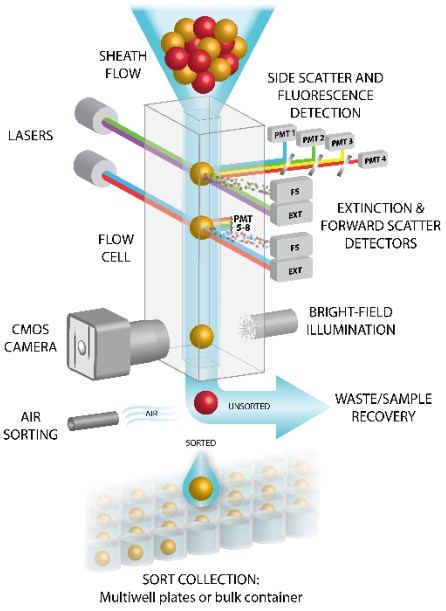
Investigation of the relationship of Aiptasia and its symbiont algae using chlorophyll autofluorescence identified by the COPAS Vision Flow Cytometer.



Objective

Since the discovery that coral systems rely on complex relationships between species, there has been a desire to better understand the symbiotic requirements to generate and maintain a robust coral system. While these relationships are still not well understood, the anemone *Aiptasia* and its symbiont algae have become a useful model in which to probe these questions. In this experiment, the COPAS Vision was used to identify the auto-fluorescence of the chlorophyll expressed by the algae residing within the *Aiptasia* larvae. In this case, the researcher was interested in identifying if algae were detectable in the *Aiptasia* larvae, if there is variation corresponding to units of algae, and can these animals be collected for downstream analysis or other use.

Introduction

The COPAS family of research instrumentation comprises a collection of large particle cytometers utilizing at least 1 excitation laser and up to 8 channels of fluorescence collection. Unique to COPAS instrumentation is the Profiling feature which graphically plots the fluorescence intensity changes along the object as it passes through the laser(s). Large particles and objects up to 1.5mm in diameter can be analyzed for physical and fluorescence characteristics and gently dispensed into a multi-well plate or other collection container for further investigation or reuse. The COPAS Vision also has equipped a camera to take an image of the object inside the flow channel. This image accompanies the cytometry data and can be analyzed using Union Biometrica's FlowPilot software or other image analysis tools.

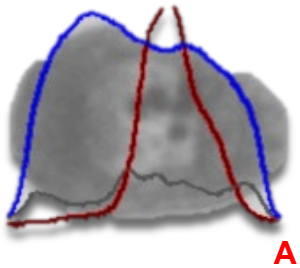
Methods

The Phillip Cleves lab at Carnegie Institute, provided 3 strains of *Aiptasia:* ssb01, mf2.2b, and BLIMP cultured for 8 days. Samples were then suspended in ocean water and run on the COPAS Vision flow cytometer utilizing the 1000um flow cell with 4 excitation lasers 405, 488, 561, 640nm and 12 channels of cytometry collection along the object's length of Time of Flight (TOF). Collected parameters include: EXT-Extinction or optical density, FS-Forward scatter, and 8 channels of fluorescence collection across the visible spectrum. The 488nm excitation laser and a 680/42nm emission filter were used to excite and detect the auto-fluorescence of chlorophyll in the algae symbiont of the *Aiptasia* larvae.

COPAS Vision Results

COPAS Vision instrument was able to identify varying levels of chlorophyll present in *Aiptasia* larvae. Figure 2A shows the image and profile graph (both collected by the Vision) of an *Aiptasia* larva with an overlay of the fluorescence profile graph--the red peak (chlorophyll auto-fluorescence) corresponds to the location of the algae contained in the larva.

Figure 3 displays the size distribution and chlorophyll auto-fluorescence peak intensity of the ssb01 sample collection. Quadrants drawn can be statistically analyzed as well as used for sorting criteria. The overlayed 4 regions comprised of various size and fluorescence levels were selected and individuals from these



A

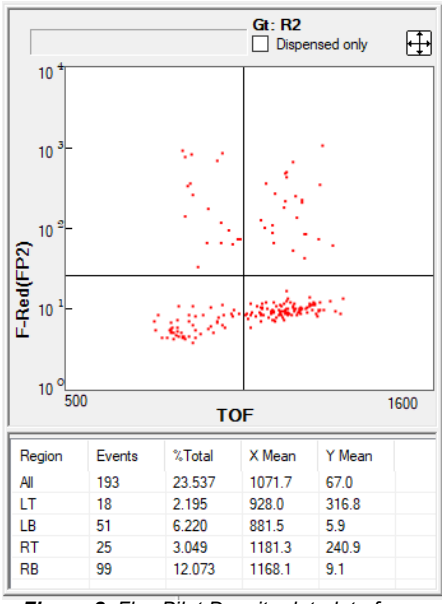
Parameter	Value
ROI width	131.03
ROI height	93.79
Area	8869.44
Perimeter	365.23
Roughness	835.54
Mean grayscale	47.94
Min. bounding box width	126.90
Min. bounding box height	89.66

B

Channel	Param...	Value
	Tof	1179
	# slices	296
	PkHt	47148
	PkHt	7594
	PkHt	87
	PkHt	175
	PkHt	495
	PkHt	47400
	Width	1128
	#Pks	N/A
	In gate	Yes
	PArea	0
	PkHt	44
	PkHt	70
	PkHt	185
	PkHt	16111
	PkHt	43514
	PkHt	11502

C

Figure 2 from FlowPilot software: *A) individual Aiptasia BLIMP larva with corresponding profile overlay of EXT (blue), FS (gray) and F-Red fluorescence at 680nm (red), B) image analysis of the larva. C) list of cytometry measurements for single larva: 12 channels: Ext, FS, and Fluorescence) and accompanying profile peak analysis (Width, #peaks, gate and peak area) if any.*



Region	Events	%Total	X Mean	Y Mean
All	193	23.537	1071.7	67.0
LT	18	2.195	928.0	316.8
LB	51	6.220	881.5	5.9
RT	25	3.049	1181.3	240.9
RB	99	12.073	1168.1	9.1

Figure 3 *FlowPilot Density dot plot of Aiptasia strain ssb01 displaying TOF (time of flight) on the x-axis and F-Red fluorescence (680/42nm) on the Y-axis in log scale.*

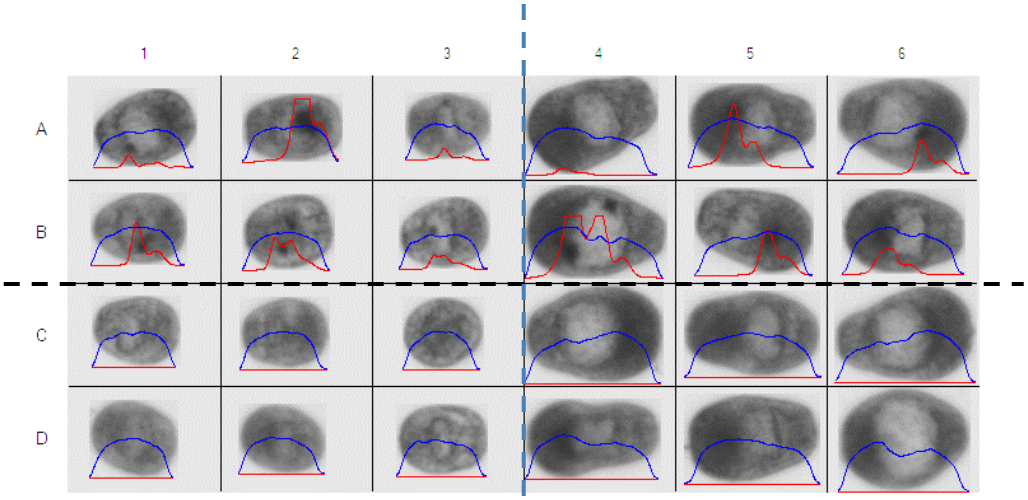


Figure 4: *Images of ssb01 strain of Aiptasia collected by the Vision instrument along with their accompanying fluorescence profile overlayed on each. 4 Quadrants corresponding to those in figure 3 are separated by the blue dashed lines.*

regions were dispensed individually to the wells of a multi-well plate. Figure 4 shows representative larvae images and their fluorescence profile graph from the 4 quadrants. We make 4 assertions based on cytometry and image collection. 1) It is clear that the size distribution corresponds to the shorter/rounder lava while those more elongated comprise a different region of the sample distribution. 2) Interestingly though, the level of fluorescence is not scaled with the length or density of the different sized larva. 3) There is a clear separation between larva with or without fluorescence regardless of size, although images of those with and without fluorescence don't appear to be morphologically different. This would suggest the fluorescence (at 680/42nm) is an attribute of the algae alone (auto-fluorescence of its chlorophyll). 4) The peak(s) of fluorescence correspond closely to the darker dense areas in the Vision collected image; it can be theorized these darker areas are the algae units visible in the image. Finally, if the algae units are separated by enough space to present as multiple peaks (such as in well B4 of Figure 3), the Profiler feature will identify and count those peaks as another distinct parameter which can be used for analysis and dispense criteria.

Conclusions

The COPAS Vision was used successfully to identify algae units residing inside *Aiptasia* larvae. Different sizes and intensities of auto-fluorescence were dispensed alive. Inspection of Vision's collected images matched to their fluorescence profile suggests the images, in tandem with fluorescence values, can be a valuable tool for investigation of the symbiotic relationship between *Aiptasia* and its symbiont. It might be possible for the COPAS Vision to be used to surveille the health or robustness of a native or lab-based collection of *Aiptasia*. Or it may be possible, having identified attributes of healthy animals, to dispense large numbers of individuals hearty enough to seed/reseed coral environments. The success of this experiment opens a range of uses for the COPAS Vision with this and other aquatic samples.

Work performed in 2022, by:
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