

ImageJ Protocol for Measuring Male Coloration

July 15, 2019

1. Open the original image (drag and drop) in Fiji/ImageJ
2. Draw a square selection around the white square on the color scale and balance the background.
Make sure not to select any white with a water glare; this will distort the light in the image.

Plugins > BIOP > BIOP SimpleColorBalance

3. Open the ROI Manager. **Analyze > Tools > ROI Manager.**
4. Select regions of interest (ROIs).

Use selection tool, select a region and add it to the ROI list by clicking “Add” button

Check “Show All” and “Labels” options in the ROI Manager window.

Select the red square

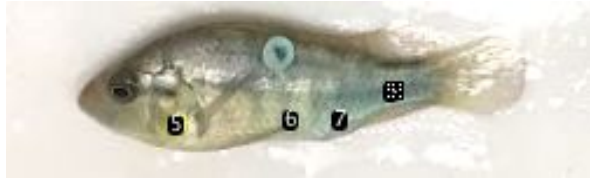
“Add” selection

Rename the selection (“red”)

Repeat 4a-4c for the yellow, light gray, and blue squares (see below)



5. Select 4 ROIs on the cichlid body



Rename ROIs - **R1, R2, R3, R4**

6. Convert the image to a multi-channel stack. **Image > Color > Make Composite**

You want statistics from the 3 color channels separately. Use the slider at the bottom of the image window to access the 3 color channels.

7. Measure the statistics of the marked regions.

Click "Measure" in the ROI manager. This will display the statistics in the Results window for the color channel currently displayed in the image window. Slide to the next channel, and click on the "Measure" button again for each color channel. It is easiest for data transfer to select each ROI in the ROI manager one at a time and measure each color channel.

8. Name the results in the Results window, **Results>Set Measurements**. Check this option after the first measurement (so that the Results window pops up), clear the results and then start over so that you will have labels for all your measurements.

Select:

- i. Area
- ii. Min and max gray value
- iii. Mean gray value
- iv. Modal gray value
- v. Display label
- vi. Median
- vii. Add to overlay

Repeat for all 3 color channels

9. Save as csv

Click on Results window and save. **File > Save As**

Save as "file name"_color.csv

10. Open csv file and edit as follows (Using example “Gar_M1_June13_color.csv

	photo	label	color channel	Area	Mean	Mode	Min	Max	Median
1	Gar_M1_June13	red_swatch	red	76960	235.743	234	189	255	235
2	Gar_M1_June13	yellow_swat	red	63168	232.717	228	215	255	232
3	Gar_M1_June13	light_grey_s	red	57024	148.409	145	131	171	147
4	Gar_M1_June13	blue_swatch	red	77480	56.284	57	34	82	57
5	Gar_M1_June13	R1	red	440	159.011	145	136	192	154
6	Gar_M1_June13	R2	red	550	192.345	186	176	221	190
7	Gar_M1_June13	R3	red	322	181.311	184	135	196	183
8	Gar_M1_June13	R4	red	462	120.957	120	91	168	120
9	Gar_M1_June13	R5	red	380	112.268	101	86	150	110
10	Gar_M1_June13	red_swatch	green	76960	95.908	90	40	243	91
11	Gar_M1_June13	yellow_swat	green	63168	238.372	234	220	255	238
12	Gar_M1_June13	light_grey_s	green	57024	157.177	154	138	178	156
13	Gar_M1_June13	blue_swatch	green	77480	120.215	120	98	144	120
14	Gar_M1_June13	R1	green	440	155.195	137	130	187	152
15	Gar_M1_June13	R2	green	550	185.633	180	171	214	183
16	Gar_M1_June13	R3	green	322	177.587	182	129	196	178
17	Gar_M1_June13	R4	green	462	130.158	129	101	177	129
18	Gar_M1_June13	R5	green	380	122.895	114	97	159	122
19	Gar_M1_June13	red_swatch	blue	76960	101.241	95	49	245	96
20	Gar_M1_June13	yellow_swat	blue	63168	97.994	93	74	153	96
21	Gar_M1_June13	light_grey_s	blue	57024	161.524	157	143	185	160
22	Gar_M1_June13	blue_swatch	blue	77480	169.046	170	142	191	170
23	Gar_M1_June13	R1	blue	440	146.477	126	120	181	145
24	Gar_M1_June13	R2	blue	550	165.327	161	146	196	163
25	Gar_M1_June13	R3	blue	322	166.056	165	112	183	166
26	Gar_M1_June13	R4	blue	462	122.067	114	94	170	121
27	Gar_M1_June13	R5	blue	380	114.697	108	90	152	113

Order by label then color channel

photo	label	color channel	Area	Mean	Mode	Min	Max	Median
Gar_M1_June13	red_swatch	red	76960	235.743	234	189	255	235
Gar_M1_June13	red_swatch	green	76960	95.908	90	40	243	91
Gar_M1_June13	red_swatch	blue	76960	101.241	95	49	245	96
Gar_M1_June13	yellow_swatch	red	63168	232.717	228	215	255	232
Gar_M1_June13	yellow_swatch	green	63168	238.372	234	220	255	238
Gar_M1_June13	yellow_swatch	blue	63168	97.994	93	74	153	96
Gar_M1_June13	blue_swatch	red	77480	56.284	57	34	82	57
Gar_M1_June13	blue_swatch	green	77480	120.215	120	98	144	120
Gar_M1_June13	blue_swatch	blue	77480	169.046	170	142	191	170
Gar_M1_June13	light_grey_swatch	red	57024	148.409	145	131	171	147
Gar_M1_June13	light_grey_swatch	green	57024	157.177	154	138	178	156
Gar_M1_June13	light_grey_swatch	blue	57024	161.524	157	143	185	160
Gar_M1_June13	R1	red	440	159.011	145	136	192	154
Gar_M1_June13	R1	green	440	155.195	137	130	187	152
Gar_M1_June13	R1	blue	440	146.477	126	120	181	145
Gar_M1_June13	R2	red	550	192.345	186	176	221	190
Gar_M1_June13	R2	green	550	185.633	180	171	214	183
Gar_M1_June13	R2	blue	550	165.327	161	146	196	163
Gar_M1_June13	R3	red	322	181.311	184	135	196	183
Gar_M1_June13	R3	green	322	177.587	182	129	196	178
Gar_M1_June13	R3	blue	322	166.056	165	112	183	166
Gar_M1_June13	R4	red	462	120.957	120	91	168	120
Gar_M1_June13	R4	green	462	130.158	129	101	177	129
Gar_M1_June13	R4	blue	462	122.067	114	94	170	121
Gar_M1_June13	R5	red	380	112.268	101	86	150	110
Gar_M1_June13	R5	green	380	122.895	114	97	159	122
Gar_M1_June13	R5	blue	380	114.697	108	90	152	113

For each label, look at median R / median (R + G + B), median G / median (R+G+B), and median B / median (R+G+B).

photo	label	color channel	Area	Mean	Mode	Min	Max	Median	R/R+G+B	G/R+G+B	B/R+G+B
Gar_M1_June13	red_swatch	red	76960	235.743	234	189	255	235	0.55687204	0.21563981	$=14/((12+13+14))$
Gar_M1_June13	red_swatch	green	76960	95.908	90	40	243	91			
Gar_M1_June13	red_swatch	blue	76960	101.241	95	49	245	96			
Gar_M1_June13	yellow_swatch	red	63168	232.717	228	215	255	232			
Gar_M1_June13	yellow_swatch	green	63168	238.372	234	220	255	238			
Gar_M1_June13	yellow_swatch	blue	63168	97.994	93	74	153	96			
Gar_M1_June13	blue_swatch	red	77480	56.284	57	34	82	57			
Gar_M1_June13	blue_swatch	green	77480	120.215	120	98	144	120			
Gar_M1_June13	blue_swatch	blue	77480	169.046	170	142	191	170			
Gar_M1_June13	light_grey_swatch	red	57024	148.409	145	131	171	147			
Gar_M1_June13	light_grey_swatch	green	57024	157.177	154	138	178	156			
Gar_M1_June13	light_grey_swatch	blue	57024	161.524	157	143	185	160			
Gar_M1_June13	R1	red	440	159.011	145	136	192	154			
Gar_M1_June13	R1	green	440	155.195	137	130	187	152			
Gar_M1_June13	R1	blue	440	146.477	126	120	181	145			
Gar_M1_June13	R2	red	550	192.345	186	176	221	190			
Gar_M1_June13	R2	green	550	185.633	180	171	214	183			
Gar_M1_June13	R2	blue	550	165.327	161	146	196	163			
Gar_M1_June13	R3	red	322	181.311	184	135	196	183			
Gar_M1_June13	R3	green	322	177.587	182	129	196	178			
Gar_M1_June13	R3	blue	322	166.056	165	112	183	166			
Gar_M1_June13	R4	red	462	120.957	120	91	168	120			
Gar_M1_June13	R4	green	462	130.158	129	101	177	129			
Gar_M1_June13	R4	blue	462	122.067	114	94	170	121			
Gar_M1_June13	R5	red	380	112.268	101	86	150	110			
Gar_M1_June13	R5	green	380	122.895	114	97	159	122			
Gar_M1_June13	R5	blue	380	114.697	108	90	152	113			

photo	label	color channel	Area	Mean	Mode	Min	Max	Median	R/R+G+B	G/R+G+B	B/R+G+B
Gar_M1_June13	red_swatch	red	76960	235.743	234	189	255	235	0.55687204	0.21563981	0.22748815
Gar_M1_June13	red_swatch	green	76960	95.908	90	40	243	91			
Gar_M1_June13	red_swatch	blue	76960	101.241	95	49	245	96			
Gar_M1_June13	yellow_swatch	red	63168	232.717	228	215	255	232	0.40989399	0.4204947	0.16961131
Gar_M1_June13	yellow_swatch	green	63168	238.372	234	220	255	238			
Gar_M1_June13	yellow_swatch	blue	63168	97.994	93	74	153	96			
Gar_M1_June13	blue_swatch	red	77480	56.284	57	34	82	57	0.16426513	0.34582133	0.48991354
Gar_M1_June13	blue_swatch	green	77480	120.215	120	98	144	120			
Gar_M1_June13	blue_swatch	blue	77480	169.046	170	142	191	170			
Gar_M1_June13	light_grey_swatch	red	57024	148.409	145	131	171	147	0.3174946	0.33693305	0.34557235
Gar_M1_June13	light_grey_swatch	green	57024	157.177	154	138	178	156			
Gar_M1_June13	light_grey_swatch	blue	57024	161.524	157	143	185	160			
Gar_M1_June13	R1	red	440	159.011	145	136	192	154	0.34146341	0.33702882	0.32150776
Gar_M1_June13	R1	green	440	155.195	137	130	187	152			
Gar_M1_June13	R1	blue	440	146.477	126	120	181	145			
Gar_M1_June13	R2	red	550	192.345	186	176	221	190	0.35447761	0.34141791	0.30410448
Gar_M1_June13	R2	green	550	185.633	180	171	214	183			
Gar_M1_June13	R2	blue	550	165.327	161	146	196	163			
Gar_M1_June13	R3	red	322	181.311	184	135	196	183	0.34724858	0.33776091	0.31499051
Gar_M1_June13	R3	green	322	177.587	182	129	196	178			
Gar_M1_June13	R3	blue	322	166.056	165	112	183	166			
Gar_M1_June13	R4	red	462	120.957	120	91	168	120	0.32432432	0.34864865	0.32702703
Gar_M1_June13	R4	green	462	130.158	129	101	177	129			
Gar_M1_June13	R4	blue	462	122.067	114	94	170	121			
Gar_M1_June13	R5	red	380	112.268	101	86	150	110	0.31884058	0.35362319	0.32753623
Gar_M1_June13	R5	green	380	122.895	114	97	159	122			
Gar_M1_June13	R5	blue	380	114.697	108	90	152	113			