Include sample sizes

Methods description:
We would airbrush collected coral fragments from each species and create a tissue homogenate consisting of Symbiodiniaceae cells and phosphate-buffered saline (PBS).
Next, we would aliquot 500 uL and centrifuge to concentrate the algal pellet. Take out supernatant, and resuspend in PBS. I would then use these aliquots to count on a haemocytometer. Then optimize side and forward scatter, and fluorescence settings on the flow cytometer (BD FACSVerse) to isolate Symbiodiniaceae cells from debris. We would repeat this process for all four species, as each species has varying types of symbionts, so we can assume the settings will be different. The BD FACSVerse flow cytometer system has a two laser system (488 nm and 640 nm) that supports 8 different parameters. We can run about 30-40 tube samples at one time, or if using microtiter plates we can use either a 96 well-microtiter plate or 384 well-microtiter plates.

Our cell standards: 1-milliliter aliquots of sample

Species we plan to use are: *Astrangia poculata, Pocillopora acuta, Montipora capitata, Porites astreoides*

Potential diving plan for astrangia: sym vs apo (brown vs white)

- Kevin has previous experience

Dilution Test Plan:
Rosenthal et al. 2017, "Cell sorting was performed with 100 μm nozzle size and sorted directly into 5 ml tubes containing 3 ml of staining media in order to minimize cellular stress. Cells (10,000–40,000) of each population of interest were sorted at a speed of 1500 cells/s."

Krediet et al 2015, "3-fold with 0.1% SDS in ASW (flow-cytometer experiment) and needle sheared...The supernatant was removed, and the algal pellet was resuspended in 0.01% SDS in dH2O using a 25 G needle and 1-ml syringe."

Symbiont cell description:

- The size of our particles of interest are
-
- Type of fluorescence

& then b/c of that type of fluorescence we will use ….

The wavelengths we should have the samples fluoresce at can be either species-specific or non-species-specific. Common wavelengths of 488or 405 nm are used

- 480 nm blue laser

Definitions of:

- Side vs forward scatter:

"Forward versus **side scatter** (FSC vs SSC) gating is commonly used to identify cells of interest based on size and granularity (complexity). It is often suggested that forward **scatter** indicates cell size whereas **side scatter** relates to the complexity or granularity of the cell." -biorad

Paper	**FlowCy Used**	**Species used**	**Laser specs**	**Wavelength**	**Forward scatter**	**Side scatter**	**Particles of interest**
Snyder et al 2020 JOVE							
Rosenthal et al 2017 BMC	BD FACS Aria II	*Pocillopora damicornis* *Aiptasia pallida (*sea anemone)	488 nm and 640 nm 405 nm on live specimen	Far-Red channels	Not sure	Not sure	Tested 30 fluorescent markers, identified 12 coral cell populations
Krediet et al 2015 PLOS ONE	Guava Flow Cytometer Also compared determinations of algal numbers with hemocytometer, Dinofinder image-analysis	Cultured nubbins of *Pocillopora damicornis*	Blue laser: 488 nm	Red flurorescence	Set to 9.93	Set to 4.0	Used similar protcol that had been previously used on sea anemones *A. pallida*

	program, and Coulter Counter						
Lee et al 2012 cytometry	MoFlo™ XDP cell sorter (Beckman Coulter®, USA)	*Pocillopora damicornis*	40 mW at 488 nm	Red and green fluorescence	Comparison of cell size	Not sure what this means	High correlations between FCM and microscopy data (very accurate)

Comparison to haemocytometer:

Statistical analyses:

For Lee et al 2012., "All statistical analyses were executed on STATISTICA v10 (Statsoft 2010) on untransformed data after checking for normality and homogeneity of variances. Two-way analysis of variance (ANOVA) was used to examine the effects of incubation duration and temperature as fixed, categorical factors on cell counts, and the relative intensities of chlorophyll and β-carotene fluorescence"
https://onlinelibrary.wiley.com/doi/full/10.1002/cyto.a.22111

For Rosenthal et al 2017, they used a LysoTracker to measure immune-associated functional assays of phagocytic cells to validate the down-stream process of data received from FACS.
https://link.springer.com/article/10.1186/s12860-017-0146-8

For Krediet et al 2015, "The data generated in each experiment were imported into Prism 6.0a (GraphPad Software). Replicates were averaged and the standard errors of the means were calculated."
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0135725

Hannah plays with flow cy 10/21/2020
Mcap396 (1 mL sample, 2 mL PBS)

- Undiluted 0.5 mL
- In future no cell strainer necessary. Just spot check under microscope for junk
- Used APC-Cy7-A and APC-A filter
- Kev & Dennis used FITC-H (by height) and FITC-A (by area) filters in 2019

- PMTV settings = FSC laser 212.1; SSC 247.7
- Identified 2 populations, p2 was clustered near 0 (probs junk), p1 was the symbiopals
- Goal is to have 10,000 “events” (aka cells/ counts)

Next steps - flow cy

- Bring a flashdrive!
- Poc, porites, mont
- No cell strainer
- Aim for 10,000 cells
- Use flow cy software to actually calculate precise # of cells

Next steps - cellometer (use 20uL of sample)

- Contact Nexelcom with goals and ask for protocol

Cost comparison

- Hemocytometer: 12/hour (human) * 2 samples an hour = $6/ sample
- Cellometer: $250 for 100 slides. $5 hour to operate machine.
- FACS: :-) . $20 hour to operate machine plus price per tubes ($75 for ??).