

# Bone segmentation workflow for murine hindpaw

Automatic recipe and description of interactive steps in Amira Software

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- Fruit of discussions between the Thermo Scientific Amira Software team and H. Mark Kenney and Ronald W. Wood from the University of Rochester School of Medicine and Dentistry, this Xtra seeks to facilitate adoption of a workflow likely to enhance translational research programs.
- It is based on the following original publication and its supplementary material:

H. Mark Kenney, Yue Peng, Kiana L. Chen, Raquel Ajalik, Lindsay Schnur, Ronald W. Wood, Edward M. Schwarz, Hani A. Awad, A high-throughput semi-automated bone segmentation workflow for murine hindpaw micro-CT datasets, Bone Reports, Vol. 16, 2022.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8816671/> (<https://doi.org/10.1016/j.bonr.2022.101167>)

- The datasets distributed within this Xtra are courtesy of the authors of the publication, for the educational purpose presented here.
  - Publications based on the ideas presented in this Xtra must cite the above-mentioned reference.
  - Please refer to the corresponding author for more information about usage.

# Overview of the workflow and proposed recipe

**1**

Selection of a Region of Interest (Optional)

**2**

Application of the automatic Recipe, to obtain:

- Binary Segmentation of all bones
- Automatic Markers for each individual bone
- Automatic watershed segmentation of individual bones

**3**

If necessary: Manual Edition of the Markers, to obtain one and only one per bone

**4**

Segmentation of Individual Bones

Project files are provided:

- 44R\_No Errors.hx: illustrates a case where the automatic recipe is sufficient to obtain an appropriate segmentation result
- 50L\_Corrections\_Applied.hx: illustrates a case where the manual correction of markers is required

# Step 1: Selection of a Region of Interest

Objective: remove bones that are not of interest from the image content

- Note: this step might be conducted together with Step 3
- Solution retained in the projects of this Xtra:
  - Create a binary mask of the region of interest:
    - Enter the (Classic) Segmentation Editor
    - Use the Magic Wand tool with 'All Slices', and masking from 2500 up to the maximum value to select and add all areas of interest in the material 'Inside'
    - Important: the original micro-CT data are calibrated, with pixel intensities representing Hounsfield Units\*.
  - Use the Mask module
    - Return to the Project View
    - Right-click on the image, create the 'Mask' module, and connect



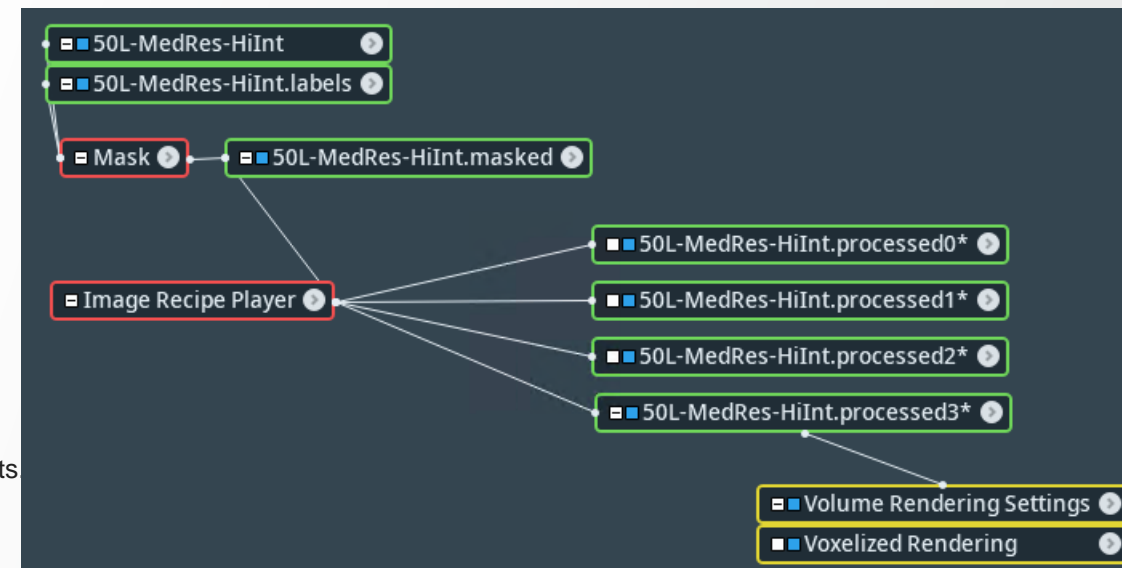
- Alternative solution: use Volume Edit to mask out areas that are not of interest
  - See supplementary videos S1 or S5 from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8816671/> <https://doi.org/10.1016/j.bonr.2022.101167>
  - or [Amira-Avizo How To | Use Volume Edit](#)

\* If you wish to apply the procedure on your own data, and if it is not calibrated, you may try using the 'Match Contrast' module first.

## Step 2: Application of the Recipe

Objective: obtain Bone Mask, Bone Markers, and Segmentation of individual bones

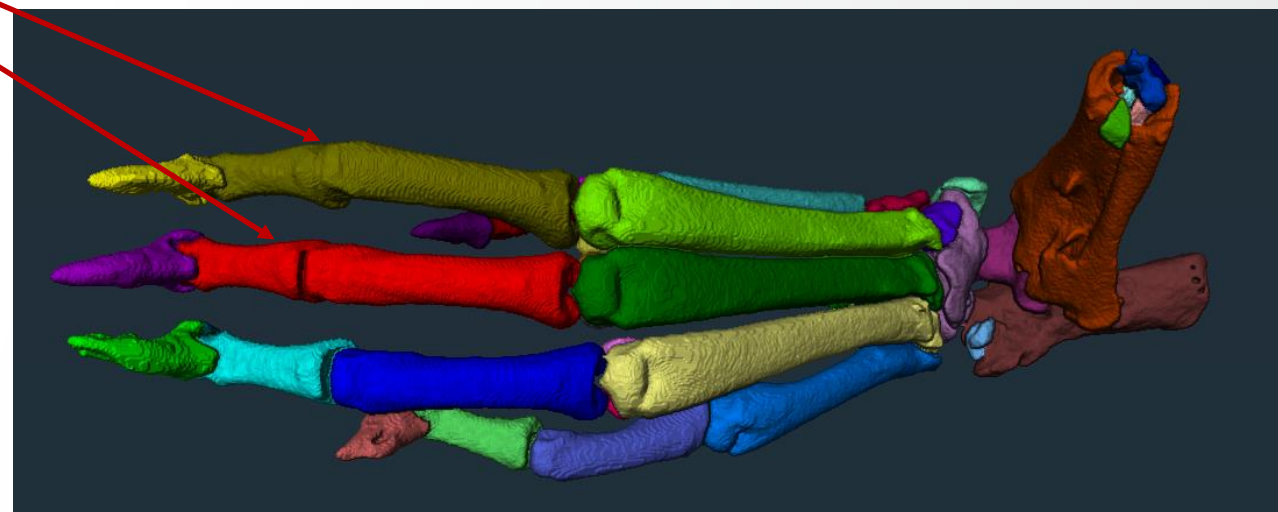
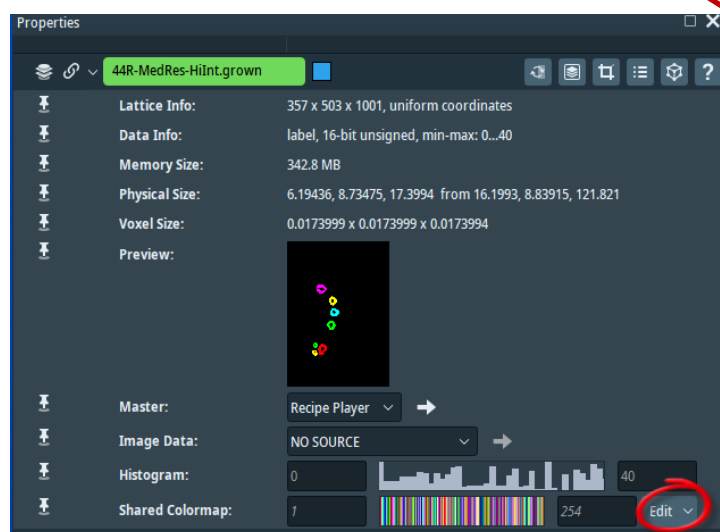
- Application of the automatic Recipe
  - Create the 'Image Recipe Player' module, and load the recipe *local\share\recipes\HindPawBoneSegmentation\_Recipe.hxisp*
  - Apply
- Recipe:
  - Input: Masked Image from Step 1
    - Important: the original micro-CT data are calibrated, with pixel intensities representing Hounsfield Units
  - 4 Outputs:
    - processed0: Filtered Image
    - processed1: Binary Bone Mask
    - processed2: Bone Markers
    - processed3: Segmented Bones
- The contents of the recipe can be observed, and modified by loading it in the 'Image Recipe Designer' workroom.
  - The default preview uses only 3 slices of the data. This may need to be increased to have more sensible outputs.
  - Please refer to the documentation of modules and to the tutorial about Image Recipes



## Step 2: Application of the Recipe

Objective: obtain Bone Mask, Bone Markers, and Segmentation of individual bones

- Visual Control of the Segmented Bones
  - Use Voxelized Rendering on the Segmented Bones (processed3).
  - It is recommended to set the Shared Colormap of the .grown to 'labels256' to reduce chances that 2 adjacent bones receive the same color
  - Under-splitting: 2 adjacent bones are displayed with the same color. Over-splitting: 1 bone appears with multiple fragments of different colors.



Result of the automatic recipe on the example dataset 50L, showing under-splitting issues



## Step 3: Manual Edition of the Markers

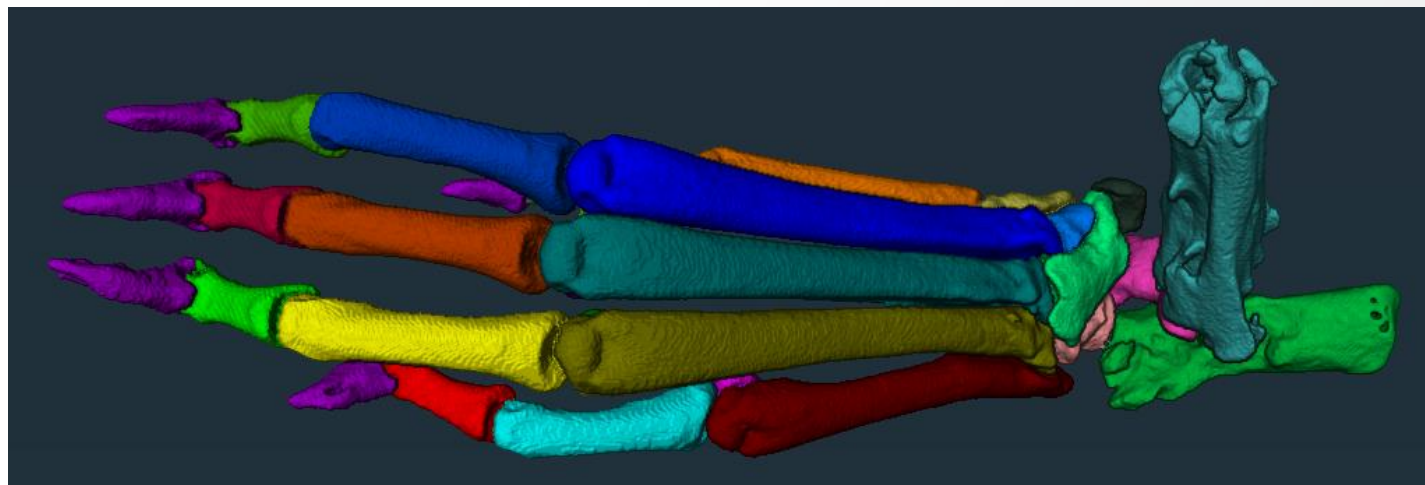
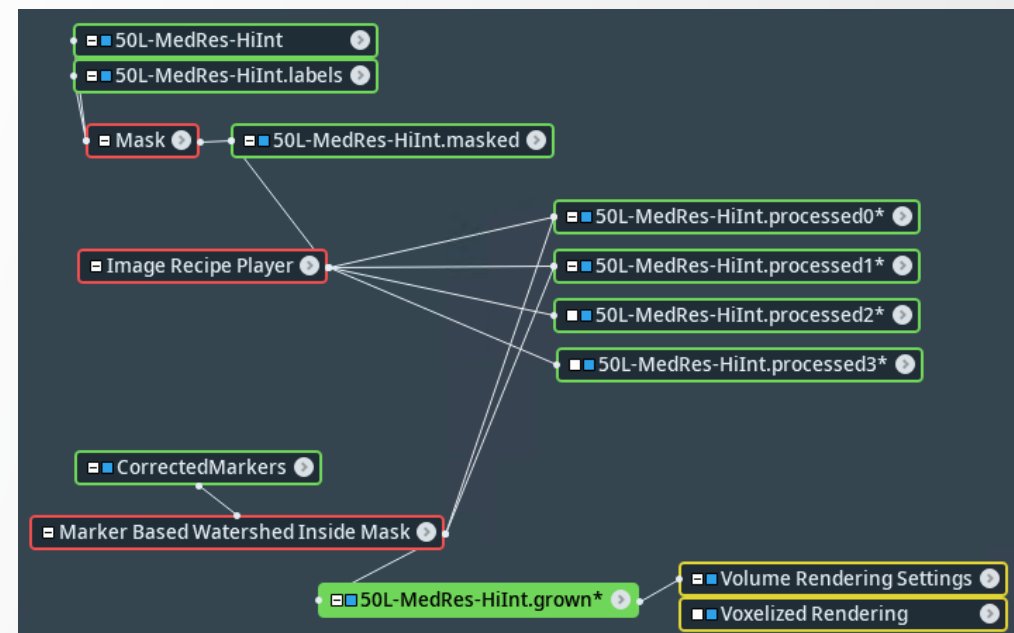
Objective: obtain one and only one marker per bone

- This step is very interactive, and best understood by following the video S3 proposed as supplementary material, from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8816671/> (<https://doi.org/10.1016/j.bonr.2022.101167>)
- Select the Bone Markers (processed2), press Ctrl+D to duplicate it, and rename the copy 'Corrected Markers'
- Enter the Segmentation Editor (Classic) and edit the Corrected Markers
  - For over-split bones:
    - Renaming a material with a name that already exists will propose to merge the 2 pieces of segmentation
    - Renaming all materials into appropriate bone names shall fix most of over-split issues
  - For under-split bones:
    - Create new materials with appropriate names for the different bones.
    - Use the brush or magic wand with 'same material only' option to select subparts, and Add it to the appropriate bones.
    - Once done, delete the initial material.

## Step 4: Segmenting Individual Bones

Objective: obtain the final segmentation

- Return to the Project View
- Reconstruct the bone segmentation:
  - Right-click on the Filtered Image (processed0) obtained at Step 2
  - Create the module 'Marker Based Watershed Inside Mask'
  - Connect the Markers to the manually Corrected Markers, obtained at Step 3
  - Connect the Binary Mask to the Bone Mask (processed1) obtained at Step 2
  - Leave the 'Split Type' to Low Intensity
  - Apply
- If necessary, continue manual corrections on the CorrectedMarkers dataset, and re-apply 'Marker Based Watershed Inside Mask' until the result is satisfying.





# Thank you

