STOVALL
LIFE SCIENCE, INC

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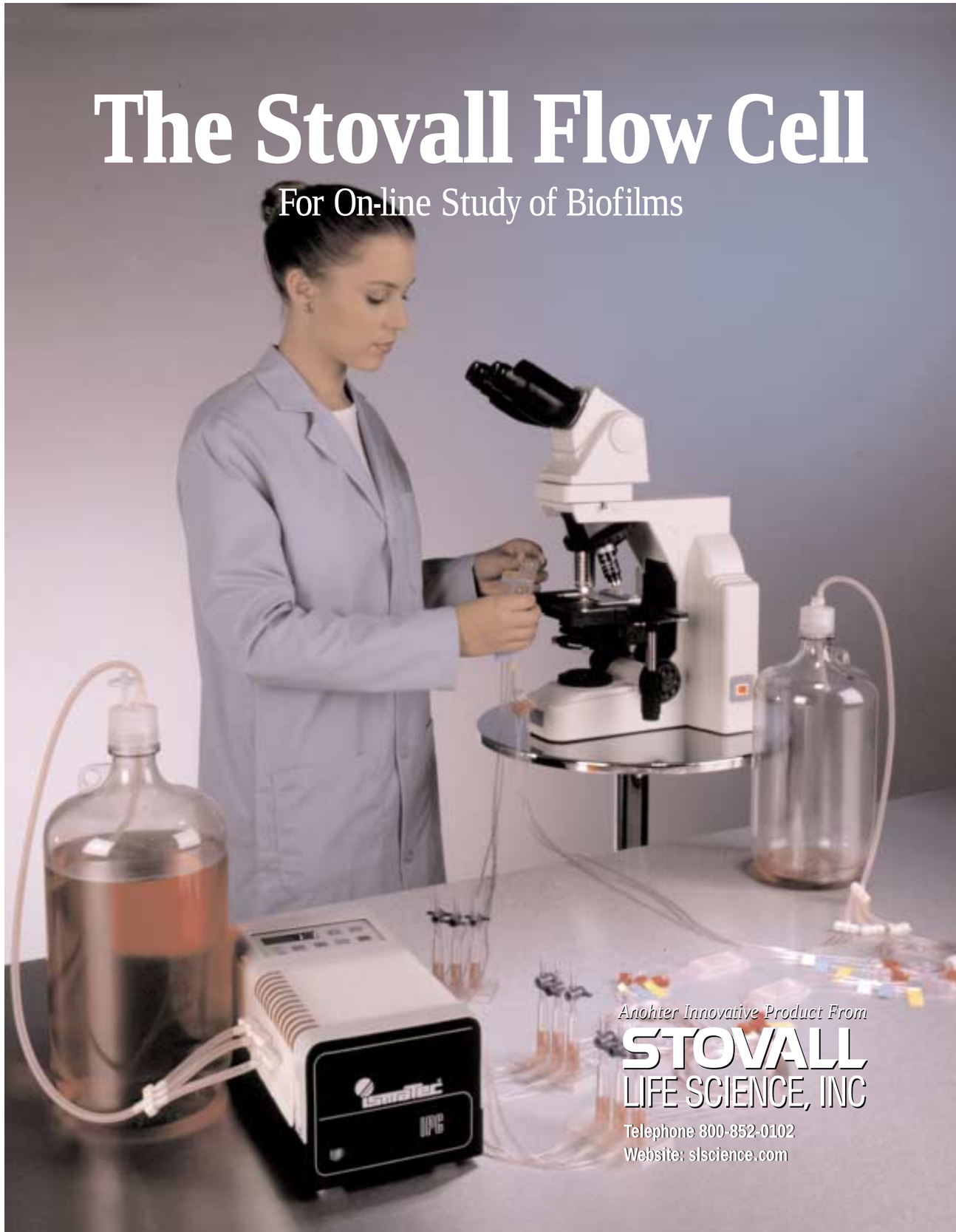
The Stovall Flow Cell

For On-line Study of Biofilms



Another Innovative Product From

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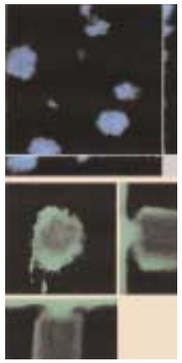
CONTINUOUS CULTURE CHAMBERS FOR REAL TIME, NON DESTRUCTIVE, MICROSCOPIC STUDY OF BIOFILMS

Stovall Life Science offers the biofilm investigator an affordable, sterile, consumable flow cell apparatus for creation of biofilms, and for direct, non-destructive, on-line microscopic examination of biofilms. The gamma irradiated flow cell, bubble trap and connective tubing necessary for experiments is completely assembled in its airtight package, and needs only to be opened and connected to a reservoir of culture medium and a peristaltic pump to start a biolfilm experiment. This single use set-up eliminates the possibility of carryover from one experiment to the next, and eliminates time consuming sterilization methods which either require equipment not easily accessible to most researchers (ethylene oxide chambers) or are, in fact, disinfection methods (hypochlorite). Moreover, the bubble trap design creates a low positive pressure on the flow of medium, thus mitigating the undesirable peristaltic pulsation in liquid delivery to the flow cell.



Traditional vs. confocal microscopy for study of biofilms.
Traditional transmission light microscopy may be used to follow biofilm development. However, as the biofilm thickness increases, it becomes more difficult to obtain good images due to the contribution from unfocused parts of the viewing field. Above, the scanning confocal laser microscope solves this problem by scanning several planes interspersed by short distances, thus reconstructing virtual three-dimensional images of the biofilm.

Bubble Trap
The triple cylinder bubble trap with air release cocks captures air bubbles released from the flowing culture medium. Inside the cylinder a "fountain" spout directs the flow of liquid upward for better release of air bubbles. The air release cocks allow the investigator to control the amount of air captured & govern the pressure on the passing liquid to help mitigate peristaltic pulsation.

Single 3 Channel flow cell with tubing attached & glass cover slip.
The triple channels of the flow cell are covered with a #1 glass cover slip, attached to the cell with acrylic adhesive. The cover slip can be scored & removed for access to the biofilm for further analysis. Each channel measures 1 mm D x 4 mm W x 40 mm L. Influent & effluent tubing is attached by barbed fittings. Write-on multicolored flags index the tubing at two junctures.

Luer Connector & Tube Rack for Effluent Capture.
The luer connectors in the effluent tubing can be unlocked briefly to collect effluent for analysis. The tube rack accessory for effluent capture is also illustrated here.

References for the Stovall Flow Cell Apparatus

Prototypes of the Stovall flow cell apparatus have been used successfully for biofilm cultures by numerous scientists over the past 3 years. Below are publications by some of these investigators who have used the flow cell apparatus:

Christensen BB, Sternberg C, Andersen JB, Palmer RJ Jr, Nielsen AT, Givskov M, Molin S.
Molecular tools for study of biofilm physiology. Methods Enzymol. 1999;310:20-42. Review.

Heydorn A, Ersboll BK, Hentzer M, Parsek MR, Givskov M, Molin S. Related Articles
Experimental reproducibility in flow-chamber biofilms. Microbiology. 2000 Oct;146 (Pt 10):2409-15.

Mathee K, Ciofu O, Sternberg C, Lindum PW, Campbell JI, Jensen P, Johnsen AH, Givskov M, Ohman DE, Molin S, Hoiby N, Kharazmi A.
Mucoid conversion of Pseudomonas aeruginosa by hydrogen peroxide: a mechanism for virulence activation in thecystic fibrosis lung. Microbiology. 1999 Jun;145 (Pt 6):1349-57.

Nielsen AT, Tolker-Nielsen T, Barken KB, Molin S.
Role of commensal relationships on the spatial structure of a surface-attached microbial consortium. Environ Microbiol. 2000 Feb;2(1):59-68.

Ramos C, Licht TR, Sternberg C, Krogfelt KA, Molin S.
Monitoring bacterial growth activity in biofilms from laboratory flow chambers, plant rhizosphere, and animal intestine. Methods Enzymol. 2001;337:21-42.

Sternberg C, Christensen BB, Johansen T, Toftgaard Nielsen A, Andersen JB, Givskov M, Molin S. Related Articles
Distribution of bacterial growth activity in flow-chamber biofilms. Appl Environ Microbiol. 1999 Sep;65(9):4108-17.

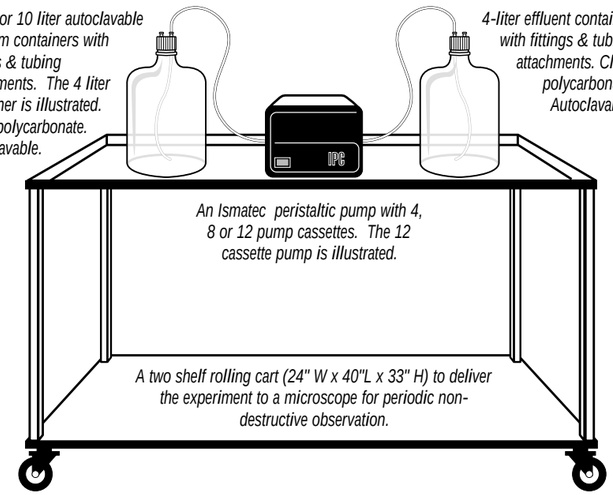
Tolker-Nielsen T, Molin S.
Spatial Organization of Microbial Biofilm Communities. Microb Ecol. 2000 Aug;40(2):75-84.

HARDWARE FOR START UP LABORATORIES

Many labs are already adequately equipped to use the Stovall flow cell perfusion system. However, for those labs needing set up equipment, Stovall offers the various hardware elements which can be purchased in part or whole for biofilm experiments.

4 liter or 10 liter autoclavable medium containers with fittings & tubing attachments. The 4 liter container is illustrated. Clear polycarbonate. Autoclavable.

4-liter effluent container with fittings & tubing attachments. Clear polycarbonate. Autoclavable.



An Ismatec peristaltic pump with 4, 8 or 12 pump cassettes. The 12 cassette pump is illustrated.

A two shelf rolling cart (24" W x 40"L x 33" H) to deliver the experiment to a microscope for periodic non-destructive observation.

Each flow cell has three separate growth channels and each channel requires a single pump cassette to supply culture medium to it. The Stovall cart is designed to accommodate a maximum of 4 flow cells (I.E.: 12 growth channels) for a single experiment.

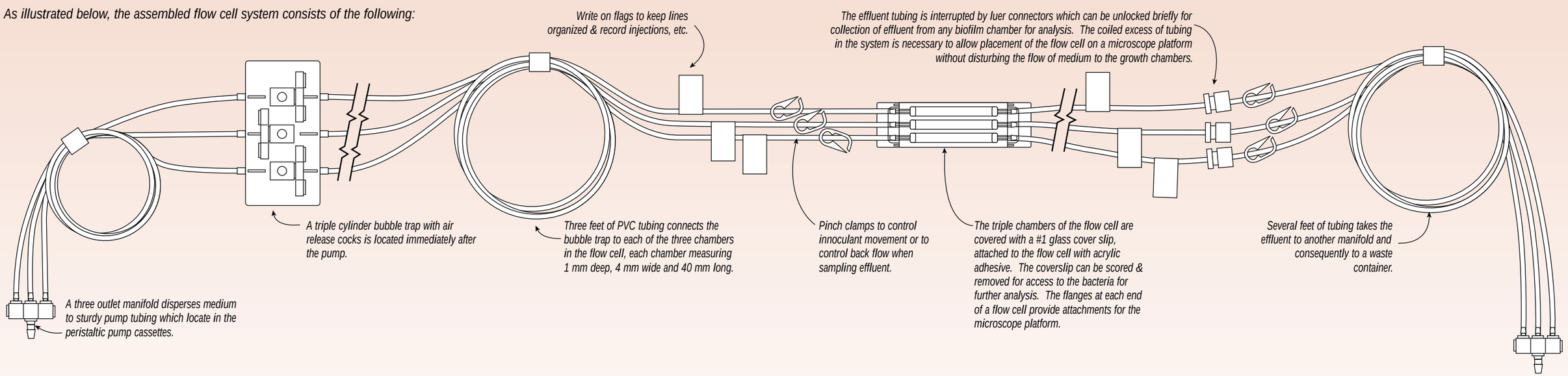
Custom Coatings for Flow Cell Glass Cover Slips.

The standard three chambered flow cell has a #1 glass coverslip (0.13 - 0.16 mm thick) attached. Stovall, in conjunction with specialized suppliers, can offer a wide range of metal, polymer and other coatings for the glass coverslips. These thin coatings do not interfere with microscope on-line observations of the development of the biofilms attached to the coated glass. Write or call us to discuss the kinds of coatings you require for your experiments.
Our e-mail address is slscience@earthlink.net.

ELEMENTS OF THE CONTINUOUS FLOW CELL SYSTEM

Telephone 800-852-0102 or visit our Website at: slscience.com

As illustrated below, the assembled flow cell system consists of the following:



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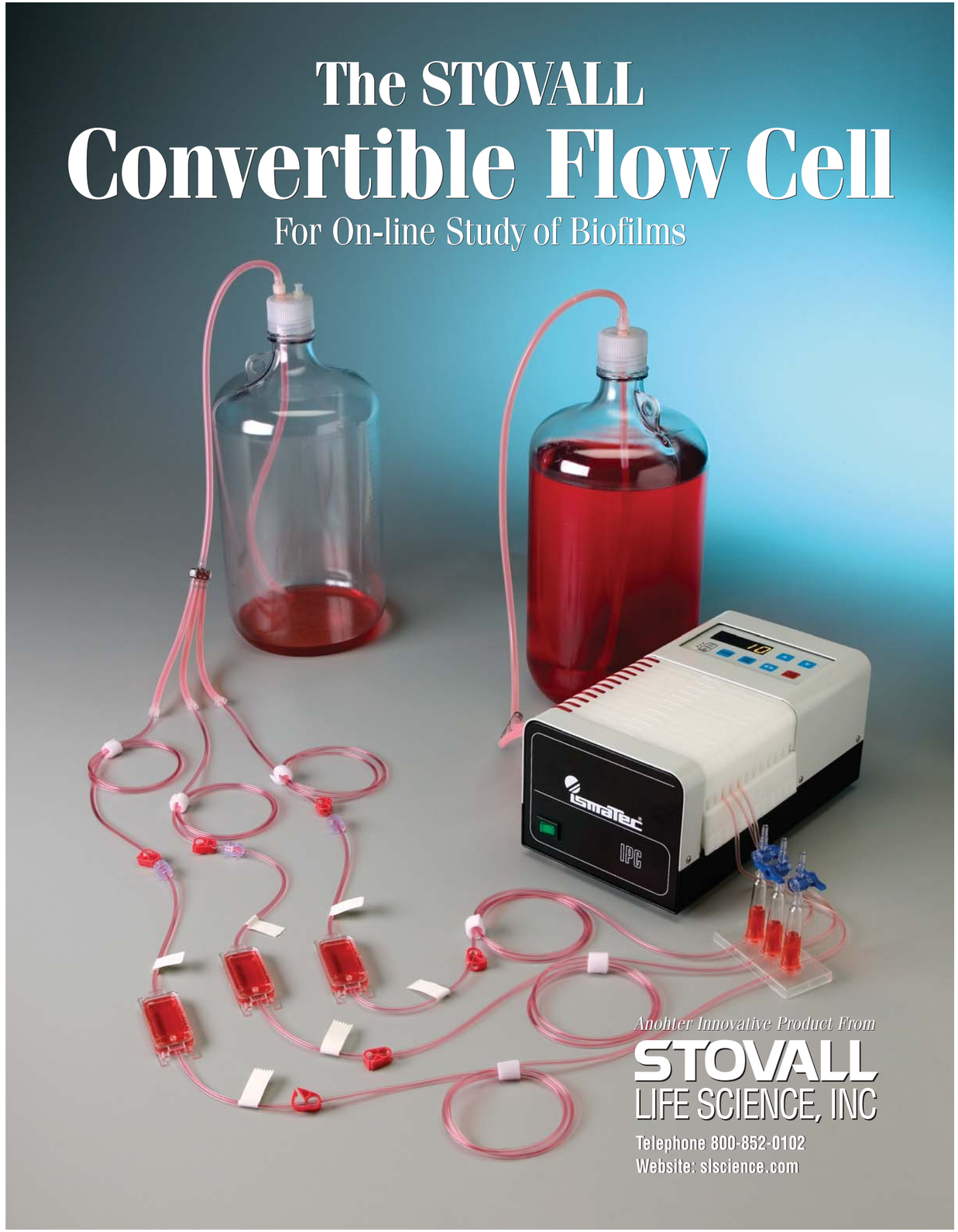

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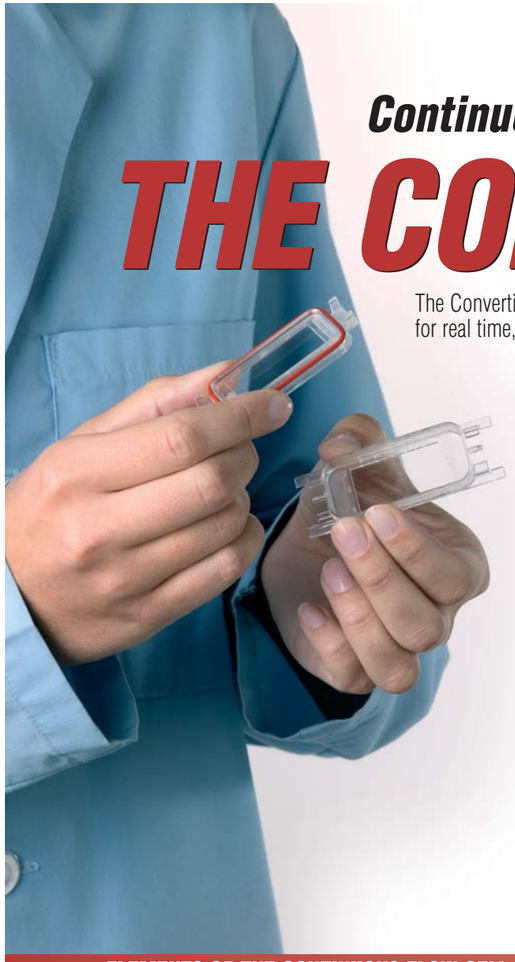

Continuous Culture Flow Cell:

THE CONVERTIBLE

The Convertible contiunous culture flow cell extends in several ways Stovall's offering for real time, nondestructive, microscopic study of biofilms:

Detachable Reattachable Top

This single chamber flow cell with a detachabe/reattachable top allows an investigator to subject test samples—polymers, metal, or other—to cells injected into the chamber through the self-sealing injection port.

Large Chamber $7.7cm^3$

The relatively large chamber, 24mm x 40mm x 8mm deep, accommodates harvesting of significant volumes of biofilms for RNA array and protein analysis. The easy access to matured biofilm facilitates this purpose.

Two Attachment Surfaces

Two glass cover slips, one on the top of the cell and one on the bottom, provide attachment surfaces for regular or inverted microscope observation.

Glass or TCT Coated Plastic Option

Alternative TCT treated APET plastic cover slips provide better attachment surfaces for some biofilms and for cell growth and yield.

SEE PRICE LIST FOR THE APET AND OTHER PRODUCTS.

Coating the Attachment Surfaces

The detachable top allows investigators to coat the glass or plastic attachment surfaces with a variety of materials—seeded cells, proteins, polymer films, ECM—for experiments testing the response of biofilms or cells to such materials.

Bubble Trap

The triple cylinder bubble trap with air release cocks captures air bubbles released from the flowing culture medium. Inside the cylinder a "fountain" spout directs the flow of liquid upward for better release of air bubbles. The air release cocks allow the investigator to control the amount of air captured & govern the pressure on the passing liquid to help mitigate peristaltic pulsation.

Self Sealing Injection Port

The self sealing injection port facilitates initial inoculation of the flow cell chamber and any additional injections an investigator wishes to add to the growth chamber in the course of an experiment.

Transmission Light and Confocal Microscopy

As with other Stovall flow cell products, The Convertible Flow Cell can be used with traditional transmission light microscopes to follow biofilm development. However, as the biofilm thickness increases, it becomes more difficult to obtain good images due to the contribution from the unfocused part of the viewing field. The scanning confocal laser microscope solves this problem by scanning several planes interspersed by short distances, thus reconstructing virtual three-dimensional images of the biofilm.

TWO CONFIGURATIONS, FOUR PRODUCTS, GAMMA IRRADIATED

The Convertible Flow Cell is offered in two different configurations:

1. *The Convertible Flow Cell by itself (CFCAS0003, CFCAS0004)*
2. *An assembled apparatus (CFCAS0001, CFCAS0002) consisting of the Convertible Flow Cell, connective tubing, pinch clamps to control innoculent movement, write-on flags to record injections, and a luer connector which can be unlocked to collect effluent for analysis. Each configuration is packaged in a sealed polybag and sterilized by gamma irradiation.*

Both Configurations are gamma irradiated and designed for a single use which eliminates the possibility of carryover from one experiment to the next, and eliminates time consuming sterilization methods which either require equipment not easily accessible to most researchers (ethylene oxide chambers), or are, in fact, disinfection methods (hypochorite).

ELEMENTS OF THE CONTINUOUS FLOW CELL SYSTEM

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