Review Article

The Utilization of Open-Source Microcontroller Boards and Single-Board Computers in Liquid Chromatography

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Abstract

Electronic system control of analytical instrumentation remains a critical aspect of modern measurement science. Within the field of liquid chromatography, this is especially relevant for automation, module operation, detection, data acquisition, and data analysis. Increasingly, home-built analytical tools used for liquid-phase separations rely upon open-source microcontrollers and single-board computers to aid in simplifying these operations. In this review, we detail literature reported within the past five years in which these types of devices were used to advance various aspects of the LC research field, including sample preparation, instrument control, and data collection.

1. Introduction

Modern chemical analysis relies not only upon specific measurements related to fundamental chemical principles, but also on the integration of computer hardware and software for data acquisition and analysis. This has been the case for analytical instrumentation used in separation science, where nearly every aspect of a given technique, from sample introduction to the final reported data figure, requires electronic components. Understanding these components can play a critical role in successful experimental design, as parameters such as autosampler movement speed can affect analytical throughput [1] and improper instrument data acquisition and filtering settings can affect the quality of the observed peaks in a separation [2,3]. Although less frequently practiced now, the highest level of instrument control that can be provided to the experimentalist is with home-built hardware and software.

Until recently, the primary option for the construction of instrument control modules and data acquisition systems, along with the design of graphical user interfaces to operate these platforms, was by employing commercial equipment and software (e.g. National Instruments boards and LabVIEW program). To provide a more accessible option to researchers, the emerging open-source movement promotes the development of scientific technology that relies less upon these commercial tools and instead uses low-cost approaches to manufacturing hardware that can be controlled by freely available software. Two major advances in this field came in 2006 with the initial release of the Arduino Uno microcontroller board and in 2012 with the release of the first version of the Raspberry Pi single-board computer [4]. These platforms, and a number of similar devices, allow for simplified integration with common electronic circuit designs through the use of General Purpose Input/Output (GPIO) pins as well as multiple communication modes (serial peripheral interface (SPI), inter-integrated circuit (I^2C), universal asynchronous receivertransmitter (UART), and universal serial bus (USB)). The simpler Arduino microcontrollers are able to perform various electronic control tasks and can be operated independently to perform uploaded routines, but typically require an external connection to a computer for coding and more complex tasks. Single-board computers, such as the Raspberry Pi, can operate as a standalone control module similar to a standard computer and provide enhanced processing power relative to microcontrollers, while still enabling the interfacing to electronic circuits through GPIO connections. The Raspberry Pi uses a Linux-based operating system (Raspberry Pi OS, formerly known as Raspbian) and can also run programs written in a variety of languages (Python, C, C++, JavaScript, etc.). An extensive review on the broader scope of microcontrollers, single-board computers, and similar electronic modules within the context of chemical research is available for readers seeking more context on the background, history, and design of these devices [4].

This review is focused specifically on the integration of open-source microcontroller boards and single-board computers into LC instrumentation and LC-focused analytical workflows. The primary ways in which they have been utilized for system control, automation, data acquisition, detection, and other specialized purposes are discussed. For broader applications within other areas of chemical measurement science, readers are directed to [4–9], and information on other low-cost aspects of instrument design is available in [10–13]. This review primarily focuses on work published within the past five years (January 2019 – December 2023), although earlier reports of specific applications are included when directly related to articles within this timeframe. Although the main focus of this review is on articles that discuss LC-based analysis, we have also included references on certain topics (sample preparation, detection, etc.) that are adaptable to LC but may have been used specifically for a different separation technique (CE, GC, IMS, etc.). For specific details on microcontrollers and single-board computers as related to other liquid-phase separation techniques, in-depth reviews on CE [14–16] and microfluidics [17–19] have also been published.

2. Sample Preparation

Sample preparation is often a critical first step in the analysis of complex samples in a variety of matrices. Recently, efforts have been made to automate sample preparation techniques to improve method throughput and reproducibility [20–22]. Frequently, microcontrollers are used to control the movement systems associated with these automated sample preparation techniques [23], especially in connection with open-source stepper motor driver boards (*e.g.* the RepRap Arduino Mega Pololu Shield, or RAMPS, board). In 2019, an open-source platform controlled by an Arduino Mega 2560 was reported that could be utilized to automate microextraction techniques that typically require micro-syringes, including single drop microextraction, hollow-fiber liquid phase microextraction, and microextraction by packed sorbent (**Figure 1**) [24]. The microcontroller was used for various aspects of the system: stepper motor control for 3-axis movement, DC motor control for magnetic stirring, solenoid valve actuation for instrument hyphenation, and relay control for instrument triggering. System operation and user control were programmed using the Arduino Integrated Development Environment (IDE) to enable compatibility with a broad array of computing systems. In one of the early demonstrations of online hyphenation between this platform and LC-MS for analysis, the amounts of simazine, atrazine, and propazine in coconut samples were measured [25]. Much of the research utilizing this system has focused on adapting various microextraction approaches to the automated sampler prior to online analysis. Some of the applications have included estrogen-like isoflavones in human urine [26], parabens in wastewater [27], sulfonamides and fluoroquinolones in surface water [28], and ring-substituted amphetamines in urine [29]. To further increase throughput for the analysis of cannabinoids in urine, six parallel syringes were mounted onto the platform for either simultaneous or independent operation of each channel [30]. This parallel system was also utilized for the measurement of endocrine disruptors in wastewater [31] and *N*-nitrosamines in losartan tablets [32].

The "Lab-In-Syringe" technique utilizes a combined flow manifold to automate the handling of samples and reagent solvents for liquid-phase microextractions [33]. This manifold can also be coupled to additional sample preparation techniques to further reduce matrix interference and/or preconcentrate samples. For automation, a Trinket M0 microcontroller board has been coupled with the manifold and connected to analytical instrumentation for a variety of applications. The Trinket M0 device is similar to an Arduino, but has a much smaller form factor, making it ideal for space-limited applications such as this integrated lab-in-syringe. Initially, the Trinket device was used to control a single solenoid valve so that an isocratic LC pump could switch between mobile phase reservoirs and provide gradient elution functionality during the post-extraction analysis of sulfonamide antibiotics in urine samples [34] and neonicotinoid pesticides in water samples [35]. Use of this microcontroller board was later expanded to include control of a magnetic stirrer motor used in the syringe manifold and for triggering instrument injection, pumps, and data acquisition for the LC-FLD analysis of fluoroquinolones in water samples [36] and the LC-MS/MS analysis of beta-blockers in serum [37].

Beyond these two general platforms that have been used for a variety of sample types, other systems have been developed to automate sample collection and/or preparation prior to LC analysis. Control of a syringe pump to draw reagents and the sample solution through an extraction membrane was achieved using an Arduino Mega to automate the extraction of chlorinated phenoxyacetic acid herbicides from sewage water [38]. Isoflavones were extracted from environmental waters on-site prior to laboratory analysis by LC-MS using an Arduino-controlled bottle-based system [39]. The cap for a standard borosilicate bottle was modified to contain a motor for sample stirring, a temperature sensor, and a conductivity sensor (**Figure 2**). A solid-supported sorptive phase was then introduced into the bottle and the collected water was agitated to enhance movement of analytes to the extraction surface, followed by downstream analysis in a standard laboratory setting. For automated sample handling of biological fluids that are collected with minimally invasive techniques, Arduino-controlled systems have been developed to aid in the analysis of inorganic ions and organic acids in exhaled breath condensate [40] and drugs of abuse from saliva samples [41]. Similarly, a pumping system used for direct *in vivo* sampling of brain interstitial fluid in animal models was designed using an Arduino [42]. For protein capture using an affinity-based extraction phase, an Arduino Mega was used to control a low-cost flow manifold for automated in-line isolation of jacalin (a galactose-binding lectin) from a jackfruit seed sample [43]. An automated membrane extractor that used a microcontroller to maintain heating temperatures was developed to remove organic solvent from samples prior to analysis, which greatly enhanced the detection limits of measuring chloramphenicol in milk [44]. Monitoring of temperature control for the thermal desorption of analytes from secondary organic aerosols captured on filters prior to downstream analysis by LC-MS has also been achieved with a microcontroller [45].

3. Autosamplers, Valving, and Other System Control

The capability of using open-source microcontrollers and single-board computers to program timed electronic signals and control movement systems has provided the opportunity to integrate their use into a variety of LC modules and platforms. One of the most common uses of these devices is in low-cost autosampler and fraction collection devices, especially in tandem with the RAMPS board, as previously described in [11]. Within the time frame focus of this review, several additional versions of these devices have been reported. For autosampling, a Raspberry Pi-based system was used to introduce samples to the injection valve of a dual-column nanoLC system [46]. A more complex movement system that also included vial movement to enable hydrodynamic injection was used in an open-source CE autosampler [47]. A system for manipulating nanoliter-scale segmented flow droplets was coupled to various analytical measurement techniques but noted that coupling to LC was more difficult due to sample injection strategies that are employed in commercial instruments [48]. A recent report on coupling segmented flow droplets directly to a high-throughput LC separation [49] could facilitate use of this platform. For post-column fluidic control, a 3-axis movement system was used to deposit the eluent from an LC column into separate vials for separation fractionation or onto a sampling plate for MALDI-MS [50]. The user interface was accessed through a Raspberry Pi with movement commands sent through a connected Arduino Uno (**Figure 3**). An Arduino Due was used to control a similar platform for MALDI spotting following a CE separation [51]. Multiple other fraction collectors with minor variations but similar implementation of microcontrollers for motor control have been reported for analytical [52] and preparative [53–55] LC separations (or similar volume scale fraction collection from samples prior to LC analysis). Motor control for a system designed for direct surface sampling from a TLC plate to an LC-MS system [56] was adapted from a similar, previously reported method for interfacing TLC directly to MS [57]. Some systems have implemented rotational motion systems to reduce the number of motors required compared to a traditional 3-axis system. A direct comparison of two platforms built using each of these approaches showed that the rotational systems cost less but provide reduced movement accuracy and precision due to some of the interfaces required for rotational axis movement [58].

Other pre-column uses of microcontrollers outside of robotic movement systems have typically involved valving control or devices to enhance mobile phase delivery to the column. An integrated platform for protein purification used a Raspberry Pi to control valve actuation, peristaltic pumping, and fraction collector movement [59]. Similar system control using an Arduino Uno was reported for a preparative-scale LC separation of crude oil samples [60]. An SPE-MS platform used for monitoring secretion of glucose regulating hormones from human islet of Langerhans samples utilized an Arduino to control the 10-port valve used for dual-loop sample introduction [61]. Actuation of a 10-port valve using an Arduino was also reported for a recycling chromatography system designed for polymer analysis [62]. Automated control of a miniaturized CE system meant for operation in extraterrestrial settings was achieved using a Teensy microcontroller [63]. The microcontroller was used to enable functions including sample loading, hydrodynamic injection, and control of the high voltage power supply (HVPS) needed for electrophoretic separations. The Teensy microcontroller, along with an I^2C multiplexer board, was operated as a "state system" in which various numerical feedback entries were monitored to determine what command to execute at a specific time. A submersible version of this CE instrument has also been developed for missions to ocean worlds [64]. A CE-MS platform with integrated control achieved with an Arduino Nano was used for the analysis of pesticides [65]. Multiple valves were controlled by a single Raspberry Pi in an on-line immunoaffinity chromatography-asymmetric flow field-flow system designed for isolating and fractionating variously sized extracellular vesicles from human plasma [66]. Both syringe pump and detector control were achieved in a compact platform designed for low-cost chromatographic analysis of urinary albumin as a diagnostic tool for chronic kidney disease [67]. To achieve mixing while sample vials were placed inside a commercial LC autosampler, a modified sample tray with embedded, motorized magnetic stirrers for each vial reservoir that included integrated user control was achieved through connection to an Arduino Pro Mini [68]. Finally, for real-time mobile phase control, a solid feeding device (controlled by a Raspberry Pi) for in-line delivery of buffering reagents [69] and an open-source version of a low-pressure gradient mixer (with full system control from an Arduino Mega) [70] have been reported. For control of the suppressor module in ion chromatography, a microcontroller-based adjustable power supply has also been described [71].

With the continued growth of multidimensional separations, certain functions to help automate these methods are being achieved with microcontroller-based platforms. In an RPLC × SEC platform for polymer separations, an Arduino Uno was used to read pump pressure sensors as a way of synchronizing pump piston movement with modulation to minimize the pressure spikes on the second dimension column [72]. Methods coupling SEC with pyrolysis-GC for polymer analysis have also been described, with an Arduino Mega used for timing valve controls between the various instrument modules [73,74]. Control of a 10-port modulation valve for RPLC × RPLC analysis of phenolic compounds in grape juices and wine was achieved using an Arduino microcontroller [75].

Miniaturized, fully integrated LC systems designed for portable operation require automated control that is simple, lightweight, and has low power consumption, all of which can be achieved with open-source microcontrollers and single-board computers. Over the past several years, a modular, portable LC platform has been in development. Early versions relied upon commercial hardware/software interfaces for control [76,77], but a more recent design has utilized a microcontroller-based electronic system (**Figure 4**) [78]. The microcontroller is used to drive syringe pumps in both gradient and isocratic modes, actuate valves for injection, and record data from a UV detector. The fully operational system can be contained within the dimensions of a briefcase with all parts under the control of a primary board and a home-built graphical user interface (GUI). The flexibility that is gained with these microcontrollers has increased the capability to connect the primary LC operation to other modules for specific applications. With this system specifically, the LC separation was coupled to a compact MS detector for on-site analysis of PFAS compounds [79] and to a synthetic reactor that contained an automated sampling device for process monitoring [80]. The system used for the latter application was recently refined with increased operating parameters for on-line synthetic reaction monitoring [81]. Similar to this LC platform, a portable system for nitrite and nitrate analysis using ion chromatography originally relied upon other commercial components for system control [82] but later utilized a Teensy microcontroller for operation [83,84]. More recent versions have been adapted for phosphate analysis with ion chromatography [85] and a general platform for operating other LC separation modes [86]. Finally, a chip-based compact system utilized an M5Stack development board for system control with connection to a separate microcontroller for data logging [87].

Because of the capability for these systems to both transmit and receive commands through Internet and/or Bluetooth connectivity, broader integration into full analytical workflows that utilize LC for analysis can be achieved. An automated system for reaction monitoring with real-time feedback provided by on-line LC-MS analysis was initially controlled using electronic signaling from commercial sampling hardware modules [88]. To further automate control of the platform, a more recent version used an Arduino to initiate sampling events [89]. A similar platform for reaction monitoring with on-line LC-DAD analysis using the Node-RED programming platform operating on a Raspberry Pi has also been demonstrated [90]. As more research groups develop home-built automated synthetic reaction platforms, the implementation of microcontrollers and single-board computers for controlling these systems will likely continue to grow.

4. Detectors and Data Acquisition

A variety of uses for these compact electronic devices related to analyte detection have also been reported. Control of hardware components (*e.g.* optical light sources, light detectors, etc.), signal acquisition and filtering, data plotting, and data processing can all be achieved with microcontrollers and single-board computers. Building upon the discussion on compact instruments in the previous section, the use of light-emitting diodes (LEDs) as absorbance detector sources in portable LC systems is frequently accomplished in tandem with compact electronic control systems [91]. Within the focused time frame of this review article, the primary developments in this approach have revolved around the development of LED-UV detection at 235 nm. A surface-mounted 235 nm UV-LED was used as an absorbance source in a version of the LC system described in [78], with control of the LED, the silicon carbide photodiode detector, and analog-to-digital conversion (ADC) circuit all achieved with a Raspberry Pi [92]. A similar design was implemented for use in a portable ion chromatography system, but the detector was controlled with an Arduino Mega in this instance [93]. To expand the detection wavelengths, a trifurcated fiber optics assembly was used to combine three LEDs (240, 255, and 275 nm) into a single source with each LED driven by an Arduino Nano [94]. To improve the performance of these multi-LED sources, high frequency switching can be implemented, although commonly used microcontroller boards and single-board computers are usually too slow to achieve the necessary switching frequencies. A Red Pitaya board that includes an integrated field programmable gate array (FPGA) can achieve much higher speeds [95] and was used to drive 235, 250, and 280 nm LEDs with improved performance during simultaneous operation [96]. The use of multiple LEDs in a single detector has also been reported in a Hadamard-transform excitation-emission-matrix fluorescence detection method for LC utilizing an Arduino for LED excitation timing [97].

A number of other optical-based detector components and related devices have been designed for use with microcontrollers and single-board computers. To identify the impact of sample flow rate on detector sensitivity for absorbance and MS detectors, an automated flow rate optimization system controlled by an Edison Compute SBC coupled to an Arduino board was constructed [98]. To improve detector signal, open-source versions of a transimpedance amplifier for low light detection of PMTs [99] and a flow-cell with integrated lock-in amplification for absorbance and fluorescence detection [100] have both been reported. An LC pump, circular dichroism spectrometer, and fluorescence spectrometer were all coupled to a Raspberry Pi-based control system to automate the characterization of proteins with both spectroscopic techniques in a single flow-based method [101].

The capacitively coupled contactless conductivity detector (C4D) remains one of the most widely-used detectors for low-cost, capillary-scale liquid-phase separation systems [14], with the "OpenC4D" that utilizes an Arduino-based control system [102] enabling easier fabrication of the standard detector [103]. In more recent years, C4D modules have been combined with 3D printed cartridge manifolds to increase functionality from the stand-alone detector. In an initial report, a 3D printed detector head was designed for on-capillary CE detection with both C4D and colorimetric (505 nm LED) modes [104]. To further expand the capabilities of the miniaturized detector, a re-designed integrated device included LIF detection as well [105]. Although these initial versions were controlled with a standard commercial hardware/software platform, a follow-up design that utilized lower wavelength (235 nm and 255 nm) UV-LEDs utilized an Arduino Uno for operation [106]. To increase the separation throughput while minimizing Joule heating using the Arduino-controlled instrument, a cooling channel can also be added [107]. In addition to these multi-mode detectors, a multi-cell array designed for parallel C4D operation with up to 64 possible detection channels partially controlled by an Arduino Nano has been described [108].

Electronic control with various microcontroller platforms has also been a strategy used in the development of ion mobility spectrometry (IMS) and MS systems. A dual polarity 20 kV HVPS designed for IMS used an Arduino Uno to control the initial input voltage in the system (**Figure 5**) [109]. An IMS system designed for use as a detector for high-throughput chromatographic separations was designed around a Xilinx-based board with an integrated FPGA [110,111]. For control of the HVPSs used for electrospray ionization (ESI), the use of both microcontrollers [112] and single-board computers [113] has been reported. To better study the ESI process, imaging systems designed using microcontroller boards have been described in the study of the effects of low frequency sound modulation [114] and plume droplet size [115] on ESI-MS signal.

Across a variety of scientific disciplines, microcontroller-based systems have been implemented as data acquisition devices (DAQs) to replace common commercial options [116]. Specifically for analytical instrumentation, an initial Arduino-based DAQ was used with both GC and CE platforms to record analog data signals [117]. Multiple low-cost ADC boards coupled to an Arduino Nano have been compared for use as DAQs for CE-C4D (**Figure 6**), with higher resolution boards connected to the microcontrollers providing better signal recording than the internal microcontroller ADC function [118]. To build upon standard data logging functionality, a Raspberry Pi-based hardware/software interface combined DAQ functionality with signal filtering and the calculation of chromatographic figures of merit for an LC separation, with results of the calculations comparable to instrument manufacturer software [119]. The processing of file types generated by these commercial software packages has also been achieving using the open-source Appia program that can be operated on a Raspberry Pi [120]. In a study comparing broadening effects from different LC detectors, a Pi was used as a data logger for the FLD that was investigated [121]. In addition to raw detector signal, Pi-based image processing of an LIF detector that added the fluorescence detection mode at the Taylor cone of ESI-MS has been reported [122]. Although programming languages such as Python, C/C++, and Java are commonly implemented for these purposes, the use of Forth has been suggested as an alternative based on the ease of developing user interfaces for control of these platforms [123].

5. Conclusions and Outlook

Progress in both automation and technique integration has provided the opportunity for microcontrollers and single-board computers to be useful in a variety of ways within the field of LC separations, as described in this paper. Prior to analysis, efforts in terms of both sample collection and sample preparation have been aided by these devices. Many components of standard commercial instruments have been improved and/or replaced by new devices that feature microcontrollers to simplify the electronic control systems needed for operation. With ongoing efforts to make access to instrumental-based separation techniques more accessible [11,13], it is highly likely that microcontrollers and single-board computers will play an essential role in the development of low-cost platforms. In some of these studies, the performance limitations of these low-cost devices required additional peripherals to fully achieve a desired analytical purpose. However, as with traditional computers, the processing power of low-cost microcontrollers and single-board computers will continue to grow over time and overcome these current challenges [124]. As has been the case with traditional computers over the past four decades, we anticipate that open-source microcontrollers and single board computers have the potential to become a mainstay in the field of liquid chromatography.

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Conflict of Interest Statement

The authors report no conflicts of interest.

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Figure Captions

Figure 1. Schematic of a multi-purpose cartesian robot utilizing Arduino Mega motor control designed to automate various sample preparation techniques, including single drop microextraction (SDME), dynamic hollow-fiber liquid phase microextraction (HF-LPME) and microextraction by packed sorbent (MEPS). Adapted with permission from Reference [24].

Figure 2. "Lab-in-a-bottle" cap containing temperature and conductivity probes (A) operated using an Arduino-controlled circuit (B). Adapted with permission from Reference [39].

Figure 3. Schematic overview of connections between an Arduino microcontroller, Raspberry Pi, and various hardware modules for operation of a 3-axis movement system used for MALDI spotting and fraction collection. Reprinted with permission from Reference [50].

Figure 4. Photograph (a) and component schematic (b) of a modular portable capillary gradient LC instrument utilizing multiple microcontrollers for system operation. Components include (as listed in original manuscript): 1) solvent pump B , 2) solvent pump A , 3) sample injection port , 4) pressure sensor, 5) refill valves, 6) mixer, 7) purge valve, 8) injection valve with loop, 9) pump home sensors, 10) column, 11) UV detector, 12) solvent reservoirs, 13) waste container 14) control electronics, and 15) constant current controller. Reprinted with permission from Reference [78].

Figure 5. Photograph of an assembled 20 kV dual polarity high voltage power supply (HVPS) controlled by an Arduino Uno. Reprinted with permission from Reference [109].

Figure 6. Three DAQ devices each comprising an Arduino Nano connected to a different ADC board with varying resolution. Reprinted with permission from Reference [118].

Figures

Figure 1.

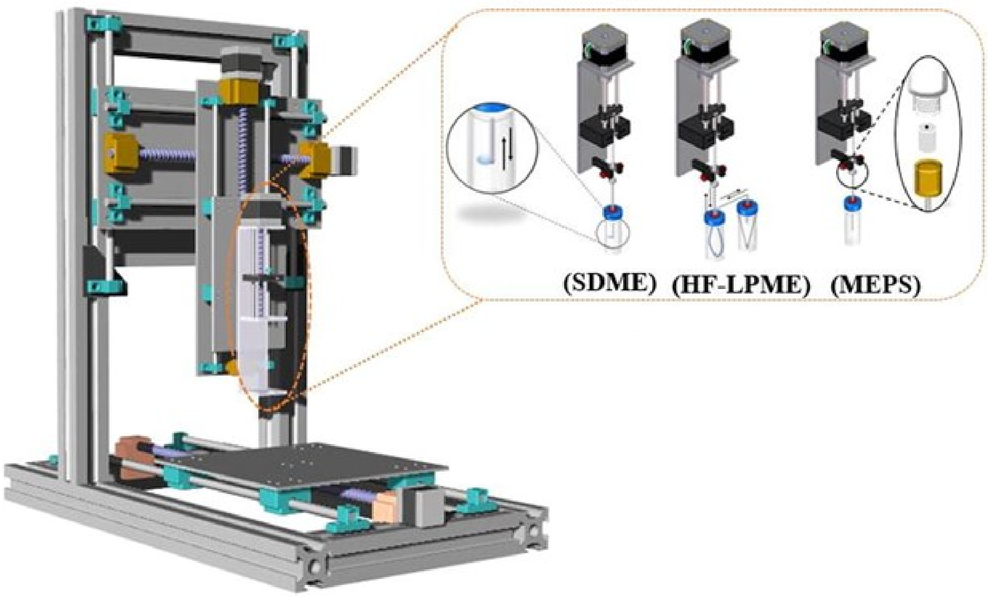


Figure 2.

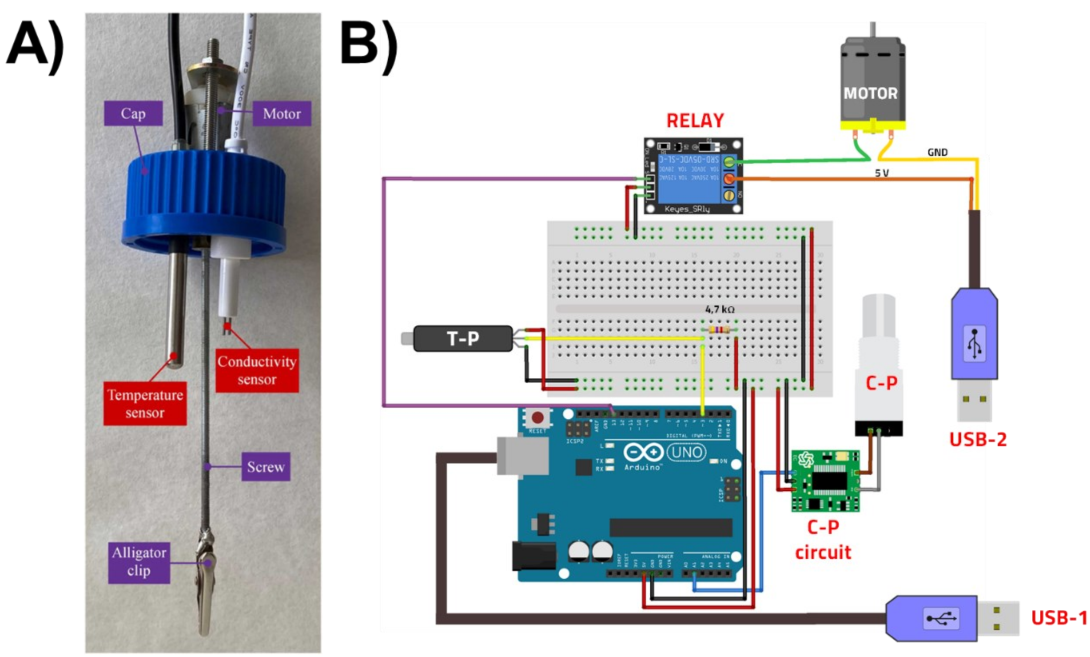
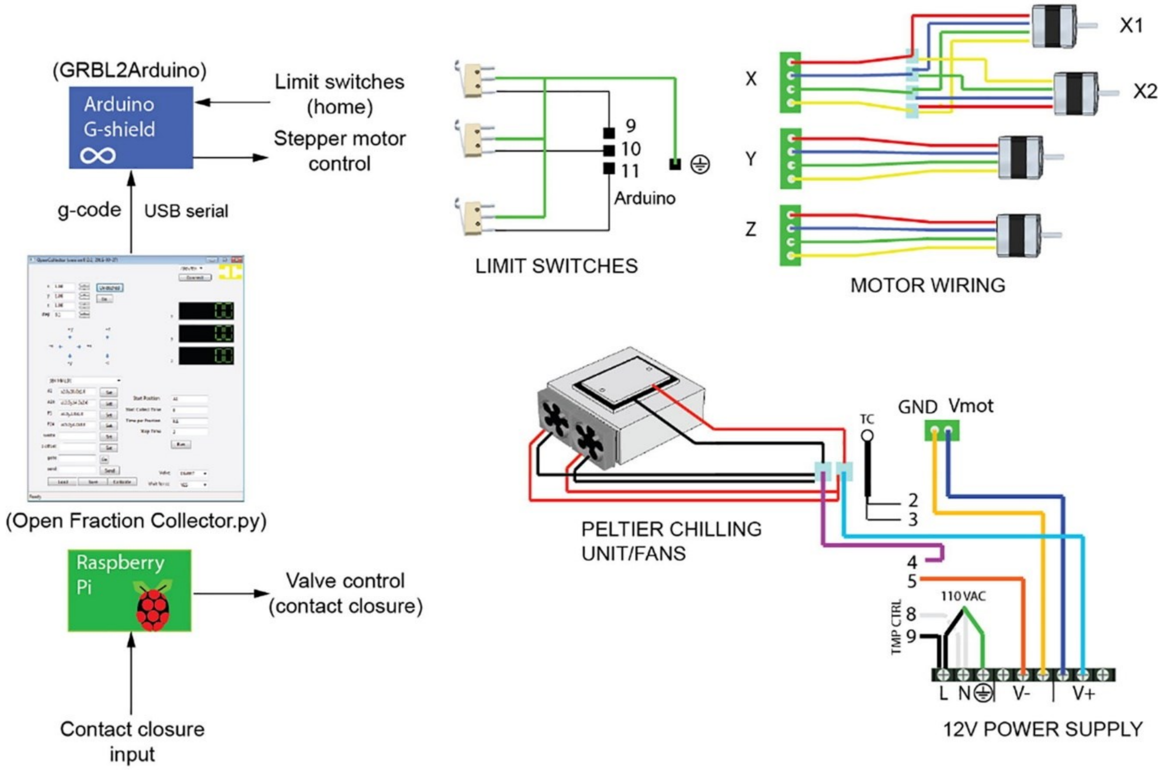


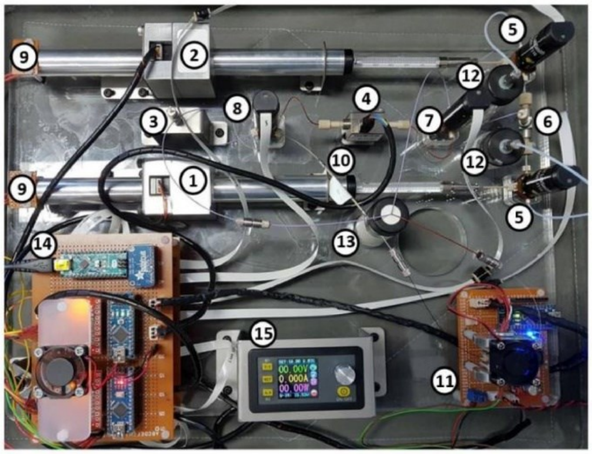
Figure 3.



SYSTEM CONTROL

Figure 4.

a)



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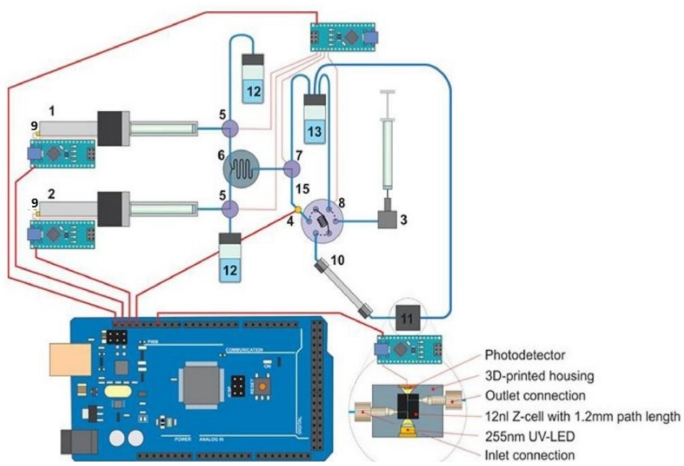

Figure 5.

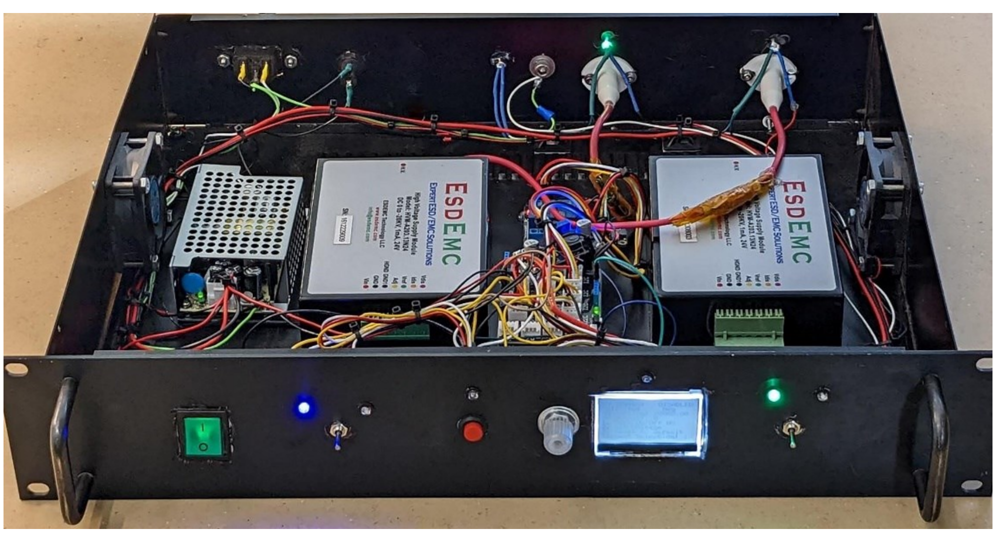

Figure 6.