Report from Dagstuhl Seminar 15352

Design of Microfluidic Biochips: Connecting Algorithms and Foundations of Chip Design to Biochemistry and the Life Sciences

Edited by

Krishnendu Chakrabarty[1], Tsung-Yi Ho[2], and Robert Wille[3]

1 **Duke University – Durham, US,** krish@ee.duke.edu
2 **National Tsing-Hua University – Hsinchu, TW,** tyho@cs.nthu.edu.tw
3 **University of Bremen/DFKI, DE, and Johannes Kepler University Linz, AT,** robert.wille@jku.at

Abstract

Advances in microfluidic technologies have led to the emergence of biochip devices for automating laboratory procedures in biochemistry and molecular biology. Corresponding systems are revolutionizing a diverse range of applications, e.g. air quality studies, point-of-care clinical diagnostics, drug discovery, and DNA sequencing – with an increasing market. However, this continued growth depends on advances in chip integration and design-automation tools. Thus, there is a need to deliver the same level of *Computer-Aided Design* (CAD) support to the biochip designer that the semiconductor industry now takes for granted. The goal of the seminar was to bring together experts in order to present and to develop new ideas and concepts for design automation algorithms and tools for microfluidic biochips. This report documents the program and the outcomes of this endeavor.

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1 Executive Summary

Krishnendu Chakrabarty
Tsung-Yi Ho
Robert Wille

Advances in microfluidic technologies have led to the emergence of biochip devices for automating laboratory procedures in biochemistry and molecular biology. These devices enable the precise control of nanoliter-scale biochemical samples and reagents. Therefore, *Integrated Circuit* (IC) technology can be used to transport a "chemical payload" in the form of micro- or nano-fluidic carriers such as droplets. As a result, non-traditional biomedical applications and markets (e.g., high-throughput DNA sequencing, portable and point-of-care clinical diagnostics, protein crystallization for drug discovery), and fundamentally new uses are opening up for ICs and systems. This represents a More than Moore-approach.

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Miniaturized and low-cost biochip systems are revolutionizing a diverse range of applications, e.g., air quality studies, point-of-care clinical diagnostics, drug discovery, and DNA sequencing. Frost & Sullivan recently predicted a 13.5% Compound Annual Growth Rate for the US biochip ("lab-on-chip") market during 2008-2015, and the market size for lab-on-chip alone (not including microarrays, biosensors, and microreactors) is expected to be over $1.6 billion in 2015. Similar growth is anticipated in other parts of the world, especially Europe and Japan. On a broader scale, the annual US market alone for in vitro diagnostics is as high as $10 billion and similar figures have been estimated for the drug discovery market. For clinical diagnostics, it has been predicted that we will soon see 15 billion diagnostic tests/year worldwide.

However, continued growth (and larger revenues resulting from technology adoption by pharmaceutical and healthcare companies) depends on advances in chip integration and design-automation tools. Thus, there is a need to deliver the same level of *Computer-Aided Design* (CAD) support to the biochip designer that the semiconductor industry now takes for granted. In particular, these CAD tools will adopt computational intelligence for the optimization of biochip designs. Also, the design of efficient CAD algorithms for implementing biochemistry protocols to ensure that biochips are as versatile as the macro-labs that they are intended to replace. This is therefore an opportune time for the software and semiconductor industry and circuit/system designers to make an impact in this emerging field.

Recent years have therefore seen growing interest in design methods and design-automation tools for the digital microfluidic platform, with special issues of *IEEE Transactions on CAD and IEEE Design & Test of Computers*, special sessions at *DAC*, *ISPD*, *ASPDAC*, and *ICCAD*, and workshops/tutorials at *ISCAS*, *ICCAD*, *SOCC*, and *DATE*. A number of CAD research groups worldwide (e.g., Duke University; Carnegie Mellon University; University of Texas at Austin; Rensselaer Polytechnic University; University of California at Riverside; University of Washington; Technical University of Denmark; Technische Universität München; University of Bremen; National Tsing Hua University; National Chiao Tung University, National Taiwan University; Tsinghua University; Indian Statistical Institute; Ritsumeikan University; Nanyang Technological University; Johannes Kepler University Linz) have initiated research projects on CAD for microfluidic biochips.

The goal of the seminar was to bring together experts in order to present and to develop new ideas and concepts for the design automation algorithms and tools for microfluidic biochips. Areas ranging from architecture, synthesis, optimization, verification, testing, and beyond have been covered. Topics which have been discussed included besides others:

- Architectural synthesis
- Behavior-level synthesis
- Cooling for integrated circuits
- Cross-contamination removal
- Cyberphysical integration
- Device modeling
- Drug-delivery biochips
- Fault modeling, testing, and protocol verification
- Light-actuated biochips
- Numerical simulation
- On-chip sensors
- Paper-based microfluidics
- Particle microfluidics
- Physical design

- Pin-constrained design
- Sample preparation

As results we received a better understanding of the respective areas, new impulses for further research directions, and ideas for areas that will heavily influence research in the domain of design automation on microfluidic biochips within the next years. The seminar facilitated greater interdisciplinary interactions between chip designers, bioengineers, biochemists, and theoretical computer scientists.

The high-quality presentations and lively discussions have been ensured by carefully selected experts who participated at the seminar. All of them have established for themselves a stellar reputation in the respective domains. While researchers working on design automation and optimization of microfluidic biochips build the majority of the participants, also some experts from surrounding research areas attended. For example, researchers working on emerging architectures and applications of microfluidic biochips provided the needed insight for the discussions about the practical problem formulation for commercialized product. Computer scientists with a focus on computer-aided design enriched the discussions about the top-down design methodology and optimization of large-scale components like mixers and routing channels. Therewith, the unique concept of Dagstuhl seminars was applied in order to bring researchers from different domains together so that the interdisciplinary topics could have been discussed and progress in these areas has been made.

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3 Overview of Talks

3.1 Hands-on Experiences on Actual Biochips

Mirela Alistar (Copenhagen)

Joint work of Alistar, Mirela; Trojok, Ruediger
URL http://www.bioflux.eu

It is not a secret that in biology laboratories hours of manual work are considered a compulsory part of the experiment. During a day of work, lab researchers have to pipette the right amounts of fluids in tubes, carry them from one machine to another, program and handle each machine individually, label and document carefully each step and then convert the results to data and analyze it. For a simple routine experiment, each of the mentioned tasks is performed at least 10 times. Past decade, a big effort has been done to produce machines (e.g., pipetting robots) that would automate some of the tasks in the lab. However, these machines were developed under the industrial mindset to maximize the throughput of a single task. Thus, these machines are of large size, task-specific, difficult to use (they usually come with dedicated drivers and software) and most importantly, extremely expensive.

Mirela Alistar and Ruediger Trojok are leading the BioFlux project, with the purpose to advance from automated biology to digital biology. In our vision, a digital lab should be: (1) fully integrated,running all the tasks on the same machine; (2) easy to use, with a web-based software for biological design of new experiments and hardware control; (3) general-purpose, allowing easy reconfiguration and design of new experiments; (4) cheap, offering open-source and do-it-yourself assembly kits.

During this workshop, we presented the common laboratory procedures for running synthetic biology applications. We showed a commercial DNA extraction kit (from Evogen Inc.) and presented the manual steps (pipetting, incubation, centrifuging) that the biologists have to take to extract the DNA.

Next, we emphasized that contamination is a significant issue by doing a microbiology experiment with one of the members of the seminary. We had talked about bacteria media, culture and growth. The participant was instructed to pour agar plates with LB-based media. After the plates set down, the participant went to wash his hands and then imprinted the plates with his fingers. The bacterial growth was monitored during the following days and all the seminar participants were updated on the progress.

The next part of the workshop consisted on a step-by-step instruction set on how to build your own biochip. We demonstrated using two versions of biochips: one that is manually controlled and one that is automatically controlled. The manual biochip was developed for a thorough study of the interaction between the fluids and the electrical potential. The automated biochip was controlled though an Arduino and can be programmed by any user due to the user-friendly Arduino interface. The seminary participants had all a chance to take photos, ask questions and test the biochips.

The last part of the workshop was dedicated to discussions. Some participants were interested in developing such low-cost DIY biochips in their groups for research purposes such as testing their own algorithms. Some discussions arose about funding possibilities, reliability of the DIY biochips, scalability of the products, end-users and applications.

Seminary participants from China, Korea, Taiwan and India expressed their desire to have such an workshop organized locally at their labs. Hence, the workshop resulted in follow-up discussions on grant applications.

3.2 Research on Biochip Architectures and EDA: Hype, Myths, and Reality (Reflections and Predictions)

Krishnendu Chakrabarty (Duke University – Durham, US)

Over the past fifteen years, significant research advances on design automation for microfluidic biochips have been reported. Early research was motivated by the considerable hype generated by technology demonstrations and the promise of a paradigm shift in molecular biology. While a sizeable research community has emerged worldwide and design automation for microfluidic biochips has now become an important component of major conferences (and the portfolio of the top journals) in the area, skepticism continues to be voiced about the practicality of design automation solutions and the relevance of this research to the broader community of biochip users. In this talk, I presented a retrospection of the early hype and some of the myths that have been exposed. A snap poll of the audience was taken with respect to a series of controversial questions. I also highlighted specific problems that design automation must tackle and led a discussion on how our community can engage in a more meaningful way with life science researchers. The discussion was lively and highly interactive. At the end, we collectively identified strategies for advancing from manipulating small volumes of liquid on a chip to accomplishing realistic biochemistry on these chips.

3.3 On-chip Logic Using Pneumatic Valves

William H. Grover (University of California at Riverside, US)

Microfluidic chips are capable of performing a wide variety of different applications faster, cheaper, and better than conventional lab-scale tools. However, the spread of microfluidic technologies is slowed by the amount of off-chip hardware required to operate microfluidic chips. This off-chip hardware is often far more expensive, bulky, and power-hungry than the chip itself, a fact that makes microfluidic instruments less suitable for use in resource-limited or point-of-care contexts. Here I describe how off-chip hardware can be reduced or eliminated by integrating the control of a microfluidic device onto the chip itself. We accomplish this using *monolithic membrane valves*, pneumatically-actuated microfluidic valves that we originally developed for controlling fluid in microfluidic chips. After finding that these valves can control air flow as well (and thereby control each other), we developed an assortment of valve-based logic gates and circuits. These principles of pneumatic logic are powerful enough to control even the most complex microfluidic chips using little or no off-chip hardware. Designing these valve-based logic circuits is not trivial, but automating their design could be a fertile area of inquiry for researchers working on microfluidic design automation.

3.4 Integrated Fluidic-Chip Co-Design Methodology for Digital Microfluidic Biochips

Tsung-Yi Ho (National Tsing-Hua University – Hsinchu, TW)

Recently, digital microfluidic biochips (DMFBs) have revolutionized many biochemical laboratory procedures and received much attention due to many advantages such as high throughput, automatic control, and low cost. To meet the challenges of increasing design complexity, computer-aided-design (CAD) tools have been involved to build DMFBs efficiently. Current CAD tools generally conduct a two-stage based design flow of fluidic-level synthesis followed by chip-level design to optimize fluidic behaviors and chip architecture separately. Nevertheless, existing fluidic-chip design gap will become even wider with a rapid escalation in the number of assay operations incorporated into a single DMFB. As more and more large-scale assay protocols are delivered in current emerging marketplace, this problem may potentially restrict the effectiveness and feasibility of the entire DMFB realization and thus needs to be solved quickly. In this research, we propose the first fluidic-chip co-design methodology for DMFBs to effectively bridge the fluidic-chip design gap. Our work provides a comprehensive integration throughout fluidic-operation scheduling, chip layout generation, control pin assignment, and wiring solution to achieve higher design performance and feasibility. Experimental results show the effectiveness, robustness, and scalability of our co-design methodology on a set of real-life assay applications.

3.5 Sample Preparation on Microfluidic Biochips

Juinn-Dar Huang (National Chiao-Tung University – Hsinchu, TW)

My recent research direction is about sample preparation in microfluidic biochips. Sample preparation on microfluidic chips actually refers to a set of problems, which can be classified in different perspectives. For example, the optimization goal can be reactant minimization, operation count minimization, waste minimization, and so on. The target microfluidic biochip can be digital (1-to-1 mixing model only) or flow-based (a mixer with N segments, $N > 2$) as well. The target concentration value of product solution can be just single one or multiple at the same time. The number of reactants in a bioassay can be at least two or more. Each different combination of the aforementioned parameters defines a unique sample preparation problem and needs to be properly solved. In the meantime, I am currently working on so-called cyber-physical sample preparation technology, which can dynamically adjust the preparation process based on real-time on-line feedback.

3.6 Using Boolean Satisifability to Design Digital Microfluidic Biochips

Oliver Keszöcze (DFKI – Bremen, DE)

Joint work of Keszöcze, Oliver; Wille, Robert; Chakrabarty, Krishnendu; Drechsler, Rolf

Advances in microfluidic technologies have led to the emergence of Digital Microfluidic Biochips (DMFBs), which are capable of automating laboratory procedures in biochemistry and molecular biology. During the design and use of these devices, droplet routing represents a particularly critical challenge. Here, various design tasks have to be addressed for which, depending on the corresponding scenario, different solutions are available. However, all these developments eventually resulted in a huge variety of different design approaches for routing of DMFBs – many of them addressing a very dedicated routing task only.

In this presentation, we show a comprehensive routing methodology which

1. provides one (generic) solution capable of addressing a variety of different design tasks,
2. employs a "push-button"-scheme that requires no (manual) composition of partial results, and
3. guarantees minimality e.g., with respect to the number of timesteps or the number of required control pins.

The approach is not to find an algorithm that solves every possible routing problem but to formally model biochips and corresponding routing problems and give that to a SMT solver (Z3 in our case) which then, in turn, produces a routing solution. This formal model consists of diverse variables that describe the system's states and constraints on these variables that model how the droplets may move as well as constraints such as the fluidic constraints.

One exemplary constraint is for the actual movement of droplets. Our approach models the movements in a backward manner. The constraints for the presence of a droplet in a specific positions means that the droplet must have been present in the neighborhood of that position in the previous time step.

This routing process then is done in an iterative manner:

1. set T to 0
2. create the model that spans T time steps
3. ask the solver to find a routing solution for that model
4. if no solution is found, increase T by one and go to 2)

Finding a solution in such a manner has two desired properties:

1. the solution is guaranteed to be minimal with respect to the amount of ime steps used in routing
2. the solution is definitely valid in the model.

In the presentation we show how to easily extend the model to consider many different aspects (e.g. fluidic constraints, pin assignment). The good thing of our approach is that there is no need to think of how the newly added problem is to be solved (the only thing to be done is to add a parameter for the amount of pins P in the iterative process described above). The solver does the main work in the background. This works especially well when to separate but interconnected tasks (i.e. droplet routing and pin assignment) are solved at the same time; no propagation of information between two different problems has to be performed by the developer.

3.7 Demo of a Visualization Tool for Digital Microfluidic Biochips

Oliver Keszöcze (DFKI – Bremen, DE)

Joint work of Keszöcze, Oliver; Stoppe, Jannis; Wille, Robert; Drechsler Rolf

There are various challenges in the development of digital microfluidic biochips Design tasks such as synthesis, routing and layouting are complex and currently being investigated by various research institutes in their ongoing endeavours. However, so far there is no tool to easily visualize the results of given approaches, making the development and analysis of approaches for these chips a tedious task.

We present a visualization engine to display a given microfluidic biochip design (e.g. the routings paths for given nets). The visualization is supposed to be easy to use, resulting in a hassle-free environment for designers to work in.

Additionally to displaying static information such as grid layout droplet and dispenser/sink position we support to visualize dynamic information such as droplet and mixer positions as well as cell actuations. To help the developer in the analysis process, the transitions between time steps (i.e. system states) is animated. This greatly helps to understand where a certain droplet game from at any given time step; this is especially helpful when moving many droplets at once. Further more, the tool displays the aggregated information of the droplet positions (i.e. the paths droplets take).

The tool has been implemented in Java using the libgdx library. Java eases the process of deploying the tool as it should run out of the box on all mayor system supporting Java. The libgdx library uses the full power of OpenGL, allowing to

(a) easily animate the system and

(b) smoothly zoom and scroll through the system under inspection.

3.8 Biochips: The Wandering of an EDA Mind (A Case Study)

Bing Li (TU München, DE)

Joint work of Li, Bing; Schlichtmann, Ulf; Ho, Tsung-Yi
Main reference T.-M. Tseng, B. Li, T.-Y. Ho, U. Schlichtmann, "Reliability-aware Synthesis for Flow-based Microfluidic Biochips by Dynamic-device Mapping," in Proc. of the 52nd Annual Design Automation Conf. (DAC'15), Article No. 141, 6 pages, ACM, 2015.
URL http://dx.doi.org/10.1145/2744769.2744899

Microfluidic biochips have revolutionized traditional biochemical diagnoses and experiments significantly by exactly manipulating nanoliter samples and reagents. This miniaturization saves expensive reagents and improves experiment accuracy effectively. With the recent advances in manufacturing technology and integration of biochips, very complex applications can now be executed on such a chip as a whole without human interference. However, the interface between this tremendous engineering advance and applications is still incomplete, and new emerging architectures are enlarging this gap further.

In this presentation, we discuss the challenges in mapping applications to several newly emerged biochip architectures. We first explain a method to improve the reliability of flow-based biochips by assigning devices dynamically on a fully programmable valve array. In a traditional flow-based biochip, valves that drive or pump fluid samples in mixers actuate

10 times more than the other valves that control flow transportation. Since the entire chip fails when any of these valves wears out, this imbalance of actuations affects the lifetime of the chip significantly. To alleviate this problem, we allow valves to change their roles during the execution of an application. In this concept, valves that have been used to pump fluid samples in a mixing operation are used to control flow transportation thereafter. Because the valves along a mixer have different roles in different mixing operations, valve actuations are distributed more evenly. Consequently, the maximum number of valve actuations in executing an application can be reduced effectively without incurring any additional cost.

In addition to reconfigurable valve arrays, we also discuss biochips printed on paper and biochips with capacitors that control electrodes in a row-column refreshing mode. Challenges in adopting paper-based biochips come from the fact that electrodes are printed only on one side of the paper. Therefore, wires providing voltages to electrodes must be routed at the same layer. The other new biochip architecture with control capacitors is based on the thin film transistor (TFT) technology. In such a chip, each pixel on the LCD plane has a capacitor-like cell. To set voltages to some pixels, a row-column write process sweeps all the capacitance cells. To use TFT pixels to manipulate droplets, challenges still remain. The first one is actually from the extremely refined electrodes. To move large droplets, multiple electrodes should be grouped dynamically for operations and transportation. The second challenge is that the voltages to electrodes should be set in the row-column mode instead of independently. It should be guaranteed that the voltage setting process does not affect the droplets on the chip.

By exploring several emerging biochip architectures, we have demonstrated challenges in mapping applications to them. To achieve a wide adoption of these new architectures in industry, a close collaboration between the chip design community and the EDA community is indispensable.

3.9 Microfluidic Large-Scale Integration and its Applications in Life Science

Sebastian J. Maerkl (Ecole Polytechnique Federale de Lausanne (EPFL), CH)

The Dagstuhl seminar #15352 on "Design of Microfluidic Biochips: Connecting Algorithms and Foundations of Chip Design to Biochemistry and the Life Science" brought together a group of scientists with backgrounds in computer science, microengineering / microfluidics, and researchers familiar with biochemistry. The Dagstuhl conference began with seminars to provide background and up to date information on the state of the various research fields represented at the conference. The second day was dominated by short as well as in depth tutorial and discussion sessions. Informal discussions were possible throughout the duration of the meeting.

I contributed an approx. 45 minute seminar providing a short review of multilayer soft lithography [1] and microfluidic large-scale integration [2], as the conference primarily focused on electrowetting based digitial microfluidic devices. This short technical introduction on MLSI was followed by a description of a proof-of-concept programmable valve-based microfluidic device including an explanation of the basic design concept, the technical developments required to achieve sufficiently high chip complexity, and the implementation of

basic fluidic operations such as on-the fly device reprogramming, fluid metering, and mixing [3]. This description was followed by two basic, proof-of-concept biological applications. The first application described the implementation of a standard immunoassay on the platform, which is commonly employed in a plethora of clinical diagnostic assays. The second application showed that the reconfigurable device could be applied to cell manipulations and on-chip culturing using *S. cerevisiae* as the model system.

During the second half of the seminar I described our recent efforts at developing methods and tools for cell-free synthetic biology. We recently developed a microfluidic chemostat device with a parallel architecture [4]. This devices allowed us to run *in vitro* transcription / translation (ITT) reactions at steady-state for up to 30 hours. Previously, such reactions were run in standard batch reaction format in test tubes, which severely limited the usability of ITT reactions for the implementation and characterization of genetic networks. Previously, only genetic cascades had been implemented in ITT reactions, primarily due to these technical restrictions [5]. With our novel microfluidic chemostat arrays we could show that genetic oscillators could be successfully implemented in a cell-free environment. We also showed that a diverse set of native biological regulatory mechanisms could be reconstituted on this platform. We then went on to show that the platform could successfully implement the repressilator, a classic synthetic network, which had been designed and implemented in *E. coli* [6]. We went on to show that we could rapidly characterize biological parts and devices, creating novel 3-node as well as the first 5-node genetic oscillators. To demonstrate that novel genetic networks implemented and optimized *in vitro* could be transferred to a cellular environment, we transferred both our 3-node and 5-node genetic oscillators to *E. coli* and characterized these two networks on the single cell level; proving that transfer is possible. We also discovered that our 3-node genetic oscillators were surprisingly synchronous as opposed to the original repressilator networks. Our current working hypothesis explaining the difference in phenotype of these two genetic oscillators, which share the exact same network architecture, lies in the fact that the molecular concentrations in our newly engineered genetic networks is likely higher than those of the original repressilator, leading to a drastic reduction in noise, which in turn results in synchronized behavior of cells in the same lineage.

Current Problems in Microengineering / Microfluidic Device Design and Implementation

Dagstuhl represented an opportunity to meet the biochip EDA community, with whose work I was only marginally familiar. This fact is probably telling and represents a significant gap between the community of microengineers / chip developers and the community of EDA computer scientist who are working on problems related to biochip design and biochip operation. The fundamental problem facing the biochip community at the moment lies in bridging the gap between developing and working on "toy" problems, which remain of low interest to the microengineering community. A similar challenge exists for the microengineering community who build new microfluidic tools and biologists and chemists for whom these new tools are being developed. It may thus be insightful to briefly describe the current challenges facing the microfluidic / microengineering community, and to describe possible approaches to maximize the impact microengineers can achieve through their work.

The first challenge for microengineers developing new microfluidic devices is to identify well-known shortcomings or limitations of currently existing technologies and to develop a novel approach that leads to a significant improvement in performance relevant for the end-users of the technology. An alternative approach involves identifying an area of biology in which no methods are available, but a clear and obvious need for novel methods exists. Development of microfluidic single cell approaches represents this second approach. A clear

set of biological questions existed, but no technologies exited that could be employed to answer these questions, or the existing methods were insufficient. A third possible strategy to develop methods for biology is to enable an entirely novel approach to conducting biology which does not serve a pre-existing community of biologists, but around which a group of scientists will form because it provides a unique and novel way of approaching a problem in biology (synthetic biology, and now cell-free synthetic biology could be considered such fields). Selecting an appropriate problem to solve unfortunately only represents the first step in the process. Even if one develops the methods and tools, impact will remain limited to the community of microengineers, and will fail to impact the biological community unless the second challenge is addressed as well.

The second challenge represents the difficulty of impacting the intended end-users of the technology (in this case biologists). This problem exists because it has proven extremely difficult to transfer microfluidic technology to biology laboratories. The entrance barrier to the field of microfluidics is sufficiently high to prevent a majority of biological labs from adopting this technology. Some biological communities have made more significant efforts in adopting microfluidic technologies and are actively involved in their development. The microbiology community is probably the community, that has made the most significant efforts in this, probably because it is obvious to this community that a number of central questions in the field of microbiology will only be answerable if microfluidic devices are employed. Most other biological communities have adopted microfluidic technology only if a commercially available system exits that meets their needs. For example, the CellAsic platform is fairly popular with microbiologists, and the Fluidigm single cell analysis devices developed for mammalian cells fill a clear need for current cell biological research. Other fields in which microfluidics is likely going to have significant impact is in personalized medicine and personalized diagnostics, through the development of next generation diagnostics platforms.

There are thus two possible approaches that can be taken if a microfluidic technology is to significantly impact the biological community. The first approach requires that technologies developed in the lab are ultimately commercialized either through start-up companies or through licensing to existing companies. In many instances, significant additional development is required to make novel microfluidic tools and methods sufficiently user friendly to allow commercialization. This area unfortunately represents a difficulty in that it rarely is of interest to an academic lab to conduct such engineering work, and in many instances investors seem to prefer more mature technologies for funding. Technology transfer is thus necessary and of utmost importance in order to maximize impact of microfluidic technologies, but is also extremely difficult.

An alternative to transferring novel technology to the commercial sector so that it becomes accessible and usable by the biology community is to directly conduct biologically relevant experiments with the newly developed technology. Impact in biology can be achieved by supplying biological datasets or novel biological insights in the form of new mechanisms, the discovery of novel molecules, or discovering novel links between existing molecules, which represent the goals classically pursued by biologists. Providing quantitative information on otherwise well known or well characterized molecules can also have considerably impact in biology [7, 8], as precise and comprehensive data can challenge existing biological dogmas derived previously based on low-quality experimental data, limited by technologies available at the time. Finally the development and characterization of synthetic biological systems on all levels is of high interest, and novel technologies are expected to facilitate such developments. The unifying characteristic of these foci is that data or biologically molecules can be easily shared with biological laboratories, and can thus readily impact biological research.

Challenges and Opportunities for Computer Science in Microengineering and Biology

The reason for describing the challenges facing the microengineering / microfluidics community is that the computer science / EDA community currently working on microfluidic devices is facing similar challenges. In order for the EDA community to impact the microengineering / microfluidics community, or the biology community requires that relevant problems are being identified and solved, and that these solutions are immediately accessible and usable by the target communities of researchers. As microfluidic platforms are becoming commercially available, biologists will likely adopt them if they provide a performance advantage over existing approaches. These advantages could be any combination of decreased cost, increased throughput, and automation. In addition, applications of microfluidic devices in the near future will remain task specific. In other words, biologists will conduct a particular task, or workflow, on a microfluidic platform such as molecular cloning, single cell analysis, or biochemical analysis. Furthermore, these tasks will generally follow a fairly well defined protocol and series of steps, with the only difference between experiments being the reagents/cells used on the devices. These requirements can be either fulfilled by valve-based or electrowetting microfluidic devices, but does not necessarily require a completely reprogrammable microfluidic device. The complexity of the needed control software therefore is likely to remain fairly limited in the foreseeable future.

Current opportunities for EDA based design and related approaches derived from computer science at the interface of microengineering / biology include the development of user-friendly control interfaces for electrowetting devices. As these devices become commercially available, better control software is needed that allows biologists to easily program their own routines on these chips. Such control software could provide an easy to use interface to defined fluid handling on the devices, and/or can be supported by more sophisticated protocol optimization algorithms. Similar control problems likely also exists for large, central robotic facilities, in which optimization is a non-trivial task. It might be of interest to contact big pharma companies to assess their needs in this domain. Finally, synthetic biology is currently facing considerable difficulties in developing rational approaches for biological network design. Although these networks still remain fairly simple, even simple networks require computer modeling to assess and optimize their performance. One the one hand, this situation is expected to become much more difficult as network size continues to grow. But, at the same time the underlying computational models and parts characterization will also drastically improve, allowing more accurate predictions to be made. There is also a clear precedence for the need and usefulness of extremely complex and sophisticated networks as found in any naturally occurring organism. It is thus almost inevitable that all biological network design in the present as well as in the near future, should or will be conducted *in silico*.

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3.10 Programming and Physical Design Tools for Flow-based Biochips

Paul Pop (Technical University of Denmark – Lyngby, DK)

Joint work of Pop, Paul; Madsen, Jan; Hassan Minhass, Wajid
URL http://www2.compute.dtu.dk/~paupo/talks/paupo_dagsthul15.v1.pdf

Microfluidic biochips are replacing the conventional biochemical analyzers by integrating all the necessary functions for biochemical analysis using microfluidics. Biochips are used in many application areas, such as, in vitro diagnostics, drug discovery, biotech and ecology. The focus of this special session is on continuous-flow biochips, where the basic building block is a microvalve. By combining these micro valves, more complex units such as mixers, switches, multiplexers can be built, hence the name of the technology, "microfluidic Very Large Scale Integration" (mVLSI). This talk has presented methods and tools for the programming and physical design of mVLSI biochips.

3.11 Algorithms for Automated Sample Preparation using Digital Microfluidic Biochips

Sudip Roy (Indian Institute of Technology – Roorkee, IN)

Main reference S. Roy, B. B. Bhattacharya, S. Ghoshal, K. Chakrabarty, "Theory and analysis of generalized mixing and dilution of biochemical fluids using digital microfluidic biochips," ACM Journal of Emerging Technologies in Computing Systems, Vol. 11, Issue 1, Article No. 2, 33 pages, ACM, 2014.
URL http://dx.doi.org/10.1145/2629578

In the last two decades, an emerging technology of "Lab-on-a-Chips (LOCs)" has been studied by the researchers of interdisciplinary fields to develop microfluidic biochips that can implement wide-range of biochemical laboratory test protocols (a.k.a. bioassays). A marriage of microelectronics and in-vitro diagnostics areas has led to this field of interdisciplinary research around LOCs or microfluidic biochips. In contrast to continuous-flow microfluidic chips, digital microfluidic (DMF) biochips are of a popular kind of microfluidic LOCs that can implement bioassays on an electrode array of a few square centimeters in size by manipulating micro/nano/pico liter volume fluid droplets. The functionality of a DMF biochip includes the following operations: dispensing the desired amount of fluids to the chip from the outside world as droplets, transporting the droplets on-chip to appropriate locations, mixing and splitting of several droplets, executing a well-defined bioassay on-chip, and finally analyzing the results at an on-chip detection site. Recent years have seen a surge in interest in design automation methods for DMF biochips. Along with several synthesis steps of DMF biochips

(like Scheduling, Module Binding, Placement, Droplet Routing, Wire Routing), protocol derivation for automatic sample preparation (dilution & mixing) using DMF biochips.

Our research envisions the algorithmic microfluidics and it expands the computer-aided-design (CAD) research to develop DMF biochips by designing algorithms for automated sample preparation (dilution and mixing) on such chips. Mixing and dilution of fluids are fundamental preprocessing steps in almost all biochemical laboratory protocols. Mixing of two or more fluids with a given ratio is often required as a preprocessing step of many real-life biochemical protocols, e.g., polymerase chain reaction (PCR). Dilution of a biochemical fluid is the special case of mixing, where only two different types of fluids, one of which is a buffer solution, are mixed at a certain ratio corresponding to the desired concentration. The dilution is commonly used in biological studies to create a variety of concentrations of the stock solution by mixing it with its diluents and it is required for sample preparation in many bioassays, e.g., real-time PCR, immunoassays, etc. For high-throughput applications, it is a challenge to determine the sequence of minimum number of mix-split steps for on-chip sample preparation. Furthermore, the production of waste droplets and/or the reactant fluid usage should be minimized. Moreover, design automation tools are necessary for optimizing the layout of the biochips.

In Dagstuhl seminar, we discussed about the basic background of DMF biochips and about several algorithms and CAD techniques for automated and on-chip fluidic sample preparation (dilution and mixing) of biochemical fluids using DMF biochips. We expect that for the betterment of our society, several low-cost, portable, automated biochemical laboratory-on-a- chips will be developed soon. In order to conduct innovative and basic research in developing of DMF biochips, it requires joining hands of experts from multiple disciplines: Computer Science, Electronics, Mechanical, Chemical, Biomedical Engineering, Microfluidics Sensor Technologies, Medical Science, etc.

3.12 Active Digital Microfluidic Paper Chips with Inkjet-printed Patterned Electrodes and their Point-of Care Biomedical Application

Kwanwoo Shin (Sogang University, KO)

Recently, our group has presented a novel paper-based fluidic chip that can enable the full range of fluidic operations by implementing an electric input on paper via an electrowetting technique [1, 2]. This powered paper-based microfluidic chip, which is known as an active paper open chip (APOC), is primarily characterized by discrete drop volumes and is an open-type chip. These active, paper-based, microfluidic chips driven by electrowetting are fabricated using inkjet printing technique and demonstrated for discrete reagent transport and mixing [1]. Instead of using the passive capillary force on the pulp in the paper to actuate a continuous flow of a liquid sample, a single, discrete drop or a group of digital liquid drops are perfectly transported along programmed trajectories. The patterned electrodes, which are designed on a desktop computer, are printed on low-cost paper, such as recycled magazine papers, with conductive CNT ink using an office inkjet printer [2], which should enable true point-of-care production and diagnostic activities. I presented our newly developed active paper open chips and their biomedical application. The solution simplifies the workflow and improves the reaction accuracy tremendously.

References

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3.13 Bioflux Technology Dagstuhl Report

Rüdiger Trojok (KIT – Karlsruher Institut für Technologie, DE)

Digital Biology

Recent advances in Synthetic biology open up new possibilities in healthcare, agriculture, chemicals, materials, energy, and bioremediation. To date this is still a very labor intensive task that requires skilled technicians and scientists. However, manual work is time consuming and wages drive development costs, thereby restricting possibilities for rapid prototyping in synthetic biology. Digital Biology is the computer aided programming of biological assays using digital microfluidic biochip devices based on electrowetting on dielectric technology. Advanced laboratory hardware will make access to biotechnogical procedures much more affordable with easy to replicate 'Do It Yourself' equipment, further also increase automation, replace time consuming labour and increase replicability and standardisation of methods. Thus, Digital Biology allows for wide scale automation of laboratory procedures in synthetic biology by improving efficiency between 1000 to 100000 fold compared to manual laboratory work, for the first time enabling wide scale rapid prototyping for the iterative creation of biological systems. This will allow even small biological laboratories in academia and industry as well as researchers in the developing world to develop synthetic biology products.

Bioflux Technology

To successfully decentralize the Digital Biology technology, we want to develop Bioflux Technology—a platform that will automate the synthetic biology flow with great medical and commercial potential. Bioflux Technology will be a combination of a software suite for biologists to plan experiments. Microfluidic device, electronics hardware to run the experiments and the required wetware (biological reagents) to perform a wide range of standardized bioassays used in synthetic biology. The hardware consists of computer controlled microchips which switch on high DC voltage on a set of electrodes. The electrodes will be printable on superhydrophically coated paper. The layout of the papers is customized to the specific bioassay. The papers can be exchanged, while the hardware setup remains the same thus avoiding contamination issues in the bioassays.Only the program in the computer and the wetware on the paper is actualized for every use case. The main users of the technology are thought to be medical personnel and biologists for field diagnostic and health treatment applications. As soon as we have developed a device that is robust and compact, the use of Bioflux Technology can be extended to a large mass of users, such as farmers (for plant treatment) or regular citizens (for rapid point of care testing). Users will be able to use Biofux Technology to design and test their desired protocols, at low cost (provided by the

small scale of biological material used) and at faster speed (enhanced by microfluidics). Bioflux Technology will be cheap, easy to distribute around the world, usable on-site where the samples are taken and connected to a global database for further analysis of the sampled data.

To render Digital Biology accessible for synthetic biology, all fundamental biological assays used in synthetic biology need to be downscaled in volume and properties to function on a Bioflux platform. This entails protocols for in vitro DNA replication and assembly, protein expression and purification and cell transformation and incubation. The fundamental assays will be integrated into composite protocols applied in synthetic biology, depending on customer needs. Each protocol will be adapted for execution on the Bioflux platform and made controllable by our specially designed software. Protocols can be flexibly created out of fundamental assays in an online user interface with a customer protocol designer. The protocols will then be loaded onto the Operating System of a Bioflux platform. The user of the device then needs to load the for the protocol required wetware input on the chip. After activation, the operating system will execute the protocol and put out the desired wetware to a designed position on the chip. Wetware output could be synthetic assembled genomes, designer proteins, cells or secondary metabolites such as specialty chemicals. Besides, the software can output measurement signals of the conducted reactions, allowing for use in medical diagnostics. The Bioflux team favours open source innovation and a global collaboration with academic and non academic partners to advance the field of Digital Biology together.

Use case: Biostrike

An overuse of the available antibiotics and subsequent evolutionary pressure led to the development of multi-resistant bacteria. By now, the situation is becoming urgent, as very few effective drugs are left to treat infections. Antibiotic resistance development is a natural process. Bacteria are under selective pressure and evolve mechanisms to avoid the antimicrobial effects of the antibiotics. Once developed, the genes for the resistance then rapidly spread even cross over between different species – a process called horizontal gene transfer. It therefore is necessary to continuously develop new antibiotics to keep up pace with resistant bacteria. However, in 1990 there were 18 companies developing new antibiotics, by 2011 there were only 4. In 1990 10 new antibiotics were licensed, in 2011 only 2. The reason for a worsening of the antibiotics problem into an antibiotics crisis is a classical market failure because there is a lack of financial incentives for the pharmaceutical industry to involve in the development of drugs like antibiotics with a small profit margin. Decentralizing the screening for antibiotics around the world using cheap and fast Digital Biology could provide a solution to this problem. On one hand to reduce the costs of research allowing more people could contribute to find a common solution and on the other hand to increase the chances to discover new compounds. In a citizen science project, people around the globe could contribute to the solution of the antibiotics problem by identifying new antibiotics in a crowd-sourced research approach using Bioflux Technology. Specialists from all fields of expertise could design the bioassays for Point of care diagnostic and treatment of multiresistant bacteria. In practise, resistant bacteria could be collected by medical personnel, screened with the Bioflux platform and the results gathered in a central online database. The databases would be accessible to a global community of researchers that shares the task to design a case specific treatment. By rational and creative design of for example Bacteriophages, entirely new antibiotics could be designed. Bacteriophages are programmable macromulecules that specifically target a multiresistant bacteria strain. To date, they can be

readily designed using synthetic biology methods. Ultimately, only the clinical trials would have to be organized by a central agency, while all other steps of the diagnosis, finding the right cure and even the production of the antibiotics could be done in a decentralized and global collaboration of scientists.

3.14 Scalable One-Pass Synthesis for Digital Microfluidic Biochips

Robert Wille (University of Bremen/DFKI, DE, and Johannes Kepler University Linz, AT)

Digital Microfluidic Biochips (DMFBs) have been proposed to automate laboratory procedures in biochemistry and molecular biology. The design of the corresponding chips received significant attention in the recent past and is usually conducted through several individual steps such as scheduling, binding, placement, and routing. This established scheme, however, may lead to infeasible or unnecessarily costly designs. As an alternative, one-pass-synthesis has recently been proposed in which the desired functionality is realized in a single design step. While the general direction is promising, no scalable design solution employing this scheme exists thus far. In this work, we address this gap by proposing an automatic design approach which follows the one-pass synthesis scheme, but, at the same time, remains scalable and, hence, applicable for larger designs. Experiments demonstrate the benefits of the solution.

3.15 Flow-based Microfluidic Biochips

Hailong Yao (Tsinghua University – Beijing, CH)

Microfluidic biochips have emerged to revolutionize the traditional biological, biochemical and biomedical experimental processes. Noticeable merits of microfluidic biochips over traditional laboratory platforms include: (1) greatly saving the assay cost by reducing expensive samples/reagents to nano-liter or pico-liter volume, (2) effectively integrating the automatic control logic for reduced human intervention and labor cost, (3) significantly increasing sensitivity, accuracy and throughput, (4) essentially facilitating portability for point-of-care diagnostics, and (5) naturally enabling microscale assays (e.g., single-cell culture, capture and analysis) that are infeasible by traditional macroscale approaches. According to Research and Markets, the global biochips market is expected to grow at a CAGR of 18.6% from 2012 to 2018, and will reach $11.4 Billion by 2018. Applications of biochips cover many different fields, such as diagnostics and treatment, drug discovery and development, biological research, forensic analysis, agriculture, environmental sensors, food inspection, etc.

Flow-based microfluidic biochips are among the most commonly used microfluidic biochips both in laboratories and hospitals. Flow-based microfluidic biochips typically consist of several functional layers, which are fabricated by elastomer material (polydimethylsiloxane, PDMS) using the multilayer soft lithography (MSL) technology. The functional layers are: (1) flow layer with microchannels for transporting sample/reagent fluids, and (2) control layer with

microchannels for transmitting control signals (i.e., hydraulic or pneumatic pressure). In flow-based microfluidic biochips, microvalves on the control layer need to be connected to control pins via control channels. In application-specific and portable microfluidic devices, critical microvalves need to switch at the same time for correct functionality. Those microvalves are required to have equal or similar channel lengths to the control pin, so that the control signal can reach them simultaneously. We present a practical control-layer routing flow (PACOR) considering the critical length-matching constraint. Major features of PACOR include: (1) effective candidate Steiner tree construction and selection methods for multiple microvalves based on the deferred-merge embedding (DME) algorithm and maximum weight clique problem (MWCP) formulation, (2) minimum cost flow-based formulation for simultaneous escape routing for improved routability, and (3) minimum-length bounded routing method to detour paths for length matching. Computational simulation results show effectiveness and efficiency of PACOR with promising matching results and 100% routing completion rate.

The past decade has seen noticeable progress in computer-aided design (CAD) methods for droplet-based (digital) microfluidic biochips. However, CAD method for flow-based microfluidic biochips is still in its infancy. There are two major stages in this CAD flow: (1) control-layer design, and (2) flow-layer design. Microvalves are the critical components that closely couple these two design stages. Inferior flow-layer design solution forces valves to be placed at unfavorable positions. This makes great trouble to the following control-layer design, or even results in design failure. I.e., separate flow-layer and control-layer design lacks a global view with degraded solution quality. We have made the first attempt on flow-control co-design methodology, which integrates the two design stages for iterative adjustments with overall design improvements.

Future microfluidic biochip will be integrated with various devices, such as photodetectors and electrochemical sensors, which forms a complicated microfluidic cyber-physical system. Promising applications of such (implantable) cyber-physical microfluidic system include real-time health monitoring along with personalized preventive health care, which benefits the whole world. Microfluidic biochips are opening a door for new exciting discoveries of the unknown world. The ever-increasing integration scale of biochips drives the urgent need for CAD tools for design, modeling, and simulation.

Participants

- Mirela Alistar
Copenhagen, DK
- Krishnendu Chakrabarty
Duke University – Durham, US
- Rolf Drechsler
Universität Bremen, DE
- William H. Grover
University of California at Riverside, US
- Tsung-Yi Ho
National Tsing Hua Univ., TW
- Juinn-Dar Huang
National Chiao Tung University – Taiwan, TW
- Oliver Keszöcze
DFKI – Bremen, DE
- Bing Li
TU München, DE
- Pietro Lio'
University of Cambridge, GB
- Jan Madsen
Technical Univ. of Denmark – Lyngby, DK
- Sebastian Maerkl
EPFL – Lausanne, CH
- Paul Pop
Technical Univ. of Denmark – Lyngby, DK
- Sudip Roy
Indian Institute of Technology – Roorkee, IN
- Ulf Schlichtmann
TU München, DE
- Kwanwoo Shin
Sogang University – Seoul, KR
- Rüdiger Trojok
KIT – Karlsruher Institut für Technologie, DE
- Steve Wereley
Purdue University – West Lafayette, US
- Robert Wille
University of Bremen/DFKI, DE, and Johannes Kepler University Linz, AT
- Hailong Yao
Tsinghua University – Beijing, CN