Development of a micro-CT mouse holder for a femur defect mouse model

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Abstract

Correct fixation of an object is essential for accurate micro computed tomography scanning. In this document, we provide a motivation for, description of, and use cases for a mouse holder appropriate for holding an externally fixated mouse in femur fracture/defect healing experiments. In addition to rigid fixation, the holder provides heating and anaesthetic gas to ensure correct anaesthetic conditions for the animal. We provide the description and design files for a Scanco viva40 scanner, but with small changes, the holder can be used with other scanners.

Introduction

During animal imaging (in particular microcomputer tomography (micro-CT) imaging) *in vivo*, correct placement and fixation of the animal is essential. Placement is often constrained via the type of scan, the type of scanner and the organ being scanned. While scanning holders are seldom reported in the literature, conventional solutions usually make use of tube-like structures in which the animal's body part is placed¹. However, this often leads to small movements within the scan as well as differing placement positions between scans. To overcome these limitations, we have constructed a mouse holder for a femur defect model that allows rigid, repeatable placement of the organ of interest (the right femur) in the correct position in a micro-CT scan.

The holder provides four core functions:

- Rigid fixation of the femur in the correct location during micro-CT scanning
- Adequate heating of the mouse during scanning
- Delivery of anaesthetic/analgesic gas during scanning
- Interface with additional devices for mechanical intervention experiments

This document provides the manufacturing and use specification of a mouse imaging and loading holder for a femur defect model in mice. The purpose is to hold a mouse, with the right femur fixated with a RISystem external fixator (RISystem, Landquart, Switzerland). This allows repeated measurements of the femur defect and prevents substantial movement between scans, facilitating image registration and longitudinal comparisons². The holder is designed for use with a Scanco viva40 micro-CT scanner (Scanco, Brüttisellen, Switzerland) but with small modifications can be used with other *in vivo* scanners. Additionally, the holder can be used with a loading machine without the need to transfer the mouse, as the holder can be removed with ease from the scanner.

Description

Figure 1: The micro-CT mouse holder

The mouse is placed on the front portion of the heated mouse holder with its nose facing towards the anaesthetic unit (nose cone can be chosen and adjusted according to animal size). The external fixator is placed in a clamp and held via tightening the side screw to a value of 8 Nmm. The fixator and the femur are slightly offset from the centre of the scanning area. Anaesthetic is provided via the anaesthetic unit, consisting of an inner tube for anaesthetic delivery and an outer tube for gas removal. Heating is provided via two 9 W (at 24 V) heating elements of Ø 10 mm flanking a temperature sensor. The unit bolts onto the standard Scanco interface block via the end plate. The full assembly dimensions are 180 mm x Ø 86 mm.

Figure 2: Femur placement in scanned region

Scanning Location

The femur is positioned off centre and slightly raised above the centre of the scan. The aim of the location is to improve mouse ergonomics, improve scan quality and prevent the muscles and fat from causing local tomography artefacts.

Part Description

Assembly:

Figure 3: Position of the femur within a micro-CT scan

Table 1: Part numbers, names and description

Index	Part Name	Description	
1	Mouse Holder Unit	The primary structural part of the mouse holder. Post-manufacture it is recommended to coat in plastic (Black EpoFlon PTFE coating at 240°C) (Epsosint, Islikon, Switzerland)
2	Anaesthetic Unit	The anaesthetic unit attaches to the mouse holder unit and acts as an interfacing part between the incoming anaesthetic pipe and the anaesthetic mask.
3	Bottom Cable Clamp Back	The bottom cable clamp back clamps the sensor and heating unit's cables.
4	Bottom Cable Clamp	The bottom cable clamp back clamps the sensor and heating unit's cables.
5	Upper Cable Cover	The upper cable cover protects the cables from the top of the mouse holder unit.

6	Cover Plate	The cover place protects the cables from the bottom of the mouse holder unit.
7	End Cap	The end cap acts as an interfacing part between the holder and the Scanco vivo micro-CT mounting device. This part also insulated the scanner mount from the heating system.
8	Clamp	The clamp holds the external fixator.

Heating and Sensing System

Two Stego PTC Heater RCE 016 (9 W at 24 V) elements are used, flanking a KS-PT1000A-1-550-3-W temperature-sensing unit. These are all controlled via a Jumo CTRON 16 control unit. Both heating elements and the sensor were covered in thermal paste before insertion.

Figure 4: Heating and sensing unit insertion holes

Examples of use

Figure 5: Mouse holder in a Scanco viva40 (left) and a Scanco viva80 (right)

Figure 6: Mouse holder in use in a Scanco viva80 with mouse

Figure 7: Mouse holder being used in conjunction with a loading device

Figure 8: An extended 2.5mm hex torque limited screwdriver is used to secure the external fixator within the clamp. 8 Nmm was used.

Figure 9: Mouse in mouse holder, top view. Padding is used to improve mouse ergonomics. Shims are used to raise mouse to correct position based on mouse size

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Appendix A: Mouse holder parts

Part List

(See accompanying drawings)

Part	Amount	Material	Manufacturing Process
Mouse Holder Unit	1	Aluminium	Selective Laser Sintering
Anaesthetic Unit	1	ABS	3D Printed
Bottom Cable Clamp Back	1	PMMA	Machined
Bottom Cable Clamp	1	POM	Machined
Upper Cable Cover	1	POM	Machined
Cover Plate	1	POM	Machined
End Cap	1	ABS	3D Printed
Clamp	1	Stainless Steel	Wire cut
M2.5x12	4	ISO 7046	Philips head
M2 x 8	2	ISO 4762	Hex slot head
M2.5 x 6	1	ISO 4762	Hex slot head
M4 x 16	3	ISO 4762	Hex slot head
M2 x 6	2	ISO 4762	Hex slot head
Shims (87x67x2mm)	2	Aluminium	Plate/Machined

Electronic Part List

(See accompanying files)

Part	Amount
Stego PTC Heater RCE 016 (9W at 24 volts)	2
KS-PT1000A-1-550-3-W	1
Jumo CTRON 16 control unit	1

Appendix B: Additional tools

Figure 10: An extended torque limited screwdriver to ensure clamp is not over tightened. 8 Nmm was used.

Appendix C: Plug and socket

Description

To facilitate easy transfer of heating and anaesthetic systems a plug and socket system was developed. The socket can be attached to a Scanco viva40 scanner (if the parts labelled **Scanco** are used) or modified appropriately for other scanners or circumstances. The ground plate should be adapted according to the scanning arrangement and scanner mounting point. Depending on the scanner, the parts under **Plug_Socket_Scanco** may need to be used in place of **Plug_Socket**. Scanco appropriate plug-socket parts are prefixed with 1000-05-XXX in their file name. Appropriate mounting plates are included (i.e. Gound_plate_Plug_Socket02-V0.1). All parts were conventionally machined. Part names align with drawing names. Fastener requirements are indicated on the drawings.

Part photos

Figure 11: Plug with cables and anaesthetic tube attached.

Figure 12: Plug socket arrangement in use

Part list general

(See accompanying drawings)

Part	Amount	Material
Plug	1	Aluminium
Plug Socket	1	Aluminium
Plug Socket Cover	1	Aluminium
Securing cylinder	1	Aluminium
Anaesthetic mating part	1	POM
Anaesthetic mating part 2	1	POM
9 pin serial female connector	1	-
Brass threaded cable joints (M12x1.5)	1	-
O ring	1	-

Part list Scanco

Part	Amount	Material
Plug-Socket-Scanco	1	Aluminium
Ground Plate-PlugSocket	1	Aluminium
Top Plate PlugSocket	1	Aluminium
PlugSocketCover	1	Aluminium