Methods and Protocols: Engine for the Next Wave of Biomedical Ultrasound Innovations

AT THE heart of a vehicle's operation is the engine. The same metaphoric relation applies between a research innovation and an experimental method. It is safe to say that innovations in biomedical ultrasonics cannot be materialized without foundational advances in experimental methods, tools, and protocols. Indeed, because biomedical ultrasonics naturally spans a diverse research space—thanks to its versatile applicability in both diagnostics and therapeutics—a broad range of investigative methods are correspondingly required to serve a multitude of experimental needs.

Let us consider innovations in ultrasound imaging as an example. In recent years, the research community has witnessed how various imaging innovations are effectively fostered by different investigative and validation tools. The Field II ultrasound field simulation toolbox [1] is one well-known investigative tool that has enabled preliminary testing of new aperture designs, beamforming approaches, and imaging algorithms. Advances in imaging phantom design, including both tissue phantoms [2] and flow phantoms [3], are at least as important, as they allow for the calibration of ultrasound scanners and the performance assessment of new imaging techniques under controlled conditions with known, reproducible medium properties. On another note, implementation of new imaging techniques is a complex engineering enterprise. This process critically relies on the advent of mature protocols for developing transducers [4], research-purpose ultrasound imaging platforms [5], and real-time computing methodologies [6]. Without any of these engineering methods, it would be challenging to bring forth a new ultrasound imaging solution from concept to practice in clinical settings, where ultrasound has been favored as a portable, safe, and inexpensive bedside imaging modality.

In the emerging field of therapeutic ultrasound, fundamental advances in engineering methods have also been making significant contributions to the discipline's technical progress. For instance, in realizing sonoporation (i.e., ultrasound-induced cell membrane perforation) for drug delivery and gene transfer purposes, it is essential to establish stable protocols for the synthesis of microbubbles [7] and nanodroplets [8] that can serve as dual-purpose agents for instigating acoustic cavitation and for carrying drug payloads. Rational design of acoustic exposure instruments [9], [10] is likewise important, both in achieving high site specificity for sonoporation-mediated drug/gene delivery and in delivering high-intensity focused ultrasound (HIFU) for ablation treatments. As well, to test the efficacy of new therapeutic ultrasound techniques, suitable experimental models need to be established [11].

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Alas, methods and protocols in biomedical ultrasound investigations have not always been reported in depth. Many technical details have been relegated as black-box processes in research articles. The Guest Editors believe that it is now time to dedicate a more concentrated effort on the reporting of the key methods used in contemporary biomedical ultrasound research. This will serve well to promote the publication and dissemination of best practices for a broad range of engineering know-how that are essential to biomedical ultrasound investigations, thus serving well as up-to-date educational resources for graduate students and researchers who are new to the biomedical ultrasonics domain.

Introduction to the UFFC Special Issue on Methods and Protocols in Biomedical Ultrasonics

As the start to a new volume of the IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control (vol. 64, 2017), we have prepared a Special Issue on the theme of "Methods and Protocols in Biomedical Ultrasonics." Here, we have compiled a collection of original tutorial-style papers on a broad range of methods, protocols, and tools that are essential to biomedical ultrasound investigations. This Special Issue is a new initiative of the UFFC Transactions to kickstart the establishment of a library of peer-reviewed literature on both seminal and novel research methodologies used in biomedical ultrasonics. A total of 23 articles have been included in this Special Issue. Each article meets one or more of the following publication objectives:

1) Pedagogically describing the technical details of a new method, tool, or protocol that can significantly benefit biomedical ultrasound research;
2) Annotating the operations and best practices of currently established simulation and experimental tools that are used either for performance validation or real-time realization of new biomedical ultrasound techniques;
3) Providing stepwise walkthroughs of well-tested laboratory procedures and fabrication protocols that are foundational to the realization of new biomedical ultrasound applications.

Papers of this Special Issue have been grouped into eight categories, as outlined in the following sections.

A. Cardiovascular Phantom Construction

Although the fabrication of tissue mimicking phantoms is a mature and well-reported topic, the design of suitable phantoms for cardiovascular investigations remains to be a nontrivial task. One particular challenge in designing cardiovascular phantoms is whether the developed experimental models can properly resemble the dynamics of cardiac contraction and arterial pulsation *in vivo*. This Special Issue has included five papers in this category to explain the technical details of different forms of cardiovascular phantoms.

The paper by Sandra Sjöstrand *et al.* (Lund University, Sweden) describes a conceptual wall phantom that is dedicated to the testing of new arterial imaging algorithms that track longitudinal wall motion. Distinguished from a typical straight tube phantom, the one reported in this paper comprises two lumen segments of different diameters with a tapering zone at the interface between the two segments.

In the paper by Tom Anderson (University of Edinburgh, U.K.), he reported a variation of the walled tube phantom that is dedicated to the testing of cardiac imaging algorithms. One unique feature of this protocol is that it has included the assembly of a customized flow pump that can support the pulsating of fast heart beat patterns (up to 600 beats per minute) that reflect the rapid cardiac contraction dynamics in rodents.

The paper by Chung Kit Ho *et al.* (University of Hong Kong and University of Waterloo, Canada) describes how it is possible to design wall-less flow phantoms with tortuous vascular geometries that are anatomically realistic based on patient information (a cerebral aneurysm model is illustrated as a case example). This type of flow phantom can serve well as an investigative tool to evaluate the performance of existing and emerging flow imaging techniques, including 3-D flow imaging.

The paper by Shiying Wang *et al.* (University of Virginia, USA, and University of Edinburgh, U.K.) reports in detail a laboratory recipe for the fabrication of tube phantoms and multipipe phantoms using different materials. The merit of this fabrication protocol is that it can support molecular target coating, thereby enabling them to be used in ultrasound molecular imaging investigations that study the binding efficacy of targeted microbubbles.

In the next paper, which is a companion paper written by the same author group, Xiaowei Zhou *et al.* explain how wall-less and walled tube phantoms for Doppler ultrasound investigations can be properly designed. It is intended to serve as a laboratory reference that documents in detail some important laboratory operations that are essential to the fabrication of tube phantoms larger than 1-mm diameter.

B. Development of Simulation Platforms

With advances in finite-element modeling (FEM) paradigms, there is an emerging interest to use this simulation approach as an intermediate step to investigate the performance of new imaging algorithms before experimentally implementing them on hardware. In this Special Issue, two papers have been included to illustrate the application of FEM simulations to the evaluation of new ultrasound-based estimation techniques.

First, Stefano Ricci *et al.* (University of Florence, Italy, and Ghent University, Belgium) have described an arterial dynamics FEM model that involves multiphysics principles. One key feature of this framework is that it can account for the fluid–structure interactions between arterial wall and pulsatile flow inside the vessel. To illustrate the use of their simulation model, the authors have applied it to assess the accuracy of a wall shear rate estimation method.

Second, Mark L. Palmeri *et al.* (Duke University and Mayo Clinic, USA) have presented an FEM model that simulates the coupling between: 1) acoustic radiation force generated by a focused ultrasound beam and 2) shear wave generation and propagation in viscoelastic media. As pedagogically described by the authors, this simulation tool can serve well as a platform for studying the performance of new estimation algorithms related to ultrasound shear wave elastography.

C. Transducer Design and Characterization

Development of transducer fabrication protocols is of critical importance to practical implementation of ultrasound imaging algorithms. On this topic, 2-D array fabrication is undoubtedly of high practical interest, as it can effectively enable the realization of real-time 3-D imaging techniques. In this Special Issue, we have included two papers on 2-D array transducer design, as well as one paper on the related topic of hydrophone measurements.

The paper by Benjamin A. Greenlay and Roger J. Zemp (University of Alberta, Canada) gives a step-by-step walkthrough of how capacitive micromachined ultrasound transducer arrays can be fabricated in academic and industrial laboratories. The authors described the fabrication of both linear arrays and 2-D arrays that operates via the top orthogonal-to-bottom electrode (TOBE) paradigm.

The paper by Emmanuel Roux *et al.* (INSA Lyon, France, and University of Florence, Italy) gives a different perspective to the 2-D array transducer design process. It explains how the number of elements on the 2-D array can be reduced through algorithmic optimization. Such work is of high relevance to 3-D ultrasound system development, because it can effectively reduce the channel count in the imaging hardware.

In the paper by Andrew M. Hurrell and Srinath Rajagopal (Precision Acoustics Ltd., U.K.), a pedagogical overview is given on the practicalities of making hydrophone measurements of transducer emissions. It is a valuable contribution that helps to establish the best practices in ultrasound metrology, thereby promoting the importance of properly taking hydrophone readings when characterizing the acoustic exposure of an ultrasound transducer.

D. Imaging and Computing Framework Implementation

Before a novel imaging method can be applied in clinical practice, it must first be tested on experimental platforms. A complex task such as this necessarily entails the establishment of engineering know-how on issues, such as real-time computing, image sequence programming, and hardware assembly. Four papers on this topic have been included in our Special Issue to provide tutorial-style illustrations on the engineering details involved in the implementation of a few different imaging techniques.

Donald VanderLaan *et al.* (Georgia Institute of Technology, USA) provided an educative tutorial on how intravascular ultrasound and photoacoustic imaging can be realized in real time. A broad scope of this discussion has been provided, covering topics such as catheter design, hardware assembly, coupling between system components, and real-time computing considerations.

Adrian J. Y. Chee *et al.* (University of Hong Kong and University of Waterloo, Canada) gave a detailed algorithmic description on how the increasingly popular eigen-processing technique can be realized in real time through devising a single-instruction, multiple-device (SIMD) computing framework. A tutorial is provided on how this parallel computing framework can be implemented on a graphical processing unit (GPU), and its applicability to ultrasound color flow imaging is demonstrated as a case example.

Yufeng Deng *et al.* (Duke University, USA) shared their in-house best practice on the implementation of shear wave elasticity imaging on an ultrasound research scanner that is commercially available. Sample scripts for image sequence programming have been included as supplementary material posted in a Github repository. This paper serves well as tutorial material for those who wish to pursue similar implementations in their own laboratory.

Kevin J. Haworth *et al.* (University of Cincinnati, USA) presented various algorithmic and implementation details on an emerging technique called passive cavitation imaging. Commentary is given on the strengths and limitations of using different ultrasound research scanners to implement the described technique. In addition, the authors have posted sample scripts to help interested users derive passive cavitation image frames.

E. Ultrasound Scanner Quality Assessment

In the preliminary testing stage, a new imaging algorithm is often evaluated using basic metrics, such as contrast-to-noise ratio (CNR) whose focus is on evaluating signal quality. However, for more mature algorithms that are already implemented on hardware, user-oriented assessment methodologies are needed to calibrate its practical efficacy. To promote the community's awareness of the need for proper quality assessment of ultrasound equipment, this Special Issue has included three papers on such a topic.

The paper by Elisabetta Sassaroli *et al.* (Harvard Medical School, USA) presents a review on performance evaluation methods for ultrasound equipment used in breast imaging. The notion of quality control is emphasized throughput this paper, and its relevance to accreditation and regulatory issues is discussed in detail to educate ultrasound technical researchers who may not necessarily be experienced in medical device regulation.

The second paper by Martin Christian Hemmsen *et al.* (Technical University of Denmark) offers a different perspective from the first one through a broad discussion on different quality assessment strategies for B-mode imaging. In particular, a multiphase assessment paradigm is presented, and it includes consideration of receiver operating characteristics that describe the sensitivity and specificity of an ultrasound equipment.

The third paper by Aiguo Han *et al.* (University of Urbana–Champaign and University of California at San Diego, USA) offers a different perspective in that its focus is on the evaluation of clinically used quantitative ultrasound (QUS) techniques. The authors gave a detailed analytical discussion on the repeatability and reproducibility of clinical QUS methods using various quantitative metrics.

F. Microbubble and Nanodroplet Fabrication Protocols

For contrast imaging and ultrasound-mediated drug delivery to flourish, it is imperative to establish mature protocols for the fabrication of microbubbles and nanodroplets. The importance of this methodological aspect is underscored in our Special Issue with the inclusion of three papers from experienced researchers who actively work in this area.

Helen Mulvana *et al.* (University of Glasgow, University of Oxford, King's College London, and Erasmus Medical Center) discussed methods that are used to characterize the properties of microbubble contrast agents. The authors have highlighted various techniques that ranged from microbubble sizing to fluorescence observation of microbubble shell composition.

In the paper by Paul S. Sheeran *et al.* (University of Toronto, University of Colorado at Boulder, University of Arizona, and University of North Carolina), a tutorial is given on how nanodroplets for contrast imaging purposes can be stably generated. This paper is considered as timely, because nanodroplets have demonstrated considerable potential since its recent inception within the context of the field.

Juan D. Rojas and Paul A. Dayton (University of North Carolina, USA) elaborated further on nanodroplet methodologies by presenting a pressure matching framework for achieving uniform acoustic vaporization of nanodroplets. With such a method, the practical efficacy of acoustic nanodroplets as contrast agents can be significantly bolstered.

G. In Vitro Methods for Ultrasound Bioeffect Studies

To help assert the therapeutic merit of ultrasound, *in vitro* experimentation based on cell models should be performed as they can offer important insight into the mechanism of action. For this purpose, effective experimental protocols need to be established. Our Special Issue has included two original papers on such a topic to illustrate related laboratory practices.

The paper by Nobuki Kudo (Hokkaido University, Japan) provides an in-depth depiction of how to develop an acoustically coupled high-speed live microscopy platform that can effectively facilitate single-cell investigations on the interactions between a microbubble and a living cell. The unique merit of this platform is that it has included optical tweezer functionality to precisely control the microbubble's position *in situ*. It can be considered as an advanced research tool for cellular-level sonoporation dynamics studies.

The paper by Sylvie Lélu *et al.* (Norwegian University of Science and Technology) reports the establishment of a cell model for studying the bioeffects of ultrasound and microbubbles on blood–brain barrier function. This model is based on the use of primary porcine brain endothelial cells. The authors have explained various laboratory skills that are needed and a few caveats that should be noted in working with this cell model.

H. Pre-Clinical Methods for Focused Ultrasound Treatments

While HIFU may already be a success story in the field of therapeutic ultrasound, much remains to be accomplished to establish its clinical efficacy and safety. On this front, effective pursuit of pre-clinical research is important. Alas, the methodology tends to vary between research groups.

In this Special Issue, one paper is included to review the current best practices in conducting pre-clinical studies on magnetic resonance imaging guided focused ultrasound (MRgFUS) treatments. Nicholas P. K. Ellens and Ari Partanen (Johns Hopkins University, USA, and Philips Medical Systems) have given an account on the methodological considerations involved in running pre-clinical MRgFUS investigations. Discussion is also provided on related system design concepts and software solutions.

Final Remarks

This Special Issue represents a publishing milestone in the UFFC Transactions. It demonstrates the commitment of the UFFC Transactions in giving due recognition to meticulous documentation and community-wide dissemination of the best practices on methods and protocols in biomedical ultrasonics. It is hoped that our Special Issue will provide thrust to cultivate the next wave of innovations in biomedical ultrasound imaging and therapy.

The Guest Editors hereby thank all the authors who have contributed excellent tutorial articles to this Special Issue. Additionally, we thank numerous anonymous reviewers for taking their time to offer detailed constructive feedback that was indispensable to improve the archival value of the accepted articles. We appreciate that documentation of methodological details is a painstaking task that requires the authors to devote significant attention to details; peer review of these manuscripts is also an intensive task, as it requires the reviewers to have a sharp eye for spotting inconsistencies and glitches in the already detailed documentation written by the authors. In these regards, both the authors and the reviewers are to be applauded for their strong dedication and effort. Last, but not least, the Guest Editors are much grateful to the Editor-in-Chief Steven Freear for his all-weather support in steering this Special Issue to success. We particularly wish to acknowledge Steven for facilitating the meeting at the Leeds Microbubble Symposium that provided the genesis of this Special Issue in June 2015.

This Special Issue had planned to include a paper by Alexander Klibanov (University of Virginia, USA) addressing canonical methods on how microbubbles, involving a variety of shell and gas compositions, can be reproducibly fabricated. This paper would also describe in detail how the microbubbles can achieve molecular targeting through the inclusion of antibodies in the shell composition. Unfortunately, due to a minor accident, Dr. Klibanov was unable to complete this paper in time for the publication deadline. The Guest Editors now plan to include this much-anticipated paper in an upcoming regular issue in the UFFC Transactions.

Looking forward, this Special Issue is by no means the end of documentation needs for methods and protocols in biomedical ultrasonics. Indeed, this Special Issue marks the start of a new, ongoing quest to promote broad dissemination of important research methods in biomedical ultrasonics. Best practices of existing methods will continue to evolve and be refined, while new protocols will be developed over time. The UFFC Transactions highly welcome prospective authors to publish detailed methods, protocols, and experimental tools that are seminal and important to spur further research in biomedical ultrasonics.

Alfred C. H. Yu, *Guest Editor*
Department of Electrical and Computer Engineering
University of Waterloo
Waterloo, ON, Canada

John A. Hossack, *Guest Editor*
Department of Biomedical Engineering
University of Virginia
Charlottesville, VA, USA

References

[1] J. A. Jensen and N. B. Svendsen, “Calculation of pressure fields from arbitrarily shaped, apodized, and excited ultrasound transducers,” *IEEE Trans. Ultrason., Ferroelect., Freq. Control*, vol. 39, no. 2, pp. 262–267, Mar. 1992.

[2] M. O. Culjat, D. Goldenberg, P. Tewari, and R. S. Singh, “A review of tissue substitutes for ultrasound imaging,” *Ultrasound Med. Biol.*, vol. 36, no. 6, pp. 861–873, Jun. 2010.

[3] P. R. Hoskins, “Simulation and validation of arterial ultrasound imaging and blood flow,” *Ultrasound Med. Biol.*, vol. 34, no. 5, pp. 693–717, May 2008.

[4] K. K. Shung and M. Zippuro, “Ultrasonic transducers and arrays,” *IEEE Eng. Med. Biol. Mag.*, vol. 15, no. 6, pp. 20–30, Nov. 1996.

[5] P. Tortoli and J. A. Jensen, “Introduction to the special issue on novel equipment for ultrasound research,” *IEEE Trans. Ultrason., Ferroelect., Freq. Control*, vol. 53, no. 10, pp. 1705–1706, Oct. 2006.

[6] H. So, J. Chen, B. Yiu, and A. Yu, “Medical ultrasound imaging: To GPU or not to GPU?” *IEEE Micro*, vol. 31, no. 5, pp. 54–65, Sep./Oct. 2011.

[7] A. L. Klibanov, “Microbubble contrast agents: Targeted ultrasound imaging and ultrasound-assisted drug-delivery applications,” *Invest. Radiol.*, vol. 41, no. 3, pp. 354–362, Mar. 2006.

[8] P. S. Sheeran and P. A. Dayton, “Phase-change contrast agents for imaging and therapy,” *Current Pharmaceutical Design*, vol. 18, no. 15, pp. 2152–2165, 2012.

[9] K. Hensel, M. P. Mienkina, and G. Schmitz, “Analysis of ultrasound fields in cell culture wells for *in vitro* ultrasound therapy experiments,” *Ultrasound Med. Biol.*, vol. 37, no. 12, pp. 2105–2115, Dec. 2011.

[10] T. Yddal, S. Cochran, O. H. Gilja, M. Postema, and S. Kotopoulis, “Open-source, high-throughput ultrasound treatment chamber,” *Biomed. Eng.*, vol. 60, no. 1, pp. 77–87, Feb. 2015.

[11] A. Alassaf, A. Aleid, and V. Frenkel, “*In vitro* methods for evaluating therapeutic ultrasound exposures: Present-day models and future innovations,” *J. Therapeutic Ultrasound*, vol. 1, Nov. 2013, Art. no. 21.

Alfred C. H. Yu (S'99–M'07–SM'12) received the bachelor's degree in electrical engineering from the University of Calgary, AB, Canada, in 2002, and the M.A.Sc. and Ph.D. degrees in biomedical engineering from the University of Toronto, ON, Canada, in 2004 and 2007, respectively.

He is an Associate Professor with the Department of Electrical and Computer Engineering, University of Waterloo, ON, Canada. He has a long-standing research interest in ultrasound imaging innovations and therapeutic ultrasound discoveries. He also interned at Philips Research North America, Briarcliff Manor, NY, USA, in 2005. Before he relocated to Waterloo, he was a Research Assistant Professor with the University of Hong Kong, where he founded and served as the Principal Investigator of HKU Biomedical Ultrasound Laboratory.

Dr. Yu is the recipient of the 2016 IEEE Ultrasonics Early Career Investigator Award. He is a Senior Member of *AIUM*.

John A. Hossack (S'90–M'92–SM'02–F'15) received the B.Eng. and Ph.D. degrees from the University of Strathclyde, Glasgow, U.K., in 1986 and 1990, respectively.

From 1990 to 1992, he was a Post-Doctoral Researcher with the E. L. Ginzton Laboratory, Stanford University, Stanford, CA, USA. His early research involved the modeling and design composite piezoelectric ceramic/epoxy transducers. Subsequently, at Acuson (a medical ultrasonic imaging equipment manufacturer in Mountain View, CA, USA), he made contributions in the fields of transducer design, beamforming, 3-D imaging methods, and image quantification/processing. Since joining the University of Virginia (Associate Professor 2000–2009, Professor 2009–), he has been working on 2-D transducer arrays for low-cost handheld ultrasound imaging, mouse heart imaging, molecular imaging using targeted ultrasound contrast agents, and developing catheters with a dual imaging and drug delivery capability for a variety of cardiovascular related applications. More recently, he has been performing research involving photoacoustic and fluorescent signal detection catheters.

Dr. Hossack is a Fellow of the American Institute of Medical and Biological Engineers.