VISUALSONICS
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Imaging Guide

Guide to Small Animal Embryonic Mouse **Image-Guided Needle Injection** using the Vevo® Imaging Systems

System Compatibility: This guide contains instructions and suggestions for work on the Vevo2100, VevoLAZR, Vevo 3100 systems and transducers from the MS, MZ and MX series.

Objective

This guide is designed to aid the user in:

- Selecting the appropriate imaging transducer
- Selecting the appropriate injection needle
- Identify optimum embryo positions to perform the injection into the anatomical targets

This guide contains images of several image-guided needle injections performed in various anatomical targets of developing embryos at E12; this technique can be performed on embryos from E6.5 – E17.5. The choice of anatomical targets for injection is dependent on the stage of gestation.

Overview of Research Areas

Image-guided needle injection into embryos is an important application in developmental biology. The delivery of various therapeutic or pathogenic compounds may be desirable to study their effect on development. Also in transgenic animals the delivery of various recovery genes into specific locations may be necessary to allow adequate development for viable embryos. The injection of specific cells into an anatomical target may also be of interest in various research areas of developmental biology.

Selecting the Appropriate Transducer for Imaging

Transducer selection for image-guided needle injection is based on the size of the embryos to be injected as well as the anatomical target. Typically the transducer used to image the embryo is the same used to perform the image-guided injection.
For example: when injecting early embryonic mice the MS550S (**40MHz**) transducer is the best choice, however if injecting into late stage embryonic mice the MS400 (**30MHz**) transducer would be more appropriate.

Injection Equipment

When injecting into externalized embryos the micro injector is used in combination with a pulled glass capillary needle which allows for very small volumes to be injected while causing little damage to the tissue.
The needle is held in a constant location and can only inject or retract along a single axis while the transducer must then be lined up parallel with the needle having an angle typically of ~45^{o}.

Animal Preparation

The animal preparation shall adhere with the Institution's Animal Care Committee approved SOP.

Reference Material: Vevo Image-Guided Injection User's Manual.

Required Materials: Petri dish, 70% Ethanol, molding clay, incision tools, cotton swabs, sterile PBS, Ringer's lactate or sterile ultrasound gel, membrane.

Injection Protocol

The following are instructions on how to externalize the embryos in order to perform image-guided needle injection:

- Everything should be sterilized with 70% ethanol
- Follow instructions on loading the needle in the Vevo Image-Guided Injection Users Manual
- Prepare a Petri dish with attached membrane. Make a small slit in the membrane that the embryos will fit through
- Place four pillars of molding clay on either side of the animal, with the height adjusted to match that of the dame
- Make an incision in the skin approximately 2 cm in length
- Make a small incision in the peritoneum
- Remove the entire horn and count the embryos. Create a diagram of the uterine horn for annotation as injections are done
- Place the uterine horn back into the abdomen
- Place the Petri dish above the dame and use cotton swabs to pull 1-2 embryos through the membrane into the Petri dish
- Fill the Petri dish with sterile PBS or Ringer's lactate. Alternatively, sterile ultrasound gel may be used
- If using PBS, fill the dish half full and then place the supporting blue membrane in the dish. If using sterile gel, place the supporting membrane in the dish prior to adding the gel
- With nothing on the screen (i.e. the embryos are out of plane), but with the probe in contact with the liquid, slowly bring the needle into view to ensure its alignment with the probe
- Retract the needle
- Bring the first embryo into view and identify the injection target
- Slowly bring the needle into view and adjust the angle and position of the needle such that the target of interest is inline for injection. The needle guide overlay may be used to help visualize the path of injection
- Perform the injection with a quick motion
- Press the inject button once the needle tip and bevel are within the target area
- Slowly retract the needle
- Bring the next embryo into frame and repeat the steps above to perform subsequent injections
- After injecting the embryos in the dish, turn the needle out of the way, push the embryos back into the dame, and carefully pull the next embryos out. Repeat the steps above until all embryos in both horns have been injected
- Once all embryos have been injected, remove the liquid or gel from the Petri dish and remove the dish from the dame
- Suture the peritoneum and suture or staple the skin
- Remove the mouse from anesthesia and place in a recovery chamber
- Monitor until mouse is fully recovered

Representative Images of Various Anatomical Targets

Before placing the needle through visualize and take notice of the trajectory the needle will go through in order to get to the target tissue and avoid injecting through the placenta as much as possible. The embryo is positioned in such a way that the desired anatomical target is in view.

Embryo Heart Ventricle Injection

In the images below the embryo is positioned in an ideal position for the injection. The examples are done on an E12 mouse embryo for image-guided injection in the heart ventricle.

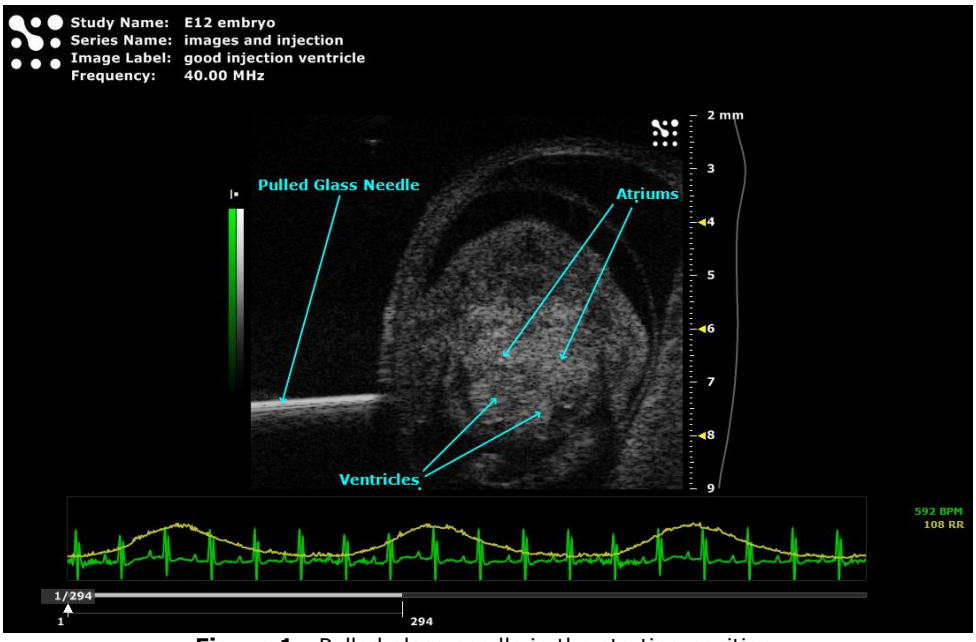

Figure 1 - Pulled glass needle in the starting position.

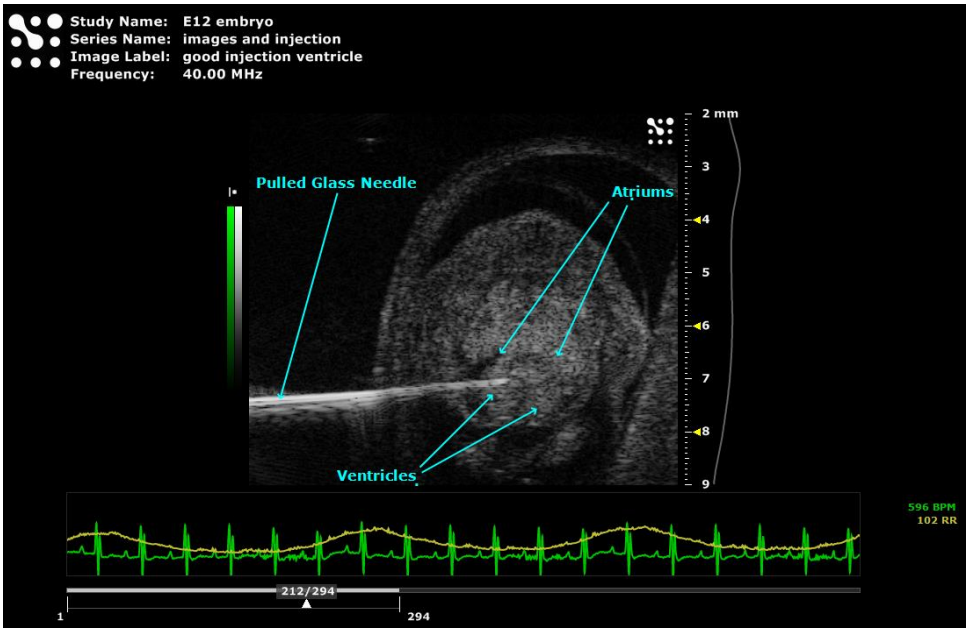

Figure 2 - Pulled glass needle placed in the heart ventricle.

Embryo Brain Injection

Examples of image-guided injection into the fourth ventricle of an E12 mouse embryo.

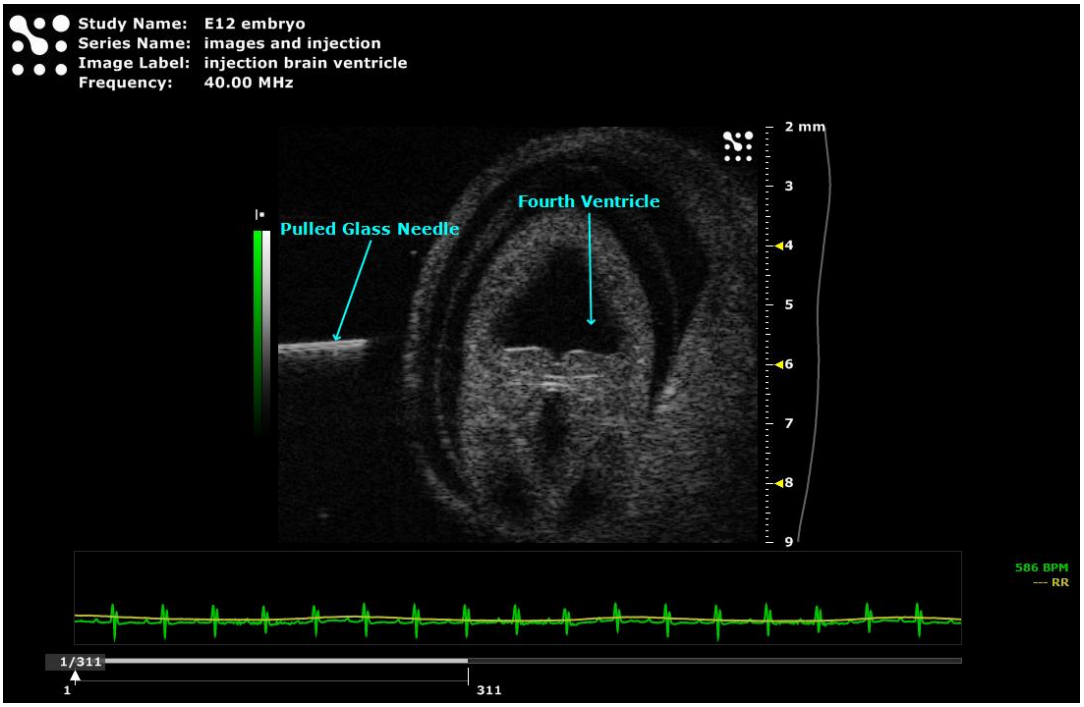

Figure 3 - Pulled glass needle in the starting position of the injection

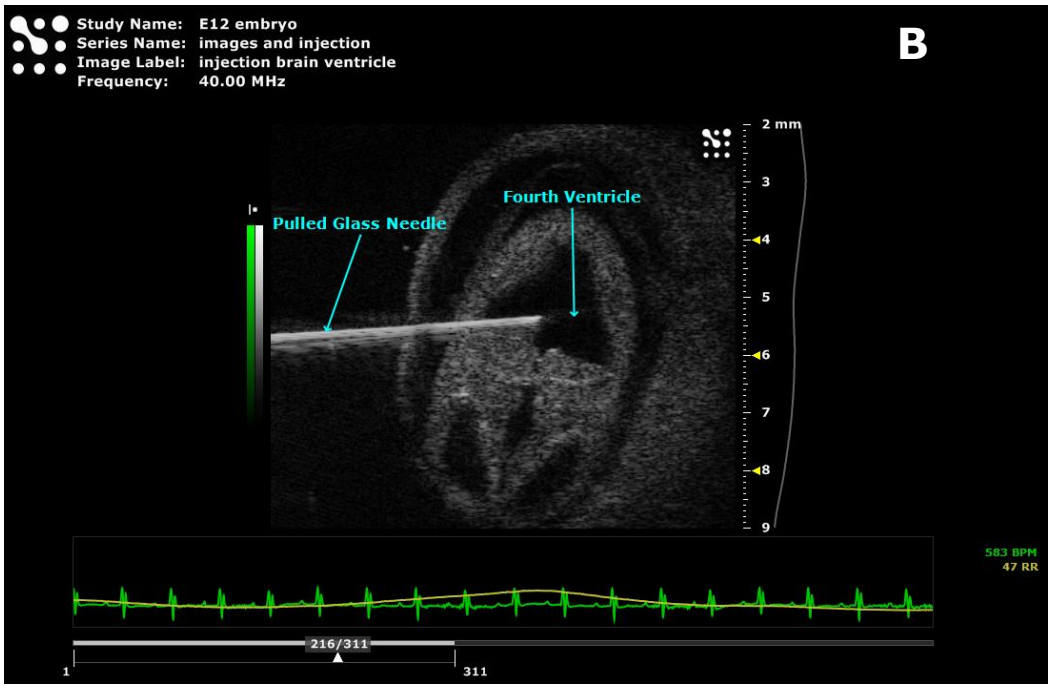

Figure 4 - Pulled glass needle placed in the fourth ventricle

Embryo Spinal Cord Injection

Example of image-guided injection into the spinal cord of an E12 mouse embryo.

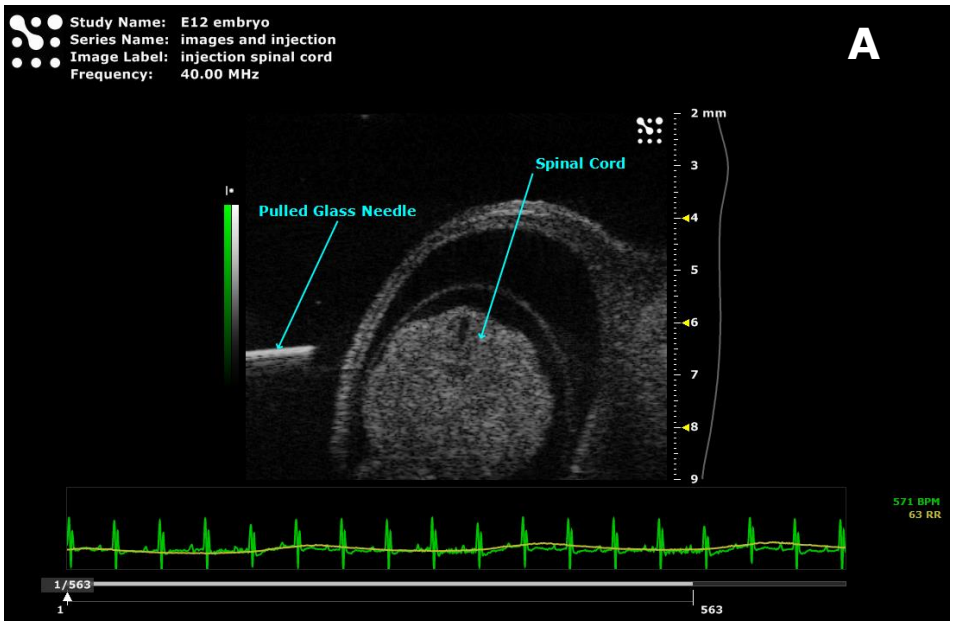

Figure 5 - Pulled glass needle in the starting position.

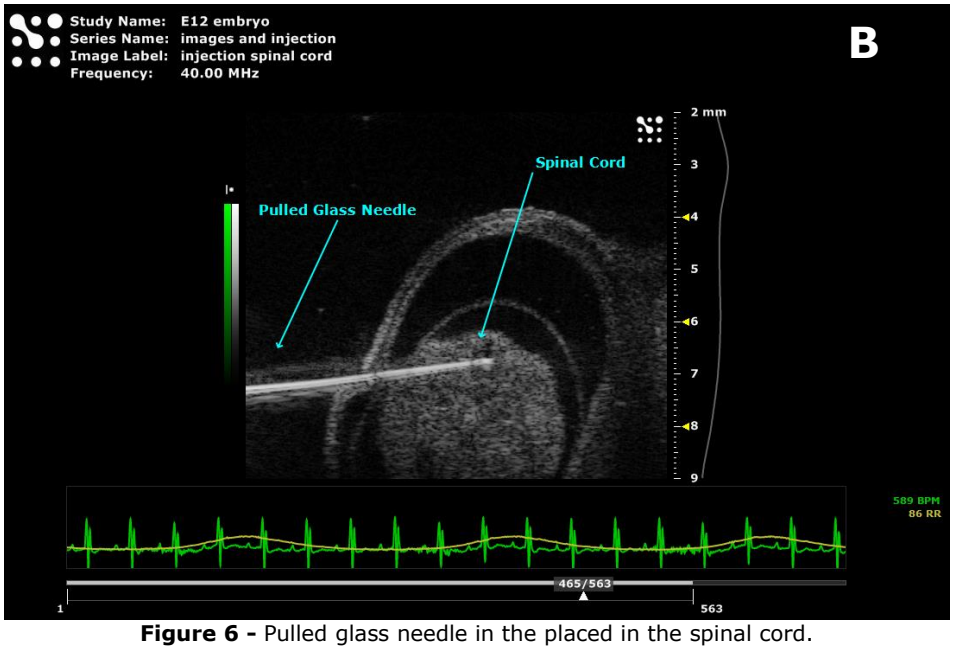

Figure 6 - Pulled glass needle in the placed in the spinal cord.

Embryo Liver Injection

Examples of image-guided injection in the liver of an E12 embryo.

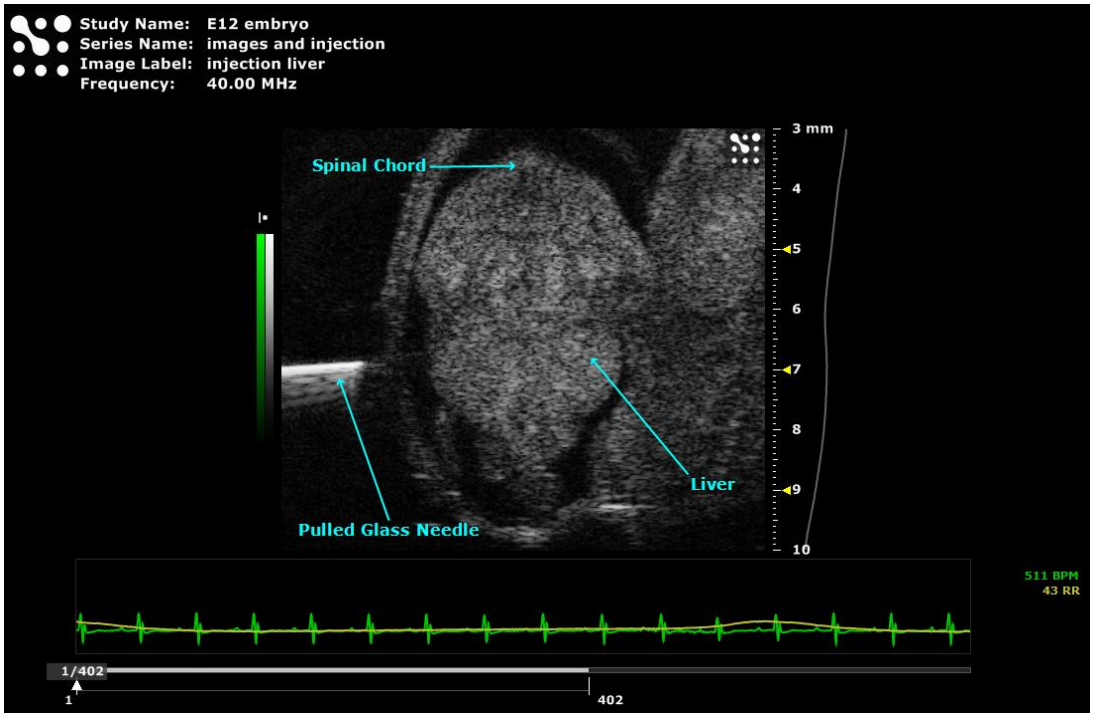

Figure 7 - Pulled glass needle in the starting position.

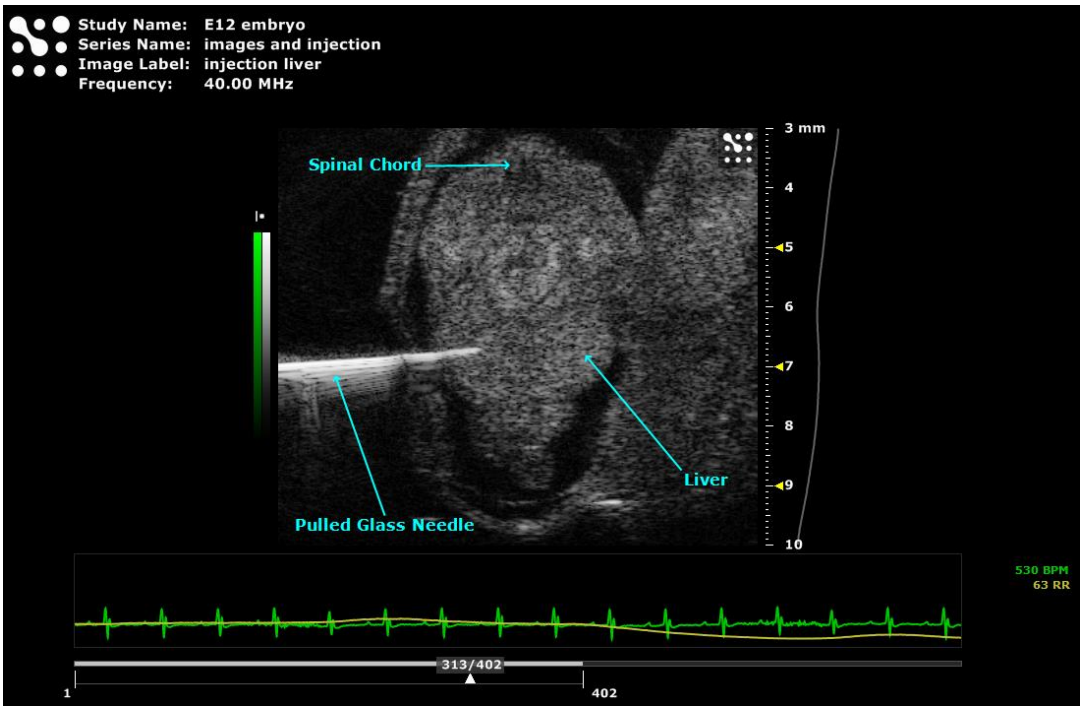

Figure 8 - Pulled glass needle in the starting position.

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