Flow Cytometry - A Specialized Analytical Skill for the Cell and Gene Therapy Industry

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Abstract: Cell and gene therapy is one of the fastest-growing fields in the biopharmaceutical industry, and Maryland is witnessing the impact of this growth first-hand. Flow cytometry, a crucial analytical tool for ensuring the quality and purity of cell and gene therapy products, has become a highly sought-after skill. However, access to flow cytometry training is often limited and expensive. Recognizing this gap, Frederick Community College (FCC) has developed an innovative Cell Therapy and Flow Cytometry course, validated by industry, to make this training accessible and affordable to local students, particularly those from underserved communities. This specialized training in cell culture and flow cytometry offers students a competitive edge in the regional job market. Integrated into various program pathways, such as the new Cell and Gene Therapy Certificate, Letter of Recognition (LOR) for Cell Therapy, digital badges, and non-credit options. Flow cytometry training caters to a diverse range of learners, including traditional degree-seeking students and incumbent workers seeking to enhance their skills. In the successful pilot run during Spring 2023, the FCC Cell Therapy and Flow Cytometry course attracted ten students from the Biotechnology Associates program and six others eager to acquire new technical skills. This initiative aims to enable employers to recruit highly qualified and diverse candidates from the local talent pool, thereby supporting the biotechnology workforce in the region. FCC anticipates that this Cell Therapy and Flow Cytometry Workforce Project will serve as a model to enable other community college biotechnology programs to meet similar workforce demands as the cell therapy industry continues to expand across the country.

Keywords: Flow Cytometry, workforce, Cell Therapy, industry-validated, Gene Therapy

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Introduction

Cell and gene therapy is the latest innovation in personalized medicine, and it holds the potential for revolutionizing patient care [1, 2]. Cell and gene therapy are related but distinct fields of practice that may be combined, e.g., to treat cancer. Cell therapy involves injecting or transplanting healthy cells into patients to trigger a medical effect. Gene therapy seeks to modify or manipulate the expression of a gene to alter the biological properties of living cells for therapeutic use. In cell therapy, cells – rather than medication or another type of therapeutic intervention – are used to treat patients. Cell and gene therapy are often combined. One example is the production of cells with Chimeric Antigen Receptors (CARs). A CAR gene contains the receptor-binding portion that binds to a protein on the surface of cancer cells

fused to the signaling portion from another gene. CAR cells are made by putting these chimeric genes into cells from the immune system, such as T cells. When a cell makes the chimeric protein, the receptor portion sticks outside of the cell and the signaling portion stays inside. When the receptor binds to a molecule on a cancer cell, it triggers the signal and tells the cell it's time to attack the cancer cell. Most clinically evaluated CAR cell products are derived from a patient's immune cells. These are called "autologous" immune cells. The possibility of engineering cells from healthy donors (allogeneic cells) is also currently being explored [3].

Flow cytometry is a crucial analytical tool for the cell therapy industry. It ensures the quality and purity of engineered cells. Flow cytometry is also used to determine if cells possess the qualities needed to help a given patient. As it is one of the primary methods of ensuring quality control, it is indispensable in the manufacturing and release of cell therapy products [4]. As noted above, many cell therapies are made by putting CAR genes inside a cell. Flow cytometry identifies the cells that produce CARs by using fluorescent antibodies to tag receptors on the cell surface. Fluorescently labeled cells are counted as they flow past single or multiple lasers [5]. FCC’s Cell Therapy and Flow Cytometry course teaches students about the cell therapy manufacturing process and gives them practice in preparing and analyzing samples using the flow cytometer. Understanding how flow cytometry works and is used to determine if cells are expressing chimeric receptors equips students with the knowledge and skills needed to enter the local workforce.

The value of the global cell therapy market is currently estimated at $9.5 billion and is projected to grow to $23 billion by 2030 [6]. About 900 firms globally are working on these advanced therapies, and approximately 1,000 cell and gene therapy clinical trials are currently underway [7]. By 2030, the U.S. Food and Drug Administration expects to approve 30-60 cell and gene therapy products [8]. The BioHealth Capital Region, encompassing Maryland, Virginia, and Washington, DC, is one of the top three bio-health clusters in the United States. With the National Institutes of Health, the National Cancer Institute's Frederick National Laboratory for Cancer Research, and the Amyotrophic Lateral Sclerosis Center for Cell Therapy and Regeneration Research at Johns Hopkins, the most advanced scientific research and advancement in the field is taking place in the local region. Maryland is home to approximately 2,700 life science companies and over 500 biotech companies. The I-270 biotech corridor, where 140 life science companies are located, is within the FCC's service area [9]. Numerous well-known clinical-stage cell therapy and gene therapy companies are located within a 40-mile radius of FCC, including Arcellx, American Gene Technologies, TCR2, REGENXBIO, Vigene Biosciences, Autolus, RoosterBio, and MaxCyte [10] (Figure 1). In April 2021, Kite, a Gilead Company, one of the top 10 CAR T cell therapy companies, finished construction on a new state-of-the-art facility in Frederick to manufacture FDA-approved products YESCARTA and TECARTUS [11]. With the increase in the number of ongoing clinical trials, the establishment of commercial manufacturing sites by major companies in the field, and the need to ensure the quality of cell therapy products, the demand for technicians skilled in flow cytometry will continue to grow.

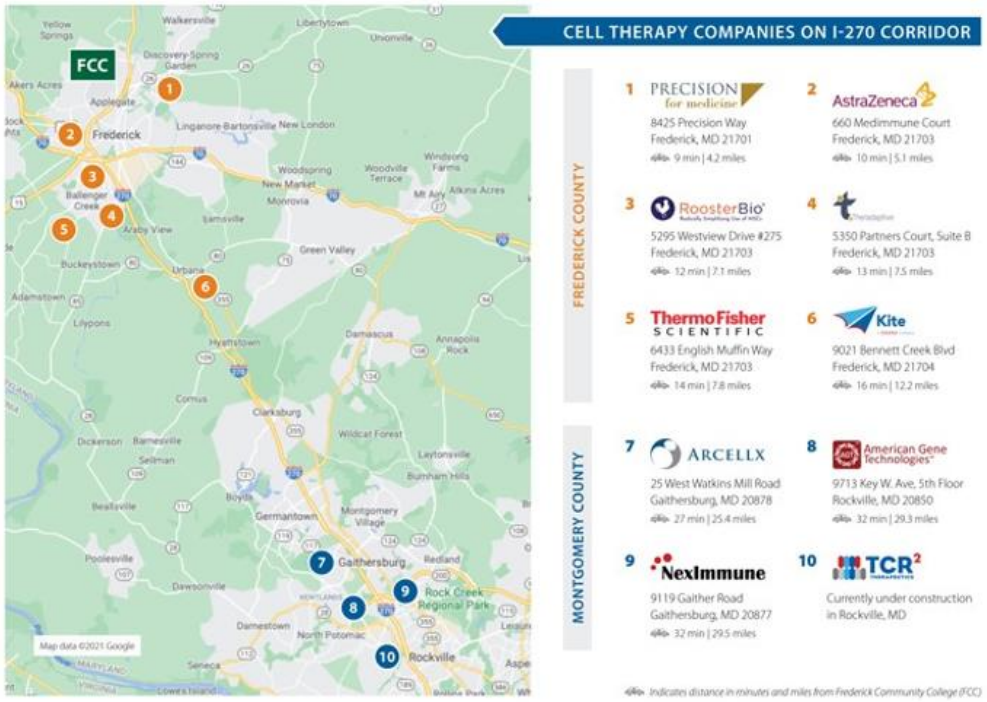

Figure. 1. Cell and gene therapy companies in the FCC service area.

Across the country, technician education programs broadly address biotechnology and cell therapy but do not focus on critical flow cytometry techniques. Aseptic technique, a set of standard procedures that prevent microorganisms in the environment from contaminating sterile cell cultures, is an important skill set for cell therapy that is commonly taught in community college biotechnology programs. Entities developing new cell and gene therapy curricula include NSF-funded projects at Shoreline Community College in Washington, Montgomery County Community College in Pennsylvania, and Montgomery College in Maryland, as well as college and industry collaboratives funded by the National Institute for Innovation in Manufacturing Biopharmaceuticals [12]. However, none of the programs focus on flow cytometry the context of the cell and gene therapy industry, nor do they offer certificates in applied flow cytometry [13-14]. A review of existing biotechnology technician education programs, compiled by InnovATEBIO, the NSF-funded National Advanced Technological Center for Biotechnology Education, indicates that flow cytometry skills are typically taught via on-the-job training in industry and research labs in specialized four-year biotechnology programs like those offered by Solano Community College and MiraCosta College (both in California) and in postgraduate level workshops [15-18]. FCC's project fills a gap in biotechnology technician education by developing a course focusing on flow cytometry as a key component of cell and gene therapy. The course complements and builds upon course currently in the FCC biotechnology program and has been developed in collaboration with industry partners. The course has become part of the FCC Biotechnology Associates degree, a new Cell and Gene Therapy Certificate, a registered apprenticeship, digital badging, and Letter of Recognition for Cell Therapy pathways.

Methods

FCC biotechnology program staff have long been aware of the growing demand for biotechnology workers with skills needed to operate a flow cytometer in central Maryland and had a vision to find a way to affordably offer a flow cytometry course as part of the biotechnology program. Intentional engagement

with the local biotechnology industry from the project's inception ensured that industry needs and insights shaped the curriculum design. Following the published business and industry leadership team or BILT model [19], FCC staff recruited a group of industry representatives to serve on the BILT. Members of the BILT represent a mix of local, national, and global employers with a presence in the FCC service area: Kite Pharma, BioNTech, American Gene Technologies, Precision for Medicine, and Frederick National Laboratories Flow Cytometry Core Laboratory. The BILT also had academic biotechnology program representatives with prior experience in developing cell and gene therapy skills standards and curriculum from Montgomery County Community College and Solano Community College. FCC program staff met with the BILT twice to obtain an industry perspective on outcomes they desired from the cell therapy and flow cytometry course including feedback on the course outline and curriculum.

FCC hired an instructor with an extensive flow cytometry training background, giving him the autonomy to devise and teach a workforce-aligned curriculum. FCC procured a BD Accuri C6 flow cytometer and lab reagents. Laboratory materials for flow cytometry were obtained from a variety of sources. Human embryonic kidney cells (HEK293) and Jurkat cells (a CD4+/CD8- T cell line) were purchased from the American Type Culture Collection (atcc.org). AAV/DJ-CMV-eGFP, an Adeno Associated Virus (AAV) vector that expresses Green Fluorescent Protein (GFP) was purchased from Vector BioLabs (Vectorbiolabs.com). The details of the fluorescence-labeled antibodies purchased from BioLegend (BioLegend.com) can be found on ATE Central [20].

The initial flow cytometry course ran as a 7.5-week class (May-March 2023) and utilized multiple modes of instruction, with online course lectures and seven 2.5 hour in-person labs (Tuesdays and Thurdays). Because only one flow cytometer was available, students were paired up for labs, with one leading the Tuesday activities and the other leading the Thursday activities. Students therefore experienced each lab twice, once as a performer and once as a verifier.

Results and Discussion

FCC has developed an industry-relevant curriculum for the Cell Therapy and Flow Cytometry course with input from its BILT. The BILT provided valuable feedback on the curriculum after the lecture and labs were developed. Marketing for the course was accomplished through BioBuzz, a local newsletter that connects the workforce and employers [21].

Overview of the curriculum

The course emphasized the following skills:

- Aseptic technique, mammalian cell culture, and sample preparation for the flow cytometer (Figure. 2-4).
- Calibration and maintenance of the instrument.
- Sample acquisition and gating.
- Data analysis of the results using FlowJo (Figure. 5).

The general steps in the experiments, as depicted in Figures 2-5, are:

Culture and maintain HEK or Jurkat cells

↓

Prepare cells for either transduction or antibody staining

↓

Calibrating the Flow Cytometer and acquiring the sample

↓

Gating the samples and collecting the sample acquisition data

↓

Analyzing the samples in FlowJo

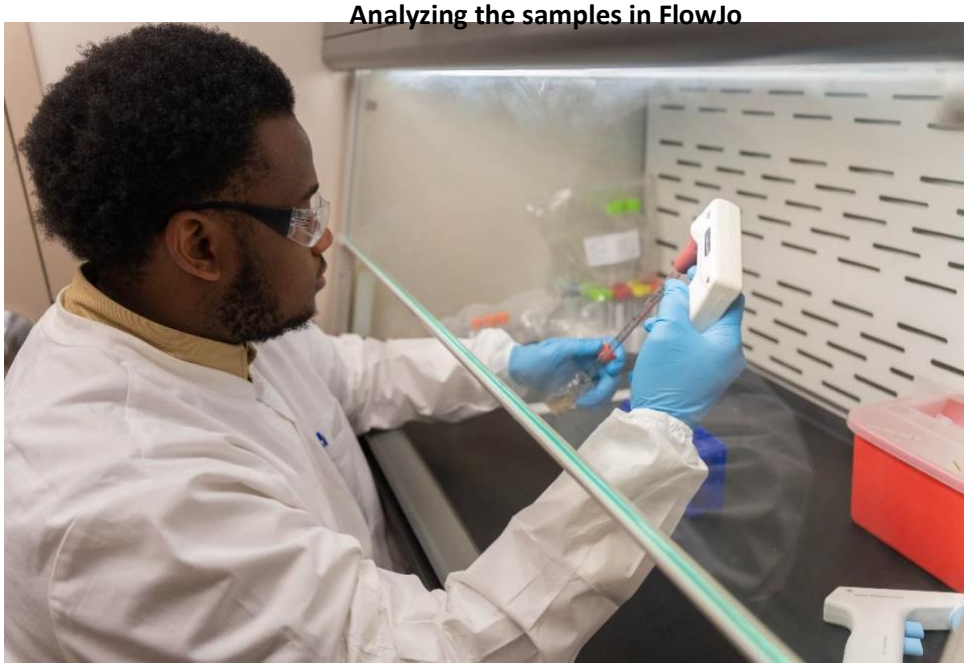

Figure. 2. Student working maintaining the Jurkat cells (T-cell line) aseptically in the Biological Safety Cabinet II A2 (BSC).

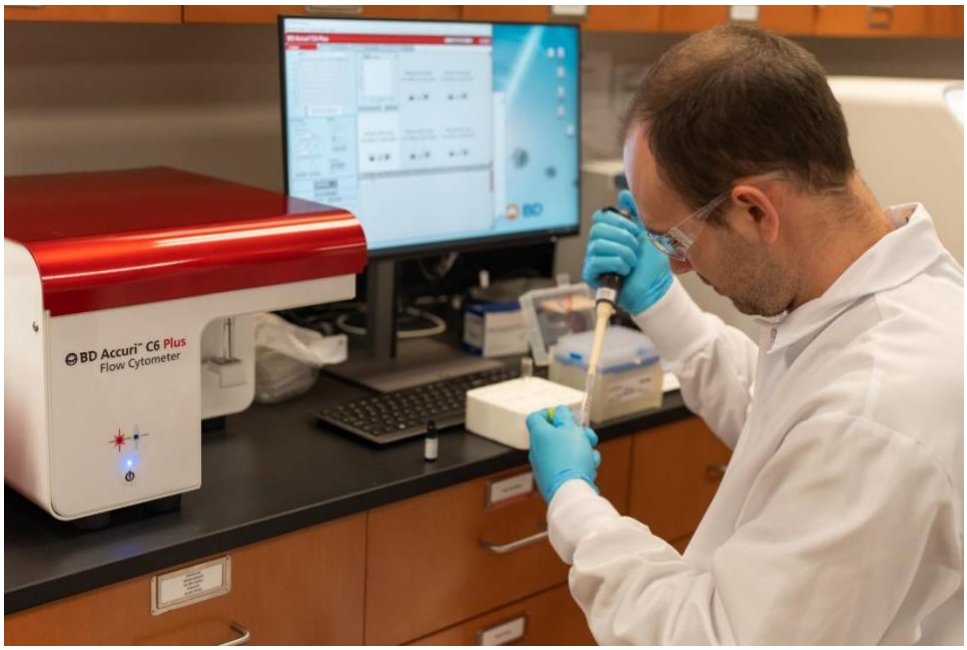

Figure. 3. Instructor demonstrating how to prepare the sample for loading on the Flow cytometer.

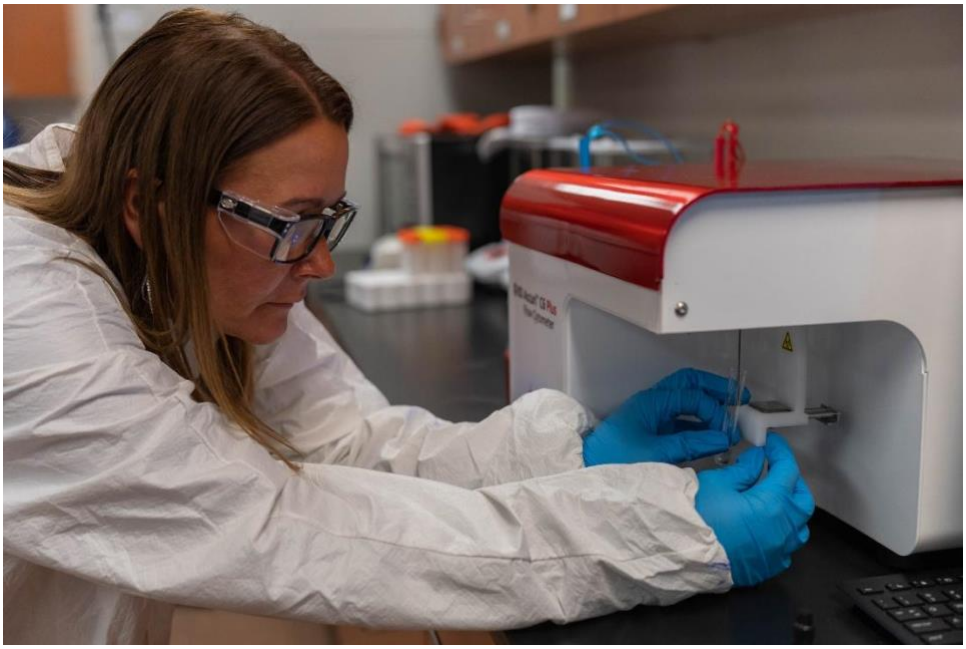

Figure. 4. A student loading a sample on the flow cytometer.

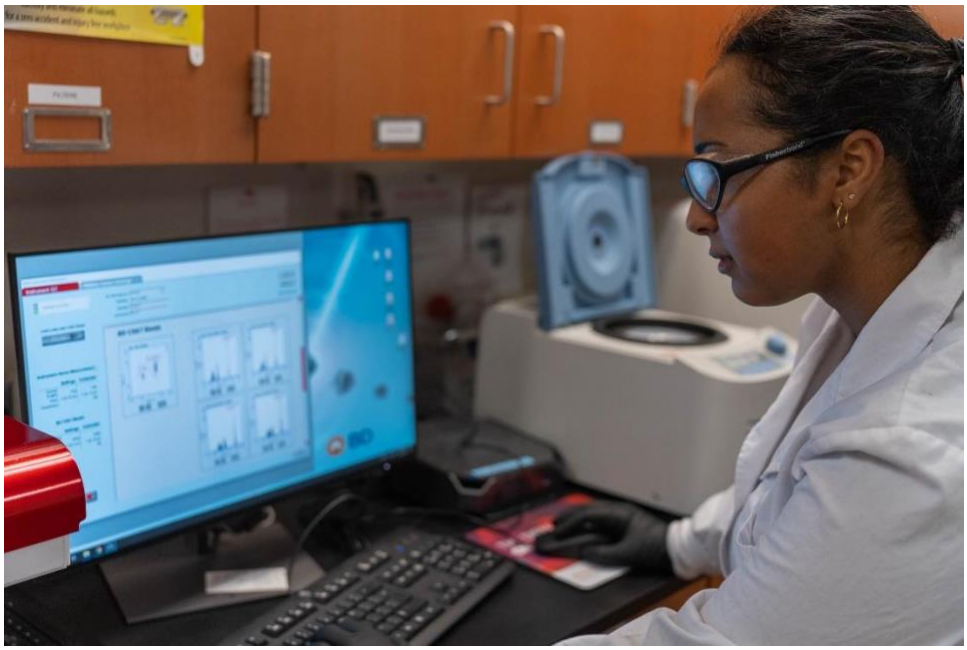

Figure. 5. Student analyzing data on the flow cytometer.

During the course, students used techniques that would be employed in manufacturing cell and gene therapies. To help studentsunderstand the application of flow cytometry in gene therapy product development, curriculum was developed to use flow cytometry to determine the effect of increasing AAV GFP viral particles, the multiplicity of infection (MOI), on transduction efficiency by evaluating the number of fluorescent cells. This experiment is described in Figure. 6B where the increase in GFP-positive cells correlates with the increase in the MOI. Details of the transduction of the HEK cells and the preparation of cells for analysis can be accessed through ATE Central [22].

To help students understand the utilization of flow cytometry for analyzing the cell therapy products, curriculum was developed using a small panel of basic T cell markers to examine the baseline characteristics of non-activated Jurkat cells which express CD3 & CD4 receptor/marker but not CD8 nor CD45RO receptors/markers. These experiments gave students the opportunity to assess multiple characteristics of the cells at once, test out a beginner-level gating strategy, and understand the variety of expression levels of different receptors.

Student data from these procedures are presented in Figures 6 and 7. Figures 6A and 7A show data from gating. In flow cytometry, gates are regions that are placed around populations of cells that share characteristics such as size. Figures 6A and 7A show oval gates in the left-hand plot that identify cells. In the left-hand plots, the gates show the Forward Scatter Area (FSC-A) plotted against the side scatter area (SSC-A) which allows debris and cell clumps to appear in the bottom left portion of the density plot. On the right, the plot shows the height of the Forward Scatter (FSC-H) plotted against the area (FSC-A). The cells in the tilted rectangle distinguish individual cells from clumps of cells. This gating process helps students identify the cells to analyze to 1) assess GFP expression levels (Figure. 6B) and 2) the presence of Jurkat cell surface markers (Figure. 7B).

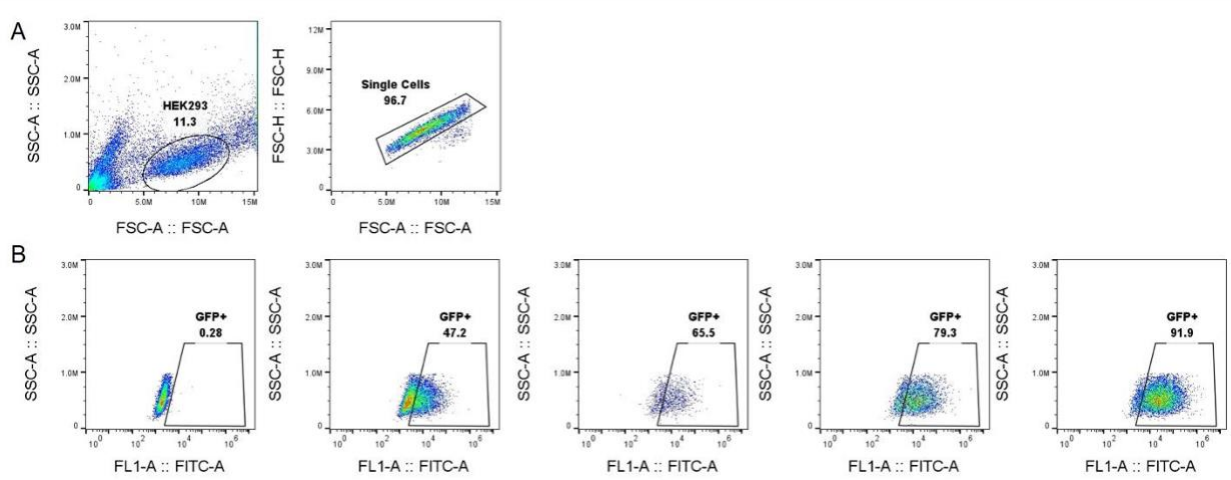

Figure 6. **Shows the effect of increased AAV GFP viral particle number on the transduction of HEK293 cells.** Cells were transduced with increasing amounts of AAV GFP virus (expressed as Multiplicity of Infection or MOI) for 48 hours. Figure 6A shows the initial gating strategy for identifying transduced cells based on forward (FSC) and side scatter (SSC). Figure 6B demonstrates increased GFP positive cells correlating with transduction with increased AAV GFP viral particles. On the BD Accuri C6 Plus, Channel FITC was used for the GFP signal. From left to right, MOI 0, 100, 200, 300, and 400, respectively.

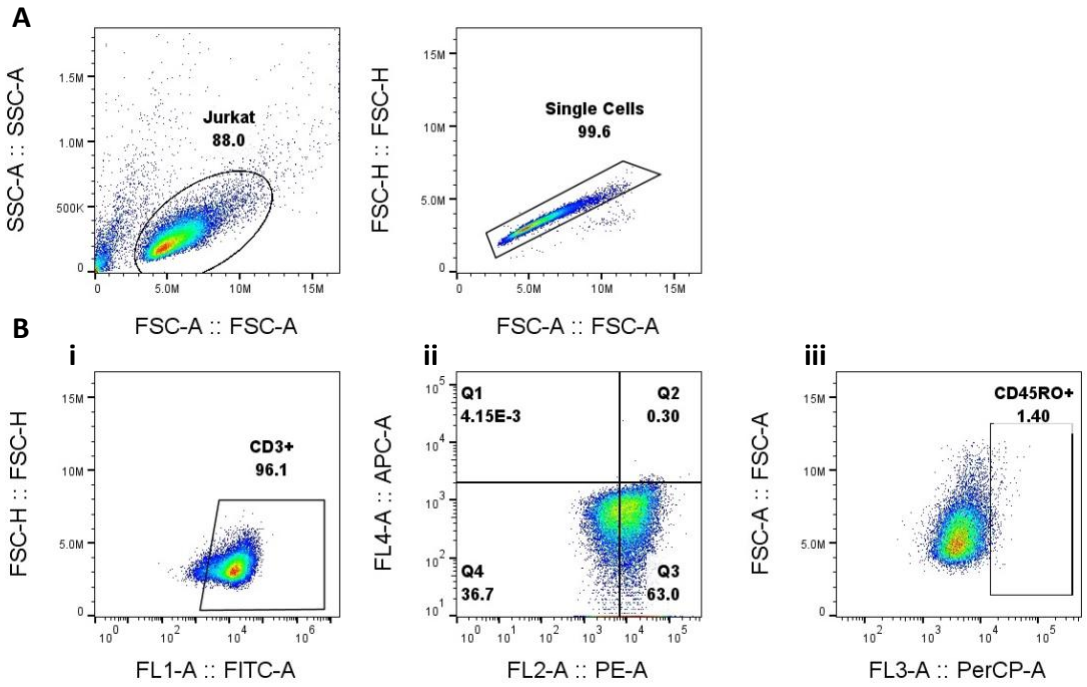

Figure 7. Multi-color panel staining of Jurkat cells. Jurkat cells classically express high levels of TCR (CD3) with low levels of CD4 and no CD8. Jurkats in culture were counted and stained with the antibody panel (CD3-FITC, CD4-PE, CD8-APC, CD45RO-PE-Cy7). Figure 7A: Cells and single cells were

identified using forward and side scatters. Figure 7B: (i) CD3+ cells labeled with FITC were gated, (ii) CD4+ cells (labeled with PE were plotted against CD8 cells (labeled with APC). Jurkat cells do not express the CD8 marker, so no staining is seen in the upper left Q1 quadrant. (iii) CD4+ cells were checked for activation marker CD45RO. Cells did not express the CD45RO marker either, as they were not activated. Gates were determined using Fluorescence Minus One (FMO) control and adjusted for spillover via compensation.

Course Outcomes

The course was initially offered in Spring 2023, and ten students successfully completed it. Seven of the ten were enrolled in the Biotechnology AAS degree program. The other three students already had associate or bachelor's degrees and took the course to gain new relevant skills.

Due to demand, FCC also offered the course as a four-week non-credit course in the Summer of 2023. The same instructor taught the class. The students met three times a week for two and a half hours; of the five students who successfully completed the non-credit course, two enrolled in the Biotechnology AAS degree program and received articulated credits towards their degree. The other three students had bachelor's or master's degrees and took the course to gain cell culture and flow cytometry skills. These three were awarded the Flow Cytometry Basics digital badge. One student did not complete the course. The assignments, labs, and final exams were the same for the credit and non-credit programs.

Table 1. Student outcomes

Course	Students in AAS program	Students with Associates or Bachelors	Total students enrolled	Course Completion	Outcomes
Cell Therapy and Flow Cytometry (credit)	7	3	10	10	2 hired 1 interviewed
Basics of Flow Cytometry (non-credit)	3	3	6	5*	1 promoted
Total	10	6	16		

* One student did not meet the completion requirements.

All students received the Cell Therapy and Flow Cytometry LOR digital badge (credit) or the Flow Cytometry Basics digital badge (non-credit). Two students were hired for research jobs that involved cell culture skills and another was promoted in her job and is now utilizing her cell culture skills at the Frederick National Cancer Laboratory for Cancer Research. One student was interviewed at the National Cancer Institute Flow Cytometry Core and received positive feedback.

Student feedback regarding the course, obtained by the evaluators via phone interviews, was overwhelmingly positive, with one saying I "loved it." Program staff designed the course with the student experience in mind and were excited to receive this feedback. The primary challenge noted by all interviewees was lab space and the amount of equipment. The lab was equipped with one flow cytometer and two biological safety cabinets. The ideal number of enrolled students was 8, but 10 students enrolled in the course. Students were therefore paired up, and they traded off who was the primary operator of the

instrument on each lab day. All students received hands-on experience with the machine for each lab, and interviewees noted that the repetition was possibly beneficial for students to learn the procedures. One student highlighted this benefit, saying that being able to "both see and do" facilitated learning instrument skills. Students felt very confident in their ability to use the flow cytometer by the end of the course. The students were assessed both for theoretical knowledge of the principle of flow cytometry as well as hands-on instrument operation and sample preparation skills. Students benefited from receiving in-demand skills and being prepared to succeed in a professional cell therapy and flow cytometry environment. Due to the small number of students in class, only qualitative data was collected.

Feedback from third-party evaluator interviews with select members of the BILT noted that the program met the local employers' needs and the students' outcome expectations. Skills of interest to employers and mastered during the program included aseptic technique, cell culture, calibration and maintenance of the equipment, sample acquisition, and data analysis. The grant Co-PI, trained by the flow course instructor during the program, will continue teaching in the program.

Conclusion

The flow cytometry instructor and students reported that every student had sufficient time to work with the flow cytometer. However, the need to pair students during labs highlights a need to add more flow cytometers in the future. This project made significant progress toward enrollment goals for the Cell Therapy and Flow Cytometry class. In the project's first year, FCC enrolled 16 students in the class. The course has been popular with older individuals working in the biotechnology industry. A future goal is to increase enrollment of 18- to 24-year-olds in the flow cytometry course. FCC's foundational work in local high schools will support higher enrollment of younger students over the next two project years.

It is rare for undergraduates to gain hands-on flow cytometry experience, especially in an affordable setting like a community college ($700 for a 7.5-week course with the possibility of student loans *vs.* $995 for a 3-day Flow cytometry workshop, https://biotrac.com/). FCC provided several complementary on-ramps into the biotechnology program (including flow cytometry) to meet student needs across varied backgrounds: a high school dual enrollment course and other programs offering lab visits, a for-credit biotechnology course, and a non-credit articulated and shortened summer course. The summer flow cytometry course demonstrates the capacity for coordination and collaboration between credit and non-credit departments to provide program offerings at various levels seamlessly. Employers frequently turn to non-credit divisions of colleges for training needs, so leveraging FCC's non-credit opportunities helps ensure a broader reach for the ATE grant investment. The Synexa Life Sciences Company and Frederick National Labs Flow Cytometry Core scientific staff have contacted us and reviewed our curriculum. The feedback indicated that the curriculum is relevant to their workforce needs, and they subsequently requested resumes from students to determine if they wanted to interview them.

In conclusion, the NSF ATE grant awarded to FCC focused on building a cell therapy and flow cytometry workforce to respond directly to local workforce needs and provide career opportunities to individuals with low incomes and marginalized communities. FCC is sharing the curriculum, lab SOPs, and batch records through the Northeast Biomanufacturing Center & Collaborative (NBC2) (Biomanufacturing.org) to facilitate the introduction of hands-on flow cytometry curriculum in other biotech programs nationwide.

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Disclosures The authors declare no conflicts of interest.

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