

WELLCOME SANGER INSTITUTE

STANDARD OPERATING PROCEDURE PACKET

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SANGER INSTITUTE STANDARD OPERATING PROCEDURE

SUBJECT: Dual Energy X-Ray Absorptiometry (DEXA) and X-Ray Imaging – V1

SOP Number: SOP0054	To be reviewed:	
Author(s):	Signed:	Date:
Editor:	Signed:	Date:
Risk Approver:	Signed:	Date:
Date of Implementation:		

INTRODUCTION:

The purpose of this procedure is to obtain high-resolution X-ray images of the mouse skeleton and to obtain body composition data by performing a DEXA scan, on wild-type and genetically altered mice.

ABBREVIATIONS:

ABR = Auditory Brainstem Response
DCF = Data Capture Form
DEXA = Dual Energy X-ray Absorptiometry
ID = Identification
IVC = Individually Ventilated Cage
LAA = Laboratory Animal Allergens
NACWO = Named Animal Care and Welfare Officer
PAF = Project Authorisation Document
PIL = Procedure Individual Licence
PPL = Procedure Project Licence
QC = Quality Control
RA = Risk Assessment
RPS = Radiation Protection Supervisor
RSF = Research Support Facility
SOP = Standard Operation Procedure
SLT = Senior Leadership Team
SMP = Sick Mouse Procedure

QUALITY CONTROL (QC) DURING PROCEDURE:

Refer to the table below for approved QC fail comments steps to be used during procedures.

If a value has been collected leave on the Data Capture Form (DCF) and then apply the fail reason from below;

Problem / Issue	QC fail reason
At any point during the procedure the mouse is deemed sick and processed through Sick Mouse Procedure (SMP)	Fail whole DCF as 'Sick mouse' – for all tests that day
Mouse incorrectly scheduled at wrong week	Fail whole DCF as 'Scheduling Issue'
Insufficient anaesthesia level affects the whole test DCF	Fail whole DCF as 'Anaesthesia Issue'
Insufficient anaesthesia level affects specific parameter(s)	Fail parameter(s) as 'Anaesthesia issue'
A welfare issue makes it impossible to collect specific parameters	Fail parameter(s) as 'Welfare issue'
Parameters affected by delays or noise due to fire alarms	Fail parameter(s) as 'Fire alarm'
An equipment failure affecting specific parameters	Fail parameter(s) as 'Equipment failure'
A software issue affecting specific parameters	Fail parameter(s) as 'Software failure'
A procedural error which affects data collection	Fail parameter(s) as 'Manual error'
Parameter cannot be assessed	Fail parameter(s) as 'Readout not possible'
Wrong value has been entered which cannot be re-measured or accounted for	Fail parameter(s) as 'Erroneous data'
Glucose meter unable to record high blood values	Fail parameter(s) as 'Meter reading HI'
Fighting occurs prior to or during data collection	Fail parameter(s) as 'Fighting during procedure'
Parameter on the current DCF is not required for that specific test/pipeline	Fail parameter(s) as 'Not required'

HEALTH & SAFETY:

This procedure is covered by the following Risk Assessment (RA):

Name: WTSI-1475

Assessment Title: DEXA X-ray recovery anaesthesia

Assessor:

Approver:

- Appropriate Personal Protective Equipment (PPE) is to be worn at all times when handling animals. This includes:
 - Overshoes
 - Gown
 - Gloves
- In addition to the above, when sources for Laboratory Animal Allergens (LAA) (animals or soiled cages) are not contained within Local Exhaust Ventilation Systems (change stations, fume hoods or downflow tables), a respiratory mask, for which you have passed a face fit test, must be worn.
- Ionising radiation is used during this test. No waste is generated from ionising radiation. Only the lowest levels required to generate image are used. Monitoring is performed and recorded monthly.

- Workers new to the process are trained and supervised by an experienced lab member until they are deemed competent to perform the task unsupervised. All operators receive local training and induction, including a radiation safety induction.
- This test poses increased level of risk to young persons and to new or expectant mothers, a separate Risk Assessment (RA) should be undertaken.
- The x-rays are contained within a shielded containment.
- The DEXA unit is shielded minimising exposure to the user and other users in the area. If the shield fails then the experiment can be stopped at any time by pressing any button on the computer and the machine can be switched off using the switch button at the back of the machine at the top.
- The Faxitron machine is internally shielded minimising exposure to the user and other users in the area. If the shield fails then the experiment can be stopped at any time by pressing the red button on the software to stop the x-rays and the machine can be turned off by switching the key to the off position.
- Lone worker alarms should be used when working alone.
- This procedure can only be performed during Research Support Facility (RSF) core hours (7:30am-7:30pm).
- All electrical equipment is to be inspected for damage before use. Both the DEXA and the Faxitron machines receive portable appliance testing (PAT) and are serviced annually.

RESPONSIBILITIES:

All staff performing this procedure are responsible for ensuring that this Standard Operating Procedure (SOP) and accompanying Risk Assessment have been read, understood and where applicable is followed in accordance with the relevant Procedure Project Licence (PPL). All staff should be trained and competent to perform the procedure, where applicable they should also be licensed to perform the procedure with a valid Procedure Individual Licence (PIL).

For secondary phenotyping, seek confirmation with project manager for deviations from this SOP. Any deviation will be detailed in the Project Authorisation Form (PAF).

RESOURCES:

Equipment:

1. Weight scale
2. 70% Ethanol - **Hazardous substance: highly flammable**
3. Hydrex Pink hand spray- **Hazardous substance: highly flammable**
4. Hydrex Hard Surface spray- **Hazardous substance: highly flammable**
5. Tecniplast mobile individually ventilated cage (IVC) recovery rack
6. Tecniplast mobile IVC transport rack
7. One clean cage and 2 nestlets per cage of mice tested
8. Diet (as defined by pipeline)
9. Clean water bottles
10. Heat mat
11. Tissues
12. 'Post Procedure Check Required' labels
13. Faxitron machine (Faxitron model MX20) and key - **Source of ionising radiation, only to be used by trained personnel with the shield in place**
14. Faxitron plates taped together (lower one with a square cut into the middle and upper of the same size without the square cut from it. The square denotes the image zone)

15. Faxitron phantom mouse
16. Surgical tape (*Supplier name; Scientific Laboratory Supplies Ltd. Supplier product code; 4702*)
17. DEXA machine: PIXImus - **Source of ionising radiation, only to be used by trained personnel with the shield in place**
18. DEXA phantom mouse
19. DEXA holding tray with securing paper (*Supplier name; Inside Outside Sales, LLC. Supplier product code; TRAY-600.*)
20. Radiation screen
21. 'Caution X-ray Procedure in Progress' sign
22. Absorptiometry and Imaging Record Sheets
23. Ruler
24. 100mg/kg Ketamine Hydrochloride, 10mg/kg Xylazine Hydrochloride (K/X) solution (Anaesthetic) - **non-hazardous in working form**
25. 1mg/kg Atipamezole hydrochloride (Antisedan) solution (Reversal) - **non-hazardous in working form**

Associated SOPs/Documentation:

- **SOP0101** – Taking and Returning Cages for Procedures
- **SOP0023** – Anaesthesia of Mice with Avertin for X-rays
- **SOP0024** – Anaesthesia of Mice with Ketamine-Xylazine + Antisedan for X-Rays
- **SOP0025** – DEXA Bodymap Analysis
- **SOP0031** – Recovery of Mice from Anaesthesia
- **SOP0032** – Preparation of Ketamine-Xylazine + Antisedan
- **SOP0035** – Preparation of 2,2,2-tribromoethanol (Avertin)
- **SOP0045** – Weigh Mice
- **SOP0055** – Analysis of High-Resolution X-Ray Images
- **SOP0057** – QC, Maintenance and Radiation Monitoring of the PIXImus
- **SOP0087** – Image & video acquisition using SONY Cybershot DSC-HX7V photo camera
- **X-ray positioning**
- **X-ray positioning cue cards X-ray Lateral Full Body Position**

Staff: This procedure can be completed by two phenotypers.

NOTE:

This procedure can be performed in conjunction with the Core Temp or Auditory Brainstem Response (ABR) assays (pipeline dependent). For Core Temp see the Xrays & Core Temp Workflow document for timings of the different assays.

In an emergency to stop the Faxitron, press the red button on the software to stop the X-rays and to turn off the machine, switch the key to the off position. The PIXImus can be stopped by pressing any button on the computer keyboard or mouse during a scan and powered down completely by turning off the switch located at the back.

PROCEDURE:

Before performing any tests verify this is the correct set of procedures at this time point in the pipeline or project, by consulting the cage card(s).

1. Display the 'Caution X-ray procedure in progress' sign on the outside of the door to the room.
2. Turn on heat mat(s).
3. Remove the anaesthetic and reversal agents (pipeline dependent) from the fridge and allow them to warm to room temperature.
4. **PIXImus preparation:**
 - 4.1. Ensure that the PIXImus machine is switched on or switch it on (at least 2 hours prior to use) via the switch located at the back of the machine next to the power cable.
 - 4.2. Login to the computer connected to the PIXImus.
 - 4.3. Double click on the LUNAR PIXImus2 software on the desktop and allow the application to initialise.
 - 4.4. Change the Data Directory
 - 4.4.1. Press F7 (System Maintenance)
 - 4.4.2. Press F6 (Research Functions)
 - 4.4.3. Press F2 (Change Data Directory).
 - 4.4.4. Select the relevant data folder.
 - 4.5. Run the QC test for the PIXImus (refer to SOP0057 – QC, Maintenance and Radiation Monitoring of the PIXImus) and record the QC values in the X-ray/DEXA log book. If the QC test fails, consult the Troubleshooting section below.
5. **Faxitron preparation:**
 - 5.1. Ensure that the computer attached to the Faxitron is turned on.
 - 5.2. Turn the Faxitron key clockwise to switch the machine on.
 - 5.3. Log into the computer.
 - 5.4. Double click on the FaxitronSR software on the desktop and allow the application to initialise.
 - 5.5. If this is the first Faxitron session of the week, wipe clean the metal plate inside the machine and perform an autocalibration:
 - 5.5.1. Click on 'Calibration and Settings'
 - 5.5.2. Ensure the inside of the faxitron is empty (i.e. no phantom mouse present)
 - 5.5.3. Select 'Autocalibrate'

- 5.5.4. Once complete record this in the X-ray/DEXA log book.
- 5.6. Run the QC test for the Faxitron:
 - 5.6.1. Click on 'Start Procedure'.
 - 5.6.2. Scroll down, find and double click to select the 'test/test' subject.
 - 5.6.3. Press 'Next'.
 - 5.6.4. Place the phantom mouse on the centre of the plate.
 - 5.6.5. Once the machine is ready (indicated by the green pilot light), run one exposure for 10 seconds at 23kV, by pressing the green button on the software to start to activate x-rays.
 - 5.6.6. At the end of the exposure, the image of the phantom mouse will appear on the screen.
 - 5.6.6.1. If this does not occur successfully or if an error message occurs, consult the Troubleshooting section and/or inform the primary phenotyper for the test and Senior Leadership Team (SLT).
 - 5.6.7. Click on the center of the image to see the Window/Level settings.

These must be pre-set at:

 - 5.6.7.1. Center: 1311
 - 5.6.7.2. Width: 2645
 - 5.6.7.3. Mouse Sensitivity: 9
 - 5.6.7.4. If these values are different, enter the above manually and press on the save icon on the menu bar.
6. Collect scheduled mice from the animal room, transport them to the procedure room and register them to the correct rack (Refer to SOP0101 – Taking and Returning Cages for Procedures).
7. Place 'Phenotyping in progress' sign on the outside of the door.
8. If performing Core Temp (Refer to SOP0028 – Core Temperature), do so now (pipeline dependent).
9. Wearing the correct PPE, begin anaesthesia of mice (refer to SOP0024 - Anaesthesia of Mice with Ketamine-Xylazine + Antisedan for X-rays or SOP0023 - Anaesthesia of mice with 2,2,2,-tribromoethanol (Avertin)).
 - 9.1. When running alongside ABR (pipeline dependent), anaesthetise the mice on the '6.5-minute call' from the ABR operator. Anaesthetise one ABR- and one non-ABR mouse at each call.
10. **Faxitron method:**
 - 10.1. Click 'Start Procedure' in the FaxitronSR application which brings up a new window. Fill out the 'Patient Name' fields as follows (mandatory field in bold):
 - 10.1.1. **First Name:** full mouse Identification (ID) (e.g. MABC7.1d)
 - 10.1.2. Middle Name: earmark (e.g. 1L1R)
 - 10.1.3. **Last Name:** genotype
 - 10.1.4. **Patient ID:** mouse barcode (e.g. M03861695).
 - 10.1.5. Enter the date of birth and sex.

- 10.2. Press 'Next' which will bring up a new window where you select the level the scan is being taken. Press 'Next' again, this opens the application to the point where you are ready to take the first scan. When mouse is fully anaesthetised begin acquisition of images.

- 10.3. Five different scans on different shelf levels are taken with the Faxitron on each mouse (see X-ray positioning cue cards): whole body (dorso-ventral), whole body (lateral), head (lateral), head (dorso-ventral) and the left forearm (dorso-ventral).

- 10.4. **Whole body (Dorso-Ventral):** (Level 1)
 - 10.4.1. Put the taped sheets on the bottom level, making sure they are as far back as possible and sitting square to the back of the Faxitron.
 - 10.4.2. Place the mouse onto the image zone of the sheets.
 - 10.4.3. Arrange the mouse ventrally so that its head is pointing towards the upper left corner of the zone and its rear towards the lower right corner with the red crosshair on its back.
 - 10.4.4. Make sure the back and neck are straight and that all of the paws are away from the body with pads down.
 - 10.4.5. Curl the mouse's tail around the body to the left, so that it is entirely within the zone without obscuring any other body part. If necessary, use some tape to keep the tail within the zone.
 - 10.4.6. Leave the sheets on the bottom level of the machine and close the door.
 - 10.4.7. Set the radiation level to 23 kV.
 - 10.4.8. Set the Time/AEC to 10 sec.
 - 10.4.9. Click on the green button to start.
 - 10.4.10. When the image is generated, click on the image to view the Window/Level settings to ensure they are on their pre-sets (as defined in step 5.6.7).

- 10.5. **Whole body (Lateral):** (Level 1)
 - 10.5.1. Put the taped sheets on the bottom level, making sure they are as far back as possible and sitting square to the back of the Faxitron.
 - 10.5.2. Place the mouse onto the image zone of the sheets.
 - 10.5.3. Arrange the mouse naturally on its side so that its head is pointing towards the upper left corner of the zone and its rear towards the lower right corner with the red crosshair on its rump.
 - 10.5.4. Ensure that shoulders are not separated and are superimposed.
 - 10.5.5. Gently pull out the right hind paw horizontally and tape it keeping the paw not flat against the plate but perpendicular to the plate.
 - 10.5.6. Gently pull out the left hind paw and tape it using a bigger loop of tape to keep the hind paw aspect as natural as possible using the above technique.
 - 10.5.7. Curl the mouse's tail behind its body along its spine, so that it is entirely within the zone without obscuring any other body part. If necessary, use some tape to keep the tail within the zone.
 - 10.5.8. Leave the sheets on the bottom level of the machine and close the door.
 - 10.5.9. Set the radiation level to 23 kV.

- 10.5.10. Set the Time/AEC to 10 sec.
 - 10.5.11. Click on the green button to start.
 - 10.5.12. When the image is generated, click on the image to view the Window/Level settings to ensure they are on their pre-sets (as defined in step 5.6.7).
- 10.6. **Head (Lateral): (Level 5)**
- 10.6.1. Put the taped sheets on the bottom level, making sure they are as far back as possible, centred and sitting square to the back of the Faxitron.
 - 10.6.2. Place the mouse on its side in the image zone with its head in the centre.
 - 10.6.3. Arrange the mouse so that the vertical red crosshair is between its eye and ear, and the horizontal crosshair is along its mouth or snout.
 - 10.6.4. Place the sheets on the second level from the top and close the door.
 - 10.6.5. Click on 'Start Procedure' bringing up the patient information window.
 - 10.6.6. Choose the current mouse and push 'Next'.
 - 10.6.7. Change the shelf level from 1 to 5 and push 'Next'.
 - 10.6.8. Set the radiation level to 23 kV.
 - 10.6.9. Keep the Time/AEC at 10 sec.
 - 10.6.10. Click on the green button to start.
 - 10.6.11. When the image is generated, click on the image to view the Window/Level settings to ensure they are on their pre-sets (as defined in step 5.6.7).
- 10.7. **Head (Dorso-ventral): (Level 5)**
- 10.7.1. Put the taped sheets on the bottom level, making sure they are as far back as possible, centred and sitting square to the back of the Faxitron.
 - 10.7.2. Place the mouse ventrally in the image zone with its head in the centre.
 - 10.7.3. Arrange the mouse so that the red crosshair is on the top of its head just above the ears.
 - 10.7.4. Place the sheets on the second level from the top and close the door.
 - 10.7.5. Keep the radiation level at 23 kV.
 - 10.7.6. Keep the Time/AEC at 10 sec.
 - 10.7.7. Click on the green button to start.
 - 10.7.8. When the image is generated, click on the image to view the Window/Level settings to ensure they are on their pre-sets (as defined in step 5.6.7).
- 10.8. **Left forepaw (Dorso-ventral): (Level 6)**
- 10.8.1. Put the taped sheets on the bottom level, making sure they are as far back as possible and sitting square to the back of the Faxitron.
 - 10.8.2. Place the mouse ventrally in the image zone with its left forelimb in the lower left quadrant of the sheets with the forepaw being in the upper left quadrant.
 - 10.8.3. Ensure that the elbow is extended away from the torso and the paw is flat with pads down.
 - 10.8.4. Arrange the mouse so that the red crosshair is just below the ear of the mouse.
 - 10.8.5. Place the sheets on the top level and close the door.
 - 10.8.6. Click on 'Start Procedure' bringing up the patient information window.
 - 10.8.7. Choose the current mouse and push 'Next'.
 - 10.8.8. Change the shelf level from 5 to 6 and push 'Next'.
 - 10.8.9. Set the radiation level to 22 kV.
 - 10.8.10. Keep the Time/AEC at 10 sec.

- 10.8.11. Click on the green button to start.
- 10.8.12. When the image is generated, click on the image to view the Window/Level settings to ensure they are on their pre-sets (as defined in step 5.6.7).

11. Allow mouse to remain in the Faxitron machine until ready to be processed by DEXA.

12. Remove the mouse from the Faxitron and prepare it for the DEXA (see step 13). Begin next mouse, performing steps 9-10 whilst first mouse completes DEXA measurement.

13. If at any point during acquisition of images; a bone breakage or another welfare issue is observed in the X-ray images of a mouse then seek advice from a Named Animal Care and Welfare Officer (NACWO) and inform the primary phenotyper for the test and SLT.

14. **DEXA method:**
 - 14.1. Measure the length of the mouse (nose to tail base) on a flat surface using a ruler.

 - 14.2. Remove the sticker from the DEXA holding tray to expose the sticky surface and position the mouse on the tray:
 - 14.2.1. Place the mouse ventrally onto the white sticky area.
 - 14.2.2. Place the head to the left with its snout in the holding slot.
 - 14.2.3. If the mouse doesn't fit into the white sticky area, move it forwards so that more of the head is in the holding slot.
 - 14.2.4. Place the paws away from the body and arrange the pads down.
 - 14.2.5. Curl the tail to the left around the body so that it is in the white sticky area without covering any other body part.

 - 14.3. Place the tray onto the machine ensuring that it slots securely into place.

 - 14.4. Close the door of the radiation shield.

 - 14.5. On the DEXA computer press F3 (Measure) to scan a new mouse. Fill out the user fields as follows :
 - 14.5.1. Subject ID: mouse barcode (e.g. M03861695)
 - 14.5.2. Description: mouse ID (e.g., MABC7.1d)
 - 14.5.3. Comments: genotype (use _ instead of /)
 - 14.5.4. Date of birth: dd/mm/yyyy (in this format exactly)
 - 14.5.5. Enter the sex, weight and length.
 - 14.5.6. Enter your name as Operator. Press OK.

 - 14.6. Press F3 (Start Measurement). The scan takes approximately 5 minutes.

15. When the DEXA scan on the mouse is complete, take the holding tray out of the DEXA machine and remove the mouse gently from the sticky surface.

16. If applicable, pass mouse for dysmorphology imaging (see SOP0087 - Image & video acquisition using SONY Cybershot DSC-HX7V photo camera).
17. Administer reversal agent if applicable (refer to SOP0024 - Anaesthesia of Mice with Ketamine-Xylazine + Antisedan for X-rays or SOP0023 - Anaesthesia of mice with 2,2,2,-tribromoethanol (Avertin)).
18. Analyse the DEXA body map (refer to SOP0025 - DEXA Bodymap Analysis).
19. Repeat steps 14-18 for all mice to be tested.
20. Monitoring of anaesthetised mice is necessary during this procedure. Initiate recovery (refer to SOP0031 - Recovery of Mice from Anaesthesia).
21. When all the mice have been tested, turn off the Faxitron by turning the key anticlockwise, but leave the PIXImus on.
22. **Data Handling DEXA method:**
 - 22.1. Once the mice have started the procedure, start the DCF by uploading the DEXA data onto the database and print the updated cage cards.
 - 22.2. Upload the DEXA data on the database.
 - 22.3. On the PIXImus computer, close the application by pressing Exit, RESTART the computer and switch off the screen.
23. **Data Handling Faxitron method:**
 - 23.1. Once the mice have started the procedure, start the DCF and print the updated cage cards.
 - 23.2. On the Faxitron computer close the application, and then press Exit before logging off and turning the screen off.
24. Clean all equipment, surfaces and the floor. **Transfer all waste to a yellow offensive waste bag or clearly labelled waste container. Place used syringes and needles into yellow sharps bins. Bind used anaesthetic and reversal solutions using chemical binding agent and dispose in a yellow offensive waste bag.**
25. **All cages must display the updated cage card. Place a 'POST PROCEDURE CHECK REQUIRED' label on all cages and register them to the correct rack whilst returning them to their destination/home rack in the animal room. (Refer to SOP0101 – Taking and Returning Cages for Procedures).**

When any major or unusual fault occurs with the equipment, please contact the SSM or Radiation Protection Supervisor (RPS) (as appropriate) responsible for the pipeline immediately and inform them of the nature and severity of the problem.

PIXImus

Problem: QC cycle failed.

Solution: Repeat it (2 times if necessary) by pressing F3 (Measure QC Phantom) as the machine will not allow you to continue without a successful QC test. Should this still fail to work, turn the PIXImus off and then turn the computer off. Next restart the PIXImus first, giving it one minute to boot up before restarting the computer. Repeat the QC cycle. Should the QC test still not pass, a field calibration may be necessary in order to fix this problem.

Problem: Error/fault with shutter.

Solution: Re-run the cycle (If this is during a mouse cycle, this will count as one extra exposure). If the problem repeats itself, restart the computer and re run the cycle.

Problem: Measurement aborted error

Solution: This occurs when any key or mouse button is pressed during the cycle and is the safety stop mechanism built into the system. Re-run the cycle ensuring the mouse or keyboard isn't used until the cycle is complete. If the measurement continues to abort, re-start the computer.

Problem: Software unresponsive

Solution: If the software becomes unresponsive and the measurement cannot be restarted, perform a full shutdown of the computer and manually turn it back on again.

Faxitron

Problem: *Camera capture error (no image on screen)*

Solution: Retake the image. Record the time of the error on the record sheet. This still counts as an extra exposure.

Problem: *No image on screen but no error message (Gray or white screen).*

Solution: Check the image contrast parameters on the left side panel. If they have fallen to 0, reset these parameters to **Center: 1311; Width: 2645; Mouse Sensitivity: 9**. If these parameters have not changed, re run the exposure using the phantom mouse and watch the "X-ray on" pilot lights. If these do not come on, this means that the X-ray tube is faulty and will require service by Qados. Faxitron cannot proceed until this has been rectified.

SANGER INSTITUTE STANDARD OPERATING PROCEDURE

SUBJECT: Dual Energy X-Ray Absorptiometry (DEXA) Body Map Analysis – V1

SOP Number: SOP0025	To be reviewed:	
Author(s):	Signed:	Date:
Editor:	Signed:	Date:
Risk Approver:	Signed:	Date:
Date of Implementation:		

INTRODUCTION:

The purpose of this procedure is to analyse body maps generated by DEXA to get accurate values for body composition of wild-type and genetically altered mice.

ABBREVIATIONS:

DCF = Data Capture Form
DEXA = Dual Energy X-Ray Absorptiometry
ID = Identification
LAA = Laboratory Animal Allergen
PAF = Project Authorisation Form
PIL = Procedure Individual Licence
PPE = Personal Protective Equipment
PPL = Procedure Project Licence
QC = Quality Control
RA = Risk Assessment
ROI = Region of Interest
RSF = Research Support Facility
SMP = Sick Mouse Procedure
SOP = Standard Operation Procedure

QUALITY CONTROL (QC) DURING PROCEDURE:

Refer to the table below for approved QC fail comments steps to be used during procedures.

If a value has been collected leave on the Data Capture Form (DCF) and then apply the fail reason from below;

In-Life Procedures:

Problem / Issue	QC fail reason
At any point during the procedure the mouse is deemed sick and processed	Fail whole DCF as 'Sick mouse' – for all tests that day

through Sick Mouse Procedure (SMP)	
Mouse incorrectly scheduled at wrong week	Fail whole DCF as 'Scheduling Issue'
Insufficient anaesthesia level affects the whole test DCF	Fail whole DCF as 'Anaesthesia Issue'
Insufficient anaesthesia level affects specific parameter(s)	Fail parameter(s) as 'Anaesthesia issue'
A welfare issue makes it impossible to collect specific parameters	Fail parameter(s) as 'Welfare issue'
Parameters affected by delays or noise due to fire alarms	Fail parameter(s) as 'Fire alarm'
An equipment failure affecting specific parameters	Fail parameter(s) as 'Equipment failure'
A software issue affecting specific parameters	Fail parameter(s) as 'Software failure'
A procedural error which affects data collection	Fail parameter(s) as 'Manual error'
Parameter cannot be assessed	Fail parameter(s) as 'Readout not possible'
Wrong value has been entered which cannot be re-measured or accounted for	Fail parameter(s) as 'Erroneous data'
Glucose meter unable to record high blood values	Fail parameter(s) as 'Meter reading HI'
Fighting occurs prior to or during data collection	Fail parameter(s) as 'Fighting during procedure'
Parameter on the current DCF is not required for that specific test/pipeline	Fail parameter(s) as 'Not required'

HEALTH & SAFETY:

This procedure is covered by the following Risk Assessment (RA):

Name: WTSI-1475

Assessment Title: Performing Dual Energy X-ray Absorptiometry and X-ray Imaging Including QC and Maintenance of the PIXImus, Use and Recovery from Ketamine e/Xylazine or Avertin Anaesthetic & Antisedan Reversal.

Assessor:

Approver:

- Appropriate Personal Protective Equipment (PPE) is to be worn at all times when handling animals. This includes:
 - Overshoes
 - Gown
 - Disposable gloves
- In addition to the above, when sources for Laboratory Animal Allergens (LAA) (animals or soiled cages) are not contained within Local Exhaust Ventilation Systems (change stations, fume hoods or downflow tables), a respiratory mask, for which you have passed a face fit test, must be worn.
- Lone worker alarms should be used when working alone.
- This procedure can only be performed during Research Support Facility (RSF) core hours (7:30am-7:30pm).
- All electrical equipment is to be inspected for damage before use.

All staff performing this procedure are responsible for ensuring that this Standard Operating Procedure (SOP) and accompanying Risk Assessment have been read, understood and where applicable is followed in accordance with the relevant Procedure Project Licence (PPL). All staff should be trained and competent to perform the procedure, where applicable they should also be licensed to perform the procedure with a valid Procedure Individual Licence (PIL).

For secondary phenotyping, seek confirmation with project manager for deviations from this SOP. Any deviation will be detailed in the Project Authorisation Form (PAF).

RESOURCES:

Equipment:

1. Lunar PIXImus2 application

Staff: This can be completed by one phenotyper.

PROCEDURE:

Before performing any tests verify this is the correct set of procedures at this time point in the pipeline or project, by consulting the cage card(s).

1. Place 'Phenotyping in progress' sign on the outside of the door and put on the correct PPE.
2. Switch on the computer and login.
3. Open the *Lunar PIXImus* application on the desktop. Allow time for the *LunarPIXImus 2.1* software to initialise.
4. Select data directory:
 - 4.1. Press F7 (System Maintenance).
 - 4.2. Press F6 (Research Functions).
 - 4.3. Press F2 (Change Data Directory).
 - 4.4. Under *Drives*, select the appropriate one. Under *Folders*, select the one of the test date to be analysed and click *OK*. The *PIXImus* will notify you that the data directory has been changed to the requested path. Click *OK*.
 - 4.5. Press F8 (Esc) twice.
5. Analyse subject:
 - 5.1. Press F4 (*Analyse Subject*).
 - 5.2. Select the file to be analysed from the appropriate folder and press *OK*. Wait for the file to load. An example of the file is shown in Figure 1.

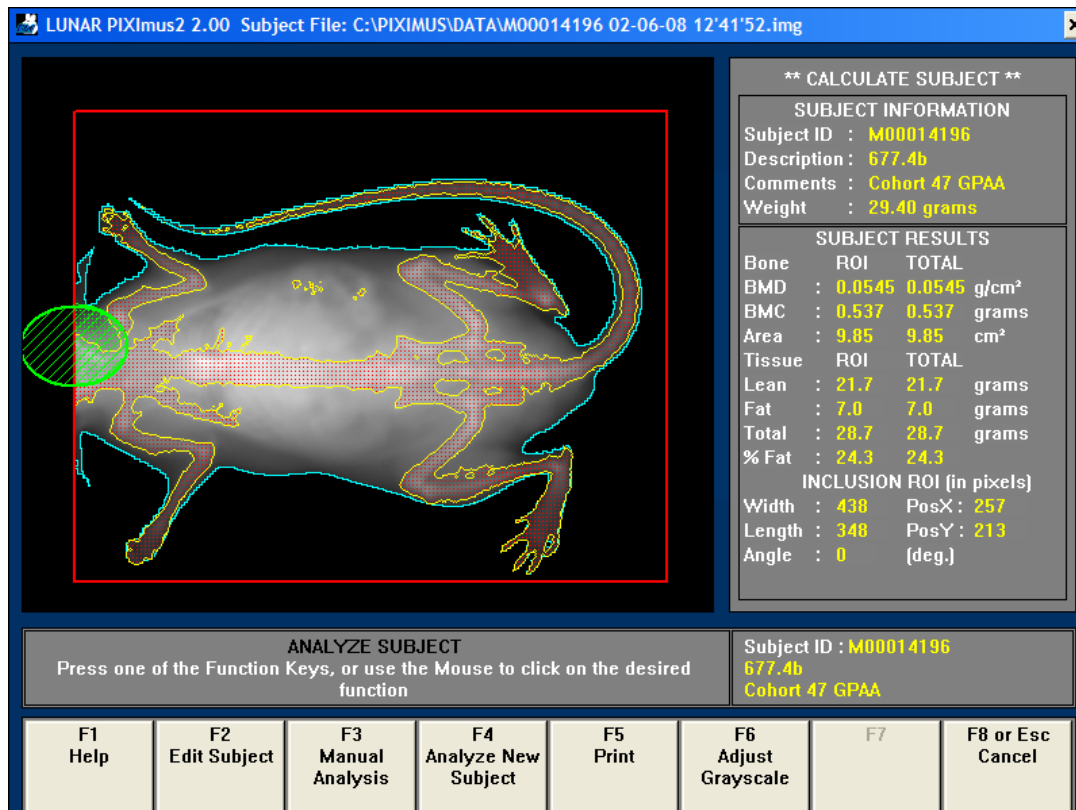


Figure 1. Screenshot of the Lunar PIXImus2 file prior to analysis.

- 5.3. Press F3 (Manual Analysis).
- 5.4. Press F3 (Adjust ROI).
- 5.5. Using the arrow keys on the keyboard, move the green shaded area to cover the head of the mouse on the image. Hold down the *Ctrl* button on the keyboard to control the size of the green shaded area whilst using the arrow keys. Hold down the *shift* button on the keyboard to increase the size of the steps. An example is shown in Figure 2.

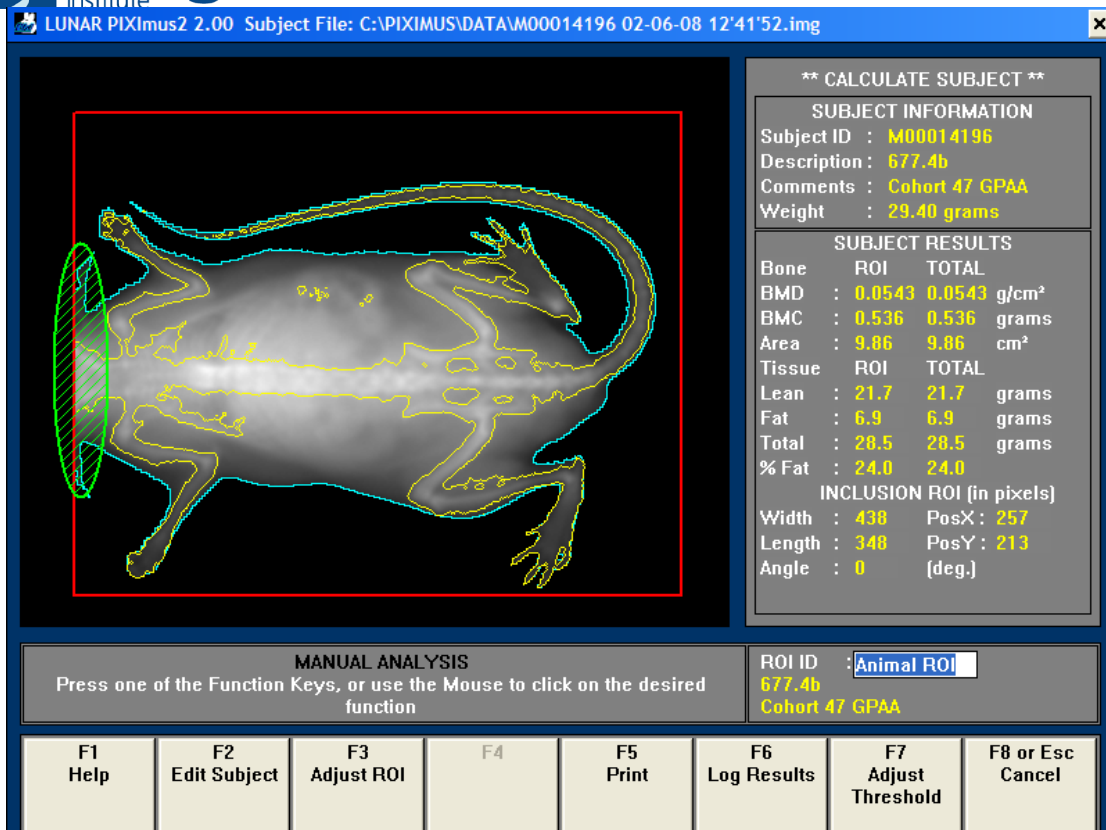


Figure 2. Screenshot of an analysed image.

- 5.6. Press *Enter* when you have successfully selected the head area to eliminate it from the analysis. Press *Enter* to confirm the ROI ID.
 - 5.7. Press *Esc*.
 - 5.8. Press F6 (*Log Results*). This confirms that the results have been logged **(This step is essential as results will not get logged in the text file if not carried out)**.
 - 5.9. Press *Esc*.
6. Repeat steps 5.1-5.9 for subsequent mice to be analysed. When all mice in that folder have been analysed, the results will be logged in a text file named **results.txt**.
 7. Upload the DEXA data on the database:
 - 7.1. When all the data have been uploaded correctly, complete the phenotyping procedures, QC failing data if necessary.
 8. On completion, close the LUNAR PIXImus2 application and shut down the computer.
 9. Clean all equipment, surfaces and the floor. **Transfer all waste to a yellow offensive waste bag or clearly labelled waste container.**

SANGER INSTITUTE STANDARD OPERATING PROCEDURE

SUBJECT: Analysis of High-Resolution X-Ray Images – V1

SOP Number: SOP0055	To be reviewed:	
Author(s):	Signed:	Date:
Editor:	Signed:	Date:
Date of Implementation:		

INTRODUCTION:

The purpose of this procedure is to make observations and annotations to X-Ray Images of wild-type and genetically altered mice.

ABBREVIATIONS:

DCF = Data Capture Form
IE = Internet Explorer
NACWO = Named Animal Care Welfare Officer
NVS = Named Veterinary Surgeon
PPL = Procedure Project License
QC = Quality Control
SMP = Sick Mouse Procedure
SOP = Standard Operating Procedure
SLT = Senior Leadership Team

Quality Control (QC) DURING PROCEDURE:

Refer to the table below for approved QC fail comments steps to be used during procedures.

If a value has been collected leave on the Data Capture Form (DCF) and then apply the fail reason from below;

In-Life Procedures:

Problem / Issue	QC fail reason
At any point during the procedure the mouse is deemed sick and processed through Sick Mouse Procedure (SMP)	Fail whole DCF as 'Sick mouse' – for all tests that day
Mouse incorrectly scheduled at wrong week	Fail whole DCF as 'Scheduling Issue'
Insufficient anaesthesia level affects the whole test DCF	Fail whole DCF as 'Anaesthesia Issue'
Insufficient anaesthesia level affects specific parameter(s)	Fail parameter(s) as 'Anaesthesia issue'
A welfare issue makes it impossible to	Fail parameter(s) as 'Welfare issue'

collect specific parameters	
Parameters affected by delays or noise due to fire alarms	Fail parameter(s) as 'Fire alarm'
An equipment failure affecting specific parameters	Fail parameter(s) as 'Equipment failure'
A software issue affecting specific parameters	Fail parameter(s) as 'Software failure'
A procedural error which affects data collection	Fail parameter(s) as 'Manual error'
Parameter cannot be assessed	Fail parameter(s) as 'Readout not possible'
Wrong value has been entered which cannot be re-measured or accounted for	Fail parameter(s) as 'Erroneous data'
Glucose meter unable to record high blood values	Fail parameter(s) as 'Meter reading HI'
Fighting occurs prior to or during data collection	Fail parameter(s) as 'Fighting during procedure'

HEALTH & SAFETY:

This procedure has been classified as not containing any hazards. It does involve the use of computers and as such a DSEasy display screen equipment assessment might be required.

RESPONSIBILITIES:

All staff performing this procedure is responsible for ensuring that this Standard Operating Procedure (SOP) has been read and understood.

RESOURCES:

Equipment:

1. Sante DICOM Viewer PRO or Sante DICOM Editor

Associated SOPs/Documentation:

- Analysis Update, X-RAY ANALYSIS WORKSHEET
- Bone and X-ray basics
- X-Ray Positioning
- X-Ray Positioning cue cards
- Commenting on Bone abnormalities
- Commenting on Transitional Vertebrae review
- Sante DICOM Viewer PRO - Instructions for Internal Use

Staff: This procedure requires 1 phenotyper.

NOTE:

Selection criteria for data sets to be analysed must be based upon priority level as indicated on the X-ray Analysis Worksheet.

When commenting on abnormalities in the X-ray DCF, a consistent format is to be adhered to. To maintain consistency, the abnormality has to be commented in the following format*:

Abnormality > Location > Bone

**This does not apply to abnormalities of spine curvature (kyphosis, lordosis, scoliosis) and if otherwise stated in "Commenting on Bone abnormalities".*

Each comment should be separated by a semicolon (;) and the entire text ended with a full stop (.). Comments of different abnormalities on the same region should be separated by a comma (,). This allows text information to be segregated if necessary.

PROCEDURE:

1. Open the X-ray analysis worksheet and select a line/cohort to analyse based on the priority system described above.
2. Open Sante DICOM viewer pro/Editor and Internet Explorer (IE)/Mozilla Firefox.
3. Select the appropriate DCFs
 - 3.1. Choose the annotator name from the dropdown menu.
 - 3.2. Click on the "Download All" button in order to download a .Zip file containing all the images and select to "Open" the file.
 - 3.3. In the new window, select all the images and right click on the top image and select open. The images should all open in the Sante DICOM software.
4. Record any abnormalities observed by selecting the correct radio button in the DCF and make a comment of it in the comments field of the current DCF.
 - 4.1. If at any point during analysis of images, a bone breakage or welfare concern is seen, then contact the person responsible for the test and the SSM responsible.
 - 4.1.1. If the mouse is still alive then seek Named Animal Care Welfare Officer (NACWO) or Named Veterinary Surgeon (NVS) advice.
5. Click on the full body dorso-ventral image.
 - 5.1. Count the number of digits.
 - 5.2. Look at the shape of the pelvis, head and limbs area, shape of spine and ribcage.
 - 5.3. When annotating the spinal column
 - 5.3.1. Observe the shape of the vertebrae and spine.
 - 5.3.2. Look for any transitional vertebrae, fusions of vertebrae and/or rib abnormality.
 - 5.3.3. Observe the transverse processes on the cervical, lumbar and caudal vertebrae.
 - 5.4. Zoom into the upper body on the full body view.
 - 5.4.1. Observe the clavicles and scapulae, elbow joints and glenohumeral joints.
 - 5.4.2. Observe the humeri, radii and ulnae.
 - 5.5. Zoom into the thorax.
 - 5.5.1. Count the number of thoracic vertebrae. This is best done by counting the number of ribs.
 - 5.5.2. Count the ribs on both sides. (A normal mouse will have 13 thoracic vertebrae with 13 pairs of ribs).
 - 5.6. Press the "Apply Default"/"0.Default" button (if needed) to return to the original contrast and brightness settings.
 - 5.7. Move down to the lumbar and pelvic area and zoom in.
 - 5.7.1. Observe the pelvis, articulation of coxae, femurs, tibia and fibulae.
 - 5.7.2. Count the number of lumbar and pelvic vertebrae (a normal mouse will have 6). The first pelvic vertebra forms a bridge joining the left and right

iliac crests (a normal mouse will have 4 pelvic vertebrae – the first 3 are fused).

- 5.7.3. To count the caudal (tail) vertebrae move the image further down to get a full view of the base of the pelvis and entire tail (the number may vary between mice – normal mice may have between 27 and 30 caudal vertebrae).
6. Open the full body lateral image.
 - 6.1. Observe the shape of the spine, head, ribcage and limbs area.
 - 6.2. Observe the joints fore and hind limbs.
7. Open the image of the lateral view of the head.
 - 7.1. Observe the overall skull shape, incisors, molars and mandibles.
 - 7.2. Use this view to confirm cervical vertebrae count (a normal mouse should have 7).
 - 7.3. View the shape of the spinous processes of the cervical vertebrae.
 - 7.4. Observe the glenohumeral joint and the bones of the ear.
8. Open the image of the dorso-ventral view of the head.
 - 8.1. Observe the overall skull shape, incisors, maxillae and zygomatic bones.
 - 8.2. Use this view to confirm cervical vertebrae count (a normal mouse should have 7).
 - 8.3. Observe the shape of the transverse processes of the cervical vertebrae as well as the overall shape of the cervical spine.
 - 8.4. Observe the bones of the ear.
9. Open the dorso-ventral image of the left forepaw.
 - 9.1. Observe the shape of the humerus, radius and ulna.
 - 9.2. Observe the glenohumeral joint, elbow joint.
 - 9.3. View the radiocarpal joint, metacarpophalangeal joints and interphalangeal joints.
 - 9.4. Observe the carpals, metacarpals and phalanges.
10. QC fail any parameters which cannot be assessed due to extremely poor positioning, movement or anaesthesia issues (use appropriate comment based on the QC failure comment table).
11. Check the correct anaesthetic combination has been selected from the *Anaesthetic* drop-down list.
12. Ensure that the correct *Mouse Status* is selected (this defaults to *Anaesthetised*. Change to *Dead* if a mouse has X-rays post-mortem).
13. Check that the *Settings (metadata)* are correctly filled (note that these settings are only logged for the full body dorso-ventral image).
 - Time of Exposure: 10s
 - Level: 1
 - Voltage: 23kV
14. Complete the DCF.
15. Repeat steps 3.1-14 for all mice to be analysed within the cohort.
16. Repeat for all the cohorts with the same colony prefix if analysing a mutant line.

17. Annotation of X-ray images: mark the images as QC passed or failed as appropriate on the database.

18. Update the X-ray analysis worksheet appropriately.

19. Publishing of X-ray images:

19.1. The publish status can only be completed **after the genotype is confirmed**. Before the genotype is confirmed, all annotation can be applied but publish status must stay as “not done”.

19.2. Once the genotype has been locked, before publishing the image must be deemed fit for publication.

19.3. If the image is fit for publication, change the publish status to “publish”.

SANGER INSTITUTE STANDARD OPERATING PROCEDURE

SUBJECT: QC, Maintenance and Radiation Monitoring of the PIXImus – V1

SOP Number: SOP0057	To be reviewed:	
Author(s):	Signed:	Date:
Editor:	Signed:	Date:
Risk Approver:	Signed:	Date:
Date of Implementation:		

INTRODUCTION:

This procedure outlines how to perform daily quality control procedures of the PIXImus, the correct way to monitor monthly environmental radiation around the lead shield employed with the PIXImus, and how to carry out a 3-monthly field calibration recommended for the routine maintenance of the machine.

ABBREVIATIONS:

LAA = Laboratory Animal Allergens
PAF = Project Authorisation Form
PIL = Procedure Individual Licence
PPE = Personal Protective Equipment
PPL = Procedure Project Licence
QC = Quality Control
RA = Risk Assessment
RPO = Radiation Protection Officer
RPS = Radiation Protection Supervisor
RSF = Research Support Facility
SLT = Senior Leadership Team
SOP = Standard Operating Procedure

HEALTH & SAFETY:

This procedure is covered by the following Risk Assessment (RA):

Name: WTSI-1475

Assessment Title: Performing Dual Energy X-ray Absorptiometry and X-ray Imaging Including QC and Maintenance of the PIXImus, Use and Recovery from Ketamine/Xylazine or Avertin Anaesthetic & Antisedan Reversal.

Assessor:

Approver:

- Appropriate Personal Protective Equipment (PPE) is to be worn at all times when handling animals. This includes:

- Overshoes
- Gown
- Gloves
- In addition to the above, when sources for Laboratory Animal Allergens (LAA) (animals or soiled cages) are not contained within Local Exhaust Ventilation Systems (change stations, fume hoods or downflow tables), a respiratory mask, for which you have passed a face fit test, must be worn.
- This procedure can only be performed during Research Support Facility (RSF) core hours (7:30am-7:30pm).
- All electrical equipment is to be inspected for damage before use.
- If the shield fails then the experiment can be stopped at any time by pressing any button on the computer and the machine can be switched off using the switch button at the back of the machine at the top.
- Ionising radiation is used during this test. No waste is generated from ionizing radiation. Only the lowest levels required to generate image are used.
- This test poses increased level of risk to young persons and to new or expectant mothers, a separate RA should be undertaken.
- The DEXA unit is shielded minimizing exposure to the user and other users in the area.
- The x-rays are contained within a shielded containment.

RESPONSIBILITIES:

All staff performing this procedure are responsible for ensuring that this Standard Operating Procedure (SOP) and accompanying Risk Assessment have been read, understood and where applicable is followed in accordance with the relevant Procedure Project Licence (PPL). All staff should be trained and competent to perform the procedure, where applicable they should also be licensed to perform the procedure with a valid Procedure Individual Licence (PIL).

For secondary phenotyping, seek confirmation with project manager for deviations from this SOP. Any deviation will be detailed in the Project Authorisation Form (PAF).

RESOURCES:

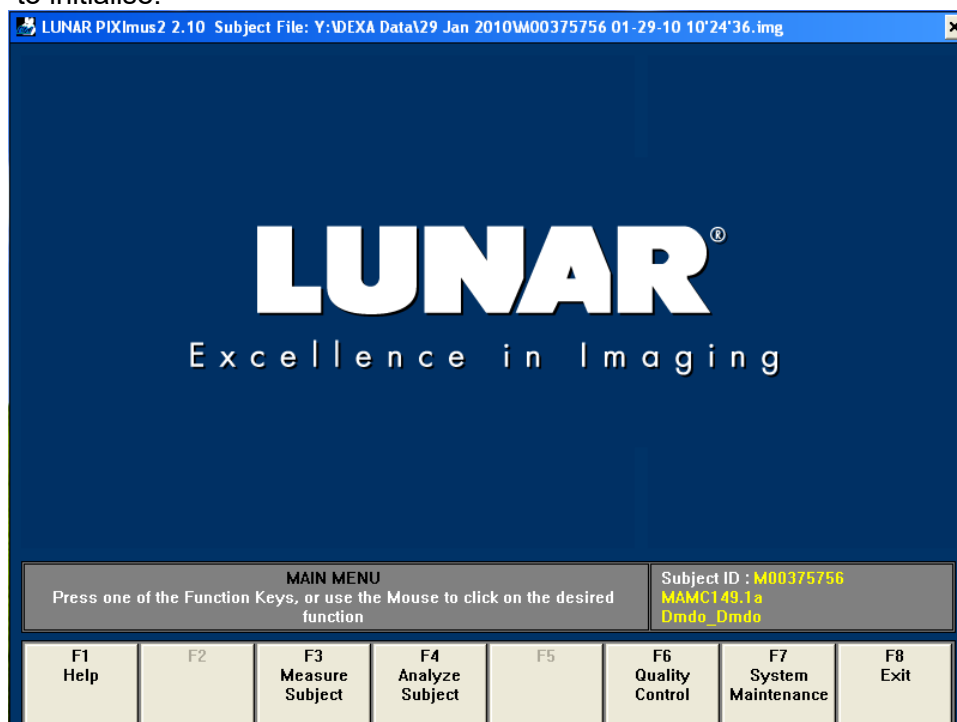
Equipment:

1. DEXA machine: PIXImus **Source of ionising radiation, only to be used by trained personnel with the shield in place**
2. Lunar PIXImus software
3. PIXImus phantom mouse
4. PIXImus specimen tray with securing paper (*Supplier name; Inside Outside Sales, LLC. Supplier product code; TRAY-600. Obtain quote from dsettergren@charter.net*)
5. Radiation shield
6. 'Caution X-ray Procedure in Progress' Sign
7. Form: DEXA shield check (for section 3 only)
8. Form: Lunar PIXImus Field Calibration Log (for section 4 only)
9. Thermo Mini 900 Ratemeter radiation counter (for section 3 only)

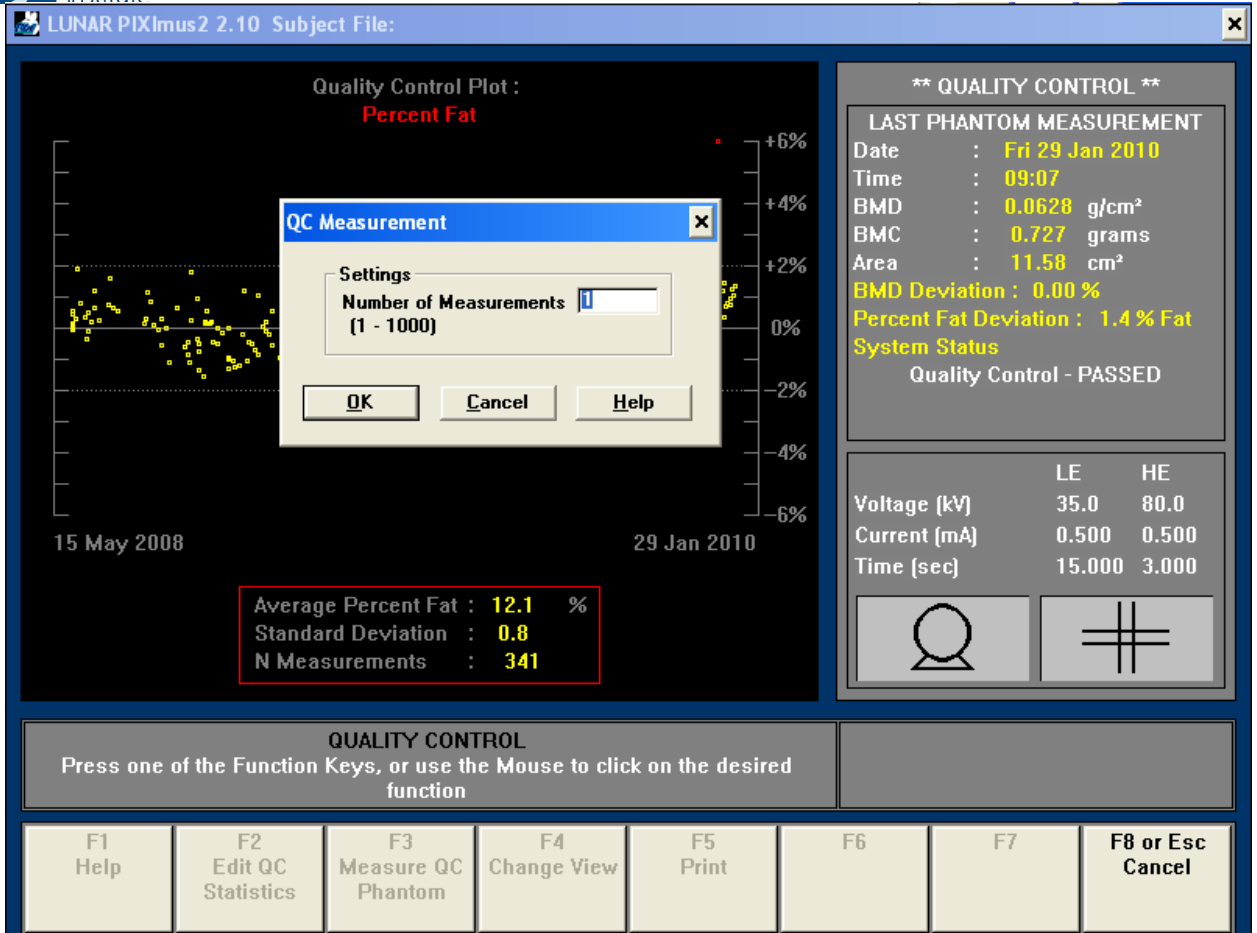
Staff: This can be completed by the Radiation Protection Supervisor (RPS), or in their absence, one phenotyper who has received appropriate training directly from the local (RPS).

Verify that this is the correct procedure at this point in time by checking when the last maintenance cycle was performed. The shield check should be performed monthly. The field calibration should be performed every 3 months.

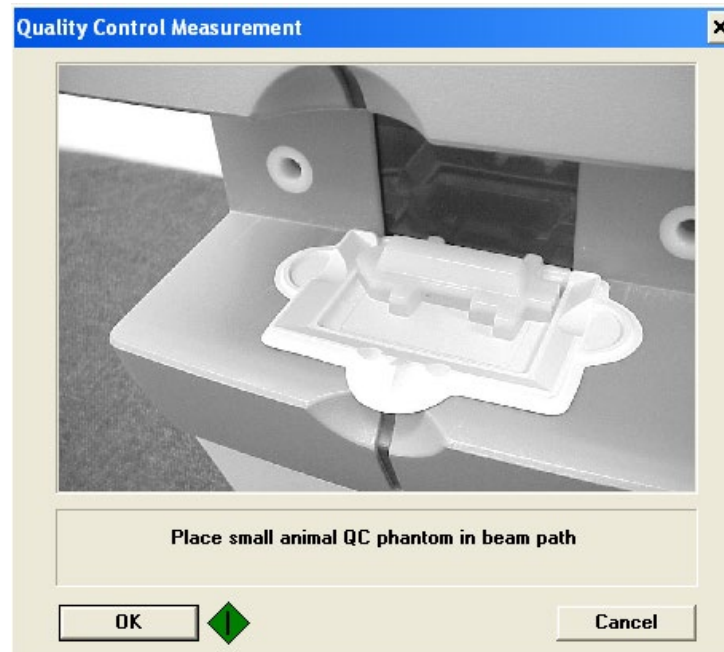
1. Place 'CAUTION X-ray procedure in progress' sign on the outside of the door and put on the correct PPE.
2. **Quality Control (QC) Cycle: Perform prior to each use of the PIXImus**
 - 2.1. Ensure that the PIXImus has been switched on for at least 30 minutes prior to use via the switch at the back of the machine.
 - 2.2. Check that the PIXImus is connected to the computer, and then turn on the computer.
 - 2.3. Open the LUNAR PIXImus2 application on the desktop. Allow the application to initialise.



- 2.4. Press F6 (*Quality Control*).
- 2.5. Press F3 (*Measure QC Phantom*).
- 2.6. Set number of measurements to '1'.

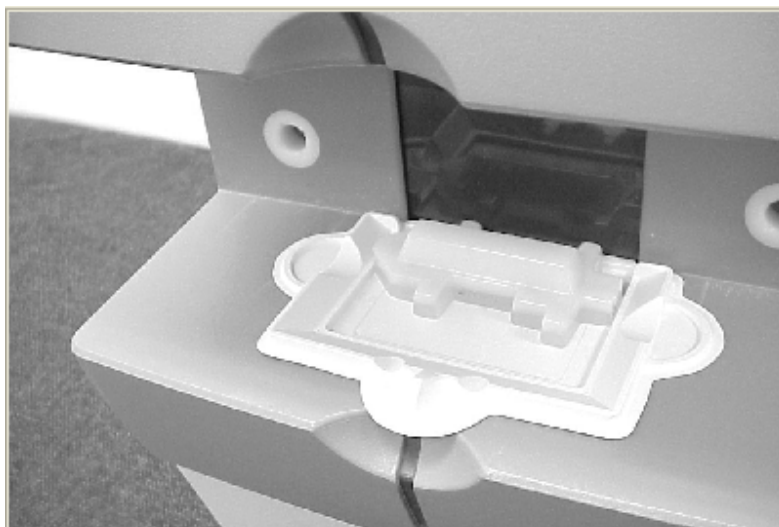


2.7. Press 'OK'. Place the Phantom mouse and specimen tray as illustrated on the software/ below:



2.8. Ensure the door of the screen is securely shut and then click 'OK'. This begins the QC cycle, which will take up to 5 minutes.

- 2.9. When the QC cycle is complete, only the green light will remain on the PIXImus, and the computer screen will display the results from the QC run and the status.
 - 2.9.1. If the status reads *Quality Control – PASSED*, proceed to use the machine.
 - 2.9.2. If the status reads *Quality Control – FAILED*, repeat.
 - 2.9.3. Should the QC fail over three times, contact the primary phenotyper responsible for the test and Senior Leadership Team (SLT) responsible for the tests before using the PIXImus.
- 2.10. Press *Esc* to return to the start-up screen.
3. **Shield check: Perform monthly** (This should take approximately 30 minutes)
 - 3.1. Ensure that the PIXImus is powered on, connected to the computer and the computer is logged on. Check that a QC cycle has been run in the last 24 hours, and if not, run a QC cycle (see section 2).
 - 3.2. On the radiation counter, turn the knob briefly to **bat** – the pointer should move to the green area of the scale. If the pointer does not move or only reaches the white area indicating replace battery, then replace the battery.
 - 3.3. If the battery on the counter is fine, move the knob to the **on** position. The counter will give a sharp beep and will begin to crackle indicating the probe is now counting radiation it is exposed to. Continue to move the knob to the **≠<** (mute) position if the noise is not desired.
 - 3.4. Press *Esc* to return to the start-up screen.
 - 3.5. Place the phantom mouse on the specimen tray (WITHOUT removing the label to expose the sticky surface), with the 'head' on the left and the 'tail' end on the right. Place the tray with the phantom mouse in the PIXImus so that it slots securely into the grooves within the area of exposure:



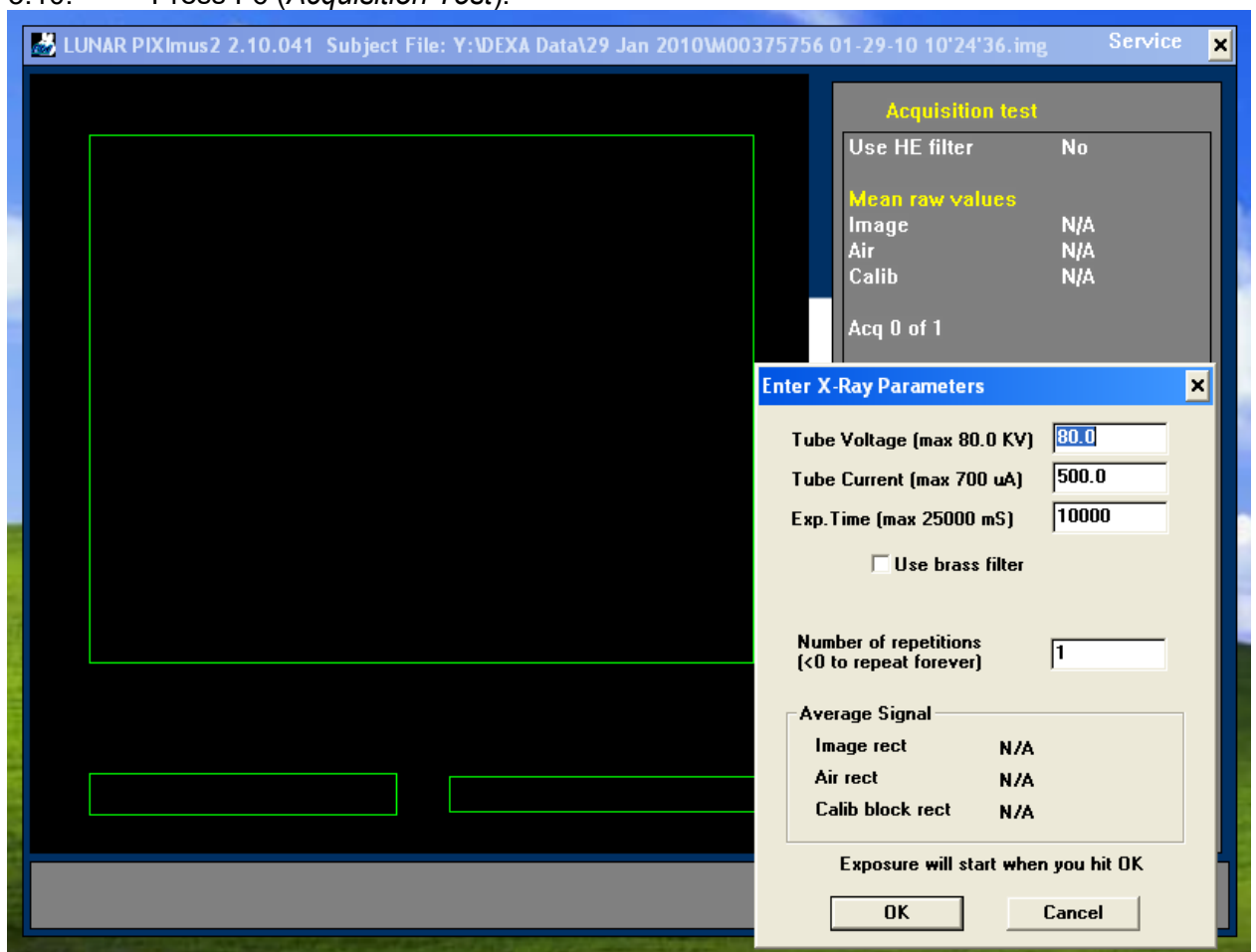
- 3.6. On the main menu of the Lunar PIXImus software, press F7 (*System Maintenance*).
- 3.7. Press F4 (*Operator Level*).



3.8. Select the **Service** radio button, type **Vail** (case-sensitive) as the password and then press **OK**.

3.9. Press F7 (*Service Functions*).

3.10. Press F6 (*Acquisition Test*).



3.11. Enter the following parameters for the test:

3.11.1. Tube voltage = 80.0

3.11.2. Tube current = 500.0

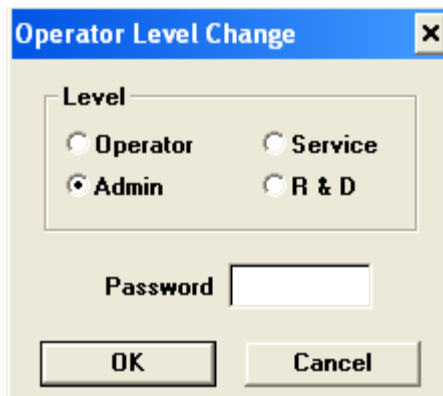
3.11.3. Exposure time = 10,000 (for 10 seconds exposure. DO NOT exceed an exposure time beyond 20,000, i.e. 20 seconds).

3.12. Remove the radiation probe from the holder on the radiation counter.

- 3.13. On the computer, press *OK* to begin the high energy X-ray exposure. When the light under the # turns yellow in the PIXImus, the exposure will begin. This exposure will be active for 10 seconds. Repeat this exposure sufficient times to allow completion of steps 3.14–3.20.
- 3.14. Place the probe on the outside of the **left wall** of the shield. Record the amount of radiation detected (in $\mu\text{Sv h}^{-1}$) on the monitoring sheet (normally only between $0.1\text{--}0.2 \mu\text{Sv h}^{-1}$ will be picked up in this position).
- 3.15. Allow the probe to settle before recording the reading.
- 3.16. Next, move the probe to the **right wall** of the shield and record the amount of radiation detected (normally only between $0.1\text{--}0.2 \mu\text{Sv h}^{-1}$ will be picked up in this position).
- 3.17. Allow the probe to settle before recording the reading.
- 3.18. Move the probe to **front lead-glass door** of the shield and record the amount of radiation detected (normally only between $0.1\text{--}0.2 \mu\text{Sv h}^{-1}$ will be picked up in this position).
- 3.19. Allow the probe to settle before recording the reading.
- 3.20. Next, place the probe on the **top right-hand side**, introducing the tip of the probe into the inner side of the shield, taking extreme care not to insert your hand into the shielded area. Record the amount of radiation detected (normally only between $0.1\text{--}0.2 \mu\text{Sv h}^{-1}$ will be picked up in this position).
- 3.21. Allow the probe to settle before recording the reading.
- 3.22. Place the probe on the **top left-hand side**, introducing the tip of the probe into the inner side of the shield, taking extreme care not to insert your hand into the shielded area. Record the amount of radiation detected (normally only between $0.1\text{--}0.2 \mu\text{Sv h}^{-1}$ will be picked up in this position).
- 3.23. Allow the probe to settle before recording the reading.
- 3.24. Next, place the probe **above** the PIXImus, introducing the tip of the probe to the area just above the shield. Record the amount of radiation detected (normally only between $0.1\text{--}0.2 \mu\text{Sv h}^{-1}$ will be picked up in this position).
- 3.25. Allow the probe to settle before recording the reading.
- 3.26. Finally, point the probe away from the machine into the surrounding area until the probe has settled to obtain the background levels of radiation.
- 3.27. If the values are significantly different from the background level, please contact RPS, SLT or RPO immediately. Do not use the machine further until clearance has been granted by the RPS or RPO.
- 3.28. When check is complete, press *Cancel* and then press F8 or *Esc* twice to return to the Main Menu on the PIXImus software.

3.29. Press F7 (*System Maintenance*).

3.30. Press F4 (*Operator Level*).



3.31. Select the **Admin** radio dial, type **Admin** (case-sensitive) as the password and the press **OK**. This brings you back from the Service mode to the safe operator level (Admin mode). This step is essential in order to avoid damage to the PIXImus, operators or subjects.

3.32. Remove the tray and the phantom mouse from within the PIXImus machine.

3.33. If further QCs or a field calibration is not scheduled, press *Esc* once, close the Lunar PIXImus 2 software and shut down the computer.

3.34. Update the 'Lead shield radiation monitoring due:' label displayed on the lead shield with the date the next check is due (+1 calendar month).

4. **Field Calibration: Perform every 3 months (This should take approximately 1.5 hours)**

4.1. Ensure that the PIXImus is powered on, connected to the computer and the computer is logged on.

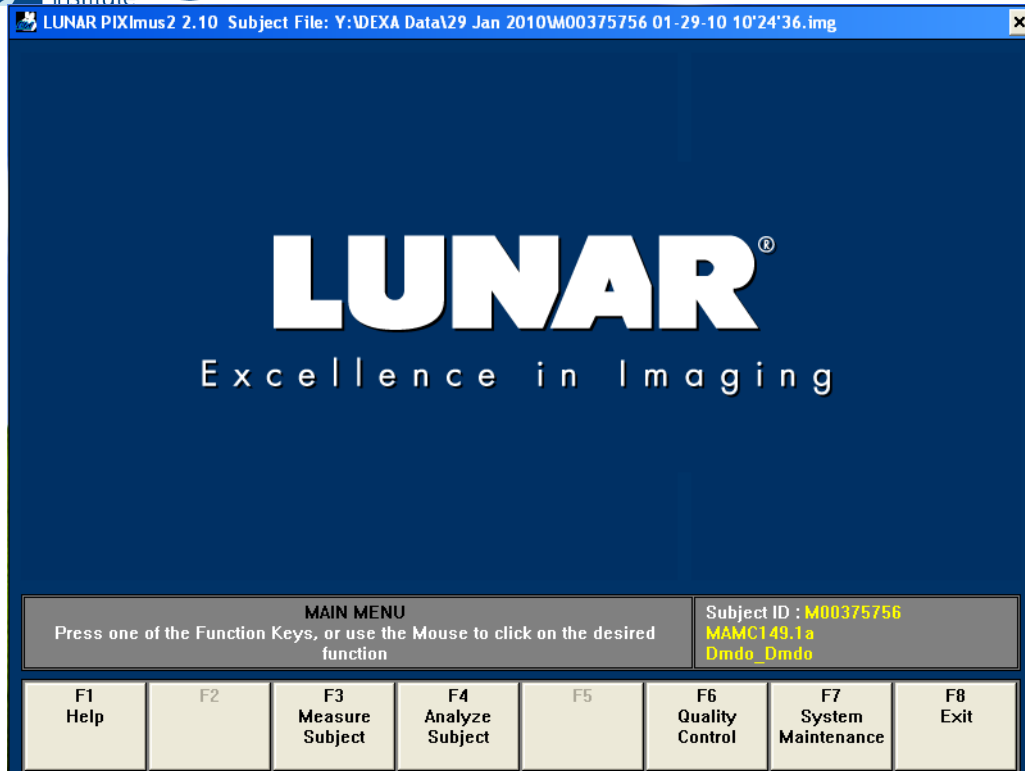
4.2. Check that a QC cycle has been run in the last 24 hours.

4.2.1. If a QC has been run, values can be found on the QC screen (from the main menu press F6 'quality control),

4.2.2. If QC hasn't been run in the last 24 hours, run a QC cycle (see section 2).

4.2.3. Write the current QC values in the Lunar PIXImus Field Calibration Log.

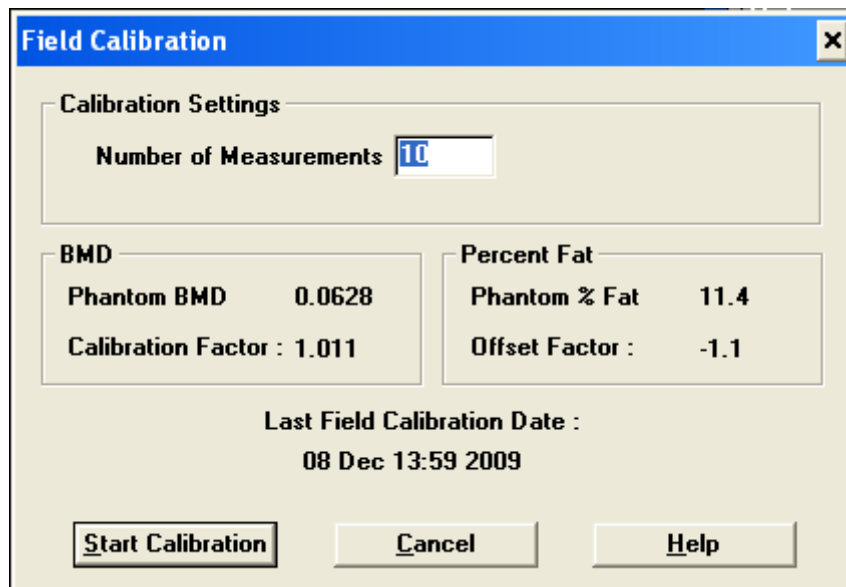
4.3. Open the Lunar PIXImus software:



4.4. Press F7 (*System Maintenance*).

4.5. Press F3 (calibration option).

4.6. Press F3 (*Field Calibration*). This opens the Field Calibration dialog box:

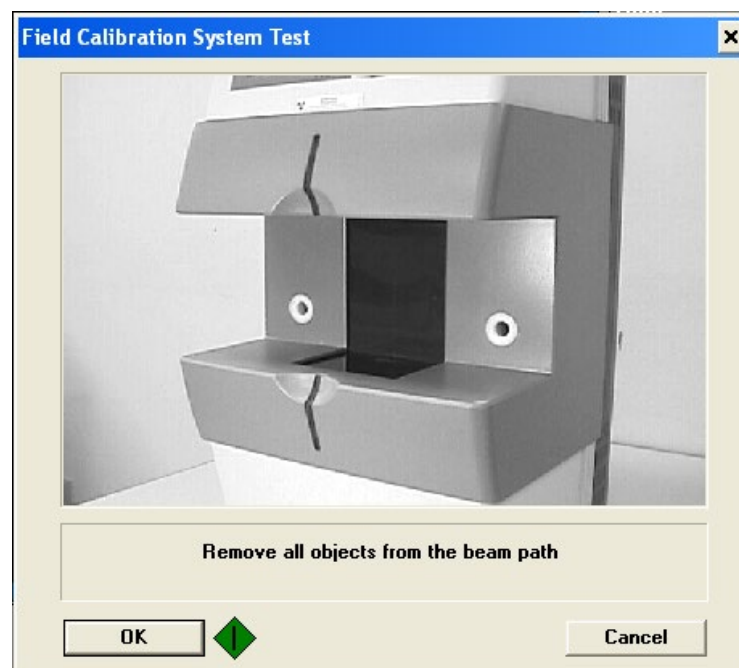


4.7. Select the number of measurements as '10'.

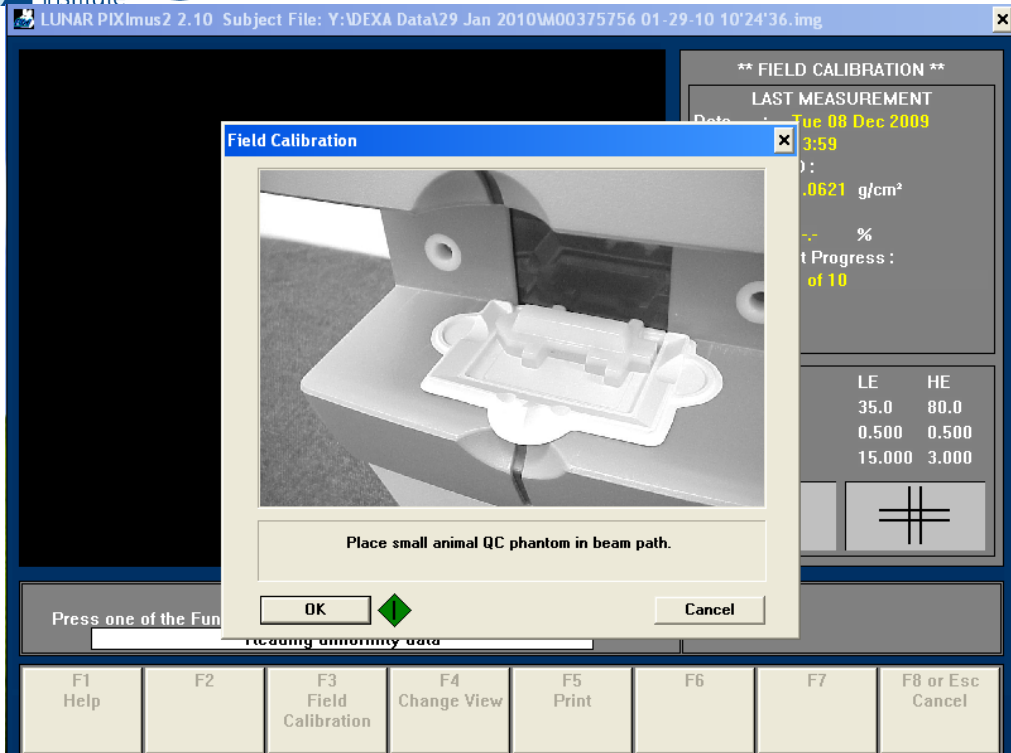
4.8. Check the values on the Phantom mouse.

4.8.1. If it matches the values on the dialog box, proceed.

- 4.8.2. If it does not, press 'Cancel' followed by esc., then press F2 (*Edit User Settings*). Enter the correct values, press OK, and then press OK. Return to main menu and restart from step 4.
- 4.9. If prompted, remove the phantom and the specimen tray from the PIXImus.
- 4.10. Ensure the door to the radiation screen is securely shut.
- 4.11. Press start calibration and then OK. This begins the alignment test, and the cycle is repeated 8 times (this should take approximately 10 minutes). Do not press the mouse button or press any key on the keyboard as this will abort the measurements and interrupt the alignment test.

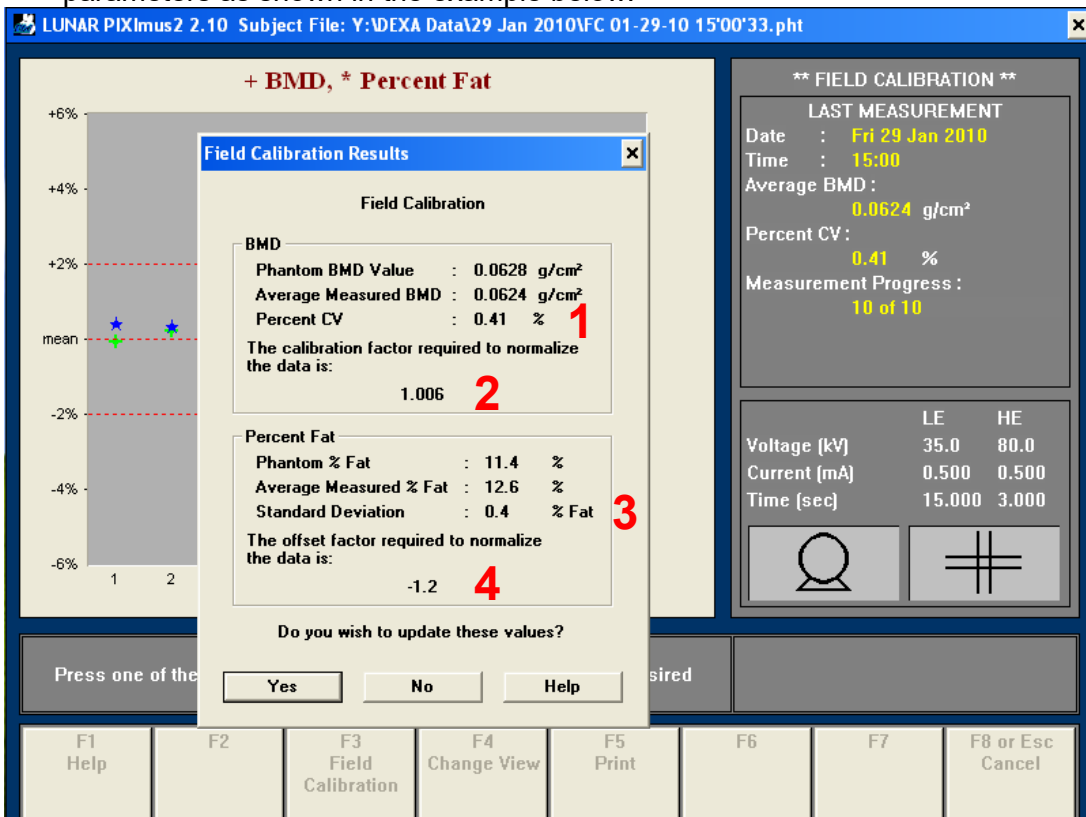


- 4.12. When alignment test is complete, place the phantom in the PIXImus with a specimen tray as shown on the software/ below:



4.13. Press OK. This begins the measurement, the cycle is repeated 10 times (approximately 50 minutes).

4.14. When complete, a dialog box will appear with the calibration parameters as shown in the example below:



- 4.15. Review the results, ensuring they fall within the ranges specified below:
- 4.15.1. BMD Percent CV result is less than 1% 1
 - 4.15.2. BMD calibration factor is between 0.8 and 1.2 2
 - 4.15.3. Percent fat standard deviation is between -1.0 and +1.0 3
 - 4.15.4. Percent fat offset value is between -5.000 and +5.000 4
- 4.16. Record these values in the *Lunar PIXImus Field Calibration Log*.
- 4.17. If the results fall within the accepted ranges, click on Yes. Press F5 (*Print*) to print the summary graph of the calibration and attach this in the X-ray log book. If results do not fall within the accepted ranges, or the calibration fails, do not update the results, make a record of the results on the log book and/ or print a screenshot of the results output screen. Immediately notify the primary phenotyper responsible for the test or SLT, who will make arrangements for GE Customer Services to carry out a full service of the equipment.
- 4.18. Press *Esc* twice to return to the main menu of the Lunar PIXImus2 2.10.
- 4.19. Run a QC procedure (See section 2).
- 4.20. When complete, note down the % BMD and % Fat values in the X-ray log book and *Lunar PIXImus Field Calibration Log*.
- 4.21. Press *Esc*, then exit the software and shut down the computer. Update the 'PIXImus field calibration due:' label displayed on the lead shield with the date the next check is due (+3 calendar months).

Clean all equipment, surfaces and the floor. **Transfer all waste to a yellow offensive waste bag or clearly labelled waste container.**