November/December 2011
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IEEE PULSE

A MAGAZINE OF THE IEEE ENGINEERING IN MEDICINE AND BIOLOGY SOCIETY

Tackling Critical Health Challenges with BioMEMS

Plus

- The Right Tool for the Job
- On a Chip
- Engineering Tissue with BioMEMS
- Beyond Proof of Concept
- Perspective on Diagnostics for Global Health

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EMB IEEE

University of Minnesota

Integrative Graduate Education and Research Traineeship (IGERT)

Systems Neuroengineering

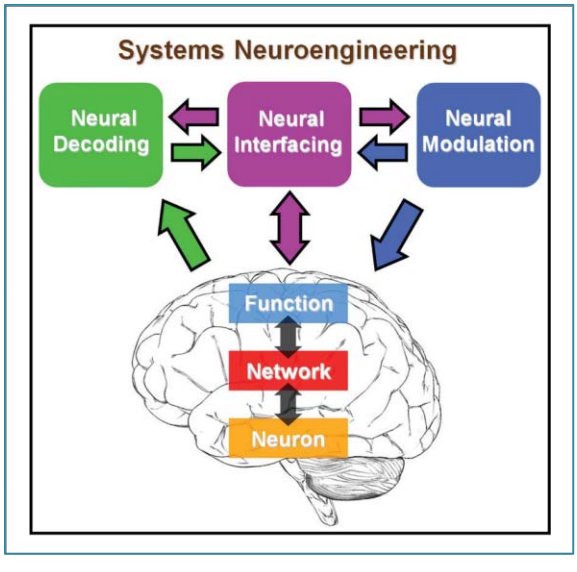
Interacting with the Brain: Mechanisms, Optimization, and Innovation

NEW Interdisciplinary Training Program!

The University of Minnesota has been awarded a National Science Foundation (NSF) grant for a new Integrative Graduate Education and Research Traineeship (IGERT) program in **Systems Neuroengineering**. Over the next five years the NSF grant will provide $3 million in funding, supporting six new graduate trainees per year. The IGERT program is designed to provide interdisciplinary graduate education and research training to exceptionally qualified doctoral students. Trainees will develop the skills to revolutionize technologies for interfacing with the brain and advance our fundamental understanding of neuroscience processes.

Program Director and Faculty

The Systems Neuroengineering IGERT Program is directed by **Dr. Bin He** (http://www.tc.umn.edu/~binhe/), a Distinguished McKnight University Professor of Biomedical Engineering and an internationally renowned scholar in neuroengineering and biomedical imaging. The IGERT program has over 40 outstanding faculty members who have made significant contributions to neural decoding, neuromodulation, neural interfacing, and neuroimaging research, and who are committed to graduate training.



Key Program Features

- Choice of research advisors from over 40 participating training faculty across engineering and brain sciences
- Joint faculty mentoring and team advising of dissertation research
- New neuroengineering graduate curriculum
- Lab rotations in engineering and basic/clinical brain sciences labs
- Industrial internship rotations
- Generous stipend ($30,000/year for two years on IGERT program) and tuition coverage

Eligibility Requirements

Trainees must be U.S. citizens or permanent residents who have been admitted to one of the four participating University of Minnesota doctoral programs: **Biomedical Engineering**, **Electrical Engineering**, **Mechanical Engineering**, and **Neuroscience**. For more information on this highly selective training program and how to apply, please visit our website at http://www.igert-ne.umn.edu/, or contact us at igert-ne@umn.edu.

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College of Science & Engineering | University of Minnesota Medical School Driven to Discover℠

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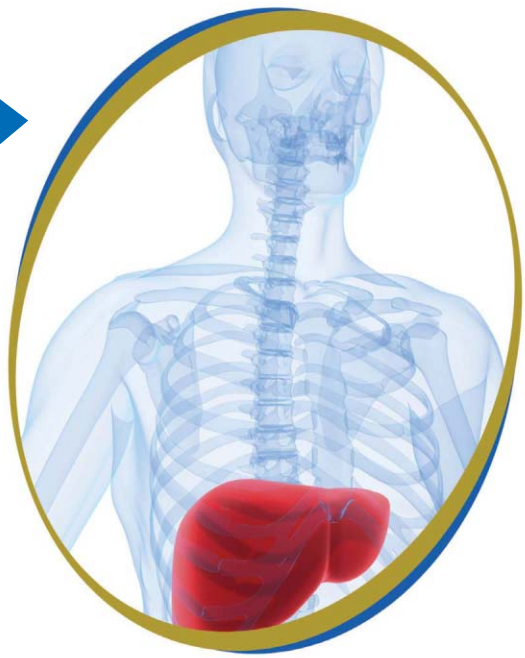

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FROM THE EDITOR

Wireless

Michael R. Neuman

Wireless technology is pervasive in our world today. Everywhere we look, we encounter some sort of wireless device—be it the mobile telephone in our pocket or the garage door opener in our car. We see wireless devices in our home in the form of remote controls for our televisions, video games, local networks for our computers, or remote controls for difficult-to-reach electric fans. Because of these and other wireless-controlled devices, our coffee tables are loaded with remote control transmitters. In its way, wireless devices have done much to contribute to our sedentary society. Perhaps this technology deserves to be recognized by receiving the Couch Potato Enhancement Award in recognition of all it has done to help us remain seated.

Based on the previous paragraph, you might think that I have a rather negative view of wireless technology and its impact on human health. It has certainly helped us to avoid getting up to switch off the television or change a compact disc in our player, but it has also made it possible for those of us who are unable to get up and move around to do these things. It has certainly improved the quality of life for individuals with movement impairments and, therefore, had a positive impact on health. Hence, maybe this technology is not so bad.

As a matter of fact, the application of wireless technology to health care and biological sciences has been one of the important contributions of biomedical engineering over the last 50 years. Some of the early pioneers in our field worked on developing miniature wireless devices for patient monitoring, animal tracking, ergonomic studies in the workplace, and making measurements that could not otherwise be done conveniently in animal and human research.

In the early days of this work, wireless technology was referred to as *biotelemetry*, and many of us still consider this to be a viable term. Pioneers in the field of biotelemetry included people such as Stuart McKay, Norman Holter, Peter Kimmich, Jap Klijn, Wen Ko, Tom Fryer, and many others whom I have failed to mention. These people developed wireless telemetry systems without, in many cases, the miniaturization and low-power advantages of present-day microelectronic technology. For example, obstetricians Orvan Hess and Edward Hon from Yale University developed the first wired fetal heart monitoring device in the 1950s. In the following decade, they developed a wireless version with Wasil Kitvenko, the chief of that medical school's electronics laboratory. This device, while small for its time, was bulky by today's standards because it used vacuum tubes as the active components and, therefore, had to have large batteries to supply the necessary power. Nevertheless, these investigators did demonstrate that wireless fetal monitoring was possible and could give the patient increased mobility.

Wen Ko's contributions included miniature telemetry transmitters using microelectronic components such as homemade tunnel diodes and commercial transistor chips. His devices were small enough to be implanted in animals, and I believe that in 1967, one of his transmitters was the first to be implanted in a human subject, Dr. Lojze Vodovnik, a visiting professor from the University of Ljubljana, in what was then Yugoslavia and now Slovenia. The telemetry device sensed electromyographic signals from the deltoid muscle of his shoulder and broadcasted them to an implantable stimulator, also developed by Prof. Ko, which was implanted in a dog's rear leg. Upon activation, when Dr. Vodovnik shrugged his shoulder, the dog lifted its leg. This was the first demonstration that wireless technology could be used in this manner to activate paralyzed muscles, and the work continued, resulting in the development of the functional electrical stimulation rehabilitation systems that we know today. Dr. Vodovnik became one of the international leaders in the field of functional electrical stimulation. He had a shoulder scar to remind him of his daring and seminal contributions to the field while at Case Western Reserve University.

I have my own memories of the early days of biotelemetry. As a master's student in electrical engineering, I developed a miniature telemetry transmitter using thin-film passive components and Prof. Ko's circuit. In later years, I believe it was 1972, I learned how wireless telemetry could be important in the care of children while I was a medical student. During that period, I was a student clerk on a pediatric ward, and one of my patients was a young girl who was experiencing cardiac arrhythmias. Because of the risk of an arrhythmia developing into ventricular fibrillation, she was connected to a cardiac monitor with a built-in defibrillator. In those days, monitors were large and looked like something that would be more appropriate for a research laboratory than a pediatric hospital room. The patient was evidently a television fan and liked to watch medical shows, as she was familiar with the defibrillator and knew how it shocked patients. Therefore, she was anxious about the fact that she might be shocked by the one sitting next to her bed and did not like being close to that ugly machine that beeped with each of her heartbeats.

Although just a medical student, I told my chief that we had a wireless device in my laboratory that would allow the nurses to monitor the patient's electrocardiogram at the nurse's station with only a small

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CALL FOR ADCOM NOMINATIONS
Submission Deadline: 1 February 2012

The IEEE Engineering in Medicine and Biology Administrative Committee (ADCOM), which establishes the policy for the Society's activities, has vacancies each year as the terms of elected members expire in rotation. This year's elections, for terms to begin 1 January 2013, will include the following geographic areas:

Asia Pacific
1 Representative (term beginning 1 January 2013, ending 31 December 2015)
Europe
2 Representatives (term beginning 1 January 2013, ending 31 December 2015)
North America (U.S.)
2 Representatives (term beginning 1 January 2013, ending 31 December 2015)
Student Representative
1 Representative (term beginning 1 January 2013, ending 31 December 2014)

Candidates are sought from all segments of the Society, including individuals from industry, universities and medical centers. To be eligible, a candidate must be a member, associate, or affiliate member of EMBS. The Nominating Committee initiates nominations.

Suggestions for nominations or self-nominations should be sent electronically to the EMB Executive Office attached with a 250 word biographical sketch and a 250-word position statement from the candidate as Word Documents in addition to a color photo (JPG).

Decisions about the final slate of candidates are the final responsibility of the Nominating Committee. However, EMBS By-Laws provide a mechanism to ensure that a particular candidate appears on the ballot: "*A petition nominating a Society member in good standing and supported by the identifiable signatures of at least 2% of the Society members eligible to vote for that position on July 1st of the previous year, or a minimum of 5 members if 2% results in a smaller number, shall automatically cause that member's name to be placed on the ballot for the specified vacancy.*"

Deadline for submitting nominations and petitions electronically to the EMB Executive Office at emb-exec@ieee.org is
1 February 2012

Petitions may be submitted in advance of the deadline to determine if the minimum number of valid signatures has been achieved.

? Questions ?
Please contact the EMBS Executive Office (emb-exec@ieee.org)

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radio transmitter attached to her electrodes and placed on her chest. The staff agreed that we should give it a try (the regulations to do clinical studies were a lot easier back then), and I went across the street to get the device from my laboratory and brought it back to the children's hospital. We attached it to the patient and placed the receiver and monitor at the nurse's station several meters away. The monitor-defibrillator was wheeled out of the young patient's room. Of course, the whole staff was interested in this new device and watched the monitor screen at the nurse's station. Over time, we noticed that the patient's arrhythmias were becoming less frequent and eventually stopped altogether. She was also sleeping better, and so we kept using this monitor with that young patient. It was at this point that I realized that wireless biotelemetry was more than just a biomedical gimmick. By eliminating the wires and overbearing electronic equipment from the patient's room, the patient's anxiety was decreased, which evidently had contributed to the cardiac arrhythmias she was experiencing. Although this was hardly a controlled scientific study, it has remained with me as an example of how wireless technology can contribute to patient care.

Today, biomedical applications of wireless telemetry abound. Wireless forms of all types of patient monitoring devices can be found in modern medical centers. For example, recently, when I had a cardiac stress test, my electrocardiogram during the test was transmitted to the monitoring equipment by a wireless link. The transmitter that I wore was a bit bigger than what the little girl in the hospital wore 37 years ago, but it transmitted all 12 leads as opposed to our single-lead device.

Wireless devices in patient's homes allow their physiological functions and activities to be monitored as they go about their daily routines. Elderly patients can wear a biotelemetry pendant that can be activated when they need help. This pendant will wirelessly transmit the information to a transponder that will contact a caregiver who can quickly respond to the patient's needs. Wildlife biologists, who study animals in their natural habitat, can observe their behavioral patterns through wireless telemetry devices worn on the animal, and the animals' locations can be followed using the global positioning system information relayed back to the researcher by satellite.

Although a lot has changed since the early days of wireless biotelemetry, still the basic idea of removing all wires so that animals or patients can be fully mobile remains the same. Biotelemetry hardware has taken much of its technology from that used in wireless consumer devices, and this has helped to make smaller, implantable, low power, and multiple channel devices possible. Over the years, biomedical engineering has played a significant role in the development of wireless biotelemetry devices, and we as patients and researchers have benefited from this. So pat yourself on the back the next time you pick up one of your remote control devices on your coffee table and say, "Good job biomedical engineer; you have contributed to making wireless technology an important tool for patient care and biomedical research even though you do not get up from your chair much any more."

PRESIDENT'S MESSAGE

Accomplishments of EMBS

Zhi-Pei Liang

With the end of the year 2011, I would like to highlight some of the activities and accomplishments of the IEEE Engineering in Medicine and Biology Society (EMBS) for this year.

First of all, despite Hurricane Irene's sweep through the northeast United States just before the start of our flagship annual meeting, IEEE Engineering in Medicine and Biology Conference (EMBC) 2011 turned out to be the largest EMBS gathering to date, attracting nearly 3,000 attendees to Boston, Massachusetts, from 30 August to 3 September 2011. Attendees from all over the world met in keynote and panel discussions, minisymposia, invited sessions, tutorials, clinical courses, and workshops to discuss the latest advances in biomedical engineering, health-care technologies, and medicine. Our Society owes a big thank you to Paolo Bonato (conference chair), Colin Brenan (conference cochair), Andrew Laine (program chair), Metin Akay (program cochair), Atam Dhawan (tutorials and workshops chair), Ming Jack Po (Web master), Caoimhe Bennis (clinical courses coordinator), and Pamela Reynolds (general program coordinator) for their able leadership and hard work to make this conference an incredible success.

We also had several highly successful topical conferences in 2011, which include the 8th IEEE International Symposium on Biomedical Imaging (ISBI'11) held in Chicago, Illinois, from 30 March to 2 April 2 2011 (conference chair: Steve Wright, program chair: Xiaochuan Pan, and program cochair: Michael Liebling); the 5th IEEE International Conference on Neural Engineering held in Cancun, Mexico, 27 April to 1 May 2011 (conference chair: Metin Akay, conference cochair: Dominique Durand, program chair: Silvestro Micera, and program cochair: Jian Tian); the 2nd IEEE AMA Medical Technology Conference held in Boston, 16–18 October 2011 (conference chair: Nicolas Chbat and program chairs: Mark Evans and Seong K. Mun). These meetings were well attended and served our members and community as well.

Our society magazine, *IEEE Pulse*, continues to rise in visibility and impact since its redesign, including winning an APEX 2011 award of excellence in the category of best redesigns.

Our publications have continued to do well this year. The following are just a couple of notes for your information. First, Dr. Nitish Thakor is finishing his two terms (six years) as editor-in-chief of *IEEE Transactions on Neural Systems and Rehabilitation Engineering (TNSRE)*. Under Dr. Thakor's able leadership, the journal has improved in all aspects: from quality and visibility to impact factor. Dr. Paul Sajda of Columbia University will serve as the next editor-in-chief of *TNSRE*. Second, the Administrative Committee (AdCom) has also passed a motion to change the title of *IEEE Transactions on Information Technology in Biomedicine* to *Journal of Biomedical and Health Informatics*. We are confident that the journal, with its new title and scope, will further enhance its impact in the field. Third, our Society magazine (*IEEE Pulse*) continues to rise in visibility and impact since its redesign, including winning an APEX 2011 award of excellence in the category of best redesigns, thanks to Dr. Neuman and his editorial staff for their outstanding work.

To enhance our member service, we have also redesigned and reactivated the EMBS Distinguished Lecturer (DL) program. For more information, please visit the EMBS DL Web site at http://www.embs.org/dl-nomination.

I am pleased to report that, after extensive discussions by the Executive Committee and AdCom, our Society has a formal five-year strategic plan in place to guide the operations of our Society for the next five years (2012–2016). Please feel free to contact the executive office for a copy of it.

I am also pleased to announce that Dr. Bruce Wheeler has been elected as president-elect (2012), Dr. Nigel Lovell as vice president (VP) conferences for a second term (2012–2013), and Dr. Gudrun Zahlmann as VP members and student activities for a second term (2012–2013). Our Society is blessed to have these extremely capable individuals to serve in the key leadership positions.

I look forward to work with you all to make our Society even more successful in 2012.

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STUDENT'S CORNER

Benefits of Starting an EMBS Student Club/Chapter at Your University

Iris Yan

Do you have an IEEE Engineering in Medicine and Biology Society (EMBS) Student Branch Chapter or club in your area? Forming a Chapter or club is much easier and beneficial than you can imagine. The following provides a brief guide on the process of opening a Chapter that will enable you to decide whether it would be worthwhile or not.

Why to Establish a Student Club/Chapter?

Free Journal Subscriptions

Your club or Chapter will receive a free annual subscription to all EMBS publications, which include *IEEE Transactions on Biomedical Engineering*, *IEEE Transactions on Medical Imaging*, *IEEE Transactions on Neural Systems and Rehabilitation Engineering*, *IEEE Transactions on Information Technology in Biomedicine*, the newly created *IEEE Transactions on NanoBioscience*, as well as *IEEE Pulse*.

Participation in the Distinguished Lecturers Program

Through the Distinguished Lecturers Program, EMB student clubs can invite a world-renowned speaker to give a talk at their own university with the financial support from the EMBS. Distinguished lecturers of the EMBS are recognized experts in their fields and are known to be wonderful presenters.

Funding for Activities

All student clubs/Chapters are eligible to apply funding for activities. For the student clubs, the EMBS will provide up to US$500 matching funds per year for approved activities, using a 2:1 matching ratio. Student Chapters have the opportunity to receive up to US$1,000 per year, using the same matching ratio. Additional funds are also available from the EMBS, the IEEE Section, and the IEEE Region with which your branch Chapter is associated.

Outstanding Performance Award

The EMBS offers awards for student clubs or Chapters demonstrating outstanding performance in promoting interest and involvement in biomedical engineering. Up to US$500 in honorarium and up to US$1,000 in travel money is awarded for a representative to attend the EMBS Annual Conference. In addition, any student club or branch Chapter that recruits ten new members will receive US$100. The Chapter with the highest number of recruits will get one free registration and up to US$1,000 for a travel stipend to attend the EMBS Annual Conference. The awarded clubs/Chapters will also be featured in *IEEE Pulse*.

Chapter or Club?

The EMBS supports two types of student groups.

- *EMB Student Clubs*: The less formal of the two, a club requires only one member (student or faculty advisor) to be the EMBS member.
- *EMB Student Branch Chapters*: These are technical subunits of an IEEE Student Branch, and as such, are generally associated with an IEEE Regional Student Branch.

How to Form and Renew an EMBS Student Club

To form an EMB student club, you need just one EMBS member who can be either a student or a faculty advisor. Then, the online petition for the establishment of an EMBS student club must be completed and submitted electronically. The faculty advisor must send an e-mail, separately, to confirm his or her role as an advisor for approval.

EMB student clubs must renew their status online by 1 November of every year. This can be done by completing and submitting the same online petition for the establishment of an EMBS student club. Don't forget to inform the EMB when you change your club contacts so that we can send you the most updated information.

How to Form an EMBS Student Branch Chapter

To form an EMB Student Branch Chapter, you will need at least 12 IEEE student members who are also EMBS members. Then, fill out the petition for the establishment of an IEEE Society Student Branch Chapter and submit it to the IEEE.

To maintain your status as a student Chapter, you must comply with the IEEE requirements of reporting on your activities each year, your plan for activities, and new officers after elections. Don't forget to create a Web page for your club or Chapter. It's a great way to advertise and promote your group.

Need Help?

Please see the EMBS student Web site http://www.embstudents.org/ and feel free to e-mail me via ennayan@gmail.com or Angela Martin via Angela.Martin@ieee.org. Does forming a Chapter or a club seem easy and rewarding to you? Get started!

Iris Yan *is currently pursuing a Ph.D. degree in the Industrial and Manufacturing Engineering Graduate Program at Pennsylvania State University.*

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PERSPECTIVES ON GRADUATE LIFE

Round Two: Doubt

Zen Liu

With the registration for fall courses complete and the arrival of new master's degree and Ph.D. students, it is hard to ignore that I am no longer new. I feel as if I had spent the last year struggling through a painfully drawn-out transition into graduate school and adult life, and with each stumble and mistake, I was able to console myself because it was my first year. Suddenly, however, I feel that my professors and colleagues see me differently ~~as~~ seasoned and somehow knowledgeable ~~and~~ I am continually surprised when new students ask me for advice. I hardly feel qualified to be doling out advice on careers or the qualifying exam or life in general. I suppose that the learning curve in graduate school never plateaus.

Besides classes and research, the biggest thing on my mind this year is the qualifying exam. Having completed a majority of my master's credits, I am on track to sit for the exam before the start of the spring semester. For myself and, I am sure, for Ph.D. students everywhere, the qualifying exam is much more than a test or evaluation: it is a major life milestone that punctuates the tireless forward push of one's drive to accomplish and achieve, of scientific advancement, and it hangs over the head of every student that dares pursue a Ph.D. degree. I have already been reassured by older students who've passed and even those who've failed their first attempts, but I think that the exam speaks to each individual's fundamental belief in their abilities as a scientist and, in my case, as a biomedical engineer.

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The funniest thing about the situation is that, in many ways, qualifying exams are quite impractical. As I discussed in my previous column regarding the exam, it could be argued that the quals, in their most traditional form, are nearly as arbitrary as many consider standardized exams in this country to be. But I know myself, and I know how much doubt I sometimes harbor about myself and my abilities ~~m~~ost likely because I idolize the faculty and senior students that have come before me ~~an~~d I know how many times I have wondered whether I can actually be successful and relevant as a research scientist in my field. I have often wondered whether I am capable of doing "good science." I have often worried that I will not live up to my potential, and then worried further that I do not actually have as much potential as everyone insisted when I first set out to build this career.

So I take back everything that I said before. The most difficult thing about graduate school has not been the transition, the move to another city, the coursework, or making new friends. It hasn't been the newfound independence in research or the raised expectations. The most difficult and thoroughly terrifying thing about graduate school is the doubt that I feel about myself on a daily basis, how utterly incompetent I sometimes feel, and the fact that it appears no one else around me feels the same way. Older students listen as I vent my fears and then brush them aside with a kind but ultimately unreassuring, "Oh, you'll be fine." I have yet to even hear my classmates confess a similar fear, though we have spent hours bemoaning midterms and finals and the impending quals. Perhaps I am alone in this neurosis.

I think that the feeling of not knowing ~~if~~ my fears are normal, if my frustrations are justified and par for the course ~~is~~ in and of itself intimidating. I have come to realize that, while guidance in research and coursework are critical, mentorship and community are necessary to overcome the emotional challenges of graduate life. It is important to know that someone felt the same way once and that they still managed to be successful and accomplish their goals.

It is for that reason that I feel so passionate about enriching student life and community building; why I have always felt passionate about these things. Even as an undergraduate, I was active in student groups, almost to a fault, and I jumped at the chance to interact with younger students, whether it was through tutoring, peer mentoring, or working as a residential advisor or a teaching assistant. I can remember how scared and lost I felt when I started college, and it was important for me to try and make it easier on the students that showed up after me; to let them know that what they were feeling was normal and that they would be just fine. I need someone to do that for me now, and I have tried to do that for the incoming students at Columbia, because unlike in college, the challenges you face in graduate school change pretty drastically from year to year. It starts with developing your thesis and preparing for the qualifying exams, moves to building your thesis committee and defending, and finally, culminates in finding a job after graduation. I suspect that everyone feels unprepared or, at the very least, suffers a certain degree of apprehension when facing each of these tasks. It's just important to know that you can do it and, no matter how you feel, you are not alone and you are ready.

Zen Liu *is currently a second-year Ph.D. student in the Department of Biomedical Engineering at Columbia University.*

The Last Summer of Your Life

Matthew C. Canver

The first year of medical school ended in early June, officially beginning what is always referred to as *the last summer of your life*. Following the end of the second year of school, all medical students will start clinical rotations in the hospital, so there is no longer an extended summer vacation. Once starting this type of clinical work in the hospital, time off essentially becomes like any other job. Even so, our summer following the end of our first year lasted for only nine weeks, which allowed the students to have one week of vacation time and then eight weeks to pursue a project or job that interested them (e.g., research and internships).

As I mentioned in my last column, I stayed in Boston for the summer to work in a cardiac-focused laboratory at the Massachusetts Institute of Technology that specializes, among other things, on tissue engineering and vascular biology. The short duration of this summer as compared to an undergraduate summer break made completing or significantly contributing to a research project a little more difficult; however, it was still valuable for learning and relaxing before the second year began.

Now begins a new school year. The second year of medical school will continue the paradigm shift in our medical education from focusing on normal function to when things go wrong, because the second-year curriculum is almost exclusively about pathology/pathophysiology. The academic schedule consists of one major course, called *human systems*, for nearly the entire year in which we learn the pathophysiology for all major systems (e.g., respiratory, renal, and gastrointestinal) in addition to a variety of other topics that were not covered last year, including nutrition, neurology/neuroscience, and pharmacology. While the second year of the curriculum has the reputation of being more difficult due to an increased amount of information, it also has the reputation for having much more interesting material. In the same vein, the information in this year is supposed to be more clinically relevant than the material from the first year, which taught a lot more basic science. The basic science was both interesting and important, but it tended to be less useful in a clinical setting.

Learning medical procedures and exams will represent a very interesting and exciting step forward in our knowledge and skill set.

A significant addition to the second year will be learning to perform a physical exam with a patient. The first year exclusively focused on taking a complete patient medical history, which just meant having a conversation with patients. It was a significant milestone in our medical education as we were instructed to purchase various medical supplies such as our own stethoscope, sphygmomanometer, and reflex hammer. Just the experience of purchasing these types of equipment was a little overwhelming because it signified our progress through the curriculum and foreshadowed the types of procedures/exams that we would be learning and performing on actual patients in the not-too-distant future. Even so, it was also exciting.

After entering medical school, it is common for friends and family members to try to ask you questions about their own medical problems. However, most people do not realize that you are not equipped to answer such questions at this point in your medical education, which is sometimes depressing as you do so much studying but still do not know enough medicine to be useful. Therefore, learning medical procedures and exams will represent a very interesting and exciting step forward in our knowledge and skill set.

In addition to the pathophysiology courses and the learning of the physical exam, the second year culminates with what is commonly referred to as *The Boards*. The boards are actually the U.S. Medical Licensing Exam (USMLE) Step I. The USMLE consists of a series of exams (Step I, Step II: clinical skills, Step II: clinical knowledge, and Step III), and all exams must be passed to receive a medical license in the United States. It is common for medical students to take the Step I exam after the end of the second year of medical school. The Step I exam tests the preclinical knowledge that was taught during the first two years of school. The exam takes place for more than eight hours (including breaks) with seven one-hour sections, each with 46 questions for a total of 322 questions. Needless to say, this exam is a major source of stress for medical students because, similar to the SAT for undergraduate college and the MCAT for medical school, it is an important component of the application when you eventually apply for residency. For Harvard Medical School, the second-year curriculum ends by March and the students generally take the Step I exam in April.

In the next issue of *IEEE Pulse*, I will talk about pharmacology, nutrition, and human development courses, about the beginning of the second year of medical school, and experiences of learning how to give a physical exam.

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Matthew C. Canver *is currently pursuing his doctor of medicine (M.D.) degree at Harvard Medical School in Boston, Massachusetts.*

The **IEEE International Symposium on Biomedical Imaging** (ISBI) is the premier forum for the presentation of technological advances in theoretical and applied biomedical imaging and image computing. ISBI 2012 will be the ninth meeting in this series and its 10th anniversary since the first edition. Previous meetings have played an important role in facilitating interaction between medical and biological imaging researchers. The 2012 meeting will continue this tradition of fostering knowledge transfer between different imaging communities and contributing to an integrative approach to biomedical imaging across all scales of observation.

ISBI is a joint initiative from the **IEEE Signal Processing Society** (SPS) and the **IEEE Engineering in Medicine and Biology Society** (EMBS). The 2012 meeting will open with a morning of tutorials, followed by a scientific program of plenary talks, invited special sessions and tutorials, and oral and poster presentations of peer-reviewed papers and abstracts. For the first time in the history of ISBI, this edition will have a double track submission system: the traditional ISBI, full-paper, review process and publication with poster/oral presentations customary and a novel, abstract-based, review process with poster presentation, without subsequent archival publication.

High-quality papers are requested containing original contributions to mathematical, algorithmic and computational aspects of biomedical imaging, from nano- to macro-scale. Topics of interest include image formation and reconstruction, computational and statistical image processing and analysis, dynamic imaging, visualization, image quality assessment, and physical, biological, and statistical modeling. Papers on structural, molecular, cellular, anatomical, and functional imaging modalities and applications are welcome.

This year's meeting will take place in the historic and cosmopolitan city of Barcelona, in the heart of the 22@ district, the city's new-technology and innovation hub along the Mediterranean sea. The social event will be hosted at the Poble Espanyol, an architectural museum conceived as a Spanish village.

Important dates

Deadline for submission of 4-page paper or abstracts:	**November 13, 2011**
Notification of acceptance/rejection:	**January 16, 2012**
Submission of final accepted 4-page papers/abstracts:	**February 15, 2012**
Deadline for author registration:	**February 15, 2012**
Deadline for early registration:	**April 1, 2012**

Venue

Barcelona International Convention Centre (CCIB), *22*@ District, Barcelona, Spain

Further information is available at http://www.biomedicalimaging.org/2012/

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GUEST EDITORIAL

Delving into BioMEMS

Rashid Bashir, Ali Khademhosseini, and Samuel Sia

We are very pleased to offer in this issue of *IEEE Pulse* a number of articles focusing on biomedical or biological microelectromechanical systems (BioMEMS) and selected applications related to this exciting field of research. Originally derived from the fields of microelectronics and microsystems, BioMEMS has experienced a tremendous growth over the last decade. It is now poised to have a significant impact on clinical and biomedical applications and can help address some of the most critical health-related challenges in the United States and around the world. In this issue, leading experts in the field come forward to present up-to-date overviews of some of the key application areas, ranging from tissue engineering, global health, clinical diagnostics, drug screening, and drug delivery.

The rising cost of health care is one of the most urgent issues today. The challenges are numerous: the largest ever aging population and a consequent rise in age-related diseases; an urgent need for novel diagnostics in global health-related communicable diseases, as well as noncommunicable diseases such as cancer, diabetes, and cardiovascular disease; the enormous costs of drug development and discovery; and a need for personalized and individualized therapeutics and diagnostics. Although these challenges may appear disparate and almost insurmountable, a common thread is that possible game-changing solutions may be achievable with BioMEMS (alongside nanotechnologies, information technology, and other promising technologies). The articles in this issue, while highlighting some of these impending challenges, also present possible solutions and a way forward for engineers in BioMEMS and related fields to address these critical health challenges.

One of the major areas where microscale applications can make a significant impact is regenerative medicine. Borenstein and Vunjak-Novakovic describe the application of BioMEMS technologies to engineering tissues with controlled microarchitectures in their article. Tissue microvasculature and cell-cell interactions can be controlled by merging microfabrication techniques with advanced biomaterials to generate microfabricated tissue structures with enhanced function or control stem-cell differentiation for generating a renewable source of cells for transplantation. In addition, Seker et al. describe the use of cell-based systems for generating tissues on a chip. In such systems, cells can be integrated within microscale systems such as microfluidic chips. Such cell-containing devices can be used for generating miniaturized systems on which cell behavior can be monitored upon exposure to various chemicals for drug discovery applications.

Microfabricated technologies can be used to generate microchips that can deliver drugs in a controllable manner.

Another area that can benefit from the use of microfabricated technology is drug delivery. In an upcoming issue of *IEEE Pulse*, Ferrari et al. will describe the use of BioMEMS approaches for drug delivery. Microfabricated technologies can be used to generate microchips that can deliver drugs in a controllable manner. Furthermore, such techniques can be used to generate micro- and nanoparticles with controllable shapes that enable more controlled delivery of drugs to particular tissues in the body for a range of diseases such as cancer.

Microscale technologies can also be used to develop cheap and portable devices for diagnostics. Two articles in this issue outline various uses of microscale technologies for global health and clinical diagnostic applications. In one, Fu et al. describe the use of microfabricated technologies for a range of diagnostic diseases. In the article by Watkins et al., the authors discuss the potential of microfluidic systems for a range of clinical and diagnostic applications including blood cell counting, detection of $CD4^+$ cells for diagnosing the progression of HIV/AIDS in patients in resource-limited settings, and bacterial detection.

Additionally, these technologies need to be translated to the market place so as to provide solutions for the betterment of the public. We also present a short discussion with two leading scientists (Stephen Quake and Shu Takayama) who have translated biomedical microtechnologies into a commercial arena.

We hope that you enjoy reading these articles. Please feel free to provide any feedback.

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IEEE EMBC 2012

August 28 - September 1, 2012
Hilton San Diego Bayfront Hotel
San Diego, CA, USA

Engineering Innovation in Global Health

Proposals for organized sessions and submissions are invited in the following themes:

- Biomedical Signal Processing
- Biomedical Imaging & Image Processing
- Bioinstrumentation: Sensors, Micro, Nano and Wearable Technologies
- Bioinformatics, Computational Biology, Systems Biology, Modeling Methodologies
- Cardiovascular & Respiratory Systems Engineering
- Neural Engineering, Neuromuscular Systems & Rehabilitation Engineering
- Molecular and Cellular Biomechanics, Tissue Engineering, Biomaterials
- Bio-Robotics, Surgical Planning and Biomechanics
- Therapeutic & Diagnostic Systems, Devices and Technologies, Clinical Engineering
- Healthcare Information Systems, Telemedicine

Plenary Keynote Speakers

William R. Brody, MD, PhD
President, Salk Institute

Eric J. Topol, MD
Vice-Chairman,
West Wireless Health Institute

Rebecca Bergman
Vice-President, CRDM
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Conference Comittee

Conference Chair: Michael Khoo, USC
Program Co-Chairs: Gert Cauwenberghs, UCSD
James Weiland, USC
International Program Chair: Shu Chien, UCSD
Tutorials, Workshops & Minisymposia Chair: Atam Dhawan, NJIT

Key Dates

Oct 15, 2011 - Jan 15, 2012	—	Proposal Submission
Feb 1, 2012 - Mar 15, 2012	—	Paper Submission
Jun 1, 2012	—	Notification of Acceptance
Jun 15, 2012	—	Author Final Submission

For more details, visit the EMBC'12 website:

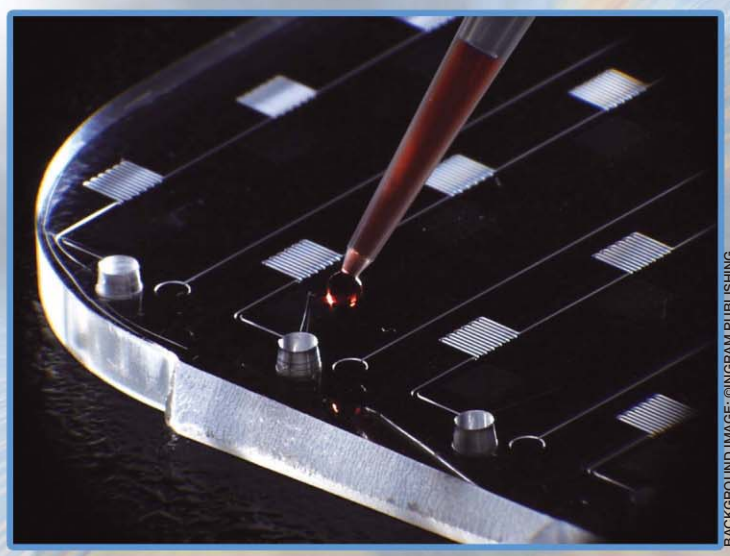
http://embc2012.embs.org

IEEE EMB

Digital Object Identifier 10.1109/MPUL.2011.943390

The Right Tool for the Job

By Leslie Mertz



BACKGROUND IMAGE: ©INGRAM PUBLISHING

From Microchips to Artificial Tissues, Experts Say BioMEMS Are Poised to Invade Medicine

In the biological world, where the typical human cell is less than 10 µm in diameter, an average bacterium a few micrometers long, and the garden-variety virus about 100 nm in length, more and more researchers view microscopic tools known as biological microelectromechanical systems (bioMEMS) as the right choice for keeping patients alive and healthy. BioMEMS are an outgrowth of MEMS—the sensors, actuators, and other microchips developed for the electronics industry. MEMS are present in everything from computers and ink-jet printers to automobile air bags and digital video projection systems.

BioMEMS are MEMS directed mainly toward biomedical uses. Today, for instance, a person goes to a medical laboratory to have blood drawn, and in a week or two, gets the results from the doctor. The costs for such tests can run into hundreds. On the other hand, with new bioMEMS technology that is on the horizon, the same person could prick a finger, deposit a drop of blood on a small testing cartridge, and read the results in a few minutes—all without ever leaving home. Not only would this eliminate a trip to the laboratory, but it would also greatly decrease the amount of blood needed for testing. In addition, it would reduce the amount of reagents required to perform the blood test, and this would help to lower costs.

"The idea is to build systems that can be used to detect cells, or to detect bacteria or viruses or proteins," said Rashid Bashir, Ph.D., Abel Bliss professor in the departments of electrical and computer engineering and bioengineering, and director of the Micro and Nanotechnology Laboratory at the University of Illinois at Urbana-Champaign. "Today, we make chips that go into every watch, every calculator, every iPad, every phone. The more we make, the lower the cost. We want bioMEMS technology to be just as pervasive."

BioMEMS are already doing or have the potential to do many medical tasks faster, better, and cheaper than traditional methods. A few examples are

- quick scanning for and diagnosis of a profusion of diseases from a single drop of blood
- generation of artificial tissues that can help a patient's damaged organ to function again

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- testing of drugs without animal experimentation
- identification of the earliest signs that cancer is spreading, allowing doctors to provide more effective treatment
- highly accurate, yet inexpensive, easy-to-use, and easy-to-interpret patient diagnostics in Third World nations, where hospitals are few and far between.

"We are at the beginning of this technology being applied to applications," said Bashir. "There has been a lot of R&D done, and there is still a lot of R&D to be done specifically when it comes to understanding the basic mechanisms of disease, but in terms of medical applications, many of them are now on the horizon, and new start-ups and other companies are beginning to adopt these technologies." Up-and-coming bioMEMS companies are diverse, but many focus on lab-on-a-chip technologies that are being designed to perform blood tests more quickly and less expensively than those offered by traditional diagnostic laboratories. He added, "We face many manufacturing and technical challenges, and we may face financial and other challenges, but the possibilities are tremendous, and it is a very exciting time to be working in this field. I believe a lot will happen in the next 10 years or so."

BioMEMS technology gives us the ability to access even rare particles, cells, and molecular species in the blood and that opens up whole new possibilities.

Diagnostics and Therapeutics

One of the hottest areas for bioMEMS is diagnostics: taking a small sample of blood or other bodily fluid from a patient and testing it for various diseases right at the bedside, rather than sending it out to a laboratory and waiting a day or more for the results.

"It turns out that today, the most prescribed tests in medicine are the blood tests," said Mehmet Toner, Ph.D., Helen Andrus Benedict Professor of biomedical engineering at Massachusetts General Hospital, Harvard Medical School, and director of the BioMEMS Resource Center. "We typically measure complete blood counts, electrolytes, glucose, several biomarkers, and other things. However, these measurements are rather nonspecific." If the blood work reports something abnormal, perhaps too-low or too-high readings of certain white blood cells, he said, a doctor typically orders additional examinations, some of which may be expensive imaging tests, such as X-rays or a magnetic resonance imaging scan.

With bioMEMS, the blood test would be enough to provide a detailed medical diagnosis. "The blood has pretty much every bit of information you need regarding your health. You can almost diagnose and monitor every single disease ~~fr~~om Alzheimer's to cancer to prenatal conditions to infectious diseases ~~fr~~om the blood," Toner asserted. "What these microtechnologies are enabling is the ability to access the cells and proteins and other disease markers that are in blood, that simply were not possible to read in the past."

An improved understanding of microfluidics, or how extremely small volumes of fluid behave, is vital to those advancements. This is because bioMEMS usually require that blood or other bodily fluids move through miniscule channels built onto a tiny electronic chip so that sensors can read the fluid's components. However, the physics of fluid movement is different at the microscale than it is at larger scales. As Toner noted, "The ability to precisely control the flow and conditions is very important, especially when you are looking for low-abundant proteins and various rare cells within the blood. BioMEMS technology gives us the ability to access even rare particles, cells, and molecular species in the blood, and that opens up whole new possibilities."

Counting Blood Cells and Bacteria

"In our group, there's a big thrust on diagnostics and biosensing, and the use of microfluidics and microelectronics in biochips," said Bashir, noting that his laboratory is interested in developing point-of-care sensors, which can analyze the sample and provide results right at the bedside. The model is the finger-prick test that people with diabetes use to check their blood-glucose levels. "That's kind of the Holy Grail in terms of taking just a drop of blood and getting some useful diagnostic information from it, but that's really the only example that you see today on the market. Why can't we have more examples like that for other important applications? Our goal is to build devices of that type with silicon or different materials." His laboratory is developing a biochip that can provide a full blood-cell count (Figure 1).

In a nonhuman project, Bashir is also working with the U.S. Department of Agriculture to create biochips that can quickly check food for bacterial pathogens, such as *Escherichia coli*, *Listeria*, and *Salmonella*. "The goal is to rapidly detect the presence of live bacteria from fluid samples," he said. Together with Laila Razouk, Ph.D., an engineer specializing in microdevices, he helped to create a company to continue developing the biochip. The company called *BioVitesse, Inc.*, is located in the San Francisco Bay area.

Finding Rare Tumor Cells

One bioMEMS project that has received considerable press is a large project on cancer diagnosis led by Toner and Daniel Haber, M.D., Ph.D., director of the Massachusetts General Hospital Cancer Center and the Kurt J. Isselbacher/Peter D. Schwartz

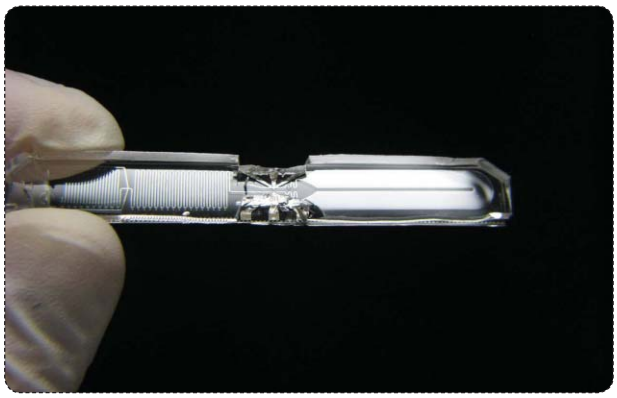

FIGURE 1 Rashid Bashir's research group developed this device, which counts white blood cells from a whole-blood sample. It is made with polydimethylsiloxane (silicone), glass, and metal electrodes. (Photo courtesy of Rashid Bashir.)

Professor of Oncology at Harvard Medical School. In this project, sensors find the elusive cancer cells that spread the disease from one part of the body to another. "Cancer doesn't kill because of the primary tumor. Nine out of 10 times, it kills because it spreads," Toner said. "We've never been able to find these cells in transit through the peripheral blood because they are so rare: They are maybe one in a billion blood cells."

Toner is working with the National Institutes of Health, health-care giant Johnson & Johnson, the charitable program Stand Up to Cancer, and a number of other organizations to develop biochips that will find the rare, circulating tumor cells in the blood. "We needed technologies that are extremely sensitive to find one cell in a billion blood cells. The question was, 'Can we develop the right technology to find them?' We can. We developed a microchip that can do that," he said.

The announcement about the development of the chip in 2007 generated great interest throughout engineering and biomedical circles, and energized the idea that bioMEMS has a wealth of applications for cancer diagnosis and therapy. "You could monitor the tumor load in circulation and know if your treatment is effective; you could look for early relapse and treat them immediately; you could use it for early detection. The applications are so broad that people believe that if this technology pans out, it has the potential to turn cancer into a chronic disease, like AIDS or diabetes."

Toner's chip is undergoing clinical trials at Massachusetts General Hospital, the Dana-Farber Cancer Institute in Boston, Memorial Sloan-Kettering Cancer Center in New York and New Jersey, and the M. D. Anderson Cancer Centers in Texas. Marketing will follow, but in the meantime, he is continuing to refine the chip. "It will afford the ability to monitor cancer patients individually, so it is personalized medicine at its best. It gets genetic as well as cellular information about the progression of their cancer, how the drugs are working on their cancer, and does that non-invasively with a simple test. It's very powerful."

Additional Cancer Work

Other bioMEMS cancer projects are underway elsewhere. Bashir, for instance, is working on a project for the National Institutes of Health to build a silicon biochip that can detect cancer markers in the blood. "The idea is to take, for example, a very small biopsy sample from a breast-cancer patient, and be able to look for the specific expression of certain proteins to indicate whether that patient's therapy is working or not." That project is still in the research and development phase.

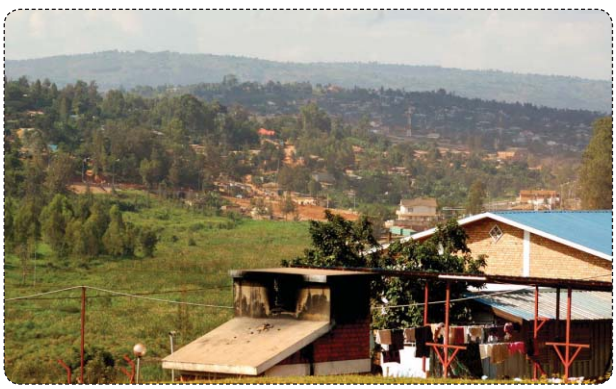

FIGURE 2 The low-cost mChip could be especially beneficial in the developing world such as this African settlement where the HIV and syphilis infection rates are high. (Photo courtesy of Samuel Sia.)

In Woburn, Massachusetts, researchers at Claros Diagnostics hope to soon market a quick and easy prostate-specific antigen (PSA) test, said Samuel Sia, a professor of biomedical engineering at Columbia University. He is one of the company's three cofounders and also the chair of its scientific advisory board. The device, which was approved in Europe last year, runs the PSA test and provides results in about 15 min. This is compared with the one to two days required for the currently used tests, which must go out to a laboratory for analysis.

Doctors routinely monitor PSA levels in men who have prostate cancer to track the disease's progression and also to follow up with those who have undergone prostate surgery to make sure the cancer hasn't returned. A faster result would benefit the health-care provider as well as anxious patients, Sia said. "The ability to perform diagnostics where the patients are would enable healthcare to be delivered more efficiently."

Claros's test consists of a hard-plastic cassette, which is lined with channels that hold the series of reagents needed for the analysis. Using vacuum, the cassette draws in the blood sample, the reagents and blood interact, and a reader provides the outcome.

Proprietary injection molding technologies allow the company to produce the intricate cassettes quickly and at a reasonable price: one cassette takes about 15 s to produce and costs around a dime, Sia said. "Injection molding is a technique commonly used to produce consumer products at a massive scale and for low cost. Traditionally, it was difficult to do with very small features," he said, but the company was able to accomplish it. "Claros has developed a suite of techniques to make the production of these cassettes rival the cost of cheap lateral-flow tests."

Fighting Disease in Developing Countries

One of the Bashir group's efforts is to fight human immunodeficiency virus (HIV) where it is most insidious: in the Third World nations of Africa (Figure 2). Specifically, he is developing a microchip that can count certain types of white blood cells, called *CD4-positive cells*, which are associated with HIV infection. HIV damages these cells, which results in a lower count of functioning CD4-positive cells. With such information in hand, doctors can track the progression of the infection and disease, and prescribe appropriate treatment for individual patients.

That research has advanced enough that Bill Rodriguez, M.D., former chief medical officer of the William J. Clinton Foundation, and Toner have started a company called *Daktari Diagnostics* in Cambridge, Massachusetts, to continue developing the CD4 device. Toner and Bashir, who are coinventors of the CD4 microchip that underlies Daktari's CD4 counting technology, also sit on the company's advisory board, along with a third coinventor, Xuanhong Cheng, Ph.D., the P. C. Rossin assistant professor of bioengineering and materials science and engineering at Lehigh University. "The company has made a lot of progress in getting to a point where we can make robust sensors,

and now in the next few years, we plan to do more testing with patient samples," Bashir said.

Sia and his colleagues have developed a microchip, called the *mChip*, to detect both HIV and syphilis (Figure 3). The device exposes a drop of blood to a series of reagents and produces easy-to-read results in a matter of minutes (Figure 4). The research group tested the chip in Rwanda, where the rates of HIV infection and syphilis are very high, and found that it could correctly identify the two conditions almost 99% of the time.

Despite the success, the mChip is not available commercially yet, Sia said. "A main hurdle is that funding is not often easily available for neglected diseases," he explained. Nonetheless, the research team is continuing to develop the chip. "We have active ongoing projects to expand the panel of infectious diseases that the mChip can detect in resource-limited settings," he said, listing potential disease candidates as hepatitis B and C, herpes, gonorrhea, and chlamydia.

Sia emphasized the importance of this work. "Take syphilis as an example. It causes stillbirths and congenital defects, but can be easily cured with penicillin if the patient is diagnosed in time. In developing countries, hundreds of thousands of people are dying every year due to the lack of a point-of-care syphilis test. The numbers are astounding," he said. The mChip, complete with the biochip, chemicals, and packaging, would run an estimated US$ 2.00–3.00 each, making it an economically viable diagnostic tool. "A low-cost test in these settings can dramatically ease human suffering."

Bashir noted that the use of bioMEMS can also easily expand to other needed medical diagnostic applications in resource-limited developing countries. "Can we have chip-based sensors where you'd need just a drop of blood to detect TB or malaria, or to sense other specific pathogens or cells?" With this biochip technology, any person anywhere could test his or her blood with a chip-containing cartridge, connect that cartridge to a cell phone or some other sort of wireless device, send the data for a quick analysis, and properly dispose of the used cartridge. "The notion of microchips used for diagnostics could be as pervasive as the use of microchips in iPads or phones," he remarked.

"One of the grand challenges that faces the U.S., the world and our society in general is health care. How do you manage healthcare? How do you deliver healthcare that is affordable to all and that is available to all? That is a basic right," Bashir noted. "We hope to use this technology to help us address some of these important issues."

FIGURE 4 When added to the mChip, a drop of blood moves through miniscule channels where it is exposed to a series of reagents. This produces easy-to-read results in few minutes. (Photo courtesy of Samuel Sia.)

Tissue Engineering for Transplantation

Tissue engineering using bioMEMS is just beginning, but it is already a vibrant research area. Some laboratories continue to perform some conventional tissue engineering without bioMEMS technology, especially those laboratories making skin and cartilage, which are already clinically available. This is possible because skin and cartilage require few blood vessels to function, while complex tissues, which occur in major organs such as the heart or liver, demand a network of capillaries, said Ali Khademhosseini, Ph.D., associate professor at Harvard Medical School. "This is where bioMEMS starts playing a role."

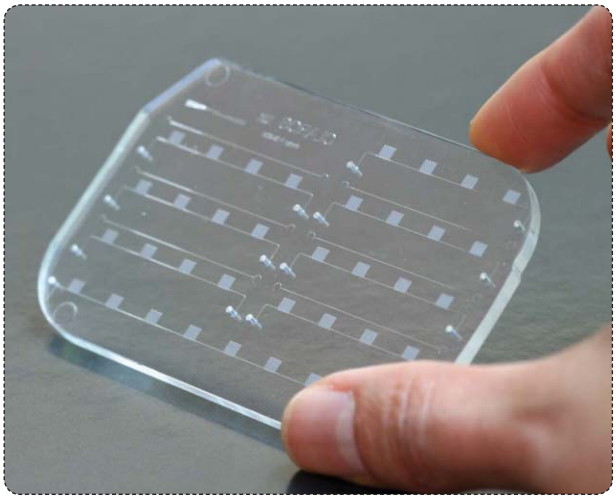

FIGURE 3 Sia and his colleagues have developed the mChip, which detects both HIV and syphilis. (Photo courtesy of Samuel Sia.)

He explained that tissue engineering may be an answer to a particularly vexing health problem: the lack of available organs for transplantation. "There is a long waiting list, and someone has to die for a patient to be able to get an organ in most cases. How do we address that? Generating artificial tissues is one way to do it." In response, his laboratory is recreating artificial tissue that works like normal human tissue. "We can't just use cells alone, because tissues actually have a lot of architecture associated with them, and this architecture affects the function," he said. "By using these microfabricated technologies, we can actually start to recreate some of these architectures so that our engineered tissues not only resemble natural tissues, but also function more like natural tissues."

One of the architectural elements is the collection of capillaries, the tiniest of blood vessels that permeate tissues and keep them alive. Using adult or embryonic cells and stem cells, along with various biomaterials, his laboratory employs bioMEMS technology to construct miniscule fluidic channels into the shape of a capillary network and add them to the engineered tissue. "We are creating the vascularization," he said. "We are applying the same technologies that the microelectronics industry uses to make cell phone and computer chips, but using degradable materials that cells will interact with. In other words, we are generating the kinds of tissue structures

that cells can integrate into and therefore have a much more functional behavior."

In addition, the microfabricated environments that his laboratory creates also help to control the body's cells, pushing them to take on the duties of specific types of organ tissue. "By doing this, we can then kind of signal the cell about what it should do and direct its behavior in a much more predictable way." The resulting engineered material can take the place of damaged, lost, or diseased tissue. "If we can transplant tissues that are compatible with the body, then we can restore a lot of a patient's lost functions. In diabetes, for example, the pancreas often does not work properly, so if we can restore that function, it would be great."

Drug Testing

Besides using artificial tissues for transplantation purposes, they show promise for drug testing. Currently, scientists develop a drug candidate, spend many, many weeks testing it on animals, and if all goes well, proceed to human testing, which can take many, many months. Negative findings along the way can send the scientists back to the drawing board. However, with artificial tissues, scientists can test a large number of drug candidates simultaneously and without the costly and sometimes inconclusive animal testing.

Khademhosseini's research group hopes to put the engineered tissues and cells on biochips for use in drug testing. "If we can make engineered human tissues that behave and function like natural human tissues, then we can do a lot of the testing of chemicals and drugs more efficiently and effectively. That will allow us, first of all, to eliminate animal testing." Not only is animal testing a long and expensive undertaking, he said, but the results often aren't translatable to humans. "A mouse does not behave in the same way to a particular chemical as a human does. So if we can actually make human tissues, then we can eliminate these differences between how an experimental animal responds to drugs and how a human responds."

As an example, researchers could construct engineered tissue to mimic human cancerous tissue. "That gives us a model of cancer. Then we can test different drugs against this cancer model in a dish and see which drugs work the best," he noted. Unlike animal testing, which is performed one drug at a time, the work with engineered tissue can proceed much more efficiently. He added, "We can not only do orders of magnitude more testing than what's currently done, but we can also do it a lot cheaper."

Drug testing with engineered tissues is a new field, but it will become more prevalent, he predicted. "For really widespread application, I think it's still going to take a few more years, but definitely I think the opportunity is there. It's become a very dynamic field that many researchers are working on and trying to push forward."

Drug delivery may also benefit from bioMEMS, Khademhosseini said. He proposed using bioMEMS technologies to generate differently shaped micro- and nanoparticles, and then encapsulating drugs within the particles. "If you inject the same drug into the circulation, but encapsulate the drug in differently shaped nanoparticles, these particles would go to different parts of the body," he said. This would allow for targeted drug delivery, which could decrease the amount of drugs patients need and also cut back on any potential side effects.

Past, Present, and Future

"If you look historically at how bioMEMS evolved, the process involved mostly understanding fluidic flow and biological sensing at the micro- and nanoscales. We didn't know the physics of fluids at that length scale very well," Toner said.

Although some questions still remain, researchers gained enough knowledge over the years to begin making actual devices. "Some of the early MEMS devices go back to the late 1970s," he said. Since then, the emphasis has been on making things tinier and tinier. "Investigators were so driven with the idea of building smaller things that it was almost like going to the Wild West and putting a flag on the ground saying, 'This is my ground.' Investigators were making the next smallest electrophoresis device or flow cytometer or chromatography device. There was a lot of miniaturization for miniaturization's sake and impressive engineering achievements, while the search for truly enabling applications was falling behind technology development." At the same time, he said, researchers were gaining insight into and experience with the manufacture of MEMS and bioMEMS, and learning about both the sensitivities and limitations of the devices.

This expanding foundation of understanding has made possible medical applications that will change the way medicine is done. As the field of bioMEMS matures, many laboratory tests will occur at home with a simple prick of the finger and have results delivered in minutes rather than days; faster test results will help doctors modify treatments for individual patients; new drugs will find their way to the bedside faster as multiple drug candidates go through screening simultaneously and without the need for animal testing; the shortage of donated organs will begin to diminish; and point-of-care devices will allow people in developing countries to receive far-improved care.

Other current or forthcoming bioMEMS applications include electrode arrays that act as artificial retinas for use in patients with macular degeneration and retinitis pigmentosa; new and improved instruments for image-guided surgery; easy-to-swallow imaging devices (endoscopes) that reveal internal organs and body cavities without patient discomfort; DNA microarrays to study gene activity and to detect genes and mutations; and sensors for environmental testing, including the rapid detection of pathogens. The possibilities are seemingly endless.

"What's happened over the last five years or so is that all of this knowledge is coming together in a very positive way: People have started exploring exciting clinical and biological applications for these devices, because we now understand it enough to manufacture them in the research lab and in the factory," Toner said.

He added, "It's taken 30 years, but the field, in my view, is at an inflection point where we are finally exploring some value-added applications. It's becoming quite exciting."

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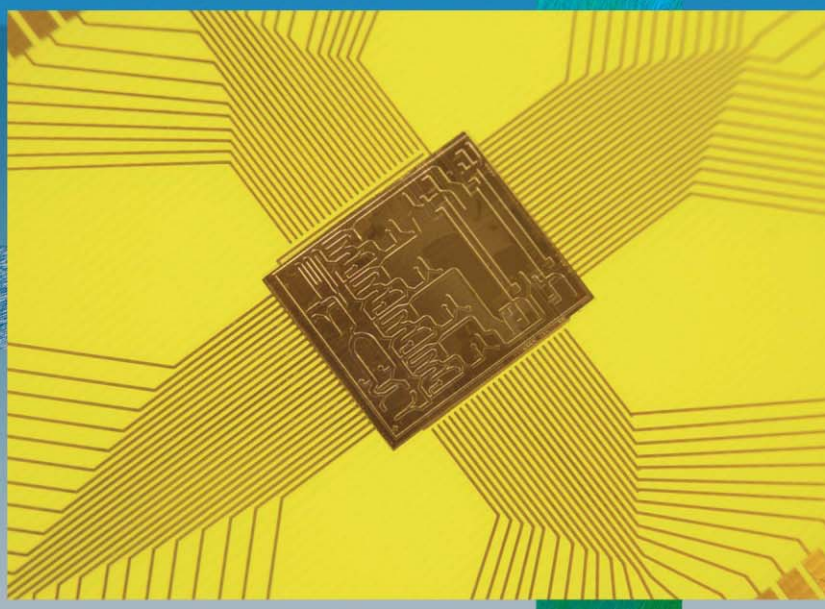
On a Chip



By Nicholas Watkins, Daniel Irimia, Mehmet Toner, and Rashid Bashir

Biological or biomedical microelectromechanical systems (BioMEMS) are poised to have a significant impact on clinical and biomedical applications. These devices ~~also~~ termed *lab-on-chip* or *point-of-care (POC) sensors* ~~re~~present a significant opportunity in various patient-centric settings, including at home, at the doctor's office, in ambulances on the way to the hospital, in emergency rooms (ERs), at the hospital bedside, in rural and global health settings, and in clinical or commercial diagnostic laboratories. The potential impact of these technologies on the early diagnosis and management of disease can be very high for sensing and reporting on parameters ranging from physiological to biomolecular. As health-care delivery and management become increasingly personalized and individualized and as genomic, proteomic, and metabolic technologies unravel the human genetic and epigenetic dispositions to disease, detection of multiple markers (at any of the Omics scale) at an individualized level to assess the state of health and disease will become even more important.

BioMEMS in Clinical and POC Applications

Various diseases afflicting the human condition worldwide and in the United States can be generally categorized into communicable or noncommunicable diseases. The eradication and management of these diseases represent one of the biggest challenges facing modern day society, both in terms of loss of human lives and monetary burdens on society. The key communicable diseases such as human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS), malaria, tuberculosis (TB), and other diseases caused by infectious agents continue to be a major cause of death and suffering worldwide. The key noncommunicable diseases such as cardiovascular, cancer, diabetes, and chronic respiratory diseases have emerged relatively unnoticed in the developing world and are now becoming a global epidemic. In 2008, 36 million people died from noncommunicable diseases worldwide, representing 63% of the 57 million global deaths that year [Figure 1(a)]. By 2030, noncommunicable diseases are projected to claim the lives of 52 million people, nearly five times as many deaths as communicable diseases worldwide, including in low- and middle-income countries [1].

POC, clinical BioMEMS can make a significant impact on these grand challenges. Cancer and cardiovascular diseases are the two leading causes of death in the United States, as shown in Figure 1(b). In 2010, about 1.5 million Americans were diagnosed with cancer and about 570,000 were expected to die of cancer [2]. The National Institutes of Health estimates the overall cost of cancer in 2010 at US$263.8 billion. Despite considerable effort, there has been limited success in reducing per capita deaths from cancer since 1950. This calls for a paradigm shift in the understanding, detection, and intervention of the

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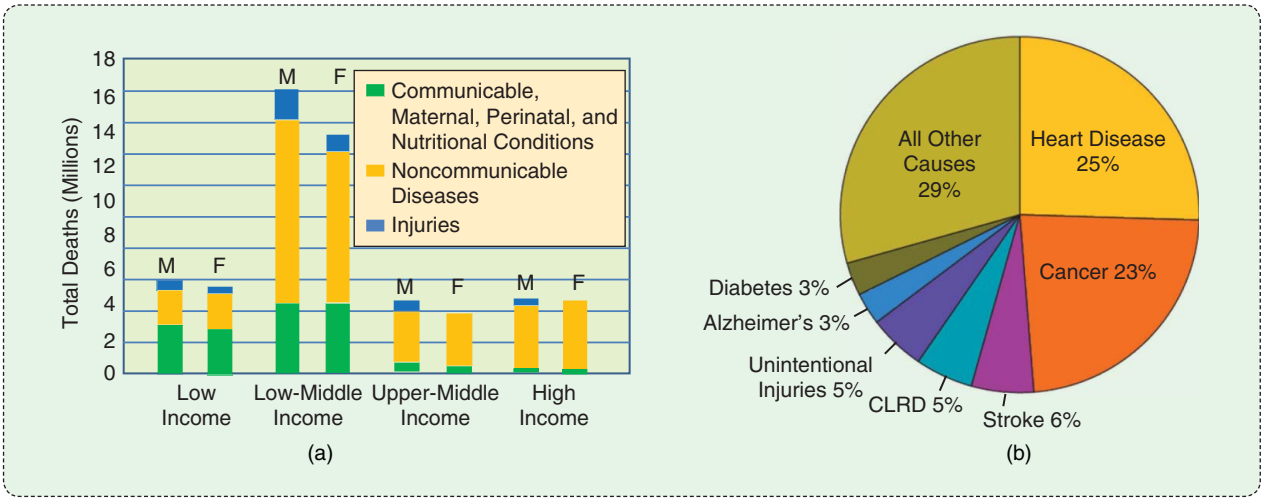

FIGURE 1 (a) Total deaths reported worldwide by Broad Cause Group, World Bank by income group and gender, 2008. (Adapted from WHO, the global status report on noncommunicable diseases in 2010 [1].) (b) Cause of death for all ages in 2007 in the United States. (CLRD: chronic lower respiratory diseases [3].)

evolution of cancer from a single cell to tumor scale. POC clinical BioMEMS can be used for the collection of circulating tumor cells (CTCs), detection of protein or deoxyribonucleic acid (DNA) cancer biomarkers from serum, collection of exosomes, and the detection of micro-ribonucleic acid (micro-RNA) for cancer detection and epigenetic analysis.

Similarly, infectious diseases cause 4% of total deaths in the United States, which appears to be much smaller than cancer and heart disease, but take a huge toll on our medical and financial system because of the need for testing in medical laboratories and hospitals. Worldwide, 14–17 million people die of infectious diseases in developing countries. The global health crisis has affected millions of people with HIV/AIDS, malaria, and TB. With more than 33 million people living with HIV/AIDS in the world, obtaining accurate helper T cell and viral load counts at regular intervals is crucial in monitoring the health of an HIV-positive patient's immune system. Rapid and point-of-use detection of these infectious diseases could dramatically change how these diseases are managed and treated.

Cancer and cardiovascular diseases are the two leading causes of death in the United States.

From the user's perspective, these BioMEMS devices for clinical, POC, and biomedical applications can potentially be used in a variety of settings as shown in Figure 2. These settings can range from individualized skin-mount devices measuring vitals and physiological parameters from the skin or beneath it to in vitro analysis of body fluids for markers or diseases or target cells and to one-time-use devices at diagnostic laboratories, doctor's office, or bedsides in the hospital. Rapid diagnostics at these aforementioned settings, in addition to transit to the hospital in ambulance or in ER settings, are also critically needed.

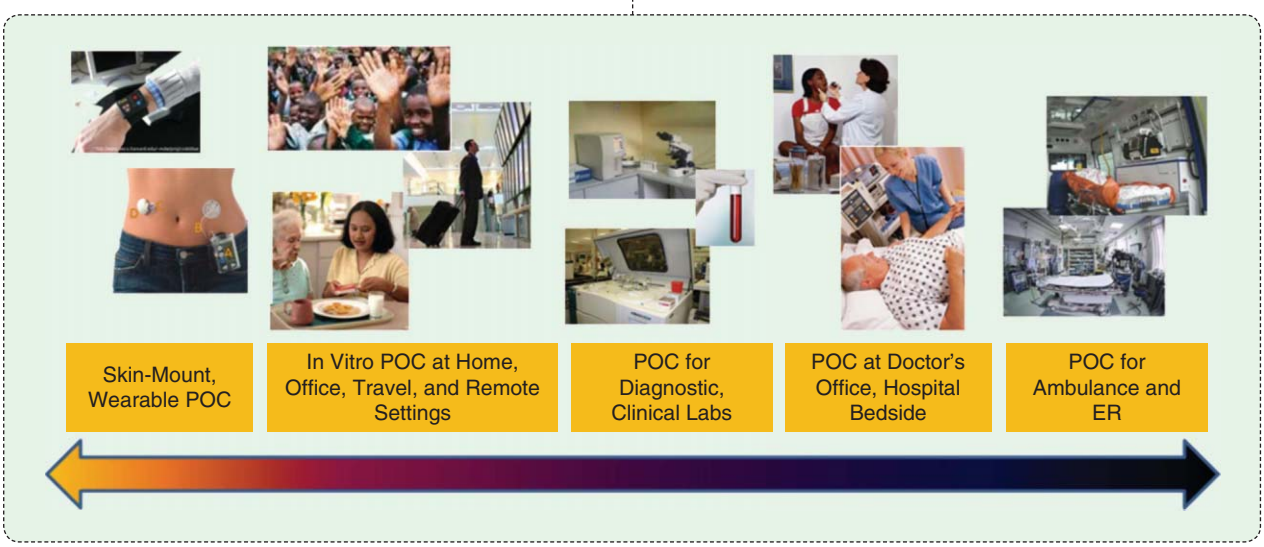

FIGURE 2 Application and settings where clinical POC can be used, ranging from personalized to hospital and ER settings.

Attributes of Biochip Sensors

These BioMEMS and biochips are built from silicon, plastics, or polymer by using micro and nanofabrication technologies. The devices include microfluidic elements such as channels and wells for fluid and sample transport and could employ a range of processing, separation, and sensing modalities (optical and electrical) [4]. These devices can also have integrated sample preparation modules and biological recognition elements such as antibodies or DNA molecules for selective capture on the same chip sensor. Clinical fluids of interest can provide a rich source of diagnostic and prognostic markers for various diseases. The possible targets from clinical fluids of interest include cells, bacteria, viruses, exosomes, protein or nucleic acid-based biomarkers, or small molecules—where many or all of these targets have applications in body fluids such as blood, urine, saliva, and cerebrospinal fluid. Blood is an especially unique tissue with target analytes that can be used for early detection or determining the progression of disease [5]. However, extracting and separating the target entities of interest from the blood can be particularly challenging. As depicted in Figure 3, separating target subpopulations of cells from whole blood requires the handling of volumes of blood that can span more than three orders of magnitude.

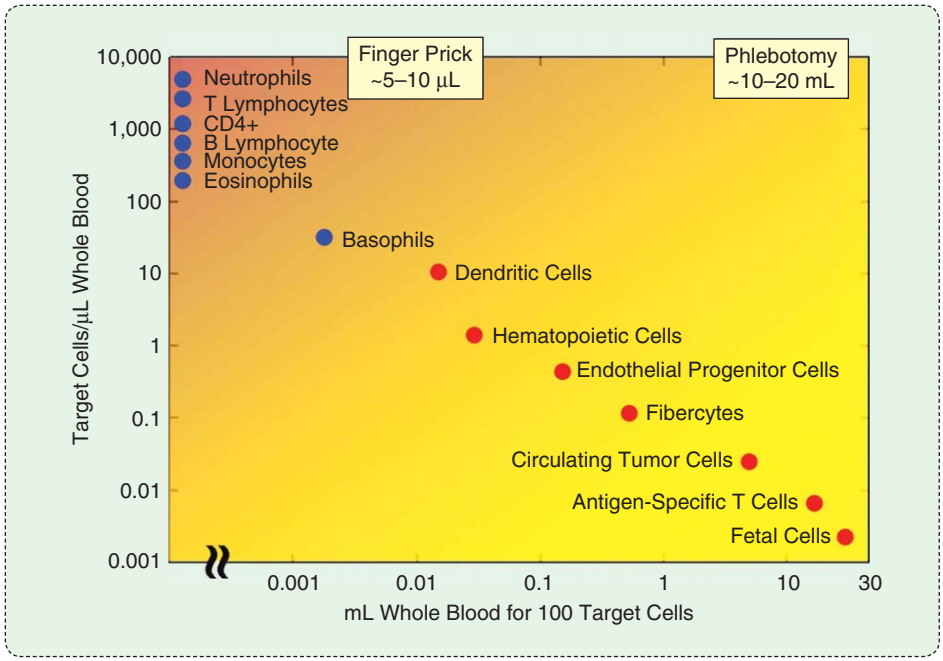

FIGURE 3 Mining blood for cellular information. Human blood contains a large variety of cells that are important for clinical diagnostic or monitoring treatment. Separating target subpopulations from whole blood requires the handling of volumes of blood that can span more than three orders of magnitude.

The diagnostics and prognostics for diseases could represent the largest and most fruitful area in BioMEMS. Figure 4 depicts a schematic overview of such a device, highlighting the various steps for detection and analysis of biological targets for clinical or biomedical applications. In general, the use of micro- and nanoscale technologies is well justified because of the following

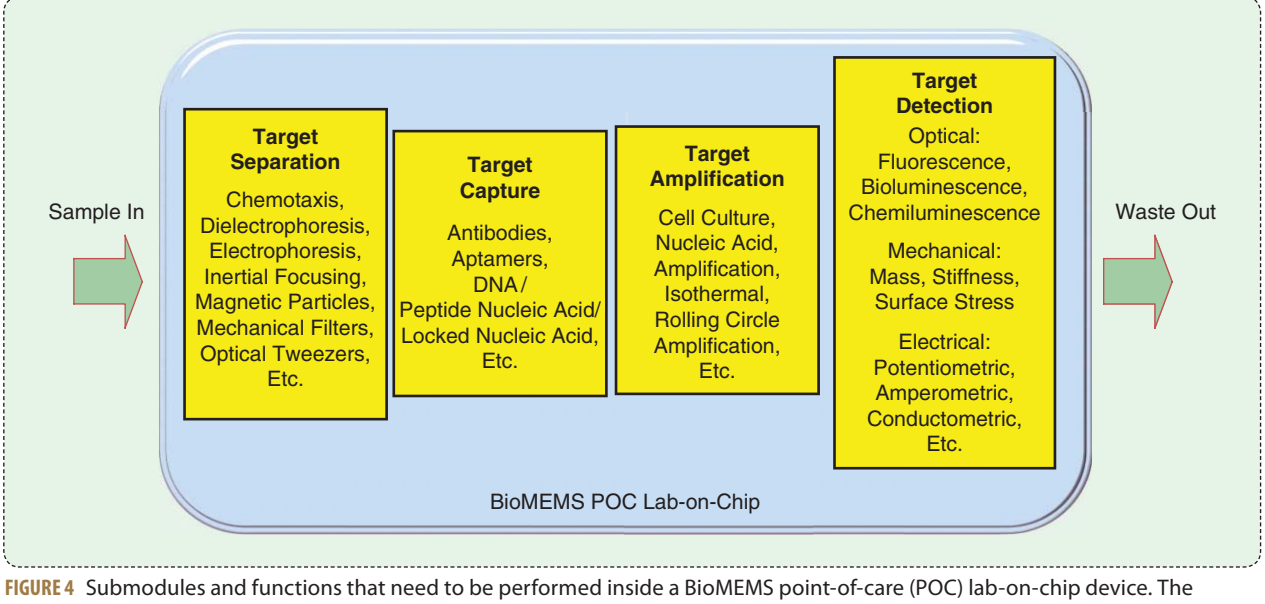

FIGURE 4 Submodules and functions that need to be performed inside a BioMEMS point-of-care (POC) lab-on-chip device. The sample is processed and target analytes, molecules, or cells are captured via recognition elements. The target molecules or the source of the target, e.g., cells, are amplified. Finally, the target is detected and identified using different possible approaches that could require a label or might be label-free.

reasons: 1) reduced time-to-result due to small volumes resulting in higher effective concentrations, 2) reducing the sensor element to the scale of the target species and hence providing a higher sensitivity, 3) reduced reagent volumes and associated costs, 4) providing one-time-use disposable sensors and cartridges, and 5) the possibility of portability and miniaturization of the entire system. Various sensing modalities are used in these devices including optical, electrical, or mechanical. Optical detection techniques are perhaps the most common because of their wide use in biology and life sciences. Optical detection techniques can be based on fluorescence or chemiluminescence.

The miniaturization of electrophoresis devices, biomolecular sensors, and detectors has been of wide interest, and as the quantity of reagents, sample, and labels are reduced, the demands on improving signal-to-noise ratio and sensitivity are increased. Mechanical detection for biochemical entities and reactions has more recently been used through the use of micro- and nanoscale cantilever sensors on a chip. These devices can be used in two modes, namely, stress sensing and mass sensing. In the stress sensing mode, the biochemical reaction is performed selectively on one side of the cantilever. A change in the surface free energy results in a change in surface stress, which results in measurable bending of the cantilever. Thus, label-free detection of biomolecular binding can be performed. The bending of the cantilever can then be measured using optical or electrical means. In the mass sensing mode, the cantilever is excited mechanically so that it vibrates at its resonant frequency. The resonant frequency is measured using electrical or optical means and compared with the resonant frequency of the cantilever once a biological entity is captured. The mass change can be found by detecting the shift in resonant frequency. One of the main advantages of the cantilever sensors is the ability to detect interacting compounds without the need of introducing an optically detectable label on the binding entities. In recent years, exciting and significant advances in biochemical detection have been made using cantilever sensors, and direct, label-free detection of a whole host of entities and mechanisms has been reported from DNA, protein, viruses, cells, and cell growth [6]–[9].

The diagnostics and prognostics for diseases could represent the largest and most fruitful area in BioMEMS.

In addition to the mechanical approaches listed earlier, electrical or electrochemical detection techniques have also been used in biochips and BioMEMS sensors. These techniques can be amenable to further portability and miniaturization, and handheld devices containing these sensors can be easily produced. Electrical detection includes the following basic types: 1) amperometric biosensors, which involves the electric current associated with the electrons involved in redox processes; 2) conductometric biosensors, which measures the conductance changes associated with changes in the overall ionic medium between the two electrodes; 3) potentiometric biosensors, which measure a change in potential at electrodes due to ions or chemical reactions at an electrode [such as an ion-sensitive field effect transistor (ISFET)]; and 4) giant magnetoresistive (GMR) sensors, where a change in electrical conductivity is measured when biomolecule-laden magnetic beads are brought close to thin GMR films. The most widely used example of amperometric sensing is the detection of glucose based on glucose oxidase, which generates hydrogen peroxide and gluconic acid in the presence of oxygen, glucose, and water. Then, hydrogen peroxide is reduced at 600 mV at a Ag/AgCl anode reference electrode. Conductometric sensors measure the changes in the electrical impedance between two electrodes, where the changes can be at an interface or in the bulk region and can be used to indicate a biomolecular reaction between DNA, proteins, and antigen/antibody reaction, or excretions of cellular metabolic products indicating cell growth [10]. And finally, potentiometric sensors use the measurement of potential at an electrode with reference to another electrode. The most common form of potentiometric sensors are the ISFETs or chemical FETs (chemFETs). These devices are commercially available as pH sensors, and many examples have been reported in literature. The potentiometric sensors have been downscaled to nanometer dimension through the use of silicon nanofabrication to take advantage of enhanced sensitivity because of higher surface area-to-volume ratio [11]. GMR sensors require a magnetic particle as a label that is functionalized with capture molecules. The particle acts as a label in a sandwich enzyme-linked immunosorbent assay (ELISA) format performed on thin microfabricated GMR films. These sensors, while requiring a magnetic label, can provide for a high sensitivity multiplexed detection of cancer markers, with high signal-to-noise ratio and wide dynamic range in clinical samples [12].

Example 1: Complete Blood Count on a Chip

A complete blood count (CBC) is a diagnostic screening tool that provides clinicians with a broad assessment of a patient's health status through a comprehensive analysis of his or her circulating blood cells. This fundamental test is normally ordered upon patient admission to obtain an initial snapshot of a patient's health status, allowing clinicians to act rapidly in determining the appropriate treatment for disorders such as infection, anemia, cancer, nutrient deficiency, and other diseases. Current CBC tests require multiple analysis tools and trained technicians, which not only make the tests prohibitively expensive for many patients but require patients to travel to overcrowded centralized clinical facilities that may provide results in several days or even weeks. A killer application for POC clinical BioMEMS could be a CBC on a chip, which could revolutionize the health-care infrastructure by not only decreasing personal health-care costs but also providing a rapid and comprehensive assessment during a physician's visit, or even at the convenience of one's own home, regardless of geographic and economic constraints.

As a basis of such a device, an HIV/AIDS diagnostics chip that selectively enumerates T lymphocytes expressing the CD4+ cell receptor on their surface, using affinity chromatography coupled with electrical impedance sensing, has been recently reported [13]. The design employs the Coulter principle to electrically count the total number of purified leukocytes entering the chip before depleting CD4+ T lymphocytes in an antibody-coated

capture chamber [14]. The CD4+ T cell count is simply obtained by subtracting the entrance count from the number of uncaptured leukocytes exiting the chip. This method has been proven to closely correlate with an optical counting method with low inherent error, showing it to be a feasible technology for portable and rapid blood diagnostics.

An electrical chip-based CBC can be realized using the differential counter module as a building block to enumerate and analyze the various blood cells (Figure 5). A whole blood sample is injected into the microfabricated chip and is equally split into two branches. Branch A selectively removes erythrocytes through a chemical reaction to lyse their membranes, leaving the leukocytes intact [15]. The purified population of leukocytes can be evenly split among several differential counting modules with capture chambers coated with antibody concoctions specific to the five main leukocyte subtypes (e.g., CD19 antibody is specific to monocytes). The total leukocyte count is obtained at the entrance of each differential counter module. Additional capture chambers with other antibody mixtures can create a more comprehensive test, such as CD4+ T cells for HIV/AIDS diagnostics. Branch B analyzes the blood sample for platelet and erythrocyte concentrations by diluting the sample to ensure that only one cell crosses the electrical sensing region at an instance [16]. In addition, the impedance pulse analysis enables the measurement of erythrocyte volume to obtain the hematocrit level (anemia) in addition to the average erythrocyte volume (iron, B12, folate deficiencies, and thalassemia) and erythrocyte distribution width (erythrocyte maturity).

Example 2: Measuring Neutrophil Motility on a Chip

Neutrophils are the first line of defense against infections. They represent the most numerous subpopulation of white blood cells in the blood and have the ability to migrate within minutes from the blood to the site of infection or injury in the tissues. They accomplish this demanding task by following chemical concentration gradients of attracting molecules released from the invading bacteria or the cells under stress. The motility function of the neutrophils is of great medical interest because failure of neutrophils to promptly arrive at sites of infection or inflammation can result in uncontrollable infections, while overzealous neutrophil infiltration can unnecessarily damage normal tissues and impair organ function, e.g., in severe forms of asthma and arthritis, acute hepatitis, or ischemia-reperfusion injury. However, measuring the motility of neutrophils is not an easy task. In a research environment, most common protocols use Boyden chambers [17]; however, these devices rely on gradients that can change in poorly controlled ways, and they only provide limited information and indirect measures of motility, e.g., the number of cells that pass through a membrane with pores.

The early application of microfluidic technologies to neutrophil motility studies helped solve the problem of gradient stability and facilitated the dynamic observation of neutrophil migration with single-cell resolution [18]. Microfluidic devices quickly become research tools for important problems in the biology of neutrophil migration [19], from the migration against gradients [20], responses to changing gradients [21], or the integration of average and slope of chemical gradients [22]. However, moving forward from these pioneering devices to robust systems to measure neutrophil motility in clinical settings requires designs that can integrate an array of distinct functions to be integrated on the same chip. Devices should be able to separate the neutrophils from whole blood quickly and, without activation, generate precise chemical gradients and measure neutrophil motility parameters automatically with minimal operation from the human operator. Recent advances in the field of microfluidic devices for neutrophil chemotaxis are encouraging, and these goals may be achieved in the near future.

First, separating the neutrophils on the chip can bypass the complex procedures for isolating neutrophils from whole blood, speeding up the assay, and eliminating the need for bulky centrifugation equipment. One recent example is a microfluidic chip that can both isolate neutrophils and monitor their migration in stable chemical gradients [23]. Only a small drop of blood from the finger is required for the assay that has orders of magnitude less than the standard assays. Neutrophils are captured inside the chip in a chamber coated with cell adhesion molecules that weakly interact with the target cells. The same weak interactions allow the cells to move at a later step when a

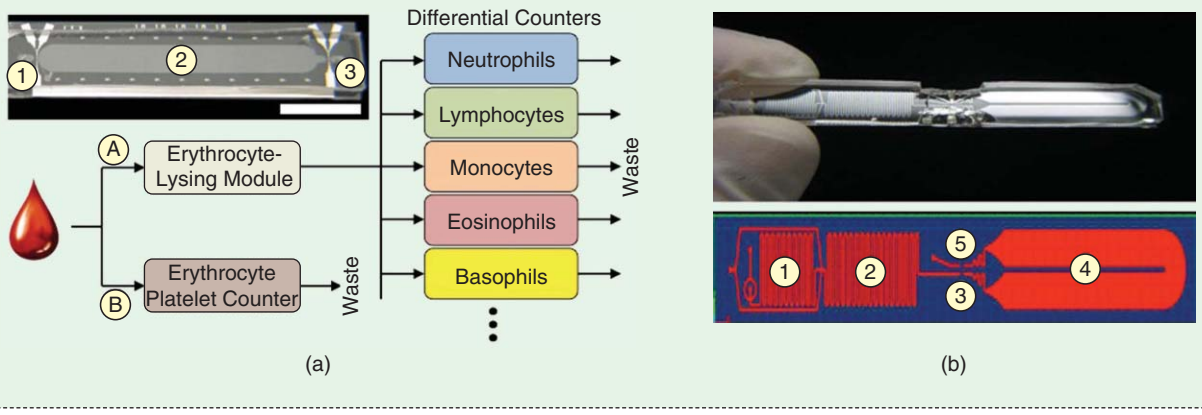

FIGURE 5 (a) Mapping of CBC on a chip. A differential counter design with 1) an entrance counter, 2) capture chamber, and 3) exit counter. The scale bar is 1 cm. (Adapted from [18].) (b) An *n* improved differential module, which has a 1) red blood cell lysis region, 2) lysis-quenching region, 3) entrance counter, 4) capture chamber, and 5) exit counter.

concentration gradient of a chemoattractant is applied. With the use of a microscope, images of the moving cells are captured and their motility is measured. Although effective, the arrangement of valves, inlets, and outlets on the chip required to accomplish the sequence of steps for the assay could become problematic for automation. Second, the precision of measuring the neutrophil motility in microfluidic devices can reach beyond the capabilities of any other method available today. Such advance was possible through the use of linear channels with cross sections smaller than the size of neutrophils (Figure 6), which could be regarded as the equivalent of speed tracks in the macroworld [24]. Using this device, for the first time, a normal range of neutrophil directional migration speed values was defined for healthy volunteers, independent of sex or age of the donor, and reproducible from the same volunteer at time points separated by weeks. The device also enabled the first precise measurement of the neutrophils motility alteration in hospitalized patients after burn injury. Although the device uses no external syringe pumps and has only one inlet and one outlet, it still requires the preliminary separation of neutrophils from the sample blood using standard techniques. Finally, high-throughput devices capable of performing more than 50 neutrophil migration assays simultaneously have been reported through the use of microfluidic technologies [25]. The new platform can operate at single-cell resolution to perform a high content analysis of motile neutrophils in a large number of independent chemoattractant conditions and screen for the effect of various drugs. However, neutrophils have to be separately purified before the assay, and the precision of measurements is limited by the physiological noisiness of speed and directionality during neutrophil migration on flat surfaces.

Although some elementary functions have already been demonstrated, the integration of efficient separation, precise analysis, and high-throughput format functions in the same device is not a trivial task, and creative new protocols and technological developments are required to overcome current obstacles. When accomplished, this integration will provide new tools for the exploration of interesting biological questions and novel applications to clinical conditions. Evaluating the risk of infections in patients at risk, modulating immunosuppressive regiments after a transplant or monitoring the resolution of acute and chronic inflammation could provide valuable insights enabled by devices for measuring neutrophil motility function in the doctor's office.

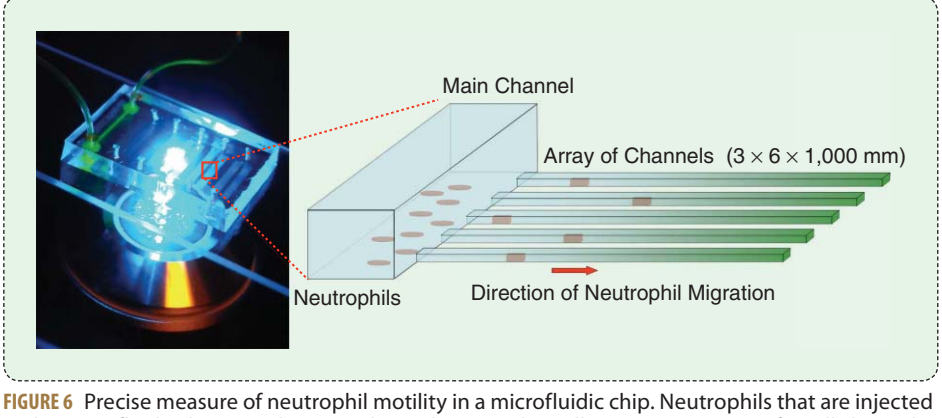

FIGURE 6 Precise measure of neutrophil motility in a microfluidic chip. Neutrophils that are injected in the microfluidic device in the main channel migrate laterally to enter an array of small channels filled with chemoattractant, toward which they move at uniform speed [24].

Example 3: Bacterial Detection on a Chip

The capture and detection of bacteria and viruses from body fluids at POC also represents a grand challenge. Figure 4 depicted the concept schematic of a lab-on-chip that conforms well to the detection of infectious agents. Such an integrated device would be capable of separating and extracting bacteria from blood, perform sorting and concentration of these microorganisms if needed, perform antibody-based capture of target organisms, possibly perform bacterial culture inside the chip to determine the presence of live or viable cells, and also to increase the number of cells needed for analysis, lyse those cells, and then perform a nucleic acid-based identification of them.

The separation of the bacteria (1 μm or so in size) or viruses (50–250 nm in size) from body fluids poses a great challenge, as discussed earlier. Cells occur at a much higher number and, hence, increase the background noise, especially from blood. The problem of viral separation is intensified because of the presence of similarly sized exosomes that are present at much higher concentrations than target viruses. Red blood cells and hemoglobin are also known to reduce the sensitivity of polymerase chain reaction (PCR) assays and, hence, separation of the target bacteria (1 μm or so in size) and viruses (50–200 nm in size) from other blood cells is critically important before the identification of these entities is performed.

For the case of identification, nucleic acid-based methods are still considered the gold standard because of their high specificity and selectivity when compared with antibody-based assays. However, the high cost of reagents and instrumentation for optical detection of the amplified PCR product and the time it takes to perform the amplification have still limited the realization of these methods as truly a POC test with total assay time of sample-to-result in, for example, less than 10 min. The grand challenge of taking a throat swab, sputum, or a sample (or drop) of blood or urine and obtaining an identification of bacteria or viruses at low concentrations for early detection still remains unfulfilled. The target bacterial counts can be just a few colony forming units (cfu) per milliliter of sample fluid at the onset of infections. In the case of elevated infection levels, the numbers can be in tens of thousands to millions of bacteria per milliliter, in which case the detection can be performed by current techniques. It should also be noted that many bacteria might survive or replicate within phagocytes. The best commercial assays for PCR claim 20 min of assay time from a 10- to 20-µL sample as long as few copies of the

target DNA are present in that volume. This means that, for detection at low concentrations (onset or early detection), either the bacteria needs to be grown or amplified by traditional petridish cultures, or separate or concentrated from a larger volume sample into smaller volumes, before they can be detected using biomolecular assays. It should be noted that, for the detection of bacteria from drinking water, environmental samples, or pharmaceutical fluids, the target concentrations are even lower at 1 cfu/100 mL to 1 cfu/250 mL. For these applications, the advantages offered by microfluidics and lab-on-chip can only be useful if the target bacteria can be concentrated from these large volumes down to 0.1–1 mL of fluids before they are introduced into these small devices.

Integrated biochip devices that can capture, trap, and detect specific bacteria based on dielectrophoresis and antibody-mediated capture [26] have been shown. In addition, to reduce the time for detection of bacteria culture and perform the detection electrically, microfluidic chips in which a small number of bacterial cells could be concentrated from a dilute sample into nanoliter volumes and then cultured have also been demonstrated. Figure 7 shows the silicon biochip platforms used for these studies. Taking advantage of the small volume of the biochip, 10^4- to 10^5-fold concentrations of bacterial cells from a dilute sample can be achieved. Similar platforms can be used to perform PCR amplification, and the detection of as few as 10–50 cells of *Listeria monocytogenes* has been performed using optical fluorescence detection [27].

Example 4: CTC-Chip and Circulating Tumor Cells for Cancer Diagnostics

Blood-borne metastasis is initiated by cancer cells that are transported through the circulation from the primary tumor to vital distant organs, and it is directly responsible for most cancer-related deaths [27]. However, CTCs are extraordinarily rare (estimated at one CTC per billion normal blood cells in the circulation of patients with advanced cancer), and they have been proven to be too difficult to isolate in sufficient numbers in order to be clinically useful. The clinical use of CTCs is very broad and includes the monitoring of individual patients with metastatic disease, noninvasive analysis of biomarkers, and acquired resistance to specific treatment regimens in targeted therapies, and, ultimately, early detection of cancer. Despite the fact that the potential of CTCs in the clinical management of cancer patients is significant, the bottleneck has been the development of highly sensitive, high-throughput, and reliable technological platforms for isolation of CTCs from the peripheral blood of patients.

The most widely used CTC isolation techniques rely on antibody-based capture of CTCs, which express epithelial cell surface markers that are absent from normal leukocytes [28]. The Toner group has developed several microfluidic approaches called *CTC chip* for single-step isolation of CTCs from unprocessed blood specimens [29]–[32]. The first-generation CTC chip is composed of etched 78,000 microposts in silicon that are coated with an antibody specific to epithelial cells (Figure 8). Whole blood flows through the chip, and the flow is precisely controlled to enhance interaction of cells with antibody-coated microposts to maximize binding of tumor cells to the surface of the microposts. Isolated CTCs are then analyzed either by direct imaging or by molecular approaches. Flow kinetics has been optimized for minimal shear stress on cells while enhancing contacts with the antibody-coated microposts. The CTC chip enables a high yield of capture (median, 50 CTCs per milliliter) and purity (ranging from 0.1% to 50%), most likely caused by the gentle one-step microfluidic processing, which may be critical when purifying rare delicate cell populations [29]. More recently, we have developed the second-generation microfluidic technology using an enhanced platform called the herringbone (HB) chip [32]. The HB chip makes use of a microvortex-mixing

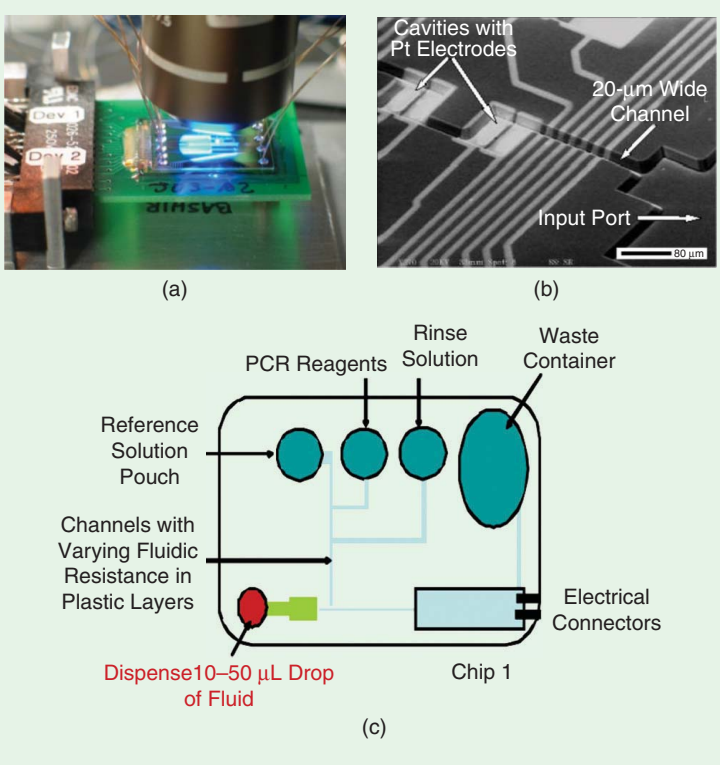

FIGURE 7 (a) The optical micrograph of a microfluidic biochip for bacterial culture and identification [10], [26], (b) scanning electron micrograph (SEM) images of the channels and wells inside the chip, and (c) concept schematic of a cartridge housing the chip along with reagents needed to perform biomolecular assays.

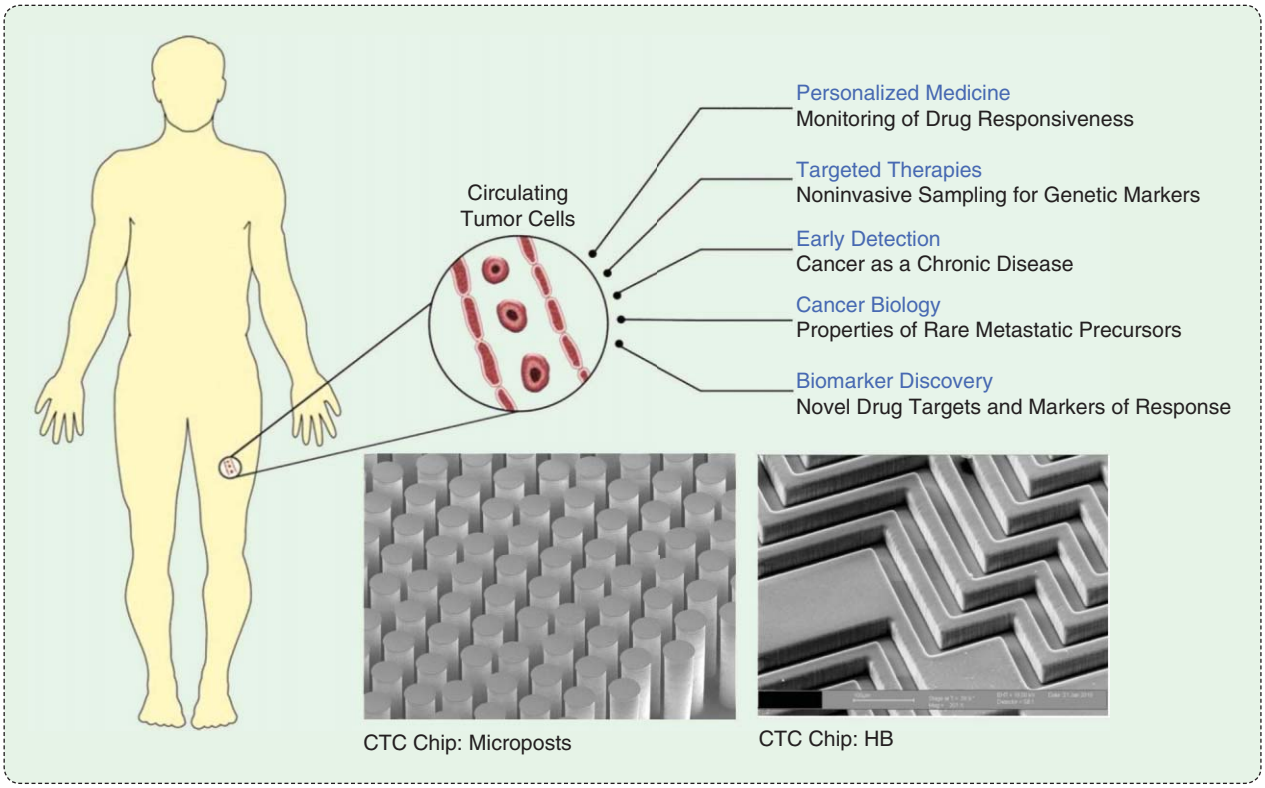

FIGURE 8 CTCs have a broad range of applications in the management of cancer patients. The two microfluidic technologies developed in the Toner group are called CTC chip and are based on chemical capture of CTCs from whole blood. The original work was based on increasing the interaction of CTCs with microposts that are chemically modified with an antibody to bind to CTCs and not to leukocytes or blood cells. The second-generation technology replaced the microposts with an HB pattern only on the top surface to achieve a gentle mixing of blood as it flows through the chip to bring CTCs in contact with the antibody-coated surfaces of the CTC chip. The key structures in both cases vary between 20 and 50 mm. The details of the chip geometry are given elsewhere [29]–[32].

device to capture CTCs from whole blood. The simple design of the HB chip is conducive to high-throughput manufacturing out of transparent materials, which greatly enhance high-resolution imaging, including the use of transmitted light microscopy. The ability to further simplify the device design and reduce the manufacturing cost while increasing reliability will ultimately enable the validation of CTC chip in multicenter clinical trials for identification and validation of clinical utility.

Summary

The future of clinical and POC BioMEMS is very bright. With an increasing emphasis on the personalization of medicine and the rising costs of health care, early detection and diagnostics at the POC will be even more important. Early detection implies early intervention, resulting in the saving of lives and reducing overall spending. The potential impact of these technologies on the early diagnosis and management of both communicable and noncommunicable diseases is very high. Many grand challenges applications are possible, e.g., routine tests such as complete blood cell count on a chip that an individual can perform at home; detection of cardiac markers from blood after a perceived heart attack; detection of cancer markers such as exosomes, CTCs from blood, or protein biomarkers in serum; and detection of infectious agents such as virus and bacteria for public health. These applications are expected to result in new diagnostic assays for home, doctor's office, clinical laboratories, and various POC settings.

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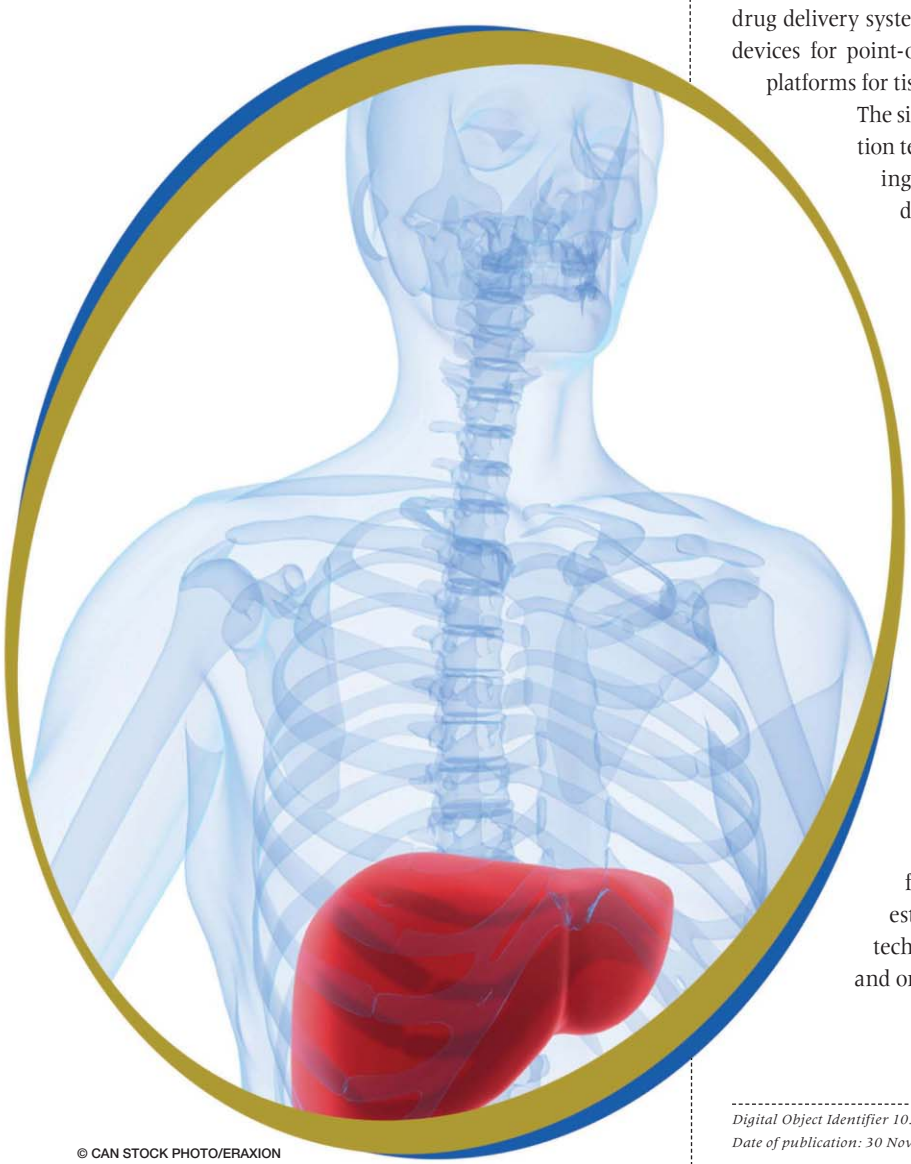
Engineering Tissue with BioMEMS

Applications of BioMEMS Technology

By Jeffrey T. Borenstein and Gordana Vunjak-Novakovic



The emergence of biological microelectromechanical systems (BioMEMS) technology has been spurred by the development of precise microscale fabrication techniques across a wide range of biomaterials substrates. BioMEMS devices are now finding application in areas ranging from genomics and proteomics to clinical diagnostics and implantable drug delivery systems. The design principles of early lab-on-a-chip devices for point-of-care diagnostics are now being extended to platforms for tissue engineering.

The simplicity, ease, and low cost of BioMEMS fabrication techniques have been principal drivers in advancing the technology in applications ranging from the development of in vitro tissue models for drug discovery and toxicity screening to artificial organ-assist devices and implantable tissue constructs. However, it is the ability of BioMEMS processing techniques to work with an enormous range of biomaterial substrates that has enabled the technology to advance rapidly in many tissue-engineering applications. BioMEMS devices can be built to mimic the mechanical and biochemical microenvironments of tissues and organs, and this is easily achieved by controlling the scaffold properties such as elastic modulus, porosity, internal architecture, and the rates of mass transport and material biodegradation.

In this article, we describe four interesting applications of BioMEMS technology in tissue engineering: the formation of vascular networks; engineering vascular beds for organs such as the liver, kidney, and lung; microfluidic bioreactors for screening stem cells; and establishment of patterned tissue interfaces. These techniques may enable the replication of the tissue and organ microenvironment on a small scale.

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Engineering Vascular Networks

One of the most significant challenges in the field of tissue engineering has been to provide an intrinsic vascular supply necessary to maintain the viability and well being of tissues at a meaningful scale for clinical use [1]. Early efforts to generate tissue constructs for highly metabolic organs, such as the heart or organs with very low oxygen diffusion coefficients (e.g., the liver), resulted in the formation of necrotic regions as the thickness of the engineered tissue increased above just a few hundred micrometers. Therefore, strenuous efforts have been focused on the methods to replicate the highly efficient oxygen and nutrient transfer of physiologic tissues using a host of microfabrication techniques.

Microfluidic Approaches for Vascularization

Vascular networks for engineered tissues have been produced using growth factors, either singly or in combination, as functionalized moieties in a variety of scaffold materials. Early work by Richardson et al. [2] demonstrated that the spatiotemporal control of growth factors introduced in a stepwise fashion in a poly(lactic-co-glycolic acid) scaffold resulted in the formation of stable microvascular networks. A functional vasculature formed from human embryonic stem cells (hESCs) in a poly(lactic acid) (PLA)-based scaffold was demonstrated by Levenberg et al. for the generation of vascularized skeletal muscle tissue [3].

More recently, the focus has shifted toward hydrogel matrices, where the coculture of endothelial cells with tissue-specific progenitor cells has produced microvascular networks. For example, micromolded collagen gels have been seeded with endothelial cells with the establishment of barrier function and resistance to leukocyte adhesion [4].

One of the principal challenges associated with BioMEMS-based microvascular networks is the inherently planar nature of MEMS fabrication techniques, leading to networks comprised of stacks of two-dimensional (2-D) capillary beds connected only by large conduits in the vertical dimension. This shortcoming has been addressed by two interesting and potentially very powerful techniques capable of generating branching networks of small channels in all three dimensions. In one case, a sacrificial spun sugar (cotton candy) technique has been developed where a polymer scaffold such as polydimethylsiloxane (PDMS) is cast around the cotton candy fiber network [5], followed by the removal of the sacrificial material to create a network of capillary-sized branching channels oriented in all directions. Ugaz [6] has reported the use of electrical discharge in polymer scaffolds to generate a similar vascular-like network architecture.

BioMEMS-Based Approaches for Vascularization

BioMEMS and microfluidic techniques are readily applicable to the formation of microchannel networks on size scales suitable for engineered vasculature. About 85% of the cross section of all human vasculature is at the capillary scale, with vessel diameters about 10 μm. These dimensions are easily accessible using conventional photolithographic approaches, whereas solid freeform methods and conventional precision machining are typically limited to an order of magnitude of larger size scales. BioMEMS approaches often utilize silicon wafer molds as masters to provide multiple replicas in an array of structural biomaterials including the common microfluidic substrate PDMS, polystyrene [7], and cyclic olefin copolymer [8]. The most common technique for patterning a silicon master mold utilizes EPON SU-8 epoxy resin as a thick photolithographic patterning layer upon which the polymeric films can be cast.

BioMEMS devices can be built to mimic the mechanical and biochemical microenvironments of tissues and organs.

The first endothelialized networks produced using BioMEMS fabrication techniques utilized PDMS as the substrate material [9]. More recently, endothelialized networks have been produced in bioresorbable polymers for implantable applications such as polyglycerol sebacate (PGS). Figure 1 illustrates an endothelialized network created in a PGS scaffold [10].

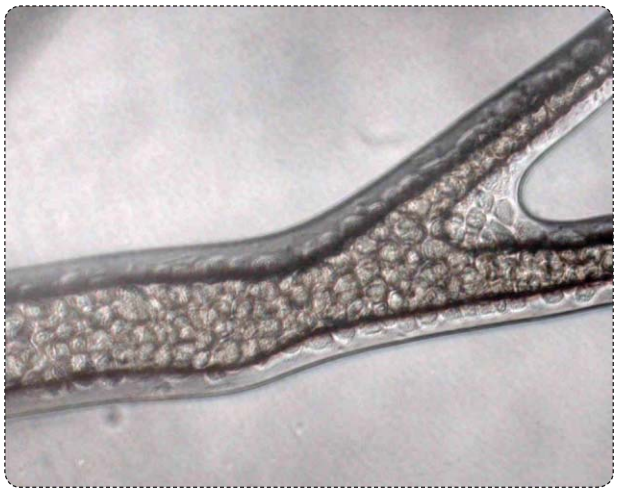

FIGURE 1 A microvascular network. Endothelialized microvascular network formed in a PGS microfluidic scaffold, showing the formation of a confluent endothelium on the inner luminal surface of the channels.

Mimicking the Structure and Function of the Vasculature

Conventional photolithographic approaches are widely used for lab-on-a-chip applications, but geometric considerations limit their applicability for vascularized tissues. Rectangular walls and sharp corners in microchannel networks lead to difficulties in cell seeding and attachment [11], and result in highly nonuniform circumferential wall shear stress profiles.

Several groups have therefore explored alternative microfabrication techniques capable of producing rounded or circular sidewalls in microchannel networks. One such approach utilizes special resist chemistries for thermally driven reshaping of the rectangular sidewalls [12]. Etching techniques, typically using xenon difluoride to produce isotropic profiles, have also been used to generate semicircular trenches in silicon wafers for vascular network applications [13]. Modification of rectangular microchannels after replica molding has been recently demonstrated by a process involving liquid PDMS dispensed within

the channels, followed by the introduction of a pressurized air stream to create rounded sidewalls [14].

While each of these techniques is capable of producing nearly perfect circular sidewalls, a remaining challenge is to vary channel diameter smoothly and gradually when transitioning from one vessel size to another and at vessel bifurcations. Conventional microfluidic fabrication techniques produce sudden transitions at intersections between vessels and in particular at junctions with varying diameter. One approach that has been explored to address this shortcoming is the use of electroplated molds with smoothly varying geometries [15]. These molds have been used to emboss polystyrene films that are assembled to form a closed microvascular network seeded with endothelial cells [12].

Vascularizing Tissues and Organs

Microfluidics and BioMEMS techniques for constructing tissue-engineering scaffolds and bioreactors are particularly well suited for applications in which the target organ comprises a highly vascularized structure, housing intricate cell and fluid spaces that perform specific fluid processing functions. Therefore, the liver, kidney, and lung are among the organ systems that can benefit the most from engineered tissue strategies that utilize BioMEMS and microfluidic technologies. In the liver, hepatocytes are arranged with sinusoidal epithelium along fluid pathways in a cylindrical fashion like the spokes of a wheel. In the kidney, the roughly four million nephrons are structured along a pathway with fluid filtration and resorption functions integrated into a tubular construct with permeable membranes housing renal epithelial cells. In the lung, the alveoli contain air spaces from the bronchial tree separated by microvasculature by a membrane only approximately 1 μm thick, resulting in an enormous surface area and highly efficient gas transfer.

FIGURE 2 A multilayer PDMS microfluidic oxygenator for artificial lung applications. Each of the ten layers of the device comprises a microvascular network and an oxygen delivery compartment separated by a thin (11 μm) membrane.

Fabricating the Liver Tissue

The liver represents the earliest focus of efforts to apply BioMEMS fabrication technology to the challenge of tissue engineering vital organ constructs. Clinically, tissue engineering of the liver has been driven by the fact that unlike the kidney and lung, where maintenance therapies are available, the only sustainable approach for treating end-stage liver failure is an organ transplant. The severe and constantly growing shortage of available donor organs has spurred strenuous efforts to develop tissue-engineered liver replacement systems. In addition, there is a critical need for improved systems for assessing drug safety relative to hepatotoxicity, a predominant cause of post-market-approval failures of new compounds. Owing to these urgent needs and the complexity of tissue architecture, the liver remains a principal target for BioMEMS technology.

Early efforts to apply BioMEMS technology to the liver focused on in vitro models for drug safety and evaluation of chemical and biological agents. One approach utilized micromachined silicon structures enabling perfusion of the liver at fluid flow and shear stress rates that mimic physiological levels [16]. A soft-lithography-based approach has been established as a screening platform for liver toxicity, where heterotypic interactions between hepatocytes and fibroblasts are governed by patterns on stamped substrates [17]. This approach has been integrated with a silicon micromachined comb to control spacing between two cell populations [18]. Microfluidic PDMS constructs have been reported by Leclerc et al. [19], whereas a membrane bilayer system has been shown to replicate the liver function by Carraro et al. [20].

The Shuler laboratory has developed a microfluidic in vitro model for the liver as part of a human-on-a-chip platform [21], whereas Lee et al. have utilized microfabrication technology to establish a perfusion barrier that mimics the sinusoidal structure in the liver [22]. Efforts to develop tissue-engineered liver replacements have explored the use of bioresorbable elastomers as a scaffolding material for implantable applications, where the use of nanopatterning to create an in vivo-like topographic basement membrane has been shown to enhance the function of primary hepatocytes [23].

Microfabricated Devices for Artificial Lungs

Pioneering efforts to develop microfabricated devices for artificial lungs by utilizing arrays of microchannels constructed using conventional MEMS techniques were reported by Mockros and coworkers [24] and Federspiel and coworkers [25]. Gas transport models for microfluidic constructs have been developed to determine whether BioMEMS approaches could achieve superior performance relative to the hollow fiber technology utilized over several decades for applications such as extra corporeal membrane oxygenation and cardiopulmonary support for coronary artery bypass grafts. Federspiel proposed the use of PDMS as a gas permeable membrane separating microchannels from an oxygen delivery compartment and

measured the efficiency of oxygen transfer as a function of membrane thickness.

Recent efforts have focused on critical issues associated with the safety and convenience of membrane oxygenator technology. Hoganson et al. [26] have reported on the development of a network with physiological shear stress. Kniazeva et al. [27] demonstrated a microfluidic oxygenator in PDMS comprising multiple layers in a design ultimately scalable for clinical use; one such device is shown in Figure 2. Potkay et al. [28] have reported on a BioMEMS-based artificial lung that may be operated using room air rather than pure oxygen, a major step toward portable and ultimately wearable systems. In vitro models of artificial lungs have been utilized to study a range of pulmonary diseases by the Takayama group [29], and Huh et al. have proposed an artificial lung unit device that may be coated with the lung epithelial cells for drug discovery applications [30].

Developing Artificial Kidneys

The progress in renal replacement systems has been more limited than for the liver and lung, in part because of the staggering complexity of the kidney in terms of structure and cellular phenotype. Many efforts to develop artificial kidneys for short-term applications have focused on the use of hollow fibers, the gold standard for kidney dialysis in the clinic and nocturnal use in the home. Recently, the Suh laboratory [31] developed an in vitro microfluidic model to investigate renal cell attachment, proliferation, and glucose uptake. A microfluidic bioreactor for the assessment of renal epithelium has been reported [32], and the efforts are being made to mimic the slitlike pores in the nephron using silicon microfabrication technology [33].

Microfluidic Bioreactors

Human stem cells are central to the development of tissue-engineering modalities for regenerative medicine, as well as models for fundamental research, study of disease, and drug development. It is well known that, both in vitro and in vivo, the cells respond to the entire context of their environment, with spatial and temporal cascades of molecular and physical regulatory factors [34]. Therefore, culture systems that faithfully replicate the cell niche found that in vivo would radically improve our ability to utilize the full potential of stem cells in tissue engineering [35].

Microtechnologies, and in particular those based on microfluidic platforms, are being increasingly used to precisely manipulate the cellular microenvironment and study cellular responses in real time and in a quantitative fashion. In these applications, the small scale of the device allows for high-throughput studies within a large experimental space with only minimal amounts of cells and materials, as well as precise environmental control due to efficient mass transport to and from the cells.

Microfluidic techniques based on soft lithography were pioneered by Whitesides and colleagues [36] and have found widespread application in the micropatterning of cells and biomaterials [37] and cell culture. For example, in cultures of hepatocytes, microscale features of these devices were used to mimic some aspects of the microstructure of the liver [38]. In another set of applications, microfluidic platforms of interconnected chambers have been proposed as living cell arrays for studying gene expression [39] and cell coculture [40].

An elegant microfluidic device was developed to culture murine embryonic stem cells (mESCs) with logarithmically scaled perfusion of medium, with more than 3,000-fold variations in flow rates across the array and study the effects of the associated hydrodynamic shear on the size of cell colonies [41]. Subsequently, a microfluidic platform was developed to study self-renewal and differentiation of hESCs over a large parameter matrix and in a semiautomated way [42].

Microfluidic systems can also be used to spatially and temporally investigate the many factors that regulate cell differentiation in high-throughput experiments. Conventional multiwell plates are certainly easy to use, but their limitation is that the composition of medium is constantly changing between medium replacements, thus limiting the control of environmental levels of nutrients and metabolites. On the other hand, perfusion bioreactors enable tight control of concentration levels in the cell environment but generally require large amounts of medium and regulatory factors, making screening studies impractical and expensive [43]. Microarray bioreactors with medium perfusion were developed to combine small scale with medium perfusion, and thereby provide versatile and tightly controlled culture environments for the screening of stem cells and factors regulating their differentiation [44]–[46].

In one set of recent studies [43], [47], a simple and practical device was developed by coupling a microfluidic platform with an array of culture wells to enable systematic and precise variation of mass transport and hydrodynamic shear in cultures of hESCs (Figure 3). This microarray bioreactor with 12 culture wells on a standard microscope slide format was fabricated using soft lithography and designed to accommodate stem cells attached to a 2-D substrate and cells encapsulated in a three-dimensional (3-D) hydrogel. Both culture formats allow for controlled perfusion of medium and tight control of medium composition and hydrodynamic shear. Using this microfluidic platform, hESCs were systematically studied for their

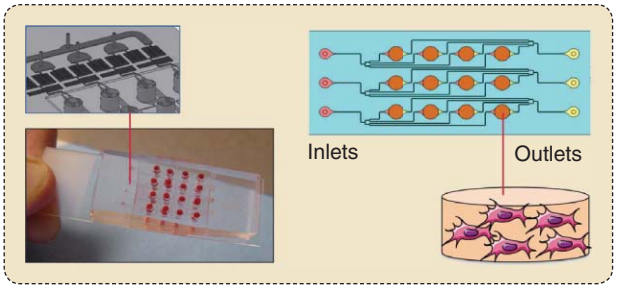

FIGURE 3 A microarray bioreactor for stem-cell screening. A simple microarray bioreactor with 12 culture wells on a standard microscope slide format was fabricated using soft lithography and designed to accommodate human stem cells attached to a 2-D substrate or encapsulated in a 3-D hydrogel. Each individual culture well is independently perfused with culture medium. Cell responses can be analyzed in real time using automated image processing [43], [47].

cardiovascular differentiation potential, by an automated image analysis of the expression of cell differentiation markers. Cell differentiation correlated to the level of hydrodynamic shear and transport rates of oxygen and growth factors with the aid of computational fluid dynamic modeling [47].

Patterning Tissue Interfaces

Another important use of microfluidic technologies is in the formation of concentration gradients of cytokines in cell cultures. In developing tissues, signaling molecules present themselves in the form of concentration gradients, which determine the migration, fate specification, and maturation of the sensing cells. Spatial gradients of diffusible signaling molecules are, therefore, of paramount interest both for identifying regulatory pathways and engineering complex tissue structures starting from undifferentiated cells.

A major advantage of microfluidic devices over other culture systems is in their capability to generate complex and well-defined concentration patterns, via tight control of fluid dynamics on a cell-level scale. Examples of gradient makers include microfluidic-photopolymerization systems for the formation of graded hydrogels [47], microfluidic devices with substrate-bound molecules [48], and membrane-based diffusion chips [49], [50].

A simple microfluidic platform was designed to generate stable concentration gradients in cell culture space by a combination of osmotic and capillary forces [53]. This system was successfully used to investigate the effects of gradients of sonic hedgehog homolog (Shh), fibroblast growth factor 8 (FGF8), and bone morphogenic factor 4 (BMP-4) on hESC-derived neural progenitors for as long as eight days. Neural progenitors differentiated into neurons and connected into a neural network.

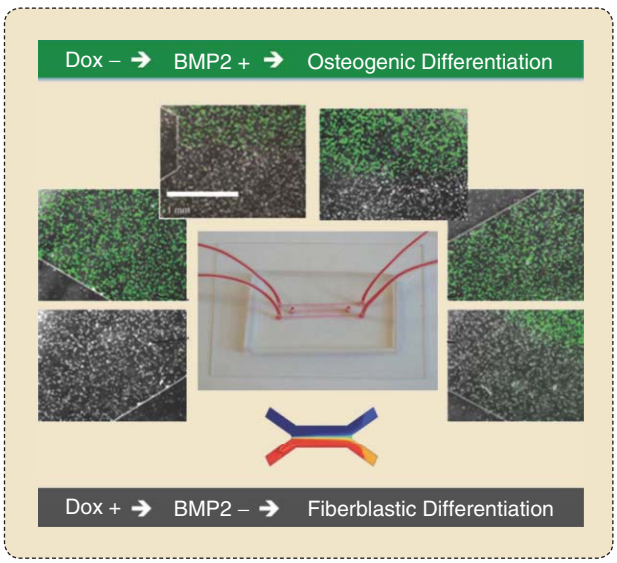

FIGURE 4 A microfluidic system for patterning a tissue interface: hMSCs with BMP-2 expression under the control of Tet-off gene were cultured between two laminar streams of culture medium, one of which contained Dox. In the areas exposed to high concentrations of Dox, BMP-2 was inactivated and the cells became fibroblastic; in the areas exposed to low concentration of Dox, BMP-2 was activated and the cells underwent osteogenic differentiation [52]. (Reproduced by permission of The Royal Society of Chemistry.)

Another microfluidic device was designed to generate stable concentration gradients over a field of cells at low hydrodynamic shear to allow the long-term culture of adherent cells [51]. To this end, a gradient of protooncogene protein (Wnt3) was established over a field of cells cultured between two parallel laminar flow streams of culture medium. Wnt3a regulation of β-catenin signaling was chosen as a case study because of its major role in stem cell proliferation, differentiation, and assembly into tissues. The activation of the β-catenin pathway in response to a gradient of Wnt3a was assessed in real time using the expression of green fluorescent protein (GFP) at a reporter gene. The exact algorithm for defining the concentration gradients was established with the aid of mathematical modeling of flow and mass transport. This simple and versatile microfluidic platform offers a high level of control over single and multiple gradients under cell-protective flow conditions.

Microfluidic platforms are now also used to pattern cell differentiation. In general, the development of transitional interfacial zones between adjacent tissues remains a significant challenge for engineering functional tissue grafts. For example, the interface between bone and soft tissue facilitates transmission of mechanical loads and minimizes stress concentration in junctions. Without adequate tissue interaction in such junctions, conventional soft tissue grafts fail at the bone insertion site. An important goal in tissue engineering is, therefore, to recapitulate tissue interfaces if the organization of an engineered tissue is to reflect that of the parent native tissue.

A novel approach to the formation of tissue interfaces was recently proposed, where cell differentiation is patterned by gene expression using a microfluidic platform [52]. The concentration gradients were verified by computational simulation and dye separation experiments. An inducible BMP-2 expressing cell line under the control of Tet-off gene expression system was investigated because of the efficient control of BMP-2 gene expression achieved by modulating the concentration of BMP-2 expression modulator doxycycline (Dox) (Figure 4). As for the gradient makers described above, the cells (human mesenchymal stem cells, hMSC) were cultured between two laminar streams of culture medium containing different levels of the regulatory molecule, in this case Dox. The regulation of hMCS differentiation is rather straightforward. In the areas exposed to high concentrations of Dox, BMP-2 was inactivated and the cells became fibrolastic; in the areas exposed to low concentrations of Dox, BMP-2 was activated and the cells underwent osteogenic differentiation. A tissue interface in the microfluidic device depended on the gradient of Dox. Therefore, by patterning the delivery of Dox to the cultured cells in the laminar flow system, one can effectively pattern the expression of BMP-2 and thereby modulate osteogenic differentiation.

Summary

In summary, microfluidic-BioMEMS platforms are increasingly contributing to tissue engineering in many different ways. First, the accurate control of the cell environment in settings suitable for cell screening and with imaging compatibility

is greatly advancing our ability to optimize cell sources for a variety of tissue-engineering applications. Second, the microfluidic technology is ideal for the formation of perfusable networks, either to study their stability and maturation or to use these networks as templates for engineering vascularized tissues. Third, the approaches based on microfluidic and BioMEMS devices enable engineering and the study of minimally functional modules of complex tissues, such as liver sinusoid, kidney nephron, and lung bronchiole. This brief article highlighted some of the unique advantages of this elegant technology using representative examples of tissue-engineering research. We focused on some of the universal needs of the area of tissue engineering: tissue vascularization, faithful recapitulation in vitro of functional units of our tissues and organs, and predictable selection and differentiation of stem cells that are being addressed using the power and versatility of microfluidic-BioMEMS platforms.

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Beyond Proof of Concept

Bringing BioMEMS to the market

Sam Sia talks with Steve Quake and Shu Takayama about commercialization

By Samuel K. Sia

Since the early 1990s, there has been an explosion of research in biological electromechanical systems (BioMEMS) across a number of academic departments. Although the potential practical uses of BioMEMS are almost unlimited, there are surprisingly few examples of BioMEMS devices used by nonspecialists. What is it that has made this critical translational step so challenging, and what can academic researchers do to overcome these challenges?

Here, two academic bioengineers who have taken this commercialization step will share their experiences and thoughts. First, we discuss this topic with Steve Quake at Stanford University. Prof. Quake has pioneered the development of multilayer microvalves as a way to control complex fluid handling at a microscale. He has spun off the technology into Fluidigm, a company cofounded by him. Fluidigm has sold many of these microfluidic chips for the research biology market.

Sam Sia (SS): Your article with Marc Unger, published in *Science* in 2000 [1], obviously made a major impact in the field (editor's note: this article has now been cited over 1,500 times), but a lot of studies have

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been published in leading journals and never found their way to having a commercial impact. You actually founded Fluidigm in 1999, a year before the article was published. Were you thinking about commercialization early on?


Steve Quake

Steve Quake (SQ): When we were doing the original research, we were just trying to make some tools to help our academic research. When we first got the valves working, we realized that there were much broader areas of application than we were originally thinking. It was serendipitous that we had founded Fluidigm before and had been exploring other microfluidic technologies. It was in the framework of the company that we realized that the valves were going to be quite useful, and they licensed it from CalTech and went to town on it.

SS: What made you decide to actually start a company? It was to help your research originally, but at some point you decided to try to build real products.

SQ: We founded the company because when you're an academic, you're developing measurement technologies, and if there's any complexity to them, there tends to be a fairly small circle of people in the world who have the ability to reproduce the work and make it work in their laboratory. So to have a maximum impact, one wants to have some kind of commercialization venue where you need not be an expert to use it and anybody can buy and use it. That's where I saw the role of the company as a way to get the technology to any biologist who wanted to use it.

SS: Can you describe the thinking of choosing protein crystallization as the initial market?

SQ: That stemmed from our academic research. As we were playing around with different things to use the plumbing for, one of the most promising research areas appeared to be protein crystallography and structural biology (Figure 1), which originated from a fantastic collaboration with James Berger at Berkeley. From all the academic work, Fluidigm was thought to have economic value and commercial impact, and licensed intellectual property and build commercial products around it. Very few structured biologists would have the wherewithal to go in and start making chips from our academic papers alone. But when Fluidigm commercialized their Topaz system, all of a sudden hundreds of biologists around the world could just buy the products and use them. Now, many structures could be solved by using the technology. They are not all out to get published because they are done by big pharma and they do keep their structures secret, but Topaz had a fantastic impact in drug discovery, which wouldn't have happened if it was just published academically.

SS: It is interesting that the choice came from your academic work initially because, in some other cases, the investors end up driving the company to a different market.

SQ: That's accurate too. The management of the company and the board, which includes the investors, were all involved in the decision of what the first product to be commercialized should be. Protein crystallography wasn't the only thing on the table, but this was the one that they thought had a lot of attractive aspects.

SS: Now it looks like the technology is taking on genomics as well as maybe drug discovery and other applications, prenatal testing. Can you speak a little bit about how much you were able to foresee the different markets from the beginning, and what would be your advice to academic researchers in choosing the initial market?

SQ: I'm totally impressed with what the people at the company have done and how they've executed on commercializing the technology. The company just issued a statement about two weeks ago announcing that they had shipped 1 billion valves to their customers—a billion micromechanical valves, which is equivalent to the total conventional plumbing in all of California, Texas, and New York put together. In my wildest dreams, I never would've thought we'd be sitting here talking about shipping a billion valves. As far as advice to other companies, you want to try to strike a balance between market opportunity, novelty, customer interest, and technical feasibility.

SS: How would you balance those factors when it comes specifically to BioMEMS?

SQ: It's very challenging to displace existing technologies from a commercial perspective, and so one of the lessons I've learned is to put a premium on novelty. For example, one of the early things they were considering about commercializing was a microfluidic cell sorter. There's a very strong established market there, and many of the customers are being served by existing instruments. We could always find new niches, but we didn't want to take on an area where there was a strong existing product already. In structural biology, there really was no good solution at that point in time. In the nucleic acid area, things like agricultural biology genotyping and single-cell gene expression analysis in the research biology market are the kind of opportunities where there wasn't an existing technology that needed to be displaced. Taking the time to try to figure out a completely new application is worth the effort in my experience.

SS: On a more general note, how do you balance your role as an academic researcher and your role in commercializing various technologies from your laboratory?

SQ: The company has always been completely separate from my academic research. I've used my one-day-a-week consulting time to spend time with them, and the rest of the time I run my laboratory. When we choose research projects, one of the criteria on the academic side is to pick something that, if it works, have the opportunity to be published in a very high-ranking journal. We don't

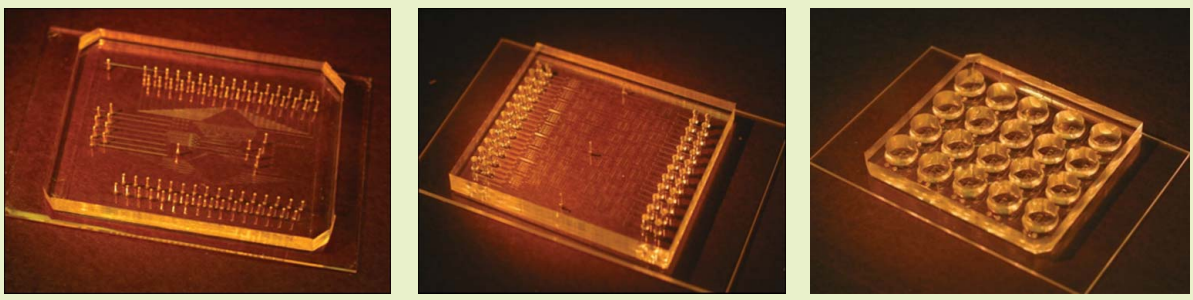

FIGURE 1 A new paradigm for structural genomics. (a) The formulator device collects solubility information and phase space maps for potential crystallizing reagents. (b) The FID screening device's free interface diffusion allows optimal screening and optimization of crystallization reaction. (c) The diffraction device offers scale-up chip to obtain diffraction data without handling crystals. (Photos courtesy of Steve Quake.)

make the decision based on the potential economic value of the project. Some of these may have economic and commercial value, and some do not. That provides a kind of menu for the company. They make their own decisions about what they want to license and commercialize, and there's a lot of things we've done that they didn't license and commercialize. They decided it wasn't something that was going to be a big market for them to build a product around and that's fine too. We have that kind of separation there in the academic world, we make our decisions based on the criteria that matter there, and in the commercial world, they make their decisions based on their criteria, and it all works reasonably well.

SS: Do you have any advice for academic researchers who are concerned about how to tackle conflict-of-interest issues?

SQ: That's something that seems to get harder and harder by the year to manage. Each institution has their rules and you've got to respect them, and it's something that waxes and wanes over time with the particular institutional administrators and leaders one has. I think that the general consensus of academics in the laboratory to have a day a week to consult is fantastic, which enables people to manage these things. I never had a really close contact between the company and my laboratory. We have never incubated a company in my laboratory, and the company has always had an independent identity from the academic research. The other point is a sort of controversy, and varies from institution to institution, but just as we faculty have the opportunity to consult, I think it's great for students and postdocs to have the ability to consult and see their research getting commercialized. We were able to do this down at CalTech, and it was really thrilling for many of the students to spend a day a week for a period to see the practical, commercial impact of their research.

SS: Another factor that inhibits a lot of faculty from starting companies is the unknown aspects of fund-raising. Academics know how to write grants, but fund-raising for companies is something that is unknown to a lot of faculty. What would you say are some similarities or differences that you can highlight?

SQ: I've been in situations where venture capitalists (VCs) have been able to move much more quickly and have been willing to take risks in ways that federal funding agencies have not. Some of the things that I've had trouble getting funded from federal grants, I've been able to fund through venture capitals and do it in a commercial context. I've seen the other way where the VC is just not that interested and we've been able to swing to federal agencies. I think it's nice that there's a multiplicity of opportunities. There's always this issue of how much time one can spend on a company versus an academic laboratory. I've never taken a leave of absence to start a company, and to make that work well, it's been important to find a chief executive officer (CEO) to run the company. If you find the right CEO, they're of great help in managing the fund-raising and business.

SS: But it's a chicken or egg issue. How do you attract a full-time person to work with you before you raise that amount of money?

SQ: That comes from a sort of networking at conferences. At CalTech, the tech-transfer office was very proactive in introducing faculty members to entrepreneurs and investors. The investors will help find a CEO. There are a lot of ways to get it done.

SS: What's the one thing you've learned through all your experience with commercialization now that you wish you would have known ten years ago?

SQ: One of the things I've learned is that I have a much finer sense of what type of project is better done in an industrial versus an academic setting. This has helped me choose my research path at the university level and has helped me decide when it's right to spin things out. That's something that one accumulates with time, I think. Being a part of two companies on the board, the discussions of investors and management has helped me understand what companies versus academics do well.

SS: In microfluidics and BioMEMS, there is a lot of research being done in academia, but still there are relatively few commercial products that are on the market. Do you think academics in this field should be placing more emphasis on translational research, or do you think they should continue to focus on basic science problems?

SQ: I think academic engineering departments, no matter what the field is, embrace both of those views. In any engineering department, you'll find that a certain segment of faculty is interested in basic science, and another segment that is interested in the practical engineering of things, so I wouldn't want the position of one to be more important than the other. They are both important parts. With respect to BioMEMS and microfluidics, I'm very pleased with the economic and commercial success of Fluidigm. We are starting to see more and more of those ventures. It takes time to grow a new field.

SS: Thank you, Steve.

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Next, we talk with Shu Takayama from the University of Michigan. Prof. Takayama has developed microfluidic methods for reproductive medicine (Figure 2), which are being commercialized by Incept BioSystems, a company he cofounded. A key requirement of a medical device for use in U.S. clinics is to conduct trials for regulatory approval from the Food and Drug Administration (FDA). The company completed its first human trials in 2010.

SS: You and your clinical collaborator, Gary Smith, started Incept BioSystems in 2005. Can you walk us through the process of founding the company, and how you were able to fit all the pieces together?


Shu Takayama

Shu Takayama (ST): This was done with a lot of help given by the tech-transfer office. There was a business school class project where students looked for the University of Michigan technology to try to commercialize. It was right about when we published the sperm sorter paper, and some students started doing market analysis studies of the sperm sorter. They came back saying that the interesting commercial opportunities were actually more in embryo culture and embryo analysis. We were also doing some embryo culture at that time, and so our clinical collaborator Gary Smith and I kept thinking about it on the science perspective. One of the business school students became very interested and committed, and so the tech-transfer office person and this business school student really pushed into the formation of a real company.

SS: So commercialization was not something you necessarily had in mind when you first published that initial paper?

ST: We had some warm fuzzy feeling that it might be interesting to commercialize, but we weren't so committed to the stage that we were considering quitting the university to spend full time on it. It really happened because the tech-transfer office and the business school person, Mike Crowley, were really committed to the project. He was one of the other cofounders and ended up working full time at the company.

SS: You raised several million dollars through VC, with some funding from the state of Michigan. Can you walk us through that fund-raising process?

ST: Some of the initial funds we received were actually from the College of Engineering Technology Transfer funds to do technology development as well as tech transfer. Initially, it was for the sperm sorter, and we actually did license the sperm sorter out to a different start-up company as well. Then, we received some state money for research and a little bit of commercialization. Also, it was just the beginning of the Coulter Foundation translational program funds. Most of it was for research in the laboratories, but some of it provided just a little bit of funding for the company as well. The business school student also went to business plan competitions and won some to provide money directly to the company. So those are the things that initially got us started, and then got us some VC funding.

SS: Given how important it is to have a person committed to the venture, what tips would you give to academic researchers for attracting people to work with you, especially at the beginning when you don't have a lot of capital or a salary to offer them?

ST: I was quite fortunate because Michigan had the environment to support a lot of these things, and there happened to be a really motivated business champion that liked our technology. That really helped to get the business going because we did not have much of an idea, between the clinician and I, and about companies and things like that. You would also want to talk to people who have connections with those types of people, such as tech-transfer people, business people, and then with people that those people might also know. It is good to, at least, do some reading and learn about how these types of business things work so that you can have some sort of conversation about the topic. I don't think it necessarily has to happen this way, but if you're excited, that makes the other person excited too, then that's the start.

SS: Fast-forwarding a little bit, last year, the company submitted 510(k) applications for FDA approval. Your company tested almost 500 embryos from 36 couples in four different sites. This is much further than most academic researchers will take their own technologies. As someone who has pushed this technology to the brink of FDA approval, how tough has this hurdle been, compared to your expectations?

ST: I have to credit people at the company and all the people that really did all the hard work, but it's definitely a lot of work ~~a~~ lot of paperwork as well as scientific work. In academia, we think about doing something new and interesting. For FDA approval, and in a way that's commercially viable, you have to think about robust, manufacturable, large-scale processes, and using materials and methods that are going to be safe in humans, and confirming that it probably should be safe in humans. So it's definitely a lot of work, and it requires a different type of innovation and thinking, perhaps, compared with laboratory research.

SS: Did you find the process to be surprising in any way?

ST: I think it was different. In academics, we wanted research laboratory devices that are programmable in a lot of different ways, versatile, and flexible. For the clinical version, we needed almost the opposite—it cannot be screwed up, cannot be changed, always does just the one thing the same way every time; so the requirements and things were quite different.

SS: Thinking about these different requirements for commercialization and still running an academic group with exciting, innovative publications, how do you balance the work between academia and translational research?

ST: First of all, I thank all the wonderful people I've been able to associate with. Even without thinking about a company, just thinking about research in the laboratory, it's because of all the great students and postdocs that these things happen in the first place. I'm relatively hands off, so maybe that lets me to do a few more things compared with if I was trying to be really hands on with everything. The other thing is that my laboratory, in general, works on technologies that are, at least conceptually and somewhat procedurally, simple, so that might have some help in being easier to commercialize. Most of the ones that got licensed or spun out were relatively simple technologies and concepts to start out with, and that seems to be helpful.

SS: That's very relevant because one can build complicated MEMS devices, but they may be too complicated in some cases for their end application.

ST: Right. The other thing is that some commercialization efforts are very synergistic with academic efforts. For example, the market analysis was actually useful in thinking about the significance, need, and areas of focus that would be academically fruitful and scientifically as well as commercially for research. If a problem has a big market, then it usually means there are technologic and scientific needs as well.

SS: What have you learned about the process of doing translational research that maybe you wish that you had known back in 2004?

ST: Now, I probably do think a little more about not just technology but whether the technology might be financially sustainable as well. I had no idea, or didn't even think about, terms like razor–razor-blade model or having a consumable component that gives you recurring revenue.

SS: Is something like that important for the translational work or does it actually give feedback to your academic work?

ST: It changes, or narrows down, what type of technology I think about for commercialization. It probably does affect a little bit some of the research that we do because we develop a lot of tools that we want to be used by others. So academically as well as hopefully, tech-transferwise, thinking about multiple ways to solve a problem might take into consideration some of those questions as we develop solutions.

FIGURE 2 Pump developed by Takayama's research group and commercialized by Incept Biosystems. (Image courtesy of Shuichi Takayama.)

SS: What do you think are the scientific areas and commercial opportunities going forward for making some of these technologies more than just an academic success but as tools that people are actually going to use?

ST: We have a three-dimensional (3-D) spheroid cell culture platform that got licensed out, and this was partly affected by thinking that we needed a more simple, user friendly, robust way to do 3-D cell culture spheroid formation that we were initially doing in microchannels. We made it into a channelless 384-plate format. Some of the newer things is the work we do with aqueous two-phase system micropatterning, where we want to do microfluidics but without any channels at all. There are opportunities for academic researchers because there are challenges with microfluidics. If you can come up with alternative ways to obtain certain results in a robust and user-friendly way, that's an opportunity. We also do some self-switching microfluidic circuit devices where we try to eliminate the computer and external controls because we make a lot of body-parts-on-a-chip-type systems. We consider these microfluidic chips to be on life support and not translatable because it's so complicated, and drug companies want to do 384 tests in parallel. Thinking about how we can take our microfluidic chip off of our life support gives us a challenge and an opportunity.

The other thing I learned is that there's a lot of innovation in terms of business models. There's a possibility to fund it through a VC, through Small Business Innovation Research (SBIR), or a nonprofit. There are different ways to bring your technology out into the world, and maybe there are special opportunities in that aspect for the academic people to think about.

SS: I think George Whitesides' nonprofit (Diagnostics for All) will be one to watch in terms of an innovative business model. Thank you, Shu.

Samuel K. Sia *(ss2735@columbia.edu) is an associate professor in the Department of Biomedical Engineering at Columbia University.*

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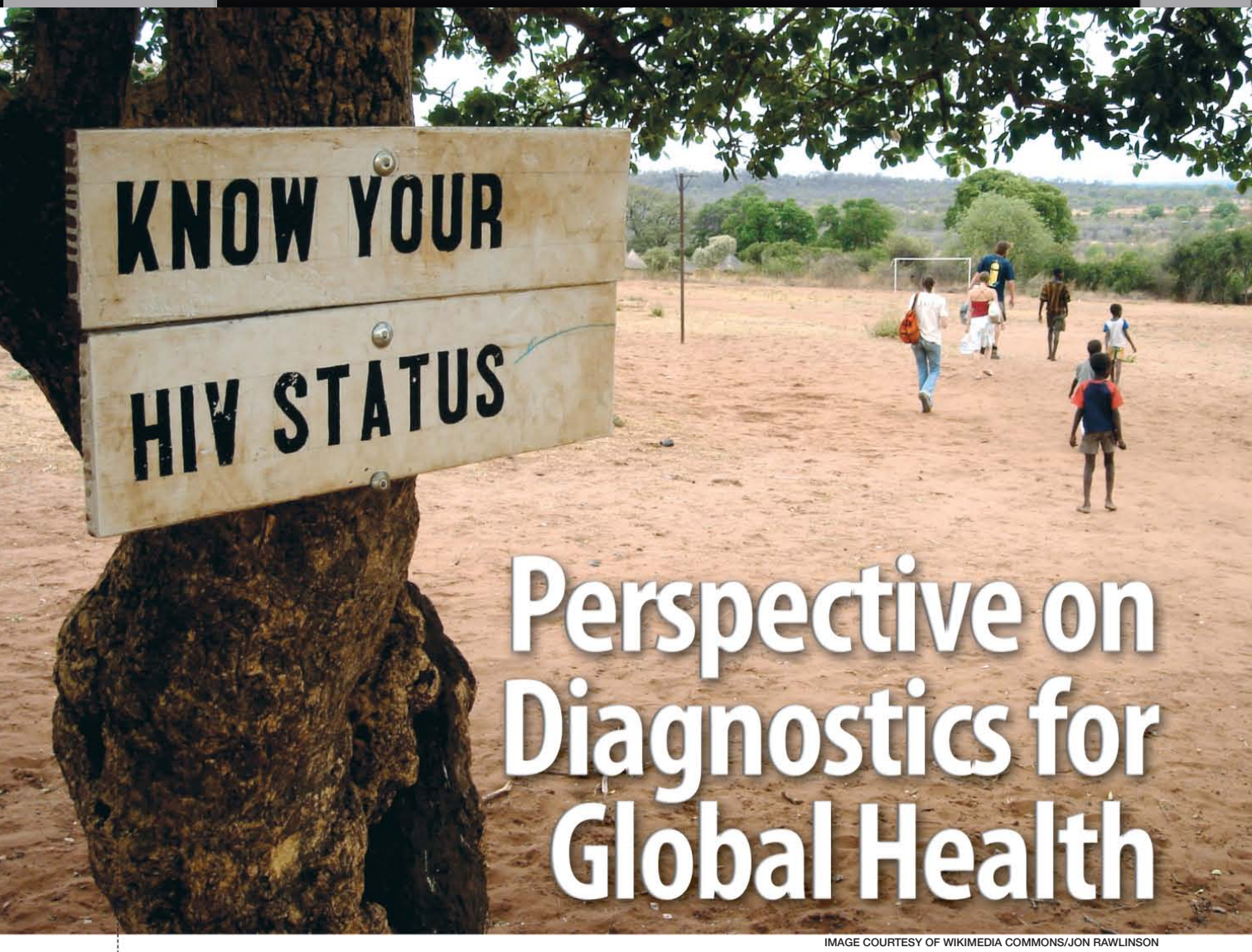

Perspective on Diagnostics for Global Health

IMAGE COURTESY OF WIKIMEDIA COMMONS/JON RAWLINSON

By Elain Fu, Paul Yager, Pierre N. Floriano, Nicolaos Christodoulides, and John T. McDevitt

Challenges in Evolving Chips-in-a-Lab to a True Lab-on-a-Chip

Diagnostic applications for global health have exploded in the past ten years. Numerous articles have been generated on global health priorities, constraints of resource-limited settings, and technological innovations for diagnostics development, including several comprehensive reviews [1]–[3]. In this article, we aim to provide 1) a focused summary of the most highly needed diagnostics, 2) a discussion of noteworthy recent developments of technologies in the field, and 3) a perspective on the evolution, challenges, and future directions of diagnostics for global health applications.

Need for Early Detection and Treatment

Disease treatment in the absence of a diagnostic test is often based on syndromic management (i.e., observing clinical symptoms and factoring in local prevalence of the disease). This situation can result in incorrect treatment of patients manifesting symptoms common to multiple diseases with local prevalence. Unnecessary treatment may compromise the patient (from harmful side effects of a treatment) or the community (through accelerated drug resistance, as has been found in

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the case of malaria [4]). Further, the patient remains untreated for the relevant condition, potentially leading to higher mortality and morbidity. A diagnostic test that can provide an accurate and timely diagnosis enables 1) earlier interventions before the appearance of advanced symptoms, 2) correct diagnosis and treatment for each patient, and 3) effective use of limited resources [4]. Thus, appropriate point-of-care (POC) diagnostics development for high-impact diseases can significantly reduce the global disease burden [4]–[6].

Unmet Needs for Diagnostic Tests

Health conditions can be divided into two broad categories (Figure 1), namely, infectious and noncommunicable diseases (NCDs). Infectious disease is a major cause of mortality in the developing world, killing nearly 15 million people each year [7]. A more comprehensive measure of disease burden is disability-adjusted life years (DALYs) and includes the effects of decreased quality of life due to disease [8]. An estimate from 2006 indicates that, for a core set of infectious diseases, the disease burden is an astounding 325 million DALYs per year [4]. Priority-infectious diseases, based on disease burden, are HIV/AIDs, tuberculosis (TB), and malaria [7]. Some progress has been made in developing POC diagnostic tests for HIV and malaria. However, there are still many unmet diagnostic needs. For example, in the case of TB, there is still no POC test appropriate for low-resource settings [9]. The gold-standard culture is highly sensitive and provides information on drug resistance but requires laboratory facilities and a long waiting time for results (e.g., ten days for rapid culture) [9]. Traditional microscopy is often used, but it requires a trained personnel and has a poor sensitivity of 70% [9] (Figure 2). High-performance nucleic acid amplification methods for the diagnosis of TB are commercially available now but are too expensive for use in low-resource settings (i.e., Cepheid GeneXpert is ~US$27,000 and ~US$30–US$85 per cartridge). With respect to HIV, CD4 counts and viral load testing remain as unmet needs that would have great value to inform treatment decisions (e.g., time to start antiviral therapy and monitoring the effectiveness of the therapy). In addition, a combined diagnostic for HIV and TB would be of value given the high rates of coinfection [9]. Within the set of neglected tropical diseases [10], there is an unmet need for an effective POC diagnostic for dengue/dengue hemorrhagic fever, human African trypanosomiasis, and leishmaniasis [9], [11]. The ability to simultaneously test for multiple disease conditions could be useful for the differential diagnosis of conditions with similar symptoms. Specifically, studies suggest that, in malaria-endemic regions, children presenting a fever but who do not have malaria often go without proper diagnosis and treatment, resulting in high mortality rates [12], [13]. Thus, a POC diagnostic that tests for a panel of fever-causing illnesses and could be adapted to a geographic area would have much value [9]. Additionally, a diagnostic for multiplexed detection of HIV, malaria, syphilis, and anemia would have specific utility for the care of women during pregnancy in the developing world [9]. In their recent review, Peeling and Mabey [9] have also highlighted the need for diagnostic tests for acute lower respiratory infections to determine the appropriateness of antibiotic treatment, and early diagnosis of the often asymptomatic sexually transmitted infections gonorrhea and chlamydia for proper patient treatment to reduce transmission.

Despite the disproportionate impact of infectious diseases in the developing world, NCDs are the leading cause of death globally, with almost 80% of deaths occurring in low- and middle-income countries [15]. For example, in 2008 there were 36 million deaths, 63% of total deaths globally, due to NCDs; the

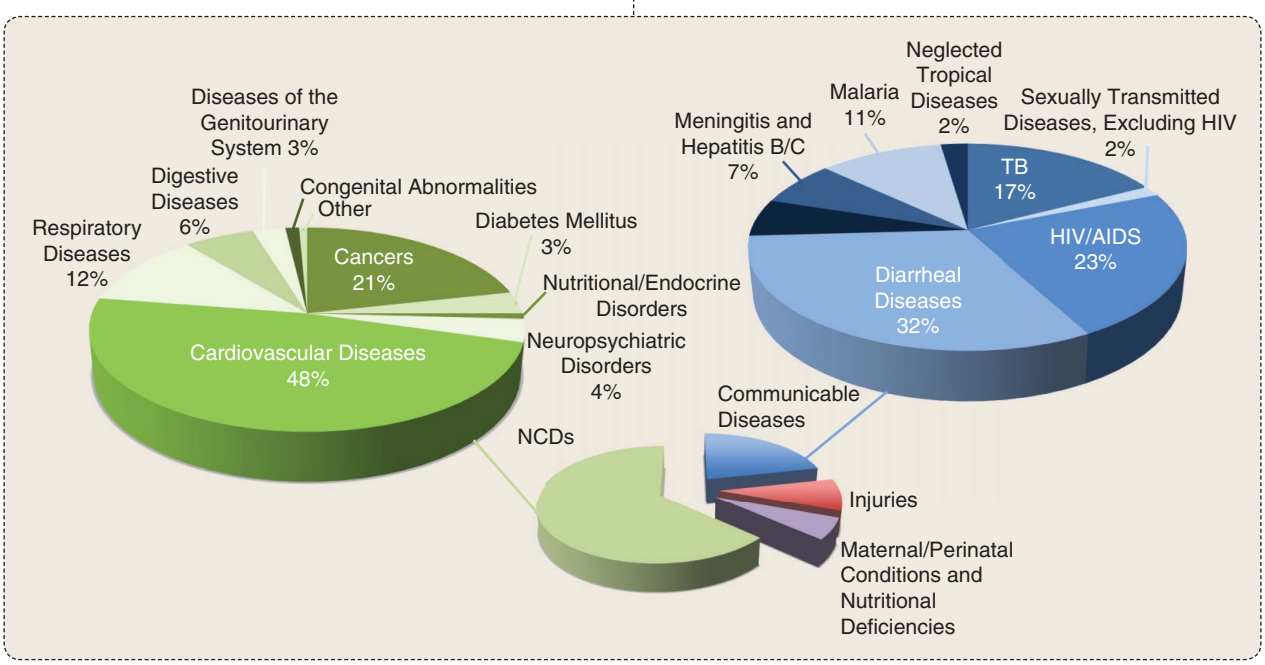

FIGURE 1 Worldwide percentage of mortality by health condition. The exploded pie chart shows the percentage of mortality in the four main categories of 1) communicable diseases, 2) NCDs, 3) maternal and perinatal conditions and nutritional deficiencies, and 4) injuries. A further breakdown of percentage of mortality worldwide for the categories of communicable diseases and NCDs is provided in the upper pie charts [14].

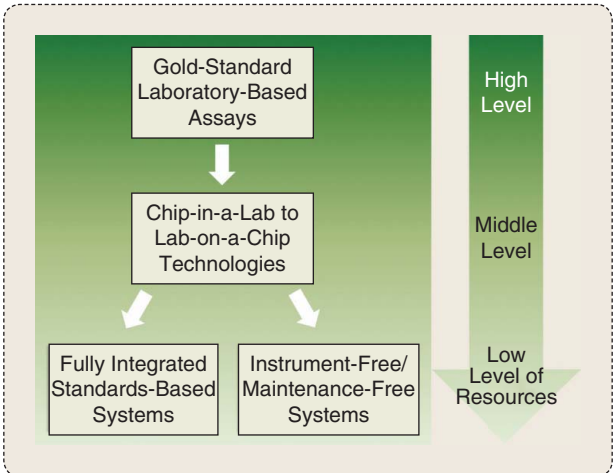

FIGURE 2 A schematic of the evolution of POC diagnostics development. The gold-standard laboratory assays are appropriate for settings with a high level of resources. There has been much progress in the development of promising chip-in-a-lab technologies that have, in some cases, been converted to true lab-on-a chip systems for use at the POC. However, the costs of the systems are often a barrier to their use in settings with lower levels of resources. One viable strategy is to push toward fully integrated standards-based systems that leverage the microelectronics and software industries. Also underway is a movement to create instrument-free diagnostics that will not only have a cost appropriate for the lowest-resource settings but will also fulfill the equipment-free requirement that is so critical to those settings.

predominant causes include cardiovascular disease (CVD), cancer, diabetes, and lung disease [15]. In the case of cancer, early detection of breast, cervical, colorectal, skin, and oral cancers, in particular, can lead to a reduction in mortality [15]. Additional NCDs of interest with respect to early diagnosis are gastrointestinal diseases and renal diseases, the latter being a related complication to both CVD and diabetes [15]. Early detection is especially compelling for certain NCDs (e.g., CVD and diabetes) because of the often simple behavioral interventions that can be implemented to improve health conditions [15].

The Continuing Challenge

Gold-standard diagnostic assays are often high-performance laboratory-based tests that require multistep protocols for complex sample processing. Tradeoffs for the high performance include long sample-processing times, long periods of time for samples to be transported to the laboratory and for the results to be transmitted back to the patient/caregiver, the need for trained personnel to run the test and interpret the results, and the need for specialized instrumentation for processing samples and detecting analytes. Also assumed is the access to electricity to power the instrumentation, maintain strict environmental conditions, and refrigerate reagents until use in the assay. The requirements of laboratory-based tests are often incompatible with the constraints of resource-limited settings. Constraints in these settings include patients with limited access to clinics and contact time while being there, limited training of test providers, lack of laboratory facilities and testing environments with uncontrolled temperatures and humidity levels, and limited local infrastructure, including a lack of cold chain for refrigeration of reagents [1], [2], [4]. The World Health Organization has coined an acronym for the characteristics of POC diagnostics that are appropriate for even the lowest-resource global health settings: affordable, sensitive, specific, user friendly, rapid and robust, equipment free, and deliverable to users (ASSURED) [16]. Thus, the overall challenge has been and continues to be to create high-performance assays that are appropriate for the various multiconstraint settings relevant for global health applications, including the lowest-resource settings.

Dedicated Global Health Lab-on-a-Chip

While significant progress has been recently made in genomics, proteomics, and other disciplines, few of the scientific discoveries have impacted clinical practice globally [17], [18]. There is a strong potential to leverage these discoveries for a broad impact in diagnostics for global heath applications using chip-based approaches. An important trend relevant here is the miniaturization of designs afforded by the small dimension scales of microfluidic-based devices. This allows for portability and the use of small sample and reagent volumes. These also enable rapid POC results at the bedside, in the ambulance, or at other remote locations [19]. The lab-on-a-chip (LOC) technology is often configured with a permanent instrument and disposable cards. The instrumentation can often be battery powered, and the cards can incorporate reagents stored in dry form to remove the immediate need for a power grid. Automation of the processes results in the ease of use for minimally trained users. In many cases, the cards can be affordable for the specific setting when scaled up for high-volume manufacturing. Key steps have been reached by numerous LOC efforts, and important goals are defined with the micro total analysis system (μTAS) paradigm. These have led to technologies suitable for dedicated global health applications at the POC, in genetic [20], [21]; proteomic [22], [23]; and cellular testing [24]–[26]. Yet, very few complete workable POC clinical devices have emerged despite tremendous progress in microelectromechanical systems (MEMS), microfabrication, microfluidics, and related areas [17], [18]. Indeed, while the core of typical LOC systems is substantially smaller than that of the bench-top counterparts, they still rely on a network of macroscopic laboratory-based infrastructure for sample processing, sample introduction, analyte detection, data processing, and reagent handling, thus limiting their utility for POC applications. The selected examples of LOC approaches described below highlight efforts to address the challenges of evolving from chips-in-a-lab to a true LOC.

The Yager group [27] has developed a microfluidic flow-through membrane immunoassay, featuring gold-antibody conjugates stored in dry form on a disposable laminate card, which works in conjunction with external pumping and imaging instrumentation. The system, demonstrated for the malarial antigen *Plasmodium falciparum* histidine-rich protein II (PfHRP2), retained high activity after a 60-day storage at elevated temperatures, with a detection limit in the subnanomolar range and a time to result below 9 min. Other approaches by Sia with MEMS and Singh using chip-based separation and quantitation have continued to increase the level of integration of diagnostic devices [28], [29].

Significant progress has been achieved in fluid handling through the implementation of innovative LOC components and actuation strategies. Another example by the Sia group demonstrated the actuation of multiple microfabricated microvalves with rapid response in an enzymatic assay via liquid-filled control channels in a handheld instrument powered by a simple 9-V battery [30].

Madou et al. have demonstrated the use of microfluidic compact disc (CD) formats [31] as a cost-effective approach to eliminate pumps, tubing, and valves, and drive fluids with centripetal force. Recently, the Liu laboratory [32] have applied this approach in detecting microparticles and cells. The device, compatible with the use of standard CD drives, was demonstrated with Chinese hamster ovarian (CHO) cells of various concentrations and could eventually be employed to perform enzyme-linked immunoassays (ELISAs). In a recent perspective article on optical biosensors, Ligler highlights the innovations that may lead to faster, smaller, and more cost-effective optical biosensor systems. Among the most promising example for facilitating integration is a new generation of polymer components, e.g., organic photodiodes (OPDs), for use as optical detectors [33].

The work done by Klapperich and coworkers has shown the suitability of plastic microfabrication methods, compatible with global diagnostics costs, to produce high-performance parts for use in continuous-flow polymerase chain reaction assays [34]. This approach may have a significant impact in the diagnosis of infectious diseases in the developing world and also can provide a cost-effective approach to DNA testing that resonates with the promise of personalized medicine in developed countries to drive down the cost and improve health care [20], [21].

Magnetic nanotechnology has been recently used to enable rapid, multiplex immunoassays for POC applications. The portable battery-powered platform, developed by the Wang laboratory [35], also has reduced requirements for operator training. Their approach has provided promising analytical results using p24 protein as a model. It is envisioned that, when demonstrated with clinical samples, this technology could be used to detect a number of infectious disease agents such as HIV, Hepatitis C virus, *Mycobacterium tuberculosis*, *Salmonella typhi*, toxigenic *Escherichia coli*, as well as swine (H1N1) flu and avian (H5N1) flu (Figure 3).

A Fully Integrated Standards-Based Systems Approach

Another viable strategy to reduce instrumentation costs for resource-limited settings, which is in development in the McDevitt laboratory, is to leverage the capabilities of a global network of diagnostic devices based on universal standards. This new approach is a significant departure from current diagnostic test systems that are fragmented in terms of specialized instruments dedicated to

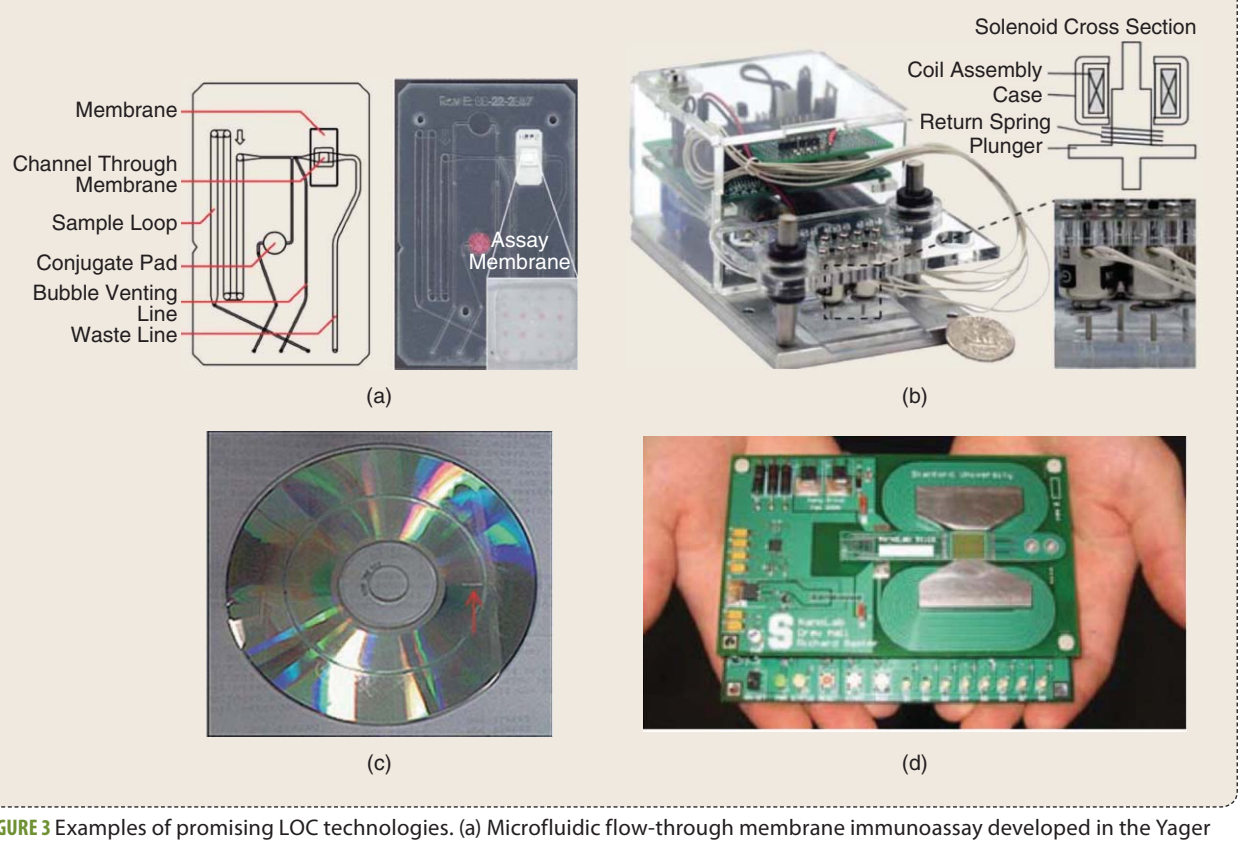

FIGURE 3 Examples of promising LOC technologies. (a) Microfluidic flow-through membrane immunoassay developed in the Yager laboratory achieves rapid and sensitive detection using dry reagents stored on the disposable card [27]. (b) The Sia laboratory has demonstrated higher-level integration that is completely battery powered [30]. (c) A CD-based approach for cell detection from the Liu laboratory reduces the requirements for pumps and valves [32]. (d) The Wang laboratory has developed a wash-free multiplexed immunoassay based on magnetic nanotechnology [35]. (All reproduced with permission from the Royal Society of Chemistry.)

Test	Gold Standard	P-BNC Chip Type	Sample Type	Results
CD4 HIV Immune Function	Flow Cytometry	CPU 2 	Drop of Whole Blood	R^2 = 0.93–0.95 (N = 200)
Oral Cancer (OSCC)	Pathologist	CPU 2 	Brush Biopsy	AUC = 0.97–1.00 (N = 52)
Cardiac AMI	CTnI, Myo, CK-MB*	CPU 1 	Oral Swab	AUC = 0.94–1.00 (N = 100)
Roadside Drug Tests	LS-MS/MS**	CPU 1 	Oral Swab	R^2 = 0.926 (N = 45)

* cardiac troponin I (CTnI), myoglobin (Myo), and creatine kinase-muscle type, brain type (CK-MB).
** liquid chromatography-tandem mass spectrometry.

FIGURE 4 The McDevitt group has developed a fully integrated programmable PBNC platform that enables new test configurations to be quickly adapted, developed, and applied for a variety of diagnostic indications through the insertion of molecular level code (or disease-specific reagents).

specific analytes as well as specific geographic and demographic sectors. For example, today, the five most active POC sectors globally are diabetes, acute coronary syndrome (ACS), coagulation, HIV, and platelet function [36]. For the area of cardiac heart disease alone, there are three major sectors involving risk, heart attack detection, and congestive heart failure with at least two qualitative and eight quantitative rapid whole blood devices deployed in POC and remote laboratory settings [36], [37].

The programmable bionanochip (PBNC) system is inspired by the microelectronics industry, in which a standard operating system is used in conjunction with modular software programs specific to a variety of applications, to provide significant cost reductions and produce increasing performance (Figure 4). The PBNC system is a platform that enables new test configurations to be quickly adapted, developed, and applied for a variety of diagnostic indications through the insertion of reagent-specific molecular level code [38], [39]. As such, the PBNC system has the capacity to serve cell counting, typing, and differentiating functions [40]–[42]. Alternatively, PBNCs can complete analysis of chemical, genomic, and proteomic analytes using bead-based microreactors [43]–[52].

These two distinct PBNC assay platforms are packaged within a disposable, single-use injection-molded plastic laboratory card comprised of a network of microfluidic components for the complete transfer and processing of biological samples. These sensors provide quick and accurate information on cellular, genomic, or proteomic biomarkers of disease at the POC. All assay steps are conducted without human intervention within the laboratory card that sits within an analyzer equipped with a light-emitting diode (LED)/charge-coupled device (CCD)-based detection system and mechanical actuators. This approach eliminates the need for external fluidics such as pumps, tubing, and connectors. The assay is performed through a sequence programmed into the controller of the analyzer with control over the flow rate, incubation time, and reagent wash achieved by the actuation of stepping motors that direct the fluid flow through the depression of fluid pouches. The sample is directed to an on-chip waste reservoir, and the entire biochip can be discarded as solid waste after the assay, facilitating biohazard waste management.

The bead-based PBNC is now moving through six major clinical trials and has been successfully applied to serve a variety of important health applications, including ovarian, prostate and oral cancer screening and monitoring [47], [48], [53], cardiac risk assessment [44], [45], and diagnosis of acute myocardial infarction (AMI) [51]. Compared with gold standard and laboratory-confined methods, most of which are based on ELISA methodology completed in bulky and expensive instruments, the miniaturized bead-based PBNCs exhibit assay times in minutes instead of hours, limits of detection two or more orders of magnitude lower [38], and a proven capacity to multiplex [38], [39], [43]–[45]. Likewise, the PBNC sensor may be programmed to detect various panels of target proteins, antibodies, toxins, and drugs of abuse in biological fluids.

The membrane-based PBNC serves as a miniaturized analysis system that mimics flow cytometry instrumentation in their capacity to complete important cell-counting applications, such as HIV immune function testing using CD4 cell counts [42]. In addition to lymphocyte enumeration in resource-limited settings, the same membrane system is now being applied for oral cancer screens for the analysis of minimally invasive brush biopsies of oral mucosal lesions [47], [48]. Here, cytomorphometric data and information about the relevant expression of molecular biomarkers of malignant potential are acquired in an automated manner using refined image analysis algorithms based on pattern-recognition techniques and advanced statistical methods. This dedicated PBNC approach has the potential to turn around biopsy results in a matter of minutes as compared to days for traditional pathology methods.

Most importantly, the results achieved with the PBNC system correlate well with those of high-performing but laboratory-confined methods. This is a feature that, when considered along with the system's modularity and advanced performance

characteristics in multiplexed capacity mode, promises to remove one of the main barriers for the ultimate acceptance and implementation of POC testing. These tests no longer have to be associated with high cost and limited performance.

Toward Instrument-Free Devices for the Lowest-Resource Settings

An especially compelling need in the lowest-resource settings is for equipment-free diagnostics, such that ongoing maintenance and repair are not required. A major challenge in creating a diagnostic device that is free of dedicated equipment is how to transport fluids within the device without the commonly used active pumping systems. The Delamarche group has developed a microfluidic capillary system with autonomous pumping capability [54]. Their silicon/polydimethylsiloxane (PDMS) microchip performs a conventional sandwich format assay on C-reactive protein (used as an indicator for myocardial infarction) using a single-step delivery of sample and conjugate (similar in operation to the chemical delivery steps in a conventional lateral flow strip test) with fluorescence-based detection [55], [56].

Capillary pumping is the method of fluid transport in the simple lateral flow tests that have been used in low-resource settings for decades. Though lateral flow tests fulfill many of the ASSURED criteria, they have been criticized for both their inability to multiplex (i.e., assay for multiple analytes from a single biosample) and their lack of sensitivity for many analytes of clinical importance [57], [58]. In 2008, the Whitesides group pioneered the use of microfluidic paper-based analytical devices (μPADS), two- and three-dimensional paper-based structures that enable colorimetric assays (e.g., for detection of glucose and protein) with multiplexing capability [59], [60]. The original μPAD structures were created by photolithography [61], but since then numerous alternative fabrication methods have been demonstrated, including wax printing [62], [63], cutting [64], and ink-jet printing [65]. Additional work in the area of paper-based assay development has focused on implementing multiplexed assays for the detection of additional biomarkers using one-step colorimetric reactions (e.g., nitrite, uric acid, and lactate) [66], [67] or performing the simultaneous analysis of multiple controls for on-device calibration [68]. Alternative detection methods in paper-based assays have been investigated, including electrochemical detection from screen-printed electrodes for metabolites and heavy metal ions [Pb(II) and Zn(II)] [69]–[71].

The second limitation of lateral flow devices is their inability to perform the controlled manipulation of multiple reagent volumes in a timed sequence of sample-processing steps characteristic of high-performance gold-standard assays. Recently, the collaboration of Yager, Lutz, and Fu [89] has addressed this issue, demonstrating two-dimensional paper networks (2DPNs) for autonomous multistep sample processing. A key feature of the 2DPN assay is the configuration of the network, composed of multiple inlets per detection region, which functions as a program for the timed delivery of multiple reagent volumes within the network. The 2DPNs that perform the processes of signal amplification [72], sample dilution and mixing [73], and small molecule extraction [73] have been demonstrated. Critical to the operation of multistep paper-based assays is a set of paper fluidic tools, i.e., analogs to the pump controls and valves of conventional microfluidics, to manipulate fluids within the network for precise timing of reagent delivery and metering of reagent volumes [72], [74], [75]. Additional tools for controlling flow via modification of the wetting properties of the paper channel [76] and simple user-activated mechanical on switches [77], [78] have been demonstrated by the Phillips, Whitesides, and Shen groups.

Also, integral to the development of ASSURED diagnostic devices is to have available robust methods for power-free temperature control. Recently, the Weigl group at the Program for Appropriate Technologies in Health (PATH) has demonstrated the use of chemical heating, e.g., hydration of CaO, and phase-change materials to perform loop-mediated nucleic acid amplification [79]. Their device achieved a controlled elevated temperature of 65 $\pm$1.5 °C for over an hour [79]. The specific combination of exothermic reactants and the composition of the phase-change material can be used to tune the thermal properties of the instrument-free heater for numerous applications, including other isothermal nucleic acid amplification methods [80], cell lysis protocols, and sample concentration methods based on temperature-responsive polymers [79].

A particularly challenging issue is how to achieve high-sensitivity or quantitative detection without dedicated instrumentation. The use of a dedicated reader in conjunction with nonvisible labels, e.g., fluorescent or magnetic particles, has been a common strategy for improving the sensitivity of conventional lateral flow tests [81], [82]. Use of a dedicated reader has also been employed for the measurement of analyte levels and quantitative readout. For example, the Whitesides group has demonstrated the use of a transmission-based reader for measurements in index-matched paper devices [83]. Alternatively, there are several commercially available readers for lateral flow tests that provide quantitative readout of fluorescence or colorimetric detection [81]. The ubiquity of cell phones (possessed by approximately 60% of people globally [84]), even in low-resource settings, provides an opportunity for higher-capability assay readout without a dedicated instrument. The use of cell phones for the acquisition, analysis, and transmission of assay data is an area of active research and development. Challenges include the acquisition of high-quality image data, given the expected wide range of lighting conditions and user variability of camera positioning [85]. The Whitesides group has demonstrated the use of a cellphone camera for direct acquisition of endpoint intensity measurements from a colorimetric paper assay [86], while the Shen group has demonstrated quantitative detection of chemiluminescence [87]. A related approach has been to develop an adapter module to interface a standard cell phone. The Ozcan group has developed a compact adapter (28 g) consisting of LEDs, lens, and filter, which couples to a cell phone camera for wide-field fluorescent and dark-field imaging capability [88] (Figure 5).

Challenges and Future Directions

Specifications Must Meet User Performance Requirements

Some technology developers have been arguing that having access to a poor-performance POC diagnostic test is better than having no diagnostic test at all. This is demonstrably false in some cases: introduction of a diagnostic test with substandard

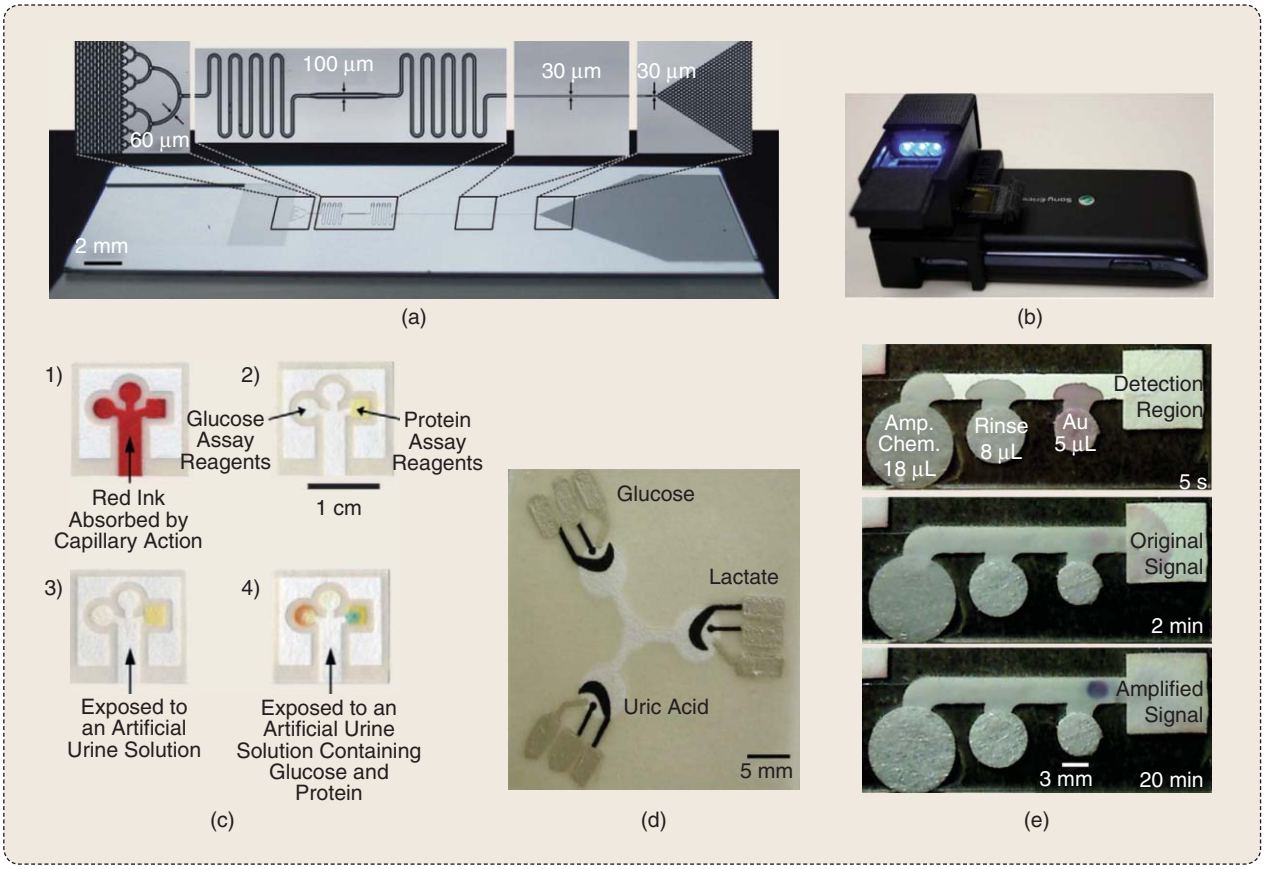

FIGURE 5 Examples of noteworthy technologies in the movement toward diagnostic devices that are free of dedicated instrumentation. (a) The Delamarche group has developed capillary-based microfluidics in a hybrid silicon/PDMS device for the pump-free manipulation of fluids [56]. (Reproduced with permission from the Royal Society of Chemistry.) (b) The Ozcan laboratory has demonstrated the use of a compact adapter that couples to a cell phone for fluorescence and dark-field imaging of assay results [88]. (Reproduced with permission from the Royal Society of Chemistry.) (c) The Whitesides laboratory has developed μPADs for multiplexed detection in paper assays [59]. (Reprinted with permission from John Wiley and Sons.) (d) The Henry laboratory has developed electrochemical detection in paper using screen-printed electrodes. (Reprinted with permission from [69]. Copyright 2009 American Chemical Society.) (e) The 2DPNs for autonomous multistep sample processing and higher performance assays have been demonstrated by the collaboration of Yager, Lutz, and Fu [89].

performance specifications can have significant adverse consequences. For example, the case of poor sensitivity of rapid lateral flow tests for influenza has been highlighted recently. Those tests generally have an acceptable clinical specificity (the number of positive cases as measured by the diagnostic test divided by the total number of true positive cases as determined by a gold standard test) of >90% but have poor clinical sensitivity (the number of negative cases as measured by the diagnostic test divided by the total number of true negatives as determined by a gold standard test) of 11–70% [90]–[93]. Low clinical sensitivity translates to false negatives, and, thus, missed opportunities to appropriately treat patients suffering from influenza with antiviral medication. The U.S. Center for Disease Control issued a statement during the influenza pandemic of 2009 that recommended discontinuing the use of those tests [94]. From the previous example, it is clear that an important factor in determining the required performance specifications for a given diagnostic test is consideration of the consequences of obtaining a false-negative or false-positive result with that test. The consequences of cases missed because of implementing a low-clinical-sensitivity diagnostic test can be severe in the context of an acute health condition with a high mortality rate. On the other hand, the consequences of implementing a low specificity diagnostic test (and the resulting high rate of false positives) can be equally problematic with respect to exposing the misdiagnosed patient to a treatment with potentially adverse side effects, undue emotional stress, and the financial burden to the health-care system of additional testing or unnecessary treatment. The latter case was highlighted recently in the context of prostate cancer screening. A false-positive result for the serum prostate-specific antigen test impacted at least one in eight men repeatedly screened [95], and has resulted in unnecessary procedures and expense to the health-care system.

Also critical to understanding the required performance specifications of a potential diagnostic test is prevalence of the disease in the population targeted for screening. The utility of a diagnostic test in a population with a given disease prevalence can be quantified by positive and negative predictive values, the proportion of positives as measured by the diagnostic test that are true positives and the proportion of the negatives as measured by the diagnostic test that are true negatives, respectively. Thus, a diagnostic test with given clinical sensitivity and specificity will have a higher positive predictive value in settings with a higher

disease prevalence, while the test will have a lower negative predictive value in settings with a higher disease prevalence. This makes clear the importance of targeted use of a diagnostic test in the most relevant populations.

Future Challenges

Rational diagnostics development for a particular health condition must begin with a thorough understanding of user requirements in the intended setting [4]; the next step is to apply a technology that can meet those user requirements of performance, ease of use, shelf life, tolerance for maintenance of instrumentation, and cost. Given the wide range of resource levels in global health applications [1] as well as varied requirements for performance that are specific to a biomarker and health condition, it is unlikely that any one technology will be appropriate for all global health applications. However, the use of numerous specialized tools, each tailored for a single diagnostic indication, also creates a challenge in terms of managing multiple tools, maintaining a supply chain, and training end users to handle multiple diagnostic aids. This dichotomy will be a central challenge for the next decade.

There are additional factors that will contribute to the success or failure of particular diagnostic tests in getting to market and to the intended end users. For example, there is generally a higher success rate for diagnostic tests that can be applied to large populations [33], such as tests with the added value of multiplexing for cost-effective testing. However, the regulatory processes for the approval of multiplexed diagnostic tests are less understood than for diagnostic tests dedicated to single measurements [96], [97]. In addition, devices for detecting more than one biomarker may suffer from a limited understanding of their relevance to the particular disease, and a resulting lack of physician awareness and backing, and/or resistance from health-care stakeholders to reimburse, especially in the cases of prevention and early diagnosis [33]. Further, diagnostics development activity in private industries has emphasized the developed rather than the developing world, resulting in the production of instrumentation that is complex and cost prohibitive to use in resource-poor settings [5].

Recent efforts in diagnostics development for global health applications are beginning to produce solutions that could be used in the low-resource setting of developing countries. In particular, diagnostic devices that are free of dedicated instrumentation have the potential to be affordable (and maintenance free) for even the lowest-resource settings. Another viable approach is to define and address the requirements of resource-limited settings in developing high-performance methods for a suite of disease applications on a common platform. With this standards-driven approach, inspired by the software and microelectronics industry, there is a strong potential to sustain the capital expansion that exploits the health-care infrastructure of developed countries while bringing the same tools to end users in the developing world.

The total LOC-based biochip market was US$2.4 billion in 2009 and is projected to increase to US$5.9 billion in 2014 [98] (part of the increasing POC market that is estimated to reach more than US$18.7 billion by 2011 [99]). This should be a powerful incentive for commercial efforts to move toward true global health solutions. The recent U.S. health-care legislation debate and consensus for reform provides additional momentum with the recognition that POC, now representing most of the growth in the in vitro diagnostics sector [100], can deliver lower costs while improving the health of patients.

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Solving Medical Problems with BioMEMS

By Erkin Seker, Jong Hwan Sung, Michael L. Shuler, and Martin L. Yarmush

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A routine laboratory exercise for an undergraduate electrical engineering student is to build a simple electronic filter circuit and determine its frequency and transient response. During the exercise, the student exposes the circuit to a range of electrical signals and captures the voltage and current characteristics. The relationship between what the circuit is exposed to and how it responds allows for developing a transfer function that provides insight into how the circuit operates. This approach, which is the basis of systems identification, can be applied to an almost infinite number of cases spanning from engineering problems to social sciences, and its application to biology is not an exception (Figure 1). For example, when a cell is exposed to a certain drug, it may respond by changing its morphology or by secreting specific molecules that can be detected by specific assays. In this case, one can produce a transfer function that couples the drug dose (input) with cell response (output) to describe the basic operation of a cell, more specifically, whether the drug produces the intended outcome.

There is a multidimensional parameter space for modulating a biological machinery. First, the type of stimulus can be biochemical, optical, electrical, or mechanical, each of which will lead to a different cellular response. Second, the temporal profile of the stimulus can be different, i.e., it can be constant or oscillatory. Finally, where the stimulus is applied, a specific location on the cell, in this case, can make a difference in the observed response. The ways in which a biological entity manifests its responses are quite complex. The stimuli can lead to changes in the regulation of certain genes, which in turn, result in the synthesis of specific proteins. These proteins may cause secretion of specialized molecules and alterations in cell morphology and intracellular processes.

Applying Micro- and Nanoscience and Engineering Principles and Tools

For the past few decades, research has produced diverse tools to modulate and monitor the activity of cells and tissues. While these tools are widely used in the biological community, some scientific questions require more sophisticated platforms. For example, a majority of conventional modulation techniques are steady state in nature, i.e., cells are exposed to a nontime-varying stimulus profile. This constitutes an obstacle for providing insight into realistic cell responses, as almost all biological processes are highly dynamic. From the monitoring standpoint, most existing assays only offer end-point measurements and are not amenable to real-time data acquisition, thereby limiting the opportunities to more accurately examine the biological response. Aside from the difficulties associated with modulation and monitoring, an enormous challenge is to accommodate the isolated biological samples in a manner that maintains their in vivo-like properties, or at least, the properties that are relevant to the biological question in hand.

An ideal testing platform would seamlessly integrate components to accommodate, modulate, and monitor cells to

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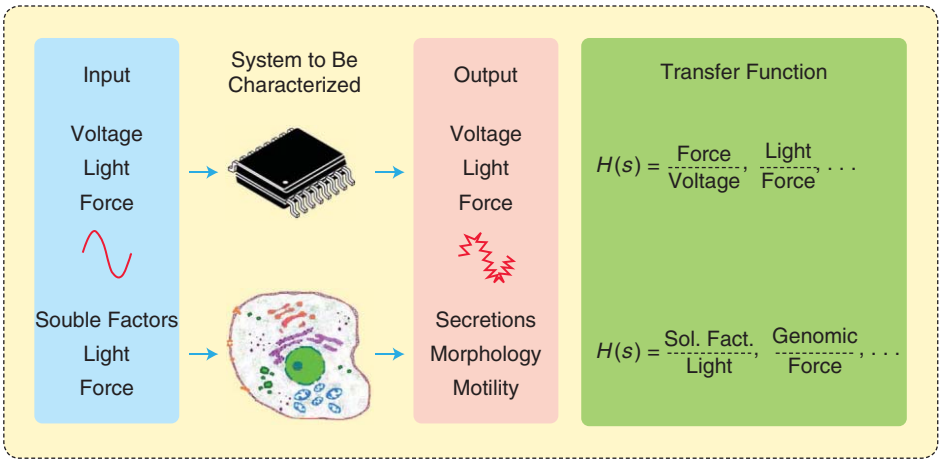

FIGURE 1 Systems identification techniques to analyze cellular processes.

better tap into biological phenomena. Over the last few decades, advancements in micro- and nanotechnology have provided unique opportunities to address some of these challenges. For example, the ability to micropattern cells into an in vivo-like configuration can improve their viability and metabolic activity. Miniaturization of conventional assays to monitor cellular function has generated more rapid assays that consume fewer reagents and allowed numerous assays to be run in parallel, thereby, increasing throughput. The recent advances in modulation allows for optically coupled electrochemical stimulation of cells with previously unattainable spatial and temporal resolution.

While there is a rich set of nomenclature describing miniature platforms and techniques, including microfluidics, microdevices, and micro total analysis systems, they can all be listed under the umbrella term *biological microelectromechanical systems* (BioMEMS). The goal of this article is to discuss several aspects of an ideal integrated platform to study the biological response of cells/tissues, with an emphasis on disease mechanics and therapeutics development.

Modulating Cell Behavior

Much of the cell response (e.g., migration [1], tumor growth [2], development [3]) is defined by a particular cellular microenvironment, which is a function of multiple variables including mechanical (physical interaction between cells and their surroundings) and biochemical cues (signaling molecules and cytokines). The techniques of modulating cell behavior are dealt with before discussing the techniques for preserving in vivo-like attributes of cells.

Chemical Modulation

Over the last few decades, microfabrication techniques adopted from the microelectronics industry have been applied to manipulate liquids and biological entities at small length scales, thereby creating the field of BioMEMS. This rapidly expanding field has enabled schemes to control cell behavior that were previously impossible. Traditionally, cells have been exposed to soluble biochemicals such as cell signaling molecules, hormones, pharmaceuticals, and nutrients. These molecules activate specific receptors on the cell membrane, or within the cell, following internalization, leading to a cascade of events that determine cell behavior. Conventionally, the metered biochemicals are added using a micropipette. In addition to the tedious task to pipette varying concentrations to mimic a time-varying stimulus, a single-step addition of biochemicals produces a stimulation profile that decreases over time due to consumption of factors by the cell or via noncellular chemical degradation.

In general, the decrease in concentration over time cannot be accurately predicted, further complicating the determination of input–output relationship. Microfluidics technology offers the critical ability to perfuse cultured cells with a well-defined stimulus pattern, thereby facilitating stimulus–response analysis [4]. A simple scheme of creating a time-varying stimulus pattern is to use many-to-one channel junctions, where different biochemicals are proportionally mixed [Figure 2(a)]. A computer can conveniently prescribe arbitrary stimulus patterns composed of desired proportions of biochemicals. Such a system, for example, can be used to simulate nutrient concentrations in blood following fasting and feeding. The cells that process and consume nutrients can then be exposed to periodic nutrient variations along with hormones, pharmaceuticals, or even biomolecules that appear during disease, with the goal of understanding the metabolic variations in various disease states, such as obesity or muscle wasting.

Perhaps, one of the most powerful advantages of BioMEMS device over traditional cell culture is the spatial and temporal control of concentration gradients at the microscale level [3], [5]–[8]. At these length scales, the fluidic flow is laminar, that is, the only mode of transport is through diffusion, unless extraordinary measures, such as chaotic mixers are incorporated [9]. This physical condition allows for establishing stable gradients of chemoattractants that play a role in how cells respond during inflammation or infection [10], [11]. The insight from these studies can help us understand the ways the cancer cells invade healthy tissue and the mechanisms of immune cells attacking foreign bodies.

Electrical Stimulation

Some cells, such as neurons and endocrine cells (e.g., pancreatic beta cells), respond to an electrical stimulation by neural transmission of signals and secretion of hormones (e.g., insulin). The ability to electrically induce cells and track their electrical activity has been the basis of electrophysiology, which is directed toward understanding how a network of neurons collectively executes high-level functions such as memory and cognition. The challenge here is to be able to individually stimulate cells. Traditionally, neurons have been selectively induced using microwires, which severely complicated the parallelized and independent stimulation of multiple cells. Micropatterned

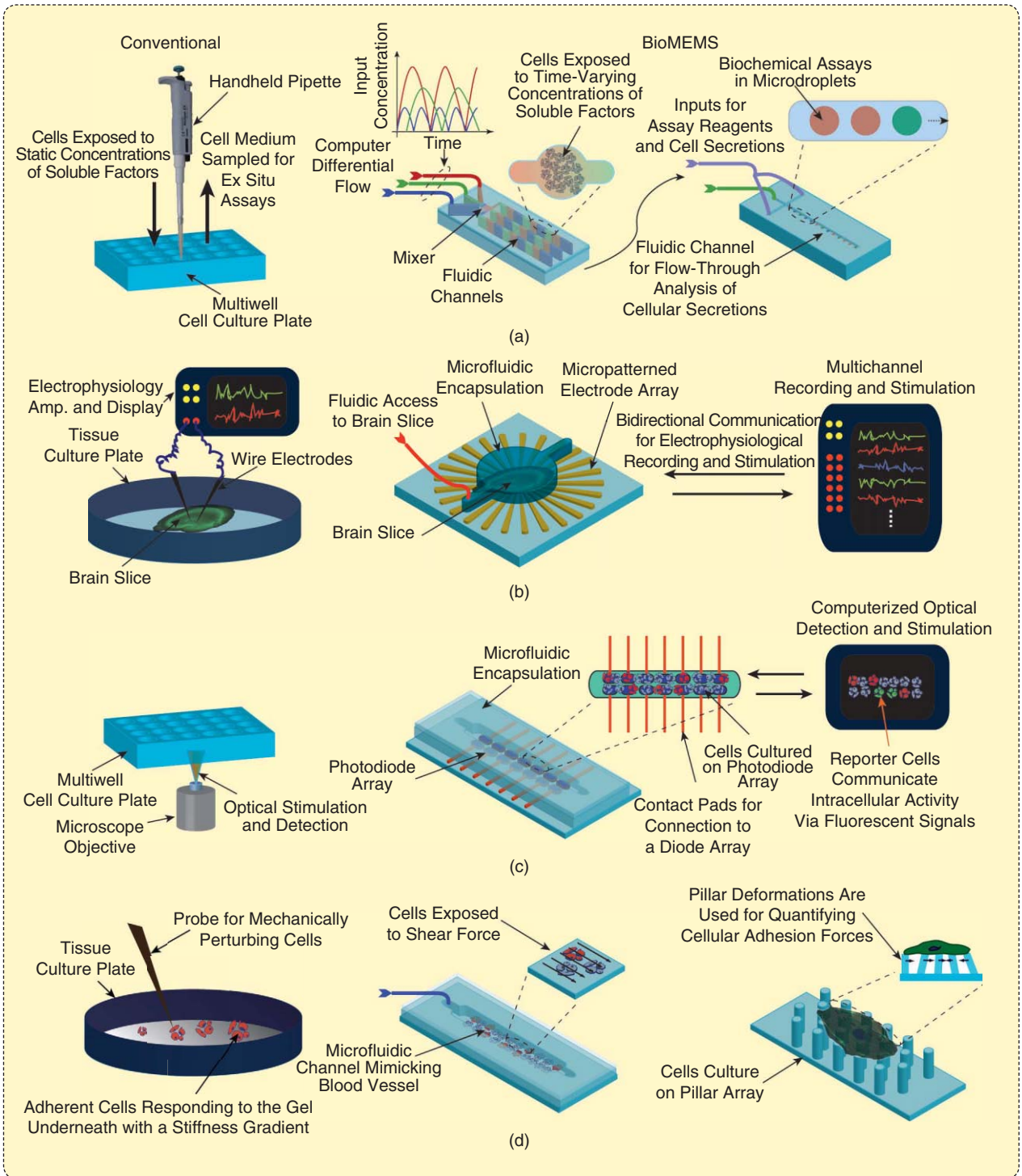

FIGURE 2 Comparison of conventional and BioMEMS approaches to monitor and modulate cellular activity: (a) biochemical, (b) electrical, (c) optical, and (d) mechanical.

electrodes on culture platforms [12], or more exotic nanoprobes [13], coupled with microfluidics for precise temporal and spatial exposure to biochemicals are powerful means to stimulate single neurons [Figure 2(b)].

Optical Methods

Recently, additional sophisticated stimulation tools have become available. Using a clever combination of genetics and optical methods, called *optogenetics* [14], investigators have modified the genetic machinery of different cell types to synthesize nonnative light-sensitive proteins (e.g., channel rhodopsins that originate from unicellular organisms such as green algae). These modified cells can then respond to light stimulation by modulating the inward/outward flow of ions through their cell membranes and consequently result in a unique cell behavior. An advantage of this technique is the

ability to stimulate cells with very high temporal and spatial resolution, which has important implications in the field of neuroscience. Although platforms that combine optogenetic tools (enable by arrays of onboard optical sources) and microfluidics are yet to be seen, we expect that this combination of techniques will yield many new and exciting opportunities for tapping into cell function.

Mechanical Perturbation

Another stimulation mode is mechanical perturbation, which affects the cell response in various ways ranging from changes in morphology to gene expression [15]. For example, endothelial cells coating the veins respond to blood flow by aligning along the direction of flow. It has been difficult to probe cells mechanically in a controllable fashion due to the absence of adequate tools. Microfluidics has been an enabling tool for applying quantifiable fluidic shear forces on cells, as channel dimensions can be precisely engineered to mimic complex vasculature and control flow rates [Figure 2(d)]. Apart from fluidic forces, variations in the substrate mechanical stiffness have been shown to dictate cell response [16]. A striking example of this is stem cells differentiating into adipocyte-like (fat) cells and osteoblast-like (bone) cells on soft and hard surfaces, respectively. Similarly, it has been observed that mesenchymal stem cells and bone marrow stromal cells tend to migrate toward mechanically stiffer surfaces [17], [18].

Monitoring Cellular Behavior

Ultimately, the power of modulation platforms is determined in part by the capabilities of the complementary platforms for monitoring biological response. The requirements for monitoring cellular behavior are the same as those for stimulation (i.e., temporal and spatial resolution), and the relative importance of these parameters depends on the application. For example, temporal resolution is important if one is interested in studying the neural firing rate, while spatial resolution is important when localizing increased neural activity. Cells have a multitude of ways to exhibit their response to a prescribed perturbation. It is important to choose a readout that is most relevant to the biological question one is attempting to answer.

Monitoring the Morphology

The most traditional readouts have involved monitoring the morphology and structural features of cells (i.e., change in shape and size), intracellular products of synthesis and metabolism (e.g., metabolites and secreted molecules), and changes in genes expressed. Morphological changes can be detected by simply observing cells under a microscope. While an experienced person can differentiate various cellular states (such as a healthy versus an unhealthy cell), it is difficult to conclusively identify a cellular state with this method alone. For example, while two cells look similar in morphology, one may be significantly less viable. Such information can be obtained through biochemical means.

Typically, special fluorescent dyes can be used to interrogate whether a cell is alive or dead. There is now a rich and expanding library of such dyes that are used for shedding light onto cellular processes including metabolism, differentiation, and proliferation. Because the majority of optical assays require bulky external equipment, there is a trend toward incorporating optical detection schemes on miniaturized platforms [19] [Figure 2(c)]. Subtle changes in morphology captured by high-content imaging can reveal key information about cellular activity, such as specific changes in cytoskeleton (the truss structure that maintains cellular form) and can offer information about stem cell fate [20]. Complementing the integrated microoptofluidic systems with the techniques of high-content imaging enable a variety of onchip measurements as well as create highly portable devices that can be used in remote settings.

Cell Secretion and Cell Surface Changes

Cells also respond to stimuli by secreting molecules that regulate their own activity as well as nearby or distant cells. For example, certain cells, when they detect an intruder, synthesize and secrete signaling molecules (e.g., interferon gamma) to alert the surrounding cells. There are established biochemical techniques that quantify the secreted proteins based on antibody or other ligand-binding schemes. Microfluidics-based assays show an assurance in improving the detection of such measurements.

A promising advancement that builds upon unique strengths of microscale geometries is the production of reproducible water-in-oil droplets [21] [Figure 2(a)]. The droplets serve as small reaction chambers that can encapsulate cellular secretions, assay reagents, and even single cells. The applications of this method include colorimetric detection of zinc in secretions from pancreatic islets [22] and fluorometric detection of cytokine secretions from encapsulated cells [23]. Electrochemical sensors can also be miniaturized to detect cellular secretions [24]. Cells exhibit changes in their surface structure via expression of different proteins, which are also commonly used for identifying cell types. There have been advances in detecting such surface markers on a single-cell level by combining microfluidic droplets and biomolecular detection methods [25]. A major challenge for monitoring cellular secretions or surface changes, however, remains as the ability to do continuous measurements.

Electrical Activity

The response of neurons is largely characterized by their electrical activity, which in turn can define normal versus abnormal activity. To probe in detail the activity of neurons, it is necessary to have electrodes that can focus on a single neuron [Figure 2(c)]. The challenge is that, as the surface area of the electrode decreases, its sensitivity begins to decrease as well. Nanostructured surfaces with high effective surface areas have alleviated this problem [26]. Microelectrode arrays have also benefited studies outside neuroscience, such as recordings of cardiac action potentials to evaluate pesticide toxicity in a high-throughput manner [27]. Recent studies have also correlated changes in electrical impedance of stem cells to their differentiated state [28], exemplifying how nontraditional readouts can provide additional information about cell behavior.

Genomic Expression

The above readouts are all palpable external to the cell. There are unique challenges associated with trying to monitor intracellular processes, such as expression of certain genes. Conventionally, messenger RNA (a set of intermediate molecules that carry a transcribed form of the gene to the ribosome to be made into a protein) is extracted from the cells and amplified. This process virtually becomes impossible when one wants to probe gene expression in many individual cells or group of cells.

One approach has been to modify the cells by introducing a synthetic DNA that produces an externally detectable signal in response to changes in some part of genetic machinery. A popular example involves the use of fusion proteins that contain green-fluorescent protein. These reporter cells can then be micropatterned into arrays enclosed with microfluidic channels to be exposed to a library of stimuli, leading to changes in cell response that can be readily observed in fluorescence [29] [Figure 2(c)]. This technology, coupled with onchip optical detection schemes, can be very powerful.

Developing Bioinformatics

The ability to modulate and monitor cell response in high throughput creates a new challenge: the necessity to process large amounts of data, preferably, in real time. This need has led to the development of complex bioinformatics algorithms [30]. Referring back to the notion of systems identification approach, the objective is to acquire adequate input-output data points at different time points to create a transfer function, which can then be used for 1) revealing intracellular mechanisms and 2) predicting a set of cellular responses (output) in response to a specific set of input. An accurate transfer function could then be used to run many experiments via computer simulations, saving enormous amounts of time and money by reserving the biological experiments for ultimate in vitro, and eventually, in vivo validation.

Accommodating Environments

BioMEMS technology offers multiple design parameters to create a more physiologically relevant environment, promoting cells to function similar to their in vivo state (Figure 3). We will discuss the differences between traditional and microscale cell culture techniques and the factors to be considered to obtain proper cell growth and normal cell functions inside BioMEMS devices.

BioMEMS for Generating Physiologically Realistic Cell Culture Environments

The flow over cells in microfluidic channels introduces shear stress. Although low shear stress can damage certain cell types, other cells require shear stress to function properly, such as vascular endothelial cells that align themselves in response to blood flow [31]. Careful modulation of flow rate and shear stress inside BioMEMS devices is thus a key factor for improving cell viability and function.

A key parameter that dictates in cell viability and function is the type of material used in BioMEMS devices. A variety of materials, including polydimethylsiloxane (PDMS), polystyrene, polycarbonate, silicon, SU-8, and glass, are available for building application-specific BioMEMS platforms. Each material comes with its own advantages and potential drawbacks. For example, PDMS, the most commonly used material for construction of BioMEMS devices that involve cell culture, offers great versatility for prototyping devices and exhibits reasonable biocompatibility. However, the inherent hydrophobicity of PDMS makes it susceptible to nonspecific adhesion of proteins and to absorbing nonpolar molecules into its polymer matrix, thereby limiting its use in biochemical assays.

Surface treatment techniques such as oxygen plasma treatment, matrix protein coatings, and various surfactants coating partially mitigate hydophobicity and the related challenges. Glass devices are significantly more suitable for biochemical assays; however, their fabrication is generally not trivial. Increasingly, evidence suggests that nanoscale surface topology of a specific material is as important as the material itself in dictating cell behavior. For example, a recent demonstration of a tissue constructed using nanotopographical cues mimicked the ventricular organization of heart and displayed electrophysiological activity similar to that of native tissues [32].

3-D Cell Culture

In animal tissues, cells reside in a three-dimensional (3-D) space surrounded by neighboring cells and an extracellular matrix (ECM). Conventional two-dimensional (2-D) cultures (e.g., cells cultured on planar geometries), therefore, may not accurately represent the in vivo state. Various 3-D cell culture systems are available for researchers to create a more in vivo-like environment. Typically, cells are encapsulated inside a 3-D matrix of hydrogel. Several hydrogels with unique chemical and physical properties exist; however, incorporating a hydrogel scaffold into a BioMEMS device is very challenging. While ultraviolet (UV)-curable hydrogels allow gel formation by simply exposing a microfluidic device to a UV source, there is a concern about the adverse effects of UV light on cell viability and function. Alginate, on the other hand, can polymerize upon contact with cationic solutions; however, slow mixing in microfluidic channels can complicate homogenous gel formation.

Natural hydrogels, such as collagen or Matrigel, can be injected into a device and polymerized by slightly increasing the solution temperature, provided that the devices are kept cold

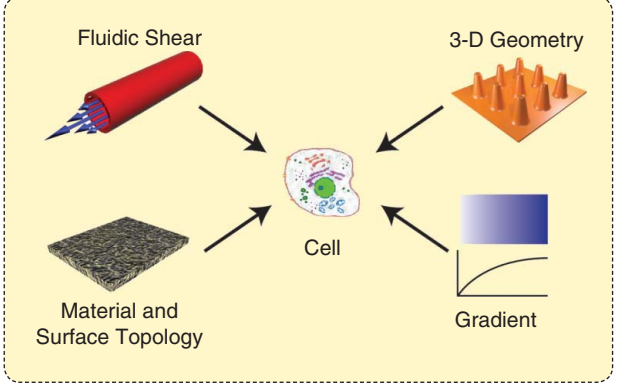

FIGURE 3 Key parameters that play a role in obtaining an in vivo-like cellular response.

beforehand to prevent premature gel formation. In addition, natural tissues consist of different types of cells, and therefore, in many cases, a coculture is necessary to elicit realistic cell function. For example, hepatocytes in the liver coexist with the supporting cell types such as Kupffer cells. Selective micropatterning of different cell types on a surface enables the creation of complex coculture systems and improves the performance of 2-D cell culture systems dramatically. However, extending these techniques to a 3-D geometry requires precise spatial manipulation of cells and is still a significant challenge to creating robust 3-D coculture systems.

Cell Seeding

The small dimensions of BioMEMS devices make uniform cell seeding a challenge. In addition, device surfaces often need to be treated with ECM proteins to promote stable adhesion of cells. Microfabrication technology allows for selective micropatterning of cells on a surface. The surface can be treated with chemical patterns that promote or repel cell adhesion in defined areas. Surface patterning techniques include micro contact printing, UV lithography, microfluidic patterning, and stencil-based patterning. While these approaches are limited to 2-D, several techniques for shaping hydrogels into 3-D shapes exist, including micromolding, UV photopolymerization, microfluidic-based patterning, and direct printing [33].

Integration of Components

Miniaturization allows the integration of components with different functions so that cell seeding, cell culture, modulation, monitoring, and analysis can be achieved on a single chip with high efficiency (Figure 4). However, exploiting this advantage to its full extent requires careful consideration of physical phenomena inside microscale systems.

Scale Up

The common approach for high-throughput platforms is to build an array of microscale wells interconnected via fluidic channels. Microfabrication techniques allow for highly integrated arrays with fluidic control for individual wells. A prominent example is the microfluidic system developed by Quake, consisting of 2,400 individual wells controlled by 7,233 valves, which was used to study the binding of transcription factors [34]. Less extensive microfluidic arrays have been developed for mammalian and bacterial cell culture with the capabilities approaching 384 multiwell plates that are traditionally used for high-throughput experiments.

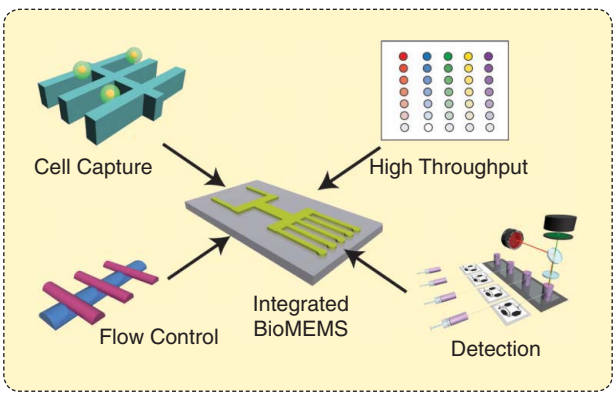

FIGURE 4 The key components of an integrated BioMEMS platform to monitor and modulate cellular activity.

Flow Control

Large-scale microfluidic systems complicate manual flow control, as nanoliter volumes need to be manipulated within microchannels. For this purpose, various miniaturized pumping and valving schemes have been introduced. The most common method of pumping fluid is the use of an external peristaltic or syringe pump. This method creates a parabolic velocity profile inside the channels, which limits uniform delivery of analytes within a channel. An alternative with a more uniform flow profile is electroosmotic flow, where the surface-induced ions displace the liquid when an electrical potential is applied at the ends of a channel. Other nonconventional pumping techniques include passive pumping, such as gravity-driven flow [35] and surface tension-driven wicking [36]. As these techniques do not typically require complicated external circuitry to operate, their integration into microfluidics could facilitate their use in resource-limited settings.

An essential requirement for fluidic control is the ability to precisely guide flow within a mazelike microfluidic structure. Following the demonstration of a PDMS-based pneumatic microvalve [37], most fluidic platforms have employed such valves for flow control. This method uses a compliant PDMS chamber adjacent to the microfluidic channel containing the fluid to be manipulated. Applying negative or positive pressure to the deformable PDMS chamber constricts or relieves the adjacent channel, thereby controlling the liquid flow. This technique is now applicable to highly complex microfluidic devices as well those that are simple, such as handheld devices or screw-actuated valves. Another interesting, but less widely utilized, method uses Braille pin displays to generate peristaltic flow [38].

Although microvalves can be actuated in various modes, including pneumatic [37], electrical [39], optical [40], or magnetic [41] with unique strengths and weakness, the universal challenge has been the excessive complexity of fluidic control. Basic passive flow control is possible by sizing cross-sectional areas and the pressure drops through channels. Simpler techniques are imperative to translate BioMEMS devices into clinical settings or biology laboratories.

While the laminar flow phenomenon in microfluidic channels is advantageous for numerous applications, low Reynolds number flows are not conducive to efficient mixing due to the absence of turbulence. Approaches to improve mixing efficiency include 1) a chaotic mixer created by etching a bas-relief pattern on the floor of a microchannel [42], 2) using magnetic particles to enhance mixing inside a microfluidic device [43], and 2) electrowetting-based mixing of droplets residing on surfaces [44].

Interface with External Components

Miniaturization creates challenges in all aspects of technology development, including detection, fluid manipulation, and stimulation. Another difficulty is interfacing the BioMEMS devices with external components such as conventional pumps, electronic instrumentation, and optical equipment. Typically, external frames, manifolds, and electrical, pneumatic, and optical setups

are used for operating BioMEMS devices. Smaller sample sizes require higher detection sensitivity and, to this end, the sensors have been integrated onto chips for more immediate detection.

Advanced flow control schemes that take advantage of micro- and nanoscale phenomena are expected to reduce the dependence on bulky external solenoids and pressure sources. As mammalian cell culture requires careful regulation of temperature and CO_2 levels, BioMEMS cell culture systems operating outside controlled environments face additional challenges. Finally, a major design question is whether to contain complexity within the BioMEMS device or within its peripheral components. Although complex BioMEMS devices may reduce the dependence on external components, reusability issues may make them less cost effective.

Application Platforms

The combination of the aforementioned BioMEMS tools and techniques allow for the development of platforms to study and control a milieu of biological phenomena. Several application areas to be highlighted have particularly attracted attention from the scientific and industrial communities.

Cell Separation

The ability to capture and enrich specific cell types from the blood stream is of huge medical importance. Physical properties such as size, density, and charge, or affinity to specific ligands, can be used to sort cells. With the ability to precisely control the flow, BioMEMS devices offer advantages over conventional cell-separation techniques such as filtration, centrifugation, and fluorescence-activated cell sorting. One prominent application of cell separation technique is the isolation of circulating tumor cells (CTCs) for diagnostic and treatment purposes, using a microfluidic device coated with antibodies against tumor cells [45]. Diverse adhesion molecules have been used for cell isolation in microchips, including peptides, proteins, aptamers, and nanostructured surfaces.

Stem Cell Differentiation

Stem cells are characterized by the capability to undergo mitotic cell division, resulting in self-renewal and differentiation into specific cell types. BioMEMS devices are ideal tools for reproducing the complex microenvironment of stem cells, inducing differentiation into specific cell types. Various aspects of cellular microenvironments and their effects on differentiation efficiency and cell function have been studied in microchips, such as soluble factors, ECM interactions, cell–cell interactions, mechanical signals, and cellular aggregate size. It is expected that these studies can provide critical insight into the optimal parameters for attaining efficient differentiation of stem cells into functional cell types for therapeutic and drug-development applications.

Artificial Organ-on-a-Chip

There have been a large number of efforts to capture the distinct characteristics of specific organs and build an in vitro system mimicking those features. These characteristics include 3-D geometries, cell–cell or cell–matrix interactions, concentration gradient of chemicals, and blood flow. For example, the liver is responsible for the biotransformation of external compounds, and recreating the function of the liver in vitro would be a tremendous achievement in biomedical engineering. There have been attempts to culture hepatocytes in a microfluidic device, re-create the oxygen concentration gradient that is known to exist in the liver [46], form spheroid-like 3-D construct of hepatocytes in a microfluidic device [47], and micropattern hepatocytes with fibroblasts [48]. Many of these attempts were reported to result in the enhancement of liver-specific metabolic activity. Other organs have been re-created as an organ-on-a-chip. These organs include lung [49], gastrointestinal tract [50], and vascular networks [51]. Recently, Huh et al. reproduced the mechanical movement of alveolar space of the lung, which was reported to enhance the inflammatory response of the lung to nanoparticles [49].

Drug Screening

BioMEMS devices can be used as in vitro platforms for various stages of the drug-development process, such as lead compound identification, toxicity/efficacy screening, and the study of drug-delivery mechanisms. In particular, BioMEMS is useful for creating differential conditions, such as a range of drug concentrations, in a single implementation. An interesting application of BioMEMS technology is the reproduction of whole-body responses to drugs. After a drug is administered, it goes through a complex process of absorption, distribution, metabolism, and elimination, which cannot be reproduced with conventional cell culture methods using a single cell line. A BioMEMS device, termed as *animal-on-a-chip*, has been developed to reproduce the dynamics of drug action in the human body. The microchip consists of multiple chambers representing organs, interconnected with fluidic channels representing the blood flow.

The main advantage of using BioMEMS is that the blood-flow pattern of the human body can be reproduced accurately, and the whole-body pharmacokinetics can be tested in vitro. It has been demonstrated that these devices can reproduce the metabolism-dependent toxicity of naphthalene and an anticancer drug, Tegafur [52], [53].

Fundamental Biological Studies

BioMEMS has been used as in vitro platforms to study various biological phenomena in a more controlled manner. Conditions mimicking a disease state or cellular environment can be better reproduced with microtechnology. The notable examples of this kind of study include chemotaxis, biomechanics, angiogenesis [54], developmental biology, and single-cell genetic networks. With microtechnology, it becomes easier to decouple a specific environmental factor from others and study the effect of a single factor on cell behavior. Similar to the drug-screening application, it is also possible to create multiple conditions in a single implementation to study the combinatorial effect of environmental factors.

Conclusions and Future Directions

The BioMEMS technology offers new possibilities for modulating, monitoring, and accommodating biological entities in unprecedented ways. These novel techniques allow researchers to create a more physiologically relevant environment, which promotes more realistic responses from cultured cells. In this article, we reviewed the recent trends and advancements in

micronanotechnologies related to disease studies and therapeutics development. While numerous proof-of-concept studies exist for BioMEMS devices to make an impact in biology and medicine, we are still far from the acceptance of BioMEMS tools by traditional biology researchers, industrial scientists, and clinicians. Complexity and unreliability in operating BioMEMS devices are two of the major obstacles preventing the widespread use of these tools. We believe that there is a need for simplifying and standardizing BioMEMS tools, as well as training biologists and clinicians for them to benefit from the advantages of BioMEMS techniques. The BioMEMS field is still growing rapidly, and we expect to see real-world applications of BioMEMS devices in the near future.

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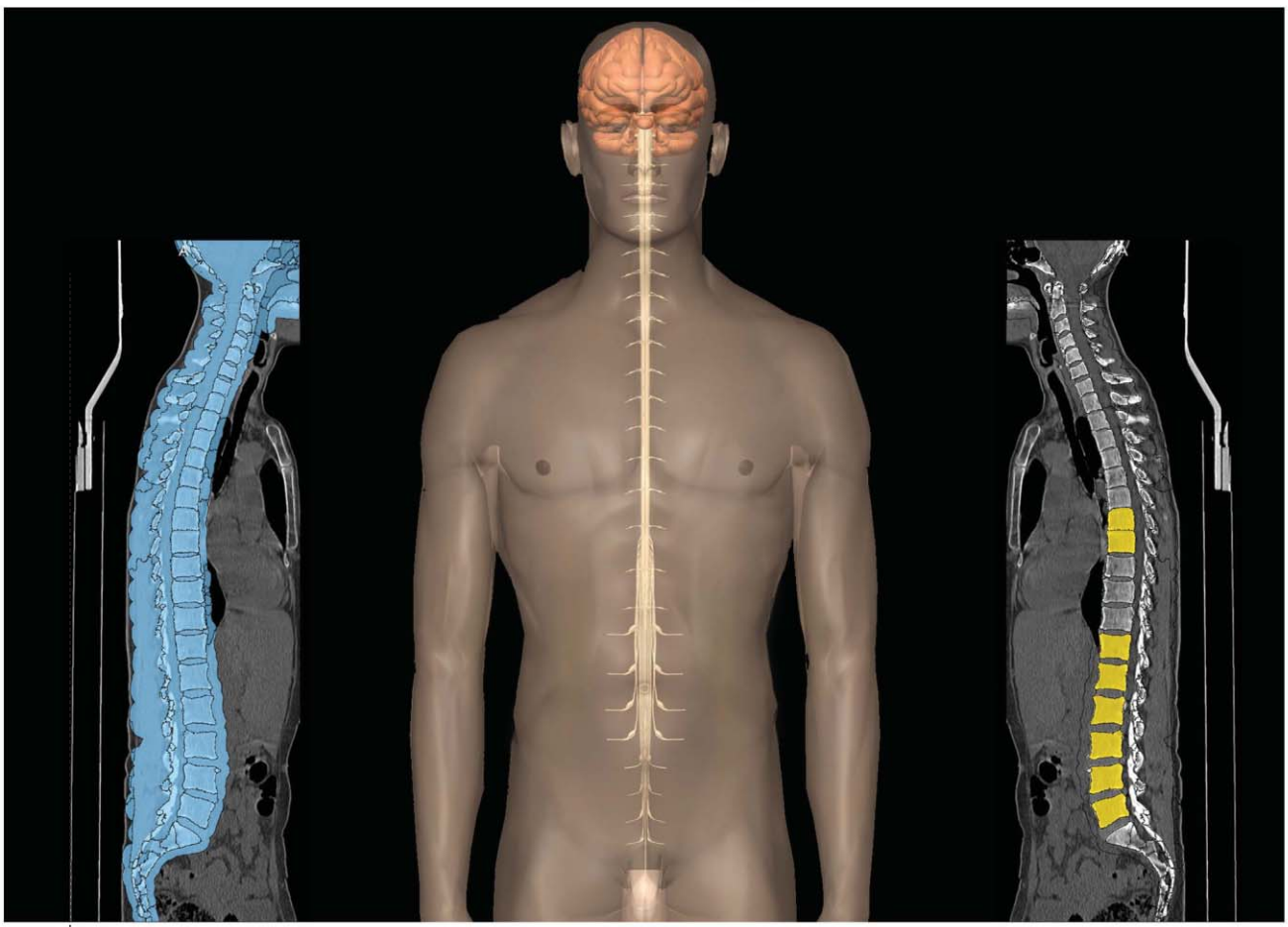
Medical Image Analysis



By Felix Ritter, Tobias Boskamp, André Homeyer, Hendrik Laue, Michael Schwier, Florian Link, and Heinz-Otto Peitgen

A Visual Approach

Since the discovery of the X-ray radiation by Wilhelm Conrad Roentgen in 1895, the field of medical imaging has developed into a huge scientific discipline. The analysis of patient data acquired by current image modalities, such as computerized tomography (CT), magnetic resonance tomography (MRT), positron emission tomography (PET), or ultrasound (US), offers previously unattained opportunities for diagnosis, therapy planning, and therapy assessment. Medical image processing is essential to leverage this increasing amount of data and to explore and present the contained information in a way suitable for the specific medical task.

In this tutorial, we will approach the analysis and visualization of medical image data in an explorative manner. In particular, we will visually construct the image-processing algorithms using the popular graphical data-flow builder MeVisLab, which is available as a free download for

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noncommercial research [1]. We felt that it could be more interesting for the reader to see and explore examples of medical image processing that go beyond simple image enhancements. The part of exploration, to inspect medical image data and experiment with image-processing pipelines, requires software that encourages this kind of visual exploration.

Image Processing

In contrast to general image processing that has primary goals, such as enhancing the esthetics of an image or creating art, the sole purpose of medical image processing is to improve the interpretability of the depicted contents. This may involve an enhancement of the image itself to increase the perception of certain features as well as the automated or manual extraction of information.

The sole purpose of medical image processing is to improve the interpretability of the depicted contents.

Since we can only discuss a few image analysis and visualization methods here, the following classification of the most important categories will provide a short reference for further investigation:

- *Image enhancement*: The removal of image distortions, such as noise and background inhomogeneities, as well as the enhancement of image contours and other relevant properties.
- *Image segmentation*: The identification of the contours of an anatomical structure, such as an organ, a vessel, or a tumor lesion.
- *Image registration*: The spatial transformation of one image such that it directly matches a given reference image. This is necessary, for example, in the combined visualization of images from different modalities (e.g., PET/CT).
- *Quantification*: The determination of geometrical properties of an anatomical structure (e.g., volume, diameter, and curvature) or physiological properties such as perfusion characteristics or tissue composition.
- *Visualization*: Two-dimensional (2-D) and three-dimensional (3-D) rendering of image data (e.g., volume rendering) and virtual models (e.g., surface models) of organs and other anatomical structures.
- *Computer-aided detection*: The detection and characterization of pathological structures and lesions, such as tumor lesions or vessel obstructions.

The term image is used here not only to refer to a conventional, 2-D image, for example, a radiograph. An image may also be comprised of a 3-D volume of parallel slices (e.g., a series of CT or MRT tomograms) and even a sequence of image volumes acquired over time, such as a dynamic MRT study, may be considered a four-dimensional image.

Images of volumetric imaging modalities, such as CT and MRT, are the most common source for medical image processing. Each volume is composed of volumetric image elements called *voxels* that represent a value on a regular, 3-D grid. The image values of CT images are of absolute value measured on the Hounsfield scale, a scale describing radio density. In our second example, we use knowledge about the radiation absorption of different materials to separate tissue, which has a value of around +30 Hounsfield units (HU) from air, approximately –1,000 HU. Such a separation is the most basic form of segmentation called *thresholding*. Often, however, materials do not differ enough in radio density to be distinguishable by using thresholding alone. For instance, the HU value of blood is around +40 HU and the value of liver tissue is between +40 and +60 HU. The perception of blood-filled vascular structures within the liver would be difficult, if not impossible, given such a small difference. This is why contrast agents are important. When applied to a patient, they will change the imaging characteristics of the aimed structures. Using a contrast agent for vascular structures will increase the HU value of blood significantly above the value of the surrounding tissue (e.g., +120 HU). The contrast-enhanced blood will not only flow through the arteries and eventually arrive in the liver but also accumulate in the liver tissue itself. Hence, timing becomes very crucial for imaging certain structures.

In contrast to the Hounsfield scale of CT imaging, the MRT imaging scale has no absolute physical meaning. But like a black and a white photograph in which you can easily distinguish a darker lawn in front of a house painted in a brighter color without knowing the exact brightness of each color, MRT images will depict different tissue characteristics in a meaningful way. In most cases, relative differences in image values are sufficient for medical diagnosis and absolute values are not required. Protocols for acquiring absolute values often need multiple scans and are therefore also more time consuming.

Generally, medical images contain noise. Noise may impede the analysis of regions based on voxel values. Smoothing the image may help in this case but will also reduce the perception and detection of contours. Like with other basic image enhancement methods such as sharpening, smoothing can be based on a fundamental image operation called *convolution*. Convolution replaces each voxel value by a weighted sum of its neighbors. The weights are specified by the convolution kernel, which can be represented by a matrix. The most basic smoothing kernel replaces each voxel with the weighted average of the direct neighbors, hence will contain a value of 1 in all elements of a $3 \times 3 \times 3$ matrix. More common, however, is a binomial (Gaussian-like) convolution kernel that applies more weight to less-distant neighbors.

A very common segmentation method for coherent regions of an image is region growing. Region growing combines homogenous, neighboring voxels to form a region starting with a given, initial voxel. Accepted values of adjacent voxels may be fixed and set in the beginning or are being adapted automatically while the algorithm spreads in the vicinity. The initial set or range of accepted voxels is often derived from the value of the initial voxel.

While looking at individual slices of a volumetric image is the most common form of diagnostic reading or inspection in the preparation of a surgical intervention, 3-D visualizations may assist the analysis of spatial relations of different structures (Figure 1). Most 3-D visualizations are 2-D projections of a 3-D image displayed on a conventional screen. By adding the ability to interactively change the point of view and reveal certain structures while hiding others, 3-D visualizations become an

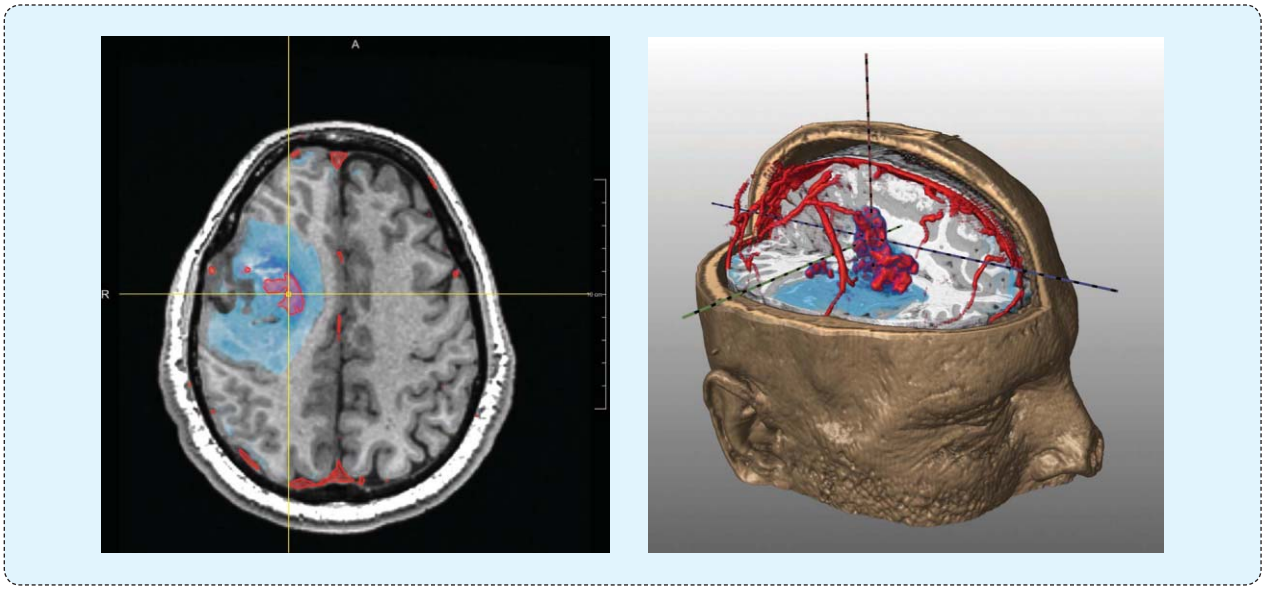

FIGURE 1 A coherent 2-D and 3-D visualizations of multimodal brain data using MeVisLab. A lesion (blue) as well as an arteriovenous malformation (red) are highlighted. The crosshair indicates the same location in both visualizations.

extremely useful tool to build up spatial understanding. There are two main 3-D visualization methods for medical image data: direct volume rendering and polygonal isosurface rendering. Direct volume rendering, as the name implies, is able to directly visualize image data. We are not required to segment structures beforehand since the whole image data are evaluated for display. Voxel values are mapped to colors and opacity values according to a transfer function. In contrast, polygonal isosurface rendering relies on the ability to extract regions from the image, which are then rendered as polygonal meshes. An isosurface represents regions of voxels of a certain value. While isosurfaces may be extracted directly from the image, this method is commonly applied to mask images of already segmented structures.

The examples discussed hereinafter will combine a number of image analysis and visualization methods to provide solutions to common medical tasks.

MeVisLab

The design and exploration of new methods in medical image processing and visualization are creative processes starting with an initial idea in need of realization and validation. In the same manner as architects sketch their ideas of a new building on a sheet of paper, making refinements on their way to the final drawing, the image-processing researcher could benefit from sketching algorithmic ideas in a visual way if this sketch can be brought to life with real medical image data.

MeVisLab provides such an environment of advanced medical image processing and visualization building blocks that can be combined visually to create new algorithms and prototypical applications, enabling the researcher or clinician to evaluate these algorithms in clinical settings. Originally designed as an internal development tool of the German Fraunhofer MEVIS research institute and its commercial spin-off MeVis Medical Solutions, MeVisLab was made publicly available in 2004 and is currently used in numerous research and development sites worldwide.

MeVisLab allows the user to create macro modules that encapsulate a network with all its modules, connections, and parameterizations.

MeVisLab includes a comprehensive, extensible library of functional components, hereinafter referred to as *modules*, for segmentation, registration, and quantitative morphological as well as functional analysis. For the visual presentation of medical data, interactive 2-D and 3-D viewing, contour, surface model, and volume representations are available (Figure 1). These modules can be combined to form module networks that implement complex image processing and visualization tasks. MeVisLab uses a graphical programming approach for the development of such networks, allowing the developer to instantiate modules in a network and draw connections in-between to define the flow of data and control information (Figure 2). Besides the MeVisLab-native MeVis image-processing library (ML), the well-known National Library of Medicine Insight Segmentation and Registration Toolkit (ITK) as well as the Visualization ToolKit (VTK) have been integrated in a manner that encourages and allows combination of their modules.

In analogy to the concept of classes in object-oriented programming, MeVisLab allows the user to create macro modules that encapsulate a network with all its modules, connections, and parameterizations. Such macro modules may be instantiated and combined with other modules in a network, allowing one to implement even more complex networks in a structured, hierarchical, and modular way.

The parameters offered by many modules to control their internal operations can be manipulated in each module's graphical user-interface (GUI) panel (Figure 2). Connections

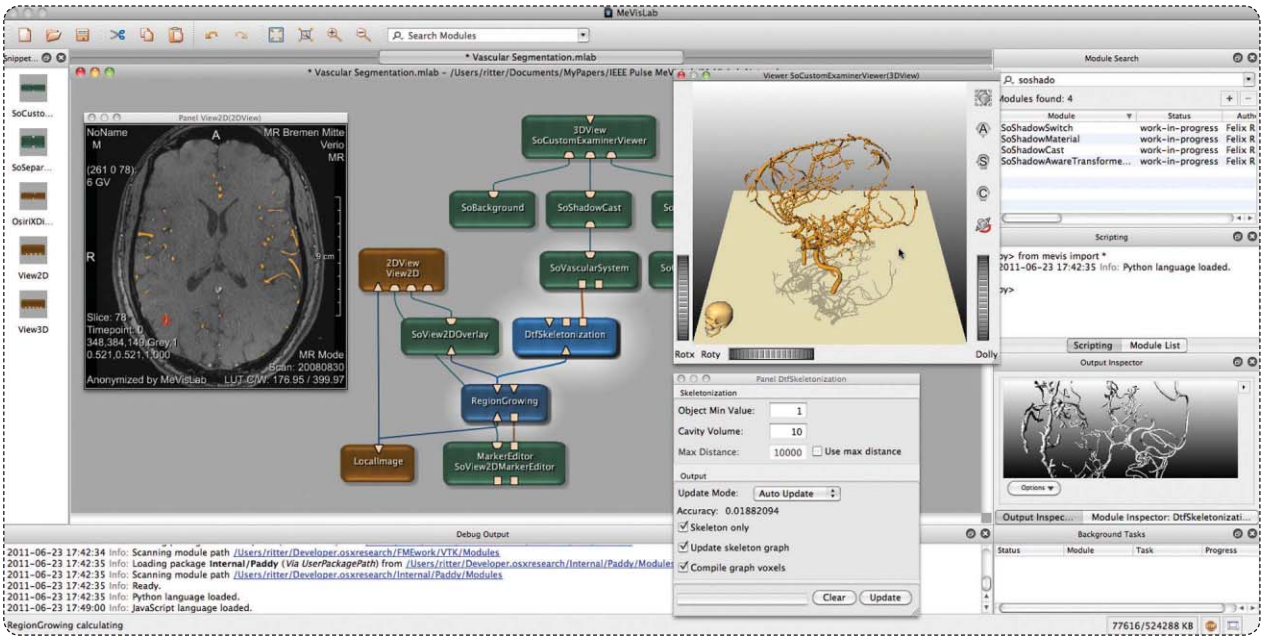

FIGURE 2 The MeVisLab visual development environment. Algorithmic building blocks displayed as boxes can be combined to form a more complex algorithm via an intuitive GUI. The parameter panel of the DtfSkeletonization algorithm is displayed to enable its configuration.

between parameters can be established to automatically transfer information between algorithms. This panel may also display result values that are generated by the module. The layout and appearance of the module panels is defined using an abstract, hierarchical scripting language. Such user-interface panels may also be defined for a complete network, giving access to only those parameters and results that are necessary for using the functionality implemented in the network.

MeVisLab offers specific modules to integrate a module network into the clinical data infrastructure. The well-accepted Digital Imaging and Communications in Medicine (DICOM) standard is used to receive image data from the imaging modalities or to transfer images from or to a clinical image data archive [picture archiving and communication system (PACS)]. DICOM tools, such as the popular OsiriX DICOM viewer, may serve as an interface for MeVisLab-based image analysis. As a result, complete software application prototypes can be created to facilitate the evaluation of image analysis or visualization method as well as dedicated workflows in a clinical setting providing valuable input for the further development of a method.

Image Processing Using MeVisLab

Two representative examples will guide our discussion on the processing of medical image data using MeVisLab. Both example networks and image data are available for download [6] and can be explored with an installation of MeVisLab [1]. The first example illustrates the segmentation of elongated structures, such as vascular systems, and their visualization in 3-D. The reconstruction of vascular structures from image data is highly useful for the diagnosis of cardiovascular diseases, in particular, the detection of abnormal variations such as aneurysms. In surgery, estimating the risk of injuring the blood supply and drainage of organs is of great importance. The second example segments, quantifies, and visualizes a round lesion that is attached to a healthy tissue. Here, we would like to calculate the maximum diameter of a metastasis in the human lung that connects to the chest wall but does not still infiltrate it. The automatic calculation of the maximum diameter in 3-D provides a much more reliable indication of shrinkage or growth in follow-up examinations than the manual measurement of a diameter in just one cross-sectional image.

MRT images will depict different tissue characteristics in a meaningful way.

Segmentation and Visualization of Vascular Structures

If you like to follow the discussion of the reconstruction of vascular structures in the MeVisLab application, please open the network file `Vascular Segmentation.mlab` in [6]. In the left viewing panel of the example network, click with your mouse in one of the bright white circular spots that represent a vascular structure. Click again in another white spot to add other vascular structures (Figure 2). To remove the markers you just placed, double-click on the module `MarkerEditor` in the network window and activate the button `Delete all`. The segmented structures are indicated in an orange color in the 2-D and 3-D views.

The reconstruction of vascular structures from a volumetric data set (stack of images) involves several steps (see Figures 3 and 4):

1) *Preprocessing*: Gaussian smoothing of noisy image data.
2) *Segmentation*: Region growing using an initial seed point.

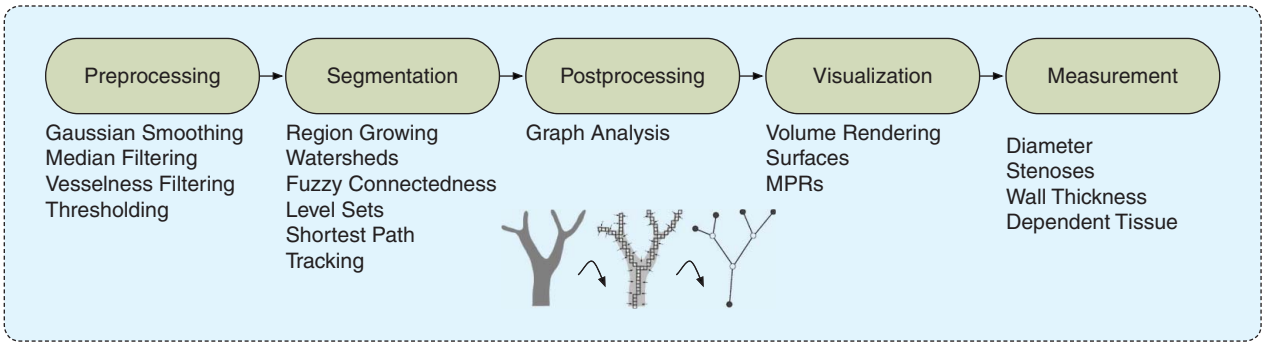

FIGURE 3 An image-processing pipeline for the visualization and quantification of vascular structures from image data.

3) *Postprocessing*: Analysis of the segmentation object and construction of a model-based representation.
4) *Visualization*: Surface rendering of the model-based representation.
5) *Measurement*: Quantification of dependent tissue for risk estimation in surgery.

For our first example, we omit the preprocessing step and start with the segmentation of the loaded image data. Loading image data can be done in several ways, depending on the nature of the data, but like the rest of the image-processing pipeline always involves a module. A series of DICOM images, which is the most basic form for medical images, can be retrieved from a disk using the `DirectDicomImport` module. The provided example data have been compacted and stored as a single file, compressed 3-D data set in the MeVisLab native format. These data are accessible via the `ImageLoad` module. We use a variant of this module called `LocalImage` to automatically locate the data in the directory of our example network. This module is a good choice for demonstration networks.

In MeVisLab, we distinguish between image-processing modules and modules that perform visualization tasks because of the different underlying frameworks. Whereas image-processing modules (colored in blue) form a pipeline, for which dedicated input and output connectors are provided, visualization modules (colored in green) are connected in a hierarchical graph called a *scene graph*. This scene graph is traversed depth

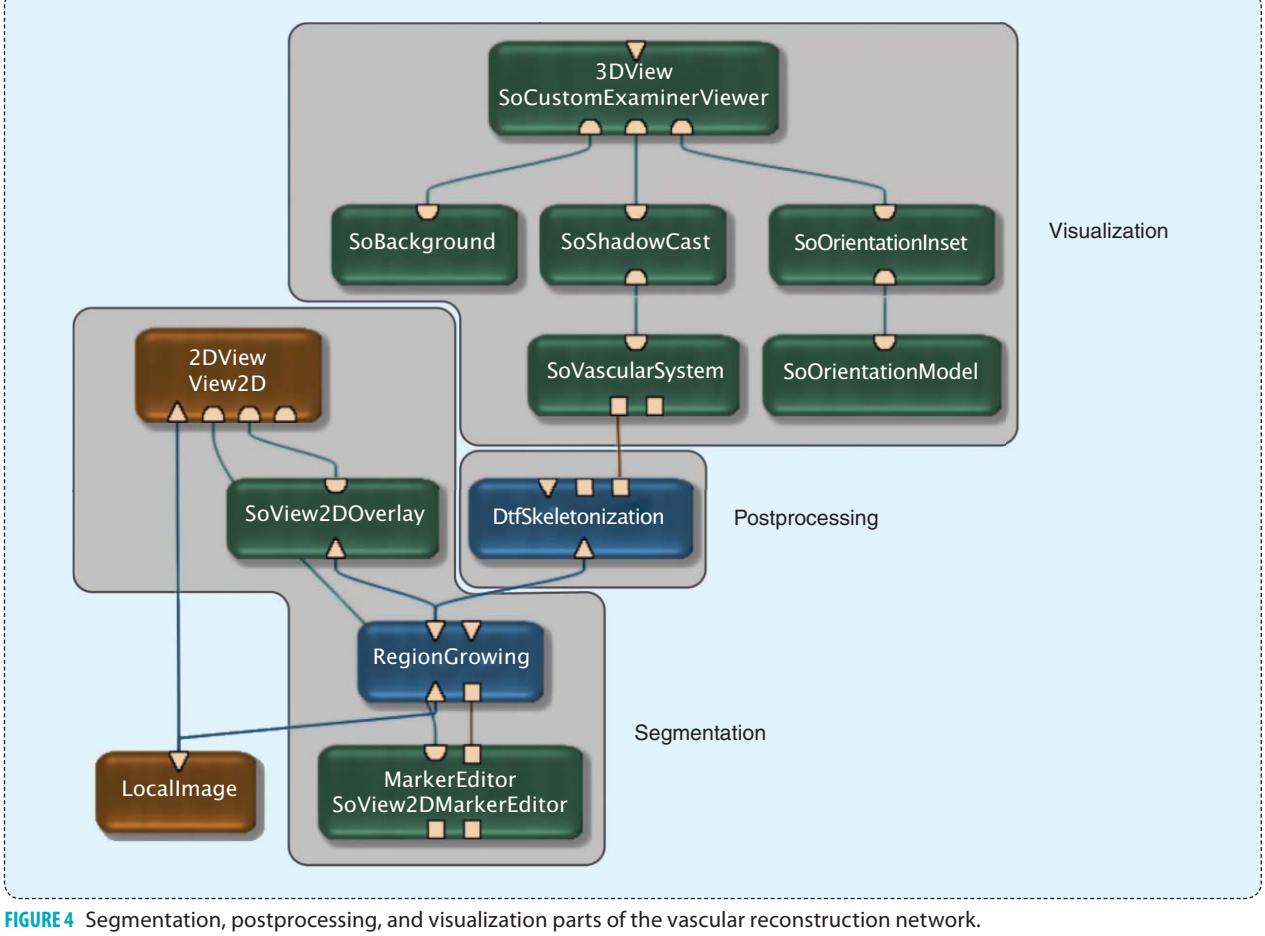

FIGURE 4 Segmentation, postprocessing, and visualization parts of the vascular reconstruction network.

first from left to right. At the root of this scene graph, we often find a viewing component displaying the visual result of the image-processing network.

Visualization and analysis often involve a separation of structures. We apply a 3-D region growing approach to the original image data to segment the vascular system. Starting with an initial voxel from the contrast-enhanced image, neighboring voxels of similar value are combined. A 2-D slice-based visualization, commonly found in medical workstations, enables the diagnostic exploration of the image set and allows the user to place a marker on an initial voxel using the mouse or trackpad. The segmented structures are highlighted on each slice to indicate the result of the region growing. Two main parameters influence the result, the lower and upper thresholds of voxel values and the initial voxel locations. Multiple markers may be placed to segment disconnected regions. The implementation tries to minimize computation by adding to or removing from already segmented regions if parameters fit.

MeVisLab provides UI building capabilities to hide the network complexity from the user.

The 3-D visualization of the segmented vascular structures supports the recognition of branching patterns as well as the inspection and analysis of their path. As outlined before, we may apply direct volume rendering and polygonal isosurface rendering. With both methods, however, small vessels tend to disappear completely. Owing to the discrete nature of the images and the segmentation mask, a model-based reconstruction algorithm that respects the thin structures of vessel trees whose diameters often grow and shrink from slice to slice will result in a much smoother visualization.

A simple but adequate model assumes that the cross section of nonpathologic vessels has a circular shape. Based on the Skeletonization algorithm with topology-preserving thinning, an exact centerline and the radius at each voxel of the skeleton are computed. The skeletonization uses the binary segmentation image and successively erodes border voxels until a center voxel is found. A subsequent graph analysis transforms the skeleton in a directed, acyclic graph where nodes represent branches (Figure 3). Further enhancements may involve pruning and smoothing of the graph. This model has been implemented in a MeVisLab module called `DtfSkeletonization`. It is directly connected to the `SoVascularSystem` module for the final graph-based visualization. The module, among other things, allows the user to limit the rendering of the vascular structures to a certain branching level. For instance, this is helpful when supporting planning of surgical interventions where branching levels indicate the volume of the dependent tissue.

The example previously shown in Figure 2 adds a shadow and a 3-D depiction of the skull to the 3-D view to facilitate orientation for nonexperts. Buttons for the three main anatomical planes, axial (aka transverse), sagittal, and coronal, are placed at the right side of the 3-D view. For an occasional analysis of image data, working directly with the MeVisLab network and individual module panels might be acceptable. However, for frequent use or studies run by a different person, a more usable and comprehensible user interface (UI) will be less error prone and also more productive. MeVisLab provides UI building capabilities to hide the network complexity from the user. Whole module panels or parts thereof can be combined with viewing panels and other supporting UI components to form a custom-tailored image-processing application. Figure 5 shows the final UI for this example network.

The UI script is written in a simple UI language (see Figure 6) that references module panels or parameter fields. For instance,

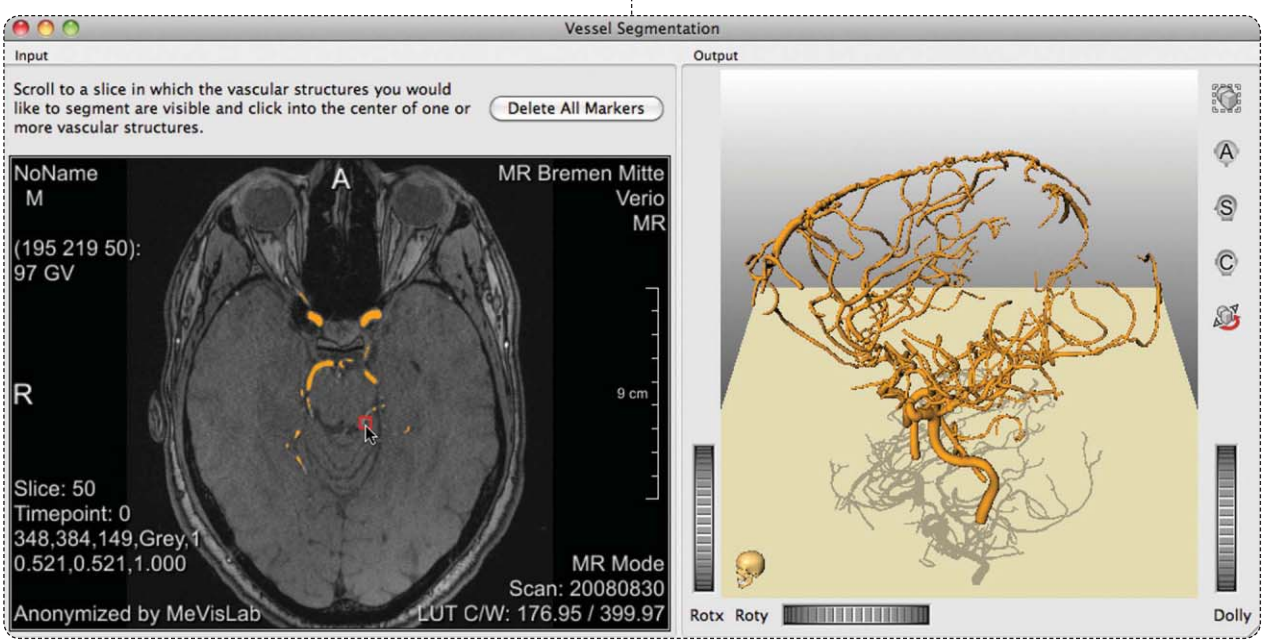

FIGURE 5 The final UI of the vascular segmentation example. The complexity of the underlying module network is hidden from the user. Choose Scripting → Start Network Script in MeVisLab to display this UI.

the Delete All Markers button in our final UI is created by referring to the parameter field `deleteAll` of the module `MarkerEditor`. `MarkerEditor` is the instance name of the `SoView2DMarkerEditor` module. The viewing panels are also referred to by the instance name but are suffixed by the special identifier .self. Layout components, such as boxes as well as horizontal or vertical alignment groups, help to arrange and semantically combine interface components. To create or open the UI script of a MeVisLab network, select Scripting → Edit Network Script from the menu bar. The script will open in the MeVisLab text editor, which provides syntax highlighting, context-sensitive help, and automatic completion for the modules and fields of the image-processing network. The result of the UI script can be tested at any time within MeVisLab by selecting Scripting → Start Network Script from the menu bar. If all module panels are closed and the MeVisLab visual development environment (IDE) has been minimized, the custom-designed UI of our image-processing application remains the only visible part.

```
1   Window {
2     style = _default
3
4     title = "Vessel Segmentation"
5
6     Horizontal {
7       expandY = yes
8       Box "Input" { expandY = yes  layout = Vertical
9         Horizontal {
10          HyperLabel { text = "Scroll to a slice in which the vascular structures
11                              you would like to segment are visible and click into
12                              the center of one or more vascular structures." }
13          Button MarkerEditor.deleteAll { title = "Delete All Markers" }
14        }
15        Viewer 2DView.self { mw = 400 mh = 400 type = SoRenderArea }
16      }
17      Box "Output" { expandY = yes  layout = Vertical
18        Viewer 3DView.self { mw = 400 }
19      }
20    }
21  }
```

FIGURE 6 The UI script defines which parameters of the image-processing network are visible and how they are presented to the user.

Segmentation, Visualization, and Quantification of Lesions

The second example segments and computes the maximum diameter of a lung metastasis. Automatic segmentation methods are often restricted to a specific type of object and data on which they operate because of need for heuristics. Here, the algorithm is based on the assumption that connected vascular structures will be of small diameter when they finally reach the lesion. Furthermore, the lesion must not infiltrate any connected tissue. The segmentation pipeline will be robust enough to ignore connections of the lesion to tissue and will also ignore connected vessel systems, in this case the pulmonary artery and vein.

To obtain a first look onto the example in MeVisLab, please open the network file `Lesion Segmentation.mlab` [6]. In the left 2-D view, scroll to slice number 46 using the scroll wheel of your mouse while pointing at the 2-D view. Place a marker into the lesion as seen in Figure 7. The segmented lesion will be instantly highlighted in the 2-D as well as in the 3-D views. To display the maximum diameter of the lesion, open the module panel of the `MaxDistance` module.

An isosurface represents regions of voxels of a certain value.

For an inspection of intermediate results in the image-processing pipeline, display the Output Inspector by choosing View → Views → Output Inspector from the menu bar. The Output Inspector will display the data available at a selected module connector.

The segmentation and quantification of the lesion involves the following steps:

1) *Preprocessing:* Thresholding and connected components analysis to compute a mask of the lung image data.
2) *Segmentation:* Region growing using an initial seed point.
3) *Postprocessing:* Dilation and computation of the border of the lesion mask image.
4) *Visualization:* Direct volume rendering of the image data and highlighting of the lesion.
5) *Measurement:* Quantification of the lesion's maximum diameter using the lesion mask image.

Large parts of a chest CT image data will contain air voxels. Air and tissue can be well distinguished in CT image data because of their different voxel values. We use an `IntervalThreshold` module with a threshold value of –200 HU for the separation since air voxel values are around –1,000 HU and tissue values are more than 0 HU. Separation of the lesion from the connected tissue is more challenging; the voxel values may not differ enough to prevent the region growing from spreading from the lesion into the adjacent regions. Since we know that the lesion will be inside the lung, we can use a mask of the lung volume to cut off the lesion at the border. This brings us the task of calculating a lung volume mask.

We already obtained a mask of all nontissue voxels from the `IntervalThreshold` module. Assuming the lung will

be the single largest object in the images, an assumption truly holding for a chest CT acquisition, a connected component analysis using the `ConnectedComponents` module will find this single largest cluster. The resulting mask image of this cluster will still contain holes and notches from the blood vessels, bronchial tubes, free-standing as well as wall-attached lesions of the lung. We are particularly interested in the boundary of the volume, in the convex hull of the lung. The `ConvexHull` module computes the convex hull for voxels of a specific value. Now that we possess a mask of tissue plus lesion and convex hull of the lung, we create a new mask of all nonair voxels that are contained within the lung using a `Mask` module. The resulting mask will still contain vascular structures. In contrast to the lesions, vascular structures are of elongated shape, a shape we eliminate from the images using a fundamental, morphological operation called *opening*. Opening uses a structural element, called the *kernel*, to eliminate structures smaller than this structural element. Hence, the kernel size parameter in the `FastMorphology` module needs to be as large as the largest structures we would like to remove. The kernel will have a global effect on the mask image and also modify the morphology of lesions; borders will be smoother and small notches will disappear. This is something to keep in mind when applying these operations.

Automatic segmentation methods are often restricted to a specific type of object and data on which they operate because of need for heuristics.

If there is only one lesion in the data set, we can stop now. The mask image will only contain this lesion. However, more often than not, the images will reveal multiple lesions. To quantify the size of a specific lesion, we opt for an interactive selection. Hence, the `RegionGrowing` module in the image pipeline will use a marker, placed on a 2-D slice, to narrow down the image mask to just the selected lesion.

The calculation of the diameter is a perfect example of using the large image processing library of MeVisLab. There is no module that computes the maximum diameter of a 3-D image object from a given mask image. One could decide to implement such a module. However, MeVisLab provides a module to calculate the maximum distance between a set of markers called `XMarkerListMaxDistance`. Fortunately, we can convert a mask image into a set of markers using the module `MaskToMarkers`. However, applying this module directly to our lesion mask image would create many inside markers, while all we need are markers on the outer border of the lesion. The `Surround` module offers a mode to calculate just the boundary of a set of voxels. Placing this module between the `RegionGrowing` and `MaskToMarkers` modules saves computation time and enables us to overlay the resultant border image onto the original image in the 2-D view to provide visual feedback about the segmentation to the user.

Strictly speaking, the 3-D visualization of the lesion is only of limited diagnostic advantage. It provides an additional means for the inspection of the segmentation result, hence the calculation of the maximum diameter. However, depending on the chosen therapy, which could, for instance, perform local

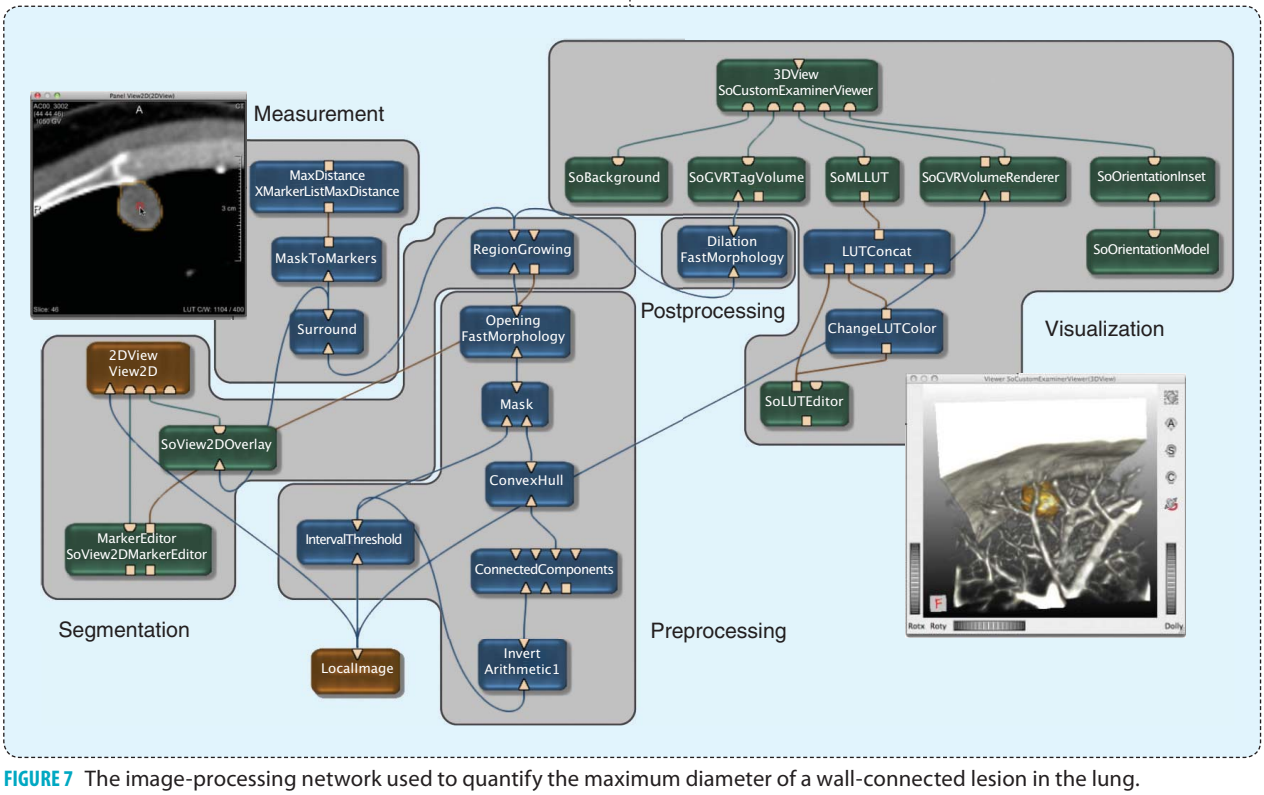

FIGURE 7 The image-processing network used to quantify the maximum diameter of a wall-connected lesion in the lung.

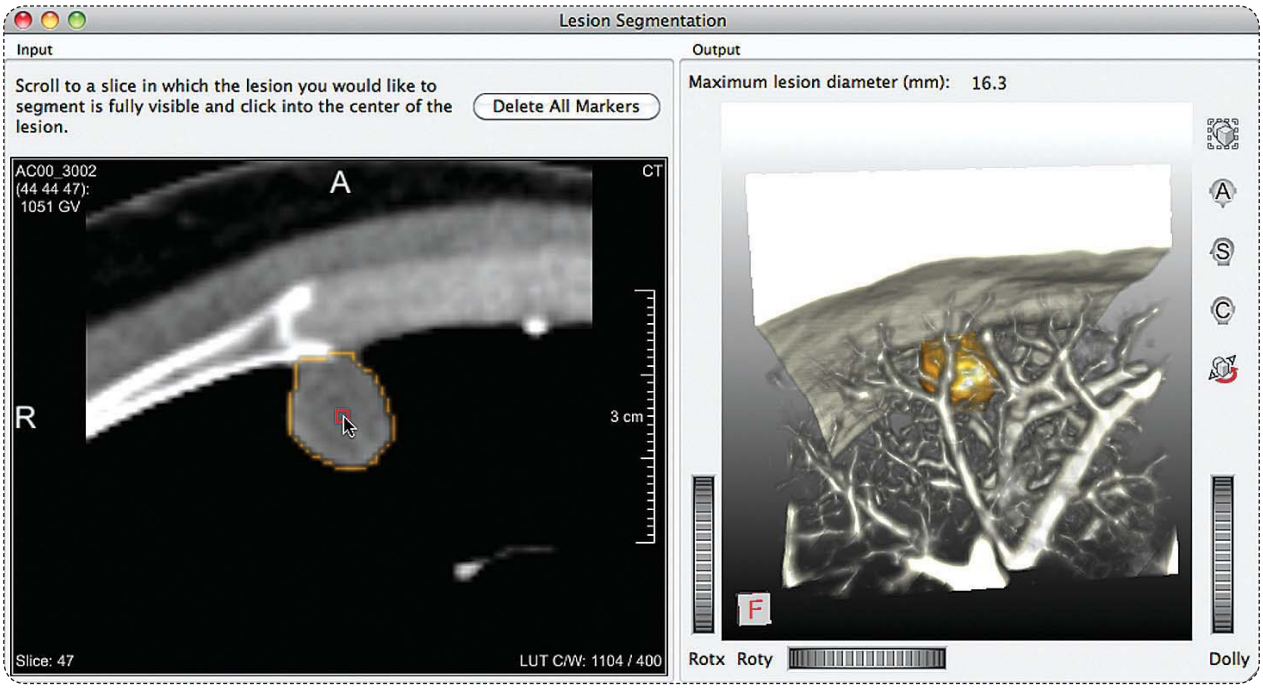

FIGURE 8 The final UI of the lesion quantification example.

destruction of the tumor, knowledge about the lesion's surroundings could prove beneficial.

Direct volume rendering is well suited for the 3-D depiction of this kind of data. Since air and tissue are represented by different values in CT and MR images, visual separation does not need an explicit mask or any additional parameters for the transfer function (e.g., derived values, such as the gradient or curvature). Highlighting the lesion itself is different. We employ the slightly enlarged lesion mask image to apply a different transfer function to the voxel values of the lesion in the original image. Voxel values with a mask value of zero are rendered using the first transfer function, designed using the `SoLUTEditor` module, whereas the second transfer function is applied to voxel values with a mask value of one. This transfer function is derived from the first by means of the `ChangeLUTColor` module. It overrides just the color values of the transfer function with a highlighting color. The volume rendering itself is implemented in the `SoGVRVolumeRenderer` module, which on its own offers various parameters to tune the visualization. The final UI of our lesion segmentation example can be seen in Figure 8.

Visualization and analysis often involve a separation of structures.

Advanced Image Processing

In this tutorial, we could only scratch the surface of medical image processing. Two more advanced image-processing topics will conclude this tutorial and hopefully spark some interest in this field. There is still a lot to explore.

Object-Based Image Analysis

Even the most sophisticated image analysis methods available today cannot cope with the remarkable pattern-recognition capabilities of human beings. For a human mind, an image represents a complex web of interrelated objects at different scales. It is prior knowledge on the characteristic properties and mutual relations of these image objects that enables a human to understand the image content. For a computer, an image is merely a rectangular grid of voxels with intensity values. Therefore, most image-processing algorithms are limited to the evaluation of voxel values in local neighborhoods. To overcome this limitation, a rich set of tools for analyzing images on the basis of objects has been created using the MeVisLab platform, similar to the way a human understands an image. This set comprises simple-to-use functionality for extracting spectral, shape, and relational object properties from the image and for the knowledge-based evaluation of this information to identify the structures of interest.

In many areas of medical imaging, object-based image analysis solves problems that are unsolvable by ordinary voxel-based image analysis. Complex image structures often do not become instantly obvious. Instead, image understanding is an iterative process of deriving information from the image and applying prior knowledge. This iterative process is demonstrated by an application for the detection of the spine in CT images [Figure 9(a)]. Voxel-based image-processing algorithms fail in this application because the spine does not exhibit unique intensity values. Also, many are too distorted or inconspicuous to be identified in isolation. Object-based image analysis solves this problem by exploiting prior knowledge that the spine is a loose chain of vertically aligned vertebrae with characteristic spectral and shape properties. A watershed-based oversegmentation creates the initial set of objects [Figure 9(b)]. The

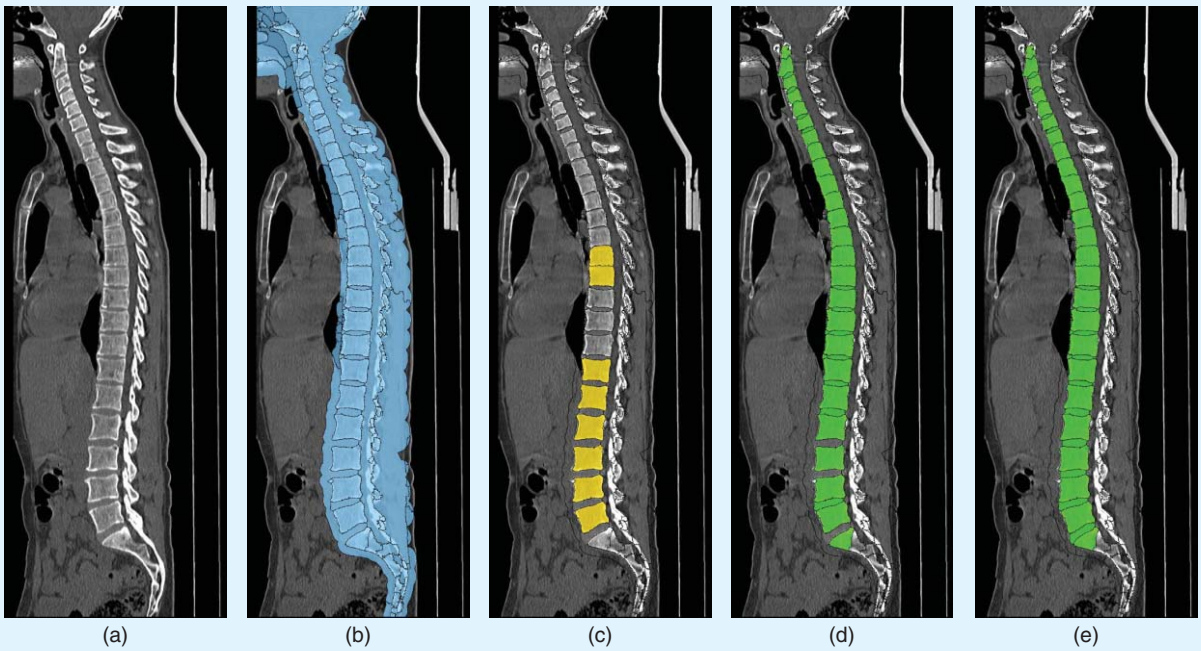

FIGURE 9 Object-based detection of the spine: (a) original image, (b) initial oversegmentation, (c) detection of certain vertebrae, (d) iterative classification of adjacent objects leading to full spine detection, and (e) refinement to close possible gaps.

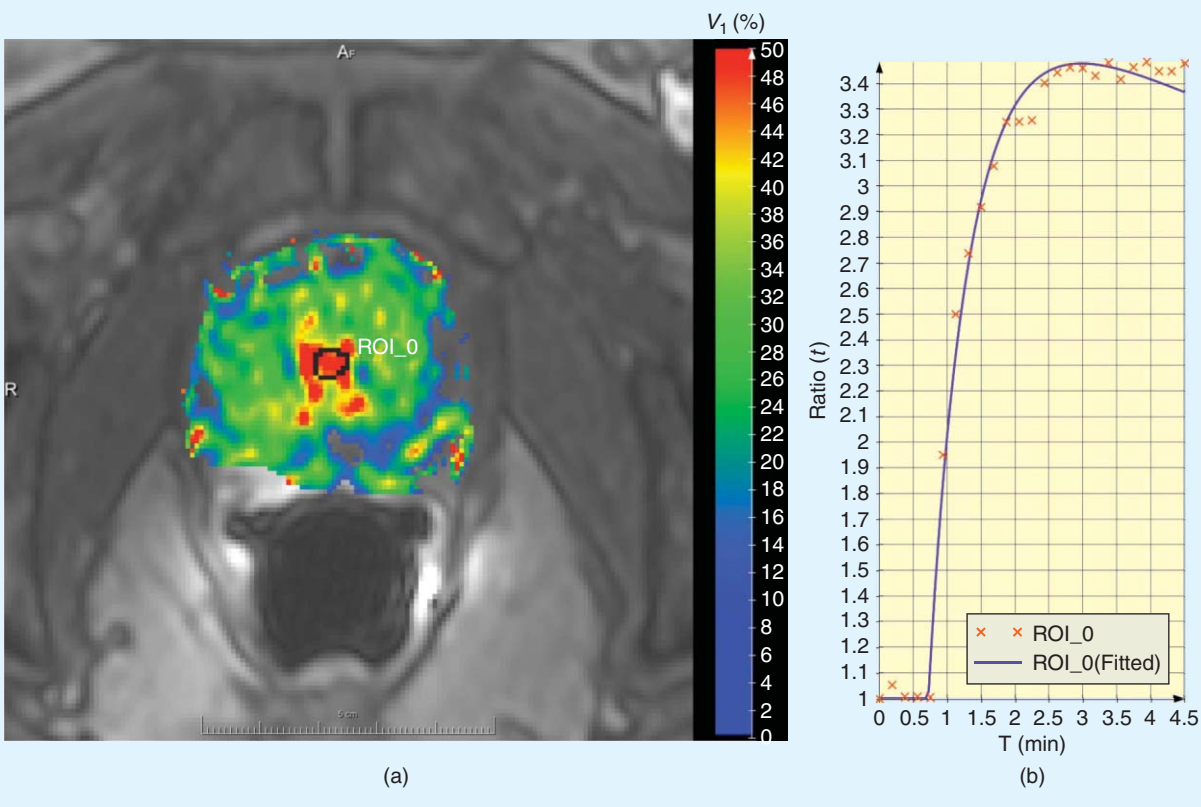

FIGURE 10 (a) Imaging the vasculature of a tumor with the help of a contrast agent. (b) The distribution of the contrast agent is measured periodically over time (red crosses), and afterward a model curve (blue curve) is fitted to the measured data. The model provides vascular information on the tissue and is superimposed onto the anatomical image data of the suspicious region as a color map.

first step of the analysis is to identify a few of these objects as vertebrae by employing strong constraints on their spectral and shape properties [Figure 9(c)]. Adjacent vertebrae, whose visual properties alone do not allow a certain classification, can now be identified by their relative location to already detected vertebrae. Repeating the last step until the whole spine is recognized yields a robust detection method that even works with noisy images and vertebrae affected by lesions [Figure 9(d)]. Remaining gaps (due to an inappropriate object presegmentation) can be closed with a subsequent refinement step [Figure 9(e)].

While we usually possess a wealth of knowledge about the objects in an image, it is often difficult to put this knowledge into words. This applies, particularly, when the knowledge has to be stated numerically, for instance, as the expected mean intensity or size of certain image objects. For this reason, our object-based image analysis methods are capable to learn. All the user has to do is to provide examples of relevant image objects, from which the computer then derives the necessary knowledge to identify further objects automatically.

A series of DICOM images, which is the most basic form for medical images, can be retrieved from a disk using the DirectDicom Import module.

Dynamic Imaging

Today, medical imaging offers a wide variety of functional imaging that can give information on human tissue beyond that on shape and structure available from the imaging information alone. These functional imaging processes can yield valuable parameters on blood supply and circulation, and oxygen consumption or cell metabolism. The parameters find a broad application in diagnosis and treatment monitoring of various diseases.

Frequently, these parameters are obtained by acquiring subsequent images over time after injecting a contrast agent into the bloodstream of the patient. Depending on the physical and biological properties of the contrast agents, the enhancement course and pattern of the contrast agent will yield information on tissue properties. For example, Fludeoxyglucose-18 is processed in cells in the same manner as glucose and therefore, is a good marker for cell metabolism. This can be exploited to measure the metabolism of tumors and differentiate tumor tissue from healthy tissue. Other contrast agents are kept confined in the blood vessels and therefore, their temporal and spatial distribution is related to blood volume and flow.

Once data are acquired, the time-to-signal-curve can be derived from the data, either by recording the signal changes over time for single voxels or averaging over regions of interest (ROI) to obtain a mean time-to-signal curve. Each curve then can be evaluated using signal-processing techniques. Commonly used are compartment models describing the processing or distribution of the contrast agent by the tissue in the voxel or ROI. The results of this analysis can either be a set of parameter maps displaying the derived parameters for each voxel or, in the ROI-based version, a table containing the parameters for the ROI. An example is shown in Figure 10, displaying the results for a very commonly used method—the dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI)—where a paramagnetic contrast agent is used to evaluate the vasculature of tumors.

MeVisLab allows the user to import series of volumes acquired over time of a number of medical imaging modalities, for instance MRI, CT, and PET. There are modules available to select an ROI in the images and generate time–intensity curves for these regions. Figure 10 shows an example evaluation created with MeVisLab. In Figure 10(a), a curve generated from the DCE-MRI data is displayed. It shows the temporal course of the ratio of signals between the contrast-agent-free first time period and time in which the contrast agent has arrived in the tissue. The ratio is proportional to the contrast agent concentration and can be used to optimize the parameters of the model describing this time course. In this case, the model describes the time course of the ratio in dependence on some basic model parameters: the vessel permeabilities and interstitial volume. The parameters are determined for all voxels in a dynamic data set by optimizing the model to the data using a least-square algorithm. After conversion by a color map, these parameters can be superimposed on top of the original image data as shown in Figure 10.

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RETROSPECTROSCOPE

Laplace's Law

Its Epistemological Context

By Max E. Valentinuzzi, Alberto J. Kohen, and B. Silvano Zanutto

The Macro-Cosmos—the Universe—is amazingly infinite; the Micro-Cosmos—cells, molecules, atoms, electrons…—seems to be infinite, too; but the Mind…oh, the Mind!… it projects further beyond…for sure! Isn't this brief musing epistemological in nature, searching for limits?

Science, technology, history, and philosophy are strongly related areas of knowledge. Perhaps, the best epistemologists are those who first were researchers in the sometimes called, and perhaps erroneously, hard disciplines (said with due respect and full recognition to pure epistemologists) because they, by force of education and training, had to be deeply involved in the intricacies of physicochemical principles and laws and technological developments to carry out measurements, and need to delve back in time for those who did it or tried to do it before, often being surprised by the ingenuity shown by predecessors in much older times. After collecting experience for a long time, the scientist falls naturally into traditional philosophical doubts and questions, the how's and what's, the up to where's, and when's. Quite interesting, children from three to five years old tend to often ask questions of this kind: Daddy, Mommy, how are we here, how was I born, what is the sky, where does the sky end, and so on. Does that mean we very early in life develop such questioning attitude that soon thereafter we lose, or maybe it is repressed by parental poor response or lack of response?

In the two preceding notes about Laplace's law [1], [2], we first recalled what it is and how it is frequently mentioned or applied in physiology, finding that in this particular case, there is an apparent separation between physiology and physics supposedly backing up the subject. Moreover, mistakes are almost a rule while amazingly and fortunately, the overall practical conclusions after very heavy simplifications are correct and well demonstrated by actual experiments and postmortem studies. The second note dealt with the mathematics of the law, and we believe that we practically exhausted all the pathways leading to the final formula, both when the wall thickness is negligible and when it is finite and significant. Now, our hat displays the epistemologist's sign, upsetting perhaps some readers, but without totally leaving out the quantitative view. Hence, the objectives of the note are established as follows:

- *general objective*: To introduce, discuss, and eventually produce answers for the epistemological aspects associated with Laplace's law
- *specific objective*: To discern if a mathematical equation has the same reach when obtained from two different physical settings (in our case, a phenomenon found in capillaries and the behavior of hollow stretchable cavities).

Common knowledge does not usually go through reflexive critical filters.

This is a good time to recall Thomas S. Kuhn's book [3] as an excellent and well-versed material to take into account when these aspects occupy our concerns. This highly cited and recognized physicist and philosopher of science (1922–1995) introduced and used the concept of scientific paradigm. Even though he never gave its precise definition, it may be described as *a very general conception of the nature of scientific endeavor within which a given enquiry is undertaken*. Ours herein is an enquiry, modest in relation with Kuhn's hugely wider environment, both in space and time, but valid as such if the physical settings given above in the objectives are considered as minor subparadigms. We could synthesize more powerfully our question by asking what is the nature of Laplace's law. The latter really comes up as the central question addressed herein.

What Is Epistemology?

Epistemology (from Greek, *ἐπιστήμη*, episteme, knowledge, and *λόγοζ*, logos, theory), as a branch of philosophy, devotes itself to scientific knowledge, clearly differentiating it from common or popular knowledge, which usually does not go through reflexive critical filters. Typical questions posed by epistemology are as follows:

- What are the necessary and sufficient conditions of knowledge?
- What sources offer possible answers?
- What is the structure of such knowledge, and what are its limits?

Broadly speaking, it may be stated that epistemology deals also with the creation and dissemination of knowledge in specific areas [4]–[10] , or perhaps better, we should speak in terms of Theory of Science. Hence, the questions posed above regarding Laplace's law clearly fall within the much wider spectrum set by

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these definitions; more specifically, what the nature and limits of these law are. Its historical development may supply some leads. We think this aspect calls at least for consideration and discussion when dealing with this more or less hidden (and even perhaps less significant) piece of physics.

Laplace's Law Based on Capillarity

Our previous two notes showed that the first contributions, starting with Laplace himself, originated in the capillary phenomenon. How does it manifest? Depending on the characteristics of the fluid (water, alcohol, mercury, or so on), on the material the tube is made of (glass, metal, ceramic, or so on), and on the gas (in general, air) forming the environment of the system, the liquid in the vicinity of the wall becomes concave or convex. In fact, the tube does not have to be a capillary to display such shapes. Quite interesting, and even surprising, is that the fluid goes either up or down; the smaller the diameter, the higher (or lower) the displacement, thus defying gravity (Figure 1).

Each liquid molecule within the liquid is attracted by the surrounding molecules and such attraction quickly decreases with the distance.

Numerical examples illustrate the following points: In a tube with a diameter of 4 m, water would barely rise 0.007 mm (negligible and essentially undetectable, but real); if the diameter is 4 cm, water goes up to 0.7 mm, but if the diameter gets down to 0.4 mm (already a capillary), the water rises up to 70 mm, giving the impression of being sucked up without an active pump! This is precisely the method clinical biochemists use to collect small amounts of blood (with density very close to that of water) from a punctured fingertip. Thus, by definition, capillary is a tube sufficiently fine so that attraction of a liquid into the tube is significant. Those use for hematocrit determination (made of glass), for example, is in the order of 1.1–1.2 mm internal diameter and 1.5–1.6 mm external diameter. There is a widely known equation to calculate the height of the column that can be found in any physics textbook or in the Web [11], [12], i.e.,

$$h = \frac{2T\cos\alpha}{\rho g r}, \qquad (1)$$

where T is the liquid–air surface tension (force/unit length), α is the angle of contact, ρ is the liquid density (mass/volume), g is the gravitational field (force/unit mass), and r stands for the tube radius (length).

To better analyze this effect and discuss it further within the context of the note, we should remember basic good old physics, the so-called surface phenomena, as described in a classic and highly recognized old textbook written by E. Perucca, in Italy, in 1932 [11]. However, we will slightly modify the derivation because, as found in other publications, the final Laplace's law appears with only one surface tension instead of two.

The contact surface between two phases is a separation surface, as between liquid and gas, solid and gas, liquid and liquid, and solid and liquid. A situation often encountered is a three-phase system formed by solid, gas, and liquid. Herein, we are interested in the latter case, where the liquid phase plays a significant role. The surface tension T of a liquid depends on its nature. By and large and as a first approximation, T does not depend much on the gas that surrounds it; however, it decreases with the temperature and is greatly modified by any contamination (ethylic alcohol in air, 22; water in air, 73; and mercury in vacuum, 435, all in dyn/cm and at 20°C). We can imagine T as the force to keep united the two edges of an ideal cut of 1 cm made over the liquid surface. The force, as said before in our previous notes, is perpendicular to the cut and tangent to the surface. Quite interesting is the fact that liquid films are contractile and cover the minimum surface compatible with the mechanical links around them and applied external forces. In other words, their potential energy is minimal (see in the following a brief description of contractile mercury droplets).

Imagine the surface separating liquid from air (or better, from vacuum). Each liquid molecule within the liquid is attracted by the surrounding molecules and such attraction quickly decreases with the distance, becoming almost zero at a distance r_m (defined as the radius of

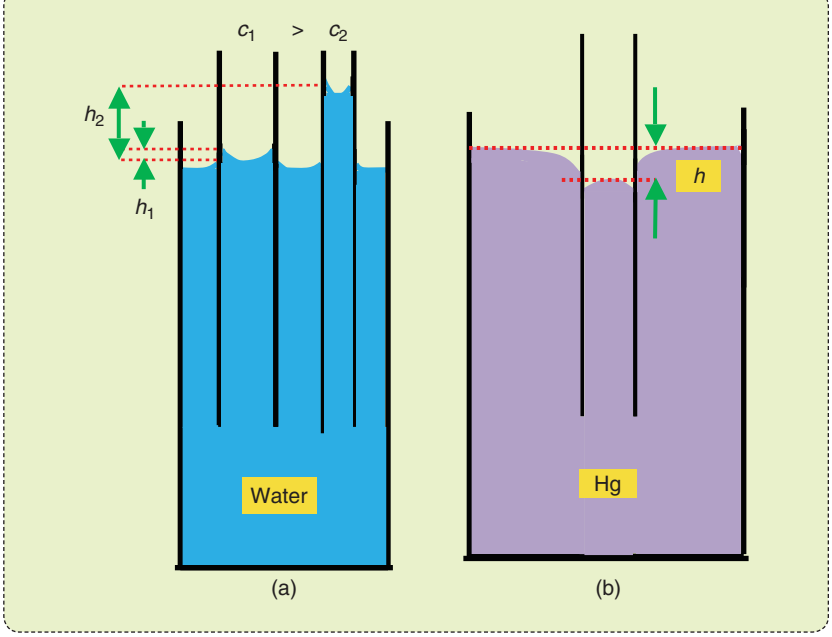

FIGURE 1 A schematic showing menisci and capillary effect. (a) Two capillary tubes C_1 and C_2 of different diameters. Menisci are concave and the larger lumen displays a lower height h_1 as compared with the smaller one h_2. (b) A capillary immersed in mercury produces a height h negative with respect to the bigger container level. Besides, menisci are convex.

molecular action), which lies in the order of about 4 nm, if it is water. Molecules fully immersed in the liquid's bulk are symmetrically attracted by the neighboring molecules, but those belonging to the surface region are attracted by the cohesion forces resultant. Such resultant force increases as the molecule gets nearer the surface. Thus, surface tension can be looked at as an indicator of internal cohesive forces of molecular origin.

A liquid in contact with a solid wall takes one of the shapes shown in Figure 2. The shaded areas and upwards, as moving in a funnel, up to the vertex A (a) or convex border (b), encompass the fluid region (say, water or mercury). By the Virtual Work Principle (for a body in equilibrium, when a virtual deformation infinitely small is applied, the virtual work of the external forces equals the inner deformation work), point A will be in equilibrium when the resultant force **R** is normal to the wall and verifies that

$$\mathbf{R} = \mathbf{T}_{12} + \mathbf{T}_{23} + \mathbf{T}_{13} = \mathbf{0}, \qquad (2)$$

where the bold face indicates vectors. Force **R** tends to bring A off the wall, which is impossible because of the mechanical link imposed by it; thus, equilibrium means

$$T_{13} = T_{23} + T_{12}\cos\alpha, \qquad (3)$$

where α stands for the angle linking wall 3 and fluid 2 (air, usually). The T's are the respective magnitudes of the vectors mentioned above. A virtual displacement is an infinitesimal change in the position of the coordinates of a system such that the constraints remain satisfied, and often, the principle is summarized by the following equation:

$$\delta W_i - \delta W_e = 0, \qquad (4)$$

where the W's stand for internal and external infinitesimal virtual works, respectively. The cosine of the angle α will be positive or negative for α smaller or larger than 90°.

Refer to Figure 3, where a small sphere with center O and radius dr cuts a nonplanar liquid surface Σ having a circumference Γ. The latter determines a differential area

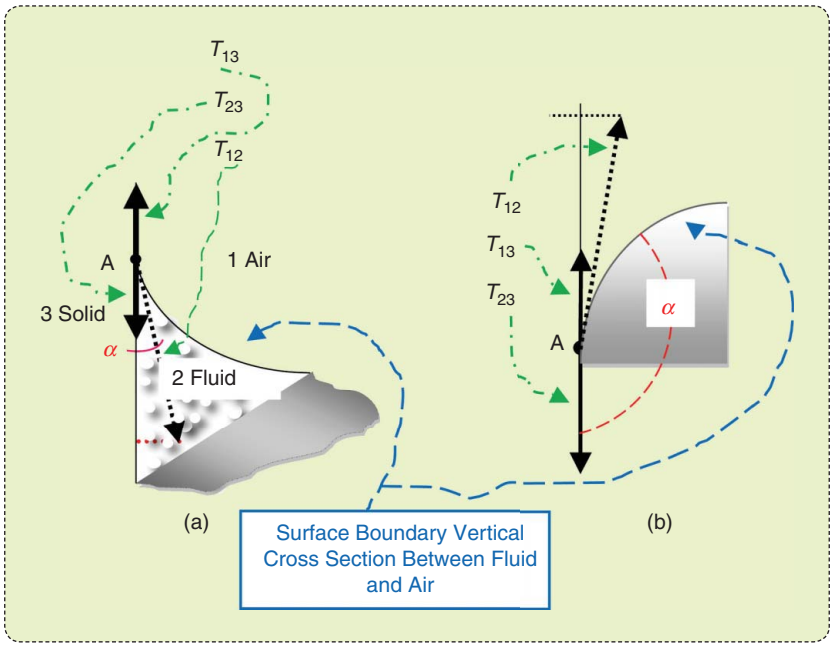

FIGURE 2 Surface tension. Two types of menisci: concave [(a) as water in glass] and convex (b), as mercury in glass. When the (a) link angle $\alpha < 90°$, it is said that the fluid wets the wall and (b) when it is > 90°, the fluid does not wet the wall. The dotted arrow represents the surface tension T_{12} between medium 1 and 2 (say, air and water). There is also a surface tension T_{23} between the solid wall (say, glass) and the fluid (downward vertical thick arrow, tangential to the inner wall surface). Finally, a third surface tension T_{13} (also tangential to the wall and pointing upward) manifests itself between air and the wall. The shaded areas on both figures mark the fluid phase (say, water or mercury).

$$dS = \pi(dr)^2. \qquad (5)$$

A diameter MM′ and a neighboring one form a differential angle $d\varphi$, thus determining over the circumference line two equal arcs $dl_1 = dl_1' = d\varphi \cdot dr$. The superficial tension applies to these two opposing arcs, respectively, forces $\tau_1 \cdot \mathbf{dl_1} = \tau_1 \cdot \mathbf{dl_1}'$, tangent to the surface Σ and perpendicular to the arcs (boldface indicates vectors). Owing to the curvature of Σ, both forces produce an infinitesimal resultant $\mathbf{dF_1}$ that points downward toward the distant center of curvature C_1, different than the small sphere's center cutting the liquid surface. Such force is given by

Molecules fully immersed in the liquid's bulk are symmetrically attracted by the neighboring molecules.

$$\mathbf{dF_1} = 2\tau_1\, dl_1 \cos\beta = -2\tau_1\, dl_1 \sin\gamma, \qquad (6)$$

$$\mathbf{dF_1} = 2\tau_1 \cdot d\varphi \cdot dr \cdot (dr/r_1) = 2\tau_1 \cdot (dr)^2 \cdot d\varphi \cdot (1/r_1), \qquad (7)$$

where r_1 is the curvature radius at point O of the section MOM′C_1; this radius will be positive when its direction coincide with the direction of the normal n and negative with the opposite direction. By the same token, a perpendicular diameter to MM′ accompanied by another neighboring one would determine two opposing arcs dl_2 and dl_2' so that an equation similar to (7) is obtained, i.e.,

$$\mathbf{dF_2} = 2\tau_2 \cdot (dr)^2 \cdot d\varphi \cdot (1/r_2). \qquad (8)$$

The four equal arcs dl_1, dl_1', dl_2, and dl_2, contribute to the perpendicular action along n in the amount

$$\mathbf{dF} = \mathbf{dF_1} + \mathbf{dF_2} = 2\tau_1 \cdot (dr)^2 \cdot d\phi \cdot (1/r_1) + 2\tau_2 \cdot (dr)^2 \cdot d\phi \cdot (1/r_2), \qquad (9)$$

$$\mathbf{dF} = \mathbf{dF_1} + \mathbf{dF_2} = 2(dr)^2 d\phi[(\tau_1/r_1) + (\tau_2/r_2)]. \qquad (10)$$

Perucca [11] states that for any pair of normal sections perpendicular to each other, the addition of their respective inverses is a constant, in turn equal to the addition of the two principal curvatures, i.e.,

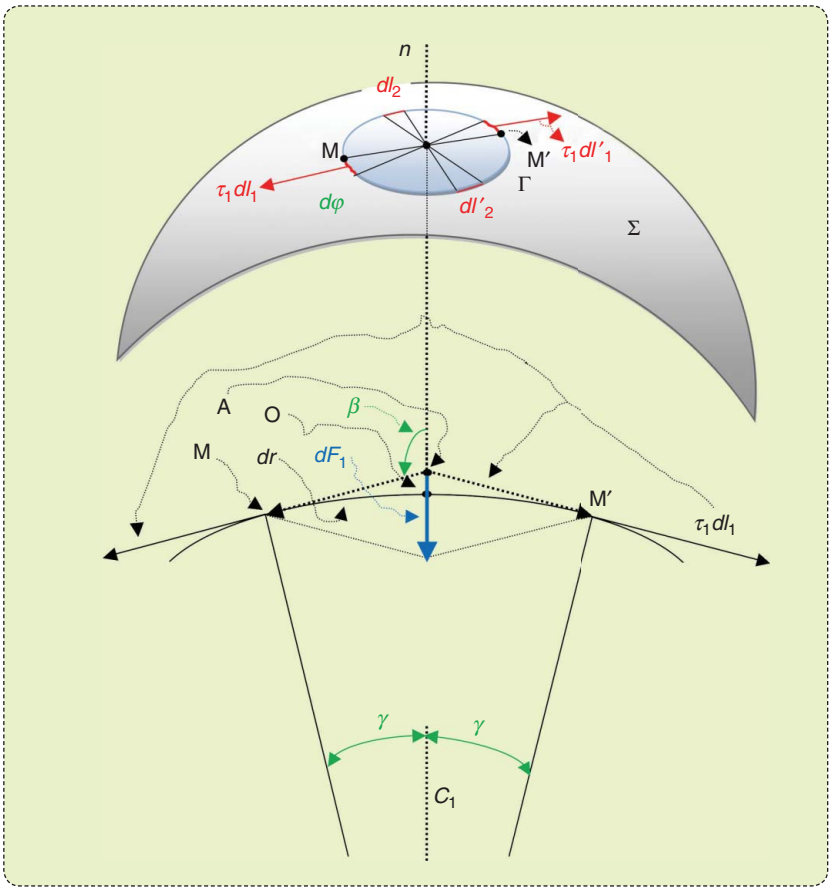

FIGURE 3 Perucca's setting. The circumference Γ above is part of a small sphere of radius *dr*. That circumference lies on and is part of surface Σ. Diameter MM′ forms an angle $d\varphi$ with another neighboring diameter.

$$[(1/r_1) + (1/r_2)] = \text{constant}, \\ = [(1/R_1) + (1/R_2)]. \quad (11)$$

Here, it must be reminded what Koiso and Palmer recently stated when recalling Thompson's expression for a system in equilibrium [13], [14],

$$T_1/R_1 + T_2/R_2 \equiv \text{constant}, \quad (12)$$

where $1/R_1$ and $1/R_2$ are the principal curvatures of the considered smooth surface, and T_1 and T_2 are orthogonally directed tensions, which depend on the material and normal direction of the surface at each point. Expression (12) is also equal to $[(\tau_1/r_1) + (\tau_2/r_2)]$, in which we emphasize that r_1 and r_2 stand for any pair of perpendicular radii different from the two principal axes. We remark that on the particular case of a sphere, the curvature itself is constant everywhere. Hence, considering Thompson's expression, r_1 and r_2 of (10) can be replaced by R_1 and R_2 leading to

$$\mathbf{dF} = 2(dr)^2 \cdot d\varphi \cdot [(\tau_1/R_1) + (\tau_2/R_2)]. \quad (13)$$

Integrating with respect to φ between 0 and $\pi/2$, i.e., adding up the normal actions **dF** generated by all the elements *dl* around the small circumference of radius *dr*, we get

$$dF = 2 \cdot (dr)^2 \cdot [(\tau_1/R_1) + (\tau_2/R_2)] \cdot \int_0^{\frac{\pi}{2}} d\phi, \quad (14)$$

$$dF = 2 \cdot (dr)^2 \cdot [(\tau_1/R_1) + (\tau_2/R_2)](\pi/2), \quad (15)$$

$$dF = dS \cdot [\tau_1/R_1 + \tau_2/R_2] \quad (16)$$

because $\pi \cdot (dr)^2 = dS$ and the 2's in (15) cancel out. If now the surface element *dS* is transferred to the left side of the equation, we end up with

$$P = dF/dS = [\tau_1/R_1 + \tau_2/R_2], \quad (17)$$

which is nothing else than our good friend Laplace's law, now showing different tensions for each radius, as it should be. Inexplicably, even though Perucca's setting of the problem is clean and well thought, the two surface tensions along the principal meridians appear as equal, losing generality and clearly violating what experience shows in pathophysiology; remember, for example, an aortic aneurism, where the dissection takes place along the longitudinal axis because only its perpendicular direction feels the pull and the former suffers no surface effect [1], [2]. To underline the concepts herein used and discussed, we must emphasize the difference between any two pairs of perpendicular radii of a small curved surface patch—such as r_1 and r_2 in (11), and how the principal radii are defined. The maximum and minimum at a given point on a surface are called the principal curvatures, and they measure the maximum and minimum bending of a regular surface at each point. To dissipate doubts, these definitions have been given by Gray in 1997 [15] and also by E.W. Weisstein [16].

Laplace's Law Based on Hollow Cavities

Our previous notes [1], [2] dealt extensively with the mathematical derivations of the law. Some were based directly on considering hollow cavities with elastic walls that, in most cases, show a finite, measurable, and nonnegligible thickness. Therefore, the concept of wall stress was introduced, often used in cardiac mechanics. Small curved patches, as small as necessary, defined by the two principal radii were the elements that any complex three-dimensional surface was decomposed into. One of the best, most direct and rigorous derivations was that produced very recently by Federico Armesto. There is no need here to repeat any of that material. It must be remarked, however, that

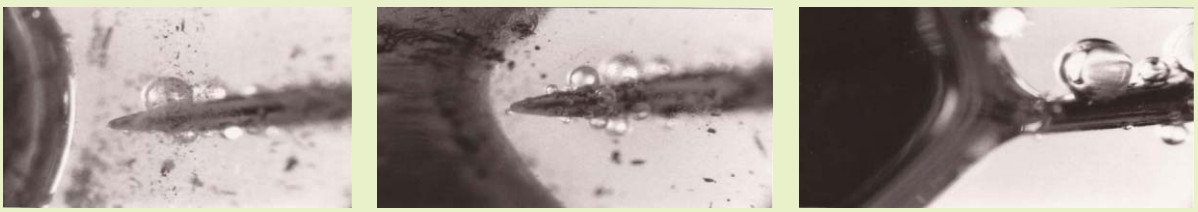

FIGURE 4 A mercury droplet immersed in a solution of dilute nitric acid and potassium dichromate. (a) A steel needle gets near the droplet surface. (b) As the needle gets closer, a local inward bending takes place. (c) When the needle touches the surface, mercury literally sticks to it.

this setting differs significantly from the capillary effect viewpoint, hence bringing up the doubt of validity of the law, even though the expression is the same.

Static and Dynamic Mercury Drops

When a drop of mercury is placed in dilute acid containing potassium dichromate and an iron wire is dipped into the liquid in close proximity to the drop, regular and rapid oscillations of the drop occur that may last for hours. At least, two related aspects can be recorded as evidences of electrochemical activity: electrical potential and impedance changes [17], [18].

When the needle is brought into contact with the droplet, oscillations stop and the impedance drops to virtually zero. The impedance increases when the droplet contracts and decreases during the expansion. Analysis of the events reveals a bistable nature that is suggestive of the electrocapillary dependence of mercury surface tension on electrode potential and polarizing current density. The needle becomes positive by approximately 0.7–0.8 V to the interior of the mercury during the second half of the expansion period, and the needle point becomes black, probably through formation of Fe_3C.

A simple explanation would suggest that the potassium dichromate decreases mercury surface tension due to repulsive forces in the double layer at the mercury–electrolyte interface. As the iron needle is advanced toward the drop, electrode current increases due to decreasing interelectrode impedance until a critical current is reached. The potassium salt then diffuses back to the surface of the drop and increases the mercury potential resulting in a change of shape. This phenomenon exhibits transition kinetics at one interface (activation and passivation of iron), which induces a mechanical change at a proximal boundary (mercury), the events being mediated by variations in electrolyte current and electrode surface potentials. Inside the droplet, a pressure must build up following Laplace's law (Figure 4).

Potassium dichromate decreases mercury surface tension due to repulsive forces in the double layer at the mercury–electrolyte interface.

Discussion

The subject we are dealing with herein deserves to be discussed within the epistemological framework. Let us see why this standing finds justification. First, looking into its historical development, we found that the capillary effect was the original motivation leading to the equation and none of the authors contributing to it (Jurin, Young, Laplace, and Gauss) ever mentioned volumetric cavities under pressure. Robert Woods was the first to apply the law to hollow organs, and Karl De Snoo appears as the first to obtain an ingenious derivation followed by actual measurements in gravid uteri under dilatation, but no reference was made by the latter to the capillary action. From a physics point of view, there is no relationship whatsoever between hollow organs or balloons and capillarity; none the less, the mathematical equation is the same. Hence, is its application valid? We should say it is because the equation has been demonstrated in the two areas, starting from the basic capillary phenomena and also from a volumetric conception (as cupolas or balloons of any shape, even including the wall thickness).

Capillaries triggered also side derivations that deserve mentioning, at least as a curiosity. Gabriel Lippmann, a physicist, showed the existence of an electric phenomenon associated with mercury when it fills capillaries. His contribution had important practical consequences in the field of cardiology, for it offered the basis for the first continuous records of cardiac electrical activity with the development of the capillary electrometer [19]. But there was more to this application. Since the capillary meniscus is a surface tension phenomenon, mercury drops under certain conditions can show an outstanding rhythmic electric and contracting activity, where surface tension plays a decisive role [17], [18]. Figure 4 illustrates such behavior. A puzzling question deserves to be posed: Does Laplace's law hold in these drops? How could this be tested? We think it does.

After Laplace's times, and in a way to be considered as his immediate continuator in capillarity studies, Gauss in 1829 clearly stands out [2]. He manifestly recognizes Le Marquis as his antecessor in this respect and, perhaps, can even be credited with indirectly naming the law. The mathematical formulation does not appear as clear enough and is rather cryptic using a notation not current nowadays. However, it is deemed as

a big step in the treatment of the subject. The principle he adopted is that of virtual velocities, gradually transformed later on into the principle of the conservation of energy. Gauss pointed out the importance of the angle of contact between the two interacting surfaces; thus, he supplied the principal defect in Laplace's work. Besides, Gauss mentioned the advantages of the method of measuring the dimensions of large drops of mercury and large bubbles of air in liquids under certain conditions by Segner and Gay Lussac, afterward carried out by Quincke [2].

Conclusion

Laplace's law explains all the capillarity phenomena as it leads to the pressure within a soap bubble or how a small bubble dumps its air into a bigger one if both are interconnected, a fact well known in certain respiratory diseases, such as atelectasis [1], [2], [11]. The demonstration given by Perucca and some of the demonstrations given in [2] clearly show that, no matter what the initial setting is (either capillary effect or hollow elastic container), the law is valid and beyond any doubt. Surface tension puts into evidence forces and generates an internal pressure within well-defined boundaries. In one sentence, it was mentioned that two fully different physical phenomena (capillarity, where three phases are components, and elastic hollow bodies sustaining pressures) converge to the same mathematical equation. As a corollary, we might add that calling Laplace's law of physiology would not be appropriate but rather DeSnoo-Barrau's because the latter was directly obtained from a hollow organ (the uterus).

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Call for Papers

Building on the success of the EMBS International Conference on Information Technology and Applications in Biomedicine (ITAB), EMBS has launched a new Special Topic Conference titled IEEE-EMBS International Conferences on Biomedical and Health Informatics (BHI), starting from this year. The inaugural meeting, BHI2012, will be held in Hong Kong and Shenzhen, China, January 5-7, 2012.

To be held in conjunction with the 8th International Symposium on Medical Devices and Biosensors and the 7th International Symposium on Biomedical and Health Engineering, the BHI 2012 will be devoted to an inter-disciplinary research area intersecting engineering, information technology and computer science with biology, medicine and health. The conference is to examine enabling technologies of devices and systems that optimize the acquisition, transmission, processing, storage, retrieval (P-STAR) of biomedical and health information as well as to report clinical studies on the novel application of health information systems. The goal of the proposed joint event is to bring together academicians, clinicians, industrial representatives, government and private research agencies and funders to facilitate a dialogue on addressing the theme "*Global Grand Challenge on Health Informatics*".

Features:

- Special Workshop on "Meet IEEE-EMBS Editors-in-Chief" sponsored by IEEE Engineering in Medicine and Biology Society (EMBS)
- Special Academician & Fellow Forum sponsored by the Key Lab for Health Informatics of Chinese Academy of Sciences (HICAS)
- Special Forum on Imaging Informatics
- Special Panel on "Grand Challenge on Health Informatics"
- Accepted papers will be included in IEEE Xplore
- Extended version of selected papers may be considered for publication in the IEEE Trans. on Information Technology in Biomedicine (SCI, EI, Scopus)

Important Dates:

- **Paper Submission Deadline: 14 Oct 2011**
- **Notification of Acceptance: 11 Nov 2011**
- **Early Registration: 2 Dec 2011**
- **Pre-conference Workshop: 5 Jan 2012 (in Hong Kong)**
- **Conference Dates: 6-7 Jan 2012 (in Shenzhen)**

The conference covers a broad spectrum of themes including but not limited to the following topics:

I) P-STAR of Biomedical and Health Information
- Wearable and implantable devices
- Body sensor/area networks (BSN /BAN)
- Diagnostic and therapeutic systems
- Internet and web solutions for healthcare delivery
- Multi-scale modeling and information fusion;
- Ambient assisted living, smart homes;
- Electronic health records, interoperability and connectivity
- Context-aware retrieval
- p-health, m-health, u-health, e-health systems

II) Biologically Inspired Informatics
- Virtual reality in medicine and surgery
- Bio-inspired robotics and biomimics
- Brain-computer interfacing and human–computer interfacing

III) Informatics in Biological Systems
- Neuroinformatics
- Genomics and proteomics
- Bioinformatics, computational biology

IV) Medical Imaging Informatics
- Realtime imaging
- Multimodal imaging
- Molecular imaging

V) Health Informatics Applications
- Cardiovascular informatics
- Applications in the early diagnosis and treatment of cancers

Organized and sponsored by:
- IEEE-Engineering in Medicine and Biology Society (EMBS)
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Technically co-sponsored by:
- The Chinese University of Hong Kong

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- IEEE-EMBS Hong Kong Chapter

Contact Us:
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Tel: +852 2609-8285

Conference Website:
http://bhi2012.embs.org

Digital Object Identifier 10.1109/MPUL.2011.943391

STATE OF THE ART

Oh Mama, Where's My Comma?

Arthur T. Johnson

I was taught in no uncertain terms that a list of words was separated by commas. If a comma did not appear between two words, then they were to be read as collective equals, both together and inseparable. So we have a list such as: French toast, ham and eggs, and coffee. The ham and eggs are to be reasoned as one ensemble, served together, such that one without the other would be a huge mistake.

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Date of publication: 30 November 2011

These days, however, for some reason unknown to me and clearly at odds with my memories of long-lost English teachers, the last comma in a list is AWOL. It just isn't there. So now, we have lists such as: fear, killing and love. Clearly, "killing" is not supposed to hang out with "love," but that's what this list says to me, being the strict traditionalist that I am.

So, why have the Machiavellian editors plotted to kill that extra comma? Is the print medium in such a tight financial crisis that they can't afford the extra ink or space that this lowly comma deserves? Or, is it just minimalism bursting upon the printed page inspired by a Phillip Glass musical pseudocomposition?

I need that comma! My eye darts to the end of a list I am reading and seeks to assuage my anxiety: is the comma there, or is it missing? If it is missing, why is it missing? Where did it go? Why are these editors mocking my high-school memories of Mrs. Veterito teaching me how to write, memories that I had not revisited until I first noticed this grammatical ravaging. That ugly scene, that missing comma, is so distracting that all I do these days is to mentally edit the piece to make it grammatically correct. The intended message of the prose fades into the background while I fuss over the comma that isn't there. Like the parable of the lost sheep, I cannot rest until I bring that comma safely back into the fold.

***Arthur T. Johnson** (artjohns@umd.edu) is with the University of Maryland.*

First Announcement

Hawaii
December 1-7, 2012

Save the date!!!!

Various biomedical grand challenges facing our society and the world can be addressed in part or in whole by interfacing biology and medicine with micro- and nanoscale technologies. The potential impact of these technologies on the early diagnosis, therapeutics, and management of disease is very high. To address this challenge, IEEE EMBS is sponsoring the first Conference on Micro- and Nanoengineering in Medicine to foster interaction between scientists, engineers and medical researchers in the context of real-world medical needs and issues. The Conference will promote vigorous and open dialogue towards the development of cutting edge technologies for faster, more quantitative, and less expensive biomedical solutions using advances in micro and nanotechnology.

Conference Chairs:
Professor Rashid Bashir, University of Illinois, Urbana-Champaign
Professor Ali Khademhosseini, Harvard University
Professor Michelle Khine, University of California, Irvine

Contact info:

Ms. Laura J. Wolf, l.wolf@ieee.org

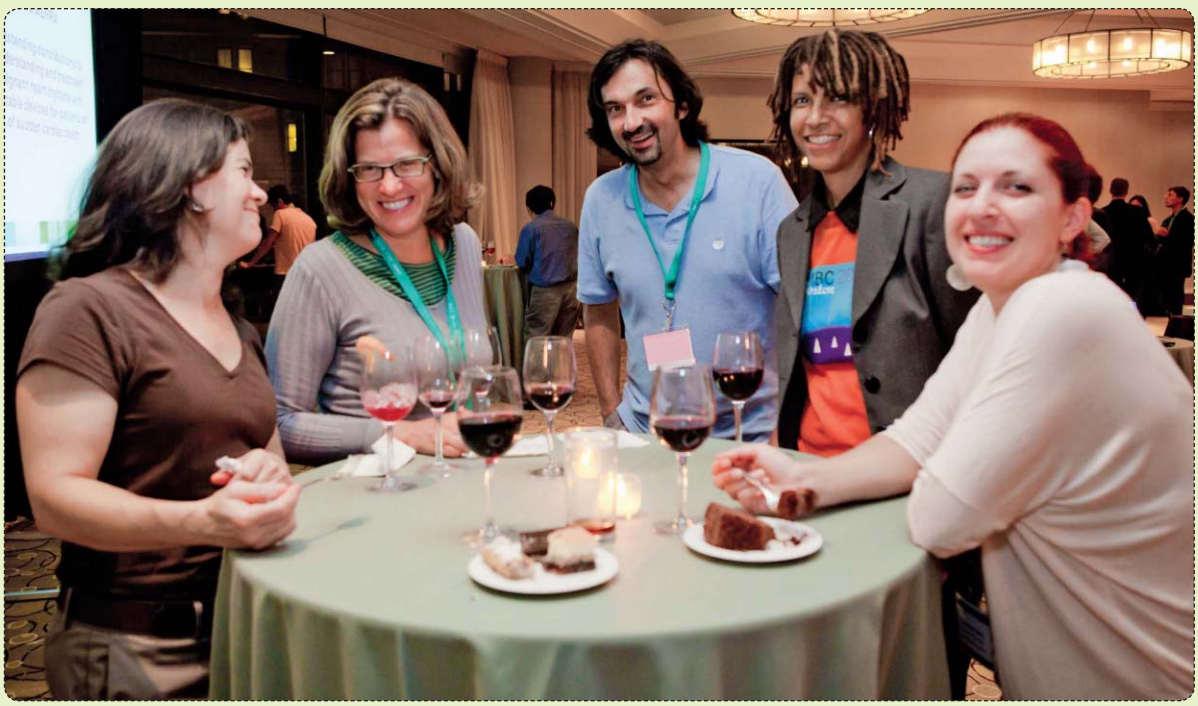
Rashid Bashir rbashir@illinois.edu,

Ali Khademhosseini alik@rics.bwh.harvard.edu

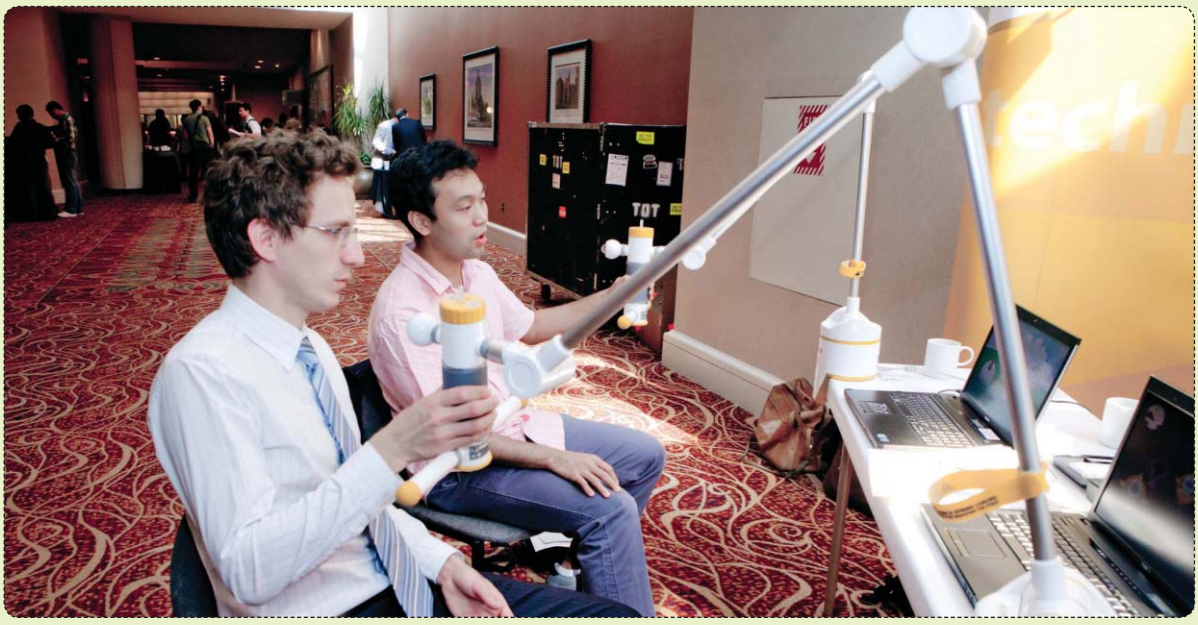
Michelle Khine mkhine@uci.edu

Digital Object Identifier 10.1109/MPUL.2011.943392

Scenes from EMBC 2011 Boston



Digital Object Identifier 10.1109/MPUL.2011.943462
Date of publication: 30 November 2011



EMBS Constitution and Bylaw Changes

The following changes to the EMBS Constitution and Bylaws was proposed and approved for recommendation to the membership by the EMBS AdCom at its Fall meeting. Members will have until 30 November 20111 to comment on the recommended changes in italics by sending an email to the Executive Director, Laura J Wolf at emb-exec@ieee.org

Constitution Article V: Management and Officers

Section 4. The Society officers shall be the President (2 year term), the President-Elect (1 year term), the immediate Past-President (1 year term), the Vice-President for Conferences (2 year term), the Vice-President for Publications (2 year term), the Vice-President for Member and Student Activities (2 year term), and the Vice-President for Financial Planning (2 year term). These officers serve as the Society Executive Committee, hereinafter referred to as the ExCom. The AdCom shall elect the Vice-President for Conferences, the Vice-President for Publications, the Vice-President for Member and Student Activities, and the Vice-President for Financial Planning from its current members and previous members (elected or appointed) who have served as AdCom members within the previous three years. The AdCom shall also elect the President-Elect from its current members and those who have served as elected ExCom members within the previous four years or as AdCom members within the previous *eight (8) years.*

Rationale: By extending the time period AdCom members are eligible for ExCom positions will provide EMBS with a larger pool of candidates.

Bylaws Section B The Annual International Conference

B.7 *The Conference Chairs of the Annual International Conference sponsored by the EMBS Society are Ex-Officio members of AdCom without vote unless they already hold an elected position on AdCom. In the latter case, the Conference Chair will instead keep his elected AdCom position, so long as bylaw 8.5 is satisfied.*

Rationale: EMBS Conference business is growing and should be represented at AdCom similarly to the EMBS Editors, who are currently non-voting members of AdCom.

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CALENDAR

HONG KONG, SHENZHEN
IEEE-EMBS International Conference on Biomedical and Health Informatics
5–7 JANUARY 2012
Contact: Y.P. Liang
Phone: +852 2609 8285
Web: http://bhi2012.embs.org
E-mail: ypliang@ee.cuhk.edu.hk

MANAUS, BRAZIL
2012 ISSNIP Biosignals and Biorobotics Conference: Biosignals and Robotics for Better and Safer Living (BRC)
8–10 JANUARY 2012
Contact: Dinesh K. Kumar
Phone: +61 3 9925 1954
Web: http://www.brc2012.org
E-mail: dinesh@rmit.edu.au

SANTA CLARA, CALIFORNIA
2012 IEEE Topical Conference on Biomedical Wireless Technologies, Networks, and Sensing Systems (BioWireleSS)
15–19 JANUARY 2012
Contact: Rizwan Bashirullah
Phone: +352 392 0622
Fax: +352 392 8381
Web: http://www.radiowirelessweek.org/biowireless/
E-mail: rizwan@tec.ufl.edu

INNSBRUCK, AUSTRIA
9th IASTED International Conference on Biomedical Engineering (Biomed 2012)
15–17 FEBRUARY 2012
Contact: Karen Lee
Phone: +403 288 1195
Fax: +403 247 6851
Web: http://www.iasted.org/conferences/cfp-764.html
E-mail: KarenL@iasted.org

BETHESDA MARRIOTT, MARYLAND
Grand Challenges in Biomedical Imaging
29 FEBRUARY–2 MARCH 2012
Contact: Dana Lynn Bernstein
Phone: +1 732 981 3451
Web: http://gcbme2011.embs.org/
E-mail: d.bernstein@ieee.org

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Date of publication: 30 November 2011

TEMPLE UNIVERSITY, PENNSYLVANIA
38th Annual Northeast Bioengineering Conference (NEBEC)
16–18 MARCH 2012
Contact: Kurosh Darvish
Phone: +1 215 204 4307
Web: http://www.nebec.org
E-mail: kdarvish@temple.edu

LA JOLLA, CALIFORNIA
6th International Symposium on Medical Information and Communication Technology (ISMICT)
25–29 MARCH 2012
Contact: Upkar Dhaliwal
Phone: +1 858 926 5839
Web: http://www.ismict2012.org
E-mail: upkar@ieee.org

VILAMOURA, PORTUGAL
International Joint Conference on Biomedical Engineering Systems and Technologies (BIOSTEC 2012)
1–4 APRIL 2012
Contact: Monica Saramago
Phone: +00351265520185
Web: http://www.biostec.org
E-mail: monica@insticc.org

SAN ANTONIO, TEXAS
Advances In Trauma Conference
9–11 MAY 2012
Contact: Pam Losefsky
Phone: +512 695 4204
Web: http://www.nationaltraumainstitute.org/meetings_and_events/meetings_and_events.html
E-mail: pam.losefsky@nationaltraumainstitute.org

BALTIMORE, MARYLAND
2012 International Conference on Imaging and Signal Processing in Health Care and Technology
14–16 MAY 2012
Contact: Karen Lee
Phone: +1 403 288 1195
Fax: +1 403 247 6851
Web: http://www.iasted.org/conferences/cfp-771.html
E-mail: KarenL@iasted.org

SHANGHAI, CHINA
6th International Conference on Bioinformatics and Biomedial Engineering, iCBBE
17–20 MAY 2012
Contact: Jie Li
Phone: +86 27 68775482
Fax: +86 27 68775482
Web: http://www.icbbe.org
E-mail: zuzujessie@yahoo.com.cn

RICHMOND, VIRGINIA
2012 International Conference on Complex Medical Engineering (ICME2012)
3–6 JUNE 2012
Contact: Kayvan Najarian
Phone: +804 828 9731
Web: http://www.people.vcu.edu/~knajarian
E-mail: knajarian@vcu.edu

BRITTANY, FRANCE
EMBS International Summer School on Biomedical Imaging
22–30 JUNE 2012
Contact: Christian Roux
Phone: +33 298 01 81 07
Fax: +33 298 01 81 24
Web: http://ieeess.enst-bretagne.fr/
E-mail: Christian.Roux@telecom-bretagne.eu

ROME, ITALY
2012 4th IEEE RAS/EMBS International Conference on Biomedical Robotics and Biomechatronics (Biorob)
24–28 JUNE 2012
Contact: Eugenio Guglielmelli
Phone: +3906225419607
Fax: +3906225419609
Web: http://www.biorob2012.org
E-mail: e.guglielmelli@unicampus.it

SAN DIEGO, CALIFORNIA
34th Annual International Conference of the IEEE Engineering in Medicine and Biology Society
29 AUGUST–1 SEPTEMBER 2012
Contact: Jessica Lotito, EMBS Executive Office
Phone: +732 981 3459
Fax: +732 465 6435
Web: http://embc2012.embs.org
E-mail: emb-conference@ieee.org

HEILBAD HEILIGENSTADT, GERMANY
15th International Conference on Bioelectrical Impedance (ICEBI), along with the 14th Conference on Biomedical Applications of Electrical Impedance Tomography
22–25 APRIL 2013
Contact: Uwe Pliquett
Phone: +49 3606 67116
Web: http://www.icebi.org
E-mail: Uwe.Pliquett@iba-heiligenstadt.de

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IEEE

CALL FOR NOMINATIONS

Submission Deadline: 15 January 2012

EMB

IEEE EMBS Achievement & Service Awards

Nominations are being sought for the following IEEE Engineering in Medicine and Biology Society Awards for 2012. Each award recipient will receive a plaque/certificate, an honorarium, and reimbursement in travel expenses associated with attending the EMBS Awards Presentation at the 34th Annual International Conference of the Society. The 2012 conference will be held in San Diego, CA, USA, 28 August – 1 September 2012 (http://embc2012.embs.org).

THE EMBS ACADEMIC CAREER ACHIEVEMENT AWARD

Honorarium $2,500 USD/Travel Reimbursement up to $1,500 USD

For outstanding contribution and achievement in the field of Biomedical Engineering as an educator, researcher, developer, or administrator who has had a distinguished career of twenty years or more in the field of biomedical engineering. Accomplishments may be technological or theoretical and need not have proceeded the award date by any specific period of time. Individual must be a current member of EMBS.

THE EMBS PROFESSIONAL CAREER ACHIEVEMENT AWARD

Honorarium $2,500 USD/Travel Reimbursement up to $1,500 USD

For outstanding contribution advancing Biomedical Engineering and its professional practices as a practicing biomedical engineer working in industry, government or other applied areas related to biomedical engineering. Accomplishments include, but are not limited to, technological advances, improvements in processes, or development of new products or procedures, and need not have preceded the award date by any specified period of time. Individual must be a current member of EMBS.

THE EMBS EARLY CAREER ACHIEVEMENT AWARD

Honorarium $1,000 USD/Travel Reimbursement up to $1,500 USD

For significant contributions to the field of biomedical engineering as evidenced by innovative research design, product development, patents, and/or publications made by an individual who is within 10 years of completing their highest degree at the time of the nomination and is a current member of EMBS.

THE EMBS DISTINGUISHED SERVICE AWARD

Honorarium $1,000 USD/Travel Reimbursement up to $1,500 USD

For outstanding service and contributions to the Engineering in Medicine and Biology Society. Accomplishments should be related to direct Society service and need not have preceded the award date by any specific period of time and individual must be a current member of EMBS.

Nomination Procedure

The required nomination packet consists of a two-page nomination form (see www.embs.org homepage), a current CV and letters from three references along with their address, telephone, facsimile number and e-mail address. *It is the responsibility of the nominator to contact the references and solicit letters of endorsement.*

The complete nomination packet must be emailed to embs-awards@ieee.org and received no later than **15 January 2012** for the nominee to be considered for 2012. It is very desirable for nominations to be submitted well before the deadline.

For questions, please contact the EMB Executive Office (embs-awards@ieee.org)

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CALL FOR NOMINATIONS

Submission Deadline: 15 January 2012

IEEE EMBS Chapter Awards

Nominations are being sought for the following IEEE Engineering in Medicine and Biology Society Awards for 2012. Each award recipient will receive a plaque/certificate, an honorarium, and reimbursement in travel expenses associated with attending the EMBS Awards Presentation at the 34th Annual International Conference of the Society. The 2012 conference will be held in San Diego, California, USA, 28 August – 1 September 2012 (http://embc2012.embs.org).

OUTSTANDING CHAPTER AWARD

Honorarium $1000 USD/Travel Reimbursement of up to $1,000 USD

For achievement in member development and delivering services to members of an EMBS Chapter during the previous calendar year. A single EMBS Chapter will be selected based on activities, community outreach and promotion of EMBS.

BEST NEW CHAPTER AWARD

Honorarium $500 USD/Travel Reimbursement of up to $1,000

For outstanding activities performed by a new EMBS Chapter within the first 12 months of Chapter formation. A single EMBS Chapter will be selected based on activities, community outreach and promotion of EMBS.

OUTSTANDING PERFORMANCE AWARD for an EMBS Student Branch Chapter or Club

Honorarium $500 USD/Travel Reimbursement up to $1,000 USD

For achievement in demonstrating outstanding performance in promoting student interest and involvement in Biomedical Engineering during the previous calendar year. A single EMBS Student Branch Chapter or Club will be selected based on activities demonstrating initiative, innovation, and creativity; areas of progress and improvement; significant impact in biomedical engineering education; and contributions to the profession.

BEST NEW STUDENT BRANCH CHAPTER or CLUB AWARD

Honorarium $300 USD/Travel Reimbursement of up to $1,000

For outstanding activities performed by a new EMBS Student Club or Chapter within the first 12 months of formation. A single EMBS Student Branch Chapter or Club will be selected based on activities demonstrating initiative, innovation, and creativity; areas of progress and improvement; significant impact in biomedical engineering education; and contributions to the profession.

Nomination Procedure

The required nomination packet consists of a one-page nomination form and supporting documentation as outlined in the nomination form (see www.embs.org homepage).

The complete nomination packet must be emailed to embs-awards@ieee.org and received no later than **15 January 2012**.

For questions, please contact the EMB Executive Office (embs-awards@ieee.org)

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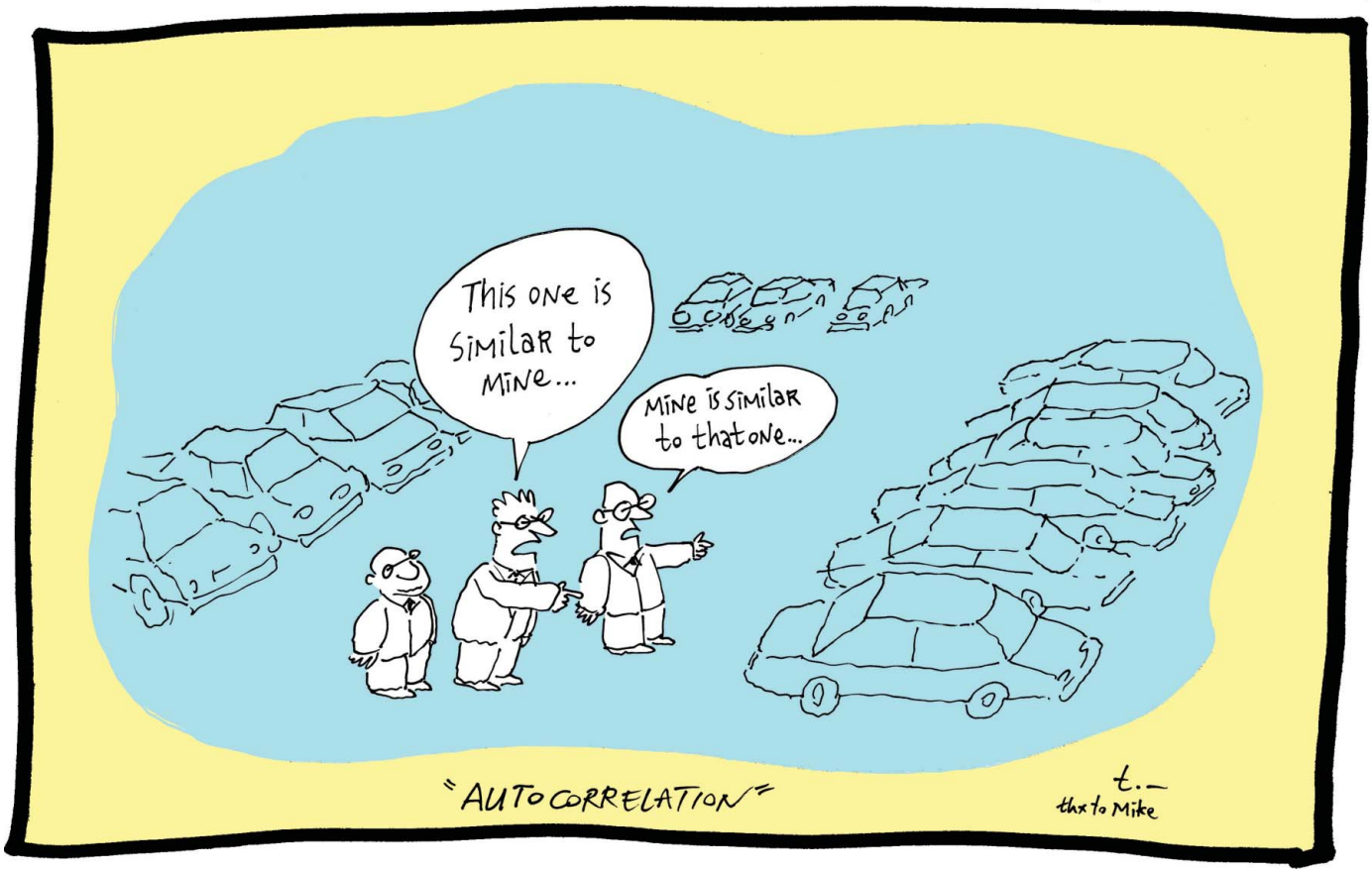

Autocorrelation (statistics): In a time series, the relationship between values of a variable taken at certain times in the series and values of a variable taken at other, usually earlier, times.

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CALL FOR NOMINATIONS

Submission Deadline: 15 January 2012

IEEE EMBS Technical Achievement Awards

Nominations are being sought for the IEEE Engineering in Medicine and Biology Society Technical Achievement Awards for 2012. Each award recipient will receive a plaque/certificate, an honorarium, and reimbursement in travel expenses associated with attending the EMBS Awards Presentation at the 34th Annual International Conference of the Society. The 2012 conference will be held in San Diego, CA, USA, 28 August – 1 September 2012 (http://embc2012.embs.org).

THE EMBS TECHNICAL ACHIEVEMENT AWARD

Honorarium $1,500 USD/Travel Reimbursement up to $1,500 USD

For outstanding achievements, contributions, or innovations in any area of bioengineering by an individual or group of individuals. Up to five awards will be selected each year.

Qualifications for the award include, but are not limited to, new technologies or significant extensions of existing technologies, research results that extend domain knowledge, and design of new hardware or software having a significant impact in any area of bioengineering.

Examples of eligible Bioengineering Technologies (additional areas will also be considered)

- Biosignal Processing
- Bioinstrumentation
- Bionanotechnology
- Cardiovascular and Respiratory Systems Engineering
- Biomaterials
- Biomechanics
- Medical Device Design and Development
- Biomedical Imaging
- Biomicrotechnology
- Computational and Systems Biology
- Molecular, Cellular and Tissue Engineering
- Biorobotics
- Therapeutic and Diagnostic Systems
- Healthcare Information Systems

Nomination Procedure

The required nomination packet consists of a two-page nomination form (see www.embs.org homepage), a current CV and letters from three references along with their address, telephone, facsimile number and e-mail address. *It is the responsibility of the nominator to contact the references and solicit letters of endorsement.*

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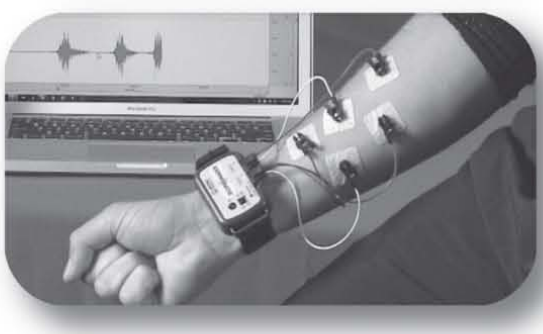

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