VivoJect™ image-guided injection accessory

Table of contents

Introduction

The VivoJect™ image-guided injection accessory integrates seamlessly with the Revvity's Vega™ ultrasound system, enhancing its capabilities for preclinical research. The VivoJect device enables researchers to perform image-guided injections in mice with increased accuracy for model instantiation without the need for invasive surgical procedures. By leveraging the high-resolution imaging of the Vega system, the VivoJect accessory allows for delivery of various payloads, including cells, into various organs and tissues.

Engineered with user-friendliness in mind, the VivoJect features a motorized design and intuitive controls, making it accessible to researchers of all experience levels (Figure 1). This combination of increased accuracy, non-invasiveness, and ease of use positions the VivoJect as a valuable tool for advancing preclinical studies and accelerating research outcomes.

This guide provides a high-level overview of the VivoJect hardware along with quick start guides for some common application areas.

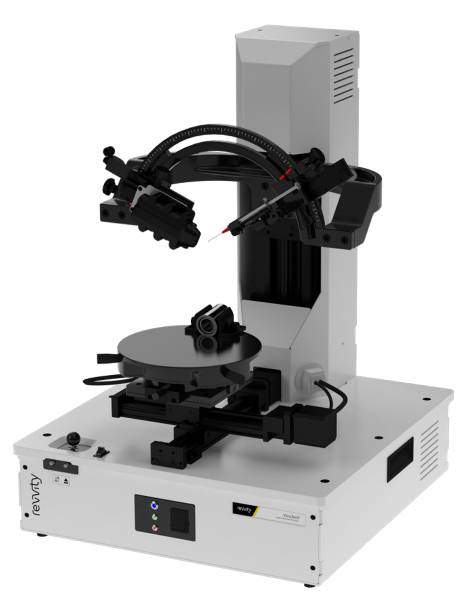


Figure 1: The VivoJect image-guided injection accessory.

Getting started

Software requirements

To get started with the VivoJect accessory, make sure that the version of SonoEQ™ software on the host computer is version 2.2.0 or later. The latest version of SonoEQ software can be downloaded for free from the Revvity *In Vivo* software page.

Connecting to the VivoJect ultrasound system

The ultrasound transducer on the VivoJect device is controlled by the SonoEQ software and is driven by the ultrasound engine on the back of the Vega system. To begin using the VivoJect, follow this checklist in order:

1. Power on the Vega instrument and the VivoJect accessory.
2. Launch SonoEQ and select acquisition mode.
3. Change the selected transducer to "Linear Array (VivoJect)" using one of the following methods:
    - Change the transducer selection in the B-Mode acquisition settings
    - Select one of the Linear Array (VivoJect) presets (Figure 3).
4. Disconnect the Vega system transducer from the ultrasound engine on the back of the Vega by unlatching the connector lock (Figure 2). Place the white transducer connector in the provided holder on top of the Vega instrument.
5. Connect the VivoJect transducer to the ultrasound engine on the back of the Vega system, making sure to note the proper orientation shown in Figure 2. The connector should seat smoothly into the socket with very little pressure.
6. Latch the connector lock highlighted in Figure 2.

Figure 2: Transducer cable and connector. The connector lock is highlighted with a red arrow. Note the orientation of the transducer relative to the connector lock.

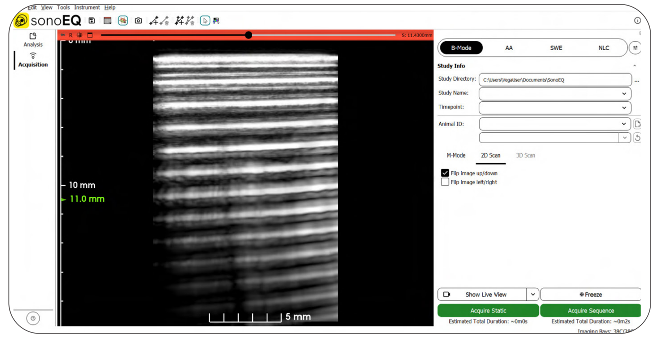


Figure 4: SonoEQ interface when the VivoJect transducer is selected. Note the axial orientation is reversed compared to the Vega system's transducer.

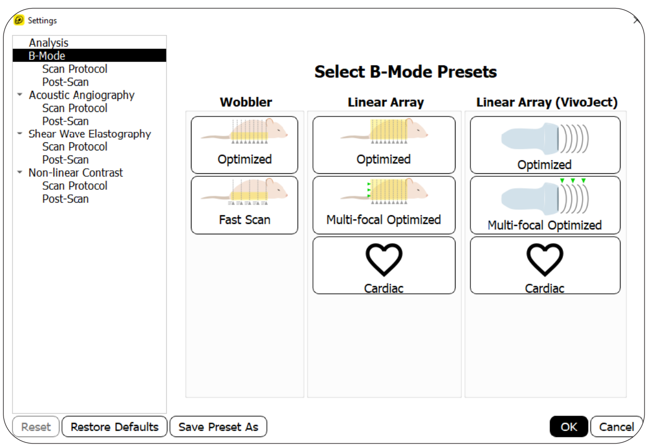


Figure 3: Preset selection menu in SonoEQ. VivoJect presets are in the column on the right.

When a VivoJect transducer is selected, the user interface of SonoEQ will have some key differences (Figure 4). The ultrasound image will be flipped to reflect the fact that the transducer is imaging in a top-down fashion, as opposed to the bottom-up approach used in the Vega system. Also, the control panel is simplified.

NOTE: The VivoJect linear array transducers can be used for B-Mode, Shear Wave Elastography (SWE), and Non-linear Contrast (NLC) imaging. It is not possible to perform Acoustic Angiography (AA) imaging with the VivoJect transducer.

VivoJect hardware overview

The VivoJect device has two primary components: a motorized animal positioning stage with a heated bed, and an arc-shaped manual stage for manipulating the transducer and needle assemblies (Figure 5). The animal platform can be moved in X and Y (left-right, forward-backward) using the motion stage control joystick. The Z stage (up-down) carrying the syringe and transducer is controlled using the thumbwheel next to the X-Y joystick. The heated animal platform can also be manually rotated about its center to facilitate animal positioning.

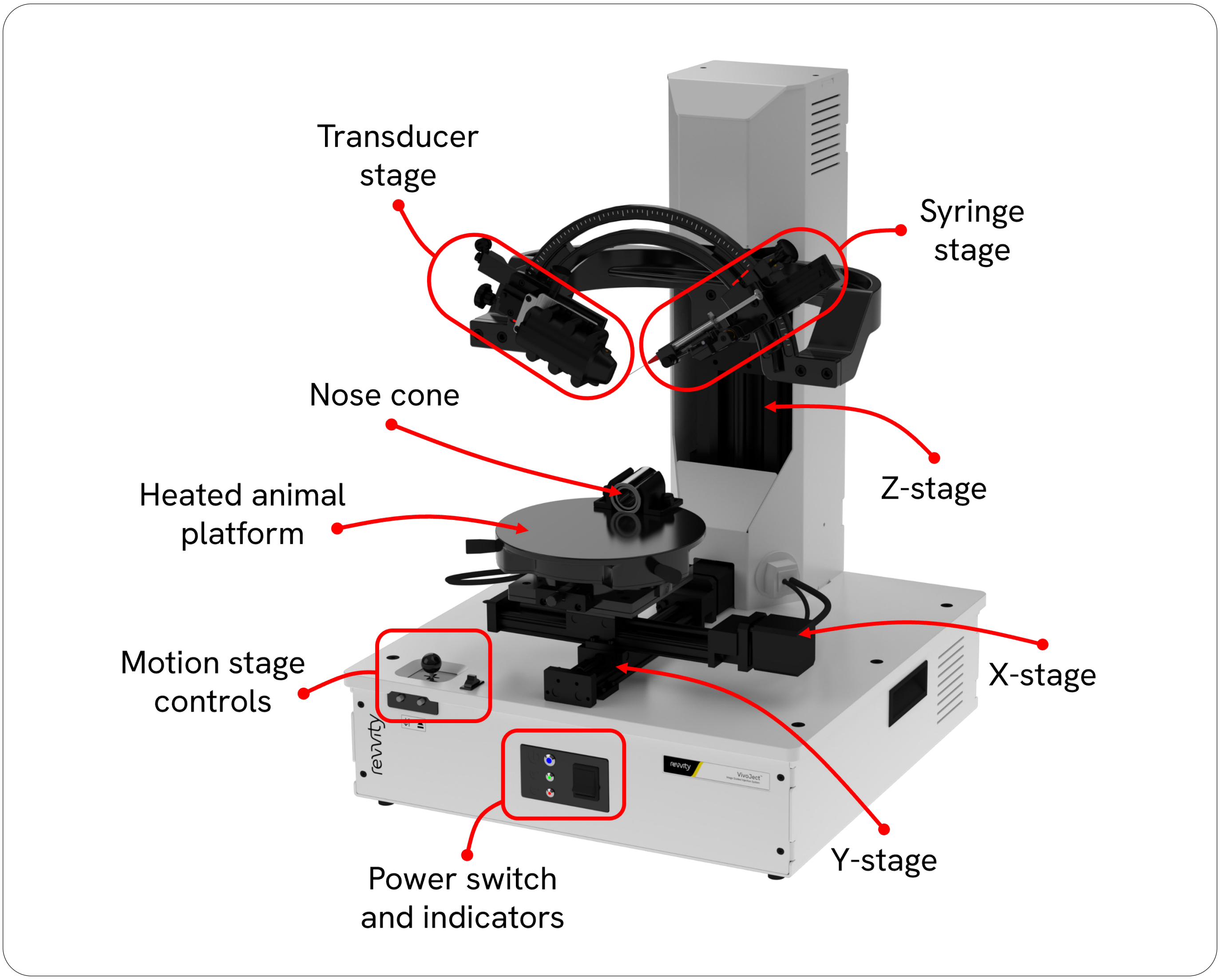


Figure 5: VivoJect hardware detail.

The transducer and syringe sliders can be manually positioned (and locked in place) at any angle on the circular rail above the animal platform. Angles on the rail are marked to ensure simple and repeatable positioning (Figure 6). Please refer to the VivoJect user manual for a detailed description of the various hardware components.

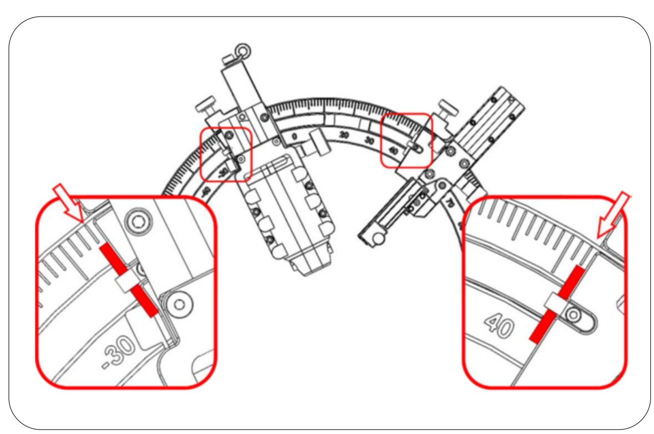


Figure 6. Example position and angle indicators for the transducer and syringe assemblies (left and right, respectively).

Materials and general recommendations

The following is a list of materials not included with the VivoJect accessory which are **required** for image-guided injections.

- Syringe: the VivoJect supports most single-use 1 mL syringes as well as many Hamilton® syringes. Please note: the VivoJect was not designed to be used with insulin syringes. It is likely that some insulin syringes may not be compatible with the VivoJect syringe carriage.
- Needle: We recommend the use of ¾″ – 1½″ needles (2 cm – 4 cm) to ensure that there is plenty of room between the hub of the needle and the transducer carriage for certain applications. We also recommend using the highest gauge needle that is compatible with your injection payload because smaller diameter needles tend to pierce the skin more easily (27g - 30g recommended).
- Ultrasound gel: Gel is used to couple sound waves to travel between the transducer and the tissue.
- Tools for shaving and depilation: Fur must be removed prior to imaging. It is recommended to first shave the mouse using a razor or electric clippers and follow up with a chemical depilation agent for 30 – 60 seconds to remove any residual fur (e.g. Nair®).

The following is a list of materials that is **recommended** but not required:

- Surgical tape: Can be useful for holding the skin taught for certain injections.
- Wipes, gauze, paper towels, etc. for cleanup.
- VesselVue™ ultrasound contrast agent: VesselVue microbubbles can be added to the injection payload to improve visualization of injection location. This is especially useful during training.

General injection protocol

Upon delivery, the VivoJect injection system is calibrated so that the needle intersects the center of the ultrasound image at 11mm. Therefore, the first step is to use the X/Y joystick and Z thumbwheel so that the target of interest is centered on the ultrasound stream at approximately 11mm in depth (distance from the top of top of the image). Once the target has been positioned, the approximate angle and track that the needle will take should be estimated to ensure that other organs will not be pierced. Monitor the needle using the live ultrasound stream and slowly translate the needle close to the skin of the mouse.

Once the needle is visible on the right side of the image, translate the needle towards and away from the skin and use a caliper to trace that path of the needle (Figure 8, green line). Adjust the needle and / or transducer angle, if necessary, and ensure that the path of the needle will intersect the target. Slowly insert the needle into the skin using the knob on the needle motion stage (there will be resistance at first) and proceed until it reaches the target site.

Small adjustments (<1mm) can be made with the X/Y joystick and Z wheel once the needle has pierced the skin, but it is recommended to fully retract the needle from the animal for making large adjustments. Once the needle tip is at the target site, lock the syringe stage in place and slowly depress the needle plunger to deliver payload.

Application areas

Cardiac (Left Ventricle) injections

Animal positioning

The animal should be placed in the supine position with the legs taped down. Rotate the animal platform so that the anesthesia nose cone is on the left side at approximately the 10 o'clock position if viewing the platform from the top down (Figure 7). The goal of this position is to align the long axis of the left ventricle with the imaging plane of the ultrasound transducer. Apply ultrasound gel to the upper abdomen from the shoulders to the bottom of the rib cage.

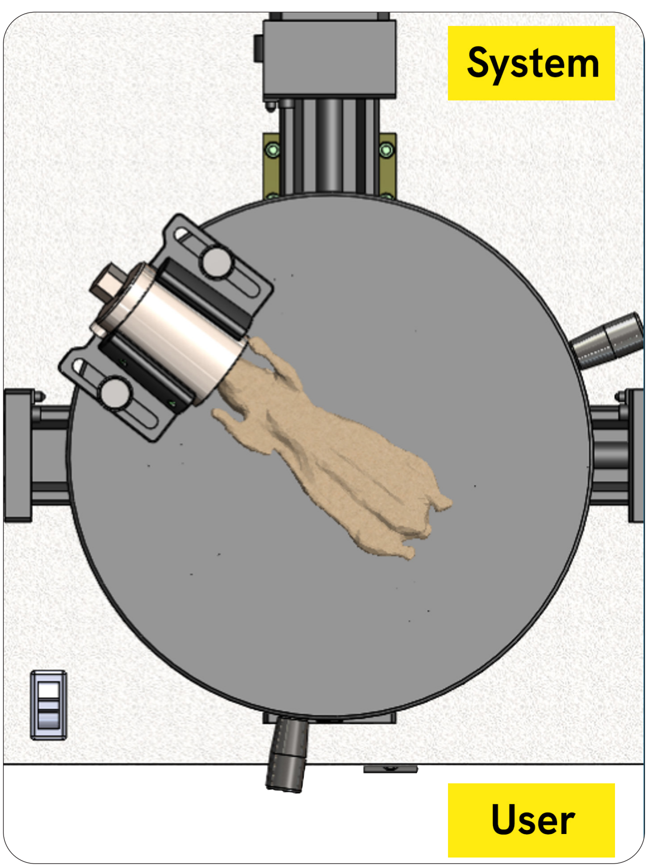


Figure 7: Recommended positioning for left ventricle injections.

Ultrasound view

Using the joystick control, locate the heart in the ultrasound livestream. Typically, the chambers of the heart are hypoechoic (dark) compared to the surrounding tissues (blood is a relatively weak scatterer). The left ventricle can be identified by its large size and the presence of the aorta (Figure 8).

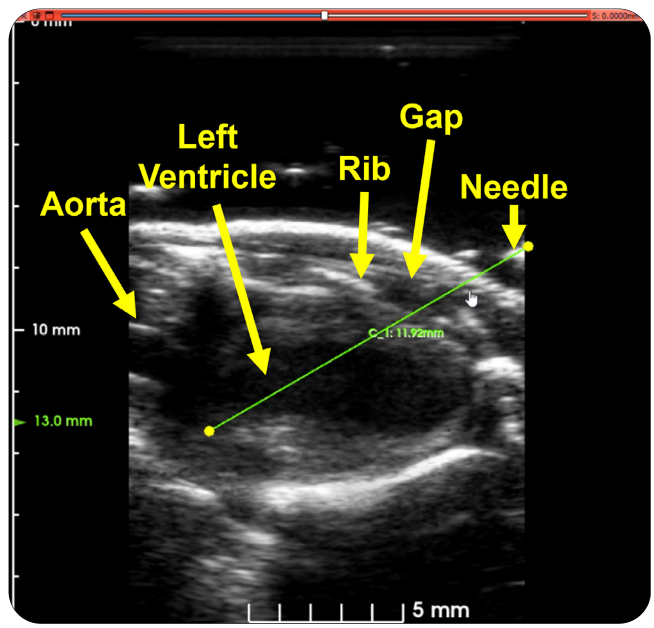


Figure 8: Example ultrasound view of the left ventricle and surrounding features.

Considerations

It can be helpful to guide the needle between two ribs to prevent any deflection of the needle. The animal's ribs will typically appear as bright objects in the ultrasound livestream (Figure 8).

Liver injections

Animal positioning

Set the rotation of the animal platform so that the nose cone is located at 12 o'clock. Place the animal in the supine position and tape the feet to the animal platform securely (Figure 9). Apply ultrasound gel to the upper abdomen. The liver is a relatively large target and there are a variety of suitable injection sites, so other animal and transducer positions can be used. Please consider your application and whether it requires a particular injection site when positioning your animal.

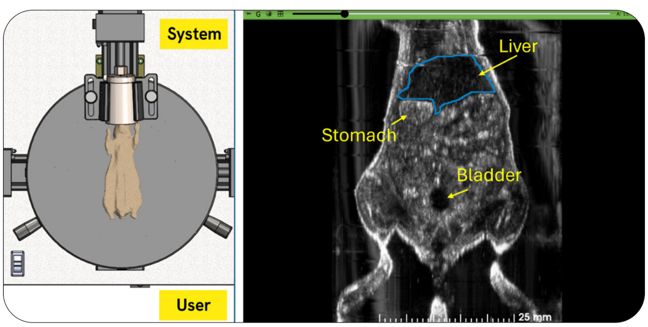


Figure 9: Recommended positioning for liver injections.

Ultrasound view

In a healthy mouse, the liver will appear as a large, dark organ directly beneath the skin. (Figure 10).

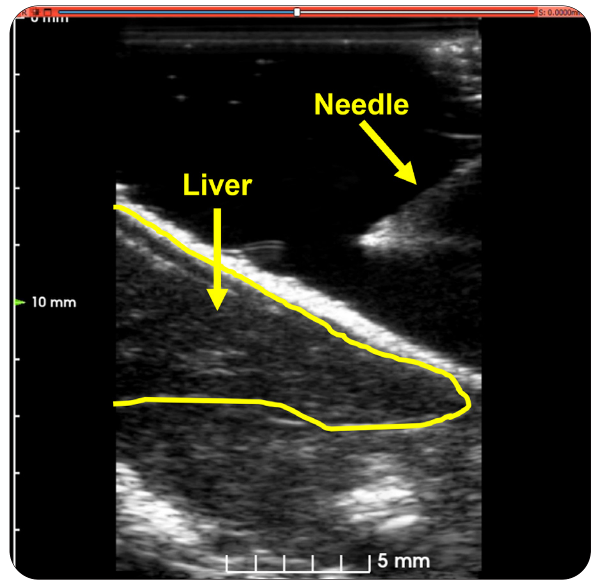


Figure 10: Example ultrasound image of the liver.

Considerations

The liver is a relatively stiff and dense tissue. Hence it is recommended to inject slowly and retract the needle slowly and carefully to avoid the injection payload leaking out of the path created by the syringe.

In healthy animals, it can be easy to confuse the spleen for part of the liver given their similar speckle characteristics. It is recommended to take the time to review the relevant anatomy and practice identifying the spleen and liver in the ultrasound livestream.

Spleen injections

Animal positioning

The goal of this positioning protocol is to align the long axis of the spleen with the imaging plane of the ultrasound transducer. Place the animal in the lateral position with its right flank down so that the spleen is facing upwards. Rotate the animal platform so that the nose cone is on the left side at approximately the 8 o'clock position if viewing the platform from the top down (Figure 11). Apply gel to the left flank of the animal spanning between the shoulder and the hip.

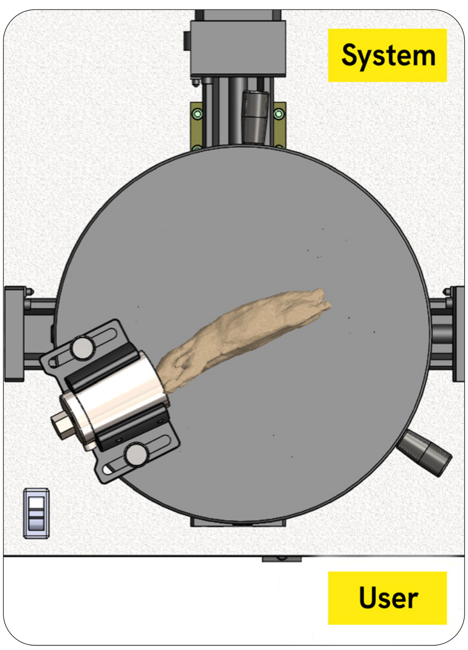


Figure 11: Recommended positioning for spleen injections.

Ultrasound view

In healthy mice, the spleen appears as a dark, oblong directly beneath the surface of the skin. Other landmarks that can be used to identify the spleen are the left kidney, pancreas, and liver (Figure 12).

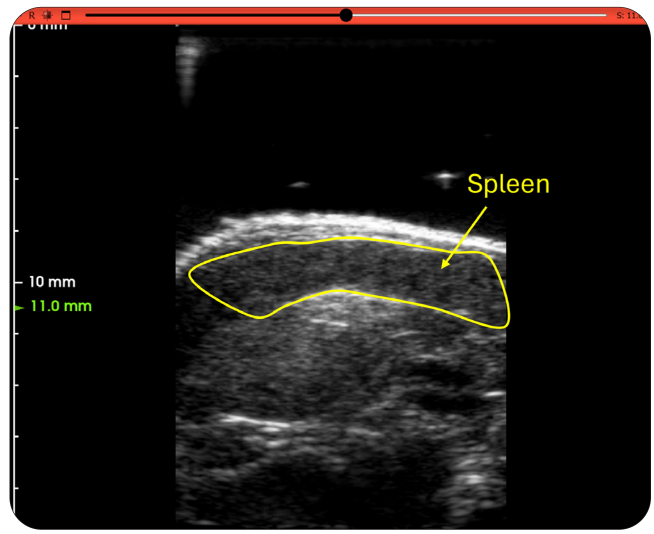


Figure 12: View of the spleen and surrounding features in the ultrasound livestream.

Considerations

Typically, the spleen will have a similar brightness compared to the liver. Given the proximity of the two organs, be sure to review the relevant anatomy to ensure that the liver is not confused for the spleen. The spleen is much smaller than the liver and can typically be visualized adjacent to the left kidney and pancreas. For many nude mice, the spleen can be visualized through the skin which can be useful for positioning.

Prostate injections

Animal positioning

In many cases, it is easiest to identify prostate tissue by its proximity to the bladder. The recommended positioning is designed to visualize the bladder and the prostate in the same imaging plane for injection planning.

Place the animal on the heated platform in the supine position, using surgical tape to fix the limbs in place. Rotate the animal platform so that the anesthesia cone is on the left side at the 9 o'clock when viewing from the top down (Figure 13).

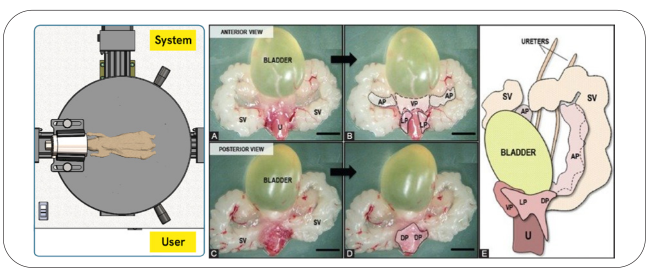


Figure 13: Left: Recommended positioning for prostate injections. Right: Mouse prostate anatomy (borrowed from https://blog.bjbms.org/the-mouse-prostate-a-basic-anatomical-and-histological-guideline/).

Ultrasound view

The bladder is one of the primary landmarks that can be used to locate the prostate. The bladder typically appears as a dark, roughly spherical object near the skin (Figure 14). It may vary in size depending on how recently the bladder has been voided. As can be seen in the photographs in Figure 13, the mouse prostate is a complex structure adjacent to the bladder.
In healthy mice, the prostate will typically present as a dark structure below and to the right of the bladder (Figure 14) when using the recommended positioning.

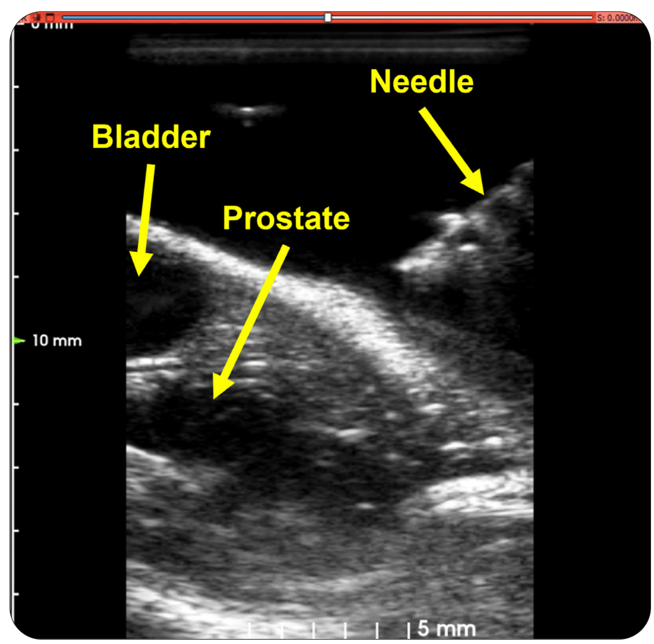


Figure 14: View of the prostate and bladder in the ultrasound livestream.

Considerations

Given the complex structure of the murine prostate, it is recommended to review the anatomy prior to injections to ensure accurate delivery to the desired tissue structures.

Pancreas injections

Animal positioning

Place the animal in the lateral position on its right side and rotate the animal platform so that the nose cone is in the 12 o'clock position (Figure 15). Roll the animal approximately 15 degrees toward its stomach so that the pancreas is facing upwards. Apply ultrasound gel to the left flank between the shoulder and the hip.

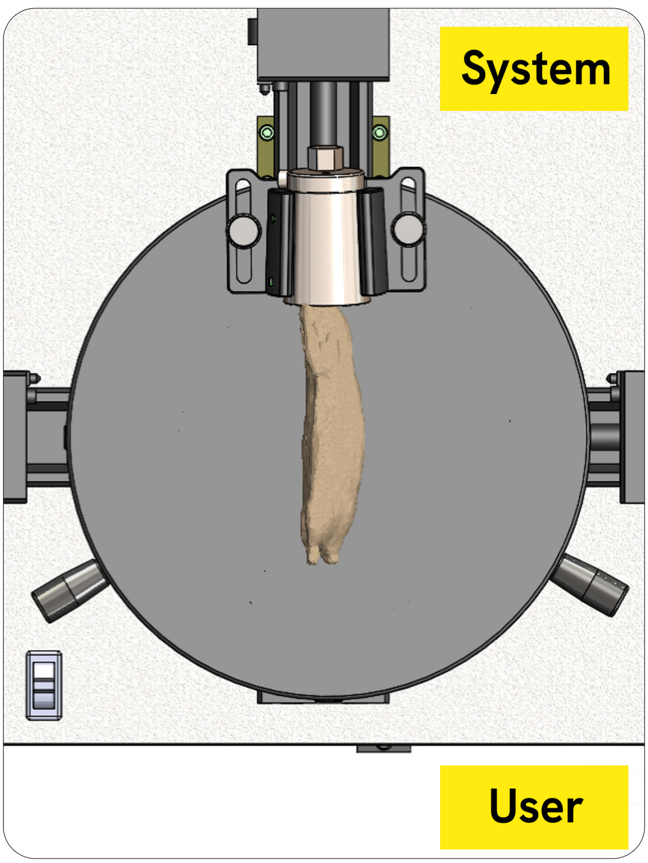


Figure 15: Recommended positioning for pancreas injections.

Ultrasound view

When using the recommended positioning described above, the pancreas will appear between the spleen and left kidney (Figure 16). In most cases, it is possible to find a view of the pancreas where there is a small gap between the spleen and pancreas that can be used for injections (see the needle guideline in Figure 16).

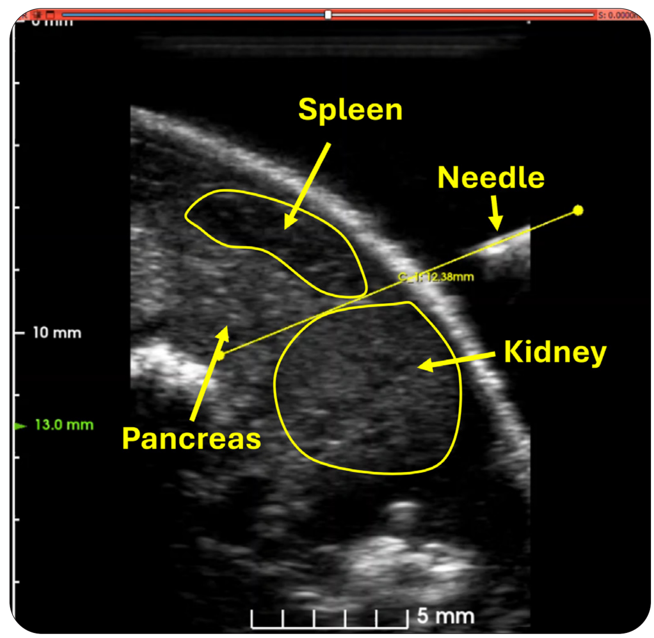


Figure 16: Ultrasound view of the pancreas and surrounding organs when using the recommended positioning.

Considerations

The pancreas is very soft compared to other organs such as spleen and liver. It can be helpful to use a short, fast motion when piercing the pancreas with the needle. If you move the needle back and forth and see the walls of the pancreas moving and stretching, it is likely that the needle tip is not inside the pancreas.

Kidney injections

Animal positioning

Place the animal in the prone position on the animal platform (Figure 17). Depending on which kidney is being targeted, it can be beneficial to roll the animal slightly to the left or right so that the target organ is facing upwards. To see the short axis of the kidney (circular cross section), set the rotation of the platform so that the nose cone is located at the 12 o'clock position. If the long axis of the kidney is preferred, rotate the platform so that the nose cone is in the 3 o'clock or 9 o'clock position.

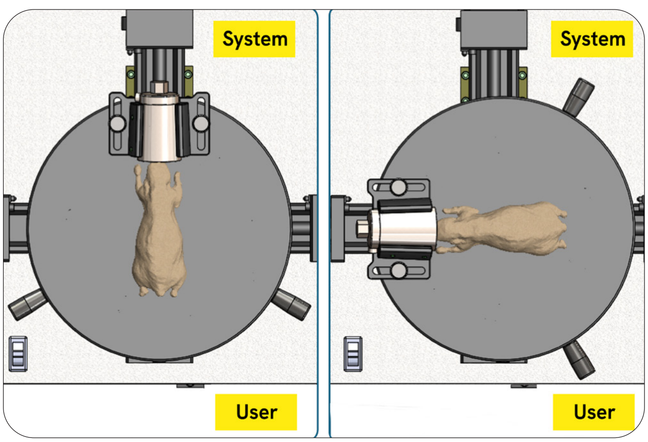


Figure 17: Recommended positioning for kidney injections.

Ultrasound view

In the short axis view (nose cone at 12 o'clock), the kidney appears as a distinctive circular structure in the ultrasound view (Figure 18). Depending on where in the kidney the imaging plane is located, it is possible to see different tissue characteristics between the medulla and cortex. In the long axis view, the kidney appears in its characteristic bean shape. In both cases, the kidney should be relatively superficial, just below the skin.

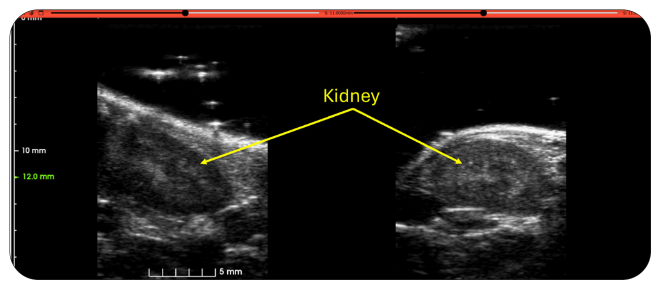


Figure 18: Ultrasound images of the kidney in the long-axis (left) and short-axis (right) views.

Considerations

The kidney is very well-vascularized, so use caution when placing your needle and retracting post-injection. It is not uncommon to see a small droplet of blood on the surface of the skin, but the animal should not bleed heavily.

Confirming your injection

Optical imaging with IVIS™

Optical imaging with the IVIS Lumina™ series is an excellent way to confirm successful injections and monitor the progression of tumor models longitudinally. In Figure 19, examples are shown of IVIS images collected immediately after VivoJect injections in the left ventricle.

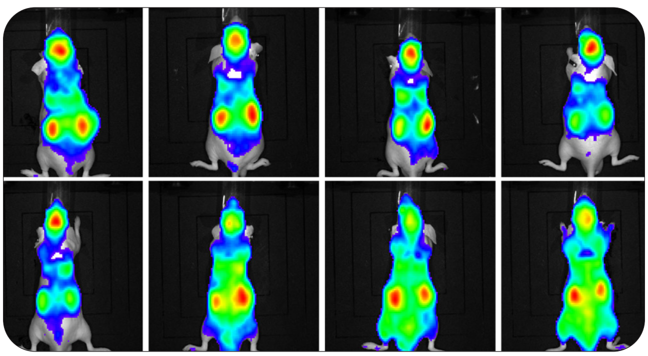


Figure 19: Examples of successful left ventricular injections resulting in systemic distribution of cells. Each of these animals received a payload of 250 µL IVISBrite™ HepG2 Red F-luc cells (Revvity, 2.5e5 cells/mL in PBS), 50 µL luciferin (15 mg/mL), and 30 µL VesselVue ultrasound contrast agent.

VesselVue microbubble contrast agent

VesselVue microbubbles can be added to injection payloads to aid in visualizing injection location. This is especially useful for applications such as left ventricular injections (Figure 19), because the contrast will be distributed systemically and immediately visible using the VivoJect ultrasound transducer. The same technique can also be used in other soft tissues to confirm successful injections (Figure 20).

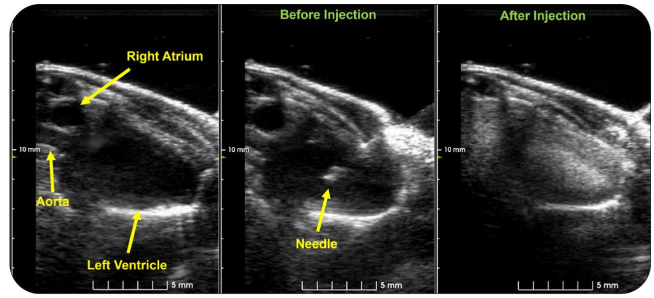


Figure 20: Example images before and after a left ventricular injection with VesselVue contrast agent in the payload. The payload consists of 250 µL IVISBrite™ HepG2 Red F-luc cells (Revvity, 2.5e5 cells/mL in PBS), 50 µL luciferin (15 mg/mL), and 30 µL VesselVue ultrasound contrast agent.

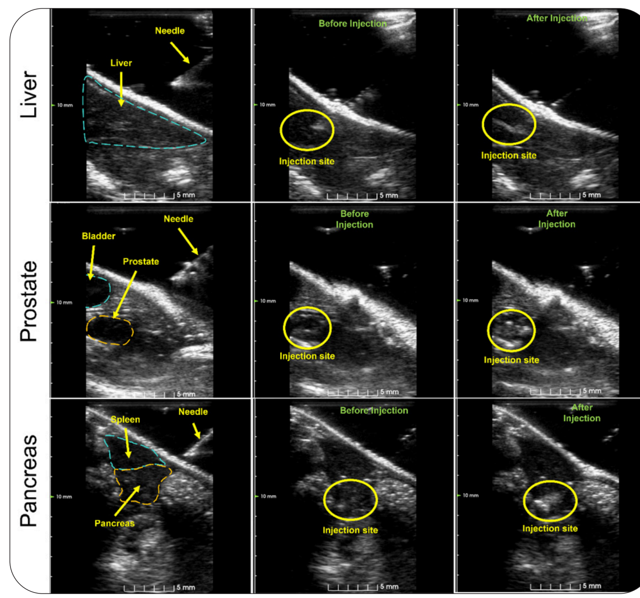
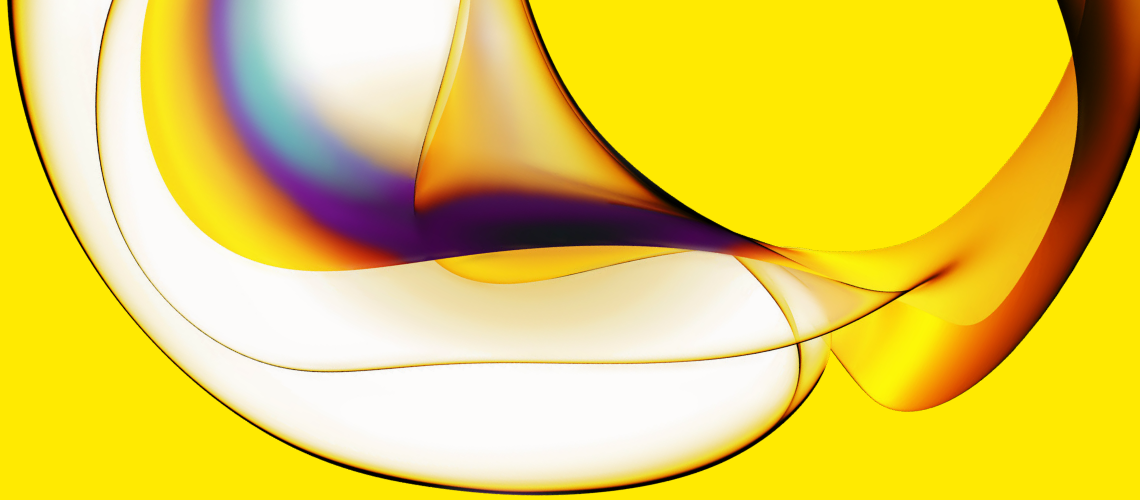


Figure 21: Example injections in the liver, prostate, and pancreas using VesselVue contrast as a visualization aid.



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