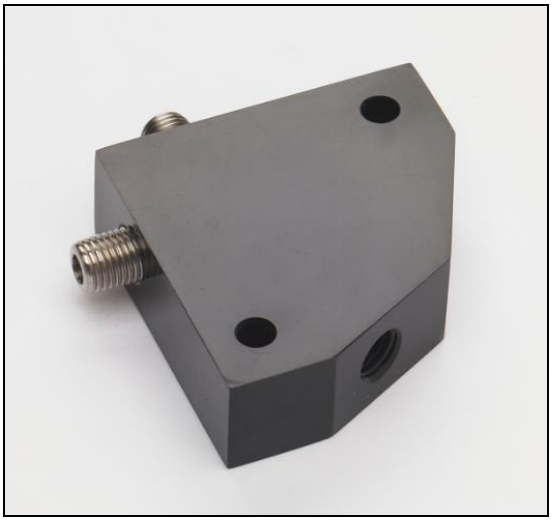
Fiber Optic SMA-FL Fluorescence Flow Cell Manual

Version 2.2



The line of fiber optic ***SMA-FL Cells*** is the top choice for many fluorescence measurements. SMA-FL cells can be made in Stainless Steel or black Ultem. The cells contain fused silica windows and are compatible with standard SMA-905 terminated fiber optic cables.

The SMA-FL cell is designed to accommodate many common optical measurements. It is ideal for use in flow injection, sequential injection and stand-alone applications and is readily disassembled for cleaning.

Please note that flow cells should not be exposed to aggressive materials for long periods of time and doing so may cause significant damage. If prolonged exposure is necessary, please consult the specification sheet in this manual to select a flow cell material most suitable to your application. It is advised to fill the flow cell with water or air when not in use.

Design:

SMA-FL Cells are designed to accommodate common fluorescence measurements. The unique fluidic path allows continuous flow up through the flow cell, minimizing bubble entrapment.

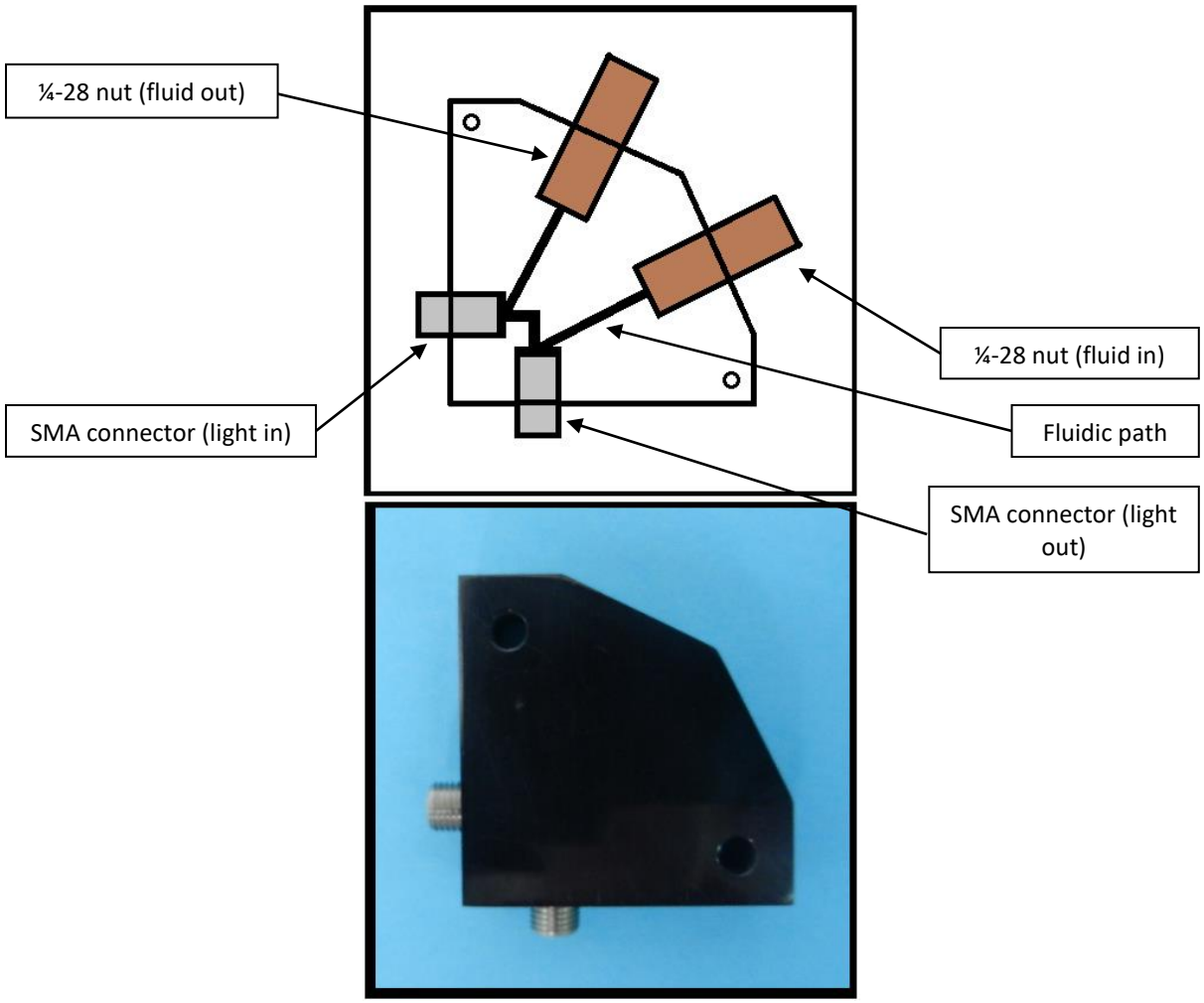


Figure 1: SMA-FL Cell Design

The flow cell optical path contains polished UV fused silica windows found at each of the cell's two fiber optic junctions. Non-corrosive and chemically resistant, these windows efficiently transmit light in the 170nm-2000nm range. Each window is sandwiched between two ring shaped Teflon seals, eliminating the possibility of fluid leakage through these ports.

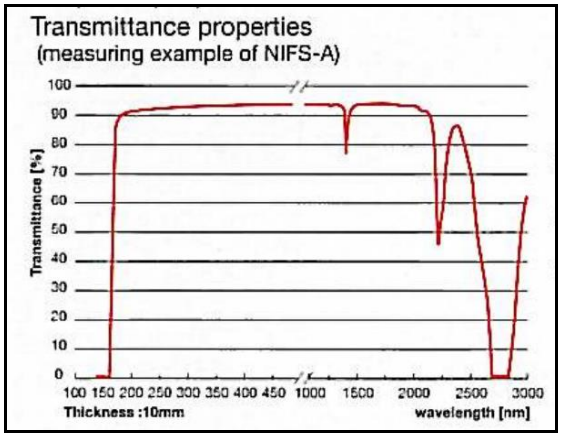


Figure 2: Transmission of UV/Silica Windows

¼-36 thread Stainless Steel SMA-905 connectors attach to the two optical ports on the flow cell and serve both to connect to external fiber optic cables and to hold the seals and windows in place.

Set up:

To use the SMA-FL cell, connect tubing to the cell's fluidic ports. Cleanly cut the supplied tubing to the desired length and place a colored nut onto the tubing following with a blue ferrule (with the cone side of the ferrule pointing towards the nut). If the dead volume in the flow cell is not an issue, screw the tubing into the flow cell so that the end of the tubing is flush with the end of the ferrule. If dead volume is an issue, screw the tubing in so that it sticks out of the ferrule and into the flow cell, but do not push it in all the way. It is CRITICAL that the tubing is not blocking any portion of the light path. To check, shine a light through one of the ports. You should be able to see the tubing if it has been pushed too far in. Repeat this procedure for tubing connecting to the other fluidic port.

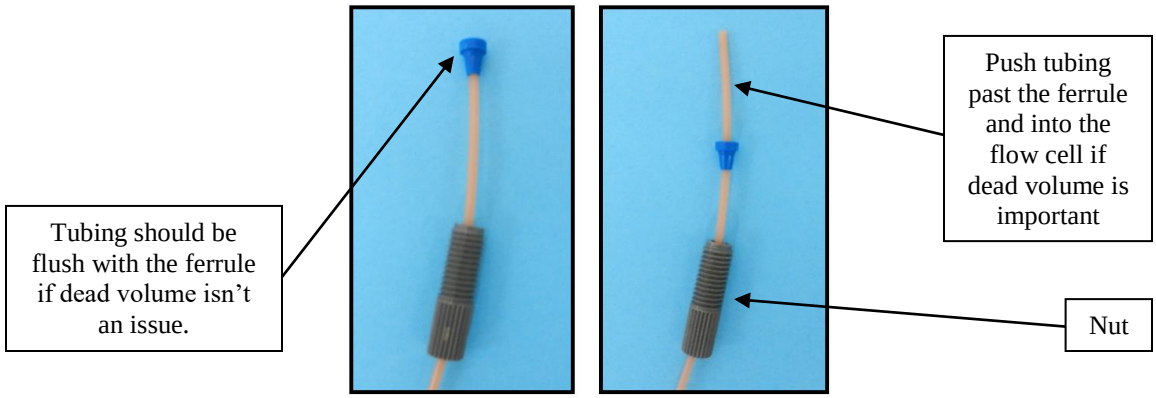


Figure 3: Inserting tubing into SMA-FL fluidic path

Fiber optic cables connect to each of the flow cell's SMA connectors.[1] One should connect to a light source and the other to a detector.

Mount the flow cell in such a way that the fluid flow path is tilted upward and fluid enters from the bottom, exiting at the top. This is done to minimize the likelihood that air bubbles will get caught in the path as measurements are being taken.

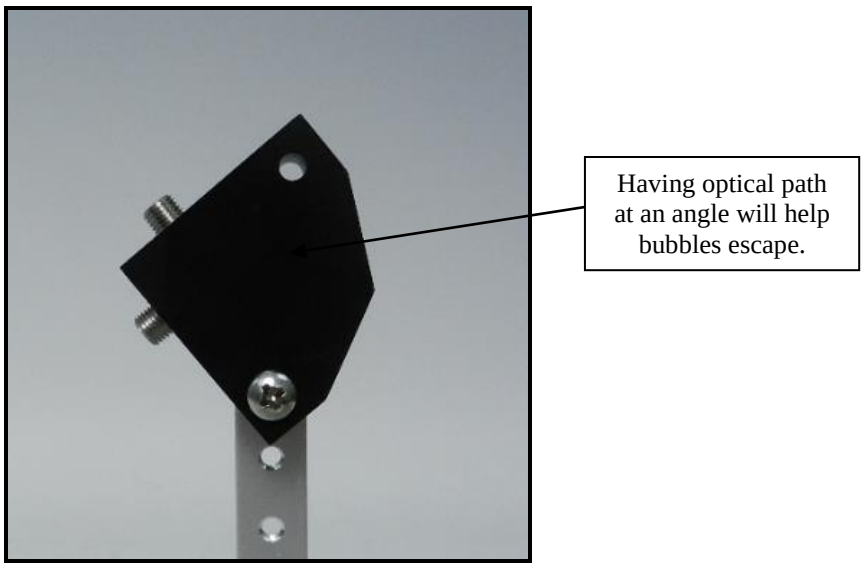


Figure 4: Mounting an SMA-FL Flow Cell

Use and Troubleshooting:

To test for leakage, before putting any chemicals through the cell, pump water through for at least ten minutes at or as close to the pressure and temperature you intend to use for your measurements. If leaks are observed coming from the fluidic ports, tighten the nut and ferrule more securely. If leaks are coming out of the junction between the SMA connector and the flow cell, tighten the SMA connector according to the instructions for replacing windows on the next page.

Whenever measurements are made, take care to make sure that the fiber optic cables don't move. If your fiber optic cables move at any point during the course of measurements, light throughput may vary.

Lastly, the most common problem with any type of flow cell is that of bubbles getting stuck in the light path. Bubbles cause significant deviations from accurate measurement. Furthermore, since bubbles do not always remain in the flow cell throughout the course of measurements, not all samples will be influenced by their presence, leading to even greater inaccuracy across the run.

The first step in troubleshooting bubbles in determining their source. Please see the end of this manual for further assistance.

[1] A FIAlab DCON-LED may be used on one side of the flow cell instead of a fiber optic cable as a direct connect light source.

Window Replacement:

When windows get stained, many users wish to replace them. Windows will also need to be replaced if for any reason they become cracked. To replace flow cell windows:

Unscrew one of the stainless steel SMA fittings from the flow cell and tap the cell against the surface of a table until the seals and window fall out. Repeat this process on the other side. If windows will not come out, soak the flow cell overnight in DI water.

Make sure to clean the flow cell well with DI water or a 0.1% solution of detergent followed by a thorough rinse before replacing windows. Dry the flow cell completely, it may be helpful to use a Kimwipe and then also leave the flow cell to air dry overnight.

When dry, insert a new Teflon seal into one of the open ports. Follow this with a silica window and then again, another Teflon seal. To hold these parts in place, gently screw in an SMA connector (the ones previously on the cell, if they have been maintained, can be reused) until it just touches the seal but make sure to not tighten. Repeat this on the other side of the flow cell.

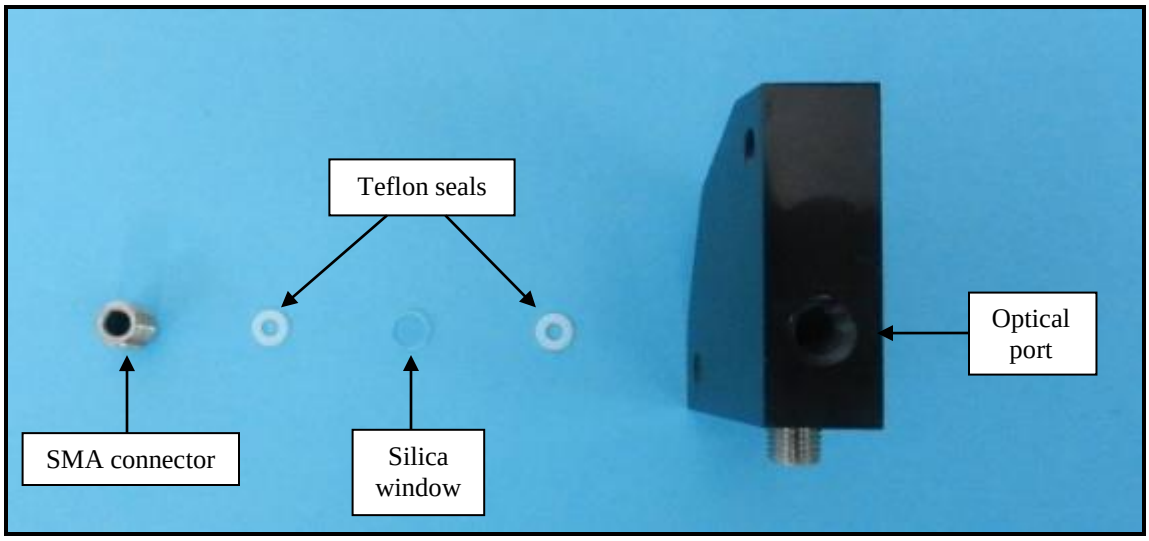


Figure 5: Components into flow cell optical port

Place a piece of large ID Tygon tubing snugly around one SMA connector and use pliers to tighten. Repeat on the other side. Insert a piece of plug tubing on one fluidic port of the flow cell and an empty syringe to the other fluidic port. Pull back the syringe's piston and check if it bounces back to ensure that the cell is airtight.

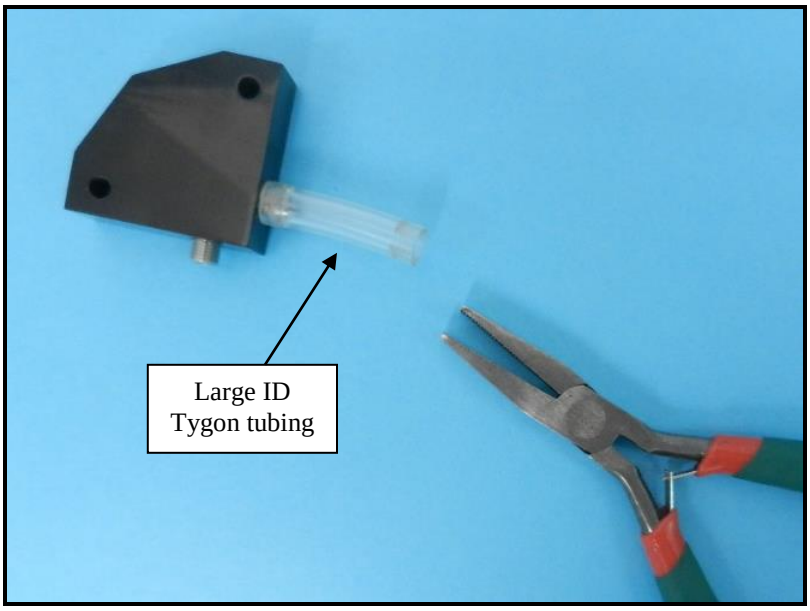


Figure 6: Tightening SMA connectors

SMA-FL Cell Specifications

Dimensions

Internal Volume [=] µL	30
*Optical Path Length [=] mm	6

*Includes both the excitation and emission regions

Physical Parameters

	Stainless Steel (303)	**Ultem (1000)**
Temperature [=] C	100	70
Pressure [=] psi	100	100

Chemical Compatibility (common solvents – room temperature)

R = recommended; NR = not recommended

	Stainless Steel (303)	**Ultem (1000)**
Acetic Acid	NR	R
Acetone	R	R
Acetonitrile	R	-
Ammonia	NR	-
Benzene	R	-
Chlorinated Hydrocarbons	-	NR
Detergent Solutions	R	R
Hydrochloric Acid	NR	R
Hydrofluoric Acid	NR	NR
Hydrogen Peroxide (<40%)	NR	-
Isopropyl Alcohol (<50%)	-	R
Methanol (pure)	-	R
Nitric Acid (20-70%)	R	R
Phosphoric Acid (<10%)	NR	R
Potassium Hydroxide (50%)	R	R
Sodium Hydroxide	NR	R
Sodium Hypochlorite	NR	R
Sulfuric Acid (<30%)	NR	R
Phosphate Buffered Saline	**NR**	R

Bubble Troubleshooting Guide

Because FIAlab flow cells are so versatile, they are used all over the world in many different configurations. Different components upstream of the flow cell in differing configurations lend to differing causes and origins of bubbles – the most common problem encountered in cell measurements.

Make sure the flow cell is oriented so that the path of the fluid always travels upward. The flow cell should always be used in this orientation because here bubbles are more likely to slip easily to the top of the flow cell and out of the optical path.

Large or small bubbles that travel through the flow cell while measurements are being taken will cause inaccuracy and imprecision. In addition, bubbles that get stuck in the optical path will likewise negatively influence results. To fix any bubble problem that may arise it is necessary to first find the bubble(s) origin. Possible origins of bubble formation follow and tips for removing stuck bubbles can be found at the end of this guide.

Chemical causes of bubbles:

Degassed Reagents:
Bubbles will come out of solution during heating as air is less soluble at higher temperatures. Rather than having this occur in a closed system, it is a good idea to degas reagent and carrier water offline prior to running experiments. To easily degas, place DI water under a vacuum and gently heat or stir for 20 minutes. Use this water to make reagents. DO NOT DEGAS SAMPLES.

Refrigerated Solutions:
As air is more soluble in cold temperatures, when a reagent is left in a refrigerator for prolonged periods of time, air will pervade the solution. Do not store solutions in the refrigerator unless necessary. If refrigeration is required, store solutions in sealed glass or metal bottles when possible as plastic containers are more susceptible to air permeation.

Reaction chemistry:
It is inevitable that certain reactions will create bubbles when mixed within the system. Certain reactions also need to take place at a high enough heat where even mostly degassed reagents will create bubbles. If solution is actively being pumped into the flow cell, adding a back pressure coil at the flow cells outlet port will increase pressure upstream and force any small bubbles that want to form to remain in solution. A simple and cost effective back pressure coil can be made by tightly coiling a 20ft length of 0.02” ID Teflon tubing.

Hydrophobic surfaces:
If bubbles get trapped at specific places, hydrophobic surfaces within the system created by salts, contaminants or oil residues may be the cause. Washing the system with a 1% detergent solution is absolutely necessary and it is best if this solution can be left in the system overnight. Rinse the system thoroughly with DI water after washing and make sure to wash out the setup at the end of each day. If compatible with the chemistry being run, adding a small amount of detergent (total 0.1%) to the carrier or reagent solutions will help prevent future bubbles by dramatically lowering surface tension.

Fluidic causes of bubbles:

Fittings:
If bubbles appear to be coming from a specific fluidic junction in the setup, it is likely that the nut and ferrule at this point is either too loose or too tight. If the fitting is loose, air can easily enter the system through the gaps at this connection. Tightening the fitting should eliminate these bubbles but DO NOT OVERTIGHTEN. Overly tight fittings can be a problem as they can collapse and crimp the tubing inside. There is also the possibility that the ferrule or nut is defective. To fix a problem fitting that cannot be remedied by tightening, unscrew the fitting, remove the nut and ferrule and trim the tubing so the portion formerly held by the fitting will no longer be used. Tubing should be cut with a square face tubing cutter. Clean out the port where the fitting was connected with a damp Kimwipe and dry thoroughly. Using a new nut and ferrule, reconnect the tubing and tighten just to the point where everything is securely held in place.

Tubing:
When dissolved gases exit a narrow constriction bubbles will often come out of the solution as the result of a drop in pressure. Upstream of the flow cell constriction is likely due to kinked or misaligned tubing. Manually push solution through the system with a syringe to test for back pressure issues and replace any tubing that has warped or reconnect any tubing that is misaligned.

Component causes of bubbles:

Selector Valves:
If a port on a component is not used but left open it creates a prime location for air to enter the system as the pressure difference between the system and its surroundings may be significant. A good example of such a situation is a selector valve used in conjunction with a syringe pump where the fluidic path may sweep past an open port when the pump is not dispensing. Make sure all unused ports are securely plugged with a nut, ferrule and solid Teflon tubing.

Syringe Pumps:
Bubbles can enter the syringe of a syringe pump if solution is aspirated into the pump too quickly. Pulling too quickly can create a vacuum that will pull dissolved gases out of the fluid and form a head space. The speed at which the pump can effectively draw solution is a function of the ID of the tubing that the solution is being pulled through. Change to a larger ID tubing on this pump port or slow down the flow rate when the pump aspirates.

Bubbles can pass through the syringe piston if the Teflon seal at the top has become worn. In this situation bubbles will be seen floating up from the plunger tip and through the syringe. Replace the syringe.

Tips to remove a stuck bubble:

Bubbles will always pass through the system when primed if the tubing in the system was left dry since its past use. If one of these bubbles gets stuck in the flow cell the following tips are easy and efficient ways to dislodge it.

Tap firmly on the side of the flow cell while solution is flowing through. FIAlab flow cells are very robust; do not be afraid to tap vigorously and with medium force. Tapping is more effective when solution is pumped through the flow cell at a high flow rate.

Introduce a large bubble to capture the stuck bubble as it travels through the cell's fluidic path. Stop any

flow of solution and with the flow cell oriented so that fluid entering the cell will travel upwards and out, loosen the fitting on the cell's inlet. Slowly a significantly sized bubble will form and begin traveling through the cell. Retighten the fitting and pump solution through as normal.

Replace the outlet fitting of the flow cell with a 1ml luer lock syringe and pump the piston up and down to make the bubble move within the flow cell path. Remove the syringe and replace the outlet fitting. As the bubble is no longer stuck in a single position, it will be more easily removed when solution is pumped through.