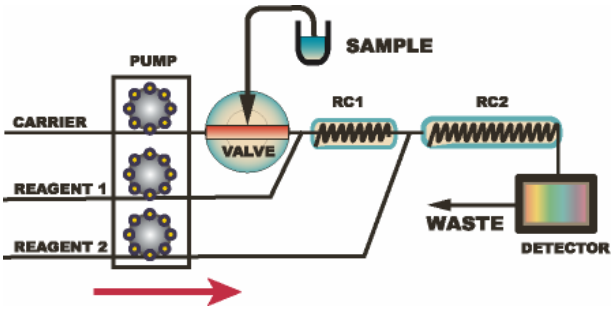
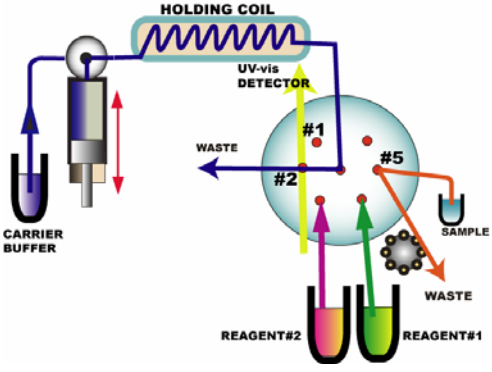
Analysis by Microfluidics

Flow Injection Analysis is an analytical technique based on microfluidic manipulation of samples and reagent(s). Injected samples are transported by carrier stream into a flow cell while desired (bio)chemical reactions are taking place. Detector response (absorbance, fluorescence, etc.) yields content of the target analyte. Continuous flow techniques, such as **Flow Injection (FI)** and **chromatography,** use constant forward flow of carrier to transport samples through the analyzer. Programmable flow techniques such as **Sequential Injection (SI)** and **Sequential Injection Chromatography (SIC)** use flow reversals and flow acceleration to mix sample with reagent, and stop flow to monitor reaction rates.



Flow Injection has been a workhorse for serial sample processing, its advantages being transparency of operation, high sample throughput and recognition of its reliability within the community of routine laboratories.



Sequential Injection uses microliter volumes of samples and reagents per assay, and its stop-go flow mode consumes reagents (and produces waste) only during the sample processing cycle. SI is the recommended approach for online and remote site monitoring, where robustness and low reagent consumption is critical.

FIAlab Instruments, Inc.

FIAlab is a leading manufacturer and distributor of Flow Injection Analysis systems and components. We offer a variety of systems for the laboratory, industrial, field, and educational environments. Our systems are utilized for such diverse applications as online fermentation monitoring, biosensor development, agricultural and environmental assays, pharmaceutical drug discovery, and chromatography.

FIAlab offers an entire range of instrumentation, including systems for **Flow Injection Analysis** (FIA), **Sequential Injection Analysis** (SIA), **Bead Injection Analysis** (BIA), and **Sequential Injection Chromatography** (SIC).

Our revolutionary **Lab-On-Valve®** manifold allows these microfluidic techniques to be further miniaturized and integrated into a transparent user friendly format.

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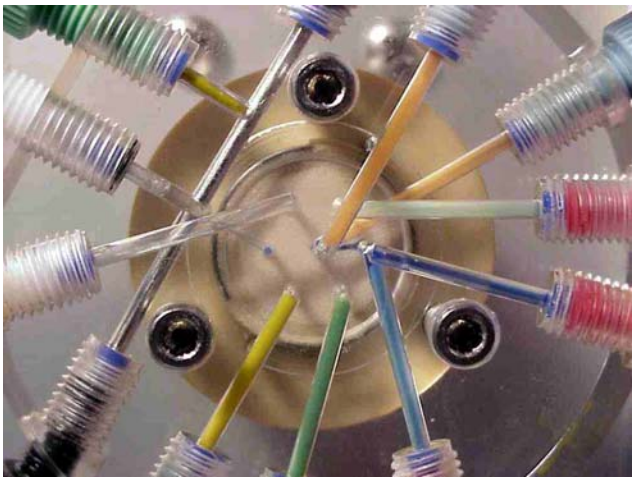
FIAlab offers **development and manufacturing services** for customized OEM systems and software. We also offer custom method development, onsite system setup and verification, and end user training.



Lab-On-Valve® for SIA, BIA, and SIC

FIAlab Instruments, Inc.
www.flowinjection.com
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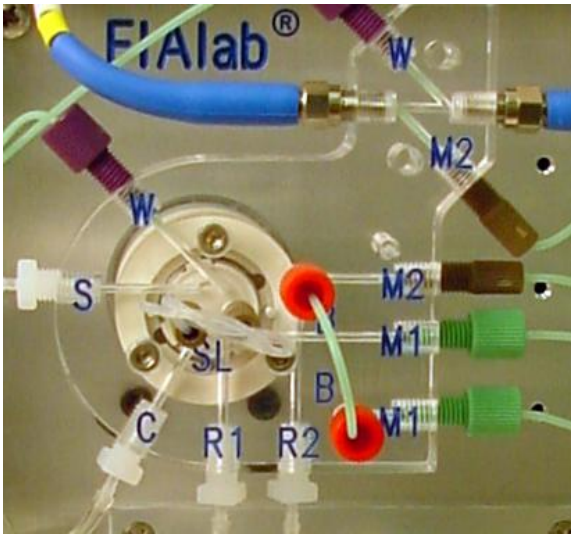
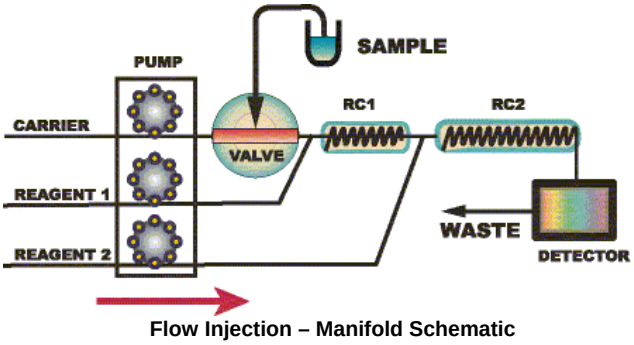
Flow Injection Analysis

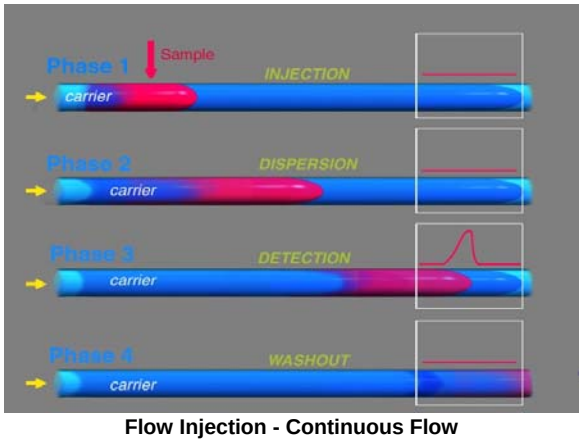
FIAlab®
Leaders in Flow Injection Technology



Overview - Flow Injection Analysis (FIA), the first generation of FIA techniques, is also probably the most widely utilized. In its simplest form, the sample zone is injected into a flowing carrier stream of reagent. As the injected zone moves downstream, the sample solution disperses into the reagent, causing the product to form. A flow through detector placed downstream records the desired physical parameter such as colorimetric absorbance or fluorescence.

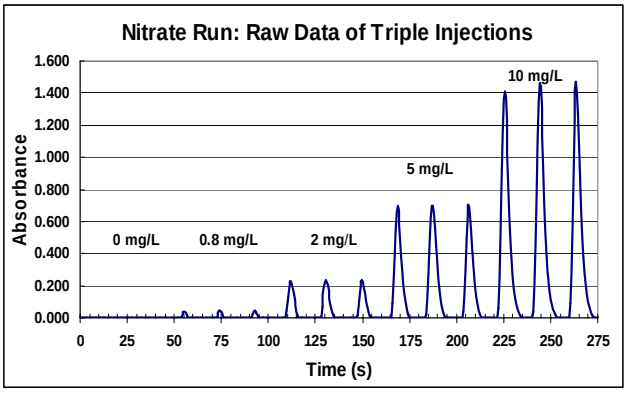


Flow Injection – Manifold Schematic



Flow Injection - Continuous Flow

FIA is a widely accepted method for serial analysis of waste water, soil testing, and other environmental/agricultural applications where measurements of Ammonia, Nitrite, Nitrate, Phosphate, Chloride, Silicate, Boron, Chromium, Copper, Iron, Zinc, Bromine, Iodine, Sulfide, Sulfate, Fluoride, Hydrogen Peroxide, Nickel, Manganese, Molybdenum, pH, and others are made. The typical laboratory based end user may have a high throughput requirement of hundreds or even thousands of samples per day.



The modern Flow Injection Analysis system usually consists of a high quality multichannel peristaltic pump, an injection valve, a coiled reactor, a detector such as a photometric flow cell, and an autosampler. Additional components may include a flow through heater to increase the speed of chemical reactions, columns for sample reduction, debubblers, and filters for particulate removal.

Agricultural and Environmental Assays

FIAlab®
Leaders in Flow Injection Technology

Agricultural and Environmental Assays are performed with FIAlab FIA systems, including; Nitrate, Nitrite, Ammonia, Phosphate, and Chloride (and many others). The following table describes a few of the more common methods, showing their concentration ranges and sample throughputs. Additional methods with lower and higher concentration ranges are available, please inquire. Some of the following methods can be performed with brackish/seawater samples. Also, these methods are suitable for off-line Fermentation and Pharmaceutical Quality Control. Multiple channel systems are available to process up to four of these methods simultaneously.

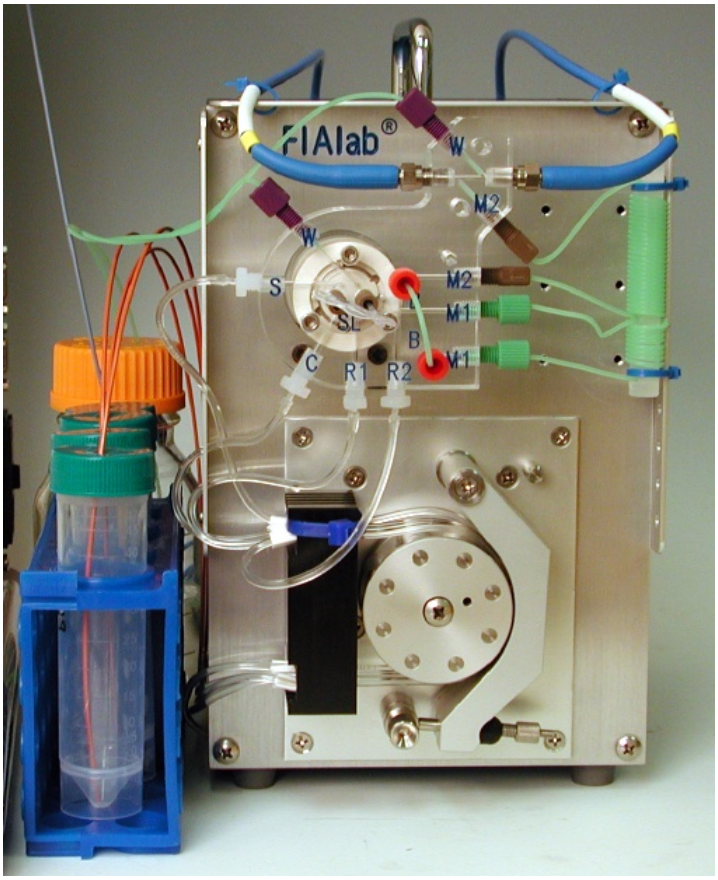
Assay	*Typical Throughput*	*Concentration Range*	*Notes*
Nitrate (Mid to High) **Nitrite (Mid to High)**	**180 samples/hour** **220 samples/hour**	**0.03 to 200 mg (N)/L** **0.005 to 100 mg (N)/L**	**Nitrites react with sulfanilamide to form azo dyes that will then couple with N- (1-Naphthyl) ethylenediamine dihydrochloride to form magenta colored solution.** **A copperized cadmium column converts nitrate to nitrite. Dynamic Range extended using selected choices of wavelengths.**
Nitrate (Low) **Nitrite (Low)**	**60 samples/hour** **80 samples/hour**	**0.003 to .9 mg (N)/L** **0.0005 to .18 mg (N)/L**	**Identical chemistry as above, however utilizes a 10 cm long optical path flow cell.**
Ammonia (Mid to High)	**120 samples/hour** **(400 samples/hour with Rapid Queuing Valve)**	**0.5 to 200 mg (N)/L NH_3**	**Ammonia in water can be determined using the standard Salicylate method. This method requires a heated reaction coil.**
Ammonia (Low)	**80 samples/hour**	**0.05 to 10 mg (N)/L NH_3**	**Identical chemistry as above, however utilizes a 10 cm long optical path flow cell.**
Ammonia (Ultra Low)	**30 samples/hour**	**0.001 to 0.5 mg (N)/L NH_3**	**Fluorometric ammonium (OPA) analysis. Required hardware includes PMT-FL and either the FIAlab-2500 or FIAlab-2700. Also requires a flowthrough heater.**
Phosphate (Mid to High) ***Method compatible with Bray and Olsen Bicarb soil extraction samples. Similar method is compatible with total phosphate assays.***	**120 samples/hour** **(400 samples/hour with Rapid Queuing Valve)**	**0.1 to 25 mg (P)**	**Orthophosphates react directly with molybdate anions to form a yellow-colored phosphomolybdate complex. This complex is then reduced by ascorbic acid to create a molybdenum blue species that has a broad absorbance range from 700nm to 900nm.**
Phosphate (Low)	**60 samples/hour**	**0.01 to 2.5 mg (P)**	**Identical chemistry as above, however requires heated reaction coil and a 10+ cm flow cell.**
Chloride	**120 samples/hour**	**1 to 50 mg Cl-/L**	**The chloride assay is based on the liberation of the thiocyanate ion (SCN) from mercuric thiocyanate, through sequestration of mercury by the chloride ion, to form un-ionized, but soluble mercuric chloride. The liberated SCN then reacts with the ferric ion to form highly colored ferric thiocyanate.**
Chloride	**60 samples/hour**	**0.1 to 5 mg Cl-/L**	**Identical chemistry as above, however utilizes a 10 cm long optical path length flow cell.**
Iron	**140 samples/hour**	**0.1 to 100 ppm**	**1,10-phenanthroline chelation chemistry to Fe^{2+}. This Fe-complex has an absorbance maximum around 510nm.**

The FIAlab-2500 System

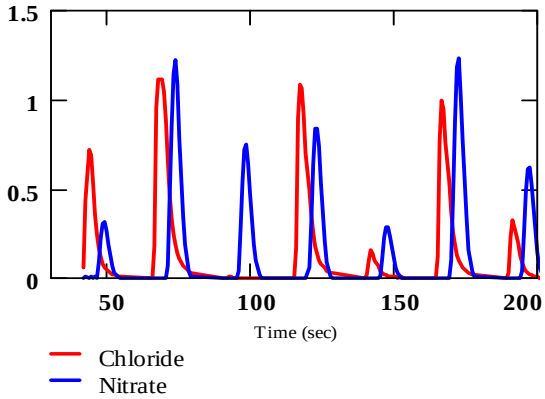
FIAlab®
Leaders in Flow Injection Technology

The FIAlab-2500 offers complete automation of flow injection analysis; including FIAlab for Windows control software, UV/VIS spectrometer, flow cell, light source, fiber optics, tubing and connectors. The low cost of the FIAlab-2500 makes this system appealing to academic teaching and research laboratories. The robust design and adaptability make it the best choice for commercial laboratories performing routine analyses. **Up to four systems can be interconnected to run four different assays simultaneously.**

The FIAlab-2500 is now available with the novel **FIA Lab-On-Valve®** which integrates all connections, sample loop, and flow cell into one simple manifold (as pictured, right). **No need to purchase separate expensive manifolds for each type of assay.**



FIAlab-2500 with the FIA Lab-On-Valve® Manifold



Example Applications

Nitrate/Nitrite Assay: Using a cadmium column, this method performs FIA assays for soil testing, drinking, ground, surface, low level seawater, domestic and industrial wastewaters.

Phosphate Assay: A low phosphate measurement method based on USEPA protocols. This method is suited for agricultural and environmental testing.

Ammonia Assay: An FIA assay for low ammonia concentrations using the well established salicylate method. Ideal for agricultural and environmental testing.

Chloride Assay: A continuous flow FIA assay for chloride measurements in the low PPM or high PPB concentration ranges.

Seawater Nutrient Level Assay: Ultra low level Nitrate and Ammonia measurements in seawater and other media using a long path flow cell.

A typical Flow Injection Analysis system includes:

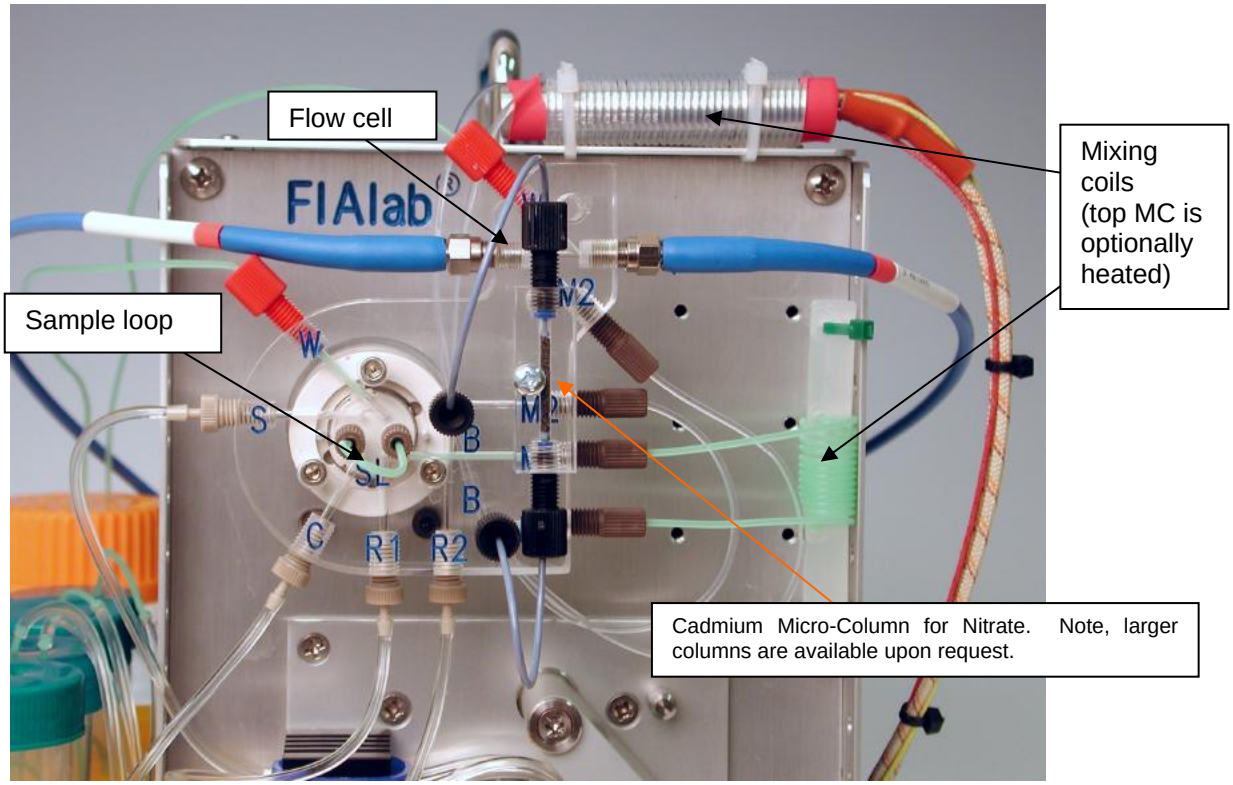
FIAlab-2500
Integrated FIA LOV Manifold (as shown)
USB4000 UV/VIS Spectrometer
Two P200-2-UV/VIS Fiber Optics
LS-1 Visible Tungsten Lamp

Optional Components include:

Autosampler
Long Path Flow cell for ultra low level assays
Integrated heater (for ammonia assays)
D2000 Deuterium UV Lamp
PMT-FL Fluorometer
SCSP-2500 Autodilutor
RQV High Throughput Valve

The FIA Lab-On-Valve® Manifold includes the complete classical FIA layout for one- or two- reagent chemistry. However, the configuration is far more compact and user friendly than the standard configuration.

The FIA-LOV Manifold, available in Plexiglas® (shown), Ultem®, and PEEK, is compatible with flow cells of 1.5 mm to 10 cm optical path lengths (user changeable).



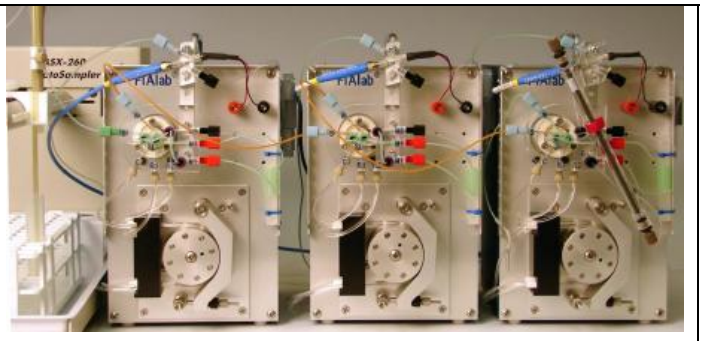
FIA Lab-On-Valve Manifold (equivalent to the classical FIA configuration)

Fittings (as labeled)	Connection	Comments
M1 to M1	Mixing Coil 1	Recommend 1.5 feet tubing (.03" ID) coiled
M2 to M2	Mixing Coil 2 (or heating coil)	Recommend 3 feet tubing (.03" ID) coiled. This coil may be replaced by the FIAlab integrated heating coil. Turn heater off when heating isn't required.
B to B	Bridge or Cadmium Column	For Nitrate use Column here, else connect B to B with short green (.03" ID) tubing.
R1	Reagent 1	First reagent to mix with sample
R2	Reagent 2	Second reagent to mix with sample
C	Carrier	Usually pure water or match of sample matrix
S	Sample	Connect to autosampler (through peristaltic pump). If an autosampler is not used, put in sample vial as needed
W	Waste	Two waste connections

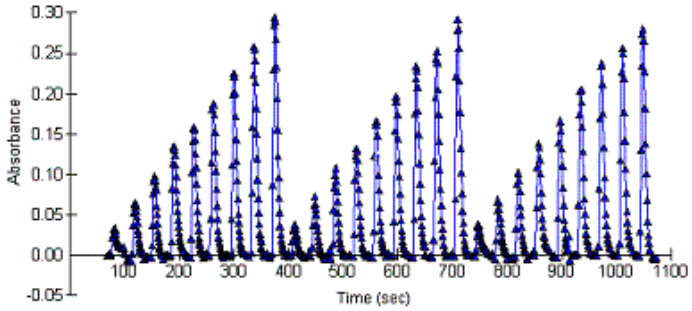
The FIAlab-2500 System

FIAlab-2500 Optional Configurations

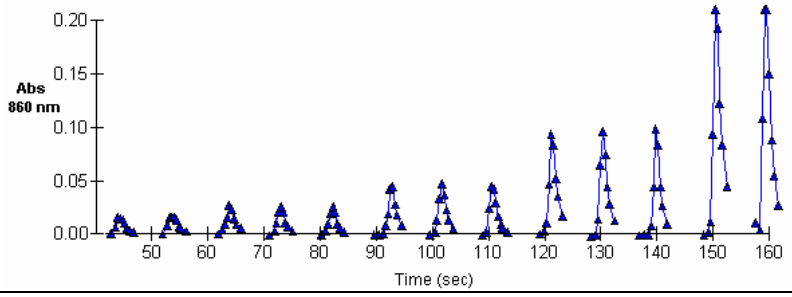
Multichannel System – Up to four Systems can be interconnected for simultaneous assays. Photo at right shows one channel for Phosphate, one for Ammonia, and one for Nitrate. Systems can be separated and run independently as needed.



SCSP-2500 Auto Dilutor - No need to prepare multiple standards, let the SCSP-2500 dilutor create the standards automatically. The SCSP-2500 aspirates standards and samples from autosampler, dilutes them to the desired level, and delivers the resulting diluted sample through the FIAlab-2500 manifold. Accurate dilutions down to twenty fold. Easily create calibration curves from a single standard, or automatically dilute samples to concentration levels within the dynamic range of the detector. With the SCSP-2500, FIAlab software will automatically detect when a high sample is "out of range", and automatically dilute and re-measure that sample.

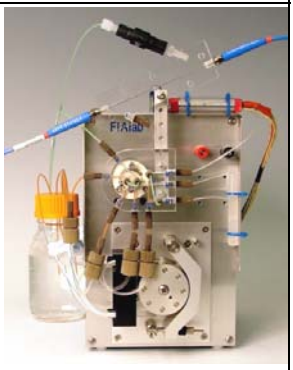


Rapid Phosphate Assays - With the new High Throughput **Rapid Queuing Valve (RQV)** option, the FIAlab-2500 can now measure Phosphate and Ammonia at around **nine seconds per sample**. This compares to 30-60 seconds per sample for other commercially available FIA systems. Note, the RQV is not compatible with all types of Phosphate assays.



Olsen Phosphate System -Olsen (Sodium Bicarbonate) extracted samples contain high quantities of sodium bicarbonate, which normally causes bubbles (foam) to be created when the sample comes in contact with the acidic reagents. This will cause significant problems with accurate absorbance measurements in the flow cell.

Solution: By utilizing a **20 PSI pressure restrictor** on the flow cell output line, the resulting overpressure prevents gas from coming out of solution. In this fashion, Olsen phosphate can be run like other phosphate extractions.



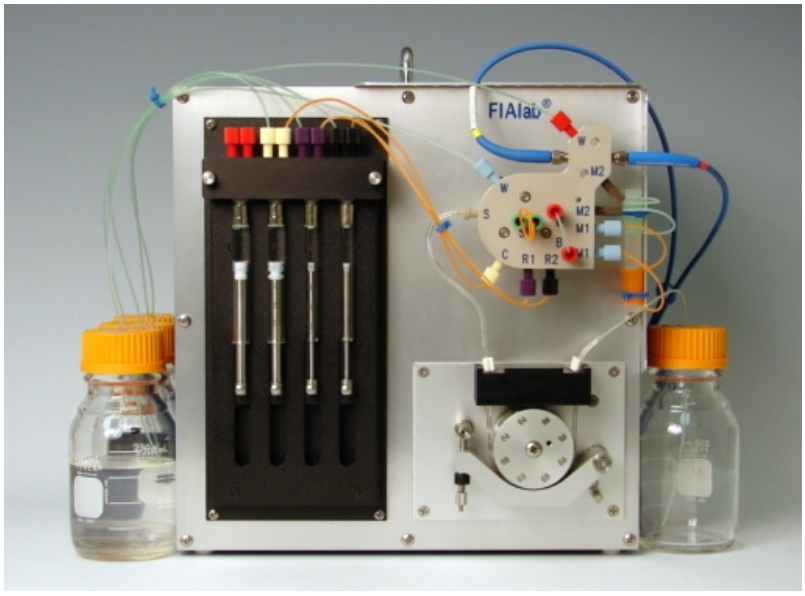
The FIAlab-2700 System

The FIAlab-2700 Multi-Syringe Flow Injection Analysis System is the first commercially available FIA system which offers a four channel syringe pump for aspirating/dispensing carrier, sample, and reagents. The FIAlab-2700 also includes the same four channel peristaltic pump as the FIAlab-2500, and can thus be run in the classical peristaltic pump based FIA mode. Or utilize both the syringe and the peristaltic pumps for more complex "hybrid" assays. This system is ideal for the research laboratory exploring new FIA approaches.

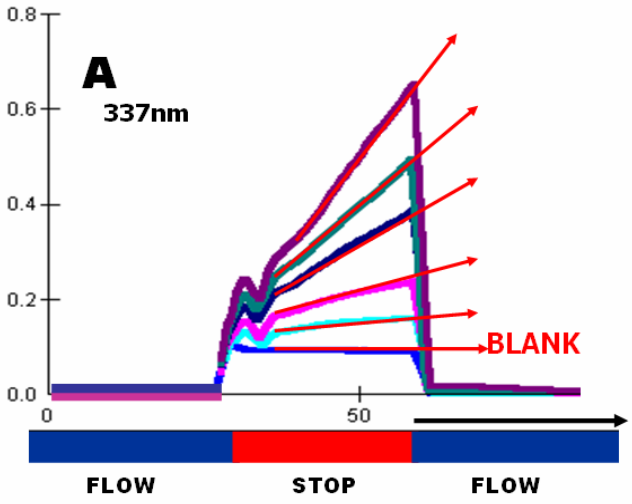
The advantages of using syringe pumps for FI applications were recognized by Japanese researchers long ago (Yoza 1977), and the use of Multi-syringe Flow Injection Systems (MSFIA) has been proposed in numerous publications. (Cerdá 1999).

Several advantages are realized by using the four syringe pump system:

- High resistance to corrosive chemicals. Wetting surfaces are limited to Teflon, PEEK, KelF and Glass.
- No need to replace expensive peristaltic pump tubing.
- Precise speed and volume control allows the user to run methods in either continuous or stop flow mode.
- Pulsation is all but eliminated, allowing smoother flow. This can be critical for low level assays.
- Relative flowrate between the channels is achieved by using different syringe sizes.
- Less reagent is used than with classical peristaltic pump based FIA.



FIAlab-2700 with the Integrated FIA Lab-On-Valve® Manifold (shown made from PEEK)



Typical Stop Flow Assay

One significant advantage of the FIAlab-2700 is the ability to run methods in stop flow mode. Referencing out the initial absorbance reading at the start of each assay removes any matrix affects such as salinity levels.

- ***Example Stop Flow Applications***

Phosphate Assay: A low phosphate measurement method based on USEPA protocols. This method is suited for agricultural and environmental testing.

Ammonia Assay: An FIA assay for low ammonia concentrations using the well established salicylate method. Ideal for agricultural and environmental testing.

Sequential Injection Analysis

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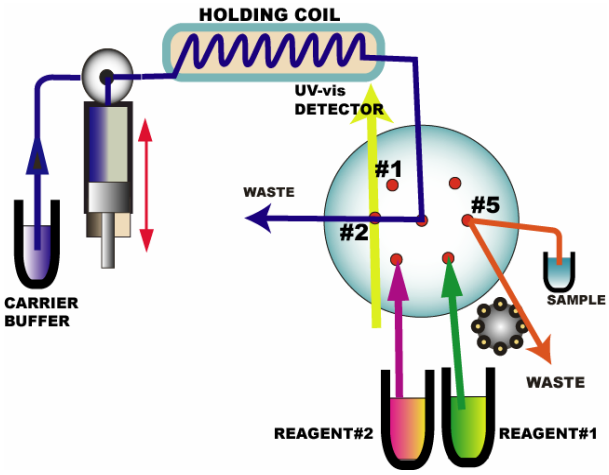
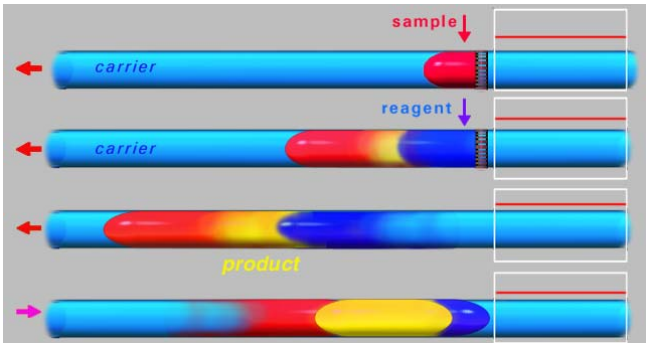
Leaders in Flow Injection Technology



Overview - Sequential Injection Analysis (SIA) is the second generation approach to FIA compatible assays. SIA usually consists of a single-channel high precision bi-directional pump, a holding coil, a multiposition valve, and a flow through detector. The system is initially filled with a carrier stream into which a zone of sample and a zone of reagent(s) are sequentially aspirated into a holding coil, forming a linear stack. These zones become overlapped due the parabolic profile induced by differences between flow velocities of adjacent streamlines. Flow reversals and flow acceleration further promote mixing. The multiposition valve is then switched to the detector position, and the flow direction is reversed, propelling the sample/reagent zones through the flow cell.



Sequential Injection Programmed Flow

The advantage of SIA over the more traditional flow injection analysis (FIA) is that SIA typically consumes less than one-tenth the reagent and produces far less waste – an important feature when dealing with expensive chemicals, hazardous reagents, or online/remote site applications.

Online process monitoring using SIA - Low reagent/sample consumption, waste production, and nearly hands-off robustness make SIA the perfect choice for many types of online processes. These applications often involve remote access, auto dosing, and alarm triggering. Examples include; 1) Monitoring of bioreactors for proteins, amino acids, ammonia, and glucose, 2) Industrial dye bath monitoring with automated dilutions and dosing, 3) Remote site environmental monitoring, 4) Aquaculture real-time monitoring. The new **FIAlab-4000 system** built to spec with an embedded PC in a NEMA 4X enclosure brings SIA to the industrial setting. Please see page 18 for FIAlab-4000 design options.

FIAlab-4000 Online Processor - Built to Spec for Custom Online Applications and OEM Customers.

Assay of Biomolecules

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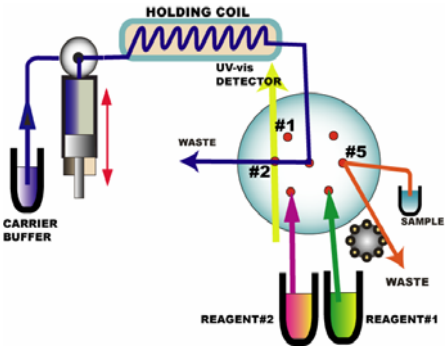
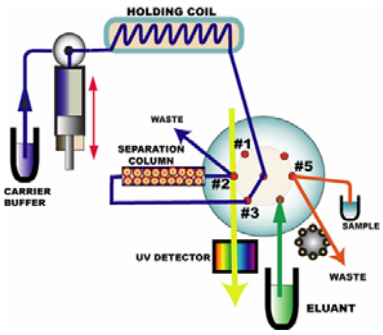
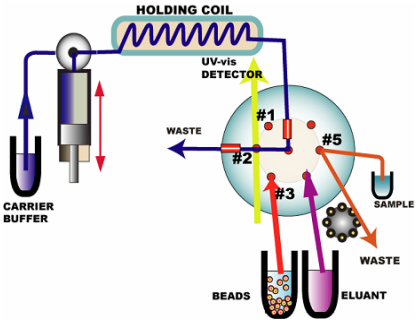
Sequential Injection Assays of Biomolecules, including Enzymatic Assays, Bioligand Interaction Assays and Chromatography of Biomolecules are usually carried out on different instruments, which necessitates mastering various types of hardware and software. This fragmented approach supports a view of an apparent incompatibility of these techniques, thus obscuring similarities of their underlying biochemical reactions and kinetics. It is the versatility of programmable flow combined with a UV/VIS (or fluorescence) detector that allows the gap between these techniques to be bridged by a single **MicroSIA, FIAlab-3200** or **FIAlab-3500 system** with FIAlab's unique **Lab-On-Valve** manifold (see next page).



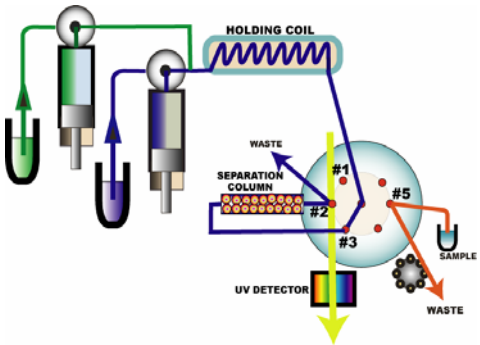
Enzymatic Assays are based on *reaction rate* measurements, carried out in a stopped-flow mode where the reaction mixture is held for monitoring within the flow through cell. Substrate assays include glucose, lactate, urea, glycerol and ethanol.



Sequential Injection microAffinity Chromatography is a redesigned variant of conventional affinity chromatography that uses the Lab-On-Valve platform to carry separation of biomolecules on automatically renewable microcolumns that are automatically packed within the LOV using Sepharose beads. Availability of many functional groups on Sepahrose make this revolutionary technique suited not only for immunoassays (Protein A or G) but for a wide variety of target biomolecules including enzymes and nucleotides.



Bioligand Interaction Assays (BIA) are based on the monitoring of UV/VIS spectra of translucent beads (Sephadex, Sepharose) during their interaction with biomolecules in the assayed solution. The protocol starts by injecting microliter volumes of beads into the flow cell, where the beads are captured and monitored while the analyte solution is passed through. Native biomolecules typically absorb at 260 and 280 nm while labeled molecules absorb visible light (and some also emit fluorescence). Thus both nonlabeled and labeled biomolecules can be measured simultaneously.



Sequential Injection Reverse Phase Chromatography is based on LC type separation of biomolecules from their interaction with stationary hydrophopbic phase. Their resolution results from the gradual change of composition of the mobile phase from polar to less polar. This forms an elution gradient in a novel way, by sequential injection of eluants, and flow reversals. The initial design of Sequential Injection Chromatography (SIC) by **Satinsky, Solich et al.,** used programmable flow and a monolithic column, to separate and assay compounds in pharmaceutical preparations**.**

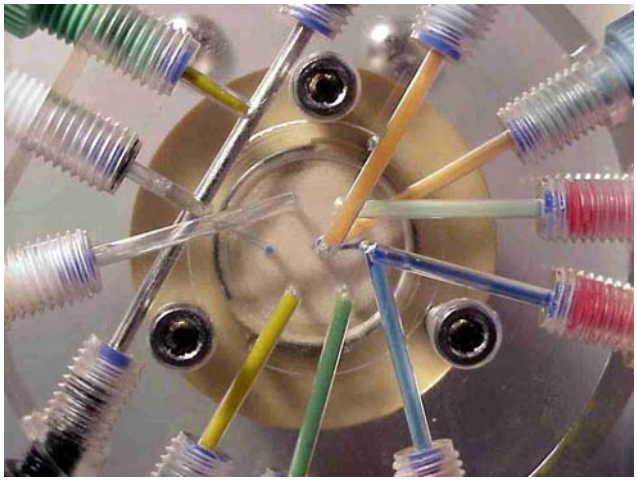
SIA in Lab-On-Valve® (LOV)

FIAlab®
Leaders in Flow Injection Technology

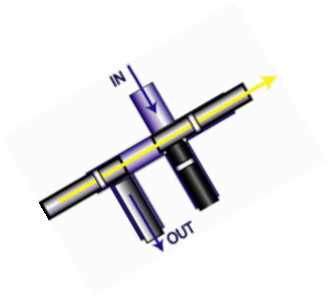
SIA in Lab-On-Valve® (LOV) format is a groundbreaking technology that has won enthusiastic acceptance in the research community for its versatility, and in the routine laboratory for its reliability. The LOV integrates manifold components, including a fiber optic coupled flow through cell, into a single unit mounted on a multiposition valve. The SIA LOV is used as a platform for **microSequential Injection** as well as for **Bead Injection** and **Sequential Injection Affinity Chromatography.**

Designed to accommodate spectrophotometric and/or fluorescence measurements, the Lab-On-Valve manifold is compatible with the **MicroSIA, FIAlab-3200** and **FIAlab-3500** systems furnished with a fiber optic spectrophotometer or photomultiplier based detector.

Downscaling of the conventional SI system is achieved by integrating the sampling conduit and flow cell into a micromachined compact structure mounted atop a conventional multiposition valve. The lab-on-valve (LOV) system operates in the microliter domain and is compatible with UV-VIS and fluorescence spectroscopy. In addition, the LOV can serve as the front end to fluorescence microscopy, electrospray mass spectrometry and capillary electrophoresis. Sample path volume is minimized by reducing the channel length, while channel diameters are kept at 0.8mm ID to prevent clogging from "real life" samples. Since the conventional sampling line has been eliminated and samples are brought into flow through port #5 by an auxiliary pump, while the syringe pump and multiposition valve process a previous sample, the sampling frequency of SIA in LOV format is higher than that of conventional SIA systems.

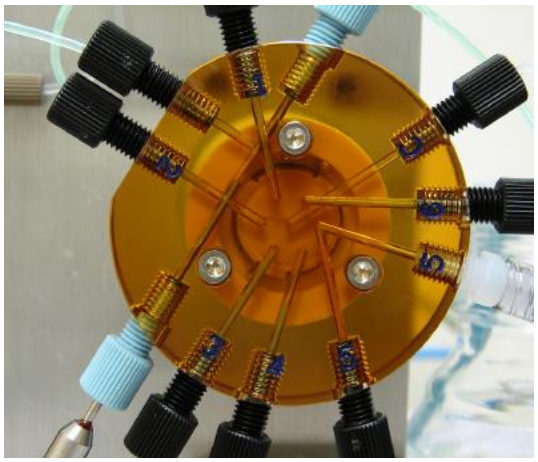


The LOV's built in flow cell can be configured for absorbance assays, fluorescence assays, as well as electrochemistry. Further enhancements can be gained by connecting the LOV to an external detector such as FIAlab's PMT-FL.



LOV Flow cell Configured for Absorbance Measurements

LOV Flow cell Configured for Fluorescence Measurements



SIA-Lab-On-Valve made from polyetherimide (Ultem) for improved chemical resistance.

The **SIA-LOV** is offered in Ultem or PEEK material, and with **six or eight ports.** Custom designs are available upon request.

Partial Compatibility list for Ultem: Acetic Acid, Acetone Alconox Acetone, Alconox , Carbon Tetrachloride, Cellosolve, Chromic Acid, Citric Acid, Clorox, Cyclohexane, Cyclohexylamine, Ethanol, Ethyl Acetate, Ethyl Ether, Formic Acid, Gasoline, Hexane, Hydrochloric Acid, Isopropanol, Kerosene, Lestoll 1, Methanol, Methylethyl Ketone (MEK), Naphtha, Nitric Acid, Phenol, Phosphoric Acid, Potassium Carbonate, Potassium Hydroxide, Propylene Glycol, Skydrol, Sodium Hydroxide, Sulfuric Acid, Tetrachloroethylene, Tin Chloride, Toluene, Trichloroethane (1,1,2-), Triethylphosphate, Xylene, Zinc Chloride

The MicroSIA System

FIAlab®
Leaders in Flow Injection Technology

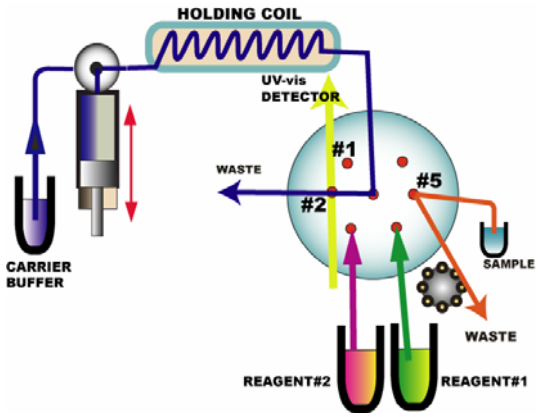
The MicroSIA system is the smallest complete Sequential Injection Analysis system available. **Only 5 inches wide, 6 inches high, 6 inches deep**, **and weighing only 8 pounds**, the unit takes less bench space than a typical laptop computer.

The MicroSIA-LOV Instrument

The portability of the MicroSIA system allows the use of the same instrument in a **laboratory** for assay development and calibration, and its subsequent deployment **in the field** or **process** monitoring. The MicroSIA is ideally suited for mobile or on-board laboratories where its robustness, small footprint and low reagent consumption makes it a valuable tool. The instrument typically uses only 25 microliters of sample per assay, and about the same volume of a reagent. Since all SIA assay protocols are based on programmable flow, the system **consumes reagents only when sample is injected.** Compared to continuous flow techniques which consume reagents continuously, micro SI uses twenty to fifty times less regent.

For **multi-component assays**, up to three microSIA instruments can be coupled in parallel, allowing three target analytes to be assayed simultaneously from the same sample stream or sample changer. An example is automated simultaneous assay of phosphate, nitrate and nitrite.

MicroSIA System Configured for Reagent Based Assays



A typical MicroSIA system includes:

MicroSIA Base Unit
Integrated SIA LOV Manifold (as shown)
USB4000 UV/VIS Spectrometer
Two P400-2-UV/VIS Fiber Optics
LS-1 VIS, DH2000 UV/VIS or LED Lamp.

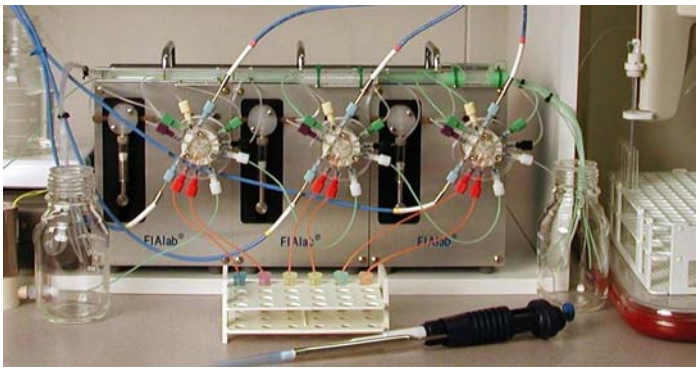
APPLICATIONS:

Reagent based assays *that use UV-VIS spectrometry, fluorescence or chemiluminescence as detection technique. Examples include* ***Ammonia, phosphate, iron, chloride, proteins, DNA.***

Enzymatic assays *carried out in reaction rate format such as* ***Glucose, Lactate, Ethanol, Glycerol***

NOTE: *Glucose and lactate can be assayed simultaneously in one microSIA system, since the enzymatic reagents are compatible and both assays use the same end colorimetry (NAD/NADH)*

Chromatography of Biomolecules for separations carried out on Sepharose and Sephadex support. Target analytes include **Antibodies, Enzymes, Nucleotides, Peptides** (*See section on SI- Chromatography).*



Three Channel MicroSIA System

The FIAlab-3200 System

The FIAlab-3200 Dual Syringe Sequential Injection Analysis system is designed for research and development projects, as well as for online applications. The utilization of **independently controllable** dual syringe pumps allows for formations of varying profiles of programmable **concentration gradients**, useful for many applications in biomolecular research. Advantages of the secondary pump include:

- The syringe pumps can be controlled simultaneously and independently with regards to direction, flowrate, and volume. Programmable gradients can thus be created.

- The system provides a wide range of flow rates. By using both a small syringe and a larger syringe, one can significantly increase the system's flow rate dynamic range.

- The dual syringe combination is ideal for auto diluting high concentration samples prior to analyses.

- The system can be run with seamless uninterrupted flow; while one syringe is refilling the other is emptying.

- The second syringe pump can be utilized to wash out the manifold after each sample assay, signifcantly increasing the system's throughput speed.

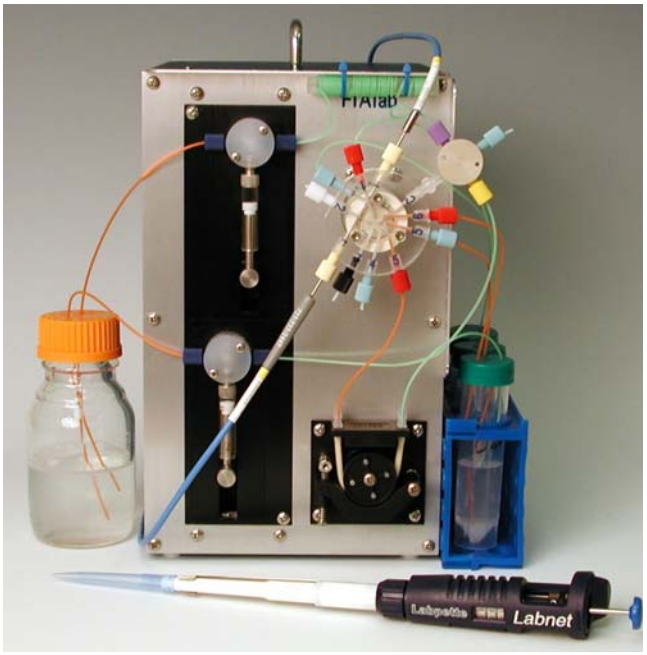
- Separate dual holding coils can be created, allowing incubation of one sample while the next sample is being processed, further increasing throughput speed.

- ***A typical FIAlab-3200 system includes:***

FIAlab-3200 Base Unit
Integrated SIA LOV Manifold (as shown)
USB4000 UV/VIS Spectrometer
Two P400-2-UV/VIS Fiber Optics
LS-1 VIS, DH2000 UV/VIS or LED Lamp.

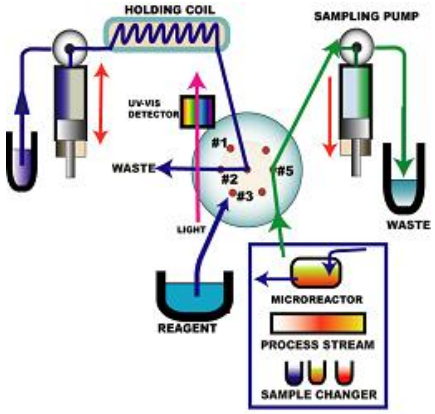
- ***Optional Components include:***

Autosampler (please inquire for configurations)
PMT-FL Fluorometer
Additional Syringe Pumps and Selector Valves



FIAlab-3200 System

Example Application



Continuous monitoring in real time is the target application of the FIAlab-3200 instrument. The second syringe pump (green line) is used to aspirate sample periodically and deliver it into the SI-LOV system for assay at a frequency selected by the operator. Thus real time monitoring of

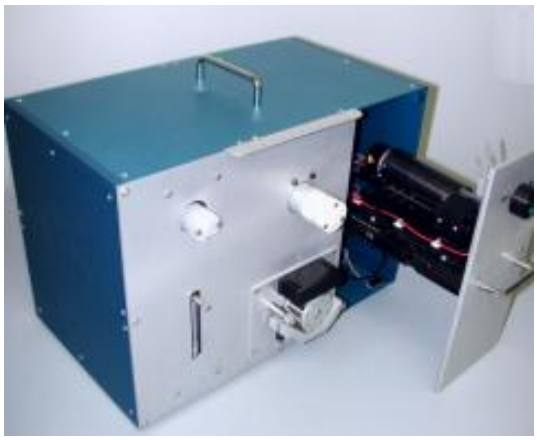
- MICROREACTORS
- PROCESS STREAMS
- SERIES OF SAMPLES

can be carried out automatically over long periods of time.

FIAlab-3500 System

The FIAlab-3500 system is a versatile instrument that performs analyses in **both SIA and FIA format.** With a multiposition valve, a high resolution microsyringe pump and a four channel eight roller peristaltic pump, the FIAlab-3500 is also compatible with many other areas of analytical chemistry, including front end fluidic processing for FT-IR, Flow Cytometry, Mass Spectrometry, and Fluorescence Microscopy.

The FIAlab-3500 system is the ideal system for developing new FIA/SIA methods and testing new sensor technologies.



The FIAlab-3500 is also available with a "built in" sensor drawer to house the optional PMT-FL or other custom based sensor systems

Example Applications:

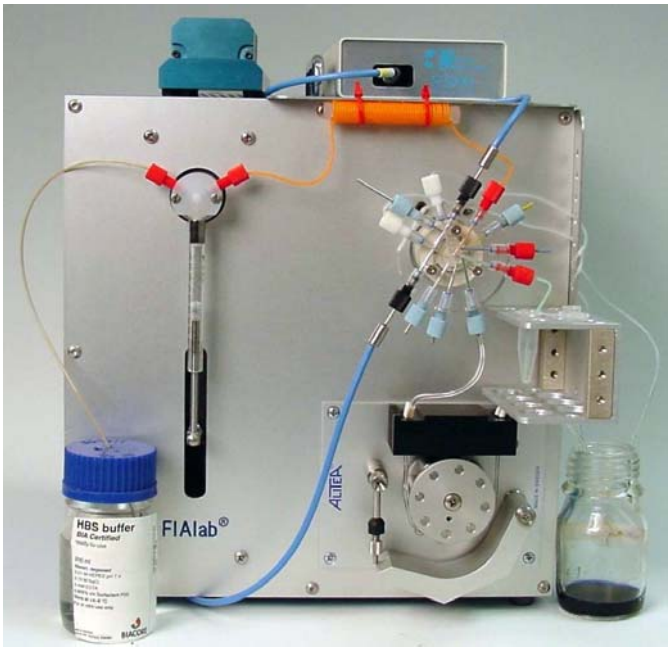
Method Development: Ideal for FIA and SIA method development.

Bead Injection Spectroscopy: Bioligand Interaction (BIA), Epitope Mapping, Elisa, Ligand Ranking

Proteomics and Drug Discovery: Merger of Biomolecular Interaction Analysis with ES-MS

Fermentation Monitoring: Continuous monitoring of ammonia, glycerol, glucose, and other assays

Mass Spectrometry: ICP-MS, Automated electro-spray MS



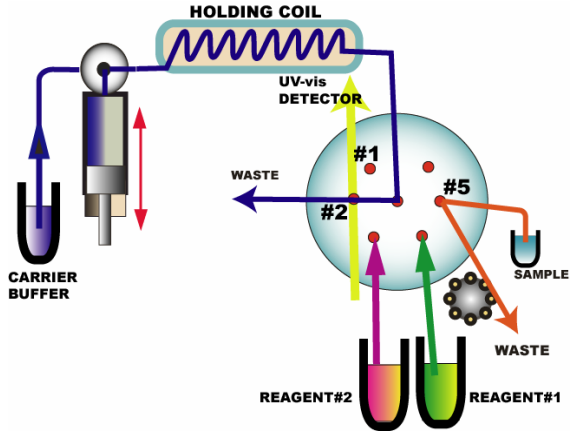
FIAlab-3500 with SIA Lab-On-Valve Manifold

A typical FIAlab-3500 system includes:

FIAlab-3500 Base Unit
Integrated SIA LOV Manifold (as shown)
USB4000 UV/VIS Spectrometer
Two P400-2-UV/VIS Fiber Optics
LS-1 VIS, DH2000 UV/VIS or LED Lamp.

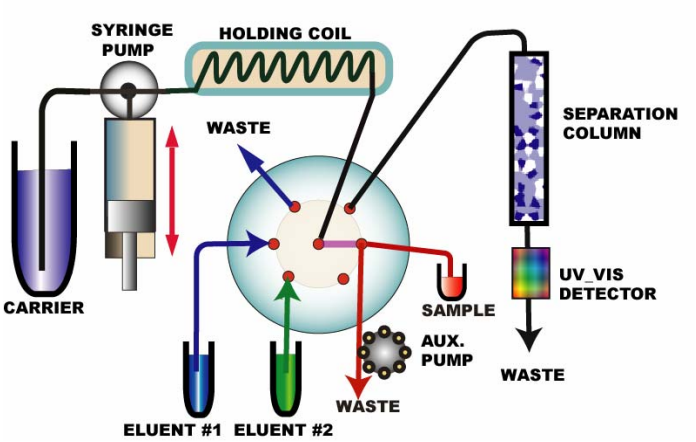
Optional Components include:

Autosampler (please inquire for configurations)
PMT-FL Fluorometer
Additional Syringe Pumps and Selector Valves



Sequential Injection Chromatography

FIAlab®
Leaders in Flow Injection Technology



SI Chromatography uses **programmble flow** instead of continuous flow, and sequential injection of sample and eluting solutions. The apparatus comprises a bidirectional syringe pump, multipostion valve, column and detector. The principal advantages of the SI Chromatography technique are:

- versatility of operation, as sample and eluant volumes can be defined by software scipt
- derivatization *prior* to separation
- abilty to accelerate auxiliary functions such as gradient formation, column conditioning and washout
- visual transparency of the entire flow path

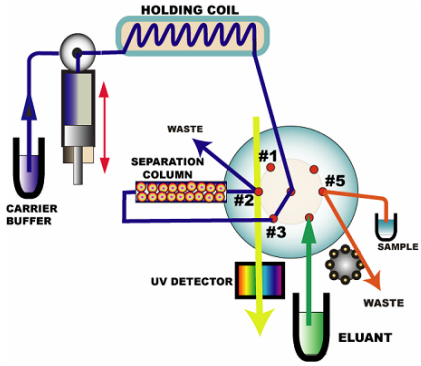
SI Chromatography can be carried out either with an **external** column (shown above) or a **column integrated into the LOV** module. Choice of a suitable instrument is determined by the flow resistance of a column selected for a desired separation. For low pressure separations (below 50 psi) on columns made of **Sepharose or Sephadex**, **the MicroSIA or FIAlab-3200** are suitable. For separation on **monolithic columns**, the **SIChrom-1000 System** has been designed to handle backpressures up to 500 psi. SI Chromatography has been applied to two types of separations:

- Affinity Chromatography and
- Liquid Chromatography

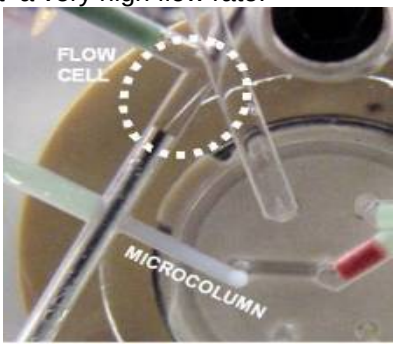
Affinity Chromatography is a powerful tool for selective separation of a target biomolecule from a highly complex biological matrix through the use of an affinity ligand that has been immobilized on a stationary phase, packed in a short column.

Liquid Chromatography on monolithic columns is suited for reverse phase separations and ion exchange separations.

SI Chromatography in Lab-On-Valve format is carried out either with an externally mounted column



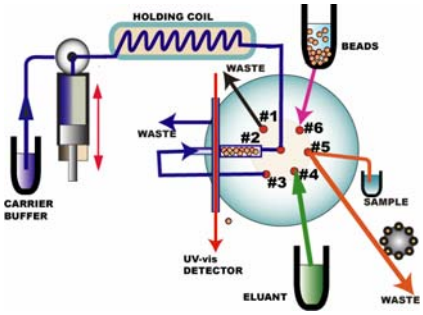
or with a column integrated within the LOV module. The **internal column is automatically renewable** using the stationary phase of Sepharose beads. The microcolumn (volume typically 10 microliters), is held in close proximity to the flow cell. This unique design allows the column to be assembled at a moderate flow rate, perfused by sample and eluted at a low flow rate, then automatically discarded at a very high flow rate.



Lab-On-Valve Closeup Showing Renewable MicroColumn and Fiber Optic Flow Cell

SI MicroAffinity Chromatography

FIAlab®
Leaders in Flow Injection Technology



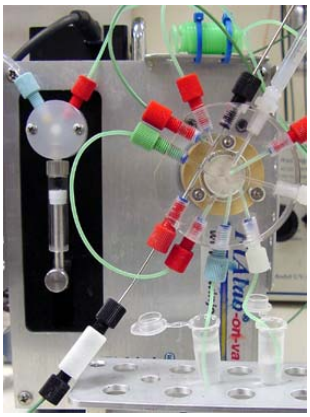
SI MicroAffinity Chromatography is a miniaturized version of conventional affinity chromatography. It uses traditional chromatographic supports: Sepharose and Sephadex, commercially available with a wide variety of bioligands. These ligands can be specific so that the antibody binds to only one epitope, or may be a group type ligand such as nucleotide analogues or Protein A or Protein G that will capture a group of selected compounds.

SI MicroAffinity Chromatography is applicable for either online **process monitoring** (e.g., cell cultures and bioreactors), or **serial assays of discrete samples** supplied manually or from an autosampler**.** Its principal advantages are:

- The internal column situated within the LOV module can be automatically filled with stationary phase and discarded after use.
- The flow cell can be automatically back flushed at the end of each assay to assure that air bubbles are removed.
- Economy of operation: column volume is 10 microliters, eluant volume 10 microliters.
- High sample throughput: one chromatographic assay often takes less than one minute.
- Versatility of selecting stationary phase, since Sephadex columns can be automatically replaced or exchanged.

Typical steps of an assay/separation protocol are:

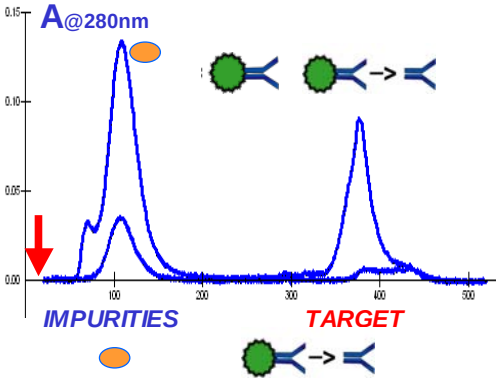
1. Sepahrose beads are aspirated from a container and packed into the microcolumn.
2. Selected sample volume is aspirated into the holding coil and by flow reversal passed through the column. At this stage **IMPURITIES** are washed out from the column.
3. Selected volume of eluant is aspirated into holding coil and perfused through the column by flow reversal. **TARGET** molecules are eluted and detected.
4. Column is discarded by fast forward flow.



SI MicroAffinity Chromatography system includes:

MicroSIA/LOV (shown above)
USB4000 UV/VIS Spectrometer
Two P400-2-UV/VIS Fiber Optics
DH2000 UV/VIS Lamp, or
FIAlab UV LED light source.

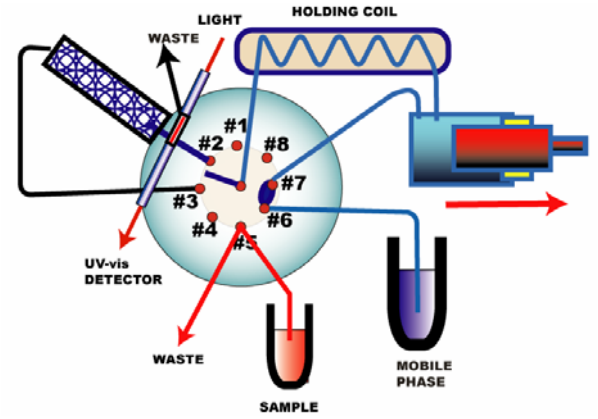
Example Assay: Production of Monoclonal Antibodies and quality control of IgG's are carried out in real time on Protein A or Protein G Sepharose 6B microcolumns. Separation and assay are completed typically within 120 to 240 seconds, with detection limit of 6ng mouse IgG, monitored at 270nm. Target analytes are eluted from a microcolumn by pulses of eluant and monitored using UV-VIS spectrometry for quantification of native IgG or labeled biomolecules. Both absorbance and fluorescence measurements can be performed. The samples to be assayed can be supplied either from an autosampler or aspirated directly from a bioreactor/cellular culture. Utilized sample volume is typically in the range of 5 to 200 microliters.



Separation of mouse IgG (TARGET) from bovine serum albumin (IMPURITIES) on renewable a microcolumn of Protein A Sepharose 6B using a MicroSIA/LOV system.

"REAL TIME MONITORING of IgG"

SI Liquid Chromatography



SI Liquid Chromatography in LOV format uses an external column that is either mounted on the LOV module adjacent to the flow cell (as shown here) or on a line between the LOV module and external detector, as described on the next page, for assay of amino acids using a fluorescence detector. The advantages of SI Liquid Chromatography: simplicity of operation, transparency of the flow system, portability and versatility result from the combination of two features that make this approach unique:

- flow programming
- use of the sol gel monolith

Flow programming – Sequential Injection offers versatility in the choice of injected sample and eluant volumes, and of sequence of their loading on the column. By changing flow rates, main operations like sample loading and analyte separation can be carried out at optimized speed, while auxiliary operations, like column washout and column conditioning can be accelerated.

In a research environment, it's common to run multiple samples under varied conditions. Therefore, the total working time of the column is not just the run time, but also the period required to re-equilibrate the column between solvent gradient runs. Flow rate acceleration reduces run time and column equilibration time, and yields increased sample throughput.

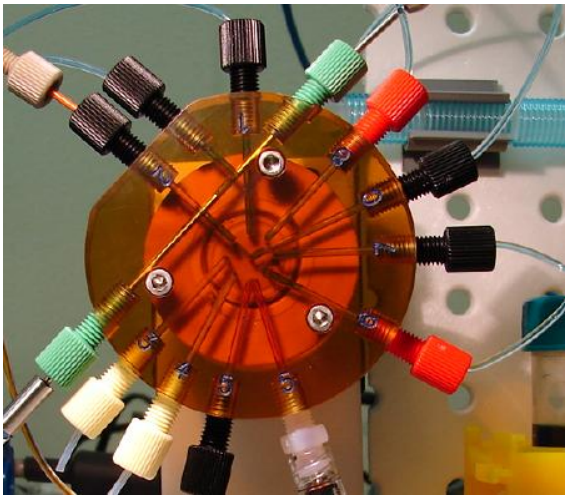
Monolith columns operate well at flow rates and pressures within the range of SI instruments. Monolithic columns that use reverse phase are finding applications such as separation of pharmaceuticals, proteins, oligonucleotides, organic acids, and enantiomeric compounds. Monoliths with ion exchanger sites have found application in ion chromatography. Monoliths are manufactured by e.g., **Phenomenex** and **Merck**.

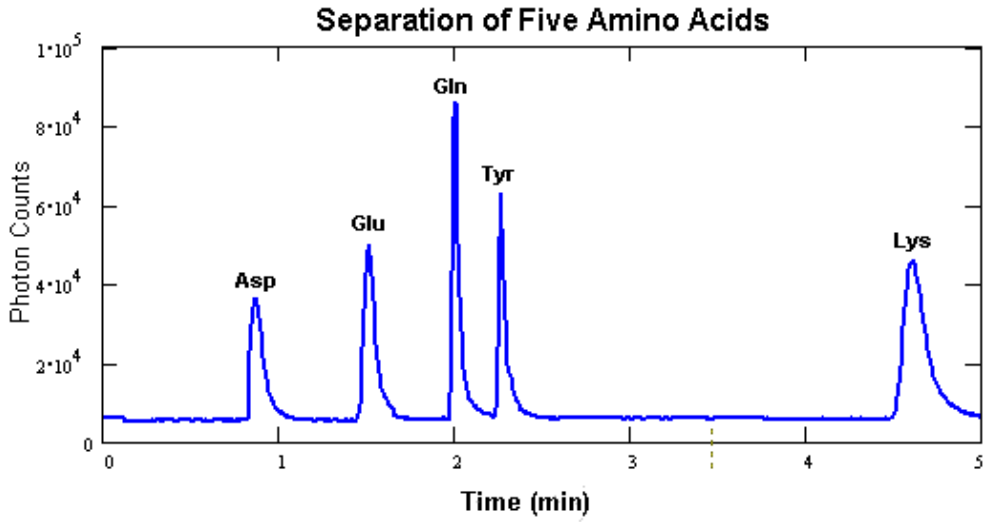
" ACCELERATED CHROMATOGRAPHY"

The SIChrom™ System is the first commercial LC instrument based on the concept of sequential injection. The instrument features a simple and robust construction, consisting of a single pump and single valve. FIAlab software allows all parameters of separation protocol and data collection to be controlled without reconfiguration of instrument hardware. A high precision, stepper motor driven syringe pump allows precise liquid metering at forward and reversed flow with pressures up to 500 psi.
All wettable components are Teflon, stainless steel or polyetherimide (Ultem).

The Lab-on-valve manifold, designed for Sequential Injection Chromatography has :

- Eight ports
- One flow through port
- Integrated fiber optic flow cell
- Ports dedicated to syringe pump refill





DERIVATIZATION for fluorescence detection has been carried by sequentially injecting sample and reagents into the holding coil. Separation was carried out by a flow reversal that brought the reacted mixture into the separation column.

SEPARATION: Conditions 25 x 4.6 mm C18 monolithic column Aq (pH 7.0) : MeOH = 87:13 > Aq (pH 7.0) : MeOH = 40:60 (gradient elution) Flow rate 11.0 mL/min

SIChrom Instrument Specifications

Detector:
Option A UV/VIS Spectrometer (for absorbance)
Option B PMT Based Detector (for fluorescence)

Light source: LED or Deuterium/Tungsten Combination
Flow cell: Adjustable Pathlength 1 mm to 1 cm, (other lengths available).
Size: 9x9x10in (22.9x22.9x25.4cm)
Software: FIAlab® for Windows
Manifold: FIAlab 8 Port Lab-On-Valve®
Pump: Piston (high-resolution stepper motor**)**

- **Pressure:** Up to 500 PSI
- **Flowrate:** 0.006 to 9.0 ml/min
- **Volume per stroke:** 4 ml

SIChrom System configured with built in PMT Detector

Online Process Monitoring/Control

FIAlab®
Leaders in Flow Injection Technology

Online process monitoring using SIA - Low reagent/sample consumption, waste production, and nearly hands off robustness make SIA the perfect choice for many types of online processes. These applications often involve remote access, auto dosing, and alarm triggering. Examples include; 1) Monitoring of bioreactors for proteins, amino acids, ammonia, and glucose, 2) Industrial dye bath monitoring with automated dilutions and dosing, 3) Remote site environmental monitoring, 4) Aquaculture and agriculture.

The new **FIAlab-4000 system,** built to customer specifications with an embedded PC in a NEMA 4X enclosure, brings SIA to the industrial setting. The internal computer and customizable software creates a complete command/control center where other external instruments can be interfaced. Electrical/communication interfaces typically include RS-232, RS-485, ADC (0-5 VDC, 4-20 mA), DAC (0-5 VDC, 4-20 mA), TTL and contact closure. The internal fluidic components typically include one or more precision syringe pumps, multiposition valve(s), flowcell(s), and UV/VIS or PMT based detector.

FIAlab-4000 Online Process Monitoring System For Custom Online Applications.

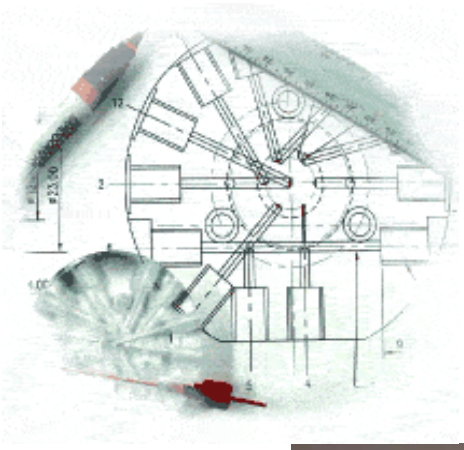
Example Application 1 – Online monitoring of industrial tanks. Single or multiple components are continuously monitored and dosed accordingly. The utilized temperature controlled flow cell has an optical path as short as 100 microns, eliminating the need for pre-measurement dilutions of optically dense samples. External components can also interfaced to the FIAlab-4000 including mass-flow, pH, and temperature sensors.

Example Application 2 – Continuous remote site monitoring of environmental streams for nutrient levels.

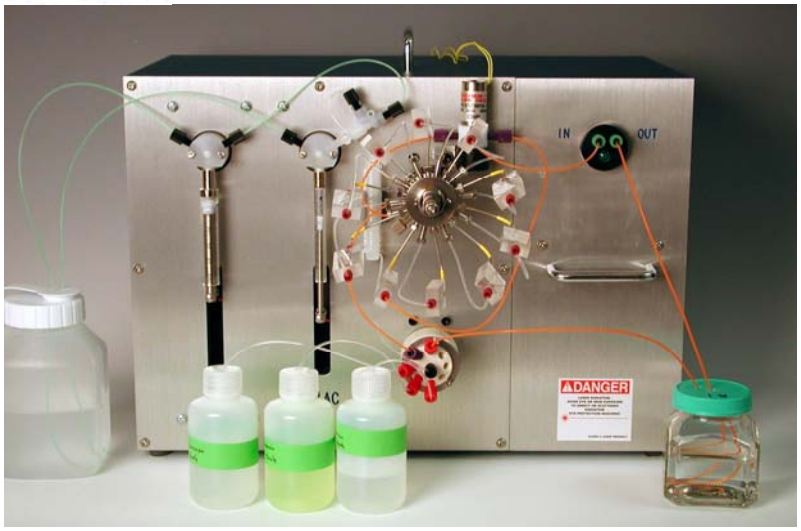
Example Application 3 – The Colifast At-line Monitor (CALM) is a stand-alone industrial autoanalyser used to determine the presence and quantity of fecal coliforms in water by monitoring Beta-galactosidase activity fluorometrically. This instrument is set up for continuous monitoring at remote sites.

OEM and Laboratory Services

FIAlab®
Leaders in Flow Injection Technology

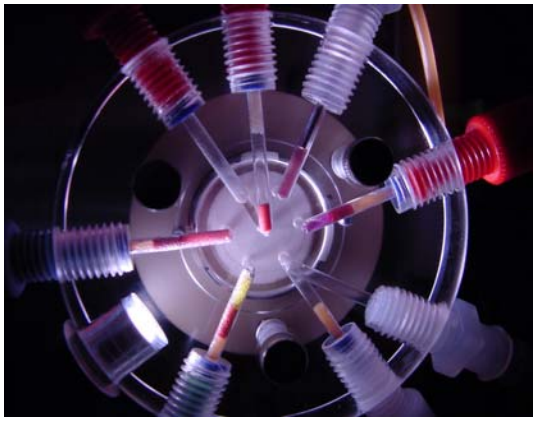


Design and Manufacturing of Custom Microfluidic OEM Services –– FIAlab Instruments designs and manufactures custom and OEM systems and components, including microfluidic manifolds, electronic controls, instrument enclosures, and software development. These systems often include built in colorimetric and/or fluorometric sensors, heaters, various custom fluidic manifolds, syringe and/or peristaltic pumping stations, and a host of valving components.



Example Custom System - Recycling Immunoaffinity Chromatography Unit with Built in Laser Induced Fluorometer

FIAlab R&D Services by the Experts



Does your laboratory have a manual assay which can be converted to FIA format? **We can develop** the method at our facilities, fully tested to your satisfaction. We will then deliver the method to your laboratory, configure the hardware for optimal throughput and sensitivity per your requirements, and perform final QC testing.

FIAlab Instruments provides affordable custom method development services. Chemists are available for consultations and both on and off site method development.

We have many years of experience in Flow Injection, Sequential Injection, and Bead Injection technology, especially as they apply to Water Quality Testing, Immunoassays, Enzymatic Assays, Automation, Proteomics, Fermentation Monitoring, and Pharmaceuticals.

Detectors and Flow Cells

FIAlab®
Leaders in Flow Injection Technology

The PMT-FL Flow Through Fluorometer is an ultra sensitive photomultiplier-based flow through detection system ideally suited for very low fluorescence, chemiluminescence or bioluminescence measurements. The heavy-duty chemical resistant housing is built to withstand industrial environments. The PMT-FL also accepts a standard 1-cm path length cuvette for manual measurements.

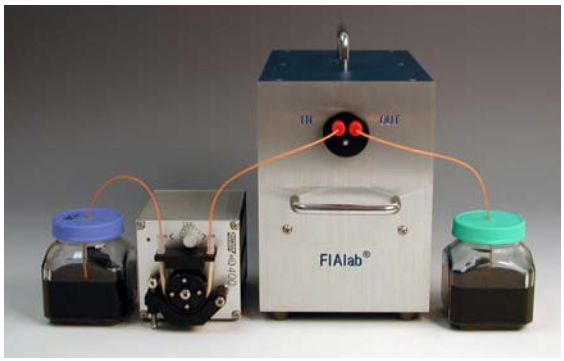
- ***Limits of Detection* - 10 parts per trillion -** measured with Fluorescein @ 250 msec integration and internal tungsten lamp

- ***Configuration Options:***

Internal excitation sources:
Quartz tungsten lamp
LEDs (available from 270 nm to 700 nm)

Fiber optic excitation sources :
All the above + Deuterium lamp

Additional options:
Flow Through Cuvette Heating system
Simultaneous absorbance and fluorescence measurements

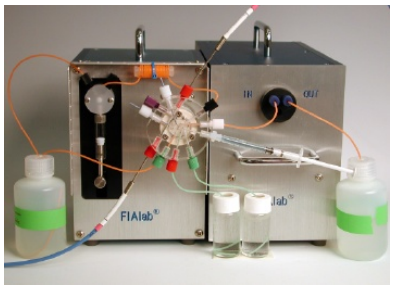


PMT-FL shown with an S2-Mini Peristaltic Pump

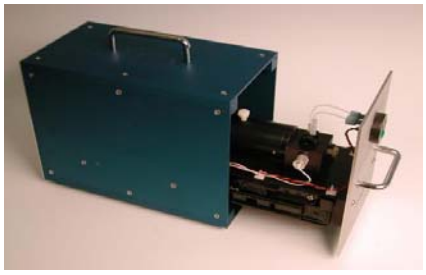
- ***Specifications:***

Photon counting PMT detector
310nm - 750nm spectral range
Pulse pair resolution: 10ns
Responsivity @ 400nm: 5 X 10^{17} cps/Watt
PMT dynamic range: 2.0E06

The PMT-FL is built to order with either an internal excitation lamp or an SMA-terminated fiber optic cable for use with an external lamp. Emission and excitation filters are mounted in user accessible slots inside the enclosure, allowing for quick removal for exchange.



The PMT-FL with the MicroSIA system


PMT-FL with sensor drawer slid open

The USB4000 Spectrometer is a miniature fiber optic UV/VIS/NIR detection system for low light level applications. The USB4000 has a compact optical bench coupled to a 3648-element linear CCD-array detector, making it especially useful for both fluorescence and absorbance applications. The USB4000 accepts light energy transmitted through single-strand optical fiber and disperses it via a fixed grating across the CCD array.

USB4000-UV-VIS Spectrometer is pre-set to 200-850 nm
USB4000-VIS-NIR Spectrometer is pre-set to 350-1000 nm

Detectors and Flow Cells

FIAlab offers flow cells for absorbance and fluorescence based assays.

The SMA-Z Absorbance Standard and Micro-Volume Flow cells have been designed for absorbance where the monitored wavelengths are **longer than 210 nm**. The flow cells are available in **1.5, 5, 10, 20, 50 and 100-mm optical path lengths, with additional sizes available as special orders**.

These flow cells utilize **UV-grade fused silica windows**, a hard, non-corrosive, chemically resistant material that also has a high UV through IR transmittance (210 nm through 2000 nm). The windows are held in place by chemically resistant Teflon seals, pressure mounted with steel SMA connectors (steel connectors do not contact the fluids). The windows are removable for cleaning or replacement with another material of different optical bandpass properties. **The flow cell can be used with backpressures up to 400 PSI.**

Available materials include chemically inert **Teflon®, PEEK®, Ultem®, steel and Plexiglas®.**

SMA-Z Product Table

Product ID	Optical Path Length (mm)	Standard Internal Volume (u l)	Micro-Volume Internal Volume (u l)
SMA-Z-1.5	1.5	NA	1
SMA-Z-5	5	13	3
SMA-Z-10	10	26	6
SMA-Z-20	20	52	12
SMA-Z-50	50	130	30
SMA-Z-100	100	260	60

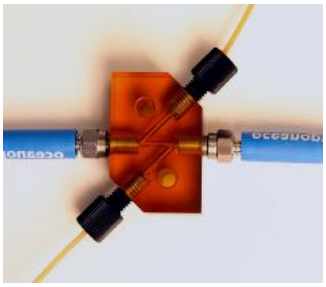
Please specify optical path length, type (SMA-Z Standard or SMA-Z Micro-volume), and material.

The SMA-USP Ultra Short Path Flow Cell is a variation to the SMA-Z flowcell which uses a thin Teflon separator to define the optical path. With this design optical path lengths are available from 0.1 to 1.0 mm. The user can take these flow cells apart for cleaning and replacement of the optical path separator. The optical windows are made from Sapphire. Wetting surfaces include Teflon, Sapphire, and Stainless Steel.

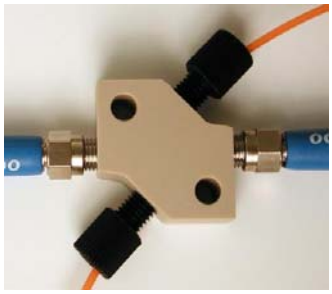
The SMA-F Fluorescence Flow Cell is ideal for moderate to high level fluorescence based assays. A fiber sends excitation energy through a fused silica window into a 2-mm inner-diameter sample compartment. Emitted energy is collected by a second fiber, oriented at 90° that connects to a spectrometer configured for fluorescence.

For the above flow cells, each order includes one flow cell, two optical windows, two steel SMA connectors, two chemically resistant Upchurch tubing connectors, ten feet of 0.0625 inch OD, 0.03 inch ID Teflon tubing, and six chemically resistant seals. Two SMA terminated fiber optic cables are required (recommended P400-2 UV/VIS or P200-2– UV/VIS). Order fibers separately.

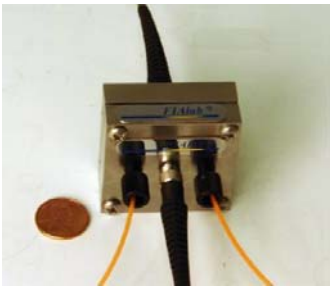
The **USB4000 spectrometer** is recommended for use with the above SMA flow cells.



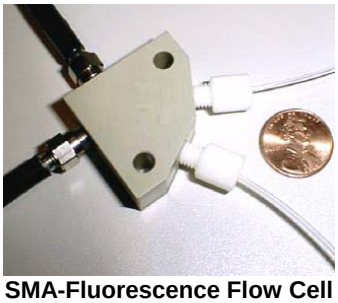
10 mm Optical Path SMA-Z Cell made in Ultem



10 mm Optical Path SMA-Z-µVOL Cell made in PEEK



SMA-USP Ultra-Short Path Flow cell available with optical paths from 0.1 to 1.0 mm.



SMA-Fluorescence Flow Cell made in PEEK

Accessories

FIAlab®
Leaders in Flow Injection Technology

LS1 Tungsten Lamp

FIAlab offers a wide selection of light sources for absorbance and fluorescence based assays.

- **LS-1 Tungsten Lamp** is a broadband light source useful from around 400 to 2000 nm. An ideal general purpose source, especially for environmental/agricultural assays such as ammonia, phosphate, and nitrite/nitrate.

- **DH-2000** Deuterium Tungsten Halogen Light Source is a powerful combination UV/VIS (215-2000 nm).

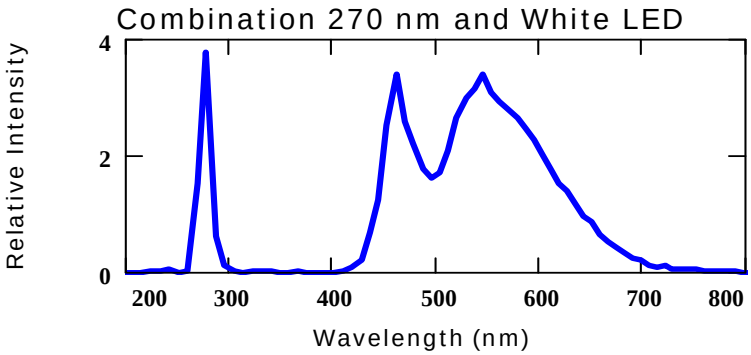
- **LED-Lamp – LED based lamps are available between 247 nm and 945 nm.** Multiple LEDs can be combined to achieve a multifunctional light source. Common combinations include:

270 nm LED with white LED - for Affinity Chromatography assays
340 nm LED with white LED – for OPA based Amino Acid assays
880 nm, 945 nm, and white LED – for Environmental/Agricultural assays

LED-lamps are often superior to other light sources for their long life and stable output. They are ideal for online applications.

DH-2000 Lamp

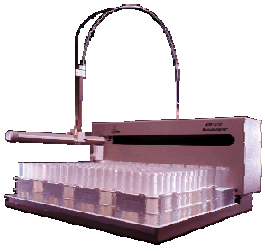
LED-Lamp



VS Peristaltic Pump

The VS-series laboratory pump is designed for a highly precise and nearly pulseless flow. The pump uses ten rollers and other special design features to provide an extremely smooth flow, making this pump ideal for FIA applications.

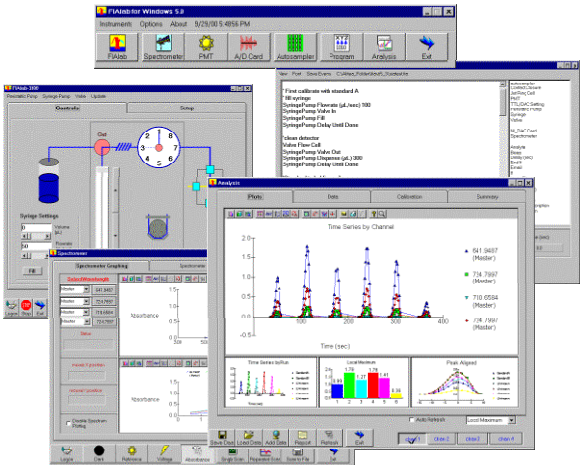
Channels: 2, 3, 4 (please specify)
Rollers: 10
Voltage: 110VAC/230VAC (please specify)
Flowrate 0.005 - 17.5 ml/min/channel (function of RPM and tube diameter)
Pressure 15 psi



ASX520 Autosampler

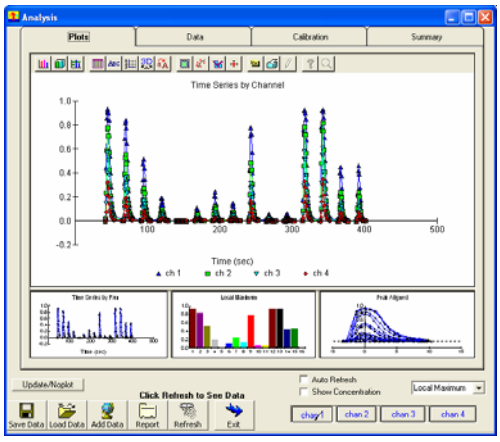
FIAlab offers several models of autosamplers, including the SV-Sampler (10 sample capacity), AIM3200 and ASX260 (180 sample capacity), ASX520 (360 sample capacity), and the M14 which is a mobile 14 rack system (1200 sample capacity).

The photo to the left shows the ASX520, which is an ideal autosampler to use in FIA/SIA applications under harsh laboratory conditions. The ASX520 includes an attached two channel peristaltic pump useful for washing and sampling. **Please inquire for the best autosampler configuration to meet your requirements.**



FIAlab for Windows 5.0 software is specifically designed to control the FIAlab series instruments. Several years were spent writing and testing this software to meet the stringent requirements of flow and sequential injection analysis. Customer response tells us these requirements have been more than met, yet the software remains easy to learn, and versatile enough for use in fields outside standard FIA and SIA, such as Mass Spectrometry, FT-IR, Fluorescence Microscopy, Flow Fluorescence, Electrochemistry, and Chemical Sensor Technology. FIAlab for Windows is also ideal for online monitoring and process control applications.

FIAlab for Windows is also compatible with a host of auxiliary devices, including multiport selector and injector valves, peristaltic pumps, syringe pumps, autosamplers, heaters, and a wide variety of sensors and detectors.



One of the key features of FIAlab for Windows is its user friendly script-based programming language. This language consists of a simple BASIC-like command structure, with all the available commands presented in a floating list box. The user simply points and clicks the desired command, automatically adding it to the procedure. With this ability, the following example FIA script can be created in just a minute or two. Furthermore, an assortment of completed scripts for FIA, SIA, and BIA are available for download from FIAlab's website.

```
' Simple Example FIA Script File

' start pump to prime lines and fill loop for 1st time
Next Sample
Injection Valve Load
Peristaltic Pump Clockwise(%) 40
Delay (sec) 25

Loop Start (#) 200
   ' inject sample, load next sample
    autosampler wash
    Analyte New Sample
    Next Sample
    Injection Valve Inject

   ' perform reference scan and start absorbance scans
    Spectrometer Reference Scan
    Spectrometer Absorbance Scanning
    Delay (sec) 11
    Injection Valve Load
    Spectrometer Stop Scanning
Loop End
```

Example FIAlab report from a short nitrate assay.

Report using calibration curve Set 1
response = 1.237494E-02 + 1.489913E-02

name	derived conc	true conc	peak amplitude	peak time (sec)
std1	37.760	37.300	0.575	44.978
std2	24.021	25.110	0.370	69.922
std3	10.473	9.440	0.168	94.811
std4	3.139	3.000	0.059	119.445
std5	0.143	0.000	0.004	144.578
unk1	1.485	?	0.034	168.562
unk2	3.976	?	0.072	193.284
unk3	2.306	?	0.047	218.188
unk4	20.428	?	0.317	242.868
unk5	0.492	?	0.020	267.863
unk6	0.397	?	0.018	292.545

Jarda Ruzicka

FLOW INJECTION ANALYSIS

3rd EDITION

Tutorial on Flow Based microAnalytical Techniques

Bibliography by E.H.HANSEN

What can be added to a topic described in almost 16,000 papers, 16 monographs and over 150 dissertations? An electronic image is worth a thousand words, and therefore these tutorials appear to comprise more pictures than text and present the field of Flow Injection in a unique way. ***Flow Injection Analysis*** (Jarda Ruzicka, **3rd Edition**, self published 2005, with bibliography by E.H.Hansen) is written in MS PowerPoint format that allows the presented material to be used in teaching and presentations. The Chapters - **Introduction, Flow Injection, Sequential Injection, Bead Injection, Biomolecular Assays, Sequential Injection Chromatography, Inventing FIA, and Key References -** comprise topics ranging from theory to system construction and from histories about its humble beginnings to applications of FIA worldwide, in academia, industry and research and for environmental assays. E.H. Hansen's comprehensive FIA bibliography comprising almost 15,000 searchable references is part of this tutorial. **Please email fialab@flowinjection.com with your name and address, and we will send you a free copy of this CD based tutorial.**