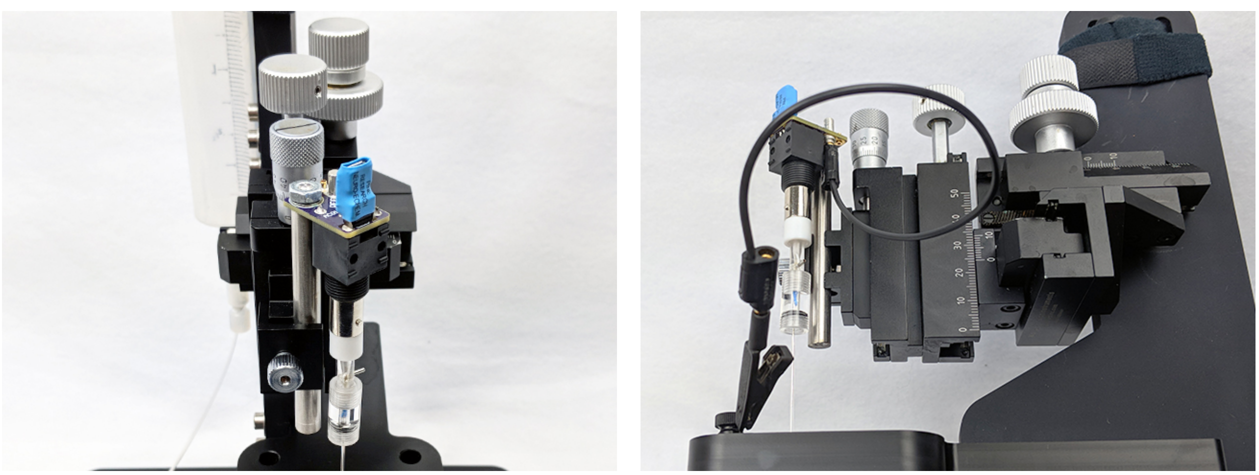


Figure 4-8. Connecting Electrodes to Potentiostat on a Malli Adapter for Flow Cell

A convenient method to determine when the CFME has reached buffer is to observe the live electrochemical response as you are lowering the CFME toward solution. When lowering the CFME, observe the *Scope* tab in HDCV. This view shows the Waveform as one trace and another trace for current sensed at the working electrode. As the electrode element (carbon fiber) makes contact with electrolyte, the waveform will apply between reference and working electrodes, giving rise to a unique response/“shape” of the of plot on the Scope tab.

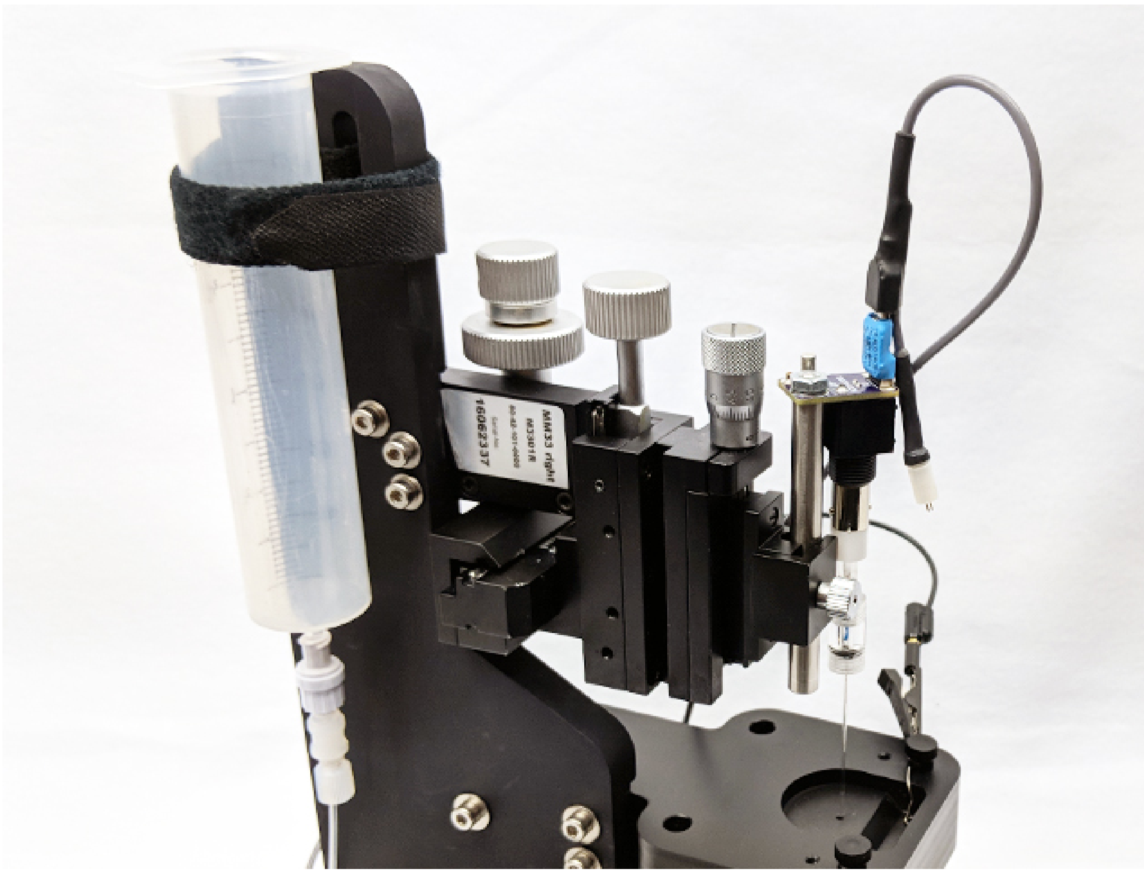


Figure 4-9. Fully Installed CFME in Using Malli Adapter in Micromanipulator

4.4 Sample Injection

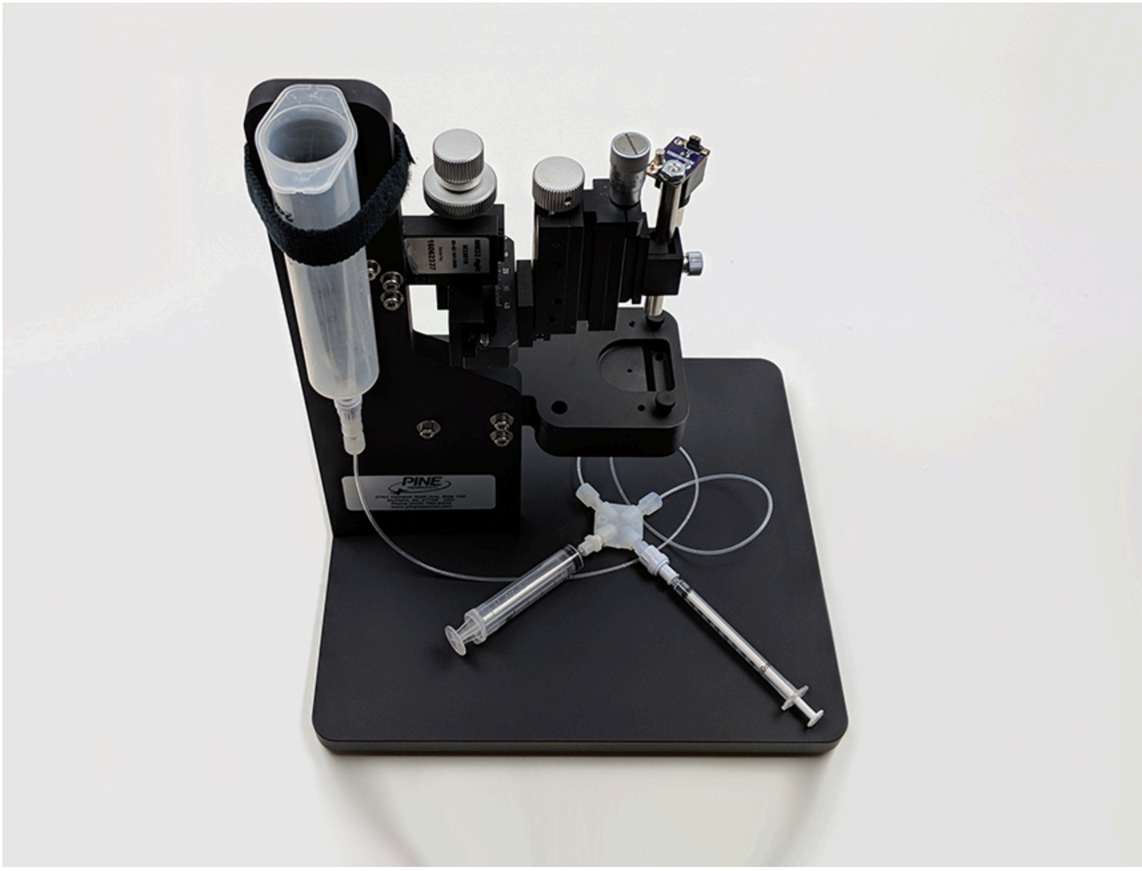


Figure 4-10. Fully Assembled Flow Cell

With run buffer flowing through solution, reference, and working electrode installed, experiments with sample injection can be successfully performed.

> **TIP:**
>
> **It is common to prepare a new CFME prior to use, in a process called "cycling." When a new CFME is cycled, the electrochemical waveform is continuously applied to the working electrode while the electrode is immersed in flowing run buffer. The new microelectrode is typically cycled for about twenty minutes before beginning the experiment to stabilize its surface chemistry. The magnitude of the change in current due to these surface changes can be monitored on the live/running color plot tab in HDCV. After cycling the electrode for about twenty minutes the surface chemistry should be stabilized and therefore the change in current response over time should stabilize (the change in current should be less than $1\ nA$ for a thirty second file).**

1. Setup your potentiostat and software accordingly. We will assume the Pine Research WaveNeuro FSCV Potentiostat with HDCV software for the remaining instructions.
2. Apply (*i.e.*, turn on) the CV waveform.
    a. For an initial period the signal for a new electrode, or an electrode recently introduced into the flow cell will encounter baseline drift. The drift arises over time as a function of changing surface chemistry at the microelectrode, which will minimize over time.
    b. Cycling the waveform at $60\ Hz$ for a few minutes may help stabilize the response.
3. Load sample into the $1\ mL$ syringe and connect it to the check valve on the four-way cross adapter.
    a. Users may employ syringes of different volumes, but in product testing, we found the $1\ mL$ syringe to provide the best injection performance.
    b. The volume in the four-way cross adapter to the flow cell body is $\sim 0.2\ mL$; therefore, the volume of sample injected should be of similar volume to maintain pre-injection flow rates.
4. With one fluid movement, inject sample by depressing syringe plunger.
5. Observe the running color plot in HDCV. Approximately $1\ s$ after injection, the electrochemical response of the sample will be apparent on the color plot (see: Figure 4-11).

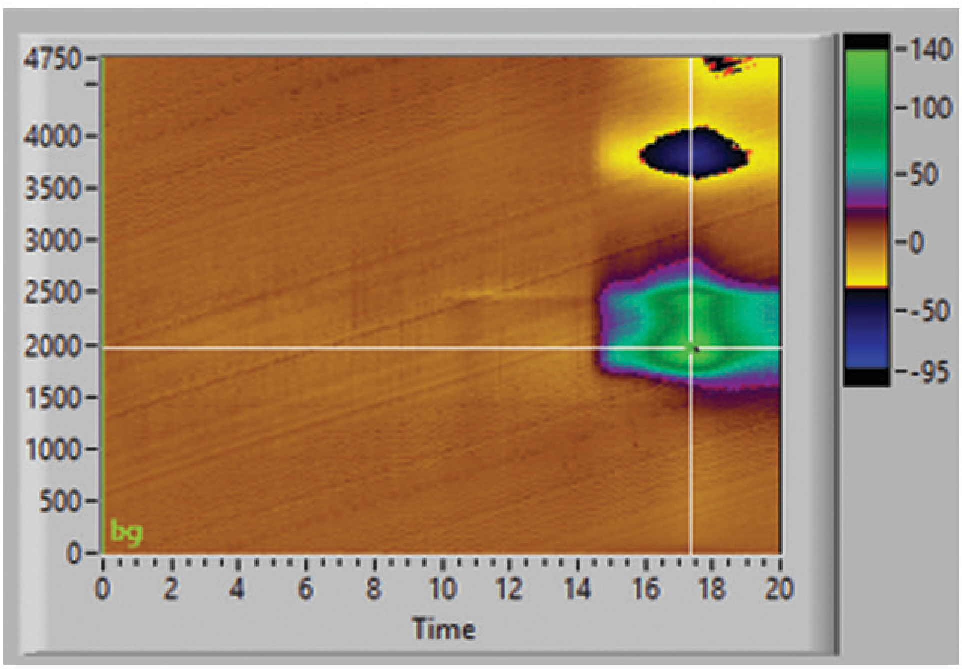


Figure 4-11. FSCV of 5-Hydroxytryptophan using Pine Research Flow Cell

5. Optional Flow Cell Capability Expansion

5.1 Automated Buffer Delivery

A gravity feed flow cell is highly cost-effective; however, the variable flow rate may present challenges to the user.

For lab-designed flow cell solutions, it is common to employ a simple syringe pump that can be set to a specific flow rate. Such a pump is fairly inexpensive and can be used directly with the Pine Research In-Vitro FSCV/Microelectrode Flow Cell without any additional tools, parts, products, or accessories required.

Contact Pine Research for a recommendation on a syringe pump. Pine Research does not current offer these products.

5.2 Automated Sample Injection

Manual sample injection is highly cost-effective and easy to use; however, it is challenging to reproducibly deliver a sample volume. It is also challenging to reproducibly inject sample at the same velocity over the same short amount of time.

For lab-designed flow cell solutions, it is common to automate the sample injection. Researchers in the FSCV community commonly use a TTL actuated HPLC valve switch to deliver sample at a defined time, in a reproducible manner.

Contact Pine Research for a recommendation on the components required to expand the capabilities of the flow cell to include automation. Pine Research does not current offer these products.

6. Support

After reviewing the content of this user guide, please contact Pine Research Instrumentation should you have any issues or questions with regard to the use of the instrument, accessories, or software. Contact us anytime by the methods provided below.

Online

Our website has a contact form which allows technical support requests to be sent directly to Pine Research. Visit www.pineresearch.com/contact.

E-mail

Send an email to pinewire@pineresearch.com. This is the general sales email, and our team will ensure your email is routed to the most appropriate technical support staff available. Our goal is to respond to emails within 24 hours of receipt.

Phone

Our offices are located in Durham, NC in the eastern US time zone. We are available by phone Monday through Friday from 9 AM EST to 5 PM EST. You can reach a live person by calling +1 (919) 782-8320.