PicoView®

Model PV-400

for the Applied Biosystems QSTAR®

Instruction Manual

NEW OBJECTIVE

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Rev 1.0

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1 Read This First

1.1 Safety Considerations

WARNING: Electrospray ionization involves the use of potentially lethal high-voltage electrical current. Observe all manufacturers' safety recommendations in the use of such equipment. No equipment modifications should be made except by trained personnel using methods approved by the manufacturer in accordance with all safety requirements. Installation of equipment should be performed by qualified personnel in accordance with all applicable electrical codes.

Never use this product with defective, damaged, or faulty equipment. Serious injury or death could result.

SAFETY PRECAUTIONS: Only qualified personnel should use this product. Provide a safe workplace equipped with all necessary safety equipment.

1.1.1 Prior to Installation

Follow all safety recommendations of the equipment manufacturer(s). All system voltages must be brought to ground potential and all high-voltage contacts disconnected from the inlet system before installation of the PicoView® system.

Inspect all equipment carefully prior to use. Any damaged, chipped, or cracked components should not be used and must be discarded or repaired.

1.1.2 Handling Fused-Silica Tubing

Handling of fused-silica or glass tubing and tips can result in serious personal injury, including eye and skin injury. Use safety glasses or goggles meeting ANSI Z87.1-1989 requirements, or the equivalent. Puncture- and chemical-resistant gloves should also be worn at all times.

1.1.3 Tip Adjustment and/or Replacement

Do not attempt to adjust or replace the tip unless the ESI high voltage and other applicable voltages are turned off and are at ground potential.

WARNING: Reduce any applicable backing pressure (liquid, gas, etc.) to ambient before loosening ESI tip fittings and removing the fused-silica tip or transfer line from the coupling union. Prior to pressurization, make sure that components are tightened to specifications to prevent separation during use. Failure to adhere to this warning could result in projectile-like expulsion of the tip from the coupling union, which could cause serious personal injury or damage to surrounding apparatus.

1.1.4 The Transfer Line

WARNING: The transfer line connecting to either a tip module or the Micro Injection Valve must not be made from an electrically conductive material. It must be fabricated from an electrically insulating material such as PEEK™ or fused silica. Otherwise, the operator may be exposed to potentially lethal voltage.

In systems where high voltage is applied directly to the ESI tip, the liquid sample inside the tip and transfer line tubing is also raised to a high voltage. To prevent exposure to potentially lethal voltages, a suitable ground point for the liquid inside the line must be provided.

Instructions for grounding are found in Section 4.4 on page 19.

WARNING: Do not operate PicoView® without the external housing and lid in place.

WARNING: Do not defeat any mass spectrometer system software or hardware safety interlocks.

1.2 Contents and Components List

PicoView® comes partially assembled, with system components contained in multiple boxes.

1.2.1 Model Number and Serial Number

Model Number Ordered: _____

Serial Number Ordered: _____

1.2.2 Carton Contents

Table 1.1: Components Checklist

	Item
☐	PicoView® system, stage, camera mount, housing, and lid
☐	Accessories box
☐	Uncoated Tip Module (UTM)
☐	Coated Tip Module (CTM)
☐	Curtain plate
☐	CCD camera
☐	Camera power supply
☐	(1) 6′ BNC cables
☐	Allen wrench set
☐	Fused-silica tubing (20, 50, 75, 100 µm ID, 2 meters each)
☐	SilicaTip™ samples
☐	TaperTip™ samples
☐	PicoFrit® column sample
☐	Illuminator box with adapter ring
☐	Monitor box with 9″ black-and-white monitor (220V adapter plug if applicable)
☐	Product literature

Inspected by: _____

1.3 Trademarks

The following trademarks are found in this instruction manual:

Teflon is a registered trademark of E.I. du Pont de Nemours and Co.; QSTAR is a trademark of Applied Biosystems, Inc.; MicroTight and SealTight are trademarks or registered trademarks of Upchurch Scientific, Inc.; PEEK and PEEKsil are trademarks of Victrex plc; PicoView, PicoTip, PicoFrit, SilicaTip, TaperTip, and PicoTip Powered are trademarks of New Objective, Inc.

1.4 Limited Warranty

DISCLAIMER

Technical information contained in this publication is for reference purposes only and is subject to change without notice. The information is believed to be reliable and accurate; however, nothing set forth herein constitutes a warranty of any kind or nature. Given the variety of experimental conditions, New Objective, Inc., cannot guarantee performance at a given flow rate; the best guide to tip selection and operation is empirical testing.

NOTICE: The user is solely responsible for complying with any patent(s) pertaining to applications or methods using the products described or mentioned in this manual.

New Objective, Inc., warrants this Product (PicoView® mounting system) to be free of defects in materials and workmanship for a period of one (1) year from the date of shipment. New Objective, Inc., warrants the accompanying SilicaTips™, PicoFrit® columns, TaperTips™, and fittings to be free of defects in materials and workmanship for a period of ninety (90) days from the date of shipment. Any item believed to be defective within the meaning of the foregoing sentence shall be returned to New Objective, Inc., and, if found by us to be defective, shall be repaired or replaced with conforming Product of like kind. Please note that a Return Authorization Number will be required. New Objective, Inc., will pay return freight on unsatisfactory items. New Objective, Inc., shall have no other liability or obligation with respect to goods alleged to be defective. The foregoing shall constitute the sole and exclusive remedy, and New Objective, Inc.'s total liability for any and all losses and damages arising out of any cause whatsoever (whether such cause be based in contract, negligence, strict liability, other tort, or otherwise) shall in no event exceed the purchase price of the Product(s) in respect of which the cause rose. New Objective, Inc., disclaims, and shall not be liable, in any event, for loss of profits, consequential or incidental damages, or punitive or exemplary damages in connection with the Product furnished hereunder.

The foregoing limited warranty (i) shall be void as to any item of Product which is in any material respect altered by the user, and (ii) does not cover misuse of the Product (for example, but not limited to, dropping or other mishandling of any components of PicoView®, improper trimming of SilicaTips™, PicoFrit® columns, or TaperTips™, damage caused by application of or exposure to excessive temperature, pressure, or voltage) or SilicaTip™, PicoFrit®, or TaperTip™ failure by reason of clogging.

The video monitor and camera are warranted to be free from defects in material or workmanship for a period of two (2) years from the date of purchase.

2 Applications

PicoView® supports a variety of operating modes, ranging from continuous infusion at nanospray flow rates to gradient run nanobore LC-MS. The two tip modules (the coated tip module (CTM) and the uncoated tip module (UTM)) included with the PicoView system afford the user the versatility to design experiments for a variety of flow rates and sensitivity requirements. With the open design and interchangeable modules, you can run the same analysis several different ways. This manual provides the most robust configurations for a given application. Following the schematic drawings, locate the desired application and the most effective PicoView configuration for that experimental design. See the referenced section for detailed directions on setting up PicoView. Techniques to optimize PicoView, including how to select the best PicoTip for your application, can be found in Section 6 on page 34.

2.1 Continuous-Infusion Nanospray

Figure 2.1: Continuous-infusion nanospray

Continuous infusion is the most straightforward method for sample introduction, with a syringe pump providing the solvent stream as illustrated in Figure 2.1. Sample is introduced directly from a syringe pump, at flow rates typically of 10–300 nL/min. The high voltage is applied through the coated tip module (CTM) to the conductive coating on the PicoTip™. Coated SilicaTips™, a type of PicoTip, are primarily used for ultra-low flow rate infusion (nanospray) to minimize electrolysis. Suggested tip sizes for continuous-infusion nanospray are 5–10 µm with a distal or standard coating. All coated tips should be handled with care, since mechanical abrasion can deteriorate the coatings. Use a pair of fine tweezers to handle the tips. Go to Section 5.3, "Coated Tip Module in the Forward Position," on page 23 for detailed instructions on setting up the CTM for continuous infusion.

2.2 Nanospray Flow-Injection

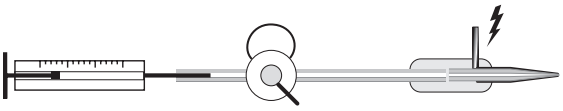

Figure 2.2: Nanospray flow-injection

Nanospray flow-injection entails injecting small volumes of sample into an established solvent stream, as depicted in Figure 2.2. An aliquot of sample (1–2 µL) is injected as a plug into the solvent stream through the micro injection valve sample loop. A syringe pump at flow rates of 10–300 nL/min is used to deliver the solvent stream that pushes the sample through the PicoTip. The flow-injection mode

allows for smaller sample sizes than continuous infusion. This type of experiment is particularly useful for the rapid analysis of desalted samples. Voltage is applied through a coated PicoTip; a SilicaTip is recommended, using the coated tip module (CTM) in the forward position. Nanospray flow-injection is best accomplished using the coated tip module (CTM) and SilicaTips with either the distal-coated tips (those with -D- in their item number) or standard coated tips (those with -CE- in their item number) coated on the "tip end." Go to Section 5.3, "Coated Tip Module in the Forward Position," on page 23 for detailed instructions on setting up the CTM for flow injection.

2.3 Microspray Flow-Injection

Microspray flow-injection, as with nanospray flow-injection, entails injecting small volumes of sample into an established solvent, as diagrammed in Figure 2.3. The difference between the two is the flow rate. Nanospray flow rates are typically below 300 nL/min, whereas the higher microspray flow rates are typically between 0.5–5 µL/min. Sample is introduced as a plug into the solvent stream from an HPLC pump or syringe at flow rates of 400–5000 nL/min. The Micro Injection Valve can be used for sample injection. Microspray flow-injection is particularly useful for the rapid analysis of desalted samples. Using a TaperTip™, a type of PicoTip, voltage is applied through a liquid junction-style contact through either the uncoated tip module (UTM) in the back position or through the conductive coating (distal-coating style) when using the coated tip module (CTM) in the forward position. For the most robust setup, it is recommended to use uncoated TaperTips and the UTM in the back position. Suggested tip sizes for microspray flow-injection are 20 to 100 µm TaperTips. Go to Section 5.4, "Uncoated Tip Module in the Back Position," on page 26, or Section 5.3, "Coated Tip Module in the Forward Position," on page 22 for detailed instructions on setting up the UTM or the CTM for microspray flow-injection. Consult Technical Note TT-1, available on our web site (www.newobjective.com), to learn more about the use of TaperTips with microspray.

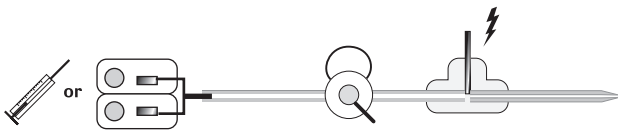

Figure 2.3: Microspray flow-injection

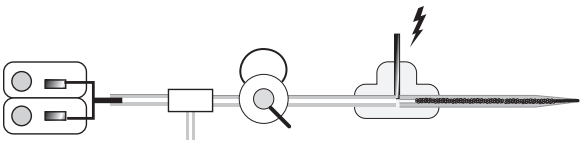

Figure 2.4: Nanobore LC-MS using a PicoFrit® column

2.4 Nanobore LC-MS Using a PicoFrit® Column

PicoView is ideal for performing nanoscale LC-MS and LC-MS/MS using PicoFrit® columns. PicoFrit columns, a type of PicoTip, are nanobore LC-MS chromatography columns with an integral tip, used at flow rates of 100–500 nL/min. In this configuration, the high voltage is applied to the back of the column through a liquid junction in the uncoated tip module (UTM) placed in the back position, as diagrammed in Figure 2.4. Solvent is delivered via an HPLC pump with a flow splitter. Suggested tip size is 15 µm with an uncoated PicoFrit column. Go to Section 5.4, "Uncoated Tip Module in the Back Position," on page 26 for detailed instructions on setting up the UTM for a PicoFrit column.

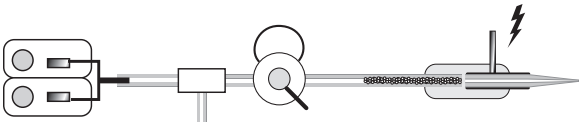

Figure 2.5: Nanobore LC using a PicoTip™ with a separate column

2.5 Nanobore LC Using a PicoTip® with a Separate Column

PicoView can be used effectively for microscale LC-MS and LC-MS/MS. When running nanoscale separations it is best to use a PicoFrit column, a combined emitter and a column, for increased sensitivity and decreased problems due to less plumbing. If experimental parameters do not allow use of a PicoFrit column, PicoView can accommodate a nanobore column connected to a PicoTip. New Objective offers IntegraFrit™ nanobore columns designed specifically for this purpose. In this application it is recommended to use the SilicaTip type of PicoTip. Sample flows through the IntegraFrit, through the SilicaTip, and into the mass spectrometer (Figure 2.5). Voltage is applied through a coated tip using the coated tip module (CTM) in the forward position. For a 75 µm ID column, the suggested tip size is 10 µm with a distal coated tip. Go to Section 5.3, "Coated Tip Module in the Forward Position," on page 23 for detailed instructions on setting up the CTM for nanobore LC using a PicoTip connected to a column.

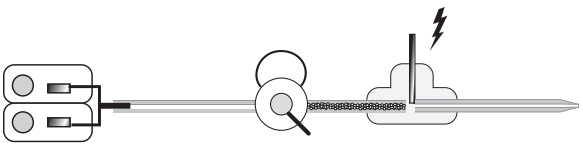

Figure 2.6: Capillary bore LC

2.6 Capillary Bore LC

Capillary bore LC uses a column with a larger diameter than used in nanobore LC. Capillary columns usually have an internal diameter of 100–320 µm and are suggested for use at flow rates of 200–1000 nL/min. Sample is introduced into a solvent stream through a capillary

bore chromatography column, which is connected to a TaperTip, a type of PicoTip (Figure 2.6). Voltage is applied through a liquid junction in the uncoated tip module (UTM) placed in the forward position. Suggested tip size is 50 µm. Go to Section 5.5, "Uncoated Tip Module in the Forward Position," on page 32 for detailed instructions on setting up the UTM for capillary bore LC using a TaperTip connected to a column.

2.7 Supplying Voltage to the Uncoated Tip Module

When using the UTM, high voltage is applied directly to the solvent stream in a liquid junction via an electrode. PicoView offers two different styles of electrodes to create a liquid junction and charge the solvent stream: a MicroTee made of PEEK™ polymer that contains an integral platinum electrode and a titanium zero dead volume (ZDV) union (ncluded in the PicoView accessory box). Figure 2.7 shows both assemblies. The MicroTee is easier to assemble and is ideal when using PicoFrit columns or other designs that require pre-column voltage contact. The MicroTee is unsuitable for low-volume post-column use, however, as it has an interior volume. Due to the greater degree of precision necessary to set up a ZDV union, it is recommended to first try the MicroTee; only use the ZDV union if post-column problems occur.

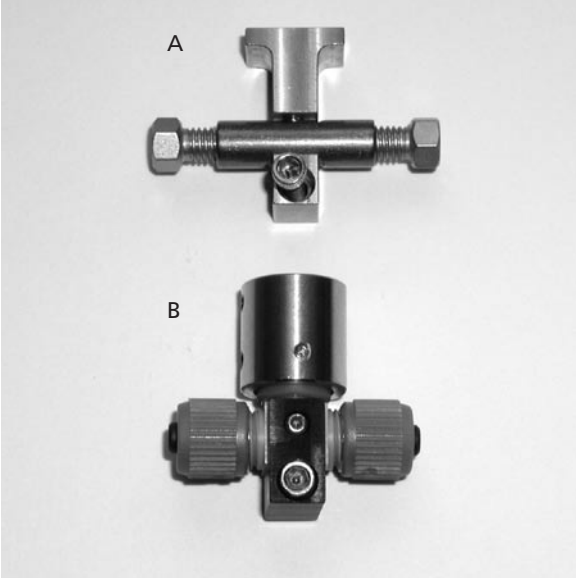

Figure 2.7: Titanium zero dead volume union (A) and MicroTee (B)

3 Quick Guide

3.1 Components of PicoView®

Figures 3.1A and B illustrate instrument- and user-side views of PicoView®, showing the general layout of the components. The translation stage and stage plate comprise the PicoView hardware used for mounting and aligning tips with respect to the inlet on the mass spectrometer. The optical stage, CCD camera, and fiber optic illuminator make up the PicoView imaging hardware necessary for optimizing spray conditions. Complete descriptions of these components can be found later in this chapter.

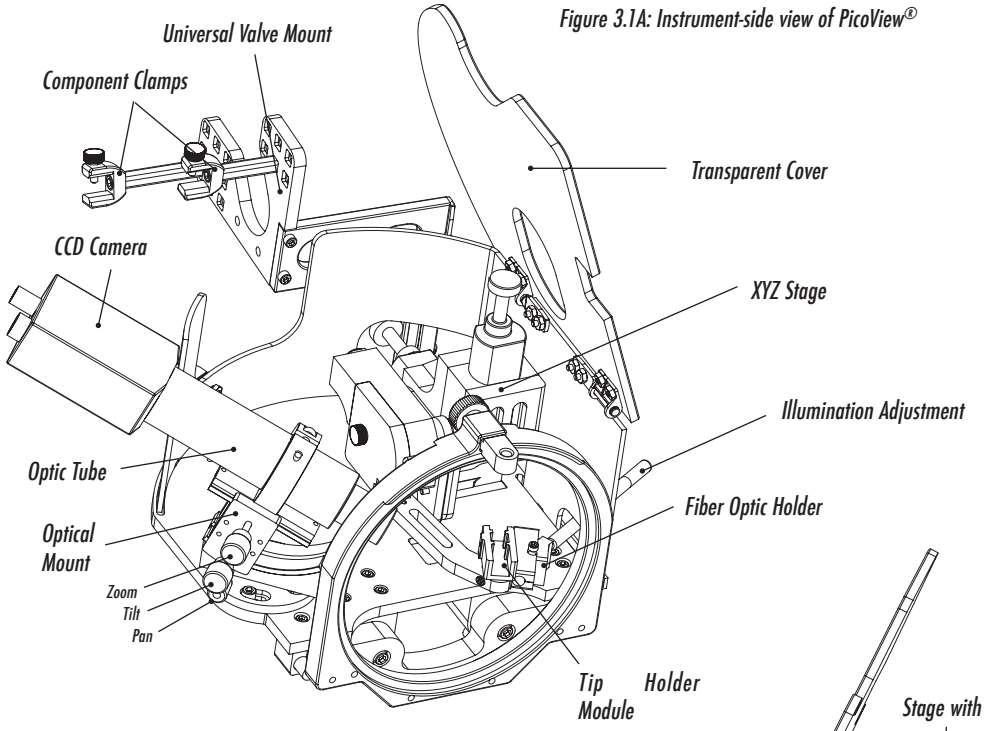

Figure 3.1A: Instrument-side view of PicoView®

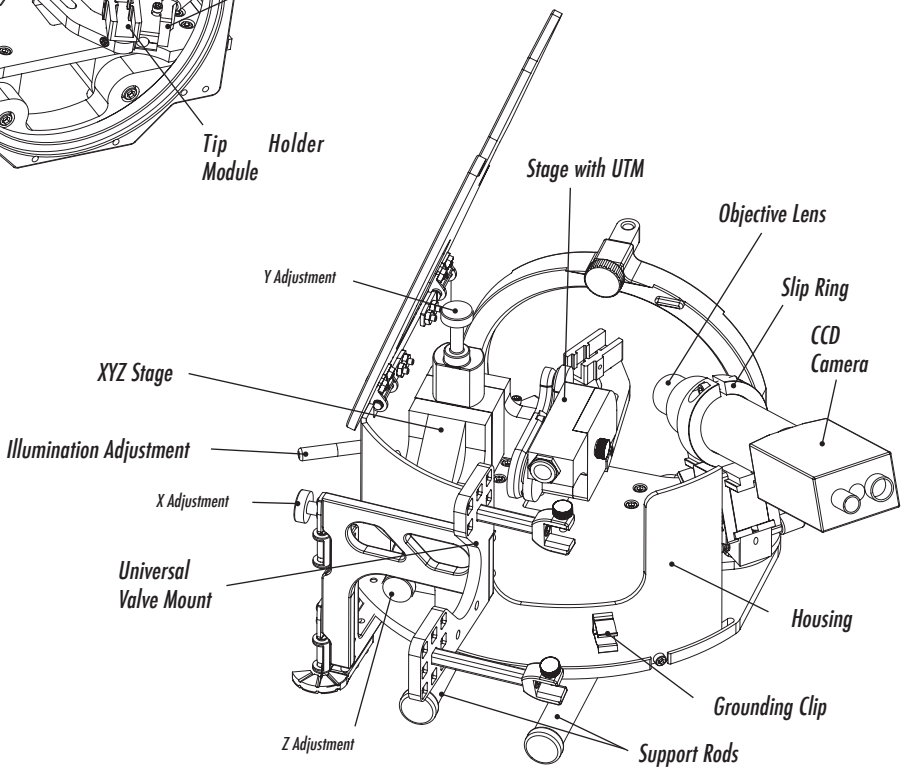

Figure 3.1B: User-side view of PicoView®

3.2 Tip Modules

The two tip modules included with the PicoView system afford the user the versatility to design experiments for a variety of flow rates and sensitivity requirements. The first two modules supply the high voltage for sample ionization through different junction styles, as dictated by the experimental design. The tip modules are each mounted on a magnetic stage plate, and each module functions uniquely to accommodate different tip and column styles. The coated tip module (CTM) is depicted in Figure 3.2, and the uncoated tip module (UTM) with tip holder in Figures 3.3 and with the sheath gas module in Figure 3.4. Detailed descriptions for the experimental configuration of the tip modules are presented in Section 5 "Setup" on page 20.

Coated Tip Module

The coated tip module (CTM), shown in Figure 3.2, connects the tip to the transfer line or column via a pass-through union. High voltage is provided to the coated tip using a conductive elastomer. The CTM is the module of choice for performing continuous-infusion nanospray and microscale flow-injection experiments at ultra-low flow rates. It can also be used with commercial nanobore columns.

Uncoated Tip Module

Within the uncoated tip module (UTM), the transfer line connects to the emitter via a MicroTee or through a zero dead volume titanium union. High voltage is applied through a liquid junction from either a platinum electrode in the MicroTee or through the body of a zero dead volume titanium union. See Section 2.7 on page 8 to learn which electrode is best for your application. The UTM can operate in modes analogous to the CTM, but it is most effectively utilized with microspray flow rates for nanobore chromatography. The UTM provides an optimal interface for PicoFrit columns to afford purification, concentration, and separation of analytes at high sensitivity.

WARNING: Turn off or disconnect all power prior to performing any service on PicoView or any devices attached to it to avoid potentially lethal electrical voltages.

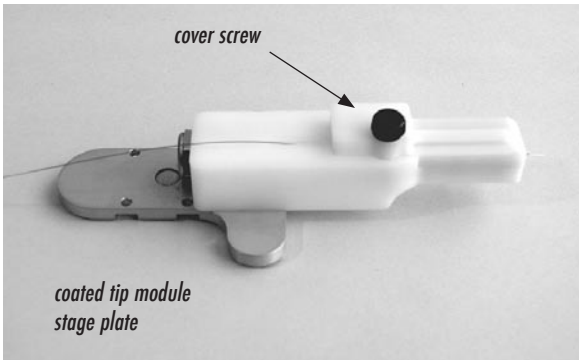

Figure 3.2: Coated tip module (CTM)

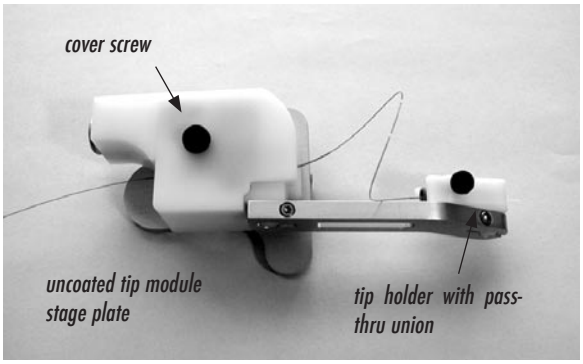

Figure 3.3: Uncoated tip module (UTM) with tip holder on magnetic stage plate

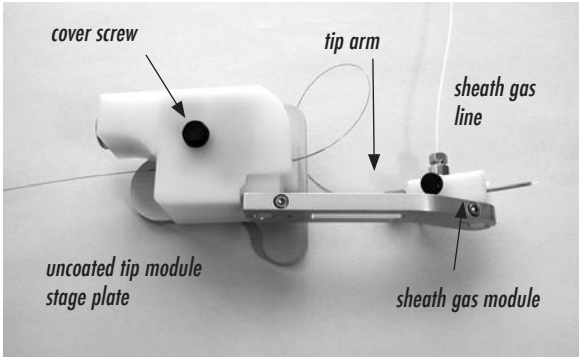

Figure 3.4: Uncoated tip module (UTM) with sheath gas module

Always use chemical- and puncture-resistant gloves and ANSI-approved safety glasses when handling fused-silica tubing.

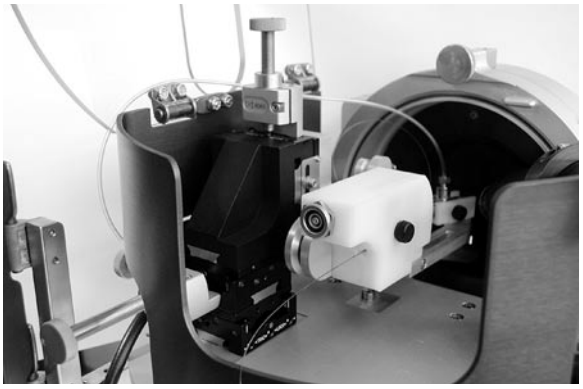

Figure 3.5: Translation stage with uncoated tip module (UTM) and stage plate

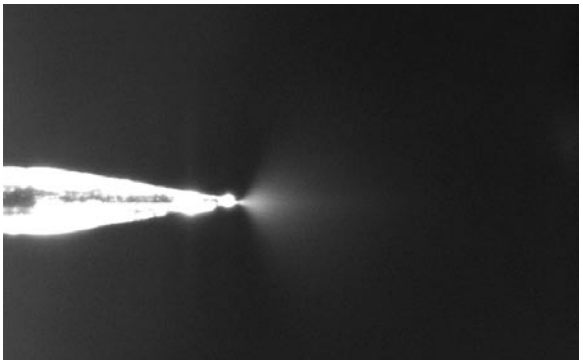

Figure 3.6: PicoFrit® and spray as seen through PicoView imaging system

3.3 XYZ Positioning Stage

Precise alignment of the tip is accomplished using the X, Y, and Z adjustment screws of the translation stage, shown in Figure 3.5. The translation stage moves the stage plate, on which a tip module is mounted. One half-inch of fine travel is available for each axis. Each axis has an additional inch of coarse travel, obtained by adjusting the stage's positioning rails. Consult the provided XYZ stage instruction manual for more information.

3.4 Imaging System

The imaging system allows the user to quickly and efficiently optimize the electrospray conditions by providing direct observation of the character of the spray. The system includes a high-resolution CCD camera and an objective lens. An optional high magnification kit is available with two different objective lenses and a 10x eyepiece which can be used in place of the CCD camera. Alignment of the imaging system is accomplished using the Focus, Tilt, and Pan adjustment screws of the optical stage. (Refer to Figure 3.1A on page 9.)

3.5 Injection Valves

A micro injection valve typically serves as the inlet for sample introduction to PicoView. The sample is loaded onto a sample loop using the injection port adapter or a syringe directly connected to the sample inlet port. Sample and solvent from an HPLC pump or syringe are transferred to a tip module and subsequently to a column or tip. Please refer to the instructions particular to your valve for information on its setup and use, as PicoView can accommodate several different valves. Section 7 "Mounting an Injection Valve" on page 40 illustrates how to mount a valve on the PicoView.

4 Installing PicoView®

4.1 Preparing the Mass Spectrometer

Before installing PicoView®, the mass spectrometer must be put into standby mode and the conventional curtain plate must be replaced with the nanospray curtin plate supplied with your PicoView.

CAUTION: Please refer to the appropriate QSTAR® Manual(s) provided by the manufacturer for all relevant safety information before attempting to remove your current ionization source and subsequent installation of the PicoView® system.

WARNING: Electrospray ionization involves the use of potentially lethal high-voltage electrical current. Observe all manufacturer safety recommendations in the use of such equipment.

4.1.1 Preparing Your Mass Spectrometer

Prepare your instrument for PicoView installation by removal of your current ionization source from the instrument chassis.

1) Place instrument into a safe stand-by condition suitable for removal of the current source (ESI, API, APCI, etc.) from the instrument. This should include lowering all applicable voltages to ground potential, cooling the source to room temperature, and turning off all applicable gases.
2) Disconnect the high-voltage cable from the instrument chassis. A PicoView-compatible cable is supplied as a replacement. Please note it should only be used with the PicoView source.
3) Disconnect the sample transfer line if applicable.
4) Disconnect the sheath gas and any applicable auxilliary gas lines.
5) Remove the source from the instrument.
6) Disconnect any extraneous sheath gas fittings from the instrument chassis. You will need to connect 1/16″ OD tubing (provided in the PicoView accessory box) to use sheath gas with the UTM assembly. An appropriate 1/16″ Swagelok® connector is also provided in the accessory box. You may opt to use suitable alternative coupling hardware, if applicable.

Switch to nanospray mode from other Ion Sources

4.2 Assembling the PicoView® Imaging System

The PicoView® imaging system consists of an optical stage for fine positioning of the camera, a slip ring through which the optical tube is inserted, the optical tube, a CCD camera, a fiber optic illuminator, and a lens. Installation of the imaging system involves attaching a lens and the CCD camera (or optional eyepiece) to the optical tube.

During assembly, be careful to avoid getting fingerprints on the imaging system lenses or inside the CCD camera.

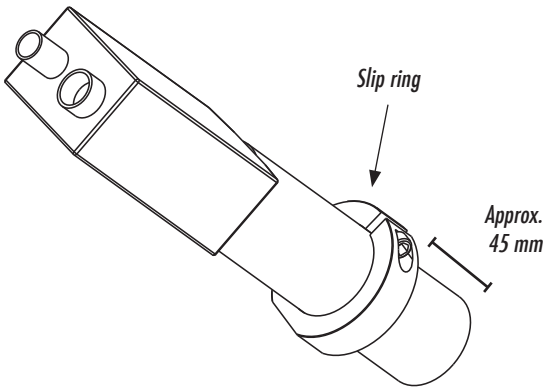

Figure 4.1: The optical tube should extend approximately 45 mm below the slip ring.

4.2.1 Connecting the Optical Tube and Lens

1) Select the 2X lens from the accessories box.
2) Screw the (male) threaded end of the lens onto the mating (female) thread on the instrument-side end of the optical tube. Turn the lens clockwise until it is finger-tight. See Figure 3.1A on page 9.

4.2.2 Attaching the High-Resolution CCD Camera

1) The CCD camera and illuminator are shipped in containers separate from PicoView. Remove the CCD camera from its box and remove the protective end cap from the camera.
2) Place the optical end of the CCD camera flush with the user end of the optical tube.
3) While supporting the CCD camera with one hand, loosen the optical tube fastener at the top of the slip ring using a 9/64 Allen wrench. Rotate the optical tube until the CCD camera is aligned horizontally, then tighten the optical tube fastener to hold the CCD camera in place. (See Figure 4.1)

4.2.3 Adjusting Camera Gain and Gamma

For optimal imaging the CCD camera's gain setting needs to be in the auto (A) position, and the gamma control needs to be turned on. The rotary gain switch has three settings: auto (A), fixed (F), and manual (M). The gamma setting (γ) is controlled with a dip switch, and has on and off positions. Both switches are located on the rear of the camera housing just below the video out connector. Proper camera settings are shown in Figure 4.2. If your camera settings are correct no action needs to be taken.

1) Using a jeweler's screwdriver or similar instrument, rotate the (brown) gain control switch clockwise until the slot points to the "A" position.
2) Using the same screwdriver, slide the gamma control to the right into the "ON" position.

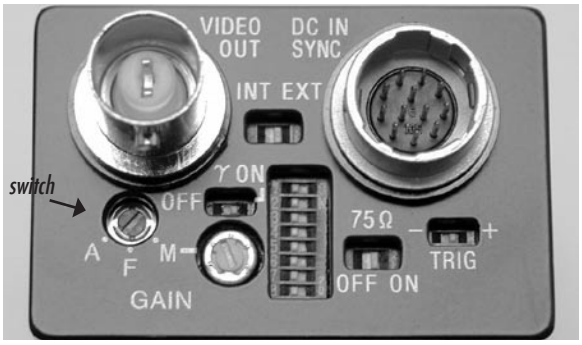

Figure 4.2: CCD camera gain switch set to automatic

4.3 Mounting PicoView® on the QSTAR®

To mount PicoView on the QSTAR system, the original curtain plate must be removed and replaced with one more suitable for nanospray. This replacement plate is included with your PicoView system.

WARNING: The curtain plate may be hot enough to cause severe burns upon skin contact. Before attempting to remove and replace the standard curtain plate, verifty that all relevant voltages are off and the plate is at room temperature.

CAUTION! Handle the curtain plate with suitable gloves to prevent possible contamination of the front and rear surfaces.

4.3.1 Replacing the Curtain Plate

NOTE: Placing a small piece of tape over the drain hole will prevent dropped screws from going down the pipe.

1) Remove the conventional ESI curtain plate by loosening the five button head screws and then pulling the plate away from the system (Figure 4.3A). Make sure that the O-rings stay in place (Figure 4.3B).

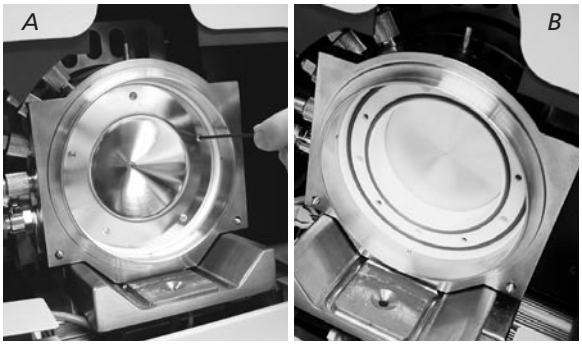

Figure 4.3: (A) Remove original curtain plate
(B) Make sure O-rings stay in place

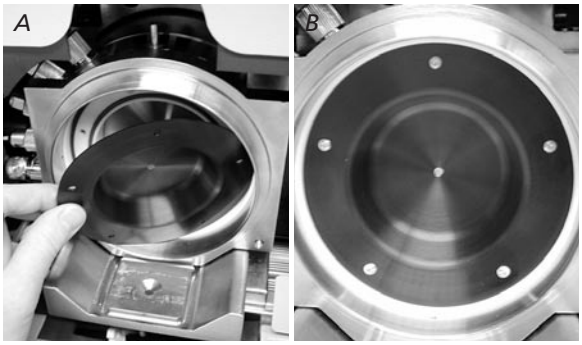

Figure 4.4: (A) Insert supplied curtain plate
(B) Newly installed curtain plate

Figure 4.5: The anchor rests on top of the ring

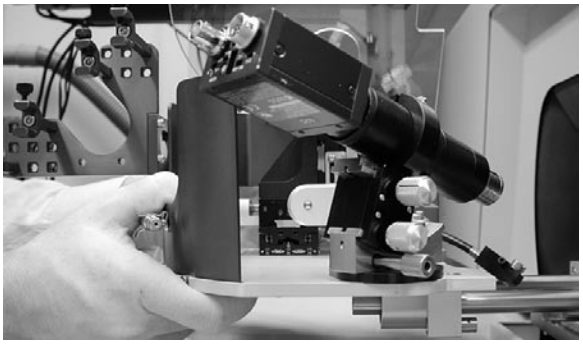

Figure 4.6: Install PicoView® and the support rods

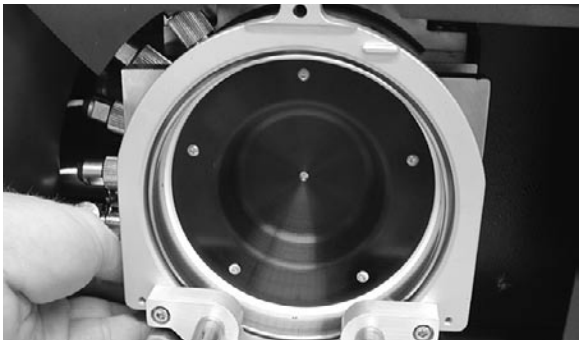

Figure 4.7: Finger-tighten the lower left and right source mounting screws

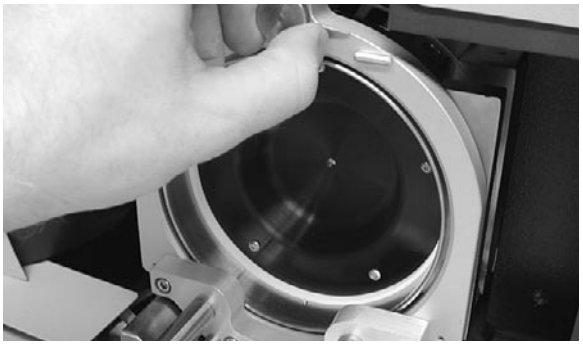

Figure 4.8: Install and finger-tighten the top anchor thumb screw

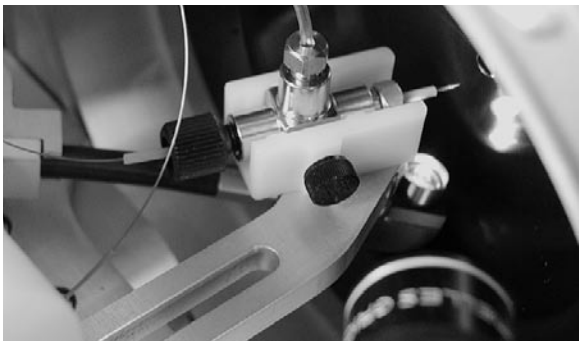

Figure 4.9: Fiber optic illuminator correctly installed

2) Insert the supplied black PicoView curtain plate against the O-rings. Lock the new plate in place using the five buton head screws (Figure 4.4).

4.3.2 Installing the PicoView® Base Support

1) Locate the source anchor pin supplied with the PicoView unit. Intall the source anchor by slipping it over the top pin (Figure 4.5).
2) Install PicoView by sliding the ring and rod assembly into place against the curtain plate. The alignment source anchor pin will align with a hole in the top of the mounting ring (Figure 4.6).
3) Finger-tighten the lower left and right hand locking screws located behind the mounting ring (Figure 4.7).
4) Insert the top anchor thumbscrew and tighten (Figure 4.8).

NOTE: When removing the PicoView from the QSTAR®, keep the source and the ring and rod assembly together (Figure 4.6).

4.3.3 Mounting the Fiber Optic Illuminator

Your PicoView is shipped with the fiber optic illuminator installed. The fiber optic may need to be adjusted within the mount to obtain optimal positioning.

1) The coupler holds a lens which focuses the illumination on the nanospray emitter and can be adjusted to accommodate for tip position using the Illumination Adjustment knob (see Figure 3.1B on page 9). The fiber optic cable is held within the mount by a spring-loaded clamp. The amount of friction holding the fiber optic can be adjusted by loosening or tightening the thumb screw.
2) Adjust the friction to enable movement of the fiber optic cable within the mount and adjust cable as necessary.
3) With the fiber optic bundle securely placed within the coupler, lock the bundle in position using the thumb screw. Do not over tighten.
4) Spool the illuminator end of the fiber optic cable through the back of the PicoView housing. Insert

the bundle adapter and the end of the fiber optic bundle into the illuminator faceplate. Tighten the set screw to finger-tight. Figure 4.9 shows the assembled fiber optic bundle and mount.

4.3.4 Locking the PicoView® Housing to the Mounting Brackets

1) Close the transparent top cover on the PicoView source.
2) Gently slide the PicoView assembly toward the curtain plate. A groove located at the tip of the mounting bracket will lock the cover down when the source is is pushed firmly against the instrument.
3) To lock the PicoView in place, insert the locking bolt into the right mounting rod under the PicoView base. Tighten bolt.

4.3.5 Connecting the PicoView® Imaging System

1) Remove the monitor from its shipping carton and place it in a convenient viewing area.
2) Plug the power cord for the monitor into an electrical outlet (preferably one protected by a suitable surge protector). Connect the video cable from the monitor to the BNC video out jack on the high-resolution CCD camera.
3) Connect the camera power supply to the high-resolution CCD camera (Figure 4.12). Plug the other end of the cable into an electrical outlet (again, preferably one with a suitable surge protector).

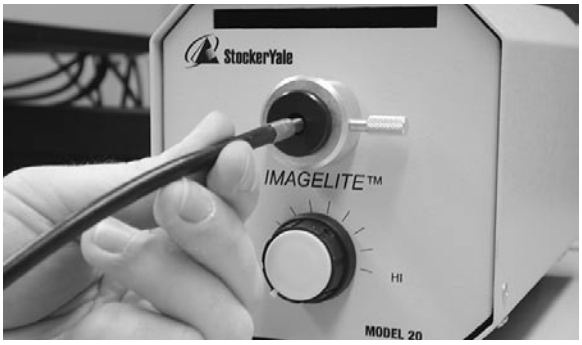

Figure 4.10: Install fiber optic bundle

Figure 4.11: Connect video input on the monitor

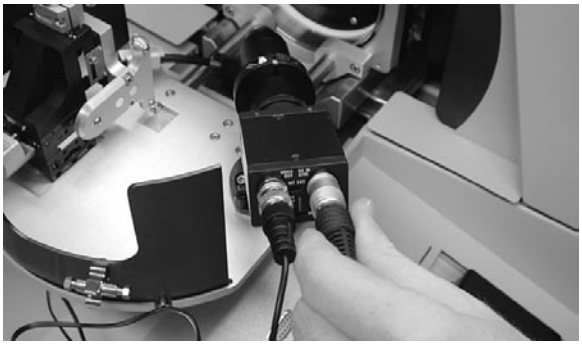

Figure 4.12: Connect power to the CCD camera

4.3.5 Using Sheath Gas

The sheath gas module can extend your available flow rates into the microspray region (1-10µL/min) or be used to assist droplet formation in highly aqueous mobile phases while running at nanospray flow rates. For use with PicoFrit® columns or uncoated TaperTip™ emitters, the sheath gas module is used with the uncoated tip module (UTM).

The sheath gas module enables the use of coaxial sheath gas when using PicoFrit® columns or TaperTip™ emitters from New Objective. The economical, easy-to-use design is based on a conventional 1/16" bore stainless steel tee. Thread the PicoFrit or TaperTip (distal end first) through the tee. Then mount the tee on the the end of the uncoated tip module (UTM) stage. Electrical contact is made at the distal end of the emitter inside the conventional UTM. The advantage of this design is that it adds no extra connections or volume to the plumbing path and allows for different length columns and emitters. For safety reasons, this module is not compatible with standard- or distal-coated PicoTips®.

4.3.5.1 Module components

IMPORTANT: Never try to feed an emitter or column through the tee tip-end first. You are likely to break the tip.

Loosen the nut securing the SealTight™ fitting by one-half to two turns and feed a PicoFrit® or TaperTip™ through the PEEK™ nozzle, distal end first, until it comes through the SealTight sleeve (Figure 4.15).

Pull the distal end of the emitter through the sleeve until the tip protrudes 0.5 to 2 mm past the end of the PEEK nozzle, as shown in Figure 4.16. Tighten the nut to lock the tip in place and ensure a gas-tight seal.

4.3.5.2 Mounting the Module on the PicoView® Stage

WARNING: Do not attempt to mount the module or replace the emitter unless the ESI voltage is turned off and the instrument is in standby mode.

IMPORTANT: Ensure that gas flow has been reduced to zero prior to connecting the sheath to the gas line.

Prepare your instrument by placing it into standby mode and reduce the ESI voltages to zero. Unlock the PicoView® mounting system and pull the PicoView base and housing into the open position. Disconnect the high voltage cable from the mounting hardware. The sheath gas module will fit into the tip arm on the UTM stage.

Load the stainless steel tee into the sheath gas module with the tip-end protruding past the slot on the face of the block, as shown in Figure 4.17. Tighten the set screw (on the right-hand side of the fixture) so that the tee is held firmly in place.

Mount the sheath gas fixture in the tip arm of the UTM stage. High voltage will be applied through the UTM to the distal end of the emitter. Remove the cover of the UTM. Trim the distal end of the TaperTip™ or PicoFrit® and connect to the MicroTee included with the UTM. Refer to Section 5.4.7.1 "Plumbing the MicroTee" on page 27 for further instructions on loading the MicroTee . The other end of the MicroTee should be connected to your sample injector or mobile phase pumping system with fused-silica tubing.

Replace the UTM cover. Connect the 1/16" OD sheath gas line to your mass spectrometer's sheath gas supply line. Reconnect the ESI high-voltage cable to the UTM. Carefully slide your PicoView system into the operating position. Use caution to make certain that the tip does not contact the mass spectrometer inlet before locking your PicoView into operating position. Adjust the position of the XYZ stage if necessary.

Tuning Hints

- For initial setup and system parameter tuning (sheath gas flow, ESI voltage, emitter position), it is best to work with a well characterized standard. Delivery of a standard by continuous infusion is preferred, as this method provides sufficient time to optimize each parameter.
- Sheath gas is best used with the emitter mounted orthogonal, or nearly orthogonal, to the MS inlet.
- Use the minimal amount of sheath gas to achieve the desired effect. Too high a gas flow setting will reduce ion current, reducing sensitivity.
- Using sheath gas will change both the optimal emitter position and the ESI voltage. Optimal emitter location is usually farther from the MS inlet (typically 20-30% greater than the non-sheath set point).

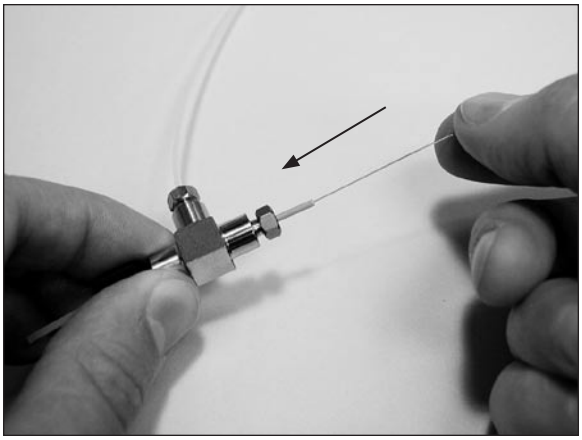

Figure 4.15: Feed the emitter, distal-end first, through the PEEK™ nozzle.

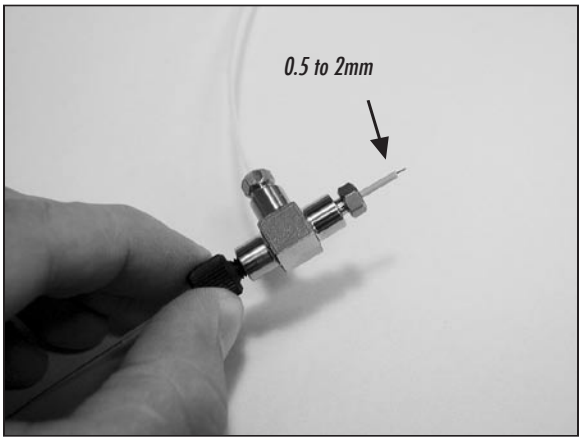

Figure 4.16: The tip of the emitter protrudes 0.5 to 2mm past the PEEK™ nozzle.

Figure 4.17: Stainless steel tee loaded into the sheath gas mounting fixture

- Optimal settings will be a function of mobile-phase flow rate. Higher mobile-phase flow rates will typically require more sheath gas and higher operating voltages.
- Optimal settings will also be a function of mobile-phase composition. For use with gradient chromatography, it is best to optimize conditions using a standard prepared in an average mobile-phase elutant, such as one prepared with 20% organic cosolvent.

WARNING: Sheath gas assembly components have been selected to work with gas at ambient temperature. No attempt should be made to use gas at an elevated temperature.

4.3.6 Adjusting the Camera Position

Optimized operation of PicoView involves adjustments of both the camera and the tip positions. Upon installation, the imaging system should only require fine adjustment. However, some course adjustment may be necessary if the lens is changed.

4.3.6.1 Fine Adjustment

Using your fingertips to move the fine adjustment knobs, you can adjust the camera position to optimally image the tip and spray pattern during operation. If a tip is not currently loaded in the PicoView source, you may use the inlet of the mass spectrometer to adjust the imagainf system. The locations of the fine tuning knobs are indicated in Figure 3.1A on page 9.

1) Move the Tilt knob up and down until the inlet is visible. At this point the inlet image will likely be very fuzzy and require focusing. Using the Tilt knob, center the inlet on the monitor (or as viewed using the optional eyepiece).
2) Adjust the Focus knob until the inlet comes into focus.
3) Move the Pan knob left or right to center the inlet.

4.3.6.2 Coarse Adjustment of the Camera Body

NOTE: Only perform coarse adjustment if the fine adjustments on the optical mount and the stage plate will not allow adequate imaging of the tip.

1) Using a 9/64 Allen wrench, loosen the optical tube fastener at the top of the slip ringholding the optical tube.
2) Looking at the monitor, slowly slide the optical tube through the slip ring until the inlet is visible and in focus. The inlet will appear on the monitor as a polished metal surface at the top of the field of view (see Figure 3.5 on page 11).
3) Tighten the optical tube fastener on the slip ring to hold the optical tube in place.
4) Use the fine adjustment protocol above to focus the image of the inlet further.

4.4 Grounding Requirements

In systems where high voltage is applied directly to the ESI tip, the liquid sample inside the tip and transfer line tubing are also raised to high voltage**. To prevent exposure to potentially lethal voltages, a suitable ground point for the liquid inside the line must be provided. It is best not to position the ground too close to the tip.** Two recommended methods for grounding the source are described below.

4.4.1 Grounding Through a Union

The ground union (Figure 4.18) is a stainless steel low dead volume union. During operation, this union is placed in the grounding clip on the housing of the PicoView, situated just under the valve mount.

To ground a sample:

1) Locate the ground union in the PicoView accessories box.
2) Remove the nut and ferrule from one side of the union by turning the fitting counterclockwise.
3) Thread fused-silica through a green SealTight™ adapter sleeve. Carefully cleave the end of the tubing.
4) Insert the sheathed tubing and fitting into the ground union receptacle. Finger-tighten the fitting by turning clockwise, making sure the tubing is firmly seated into the bottom of the fitting. (Figure 4.19)

Repeat steps 1–3 for the tubing leading from the installed tip module. Complete descriptions of the tip modules can be found in Section 5 on page 22.

4.4.2 Grounding Guidelines with the CTM or UTM

For the highest level of safety it is recommended that the grounding union be spliced into the plumbing between the Micro Injection Valve and the CTM or UTM, as shown in Figure 4.20A. This configuration is recommended for continuous-flow microspray or nanospray operation. With nanobore chromatography, however, the interior volume of the union may deteriorate chromatographic peak

Figure 4.18: The grounding clip

Figure 4.19: Grounding union in place

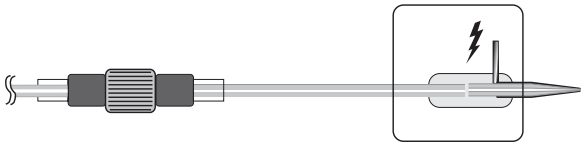

Figure 4.20A: Optimal location for grounding union for continuous-flow microspray or nanospray

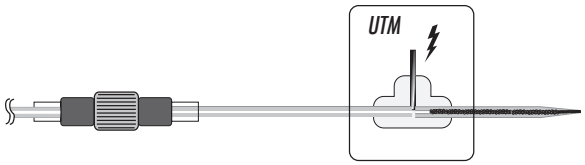

Figure 4.20B: Optimal location for grounding union for nanobore chromatography with a PicoFrit® column

shape. In this event, the optimal place for the grounding union is between the injection valve and the HPLC or syringe pump (Figure 4.20B).

If your mobile phase contains a high concentration of TFA or formic acid, the electrical conductivity of the liquid may rise to the point where the electrospray power supply is partially shorted to ground. If this occurs, it becomes impossible for a stable spray to form because the voltage at the end of the tip is too low, and the total spray current displayed on your monitor will read abnormally high (in the multi-microamp range). This can only be corrected by reducing the conductivity of the mobile phase or by replacing the tubing between the ground point and the tip module. The new splice should be of longer length, of smaller inside diameter, or both. Good performance can be obtained with a 25–30 cm length of fused silica of either 20 or 50 µm ID.

4.4.3 Grounding to a Syringe Needle or HPLC

If the syringe pump is being used to deliver solvent to PicoView, use an insulated wire with alligator clips on each end for grounding. Clip one end to the stainless steel syringe needle. Clip the other end to a ground point such as the loop on the injection valve located on the front panel of the mass spectrometer.

If an HPLC is being used to deliver solvent to PicoView, please refer to the manufacturer's directions for grounding the pump.

WARNING: Transfer lines connected to either the tip module or the micro injection valve must not be made from an electrically conductive material such as steel or some varieties of PEEKsil™. These transfer lines must be fabricated from an electrically insulating material such as PEEK™ or fused silica. Otherwise, the operator may be exposed to potentially lethal voltage.

CAUTION: Do not attempt to use the injection valve as a grounding point. The interior of the valve is fabricated from ceramic materials and is electrically insulating; therefore, a proper ground will not result.

5 Setup

5.1 Cutting Fused Silica

Proper cleaving of the fused-silica tubing is critical for achieving optimal performance of the PicoView® system. A flat, smooth cut is essential for maintaining low dead volume connections. Cleaving is best accomplished with a high-quality diamond chip scribe, available from many chromatography supply houses. New Objective offers a high-quality scribe as part of a nanospray tool kit (stock number TIP-KIT). Inexpensive carbide or ceramic scribing tools are not recommended, since they generally result in poor-quality (i.e., ragged) cleaved ends, generating fine particles that lead to clogging.

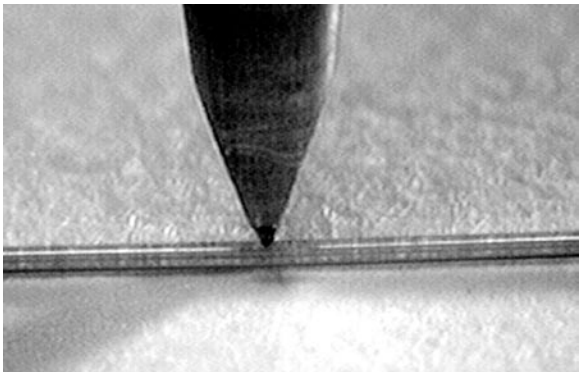

Figure 5.1: Cleaving fused-silica tubing

1) Place the tubing to be cut on a clean, flat surface and position the cleaving tool blade perpendicular to the tubing bore, as illustrated in Figure 5.1.
2) Press down with a gentle force. DO NOT saw or move the blade laterally; simply nick the surface of the polyimide coating to score the fused silica.
3) Pull gently on the tubing along its axis; it should easily separate at the point of scoring. If it does not, repeat the procedure with slightly more force. Be careful not to force the blade through the tubing, as this will create a ragged end.
4) It is best to check the cleaved tubing ends for particle contamination prior to use. This is most effectively done using a light microscope at 100x magnification.

5.2 Connectors

Making leak-free connections is important for limiting sample loss and for reducing turbulent flow. PicoView comes with two different systems of microconnectors for plumbing fused silica to the various fittings. Assembling the connectors is covered in detail later in this section. The two different connector systems differ by size and are not interchangeable. The MicroTight® system is used with the MicroTee and MicroTight unions in the CTM and UTM tip modules and with the tip holder. The SealTight™ system is used to connect fused silica to any standard 1/16" port, such as the titanium zero

dead volume (ZDV) union and the grounding union. Each union uses specific ferrules, nuts, and sleeves to seat the fused silica. Do not use a MicroTight® sleeve with a SealTight™ fitting and vice versa. Table 5.1 lists the different locations of connectors within PicoView and describes which ferrules and nuts are used. All of the listed components are found in the PicoView accessory box. Replacement unions, ferrules, nuts, and sleeves should be ordered directly from Upchurch Scientific (toll free 800-426-0191 or www.upchurch.com). The corresponding Upchurch part numbers are provided.

Table 5.1: Connectors

Connector	System	Upchurch Part Number Ferrule	Nut
CTM	MicroTight®	Combined F-125	
UTM (MicroTee) (ZDV)	MicroTight® SealTight™	F-172 F192	P416 F194
Tip holder	MicroTight®	Combined F-125	
Grounding union	SealTight™	F192	F194
Injection port adapter	MicroTight®	F-152	P416

Sleeves are used within unions to ensure a reliable connection. As with the ferrules and nuts, it is very important to use the appropriate sleeve for the connection system. Table 5.2 lists the different sleeves supplied with PicoView and the sleeves' applications. Always use MicroTight® sleeves with MicroTight® unions and SealTight™ sleeves with 1/16" diameter fittings.

Table 5.2: Sleeves

System	Sleeve Color	Upchurch Part No.	Sleeve OD (inches)	Sleeve ID (µm)	Connector
MicroTight®	Blue	F-183	0.025	280	CTM, UTM, Tip holder
MicroTight®	Green	F-185	0.025	380	CTM, UTM, Tip holder
SealTight™	Blue	F-240	1/16	280	ZDV, Grounding union
SealTight™	Green	F-242	1/16	380	ZDV, Grounding union

The sleeve ID should be approximately equal to the tubing OD. Other sleeve sizes are available from Upchurch Scientific. 380 µm ID sleeves are compatible with New Objective SilicaTips.

NOTE: Always select proper ferrules, nuts, and sleeves for the union. Mismatching of union components will result in spaces with dead volume or a leaky seal.

NOTE: Always cleave the fused silica, either tubing or the back end of a PicoTip®, after pushing it through a sleeve. The fused silica will collect dirt and particles that were in the sleeve, which, if left in the fused silica, may cause clogs downstream. Cleaving the fused silica will help prevent particle contamination.

5.3 Coated Tip Module

This section provides instructions on connecting standard-length 5 cm distal or standard coated PicoTips to a transfer line or capillary column. "PicoTip" refers to any of New Objective's high-quality tips for electrospray ionization, such as SilicaTips™, PicoFrits™, and TaperTips™.

NOTE: Users must take care when tightening the MicroTight® fittings, making sure to only tighten enough to prevent leaks from occurring. Due to the delicate nature of some fused-silica tubing, it is possible to damage the tubing if the fittings are overtightened.

5.3.1 Loading the MicroTight® Union

1) Remove the MicroTight Union from the PicoView components box. Unscrew and remove the compression fittings from both ends of the union.
2) Screw the white gauge plug finger-tight on to one end of the union. Thread the fused-silica transfer line through a green MicroTight sleeve. The appropriate sleeve size is 0.002–0.003 inches greater than the OD of the capillary tubing. Use the green MicroTight sleeves with 360 µm OD tubing. After the transfer line passes through a green sleeve, thread it through one of the compression fittings. Figure 5.3 shows the union loaded with the white gauge plug on the right and fused-silica tubing threaded through a tubing sleeve and a compression fitting. Cleave the end of the tubing and slip it into the union until both the tubing and the sleeve ends seat against the gauge plug inside the union. Screw the compression fitting finger-tight into the union, as shown in Figure 5.4.
3) Remove the gauge plug and return it to the PicoView components box.
4) Carefully trim a new green MicroTight sleeve to a length of approximately 14 mm, as shown in Figure 5.5. The shorter sleeve will allow the coating on the PicoTip to contact the conductive elastomer inside the CTM.

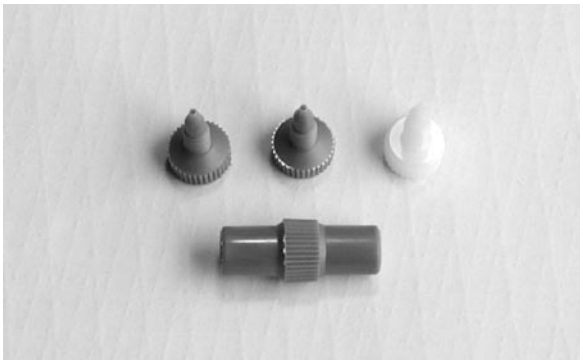

Figure 5.2: MicroTight® union, gauge plug, and compression fittings

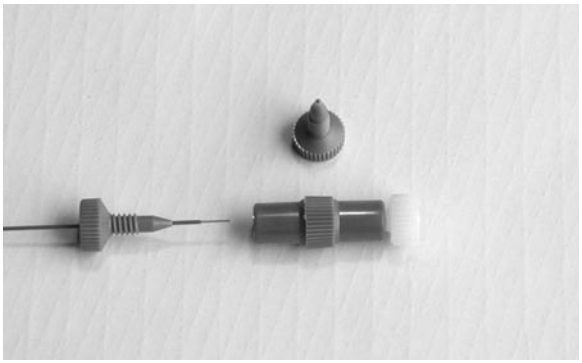

Figure 5.3: MicroTight® union with gauge plug and compression assembly ready to load

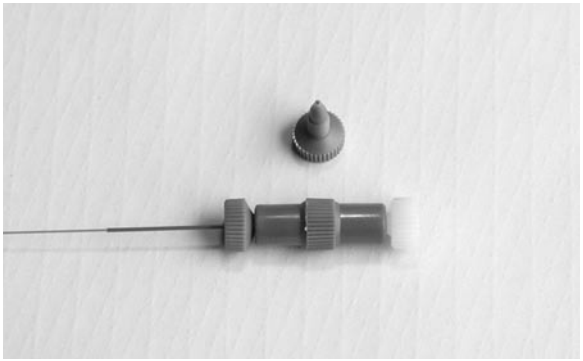

Figure 5.4: MicroTight® union with gauge plug and compression assembly loaded

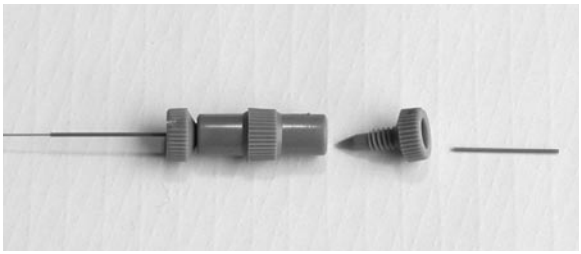

Figure 5.5: Union, compression fitting, and MicroTight® sleeve cut to 14 mm

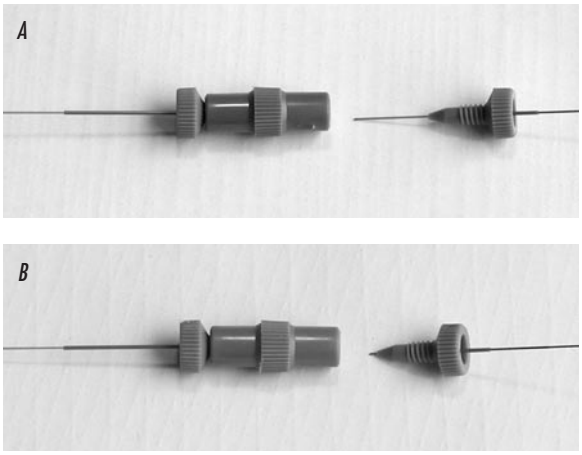

Figure 5.6: PicoTip® in fitting, before (A) and after (B) trimming to length

Figure 5.7: A fully assembled MicroTight® union

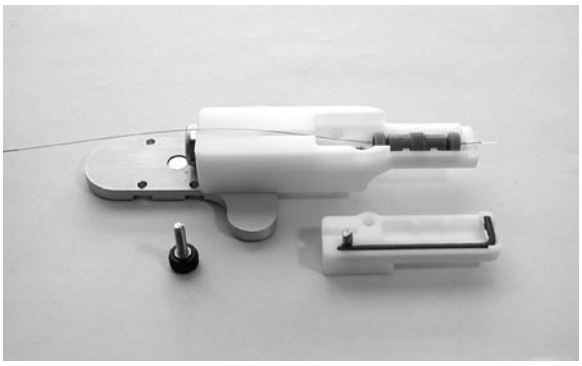

Figure 5.8: MicroTight® union inserted in the CTM base

5) Choose a PicoTip from the assortment sent with PicoView. Although either coating style, the standard coating (-CE-) or the distal coating (-D-), will work, if flow rates permit, the distal coating is recommended due to its immunity to arcing.
6) Insert the back, or distal, end of the PicoTip through the trimmed sleeve and through the other compression fitting. When properly installed, the tip end should extend 15–20 mm past the end of the fitting when it is tight. This will afford optimal positioning of the PicoTip within the adjustment range of the stage plate. Using a ruler, measure and note the distance the tip extends from the fitting. Remove the fitting/sleeve/PicoTip assembly and carefully trim the back end of the PicoTip so the extension of the tip beyond the fitting is 15–20 mm. Cleave the remaining portion from the back end of the PicoTip. See Figures 5.6A and B. Refer to Section 5.1 “Cutting Fused Silica” on page 20 for cleaving instructions.
7) After trimming, reinsert the assembly into the union, seat the PicoTip and sleeve against the transfer line or column tubing, and tighten the compression fitting finger-tight. Pull gently on the tubing to ensure the connection is tight. Figure 5.7 depicts the fully assembled union.

CAUTION: Always use chemical- and puncture-resistant gloves and ASNI-approved safety glasses when handling fused-silica tubing.

5.3.2 Loading the Coated Tip Module

1) Locate the CTM from the PicoView shipping box. Disassemble the CTM by unscrewing and removing the cover screw.
2) Lower the assembled union and PicoTip into the cavity in the base of the CTM, as shown in Figure 5.8.

NOTE: The PicoTip must make direct contact with the conductive elastomer inside the cover of the CTM.

3) Replace the top of the CTM. Replace the cover screw through the hole in the top and fingertighten. Be careful not to touch the tip of the emitter to any surface.

WARNING: It is very easy at this point to break the tip. Be careful not to touch the tip to any surface.

5.3.3 Attaching the High-Voltage Cable

Holding the CTM firmly, attach the high-voltage cable as follows:

1) Connect the cable by inserting it into the cable jack and turning the locking ring clockwise until it is tight.
2) Place the magnetic stage back onto the XYZ stage as shown in Figure 5.9.

5.3.4 Adjusting the Tip Position

Carefully slide the source into operating position while observing the tip position. If it looks like the tip may contact the inlet, adjust the Z-axis to prevent contact. When certain that contact will not occur, attach the locking bolt to the PicoView base.
By visual inspection, adjust the stage plate position using the knobs on the X, Y, and Z translation elements (see Figure 3.1A on page 9) until the tip is approximately 4 mm from the mass spectrometer inlet. Focus the camera by following the instructions in Section 4.3.9 “Adjusting the Camera Position” on page 17. Instead of focusing on the inlet, focus on the tip, which may appear as a fuzzy line prior to fine focusing. Do not let the tip contact the inlet.

Refer to Section 6.7 “Optimizing the Spray” on page 38 for information on optimizing the spray.

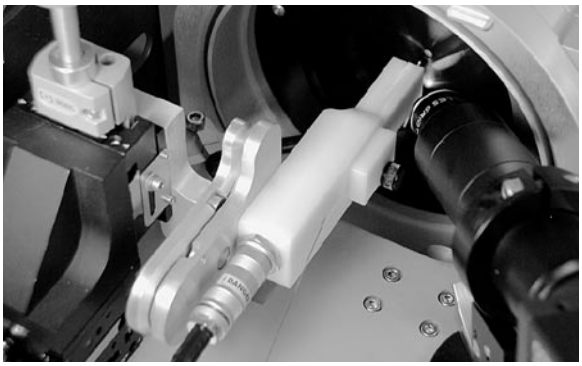

Figure 5.9: CTM assembly positioned correctly

5.4 The Uncoated Tip Module

The uncoated tip module uses a MicroTee or titanium zero dead volume (ZDV) union to supply the high voltage. Which connector you use depends on the application. Please go to Section 2.7 on page 8 for a discussion of the two types of junctions. Both set up instructions are included in this section. "PicoTip®" refers to any of New Objective's high-quality tips for electrospray ionization, such as SilicaTips™, PicoFrits™, and TaperTips™. When using PicoView with long PicoTips, such as PicoFrits or uncoated TaperTips, a tip holder is needed to position the end of the tip toward the inlet. This orientation is accomplished using a MicroTight® union within the tip holder. If using a TaperTip, proceed to Section 5.4.3 on page 28.

5.4.1 Using a PicoFrit® Column

PicoView is ideal for performing nanoscale LC-MS and LC-MS/MS using PicoFrit columns. In this configuration, the high voltage is applied to the back of the column through a liquid junction in the uncoated tip module. The PicoFrit column is packaged as a loop, as shown in Figure 5.11, to keep both ends of the column at the same pressure during storage and shipping. Do not remove either the orange-colored or the clear sleeve of snug-fitting PEEK™ and FEP (Teflon®) tubing holding the ends of the loop together until the column is being prepared for installation and use.

There are some important considerations in the handling and use of PicoFrit columns that make them very different from any other chromatography columns on the market today. PicoFrit columns are fabricated from 360 µm OD, polyimide-coated, fused-silica tubing. The column has a specially tapered tip with an integral high-porosity frit. Behind the frit is the packed chromatography bed. There is no frit at the back end of the bed, only unpacked fused-silica tubing. Mobile phase flow must always be directed toward the tip. Reversing the flow may result in partial or complete unpacking of the chromatography bed.

The column is shipped filled with methanol and needs to be conditioned with an appropriate mobile phase for your gradient. Since there is no distal frit, the distal end of the column bed may loosen with time; this is not a problem, as the bed will repack when the column is pressurized.

Note the label attached to the opaque orange PEEK sleeve that slides along the length of the column. The arrow on the label points toward the tip end. The tip end may also be identified by the charred section of polyimide coating just prior to the tapered region of the tip.

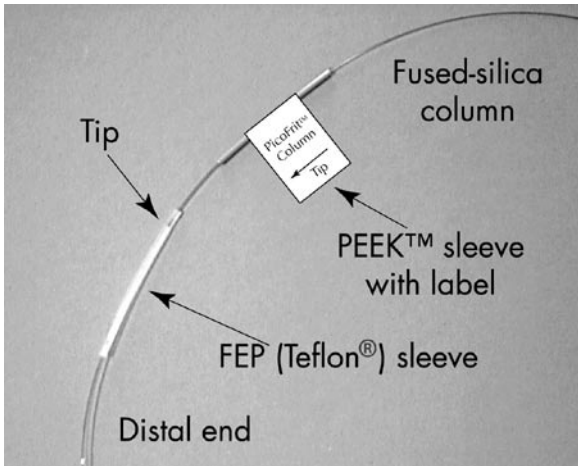

Figure 5.11: PicoFrit® column packaged for shipping

5.4.2 Removing a PicoFrit® Column from the FEP Sleeve

Care must be taken to properly remove the column from the FEP (Teflon) sleeve to prevent damaging the fragile tip and frit. Use the free-sliding orange PEEK sleeve to push the clear FEP sleeve off the fused-silica tubing without damaging the tip.

1) Remove the distal end of the column from the FEP sleeve by either pulling it free of the sleeve or (preferably) by cleaving the fused-silica tubing near the terminus.

2) Slide the PEEK sleeve toward the tip end until it butts up against the FEP sleeve, as in Figure 5.12.

3) Orient the FEP sleeve vertically, with the tip facing toward the floor. This way, when the FEP sleeve slides free, it will fall toward the ground without damaging the tip.
4) Carefully push the PEEK sleeve against the FEP sleeve until the FEP sleeve falls off, as shown in Figure 5.13. This may take a LOT of force. You will have to move the PEEK sleeve about 3–5 mm. Grasp the column in one hand while pushing the sleeve off with the other hand.
5) Do not let the PEEK sleeve slide off the tip end. Instead, remove it by sliding it over the back end.

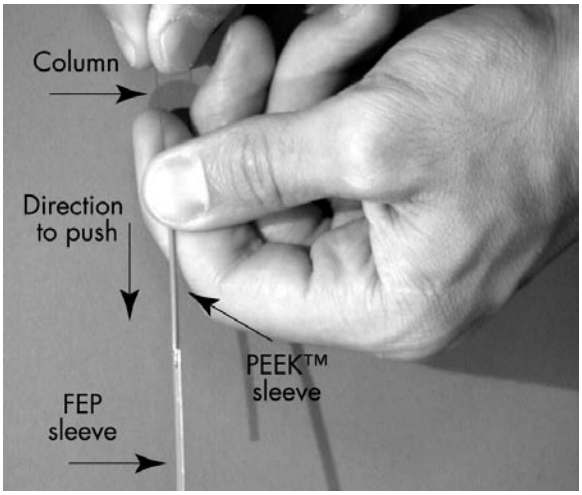

Figure 5.13: Pushing the PEEK™ sleeve against the FEP sleeve

5.4.3 Using the Uncoated Tip Module with Sheath Gas

Please refer to Section 4.3.5 "Using Sheath Gas" on page 17 for information on using sheath gas with PicoView.

5.4.4 Using the UTM with the Tip Holder

Although sheath gas is recommended for most uses of PicoFrit columns on the QSTAR®, the UTM can also be used without sheath gas. The tip holder, on the end of the UTM swing arm, can be replaced with a unit suitable for mounting a MicroTight® union. The tip holder and union may be found in the PicoView accessory box, see section 6.7.1.3 for further discussion.

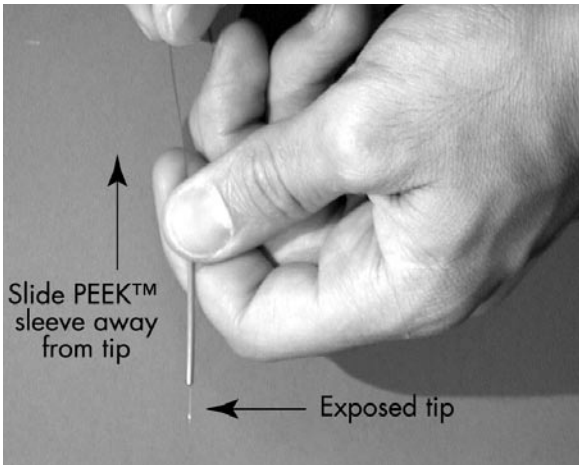

Figure 5.14: Pushing the FEP sleeve off the tip end of a PicoFrit® column

5.4.5 Loading the MicroTight® Union

1) A MicroTight union within the tip holder is used as a pass through to position the tip toward the inlet. Remove the union from the PicoView components box.
2) Unscrew and remove the compression fittings from the end of the union. Thread the distal end of the PicoFrit® through a green MicroTight sleeve and through a MicroTight fitting. Gently screw the fitting into the union, but do not tighten. Push the distal end of the PicoFrit all the way through the union. The PicoFrit should freely move within the union.
3) When properly installed into the MicroTight union, the tip end should extend 15–20 mm

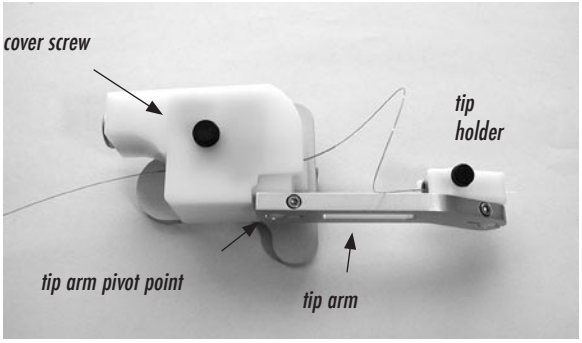

Figure 5.15: UTM and tip holder attached to stage plate

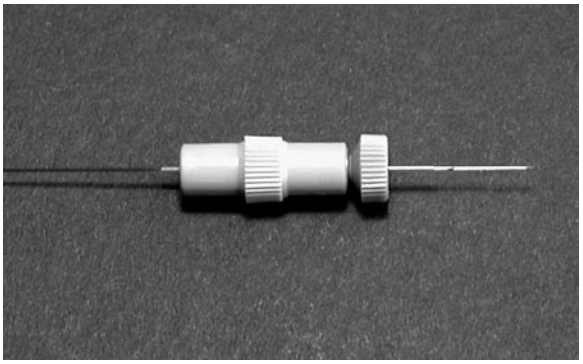

Figure 5.16: PicoFrit® column loaded into MicroTight union

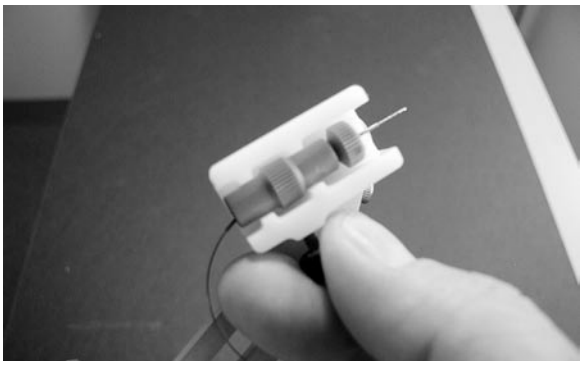

Figure 5.17: Firmly press union into tip holder and tighten tip holder screw

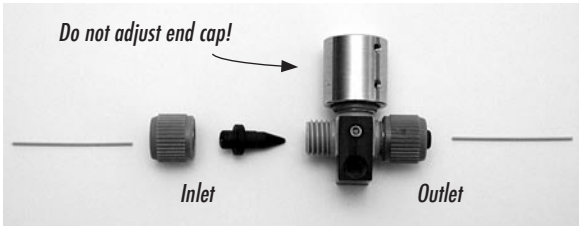

Figure 5.18: MicroTee with sleeves

past the end of the fitting when it is tight. This will afford optimal positioning of the tip within the adjustment range of the stage plate. Using a ruler, measure and note the distance the tip extends from the fitting. Gently move the PicoFrit until the tip extends 15–20 mm past the end of the fitting. Finger-tighten the compression fitting to hold the tip in place. Figure 5.16 shows a correctly loaded union.

5.4.6 Installing the MicroTight® Union into the Tip Holder

WARNING: It is very easy at this point to break the tip. Take care not to touch the tip to any surface.

1) Being careful not to put mechanical stress on the fused-silica tubing, insert the MicroTight union into the recessed area of the tip holder. tighten the tip holder finger screw. (Figure 5.16).

5.4.7 Supplying High Voltage

The uncoated tip module uses a MicroTee or titanium zero dead volume (ZDV) union to supply the high voltage. Which connector is used depends on the application. Please go to Section 2.7 on page 8 for a discussion of the two types of junctions. Instructions for both set ups are included in this section.

5.4.7.1 Plumbing the MicroTee

1) The MicroTee joins the transfer line to the PicoTip® and supplies the high voltage. Remove the MicroTee from the PicoView components box. Orient the MicroTee as shown in Figure 5.18 so that the platinum electrode is facing away from the user and the setscrews are visible. Unscrew the nuts and remove the black MicroFerrules from the posts of the MicroTee.

WARNING: Do not loosen the setscrews or remove the electrode cap, as this may damage the electrode. The solvent will not become charged and an electrospray will not form.

2) Thread the end of the PicoTip tubing through a green MicroTight sleeve, which is used for assembly with 360 µm OD tubing. Make sure the PicoTip does not extend past the tubing sleeve end that will be inserted into the MicroTee. Thread the sleeved PicoTip through the fitting nut and a black MicroFerrule, as shown in Figure 5.19.
3) Cleave the end of the PicoTip after the tubing is threaded through the sleeve, nut, and ferrule. Instructions for cleaving fused silica may be found in Section 5.1 on page 21. Slip the end of the tubing through the right post of the MicroTee, as viewed in Figure 5.20A, until the tubing and sleeve seat against the bottom ledge inside the post, as shown in Figure 5.20B. Screw the nut finger-tight onto the MicroTee.
4) Insert the distal end of the fused-silica transfer line through a green MicroTight sleeve, then through the nut and the black MicroFerrule, as shown in Figure 5.21A. Carefully trim the end of the transfer line. After trimming, insert the assembly back into the MicroTee, seat the transfer line, ferrule, and sleeve against the PicoTip, and finger-tighten the nut, as shown in Figure 5.21B. Gently pull on the tubing ends to ensure the connection is tight. Check for leaks by running solvent through the tubing at the expected operating pressure. Leaks will be apparent if solvent collects at the exposed ends of the sleeves.

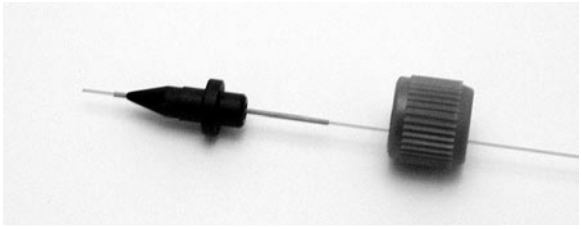

Figure 5.19: Fitting nut, MicroFerrule, and sleeve threaded by PicoTip

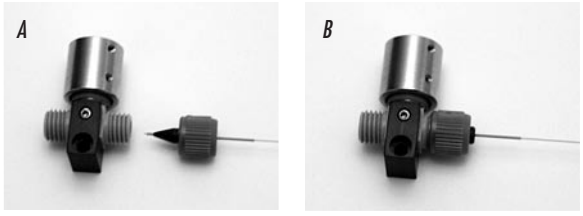

Figure 5.21: Assembling the nut, ferrule, sleeve, and PicoTip® (A) and securing finger-tight into the MicroTee (B)

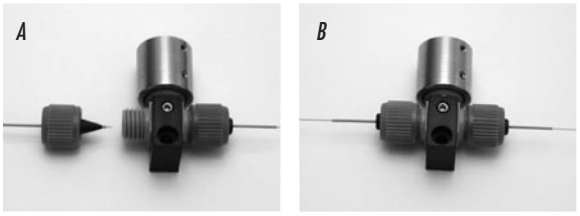

Figure 5.22: (A) Assembling the nut, ferrule, sleeve, and transfer line and (B) securing into the MicroTee

5.4.7.2 Plumbing the ZDV Union

NOTE: This option is not recommended with PicoFrit® columns.

1) Remove the ZDV union from the PicoView components box. Orient the union as shown in Figure 5.24 so that the "T" bracket is facing away from the user. Unscrew the nuts and remove the ferrules from the union.
2) Insert a green SealTight™ sleeve through the ferrule and nut. Thread a PicoTip through the sleeve/ferrule/nut assembly, as shown in Figure 5.24A. Cleave the end of the PicoTip after the tubing is threaded through the sleeve, ferrule, and nut. Slip the end of the tubing into the

Figure 5.23: ZDV union assembly

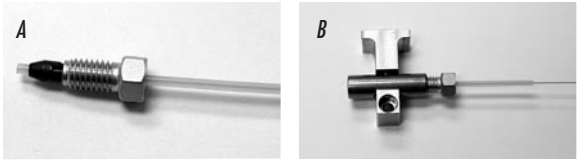

Figure 5.24: Assemble the nut, ferrule, sleeve, and PicoTip (A) and secure finger-tight into union (B)

Figure 5.25: Fully assembled ZDV union

right side of the union, as viewed in Figure 5.24B, until the ferrule and sleeve seat against the bottom ledge inside the union. Screw the nut finger-tight into the union. Do not tighten enough to compress the sleeve.

NOTE: Be careful not to touch the tip to any surface.

3) Trim 5 mm from the end of a green SealTight™ sleeve and insert the sleeve through the ferrule and nut. Thread the back end of the fused-silica transfer line through the sleeve/ferrule/nut assembly. Cleave the back end of the transfer line and slip it into the left side of the union until the transfer line/ferrule/sleeve assembly seats against the PicoTip. Tighten the nuts on both ends of the union. Pull gently on the tubing to ensure the connections are tight. Figure 5.26 depicts a fully assembled ZDV union.

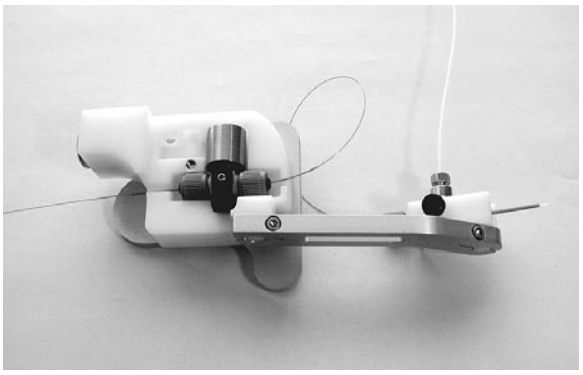

Figure 5.26: Plumbed MicroTee loaded in the UTM

5.4.8 Loading the Assembled MicroTee or ZDV Union into the UTM

1) Lower the assembled MicroTee or ZDV union into the cavity in the base of the UTM. (See Figure 5.26). If using the MicroTee, the setscrews on the black cap should be visible when the MicroTee is properly in place. If using the ZDV union, angle the union slightly so the right side of the "T" bracket presses against the high-voltage connector. Twist and lower the assembled union into the cavity in the base of the UTM. An optional mounting screw can be used to further secure the union into the UTM.
2) Replace the UTM module top. Replace the cover screw through the hole in the module top and align it with the mating hole in the module base and tighten. Figure 5.27 shows properly mounted UTM and tip holder.

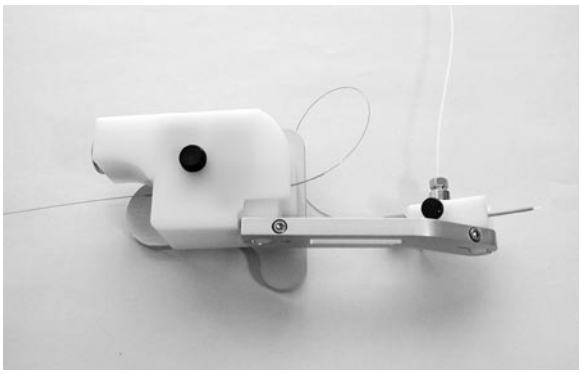

Figure 5.27: Plumbed UTM with cover in place

NOTE: Alignment of the UTM cover is critical. Misalignment of cover may damage the internal spring contact mechanism. Make sure cover is correctly aligned with base before applying pressure and securing cover.

5.4.9 Connecting the High Voltage

Run the high-voltage cable from the mass spectrometer through the opening in the rear housing. Connect the cable by pushing it into the cable jack and turning the locking ring clockwise until it is tight.

5.4.10 Adjusting the Tip Position

By visual inspection, adjust the stage plate position using the knobs on the X, Y, and Z translation elements (see Figure 3.1A on page 9) until the tip is approximately 4 mm from the inlet. Alternately, the tip arm and sheath gas or tip holder can be rotated for optimum positioning. Focus the camera by following the instructions in Section 4.3.9 on page 17. Instead of focusing on the inlet, focus on the tip, which may at first appear as a smudged line.

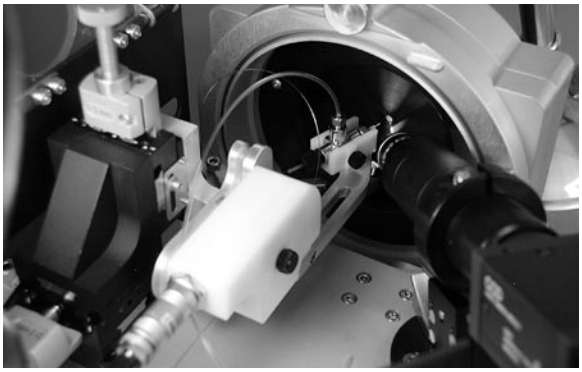

Figure 5.28: UTM with MicroTee in place on magnetic stage

6 Techniques to Optimize PicoView®

This section provides techniques to assure successful use of PicoView®, along with hints on ways to optimize the spray to consistently generate high-quality data. Also discussed are the interdependent parameters involved in creating stable electrospray.

6.1 Selecting a PicoTip®

Low flow rate ESI (under 1 µL/min) differs substantially from conventional ESI in that signal levels in the mass spectrometer are more sensitive to solvent composition, flow stability, and applied voltage. It may take a little time to learn how to obtain consistent results at low flow rates. A little patience and practice early on can pay off greatly in terms of success.

An effective way to begin working at low flow rates is by starting with larger (15–30 µm) ID tips and using flow rates at the higher end of the recommended range of operation for a given tip style. Once you are comfortable working at a certain level of performance, you can more easily switch to emitters with smaller diameters and operate at lower flow rates. "PicoTip®" refers to any of New Objective's high-quality tips for electrospray ionization, such as SilicaTips™, PicoFrits®, and TaperTips™. TaperTips are used for higher flow rate applications and may be a good starting point when learning to use PicoView. Once basic performance levels are established, you can switch to SilicaTips with smaller tip sizes. For highly aqueous mobile phases, however, tip sizes of 10–15 µm and smaller are recommended.

6.2 Choosing Tip Size and Coating Style

New Objective SilicaTips are available in sizes from 2 µm ID to 30 µm ID, in three different coating styles: standard (-CE-), distal (-D-), and uncoated (-N-). Coated PicoTips have a special enhanced conductive multilayer coating (U.S. Patent 5,788,166) that provides for excellent electrochemical stability and durability against ESI solvent exposure. All coated tips should be handled with care, since mechanical abrasion can deteriorate the coatings. Use a pair of fine tweezers to handle the tips.

Coated tips are typically employed for the lowest flow rate applications requiring the highest degree of performance from the system. For lowest flow rate experiments, the standard (-CE-) coating is recommended, but it may be subject to damage from arcing at high voltage. The use of distal (-D-) coated tips is encouraged due to their immunity to arcing. Use uncoated (-N-) PicoTips when a liquid junction is required, typically for nanoscale flow injection applications or when using LC columns larger than 100 µm ID. The choice of tip size and coating style will be influenced by the desired flow and application (continuous-flow nanospray, capillary LC, CE interfacing, etc.). See Section 2 on page 5 for assistance in choosing tip size and coating style based on the desired application. Since the size of the Taylor cone grows with flow rate, it is proportional to tip size. Use Table 6.1 as a guide in choosing the proper tip size.

Follow the flowrate guidelines in Table 6.2 to obtain the best results. Smaller diameter tips will operate most effectively at the lower end of the flow rate range, and larger diameter tips require higher flow rates to establish a stable spray (see Table 6.1).

Table 6.1: Recommended Flow Rate Ranges for Different Tip IDs

SilicaTip™ Tip ID (µm)	Flow Rate* (nL/min)	TaperTip™ Tip ID (µm)	Flow Rate* (nL/min)
5	20–200	20	200–500
8	50–300	75	300–2000
10	100–350	100	400–3000
15	150–500		
30	300–1000		

** Approximate range of flow rates over which the tips will generate a stable electrospray plume without sheath gas assistance. Actual performance may differ considerably due to variations in experimental parameters (mobile phase composition, applied voltage, source design, etc.).*

Table 6.2: Recommended Flow Rate Ranges for Different Coating Styles

Tip Coating	Recommended Flow Rate Range*
Standard (-CE-) Coated	10 nL/min–250 nL/min
Distal (-D-) Coated	100 nL/min–5 µL/min
Uncoated (-N-)	100 nL/min–5 µL/min

** Operable flow rate is a function of tip size.*

6.3 In-Line Filtration

Loss of spray due to clogging by fine particles is the most common cause of tip failure. Installing an in-line filter in the solvent transfer line is highly recommended to minimize this problem, even if using HPLC-grade solvents.

6.4 Air Bubbles

Air bubbles in your emitter have three different possible origins. The first is a leaky HPLC fitting. A fitting, even if under high pressure, will leak air into the system if liquid is leaking out. Small leaks, even those in which no liquid is visible, can admit significant quantities of air into the system. Always make sure to tighten the fittings.

The second possible origin of air bubbles is outgassing of the mobile phase. If gasses are forced into the mobile phase at high pressure, air bubbles will form as the mobile phase exists the column. The use of strictly degassed solvents is strongly recommended. If you are connecting a SilicaTip or a TaperTip to the outlet of a conventional LC column, air bubbles from outgassing can adversely affect the stability of the electrospray, especially at lower flow rates. Using a smaller tip size can also reduce this effect since the increase in back pressure generated by the tapered restriction can significantly reduce bubble formation. You may end up using a smaller tip than your required flow rate would suggest, but stability will improve.
The third possible cause is outgassing due to electrolysis of mobile phase at the high-voltage electrode. Since

the electrode is at a high potential there are always re-dox reactions occurring on its surface. Air bubbles may form at the liquid junction interface in the UTM as a result of electrolysis; the use of higher flow rates, however, tends to minimize the effects of air bubbles. These relatively higher flow rates used with the UTM match well with the flow rate requirements of most LC delivery systems. If air bubbles persist and you believe they are due to electrolysis, the most effective way to completely eliminate the phenomenon is to switch to a pre-column high-voltage contact with the UTM in combination with a PicoFrit column. The even backpressure gradient created by the column will eliminate air bubble formation due to electrolysis. If a post-column set-up must be used, fewer air bubbles are produced when using the CTM equipped with coated PicoTips. The use of distal coated (-D-) PicoTips is recommended due to their immunity to arcing at higher voltages.

6.5 Operating Parameters

Parameters that require adjustment to optimize the electrospray are the position of the tip with respect to the heated capillary inlet, the applied voltage, and the flow rate.

6.5.1 Tip Position

The transfer of ions created in the electrospray process is maximized when the flow rate is minimized and when the tip is positioned closest to the mass spectrometer inlet. The optimal gap between the tip and the inlet varies from 0.5 mm to 5 mm. Too close and the tip may arc; too far away and the electric field strength drops to a point where the Taylor cone collapses.

Tuning becomes somewhat difficult at distances less than 0.5 mm due to the large effect on the spray pattern of relatively small changes in voltage. Most tips can be used at distances of 2–5 mm with a minimal loss in ion current (under 10 percent). For the majority of operating conditions, a distance of 1–2 mm appears to be an optimal compromise between sensitivity and ease of tuning.

6.5.2 Applied Voltage

Applied voltage is perhaps the most important parameter for stable, efficient operation. Never use a “turn on” voltage above 500 volts unless stable ESI has been previously established. Direct application of a high voltage (e.g., 2.0 kV) can cause a corona discharge or an arc between the tip and inlet. This can destroy the conductive coating, disrupt the fine structure of the tip, and cause the formation of air bubbles within the tip. Once stable operation has been achieved, however, the voltage can be turned on and off at the same level with no fear of arcing, provided no other operating parameters have changed.

6.5.3 Solvent Flow Rate

The optimal flow rate of solvent delivered to PicoView depends on the tip size and the applied voltage. A flow rate too low or too high may result in an unstable spray pattern.

Using a flow rate that is too low leads to large fluctuations in the ion current. Ion current will likely drop to zero frequently, perhaps even periodically. To improve the spray quality, raise the flow rate by 25 percent and look for an improvement in signal stability.

Using a flow rate that is too high is generally not as problematic; ion current will fluctuate but will probably

not drop to zero. General chemical noise may increase, and the quality of the mass spectra may be diminished. To minimize the ion current fluctuations, reduce the flow rate by 25 percent and look for improvements in signal stability. Raising the applied voltage can sometimes compensate for a flow rate that is too high, provided the voltage is not above the threshold for corona discharge.

6.5.4 Electric Field Strength and Flow Rate Interdependence

In low-flow ESI, it is important to recognize the interdependence of flow rate and the applied electric field. The optimal value of the flow rate is altered when a parameter is changed that affects the "strength" of the electric field (e.g., the size of the tip, the distance from the tip to the heated capillary inlet, or the applied voltage).

For a given tip size, stable ESI can occur over a wide range of flow rates but only over a narrow range of field strength (100 volts or less). Raising the flow rate requires a higher field strength, and vice versa.

In the static nano-electrospray mode, where there is no external flow source, the system is self-regulating in that the electric field actually dictates its own optimal flow rate.

6.5.5 Sheath Gas

The use of sheath gas for nanospray on the QSTAR® is typically beneficial for both sensitivity and stability. Sheath gas can be especially useful at flow rates between 200 and 500 nL/min, the optimal flow range for PicoFrit® columns. The important thing to keep in mind is that a little sheath gas is beneficial, but too much sheath gas will hurt sensitivity. Care should be exercised in making small adjustments.

6.5.6 Curtain Gas

Much like sheath gas, using curtain gas can improve both stability and sensitivity. Also like sheath gas, too much gas flow will lower sensitivity. A high gas-flow setting appears to direct most of the spray plume away from the inlet. The minimal amount of gas necessary to generate a stable signal should be used.

6.6 Voltage Tuning Procedure

1) For a continuous-infusion experiment, prepare a standard sample and load 100–200 µL into a 250 µL syringe. Peptide and protein standards should be prepared at 1 µM in a suitable solvent system, such as 1:1 0.5% formic acid:acetonitrile. Secure the syringe to the mass spectrometer syringe pump using the syringe holder. Ground the needle with an alligator clip as described in Section 4.4.3, "Grounding to a Syringe Needle or HPLC," on page 20.
2) Lock PicoView to the mass spectrometer using the mounting brackets. Start with an initial tip position of approx. 4–7 mm away from the inlet.
3) Turn on the mass spectrometer and initiate source parameters.
4) Set the initial ESI Voltage to 500 volts, use a curtain plate voltage typical for your instrument. Set the initial sheath gas flow to zero. Set the curtain gas flow to a typical setting.
5) Start your syringe pump and let the flow rate stabilize. If the CTM is being used, the flow rate should be set between 50 nL/min and 250 nL/min. If the UTM is employed, the flow rate should be set to a value

greater than 100 nL/min, 300-500 nL/min is a good starting point.

6) Check the imaging system monitor to see if solvent is flowing, forming a droplet at the tip of the emitter. If it is, increase the voltage in 100-volt increments until ESI current is observed. If solvent is not flowing, try increasing the flow rate setting until a solvent droplet comes into view. If a solvent droplet does not appear, a clog may have formed. If necessary, change tips.
7) You may need to adjust the illuminator direction and angle to get a good image. For an optimal image, the background behind the tip should be as black as possible, with the tip appearing a bright white. Refer to Section 6.7.2 on page 38 for optimization using the imaging system.
8) If the ion current does not stabilize, optimal field strength may been exceeded. Lower the voltage 200 volts below the point at which current was first observed, then raise the voltage in 50-volt increments.
10) The sheath gas should be adjusted if stable ESI current cannot be achieved. Start with a low setting, and go through the voltage ramp procedure (5-8), optimizing current along the way.
11) If no signal is observed, turn off the applied voltage and look for droplet formation at the tip to visually verify solvent flow-through. If a clog has formed, change the tip.

Do not be concerned if the applied voltage required to generate an electrospray is greater than that normally required for nano-electrospray methods (e.g., nano-ESI may only require 600 volts for proper operation, while PicoTip® operation using a 15 µm ID may require 2 kV or more). The applied voltage should still be well below that required for conventional ESI. Voltages above 4 kV are generally considered excessive with most PicoTips. A corona discharge may occur if the applied voltage is too high, even when the distance between the tip and the heated capillary is large (greater than 5 mm).

6.7 Optimizing the Spray

PicoView® is ready for use once the tip or column is in position, fluid transfer has been established, and electrical contact has been made. Bringing the system to stable ESI conditions will involve adjusting the solvent flow rate and the ESI spray voltage and using the imaging system to visualize the resulting spray pattern.

It is recommended that a procedure, such as that in Section 6.6, be followed for initial set-up. Using a known standard pumped by continuous infusion will provide enough time to determine optimal values for tip position, applied voltage(s), sheath gas (if used), and curtain gas settings.

6.7.1 Selecting a Flow Rate

6.7.1.1 Using the Coated Tip Module

The CTM is most effective at flow rates below 250 nL/min when used with SilicaTips™ and above 500nL/min with TaperTips™. The user will need to empirically determine the best flow rate based on specific sample conditions and PicoTip placement. Use the lowest possible flow rate to generate a stable electrospray when low sample volumes and/or extremely dilute samples are analyzed. When the pumping element is a syringe pump, a 10–100 µL syringe is recommended.

6.7.1.2 Using the Uncoated Tip Module

The UTM is most effectively used at flow rates above 100 nL/min and with PicoFrit columns at flow rates ranging between 150 and 350 nL/min. The user will need to empirically determine the best flow rate for the specific experimental design. When the pumping element is a syringe pump, a 100–250 µl syringe is recommended.

6.7.1.3 Using Sheath Gas

Using sheath gas is a subjective choice, and while consistent results are often easier to obtain with sheath gas, poor tuning of gas flow can destroy sensitivity. If you plan to use tips smaller than 10 µm, sheath gas is unlikely to yield an analytical benefit.

The amount of sheath gas used will depend both on the tip-curtain plate distance and the amount of curtain gas being used. Increasing the amount of curtain typically requires an increase in the amount of sheath gas used. If both are set too high, turbulence may occur in the source, which is generally not beneficial for nanospray operation.

6.7.1.4 Tip Position

There is a general misconception that for high sensitivity, the tip should be as close to the inlet as possible. While this may hold true for some inlet designs, the curtain gas arrangement on the QSTAR® does not function correctly in that manner. If the tip is too close (less than approx. 1 mm), desolvation is incomplete, and the mass spectrum will have a high level of broadband chemical noise. If the tip is too far (greater than 10 mm), mass transfer of droplets into the inlet is minimal, and S/N is lost. Starting at 4-5 mm is a good initialization distance. Lower flow rates (less than 100 nL/min) will require a closer tip position (2-3 mm).

6.7.1.5 Curtain gas considerations

High temperature curtain gas plays a critical role in the desolvation of electrospray droplets. Without curtain gas, mass spectra are typically characterized by a high degree of chemical noise and sample adducts. Much like sheath gas, the minimal amount of curtain gas should be employed. A closer tip position will require a lower curtain gas setting, as will a lower flow rate.

6.7.2 Using the Imaging System to Optimize the Spray

The high-resolution imaging capabilities of PicoView® allow the user to fine tune the electrospray and easily optimize the distance/voltage/flow rate parameters. The figures below depict characteristics of unstable and stable spray patterns.

If the applied voltage is slightly low, an unstable spray will form with droplets forming periodically at the tip. This stream of large droplets normally seen under these conditions is not ideal (Figures 6.1 through 6.4) and will result in poor and unstable ion signal for the mass spectrometer. This spray mode is most commonly seen in gradient chromatography during highly aqueous portions of the gradient. Tuned correctly, the spray will stabilize as the gradient becomes more organic. Typically, the spray should be optimized at 30% organic.

When the voltage is correct, a stable fine spray will form, as shown in Figure 6.5, and the best-quality data, given the experimental conditions, will be generated.

If the voltage is too high, multiple jets can form around the tip, as shown in Figures 6.6 and 6.7. Decrease the voltage until a stable spray is formed.

Throughout an experiment, periodically check the imaging system monitor to confirm that the spray is stable. Clogs can form in the tip during infusion, causing the spray to fail. Changes in the organic composition of the solvent gradient may affect the solvent flow rate during micro-LC applications, and it may become necessary to adjust the position of the tip to maintain an optimal spray. It is generally not necessary to change any of the other parameters.

It may take some time to master electrospray at low flow rates. With careful tuning, PicoView can be used to generate consistent, high-quality data for a variety of chemical and biochemical applications.

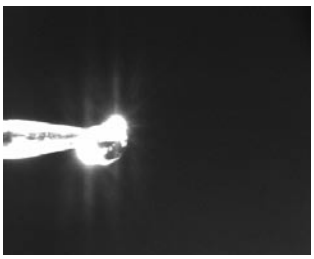

Figure 6.1: 1200V
Drops stick to tip, no spray

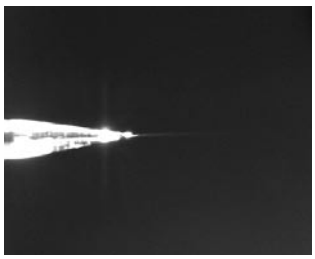

Figure 6.2: 1300V
Droplet stream

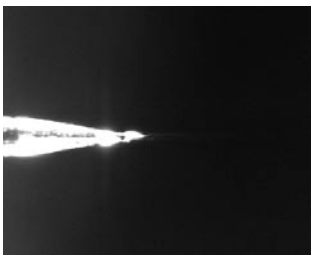

Figure 6.3: 1400V
Droplet stream and unstable spray

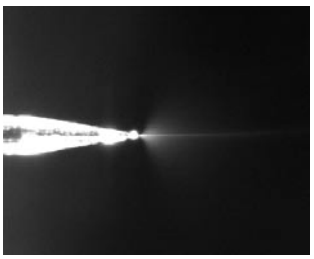

Figure 6.4: 1800V
Droplet stream and stable spray

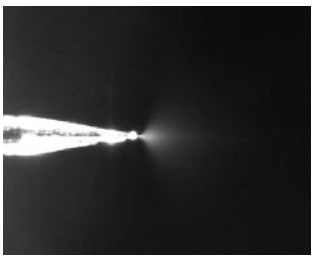

Figure 6.5: 2200V
Well tuned spray

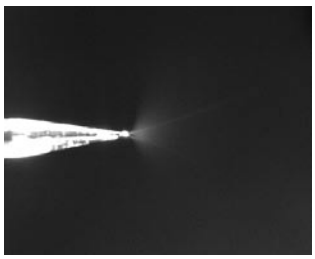

Figure 6.6: 2400V
Multiple sprays formed

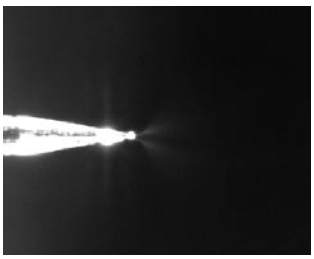

Figure 6.7: 2800V
Multiple sprays formed

Tip size FS360-75-15 Flow rate: 350 nL/min. Composition: 50% methanol, 0.1% formic acid.

7 Mounting an Injection Valve

The valve mountof the PicoView® has been designed to accommodate a variety of valve sizes (6 or 10 port) and from different vendors. A brief set of instructions are listed below showing the mounting of two popular valves: the Upchurch Scientific® manual micro-injection valve and the actuated Valco valve.

7.1 Mounting an Upchurch Manual Valve

1) An adaptor backing plate is used to mount the manual Upchurch valve to the PV-400. It can be found in the accessories kit. To fasten the plate, first place the valve with the port side on the table and the handle pointing away from you. Attach the plate as shown in Figure 7.1 using the two 8-32 cap screws with an allen wrench.
2) Attach the backing plate with the Upchurch valve to the PV-500 valve mount using the two 6-32 cap screws as shown in Figure 7.2 and 7.3. Make sure the valve ports face the opening in the housing.

Figure 7.1: Upchurch valves require the included backplate be attached prior to mounting

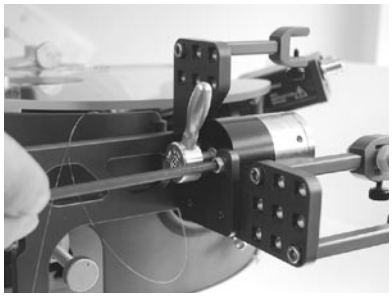

Figure 7.2: Attach the backplate to the mount using two mounting screws

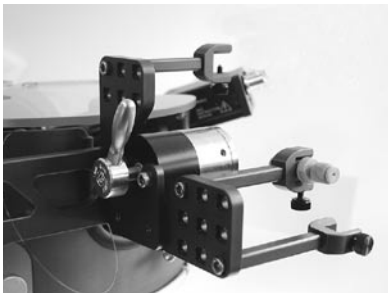

Figure 7.3: Properly mounted Upchurch valve

7.2 Mounting a Valco Valve

The Valco valves can be attached directly onto the PicoView valve mount. The two holes on the valve mount directly match two 8-32 threaded holes on the face of the Valco valve. Using two 8-32 cap screws, fasten the Valco valve to the valve mount as shown in Figure 7.4. Make sure the valve ports face the opening in the housing.

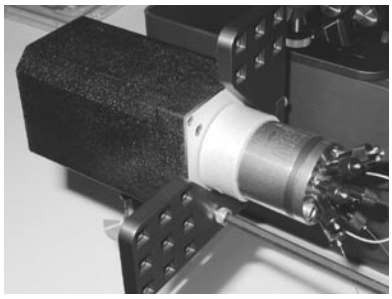

Figure 7.4: Attach the Valco valve directly to the mount using two mounting screws, as shown

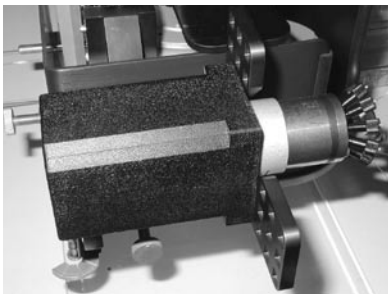

Figure 7.5: Properly mounted Valco valve

7.3 Components and Component Clamps

PicoView® has four (4) component clamps that have been designed to hold an injection adapter (included in the accessories kit), columns or unions that may be required in your experiment. The thumb screws in the component clamps will gently hold items in place. Alternately, these clamps can be removed and set aside when they are not needed.

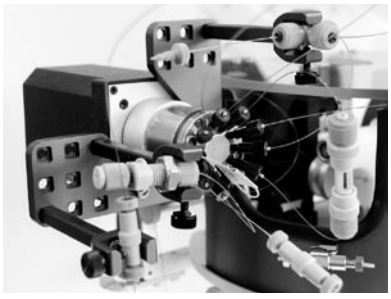

Figure 7.6: Component clamps with injection adapter and an IntegraFrit™ trap column

Figure 7.7: Micro injection adapter with mounting nut