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1  macro "PAGIS_v09_i"{
2
3      //THANK YOU FOR DOWNLOADING THE THESIS ITERATON OF THE
4      //PHASE ANALYSIS OF GRAYSCALE IMAGES CODE, PAGIS
5      //VERSION: 09.i.
6      verNo = "09_i";
7      //PLEASE DO NOT RE-DISTRIBUTE
8      //WILL BE DULY RELEASED UNDER:
9      //Attribution-NonCommercial-ShareAlike 4.0 International (CC BY-NC-SA 4.0)
10
11
12     //THIS CODE CAN BE RUN WITH AN IMAGEJ PACKAGE SUCH AS FIJI
13     //VIEWING OF CODE IS RECOMMENDED WITH A TEXT READER SUCH AS NOTEPAD++
14     //JavaScript LANGUAGE SETTINGS PROVIDE A SUITABLE MARKUP STYLE
15
16
17
18
19     //Feature request: CHECK HERE FOR VALID STARTING FILE TYPE
20     myFileName=getInfo ("image.filename");
21
22     //get path to file dir
23     myFileDir=getDir("image");
24     ogDirNames = getFileList(myFileDir);
25     myFilePath=myFileDir + myFileName;
26
27     //Create a local temp dir and save image, clearing any redundant one
28     tmp = getDirectory("temp");
29     if (tmp=="")
30         exit("No temp directory available");
31     tempDir = tmp+"fijiTemp"+File.separator;
32
33     //Old temp folder check and deletion
34     if (File.exists(tempDir)){
35         oldTempFileNames = getFileList(tempDir);
36         for (index= 0; index < oldTempFileNames.length; index++) {
37             File.delete(tempDir+oldTempFileNames [index]);
38         }
39
40         File.delete(tempDir);
41     }
42     File.makeDirectory(tempDir);
43     if (!File.exists(tempDir))
44         exit("Unable to create temp directory");
45
46     //Remove scalebar, adjustable for any user image resolution/edge scalebar location
47     print("\\Clear");
48     run("Close");
49     setTool("rectangle");
50     waitForUser("Select image area excluding scalebar and hit OK");
51     getSelectionBounds(x,y,width,height);
52     makeRectangle(x, y, width, height);
53     run("Crop");
54     run("Options...", "iterations=1 count=1 black");
55
56     //Histogram generation
57     getStatistics(area, mean, min, max, std, histogram);
58     values = newArray(256);
59     histoLog = newArray(256);
60     histoLogNorm = newArray(256);
61     histoNorm = newArray(256);
62     value = min;
63     binWidth = (max-min)/256;
64
65     //Generaion of array with log data
66     for (i=0; i<256; i++) {
67         values[i] = value;
68         value += binWidth;
69         //check + ignore 0/-ve values
70         if (histogram[i] < 1) {
71             histoLog[i]= 0;

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72         }
73         else{
74             histoLog[i]= log(histogram[i]);
75         }
76     }
77
78     //Normalize both arrays
79     Array.getStatistics(histoLog,min,LogMx);
80     Array.getStatistics(histogram,min,HistoMx);
81     for (i=0; i<256; i++) {
82         histoLogNorm[i]= (histoLog[i]/LogMx)*100;
83         histoNorm[i]= (histogram[i]/HistoMx)*100;
84     }
85     Plot.create("Detecting Histogram Minima", "Gray Value", "Normalised Pixel Count",
values, histoNorm);
86     Plot.setFrameSize(600, 450);
87     Plot.setLimits(0, 255, 0, 100)
88     Plot.setLineWidth(2);
89     Plot.setColor("blue");
90     Plot.add("line",histoLogNorm);
91     Plot.setColor("black");
92     Plot.setFontSize(24, "bold");
93     Plot.setAxisLabelSize(24, "bold");
94     run("RGB Color");
95     Plot.show;
96     Plot.setLegend("Histogram\nlog Histogram");
97
98     //Phase number definition and refinement
99     Dialog.create("Number of phases");
100    Dialog.addMessage("Please define the number of phases,\nincluding things like
pores\n present in the sample");
101    Dialog.addNumber("Number Present",3,0,1,"Phases");
102    Dialog.show();
103
104    //Timing bookmark to measure analysis duration
105    t1 = getTime();
106
107    //Calculation of histogram minima
108    PhaseNum = Dialog.getNumber();
109
110    //Feature request: ADD USER ADJUSTABLE SLIDER AND INTERATIVE CONFIRMATION OF
PHASE SELECTION
111    //Minima sensitivity paramater
112    SensT = 100;
113
114
115    minLocs= Array.findMinima(histogram, SensT);
116    Array.sort(minLocs);
117    print("\Clear");
118    print("Minima:");
119
120    //Plot minima locations on histogram
121    for (index= 0; index < minLocs.length; index++) {
122
123        x= minLocs[index];
124        y = histoNorm[x];
125        print("x= ", x);
126        toUnscaled(x,y);
127        setColor("RED");
128        fillOval(x-10, y-10, 20, 20);
129    }
130
131    //Duration uptdate for analysis time
132    t2 = getTime();
133    print("\nProcessing time 1 =", d2s((t2-t1)/1000,3), "seconds");
134
135    //Create array to store area results
136    areaResults = newArray(minLocs.length - 1);
137
138    //Threshold and area measurement loop
139    for (index= 0; index < minLocs.length -1; index++) {

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140
141 //Fresh file open and scalebar removal***
142 open(myFilePath);
143 makeRectangle(0, 0, width, height);
144 run("Crop");
145 run("Options...", "iterations=1 count=1 black");
146
147 //Set threshold variables from minima calculations
148 leftThreshold = minLocs[index];
149 rightThreshold = minLocs[index+1];
150
151 //Set to correct range to measure
152 setThreshold(leftThreshold, rightThreshold);
153 setOption("BlackBackground", true);
154 run("Convert to Mask");
155
156 //Save to temp directory
157 Layer_name = myFileName+"_"+index;
158 saveAs ("Tiff",tempDir+Layer_name);
159
160 //Measurement and storage of results in an array
161 run("Set Measurements...", "area mean min area_fraction limit display
162 redirect=None decimal=3");
163 run("Measure");
164 areaResults [index] = getResult("%Area",index);
165 run ("Select None");
166
167 }
168
169 //Store names of temp files in an array
170 layerFileNames = getFileList(tempDir);
171
172 //Merge composite, flatten and save to source dir
173 bigString = "";
174
175 for (index=0; index<layerFileNames.length; index++) {
176     bigString = bigString+" c"+index+1+"="+layerFileNames[index];
177 }
178 run("Merge Channels...", bigString+" create keep");
179 run("Flatten");
180
181 //1st processing step time check
182 t2 = getTime();
183
184 //User phase labelling
185 colourLbIs = newArray("RED", "GREEN", "BLUE", "GRAY", "CYAN", "MAGENTA", "YELLOW");
186
187 Dialog.create("Assign a label to each identified phase");
188 for (index=0; index<layerFileNames.length; index++) {
189     Dialog.addString("Label for "+colourLbIs[index]+" phase:", "");
190     Dialog.addToSameRow();
191     Dialog.addCheckbox("Is real", true);
192 }
193
194 Dialog.show();
195
196 userReality = newArray(layerFileNames.length);
197 userLabels = newArray(layerFileNames.length);
198
199 //Stores and deletes labels wrt "reality index"
200 t3 = getTime();
201 for (index=0; index<layerFileNames.length; index++) {
202     userReality [index] = Dialog.getCheckbox();
203     userLabels [index] = Dialog.getString();
204 }
205
206 //Draws labels and keys
207 lpCntr = 0;
208 for (index=0; index<layerFileNames.length; index++) {
209     if (userReality [index] > 0){
210         makeRectangle(50, 50+(lpCntr*150), 120, 120);

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210         setColor("black");
211         run("Fill", "slice");
212         setColor(colourLbls [index]);
213         makeRectangle(60,60+(lpCntr*150),100,100);
214         run("Fill", "slice");
215         setColor("black");
216         setFont("Arial",60,"Bold");
217         setFont("Arial",60,"Bold");
218         drawString(userLabels [index],200,150+(lpCntr*150),"white");
219         lpCntr = lpCntr + 1;
220     }
221     else {
222         close(layerFileNames [index]);
223         File.delete(tempDir+layerFileNames [index]);
224     }
225 }
226
227 //Flatten and save version of first composite image
228 saveAs ("Tiff", myFileDir+myFileName+"_phasecompositelabel");
229 run("Images to Stack", "name=Stack title=[] use");
230
231 rowCntr = 2;
232 if (lpCntr < 2) {;
233 }
234     else if (lpCntr < 4) {
235         rowCntr = 3;
236     }
237     else if (lpCntr < 6) {
238         rowCntr = 4;
239     }
240     else if (lpCntr > 5) {
241         rowCntr = 5;
242     }
243 run("Make Montage...", "columns=2 rows="+rowCntr+" scale=0.50 border=5 label");
244 close("\\Others");
245 saveAs ("Tiff", myFileDir+myFileName+"_phaseselection-montage");
246
247 //Processing time checkpoint
248 t4 = getTime();
249 print("Image 1 processing time =", d2s(((t4-t3)+(t2-t1))/1000,3), "seconds");
250 print("\n");
251
252 //Update temp directory file list
253 layerFileNames = getFileList(tempDir);
254
255 //normalization factor and results printing
256 areaResNorm = areaResults;
257 normFactor = 0;
258 phaseCounter = 0;
259 phaseNumber = newArray(areaResults.length);
260 Array.fill(phaseNumber,0);
261
262 //Delete data from non-real phases
263 for (index=0; index<areaResults.length; index++) {
264     if(userReality [index] <1){
265         areaResNorm[index] = 0;
266     }
267     else{
268         normFactor = normFactor + areaResNorm[index];
269         phaseCounter = phaseCounter + 1;
270         phaseNumber[index] = phaseCounter;
271     }
272 }
273
274 //Normalize remaining data and dialogs with labels, confirmation window presented
275 Dialog.create("Confirm phase selection and set source");
276 for (index=0; index<areaResults.length; index++) {
277     if(userReality [index] >0) {
278         areaResNorm[index] = (areaResNorm[index]/normFactor)*100;
279         Dialog.addMessage("Phase "+phaseNumber[index]+", "+colourLbls[index]+": "+
280 userLabels[index]+", "+d2s(areaResNorm[index],1)+"% (norm.)");

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280     }
281 }
282 Dialog.addDirectory("Please select the directory of image set", myFileDir);
283 Dialog.show();
284 dataSource = Dialog.getString();
285
286 //Time marker
287 t5 = getTime();
288
289 //Collect filenames in datasource folder, open all and create stack
290 dataList = getFileList(dataSource);
291 close("*");
292
293 //Measuere stack
294 //Feature request: CHECK FOR VALID PHASES ONLY
295 dataSetResults = newArray(areaResults.length);
296
297 for (index= 0; index < minLocs.length-1; index++) {
298     for (j=0; j<dataList.length; j++) {
299         open(dataSource+"/"+dataList[j]);
300     }
301
302     run("Images to Stack", "name=Stack title=[] use");
303
304     makeRectangle(0, 0, width, height);
305     run("Crop");
306     run("Options...", "iterations=1 count=1 black");
307
308     //Set threshold variables from minima calculations
309     leftThreshold = minLocs[index];
310     rightThreshold = minLocs[index+1];
311
312     //Set to correct range to measure
313     setThreshold(leftThreshold, rightThreshold);
314     setOption("BlackBackground", true);
315     run("Convert to Mask", "method=Default background=Dark black");
316
317
318     //Measurement and storage of results in an array
319     run("Set Measurements...", "area mean min area_fraction limit display
320     redirect=None decimal=3");
321     run("Measure");
322     //Feature request: ADD (SLOWER) INDIVIDUAL MEASUREMENT AS TO ALLOW FOR AVERAGE
323     //OF STACK AND ERROR CALC
324     dataSetResults [index] = getResult("%Area",index);
325     print(userLabels[index]+" phase, measured between ("+leftThreshold+"-"+
326     rightThreshold+"): " +d2s(dataSetResults[index],1)+"%");
327     run ("Select None");
328     close("*");
329 }
330
331 //Total set processing time checkpoint
332 t6 = getTime();
333 print("\nImage SET processing time =", d2s((t6-t5)/1000,3), "seconds");
334
335 print("\nThank you for using\nP.hase A.nalysis G.rayscale I.image
336 S.cript\nVersion_ "+verNo);

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