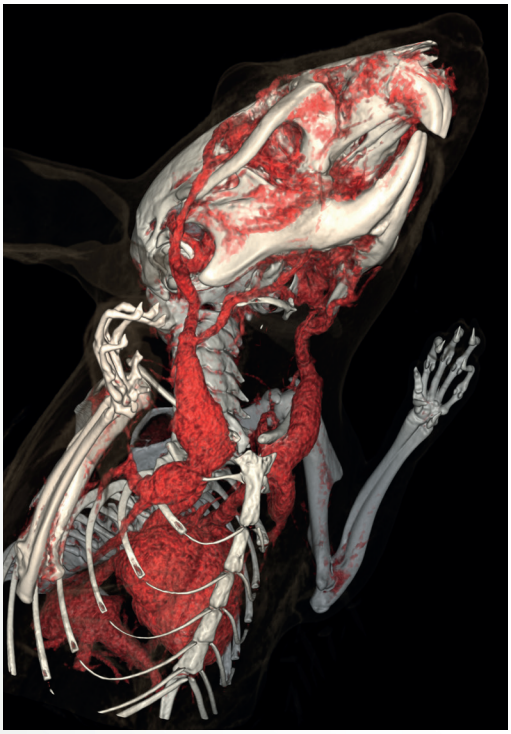
enestra®

Vision without Sacrifice



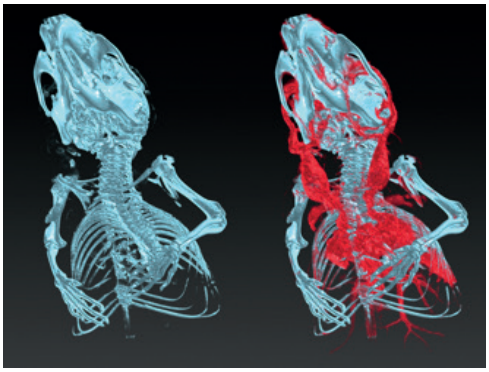
Imaging Agents for Preclinical Computed Tomography

enestra® LC

Hepatobiliary
Contrast Enhancement

enestra® VC

Long-Lasting Vascular
Contrast Enhancement



Fenestra® nano-emulsions for soft tissue micro-CT provides flexible, long-lasting contrast enhancement for a wide range of imaging applications for research on detection and treatment of human diseases

FOR NON-HUMAN RESEARCH USE ONLY

Visualize

Researchers and scientists interested in visualizing anatomy in living animals using CT imaging techniques are often faced with the challenge of discerning structures with similar or identical contrast properties. With the Fenestra® line of imaging products, you can easily achieve soft tissue or vascular contrast from a single administration.

Quantify

Fenestra® provides the opportunity for quantification of soft tissue tumor burdens and disease processes. With this capacity for quantification, you can assess the physiologic response to a therapeutic intervention or follow the natural etiology of a disease process.

Imaging Over the Life of the Animal

Since Fenestra® can be administered repeatedly to animals, the imaging data you want most can be collected in the same animal over the entire course of a study. By eliminating the need to sacrifice and assay animals at individual time points, Fenestra® saves you both time and money.

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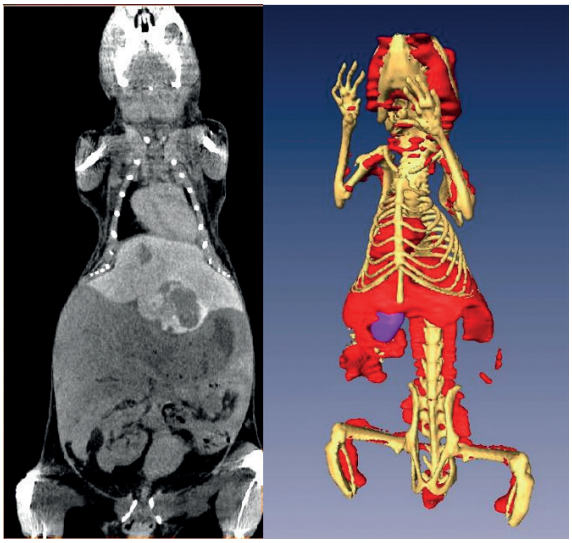
Fenestra® Applications

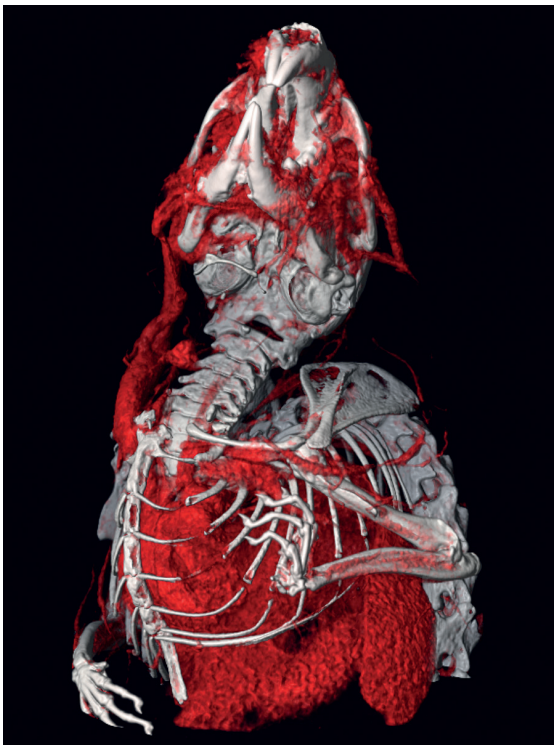
The following section highlights some of the applications of Fenestra®.

Quantitation of Tumor Burdens



Coronal view, and 3D reconstruction of male Balb/c mouse with hepatic CT-26 adenocarcinoma 2 hr after IV injection of Fenestra LC. At this time point the vascular system is still enhanced as evidenced by the lack of hepatic vascular detail and isodensity of the ventricles of the heart. The hypodense gall bladder near the dome of the liver is readily visualized in the coronal view. The CT-26 tumor is observed at the lower edge of the liver with a narrow rim of enhanced liver below the tumor and was colorized (purple) in the 3D reconstruction. The absence of artefacts in the lower abdomen is a result of the mouse having been fed a soft, non-chow diet for 48 hours prior to imaging.

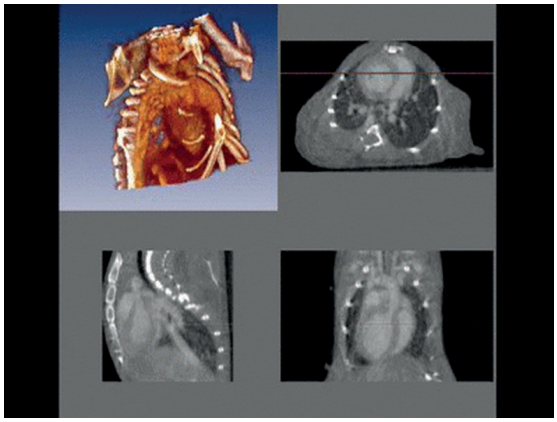
Vascular Anatomy and Angiogenesis



Courtesy of Dr. Charles Keller, OSHU

Vascular CT (Fenestra® VC) of neck tumor in transgenic mice harboring a conditional knock-in of the Pax3:Fkhr oncogene causing alveolar rhabdomyosarcomas. Neck tumor aggressively displaces adjacent vessels increasing volume of blood feeding tumor, an effect not seen from right sternocleidomastoid.

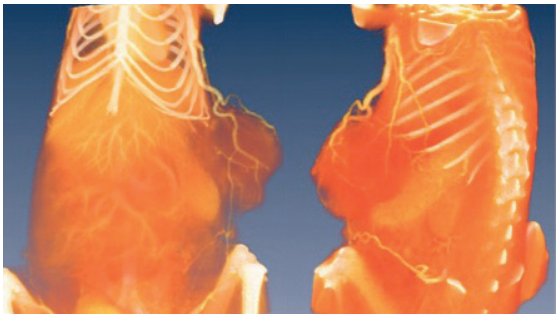
4D Cardiovascular Imaging



Courtesy of Dr. Timothy Doyle, Stanford University

4D imaging of wild type mouse (Fenestra® VC) gated on both cardiac and breath cycle with all images acquired in the exhaled breath phase. Prolonged residence time of 3 hours for Fenestra® VC facilitates acquisition of gated image which takes approximately 5 minutes for one time frame and one hour for entire cycle.

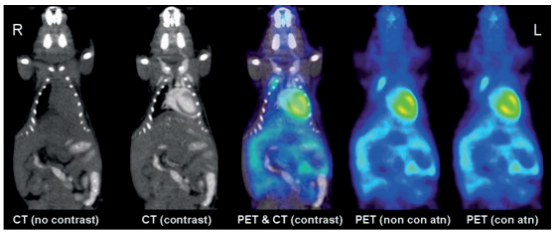
Preclinical Angiography



Courtesy of Dr. Jamey Weichert, University of Wisconsin

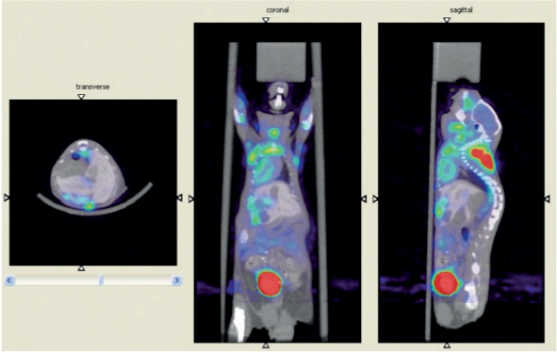
Angiogenesis in Colon-51 Tumor Model. Frontal and lateral views of vascular supply to colon-51 xenograft tumor in live mouse following injection of Fenestra® VC .

Soft-Tissue CT Data for Multimodal Molecular Imaging



Courtesy of Dr. David Stout, UCLA

Micro-CT without contrast, micro-CT with Fenestra® VC, co-registered contrast micro-CT and micro-PET images, micro-PET image corrected for attenuation using the non contrast enhanced CT data and micro-PET image corrected for attenuation using the Fenestra® VC enhanced CT data. The presence of Fenestra® VC does not significantly effect CT-based attenuation correction of the micro-PET images.



Courtesy of Dr. David Stout, UCLA

Mouse with liver tumors imaged with ~200 uCi FDG and contrast enhanced micro-CT after injection of 300 uL Fenestra® LC.

Getting great results with Fenestra® nano-emulsions

Fenestra® nano-emulsions provide long-lasting contrast enhancement for a wide range of applications in preclinical computed tomography and molecular imaging.

Using Fenestra®

The guidelines and recommendations in this publication are provided to help you optimize image quality and comply with established regulations for animal studies.

Fenestra® LC and VC

Because of their comparatively long and stable in vivo residence times, Fenestra® contrast agents can be used to access anatomy and function in a wide range of microCT imaging applications. Prolonged enhancement of the entire hepatobiliary system is possible from a single intravenous administration of Fenestra® LC, while the intravascular formulation of Fenestra® VC provides superior vascular characterization and enables 4D cardiac imaging.

Fenestra® LC enables visualization of the hepatobiliary system and liver function by mimicking chylomicron remnants and localizing contrast-producing lipids into the liver's parenchyma. In normal animals, hepatic contrast enhancement lasts for up to several hours after injection.

Fenestra® VC is a refined version of Fenestra® LC in which the surface of the lipid emulsion particles is modified to alter recognition of the particle by the receptors on the hepatocytes that are responsible for uptake into the liver. This delayed uptake produces contrast enhancement of the entire vascular system that can last for several hours. Fenestra® VC remains intravascular as long as the endothelial integrity of the vessel is maintained. Like its liver-selective counterpart, Fenestra® VC is eventually metabolized and eliminated through the hepatobiliary system.

Storage and General Use

Fenestra® contrast agents are non-toxic, non-radioactive, and do not contain active or biological products. Special care is NOT required for storage, handling, or disposal. However, you should wear goggles and disposable gloves as well as protect clothing whenever you use Fenestra®.

Storage

Always store Fenestra® at room temperature. Refrigeration is NOT recommended. Even partial freezing, which is likely to cause phase separation, can render the product ineffective as well as toxic. Do NOT transfer to any other container.

Expiry Date

Check the expiration date prior to administration. Do NOT use expired Fenestra®.

Appearance

Examine the liquid prior to use. Fenestra® should appear as a homogenous white or slightly off-white milky fluid, without any solids or separation into distinct liquid phases. Do NOT use if the formulation appears to have separated into oil and water phases or appears to have solidified.

Usage

Record the date of first entry into the vial prior to use. Discard the remaining material 30 days after the first entry. Do NOT dilute or combine with any other product.

Unadulterated Fenestra® should be injected directly. Disinfect the vial with an alcohol swab before each usage.

Dosage

Fenestra® is typically administered intravenously via a lateral tail vein. CT enhancement profiles of Fenestra® will vary with dose. Refer to review article MicroCT liver contrast agent enhancement over time, dose, and mouse strain (Mol Imaging Biol. 2008 Mar-Apr;10(2):114-20. doi: 10.1007/s11307-007-0128-x. Epub 2008 Jan 16 available at *http://www.ncbi.nlm.nih.gov/pubmed/18204990)*

Preparation

Mix Fenestra® by gentle inversion of the vial before administration. Avoid shaking, which can create air bubbles. If the agent is not used within five to ten minutes of drawing the dose, the contents of the syringe should be remixed by gentle inversion prior to injection. You should be aware that it can be difficult to see bubbles in Fenestra®, which is opaque.

Elimination

Fenestra® is normally eliminated in the feces with only a fraction of the product will be eliminated in the urine of normal animals.

Animal Preparation and Other Considerations

You should consider implementing the following recommendations for preparing and anesthetizing animals to help optimize your imaging studies. All studies should be preceded by a preliminary investigation to validate experimental parameters. You should note that animal strain, diet, housing, dosage, experiment design, and other factors may affect the metabolism of Fenestra®.

Always follow your approved experiment protocol and the accepted standards for the care and use of laboratory animals whenever you prepare, anesthetize, or dose an animal.

Minimize Stress

Laboratory animals, particularly mice, are easily stressed if they are not accustomed to being handled. Shielding rodents from extremes of light, temperature variations, and loud noises can also make a noticeable difference in how well experimental procedures are tolerated. Some investigators opt for administering a short-acting inhalant anesthetic, such as isoflurane, to reduce or eliminate anxiety associated with handling and dose administration.

Diet and Fasting

Most commercial laboratory animal chow contains considerable quantities of radio-opaque minerals that can cause significant image artifacts. If possible, fast animals prior to imaging studies or, preferably, place animals on a liquid or soft vegetable diet 24 to 48 hours prior to imaging.

Benadryl Pretreatment

Some users report that pretreatment with Benadryl, diphenhydramine hydrochloride, improves tolerance to Fenestra®; presumably similar to the way Benadryl can reduce the symptoms of iodine allergy in humans receiving iodinated contrast media. MediLumine cannot recommend a specific pretreatment procedure, but the literature suggests that mice can tolerate Benadryl up to at least 10 mg/kg body weight delivered via intraperitoneal injection.

Hydration

Evidence suggests that mice tolerate Fenestra® better when they are well-hydrated. This is not surprising as most protocols involve large doses of agent relative to total blood volume and Fenestra® agents comprise over 20 percent lipids by weight. Achieving optimum hydration includes allowing unlimited access to water up to the initiation of anesthesia and as soon as animals return to consciousness. As an alternative, some users inject a 0.2 to 0.5 ml isotonic saline solution subcutaneously between the shoulder blades shortly before IV administration of Fenestra®. Other users habitually administer liposyn intraperitoneally prior to administration of Fenestra®.

Keep Animals Warm

It is vital that the animal's body temperature is maintained within acceptable limits at all times. Careful use of a mild heat source, such as a heating pad or heat lamp, during animal preparation and waiting periods can help prevent hypothermia. Control of body temperature is especially important while animals are anesthetized and during recovery. Wrapping the anesthetized mouse in a single layer of bubble wrap can help maintain body heat during the scanning regimen.

Anesthesia

The choice of an anesthetic or other agent for immobilization should always be based upon individual protocols and local guidelines. When determining your anesthetic needs, you should consider the duration of anesthesia required for completion of the scan, the desired level of anesthesia, and how anesthetic affects on respiration rate and cardiac function may influence image quality. You should also be aware that some anesthetic agents, such as pentobarbital, may alter the pharmacokinetics of Fenestra®. Consult a veterinarian and your local guidelines when considering the use of any agent.

Isoflurane inhalation is a preferred approach for anesthesia, as it allows animals to recover quickly and is amenable to multiple same-day scanning sessions. A typical procedure calls for inducing mice at 4 to 5% and then maintaining anesthesia at 1.5 to 2% isoflurane. The animals should be fully anesthetized and ready for injection and scanning within a few minutes. As an alternative, some researchers use an intraperitoneal injection of a mixture of ketamine (80 mg/kg body weight) and xylazine (5 mg/kg body weight). This technique usually affords 45 to 60 minutes of anesthesia.

Maintenance of anesthesia can be achieved with quarter dose increments as needed.

You should note that when animals are to be scanned at multiple time points on the same day, it is usually preferable to bring them back to consciousness between scanning procedures. You should also note that animals are particularly prone to hypothermia when anesthetized and should be kept warm until they can resume normal activity.

Cardiac and Respiratory Gating

Gating may be necessary for some cardiothoracic imaging studies if normal respiration affects image quality. Refer to the review article Gating in small-animal cardiothoracic CT (Methods. 2010 Jan;50(1):42-9. doi: 10.1016/j.ymeth.2009.07.006. Epub 2009 Aug 3 available at *www.ncbi.nlm.nih.gov/pubmed/19651213*).

Injection Techniques

Fenestra® is typically administered intravenously via the lateral tail vein, but can be introduced into any viable vessel. The use of a catheter, such as Strategic Applications' 12-inch mouse tail vein catheter MTV-01, is strongly recommended. Terumo tuberculin syringes can also be used. Because Fenestra® persists in the animal for several hours, rapid injection is not required. A slow bolus injection rate of about 1 ml/minute is optimal. If injected properly, the agent should flow smoothly into the vessel with virtually no resistance. Do NOT inject Fenestra® unless you are sure that the catheter or needle is in the vein.

You should note that tail vein injection requires considerable skill. Ask for assistance if your own experience is limited. Successful tail vein injections can be facilitated by dipping the tail in warm water to induce vasodilation, cleaning hair and scales off the tail at the injection site, using a magnifying light to properly visualize the vessel, and inserting the needle with the bevel facing up and then rotating once inside the vessel.

Troubleshooting

Why didn't I get good vascular contrast?

Contrast enhancement can vary for a number of reasons. The most common source of poor contrast is complete or partial extravasation of the dose during injection. If in doubt, scan the tail of the animal to determine if your injection was successful. If the tail is bright, the injection was extravasated. For recommendations about administration, refer to Injection Techniques on page 8.

When should I be imaging?

When using Fenestra®VC in mice, optimal contrast enhancement in the blood is provided for up to four hours after injection. When using Fenestra®LC, optimal liver contrast enhancement is provided at approximately four hours after administration, but earlier and later time points can also provide adequate contrast enhancement. For other species, optimal timing may be different.

You should note that optimal timing can vary whenever hepatic function is altered, for example during disease progression and treatment, in alcohol studies, with the introduction of special diets, or if transgenic mice are used.

Why are there so many image artifacts?

Inappropriate selection of anesthesia or inadequate anesthetic dosing can have a dramatic effect on image artifacts. Inhalation anesthesia normally results in the animal having greater respiratory motion than if an injectable anesthetic, which can induce respiratory depression, is used. However, injectable anesthetics may introduce other undesirable effects that range from periodic respiratory gasping to altered metabolism and susceptibility to overdose death. Conversely, inadequate anesthetic dosing may yield significant motion artifacts.

You should note that cardiac and respiratory gating can increase the resolution of the thoracic region, as indicated in the topic Cardiac and Respiratory Gating on page 8.

Contents in the gastrointestinal tract can also introduce significant artifacts. Refer to the topic Diet and Fasting on page 7 for information about animal preparation prior to scanning.

How can I get additional help?

For more information about applications, experimental design, or for any other technical question, you can contact technical support for Fenestra®

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