Contrast-enhanced ultrasound (CEUS) uses microbubble (MB) contrast agents that are con ned to the vascular space to improve visualization of blood ow and the measurement of tissue perfusion kinetics[17]. It is now known that during the early stages of NAFLD development, fat-laden hepatocytes become swollen, and in NASH, further swelling occurs due to hydropic change (ballooning) of hepatocytes leading to sinusoidal distortion. Consequently, both intrasinusoidal volume and microvascular blood ow are reduced up to 50% of control[18,19]. is observation was supported by a recent 2019 study that also demonstrated CEUS imaging was more sensitive in diagnosing early stage fatty in ltration-mediated microvascular changes in liver parenchyma

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e overarching goal of our preclinical research was to evaluate the use of in vivo mpUS imaging for classifying normal from steatotic liver. Starting with SWE measurements, Fig. 2 illustrates a temporal sequence of propagating shear waves (0.2–3.2 ms a er pulsed US excitation) in control and MCD diet liver. Inspection of these SWE images reveals that shear waves exhibit a slightly lower speed and are more attenuated at distance in MCD die fed animals at 6 weeks compared to the age-matched controls. From these example cases, the shear wave sp was estimated to be 1.52 and 1.36 m/s for control and MCD diet animals, respectively, whereas the shear wav attenuation was found to be 94.8 and 113.9 Np/m.

B-scan and H-scan US imaging gives insight into backscattered signals from tissue. Representative B-scan U images with an H-scan US colored overlay from the liver parenchyma is presented in Fig. 3. Note the blue color describes local backscatotimEeetl999809 frla)-10.39()12.699999809(e)11.699999809(m s.5(s)m).7(a)95.3(l)- adeic-6(t id) .9erii-2.9(a)14(l)erietsor 4( )ae 19.tu-3(a)99.tioiom4(e i)12.nfM(TTJ /T1_011 Tf . Tc 0(BTj /CT110 typical TIC describes US image intensity values over time in a ROI. As the MB contrast agent was administered via a tail vein catheter, CEUS image enhancement first occured in the IVC, aorta, porta vein, and then the liver parenchyma, Fig. 4

utilized, which is not shown since 6 features cannot be visualized. To validate the classi ciation, when dividing the features into training and testing sets, classi cation accuracies were 100% and 99%, respectively, for the two-category approach. For the three-category classi cation, the accuracies were 93% and 82%, respectively.

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Quantitative detection of liver fat and brosis content is of great importance in the evaluation and staging of NAFLD. e two most prevalent imaging techniques to examine NAFLD patients include magnetic resonance agreement and the subjective nature of traditional US imaging[40], more quantitative US-based measurements have been explored. More specifically, several elastography studies have reported sensitivity to identifying NASH with fibrosis in patients with biopsy-proven NAFLD[41–43]. However, recent reports have shown that the sensitivity is improved considerably when SWE information is combined with quantitative US measures of tissue scattering[44]. Franceschini et al. combined spectral-based quantities with SWE to improve classification performance[45]. It was also been shown that the combination of three US parameters (stiffness, effective scatterer size, and acoustic concentration) provides the best classification performance when compared to classifications obtained from the spectral-quantitative US or stiffness parameters alone. However, this study was conducted on ex vivo liver samples and did not demonstrate the ability to distinguish NASH from fibrotic tissue. In another study, it was demonstrated that the classification of NASH can be improved when SWE is combined with quantitative US parameters[44]. The area under the receiver-operating characteristic curve (AUROC) increased from 0.63 for SWE

Herein we introduced an in vivo mpUS imaging approach that incorporated shear wave parameters to deter-mine viscoelasticity, CEUS to evaluate liver vascularity and perfusion, and H-scan US to estimate tissue micro structural information. e formation of steatosis leads to a decrease in liver tissue shear wave speed measures and an increase in attenuation[32–46]. However, the shear wave speed also increases with increasing sti ness, where this can be observed from control measures where the liver appeared to stiffen with age[47,48]. H-scan US imaging was used to obtain the microstructure properties of liver tissue by analyzing backscattered US signals. Progressiv accumulation of fatty deposits in the liver altered the spectral content, leading to a signi cant blue shi in the

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