Leveraging Flexible Pipette-based Tool Changes to Transform Liquid Handling Systems into Dual-Function Sample Preparation and Imaging Platforms

Authors

Mohammad Nazeri[1 †], Jeffrey Watchorn[2 †], Sheldon Mei[1], Alex Zhang[4], Christine Allen[5], Frank Gu[1,3 *]

Affiliations

[1] Institute of Biomedical Engineering, University of Toronto, 164 College St, Toronto, ON M5S 3G9
[2] Acceleration Consortium, 700 University Avenue, Toronto, ON M5G1X6
[3] Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College St, Toronto, ON M5S 3E5
[4] Department of Mechanical & Industrial Engineering, University of Toronto, 5 King's College Rd, Toronto, ON M5S 3G8
[5] Leslie Dan Faculty of Pharmacy, University of Toronto, 11 College St, Toronto, ON M5S3M2

Corresponding author's email address

f.gu@utoronto.ca

Abstract

In this study, we present an advanced system that integrates simultaneous pipetting and in-situ imaging using the Opentron OT-2 liquid handling robot. This system enables real-time monitoring and characterization of dynamic processes, such as hydrogel crosslinking, without any manual intervention. The platform's modular design maintains cost-effectiveness and high-throughput capabilities while expanding the versatility of the OT-2 robot by incorporating imaging functionalities into the experimental workflow using a pick-and-place apparatus. Due to its modular architecture, based on an OT-2 robot, the system can be adapted for a wide range of laboratory applications at an affordable cost. This real-time imaging solution offers a practical approach to laboratory automation, leading to more efficient and data-driven experimentation. Although ionically crosslinked hydrogels were used as a proof-of-concept, this platform has potential applications across various materials systems, including crystallization dynamics, polymerization kinetics, and drug delivery system development.

Keywords

Real-Time Imaging Integration, Liquid Handling Robot, Automated Hydrogel Formulation, Closed-Loop System, Bayesian Optimization for Experimentation, High-Throughput Material Characterization

Specifications table

Hardware name	Automated Hydrogel Imaging System
Subject area	• Engineering and materials science • Medical (e.g., pharmaceutical science)
Hardware type	• Imaging tools • Biological sample handling and preparation • Measuring physical properties and in-lab sensors
Closest commercial analog	No commercial analog is available
Open source license	Non-commercial license: CC BY-NC 4.0
Cost of hardware	$10,000 USD (OT-2) + $110 USD (Hardware Module)

1. Hardware in context

Imaging during material synthesis is crucial for uncovering properties that are otherwise difficult to measure, such as reaction kinetics, structural changes, diffusion processes, and phase transitions [1], [2], [3], [4], [5]. This is particularly important for semi-solid or gel-like systems [6], [7], [8] where dynamic properties or insufficient mechanical strength make transferring samples to other instruments challenging [9]. Traditional imaging systems, such as microscopes, typically require moving samples from their synthesis location to the imaging system and involve significant manual intervention to produce high quality images [10], [11]. This manual intervention can have a significant influence on the accuracy of quantitative property measurement and can limit the system's ability to handle additional sequential reactions, making it less efficient for comprehensive material characterization.
To overcome these challenges, we developed an imaging platform using the Opentrons liquid handling system. Opentrons was chosen for its open-source nature, which allows for extensive customization [12], and its affordability compared to other commercial liquid handling systems [13], [14], [15]. This integration enhances the efficiency and precision of imaging during synthesis, enabling more accurate and reliable material characterization.

Imaging systems are widely used across various fields and industries [16], [17], [18]. As such, there has been significant interest in automating imaging systems for several applications, such as histology, drug delivery, and soft materials science [19], [20], [21], [22], [23]. Typically, these automated systems focus on autonomous image capture and processing across a sample area (such as a microscope slide). While there are some recent examples of gantry-based image capturing solutions, which enable capturing of individual sample images, these solutions do not support dynamic experimentation (i.e., the addition of solvent or other materials to the sample stage) as they remove any liquid handling capacity in favor of imaging [24], [25]. The current hardware platform aims to address these limitations and challenges by developing a low-cost, high-throughput automated image capture and analysis platform in a manner that enables continued experimentation by maintaining pipetting ability while orienting the camera in multiple different possible geometries. This makes it an ideal solution for laboratories seeking to leverage advanced automation without the associated high costs and inflexibility of commercial systems.

2. Hardware description

The hardware platform developed for this study is a custom, yet generalizable solution designed to overcome the limitations of existing imaging systems, particularly for dynamic monitoring during material synthesis. The core innovation of this hardware lies in its modular design, which allows for flexible imaging and experimentation. Unlike traditional imaging systems that are fixed in design and require transporting samples to the imaging station, our system integrates a robotic pipetting gantry with a custom 3D-printed pick-and-place apparatus. This includes a holder for a pipette tip and a vertical camera mount. The pipette can automatically engage the apparatus by picking up the tip, and subsequently handling the attached camera. By combining the dual functionalities of liquid dispensing and real-time imaging, we are able to monitor the evolution of the physical properties of materials systems effectively.

This characteristic is especially helpful for researching semi-solid or gel-like systems, since the properties of the material might vary depending on environmental conditions or experimental manipulations. Additionally, the integrated liquid handling system allows for the addition or removal of solvents and reagents during imaging, which is important for monitoring dynamic processes such as hydrogel crosslinking [26], [27] and disintegration [28].

The primary components of the innovative hardware platform are as follows:

1) Robotic pipetting gantry: The pipetting gantry is based on an Opentron OT-2 liquid handling robot. This robot can be controlled by designing the experiment either using the Opentrons Protocol Designer or the Opentrons Python API, enabling flexible and precise automation of pipetting tasks.

2) Custom pick-and-place apparatus: The current embodiment of this hardware uses a pipette tip and a vertical camera mount. This adaptor enables the camera to be in a vertical position and image directly beneath the gantry system. The camera model used is the low cost Opti-Tekscope OT-HD, which connects to an experiment orchestrator server via USB. The distance between the camera and samples in a 96-well microplate on the OT-2 deck can be adjusted via adjusting the camera position or the length of the holder apparatus.

The design of the camera holder, which is attached vertically and picked up by the pipetting gantry, is important for capturing high-resolution images of the hydrogels in the 96-well microplate. The design process began with identifying the initial requirements and constraints. The camera holder needed to be compatible with the existing pipette gantry system and securel the camera in a fixed vertical position. This allows for easy adjustments of the camera focus and provides stability to minimize vibrations, ensuring clear image capture for the automated hydrogel imaging.

The hardware platform provides valuable support to researchers in the following ways:

- **Real-Time Analysis:** The ability to image simultaneously while pipetting liquids and forming semi-solids is a powerful tool. It can be used not only in the biomaterials field but also for in-situ characterization of various materials during synthesis.

- **High-Throughput Screening:** This hardware has been utilized to analyze the formation of ionically crosslinked hydrogels. It can also monitor various other combinations, enabling researchers to determine, through integrated imaging, whether those combinations result in gel formation and identify which combinations produce gels according to the desired outcome.

- **Cost-Effective Automation:** Instead of relying on expensive characterization techniques to analyze gel formation, this custom system equipped with a low-cost camera and camera holder on an automated platform allows for effective monitoring of gel formation. It also provides insights into how cross-linking occurs over time and under various conditions, offering a preliminary understanding of the system's performance. This approach enables the selective identification of the most promising combinations, which can then be subjected to more advanced and costly characterization techniques for thorough analysis.

High-Throughput Screening Capability

The custom hardware platform allows for high-throughput screening of hydrogel formulations within the OT-2 liquid handling robot. As shown in Figure 1, the deck configuration accommodates up to six microplates, allowing for the monitoring of 576 unique combinations in a single experiment. This setup enables the automated preparation and analysis of hydrogels, with an integrated camera capturing images at different time intervals. Additionally, the deck includes a 300μL tip rack and a custom 3D-printed holder that can house 54 vials of 2 mL each. By systematically varying parameters and observing the gelation process, this platform significantly accelerates the discovery and optimization of hydrogel materials. This capability is particularly valuable for applications requiring extensive combinatorial testing, such as developing new biomaterials or optimizing cross-linking conditions for hydrogels.

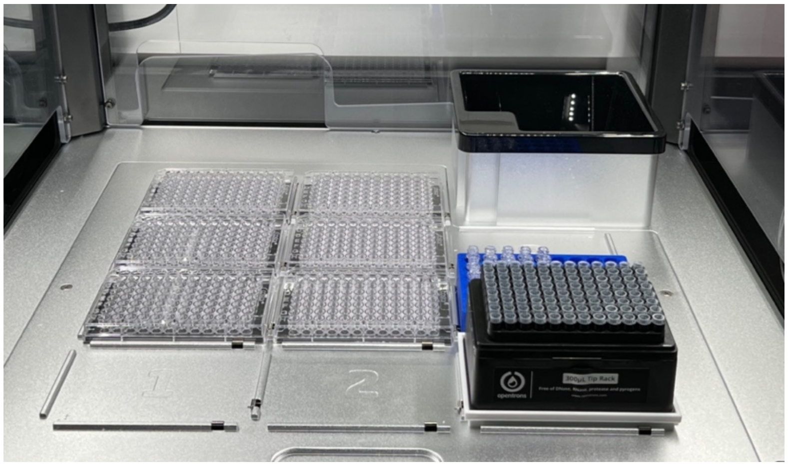

Figure 1. Deck configuration showing the high-throughput screening capability within the OT-2 robot

3. Build instructions

- *Design a pick-and-place tool including a tip place and vertical camera holder*

A 3D model of the camera holder was created using SolidWorks, as shown in Figure *2*. The design includes a pipette tip holder that securely positions the tip, allowing the pipette gantry to pick up the entire 3D-printed structure. Additionally, a horizontal cylindrical holder keeps the pen-like camera at the appropriate angle for capturing the hydrogel beads inside each well. The horizontal holder also features a few screw holes to securely fasten the camera, preventing it from rotating during the movement of the pipette gantry.

Once the design was finalized, the 3D model was exported as an STL file and loaded into the Cura slicing software, compatible with the Prusa i3 Mk3s printer. The holder was printed using a polylactic acid (PLA) filament, chosen for its durability and ease of printing. Post-processing involved removing support structures and sanding any rough edges to ensure a smooth finish.

Calibration was then performed by adjusting the camera's level and focus to achieve the optimal imaging of the hydrogel beads. Initial tests included capturing images of empty wells to validate the accuracy and stability of the setup. Based on the test results, adjustments were made to ensure the holder provided consistent and clear images.

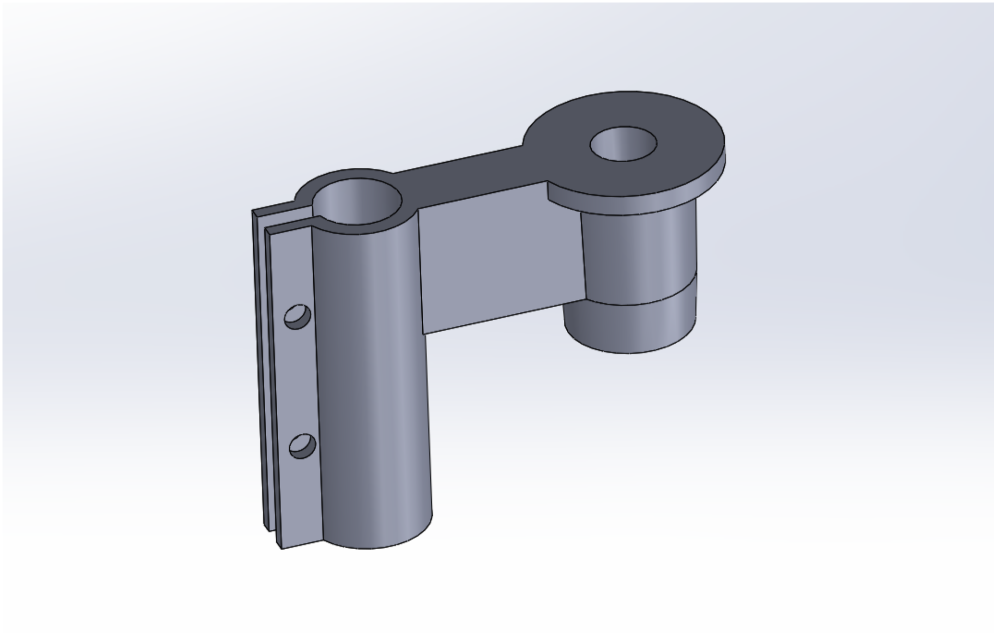

Figure 2. Detailed view of the pick-and-place apparatus holding a camera vertically used by the OT-2 liquid handling robot.

- *Integrating a camera module to OT-2*

The camera module (Opti-Tekscope OT-HD) connects to an experiment orchestrator server via USB. The USB cable was secured by taping it to the moving gantry of the OT-2, keeping it suspended above the labware on the deck to prevent tangling during gantry movement. The server runs a Python-based program that is responsible for controlling operations of the OT-2 and data acquisition from the camera module. The orchestrator uses a Python wrapper of the OpenCV-Python library, a free and open-source computer vision tool, to read image data from the camera module. The camera captures raw RGB images with 640x480 pixel resolution. To view a live camera feed, additional Python libraries, including Pillow and Tkinter, were utilized to display the live images on screen. The assembly of the camera into the holder and the integration of the entire setup into the OT-2 liquid handler are demonstrated in Figure *3*.

Figure 3. Close-up of the robotic pipetting gantry with a custom 3D-printed pick-and-place apparatus, holding a pipette tip and camera mount.

4. Operation instructions

- *Controlling OT-2*

The OT-2 liquid handler was controlled using the Opentrons Python API. Two pipettes are used in the OT-2 system. One pipette is responsible for picking up and dropping tips, as well as transferring, distributing, aspirating, and dispensing liquids. The other pipette, as shown in Figure 3, is designed to pick up the pick-and-place apparatus by first attaching a 1000 µL tip and then moving to different locations above the well-plate, allowing the camera to capture images inside the wells.

- *Bayesian Optimization and Closed-Loop Systems*

The core of our automated platform is the main feedback loop, which links numerous important components, including Bayesian Optimization (BO), the MQTT Message Broker, the Experiment Orchestrator, and the Database. This feedback loop serves as the decision-making engine of the closed-loop system. BO explores the parameter space of the experiment to identify the global optimum, while the MQTT Message Broker serves as the communication hub, transferring commands from the BO to the Experiment Orchestrator. The OT-2 liquid handler and its attached camera are then managed by the Experiment Orchestrator, which also performs the experimental protocols and returns the gathered data to the database. Real-time, ongoing adjustment of the experimental settings is made possible by feeding the processed data back into the BO algorithm.

5. Validation and characterization

The custom hardware platform was validated and characterized for its performance in the high-throughput screening and analysis of hydrogel formulations. The study focused on ionically crosslinked hydrogels formed through the interaction between alginate and calcium chloride ($CaCl_2$) solutions. While numerous studies have investigated alginate and $CaCl_2$ hydrogels [29], [30], [31] this work uniquely combines automated hydrogel synthesis with real-time imaging of hydrogel beads, enabling in-situ characterization of the cross-linking process. Alginate is a natural biopolymer derived from brown seaweed, and widely used in biomedical applications [32], [33] due to its ability to form gels in the presence of divalent cations such as calcium. Alginate gels are commonly used for drug delivery [34], tissue engineering [35], and as a thickening agent [36]. Clinically, alginate hydrogels are widely used in wound dressings [37] and have been explored in both preclinical and clinical trials for applications in drug delivery and cell encapsulation [38], [39].

This study aimed to investigate the gel formation process and cross-linking efficiency under varying concentrations of alginate and $CaCl_2$, using the integrated imaging system to capture real-time data at multiple time intervals. The platform's performance was assessed based on its ability to automatically prepare and monitor the gelation process across different concentrations of alginate (0.5%, 1.0%, 1.5%, and 2.0%) and $CaCl_2$ (0.1%, 0.5%, 1.0%, 1.5%, 5.0%, and 10.0%). As shown in the Figure *4* (a), images were captured automatically 30 minutes after combining different concentrations.

At lower $CaCl_2$ concentrations (0.1%), the hydrogel beads were difficult to visualize. As the concentration increased, the beads became more opaque. However, when the concentration exceeded 1.5%, reaching 5% and 10%, the beads became less opaque and more transparent. As shown in Figure *4* (b), image analysis confirmed these trends for different alginate concentrations, with a decrease in pixel intensity observed at higher $CaCl_2$ concentrations.

Some reports have indicated that for alginate and $CaCl_2$ combinations, there is a critical value known as R, defined as ($2[Ca^{2+}]/[COO^-]$). Below this R threshold, increasing the Ca^{2+} concentration makes the alginate gel stiffer. However, when this ratio exceeds the R value, further increases in calcium concentration result in a less stiff gel [40], [41]. The image analysis conducted with this automated platform confirmed that the system can identify the critical point beyond which gels become less opaque at higher $CaCl_2$ concentrations, a change that correlates with a reduction in gel stiffness.

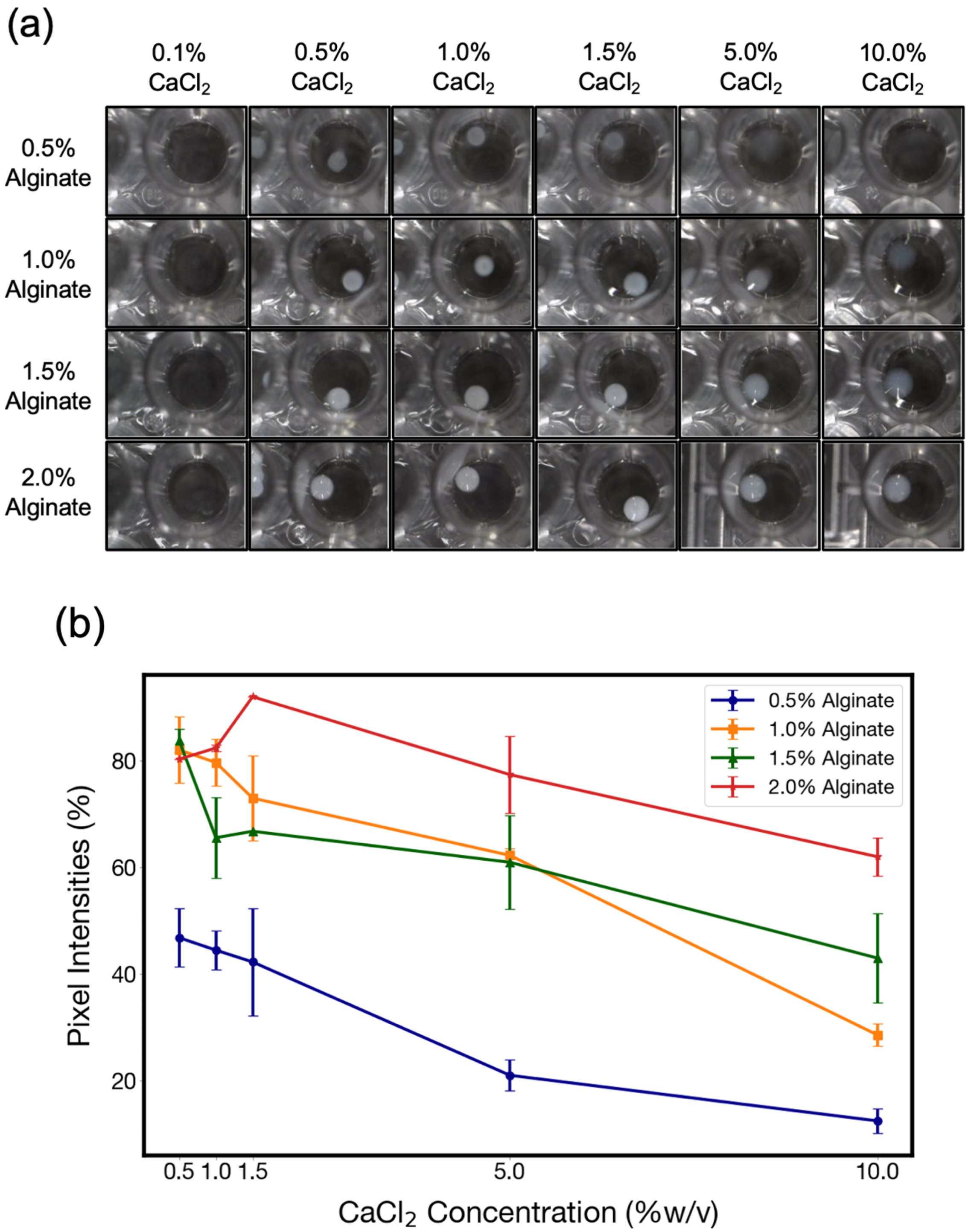

Figure 4. (a) Images of alginate beads formed at varying concentrations of alginate (0.5%, 1.0%, 1.5%, 2.0%) and $CaCl_2$ (0.1%, 0.5%, 1.0%, 1.5%, 5.0%, 10.0%). (b) Pixel intensity analysis of the alginate beads that shows a decrease in intensity at higher $CaCl_2$ concentrations, particularly beyond the critical R value, confirming reduced opacity and stiffness in the gels. The error bars represent the distribution of pixels intensity values.

6. Design files summary

Design file name	File type	Open-source license	Location of the file
Pick-and place apparatus	CAD file	Non-commercial license: CC BY-NC 4.0	Available with Supplementary Information file
2mL vials holder	CAD file	Non-commercial license: CC BY-NC 4.0	Available with Supplementary Information file

Pick-and Place Apparatus (CAD file): The file includes the 3D design of a pick-and-place apparatus that can carry a vertical camera in sync with the pipetting gantry's movement.

2mL Vials Holder (CAD file): The file includes the 3D design of a holder specifically to securely position 2mL vials during experimental procedures.

7. Bill of materials summary

Designator	Component	Number	Cost per unit - currency	Total cost - currency	Source of materials	Material type
Liquid Handler Robot	Opentron (OT-2)	1	$10,000	$10,000	LabX	Other
Camera	Opti-Tekscope OT-HD	1	$88.08	$88.08	Amazon	Metal
Pick-and-Place Apparatus	3D printed PLA	1	$18.99	$18.99	Overture	Polymer
Screw	Super-Corrosion-Resistant 316 Stainless Steel Socket Head Screw	3	$0.72	$2.16	Amazon	Metal

CRediT author statement

Mohammad Nazeri: *Conceptualization, Methodology, Data Curation, Formal Analysis, Investigation, Writing – Original Draft, Visualization* ***Jeffrey Watchorn****: Conceptualization, Methodology, Software, Validation, Writing – Review and Editing* ***Sheldon Mei****: Methodology, Software, Validation* ***Alex Zhang:*** *Resources, Methodology, Visualization, Software* ***Christine Allen:*** *Writing – Review and Editing* ***Frank Gu****: Conceptualization, Supervision, Project Administration, Funding Acquisition, Writing – Review and Editing*

M.N and J.W contributed equally to this work.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors acknowledge the use of the Formulation Self-Driving Laboratory (SDL-5) at the Acceleration Consortium for providing essential facilities and technical support.

This research is part of the University of Toronto's Acceleration Consortium, which receives funding from the Canada First Research Excellence Fund (CFREF). NSERC Discovery Grant (Spon Ref: RGPIN-2019-06441, Fund# 506723) - Canada First Res Excellence Fund - AC: Scientific Strategy (Spon Ref: AC-CFREF-2022-00042, Fund# 516045)

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