

Flow Cytometry for Undergraduate Students

John T. Boothby and Ruthann Kibler, San Jose State University, San Jose, CA

Why Teach Flow Cytometry to Undergraduate Students?

Flow cytometry has revolutionized our understanding of cells, the basic unit of life. The technology combines physics, chemistry, engineering, information science, and cell biology and provides students with an opportunity to integrate the many disciplines that form the foundation of undergraduate education in biology. Presently, more cells are individually analyzed using flow cytometry than all other methods combined; yet few undergraduate students understand the basics of this instrument or how to interpret the data it generates. Textbooks often describe what a flow cytometer is, but none tells how it works or what the data mean (1,2). Most graduates from biology programs are able to identify the salient features of a transmission electron micrograph (TEM) of a cell (Figure 1A). Some students know something about TEM and perhaps can properly identify the cell. Few biology graduates could summarize flow cytometry data (Figure 1B).

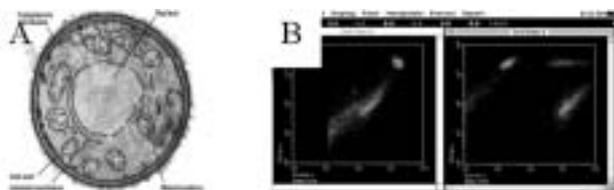


Figure 1. TEM of cell (A) and flow cytometry dot plot (B) (side scatter versus fluorescence) of a mixture of fluorescent-labeled yeasts and phagocytic protozoa.

The flow cytometer is now a cornerstone instrument for analyzing cells (Figure 2) (3–5). Paradoxically, although many bacterial, fungal, protozoal, algal, mammalian, and plant cells are routinely analyzed using flow cytometry, the instrument and its data remain enigmatic to most biologists. A search of biological abstracts for “flow cytometry” yielded more than 30,000 citations since 1980 and more than 3,000 in the last 12 months alone (6). However, our published report is one of two describing flow cytometry in undergraduate teaching, and it is the only one describing its use in laboratory courses (7,8). We believe all graduates from biology programs should understand the basics of flow cytometry and how to interpret the data it generates in the same way they understand the light microscopy and electrophoresis.

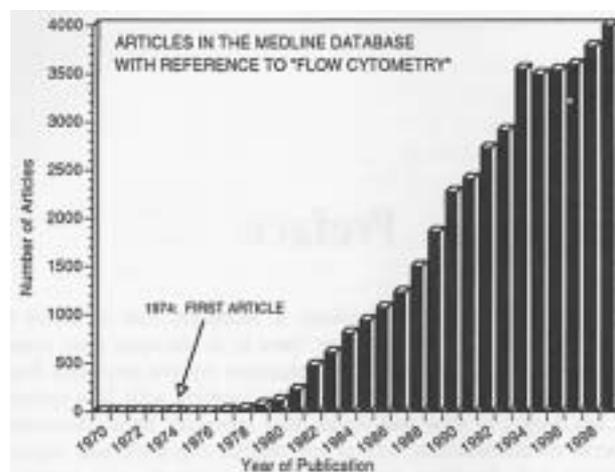


Figure 2. Yearly incidence of citations with reference to flow cytometry (1970–2000) (3).

What Is Flow Cytometry?

The flow cytometer works by focusing a laser beam on individual cells as they flow through an interrogation point. An "optical fingerprint" is generated for each cell within a population as it passes through the beam. When cells are labeled with fluorescent probes, several parameters of each cell can be determined simultaneously. Since the cells are interrogated at a rapid rate, data on thousands of cells can be acquired in seconds (Figure 3).

Our Experiences Teaching Flow Cytometry

Teaching flow cytometry to undergraduate students

Flow cytometry instruction is now integrated into our lower-division and upper-division core courses at San Jose State University (SJSU) and has been introduced throughout the country through flow cytometry teaching workshops for teachers (9). In our general microbiology laboratory course at SJSU, we use an inquiry-based approach to study factors

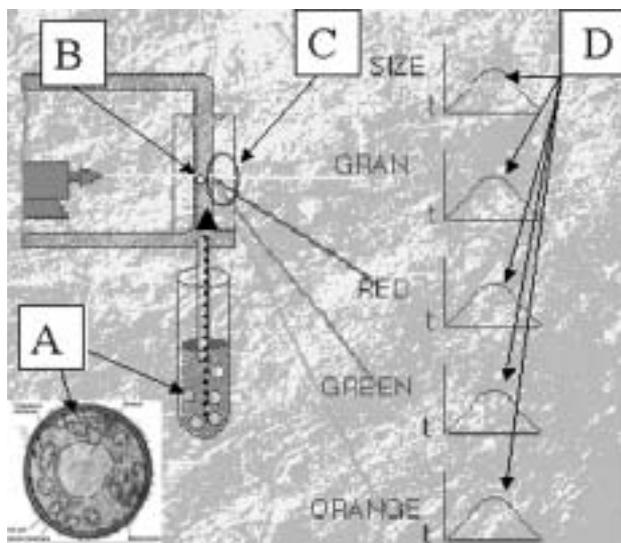


Figure 3. Flow cytometry schematic. Cells in a sample (A) are guided to an interrogation point (B), where they are excited with a laser beam. The emitted light (C) is channeled to photodetectors, where the light intensity of the emitted light in each channel by each cell is measured (D). A computer compiles the data for analysis into a graphical format such as a dot plot. The process of measuring and recording these values is termed "data acquisition." The process of deriving information from the acquired data is termed "data analysis" and does not require a flow cytometer.

affecting phagocytosis of fluorescent-labeled yeast by *Tetrahymena sp.* (7,8). Students in small groups design experiments; the class discusses them and chooses one to perform as a class experiment. The instructor produces a completed protocol using the variables and controls chosen by the students. In past semesters, we have studied ingestion time and digestion time, as well as the effect of temperature, media composition, prey viability, prey size, pH, salt concentration, motility, and culture age (7,10). Importantly, the experiments are not pre-run, and neither students nor instructors know the outcome beforehand. These experiments are simple, fast, and inexpensive, and they are *designed and conducted by students*, placing the *student* at the center of the decision-making process (11–18).

Flow cytometry has been established as a core component of our undergraduate curriculum in biological sciences (Table 1). Each year, approximately 400 students use the cytometer and analyze their data files. When outreach efforts across the country are included, thousands of student educational experiences have benefited from flow cytometry instruction (9).

We have shown that these educational experiences enhanced the ability of undergraduate students to understand biology, analyze data, and appreciate computer-aided data analysis (7,9). Student perceptions of their learning experiences were assessed using questionnaires after the flow cytometry exercises in their General Microbiology course at SJSU (Table 1) over a 3-year period (2001–2003). Students felt that these flow cytometry experiences had enhanced their understanding of biology, their ability to analyze data, and their appreciation for computer-aided data analysis (Table 2). Responses indicated that students had learned about flow cytometry and microbiology, that instructional and instrument support for the activities enhanced their experience, and that they would like to see flow cytometry incorporated into other classes (Table 3). For each of the 3 years included in the table, the overall median for the ratings is 5.0 (data not shown). Thus, the evidence presented here demonstrates that students viewed their exposure to the flow cytometry curricula as a highly positive experience.

Flow cytometry workshops for teachers

More than 80 faculty and staff have been trained in teaching flow cytometry to undergraduate students through a series

Table 1. Overview of Courses and Class Time for Flow Cytometry Instruction at SJSU, 2001–2003

Course	Student population	Experimental problem(s)	Instructional time (hours)
Introduction to Cell Biology	Biology and biochemistry majors	Plant cell cycle Location of the meristem	4 (1, 3)*
General Microbiology	Biology and biochemistry majors	Tetrahymena phagocytosis	11 (2, 9)
Immunology	Microbiology/molecular/physiology/general biology majors MA/MS students	Live/dead discrimination Phagocytosis/opsinization Cell surface molecule identification Differential counts	5.5 (1.5, 4) 7 (2, 5) 3 (1, 2) 3 (1,2)
Microbial Physiology	Microbiology majors	Cell growth, GFP expression	4 (1, 3)
Hematology	Microbiology majors	Immunophenotyping	7.5 (2.5, 5)
Immunologic Techniques	MA/MS students Undergraduates	Isotype determination	5 (2, 3)
Flow Cytometry Analysis	MA/MS students Undergraduates	Analysis of data files of experimental problems—flow cytometry without a cytometer	30

*Lecture, laboratory/analysis hours.

Table 2. Student Assessment of Flow Cytometry Educational Experiences over a 3-Year Period (2001–2003)

Has your experience with flow cytometry enhanced your:	Year 1		Year 2		Year 3		All Years	
	Yes	No*	Yes	No*	Yes	No*	Yes	No*
Ability to understand biology?	43	6	50	2	118	11	211	19
Ability to analyze data?	49	0	51	1	120	9	220	10
Appreciation for computer-aided data analysis?	48	1	52	0	115	14	215	15

*Answer either “no” or “not applicable.”

Table 3. Areas to Measure Students' Perceptions of Flow Cytometry Laboratory Experiences that Were Included on Post-Lab Assessment for 3 Years (2001–2003)

Questions*	Year 1 (mean + SD)	Year 2 (mean + SD)	Year 3 (mean + SD)	All years (mean)
This class enhanced my general knowledge of flow cytometry.	4.59 + 0.76	4.33 + 0.81	4.33 + 0.88	4.42
This class enhanced my ability to understand microbiology.	4.27 + 0.78	4.35 + 0.40	3.64 + 1.20	4.09
The written material for this class was useful.	4.49 + 0.68	4.46 + 0.80	4.20 + 0.87	4.38
The instructors were well prepared and knowledgeable.	4.69 + 0.55	4.56 + 0.83	4.57 + 0.73	4.61
The facilities were adequate to support the class.	4.59 + 0.73	4.44 + 0.80	4.25 + 0.89	4.43
I would like to see flow cytometry used in other classes that study cells.	4.73 + 0.72	4.61 + 0.60	4.29 + 0.90	4.54
Overall rating	4.56 + 0.70	4.46 + 0.80	4.22 + 0.96	4.41

*Responses were on a five-point scale that varied from strongly disagree (1) to strongly agree (5).

of seven workshops across the country. These educators received hands-on training in flow cytometry procedures and explored strategies for integrating this technology into their teaching. We predict that these teachers will enhance the educational experiences of their students as a result. We are continuing to collaborate with many of them to further develop materials for teaching flow cytometry, and several make use of our flow cytometer and teaching materials in their courses at nearby 2- and 4-year institutions.

Assessment of workshop activities was accomplished using a post-workshop questionnaire. The questionnaire contained 19 items. Thirteen of these were yes or no questions used to assess the previous flow cytometry experience of the participants and their degree of interest in developing flow cytometry teaching modules. Responses to six of the questions were assessed on a five-point scale that ranged from 1 (low) to 5 (high) (Table 4). The median score for each of these items for the workshop participants was 5.00 (data not shown), and the overall mean for all of the responses to these questions was 4.68 +/- 0.57. Assessment showed

consistent, highly positive ratings. The workshop participants all believed that what they had learned had enhanced their ability to teach flow cytometry. They indicated that they were highly motivated to collaborate in developing flow cytometry teaching modules.

Presenting flow cytometry at professional meetings

Our work has been well received at regional and national meetings (10) and in a peer-reviewed publication (7). These experiences presenting our work at meetings of professional societies through eight oral presentations, seven workshops in teaching flow cytometry for faculty and staff, and five posters have convinced us of the value of both science education-based dissemination and academic discipline-based dissemination. Professional networking through regional and national meetings, although difficult to document, represents a powerful dissemination opportunity. Through our professional networking and flow cytometry workshops for teachers, we are in contact with dozens of educators throughout the country who intend to use flow cytometry

Table 4. Assessment of Flow Cytometry Teaching Workshops for Teachers (2001–2003)

For the following statements, circle the number on the 1–5 scale provided that best describes your workshop experiences:

	Strongly disagree	1	2	3	4	Strongly agree	Not applicable
This workshop enhanced my general knowledge of flow cytometry.	1	2	3	4	5	NA	
This workshop enhanced my ability to integrate flow cytometry in my teaching.	1	2	3	4	5	NA	
The written material used in this workshop was useful.	1	2	3	4	5	NA	
The instructors were well prepared and knowledgeable.	1	2	3	4	5	NA	
The facilities were adequate to support the workshop.	1	2	3	4	5	NA	
I want to collaborate to develop future teaching modules for my courses.	1	2	3	4	5	NA	

teaching materials when they become available (9).

We have successfully addressed numerous challenges in establishing flow cytometry as a centerpiece technology for undergraduate education (Table 5).

In meeting these challenges, we have discovered solutions that are attainable and will bring this important, integrative technology into biology classes across the country. Given the preeminence of flow cytometry in analyzing cells from such a wide array of sources, why isn't flow cytometry commonly used to teach undergraduate students about cells? The major challenges to teaching flow cytometry include the high cost of a flow cytometer, limited expertise among teaching faculty, and a paucity of good teaching materials. Our collective 12 years of experience teaching flow cytometry has led us to attainable solutions to these challenges.

Challenge #1: Most college biology teachers have insufficient flow cytometry experience to teach the principles of flow cytometry.

Solution: Develop supportive instructional materials using simple concepts and applications for flow cytometry. We have developed and used our materials in workshops for 2- and 4-year college teachers who have introduced flow cytometry into their courses.

Challenge #2: Most colleges don't have a flow cytometer.

Solution: Teach flow cytometry analysis *without a flow cytometer* using archived flow cytometry data files and computer analysis programs. Although these students may not perform lab work or acquire data, they learn what a flow cytometer does, how it is done, and what the data mean. This approach uses discovery, active learning, and critical thinking. Integrative concepts from chemistry, physics, and biology can be used.

Challenge #3: Flow cytometry analysis software currently available is too expensive and/or too complex. These programs are often not intuitive and not compatible with both Mac and PC operating systems.

Solution: We have developed an intuitive, easy-to-use flow cytometry analysis program using the Java platform. The program is small, compatible with virtually all operating systems, and free. Students can analyze their data files at home.

Challenge #4: Flow cytometry can be complex.

Solution: We have developed flow cytometry laboratory modules and flow cytometry analysis modules (for classroom use without a flow cytometer) for a wide range of academic levels from basic plant biology or cell biology classes, to three-color lymphocyte immunophenotyping modules for

Table 5. Accomplishments of DUE 0088141: Laboratory Instruction in Flow Cytometry

- Purchased a flow cytometer and established a flow cytometry laboratory
- Trained 14 SJSU faculty and staff in flow cytometry
- Conducted seven regional and national workshops for 70 faculty and staff in teaching flow cytometry
- Developed six flow cytometry teaching modules at SJSU for use with a flow cytometer
- Developed eight flow cytometry teaching modules at SJSU for use without a flow cytometer
- Integrated flow cytometry into the biology curriculum (five courses)
- Enhanced the education of thousands of students nationally through workshops for faculty (9)
- Acted as a resource for flow cytometry in our region
- Created web-based instructional aids (http://www.sjsu.edu/depts/Biology/specialprogs/flow_cytometry.html)
- Produced an easy-to-use flow cytometry analysis program designed for undergraduate teaching
- Established a consortium of faculty interested in developing flow cytometry curriculum
- Made eight flow cytometry educational presentations at professional meetings
- Presented five posters on flow cytometry education at regional and national meetings
- Published a paper on teaching flow cytometry to undergraduates in a peer-reviewed professional journal

advanced cell biology or immunology upper-division classes. In our experience, introducing flow cytometry incrementally, starting with simple but stimulating modules, makes learning advanced concepts easy.

Conclusions

These solutions will produce teachers and students who understand flow cytometry. We have demonstrated that undergraduate students are capable of understanding flow cytometry, analyzing data files, and applying these skills to draw scientific conclusions (7,9,10). Flow cytometry laboratory exercises have proven to be ideal for teaching about cells using inquiry-based formats. The flow cytometer provides rapid acquisition of experimental data and is easy for students to operate in simple applications once the flow cytometer is calibrated. Our newly developed flow cytometry analysis software was designed specifically for teaching undergraduate students and is intuitive. Extracting meaningful data from thousands of cells for up to four parameters is straightforward. This exciting technology should be a part of every biology student's undergraduate experience.

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