Instructions for the exercise session

"Extract structural parameters from real data using ImageJ"

ImageJ (or Fiji which is "just imageJ") is a powerful image analysis program that was created at the National Institutes of Health. ImageJ is an open-source software widely used in the biological community to extract information from images. It is in the public domain, runs on a variety of operating systems and is updated frequently.

The idea of the present exercise session is to give you a brief introduction on the software, with some examples of what can be done with 3D tomographic images of bone tissue.

This has not the pretention whatsoever to be exhaustive, and the software presents huge possibilities, so I encourage you to dig in and try out! The community is also quite active in creating new plugins (addition a specific codes/methods) as well as describing online the available features.

Aims

- To get hands-on/discover software ImageJ, open-source software for image analysis
- Compare x-ray tomography to neutron tomography on bone specimens by extracting some structural parameters

Literature & useful documentation

Doc 1: Basics of ImageJ (found at https://imagej.nih.gov/ij/docs/pdfs/ImageJ.pdf

Internet has a lot of help for you too, look for example at the online tutorial:
http://www.icmr.ucsb.edu/programs/3DWorkshop/Uchic-2015_FIJI_Tutorial.pdf

Preparation

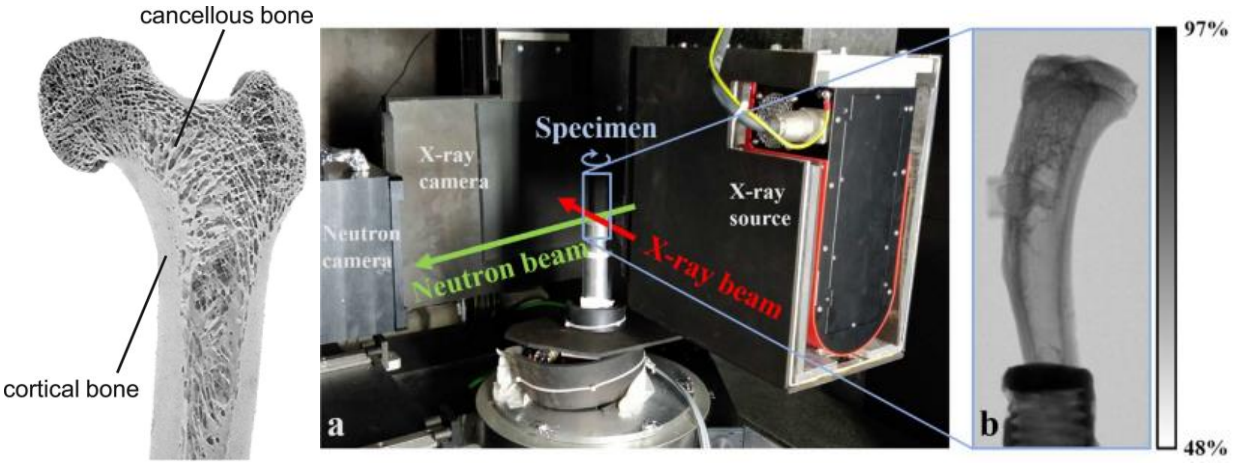
- If not done, install ImageJ from https://imagej.net/software/fiji/downloads (it's better to install Fiji which is a distribution of ImageJ including many useful plugins)
- Download input data: 2 tiff stacks of images, Boneplug_neutron.tiff and Boneplug_xrays.tiff
- Some basic definitions:
    - Stack: compilation of 2D images which form a 3D stack/image volume
    - Plugin: addition of codes with specific function

Some context of the input data

Bone tissue can be separated into two types: cortical and cancellous bone. The first one is dense, on the outside of the long bone, acting like a protective shell. The latter is more porous, present inside the bones, and has a specific micro-architecture optimised to compromise weight/energy needed for locomotion and mechanical strength.

The specimen you are analysing is a cancellous bone plug, extracted from the femoral heal of a bovine hip bone.

They have been imaged at ILL (Grenoble, France) at the D50 instrument NeXT-Grenoble, which enables to image specimens both with neutron and x-rays microtomography, without moving the specimens.



The plugs were extracted from the femoral head (left) and imaged with both neutron and x-rays tomography at Next-Grenoble (right, here presented for another specimen)

Go!

1. Basic manipulation of 3D data set

Let us start with the x-ray data. Open the image stack (Boneplug_xrays.tiff).

Wander around in the menu or use the document 1_basics_ImageJ.pdf

Play around with the mouse: scroll with the middle button, ctrl+scroll to zoom, etc

1: Z length = number of slices / 2D images

2: 2D image (slice) size (X*Y) in pixel

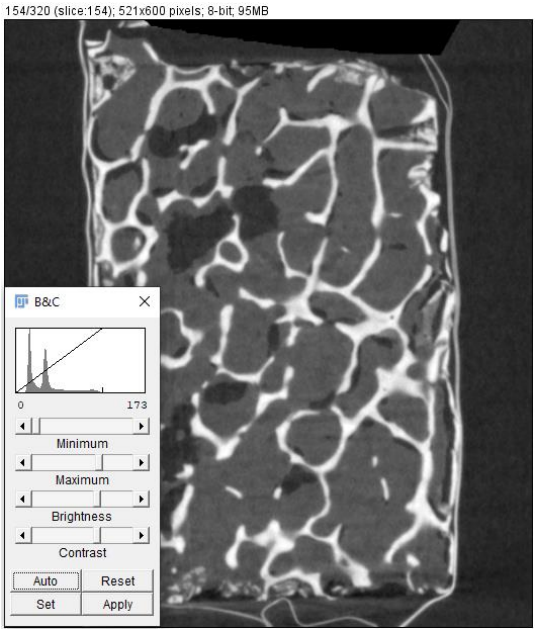
3: type of images, here 8-bit it means grey value of the pixels range from 0 to 255 *(FYI: 16-bit images have more information, with a grey level range from 0 to 65535)*

4: size of the image stack

5: scroll bar so you can navigate among slices of the specimen

To visualize the 3D stack, you can use the menu *Image->Stack->Orthogonal views*.

We do not see well the bone -> try to **adjust the contrast** (auto and apply)



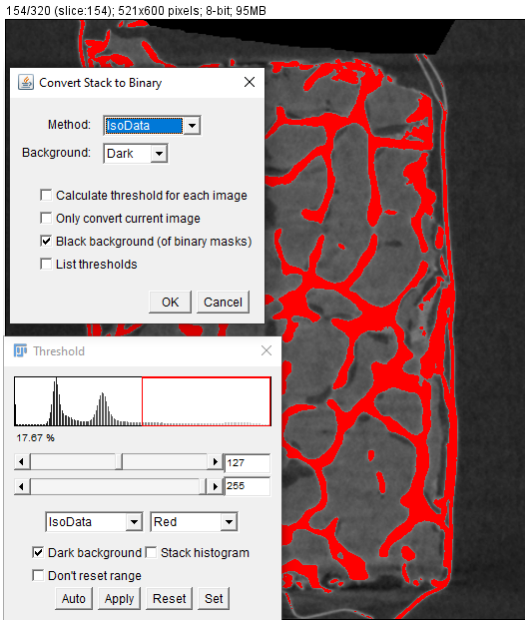
Duplicate (menu Image) the slice 184 and save as a tiff file (such as *bone_plug_xrays_184.tiff*), we will come back to it.

2. Isolate the bone structure

We are interested in the bone structure, appearing here in bright grey pixels. To isolate it, we will convert the image to binary (e.g. black and white) via **thresholding**.

Refer to document 1_basics_ImageJ.pdf for information. Various threshold techniques exist, we will here use a basic one: IsoData. Tick “Dark bacground”, “Apply” and untick “calculate threshold for each image”.

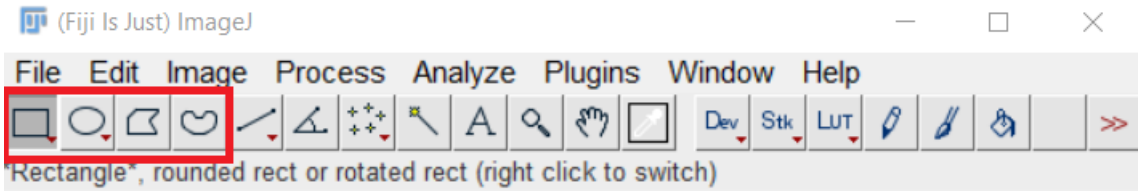
Once segmented we have a binarized image => check that bone has a pixel value of 255 (white) and the background 0 (black).



Duplicate the slice 184 and save it as a tiff file (such as *bone_plug_xrays_184_segm.tiff*), we will come back to it.

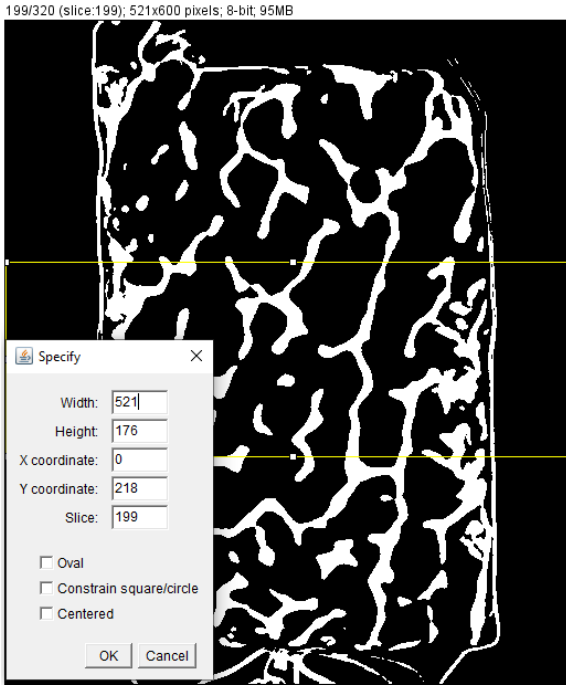
To further investigate the structure, we will focus on a clean portion of the plugs -> i.e. we are going to **crop the images**, to keep only a cube, within the plug.

For this you will need the drawing tools and the cropping function (menu *Image*)

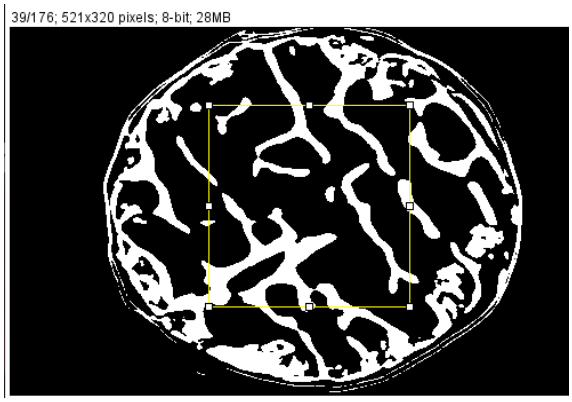


First, draw a rectangle in the middle portion. With the menu *Edit->Selection->Specify*, you can specify position and size. Take a third of the height (write it down because we want a cube at the end), and place it in the middle of the plug.

Once your drawn region (yellow) is well placed, **Crop** the image.



Reslice the stack from the top (*Image->Stack->reslice*) – we now visualize the plug "from the top".

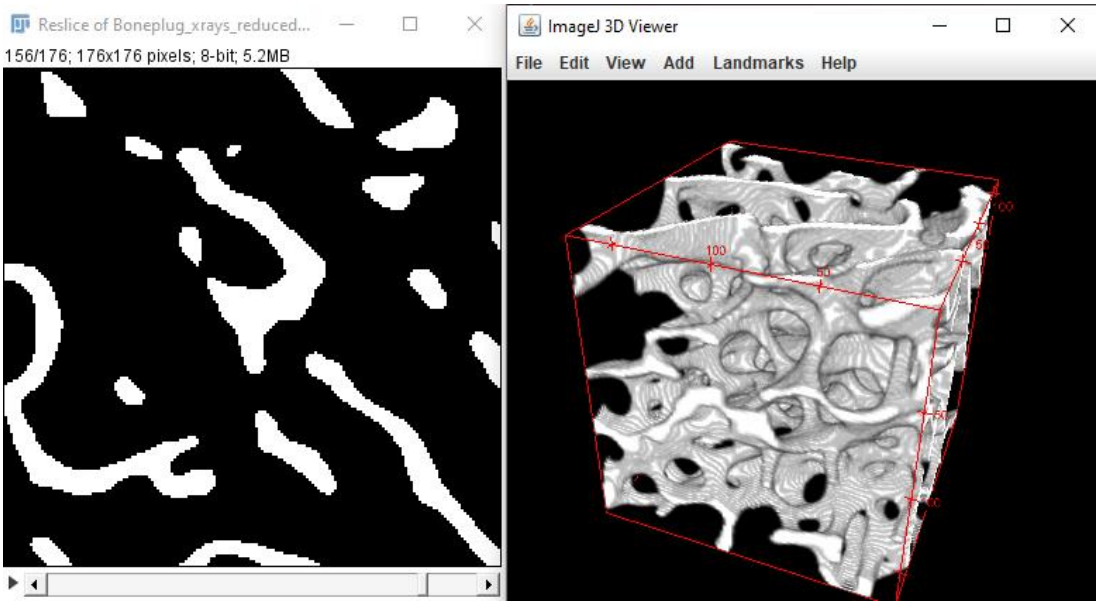


Draw then crop a square inside the plug, of side the same length as your previous height *(if you hadn't written it down, try looking at the top info of your previously cropped stack, the info you need is there…)*. This is to avoid the bone debris which you can see at the edges of the plug, coming from the extraction of the plugs.

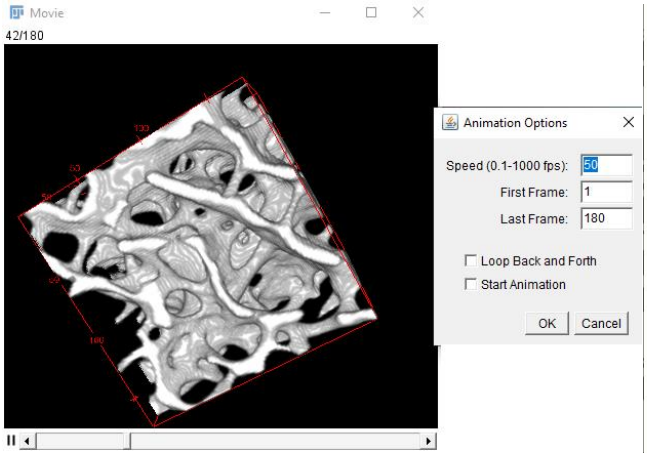
→ You have now a cube of cancellous bone!

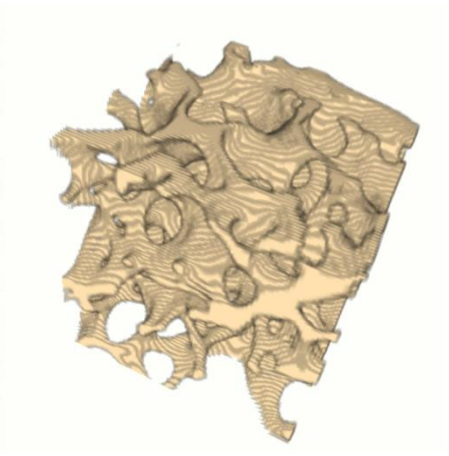
3. Visualize the portion of bone isolated

3D visualization -> use the plugin 3D Viewer. In the popup window, decide which "Image" to visualize.



Try to create a 360° rotation movie. Adapt the rotation speed with right clicking on play/pause. You can save your video as AVI. Play around, lots of options exist: change axis of rotation, change background colour, change bone colour…





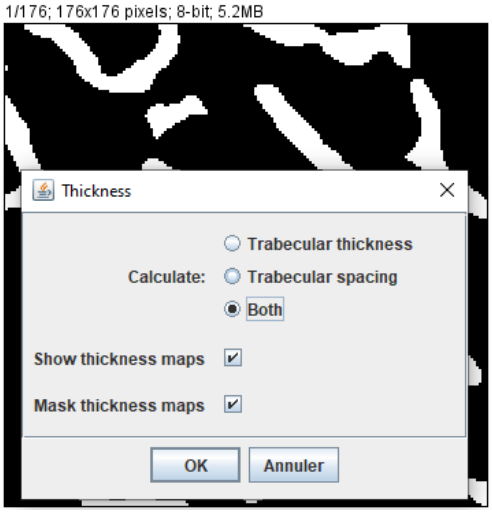
4. Measure structural parameters

You need to install the plugin (BoneJ) *https://imagej.net/plugins/bonej*

You may need to restart Fiji, before doing so, save your cube of segmented bone as Tiff: e.g. "*Boneplug_neutron_cropped.tiff*"

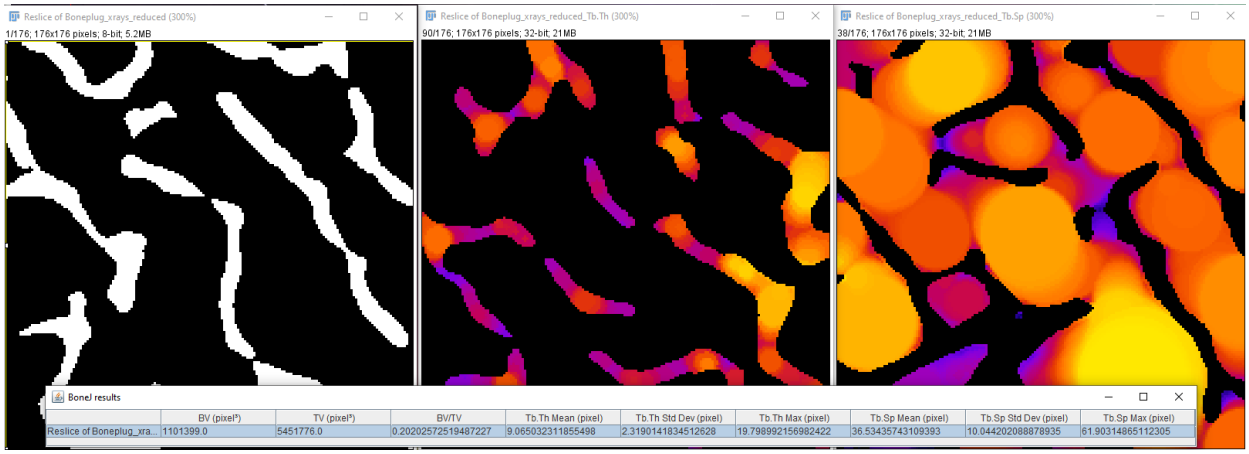
Calculate the bone volume fraction, usually called BV/TV (bone volume over Total volume). This gives an estimation of the density/bone content.

You should have values around 0.2 which means 20% of the volume is bone. What do you think is the rest?



Play around with the plugin, especially to extract maps of Trabecular thickness (the thickness of the "bone trabeculae", the structures you observe), and trabecular spacing (the distance in between trabeculae).

This information is commonly used to characterise bone, it is for example known that with aging, the trabeculae get thinner, hence weakening the whole bone structure.



5. Comparison with neutron data

Open the neutron stack.

We can note some differences already: the images look different but it's the same specimen!

Duplicate the slice 146 and save as a tiff file (such as bone_plug_neutron_146).

You should have two slices of the X-ray data saved (184 original and 184 segmented). The idea here is better grasp visually the differences, by **overlapping the images**.

Close all but those three slices: 146 original neutron, 184 original X-ray and 184 segmented xrays.

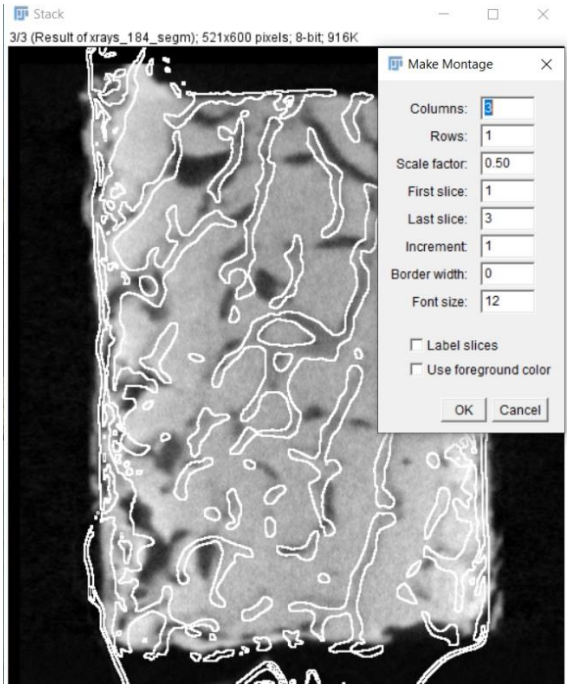
Using the segmented x-rays, **find edges** (process).

The images are not yet perfectly aligned, translate the neutron slice (*Image->Transform*) by +10 pixels on X.

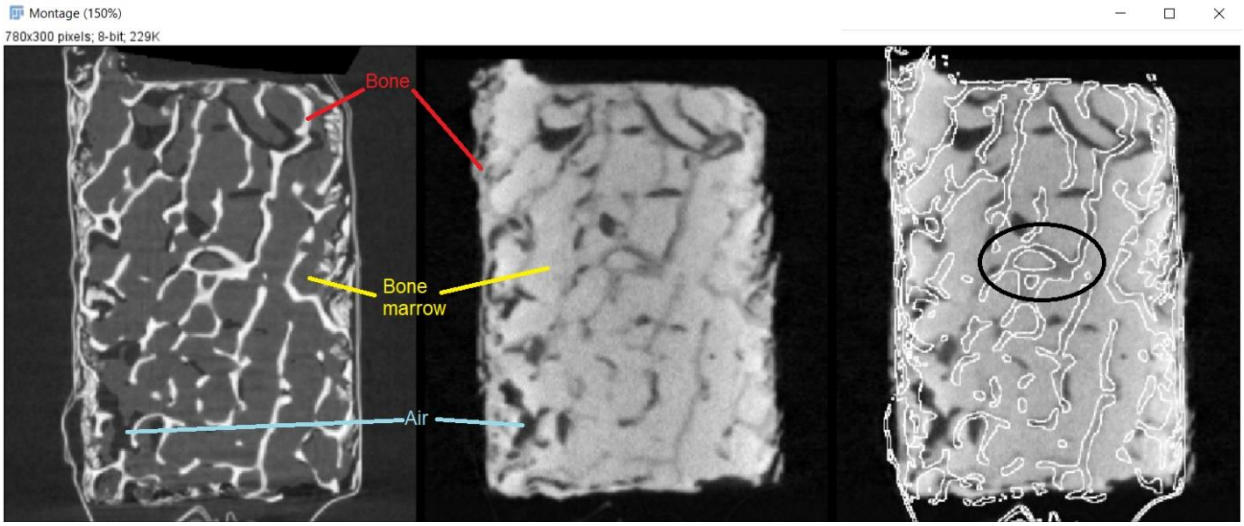
Using the **Image Calculator** (menu *Process*), overlap (add) the edges and the neutron slices (create new window).

Close the edge-image, you should have three images left: original x-rays, original (translated) neutron and the overlapped one. Convert the image to a stack (*Image->Stack->Image to stack*) and make a **Montage** *(Image->Stack->Make montage)* to print the three images one tot the other.

What can you say about the "rest" of the bone?



6. Conclusions and some explanations



Example of the montage to obtain. Left: x-rays, centre: neutron and right the overlap.

The images are not yet perfectly aligned but in the black circled zone, you can see the relatively good overlap and grasp the differences in absorption between the two techniques.

We can see three phases in our bone plug: the bone, the bone marrow and air. The bone marrow, located within the pores of the bone, is a fatty substance which produces bone marrow stem cells and later blood cells. We can see here that the bone marrow disturbs the contrast in the neutron images (because it highly absorbs the neutrons) and makes the analysis of the bone structure tricky. Thus, it is important to work on preservation techniques and/or contrast agent to enhance contrast in such specimens.

However, bone marrow was not as clearly visible in the x-rays data, and has grey level close to the air, so the use of neutron imaging here opens the door to more extensive analysis and investigation of the bone tissue growth and remodelling.

Congrats if you reached the end, and I hope you enjoyed the tutorial!

Do not hesitate to give me feedback at sophie.le-cann@u-pec.fr