System for Automated Cell Culture

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Doktor Ingenieur (Dr. Ing.)

of the Faculty for Computer Science and Electrical Engineering

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Applied by: Dipl. Ing. Steffen Junginger

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Surveyors: Prof. Dr. Ing. habil Kerstin Thurow, University of Rostock

Dr. Ing. habil Mohit Kumar, University of Rostock

Prof. Robin A. Felder, Ph.D., University of Virginia, Charlottesville, Virginia, USA

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Dedicated to Nele and Anni -

That you may continue developing your potential.

Expression of thanks

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List of Abbreviations

ACCF	Automated Cell Culture Flask
ALP	Automated Labware Positioner
BRT	Biomek Robotic Transport
CAD	Computer Aided Design
CAN	Controller Area Network
CCD	Charge-Coupled Device
CFR	Code of Federal Regulations
CHO	Chinese Hamster Ovary Cells
CHO-K1	Subclone of CHO cell line (Chinese Hamster Ovary)
COM	Component Object Model
DDE	Dynamic Data Exchange
DIN	Deutsches Institut für Normung
DMEM	Dulbecco's Modified Eagle Medium
EDTA	Ethylenediaminetetraacetic Acid
FBS	Fetal Bovine Serum
FDA	U.S. Food and Drug Administration
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GUI	Graphical User Interface
HCS	High Content Screening
HEK 293	Human Embryonic Kidney 293 cells
HeLa	Human cervical cancer cell line
HEPA	High Efficiency Particulate Airfilter
HTS	High Throughput Screening
I/O	Input / Output
JALA	Journal of the Association for Laboratory Automation
LED	Light-Emitting Diode
LIMS	Laboratory Information & Management System
MEMS	Micro-Electro-Mechanical Systems
MTP	Micro Titer Plate
OCX	OLE custom control, ActiveX control
OLE	Object Linking and Embedding
ORCA	Optimized Robot for Chemical Analysis
OS	Operating System

PBS	Phosphate Buffered Saline
PC	Personal Computer
PLC	Programmable Logic Controller
PTFE	Polytetraflourethylene
PVC	Polyvenylchloride
RGB	Red / Green / Blue
SAMI	Sagian Automated Method Interface
SBS	Society for Biomolecular Sciences
SCARA	Selective Compliance Assembly Robot Arm
TCP / IP	Transfer Control Protocol / Internet Protocol
TIS-10	Stably transfected HeLa cell line with Tetracyclin
uHTS	Ultra High Throughput Screening
ULPA	Ultra Low Penetration Air
USB	Universal Serial Bus
UV/VIS	Ultra Violet / Visible

1 Introduction

In life sciences automation is a widely established technology today. Automation in life sciences is established today in all areas from basic research, applied research, development over production and therapy. In the focus is there not the substitution of human staff in the process by machines, but the increasing of efficiency and effectiveness of resources in all fields of life sciences. The investigation to find new active substance combinations in pharmaceutical research to find new drugs needs today a multiple effort compared to the 1970s. [1;2] Another aspect is the enhanced reproducibility of processes and the complete documentation and traceability of life science processes which is supported by process data management, LIMS systems and workflow management solutions as it is ruled by the U.S. Food & Drug Administration, GMP and GLP guidelines. Furthermore the goal of higher degrees of automation is to disburden skilled laboratory staff from monotonous recurring manual work. So the human resources in the laboratories can be used focused more on sophisticated tasks and to data analysis.

Automation of animal and human cells is the topic of this dissertation. As result of this dissertation there exists a full functioning prototype of an automated system for culture of adherent growing cell lines for applications in scientific laboratories where cellular applications like screening, safety pharmacology or tissue engineering are ran.

1.1 Importance of Cell Culture in Life Sciences

The application spectrum for living animal and human cells extends to a wide area of the life sciences. Cells are matter of analysis in pharmacologic research and drug discovery, in primary screening, secondary screening and in later stages of the development of drugs like in safety pharmacology. Furthermore cells are used in the fields of cosmetic research and product development where they often replace experiments on animals. Besides this living cells are used in

medical research, in therapy for many diseases and in the new reconstructive medical technologies like tissue engineering.

One more application field of cells is the production of basic pharmaceutical components. Here cells grow in bioreactors and metabolic products of living cells are harvested which are used for therapeutic purposes and for production of active agents.

1.1.1 Primary Screening / HTS

In primary screening large numbers of test samples – ten thousands to millions - are tested on their influence to test objects like living cells in target based screening using laboratory automation lines. Those cell based assays in high throughput screening (HTS) and ultra high throughput screening (uHTS) are performed in micro titer plate labware. The main purpose of primary screening is to find targets for further investigations and detailed tests. [3-5] Due to the high number of samples to be tested there is the need for automation in screening on the one hand. On the other hand for HTS are needed cells in sufficient quantity and in constant quality.

Figure 1 High Throughput Screening System

1.1.2 Secondary Screening / HCS

In secondary screening the hits found in primary screening / High throughput technologies are tested more detailed. Often the technology of High Content Screening (HTS) is used, which combines modern cell biology methods, automated microscopy (fluorescence microscopy, confocal imaging) and robotic handling. Combination of fluorescence tagging, dying and other techniques allow methods generating multiple informational vectors in parallel. Integration of incubation chambers allow technologies like live cell imaging, neurite outgrowth and other complex assays. Also in high content screening cells, cell tissues and the components of cells are the objects of interest. To allow for high quality results in HCS cells of constant quality and sufficient number are needed. [6;7]

Figure 2 High Content Screening Imager source: BD Biosystems

1.1.3 Safety Pharmacology

In the stage of preclinical testing and safety pharmacology there are cells used in methods like ion channel assays which generate dose-response curves using patch clamp techniques for example. [8;9] Therefore especially modified cells

with amplified ion channels and different cell types are needed. Currently there are new methods like ‚instant frozen cells' in development which allow thawing of frozen cells and immediate usage in assays.

1.1.4 Cell Based Protein Production

Cells are widely used as providers of their metabolism products. So cell cultures can be used to produce proteins like antibodies for research and therapeutic use. This gets more important in new forms of therapy. Specific Antibodies can be used i.e. in cancer therapies instead of chemotherapy. The antibody production is a fast developing field in Biotechnology. Automated cell culture can help developing those technologies in research departments as well as in production. [10;11]

1.1.5 Stem Cell Research and Cell Therapy

Because of the ability to differentiate embryonic stem cells and adult stems cells are in the focus of many fields in research for medical therapy and reproductive medicine. Stem cells have the potential to regenerate affected tissue, for instance after myocardial infarction. Because of the special treatment of stem cells in vitro and the sensitivity of stem cells in culture it is worth to investigate in automated processes for stem cell culture with stable environmental conditions, reproducible techniques and complete automated documentation. [12;13]

Cells and products of cells play an important role today in many therapeutic techniques in oncology, neurologic repair, cardio repair, vaccination etc. Therefore cells are cultured in vitro. Besides this isolation and separation processes play an important role in cell therapy. Because of the high safety constraints and regulations like obligatory sterility and safe exclusion of cross contamination when working with cells for patient therapy cell handling automation seems to be suited for those therapeutic technologies. [13]

1.1.6 Tissue Engineering

Tissue Engineering is one of the important technologies in reconstructive medicine. The methods of tissue engineering are based on cultivation of living cells in 3D constructs with the aim to re-implant tissues into patient organs, to re-implant skin or to rebuild cells taken out of patient. [14;15] So for tissue engineering, where cells taken from patients are grown in vitro and are used to be re-implanted, stable sterile conditions of highest quality ant maintenance of reproducible condition are necessary. In addition, the data tracking, data management and online quality control mechanisms of cell handling automation have the potential to support those therapies.

Besides this there are developing new fields in tissue engineering where artificial organs like skin reproductions are designed in vitro for clinical trials, product testings in medicine and cosmetics, where a high degree auf automation is the aim. [16]

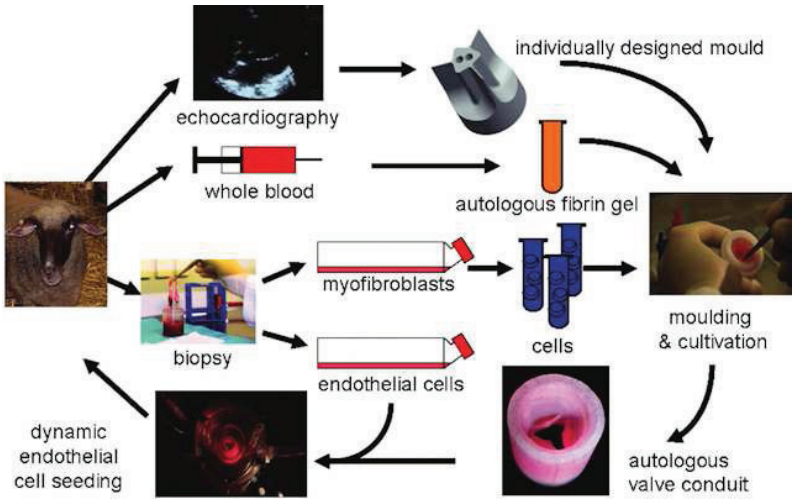


Figure 3 Tissue Engineering of Heart Valve **Source: Flanagan, TC;AME; RWTH Aachen**

2 Cell Culture

"Cell Culture — Growing the cells in an artificial environment, to allow them to multiply and increase their numbers." [17]

Cell culture means to preserve living animal or human or crop cells in vitro where the goal is to maintain the cells in constant condition and quality and/or to grow them to expand the number of cells to a desired amount. Therefore cells are kept in dedicated environmental conditions to support life of the cells and to allow for continuous cell division. Environmental conditions contain temperature, air humidity and gas atmosphere. Typically, cells are grown in liquid media. Cell culture medium is a complex combination of cell nutrition, amino acids, salts, vitamins, buffer substances, and serum or serum replacements to rebuild a near to in vivo conditions in vitro. [18;19] In addition drugs like antibiotics can be added to simulate immune system. The tasks of cell culture media are nutrition, reproduction of in vivo conditions, and in addition, maintenance of stable conditions by buffers which neutralize cell metabolism products for a growth period.

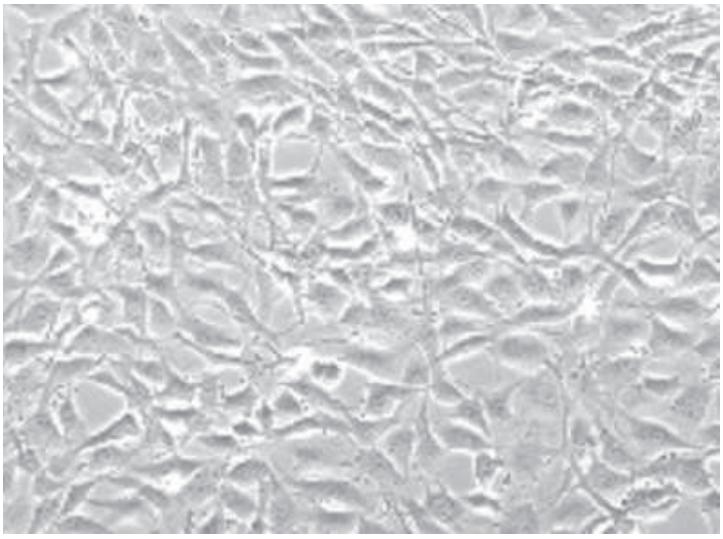


Figure 4 Monolayer of cells grown adherent on plastic surface

For cell culture today there is available a wide range of containers like culture flasks, culture bags, bioreactors, spinner flasks etc. depending on the desired application and the type of culture.

2.1 History of Cell Culture

First tries to culture tissues and organs are long time ago. The beginning of the age of cell culture and tissue culture is dated today to the beginning of the 20th century. At that time a few scientists began to work on this field. One of the main challenges at that time was the numerous types of contamination, which were very difficult to be handled at that time. The discovery of Penicillin at the end of the 1920s and the development of antibiotics and antimycotics helped to cope with contamination issues. At that time standard cell growth media, as we know them today, were not available and first culture trials were performed by using Ringer's solution. [20]

With the development of cell type specific culture media in the middle of the last century cell culture became a triumphal technology in life sciences. Since that time cell culture grew not only to the widespread technology replacing animal experiments, but also developed to an essential tool for biotechnological research, drug discovery and medical therapies.

Nobel price owner Alexis Carrel is called the 'father' of tissue culture. He developed the theory, that cell cultured in vitro are able to divide endlessly. He has cultivated a tissue cut of the heart muscle of a chicken embryo. The fibroblasts of this primary tissue grew continuously for 34 years. Leonard Hayflick and Paul Moorhead showed later in 1961 that the theory of endless division of cells in vitro is false.

Figure 5 Alexis Carrel, source: The Library of Congress, PPOC

In the late 1940s Earle, Hanks, Eagle, Dulbecco and Ham performed a lot of pioneer work in the development of defined standard cell culture media that are used still today in cell culture of primary culture and established cell lines.

In 1951 Alma Howard and Stephen Pelc discovered the four phase cell cycle .

The HeLa was the first cell line taken from human tissue in 1952. Donator was the 31 year old Henrietta Lacks from Baltimore who suffered from cervix carcinoma. That cell line is still available up to today as in vitro culture. It is the worldwide most discovered cell line. [21]

In 1975 first cell hybrids were cultured constantly. Those Hybridoma cells are used in antibody production.

Further milestones in cell culture were the exploration of the sequence of the telomers, the finding of the Telomerase and many more which cannot be mentioned in their full scale. Many of them helped to make cell culture easier, more reproducible, more stable and safer. [22;23]

2.2 Classification of Cell Culture

Types of cell culture can be separated by means of different characteristics of cell cultures. So types of cell culture can be divided into primary culture and culture of cell lines. Another classification is the division into adherent growing culture, the suspension culture and the 3D culture where the types relate to the way cells are growing in the in vitro environment. Furthermore the separation into the duration or number of passages is a classification of cell culture. Some cell types can only be passed a few times – the finite cultures. Other cell lines can be cultured permanently, especially various tumor cells, transformed cells and stably transfected cells. Following the technologies of adherent growing, in suspension growing and in 3D structures growing cultures will be explained slightly more in detail because this classification and the treatment of the cells in this technologies is important for cell culture automation.

2.3 Adherent Culture

Standard form of in vitro cell culture is adherent culture where cells grow in a monolayer on an artificial substrate. To allow cell adhesion the substrates have to be correctly pretreated and charged. Although many types of cells can be grown in suspension or can be adapted to suspension growth, most normal cell lines need to spread out on a substrate to allow proliferation. The original substrate for adherent cell culture was glass, but today it is substituted almost completely by disposable plastics. Sterile Polystyrene in good optical quality is suited very well for disposable plastic substrates used for monolayer cell cultures. Polystyrene is hydrophobic after production. Treatment by gas plasma, γ-irradiation, corona discharge or chemical treatment provides a wettable surface. Other materials of disposable labware for adherent cell culture are PVC, Polycarbonate, PTFE and others. Most common plastic labware for adherent culture are cell culture flasks – so called T-flasks, micro titer plates treated for cell adhesion and the newer automation friendly cell culture flasks in microplate footprint. [24;25]

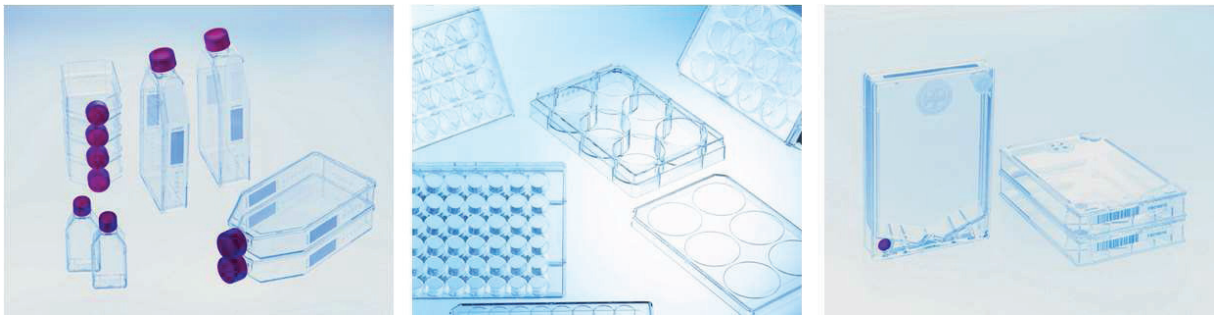


Figure 6 Adherent cell culture labware in microplate format ©Greiner Bio-One GmbH, Germany

2.3.1 Cell Culture Process in Adherent Monolayer Culture

Figure 7 shows a scheme of a typical cell culture process for adherent grown monolayer cultures in automation friendly SBS microplate footprint culture flasks.

Culture process starts with seeding of cells into culture flasks and addition of cell growth media, and depending on cell lines and culture conditions of growth factors, antibiotics etc.

After liquid handling the cells incubate under defined environmental conditions (temperature, humidity, atmosphere). To maintain a stable ph value regardless of consumption of media and addition of cell metabolism products into suspension cell growth media are buffered solutions which are able to balance pH value by using CO_2 atmosphere. The cells remain incubated for a time of 2 days to 10 days depending on growth rate and other cell properties as long as there has grown a confluent monolayer of cells on the substrate.

After the incubation period cell culture medium is removed from culture labware, the cell layer stays adherent on the substrate. After this step the cell are washed by using a buffer solution to remove the rest of the media from cells and culture vessel.

The harvesting of the cells – cell passage – the loosing of the cell layer from the substrate is done by adding cell detachment solution which consists of enzymes like Accutase or Trypsin. After a short incubation period of some minutes the

cells have detached and the enzymatic activity is stopped by adding fresh media (when using Trypsin). As result the cell culture vessel contains a solution with an appropriate number of cells for splitting and seeding into new culture labware or for usage in the desired application, or a combination of both. [18-20]

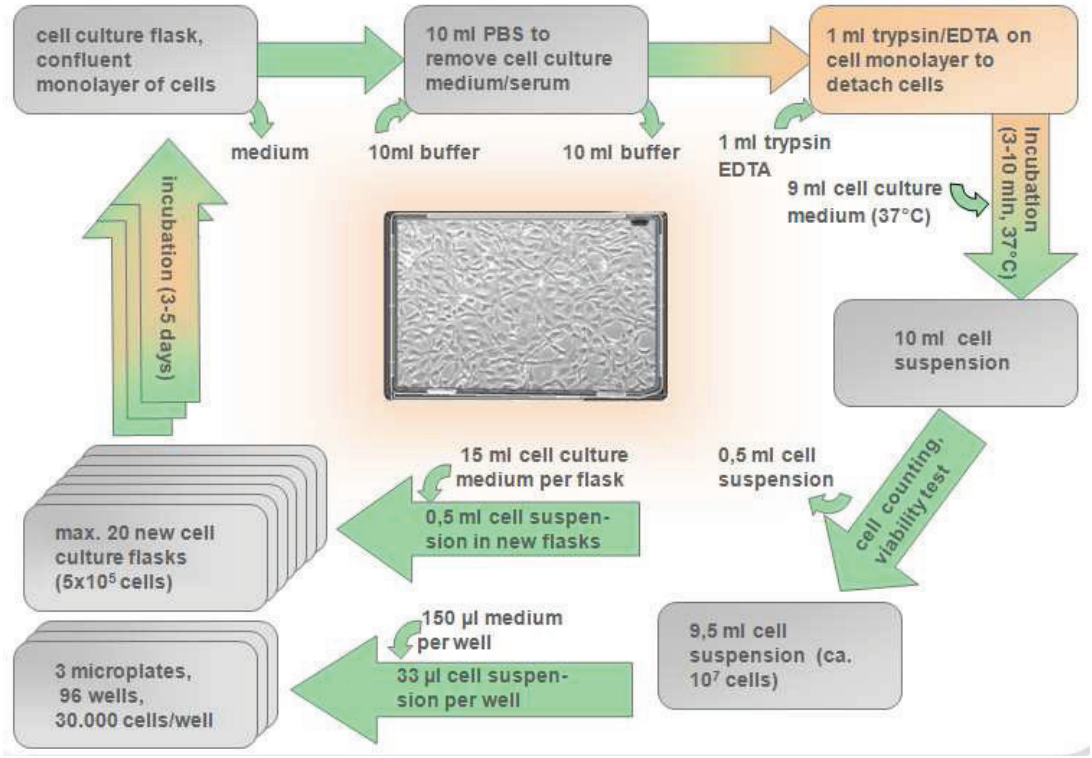


Figure 7 Cell culture process for adherent monolayer cultures

2.4 Suspension Culture

In general cell of the hematopoietic systems grow in suspension while tissue cells grow adherent. However, some tumor cell lines and many transformed cell lines can be adapted to grow in suspension and can be made independent from surface charge.

Suspension cultures can be done in any kind of sterile flask, plate or petri dish. Some suspension cultures need to be kept in agitated suspension. For those applications there are available stirrer flasks, spinner flasks or orbital and wave shakers to keep suspension in motion. Here has to be taken care on slow motion because of cells sensitivity against shear forces. [26;27]

Figure 8 Spinner flasks for cell culture

The main aspect of suspension culture in reference to automation is, that suspension cells need to be centrifuged for cell harvesting, media exchange etc. For that purpose, in a cell culture automation there have to be available automation friendly culture flasks that are suited to be centrifuged. In addition an automated centrifuge has to be integrated into the cell culture line.

2.5 3D Culture

A special form of cell culture which combines the advantages of adherent and suspension culture is the culture of cells on microcarriers. These microcarriers are kept in a continuously stirred suspension. On microcarriers cells are able to grow in 3D structures which are nearer to tissues as they grow in vivo compared to monolayer cultures or common suspension cultures. This is due to interaction of neighboring cells and extracellular matrix. So growing cells on microcarriers combines the advantages of adherent culture and suspension culture. The growth of cells on microcarriers was performed in large scale from liters to thousands of liters up to now. The investigation on new and cost efficient technologies for microcarrier based culture evens the way to microcarrier 3D cell culture also in research lab environment and cell based screening facilities. The design of new technology magnetic microcarriers without autofluorescense allows for using this 3D cell objects which are near to in vivo cells directly into assays with optical

readers. If required, the cells can also be removed from the microcarriers when harvesting. [28-31]

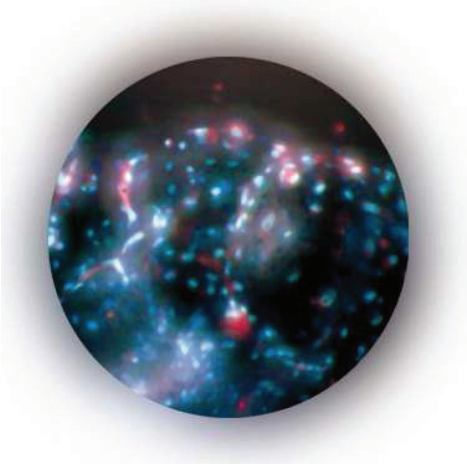


Figure 9 Cells in 3D on microcarrier source: Global Cell Solutions Inc.

The magnetic microcarrier design enables automated 3D culture in small scale for scientific laboratory use, research and screening. 3D cells can be cultured in actuated tubes operated by benchtop devices. The principle is to keep the microcarriers soaring in the solution by influencing them with magnetic fields. This technology was evaluated among others during this dissertation work.

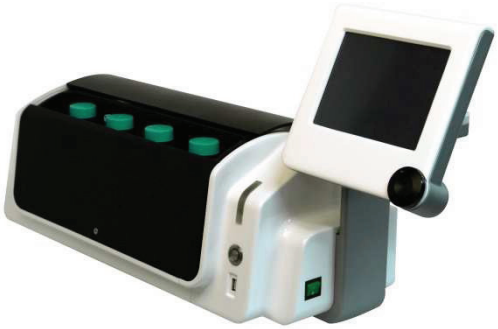


Figure 10 3D culture system using tubes source: Hamilton Company

3 Life Sciences and Automation

In the field of laboratory automation the challenges grow continuously. One fundamental requirement to automation solutions is high throughput. Others are flexibility, reproducibility, throughput, ease of control and riddance of monotonous manual work steps of laboratory staff. All these aspects have to be considered when designing solutions in cell handling automation.

For higher throughput and cost efficiency parallelization is an established way in life science automation. The development of the micro titer plate technology replaced the use of test tubes more and more. Today liquid handling robots are mostly designed for microplate format handling. By development of microplate format cell culture flask the labware vendors prepared the way to cell culture automation based on microplate liquid handling robot technology.

The rapidly growing fields of Biotechnology, Life Sciences and its fields of Drug Discovery, Genomics, Proteomics etc. provided besides the necessity of higher parallelization and fast analysis also a high flexibility in automation systems regarding hardware and application. Lab automation systems should designed in a way that allows for easy integration of additional components and adaption of applications and assays. So automation platforms with open architecture in hardware and control systems deliver advantages in laboratory automation and life science automation. Those systems are always adaptable, expandable and thus, compatible for future developments. In higher stages of this technology there are available components and devices that can be operated ‚plug and play'. [32;33]

By continuous miniaturization in laboratory automation the requirements to liquid handling robots and, especially for detection platforms have been higher and higher within the last years. Besides liquid transfers in sub microliter ranges the systems must be very precise for accessing 1536well plates, 3456 plates, and in addition for tube access, tube piercing etc. [34]

Continuous growing sample numbers and data points generate a new challenge to data storage and data processing technologies. While Data Storage

technologies are well developed and available as cluster server and database solutions, data analysis is often still manual work and very time consuming, what makes it a limiting factor in laboratory automation solutions. [35;36]

Therefore intelligent software solutions for measurement are needed which analyse automatically process data and measurements, so that systemic errors in measurement data and changes in application quality can be registered automatically. [35]

Due to the subject of this thesis – animal and human cell handling and cell culture automation - the following chapters focus especially on the fields of cell based applications and screening in laboratory automation.

3.1.1 Fully Automated Systems

Full automation in laboratory environments requires system integration of devices for different functions like sample preparation, liquid handling, sample treatment, data acquisition sample transport etc. Central system integrator commonly is a robotic system that connects the stations by transporting sample containing labware, reagents and i.e. cell suspensions. By combination of stations like pipetting robots, incubators, detection platforms and treatment stations like shakers, washers etc. full automation can be obtained to processes like cell based assays, cell culture and so on. There are different vendors who offer full automation solutions for drug discovery, screening and cell handling. [37;38]

3.1.2 Robotic Systems

Based on their mechanic design robots can be classified into following categories:

- Cartesian robots
- Gantry robots
- Cylindrical robots

- Articulated robots
- Scara robots

Cartesian and Gantry robots are found especially in liquid handling systems like pipetting robots. By combining three orthogonal mounted axes the needed degrees of freedom for pipetting task on a rectangular work surface can be obtained. In addition to pipetting tasks these robots can contain further vertical axes for labware grippers. There are different configurations with gantry and axis designs.

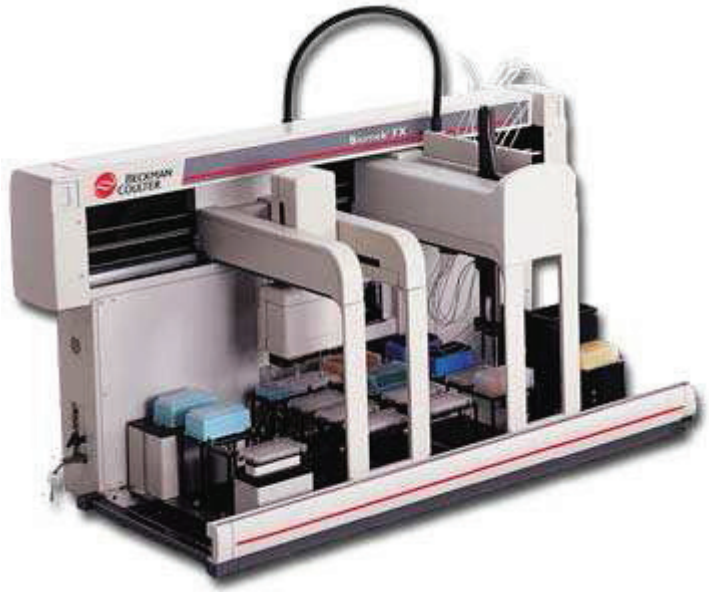


Figure 11 Pipetting Robot including single channel and multi channel pipetting

Cylindrical Robots are placed in a central position inside multi component systems. These robots have linear axes in horizontal and vertical directions. These axes are mounted on a rotating column so that the work area of this type of robot is a cylinder. Cylindrical robots are found as central system integrator and as pick and place devices. Pick and Place Robots are i.e. Plate Crane EX (Hudson Robotics, Inc.; pick & place), Twister II (Caliper Life Sciences, Inc.; pick & place), Orbitor RS Microplate Mover (Thermo Fisher Scientific; pick & place) and XP robot (Caliper Life Sciences, Inc.; central system integrator).

source: Hudson Robotics, Inc.

source: Caliper LS

source: Thermo Fisher Scientific

Figure 12 Cylindrical robots in laboratory automation applications

Articulated robots are able to operate in a 3D work area with a high flexibility. The number of joints and axes causes a high number of degrees of freedom. Five to six degrees of freedom are common and allow performing complex motion sequences with its end effector. Thus articulated robots are suited very well to connect different types of devices with constraints regarding labware put and get accessibility. So articulated robots, often mounted on a linear rail to enhance the accessible workspace, are the first choice as system integrator in complex laboratory automation processes. There are some types of robots especially made for laboratory automation. (i.e. ORCA® robot, Beckman Coulter, Inc.) In the past suppliers for complex laboratory automation systems tend to integrate industrial robots (Motoman HP3JC integrated by Beckman Coulter, Inc.; Stäubli integrated by GNF Systems)

Figure 13 Articulated 6-axis robot in lab automation work cell, source: Beckman Coulter, Inc.

SCARA robots – selective compliant assembly robot arm – offer less degrees of freedom and herewith less flexibility to operate in a 3D work area compared to articulated robots. However they are capable to perform very fast pick and place operations and are applicable for less complex systems integration and for pick & place tasks like i.e. tube sorting. SCARAs are integrated by companies like Adept Technology GmbH, Manz Automation AG and i.e. Agilent Automation.[39-42]

Figure 14 Scara robot 'direct drive robot', source: Agilent Automation

3.1.3 Liquid Handling Robots

Liquid Handling in laboratory automation means transfer of liquids between labware and includes labware to labware transfers, dispensing, aspiration and transfers to waste.

In modern life science laboratories liquid handling robots are used for microplate pipetting, single tube liquid handling and handling of custom formats. There is a wide range of applications from simple pipetting tasks like plate to plate transfers, media addition serial dilutions etc. to complex operations like running of complex assays. Many liquid handlers support on deck integration of additional devices like barcode readers, heaters, thermocyclers, shakers, washers, plate readers and so on.

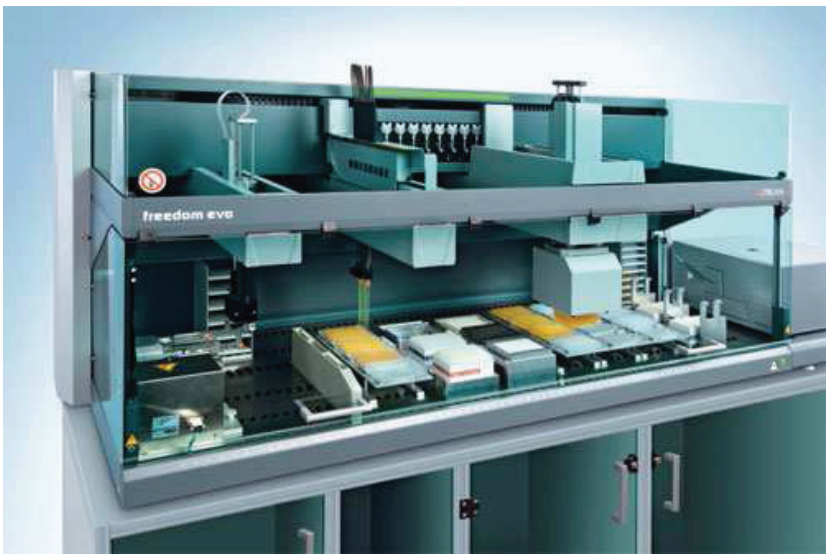


Figure 15 Liquid Handling Robot source: www.tecan.com

The necessity of cost efficiency, miniaturisation as well as continuous growth of sample number in diagnostics and drug discovery have driven the development of pipetting robots in the past and still today. Liquid handling robots are mostly capable to operate microplates in all formats up to 1536 well plates. The most used microplate format in high throughput screening today is the 384 well plate.

Regarding their liquid handling capabilities there are two forms of pipetting systems:

Multichannel pipetting heads are available as 96 well or 384 well types. These pipetting heads operate all channels equally at a time and are used for plate replication, media addition and so on. Some vendors offer special solutions to work with custom tip loading option to provide the opportunity to perform individual single well or single column transfers with multichannel pipetting heads. (i.e. Beckman Coulter Biomek FX^{P} / NX^{P} Multichannel head selective tip pipetting option)

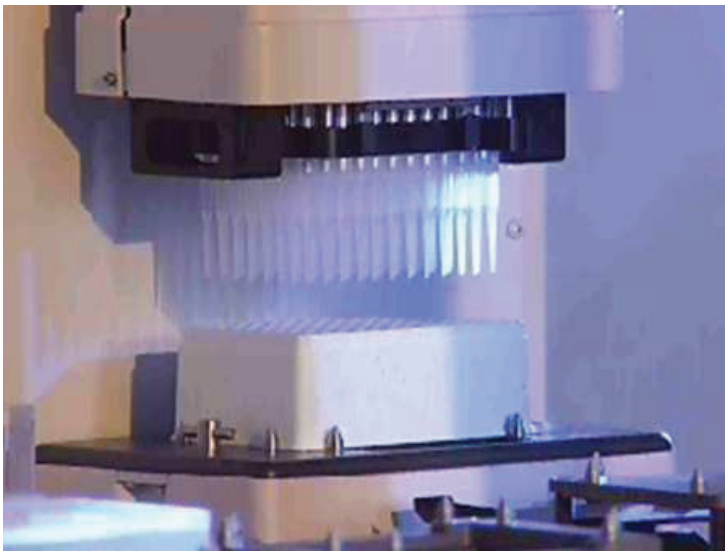


Figure 16 Multichannel Pipetting Head

Single channel pipetting heads are used for individual liquid transfers, hit picking transfers, tube liquid handling and other operations where multichannel heads don't work. Actually there are real single channel heads available and other systems with four or eight pipettes. Some suppliers of liquid handlers offer systems where the four or eight pipetting channels work independent in pipetting height, span between the pipetting channels and volume. An additional liquid level sensing technology allows high precision pipetting in labware with unknown liquid levels.

There are different technologies for pipetting. Most systems work with system liquid technology. There are also systems available which use air displacement

piston technology coupled with pressure sensing (Hamilton monitored air displacement technology), others work with micro annular gear pumps (Lissy – Zinsser Analytic GmbH, Xantus – Sias AG) [43-45]

Figure 17 Independent 8-channel pipetting head

Systems vary in deck space and ability to integrate 3rd party devices. In addition an important aspect is software functionality. In general the trend goes to intuitive graphical user interfaces (GUI) that can be operated by laboratory personnel without having technical background. However, in a more detailed view software abilities are quite different between vendors of liquid handling systems. The best equipped packages come today with easy to operate interface that allow fast, easy and intuitive method development on the one hand, on the other hand they support adaptations in liquid handling details down to the machine control layer for specialized techniques. Functions like liquid level tracking, liquid types etc. are found only in the software solutions of the leading vendors of liquid handling systems. [46;47]

3.1.4 Detection Systems

3.1.4.1 In High Throughput Screening

In life science automation and screening there is a wide range of detection platforms. In high throughput and highly parallelized systems fast detection is essential. In this field microplate readers are often used. Microplate readers are analytic devices with optical sensing systems for measurement of absorption, luminescence, fluorescence (fluorescence intensity, fluorescence polarization, time resolved fluorescence), UV/VIS-spectrums and other. [48-51]

So called Multi Mode Readers unite different detection technologies in one device. Modern Multimode Readers are flexible in configuration. The outperforming products contain different optical cartridges for different detection modes which are changed automatically. In addition the readers can be configured with varying sets of optical cartridges. Differences can be found here regarding light source: Some Multimode readers with optical cartridges have one common light source and only the detection cartridges are exchangeable. (i.e. PHERAstar, BMG Labtech GmbH) others contain detection system as well as light source inside the exchangeable cartridges. (i.e. PARADIGM Detection Platform, Beckman Coulter, Inc.) [52;53]

3.1.4.2 In High Content Screening

As already mentioned in chapter 1.1.2 in High Content Screening analyses go deeper in detail then in High Throughput Screening. Therefore detection systems are more complex and allow for deeper insights into mechanisms of cells. High content analysis is performed often by using automated fluorescence microscopy. The higher level imagers are capable of confocal imaging and image processing so that several different-layer-images can be coupled together to provide a sharp image with higher depth of field. Some imagers contain live cell chambers for live cell imaging. Selected imagers are equipped with integrated automated pipettes to allow sample addition etc. in runtime. (i.e. BD Pathway 855). The high end imagers include fluorescence intensity measurements, kinetic imaging (i.e. for

neurite outgrowth), and morphological analysis, including sub-cellular imaging.[54]

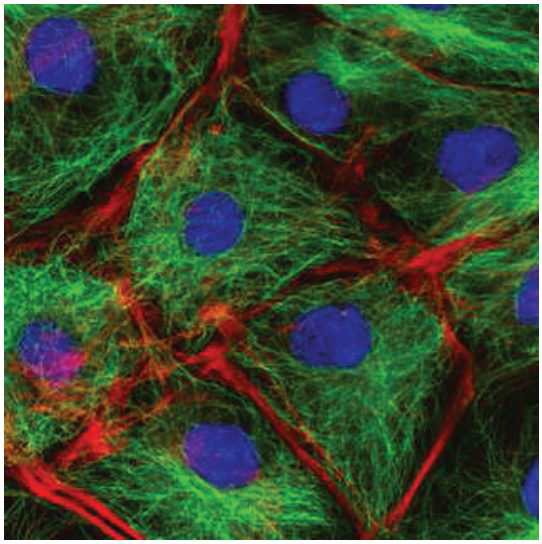


Figure 18 Confocal image showing nuclei and cytoskeleton (microtubules, filaments), source: http://sites.google.com/site/scienceprofonline/

3.1.4.3 In Automated Cell Culture

In automated cell culture detection focuses on cell quality parameters like cell viability, cell number per milliliter, cell size, clustering etc. Therefore on the some suppliers for laboratory devices offer so called cell counters which work with different principles. Most cell counters work with optical methods (Fluorescence microscopy with fluorescent dyes – i.e. Chemometec NucleoCounter®, Trypan blue dye and microscopy – i.e. Innovatis Cedex, Beckman Coulter Vi-CELL™ series), some work with special technologies like current measurement and pulse area analysis (Innovatis CASY series). In addition, measurement of media quality makes sense. Therefore optical technologies like colour measurement (ph value) and / or light scatter measurement can be used. [55] Depending on the technology some devices are more or less suited for automation. I.e. devices that work with disposable cartridges where cell suspension has to be pipetted in before inserting for measurement cause automated handling of additional labware which is a challenging issue often. Some devices that are designed for manual use are available in versions adapted for remote control and automated cell suspension aspiration. [56;57]

Figure 19 Automated cell counting device; source: www.innovatis.com

3.1.5 Complex Automated Systems

There are several concepts for laboratory automation that base either on smaller workstations or on complex laboratory automation systems, depending on application and the needed degree of flexibility, throughput, reliability and obtainability.

For systems with fixed applications and the need of high throughput and less flexibility systems with linear automation and linear workflow can be used. In those systems all devices are combined in a linear one-way structure where the stations are connected by conveyor belts or similar. Devices that are needed more than one time in the application have to be duplicated and to be placed in appropriate position. Software of those systems can be kept quite simple because there is no scheduling and ease of method design needed. Assays are easy applicable to linear automation systems. But with linear automation systems there is always a lack of flexibility. For this reason those systems are used more in routine applications and less in research and discovery. Some systems are capable of labware transport in two directions that causes in relevant reduction of throughput. Examples for linear systems are PlateTrak® (Packard Bell), MAP® (Titertek), Allegro® (Zymark). At present linear systems are substituted more and more by flexible integrated systems. [58-60]

Figure 20 Linear automation system; source: www.biodirectusa.com

Other integrated Systems were designed in the past for a specific application and were not fully open for further integrations. The advantage of these systems was the reduction of mechanical and software interfaces between systems and sub systems. There were systems used in screening like EVOsccreen® (Evotec Technologies), UHTS Platform (Pharmacopeia) and UHTSS® (Aurora Biosciences).[61;62]

There are two applications, where those closed systems are still used today: sample and compound storage systems and routine cell culture. Suppliers of storage solutions are i.e. REMP AG (LSS, MSS, SSS), Hamilton Storage Technologies, Inc. (SAM, ASMST), Matrical (Nitrostore Mars, MiniStore™, MatriStore™). For routine cell culture there are all-in-one cabinet systems available which integrate cell culture incubation, robotic culture flask handling, liquid handling and quality control like cell count, viability measurement etc. Often those systems tend to reproduce manual operator's cell culture steps. So the robot as central system integrator does not only perform transportation task, but also things like pouring, operation of Pasteur pipettes, flask shaking etc. Systems of this type were seen from The Automation Partnership (CompacT SelecT, ComacT CellBase, Cello, Cellmate), Matrical (MACCS™), GNF Systems and others. [63;64]

Figure 21 Compound Storage System

Figure 22 Routine Cell culture system source: http://www.biotechno.ru

Automation lines with an open architecture, allowing easy integration of further components, are best suited and most cost effective for applications in research and discovery environments where type of tasks change and grow continuously. These systems are easily and fast adaptable to changed requirements and offer adaptability to new developed technologies. For integration of third party components there are software developer's kits available to allow seamless integration. Those systems are known from Beckman Coulter, Inc. (Integrated Robotic Solutions – Industrial Robot as system integrator, Sagian™ Core systems with ORCA® Robot, Workstation automation using direct on deck integration, integration by conveyors and BRT Biomek® robotic transport), Thermo Fisher (Dimension4™ Modular Automation Platform, Catalyst system, F3/F3T robotic system), Hamilton Robotics (Hamilton integrated instruments), HighRes Biosolutions (MicroStar, NanoCell) [65-68]

Figure 23 Thermo Scientific modular automation system, source: www.thermo.com

Figure 24 HighRes Biosolutions modular automation platform
source: http://www.highresbio.com/systems/microstar.php

4 System for Automated Cell Culture

When going over from automated cell culture processes to automated cell culture there is first the necessity of an analysis of work steps in manual cell culture. This has to be performed from the perspective of the manual operator, as well as from the perspective of cell treatment and handling to change from manual operation to automation. This implicates, that not only patently seen work steps like pipetting, media exchange, incubation, measurement of cell count and viability have to be analyzed. In addition there has to be focused on small steps and handlings that are performed as an automatism by the manual working laboratory staff.

For partially processes in cell culture there is a number of devices available on the market suited to be integrated into automated cell handling systems. To be successful integrating a complex laboratory automation system for cell culture and to provide functioning applications with growing cells, engineering know how and scientific biologic know how has to be combined. Culture of animal and human cells in scientific research environment can be divided into categories with different complexity, that all contain various work steps. Some of these steps are the same in all these different categories, others vary and for this reason they need sets of variable parameters in an automated environment. Specific parameters are directed by the specific properties of the cell line. This can be done with easy to handle robust cell lines like cancer cell lines as well as for very sensitive cells like embryonic stem cells. [69;70]

4.1 Work Steps in Cell Culture

From the cell handling point of view the generalized steps of liquid handling, incubation and measurement of cell count and viability are to be executed. Liquid handling contains here transfer of cell suspensions, transfer of growth media, buffers, growth factors and medicals, furthermore aspiration of liquids and transfer to waste, cell harvesting and distribution of readily grown cells in destination containers like, tubes, flasks and micro titer plates. Incubation is

done mostly at 37°C in controlled CO_2 atmosphere. In addition there is a number of handling steps that are performed by the laboratory staff in manual culture that has to be adapted to automated processes now. These contain handling and transport of all kinds of used labware, opening / closing / delidding of labware, pivoting of labware containing cells, tapping of culture labware for cell passage etc. Moreover there are tasks like time management, run time decisions and calculations like distribution of cell count per volume in destination labware, storage of labware, labware labelling, labware label reading, tempered storage of liquid media, data logging, documentation and many more. [71]

4.2 Requirements to Automated Cell Culture Systems

In addition to the transformation of work steps from manual cell culture to fully automated culture and analysis of further handling steps of manual operators there are more factors to take attention on. For an automated cell culture system, that works in research lab, as well as in routine tasks there are following requirements:

- **High Degree of Automation**

The aim of an automated cell culture system should be, to achieve the highest possible degree of automation. This spans from preparation of liquids like growth media, preparation of cell solutions over liquid handling, labware transport, preparation of assay plates, quality control, data logging to integration into process management environments. [72]

- **Compactness**

Laboratory space is always critical to complex automation solutions. The need of compactness is driven by space saving issues and, in addition necessary from the workflows perspective.

- **Capacity**

Batch size of cell culture processes is always limited by labware storage capacity and liquid capacity of the system. Depending on the application cell culture systems should be able to process up to thousands of plates for routine

production purposes or just about one hundred for research and discovery purposes.

- **Flexibility**

Except in routine fixed applications flexibility is of enormous importance in automated cell culture. Different cell lines require different treatment, constraints by the following processes demand flexibility in automated culture processes. Besides system design for flexibility particularly the method editor- and control software has to be able to support changes comfortable and intuitive. This is an important issue because it is a major challenge to provide editing and control software meeting the needs of complex control, adaptability, flexibility and intuitive graphical user interfaces (GUI's). [73]

- **Sterility**

Sterility and continuous maintenance of sterility is unavoidable requirement in cell handling and thus in automated cell culture. Continuous expansion of cell lines requires operation under stable sterile conditions for months. Contamination by microorganisms like Bacteria, Viruses and in worse cases Mycoplasms. [74]

4.3 State of the Technology

As earlier mentioned the focus here is reduced to cell culture in cell culture flasks and micro titer plates leaving cell culture in bioreactors and bags disregarded. All existing cell culture platforms availably base on laboratory robotic components. At minimum the labware transport is performed by robotic transports. Due to the biological needs all platforms are enclosed in cabins or hoods with HEPA air filtration to maintain sterile conditions. All systems have in common, that they use automated CO_2 incubators, media storage. In addition automated liquid handling is integrated, partially performed by microplate liquid handlers, others use robotic arms holding Pasteur pipettes or similar for liquid handling. All systems include at least a cell counting / cell viability measurement. Some are equipped with further detection devices i.e. to select transfected clones etc. Differences are also found in the scope of functions. Some devices are designed

generally for production of cells in mid to high capacity range. These systems are mostly reduced in flexibility in terms of hardware changes and software capabilities. In general the systems for routine production come with simple user interfaces providing ease of use but implicated limited method and parameter editing capabilities. Table 1 shows a selection of automated cell culture systems currently available. [75]

Company	System	Comments
The Automation Partnership	CompacT SelecT Cello Piccolo Cellmate SelecT etc.	• large selection of systems in different sizes, for different labware and with different functionalities • industrial robot performs work steps besides transport (liquid transfer, shaking, pouring of flasks, capping) • turnkey solution
Matrical Bioscience	MACCS™	• one general platform, expandable modules, integrated articulated robot performs work steps • proprietary labware (MatriMix™) • turnkey solution
Thermo Scientific	CGD workCell™	• one platform for cell expansion and assay execution • turnkey solution
GNF systems		• large scale cell culture connected to uHTS solutions
Tecan	Cellerity	• based on automated microplate liquid handler • designed for Corning® RoboFlask™
Hamilton Robotics	Cell^{host}	• based on automated microplate liquid handler • evaluated with embryonic stem cells
RTS Life Science	acCelerator	• mid size system
Agilent Automation	BenchCel Workstation, BioCell Workstation	• custom designed cell maintenance and cell expansion, modular system

Table 1 Examples for suppliers of automated cell culture systems

4.3.1 Patents and Publications in Automated Cell Culture

Automating cell culture procedures has a history of about 20 years of development. A first cell culturing apparatus has been patented in 1990 [76]. The rack supporting apparatus includes a loop tracking and a plurality of

culturing racks connected in series. Each culturing rack accommodates culturing containers therein the cell culturing during their travel on the loop tracking. Each rack is through conveyors accessible to a container handling station where culture medium is filled and cell inoculation is carried out. Culturing containers in which cell culturing in finished, are removed by a discharge station. Kearney patented in 1995 an automated cell culture and testing system [77]. The system is maintained at a slightly positive pressure to prevent contamination from the surrounding. It includes three levels of containment to completely isolate living tissues from ambient surroundings. The system has successfully been tested under conditions of zero gravity. The cells are cultured in a plurality of bioreactors containing an inlet and an outlet for supplying nutrition media or tissue testing media. Also included are a gas pathway for supplying filtered humidified gas and an exhaust of the spent media. Another automated, multicompartmental cell culture system was published in 1997 [78]. The invention relates to an in vitro system for physiological and metabolic investigations of potential drugs. It includes different cell chambers containing cells in a culture medium and a gas-liquid exchange device for providing oxygen to the cells. The substances to be tested are added to culture medium and circulated through the system. The cells in the cell chambers are then evaluated for effects of the added substances.

An automated cell management system for growth and manipulation of cultured cells has been described in 2004 [79]. Primary goal of the system is its use for high-throughput cell culture as well as the integration of dissociated operations of cell culturing and cell manipulation in one unit. Main unit of the system is a biochamber to provide controlled environmental conditions in which the cells may be cultured and manipulated. Temperature, atmospheric gas content, humidity and airflow are controlled. The storage array can accommodate a number of cell culture devices (50-1000). A mechanical gripper and a mechanical arm are integrated for the transportation of the cell culture devices within the system. A centrifuge as well as a harvesting station and pipette system with a plurality of reservoirs are integrated into the system.

In 2007 an automated bioculture and bioculture experiment system has been patented [80]. The system allows feedback control for bioculture platforms and can be used as a precision cell biology research tool and for clinical cell growth

and maintenance applications. The claimed invention is a cell culture apparatus for use within an incubator including different sensor for monitoring the cells such as pH sensor, glucose content sensor, oxygen sensor and a spectroscopy sensor.

Devices for in vitro cell culture and methods for a microscale cell culture analog device have been reported by Shuler et al. [81]. The devices allow for the maintenance of cells in vitro with pharmacokinetic parameter values similar to in vivo. The used microscale device comprises a fluidic network of channels segregated into discrete but interconnected chambers. Different chambers are designed to provide specific conditions such as liver or fat pharmacokinetics. In 2007 a patent has been granted for a human cell/tissue culturing system [82]. The system includes a plurality of incubators for aseptically storing and culturing cells or tissues derived from a human individual. A bioreactor apparatus and cell culturing system for the automated cultivation and processing of living cells on earth and in low gravity has been developed by Vellinger et al. [83]. They use a cylindrical reactor vessel which can be optionally rotated about its cylindrical axis while allowing the entrance or removal of fresh / spent media and the collection of samples. A plurality of reactor tubes is also used by Choi et al. to simultaneously apply compressive strain for cell differentiation and shear strain for cell proliferation to cells [84].

The automation of small cell culture at an experimental level on research has been reported in 2008 [85]. The apparatus permits the execution of a medium replacement process and a passage culture process. A pipetting unit as well as a centrifugal unit is integrated in the system together with a handling unit for moving the culture vessels and the centrifuge tube to the different units. The apparatus can include a housing for providing sterile conditions.

Recently Hibino et. al reported a cell culture system apparatus for checking cultured cells [86]. This system enables the culturing of predetermined cells collected from patients and supplies cultured cells to the original patient. Cells are transferred to culture containers. Important part of the invention is a system for unique identification information.

A patent application for an automated method and apparatus for embryonic stem cell culture has been provided in 2009 [87]. The invention covers methods for

the automated culture of embryonic stem cells (ESCs) and automated systems for passage and expansion of ESCs. The system uses 4-, 6- or 8-well plates for the culturing and maintenance of the cells. It is housed in a class 100 clean room compliant with biosafety level 2. The automated maintenance of embryonic stem cell cultures based on a Cellhost system has been evaluated by Brüstle et al. [88].

One of the latest patents in the field of automated cell culturing has been granted to Suzuki et al. for a device for cell culture in 2010 [89]. The system is capable of automatically performing operations for cell culture over several days to several months. The state of the culture in the system is observed with a camera. Wastewater / old culture medium can be automatically discharged from the system. Robbis et al. reported in 2010 a perfusion bioreactor for culturing cells [90]. The system comprises a multi-well platform including an array of bioreactor units. The system includes a perfusion unit with a multi-well plate.

The miniaturization of cell processes is currently an interesting research field. Quake et al. reported a versatile fully automated microfluidic cell culture system. Main part of the automated cell culture platform is a ODMS microfluidic chip with 96 individually addressable culture chambers (volume 60 nl each) [91]. A microfluidic system fabricated using MEMS techniques was reported by Huang and Lee. The system is capable of automating cell cultures and has successfully been tested for the cultivation of human lung cancer cells (A549) [92]. The merging of surface tension driven passive pumping with traditional fluid handling tools to create a new microfluidic platform for cell culture has been described by Meyvantsson et al. [93]. Hung et al. presented a high aspect ratio microfluidic device for culturing cells inside an array of microchambers with continuous perfusion of medium [94]. The microchambers have the same cell growth area as a typical well in 1,536 well plates. The system was successfully tested for the cultivation of human HeLa cell line.

4.4 Motivation

Based on an overview on the state of technology and on investigation on the specific requirements for cell culture in the research environment of the Center for Life Science Automation following aspects influenced the decision to develop a proprietary automated cell culture system.

- **research environment**

  Life Science automation requires special properties to processes and systems. Especially in research, screening and discovery the conditions change often and functionalities need to be able to grown continuously. Therefore a system with an open architecture is needed on the one hand, and the skills to integrate new features and components help on the other hand.

- **small footprint**

  Conventional systems most span mainly horizontal. A sandwich architecture where components are stacked over each other seemed to be more lab space efficient and room effective.

- **mobile option**

  To increase the use of the system and to bring it near to the points of application for the cultured cells, one of the goals was to be able to transport the automated cell culture system from one laboratory to another. The stacked design supports this idea by providing small footprint.

- **flexibility**

  Especially in the exploring environment of research and discovery a flexible system design and easy method change is essential. Compared to other solutions with easy to use, but limited software interfaces the aim for the automated cell culture machine was, to support flexibility by state of the art method editing software providing illimitable capabilities from easy intuitive programming of standard procedures to full detailed control of work steps.

  Main approaches to meet these requirements on flexibility are:

- Universal functionality by using open hardware and software platforms
- Complete data tracking of process parameters and variables and herewith potential for nested processes operation
- Intuitively controllable method editor
- Process planning and process editing using process management software supports comfortable planning of multiple processes in advance
- Different processes are able to be planned and executable
    - cell line maintenance
    - expansion of cells
    - seeding of cells into destination labware
    - further liquid handling processes and performing of assays

- **proven technology**

The concept was to integrate available devices that already have been proven in laboratory automation and to develop and integrate proprietary components to complete the automated system in terms of special functions and special handling steps in cell culture.

- **software features**

Software for laboratory automation systems varies from text based editors, over graphical user interfaces with standard operation sets to intuitive GUIs for fast and easy method editing including options for detailed influence of operation. To open all features and flexibility in method design the software control system has to be state of the art including intelligent scheduling, complex process planning and supporting handling of parallel cell culture processes.

- **scientists and engineering**

For automation projects in fields of life sciences combination of scientific and engineering know how is essential for success in application. The structure of the Center for life science automation with the combination of cell biology

scientists and engineers allows for this in a good manner an secures for reaching the goal, not only to develop automated systems, but also establish stable and steady running of cell culture applications in automation.

4.5 Concept for Automated Cell Culture System

When designing custom laboratory automation systems, a number influencing factors from different aspects have to be included in system design and control philosophy. Main aspects for design of a laboratory automation system are:

- Control system aspects
- Sample and biology or chemistry aspects
- Systems architecture aspects

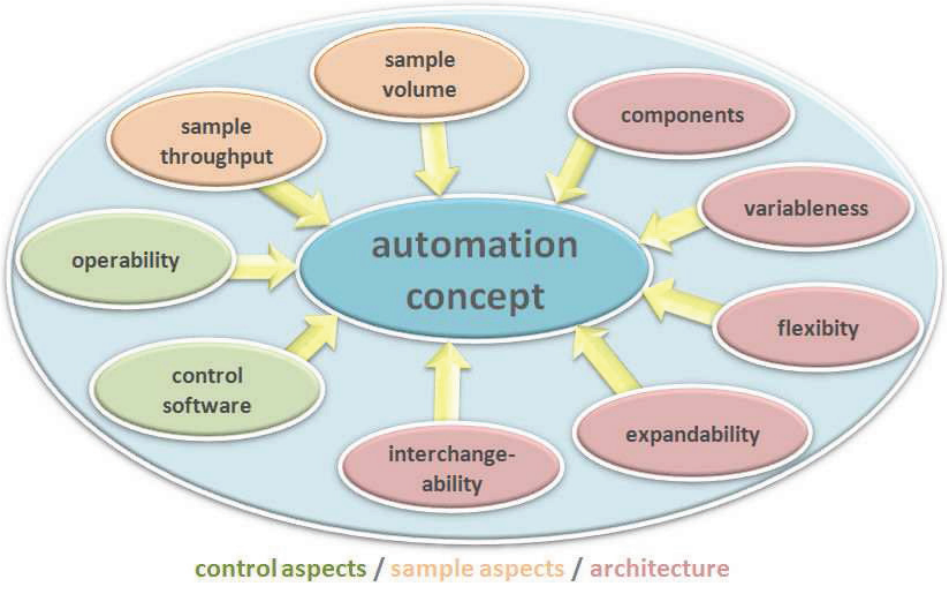


Figure 25 Influencing factors to laboratory automation concept

In detail following attributes were included into the development of the automation concept:

Throughput – number of samples, labware items and cells influences storage capacity, as well as liquid handling capabilities, liquid stock volumes etc.

Liquid volumes – Intended use of the automation platform as a flexible cell culture automation system in research environment implicates the decision for

micro titer plate format labware, microplate format liquid handler and other devices.

Variableness – processes in research and discovery require a high grade of variableness in terms of automated process design

Components – Biological processes and desired functionality of the automated system requires integration of certain components and functions

Flexibility – design of the system for different applications like cell growth, maintenance, expansion as well as seeding into assay plates and running of assays

Expandability – ability to add further components for enhanced scope of functions

Interchangeability – ability to replace components by others

Control software – technical requirements to process management and control software like systems control, 3rd party integrations, data management and data handling, optimizing scheduling etc.

Operability – intuitively programmable processes, graphical user interfaces, suited for not-technical-trained operators

All of these attributes were kept in focus when designing the system. Following pages show the way of conception and systems design for the automated cell culture system.

4.5.1 Flexible Automation in Laboratory Research and Discovery

Common industrial automation processes operate with a linear workflow architecture which fits for linear processes that doesn't change often. In opposition to that in laboratory automation systems for research environments more flexibility is required. Figure 26 illustrates the number of different variations in a flexible automation system with two steps in process and four different versions of treatment or process device for each step.

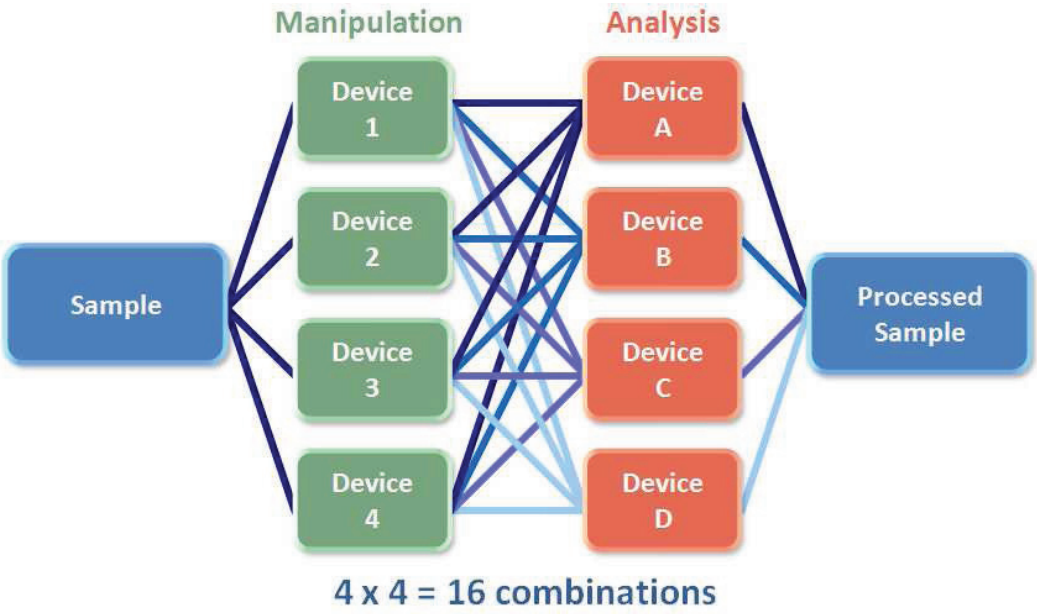


Figure 26 Example for number of combinations in process planning

In automated cell culture different cell lines are treated in different ways, different devices are used in different processes. Besides this cells are to be analyzed with different methods, like Trypan blue dying for cell count and viability, confluency measurement etc. Therefore in laboratory automation in explorative environment flexibility in method design, process workflow and labware transportation is essential. The control system has to able to change methods and workflow parameters easily and fast. These requirements can be met by a high degree of modularity in devices, open structures for device integration and graphically oriented user interfaces.

4.5.2 Systems Approach

As mentioned in 2.3 cell culture processes in monolayer require as main operations liquid handling, incubation and readout / quality control. Devices that can be bought from the marked were decided to not being developed from scratch, but used as available or adapted to the specific needs of the desired application. Starting from the approach of a small footprint system with stacked components there was generated as a vision simulation in 3D CAD. Figure 27

shows the first concept for a sandwich style stacked culture system containing an automated liquid handler for microplate format labware, a reading device for cell vitality parameters like viability, cell count etc. and a cell growth incubator. To integrate these components a vertical transport robot was planned. For continuous sterile conditions a sterile enclosure was planned for the system.

Based on this idea there was made a selection for devices to use for liquid handling, incubation and quality control. This selection was influenced by following facts:

- availability
- state of the technology
- applicability
- integration interface
- flexibility

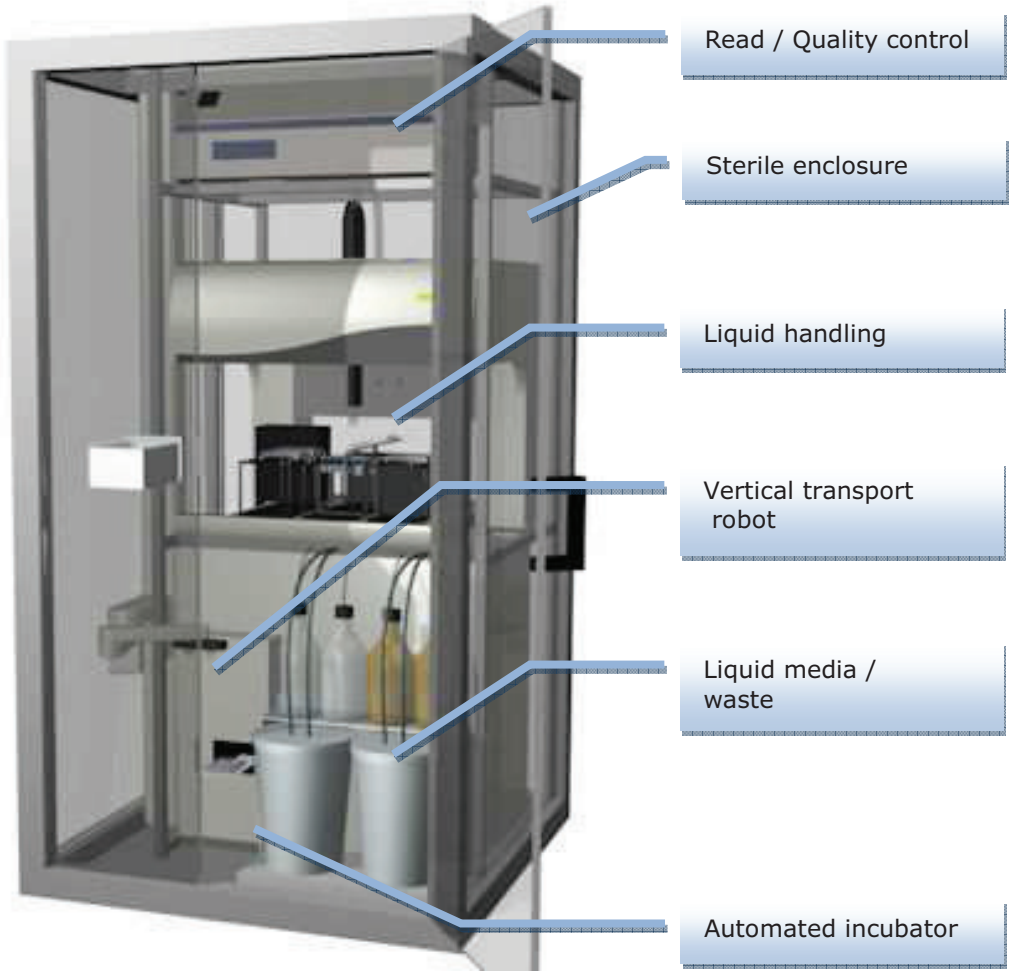


Figure 27 3D CAD Study for an automated cell culture system

As result of these initial simulations and based on the initial thoughts on automated cell culture processes following attributes were set to be implemented:

- Cell culture in micro titer plates and culture flasks in micro plate format
- Automated CO2 incubator
- Pipetting platform for seeding, feeding, harvesting etc.
- Integrated measurement of cell concentration and cell viability
- Transport robot as central system integrator
- Set up a working functional model

The following chapters will document the technical development, integration and assembly of the automated cell culture device.

4.5.3 From Manual Process to Full Automation

When transferring manual processes to automated operation besides the main steps a number of supporting steps have to be implemented. In addition for automated cell culture labware designed for automated handling has to be found. If those labware is not available, strategies have to be developed to handle labware that is not designed for automated use and to handle biological material in vitro.

4.5.3.1 Labware Handling

Because of the complex number of steps in cell culture automated transport of labware between stations of the automation line is necessary. Typical labware for adherent cell culture are besides multiwell plates in micro titer plate format the de facto standard of the cell culture flasks, also T-flask. (Please refer to chapter 2.3) With the development of automation friendly cell culture flasks in micro titer

plate format the labware suppliers prepared the way to cell culture automation based on microplate format liquid handlers.

Many liquid handling robots for microplate labware include robotic grippers to transport plates and lids on their workspace. Depending on the complexity of the automated system additional robotic transports are needed to integrate further components into the automation line where the liquid handler often is the central element. For simpler architectures conveyors or linear transport stages can be used, for more complex integrations pick and place robots and articulated robots are used.

In terms of copying manual treatment to automation, sometimes robots are used to rebuild manual operator's labware handling i.e. for plate tapping for cell passage or plate shaking for media distribution on the cell growth surface.

4.5.3.1.1 Capping Decapping

When using standard cell culture flask containing screw caps for liquid addition and removal automated capping and decapping is necessary. Due to the availability of automation friendly microplate footprint culture flasks and successful initial biological tests with those types of culture flasks (refer to 4.7.1) there was taken the decision to focus on this labware instead of using standard T-flask. Because of the design with pierce-able septa for liquid transfer of these labware there is no need for automated capping / decapping integration in the planned application. For lidding and delidding of labware it was aspired to use a liquid handling robot with integrated gripper.

4.5.3.1.2 Tapping of Labware

Regardless of the cell passage method by using Trypsin or Accutase in manual cell culture after enzyme incubation the cell growth labware is tapped manually by the operator to loosen cells from the growth surface and to bring the cells into solution. Because of a lack of this function on standard microplate liquid handlers

the tapping step was one of the needed steps in the process of adherent monolayer cell culture. After analyzing further necessary steps there was made the decision to develop an on deck labware manipulator including a tapping function.

4.5.3.1.3 Pivoting

Manual operators commonly pivot cell culture flasks to distribute media on the whole growth surface. Often in culture flasks with an area of 75cm^2 to 100cm² cell suspension volumes of about 10 milliliter are used. Enzyme volumes for cell passage is often only in a 2-4 milliliter range. To ensure sufficient liquid distribution also in automated cell culture process, it was planned to add a pivoting function for microplate labware in the proprietary on deck labware manipulator.

4.5.3.1.4 Tilting

Microplate labware with bigger well squares, as well as certain types of microplate footprint automation cell culture flasks need to be tilted while media aspiration to empty the labware completely. Media addition can be required also in a labware-tilted manner. To offer this also in automation the custom on deck culture plate manipulator should also provide a flexible tilting function in different directions. In addition, for easier to handle multiwell plates it was planned to integrate a more simple tilting device for only one tilting direction and a manual adjustable, fixed tilting angle. [95]

4.5.3.1.5 Flipping

Corning® RoboFlask™ and BD Falcon™ Automated Cell Culture Flask require by design pipetting through a septum while plates are flipped to 90° as shown in

Figure 28. For cell growth and robotic transports the plates have to be flipped back into a flat position. Because of the mechatronic complexity this function cannot be integrated in the proprietary on deck labware tilting / clamping / knocking manipulator. Therefore was decided to design a flipping labware positioned for handling of RoboFlask™ and BD Falcon™ Automated Cell Culture Flask. [96;97]

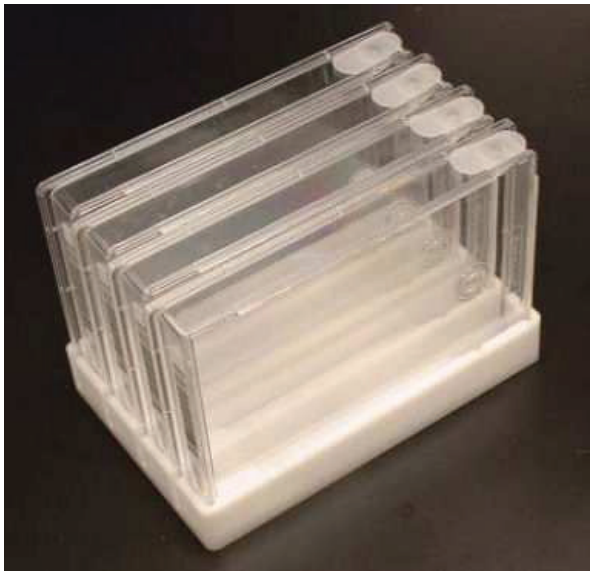


Figure 28 BD Falcon™ Automated Cell Culture Flasks in pipetting position; source: BD Biosciences

4.5.3.1.6 Clamping

In automated liquid handling of standard microplates labware positioning on flat platforms with some X-Y-alignment edges fits to the needs of stable automated operations. For pipetting of 1535-well plates and plates containing more wells plates are often fixed in X and Y direction to guaranty precision in positioning for pipetting. For the special case in automated cell culture where culture flasks with septa are operated, there is the special need for fixation of flask against vertical movement. Due to the attachment of the septum to the pipetting tip the plate would tend to move with the upward moving pipetting tip when travelling vertically out of the plate position. To ensure reliable and safe operation so every position for where septa of culture flasks are pierced, the plates have to be fixed

by clamping. This is essential for the proprietary on deck labware manipulator as well as for the plate flipping device.

4.5.3.1.7 Shaking

In micro titer plate handling shaking is a step that is often performed to mix a liquid, to homogenize or to dissolute powders or salts in liquid. Shaking can also imitate manual pivoting steps as performed in manual culture. On the laboratory device market there are several shakers for microplates and for automation available. So integration of a microplate shaker, and - especially for cell harvesting – it was planned to integrate an on deck heating incubator / shaker.

4.5.3.2 Data Handling

Changing from manual cell culture to full automation requires extensive process data handling, seamless data logging, as well as data processing. In automation, on paper written laboratory journals are replaced by automated reporting. So the overall control system including all the custom integrations has to support complex process data management. This requirement is important for control software decision and for concept of system integration. Figure 29 shows examples starting from biological sample information, sample container information over device data, system information up to whole automation process data.

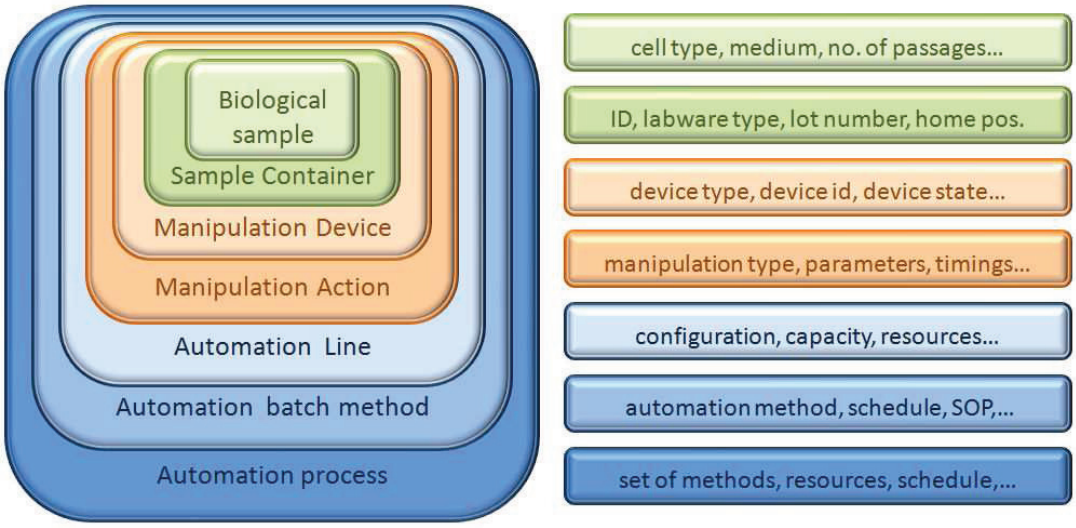


Figure 29 Content and process information structure

4.6 Systems Engineering

Following chapters focus on System design, custom developments and system integration.

4.6.1 Software

In complex laboratory automation systems there are different layers of control software beginning from firmware inside single devices over device specific control software running on pc up to the highest level software components – the all-over process editor and process control software. In addition for complex automation processes like in cell culture further software can be used that connects multiple automation run lines and supports in multiple method scheduling.

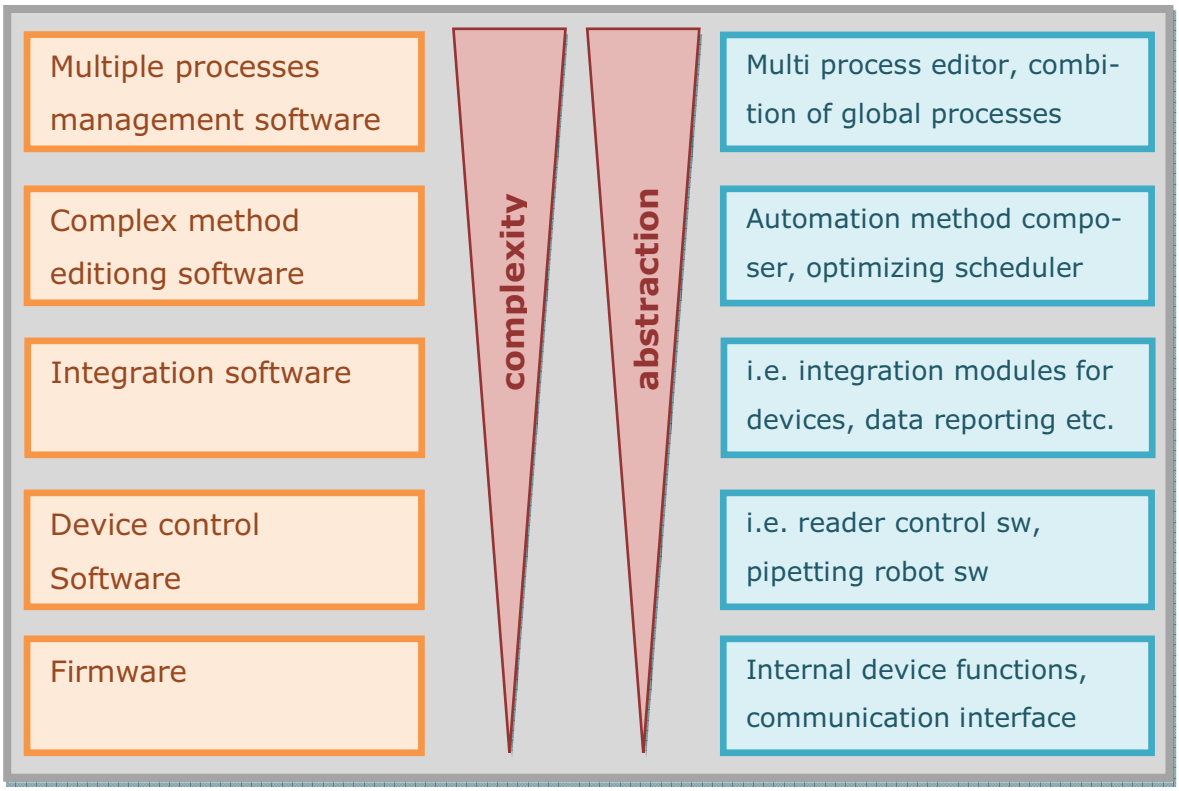


Figure 30 Software levels in laboratory automation

4.6.1.1 Software Architecture for Automated Cell Culture System

Flexibility in automation always causes complexity. Figure 31 shows the concept modularity and flexible integration of a complex automation system. Based on the automation controller's operating system a single process automation software controls dedicated system processes. The software contains method editing, method scheduling and method runtime control components. The single process automation software communicates with device integration modules, which are interfaced to the various automation devices. Automation devices are controlled by device-specific firmware. For the device integration there are three different cases:

Direct integration: The automation device is connected directly to the device integration module via hardware interface like Ethernet, CAN, RS232 etc.

Device software integration: The automation device has its own proprietary host controller software that communicates via hardware

interface to the automation device. The device integration module here communicates to the device controller software running on the same automation controller (PC, PLC, …)

Additional controller integration: If the automation controller requires a separate automation or devices have to be doubled and device software cannot run in more than one instance on the automation controller, the device can be integrated with its own automation controller, where the device integration module communicates to the automation device software via hardware interface. In some cases the original device software has to be added by a remote control module which supports control and data exchange from a higher instance.

Support of such modular architecture supports the requirements to flexibility and expandability.

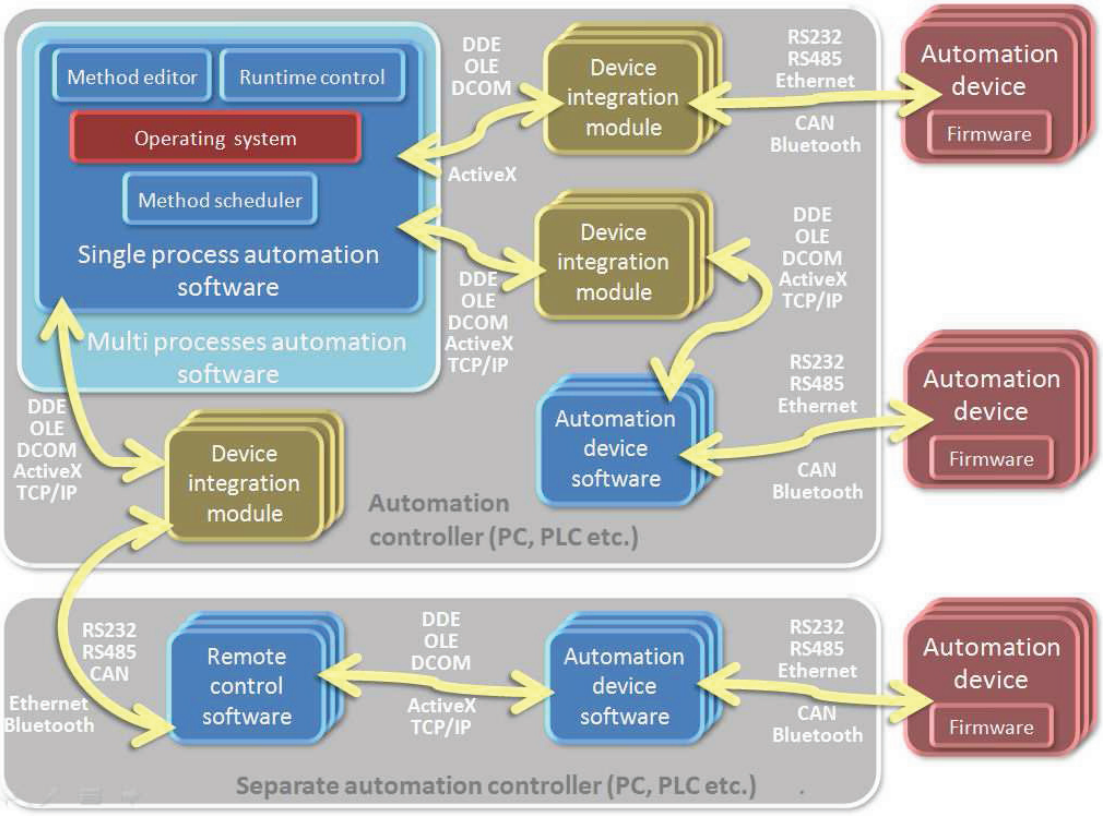


Figure 31 Automation control topography examples

Together with the decision to use the liquid handler from Beckman Coulter, also the decision for the complex method editor and upper level software was made. In the automated cell handling system all of the categories shown in Figure 30 are present. Beginning on the device level software categories should be listed below.

4.6.1.2 Firmware

Almost every device used in automated processes is controlled internally by microcontrollers or microprocessors running firmware. Firmware controls the elemental functions of the devices and provides interface connectivity to upper level control software. In proprietary device developments for the automated cell handling systems firmware was developed in the process of system design. These firmware components are described more in depth in the chapters documenting the proprietary developments.

4.6.1.3 Device Control Software

More complex devices in laboratory automation are controlled externally by device control software running on external computers, i.e. optical readers, pipetting robots. In general all devices with a higher functionality, flexibility and a high number of parameters need their own external control software. Commonly these devices are sold together with their software often containing an external computer too.

4.6.1.4 Integration Software

When integrating third party devices into complex automation lines always the device and its control software has to be interfaced to the upper level automation control software. Therefore drivers are written that translate data and commands

from upper control instance to device or device software and vice versa. For custom integrations into the developed cell handling system and for proprietary developed devices these integration drivers were programmed during the system development of the cell culture system. The developed integration drivers are detailed described in the chapters reporting on these devices.

4.6.1.5 Complex Method Editing and Control Software

Laboratory automation workstation software commonly includes graphical method editing interfaces and capabilities to integrate third party devices. For more complex applications often additional hardware platform independent method editing, scheduling and run time control software is necessary to enable high throughput, time efficient run and cost effective system usage. For the automated cell handling system SAMI® EX from Beckman Coulter is used, an open architecture state of the technology method editing and runtime environment.

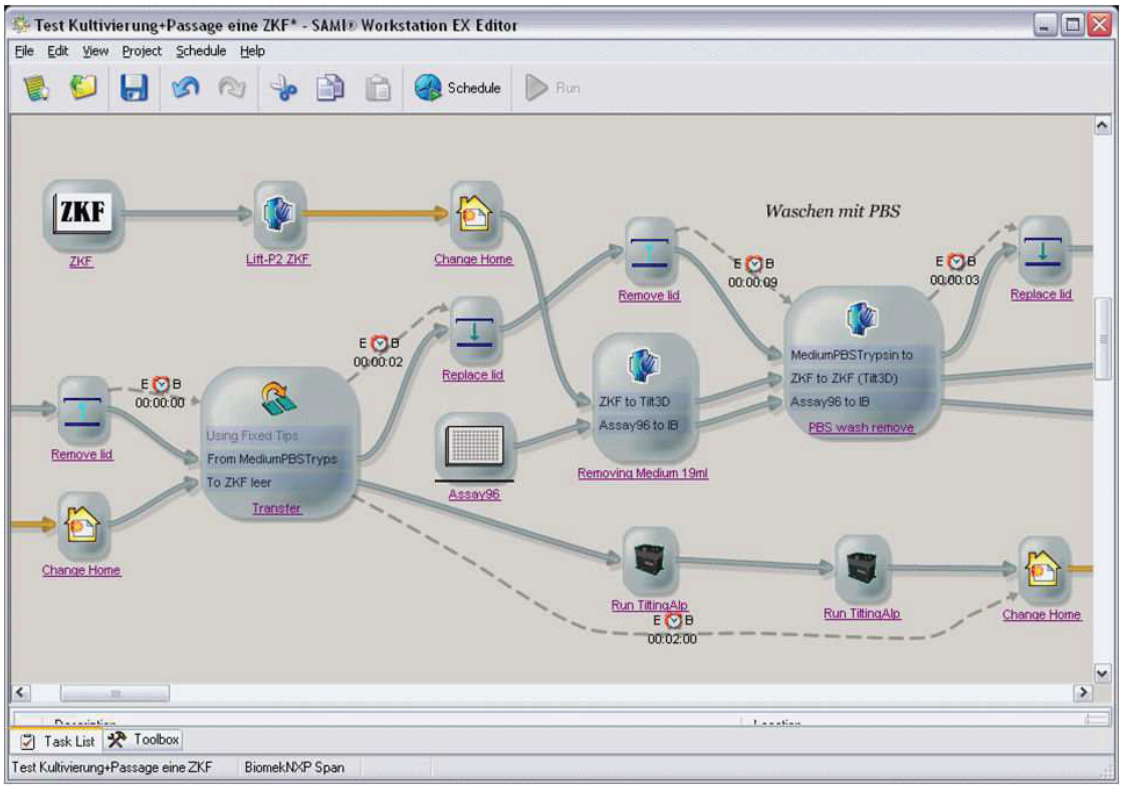


Figure 32 SAMI® EX method editor

In addition the systems software includes an optimizing scheduler with a data driven dynamic rescheduling functionality for pre validated schedules. Further benefits of this software:

- Intuitive Method Editor
- 21 CFR Part 11 Tools
- Schedule Optimization
- Dynamic Rescheduling
- Rigid and Flexible Timing
- Tip Tracking
- Microsoft Windows XP OS
- Graphic Run Time Environment
    - Status information
    - Live method view
    - generate labware report
    - Live system view
    - plate location in system
    - System activities

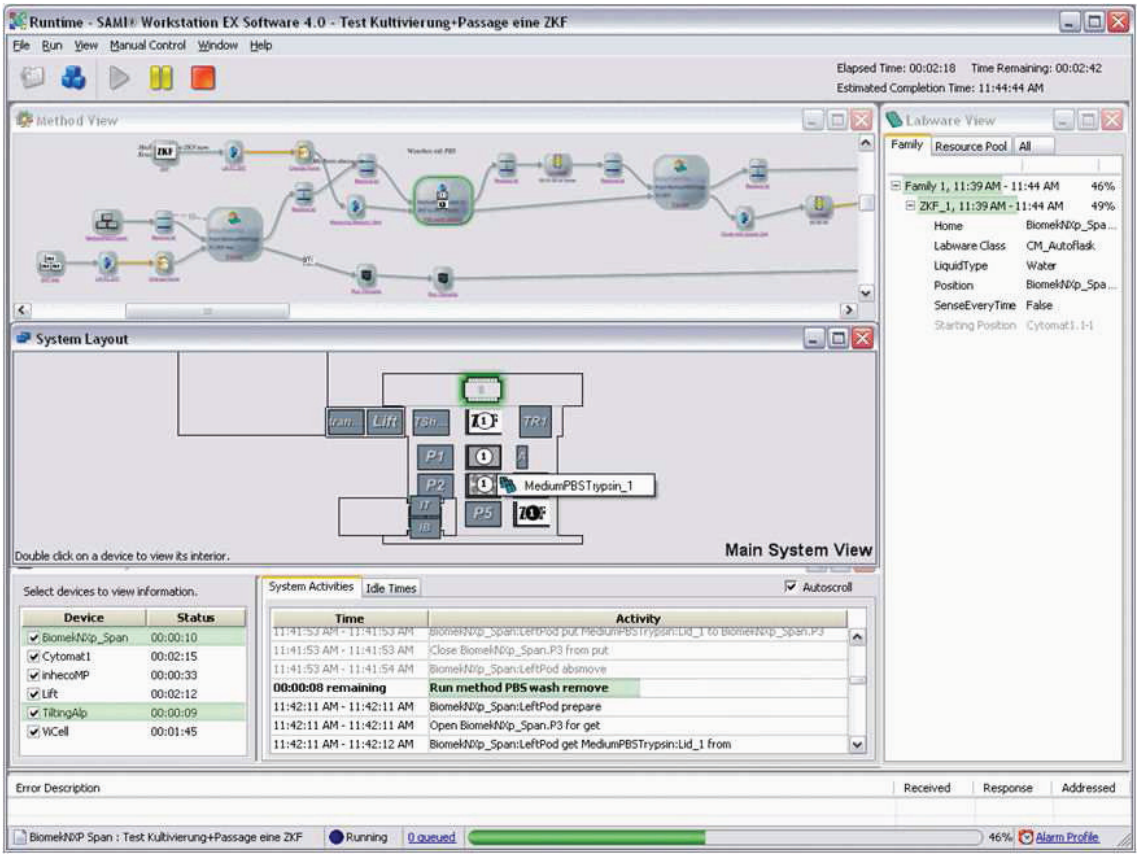


Figure 33 Runtime control software live view

Because of various new custom integrations into the system control environment during the development of the automated cell culture system the following chapter gives a deeper insight into the communication and control technology behind SAMI.

4.6.1.5.1 SAMI® EX Integration / SILAS

Integral content of the SAMI® EX is a standardized protocol 'SILAS' that supports custom integrations into automated systems controlled by SAMI®. SILAS is based on Microsoft ActiveX messaging. SAMI EX and the integration modules normally run on PCs with Microsoft Windows XP. Integration of devices running under other platforms is possible, i.e. via network communication. So far any device controllable via electronic interfaces like RS232, USB, Firewire, Ethernet etc. is possible and, in addition any device controlled by its own software can be integrated, if the device software running on a separate controller has a remote control interface like COM, ActiveX, OLE, DDE or TCP/IP communication.

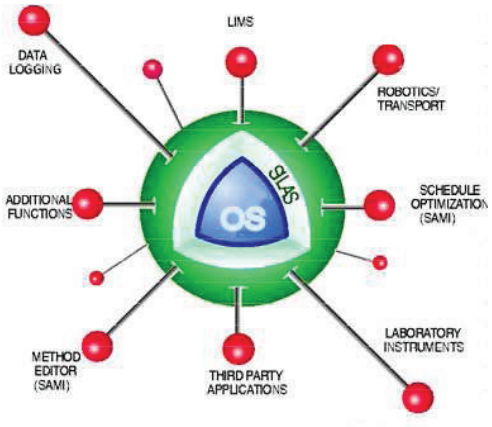


Figure 34 SILAS open architecture

For every device to be custom integrated there has to be developed a device specific executable module that communicates with the SILAS environment on the one side and directly with the device or with the device control software on the other side. Selection of a programming language is free of choice for the developer. So for every subsystem or device in the automation system there is

its own SILAS integration module. Communication in the SILAS level is message based and controlled by the SILAS router.

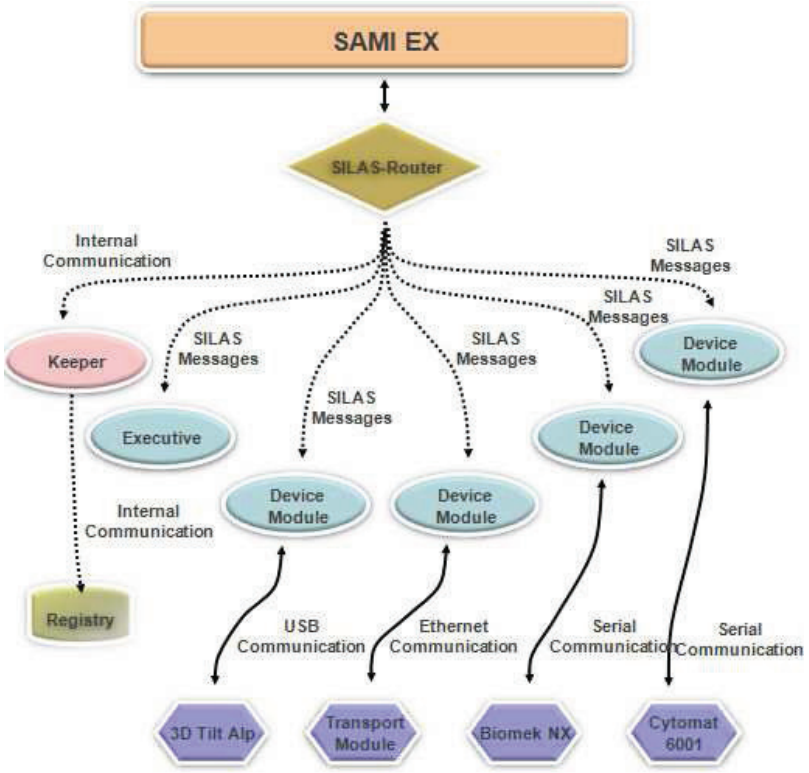


Figure 35 SAMI EX / SILAS communication

The main construct of SILAS consists of three parts: Keeper registry, SILAS executable program and 'Message Control' ActiveX control.

SILAS.exe contains keeper to access the keeper registry database (Keeper.rrg) and to load and unload modules. In addition the SILAS.exe contains the message router.

Keeper.rrg – the all-in-one database contains system information on all components of the automation system in terms of relevant parameters. This begins with module specific information, over communication details (type of port, port number etc. up to transportation information for labware related to the device.

SILAS.ocx is embedded into any custom integration and provides the general SILAS communication features and rules. The SILAS OCX is the direct interface

to the internal router. The SILAS software developer kit contains a pre built module framework as basis for custom integrations.

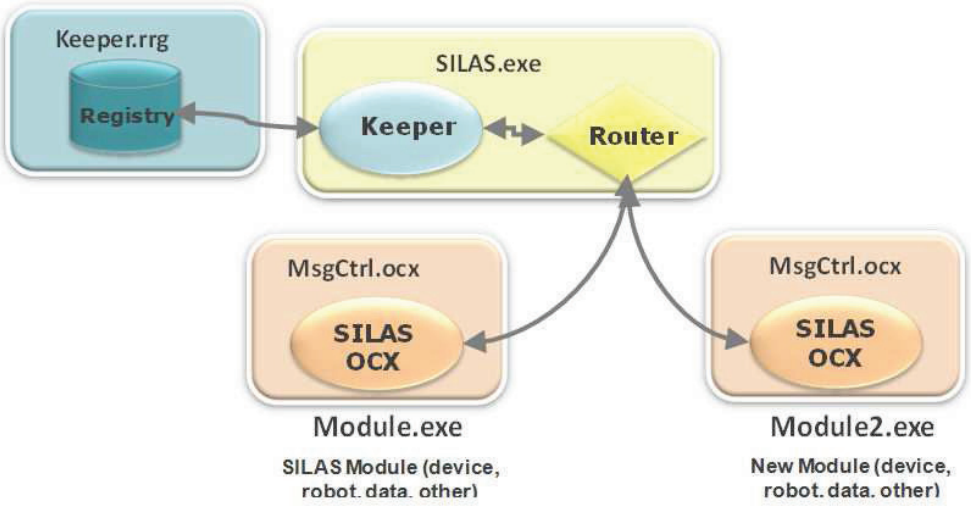


Figure 36 SILAS Modularity

Besides the internal communication over by SILAS protocol every device needs a user interface to parameterise function of the device while editing methods for the automation line. These action config dialogues allow direct setting of operation parameters for more simple devices or selection of pre defined methods for more complex devices (liquid handlers, readers etc.) [98]

4.6.1.6 Multiple Processes Management Software

Cell culture processes consist of a number of activity steps and longer incubation periods. In standard method programming, incubation times are active integral sections of the automated runs. On the other hand these – sometimes several days lasting – timeframes could used by the system to perform others task, like processes with different cell lines etc. This can be achieved by splitting cell culture processes in different methods. Management of these multiple processes is supported by custom developed Process Management Software, a custom tool that supports planning and combining of automation system methods. Therefore following components are integral part of Process Management Software:

- Process editor to plan multiple methods
- Calendar to support scheduling of multiple methods
- Overall multiple process labware property data and labware position linkage and report generation over different automation line processes using data acquisition and reporting tools

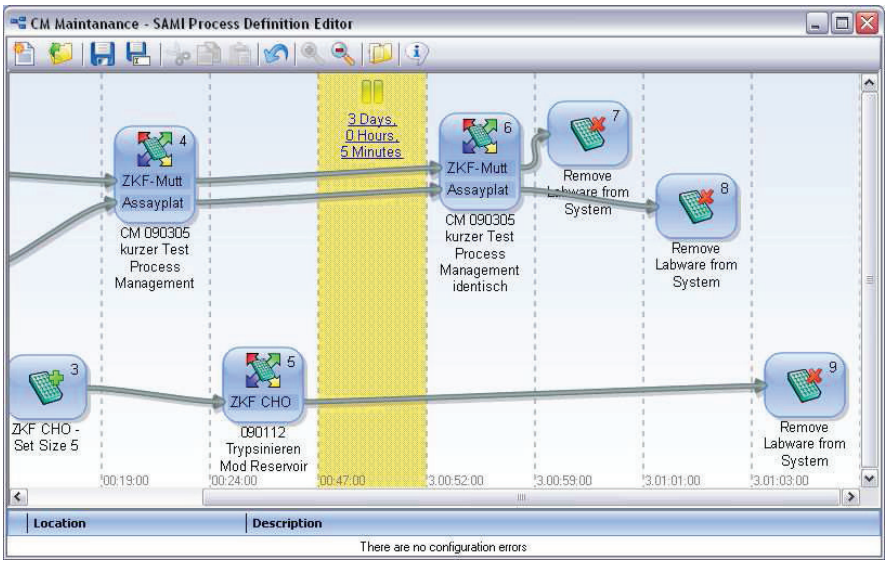


Figure 37 Process Management - Process definition editor

In the process definition editor multiple automated methods are combined to a type of process. In automated cell culture those processes can be different culture methods for diverse cell lines. The process definition includes labware addition and location, automation line runs, incubation times and labware removal.

These pre-defined processes that can consist of several automation line runs can be scheduled in the Process Management Calendar. In the calendar for every process the timings and the number of families to be processed in the process are scheduled. The calendar supports the user with hints on scheduling problems, off-workday events and irresolvable schedules. Besides this the Process Management keeps together process data and labware locations over multiple automation line runs.

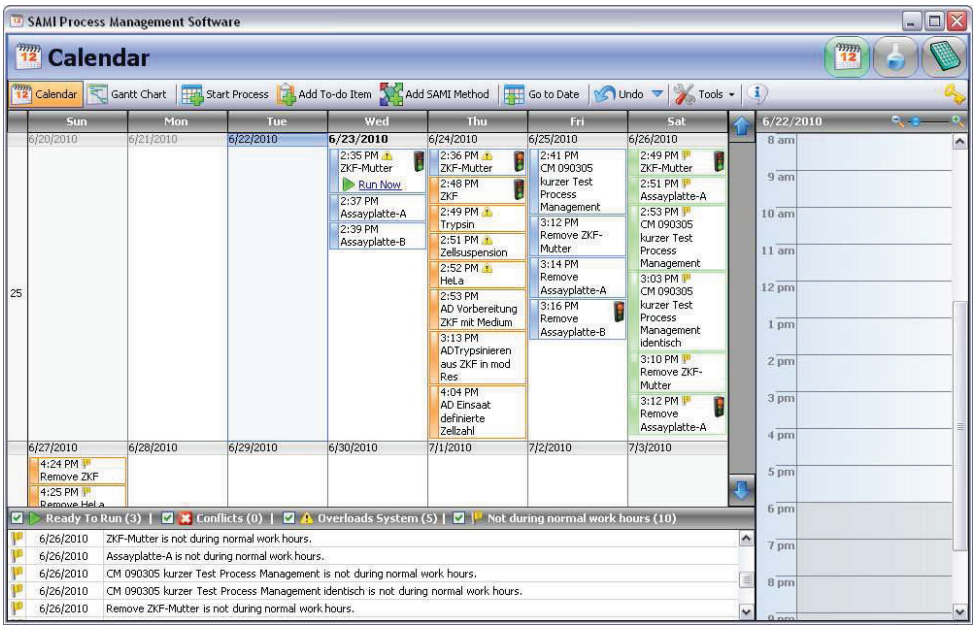


Figure 38 Process Management Calendar

4.6.2 Hardware

The automated cell handling system is composed of devices available on the market and of custom designed devices for the specific tasks in cell culture and for specific automated handling of cell culture flasks. As shown in Figure 27 the concept for the automated system was to stack the main components to design space saving. The central element of the automation system is the liquid handling robot that was planned to be in a height as placed on normal desktop or workbench height. This is import because of the necessity to access the liquid handler deck by operators for manual labware placement and to observe automated plate handling and liquid handling in method evaluation and method development. The incubator was planned to be placed below the liquid handler, integrated by a linear transport robot. In the first stages of the design it was planned to place the reading device on top of the liquid handler. Refer also to Figure 27.

4.6.2.1 Main Components Purchased from the Market

As far as possible the automated cell handling system was designed from components purchased from laboratory automation providers. This chapter list devices that were purchased already designed for automation purposes and already equipped with integration interfaces in hardware and software.

4.6.2.1.1 Liquid Handler Biomek® NX^{P} Span8

The liquid handling robot is the central element of the cell culture automation system. Decision was made to purchase a Biomek® NX^{P} Span8 from Beckman Coulter, Inc. Requirements to special requirements to the liquid handling system were:

- One to 8 independent pipetting channels
- Fixed tip and disposable tip support
- Capability of tip washing
- Sufficient on deck space for labware and on deck integrations
- Capability of high volume bulk dispensing
- Pipetting volumes in a 5ml range or higher
- Integrated gripper for labware transport

Besides the general requirements to the liquid handler following properties of Beckman Coulter influenced the purchasing decision:

- Proven technology
- Open architecture
- Possibility to integrate custom devices, Software developers kit for integration available
- State of the art method editing software, support of customized pipetting templates
- Extensive data handling, data management and advanced reporting tools

Figure 39 Biomek NXP Span8

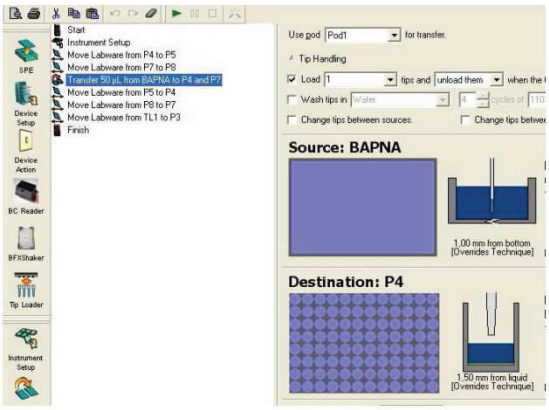


Figure 40 Biomek Software Screenshot

The configuration of the Biomek® includes 8 independent pipetting channels, four fixed tip channels, each 1000ml pipetting volume and four disposable tip channels, each 5000ml pipetting volume. Together with the Biomek® comes the Biomek® software a comfortable intuitive method editing and operating program for standalone and workstation operation supporting custom integrations. [99;100] Further specifications of the Biomek® can be found in the Appendix.

4.6.2.1.2 Cell Growth Incubator – Cytomat 6001

From the variety of cell growth incubators only a few companies offer automated incubators with plate delivery and transfer gate. The two bigger vendors for those incubators are Liconic and Thermo. Due to capacity and compatibility to existing incubators in the institutes environment for the automated cell culture system the Cytomat 6001 incubator was selected. The incubator has a capacity of 189 shallow well plates and is equipped with temperature control and CO_2 control. For automation integration the incubator is equipped with an automated carousel, internal pick and place robotic arm, transfer gate and transfer station for external robotic access. For remote control and integration the Cytomat 6001 is equipped with a serial RS232 port. [101-103]

Figure 41 Cytomat 6001 Incubator

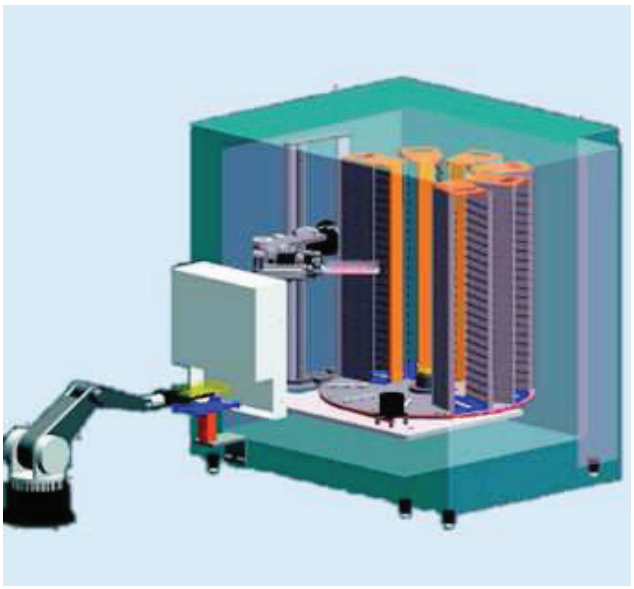


Figure 42 Cytomat 6001 3d CAD view; source: JALA [101]

4.6.2.1.3 Detector – Vi-CELL™ XR

During concept development there was made the decision to integrate a cell counter as measuring and quality control device into the automation line. Most important parameters for cell culture automation are cell density and viability. The cell counter Vi-CELL™ XR from Beckman Coulter offers viability measurement based on Trypan Blue dye. Besides the parameters cell density and viability the Vi-CELL™ XR registers parameters like cell size, clustering by using automated microscopy and image processing. In addition the Vi-CELL™ XR provides real time cell images for documentation. It is a proven device in cellular analysis. On aspect for deciding to purchase the Vi-CELL™ XR is the availability of a ready to use integration interface for the central liquid handler Biomek® NX^{P} and its control software. As a special feature the runtime cell count and viability data provided by the Vi-CELL™ XR can be used for event driven decisions in automated runs like calculation of pipetting volumes for specified well numbers in assay plates or for decisions on cell use based on viability parameters. [104]

Figure 43 Vi-CELL™ XR, Beckman Coulter, Inc.

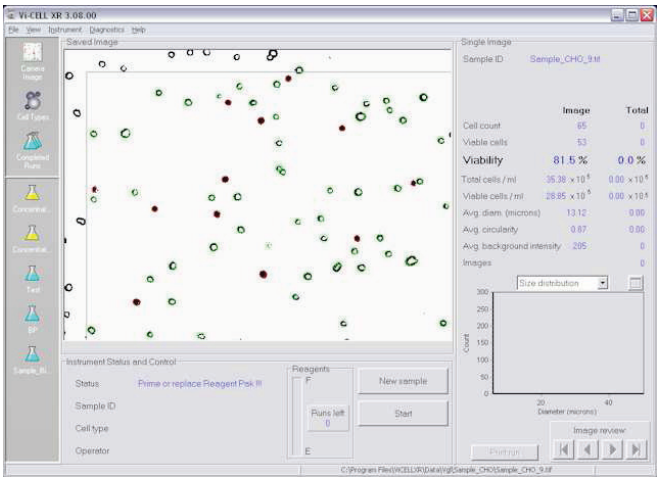


Figure 44 Software Screenshot Vi-CELL™ XR, Trypan blue dyed cells marked red (dead)

4.6.2.2 Custom Developments

Following chapters describe the system design, assembly of the cell workstation and development of custom devices to meet the demands of automated adherent cell culture.

4.6.2.2.1 Frame and Enclosure

Because of the necessity of sterile environment the workspace of the liquid handling area of cell handling system must be kept under clean-room conditions. Therefore a frameset for all components of the cell handling system was designed together with a sterile enclosure with HEPA filtrated air flow.

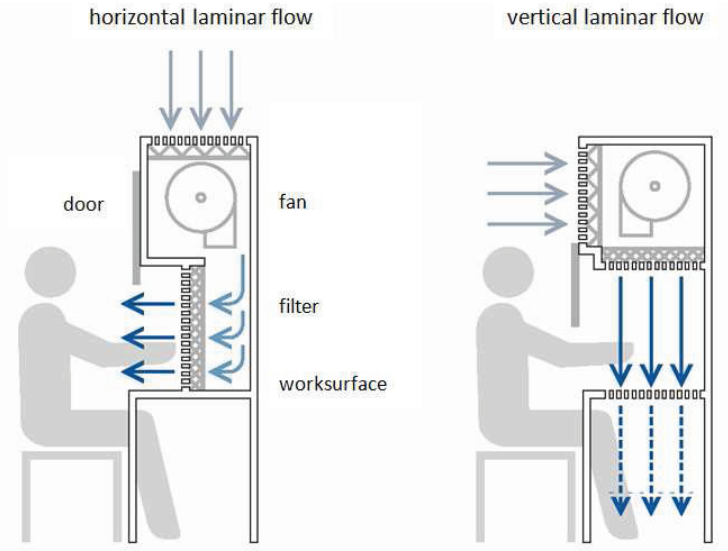


Figure 45 Laminar flow hoods

Clean-room environments are needed in all situations, where smallest particles can influence processes and products in a negative manner. Life science applications, production processes with biologic material and pharmaceutical productions are only a few examples where clean-room environment is needed. Continuous air filtration and working under positive air pressure maintenance the work area free of particles and reduces so contamination. For modular enclosure design combined filter-fan-units are available where laboratory air is drawn into the module by fans and the air particles are separated by micro glass fibre filters. Depending on the filter class so separation degrees of up to 99.999995% can be reached. Continuous positive pressure inside the clean work area ensures that the environment stays free of particle contamination also through opened access doors or gates. [105] Table 1 lists the classes of filters used in Germany and the EU and their degrees of separation.

Filter	Filter Class DIN EN 1822-1	Filter Class DIN 24183-1	Average separation degree (gravimetric, %)
HEPA	H 10	EU 10	85
HEPA	H 11	EU 11	95
HEPA	H 12	EU 12	99,5
HEPA	H 13	EU 13	99,95
HEPA	H 14	EU 14	99,995
ULPA	U 15	EU 15	99,9995
ULPA	U 16	EU 16	99,99995
ULPA	U 17	EU 17	99,999995

Table 2 Filter classes and degree of separation

The combination of flat panels and an aluminum frameset together with a HEPA filter-fan-unit is the base for a clean-room condition on the work area of the liquid handler deck.

All components of the frameset and the sterile enclosure had to be customized designed for the dimensions of the automated cell handling system where the liquid handler, the incubator and the transport lift which connects incubator and liquid handler are the central components. For the design of the frameset and enclosure all important devices were modeled in 3D CAD software so that the components could be aligned with each other virtually in advance. This helps designing the frameset in 3D and ensures optimal design and dimensions.

Orientation of Liquid Handler and Incubator

In the phase of system planning there was the idea to connect the liquid handler and the incubator, which is placed under the liquid handler, by a simple single axis lift. In CAD simulation an optimal orientation was found where such a type of lift can connect the incubator and the liquid handler. The reach and workspace of the liquid handler and the position of the transfer gate of the incubator was essential here. Figures 46 to 48 illustrate orientation approach in CAD.

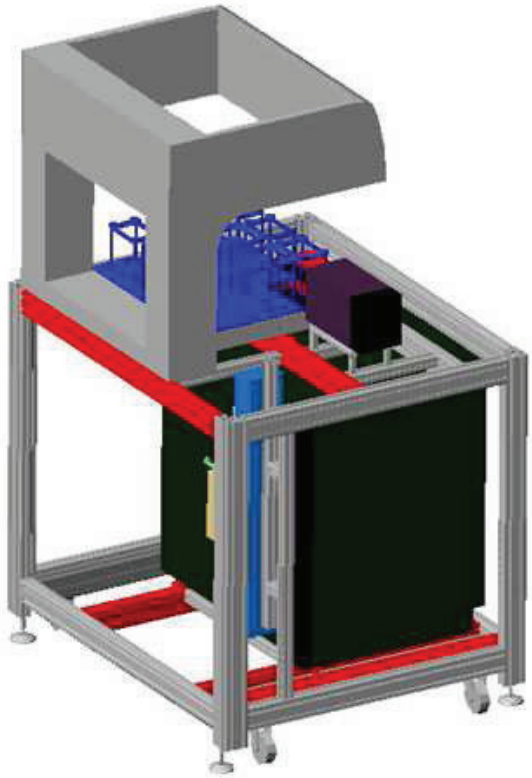


Figure 46 CAD placement of main components

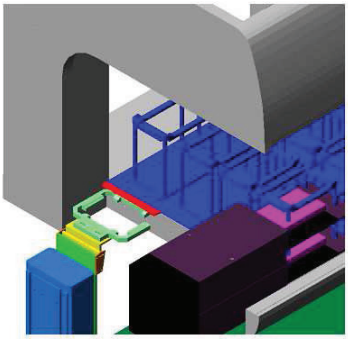


Figure 47 CAD lift detail

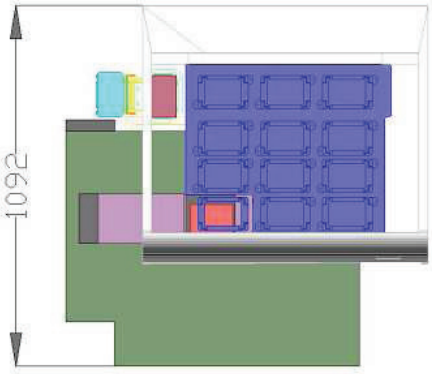


Figure 48 CAD footprint simulation

Stand-Table

Based on the CAD simulations the frameset for the stand-table was designed. The aluminum profiles used are content of a systems profile that can be ordered pre configured in profile type, length and connector options. Figure 49 illustrates the final design of the frameset for liquid handler and incubator considering lift slot for labware transport.

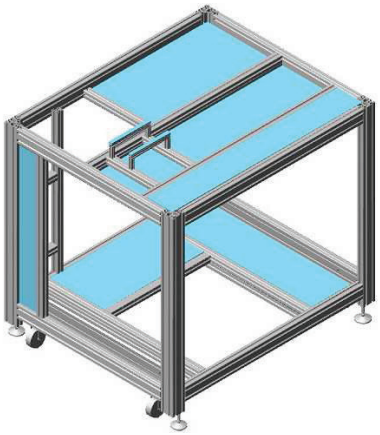


Figure 49 CAD design frameset for liquid handler and incubator

Housing

The laminar flow housing is designed as a removable hood. Due to limited room height in laboratories it is designed to be able to remove it horizontally for inspection, service and cleaning. At the front side and at the rear side there are doors for operator access to place labware and to allow fast access to the work deck. The filter fan unit is a compact box on top of the housing especially designed for laminar flow conditions. It is made from stainless steel and has an integrated HEPA H 14 filter with a separation degree of 99,995%. The filter fan unit is capable of providing about 0.5 cubic meters air per second. The flow rate can be adjusted manually.

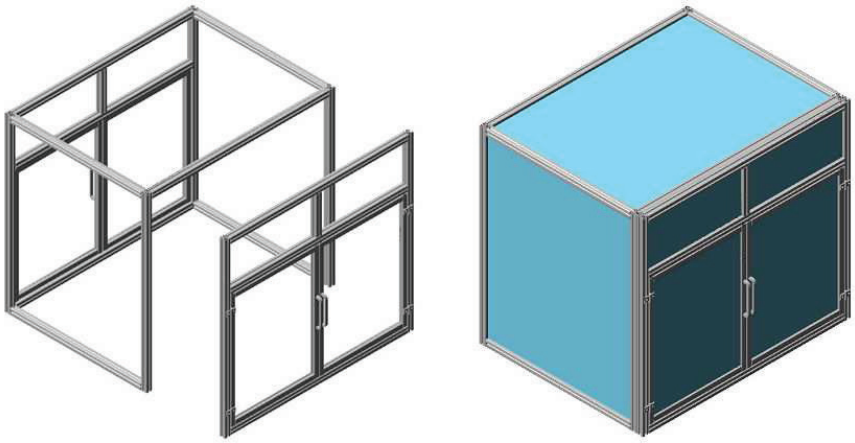


Figure 50 Housing

Filtered air flows from the Top side of the system through the work area of the liquid handler and flows out through openings in the bottom section and through door slots. The main unit of the frameset is equipped with wheels to allow moving of the system from one laboratory to another. [106]

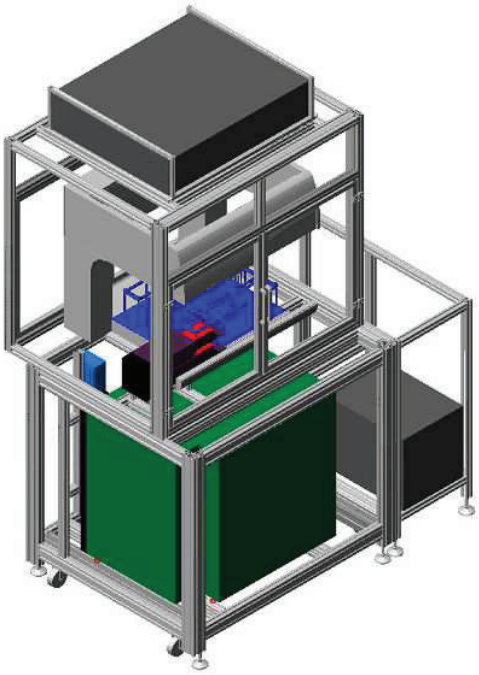


Figure 51 CAD 3D plot frameset and housing

Figure 52 Frameset and housing completed

4.6.2.2.2 Vertical Transport Lift

The orientation of incubator and liquid handler offered the opportunity to integrate a quite simple transport lift for labware transport between these stations. All other positions for microplates can be reached by the liquid handler transport gripper.

Device design

For the transport lift a pre configured linear spindle drive unit was selected: The spindle is actuated by a servo controlled drive. The linear motion unit is suitable for clean-room applications. In order to support easy integration into the liquid handler deck the labware coming from the incubator has to be twisted by 90 degrees to allow full functionality in method design. Without twisting the labware coming from the incubator would be presented in portrait format to the liquid handler. This is supported by the mechanics of the liquid handler gripper and by the Biomek® software, but usage in SAMI would cause some additional effort. The 90 degree twisting is designed by a pneumatic twisting mechanism that is

attached to the lifts travelling stage. Mechanical design for the lifts custom parts was made completely in 3D CAD. [107;108]

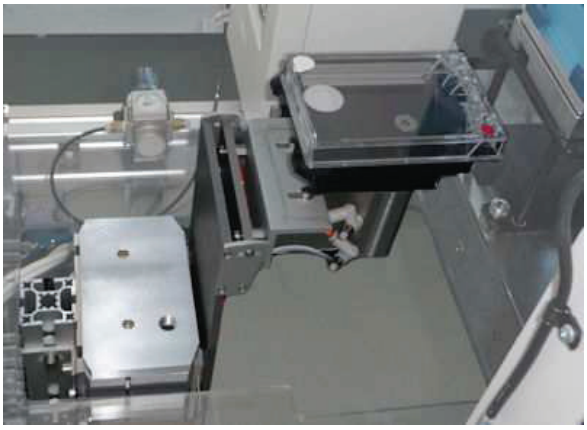


Figure 53 Vertical lift in upper position for robotic gripper access

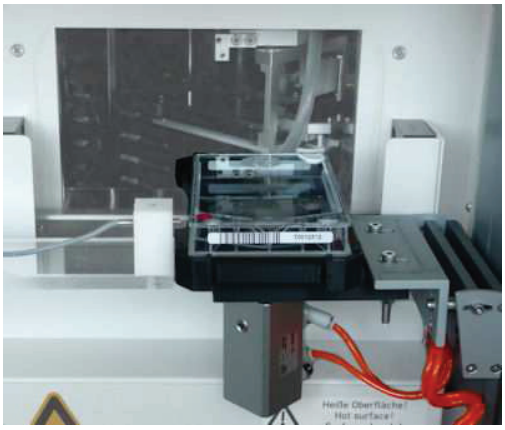


Figure 54 Lift in lower position for incubator access

SILAS integration

The SILAS integration for the transport lift includes following functions:

- Communication to servo controller

  The SILAS integration module communicates to the servo controller and translates commands from the SILAS level to the drive. In the other direction position information and status info from the servo controller is translated to SILAS protocol by the integration module.

- Action config dialog for lift module
  Every integration needs a user configuration dialog where the operation to perform in runtime can be parameterized. For the vertical lift the parameters are the desired positions for the lift traveler. In the automated operation the selection of the position is done automatically. In the action config dialog the user can move the lifts traveler by selecting move function and selection of the position. In addition the ramp speed for acceleration and deceleration can be selected. A further necessity for integration module is the prediction of the duration of the configured operation. This 'time estimate' is used by the internal SAMI scheduler to calculate and schedule a time optimal run. The time estimate values are written in the SILAS Keeper registry when performing an action of the

integration device manually. A separate options dialog is available for the lift integration to set communication port of the pc to communicate with the Servo controller o the lift. In addition the absolute positions of the lift traveler for liquid handler access and for incubator access can be set in the options dialog.

- SILAS communication.
  The integration module automatically converts user inputs and servo controller data into SILAS format and vice versa. In method editing the user does not have to take care on the lift operation. Selecting the incubator as a source and selecting a following action on the liquid handler deck implicates a lift transport. This is scheduled automatically by SAMI. In general labware transports do not have to be programmed by the user. The user only selects the locations or the devices where labware has to be processed. The transports to the locations and devices are calculated and performed automatically. [109]

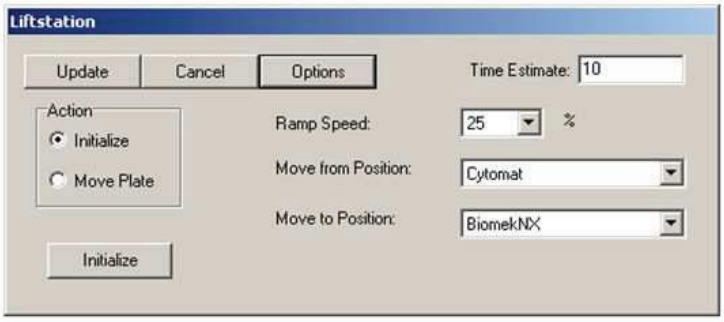


Figure 55 Action config dialog for lift station

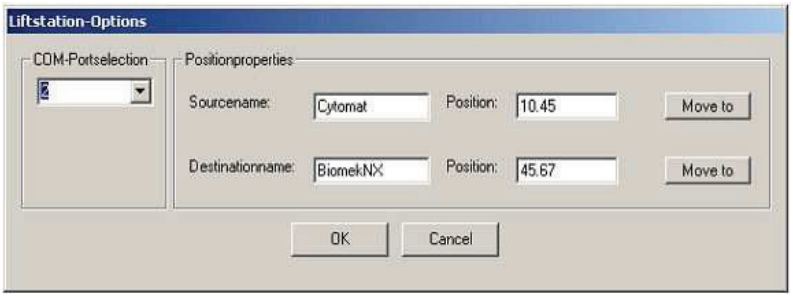


Figure 56 Options dialog for lift station

4.6.2.2.3 3D Tilting Device

When designing on-deck-components for liquid handlers it is very important to be very space saving. A device for operation with only one micro titer plate footprint labware should also keep its dimensions in the area of a micro titer plate footprint. The Biomek deck design with lifted platforms for all positions ensures to have some room under the microplate platforms for the device components. So the goal for the 3D tilting device was to use only one microplate footprint position on the Biomek deck. This was a challenge because achieving all the desired functions of the 3D tilting rack requires a complex mechanical and electronic design. So the 3D tilting rack was the most complex custom device development for the automated cell culture system. As an example the development and the operation of the 3D Tilting rack is documented more detailed. The design of the 3D Tilting Rack included following steps:

- Mechanical design – 3D CAD
- Pneumatic design – 3D CAD
- Electronic design – Electronic CAD
- Firmware design – software development
- SILAS integration – software development
- Biomek ALP installer
- Biomek liquid handling techniques / Pipetting templates
- Biologic evaluation – application tests

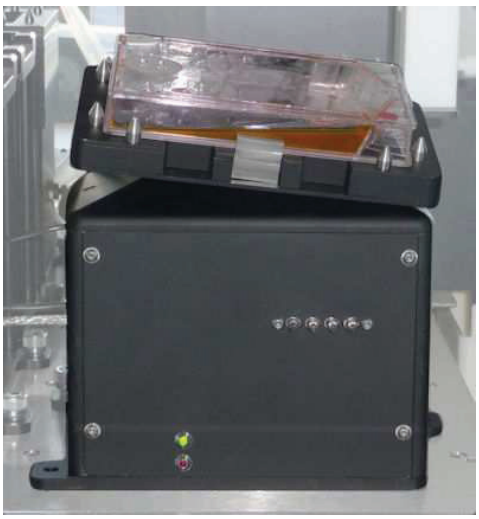


Figure 57 3D Tilting Rack

All of these development steps have to be performed joined together in a synchronized manner.

The functions to be implemented into the 3D Tilting Rack were:

- Labware clamping (for septum piercing)

- Labware tapping (for detachment of cells)
  - Parameters: number of taps

- Labware tilting (for Aspiration or dispensing or other)
  - Parameters: tilting direction (x, y or x-y-direction)
    tilting angle
    tilting speed

- Labware pivoting (for media distribution)
  - Parameters: pivoting angle
    pivoting speed
    pivoting duration

In the initial phase of the device development decision was made to have a tripod design to bear the tilting platform. So the basic idea was to have a tilt able cardan joint in the center of the platform and two further pods with variable dimensions for changing tilting angles in x-, y- or combined in x-y- direction. This was designed with stepper motor driven spindles. For accuracy and reliable operation an angle sensor was integrated into the labware tilting platform to allow closed loop tilting angle control.

For clamping and knocking miniaturized pneumatic cylinders are used. Together with a sophisticated pneumatic diagram both – clamping and knocking can be operated by using three clamps.

Mechanical design was made in AutoCAD® and SolidWorks. All components including motors and solenoid valves for pneumatics were modeled in 3D.

For the electronic control a microcontroller unit based on a Freescale MC68HC908GT16 is used. For internal communication and wiring a printed circuit board was designed docking the microcontroller board and all of its peripherals. Internal control is interfaced by analog-digital-conversion and I²C bus communication. [109-111]

The microcontroller unit contains the firmware and is programmed in C.

The SILAS integration software is programmed in Microsoft® Visual C++.

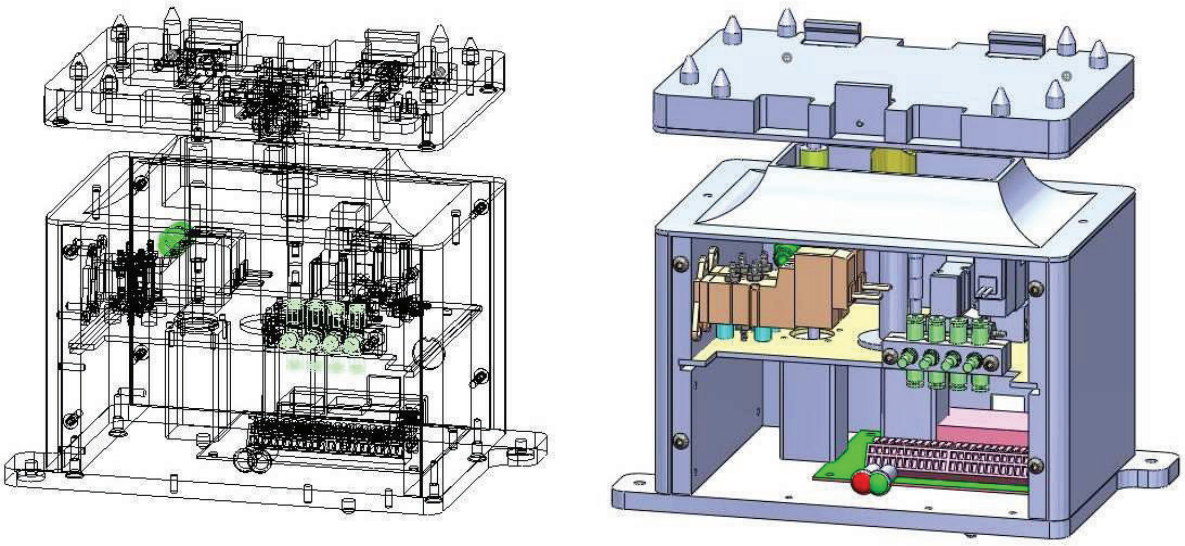


Figure 58 3D models of the 3D TiltRack

4.6.2.2.3.1 Installation and Operation with SAMI 3D Tilting Device Module

The 3D Tilting Rack can be used in SAMI EX and in Biomek software. The easiest way to use the 3D Tilting rack is to use it in SAMI EX. When used in SAMI EX the open and close commands for the clamps of the 3D Titling rack are executed automatically.

When used in Biomek software, Open Steps have to be built in as part of the Biomek methods. Open Steps have to be performed before a Labware transport to 3D Tilting rack can be done. Clamps will be closed automatically before the

rack performs tilt or pivot actions. For closing the clamps only the Tilt step should be used with angle 0º. An additional Open Step has to be performed before removing labware from 3D Tilting rack.

It has to be ensured, that the 3D Tilting rack is in tilted position only for pipetting steps. At any labware transportation to or from the rack by Biomek Pods the 3D Tilting rack must be in horizontal position. In SAMI EX the automatically sent 'Open' command tilts back to 0°. In Biomek software an Open Step inserted before labware move ensures that the rack tilts back to 0°.

Software Installation

For installation of the 3D Tilting Rack integration to the automation line there are several modules to be configured and installed as follows:

- SILAS integration module
- Biomek deck ALP definition (dimensions, positions etc.)
- Custom Pipetting Templates for tilted Labware
- Biomek method step for 3D Tilting Rack
- USB driver
- Hardware Setup and evaluation tool
- User Manual (pdf document)

For that purpose there was programmed an installation tool guiding the operator through the installation process.

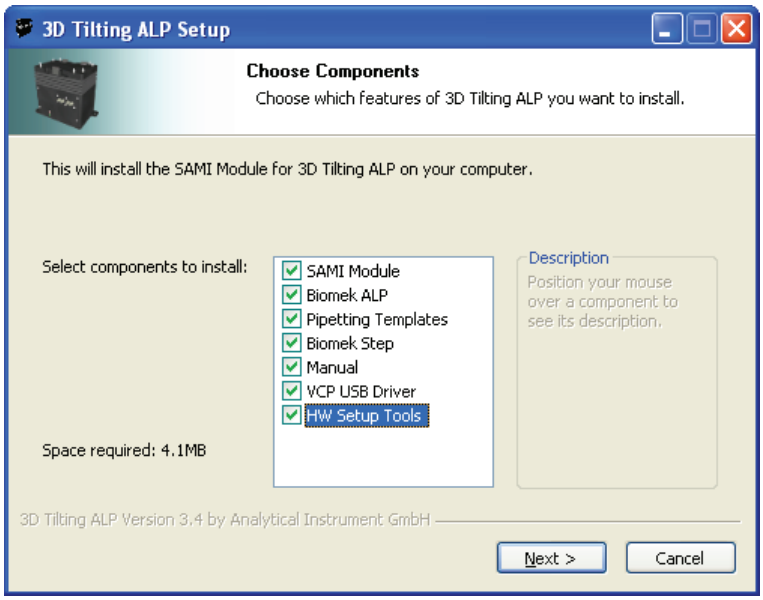


Figure 59 3D Tilting Rack installation

ALP Installation / Framing

The 3D Tilting rack fits on Biomek deck on any OneByOne ALP position. By performing software installation a new ALP Type ‚Tilt3DAlp' is generated in Biomek software. This ALP type can be selected in Biomek deck editor and can be installed on deck like other standard ALPs. Framing of 3D Tilting rack is performed like framing with framing tool of standard Biomek ALPs.

Action Config dialog

The Action Config dialog of the 3D Tilting rack allows for selection of operating mode and parameterization of tilting angles etc. Additionally the Action Config dialog offers some features for manual processing like 'Clamp on', 'Clamp Off' and 'Tilt to 0'. Figure 60 shows a screenshot of the 3D Tilting rack Action Config Dialog, where the tilting function is selected.

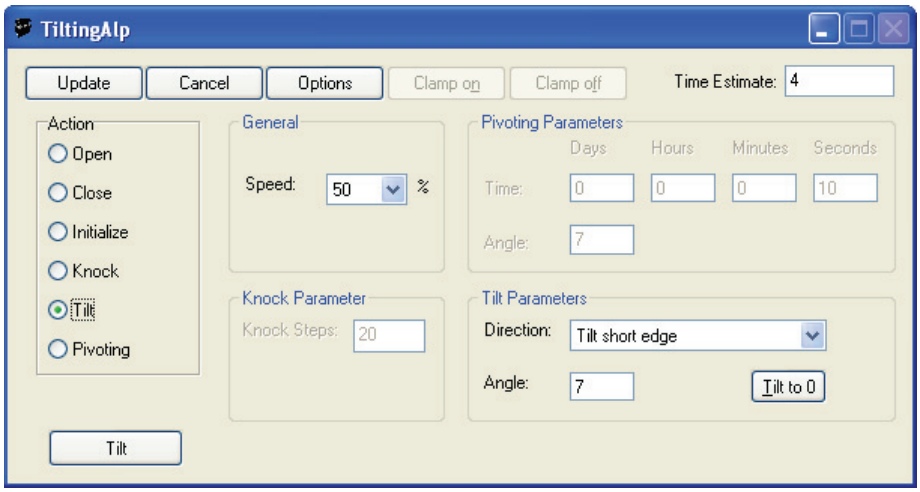


Figure 60 3D Tilting Rack Action Config Dialog

Knock

In knock mode the number of knock steps can be modified. Number of knocks can be in range from 1 to 99.

Tilt

In Tilt Mode the user can select the tilting direction:

- Tilt short edge
- Tilt long edge
- Tilt diagonal edge

Furthermore the tilting angle can be set in the 'Angle' field. Angles from -15 to 15 degrees are possible.

The tilting speed can be set by modifying dropdown field 'Speed'

Pivot

When pivoting is selected, the user can put in pivoting time, angle and speed.

Pipetting

Operation of 3D Tilting rack causes changing XYZ positions of Labware wells. To adapt pipetting coordinates to tilted plates there was developed special technique

and pipetting template using a script that is embedded into Biomek software. The tilt angle information is forwarded to the template via labware properties. There are three angle properties:

- TiltAngleX
- TiltAngleY
- TiltAngleXY

In Biomek software the Tilt rack steps will set these properties. The SAMI module sends out these angles as labware properties via Touch and Data messages. This enables the use of the '3D Tilt' technique in SAMI EX transfer steps.

Biomek Tilting rack Steps

There are four Biomek steps for the 3D Tilting rack. These Biomek steps of the 3D Tilting ALP integrate into the Biomek software environment the same way as original Beckman Coulter steps are embedded into the software.

The Open step is intended to be used to transport labware to or from the rack. This step will open the clamps. To close the clamps the Tilt step with an angle value of 0º can be used.

The Tilt step tilts the rack according to the given angle and direction. Clamps are closed before the rack starts tilting.

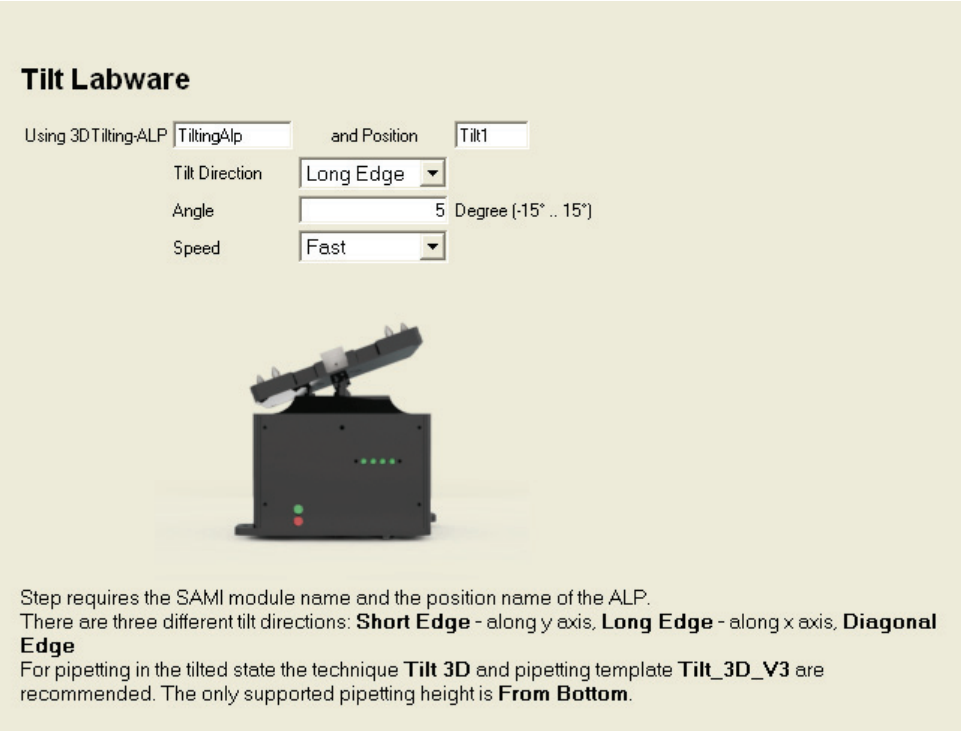


Figure 61 3D Tilt Step in Biomek software

Pivoting labware can be done with the Pivot step. The Knock step allows knocking on labware. This step requires tilt angle 0º and opened clamps.

After describing the 3D Tilting device more detailed the following device developments and their integration will be described more briefly.

4.6.2.2.4 Tilting Device

The Tilting device is made for multiwall micro titer plates. When cell are cultured i.e. in six-well plates the Tilting device can be used to support complete media aspiration or washing steps over the adherent cells. The device works with a fixed tilting angle that can be adjusted manually. It is designed in 3D CAD. The Tilting Mechanism is actuated by a pneumatic drive, switched by a solenoid valve. The valve is switched via Biomek FX Device Controller low Power output.

Figure 62 Tilting Rack

A feedback contact can sense the horizontal position. This sensor can be connected to Biomek Device Controller Inputs so that the correct horizontal position can be sensed by the Biomek workstation.

The tilting angle can be set up manually by sliding the position of the pneumatic pusher:

- bring pneumatic actuator in position 'Tilted' by using Manual Control of Biomek FX (Device Controller – Digital Outputs)
- Loosen knurled nut of pneumatic pusher
- slide pneumatic pusher, so that desired tilting Angle is set up
- fasten knurled nut of pneumatic pusher
- bring pneumatic actuator in position 'Horizontal' by using Manual Control of Biomek FX (Device Controller – Digital Outputs)

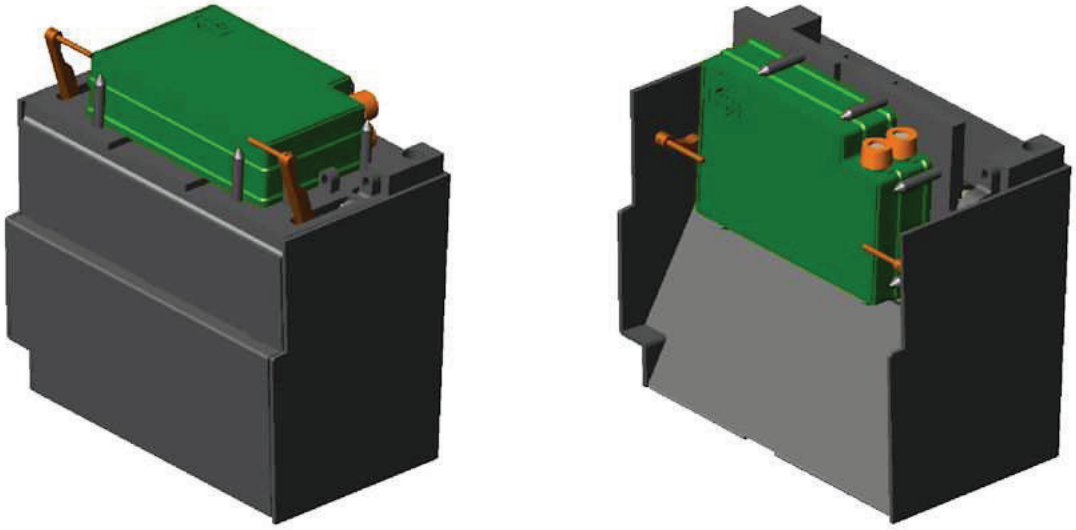
For pipetting in tilted positions there is a pipetting template for tilted plates. When changing the default tilting angle this template always has to be adapted to the set angle of the tilting ALP.

4.6.2.2.5 Flipping Device

Because of the geometry and the position of the septum the Corning® RoboFlask™ and the Automated Cell Culture Flasks from BD Falcon™ cannot be handled on the 3D Tilting device. For cell growth the flasks are to be stored in flat orientation as micro titer plates. For pipetting tip access the flasks have to be flipped to upright manner as shown in Figure 63. In order to support all currently available automation friendly cell culture flasks in microplate format on the automated cell culture system, decision was made to develop a Flipping device to allow tip access for Corning® RoboFlask™ and for BC Falcon™ Automated Cell Culture Flask.

Figure 63 Corning® RoboFlask™; source: www.corning.com

The device is designed as an automated labware positioner on the deck of the Biomek® NX^{P} Span8 liquid handler. Because of the reduced set of parameters for a 90° flipping device – just tilting and clamping of plates in a fixed manner – the device was designed to be controlled by digital I/Os of the Biomek® device controller. The device is operated by pneumatic cylinders switched by solenoid valves. Feedback switches to the Biomek® device controller ensure for operation feedback. For higher throughput the device is designed to hold up to two RoboFlasks at a time. Extensions for up to 4 RoboFlask at a time would be possible.



Transport access position Pipetting position

Figure 64 CAD study Flipping device for Corning® RoboFlask™

4.6.2.2.6 Automated Self Refilling Reservoir

Standard liquid handlers for microplates are designed for volume transfers in the microliter range for every pipetting tip and microplate wells. In cell culture larger volumes are handled in the cell culture flasks. Standard disposable reservoirs in microplate footprint would limit the available liquid volumes on deck in an unacceptable manner. To compensate this shortcoming, during the development of the automated cell culture system different self refilling reservoirs were developed. In addition to the self refilling function these reservoirs are able to be tempered by circulating thermostats. Depending on the type of liquids to be held in the reservoirs and the desired application there are three versions now:

- Multichannel reservoir, optical sensing

  For optical clear media the optical sensing version can be used. A light beam is sent through the disposable reservoir where liquid inside the reservoir changes the light refraction and deflects the light beam. The optical sensing unit working with optical fiber sensors is adjustable for the liquid type. In addition threshold values can be configured.

- Multichannel reservoir, gravimetric sensing

For non clear media or when cooling and water condensation might be an issue, there was developed a gravimetric sensing self refilling reservoir. The switching force and so the volume of liquid held anytime in the reservoir is adjustable.

- Quarter Span8 reservoir, ultrasonic sensing

If more different liquids are needed and space saving is required, for single channel to eight channel pipetting there was developed the quarter self refilling reservoir. The sensing principle is based here on an ultrasonic distance measurement which checks the filling height of the reservoirs. This type of reservoir is equipped with four independent channels for four different media.

Each of the three types of reservoirs itself needs only one deck position for microplates on the liquid handler. All the reservoirs have a reservoir bottle. They all work by using filtered pressurized air. Liquid dispensing is initiated either by automatic switching air pressure to the reservoir bottle or by keeping air pressure to the bottle and switching the liquid path to the reservoir be inert solenoid valves.

The multichannel reservoirs additionally can function as microplate platforms. So there is more room for labware on the one hand and the reservoir is lidded on the other hand. For access to the multichannel reservoirs liquid the microplate or lid or tip-box on top has to be removed by the liquid handler gripper.

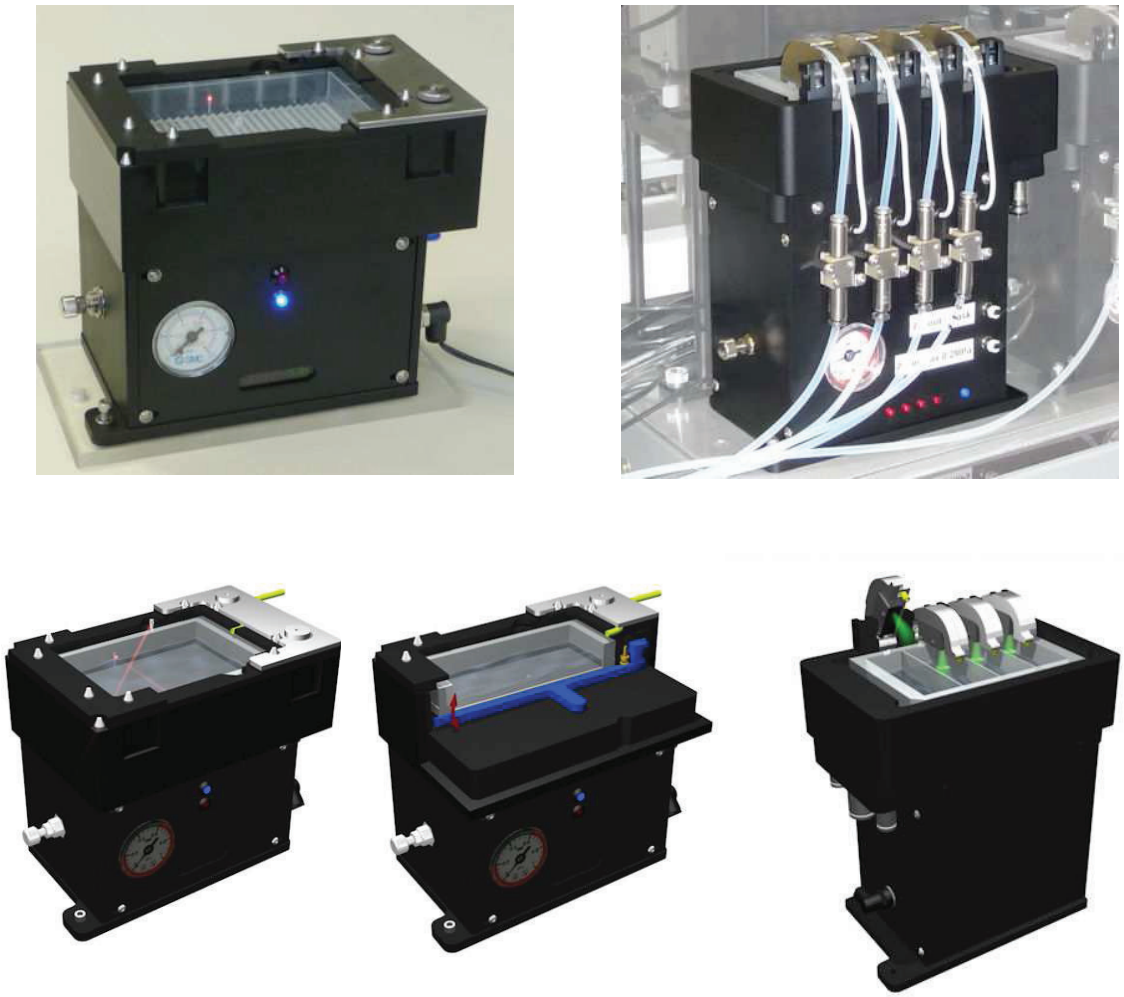


Figure 65 Self Refilling reservoirs, photographs and CAD studies

All the reservoirs are equipped with exchangeable disposal reservoirs, the multichannel reservoir is a Thermo Matrix and the quarter reservoir insert comes from FluidX.

4.6.2.2.7 Media Quality Sensing

A constant media quality and a constant pH value in cell growth media are essential for successful cultivation. Therefore buffer solutions are content of cell growth media which regulate the pH value by CO_2 consumption. In addition many cell culture growth media contain a pH indication dye. Mostly phenol-red (Phenolsulfonphtalein) is used for that purpose. Phenol red is suited well for this

use because of its low level of cytotoxicity and its colour changing interval. Phenol-red is red in fresh media with a pH value of 7.4. Acidic solution change to yellow, pH values above 7.4 change to pink / purple.

A fast decrease of the pH value in cell growth media indicates problems in the current culture. So waste products produced by dying cells or fast growth of bacterial contaminants cause a decrease in pH, which results in a change of indicator colour. In addition metabolism waste products produced by the growing cells slowly decrease the pH, gradually turning the solution orange and then yellow.

For the automated cell handling system there was decided to provide the option to automatically observe the colour of the cell growth media and so to provide a further parameter for quality control in automated cell culture. Therefore a special Colour sensing device was developed that measures optically the colour of the media in cell culture flasks and helps observation on media quality and support of decisions regarding media change or rejection of contaminated cell culture flasks.

Figure 66 Tilted flask on color sensing device

Media quality sensing device design

The sensing device is designed as an ALP that can hold a microplate format labware. For quality sensing a cell culture flask is put on the device by liquid handler transport arm. After tilting the flask a white light led lights the media

and on the opposite a RGB colour sensor converts the intensities of the components of red, green and blue light into electrically measureable signals. By using analogue-digital converters and microcontrollers the signals are computed to colour information of media.

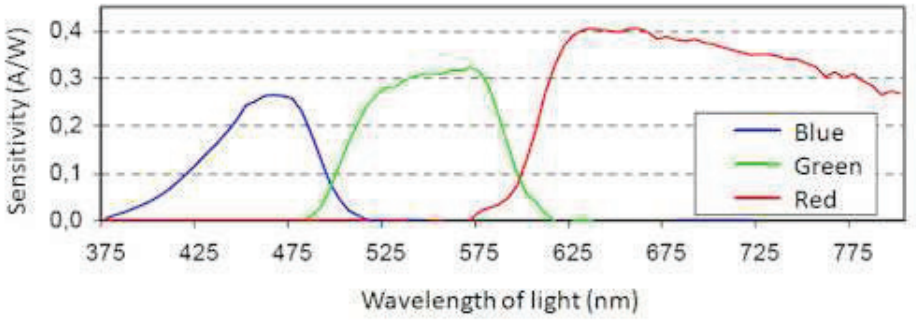


Figure 67 Sensitivity of RGB channels of colour sensor

Because of different color and different color intensities of diverse cell growth media, all types of media used on the cell culture system has to be taught with the media quality sensing device. For every media samples with 3 different ph values are needed to be taught into the sensing device.

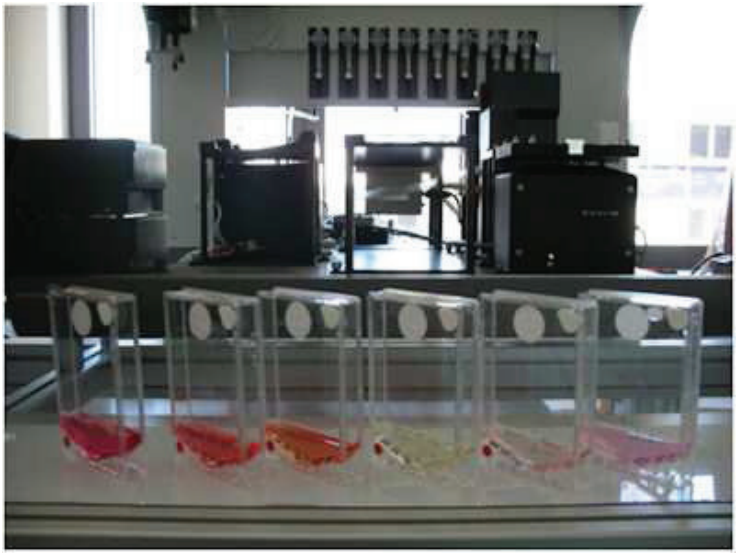


Figure 68 different colors with different pH values for Ham's F12 and DMEM

For indications of pH values the 3 RGB values are used as 3 coordinates in a 3 dimensional room. The pH value can be indicated by calculation of the length and algebraic sign of the vector from original ph value to current pH value.

By knowing type of media, new media value and thresholds for acidic and basic solutions the Media quality sensing device provides information for run time decisions of the automated cell handling system.[112]

Another option is the measurement of light scatter. Often micro organism contamination is indicated by obfuscation of growth media which can be sensed my light scatter measurement. It is assumed, that the developed hardware can measure light scatter too. Currently there are not yet data available on light scatter. In addition the software and firmware of the device would have to be adapted for that purpose.

4.6.2.2.8 On Deck Incubation

For short incubation times on deck without time consuming transports a two position on deck heating / shaking incubation station was integrated into the automated cell culture system. Therefore two single position incubators 'Incubator Shaker MP' from Inheco GmbH are used. Adaption frames make the incubators fitting on liquid handler deck space for direct gripper access.

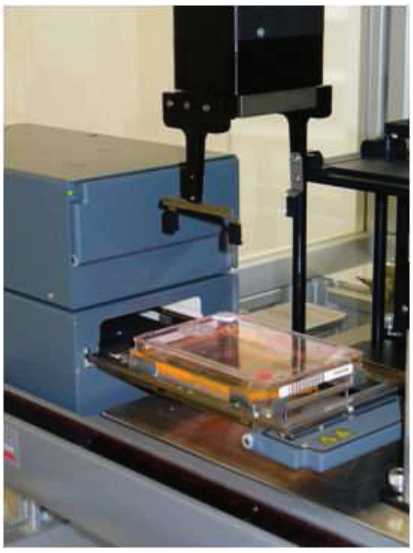


Figure 69 Dual Inheco Incubator Shaker MP integrated on deck

The Incubator Shaker MP is designed for use in automated environments for SBS footprint microplates. It has a compact footprint and allows easy robot gripper access. Several incubators can be stacked to a tower of incubators. All incubators in one tower a controlled via one USB port, working with a master-slave principle.

The Incubator Shaker works with a unique vibration reduced shaking principle, enabling free programmable shaking curves (linear, circle, elliptic, eight).

In the automated cell culture system the on deck incubators are used for short time heating incubations i.e. for Trypsin influence in cell passage.

The Inheco Incubator Shaker MP comes with a library for software integration. For SILAS integration a software module was programmed as it is performed for any custom integrations adapting to the SILAS communication protocols.

The user can select incubation temperature, shaking speed, device and duration of incubation. The design of the action config dialog is as known from SILAS integrations.

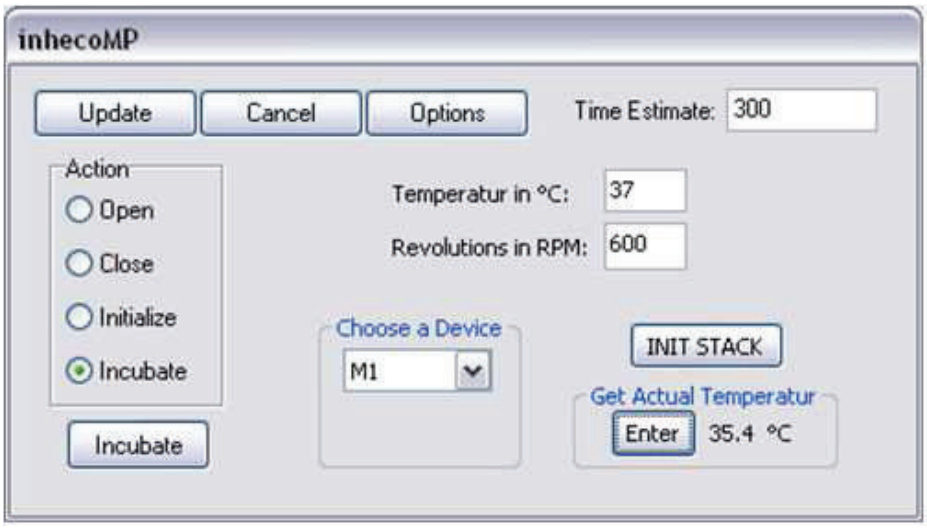


Figure 70 Inheco Incubator / Shaker integration action config dialog

4.6.2.2.9 Bulk Media Dispense

Smaller volumes of liquid media are held on deck of the liquid handler and are handled by normal single-channel up to eight-channel pipetting. If there are larger volumes to be handled this can be done through bulk media dispense. The Biomek® NX^{P} Span8 has a built in functionality to dispense through the system liquid channels. To be able to change system liquid from standard (degassed water) to other liquids like cell growth media, buffers, cleaning- and sterilization solutions a multiport valve is integrated for liquid selection. This enables working the system liquid path as a large volume dispensing path. The standard operation here is to work with the standard embedded syringe pumps combined with port selection valves. Because of the risk of contaminations not easily to be fixed by flushing with complex liquid paths, syringe pumps and valves it was decided to modify one system liquid channel to a more simple and robust design: One channel is equipped with a peristaltic pump where the channel can be used for pipetting similar to standard pipetting with system liquid and syringe pumps. In addition this channel can be used as a bulk dispense channel, where the number of valves, tube connectors etc. is reduced to a minimum for prevention of contamination and ease of tube replacement.

The integrated peristaltic pump is an 'Ismatec Reglo Digital' with a RS232 interface for automated control. The pump is designed for precision media dispensing and can be calibrated. The "click'n go" easy exchange tube cassettes support comfortable and fast tube set changing.

For system integration of the 'Reglo Digital' there was developed a SILAS module integrating the pump directly into Biomek software.

4.6.2.2.10 Media Storage and In-Line Warming

From a certain point of view cell growth media and buffers should be kept in refrigerated conditions for longer periods. On the other point of view cells need to be treated under stable temperature conditions and need to be fed by warmed media. To meet both needs the media storage bottles are kept in a refrigerator

embedded into the automated cell culture system. When dispensing media to labware on deck, the liquid is pumped through an inline heat exchanger which warms the liquid from approx. 5°C to about 35°C. This ensures not to shock the cells by chilled media and helps maintaining cell growth friendly conditions.

4.7 Biologic Application Development

Hardware design and systems engineering is only one part in laboratory automation. Another part is application development which includes automated method development, biologic application evaluation, turning manual processes into automated operation, maintenance of sterility etc. Together with the system design different approaches were made on application development as described in the next chapters.

4.7.1 Automation Friendly Cell Culture Labware Survey

As a pre-automation study the automation friendly cell culture flasks from different vendors were evaluated on cell growth rates. Currently there are microplate format cell culture flasks available from three labware product companies:

- 'RoboFlask™' from Corning®
- 'CELLSTAR® AutoFlask™' from Greiner Bio-One
- 'Automated Cell Culture Flask' from BD Falcon™

RoboFlask AutoFlask Automated Cell Culture Flask

Figure 71 Automation friendly cell culture flasks

4.7.1.1 Corning® RoboFlask™

The RoboFlask™ was designed as the first microplate format automation friendly cell culture flask by Corning®. It was first used on the Tecan Cellerity™ system. It is available tissue culture treated, which makes the growth surface hydrophilic. In addition a version with Corning® CellBIND® Surface is offered. These flasks are treated with a vacuum plasma technology. CellBIND® surfaces have higher levels of oxygen in the surface that results in very stable and consistent material property. According to documentations of the vendor the benefits of Corning® CellBIND® are[113]:

- Even, more consistent and stronger cell attachment for increased growth and yields
- Better and more quick cell adaptation to reduced-serum or serum-free conditions
- Elimination of the necessity of additional coatings
- No refrigeration or special handling required, stable at room temperature

Further basic properties of the RoboFlasks are:

- Removable cap with or without pierceable septum for manual and for automated handling
- Microplate footprint for automation support
- Barcoding
- permeable membrane for gas and liquid exchange
- cell growth area: 92,6cm²
- stackable
- media recovery slope

4.7.1.2 Greiner Bio-One AutoFlask™

The CELLSTAR® AutoFlask™ from Greiner Bio-One was the second automation friendly cell culture flask in microplate dimensions. It was used first with the

Automated Compound Profiling System (ACP) of the Genomics Institute of the Novartis Research Foundation (GNF). The AutoFlask™ is the only microplate format cell culture flask that can be operated completely in horizontal position because of the orientation of the septum. In addition the AutoFlask™ is the only one having an integrated centrifugation pocket for media exchange or harvesting of suspension cultures. There are available versions for suspension cells as well as for adherent culture. These plates are treated with a proprietary physical process to make the cell growth surface hydrophilic for cell adhesion.

Greiner offers a protein coating of cell growth surfaces called CELLCOAT® which enhances adherence of the cells which is necessary in methods with washing steps.

A new development of Greiner is Advanced TC™ which was made for the cultivation of sensitive cell lines. Greiner states, that a newly modification of polymers in the high grade optical clear polystyrene changes the cell growth surface in a way, that primary and long-term adherence of the cells is influenced in a positive manner. This supports higher proliferation and faster expansion of cells. Furthermore Greiner reports that also the culture conditions of highly sensitive cells are optimized with Advanced TC™ and that the transgene activity of transfected cells is higher on Advanced TC™ surfaces. [95;114-116]

Applications for Advanced TC™ are:

- Culture of sensitive primary cells and standard cell lines
- Usage of serum-reduced or serum-free growth media
- Differentiation of semi-adherent cells
- Transfection
- Transduction
- Automation, High Throughput

Furthermore Greiner CELLSTAR® AutoFlask™ comes with following properties:

- Growth area: 83,6 cm²
- Stacking support for low room consuming incubation
- PTFE coated filter membrane for prevention from wetting

- Microplate footprint for automation support
- Barcoding

4.7.1.3 BD Falcon™ Automated Cell Culture Flask

BD Biosciences offers the Automated Cell Culture Flask for cell culture automation. This type of flask is to be handled the same way as the Corning® RoboFlask™. The Falcon flask was designed for use with the TECAN Cellerity™ system. As the other automation cell culture flasks the Falcon flask has a pierceable septum and a gas exchange membrane. [117]

Features:

- Sterile, non-pyrogenic
- 94cm² growth surface
- Recommended medium volume: 20...25ml
- Optically clear virgin polystyrene
- Unique barcode on every flask
- Removable septum cap
- BD BioCoat™ available

4.7.1.4 Cultivation of CHO_K1 Cells in Automation Friendly Cell Culture Flasks Without Pre-Cultivation

As an initial study the automation friendly cell culture flasks were tested for growth rate of CHO-K1 cells without pre cultivation. That means the cells were seeded into the flasks without a pre-cultivation in labware of the same vendor.

Cultivation was performed in standard procedures of animal cell culture with incubation und 37°C and 5% CO_2. Flasks were washed before passage with 10ml PBS buffer and were harvested by using 2ml Trypsin / EDTA solution. Cell culture

medium was Hams F-12 w 10% FBS, Gln, P/S. The cell count was measured by using a Nucleocounter.

4.7.1.4.1 Cultivation in Greiner AutoFlask™

Table 3 shows cell counts in AutoFlask compared to T75 flask (Nunc) as used in current culture of CHO cells.

Number of approach	Flask type	Seeded cells (10E5c)	Volume of media (ml)	Growth period	Cell count after culture (10E6c)
1	T-75	3	25	2 days	1,4
					2
	AutoFlask				0,87
					0,75
2	T-75	3	25	2 days	2
		2,4			1,9
	AutoFlask	3			1
		2,4			1,8
3	T-75	3	25	3 days	11,3
					10,2
	AutoFlask				4,32
					3,36
4	T-75	3,175	25	5 days	32,16
	AutoFlask				15,9
					25,8
5	T-75	3,2	20	5 days	40,29
	AutoFlask				39,51
					28,8

Table 3 Cultivation of CHO K-1 cells in Greiner AutoFlask

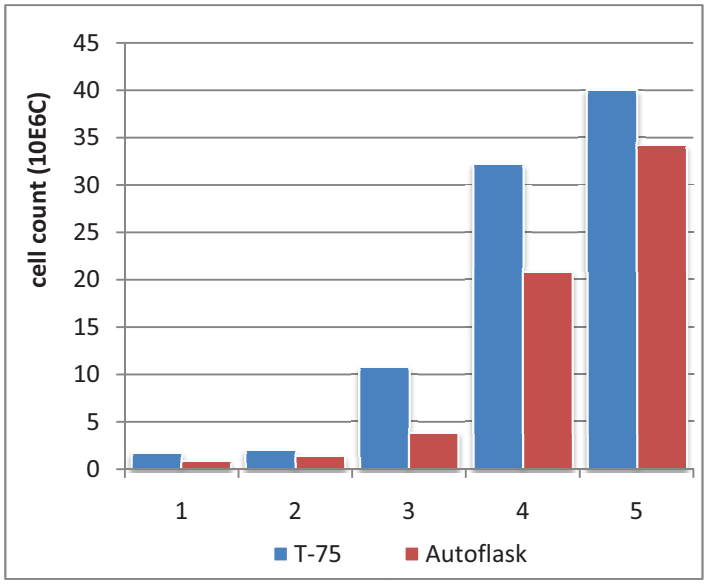


Figure 72 CHO cells in AutoFlask, five approaches

Diagram in Figure 72 shows the cell counts in the 5 approaches. Cell counts for AutoFlask are significant lower than in T75 flasks. Analysis of these effects follows in chapter 4.7.1.4.4.

4.7.1.4.2 Cultivation in Corning RoboFlask

Table 4 shows cell counts in RoboFlasks compared to T75 flask (Nunc) as used in current culture of CHO cells.

Number of approach	Flask type	Seeded cells (10E5c)	Volume of media (ml)	Growth period	Cell count after culture (10E6c)
1	T-75	3	20	3 days	8,97
					8,67
	RoboFlask				5,61
					8,04
2	T-75	3,175	25	3 days	11,3
					10,2
	RoboFlask				9,66
					8,88
3	T-75	3,2	20	2 days	3,45
					2,9
	RoboFlask				3,15
					3,24

Table 4 Cultivation of CHO K-1 cells in Corning RoboFlask

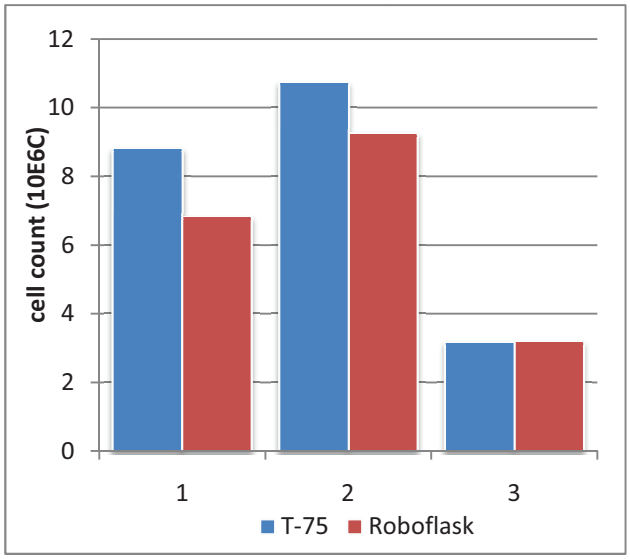


Figure 73 CHO cells in RoboFlask, three approaches

Diagram in Figure 73 shows the cell counts in the 3 approaches. Cell counts for RoboFlask are significant lower than in T75 flasks. Analysis of these effects follows in chapter 4.7.1.4.4.

4.7.1.4.3 Cultivation in BD Falcon Automated Cell Culture Flasks

Table 5 shows cell counts in BD Falcon Automated Cell Culture Flasks (ACCF) compared to T75 (Nunc) flask as used in current culture of CHO cells.

Number of approach	Flask Type	Seeded cells (10E5c)	Volume of media (ml)	Growth period	Cell count after culture (10E6c)
1	T-75	3,4	20	3 days	9,42
					9,51
	ACCF				11,25
					9,45
2	T-75	3,175	25	3 days	11,3
					10,2
	ACCF				10,6
					13,1
3	T-75	3	20	3 days	8,97
					8,67
	ACCF				9,99
					8,04

Table 5 Cultivation of CHO K-1 cells in BD Falcon Automated Cell Culture Flask

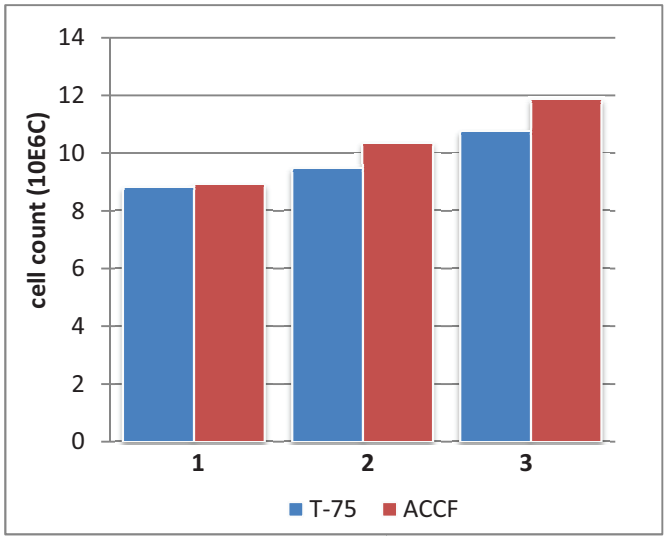


Figure 74 CHO cells in BD Falcon Automated Cell Culture Flasks, three approaches

Diagram in Figure 74 shows the cell counts in the 3 approaches. Cell counts for Automated Cell Culture Flask are higher than in T75 flasks. Analysis of these effects follows in chapter 4.7.1.4.4.

4.7.1.4.4 Summary – CHO

The cultures with CHO cells in AutoFlasks and RoboFlask delivered significant lower cell counts than the culture of cells in Nunc T75 flasks as used usually in manual cell culture in the institute although the growth area of all automation friendly cell culture flasks is bigger than the area in T75 flasks . On the other side the culture in BD Falcon delivered higher cell counts in the BD Falcon Automated Cell Culture Flasks than in the Nunc T75 flasks. Figure 75 shows the growth in the three flasks in relation to the Nunc T75 flask (100%). The lower cell numbers in RoboFlask and AutoFlask is caused by adaptation of cells to new labware. The change of cell culture vessel material means changed conditions for the cells and for this reason the cells need more time to adapt to the environment so that they proliferate slower. The significant higher growth rate in the BD Falcon Automated cell culture flask is explained in the way, that the BD Falcon cell culture flask material is most similar to the material of the Nunc flasks. In later performed tests with pre-cultivation in labware of the same manufacturer these recognition is confirmed to be right.

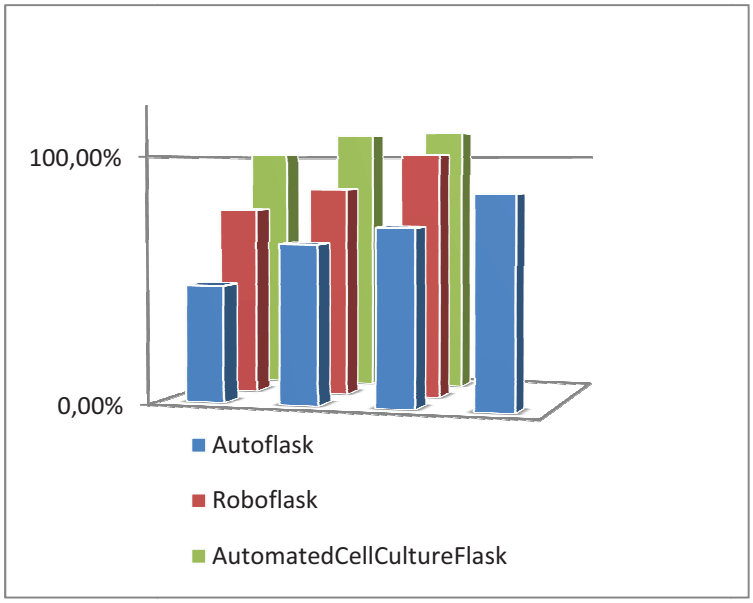


Figure 75 Comparison of cell counts (CHO) in automation friendly cell culture flasks in relation to T75 flasks (100%) without pre cultivation

4.7.1.5 Cultivation of HeLa Cells in Automation Friendly Cell Culture Flasks Without Pre-Cultivation

The initial test for cultivation of HeLa cells was performed the same way as the test with CHO cells before. (Please refer to 4.7.1.4)

4.7.1.5.1 Cultivation in Greiner AutoFlask™

Table 6 shows cell counts in AutoFlask compared to T75 flask (Nunc) as used in current culture of HeLa cells.

Number of approach	Flask Type	Seeded cells (10E5c)	Volume of media (ml)	Growth period	Cell count after culture (10E6c)
1	T-75	3	25	5 days	2,1
					2,5
	AutoFlask				0,78
					0,3
					0,69
					0,6

2	T-75	3	25	3 days	1,2
					1,6
	AutoFlask				0,54
					0,6
					0,84
					0,81
3	T-75	3,2	25	5 days	2,7
		3			2,3
	AutoFlask	3,2			0,51
					0,45
		3			0,84
					0,45
4	T-75	5,4	20	7 days	12,5
	AutoFlask				8,46
					8,82

Table 6 Cultivation of HeLa cells in AutoFlasks compared to T75 flasks (Nunc)

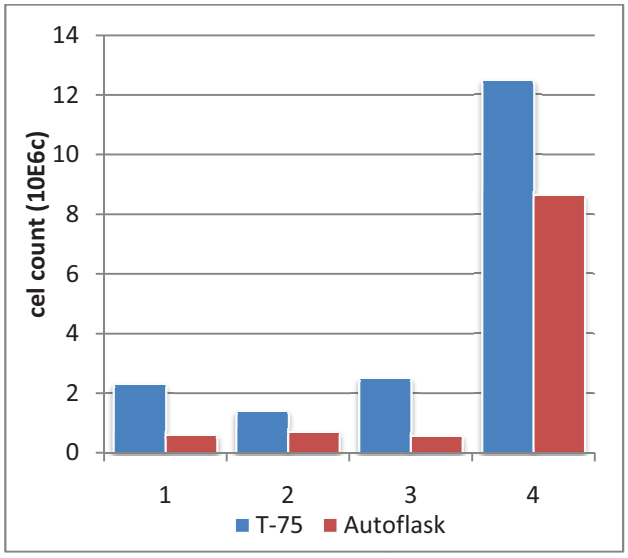


Figure 76 HeLa cells in AutoFlask, four approaches

Diagram in Figure 76 shows the cell counts in the 4 approaches. Cell counts for AutoFlasks are significant lower than in T75 flasks. Analysis of these effects follows in chapter 4.7.1.5.4.

4.7.1.5.2 Cultivation in Corning RoboFlasks

Table 7 shows cell counts in RoboFlasks compared to T75 flask (Nunc) as used in current culture of HeLa cells.

Number of approach	Flask Type	Seeded cells (10E5c)	Volume of media (ml)	Growth period	Cell count after culture (10E6c)
1	T-75	5,4	20	4 days	2,7
					3,15
	RoboFlask				3,09
					4,58
2	T-75	4,7	20	4 days	2,9
					2
	RoboFlask				4,83
					2,7
3	T-75	6,25	20	4 days	2,5
					3,84
	RoboFlask				3,75
					3

Table 7 Cultivation of HeLa cells in RoboFlasks compared to T75 flasks (Nunc)

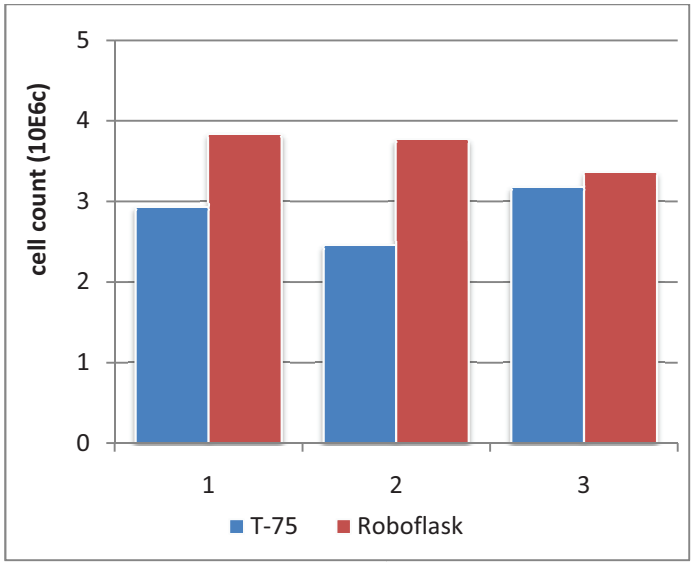


Figure 77 HeLa cells in RoboFlasks, three approaches

Diagram in Figure 77 shows the cell counts in the 3 approaches. Cell counts for RoboFlasks are mostly significant higher than in T75 flasks. Analysis of these effects follows in chapter 4.7.1.5.4.

4.7.1.5.3 Cultivation in BD Falcon Automated Cell Culture Flasks

Table 8 shows cell counts in BD Falcon Automated Cell Culture Flasks (ACCF) compared to T75 (Nunc) flasks as used in current culture of CHO cells.

Number of approach	Flask Type	Seeded cells (10E5c)	Volume of media (ml)	Growth period	Cell count after culture (10E6c)
1	T-75	5,4	20	4 days	2,7
					3,15
	ACCF				5,22
					5,19
2	T-75	4,7	20	4 days	2,9
					2
	ACCF				4,77
					4,32
3	T-75	6,25	20	4 days	2,5
					3,84
	ACCF				3,66
					3,06

Table 8 Cultivation of HeLa cells in BD Falcon Automated Cell Culture Flasks compared to T75 flasks (Nunc)

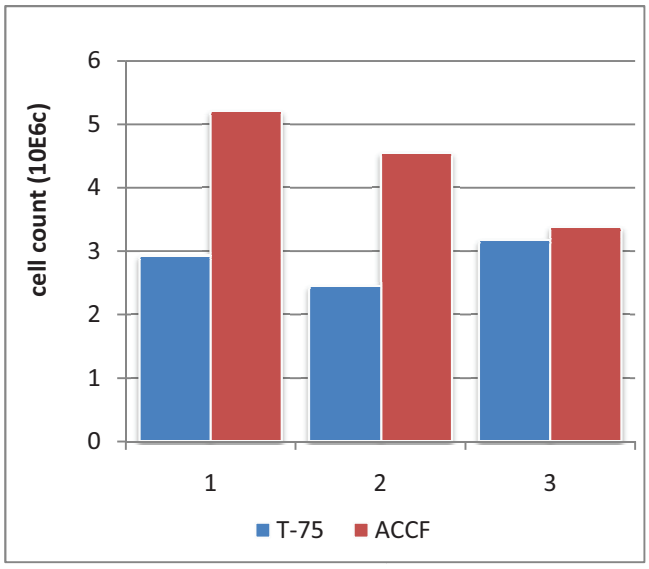


Figure 78 HeLa cells in BD Falcon Automated Cell Culture Flasks, three approaches

Diagram in Figure 78 shows the cell counts in the 3 approaches. Cell counts for BD Falcon Automated Cell Culture Flask are mostly significant higher than in T75 flasks. Analysis of these effects follows in chapter 4.7.1.5.4.

4.7.1.5.4 Summary – HeLa

In general the HeLa cells grow slower compared to the CHO cells. In AutoFlasks the cell count is significant lower for HeLa cells the same way as it was with CHO cells. In RoboFlasks and in BD Falcon Automated Cell Culture Flasks cultivation of HeLa cells result in higher cell numbers compared to T75 flasks (Nunc). It seems that different cell lines tend to adapt differently to changes in cell growth vessel material. Growth in Autoflask shows a reduced growth within the first days of culture. More detailed studies on this issue would go beyond the scope of this dissertation with complex system design, hardware and software development and first initial cell culture evaluations.

In result of the initial test without precultivation and the adaptation effects of different cell lines following studies were made with precultivation in labware of the same vendor, as used in automated cell culture system later.

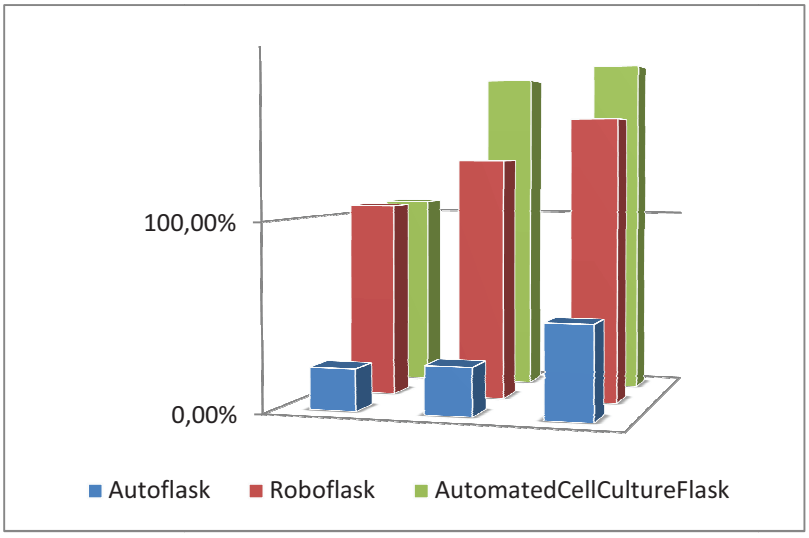


Figure 79 Comparison of cell counts (HeLa) in automation friendly cell culture flasks in relation to T75 flasks (100%) without pre cultivation

4.7.1.6 Cultivation with Pre-Cultivation in Cell Culture Flask of the Same Manufacturer and Same Material

In a further study the automation friendly cell culture flasks were tested for growth rate of CHO-K1 cells with pre cultivation in labware of the same manufacturer and the same material. In parallel cells were cultivated in T75 flasks of each of the manufacturers.

Cultivation was performed in standard procedures of animal cell culture with incubation und 37°C and 5% CO_2. Flasks were washed before passage with 10ml PBS buffer and were harvested by using 2ml Trypsin / EDTA solution. Cell culture medium was Hams F-12 w 10% FBS, Gln, P/S. The cell count was measured by using a Nucleocounter. Because of the high growth rate of CHO-K1 cells cultivation period was limited to 2 days.

Table 9 illustrates an example of cell growth rates in automation friendly cell culture flasks of the three different manufacturers. In addition cell growth rates in corresponding T75 flasks of the three manufacturers were monitored. In difference to the initial culture experiments without pre-cultivation in labware of the same manufacturers the growth rates in all labware types are in the same range. This seems to confirm the assumption that cells need to adapt to need cell growth surfaces before they can develop a significant growth.

manufacturer	flask type	growth area [cm²]	seeded cells x10^5	seeded cells per cm² x10^5	media volume [ml]	cells after culture x10^5	cells after culture per cm² x10^5	multiplication
Greiner	Cellstar	75,0	5	0,067	5,0	60,00	0,80	12,00
Greiner	Autoflask	83,6	5	0,060	5,0	60,50	0,72	12,10
Corning	Culture Flask (cellbind surface)	75,0	5	0,067	5,0	58,00	0,77	11,60
Corning	Roboflask (cellbind surface)	92,6	5	0,054	5,0	61,50	0,66	12,30
BD Falcon	Cell Culture Flask	75,0	5	0,067	5,0	51,50	0,69	10,30
BD Falcon	Automated Cell Culture Flask	94,0	5	0,053	5,0	52,00	0,55	10,40

Table 9 Cell growth in labware of different manufacturers with pre-cultivation

In Figure 80 the average cell densities per area are displayed for the culture flasks of the three manufacturers. Opposite to the T75 flasks where the cell densities are nearly the same for the three manufacturers, the densities in the automation friendly vary and the densities are lower compared to the T75 flasks. Analyzing this would exceed a separate issue. Possible reasons for the lower densities are smaller gas phase volume combined with lower gas exchange through liquid proof membranes in the automation friendly cell culture flasks. In following experiments in automated cell culture cell were always pre cultured in labware of the same manufacturer.

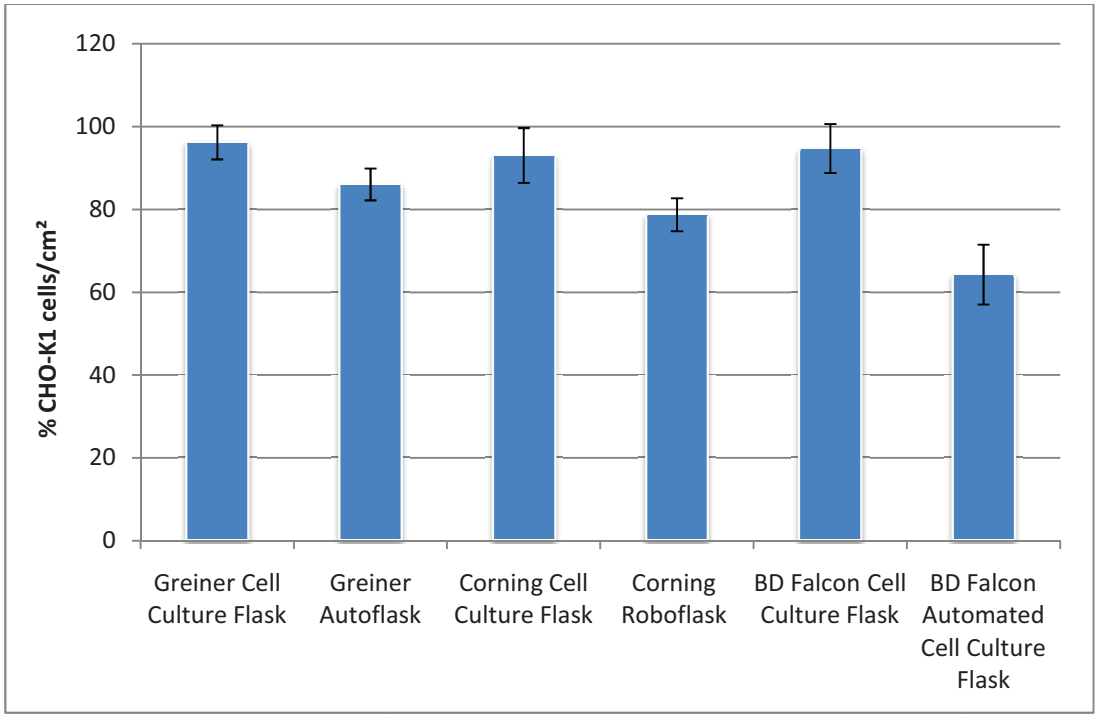


Figure 80 Average cell densities after cultivation for different cell culture flasks

4.7.2 Automated Cell Culture Evaluation

In the automated cell culture evaluation initially cell were handle and cultivated on the new developed automated cell culture system. Therefore common cell lines growing in adherent culture were used. First of all automated methods were

programmed using ViCell XR software, Biomek Software, SAMI EX and SAMI process management.

Figure 81 illustrates a typical example of performing multiple cell line cultivation runs. Multiple ViCell measurement methods, Biomek Liquid Handler Methods, SAMI Methods and whole cell culture processes are combined on the automated cell culture system. Running multiple nested processes is supported by this system by using modular methods and overall process- and labware data management provided by Data Acquisition and Reporting Tool and SAMI Process Management.

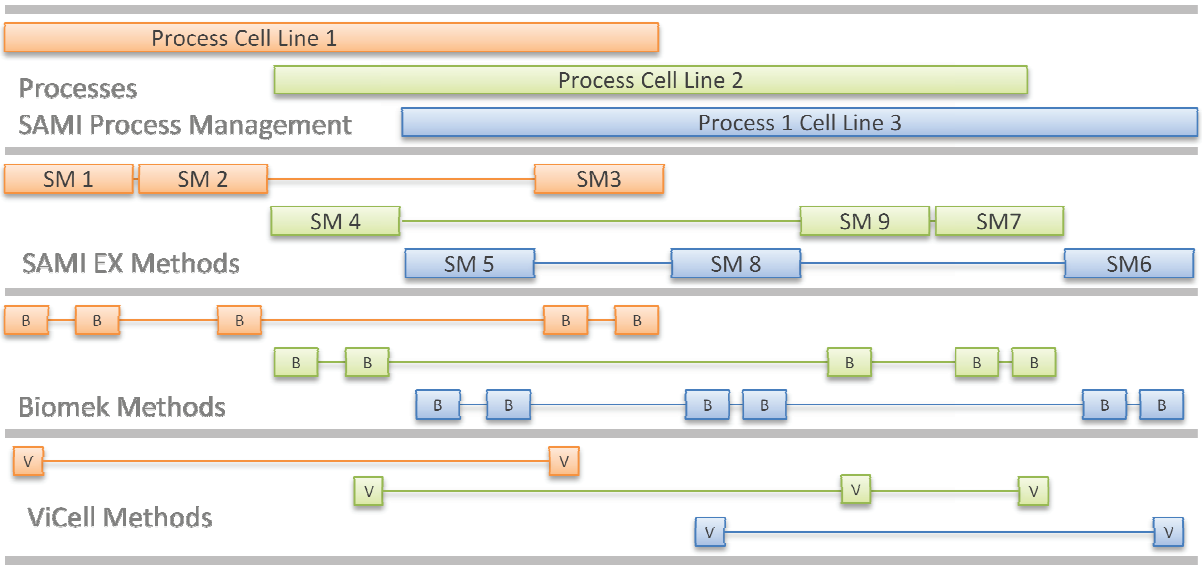


Figure 81 Multiple Methods running on automated cell culture system

4.7.2.1 ViCell Methods

The ViCell XR cell counter has its own PC software where multiple parameters and cell count/cell viability methods are to be programmed. Different cell types and cell lines need different measurement methods. ViCell methods are programmed and are saved in cell type specific method files. Through the ViCell SILAS integration module the device is integrated into Biomek software. By using data sets results of ViCell measurements can be used while run time for further processing like creation of defined cell counts for assay plates, new cell expansion processes etc.

4.7.2.2 Biomek NX^{P} Methods

The Biomek software is used for Biomek method development and runtime control of the Biomek and its on deck integrations. Biomek software uses SILAS communication to interact with on-deck integrated devices like ViCell XR, 3D tilting ALPs, on-deck incubators, barcode reader etc. Biomek methods contain sub processes of cell culture which are controlled on the liquid handler level without complex time optimizing scheduling. Run time data of on deck integrated devices can be processed by Biomek software through SILAS and can be provided to the higher level control software modules SAMI EX and SAMI Process Management.

4.7.2.3 SAMI EX Methods

In SAMI EX more complex methods including all device integrations like Biomek, cell growth incubator, transport lift and Biomek integrated devices are programmed. SAMI EX supports multiple labware family scheduling and resource pools. The integrated time optimizing scheduler calculates multiple-labware-family runs. In SAMI EX coherent processes of cell culture are programmed in methods like cell seeding, cell passage, quality control, media exchange, preparation of assay plates etc. Longer periods of cell growth incubation are not included in SAMI EX for this system, because incubation with long term device inactivity would block the system for other processes, when included in SAMI EX. The methods in SAMI EX contain Biomek methods and all separate integrations.

4.7.2.4 SAMI Process Management

SAMI Process Management contains a Process definition Editor where complete culture processes are defined. This includes controlling of labware addition,

labware removal and labware storage as well as multiple SAMI methods, resource planning and long term incubation times.

The Process management Calendar tool supports planning and running of pre-defined culture processes and nesting of processes. It observes on storage capacities, time schedule collisions etc. In runtime it interacts with the operator supporting for labware changes, method running etc.

In the background SAMI Processes collect data from multiple SAMI runs that are related to each other in one culture process. In addition it prepares and configures data reports.

4.7.2.5 Multi Processes in Automated Runs

Figure 82 illustrates a typical cell culture run with cell seeding, incubation, harvesting, cell count, cell density normalisation and seeding into assay plates. All levels of control software are active in such a process. The diagram shows the activities of SAMI Process Management Software, SAMI EX, Biomek Software and Device specific software. Due to clearness issues the diagram contains only major steps. Sub steps and labware transport steps are left out in the diagram. What looks somewhat complicated in the diagram is quite easy in practice. The operator does only interact with the Process Management Software calendar module and with SAMI Runtime. The other levels are controlled automatically through SILAS. Only for method development highly skilled staff needs to modify integration module software like ViCell software and Biomek software, SAMI EX Method Editor, as well as SAMI Process Management Process Definition editor.

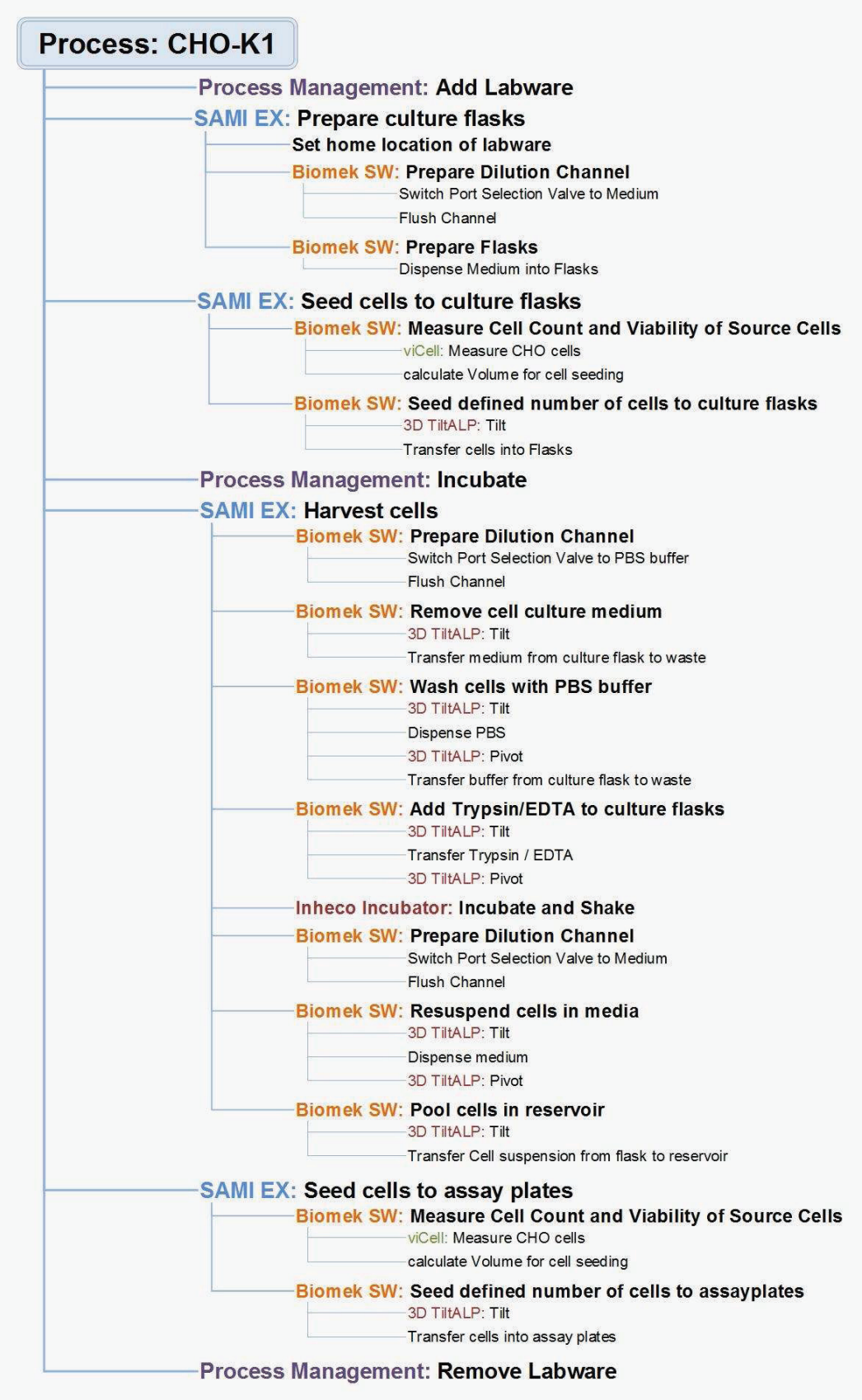


Figure 82 Cell culture process as performed on automated cell culture system

4.7.2.5.1 Cell Pooling and Pipetting of Sedimented Cells

In cell culture processes cell often are pooled into one reservoir. That means, all harvested cells of the same culture approach and of the same cell line from several cell culture flasks are put together in one reservoir. This ensures an equal cell count per volume and an equal viability for all labware cells are seeded to after passage. So in this way, cells remain for some time in a reservoir because of time consumption of harvesting cells from multiple cell culture flasks and, on the other side, because of time consumption of seeding cells into assay plates or seeding cells into culture flasks for further cultivation. Within this time cells in solution tend to sediment on the bottom of the reservoir. Solutions to prevent cells from sedimentation and attachment to the bottom of reservoirs could be stirring or shaking of the reservoir. In order to keep the process simple and because of cell stress issues when kept in continuous shaking or stirring there was evaluated a mix step in the reservoir performed by pipetting tips prior to cell aspiration for further processes.

Here the capabilities of the Biomek software package were used, to design custom pipetting templates. This includes x-y-z- moving mix functions as well as repeated mixing etc. Diagram in Figure 83 shows results of different pipetting techniques to resuspend sedimented cells for seeding from a static quarter reservoir to assay plates or cell culture plates. The results are displayed in relation to manual resuspension with a cell count of 100%. Cells were resuspended by prior custom mix steps of the liquid handler and then samples of the cell suspension were transferred to the ViCell XR and cell count was measured. Clearly to be seen the number of resuspended cells vary from 23% cells to 97% cells compared to manual resuspension. This example shows that detailed and diligent method development is essential when transforming manual laboratory processes to complex automation.

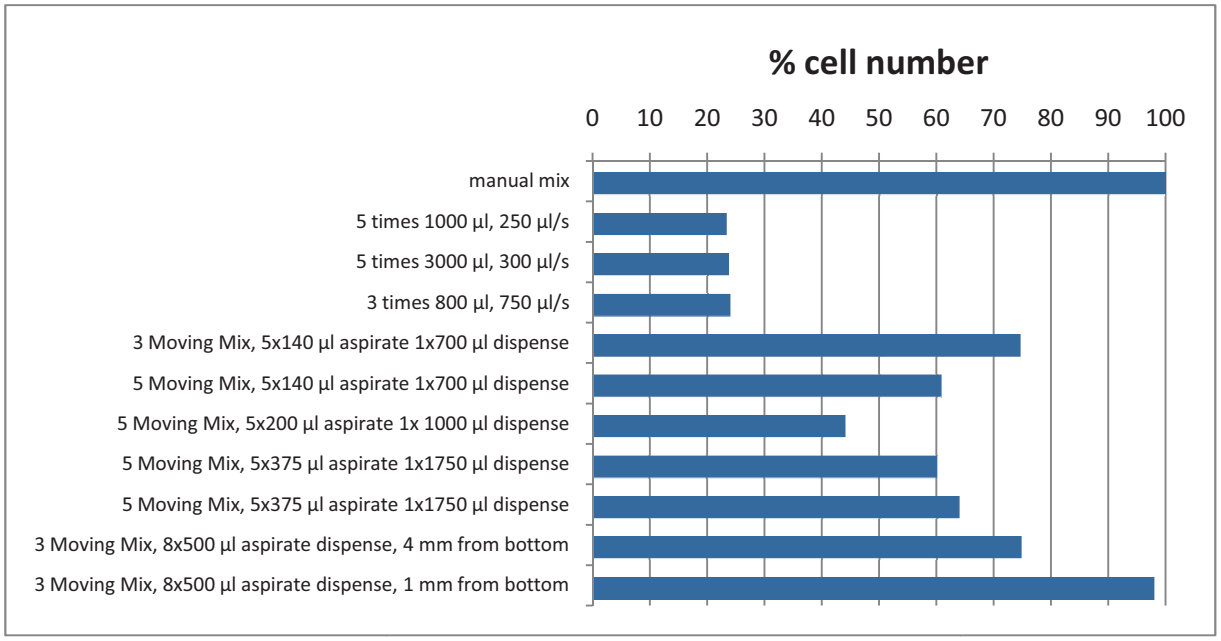


Figure 83 cell reservoir pipetting tests to prevent sedimentation

4.7.2.6 Cell Culture on Cell Handling Workstation

To evaluate the feasibility of continuous automated cell culture on the developed automation system several modular methods for cultivation of different adherent growing cell lines were developed and tested in multiple automated cell culture approaches. All methods were developed beginning from the technology of manual adherent cell culture transforming into automated processes.

4.7.2.6.1.1 SAMI Methods

Figure 84 and following show examples of SAMI EX methods for preparation of labware, harvesting of cells and cell count / normalization. The combination of different software platforms was already illustrated in 0.

The preparation consists of following steps (Figure 84):

- culture flask transport from the incubator to the liquid handler deck

- if first family processed, change liquid dispense channel to Medium and flush channel
- add medium to cell culture flask
- transport labware to incubator

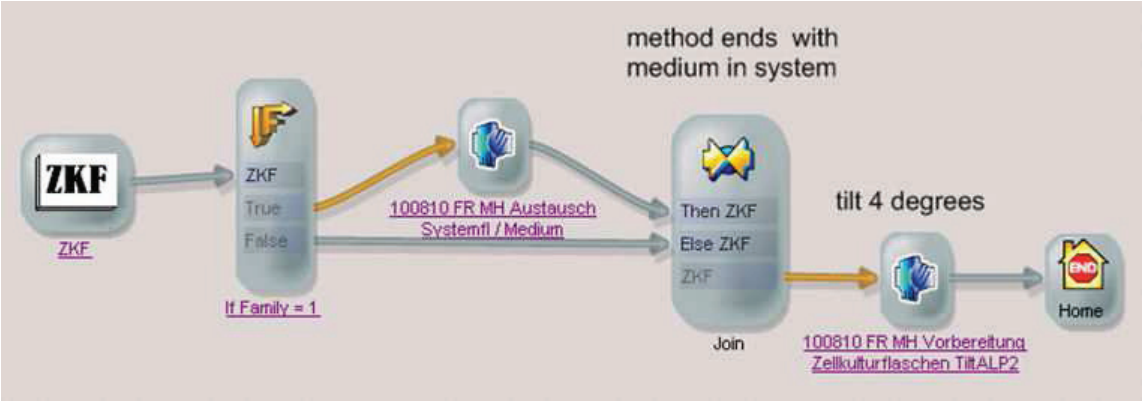


Figure 84 SAMI EX method for preparation of cell culture flasks

The cell harvesting method, shown in Figure 85, contains several Biomek steps, resource pools and device actions.

- Robotic transport of confluent grown cell culture flask from incubator to liquid handler deck, 3D Tilting device
- change liquid dispense channel to PBS buffer
- remove medium from tilted cell culture flask to waste
- Washing of cell monolayer with PBS buffer
- Removal of PBS buffer
- Addition of Enzyme (Trypsin / EDTA) to cells
- transport cell culture flask to on-deck incubator
- shaking incubation for enzymatic cell detachment, 37°C
- change liquid dispense channel to Medium
- transport cell culture flask to 3D Tilting device
- Stop enzymatic reaction, resuspend cells in Medium
- Transfer cells from culture flask to cell pooling reservoir
- Transport culture flask to labware trash

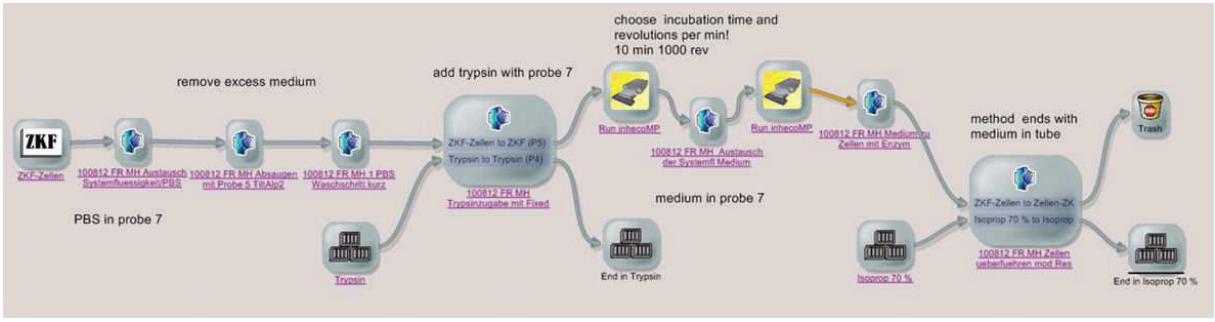


Figure 85 SAMI EX method for Cell harvesting

The run time calculation functions, the support of variables and the run time rescheduling option of SAMI EX and Biomek software allows for seeding defined cell numbers into assay plates or into new culture flasks. Therefore a method was programmed using live cell count data from the ViCell. The cell counts are calculated to pipetting volumes for the destination labware. Rescheduling nodes apply new pipetting volumes for seeding steps. Figure 86 shows the SAMI method for cell count and normalization of pipetting. It contains following steps:

- culture flask transport from the incubator to the liquid handler deck
- resuspend cell solution in reservoir by custom moving mix
- if first family processed transfer sample of cell suspension to ViCell XR
- measure cell count and viability
- broadcast cell count to Biomek software for normalization
- reschedule run with run time cell count data for pipetting volumes
- transfer cell suspension to new labware
- transport labware to incubator

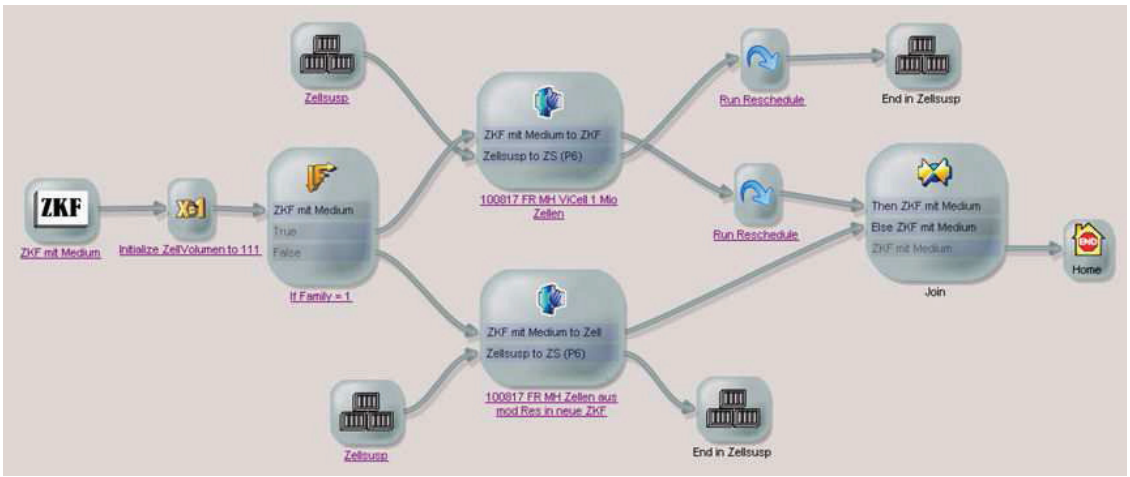


Figure 86 SAMI EX method for cell count and normalization

The ViCell measurement result is put into a dataset that is valid through the runtime of the whole SAMI EX method. From the dataset the pipetting volume for transfers of cell suspension is calculated and broadcasted to the volume parameter settings of the liquid transfer step of the Biomek method (Refer Figure 87)

Figure 87 Biomek Method for normalization step

4.7.2.6.2 Optimization and Results of Automated Culture Experiments

Within a time period of one year multiple automated cell culture experiments were performed with the automated cell culture system. Therefore four different adherent growing cell lines were used:

- HeLa Human cervical cancer cell line
- HEK 293 Human Embryonic Kidney 293 cells
- TIS-10 Stably transfected HeLa cell line with Tetracyclin
- CHO-K1 Subclone of CHO cell line (Chinese Hamster Ovary)

After a series of experiments from time to time microbiologic contaminations were noticed when working without antibiotic pharmaceuticals in medium. To maintain sterile conditions a cleaning and disinfection routine was embedded were all liquid channels of the system were flushed with 4% Korsolex solution for device disinfection. In parallel surfaces of the system inside the laminar flow hood were cleaned with 70% Isopropanol. These approaches were sufficient in order to maintain sterile conditions inside the system.

While performing these experiments method steps were optimized and hardware changes were made to establish optimal condition for cell culture and cell yield. Pooled cell resuspension was already mentioned in 4.7.2.5.1. Further modified parameters were:

- Cell culture media volume
- Harvesting method
    - Tests with Trypsin vs. Accutase
    - Enzyme volume
    - Harvesting incubation
- 3D Tilting ALP – tilting angle span extension
- 3D TiltALP – pivoting angle / speed optimization
- Pipetting speed of cell suspensions
- Etc.

Table 10 shows the results of one experiment where 4 cell lines were cultured in the automated cell culture system and, in parallel cells were cultured in manual procedure in a laboratory with clean bench and manual incubator. In this experiment the cell growth in the automated processes was higher than in compared manual culture, in AutoFlasks as well as in T75 flasks. This as a result of a single experiment and could not reproduced in all approaches.

cell line	culture flask	measured in	cell count x10^6 c/ml	cells seeded x10^6	incubation time /days	media volume /ml	multiplication per day
HeLa	Autoflask	Nucleocounter	1,33	1	3	6	2,33
		Vi-Cell	1,386	1	3	6	2,44
	Sarstedt T75	Nucleocounter	1,33	1	3	6	2,33
		Vi-Cell	1,426	1	3	6	2,52
	Autoflask automated		**2,0212**	**1**	**3**	**6**	**3,71**
293 HEK	Autoflask	Nucleocounter	3,963	1	3	6	7,59
		Vi-Cell	3,698	1	3	6	7,06
	Sarstedt T75	Nucleocounter	3,459	1	3	6	6,58
		Vi-Cell	3,44	1	3	6	6,55
	Autoflask automated		**4,586**	**1**	**3**	**6**	**8,84**
TIS-10	Autoflask	Nucleocounter	1,71	1	3	6	3,09
		Vi-Cell	1,642	1	3	6	2,95
	Sarstedt T75	Nucleocounter	1,99	1	3	6	3,65
		Vi-Cell	1,862	1	3	6	3,39
	Autoflask automated		**2,088**	**1**	**3**	**6**	**3,84**
CHO-K1	Autoflask	Nucleocounter	3,42	0,5	3	6	6,67
		Vi-Cell	4,162	0,5	3	6	8,16
	Sarstedt T75	Nucleocounter	3,231	0,5	3	6	6,30
		Vi-Cell	4,708	0,5	3	6	9,25
	Autoflask automated		**4,38**	**0,5**	**3**	**6**	**8,59**

Table 10 Example of cell culture experiment, automation vs. manual culture

Diagram in Figure 88 displays the growth rates of experiment shown in Table 10 for all in automation tested cell lines. For a more significant statement further approaches including multiple culture experiments are to come in separate studies.

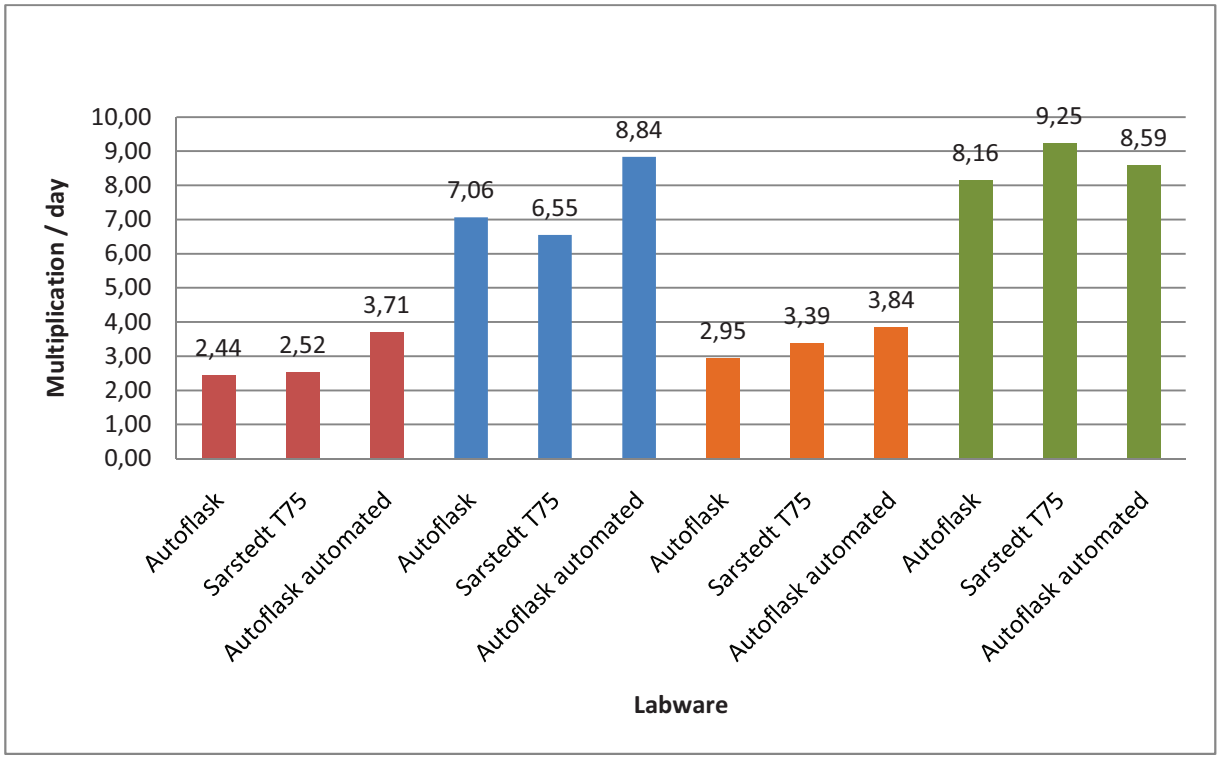


Figure 88 Growth rate automated cell culture vs. manual cell culture

5 Conclusion and Results

The development of an automated cell culture system is an interdisciplinary undertaking including complex tasks from automation engineering, electrical engineering, mechanical engineering, software developments as well as nature sciences like cell biology. The challenge in laboratory automation projects is to accomplish the challenges of reliable and stable automated operation together with biologic processes adapted to procedures on automated systems. Those approaches require broad engineering skills combined with understanding of cell biology processes. On the other hand substantiated cell biology knowledge is required with understanding of technical processes and automation is necessary in such a multi-disciplined topic. The infrastructure and composition of scientific and engineering staff at the University of Rostock, College of Computer Science and Electrical Engineering, Institute for Automation and the Center for Life Science Automation facilitates this challenge in a positive manner.

As result of this approach a working prototype of a flexible automated cell culture system is present that functions as an integrated system. In addition the system design automated methods were programmed and evaluated in initial experiments by culturing of different adherent growing cell lines.

5.1 Automation Line

Resulting from the system concept an integrated system of components available on the market combined with proprietary new developed devices for the specific cell culture application was designed and assembled including following properties:

- System for cell culture in micro titer plates and culture flasks in micro plate format
- Automated CO_2 incubator
- Pipetting platform for seeding, feeding, harvesting etc.
- Integrated measurement of cell concentration and cell viability
- Transport robot as central system integrator

- Suited for research environment – high grade of flexibility
- small footprint, mobile option
- Universal functionality by using open hardware and software platforms
- Complete data tracking of process parameters and variables and herewith potential for nested processes operation
- Intuitively controllable method editor
- Process planning and process editing using process management software

By finishing the development a stable working prototype meeting the requirements of automated cell handling processes and automated cell culture is present. The development included system design components from different areas of engineering as well as a priori cell biology knowledge and requirement specifications. The developed system is intuitively controllable by laboratory staff without highly technical background.

5.2 Application

With focus on the goal to develop a functioning automated cell culture process in addition to systems design and assembly automated processes were set up by transferring manual procedures to automated methods. For evaluation of these methods initial experiments with automation friendly cell culture labware and with different adherent growing cell lines were performed. As the result of these experiments it can be stated, that cells grow with satisfying results in the automated cell culture system by using automated methods. Cell growth behaviour is comparable to established manual adherent cell culture methods. Differences in cell growth rates are caused by differences in labware design.

The cell culture survey showed that all software components in different layers, supplied modules and customized developed integrations work stably together. Although the complexity of processes to run, the software package is operable intuitively. On the one hand the package is to be operated by laboratory staff with basic knowledge; on the other hand trained operators can modify the

processes down to the machine level guaranteeing usage of the full capabilities of the components of the automation line.

Due to the complex range of this project ‘System for Automated Cell Culture’ the application experiments were reduced to initial cell culture trials. Further attempts in routine culture and on further options are to be made in separate investigations.

6 Outlook

The System for Automated Cell Culture is ready to work with adherent cells today. Nevertheless there are some surveys to be performed to make the system ready for routine work.

6.1 Continuous Culture

In the initial cell culture experiments repeatedly cell lines were cultured on the automated systems. For validation of long term operation the process of continuously passaged cell lines are to be evaluated. In the focus of a long term continuous culture survey there are following aspects:

- Continuous cell proliferation and viability over several passages
- Maintenance of sterility and continuous growth conditions
- Development of standard system maintenance steps (cleaning, flushing, liquid media exchange)
- Integration of system maintenance steps, like tubing disinfection and cleaning, into automated cell culture processes

The automated cell culture system, as it is today, is ready for those cell culture surveys. It is planned to start those experiments in the near future to show the feasibility of long term continuous culture.

6.2 Parallel Culture

One demand for cell culture in research and discovery is to produce different cell lines in series and, more challenging for an automated cell culture system, in parallel or time shifted in parallel. From the device design point of view the system supports parallel cell culture with different cell lines. Figure 89 shows a possible scenario with culture of different adherent growing monolayer cell cultures. The most important aspect when handling different cell lines at a time in one cell culture system is prevention from cross contamination with cells. The addition of low numbers ore only single cells to another cell line causes pollution

of the cell line and continuous amplification and mixture of cells of different type in one line.

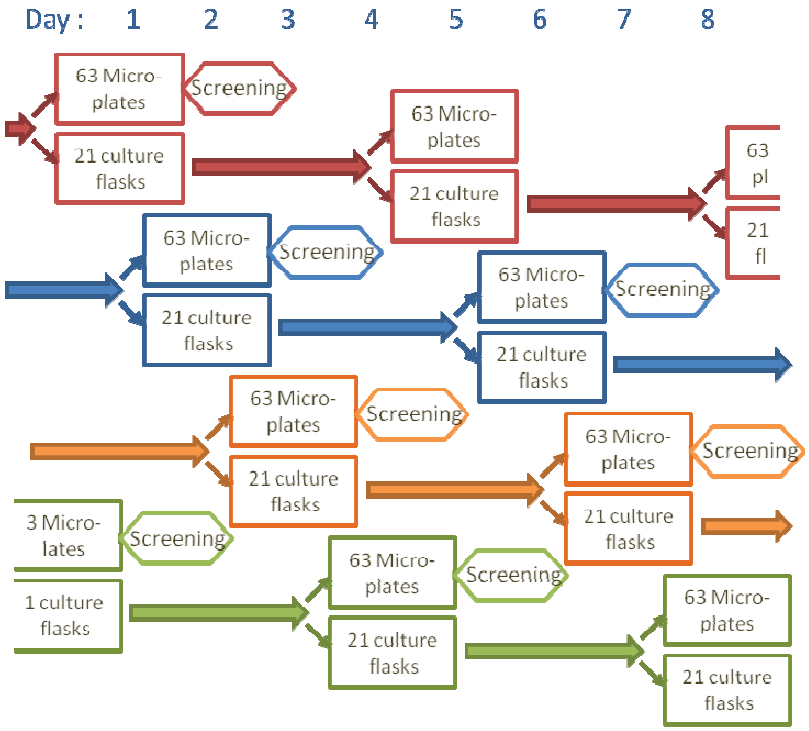


Figure 89 Parallel culture scenario

Technologies to prevent from cell cross contaminations are:

- Working with disposable labware and disposable pipetting tips
- Tip washing
- Fixed tip and cell liquid path flushing with disinfection solution
- Continuous quality control of cell lines.

An applicable technology to evaluate parallel cell cultures regarding cross contamination could be used as follows:

In parallel to a standard cell line in culture a cell line that stably expresses a fluorescence marker could be cultured. In case of contamination of the first cell line with fluorescent cells these cell can be found easily by using a fluorescence microscope. This method can be varied by changing location of the fluorescent cells in the all over process.

6.3 Additional Functionality

Because of the flexibility and modularity of the developed automated cell culture system there is a wide range of options to expand the functionality of the cell handling system in terms of capacity and integration of additional devices. There is a wide range of measuring devices like optical readers, imagers etc. Following some examples should be mentioned that could enlarge an automated cell handling system to a wider scope of function.

6.3.1 3D Culture

The 3D cell culture on magnetic microcarriers as already mentioned in chapter 2.5 is a promising technology of culturing cells. As soon as available, a liquid handler integration version of the current 'Biolevitator™' benchtop device, would broaden the cell handling system by 3D GEM™- Global Eucarotic Microcarrier - culture. By integrating this device the cell yield could be enhanced for cell lines growing on GEMs and adherent culture in cell culture flasks and 3D culture on microcarriers in tubes could integrated in one device.

6.3.2 Integration of Confluence Measurement and Optical Readers

One problem in the automated cell culture process is that in the current configuration the cell density can only be measured in cell suspension. In manual culture the lab assistants often check the cell confluence by checking the cell monolayer on a microscope. Currently available microscopes for automation are quite expensive and the high price doesn't justify the only use for confluence measurement inside the automated cell culture system. A current development of the reader branch of Beckman Coulter, Inc. shows a promising alternative. The Paradigm multimode reader works with optical modules for different measurement modes. A new, currently in development being, module will

broaden the application spectrum of the paradigm reader by a CCD camera module, that will be capable to measure confluence. So by integration of the paradigm the automated cell handling system could be added by the cell confluence measurement in cell culture flasks. In addition the integrated paradigm reader could transform the cell culture system to a cell culture and screening system.

6.3.3 More Detailed Cell Analysis

For more specific investigations in cells integrated into the system cell culture there are options to integrate higher resolution cell analyzers. The Cellavista system from Innovatis is a very compact image based device combining brightfield and fluorescence imaging that is widely used in cellular research, drug discovery and cell line development. In addition, the device is ready for automation integration.

Summing it up, a wide range of additional devices can be integrated, transforming the automated cell culture system from a cell-culture-only-device to a cell culture and discovery device.

Appendix

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Cell Culture System – Configuration

Component	Sub Component	Remarks
Biomek NX workstation	Biomek NX^{P} Span8	Liquid handling robot
	4x 1ml Syringe Pumps	Disposable Tips 20µl to 1000µl
	4x 5ml Syringe Pumps	Fixed Tips 100µl, 3 Tips used with Syringe pumps
	Peristaltic Pump channel	Fixed Tip 100µl, for large volume dispensing and large volume transfers
	Biomek Device Controller	For wash station pump control, for Tilting ALP control, for Indicator device control
	Biomek Span 8 Wash station	Tip washing, includes peristaltic pump
	2x 3D Tilting Device	For AutoFlask operation
	In Line media warming heat exchanger	Includes recirculating thermostat
	Tilting device	For tilted microplate operation
	Fly by barcode reader	For microplate and cell culture flask barcode identification
	Accu frame	For deck framing
	Self refilling reservoirs	Liquid media reservoirs
	H+P Shaker	For Labware shaking
	Microplate position ALPs	On deck labware holder
	Span 8 Thrash ALP	For tips and labware trash
	Automation controller	Windows operated pc running all software modules
	2x Inheco Incubator MP	For short term on deck incubation
	Port selection valve	Media selection for high volume peristaltic channel
ViCELL XR	Analyzer including transfer line from Biomek	Cell count, viability, further parameters
Plate lift		Integrated microplate incubator into liquid handling workstation
Cytomat 60001 incubator	Automated incubator, includes plate shuttle	For cell growth incubation

Theses

1. Automation is established in Life Sciences and is used in a continuous growing number of fields.

2. Because of the high number of different applications and the various demands of life science applications standard automation technology is not always suited for life science processes without customization.

3. Effort for finding new drugs, new active compounds or new explorations in the field of laboratory automation is multiples higher compared to the 1980ies.

4. Life science discovery continuously reduces sample volume and compound volumes in addition to a continuously paralleled sample handling, readout and data analysis.

5. SBS micro titer plate standardization has provided a unique and widely used format in life sciences and life science automation.

6. A majority of life science automation focuses on micro titer plate footprint labware.

7. Automation helps successfully managing the increased effort finding new targets in life sciences.

8. In research and discovery environments a high grade of flexibility is required to laboratory automation in contrast to standard industrial automation.

9. An open systems architecture and modular control software supports flexibility in laboratory automation systems.

10. Standardized communication interfaces of laboratory devices for automation would increase ease of systems flexibility.

11. In automation projects with biological processes application specific demands play an important role in addition to technical parameters.

12. Establishing automated processes in laboratory automation from manual procedures requires comprehensive application development work.

13. Different cell lines growing in vitro need individual treatment and different condition to grow optimal.

14. In life science automation process development interfaces have to provide functionalities to adapt applications down to the machine level for exact biologic process reproduction. At the same time operating interfaces have to be handled easily and intuitively.

15. Schedule optimization and dynamic rescheduling helps rebuild manual procedures into automation and guarantees for time optimized system running.

16. Cells cultured in the automated cell handling system developed in this dissertation proliferate comparably to cells cultured in manual culture.

Conclusion

Scope of this Dissertation Thesis is the development of an automated cell culture system for adherent growing cells including fields of automation engineering, electrical engineering, mechanical engineering, software development as well as nature sciences like cell biology. As result of this approach a working prototype of a flexible automated cell culture system is present that functions as an integrated system. In addition the system design automated methods were programmed and evaluated in initial experiments by culturing of different adherent growing cell lines. The developed system is intuitively controllable by laboratory staff without highly technical background. As the result of cell culture experiments it can be stated that cells grow with satisfying results in the automated cell culture system by using automated methods. The cell culture survey showed that all software components in different layers, supplied modules and customized developed integrations work stably together.

Zusammenfassung

Inhalt dieser Arbeit ist die Entwicklung eines Systems zur automatisierten Kultivierung von adhärent wachsenden tierischen und humanen Zellen. Die Thematik beinhaltet Aspekte aus den Feldern der Automatisierungstechnik, des Elektroingenieurwesens, des Maschinenbaus und der Konstruktion sowie Softwaretechnologie und Naturwissenschaften, wie Zell- und Molekularbiologie. Als Ergebnis dieser Arbeit liegt ein funktionsfähiges Laborautomationssystem vor, das als kompaktes voll integriertes System seine Funktion in Untersuchungen mit diversen Zelllinien gezeigt hat. Alle Komponenten des Systems sind vollständig integriert und arbeiten stabil und zuverlässig zusammen. Das System ist intuitiv bedienbar. Als Ergebnis von Zellkultur Experimenten kann die Aussage getroffen werden, dass mit das vorliegende Funktionsmuster geeignet ist, adhärent wachsende Zelllinien automatisiert zu kultivieren.

List of Publications

Presentations on Scientific Conferences

Junginger, S.; Thurow, K.; Diener, A.: Automated Cell Culturing For High Throughput Applications, MipTec Conference, Basel, Switzerland, 2009

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