Perelman School of Medicine, University of Pennsylvania — Penn Center for Musculoskeletal Disorders

PCMD MicroCT Imaging Core Learning Lunch Series

In Vivo µCT Imaging of Live Rodents
+
Image Registration

Presenter: Vincent and Wen

Dec. 18, 2023
PCMD MicroCT Imaging Core

PENN Center for Musculoskeletal Disorders

Penn Center for Musculoskeletal Disorders (P30-AR069619)

McKay Orthopaedic Research Lab, University of Pennsylvania

Outlines

- Brief introduction of our core facility
- In vivo µCT imaging of rodents
- Image registration software development - CTPros
- Q & A

µCT Imaging Core Resources

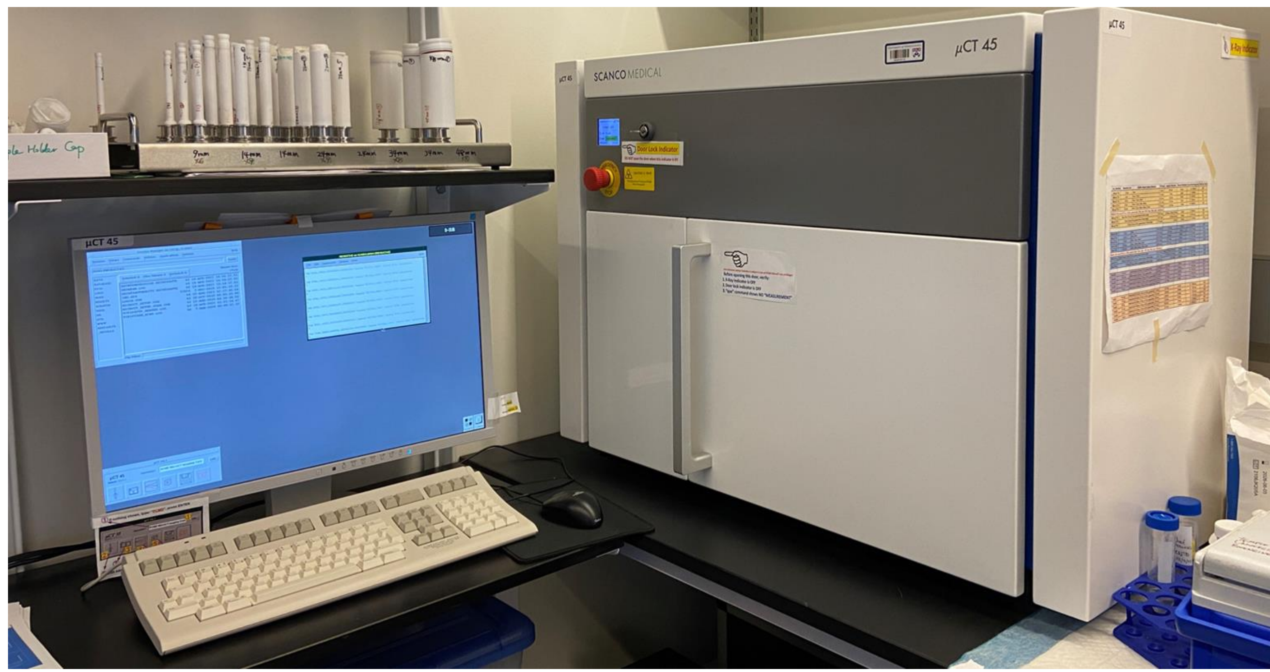
Model	Location	Scan Size (ØxL;mm)	Voxel Size (µm)	Applications
µCT 35	Stemmler Hall	37.9 x 120	3.5-72	High resolution *ex vivo* scans
µCT 45	Stemmler Hall	50 x 120	3.0-100	High resolution *ex vivo* scans
vivaCT 80	Stemmler Hall	80 x 145	10.4-76	High resolution *in vivo* scans for small animals
µCT 50	PVAMC/TMRC	50 x 120	0.5-100	Ultra high resolution (sub-micron) *ex vivo* scans
vivaCT 75	PVAMC/TMRC	79.9 x 145	21-150	*In vivo* scans for small animals; *Ex vivo* scans for large specimens
XtremeCT II	CTRC	140 x 200	60-82	Clinical scans for peripheral skeleton

Ex vivo (Specimen) Scanners

- Scanco µCT 35 (Purchased in 2012)
  - Native voxel sizes: 3.5 µm, 6 µm, 10 µm, 15 µm, 18.5 µm

Ex vivo (Specimen) Scanners

- Scanco µCT 45 (Purchased in 2019)
  - Native voxel sizes: 3 µm, 4.5 µm, 7.4 µm, 10.4 µm, 14.6 µm
  - Carousel system supporting 20 sample holders
  - "Air" filter for scanning low density materials
  - "Copper" filter for scanning specimen with metal implants



In vivo Scanner

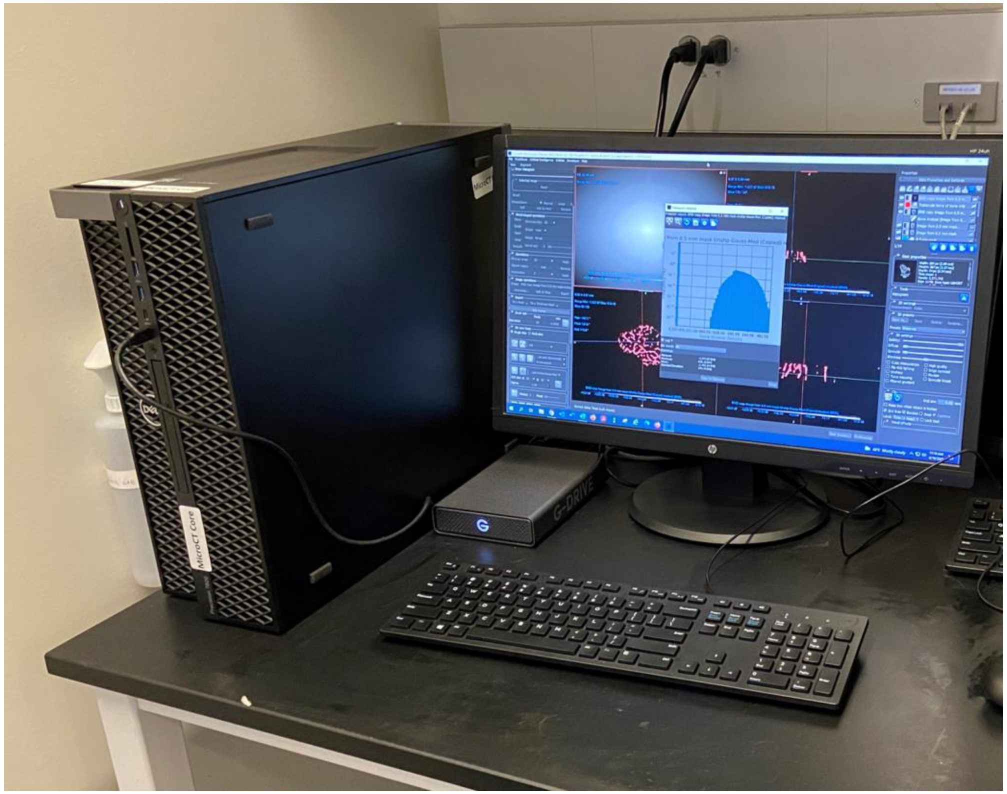
- Scanco vivaCT 80 (Purchased in 2018)
  - Voxel sizes: 10.4 μm, 11.6 μm, 13 μm, 16.1 μm, 20.8 μm, 26 μm
  - Internal heating device to keep animal warm
  - Internal camera to monitor animal's breathing
  - Ex vivo scan for specimen from large animals or human cadaver

MicroCT Analysis PC

- 2 PCs for MicroCT Analysis (315 Stemmler)
  - Windows 10 platform
  - Either remote or onsite access
  - Scanco software

Dragonfly Workstation

- Workstation for Dragonfly software (324 Stemmler)
  - Windows 10 platform
  - PMACS account required (either remote or onsite access)
  - Deep learning assisted analysis
  - Training videos https://www.theobjects.com/dragonfly/tutorials.html



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Why *in vivo* µCT?

- µCT provides 3D imaging with sufficient spatial resolution for the assessment of rodent bone microarchitecture
- *In vivo* µCT: Longitudinal studies of bone morphology
  Waarsing+2006 Brouwers+2007, Brouwers+2008, Brouwers+2009, Klinck+2008, Bouxsein+2010, Lan+2013, Boyd+2006, Campbell+2008, Buie+2008, Lambers+2011, Schulte+2011
  - Skeletal responses to various diseases and treatments
    - Bone loss associated with disuse or surgery
    - Increased bone mass due to pharmacologic treatment or mechanical loading
- Input to micro finite element (µFE) models to track the mechanical properties of bone van Rietbergen+1998, Schulte+2011
- Reduction in number of animals Bouxsein+ 2010

vivaCT 80

- vivaCT 80 (Purchased in 2018)
  - Best resolution:
    10.4 µm isotropic voxel size
  - X-Ray Source:
    30 - 70 kVp
  - Max Scan Size:
    80 x 145 mm (Ø x L)
  - Capacity to scan:
    All tissues on mice
    All tissues on rat
    (body weight < 700g)

In Vivo µCT Imaging

1. Animal Model (Rats, mice)
2. Scanning Resolution
3. Sample Holder
4. Before Scanning: Anesthesia
5. During Scanning: Monitor animal breathing
6. After Scanning: Animal waking up

In Vivo µCT Imaging

Animal Model (Rats, mice)

↓

Scanning Resolution

↓

Sample Holder

↓

Before Scanning:
Anesthesia

↓

During Scanning:
Monitor animal breathing

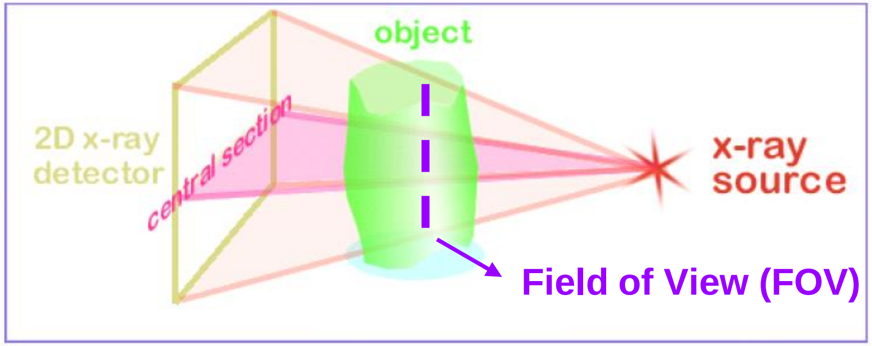
↓

After Scanning:
Animal waking up

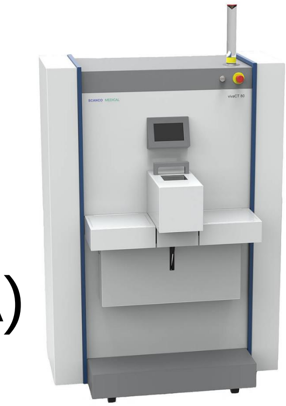
How to Choose Image Resolution (vivaCT 80)

- Image resolution is determined by FOV and number of projections

vivaCT80 Field of View (mm)	Proj./180°	Best Resolution (µm)
31.9	1500	10.4
35.9	1500	11.6
39.9	1500	13.0
49.8	1500	16.1
63.9	1500	20.8
79.9	1500	26.0



Radiation Dose – VivaCT 80



- Computed Tomography Dose Index (CTDI): Proportional to the integration time (s), current (µA) and number of projections

Energy (KV)	Integration time (ms)	Current (µA)	Field of View (mm)	Proj./180°	CTDI (mGy)	Resolution (µm)
55	300	145	32	1500	1537	10.4
55	300	145	40	1500	998	13.0
55	300	145	50	1500	615	16.1
55	300	145	64	1500	368	20.8
55	300	145	80	1500	230	26.0

Concerns – Radiation Exposure

- **In vivo scan on Wistar rats** Klinck+ 2008
  - 8 month old, female rats
  - 12.5 μm isotropic voxel size, 55 kV voltage, 109 μA current, 200 ms integration time, 2000 projections
  - Scanned right tibia at wk0, 2, 4, 6, 8, 12
  - Radiation dose: 502.5 mGy

  → No radiation effect

- **In vivo scan on Wistar rats** Brouwers+ 2007
  - 30 week old, female rats
  - 15 μm isotropic voxel size, 70 kV voltage, 85 μA current, 350 ms integration time, 2000 projections
  - Scanned right tibia at wk0, 1, 2, 3, 4, 5, 6, 8; left tibia at wk0 and 8
  - Radiation dose: 939 mGy
  - Determined cell radiation damage using a cell viability test

  → No radiation effects on bone microarchitecture and marrow cells

Concerns – Radiation Exposure

- **In vivo scan on BL6 mice** Laperre+2011
  - 10 weeks old, male mice
  - 9 µm isotropic voxel size
  - In vivo scanned left tibia at wk0, 2, 4; ex vivo scanned on both tibia after sacrifice (wk4)
  - Radiation dose: 776 mGy

  → Negative effects on BV/TV and Tb.N and increased Oc.S/BS

- **In vivo scan on BL6 mice** Laperre+2011
  - 4 and 16 weeks old, male mice
  - 9 µm and 18 µm isotropic voxel size
  - In vivo scanned left tibia at wk0, 2, 4; ex vivo scanned on both tibia after sacrifice (wk4)
  - Radiation dose: 434 mGy (9 µm) and 166 mGy (18 µm)

  → No radiation effect on both trabecular and cortical bone architecture in all mice

Concerns – Radiation Exposure

- **In vivo scan on C3H, BL6, and BAL mice** Klinck+ 2008
  - 8-10 weeks old, female mice
  - 10.5 µm isotropic voxel size, 55 kV voltage, 109 µA current, 200 ms integration time, 2000 projections
  - Scanned right tibia at wk0, 1, 2, 3
  - Radiation dose: 712.4 mGy

  → Negative effects on trabecular microarchitecture

- **In vivo scan on BL6 mice** Zhao+ 2016
  - 12 weeks old, female mice
  - 10.5 µm isotropic voxel size, 55 kV voltage, 109 µA current, 200 ms integration time, 2000 projections
  - In vivo scanned right femur and L4 at wk0, 3, 6; ex vivo scan on both femurs, L3 and L4 after sacrifice (wk9)
  - Radiation dose: 639 mGy (femur) and 310 mGy (vertebra)

  → No effect on BV/TV and cellular activities; Negative effects on trabecular microarchitecture (~10-20%)

Conclusion: Radiation Exposure

- Minimal impact on rat bone mass and bone microarchitecture

- Compared to rats, mice are more sensitive to radiation exposure
  - High resolution scans (10-15 µm) leading to 10-20% deterioration of trabecular bone microarchitecture compared to non-radiated sites
  - ***Suggestion*** to reduce radiation exposure:
    - Reduction in scan frequency and Increase in interval time between repeated scans
    - Reduction in scan resolution

In Vivo µCT Imaging

Animal Model (Rats, mice)

↓

Scanning Resolution

↓

==Sample Holder==

↓

Before Scanning:
Anesthesia

↓

During Scanning:
Monitor animal breathing

↓

After Scanning:
Animal waking up

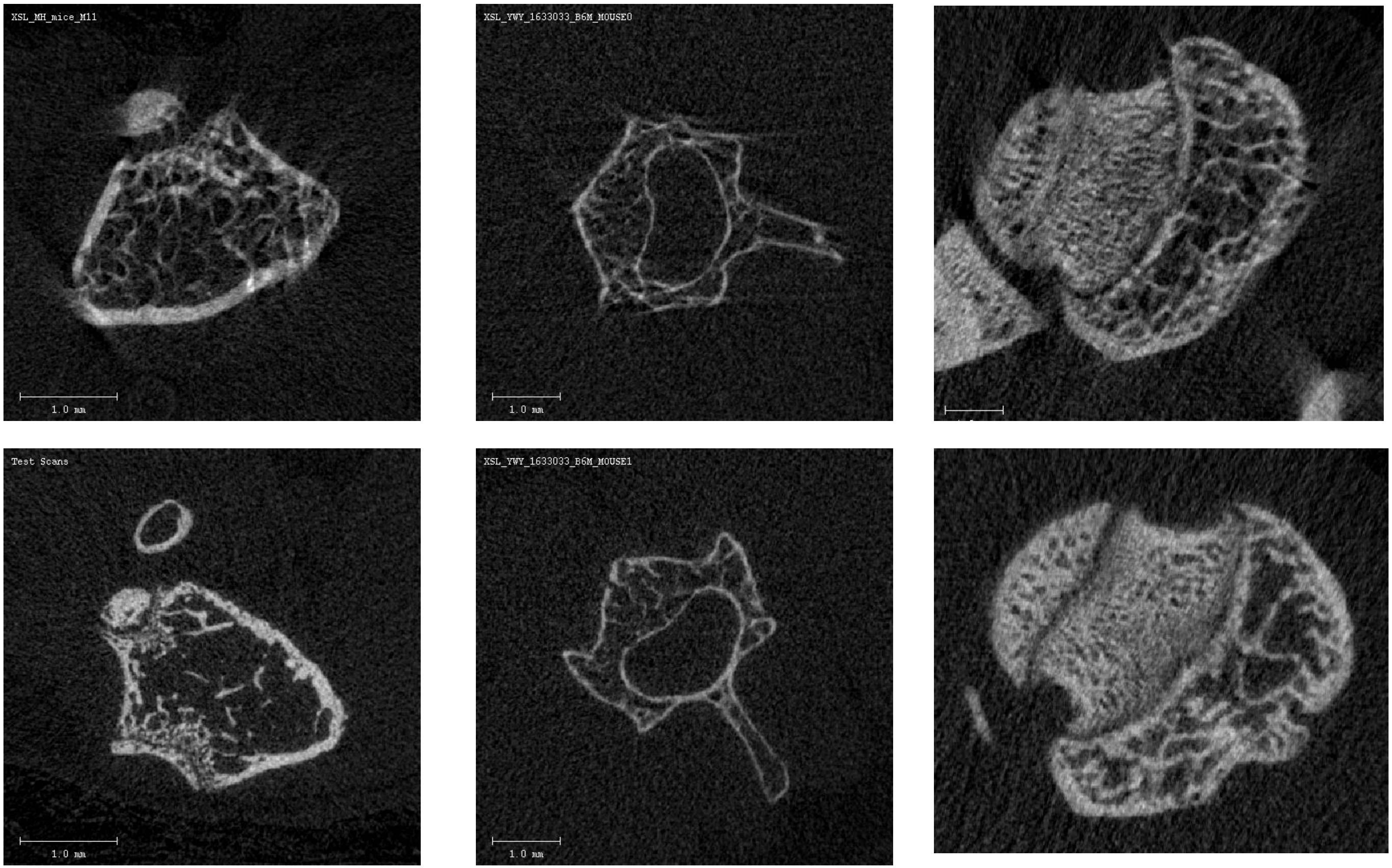
Why Need Holder? Movement Artifacts

- Movement Artifacts caused by **animal breathing**

Why Need Holder? Movement Artifacts

- Movement Artifacts due to **animal breathing**

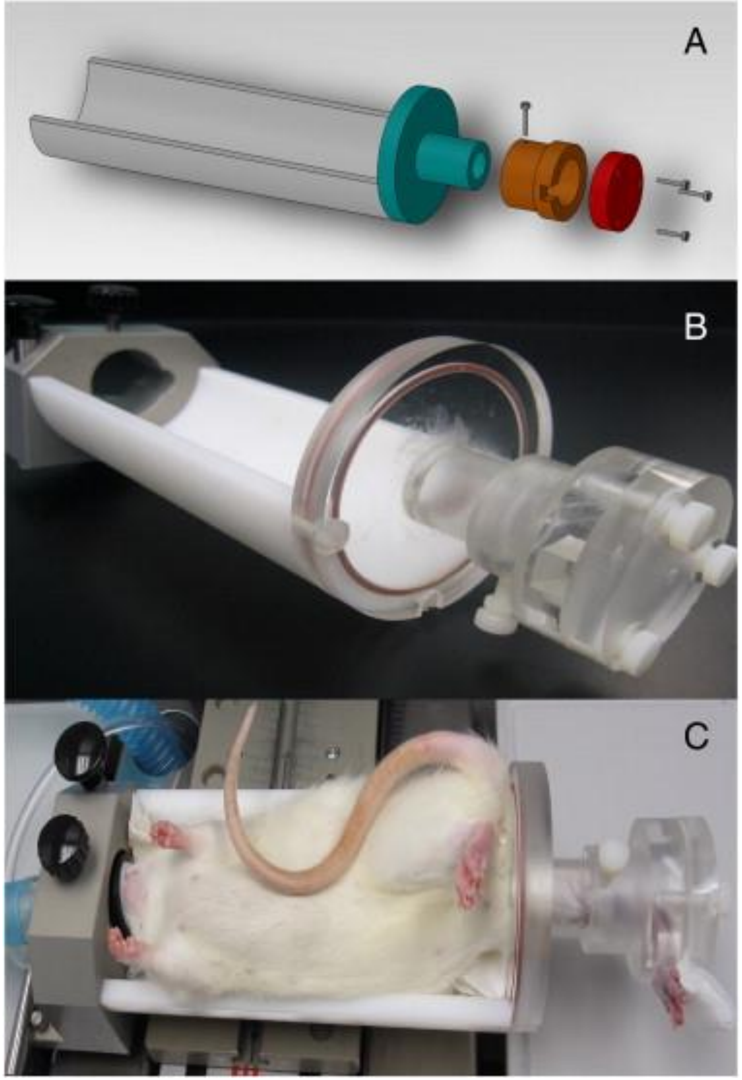
Distal Femur | **L2 Vertebrae** | **Humerus**



Customized Holders - 3D Printing

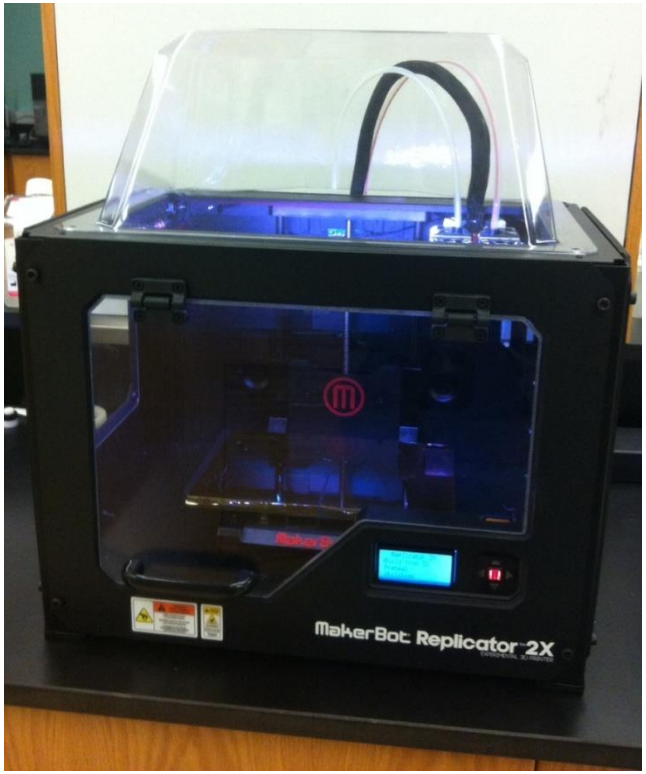
- Minimize the movement of the skeletal site of interest

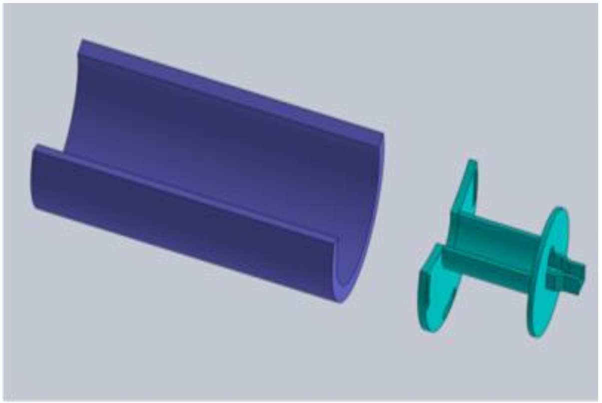
- Minimize the reposition error induced by repeat scans

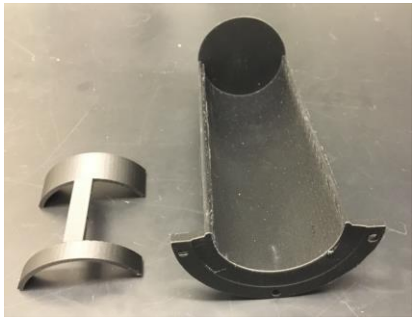


Rat tibia holder

Customized Holders - 3D Printing







In Vivo µCT Imaging

Animal Model (Rats, mice)

↓

Scanning Resolution

↓

Sample Holder

↓

Before Scanning:
Anesthesia

↓

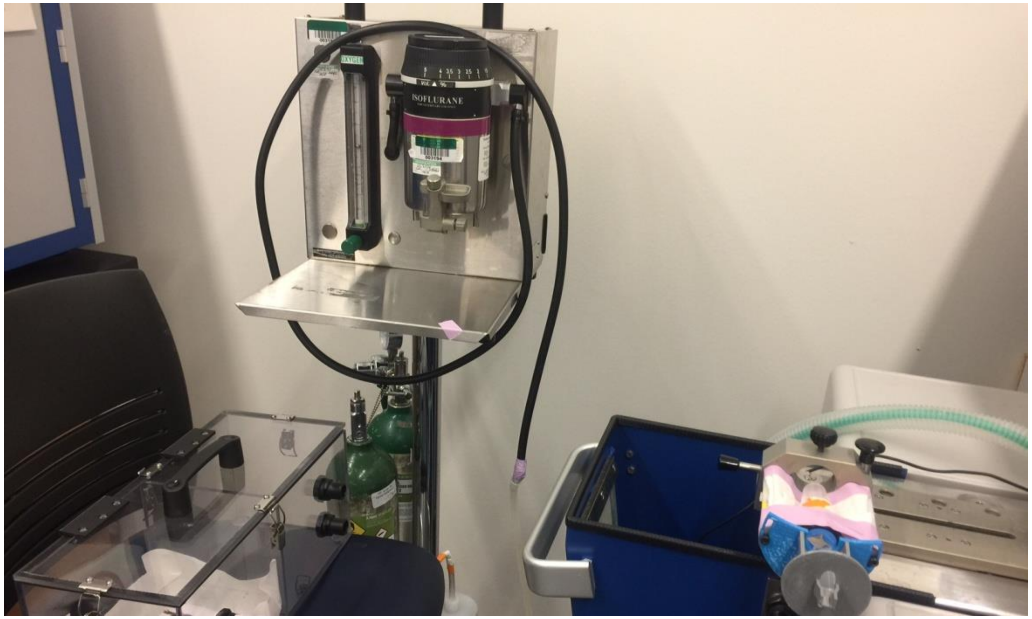
During Scanning:
Monitor animal breathing

↓

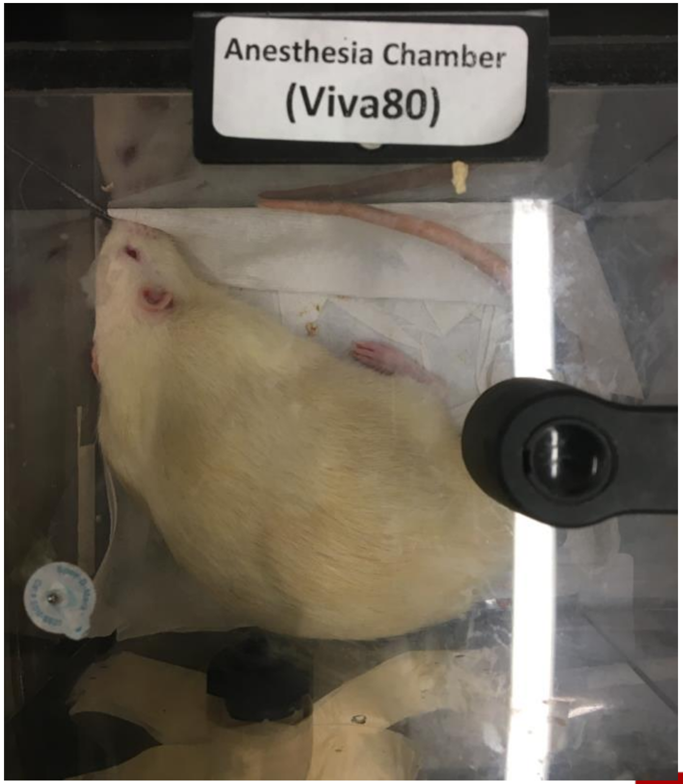
After Scanning:
Animal waking up

Before Scanning - Anesthesia

- Non-painful procedures (Penn IACUC Guideline)
  - Isoflurane
    - Mice: 3-4% for induction and 1-3% for maintenance
    - Rats: 3-5% for induction and 1-3% for maintenance



Anesthesia chamber



http://www.upenn.edu/regulatoryaffairs/Documents/iacuc/guidelines/IACUCGuideline-MouseAndRatAnesthesiaAndAnalgesia.pdf

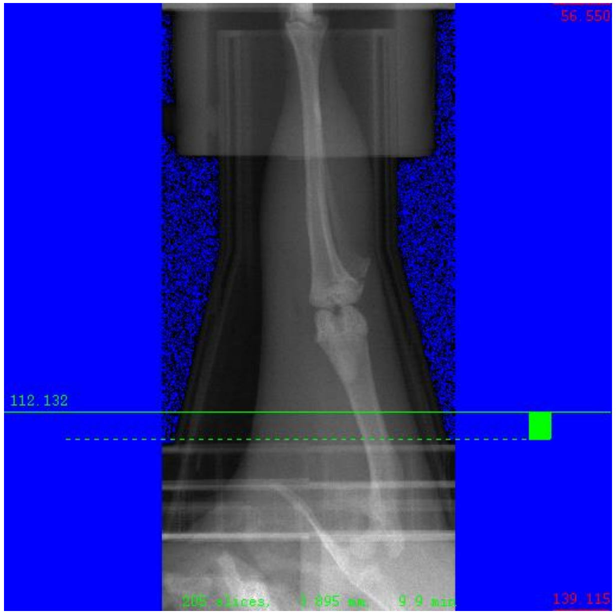
Before Scanning - Anesthesia

- Advantages of Isoflurane (vs. Ketamine/xylazine)
  - Safer
  - Faster (induction, adjusting depth and recovery)
  - No need for reversal agents

http://www.upenn.edu/regulatoryaffairs/Documents/iacuc/guidelines/IACUCGuideline-MouseAndRatAnesthesiaAndAnalgesia.pdf

During Scanning

- In vivo µCT scan
  - 19 µm isotropic voxel size
  - 2 mm bone segment of femur midshaft ~~and muscle~~
  - Average scan time: 10 mins



During Scanning

- Monitor animal's breathing

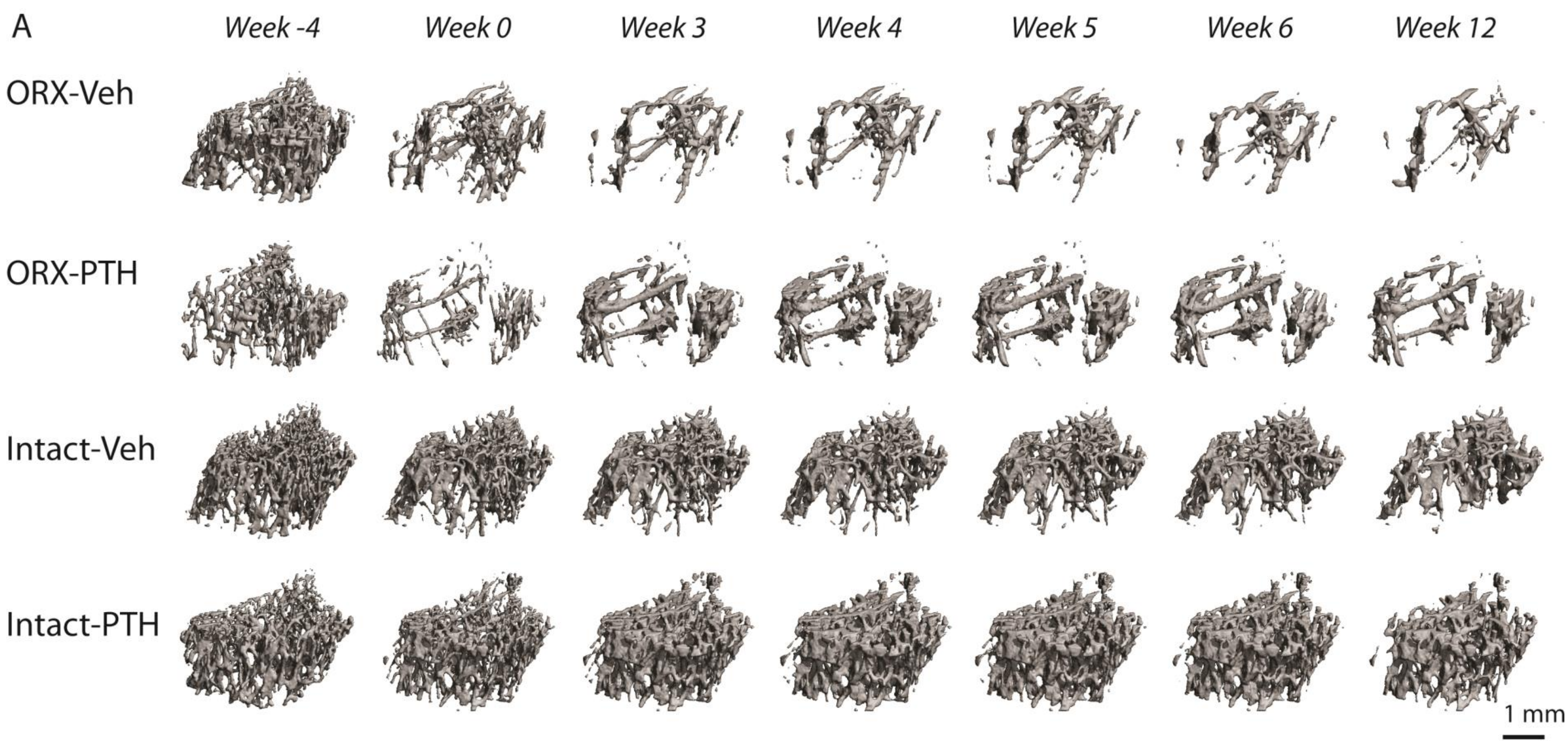
After Scanning

- Waking up the animal: Heating lamp

Precision

- Precision is affected by **reposition** of animals at each follow-up scan
  - Short term precision study (same day, multiple scans)
    - 12.5 µm, Precision: 1-6% in rats Nishiyama+2010
    - 10.5 µm, Precision: 1-7% in rat tibia Lan+2013
    - 10.5 µm, Precision: 1-8% in BL6 or C3H mice tibia Nishiyama+2010
    - 10.5 µm, Precision: 4-12% in femur and 6.5-17.6% in L4 of BL6 mice Chang+2016 SB3C
- To minimize the reposition error
  - Customized animal holders for the scan
  - Image registration

16 Weeks longitudinal scans of male rats



ORX=Orchiectomy surgery; PTH=Parathyroid hormone; Veh=Saline

(Scanned by Vincent)

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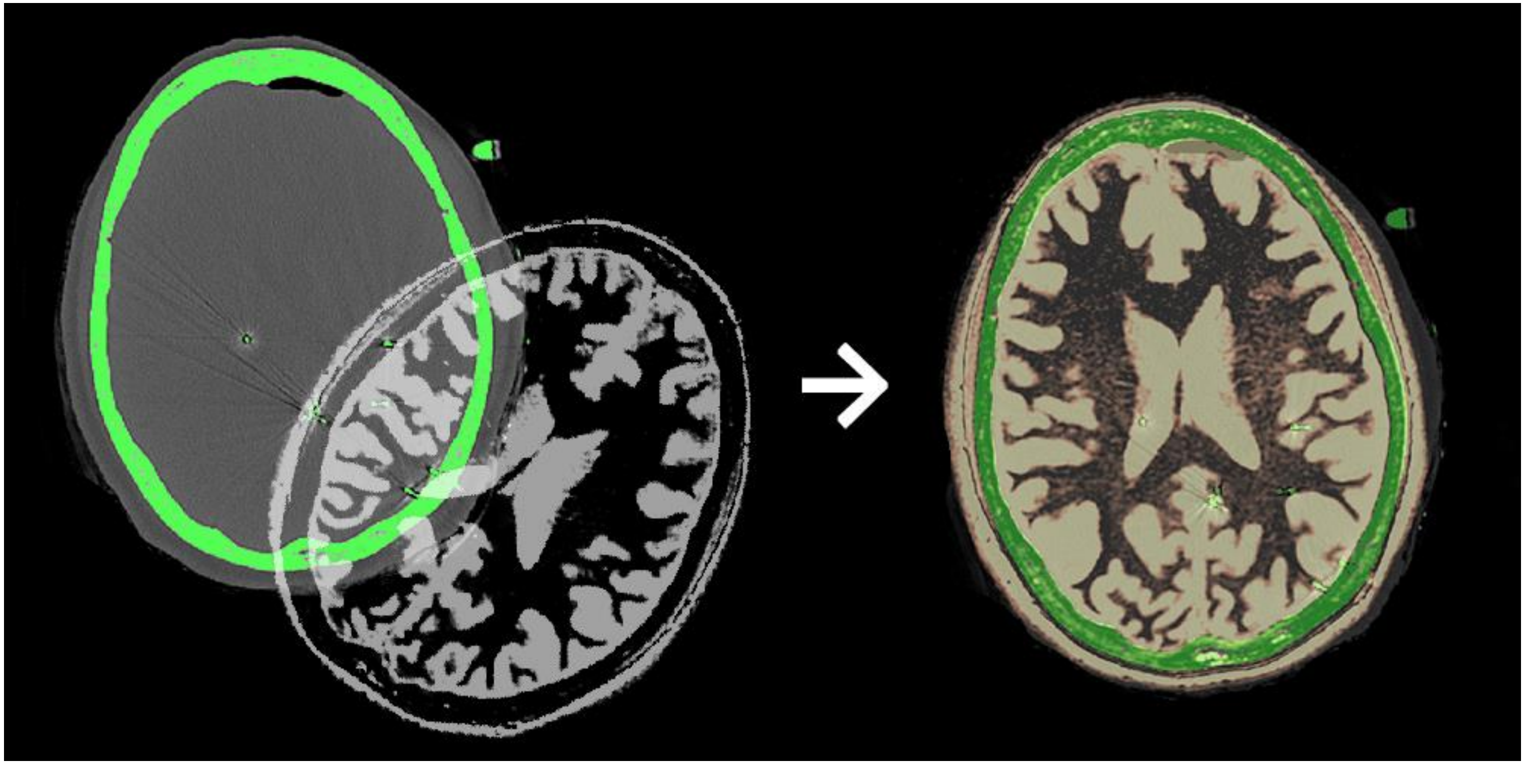
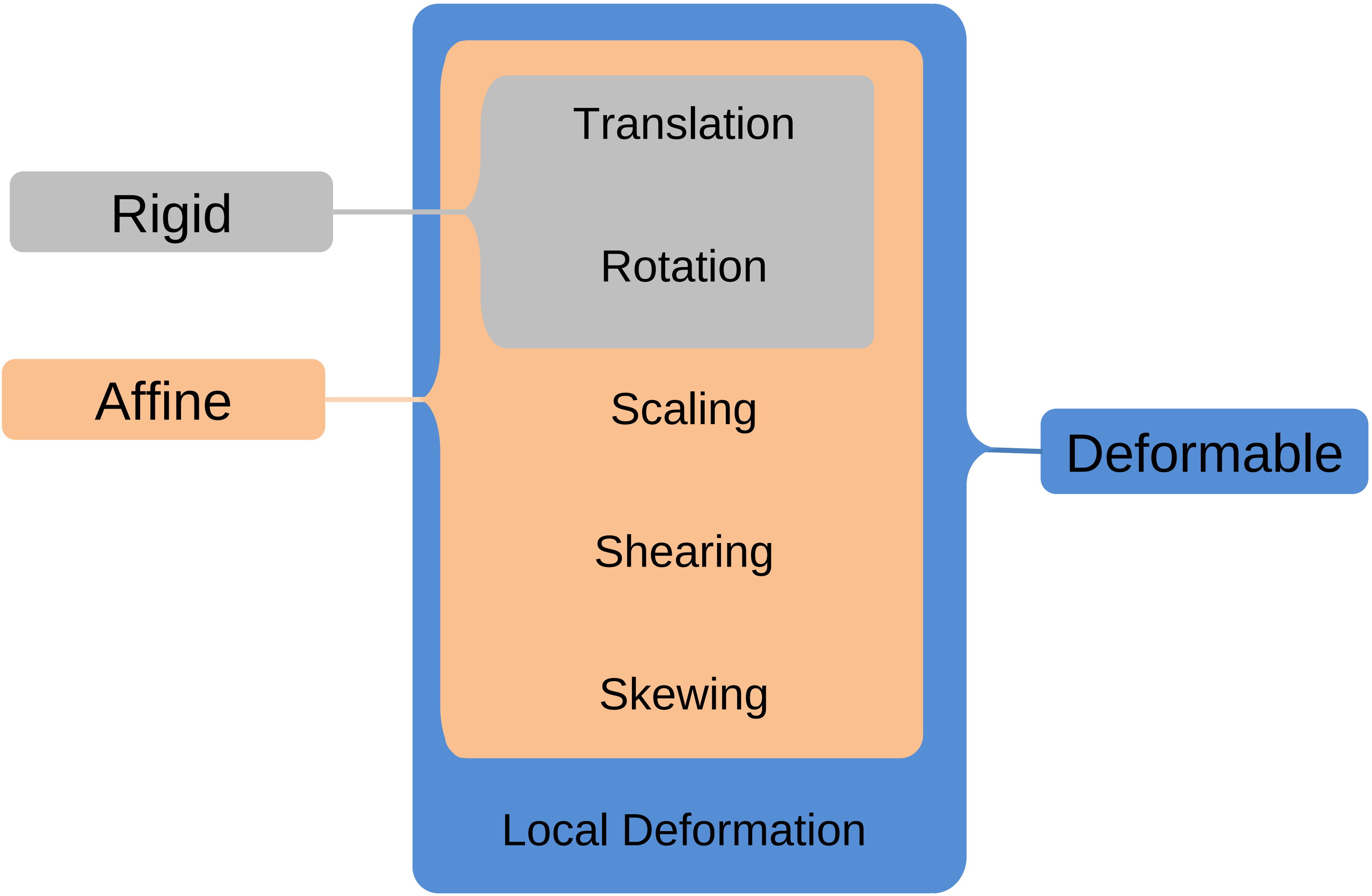
Medical Image Registration

- A process in medical imaging where multiple images from different modalities (X-ray, MRI, *etc*.) or **time points** are aligned
- Enables better understandings of the spatial relationships between structures or temporal changes over time.



Medical Image Registration

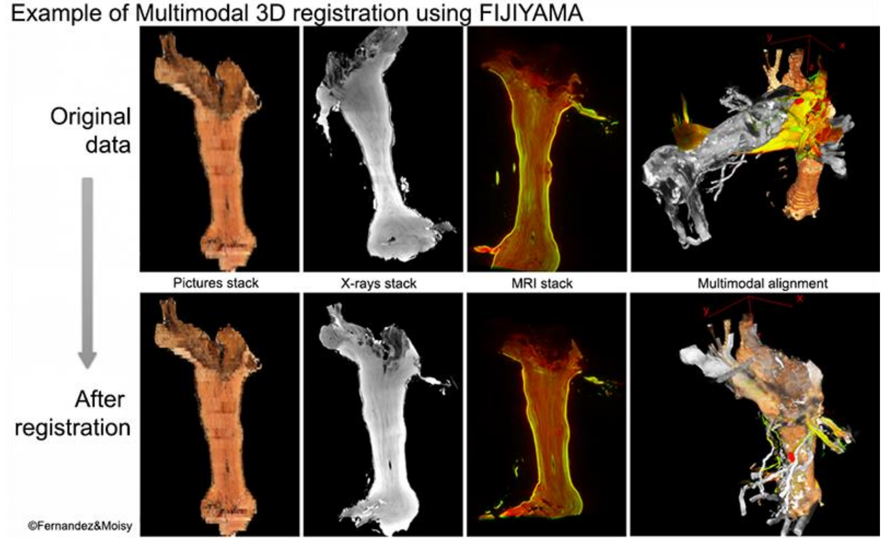


Registration Tool

- *Rigid registration* - Required when the two images, volumes, or surfaces differ by a global shift and a global rotation

MATLAB
(Medical Imaging Toolbox)

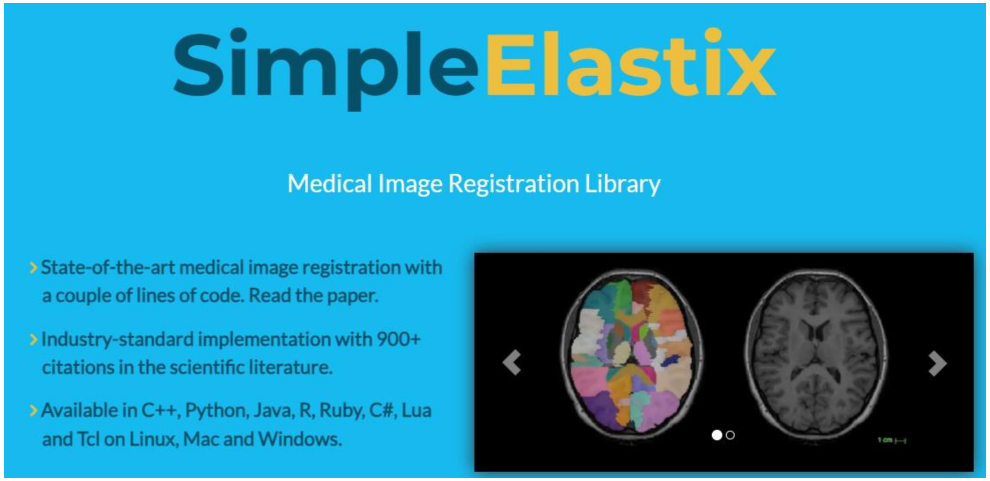
Example of Multimodal 3D registration using FIJIYAMA



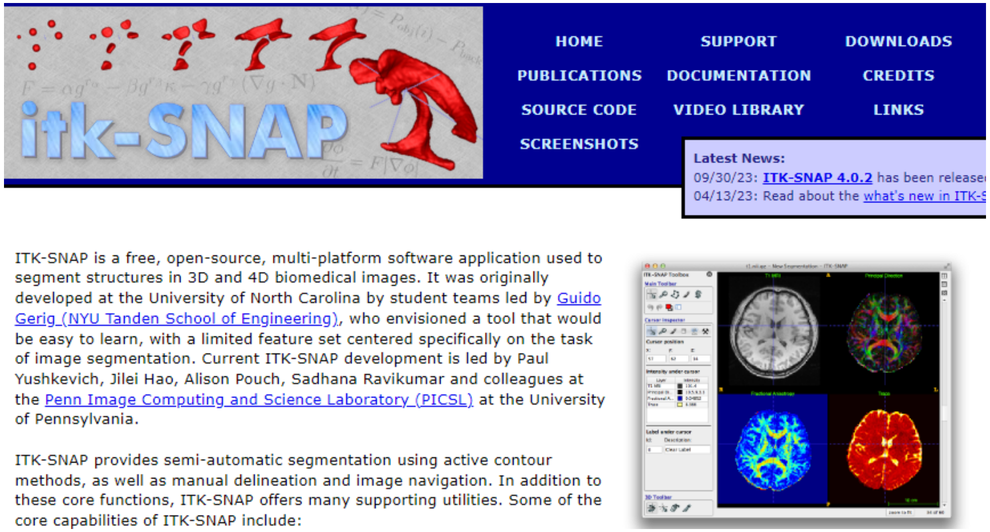
FiJi plugins

Registration Tool

SimpleElastix
(SimpleITK extension)

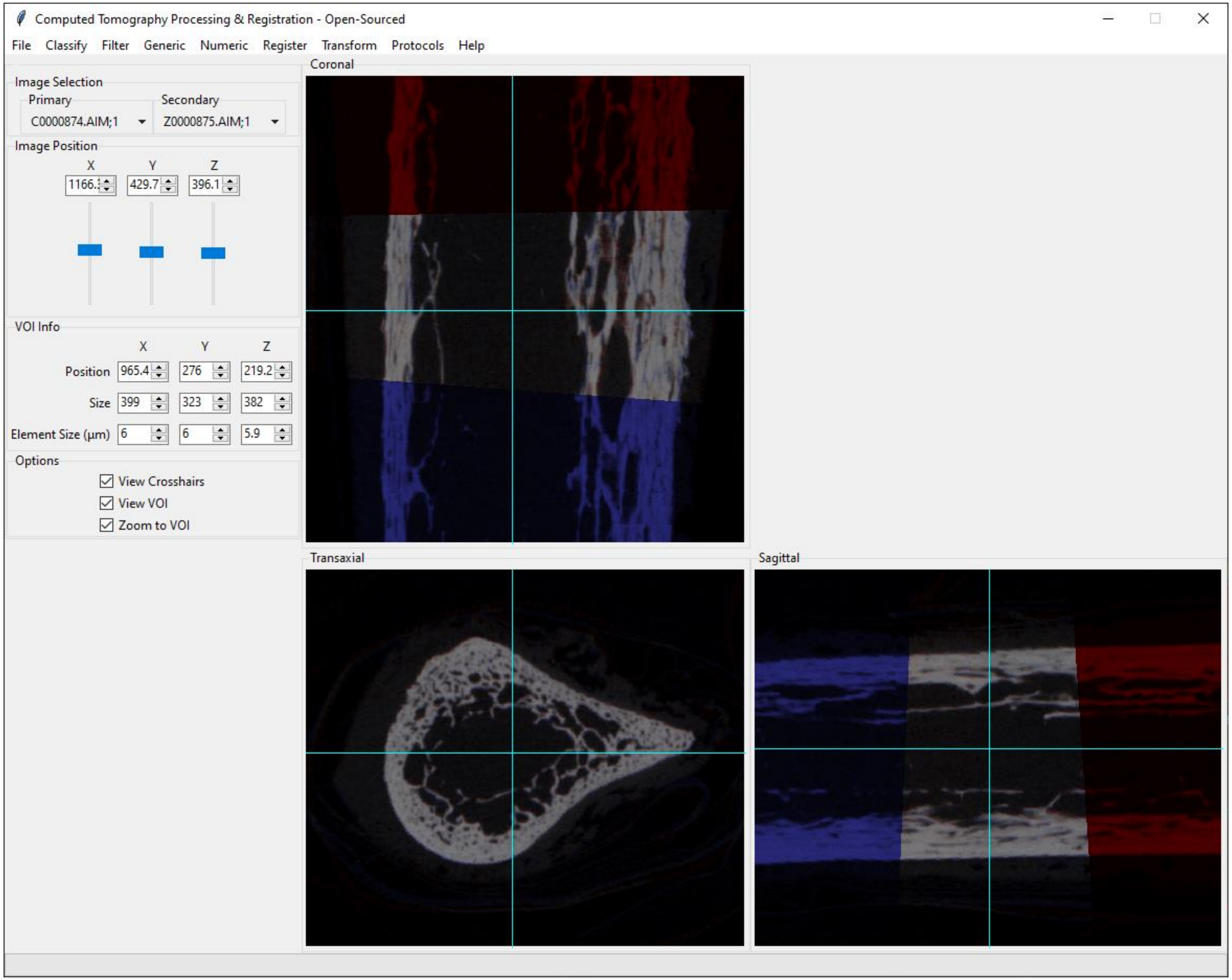


ITK-SNAP



Computed Tomography: Processing, Registration, Open Sourced

CTPros



Carlos Osuna

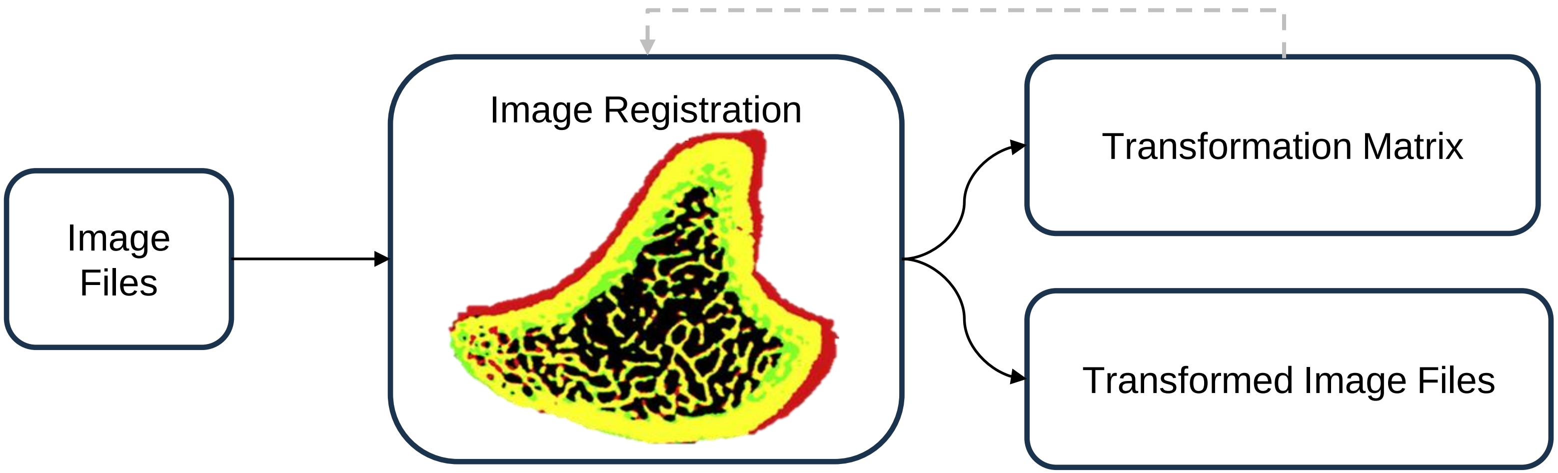
Input and Output

- Image files:

  *.aim, *.dcm, *.tif, *.jpg, *.rsq, …

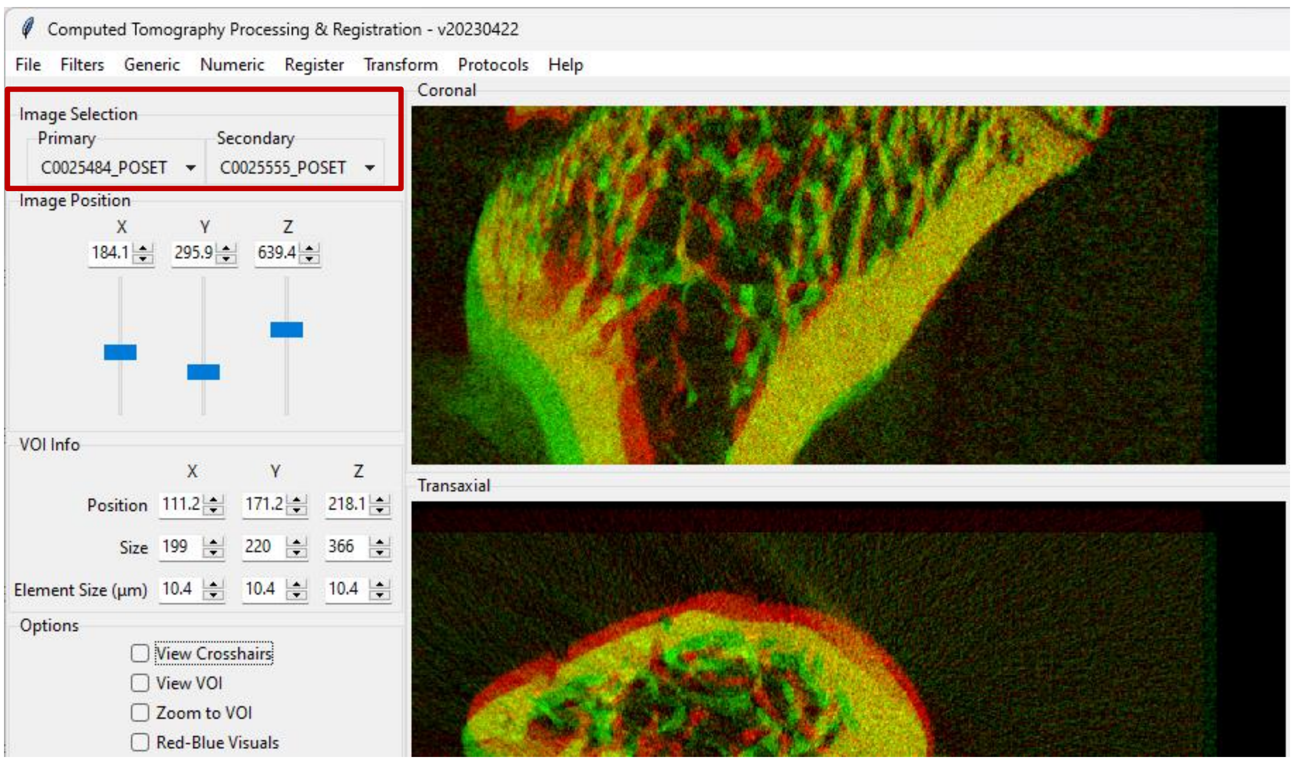
- Transformation matrices :

  *.tfm (binary), *.tfmtxt



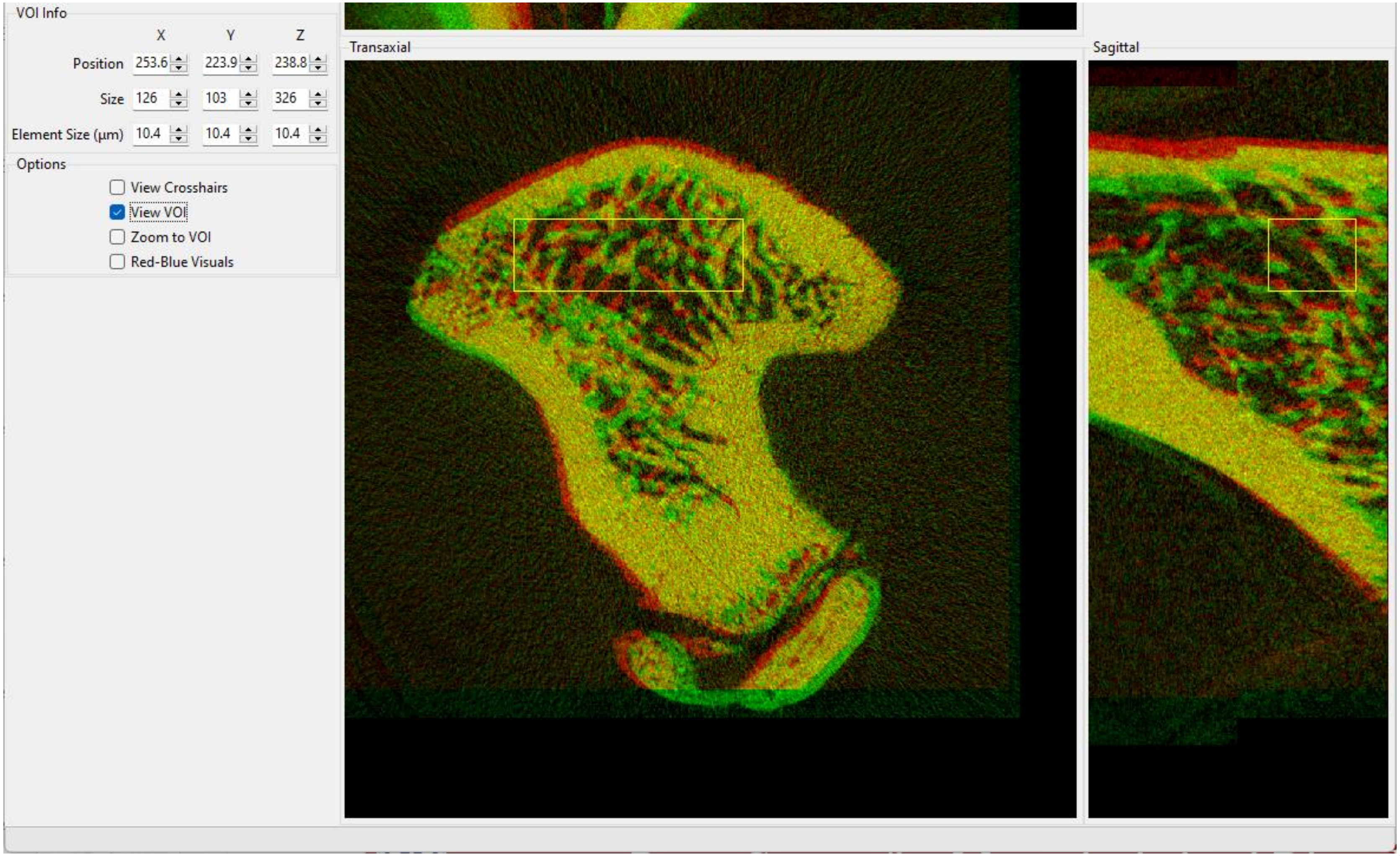
Registration Workflow

- Load images
  - Primary image (moving image)
  - Secondary image (fixed image)



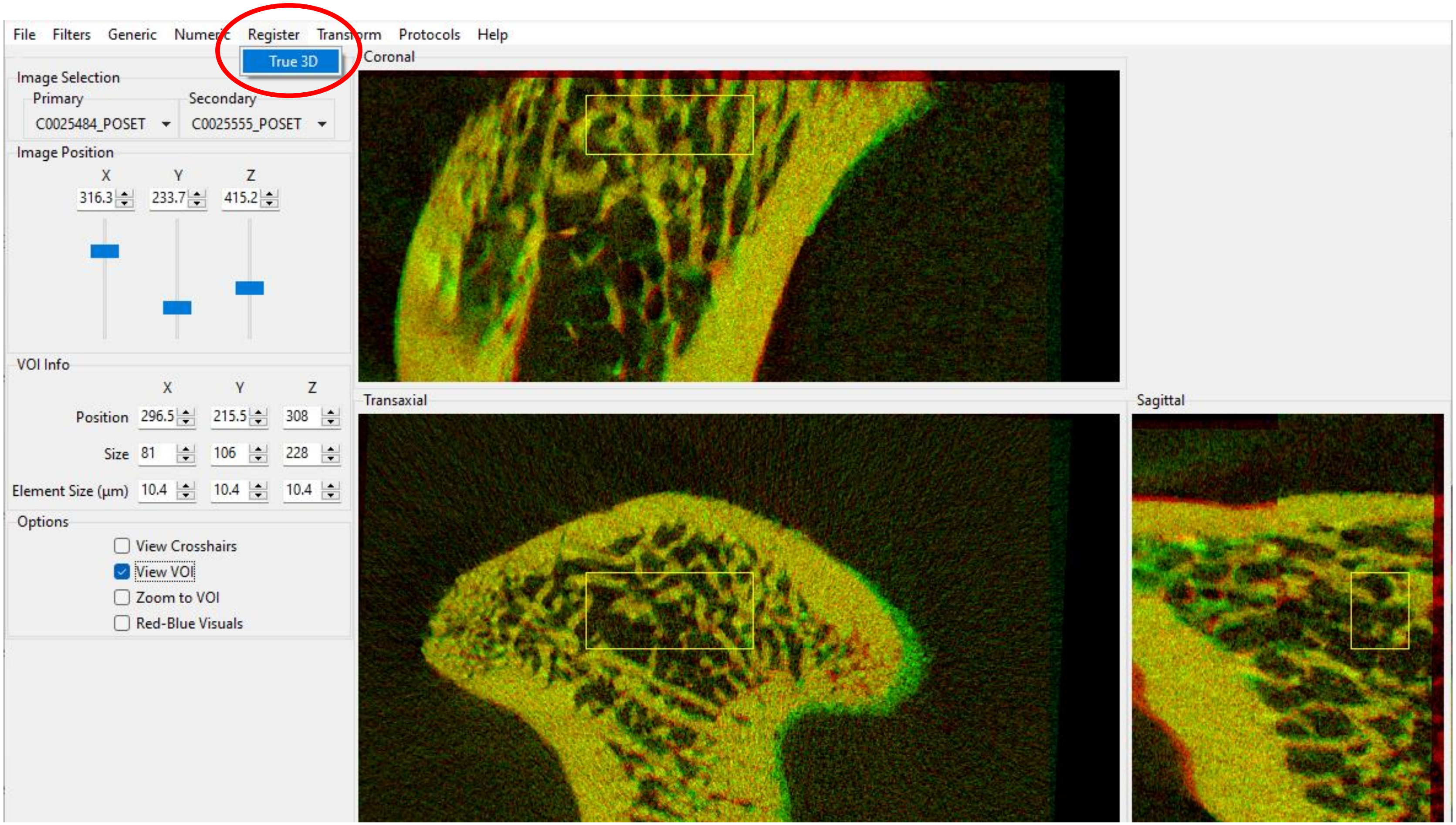
Registration Workflow

- Manual alignment and VOI selection



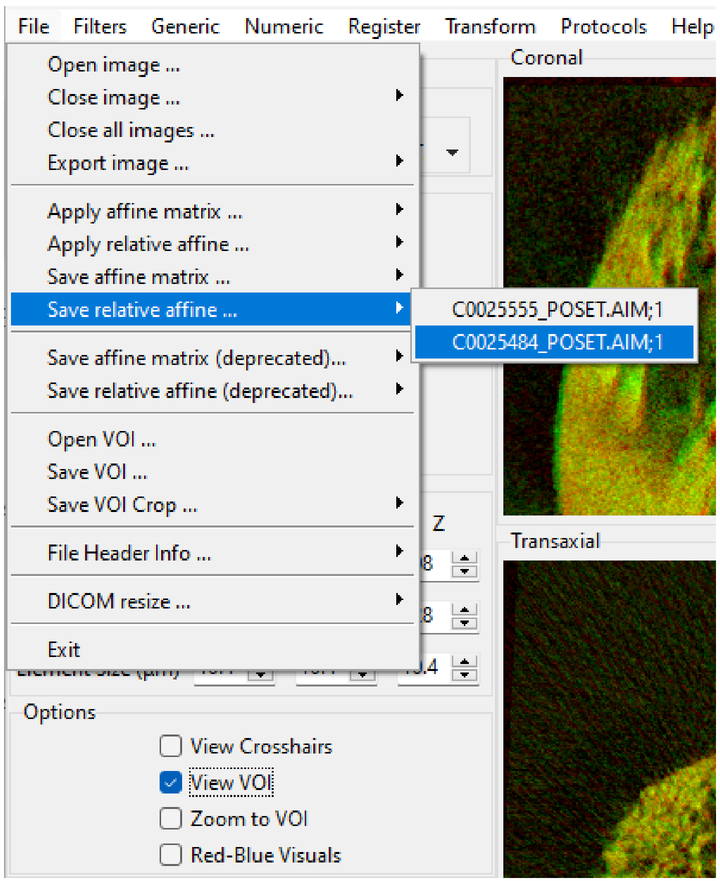
Registration Workflow

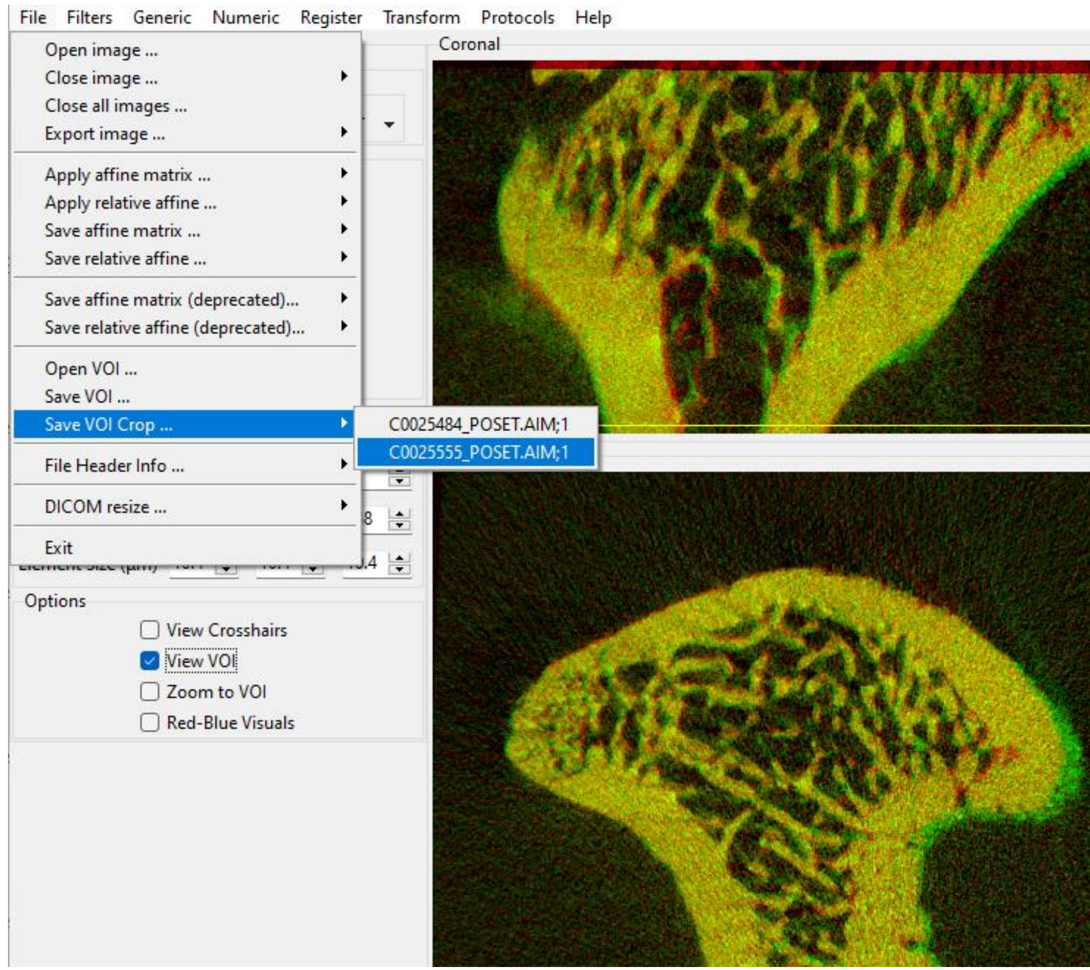
- Perform (rigid) image registration



Registration Workflow

- Output transformation matrix and registered moving image





Future Development

- Future development:
  - Manual contour/segmentation
  - Numerical analysis/evaluation
  - VOI identification / Automatic segmentation
  - Other…