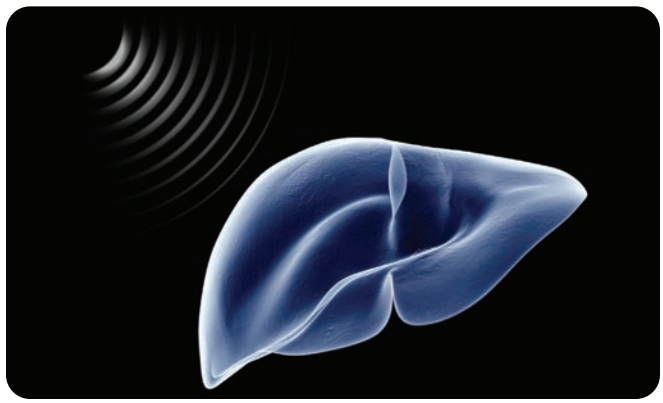
CASE STUDY

Temporal tracking of an effective intervention in rodent liver fibrosis.

A case study using a new ultrasonic research tool for rapid and non-invasive 3D tissue assessment

The liver, as the largest solid internal organ of the body, plays a central role in multiple physiological processes, including metabolism, detoxification, nutrient storage, and protein synthesis. Damage to the liver, whether arising from metabolic disorders, viral infections, or exposure to toxic substances, can manifest in various forms and severity, ranging from abnormal fat accumulation (steatosis) and mild inflammation to the development of scar tissue (fibrosis), progressing to more severe conditions such as cirrhosis or liver failure. The repercussions of liver injury span across various research domains, including metabolic disease, toxicology, cancer, infectious disease, alcoholic disease, and rare genetic conditions like cystic diseases.

Since nearly all chronic liver diseases result in fibrosis, which later causes even worse downstream prognosis (e.g., cirrhosis and liver failure), many different therapeutic approaches to treating liver disease focus either wholly or in part on addressing and reversing fibrosis. Despite the urgency and absence of a cure for liver fibrosis, preclinical research tools for liver studies have been lacking, hindering progress in therapeutic development. The lack of widespread research tools to non-invasively observe critical phenotypes in liver tissue echoes the challenges researchers in oncology faced in the early 2000s; at that time, tracking tumor growth and therapeutic response relied on very crude assessments (e.g., caliper measurements or *ex vivo* tumor weights). The oncology research field struggled with these crude measurements until the introduction of *in vivo* bioluminescence imaging systems, which offered a non-invasive, real-time observation platform, revolutionizing preclinical cancer research.



In a similar vein, a new and fundamentally enabling tool has now become available to preclinical liver disease researchers. The Vega® system has emerged as a significant advancement in non-invasive *in vivo* technologies, enabling researchers to gain a deep understanding of critical liver disease phenotypes. Functioning as a hands-free, automated, high-throughput preclinical ultrasound imaging system, the Vega has demonstrated effectiveness in noninvasively staging liver disease phenotypes across an array of diverse liver disease models. The list of rodent models tested with the Vega includes chemical injury models (e.g., CCl_4[1]), genetic models such as cholestasis,[2] and metabolic disease models (e.g., western diet,[3] high fat diet and choline-deficient high fat diet[4]) providing critical insights into the natural progression of liver disorders. While the Vega has been used to track these different liver disease models evolving longitudinally, until recently it had not been thoroughly tested tracking *interventions* to these diseases. This ability to not only observe the onset of liver disease phenotypes but also their resolution after therapeutic intervention is a critical piece of the puzzle for labs looking to implement this technology on their own novel therapies for liver disease.

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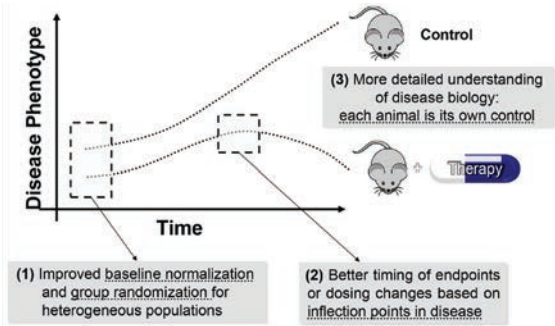
In this study, researchers set out to evaluate the efficacy of the Vega system's automated ultrasound for non-invasively monitoring therapy response in rodent models of non-alcoholic steatohepatitis (NASH). The system, equipped with 3D B-mode and Shear Wave Elastography (SWE) modes, facilitates the tracking of changes in liver echogenicity (brightness) and stiffness, respectively, providing valuable insights into fat accumulation and fibrosis severity.

This study not only confirms the Vega's ability to provide non-invasive insights into disease progression, but also underscores its capacity to monitor disease regression, which is important for evaluating drug efficacy and therapeutic interventions.

Rationale for using *in vivo* imaging

- Provides non-disruptive insights into longitudinal disease progression within individual mice.
- Essential for studies with extended diet-induced models where mistimed endpoints could mean thousands of dollars wasted in lab resources and months of time.
- Helps in eliminating outliers and reducing variability within experimental groups.
- Ensures good baseline normalization between animals or cages, ensuring they are at the same level of disease severity at the commencement of the study thereby reducing downstream biological variability.

Motivation for longitudinal *in vivo* imaging



Methodology and study design

To assess the Vega system's efficacy for monitoring therapy response, the researchers tested two different therapeutic interventions for NASH: diet reversal and CRISPR therapy.

In the first study, mice were fed choline deficient high-fat diets (CDAHFD) for 8 weeks. At this timepoint, half of the mice were transitioned to a less damaging standard high fat diet, simulating effective therapy, while the remaining mice continued on the CDAHFD. A third control group received a standard chow diet for the whole study period. Imaging was performed every 2 weeks for 18 weeks. In the second study, mice were fed a Gubra Amylin NASH diet (GAN) and treated with liver-directed AAV-CRISPR against three candidate genes. Two control groups were included, representing a GAN-only and a vehicle injection group, and imaging was performed every 8 weeks for 32 weeks.

The diets in both treatment approaches were selected for their known ability to induce liver damage, providing a platform for assessing therapeutic responses. Longitudinal imaging using 3D ultrasound was utilized to measure liver echogenicity and stiffness, enabling the researchers to evaluate the impact of therapeutic interventions on liver health over time.

Results

Diet reversal

Researchers presented compelling evidence that liver disease instantiation and resolution can be tracked within individual mice noninvasively by the Vega system. Both qualitative and quantitative liver assessments are shown in Figures 1 and 2, respectively. Figure 1 demonstrates how mice on the CDAHFD exhibited increased liver stiffness over time, which was clearly reversed following the dietary switch at 8 weeks. This reversal is further illustrated in Figure 2, where both liver echogenicity and stiffness rapidly decline following the timepoint where the diet was switched.

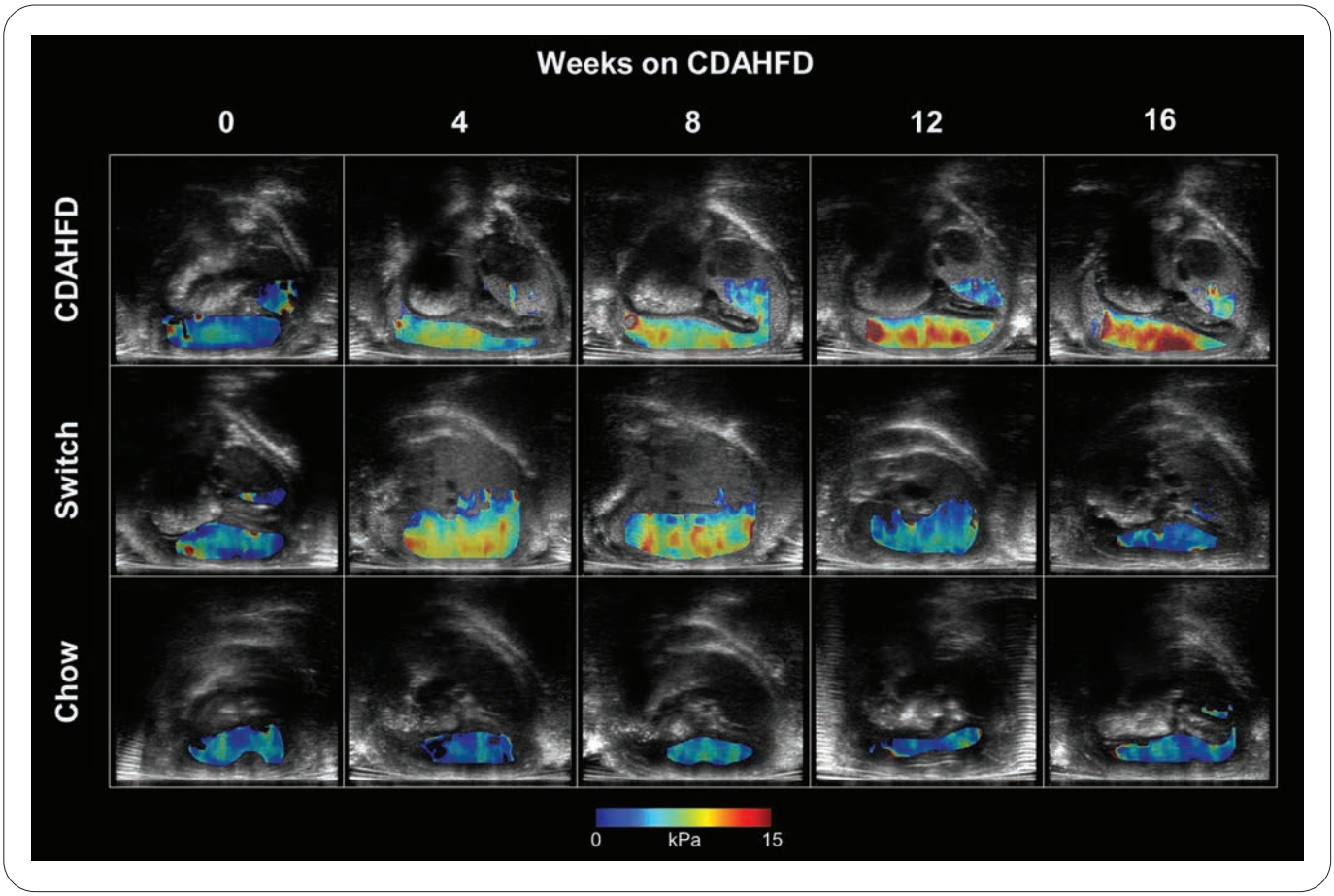


Figure 1: CDAHFD Image Data. Representative liver images in the three groups across the course of the CDAHFD diet-reversal study. CDAHFD elicited a strong response in noninvasive markers of liver echogenicity and stiffness, with clear reversal at the diet switch timepoint.

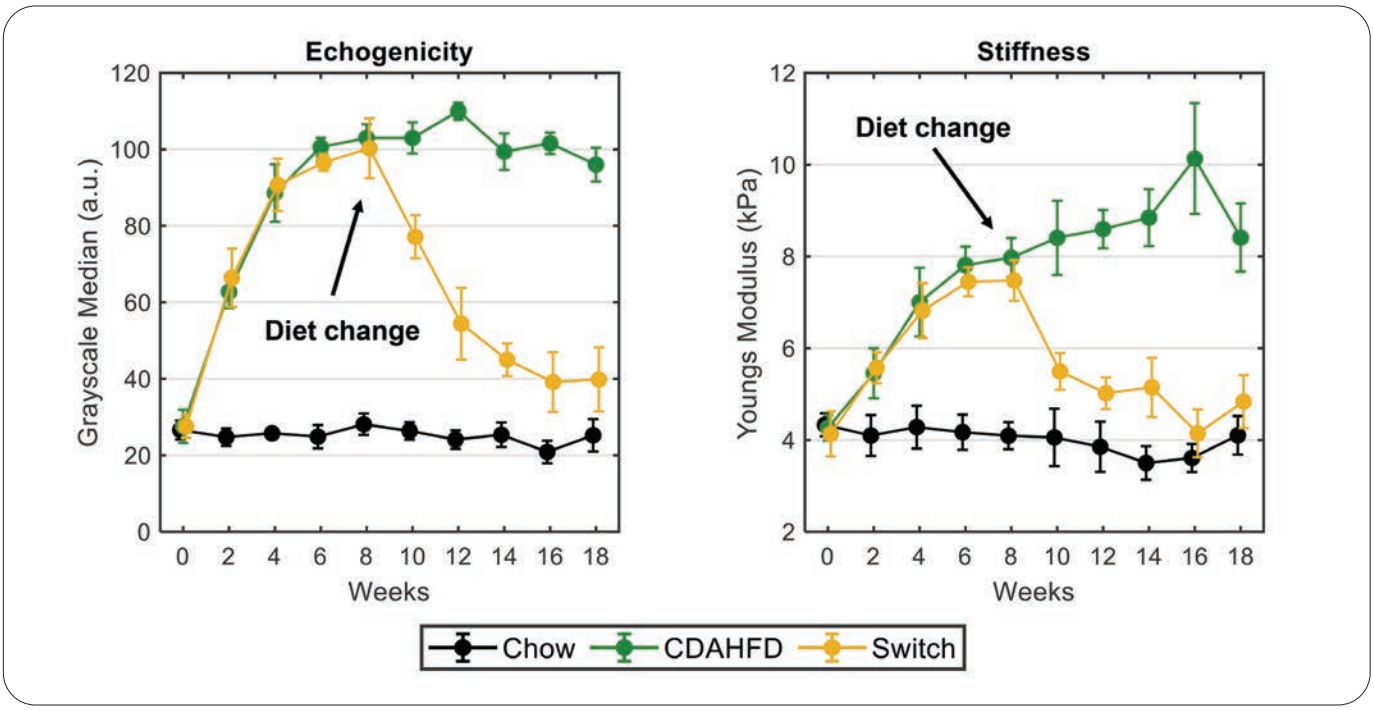


Figure 2: CDAHFD Noninvasive Quantification. Longitudinal progression of liver echogenicity and stiffness over 18 weeks in mice fed CDAHFD, standard chow, or switch from CDAHFD → chow. Error bars represent mean ± std.

CRISPR therapy

Figure 3 shows how both brightness and stiffness of the livers of mice with no CRISPR treatment increased over time in response to GAN diet feeding, confirming the Vega's ability to track changes in liver disease phenotypes over time.

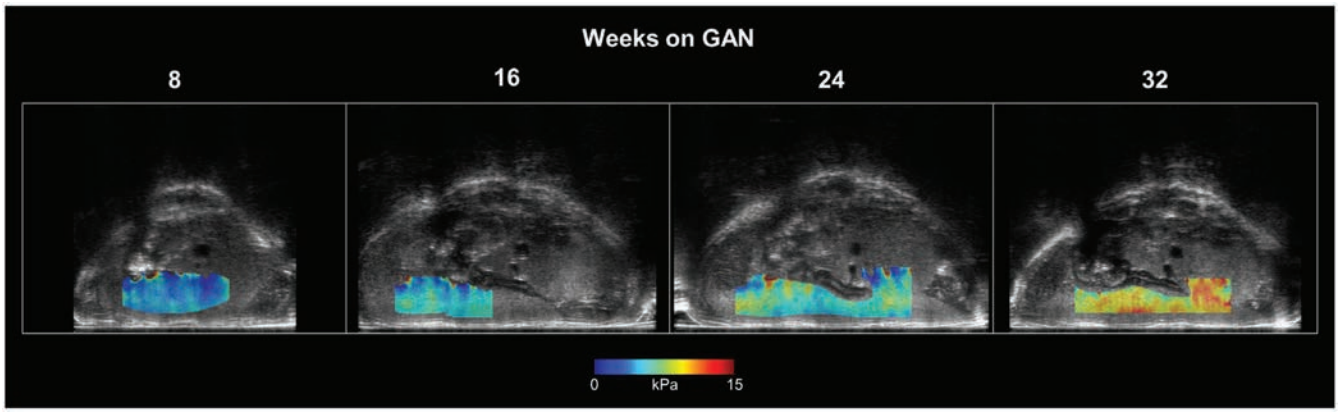


Figure 3: GAN + CRISPR Image Data. Representative images showing both brightness and stiffness images of the livers in mice on a GAN diet with no CRISPR treatment ("Control" group). Body weight, liver volume, echogenicity, and stiffness all increased substantially in response to GAN diet feeding.

While liver echogenicity readings (a marker of fatty liver) increased over time in all groups, Figure 4 demonstrates how differences in liver stiffness readings were identified between the CRISPR treatment groups. Specifically, liver stiffness was delayed and lower in the CRISPR-2 treatment group, suggesting that this treatment knocked out or edited a gene that protected against liver stiffness associated with the GAN diet.

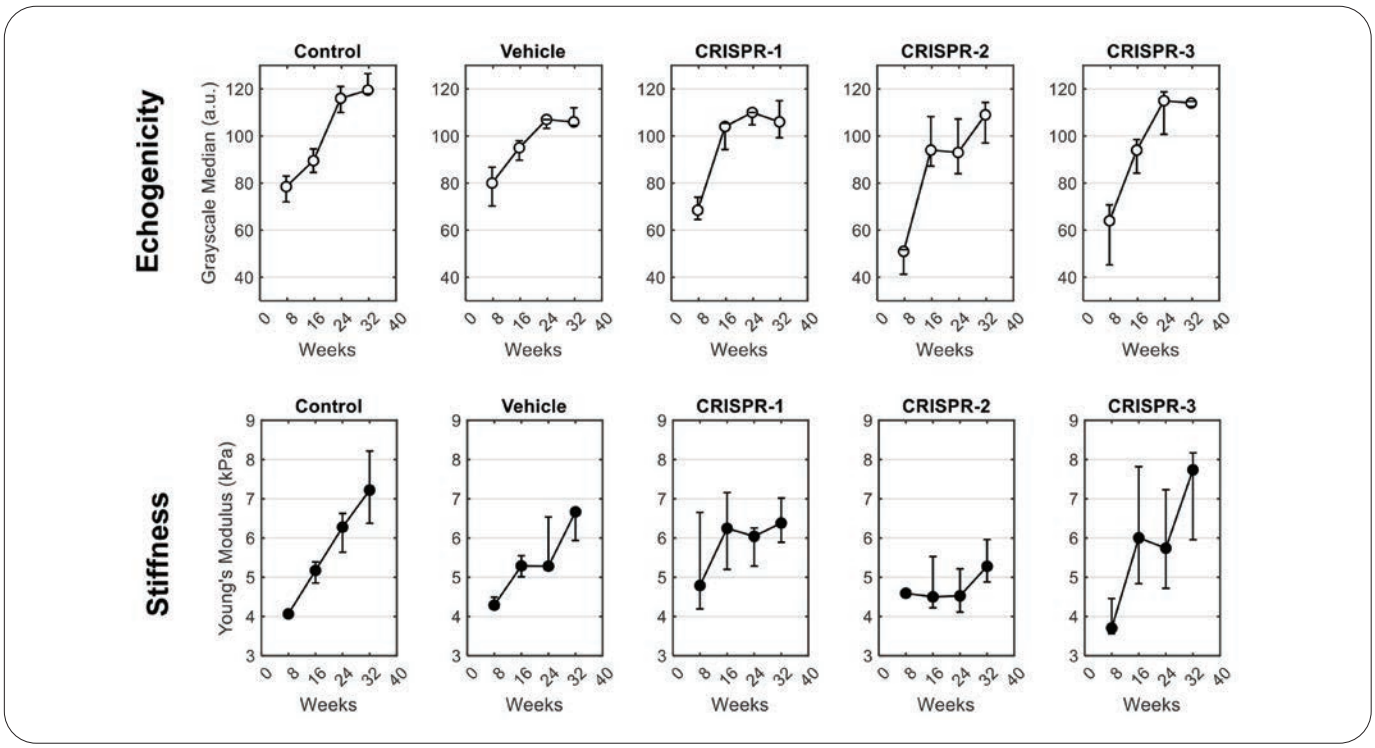


Figure 4: GAN Noninvasive Quantification. Longitudinal progression of liver echogenicity and liver stiffness readings over 32 weeks in mice fed a GAN diet with CRISPR treatment. Echogenicity increased over time across all groups in a similar fashion, plateauing after 24-32 weeks. Stiffness also increased over time, however CRISPR-2 treatment appeared to delay the onset of stiffness rise, suggesting less liver damage. Error bars represent median ±IQR.

Importantly, Figure 5 illustrates the Vega's ability to identify mice that developed spontaneous tumors following CRISPR treatment (an unintended consequence of the genetic alterations in the treated groups). At week 8, there is no visible tumor but the echotexture of the liver appears heterogenous and irregular, hinting at underlying changes. By week 16 a small mass is visible and by week 24 the mass undergoes significant expansion. This information is essential in the field of cell and gene therapy, so that unintended outcomes can be identified early and to ensure that that genetic interventions are refined.

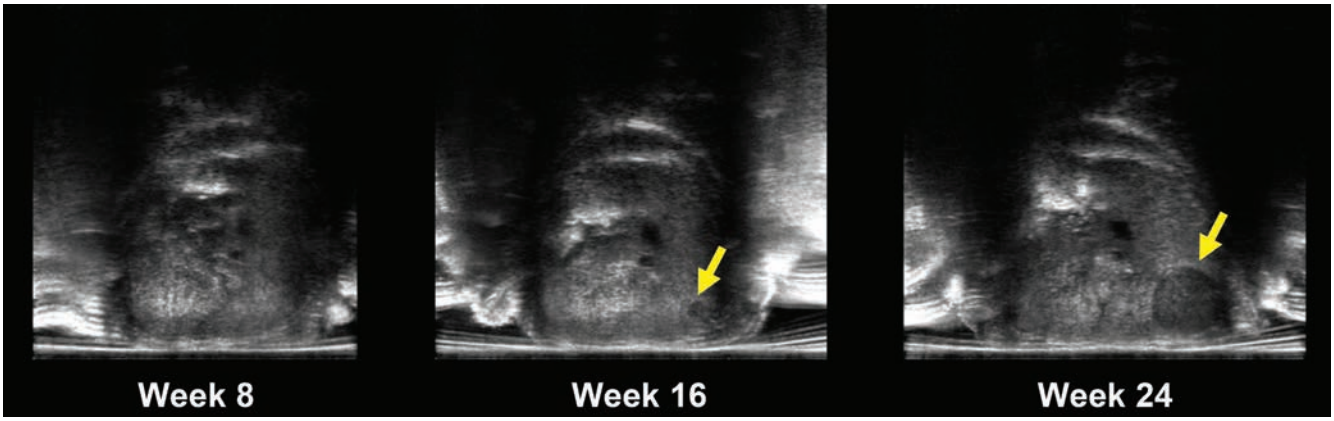


Figure 5: Spontaneous Liver Tumors. Representative images of a spontaneous liver tumor developing over time. At week 8, the tumor is not visible however the echotexture of the liver appears heterogenous and irregular. By week 16, a small echolucent mass is visible (yellow arrow, <1 mm^3), and by week 24 the mass had expanded significantly (716 mm^3).

Study relevance

Visualizing and quantifying the progression and regression of liver echogenicity and stiffness through non-invasive imaging offers a valuable means to monitor liver disease over time in response to therapeutic interventions. The diet intervention study, which illustrated disease progression and regression, highlighted the Vega system's potential for showing the dynamic changes in liver phenotypes in response to effective therapy. The delayed rise in liver stiffness following CRISPR-2 treatment in the second study not only supports the Vega's ability to identify effective treatments, but also emphasizes how this finding might not have been evident from echogenicity changes alone. The Vega also proved invaluable in detecting unintended side effects, particularly the development of spontaneous liver tumors following CRISPR treatment. This capability is crucial in the context of genetic interventions, where unexpected consequences need to be promptly identified and addressed.

Conclusion

Overall, this study marks a significant step forward in validating a cutting edge tool for liver disease researchers, namely its ability to noninvasively assess liver fibrosis and steatosis in response to effective therapeutic interventions. The study demonstrates the ability of the Vega system to provide valuable, noninvasive insights into liver disease progression and the efficacy of therapeutic interventions in mouse models.

SWE is an ultrasound-based stiffness quantification technology that is used routinely for noninvasive liver fibrosis assessment in the clinic. However, it is not widely utilized in rodent models. As demonstrated in this study, the combination of echogenicity and stiffness measurements enables more informed decisions regarding treatment efficacy, disease progression, and potential interventions. This dual approach proves instrumental in identifying treatments that positively influence both echogenicity and stiffness, offering a more comprehensive understanding for managing liver diseases effectively.

Looking ahead, future work will focus on further automating the workflow, leveraging machine learning approaches to streamline data acquisition and analysis. This move reflects the commitment of the Revvity team to enhance the efficiency and precision of non-invasive assessments in liver research, ensuring the continued advancement of therapeutic development.

https://www.revvity.com/category/ultrasound-imaging

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