



Kaunas University of Technology

Faculty of Mechanical Engineering and Design

Development of Chemical Synthesis and Analysis Automation System

Master's Final Degree Project

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Project author

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Supervisor

Kaunas, 2024



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Mechatronics (6211EX017)

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Development of Chemical Synthesis and Analysis Automation System

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Task of the Master's Final Degree Project

Given to the student – Arnas Vaškevičius

1. Title of the Project

Development of Chemical Synthesis and Analysis Automation System

(In English)

Cheminės sintezės ir analizės automatinės sistemos kūrimas

(In Lithuanian)

2. Aim and Tasks of the Project

Aim: to develop an automation system capable of performing chemical synthesis and analysis.

Tasks:

1. to compare existing chemical synthesis and analysis automation systems;
2. to design and make parts for the chemical synthesis and analysis automation system;
3. to assemble mechanical and electrical parts for the chemical synthesis and analysis automation system;
4. to write a control software for the chemical synthesis and analysis automation system;
5. to evaluate the capacity of the developed automation system to perform chemical synthesis and analysis;
6. to calculate the cost of developed automation system parts and raw materials.

3. Main Requirements and Conditions

Automation system should cost less than 8000 euros in parts and raw materials. Chemical synthesis of at least two liquid reagents can be performed at room temperature with mixing speed of at least 200 RPM using the automation system. At least 0.5 ml of the reaction product can be transferred to be analysed using thin-layer chromatography method. Analysis is performed on plates of at least 20 mm width and 50 mm length. Staining of the plate covers at least 50 percent of the plate lengthwise. Imaging of the plate is under visible or ultraviolet 254 nm lighting conditions.

4. Additional Requirements for the Project, Report and its Annexes

Not applicable.

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Summary

In effort to combine chemical synthesis and analysis processes under one automation system, a low-cost system was designed, developed, and tested for automating both chemical synthesis and thin layer chromatography analysis processes. Two delta robots were used for chemical liquid dispensing that employed a plunger-operated air displacement dispensing method, and one delta robot was used for the manipulation and transferring of thin layer chromatography plates. Four steps of thin layer chromatography analysis method were fully automated: spotting, development, imaging, and staining. The total cost of parts and materials of the system was calculated to be 7275.6 euro. Systematic and random errors of liquid handling were tested according to ISO 8655 and ISO 23783 standards using a gravimetry method. Obtained systematic errors conformed to the standards, however random errors conformed to the standards only for the dispensing of the 10% of maximum volume, therefore the liquid handling capabilities of the system were determined to be accurate but not precise. Furthermore, the repeatability of the system to produce thin layer chromatography analysis data was tested. Values for the coefficients of variation of the retention factors of different compounds of the developed plates across multiple tests ranged from 4.51% to 11.27%. A comparison of obtained coefficients of variation showed that the automation system had enough repeatability to analyse chemical samples using thin layer chromatography. Lastly, using the system, ongoing chemical synthesis reactions were monitored with thin layer chromatography method for periods ranging from 14 to 36 hours. The formation of products and usage of reagents were visible on the images of the developed and stained plates indicating that the automation system was capable of performing chemical synthesis and synthesis reaction monitoring using thin layer chromatography analysis method.

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Reikšminiai žodžiai: cheminė sintezė; cheminė analizė; plono sluoksnio chromatografija; automatinė sistema; robotika.

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Santrauka

Nebrangi sistema, naudojama cheminės sintezės ir plono sluoksnio chromatografijos procesams automatizuoti, buvo suprojektuota, išvystyta ir ištestuota, siekiant sujungti cheminės sintezės ir analizės procesus vienoje automatinėje sistemoje. Cheminių skysčių dozavimui buvo panaudoti du delta robotai su stūmuokliu valdomu oro poslinkio dozavimu metodu. Plono sluoksnio plokštelių manipuliavimui ir transportavimui buvo panaudotas vienas delta robotas. Keturi plono sluoksnio chromatografijos žingsniai buvo pilnai automatizuoti: mėginio užnešimas, plokštelių kildinimas, vizualizacija ir derivatizavimas. Buvo paskaičiuota bendra sistemos dalių ir medžiagų kaina, kuri siekė 7275.6 eurus. Dozavimo robotų skysčio manipuliavimo sisteminės ir atsitiktinės paklaidos buvo apskaičiuotos naudojant gravimetrinį metodą remiantis ISO 8655 ir ISO 23783 standartais. Apskaičiuotos sisteminės paklaidų vertės atitiko ISO standartus, tačiau apskaičiuotos atsitiktinės paklaidų vertės standartus atitiko tik dozuoiant 10% didžiausio dispensijuojamo tūrio. Įvertinus sisteminės ir atsitiktines paklaidas ir palyginus su leistinomis paklaidomis pagal ISO standartus, automatinės sistemos cheminio skysčio dozavimas buvo nustatytas tiksliai, tačiau nepreciziniu. Buvo ištestuotas matavimo rezultatų atsikartojamumas atliekant plono sluoksnio chromatografijos analizę. Variacijos koeficientų vertės, apskaičiuotos skirtingų plono sluoksnio chromatografijos plokštelių iškildintų junginių sulaikymo koeficientams, siekė nuo 4.51% iki 11.27%. Bandymų metu gautų variacijos koeficientų verčių palyginimas su vertėmis iš literatūros parodė, kad automatinė sistema turėjo atsikartojamumą atliekant cheminio mėginio analizę naudojant plonasluoksnę chromatografiją. Buvo ištirtas automatinės sistemos gebėjimas atlikti cheminę sintezę ir sintezės reakcijos stebėjimą naudojant plono sluoksnio chromatografiją. Naudojant sistemą, vykstančios cheminės reakcijos buvo monitoruojamos plonasluoksnio chromatografijos metodu nuo 14 iki 36 valandų. Cheminių produktų susidarymas ir reagentų sunaudojimas buvo pastebimas iškildintų ir derivatizuotų plokštelių nuotraukose, kas parodė, kad automatinė sistema gebėjo atlikti cheminę sintezę ir monitoruoti cheminės reakcijos procesą naudojant plono sluoksnio chromatografijos analizės metodą.

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List of Abbreviations and Terms

Abbreviations:

3D – 3-dimensional;
ALHS – automated liquid handling system;
CV – coefficient of variation;
DART-MS – direct in real-time - mass spectrometry;
Flow-FTIR – flow chemistry - Fourier transform infrared;
Flow-NMR – flow chemistry - nuclear magnetic resonance;
GC – gas chromatography;
HPLC – high-performance liquid chromatography;
HPLC-MS – high-performance liquid chromatography – mass spectrometry;
HTE – high-throughput experimentation;
ID – inner diameter;
LC-MS – liquid chromatography – mass spectrometry;
M – molar concentration;
MS – mass spectrometry;
NEMA – National Electrical Manufacturers Association;
NIR – near-infrared spectroscopy;
NMR – nuclear magnetic resonance;
OD – outer diameter;
PETG - poly(ethylene terephthalate glycol);
PLA – poly(lactic acid);
POM – poly(oxymethylene);
POVA – piston-operated volumetric apparatus;
PP – poly(1-methylethylene) (polypropylene);
PTFE - poly(1,1,2,2-tetrafluoroethylene);
RPM – rotations per minute;
SLA – stereolithography;
TLC – thin layer chromatography;
TOF – time-of-flight;
USB – Universal Serial Bus;
UV – ultraviolet light.

Introduction

Chemical synthesis is used in various fields of manufacturing, pharmaceutical, and research for making chemical products. Chemical analysis process is used together with chemical synthesis for separation, identification, and characterization of different compounds in a chemical sample. Spectroscopy and chromatography are the two main types of analytical methods that are used together with chemical synthesis processes. A method where a large amount of analytical data is obtained is called high-throughput experimentation and usually, automation systems are used to achieve the high-throughput. Commercially available automation systems used for chemical synthesis processes are called liquid handlers or dispensers. These systems usually employ a Cartesian robot and a pipette or a syringe as an end effector. Chemical liquid dispensing is achieved using piston or plunger-operated and positive or air displacement methods. Chemical analysis is usually automated by employing a chemical liquid sampler coupled to an analytical device: the liquid sampler dispenses a chemical sample into the analytical device for analysis. Chemical synthesis and chemical analysis automation systems are usually not coupled, meaning that the products obtained during chemical synthesis must be transferred manually to the liquid sampler of the analysis automation system. A fully automated system includes automation of both chemical synthesis and analysis processes allowing for faster analytical data acquisition, minimization of human error, standardized acquisition of analytical data, ability to construct closed-loop setups for optimization of chemical synthesis reaction parameters using collected data, and the ability to remotely operate a system that has synthesis and analysis capabilities. Many analysis automation systems can be used together with chemical synthesis processes, although thin layer chromatography is a great choice due to its versatility, and low cost. Thin layer chromatography analysis method is a separation of chemical samples into different compounds based on the different affinities between chemicals and the medium allowing the monitoring of the synthesis process. In this work, a low-cost fully automated system combining chemical synthesis and thin layer chromatography analysis with spotting, development, imaging and staining steps was designed, developed and tested. Conventional manufacturing techniques such as 3D printing and CNC machining were employed for the manufacture of parts in order to reduce the cost of the system. Additionally, Raspberry Pi microprocessors, Arduino microcontrollers, and non-industrial robots were used. The liquid handling capabilities of the system were tested according to ISO standards by comparison of obtained systematic and random dispensed volume error values using a gravimetry method. Additionally, the repeatability of the system to produce analytical data was evaluated by calculating coefficients of variation of retention factors of different compounds from the thin layer chromatography plates. Lastly, the ability to perform chemical synthesis processes and the ability to monitor synthesis reactions was evaluated by comparing obtained images of thin layer chromatography developed plates.

Aim: to develop an automation system capable of performing chemical synthesis and analysis.

Tasks:

1. to compare existing chemical synthesis and analysis automation systems;
2. to design and make parts for the chemical synthesis and analysis automation system;
3. to assemble mechanical and electrical parts for the chemical synthesis and analysis automation system;
4. to write a control software for the chemical synthesis and analysis automation system;
5. to evaluate the capacity of the developed automation system to perform chemical synthesis and analysis;

6. to calculate the cost of developed automation system parts and raw materials.

Hypothesis: a low-cost automation system for chemical synthesis and thin-layer chromatography analysis can be developed.

1. Chemical Synthesis and Analysis Automation

In this chapter, theoretical descriptions of chemical synthesis and analysis are described, fields where chemical synthesis is used are listed, and the types and applications of chemical analysis are given. Thin layer chromatography (TLC) analysis method is provided in more detail, as this method will be used in further chapters of this work. High-throughput experimentation method (HTE) and its application in chemistry is explained. Existing automation systems for chemical synthesis and chemical analysis processes are provided, in addition, examples of automation systems that combine both chemical synthesis and analysis are given.

1.1. Chemical Synthesis

Chemical synthesis is the combination of several chemicals (reagents) in order to synthesize a different compound (product), which is usually more complex than the reagents. During the chemical synthesis process, a chemical reaction occurs: the chemical bonds between different molecules break and rearrange forming new chemical structures. Chemical synthesis is employed in many industries such as pharmaceutical, research, and manufacturing. In the pharmaceutical sector, chemical synthesis is used for the manufacture and discovery of drugs. In the research sector, chemical synthesis is used for manufacturing novel molecules, finding new synthesis pathways, understanding and optimizing reaction parameters and mechanisms, and testing the different properties of synthesized products. In the manufacturing sectors, chemical synthesis is used to manufacture polymers and plastics, various construction materials, chemicals that are used in agriculture, dyes, explosives, and various consumer products. In chemical laboratories, where chemical synthesis is performed manually, various equipment and techniques are used: for a typical chemical reaction, a reaction flask placed on a heating mantle acts as a vessel in which liquid or solid chemicals are placed during the process, additionally, a reflux condenser is sometimes attached to the reaction vessel in order to condense the produced vapours during the heating of the chemical reaction mixture. During a synthesis reaction, in order to initiate or accelerate the reaction process, heat can be applied to the reaction vessel. On the other hand, cooling can be used to reduce the rate of the reaction to prevent the formation of unwanted products, or to prevent the decomposition of certain products due to an uncontrolled high temperature in the reaction vessel. After the chemical reaction is finished, sometimes the reaction mixture needs to be separated to extract different products: filtering or decanting can be used in order to separate a chemical mixture that is made up of liquid and solid materials, however, if the reaction product mixture is only liquid, a different extraction technique called liquid-liquid extraction is then used. Another method for separating compounds in a mixture is called chromatography: in column chromatography, by using gravity or vacuum a chemical mixture is passed through a column packed with an adsorbent material. Different components in the chemical mixture pass through the column at different rates due to varying affinities of the chemicals with the chromatography development solvent and the packed material of the column. The chemical compounds that are passed through the column are called fractions and are collected in different containers, separating the mixture into its components. Chromatography method can also be used not only for mixture separation but for chemical analysis [1-6].

1.2. Analysis

Analysis in chemistry is a quantitative and qualitative characterization of chemical compounds. Qualitative analysis involves the identification of chemical compounds in a chemical mixture, and

quantitative analysis involves the identification of the quantity, for example, concentration of a compound. Chemical analysis is used together closely with chemical synthesis: after or during the synthesis process, the chemical products are analysed in order to gain information about the different compounds in the mixture. Analysis is important in many sectors: pharmaceutical, research and manufacturing. In the pharmaceutical sector, the synthesized drugs are tested so that no adverse health effects are caused to the consumers. For pharmaceutical and research sectors, during the discovery of chemicals or new synthesis pathways, chemical analysis is used for identification of different compounds in a chemical mixture. In the manufacturing sector, analytical chemistry identifies manufactured chemicals and ensures that quality standards are met. The most commonly used techniques for chemical analysis are spectroscopy and chromatography. Firstly, spectroscopy can be used for both qualitative and quantitative analysis of chemical compounds. Spectroscopy uses light, usually ultraviolet (UV) or visible, to illuminate the chemical compound, the detector of the spectrometer then measures the absorbance spectra of the sample. By comparing the obtained absorption spectra with standards, the different compounds in the chemical mixture can be identified. Additionally, quantitative analysis can be performed by using Beer's law for calculating concentrations of compounds. If spectroscopy is performed during an ongoing chemical synthesis process, the changes in adsorption spectra over time throughout the chemical reaction process can identify the different stages of the chemical reaction, for example, if the reaction stopped. Another analytical technique is chromatography, which is the separation of chemical compounds in a chemical mixture based on the affinities of the chemicals and the medium in which they are being separated. The most commonly used methods of chromatography are column chromatography, thin layer chromatography (TLC), and high-performance liquid chromatography (HPLC). During column chromatography, different fractions of the chemical sample are collected and can be analysed using other methods. In TLC analysis method, a thin layer of absorbent material, such as silica gel deposited onto a plate is used to separate the chemical sample into its compounds. An illustration of TLC spotting and development steps can be seen in Fig. 1.

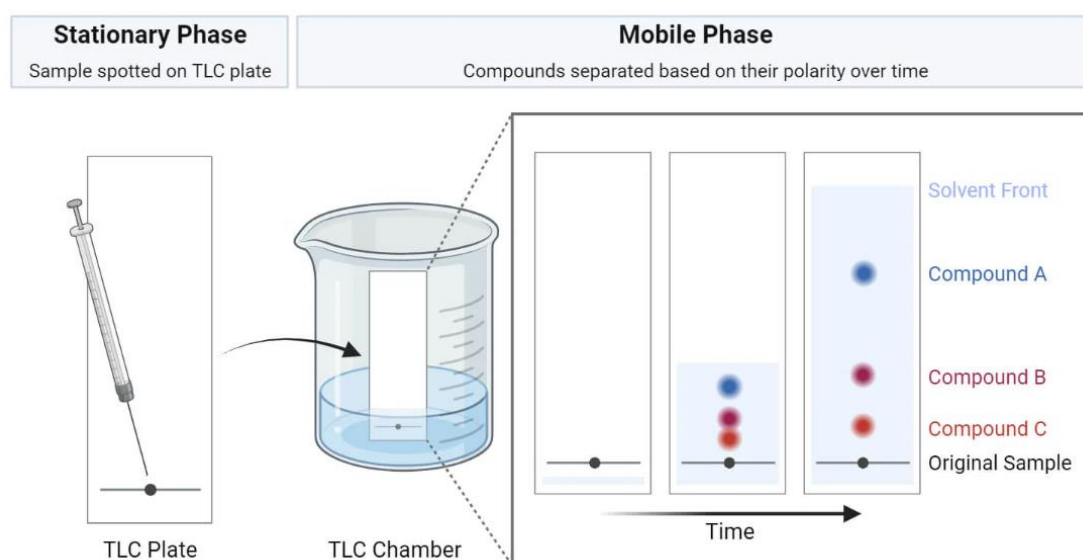


Fig. 1. Thin layer chromatography (TLC) spotting and development procedure [7]

During the spotting step, a chemical sample is spotted onto the absorbent material. If the process is performed manually, usually a glass capillary tube (Fig. 2B) or a pipette is used. The development step is performed after spotting: the plate is immersed into a chamber containing a development

solution, an example of a horizontal development chamber can be seen in Fig. 2A. The solution travels on the adsorbent material using capillary action, and due to the different affinities of the spotted sample with the development solvent and with the adsorbent material, compounds of the chemical mixture separate at different locations along the TLC plate. Conclusions can be drawn based on the separation lengths (retention factors) of different compounds to allow the identification of the synthesis reaction progression, identification of a number of compounds in the sample, as well as comparison of concentrations of compounds based on the brightness and size of the spots [5, 6, 8-12].



Fig. 2. Manual TLC development and spotting equipment: A) horizontal plate development chamber [13] B) capillary tubes for spotting chemical samples onto plates [14]

Lastly, another method of chromatography is called high-performance liquid chromatography (HPLC), during which a chemical sample and a solvent are injected into an injection port of the chromatography device, the solvent carries the chemical mixture through a packed column. Due to the different affinities of the chemical compounds with the solvent as well as different affinities of the chemical compounds with the adsorbent material in the packed column, the chemical compounds travel through the packed column at different rates. The detector of the HPLC analyses the passed liquid at the end of the column and produces a chromatograph, which characterizes the chemical sample. The most commonly used detectors use light or mass spectrometry. Column chromatography, TLC and HPLC methods can be used to obtain a large amount of analytical data required during research or manufacture, and such methods are characterised as high-throughput experimentation (HTE) methods [5, 6, 8-12].

1.3. Automation

HTE is a method used for producing a large number of experimental results. It involves rapid chemical sampling, collection and storage of analytical data in order to increase the productivity in finding the correct chemical samples used for different applications. Due to the large search space in chemistry that comes from many available chemical reagents and different synthesis parameters, HTE is widely used in various sectors, such as pharmaceutical, research and manufacturing. In order to achieve high throughput, automation tools are used for chemical synthesis and analysis. Usually, HTE involves the chemical synthesis of different mixtures in an array of containers. In each place of the array, a different chemical synthesis is performed by either combining different chemical reagents or by combining the same chemical reagents but with different concentrations. Automation systems used for chemical synthesis are called liquid dispensers or handlers. After the chemical synthesis is performed, each sample in the array is analysed using analytical devices, analytical data is then

collected, documented and stored. Furthermore, closed-loop systems can be constructed by using the obtained results from the analytical part of the HTE automation setup for the optimization of chemical synthesis parameters. There are existing commercially available automation systems separately for chemical synthesis and for chemical analysis automation [15-17].

Chemical synthesis automation involves either the dispensing of chemicals in liquid or solid form from predefined locations into chemical reaction containers. Chemical synthesis automation systems that dispense liquid chemicals are called liquid dispensing, handling, dosing or pipetting systems. Some examples of these systems are: Liquid Handling With Modular Dosing System by Festo [18], Assist Plus Pipetting Robot For Full Workflow Automation by Integra [19], Fluent or Freedom EVO liquid handling platforms by Tecan [20], OT-2 Robot by Opentrons [21], Bravo Automated Liquid Handling Platform by Agilent [22], epMotion 5000 series pipetting robots by Eppendorf [23], Biomek Ngenius Library Prep System and Biomek i-Series Automated Workstations from Beckman [24], Microlab Prep, Microlab NIMBUS, Microlab STAR, Microlab STAR V automated liquid handlers by Hamilton [25]. The liquid handling automation system Assist Plus by Integra can be seen in Fig. 3A. The end effector of the liquid handler is a pipette which uses piston air displacement technology. The pipetting module has different accuracy and precision values depending on the size of the pipette volume as well as the amount of volume transferred (Fig. 3B).

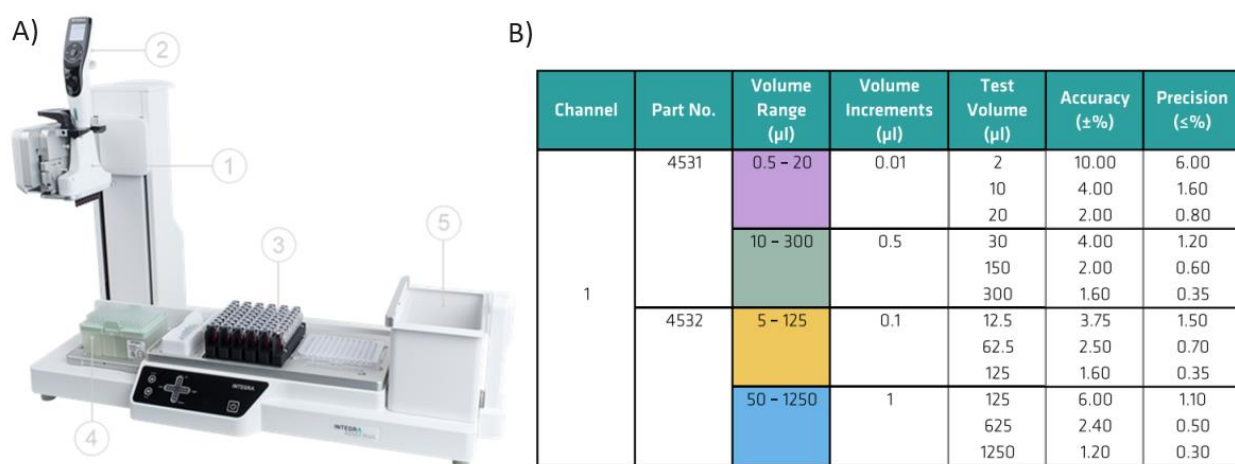


Fig. 3. Liquid handling automation system: A) Assist Plus by Integra 1) pipetting end effector, 2) user interface and control panel, 3) chemical container rack, 4) pipette tip storage container, 5) pipette tip waste container [26], B) specifications of D-ONE pipetting modules [27]

A system called Liquid Handling With Modular Dosing System by Festo can be seen in Fig. 4A. The pipetting system uses pump air displacement technology and is reported to have pipetting accuracies of $\pm 5\%$ for dispensed volumes of 10 μl , $\pm 2\%$ for 50 μl , $\pm 1\%$ for 500 μl and $\pm 0.5\%$ for 1000 μl [28]. Microlab Star V by Hamilton (Fig. 4B) automation system uses one manipulator arm which is capable of both pipetting and transporting container racks and bottles. The dispensing for this system is achieved by utilizing air displacement method using a magnetic piston controlled by a magnetic field. The accuracy of the dispensing pipette ranges from 1% to 10% and precision from 0.75% to 6% for different volume ranges [27, 29, 30]. The Freedom EVO 200 by Tecan (Fig. 4C) automation system is the largest platform from the Freedom EVO product lineup and has the ability to control three mounted robotic arms. Liquid dispensing pipettes are either of piston air displacement or pump air displacement types. For the pump pipette, the liquid transfer accuracies and precisions are reported to be $\pm 9.5\%$ and 6% for 10 μl , $\pm 1.0\%$ and 0.5% for 1000 μl [31].

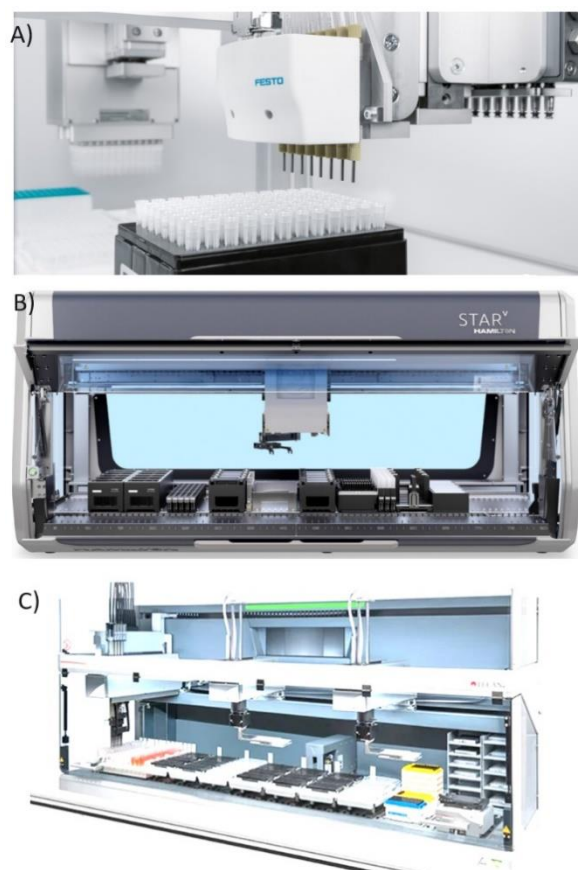


Fig. 4. Liquid handling automation systems: A) Liquid Handling With Modular Dosing System by Festo [28], B) Microlab Star V by Hamilton [29], C) Freedom EVO 200 by Tecan [31]

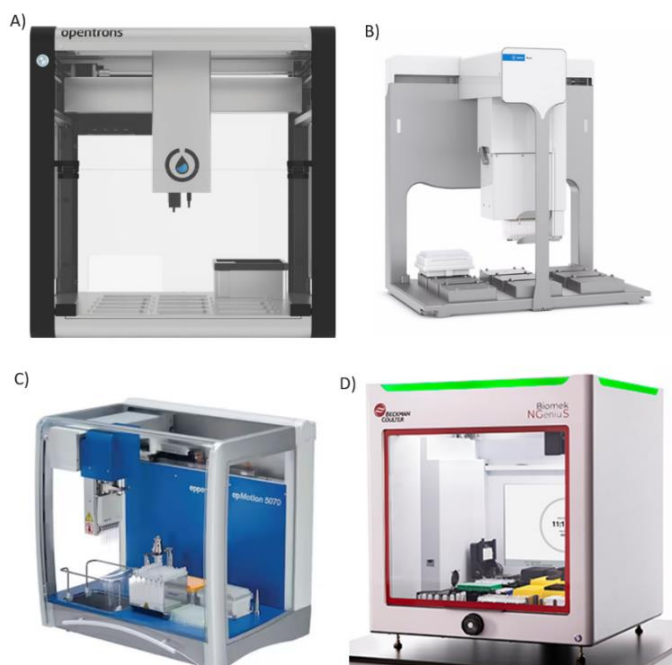


Fig. 5. Liquid handling automation systems: A) OT-2 by Opentrons [32], B) Automated Liquid Handling Platform Bravo by Agilent [33], C) epMotion 5070 by Eppendorf [34], D) Biomek NGeniusS Next Generation Library Prep System by Beckman Coulter [35]

OT-2 by Opentrons system is open-sourced and can be seen in Fig. 5A. All available pipettes for this automation system use piston air displacement technology, for which the accuracies and precisions

are $\pm 15\%$ and 5% for $1\ \mu\text{l}$, $\pm 1.5\%$ and 0.8% for $20\ \mu\text{l}$ with P20 GEN2 pipette, and $\pm 2\%$ and 1% for $100\ \mu\text{l}$, $\pm 0.7\%$ and 0.15% for $1000\ \mu\text{l}$ with P100 GEN2 pipette [32, 36]. Automated Liquid Handling Platform Bravo by Agilent (Fig. 5B) has the ability to use 8, 16, 96 or 384 channel pipettes. The accuracies and precisions of the 96 channel pipette are reported to be $\pm 4.4\%$ and 3.9% for $0.1\ \mu\text{l}$, $\pm 2.1\%$ and 2.1% for $1.0\ \mu\text{l}$ [33, 37]. Automation system epMotion 5070 by Eppendorf (Fig. 5C) uses piston air displacement method for liquid dispensing. The reported systematic errors and random errors respectively are $\pm 5.0\%$ and 1.5% for $40\ \mu\text{l}$, $\pm 0.7\%$ and 0.15% for $1000\ \mu\text{l}$ with the single-channel pipette TS 1000 [34, 38]. The Library Prep System by Beckman Coulter (Fig 5D) uses 8-channel pipettes. The accuracies and precisions respectively are 5% and 5% for $2\ \mu\text{l}$, 3% and 3% for $100\ \mu\text{l}$ [24, 35]. All mentioned chemical dispensing systems use Cartesian robotic platforms, with end effectors mounted on the z-axis of the robots. Some automation systems have different types of equipment used for mixing or heating reagents in the chemical vessel. These automation systems are only used for chemical synthesis, different systems are needed to automate chemical analysis [18-25].

Chemical analysis automation systems involve the intake of the chemical sample from predefined locations and the injection of the sample into an analytical device or onto a consumable. Analytical techniques used in chemical analysis automation systems are varied, but most often use chromatography or spectrometry, such as TLC, HPLC, UV-visible spectrophotometry, mass spectrometry (MS), or nuclear magnetic resonance (NMR) spectrometry [39]. Examples of chemical analysis automation systems include a TLC spotting semi-automatic system called Linomat 5 by Camag [40], TLC spotting automation system called Automatic TLC SAMPLER 4 by CAMAG [41], an HPLC sampler called HT4000L Autosampler by HTA [42] connected with an HPLC analytical device, UV-visible and near-infrared spectroscopy (NIR) autosampler called HT1500U by HTA [43] connected with a spectrophotometer device, a liquid chromatography - mass spectrometry (LC-MS) autosampler called QS-Works Autosampler by PerkinElmer [44] connected with an LC-MS analytical device, an NMR spectrometer autosampler called SampleCase Plus by Bruker [45] connected with a NMR spectrometry analytical device. HT4000L Autosampler by HTA connected to an HPLC device can be seen in Fig. 6A. The autosampler injects a chemical sample from a container into the HPLC device [46]. HT1500U Autosampler by HTA connected to a chromatograph device can be seen in Fig. 6B. HT1500U autosampler takes chemical samples from up to 40 10ml tubes and injects into the chromatograph [47]. QS-Works Autosampler by PerkinElmer connected to an LC-MS analytical device can be seen in Fig. 7A. The QS-Works autosampler takes in the chemical sample from 420 1ml, 324 2ml or 90 10ml vials and injects the sample into the LC-MS device [48]. SampleCase Plus autosampler connected to an Ascend 300 NMR analytical device can be seen in Fig. 7B. SampleCase Plus autosampler can heat 24 sample containers to $125\ ^\circ\text{C}$ or cool the samples to $4\ ^\circ\text{C}$. The autosampler takes in the chemical samples from the containers and injects into the NMR analytical device [49]. The automatic TLC SAMPLER 4 by CAMAG can be seen in Fig. 15. The sampler takes in chemical samples from up to 66 2ml vials and sprays the samples onto a TLC plate using pressurized nitrogen utilizing a jet dispensing method [41]. All chemical analysis automation systems except the Linomat 5 and TLC SAMPLER 4 use an autosampler connected to an analytical device. Autosampler takes chemical samples from predefined locations and dispenses into the analytical device. The analytical device performs analysis of the injected chemical samples, records and documents the analysis results. If chemical synthesis and analysis processes are not connected under one system, the analysis results can be examined and only then changes can be made in how the samples are prepared. In most setups, the chemical synthesis and analysis parts are only connected

indirectly: the results from the analytical part need to be manually processed and applied to the synthesis part, since the analysis is done on a separate automation system to the chemical synthesis automation system [40, 42-45, 50].

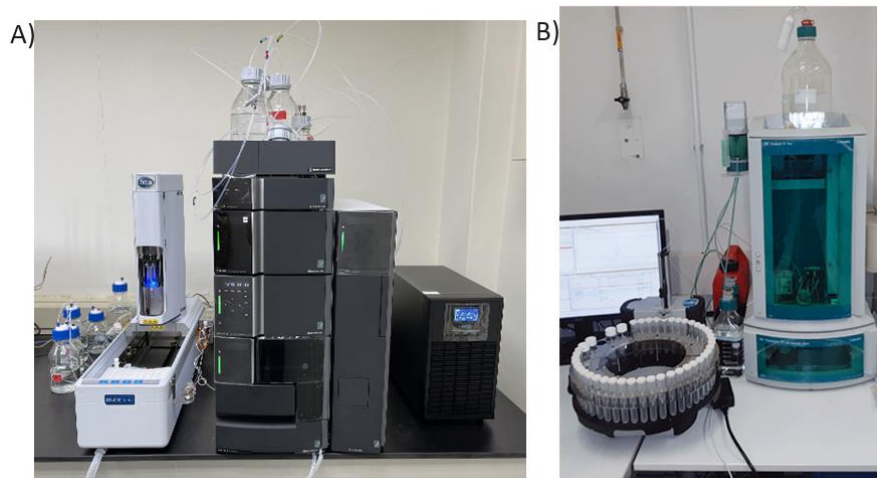


Fig. 6. Autosamplers coupled to analytical devices: A) HT4000L Autosampler by HTA connected to an HPLC device [46], B) HT1500U Autosampler by HTA connected to a chromatography device [47]



Fig. 7. Autosamplers coupled to analytical devices: A) QS-Works Autosampler by PerkinElmer connected to an LC-MS analytical device [48], B) SampleCase Plus autosampler connected to an Ascend 300 NMR analytical device [49]

Currently, there are no mass-produced commercially available fully automated systems that combine both chemical synthesis and analysis processes. Fully automated systems are only being developed for internal use inside chemical laboratories, and research is still ongoing for the development of such automation systems, with the only exceptions being that some companies provide chemical synthesis and analysis automation solutions by allowing the users to remotely control the laboratory devices for custom applications. There are multiple chemical synthesis and analysis automation solutions that researchers have explored for the development of a fully automated system combining both chemical synthesis and analysis. One research group developed an automation system containing a collaborative robot that is able to handle chemical samples from microfluid reactors where synthesis takes place and move those samples to a spectrometer analytical device [50, 51], the system can be seen in Fig. 8.

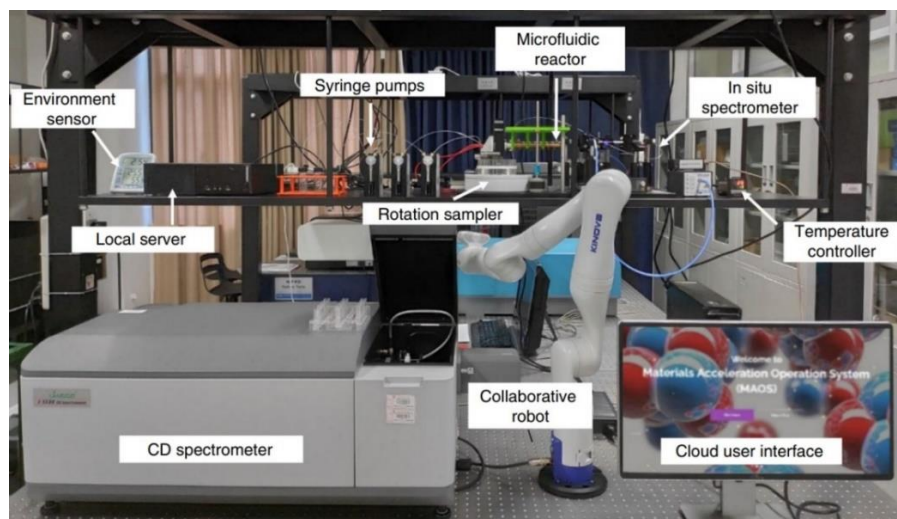


Fig. 8. A fully automated system using a collaborative robot arm and a circular dichroism spectrometer analytical device [51]

Using this automation system a closed-loop chemical synthesis of inorganic perovskite nanocrystals was performed by using a microfluidic reactor and a spectrometer that measured the absorbance spectra of samples as well as the circular dichroism spectrometer that measured the chirality of the samples. Obtained data from the spectrometers was used to optimize the synthesis reaction by planning the experiment conditions for the next synthesis cycle [51]. Another research group developed an automation system combining an automated synthesis using flow chemistry with direct analysis in real-time – mass spectrometry (DART-MS) analytical device, flow chemistry nuclear magnetic resonance (flow-NMR) spectrometer analytical device, and a high-performance liquid chromatography-mass spectrometry (HPLC-MS) analytical device (Fig. 9).

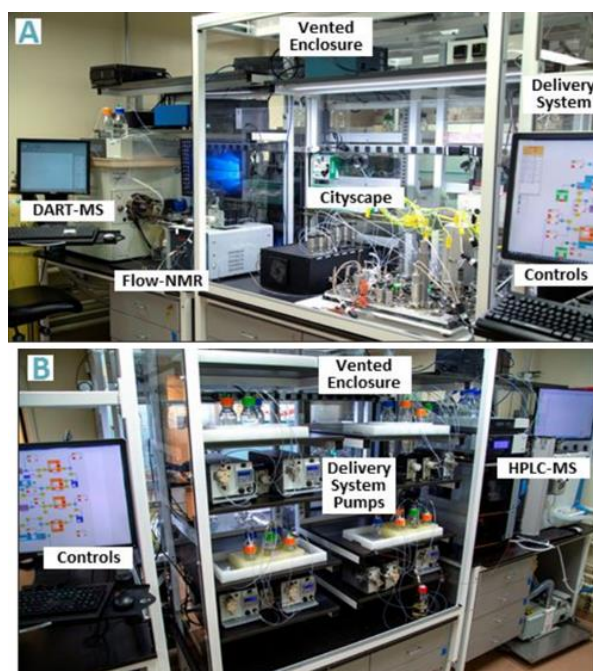


Fig. 9. A fully automated system: A) using flow chemistry for synthesis and flow-NMR, DART-MS and B) HPLC-MS analytical devices [52]

Fig. 9A shows the synthesis part of the automation system called Cityscape connected to flow-NMR and DART-MS analytical devices, Fig. 9B shows the delivery system for the HPLC-MS and the

HPLC-MS device itself. The analytical devices provided the capability to monitor the route of the synthesis reaction processes, as well as the capability to perform quality control of the samples once the synthesis reaction was finished [52]. Another research group developed an automated flow chemistry synthesis platform combined with an in-situ flow chemistry Fourier transform infrared (flow-FTIR) spectroscopy analytical device and flow ultrasonic velocity sensor (Fig. 10). Fig. 10A shows the synthesis part of the automation system and Fig. 10B shows the parts of the automation system where precipitation, crystallization, and formulation processes took place. An ultrasonic velocity sensor was used to measure the concentration of the liquid chemical samples. Flow-FTIR was used to monitor the progress of the synthesis reactions [53]. Furthermore, other authors developed a procedure that allows the automation of chemical synthesis and analysis using standard equipment and analytical devices found in the laboratory by connecting these devices using valves and pumps to provide the transfer of the required chemicals from one device to another (Fig. 11).

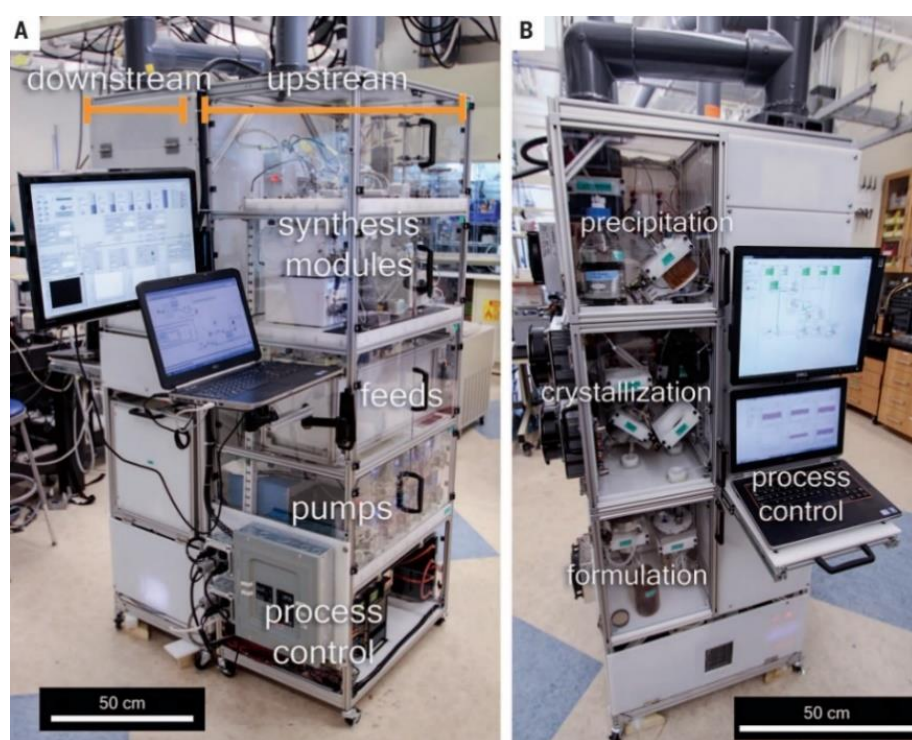


Fig. 10. A fully automated system using flow chemistry with a flow-FTIR analytical device and flow ultrasonic velocity sensor: A) place where chemical synthesis takes place, B) place where precipitation, crystallization and formulation takes place [53]

Fig. 11A shows the fluid connections and flow paths of the system and Fig. 11B shows the chemical laboratory equipment used in the automation system. The chromatography device used for the automation system was employed for the purification of the chemical mixtures: analytical data from the UV-visible spectrophotometer was used to optimize the retention rates used for the flash column chromatography device in order to collect different fractions from the chemical mixture [54]. Additionally, another research group achieved chemical synthesis and analysis automation by using a mobile robot in a chemistry laboratory (Fig. 12). Fig. 12A shows the robotic arm loading chemical samples into the photocatalytic activity measuring device (photolysis station), Fig. 12B shows the robotic arm loading empty vials into a solid dispensing station, and Fig. 12C shows the robotic arm loading the samples into a gas-chromatography (GC) analytical device. The photolysis station was used to illuminate the synthesized samples, after the illumination the samples were transferred to a GC analytical device where the hydrogen evolution rate was measured [55, 56].

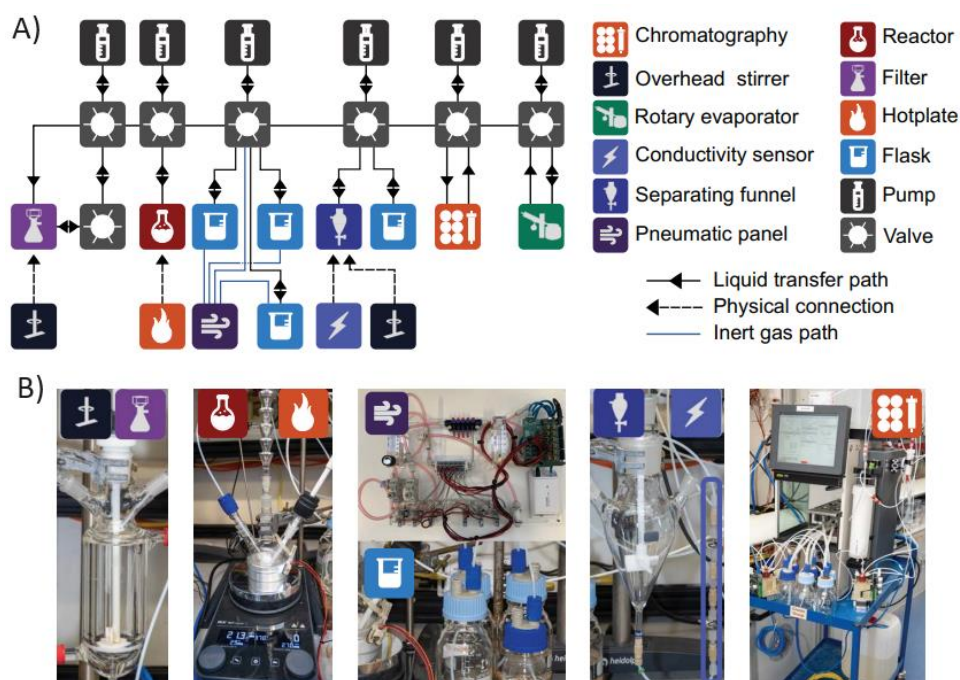


Fig. 11. A fully automated system using ChemPu platform connected to various chemical laboratory equipment including a chromatography analytical device: A) fluid connection and flow paths of the system, B) chemical laboratory equipment connected to the automation system [54]

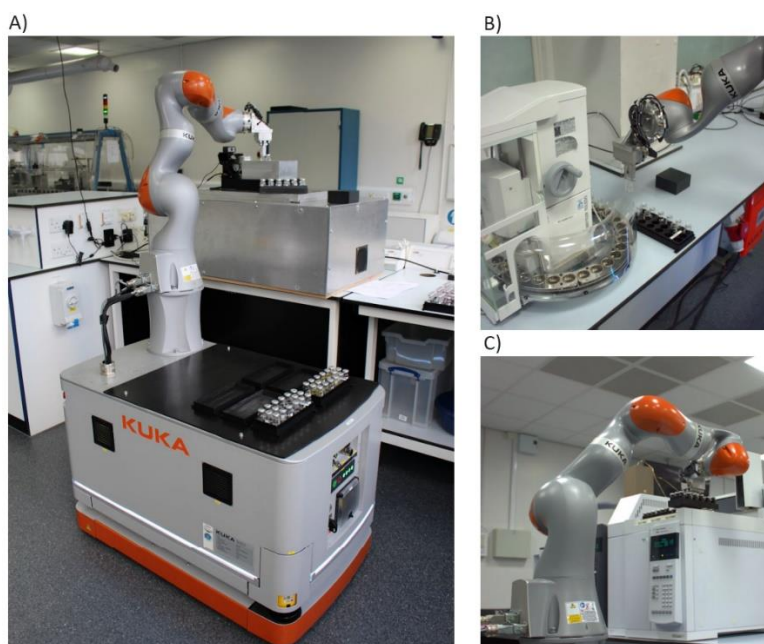


Fig. 12. A fully automated automation system using a Kuka robotic arm mounted on a mobile platform, GC analytical device, and a photocatalytic activity measuring device: A) robot arm loading samples into the photocatalytic activity measuring device, B) robot arm loading empty vials into a solid dispensing station, C) robot arm loading samples into a GC analytical device [56]

Furthermore, synthesis and analysis automation solutions are provided by a company called Strateos [57], which offers access to remotely controlled prebuilt automated workflows for various synthesis and analysis applications, as well as provides a service for building custom workflows. After that, the users can execute the experiments remotely, watch the experiments take place in real-time through video streaming, and view analytical data [57]. In the Strateos laboratories, fully automated systems

are used: from the virtual laboratory tour videos which are available on the Strateos website, it can be seen that the delivery systems for moving chemical samples and other consumables between chemical synthesis and analysis stations are automated by using robotic arms, and other transfer systems [58]. Lastly, another company providing remote chemical laboratory services is called Emerald Cloud Labs [59]. Chemical synthesis and analysis equipment are used in the laboratory, for which the control of the protocols and data visualization is done remotely by the users. From the instrumentation list as well as the virtual tour video which are available from the company's website, it can be seen that the majority of the laboratory is not fully automated, meaning that the samples are transferred between the different laboratory equipment without automation, except for one type of automation system containing Hamilton Star for chemical synthesis, a Fluostar Omega for absorbance measurements and a Clariostar for fluorescence measurements [60, 61]. The lowest tier pricing from the Emerald Cloud Labs for renting three experiment setups costs about 22000 euros per month [62]. Fully automated systems that combine both chemical synthesis as well as analysis are either being developed internally in some laboratories, are still in the research stage and are not sold commercially, or only a rentable service is provided for fully or semi-automated systems that can only be controlled remotely [50-55, 57, 59].

1.4. Chapter Summary

Chemical synthesis is a process where different reagents are combined to produce products and is used in various fields of manufacturing, pharmaceutical, and research. Chemical analysis is a process for qualitative and quantitative characterization of chemical compounds. The most commonly used analytical methods involve spectroscopy or chromatography. The most basic chromatography analytical method is TLC where a chemical sample spotted on a coated plate is developed, during which the different chemical compounds of the chemical sample separate. HTE is a method used for producing a large quantity of experimental data, and usually, automation systems are used to achieve this. There are commercially available automation systems for chemical synthesis that involve a liquid dispensing robot. These systems utilize a Cartesian robotic platform and a pipette as the end effector to transfer chemicals from one place to another. Examples of commercially available systems include Liquid Handling With Modular Dosing System by Festo, Assist Plus Pipetting Robot For Full Workflow Automation by Integra, Fluent or Freedom EVO liquid handling platforms by Tecan, OT-2 Robot by Opentrons, Bravo Automated Liquid Handling Platform by Agilent, epMotion 5000 series pipetting robots by Eppendorf, Biomek Ngenius Library Prep System and Biomek i-Series Automated Workstations from Beckman, Microlab Prep, Microlab NIMBUS, Microlab STAR, Microlab STAR V automated liquid handlers by Hamilton. There are commercially available chemical analysis automation systems which usually involve an autosampler coupled to an analytical device. Examples of analysis automation systems include: Linomat 5 by Camag, Automatic TLC SAMPLER 4 by CAMAG, HT4000L Autosampler by HTA coupled to an HPLC, HT1500U by HTA coupled to a spectrophotometer, QS-Works Autosampler by PerkinElmer coupled to an LC-MS device, SampleCase Plus by Bruker coupled to an NMR spectrometer. There are no mass-produced commercially available automation systems that combine both chemical synthesis and analysis processes, however, these automation systems are being created by various research groups or companies that provide chemistry laboratory services remotely.

2. Automation System Design

In this chapter, the working principles and design choices of commercially and non-commercially available chemical synthesis automation systems and TLC analysis automation systems are explored. The design of an automation system combining both chemical synthesis and analysis using TLC method is provided and the methodology for testing the automation system is given.

2.1. Existing Automation Solutions

In a fully automated system, both processes of chemical synthesis and analysis need to be automated. The design process of the system can be split into multiple parts: the design of the chemical synthesis process automation, the design of the analysis process automation and the interface that connects the synthesis process with analysis. Typically, chemical analysis only requires a small amount of chemical sample taken from the chemical synthesis process, therefore a sample transfer mechanism can be utilized between the two processes of chemical synthesis and analysis in order to combine them under one automation system. The design choices of other automation systems can be explored by looking at the automation systems that automate only chemical synthesis and systems that only automate chemical analysis separately. Firstly, the working principles and design choices of the chemical synthesis automation systems are explored. A Microlab STAR automated liquid handler by Hamilton [63] is a commercially available product that automates chemical synthesis and can be seen in Fig. 13.



Fig. 13. Microlab STAR automated liquid handler by Hamilton [64]

A brochure is provided by Hamilton detailing the hardware and processes of the system [64]: a Cartesian robotic platform is used, for which the end effector is 8 vacuum channels, on which multiple tools can be mounted, such as pipettes for transferring chemical liquids, vacuum grippers for transporting racks of chemical bottles, smaller vacuum grippers for opening and closing chemical bottles, and suction cups for moving a single chemical bottle. Multiple-sized pipettes can be mounted onto the end effector: 1 ml and 5 ml. Consumable pipette tips are used when transferring liquids to avoid cross-contamination. After the liquid transfer process, the automation system either ejects the pipette tip to a waste container or returns the tip to its resting place on the rack, these procedures are determined by defining the behaviour in the proprietary automation system software. A vacuum pump is connected to the pipette that controls the intake and outtake of liquid during dispensing. A pressure sensor is located inside the pipette, which allows the monitoring of the pressure changes during liquid transfers. As a result of the pressure monitoring, the pressure profile over time can be obtained allowing the automation system to detect these events: no liquid is dispensed, a foam or solid

formation inside a chemical bottle, or the syringe tip is clogged. Pressure profile monitoring also allows the automation system to intake or dispense near the surface of the liquid, and prevent dripping during the transfer of volatile liquids, for example, acetone, by providing an additional negative pressure to the pipette. The physical dimensions of the automation system are 166.4 cm in width, 78.1 cm in depth, and 90.3 cm in height. The time it takes to fill one bottle with a 0.1 ml liquid including changing the tip takes 320 seconds. The accuracy of the Cartesian robotic platform is 0.1 mm. On the baseplate, various racks for holding differently-sized bottles can be placed in their predefined locations. The front end of the propriety software includes a user interface, in which a visual depiction of the current setup of the automation system is shown, visualizing where each rack is placed on which part of the baseplate. A sequence of liquid dispensing procedures can be set for a current job, the pipette tip types, the amount of dispensed liquid, and other various pipetting settings as well as error-handling parameters can be selected [64].

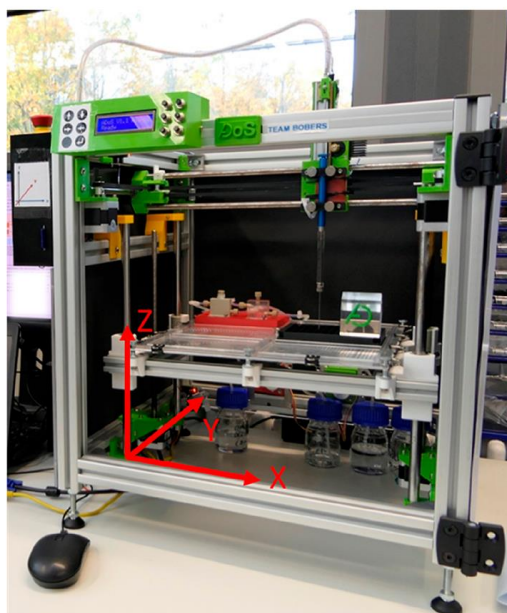


Fig. 14. Automated Dosage System [65]

Another chemical synthesis automation system is called an automated dosage system (AdoS) (Fig. 14) which was developed by a research group and is not commercially available [65]. The automation system uses an existing open-sourced 3D printer which is a Cartesian robotic platform. The frame of the automation system is built using aluminium extrusion profiles, and the joints for the frame were 3D printed using polylactic poly(lactic acid) (PLA) material. The size of the automation system is 280 cm in width, 280 cm in depth, and 200 cm in height. National Electrical Manufacturers Association (NEMA) stepper motors are used for the movement of each axis. The end effector is a gas-tight glass syringe mounted on a linear actuator, a syringe needle that comes in contact with chemical liquids during dispensing is mounted at the end of the syringe. The control for the stepper motors of the automation system is performed using an Arduino Mega microcontroller using G-code-based commands. The automation system does not use stepper motor encoders, the home positions are set only by using limit switches at each axis end of the Cartesian robot. The pumps for transferring cleaning liquid solutions are controlled by an additional Arduino microcontroller. Racks for holding variously sized chemical bottles can be placed on a baseplate in predefined locations. Racks were either 3D printed using PLA material or machined using poly(1-methylethylene) PP (polypropylene) material. The cleaning of the syringe and syringe needle is done by an intake of a cleaning solution

and dispensing to a waste container. The authors noticed syringe clogging after prolonged use and recommend cleaning the syringe with concentrated hydrochloric acid, as well as filling the syringe with water if it is not being used for longer periods of time [65].

For the analysis part of the fully automated system, a TLC analysis method is the most suitable, due to the low cost of consumables as well as the versatility of the method [66]. The TLC analysis method is separated into multiple parts: spotting, developing, staining, and imaging [67]. There are commercially available automation devices for each step of the TLC analysis method, but automation systems that automate more than one of the TLC steps are still being researched, only developed internally and are not commercially available [41, 68-70]. For the spotting step of the TLC analysis method, there is an automation system called Automatic TLC sampler 4 by CAMAG [41] (Fig. 15). The automation system is able to perform the spotting step, which involves taking in a chemical sample and then dispensing it onto a TLC plate so that the sample forms a spot. The automation system uses a Cartesian robot with a syringe capable of dispensing chemical liquids as an end effector. A syringe needle is attached to the syringe, which comes in contact with the chemical liquids and the spotting of the chemical samples is achieved by using pressurized nitrogen gas to spray the chemical sample onto the TLC plate. There are predefined positions on the baseplate where racks for holding chemical bottles, as well as the TLC plates, can be placed.



Fig. 15. Automatic TLC SAMPLER 4 by CAMAG [41]

For the development stage of the TLC analysis, there is a commercially available automation device called Automatic Developing Chamber 2 by CAMAG [68] (Fig. 16A). The spotted TLC plates can be placed inside the developing chamber and a developing solution can be poured into the chamber. Chamber is then saturated with the solution gas and the development liquid is dispensed at the bottom of the plate starting the development process. The temperature in the chamber is controlled to be at the set temperature. After the set time, once the development is finished, the TLC plate is dried. The parameters for the development can be controlled through a proprietary software. The main application of this developing chamber is that the same conditions for development are being applied to all development cycles to achieve consistent analysis results [68]. For the imaging stage of the TLC analysis, there is a commercially available automation device called TLC Visualizer 3 by CAMAG [69] (Fig. 16B). A spotted and developed TLC plate can be placed inside the imaging device, and an image of the developed plate is then taken under different lighting conditions, such as visible light, UV of 366 nm and UV of 254 nm. The taken images are sent to a proprietary software

where they can be analysed by the user. The application of this device is to provide the same lighting conditions during every plate imaging so that the analytical results are consistent and can be compared from one plate to another [69]. For the staining (derivatization) part of the TLC analysis, there is a commercially available automation device called Chromatogram Immersion Device 3 by CAMAG [70] (Fig. 16C). A spotted and developed TLC plate and a staining solution can be added to the device, the device immerses the plate for a set amount of time and then dries the plate. The parameters of the device can be controlled through a proprietary software. The application of this device is the derivatization of plates using the same parameters for all plates so that the analytical results are consistent and can be compared to each other [70].

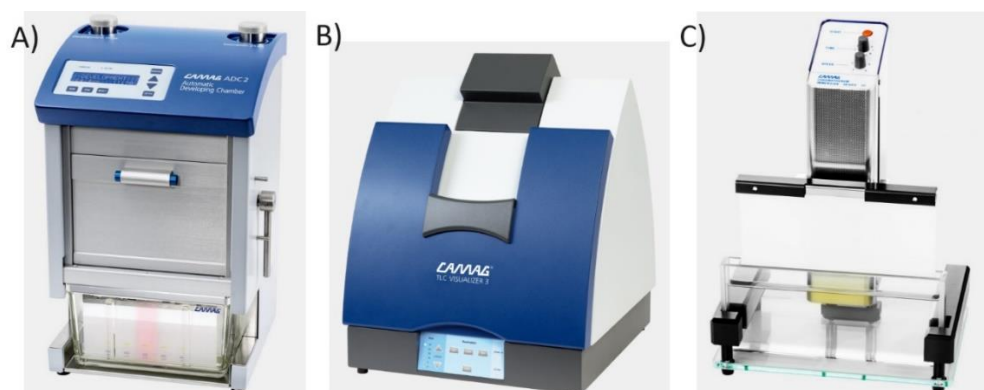


Fig. 16. Commercially available TLC automation systems: A) Automatic Developing Chamber 2 by CAMAG [68], B) TLC Visualizer 3 by CAMAG [69], C) Chromatogram Immersion Device 3 by CAMAG [70]

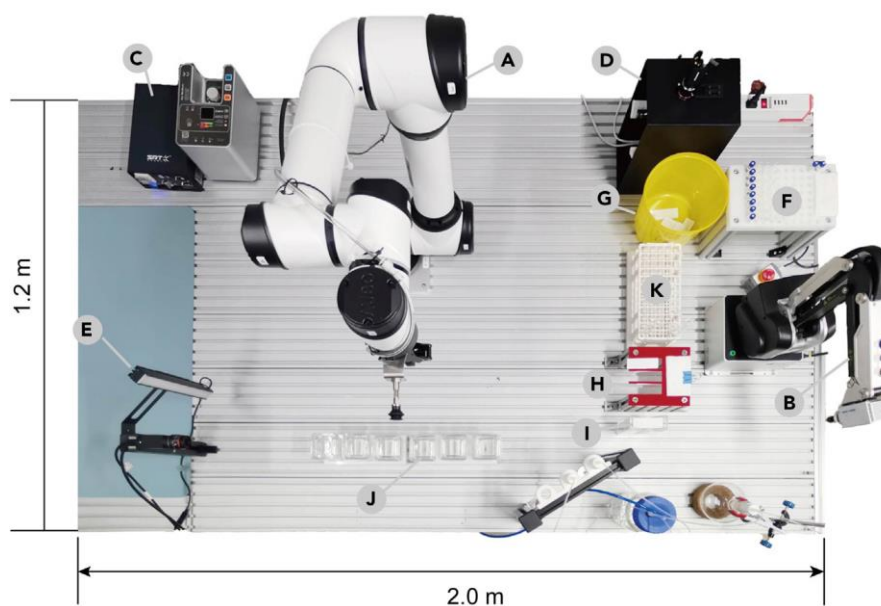


Fig. 17. TLC spotting, development and imaging automation system using two arm robots: A) robotic arm AUBO i5, B) robotic arm MG400, C) vacuum pumps, D) UV photo station, E) visible light photo station, F) chemical sample container rack, G) waste container, H) plate position changing station, I) plate storage rack, J) vertical development chamber, K) chemical sample container rack 2 [71]

Fully automated systems that combine both synthesis and TLC are not commercially available but are being researched. One research group developed an automation system capable of TLC spotting, developing, and imaging in one system [71] (Fig. 17). This automation system uses two robotic arms:

AUBO i5, which has 6-axis and a DOBOT MG400 which has 4-axis. The AUBO i5 robot has both a vacuum and a mechanical gripper for the manipulation of TLC plates. The DOBOT MG400 robot has a glass capillary tube mounted onto the end effector, which is used for spotting the chemical samples onto the TLC plates. Two robotic arms are mounted on a base plate of 200 cm in length and 120 cm in width. A rack for storing chemical samples is mounted onto predefined places from which the DOBOT MG400 robot takes chemical liquid samples using capillary action and spots the chemical samples onto the TLC plate. After the spotting process, using the mechanical gripper the AUBO i5 arm immerses the plate into a vertical development chamber. After the plate development is finished, the plate is carried to an imaging station and images are taken under UV illumination conditions. The research authors report encountering problems of some spots being hardly visible, overlapping or tailing and propose solutions for the system to have the ability to increase or reduce the concentration of samples before spotting them onto the plate, these problematic TLC plates are discussed further in Chapter 3.4.

Taking into account the working principles and the design choices of the automation systems of chemical synthesis and TLC analysis, the functional requirements and restrictions for a fully automated system that combines both chemical synthesis and TLC analysis processes can be constructed. Starting with the design for the chemical synthesis part of a fully automated system: the process of chemical synthesis from an automation system's perspective is a liquid transfer from one predefined place to another and mixing of the liquids. Equipment needed for synthesis is a reaction flask and containers for storing reagents. The reaction flask should be placed on top of the stirring mantle, and a magnet bar should be placed inside the reaction flask. The liquid transfer of various chemicals needs to be achieved in order for chemical synthesis to take place, therefore a liquid handler robot can be used. During the liquid transfer, the intake of the liquid needs to be performed from the containers containing chemical reagents, the reagents from the containers could be reached by using a syringe needle and utilizing either a piston positive displacement or air displacement dispensing method. The syringe movement could be achieved by mounting a plunger-operated syringe into a linear actuator that operates the plunger of the syringe, the syringe can then be connected via tubing to the syringe needle. Since a single syringe needle comes in contact with multiple different reagents, cleaning procedures should be performed in order to avoid cross-contamination, as well as to prevent clogging of the syringe due to the sediment formation in the syringe needle. Each chemical container, as well as the stirring mantle, should have a designated place on the automation system since the containers will be removed during cleaning and refilling of the chemical reagents. For the manipulation of the end effector, multiple different robotic platforms could be selected: Cartesian, arm, or delta. The robots should have closed-loop movement to achieve acceptable accuracy when transferring and dispensing chemicals from containers. Due to the constraint of the budget being lower than 8000 euros for the whole automation system, which was raised in the requirements of this work, the robot has to be non-industrial due to the industrial robots being too expensive for this budget. This requirement excludes arm robots having closed-loop control, due to their price exceeding the cost constraint of the automation system [72].

For the analysis part of a fully automated system, the analysis of the samples from the chemical synthesis part could be analysed by using TLC analytical technique due to its versatility and low cost compared to other chemical analysis methods [66]. TLC is divided into several steps, which include three mandatory steps: spotting, development, and imaging, and an optional step of staining (derivatization). Staining can be used if the compounds on the developed TLC plate under different

illumination conditions are difficult to see. During staining, the derivatization agents react with the separated compounds on the TLC plate and the compounds can become more visible. From the automation system's perspective, spotting is the transfer of liquid from a chemical container onto a TLC plate in a predefined location on the plate. Development is a liquid transfer of one or more development chemical solutions onto the spotted TLC plate using capillary action from one side of the plate. Imaging is taking a photo of a developed TLC plate under different lighting conditions, such as visible or UV. Staining is the immersion of the developed plate into a staining solution up to a certain part of the plate lengthwise, additionally imaging after staining needs to be performed that involves imaging of the stained plate but only under visible light, since the staining solution usually prevents visualization of the spots under UV lighting conditions. In order to automate all mentioned parts of the TLC method, multiple stations performing spotting, development, staining and imaging are needed. For the spotting station, a liquid handler robot is needed in order to dispense chemical samples onto the TLC plate. Similar design choices used for the liquid handler for the chemical synthesis part of the automation system can be used for the liquid handler in the analysis part. The spotting mechanism of the chemical sample onto a TLC plate needs to be considered: the TLC plate is usually a glass plate coated with a thin layer of silica gel, therefore the robot must carefully dispense the chemical onto the plate in order not to break the silica gel. In addition, the spots must not overlap with other spots within the plate, therefore liquid handler needs to produce small enough spots, so they do not overlap in addition to not breaking the silica-gel. This may be achieved by using a small gauge syringe needle with a blunt tip, as well as making sure that the needle touches the silica gel without breaking it. The liquid handler robot can also be used for the development part of the TLC analysis. During the TLC development stage, the liquid handler needs to prepare a development solution mixture out of one or more development solvents and mix them, therefore a rack for holding containers for the development solvents is needed. In the development station, there should be a mechanism for performing horizontal development of the TLC plate, which involves the placement of the plate onto a horizontal surface, pouring the development solution mixture into a reservoir, contacting the liquid of the reservoir to the end of the plate, waiting for the development solution to travel up the plate using capillary action, and removal of the plate from the development station. The horizontal development chamber would be more suited compared to a vertical development chamber because the plate can be placed onto the horizontal chamber and would not need to be held using a robot during the development process. The liquid handler could also be used for the cleaning of the syringe needle since it will be in contact with multiple different chemical solvents, as well as chemical samples. Cleaning is needed to avoid cross-contamination and clogging of the syringe needle due to sediment or crystal formation in the needle. For the imaging station, there needs to be an enclosure, in which TLC plates could be placed and imaged under different lighting conditions such as visible and UV. An enclosure would provide cover so that the lighting would be uniform and consistent throughout the imaging process of different plates, this way, the analytical results would be more standardized across different plates and the analytical results could be compared with each other. For the staining station, the solutions are usually very concentrated in order to stain the TLC plates, therefore it can be difficult for the liquid handling robot to transfer staining liquids, as the chemicals could clog the syringe needle due to rapid crystallization in the syringe needle. Therefore, it would be easier to prefill the chemicals into containers before starting the analysis process, and the plate could be immersed into the prefilled containers in order to be stained without the need of dispensing any staining chemical solutions. TLC plates need to be transferred between different stations: spotting, development, imaging and staining, as well as picked up from a plate storage location and placed into a waste pile after they have been used for analysis. For the transportation of the plates, an

additional robot can be used that has a vacuum suction cup and a mechanical gripper as end effectors. Two end effectors are needed in order to manipulate the plate when the plate is in the horizontal position (with the vacuum suction cup) for the spotting, developing and imaging processes, and in the vertical position (with the mechanical gripper) for the staining process when immersing the plate into the staining solutions. The vacuum pressure of the suction cup needs to be low enough so that the silica gel that is coated on the plates does not break. In addition, a filter for the vacuum lines needs to be installed to prevent particles of silica gel from entering the vacuum lines.

The automation system is fully automated, meaning that both chemical synthesis and analysis parts are connected. Chemical analysis using TLC requires only a small volume amount of sample from the chemical reaction flask, however, the volume needs to be large enough so that a spot on the TLC plate can be made. The chemical synthesis, on the other hand, can be performed in larger volume scales, for example from 1ml to multiple liters. This difference in the volume scale is also reflected in chemical containers used for synthesis and analysis: containers used for synthesis can be larger than the ones required for analysis. Therefore, the design of an automation system can be such, that the synthesis part of the automation system works on a separate scale of volume compared to the analysis, and there is only an interface between the two parts of the automation system where the chemical liquid is transferred from the chemical synthesis to the analysis parts. The transfer of liquid the other way is not meaningful, since the chemical synthesis part does not require samples from the analysis part. The interface can be a moving platform that travels between the two parts of the automation system. Onto the moving platform, a rack containing chemical bottles can be mounted in such a way that the liquid dispensing robot of the chemical synthesis side can dispense chemicals from the reaction flask, as well as the liquid handling robot of the analysis side can take in the chemical samples and use them for TLC analysis. Additionally, the chemical containers require that both end effector syringe needles of the liquid handling robots can reach the chemical bottles.

For the structure of the automation system, everything should be mounted onto a baseplate for easier system relocation. The baseplate should contain predefined places where different racks for various consumables used for both synthesis and analysis processes could be placed. The chemical synthesis part of the automation system should contain at least 15 chemical bottle places, where different chemical reagents could be stocked, that way a chemical synthesis reaction could be performed that requires a larger number of chemical reagents. For the analysis part of the automation system, there should be racks for holding different development solutions so that those solutions can be mixed to create a mixture used for the development process during TLC analysis. In addition, there should be racks for storing staining solutions which would be used for the staining process during TLC analysis. All different racks could be mounted on the baseplate of the automation system. The frame of the automation system mounted to the baseplate could be made out of extruded aluminium profiles, which would provide a structure to the automation system, in addition, the robots could be mounted onto rails which would extend the working areas of the robots. For the operation of linear actuators and rails, stepper motors and stepper motor controllers could be used. In order to operate the different mechanical parts in the development station, as well as the imaging station, servo motors could be used. Various devices that require to be turned on or off during the operation of the automation system, such as fans, UV and visible lights could be controlled by using relay switches. Stepper motor controllers, servos and relays could be controlled using Arduino microcontrollers. The Arduino microcontrollers, robots and heating mantle could be controlled through serial communication through universal serial bus (USB) connections using a Raspberry Pi microprocessor. The choices of

the Arduino microcontroller and the Raspberry Pi microprocessor are because of their availability, versatility and low cost.

The control software of the automation system needs to be able to control the movement of all robots including the liquid handling robot for the chemical synthesis part, the liquid handling robot for the analysis part, the robot used for TLC plate handling for the analysis part of the automation system, as well as other equipment employed in the system. A user interface needs to be created for the control software so that various functions of the automation system can be performed to operate the system. In addition, the control software needs to be able to take instructions from the web server, and send monitoring results back. The control software could be written in Python language due to its simplicity and development speed.

2.2. Design of the Automation System

The automation system requires three robots: a liquid handling robot used in the chemical synthesis part, a liquid handling robot used in the analysis part, as well as a TLC plate handler robot used for the analysis part of the automation system. A commercially available non-industrial delta robot was chosen for this system. The delta robot is called DeltaX2 from Delta X [73], which can be seen in Fig. 18A.

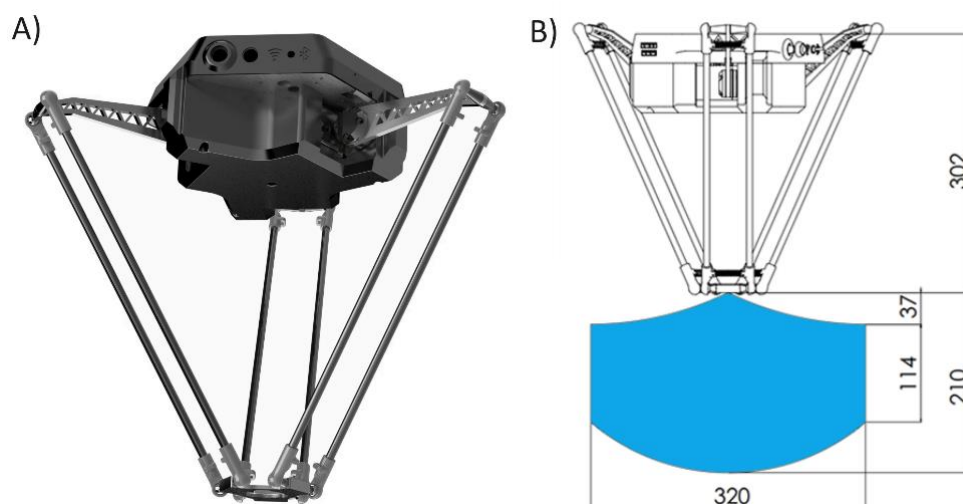


Fig. 18. Delta robot: A) DeltaX2 by DeltaX [73], B) robot dimensions in mm [74]

The delta robot uses closed-loop stepper motors, therefore the robot will provide more accuracy and repeatability when dispensing liquids as well as handling TLC plates. The price of the robot is about 641 euros at the time of writing this work without the shipping and tax costs from Vietnam. The DeltaX2 robot comes with a vacuum suction cup end effector and a vacuum pump, therefore these parts will be used for the manipulation of the TLC plates. The robot has an additional connection for the 4th-axis control, which will be used to control the stepper motors of the linear actuator that actuates the syringe plunger for the liquid dispensing robots. The robot has an accuracy of 0.15 mm, which is enough for the robot to perform applications required by the automation system. The max payload of the robot is 700 g, which is enough to manipulate the end effector for the liquid handling robots and the plate transporter robot. The mass of the robot is 4 kg, maximum speed is 0.8 m/s, maximum acceleration is 20 m/s², and the robot runs on a 12 V power supply. The robot uses a control board that has ARM Cortex-M3 processor. A control software is provided by the suppliers, which

accepts G-code commands, which are listed on the supplier's website references page [75]. The controller of the robot can be interfaced using a USB communication protocol. The inner arms, the base platform, and the motor mounts of the robot are made from aluminium plates, the outer arms are made from carbon fiber tubing, and the cover is 3D printed using plastic. The dimensions of the robot and its working area in mm are presented in Fig. 18B.

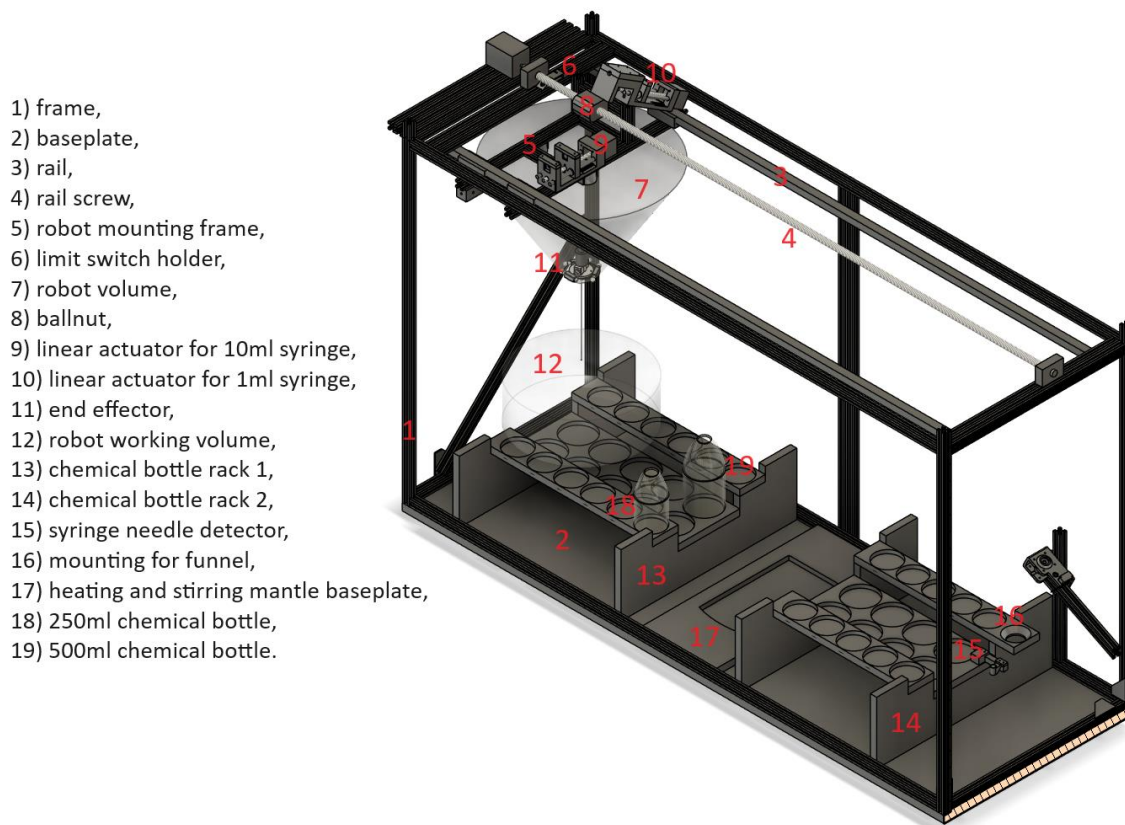


Fig. 19. Model of the synthesis part of the automation system

Different parts of the automation system were made using Autodesk Fusion 360 software [76]. The model for the synthesis part of the automation system can be seen in Fig. 19. The synthesis part of the automation system has a DeltaX2 robot mounted on a rail using a robot mounting frame (Fig. 19-5). Robot volume can be seen in Fig. 19-7. The rail is driven by a stepper motor coupled to a rail screw (Fig. 19-4), and the rail screw is coupled to the robot mounting frame using a ballnut (Fig. 19-8). The robot mounting frame is designed to be able to calibrate its home position using a limit switch mounted on the limit switch holder (Fig. 19-6) on the frame, this setup gives the robot more working volume. The working volume of the robot at a single rail position is depicted in Fig. 19-12 which was made according to the specifications of the DeltaX2 robot. The height of the robot mount was designed using the provided working area dimensions of the robot, as well as the robot dimensions from the DeltaX2 robot specifications page [77]. Two linear actuators are mounted on top of the robot. One linear actuator (Fig. 19-9) drives the plunger of a 10 ml syringe, and the other linear actuator (Fig. 19-10) drives the plunger of a 1 ml syringe. The 10 ml syringe is used for dispensing chemical liquids, while the 1 ml syringe is used to provide a constant vacuum in the tubing when the chemical liquids are being transferred, this way preventing the liquids from leaking from the end of the syringe needle. This is particularly important when dispensing volatile solvents as they tend to leak more compared to less volatile ones. The end effector is a buffer tank mounted onto the end effector's base (Fig. 19-11). The model for the buffer tank can be seen in Fig. 20A and Fig. 20B.

The buffer tank is used to store the liquid that is being dispensed. The main body of the buffer tank (Fig. 20B-1) is designed to be mounted to the end effector's body (Fig. 20B-4) by using a screw and a nut (Fig. 20B-6). The end effector body is mounted to the end effector base (Fig. 20B-5). The top of the buffer tank is an M6 hole in which a flangeless fitting of 1/16 inch to M6 is connected (Fig. 20B-2). The bottom of the buffer tank also has an M6 hole in which a Luer lock adapter M6 is connected (Fig. 20B-5). A syringe needle can be connected to the Luer lock adapter M6. The buffer tank on one end is designed to be connected via tubing to the tubing splitter and the tubing splitter is designed to be connected to the 10 ml and 1 ml syringes. A frame (Fig. 19-1) is designed to support and allow the movement of the rail (Fig. 19-3) and the robot, the frame is mounted onto a baseplate (Fig. 19-2). Two racks for storing bottles of 250 ml (Fig. 19-18) and 500 ml (Fig. 19-19) volume used for storing chemical reagents are placed on the baseplate (Fig. 19-13, Fig. 19-14). In the middle of the two bottle storage racks, a place is designed to hold a heating and stirring mantle, on which a chemical reaction flask can be placed. At one end of the chemical bottle storage rack a syringe needle detector (Fig. 19-15) is placed, which is designed to detect if the end effector syringe needle is in the right position, as well as to check if the rail movement is correct. Furthermore, at one end of the second rack, a place is designed to hold a glass funnel (Fig. 19-16), which is used to collect all waste chemicals into a designated chemical waste container for further disposal.

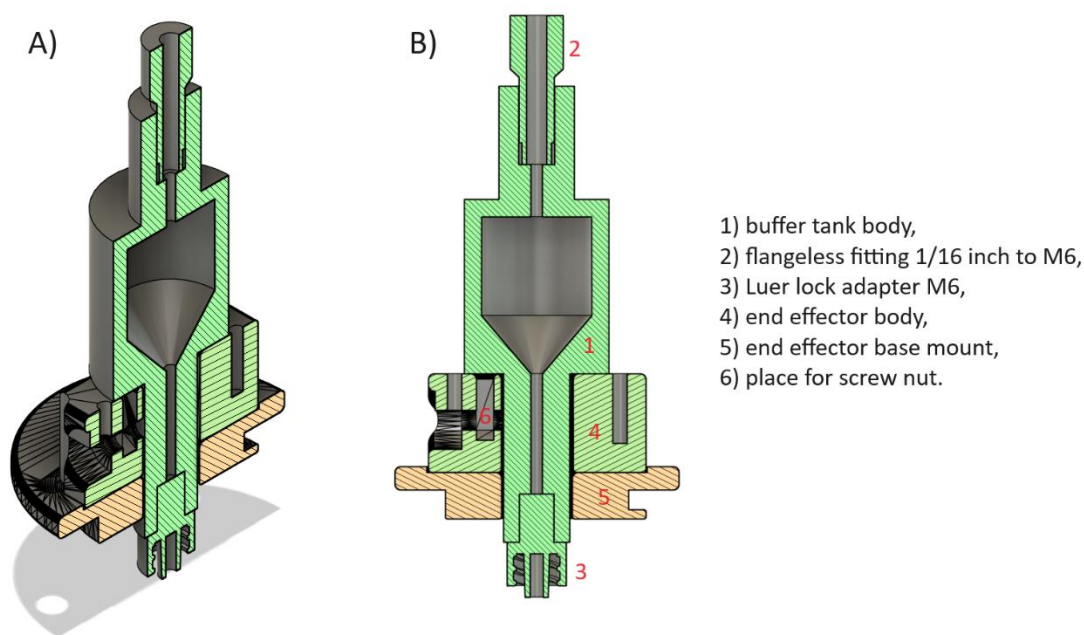


Fig. 20. Model of the liquid handling robot end effector part: A) isometric cross-section view, B) side cross-section view

The model for the analysis part of the automation system can be seen in Fig. 21. This part of the system has a frame (Fig. 21-1) mounted onto a baseplate (Fig. 21-2). The analysis part of the system includes two DeltaX2 robots mounted using mounting frames (Fig. 21-8, Fig. 21-10) on the same set of rails (Fig. 21-3) but with two different rail screws: one connected to the analysis liquid handling robot (Fig. 21-4) and the another connected to the plate handling robot mounting frame (Fig. 21-5), allowing to drive the two robots on the rails by separate stepper motors. One delta robot is a liquid handler, its volume can be seen in Fig. 21-9. A linear actuator is mounted on top of the robot which drives a 5 ml syringe plunger. The end effector of the liquid handler robot (Fig. 21-12) is a buffer tank which is connected to a 5 ml syringe on one end and to a syringe needle on the other end. The other delta robot is a plate transporter which is used to transport the TLC plates from one analysis

station to another, and its volume can be seen in Fig. 21-11. The end effector of the plate transporter robot (Fig. 21-13) is a vacuum suction cup as well as a mechanical gripper. The vacuum suction cup allows the transportation of the plates without damaging their silica gel coating and the gripper allows the grabbing of the plate vertically in order to immerse it into the staining solution used in the staining part of the TLC analysis method. A vacuum pump is mounted on top of the plate transporter robot providing the vacuum needed for the vacuum suction cup operation. A vacuum filter is mounted between the suction cup and the vacuum pump tubing, to act as a filter, which prevents the silica gel from entering from the suction cup to the vacuum pump.

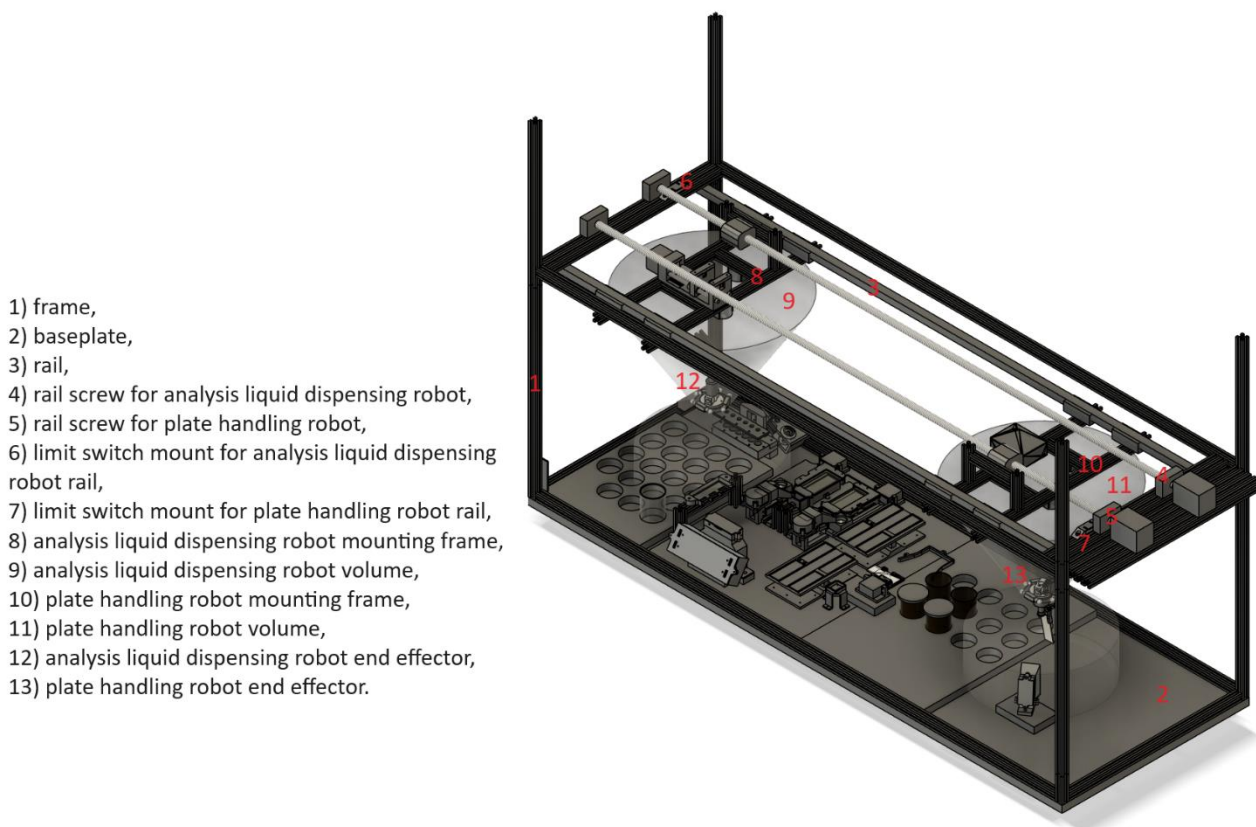


Fig. 21. Model of the analysis part of the automation system

Equipment of the analysis part of the automation system can be seen in Fig. 22. There are multiple stations for TLC method: spotting station, development station, imaging station, staining station, stained plate imaging station, and plate storage area. The plate storage area consists of two identical racks (Fig. 22-14) from where the TLC plates are picked up to be used in the analysis. The racks are designed in such a way as to allow the picking of the plates using the plate transporter vacuum suction cup. The racks have a load cell that measures when the plate handler touches the plates, allowing both: to always pick the highest plate in the plate pile without the need to define the pickup height to the control system and to check if the plate was picked correctly. Two of these plate storage racks are designed to be placed on the baseplate, allowing for storing up to 8 piles of 20 plates for a total of 160 plates. This is done so that multiple types of plates can be used for the TLC analysis. The spotting station includes a plate guider (Fig. 22-1), onto which the plate transporter places a TLC plate for the spotting step of TLC. A mounting for placing cotton pads (Fig. 22-8) is used to clean the syringe needle just before spotting during the spotting process, which is done to prevent the dripping of chemical samples onto the plate. The development station includes a baseplate (Fig. 22-5) for placing 120 ml volume chemical bottles (Fig. 22-6) used for storing chemical solvents which will be used for

development solution creation. This station also includes a horizontal development chamber (Fig. 22-9), a mixing container (Fig. 22-12), and a glass lid (Fig. 22-10) to act as a barrier so that the development liquid does not evaporate and the development process happens more quickly. The glass lid movement is controlled by a servo motor (Fig. 22-11). Additionally, a fan with airflow guiding channels (Fig. 22-13) is attached to the development chamber in order to dry out the development solution from the chamber. Drying of the development solution is needed so that on the next analysis cycle, a different development solution could be made using different analysis parameters. Since the chemicals used for making the development solution are always pure solvents, drying is enough to clean the development chamber and no further cleaning procedures are required. An imaging station contains an imaging enclosure (Fig. 22-7) which has a movable arm to move the plates into the closed chamber, where UV light is turned on and a picture is taken of the plate during the imaging step of TLC. A time-of-flight (TOF) sensor (Fig. 22-15) is mounted to the baseplate and is used to detect if the plates were picked up by the plate handling robot using the suction cup end effector. A staining station contains a vertical lift (Fig. 22-20) to allow changing the plate's position from horizontal to vertical. Staining solution containers (Fig. 22-17) can be placed on the staining bottle baseplate (Fig. 22-16). The staining 120ml containers have a lid (Fig. 22-18) that is placed on top of them, and the lid is designed to be picked up by the plate handling robot using the suction cup end effector and moved to a staining bottle lid guider (Fig. 22-19) during the staining process. Imaging after staining station includes a mount onto which a visible light and a camera are placed (Fig. 22-21). The plate is transferred to this station using the mechanical gripper of the plate transporter robot, the light is turned on and the image of the stained plate is taken, after that, the plate is dropped into a plate waste pile.

- 1) spotting station plate guider,
- 2) interface carriage bottle rack,
- 3) interface carriage,
- 4) interface carriage mounting to frame,
- 5) development station container baseplate,
- 6) 120ml bottles for development solvents,
- 7) imaging station imaging enclosure,
- 8) cotton pads mount,
- 9) horizontal development chamber,
- 10) development chamber glass lid,
- 11) development chamber glass lifting control servo,
- 12) development solution mixing container,
- 13) development station drying fan and airflow guider,
- 14) plate storage rack,
- 15) time of flight (TOF) plate detector,
- 16) staining bottles baseplate,
- 17) 120ml bottles for staining solutions,
- 18) staining bottle lid,
- 19) staining bottle lid guider,
- 20) vertical lift,
- 21) imaging after staining imaging enclosure.

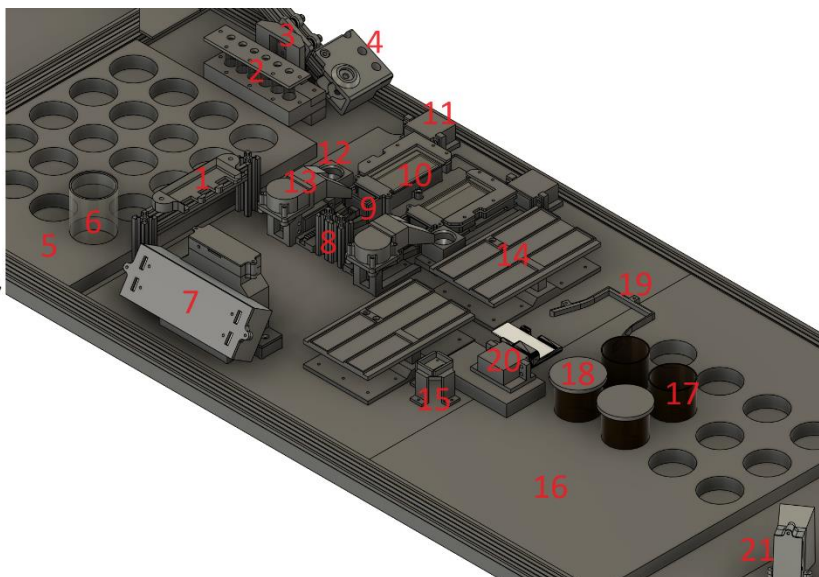


Fig. 22. Model of the different stations for the analysis part of the automation system

For the chemical sample transfer between the synthesis and analysis parts of the automation system, an interface was designed. The interface model can be seen in Fig. 23, it contains a carriage (Fig. 22-3, Fig. 23-1) which allows the movement of a rack (Fig. 22-2, Fig. 23-2) containing six 4 ml chemical bottles (Fig. 23-4) between the synthesis and analysis parts of the automation system. A rack is mounted to the carriage by using a mounting part, which can be seen in Fig. 23-5. On top of the rack, a guiding plate (Fig. 23-3) is mounted to provide the guiding of the syringe needles of both the

synthesis liquid handler end effector syringe needle as well as the analysis liquid handler end effector syringe needle into the 4 ml chemical bottles, as the entrance of the chemical bottle is small. The carriage is designed to move on a 20x20 aluminium frame using a belt that is driven by a stepper motor. The aluminium frame on which the carriage moves is mounted to the frame of the automation system using mounting parts (Fig. 22-4, Fig. 23-7, Fig. 23-8) at each end of the carriage frame. The home position of the carriage is set by using a limit switch on one side of the aluminium frame (Fig. 23-6).

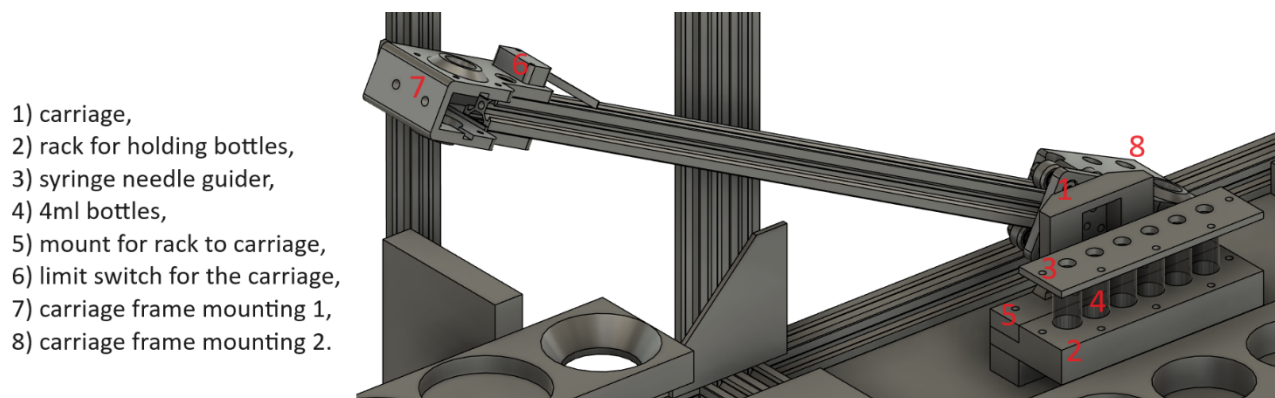


Fig. 23. Model of the interface between the synthesis and analysis parts of the automation system

The whole automation system combines a synthesis part, a TLC analysis part and an interface between the two parts. The model for the whole automation system can be seen in Fig. 24. The main automation system dimensions in mm can be seen in Fig. 48 in Appendix 1. The synthesis and analysis baseplate lengths are 1500 mm each. The total length of the automation system is 3200 mm due to the frame extending beyond the baseplate length for both synthesis and analysis parts in order to mount the stepper motor that is coupled to the rail screws. The width of the baseplate for both synthesis and analysis parts is 500 mm. The largest height of the automation system is 1072.42 mm from the baseplate bottom to the top point of the 2nd linear actuator (without the syringe attached) mounted onto the synthesis liquid dispensing robot mounting frame. The working volumes of the robots are also presented in Fig. 48 in Appendix 1, and have a diameter of 320 mm and height of 114 mm to the upward concave part of the volume. The total height of the working volume of the robot is 209.95 mm. The syringe needle length of the synthesis liquid dispensing robot end effector is 200 mm and the syringe needle length of the analysis liquid dispensing robot end effector is 50 mm.

After the automation system was designed, all required custom parts were manufactured using extrusion-based, and stereolithography (SLA) 3-dimensional (3D) printers, as well as computer numerical control (CNC) machining. Ender-5 Plus 3D printer by Creality [78] was used for the extrusion printing using PLA material. Photon by Anycubic [79] printer was used for stereolithography 3D printing using UV-curing resin. CNC machining was done using X9-2200 CNC Desktop Engraving Machine by OMIO CNC [80] using plywood, PP, poly(oxymethylene) (POM), and phenolic paper laminate materials. After all required parts were made and bought, the automation system was assembled, for which the process is described in Chapter 3.1. A control software was written for the automation system in Python language [81] using Visual Studio software [82] and Arduino language [83] using Arduino IDE [84]. Control software process flow diagrams were prepared using Draw.io software [85] and the software working principles are presented in Chapter 3.2. The working principles of the automation system are presented in Chapter 3.3.

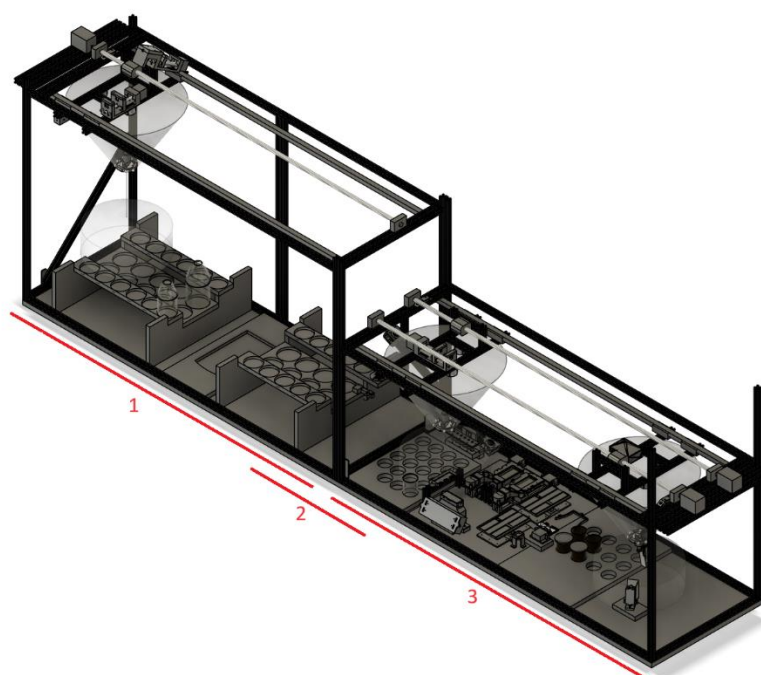


Fig. 24. Model of the automation system: 1) synthesis part, 2) interface between synthesis and analysis parts, 3) analysis part

2.3. Automation System Testing Methodology

In order to evaluate the developed automation system multiple tests were designed. Two ISO standards could be applied to the liquid handling robots used in the automation system: ISO 8655 and ISO 23783. ISO 8655 standard document describes the requirements for piston-operated volumetric apparatuses (POVA) [86]. In this work liquid dispensing in the automation system is achieved by using POVA called laboratory syringe. ISO 8655-9:2020 document provides the range of values of the maximum permissible systematic errors as well as the maximum permissible random errors for which the laboratory syringes need to be in range to meet the ISO standard. The type of pipettes used by the liquid handling robots is called single-channel air displacement, since only a single pipetting process can be performed at one time, as well as the syringes are connected to the needles by tubing which is filled with air [87], ISO 8655-6:2022 document describes the procedure for evaluating such pipettes [88]. However, since the syringes are part of the liquid handling robots, and in turn, the liquid handling robots are part of an automation system, the ISO 23783 standard methodology was used for evaluation of the syringes. Document ISO 23783-2:2022 describes the requirements for an automated liquid handling system (ALHS). In this case, the type of ALHS was piston operated using air displacement, since the syringe has a plunger and the syringe is connected to the needle using tubing which is filled with air [89]. ISO 23783-2:2022 describes the methodology for testing the volumetric dispensing capabilities of the liquid handling system using a gravimetric testing procedure for single channel pipettes [90]. In other research works, this type of testing procedure was used for determining the accuracy of ALHS systems used for chemical synthesis. One research group employed a commercially available chemical synthesis automation system by Chemspeed: hexane was transferred from one container into another that was weighted on a scale. After the transfer of hexane, the container was weighed again and the transferred volume was calculated since the density of hexane was known. For six liquid dispenses of the same volume that were done one after another, a 5% systematic error was calculated for the Chemspeed automation system [91]. In another work, for

the already mentioned ADoS system (Fig. 14), the accuracy of the dispensing syringe was tested in order to determine if the syringe met the ISO 8655 standard. In that work, the authors found that the syringe did meet the standard except for low values of dispensed liquid of 50 µl, which the authors attribute to the limited resolution of the stepper motor driving the linear actuator onto which the syringe was mounted [65].

The methodology for two tests called Test 1 and Test 2 which are for testing the liquid handling capabilities of both synthesis and analysis liquid handling robots of the automation system were prepared according to methodology provided in ISO 8655 and ISO 23783 standards. For Test 1 the liquid handling robot in the synthesis part of the automation system was used and for Test 2 the liquid handling robot in the analysis part of the automation system was used. As per instructions of ISO 8655, both tests were performed at 100%, 50% and 10% of nominal pipetting volume, which is 6 ml, 3 ml and 0.6 ml for Test 1 and 3 ml, 1.5 ml and 0.3 ml for Test 2. The nominal volumes of both liquid handling robots are lower than the syringe nominal volumes due to the pressure loss in the tubing, connectors, buffer tank and the tubing splitter. As per instructions of ISO 23783, the automation system was instructed to bring a set amount of volume of distilled water from a liquid container to a weighing vessel, the vessel was weighed using precision scales of 0.0001 g accuracy. For each set amount of volume, 20 measurements were performed. The density value of the water was calculated using Tanaka equation for the density of pure water [90] (Eq. 1). The value for the temperature of water (t_w) was taken to be 25°C. The weights obtained using the scales were converted to volumes using Eq. 2, and the average volume values were calculated using Eq. 3. The systematic errors in µl were calculated using Eq. 4 and the systematic errors in µl were calculated using Eq. 5. The results of the Test 1 and Test 2 are presented in Table 1 and Table 2.

$$\rho_w = a_5 \left[1 - \frac{(t_w + a_1)^2 (t_w + a_2)}{a_3 (t_w + a_4)} \right], \quad (1)$$

where: ρ_w is the density of water (g/ml); t_w is the temperature of water (°C); $a_1 = -3.98$ (°C); $a_2 = 301.797$ (°C); $a_3 = 522528.9$ ((°C)²); $a_4 = 69.34881$ (°C); $a_5 = 0.99$ (g/ml).

$$V_i = \frac{m_i}{\rho_w}, \quad (2)$$

where: V_i is the volume of the water for the i-th measurement (ml); m_i is the mass of the water of the i-th measurement (g).

$$V_{avg} = \frac{1}{20} \sum_{i=1}^{20} V_i, \quad (3)$$

where: V_{avg} is the average volume of water (ml).

$$e_s = 1000(V_{avg} - V_{set}), \quad (4)$$

where: e_s is the systematic error (µl); V_{set} is the set amount of volume to be delivered by the automation system.

$$s_r = 1000 \sqrt{\frac{\sum_{i=1}^{20} (V_i - V_{avg})^2}{n-1}}, \quad (5)$$

where: s_r is the random error (µl).

There are no ISO standards for an automation system performing chemical synthesis and TLC spotting, development, imaging, staining and imaging after staining processes, therefore custom methodologies were used for evaluating the developed system. Methodologies for five tests called Test 3, Test 4, Test 5, Test 6 and Test 7 were prepared in order to test the capabilities of the system. Test 3 and Test 4 were designed to evaluate the capability of the automation system to produce repeatable analysis results using TLC. In those tests, the same chemical sample, in which no chemical synthesis process was taking place, was analysed and the analytical data from multiple analysis cycles was compared. The expected result was that the images of the developed TLC plates were similar to each other since the chemical sample and the analysis parameters were the same. On the other hand, Test 5, Test 6 and Test 7 were designed to test the capability of the automation system perform chemical synthesis and to monitor a synthesis reaction using TLC. During these tests, a synthesis reaction took place in the synthesis part of the system and multiple analysis cycles were performed in the analysis part of the system taking chemical samples from the synthesis part via the interface in order to obtain data on how the chemical reaction evolves over time. The expected results of these tests were that the images of the developed plates have different compound separations of spotted chemical samples which correspond to the formation of intermediate or final products as well as the consumption of reagents during the chemical synthesis reactions.

For Test 3, 1 molar concentration (M) of benzaldehyde in methanol was manually mixed with 1M of sodium borohydride using 1:1 volume ratio, this chemical reaction can be seen in Fig. 25. The synthesis reaction was stirred at 200 rotations per minute (RPM) at room temperature for 24 hours. The reaction mixture was then transferred to the automation system chemical reaction flask in the synthesis part. The automation system was set to perform an analysis process, analysis process parameters were set to use a development mixture of hexane/ethyl acetate 90%:10% by volume for analysis of the chemical sample 19 times every 40 minutes spotting one spot per plate of the transferred chemical sample from the reaction flask to the analysis part of the system via the interface.

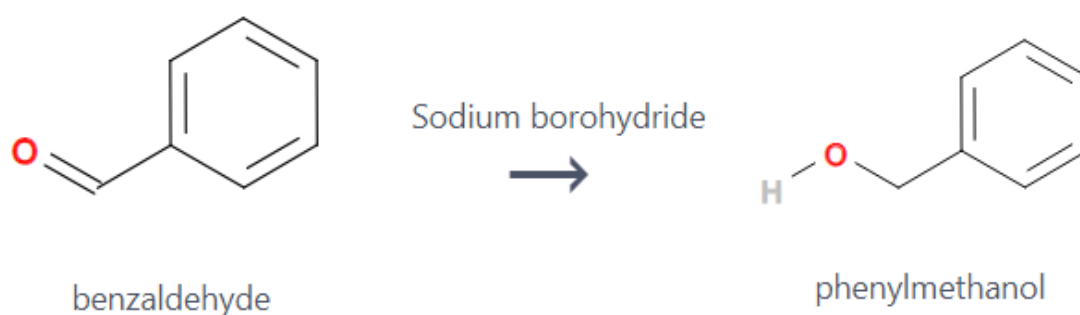


Fig. 25. Chemical reaction of Test 3 and Test 6

For Test 4, 1M of (1E,4E)-1,5-diphenylpenta-1,4-dien-3-one in acetone was manually mixed with 0.5M of (1E)-2,6-dimethylhepta-1,5-dien-1-yl methanesulfonate using 1:1 volume ratio, this chemical reaction can be seen in Fig. 26. The reaction mixture was stirred at 200 RPM at room temperature for 24 hours. The reaction mixture was then transferred to the automation system chemical reaction flask in the synthesis part. Analysis process was set to be performed by the automation system, analysis process parameters were set to use a development mixture of 1-butanol/1-propanol 80%:20% by volume for analysis of the chemical sample 30 times every 40

minutes spotting one spot per plate of the transferred chemical sample from the reaction flask to the analysis part of the system via the interface.

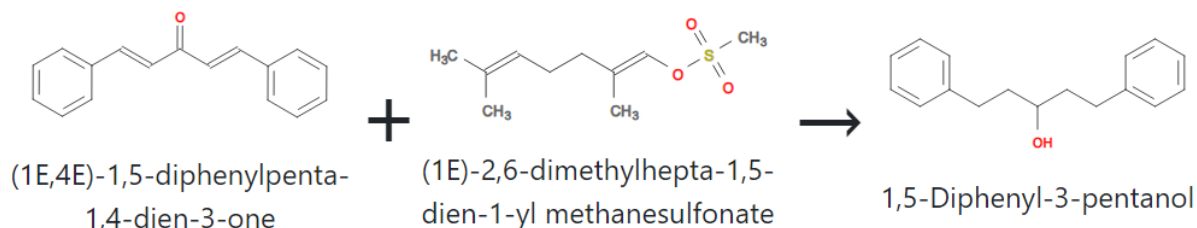


Fig. 26. Chemical reaction of Test 4

For Test 3 and Test 4, the retention factors (R_f) for each compound of each plate were calculated using Eq. 6. The retention factor allowed to quantify the separation lengths of different compounds of the spotted chemical sample on the plate that occurred during the development process of TLC analysis. Retention factors were then used for the calculations of coefficients of variation (CV_{R_f}) (Eq. 7) in order to quantify the repeatability of the automation system for producing TLC analysis data. Coefficients of variation were calculated for every retention factor of each unique compound for both Test 3 and Test 4.

$$R_{f_{ij}} = \frac{a_{ij}}{b_i}, \quad (6)$$

where: $R_{f_{ij}}$ is the retention factor of the j-th compound and i-th plate; a_{ij} is the length from the base line where the chemical sample was spotted onto the TLC plate to the compound place after the development of the plate for the j-th compound and i-th plate (pixels); b_i is the length from the base line to the solvent line to which the solvent travelled up during the development process of the i-th plate (pixels).

$$CV_{R_{f_j}} = 100 \sqrt{\frac{\frac{\sum_{i=1}^k \left(R_{f_{ij}} - \frac{1}{k} \sum_{i=1}^k R_{f_{ij}} \right)^2}{k-1}}{\frac{1}{k} \sum_{i=1}^k R_{f_{ij}}}}, \quad (7)$$

where: $CV_{R_{f_j}}$ is the percent coefficient of variation of the retention factors for the j-th compound (%); k in the number of plates acquired during Test 3 and Test 4.

For Test 5, reagents of 1M 250 ml benzaldehyde in ethanol, 1M 250 ml of 1-phenylethan-1-one in ethanol and 1M 250 ml of sodium hydroxide in ethanol were added to chemical flasks in the synthesis part of the automation system. The automation system was set to perform a synthesis process by adding 20 ml of benzaldehyde, 20 ml of 1-phenylethan-1-one and 4 ml of sodium hydroxide to the reaction flask. The stirring of the reaction flask was set to 200 RPM and the reaction took place at room temperature. The synthesis reaction that took place in the automation system can be seen in Fig. 27. The automation system was also set to perform analysis after all the reagents were added to the reaction flask. The analysis was set to be performed using a development mixture of hexane/ethyl acetate 95%:5% by volume for 36 times every 60 minutes spotting one spot per plate of the transferred chemical sample from the reaction flask to the analysis part of the system via the interface.

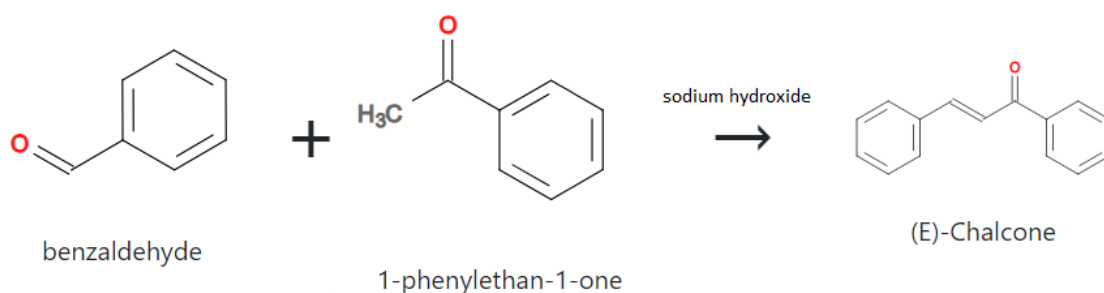


Fig. 27. Chemical reaction of Test 5

For Test 6, reagents of 1M 250 ml benzaldehyde in methanol, and 1M 250 ml of sodium borohydride in methanol were added to chemical flasks in the synthesis part of the automation system. The automation system was set to perform a synthesis process by adding 15 ml of benzaldehyde and 10 ml of sodium borohydride to the reaction flask. The stirring of the reaction flask was set to 200 RPM and the reaction took place at room temperature. The synthesis reaction that took place in the automation system is the same as for the Test 3 and can be seen in Fig. 25. The automation system was also set to perform analysis after all the reagents were added to the reaction flask. The analysis was set to be performed using a development mixture of hexane/ethyl acetate 90%:10% by volume for 14 times every 60 minutes and spotting 2 spots per plate: the first spot of benzaldehyde reagent from the reagent chemical flask and the second spot of the chemical sample taken from the reaction flask, all transferred to the analysis part of the system via the interface.

For test 7, reagents of 1M 250 ml benzaldehyde in methanol and 1M 250 ml of propanedinitrile in methanol were added to chemical flasks in the synthesis part of the automation system. The automation system was set to perform a synthesis process by adding 25 ml of benzaldehyde and 25 ml of propanedinitrile. The stirring of the reaction flask was set to 200 RPM and the reaction took place at room temperature. The synthesis reaction that took place in the automation system can be seen in Fig. 28. The automation system was also set to perform analysis after all the reagents were added to the reaction flask. The analysis was set to be performed using a development mixture of hexane/ethyl acetate 80%:20% by volume for 19 times every 60 minutes and spotting 3 spots per plate: first spot of benzaldehyde reagent from the reagent chemical flask, the second spot of propanedinitrile reagent from the reagent chemical flask, and the third spot of the chemical sample taken from the reaction flask, all transferred to the analysis part of the system via the interface. Additionally, the automation system was set to perform staining and imaging after staining processes using potassium permanganate staining solution every 360 minutes.

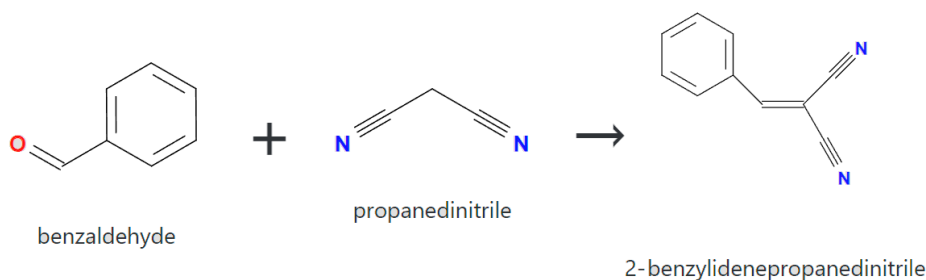


Fig. 28. Chemical reaction of Test 7

For Test 3, Test 4, Test 5, Test 6, and Test 7 custom-made TLC plates were used by combining a normal phase silica gel, a binder agent, and a fluorescent indicator and coated onto glass plates of 1 mm thickness, 25.5 mm width and 75 mm length. For Test 3, Test 4, Test 5, Test 6 and Test 7 1 ml of chemical sample for each required spot was transferred from the synthesis part to the analysis part of the automation system using the interface. Images for the chemical reactions in Fig. 25, Fig. 26, Fig. 27, Fig. 28 were made using the web page from which the automation system was controlled. All testing results are presented in Chapter 3.4.

Test 5, Test 6 and Test 7 tested the capability of the automation system to perform chemical synthesis that was evaluated by observing the appearance, disappearance, brightness and size increase and decrease of compounds on the obtained TLC plate images. However, a deeper evaluation of the capability of the automation system to perform chemical synthesis processes could be done by using a quantitative analysis method of the chemicals throughout the process of the synthesis reaction using an HPLC or an MS analytical device. Lastly, research in this work was not performed on humans or animals, therefore there are no ethical concerns regarding tests done by using the automation system. Chemical reagents, products, cleaning solvents, development solvents and staining solutions obtained and used during Test 1, Test 2, Test 3, Test 4, Test 5, Test 6 and Test 7 were disposed according to the disposal recommendations of each chemical.

2.4. Chapter Summary

The design process for the fully automated system combining both chemical synthesis and analysis, was split into multiple parts. One part was the design of the automation system for the chemical synthesis process, the second part was the design of the automation system for the TLC analysis process, and the last part was the design of the interface between the chemical synthesis and analysis parts of the automation systems. For the first part of the design, the working principles and design choices of existing commercially available as well as non-commercially available automation systems for chemical synthesis were explored. For the second part of the design, the working principles and design choices of existing commercially available as well as non-commercially available automation systems for TLC analysis process were explored. After that, the functional requirements, design choices and restrictions were raised for the automation system that combines both chemical synthesis and TLC analysis processes which included: two liquid dispensing systems using a plunger-operated air displacement dispensing method, TLC plate handling transporter, heating mantle for synthesis reaction flask, TLC spotting, development, imaging, staining and imaging after staining stations, an interface between the synthesis and analysis parts of the system, no use of industrial robots to keep the cost lower than 8000 euro for the parts of the system. Automation system was modelled in Fusion 360 software and parts were manufactured using extrusion and SLA 3D printing, and CNC machining. Two ISO standards were considered for designing the methodology for the automation system tests: ISO 8655 for liquid handling using syringes and ISO 23783 for using liquid handling systems. Methodologies of Test 1 and Test 2 were described which involved the acquisition of data using gravimetry method for systematic and random error calculations. Methodologies for Test 3 and Test 4 were described which involved using analysis process of the automation system for TLC analysis of a chemical sample that does no longer undergo a chemical reaction in order to obtain data for evaluation of the repeatability of the automation system to produce TLC analysis results. Formulas for calculating systematic and random errors, retention factors and coefficient of variation of the retention factors were provided. Methodologies for Test 4, Test 5, and Test 6 were described which involved using both synthesis and analysis processes of the

automation system to obtain data used for evaluation of the capabilities of the system to perform chemical synthesis and monitor an ongoing synthesis reaction.

3. Automation System Setup and Testing

In this chapter, the assembly of the synthesis, analysis and interface parts of the automation system is presented. The working principles of the written control software of the automation system are provided, the user interfaces for the control software are described. The working principles of synthesis, heating and stirring, and analysis processes of the automation system are described. Lastly, the testing results performed using the automation system, discussions and comparisons are given.

3.1. Automation System Assembly

Parts for the synthesis section of the automation system were manufactured using 3D printing, CNC machining, and other required parts were bought and assembled. The assembled synthesis part of the automation system can be seen in Fig. 29.

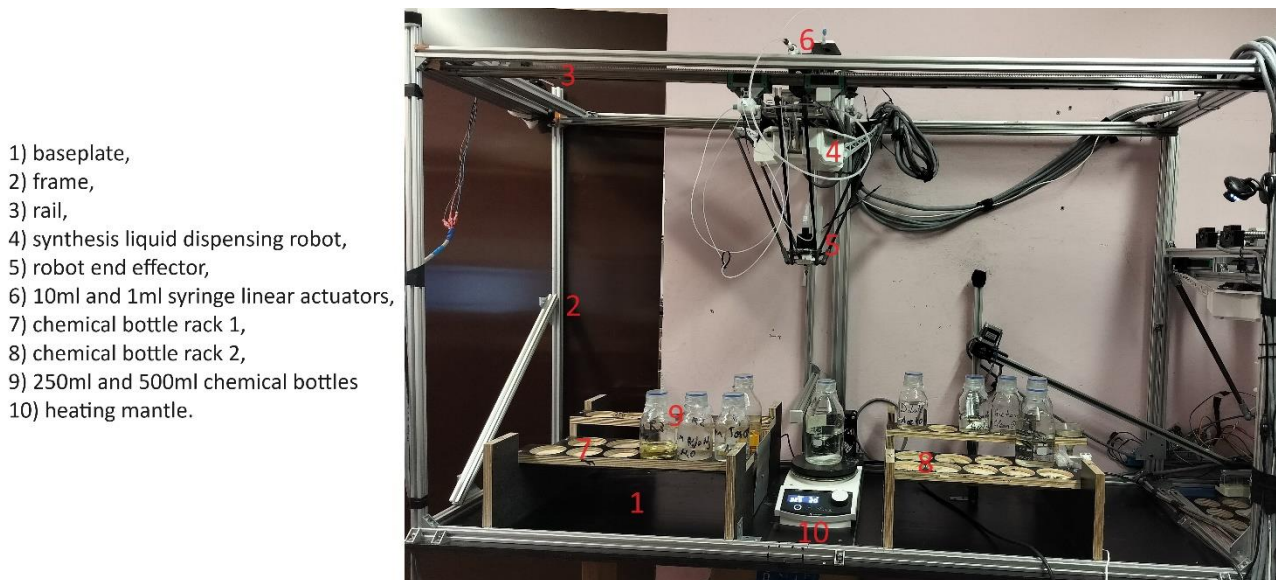


Fig. 29. Assembled synthesis part of the automation system

The frame (Fig. 29-2) was made from aluminum 20x20 mm V-slot profiles and connected using 20x20 corner brackets with M5 screws and M5 T-Nuts. The frame was mounted to a wooden 18 mm thickness baseplate (Fig. 29-1), which was cut to size manually. The rail (Fig. 29-3) was mounted onto the frame, an HGR20 linear rail was used with a SFU1605 ball screw rod of 1500 mm in length, the screw was coupled to the robot mounting frame using a ballnut. The rail screw was mounted using a 6.35x10 mm coupling to a JK57HS76-2804 stepper motor, which was connected to a DM556 stepper motor controller set to provide 2.1A peak current when active, and half current during stationary periods, the stepper motor controller was connected to an Arduino Mega microcontroller. A limit switch mount was 3D printed, and mounted with a limit switch onto the automation system frame. The mounting frame for the DeltaX2 robot of the liquid dispenser (Fig. 29-4) was made and mounted onto the frame. The mainboard for the DeltaX2 robot was separated from the rest of the robot, additional wires were soldered to the connections of the limit switches and stepper motors of the robot to the control board. This was done in order to keep the control board of the robot separate so that the chemicals used during the chemical synthesis processes would not be able to corrode the main board of the robot. The robot was then mounted to the robot mounting frame using 3D printed spacers in between the frame and the robot to allow the movement of the delta robot arms, as well as providing space for the connections of the robot. Two linear actuators (Fig. 29-6) were constructed

using mounting parts that were 3D printed, 8 mm stainless steel rods as linear actuator guides, 8 mm lead screw, lead screw nut, and a 5x8x25 mm coupling between the screw and the 17HS19-2004S1 stepper motor. Stepper motors were connected to DM556 stepper motor controllers set to provide 1.4A of peak current at all times. One stepper motor controller that controlled the stepper motor for the linear actuator containing a 10 ml syringe was connected to the DeltaX2 control board's 4th-axis connection, and the other stepper motor controller was connected to an Arduino Mega microcontroller. Hamilton 1010 TLL gas-tight glass syringe was mounted onto the 10 ml linear actuator, and ILS RQ Replacement Syringe 1 ml gas-tight glass syringe was mounted onto the 1 ml linear actuator. The syringes were connected to a 1.58 mm outer diameter (OD) and 0.5 mm inner diameter (ID) poly(1,1,2,2-tetrafluoroethylene) (PTFE) tubing using a female Luer to 1/4-28 female connector and a flangeless fitting of 1/16 inch. The tubing was then connected to a tubing splitter using the flangeless fitting screwed into an M6 hole. The tubing splitter was made using POM material by manually machining the inner flow channels and tapping the required holes. The tubing splitter was connected via PTFE tubing to an M6 hole in the buffer tank using a flangeless fitting and an adapter of 1/16 flange to M6. The buffer tank, mounted to the end effector of the robot (Fig. 29-5) was made by a third party using the author's design from POM material. The other end of the buffer tank was connected using a Luer lock adapter screwed into an M6 hole. A 14-gauge 20 cm length stainless steel syringe needle was connected to the Luer lock. Two racks (Fig. 29-7, Fig. 29-8) for holding the 250 ml and 500 ml glass chemical bottles (Fig. 29-9) were CNC machined out of plywood, assembled, and connected to the baseplate. A glass funnel was added to the second rack, and the end of the funnel was connected via silicon tubing to a 1-liter glass chemical bottle which functioned as a waste container for the chemicals used during the chemical synthesis processes. The parts for the syringe needle detector were 3D printed, the detector was assembled using a 5 kg load cell and mounted on the second rack. The load cell was connected to an HX711 force sensor interface module, which was connected to an Arduino Mega microcontroller. The syringe needle detector allowed the detection if the syringe needle was not bent and if the rail position was correct. A baseplate for mounting the heating mantle (Fig. 29-10) was CNC machined using plywood, a Heidolph Hei-Connect heating and magnetic stirrer was added to the baseplate, and a chemical reaction flask of 500 ml was added on top of the heating mantle.

Parts for the analysis section of the automation system were manufactured using 3D printing, CNC machining, and other required parts were bought and assembled. The zoomed-in images for the analysis liquid dispensing robot can be seen in Fig. 30A, plate handler robot can be seen in Fig. 30B and the zoomed-in view of the plate handler robot end effector can be seen in Fig. 30C. The assembled analysis part of the automation system can be seen in Fig. 31. The different stations of the analysis part of the automation system are the spotting station, development station, imaging station, staining and imaging after staining stations, and plate storage racks (Fig. 31-12). For the analysis part of the automation system, the frame and the baseplate were constructed from the same materials as for the synthesis part of the automation system. One set of rails was mounted onto the frame, but two screws were added and each were coupled to one of the two DeltaX2 robots in the analysis part of the automation system. For the first delta robot, which was used for the liquid dispensing (Fig. 30A), mounting was made and connected to the rails using the same type of spacers as in the synthesis part of the automation system. Using a ballnut (Fig. 31-3) the rail screw was coupled to the mounting frame on which the analysis liquid handling robot was mounted. Two JK57HS76-2804 stepper motors were added for the driving of the screws of the rail, which were connected to two DM556 stepper motor controllers and were set to provide 2.1A peak current when active, and half current during

stationary periods. Like the synthesis liquid dispensing robot, the control board for the analysis liquid dispensing robot was separated from the rest of the robot and connection extensions were added.

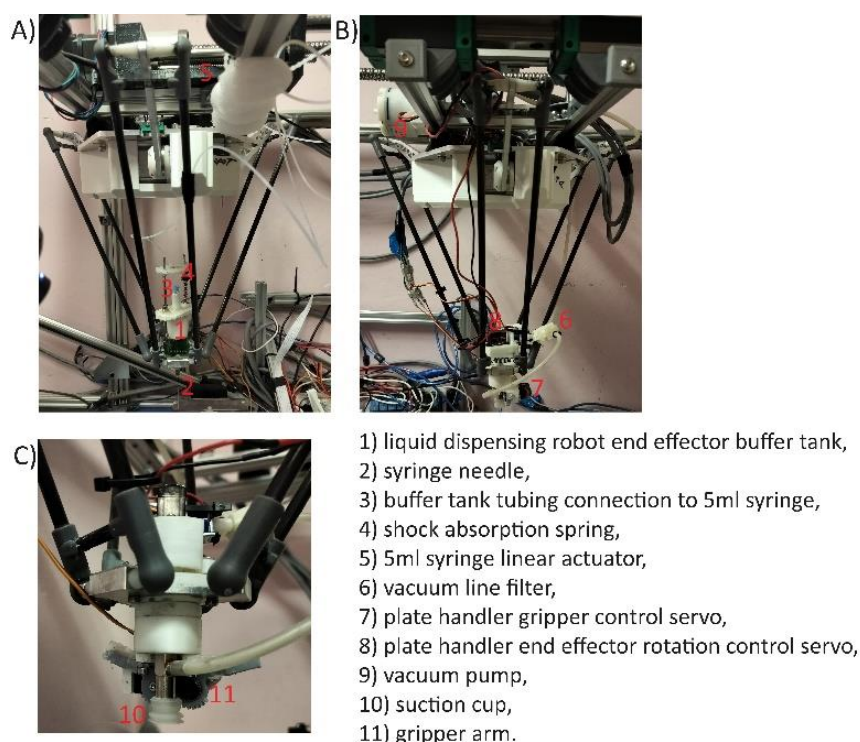


Fig. 30. Assembled robots of the analysis part of the automation system: A) liquid dispensing robot, B) plate handler robot, C) plate handler robot end effector

A 5 ml linear actuator (Fig. 30A-5, Fig. 31-2) was made using the same materials as for the synthesis part of the automation system. The same type of stepper motor and stepper motor controller was used for connecting the linear actuator to the DeltaX2 robot control board's 4th-axis connections. A Hamilton 1050 TLL gas-tight glass syringe was mounted onto the linear actuator. The syringe was connected to a connector of female Luer to a 1/4-28 female, then to a flangeless fitting of 1/16 inch, and via 1.58 mm OD x 0.5 mm ID PTFE tubing to a flangeless fitting of 1/16 inch (Fig. 30A-3) which was screwed into an M6 hole into the buffer tank (Fig. 30A-1). The manufacturing of the buffer tank was outsourced and made using the author's design from POM material, the buffer tank was mounted to the end effector (Fig. 31-1) of the analysis liquid dispensing robot. The end of the buffer tank was connected via an M6 hole to a Luer lock adapter and then to a syringe of 22-gauge and 50 mm length (Fig. 30A-2). The end of the syringe needle was machined to achieve a blunt tip, this way spotting onto the TLC plates could be performed using the syringe. The buffer tank mounted in the analysis liquid dispensing robot end effector base was not screwed in, therefore the buffer tank could move in one direction. Two 3 mm steel rods (Fig. 30A-4) were mounted to the end effector body and the buffer tank was mounted to the 3 mm steel tubes using LM3UU linear bearings. A spring was added to one of the 3 mm steel tubes so that the buffer tank had shock-absorbing properties when spotting the chemical sample onto the plate. Shock absorption minimized the damage done to the silica gel coating of the plate, and in turn, multiple spottings could be performed to the same place on the plate to increase the concentration of the spotted sample without destroying the silica gel. Another mounting frame was made for the other DeltaX2 robot which was used as a TLC plate transporter (Fig. 30B) and mounted to the rail using the same 3D printed spacers that were used to mount the previous two DeltaX2 robots to the rail. The second rail screw was coupled to the plate handling robot

mounting frame using a ballnut (Fig. 31-6). Similarly to the previous two robots, the control board was separated from the rest of the robot, and extension cables for the connections were added. The end effector of the plate transporter robot (Fig. 30C) was mounted which was a vacuum suction cup (Fig. 30C-10) that was obtained from the same vendors from where the DeltaX2 robots were bought. The connections for the vacuum suction cup end effector were connected to the DeltaX2 robot control board. A silica gel filter (Fig. 30B-6) was made using SLA 3D printing, assembled, and connected between the vacuum lines coming from the suction cup to the vacuum pump (Fig. 31-5), that was mounted on top of the robot. Mechanical gripper parts were 3D printed, and the gripper was assembled using a Tower pro MG90S servo (Fig. 30B-7) for controlling the gripper arm (Fig. 30C-11). Furthermore, an additional Tower pro MG90S servo (Fig. 30B-8) was attached to control the plate handling robot end effector rotation so that the plates picked up by the suction cup end effector could be rotated in order to be placed them in the different stations. Both servos were connected to a PCA9685 servo control board that was connected to an Arduino Uno microcontroller.

- 1) liquid handler end effector,
- 2) 5ml syringe linear actuator,
- 3) ballnut 1,
- 4) plate handler end effector,
- 5) vacuum pump,
- 6) ballnut 2,
- 7) development station container baseplate,
- 8) spotting station plate guider,
- 9) horizontal development chamber,
- 10) cotton pads for syringe needle cleaning,
- 11) imaging station,
- 12) plate storage racks,
- 13) TOF plate detector,
- 14) vertical lift,
- 15) staining bottle lid guider,
- 16) staining station containers,
- 17) plate waste area,
- 18) imaging after staining station.

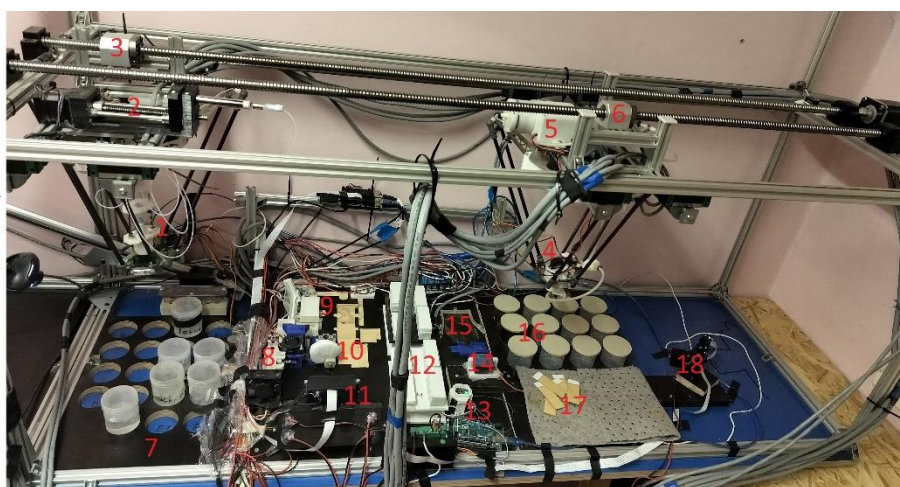


Fig. 31. Assembled analysis part of the automation system

Different stations for the analysis part of the automation system were 3D printed, CNC machined, bought, and assembled, the assembled picture of the stations can be seen in Fig. 31. For the spotting station, a plate guider (Fig. 31-8) was CNC machined using PP material and mounted to the baseplate, a 12 V direct current brushless fan was mounted on top of the guider. The fan was connected to an 8-channel relay module, which was connected to an Arduino Mega microcontroller. A mounting for the cotton pads (Fig. 31-10) used for cleaning the syringe needle just before spotting, was made using 20x20 aluminum extrusion frames, and the cotton pads were attached using a clamp made from PP material using CNC machining. The parts for the plate storage racks (Fig. 31-12) for the TLC plates were 3D printed and two racks were assembled by adding 5 kg load cells which were connected to an HX711 interface, the interfaces were connected to an Arduino Mega microcontroller. The loadcells allowed the measurement that the plate handler robot exerted on the placed plates, which enabled the control system to determine when the vacuum suction cup touched the topmost plate in the plate pile without needing to specify the height of the plate pile to the control software and without needing to keep track of the changing plate pile height throughout the analysis processes. In addition, the loadcells allowed the checking if the plate was successfully picked up from the plate pile.

For the development station of the analysis part of the automation system, firstly, a rack for placing 120 ml volume containers (Fig. 31-7) was CNC machined using plywood, and the rack was mounted onto the baseplate. The rack contained 19 places where 120 ml containers could be placed. Secondly,

the parts for the horizontal TLC development chamber (Fig. 31-9), and the development solution mixing container were CNC machined using POM material as well as 3D printed, the parts were assembled with an MG90S micro servo motor for controlling the horizontal development chamber and a COM-Motor02 servo motor from JOY-IT for controlling the glass lid that covers the plate during the development process. Both servo motors were connected to a PCA9685 servo control board, the board was connected to an Arduino Uno microcontroller. A 12 V 40x40x20 mm direct current brushless fan was added together with a 3D printed airflow guiding channel for the drying of the development and development solution mixing chambers after the development process. The fan was connected to an 8-channel relay module which was connected to an Arduino Mega microcontroller. For the imaging station (Fig. 31-11), the parts were 3D printed and assembled. An MG90S servo was added for the control of the imaging station's imaging enclosure arm. The servo was connected to a PCA9685 servo control board, the board was connected to an Arduino Uno microcontroller. An alternating current UV 254 nm lamp was mounted into the imaging station, the lamp was connected to a relay that was connected to an Arduino Mega microcontroller. A Raspberry Pi Camera Module V2 was mounted inside the imaging station, the camera was connected via ribbon cable directly to the Raspberry Pi 4 microprocessor. For the staining station of the analysis part of the automation system, firstly, a rack for placing 12 120 ml bottles (Fig. 31-16) was CNC machined using plywood, and the rack was mounted to the baseplate. Secondly, the lids for the 120 ml bottles were CNC machined using PP material and placed on top of the 120 ml bottles. The lids were used so that the plate transporter robot could remove the lids when the staining process was needed to be performed and add the lids onto the 120 ml bottles when the staining process was finished, that way the staining solutions inside the 120 ml evaporated more slowly. Thirdly, a lid guider (Fig. 31-15) part for the lids was made using SLA 3D printer and mounted onto the baseplate, the guider acted as storage for the lids during the staining process. A vertical lift (Fig. 31-14) for the TLC plates was 3D printed and the parts were assembled, a COM-Motor02 from JOY-IT servo motor was added for the lifting of the lift arm. The servo was connected to a PCA9685 servo control board, the board was connected to an Arduino Uno microcontroller, and the assembled vertical lift was mounted onto the baseplate. For the imaging after staining station (Fig. 31-18), the parts were 3D printed and assembled, a 3W STAR from OptoSupply visible light diode was mounted inside the imaging station, the diode was connected to an 8-channel relay module which was connected to an Arduino Mega microcontroller. A Raspberry Pi Camera Module V2 was mounted inside the imaging after staining station, and the camera was connected via ribbon cable directly to the second Raspberry Pi 4 microprocessor. In addition to all the stations of the analysis part of the automation system, a sensor for detecting TLC plates was installed, the mounting for the sensor was 3D printed, and a TOF sensor (Fig. 31-13) VL53L4CD was added. The TOF sensor was connected to an Arduino Mega microcontroller, and the mounting for the TOF sensor was mounted to the baseplate. The TOF sensor allowed the detection of errors when the automation system failed to pick up a plate and was used for stopping or pausing the automation system. The waste area where the used plates during the analysis process were placed can be seen in Fig. 31-17.

Three power supplies of 5 V, 12 V, and 24 V were installed in the system: 5 V power supply was used to power the MG90S micro servos and COM-Motor02 JOY-IT servos through the PCA9685 board, 12 V power supply was used to power all fans through the 8-channel relay and all three DeltaX2 robots, 24 V power supply was used to power all stepper motors except for the DeltaX2 robot stepper motors through the DM556 stepper motor controllers. TOF VL53L4CD sensor,

PCA9685 servo control board, and HX711 interfaces were powered through the Arduino microprocessors.

For the interface between the synthesis (Fig. 32A-5) and analysis (Fig. 32A-6) parts of the automation system, the required parts were 3D printed, CNC machined, bought, and assembled. The assembled and connected interface to the synthesis and analysis parts of the automation system can be seen in Fig. 32A and Fig. 32B. The interface was assembled by adding a 17HS19-2004S1 stepper motor to drive a carriage (Fig. 32A-1) mounted on a 20x20 aluminum frame (Fig. 32A-2, Fig. 32B-12) using a carriage rail (Fig. 32B-11) which was driven by a belt (Fig. 32B-13). The aluminum frame was mounted to the frame of the automation system using two mounts: at one end (Fig. 32A-3) and the other end (Fig. 32A-4) of the carriage frame. The rack (Fig. 32B-8) for holding 6 4 ml bottles (Fig. 32B-9) was CNC machined out of PP material, the rack was mounted to the carriage using a mounting part (Fig. 32B-7) which was 3D printed. CNC machined syringe needle guiding plate (Fig. 32B-10) that was made from phenolic paper laminate material was mounted onto the rack. The stepper motor that drove the belt of the carriage rail was connected to a DM556 stepper motor controller set to provide 1.4A of peak current at all times since the carriage moved on an incline, the stepper motor controller was connected to an Arduino Mega microcontroller.

- 1) interface carriage,
- 2) interface frame,
- 3) interface mounting to automation system frame 1,
- 4) interface mounting to automation system frame 2,
- 5) synthesis part of the automation system,
- 6) analysis part of the automation system,
- 7) rack to carriage mount,
- 8) rack for holding bottles,
- 9) 4ml bottles,
- 10) syringe needle guider,
- 11) carriage,
- 12) frame on which carriage moves,
- 13) belt.

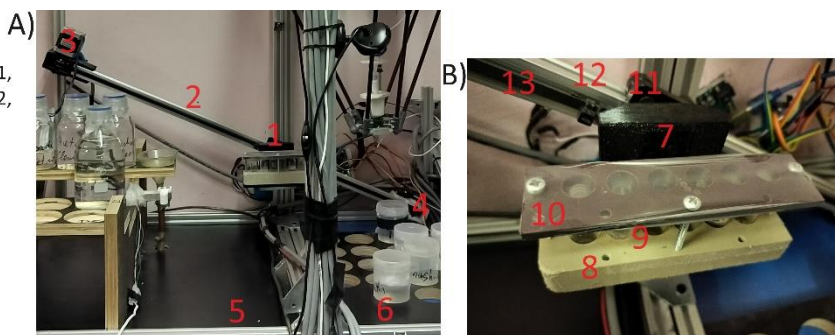


Fig. 32. Assembled interface between the synthesis and analysis parts of the automation system: A) zoomed-out view, B) zoomed-in view of the carriage

The automation system used three adaptive systems: a plate detector (Fig. 31-13) a plate storage rack with a weight sensor (Fig. 33A), and a syringe detector (Fig. 33B). The plate detector used a TOF sensor to detect if the plate handling robot successfully picked up a plate using the robot's suction cup end effector (Fig. 31-4). The plate storage rack with a weight sensor used a load cell to measure the weight of the plates allowing the detection when the plate handling suction cup end effector touched the topmost plate in the plate pile on the storage rack, as well as checked if the plate was successfully picked up by calculating the difference of the weights before and after the pickup process. The plate storage rack zoomed-in view can be seen in Fig. 33A, the rack was made up of a bottom part of the rack (Fig. 33A-1) which was mounted to the baseplate, the top part of the rack (Fig. 33A-2) on which the plates (Fig. 33A-3) were placed, and a loadcell (Fig. 33A-4) in between the bottom and the top parts of the rack for weight measurements. The last adaptive process was the syringe detector which also used a load cell to detect if the synthesis liquid dispenser robot end effector syringe needle touched the contact part of the detector which was mounted on one side of the load cell. This detector allowed to check if the syringe needle of the synthesis liquid dispenser was attached, not bent, and if the rail of the liquid dispenser robot was working correctly. The syringe detector zoomed-in view can be seen in Fig. 33B, the detector was made from a syringe needle detector mounting part (Fig. 33B-5) which was mounted to the chemical reagent bottle rack, a loadcell

(Fig. 33B-6) for measurement of the force exerted by the syringe needle, and the syringe needle detector part (Fig. 33B-7) in which the syringe needle came in contact with the detector.

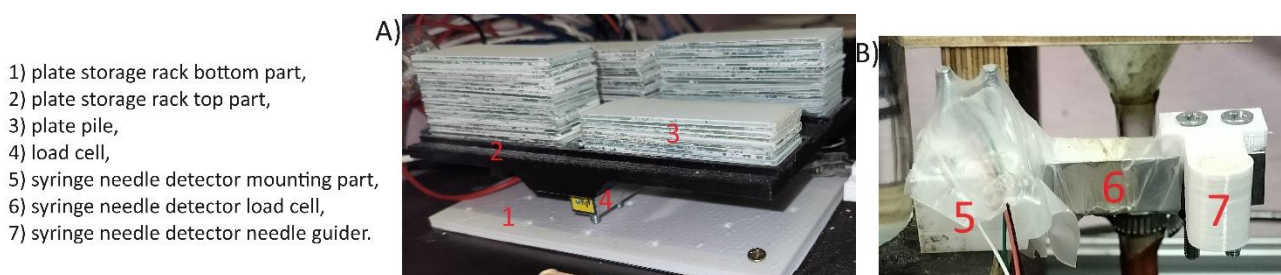


Fig. 33. Adaptive systems of the automation system: A) plate storage rack, B) syringe needle detector

The two parts of the automation system: synthesis and analysis were attached and the interface between the two parts was installed, the whole assembled automation system can be seen in Fig. 34, The synthesis part can be seen in Fig. 34-1, the analysis part can be seen in Fig. 34-2, and the interface between the two parts can be seen in Fig. 34-3.

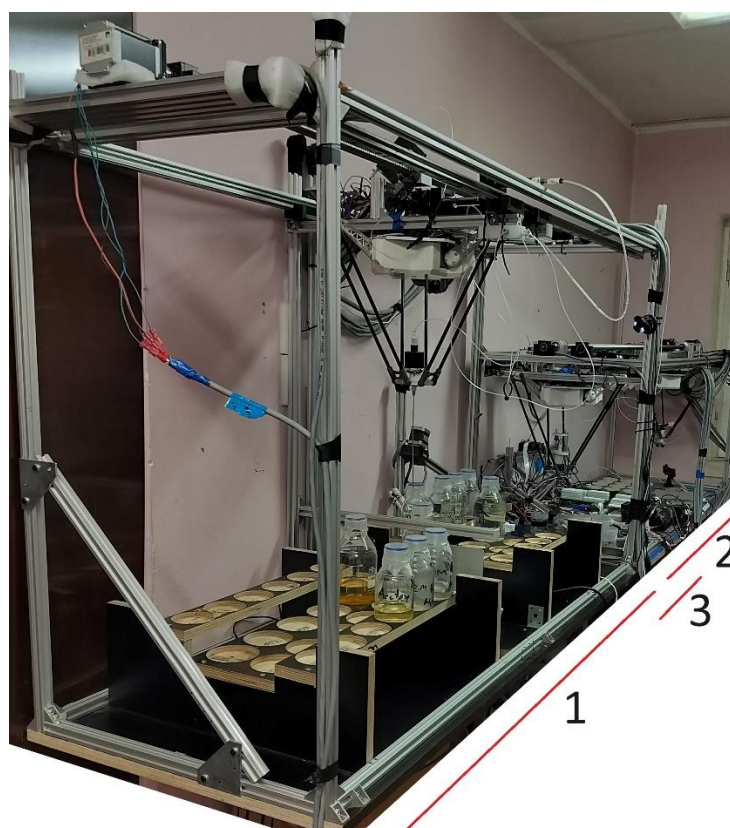


Fig. 34. Automation system: 1) synthesis part, 2) analysis part, 3) interface between synthesis and analysis parts of the automation system

3.2. Control Software Working Principle

Two Raspberry Pi 4 microprocessors, two Arduino Mega microcontrollers and one Arduino Uno microcontroller were used for the automation system due to the availability and cost of these parts as well as the capability to satisfy the control requirements for the automation system. The use of multiple Arduino microcontrollers was not necessary, three microcontrollers were used in the system due to easier and faster setup in order to build a working prototype, as well as to ensure future

scalability, the ability to break down the system into independent modules, and the ability to perform parallel processing. The data flow diagram of the automation system can be seen in Fig. 49 in Appendix 2. The main Raspberry Pi 4 microprocessor was connected to the three Arduino microcontrollers through USB ports. The fourth USB port of the Raspberry Pi 4 was connected to a 4-channel Logilink USB hub, the USB hub was connected to three DeltaX2 robots using USB ports, and the fourth USB hub port was connected to the Heidolph Hei-Connect heating mantle. The secondary Raspberry Pi 4 was used for taking images and was connected via a two-way connection using RabbitMQ [92] messaging broker which was implemented through Pika [93] library in Python. The RabbitMQ server for this two-way communication between the Raspberry Pi 4 microprocessors was hosted on the main Raspberry Pi 4. Both Raspberry Pi microprocessors were connected to a web server, the server was made by outsourcing its development to a third party using the author's design. The main Raspberry Pi 4 communicated with the web server using two-way communication, and the secondary Raspberry Pi 4 communicated with the web server using only one-way communication to the web server (message consumption acknowledgement still took place) due to the reasons that the secondary Raspberry Pi 4 only streamed images and the server did not need to monitor the processes taking place in the control software that ran on the second Raspberry Pi 4 microprocessor.

The control diagram of the automation system can be seen in Fig. 50 in Appendix 2. The main Raspberry Pi 4 microprocessor controlled the first, second, and third Arduino microcontrollers, took images of plates in the imaging station, controlled all three DeltaX2 robots for the synthesis liquid dispenser robot, the analysis liquid dispenser robot and the plate handler robot, as well as the heating mantle, and the secondary Raspberry Pi 4 microprocessor. The first Arduino controller controlled the stepper motors of the rails on which the three robots moved, the interface carriage movement, all relays, two load cells for the plate storage rack weighing, and the loadcell for the syringe detector. The second Arduino microcontroller controlled all servo motors, and the third Arduino microcontroller controlled the TOF sensor and the 2nd linear actuator for the 1 ml syringe of the synthesis liquid handling robot. The secondary Raspberry Pi 4 microprocessor took images of plates after staining in the imaging after staining station. The synthesis liquid dispensing DeltaX2 robot controlled the linear actuator for the 10 ml syringe of the synthesis liquid dispensing robot through the robot's 4th axis connection, and similarly, the analysis liquid dispensing DeltaX2 robot controlled the linear actuator for the 5 ml syringe of the robot through the robot's 4th axis connection. The plate handler DeltaX2 robot controlled the vacuum pump for the vacuum suction cup end effector through its auxiliary connections.

The control software for all three Arduino microcontrollers was written in Arduino language using Arduino IDE. The code snippet of the code for the Arduino microcontroller being edited in Arduino IDE can be seen in Fig. 51B in Appendix 2. For the first Arduino microcontroller which was an Arduino Mega, the control software was 572 lines and contained the code for the communication between the Arduino and the main Raspberry Pi 4 through USB ports, control of the stepper motors for the rail of synthesis, and the rail of analysis parts of the automation system, control of the relays, and control of the load cells through the HX711 interface. For the second Arduino microcontroller which was an Arduino Uno, the control software was 102 lines long and contained the code for the communication between the Arduino and the main Raspberry Pi 4 through USB ports, and control of the servos through the PCA9685 board. For the third Arduino microcontroller which was an Arduino Mega, the code was 409 lines long and contained the code for the communication between the Arduino and the main Raspberry Pi 4 through USB ports, the control of the TOF VL53L4CD sensor,

and the control for the linear actuator containing the 1 ml syringe for the synthesis liquid handler robot. The main control boards of the DeltaX2 robots ran a proprietary control software, for which the source file was not available, only the firmware hex file was provided through the robot seller's website [94]. The control software of the DeltaX2 robots included the functionality of communication through the USB serial ports and the performing of the G-code commands which were listed on the DeltaX2 resources website [75]. In this automation system, the DeltaX2 robots received a G-code command sent by the main Raspberry Pi 4 microprocessor using USB serial ports, performed the command, and returned an answer to the main Raspberry Pi 4. The answers from the DeltaX2 robot were used to control the timing of the instructions sent to the DeltaX2 robots from the main control software. The main control software that ran on the main Raspberry Pi 4 microprocessor was written in Python language using Visual Studio software and contained 17495 lines of code. The code snippet of the control software being edited in Visual Studio software can be seen in Fig. 51A in Appendix 2. The control software contained the code for the serial communication and control of different processes to all three DeltaX2 robots, to the Heidolph heating mantle, to three Arduino microcontrollers, as well as communication using RabbitMQ message broker to the web server and to the second Raspberry Pi 4 microprocessor. The control software was responsible for taking the requests from either the user interface or the web server and executing the set instructions with set parameters by generating G-codes for the DeltaX2 robots as well as sending commands for the Heidolph heating mantle, three Arduino microcontrollers and to the secondary Raspberry Pi 4 microprocessor. The main control software reported the automation system's state and sent analysis results back to the web server. The control software had a file structure, containing configure, coordinate mapping, message logging files, folders for storing images taken during analysis and other miscellaneous tasks, the control software file structure can be seen in Fig. 52B in Appendix 2. The config file had 635 changeable parameters, the snippet of the config file opened in Visual Studio software can be seen in Fig. 52A in Appendix 2. The config files and coordinate mapping files could be modified if some parameters of the automation system or the coordinates for the different parts of the system needed to be adjusted or changed for different applications. In addition, multiple automation systems could have different config and coordinate mapping files but the same control software file, making the updates to the software easier to apply. The control system outputted different parameters to the console while the software was running for debugging, in addition, the software logged important alerts as well as the received messages through the web server in the log files so that the files could be analyzed. Lastly, the control software for the second Raspberry Pi 4 microprocessor was written using Python in Visual Studio software. The code was 800 lines and contained the communication to the main Raspberry Pi 4 microprocessor as well as to the web server through RabbitMQ messaging broker implemented using Pika library, and the instructions for capturing, formatting and streaming images using a camera. The communication of the second Raspberry Pi 4 microprocessor to the web server was only one way, as there were only images being streamed to the web server.

In order to achieve the desired control system for the automation system, process threading and message queue system was implemented for the control software which ran on the main Raspberry Pi 4 microprocessor. The control software flow diagram for the initialization, message-consuming thread processes, and function queue thread processes can be seen in Fig. 53 in Appendix 2. The control software flow diagram of the main function slot thread and interrupt function slot thread can be seen in Fig. 54 in Appendix 2. The control system initialization was started by starting the control software in the Raspberry Pi 4 microprocessor and pressing the initialization buttons in the user

interface of the control software. The system connected to the web server as well as the secondary Raspberry Pi 4 microprocessor through the RabbitMQ messaging broker, then started thread 4, thread 3, thread 2, and thread 1, after thread 1 was started, the initialization process was finished. Thread 1 was the message-consuming thread, which listened and consumed messages coming from the web server and added the received messages to a message queue array. Thread 2 was the message queue thread, which periodically went through the message queue array and checked the type of the messages. The message was added to the main function slot if all of the following conditions were met: the message in the queue was of a non-interrupt type, the message was the first in the queue array, and the main function slot of the control system was empty. Once the message was added to the main function slot, the message was deleted from the message queue array. If the message in the message queue array was of interrupt type, and if the interrupt function slot was empty, then that message was added to the interrupt function slot and then deleted from the message queue array. The non-interrupt type message could only be added to the main function slot if that message was first in the message queue, but an interrupt-type message could be added to the interrupt function slot even if it was not first in the message queue array. Emergent properties of thread 1 and thread 2 were that non-interrupt messages and interrupt messages are processed in the order they were consumed from the web server allowing to send sequences of instructions to the automation system. Thread 3 was called the main function slot thread, the main function slot was an array in which the messages get appended by the thread 2 process. Thread 3 process checked if the main function slot had a new appended message, the message was decoded to extract the function and the function parameters, and then that function was called. When the called function ended, the main function slot deleted that message. Thread 4 is similar to thread 3, only that interrupt functions were processed instead of the non-interrupt functions like in thread 3. Emergent properties of thread 3 and thread 4 were that two simultaneous processes, one non-interrupt, and one interrupt could be performed by the automation system. An example of the use of this threaded control system was if thread 3 was processing a synthesis function, and the user of the automation system wanted to change the temperature of the heating mantle, the request for the change of the temperature could be an interrupt function which was processed on thread 4, this way the temperature of the heating mantle was changed without the need to stop the synthesis function. Another example is the emergency stop message being sent from the web server as an interrupt function, this way the automation system software can stop all the processes faster without the need to stop the current function being processed in the main function slot. However, careful considerations had to be made when defining interrupt functions, in order not to allow two simultaneous processes to interact in a way that could be damaging to the automation system.

The first user interface of the control software was made using Tkinter [95] library, the main page of the user interface contained 9 buttons which can be seen in Fig. 55 in Appendix 2. The buttons were color-coded: blue color meant that the button needed to be pressed before the automation system could be used. After the blue-colored buttons were pressed and the respective function was performed, the button turned to green color, indicating that the button was no longer needed to be pressed. Blue-colored buttons included “Initiate robot“, which initiated all USB connections of the main Raspberry Pi 4 microprocessor and performed home position calibration for all the rails of the robots and the robots themselves. The “Calibrate SS2_1“ button moved the analysis liquid dispensing robot end effector to a specific place where the end effector syringe needle could be adjusted manually, similarly “Calibrate SS1“ button performed the same function but for the synthesis liquid dispensing robot. “Internal queue start“ button started thread 4, thread 3, thread 2, and thread 1. The

“Connect to server“ button initialized RabbitMQ connections to the web server and between the main and secondary Raspberry Pi 4 microcontrollers. Yellow buttons meant that the button could be pressed at any time, and the functions of the button were usually a toggle for showing or hiding a window to display additional information or to allow the user to set additional parameters for the automation system. The “Show cleaning/system state“ button was yellow and upon pressing called a function to toggle the showing and closing of a new page in which cleaning parameters for the automation system could be modified. Orange buttons meant that the button needed only to be pressed when specific conditions took place in the automation system. “SS2 resume“ button was an orange button and was used for cancelling the pause process that occurred for the analysis part of the automation system if the TOF sensor did not detect a TLC plate during the imaging process part of the analysis process. Similarly, the “SS1 resume“ button cancelled the pause process that occurred for the synthesis part of the automation system if the syringe needle detector did not detect the syringe during the synthesis process. The red-colored button meant that the buttons should only be pressed in an emergency because the function of the button could disrupt the automation system process. A red button was called “Emergency stop“, and when pressed, the control software turned off all stepper motor controllers and all relay switches. Other pages of the user interface contained buttons, labels, entries and checkboxes for monitoring and controlling the automation system during calibration, testing, and maintenance.

The second interface of the system was a webpage from which the web server could control the automation system, the webpage had a user panel and an admin panel. The user panel was used for the user to set up a sequence of steps, which included Stirr/heat, Add, and Analyse steps with their corresponding parameters. Stirr/heat step corresponded to the stirring and heating of the heating mantle in the synthesis part of the automation system. Add step corresponded to the addition of reagents for the synthesis process that was performed in the synthesis part of the automation system. Analyse step corresponded to the TLC analysis process performed in the analysis part of the automation system that analysed a chemical sample from the synthesis part of the system. The user panel for the setup of the sequence to be performed by the automation system can be seen in Fig. 56 in Appendix 2. Once the user set the sequence of steps and pressed the Start button, the information of this sequence of steps was then viewable in the admin panel, the admin panel can be seen in Fig. 57 in Appendix 2. The admin then checked if the set sequence was acceptable from the perspective of the involved chemical reagents and the chemical processes involved and either approved or cancelled the sequence. This was done to prevent illicit or extremely dangerous product synthesis done by the automation system from users that could cause harm accidentally or intentionally. The admin then brought the required solvents and reagents for the set sequence of steps and parameters and put the solvents and reagents into the automation system simultaneously mapping the chemicals to coordinates on the automation system, for example in Fig. 57 in Appendix 2 it can be seen that benzenesulfonyl chloride in Acetone was put into the Synthesis 1 D4 coordinate of the automation system, and Toluene was put into Development A3 coordinate. This was done so that a map of reagent to a predefined chemical liquid container place was formed, which was going to be used during the operation of the automation system. After the admin put all the required solvents and reagents, mapped each chemical to a coordinate, then added all the required consumables of the automation system, for example: plates for TLC analysis, cotton pads for the analysis liquid dispensing robot syringe needle cleaning, required cleaning solvents, the admin then started the sequence. After the sequence was started, the web server converted the mapping of chemical liquids to a coordinates file in a .csv format file and sent it to the automation system, then encoded all the steps in the sequence

and sent to the automation system in string format. Encoding involved converting the steps and their parameters into a string using predefined encoding rules. The automation system then received the messages by using the thread 1 process of the control software and executed the decoded steps using thread 2, thread 3, and thread 4 processes. The web server periodically sent an interrupt message to the automation system asking it to report its current state, the automation system received the interrupt message and sent the internal state report of the automation system to the web server. This way the process tracking could be done of the automation system which was visualized to the user and admin panels on the webpage. This can be seen in Fig. 58 in Appendix 2, the progress state of each step of the sequence was displayed: if the step was still in a queue, if it was being performed by the automation system, or if it was already performed and finished. In addition, the automation system sent back the images that were produced during the analysis process which were then displayed on the user and the admin pages, this can also be seen in Fig. 58 in Appendix 2. This allowed both the user and the admin to monitor the progress of the synthesis and analysis processes done by the automation system.

3.3. Automation System Working Principle

There were three main processes that the automation system could perform: heating/stirring, synthesis, and analysis. The heating and stirring process involved the Heidolph Hei-Connect heating and stirring mantle which was located in the synthesis part of the automation system. The flow diagram of this process is presented in Fig. 59 in Appendix 3. During the process, the control software sent the parameters, started the rotation, started the heating and started the timer for a set amount of time. Once the timer finished, the heating was stopped, the rotation was stopped, and the process of heating and stirring ended.

The synthesis process was done at the synthesis part of the automation system and only the liquid handling robot for the synthesis part was used. The flow diagram of the synthesis process is presented in Fig. 60 in Appendix 3, and images of the automation system performing the synthesis process can be seen in Fig. 35. When the synthesis process started, the robot moved out of the designated chemical bottle filled with a cleaning solvent, this place was called 'bath' in the process flow diagram because the syringe needle of the liquid dispensing robot was immersed in the cleaning solvent when the dispensing robot was not working in order to keep syringe needle clean and prevent crystal formation. Then the robot arms moved to their home positions by using the limit switches on the DeltaX2 robot, as well as the rail of the synthesis part of the automation system moved until the limit switch mounted on the frame was hit. After that, the robot moved to a syringe needle detector which was located at the second chemical bottle rack (Fig. 35A). The syringe needle was checked by an adaptive process using a weight sensor. The needle-checking algorithm is described in more detail after this process. If the needle was not detected, then the synthesis process was paused until a resume button was pressed in the user interface of the control software, if the resume button was pressed then the synthesis process resumed, however, if the needle was detected, then the synthesis process continued. The robot syringe needle and buffer tank were prewetted (Fig. 35B) by taking in a set amount of chemical cleaning solvent and dispensing it into a chemical waste container (Fig. 35C). Prewetting was done so that the syringe needle and the buffer tank were cleaned as well as the air in the tubing was saturated with a solvent to prevent dripping. Then the robot took chemical liquid from a set chemical bottle in the bottle rack (Fig. 35D) and dispensed it into the chemical reaction flask which was located on the heating and stirring mantle (Fig. 35E). There was a maximum volume of liquid that could be dispensed per cycle, therefore if the set amount of liquid that was needed to be dispensed

for the same reagent was larger than the maximum amount per single cycle, then the intake of the reagent and dispensing into the chemical reaction flask was repeated until the set amount of transferred liquid was reached. After that, the robot performed the cleaning process, which involved the intake of cleaning solution from a set bottle and dispensing the cleaning solution into the waste container, after cleaning, the syringe needle was then immersed into the ‘bath’ (Fig. 35F). Once that step was performed, the synthesis process finished. The result of one cycle of the synthesis process was a set amount of chemical reagent transferred from one chemical bottle into the reaction flask. If multiple reagents were needed to be transferred to the chemical reaction flask, the synthesis function was called multiple times with different parameters.

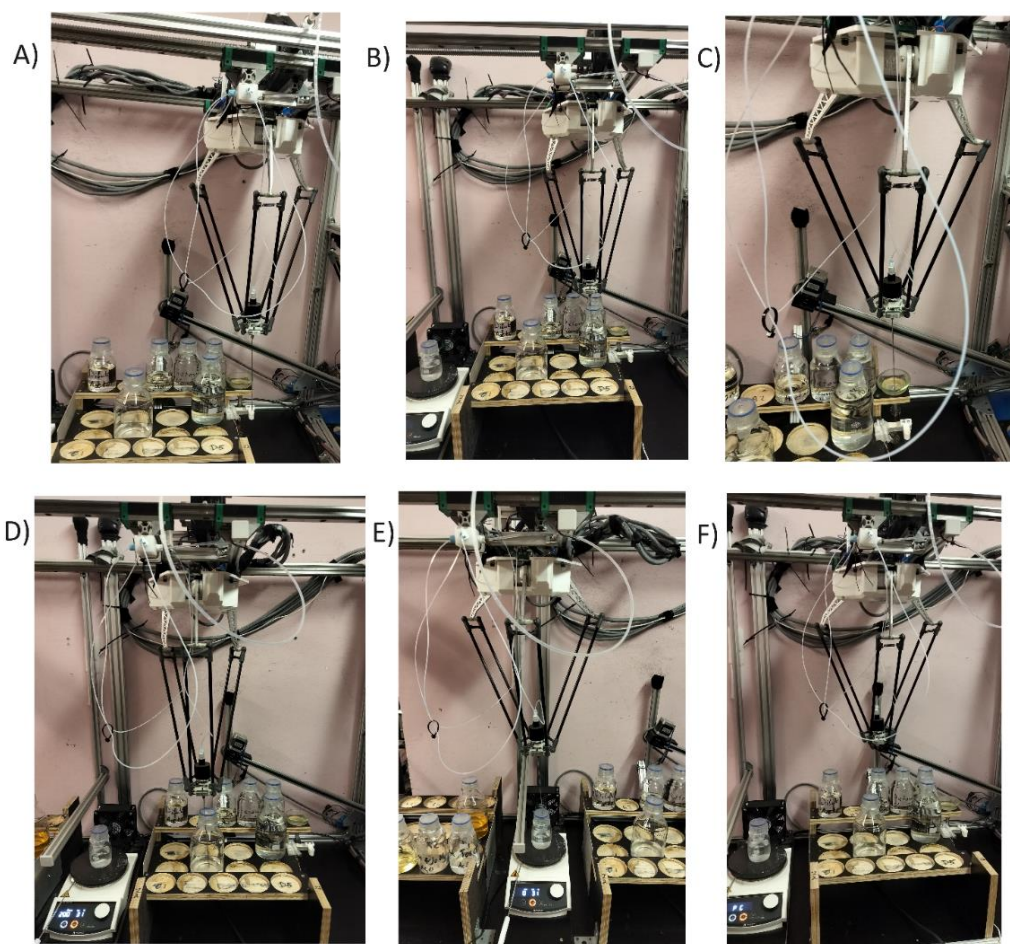


Fig. 35. Synthesis part of the automation system performing synthesis process: A) syringe needle detection, B) intake of cleaning solvent for prewetting, C) dispensing solvents into a waste container, D) intake of reagent, E) dispensing reagent into a chemical reaction vessel, F) idle position of liquid dispensing robot

The syringe needle detection flow diagram is presented in Fig. 61 in Appendix 3. The syringe needle check process performed during the synthesis process happened the following way: firstly, the robot moved to a set location near the syringe needle detector which was located at the second chemical bottle rack in the synthesis part of the automation system. The value of the load cell in the syringe needle detector was measured, then the robot moved in the Z direction which resulted in the syringe needle moving closer to the syringe needle detector by a set amount of distance. The value of the Z coordinate for the robot was tracked by the control software and as compared to a set threshold. If the Z coordinate exceeded the set threshold, the needle check process, and in turn the synthesis process were paused. Only by pressing the resume button in the user interface of the control software, the process was continued. The Z coordinate exceeding the set threshold meant that the syringe needle

missed the syringe needle detector, which could happen due to the syringe needle not attached, bent, the syringe detector malfunctioning, or the rail of the robot not moving to a correct place. In any of the described cases, the process was paused and the problem had to be solved before the synthesis process could continue. However, if the Z coordinate was not exceeded, then the process continued: the loadcell value was measured again and a difference between the two latest loadcell measurement values was calculated and compared to a set threshold. If the threshold was exceeded, that meant that the syringe needle successfully touched the syringe needle detector, and the process of the syringe needle checking ended, and the synthesis process continued. If the threshold was not exceeded, then the robot moved again by a set amount in the Z direction. The process repeated until the Z value threshold was exceeded or the difference between the two latest loadcell values exceeded their set threshold.

The third main process of the automation system was called analysis which involved the analysis of a chemical sample using TLC method from the reaction flask in the synthesis part of the system transferred to the analysis part of the system via the interface. The flow diagram of this process is presented in Fig. 62 in Appendix 3. The process flow diagram was too large to fit into one page, therefore the process flow of the analysis process was split into four parts: spotting process, development process, imaging process, and staining and imaging after staining process. When the analysis process was started, spotting was performed first, then development, and imaging processes. If the staining process was not set to be performed, then the analysis process ended, otherwise, the staining and imaging after staining processes were performed and only then the analysis process ended.

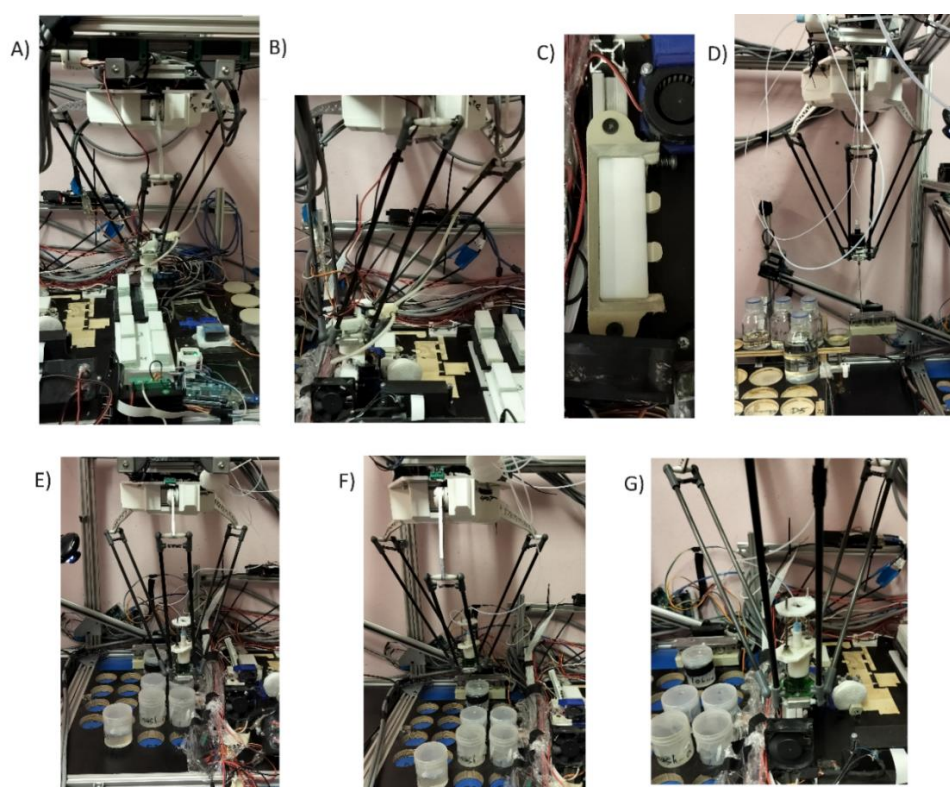


Fig. 36. Automation system performing spotting step of the analysis process: A) plate pickup from plate storage racks, B) plate placement on the plate guide, C) placed plate on the plate guide, D) dispensing of chemical sample for analysis from synthesis part of the automation system to the interface carriage bottles, E) intake of cleaning solution for cleaning of the analysis dispensing robot, F) intake of chemical sample from the interface carriage bottles, G) spotting of the chemical sample onto the plate

The flow diagram of the spotting process part of the analysis process is presented in Fig. 63 in Appendix 3 and the pictures of the automation system performing the spotting process are presented in Fig. 36. The spotting process used all three DeltaX2 robots. In the process flow diagrams the liquid handling robot in the synthesis part of the automation system is referred to as 'Robot 1', the liquid handling robot in the analysis part of the automation system is referred to as 'Robot 2', and the plate transporter robot in the analysis part of the automation system is referred to as 'Robot 3'. The carriage between the synthesis and the analysis parts of the automation system is referred to as 'Interface'. When the spotting process was started, Robot 1 moved out of the cleaning solution chemical bottle which was referred to as 'bath', then the arms of the Robot 1 moved to their home positions by using the limit switches on the DeltaX2 robot, as well as the rail on which the Robot 1 was mounted moved to its home position by hitting the rail limit switch. The syringe needle of the Robot 1 was checked by using the needle detector, the process for this step was the same as in the synthesis step and was detailed previously. Then Robot 1 was prewetted by taking in a set amount of chemical cleaning solution and dispensing it into the waste container. Robot 3 moved to its home positions by hitting the limit switches on the DeltaX2 robot as well as the rail for the Robot 3 moved to its home position by hitting the limit switch mounted on the rail. The Robot 3 took a TLC plate from the plate storage rack located in the analysis part of the automation system by an adaptive process (Fig. 36A), which is detailed after the spotting process. Robot 3 used the suction cup end effector for the transportation of the plate to the spotting plate guider (Fig. 36B, Fig. 36C) in the spotting station of the analysis part of the automation system. The Interface moved to the synthesis part of the automation system, Robot 1 then took 1 ml chemical sample from the chemical reaction flask and/or reagents from the reagent containers and dispensed that sample into the Interface 4 ml volume bottle (Fig. 36D). Robot 1 cleaned its syringe and buffer tank by taking in a cleaning solution and dispensing it into a waste container, then performed dilution of the chemical sample by taking in dilution chemicals and dispensing into the 4 ml volume bottle located in the Interface, mixing the chemical solutions, and then cleaning its syringe and buffer tank. The dilution was performed so that the concentration of the chemical sample could be reduced for better results during the TLC analysis: high-concentration sample spots can overlap with other spots, making the separated spots difficult to discern. The dispensing of the chemical sample and dilution was repeated until one, two, or three 4 ml bottles were filled depending on the set parameters of the spotting process. After that, Robot 1 placed the syringe needle into the 'bath' bottle, then the Interface moved from the synthesis to the analysis part of the automation system. The Robot 2 moved out of the 'bath' and moved to its home positions by using the limit switches of the DeltaX2 robot, in addition, the rail onto which the Robot 2 is mounted, moved to its home position by hitting the limit switch mounted on the frame. Robot 2 was prewetted by taking in chemical solvent from the spotting station container rack (Fig. 36E) in the analysis part of the automation system and dispensed it into a waste container. Then Robot 2 took the chemical sample from the Interface's 4 ml bottle (Fig. 36F) and cleaned its syringe needle by touching a cotton pad mounted near the spotting plate guider. The cleaning of the syringe was done so that the droplets formed during the movement of the Robot 2 end effector were wiped off and the spot on the plate during spotting was uniform across multiple spots, the droplets could make the spot on the plate much bigger due to excess chemical sample liquid. Robot 2 then made a spot on the plate (Fig. 36G), cleaned the syringe needle by taking in a cleaning chemical solution and dispensed it into a waste container. A drying fan which was mounted on the spotting plate was turned on in order to dry the spot on the plate. After a set amount of time, the drying fan was turned off. The process of spotting a chemical sample was done multiple times depending on if 1, 2, or 3 chemical samples were set to be

spotted onto the plate. Once all of the set spots were spotted on the plate, the spotting process ended and the development process was started.

The process for the TLC plate pickup that occurred in the analysis part of the automation system from the plate storage racks by using Robot 3 was an adaptive process involving a loadcell for both sensing the end effector of the Robot 3 and the measurements of the weight of the plates to determine if the plates were picked up. The flow diagram for this process can be seen in Fig. 64 in Appendix 3. This adaptive process allowed the placement of any number of plates, with a maximum number of 20 plates per pile, onto the plate racks without needing to define a height position in the control system as well as without the need to keep track of the changing pile height due to the plates being removed from the pile during analysis process. Using this adaptive process Robot 3 was able to find the position of the topmost plate in the plate pile in order to pick it up. In addition, checking whether the plate was picked up allowed having multiple plate piles by detecting that the plate was not picked up from a certain pile in a case where there were no more plates in that pile, and then moving on to a different pile to check if there might be more plates to be picked up. The whole adaptive process started when Robot 3 moved to the plate storage rack to pick up the plate used for the spotting process part of the analysis process. The Robot 3 arrived at the set coordinate where a plate pile was put onto one of the plate racks, and the load cell of the plate rack measured its value. The Robot 3 moved in the Z direction by a set amount. The Z direction value was tracked by the control software and was compared to a threshold, if the set Z value threshold was exceeded meaning that the suction cup of the Robot 3 end effector reached the plate rack itself and there were no plates in this particular pile, or missed the plate rack due to some error. Then if there were more plate piles to check, the Robot 3 moved to a different plate pile coordinate, the loadcell value was measured again, and the robot moved in Z direction. However, in a case where the Z value threshold was exceeded, and there were no more different plate piles to check, meaning that there were no more plates on the plate racks in every pile, the process was paused until the resume button was pressed in the control software user interface. If the button was pressed, the plate pickup check process was ended. In the case where the Z value threshold was not exceeded upon the movement of Robot 3 in the Z direction, the loadcell value was measured and the difference between the two latest loadcell values was calculated and compared against a set threshold. If the compared value did not exceed a threshold, meaning that the suction cup of Robot 3 was still approaching the plate pile, Robot 3 moved again in the Z direction and the difference of loadcell values were compared. If the difference between the two latest loadcell values was exceeded, meaning that the suction cup of Robot 3 touched the topmost plate in the plate pile, Robot 3 then picked up the plate and moved away from the plate storage rack. After that, the load cell took a measurement and calculated the difference between the two latest measurement values. If the difference was lower than a set threshold, the plate check process ended. The difference being lower than a set threshold meant that the plate was successfully picked up from the pile, therefore the value of the loadcell measurement decreased. If the compared value was not lower than a set threshold, that meant that no plate was picked up from this specific plate pile, control software then checked if there were more different plate piles, if there were, then the robot moved to a different pile, if there were not, then the process was paused, because there were no more plates in any plate pile.

For the development process part of the analysis process, all three robots were used. The flow diagram of this process is presented in Fig. 65 in Appendix 3, and images of the automation system performing this process are presented in Fig. 37. Firstly, Robot 3 took a plate from the spotting guider in the spotting station and put the plate into a horizontal development chamber (Fig. 37A) located in the

development station of the analysis part of the automation system. The development chamber glass lid was closed (Fig. 37B), so that during the development of the plate, the development chemical solution would not evaporate easily. Evaporation increased the development time or prevented the development solution from travelling up the TLC plate by capillary action. Robot 2 took solvent from the development solution rack in the development station and dispensed the solvent into the development solution mixing container (Fig. 37C). Robot 2 then cleaned its syringe and buffer tank by taking in and dispensing cleaning solution into a waste container. Robot 2 then repeated the dispensing of development solvents into the mixing container for all set development solvents.

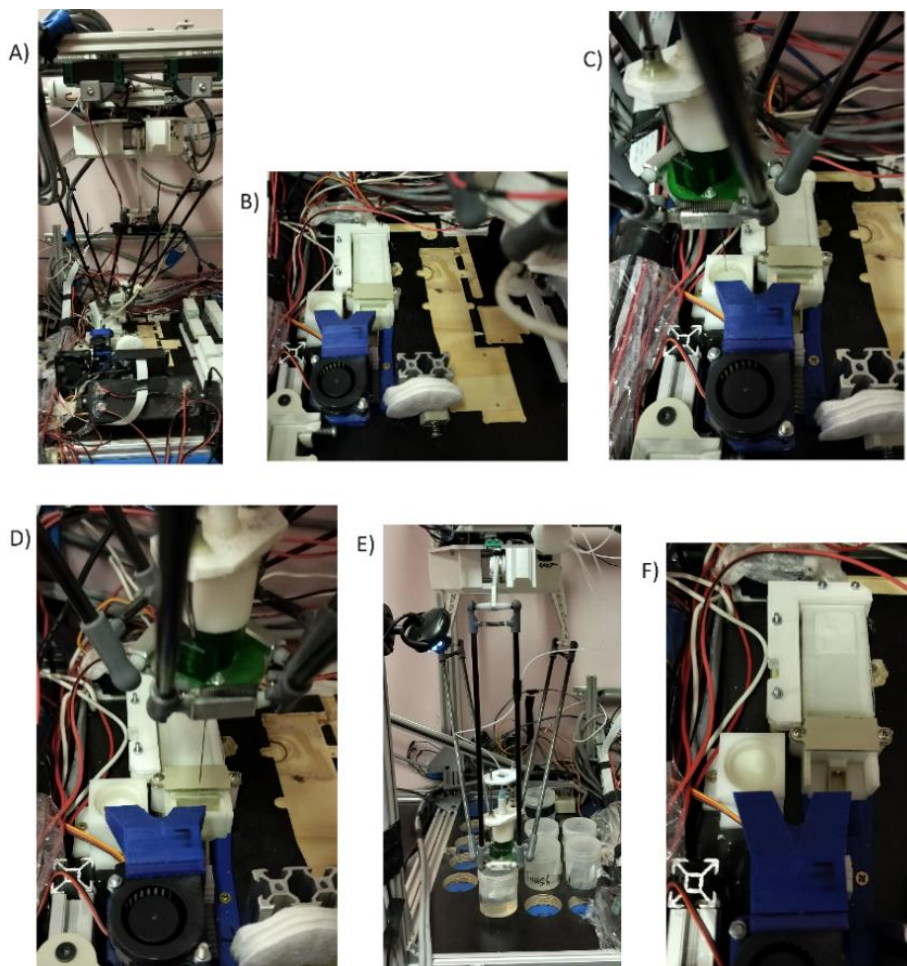


Fig. 37. Automation system performing development step of the analysis process: A) plate placement into horizontal development chamber, B) closed position of the development chamber lid, C) dispensing of development solvents into the development solvent mixing container, D) dispensing of development mixture into the development chamber, E) analysis liquid dispenser robot idle position, F) closed development chamber

After that Robot 2 mixed the solvents in the mixing container, took the mixed development solution, and dispensed the mixture to the horizontal development chamber (Fig. 37D). Robot 2 then cleaned its syringe needle and buffer tank and placed the needle into a chemical solution bottle which was designated as ‘bath’ (Fig. 37E). The horizontal development chamber was closed, meaning that chamber in which development solution touched the plate and the solution started to travel up the plate by using capillary action (Fig. 37F). A timer was started for a set amount of time, and when the timer finished the development chamber was opened together with the development chamber glass lid to stop the development process and start the drying process of the plate. At the same time when

the development chamber was closed and the development timer was started, the Interface moved to the synthesis part of the system, then Robot 1 took the remaining chemical sample liquid that was not used for the analysis from the Interface 4 ml bottles, dispensed into a waste container, took in cleaning solution, dispensed into the Interface bottles and mixed, took out the mixed solution and dispensed into a waste container, this way cleaning the Interface bottles to prepare them for the next analysis process cycle. All Interface bottles were cleaned depending on if 1, 2, or 3 bottles were used for the analysis process, after that, Robot 1 immersed its cleaned syringe into a designated bottle named 'bath'. Once both steps: cleaning of the Interface bottles by Robot 1 (for which its end was marked by the immersion of Robot 1 into the 'bath') and the development of the plate in the development station (for which its end was marked by the opening of the development chamber glass lid) were performed, the development process ended and imaging process started.

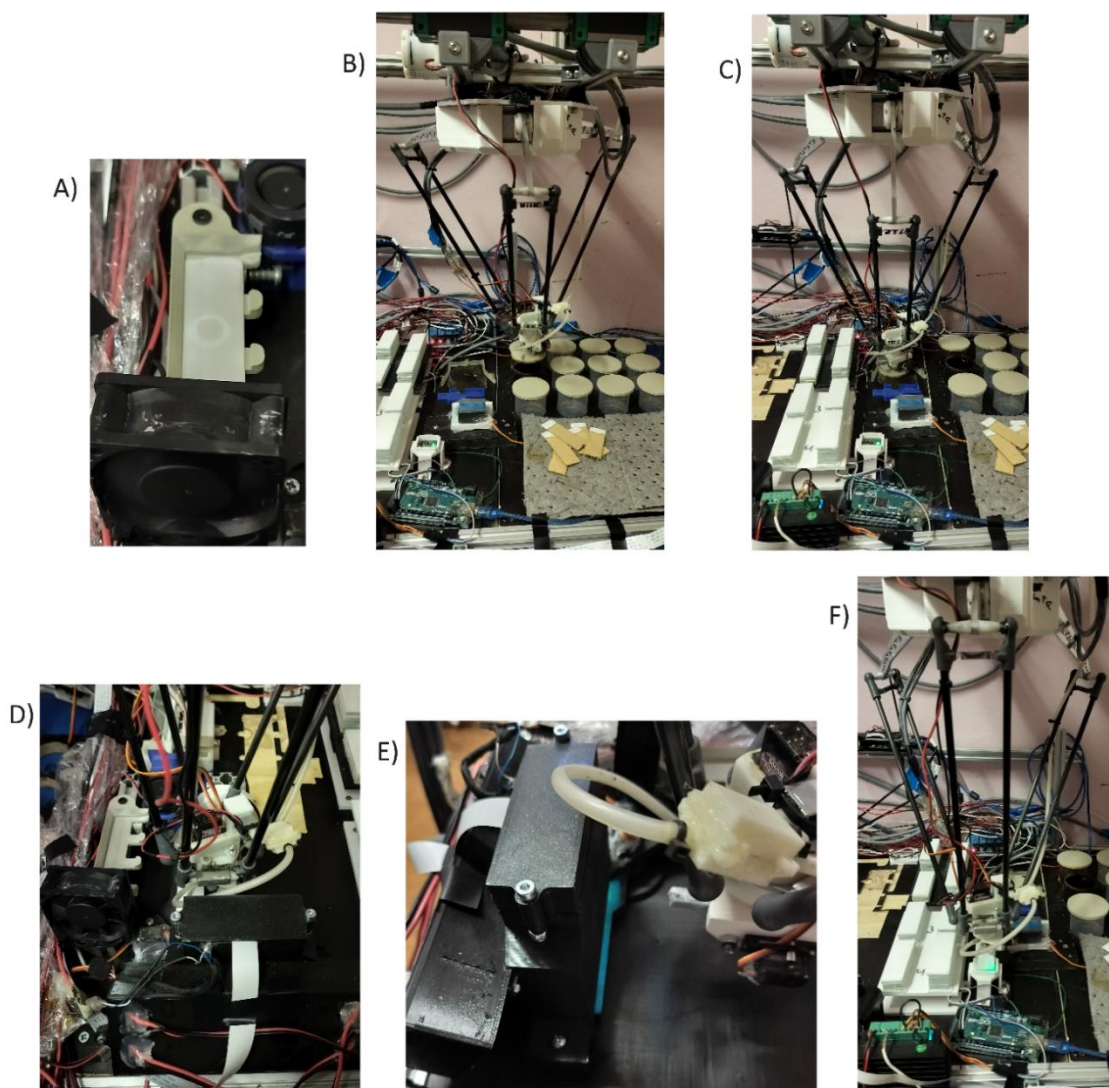


Fig. 38. Automation system performing imaging part of the analysis process: A) drying of the developed plate, B) taking off the staining bottle lid, C) placement of the staining bottle lid into the lid guider, D) placement of the plate onto the imaging station moving arm, E) imaging of the plate in the imaging station enclosure under UV lighting conditions, F) placement of the plate under TOF sensor

For the imaging process part of the analysis process only Robot 3 was used. A flow diagram of this process is presented in Fig. 66 in Appendix 3, and images of the automation system performing the imaging process are presented in Fig. 38. Robot 3 took the plate from the development chamber in

the development station of the analysis part of the automation system and placed the plate onto the spotting station plate guider. The drying fan that was mounted onto the plate guider in the spotting station was turned on (Fig. 38A) so that the recently developed plate was dried out and could be imaged. A wet plate provided unusable results when imaged, the compound distribution across the plate was hardly visible due to the development solution blocking the view of the chemical compounds under UV lighting conditions. The drying timer was started, and once finished, the drying fan was turned off. At the same time, the drying fan was turned on, Robot 3 took the staining bottle lid from the staining bottles that were placed in the staining bottle rack in the staining station (Fig. 38B) and placed the lid at the lid guider in the staining station (Fig. 38C). The lid guider provided a place for the robot to place and pick up the lids, increasing the repeatability of the lid placement. Once both steps were finished: the drying of the plate (for which its end was marked by turning off the drying fan) and placing of the staining bottle lid at the lid guider, the next step started. The Robot 3 then took the dried plate from the plate guider at the spotting station and placed the plate on the imaging station's arm. The imaging station arm was closed, that way moving the plate into the imaging station enclosure. The UV 254 nm was turned on and the image of the plate under UV lighting conditions was taken using the Raspberry Pi camera module (Fig. 38E). An example of an image taken of a development plate in the imaging station using the automation system can be seen in Fig. 39A.

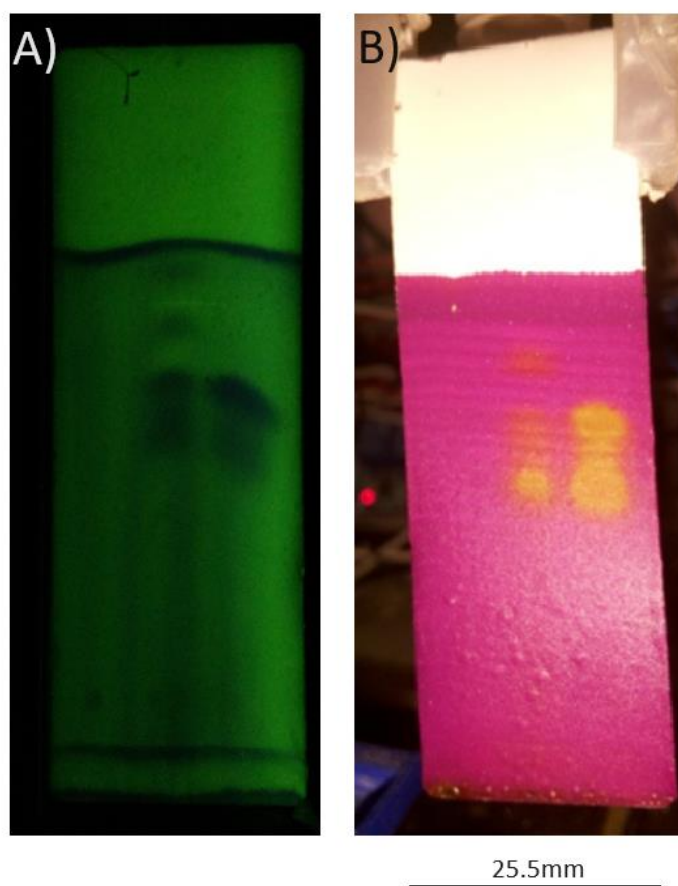


Fig. 39. Images produced in the automation system: A) imaged plate after development under UV 254 nm lighting conditions, B) imaged plate after staining under visible lighting conditions

The UV light was then turned off, the arm of the imaging station was opened, and the Robot 3 took the plate from the imaging station and moved the plate to the TOF sensor (Fig. 38F). The TOF sensor

measured its value and compared if the measured value was lower than a set threshold. If the plate was detected, then the imaging process ended. If staining and imaging after staining process was set to be performed, then that process was started, otherwise, the whole analysis process ended. However, in a case where the plate was not detected by the TOF sensor, the process was paused. The paused process could be resumed if a resume button was pressed in the control software user interface. The TOF sensor could not detect the plate due to several reasons: the plate got left at some other steps of the process due to an error, the TOF sensor was not working properly, the Robot 3 was not working properly, and in any of these cases, the problem needed to be solved before resuming the process.

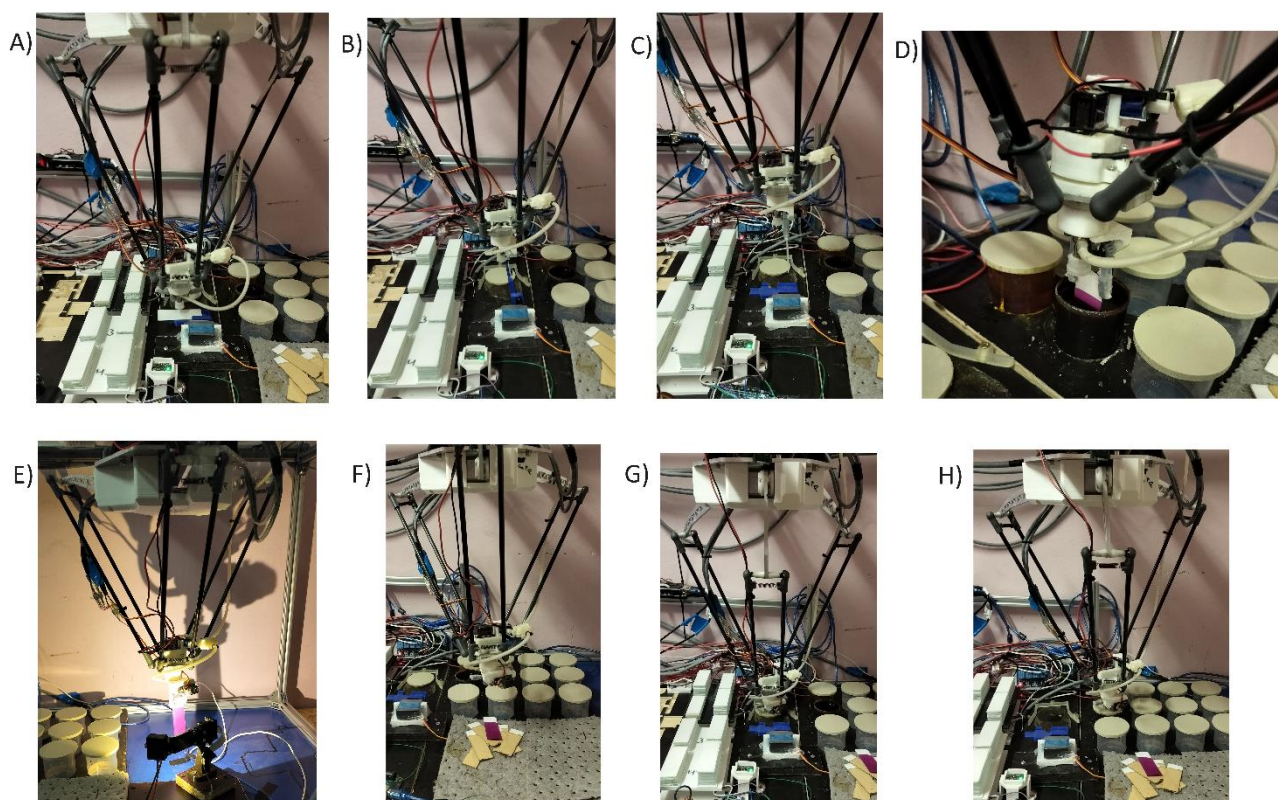


Fig. 40. Automation system performing staining and imaging after staining of the analysis process: A) placement of the plate onto the vertical lift, B) vertical lift lifting plate to vertical position, C) plate grabbed using mechanical gripper, D) plate is immersed into staining solution, E) plate is imaged after staining, F) plate is dropped into plate waste area, G) staining bottle lid is picked up from the lid guider, H) staining bottle lid is placed onto the staining bottle

The staining and imaging after staining processes of the analysis process involved only Robot 3. The flow diagram of this process is presented in Fig. 67 in Appendix 3, and images of the automation system performing this process are presented in Fig. 40. Robot 3 took the plate away from the TOF sensor and placed the plate into the vertical lift in the staining station (Fig. 40A). The vertical lift lifted the plate from the horizontal position to a vertical position (Fig. 40B). The Robot 3 then grabbed the plate using the mechanical grippers end effector from the vertical lift (Fig. 40C), brought to the staining solution bottle and immersed the plate into the staining solution bottle that was filled with a desired staining solution (Fig. 40D). Robot 3 took out the plate from the staining solution and moved the plate to the imaging after staining station. The visible light diode was turned on in the imaging after staining station and the image was taken of the stained plate (Fig. 40E). An example of the taken image in the imaging after staining station using the automation system can be seen in Fig. 39B. The

visible light diode was turned off and Robot 3 moved the plate to the plate waste pile (Fig. 40F). After that, the Robot 3 took the staining bottle lid from the lid guider in the staining station (Fig. 40G) and placed the staining bottle lid onto the staining bottle in the staining bottle rack (Fig. 40H). Robot 3 then moved back to its home position and the staining and imaging after the staining process ended, ending the whole analysis process as well. The results of the whole analysis process are either an image of a spotted and developed plate (Fig. 39A) or two images: one of a spotted and developed plate and the other image of a spotted, developed and stained plate (Fig. 39B) if the optional stained process was set to be performed.

3.4. Automation System Testing Results

Results of Test 1 are presented in Table 1 and results from Test 2 are presented in Table 2. $e_{s_{max}}$ is the maximum systematic error and $s_{r_{max}}$ is the maximum random error allowed for a liquid handling system to be considered conforming to the ISO standards. The values for both $e_{s_{max}}$ and $s_{r_{max}}$ were taken from the ISO 8655-9:2020 document [87]. By looking at Table 1 and Table 2, it can be seen that both liquid handling robots systematic errors fall between the allowed ranges for all 100%, 50%, and 10% of nominal dispensed volume, indicating that on average the liquid handling dispensed volume is accurate according to the standards raised by the ISO 8655. However, the results from Table 1 and Table 2 also show that the random error for both liquid handling robots when dispensing 100% and 50% of nominal volume were not in the range of the maximum random error, only at 10% of the maximum dispensed volume both liquid handling robots conformed to the standard, indicating that the liquid handling capabilities are not precise as they do not conform the standards raised by the document ISO 8655.

Table 1. Test 1 results for the accuracy and precision of the liquid handling robot in the synthesis part of the automation system

V_{set} (μl)	V_{avg} (μl)	e_s (μl)	$e_{s_{max}}$ (μl)	e_s (%)	s_r (μl)	$s_{r_{max}}$ (μl)	s_r (%)
6000	5966.71	-33.2918	±36	-0.55486	69.6857	18	1.16143
3000	3002.56	2.55860	±72	0.085287	44.6945	36	1.48982
600	610.693	10.6933	±360	1.78222	47.2376	180	7.87293

Table 2. Test 2 results for the accuracy and precision of the liquid handling robot in the analysis part of the automation system

V_{set} (μl)	V_{avg} (μl)	e_s (μl)	$e_{s_{max}}$ (μl)	e_s (%)	s_r (μl)	$s_{r_{max}}$ (μl)	s_r (%)
3000	3000.08	0.07980	±24	0.00266	45.9224	9	1.53075
1500	1539.18	39.1784	±48	2.6119	28.7709	18	1.91806
300	304.176	4.17592	±240	1.39197	8.95061	90	2.98354

An interesting thing to note is that the random error for the liquid handling robot in the analysis part of the automation system decreased as the dispensed volume decreased, which should be reversed as suggested by the ISO standard (ISO standard allows larger permissible errors as the dispensed volume decreases). This indicates that the liquid handling with larger volume amounts compared to lower amounts is less precise, which could be due to the construction of the linear actuator, buffer tank, tubing, tubing fittings, or syringe. Comparison of the liquid handling robots with existing commercially available liquid handling automation systems is difficult since those systems do not usually have pipetting capabilities for over 1ml, with the exception of Assist Plus by Integra having a pipette for 1.25 ml dispensing capability, and the dispensing volume of the liquid handling systems

developed in this work ranged from 0.3 ml to 6 ml. Keeping that in mind, the least accurate of the commercially available liquid handling systems was achieved by Assist Plus by Integra for 1.25 ml having 1.2% accuracy, and the highest accuracy was achieved by Liquid Dosing System by Festo for 1 ml of 0.5%. The absolute values of the percentage of systematic errors achieved in this work ranged from 0.03% to 2.61%. The least precise commercially available system was Microlab STAR V by Hamilton for 1 ml having 0.75% and the most precise systems were OT-2 by Opentrons and epMotion 5070 by Eppendorf for 1 ml having 0.15%. The percentage of random errors achieved by liquid handling robots in this work ranged from 1.16% to 7.87%. Results from Table 1 and Table 2, and the comparison of the accuracy and precision values with the existing commercially available liquid handling systems indicate that both liquid handling robots developed in this work were accurate but not precise. Precision could be improved by modifying the linear actuator of the syringe plunger to be made of sturdier materials, installing a more accurate screw lead, having a better stepper motor resolution, reducing the length of the tubing between the syringe and the syringe needle, improving the quality or changing the design of the buffer tank so that liquid easily flows in and out of the tank, ensuring gas-tightness in the liquid handling tubing, changing the liquid dispensing method from air displacement to positive displacement.

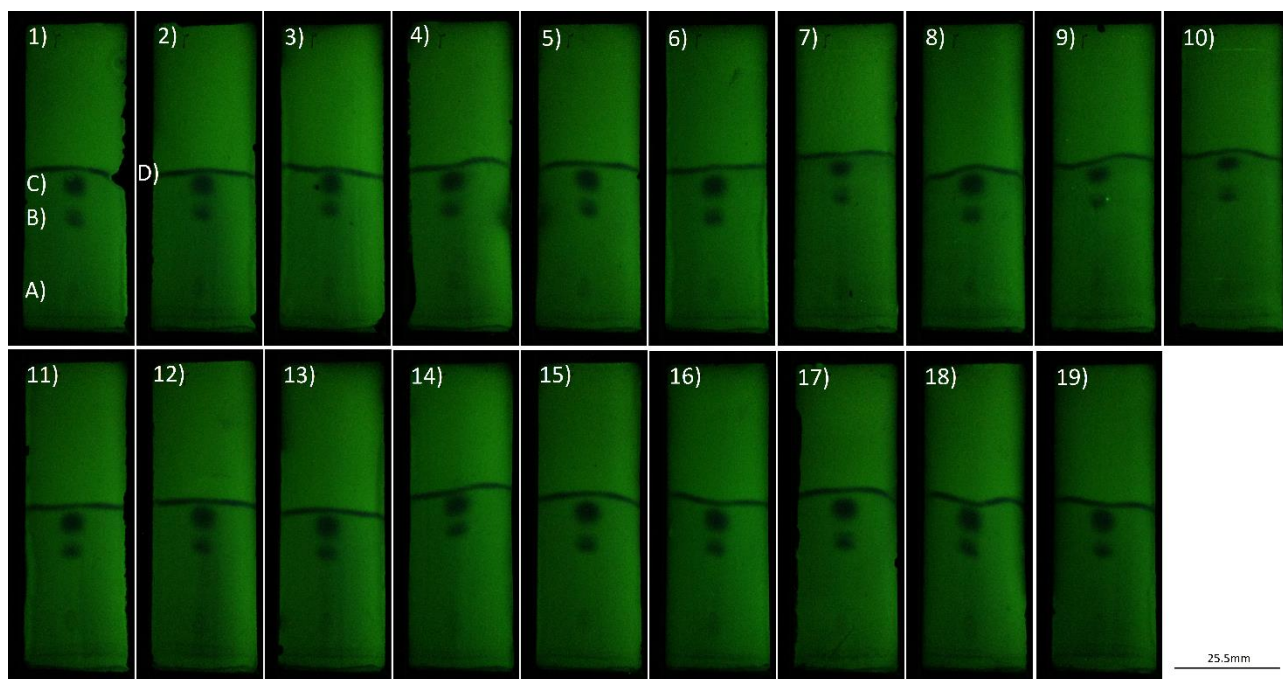


Fig. 41. Images of plates taken during Test 3: 1-19) images from 1st to 19th, A) sample spot, B) compound 1, C) compound 2, D) front line

Results of Test 3 are presented in Fig. 41, the images of the plates are presented in a sequence they were taken using the automation system from the 1st to the 19th. The initial spot can be seen in Fig. 41-A, the separated compounds of the chemical sample can be seen in Fig. 41-B and Fig. 41-C. In Fig. 41-B there are two very closely spaced compounds although they are difficult to see in the figure. The front line can be seen in Fig. 41-D, which results from the compounds that make up the plate coating also traveling with the development solution during the plate development process. The calculated coefficient of variation (CV) for compound 1 was 7.59% and 6.63% for compound 2. CV of retention factor values for compounds 1 and 2 were similar indicating that both compounds varied similarly between the plates. In literature, the CV of retention factors for plates obtained in the same laboratory range from as low as 0.2% (for example for methoxychlor, chlorfenvinphos active

ingredients) to 15.7% (for example for bromoxynil) or even higher values, even though higher values than 15% are considered atypical [96, 97]. The obtained values of CV of retention factors did not exceed the values for which in literature the repeatability would be considered atypical. From Fig. 41, it can be seen that all 19 plates were produced successfully, since for all the plates the initial spots were spotted successfully, two compounds were present in all the plates, and the front line was closely at the same place across the plates. Due to the similarity of the separated compound locations as well as the front line position on all of the plates, CV of the retention factor values not exceeding a threshold value which would be considered atypical when compared to the data in literature, it can be concluded that the automation system had enough repeatability throughout multiple analysis cycles to produce TLC analysis results.

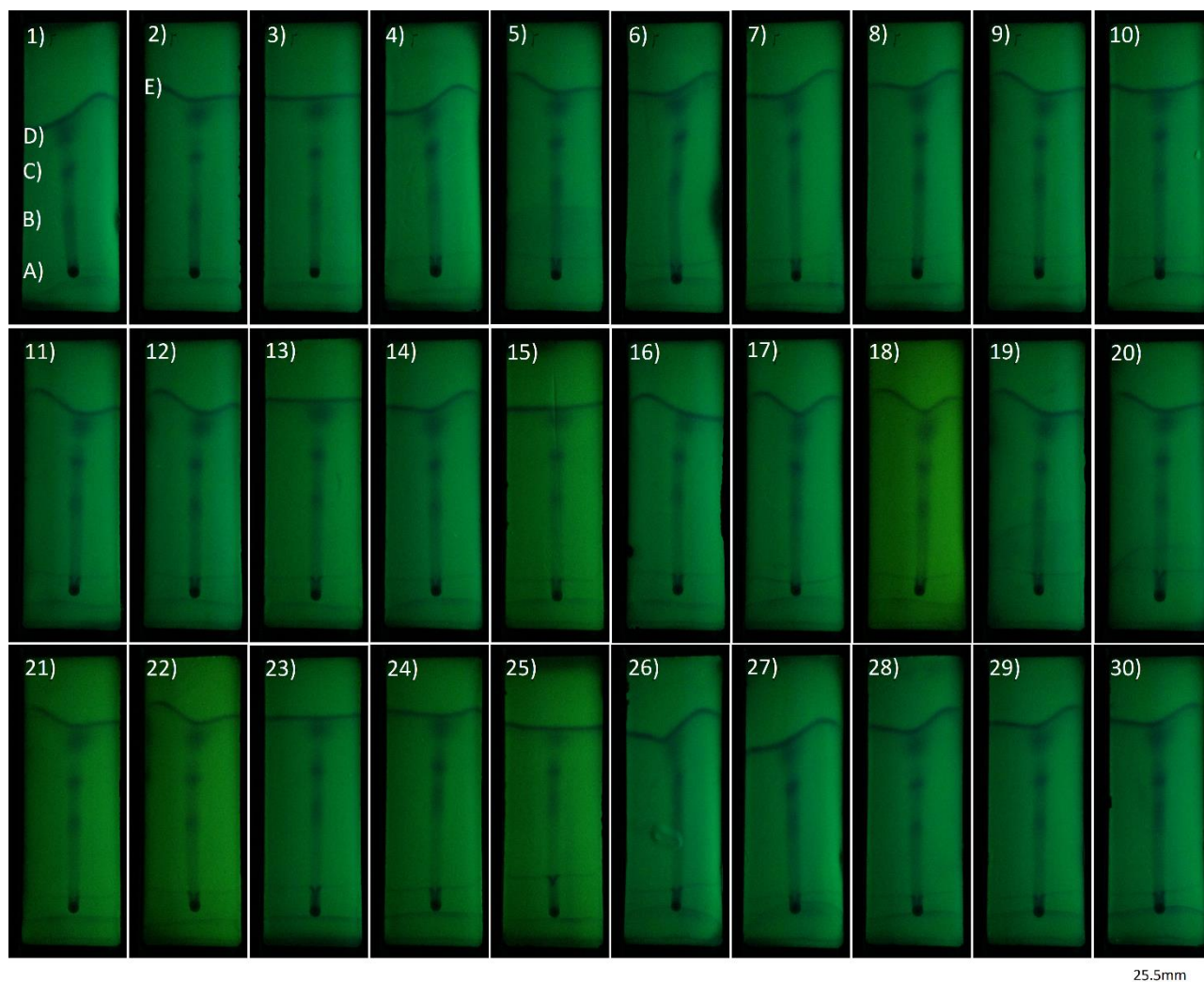


Fig. 42. Images of plates taken during Test 4: 1-30) images from 1st to 30th, A) sample spot, B) compound 1, C) compound 2, D) compound 3, E) front line

Results of Test 4 are presented in Fig. 42, the images are presented in a sequence in which the data was taken using the automation system from 1st to 30th. The initial spot can be seen in Fig. 42-A, the three compounds of the separated chemical sample can be seen in Fig. 42-B, Fig. 42-C, and Fig. 42-D, and the front line can be seen in Fig. 42-E. CV of the retention factors for compound 1 was calculated to be 11.27%, 6.24% for compound 2, and 4.51% for compound 3. CV of retention factor value for compound 1 being 11.27% which was higher compared to compound 2 and compound 3 indicates that compound 1 location on the plate varied the most out of all the compounds. This is

evident in Fig. 42 for the 1st, 2nd, and 3rd plates: compound 1 was slightly lower compared to other plates. CV of retention factor values for compounds 2 and 3 are similar being 6.24% and 4.51% indicating that both these compounds varied similarly for all the plates. Because 30 plates were successfully developed, the initial spots, the three compounds, and the front line were similarly distributed across all of the plates, CV retention factor values did not exceed a threshold value which would be considered atypical according to the data in literature, the automation system had the repeatability required to produce meaningful analytical TLC data throughout multiple analysis cycles.

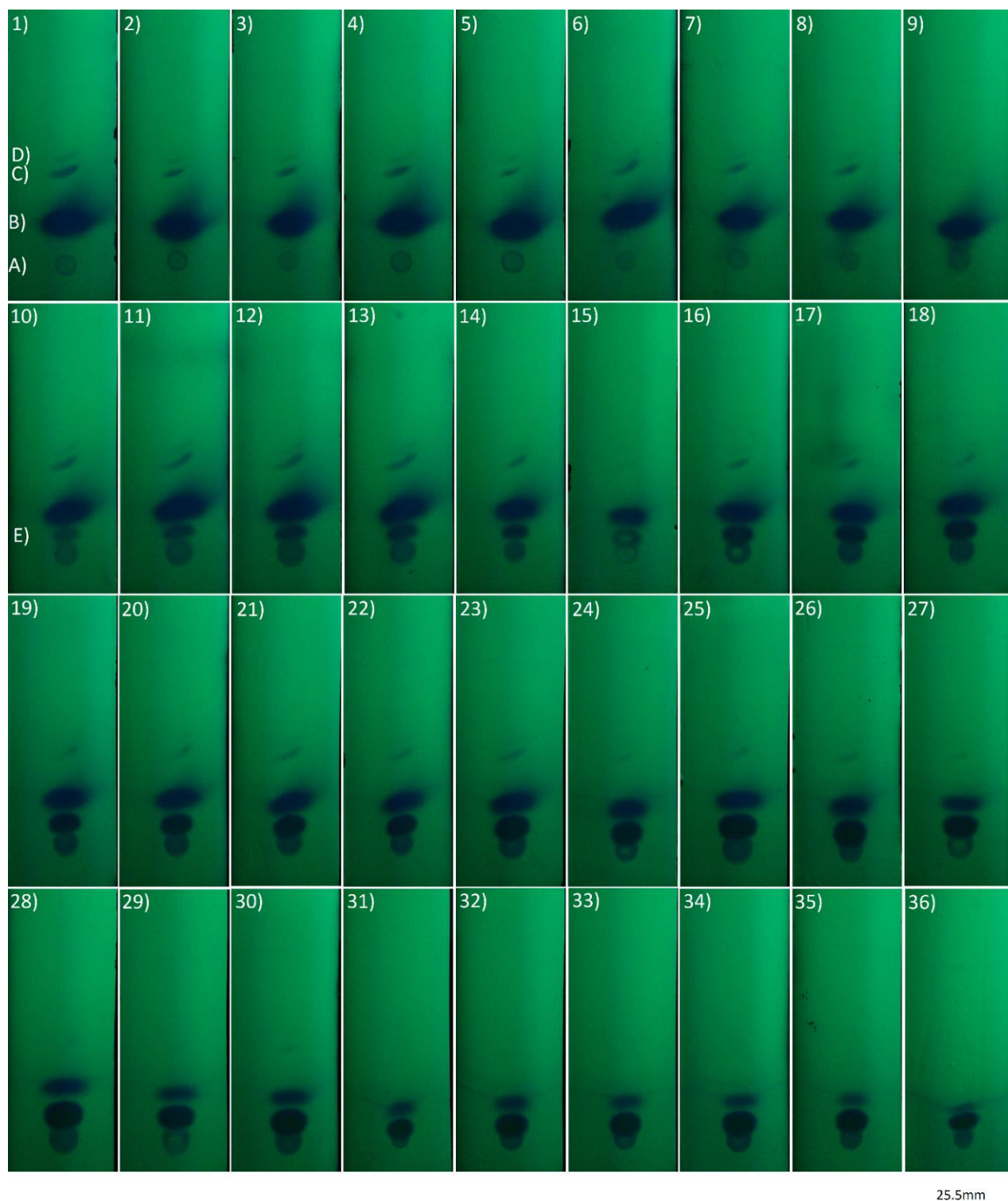


Fig. 43. Images of plates taken during Test 5: 1-36) images from 1st to 36th, A) sample spot, B) compound 1, C) compound 2, D) compound 3, E) compound 4

Results of Test 5 are presented in Fig. 43, the images are presented in a sequence that the automation system had acquired from the 1st to the 36th. The initial spot can be seen in Fig. 43-A, and the separated compounds can be seen in Fig. 43-B, Fig. 43-C, Fig. 43-D, and Fig. 43-E, the front line is not very clear but can be seen near the first compound (Fig. 43-B). In this test, a synthesis reaction was monitored using TLC. When the chemical reaction progresses, changes in the separation of the spots change, as well as new spots appear and previously visible spots disappear, as the composition of the chemical compounds changes due to the synthesis process. These changes are visible in Fig. 43: at the 8th plate, a formation of the chemical product can be seen, which is marked as compound 4 (Fig. 43-E). Additionally, compound 3 (Fig. 43-D) starts to disappear around the 18th plate, which corresponds to some chemical compound being used up in the chemical reaction. Similarly, compound 2 (Fig. 43-C) starts to disappear at around the 31st plate, also indicating that some chemical compound was used in the synthesis process. Compound 1 (Fig. 43-B) can be seen to reduce in brightness and size throughout the whole sequence of plates from 1st to 36th, indicating that the quantity of compound 1 reduced, therefore it was being used during the reaction. On the other hand, compound 4 (Fig. 43-E) can be seen to increase in brightness and size indicating that the quantity of that chemical increased, therefore a product was synthesized in the chemical reaction. All of these changes seen throughout the analysis process in Test 5 indicate that the automation system was capable of monitoring changes in an ongoing chemical synthesis reaction using the TLC analytical method. Additionally, the successful collection of the 36 analytical data points shows that the automation system has enough repeatability to monitor a synthesis reaction and to perform chemical synthesis throughout the data collection period.

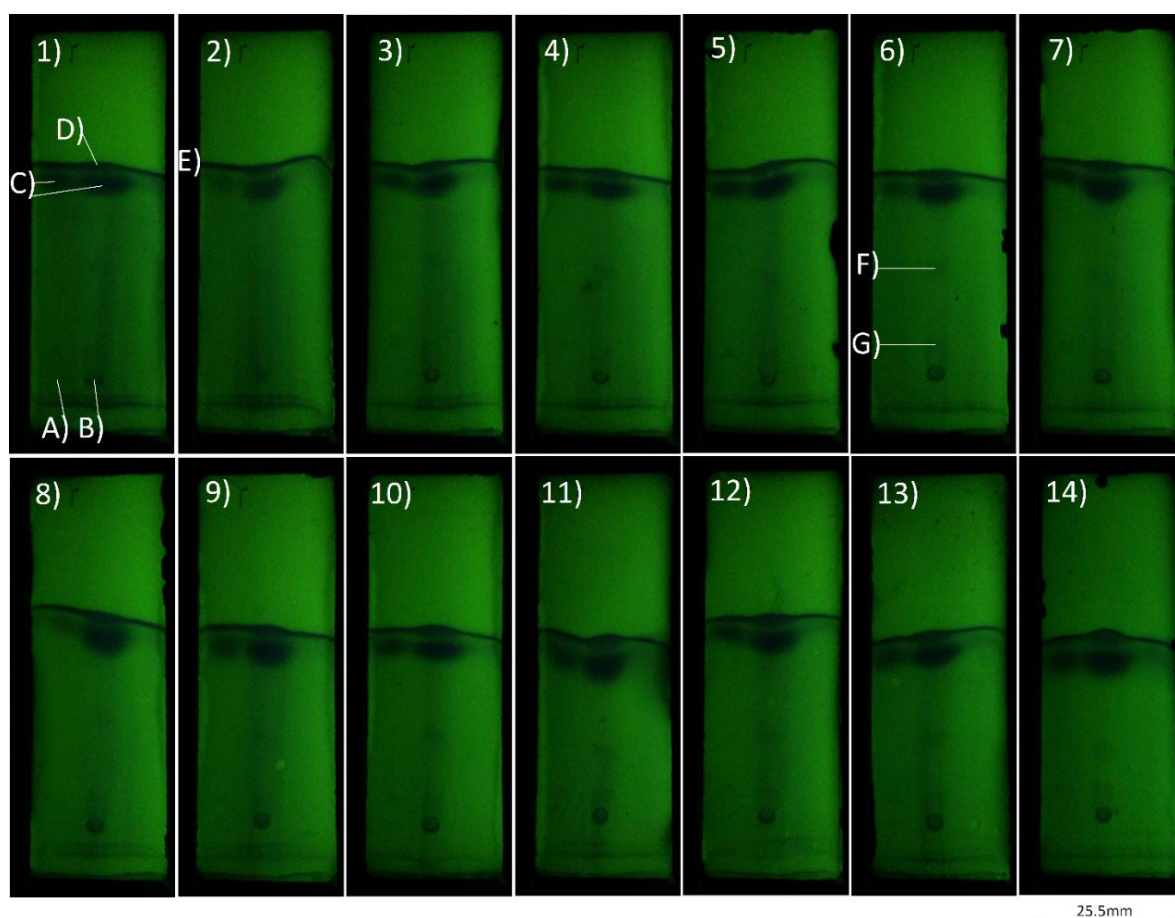


Fig. 44. Images of plates taken during Test 6: 1-14) images from 1st to 14th, A) sample spot 1, B) sample spot 2, C) compound 1, D) compound 2, E) front line, F) compound 3, G) compound 4

Results of Test 6 are presented in Fig. 44, the images are presented in a sequence in which the automation system acquired them from 1st to 14th. The 1st spot was spotted using a sample from a chemical reagent and can be seen in Fig. 44-A. The 2nd spot was spotted using a sample from the chemical reaction flask where the synthesis process was taking place and can be seen in Fig. 44-B. The compound 1 for both the 1st and 2nd spot can be seen in Fig. 44-C. The compound 2 of the 2nd spot can be seen in Fig. 44-D. The front line can be seen in Fig. 44-E. During the chemical reaction process, new compounds appeared around the 5th plate and can be seen in Fig. 44-F and Fig. 44-G, corresponding to compound 3 and compound 4. The appearance of these compounds indicates that intermediate products were formed during the synthesis process. Spots of compound 3 and compound 4 are not bright and are small in size, indicating that the synthesis process was slow and only a small quantity of these compounds were produced in the chemical reaction. The results from Fig. 44 indicate that the chemical synthesis reaction parameters or reagent concentrations need to be adjusted in order to accelerate the desired chemical product synthesis, additionally, the results also show that the automation system had the capability and enough repeatability to monitor an ongoing chemical reaction using TLC and to perform chemical synthesis.

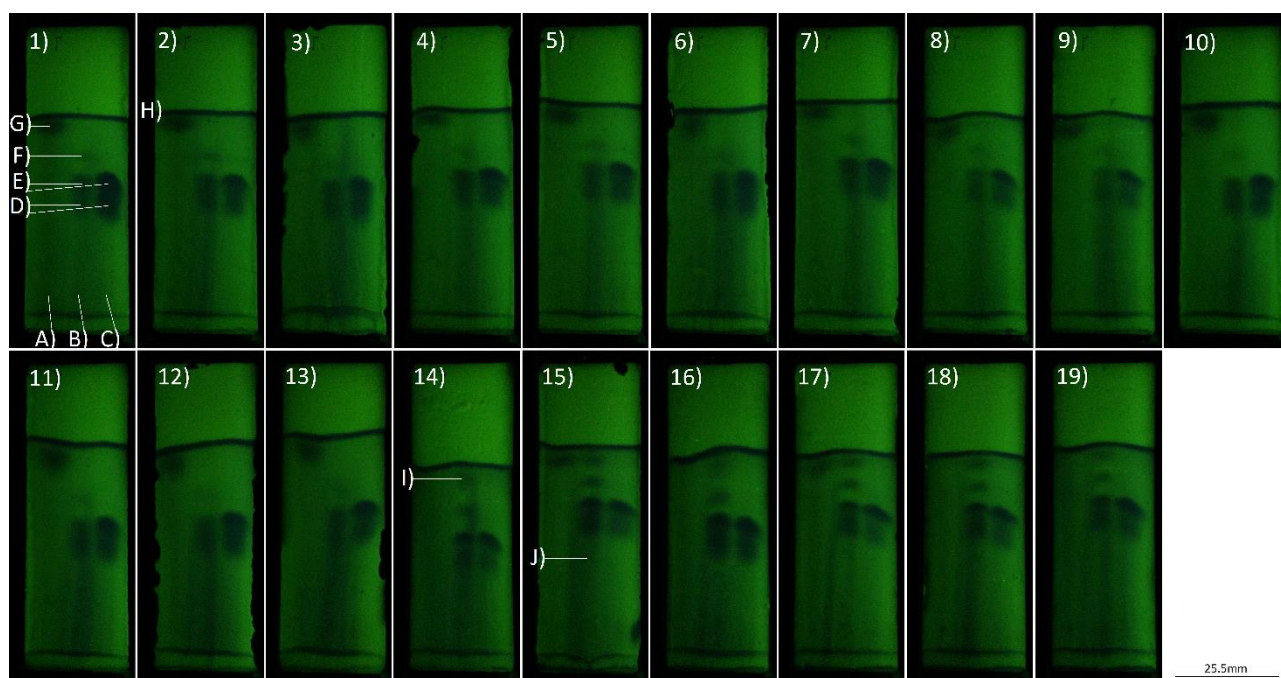


Fig. 45. Images of plates taken during Test 7: 1-19) images from 1st to 19th, A) sample spot 1, B) sample spot 2, C) sample spot 3, D) compound 1, E) compound 2, F) compound 3, G) compound 4, H) front line, I) compound 5, J) compound 6

Results of Test 7 are presented in Fig. 45, the images are placed in the sequence as they were acquired using the automation system from the 1st to the 19th. Three spots were spotted onto the plate: the 1st spot in Fig. 45-A, 2nd spot in Fig. 45-B, and the 3rd in Fig. 45-C. The 1st and 3rd spots were of the chemical reagents and the 2nd spot was the chemical sample taken from the chemical reaction flask in which the synthesis process took place. Only one compound of the 1st spot is visible and can be seen in Fig 45-G. The 3rd spot has two visible compounds in Fig. 45-D and Fig 45-E. The 2nd spot initially has three compounds in Fig. 45-D, Fig. 45-E and Fig. 45-F. At the 14th plate, another compound (compound 5) of the 2nd spot appears and can be seen in Fig. 45-I, and at the 15th plate, the final compound (compound 6) of the 2nd spot appears and can be seen in Fig. 45-J. The appearance of compounds 5 and 6 indicates that some products were produced during the chemical

synthesis reaction. Additionally, compound 3 (Fig. 45-F) throughout the process becomes brighter indicating an increase in the quantity of that compound, which indicates that compound 5 was produced in the chemical synthesis. Compound 4 (Fig. 45-G) is not visible in plates 14th, 16th, 18th, and 19th and it was determined that the reagent had run out during the process due to evaporation in the Interface 4 ml bottle, therefore refilling algorithms were updated in the control software to prevent such case from happening in the future. Results from Fig. 45 indicate that the automation system had the capacity to perform chemical synthesis and obtain TLC analysis data of an ongoing synthesis reaction.

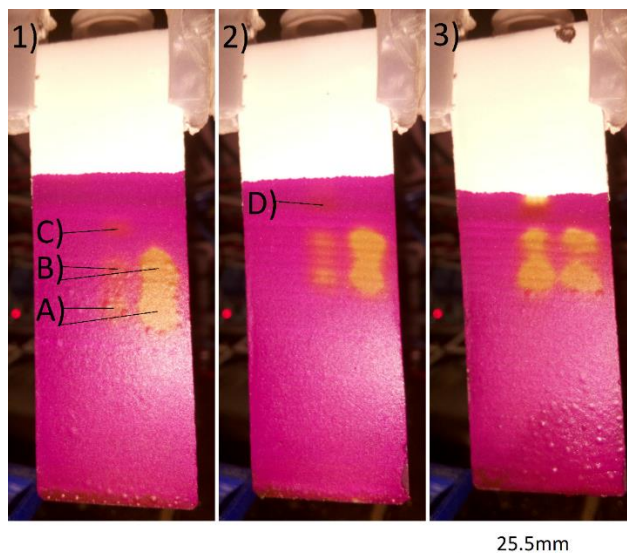


Fig. 46. Images of stained plates taken during Test 7: 1-3) images from 1st to 3rd, A) compound 1, B) compound 2, C) compound 3, D) compound 4

The analysis staining step results from Test 7 are presented in Fig. 46, the plate in Fig. 46-1 was obtained around 6 hours from the start of the synthesis, Fig. 46-2 was obtained around 12 hours, and Fig. 46-3 around 18 hours. Only specific spots and compounds are visible in the stained images that react with the staining solution, therefore for the 3rd spot two compounds are visible: compound 1 (Fig. 46A) and compound 2 (Fig. 46B). For the 2nd spot in the first plate all three compounds are visible: compound 1 (Fig. 46A), compound 2 (Fig. 46B), and compound 3 (Fig. 46C). In the 2nd and 3rd plates another compound becomes visible: compound 4 (Fig. 46D). The appearance of compound 4 indicates and confirms that products were synthesised during the reaction process. Compound 4 becomes much brighter in the 3rd compared to the 2nd image indicating that the quantity of compound 4 chemical increased. The images of the stained plate were produced under visible light illumination and from Fig. 46 it can be seen that more than 50% of the plates lengthwise were stained during the derivatization process. Results from Fig. 46 indicate that the automation system was capable of performing a synthesis reaction and was capable of monitoring a live chemical synthesis reaction using TLC with the staining step.

The comparison of the obtained normal plates and problematic plates during the testing of the automation system with the obtained plates from another research work [71] can be seen in Fig. 47. Fig. 47A shows the plates obtained using a platform that automated the TLC spotting, development and imaging processes, and was described in Chapter 2.1 (Fig. 17). Fig. 47B shows the plates obtained using the automation system that was made by the author. In Fig. 47 the plates are labeled either normal, tailing, overlapping, or having a paler color. Normal plates are those plates, in which the

spots are clearly separated and visible, and in both A and B, the normal plates are very similar. Tailing for the problematic plates occurs when the spot leaves a visible trail when the sample travels along the plate with the trail obstructing other separated spots, this effect can be seen in both A and B, however in B the effect is less visible. Overlapping occurs when the spots are very large due to the high concentration of the chemicals or too much sample in that region, and in turn, the large spots can cover other spots, this effect can be seen in both A and B. The paler color refers to the spots that are very dim and small and therefore the correct place of such spots is difficult to determine accurately, this can be seen in both A and B. Comparison of the plates obtained by the author and the plates obtained by other research group suggests that there are intrinsic issues using TLC analysis method in automation systems: tailing, overlapping and paler color of spots. These issues need to be mitigated by implementing algorithms in the automation system for correction so that the obtained data is as close to the normal TLC plate as possible: the ability to increase the concentration of the chemical samples for paler color spots, decrease the concentration of the chemical samples in spots when tailing and overlapping occurs, the addition of a recognition algorithm for detection of these problems, the addition of tailing-suppressing reagents during the analysis process to avoid tailing effect.

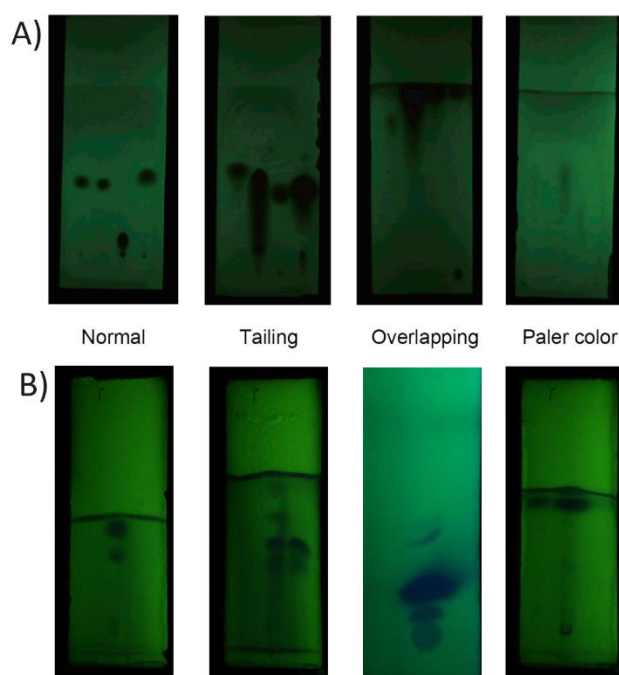


Fig. 47. Comparison of normal and problematic plates: A) plates obtained during the automation system testing, B) plates obtained by other authors using an automated platform for TLC [71]

3.5. Chapter Summary

Parts for the automation system were manufactured using extrusion and SLA printing, CNC machined, other parts were bought and the system was assembled. The control software was written in Python and Arduino languages to control Raspberry Pi microprocessors, Arduino microcontrollers, three delta robots, heating mantle and other equipment. The working principles of the automation system were described for the main processes of heating/stirring, synthesis, and analysis. Automation system testing results for Tests 1-7 were provided. The values of systematic and random errors during liquid handling obtained during Test 1 and Test 2 and the comparison of these values with other existing automation systems and ISO standards showed that the automation system was accurate but

not precise in terms of liquid handling. The images and calculated values of variation coefficients of the retention factors obtained during Test 3 and Test 4 and comparisons with variation coefficient values from literature showed that the automation system had enough repeatability to produce meaningful TLC data. Data obtained during Tests 4, 5, and 6 showed that the automation system was capable of performing chemical synthesis and monitoring of ongoing synthesis reactions using the TLC method. A comparison of TLC plates obtained using the automation system with an automation system for TLC from other authors showed that similar normal and problematic plates were obtained in both systems.

4. Cost Calculation

The cost calculation for the different parts of the automation system was performed and can be seen in Table 3. Prices of each part were taken on April 16th 2024. Total cost was calculated by looking up the price of the unit of a particular part and then multiplying it by the unit amount that was required for the assembly of the system. If a part was obtained in Lithuania, the total cost does include tax, if a part was obtained not in Lithuania, the cost is calculated with the value that the seller provided. All costs were calculated excluding shipping and import costs. The total cost values for the 3D printed parts with extrusion were calculated by using the estimated mass values of the supported and sliced parts in Cura software [98]. In Cura, for all the parts the same parameters of 60% infill and 40% support infill were used. The price of the poly(ethylene terephthalate glycol) (PETG) filament was taken to be 20.9 euro for 0.85 kg from Anodas website [99]. The total cost values of 3D printed parts with SLA were calculated by using the estimated values of the supported and sliced parts in Chitubox slicer software [100]. The density of the SLA resin was taken to be 1.1 g/ml and the cost was taken to be 43.9 euro for 1100 g. The cost was taken from Anodas website for a 1000 ml water-washable UV resin [101]. For the calculations of the cost of materials for the CNC machined parts, the volume ratio of the part with the total volume of the material was multiplied by the cost of the material. For the POM material of 30 mm thickness, the dimensions of the material were taken to be 30x1000x2000 mm plate costing 1380.07 euro, cost taken from e-Plastena website [102]. For phenolic paper laminate, the dimensions of the material were taken to be 5x1050x2050 mm costing 181.43 euro, price taken from e-Plastena website [103]. For the PP material of 20 mm thickness, the dimensions were taken to be 20x1500x3000 mm costing 516.79 euro, price taken from e-Heliopolis website [104]. For the PP material of 12 mm thickness, the dimensions were taken to be 12x1500x3000 mm costing 310.55 euro, cost taken from e-Heliopolis website [105]. For the PP material of 10 mm thickness, the dimensions were taken to be 10x1500x3000 mm costing 258.84 euro, cost taken from e-Heliopolis website [106]. For the PP material of 5 mm thickness, the dimensions were taken to be 5x1500x3000 mm costing 157.04 euro, the price taken from e-Heliopolis website [107]. The cost calculations do not include the electrical power cost of the 3D printers and the CNC machine, nor the consumables cost for the 3D printing post-processing or CNC machine parts like end mills, or the cost for the development of the web server. The only part that includes the labour cost is the liquid dispensing robot end effector buffer tanks (number 47 in Table 3) since the manufacture of these parts was outsourced.

Table 3. Part list with total costs of the automation system

	Automation system part	Material / part name	Material / part description	Unit size	Unit amount	Total cost, eur
1	frame type 1	aluminum extrusion profile	20x20 mm V-slot	1 m length	33.5	461.30
2	frame type 2	aluminum extrusion profile	20x40 mm V-slot	0.5 m length	3	22.50
3	frame type 3	aluminum extrusion profile	20x80 mm V-slot	0.5 m length	1	11.00
4	frame mounting part: brackets	bracket	JJ-2020	1	200	56.00
5	frame mounting part: slide T-nuts	slide T-nut	LE-20-M5	1	400	44.00
6	frame mounting part: profile connection L plate	L plate	L plate 20S	1	5	8.15

7	frame mounting part: screws	screw	M5x8 mm	25	16	49.60
8	rack to baseplate mounting brackets	L-bracket	40x40x2 mm stainless steel L- bracket	100	0.06	6.72
9	wood screws	wood screw	3.5x25 mm	500	1	9.45
10	screws type 1	low profile M5 screw	M5x15 mm	25	1	3.80
11	screws type 2	low profile M5 screw	M5x25 mm	25	1	4.00
12	screws type 3	low profile M5 screw	M5x50 mm	25	1	5.36
13	screws for interface 4 ml bottle rack guiding plate	M4 screw	M4x40 mm	100	0.03	0.21
14	electrical wires type 1	control cable	LIYCY 2X1.5 mm ² , flexible, shielded	1 m length	48	62.40
15	electrical wires type 2	control cable	LIYCY-JZ 4X1.0 mm ² , flexible, shielded	1 m length	48	72.00
16	electrical wires type 3	control cable	BIT500 10X1.0 mm ² (YSLY), flexible, shielded	1 m length	24	71.76
17	electrical wires type 4	M-F terminals electrical wire	M-F, 40 mm	40	3	10.80
18	electrical wires type 5	copper cable, black color	H07V-K 1,5 mm ² , flexible	1 m length	18	6.12
19	electrical wires type 6	copper cable, red color	H07V-K 1,5 mm ² , flexible	1 m length	18	6.12
20	electrical wires type 7	signal wire	CA-40/22 4X0,22 mm ²	1 m length	18	4.50
21	electrical wires type 8	control cable	LIYCY 3X1.0 mm ²	1 m length	54	70.20
22	electrical wires type 9	M-M terminals electrical wire	M-M, 40 mm	40	3	10.80
23	500 ml bottles	chemical reagent bottle of 500 ml	borosilicate glass, autoclavable, polipropilene ring seal	1	18	99.54
24	250 ml bottles	chemical reagent bottle of 250 ml	borosilicate glass, autoclavable, polipropilene ring seal	1	22	100.98
25	chemical reaction flask type 1	chemical reagent bottles of 100 ml	borosilicate glass, autoclavable, polipropilene ring seal	1	1	4.07
26	chemical reaction flask type 2	chemical reagent bottles of 250 ml	borosilicate glass, autoclavable, polipropilene ring seal	1	1	4.59
27	chemical reaction flask type 3	chemical reagent bottles of 500 ml	borosilicate glass, autoclavable, polipropilene ring seal	1	1	5.53

28	4 ml bottles	chemical reagent bottles of 4 ml	borosilicate glass, 15 mm diameter opening, 45 mm height	100	0.06	1.42
29	120 ml bottles	container for chemicals of 120 ml	plastic, 52 mm diameter opening	1	31	11.47
30	heating/stirring mantle	heating/stirring mantle	Heidolph MR Hei-Connect	1	1	1307.00
31	rail	linear guide set	HGR20 and SFU1605, 1500 mm	1	3	436.20
32	stepper motors type 1	stepper motor	JK57HS76-2804, 1.9Nm, NEMA 23	1	3	94.50
33	stepper motor controllers	stepper motor controllers	DM556	1	7	188.30
34	limit switches	limit switch	VT16031C	1	4	6.68
35	linear actuator parts: couplings to stepper motor	lead screw coupling to a stepper motor	Flexible Coupling for Stepper Motor 5x8x25 mm	1	3	10.20
36	linear actuator parts: bearing	linear bearing	LM8UU	1	6	13.80
37	linear actuator parts: nuts	brass nut for T8 lead screw	T8	1	3	14.40
38	linear actuator parts: guiding rods	guiding rod	T8x1000 mm Smooth Rod	1000 mm length	0.9	10.80
39	linear actuator parts: lead screws	lead screw	T8x600 mm Trapezoidal Lead Screw	600 mm length	0.67	8.71
40	delta robots	delta robot	DeltaX2 from DeltaX	1	3	1954.80
41	stepper motors type 2	stepper motor	17HS19-2004S1, Nema 17	1	4	56.92
42	1 ml syringe	gas-tight glass syringe of 1 ml	ILS RQ Replacement Syringes, 1.0 ml	1	1	95.00
43	10 ml syringe	gas-tight glass syringe of 10 ml	Hamilton 1010TLL, 10 ml	1	1	170.61
44	5 ml syringe	gas-tight glass syringe of 5 ml	Hamilton 1005TLL, 5 ml	1	1	153.67
45	load cells	metal deformation weight sensor	5kg maximum value, 80x12.7x12.7 mm, analog, 5-10V DC	1	3	17.10
46	glass funnel for chemical waste collection	glass funnel	glass, 75 mm diameter top	1	1	2.52
47	liquid dispensing robot end effector buffer tanks	buffer tank	machined using a lathe by a third party from POM material	1	2	120.00
48	flangeless fittings	flangeless fitting	1/4-28 male Upchurch nut and ferrule for 1/16 in. O.D. tubing	5	1.6	120.00

49	tubing type 1	PTFE tubing	50 ft L, 1/16 in. O.D., 0.023 in. I.D.	50 ft length	0.23	21.76
50	tubing type 2	silicone tubing for chemical waste collection	8 mm O.D., 5 mm I.D.	5 m length	0.4	8.15
51	syringe needle type 1	metal syringe with Luer lock	14 G, 20 cm	1	1	12.99
52	flangeless fitting connectors to syringe	HPLC fittings	Luer adapter, female Luer to 1/4-28 FEM	1	3	38.70
53	waste container	chemical bottle of 2000 ml	borosilicate glass, autoclavable, polipropylene ring seal	1	1	13.65
54	clamp for tubing	tube clamp	8-10 mm clamp	2	0.5	1.00
55	rail stepper motor mounts	stepper motor mount L-type	NEMA 23, L Type	1	3	39.60
56	buffer tank to needle adapter	adapter from male Luer lock to M6	adapter male Luer lock, screw thread M6, Delrin	1	2	25.70
57	belt	belt	GT2 6 mm rubber belt	1 m length	1.6	3.52
58	carriage	carriage	Mini V Gantry Set	1 set	1	26.50
59	carriage drive pulley type 1	timing drive pulley	GT2 Aluminum Timing Drive Pulley 20 Teeth – 5 mm Bore	1	1	3.70
60	carriage drive pulley type 2	timing pulley	GT2 Timing Pulley 20 Teeth - 6.35 mm Bore	1	1	2.10
61	plate handler gripper parts 1	bearing	Xtreme Mini V Wheel Kit	1	1	5.00
62	servo motor type 1	servo motor	Servo TowerPro SG90 - Micro	1	4	14.00
63	spotting station plate fan	direct current centrifugal fan	Centrifugal Blower Fan - 40x40x20 mm – 24 V DC	1	1	7.92
64	camera	Raspberry Pi camera	Raspberry Pi Camera V2 8 MP	1	2	63.20
65	imaging station UV lighting	UV lamp	4 W 254 nm UV lamp	1	1	3.48
66	servo motor type 2	servo motor	Servomotor 18.3 kg/cm (6 V) 180°	1	2	35.98
67	chromatography plate detector parts 1	TOF sensor	VL53L4CD TOF distance sensor - 1-1300 mm, Adafruit 5396	1	1	27.30
68	imaging after staining station visible lighting	led	LED, power 3 W warm white, 3000 K 210 lm, 140°, STAR	1	1	3.50

69	camera cables	ribbon cable	Raspberry Pi Zero Camera Adapter – 150 mm	1	2	7.00
70	UV lamp driver	UV lamp driver	QT-ECO-1X4-16/220-240-S - Electronic Control Gear 16 W, Osram	1	1	20.66
71	microprocessor	Raspberry Pi	Raspberry Pi 4B WiFi 4GB RAM set with accessories	1	2	257.40
72	microcontroller type 1	Arduino Uno	Arduino Uno R3	1	2	62.80
73	microcontroller type 2	Arduino Mega	Arduino mega R3 2560	1	1	59.00
74	servo motor controller	servo motor controller board	PCA9685, 16-channel, 12-bit PWM I2C, Adafruit 815	1	1	32.20
75	USB hub	USB hub	USB A socket x4, LOGILINK, external power supply	1	1	33.19
76	5 V power supply	5 V power supply	5 V, 22 A, 110 W	1	1	28.30
77	24 V power supply	24 V power supply	24 V, 13.4 A, 320 W	1	1	56.70
78	uninterruptible power supply	UPS	UPS Tecnoware ERA PLUS 1200, 840 W	1	1	85.50
79	emergency button	emergency stop mushroom-type button	turnable ON-OFF, HIGHLY	1	1	5.57
80	electrical enclosure	electrical enclosure	500x500x250 mm, IP66, 1.5 mm thickness steel	1	1	98.99
81	relays	8-channel relay module	logic signal voltage: 5 V DC	1	1	7.80
82	analog-to-digital converter	ADC for loadcell	HX711 load cell module	1	3	9.30
83	USB extension cable	USB extension cable	USB AM - USB AF (2.0)	1	2	9.20
84	syringe needle type 2	hypodermic needle	22 G, 50 mm	100	0.01	0.22
85	baseplates	plywood	film-faced plywood, 18 mm thickness		1	32.59
86	chemical containers racks	plywood	film-faced plywood, 18 mm thickness		1	18.69
87	baseplate for heating mantle	plywood	film-faced plywood, 18 mm thickness		1	1.74
88	baseplates for the analysis part of the automation system	plywood	film-faced plywood, 18 mm thickness		1	11.95
89	limit switch mounts	3D printed using extrusion	PETG material		4	1.08

90	spacers for mounting delta robots	3D printed using extrusion	PETG material		9	3.32
91	10 ml syringe linear actuator mount parts	3D printed using extrusion	PETG material		1	3.91
92	1 ml syringe linear actuator mount parts	3D printed using extrusion	PETG material		1	7.47
93	5 ml syringe linear actuator mount parts	3D printed using extrusion	PETG material		1	4.11
94	syringe needle detector mount	3D printed using extrusion	PETG material		1	0.44
95	interface carriage to frame mounting parts	3D printed using extrusion	PETG material		1	2.29
96	interface rack to carriage mounting part	3D printed using extrusion	PETG material		1	3.29
97	plate handler gripper parts 2	3D printed using extrusion	PETG material		1	0.81
98	spotting station fan guider	3D printed using extrusion	PETG material		1	1.20
99	imaging station enclosure	3D printed using extrusion	PETG material		1	12.61
100	development station horizontal development chamber parts 1	3D printed using extrusion	PETG material		1	1.43
101	development station horizontal development chamber lid mount	3D printed using extrusion	PETG material		1	1.18
102	chromatography plate rack parts	3D printed using extrusion	PETG material		2	14.16
103	vertical lift parts	3D printed using extrusion	PETG material		1	0.74
104	chromatography plate detector parts 2	3D printed using extrusion	PETG material		1	0.91
105	imaging after staining station mounting parts	3D printed using extrusion	PETG material		1	3.00
106	vacuum line silica-gel filter	3D printed using SLA	SLA resin material		1	0.24
107	stain bottle lid guider	3D printed using SLA	SLA resin material		1	0.66
108	interface 4ml bottle rack guiding plate	CNC machined	Phenolic paper laminate material, 5 mm thickness		1	0.39
109	tubing splitter	CNC machined	POM material, 30 mm thickness		1	1.13
110	development station horizontal development chamber parts 2	CNC machined	POM material, 30 mm thickness		1	5.92
111	interface 4 ml bottle rack	CNC machined	PP material, 20 mm thickness		1	0.86
112	spotting station plate guider	CNC machined	PP material, 12 mm thickness		1	0.34
113	spotting station cotton pad mount	CNC machined	PP material, 12 mm thickness		1	0.23
114	development station horizontal development chamber parts 3	CNC machined	PP material, 10 mm thickness		1	0.07

115	staining solution container lids	CNC machined	PP material, 5 mm thickness		1	1.66
					Total:	7275.60

From Table 3 it can be seen that the largest cost of the automation system were the three delta robots used for liquid handling and plate transportation costing a total of 1954.8 euros. The cost of the delta robots cannot be easily reduced since already the cost of these delta robots with closed-loop stepper motors was much lower compared to other smaller-sized delta robots with minimal payload (for example one 3-axis delta robot for 5 kg payload costing 3732.62 euro from Igus [108]). The type of robots used could be changed from delta to Cartesian for reduction of the total cost of the system. The second biggest cost was the stirring/heating mantle for 1307 euros. The heating mantle used in this work was Heidolph MR Hei-Connect which had stirring RPM and temperature parameter control through an RS-232 interface. The cost of the mantle could be reduced by making a custom mantle that uses a motor with an RPM control for stirring and a heating element with temperature control for heating. Cost for other parts cannot be easily reduced, since either the parts were already made using 3D printing technologies, CNC machined or are functionally important, like gas-tight syringes and chemically resistant tubing and fittings.

The cost of the automation system developed in this work is difficult to compare with other existing commercially available and non-commercially but open-sourced automation systems for liquid handling and for the TLC analysis since there are no commercially available fully automated systems combining both synthesis and TLC analysis, therefore the cost comparison was done by comparing the costs of chemical synthesis automation systems and TLC analysis automation systems that automate one or multiple steps of TLC method separately. The cheapest commercially available liquid handling system that could be used for chemical synthesis is OT-2 Robot by Opentrons with a single channel pipette configuration, costing around 12500 euros [21]. The most expensive of the liquid handling systems explored in this work was Microlab Star V from Hamilton, costing around 160000 euro [109]. The automation system for the TLC spotting step called Automatic TLC SAMPLER 4 by CAMAG [41] costs around 40000 euros [110]. The estimated cost of the TLC spotting, developing, and imaging automation system that uses two arm robots [71] that was explored in Chapter 2.2 was calculated by combining the cost of both arms and the gripper. The cost of AUBO Robotics i5 is 18000 euros [111], DOBOT MG400 is around 3200 euros [112] and the gripper is around 3200 euros [113], therefore the total cost of the automation system is around 24400 euros. The total cost of the automation system developed in this work is 7275.6 euro, which is lower than the cheapest commercially available option for the automation system of liquid handling for performing chemical synthesis and the cheapest non-commercially available but open-source platform for TLC analysis spotting, development and imaging steps. Based on that, the automation system developed in this work can be considered low-cost.

For the operational costs of the automation system, firstly the cost of consumables should be considered. The first consumables are the cotton pads used for cleaning the syringe tip during the spotting of the sample onto the TLC plate. 3 pads are required to be mounted onto the automation system and then changed after 24 hours. The total cost of the pads is 0.085 euro for a 24-hour period. The second consumable is a film called parafilm that is put on top of the chemical containers so that the chemicals evaporate more slowly. To cover every container in the automation system, 1.85 m of 10 cm width parafilm is needed, which costs 2.47 euro. Parafilm can only be used once and needs to be changed if the chemical container is refilled or changed to another. Thirdly, the automation system

requires TLC plates in order to function. The system can perform about a maximum of 40 plates during a 24-hour period. The author in this work used custom-made plates by combining silica gel, a binder, and other chemicals to manufacture a TLC plate. However, to estimate a price, commercially available plates were used for the price calculations. Using 25x70 mm sized plates from Supelco [114], the cost of 40 plates for a 24-hour period of using the automation system would cost about 41.2 euro. Fourthly, the system requires 2970 ml of some chemical solution which the system will consume during its operation for cleaning, as well as for dilution of chemical samples for analysis for a period of about 24 hours. Many different chemical solutions can be used, for example, acetone, ethylacetate, or methanol. 2970 ml of acetone would cost about 7.72 euros, 14.84 euros of ethylacetate, or 3.86 euros of methanol. The costs for the solvents were taken from Lerochem website [115]. Lastly, the system requires reagents used for the chemical synthesis and analysis. Since the reagents are specific to a particular synthesis reaction and TLC analysis, the cost of the chemicals would need to be looked at on a case-by-case basis and calculated depending on the application.

4.1. Chapter Summary

The cost calculation was performed for every part of the automation system developed in this work including purchased, outsourced, 3D printed, and CNC machined parts. The total cost was 7275.6 euro. Cost reduction could be done by manufacturing a custom heating and stirring mantle, and changing the robot types from delta to Cartesian. Operational costs of the automation system included cotton pads, parafilm, TLC plates, chemical solutions for cleaning and chemical sample dilution, and chemical reagents for synthesis and analysis. The cost of the automation system was compared to existing commercially available liquid handling systems as well as non-commercially available TLC automation systems. The cost of the system developed in this work was lower compared to other systems, therefore the automation system developed in this work was considered low-cost.

Conclusions

1. Commercially available liquid handling automation systems used for chemical synthesis had dispensing accuracies from 0.5% to 1.2% and precisions from 0.15% to 0.75% for 1 ml dispensing volumes. Commercially available and non-commercially available systems used piston air displacement or piston positive displacement dispensing methods, Cartesian robots, and consumable pipetting tips or syringe needles. The main analytical methods employed in commercially available chemical analysis automation systems were spectrometry or chromatography. For automation of thin layer chromatography, commercially available systems automated each step of the analysis method separately: spotting, development, imaging or staining, and a non-commercially available but open-sourced system combined three processes: spotting, development, and imaging by utilizing two robotic arms.
2. Restrictions and functional requirements were raised for the automation system: a budget of 8000 euros, the use of non-industrial robots, chemical synthesis automation using a liquid handler and a heating mantle, thin layer chromatography analysis automation with spotting, development, imaging and staining steps using a liquid handler and a thin layer chromatography plate transporter and manipulator, and an interface capable of transferring liquid chemical samples between the synthesis and analysis parts of the system. The automation system was designed using Fusion 360 software, and parts were manufactured using 3D printing and CNC machining for cost reduction.
3. The mechanical and electrical parts were assembled to combine the synthesis, analysis and interface in one automation system. The automation system used three delta robots mounted on rails, two robots were used for liquid handling employing piston air displacement dispensing method and one robot was used for the thin layer chromatography plate transportation utilizing two end effectors: a vacuum suction cup and a mechanical gripper. Three adaptive processes using a distance sensor and scales were used for detecting end effectors. A heating mantle, fans, cameras, lights, and servo motors were utilized for the different parts of the system to automate chemical synthesis and thin layer chromatography analysis processes. Raspberry Pi microprocessors and Arduino microcontrollers were used for the control of the system equipment.
4. A control software was written in Python and Arduino languages to include the serial communication between the microprocessors and microcontrollers, communication with the web server, generation of G-code and tracking of three delta robots, control for the heating mantle, stepper motors, relays, cameras, distance and weight sensors. Control software of the main microprocessor included process threading and a message queue system allowing the automation system to perform a sequence of received instructions and providing the ability to perform multiple instructions simultaneously. The control software could be interacted with through a user interface for initialization, debugging and maintenance of the automation system and through a web server for instruction creation, automation system process tracking, and analytical data visualisation.
5. Testing of the accuracy and precision of the liquid handling robots used in the automation system was done according to ISO 8655 and ISO 23783 standards. Calculated systematic error values ranged from 0.03% to 2.61% which conformed to the ISO standard, random error values ranged from 1.16% to 7.87% and only conformed when dispensing 10% of the maximum volume, indicating that liquid handling was accurate but not precise. Furthermore, the repeatability of the system to perform analysis of chemical samples using thin layer chromatography was tested by calculating coefficients of variation of retention factors obtained from the images of the developed

plates. The obtained coefficient of variation values for different compounds ranged from 4.51% to 11.27% which were not atypical compared to different literature sources, indicating that the automation system had repeatability for obtaining thin layer chromatography analytical data. Lastly, the capability of the system to perform chemical synthesis and to monitor a synthesis reaction using thin layer chromatography was tested. Formation of synthesis products and usage of chemical reagents was identified from the obtained chromatography analytical data, indicating that the automation system was capable of performing synthesis and chemical reaction monitoring using thin layer chromatography.

6. The cost of the automation system parts and materials was calculated to be about 7300 euro. The cost of the system was compared to other commercially available synthesis automation systems ranging from 12500 to 160000 euros, to commercially available thin layer chromatography spotting step automation system costing 40000 euros, and to a non-commercially available but open-sourced thin layer chromatography spotting, development and imaging automation system costing 24400 euros. The cost of the system developed in this work was lower than every other system, therefore it was determined to be low-cost.

Recommendations

1. Robots for liquid handling could be changed from delta to Cartesian and the dispensing method could be changed from air displacement to positive displacement. After these changes, the system could be evaluated using methodology from ISO 23783 and checked if the liquid handling capabilities conform to the ISO 8655 standards.
2. A thin layer chromatography – mass spectrometry interface could be added to allow the selective chemical compound characterization using mass spectrometry from the developed thin layer chromatography plates. This would allow quantitative evaluation of the capabilities of the automation system to perform chemical synthesis and would expand the applications of the system to include mass spectrometry of selective compounds.
3. The cost of the automation system could be slightly reduced by manufacturing a custom heating and stirring mantle that uses a motor with an RPM control for stirring and a heating element with temperature control for heating.
4. A reflux condenser could be added to the chemical reaction flask in order to allow the chemical synthesis reactions to occur at higher temperatures extending the applications of the automation system.
5. A cover for the automation system could be made to allow the injection of nitrogen gas for the creation of an inert atmosphere. An inert atmosphere would minimize the contamination from reactive gases as well as ensure that substances are below their flammability limits.
6. The reliability of the automation system could be determined by evaluating which parts of the system needed the most maintenance over longer periods of use (over 1 year).
7. The user and admin experiences using the user interface of the control software and the web page could be analyzed to find areas of improvement.

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Appendices

Appendix 1. Automation System Drawings

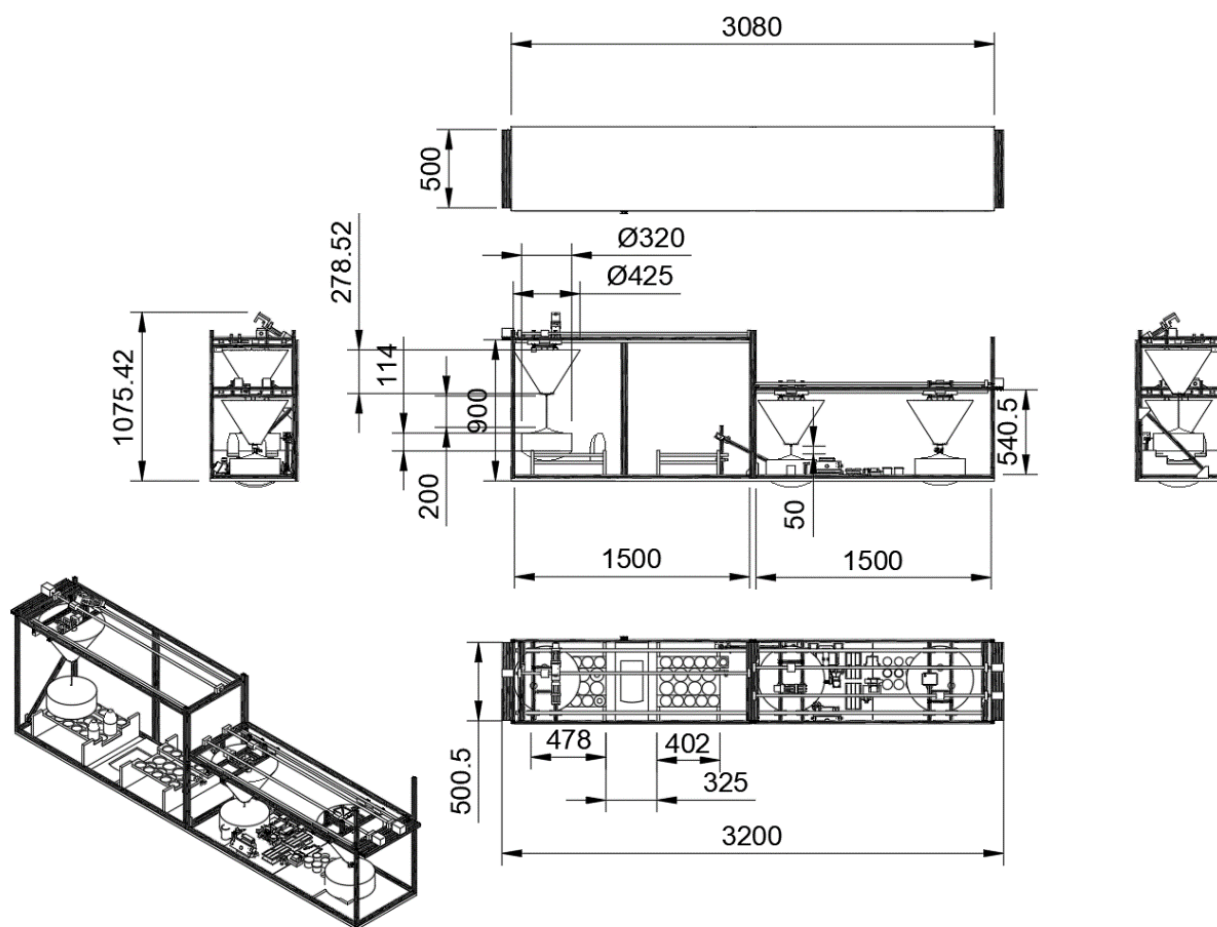


Fig. 48. Main dimensions in mm of the automation system

Appendix 2. Automation System Control Software

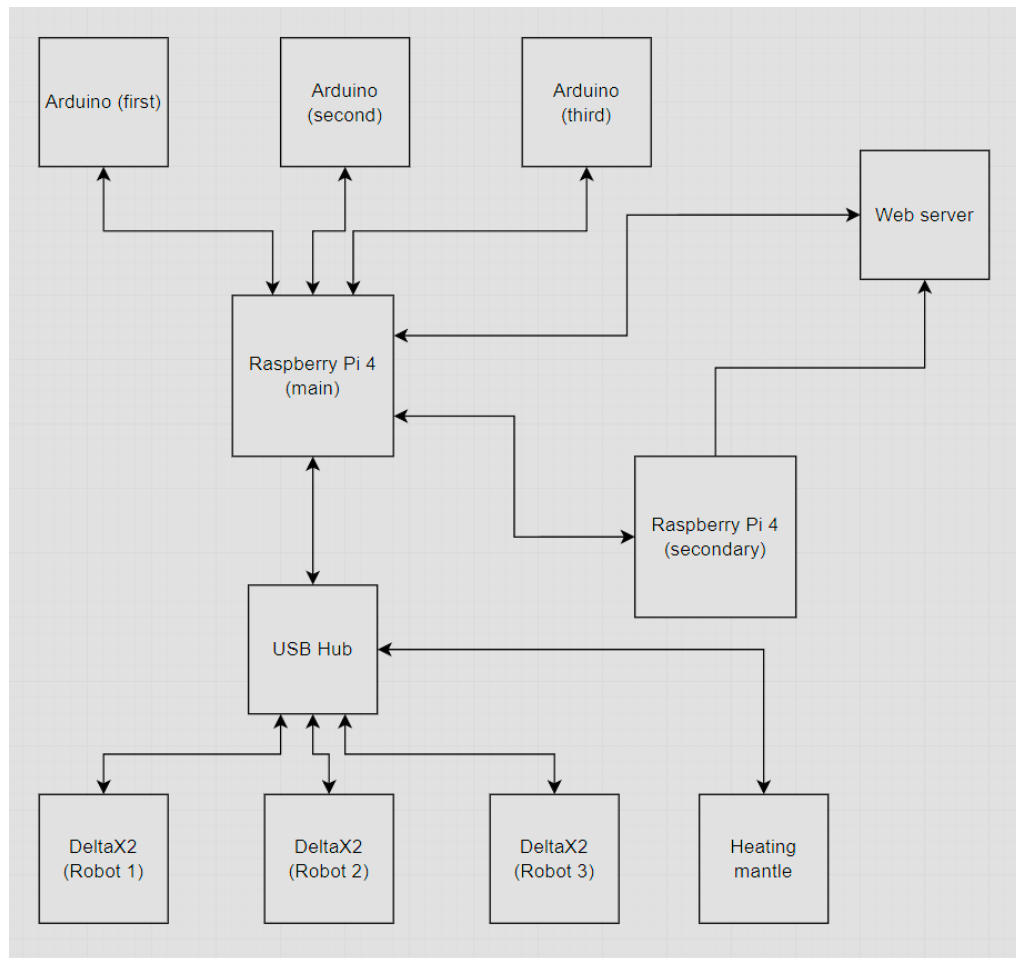


Fig. 49. Data flow diagram of the automation system

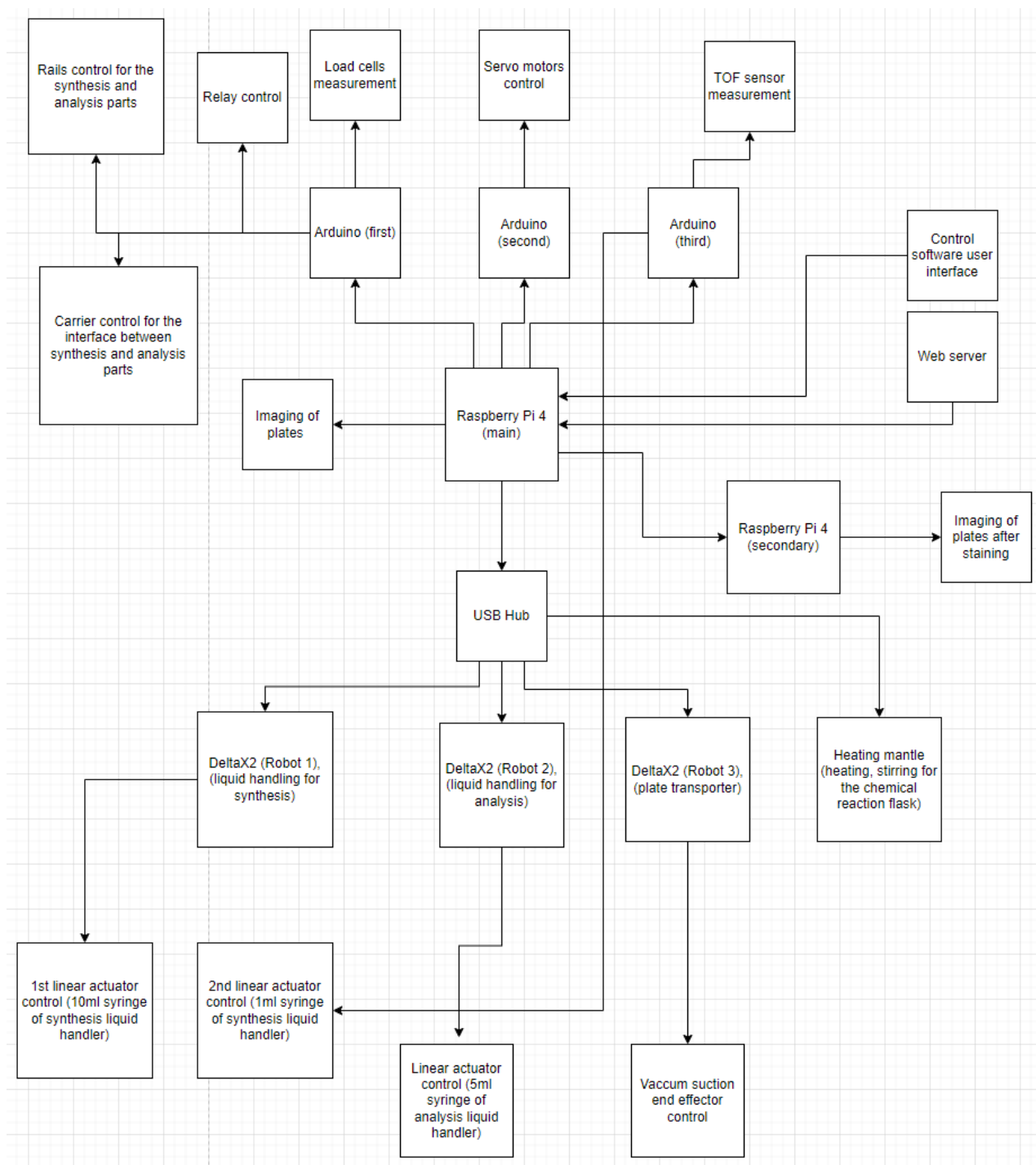


Fig. 50. Control diagram of different parts of the automation system

A)

```

4681 #
4682 global bool_before_mixing_skip_S51_return
4683 #S51: For all inputs, mix all inputs
4684 temp_calculated_total_liquid_amount_in_bottle_arr = [] #keeps track of calculated total liquid amount in bot
4685 for k in range(len(bottle_index_arr)):
4686     if bottle_index_arr[k] != '': #skip
4687         temp_total_liquid_amount_in_bottle = 0
4688         if refill_arr[k] == True: #do refills only if specified (used for when reagents in bottles 1 and 2 are
4689             for i in range(len(input_location_arr_nested[k])):
4690                 if input_amount_arr_nested[k][i] > 0: #do only if set amount is non 0, because if -, means
4691                     #P0003 liquid amount loop
4692                     print('just before calling sub:')
4693                     print('k:', k)
4694                     print('i:', i)
4695                     print('input_location_arr_nested[k][i]:', input_location_arr_nested[k][i])
4696                     print('input_location_arr_nested[k][i]:', input_location_arr_nested[k][i])
4697                     print('bottle_index_arr[k]:', bottle_index_arr[k])
4698                     print('input_amount_arr_nested[k][i]:', input_amount_arr_nested[k][i])
4699                     print('input_amount_arr_nested[k][i]:', input_amount_arr_nested[k][i])
4700
4701                     bool_before_mixing_skip_S51_return = False
4702                     #print('len(input_location_arr_nested[k]):', len(input_location_arr_nested[k]))
4703                     if i == len(input_location_arr_nested[k]) - 1: #if this is the last dispense for k bott
4704                         if bool_to_mix_arr[k] == True: #we are mixing after this dispense,
4705                             bool_before_mixing_skip_S51_return = True #prevents robot from certain movement
4706                             print('debug91 f TLC_develop_plate')
4707                             bool_skip_cleaning_in_f_move_sub_2 = True
4708                             f_move_sub_2(1, input_location_arr_nested[k][i], input_location_arr_nested[k][i], 'Z_Carrie
4709                             bool_skip_cleaning_in_f_move_sub_2 = False
4710                             if bool_skip_f_clean_S51_at_liquid_change == False:
4711                                 #f_clean_V2('Z', '40')
4712                                 if bool_before_mixing_skip_S51_return == True: #prevents case when needs mixing, but
4713                                     f_clean_S51_at_liquid_change_V2('Z2', quant_tracking_current_at_liquid_change_c
4714                                     if bool_skip_f_clean_V2_at_f TLC_develop_plate == False:
4715                                         if bool_before_mixing_skip_S51_return == True: #prevents case when needs mixing, but
4716                                             f_clean_V3('Z2', '04')
4717
4718                                 if input_amount_arr_nested[k][i] not in [0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9]:
4719                                     temp_total_liquid_amount_in_bottle += input_amount_arr_nested[k][i]
4720                                     #because the
4721                                     print('temp_total_liquid_amount_in_bottle:', temp_total_liquid_amount_in_bottle)
4722
4723                                 temp_calculated_total_liquid_amount_in_bottle_arr.append(temp_total_liquid_amount_in_bottle)
4724                                 if bool_to_mix_arr[k] == True:
4725                                     #print('temp_total_liquid_amount_in_bottle:', temp_total_liquid_amount_in_bottle)
4726                                     #print('temp_total_liquid_amount_in_bottle:', temp_total_liquid_amount_in_bottle)
4727                                     if bool_use_f_liquid_mix_S51:
4728                                         if float(temp_total_liquid_amount_in_bottle) == float(input_amount_arr_nested[k][i]) and
4729                                             float(temp_total_liquid_amount_in_bottle) == float(input_location_arr_nested[k][i]) and
4730                                             float(temp_total_liquid_amount_in_bottle) == float(bottle_index_arr[k]):
4731                                             if analysis_more_dilution_bool_plate == False:
4732                                                 if analysis_more_dilution_bool_plate == False:
4733                                                     bool_skip_f_clean_V3_at_f_liquid_mix_S51_bubble = True
4734                                                     f_liquid_mix_S51_bubble('Z_Carrier', bottle_index_arr[k], temp_total_liquid_amount
4735                                                     bool_skip_f_clean_V3_at_f_liquid_mix_S51_bubble = False
4736                                                     if bool_do_more_dilution_at_f TLC_develop_plate: #global toggle
4737                                                         if analysis_more_dilution_bool_plate:
4738                                                             print('more dilution-starting')
4739                                                             for more_dilution_loop_index in range(int(analysis_global_more_dilution_loo
4740                                                             print('more dilution loop index:', more_dilution_loop_index)
4741                                                             f_dilute_syr(bottle_index_arr[k], analysis_more_dilution_tube_amount_at
4742                                                             more_dilution_loop_index = 0 #reset this, otherwise if there are >1 spots, a
4743                                                             print('more dilute loop:exit')
4744
4745                             else:
4746                                 print('refill -> skipped')
4747                                 temp_calculated_total_liquid_amount_in_bottle_arr.append(temp_total_liquid_amount_in_bottle) #S
4748
4749

```

B)

```

44
45 void checkSerial()
46
47 if (Serial.available() > 0)
48 {
49     receivedCommand = Serial.read();
50     newData = true;
51
52     if (newData == true)
53     {
54         switch (receivedCommand)
55         {
56             case 'r':
57                 receivedSwitchValue = Serial.parseFloat(); //index of the servo
58                 receivedSwitchValue2 = Serial.parseFloat(); //angle of the servo to go to
59                 receivedSwitchValue3 = Serial.parseFloat(); //speed from 1 to 30, corresponding to (3
60                 Servo_moveTo(receivedSwitchValue, receivedSwitchValue2, receivedSwitchValue3);
61                 break;
62             default:
63                 break;
64         }
65     }
66     newData = false;
67 }
68
69 int angleToPulse(int ang){
70     int pulse = map(ang,0, 180, SERVOMIN,SERVOMAX); // map angle of 0 to 180 to Servo min and Ser
71     return pulse;
72 }
73
74 void Servo_moveTo(int servo_index, int position, int speed) {
75     //long mapSpeed = map(speed, 0, 1000, 0, 0);
76     for (servo_positions_arr[servo_index] <= position; servo_
77         pwm.setPWM(servo_index, 0, angleToPulse(servo_positions_arr[servo_index]));
78         delay(speed);
79     }
80     servo_positions_arr[servo_index]++; //gets added when looping, but no movement is done
81     Serial.println(String(servo_index) + ", " + String(servo_positions_arr[servo_index]));
82 }
83 else if (position < servo_positions_arr[servo_index]) {
84     for (servo_positions_arr[servo_index]; servo_positions_arr[servo_index] >= position; servo_
85         pwm.setPWM(servo_index, 0, angleToPulse(servo_positions_arr[servo_index]));
86         delay(speed);
87     }
88     servo_positions_arr[servo_index]--; //gets subtracted when looping, but no movement is don
89     Serial.println(String(servo_index) + ", " + String(servo_positions_arr[servo_index]));
90 }
91 else {
92     pwm.setPWM(servo_index, 0, angleToPulse(position));
93     Serial.println(String(servo_index) + ", " + String(servo_positions_arr[servo_index]));
94 }
95 }
96
97
98
99
100
101
102

```

Fig. 51. Code snippets of: A) the main control software, B) Arduino microcontroller code

A)

```

496 [SS2_2_TOF_V2]
497 SS2_2_TOF_V2_cords_rail = 50000
498 SS2_2_TOF_V2_cords_x = -11
499 SS2_2_TOF_V2_cords_y = -115
500 SS2_2_TOF_V2_cords_z = -350
501 SS2_2_TOF_V2_cords_z_safe = -280
502 SS2_2_TOF_V2_measure_measurement_index = 3
503 SS2_2_TOF_V2_threshold_plate = 25
504 SS2_2_TOF_V2_threshold_lid = 150
505
506 SS2_2_TOF_V2_cords_lid_rail = 55000
507 SS2_2_TOF_V2_cords_lid_x = -5
508 SS2_2_TOF_V2_cords_lid_y = -15
509 SS2_2_TOF_V2_cords_lid_z = -351
510
511 [f_pickup_TLC_plate]
512 z_height_to_offset_dict_z_height_value_Layer_5 = -320
513 z_height_to_offset_dict_z_height_value_Layer_4 = -333
514 z_height_to_offset_dict_z_height_value_Layer_3 = -346
515 z_height_to_offset_dict_z_height_value_Layer_2 = -359
516 z_height_to_offset_dict_z_height_value_Layer_1 = -372
517 z_height_to_offset_dict_z_height_value_Layer_0 = -500
518
519 [misc_for_bring_input_to_liquid_SS2_1_syr]
520 syr_SS2_1_percentage_to_step_dict_coefficient = 1
521
522 [Liquid_quant_tracking]
523 quant_tracking_Z3_max_step_value = 3200
524
525 quant_tracking_Z2_max_step_value_1 = 4450
526 quant_tracking_Z2_max_step_value_2 = 8900
527
528 [holding_tlc_plate_with_SS2_2]
529 holding_tlc_plate_with_SS2_2_rail = 69500
530 holding_tlc_plate_with_SS2_2_x = -63
531 holding_tlc_plate_with_SS2_2_y = 146
532 holding_tlc_plate_with_SS2_2_z = -380
533
534 [container_lid_servo_angles]
535 container_lid_1_servo_angle_initial = 130
536 container_lid_2_servo_angle_initial = 130
537 container_lid_1_servo_angle_close = 30
538 container_lid_2_servo_angle_close = 20
539
540 [f_clean_S51_at_liquid_change]
541 f_clean_S51_at_liquid_change_clean_amount = 6
542 f_clean_S51_at_liquid_change_repeat_index = 2
543
544 [f_clean_S51_at_liquid_change_V2]
545 f_clean_S51_at_liquid_change_V2_cords_z_value_when_moving
546 f_clean_S51_at_liquid_change_clean_V2_amount = 3
547
548 [SS1_syr_antidrip]
549 SS1_syr_antidrip_acceleration = 4000
550 SS1_syr_antidrip_syr_step_amount = 22000
551 SS1_syr_antidrip_syr_step_speed = 1000
552 SS1_syr_antidrip_acceleration_reset = 4000
553 SS1_syr_antidrip_syr_step_amount_reset = 0
554 SS1_syr_antidrip_syr_step_speed_reset = 4000
555
556 SS1_syr_antidrip_microleter_syringe_speed = 4000
557
558 [SS1_syr_bath]
559 SS1_syr_bath_cords_rail = 70000
560 SS1_syr_bath_cords_x = 0
561 SS1_syr_bath_cords_y = 116
562 SS1_syr_bath_cords_z_near = -262
563 SS1_syr_bath_cords_z_low = -400
564
565 [SS2_1_syr_bath]
566 SS2_1_syr_bath_cords_rail = 0

```

B)

```

Search Solution Explorer - Folder View (Ctrl+;)
C:\Users\arnas\Source\Repos\ArnasVask\Sy
├─ Arduino_control
├─ data
│  ├─ backup
│  ├─ Cam
│  ├─ config
│  │  ├─ config.ini
│  │  ├─ config_backup.ini
│  │  └─ config_backup_old.ini
│  ├─ coordinates
│  └─ liquid_to_container
├─ plate_pile
│  ├─ plate_pile_in.txt
│  ├─ plate_pile_in_2.slot.txt
│  ├─ plate_pile_in_3.slot.txt
│  └─ plate_pile_out.txt
├─ robot_synthesis
│  ├─ big_rack
│  └─ small_rack
│     ├─ cords_robot_synthesis_small_rack_x.txt
│     ├─ cords_robot_synthesis_small_rack_y_rail.txt
│     ├─ cords_robot_synthesis_small_rack_y.txt
│     ├─ cords_robot_synthesis_small_rack_z_low.txt
│     └─ cords_robot_synthesis_small_rack_z_near.txt
├─ tlc_development
├─ zone_Carrier01
├─ zone_1
├─ zone_2
├─ zone_3
├─ zone_4
├─ Errors to fix
├─ external_monitoring
├─ legacy
├─ log
├─ misc
├─ notes
├─ OpenCV
├─ testing
├─ images
├─ Readme
├─ RP2_control
├─ server_test
├─ volatile
├─ PY
├─ main.py
├─ readme.txt
└─ rules.txt

```

Fig. 52. Control software: A) config file, B) file structure

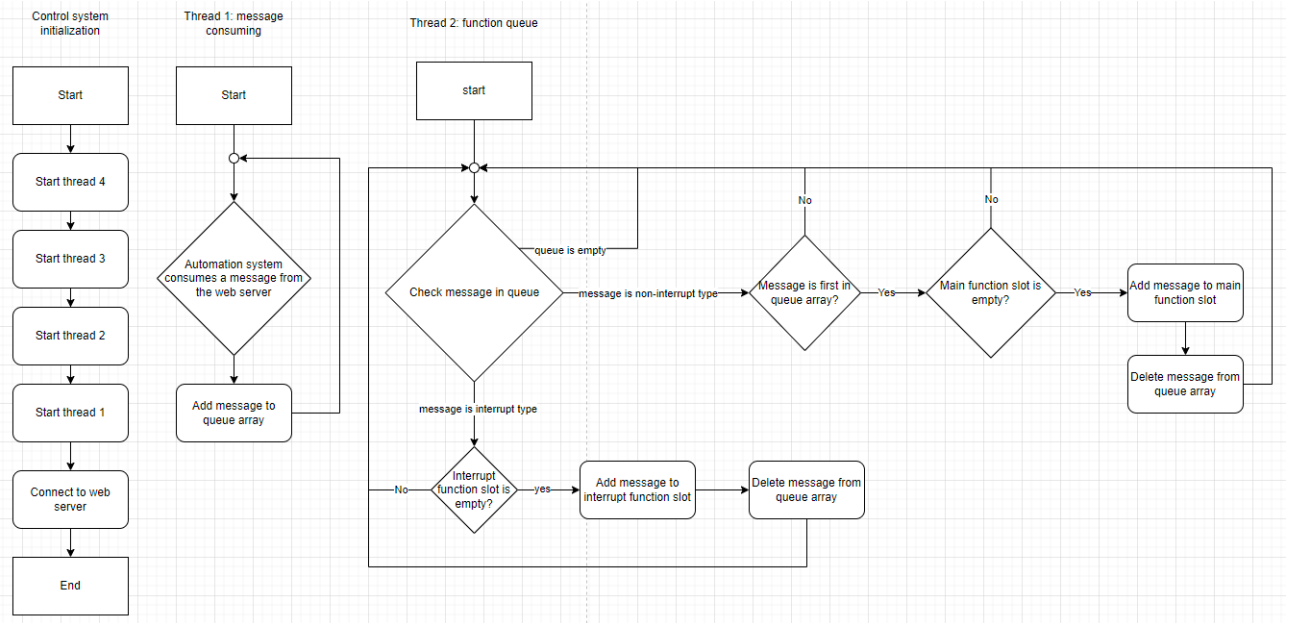


Fig. 53. Automation system control software flow diagram of initialization, message consuming thread, and function queue thread

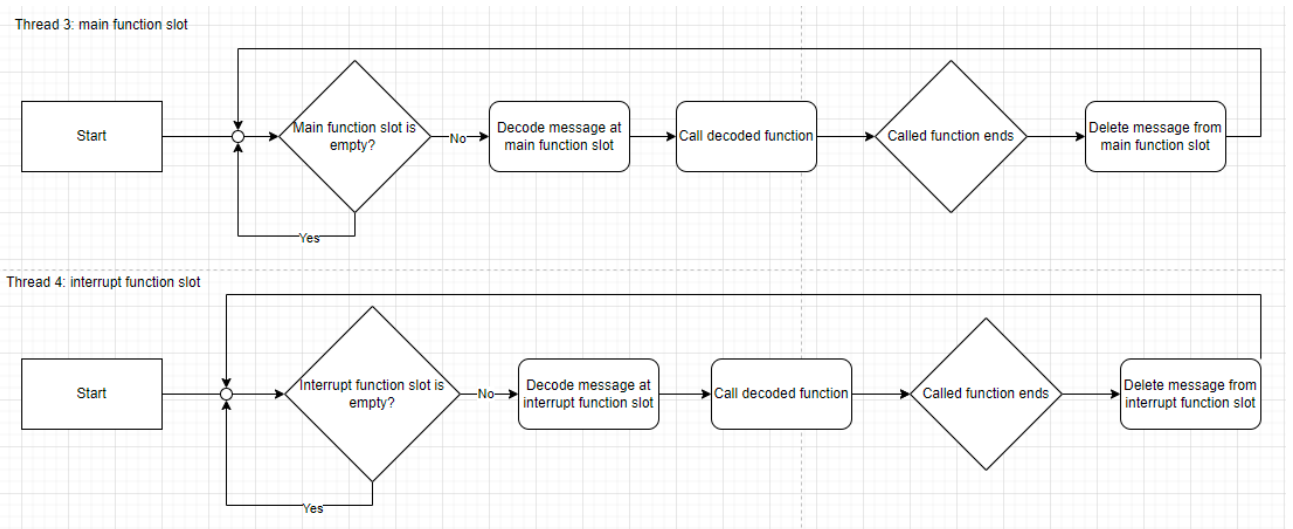


Fig. 54. Automation system control software flow diagram of main function slot thread and interrupt function slot thread

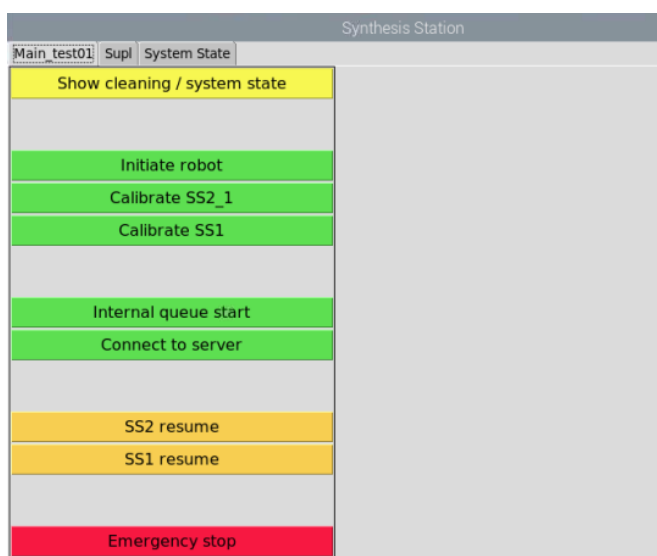


Fig. 55. User interface of the control software of the automation system

View reaction "TosN3"

Chemical reaction scheme for "TosN3":

ClS(=O)(=O)c1ccccc1.[Na-].[N-]=[N+]=[N-].CC(=O)CC>>CC(=O)CCS(=O)(=O)c1ccc(cc1)[N-]=[N+]=[N-]

benzenesulfonyl chloride (R1) + Sodium azide + butan-2-one + Add reagent → TosN3 + Add product

#	Operation	Temp.	Time	Reagent	Molar conc. (M)	Solvent	Volume of solvent	Countdown timer
1	Stir/Heat	25 °C	2000 min	Continuous				0:00:00
2	Add	1 min	butan-2-one	-	1	Acetone	25 ml	
3	Add	1 min	benzenesulfonyl chloride	-	0.5	Acetone	25 ml	
4	Analyze	Single analysis	TLC SiOH	2 spots (reaction)	2 selected	Frequency: 40	Rem. analysis: No c	Dilution/spotting: No c
5	Add	1 min	Sodium azide	-	0.5	Water	1 ml	
6	Analyze	Single analysis	TLC SiOH	2 spots (reaction)	2 selected	Frequency: 39	Rem. analysis: No c	Dilution/spotting: No c

Additional parameters for TLC analysis (rows 4 and 6):

Mobile phase solvent	Ratio
Tolu	[Nor] [Nor] 100 %

Fig. 56. User interface of the webpage used to setup instructions to control the automation system

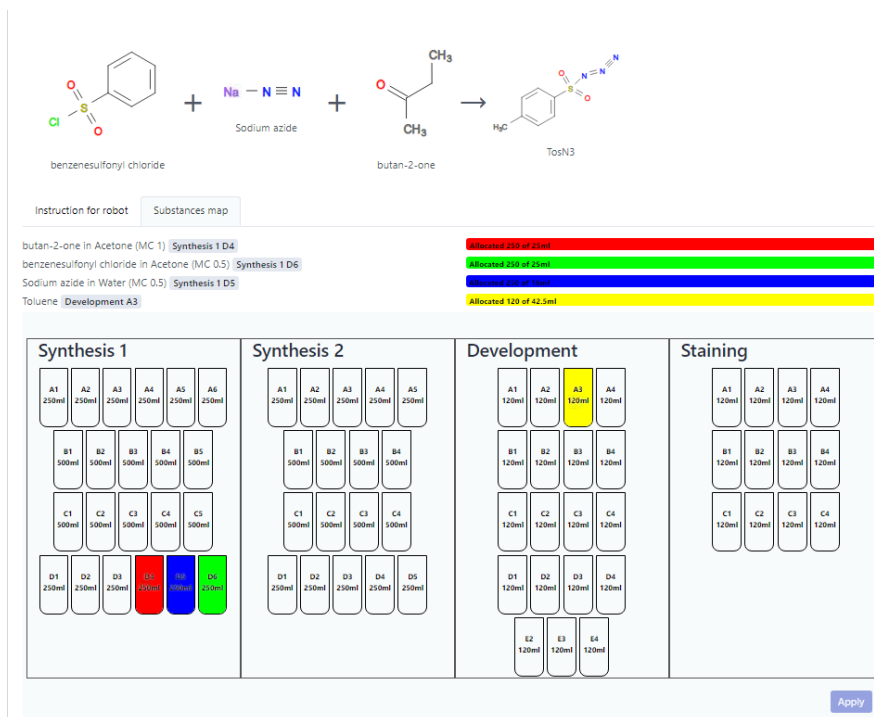


Fig. 57. Admin interface of the webpage used to control and monitor the automation system

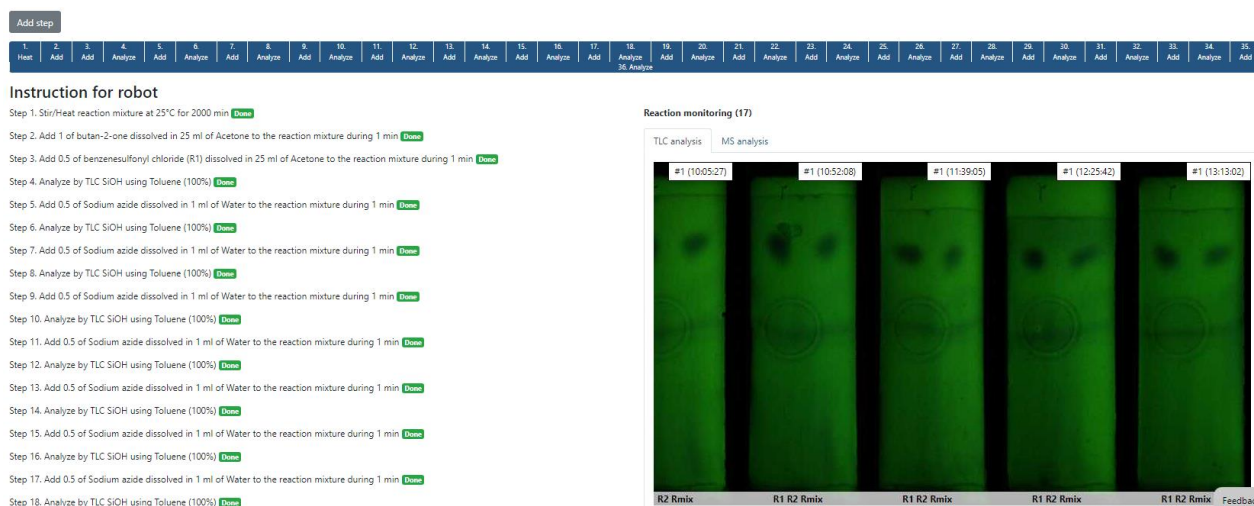


Fig. 58. User interface of the webpage used monitor processes of the automation system

Appendix 3. Automation System Working Principle Flow Diagrams

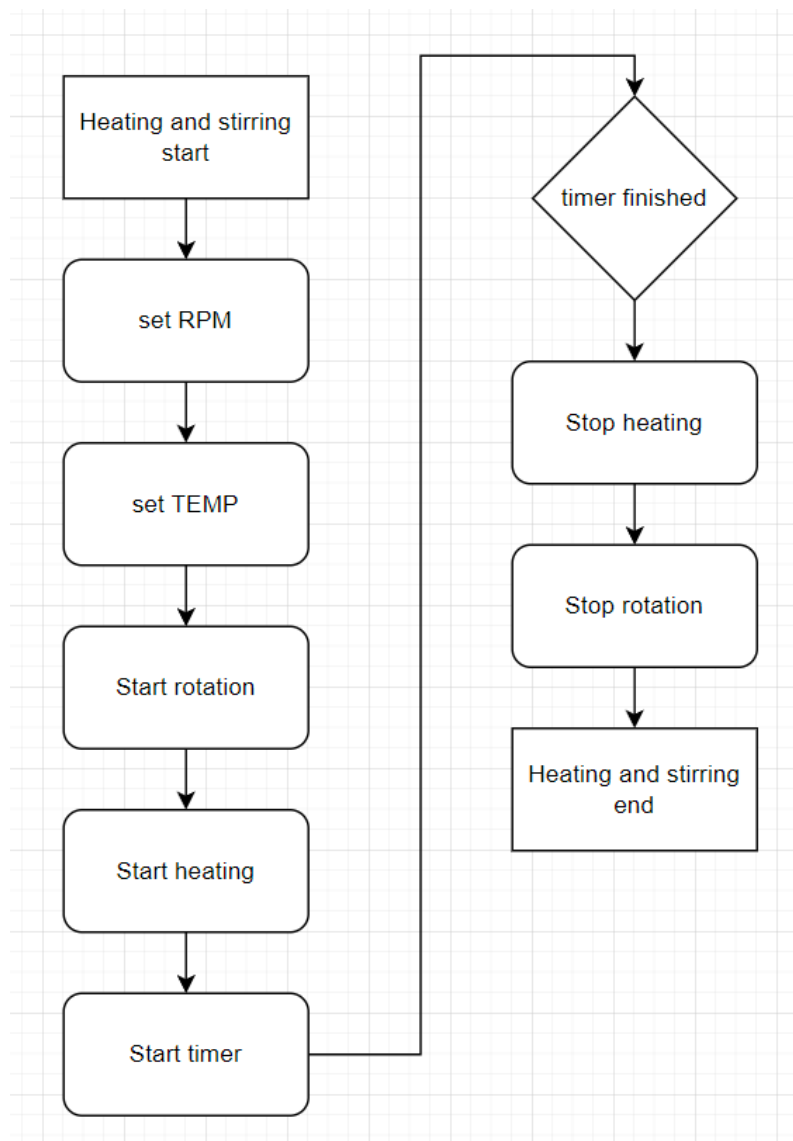


Fig. 59. Flow diagram of heating and stirring process of the automation system

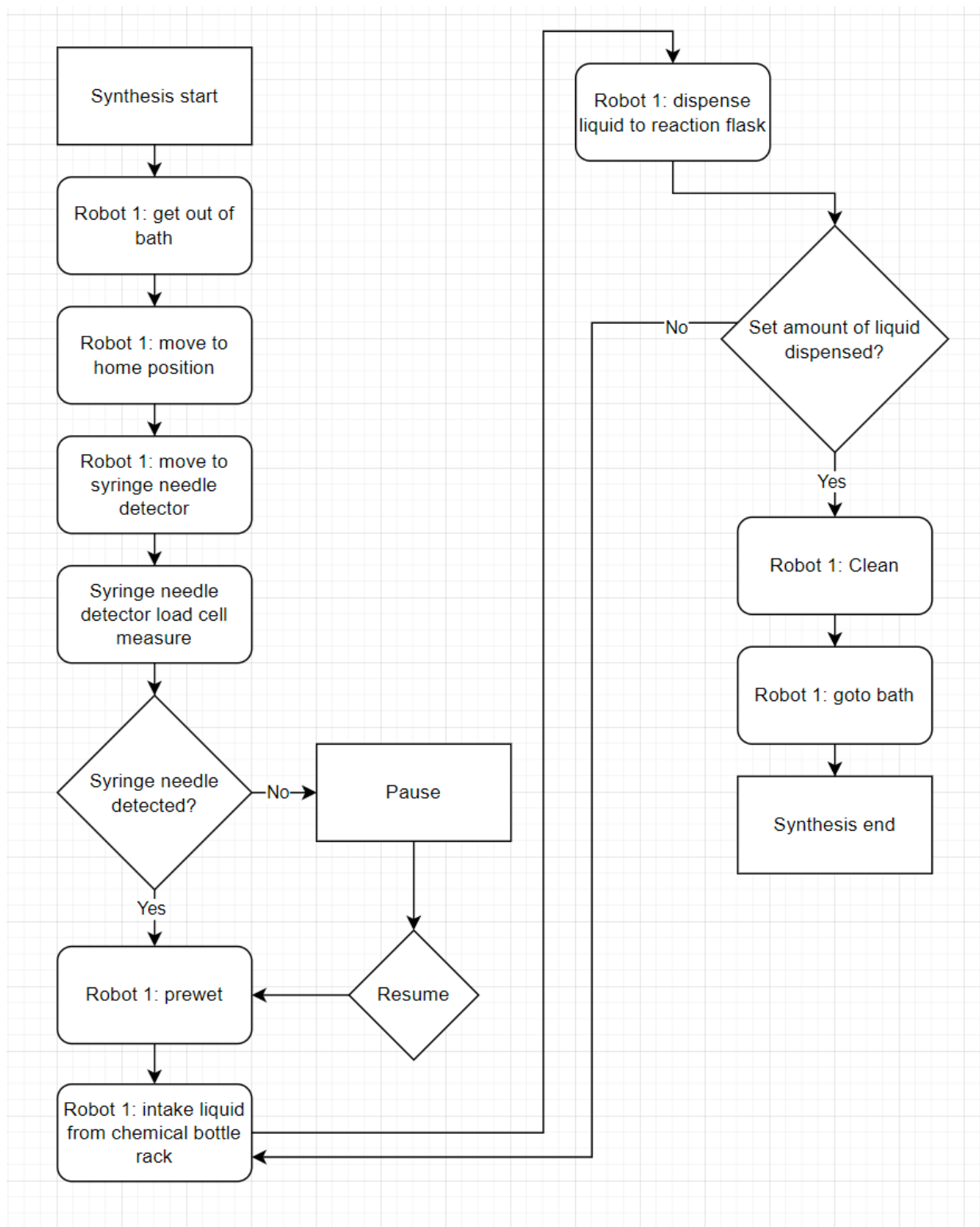


Fig. 60. Flow diagram of the synthesis process of the automation system

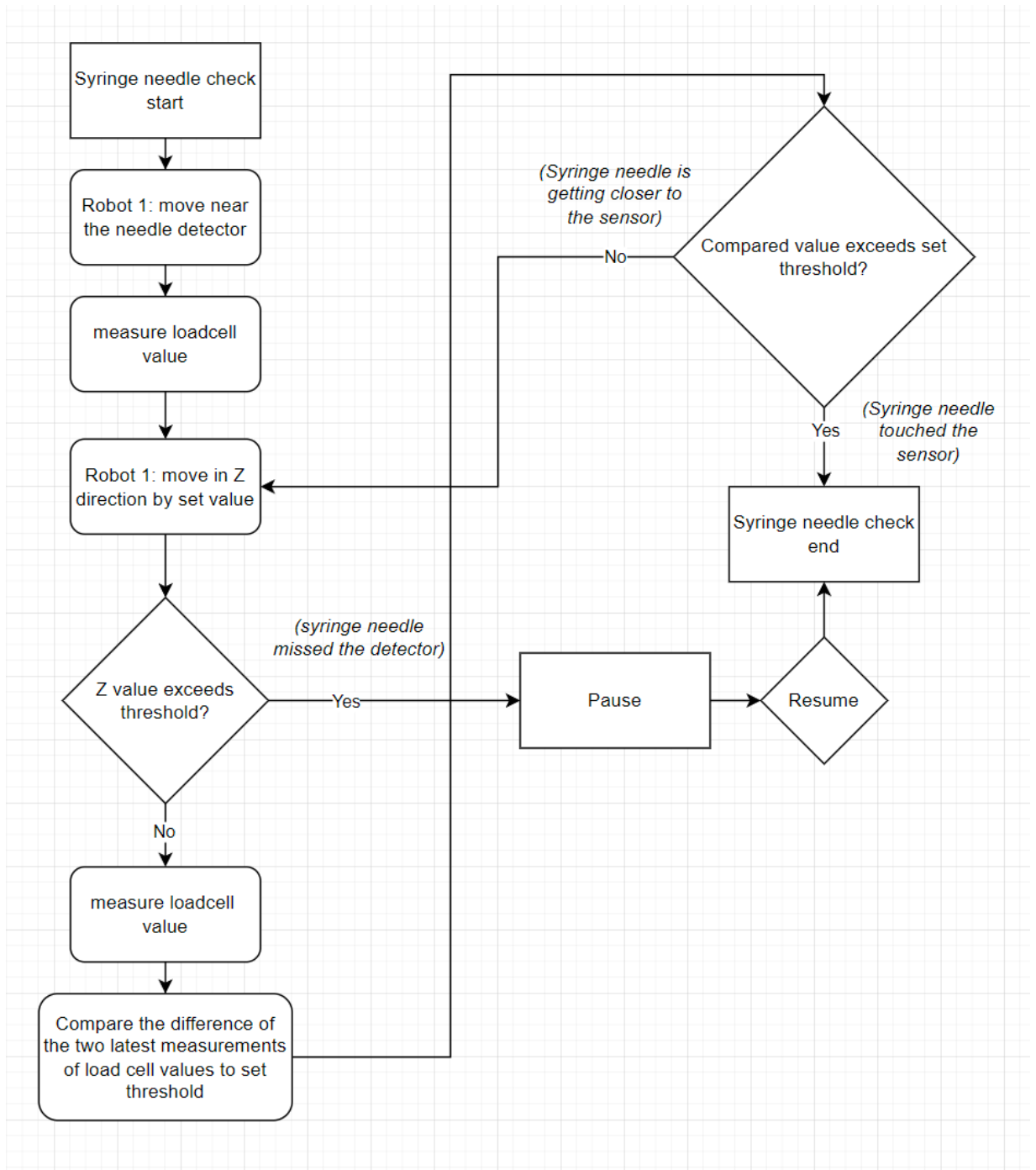


Fig. 61. Flow diagram of the syringe needle check algorithm of the control system

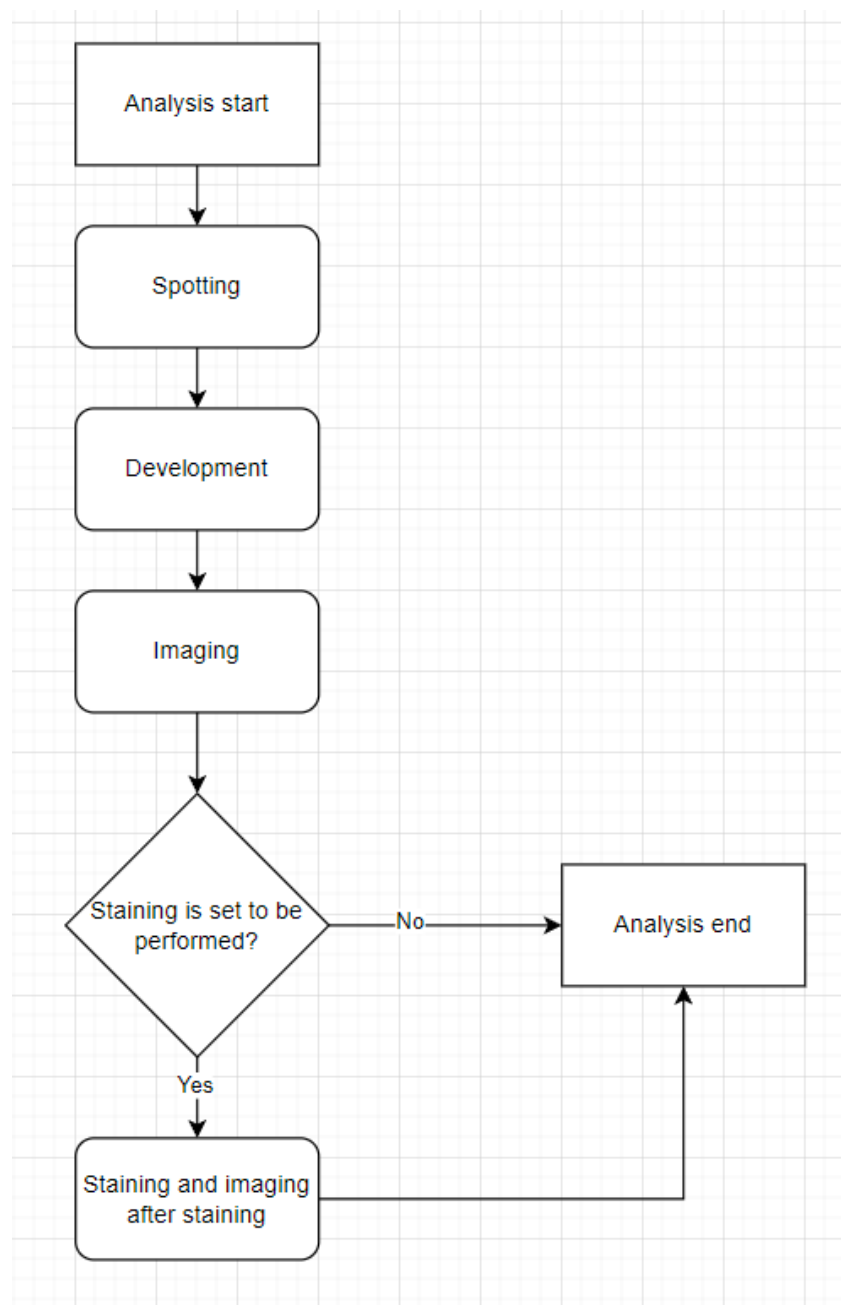


Fig. 62. Flow diagram of analysis process of the automation system

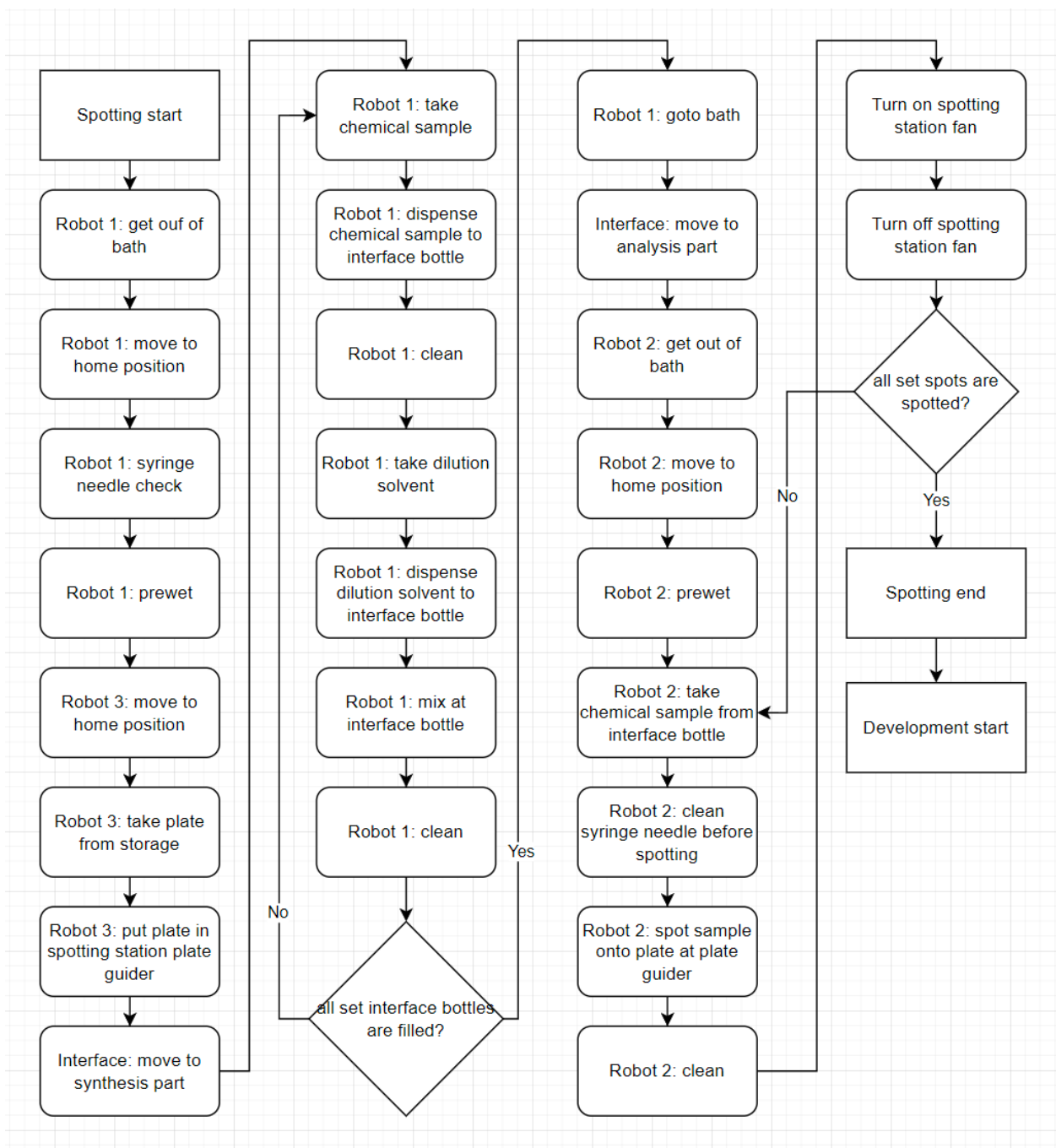


Fig. 63. Flow diagram of spotting process of the automation system

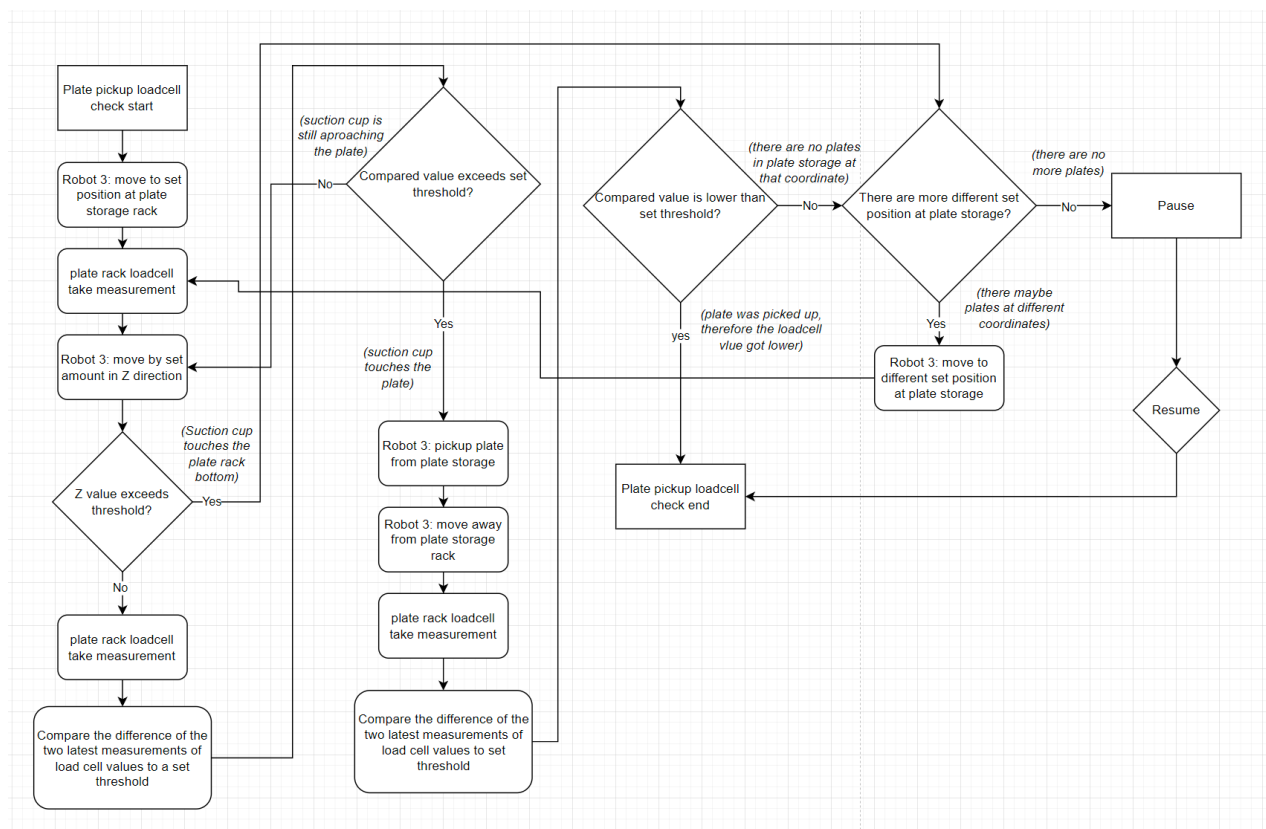


Fig. 64. Flow diagram of the adaptive process during plate pickup from the plate storage rack.

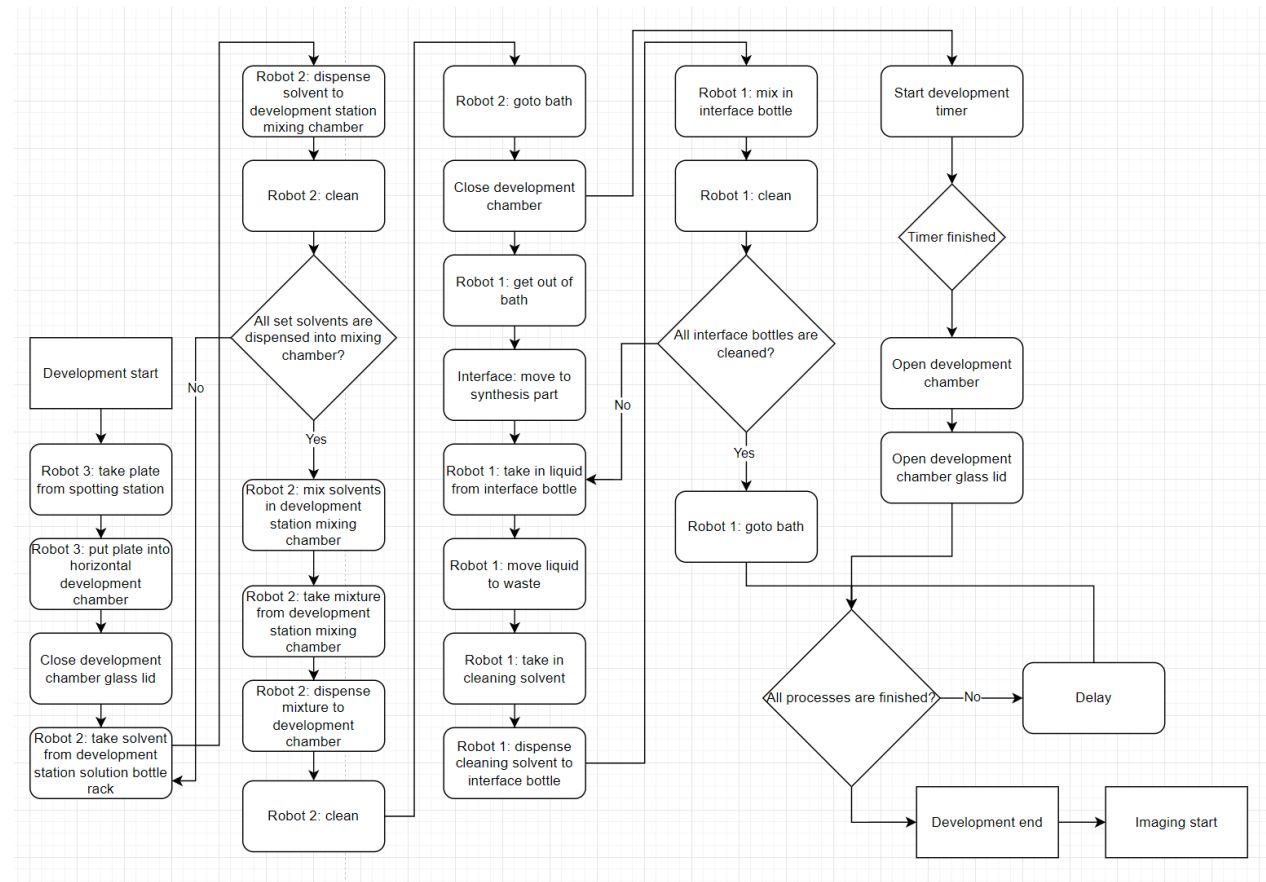


Fig. 65. Flow diagram of the development part of the analysis process of the automation system

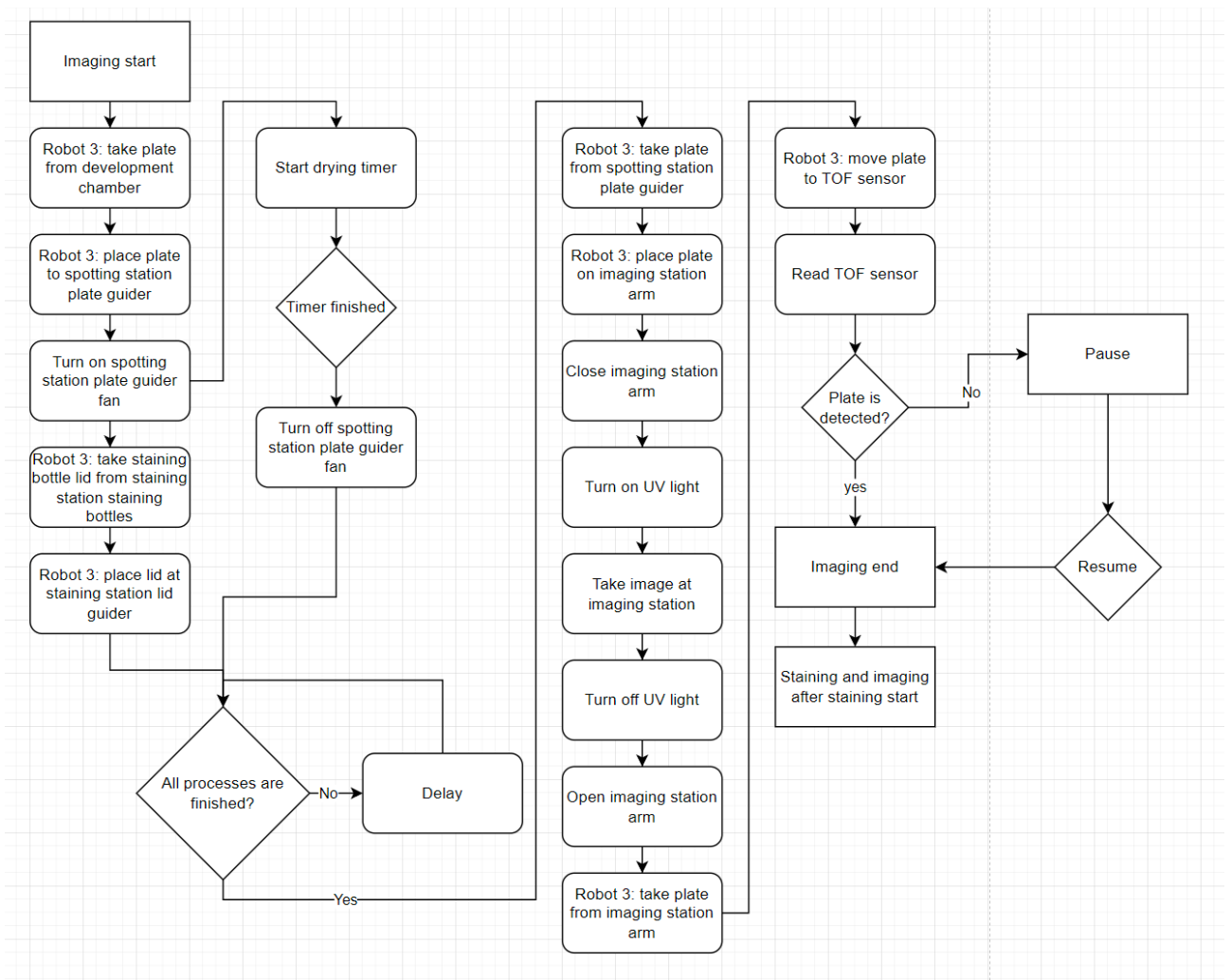


Fig. 66. Flow diagram of imaging part of the analysis process of the automation system

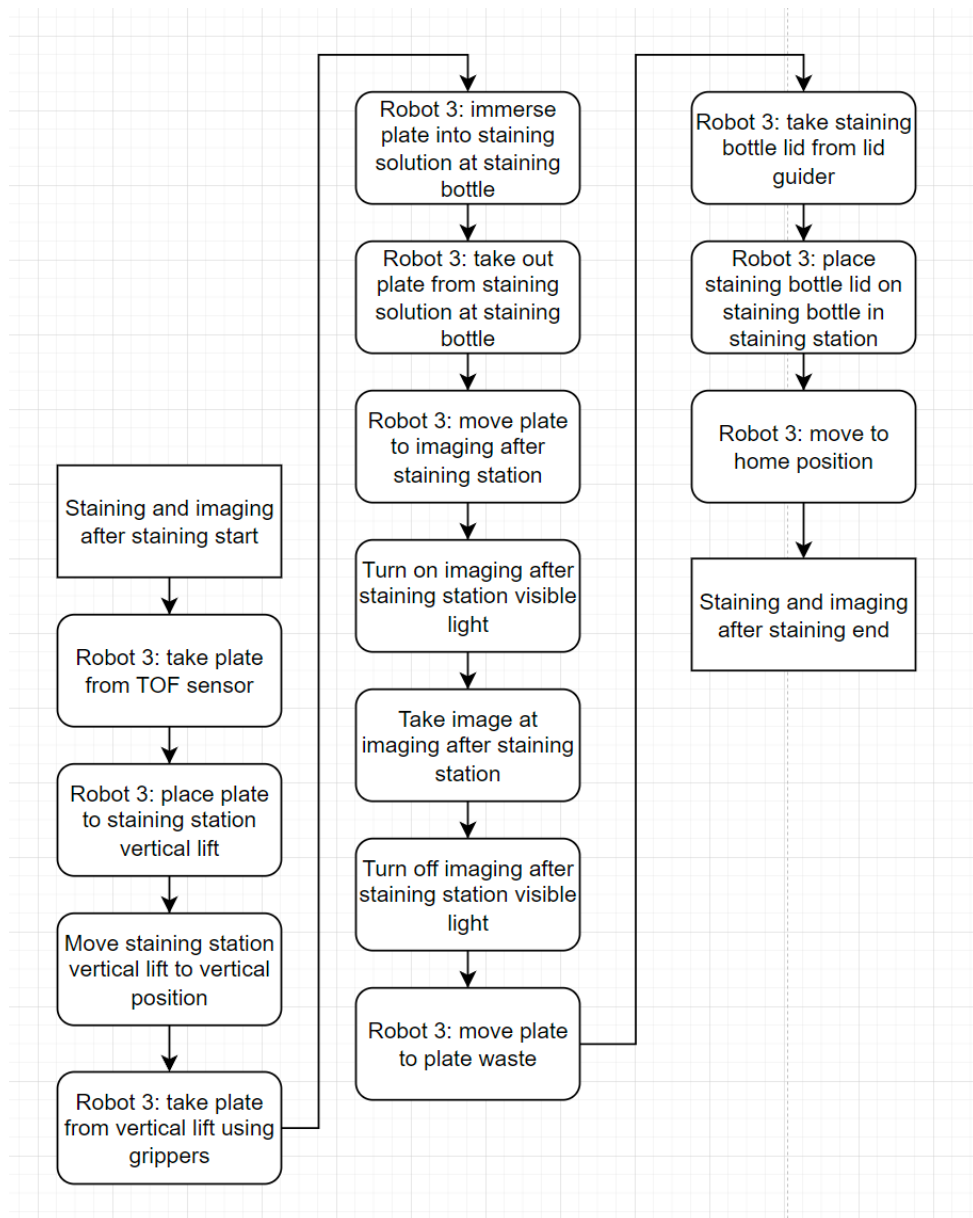


Fig. 67. Flow diagram of staining and imaging after staining parts of analysis process of the automation system