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Quantification of Marine Virus by Flow Cytometer

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PURPOSES

- Rapid quantification of viral abundance with high precision is essential to the better understanding of marine viral ecology, including viral production, viral lysis, and burst rate.
- Flow cytometry analysis is efficient and precise to quantify nanoparticles in water, representing a promising tool in marine virology.

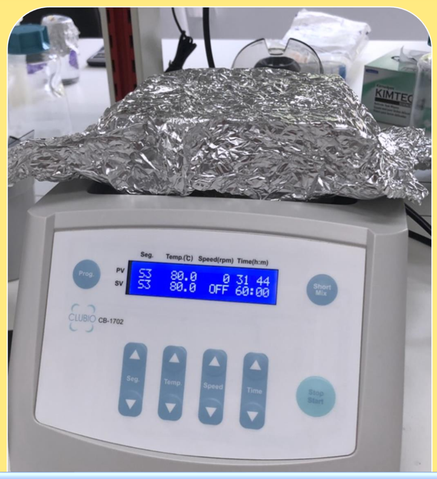
MATERIAL AND METHODS

Sample Treatment by

- Staining sample : 1% commercial stock of SYBR GREEN 1
- Diluting sample : TE buffer pH 8.0

Sample Dilution and Staining

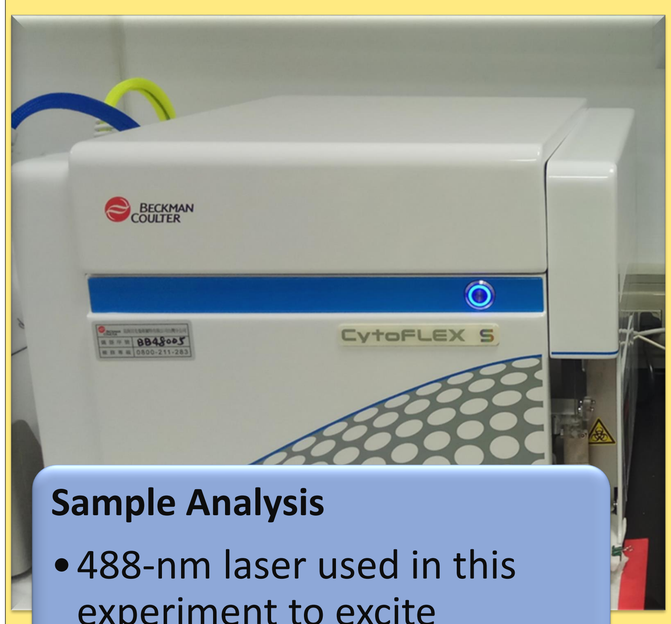
- 10x Sample Dilution : 895.5μl TE Buffer + 99.5μl sample
- Staining: 5μl (1%) SYBR GREEN I + 995μl diluted sample



Incubation

- Using thermo-shaker to heat up stained sample in 80℃ for 10min in the dark.
- Cooling up the sample prior analysis required

INSTRUMENT



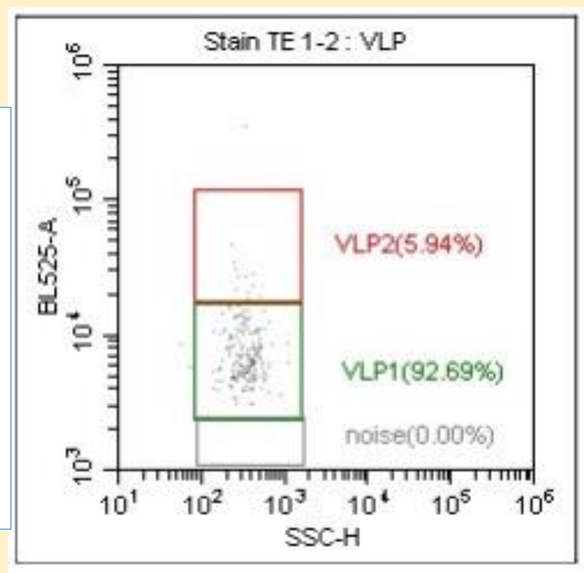
Sample Analysis

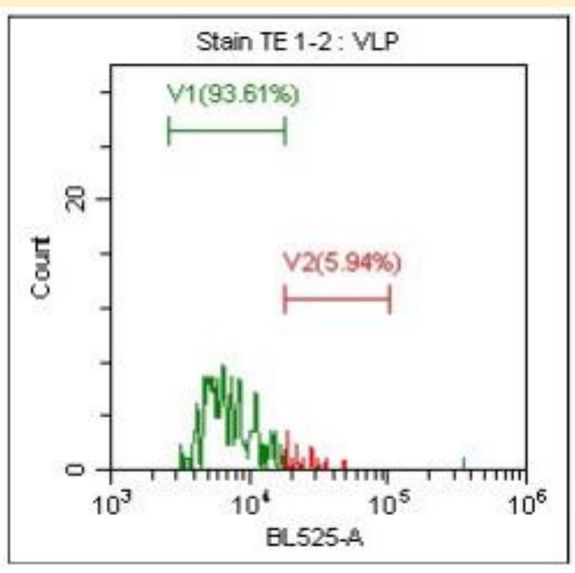
- 488-nm laser used in this experiment to excite fluorophore
- 525-nm laser was emitted after excitation of fluorophore

RESULT AND DISCUSSION

Blank (TE buffer + SYBR Green I)

Blue Light 525 – Area (BL525-A) is indicating the intensity of green light emission by fluorophore (SYBR GREEN I). Side scatter (SSC) indicates the size of particles.

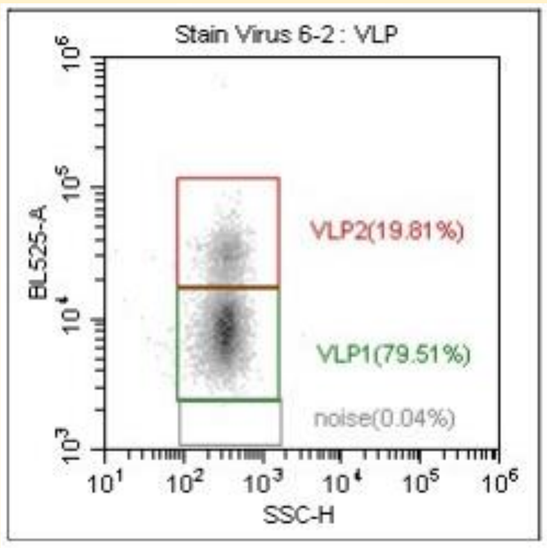


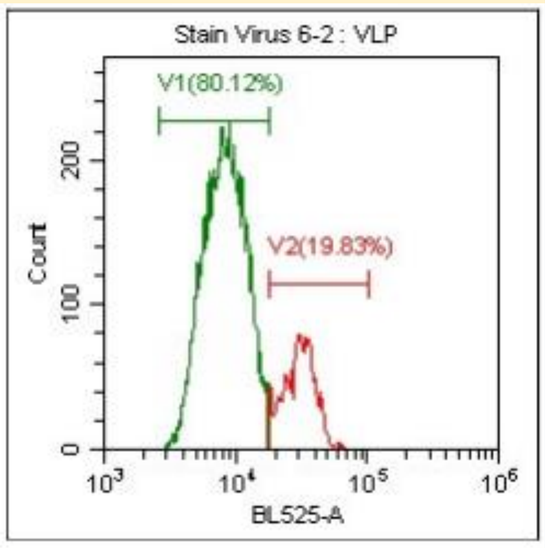


Count (number of events), collected from the VLP1 and VLP2 window mark as the interest particle region.

Marine Viruses Collected near Orchid Island

From this graph we can clearly separate Virus-like particles in the sample into 2 different virus groups by comparing their SYBR GREEN I signal intensity: VLP1 population have lower concentration of nucleic acids and VLP2 have higher concentration of nucleic acids.





Total virus total counts were obtained by correcting the total counts with TE buffer noises. Data gathered and analyzed by Cytoflex (software) , indicate the total virus count for this sample is 1.6 virus (x 10^6 particles/mL)

APPPLICATION OF VIRUS QUANTIFICATION BY FLOW CYTOMETRY

- Viruses are abundant in every environment on the Earth. They are important to the production and biogeochemistry in the oceans since they are one of the major sources of bacterial and protist mortality.
- With flow cytometer detection and counting: accuracy and efficiency of viral counting and analysis is improved

FUTURE WORK

- Applying flow cytometer to evaluating the balance of viral production and viral decay.
- Analysing viral life strategies within the host.