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THE LIVERMORE FLOW CYTOMETER

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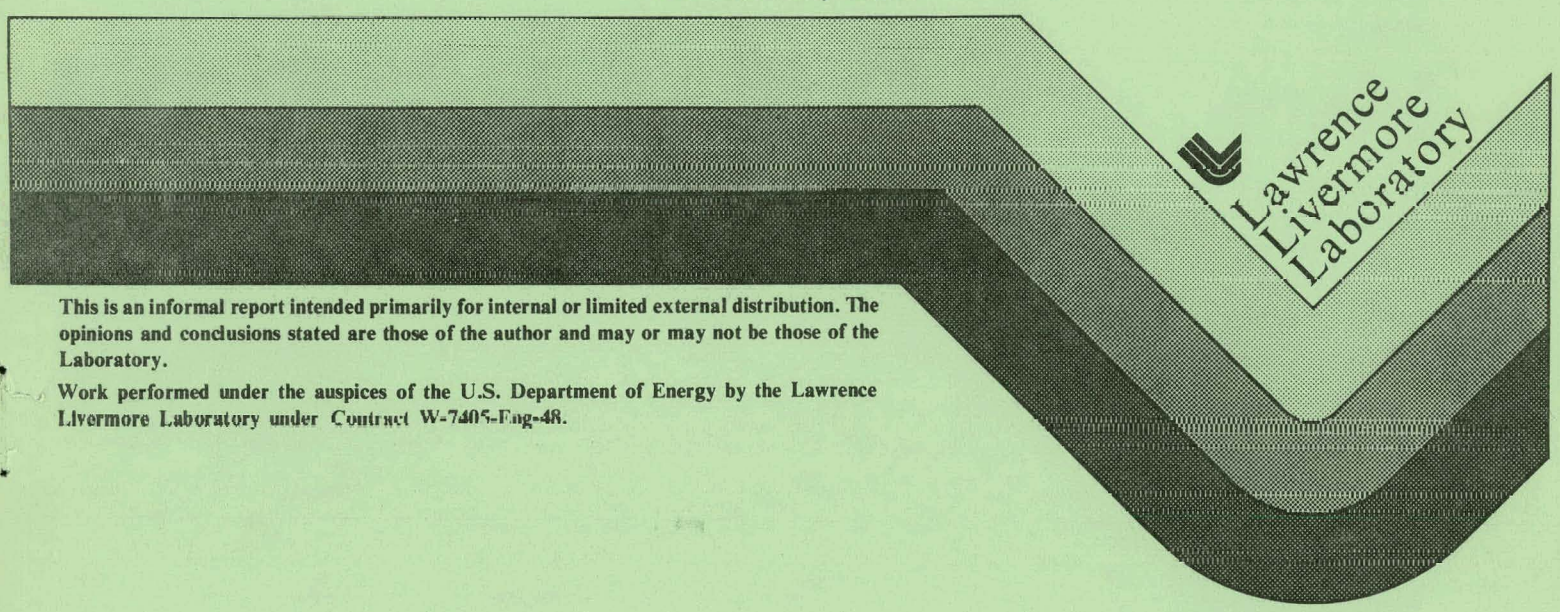
Phillip N. Dean
and
Daniel Pinkel

BIOMEDICAL SCIENCES DIVISION

November 19, 1981



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DISCLAIMER

THE LIVERMORE FLOW CYTOMETER

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ABSTRACT

A stable, easy to operate, flow cytometer was built for routine use by research personnel in a biology laboratory. A 5 watt argon-ion laser excites the fluorescent dyes in cells flowing through a quartz flow chamber. A f/0.95 TV camera lens collects the fluorescent and scattered light and directs it through color separation filters and a beam splitter onto two photomultiplier tubes. Two 3-axis positioners are used to align the focused laser beam and the flow chamber with the light collection optics. All of the components, except the laser, are mounted on a rigid aluminum plate. Standard electronics modules are used. A coefficient of variation of about 1% can be obtained with fluorescent microspheres.

Flow cytometers make high-speed measurements of components and properties of individual cells in a liquid suspension. Most of these instruments use a laser to illuminate biological objects that have been stained with fluorescent dyes. Light detectors produce electrical signals proportional to the fluorescence and scattered light intensity from each object. The signal amplitudes are stored in a computer as a histogram of fluorescent or scatter intensity versus number of objects. Routine use of such a system by research personnel in a biology laboratory requires a flow cytometer that is stable with time and easy to operate and maintain. This report is a description of a flow cytometer (FCM) we developed that meets these requirements. The report is intended to be a technical reference for users of the system and will contain no data.

The FCM is drawn to scale in Figure 1a (top view) and 1b (front view). The axes of the flow stream, incident laser beam and emitted light are mutually orthogonal. Cells flow upwards through the flow chamber, reducing trapped air bubble problems. A 5 watt argon-ion laser (Spectra Physics Model 164-05) is normally used. Table I lists the available wavelengths and intensities. The laser beam passes through a 360 mm focal length cylinder lens (L1), used to generate an elliptical beam cross-section. The beam is then focused on to the flow chamber (FC) with lens L2. L2 is normally a 32X, 0.60 numerical aperture, microscope objective lens (Leitz) with an 8 mm focal length. For experiments using high power U.V. excitation (1 watt at 351 and 364 nm from the Spectra Physics 171-05 laser), which damages this lens, a 25 mm focal length plano convex quartz spherical lens (Melles Griot) is used instead. Lens L2 and the flow chamber are suspended below the 3-axis precision positioners P1 and P2 (Micro-Swiss of Horsham, Pa.).

The fluorescent and scattered laser light is collected by a 25 mm, f/0.95 TV camera lens (L3, Ercona), with the object (sample stream) in the back focal plane. This lens was selected for its high numerical aperture, long working distance and very good imaging properties over the visible spectrum. The light is focused through a 0.5 mm pinhole (PH) onto photomultipliers PMT 1 and PMT 2 (EMI 9798B). The beam splitter (BS) can be rotated such that 50% of the light passes to each photomultiplier or all of the light passes to PMT 2. The filters F1, F2, and F3 are selected based on the fluorescent dye and laser wavelength in use and they can be either colored glass, neutral density, or dichroic. The filters, beam splitter, and pinhole are in an assembly purchased commercially (Becton Dickinson FACS Systems, Sunnyvale, California).

A 12 x 24 x 3/4 inch aluminum mounting plate (MP) supports all of the components, except the laser. The laser light beam is surrounded by a light shield (SH) and absorbed by a beam dump (BD). Not shown is a utility microscope (American Optical) for viewing the laser beam-sample stream interaction.

The system is aligned by positioning the flow chamber and lens L2 such that the focused laser beam intersects the sample stream at the image of the pinhole formed by lens L3. The positions of lenses L1 and L3 are fixed. The alignment can be checked by back lighting the pinhole and viewing it's image and the laser beam-sample stream intersection with the utility microscope. Fine tuning of the alignment is achieved by adjusting the positioners for maximum signal amplitude and minimum signal width from the photomultipliers, as viewed on an oscilloscope.

The lower portion of Figure 1b shows the control panel and sample holders. The sample holders and the sheath water tank (not shown) are

pressurized with air that is controlled by air pressure regulators on the panel. The two gages monitor air pressure. Switches S1 to S4 are air valves (Clippard Instrument Laboratory, Cincinnati, Ohio). Switches S1 and S4 turn on the sheath and sample air, respectively. S2 actuates a laser beam block, used when the light shield is removed. Switch S3 controls a solenoid and pinch valve and allows the sheath water to flow, its rate being monitored by a ball flow meter.

The flow chamber is drawn to scale in Figure 2. The quartz flow cell (Precision Cells, Hicksville, NY) has a round entrance section and a 2 mm square exit section (Figure 2c). The flow channel inside the exit section is 0.25 mm square. A square section, rather than round, is used for the optical measurements because it does not act like a cylindrical lens, as does a cylindrical flow chamber. The brass cap and end piece and the rubber bushing (Figure 2b) were purchased commercially (Becton Dickinson, Rutherford, NJ). A 26 gauge syringe needle, with the tip removed, was used for the sample injection tube. The flow cell can easily be removed for cleaning.

NIM (Nuclear Instrumentation Module) standard electronics are used for the power supplies and signal processing, except for the photomultiplier pre-amplifiers which are made locally. The signals from the photomultipliers can be integrated, or not, depending on the amplifiers that are used. Integration is used for measuring the total amount of light emitted by each object during the time the object passes through the laser beam. The signal pulse length is proportional to the length of the object. Integration would not be used when measuring object length. Pulse height analysis is accomplished through the use of a two-parameter, computer-based analyzer (ND-620, Nuclear Data, Inc., Schaumberg, Illinois).

The system alignment is stable over at least periods of days. It did not require realignment after a strong earthquake shook the laboratory. The instrument can yield a coefficient of variation of about 1% for fluorescent microspheres (1.75 μm diameter No.106B provided by Polysciences, Inc.) excited at 458 nm.

ACKNOWLEDGMENTS

The flow cytometer that we have described was patterned on the design of a dual laser cytometer developed by P.N. Dean and D. Pinkel of this laboratory (1).

REFERENCES

1. Dean, P.N., Pinkel, D.: High resolution dual laser flow cytometry. J. Histochem. Cytochem. 26: 622, 1978.

TABLE 1

Beam Wavelengths Available on the FCM, from the Spectra Physics 164-05 Laser

Wavelength (nm)	Intensity (Watts)
351, 364	.08
454	.12
458	.35
466	.20
473	.30
476	.75
488	1.5
496	.70
502	.40
514	2.0

FIGURE LEGENDS

Figure 1 Scale drawing of the top view (1a) and front view (1b) of the FCM. The two three-axis positioners (P1 and P2) support, through a hole in mounting plate (MP), the laser beam focussing lens L2 and the flow chamber (FC), respectively. L1 is a fixed cylindrical lens used to give the focussed laser beam an elliptical cross-section. The laser beam path is surrounded by a light shield (SH) and stopped by a beam dump (BD). The laser beam-sample stream intersection is imaged by lens L3 onto pinhole (PH). Light passing through PH is converted to an electrical signal by photomultiplier tubes (PMT) 1 and 2. F1, 2, and 3 are optical filter holders and BS is a beam splitter. The sheath and sample controls and the sample holders are beneath the light shield. Switches S1-4 control air pressure to the sheath reservoir, laser beam block piston, sheath flow valve, and sample holder, respectively.

Figure 2 a) Flow chamber drawn at two times actual size. b) Exploded view of individual parts. c) The quartz flow cell showing the round inlet section and the square outlet section. The laser beam intersects the square section.

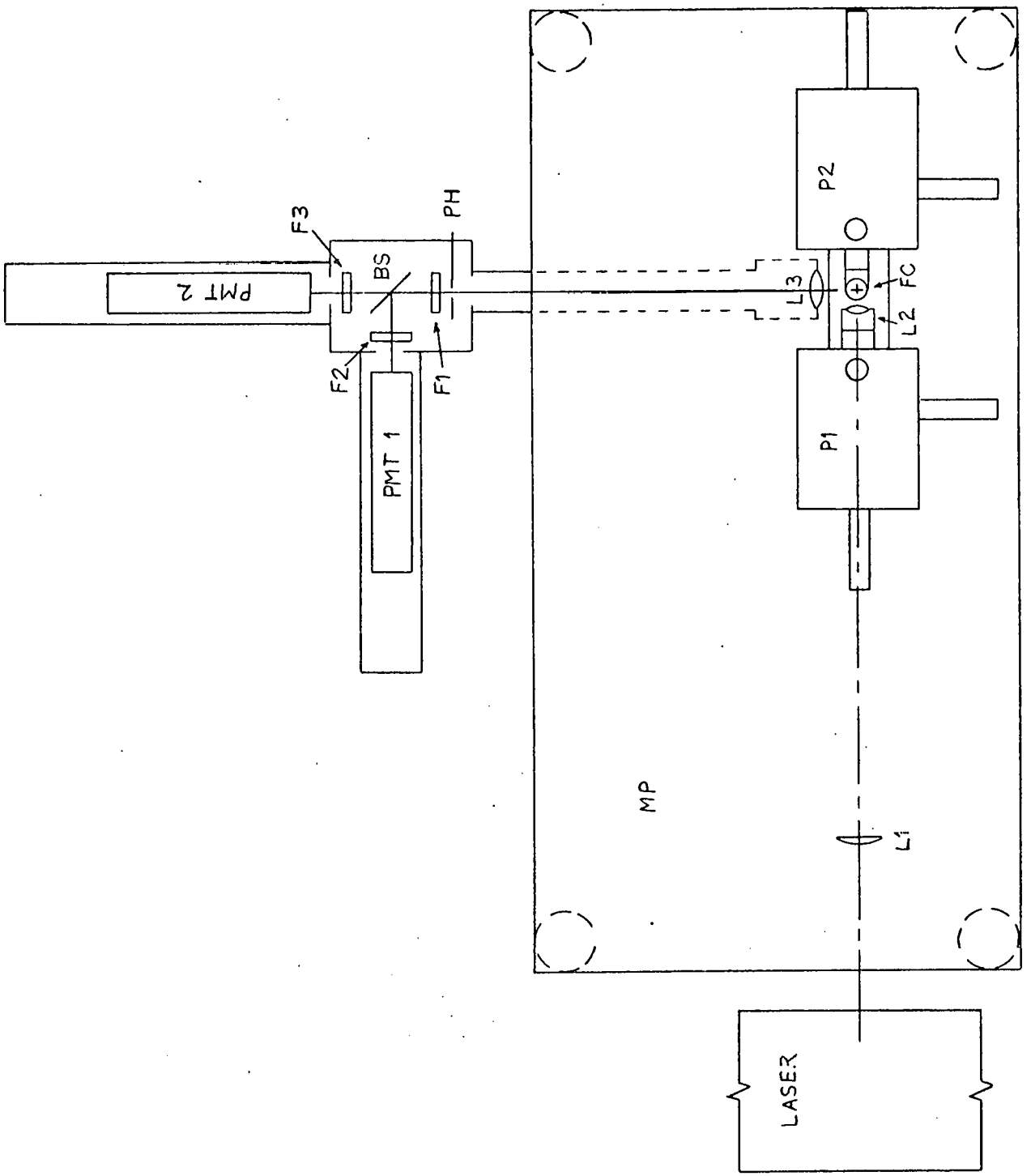

Figure 1a

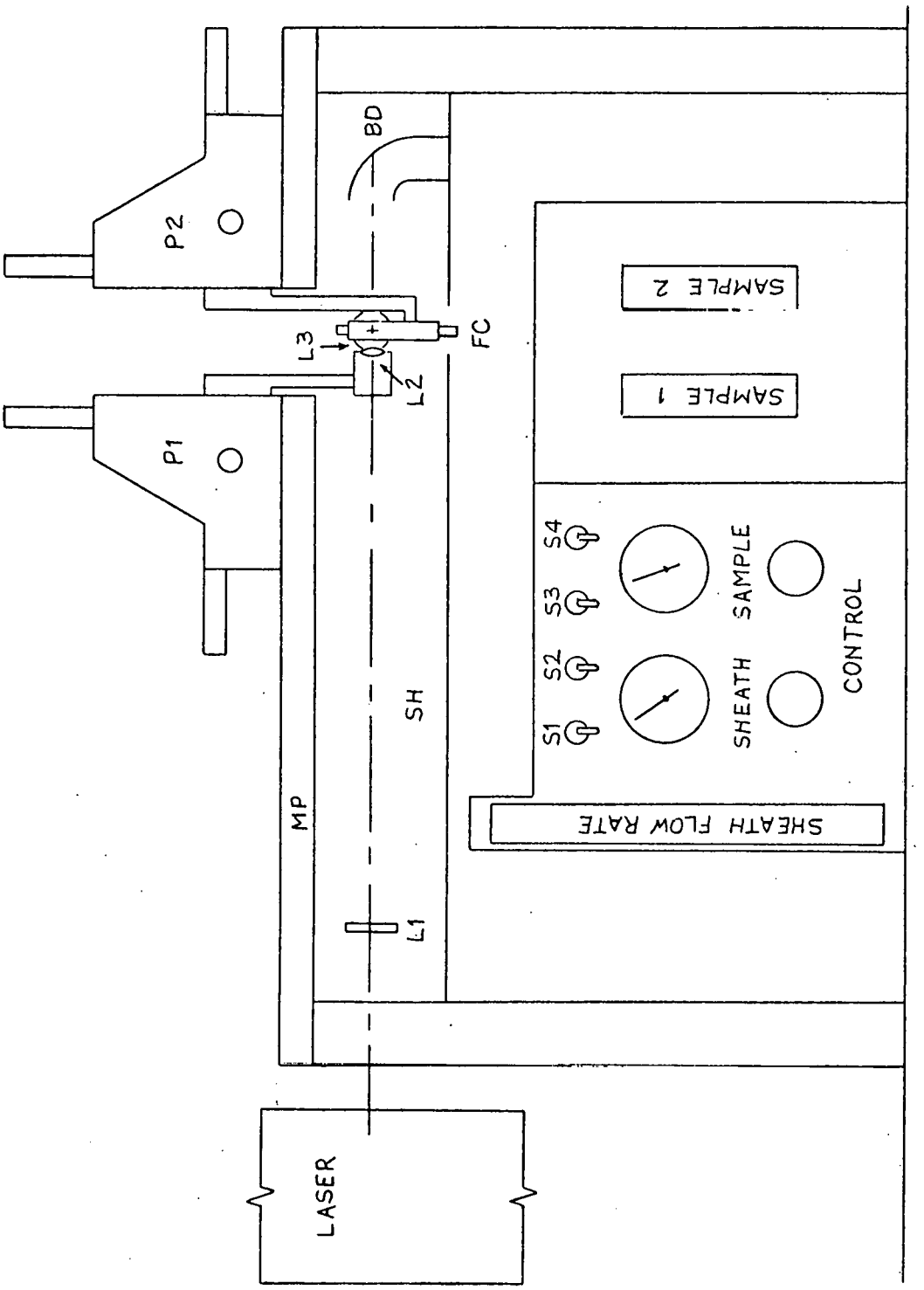
BD
P2
SAMPLE 2
SAMPLE 1
FC
L3
L2
P1
S4
S3
S2
S1
SAMPLE
SHEATH
CONTROL
SH
MP
SHEATH FLOW RATE
L1
LASER

Figure 1b

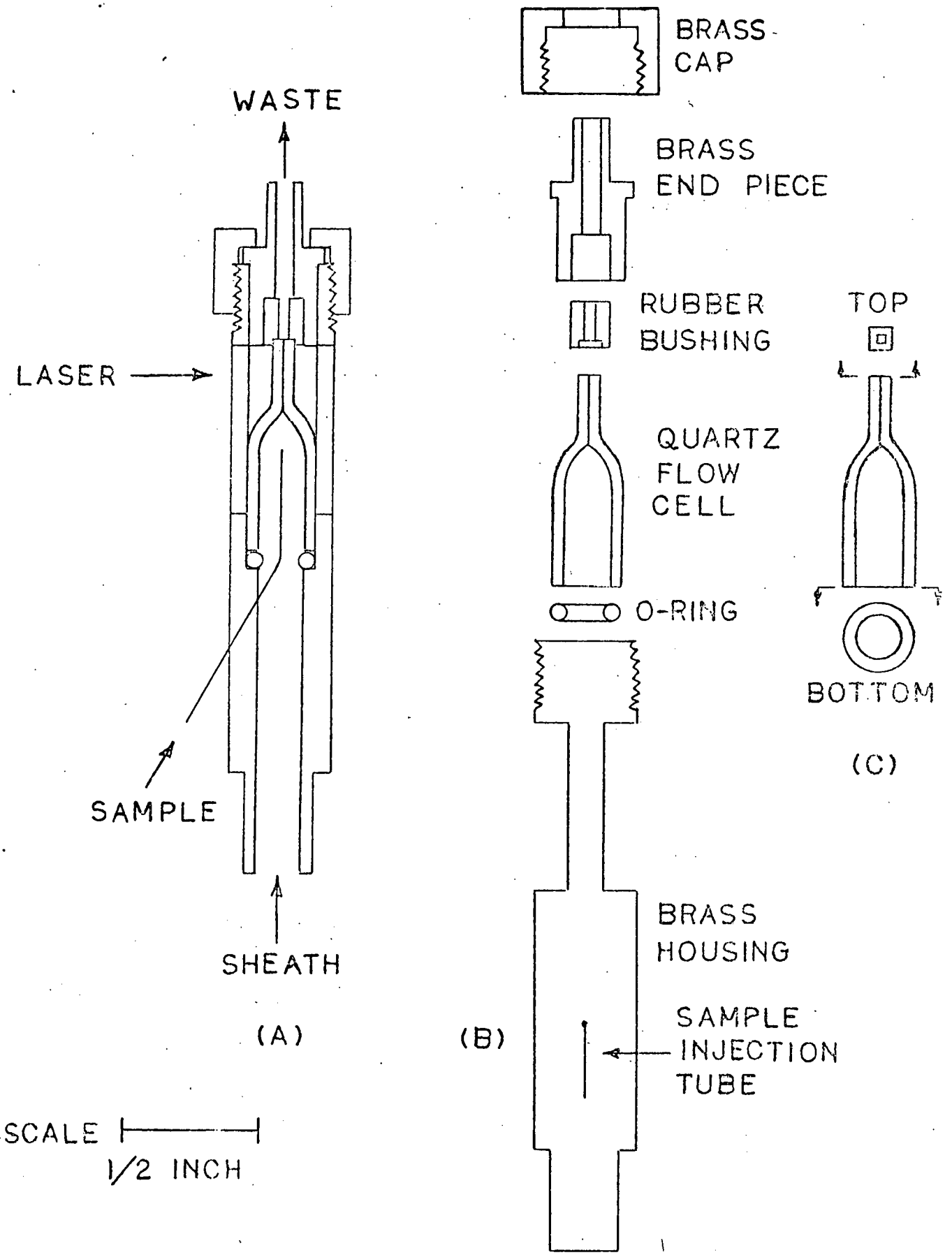
WASTE
LASER
SAMPLE
SHEATH
(A)
SCALE
1/2 INCH
BRASS CAP
BRASS END PIECE
RUBBER BUSHING
QUARTZ FLOW CELL
O-RING
BRASS HOUSING
SAMPLE INJECTION TUBE
(B)
TOP
BOTTOM
(C)

Figure 2

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