

Research Progress and Clinical Translational Application of Ultrasound Localization Microscopy in Microcirculation Imaging

Xuedi Han^a, Ligang Cui^{a,*}

^a Department of Ultrasound, Peking University Third Hospital, Beijing, China

Received September 16, 2026; revision requested September 30, 2026; accepted November 24, 2026

Abstract: As an emerging super-resolution ultrasound imaging technology, ultrasound localization microscopy (ULM) breaks the acoustic diffraction limit of conventional ultrasound. By localizing and tracking individual microbubble contrast agents at the sub-wavelength scale, ULM achieves high-precision visualization and quantitative evaluation of micron-level microvascular structures and hemodynamic parameters. Compared with traditional color Doppler flow imaging and contrast-enhanced ultrasound, ULM possesses superior spatial resolution and microcirculation quantification capability, enabling in-depth analysis of microvascular morphological remodeling and functional perfusion abnormalities. This review summarizes the core technical principles, optimized algorithms, and key technical bottlenecks of ULM, including microbubble signal separation, single-bubble localization accuracy, physiological motion artifacts, and prolonged acquisition duration. Furthermore, we comprehensively elaborate the preclinical and clinical application progress of ULM in multiple systems, covering neurovascular, renal, tumor, cardiovascular, endocrine and cardiac microcirculation diseases, with a specific focus on its novel clinical value in identifying vulnerable carotid atherosclerotic plaques. We also discuss the current limitations restricting large-scale clinical translation, standardized protocol establishment, and deep-tissue imaging performance of ULM, noting that the majority of existing human ULM evidence is still at the feasibility stage and large-scale prospective multi-center clinical validations are still needed. Targeting the above-mentioned translational bottlenecks, finally, this review prospects the future development directions of ULM, including artificial intelligence optimization, three-dimensional whole-organ imaging, multi-modal and multi-omics fusion. With continuous technical iteration, ULM is expected to become a novel non-invasive imaging modality for early diagnosis, risk stratification and therapeutic efficacy evaluation of microcirculation-related disorders.

Key words: Ultrasound localization microscopy; Super-resolution ultrasound; Microbubble contrast agent; Microcirculation; Microvascular imaging; Clinical application

Advanced Ultrasound in Diagnosis and Therapy 2026; 04: 298-316

DOI: [10.26599/AUDT.2026.260040](https://doi.org/10.26599/AUDT.2026.260040)

Conventional ultrasound is limited by acoustic diffraction, yielding a spatial resolution around 500 μm and failing to visualize microvessels below 100 μm [1]. However, microvascular morphological and hemodynamic data are clinically vital for early tumor detection, renal function assessment and cerebrovascular disease surveillance. Drawing on optical super-resolution techniques such as stochastic optical

reconstruction microscopy and photoactivated localization microscopy [2], ultrasound localization microscopy (ULM) reconstructs sub-diffraction microvascular maps by accumulating localization coordinates of sparse microbubbles across massive image frames. Since its introduction in 2011, ULM has reached 40 μm resolution, an order-of-magnitude improvement over conventional ultrasound [3]. Despite this, its clinical translation

* Corresponding author: Department of Ultrasound, Peking University Third Hospital, 49 North Garden Road, 100191, Haidian district, Beijing, China. (Ligang Cui)
e-mail: cuiligang_bysy@126.com (LG C)

faces multiple barriers: tissue clutter interference, high-density microbubble localization errors, motion artifacts, long scanning time and resolution loss at depth [4-6]. Emerging signal processing, deep learning and hardware upgrades have alleviated these challenges [7,8]. This review summarizes recent ULM advances in technical approaches and clinical applications.

Conception, Basic Principles and Imaging Pipeline of ULM

Super-resolution ultrasound (SRUS), first proposed in 1979, describes ultrasound microvascular imaging techniques that break the classical diffraction limit [2]. ULM is one subtype of SRUS, originally proposed by Mickael Tanter's group [9,10], where individual microbubble passage inside tumor vessels were visualized on high-frequency B-mode images. Sono-activated ULM as a sub-variant of ULM, the acoustic counterpart of fluorescence photoactivation localization microscopy, relies on ultrafast ultrasound imaging and microbubbles serving as acoustically activated point sources [10]. Graphical processing unit based computing has enabled clinical translation of ultrafast imaging, which delivers frame rates above 1000 fps over a broad field of view to capture rapid microbubble motion and disruption [11]. Under ultrafast scanning, microbubbles act as discrete acoustic point emitters, their echoes can be localized to generate sub-diffraction images [3,10,11]. Sono-activated ULM achieves 13-fold higher resolution than conventional ultrasound, with stronger image correlation to optical microscopy [10]. Multiple synonymous terms including ultrafast ULM, contrast-enhanced super-resolution ultrasound and ultrasound super-resolution imaging have also been adopted across different studies [7,12,13].

Color Doppler flow imaging (CDFI) only visualizes millimeter-scale vessels, while Contrast-Enhanced Ultrasound (CEUS) enables semi-quantitative microcirculation assessment. Limited by acoustic diffraction, both CEUS and superb microvascular imaging cannot resolve sub-wavelength structures, and conventional high-frequency ultrasound (20-50 MHz) fails to detect 10 μm microvessels and capillary slow flow (1 mm/s) [14]. As a super-resolution technique, ULM achieves sub-diffraction vascular imaging, yet its resolution is not a fixed value but modulated by acquisition parameters including transducer frequency, geometry, signal-to-noise ratio, echo bandwidth and beamforming array elements. With a 128-element receiving matrix, resolution exceeding $\lambda/10$ is attainable; microbubble localization precision can approach 6 μm [12], revealing capillary-level vascular networks in deep organs. Phantom experiments demonstrated lateral and axial resolution improvements of 5.1-fold and 2.2-fold over standard ultrasound, and a 2 MHz clinical scanner could theoretically reach 2-4 μm resolution for ideal stationary scatterers [1]. In vivo ULM studies have achieved 9 μm in mouse ear and 19 μm resolution in rat brain, delivering roughly 10-fold resolution gains relative to conventional ultrasound, alongside 4 cm/s velocity mapping for microvessels [15-17].

The standard ULM pipeline comprises six key stages in figure 1: (1) microbubble contrast agents are administered intravenously to achieve sparse intravascular distribution; (2) continuous ultrasound image sequences are captured at high frame rates (generally > 100 Hz); (3) frame-by-frame microbubble signal extraction is performed to suppress tissue background and clutter; (4) subpixel localization is conducted to pinpoint individual isolated microbubble echoes; (5) motion compensation is applied, and localization results from numerous

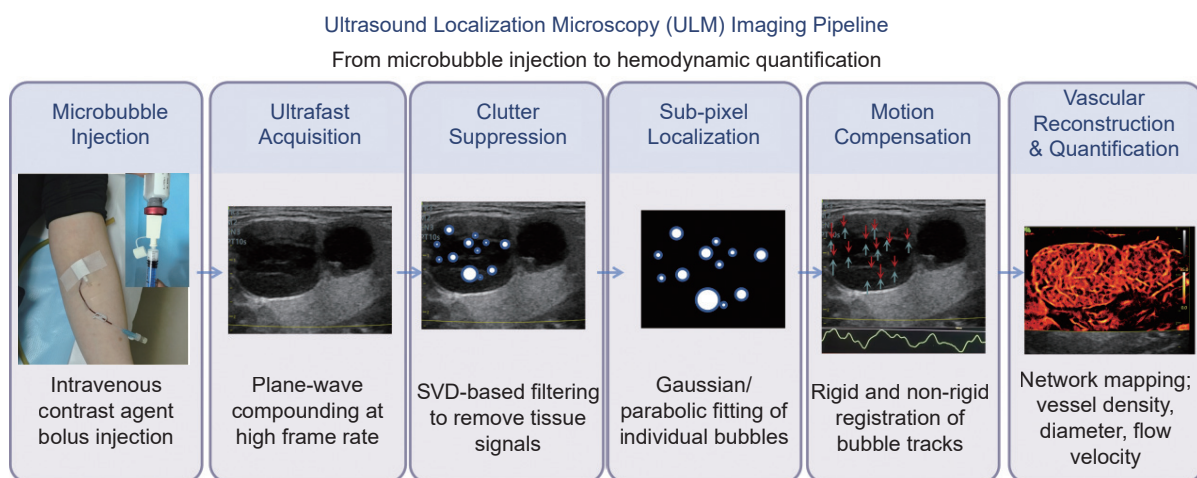


Figure 1 Conceptual schematic of the ULM imaging pipeline. ULM involves intravenous microbubble injection, ultrafast ultrasound data acquisition, clutter suppression, sub-pixel microbubble localization, motion compensation, and final vascular reconstruction with hemodynamic quantification.

frames are integrated to reconstruct super-resolution vascular density maps; (6) inter-frame microbubble tracking is implemented to quantify blood flow velocity and flow direction.

Technical Optimization and Algorithm Innovation of ULM

Various modules within the ULM pipeline, such as microbubble separation, localization algorithms, motion correction and reconstruction frameworks, have undergone extensive optimization, which will be discussed in the following sections.

Optimization of Microbubble Signal Separation and Clutter Suppression

Microbubble signal separation, the front-end step of ULM, extracts nonlinear microbubble signals from raw ultrasound data masked by tissue echoes and clutter. Common techniques include pulse inversion, differential imaging (DI) and singular value decomposition (SVD) filtering [18]. To improve signal extraction and localization accuracy, a modified MCMCDA algorithm has been proposed for robust microbubble tracking under high bubble density [14]. The lightweight deep-learning Angle-Fused U-Net achieves 20-fold faster clutter filtering than SVD while preserving microvascular features, facilitating real-time ULM implementation [19]. And signal onset-based axial localization substantially enhances imaging contrast and spatial precision [20], paving the way real-time clinical ULM. The key performance metrics of representative clutter-suppression and localization algorithms are summarized in table 1.

Clinically, conventional ULM separates microbubble detection and tracking into independent sequential steps, which easily induces matching errors under dense microbubbles or low frame rates. Advanced tracking algorithms have been developed to address this limitation: hierarchical Kalman trackers adapt to varying microbubble states and flow velocities to refine vascular reconstruction [21].

Single-microbubble localization optimization and performance benchmarking

Accurate single-microbubble localization is a key bottleneck restricting the clinical translation of ULM. In medical ultrasound, encapsulated gas microbubbles (average diameter < 5 μm) act as acoustic fluorescent probes, while inherent trade-offs exist among imaging sensitivity, transmitted ultrasound energy and microbubble concentration. A mouse skeletal muscle microvascular study systematically verified that microbubble dosage, ultrasound frame rate and acquisition duration

are core influencing factors [22]. Distinct from conventional CEUS, ULM relies on ultra-high frame rates to shorten acquisition time, which exposes microbubbles to prolonged and frequent ultrasound pulse irradiation. Although higher microbubble concentrations strengthen backscatter signals, dense microbubble distribution in large vessels easily causes overlapping signals that cannot be completely resolved by spatiotemporal filtering, severely degrading single-bubble localization accuracy and imaging sensitivity [23,24]. In vivo rat tumor experiments further demonstrated that microbubble size dominates ULM detection sensitivity: larger microbubbles achieve up to 8.7-fold sensitivity improvement and possess longer intravascular circulation persistence, highlighting the critical value of optimizing single-bubble-level imaging performance [25]. As the core determinant of ULM super-resolution quality, sub-pixel centroid localization of microbubble point-spread functions directly dictates the final spatial resolution [26]. Classical localization algorithms, including Gaussian fitting, radial symmetry, center-of-mass and peak detection, exhibit parameter-dependent performance, with optimal point-spread functions-pixel matching and patch settings varying across different methods [27].

A open-source benchmark framework was established to systematically evaluate mainstream localization algorithms across multiple in vivo datasets, confirming radial symmetry and Gaussian fitting deliver optimal accuracy and detection performance, despite the high computational cost of Gaussian fitting [28]. Beyond parameter optimization, multiple innovative strategies have improved localization performance: convolutional neural network (CNN) object-detection frameworks were introduced for bubble localization and benchmarked against traditional Gaussian fitting [29], and optimized tracking algorithms can suppress false microbubble connections to generate smoother vascular trajectories [30]. Furthermore, CNN-based deep learning frameworks outperform traditional Gaussian fitting in localization accuracy and speed, showing strong robustness against microbubble overlap and noise, though their cross-scenario generalization ability still needs further validation. Additionally, high-precision beamforming is also essential for accurate microbubble localization in ULM imaging.

Motion correction and super-resolution image reconstruction strategies

Motion correction and image reconstruction are critical prerequisites for reliable clinical and in vivo ULM application. Physiological disturbances including respiration, heartbeat and spontaneous organ movement cause spatial deviation of microbubble coordinates dur-

Table 1 Comparison of representative microbubble signal extraction and localization algorithms for ULM.

Algorithm	Paper	Computational complexity	Localization accuracy	Capacity for high-density overlapping microbubbles	Dependence on SNR	Real-time performance	Main merits and limitations
Clutter-suppression algorithms							
SVD filtering	Brown 2019	High	–	Poor	High	Poor	Delivers effective tissue clutter suppression. Suffers heavy matrix-decomposition computational cost; fails for stationary microbubbles and may introduce hundreds-of-microns localization errors under 0.2 mm/s flow; mostly limited to offline processing.
Pulse inversion	Brown 2019	Moderate	–	Moderate	Moderate	Moderate	Insensitive to flow direction and velocity, suitable for slow-flow and tortuous microvasculature. Requires multiple transmit pulses and cannot separate overlapping microbubbles inherently.
Differential imaging (DI)	Brown 2019	Low-Moderate	–	Moderate	High	Good	Low computational overhead. Only functional for flow rates > 0.5 mm/s under axial-dominated blood flow; performance deteriorates for slow or lateral-oriented perfusion.
AF-UNet	Zhang 2026	Low	–	Moderate	Moderate	Good (GPU-dependent)	Lightweight multi-angle-fusion network achieving > 20-fold speedup relative to SVD with favourable cross-organ generalization. Better preserves microbubble point-spread function (PSF) than 3D U-Net variants but cannot intrinsically resolve spatially-overlapping microbubbles; faint microbubble signals may be attenuated. Real-time capability depends on GPU hardware, without native scanner-side implementation. relies on high-quality training datasets
Localization algorithms							
Gaussian fit (GF)	Sobolewski 2024, Heiles 2022	High	High	Limited-test	Moderate-high	Poor	Iterative nonlinear least-squares fitting. Reaches precision close to the Cramér-Rao lower bound with properly selected patch size and sampling parameters and yields the lowest standard deviation among classical approaches. Performance degrades under Rician noise and oversized patches; bimodal error occurs with undersampling; computationally expensive. Only single-microbubble simulations in cited literature; overlapping-bubble scenarios remain unevaluated.
YOLO-modified object-detection-based CNN	Liu 2023	Moderate-High	High	Limited-test	Moderate	Moderate	Modified YOLO-style convolutional-neural-network detector outputting microbubble coordinates and confidence scores. Achieves 77% Iaccard index and 20 μ m localization error with 7-fold speed-up against Gaussian fitting on ULTRASR-Challenge simulated datasets; confidence filtering suppresses false detections. Performance heavily relies on training-data distribution; lacks systematic overlapping-microbubble and in-vivo validations and requires GPU acceleration. Only simulated flow-like dataset in original publication; dedicated overlapping-microbubble tests are missing.
Tracking-association algorithms							
Modified MCMCDA	Ackermann 2016	High	–	Good	Moderate	Poor	Designed for high-density microbubble track linking, accounting for false alarms and track birth-death events; validated via phantom and in-vivo mouse-tumor measurements. Heavy computational overhead prevents real-time deployment.
Hierarchical Kalman tracker (HK)	Taghavi 2022	High	–	Good	Moderate	Poor	Hierarchical Kalman framework adopting velocity-stratified initialization parameters. Delivers superior reconstruction accuracy under high microbubble density and large localization uncertainty. Multi-level iteration imposes high computational cost and supports only offline workflows (3 million MB positions, 5-level HK, with 35.79 min runtime).

note: “–” indicates this metric is not applicable the corresponding algorithm.

ing scanning, inevitably resulting in vascular blurring, imaging artifacts and biased hemodynamic quantification without effective compensation. Multiple targeted motion correction strategies have been developed to address physiological motion interference in different organs. In rat renal ischemia-reperfusion models, combined motion compensation and microbubble tracking eliminates respiratory and cardiac renal displacement, enabling clear visualization of 15–20 μm vasa recta and reliable identification of post-reperfusion vascular changes [31]. For brain imaging vulnerable to cardiac pulsation artifacts, phase-correlation-based rigid motion correction achieves micrometer-level displacement resolution, correcting tissue deviations far exceeding intrinsic ULM resolution and realizing super-resolution imaging in moving cerebral tissues [5]. Combined rigid-body compensation and nonlinear phase inversion further enables accurate tracking of slow-flow microvessels with velocity below 2 mm/s, supporting precise low-speed hemodynamic evaluation [4]. Harput S et al. [32] proposed a two-stage motion-correction algorithm combining affine and non-rigid motion estimation for ULM. When deployed on clinical in-vivo datasets acquired with patient derived motion, this two-step pipeline reduced the width of motion-blurred microvessels by approximately 1.5-fold, demonstrating its capability to mitigate motion-induced blurring for human ULM data.

To overcome the long acquisition time of conventional frame-by-frame ULM accumulation, deep learning-based reconstruction methods have achieved groundbreaking progress. Innovatively optimized motion model ultrasound localization microscopy realizes ultra-fast ULM imaging within one minute on conventional clinical scanners. It not only acquires high-definition tumor microvascular images and quantifies perfusion parameters, but also provides unique morphological and functional biomarkers to distinguish tumor vascular phenotypes. Clinical breast cancer studies validate its feasibility for dynamic monitoring of tumor vascular changes and early evaluation of chemotherapy response [33]. In atherosclerotic rabbit models, optimized motion-adaptive ULM successfully resolves 41 μm microvessels under cardiac motion interference [34]. Despite these advances, clinical ULM still faces challenges from complex in-plane and out-of-plane tissue motion, unstandardized microbubble delivery and suboptimal scanning parameters. Relevant optimization studies and saturation model-based vascular assessment methods have standardized contrast agent administration and acquisition protocols, verifying that qualified clinical super-resolution vascular imaging can be completed within a single contrast injection and less than five minutes of scanning, laying a solid foundation for large-

scale clinical translation of ULM [35].

While these innovative algorithms exhibit compelling performance on phantom and pre-clinical animal datasets, their generalizability toward heterogeneous real world human in vivo data has not been sufficiently validated.

Technical improvements of acquisition duration and imaging saturation

Acquisition duration, imaging saturation and resolution limitations constitute another major barrier restricting clinical ULM translation, with inherent physical and probabilistic mechanisms fundamentally limiting scanning efficiency and vascular reconstruction quality. ULM imaging performance is depth-dependent and constrained by unavoidable trade-offs among imaging speed, spatial fidelity and resolution [36]. Successful microvascular reconstruction requires accurate single microbubble detection and tracking, yet microbubbles are extremely sparse in circulation compared with red blood cells, leading to rare microbubble transit events, particularly in slow-flow microvessels, which substantially prolongs reconstruction of tiny vascular structures [37–39]. Practically, ULM acquisition time strictly determines imaging resolution and vessel recoverability, presenting a clear time-resolution gradient: 10-second scanning acquires 100 μm resolution vascular images, 60 seconds enables 20 μm resolution, and nearly 4 minutes is required for 5 μm ultra-fine reconstruction. Similarly, vessel size and regional blood flow dominate reconstruction efficiency, as microvessels of 30–50 μm require a minimum of 160 s scanning, while capillary-level structures demand far longer acquisition [40]. Christensen-Jeffries et al. [41] established a comprehensive model to elaborate how imaging configuration, microbubble density and hemodynamic conditions jointly determine the minimal acquisition time for saturated super-resolution reconstruction. Huang C et al. [42] proposed an algorithm that segregates overlapping microbubbles by flow features for subgroup-based localization-tracking, alleviating the trade-off between microbubble concentration and acquisition duration. Validated in phantoms and chick-embryo models, it achieves robust super-resolution microvascular imaging within seconds with high-dose microbubbles.

Complementing the empirical saturation model, probabilistic kinetic theories explain the inherent necessity of long ULM acquisition from microbubble perfusion dynamics. Different from the saturation model that predicts achievable imaging quality under fixed scanning duration, Markov-chain modeling and Monte-Carlo simulation-based research reveals the physical origin of prolonged acquisition. Validated by confocal

microscopy, the study verifies ULM's inherent sparsity constraint, with consistent microbubble counts detected in experimental fields and simulated frames [43]. Heterogeneous microvascular hemodynamics are confirmed as the core cause of delayed perfusion: homogeneous flow only requires 24.5 s for full capillary network reconstruction, while heterogeneous low-perfusion regions extend the duration to approximately 182 s, close to experimental results of 193 s.

Heterogeneous perfusion delays microbubble arrival in low-flow vascular segments, and frame-level microbubble sparsity further establishes a physical lower bound for ULM scanning time. Notably, simply increasing microbubble dosage or frame rate cannot fundamentally solve this problem, indicating the necessity of developing adaptive acquisition strategies and targeted enhancement techniques for low-perfusion regions. To mitigate long-acquisition limitations and promote clinical translation, multiple algorithmic optimizations have been proposed. The sparsity-based ultrasound super-resolution hemodynamic imaging achieves remarkable resolution improvement within tens of milliseconds and high temporal resolution by exploiting vascular sparsity and signal independence, effectively reducing deep-tissue motion artifacts, though it lacks clinical validation [6]. Moreover, sparsity-enhanced ULM combined with microbubble flow kinematics improves microbubble detection recall under high-concentration and low-frame-rate conditions, enabling robust vascular reconstruction against severe microbubble overlap. Validated on clinical prostate ultrasound data, this method exhibits superior reconstruction accuracy and great potential for real-time clinical ULM implementation [44].

Huang C et al. [45] present a robust human-applicable ULM on a high-frame-rate clinical scanner with acquisition < 10 s, utilizing motion registration, microbubble separation and Kalman-filter tracking. Tested across multiple human normal and pathological tissues including healthy liver and diseased liver, kidney, pancreatic tumor, it achieved 5.7-fold resolution improvement over power doppler and angle-independent flow measurement. Gold-standard validation for tiny microvessels is still required for clinical deployment. Apart from intrinsic spatio temporal trade offs, minutes long acquisition also introduces practical clinical challenges including higher patient-motion-artifact risk, progressive microbubble bolus decay, and poor compatibility with time-limited clinical ultrasound workflows.

Pre-clinical and Clinical Applications

Microcirculation consisting of microvessels smaller

than 100 μm dominates tissue blood perfusion and material exchange, serving as a core physiological basis for maintaining organ function and reflecting early pathological alterations. Current preclinical and clinical ULM studies cover multiple organs including brain, spinal cord, kidney, tumor, skeletal muscle and heart, forming a solid translational research foundation (Table 2).

Cerebral and spinal microcirculation imaging

Cerebrovascular and spinal vascular diseases are major causes of global disability and mortality, and early quantitative assessment of microcirculatory perfusion is crucial for early diagnosis, pathological mechanism analysis and prognostic evaluation of neurovascular lesions. Traditional imaging techniques are difficult to visualize intracranial and spinal microvascular lesions at the microscopic level, while trans-cranial ULM realizes high-precision in vivo detection of cerebral microcirculation, providing a novel tool for neurological disease research.

ULM demonstrated reduced blood velocity and elevated vascular tortuosity throughout all brain regions in mouse model of aging, alongside diminished cerebral-cortex blood volume. These findings represent the first in vivo ULM characterization of subcortical microvascular alterations with aging and highlight aging-related perturbations of microcirculatory dynamics [46].

In juvenile porcine hydrocephalus models [47], CEUS combined with microbubble tracking successfully adopted cerebral microcirculation indices to evaluate microvascular perfusion status. The cerebral microcirculation parameters exhibited a high correlation coefficient above 0.85 with intracranial pressure. Specifically, cerebral perfusion decreased by an order of magnitude when intracranial pressure exceeded 50% of the mean arterial pressure, identifying a critical perfusion-pressure threshold for cerebral microcirculation collapse. This finding verifies the great potential of ULM for bedside dynamic monitoring of cerebral microcirculation in patients with hydrocephalus and traumatic brain injury.

For functional brain imaging, the novel localization with context awareness ULM deep learning pipeline integrating microbubble simulation and localization effectively solves the high bubble concentration interference problem that restricts conventional ULM. In-silico tests achieves 97.8% microbubble detection accuracy and 23.8% missing rate under physiological high-density microbubble conditions, successfully reconstructing dense rat cerebral vascular networks [48].

ULM also shows unique advantages in spinal cord and cerebral small vessel disease research. In spinal cord injury studies, ULM realizes comprehensive in vivo analysis of macroscopic and microscopic vascular

Table 2 Summary of preclinical and clinical application studies of ULM.

Paper	Study design	Cohort size	Application field	Research model	Research focus	Disease background	Reference standard	Core research findings	Evidence maturity
Lowerison 2020	Simulation assisted pre-clinical observational	5	chicken embryos	renal cell carcinoma xenograft model grown in the chorioallantoic membrane of chicken embryos	To provide a quantitative measures of tissue hypoxic status of ULM	Tumor	fluorescent histology	ULM derived inter vessel distance strongly correlates with histological microvascular density, and vascular tortuosity reliably reflects tissue hypoxia levels	Pre-clinical animal
Lowerison 2022	Pre-clinical observational	99	chicken embryos	human colorectal carcinoma tumor xenograft model engrafted into the CAM of chicken embryos	To indicate the functional microvascular status of chemotherapy sensitivity	Tumor	histology	Elevated vascular fractal dimension on ULM images is closely associated with tumor therapeutic resistance	Pre-clinical animal
Huang 2020	phantom + in vivo chicken embryo proof of concept study	biological replicate number not reported, representative datasets shown	chick chorioallantoic membrane, embryo tumour xenograft	chicken embryo tumor xenograft model and chicken brain	To resolve the inherent trade-off between microbubbles concentration and acquisition duration	Tumor	optical imaging	This novel algorithm separates overlapping microbubbles based on flow velocity and direction differences, enabling robust high-resolution microvascular imaging within seconds under high microbubble dosage	Pre-clinical animal
Chen 2020	Pre-clinical observational	15	mice	mouse acute kidney injury model	To evaluate early acute kidney injury	Kidney disease	histology and immunohistochemistry (CD31)	ULM identifies early renal microcirculatory disorders prior to serum creatinine elevation, supporting early warning of acute kidney injury	Pre-clinical animal
Lowerison 2022	Pre-clinical observational	27	mice	aging mouse model	To varify differences of aged brain and normal brain	Aging	not applicable	Aged brains exhibit reduced blood velocity, increased vascular tortuosity, and decreased cortical blood volume across whole-brain regions on ULM	Pre-clinical animal
Ma 2022	Pre-clinical observational	8	rat	stroke events and Alzheimer's disease	To assess microvascular features of cerebral small vessel disease	Cerebral small vessel disease	H&E staining, PTAH staining, TB staining, MRI	A controllable cerebral small vessel disease model exhibits reduced microvascular number, decreased flow velocity and diminished blood volume in sonicated lesions	Pre-clinical animal
Andersen 2020	Pre-clinical interventional	10	rat	Sprague-Dawley rat	To identify early ischemic damage to the renal microcirculation	Kidney disease	Hematoxylin and eosin staining, Ex-vivo MRI	ULM reliably resolves 15–20 µm ascending vasa recta and distinguishes microvascular alterations after renal ischemia-reperfusion injury	Pre-clinical animal

(To be continued on the next page)

(Continued)

Paper	Study design	Cohort size	Application field	Research model	Research focus	Disease background	Reference standard	Core research findings	Evidence maturity
Andersen 2022	Pre-clinical interventional	7	rat	normal rat	To assess renal vascular velocity responses to pharmacological intervention	Kidney disease	not applicable	Pharmacological intervention with prazosin induces velocity reduction specifically in cortical arterioles and outer-medullary vasa recta, enabling compartment-specific renal hemodynamic evaluation by ULM	Pre-clinical animal
Beliard 2022	Pre-clinical interventional	10, 5 per group (4 week post contusion SCI, 8 week post contusion SCI)	rat	chronic spinal cord injury	to explore changes in spinal cord blood vessels	Spinal cord injury	immunohistochemistry (laminin and for SMI-71), BBB locomotor behavioural scoring	Spinal cord injury presents reduced perfusion velocity and asymmetric hypoperfusion, with ULM-derived vascular biomarkers closely correlating with injury severity and supporting longitudinal vascular function assessment	Pre-clinical animal
Qiu 2022	Pre-clinical observational	11	rat	spontaneously hypertensive rats and normal Wistar-Kyoto rats	To detect features of CKD in early stage	Kidney disease	phantom experiments, Pathology (H&E staining)	ULM detects altered interlobular arterial flow velocity, allowing early diagnosis of hypertensive nephrosclerosis.	Pre-clinical animal
Søgaard 2022	Pre-clinical observational	8 male Zucker rats: 4 obese Zucker rats + 4 age matched lean Zucker rats	rat	obese Zucker rats	To quantify renal vascular density and tortuosity in vivo	Kidney disease	renal functional markers, Histopathology (H&E, periodic acid-Schiff, Sirius Red, and Jones Silver staining)	Obese rats show increased renal vascular density and subclinical renal dysfunction without apparent histological damage, which can be non-invasively identified by ULM	Pre-clinical animal
Demeulenaere 2022	Pre-clinical observational ex-vivo + in-vivo animal study	9 Sprague Dawley male rats: 6 isolated Langendorff-perfused beating hearts (ex-vivo), 3 closed chest in-vivo anaesthetized rats	rat	isolated beating rat hearts	To visualize whole heart coronary microvasculature beyond the acoustic diffraction limit (sub-20 µm resolution)	Heart	Ex-vivo micro computed tomography of fixed hearts; aortic perfusion flow measurements from Langendorff perfusion system	ULM enables sub-20 µm three-dimensional visualization of beating-heart coronary microvasculature and accurately quantifies adenosine induced coronary flow augmentation.	Pre-clinical animal
Qian 2022	Pre-clinical observational: in-vitro phantom + single animal in-vivo proof of concept study	Custom built flow channel phantoms; 1 New Zealand white rabbit imaged under three IOP levels (10 mmHg, 30 mmHg, 50 mmHg)	rat	pre-designed flow phantoms in vitro; New Zealand white rabbit eye in vivo	To monitor alterations of ocular blood flow of the deep eye vasculature	Retinal and optic nerve diseases	Phantom: preset pump driven ground truth flow speeds; in-vivo: calibrated manometer controlled intraocular pressure; literature reported OCT-A performance as comparative benchmark	Elevated intraocular pressure reduces ocular vascular density and flow velocity, and ULM provides high-resolution deep ocular imaging comparable to optical coherence tomography angiography	Pre-clinical animal

(To be continued on the next page)

(Continued)

Paper	Study design	Cohort size	Application field	Research model	Research focus	Disease background	Reference standard	Core research findings	Evidence maturity
Zhang 2021	Pre-clinical interventional longitudinal in-vivo animal feasibility study	8 male New Zealand white rabbits; 6 rabbits with successfully implanted VX2 liver tumors for longitudinal baseline/post sorafenib therapy imaging	Rabbit	liver tumor model	To present a novel anesthesia protocol to enable the long image acquisition times	Tumor	Post mortem histopathology including H&E staining, CD31 immunohistochemistry for microvascular endothelium, Hypoxyprobe pimonidazole staining for intratumoral hypoxia; in-vivo physiological vital sign monitoring	Optimized apneic imaging protocols eliminate motion artifacts, enabling clear visualization of hypovascular tumor cores and intact tumoral microvascular architecture	Pre-clinical animal
Lei 2022	Pre-clinical observational longitudinal in-vivo animal feasibility study (endoscopic ULM)	6 female New Zealand white rabbits; longitudinal imaging at baseline, 7, 14 and 21 days after rectal VX2 tumour implantation	rabbit	gastrointestinal tract model	Feasibility assessment of endoscopic ULM for gastrointestinal tumour microvascular evaluation	Tumor	In-vitro flow phantom ground truth tube dimensions; ex-vivo micro-CT angiography of resected rectal tumour tissue for vascular pattern validation	Endoscopic ULM reveals significant microvascular pattern and density differences across distinct tumor growth stages	Pre-clinical animal
Yu 2018	Pre-clinical observational proof of concept in-vivo animal study	1 New Zealand white rabbit; balloon injured femoral artery atherosclerosis model, with contralateral non-injured artery as internal control	New Zealand white rabbit	atherosclerosis model	To develop deconvolution combined ULM with STIC synchronization to tolerate cardiac related physiological motion	Atherosclerosis	In-vitro synthetic phantom for algorithm validation; post mortem H&E histology and vWF immunofluorescence staining for vasa vasorum assessment	A customized deconvolution and spatiotemporal correlation workflow achieves 41 µm microvascular visualization in rabbit atherosclerotic models under cardiac motion interference	Pre-clinical animal
Zhu 2019	Pre-clinical observational feasibility in-vivo animal study	3, 2 male New Zealand white rabbits for in-vivo popliteal LN imaging; one extra rabbit for ex-vivo micro-CT vascular corrosion cast reference	rabbit	lymph node	Feasibility of 3D ULM for in-vivo lymph node microvascular and flow assessment	Lymph node disease	Ex-vivo micro-CT vascular corrosion cast (separate animal) for vessel size distribution benchmark; in-vivo ASAP enhanced power doppler as imaging comparator	3D-ULM resolves vessels separated by 30 µm (~15-fold resolution improvement vs Doppler); most ULM detected vessels < 80 µm (PD mostly > 100-250 µm); predominant intranodal blood-flow velocity < 5 mm/s	Pre-clinical animal
Zhu 2022	Prospective human feasibility pilot clinical study	10 lymph nodes from 10 patients (4 reactive benign LNs, 6 metastatic LNs); screened from 44 patients	human	metastatic and reactive LNs	To detect and distinguish metastatic lymph nodes from benign lymphadenopathy	LN's disease	Ultrasound guided core needle biopsy histopathology	LFDI, a metric reflecting local blood flow direction disorder, is a promising ULM derived biomarker for identifying metastatic lymph nodes. This pilot study highlights major practical challenges including patient motion and scanner frame rate constraints	Human feasibility

(To be continued on the next page)

(Continued)

Paper	Study design	Cohort size	Application field	Research model	Research focus	Disease background	Reference standard	Core research findings	Evidence maturity
Bodard 2023	Prospective human feasibility pilot study including method optimization and proof of concept	49 kidney transplant recipients; 35 for imaging protocol optimization; final analysis on 7 kidney allograft patients	human	human kidney allografts	Evaluate feasibility of ULM implemented on conventional low frame rate clinical ultrasound scanner for renal microvascular assessment	Kidney disease	Comparative imaging benchmark versus SMI, advanced dynamic flow (ADF) and CDFI	ULM resolved vessels 2–4-fold thinner than Doppler modes, with the minimum analyzable vessel cross-section averaging 0.3 ± 0.2 mm, and enabled quantitative cortical blood velocity measurement	Human feasibility
Huang 2021	Prospective multi-organ human feasibility proof of concept study	Multiple human subjects; imaging of healthy liver, healthy kidney, pancreatic tumour and DCIS breast tumour; exact individual subject count not explicitly reported	human	a healthy liver and a diseased liver with acute-on-chronic liver failure, a kidney, a pancreatic tumor	To study a robust in human ULM on a high frame-rate clinical ultrasound scanner to achieve short acquisition time	Human multiple-organ in-vivo model (Liver disease, kidney disease, and tumor)	Contrast enhanced power doppler reconstructed from identical microbubble dataset for imaging performance comparison	Clinical high frame rate ULM enables ultrafast human microvascular imaging across multiple tissues and shows broad clinical potential, though rigorous gold-standard validation for tiny vascular structures remains necessary	Human feasibility
Zhang 2022	Prospective human clinical cohort study	46 breast masses from 46 female patients (20 benign, 26 malignant)	human	different breast masses	To evaluate its feasibility and value for the differentiation of breast masses	Breast lesions	Ultrasound guided core needle biopsy or surgical resection	ULM provides supplementary morphological and functional microvascular information for breast lesions, and MVD serves as a reliable biomarker for differentiating breast malignancy	Human feasibility
Li 2025	Prospective human clinical cohort study	31 breast lesions (14 benign, 17 malignant), confirmed by pathology	human	Human breast lesions	Evaluate qualitative and quantitative ULM parameters for differentiating benign and malignant breast lesions; assess intra and inter operator reproducibility of ULM quantitative indices	Benign and malignant breast lesions	Post biopsy surgical histopathology for global lesion diagnosis	Qualitative vascular morphology patterns and quantitative ULM metrics show high diagnostic performance to discriminate benign and malignant breast lesions; ULM-derived quantitative parameters exhibit excellent intra and inter operator reproducibility, supporting its diagnostic potential for breast lesion evaluation	clinical cohort
Zhang 2022	Prospective human feasibility pilot clinical study	24 thyroid nodules from 24 patients (12 benign, 12 malignant)	human	different thyroid nodules	Pilot evaluation of ULM derived vascular metrics for distinguishing benign and malignant thyroid nodules	Thyroid lesions	FNA or surgical resection histopathology for global nodule diagnosis	Benign thyroid nodules exhibit significantly higher MVD and MFR values than malignant thyroid lesions on ULM	Human feasibility
Zhang 2025	Prospective human clinical cohort study	135 cervical LNs from 135 patients (61 reactive hyperplastic LNs, 74 metastatic LNs)	human	metastatic and reactive lymph nodes	To evaluate ULM for discriminating reactive hyperplastic versus metastatic cervical lymph nodes	Lymph nodes	Ultrasound guided core needle biopsy histopathology	integrating ULM with routine ultrasound improves diagnostic accuracy for metastatic cervical lymph nodes	Human feasibility

(To be continued on the next page)

(Continued)									
Paper	Study design	Cohort size	Application field	Research model	Research focus	Disease background	Reference standard	Core research findings	Evidence maturity
Li 2024	Prospective human clinical cohort study	58 Patients with obstructive azoospermia (OA) and 36 non-obstructive azoospermia (NOA)	Human	Obstructive and non-obstructive azoospermia	To evaluate testicular microcirculation via ULM; to explore correlations between ULM haemodynamic metrics and spermatogenic function; construct combined diagnostic model for differentiating NOA and OA	Human testis of azoospermia patients	Clinical diagnosis of OA/NOA; no pixel wise spatial registration between ULM vascular indices and testicular immunohistochemistry	ULM enables non-invasive detailed assessment of human testicular microvascular haemodynamics. ULM-quantified vascular parameters reflect links between testicular microcirculation and spermatogenic function. Combining serum FSH with ULM derived mean vascular diameter greatly improves diagnostic performance for discriminating NOA from OA	clinical cohort
Papanikolaou 2026	Prospective case control study	Human cohorts: 10 healthy men, 9 HH patients receiving testosterone, 9 gonadotrophins, 12 untreated; 7 HH patients for 12 month fertility response follow-up; 5 rodent pubertal blockade model	human	HH and male infertility related testicular disorders	To assess ULM as a treatment responsive biomarker for testicular function in hypogonadotrophic HH; to evaluate its capability for early detection of therapeutic response	Human testis and rodent pubertal blockade testis patients	Clinical endocrinological laboratory markers	ULM yields biologically meaningful, treatment responsive vascular biomarkers for testicular function. It enables patient stratification and earlier identification of therapeutic response in HH compared with conventional clinical markers, with supporting pre-clinical evidence from rodent pubertal blockade experiments.	clinical cohort
Li 2026	Prospective observational human clinical study	20 traumatic brain injury patients after decompressive craniectomy	human	Human brain of traumatic brain injury patients after decompressive craniectomy	To evaluate bedside ULM cerebral microvascular imaging performance using a multihypothesis multiframe global optimization algorithm; investigate post-injury cerebral microvascular remodelling and prognostic value of ULM composite metrics	Traumatic brain injury following decompressive craniectomy	Invasive intracranial pressure(ICP) monitoring and 6 month clinical functional outcome assessment	A multihypothesis multiframe global optimization algorithm enables bedside cerebral ULM on clinical grade systems without strict ultrafast sequence prerequisites. Bedside ULM can depict post traumatic cerebral microvascular remodelling, correlate with invasive ICP, and stratify long term clinical outcomes for neurocritical care patients	

remodeling during chronic SCI progression. It accurately detects reduced regional perfusion velocity and asymmetric hypoperfusion in injured spinal cord tissues, and the extracted vascular dysfunction biomarkers are significantly correlated with spinal cord injury severity [49]. For cerebral small vessel disease (CSVD), a disease closely related to cognitive decline, stroke and Alzheimer's disease, ULM effectively quantifies CSVD related hemodynamic changes. Controllable CSVD model experiments confirmed that targeted ultrasonic stimulation leads to significant reductions in microvessel density, blood flow velocity and blood volume in sonicated brain areas, verifying ULM's capability for early in vivo evaluation of CSVD microvascular injury [50].

Besides, traumatic brain injury patients were studied. The moderate injury patients showed better microvascular structural recovery, severe injury patients presented elevated early vascular resistance and persistent flow deficits. Composite ULM metrics were negatively correlated with invasively measured intracranial pressure. The microvascular perfusion efficiency coefficient effectively stratified 6 month clinical patient outcomes [51].

Renal microcirculation imaging and nephropathy evaluation

The kidney has a delicate and hierarchical microvascular network, and renal microcirculation dysfunction is the initiating and core pathological change of almost all acute and chronic kidney diseases, occurring far earlier than the elevation of serum creatinine and other routine biochemical indicators [52]. ULM can visualize renal microvessels at the micron level to clear display of tiny microvessels around the renal capsule [45] and quantitatively evaluate structural and functional microcirculation abnormalities [53], providing early biomarkers for the diagnosis and intervention of various nephropathies.

Using a chicken embryo chorioallantoic membrane renal cell carcinoma xenograft model, a study employed

ULM to characterize tumour microvascular architecture, perfusion and hypoxia. Quantitative ULM biomarkers, including intervessel distance and vascular tortuosity, correlated well with histological microvascular density and hypoxyprobe-detected hypoxia, respectively. ULM provides non-invasive in vivo microvascular readouts and is promising for solid tumour diagnosis and treatment monitoring [54].

In acute kidney injury (AKI) research, ULM imaging sensitively captures early renal microcirculation disorders in mouse AKI models, which precede the elevation of routine renal function indicators [55]. Standardized CEUS acquisition combined with super-resolution post-processing can effectively identify early renal microvascular injury, making up for the deficiency of conventional ultrasound in early AKI diagnosis. For renal ischemia-reperfusion injury, ULM clearly visualizing 15-20 μm vasa recta and accurately detecting microvascular structural and hemodynamic alterations after ischemia-reperfusion [31].

ULM also has important application value in chronic kidney disease evaluation. In diabetic kidney disease, ULM derived microbubble event count, microvessel density (MVD) and marginal renal blood flow velocity can effectively distinguish diabetic renal microvascular damage from normal tissues [56]. Figure 2 shows the MVD comparison between normal people and diabetes kidney disease (unpublished research results). Regional hemodynamic analysis confirmed that cortical arterioles and outer-medullary descending vasa recta present specific hypoperfusion changes under pathological conditions, and ULM can accurately differentiate hemodynamic responses of different renal vascular compartments to pharmacological intervention. In obese Zucker rat models with early renal damage, ULM detects increased renal vascular density and subclinical renal dysfunction without obvious histological injury, demonstrating its unique advantage in identifying early reversible renal vascular remodeling [57]. For hypertensive nephrosclerosis, ULM sensitively captures

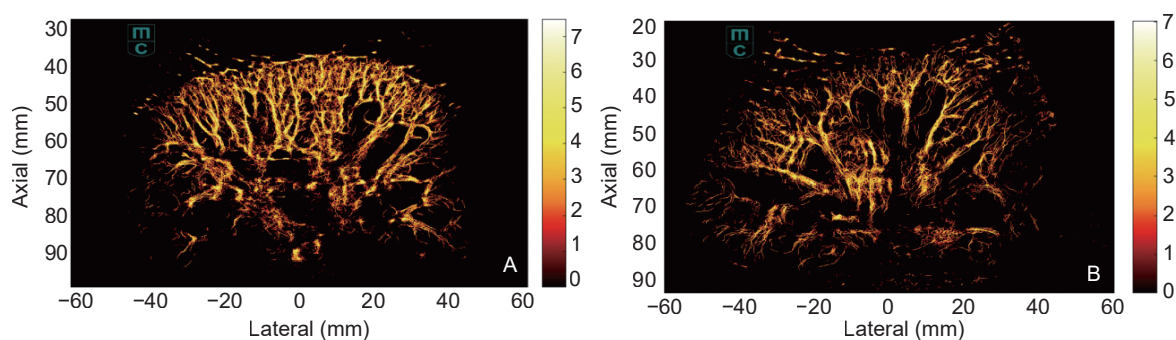


Figure 2 Comparison between normal kidney and diabetes nephropathy. (A) shows the normal kidney image of a 38 year old male; (B) shows a diabetes nephropathy of a 58 year old female which were diagnosed by histopathology (unpublished research results).

decreased interlobular arterial blood flow velocity in spontaneously hypertensive rats, achieving early differential diagnosis of hypertensive renal vascular lesions superior to conventional ultrasound [58]. In addition, ULM also has potential application prospects to detect and differentiate regional hemodynamic responses to pharmacological intervention across distinct renal vascular compartments [59].

Tumor microcirculation and oncological application

Tumor angiogenesis is a key driver of tumor growth, invasion and metastasis. Tumor microvascular morphology, density and hemodynamic characteristics are closely correlated with tumor malignancy grade, clinical staging, therapeutic response and long-term prognosis. Benefiting from ten-fold higher resolution than conventional ultrasound, ULM can visualize microscopic tumor vascular structures that are invisible to CDFI and CEUS, realizing multi-dimensional evaluation of tumor angiogenesis and clinical translational application in tumor differential diagnosis, metastasis prediction and efficacy monitoring.

In basic tumor models, ULM successfully depicts intricate microvascular architectures in VX2 liver tumor model [60], pancreatic tumor [45], and renal tumor [61], and breast tumor [62], verifying its capability for ultra-fine tumor angiogenesis imaging. Besides, ULM can identify early microcirculation changes in gastrointestinal tumors, supporting early screening and auxiliary diagnosis of gastrointestinal malignancies [63].

In terms of benign and malignant tumor differentiation, ULM provides abundant morphological and functional microvascular biomarkers for lesion identification.

In a clinical study of 46-patients who were assessed as Breast Imaging Reporting and Data System (BI-RADS) Category 4 or 5 with ultrasound [64], malignant breast lesions exhibited significantly higher peak intensity, area under the curve (AUC), MVD and MFR but lower time to peak (TTP) than benign lesions. Similar results are shown in [figure 3](#) (unpublished research results). Among all quantitative parameters, MVD showed the strongest positive correlation with breast malignancy, serving as a core imaging marker for breast lesion differential diagnosis. Differently, in thyroid nodules, benign lesions present higher MVD and MFR than malignant ones, indicating that ULM parameters have targeted differential diagnostic values for different tumor types [13]. In terms of tumor metastasis prediction and therapeutic monitoring, ULM has unique early evaluation advantages. For breast cancer sentinel lymph node metastasis, ULM achieves a diagnostic accuracy of 0.824, which is significantly higher than conventional ultrasound; the combined application of ULM and conventional ultrasound further improves the accuracy to 0.844, providing an effective non-invasive tool for preoperative metastasis risk assessment [65].

For tumor treatment efficacy evaluation, longitudinal ULM monitoring of colorectal carcinoma xenografts treated with sorafenib, FOLFOX-6 and combined regimens confirms that tumor microvascular fractal dimension is closely related to tumor hypoxia and therapeutic resistance [66]. Decreased vascular fractal dimension indicates good treatment response, which can serve as a novel imaging biomarker for predicting tumor treatment prognosis. [Figure 4](#) shows a patient diagnosed with lymphoma had significantly reduced MVD after one cycle

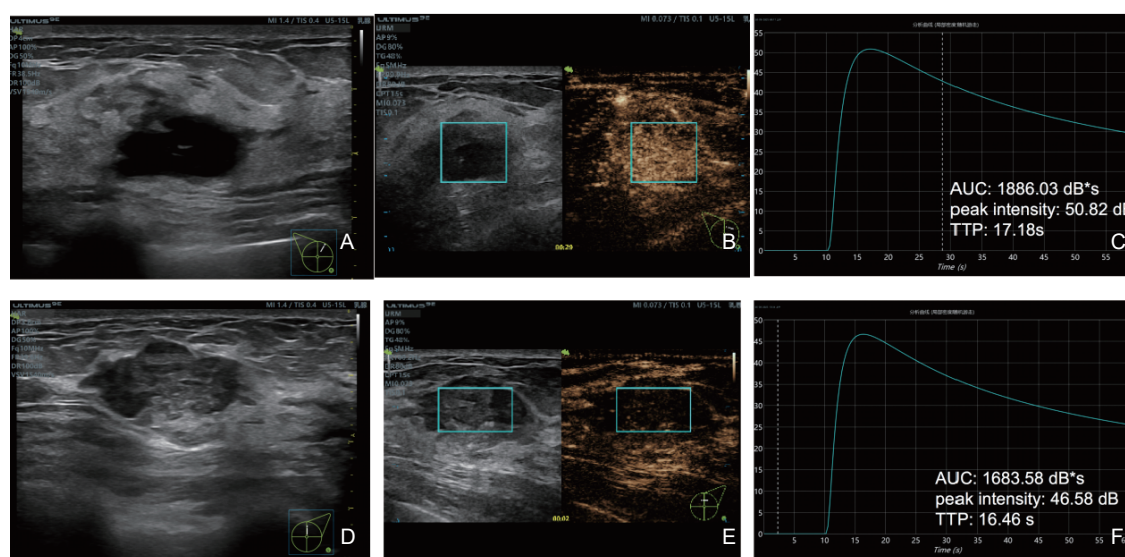


Figure 3 CEUS quantitative parameters of malignant breast lesions and benign lesions. The malignant breast lesions diagnosed as exhibited significantly higher AUC, peak intensity, but lower TTP than benign lesions (unpublished research results). (A-C) shows a poorly differentiated invasive breast cancer of 50 year old female; (D-F) shows a fibroadenoma lesion of 38 year old female.

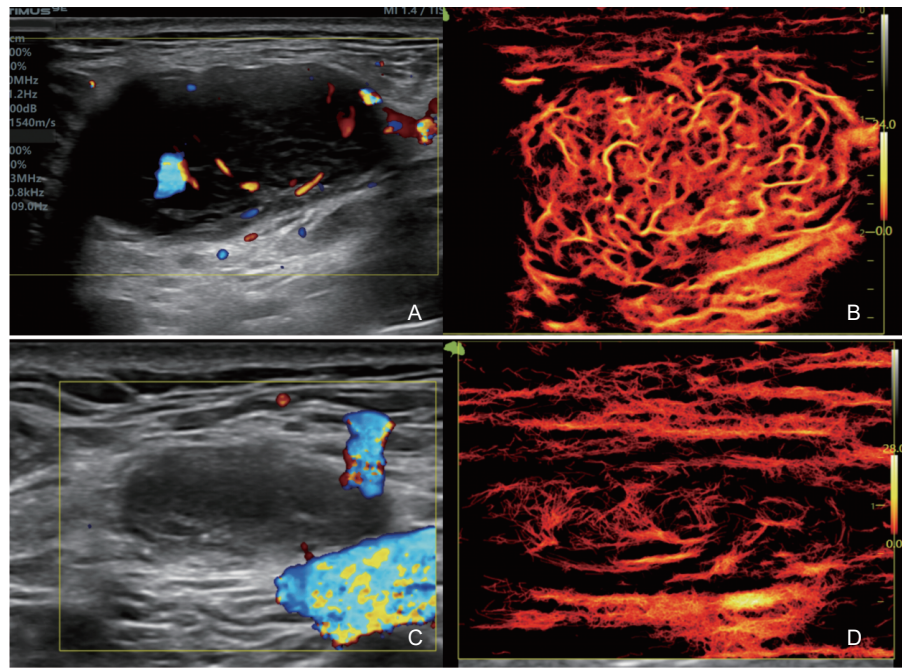


Figure 4 Comparison of ULM imaging before and after follicular lymphoma treatment of a LN. (A,B) showed the color images and microvascular density of LNs before treatment; (C,D) showed the corresponding results after treatment. The MVD decreased from 60.8% to 27.2% after treatment (unpublished research results).

of treatment compared to MVD before treatment, and showed a good prognosis finally (unpublished research results). ULM can capture early microcirculatory changes after anti-angiogenic therapy, realize tumor vascular subtype classification and high-risk subgroup screening, and support precise and individualized tumor treatment.

Endocrine and cardiac application

In cardiac microcirculation research, ULM realizes unprecedented high-precision imaging of beating-heart coronary microvasculature [67]. It achieves dynamic three-dimensional visualization of coronary vessels down to sub-20 μm resolution, covering capillary-level microvascular structures. Pharmacological stimulation experiments confirmed that adenosine can double coronary microvascular blood flow, and the ULM quantified microcirculatory flow changes are highly consistent with aortic perfusion detection results, fully verifying the accurate and reliable functional quantification capability of ULM for cardiac microcirculation. This technique breaks through the limitation that conventional imaging cannot dynamically evaluate beating-heart microvascular hemodynamics, providing a novel tool for the study of coronary microcirculation dysfunction and early diagnosis of myocardial microvascular lesions. These research directions remain in the early exploratory phase with limited human in-vivo evidence, and pre-clinical results cannot be directly extrapolated to routine human clinical practice.

Clinical application in carotid atherosclerotic plaque

Intra-plaque neovascularization (IPN) is a hallmark of unstable carotid atherosclerosis and closely correlates with plaque rupture and ischemic stroke risk. Conventional CEUS can only achieve limited semi-quantitative assessment of superficial plaque neovascularization, lacking microscale morphological and hemodynamic quantification. In a cohort of 65 patients with 78 carotid plaques, ULM enabled sub-diffraction microbubble localization and tracking, extracting quantitative microvascular morphological and hemodynamic parameters including MVD, vascular length, tortuosity and intra-plaque flow velocity. Compared with asymptomatic plaques, symptomatic lesions exhibited significantly higher microbubble counts, track numbers, microbubble path lengths and elevated blood flow velocity [68].

Others clinical application fields

ULM provides high-resolution deep-ocular imaging comparable to optical coherence tomography angiography in New Zealand white rabbit eye in vivo [69]. High-resolution visualization of retinal-choroidal vessels and major ocular arteries-veins was achieved. Elevated intraocular pressures reduced vascular density and flow velocity.

Distinguishing metastatic lymph nodes (LNs) from benign lymphadenopathy remains a major clinical challenge for cancer diagnosis and prognosis. Leveraging microbubble localization and tracking, ULM overcomes the acoustic diffraction limit to provide non-invasive in-

vivo microscopic visualization of vascular morphology and flow dynamics. The apparent size distribution of most vessels in the ULM images was below 80 μm . Blood flow velocity was lower than 5 mm/s [70]. In a pilot clinical investigation [71] involving 10 patients (6 with metastatic LNs, 4 with benign reactive lymphadenopathy), ULM quantitative biomarkers including MVD, fractal dimension, mean flow speed, and Local Flow Direction Irregularity (LFDI) were extracted and compared against pathological gold standards. Statistically significant elevations in LFDI were observed in metastatic LNs, with LFDI values 60% higher than those of reactive LNs. This pilot work verified the clinical feasibility of ULM for LNs assessment and highlighted the potential of LFDI for differentiating pathological lymphadenopathy.

Another pilot study [72] of 54 enlarged superficial LNs (34 malignant, 20 benign) reported that benign LNs had significantly higher MVD, flow weighted density of the vessel and perfusion index, as well as lower velocity variance, relative to malignant LNs. The authors also explored optimal contrast agent dosage and acquisition duration. Under a 0.5 mL microbubble bolus, significant inter-group differences emerged within the 6 s acquisition period and amplified progressively over time. By contrast, no parameters showed statistically significant differences between groups when using the 1.0 mL dosage. Furthermore, a study including 135 LNs also verified the clinical feasibility of ULM for superficial LNs differentiation [73]. Of note, heterogeneous microbubble dosage, circulation half life and injection protocols across studies act as important confounders that limit cross study comparability of ULM quantitative vascular parameters.

When applied to human liver tissue, ULM uncovered fine vascular architecture and hemodynamic features for both healthy livers and livers affected by acute-on-chronic liver failure. Compared with the organized, hierarchical vasculature in healthy liver, ULM visualized prominent pathological alterations in diseased liver, including distorted, highly tortuous main vascular branches with tapering morphology, changes presumably associated with liver-failure-related tissue remodeling. These findings illustrate ULM's capacity to resolve microvascular morphological and hemodynamic deviations in human liver disease that are inaccessible to conventional power-Doppler ultrasound [45].

In human testicular disease research, ULM has been utilized to characterize testicular microcirculation in patients with obstructive azoospermia (OA) and non-obstructive azoospermia (NOA). Significant reductions in mean vascular diameter, MVD and fractal number were observed in NOA patients compared with OA

counterparts. Correlation analyses revealed associations between ULM derived microvascular metrics, testicular volume, age and reproductive hormonal markers. A combined diagnostic model integrating serum follicle stimulating hormone (FSH) and ULM-measured mean vascular diameter yielded a high diagnostic performance (AUC = 0.968) for differentiating NOA from OA [74]. A prospective case control study verified that ULM vascular metrics could discriminate hypogonadotrophic hypogonadism from healthy controls, differentiate treatment subgroups, and detect testicular functional activation earlier than conventional markers [75].

Caveats for interpretation of ULM quantitative biomarkers

Different from semi-quantitative perfusion assessment, ULM establishes a comprehensive morphological and functional microcirculation evaluation system covering multiple core quantitative indicators [76], which can sensitively capture early microvascular lesions that cannot be detected by traditional imaging.

Key ULM quantitative parameters and their clinical implications are clarified as follows. Vessel density (VD) or MVD, defined as the frequency of microbubbles passing through unit vascular space within a fixed period, directly reflects the richness of regional microvascular perfusion and vascular patency, and its abnormal increase or decrease is closely associated with angiogenesis, microvascular injury and hypoperfusion state [64]. Blood flow velocity and microvascular flow rate (MFR) accurately characterize microcirculatory hemodynamic status, enabling sensitive detection of regional hypoperfusion, vasoconstriction and hemodynamic disorders [59]. Microvascular diameter distribution achieves precise delineation of hierarchical microvascular structures from arterioles to capillaries, supporting fine classification of vascular injury besides, microvascular diameter is also helpful for distinguishing benign from malignant breast lesions [62].

Although multi-dimensional quantitative parameters provide promising microvascular insights, several important confounding factors and limitations should be kept in mind when interpreting these metrics. First, these quantitative values are susceptible to variations in acquisition scan parameters, physiological or transducer-induced motion artifacts, and micro bubble concentration [35]; all these variables can substantially alter final biomarker outputs across different datasets. There is notable risk of over-interpreting these indices under sub optimal image reconstruction quality. Second, well-established normal reference-value ranges for most human organs have not yet been built. Published cut off thresholds are mostly derived from single center pilot

cohorts and lack multi centre external validation [68,71,72]. Collectively, these caveats indicate that ULM-derived quantitative biomarkers are not ready for standalone clinical diagnostic use at the current stage. They should rather be treated as exploratory research oriented imaging read-outs and interpreted together with morphological imaging findings and clinical pathological information.

In quantitative ULM analysis, reconstruction saturation represents one of the most prominent technical confounders that bias derived vascular metrics. Scan parameters such as spatial resolution and voxel size directly govern the capability to resolve fine vascular features. Insufficient spatial resolution can result in small microvessels being missed, leading to underestimation of both MVD and FD. Increasing frame rate improves micro bubble tracking accuracy, yet it requires joint optimisation with microbubble concentration.

Microbubble concentration exerts profound impacts on the completeness of reconstructed vascular networks. At low microbubble concentrations, sparse detected bubble events produce incomplete vascular reconstruction, causing underestimation of MVD and lowered FD values. At excessively high concentrations, overlapping point spread functions (PSF) of adjacent microbubbles trigger localization saturation: false bubble localization emerge, distorting vascular morphological complexity, which may produce artificially elevated or anomalous FD readings, while MVD can also be underestimated [23,24].

Tissue motion represents another major barrier for quantitative ULM, originating from respiration, cardiac beating and organ displacement. Imperfect motion correction artefactually overestimates vessel density, vessel diameter and tortuosity, inflating calculated FD values and severely impairing MVD quantification. Biological tissues commonly exhibit complex non-rigid deformation during scanning [32]. In clinical scenarios, however, ULM cannot be adequately performed in several subjects who are unable to hold their breath [61]. CorULM multi-scale three dimensional motion correction [67] can compensate for global displacement and partial sub wavelength local deformation induced by cardiac beating. Nevertheless, this approach relies on gating to select diastolic windows. It has only been validated in isolated Langendorff rat hearts, and substantial hardware related and algorithmic challenges remain before cardiac ULM can be translated to human clinical applications.

Current Clinical and Technical Limitations of ULM

Despite encouraging pre-clinical findings and preliminary human feasibility results of ULM technology, sev-

eral critical barriers still hamper its robust clinical implementation. The majority of existing human ULM investigations are small-sample pilot or feasibility studies rather than large scale well-powered clinical cohorts. To date, there remains a lack of universally-accepted standardized scanning protocols, unified post-processing pipelines, and established reference-value ranges for core quantitative biomarkers. Furthermore, diagnostic cut-off thresholds derived from single-center pilot datasets have not undergone independent external validation across multi-center cohorts. For emerging algorithm-driven innovations including deep-learning-powered microbubble localization, Trans-ULM, and SUSHI frameworks, superior performance has been repeatedly demonstrated using phantom and pre-clinical animal datasets. Nevertheless, their generalization capacity against heterogeneous real-world human in-vivo data remains insufficiently validated, constituting a notable translational gap between pre-clinical performance and clinical utility.

Multi-omics [77] and multi-modal integration will further expand the clinical application boundary of ULM. While most early ULM implementations depended on research-mode ultrafast ultrasound sequences and offline post-processing workflows without native reconstruction modules on routine clinical scanners, integrated on-board super resolution reconstruction and quantitative analysis tools have become available on certain commercial clinical ultrasound platforms (e.g., VINNO ULTIMUS series with URM technology and selected Mindray systems) [51,65], enabling in-device ULM processing for human clinical investigations. Nevertheless, broad availability across mainstream ultrasound vendors remains limited.

As ULM requires intravenous microbubble contrast agent injection, relevant safety considerations and contraindications should be noted. Although the safety of intravenous microbubble contrast agents has been verified [78], special caution should be exercised for vulnerable groups including pregnant people, lactating women, and patients with severe pulmonary hypertension or unstable cardiopulmonary status. Adequate emergency preparedness against rare hypersensitivity-related adverse reactions is mandatory prior to contrast agent injection [79].

Furthermore, multi-level reproducibility evidence remains incomplete for ULM quantitative biomarkers in human cohorts. Large-scale studies systematically evaluating inter-scan, inter-operator, and inter-scanner reproducibility of vascular metrics (including microvessel density, fractal dimension and flow-derived indices) are still under-represented in published literature, which constitutes an important barrier before widespread rou-

tine clinical adoption.

In summary, with continuous breakthroughs in algorithm optimization, multi-modal integration and intelligent transformation, ULM is expected to become a routine clinical imaging modality for early diagnosis, efficacy monitoring and prognostic evaluation of microcirculation-related diseases.

Acknowledgements

We are grateful to Dr. Chen Luo for assistance in kidney ULM analysis.

Authors' Contributions

Concept and design: CLG. Literature Search: HXD. Drafting of the manuscript: HXD. Supervision: CLG. All authors have read and agreed to the published version of the manuscript.

Funding

The study was funded by National Natural Science Foundation of China (82672533).

Data Availability

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Conflict of interest

The authors have no conflict of interest to declare.

Consent for publication

Not applicable.

References

- [1] Viessmann OM, Eckersley RJ, Christensen-Jeffries K, Tang MX, Dunsby C. Acoustic super-resolution with ultrasound and microbubbles. *Phys Med Biol* 2013;58:6447-6458.
- [2] Ikeda O, Sato T, Suzuki K. Super-resolution imaging system using waves with a limited frequency bandwidth. *JASA* 1979;65:75-81.
- [3] Couture O, Besson B, Montaldo G, Fink M, Tanter M. Microbubble ultrasound super-localization imaging (MUSLI). IEEE international ultrasonics symposium 2011[Conference abstract].
- [4] Foiret J, Zhang H, Ilovitsh T, Mahakian L, Tam S, Ferrara KW, et al. Ultrasound localization microscopy to image and assess microvasculature in a rat kidney. *Sci Rep* 2017;7:13662.
- [5] Hingot V, Errico C, Tanter M, Couture O. Subwavelength motion-correction for ultrafast ultrasound localization microscopy. *Ultrasonics* 2017;77:17-21.
- [6] Bar-Zion A, Solomon O, Tremblay-Darveau C, Adam D, Eldar YC. SUSHI: sparsity-based ultrasound super-resolution hemodynamic imaging. *IEEE Trans Ultrason Ferroelectr Freq Control* 2018;65:2365-2380.
- [7] Espindola D, Lin F, Soulioti DE, Dayton PA, Pinton GF. Adaptive multifocus beamforming for contrast-enhanced-super-resolution ultrasound imaging in deep tissue. *IEEE Trans Ultrason Ferroelectr Freq Control* 2018;65:2255-2263.
- [8] Wang R, Lee WN. Automated microbubble discrimination in ultrasound localization microscopy by vision transformer. *IEEE Trans Ultrason Ferroelectr Freq Control* 2025;72:1134-1146.
- [9] Errico C, Pierre J, Pezet S, Desailly Y, Lenkei Z, Couture O, et al. Ultrafast ultrasound localization microscopy for deep super-resolution vascular imaging. *Nature* 2015;527:499-502.
- [10] Desailly Y, Couture O, Fink M, Tanter M. Sono-activated ultrasound localization microscopy. *Applied Physics Letters* 2013;103:174107.
- [11] Tanter M, Fink M. Ultrafast imaging in biomedical ultrasound. *IEEE Trans Ultrason Ferroelectr Freq Control* 2014;61:102-19.
- [12] Desailly Y, Pierre J, Couture O, Tanter M. Resolution limits of ultrafast ultrasound localization microscopy. *Phys Med Biol* 2015;60:8723-8740.
- [13] Zhang G, Yu J, Lei YM, Hu JR, Hu HM, Harput S, et al. Ultrasound super-resolution imaging for the differential diagnosis of thyroid nodules: a pilot study. *Front Oncol* 2022;12:978164.
- [14] Ackermann D, Schmitz G. Detection and tracking of multiple microbubbles in ultrasound B-mode images. *IEEE Trans Ultrason Ferroelectr Freq Control* 2016;63:72-82.
- [15] Christensen-Jeffries K, Browning RJ, Tang MX, Dunsby C, Eckersley RJ. In vivo acoustic super-resolution and super-resolved velocity mapping using microbubbles. *IEEE Trans Med Imaging* 2015;34:433-440.
- [16] Hou C, Wang Y, Wang L, Yang L, Li S, Nie F, et al. Super-resolution ultrasound imaging indirectly reveals neurovascular uncoupling in substantia nigra of a Parkinson's disease model. *NPJ Parkinsons Dis* 2026;12(1):130.
- [17] Denis L, Bodard S, Hingot V, Chavignon A, Battaglia J, Renault G, et al. Sensing ultrasound localization microscopy for the visualization of glomeruli in living rats and humans. *EBioMedicine* 2023;91:104578.
- [18] Brown J, Christensen-Jeffries K, Harput S, Zhang G, Zhu J, Dunsby C, et al. Investigation of microbubble detection methods for super-resolution imaging of microvasculature. *IEEE Trans Ultrason Ferroelectr Freq Control* 2019;66:676-691.
- [19] Zhang X, Liu P, Qian X. A cost-efficient multi-angle fusion deep learning for ultrasound localization microscopy. *IEEE Trans Biomed Eng* 2026;73:2123-2132.
- [20] Christensen-Jeffries K, Harput S, Brown J, Wells PNT, Aljabar P, Dunsby C, et al. Microbubble axial localization errors in ultrasound super-resolution imaging. *IEEE Trans Ultrason Ferroelectr Freq Control* 2017;64:1644-1654.
- [21] Taghavi I, Andersen SB, Hoyos CAV, Schou M, Gran F, Hansen KL, et al. Ultrasound super-resolution imaging with a hierarchical Kalman tracker. *Ultrasonics* 2022;122:106695.
- [22] Ghosh D, Xiong F, Sirsi SR, Shaul PW, Mattrey RF, Hoyt K, et al. Toward optimization of in vivo super-resolution ultrasound imaging using size-selected microbubble contrast agents. *Med Phys* 2017;44:6304-6313.
- [23] Lerendegui M, Yan J, Stride E, Dunsby C, Tang MX. Understanding the effects of microbubble concentration on localization accuracy in super-resolution ultrasound imaging. *Phys Med Biol* 2024;69.
- [24] Zhang G, Hu X, Ren X, Zhou B, Li B, Li Y, et al. In vivo ultrasound localization microscopy for high-density microbubbles. *Ultrasonics* 2024;143:107410.
- [25] Lin F, Tsuruta JK, Rojas JD, Dayton PA. Optimizing sensitivity of

- ultrasound contrast-enhanced super-resolution imaging by tailoring size distribution of microbubble contrast agent. *Ultrasound Med Biol* 2017;43:2488-2493.
- [26] Tafast A, Benoudjit N, Bouakaz A. Comparative analysis of semi-adaptive beamforming algorithms for ultrasound localization microscopy. ICAECCS 2025[Conference abstract].
 - [27] Sobolewski J, Dencks S, Schmitz G. Influence of image discretization and patch size on microbubble localization precision. *IEEE Trans Ultrason Ferroelectr Freq Control* 2024;71:1823-1832.
 - [28] Heiles B, Chavignon A, Hingot V, Lopez P, Teston E, Couture O. Performance benchmarking of microbubble-localization algorithms for ultrasound localization microscopy. *Nat Biomed Eng* 2022;6:605-616.
 - [29] Liu X, Almekkawy M. Ultrasound microbubbles localization using object detection model. *IEEE IUS* 2023;1-4.
 - [30] Ramilo U, Garay G, Barolin J, Sorriba V, Bube M, Damián JP, et al. Comparison of microbubble tracking algorithms for ultrasound localization microscopy. GMEPE/PAHCE 2026[Conference abstract].
 - [31] Andersen SB, Taghavi I, Hoyos CAV, Sogaard SB, Gran F, Lönn L, et al. Super-resolution imaging with ultrasound for visualization of the renal microvasculature in rats before and after renal ischemia: a pilot study. *Diagnostics (Basel)* 2020;10:862.
 - [32] Harput S, Christensen-Jeffries K, Brown J, Li Y, Williams KJ, Davies AH, et al. Two-stage motion correction for super-resolution ultrasound imaging in human lower limb. *IEEE Trans Ultrason Ferroelectr Freq Control* 2018;65:803-814.
 - [33] Opacic T, Dencks S, Theek B, Piepenbrock M, Ackermann D, Rix A, et al. Motion model ultrasound localization microscopy for pre-clinical and clinical multiparametric tumor characterization. *Nat Commun* 2018;9:1527.
 - [34] Yu J, Lavery L, Kim K. Super-resolution ultrasound imaging method for microvasculature in vivo with a high temporal accuracy. *Sci Rep* 2018;8:13918.
 - [35] Dencks S, Piepenbrock M, Opacic T, Krauspe B, Stickeler E, Kiessling F, et al. Clinical pilot application of super-resolution US imaging in breast cancer. *IEEE Trans Ultrason Ferroelectr Freq Control* 2019;66:517-526.
 - [36] Kanoulas E, Butler M, Rowley C, Voulgaridou V, Diamantis K, Duncan WC, et al. Super-resolution contrast-enhanced ultrasound methodology for the identification of in vivo vascular dynamics in 2D. *Invest Radiol* 2019;54:500-516.
 - [37] Schneider M. Characteristics of SonoVue trade mark. *Echocardiography* 1999;16:743-746.
 - [38] Bianconi E, Piovesan A, Facchin F, Beraudi A, Casadei R, Frabetti F, et al. An estimation of the number of cells in the human body. *Ann Hum Biol* 2013;40:463-471.
 - [39] Ivanov KP, Kalinina MK, Levkovich YuI. Blood flow velocity in capillaries of brain and muscles and its physiological significance. *Microvasc Res* 1981;22:143-155.
 - [40] Hingot V, Errico C, Heiles B, Rahal L, Tanter M, Couture O. Microvascular flow dictates the compromise between spatial resolution and acquisition time in Ultrasound Localization Microscopy. *Sci Rep* 2019;9:2456.
 - [41] Christensen-Jeffries K, Brown J, Harput S, Zhang G, Zhu J, Tang MX, Dunsby C, Eckersley RJ. Poisson statistical model of ultrasound super-resolution imaging acquisition time. *IEEE Trans Ultrason Ferroelectr Freq Control* 2019;66:1246-1254.
 - [42] Huang C, Lowerison MR, Trzasko JD, Manduca A, Bresler Y, Tang S, et al. Short acquisition time super-resolution ultrasound microvessel imaging via microbubble separation. *Sci Rep* 2020;10:6007.
 - [43] Dencks S, Piepenbrock M, Schmitz G. Assessing vessel reconstruction in ultrasound localization microscopy by maximum likelihood estimation of a zero-inflated poisson model. *IEEE Trans Ultrason Ferroelectr Freq Control* 2020;67:1603-1612.
 - [44] Solomon O, van Sloun RJG, Wijkstra H, Mischi M, Eldar YC. Exploiting flow dynamics for superresolution in contrast-enhanced ultrasound. *IEEE Trans Ultrason Ferroelectr Freq Control* 2019;66:1573-1586.
 - [45] Huang C, Zhang W, Gong P, Lok UW, Tang S, Yin T, et al. Super-resolution ultrasound localization microscopy based on a high frame-rate clinical ultrasound scanner: an in-human feasibility study. *Phys Med Biol* 2021;66:10.1088/1361-6560/abef45.
 - [46] Lowerison MR, Sekaran NVC, Zhang W, Dong Z, Chen X, Llano DA, et al. Aging-related cerebral microvascular changes visualized using ultrasound localization microscopy in the living mouse. *Sci Rep* 2022;12:619.
 - [47] Zhang Z, Hwang M, Kilbaugh TJ, Sridharan A, Katz J. Cerebral microcirculation mapped by echo particle tracking velocimetry quantifies the intracranial pressure and detects ischemia. *Nat Commun* 2022;13(1):666.
 - [48] Shin Y, Lowerison MR, Wang Y, Chen X, You Q, Dong Z, et al. Context-aware deep learning enables high-efficacy localization of high concentration microbubbles for super-resolution ultrasound localization microscopy. *Nat Commun* 2024;15:2932.
 - [49] Beliard B, Ahmanna C, Tiran E, Kanté K, Deffieux T, Tanter M, et al. Ultrafast Doppler imaging and ultrasound localization microscopy reveal the complexity of vascular rearrangement in chronic spinal lesion. *Sci Rep* 2022;12:6574.
 - [50] Ma H, Yang Y, Gao M, He Q, Zhao D, Luo J, et al. A novel rat model of cerebral small vessel disease and evaluation by super-resolution ultrasound imaging. *J Neurosci Methods* 2022;379:109673.
 - [51] Li M, Wang W, Su Z, Wu J, Yang Y, Wang L, et al. Clinically translatable ultrasound localization microscopy reveals cerebrovascular remodelling and prognosis in patients with traumatic brain injury. *Nat Biomed Eng* 2026.
 - [52] Kwiatkowska E, Kwiatkowski S, Dziedzicko V, Tomaszewicz I, Domański L. Renal microcirculation injury as the main cause of ischemic acute kidney injury development. *Biology (Basel)* 2023;12:327.
 - [53] Bodard S, Denis L, Hingot V, Chavignon A, Hélén O, Anglicheau D, et al. Ultrasound localization microscopy of the human kidney allograft on a clinical ultrasound scanner. *Kidney Int* 2023;103:930-935.
 - [54] Lowerison MR, Huang C, Lucien F, Chen S, Song P. Ultrasound localization microscopy of renal tumor xenografts in chicken embryo is correlated to hypoxia. *Sci Rep* 2020;10:2478.
 - [55] Chen Q, Yu J, Rush BM, Stocker SD, Tan RJ, Kim K. Ultrasound super-resolution imaging provides a noninvasive assessment of renal microvasculature changes during mouse acute kidney injury. *Kidney Int* 2020;98:355-365.
 - [56] Zhang H, Huang L, Yang Y, Qiu L, He Q, Liu J, et al. Evaluation of early diabetic kidney disease using ultrasound localization microscopy: a feasibility study. *J Ultrasound Med* 2023;42:2277-2292.
 - [57] Sogaard SB, Andersen SB, Taghavi I, Hoyos CAV, Christoffersen C, Hansen KL, et al. Super-resolution ultrasound imaging provides quantification of the renal cortical and medullary vasculature in obese Zucker rats: a pilot study. *Diagnostics (Basel)* 2022;12:1626.
 - [58] Qiu L, Zhang J, Yang Y, Zhang H, Lee FF, He Q, et al. In vivo assessment of hypertensive nephrosclerosis using ultrasound localization microscopy. *Med Phys* 2022;49:2295-2308.

- [59] Andersen SB, Taghavi I, Sogaard SB, Hoyos CAV, Nielsen MB, Jensen JA, et al. Super-resolution ultrasound imaging can quantify alterations in microbubble velocities in the renal vasculature of rats. *Diagnostics (Basel)* 2022;12:1111.
- [60] Zhang W, Lowerison MR, Dong Z, Miller RJ, Keller KA, Song P. Super-resolution ultrasound localization microscopy on a rabbit liver VX2 tumor model: an initial feasibility study. *Ultrasound Med Biol* 2021;47:2416-2429.
- [61] Zhang L, Tian J, Chen Y, Hua Q, Yuan C, Zhang Y, et al. Super-resolution ultrasound combined with contrast-enhanced ultrasound: an exploratory approach for differentiating benign from malignant renal tumors based on microvascular characteristics. *Acad Radiol* 2026;S1076-6332-00550-7.
- [62] Li J, Wei C, Ying T, Liu Y, Wang R, Li M, et al. Differentiation of benign and malignant breast lesions by ultrasound localization microscopy. *Insights Imaging* 2025;16:128.
- [63] Lei S, Ma T, Gao Z, Zhang Q, Wang S, Hu D, et al. Endoscopic ultrasound localization microscopy for the evaluation of the microvasculature of gastrointestinal tract tumors in rabbits. *IEEE Trans Biomed Eng* 2022;69:3438-3448.
- [64] Zhang G, Lei YM, Li N, Yu J, Jiang XY, Yu MH, et al. Ultrasound super-resolution imaging for differential diagnosis of breast masses. *Front Oncol* 2022;12:1049991.
- [65] Xia S, Hua Q, Song Y, Yuan C, Zheng Y, Tao R, et al. Super-resolution ultrasound imaging of intranodal lymphatic sinuses for predicting sentinel lymph node metastasis in breast cancer: a preliminary study. *Eur Radiol* 2025;35:6079-6088.
- [66] Lowerison M, Zhang W, Chen X, Fan T, Song P. Characterization of anti-angiogenic chemo-sensitization via longitudinal ultrasound localization microscopy in colorectal carcinoma tumor xenografts. *IEEE Trans Biomed Eng* 2022;69:1449-1460.
- [67] Demeulenaere O, Sandoval Z, Mateo P, Dizeux A, Villemain O, Gallet R, et al. Coronary flow assessment using 3-dimensional ultrafast ultrasound localization microscopy. *JACC Cardiovasc Imaging* 2022;15:1193-1208.
- [68] Wang T, Zhang Y, Chen J, Chen P, Kandi F, Yao J, et al. Ultrasound localization microscopy in identifying high-risk carotid plaque using low-frame-rate ultrasound imaging. *Ultrasound Med Biol* 2026;52:123-132.
- [69] Qian X, Huang C, Li R, Song BJ, Tchelepi H, Shung KK, et al. Super-resolution ultrasound localization microscopy for visualization of the ocular blood flow. *IEEE Trans Biomed Eng* 2022;69:1585-1594.
- [70] Zhu J, Rowland EM, Harput S, Riemer K, Leow CH, Clark B, et al. 3D super-resolution US imaging of rabbit lymph node vasculature in vivo by using microbubbles. *Radiology* 2019;291:642-650.
- [71] Zhu J, Zhang C, Christensen-Jeffries K, Zhang G, Harput S, Dunsby C, et al. Super-resolution ultrasound localization microscopy of microvascular structure and flow for distinguishing metastatic lymph nodes - an initial human study. *Ultraschall Med* 2022;43:592-598.
- [72] He J, Yi H, Tang D, Zhang X, Cui X, Zhang W, et al. Differentiating benign and malignant superficial lymph nodes using super-resolution contrast enhanced ultrasound - a pilot study. *BMC Med Imaging* 2025;25:170.
- [73] Zhang J, Hu Y, Huang X, Huang X, Deng Q, Zhou Q. Ultrafast ultrasound localization microscopy for differential diagnosis of reactive hyperplasia and metastatic cervical lymph nodes: a pilot study. *Quant Imaging Med Surg* 2025;15:10204-10214.
- [74] Li M, Zhang X, Yan J, Shu H, Li Z, Ye C, et al. Non-invasive ultrasound localization microscopy (ULM) in azoospermia: connecting testicular microcirculation to spermatogenic functions. *Theranostics* 2024;14:4967-4982.
- [75] Papanikolaou N, Yan J, Huang B, Davies R, Dunsterville C, De Silva NL, et al. Ultrasound localisation microscopy tracks testicular microvascular adaptations to endocrine function in male infertility. *EBioMedicine* 2026;129:106333.
- [76] Smith CAB, Wilson H, Yan J, Tang MX. Quantitative image markers of super-resolution ultrasound. *EBioMedicine* 2026;124:106108.
- [77] Xia S, Zheng Y, Hua Q, Wen J, Luo X, Yan J, et al. Chinese artificial intelligence alliance for thyroid and breast ultrasound. Super-resolution ultrasound and microvasculomics: a consensus statement. *Eur Radiol* 2024;34:7503-7513.
- [78] Piscaglia F, Bolondi L; Italian Society for Ultrasound in Medicine and Biology (SIUMB) Study Group on Ultrasound Contrast Agents. The safety of Sonovue in abdominal applications: retrospective analysis of 23188 investigations. *Ultrasound Med Biol* 2006;32:1369-1375.
- [79] Hu C, Feng Y, Huang P, Jin J. Adverse reactions after the use of SonoVue contrast agent: characteristics and nursing care experience. *Medicine (Baltimore)* 2019;98:e17745.