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//Virtual Slice Islet Macro
//DECEMBER 28, 2009

function prepare_dir (myDir, regional) {
print("starting in " +myDir);
list = getFileList(myDir);
  for (i=0; i<list.length; i++){
    if (endsWith(list[i],".tif")){
      filename=list[i];
      path=myDir;

      mkdir=path+"clean/";
      out_file=filename+" clean.tif";
      if (!File.exists(mkdir+out_file)) {
        open(path+filename);
        waitForUser("Remove bubbles and reflective tissue edges\nPress 'OK'
to save");

        if (!File.exists(mkdir))
          File.makeDirectory(mkdir);
        if (!File.exists(mkdir))
          exit("Unable to create directory");
        saveAs("TIF", mkdir+out_file);
      } else {
        //waitForUser(mkdir+out_file+"is clean");
        print(mkdir+out_file+" is already clean");
        open(mkdir+out_file);
      }
      run("Set Scale...", "distance=1 known=1 pixel=1 unit=micron
global"); //why not?
      if (regional == 1) {
        setOption("Show All",true);
        waitForUser("Select the head region");
        roiManager("Add");
        roiManager("Select", 0);
        roiManager("Rename", "head");
        wait(10);
        waitForUser("Select the body region");
        roiManager("Add");
        roiManager("Select", 1);
        roiManager("Rename", "body");
        wait(10);
        waitForUser("Select the head region");
        roiManager("Add");
      }
    }
  }
}

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roiManager("Select", 2);
roiManager("Rename", "tail");

roiManager("Deselect");
roiManager("Save", mkdir+filename+".zip");
roiManager("Delete");
}
run("Select None");

run("Gaussian Blur...", "sigma=3");

//BG Level
waitForUser("Determine background level\nPress 'OK' to input");
Dialog.create("Parameters");
Dialog.addMessage("What is the background level?");
Dialog.addNumber("Background:",0);
title="What is the background level?";
Dialog.show();
sub = Dialog.getNumber();
run("Subtract...", "value="+sub);

//Background subtraction
waitForUser("Use the line tool to select the radius of the largest
particle\nPress 'OK' to record");
getLine(x1, y1, x2, y2, lineWidth);
if (x1==-1)
    exit("This macro requires a straight line selection");
print("Starting point: (" + x1 + ", " + y1 + ")");
print("Ending point: (" + x2 + ", " + y2 + ")");
dx = x2-x1; dy = y2-y1;
ball = sqrt(dx*dx+dy*dy);
print("radius:", ball);
run("Subtract Background...", "rolling="+ball+"");

//Thresholding
run("Enhance Contrast", "saturated=2.0");
run("Threshold...");
waitForUser("Pick a low threshold value\nPress 'OK' to input");
getThreshold(tlow, upper);
wait(10);
waitForUser("Pick a high threshold value\nPress 'OK' to input");
getThreshold(thigh, upper);
wshed_thold=getString("Low Threshold Cutoff:", "30000");
scatter=getString("Pixels of light scattering:", "30");

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//function analyze
(path,filename,wshed_thold,sub,tlow,thigh,ball,scatter) {
File.append("analyze(\""+mkdir+"\",\""+out_file+"\","+wshed_thold
+",""+sub+",""+tlow+",""+thigh+",""+ball+",""+scatter+"");\n",path
+"parameters.txt");
blur=3;
if (getBoolean("Analyze?")){
setBatchMode(true);
  path=mkdir;
  filename=out_file;

  out_path=path+filename+" sub-"+sub+" "+tlow+"-"+thigh+" blur-"+blur
+" ball-"+ball+" scatter-"+scatter+" analysis/";
  tmid=round((tlow+thigh)/2);

  mkdir=out_path;
  File.makeDirectory(mkdir);
  if (!File.exists(mkdir))
    exit("Unable to create directory\n"+mkdir);
  print(mkdir);

  rename("original");
  run("Set Measurements...", "area perimeter circularity feret's
centroid center limit redirect=None decimal=3");
  run("Set Scale...", "distance=1.549 known=1 pixel=1 unit=micron
global");

//TriPLICATE
  run("Duplicate...", "title=original2");
  run("Duplicate...", "title=original3");

//Generate low threshold mask
  selectWindow("original");
  setThreshold(tlow,255);
  run("Convert to Mask");
  //saveAs("PNG", out_path+filename+"_"+tlow+"_full_mask.png");
  rename("low_mask_all");
  run("Analyze Particles...", "size=170-"+wshed_thold+"
circularity=0.00-1.00 show=[Masks] display clear summarize");
  //saveAs("PNG", out_path+filename+"_"+tlow+"_thold_mask.png");
  rename("low_mask");
  selectWindow("low_mask_all");

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close();

//Generate high threshold mask
selectWindow("original2");
setThreshold(thigh,255);//thigh was originally 43
run("Convert to Mask");
//saveAs("PNG", out_path+filename+"_"+thigh+"_full_mask.png");
rename("high_mask_all");
run("Analyze Particles...", "size="+wshed_thold+"-Infinity
circularity=0.00-1.00 show=[Masks] display clear summarize");
//saveAs("PNG", out_path+filename+"_"+thigh+"_thold_mask.png");
setThreshold(tlow,255);
rename("high_mask");
selectWindow("high_mask_all");
close();

//Generate gap mask
selectWindow("high_mask");
run("Duplicate...", "title=high_mask_blur");
run("Gaussian Blur...", "sigma="+scatter+" scaled");
rename("high_mask_blur");
imageCalculator("Subtract create", "original3", "high_mask");
rename("temp");
imageCalculator("Subtract create", "temp", "high_mask_blur");
rename("temp2");
imageCalculator("Subtract create", "temp2", "low_mask");
//saveAs("PNG", out_path+filename+"_et_"+wshed_thold+"_gap.png");
setThreshold(tmld,255);
run("Convert to Mask");
//saveAs("PNG", out_path+filename+"_"+tmld+"_full_mask.png");
rename("mid_mask");

//Clean up some windows
selectWindow("temp");
close();
selectWindow("temp2");
close();
selectWindow("high_mask_blur");
close();
selectWindow("original3");
close();

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//segregate particles w circularity 0-circ for all thresholds into
masks
  selectWindow("low_mask");
  run("Analyze Particles...", "size=170-Infinity circularity=0.00-1.0
show=[Masks] display clear summarize");
  rename("low_mask_ws");
  selectWindow("mid_mask");
  run("Analyze Particles...", "size=170-Infinity circularity=0.00-1.0
show=[Masks] display clear summarize");
  rename("mid_mask_ws");
  selectWindow("high_mask");
  run("Analyze Particles...", "size=170-Infinity circularity=0.00-1.0
show=[Masks] display clear summarize");
  rename("high_mask_ws");

run("Clear Results");

selectWindow("low_mask");
close();
selectWindow("mid_mask");
close();
selectWindow("high_mask");
close();

//Watershedding disabled:
/*
selectWindow("high_mask_ws");
setThreshold(thigh,255);
run("Watershed");
selectWindow("low_mask_ws");
setThreshold(tlow,255);
run("Watershed");
selectWindow("mid_mask_ws");
setThreshold(tmids,255);
run("Watershed");
*/
//Analyze WS particles
selectWindow("low_mask_ws");
run("Analyze Particles...", "size=170-Infinity circularity=0.00-1.00
show=[Outlines] display summarize");
//saveAs("PNG", out_path+filename+"_low-mask-ws.png");
rename("low_result");

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```
selectWindow("mid_mask_ws");
run("Analyze Particles...", "size=170-Infinity circularity=0.00-1.00
show=[Outlines] display summarize");
//saveAs("PNG", out_path+filename+"_mid-mask-ws.png");
rename("mid_result");
selectWindow("high_mask_ws");
run("Analyze Particles...", "size=170-Infinity circularity=0.00-1.00
show=[Outlines] display summarize");
saveAs("Measurements", out_path+filename+".xls");
//saveAs("PNG", out_path+filename+"_high-mask-ws.png");
rename("high_result");
print(filename+" completed.");
```

```
//Clean-up
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```
selectWindow("low_mask_ws");
close();
selectWindow("mid_mask_ws");
close();
selectWindow("high_mask_ws");
close();
```

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//Sum up outlines
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```
selectWindow("low_result");
rename("temp");
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```
imageCalculator("Add create 32-bit", "temp", "mid_result");
rename("temp2");
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```
selectWindow("temp");
close();
selectWindow("mid_result");
close();
```

```
imageCalculator("Add create 32-bit", "temp2", "high_result");
```

```
selectWindow("temp2");
close();
selectWindow("high_result");
close();
saveAs("PNG", out_path+filename+"_outlines.png");
setBatchMode(false);
```

```
}  
  
//Close windows  
while (nImages()>0) {  
    selectImage(nImages());  
    run("Close");  
  
    }  
}  
}  
  
}  
print("done");  
  
prepare_dir("/home/mhara/Desktop/neonate wt split/P14/",0);
```