

Additional Supporting Information for: Overall bone structure as assessed by slice-by-slice profile.

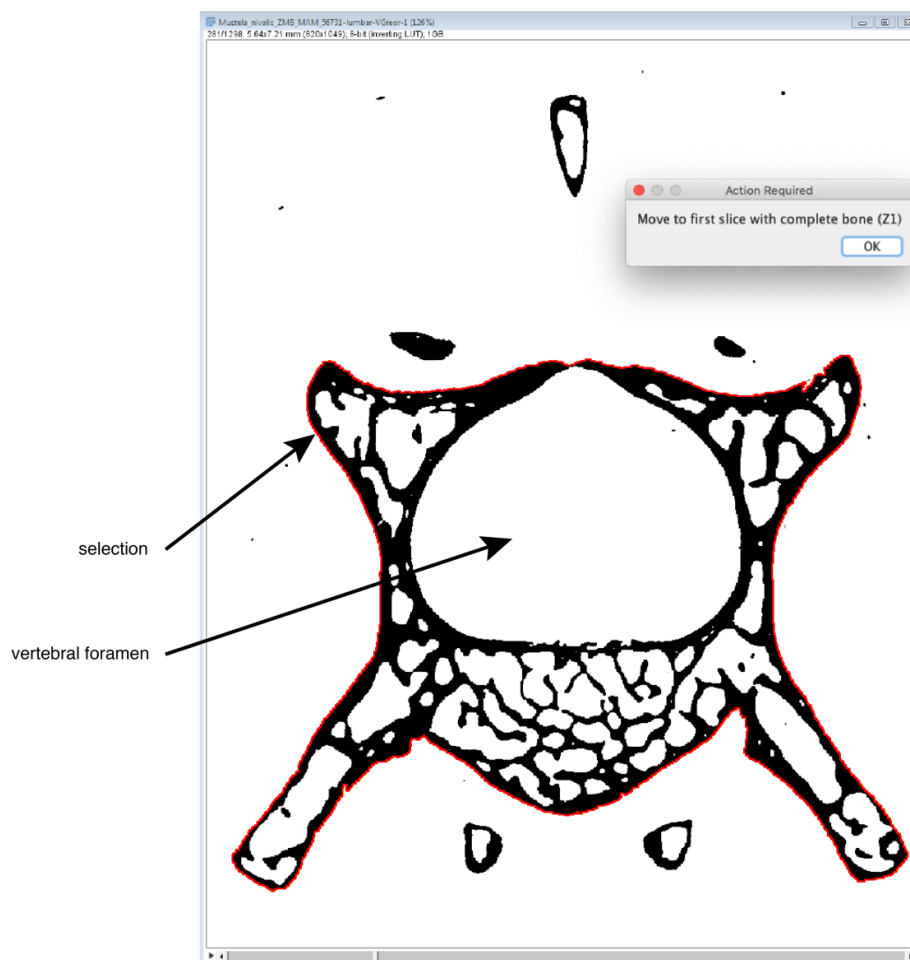
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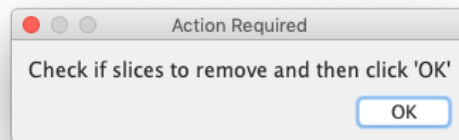
Methods S1. Workflow of the ImageJ macro

The macro itself is available at <https://github.com/eliason/sliceByslice>. The example provided here was performed with the mid-lumbar vertebra of *Mustela nivalis* (ZMB Mam 56731; downgraded file available at <https://figshare.com/account/projects/63800/articles/8143631>). The unit of length associated with the stack should either be mm or inch (the latter will be converted into mm).

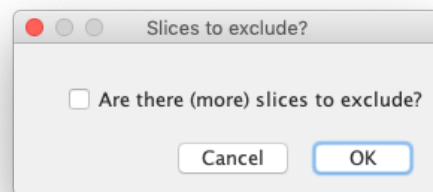
After having opened the stack and started to run the macro, first the whole stack is binarized (if the stack is not already binarized), and parameters are automatically acquired for all slices. Then, for the first manual operation, a prompt asks the user to define the first slice of interest (Z1). In this test case dealing with vertebrae, it corresponds to the anterior-most slice for which the vertebral canal is complete (see image below).



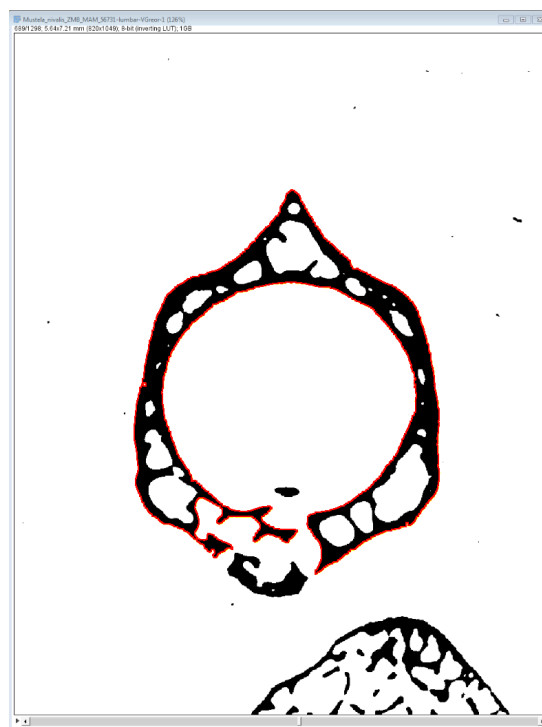
Once the user has moved to the desired slice and clicked ‘OK’, the second prompt invites the user to check whether slices need to be manually excluded. The user can then scroll along the stack in order to make such an assessment.



After clicking ‘OK’, the user is asked whether there are indeed slices to exclude, which can be specified by ticking the dedicated box.

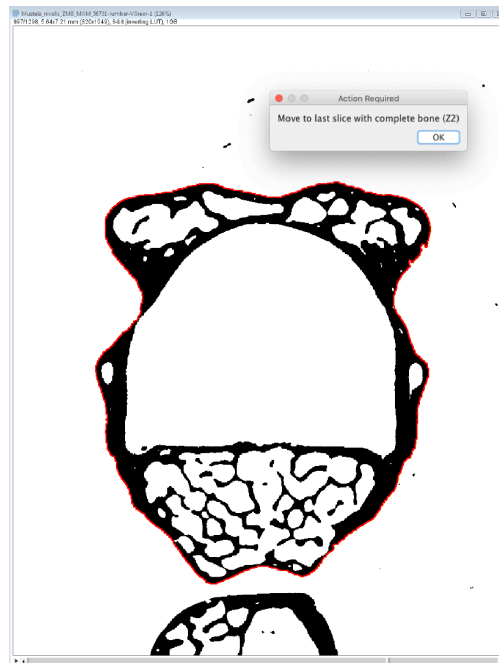


If there are indeed some slices that the user would like to manually exclude, then two subsequent prompts will invite the user to define the first and the last slices to exclude (as for Z1, moving to the desired image and clicking ‘OK’). For instance, in the case of vertebrae, it is very common for large vascular canals to connect the periosteal surface to the vertebral canal, which prevents accurate automatic selection of the whole cross-section. See the red selection line on the image below, which does not comprise the vertebral canal and part of the centrum.



If only one slice is to be excluded, then the “last slice to be excluded” should be set as the same one as the “first slice to be excluded.” The two last prompts are then repeated until the user

specifies that no more slices are to be excluded (clicking ‘OK’ without ticking the box for the previous prompt). With a last prompt, the user is finally asked to define the last slice of interest (Z2), which should be done in the same manner as for Z1.



As a result, the mean total cross-sectional area (Tt.Ar) and global compactness (Cg) are given in the Log window. The Results table can be saved for further analysis. The saved data table can be read for instance by the R software (see the accompanying R script, also available at <https://github.com/eliason/sliceByslice>).

Results			
File	Edit	Font	Results
	ResArea (mm)	ResC	ToRem
135	6.810	56.439	0
136	6.604	58.154	0
137	6.582	57.858	0
138	9.859	38.348	0
139	9.871	37.631	0
140	9.797	37.926	0
141	9.798	37.974	0
142	9.796	38.058	0
143	9.922	37.313	0
144	10.008	36.867	0
145	10.118	36.354	Z1
146	10.120	36.153	0
147	10.130	35.781	0
148	9.859	36.411	0
149	9.843	35.993	0
150	10.079	34.728	0
151	10.075	34.279	0
152	6.295	54.127	0
153	10.111	33.584	0
154	9.792	34.552	0
155	6.399	52.761	0
156	9.210	36.824	0
157	9.219	36.750	0
158	9.807	34.291	0
159	9.803	34.036	0
160	9.804	33.673	0
161	9.795	33.162	0
162	9.107	35.265	0