Bone class script

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March 2022

1 Description

The following scripts has been designed for semi-automatic quantification of bone states in response to implantation. The states covered by the script are; degrading, resting, developing and NA, where NA is an option for undefinable bone segments. Bone states are to be quantified in a radius of 3mm around the implant, posterior and anterior, each divided into 3 sub areas, of 1, 2 and 3mm radius. Additionally a grid is placed on the entire image. Quantification is performed by placing points at grid-bone intersections, matching the above mentioned bone states. The script will automatically record the position of the points, as well as the class by the selected point colour, thus, letting the researcher focus entirely on evaluating the bone state.

Maybe insert image here?

2 Installation and setup

The scripts are written in the ImageJ Macro language(ijm), for ImageJ/FIJI version 1.53o. First of all, either install or update ImageJ/FIJI to this version. The script should also work on versions released in the future, but if any problems arise, downgrade FIJI to this version. FIJI can be downloaded here: `https://imagej.net/software/fiji/downloads`. Follow the setup, and start FIJI. Next go to `https://github.com/jtranbergjensen/BoneClassScript`, and download the two files *BoneClassScript* and *BoneClassMacros*. To download the files, press the green code button, and chose to download them as .zip. Unpackage them and they should now be available.

2.1 Install macros

- Open the macro script.
  - In FIJI → File → New → Script.
  - A scripting window will open.
  - In the scripting window → File → Open.
  - Find the *BoneClassMacros.ijm* and open it.
- Open the start up macro collection.
  - In the FIJI menu, go to Plugins → Macros → Startup Macros.
  - A script window will open with all of the preset startup macros. Make sure to not make any changes to the existing macros here.
  - Copy paste the contents of *BoneClassMacros.ijm* to the bottom of the start up macro script and save it.
  - Restart FIJI.

The hotkeys defined in *BoneClassMacros.ijm*, should now be operational.

2.2 Install script

- Find the folder Fiji.app
- In the subfolders go to scripts → Plugins
- Copy *BoneClassScript.ijm* to this folder
- Restart FIJI.

The BoneClassScript can now be initiated by navigating the FIJI task bar to the bottom of the Plugins menu.

3 How to start the data collection

To begin the semi-automatic quantification of the data set, the image data needs to be collected in the the same folder and an output folder needs to be created.

3.1 Setting up the data and starting the script

- Combine all the image data in one folder and create an empty folder. The empty folder is where all the data will be saved.
- Open FIJI and run the script from the plugins menu.
- A dialog box will open and ask for the location of the images to process, as well as a place to store the output. Select the recently created folders (Figure 1).
- Press OK

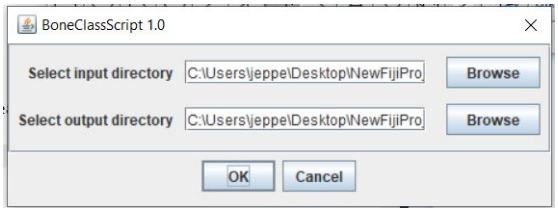

Figure 1: View of the input and output directory menu. Use the browse button to find the folders. Input is the folder containing the image data. Output is the newly created and empty folder.

3.2 Automatic centering and grid placement

- A black and white image will appear along with a dialog box instructing the user to make a small rectangle enclosing the implant (Figure 2).
- A series of segmentation steps will then be shown, after which the center of the implant has been defined.
- The same image will then appear with a grid and concentric circles afterwards.

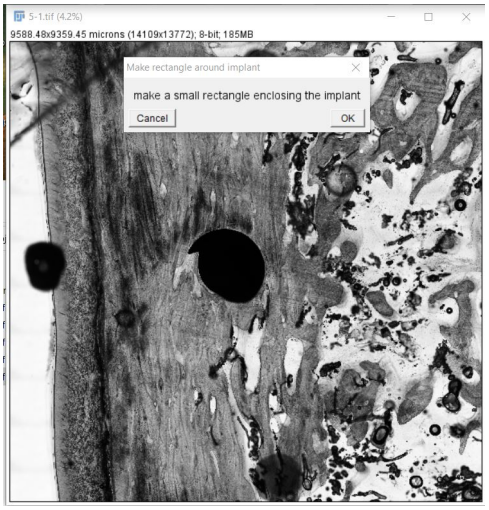

Figure 2: View of the centering step. After a rectangle has been made around the implant, press OK. The rectangle tool is automatically sett before this step.

3.3 Data collection

The semi automatic data collection will now begin:

- A series of dots can now be placed in grid-bone intersections (Figure 3). Use the keybord + and - to zoom and zoom out, respectively.
- After placing a series of dots, their collective class can now be changed by pressing the keyboard keys: *q, w, e, r.*
- Thus, only place dots of a single category, then change the bone class.
- Now press *m*, to log the measurements and continue with a new bone-class.
- The script will automatically note the location and match the dot colour with the designated bone-class.
- The measurements will automatically be saved.
- After the last classification has been made to the image, press OK. The next image in the data set will now open, and the above processes will begin

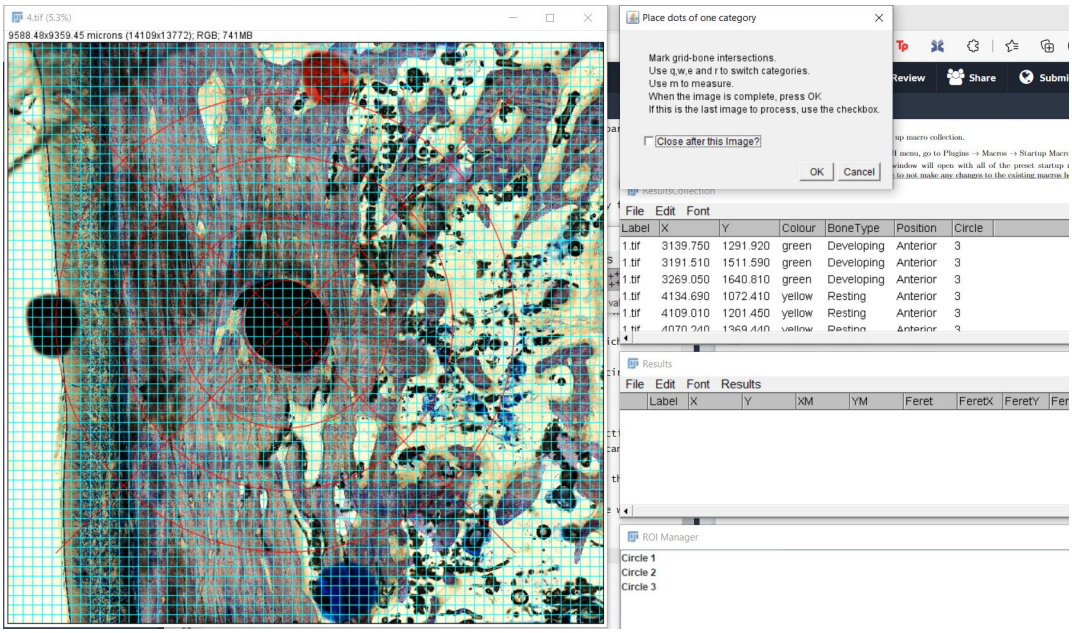

Figure 3: View of the data collection step. Place dots at grid-bone intersections and classify the dots collectively by pressing *q, w, e, r.* Press *m*, to log the measurements.

3.4 End session and output

- If the opened image is the last to process for this session, check the box "Close after this image", whereafter the macro will end, and the measurements saved.
- The macro logs the images it has already processed, thus, when resuming the data collection, the macro can just be run. If a mistake has happend or a re-analysis is needed, simply delete the image from the log file in the output folder. The file is called *FileLog*, and can be opened with Notepad.
- The processed images can also be found in the output folder, open them with FIJI to display the grids and dots.
- The results txt file can also be found in the output folder, it is called *ResultsCollection.txt.* It is a tab-delimited txt file, which can easily be pasted into excel or another statistics software such as R. Right click to select all the contents of the file and simply copy paste.

4 Additional specifications

- Grid area: 10.000 μm^2.
- Concentric circle size:
  - The center and area of the implant is approximated in the first step. These measurements are used to replicate the implant as a perfect circle, the inner circle shown in the window. From this inner circle, the 3 outer concentric circles are defined, but all with an increasing radius of 1, 2 and 3mm.
  - Because the outer circles are defined by the size of the implant, if the implant varies a lot in size, so will the measurement area.
  - The distance from the approximated implant to the outer circles, are however constant.
- Input format: The preferred input format is TIF. From OMERO, the data can be downloaded as tif, which is recommeneded. The meta data is not shown in these files, when loaded into FIJI, but the script will automatically retrieve it.
- Preferably the images should all be collected with anterior up, posterior down.