Submission ID #: 61269
Scriptwriter Name: Anastasia Gomez
Project Page Link: https://www.jove.com/account/file-uploader?src=18687093

Title: A High-Throughput Image-Guided Stereotactic Neuronavigation and Focused Ultrasound System for Blood-Brain Barrier Opening in Rodents

Authors and Affiliations:

Rianne Haumann[1,2*], Elvin 't Hart[2*], Marc P. P. Derieppe[2], Helena C. Besse[3], Gertjan J. L. Kaspers[1,2], Eelco Hoving[2], Dannis G. van Vuurden[1,2], Esther Hulleman[1,2], Mario Ries[3]

[1] Amsterdam UMC, Vrije Universiteit Amsterdam, Pediatric Oncology, Cancer Center Amsterdam, The Netherlands
[2] Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands
[3] Imaging Division, Utrecht University, Utrecht, The Netherlands

* Co-authors contributed equally to this study

Corresponding Authors:
Esther Hulleman
E.Hulleman@prinsesmaximacentrum.nl

Email Addresses for All Authors:
r.haumann1@amsterdamumc.nl
H.C.Besse-3@prinsesmaximacentrum.nl
M.P.P.Derieppe-3@prinsesmaximacentrum.nl
E.tHart-6@prinsesmaximacentrum.nl
E.W.Hoving@umcutrecht.nl
E.Hulleman@prinsesmaximacentrum.nl
G.J.L.Kaspers@prinsesmaximacentrum.nl
M.Ries@umcutrecht.nl
D.G.vanVuurden@prinsesmaximacentrum.nl
E.Hulleman@prinsesmaximacentrum.nl

Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

   If **Yes**, we will need you to record using screen recording software to capture the steps. If you use a Mac, QuickTime X also has the ability to record the steps. Please upload all screen captured video files to your project page as soon as possible.

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 14
Number of Shots: 40

Videographer: Film the screen for all SCREEN shot as a backup.

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Dannis van Vuurden:** Many high-grade gliomas such as diffuse intrinsic pontine gliomas are invasive growing tumours with a high mortality rate, despite intensive research. The blood-brain barrier limits the efficacy of chemotherapy, but fortunately FUS with microbubbles is able to temporally and locally open the BBB for treatment.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Dannis van Vuurden:** We have developed a small and cost-effective neuro-navigated FUS-BBBD system. The system is based on commercially available components with an emphasis on a high degree of automation suitable for high-volume preclinical drug evaluation studies.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

OPTIONAL:

1.3. **Dannis van Vuurden:** Precision Medicine for HGG has been defined as “identifying the right drug, for the right patient, at the right dose, at the right time”. However, in order to achieve this, many pre-selected drugs have to be screened. This compact high-throughput FUS-BBBD system allows us to do that.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Introduction of Demonstrator on Camera

1.4. **Dannis van Vuurden:** Rianne Haumann and Elvin ’t Hart, two PhD students from my research group, will demonstrate the procedure.

1.4.1. INTERVIEW: Author saying the above.

1.4.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Ethics Title Card

1.5. Procedures involving animal subjects have been approved by the Animal Welfare Body of the Vrije Universiteit Amsterdam, the Netherlands.

Protocol

Videographer: For clarification, names of presenters were added to the shot descriptions in bold (replacing our usual "talent" label).

2. Focused Ultrasound System

2.1. The workflow for image guided stereotactic neuronavigation focus ultrasound includes interventional imaging and acoustic coupling for sonoporation at the ultrasound platform. Afterwards, follow-up imaging can be performed **[1]**.

2.1.1. WIDE: shot of the ultrasound system, laptop and imaging module.

2.2. Select a suitable transducer based on the requirements of the designed experiment. Place it in a 3D printed cone, filled with degassed water and closed at the bottom with an acoustically transparent mylar membrane **[1]**. Connect the transducer to a function generator and a power amplifier **[2]**.

2.2.1. **Rianne** shows in one shot the water filled cone, with the transducer inside and at the bottom the acoustically transparent mylar membrane.

2.2.2. **Rianne** shows the function generator with connection to the power amplifier and the transducer

2.3. Mount the transducer on a motorized linear stage for automatic vertical positioning **[1]** and connect it to the power amplifier **[1-added]**. Use a detachable stereotactic platform **[2]** that includes temperature regulated heating, bite and ear bars, anesthesia, and multi-modality fiducial markers **[2-added]**. Attach the platform to the 2D linear stage **[3]**. *Videographer: This step is important!*

2.3.1. **Rianne** mounts the transducer on the linear stage and

ADDED SHOT: connects the transducer to the power amplifier.

2.3.2. WIDE: the stereotactic platform

ADDED SHOT: with temperature regulated heating, bite and ear bars, anesthesia, and multi-modality fiducial markers.

2.3.3. **Rianne** mounts the platform on the stage.

2.4. Before the FUS system can be used, calibrate it and determine the focus point of the transducer **[1]**. The focus point corresponds to the vertical position of the animal on the stereotactic platform **[2]**.

2.4.1. **Rianne** shows the movement of the transducer.

2.4.2. **Talent** at the computer, setting up the calibration.

3. Animal Preparation

3.1. Acclimatize the animal to the facility for at least one week after arrival and weigh it regularly **[1]**. On the day of the experiment, administer buprenorphine via a subcutaneous injection 30 minutes prior to FUS treatment **[2-TXT]**. After anesthetizing the mouse, apply eye ointment to prevent dry eyes **[3]**.

3.1.1. Mouse in the cage.

3.1.2. **Rianne** administers buprenorphine via s.c. injection. **TEXT: 0.05 mg/kg**

3.1.3. **Rianne** applies eye ointment.

3.2. If necessary, remove the hair on the top of the animal's head with a razor and depilatory cream. Wash the skin with water to remove any residues **[1]**. For experiments with BLI tumor models, inject 150 microliters of D-luciferin intraperitoneally with a 29-gauge insulin syringe **[2-TXT]**.

3.2.1. **Rianne** shows the anesthetized mouse and shows the head

3.2.2. **Rianne** injects D-luciferin with an i.p. injection. **TEXT: 30 mg/mL**

3.3. For the administration of microbubbles for BBB opening, insert a 26 to 30-gauge tail vein catheter **[1]** and flush the vein with a small volume of heparin solution **[2-TXT]**. Secure the catheter with tissue tape to prevent detachment **[4]**. If the catheterization is successful, fill the catheter with the heparin solution to avoid blood clotting **[3]**. *Videographer: This step is difficult and important!*

3.3.1. **Rianne** places a warm glove on the tail of the animal to prepare for catheter placement and inserts the tail vein catheter. NOTE: Use take 1

3.3.2. **Rianne** flushes the vein with heparin. **TEXT: 5 UI/mL** NOTE: Use take 1

3.3.3. **Rianne** fills the catheter with heparin solution and closes the catheter with a cap. NOTE: Use take 2

Videographer NOTE: shot 3.3 + 3.4 is only 3.3.1 + 3.3.2 + 3.3.4, 3.3.3 is separate

3.3.4. **Rianne** tapes the catheter to secure it. NOTE: Move step 3.3.4 before 3.3.3 and use TAKE1

3.4. Place the animal on the temperature regulated stereotactic platform to avoid hypothermia **[1].** Fix the head of the animal using a bite bar and ear bars **[2]** and tape the tail and catheter to the platform **[3]**. Fix the body of the mouse with a strap **[4]**. *Videographer: This step is important!*

3.4.1. **Rianne** places the animal on the platform. Videographer NOTE: There is no 3.4.1 take 1

3.4.2. **Rianne** tapes the mouse's tail and catheter to the platform.

3.4.3. **Rianne** fixes the animal's head with the ear and bite bars. NOTE: Move step 3.4.3 before step 3.4.2.

3.4.4. **Rianne** fixes the mouse's body with a strap.

4. In Vivo Image-guided Focused Ultrasound

4.1. Place the stereotactic platform with the mounted mouse in the imaging modality **[1]** and take images of the animal **[2]**. Determine whether the animal is correctly placed on the platform **[extra take]**.

4.1.1. **Elvin** places the platform in the imaging modality.

4.1.2. **Elvin** takes images (BLI/X-ray) of the animal.

EXTRA TAKE: Zoomed in shot of X-ray

4.2. Mark the position of the animal using the multi-modality fiducial markers in combination with the image-processing pipeline according to the focus point of the transducer **[1]**. Determine the target area by placing a brain outline over the acquired X-ray image or by using BLI images to determine the center of the tumor **[2]**. *Videographer: This step is important!*

Videographer: Film the screen for all SCREEN shots as a backup.

4.2.1. SCREEN: **Elvin** marks the position of the animal. NOTE: All SCREEN shots uploaded to project page and files are titled according to shot number.

4.2.2. SCREEN: **Elvin** determines the target area.

4.3. Before positioning the transducer, protect the animal's nostrils and mouth with tape to prevent ultrasound gel from interfering with breathing **[1]**. Apply ultrasound gel on top of the mouse's head for proper sound conduction **[2]**.

4.3.1. **Elvin** covers the mouse's nostrils and mouth with tape.

4.3.2. **Elvin** applies ultrasound gel to the head.

4.4. Record microbubble cavitation with the needle hydrophone, which is placed in the direct vicinity of the occipital bone **[1]**.

4.4.1. **Elvin** retracts the skin on the mouse's neck and positions the hydrophone needle. NOTE: Use take 3

4.5. Based on the calibration values, guide the transducer to the correct position above the animal **[1]**. Apply preconfigured settings to all attached devices to target the brain region of interest **[2]**. *Videographer: This step is important!*

4.5.1. **Elvin** guides the transducer to the correct position at the computer.

4.5.2. SCREEN: 4.5.2..mp4. 0:10 – 0:23. **Elvin** applying the preconfigured settings.

4.6. After the transducer is placed in the right position, dissolve and activate the microbubbles as described by the manufacturer **[1]**. Before microbubble injection, flush the tail vein catheter with saline to confirm tail vein access **[2]**. *Videographer: This step is important!*

4.6.1. **Elvin** activates the microbubbles, by slowly injecting the saline into the microbubble flask.

4.6.2. **Elvin** flushes the catheter with saline.

4.7. Start the insonication trajectory **[1]** and simultaneously inject the microbubbles **[2-TXT]**. At the same time, the needle hydrophone will detect and verify microbubble cavitation in real time **[3]**.

4.7.1. **Elvin** starts the insonication and shows the trajectory of the transducer

4.7.2. **Elvin** injects the microbubbles **TEXT: two bolus of 120 µL (5.4 µg)** NOTE: There are 2 takes of two boli injections

4.7.3. SCREEN: 4.7.3..mp4. 0:15 – 0:40. Microbubble cavitation displayed.

4.8. If desired, administer an intravascular contrast agent **[1]** or drug after sonoporation. Monitor the animal until a predetermined time point **[added]**.

4.8.1. **Elvin** administers contrast agent ~~and/or drug~~ to the animal.

4.8.2. ~~**Elvin** takes the platform with the animal off of the imaging modality~~.

4.8.3. **Added: Elvin administers** drug to the animal

Results

5. Results: BBB Opening with FUS

5.1. Acoustic pressure with a mechanical index of 0.4 in combination with microbubbles provided a very sensitive and reliable means of stable cavitation detection **[1]**, in comparison to no detection of subharmonics when no microbubbles were injected **[2]** or the observation of inertial cavitation when an MI of 0.6 was applied **[3]**.

5.1.1. LAB MEDIA: Figure 3 B.

5.1.2. LAB MEDIA: Figure 3. *Video Editor: Emphasize A.*

5.1.3. LAB MEDIA: Figure 3. *Video Editor: Emphasize C.*

5.2. While acoustic pressure with an MI of up to 0.6 resulted in no macroscopic damage **[1]**, microscopic damage was evidenced histologically at an MI of 0.6 **[2]**. A pressure amplitude with an MI of 0.8 resulted in a macroscopic brain hemorrhage of larger vessels and wide-spread tissue lysis with the extravasation of erythrocytes **[3]**.

5.2.1. LAB MEDIA: Figure 4 A and B.

5.2.2. LAB MEDIA: Figure 4 A and B. *Video Editor: Emphasize B.*

5.2.3. LAB MEDIA: Figure 4. *Video Editor: Emphasize C.*

5.3. Intravenous Evans blue was injected to validate the opening of the BBB in the pontine region. Extravasation of Evans blue-conjugated albumin was observed at the level of the pons and the cerebellum in the mouse treated with FUS and microbubbles, demonstrating precise targeting of the region of interest **[1]**.

5.3.1. LAB MEDIA: Figure 5.

Conclusion

6. Conclusion Interview Statements

6.1. **Dannis van Vuurden:** The most important steps of this protocol are the correct placement of the tail vein catheter, the positioning and targeting of the animal, and microbubble mediated focused ultrasound and cavitation detection.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.1, 4.2.2, 4.6.1.*

6.2. **Dannis van Vuurden:** This self-made focused ultrasound system can be adapted and redesigned for each research question, all while being cost effective and having an automated workflow that can be followed by inexperienced researchers.

6.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.