Super-resolution ultrasound microvascular imaging: Is it ready for clinical use?

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Abstract

The field of super-resolution ultrasound microvascular imaging has been rapidly growing over the past decade. By leveraging contrast microbubbles as point targets for localization and tracking, super-resolution ultrasound pinpoints the location of microvessels and measures their blood flow velocity. Super-resolution ultrasound is the first in vivo imaging modality that can image micron-scale vessels at a clinically relevant imaging depth without tissue destruction. These unique capabilities of super-resolution ultrasound provide structural (vessel morphology) and functional (vessel blood flow) assessments of tissue microvasculature on a global and local scale, which opens new doors for many enticing preclinical and clinical applications that benefit from microvascular biomarkers. The goal of this short review is to provide an update on recent advancements in super-resolution ultrasound imaging, with a focus on summarizing existing applications and discussing the prospects of translating super-resolution imaging to clinical practice and research. In this review, we also provide brief introductions of how super-resolution ultrasound works, how does it compare with other imaging modalities, and what are the tradeoffs and limitations for an audience who is not familiar with the technology.

Keywords: Super-resolution; Ultrasound; Microvascular imaging; Ultrasound localization microscopy

Introduction

Ultrasound is one of the most popular modalities for vascular imaging. Thanks to a fast imaging speed and high sensitivity to motion, ultrasound uniquely provides real-time imaging of blood flow with rich spatiotemporal information. To date, established techniques such as pulsed wave (PW) Doppler, color flow imaging (CFI), and power Doppler (PD) are ubiquitous on ultrasound scanners and widely used in clinic.

In recent years, a trending direction in ultrasound vascular imaging development is increasing the sensitivity to small vessels and the microvasculature. The key enabling technology behind these developments is ultrafast ultrasound imaging, which uses massive, parallel receive beamforming (e.g., plane wave imaging) to achieve an ultrafast imaging speed of tens of thousands of frames per second [1,2]. Similar to the effect of long exposure photography, an ultrafast imaging speed allows rapid accumulation of blood flow signals within a short period of time, significantly boosting the sensitivity to small vessels that are otherwise invisible via conventional imaging methods [3,4] (e.g., Fig. 1(a) vs. (b)). When combined with advanced tissue clutter filters such those based on singular value decomposition, these ultra-sensitive Doppler techniques become even more effective at extracting small vessel signals from the moving tissues [5,6].

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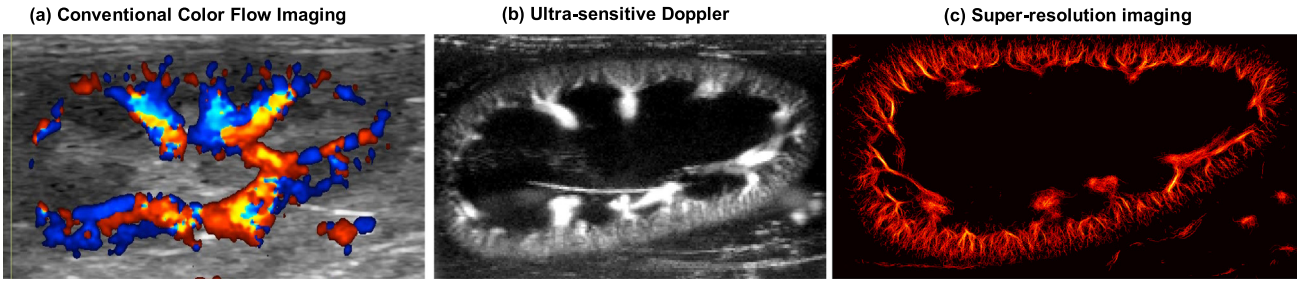

Figure 1. Example ultrasound blood flow images taken from a rabbit kidney. (a) Conventional color Doppler flow imaging is limited to the larger vessels, predominantly in the medullary region. (b) Ultra-sensitive Doppler, enabled by ultra-fast ultrasound imaging, reveals small vessels in the kidney cortex (reprinted from [76]). (c) Super-resolution ultrasound imaging can resolve these cortical microvessels without sacrificing penetration depth (reprinted from [77]).

More recently, this pursuit of higher sensitivity to small vessels has entered a new era – super-resolution ultrasound microvascular imaging (Fig. 1(c). We will use the term "super-resolution ultrasound" or "super-resolution imaging" for the rest of the paper). Different from ultra-sensitive Doppler which is contrast-free, super-resolution ultrasound primarily relies on contrast microbubbles to gain sensitivity to microvessels, which is similar to the principles of contrast enhanced ultrasound (CEUS). In addition to the high sensitivity to small vessels, super-resolution ultrasound is also capable of resolving individual microvessels that are indiscernible with conventional ultrasound such as ultra-sensitive Doppler and CEUS. At present, super-resolution ultrasound remains the only imaging modality that offers micron-scale spatial resolution at a clinically relevant imaging depth (e.g., centimeters). The unique combination of high spatial resolution and deep imaging penetration has opened new doors for many enticing preclinical and clinical applications based on vascular and microvascular biomarkers. Here, we will provide a brief review of the principles of super-resolution ultrasound imaging and state-of-the-art techniques. We will then discuss trade-offs, challenges, and limitations of super-resolution imaging, followed by an overview of existing applications and future directions. For an extensive review of the super-resolution imaging technology, readers are referred to two seminal review papers by Couture *et al*. [7] and Christensen-Jeffries *et al.* [8].

Principles of super-resolution ultrasound microvascular imaging

Inspired by optical super-resolution imaging techniques such as PALM [9,10] (Photoactivation Localization Microscopy), Couture *et al*. first introduced the concept of super-localizing disrupted contrast microbubbles to break the diffraction limit of ultrasound and achieve super-resolution [11]. Around the same time, Siepmann *et al*. used microbubble positions in tumor mouse models to improve microvascular imaging spatial resolution [12]. These early works were rapidly followed by various groups around the world [13–15], and the seminal works by Errico *et al*. [16] and Christensen-Jeffries *et al*. [17] in 2015 served as catalysts for the rapid growth of the super-resolution imaging field [18–23]. The principle behind PALM and super-resolution ultrasound is straightforward (Fig. 2): by localizing the position of a point target (i.e., fluorophore in PALM, microbubble in ultrasound), which is much smaller than the size of the interrogating waves, one can effectively deblur the image and achieve super-resolution. For example, in optics, a fluorophore is a few nanometers in size while an emitted photon wavelength is typically on the scale of hundreds of nanometers; for ultrasound imaging, as illustrated in Fig. 3, a microbubble is a few microns in diameter and the wavelength of clinical ultrasound is several hundreds of microns. Without super-localization, a direct accumulation of microbubble signal (i.e., power Doppler) generates blurred vascular images (Fig. 2(d)). In contrast, by super-localizing and accumulating the location of microbubbles, one can generate super-resolved vascular images with much enhanced spatial resolution (Fig. 2(e)). The branch of super-resolution ultrasound imaging techniques that relies on microbubble localization to break the diffraction limit and achieve super-resolution is also commonly referred to as ultrasound localization microscopy (ULM).

The key condition for super-resolution imaging to work is to have spatially isolated point targets (e.g., Fig. 2(b)). In PALM, this condition is fulfilled by activating and deactivating photoactivatable molecules so that their signals become uncoupled in space. In super-resolution ultrasound, uncoupling is primarily achieved by the movement of microbubbles in the blood stream. For example: if the ultrasound signal from microbubble A overlaps with that from microbubble B in time t, they may become separated in time $t+\Delta t$ if they move relative to one another. Although several studies have reported methods of active manipulation of microbubble signals for super-resolution [13,24,25], the

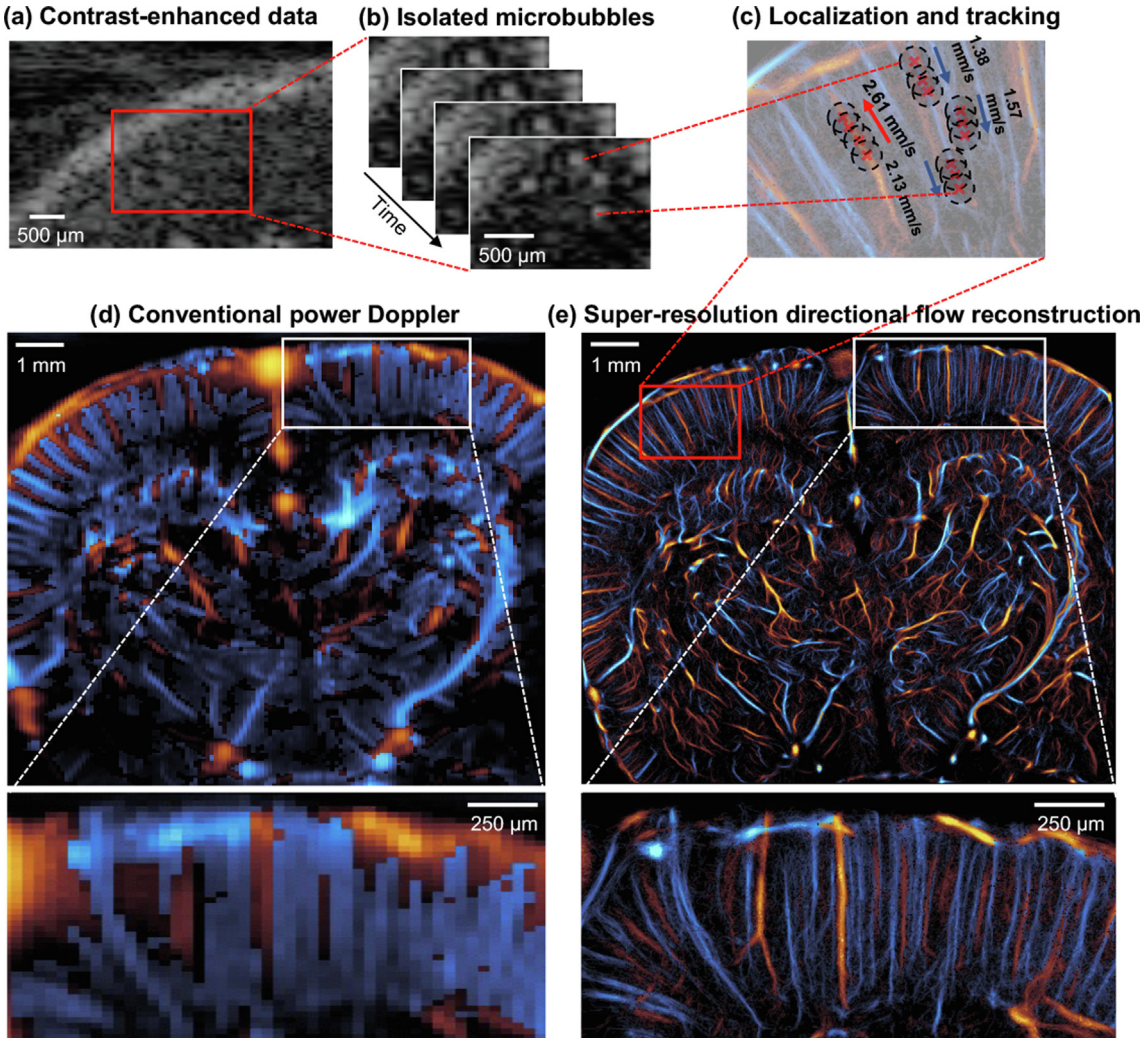

Figure 2. Super-resolution ultrasound imaging workflow. (a) Contrast-enhanced ultrasound imaging data undergoes clutter filtering to reveal (b) isolated microbubble signals. (c) Microbubble localization, frame-to-frame pairing, and tracking is performed to estimate super-resolved blood flow trajectories. (d) A conventional power Doppler processed coronel section of a rat brain is compared to a (e) super-resolved directional flow map of the same dataset.

majority of the existing techniques rely on the passive and stochastic process of blood flow-induced microbubble movement to achieve super-resolution.

Another unique feature of super-resolution ultrasound imaging is blood flow velocity quantification in small vessels. For example, as shown in Fig. 2(c), by tracking microbubble movement in the blood stream, one can infer blood flow speed and direction. This feature is distinct from conventional Doppler ultrasound which uses the Doppler frequency shift induced by the movement of red blood cells to measure blood velocity [26]. As such, super-resolution ultrasound is not subject to common sources of errors in Doppler ultrasound such as Doppler angle and spectral broadening [26]. However, super-resolution ultrasound may be less robust in measuring blood velocity in bigger vessels because of the higher microbubble concentration (i.e., less likely to localize isolated microbubbles) and faster blood flow (because it is difficult to track multiple microbubbles that are fast moving). In practice, as detailed below, blood flow measurements provided by super-resolution ultrasound is the time-averaged velocity during data acquisition. Instantaneous velocity measurements are available during the microbubble tracking process [27] but they may not be as robust as time-averaged estimates [16].

The big picture

How does super-resolution ultrasound compare with existing imaging modalities in terms of imaging resolution versus penetration? Fig. 4 shows the comparison. Compared

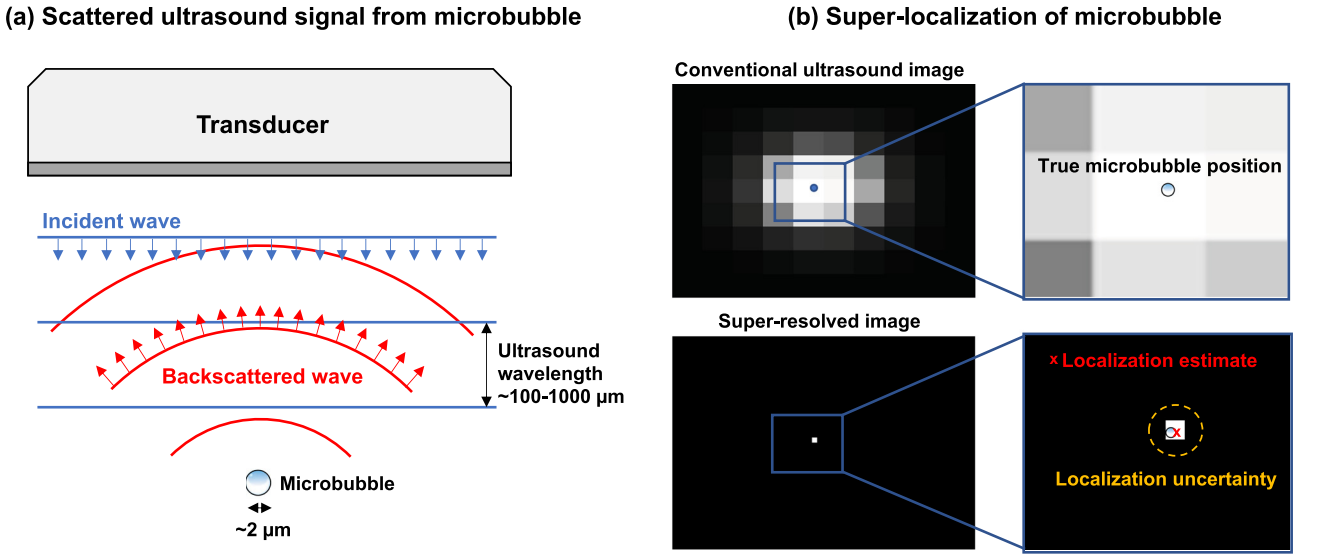

Figure 3. Principles of microbubble super-localization. (a) The scattered ultrasound wave is several orders of magnitude larger that the microbubble diameter. (b) Super-localization strategies provide microbubble localization information that is much smaller than the ultrasound wavelength. The localization accuracy is inherently limited by the Cramér-Rao lower bound of the ultrasound imaging system and beamforming process which include factors such as ultrasound frequency, bandwidth, signal-to-noise-ratio (SNR), and correlation between the true microbubble signal and the estimated template [78].

to optical imaging and photoacoustic microscopy, super-resolution ultrasound offers better imaging penetration. Super-resolution ultrasound has comparable imaging depth as conventional ultrasound because the localization process does not compromise imaging penetration (i.e., it uses the same data as conventional ultrasound imaging). Compared to non-optical imaging methods such as magnetic resonance imaging (MRI), super-resolution ultrasound offers significantly higher spatial resolution. As indicated in Fig. 4, super-resolution ultrasound begins to occupy the penetration-resolution gap of existing biomedical imaging modalities.

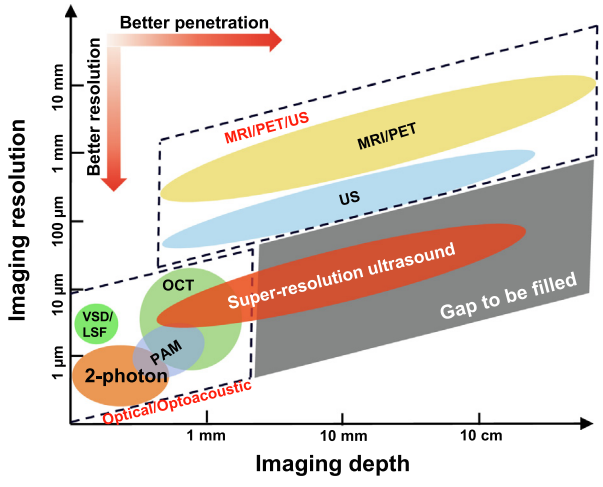

Figure 4. Comparison of imaging resolution and imaging penetration depth for different modalities that are capable of vascular imaging. (2-photon: two-photon microscopy; LSF: laser-speckle flow imaging; MRI: magnetic resonance imaging; OCT: optical coherence tomography; PAM: photoacoustic microscopy (optical resolution); PET: positron emission tomography; US: ultrasound; VSD: voltage sensitive dye imaging).

What are the tradeoffs and limitations?

Invariably, there is a tradeoff. Fig. 5 helps to illustrate: since microbubble localization depends on the presence of spatially separated microbubble signals, the time it takes to generate a complete microvessel image is controlled by how quickly one can localize at least one microbubble at each spatial location where there is a vessel [28–30]. In practice, this process takes a long time because of the limited perfusion rate of microbubbles in the blood stream [31] (especially for smaller vessels where microbubble events can be rare) and the stochastic nature of microbubble uncoupling (e.g., being spatially isolated). In theory, increasing the microbubble concentration, which increases the perfusion rate (Fig. 6(a)), should decrease imaging time; however, more microbubbles decrease the likelihood of uncoupling and makes the localization process more challenging (Fig. 6(b)). As such, using the appropriate microbubble concentration is essential for successful super-resolution imaging. Unfortunately, the appropriate concentration is

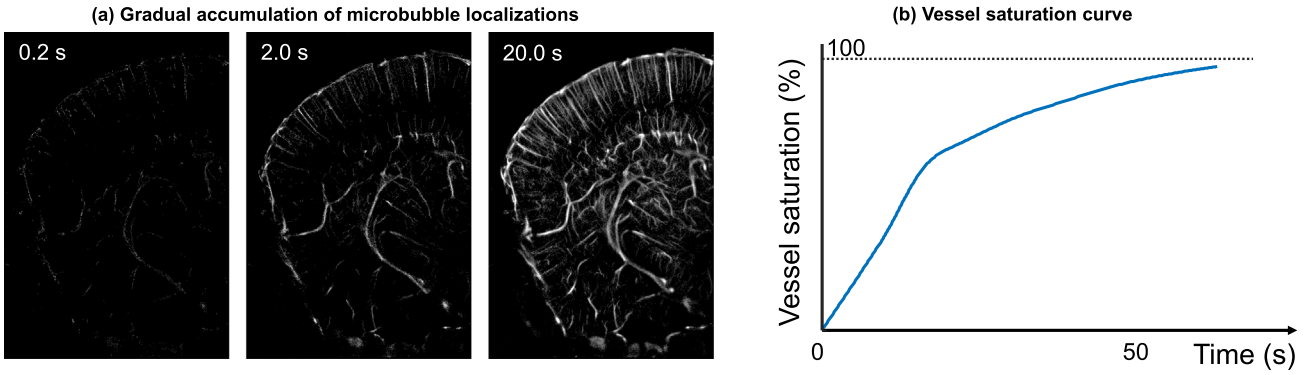

Figure 5. Illustration of super-resolution ultrasound accumulation process limited by microbubble perfusion rate. (a) Gradual accumulation of microbubble localizations in a coronel section of a mouse brain. (b) Example saturation curve for reconstructed cerebrovasculature from images taken in (a).

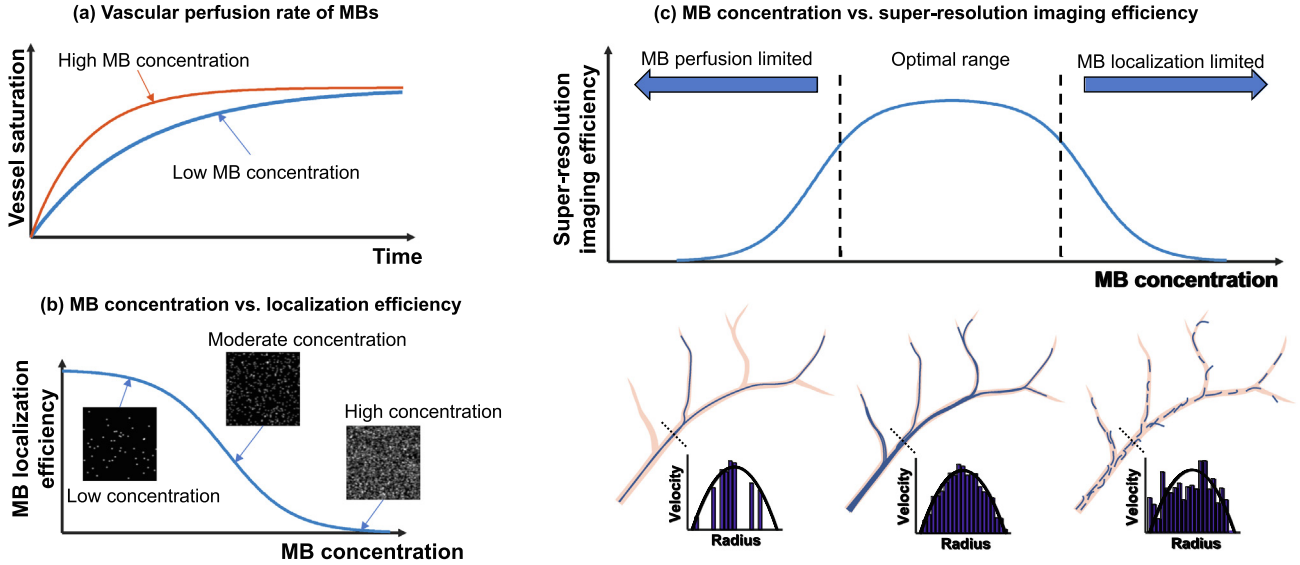

Figure 6. Schematics for illustrating the relationships among microbubble (MB) concentration, vessel saturation rate, microbubble localization efficiency, and microvessel reconstruction fidelity. (a) A higher concentration of microbubbles will perfuse more vessels per unit time, leading to a faster vascular saturation rate. (b) Lower concentrations of microbubbles are more spatially sparse, making detection and localization easier. (c) Super-resolution ultrasound requires a "sweet spot" of microbubble concentration to enable accurate vessel reconstruction within a pragmatic amount of time.

typically lower than the standard clinical dose of microbubble administration. Additionally, infusion of microbubbles is generally preferred over bolus injections for a stable microbubble concentration. Collectively, these factors contribute to a long data acquisition time for super-resolution imaging that ranges from several seconds to several minutes depending on the tissue or organ and the desired reconstruction fidelity.

Recognizing slow imaging speed as the key limitation of super-resolution ultrasound, the research community has proposed and developed many methods and techniques to address this challenge. These solutions span basic filtering techniques [32], advanced sparsity-based algorithms [22,33] and recently, deep learning [22,34–38]. Some of the solutions push the temporal resolution of super-resolution ultrasound to one second and even sub-seconds [22,38,39]. However, most of the existing fast super-resolution imaging techniques trade spatial resolution and completeness of the vessel structure for imaging speed. Also, some techniques do not offer the capability of blood flow velocity measurement provided by conventional super-resolution imaging (Fig. 2). Nevertheless, super-resolution

imaging acceleration remains an active area of research and solutions for fast and robust imaging are anticipated in the near future.

Another limitation of super-resolution ultrasound imaging is the need for contrast injection, which diminishes the attraction of this otherwise excellent methodology. Areas already accustomed to contrast use may show faster transition to super-resolution imaging than those currently relying on intrinsic mechanisms such as scattering from moving red blood cells. Unfortunately, since the majority of clinical vascular imaging applications are non-contrast-based, the rate of adoption for super-resolution ultrasound may be limited. Compelling applications of super-resolution ultrasound with clear clinical evidence of effectiveness are necessary to facilitate the translational process. In the meantime, contrast-free techniques based on backscattering of native blood cells are also being actively developed [40–42]. Because of the absence of spatially isolated point targets such as microbubbles, contrast-free techniques do not provide spatial resolution as high as conventional super-resolution ultrasound. However, they may become more attractive alternatives to conventional super-resolution imaging for pragmatic reasons.

What are the existing applications?

To date, most super-resolution ultrasound applications are concentrated on preclinical animal models and pilot clinical studies. Table 1 summarizes representative existing applications of super-resolution ultrasound, which involve clinical areas including neurology, oncology, nephrology, and cardiology. Figs. 7-10 presents representative examples of *in vivo* human super-resolution ultrasound imaging.

For applications in the brain, super-resolution ultrasound found a niche in small animal disease models because it allows the probing of deep brain regions with high spatial resolution, which complements mainstream brain imaging modalities including optics and MRI. Several studies have also demonstrated intact skull imaging with super-resolution ultrasound (in animals [43] and humans [44]). For applications in stroke [45,46], the high spatial resolution and quantitative hemodynamic measurements of microvessels (e.g., diffusion index, flow speed) allowed detection of deep seated aneurysm and separation between ischemic and hemorrhagic stroke. For applications in aging and Alzheimer's disease [47,48], super-resolution ultrasound indicated global reduction of vascularity and microvascular flow speed in mouse models. Super-resolution ultrasound also revealed early functional impairment of the cerebral microvasculature (e.g., reduced blood velocity) that preceded structural variations (e.g., reduced vascularity) in Alzheimer's disease.

Cancer is the next major application of super-resolution ultrasound where noninvasive imaging of tumor microvasculature is significant for early detection, diagnosis, and prognosis. The ability of resolving detailed tumor microvasculature and providing quantitative metrics such as microvessel density, tortuosity, and flow velocity is essential for identifying microvessel phenotypes and characterizing the tumor vascular microenvironment, which is highly correlated with tumor invasiveness and metastatic potential [21,49,50]. Some of the assessments such as small vessel tortuosity and flow velocity are not readily available from other imaging modalities and unique to super-resolution ultrasound. These measurements reflect the structural and functional abnormalities of the tumor microvascular environment, which are useful for applications such as evaluating and predicting antiangiogenic therapy response [51,52]. Nevertheless, the usefulness of super-resolution ultrasound for cancer and the advantages of super-resolution imaging over CEUS still need to be demonstrated by following existing CEUS cancer studies [51,53–55]. In addition, as suggested by clinical evidence [56,57], tumor perfusion measurements are sensitive biomarkers for evaluating cancer treatment response. In theory, super-resolution ultrasound is capable of evaluating tumor perfusion when combining the blood velocity measurements with vessel geometry. The emerging 3D super-resolution imaging techniques greatly facilitate the development of this new capability in the future [43,45,58].

Is super-resolution ultrasound ready for the clinic?

Although super-resolution ultrasound is likely ready for research investigations in clinic, a pressing issue for its application for large-scale clinical use is the lack of demonstrated clinical value of super-resolution imaging over conventional contrast-enhanced ultrasound (CEUS). This raises further questions: can super-resolution imaging provide better quantification (than CEUS) which translates to more accurate diagnoses? Is super-resolution imaging less susceptible to the sources of variabilities in CEUS [59]? Is super-resolution imaging repeatable? Since CEUS is a much simpler and faster technique than super-resolution imaging, the added benefit must be significant for super-resolution to gain traction in the clinic.

To answer these questions, one needs to have established super-resolution imaging solutions that are readily available in a clinical imaging setting. Unfortunately, super-resolution imaging largely remains as a research tool at present due to the aforementioned imaging speed limitation (and associated issues including expensive computational cost, tissue motion due to handheld scanning under elongated data acquisition

Table 1
Summary of existing *in vivo* preclinical and clinical studies using super-resolution ultrasound imaging

Organ	Condition	Imaging setting and model	Publication	Likelihood for transition to clinical research and practice
Brain	Stroke	Rat	Chavignon *et al.*, 2022 [45]	Medium (non-neonates): • Transcranial imaging through intact skull is challenging in adults. • Possible through temporal window but limited FOV. • Intraoperative application is possible. High (neonates): • Ultrasound provides a viable noninvasive imaging solution on newborns before fontanelle closure.
Brain	Stroke	Human	Demené *et al.*, 2021 [44]	
Brain	Alzheimer's disease	Mouse	Lowerison *et al.*, 2022 [47]	
Brain	Aging	Mouse	Lowerison *et al.*, 2022 [48]	
Brain	Hydrocephalus	Pig	Zhang *et al.*, 2022 [79]	
Brain	Normal animal for functional neural activity imaging; normal animal for dynamic pulsatility measurement	Rat	Renaudin *et al.*, 2022 [39]; Bourquin *et al.*, 2022 [67]; McCall *et al.*, [80]	
Spinal cord	Normal	Rat	Claron *et al.*, 2021 [81]; van Sloun *et al.*, 2021 [34]	Low-medium: • Noninvasive imaging challenged by limited acoustic window around vertebrate. • Intraoperative application is possible.
Breast	Cancer	Human	Opacic *et al.*, 2018 [21]; Huang *et al.*, 2021 [62]	High: • Ultrasound is routinely used in clinic for managing breast cancer. • Shallow and stationary tissue provides an ideal imaging condition.
Lymph Node	Normal	Rabbit	Zhu *et al.*, 2019 [82]	High: • Ultrasound is routinely used in clinic for managing suspicious lymph nodes. • Shallow and stationary tissue provides an ideal imaging condition.
Lymph Node	Metastatic cancer	Human	Zhu *et al.*, 2022 [61]	
Tumor xenografts	Colorectal cancer; renal cell carcinoma; fibrosarcoma; lung carcinoma; epidermoid carcinoma; myxoid liposarcomas	Chicken embryo chorioallantoic membrane; rat; mouse	Lowerison *et al.*, 2022 [50]; Lowerison *et al.*, 2020 [49]; Lin *et al.*, 2017 [18]; Opacic *et al.*, 2018 [21]	N/A
Pancreas	Cancer	Human	Huang *et al.*, 2021 [62]	Medium: • Poor visibility of pancreas under ultrasound is the main challenge.
Liver	Cancer	Rabbit	Zhang *et al.*, 2021 [64]	Medium – High: • Ultrasound is routinely used in clinic for liver imaging. • Respiratory and cardiac motion can be significant. • Deep liver region may be challenging to image because of ultrasound attenuation.
Liver	Normal and acute-on-chronic liver failure	Human	Huang *et al.*, 2021 [62]	
Kidney	Ischemia	Rat	Andersen *et al.*, 2020 [23]	Medium – High: • Ultrasound is routinely used in clinic for kidney imaging. • Respiratory motion can be significant. • Depth of penetration can be an issue for kidney ultrasound.
Kidney	Acute injury	Mouse	Chen *et al.*, 2020 [83]	
Kidney	Normal	Human	Huang *et al.*, 2021 [62]	

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Kidney	Transplant allograft	Human	Bodard *et al*., 2023 [73]	High: • Shallow and accessible tissue with little motion • Contrast microbubbles are not associated with nephrotoxicity.
Prostate	Unknown	Human	Solomon *et al*., 2019 [60]	High: • Transrectal ultrasound is commonly used in clinic for prostate imaging. • Shallow and stationary prostate tissue from transrectal imaging provides ideal imaging condition.
Heart	Normal	Isolated rat heart; *in vivo* rat heart (transthoracic imaging)	Demeulenaere *et al*., 2022 [65]; Cormier *et al*., 2021 [66]	Low-Medium: • Cardiac motion is significant. • Limited data acquisition window (e.g., during diastole) • Depth of penetration and suboptimal imaging quality can be issues for echocardiography
Lower limb muscle	Normal	Human	Harput *et al*., 2018 [84]	High: • MSK ultrasound is routinely used in clinic. • Low level of tissue motion in lower limbs
Vasa vasorum	Atherosclerotic plaques; Takayasu arteritis	Rabbit and human	Chen *et al*., 2020 [85]; Goudot *et al*., 2023 [86]	Medium - High: • Ultrasound is routinely used in clinic for imaging atherosclerotic plaques. • Tissue motion can be significant.
Eye	Elevated intraocular pressure	Rabbit	Qian *et al*., 2022 [87]	Medium: • Low level of tissue motion. • Microbubbles are not commonly used in eye ultrasound.

time, etc.), which impedes the implementation of the technique on existing clinical scanners. However, studies have shown that super-resolution can be retrospectively performed using conventional CEUS data acquired from existing clinical scanners [60,61]. Although the super-resolution imaging quality may be suboptimal because of the low imaging frame rate (e.g., 10-50 Hz) that is typical available on clinical imaging systems (low frame rate makes it more difficult to track and link the microbubbles to form microvessels), increasing numbers of newer generation ultrasound scanners have started to support high frame-rate imaging that is ideal for super-resolution [44,62].

For clinical research investigations using super-resolution ultrasound, the widely available research ultrasound scanners (e.g., Verasonics) and open-source super-resolution data and processing codes (e.g., PALA [27] and ULTRA-SR [63]) provide a robust platform for the dissemination of the technology. Research investigations can follow existing clinical applications of CEUS to identify the unmet clinical needs and test whether super-resolution imaging can fill the penetration-resolution gap.

Finally, Table 2 summarizes the roadblocks for clinical translation of super-resolution ultrasound imaging.

What about basic research on animal models?

Preclinical applications of super-resolution ultrasound are associated with fewer pragmatic challenges than clinical imaging. For example, long data acquisition time is not a significant issue on animals because ultrasound probes can be mechanically fixed instead of handheld. Respiratory and cardiac motion can be mitigated by anesthesia, mechanical ventilation, and prospective or retrospective gating [64–67]. Microbubble infusion is also convenient to implement for stable microbubble concentration in the blood stream. By providing on demand, noninvasive imaging of tissue microvasculature, super-resolution ultrasound offers an attractive alternative to histology in longitudinal study designs because repeated imaging on same animals can be conveniently achieved.

In neuroscience based on animal models, super-resolution ultrasound has rapidly gained traction because it allows

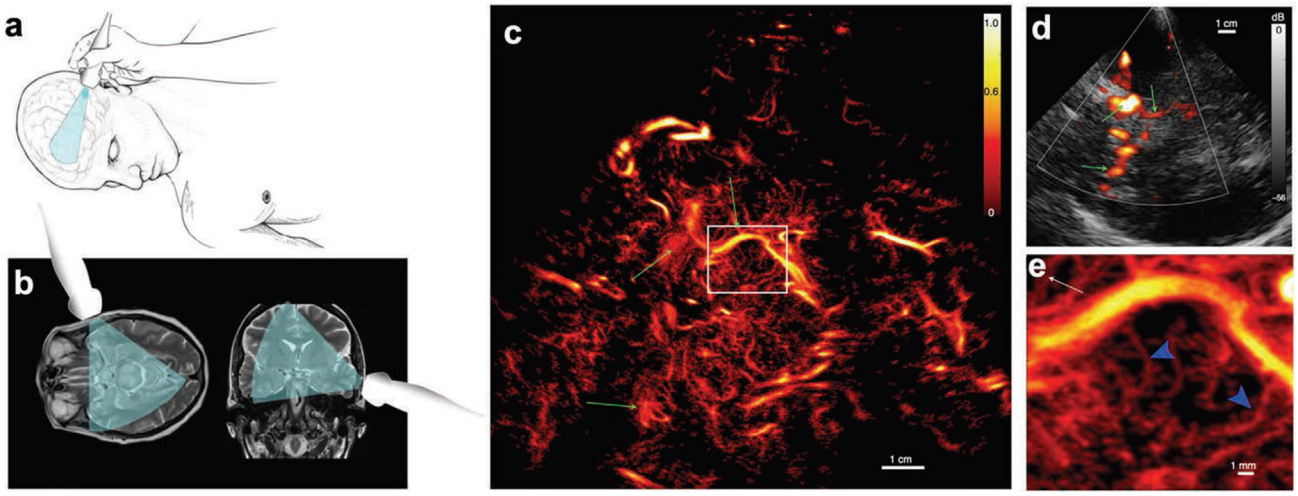

Figure 7. *In vivo* human brain super-resolution imaging. (a) Schematic diagram indicating the handheld ultrasound imaging setup; (b) diagram indicating the ultrasound imaging field-of-view with respect to brain MRI images; (c) reconstructed super-resolution microvessel density map. The white box indicates the location of the magnified view in (e); (d) conventional power Doppler image of the same brain region as in (c). The green arrows serve as landmarks for comparison between (c) and (d). (Images adapted from Demené *et al*., 2021 [44]).

whole-brain, micron-scale mapping of the cerebral vasculature [16,43], which is unmatched by existing imaging modalities. This feature allows *in vivo* noninvasive imaging of deep brain regions with a spatial resolution that has never been achieved before, which is significant for many neurological disease applications including aging, Alzheimer's disease, and ischemic and/or hemorrhagic stroke [45,47,48]. When combined with functional ultrasound [4,39,68] (fUS), super-resolution imaging provides an exciting new tool that allows neuroscientists to explore beyond the brain cortex and integrate the functional brain organizations across various brain regions at different scales (Fig. 11).

Whats next?

Because of the large discrepancies in imaging settings and safety regulations between preclinical and clinical imaging, the path of development of super-resolution ultrasound can be split into preclinical and clinical routes. Preclinical and clinical applications of super-resolution imaging present different technical challenges as well as different opportunities that lead to different possibilities. For example, as discussed above, for preclinical animal studies, longer data acquisition time may not be as significant of an issue as in clinical imaging. Therefore, reducing data acquisition time is not as critical in animals than in humans. However, improving the temporal resolution of super-resolution ultrasound is a common goal between preclinical and clinical settings. As another example, in animal studies, one can use non-FDA-approved contrast agents such as long-persistence microbubbles and nanodroplets to enhance the imaging performance of super-resolution ultrasound [24,69]. The use of nanodroplets also provides opportunities to probe extravascular space by leveraging extravasation [70]. For clinical applications, the task of developing robust super-resolution imaging solutions that provide real/quasi-real-time imaging takes priority. If longer data acquisition is needed, the duration should not exceed 10-15 seconds (i.e., within a single breath hold). Tissue motion correction [71] is another priority for *in vivo* human imaging to account for handheld probe movement and other physiological motion. In addition, super-resolution ultrasound will also benefit from the development of newer and faster 3D ultrasound imaging techniques because 3D imaging is indispensable for localization and tracking microbubbles that travel in-and-out of the imaging plane, which results in biased flow velocity estimation using 2D imaging [43,45,58,72].

For future clinical applications, one area that is potentially ripe for exploration with super-resolution ultrasound is to evaluate organ transplant rejections (e.g., kidney, pancreas, and liver). A new proof-of-concept study on human kidney allografts has been reported by Bodard *et al* [73]. Vascular rejection, manifesting as an arteritis which ranges of mild intimal inflammation and intimal thickening to necrosis of the intima with deposition of platelets, fibrin, and inflammatory cells requires biopsy for diagnosis [74]. The high spatial resolution of super-resolution ultrasound provides a powerful tool for detecting dysfunctional and morphologically damaged vessels *in vivo* without a biopsy. Such evaluations could be performed over time monitoring changes in vessel morphology with treatment. This would

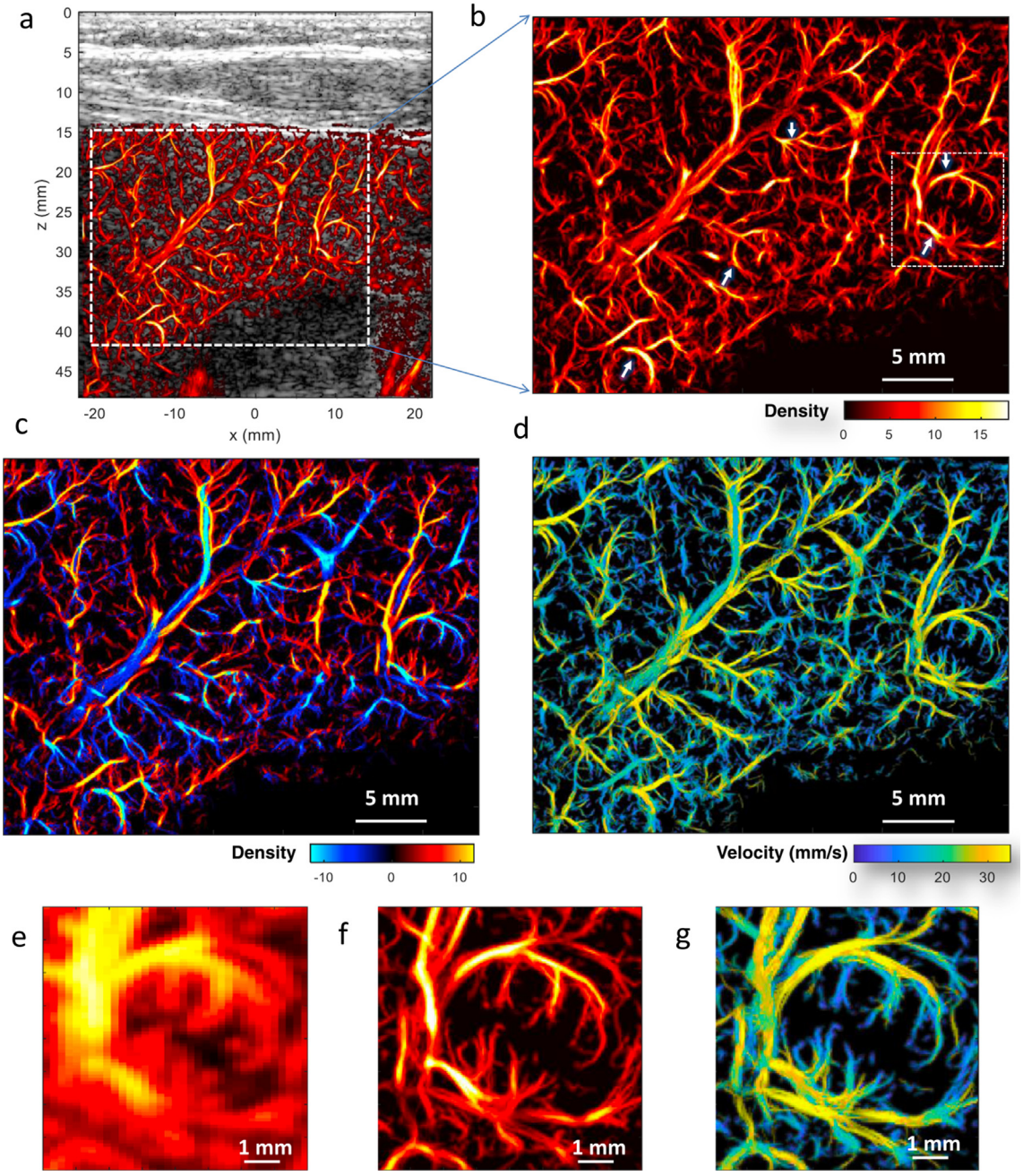

Figure 8. *In vivo* human liver super-resolution imaging from a 38 year old patient with acute-on-chronic liver failure. (a) Super-resolved liver microvascular image overlaid on the B-mode image; (b) magnified view of the region indicated by the white dashed box in (a); (c) directional microvessel density map (red color indicating bottom-top flow direction and blue color indicating top-bottom flow direction); (d) super-resolved flow velocity map; (e) – (g) power Doppler, super-resolved vessel density, and flow velocity map of a local region marked by the white dashed box in (b). The white arrows denote distorted vessels with tortuosity and/or tapering in the main branches. (Images reprinted from Huang *et al*., 2021 [62]).

be a major advance in vascular rejection diagnosis and assessment. Further, transplant organs such as kidney and pancreas also provide a relatively benign environment for super-resolution ultrasound imaging: they do not present significant tissue motion and are easy to access, which is ideal for the prolonged data acquisition of super-resolution. Con-

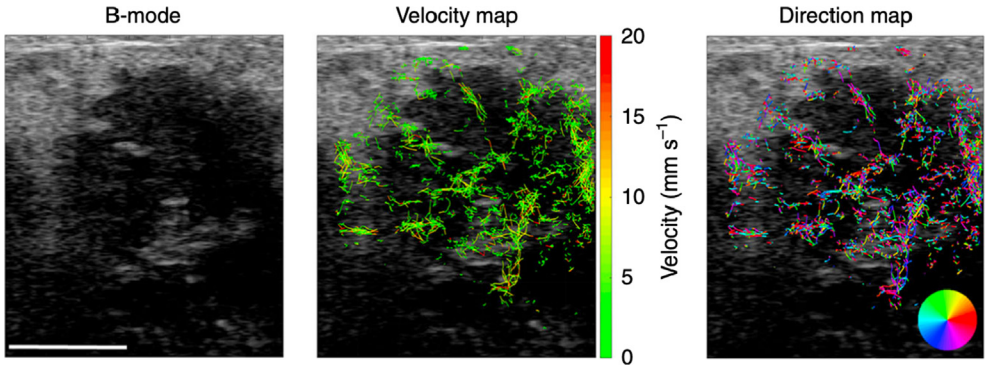

Figure 9. Super-resolution ultrasound imaging of a patient with HER2 positive breast cancer. The vascular map reveals heterogeneously distributed microvessels throughout the tumor. (Images adapted from Opacic *et al*., 2018 [21]).

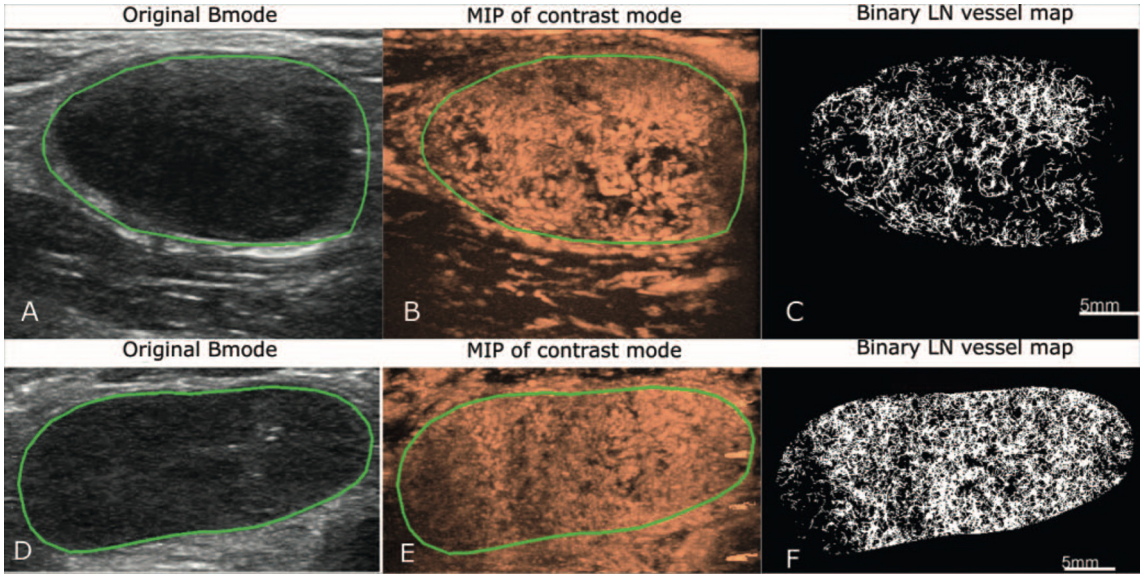

Figure 10. *In vivo* human lymph node super-resolution imaging. (A-C) images from a reactive lymph node; (D-F) images from a metastatic lymph node. The first column presents the B-mode images, the second column presents the maximum intensity projection (MIP) images of the microbubble data, and the third column presents the reconstructed super-resolution microvessel density maps. (Images reprinted from Zhu *et al*., 2002 [61]).

Table 2
Summary of roadblocks for clinical translation of super-resolution ultrasound imaging

	Name of the challenge	**Solutions**
Critical roadblocks for clinical translation	Slow imaging speed/long data acquisition	Technical development for fast super-resolution imaging
	Tissue motion (especially for out-of-plane motion)	• Improve imaging speed to mitigate tissue motion • Use of 3D imaging to track 3D tissue motion • Develop new motion correction techniques
	Use of contrast microbubbles	Contrast-free solutions that provide enhanced spatial resolution (not as high as conventional super-resolution ultrasound that is localization-based)
"Soft" roadblocks for clinical translation	Slow imaging frame rate on conventional ultrasound scanners	• Designated high frame-rate imaging mode for super-resolution data acquisition; • Algorithm development for super-resolution imaging with low frame rate, conventional CEUS data
	Computational cost associated with beamforming and post-processing	Algorithm acceleration, optimization, and parallelization (e.g., GPU-based)

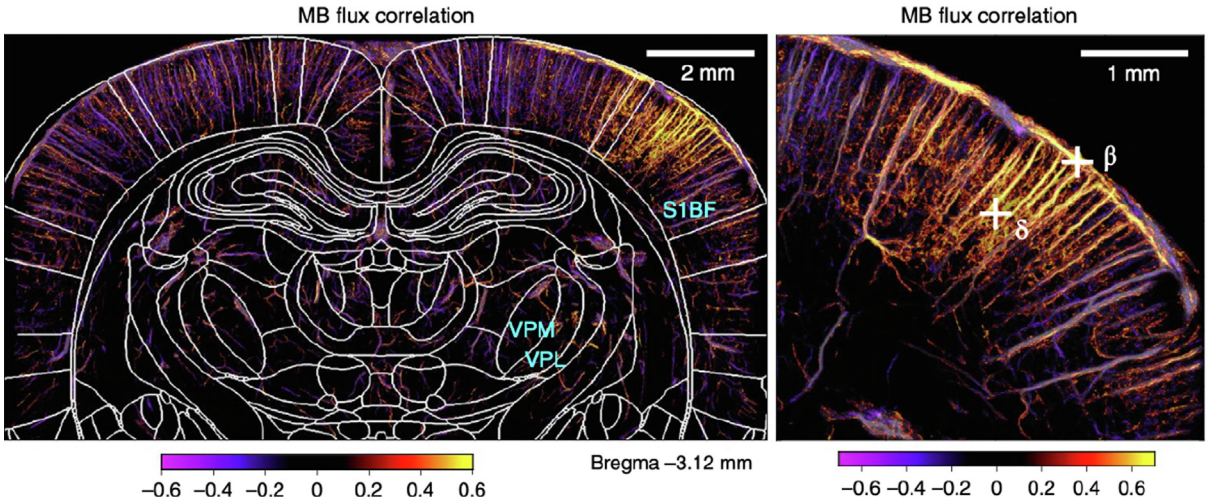

Figure 11. Functional super-resolution imaging of the rat brain undergoing whisker stimulation. The microbubble (MB) flux correlation map demonstrates elevated microvascular blood flow in the S1 barrel field (S1BF) and ventro-posterio-median (VPM) thalamic nucleus for whisker stimulation. (Images adapted from Renaudin *et al.*, 2022 [39]).

trast microbubbles also have no organ toxicity, which is ideal for applications in transplanted organs.

Another similar target would be direct evaluation of microvessel damage as a means of diagnosis and assessing graft vs host disease (GVHD) after bone marrow transplantation. Without a tissue biopsy, anti-endothelial GVHD can be very hard to diagnose [75]. A method such as super-resolution that can actually visualize microvessel morphology in a benign way could have great utility in the management this condition. Bowel motion could be problematic, but the unique strengths of the method to visualize microvascular anatomy would make the method worth trying.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [P.S. and M.R.L. have patents in the field of super-resolution ultrasound imaging, some of which have been licensed.].

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