TECHNICAL NOTE

Non-linear contrast mode using the Vega ultrasound system.

Introduction

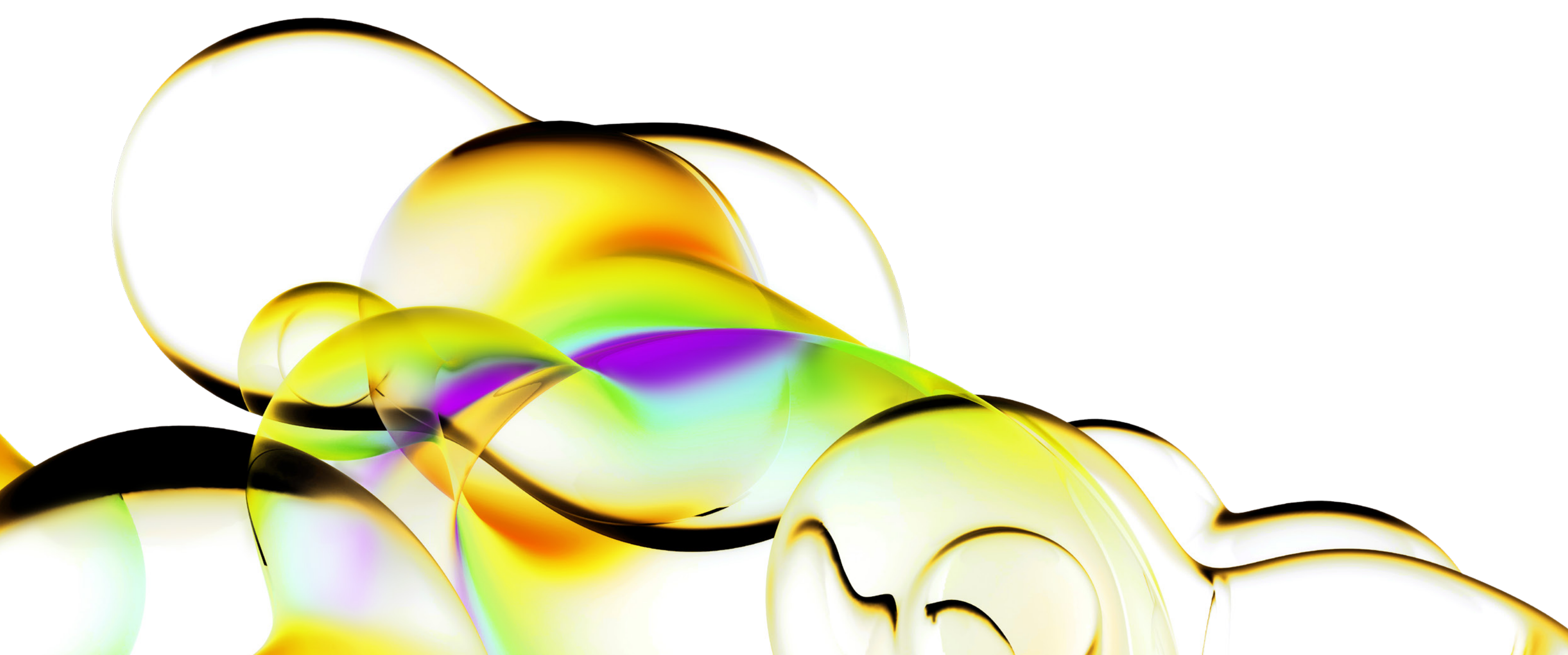
Non-linear contrast (NLC) imaging using the Vega™ system leverages the unique acoustic properties of VesselVue™ microbubble contrast agents to produce high-resolution, highly sensitive images of blood flow and tissue perfusion. By exploiting the non-linear oscillations of these microbubbles in response to ultrasound waves, SonoEQ™ analysis software can differentiate contrast agent signals from surrounding tissue with high clarity.

This technical note highlights various aspects of the Vega ultrasound system's NLC mode including:

1. What is NLC and what does it do?
2. What are the major application areas?
3. Scanning modes and acquisition settings
4. New analysis workflows using SonoEQ software
5. Additional resources for VesselVue microbubble contrast agent

What is NLC and how does it work?

NLC imaging in ultrasound leverages the unique acoustic properties of microbubble contrast agents, which are tiny gas-filled spheres that respond nonlinearly to ultrasound waves. Unlike tissue that reflects sound waves linearly, microbubbles produce harmonic frequencies when excited with ultrasound. In other words, microbubbles "sound" different than normal tissues, and it is possible to "listen" for this unique acoustic signature to help separate signals originating from tissue and the contrast agents.



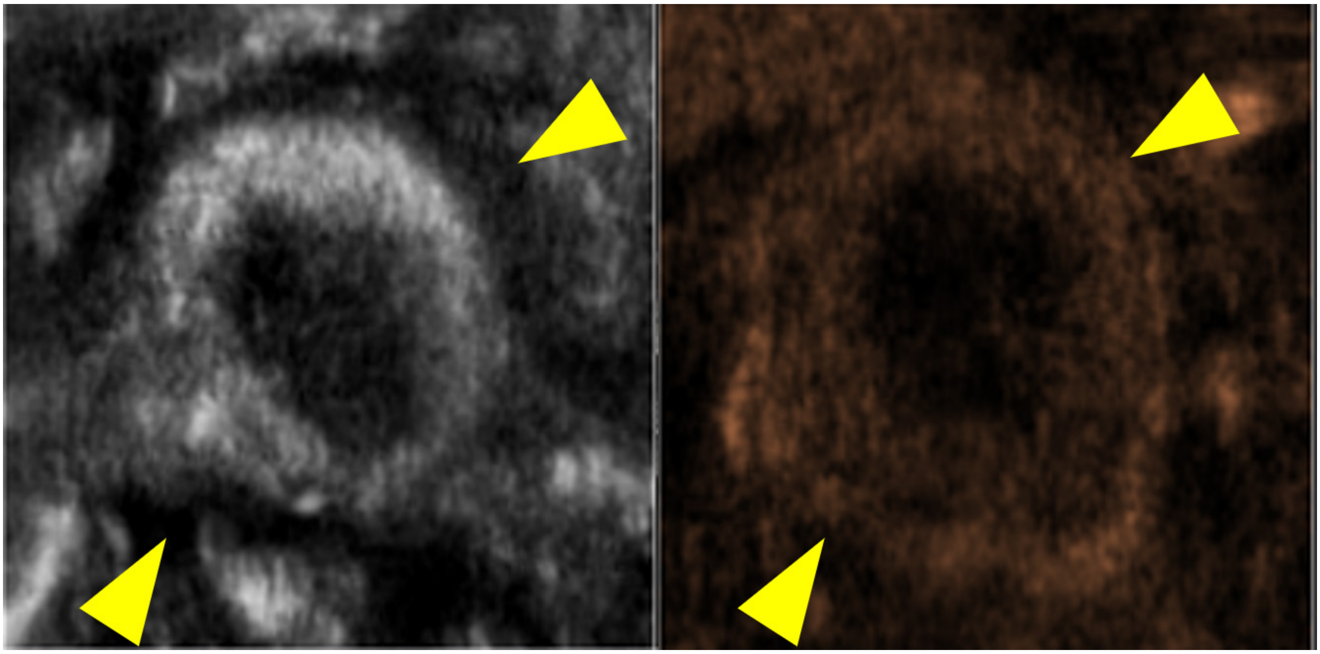


Figure 1: B-mode (left) and NLC (right) image of a tumor. The border is easier to recognize in the NLC image due to the enhanced blood flow at the periphery of the tumor. This enhances the true border of the tumor and makes volumetric measurements more accurate.

NLC mode on the Vega ultrasound system exploits this nonlinear behavior to create high-contrast images of the blood pool with excellent spatial and temporal resolution. NLC significantly reduces tissue associated background, enables better visualization of blood vessels, and enhances capabilities in various applications such as tumor assessment (Figure 1), organ perfusion evaluation (Figure 2), and cardiovascular imaging.

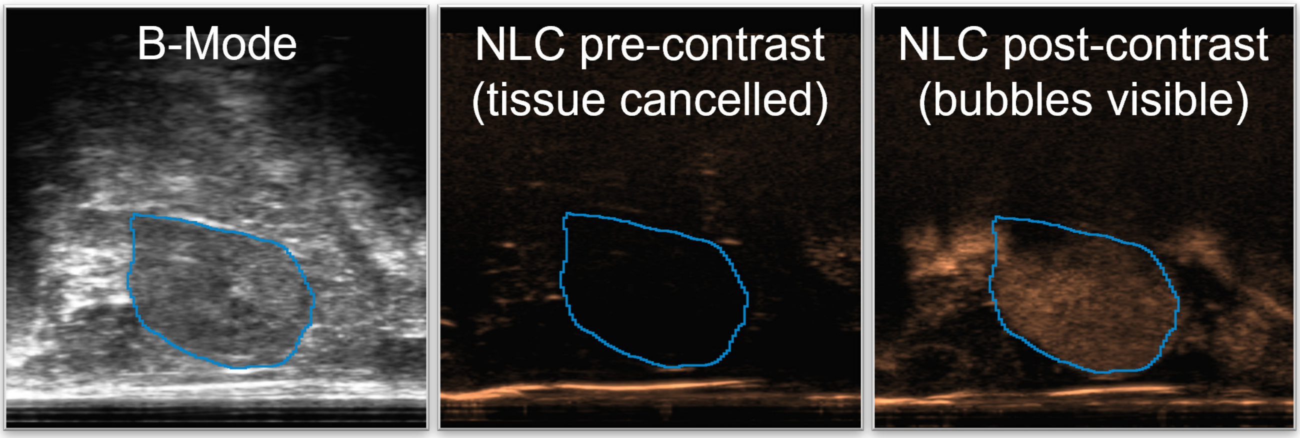


Figure 2: Demonstration of tissue cancellation and contrast imaging with NLC. (Left) A reference B-mode of a mouse kidney. (Center) The NLC mode achieves excellent suppression of tissue associated background signal. (Right) NLC is very sensitive to the VesselVue contrast agent.

NLC scan modes

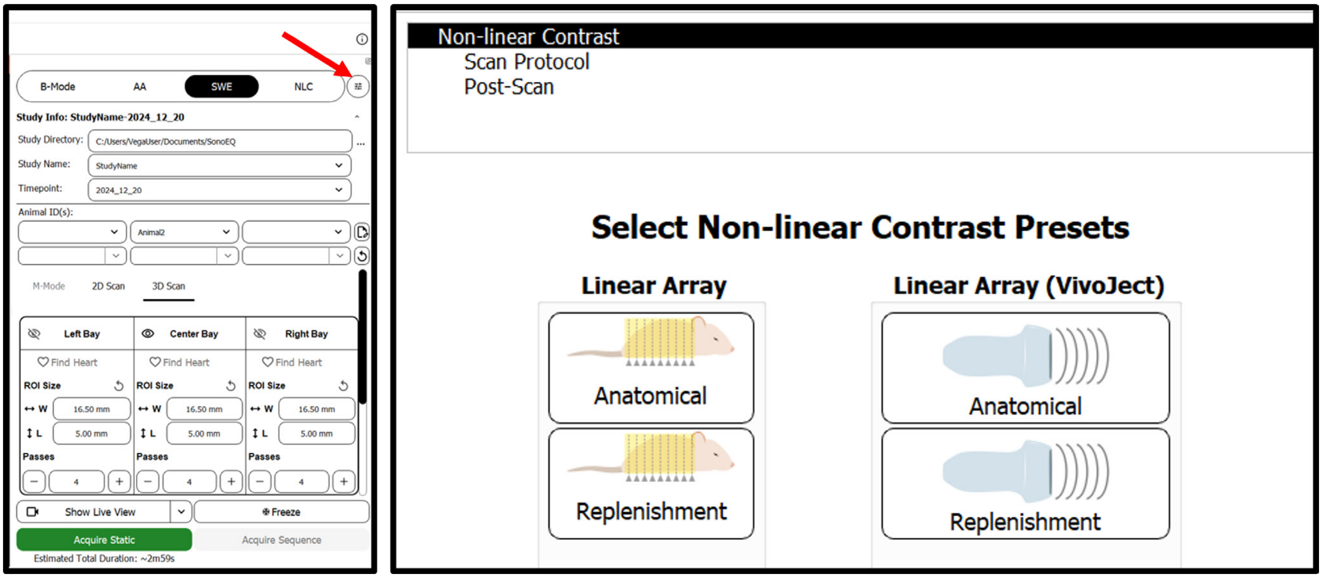


Figure 3: To apply an NLC preset, first open the settings tray by clicking the settings button (left). Then, select one of the factory presets (right).

NLC supports two types of acquisitions: anatomical and replenishment. Anatomical images help-define tissue borders while replenishment images provide perfusion information. Both image types can be acquired in 2D or 3D on the Vega system or on the VivoJect™ image-guided injection system (2D only). Regardless of how they are acquired, the general workflow is the same (Figure 4).

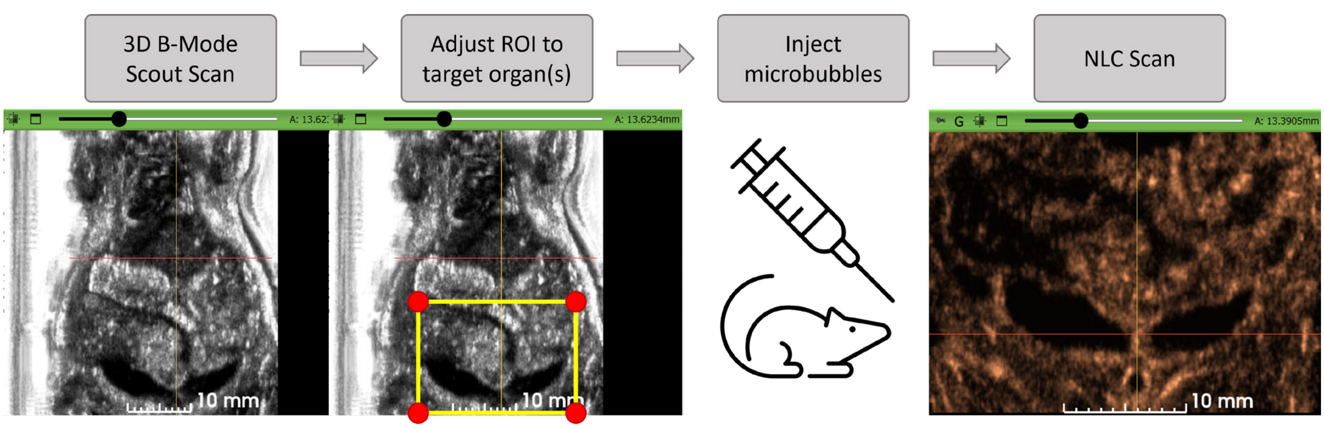


Figure 4: Workflow of NLC on the Vega system.

Anatomical

The Anatomical preset (Figure 5) is designed to acquire high-resolution 2D and 3D scans of microbubble contrast agents. These images can be analyzed in much the same way as B-mode static images to compute volumes, brightness, vessel density, and more. As with other modes on the Vega system, these images are automatically and precisely reregistered to one another, allowing for multi-modal analysis (e.g., measuring organ stiffness with shear wave elastography, organ volume and vessel density using nonlinear contrast).

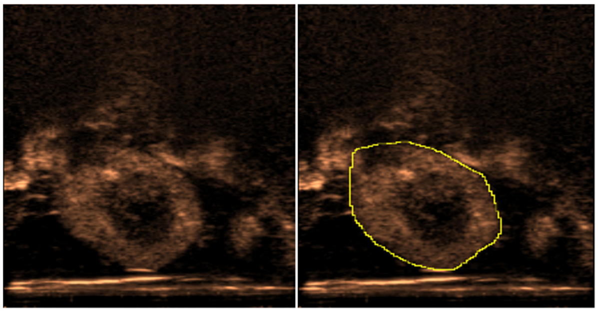


Figure 5: NLC of kidney showing a clearly defined border. The same image with (right) and without (left) a border highlight is shown.

Acquiring an anatomical NLC image (2D or 3D)

Static NLC images are captured the same way 2D or 3D B-mode images are, and take 3 easy steps:

1. Select NLC at the top.
2. Select the **Anatomical** preset (Figure 3) and set your scan region.
3. Hit **Acquire Static** to get your 2D or 3D NLC image.

Replenishment

The Replenishment preset allows the user to collect 2D and 3D flash-replenishment sequences to measure the kinetic properties of different targets. Each acquisition consists of 3 stages: a baseline acquisition, microbubble destruction, and a replenishment acquisition (Figure 6). One or more markups can be drawn on these images to compute kinetic statistics within different regions of interest.

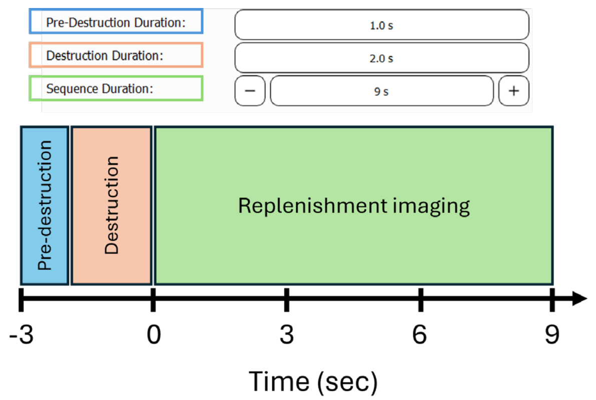


Figure 6: Visual summary of a flash-replenishment acquisition.

Acquiring a flash-replenishment sequence with NLC

To gather perfusion rates, you'll want to use the flash-replenishment preset. This method can also be acquired in 2D, 3D and on the VivoJect system in 3 easy steps:

1. Select NLC at the top.
2. Select the **Replenishment** preset (Figure 3) and set your scan region.
3. Hit **Acquire Destruction Sequence** to get your 2D or 3D NLC image.

Analyzing NLC images

Anatomical NLC images can be used to find borders for 2D or 3D segmentations and are analyzed in the same manner a static B-mode image. With the addition of sequence data to NLC replenishment acquisitions, SonoEQ software incorporates a new analysis tool that enables plotting, curve fitting, and perfusion statistic calculations instantly. The mode utilizes the existing segmentation tools and SonoEQ software automatically recognizes the NLC dataset. The user would simply:

1. Segment the region you wish to analyze.
2. Change the view to show the Red-Plot (Figure 7) pane to show the raw signal, filtered signal (breath artifacts removed), and curve fit.
3. Click the Quantify tab to see the calculated values.

Note: For detailed information please refer to the Vega user manual.

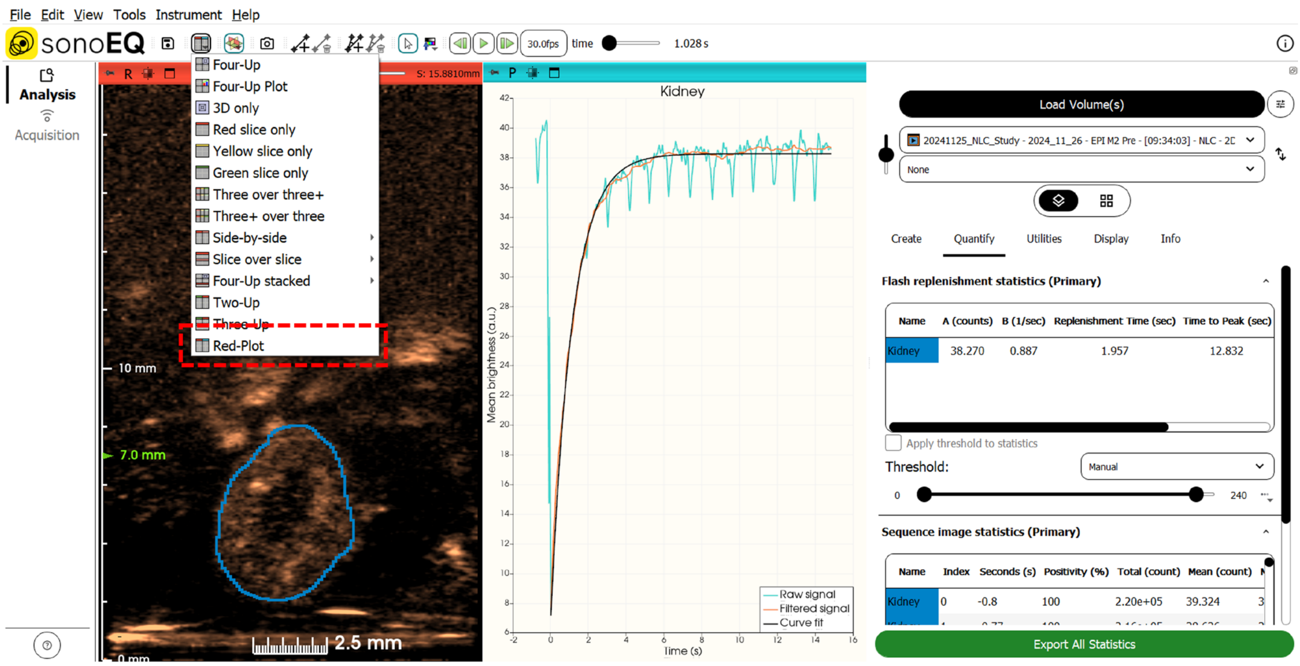


Figure 7: How to open the plot view.

Applications

Angiogenic NLC

Non-linear contrast ultrasound imaging is a powerful tool for monitoring angiogenesis, particularly in tumor growth and metastasis (Ferrara et al., 2007; Cosgrove & Lassau, 2010). By leveraging microbubble contrast agents and their non-linear acoustic properties, this technique provides high-resolution, real-time visualization of tumor vasculature and blood flow dynamics (Deshpande et al., 2010). NLC ultrasound excels in detecting and imaging small, newly formed blood vessels characteristic of tumor angiogenesis, quantifying blood flow volume and velocity, and revealing abnormal vascular patterns (Lassau et al., 2007). Its non-invasive nature allows for longitudinal studies, making it invaluable for assessing treatment response to anti-angiogenic therapies (Willmann et al., 2008). Furthermore, when combined with targeted microbubbles, NLC can detect specific molecular markers associated with angiogenesis (Gessner & Dayton, 2010).

Applications of tissue border enhancement

In addition to tumors, NLC can be applied to highlight borders of other tissues and remove artifacts. The GI tract is often associated with bright spots due to the food, excrement and gas in the stomach and intestinal track but with NLC tissue artefacts are reduced and the gastrointestinal wall can be more clearly visualized (Figure 8). This same contrast enhancement can also be utilized in other tissues like the liver (highly vascularized) and spleen (less vascularized) to help delineate borders between overlapping tissues.

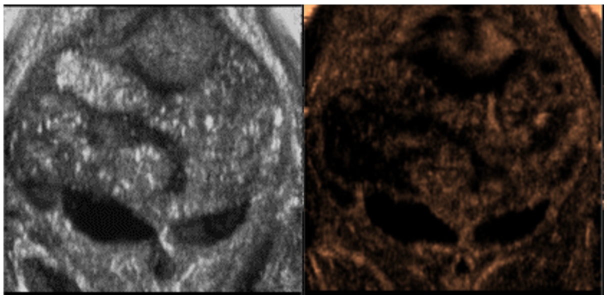


Figure 8: B-mode image (left) and NLC image (right) of GI tract. The white speckling artefacts from food are significantly reduced in NLC mode, show clearer borders of the intestinal wall.

Kinetic imaging via flash-replenishment

NLC images can be acquired and analyzed as sequences, allowing users to measure and quantify the kinetics of various tissues *in vivo*. The Vega system uses a technique known as flash-replenishment imaging. Briefly, a strong pulse is transmitted to disrupt microbubbles within the imaging window, and then a video is recorded as the contrast agent flows back into the tissue. Several perfusion parameters can be derived by analyzing the mean intensity curve for a region of interest (Figure 9). Measuring tissue perfusion rates has many applications, including measuring response to therapy, injury, or various disease states.

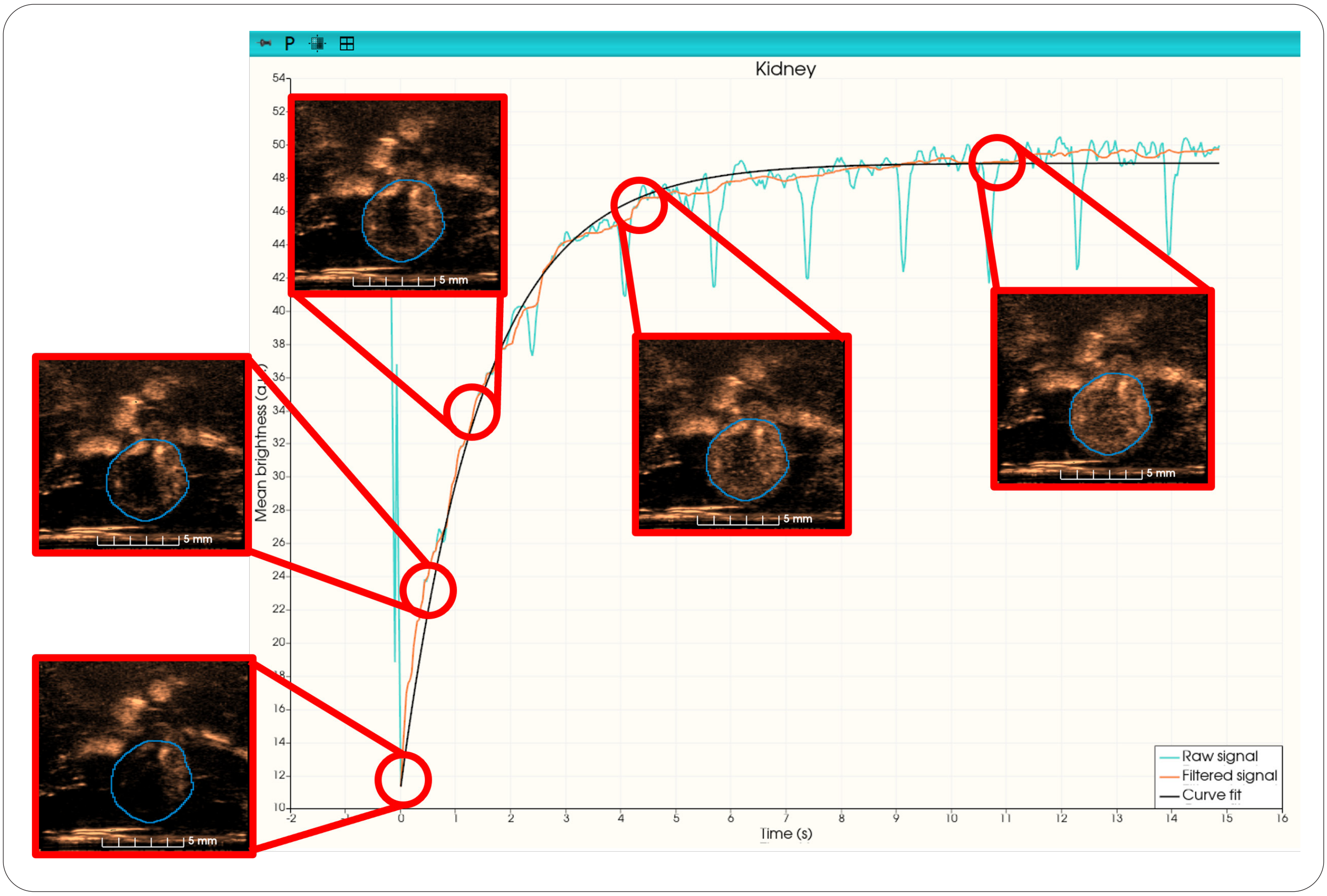


Figure 9: Example of a time intensity curve for a flash-replenishment acquisition.

Improved visualization of injection payload

The VivoJect* device is an accessory for use with the Vega ultrasound system to administer targeted injections in small animals (e.g., mice) for applications such as orthotopic tumor injections, intra-cardiac injections, in utero injections, and more with increased accuracy. NLC on the VivoJect accessory can be used to confirm delivery of cells, therapeutics, etc. in real time using VesselVue microbubble contrast agent. For example, a successful left ventricular injection should result in systemic distribution of the injection payload and can be easily visualized using NLC (Figure 10). VesselVue agent is compatible with most injection payloads, including cells, Matrigel, etc.

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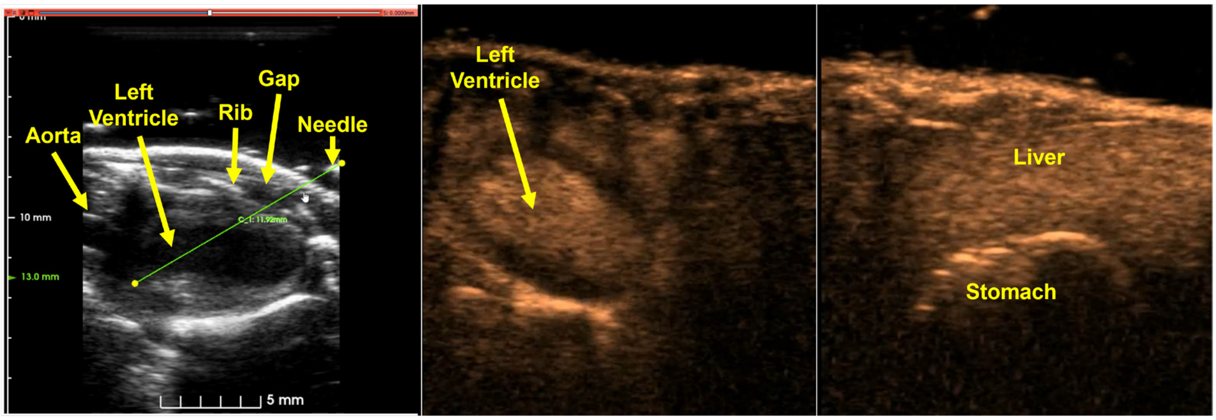

Figure 10: NLC and VesselVue agent can be used to confirm successful injections. Left: B-Mode image of the left ventricle prior to injection. Center: NLC image with the VivoJect systems' transducer shows strong contrast signal in the left ventricle after injection. Right: Contrast signal is also present in the liver, suggesting systemic distribution of the injection payload.

A note on injections

NLC is optimized to work with VesselVue microbubble contrast agents. Bolus injections (intravenously or retro-orbitally administered) and continuous infusions are suitable for NLC acquisitions. A bolus injection will provide approximately 5 minutes of use for multiple readings however, larger scan areas are difficult within this timeframe especially for 3D acquisitions. For larger areas and a more economical usage of microbubbles, we suggest cannulation and slow administration with an infusion pump.

References

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