PROPOSAL:
Environmental Enclosure for a Single-Cell Inkjet Printer

Leanna Hogarth, Natkamol Limapichat,
Sadan Wani, Andrew Yan, and Wenting (Wendy) Zhou

University of British Columbia
Electrical and Computer Engineering

October 14, 2019

Executive Summary

The BioMEMS Lab has developed a proof-of-concept for a novel single-cell inkjet printing system that features high throughput and high accuracy of single-cell isolation. To facilitate the dispensing process in practical applications, the printing system requires proper environmental regulation. Factors such as substrate temperature, relative humidity, and airborne particle content must be dynamically controlled during the printing operation for the sensitive downstream measurements. An enclosure system is proposed to meet this need. The proposed system contains: a temperature control unit which regulates the temperature of the printing plate with high precision and accuracy; a humidity control unit which maintains the humidity level inside the enclosure to avoid condensation and evaporation at the plate; and an enclosure with a filtration system to maximize sterility. A timeline and budget plan are created to layout the steps towards completion of the project by April 2020. Roles, responsibilities, and individual team member expectations are also outlined.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ACRONYMS AND ABBREVIATIONS

BioMEMS	Bio-Medical Micro Devices
C	Capacitor
DC	Direct current
HEPA	High-efficiency particulate air
LCD	Liquid crystal display
MDF	Medium-density fibreboard
Misc.	Miscellaneous
MSDS	Materials Safety Data Sheet
PCB	Printed circuit board
PPE	Personal protection equipment
PSU	Power supply unit
R	Resistor
SLA	Stereolithography Apparatus
TA	Teaching assistant
TEC	Thermoelectric cooler
Temp.	Temperature
WDM	WDM

1 BACKGROUND AND CONTEXT

The BioMEMS Laboratory is a research group at UBC that focuses on the miniaturization of systems and devices, particularly for biomedical applications. Currently, the BioMEMS Lab is developing a novel inkjet printing system for isolation of single cells. Inkjet printing technology provides a flexible, cost-effective, and efficient means of micropatterning living cell samples and can be used for a variety of applications including genomic sequencing, antibody discovery, and stem cell research. While existing cell printers struggle to balance the high accuracy necessary for single-cell isolation with sufficient throughput, the BioMEMS' system uses machine vision and deep learning to facilitate a single-cell dispensing process at high rates.

One challenge with single-cell isolation processes is that they must be performed within meticulously controlled environments. External factors such as temperature, humidity, and sterility (i.e. airborne particles and bacteria) can be detrimental to the health and usability of cell samples; without proper regulation, downstream analysis cannot be performed. In order to evolve the current single-cell inkjet printer from a proof of concept to a practical technology for end users, a controlled environmental enclosure must be designed. A market analysis is provided in the Appendix to review some of the existing alternatives.

2 OUTCOME

The main goal of this project is to design and build a strictly controlled environmental enclosure to complement the existing single-cell inkjet printing system of the BioMEMS Lab. This is necessary to mitigate the negative impacts of the environment on biological samples and to allow downstream analysis for future end-users. Upon completion, this project will serve as an initial prototype with fully integrated humidity, temperature, and sterility control systems; it will act as supporting equipment for the single-cell isolation printer.

3 DELIVERABLES

As mandated by the dispensing process, the resultant project must maintain precise control of two main variables: temperature and humidity. Temperature and humidity are particularly important because they determine the health and evaporation rates of cell samples. Sterility is also essential as to avoid contamination; however, due to the availability of economical filtration systems on the market, sterility is a secondary focus for this project. Therefore, the project can be split into the following deliverables.

3.1 Local Temperature Control

The purpose of local temperature control is to keep the cells alive and to sustain a desired cell state. By maintaining control at the well-plate itself, temperature can be quickly adjusted in the wake of any sudden changes. Local temperature control also allows for greater certainty with regard to the true temperature of the samples.

Since well plates vary across applications, this project will demonstrate local temperature control for two different scales: the 5,184 Nanowell Plate (small-scale plate) and the 96-Well Plate (larger-scale plate). Two separate local temperature control platforms will be developed to accommodate the different sized well plates used by the BioMEMS Lab. The sizing specifications are as follows:

Sizing Requirements

- The total width of the temperature control platform must not exceed 120 mm and the total height of the platform must not exceed 40 mm.
  5,184 Nanowell Plate:
  - The platform for the plate must have an embedded cavity of inner dimensions 60 mm by 60 mm and depth 3.97 mm to secure the plate

  96-Well Plate:
  - The platform for the plate must have an embedded cavity of inner dimensions 127.71 mm by 85.43 mm and depth 14.10 mm to secure the plate

Aside from structural differences, the temperature controllers will be expected to meet the same requirements and specifications:

Temperature Requirements

- At the well plate, the controller must be able to produce and maintain the following temperature setpoints while the inkjet printer is operating:
  - 2.0 °C - 4.0 °C (cell stasis temperature)
  - 37.0 °C (incubation temperature)
- Outside of active operation of the inkjet printer, the controller must be able to produce and maintain at temperature setpoint of 80.0 °C for sterilization of well plate
- The temperature measurement must be precise to 0.1 °C and the controlled temperature should be accurate to within 0.5 °C for each setpoint
- The temperature setpoints must be reached within 1 minute
- When reaching the setpoint, the controller must not overshoot the temperature more than 0.5 °C before settling

3.2 Humidity Control

The main purpose of the humidity control component is to minimize droplet evaporation– a significant concern when samples can be less than a few nanoliters in volume. The humidity controller will also serve to prevent condensation of water on the well-plates and avoid contamination. To provide these functions, the humidity controller must conform to the requirements and specifications below:

Humidity Requirements

- The humidity of the enclosure must be controlled to 95.0% relative humidity at the operating setpoint temperatures of 2.0 °C - 4.0 °C and 37.0°C
    - This is to prevent evaporation of samples as well as condensation on the well plates
- The humidity must be measurable to the nearest 0.1 % and accurate to within 2.0% for the two distinct operating setpoint temperatures of 2.0 °C - 4.0 °C and 37.0°C
- The setpoint must be reached within 5 minutes

3.3 Enclosure and Filtration

The purpose of the enclosure is to provide a sealed environment to properly regulate the various conditions. HEPA filtration[1] will be incorporated after the primary deliverables have been accomplished. The following table outlines some of the key requirements for the enclosure and filtration system:

Sizing Requirements

- The enclosure must encase the single-cell inkjet printer, therefore the minimum inner dimensions of the enclosure are 850 mm x 700 mm x 500 mm

Structural Requirements

- The enclosure must have a sealed door(s) to provide access to both the front and top of the inkjet printer. This is to allow access to the printer mechanics as well as the well plate itself.
    - The minimum dimensions of this door are 210 mm x 160 mm
- The enclosure must have sealed conduits to allow passage of tubes and cables to the external environment

[1] Filtration is a secondary priority; it will be developed after the main deliverables of temperature and humidity control are functional.

Material Requirements[2]

- The materials used for the enclosure must be able to sustain a continuous temperature range of 0 °C to 40.0°C without degrading, deforming, or changing physical/chemical properties*

- The materials used for the enclosure must not:
    - Dissolve in ethanol (as it is needed for sterilization)
    - Release chemicals as particulate matter or gas (no off-gassing)
    - Be hygroscopic[3] (humidity sensitive)

Filtration Requirements

- The air within the enclosure must be sterilized prior to the start of printing to catch 99.97% of the 0.3-micron particles
    - *Note:* this is the standard for HEPA filtration

3.4 Constraints

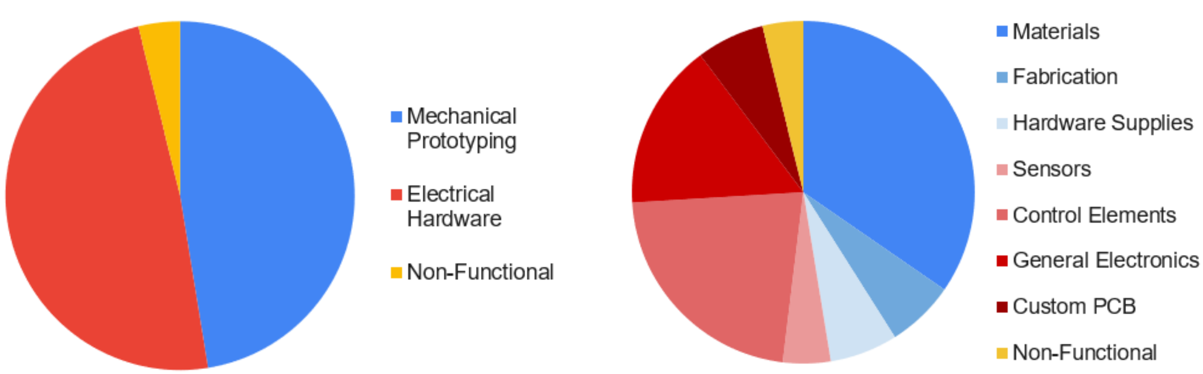
One of the most significant constraints for this project is the need to maintain an environment of high relative humidity without producing large water aerosols. This prevents us from using technologies such as ultrasonic humidification directly because they generate water aerosols instead of vapor, leading to potential sample contamination through trapped dust, minerals, or microorganisms. Another challenge is maintaining local temperature without producing condensation. In absence of proper ambient control, changes in the temperature of the well plate could result in the formation of water droplets on the surface, polluting the samples. Because these concerns may require more precise and unique solutions in regard to sensor and control elements, the budget may also be a limiting factor.

[2] Some possibilities that fit these requirements include: **Polyethylene, Polypropylene**
[3] In order to produce a RH of 95% at operating setpoint temperatures of 2.0 °C - 4.0 °C and 37.0°C, ambient temperature may need to be adjusted accordingly

4 BUDGET

The budget consists of all projected expenses for the project timeline. Some of the main costs for this project are associated with mechanical prototyping (materials and utilities) and electrical hardware. Because the enclosure will be quite large and has strict requirements for sterility, the material costs take up a significant portion of the budget. Cheaper materials like wood and MDF will be used for initial structural prototyping, however they may not be suitable for the final product due to robustness and sterility requirements.

Electrical hardware also incurs a considerable expense for this project. The system requires an array of different sensors and control elements to regulate environmental conditions within the enclosure; it also requires supplementary electronics to support general operation. Since there is typically a tradeoff between precision and cost, these expenses may vary significantly depending on the chosen design solution. The expense summary is therefore a middle-range estimate to account for uncertainty in the design solutions. Figure 1.a) depicts a general budget distribution for the project while Figure 1.b) provides a more detailed breakdown of this budget. Table 1 provides a full estimate for the expenses, listing individual expenses and their estimated costs; sources for the cost-estimates can be provided upon request.



(a) (b)

Figure 1: (a) General budget distribution (b) Detailed budget distribution

Mechanical Prototyping			
CATEGORY	SUBCATEGORY	SUBTOTALS	TOTAL
Materials *(3D printed, sheets, etc.)*	Initial Materials *(MDF, wood)*	$140.00	$540.00
	Robust, sterilizable plastic *(final)*	$400.00	
	Aluminum cast(s) for local temp. control	-	
Fabrication *(3D printer, laser)*		$100.00	$100.00
Mechanical Hardware Supplies	Cables	$10.00	$100.00
	Door Latch	$20.00	
	Door Gaskets	$20.00	
	Door Hinges	$20.00	
	Conduit supplies *(Tubing, waterproof cable glands, silicone Sealant, etc.)*	$20.00	
	Screws/Bolts	$10.00	
MECAHNICAL SUBTOTAL			**$740.00**

Electrical Hardware			
CATEGORY	SUBCATEGORY	SUBTOTALS	TOTAL
Sensors	Humidity *(hygrometers)*	$30.00	$70.00
	Temperature *(thermistors, thermocouple, etc.)*	$30.00	
	Air Pressure	$10.00	
Control Elements	Fans	$80.00	$345.00
	Humidifier/Dehumidifier Components	$150.00	
	Peltier element for temp. control	$30.00	
	Cooling Element for ambient air *(i.e. peltier elements)*	$60.00	
	Heating Element for ambient air *(i.e. lightbulb + socket)*	$25.00	
General Electroni	Fan driver circuit	$15.00	$245.00
	Buck Converter	$20.00	
	Misc. *(C's, R's, etc.)*	$30.00	
	PSU	$100.00	
	Microcontroller(s)	$50.00	
	Interface *(i.e. LCD + buttons or touch)*	$30.00	
Custom PCB *(i.e. PCBWay)*		$100.00	$100.00
ELECTRICAL SUBTOTAL			**$760.00**

Non-Functional	
CATEGORY	TOTAL
Misc. *(Paints for aesthetics, Google Drive storage,*	$20.00
Poster *(final)*	$40.00
OTHER SUBTOTAL	**$60.00**
TOTAL EXPENSES	**$1,560.00**

Table 1: Detailed budget distribution with cost-breakdown

The starting budget for this project is $650, provided by Capstone. Initial calculations suggest that this project will require additional funding to meet the strict requirements proposed (see Table 1). The BioMEMS lab has expressed its' willingness to aid the project financially, up to a dollar amount of $1000 if the costs are deemed reasonable. Larger purchases will require the development of a cost-comparison document to outline the expenses associated with different design alternatives and their trade-offs. This procedure is enacted to ensure that the budget is spent wisely.

If additional unexpected expenses are incurred, design choices may require adjustment to accommodate the deficit. Some unexpected costs may include sunk costs from purchases that fail to meet project requirements as well as expenses incurred from the mishandling or poorly planned use of materials and hardware.

5 PROJECT MANAGEMENT

The following section outlines the general management plan for this project. Details including the project timeline, risks and dependencies, team member responsibilities, and conflict resolution are all outlined below. By clearly defining these aspects early on, team members become accountable to the project and its' objectives, increasing the likelihood that the project stays on course.

5.1 Timeline / Milestone Summary

A Gantt chart (Figure 2) is used to illustrate the timeline of the project. The project timeline is split into Milestone tasks as well as individual tasks for each sub-team. Each sub-team is responsible for one of the three main deliverables: Enclosure and Filtration, Humidity Control, and Local Temperature Control; the initials of the team members responsible for each subsystem are clearly denoted.

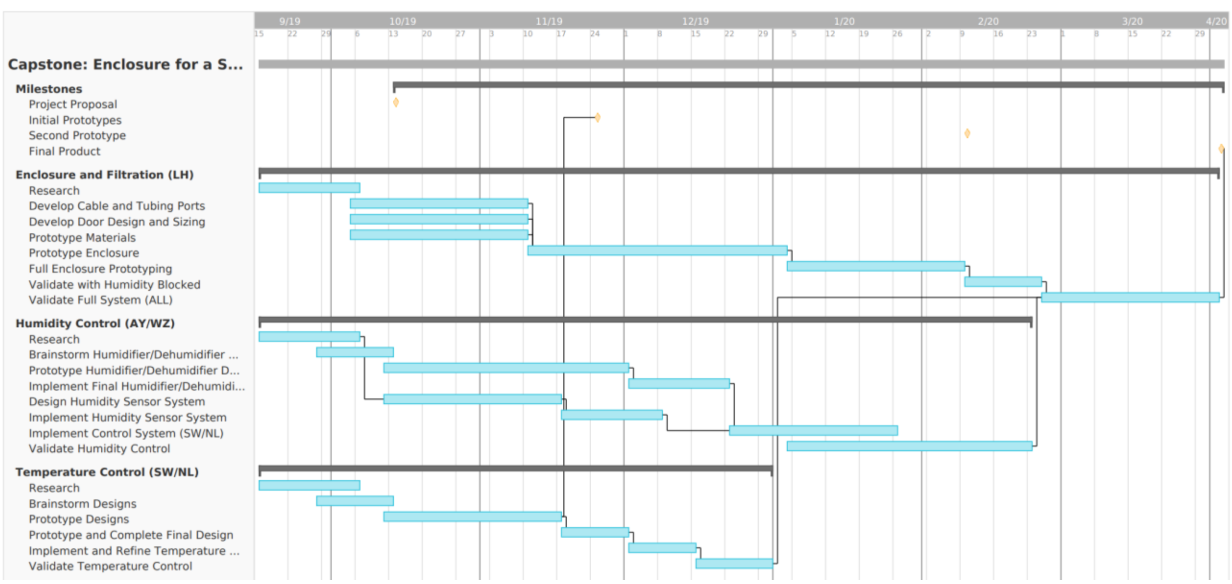


Figure 2: Gantt Chart outlining project tasks and their respective deadlines

The project tasks outlined in the Gantt chart correspond to the 4 primary milestones established by Capstone itself. Table 2 summarizes how the Capstone Milestones are integrated in the project timeline.

Date	Milestone	Goals
October 15, 2019	Project Proposal	● Have brainstormed potential designs for each subsystem ● Have begun prototyping potential designs for each subsystem
November 25, 2019	Initial Prototype	● Have a completed prototype for each subsystem ● Have determined the final method for controlling enclosure humidity
February 10, 2020	Second Prototype	● Have a full system prototype with all subsystems integrated ● Have a second prototype of the humidity control system and begun subsystem validation ● have a completed temperature control system, including subsystem validation
April 3, 2020	Final Product	● Have a fully integrated system with final designs for each subsystem ● Have validated the product to ensure that it meets the specifications and requirements

Table 2: Summary of project timeline according to Capstone Milestones

Given the interdependent nature of this project, it is understood that tasks for different subsystems may be delayed as one subsystem encounters new and unexpected challenges. Team members from each subsystem will be expected to collaborate and aid each other to minimize bottlenecking.

5.2 Risks and Dependencies

Project risks are uncertainties that have the potential to negatively affect the deliverance of a project's objectives. In order to prevent project objectives from failing, mitigation plans must be established.

Risk Category	Description	Mitigation
Financial	The project budget of $1650 is exceeded.	• A purchasing procedure is enacted to prevent unnecessary or negligent purchases. • This procedure requires the production of a cost-comparison document for larger purchases, outlining the expenses associated with different design alternatives and their trade-offs. • The cost-comparison documents will be presented to the client for purchasing feedback.
Performance and Schedule	The final product does not meet the established requirements and specifications within the allotted time.	• Gantt charts are created and will be updated to ensure the project stays on schedule. • The client (Eric Cheng, Dr. Karen Cheung) and other external professionals will be consulted regularly to provide feedback and advice on the complex hardware and software subsystems.
Integration	Integration of the subsystems is initiated too late in the project timeline, producing an incomplete enclosure system.	• Team members working on the different subsystems will be required to provide weekly updates on their progress to-date. • Sub-teams will be required to collaborate weekly, ensuring that subsystems are designed to work together and in harmony. • Once basic operation has been achieved for each-system, initial integration and systems-level controls will commence.

Safety	Exposure to hazardous materials	• Team members will all receive proper training for any machinery or equipment used throughout the project. • Team members will wear the appropriate PPE and follow safety guidelines. • Team members will consult MSDS for any new materials used and direct any questions to the appropriate professionals
	Injury from heavy machinery	
	Electric shock	
	Frostbite/Burns	

Table 3: Risk categories and mitigation procedures

5.2.1 Project Dependencies

As outlined in the Timeline/Milestone Summary section, the localized temperature, ambient humidity, HEPA filtration, and enclosure control systems are all highly interdependent. An initial prototype of the enclosure must be built before controls can be implemented for the ambient humidity system. Fine tuning of the humidity system will also be dependent on when the local temperature control system is integrated, as humidity, condensation, and evaporation are all highly susceptible to temperature changes. To maximize efficiency, team members of the different sub-teams will be expected to assist other teams when unexpected challenges arise. Time associated with the delivery of parts and components may also delay progress for individual sub-teams and should be accounted for within the project timeline.

5.3 Roles and Responsibilities

Roles and responsibilities are defined to ensure that each team member is assigned an equal portion of the project and its' deliverables. Each team member will be held accountable for any lack of initiative in the completion of their assigned tasks. The primary responsibilities are split into two categories: Design and Administrative responsibilities.

5.3.1 Design Responsibilities

Based on the Deliverables, the project can be split into three broad categories: local temperature control, humidity control, and environmental enclosure. The sub-team design distribution is outlined in Table 4.

Team Members	Design Responsibilities
Natkamol Limapichat, Sadan Wani	• Design and implementation of the localized temperature control system for the microwell and nanowell plates • Devise a mounting mechanism for both plates
Andrew Yan, Wendy Zhou	• Design and implementation of the ambient humidity control system • Consult with client more regularly for
Leanna Hogarth	• Design and construction of the enclosure • Implementation of filtration system*

Table 4: Design role assignments

This project will require a lot of collaboration between members as all the subsystems are interdependent and must work in unison for the product to function.

5.3.2 Administrative Responsibilities

Due to the scale and complexity of the project, team members will take on further administrative responsibilities to ensure smooth operations. Table 5 summarizes the task distribution for administrative responsibilities.

Administrative Responsibilities	Subcategories and Descriptions	Team Member
Finance	• ***Purchases*** - Responsible for maintaining an account of the project expenses and making purchases on behalf of the team. Will seek reimbursement as needed	Wendy Zhou
	• ***Budgeting*** - Responsible for maintaining an account of the project expenses and management of the budget provided to the team. Purchase decisions made via a WDM. Will seek reimbursement as needed	Sadan Wani
Communications	• ***Internal*** - Responsible for communication between team members and clearly outlining expectations throughout the project timeline. Arranging meetings or discussions as needed.	Andrew Yan
	• ***External*** - Responsible for communication between team members and other stakeholders. Keep stakeholders informed on progress or reach out for help as needed.	Leanna Hogarth
Safety and Support	• Ensure that all team members follow safety protocol and best practices. • Assist other team members in their administrative responsibilities as needed	Natkamol Limapichat

Table 5: Administrative responsibilities assignment

5.3.3 Individual Team Member Expectations

All members of the team are expected to dedicate time and effort to the project to the best of their abilities. In general, members are expected to spend at least eight hours per week on the project. Table 6 summarizes the fundamental expectations for all team members.

Expectation	Description
Communication	● Members should communicate frequently in exchanging thoughts and ideas, and the general information of the project should be kept transparent and reachable by all members.
Group meetings	● Group meetings should be held weekly and all members are required to attend unless given reasonable excuses. ● During the meetings all members should discuss the research documents they have found useful, progress they have made, and obstacles they have encountered. ○ If the problems addressed cannot be solved by other team members, the team should seek help from instructors, TAs, and clients.
Response time	● Emails or other notifications received from the instructors, clients, and other team members should be responded within 12 hours.
Meetings with clients	● The meetings with clients should be scheduled at least two days ahead and all members of the team are expected to attend.

Table 6: Summary of team member expectations

5.4 Conflict Resolution

Conflict typically arises internally, from disputes between individuals in a team. Common conflicts are attributed to a difference in opinion regarding project design solutions or to individuals not fully fulfilling their assigned tasks and duties.

When team members have conflicting ideas on the methods or solutions they believe are best suited to solve a design problem, team members will be required to provide proper evidence outlining the various advantages and disadvantages of their solution. These alternatives will then be compared within a WDM. If no solution is clearly superior, initial validation may be required in the form of early prototyping. All team members will then express their thoughts and concerns during a group meeting, and a vote will be cast to make a final decision.

In the case where a team member consistently fails to meet expectations, the affected team members should tactfully approach the individual to see if the problem can be resolved directly. If an agreement still cannot be achieved, the team should report this incident formally to the instructor and the instructor will deem if further action is necessary.

In the event that an external conflict arises between the project team and the client that cannot be resolved through formal discussion, Capstone instructors may be asked to intervene.

APPENDIX: MARKET ALTERNATIVES

Some of the market alternatives can be found in Table 7 below:

Specification	Molecular Devices: CloneSelect Single-Cell Printer Series	Bio-ink for Inkjet Printing of Living Cells	Wafergen Biosystems: ICELL8	Fluidigm: C1
Relative Humidity (RH) Range	Not Publicly Available	"Humidified"	Non Publicly Available	Not Publicly Available
Temperature	Not Publicly Available	37 °C	37 °C	4 °C - 99 °C
Well Plate Types	96- or 384-	N/A	5184 Nanowell	Integrated 96-cell
Sterilization	Ethanol and Isopropanol, Autoclaved (121 °C)	70% Ethanol, UV Light	Not Publicly Available	Not Publicly Available

Table 7: Market Analysis Comparison

BIBLIOGRAPHY

1. Ferris, C.J. & Gilmore, Kerry & Beirne, Stephen & Mccallum, Don & Wallace, Gordon & in het Panhuis, Marc. (2013). Bio-ink for inkjet printing of living cells. Technical Proceedings of the 2013 NSTI Nanotechnology Conference and Expo, NSTI-Nanotech 2013. 2. 261-264.

2. "Molecular Devices." Molecular Devices, http://www.moleculardevices.com/.

3. "Overview of the ICELL8 Cx Single-Cell System." Takara Bio-Home, https://www.takarabio.com/learning-centers/automation-systems/icell8-introduction/icell8-technology-overview.

4. "The Expression You Seek. The Workflow You'll Love." Fluidigm, http://www.fluidigm.com/.

5. "The University of British Columbia." *UBC*, https://www.ece.ubc.ca/research/biomems.