

# The Olis<sup>®</sup> USA Stopped-Flow Mixing Units



## Useful, Simple, and Affordable

**Stopped-flows for absorbance, fluorescence, and circular dichroism.**

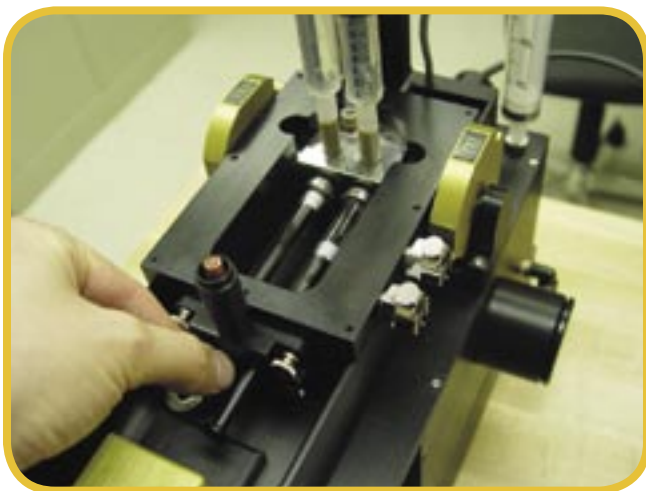
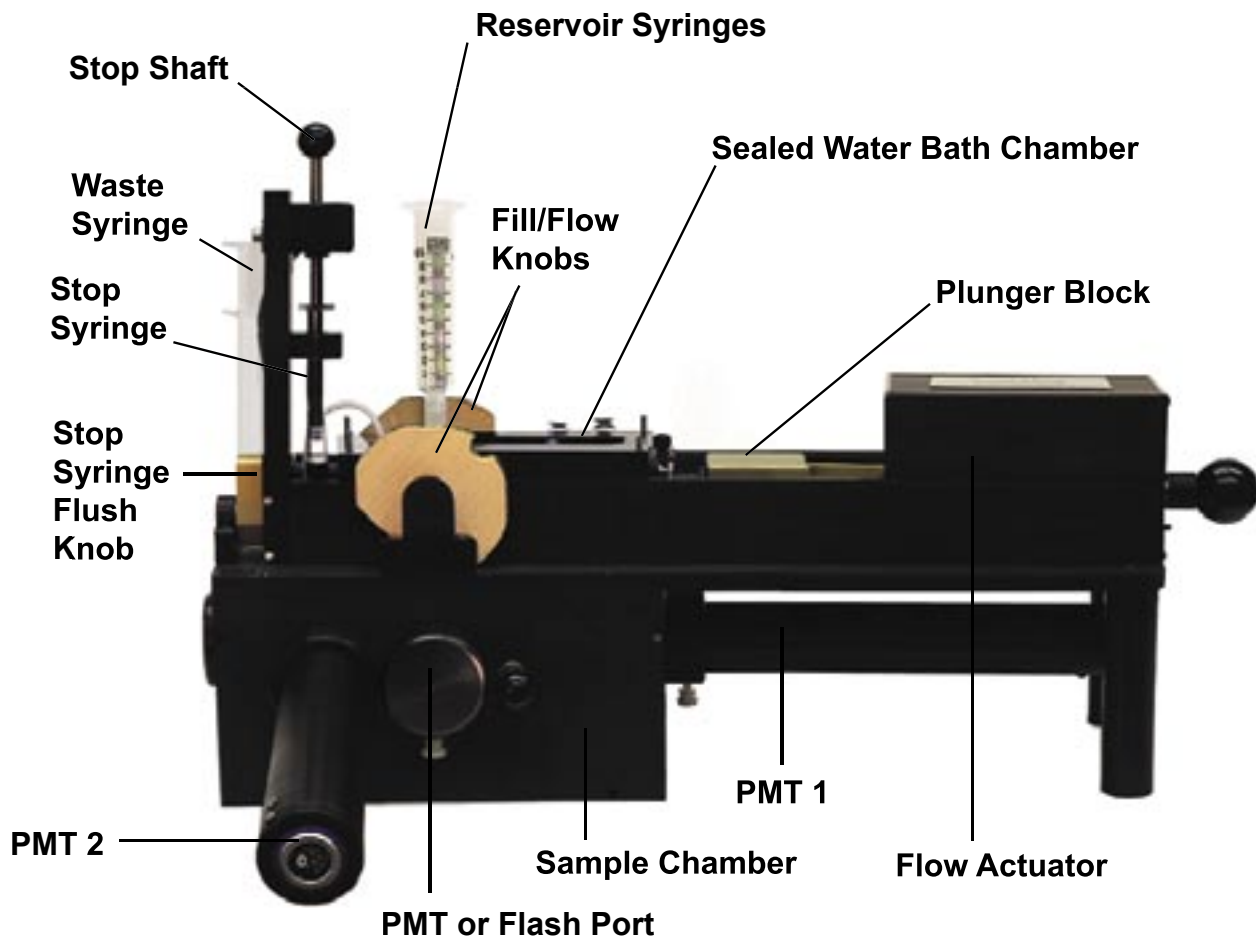
When designing the first Olis stopped-flow, our goal was to make an accessory which people would be comfortable using. We wanted it to be free from spurious complicating features. And we wanted it to be affordable. The stopped-flow we introduced in 1992 met these goals, deserving the moniker Useful, Simple, and Affordable, or U.S.A.

Fundamental attributes of the Olis USA stopped-flow are ease of correct use, sample security, operator safety, reproducibility, durability, mixing quality, and dead-time. With computerized monitoring of the hardware, we guarantee correct use, sample security, and operator safety. With ceramic valves, we guarantee durability.

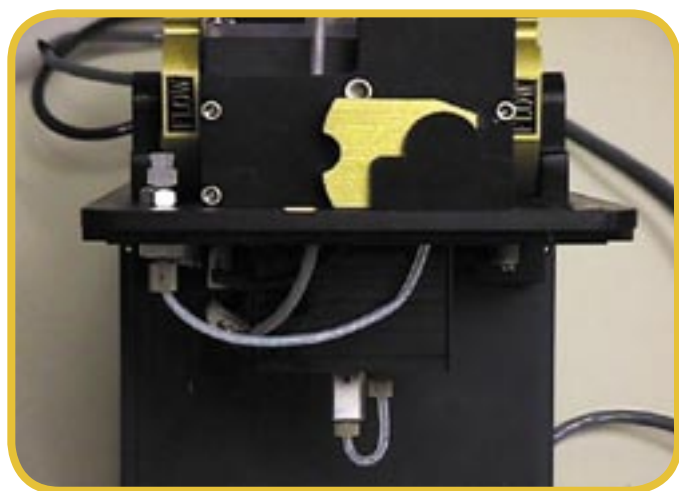
With pneumatic drive, we ensure reproducibility and mixing quality. And with four distinct observation cell designs, we provide the dead time and sample volume suited to research grade stopped-flow absorbance, fluorescence, and circular dichroism spectrophotometry.

Ultimately, the results from a stopped-flow are only as good as the spectrophotometer providing the light, detection, and acquisition. It is the speed, sensitivity, and precision of the spectrophotometer which determines the final volume of sample required for publication-ready result. Olis spectrophotometers range from the useful and inexpensive to the premier in performance.

# Naming the Parts of an Olis Stopped-Flow



Filling the drive syringes



Drive and stopping syringe valves and observation cell

# Parts of the Stopped-flow Mixer

**Reservoir Syringes:** These two syringes hold the unmixed reagents before they are introduced into the drive syringes. These syringes can be plastic (as shown), tonometers, or specialized fluid holders.

**Fill/ flow Knobs:** We machine heavy-duty knobs for opening and closing the fill-flow valves, which are comfortable to use and a long-lasting alternative to the tiny handles often found on other stopped-flows. There are two fill/ flow knobs, one for each drive syringe. Putting the knobs in “fill” position opens the valve between the reservoir and drive syringes to allow the drive syringes to be filled with reagent and closes the valve between the drive syringes and the mixing chamber to keep the reagents in their respective syringes prior to the firing of the stopped-flow.

Computerized operation of the fill/ flow valves can be added at additional cost and is desirable when the Olis Auxiliary Mixer is used.

**Drive Syringes:** To achieve anaerobic stability, gas tight syringes are used. These drive syringes are oriented horizontal to the table and are immersed in a floodable water bath chamber. This chamber is sealed, so that an open-cycle water bath can be used. It is also purgeable with gas, to maintain an oxygen-free environment for anaerobic work.

As shown, one can look down and immediately view the contents and hence remaining volume. Scientists skilled in filling syringes will not introduce bubbles regardless of the orientation of the syringe. Horizontal drive syringes are easy to fill and easy to view.

A pair of 2.5 mL syringes are provided by default. Alternative choices include 0.5, 1, 5, and 10 mL syringes. The pair can be the same sizes, to produce 1:1 mixing, or different, for ratios up to 1:20. These two syringes are actuated by the pneumatic drive using 70-100 psi (5-7 ATU) supplied by an air or other gas tank.



Reservoir Syringes



Fill/ Flow Knobs

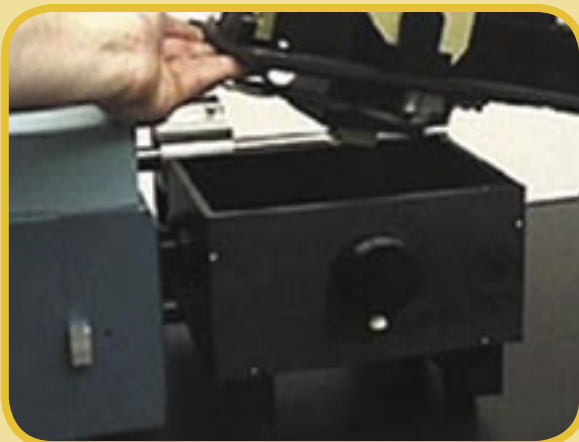


Drive Syringes

## Parts, Continued



**Flow Actuator & Plunger Block**



**Sample Compartment**



**Light guide attached Olis USA stopped-flow to an Aminco DW-2**

**Flow Actuator & Plunger Block:** The stopped-flow is pneumatically driven for consistent performance. The Plunger Block makes contact with the syringe and drives them forward when gas is admitted to the pneumatic cylinder.

**Sample Compartment:** The sample compartment shown at left is common to most Olis spectrophotometers. The Olis stopped-flow fits exactly to this 19 x 14 x 10 cm box. The light-tight fit is assured with machined beveling on the underside of the stopped-flow. Light travels from the spectrometer through the observation cell assembly of the stopped-flow (shown facing page) to detectors mounted at a round port on the sample compartment or after a (emission) monochromator.

**PMT 1 and PMT 2:** There are several positions for the detectors. The detector mounted at PMT 1 position is detecting the light in what is the “absorbance” position. The detector at PMT 2 is used as the reference beam in dual beam absorbance readings. If a PMT is positioned at the “PMT or Flash Point,” right angle fluorescence and/or scatter will be detected.<sup>1</sup>

**Detectors** can be high-speed photomultiplier tubes, useful in the UV/VIS regions, or high-speed InGaAs detectors, useful in the Vis/NIR regions. Photon counters could be used, but these digital detectors are optimized for sensitivity, not speed.

The ‘**PMT or Flash Port**’ position is for a flash lamp to photolyze freshly mixed solution, a detector to acquire full fluorescence, or for an emission monochromator and detector to be mounted.

<sup>1</sup> Mounting an Olis stopped-flow to other manufacturers' spectrophotometers is practical if and only if the non-Olis instrument has a fast acquisition mode. One cannot do stopped-flow speed kinetics without collecting at least 100 points per second. Olis spectrophotometers generally support collection of 1000 points per second; the Olis RSM 1000 collects 1,000 scans per second.

# Introducing the Four Cell Assemblies

Exactly as one chooses a cuvette with a particular volume, diameter, and pathlength for the particular measurement, so one will choose an observation cell for a stopped-flow mixing unit. As shown, Olis offers four choices.

## **Classic<sup>1</sup> Absorbance Observation Cell, 20mm x 2 mm**

Sample flows through a cylindrical optical-grade quartz tube, 20 mm in length and 2 mm in diameter. This was the original design of the Gibson/ Durrum stopped-flow and remains a popular choice for work done at near-ambient temperatures. It is suitable for absorbance (20 mm) and fluorescence (2 mm) use.

## **Modern Absorbance Observation Cell, 10 mm**

This observation chamber is solid stainless steel, supporting low temperature operation with a 10 mm pathlength.

## **Modern Fluorescence and Absorbance Observation Cell, 4 mm x 4 mm**

This observation cell is a 4 mm diameter quartz<sup>2</sup> cylinder, open on all four sides and useful to low temperatures.

## **Modern Circular Dichroism and Absorbance Observation Cell, 4 mm x 4 mm**

Developed for circular dichroism measurements, this chamber has a cylindrical window with a 2 mm pathlength; it is useful for absorbance and CD and can be used at low temperatures.

<sup>1</sup> Identical flow design as the original Gibson/ Durrum/ Dionex models.

<sup>2</sup> UV-enhanced fused silica.



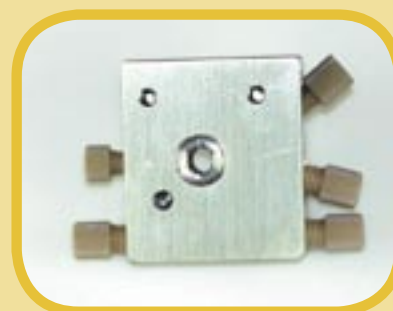
**Classic Absorbance Observation Cell**



**Modern Absorbance Observation Cell**



**Modern Fluorescence and Absorbance Observation Cell**



**Modern Circular Dichroism and Absorbance Observation Cell**

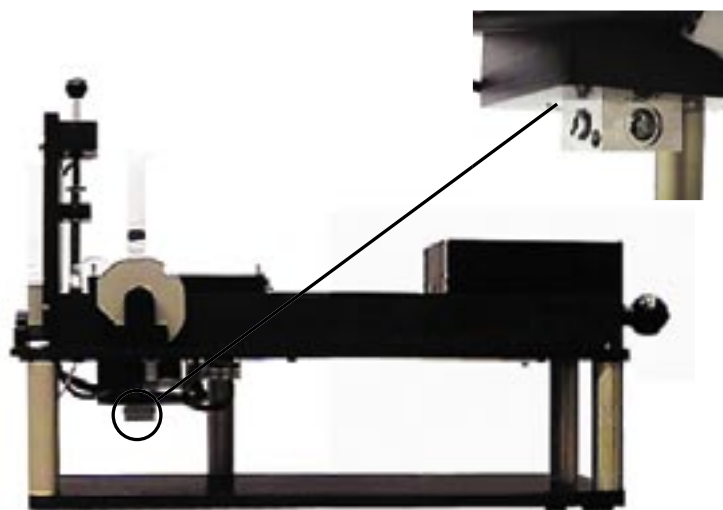
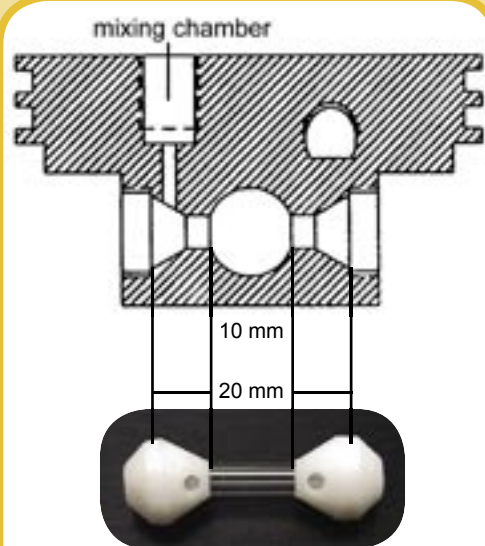
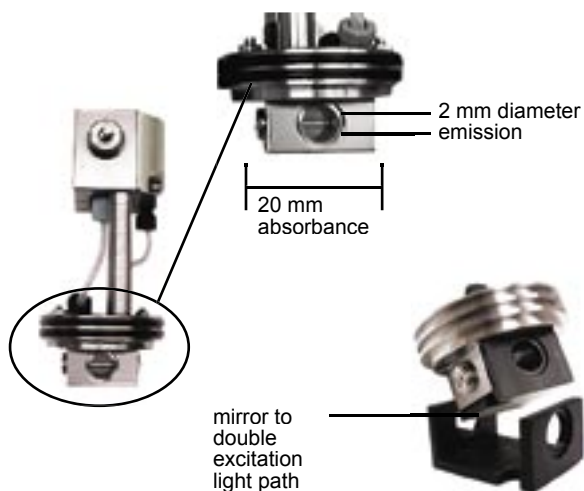
# Classic Absorbance Observation Cell Assembly

The classic stopped-flow cell assembly supports both absorbance and fluorescence measurements at moderate temperatures; because Teflon is used in the cell, temperatures below approximately 10°C are impractical.

The classic cell assembly with a close up of the flow cell is shown upper left. The light path for absorbance is through the 20 mm cylinder. Fluorescence and scatter, measured at 90 degrees, has a 2 mm pathlength.

For fluorescence measurements, a mirror attachment is available to maximize emission collection. The mirror attachment slides over the cell assembly (shown above) such that the mirror is positioned directly behind the rear window; emissions that would be lost through the rear window are thereby reflected towards the detector.

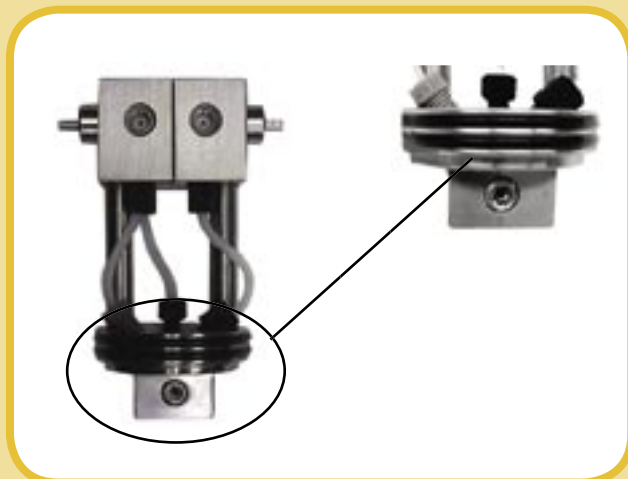
**The optical pathlengths for the two measurements are indicated in the photo and diagram.**



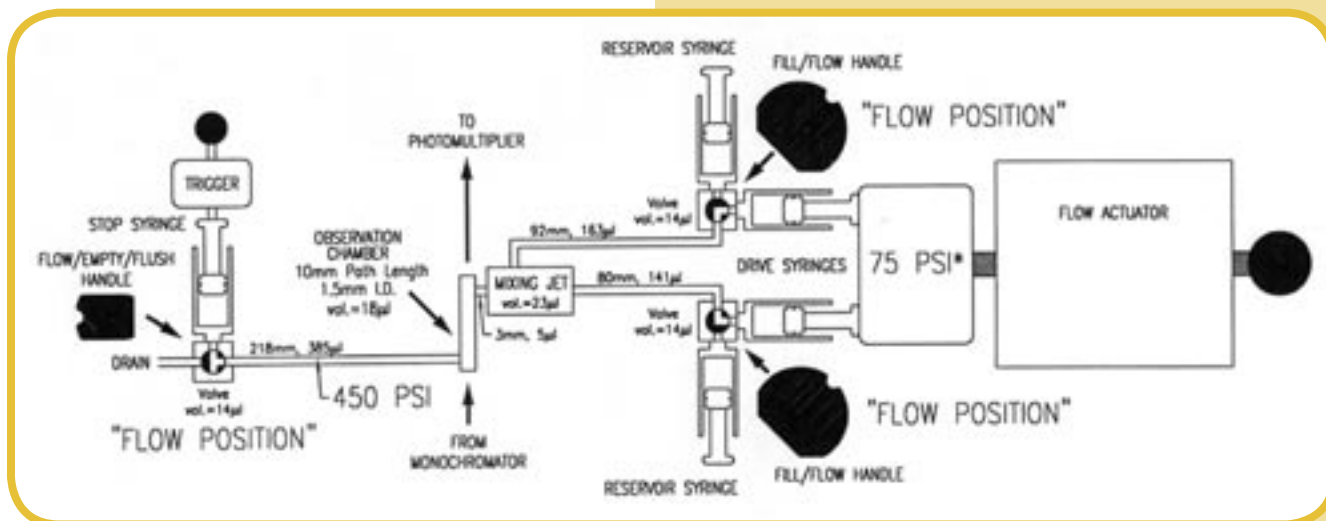
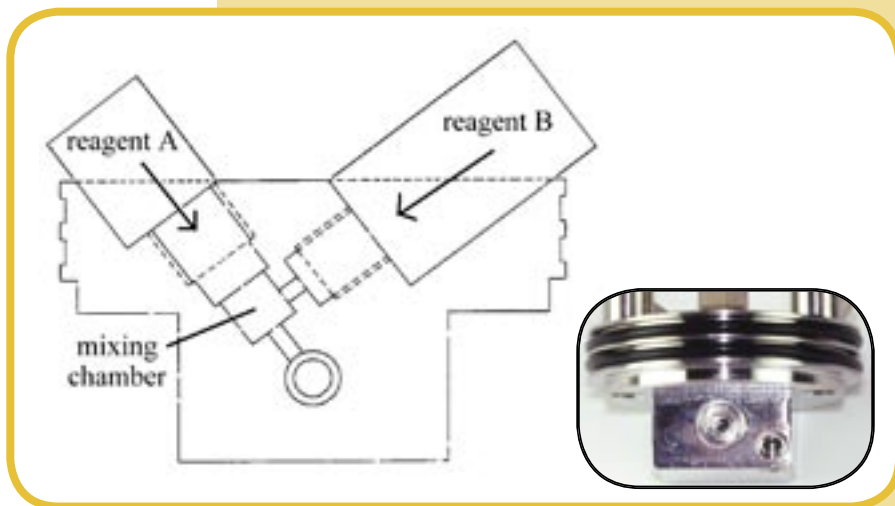
This cell assembly remains a very useful "classic" choice. A great deal of stopped-flow work has been done using this geometry cell, so it is easy to compare results today with earlier work. Also, the signal from such relatively large volume is relatively strong, so that (very) few multiple shots are necessary to improve signal to noise. With these positives comes longer dead time than on other Olis models, upwards of 4 milliseconds (see also page 10).

# Modern Absorbance Observation Cell Assembly

One of two OLIS stopped-flows optimized for low-temperature experiments, the low-temperature absorbance stopped-flow has a stainless steel flow cell assembly with a 10-mm optical pathlength. The low-temperature absorbance cell assembly is pictured at near right with a close-up of the flow cell at far right.



The block diagram shows a cross section of the modern absorbance cell assembly, illustrating how the reagents enter and are mixed at the Ball-Berger section ("mixing chamber").



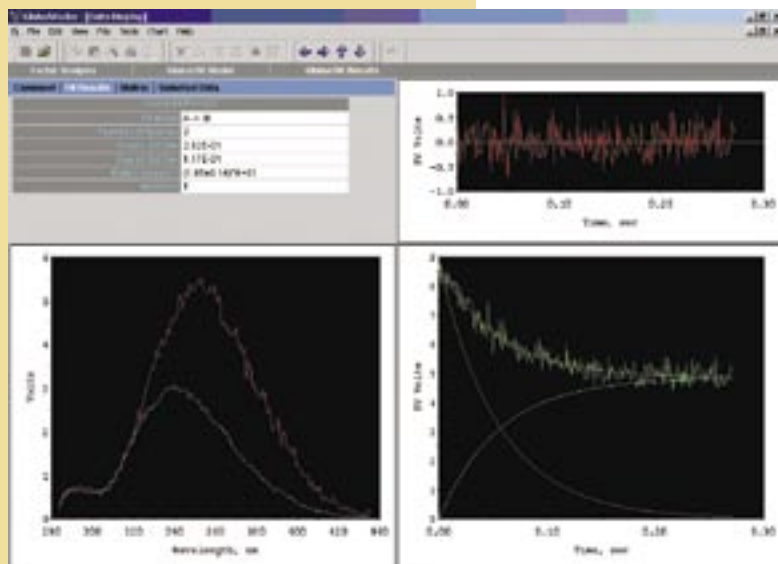
# Modern Fluorescence and Absorbance Observation Cell Assembly



The fluorescence-optimized stopped-flow cell assembly is a 4 mm diameter cylinder of UV-optimized fused silica (Suprasil) suitable for both absorbance and fluorescence. Work at lower temperatures can be done, if appropriate third-party syringes are used.

As noted earlier, working below 8 or 10°C necessitates syringes machined for this purpose. Because of the very low interest in these syringes, manufacturers rarely keep them in stock, making them very difficult to obtain. Please inquire for further details.

Approximately 50  $\mu\text{L}$  is used to fill the observation chamber. This Olis USA stopped-flow has the shortest dead time of the four Olis models, at approximately 1.5 milliseconds.



Results of one stopped-flow shot using an Olis RSM 1000, which acquired 250 multiple wavelength data points (scans) in 250 milliseconds. Less than 300  $\mu\text{L}$  was used.

# Modern CD and Absorbance Observation Cell Assembly

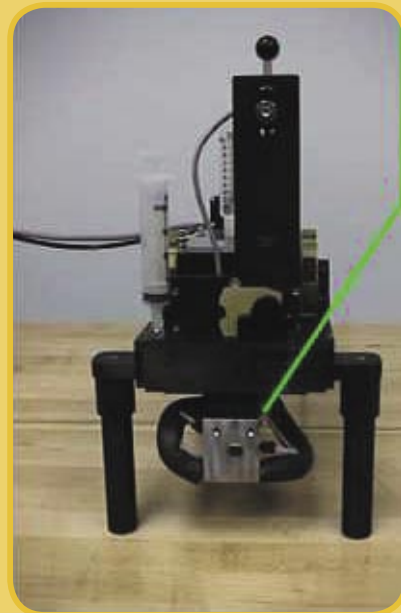
Combining circular dichroism (CD) with stopped-flow speed mixing means detecting (sub)milliabsorbance signals on a millisecond time scale. Rendering this very difficult measurement more practical is the Olis CD stopped-flow system.

The cylindrical observation cell has a 2 mm pathlength. Both beams of light from the Olis DSM CD spectrophotometer pass completely through this window, so that all of the benefits of digital CD data acquisition accrue. (See a separate CD spectrophotometer document for complete details.)

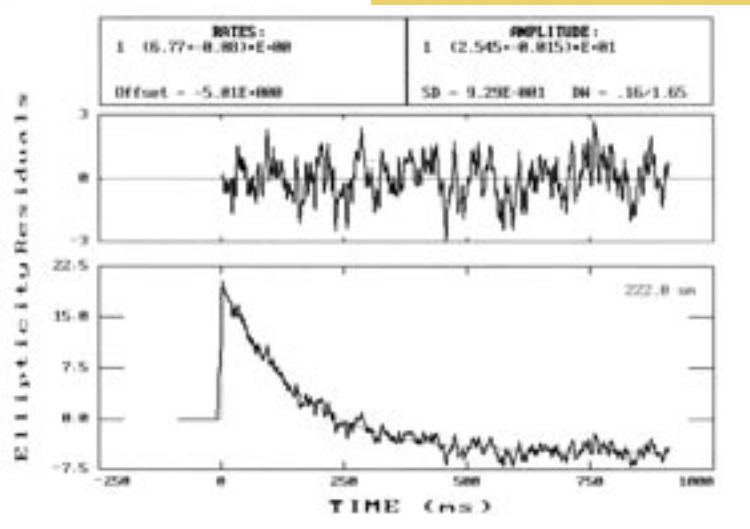
Only 18  $\mu\text{L}$  of fluid fill the chamber. The dead time is between 3 and 5 milliseconds.

All three Olis DSM CD spectrophotometers collect up to 1,000 points per second in true dual beam mode during fixed wavelength stopped-flow data acquisition.

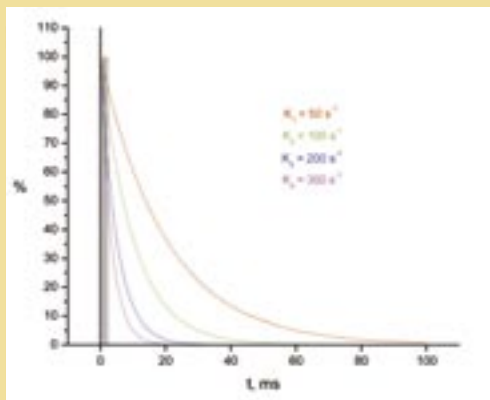
The unique Olis DSM 1000 CD can collect up to 60 CD scans per second, which is practical when the CD signal is reasonably large and the light levels are reasonably high. Rapid-scanning CD stopped-flow in the 260-190 nm is rarely practical, due to the very small signal and the very low light levels below 200 nm.



Single unaveraged CD stopped-flow shot acquired on an Olis DSM 1000 CD spectrophotometer. Less than 300  $\mu\text{L}$  was used to obtain this very fittable trace.

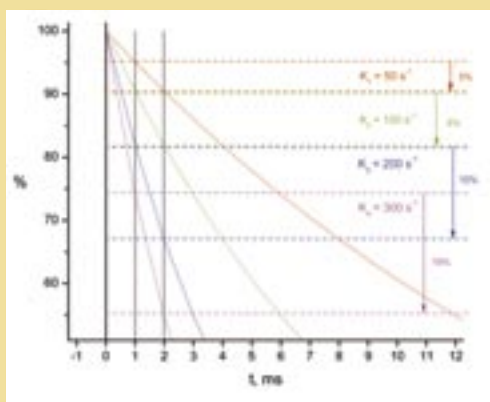


# Dead Times range from 1.4 to 4.1 Milliseconds



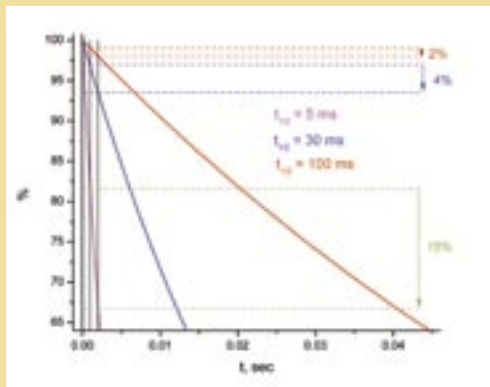
“Dead-time” of the stopped-flow is the age of the mixture when it is first available for measurement. To achieve short dead-times, very small volumes of solution must be moved. If too little volume is used, the signal obtainable will be very noisy, necessitating repeat shots to improve S/N.

**The associated plots graphically compare a 1 and 2 millisecond dead time. Clearly, most stopped-flow experiments will not require a “1 msec dead time.”**



The first graph shows what traces look like for reactions with rate constants of 50 to 300 per second. Two vertical black lines are drawn to mark 1 and 2 milliseconds.

The second graph is an expanded view of the first 12 milliseconds. The percentages shown on the right (5, 8, 15, and 19) are the percentage less amplitude that the 2 millisecond dead time stopped-flow would capture over the 1 millisecond dead time apparatus.<sup>1</sup>



The third graph shows a situation closer to typical, wherein the reaction half times<sup>2</sup> are 50 to 100 milliseconds. Here, one can see that dead times as long as 5 or 10 milliseconds impose little deleterious effect on the final answer.

A sensible compromise is made between signal strength and dead-time in each Olis stopped-flow, with the times ranging from 1.4 ms for the fluorescence cell assembly (23 uL) to 4.1 for the classic cell assembly (63 uL).

**If a given reaction will fail with dead-times longer than a few milliseconds, faster perturbation methods which do not require mixing, such as flash photolysis and T-jump, must be considered.**

<sup>1</sup> Zero second is the theoretical start of the reaction, 100% amplitude.  
<sup>2</sup> The relationship of rate constant (k) to half time ( $t_{1/2}$ ) is  $t_{1/2} = 0.69/k$ .

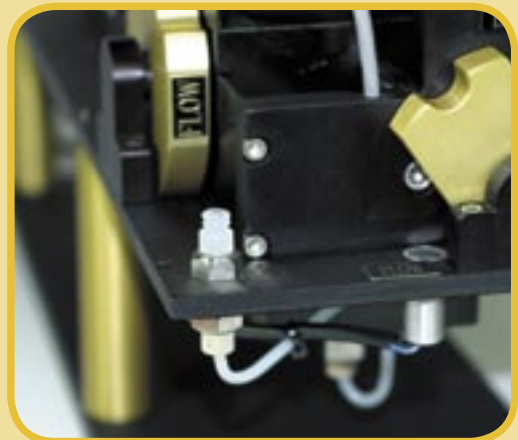
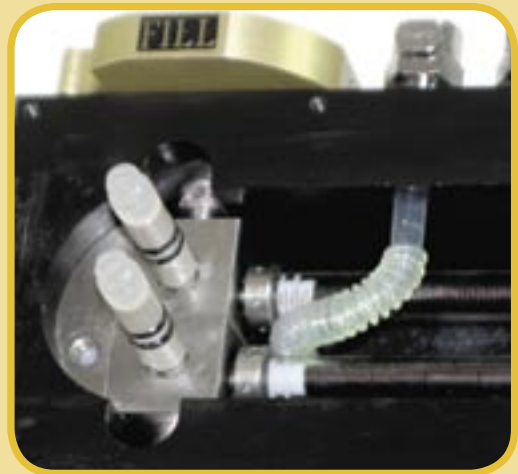
# Doing Anaerobic Work

The most important factor to achieve good anaerobic work is care in introducing one's sample into the stopped-flow. The best situation is to prepare one's sample near by the stopped-flow and to provide connection to the stopped-flow input, which can remain intact throughout the transfer of reagents. Such as a tonometer, shown below.

Potentially useful strategies to further insure success include keeping the stopped-flow filled with oxygen scavenging solutions (e.g. dithionate) when it is not in use, and to flush nitrogen over the syringe ends, and into the water bath system (Olis provides connections to facilitate this).

Instead of, or in addition to, one has the option of putting the entire spectrophotometer or just the stopped-flow apparatus within a glove box or glove bag. Use of light guides to transmit light from the host spectrophotometer to the mixing chamber is a means of separating the hardware, allowing just the sample handling portion to be enclosed.

Stainless steel tubing can replace the standard Telzel tubing, by request.



# Temperature Range and Control



Shown is the Julabo F-30C water bath for which we have drivers, which allows computer control of the temperature(s) by the Ollis software. Manual water baths from any supplier can be used if computer control is not required.

Two of our stopped-flow operate successfully below 10°C. Temperatures approaching zero are sustainable, as well as high temperatures to 100°C. The component limiting our stopped-flows from even subzero temperature is the syringe pair. The syringes have different thermal properties than the high-density plastic of its pistons. Work to 5°C can be done with care; work to 10°C can be guaranteed. If and when true low-temperature syringes are found, work to -30°C could be guaranteed.

**As a rule of thumb, for every 10°C drop in temperature, one realizes a 2-fold reduction of the rate constant. Thus, doing a stopped-flow shot at 20°C rather than 30°C will reduce the rate constant from 10 to 5 (for instance).**

## Solvent-Impervious Ceramic Valves

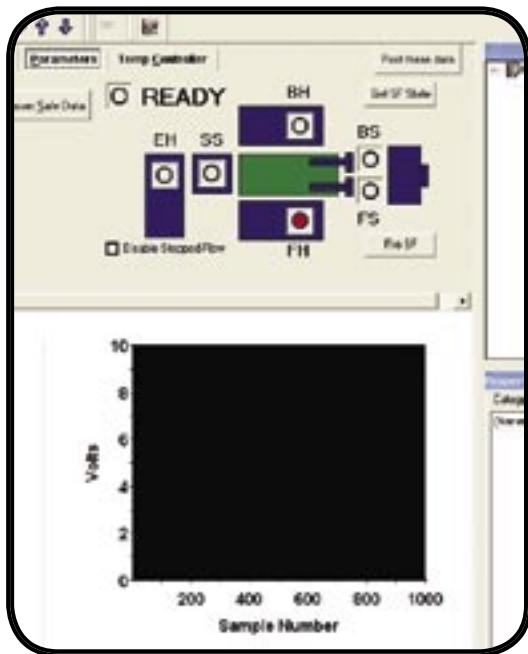
Our stopped-flow has ceramic valves. Only diamond is more impervious to solvents, temperature, and pressure than ceramic (fired sapphire).

Our exclusive use of ceramic valves has significant consequences for the robustness and longevity of the flow system, rendering it impervious to abuse which renders other stopped-flows useless. Any damage to the stopped-flow will happen from external spills onto it or into the water bath, but never from leaks outward.



# Fail-safe Sensors

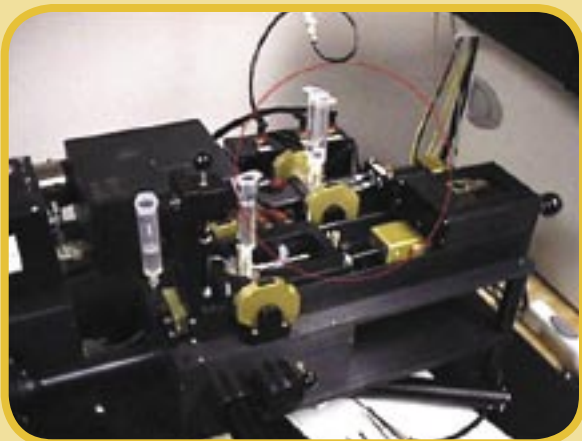
Unique to the Olis stopped-flow are five sensors which provide 100% security against misfiring, pre-mixing, and damage to the hardware and operator. Feedback from these sensors comes from both the trigger box (LED display) and Olis software. This advance has significant consequences for the security of the hardware and safety of the human operator. Syringe damage is prevented, as is pre-mixing of solutions. The stopped-flow can be fired only if it is set correctly for use.



When the instruction to “fire” is initiated and all settings are not perfect for firing, this block diagram appears on the screen, highlighting the incorrect setting in red (the correct positions are denoted with green). The stopped-flow cannot be fired until the positions of the drive syringe, drive syringes’ fill/ flow valves, and the stopping syringe valve are correct. Once the errant setting is corrected, the stopped-flow can be fired safely.

Misfiring, pre-mixing, and breaking syringes are made impossible with this “safety interlock” feature.





## Mixing more than Two Solutions

One can add the Olis Auxiliary Mixer (OAM) for those cases wherein one of the reagents is unstable and will be created in situ to be subsequently mixed with another reagent already in the stopped-flow

Upon flow and mixing, the homogenous A+B mixture produced by the OAM flows directly into one of the drive syringes of the stopped-flow. That is, the solution in syringe 1 of the Olis USA stopped-flow comes from the Auxiliary Mixer, with syringe 1 acting as the stopping syringe for the OAM. After loading the syringe 1 with the A+B mixture, and syringe 2 already containing (same volume) reagent C, the stopped-flow is fired in the usual way to create the (A+B) + C reaction.

Ultimately, the OAM acts as a means to load the syringe with the newly created mixture. Then, the Olis USA Stopped-Flow is used in the normal way, with one of its reagents incidentally having come from the OAM.

Multiple stopped-flow runs can be performed; their repetition rate (and thus the age of the (A+B) mixture) is limited by the time required to collect the data for each shot.

Full automation of the stopped-flow valves is optionally available when Auxiliary Mixing is added. With this, sequential stopped-flow shots can be taken with no user intervention, so that the reactive A+B in one of the syringes is used as quickly as possible.

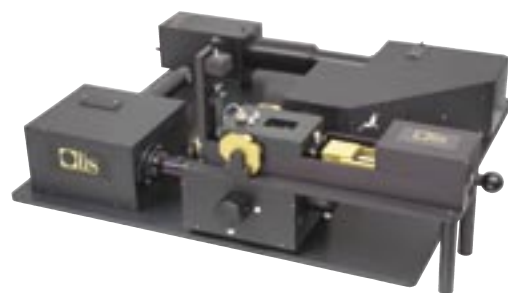
# Olis USA Stopped-flows mounted to Olis Spectrophotometers

Classic Absorbance Observation Cell Assembly model used with an **Olis RSM 1000 Rapid-scanning Absorbance and Fluorescence Spectrophotometer**, supporting millisecond spectral acquisition rates in both modes.

Modern Absorbance Observation Cell Assembly model used with an Olis modernized Cary Spectrophotometer, supporting **NIR stopped-flow** as well as UV and Visible.

Modern Fluorescence and Absorbance Observation Cell Assembly model used with the **Olis DM 245 spectrofluorimeter**, supporting optional simultaneous absorbance and fluorescence detection.

Modern Circular Dichroism and Absorbance Observation Cell Assembly model used with the **Olis DSM 20 CD Spectrophotometer**, supporting optional simultaneous absorbance and CD detection.



"[From the first] I was impressed by the company's dedication to customer service, product development and the fact that the instrumentation, data acquisition and data processing was all developed by a kineticist! This aspect makes OLIS kinetic equipment imminently logical and useful to use and apply.

In my opinion, the technical support that OLIS provides is without question way beyond what would be expected by any company. I now own two stopped-flow collection systems, a laser flash unit and an RSM 1000...all from OLIS! Even now, when I have a question about how to use a particular feature or when the rare "down-time" occurs, I know that help is only a phone call away. It is reassuring for me to know that I am not "in it alone" when it comes to instrumental support of my kinetic work..."

**Michael D. Johnson, Ph.D.**  
**Professor, NMSU, Chemistry Department**



**For more information on this and other Olis products:**

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**1-706-353-6547** worldwide