

1 **Supplement to:**

2 **A novel application of Gini coefficient for the quantitative measurement of** 3 **bacterial aggregation**

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14

15 **Supplementary Fiji macro 1: Biovolume calculation in each divided sub-stack** 16 **within defined folders**

17

```
18    dir1 = getDirectory("Choose source folder");  
19    dir2 = getDirectory("Create and select a folder for .txt results");  
20    dir3 = getDirectory("Create and select a folder for binary images");  
21    listAll = getFileList(dir1);  
22  
23    setBatchMode(true);  
24  
25    listImage = newArray();  
26    for(i = 0; i < listAll.length; i++){  
27        if(endsWith(listAll[i], ".tif")){  
28            listImage = Array.concat(listImage, listAll[i]);  
29        }  
30    }  
31    Array.sort(listImage);  
32  
33    for(i=0; i<listImage.length; i++){  
34        open(dir1+listImage[i]);  
35        cubeTitle = getTitle();  
36        run("8-bit");  
37  
38        run("Duplicate...", "title=B duplicate");  
39        duplicateTitle = getTitle();  
40        run("Set Measurements...", "area limit display redirect=None decimal=3");
```

```

41
42     selectWindow(duplicateTitle);
43     NbSlice = nSlices;
44     for(n = 1; n <= NbSlice; n++){
45         setSlice(n);
46         setAutoThreshold("Otsu dark no-reset");
47         //run("Threshold...");
48         setAutoThreshold("Otsu dark no-reset");
49         run("Measure");
50     }
51     selectWindow(duplicateTitle);
52     close();
53     selectWindow("Results");
54     for(n = 0; n<nResults; n++){
55         resultArea = getResult("Area", n);
56         if(resultArea > 0){
57             Object3D = 1;
58         }
59         else {
60             Object3D = 0;
61         }
62     }
63
64     if(Object3D == 0){
65         selectWindow("Results");
66         saveAs("Text", dir2+"M_"+cubeTitle+"_Results.txt");
67         run("Close");
68         print(cubeTitle+": no 3D objects found");
69         print("-----");
70     }
71     else {
72         selectWindow("Results");
73         run("Close");
74     }
75
76     if(Object3D == 1){
77         selectWindow(cubeTitle);
78
79         setAutoThreshold("Otsu dark no-reset");
80         //run("Threshold...");
81         setOption("BlackBackground", false);
82         run("Convert to Mask", "method=Otsu background=Dark calculate");
83         saveAs("Tif", dir3+cubeTitle+"_binary.tif");
84
85         // some options in Manager3D Options, select Plugins/Record to record
86         run("3D Manager Options", "volume sync
87 distance_between_centers=10distance_max_contact=1.80");
88         // run te manager 3D and add image
89         run("3D Manager");
90         // segment the signal at a defined threshold range

```

```

91         Ext.Manager3D_Segment(250, 255);
92         // add the segmented signal (3D objects) to ROI manager
93         Ext.Manager3D_AddImage();
94         // Measure the Volume of 3D objects and save as csv file
95         Ext.Manager3D_Measure();
96         Ext.Manager3D_SaveResult("M", dir2+cubeTitle+"_Results3D.txt");
97         // close the result window and 3D Manager
98         Ext.Manager3D_CloseResult("M");
99         //Ext.Manager3D_Reset();
100        Ext.Manager3D_Close();
101
102        run("Close All");
103        print("-----");
104    }
105}
106print("All images have been processed!");
107selectWindow("Log");
108saveAs("Text", dir2+"Log.txt");
109run("Close");
110exit("ALL IMAGES HAVE BEEN PROCESSED");
111
112
113

```

114 **Supplementary Fiji macro 2. Fiji macro 2 for extracting ‘volume’ data from txt**
115 **result files of all sub-stacks into a summary file.**

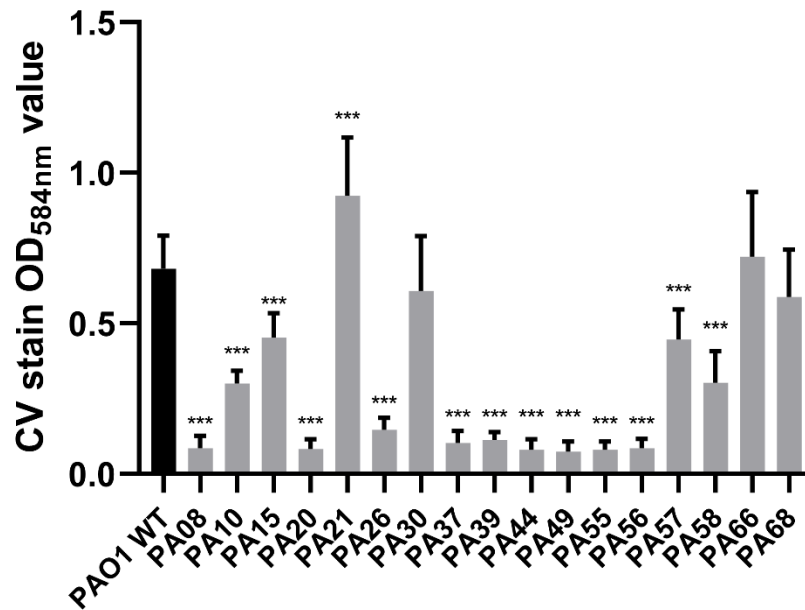
```

116
117 dir2 = getDirectory("Select folder with results files");
118 folderName = File.getName(dir2);
119 listTxt = getFileList(dir2);
120
121 listResults = newArray();
122 for(i=0; i<listTxt.length; i++){
123     if(endsWith(listTxt[i], "_Results.txt") | endsWith(listTxt[i],
124 "_Results3D.txt")){
125         listResults = Array.concat(listResults, listTxt[i]);
126     }
127 }
128 Array.sort(listResults);
129
130 volumeTot_array = newArray();
131
132 for(i=0; i<listResults.length; i++){
133     filePath = dir2+listResults[i];
134     run("Results... ", "open=filePath");
135
136     valueTot = 0;
137

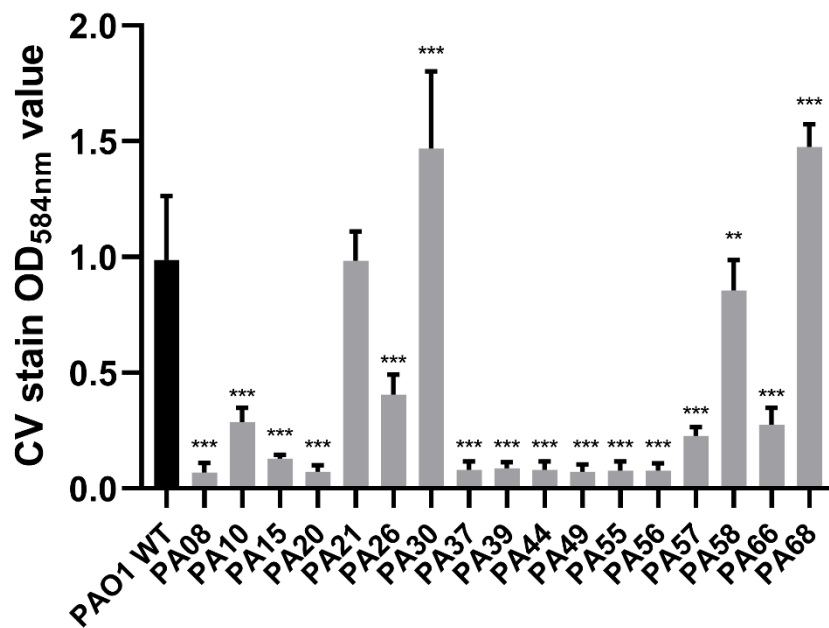
```

```
138         if(endsWith(listResults[i], "Results.txt")){
139             for(n=0; n<nResults; n++){
140                 value = getResult("Area", n);
141                 valueTot = valueTot + value;
142             }
143         }
144         else {
145             for(n=0; n<nResults; n++){
146                 value = getResult("Vol (unit)", n);
147                 valueTot = valueTot + value;
148             }
149         }
150         volumeTot_array = Array.concat(volumeTot_array, valueTot);
151         run("Close");
152     }
153
154     if (nResults>=0){
155         run("Clear Results");
156     }
157     i = nResults;
158
159     for(i=0; i<volumeTot_array.length; i++){
160         setResult("Cube", i, listResults[i]);
161         setResult("Volume total", i, volumeTot_array[i]);
162     }
163     updateResults;
164     saveAs("Results", dir2+folderName+"_Results.txt");
165     run("Close");
166
167
168
169
```

(a) **Microtiter plates 24 hrs biofilm in BHI**

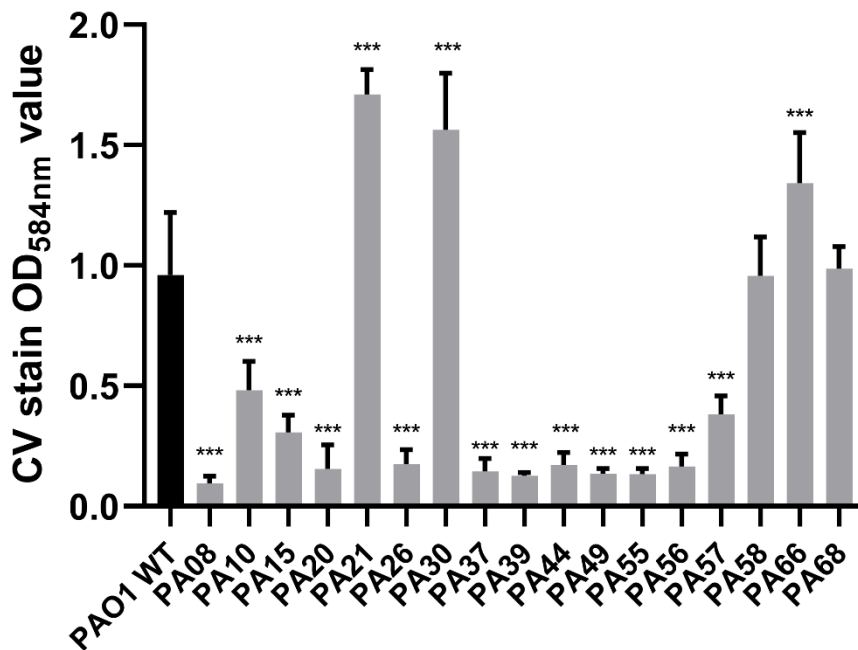


(b) **Microtiter plates 24 hrs biofilm in LB**

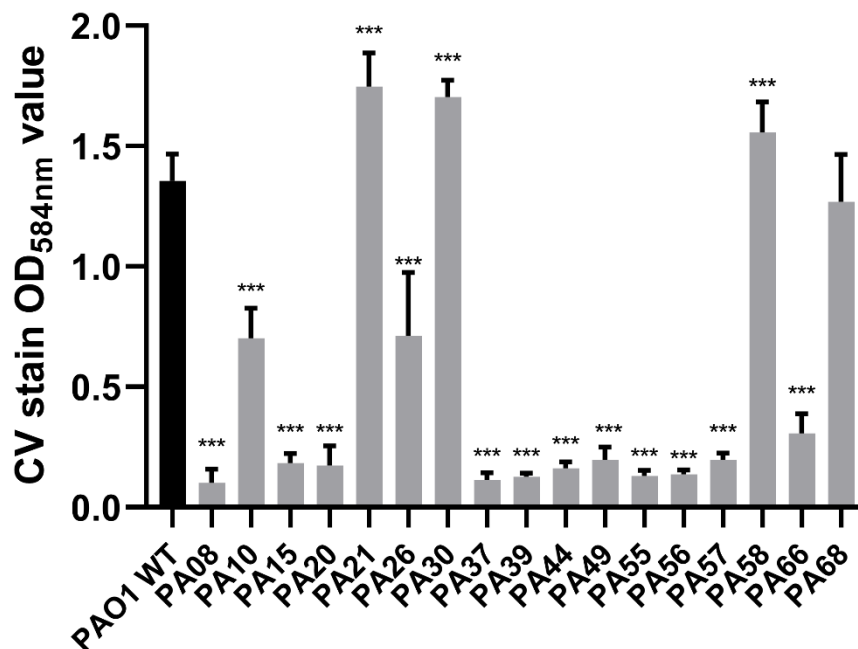


Supplemental Figure S1. 24 hrs CF-PA isolates biofilms in BHI (a) and LB (b) medium in microtiter plates. The biofilm formation of each CF-PA strain is compared to PAO1 using two-tailed Student T test. *** denotes $P < 0.01$, ** denotes $0.01 < P < 0.05$. Data acquired from 3 independent experiments and 6 technical replicates.

(a) **Microtiter plates 72 hrs biofilm in BHI**



(b) **Microtiter plates 72 hrs biofilm in LB**



Supplemental Figure S2. 72 hrs CF-PA isolates biofilms in BHI (a) and LB (b) medium in microtiter plates. The biofilm formation of each CF-PA strain is compared to PAO1 using two-tailed Student T test. *** denotes $P < 0.01$, ** denotes $0.01 < P < 0.05$. Data acquired from 3 independent experiments and 6 technical replicates.