

### **Supplemental Material: Fiji/ImageJ Macro**

waitForUser("Halt!", "Make a selection(s) and then proceed by clicking [OK].\nYou can have MULTIPLE selections by holding down [Shift] when making a new selection.\nYou can EXCLUDE areas by holding down [Alt] when selecting.\nTip: You can make a large selection with the [Freehand Selection Tool] then remove areas using [Alt]-[Selection Brush Tool].");

```
SEL=selectionType();
```

```
if (SEL>-1) {
```

```
    original=getTitle();
```

```
    output = getDirectory("Where should the processed files go?");
```

```
    run("Duplicate...", "title=Area");
```

```
    selectWindow("Area");
```

```
    run("Line Width...", "line=10");
```

```
    run("Draw");
```

```
    saveAs("Jpeg", output + original + "-Area.jpg");
```

```
    close();
```

```
    run("Select None");
```

```
    saveSettings();
```

```
    setForegroundColor(255,0,0);
```

```
    w = getNumber("How wide (in pixels) is the box", 640);
```

```
    h = getNumber("How high (in pixels) is the box", 480);
```

```
    width = getWidth()-w;    // width of the randomly placed ROI
```

```
    height = getHeight()-h;  // height of the randomly placed ROI
```

```
    RoisN = getNumber("How many ROIs should be made?", 20); // number of ROIs
```

```
    Runs = getNumber("How many attempts should be made? (The higher the number, the longer it will take)", 50); //Runs
```

```
    trials=RoisN*Runs; //maximum trials to avoid infinite loop (originally 1000)
```

```
    i=0;
```

```
    j=0;
```

```
    xa=newArray(RoisN);
```

```
    ya=newArray(RoisN);
```

```
    run("Duplicate...", "title=Reference");
```

```
    selectWindow("Reference");
```

```
    run("8-bit"); //makes it greyscale
```

```
    run("RGB Color"); //RGB to display colours
```

```
    run("Restore Selection");
```

```
    run("Make Inverse");
```

```
    run("Fill");
```

```
    run("Select None");
```

```
    while (i<RoisN && j<trials){
```

```
        x = random()*width;
```

```
        y = random()*height;
```

```
        j++;
```

```
        //Check for pixels with value (255,0,0):
```

```
        flag= -1;
```

```
        makeRectangle(x, y, w, h);
```

```
        //Scanning the rectangle perimeter should be faster than scanning the whole box.
```

```
        //This is slower, as checks all the points in the box:
```

```
        for (xs=x;xs<x+w;xs++){
```

```
            for (ys=y;ys<y+h;ys++){
```

```
                if (getPixel(xs,ys)==-65536) // pixel is (255,0,0)
```

```

        flag=0;
    }
}
if (flag==--1){
    xa[i]=x;
    ya[i]=y;
    run("Fill");
    i++;
}
}

close();
run("ROI Manager..."); //open ROI manager
selectWindow(original);
setForegroundColor(255,255,0);
run("Set Measurements...", "centroid bounding display add redirect=None decimal=0"); //to
ensure measurements are correct.
for (j=0;j<i;j++){
    makeRectangle(xa[j], ya[j], w, h);
    roiManager("Add");
    run("Duplicate...", original + "-" + j+1 + ".tif");
    saveAs("Jpeg", output + original + "-" + j+1 + "-ROI.jpg");
    close();
}

roiManager("deselect");
roiManager("measure");
roiManager("Save",output + original + "-ROI.zip");
saveAs("Results",output + original + "-RESULTS.csv");
roiManager("show all with labels");
run("Line Width...", "line=5");
roiManager("Show All");
roiManager("Draw");
saveAs("Jpeg", output + original + "-MAP.jpg");
restoreSettings();
run("Select None");
run("Close All");
selectWindow("Results");
run("Close");
selectWindow("ROI Manager");
run("Close");
showMessage("All Done! Have a nice day :)");
}
else {
    showMessage("No selection was made");
}
}

```