

## A Miniature Flow Cell Designed for Rapid Exchange of Media Under High-power Microscope Objectives

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(Received 29 May 1984)

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The design, fabrication and use of a flow cell that allows rapid displacement of media viewed by short working distance, high power objectives are described. The cell has a small internal depth (about 0.04 cm), small volume (about 0.05 ml), and is chemically inert. It has been tested extensively in studies of tethered bacteria.

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### INTRODUCTION

A bacterial flagellum is driven by a reversible rotary motor powered by a protonmotive force. The direction of rotation of the motor depends, in part, on signals generated by sensory systems, e.g. those that analyse chemical stimuli. The rotation of the motor can be observed directly by fixing the flagellar filament to a glass slide or cover slip; the cell body spins alternately clockwise and counterclockwise (Silverman & Simon, 1974). These cells are called tethered cells. In using this technique to study the dynamics and energetics of the flagellar motor (e.g. Manson *et al.*, 1980; Khan & Berg, 1983) and the chemotactic response (e.g. Berg & Tedesco, 1975; Block *et al.*, 1983), it is convenient if the medium bathing the cells can be displaced in a controlled manner. To this end, we have developed a miniature stainless steel flow cell. It has a small internal volume and means for dispersing fluid uniformly from side to side, so media can be exchanged rapidly; it is shallow, so that the inner surfaces of both windows can be viewed with short working distance objectives; and it is chemically inert, so that it does not generate spurious chemical stimuli yet can be cleaned thoroughly. Interest in this device by others prompts this report.

### METHODS

*Flow cell design.* Top, side and bottom views of the cell are shown in a scale drawing (Fig. 1). The cell is enclosed on the top and bottom by two glass cover slips, one 12 mm in diameter (no. 1, Carolina Biological Supply, Burlington, NC, USA), the other 22 mm in diameter (no. 2, widely available). Solutions are drawn into the cell through a stainless steel pipe (a, Fig. 1) and then via a connecting hole (b) into a transverse channel (c), and finally through a thin longitudinal channel (d) into the centre of the cell. The solutions leave by a symmetrical pathway. The details of these interconnections are given in an expanded side view (Fig. 2). The transverse channel is relatively deep (0.041 cm, plus the thickness of the cement used to attach the bottom window), and the longitudinal channel is relatively shallow (0.008 cm, plus the same thickness). For laminar flow, the pressure drop per unit length of a rectangular channel carrying fluid at a constant rate is inversely proportional both to the width of the channel and to the cube of its depth. Therefore, the pressure drop per unit length of the transverse channel is about 4% of that of the longitudinal channel, and the transverse channel acts as a constant pressure source that feeds fluid into the longitudinal channel along its entire width. The flow through the cell is nearly uniform from side to side.

*Flow cell fabrication.* Corrosion-resistant stainless steel is difficult to work; the services of an accomplished instrument maker are recommended. The fixture shown in Fig. 3 was developed so that a number of units could be made with the same setup (for reasons of economy) and the work would be supported from the back when turned or milled from the front (to prevent bending). The following fabrication steps are suggested (repeated, as necessary, for multiple units); see the dimensions in Figs 1 and 2. (1) Mill or grind 316-stainless plates to the finished length, width and thickness. (2) Centre the fixture in a four-jaw chuck in a lathe and indicate all surfaces true. (3) Clamp a plate in the fixture, bore the centre hole (0.762 cm diameter), and sink the recess for the bottom

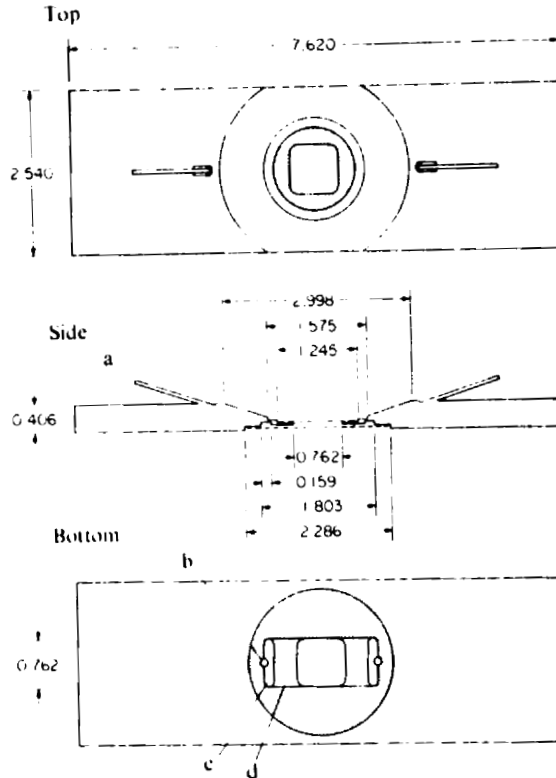


Fig. 1. Scale drawings of the flow cell (top, side, and bottom views, with dimensions in cm). The cell is enclosed on the top and bottom by glass windows, shown schematically in the side view by dashed lines. The seats for these windows are shown shaded. The cell is made from 316-stainless steel plate and 22-gauge stainless steel tubing (0.071 cm o.d.). Fluid moves into the cell through pipe a, access hole b, transverse channel c, and longitudinal channel d. See the expanded side view (Fig. 2).

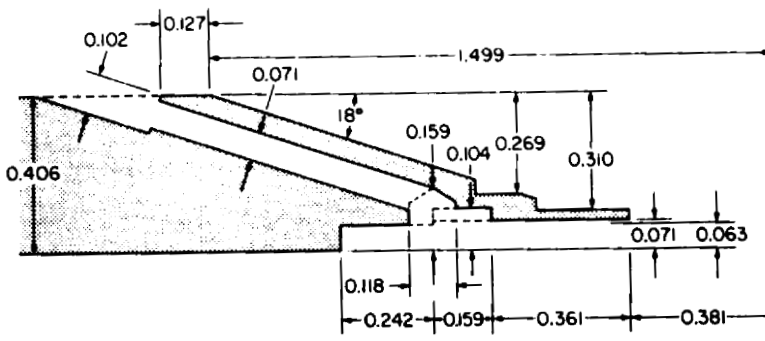


Fig. 2. A partial side view of the flow cell showing the slant-hole for the pipe and its connection to the transverse channel (dimensions in cm). Note that the inside depth of the cell at its centre is only 0.033 cm (ignoring the thickness of the material used to seal the top and bottom windows).

window. (4) Remove the piece, turn it over, insert support plug B (Fig 2), and clamp the piece once again. (5) Bore and turn the top two recesses. The smaller recess, the one for the top window, must be carefully worked, because the final thickness of its floor is quite small; use a finely-honed tool bit with no more than a 5° top rake and take very light cuts. (6) Turn the 18° clearance angle. (7) Transfer the chuck with the fixture in place to a dividing head on a milling machine and indicate the fixture level and square. (8) Tilt the dividing head 18° from the horizontal

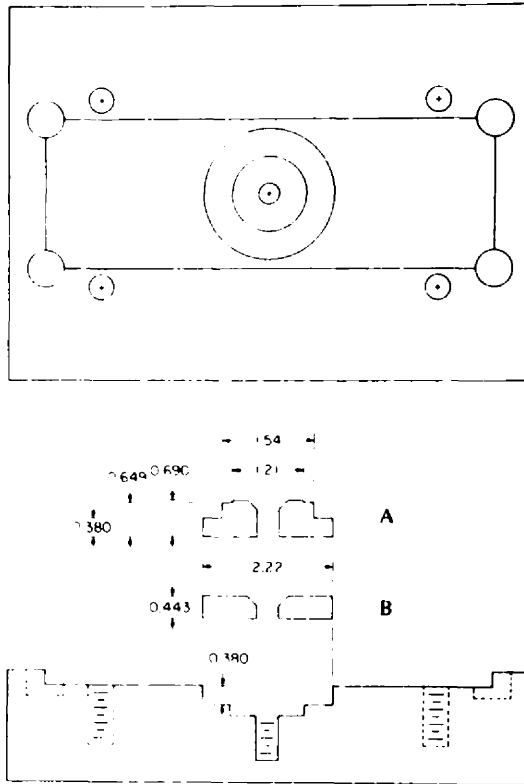


Fig. 3. The fixture used in the fabrication of the flow cell (top and side views, with dimensions in cm), including interchangeable support plugs A and B. The fixture is made of aluminium alloy; the plugs are made of stainless steel. The flow cell is held in the fixture by dogs (not shown) with bolts that fit into the four threaded holes. The plugs are held in place by a flat-headed screw (not shown).

and use a 1.5 mm ( $\frac{1}{16}$ -inch) carbide endmill to sink the troughs for the inlet and outlet tubing; remove the burrs. (9) Return the dividing head to horizontal, remove the piece, turn it over, change from support plug B to support plug A (Fig. 3), and clamp the piece once again. (10) Use a 3 mm ( $\frac{1}{4}$ -inch) carbide endmill to square the centre hole; leave the corners round. (11) Use a 1.2 mm (no. 56) drill to sink connecting hole b (Fig. 1). (12) Use a 1.5 mm ( $\frac{1}{16}$ -inch) carbide endmill to sink lateral channels c (Fig. 1); use light cuts. (13) Use a 3 mm ( $\frac{1}{4}$ -inch) carbide endmill to sink longitudinal channels d (Fig. 1); use light cuts and a coolant. (14) Remove the piece and plug A. (15) Remove the fixture from the chuck and clamp it in a vice in a small drill press with the long axis  $18^\circ$  from the vertical. (16) Clamp the piece in the fixture and locate the starting point for the drill in the centre of the bottom of the trough. (17) Use a 0.7 mm (no. 70) drill with lubricant (e.g. Westlube, Westland Products, San Leandro, Calif., USA) to drill through to connecting hole b (Fig. 1); use medium speed, light feed, and frequent withdrawal to clear chips. If the drill breaks, dissolve it out by soaking the piece in a solution of concentrated aluminium ammonium sulphate (aluminium alum) at about  $100^\circ\text{C}$ ; add water to maintain the level; probe the hole with a sharp pick to expose the undissolved part of the drill. (18) Check the flatness of the seat for the top window; if necessary, lap it by hand. (19) Install the inlet and outlet pipes (22-gauge stainless steel tubing) with a light press fit, and fill the access troughs with clear epoxy. (20) Install the bottom window by applying a thin layer of translucent silicone cement (General Electric RTV 108) to the seat with a narrow applicator. Place a pair of 22 mm cover slips on this surface and press them home with both thumbs. Take care not to touch the inner surface of the cell. The cement should spread out in a nearly transparent film and not spill over into the flow channels; check for complete coverage. Remove and discard the outer (soiled) cover slip. If things are not right, pry the window off at once, and wipe the cement off with tissue soaked in ethanol ( $95^\circ$ ). Otherwise, let the cement set overnight. Later, if the window breaks soak the cell overnight in *n*-heptane, pry up the broken glass, and rub off the remaining cement.

**Flow cell use.** The flow cell should be clean. Particles stuck in the inlet or outlet pipes can be removed with a piece of fine wire (e.g. a 30-gauge syringe needle), and debris in channel d (Fig. 1) can be teased out with a strip of paper. Excess grease (see below) can be removed with tissue dipped in *n*-heptane and/or by drawing *n*-heptane through the cell; limit this exposure to about 1 min and chase the heptane with ethanol ( $95^\circ$ ) and then with

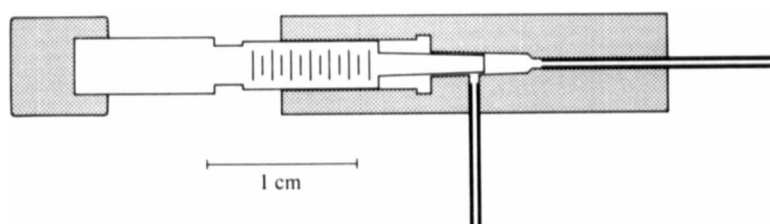


Fig. 4. A miniature needle valve used to restrict the rate of flow from the cell. The shaft and pipes are stainless steel, the shaded parts brass. The shaft and body mate with a fine thread (22 threads per mm) sealed with silicone high vacuum grease (Dow-Corning). The tapered hole was finished with a no. 7/0 taper pin reamer, and the tapered part of the shaft was turned to match. The pipes were soldered in place with a tin-silver solder.

distilled water. For work with tethered cells, apply a thin film of high-vacuum grease (Apiezon L) to the top seat, connect 15 cm or more of polyethylene tubing (Intramedic PE-50, 0.058 cm i.d., 0.097 cm o.d.; Clay Adams Div. of Becton, Dickinson, Parsippany, NJ, USA) to the pipes at either end of the cell, and backfill both ends of the cell with the desired medium using disposable glass syringes (B-D Glaspak, 2.5 ml, with 22-gauge needles shortened and honed smooth). Apply a thin film of grease to the edge of a 12 mm coverslip – one way to do this is to transfer grease from the end of a brass cylinder machined to about the same inside and outside dimensions as the top seat (Fig. 1) – tether cells to the cover slip in a drop of medium in the middle of the cover slip, wick away most of this liquid with a piece of tissue, place the cover slip on top of the brass cylinder (now free of grease), invert the flow cell over the cover slip so that the drops of medium coalesce, press gently downward to seat the cover slip, and turn the cell right-side up. Press the window home gently with forceps, rinse and wick away any medium that has spilled onto the outside of the cover slip, and place the cell on the microscope stage. A convenient way to control the flow through the cell is to connect the outlet tubing to a side port of a small three-port valve (Hamilton no. 1XP-3-NNN-2) which is connected by its centre port to a vacuum trap via a miniature needle valve (Fig. 4). The other side port is left open so that liquid can be blown out of the vacuum line. Insert the inlet tubing of the cell into one of a series of test tubes containing the appropriate medium. Switch the valve to the side port connected to the cell and adjust the needle valve to the desired rate of flow ( $0.05 \text{ ml s}^{-1}$  or less). When the three-port valve is closed, the input tubing can be switched to other test tubes at will. Alternatively, the flow can be controlled with a peristaltic pump (e.g. Minipuls II, Gilson, Middleton, Wis., USA, but see below). During flow, the pressure of the medium inside the cell is less than atmospheric, so flexing of the cover slips occurs. This alters the focus of tethered cells. Changes due to roller-to-roller transitions of peristaltic pumps can be minimized by insertion of surge filters (Block *et al.*, 1983). Also, bubbles of air may eventually appear, even though the cell is completely airtight. This problem can be serious during long experiments or with high flow rates. It can be minimized by autoclaving the medium (to remove dissolved air) and, if oxygen is required, bubbling through 80% He/20% O<sub>2</sub> (Block *et al.*, 1983).

*Flow cell calibration.* The exchange properties of the cell were measured by drawing solutions of blue dye through the cell and monitoring the transmittance at its centre with a microscope modified to serve as a spectrophotometer. We used Blue Dextran 2000 (0.4% in water) and bromophenol blue (0.08% in 1 mM-NaOH), a Nikon S-Ke scope equipped with a 50 W tungsten-halogen lamp powered by a regulated direct current supply, a 620 nm bandpass filter (Esco Optical Products, 50% T, 10.6 nm half-width), a 20× objective, a 20× ocular, and a photodiode detector (United Detector Technology PIN-SDP with National operational amplifier LF356) linked to a strip chart recorder.

## RESULTS

The time courses of change in optical thickness of Blue Dextran and bromophenol blue are shown in Fig. 5(a, b). The curves for Blue Dextran are somewhat irregular, and wash in is more rapid than wash out; the curves for bromophenol blue are quite regular and symmetrical; both experiments were highly reproducible. The differences between the two sets of curves are due to diffusion; Blue Dextran has a molecular weight of two million, bromophenol blue of about 600; their diffusion coefficients differ by a factor of about 15. Thus, irregularities in concentration smooth out more rapidly with bromophenol blue. However, with either dye, the exchange is complete sooner than expected for a parabolic flow profile (indicated in Fig. 5 by the dashed and dotted line). While we do not understand this anomaly, it is a favourable one, because bacteria

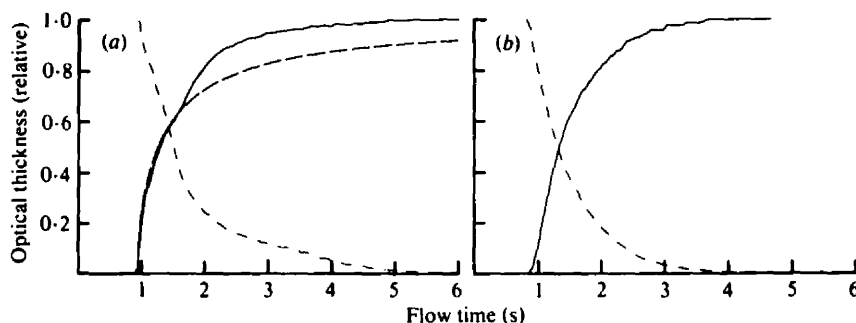


Fig. 5. Time course of the change of optical thickness of blue dye near the centre of the flow cell with inlet tubing 15 cm long and a flow rate of  $0.05 \text{ ml s}^{-1}$  generated by connection to a vacuum line. (a) Blue Dextran, (b) bromophenol blue. Wash in is shown by the solid curves, wash out by the dashed curves. These curves were obtained from strip chart recordings of the intensity of transmitted light,  $I$ , assuming the validity of Beer's law, i.e. that  $I(d) = I(0) \exp(-\epsilon Cd)$ , where  $\epsilon$  is the extinction coefficient of the dye,  $C$  is its concentration, and  $d$  is the optical thickness of the layer of dye along the axis of the microscope. Since  $\epsilon$  is concentration dependent, this calibration is more accurate than one based on measurements of serial dilutions. The high-frequency fluctuations in the curves are due primarily to errors in digitizing the strip-chart records. Parabolic flow would generate a curve (shown for wash in as a dashed and dotted line in a) of the form  $h(1 - x/v_m t)^2$ , where  $h$  is the cell depth,  $x$  the longitudinal displacement at the point of observation,  $v_m$  the maximum fluid velocity, and  $t$  the time (cf. Landau & Lifshitz, 1959, p. 56, or Berg, 1983, pp. 53–54).

tethered to either coverslip can be exposed to reagents more rapidly than would be possible otherwise (see below).

The situation is more complicated when the flow is driven by a peristaltic pump. With the Gilson Minipuls II (without surge filters; pulsation frequency 3.5 Hz at a flow rate of  $0.05 \text{ ml s}^{-1}$ ) wash in of bromophenol blue was similar to that shown in Fig. 5(b) but delayed about 0.3 s, while wash out was delayed initially by a similar time but was completed much sooner (95% at 2.2 s; data not shown). This asymmetry was smaller at smaller flow rates. Evidently, these effects have to do with non-linearities in the flow that couple the periodic motion introduced by the peristaltic pump, which tends to give blunt flow profiles (cf. Landau & Lifshitz, 1959, pp. 88, 89, 95), to steady motion, which normally gives parabolic flow (ibid, pp. 55–57). These effects are not due to turbulence, because the Reynolds numbers are too small (at a flow rate of  $0.05 \text{ ml s}^{-1}$ , 115, 52, 14 and 13, in the polyethylene tubing, the transverse channel, the longitudinal channel and the centre of the cell, respectively; cf. Goldstein, 1965, Chapter 7). Given these complications, if the exchange properties of a cell need to be known in detail, it should be calibrated with a dye having a diffusion coefficient roughly equal to the substance of interest under the conditions of the relevant experiments. Do not use methylene blue, except for wash in experiments (Berg & Tedesco, 1975), because it adsorbs strongly to surfaces and washes out slowly.

Diffusion does play an important role for work with tethered cells, because fluid near the cover slips moves more slowly than fluid farther away and, hence, exchanges more slowly (see also Kerner & Koshland, 1984). Note (Fig. 5b) that the exchange of bromophenol blue is 95% complete after about 2.7 s. If layers of fluid near the cover slips have not been affected by this time, they soon will be, because these layers are at most  $y = 0.5 \times 0.05 \times 0.033 \text{ cm} = 8 \times 10^{-4} \text{ cm}$  thick. Diffusion of a molecule with diffusion coefficient  $D = 5 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$  will occur over this distance in a time of about  $t = y^2/2D = 0.07 \text{ s}$  (cf. Berg, 1983, Chapter 1). At earlier times, say 1.8 s, this is not the case, because then the exchange is only about 75% complete, and diffusion must occur over a distance 5 times as great. This takes  $5^2 = 25$  times as long, or about 1.8 s. Thus, even with a flow cell as small as this one, it is difficult to expose tethered cells to variations in concentration in periods shorter than a second. One can change concentrations much more rapidly with iontophoretic techniques (Segall *et al.*, 1982; Block *et al.*, 1982); however with iontophoresis, absolute concentrations are not as well determined.

We thank Jim Gilliam and Gene Nettesheim for perfecting the fabrication techniques and Markus Meister and Ted Wu for discussions of hydrodynamics. This work was supported by grants PCM-8215126 from the US National Science Foundation and AI-16478 from the US National Institutes of Health.

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