

## Supplementary Text

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import ij.*;
import ij.gui.*;
import ij.process.*;
import ij.measure.ResultsTable;
import ij.measure.Measurements;
import ij.plugin.*;
import ij.plugin.filter.*;
import ij.plugin.filter.ParticleAnalyzer;
import ij.plugin.frame.ThresholdAdjuster;
import java.awt.*;
import java.awt.image.*;
import java.lang.Math.*;

public class Enumerate_Cells implements PlugInFilter {
    private boolean LOG=true;
    ImagePlus imp;
    ImageStack stack=null;
    int histMax=5000; int histMin=0; int nBins=100;
    double maxThres=103.0f;

    public int setup(String arg, ImagePlus imp) {
        this.imp = imp;
        return DOES_ALL;
    }

    public void run(ImageProcessor ip) {
        int whichObject = 2;
        GenericDialog gd = new GenericDialog("Enumerate cells and Count debris");
        String radioList[] = {"Cells", "Debris", "Both"};
        gd.addRadioButtonGroup("Which objects enumerate ?",
            radioList, 1, 3, radioList[whichObject]);
        gd.addNumericField("maxThres for debris: ", maxThres, 0);
        gd.addCheckbox("Show logY", LOG);
        gd.showDialog();
        if (gd.wasCanceled()) return;

        String radio = (String)gd.getNextRadioButton();
        maxThres = (double)gd.getNextNumber();
        LOG = (boolean)gd.getNextBoolean();

        whichObject=0;
        while(!radio.equals(radioList[whichObject])){ whichObject++; }
        int WH=100;

        ByteProcessor bp = ip.convertToByteProcessor();
        ColorModel cm = LookUpTable.createGrayscaleColorModel(false);
        stack = new ImageStack(WH, WH, cm);

        Plot pt;
        if (LOG)
            pt = new Plot("Histogram of area", "Area (pixels)", "# of cells (LOG)");
        else
            pt = new Plot("Histogram of area", "Area (pixels)", "# of cells");
        pt.setLogScaleY();
        double xmin=0; double xmax=histMax;
        if (LOG) pt.setLimits(xmin, xmax, 0, 10);

        ResultsTable rt1=null;
        ResultsTable rt2=null;
        // For Cells
        if (whichObject==0||whichObject==2) {
            rt1 = extractCells(bp);
            if (rt1.getCounter()==0){IJ.showMessage("rt1 is 0");return;}
        }
    }
}
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IJ.log("rt1: "+rt1.getCounter());
float[] x1 = new float[nBins];
float[] y1 = new float[nBins];
makeAreaHistogram(rt1, x1, y1, LOG);
pt.setColor(Color.blue); pt.setLineWidth(2);
pt.addPoints(x1, y1, Plot.LINE);
if (whichObject==0) IJ.showMessage("Cells: "+rt1.getCounter());
}
// For Debris
if (whichObject==1||whichObject==2) {
rt2 = extractDebris(bp);
if (rt2.getCounter()==0) {IJ.showMessage("rt2 is 0");return;}
float[] x2 = new float[nBins];
float[] y2 = new float[nBins];
makeAreaHistogram(rt2, x2, y2, LOG);
pt.setColor(Color.red); pt.setLineWidth(3);
pt.addPoints(x2, y2, Plot.LINE);
if (whichObject==1) IJ.showMessage("Debris: "+rt2.getCounter());
}
if (whichObject==2)
IJ.showMessage("Cells:"+rt1.getCounter()+"\n"+
"Debris: "+rt2.getCounter());
pt.show();

// For Cells
if (whichObject==0||whichObject==2) {
if (stack.getSize()!=0) {
int nSlices = stack.getSize();
int columns = (int)Math.sqrt(nSlices);
int rows = columns;
int n = nSlices - columns*rows;
if (n>0) columns += (int)Math.ceil((double)n/rows);
double scale = 1.0;
ImagePlus imp = new ImagePlus("stack", stack);//imps.show();
MontageMaker mm = new MontageMaker();
mm.makeMontage(imp, columns, rows, scale, 1, nSlices, 1, 3, false);
}
}
}

void makeAreaHistogram(ResultsTable rt, float[] x, float[] y, boolean flag){
float[] fArea = rt.getColumn(0);
short[] sArea = new short[fArea.length];
for(int i=0;i<sArea.length;i++) sArea[i] = (short)fArea[i];
ColorModel cm = LookUpTable.createGrayscaleColorModel(false);
ShortProcessor sp = new ShortProcessor(1, sArea.length, sArea, cm);
ImagePlus imp1 = new ImagePlus("area", sp);
int mOptions = 0;
ImageStatistics stats=
imp1.getStatistics(mOptions, nBins, histMin, histMax);
long[] his = stats.getHistogram();
for(int i=0;i<x.length;i++) {
x[i]=(float) i*(float) (histMax-histMin)/(float)nBins;
if (flag) y[i]=(float)Math.log10((double)his[i]);
else y[i]=(float)his[i];
}
}

ResultsTable extractCells(ImageProcessor bp) {
ImagePlus imp2 = new ImagePlus("Cells", bp);
ResultsTable rt = new ResultsTable();
int options = ParticleAnalyzer.SHOW_RESULTS|
ParticleAnalyzer.EXCLUDE_EDGE_PARTICLES|
ParticleAnalyzer.CLEAR_WORKSHEET;
//ParticleAnalyzer.INCLUDE_HOLES;

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int measurements = Measurements.RECT|Measurements.AREA;

bp.setAutoThreshold(AutoThresholder.Method.Triangle,true);//true=Dark Background
int minSize=100; int maxSize=5000;
ParticleAnalyzer pa =
    new ParticleAnalyzer(options, measurements, rt, minSize, maxSize,0.2,1.0);
pa.setHideOutputImage(true);
pa.analyze(imp2);
int WH=100;
int width=bp.getWidth(); int height=bp.getHeight();
for(int i=0;i<rt.getCounter();i++){
    int bx = (int)rt.getValue("BX",i);
    int by = (int)rt.getValue("BY",i);
    int w = (int)rt.getValue("Width",i);
    int h = (int)rt.getValue("Height",i);
    if ( (w<=WH) && (h<=WH) ){
        if ( (bx+w/2+WH/2<=width) && (by+h/2+WH/2<=height) ){
            int cbx = (bx+w/2)-WH/2;
            int cby = (by+h/2)-WH/2;
            if ( cbx>=0 && cby>=0 ){
                bp.setRoi(cbx, cby, WH, WH);
                ImageProcessor crop = bp.crop();
                stack.addSlice(crop);
            }
        }
    }
}
imp2.close();
return rt;
}

ResultsTable extractDebris(ByteProcessor bp) {
    ImagePlus imp2 = new ImagePlus("Debris",bp);
    double minThres=0.0f;
    bp.setThreshold(minThres, maxThres, ImageProcessor.RED_LUT);
    int options = ParticleAnalyzer.SHOW_RESULTS|
        ParticleAnalyzer.EXCLUDE_EDGE_PARTICLES|
        ParticleAnalyzer.CLEAR_WORKSHEET|
        ParticleAnalyzer.SHOW_MASKS|
        ParticleAnalyzer.INCLUDE_HOLES;
    int measurements = Measurements.RECT|Measurements.AREA;
    ResultsTable rt = new ResultsTable();
    int minSize=5; int maxSize=100;
    ParticleAnalyzer pa = new ParticleAnalyzer(options,
        measurements, rt,minSize,maxSize,0.2,1.0);
    pa.setHideOutputImage(false);
    pa.analyze(imp2);
    imp2.close();
    return rt;
}
}

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