



Tutorial on Zone Fluidics

Zone Fluidics (ZF) is defined as the precisely controlled physical, chemical, and fluid-dynamic manipulation of zones of miscible and immiscible fluids and suspended solids in narrow bore conduits to accomplish sample conditioning and chemical analysis. ZF is a modern evolution of in-line sample processing techniques of flow injection analysis (FIA), sequential injection analysis (SIA) and injection segmented flow analysis (*i*SFA).

Stepping back for a moment to briefly review the progression of these technologies, FIA is a continuous flow technique that exploits controlled dispersion in narrow bore tubing to achieve automation of wet chemical analysis. SIA is an intermittent, bi-directional flow technique that also makes use of controlled dispersion. FIA utilizes a sample injection valve to introduce a sample into a mono-directional flowing stream, add reagents at T-fittings, perform mixing under laminar flow conditions and deliver the reaction product to a detector. SIA utilizes bi-directional flow and a selection valve for the same purpose. The centrally-located selection valve which characterizes SIA provides a convenient means of selecting among standards, samples and multiple reagents. SIA expanded the capabilities of flow-based analysis and remained tied to its roots in FIA by focusing on dispersion as a primary means of mixing. Finally, *i*SFA is a version of flow injection analysis, but samples are injected into a stream segmented by a non-aqueous phase such as a gas or solvent to improve mixing and reduce band-broadening.

Zone Fluidics is the next step to a new generation of flow-based analysis which focuses on the creation and manipulation of zones to automate complex sequences of sample processing operations. As with *i*SFA, ZF involves the addition of air or solvent segmentation to create Taylor flow conditions to improve mixing and decrease sample dilution by reducing band-broadening. However, unlike FIA or *i*SFA, ZF employs a pulseless, bi-directional positive displacement pump to transport fluids accurately and precisely instead of using multiple peristaltic pump tubes which are subject to wear and are not inert.

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Where FIA and SIA focus on dispersion, ZF builds on these techniques and adds phase segmentation, as with *i*SFA, to overcome diffusion-based molecular transfer. When adopting this approach, the capabilities of flow-based analysis are found to significantly expand and ZF becomes a general-purpose fluid handling tool, allowing the precise manipulation of gases, liquids and solids to accomplish very complex preparative and analytical manipulations with relatively simple hardware.

A *zone* is a volume region within a flow conduit containing at least one unique characteristic.

The term *fluid* refers to a substance tending to flow or conform to the outline of its container and includes liquids, gases, aerosols, and suspensions.

From an operational point of view, ZF is an approach to sample handling where zones are shuttled between and within an assembly of one or more unit operations where different sample processing steps are performed. ZF combines elements from other flow-based techniques including FIA, SIA, and *i*SFA to create a unique, flexible and powerful technique to perform complex sample processing unit operations.

A unit operation in ZF is comprised of a set of dynamic or static fluid handling steps carried out in a device intended to contribute to the transformation of the sample into a detectable species. Examples of unit operations include, but are not limited to sample filtering, dilution, digestion, distillation, solvent extraction, membrane separation, headspace sampling, matrix elimination, hybridization and reaction. In current analytical practice, many of these steps are handled manually prior to analysis or in isolated pieces of equipment with minimal integration and there is a high degree of analyst involvement. In ZF, the sample zone is subjected to these unit operations in a sequential (and sometimes parallel) manner being transported within or from one unit operation to the next under precise fluidic control.

ZF offers an alternative approach to automation whereby unit operations are performed in narrow bore conduits and the transportation means, instead of being mechanical as in robotics, is fluidic. ZF also makes use of concepts employed in robotics where samples are carried from one workstation to the next. In a sense, ZF is a sort of fluidics robot which transports a sample from station to station via conduits rather than a mechanical arm.

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We will now review how Zone Fluidics actually works. Figure 1 (below) provides a conceptual diagram of the general configuration of a ZF-based system with its multiple options (unit operations) for sample processing.

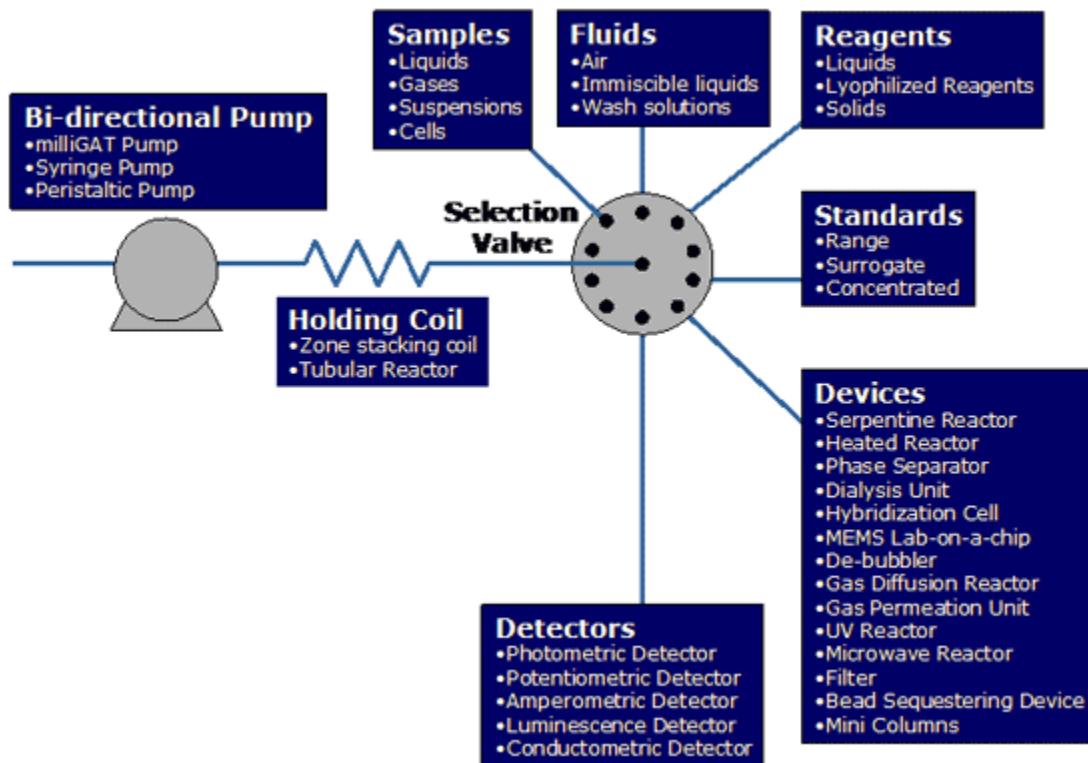


Figure 1

There are numerous possible configurations of ZF, but the configuration in Figure 1 is preferred because it combines great versatility with simple design. At the heart of a ZF manifold is a multi-position selection valve coupled with a precise bi-directional flow pump. A holding coil between the pump and valve performs a similar role as in SIA. The ports of the multi-position valve are coupled to various reservoirs, reactors, unit operators, manifold devices and detectors as indicated. Narrow bore conduits comprise the flow channels and provide fluid contact between manifold devices and components.



In many respects, the prototypical ZF manifold is reminiscent of an SIA manifold and the point must be made that SIA is an important precursor to ZF. However, by contrasting characteristics of SIA with ZF, to be discussed in more detail, the broader scope of ZF will become evident.

In ZF, unit operations are assembled around the selection valve and the sample is transported from one unit operation to the next to achieve the desired analytical methodology. Usually SIA procedures are comprised of one or at most two sample manipulations (typically sample-reagent stack building) prior to detection.

ZF extends beyond SIA in employing, when necessary, immiscible zones (for example, air-liquid or aqueous-organic) to facilitate zone manipulation. In some cases dispersion is used as part of the zone manipulation protocol. In other instances, separating zones with immiscible fluids prevents dispersion and allows the sample zone to be carried from one unit operation to the next for processing without dilution. Such bracketed zones experience mixing patterns typically found in segmented flow systems.

ZF borrows from the power of SFA in using air bubbles to separate one sample from the next and create a miniaturized well-mixed environment. In ZF, sample zones are pumped from one unit operation to the next, the sample integrity often being maintained by interspersed immiscible zones, usually air or nitrogen. To introduce precise air bubbles, one port on the stream selection valve is left open to allow air intake. In segmented flow analysis, bubbles are merged with the sample stream continuously. ZF goes beyond simple air segmented flow systems to judiciously use air zones (or other immiscible fluids) *when required* to achieve desired zone manipulations. In ZF, bubbles are purposefully metered into the zone stack as required in both placement and size according to the requirements of the specific analytical sequence or unit operation to be carried out. Wash solutions and other operational fluids are introduced to the fluidics manifold via the selection valve.

Samples in ZF are not limited to liquids. Gases, aerosols and suspensions containing solids or cells can also be included. Where solid samples are used, particles are limited to a size that ensures no blockages of tubing or valves.



While in most cases, reagents are prepared and then coupled to the ZF manifold, the metering capability of the pump and ZF mixing unit operations allow for reagents and standards to be prepared *in situ*. Reagents can therefore be presented to the ZF manifold in an appropriately designed cartridge as ready-made, reagent concentrates, lyophilized, or crystalline form. This capability is particularly advantageous when reagent solutions are relatively unstable but have a long shelf life in solid or dry form.

Individual standards can be connected to the multi-position valve as discrete reservoirs providing the required range of concentrations. However, as with reagents, a series of standards can also be prepared by in-line dilution of a stock standard to allow calibration over a wide dynamic range.

A wide range of devices can be coupled to the ZF manifold to provide the needed unit operations. Most of these devices have been reported in use in FIA and SIA. Again ZF provides for the extension of these devices by providing sample manipulation capabilities and highlighting the possibility of manifolds with an assembly of devices linked to the multi-position valve.

In some cases, the focus of a unit operation is a static zone such as a separation column or immobilized enzyme reactor where the sample interacts with the packed separation media or immobilized enzyme reagent. In other cases, instead of being confined to non-equilibrium dynamic processes as is the usual case in FIA, batch processing with equilibrium conditions can be employed.

The first step in developing a ZF assay involves identifying the critical fluidic components in the assay. A good place to start is simply to look at the reagent list. These components form the core of the zones that will be manipulated in the ZF manifold. Other zones that could play a role are air zones, immiscible liquids, and solid reagents such as packed reactors or enzyme reactors.

The next step involves looking at the method's procedure and identifying each unit operation. This means asking the question: What do we want to do to these zones?

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Then, one-by-one, the zone manipulation sequence for each unit operation is developed, optimized and tested. By isolating and optimizing each unit operation, it is possible to assemble a library of analytical processors that are then assembled into a method protocol.

Finally all the steps are integrated and compiled into the required assay.

We will now illustrate how Zone Fluidics can be applied to create complex sample processing operations starting with liquid-liquid solvent extraction.

Solvent extraction is a technique that has been used in the laboratory for many decades and is still in wide spread use. Most solvent extractions are carried out by a tedious and time-consuming manual process, although there are some robotic automated systems that are bulky, slow, and expensive. With both manual and robotic systems, shaking the two phases can generate turbulent mixing that often causes emulsions that are generally slow and difficult to break and settle. With ZF, no emulsions are formed.

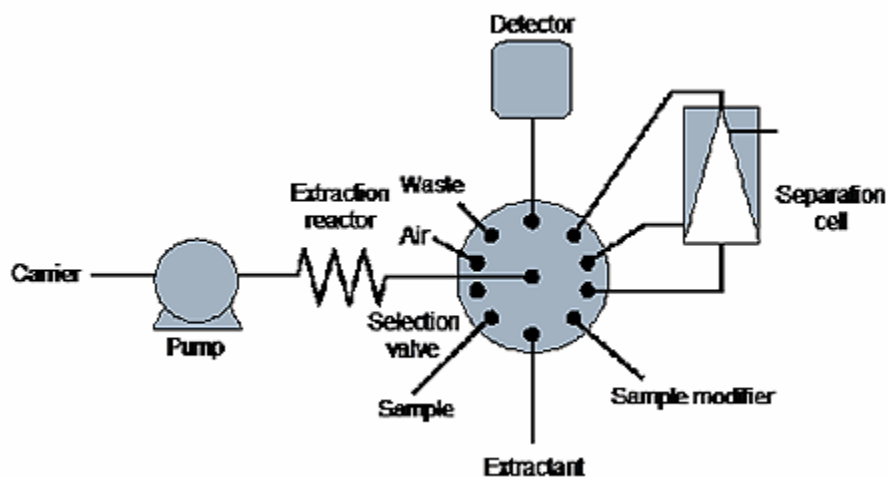


Figure 2



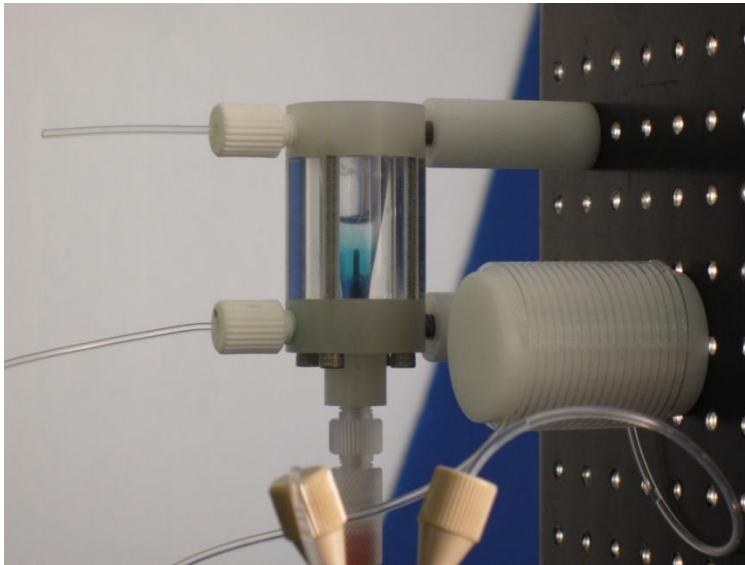
Figure 2 depicts a ZF manifold configured for solvent extraction. The system has two unit operations. The first automatically performs the steps of chemically modifying the sample (if necessary), exposing the chemically-modified sample to a suitable extractant, and mixing the sample and extractant to ensure phase transfer of the component of interest.

The second step separates the immiscible sample and extractant zones. The extractant zone can then be transported to a suitable detector or to additional unit operations and the unwanted phase is discarded. With no changes to the ZF manifold, further processing may involve performing a back extraction with a second extractant (another unit operation).

The following steps are performed in the application of solvent extraction to ZF. The steps described include sample modification. We include a full listing of the fluidic operations to illustrate the complexity of fluidic tasks that can be accomplished with simple ZF hardware.

- A repeated stack of multiple zones containing sample sandwiched between sample modifier and extractant zones is assembled in the extraction reactor. The size and order of each zone is controlled accurately and precisely.
- Once formed, this stack of zones is then slowly drawn back into the extraction reactor that is designed to ensure efficient secondary flow patterns. As the zone stack is withdrawn into the extraction reactor, it is followed by a large air zone to prevent further dispersion at the trailing edge. The efficient, but non-turbulent mixing, together with the tube wetting characteristics of the extractant, ensures rapid and efficient phase transfer.
- Once the zone stack has reached the pump end of the extraction reactor, flow is reversed and the zone stack is transferred to the separation cell where rapid separation of the two phases occurs under static conditions.

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- After phase separation is completed, the desired phase is drawn out of the separation cell (the separation cell is designed to allow either the top or the bottom phase to be recovered for detection), transferred to the desired analytical finish and measured or to a fraction collector or autosampler. Any residual liquid is then withdrawn from the separation cell and discarded to waste and the manifold is prepared for the next measurement. This preparation involves flushing the separation cell and manifold conduits and filling certain conduits with air.

The key features distinguishing the ZF solvent extraction approach from other flow-based extraction methodologies are: 1) accurate and precise control of the size and order of chemical conditioning, sample, and extraction solvent zones, 2) phase separation of the two immiscible phases under static rather than dynamic conditions, and 3) the deliberate manipulation of air as a third phase mutually immiscible with the immiscible liquid phases. Critically, ZF employs a pulseless, self-priming, bi-directional positive displacement pump rather than peristaltic pump tubing which is liable to rapid degradation leading to flow deterioration.



The key advantages of the ZF approach to solvent extraction include the following:

- Total automation of the solvent extraction process including pre-extraction chemical modification.
- Pulseless positive displacement versus peristaltic pumping
- No emulsion formation.
- Separation of phases occurs under static rather than dynamic conditions, thus eliminating the need for dynamic phase separation as described in previous FIA based extractions. The extractant is automatically delivered to the detector or introduced into an instrument such as a GC or HPLC.
- Substantial reduction in solvent, reagent, and sample usage.
- Substantial reduction in waste generation.
- Amenable to unattended process analytical applications.
- Complex fluid manipulations automated with very simple hardware.

We will now look at a second example of ZF, reagent preparation. Usually in flow-based analysis, reagents are prepared by dissolving appropriate chemical compounds in an appropriate solvent. Because the ZF manifold is equipped with a high precision pump that enables accurate volumetric metering, the capability exists for preparing reagents and *in situ*. By treating the reagent preparation step as a unit operation in the ZF manifold, there are several benefits. The computer-controlled fluidics manifold is tasked with the mundane task of reagent or standard preparation. Where required, reagents or standards can be altered in real-time to better match the requirements of the assay.

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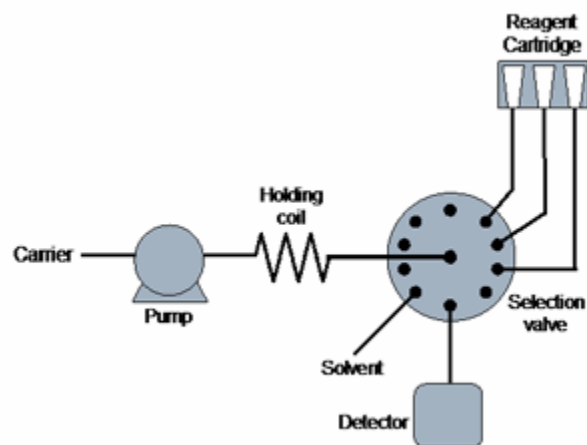


Figure 3

Figure 3 depicts a simple manifold that can be used for this purpose. Reagent cartridges are constructed from disposable syringe barrels with porous frits pressed into the bottom to promote mixing as required by the described sequences. Tubing lengths to the reagent cartridges are kept as short as possible to minimize prime volumes and reduce reagent consumption.

The pump is used to meter the solvent into the reagent cartridge. Two mixing techniques can be used to achieve dissolution and homogenization of the dissolved reagent; mixing as the solution is drawn through the frit and passing bubbles through the mixture.

In the first sequence, the solvent is metered into the reagent cartridge where it dissolves most of the powder. The solute is then drawn through the frit to the holding coil. This pump action further dissolves remaining reagent and begins the mixing process. A certain degree of vertical stratification may be noticeable and this is disrupted by dispensing several large zones of air to the reagent cartridge. Passage of the bubbles through the liquid bulk achieves good mixing of the vertical concentration gradient.



In another sequence, the power of the frit to assist in dissolution and mixing can be exploited by adopting a two steps forward, one step back approach. The solvent is metered to the cartridge by dispensing 200 μL and then withdrawing 100 μL . This sequence is repeated as many times as required to get the desired volume. With each iteration, the reagent is dissolved, diluted, and mixed. Finally, bubbles are employed to disrupt stratification in the reagent column.