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An active, collaborative approach to learning skills in flow cytometry

Kathryn Fuller,[1] Matthew D. Linden,[1,2] Tracey Lee-Pullen,[3,4] Clayton Fragall,[1] Wendy N. Erber,[1] and Kimberley J. Röhrig[1]

[1]*School of Pathology and Laboratory Medicine, University of Western Australia, Crawley, Western Australia, Australia;* [2]*Centre for Microscopy, Characterisation and Analysis, University of Western Australia, Crawley, Western Australia, Australia;* [3]*Bendat Family Comprehensive Cancer Centre, St John of God Subiaco Hospital, Perth, Western Australia, Australia; and* [4]*School of Surgery, The University of Western Australia, Crawley, Western Australia, Australia*

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Fuller K, Linden MD, Lee-Pullen T, Fragall C, Erber WN, Röhrig KJ. An active, collaborative approach to learning skills in flow cytometry. *Adv Physiol Educ* 40: 176–185, 2016; doi:10.1152/advan.00002.2015.—Advances in science education research have the potential to improve the way students learn to perform scientific interpretations and understand science concepts. We developed active, collaborative activities to teach skills in manipulating flow cytometry data using FlowJo software. Undergraduate students were given compensated clinical flow cytometry listmode output (FCS) files and asked to design a gating strategy to diagnose patients with different hematological malignancies on the basis of their immunophenotype. A separate cohort of research trainees was given uncompensated data files on which they performed their own compensation, calculated the antibody staining index, designed a sequential gating strategy, and quantified rare immune cell subsets. Student engagement, confidence, and perceptions of flow cytometry were assessed using a survey. Competency against the learning outcomes was assessed by asking students to undertake tasks that required understanding of flow cytometry dot plot data and gating sequences. The active, collaborative approach allowed students to achieve learning outcomes not previously possible with traditional teaching formats, for example, having students design their own gating strategy, without forgoing essential outcomes such as the interpretation of dot plots. In undergraduate students, favorable perceptions of flow cytometry as a field and as a potential career choice were correlated with student confidence but not the ability to perform flow cytometry data analysis. We demonstrate that this new pedagogical approach to teaching flow cytometry is beneficial for student understanding and interpretation of complex concepts. It should be considered as a useful new method for incorporating complex data analysis tasks such as flow cytometry into curricula.

active learning; collaborative learning; inquiry-based learning; experiential learning; next generation learning spaces; flow cytometry; hematology; cell biology; science, technology, engineering, and mathematics education

ADVANCES IN SCIENCE EDUCATION RESEARCH have the potential to improve the way we teach methods of scientific analysis to students in higher education. In science programs in particular, there have been widespread calls to change teaching methods to be more aligned with evidence-based educational research (1, 12, 18).

Flow cytometry is a technology widely used in biological science applications, where cells or other particles are suspended in a stream of liquid and passed through a detection mechanism. The technology employs fluorescent-labeled molecular probes to characterize the cells of interest, and as these cells pass through the detector, the flow cytometer records the fluorescent signature detected for each cell. The output is in the form of dot plots and histograms, which indicate what proportion of the cells present are positive for a given marker. By using multicolored combinations of probes in a single sample, complex sequential gating analyses can be performed to describe numerous cell populations, for example, to characterize leukocyte subpopulations in human blood during an investigation for malignancy. Several authors have described the use of flow cytometry as a tool to teach concepts, for example, in microbiology (3, 9) and pharmacology (22), but no studies have explored new methods to teach students the skills of using flow cytometry data.

Increasingly universities are using active, collaborative methods to teach students to perform scientific interpretations and understand science concepts (11, 23, 24). Most recently, laboratory classes in science subjects are being rewritten to include more student inquiry (15, 28). In inquiry-based classes, students devise their own strategies to solve scientific questions. Inquiry-based activities complement experiential learning, a pedagogically effective and powerful method that focuses on the learning processes of the individual (10). Such inquiry-based approaches promote deep learning of scientific concepts (8, 19), develop critical thinking skills (17), and can positively influence interest in science as a career (2). The benefits of group learning are well established (13), and group work is a hallmark of active and inquiry-based classes.

While the benefits of practical, experiential, and inquiry-based approaches on the learning process are therefore well known, applications in cytometry are often limited by instrument access and the ability to accommodate large classes. However, alongside improvements in pedagogy, advances in learning space design are also driving change within higher education science programs. Active, collaborative learning spaces, sometimes called next-generation learning spaces, are specifically designed to support active learning and cooperation between students (26, 27). Key features of active classrooms are decenteredness, where the emphasis is placed on students rather than on the teacher, and the ubiquitous presence of technology in the form of computer terminals and support for mobile and laptop devices (5).

We developed two short programs, one program tailored for undergraduate student education in the context of a pathology course and one program for researcher training in the context of a core facility induction, to teach concepts and skills in manipulating flow cytometry data using FlowJo software in an active learning space. The programs drew on recent develop-

Address for reprint requests and other correspondence: K. J. Roehrig, The Univ. of Western Australia, 35 Stirling Highway, M504, Crawley, WA 6009, Australia (e-mail: kimberley.roehrig@uwa.edu.au).

ments in educational research, incorporated active collaboration between students and elements of inquiry-based learning, and were evaluated using student and teacher surveys.

METHODS

Students/participants. The University of Western Australia Human Research Ethics Committee approved the research described in this study (RA/4/1/6051 and RA/4/1/6498).

Undergraduate students enrolled in the Pathobiology of Human Disease unit were given a compulsory tutorial in flow cytometry as part of their third-year curriculum. A single 3-h tutorial on flow cytometry was included in a semester of study of pathology. Two separate undergraduate cohorts were assessed using slightly different survey methods (see *Surveys* below).

The research trainee cohort enrolled in a 3-day course to learn the basics of flow cytometry, forming a prerequisite for induction into the flow cytometry core facility (Centre for Microscopy, Characterisation and Analysis, University of Western Australia). The 3-h active tutorial was delivered within the 3-day program. These students were primarily Honours students, PhD students, or staff members who intended to apply flow cytometry analysis to their own projects.

Facility. An active, collaborative learning facility was constructed at the University of Western Australia in 2013 for the education of students studying the biomedical sciences. The facility contains 29 tables, each accommodating six students, and each table is fitted with two built-in Mac Minis operated by wireless keyboards and mice, power outlets, network points, audio/visual input ports, and two wall-mounted LCD screens. High-capacity WiFi access is fitted throughout the facility.

FlowJo (version X) flow cytometry analysis software (FlowJo LLC, Ashland, OR) was installed on the Mac Minis built into the collaborative desks for students to perform analysis, and teachers used laptops fitted with FlowJo license dongles to do broadcasted demonstrations.

Activities. Two separate activities were tailored for undergraduate students and research trainees using the FlowJo software. In past years, students would learn the principles of flow cytometry through didactic demonstration and be asked to interpret flow cytometry data in the form of dot plots. The new activities, made possible by the installation of FlowJo software in an active learning facility, required students to interrogate data using a guided inquiry approach: data were supplied in the form of FCS files and students were asked to design their own gating strategy, draw gates, perform compensation (research trainee cohort only), and draw conclusions from the data. The tutorials commenced with brief introductions from tutors experienced in using flow cytometry, and students were then asked to manipulate data on their own. Throughout the class, tutors were present in ratios of ~10–15 students per tutor to answer questions and provide prompts if students were stuck or unsure what to do next. The learning outcomes of the new activities are shown in Table 1.

The undergraduate activity focused on the use of flow cytometry in medical practice. Compensation and antibody optimization were performed for the students, and deidentified clinical data were provided by PathWest Laboratory Medicine. Students were asked to construct a sequential gating strategy to diagnose three patients with different hematological malignancies on the basis of their immunophenotype. In a single lesson lasting ~3–4 h, students were first introduced to the principles of flow cytometry, given three sets of unknown patient data, and then asked to design a gating strategy and make a diagnosis. The antibody panels used are shown in Table 2. Students had previously been given a lecture on hematological malignancies and were provided with reference tables for characteristic immunophenotypes of acute lymphoblastic leukemia, acute myeloid leukemia, and chronic lymphocytic leukemia (Table 3). Students also had access to recommended course texts for further information on the immunophenotypic markers used in routine diagnostic analysis (20). The three patient cases increased in complexity: the first patient case consisted of 3 files with 3 flow cytometry panels providing data on 17 antigens, the second patient case consisted of 3 files and 22 antigens, and the third case of files with 6 panels and 30 antigens. Students were given the task of completing the first two patient cases, with the third patient case being optional for groups who wanted to extend themselves further.

The research trainee activity had a vocational focus and was targeted towards students using flow cytometry in research projects. Uncompensated FCS files were collected using a FACS Canto II flow cytometer (Becton Dickinson) and provided by the Centre for Microscopy, Characterisation and Analysis at the University of Western Australia. Students were required to perform their own compensation and antibody titration analysis. The samples and antibodies used are shown in Table 4. Tasks increased in complexity, and students progressed toward greater autonomy over three activities. In the first activity, students were provided with listmode files of three single-stained compensation controls and an uncompensated sample of peripheral blood mononuclear cells (PBMCs) labeled with all three antibodies. Students were asked to identify positive and negative staining peaks and to perform both automatic and manual compensation matrixes and apply them to the PBMC sample. In the second activity, students were provided with listmode files of PBMCs stained with five 1:2 serially diluted concentrations of tandem peridinin chlorophyll-cyanine 5.5-conjugated anti-human CD8a and asked to gate single lymphocytes, identify positive and negative peaks, calculate the staining index using a formula function, and determine the optimal antibody staining concentration. In the third activity, students

Table 1. *Learning outcomes for inquiry-based activities tailored to undergraduate students and research trainees*

	Undergraduate Students	Research Trainees
	By the end of this tutorial, students will be able to:	By the end of this tutorial, students will be able to:
Outcomes addressed in past tutorials limited to theoretical concepts and analysis of dot plots	Give examples of how flow cytometry can be used in medical applications	Give examples of the diversity of applications in flow cytometry
	Explain how flow cytometry is used to distinguish cell types in a mixed sample	Explain the relationship between voltage pulse, list mode data, and dot plots and histograms
	Interpret positive and negative populations in flow cytometry dot plots and histograms	Describe the process of automatic compensation
		Describe the process of titrating antibodies
Additional outcomes included in the active tutorial	Use flow cytometry software (FlowJo) to plot data, draw gates, and display statistics	Use flow cytometry software (FlowJo) to plot data, draw gates, and display statistics
	Construct a sequential gating strategy to identify and quantify cell subtypes in a sample	Construct a sequential gating strategy to identify and quantify cell subtypes in a sample
	Compare experimental flow cytometry data with known characteristic disease immunophenotypes to draw conclusions about a patient	Perform automatic and manual compensation using FlowJo software
		Calculate the staining index and determine optimal antibody concentrations using FlowJo software

Table 2. *Examples of flow cytometry panels supplied to undergraduate students to diagnose unknown patients on the basis of cell surface and intracellular marker expression*

Panel 1	*Panel 2*	*Panel 3*	*Panel 4*	*Panel 5*	*Panel 6*
CD7-FITC	κ-FITC	IgM-FITC	CD10-FITC	CD7-FITC	MPO-FITC
CD2-PE	λ-PE	CD23-PE	CD13-PE	CD64-PE	CD79a-PE
CD3-PerCP-Cy5.5	CD5-PerCP-Cy5.5	CD45-PerCP-Cy5.5	CD45-PerCP-Cy5.5	CD45-PerCP-Cy5.5	CD45-PerCP-Cy5.5
CD16/CD56-PE-Cy7	CD19-PE-Cy7	CD5-PE-Cy7	CD33-PE-Cy7	CD117-PE-Cy7	CD34-APC
CD5-APC	CD10-APC	CD79b-APC	CD34-APC	HLA-DR-APC	CD3-APC-H7
CD8-APC-H7	CD3-APC-H7	CD19-APC-H7	CD19-APC-H7	CD3-APC-H7	MPO
CD4-V450	CD20-V450				
CD45-V500	CD45-V500				

MPO, myeloperoxidase.

were provided with listmode files of single stained compensation controls for six anti-mouse antibodies (Table 4) as well as a sample of mouse PBMCs stained with all. Students were asked to perform compensation for spectral overlap as per *activity 1* and then undertake sequential gating to solve a series of problems by interrogating the data set. These tasks increased in complexity from determining the proportion of lymphocytes that were CD4-positive T cells through to determining the proportion of regulatory T cells that were proliferating.

Surveys. Three separate cohorts of students (*undergraduate cohort A*, *undergraduate cohort B*, and the research trainees) were asked to complete anonymous surveys. All surveys asked how much experience students had with flow cytometry before the class and how confident they felt working with flow cytometry data. *Undergraduate cohort A* was surveyed twice in class, once before the new curriculum and once after. In addition to confidence, students were also required to perform a task to ascertain their achievement of the learning outcomes (see *Student achievement of the learning outcomes* below for details) and asked to rate their perceptions of flow cytometry and, in particular, indicate whether they would consider choosing a career in flow cytometry. From 154 students enrolled, the 2 surveys before and after the tutorial received 93 responses (60%) and 117 responses (76%), respectively. To eliminate the possibility that prior exposure to the survey would alter student success rates in the achievement task, one-third of students were not surveyed before the class. This had no effect on achievement outcomes (51% of students not previously exposed to the survey answered the final task correctly compared with 49% and 52% in the remaining cohorts exposed to the survey before the curriculum).

Undergraduate cohort B and the research trainees were both surveyed once only, after the tutorial. The survey for *undergraduate cohort B* students was conducted online, and 35 responses were received from 72 enrolled students (49%). The research trainee survey was conducted in class at the end of the tutorial, and all 20 students responded (100%). These surveys also included an additional question asking students to provide written qualitative feedback on the tutorial.

The survey used for *undergraduate cohort A* is presented in APPENDIX A, and the survey used for both *undergraduate cohort B* and the research trainee cohort in presented in APPENDIX B.

Student achievement of the learning outcomes. Student achievement of the intended learning outcomes was measured in *undergraduate cohort A* using two multiple-choice questions (APPENDIX A). The first of these questions, *question 6* (dot plots), was used to determine whether students could interpret pregated dot plots to identify discrete cell populations. Only one of the four options presented was correct, with each of the three distractors being potential answers that the students might choose through errors in interpretation of the plots. For the purpose of our analysis, we designated the least correct distractor (that is, the one with the most errors in logic) as *answer A*, followed by progressively more correct *answers B* and *C*, and correct *answer D*. In the survey, these answers were randomly assigned (see APPENDIX A).

The second achievement question was used to determine the students' ability to construct a sequential gating strategy. Students were shown four plots and asked to select from five potential ways that these could be used as a gating strategy to measure the abundance of a discrete cell subset. The least correct distractor, which we designated *answer A*, contains numerous flaws in logic. Students who selected this option failed to choose the plot that gates the parent cells and none of the steps in this strategy select for the specific subset. Likewise, in *answer B*, the parent cells have not been gated and the specific subset marker is missing. *Answers C* and *D* contain the parent and subset populations but are out of logical sequence. *Answer E* is the only correct response.

Statistical analysis. The surveys used in this study used Likert-type questions with graded responses. The choice of statistical tests for Likert items and Likert scales is a subject of discussion in the literature. We adopted the nonparametric statistical approaches advocated by de Winter (7) for differences between items and Clason (6)

Table 3. *Examples of hematological malignancy phenotypes supplied to undergraduate students to diagnose unknown patients on the basis of cell surface and intracellular marker expression*

	Description
Acute lymphoblastic leukemia	
Phenotype	Lymphoblastic leukemia of B or T cell origin
Precursor B cells:	CD10, CD19, and cCD78a; TdT: positive
Early T cells	cCD3 and CD7; TdT: positive
Thymic T cells	CD1a, CD2, CD5, CD7, CD4, and CD8; TdT: positive
Mature T cells	CD2, CD3, CD4 or CD8, CD5, and CD7; TdT: positive
Acute myeloid leukemia	
Phenotype	CD13, CD33, CD117, MPO, and HLA-DR
	CD45 weak and intermediate side scatter
Chronic lymphocytic leukemia	Chronic lymphocytic leukemia is a neoplasm of mature B cells that express CD5 and CD23 antigens
Phenotype	B cell lineage: CD20 (weak) and IgM (weak)
	CD19, CD79a, CD23, and CD5; positive

Table 4. *Files supplied to students in the research trainee cohort*

Activity 1: Compensation	*Activity 2*: Antibody Titration	*Activity 3*: Compensation and Sequential Gating
Single stained PBMCs	Single stained PBMCs	Single stained mouse PBMCs with anti-mouse antibodies
CD3-FITC	CD8a-PerCP-Cy5.5	FoxP3-FITC
CD8-PE	Five 1:2 serial dilutions	Ki67-PE
CD4-PE-Cy5		Thy1.1-PerCP-Cy5.5
Multicolored sample		Nrp-1-APC
PBMCs stained with all of the above		CD4-Pacific blue
		CD3-V500
		Multicolored sample
		Mouse PBMCs stained with all of the above

PBMCs, peripheral blood mononuclear cells.

for correlation analysis, as these methods deal explicitly with the use of individual Likert items as opposed to multi-item Likert scales and thus are most appropriate for our data.

Responses on the four-point scale were assigned values from *0* to *3*. Mean values and confidence intervals are reported. Gains in confidence and comparisons of confidence between groups were measured using a Mann-Whitney test (7). Correlation coefficients were determined using a Spearman rank (6). Student achievement of the set tasks before and after the curriculum were compared using a one-tailed Fisher's exact test.

All statistical analyses were performed using Graphpad Prism software (Graphpad Software, La Jolla, CA).

RESULTS

Students' prior experience and confidence with handling flow cytometry data. In *undergraduate cohorts A* and *B*, 45% of students had never encountered flow cytometry before this tutorial (Table 5). In the research trainee cohort, 80% had no previous training in flow cytometry. Even among those with prior experience, most reported their experience as fewer than four classes.

All cohorts reported increasing confidence with manipulating flow cytometry data from before to after the tutorial (*undergraduate cohort A*: 1.04 to 1.71, *undergraduate cohort B*: 0.97 to 2.11, and research trainee cohort: 0.30 to 1.80; Fig. 1) on a four-point scale (where *0* = not confident at all, *1* = I can manage some things but I frequently feel lost, *2* = I am getting the hang of it, and *3* = I can confidently construct a gating strategy to draw conclusions about a patient). Greater gains in confidence were reported by *undergraduate cohort B* and the research trainee cohort than *undergraduate cohort A*. Within each cohort, the greatest gains were seen among students who had never before experienced classes in flow cytometry (Table 5).

Student achievement of the learning outcomes. The survey given to *undergraduate cohort A* included two questions to assess student achievement of the learning outcomes. The first question required students to correctly identify cell populations on labeled dot plots, and the second question required both interpretation and the correct gating sequence of plots (see APPENDIX A for the original questions).

The cohort showed no significant improvement in interpreting dot plots ($P = 0.164$; Fig. 2, *A–C*), with 74% correctly interpreting the plots after the new curriculum compared with 67% before the tutorial. In contrast, great gains were seen in being able to apply a sequential gating strategy to a series of plots (50% correct compared with 25%, $P < 0.001$; Fig. 2, *D–F*). Among the incorrect responses, there was also improvement toward answers that had fewer errors (Fig. 2, *E* and *F*). In particular, there was a stark reduction in the number of students who chose *answer C* from 36% to 13%. In *answer C*, popula-

Table 5. *Mean scores and 95% confidence intervals of prior experience with flow cytometry, confidence before and after the tutorial, and perceptions of flow cytometry as a career for undergraduate students and research trainees exposed to the new curriculum*

	n	Confidence Before the Tutorial	Confidence After the Tutorial	Perception of Flow Cytometry
Undergraduate cohort A				
No prior experience	52	0.87 (0.63–1.12)	1.71* (1.52–1.91)	1.71 (1.50–1.91)
Prior experience	63	1.29 (1.06–1.52)	1.76* (1.61–1.92)	1.45 (1.25–1.65)
Undergraduate cohort B				
No prior experience	16	0.50 (0.16–0.84)	1.81* (1.41–2.21)	1.63 (1.24–2.01)
Prior experience	19	1.37 (1.04–1.70)	2.37* (2.08–2.66)	2.05 (1.80–2.31)
Research trainee cohort				
No prior experience	16	0.07 (−0.01 to 0.20)	1.69* (1.37–2.01)	N/A
Prior experience	4	1.25 (−0.75 to 3.25)	2.25 (1.45–3.45)	N/A

Values are means with 95% confidence intervals in parentheses; *n*, number of students/trainees. Data for *undergraduate cohort A* were collected from two surveys, one survey conducted before the new curriculum ($n = 93$) and one survey after ($n = 117$). Data for *undergraduate cohort B* and the research trainee cohort were collected in a single survey at the end of the tutorial. The prior experience scale was as follows: *0* = less than 1 class, *1* = 1–4 classes, *2* = 5–10 classes, and *3* = more than 10 classes. The confidence scale was as follows: *0* = not at all confident, *1* = I can manage some things but I frequently feel lost, *2* = I'm getting the hang of it, and *3* = I can confidently construct a gating strategy to draw conclusions about a patient. The perception of flow cytometry scale was as follows: *0* = I strongly dislike flow cytometry and would prefer not to learn this content, *1* = I can understand the relevance but I prefer other activities in the unit, *2* = I enjoy flow cytometry as much as other activities in the unit, and *3* = I like flow cytometry more than other activities in the unit and might consider it as a career. Two students from *undergraduate cohort A* ($n = 117$) did not answer the question relating to prior experience. N/A, not applicable. *$P < 0.0005$ compared with confidence before the tutorial by an unpaired Mann-Whitney test.

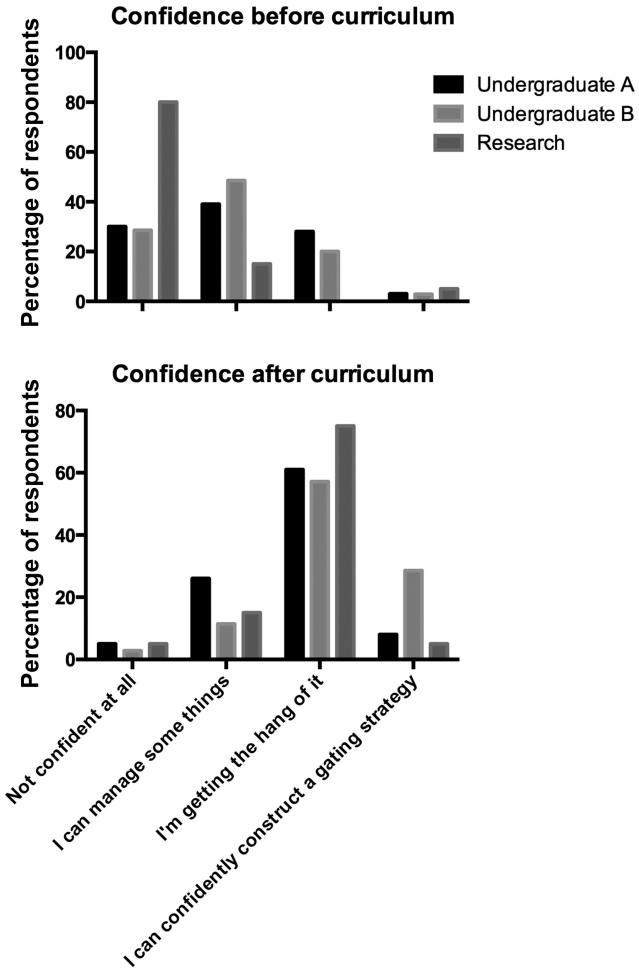

Fig. 1. Self-reported confidence with handling flow cytometry data on a four-point scale. Data for *undergraduate cohort A* were collected from two surveys, one survey conducted before the new curriculum ($n = 93$) and one survey after ($n = 117$). Data for *undergraduate cohort B* and research trainee cohort were collected in a single survey at the end of the tutorial.

tions are selected out in one step but then reappear in a subsequent panel so that the plots are correct but out of sequence. The proportion of students that selected *answers A* and *B*, which reflect a lack of understanding of dot plots, was similar before and after the curriculum at 15% and 12%, respectively.

Choice of flow cytometry as a career. Correlation analysis revealed that student perceptions of flow cytometry were related to confidence after the tutorial (correlation coefficient: 0.456, $P < 0.001$; Table 6 and Fig. 3), in particular among those students with the least prior experience with flow cytometry data (correlation coefficient: 0.532, $P < 0.001$; Table 6). There was no correlation between perceptions of flow cytometry and achievement of the gating task or between student confidence and achievement (Table 6). Students who chose the highest confidence rating ("I can confidently construct a gating strategy to draw conclusions about a patient") were most likely to consider a career working with flow cytometry (33% of students in this category compared with 6% across the remaining categories). Compared with achievement, 14% of students who were able to choose a valid gating strategy indicated they would consider a career in flow cytometry compared with 4% of students who made errors in their gating.

Student and teacher perceptions of the tutorial. Both student and teacher feedback on the tutorial were extremely positive. Figure 4 shows a graphical representation of the themes that emerged from the student comments.

In the free comments, students described the tutorial as enjoyable, informative, and an effective way to learn:

> I think this is an extremely important research skill, but is also one of the topics that sends students to sleep easily. I cannot imagine learning flow cytometry data analysis a better way than it was presented to me. The e-learning suites made this an enjoyable collaborative environment to learn an otherwise 'dry' topic.
>
> PS: Personally I think flow cytometry is awesome!
>
> Undergraduate student

Research trainees specifically commented on how the facility design contributed to their learning:

> This was a great introduction but a little overwhelming. Having the two computer screens together where we were able to follow the lecturer helped a lot.
>
> Research trainee

Teachers valued giving their students learning activity with greater opportunities for student interaction.

> Normally you have to break into small groups so you can all fit around the instrument and even then it's not particularly interactive for everyone, but this was an opportunity for everyone to get stuck into the data and really start to understand what it means.
>
> Teacher, research trainee cohort

Some students offered suggestions for change. In particular, undergraduate students felt they could have used more direct instruction at the beginning of the class both on how to use FlowJo and how they might go about designing a gating strategy. The main negative comment offered by students in the research trainee cohort was that the tutorial was too fast for them and some were concerned that they might not remember what they had learned in future applications.

> The pre-lab was good but it needed some 'steps' for how to go about diagnosing because it took our group ages to get the hang of it and I still didn't understand what we were doing most of the time.
>
> Undergraduate student
>
> I'm concerned that I will forget what I have learned (I may not use this new knowledge immediately) and not sure where to go for help using FlowJo at a later date.
>
> Research trainee

Overall, the comments received from teachers and both undergraduate and research trainees were positive and enthusiastic for this mode of teaching.

DISCUSSION

We designed active, collaborative activities to teach students how to manipulate flow cytometry data. This approach allowed students to achieve learning outcomes we had not previously been able to provide (Fig. 2), such as having students perform compensation on data or design their own gating strategy without forgoing essential outcomes, such as the interpretation of dot plots. Students found the activities enjoyable and informative (Fig. 4) and reported increased

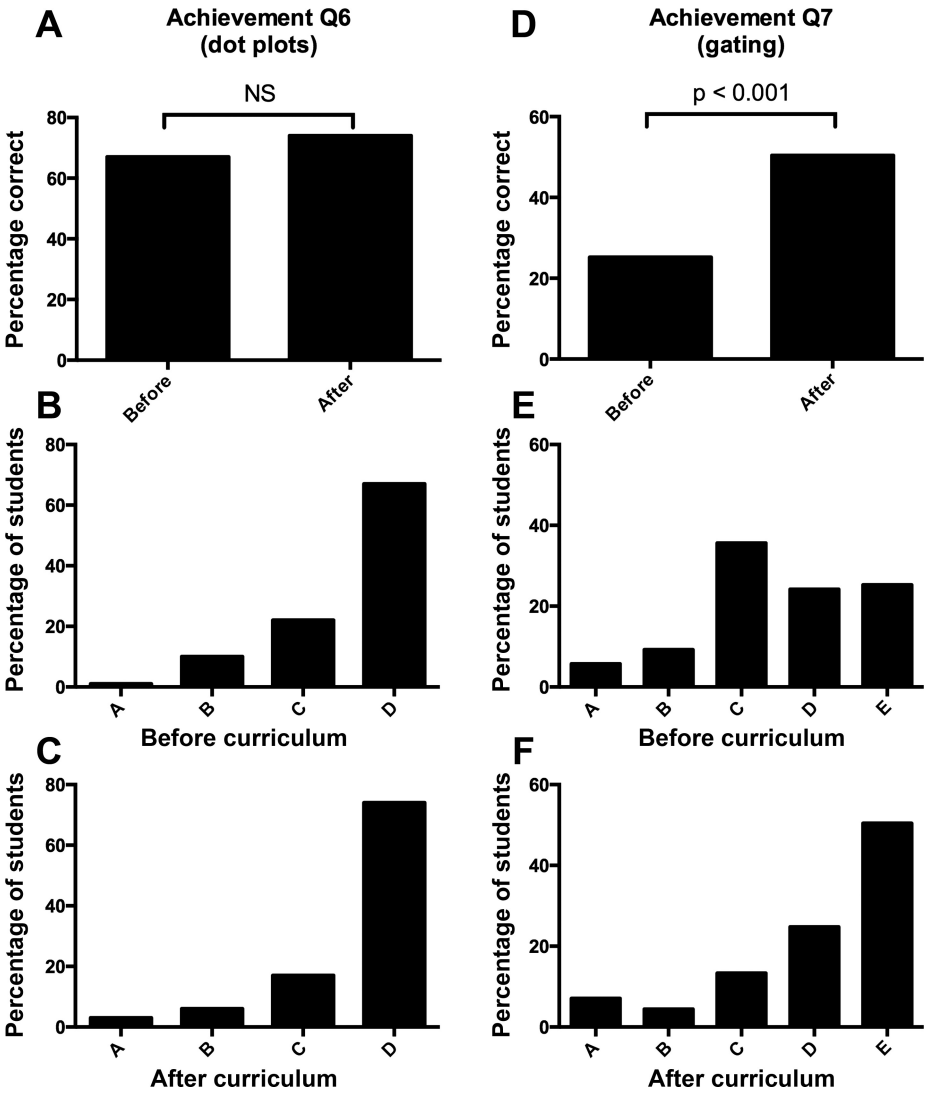

Fig. 2. Student achievement at interpreting dot plots (*A–C*) and designing a gating strategy (*D–F*). *A* and *D*: percentages of students who chose the correct answers before and after the tutorial. Student achievement before and after the tutorial was compared using a Fisher's test. *B* and *E*: percentage breakdown of responses before the tutorial. *C* and *F*: percentage breakdown of responses after the tutorial. *Answer A* represents the answer with the most flaws in logic. The other answers have progressively fewer flaws, with *answer D* (dot plots) and *answer E* (gating) representing the correct answers. NS, not significant.

confidence with manipulation and interpretation of flow cytometry data (Table 5 and Fig. 1), and high levels of confidence were related to favorable perceptions of flow cytometry (Fig. 3 and Table 6).

The new curriculum saw a considerable improvement in students' ability to construct a sequential gating sequence (Fig. 2). In particular, there was a strong shift away from students selecting *answer C* and toward the correct option. *Answer C* in particular relates to the sequence of the plots, as a population is gated out in one step but then reappears as a subsequent step so that students who choose this option interpret the dot plots correctly but misunderstand the sequential nature of gating. The shift of answers from *answer C* to *answer E* (the correct option) demonstrates that students have gained the ability to construct a gating sequence.

However, the learning gains observed for gating were not accompanied by large gains in students' ability to interpret dot plots (Fig. 2). This was partly because most students were already able to interpret dot plots before they attended the tutorial. Given that almost half of these students had never encountered classes in flow cytometry before (Table 5), the relatively high success rate in interpreting dot plots before the class might indicate that the interpretation of dot plots is an intuitive skill that undergraduate science students might pos-

Table 6. *Correlation coefficients for undergraduate cohort A student confidence after the tutorial, achievement of the set task, and perceptions of flow cytometry*

	n	Confidence Versus Achievement (Gating)	Perceptions Versus Achievement (Gating)	Perceptions Versus Confidence
No prior experience	52	0.010 (0.02 ± 0.08)	0.076 (0.02 ± 0.08)	0.532* (0.55 ± 0.13)
Prior experience	63	0.183 (0.10 ± 0.07)	0.158 (0.11 ± 0.09)	0.356* (0.42 ± 0.15)
All *undergraduate cohort A*	117	0.149 (0.10 ± 0.05)	0.150 (0.08 ± 0.06)	0.456* (0.50 ± 0.10)

n, number of students. Correlation analysis was performed by Spearman rank, as suggested in Ref. 6. *$P < 0.001$. Slopes were determined by linear regression with ±SEs (in parentheses). Two students from *undergraduate cohort A* ($n = 117$) did not answer the question relating to prior experience

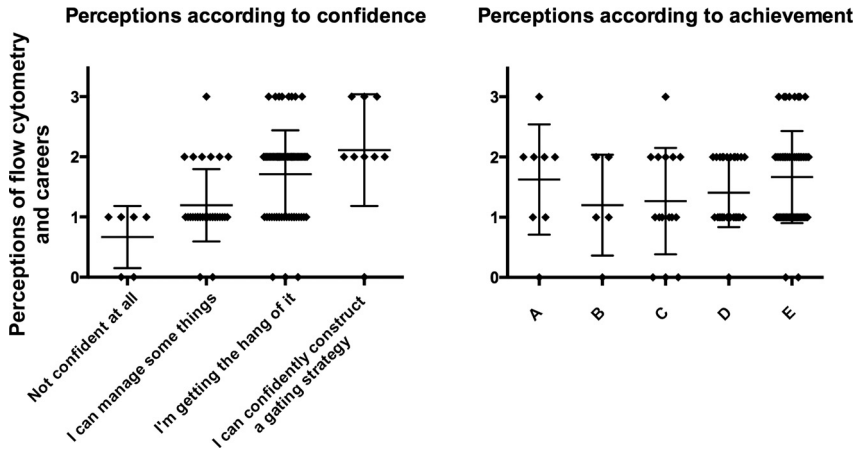

Fig. 3. Undergraduate perceptions of flow cytometry as a subject, related to self-reported confidence and achievement of the set task. Perceptions of flow cytometry were gauged by student responses to the following question "How do you feel about flow cytometry as a subject?," where *0* = I strongly dislike flow cytometry and would prefer not to learn this content, *1* = I can understand the relevance but I prefer other activities in the unit, *2* = I enjoy flow cytometry as much as other activities in the unit, and *3* = I like flow cytometry more than other activities in the unit and might consider it as a career. Achievement of the set task was determined using a question asking students to select a gating strategy from available scatterplots. *Answer A* represents the answer with the most flaws in logic, and the other answers have progressively fewer flaws, with *answer E* representing the correct answer.

sess generally without the need for explicit tuition. These results have implications for curriculum design, as teachers might make best use of limited classroom time by spending more time teaching specific, complex skills such as gating and less time teaching intuitive skills such as the interpretation of dot plots.

A small proportion of students were still making fundamental errors in interpreting dot plots at the end of the tutorial. While this curriculum did not address the interpretation of dot plots in particular, objective measures like those used in this study could be applied to identify such students before the gating exercise and provide extra tuition to ensure that everyone had the basic skills needed to go on to more complicated learning outcomes such as gating.

The objective measures of learning gains used in this study were insightful but had some limitations. The questions were used in isolation and therefore have not been validated against a bank of established questions or across multiple cohorts. Furthermore, we did not compare the learning gains of the new curriculum with what would be achieved with traditional paper-based classes limited to the interpretation of pregated dot plots alone or with a more scripted exercise using FlowJo. These measures would be of interest in future experiments, including broadening the set of questions available to interrogate skills in flow cytometry and validating those questions. The methods used in this report could then be applied to answer additional research questions.

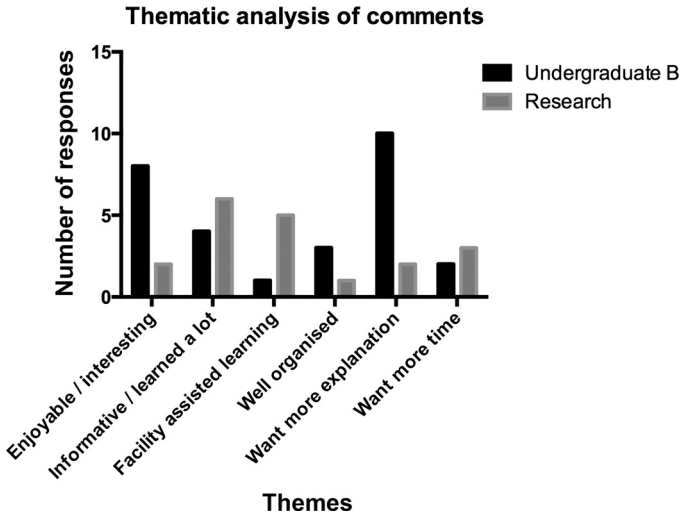

Fig. 4. Thematic analysis of student comments regarding the flow cytometry tutorials. Of the 35 *undergraduate cohort B* respondents to the survey, 22 respondents provided comments. Of the 20 research trainee respondents, 13 respondents provided comments. Some student comments covered more than one theme, for example, where students commented that the tutorial was enjoyable and they learned a lot.

Undergraduate students were asked for their perceptions of working with flow cytometry as a career. All cohorts gained confidence, but greater gains were observed in *undergraduate cohort B* and the research trainee cohort than in *undergraduate cohort A*. This may reflect differences in the survey methods. *Undergraduate cohort A* was surveyed twice, once before and once after the tutorial, whereas *undergraduate cohort B* and the research trainee cohort were asked in a single survey to reflect on their confidence before and after the tutorial. It is possible that students asked to rate their confidence by reflection may overstate the contrast between confidence before and after the tutorial.

Favorable perceptions of flow cytometry were correlated with student self-reported confidence ratings but not with objective measures of how well students could perform flow cytometry data analysis. These results may be of interest to teachers who are also hoping to recruit students to the field of flow cytometry. While it is important to increase students' skill levels, recruitment to careers in flow cytometry will be driven more by student confidence than ability. The perception confidence correlation was strongest in those students who did not have prior experience working with flow cytometry (Table 5) and thus was not likely to be due to sampling of students who already prefer flow cytometry-like tasks. These results suggest that activities that increase confidence with handling flow cytometry data can influence students' desire to choose flow cytometry as a career.

Interestingly, the undergraduates who participated in this study were more likely to have had prior experience working with flow cytometry than those in the research trainee cohort (Table 5). This might be a reflection of the undergraduate curriculum: the undergraduate cohort was relatively uniform, having common first- and second-year subjects, and most would have been exposed to flow cytometry data in the form of supplied dot plots in second-year pathology classes. In contrast, the research trainees came from a diverse range of backgrounds and might not have been exposed to flow cytom-

etry data at all. Although we did not ask the research trainee cohort for their perceptions of flow cytometry (as it was assumed that their enrollment in such a course already indicated a preference for flow cytometry work in their research careers), this relatively inexperienced cohort might stand to benefit the most from increasing levels of confidence, as it was in inexperienced students that we observed the greatest link between confidence after the tutorial and perceptions of flow cytometry (Table 6).

The most common suggestion for change, especially among undergraduates, was a request for more explicit instruction on how to use the FlowJo software and how to construct a gating strategy. It is debatable how much instruction is optimal for learning. Some authors have cautioned against giving too little instruction (14), and previous studies have shown that students can feel more anxious and stressed when active or inquiry-based methods are used (16, 25). Such negative responses do not necessarily mean that students are not learning. Indeed, some dissatisfaction among students has been observed even when those same students are in fact achieving learning outcomes at least as well as or better than when presented with traditional instruction (4, 21, 25). With our curriculum, objective assessment showed a clear improvement in achieving ambitious outcomes (designing appropriate gating strategies) and students self-reported feeling more confident that they could manipulate flow cytometry data. Although students might have felt "thrown in the deep end" at times during the learning activity, the explorative as opposed to instructional nature of active learning is undoubtedly more realistic preparation for real-world scenarios and may be beneficial to the overall learning process. In future iterations of these classes, we will focus on adding scaffolding, such as that suggested by one student ("guiding questions, like how do you determine whether cells are T or B cells?") to support students through their learning without impacting the exploratory nature of the classes.

Collaborative learning in this study took place in an active learning facility designed for the purpose, and research trainees especially commented that the facility contributed to their learning of the topic. Not all educators will have access to such a facility; however, there are a few minimum requirements that could be used to adapt these activities to other settings. To explore compensation and gating of data on their own, students need access to computers and flow cytometry software. We recommend that students work in groups. In addition to the learning benefits (13), group-based learning has the advantage that fewer computers and software licenses are required, which can help keep costs low. Our recommendation is that teachers offer students the opportunity to engage in inquiry-based activities (such as designing their own gating strategy) in groups that have shared access to flow cytometry data manipulation software.

In conclusion, this report describes active, collaborative activities that can be used to teach students how to manipulate flow cytometry data. This approach had numerous advantages over previous teaching activities (limited to dot plot interpretation), including additional and more advanced learning outcomes, increased student enjoyment, and greater student confidence with handling flow cytometry data. Higher levels of student confidence were associated with better perceptions of flow cytometry and potential selection of flow cytometry as a career choice.

APPENDIX A

Survey questions were administered to students in *undergraduate cohort A* before and after their tutorial in flow cytometry. Values in parentheses indicate numeric ratings or alphabetic designations assigned to each option.

Question 1. Have you completed the tutorial entitled "Flow Cytometry Part 2" using the FlowJo software?

A. Yes

B. No

Question 2. Have you completed this survey previously?

A. Yes

B. No

Question 3. How much prior experience have you had using flow cytometry in classes?

A. Very little, less than 1 class (0)

B. A module within a unit, 1–4 classes (1)

C. An extended module, 5–10 classes (2)

D. More than 10 classes (3)

Question 4. Please rate your confidence in manipulating flow cytometry data.

A. Not confident at all (0)

B. I can manage some things but I frequently feel lost (1)

C. I'm getting the hang of it (2)

D. I can confidently construct a gating strategy to draw conclusions about a patient (3)

Question 5. How do you feel about flow cytometry as a subject?

A. I strongly dislike flow cytometry and would prefer not to learn this content (0)

B. I can understand the relevance but I prefer other activities in the unit (1)

C. I enjoy flow cytometry as much as other activities in the unit (2)

D. I like flow cytometry more than other activities in the unit and might consider it as a career (3)

Question 6. Consider the following data (Fig. A1). Which population represents $CD3^+CD4^-$ cells?

A. *Cells 1* (*answer C*)

B. *Cells 2* (*answer A*)

C. *Cells 3* (*answer B*)

D. *Cells 4* (*answer D*)

Question 7. Consider the following data (Fig. A2). Suppose you wanted to answer the following question: "What proportion of $CD3^+$ lymphocytes are $CD8^+$ in this sample?." Using this data set, which of the following would be a sensible gating strategy?

A. Gate the $CD3^+$ cells in *plot D* and then count *cells 2* in *plot C* (*answer A*)

B. Gate the $CD3^+$ cells in *plot D* and then count *B2 cells* in *plot B* (*answer B*)

C. Gate the lymphocytes from *plot A*, gate *B1 cells* in *plot B*, and then count $CD3^+$ cells in *plot D* (*answer D*)

D. Gate the lymphocytes from *plot A*, gate *B1 cells* in *plot B*, and then count *cells 4* in *plot C* (*answer C*)

E. Gate the lymphocytes from *plot A*, gate $CD3^+$ cells in *plot D*, and then count *B1 cells* in *plot B* (*E*)

APPENDIX B

Survey questions were administered to students in *undergraduate cohort B* and the research trainee cohort after their tutorial in flow cytometry. Numbers in parentheses indicate numerical ratings assigned to each option.

Question 1. How much prior experience have you had using flow cytometry in classes?

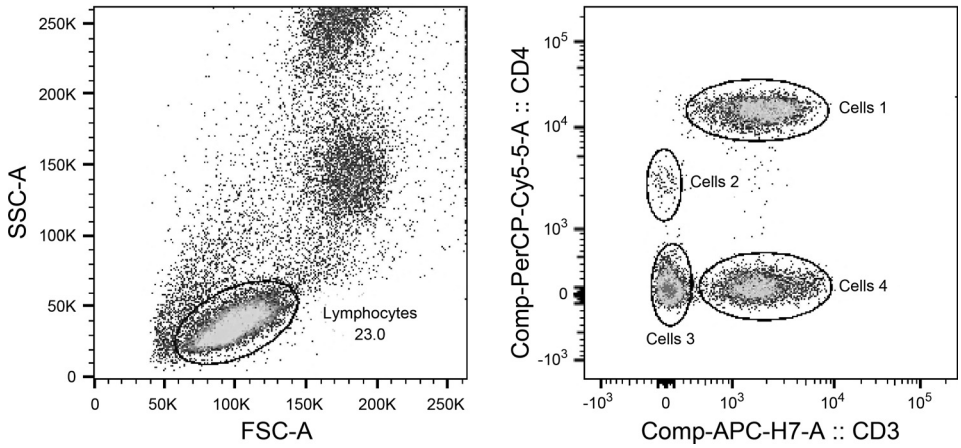

Fig. A1. Data set for *question 6*: which population represents $CD3^+CD4^-$ cells? SSC, side scatter; FSC, forward scatter; COMP, compensated data; PerCP-Cy5, peridinin chlorophyll-cyanine 5.5; APC-H7, allophycocyanin-H7.

A. Very little, less than 1 class (0)
B. A module within a unit, 1–4 classes (1)
C. An extended module, 5–10 classes (2)
D. More than 10 classes (3)

Question 2. Please rate your confidence in manipulating flow cytometry data BEFORE the tutorial this week.

A. Not confident at all (0)
B. I can manage some things but I frequently feel lost (1)
C. I'm getting the hang of it (2)
D. I can confidently construct a gating strategy to draw conclusions about a patient (3)

Question 3. Please rate your confidence in manipulating flow cytometry data AFTER the tutorial this week.

A. Not confident at all (0)

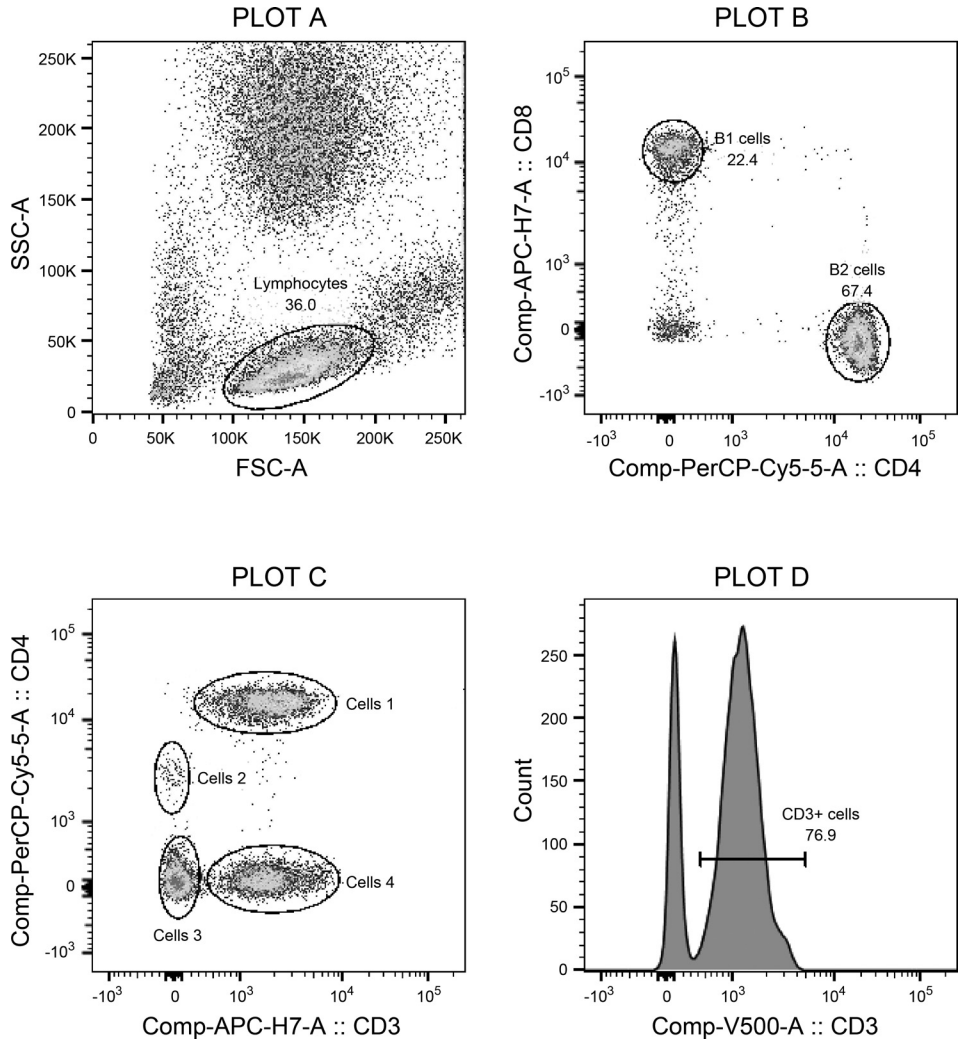

Fig. A2. Data set for *question 7*: what proportion of $CD3^+$ lymphocytes are $CD8^+$ in this sample? SSC, side scatter; FSC, forward scatter; Comp, compensated data; APC-H7, allophycocyanin H7; PerCP-Cy5.5, peridinin chlorophyll-cyanine 5.5.

B. I can manage some things but I frequently feel lost (1)

C. I'm getting the hang of it (2)

D. I can confidently construct a gating strategy to draw conclusions about a patient (3)

Question 4. How do you feel about flow cytometry as a subject? (This question was offered to undergraduate students only.)

A. I strongly dislike flow cytometry and would prefer not to learn this content (0)

B. I can understand the relevance but I prefer other activities in the unit (1)

C. I enjoy flow cytometry as much as other activities in the unit (2)

D. I like flow cytometry more than other activities in the unit and might consider it as a career (3)

Question 5. Do you have any further comments to add?

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: K.F., M.D.L., T.L.-P., C.F., and K.J.R. performed experiments; K.F., M.D.L., and K.J.R. analyzed data; K.F., M.D.L., T.L.-P., C.F., W.N.E., and K.J.R. interpreted results of experiments; K.F. and K.J.R. drafted manuscript; K.F., M.D.L., T.L.-P., C.F., W.N.E., and K.J.R. edited and revised manuscript; K.F., M.D.L., T.L.-P., C.F., W.N.E., and K.J.R. approved final version of manuscript; M.D.L. and K.J.R. prepared figures; K.J.R. conception and design of research.

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