Standard Operating Procedure
7T MR Surface coil scanning of a tumor-bearing mouse

[Blocker S.J., 2018]

This SOP is designed as a general guide for in vivo MR imaging of small animals as part of a preclinical cancer study, using a volume transmit coil + surface receive coil at high fields. Note that this protocol begins with information that is not machine or project specific. This portion (Section I) focuses on the general requirements for setting up, supporting, monitoring, and scanning a live animal in the context of high-throughput MR studies. A more detailed example, based on our U24 protocol on a 7T Bruker system is provided afterwards (Section II). The authors use a 7T Bruker BioSpec 70/20 magnet as part of the Duke University CIVM, utilizing the associated volume transcieve and brain surface coils. The software associated with this magnet is ParaVision 6.0.1. Animals were scanned on house-made 3D printed beds with temperature support and breath monitoring. Any demonstrative images in this document were acquired on this setup. For project specific design, detailed information on sequence selection, data reconstruction, and data transfer should be available in supporting documentation specific to the user's magnet and software platform.

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SECTION I – General steps for scanning and animal support

PROTOCOL

1. Animal anesthesia induction

- ❖ Always consult the policies and procedures of the institution when designing in vivo imaging studies.

a. Prior to scanning, the scan bed/platform must be prepared, and the animal must be anesthetized and positioned securely.

b. Prior to anesthetizing the animal, the scanner must be prepared for acquisition. In this case, both the volume transmit coil and the surface receive coil must be plugged in. The volume coil is aligned in the bore, where the center of the coil aligns with the center of the magnet. It is very useful to know the distance between the center of the volume coil/bore and the exterior of the magnet once the volume coil is placed. This can make proper positioning of the surface coil much easier once the animal is in the scanner.

c. Temperature maintenance is important for the health of animals being scanned. This can be achieved via integrated warm water piping in the scan bed, or via forced air through the bore of the magnet. Monitoring of animal body temperature during scans can inform the user for manual temperature adjustment, or monitoring/adjustment can be automated.

d. Anesthesia via inhaled isoflurane can be achieved via nose cone attached to the scan bed. Breath rate is monitored via pneumatic pillow, and isoflurane can be adjusted manually or automatically during the entirety of scanning.

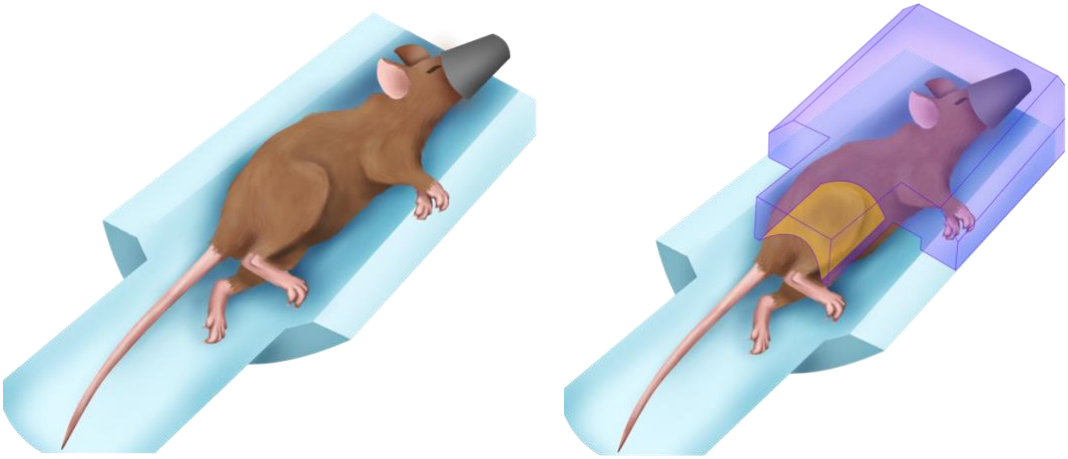
e. Note: Animals must be monitored during the entirety of scanning to confirm appropriate anesthesia administration, as well as ensure healthy vital signs.

f. If a tail vein catheter is needed for contrast agent injection during scanning, a tail vain catheter must be place prior to scanning, but after anesthesia has been induced.

    i. ***IMPORTANT NOTE:*** *the needle/catheter must be free of any and all metals that cannot enter the magnet proximity! Even if only slight, motion due to magnetic force can dislodge a needle from the vein and cause injury and harm to the animal.*

2. Animal positioning and coil placement

a. Once the animal is properly sedated (and catheter, when applicable, is placed), the animal must be positioned on the scanner bed in an orientation which exposes the tumor-bearing region for proper placement of the surface coil. In the case of a right hind limb, the mouse is positioned on its left side with the isoflurane nose cone adjusted accordingly. The tumor-bearing limb is easily accessed in this position for placement of the surface coil (see below):



b. A pneumatic pillow must be securely fastened on/under the thorax of the animal for proper breath monitoring. Temperature monitoring can be achieved via external thermometer placement under the body, or more accurate measures can be achieved with internal monitoring, such as with rectal thermometry.

c. The surface coil unit should be placed such that the tumor-bearing region is centered under the coil area. The unit must then be securely fastened to the bed, such that the coil will not move at any point during acquisition. This can be achieved with custom bed printing, or by securing with a reliable laboratory tape. Note: the animal does not have to be able to move under the coil, but the coil ***must not*** impede breathing. Breath obstruction is immediately recognizable by a sudden drop in breath rate, as measured with the pneumatic pillow, upon coil placement.

d. *Remember to <u>always continue monitoring</u> the animal vitals when it is on the scanner, especially when going through set up steps.* It may be useful for some users to designate a person for health monitoring for the duration of the scan. During position adjusting, sequence setup, and adjustment running, it can be difficult to simultaneously manage animal vitals. Consider having an animal monitor, especially in the beginning of experiments.

e. Once the animal is positioned and secured on the bed, and vital sign monitoring ensures that the animal is properly anesthetized, the bed can be moved into the bore of the magnet. The center of the active surface coil area should be aligned with the center of the volume coil.

    1. Achieving proper alignment of the surface coil within the volume coil is critical in obtaining good images. It may be easiest to mark the center of the surface coil on the unit.

    2. Once the coil is placed on the animal, use a non-metal yardstick to measure the center-to-exterior distance (mentioned in the first step) and mark this length on the bed apparatus.

    3. Then, when placing the bed in the magnet, align the mark with the exterior magnet. This should get the active area close enough to the center to acquire a useful localizer.

3. Initiating a localizer

a. *Remember to always continue monitoring the animal vitals when it is on the scanner, especially when going through set up steps.*

b. To initiate a localizer, you must locate the appropriate protocol in the scanner interface/software. Accessing and operating scan protocols may differ between machines, so consulting the manual should provide necessary insight in operating a localizer scan.

   i. Generally, a simple short T1-weighted image with a single slice in each orthogonal direction (converging at the center/origin) should provide enough spatial information to ensure that the animal is properly placed underneath the coil.

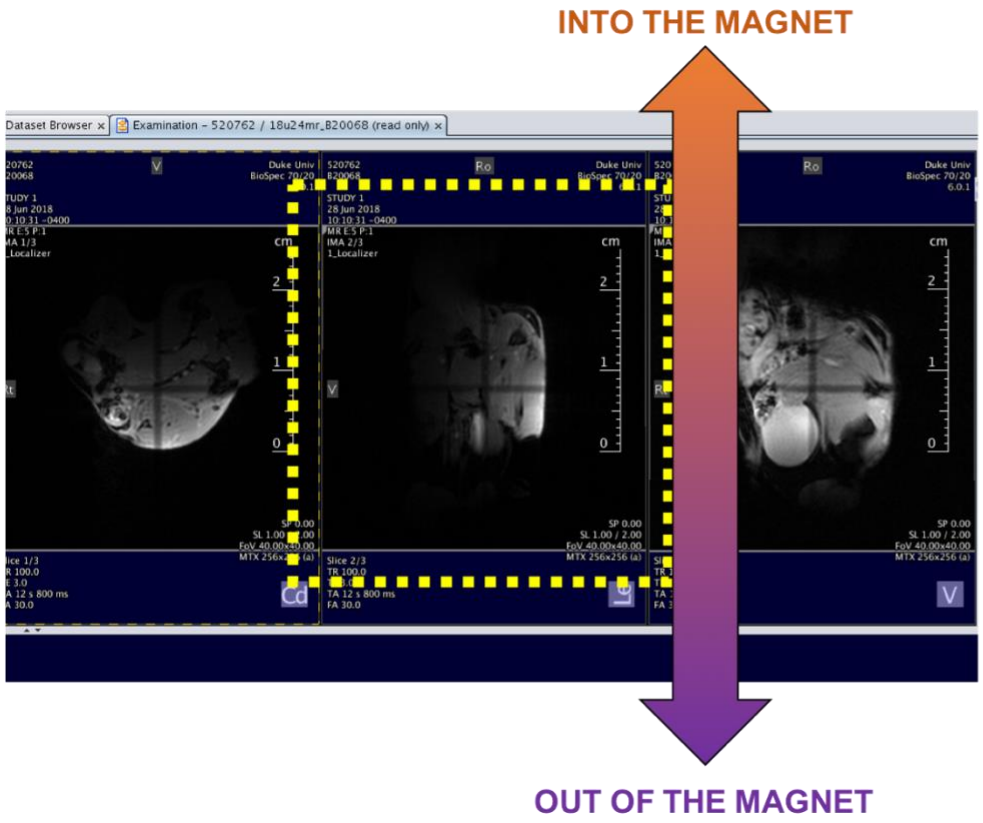
   ii. If using a pre-programmed localizer protocol, follow the instructions provided in the operation manual. If not, prior to scanning, it may be useful for the user to set the basic frequency, operate a simple shim, and set the reference power.

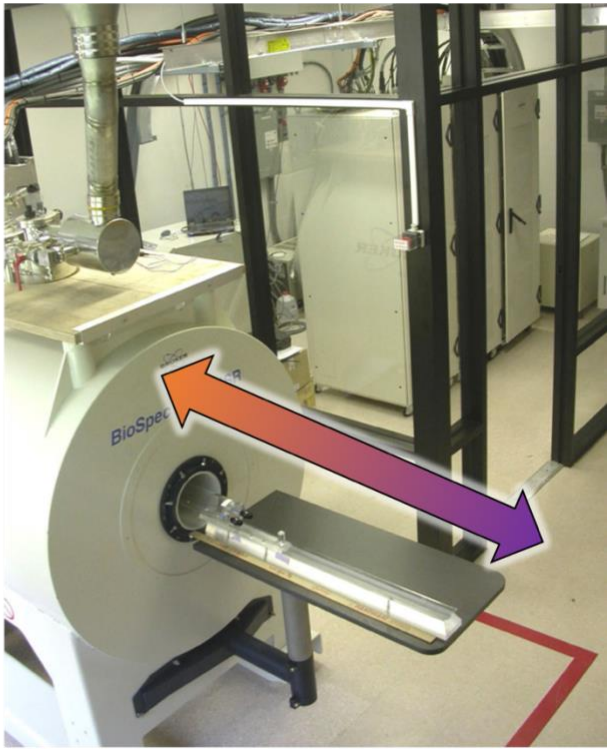
   iii. Upon running the localizer, the user should be able to see the animal in 3 orthogonal views and determine if the coil or bed needs to be repositioned. Types of repositioning:

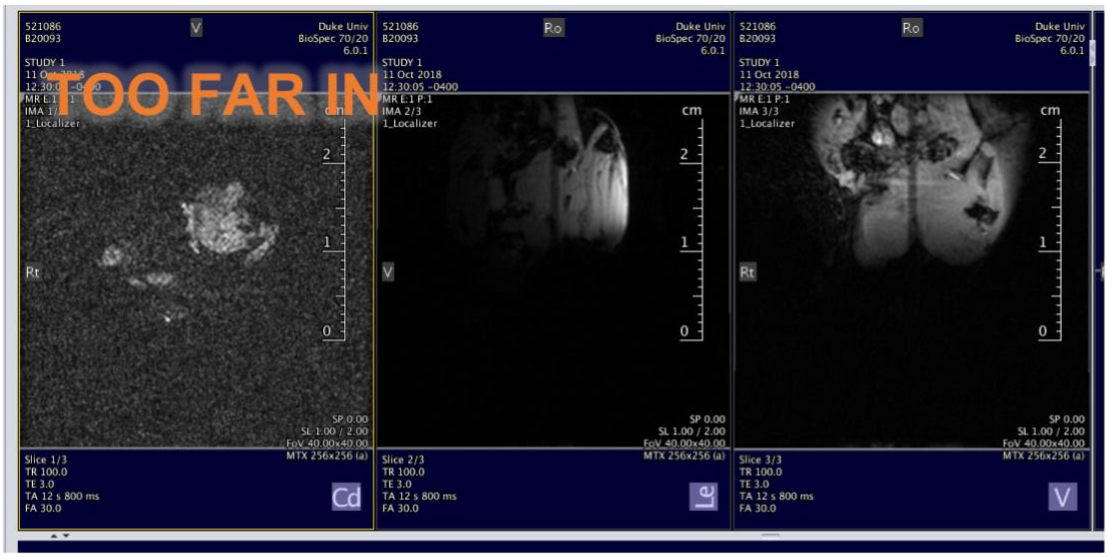
      1. **Good signal, incorrect anatomical area.** In this case, the image shows plenty of bright, visible areas of the body, but the part of the body shown is not the tumor-bearing area. This requires a repositioning of the surface coil on the animal and bed. The bed must be removed from the bore of the magnet, and the surface coil must be repositioned on the animal. Secure, replace, center and re-scan.

      2. **Poor signal, correct anatomical area.** In this case, the surface coil is likely sitting over the correct spot on the animal, but the signal is weak and appears off-center in the localizer. This is a product of a misaligned coil. Remember that the surface coil must be centered in the volume coil, which must be

centered in the magnet. First, try repositioning the bed, in an attempt to align the animal/surface coil in the center of the volume coil. Scan again with the localizer protocol. As the area of interest gets closer to centered, the signal will improve, and the anatomy will become centered in the localizer image. An example of a localizer showing a bed position error is shown below (acquired on a Bruker 7T magnet, with ParaVision 6.0.1 software):


INTO THE MAGNET
Dataset Browser
Examination – 520762 / 18u24mr_B20068 (read only)
OUT OF THE MAGNET




TOO FAR IN
Duke Univ
BioSpec 70/20
6.0.1
STUDY 1
11 Oct 2018
Slice 1/3
Slice 2/3
Slice 3/3
TR 100.0
TE 3.0
TA 12 s 800 ms
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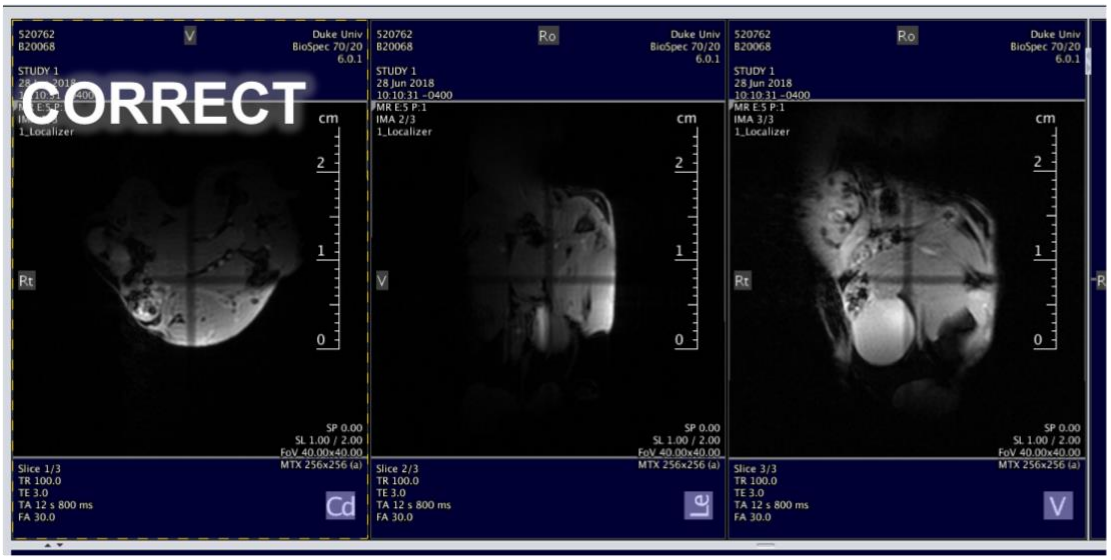
CORRECT
Duke Univ
BioSpec 70/20
6.0.1
STUDY 1
28 Jun 2018
Slice 1/3
Slice 2/3
Slice 3/3
TR 100.0
TE 3.0
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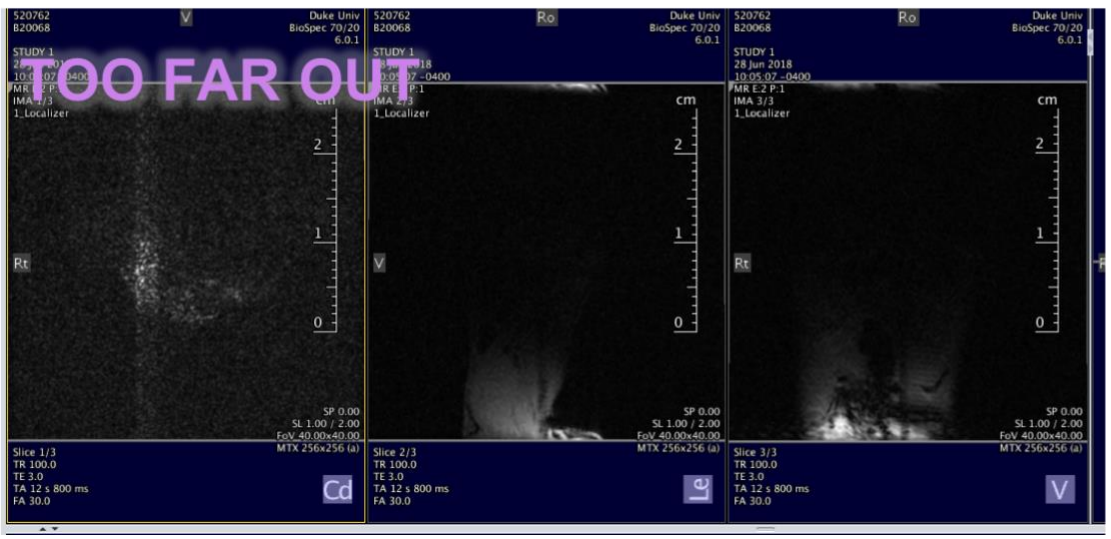
TOO FAR OUT
Duke Univ
BioSpec 70/20
6.0.1
STUDY 1
Slice 1/3
Slice 2/3
Slice 3/3
TR 100.0
TE 3.0
TA 12 s 800 ms
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V

3. **No signal and/or acquisition failure.** This is likely due to a complete failure in alignment, if all other adjustments (basic frequency, shim) ran without failure. Acquisition fails if there is no signal to detect. This can be caused by lack of sample, off-center surface coil (the bed is too far in or out), or incorrect volume coil alignment. In vivo samples have adequate sample for proton MRI, so misalignment is likely the cause of failure. Adjust the bed position first. If this is unsuccessful, the volume coil may need to be re-centered and secured.

iv. Once a successful localizer had been acquired, the position of the bed/animal can be adjusted until the region of interest is centered. An example acquired on a Bruker 7T magnet, with ParaVision 6.0.1 is shown here:

v. From here, the scan protocol can be set up and initiated.

vi. Note: Identifying specific regions of interest, such as tumor, may be difficult to do in a simple localizer. If a tumor is of reasonable size compared to the localizer voxel, tumors are often visible when near the center/origin of the scan. However, it is usually difficult to know with certainty the 3-dimensional boundaries of that tumor, which may inform the field of view (FOV) of subsequent scans. There are two means of overcoming this limitation:

    1. Use a larger FOV in the subsequent scan program (non-localizer acquisitions). If time/resolution restraint permit, a larger FOV increases the chances of including all relevant anatomy without excessive localizer scanning and re-positioning. This is extremely useful in the context of high-throughput, multi-animal studies, where scan protocols are standardized and time is an important factor. It also allows the same pre-programmed scan to be used each time, instead of customizing a scan for each animal, improving reproducibility and streamlining workflow.

    2. Use a more complex localizer. If the subsequent acquisitions are spatially limited, the specific region of interest may need to be identified. In this case, using a multi-slice and/or T2-weighted localizer may be of value. This depends on the needs of the experiment, and is not as useful for high-throughput studies.

4. Initiating a scan protocol and defining the FOV

a. *Remember to <u>always continue monitoring</u> the animal vitals when it is on the scanner, especially when going through set up steps.*

b. Once the animal is positioned appropriately, set up the first scan of the experimental scan protocol. This may be a pre-programmed sequence, or a house-written protocol. Consult with the manual of the specific machine/software for details on choosing, modifying, or building sequences.

c. Select a FOV that incorporates all of the anatomy of interest. Note that the FOV is most often limited by resolution and time. Selection of FOV, resolution, etc. should be based on the specific sequence, the desired output, and the needs of the user. The desired FOV should be placed over the tissue, such as the tumor, as it appears in the localizer. If, to achieve this, the FOV is significantly off-center from the origin, it may be useful to continue repositioning with localizers until the region of interest is reasonably centered.

d. If running multiple scans as part of a scan program, prepare and assemble these sequences for successive acquisition. Adjust the FOV of these images accordingly.

e. Prior to initiating the scan, it is often useful to perform a series of adjustments to ensure better acquisition. These include, but are not limited to:

    i. **Wobble –** tuning and matching of the volume coil.

    ii. **Set the basic frequency**

    iii. **Perform necessary shims.** A variety of shim techniques can improve scan quality. The order of shim and shim technique may vary based on the needs of the imaging and the user. Consult with scanner/program documentation for shim implementation details.

    iv. **Setting the reference power**

v. **Generating a B0 inhomogeneity map.**

f. Once adjustments are performed, initiate the scan program. Monitor the animal's vital signs, adjusting temperature and isoflurane as needed. For further information on animal support, it is recommended that the user consult with veterinary and/or DLAR staff at their institution.

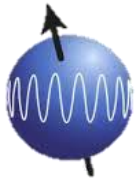
SECTION II – Detailed (machine-specific) protocol based on U24 studies at Duke University CIVM

PROTOCOL

1. Opening ParaVision 6.0.1 and generating a new study

a. Log into the designated user on the computer lock screen.

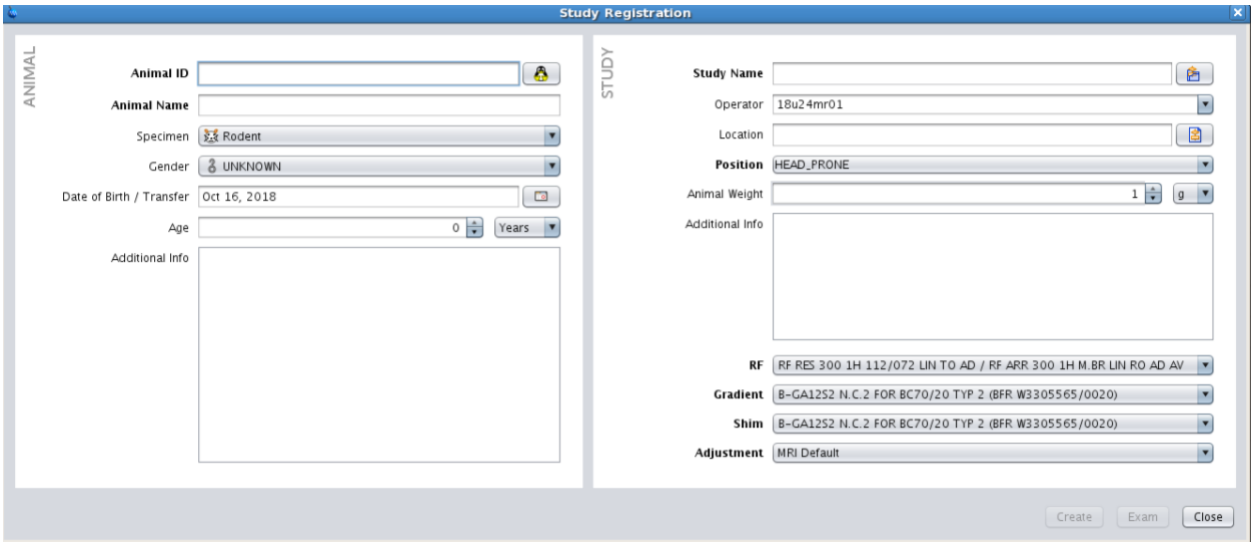
b. Locate the ParaVision 6.0.1 icon on the desktop and launch:



c. Once the software had finished loading (orange bar at top of screen will disappear), you can generate a new study.

d. Choose File → New → Study

e. The Study Registration box will appear:



f. Fill in the study information as described in the labeled figure on the following page.

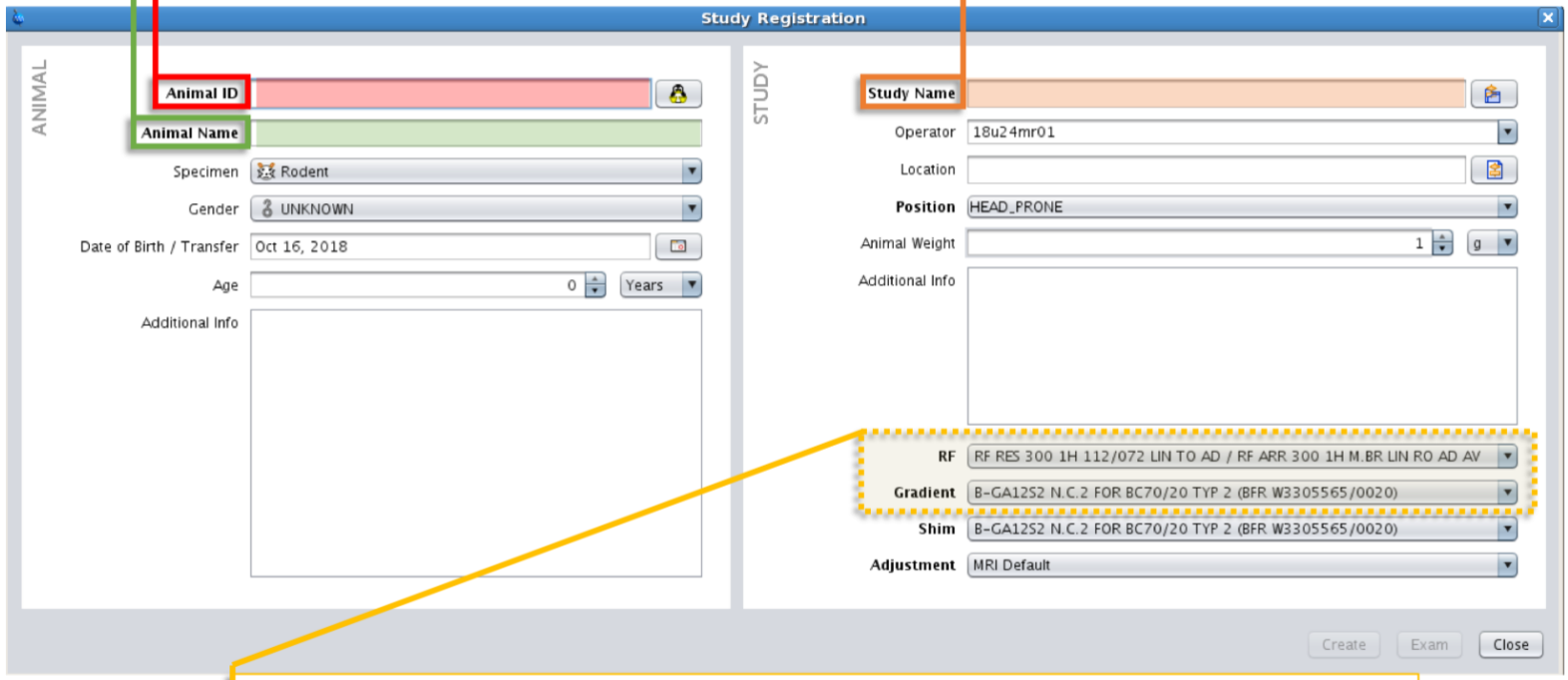
g. Once the information has been entered, select “Create”, followed by “Exam”

h. This will open up the Examination Window. This is comprised of the protocol list on the left, the viewing panels on the top, and the Instruction Cards on the bottom (where you execute protocols). The Acquisition screen will be on the right monitor and will be blank prior to starting.

Animal ID: Enter the next sequential run number.

Animal Name: Enter the designated animal number or identifier.

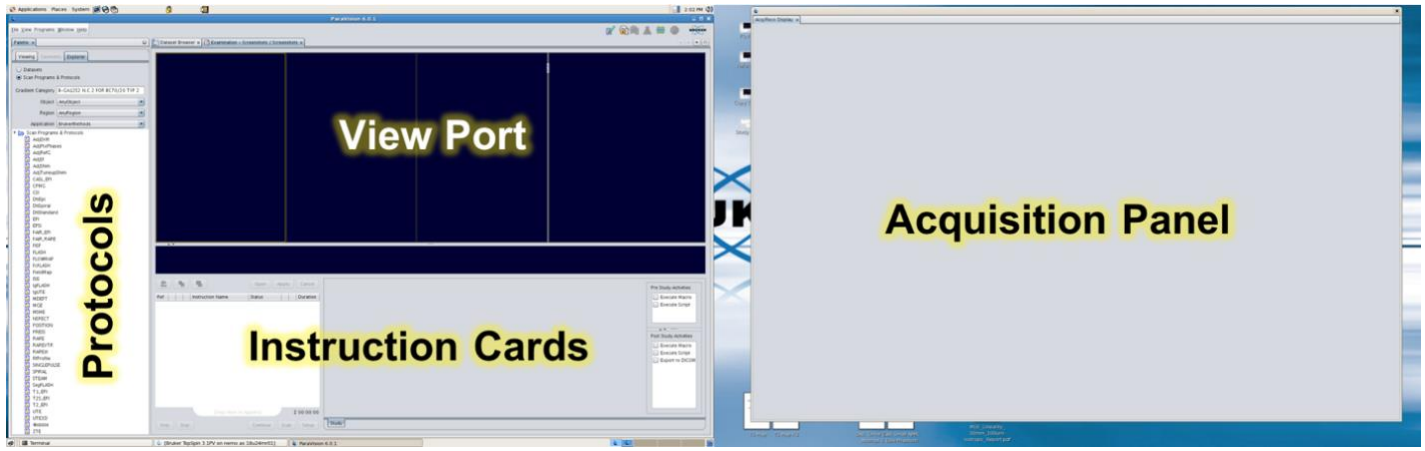
Study Name: Enter the study code for project identification. Particularly useful when specifying billing for a project.



Ensure that the RF and Gradient configurations are correct. They should be set automatically, so just check to make sure:

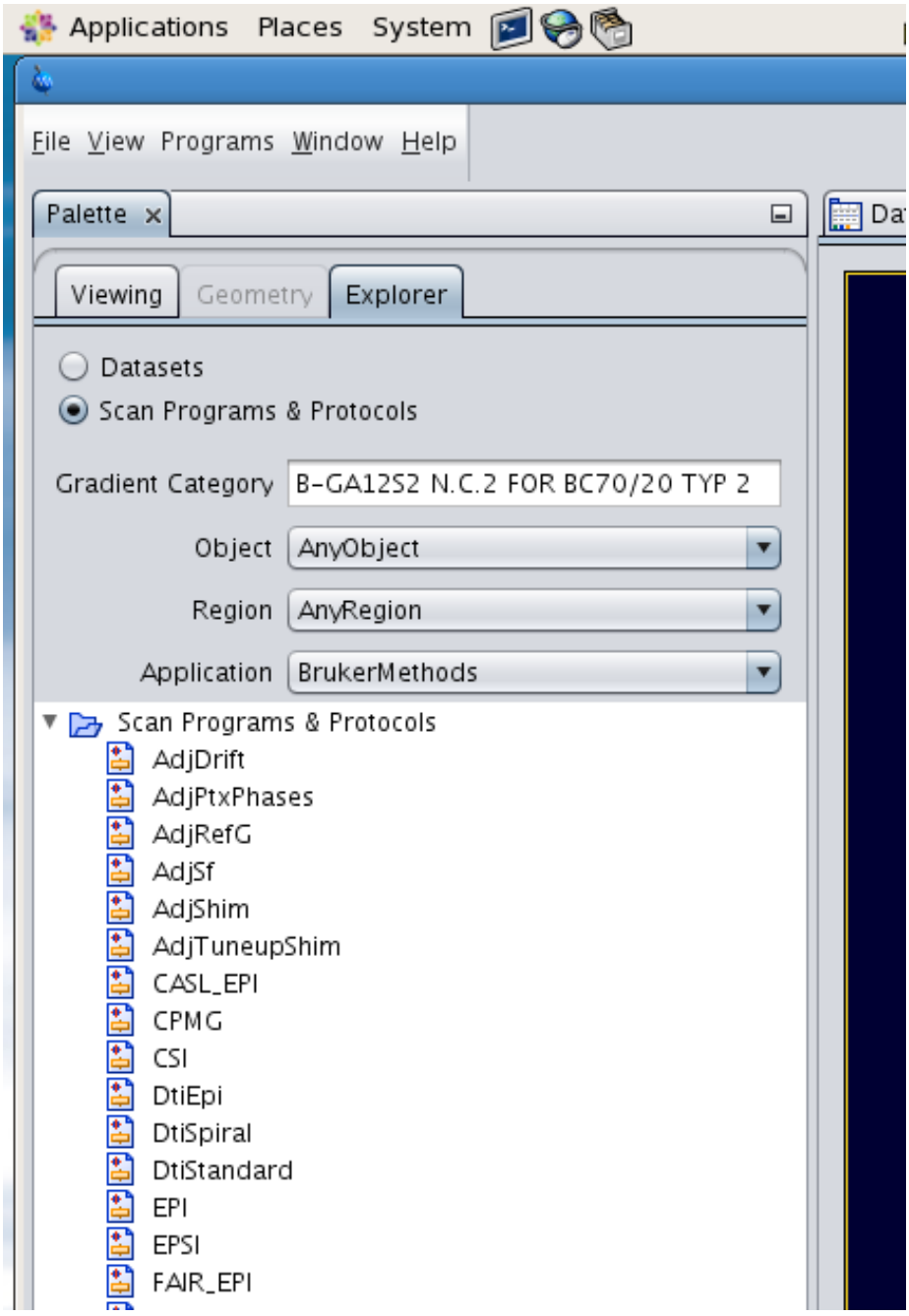
RF: **112/072 LIN** / **R.BR**

Gradient: B-GA12S2 N.C.2 FOR BC70/20 TYP 2 (BFR W3305565/0020)


View Port
Protocols
Instruction Cards
Acquisition Panel

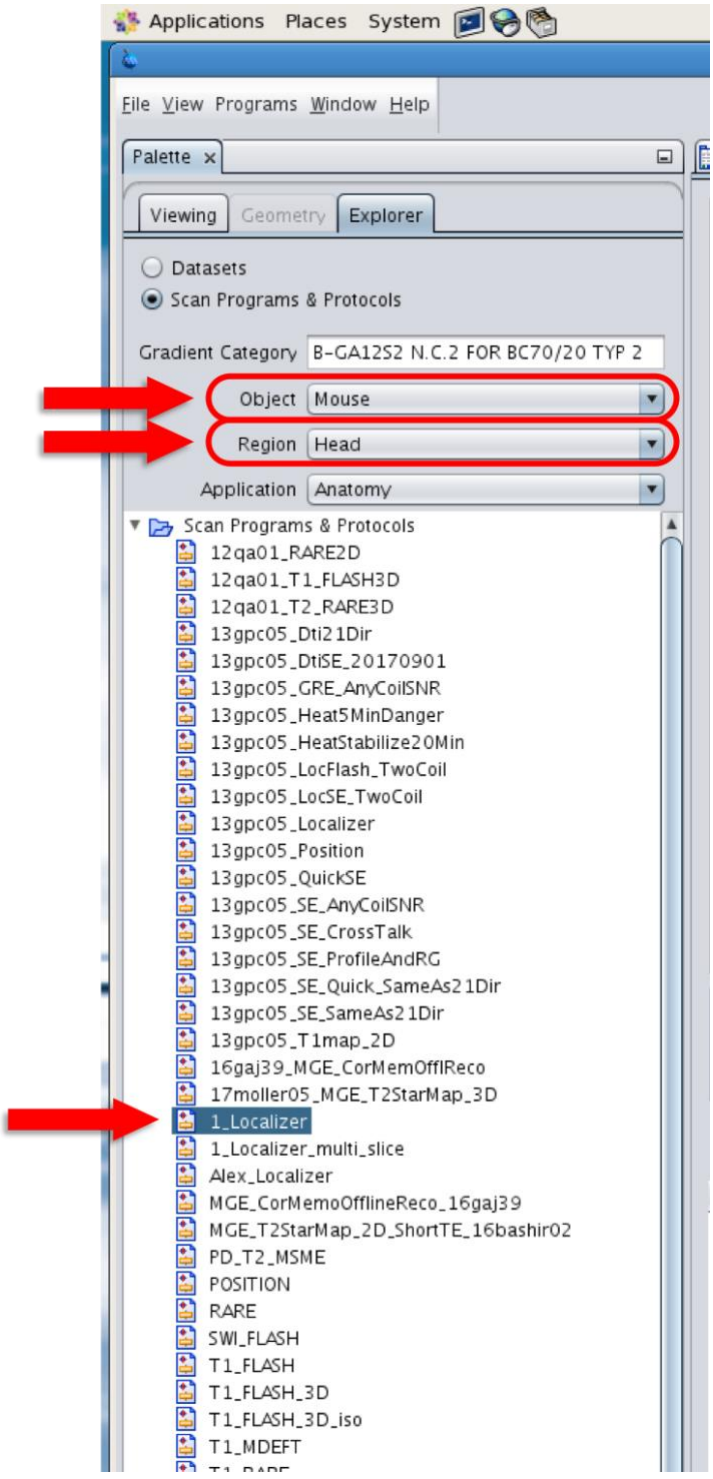
2. Initiating a localizer

a. To initiate a localizer, you must locate the protocol in the explorer on the left side of the screen. This area will display a list of protocols from which you can choose:

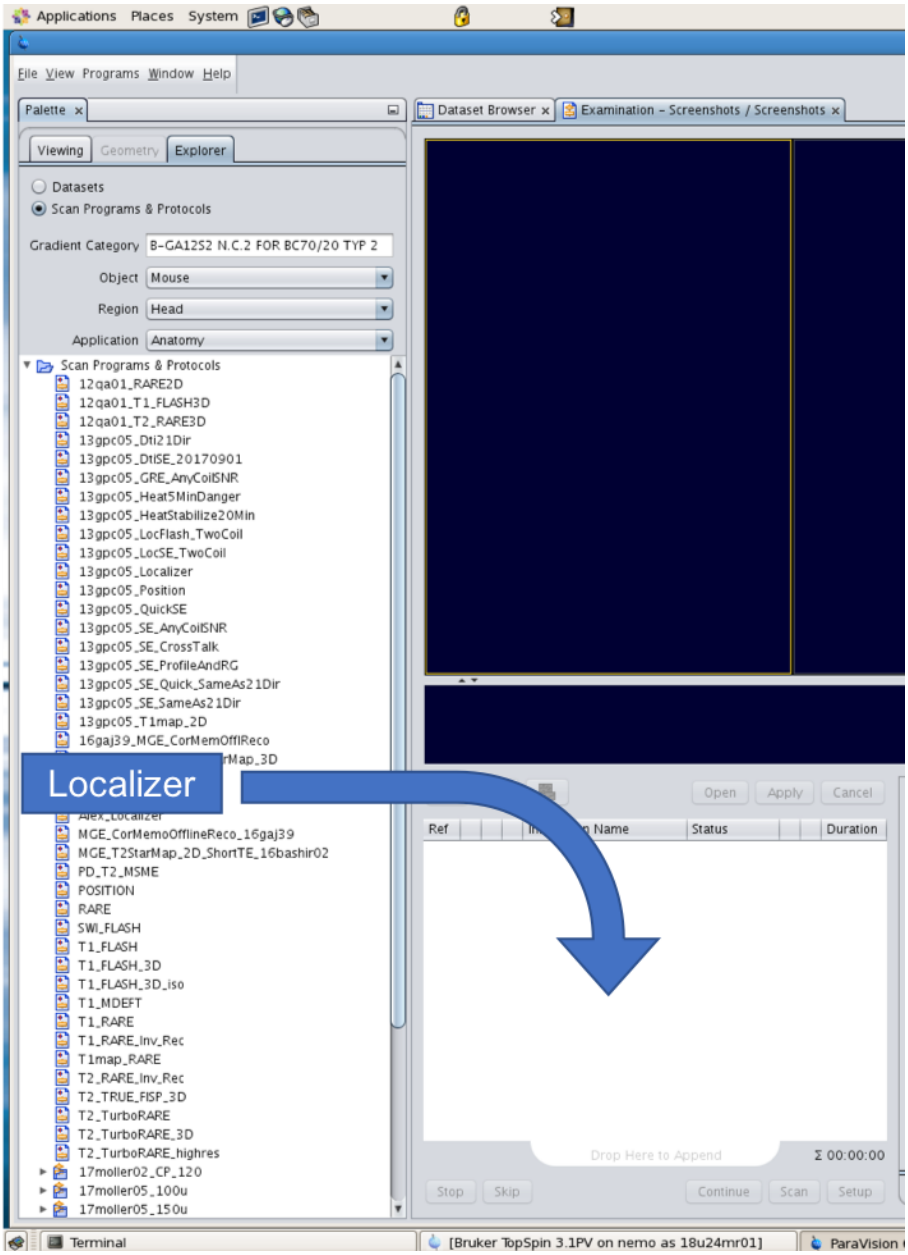


b. The default list will be “Any Object” and “Any Region”

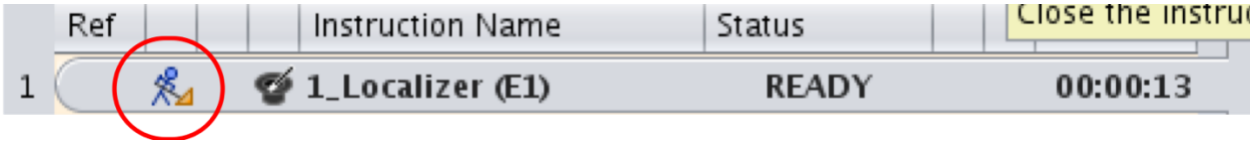
c. A general localizer will be listed under “Mouse” and “Head”, as shown here:



d. Click on “1_Localizer” until it is highlighted blue. Using the mouse, drag and drop the localizer command into the instruction panel (white box at the bottom of the screen):

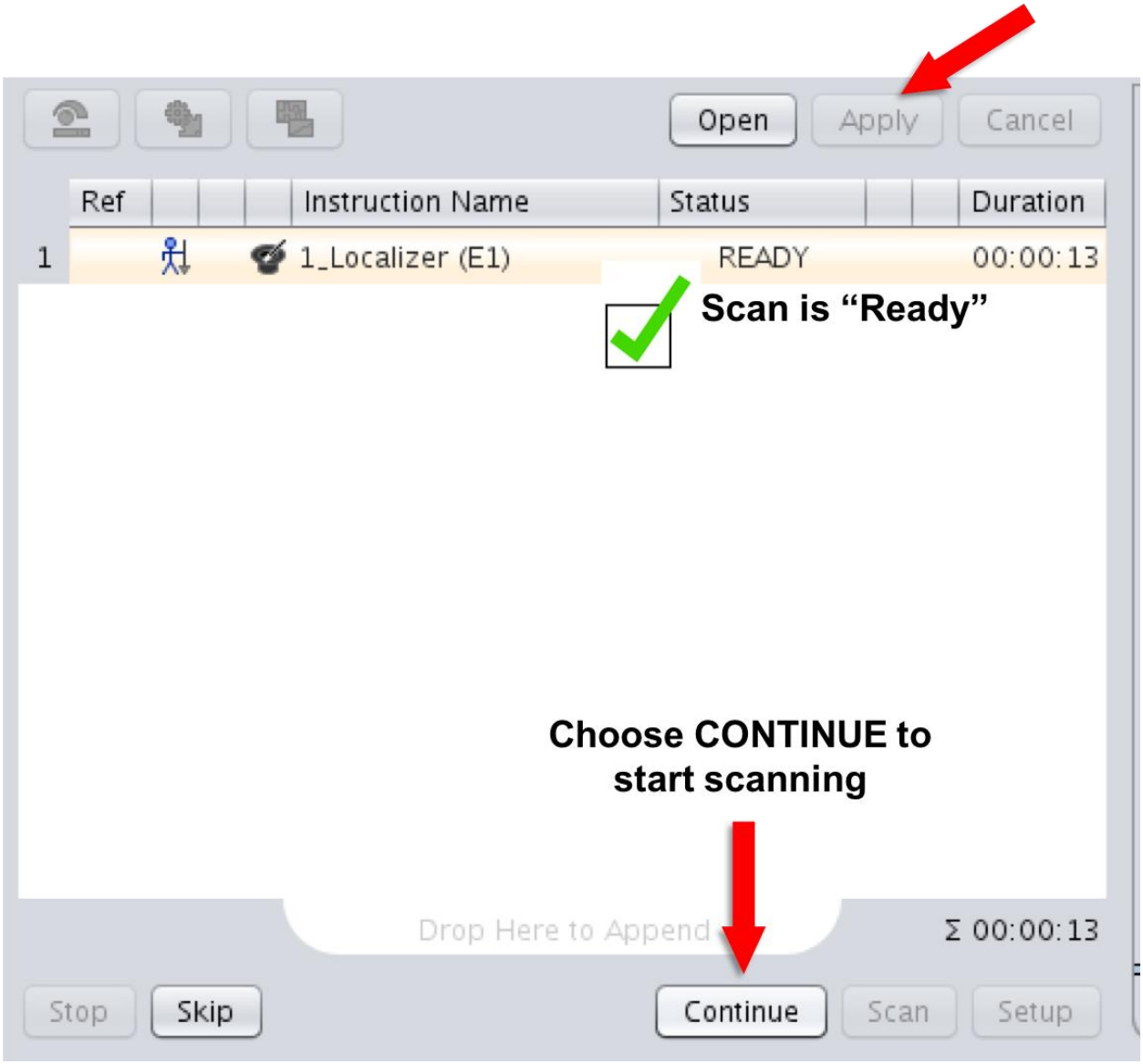


e. The localizer will appear in the panel with a shoveling stick figure next to it. This means “requires user input”.



f. In this case, the only user input required is to click “Apply” above the instruction card. The stick figure should then change

to be standing up straight, and the scan will say “Ready”. To start the localizer, click “Continue”:



g. This will begin the scan. First, the scanner will automatically run certain adjustments, including setting the basic frequency, shimming, and setting the reference power. As they run, each adjustment will be noted at the top of the screen, and the spectra appear in the acquisition window.

h. Once completed, three orthogonal images will appear in the view ports:

i. At this point, the animal/bed may need to be adjusted to center it in the scanner, as described in detail in the next section.

3. Positioning the animal for scanning

a. The leg will appear as a dome (pointing downward) in the first panel, and a lump (pointing to the right) in the middle panel.

b. If the coil was positioned properly on the leg, the only adjustments that should need to be made are movement of the bed further in or out of the magnet bore. The center panel is best for determining depth of the animal into the magnet.

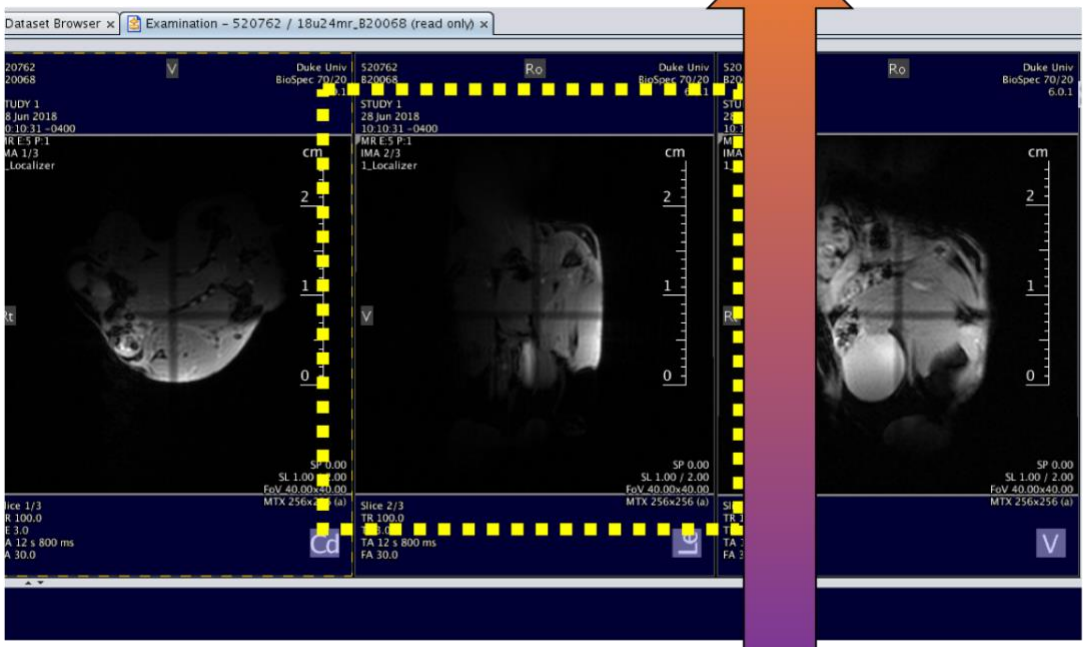
c. If the bed position needs to be adjusted, you will want to run another localizer to ensure proper placement. Duplicate the same localizer by right-clicking on the completed localizer instruction and selecting “Duplicate instruction”.

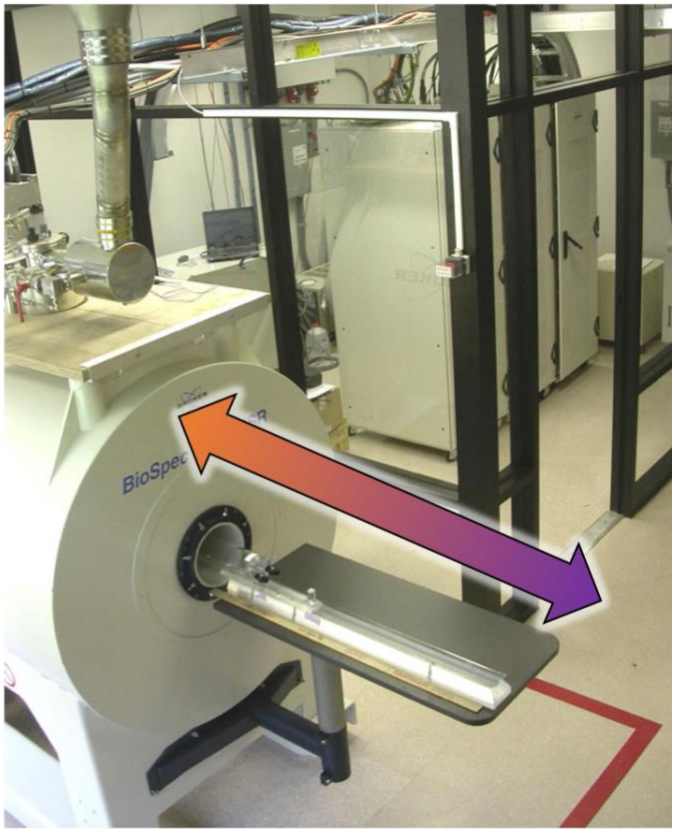
d. This will create another identical localizer in the instruction panel. Click “Apply” and then “Continue” to run the next localizer.

e. Repeat steps C and D until the animal is in the best position for scanning.

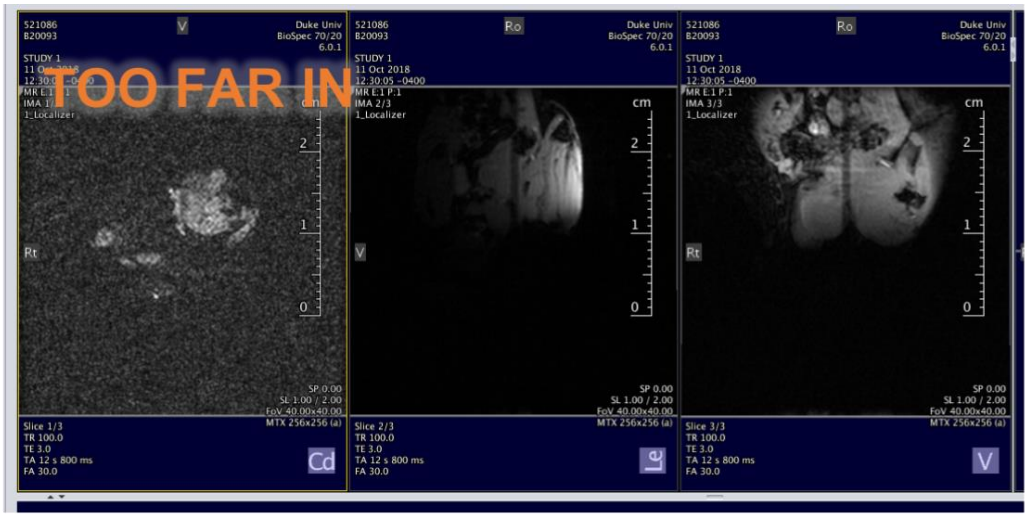
f. **See the next two pages to demonstrate positioning of the bed based on information seen in the localizer.**

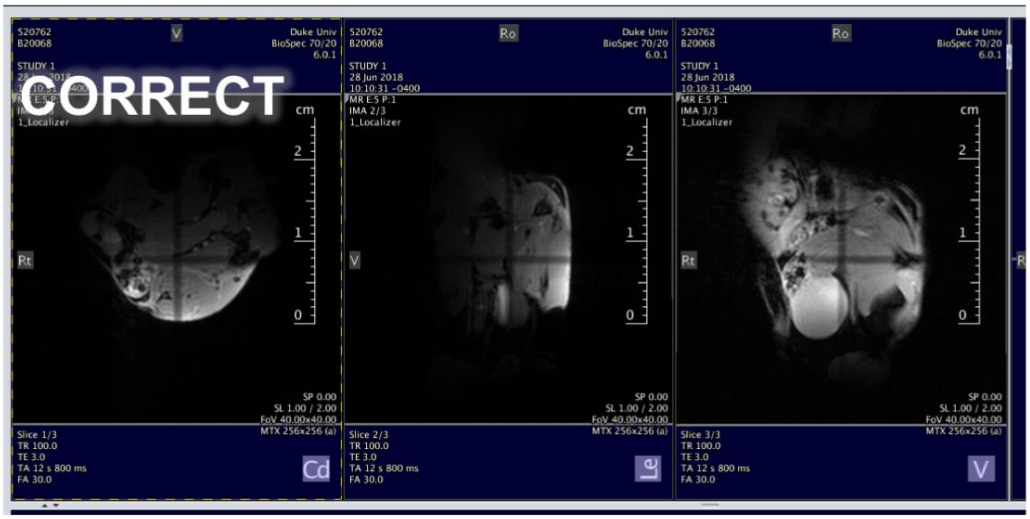
INTO THE MAGNET

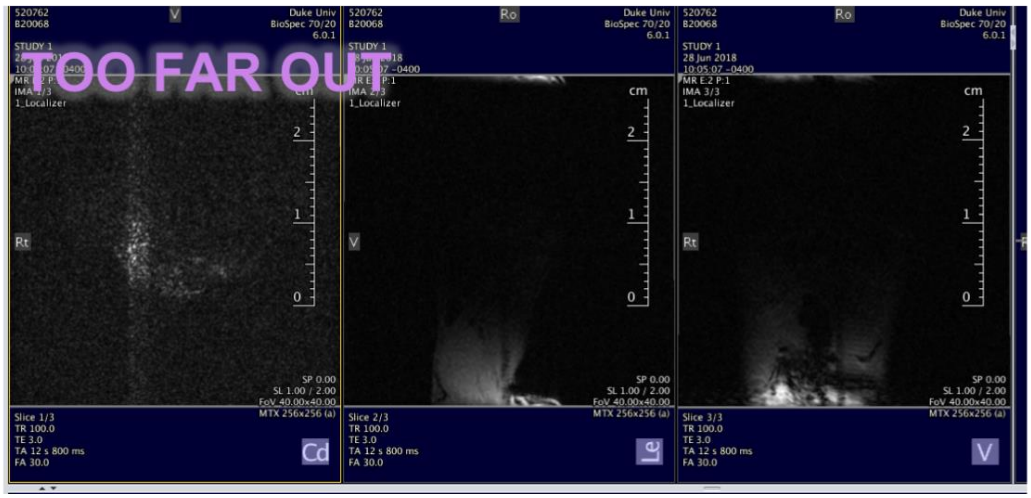


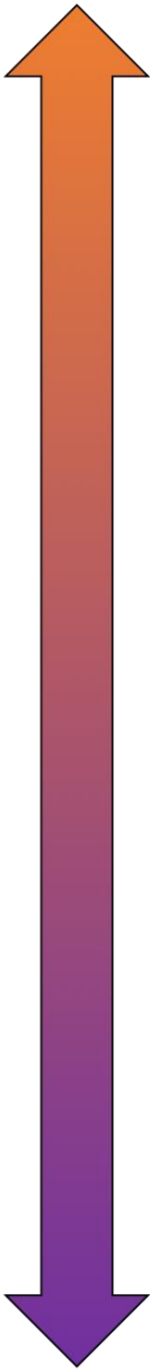


OUT OF THE MAGNET


TOO FAR IN

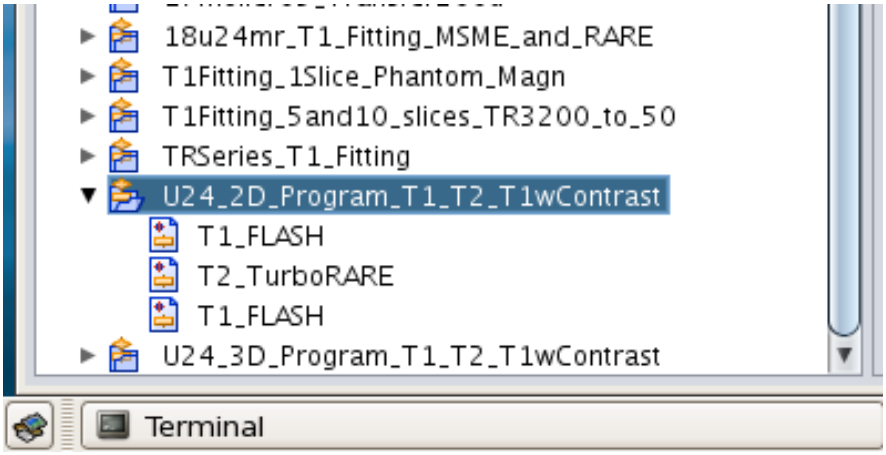

CORRECT


TOO FAR OUT



4. **Selecting the first study protocol (example: U24 project, T1_FLASH protocol) and field of view**

   a. Once the animal is positioned appropriately, select the first study (U24) scan protocol from the protocol list on the left. In this case, the scan protocols have been saved with pre-defined input parameters. Once a useful program has been set up, it can be saved in a study folder. In this case, the folder begins with 2D_U24… (shown below).



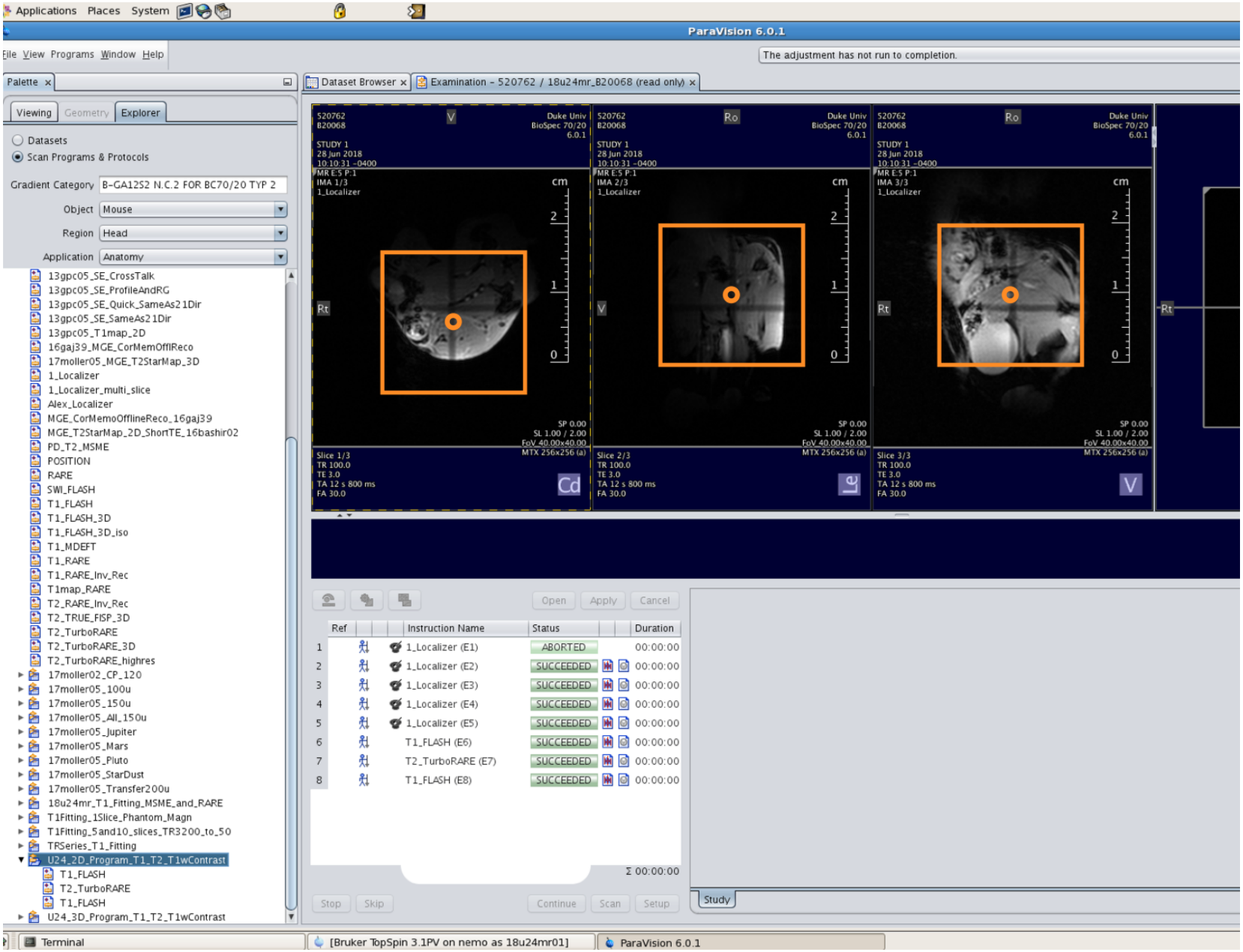
   b. Drag and drop the desired protocol into the instruction panel. For the U24 study, the first sequence in the U24 program is a T1_FLASH sequence.

   c. An orange rectangle should appear in each section of the viewport. (If it doesn't for some reason you can click "Geometry" tab in the upper left-hand corner and then click back to the "Explorer" tab. For some reason, this activates the geometry to appear).

   d. The orange box represents the FOV of the scan. It can be moved around using the mouse. Position the FOV over the "lump" that is the mouse's leg (see next page).

   e. If a mistake is made with the geometry that can't seem to be fixed (such as an accidental rotation), simply click "Cancel" on the instruction panel and try again.

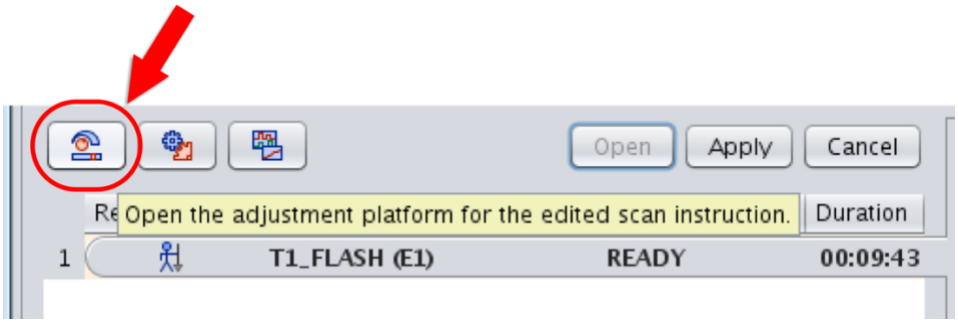
   f. Once the geometry is correct, click "Apply" to save the changes.


Applications Places System
ParaVision 6.0.1
File View Programs Window Help
The adjustment has not run to completion.
Palette
Dataset Browser
Examination - 520762 / 18u24mr_B20068 (read only)
Viewing
Geometry
Explorer
Datasets
Scan Programs & Protocols
Gradient Category B-GA12S2 N.C.2 FOR BC70/20 TYP 2
Object Mouse
Region Head
Application Anatomy
13gpc05_SE_CrossTalk
13gpc05_SE_ProfileAndRG
13gpc05_SE_Quick_SameAs21Dir
13gpc05_SE_SameAs21Dir
13gpc05_T1map_2D
16gaj39_MGE_CorMemOffReco
17moller05_MGE_T2StarMap_3D
1_Localizer
1_Localizer_multi_slice
Alex_Localizer
MGE_CorMemoOfflineReco_16gaj39
MGE_T2StarMap_2D_ShortTE_16bashir02
PD_T2_MSME
POSITION
RARE
SWI_FLASH
T1_FLASH
T1_FLASH_3D
T1_FLASH_3D_iso
T1_MDEFT
T1_RARE
T1_RARE_Inv_Rec
T1map_RARE
T2_RARE_Inv_Rec
T2_TRUE_FISP_3D
T2_TurboRARE
T2_TurboRARE_3D
T2_TurboRARE_highres
17moller02_CP_120
17moller05_100u
17moller05_150u
17moller05_All_150u
17moller05_Jupiter
17moller05_Mars
17moller05_Pluto
17moller05_StarDust
17moller05_Transfer200u
18u24mr_T1_Fitting_MSME_and_RARE
T1Fitting_1Slice_Phantom_Magn
T1Fitting_5and10_slices_TR3200_to_50
TRSeries_T1_Fitting
U24_2D_Program_T1_T2_T1wContrast
T1_FLASH
T2_TurboRARE
T1_FLASH
U24_3D_Program_T1_T2_T1wContrast
520762
B20068
Duke Univ
BioSpec 70/20
6.0.1
STUDY 1
28 Jun 2018
10:10:31 -0400
MR E:5 P:1
IMA 1/3
1_Localizer
cm
Slice 1/3
TR 100.0
TE 3.0
TA 12 s 800 ms
FA 30.0
SP 0.00
SL 1.00 / 2.00
FoV 40.00x40.00
MTX 256x256 (a)
Slice 2/3
IMA 2/3
Slice 3/3
IMA 3/3
Open
Apply
Cancel
Ref
Instruction Name
Status
Duration
1 1_Localizer (E1) ABORTED 00:00:00
2 1_Localizer (E2) SUCCEEDED 00:00:00
3 1_Localizer (E3) SUCCEEDED 00:00:00
4 1_Localizer (E4) SUCCEEDED 00:00:00
5 1_Localizer (E5) SUCCEEDED 00:00:00
6 T1_FLASH (E6) SUCCEEDED 00:00:00
7 T2_TurboRARE (E7) SUCCEEDED 00:00:00
8 T1_FLASH (E8) SUCCEEDED 00:00:00
Σ 00:00:00
Stop
Skip
Continue
Scan
Setup
Study
Terminal
[Bruker TopSpin 3.1PV on nemo as 18u24mr01]
ParaVision 6.0.1

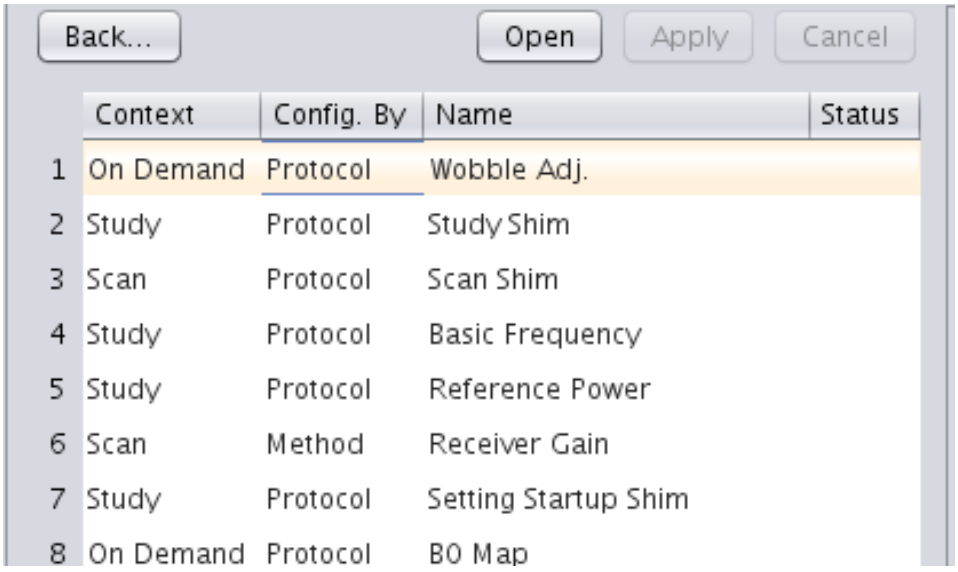
5. Running adjustments

a. Prior to running the first scan, you must re-run some of the adjustments to account for any repositioning that occurred after the very first scan.

b. Adjustments are run by launching the “Adjustments” platform, which is the Left-most button just above the instruction panel:

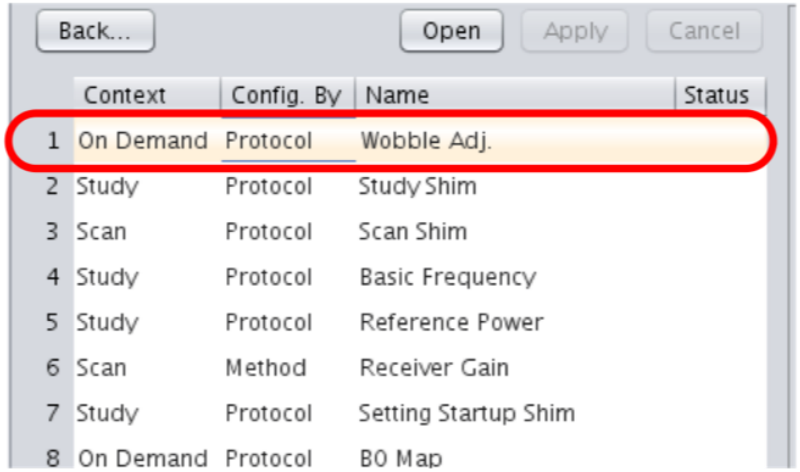


c. This will open the Adjustments applicable to the scan protocol (the T1_FLASH protocol), and list them in the instruction panel:



d. Step-by-step diagrams for Adjustment completion are shown in the following pages:

First Adjustment: Wobble
(Tune and match)

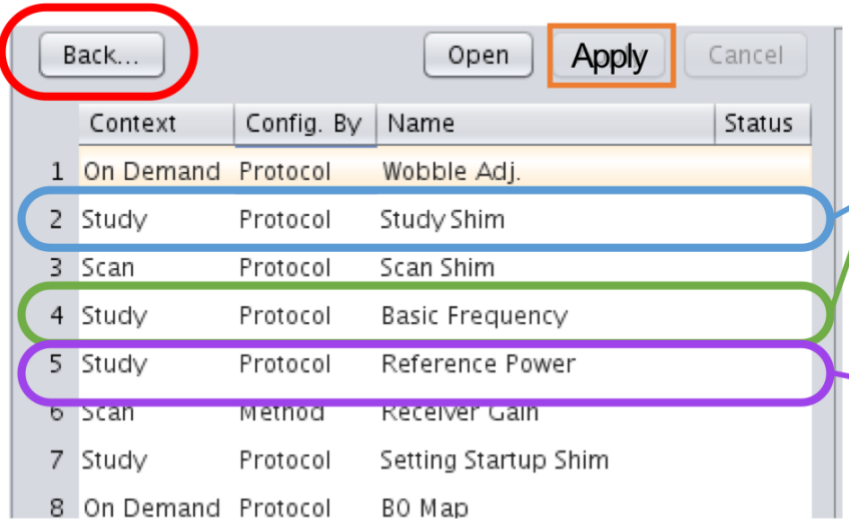


1. Double click on Wobble Adj.
2. Select “Setup” at the bottom-right of the adjustment panel.
3. Wait for the spectrum to appear on the right monitor.

4. CAREFULLY AVOID ANY TAIL VEIN CATHETER LINES WHEN MOVING ABOUT THE SCANNER

5. Use the rods in on the back of the volume coil to tune and match. The lights in the back of the machine can be used to tune and match, since the screen is nearly impossible to see.
6. *Note: moving the cables of the surface coil can alter the position and shape of the peak. If you are having trouble T/M-ing, try shifting the cables.*
7. When you are reasonably satisfied with the T/M, select “Stop”.
8. Once it stops processing, select “Apply”.

Other Adjustments:



1. **Basic Frequency**
   - Double-click “Basic Frequency”
   - Select “Start”
   - Allow to run
   - When finished, click **“Apply”** at top.

2. **Study Shim**
   - Double-click “Study Shim”
   - Select “Start”
   - Allow to run
   - When finished, click **“Apply”** at top.

3. **Reference Power**
   - Double-click “Reference Power”
   - Select “Start”
   - Allow to run
   - When finished, click **“Apply”** at top.

When all adjustments have been applied, choose “Back”

e. By choosing “Back”, you will be taken back to the instruction panel which will be displaying the selected protocol (i.e. the T1_FLASH protocol). Select “Apply”. The scan is now ready to run.

f. Select “Continue” to run the sequence.

- If at any point you need to cease the scan and start over, choose “Stop” at the bottom left of the instruction panel.
- Even a scan which was aborted can be duplicated and started again by right clicking the aborted scan and selecting “Duplicate Instruction”, followed by selecting “Apply”

6. **Selecting the second study protocol (example: U24 project, T2_TurboRARE) and "copying" FOV geometry**

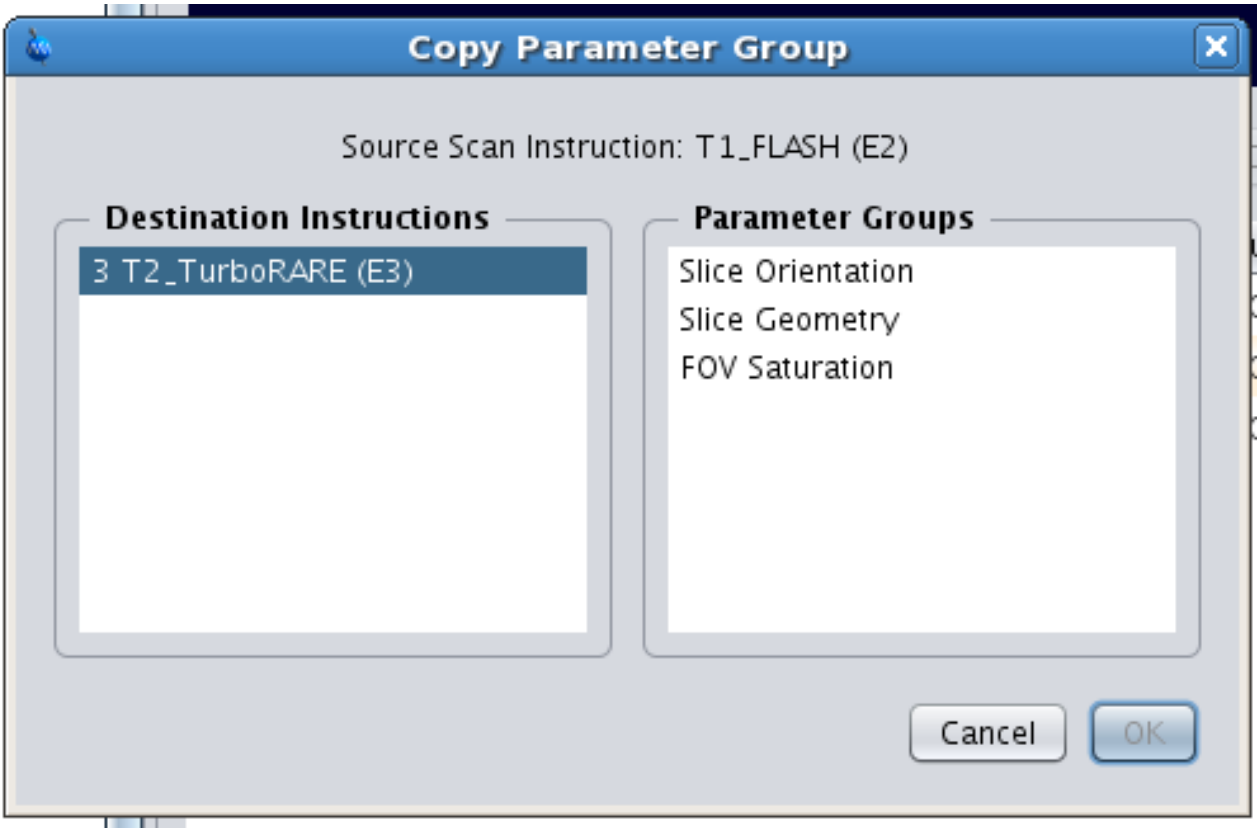
    a. As the first scan is running, the second sequence can be set up.

    b. From the protocol list, drag and drop the desired sequence (ex: T2_TurboRARE sequence) into the instruction panel underneath the first sequence. Click "Apply"

    c. The geometry from the first scan should be maintained in the second scan. To achieve this, highlight the first (T1_FLASH) sequence.

    d. While holding down the left mouse key, drag the highlighted first sequence (T1) over the second sequence (T2) until the second sequence turns a light blue.

    e. Drop the first onto the second. This will initiate the "Copy Parameter Group" window:



f. Under “Destination instructions”, the second sequence (T2_TurboRARE) should be listed.

g. From the “Parameter Groups” column, select Slice Orientation so it is highlighted. While holding down the Shift key, select FOV Saturation. Now ALL THREE should be highlighted.

h. Click OK.

i. Once back at the instruction panel, select Apply.

j. Now, both scans can be allowed to run to completion.

7. **Injection of contrast (+ wait 3 minutes), and duplicating the T1 Sequence**
    a. In the U24 protocol, once the T2 sequence has completed running, contrast agent is injected via the tail vein. If the scan protocol is relatively short, there will likely be a timed delay required prior to scan initiation. This is because the scan should be acquired at the time of peak contrast infiltration in the tissue of interest (in this case, the tumor). This timeline was determined previously by performing a time-course contrast study.

    b. Contrast enhancement is usually performed with a scan sequence that is duplicated from a non-contrast scan. In other words, the same sequence is run twice, once before and once after contrast injection. In the U24, this is the T1_FLASH protocol. The scan should be run with the same parameters. Right click on the previously-run sequence (T1_FLASH sequence). Select “Duplicate Instruction”

    c. Now a new scan with the same geometry as the first one will appear in the instruction panel. Select “Apply”. The final scan is now ready.

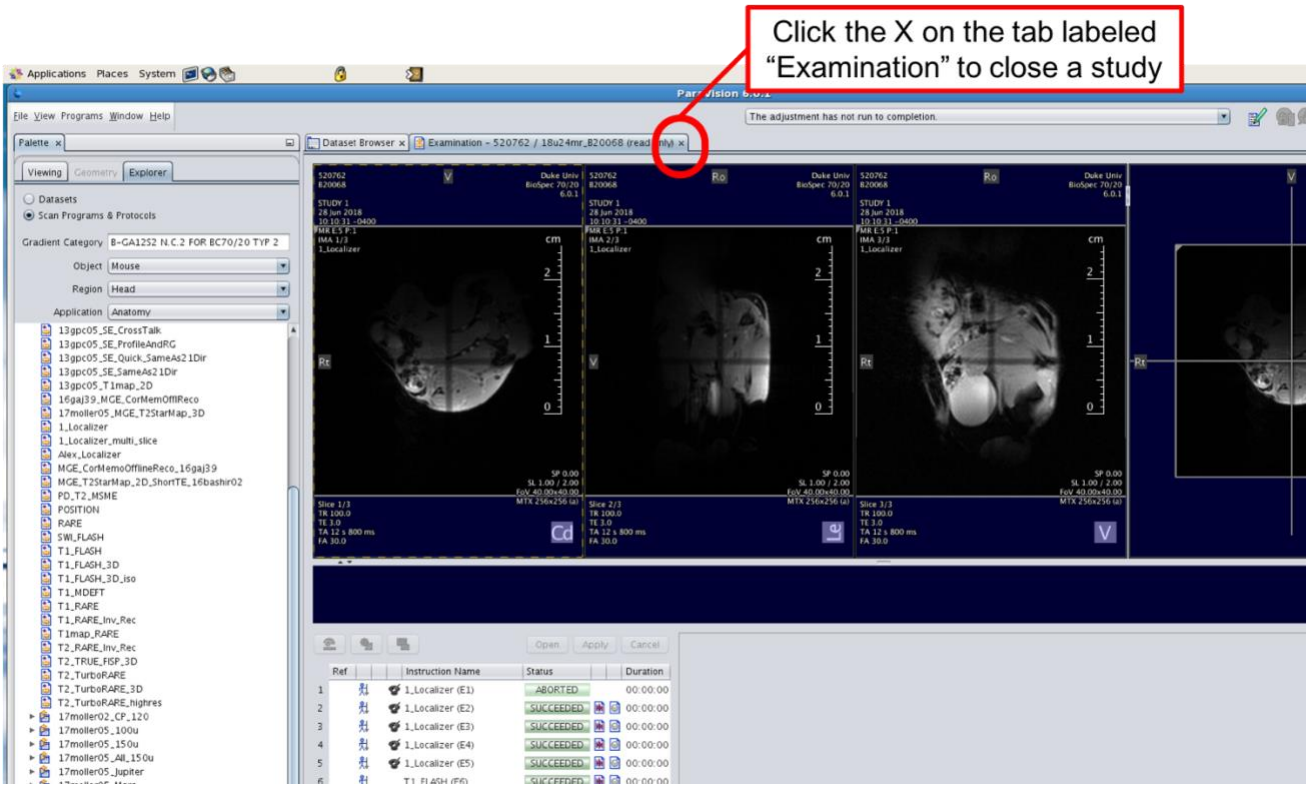
    d. Once injected, take note of the time and wait the appropriate amount of time prior to initiating the contrast-enhanced scan protocol.

    e. When the appropriate post-injection time has elapsed, select “Continue” to begin scanning.

8. Completion

a. When the final sequence has run to completion, the study is complete.

b. If you are running another mouse after the current animal, you can simply close the current examination card by clicking the X on the tab (see below). *Note: no new animals can be run while the current examination card is open. Also, an examination card cannot be reopened to continue scanning – only to read.*



- To run the next animal, close the examination card, and select File → New → Study. Return to Step 1.
- If you are finished with animals, close ParaVision, allowing it to undergo its entire closing procedure. Once it is finished, you can log out of the user on the scanner.