

# Detecting, Mapping, and Quantifying Bubble Activity in Therapeutic Ultrasound Workshop

**Virtual Meeting**

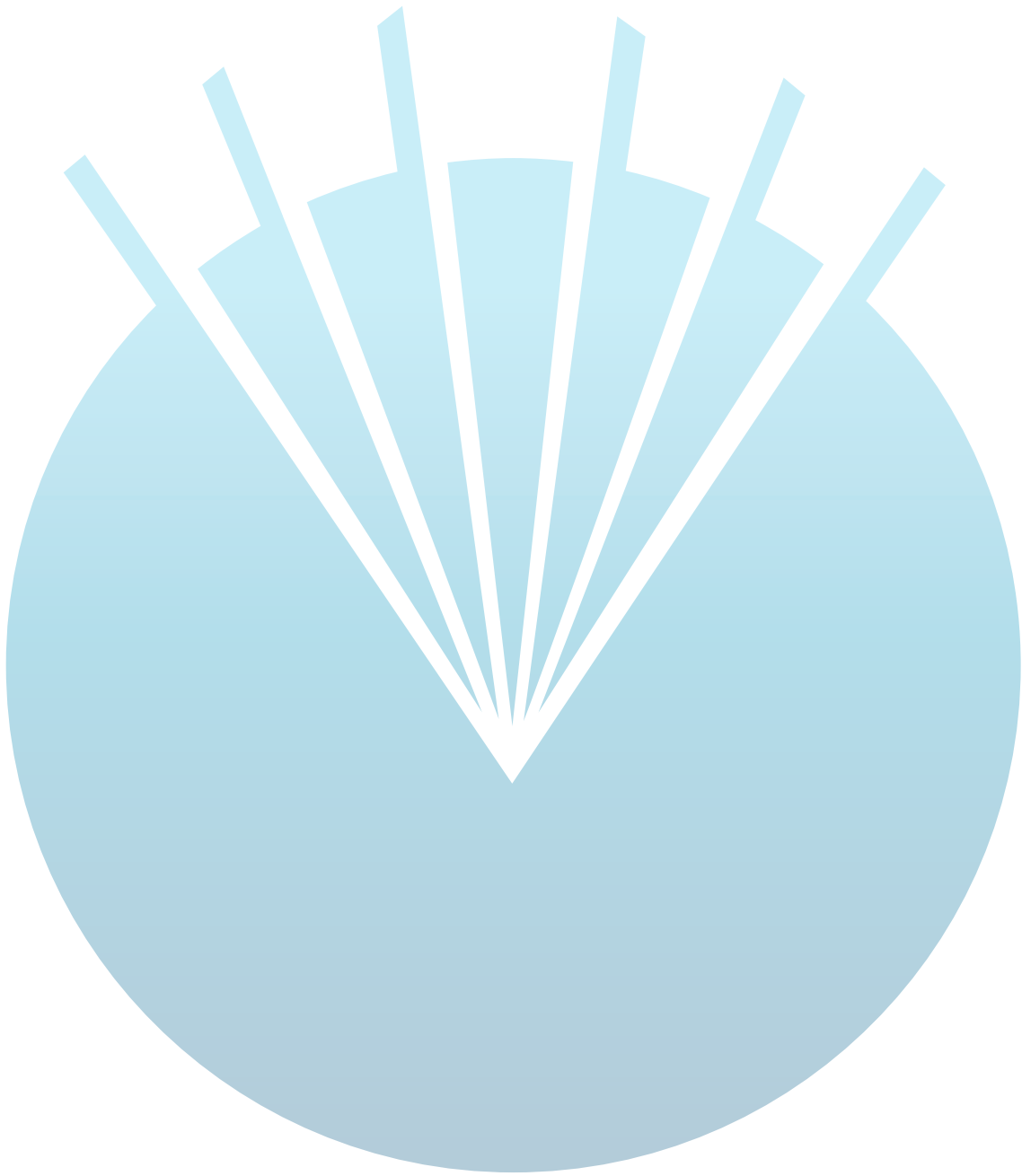
September 24, October 1, 8, 15, 22, and 29

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## Executive Summary

The Focused Ultrasound Foundation in partnership with American Institute of Ultrasound in Medicine's Future Fund hosted a series of virtual workshops on detecting, mapping, and quantifying bubble activity in therapeutic ultrasound on September 24, October 1, 8, 15, 22, and 29, 2021.

Planning of the session was performed by an organizing committee including researchers, engineers, physicians. The meetings brought together critical stakeholders, including researchers, clinicians, industry, government, and others, to share and combine knowledge to advance the field. The event was held to review and discuss the levels of knowledge, gaps in knowledge, and best practices in monitoring and quantifying cavitation (and more generally bubble activity) for ultrasound therapy. The ultimate objective was to propose guidelines on how to best detect, monitor, map and quantify bubble activity for ultrasound therapy.

Sessions were accessed via live open meetings, and participants were invited to submit questions through a Q&A interface as talks were given. (Note: Not all speakers were available for the Q&A sessions.) The live virtual sessions centered around a specific topic during each session:

- Cavitation equipment
- Cavitation localisation and mapping
- Cavitation thresholds and dose
- Cavitation agents
- Clinical experiences and regulatory considerations
- Summary and working session

The attendees were asked to continue thinking and collaborating on these issues, and to share any additional thoughts with their colleagues, the Focused Ultrasound Foundation (FUSF) and the AIUM. The organizing committee plans to create a consensus paper for dissemination that highlights the topics covered during the workshop series and identify the remaining challenges.

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## Organizing Committee

**Costas Arvanitis, PhD**

Georgia Institute of Technology

**Brian Fowlkes, PhD**

University of Michigan

**Kevin Haworth, PhD**

University of Cincinnati

**Frédéric Padilla, PhD**

Focused Ultrasound Foundation

**Zhen Xu, PhD**

University of Michigan

**Constantin Coussios, PhD**

University of Oxford

**Michael Gray, PhD**

University of Oxford

**Meaghan O'Reilly, PhD**

Sunnybrook Research Institute

**Graeme Woodworth, MD**

University of Maryland

**Timothy Ziemlewicz, MD**

University of Wisconsin

## Acknowledgements

The Detecting, Mapping, and Quantifying Bubble Activity in Therapeutic Ultrasound workshop was sponsored by The Focused Ultrasound Foundation, in partnership with the American Institute of Ultrasound in Medicine's Future Fund.

The sponsors wish to thank the organizing committee for their participation and contributions which led to the successful execution of this event. It was produced by the AV Company using the EventMobi Platform. This summary was written by Heather Gorby, PhD. Each speaker reviewed and approved the content from their presentation and their discussion comments. Emily Whipple, PhD, MBA and Frédéric Padilla, PhD provided final approval of the summary.

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To view the webinar a link can be found on Foundation's [website](#) to the Foundation's [YouTube channel](#).

# Abbreviations

<b>ACT</b>	Acoustic cluster therapy
<b>AD</b>	Alzheimer's disease
<b>AE</b>	Adverse events
<b>AIUM</b>	American Institute of Ultrasound Medicine
<b>AU</b>	Arbitrary units
<b>BBB</b>	Blood-brain barrier
<b>BBBo</b>	Blood-brain barrier opening
<b>DMG</b>	Diffuse midline glioma
<b>DVT</b>	Deep vein thrombosis
<b>ELIP</b>	Echogenic liposomes
<b>FLAIR</b>	Fluid-attenuated inversion recovery
<b>FUS</b>	Focused Ultrasound
<b>FUSF</b>	Focused Ultrasound Foundation
<b>HIFU</b>	High intensity focused ultrasound
<b>HITU</b>	High intensity therapeutic ultrasound
<b>IC</b>	Inertial cavitation
<b>IEC</b>	International Electrotechnical Commission
<b>MB</b>	Microbubble
<b>MI</b>	Mechanical index
<b>NIST</b>	National Institute of Standards and Technology
<b>PAM</b>	Passive acoustic mapping
<b>PCD</b>	Passive cavitation detection
<b>PRF</b>	Pulse-repetition frequency
<b>PSF</b>	Point-spread function
<b>RBC</b>	Red blood cell
<b>SNR</b>	Signal-to-noise ratio
<b>SWL</b>	Shock wave lithotripsy

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September 24, 2021

## Welcome from Focused Ultrasound Foundation and American Institute of Ultrasound Medicine

**Frédéric Padilla and J. Brian Fowlkes**

The workshop was a collaborative effort between American Institute of Ultrasound in Medicine (AIUM) and the Focused Ultrasound Foundation (FUSF). The aims of the workshop were to review the state of the art of cavitation, and more generally, bubble activity; and to identify level of knowledge, gaps in knowledge, and best practices. The objective is to propose guidelines on how to best detect, monitor, and quantify bubble activity for ultrasound therapy.

There were 6 sessions held from September 24 to October 29th. There were 5 technical sessions covering the detection, mapping, threshold and dose, cavitation agents, clinical experiences, and regulatory vision. The last working session was to develop a summary on how and what to measure and report and determine next steps.

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## Cavitation Detection Equipment

### *September 24, 2021*

This session focused on the technology and equipment used for cavitation including passive cavitation detection systems, calibration methods, and signal processing and analysis methods along with their clinical implementation for brain applications. Costas Arvanitis (Georgia Institute of Technology) and Constantin Coussios (Univ. of Oxford) moderated the session, with presentations given by Kyle Morrison (President, Sonic Concepts), Kevin Haworth (Univ. of Cincinnati), Michael Gray (Univ. of Oxford), Nathan McDannold (Harvard Medical School), Elisa Konofagou (Columbia Univ.) and Itay Rachmilevitch (Insightec).

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### Passive Cavitation Detectors *Design & Calibration Methods*

**Kyle Morrison | Sonic Concepts**

Kyle Morrison presented an overview on passive cavitation detectors and the design and calibration methods used at Sonic Concepts. Passive cavitation monitoring is the recording of signals emitted during bubble events. There are a range of cavitation types from stable cavitation, which emit super- and sub-harmonic content of a bubble's resonant frequency, to inertial cavitation, the collapse of those bubbles, which emit wide-band, high-frequency content. To detect both cavitation types, the solution is to have sensitive and wide-band passive receiver sensors, often called passive cavitation detectors, to perform passive cavitation detection (PCD). The PCD receiver can be coaxially aligned with therapeutic transducer with a central opening to overlap with the focal site and can be used for minimally invasive or noninvasive applications. There are high-intensity focused ultrasound (HIFU) transducers that also can be coupled with an imaging probe so that PCD can be performed along with imaging in real time for guidance and monitoring of HIFU bubble cavitation events.

There are various kinds of PCD's. For wide passband PCD, a 10 MHz PVDF piezopolymer material with a low Q (the quality factor, a measure of the sharpness of the frequency response) can be used. The goal is to have high sensitivity over a large bandwidth. Low density materials lower the longitudinal impedance, which lowers Q. This is 3,000 times more sensitive than a 200-micron needle hydrophone, which helps with signal-to-noise ratio (SNR). However, piezopolymers do not make a good transmitter. For pulse-echo sensitivity, a 10 MHz 1-3 piezo-composite was created. The density was increased, which allows a 4 times higher Q. The composite was eighteen times more efficient than typical piezopolymers. There are a variety of PCD types developed for FUS.

### Cavitation Detection Equipment

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### Q & A

- There was a question on how the directional capability of the PCD was affected by its aperture. Morrison responded that the larger the aperture, the smaller the directivity becomes. If a larger field of view is desired, a smaller sensor is better to provide greater directivity, but you lose sensitivity.
- A question was asked about the dependence of the focal gain of a spherical concave aperture as a function of frequency content, and how this will affect measurements throughout the axial plane. Morrison replied focused PCD's are difficult to calibrate within a reasonable uncertainty. The spatial resolution across the frequency spectrum is very difficult to quantify. The focused PCD's geometric focus cannot be assumed to be in phase. It is possible to attempt calibration of the geometric focus and disregard the phasing variability, but this is complex. Instead, we rely on the electrical impedance and axial focusing simulations to approximate the sensitivity.
- A participant asked if it was possible to tune the system to detect sub-harmonics. Morrison commented that it is possible to narrow the frequency range specific to a frequency range of interest with a higher Q composite or ceramic to increase sensitivity.
- There was a question on the sensitivity of a ring-transducer. The surface area and  $f/\text{number}$  of the PCD aperture dictates the sensitivity for that given piezomaterial being used. When introducing a central opening (hole) in the middle, you spread the field from the primary lobe into the secondary lobes, so effectively lose peak sensitivity.
- A question was asked on design considerations regarding a single element versus a multi-element array. Morrison responded that a multi-channel array allows the user to steer the ultrasound to leverage more aperture & increasing the sensors sensitivity, while narrowing the monitoring zone.

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## Receiving and Processing Passive Signals

Kevin Haworth, PhD | Univ. of Cincinnati

Kevin Haworth described passively receiving and processing signals for cavitation detection. There is a common set up of a therapy transducer insonifying the tissue and a PCD used for recording the cavitation emissions, although specific details may vary. The cavitation induced by the therapy transducer causes bioeffects. The analog received signal may be amplified with a preamplifier. Data acquisition boards digitize the signal, which can be further processed to obtain relevant information, such as from a power spectrum. Information may be desired for knowing cavitation at both the target tissue to determine if the desired cavitation occurred, or for non-target tissues to determine if off-target cavitation occurred. The choice of the PCD can be designed to be sensitive over a relatively large area by using an unfocused transducer, or over a relatively small area by using a focused transducer with the application dictating the selection. The selection of the transducer, preamplifier, and data acquisition tool should be designed with the frequency bandwidth of interest in mind. Other considerations were whether to include the fundamental insonation frequency within the bandwidth of interest, which can be a large signal that can affect the SNR of other frequencies that are much smaller, such as ultra-harmonics or broadband emissions.

Data truncation and signal processing are important considerations when analyzing signals. Haworth cautioned that it is important to account for the fact that real-life data is discretized on a computer. For example, when comparing a continuous Fourier transformation and discrete Fourier transformation there are scaling factors that involve sampling frequency and phasers that involve the frequency step size. The resulting power spectrum is only useful for comparing within that same power spectrum. To compare between different experimental groups and setups, we need to move to calibrated systems that report results that account for discretization and calibration factors. Other transformations include the wavelet transformation and singular value decomposition for isolating different signal components.<sup>1</sup>

PCDs are significantly larger than a wavelength to increase the sensitivity of the system. However, phase averaging across the face of the PCD will occur. For a PCD with a focus on a single-known source there is good coherence for the received signal on the PCD. When the cavitation source is an ensemble of unknown sources that dynamically change over time, the received signal will be the interference of these sources. A diffraction correction factor could be used to characterize radiated power from cavitation sources if they are treated as a random ensemble.

As the field moves forward, there are several key opportunities and challenges to consider: uniform approaches to reporting methods, including processing code;<sup>2</sup> practical methods

for calibrating systems; and development of gold-standard techniques to enable comparison of different approaches.

### Q & A

- There was a question on what a typical number of cycles would be for a short therapeutic pulse. The number of cycles will depend on the application. Intrinsic threshold histotripsy has short pulses of just a half-cycle. For drug delivery applications, short pulses would be ‘tens’ of cycles.
- A participant asked about gold-standard calibration methods. Haworth responded that this is a challenge and something that requires consideration. Bubble activity is dynamic, stochastic, and changing. A gold-standard calibration method is something the community as a whole should create.
- The methods presented require a great amount of expertise, a participant asked if there was a repository or open-source software for non-expert users. Haworth stressed that creating a code-repository is particularly important. Researchers should also document their system pathways in publications and industry could also create turn-key systems for non-experts.
- A question was posed on the appropriate metrics for measuring energy. The answer on what metric to use was that the best metric depends on the bioeffect. For example, for thermal ablation time-averaged power would be the most relevant. Pulse-averaged energy or amplitude might be better for drug delivery with microbubbles. Haworth suggested that bioeffects should be linked to cavitation in order to develop gold-standard metrics.

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## Considerations for Calibrated Cavitation Monitoring

Michael Gray, PhD | Univ. of Oxford

Dr. Gray presented on calibrated cavitation monitoring. A framework for cavitation generation and monitoring was briefly described. A critical point was that full calibration involves more than hardware: proper accounting must be also made for signal propagation through tissue, received signal processing, and beamforming. Cavitation imaging, like all other medical imaging modalities, is based on limited observations of the physical phenomena of interest. The goal is to make the best use of the data obtained, and calibration can play a key role.

In terms of tissue propagation, the ideal and often-assumed situation is that of a straight and lossless path between the emitter and the receiver. However, in real-world situations refraction, diffraction, reflection, and attenuation result in distortion and order-of-magnitude signal loss.

Signals propagating through tissue and arriving at the receiver may be affected by both the device's electroacoustic response (set by device construction) and diffraction response (set by element size, shape, and focusing). The latter is dependent on relative position of the emitter and the receiver, which can make proper calibration challenging over the full imaging field of view.

Processing of received signals typically removes unwanted frequency bands and separates signal types depending on what is of interest (e.g., ultra-harmonics or broadband spectra). A key consideration here is simply that of rigorously reporting what signal components are being retained in the analysis.

An essential descriptor of beamformer performance is the point-spread function (PSF), which shows how a finite aperture receiver interprets a point emitter at a specific location. The PSF may be strongly sensitive to emitter location and receiver geometry, and different beamformers will have different PSFs. Moreover, adaptive beamformers may change the PSF in a single fixed location with only minor changes in input data. All of this is important when trying to assess reliability of energy and dose estimation.

Gray presented a few examples and methods to alleviate some of the issues with calibrating PCDs.<sup>3,4</sup> This discussion was followed by a set of passive mapping examples that illustrated how images of a fixed emitter can vary substantially in size, level and location as receiver depth and geometry were varied. Examples of array calibration were also presented.<sup>5,6</sup> Finally, transcranial imaging was discussed with an emphasis on how different forms of aberration correction may change the amplitude, location, and detailed shape of cavitation.<sup>7,8</sup>

In review, it was proposed that there are no glaring holes in the available methodologies for full path (tissue, receiver, beamformer) calibration. The individual technological capabilities are largely available but putting all the pieces together will be challenging. Standardization, adoption, and definition of methods by application and monitoring type remain unresolved issues. It was noted that there are lessons the cavitation community can learn from contemporary radiological practice, specifically in diagnostic ultrasound safety metrics and PET/CT dose calibration.

### Q & A

- Gray was asked to describe the key components that every lab should be using for calibration. First, there are several internal biasing factors within a single system, and there is no “one-size-fits-all” solution. It remains to be defined what is ‘good enough’ for internal references versus establishing community-wide dose metrics.

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## Transcranial Hardware

**Nathan McDannold, PhD | Harvard Medical School**

Nathan McDannold discussed transcranial cavitation monitoring. Transcranial thermal ablation can be performed with the ExAblate Neuro system from Insightec via closed-loop feedback control in an FDA-approved treatment for essential tremor (thalamotomy with FUS).<sup>7</sup> Cavitation detection is built into the system and automatically detects cavitation and lowers the power for safety. Recent data suggest that by using acoustic models based on CT scans, the system can perform aberration corrections for transcranial propagation.<sup>7</sup> If implemented, this will allow more patients access to treatment, which is limited by the variability of the skull. Passive acoustic emission monitoring is used to verify BBBo.<sup>8</sup> Staying within the harmonic emission range is one strategy to maintain safety and prevent inertial cavitation.<sup>8</sup> Another method is to increase pressure until ultra-harmonic emissions are detected, and then lower it to harmonic levels.<sup>9</sup> Harmonic emissions also correlate to the amount of drug delivery in the brain.<sup>10</sup>

McDannold discussed the ongoing multicenter trial that is delivering chemotherapy via blood-brain-barrier disruption to patients with glioblastoma following tumor resection. As part of the trial, McDannold’s team is performing cavitation monitoring in these patients. The target is white matter, that has a low vascular density. There is a clear relationship between the amount of BBBo and the amount of petechia as a function of cavitation dose.

## Cavitation Detection Equipment

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An experimental helmet has been developed at Sunnybrook Research Institute that has both transmission and receiving capabilities.<sup>11</sup> The helmet has a large number of detectors to increase sensitivity and account for beam steering with higher quality for detection of strength and location of the emissions. Passive acoustic mapping can also be used to detect microbubble concentrations.<sup>12</sup>

Future directions for transcranial cavitation detectors include reporting calibration values for detectors in publications and elsewhere. Improved treatment planning will include effects of skull attenuation, beam steering, and vascular density for cavitation monitoring and control. To coincide with the lower microbubble (MB) doses used clinically, detector sensitivity needs to be increased to improve measurements in white matter. If transcranial BBBo is to be successful, the field needs to move from cavitation monitoring to cavitation imaging.

### Q & A

- There was a question on how to get rid of arbitrary units that appear in cavitation measurements for BBBo and brain treatments. McDannold replied that they drive the pressure until a threshold event is observed, such as an ultra-harmonic emission or an increase in harmonic emission. For calibration, the sensitivity of the detector needs to be determined. Determining cavitation dose is challenging as it requires modeling skull effects on both the transmit and receive signals. MB dose is also affected by vascular density.
- A question was asked regarding using the same MB dose in both preclinical and clinical studies to achieve better compatibility and predictive ability of animal studies in humans. McDannold agreed that this would help; in the US, the FDA was allowing a wider range of MB dosing in humans. Alternatively preclinical research studies should be conducted with lower (physiologically) relevant doses.
- A participant asked what the optimal unit of calibration would be for BBBo. McDannold stated that detecting a threshold event that has physical meaning is a good place to start, but this will be challenging.
- There was a request to expand on the variability between patients regarding cavitation dose. McDannold responded that it depends on the exact tumor location in the head. For future treatments, including additional information from treatment planning to the cavitation monitoring should improve prediction.

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## Clinical Implementation of Single-element PCD

Elisa Konofagou, PhD | Columbia Univ.

Elisa Konofagou presented on experience with clinical implementation of cavitation detection. Her laboratory has their own system for BBBo with a neuronavigation-guided single-element FUS transducer.<sup>15</sup> One advantage of this system is that a previously acquired MRI image can be used to identify the target during treatment planning.<sup>15</sup> During treatment, harmonic and ultra-harmonic emissions are monitored in real time. A spectrogram is created to allow frequency monitoring. BBBo can be confirmed without contrast.

There are 2 ongoing clinical trials at Columbia University. One is a clinical trial for patients with Alzheimer's disease (AD, NCT04118764) for BBBo alone and the other is to use FUS in combination with oral panobostat in children with progressive diffuse midline glioma (DMG) (NCT04804709).<sup>16,17</sup> In the AD trial, the prefrontal cortex is the FUS target. Real-time cavitation monitoring is performed throughout treatment.<sup>18</sup> Harmonic, ultra-harmonic, and inertial cavitation is monitored, the cavitation dose is calculated with harmonic emissions during the procedure.<sup>18</sup> In the pediatric trial, the PCD is placed at the back of the head, and gravity can make the set up challenging. Cavitation monitoring can also be done in these patients. Passive acoustic mapping (PAM) was also demonstrated with pediatric patients with an array along with a Verasonics system. PAM allows the spatial identification of the location of highest cavitation in the brain.

### Q & A

- A question was asked whether absolute or relative measurements for calibration were more important. Konofagou replied that there is still a lot of uncertainty with FUS for brain treatments. For example, the incidence angle on the skull is unknown; the skull is heterogenous, and thickness varies. The harmonic versus broadband ratio may be useful for comparison between participants, but the cavitation dose is highly variable and is not a useful comparative metric.

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## Implementation and Use of PCD— The ExAblate System

Itay Rachmilevitch | Insightec

Itay Rachmilevitch presented on the management and usage of the ExAblate system for cavitation detection. Cavitation monitoring is a key safety element in any FUS therapy. Early brain FUS systems did not include cavitation detection, which could cause adverse events. Local tissue heterogeneity leads the cavitation signal to appear under widely different acoustic parameters and needs to be monitored in real time. The cavitation detector response is used for closed-loop power modulation for cavitation control. If cavitation levels are above a critical threshold, the sonication is halted. There is an ongoing effort for multi-element-based cavitation detection and localisation. An echo-imaging concept is being developed where all transducer elements are used as both transmitters and receivers.

In over 5,000 transcranial treatments, cavitation safety thresholds proved robust and effective. Therapies based on cavitation (i.e., BBB0) require real-time control mechanisms for controlling efficacy and safety with proper spatial localisation technology. Future development will continue to improve spatial and temporal resolution.

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## Panel Discussion

Following the presentations, a panel discussion took place led by Ron Roy (Univ. of Oxford) and Tom Matula (Univ. of Washington), where consensus was reached on many challenging topics.

These include:

1. PCD is essential for any bubble mediated therapy. Even though cavitation is a threshold phenomenon, there was consensus that merely identifying the threshold qualitatively would be insufficient, because the level of cavitation activity above the threshold directly impacts the observed bioeffects. The development of ubiquitous methods and techniques for calibrated PCD was therefore considered highly desirable.
2. The development of calibrated point sources that can be easily deployed across labs and clinics for easily referenceable free-field calibration of the PCD systems is highly desirable, as it will allow comparison of the signals recorded across

different systems and help to set up standards for the different applications. Both existing hydrophones that could be used in transmit mode and photo-acoustically driven sources were proposed as potential ‘true’ point sources, and the possibility of manufacturing them as single-use disposables that would not require re-calibration was also discussed. Several challenges were identified that will need to be addressed in developing such point sources, including frequency response, directivity, and the careful consideration of the presence of side lobes and the need for careful instructions to ensure reproducible, far-field measurements.

3. The development of phantoms that capture disease or organ specific characteristics could enable further refinement and standardization of measurements obtained with calibrated systems; in particular, for systems intended for use with artificial cavitation nuclei (microbubbles or other sonosensitive particles), the inclusion of flow channels or flow compartments with known nuclei concentrations could be used to enable complete system validation using standardized procedures.
4. In general, there was consensus that the design of standard calibration systems with clear instructions and the creation of a repository of standard signal processing codes would assist users who are not expert in cavitation, enabling them to create measurements that are aligned in quality and reliability with those of the more expert community. This was seen as particularly important in the context of enabling ultrasound and cavitation to become research tools that are increasingly used by wider diverse scientific communities.
5. Lastly, the development of methodologies for self-calibration using in-patient reference measurements (rather than additional/extracorporeal calibration tools) will accelerate clinical translation and potentially bridge the gaps between preclinical and clinical investigations and practice. In their simplest form, these measurements could be cavitation threshold measurements in the absence and presence of exogenous nuclei or making it necessary to record baseline noise levels before and after cavitation events to provide standardized reporting of noise reference levels.

The panel discussion ended with the recommendation to establish a program that will bring together academia, industry and National Institute of Standards and Technology (NIST), among others, to facilitate the implementation, refinement, and subsequent dissemination of these methods.

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## Cavitation Localisation and Mapping

### *October 1, 2021*

### Welcome from Focused Ultrasound Foundation and American Institute of Ultrasound in Medicine

Frédéric Padilla and J. Brian Fowlkes

Frédéric Padilla welcomed participants and introduced the day's topic, cavitation imaging and mapping. The goal of the session was to review the state of the field, what is known and unknown, and produce a plan for future directions. The session was moderated by Michael Gray (Univ. of Oxford) and Kevin Haworth (Univ. of Cincinnati) with presentations by Constantin Coussios (Univ. of Oxford), Meaghan O'Reilly (Sunnybrook Research Institute), Jonathan Sukovich (Univ. of Michigan), Julianna Simon (Pennsylvania State Univ.), and Costas Arvanitis (Georgia Institute of Technology).

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### Passive Acoustic Mapping for Cavitation Imaging *From Where the Bubble Tolls*

Constantin Coussios, PhD | Univ. of Oxford

#### Cavitation Localisation and Mapping

October 1, 2021

Constantin Coussios discussed Passive Acoustic Mapping (PAM) for cavitation imaging. Passive acoustic mapping (PAM) is a promising imaging method that enables real-time two-dimensional or three-dimensional spatio-temporal monitoring of ultrasound therapy through the reconstruction of acoustic emissions passively received on an array of ultrasonic sensors.

Many of the lectures from the previous week were focused on single-element Passive Cavitation Detectors (PCD). While this method can classify bubble activity (inertial and non-inertial), it can only detect bubble activity along the axis of the PCD transducer. This method can also only reliably localise the cavitation event most proximal to the transducer. Prior work investigated the synchronous investigation of cavitation with single-element PCDs synchronously with conventional B-mode imaging and found that substantial cavitation could exist long before it was detected on a B-mode image.<sup>19-21</sup> These findings led to the conclusions that passive cavitation detection was necessary for the detection of clinically relevant cavitation activity, and that the use of multi-element arrays used as passive detectors could enable mapping of cavitation in real time with increased SNR compared to single-element systems.

PAM for cavitation imaging was first developed in 2008.<sup>22,23</sup> PAM localises sources of non-linearity in general, and cavitating bubbles in particular, through the application of coherence metrics applied to passively received signals across several detectors. Unlike B-mode, PAM does not require prior knowledge of event timing nor prior knowledge of the signal or energy that caused the sound source. It does not image the presence of bubbles but is solely sensitive to bubbles that exhibit nonlinear behavior (non-inertial or inertial cavitation) and produce a secondary acoustic emission. Whilst the axial spatial resolution of B-mode depends on pulse length, both the axial and transverse resolution of PAM depend on the point spread function and are thus determined by the ratio of imaging depth to array aperture size and by the wavelength. PAM also presents superior frequency-domain resolution because bubbles are mapped in response to quasi-continuous rather than short-pulse excitation. Most importantly, both B-mode and PAM co-exist within the same hardware, therefore users do not have to choose between them.

The original PAM algorithm, known as Time Exposure Acoustics, was inspired from the field of seismology (details were provided in the presentation) and implemented in the time-domain. Significant improvements in ease and speed of processing were shown shortly thereafter to be achievable by performing the processing in the frequency domain.<sup>33,77</sup> The limitations of these early PAM algorithms include artefacts arising from interference from multiple bubbles, variations in element sensitivity, small receiving aperture and inadequate compensation for spherical spreading with distance, attenuation, variations in speed of sound, and array diffraction.<sup>24</sup> Adaptive beamformers enable the use of different weighting coefficients for signals received on each array elements to minimise the contributions of noise and of any signals outside the region of interest, all without distorting the signals that comes from within the region interest.<sup>24,78</sup> Recently, additional beamforming and processing developments have been made including short pulse methods, Doppler tracking, processing acceleration, and time windowing effects that have yielded further improvements in time and spatial resolution. Taking these 15 years of improvements into account, commercial systems are currently in development that can perform real-time PAM for monitoring treatments, using either CPUs or GPUs to achieve frame rates in excess of 20 Hz. However, much work remains to enable the direct correlation of quantitative PAM-derived cavitation doses to specific desirable or undesirable bioeffects.

### Q & A

- There was a question on the next challenge for PAM. Coussios replied that the next challenge is to map cavitation activity in a consistent way across labs and report results in a manner that is energy preserving and represents real units irrespective of the system used. The next advances will come from computational efficiency as well as transducers that allow reconstruction of images in 3D instead of segmented planes.

- A question on whether PAM can help resolve individual events within cavitation clouds was asked. Coussios responded that we are unable to gain information inside the bubble cloud and can only quantify the energy radiated from the outer boundary of the bubble cloud, because there is no unique solution to the inverse problem.
- A participant asked if dedicated imagers were required for PAM? Coussios responded that any technology that provides access to raw RF data can be used to build PAM images, in combination with either CPUs or GPUs. Over time, it will likely become a low-cost technology with processors that can be 'plug and play.'

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## Localisation, Mapping and Quantification of Bubble Activity in Transcranial and Transvertebral Applications

Meaghan O'Reilly, PhD | Sunnybrook Research Institute

### Cavitation Localisation and Mapping

October 1, 2021

Meaghan O'Reilly presented on the quantification of bubble activity in transcranial and transvertebral applications. There is a need for localisation and mapping to quantify bioeffects. With a single-element receiver, the frequency-dependent attenuation of the skull bone can be used to as a predictor of whether cavitation occurs within or outside the skull.<sup>25</sup> With a limited number of receivers, 3 hydrophones for example, cavitation can be localised within a few millimeters in water.<sup>26</sup> With 4 receivers, cavitation localisation can be detected through the human skull within a few millimeters.<sup>27</sup> With these methods, accuracy deteriorates with increasing pulse length and number of sources. With linear array, passive mapping can visualize acoustic emissions in two dimensions.<sup>7</sup> Large aperture phased arrays that are custom built result in tight focal spots and three-dimensional reconstruction with PAM.<sup>13,28</sup> Phase correction is necessary with PAM through the skull.<sup>8</sup> Ultrafast image capture can better predict the tissue volume distribution during nonthermal ablation than averaging the bubble activity maps over long pulses.<sup>29</sup>

There are several challenges with FUS for spine including proximity of target tissue to bone, high probability of cavitation events in pre-laminar region, and aperture limited by anatomy. Current work aims to address some of these challenges. In terms of unresolved challenges, quantification of skull loss and receiver sensitivity are important questions to address. Identifying a metric that relates to a specific bioeffect is another key challenge. Hardware limitations and beamformer speed remain unmet needs in the field.

### Q & A

- A question was asked on the type of phase correction necessary for intracranial cavitation. O'Reilly responded that a distinct signature is needed and that these should be unique time-domain signals.

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## Active and Passive Cavitation Localisation and Mapping (Including Histotripsy)

Jonathan Sukovich, PhD | Univ. of Michigan

### Cavitation Localisation and Mapping

October 1, 2021

Jonathan Sukovich discussed the fact that localising and mapping cavitation is critical to ensure accurate delivery and to identify the extent of treatment. B-mode imaging works well to image cavitation events with boiling histotripsy.<sup>30</sup> Doppler imaging of bubbles can be used to monitor transient images of bubble clouds.<sup>31</sup> Ultrafast active imaging can also be used to monitor cavitation by synchronizing the imaging acquisition to the timing of the bubble cloud.<sup>32</sup> However, these active methods may require the removal of overlying tissues (e.g., the skull) to ensure that they can “see” the generated bubbles, and can be “blinded” by interference from the histotripsy pulses themselves. Passive methods can capture acoustic emissions from bubbles and rely on detecting harmonics/sub-harmonics of the input acoustic signals for stable bubbles, or broadband noise emissions for inertially cavitating bubbles. With passive techniques, frequency filters can remove the frequency of the driving acoustic histotripsy pulse to isolate the cavitation signals and localize the bubbles.<sup>33</sup>

Intrinsic threshold histotripsy transducer array elements can transmit and receive acoustic signals. The array elements are only narrow band receivers though, which prevents the use of frequency filtering to identify the cavitation signals. However, because histotripsy pulses are such short duration (<5us) the shockwave emissions from the collapses of generated bubbles are temporally well isolated from them. The shockwave emissions can thus be readily identified in acquired signals without requiring significant signal processing or frequency filtering steps. Intrinsic threshold histotripsy can be localised based on these shockwave emissions with an accuracy around 1.5 millimeters.<sup>34</sup> Nevertheless, overlying tissues can still present challenges and cavitation events can be difficult to detect. The sensitivity of the receivers is an important consideration.

### Q & A

- There was a question on the importance of interrogation for segmenting active mapping. Sukovich responded that it depends on the type of histotripsy. For boiling histotripsy, the cavitation event lasts longer. For intrinsic threshold histotripsy, the pulse lasts only a few microseconds.

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**Cavitation Localisation and Mapping**

October 1, 2021

**Monitoring of Kidney Stone and DVT Therapies**

**Julianna Simon, PhD | Pennsylvania State Univ.**

Julianna Simon presented on the monitoring of procedures to break up kidney stones and lyse deep vein blood clots. Kidney stones are influenced by the structures surrounding it as it develops. Kidney stones can be detected by plain film x-ray, computed tomography, or ultrasound. The twinkling artifact improves the sensitivity/specificity of kidney stone detection by ultrasound. Small stable bubbles on kidney stones are a likely source of the twinkling effect.<sup>35</sup> Kidney stones may be treated with shock wave lithotripsy (SWL), which uses cavitation and stresses created by the elastic wave to break stones. PCD has been used for real-time evaluation of stone targeting in SWL. PCD has also been used to monitor treatment progress as energy in 0.5-2 MHz frequency band increases as stones fracture. PCD in conjunction with B-mode imaging can monitor for tissue injury from cavitation in SWL.<sup>36</sup> The combination of active and PAM can be combined to evaluate the spatiotemporal distribution of bubbles in SWL.

Deep vein thrombosis (DVT) can be treated with thrombolytics, filters, catheter interventions, or sonothrombolysis. Sonothrombolysis uses the thermal and/or mechanical effects of ultrasound with or without echo-contrast agents to lyse clots. Clots can be lysed through stable and/or inertial cavitation including the use of circulating microbubbles or nanodroplets. Additionally, thrombolytics enhance clot dissolution. PCD has been used to evaluate lytic rate for stable cavitation of Definity microbubbles with freely circulating rt-PA in vitro.<sup>37</sup> Nanodroplets with tPA can be insonated by an intravascular transducer to lyse retracted clots. The Doppler shift from superharmonics has been used to characterize microbubble translation. Combined photoacoustic and B-mode imaging of microbubble-mediated sonothrombolysis allows for better monitoring. Doppler ultrasound with phase-change nanodroplets can evaluate clot boundaries and monitor dissolution. Currently, integrated therapy-monitoring systems are being designed and evaluated to improve cavitation monitoring in DVT. Remaining challenges include the standardization of PCD/PCI collection and processing and the identification of therapeutic cavitation versus tissue injury.

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### Feedback Control Methods on Cavitation/ Microbubble-based Ultrasound Therapy

Costas Arvanitis, PhD | Georgia Institute of Technology

#### Cavitation Localisation and Mapping

October 1, 2021

Costas Arvanitis discussed feedback control methods to regulate MB activity. Feedback control methods for microbubble-based ultrasound therapy help to estimate focal pressure behind bones, where inertial cavitation must be limited to prevent unwanted damage, account for human error, and a wide range of other factors. The ideal controller should directly control the microbubble radius as a function of time,<sup>38,39</sup> however in in vivo conditions this is not possible. Hence, current implementations are based on indirect estimates of microbubble dynamics based on microbubble acoustic emissions. Although this is a highly sensitive and translational method, challenges in i) attaining reproducible emissions/recordings, ii) factoring in microbubble concentration (dose and vascular density) and properties (shell and size) that might affect the emitted signal, iii) accounting for microbubble kinetics (clearance) that leads to a time-varying signal, and iv) monitoring the presence of off-target microbubble activity that may render the recorded emission level unfit for tuning the exposure, remain.<sup>40</sup>

Arvanitis talked about microbubble-based acoustic controllers and how they may address some the above challenges. These controllers use the spectral content of MB dynamics as a proxy measurement, and then enforce a control law based on an algorithm using the value of the state observer, to change the applied acoustic energy to obtain a desired level of microbubble activity.<sup>40</sup> The goal of open-loop controllers is often to meet an acoustic emissions threshold. Open-loop controllers based on harmonic emissions for calibration offer high sensitivity,<sup>10</sup> while controllers that are based on ultra-harmonic,<sup>11</sup> trade sensitivity for specificity, as these emissions depend only on MB dynamics, albeit their strength is not as high. Recently, an open-loop controller based on sub-harmonic emissions was introduced.<sup>41</sup> This controller has high specificity and may offer more sensitivity, as lower frequencies are attenuated less. The ExAblate system, which employs such control method, the acoustic power is titrated once per target for each patient using a power ramping process.<sup>42, 43</sup> Open-loop controllers based on a combination of emissions and averaged baseline spectra have also been proposed.<sup>44</sup>

Closed-loop controllers that aim to maintain the acoustic emissions at a target level are also in development. A closed-loop controller based on harmonic emissions provides proportional feedback based on harmonic emissions and integral component based on cavitation dose.<sup>12, 45</sup> Also in development is a closed-loop controller that accounts for MB kinetics and adjusts the pressure input according to the third harmonic emission level and a predetermined trajectory based on calibration curves assembled prior to the experiment. Another closed-



loop controller has been designed using PAM based on the angular spectrum method, offering a spatial and temporal control of MB dynamics.<sup>46</sup>

Despite progress, several open questions remain to control acoustic cavitation in clinical situations. For example, the calibration methods needed to attain system-independent quantification of the MB acoustic emissions. The ability to directly control the MB dynamics and whether we can control the forces exerted during stable/inertial cavitation remain unknown.

### Q & A

- A participant asked about controller design for applications outside the brain, particularly when movement is involved. Arvanitis stated that mapping might be useful. The challenge is localising cavitation, and then these techniques can be applied. This issue is not about the controller but localising the cavitation.
- There was a question on whether closed-loop controllers use relative measurements, and the importance of the precision and calibration of the instrument. Arvanitis replied that relative measurements are desired so that any background signal can be removed. This is a challenge that has to be addressed.
- There was a question on the surrogate pulses used to track the microbubble kinetics; given that they last for around 10 milliseconds, were they contributing to bioeffects. Arvanitis responded that these were based on prior measurements of vessel permeability (ktrans) and these pulses resulted in no changes in ktrans with respect to baseline values. This was only a proof-of-concept study and further refinements could be made.

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### Cavitation Localisation and Mapping

October 1, 2021

## Panel Discussion

Following the 5 presentations, Kathy Ferrara (Stanford Univ.) and Joan Vidal-Jove (Institute Khwab for Interventional Oncology) joined the moderators (Michael Gray and Kevin Haworth) and speakers for a lively discussion that centered around the following topics.

### Calibration

Building on the first session of the workshop, there was consensus that calibration of cavitation imaging and mapping approaches is needed. However, it was recognized that the nature of the calibration (such as what is calibrated and whether the calibration provides absolute or relative information) is going to be application specific. Furthermore, for most applications additional data are needed to build consensus around best practices and determining what cavitation metrics are needed to identify the desired bioeffects or be cognizant in avoiding deleterious bioeffects. Finally, the issue of accuracy goals must be addressed, both in terms of emission quantification errors and localisation (and by extension co-registration).

### Consistency

The systems used need to be robustly described in a standardized way and consistently reported so that data coming from different laboratories can be compared to each other. Currently, in several application areas, different research groups are finding different metrics for cavitation-based monitoring and these differences may be arising from different systems having different sensitivities.

### Collaboration

Overall, there is a substantial number of tools, techniques, and algorithms available to the field and although for most applications we are not at a mature enough state to build consensus on which are the best to use, there are an extraordinary number of opportunities for the field to pursue. This pursuit will require collaboration between laboratories to be successful in developing means of mapping bubble activity for providing guidance for safety and efficacy of treatments in a way that will satisfy regulators and clinician-practitioners. The collaboration will require working together to use consistent techniques and also sharing data sets in a way that builds the rigor and reproducibility of the field. Furthermore, as companies move into these spaces to translate the findings to the development of clinical devices, that collaboration should continue with researchers playing an active role in using the data to continue advancing the field. The clinical trials can produce a great deal of productive data for understanding of how the therapies are working and best practices for monitored.

### Clinical Perspective

Clinicians need to be active collaborators in developing the cavitation localisation and mapping techniques to ensure that the real-time methods implemented provide images of sufficient quality to be useful and also that clinicians can read the types of information they need out of the images. We should also examine how to build multimodality platforms that provide the information needed to clinicians, which could be as simple as duplex B-mode and cavitation maps but could be far more complex such as fusing our data into pre- or simultaneously acquired MRI data sets or with other ultrasound-based modalities.

### 3D Considerations

As the hardware and processing throughput evolves to better facilitate 3D imaging, several concerns must be addressed. These include a) understanding how receiver arrangements can be optimized for different monitoring scenarios, b) transfer and handling of the resulting data sets, c) display of processing output to effectively guide treatment, and d) interpretation of cavitation data in the presence of physiologic motion.

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## Cavitation Thresholds and Dose

### *October 8, 2021*

### Welcome from Focused Ultrasound Foundation and American Institute of Ultrasound in Medicine

Frédéric Padilla and J. Brian Fowlkes

Frédéric Padilla welcomed participants and introduced the day's topic, cavitation thresholds and dose. Elisa Konofagou (Columbia Univ.) and Meaghan O'Reilly (Sunnybrook Research Institute) moderated the session. Speakers included Cameron Smith (Univ. of Oxford), Eli Vlaisavljevich (Virginia Polytechnic Institute and State Univ.), Hao-Li Liu (National Taiwan Univ.), Christy Holland (Univ. of Cincinnati), and Adam Maxwell (Univ. of Washington).

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### Cavitation Dosimetry with Passive Acoustic Mapping

Cameron Smith, PhD | Univ. of Oxford

Cameron Smith discussed the opportunities for passive cavitation strategies to provide a quantitative measure of cavitation dose that can be directly related to the safety and efficacy of cavitation-based ultrasound therapies.

Single-element passive cavitation detectors (PCDs) have been previously used to derive a measure of inertial cavitation dose within their sensitive volume. Inertial cavitation dose was previously shown to correlate with endothelial cell damage and blood cell lysis (hemolysis).<sup>79,80</sup> However, PCDs have a limited focal volume at a fixed location and are therefore unable to determine where the cavitation has occurred, or to quantify cavitation activity outside their focal region.

As evidenced by previous talks on Passive Acoustic Mapping, a linear array can be used to map cavitation activity in real time and could enable spatio-temporal correlations of cavitation dose with desirable or undesirable bioeffects. Until recently, PAM had only been used qualitatively, but not to derive quantitative measures of spatially dependent cavitation dose that related to spatially variable bioeffects. Smith presented in vitro experiments which suggest that a PAM-derived spatially varying cavitation dose directly correlates with hemolysis in blood samples exposed simultaneously but in different locations of a complex sound field.<sup>80</sup> This PAM-derived cavitation dose was obtained using calibrated ultrasound arrays

### Cavitation Thresholds and Dose

October 8, 2021

on receive in units of acoustic Joules per unit volume, and was directly predictive of cellular damage irrespective of the cavitation nucleation agent or ultrasound exposure parameters (pulse length, pulse repetition frequency) used.

However, one of the key challenges in achieving better spatiotemporal correlations of PAM-derived cavitation doses with bioeffects is the fact that most PAM algorithms are not energy-preserving, ‘spreading’ the energy of secondary acoustic emissions associated with cavitation over several pixels adjacent to the true source location. In order to address this limitation, Smith proposed a novel algorithm based on Lucy-Richardson deconvolution (LRD) of the PAM cavitation image obtained with the point spread function attributable to the imaging array at that location. Improved cavitation source localization and appropriate confinement of the absolute energy associated with its acoustic emissions was shown to enable significant enhancements in the predictive ability of PAM-derived spatially-varying cavitation doses and their relationship to cellular bioeffects.

In conclusion, PAM can be used quantitatively to monitor cellular safety. If appropriately derived using well-characterized arrays on receive, the relationship between cavitation dose and cellular safety is independent of how that cavitation dose is generated. LRD-PAM and calibration allows for universal estimates of cavitation dose and computationally efficient cavitation monitoring. However, several challenges remain, including the fact that prefocal shielding of the receiver array by proximal cavitation could limit the field of view and accuracy of cavitation dose estimates. Linear arrays are also presently limited in that they are unable to provide out-of-imaging-plane information, and significant improvements could thus be achieved with 2D arrays for 3D characterization of cavitation activity. It was noted that calibrated cavitation reporting is still sparse in the literature, and that laboratories should be encouraged to report calibrated-receiver values in their publications to facilitate comparison of safety and efficacy cavitation dose values across research groups and applications.

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### **Intrinsic Thresholds, Bubble Cloud Dynamics, and Dose for Single Cycle Histotripsy**

**Eli Vlaisavljevich, PhD | Virginia Polytechnic Institute and State Univ.**

#### **Cavitation Thresholds and Dose**

October 8, 2021

Eli Vlaisavljevich talked about cavitation generation and intrinsic threshold histotripsy. Cavitation events were detected using high-speed optical imaging and backscatter PCD. Cavitation probability curves showed consistent cavitation threshold for degassed water and water-based media using single-cycle acoustic pulses.<sup>47</sup> This “intrinsic threshold” significantly decreased with increasing temperature but was independent of tissue phantom stiffness and only slightly dependent on frequency. Histotripsy intrinsic threshold was independent of

changes in transducer f-number. Bubble expansion is reduced for phantoms with increasing Young's modulus and increasing frequency.<sup>48</sup> Theoretical models have led to the proposal that the enhanced expansion of bubbles under different conditions induces larger strain that can enhance tissue ablation. When pressure is increased above the intrinsic threshold, larger clouds are formed without an increase in individual bubble size.<sup>48</sup> Bubble clouds generated with intrinsic threshold histotripsy match the location of the focal region that exceeds the intrinsic threshold. Bubble clouds form in the region of the focus above the intrinsic threshold, resulting in larger clouds at lower frequency. Bubble density is increased at lower f-number and high frequency.<sup>49</sup>

Intrinsic threshold histotripsy, or "microtripsy," can create precise and predictable ablation in red blood cell (RBC) phantoms and ex vivo tissues matching the region of focus about the threshold. There was a slight increase in efficiency when ablation occurred at a lower frequency. Lower f-number transducers also resulted in more efficient and well-defined lesions. Predictable ablation volume closely matches region above intrinsic threshold. Ablation depends on bubble density, bubble expansion, and tissue type.

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## Neuronavigation-guided Focused Ultrasound *A Platform for Accelerating the Treatment of CNS Disease*

Hao-Li Liu, PhD | National Taiwan Univ.

### Cavitation Thresholds and Dose

October 8, 2021

Hao-Li Liu presented on threshold and cavitation-based manipulation for BBBo. To translate BBBo for wider clinical use, a greater understanding of BBBo threshold, monitoring indicators of BBBo, and means to control BBBo stability are needed. BBBo threshold increases with higher frequencies. The mechanical index (MI) (0.46–0.60) can consistently indicate BBBo across species.<sup>50</sup> A higher microbubble volume induces a higher degree of BBBo. Commercial MBs seem to result in similar BBBo effects. Using a neuronavigation device (NaviFus) in human patients, the MI was similar to prior reports for BBBo.<sup>51</sup> Preclinical work using PCD to monitor cavitation showed that sub-harmonics can be used as a metric to control BBBo. BBBo based on PCD-feedback control using sub-harmonics, harmonics, and ultra-harmonics is in development, which can be done without MRI.

In summary, a fixed power-level scheme at a suggested threshold level of MI (0.4–0.8) with commercial microbubbles for BBBo is straightforward and feasible. For a PCD-based power control scheme, sub-harmonics, harmonics, and ultra-harmonics can all serve as effective indicators and sonication can be tailored to the desired BBBo effect. However, different

transducers have independent performance, sensitivity, and bandwidth that make it difficult to define a dose or threshold for BBBo.

### Q & A

- There was a question on inertial cavitation suppression. Liu mentioned that the amount of inertial cavitation matters because it could cause tissue damage. Stable cavitation is usually more important to control, followed by controlling for inertial cavitation.
- Some discussion centered on the optimal MB dose, and this has yet to be defined.

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## Thresholds for Thrombolysis and Drug Delivery

Christy K. Holland, PhD | Univ. of Cincinnati

### Cavitation Thresholds and Dose

October 8, 2021

Christy K. Holland presented on cavitation thresholds for thrombolysis and drug delivery. Nuclei are necessary for the nadir of the cavitation threshold.<sup>52</sup> If nuclei of the optimal size are present in blood, the MI thresholds for inertial or stable cavitation are quite low, 0.13 and 0.9, respectively.<sup>53</sup> At MI values exceeding these thresholds with nuclei present, both types of cavitation are generated and detected. In a porcine thromboembolism model, sonothrombolysis with rt-PA, Definity and intermittent continuous wave 220 kHz ultrasound accelerated reperfusion.<sup>54</sup> In an in vitro flow model of DVT, human whole blood clots exposed to histotripsy alone contained more fibrin compared with clots exposed to histotripsy and rt-PA.<sup>55</sup> The location and amount of stable and inertial cavitation nucleated by Definity or drug-loaded echogenic liposomes infused through the EkoSonic endovascular system was explored in an in vitro flow phantom.<sup>56</sup> Both inertial cavitation and stable cavitation were sustained throughout the infusions. To move beyond cavitation detection to determine bioeffects, partnerships with experts in vascular biology, thrombolysis, and clinical treatment of cardiovascular disease will be necessary.

### Q & A

- A participant asked about the lower thresholds for cavitation with MB relate to cavitation thresholds for histotripsy. Holland replied that the difference is in the nucleation and the presence of exogenous MBs. Most echo-contrast agents are FDA-approved for venous injection and not arterial injection and the effects of arterial administration should be studied.

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### Thresholds for Multicycles-Long-Burst Histotripsy, Shock Scattering Histotripsy, Burst Wave Lithotripsy

Adam Maxwell, PhD | Univ. of Washington

#### Cavitation Thresholds and Dose

October 8, 2021

Adam Maxwell discussed the characteristics of bubbles in multicycle histotripsy treatments. Maxwell reviewed the distinct types of histotripsy based on pulse duration. A gel phantom study demonstrated that exposure with N-waves (waves with greater negative peak pressure vs. positive peak pressure) immediately followed by P-waves (greater positive peak pressure vs. negative peak pressure) could generate clouds of cavitation bubbles.<sup>57</sup> Thresholds for cloud formation by shock scattering are dictated by frequency, shock amplitude, peak negative pressure, transducer geometry, and properties of the medium. Modeling and measurements indicate the nuclei for the cloud cavitation generated during shock scattering have the same threshold as intrinsic nucleation. Increased tissue stiffness minimizes growth of individual bubbles and mitigates shock scattering. At high pulse-repetition frequency (PRF) bubbles do not dissolve between pulses and function as nuclei for subsequent pulses. For boiling histotripsy, shock-induced heating is important to create the effect. Bubble nuclei must be generated in the focus. A combination of temperature and pressure are required to generate the nucleus. Boiling bubbles undergo explosive rectified growth near 100°C and can grow to millimeters in diameter.<sup>58</sup> Growth may be further enhanced by the nonlinear shape of the waveform.

Several interactions contribute to histotripsy, but it has been recognized that bubble expansion creates circumferential tension on nearby cells or tissues and the strain produces mechanical disintegration of cells in bulk tissue.<sup>59</sup> Lesions recorded in RBC phantoms correlate with maximum bubble diameter. However, both circumferential (growth) and radial (collapse) tensile strains can produce cell deformation and damage. For example, cloud collapse was observed to result in the greatest strains in human breast cancer cells in fibrin matrix.<sup>60</sup> During boiling histotripsy, cavitation can also be generated by shock scattering from boiling bubbles, tending to form a tadpole shape with the ‘head’ being formed by cavitation and the ‘tail’ formed by boiling histotripsy. There are many challenges and opportunities that remain. Thresholds have only been studied in vitro and more precise measures in vivo will help translate these models for clinical use. Additional modeling of nonspherical bubbles, bubble translational motion and propagation, and cloud dynamics should be performed. Additionally, identifying the dominant mechanisms for ablation under different exposure conditions could add greater understanding.



### Q & A

- A participant asked whether the cavitation threshold was independent of emission frequency, this information can sometimes be contradictory. Maxwell replied that it seems contradictory between different modes of histotripsy. For intrinsic histotripsy, bubble nuclei are nearly independent of frequency, and are set by the threshold for nanoscopic nuclei at 25–28 MPa in most tissues. Shock scattering or boiling histotripsy are dependent on frequency since frequency affects the heating and size of cavitation bubbles, which contribute to further bubble formation.

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### Panel Discussion

#### Cavitation Thresholds and Dose

October 8, 2021

Following the presentations, Konofagou, O'Reilly, Liu, Holland, and Maxwell were joined by Jon Cannata (Histosonics), Constantin Coussios (Univ. of Oxford), Hong Chen (Washington Univ. St. Louis), Gail ter Haar (Institute of Cancer Research, U.K.), and John Eisenbrey (Thomas Jefferson Univ.), and a lively discussion followed. Although many questions remain unresolved, the group reached consensus on the following topics:

- 1. Thresholds.** Knowledge of thresholds has its uses, although more limited than previously thought. The cavitation threshold is important for treatment planning and the cavitation dose is important for control and feedback. For cavitation-based treatments, both threshold and dose are needed for treatment planning, to ensure that appropriate cavitation levels are achieved. For both cavitation-based and 'hybrid' (heat + cavitation) treatments knowing the cavitation threshold outside the treatment volume is important for ensuring safety and defining the spatial limits of cavitation effects.
- 2. Dose.** The concept of dose is important for understanding and prediction of bioeffects, whether wanted or unwanted. It is also necessary for describing and comparing treatments. A dose unit is needed, and a dose map will be important as well. The challenge is to find a definition of dose that will work across many transducer/system configurations and treatment regimes. The ultrasound world should look closely at how similar approaches in dose monitoring have been employed, standardized, and reported in other fields. Relevant fields include radiation oncology, hyperthermia, and interventional oncology. These examples can be used to guide standardization and dose monitoring, but also as to what level of treatment precision is necessary.
- 3. Application Dependency.** It was agreed that cavitation thresholds and dose, and the units or metrics for reporting these will be highly application specific.

There was agreement that classification by cavitation regime has utility across applications. However, the idea of a unifying parameter for reporting dose was dismissed as not feasible by several participants, with too many considerations specific to each application (tissue type, cavitation regime, burst length). Instead, consensus on dose reporting should be sought within each application.

**4. Calibration.** Echoing the discussion from the first 2 sessions of the workshop, it was agreed that standardization of reporting and of calibration techniques is needed. It has been hard to define unified “cavitation threshold” and “cavitation dose,” even within a given application. One major challenge is that different cavitation detectors and algorithms have been used by separate groups. A calibrated and standardized “hydrophone for cavitation detection” would be one solution that could be used by research groups to benchmark and compare different measurements.

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## Cavitation Agents

### *October 15, 2021*

## Welcome from Focused Ultrasound Foundation and American Institute of Ultrasound in Medicine

**Frédéric Padilla and J. Brian Fowlkes**

Frédéric Padilla welcomed participants and introduced the day's topic, cavitation agents. The session was moderated by J. Brian Fowlkes (Univ. of Michigan) and Tyrone Porter (Univ. of Texas at Austin). The session featured presentations from Kang-Ho Song (Univ. of Colorado at Boulder), Eleanor Stride (Univ. of Oxford), Kullervo Hynynen (Sunnybrook Research Institute), and Christian Coviello (OxSonics Therapeutics).

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## Pro and Cons of Microbubbles as a Cavitation Agent for Therapeutic Ultrasound

**Kang-Ho Song, PhD | Univ. of Colorado at Boulder**

Kang-Ho Song presented on microbubbles as cavitation agents. In this context, microbubbles (MBs) are (lipid-, protein-, polymer-) encapsulated gas spheres typically 1–10  $\mu\text{m}$  in diameter. MB-assisted FUS (MB-FUS) is efficient, tunable, highly specific, noninvasive, transient, non-heating, cost-effective, and versatile. However, a potential barrier to MB-FUS adoption is the considerable permutation of possible MB and ultrasound parameters, as well as their interaction with physiological parameters. In commercially-available MBs, batch-to-batch variations in both microbubble concentrations and size distributions represent a source of variability. Additionally, MB bioavailability is largely limited to the injection cavity, and adding MBs to an existing protocol represents additional regulatory burden. Safety is a critical direction of study for MB-FUS, particularly in the context of neuroinflammation.<sup>61–63</sup> MB size has effects on almost all MB behaviors, from passive dissolution to response to ultrasound. More characterization is needed to discover any potential bioeffects variations with MB size. MB clearance from blood is very quick, less than 10 minutes in a pilot study. Preliminary research with non-lipid shell MBs aims to decrease immunogenicity and inflammatory effects with MBs, but more data is needed. Song concluded that many of the drawbacks of MBs are surmountable.

## Cavitation Agents

October 15, 2021

### Q & A

- A question was asked about the dependence of bioeffects on the size of the MB and whether these factors were also dependent on ultrasound frequency. Song responded that the only frequency used in the described experiments was 1 MHz. However, other frequencies could destroy MBs more quickly.
- A question was asked if monodisperse MBs may be a better alternative, and how to select MB size for a given application. Song replied that there are different approaches. For example, collapsing MB dosing parameters and use ultra-harmonics to look at MB response may simplify protocols. Engineering uniform MB size could remove the need to consider how variations in MB size would affect the tissue.
- A participant asked if Song had any suggestions on how to engage with manufacturers of commercial MBs for diagnostic purposes to encourage the creation of MBs for therapeutic purposes. Song stated that this is an ongoing issue and should be discussed later in the workshop.

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## Pro and Cons of Cavitation Agents (Other than Microbubbles) for Therapeutic Ultrasound

Eleanor Stride, OBE, FREng, PhD | Univ. of Oxford

### Cavitation Agents

October 15, 2021

Eleanor Stride discussed cavitation agents. She outlined a set of criteria for the ideal cavitation agent which included: excellent stability both before and after injection, strong acoustic response enabling them to be detected before and during therapy and a low and predictable activation threshold. Cavitation agents should be convenient and compatible with existing clinical pathways and hardware as well as easy to manufacture. They should also be able to penetrate extravascular tissue and be activated and detectable outside the bloodstream. It should also be possible to functionalize cavitation agents to enable drug-loading and molecular targeting. They should be formulated from biocompatible materials and produce a consistent and reliable response to make them acceptable to regulatory authorities.

Microbubbles meet most of these requirements, but they have a short circulation time in the bloodstream, they are rapidly destroyed by ultrasound exposure, and they do not extravasate. Solid, liquid and gas “nano” particles have therefore been widely investigated as alternative agents as offering better stability and circulation times<sup>64</sup> and continuous cavitation over several minutes.<sup>65</sup> Stride noted, however, that extravasation may not be solely controlled by the size

of a particle; recent studies suggest that inter-endothelial gaps are not as important for the transport of nanoparticles into solid tumors as active transport through endothelial cells.<sup>66</sup> The disadvantages of nanoparticles are that typically require higher ultrasound pressures for activation than microbubbles, they have smaller drug-loading capacity and cannot be easily detected prior to activation. Their activation thresholds are also less predictable due to the stochastic nature of cavitation.<sup>67</sup>

In summary, there is no one-size-fits all approach. Cavitation agents should be selected for their specific purpose. Agents for drug delivery may be quite different from those designed for mechanical ablation. Stride suggested that different particles should be engineered for different FUS applications.

### Q & A

- There was a question on the half-life of nanodroplets and nanocups. Stride responded that this is still under investigation and estimates range from a few hours to a few days and is dependent on the detection technique.
- A participant asked about the combination of several types of cavitation agents. Stride replied that early evidence suggests that microbubbles are unnecessary to activate nanodroplets because nanodroplets vaporize without MBs. However, the presence of MBs may accelerate the vaporization of nanodroplets.
- There was a brief conversation cautioning on the use of the term ‘nano’ for these particles. The particles do not meet the size requirement (less than 100 nm) for nanoparticles, and regulatory bodies do not like the term

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## Microbubbles as Cavitation Agents for BBBo

### Kullervo Hynynen, PhD | Sunnybrook Research Institute

Kullervo Hynynen discussed MBs as cavitation agents for BBBo. The BBBo is a major barrier for therapeutics. FUS BBBo can enhance the delivery of trastuzumab to HER2+ brain metastases in human patients.<sup>68</sup> Hynynen reviewed 20 years of research on MBs for BBBo. Bigger bubble sizes induce greater shear stress. MB-mediated permeability enhancement of the BBB results in the release of proinflammatory mediators.<sup>69</sup> Lipid pluronic nanobubbles were comparable to commercial MBs for producing BBBo.<sup>70</sup> Acoustic cluster therapy (ACT) comprises administration of free flowing clusters of negatively charged microbubbles and positively charged microdroplets.<sup>71</sup> When exposed to ultrasound, the microdroplets are vaporized.

## Cavitation Agents

October 15, 2021

In summary, FUS plus MBs enhances the permeability of the blood-brain barrier opening (BBBo) in clinical treatments. The majority of the work has been done with Definity MBs, but other bubbles can be used. There is research with nanobubbles and large bubbles. The induction of proinflammatory mediators correlates with ktrans. Bubble size has an impact on the enhancement, larger bubbles create a larger enhancement, which is possibly dependent on gas volume. Large, monodispersed bubbles produce greater pre-inflammatory mediators.

### Q & A

- There was a question on whether vessel leakage, fast and slow, was dependent on bubble size. Hynynen responded that this is unknown, and no experiments have been done to determine this. There may be a relationship between bubble size and leakage. The time to healing for fast and slow leakage is unknown.
- A participant asked about the fact that commercial MBs are indicated and approved only for IV administration, and whether it was possible to inject arterially without negative consequences on the brain. Hynynen replied that there are concerns with intra-arterial injection and it is not recommended.
- There was a question on particle recognition of different systems for nanobubbles and could this explain differences observed with bubble response, etc. Hynynen stated that this is a good question, and more research is needed to look at this.
- There was a question on the most important parameters for BBBo safety. Hynynen stated that low frequency is important for safety and for considering skull differences. Recent research suggests that the type of bubble matters and bioeffects depend on the type of bubble administered. Greater understanding of downstream biological effects is needed.

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## SonoTran Particles as Cavitation Agents for Drug Delivery

Christian Coviello, PhD | OxSonic Therapeutics

### Cavitation Agents

October 15, 2021

Christian Coviello discussed the need to improve drug delivery in oncology. Many drugs have poor delivery and penetration into human tumors, and accumulation is often only at the periphery of the tumor. OxSonic has developed a cavitation particle, SonoTran, that creates a microstreaming and micropumping effect when excited with ultrasound. The particles have a unique size, morphology, and cavitation characteristic. These particles are cup-shaped particles, sometimes called nanocups, that are 450 nm in diameter with a 200nm cavity and are monodisperse with a low polydispersity index. These particles have broadband emissions. The SonoTran particles do not have much acoustic contrast with conventional B-mode. These particles can extravasate with active pumping.<sup>72</sup>

OxSonic is in the process of scaling the SonoTran particles for commercial development. The particles have undergone extensive development from scale up, manufacture, preclinical toxicology, safety/validation testing, and regulatory documentation to support clinical use. OxSonic has also developed a proprietary SonoTran System to optimize the use of these particles. The system has a multi-function probe that can use the same aperture for B-mode imaging and detect acoustic emissions in real time (PAM) with a second set of elements that can transmit focused ultrasound. Coviello mentioned that the field should research coupling cavitation dose with bioeffects. The system has been validated in porcine models. In these experiments, cavitation can be sustained for treatment times of up to 1 hour, and no adverse events have been detected. The first-in-human clinical trial commenced early 2022.

### Q & A

- There was a question on using injections (bolus vs. infusion) of MBs to get consistent cavitation over time. Coviello mentioned that they use a slow infusion of particles over time, and the stability of the particles in the infusion bag was good.
- A question was asked on the differences in MB behavior between SonoTran particles and droplets. Coviello responded that the SonoTran particles have a solid core, the nonsymmetric nature of the particle results in differences. The particles result in reliable cavitation and activation.

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### Panel Discussion

#### Cavitation Agents

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The presenters were joined by a panel of experts on exogenous cavitation nuclei to answer questions from attendees and discuss the present state of the field. The panelists included Christy Holland (Univ. of Cincinnati), Paul Dayton (Univ. of North Carolina Chapel Hill), Michael Canney (CarThera), Alexander Klibanov (Univ. of Virginia), and Alfred Yu (Univ. of Waterloo). Moderated by J. Brian Fowlkes and Tyrone Porter, the panel discussion was lively and informative. The panelists discussed design considerations for exogenous cavitation nuclei, exciting applications, and challenges to clinical translation. There were several salient points made by the panelists. Some of the highlights include:

1. Microbubble-based contrast agents originally designed for diagnostic applications are now being readily used for therapeutic applications. Resulting bioeffects from cavitation activity can vary widely depending on location of application as well as acoustic parameters used. Some metrics such as sub-harmonic emissions seem to work well for specific applications but there remains significant need for metrics for monitoring cavitation activity as well as biological response.
2. The panel expressed interest in an index that could relate cavitation activity to bioeffects as well as standardization in how cavitation dose is calculated and communicated. The potential for metrics based on ratios of measured parameters (e.g., harmonics to broadband noise) were discussed in analogy to such metrics as pulsatility index in Doppler ultrasound.
3. Variation in nuclei type such as gas, liquid, solid can lead to different pros and cons for the application of agents. Attributes such as circulation time, extravasation, activation thresholds, and activation duration, etc. all contribute to the utility of the agent in separate ways for different applications.
4. The panel urged deeper study of the biological interactions and engagement of expertise in related fields to expand our understanding of bioeffects of the agents and their use in therapy.
5. Design of therapy-specific agents will be desirable but subject to the hurdles of regulatory approval. This landscape may be affected by recent litigation on how agents may be regulated as a drug or device going forward.

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## Clinical Experiences and Regulatory Considerations

*October 21, 2021*

### Welcome from Focused Ultrasound Foundation and American Institute of Ultrasound in Medicine

**Frédéric Padilla and J. Brian Fowlkes**

Frédéric Padilla welcomed participants and introduced the day's topic, clinical experiences, and regulatory considerations. The session was moderated by Alexandra Golby (Brigham and Women's Hospital) and Keith Wear (US Food and Drug Administration). Speakers were Graeme Woodworth (Univ. of Maryland), Timothy Ziemlewicz (Univ. of Wisconsin), Yuexi Huang (Sunnybrook Research Institute), and Subha Maruvada (US Food and Drug Administration).

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### Clinical Experiences with BBBo

**Graeme Woodworth, MD | Univ. of Maryland**

Graeme Woodworth discussed detecting, mapping, and quantifying bubble activity with therapeutic ultrasound. Over the past few years, new clinical tools have been developed including the ExAblate Neuro, CarThera SonoCloud, and NaviFUS.<sup>73</sup> The biological effects of FUS in thermal modes and mechanical modes has continued to advance as more research is conducted. FUS is under consideration for a variety of treatment opportunities including BBBo, FUS-assisted drug delivery, neuromodulation, thermal ablation, radiosensitization, sonodynamic therapy, and immunomodulation.<sup>74</sup> Real-time monitoring is key to measure the effects of FUS. MB-enhanced FUS has some regulatory challenges, i.e., the FDA considers the specific MB used as a new drug in the setting of therapeutic FUS. An early clinical trial of MB-FUS in patients was conducted in patients with glioblastoma undergoing standard chemotherapy (NCT03551249). MB-FUS was applied early in the 5-day cycle of temozolomide treatment. Preliminary results showed the safety and feasibility of monthly, large volume MB-FUS treatments during these standard chemotherapy cycles. Acoustic emissions were used for real-time monitoring of safety and efficacy (new MRI contrast enhancement) in this trial. Dose response for acoustic energy to achieve desired bioeffects are under investigation. This has led to further research into closed-loop systems based

### Clinical Experiences and Regulatory Considerations

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on imaging and/or acoustic emissions feedback monitoring that are likely to accelerate clinical translation with greater control of energy delivery, prescribed doses, and tuning of desired bioeffects.

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## Clinical Experiences with Histotripsy

Timothy Ziemlewicz, MD | Univ. of Wisconsin

Timothy Ziemlewicz presented on the clinical experience with histotripsy. An early study in patients with benign prostatic hyperplasia using histotripsy decreased symptoms but was not able to achieve debulking.<sup>75</sup> Thermal ablation techniques are standard of care for early stage hepatocellular carcinoma and are included in guidelines for treating hepatic metastases from multiple primaries when patients cannot undergo surgery (National Comprehensive Cancer Network (NCCN)). The current field of thermal ablation has several limitations, including that the treatment effect is not always visible or does not correlate with the area of irreversible damage. There has been variability between ex vivo treatment guides and clinically achieved ablations, leading to unpredictability. Due to these limitations, thermal ablation in the liver has been associated with local recurrence rates of 7% to 40%. Histotripsy has the potential to overcome these limitations due to continuous, real-time visualization of the treatment effect, histologic precision of planned treatments, and lack of thermal damage.

The THERESA study is a phase I, open-label, nonrandomized, first-in-human study to evaluate the safety and short-term efficacy of hepatic histotripsy in patients with primary or metastatic liver cancer to determine the viability for larger trials. An initial treatment with histotripsy in a human patient achieved well-defined ablation and sharply demarcated regions of the treatment zone. One day after treatment there were patent blood vessel running through the treatment zone, consistent with preclinical studies. Another patient presented had metastatic colorectal cancer and real-time video of the treatment was shown, demonstrating the clear visualization of the treatment effect. In this initial human study, histotripsy achieved treatment zones within millimeters of the plan, confirming the precision seen in preclinical models. There was 100% technical success for planned ablations with local tumor control in appropriately targeted tumors. There were no device-related serious adverse events. These results support moving to larger clinical trials and these trials are ongoing trials in the US and Europe.

### Q & A

- There was a question on whether there is a real-time metric for measuring whether ablation was achieved. Ziemlewicz replied that the presence of a bubble cloud

indicates treatment success. The bubble cloud correlates with an area where cell death is occurring.

- A participant asked whether the system could account for respiratory motion. Ziemlewicz responded that the system does not account for respiratory motion, though the continuous visualization of the treatment effect allows monitoring for any significant changes in respiratory excursion. Ongoing research is studying how to predict and account for respiratory motion in real time.

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## Clinical Experiences from the Engineer/ Physicist Viewpoint

Yuexi Huang, PhD | Sunnybrook Research Institute

### Clinical Experiences and Regulatory Considerations

October 21, 2021

Yuexi Huang presented on clinical trials from the physicist perspective. Hundreds of FUS procedures have been conducted at Sunnybrook Health Sciences Centre and Research Institute over the years. Cavitation control in thermal ablations has been developed for safety. Preclinical work for BBBo in a porcine model with a human skull was conducted in preparation for human clinical trials.<sup>76</sup> The ultrasound beam was steered during sonications over a  $3 \times 3$  grid at 3 mm spacing at acoustic power levels of 3 to 20 W with bolus injections of microbubbles at 4  $\mu\text{L}/\text{kg}$ . Results suggested that an emission-based controller was needed for safety. In 2019, Insightec upgraded the ExAblate system to allow for 32 sub-spots with 10ms pulses at 1Hz PRF per subspot and the software could calculate the accumulated dose. Manual control is often used to improve the dose distribution. Echo focusing images microbubbles with a receiver that has 1,024 elements. Echo focusing has the potential to expand the treatment envelop for off-center targets.

### Q & A

- A question was asked on the cavitation metric derived for thermal ablation and the safety threshold. Huang replied that Insightec derived the safety threshold based on preclinical work.

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## Regulatory Considerations for Cavitation Detection in Therapeutic Ultrasound

Subha Maruvada, PhD | US Food and Drug Administration

### Clinical Experiences and Regulatory Considerations

October 21, 2021

Subha Maruvada discussed regulatory considerations for high intensity therapeutic ultrasound medical devices. Unique features of medical devices and the device industry were described. Since devices span a broad array of products, there are 3 different mechanisms to market:

- Class I: general controls (minimal FDA involvement)
- Class II: premarket notification – 510(k) (similar to existing device), de novo (general/special controls provide safety and efficacy but no legally-marketed predicate exists)
- Class III: premarket approval (PMA) application for high risk/new technology devices new intended use or a new type of safety effectiveness question

Cavitation with respect to medical ultrasound devices has several technical considerations. Cavitation microbubble formation is unpredictable, accurate knowledge of location of cavitation event is crucial for safety determination, and formation of pre-focal bubble cloud can block the focus. There is no specific FDA guidance devoted to cavitation detection methods for therapeutic ultrasound. However, cavitation detection is mentioned in 2 standards from the International Electrotechnical Commission (IEC). These are IEC 60601-2-62:2013, Medical electrical equipment - Part 2-62: Particular requirements for the basic safety and essential performance of high intensity therapeutic ultrasound equipment, and IEC TS 63001:2019 Measurement of cavitation noise in ultrasonic baths and ultrasonic reactors.

Cavitation effects are important considerations because of the high pressures and temperatures in surrounding tissues. There are several potential adverse effects associated with cavitation including microbubble expansion and collapse resulting in cellular damage, hemorrhage, or hemolysis. Another concern is that bubble cloud formation can block acoustic energy needed for effective treatment.

Cavitation detection equipment includes PCD, ultrasound imaging, passive cavitation mapping, and active cavitation mapping. Once the cavitation detection method is determined bench testing should be performed, typically using a phantom for cavitation detection. Sometimes in-vivo studies are also required with the choice of animal model and detection method made by the sponsor. Threshold determination is required for the justification of clinical parameters. Engineering mitigations to avoid adverse events (AEs) include details of cavitation detection and monitoring equipment, as well as the controls in place for patient safety and treatment efficacy.

Moving forward there is a great need to standardize cavitation detection methods. This includes a need for uniform approaches to reporting methods, including processing codes, practical methods for calibrating systems, development of gold-standard techniques to enable comparison of different approaches. There is also a need for reporting methods for cavitation detection sensitivity in water or phantoms at baseline. Characterization and calibration of cavitation detection sensitivity is necessary.

### Q & A

- A question on cavitation detection as a measure of safety or efficacy and what the kind of information would the FDA require to approve or clear that aspect of the device? Maruvada replied that review is on a case-by-case basis. Both the device and application along with the proposed method for cavitation monitoring are considered. The field is so broad that it is difficult to provide general advice.
- There was a question on whether calibration of cavitation detection equipment was necessary. Maruvada responded that both points of view have been argued, and further discussion on when calibration is necessary should be conducted.

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### Panel Discussion

Following the presentations, the moderators and speakers were joined by Jon Cannata (HistoSonics), Dinah Parker (OxSonics), Michael Canney (CarThera), and Ryan Jones (Sunnybrook Research Institute). Some highlights include:

1. Cavitation Monitoring During BBB Disruption (InSightec). The Insightec ExAblate has 8 cavitation receivers to detect sub-harmonic and broadband signals. Closed-loop controllers based on acoustic emissions offer feedback to guide and control therapy. Manual control can be used for improving cavitation dose homogeneity.
2. Cavitation Monitoring During Histotripsy (HistoSonics). B-mode ultrasound imaging is currently the most direct and real-time method for monitoring bubbles in abdominal histotripsy. Some experts can hear cavitation. Sometimes deeper lesions are easier to see because artifacts from the abdominal wall (due to reverberations in the standoff device) can interfere with the shallow portion of the B-mode image. Overall, however, there is confidence that treatment is applied to the appropriate location. Degassed water in the standoff device is used to prevent pre-focal cavitation in histotripsy. Oils have also been used to prevent pre-focal cavitation in other applications.

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3. Cavitation Monitoring for Skull-Implantable Transducer (CarThera). There is no real-time cavitation monitoring during sonication with transducer implanted in skull for BBBo. Over 80 patients (several hundred sonications) have been treated. Excellent BBBo has been documented, with a good safety record, as documented with MRI following treatment. Although the implanted transducer has a small aperture, it is targeted to glioblastoma region because the surgeon places transducer in proximity to the desired treatment region after surgical resection of as much of the tumor as possible.
4. MRI Monitoring in Brain. For MRI monitoring, gadolinium contrast agent is not administered at the time of sonication, but afterwards because of safety concerns. Gadolinium enhancement is used to document BBBo efficacy while T2\* is used to monitor for AEs such as petechial or other hemorrhage. Fluid-attenuated inversion recovery (FLAIR) can be used after sonication to monitor for inflammatory processes associated with BBBo. The correlation between gadolinium extravasation and drug delivery is an area that deserves more investigation.
4. FDA Regulation for Cavitation Monitoring. FDA recommends cavitation monitoring (and mitigation strategies for AEs, if applicable) for therapeutic ultrasound devices capable of producing cavitation. However, this is very device-dependent and application specific. Cavitation is monitored during lithotripsy to ensure that there is no pre-focal cavitation that would impede treatment. A standard (e.g., IEC standard) should be developed for cavitation monitoring during therapeutic ultrasound.

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## Summary and Working Session

### *October 29, 2021*

## Welcome from Focused Ultrasound Foundation and American Institute of Ultrasound in Medicine

**Frédéric Padilla and J. Brian Fowlkes**

Frédéric Padilla welcomed participants and introduced the day's topic, summarizing and discussing the prior 5 sessions.

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## Panel Discussion

### Summary and Working Session

October 29, 2021

#### Session 1

### Cavitation Detection Equipment

The panel discussed cavitation detection and equipment, and whether a standardized point source to calibrate single-element and phased array can help to compare results obtained across laboratories and cavitation-based therapies. The group agreed that the field should work towards standardization. One issue is cost, and another issue is how a single point source could be used across varying frequency ranges. Once the signal is digitized there are many ways to process it. Signal processing standardization will also be necessary.

The group also discussed the potential for establishing standardization or best practice for processing, quantification, and reporting of results. Application-specific working groups should be formed for this purpose, particularly with regards to signal processing. It is also important to create separate metrics for single-element detectors and phased arrays.

As part of this process, when a system is calibrated, differences in bioeffects should be analyzed for bias and error in measurements to improve standardization.

#### Session 2

### Cavitation Localisation and Mapping

The panel discussed calibration approaches. One suggestion was to provide recommendations with various levels in the approach. A good calibration source could eliminate difference among monitoring systems and equipment set ups. A first step is to measure the sensitivity of the equipment. One suggested method was to measure the shockwave from MBs.

Standardized scatter is also useful for calibration. It is important to calibrate transducers, but there are several technical challenges. There was a suggestion to find a method of processing the data so that the electrical aspects between the transducer and the system are understood. Processing of acoustic fields is different across laboratories; the field needs a standardized method to compare across laboratories and different systems. There was a suggestion to standardize between single element and phased-array transducers by using the beamforming of the array to approximate a single-element PCD. An additional suggestion was made to start publishing the scripts used to process signals, in addition to the mathematical equations, as a supplemental file. Documentation on the point source characteristics would also be helpful.

### Session 3

## Cavitation Thresholds and Dose

Detector sensitivity is an issue for cavitation detection; cavitation could be present, and the detector is not sufficiently sensitive. A given threshold also needs to be well defined as part of this process. Stable cavitation should be measured in terms of average energy and inertial cavitation should be calculated with reference to occurrences and amplitude, not averages. There is also the question of units as there needs to be some consideration on how to report these measurements. Creating a data bank or repository with details such as bioeffects and emission data that would allow for future data mining was suggested.

### Session 4

## Cavitation Agents

There was agreement that companies that already make imaging agents could be approached to make MBs for therapeutic purposes. There is some controversy on how the FDA categorizes MBs, whether they are a device or a drug. Because of a legal matter, the FDA is re-evaluating all contrast agents as either a device or a drug depending on whether they meet the definition of a drug or a device. There was a comment that if it makes sense from a functional perspective, it is better to use the existing MBs because of regulatory challenges. However, if it does not make sense from a functional perspective, a new agent should be developed along with a plan for a regulatory pathway to be able to use the agent. One major issue with the currently FDA-approved imaging MBs is the issue of polydispersion. Further discussion on the development of new MBs will be vital going forward and the field should not rely only on the currently available agents.

Developing precise monodispersed agents will also help develop better monitoring systems for cavitation. Participants also stressed the importance of reporting the details of experiments, including dose, method of administration (bolus versus infusion), MB size and size distribution, material, and kinetics of the agent. Agents may also need to come in varied sizes to achieve the desired bioeffect, inertial cavitation versus BBBo for example.



### Session 5

## Clinical Experiences and Regulatory Considerations

Commonalities across modalities for any therapeutic device is important to understand from a regulatory perspective. Mapping or imaging for cavitation detection could be a commonality. There was a suggestion on characterizing bubbles in order to adapt the ultrasound field to take advantage of specific kinds of populations of MBs. Additionally, once MBs are liberated, the population has changed, and being able to characterize those MBs would also be useful for future applications.

## Discussion on Potential Partner Organizations

Subha Maruvada provided a brief overview on the IEC Technical Committee (TC) 87 Ultrasound Organization and Standards. The scope is to prepare standards related to the characteristics, methods of measurement, safety, and specifications of fields, equipment, and systems in the domain of ultrasonics. The committee has several working groups that convene to work on standards.

Stephen Russek gave a brief overview of the NIST/NIBIB medical phantom lending library (<https://www.nist.gov/programs-projects/nistnibib-medical-imaging-phantom-lending-library>) and MRI biomarker calibration service. The phantom lending library provides access to common standards for round-robin testing and validation. Phantoms are calibrated and traceable to fundamental standards. The library is expanding from MRI-based phantoms to CT, PET/SPECT, and optical imaging with future consideration of ultrasound. Biomimetic standards are in development that can be used across many imaging platforms. The goal is to support development with accurate standards without biases. FUSF provided a list of potential partnerships and asked for feedback from attendees on the best path forward.

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### Summary and Working Session

October 29, 2021

## Open Discussion

### Standards Development

Participants discussed next steps. One important consideration is how to create standards for FUS when the field is diverse and multidimensional. There was a suggestion to look at hydrophone standards. These cover bandwidth and how to report, IEC 62127-1, -2 and -3. These are a useful source for how to organize the material. IEC 63001 cavitation standards for ultrasonic cleaners could also be useful for terminology. Prioritization to forming a working a group to work on terminology should be started quickly. A suggestion was made to separate calibration in tissue from calibration in water. Bubbles should be characterized with a multimodal approach, i.e., ultrasound along with other modalities (MRI, CT, etc.). There was a suggestion that spectral content may contain signatures that could be indicative of types of bubble activity or might correlate to a particular therapeutic endpoint. A list of IEC documents that mention cavitation will be created and shared with the group and an additional working group should be formed to gather all existing cavitation standards. There was also a suggestion to collaborate with other organizations outside the US that focus on standards.

### Workshop Outputs

Participants discussed potential publications based on the workshop series. The output from the meeting will be a white paper to give a broad overview of each topic, state of knowledge, gaps, and future actions. An emphasis on an additional peer-reviewed review paper was stressed by participants in order to facilitate the future development of standards. Participants also encouraged the foundation to archive the videos for students to review.



To view the videos on the Foundation's [YouTube channel](#).

### Resourcing

Participants suggested that if an informational portal is created, there also needs to be additional support to maintain the repository and keep it up to date with current publications and standards. Another suggestion was made to fund the creation of a prototype for calibration and other associated needs and support 'round-robin' experiments at several laboratories. Larger experiments will need additional funding sources beyond FUSF to facilitate further development. There was a comment that the workshop did not discuss bioeffects and interactions as well as clinical endpoints. Future work will need to determine the downstream consequences of cavitation.

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## Workshop Presenters & Moderators

### **Brigham and Women's Hospital, Harvard Medical School**

Nathan McDannold, PhD  
*Professor of Radiology*

### **Columbia University**

Elisa Konofagou, PhD  
*Professor*

### **Focused Ultrasound Foundation**

Frédéric Padilla, PhD  
*Director of Applied Physics Research*

### **Fondazione IRCCS Istituto Neurologico Carlo Besta**

Francesco Prada, MD  
*Assistant Professor*

### **Georgia Institute of Technology**

Costas Arvanitis, PhD  
*Assistant Professor*

### **HistoSonics Inc.**

Jon Cannata, PhD  
*Director of Research and Advanced  
Development*

### **Insightec**

Itay Rachmilevitch  
*Neuro Applications Manager, Asia  
& Research Projects Manager*

### **Institut Khuab for Interventional Oncology**

Joan Vidal-Jove, MD, PhD  
*Director, Comprehensive Tumor Center*

### **Institute of Cancer Research**

Gail ter Haar, PhD  
*Team Leader*

### **National Taiwan University**

Hao-Li Liu, PhD  
*Professor*

### **OxSonics Therapeutics**

Christian Coviello, PhD  
*Chief Technology Officer*  
Dinah Parker, PhD  
*RAQA Director*

### **Pennsylvania State University**

Julianna Simon, PhD  
*Assistant Professor*

### **Sonic Concepts, Inc.**

Kyle Morrison  
*President*

### **Sunnybrook Health Sciences Centre and Research Institute**

Yuexi Huang, PhD  
*Research Associate*

Kullervo Hynynen, PhD  
*Vice President Research and Innovation*

Ryan M. Jones, PhD  
*Research Associate*

Meaghan O'Reilly, PhD  
*Senior Scientist*

### **Thomas Jefferson University**

John Eisenbrey, PhD  
*Assistant Professor*

### **US Food and Drug Administration**

Subha Maruvada, PhD  
*Acoustics Research Engineer*

Keith A. Wear, PhD  
*Research Physicist*

### **University of Cincinnati**

Kevin J. Haworth, PhD  
*Associate Professor*

Christy K. Holland, PhD  
*Professor*

### **University of Colorado at Boulder**

Kang-Ho Song, PhD  
*Research Associate*

### **University of Maryland School of Medicine**

Graeme Woodworth, MD  
*Professor and Chair, Department of  
Neurosurgery*

### **University of Michigan**

J. Brian Fowlkes, PhD  
*Professor*

Zhen Xu, PhD  
*Professor*

### **University of North Carolina**

Paul Dayton, PhD  
*Professor & Associate Chair*

### **University of Oxford**

Constantin Coussios, FEng  
*Director, Institute of Biomedical Engineering*

Michael Gray, PhD  
*Senior Research Fellow*

Ronald A Roy, PhD  
*Professor and Head of Department*

Cameron Smith  
*DPhil Student*

Eleanor Stride, OBE, FEng, PhD  
*Professor of Bioengineering*

### **University of Texas, Austin**

Tyrone Porter, PhD  
*Professor*

### **University of Virginia**

Alexander L Klibanov, PhD  
*Associate Professor*

### **University of Washington**

Tom Matula, PhD  
*Director, Center for Industrial and  
Medical Ultrasound (CIMU)*

Adam Maxwell, PhD  
*Research Assistant Professor*

### **University of Waterloo**

Alfred Yu, PhD  
*Professor*

### **University of Wisconsin School of Medicine and Public Health**

Timothy Ziemlewicz, MD  
*Associate Professor of Radiology*

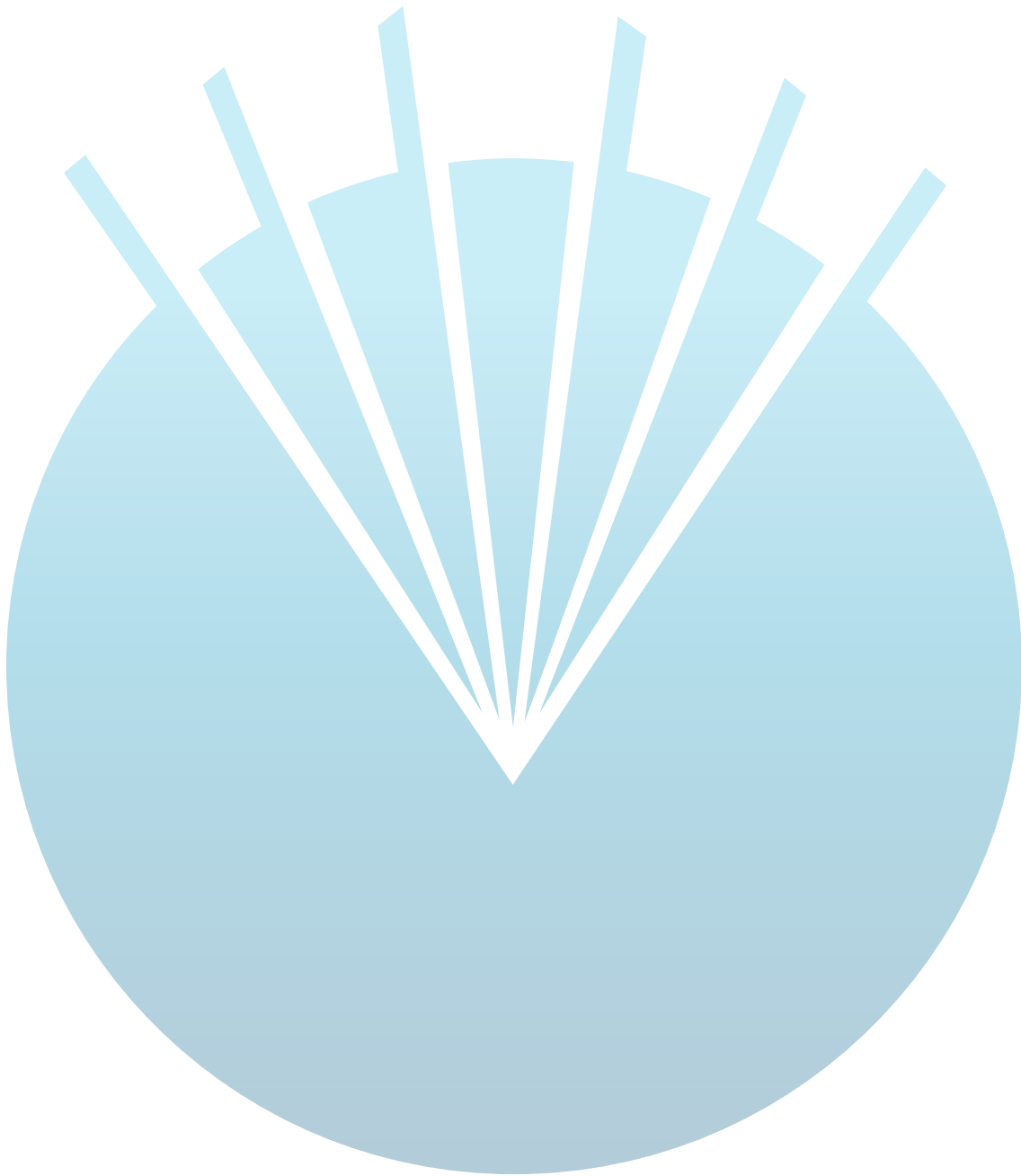
### **Virginia Polytechnic Institute and State University**

Eli Vlasisavljevich, PhD  
*Assistant Professor*

### **Washington University**

Hong Chen, PhD  
*Assistant Professor*







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