Launching Omero at the DRFZ Berlin - the setup and our plans

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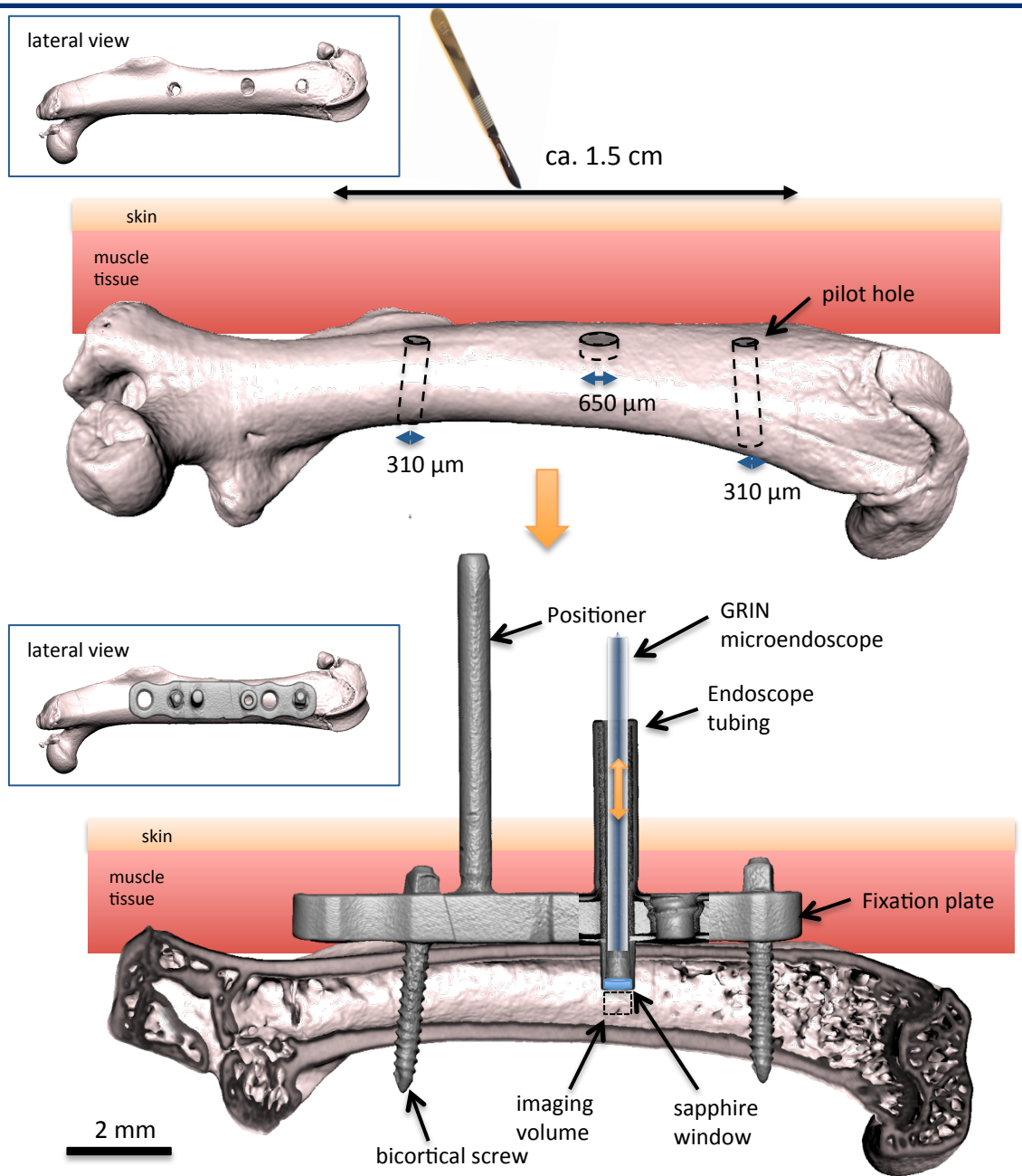
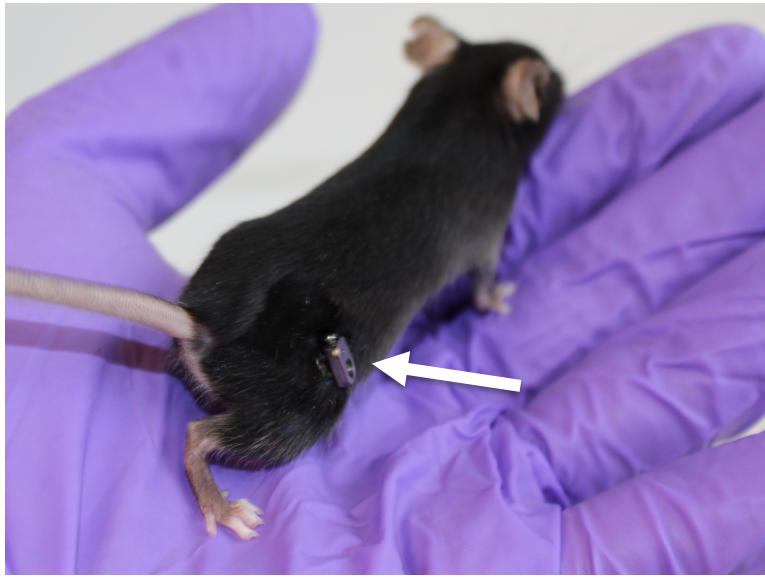
10th Annual Users Meeting
June 2015

DRFZ BERLIN
Deutsches Rheuma-Forschungszentrum
Ein Institut der Leibniz-Gemeinschaft

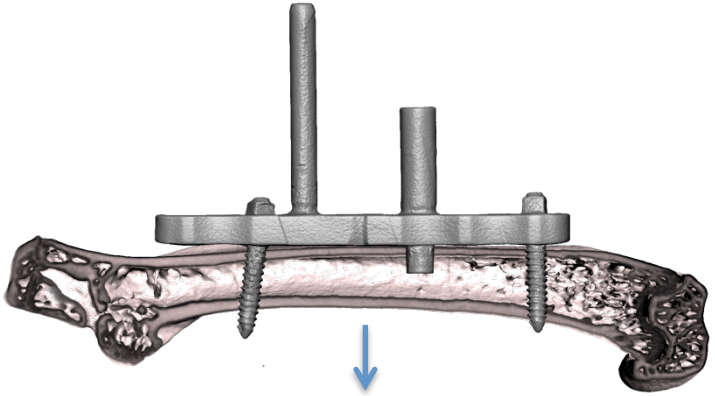
Longitudinal intravital microendoscopy in the murine bone marrow (LIMB)



- Miniaturized version of conventional fixator as used in clinics
- Internal System
  →Stable fixation close to the cortex
- Medical grade Ti-alloy
  →Biocompatibility
  →high rigidity and low density



Characterization via *ex vivo* methods
- µCT, Histochemistry and Immunofluorescence



Fixation (4 % PFA, 10-30 % Sucrose Sol.)

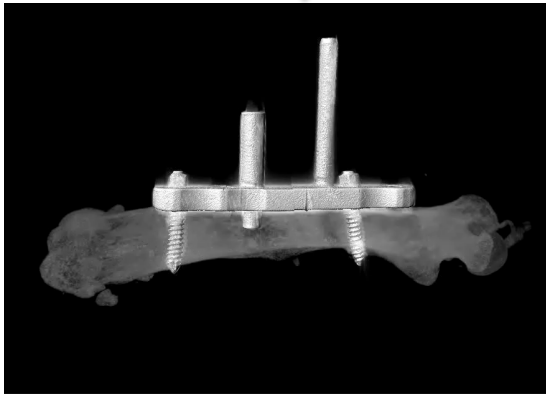
Explantation

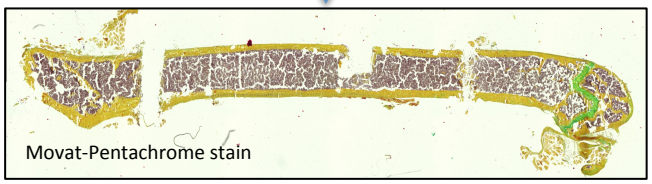
7 µm freeze-sections (*T. Kawamoto, 2012*)

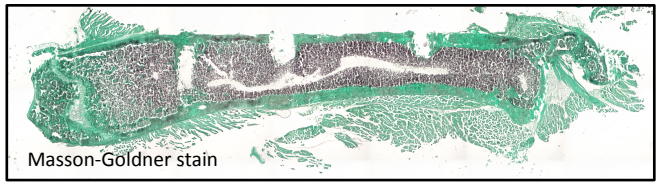
µCT

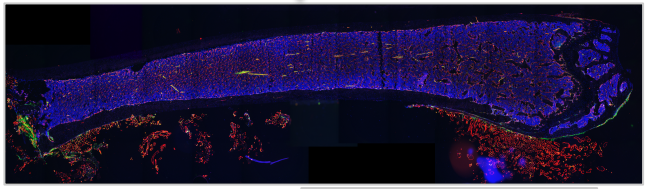
Histochemistry

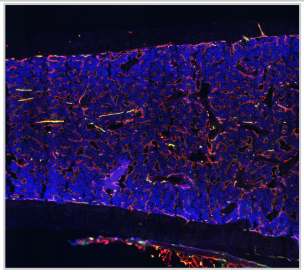
Immunofluorescence



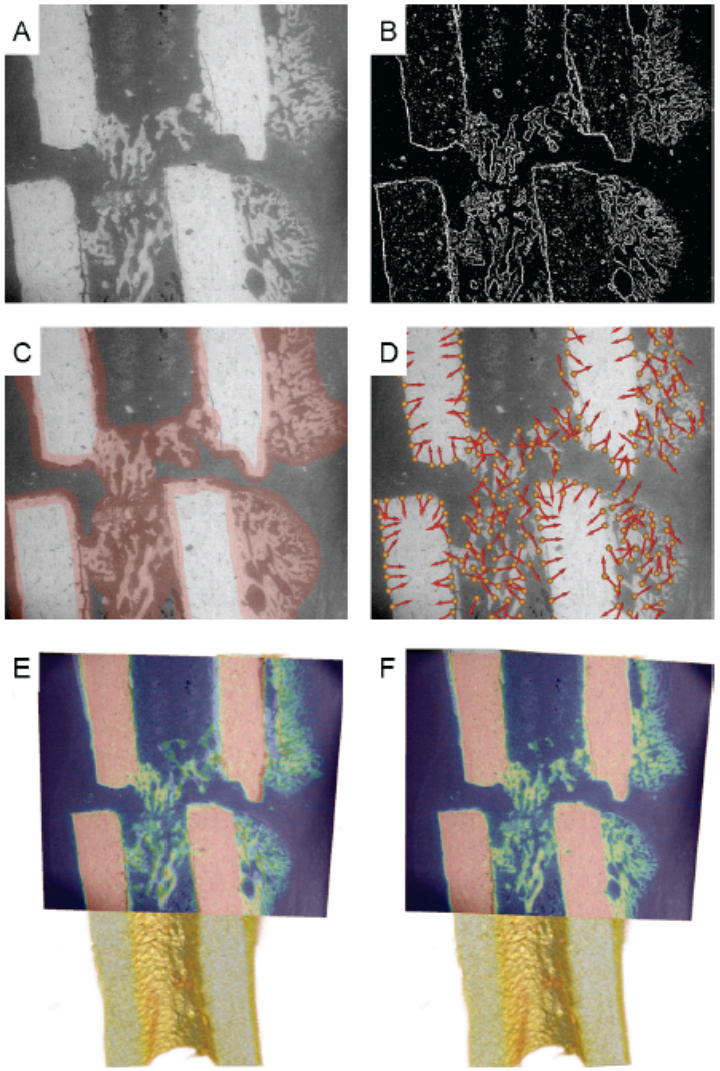


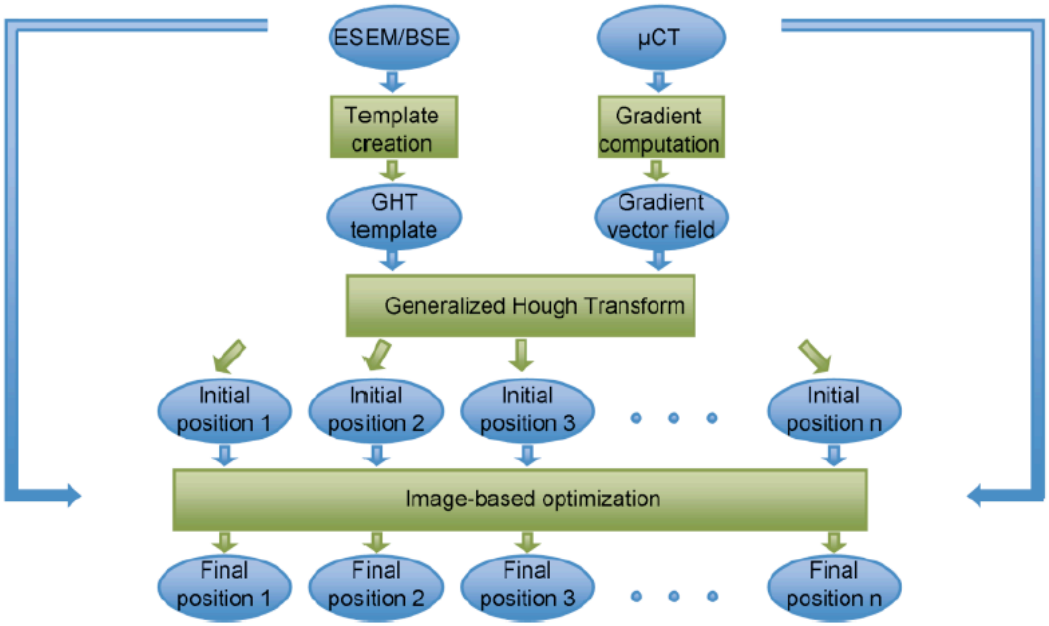






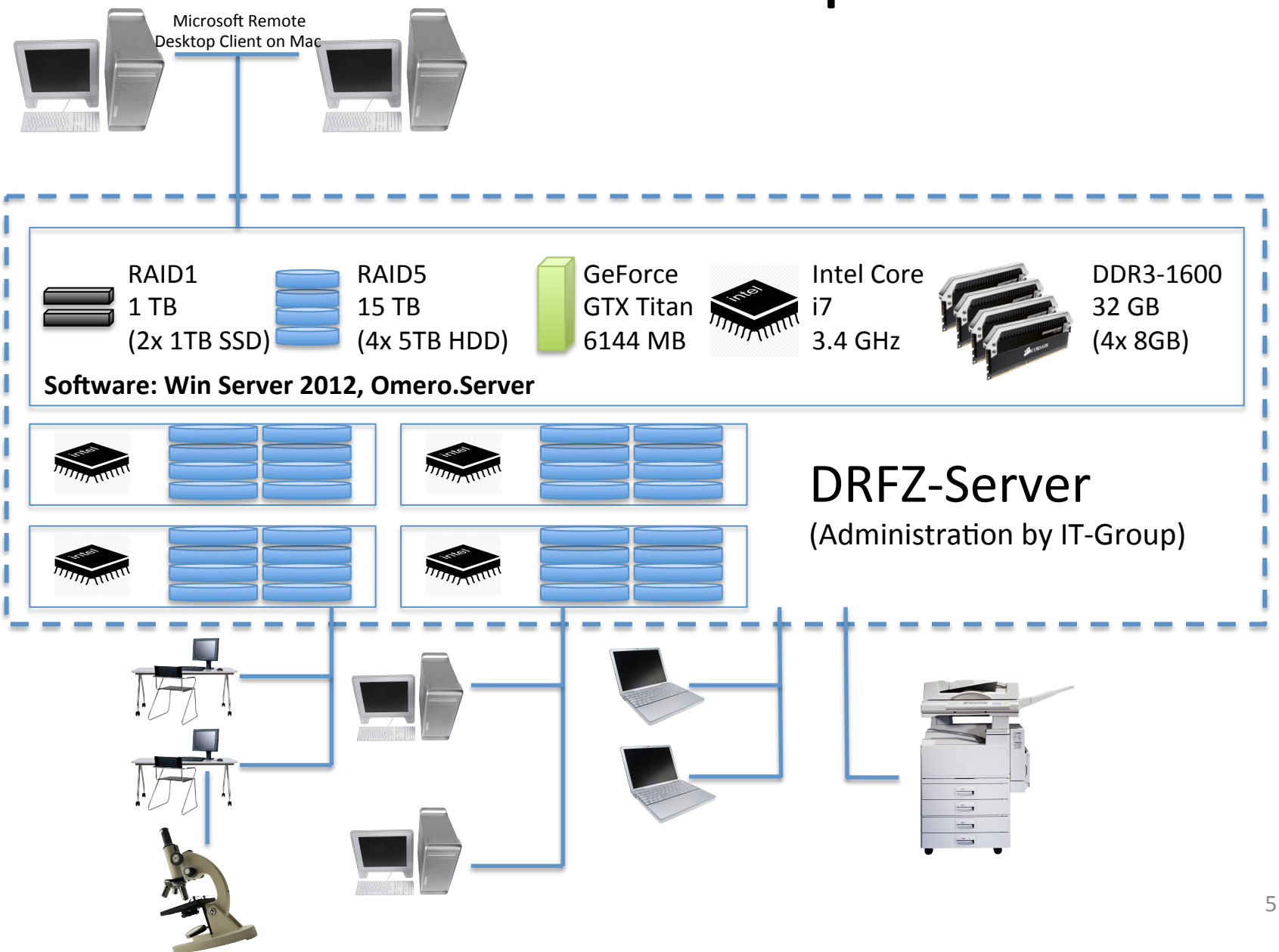
Registering of 2D and 3D imaging data of bone - a new tool for Amira



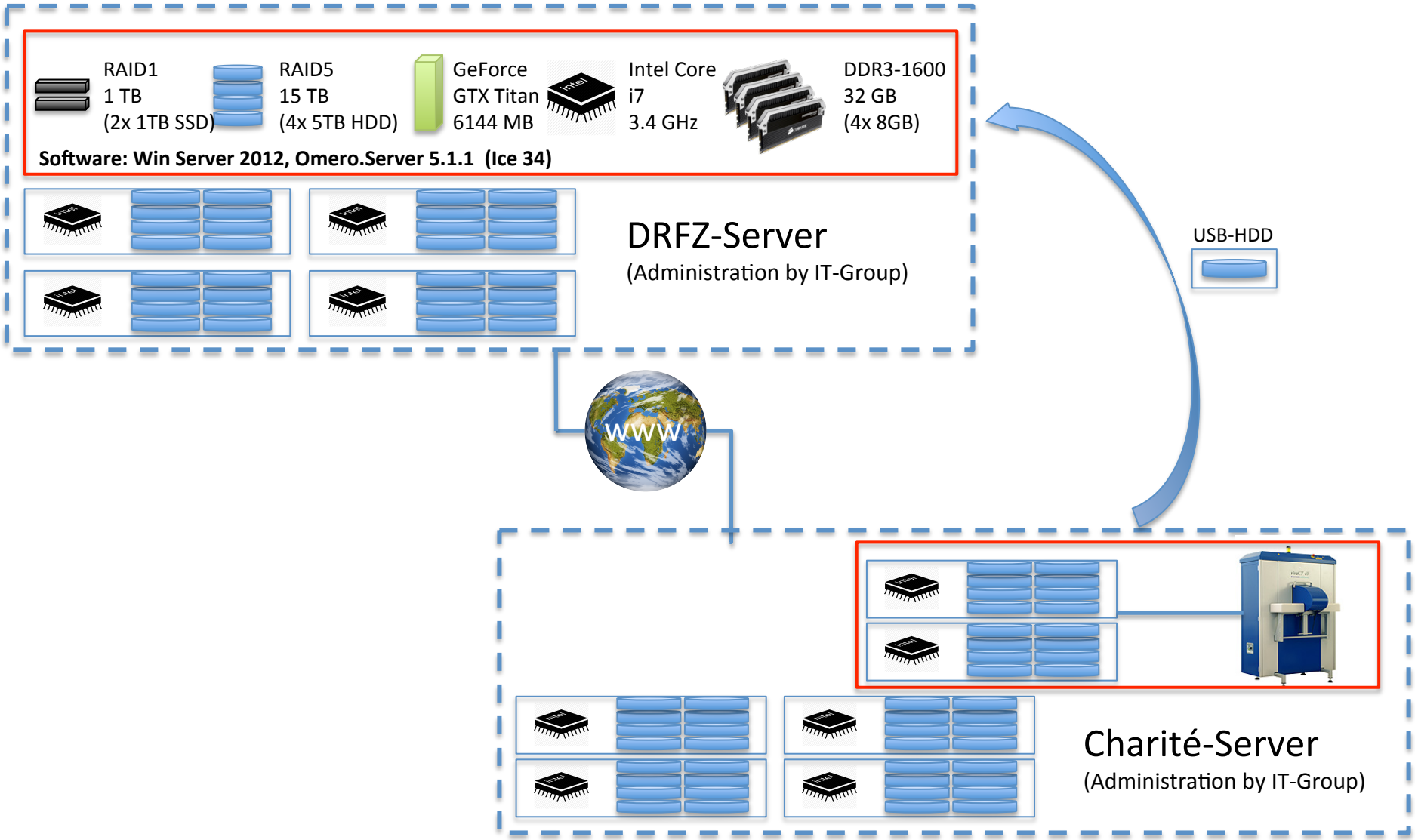


Rebecca M. Hoerth, Daniel Baum, David Knötel, Steffen Prohaska, Bettina M. Willie, Georg N. Duda, Hans-Christian Hege, Peter Fratzl, Wolfgang Wagermaier; **ZIB Report 15-01 (January 2015)**

Server Setup



Server Setup



Open Questions

1. How to link Amira-tools to Omero?
2. Can Omero handle (raw) µCT-files like .isq and **.rsq**?
3. How to prepare the histo-images for the creation of a binary template?
4. Which tools are best suited for stitching of 3D tilescans with rotations and translations?
5. …

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Georg Duda
Mario Thiele
Katharina Schmidt-Bleek
Marzena Princ

RISystem

Romano Matthys
Reto Nützi

DFG Deutsche Forschungsgemeinschaft

FOR 2165
Regeneration in Aged

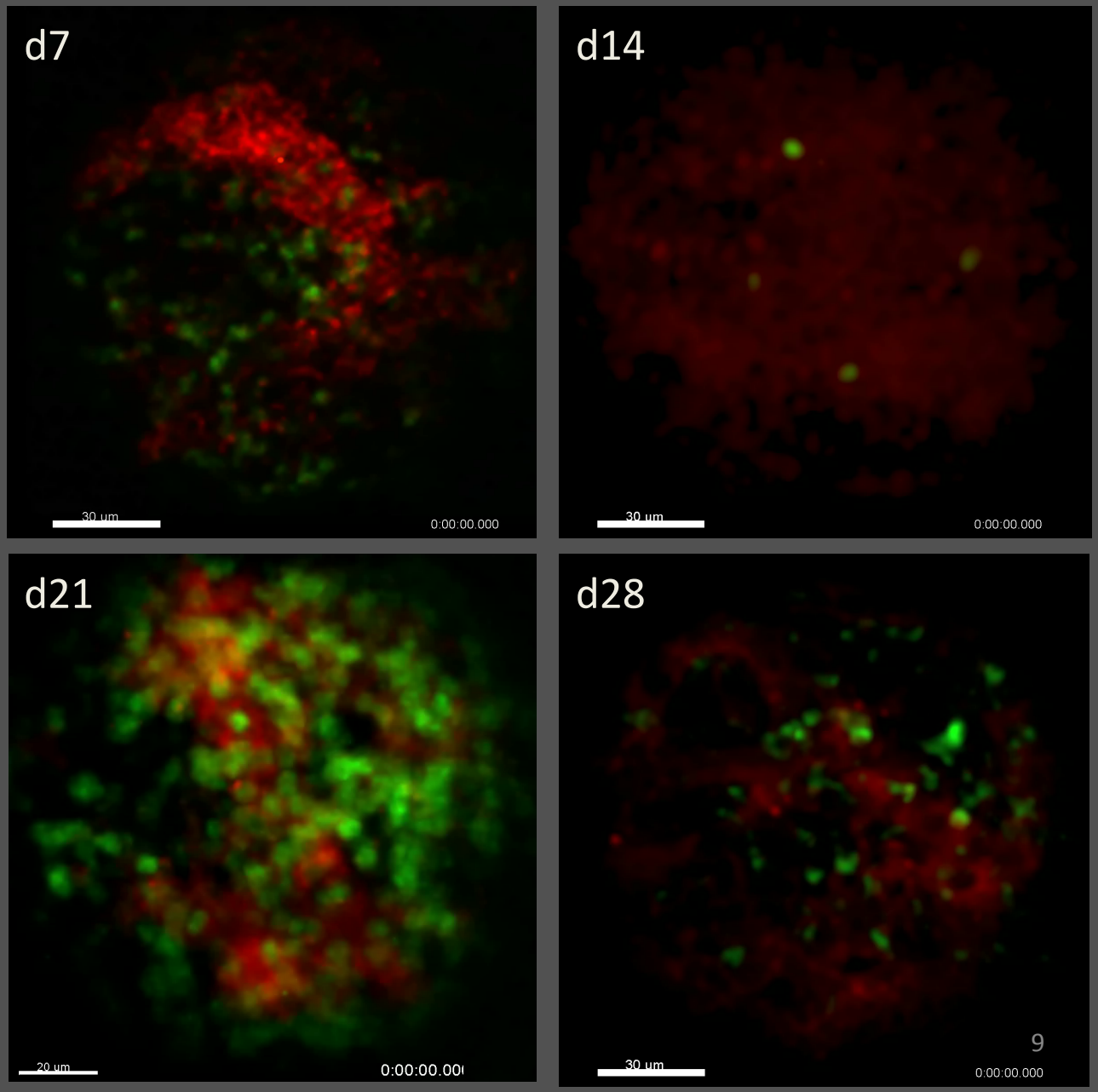
Jimia
A Joint Intravital Microscopy and Imaging Platform

Thank you for your attention!

Longitudinal intravital imaging of CD19-tdRFP mouse

Imaging parameter
Ex.: 1100 nm (OPO)
Image size: 150 x 150 µm
z-Stack: 54 µm
Averaging: 2

Post processing:
smoothing (Gaussian)
deconvolved (Huygens, SVI)



CD19-tdRFP
Vessels (Qdots 655 i.v.)

Omero diagnostics

Administrator: Windows PowerShell

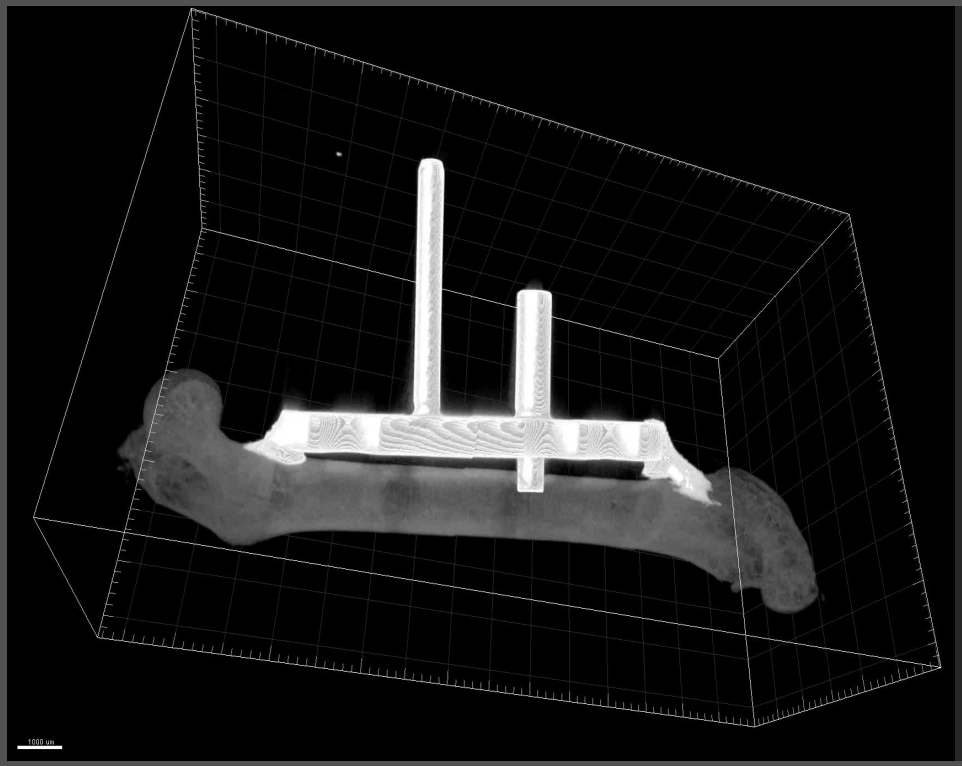
```
Windows PowerShell
Copyright (C) 2014 Microsoft Corporation. All rights reserved.

PS C:\Users\Administrator> cd c:\omero.server
PS C:\omero.server> bin\omero admin diagnostics

================================================================================
OMERO Diagnostics 5.1.1-ice34-b43
================================================================================

WARNING:omero.util.UpgradeCheck:UPGRADE AVAILABLE:Please upgrade to 5.1.2 See http://trac.openmicroscopy.org.uk/omero fo
r the latest version

Commands:   java -version                  1.8.0     (C:\ProgramData\Oracle\Java\javapath\java.EXE)
Commands:   python -V                      2.6.6     (C:\Python26\python.EXE)
Commands:   icegridnode --version          3.4.2     (C:\Ice-3.4.2\bin\icegridnode.EXE)
Commands:   icegridadmin --version         3.4.2     (C:\Ice-3.4.2\bin\icegridadmin.EXE)
Commands:   psql --version                 9.4.1     (C:\Program Files\PostgreSQL\9.4\bin\psql.EXE)

Server:     icegridnode                    running
Server:     Blitz-0                        active (pid = 2676, enabled)
Server:     DropBox                        inactive (disabled)
Server:     FileServer                     active (pid = 2716, enabled)
Server:     Indexer-0                      active (pid = 2732, enabled)
Server:     MonitorServer                  active (pid = 2748, enabled)
Server:     OMERO.Glacier2                 active (pid = 2776, enabled)
Server:     OMERO.IceStorm                 active (pid = 2820, enabled)
Server:     PixelData-0                    active (pid = 2840, enabled)
Server:     Processor-0                    active (pid = 2860, enabled)
Server:     Tables-0                       inactive (disabled)
Server:     TestDropBox                    inactive (enabled)
Server:     OMERO.master                   active (running as LocalSystem)

Log dir:    C:\OMERO.server\var\log        exists

Log files:  Blitz-0.log                    29.0 MB       errors=12   warnings=19
Log files:  DropBox.log                    2.0 KB        errors=9    warnings=0
Log files:  FileServer.log                 0.0 KB
Log files:  Indexer-0.log                  394.0 KB
Log files:  MonitorServer.log              0.0 KB
Log files:  OMEROweb.log                   556.0 KB      errors=64   warnings=2
Log files:  OMEROweb_request.log           28.0 KB       errors=58   warnings=0
Log files:  PixelData-0.log                136.0 KB
Log files:  Processor-0.log                4.0 KB        errors=0    warnings=1
Log files:  Tables-0.log                   n/a
Log files:  TestDropBox.log                n/a
Log files:  master.err                     0.0 KB
Log files:  master.out                     0.0 KB
Log files:  Total size                     30.64 MB


Environment:OMERO_HOME=(unset)
Environment:OMERO_NODE=(unset)
Environment:OMERO_MASTER=(unset)
Environment:OMERO_TEMPDIR=(unset)
Environment:PATH=C:\Program Files (x86)\NVIDIA Corporation\PhysX\Common;C:\Python26;C:\ProgramData\Oracle\Java\javapath;
C:\Windows\system32;C:\Windows;C:\Windows\System32\Wbem;C:\Windows\System32\WindowsPowerShell\v1.0\;C:\Ice-3.4.2\bin;C:\
Program Files\PostgreSQL\9.4\bin;
Environment:PYTHONPATH=C:\Ice-3.4.2\python\
Environment:ICE_HOME=(unset)
Environment:LD_LIBRARY_PATH=(unset)
Environment:DYLD_LIBRARY_PATH=(unset)

OMERO SSL port:4064
OMERO TCP port:4063
OMERO data dir:'D:\\OMERO'                    Exists? True      Is writable? True
OMERO temp dir:'C:\Users\Administrator\AppData\Roaming\omero\tmp' Exists? True  Is writable? True   (Size: 0)

JVM settings: Blitz                        -Xmx5140m -XX:MaxPermSize=1g
JVM settings: Indexer                      -Xmx3427m -XX:MaxPermSize=1g
JVM settings: Pixeldata                    -Xmx5140m -XX:MaxPermSize=1g
JVM settings: Repository                   -Xmx3427m -XX:MaxPermSize=1g

OMERO.web status... DEVELOPMENT: You will have to check status by hand!
PS C:\omero.server> _
```

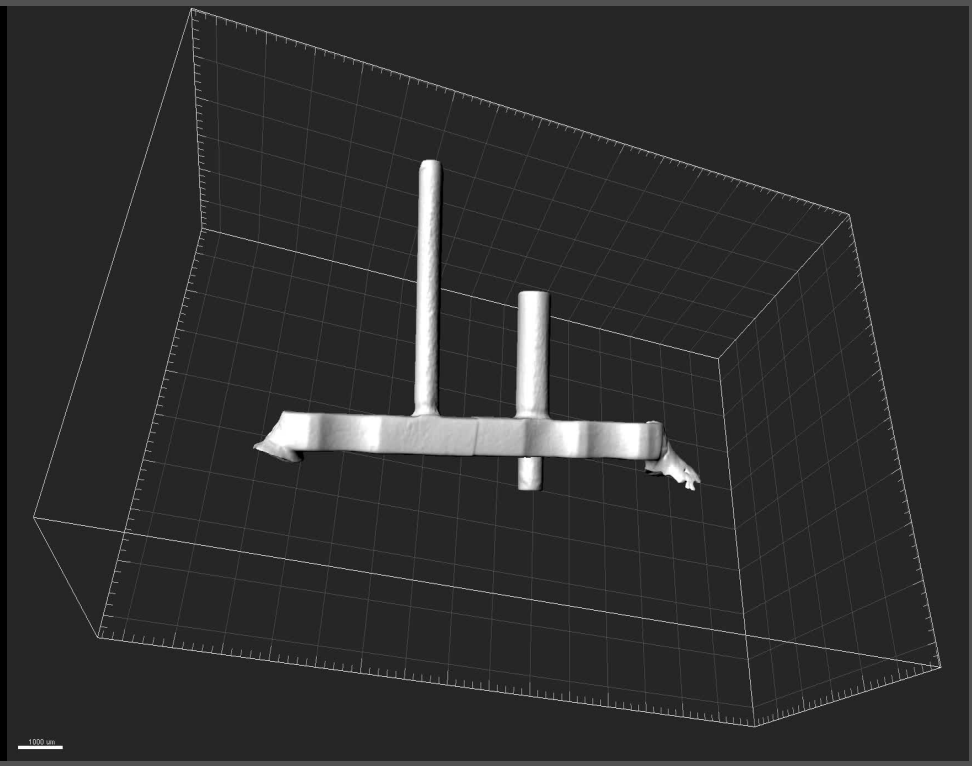
vivaCT 40

Specifications
Type Cone-beam *in vivo* microCT
Fully shielded
No external shielding required
X-ray source Sealed
Air-cooled
Maintenance-free
30 - 70 kVp
Detector 2048 x 256 elements, 26 µm pitch
Resolution ‹ 14 µm (10% MTF)*
5 - 76 µm nominal isotropic (pixel size)*
Image matrix 512 x 512 to 4096 x 4096 pix
Max. scan size 38.9 x 145 mm (ØxL)
Max. specimen size 80** x 500 mm*** (ØxL)
Min. scan time 30 s
Electrical 100 - 230 V / 50 - 60 Hz
370 W
Dimensions 1650 x 1090 x 920 mm (HxWxD)
Weight 258 kg

Outlook
…reduce iatrogenic damage to the BM

→ Use of dental bone adhesive

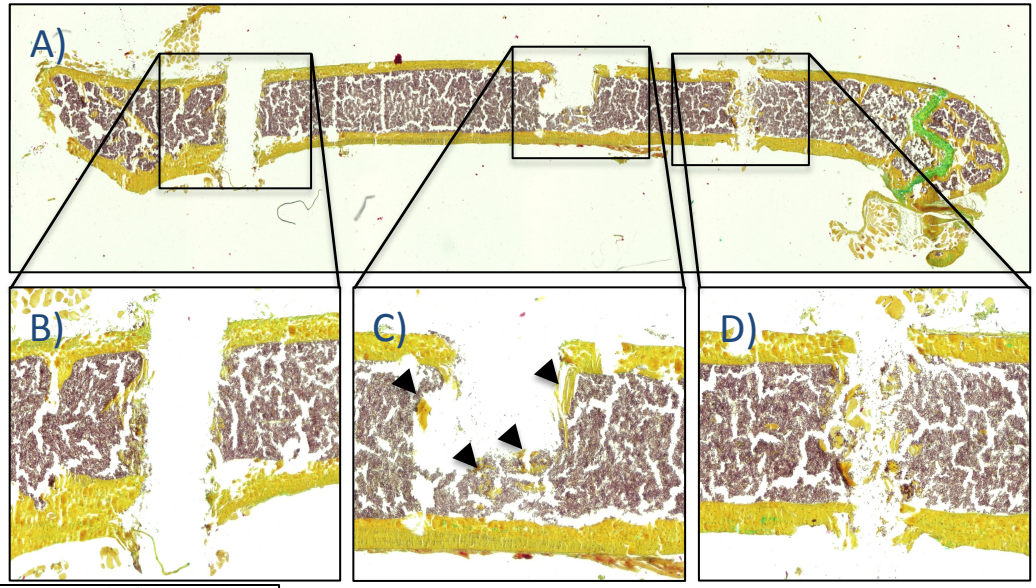


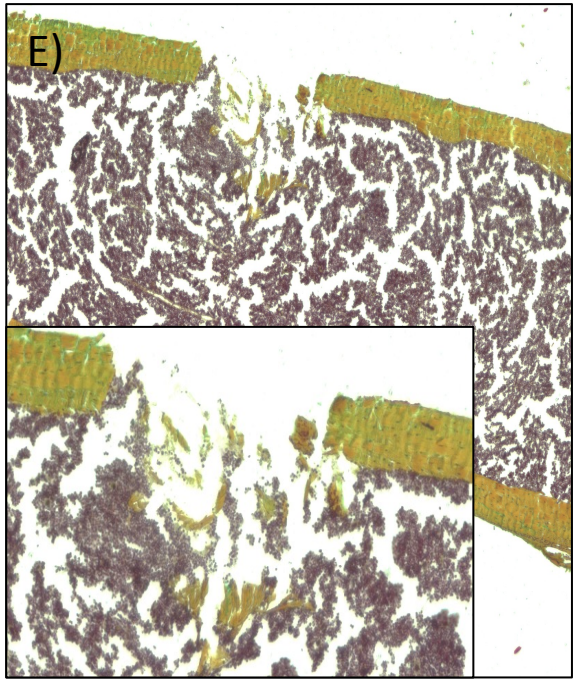


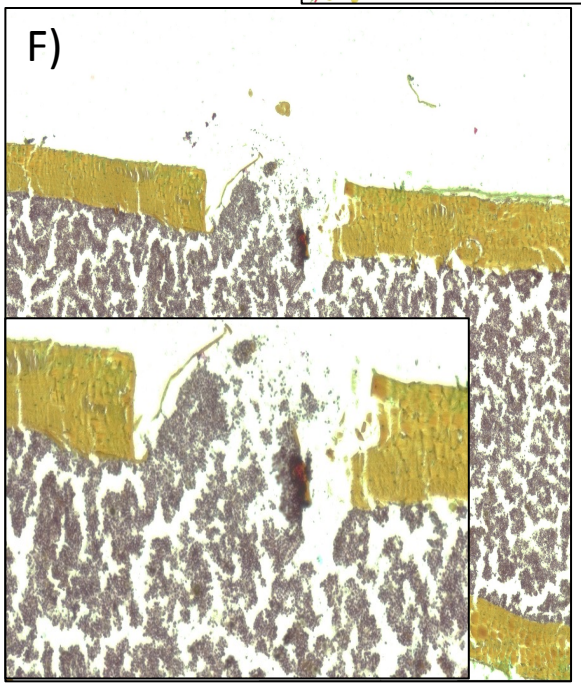
Challenges:

- Mechanical stability
- Application of adhesive and implant

Manual drilling causes iatrogenic distortion of the BM - introduction of bone fragments

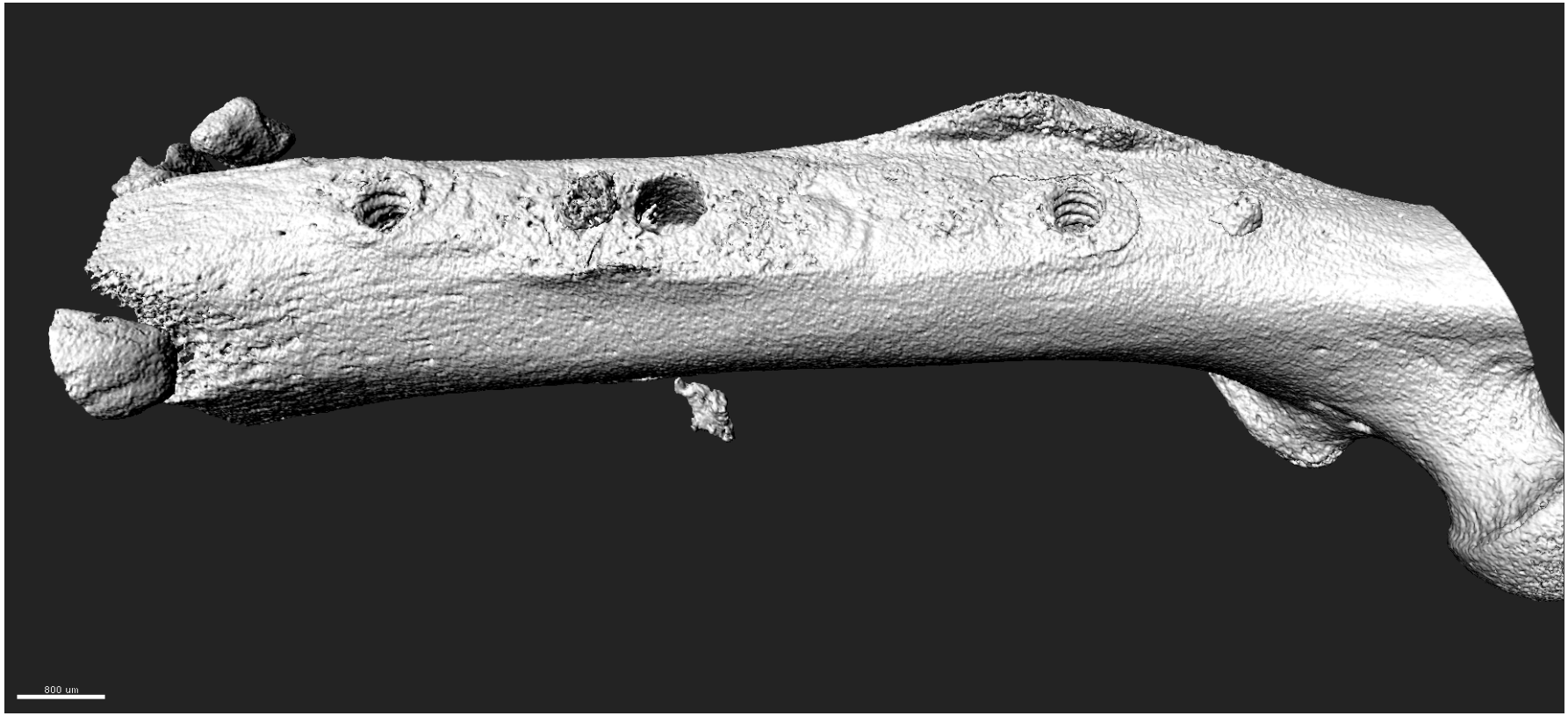
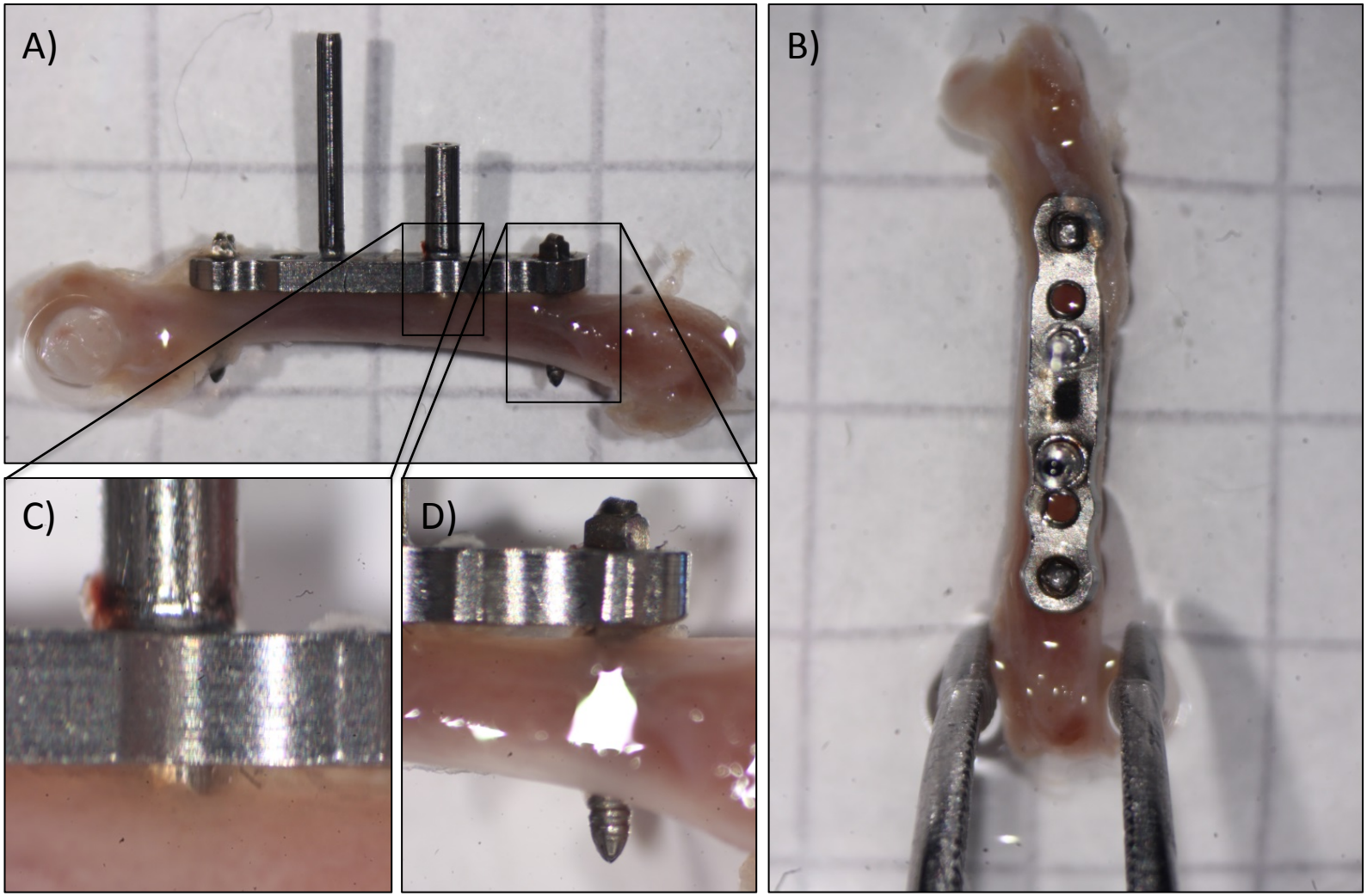






cancellous bone
Cytoplasm
cell nuclei
mineralized cartilage

3D reconstruction of µCT scan w/o implant
- the bone adapts due to bone-to-plate contact



Preliminary experiments
- Identification of hotspots