ReadPlate

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History:
August 15, 2020: ReadPlate 3.0 version incorporates the capability of reading multi-well plates of 6 (3x2), 12 (4x3), 24 (6x4), 48 (8x6) or 96 (12x8) wells. A new user-friendly interface facilitates image analysis. This version allows new user-defined features to optimize blank correction. Multiple readings of the same plate have also considerably been facilitated, appending the full parameter file to the results and log file
February 16, 2018: ReadPlate2.1 incorporates an improved blank correction algorithm
April 15, 2016: ReadPlate2 introduces a correction for blank measurements
March 10, 2016: Inclusion of alpha numeric labeling for the wells and calculation of absorbance values
December 16, 2015: Original version
With thanks to Sandra Verstraeten, Pablo Carabias, Irene Mangialavori and Gabriela Gomez for their enthusiasm, suggestions and help with this project

Key words:
Plate reader, microplate reader, microtiter plate photometry, absorbance, digital camera, cell-phone camera

Relevant literature reference:
Carla R. Angelani, Pablo Carabias, Karen M. Cruz, Jose M. Delfino, Marilina de Sautu, Maria V. Espelt, Mariela S. Ferreira-Gomes, Gabriela E. Gomez, Irene C. Mangialavori, Malena Manzi, Maria F. Pignataro, Nicolas A. Saffioti, Damiana M. Salvatierra Frechou, Javier Santos, Pablo J. Schwarzbaum
"A Metabolic Control Analysis Approach to Introduce the Study of Systems in Biochemistry: the Glycolytic Pathway in the Red Blood Cell"
Biochemistry and Molecular Biology Education, Volume 46, Issue5, September/October 2018, Pages 502-515 https://doi.org/10.1002/bmb.21139

Source:
The source code of ReadPlate3.0 version is available at
http://imagej.nih.gov/ij/macros/ReadPlate3.0.txt

Installation:
Download ReadPlate3.0.txt and do Plugins > Install. The plugin is ready to be launched by clicking Plugins >ReadPlate3.0
An example image of a 96-well plate is available at http://imagej.nih.gov/ij/macros/images/plate.jpg

Description:

Image acquisition:
The multi-well plate is located on top of a home-built trans-illuminator: a white 7 x 10 LED array, powered by a 12V DC power supply, covered with an acrylic plate that acts as a light-diffusing base (**Figure A**). To avoid the influence of stray light, the device is covered with a tall black plywood pyramidal box equipped with a central hole at the top (**Figure B**), through which the zoom lens of the camera is located (Nikon CoolPix S6300 in our case, but cell-phone cameras can be used as well). The vertical optical axis passes through the center of the plate. To minimize parallax error that would affect the light path through the samples, pictures are taken at a minimal distance of 70 cm. The plate

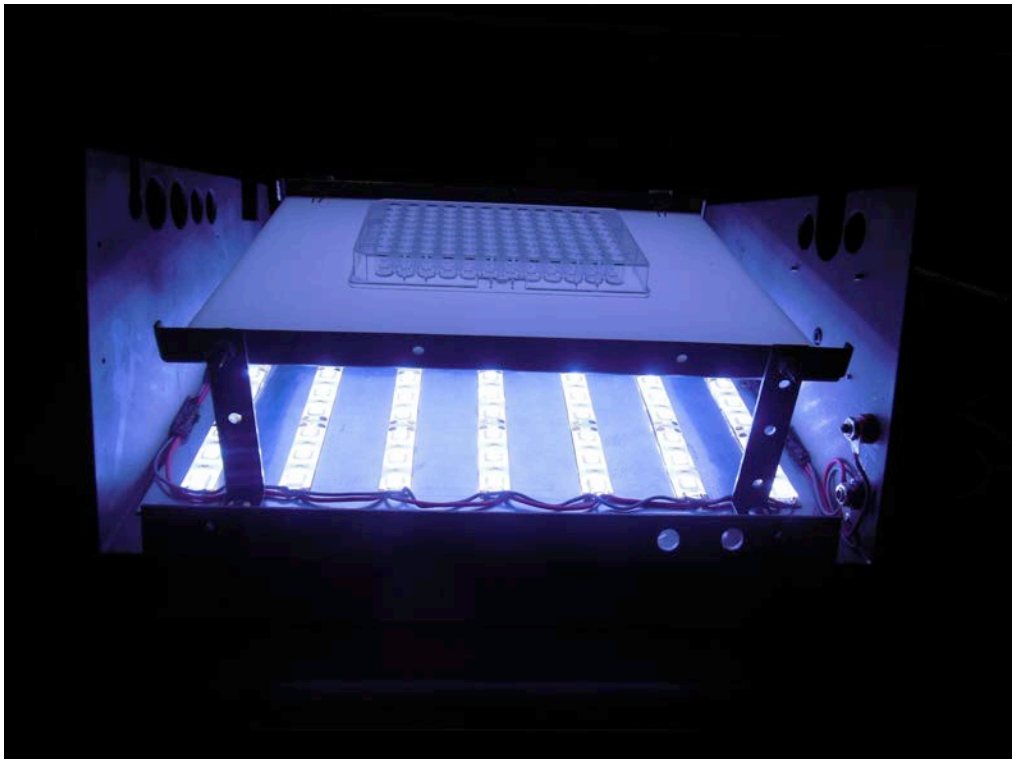
borders should be parallel to the frame of the picture. The example photograph (stored as a .jpg file) corresponds to a 96-well plate with samples of a colorimetric assay for lactate (120 μL per well, maximum of absorbance at 555 nm). In this case, the green channel shows the highest sensitivity, due to maximal spectral overlap between the absorption spectrum of the chromophore in the sample and the green window.

Plugin use:
Before starting (very important!), please set the following parameters for measurements: (Analyze > Set Measurements). The following should be selected: Area / Standard Deviation / Min & Max Gray Value/ Mean Gray Value/ Modal Gray Value/ Add to Overlay / Redirect to None / Decimal Places (0-9): 3.

ReadPlate measures the color intensity (RGB) of an image (.jpg file) of a multi-well plate of 6 (3x2), 12 (4x3), 24 (6x4), 48 (8x6) or 96 (12x8) wells. The color photograph of the plate should be centered at the middle point. For further details and validation against a commercial plate reader, see the Supplementary Material (Figure S3) of the literature reference cited above. Open the .jpg image from within the ImageJ software (tested with version 1.53c, Wayne Rasband, NIH, 26 June 2020). Run the plugin ReadPlate (by launching Plugins > ReadPlate).

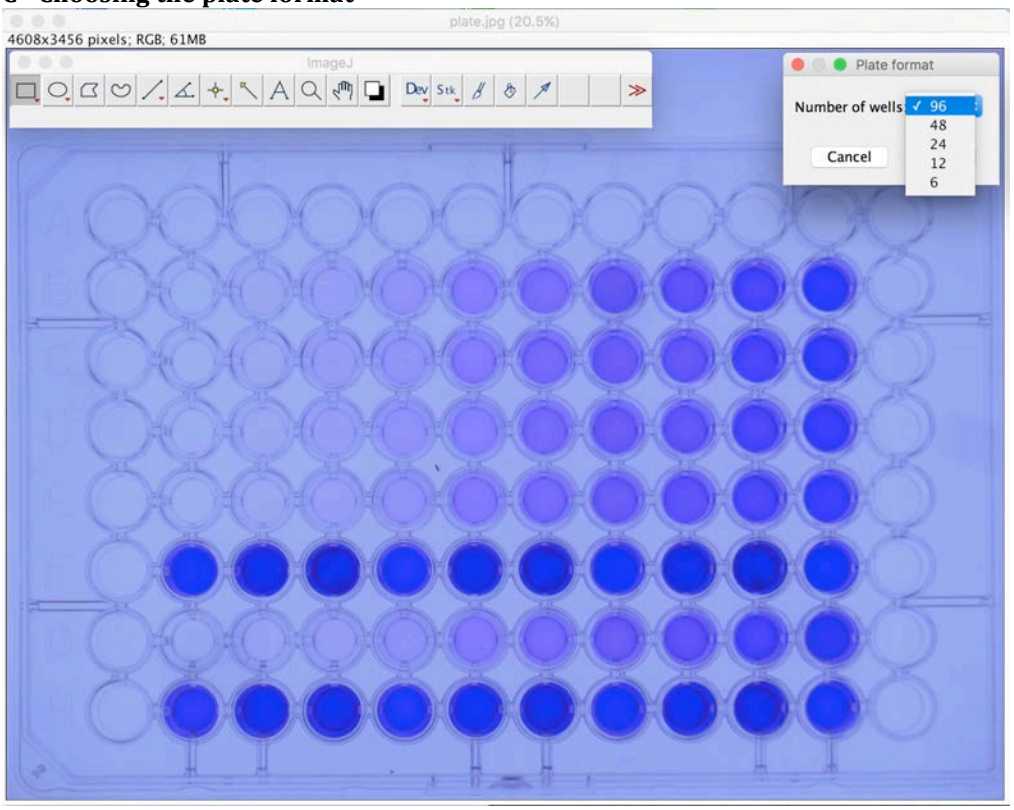
Select the correct plate format: 6(3x2), 12(4x3), 24(6x4), 48(8x6) or 96(12x8) wells (**Figure C**). Make a center-to-center rectangular selection of wells (upper-left and lower-right corners, **Figure D**). Select the desired color channel (Red, Green, Blue or Gray) for measurements (**Figure E**). Choose the appropriate parameters for the grid of circles to be measured (**Figure F**). Each circle needs not be too large, because enough color information is coded in pixels covering a relatively small area. The final grid overlaid on the plate should show main circles centered on each well surrounded by ancillary circles located outside (**Figure G**). The latter are used by the blank correction algorithm to compensate for any difference in local light intensity (**Figure H**). Check the fit of the final grid onto the plate image. If satisfactory, the user proceeds to collect measurements. A table of results will appear next (**Figure I**). This includes number, alphanumeric label and area of each well, light intensity measurements (Mean, StdDev, Mode, Min, Max), and absorbance values. Corrected absorbance values (Acorr) result from applying the blank suppression algorithm. Note that *all measurements are taken on a single image of the plate*, thus eliminating the need for taking parallel measurements on an empty plate. By default, results are formatted as .csv files, readily interpretable by Excel (**Figure J**).

A - The trans-illuminator



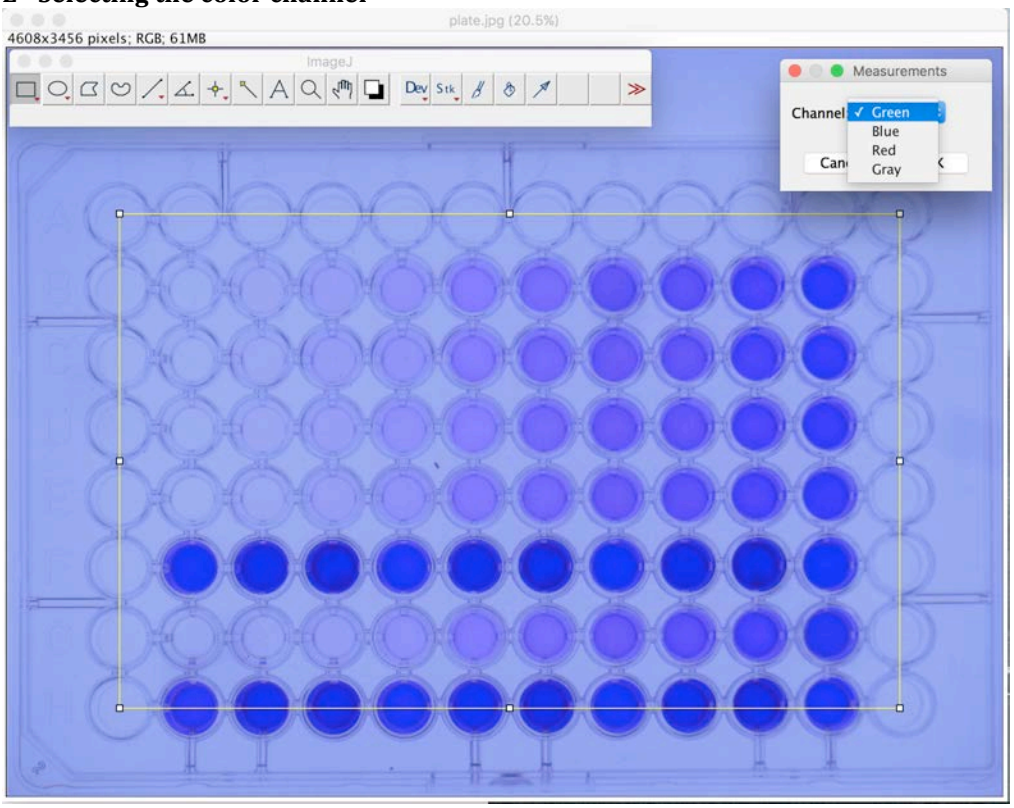
B - The black box and cameras

C - Choosing the plate format

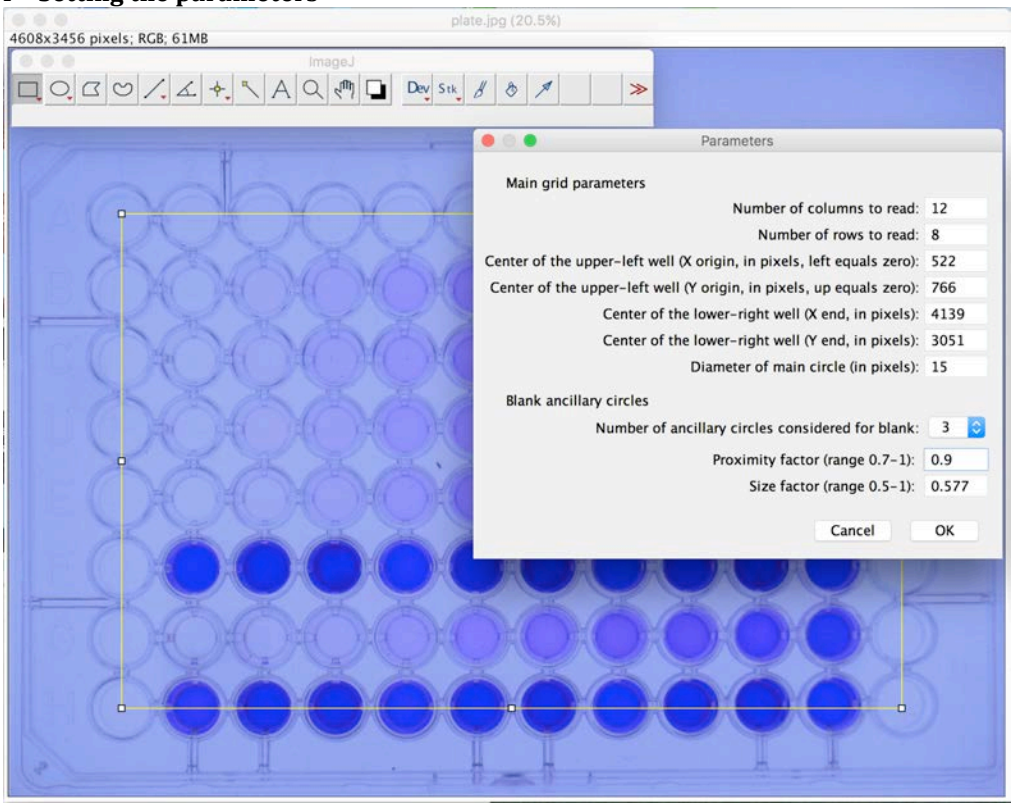


D - Making the rectangular selection

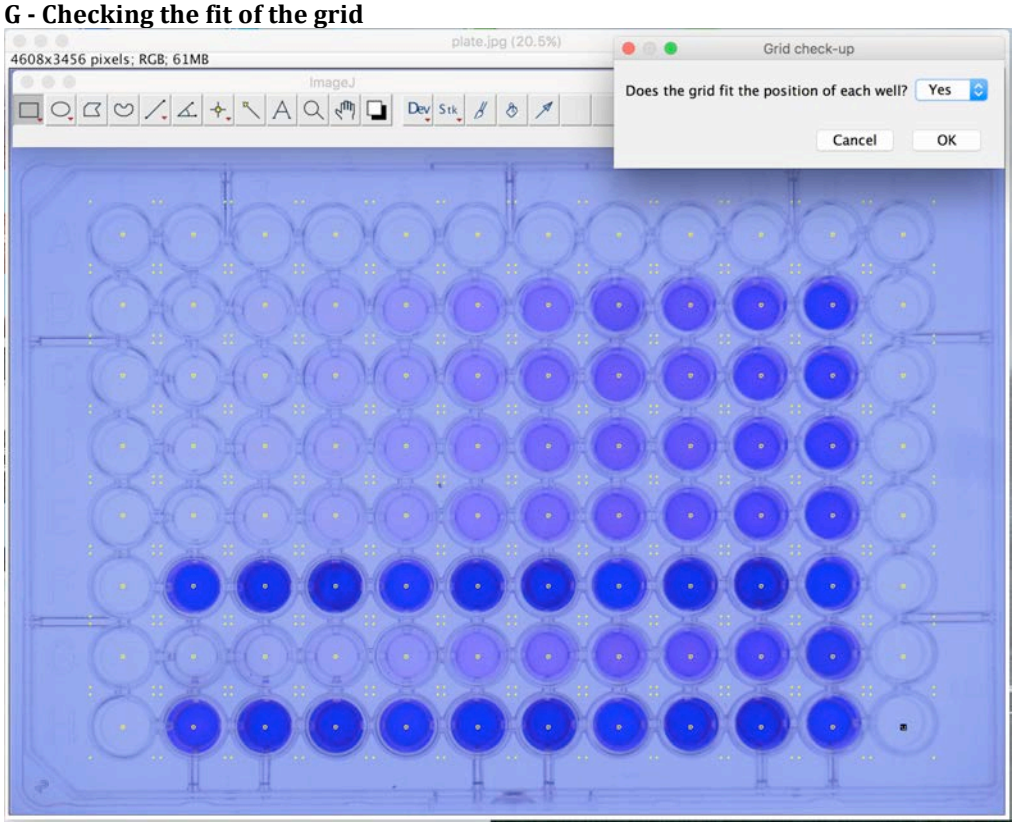
E - Selecting the color channel



F - Setting the parameters

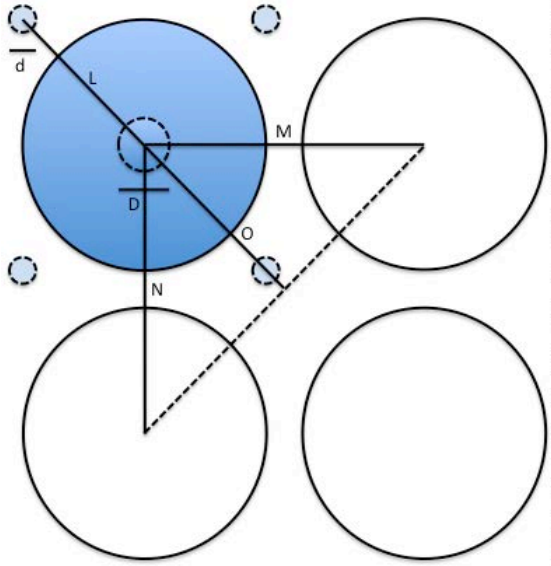


G - Checking the fit of the grid



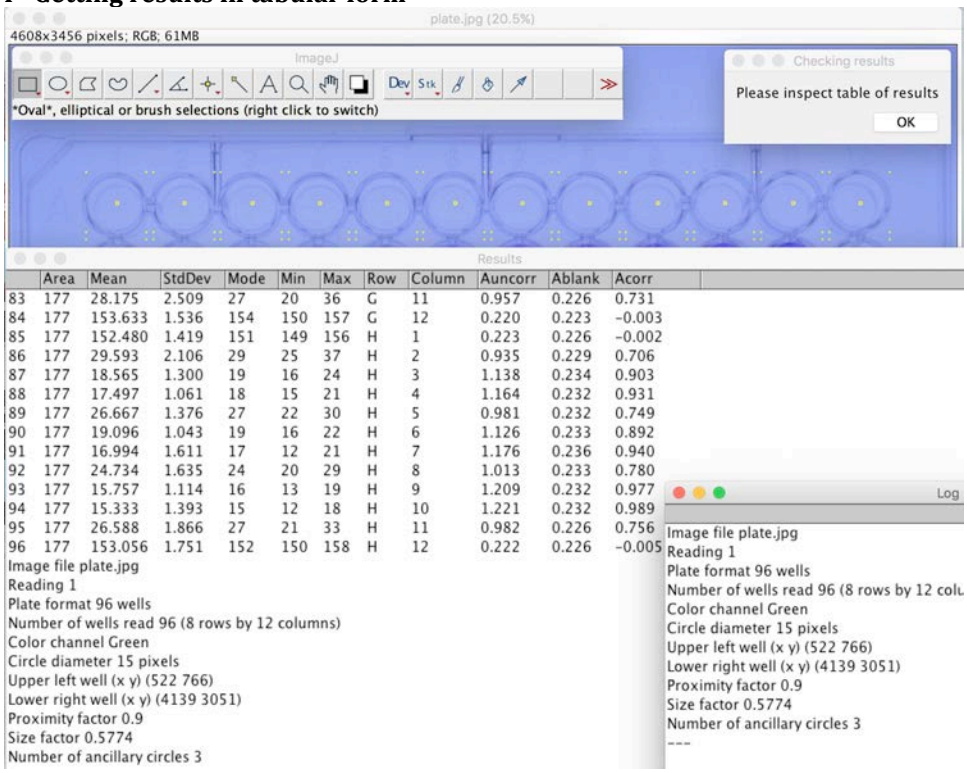
H - The blank suppression procedure

Blank suppression procedure implemented in ReadPlate3.0



The main purpose of this procedure is to correct for differences in illumination across the plate. The user defines the grid of main circles (of diameter D) to be measured. In addition, for each well the program measures light intensity in all 4 ancillary circles (of diameter d). The size and proximity to the well of the latter are set by factors g (=d/D) and f (=L/O), respectively. In addition, the user can choose any number (0-4) of ancillary circles for calculations. The mean of the higher intensity values is always used. In this fashion, artifacts that may cause abnormally lower intensity readings due to obstruction elements present by design in the plastic plate can be circumvented. The mean value of light intensity is used to calculate the absorbance of the blank (Ablank), that is subtracted from the absorbance of the main circle (Auncorr) to yield the corrected absorbance value (Acorr).

I - Getting results in tabular form



	Area	Mean	StdDev	Mode	Min	Max	Row	Column	Auncorr	Ablank	Acorr
83	177	28.175	2.509	27	20	36	G	11	0.957	0.226	0.731
84	177	153.633	1.536	154	150	157	G	12	0.220	0.223	-0.003
85	177	152.480	1.419	151	149	156	H	1	0.223	0.226	-0.002
86	177	29.593	2.106	29	25	37	H	2	0.935	0.229	0.706
87	177	18.565	1.300	19	16	24	H	3	1.138	0.234	0.903
88	177	17.497	1.061	18	15	21	H	4	1.164	0.232	0.931
89	177	26.667	1.376	27	22	30	H	5	0.981	0.232	0.749
90	177	19.096	1.043	19	16	22	H	6	1.126	0.233	0.892
91	177	16.994	1.611	17	12	21	H	7	1.176	0.236	0.940
92	177	24.734	1.635	24	20	29	H	8	1.013	0.233	0.780
93	177	15.757	1.114	16	13	19	H	9	1.209	0.232	0.977
94	177	15.333	1.393	15	12	18	H	10	1.221	0.232	0.989
95	177	26.588	1.866	27	21	33	H	11	0.982	0.226	0.756
96	177	153.056	1.751	152	150	158	H	12	0.222	0.226	-0.005

Image file plate.jpg
Reading 1
Plate format 96 wells
Number of wells read 96 (8 rows by 12 columns)
Color channel Green
Circle diameter 15 pixels
Upper left well (x y) (522 766)
Lower right well (x y) (4139 3051)
Proximity factor 0.9
Size factor 0.5774
Number of ancillary circles 3

Image file plate.jpg
Reading 1
Plate format 96 wells
Number of wells read 96 (8 rows by 12 colu
Color channel Green
Circle diameter 15 pixels
Upper left well (x y) (522 766)
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Proximity factor 0.9
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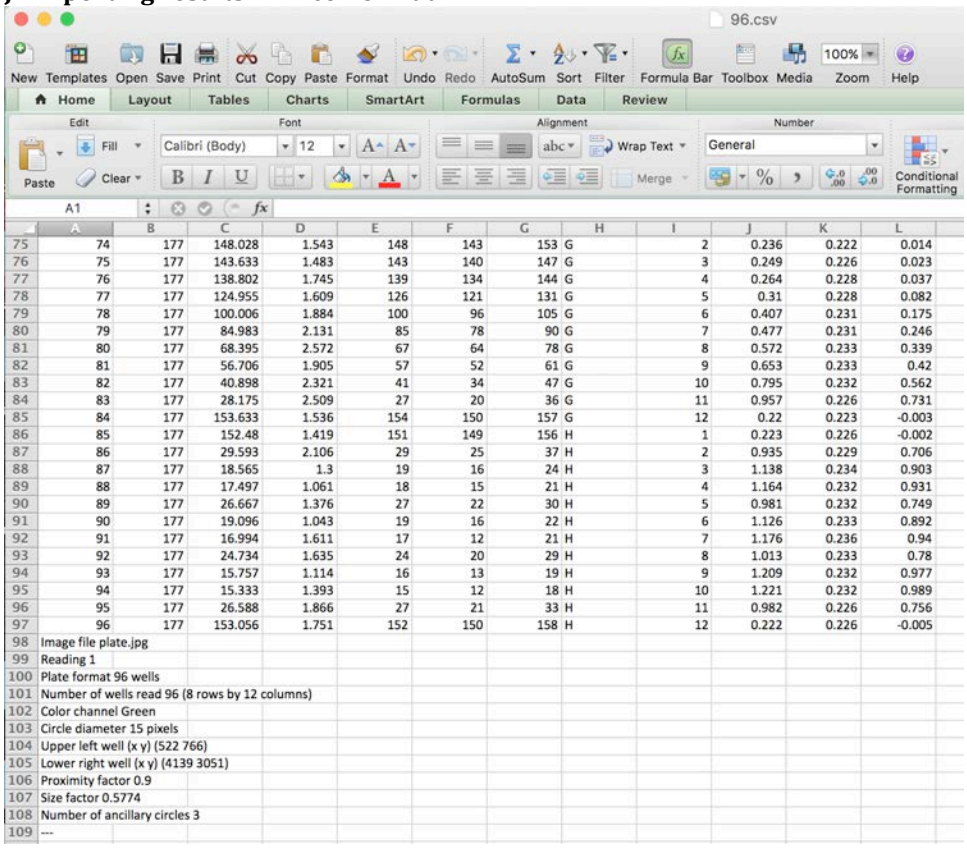
J - Exporting results in Excel format



	A	B	C	D	E	F	G	H	I	J	K	L
75	74	177	148.028	1.543	148	143	153	G	2	0.236	0.222	0.014
76	75	177	143.633	1.483	143	140	147	G	3	0.249	0.226	0.023
77	76	177	138.802	1.745	139	134	144	G	4	0.264	0.228	0.037
78	77	177	124.955	1.609	126	121	131	G	5	0.31	0.228	0.082
79	78	177	100.006	1.884	100	96	105	G	6	0.407	0.231	0.175
80	79	177	84.983	2.131	85	78	90	G	7	0.477	0.231	0.246
81	80	177	68.395	2.572	67	64	78	G	8	0.572	0.233	0.339
82	81	177	56.706	1.905	57	52	61	G	9	0.653	0.233	0.42
83	82	177	40.898	2.321	41	34	47	G	10	0.795	0.232	0.562
84	83	177	28.175	2.509	27	20	36	G	11	0.957	0.226	0.731
85	84	177	153.633	1.536	154	150	157	G	12	0.22	0.223	-0.003
86	85	177	152.48	1.419	151	149	156	H	1	0.223	0.226	-0.002
87	86	177	29.593	2.106	29	25	37	H	2	0.935	0.229	0.706
88	87	177	18.565	1.3	19	16	24	H	3	1.138	0.234	0.903
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109	---											