IMOS Flow Cytometry Instrument Comparison

FacsCanto II and CytoSub flow cytometers

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Executive summary

As part of the Integrated Marine Observation System (IMOS), the National Reference Station (NRS) network involves regularly repeated (monthly to quarterly, depending on the NRS) seawater sampling from multiple locations around Australia. Since 2009, samples have been analysed by flow cytometry along with other biogeochemical analyses. During 2016 a change occurred in the instrument used to analyse these flow cytometry samples, from a BD FacsCanto II to a CytoBuoy CytoSub. These instruments are very different in their specifications, with each having their own strengths and weaknesses for the purposes of measuring IMOS NRS samples. As a result of this change, it is important to calibrate between instruments to ensure consistency of data.

To this end, selected IMOS samples collected between 2013 and 2016 were analysed in parallel using both the FacsCanto II and the CytoSub instruments. The resulting data comparison has been used to explain the differences between instruments and to ascertain if, and to what extent, conversion factors could be applied when using the IMOS NRS flow cytometry data set across the temporal range where results from both instruments have been reported. In determining any conversion factors that may be applied, several levels of data were interrogated: all NRSs combined; by NRS only; by taxonomic grouping only; and by NRS and taxonomic grouping.

When all NRS data was combined or when data was separated by NRS, conversion factors were very small – less than 10% apart from Port Hacking Bay (PHB) which was just above 20%. When data was separated by taxonomic grouping, a distinct difference in the measured values was seen between the two instruments and therefore the scale of conversion factor from *ca.* 5% for *Synechococcus* to *ca.* 40% for *Prochlorococcus* and the Picoeukaryotes. Interestingly, the CytoSub underestimated *Prochlorococcus* relative to the FacsCanto whereas the FacsCanto underestimated the Picoeukaryotes relative to CytoSub by a similar magnitude.

Conversion factors calculated when data was separated by a combination of NRS and taxonomic grouping were similar to taxonomic grouping-only conversion factors, with one or two exceptions per grouping: for *Prochlorococcus* and *Synechococcus* the exception was North Stradbroke Island (NSI) whereas for the Picoeukaryotes the exceptions were Maria Island (MAI) and Darwin (DAR). Possible reasons for these differences are discussed and include (not exhaustive): instrument noise affecting signal discrimination; flow cell and tubing sizes allowing more cells and/or different cell morphologies through the flow cell; flow rates and sample-sheath properties; laser, detector and/or optical differences; analytical software functionality and population gating; sample age at date of measurement; and sample storage. The table shown below provides the critical summary of the conversion factors that should be applied if data users desire consistency of flow cytometry data over the temporal range when the change in analytical instrument occurred.

While the time period from which samples were considered in this instrument comparison spanned June 2013 to June 2017, the official switch from FacsCanto- to CytoSub-measured data occurred during 2014/2015 due to the timing and availability of samples from the NRSs. Specific details of when this switch occurred for each NRS are contained within.

Factors for converting between CytoSub and FacsCanto flow cytometry measurements by IMOS NRS and taxonomic grouping.

FLOW CYTOMETER CONVERSION FACTORS (*CF*) FOR IMOS DATA				
Converting:		**CytoSub (CS) to FacsCanto (FacsC)**	**FacsCanto (FACS) to CytoSub (CS)**	**R^2**
Equation:		**FacsC# = CS# x *CF***	**CS# = FacsC# x *CF***	
Prochlorococcus*	DAR	1	1	-**
	KAI^	1.407	0.716	0.75
	MAI	1.312	0.735	0.73
	NSI	1.574	0.635	0.67
	PHB	1.332	0.751	0.89
	ROT	1.247	0.802	0.41
	YON	1.360	0.735	0.73
	ALL	**1.400**	**0.714**	**0.64**
Synechococcus	DAR	1.032	0.962	0.95
	KAI^	1.021	0.979	0.81
	MAI	1.065	0.939	0.94
	NSI	0.962	1.040	0.40
	PHB	1.060	0.943	0.74
	ROT	1.095	0.913	0.65
	YON	1.062	0.942	0.61
	ALL	**1.046**	**0.956**	**0.79**
Picoeukaryotes	DAR	0.959	1.042	0.50
	KAI^	0.795	1.317	0.65
	MAI	0.593	1.687	0.90
	NSI	0.666	1.501	0.58
	PHB	0.865	1.156	-0.90
	ROT	0.856	1.169	0.41
	YON	0.706	1.416	0.36
	ALL	**0.728**	**1.374**	**0.82**

*Conversion factors should only be applied to *Prochlorococcus* values above 1000 cells/mL
**All values were below 1000 cells/mL and should be taken as measured
^KAI conversion factor based on all-NRS average due to no data available for comparison

1 Introduction

As part of the Integrated Marine Observation System (IMOS), the National Reference Station (NRS) network involves regularly repeated (monthly to quarterly depending on the NRS) marine sampling from multiple locations around Australia. Since 2009, samples have been analysed by flow cytometry along with other biogeochemical analyses. Between July 2009 and June 2014 these flow cytometry samples were analysed exclusively by Dr. Paul Thomson – initially using a FACSCalibur (BD Biosciences, USA) instrument located at the Australian Antarctic Division (AAD) followed by a FacsCanto II (BD Biosciences, USA) instrument located at the University of Western Australia (UWA) Centre for Microscopy and Critical Analysis (CMCA).

During late 2016, CSIRO Oceans & Atmosphere acquired a CytoSub (CytoBuoy, Netherlands) flow cytometer which has been used to analyse the IMOS flow cytometry samples from mid-2016 onward. Unlike the BD instruments which are ideally laboratory-bound due to their sensitivity to external motion, the CytoSub is designed to be interchangeable between laboratory, ship-board and submersible sample analysis modes. These modes potentially give users the ability to make *in situ* or "on location" analyses and compare these with analysis of fixed and stored samples – the standard method for IMOS samples. The CytoSub is primarily located in the BC2/PC2 Algal Laboratory at CSIRO's Battery Point, Tasmania site – the same laboratory that processes all Maria Island NRS samples and also receives IMOS samples from all other NRSs for pigment, microscopy, microbial and cell analysis.

With this change of instruments, it is important to calibrate between instruments to ensure consistency of data. To this end, selected IMOS samples collected between 2013 and 2016 were analysed in parallel using both the FacsCanto II and the CytoSub instruments and the data compared. This comparison explains the observed differences between instruments and to ascertain if, and to what extent, conversion factors could be applied when using the IMOS NRS flow cytometry data set across the temporal range where results from both instruments have been reported.

2 Comparison of instrument specifications

The pathways to development of the FacsCanto II and CytoSub flow cytometers are very different and, as a result, have distinctly different features (Table 1). A comparison of instrument specifications therefore may serve as a primary reference in such cases when differences in measurements are generated by the two instruments. The FacsCanto II was developed to cater for medical applications, in particular for measuring blood cells which have a relatively uniform and narrow range of size and shape, as well as for measuring non fluorescent cells that have been stained with fluorophores. On the other hand, the CytoSub was developed for aquatic science applications, in particular for a broad and irregular range of "particle" sizes and shapes – including pigment-containing autofluorescent cells.

The main advantages of using an instrument such as the FacsCanto II for analysis of IMOS NRS samples include: easier cross-comparison of use and methods across flow cytometry applications due to high market penetration of this kind/manufacturer of instrument; ease of access to local technical support; and the ability to "batch" process with up to 96 samples being autoanalysed in a batch resulting in less operator hands-on time. The main advantages of using the CytoSub include: a high degree of functional customisation; a higher size allowance for cells to enter the flow cell (~800 µm *c.f.* ~50 µm); additional software functionality, whereby fluorescence profiles are generated for every particle that passes through the flow cell allowing greater scrutiny of particle characteristics and ultimate taxonomic classification.

Some other features are also worth specifically noting in the comparison between instruments:

- *Sheath fluidics* – The FacsCanto II is permanently in an open sheath mode that requires a continuous supply of proprietary ionic sheath fluid to be pumped through the instrument during all sample analyses. The CytoSub on the other hand has two sheath modes, the first and most commonly used being a closed sheath mode. In this mode sheath fluid is continuously recirculated within the instrument sheath loop. Two 0.22 µm dialysis filters, that form part of the sheath loop, continuously filter the sheath fluid during closed sheath operation. After a sample is taken into and passes through the flow cell, it is then incorporated into the sheath fluid and a small and proportionate amount of original sheath is displaced. The second mode is an open sheath mode that is equivalent to that of the FacsCanto II, whereby 0.22 µm pre-filtered seawater is used as the sheath fluid. Closed sheath mode is the preferable operating mode when using the CytoSub to measure picoplankton and other autofluorescing cells, whereas stained samples are measured in open sheath mode in order to minimise contact time between the stain and instrument components (tubing, filters, flow cell, etc.) that could potentially act as a contaminating fluorescence source for subsequent measurements.
- *Submersible sampling* – The CytoSub has a high-pressure by-pass loop – as opposed to the normal low-pressure loop for laboratory measurements – that operates in a closed sheath mode and allows the instrument to measure samples *in situ* up to 200 m in depth.
- *Green fluorescence* – When measuring cells that have been stained with a fluorophore that fluoresces in the green range of the spectrum, such as heterotrophic bacteria stained with SYBR Green II fluorescent dye, the FacsCanto II has a green fluorescence detector as a factory standard. The CytoSub is not currently equipped with a green fluorescence detector. Instead, the CytoSub has a yellow fluorescence detector and the ability to critically analyse ratios of selected combinations of the six detectors in order to serve as a proxy for direct green fluorescence detection. Nonetheless, an extra detector for direct green fluorescence detection is the best scenario and efforts are being made in this regard.
- *Sample handling* – The CytoSub requires an analyst to be present when measuring samples as there is no automated sample injection and mixing functions.

Table 1 Comparison of instrument specifications

INSTRUMENT SPECIFICATION	FACSCANTO II	CYTOSUB
Manufacturer and general instrument information	Becton-Dickinson (BD; USA) Common usage across medical fields; increasingly used in aquatic applications	Cytobuoy (Netherlands) Developed with aquatic research community being the primary focus
Configurability	Low/medium; user choices limited by manufacturer restrictions	High; instrument designed to be highly configurable to user's requirements
Power and computing requirements	Dedicated benchtop PC for command instructions and transfer of raw files to analytical software CPU	Laptop CPU for command instructions, analysis and transfer of raw files from internal CPU; some analytical processing possible on internal CPU
Number of lasers	1	2
Laser specifications	488 nm; 30mW?	488 nm; 120 mW dialled down to 60 mW 552 nm; 60 mW dialled down to 30mW
Emission detection	Side-scatter Forward-scatter (software interpolation required for "shadow" region) Fluorescence: Red; Orange; Green	Side-scatter Forward-scatter (dual detectors for true measurement integration of "shadow" region) Fluorescence: Red; Orange; Yellow
Sampling location utility	Laboratory use only. Instrument requires dedicated bench and associated PC space; footprint ~3m^2	Laboratory, ship-board and in-situ deployable Instrument is portable; footprint ~1m^2. Can be switched between standard lab housing and high-pressure housing (rated to 200 m operating depth)
Sample handling	Single sample OR multi-well (36) plate Each sample individually aspiration-mixed by instrument	Single sample only Multi-well sampler (12 samples) add-on available from manufacturer (not in current configuration) Manual sample mixing only. Programmable for automatic sampling when in field mode
Sheath mode and characteristics	OPEN (flow-through) only Single-use, manufacturer-specific sheath recommended; sheath usage rate ~100 mL/sample	Optional OPEN or CLOSED modes User choice of sheath properties. OPEN mode: sheath usage rate ~250 mL/sample. CLOSED mode (normal operating): sheath usage negligible; sheath constantly passed through internal 0.22 µm filters
Sample volumes and cell size range	User-defined; small sample loop so little more than actual sample volume required ≤50 µm cell size upper limit	User-defined; relatively long sample loop, minimum of 600 µL required per sample 800 µm cell size upper limit
Pumping	Vacuum	Peristaltic. Multiple user options involving avoidance of roller "pinch spots" during analysis
Analysis software	FlowJo. Batch analytical processing	Cytoclus. Individual particle profiling
Other attributes	Green fluorescence detector allows for direct enumeration of particles stained with fluorescent dyes e.g. SYBR Green	Particle imaging: contains an internal camera; individual particles can be scrutinised using images aligned to fluorescence information

3 Sample details

The samples used for this instrument comparison were mostly opportunistic replicates, with some exceptions (e.g. MAI and YON) where a dedicated effort was allowed for in sampling to cater for these purposes. Hence, the data set is somewhat sporadic between stations.

All samples were collected and dealt with in accordance with recognised and published protocols (Marie et al, 2000, 2005; Ribeiro et al, 2016) and the National Reference Stations Biogeochemical Operations Manual (Davies et al., 2019). In brief, 1 mL aliquots were taken from each pooled depth sample in triplicate or quadruplicate and decanted into 2 mL cryovials, to which 10 µL of 25% glutaraldehyde solution was added. Samples were then gently mixed and stored at room temperature for 15 minutes before being placed in either liquid nitrogen or in a -80 ^{o}C ultrafreezer. Samples requiring shipping were done so in a fully saturated liquid nitrogen dry shipper and then quickly placed in a -80 ^{o}C ultrafreezer until analysis. Before being analysed, samples were thawed in a 25 ^{o}C water bath for no more than 15 minutes and then kept in low or no light until measurement.

Table 2 Details of IMOS NRS samples included in the flow cytometer instrument comparison

NATIONAL REFERENCE STATION (NRS)	SAMPLING TIME PERIOD	NUMBER OF SAMPLES INCLUDED IN ANALYSIS
Darwin	September 2013 – July 2014	6
Kangaroo Island	N/A	0
Maria Island	September 2013 – June 2017	35
Nth Stradbroke Island	July 2013 – June 2017	33
Port Hacking Bay	October 2013 – March 2015	9
Rottnest Island	July 2013 – June 2017	33
Yongala	June 2014 – June 2017	27
TOTAL		**143**

Samples from all NRSs except for KAI were included in the instrument measurement comparison. Sample collection dates ranged from June 2013 to Jun 2017 and depended on availability of replicate samples (Table 2). Unfortunately, no samples were available from KAI for the comparison period and therefore KAI

will not be included in further details regarding samples. A total of 152 samples were used in the instrument comparison, ranging from a low of 6 from DAR and a high of 36 from YON. For all stations, excluding DAR, more than one full calendar year of samples was covered.

As much as possible, flow cytometer operation was kept consistent between instruments, e.g. analysis volumes, although for many functions such as sheath speed and detector voltage gain, the optimal instrument operating parameters were particular to the instrument in order to obtain the best possible results.

4 Comparison of analysed samples

4.1 All data comparisons

4.1.1 ALL NRS DATA COMBINED

Regression analysis for all data combined from the 152 parallel samples measured by each flow cytometer showed that there was a strong correlation between instruments (R^2 = 0.83; Fig. 1). This suggests that both flow cytometers produced close results over the range of cell concentrations from under 100 cells/mL to over 175000 cells/mL.

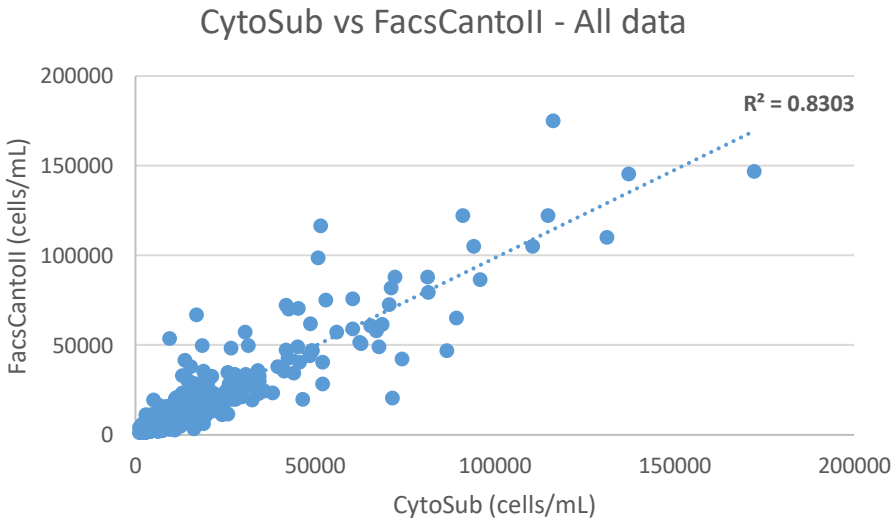

Figure 1 CytoSub versus FacsCanto II flow cytometer measurements for all NRS data used in the instrument comparison analysis. *Prochlorococcus* data was excluded from analysis when cell densities of less than 1500 cells/mL were measured by either instrument due to potential instrument low-level detection issues.

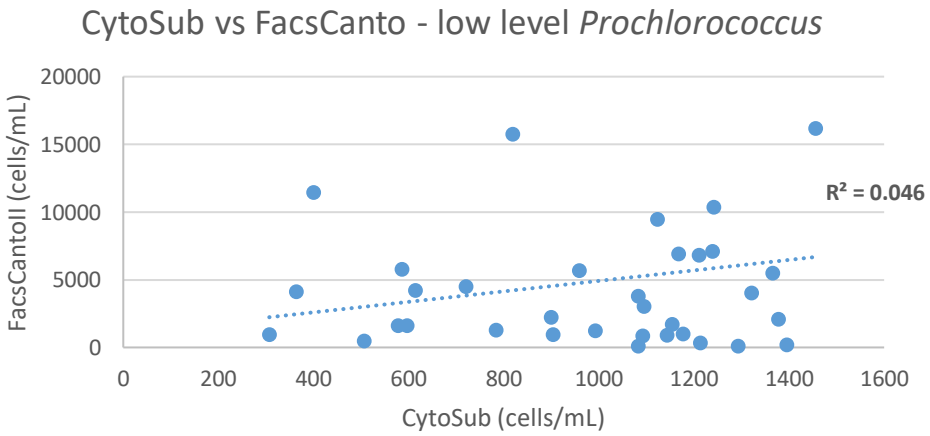

Figure 2 Regression of CytoSub versus FacsCanto measurements for *Prochlorococcus* values estimated as being less than 1500 cells/mL for either instrument.

Prochlorococcus cells in concentrations of less than 1500 cells/mL, as measured on either instrument (*n* = 35), had no correlation (R^2 = 0.046; Fig. 2) and therefore this data was omitted from the instrument comparison analysis and subsequent conversion factors.

The following equations have been determined if data were to be considered for a single conversion from one instrument to another (measurements in cells/mL):

1. CytoSub = FacsCanto x 1.063
2. FacsCanto = CytoSub x 0.940

While this single conversion factor is very close to 1:1, i.e. both instruments are measuring very similar picoplankton abundances, the above conversion equations are a simplification of the situation and better resolution can be gained from separating data by NRS, by taxonomic grouping, or most precisely by a combination of NRS and taxonomic grouping, as the following analysis and discussion suggests.

4.1.2 DATA SEPARATION BY NRS

When data was separated by NRS, a large variation in correlation between the two instruments was seen (Fig. 3). NSI had the worst correlation (R^2 = 0.46) while DAR had the best (R^2 = 0.96).

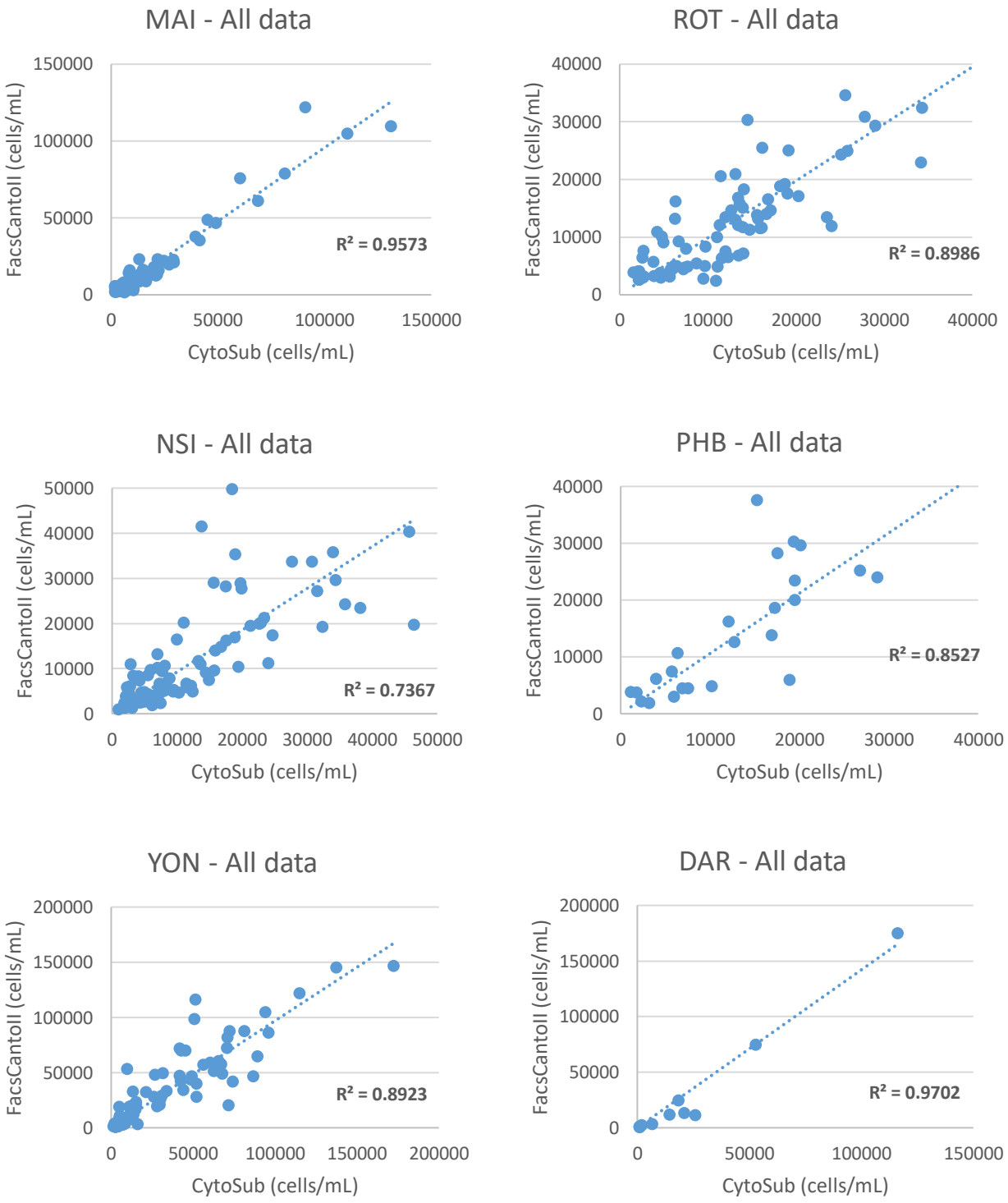

Figure 3 CytoSub versus FacsCanto flow cytometer measurements of all data separated by NRS. KAI has been omitted due to a lack of sample availability for comparison purposes.

In terms of relative data "closeness" between the two instruments – though not necessarily the quantum of the final conversion factor – it appears that the sites with the largest concentrations and/or range had the best correlations. DAR, MAI and YON all had a data range spanning more than 100000 cells/mL and the closest correlation in data between the two instruments (R^2 values of 0.96, 0.94 and 0.82 respectively),

while ROT, PHB and NSI all had a data range spanning less than 50000 cells/mL and lower correlation in data between the two instruments (R^2 values of 0.74, 0.65 and 0.46 respectively).

The following equations have been determined if data were to be converted from one instrument to another based on NRS alone (all measurements in cells/mL):

DAR

3. CytoSub = FacsCanto x 0.996
4. FacsCanto = CytoSub x 1.004

MAI

5. CytoSub = FacsCanto x 0.984
6. FacsCanto = CytoSub x 1.017

NSI

7. CytoSub = FacsCanto x 1.090
8. FacsCanto = CytoSub x 0.918

PHB

9. CytoSub = FacsCanto x 1.233
10. FacsCanto = CytoSub x 0.811

ROT

11. CytoSub = FacsCanto x 1.086
12. FacsCanto = CytoSub x 0.921

YON

13. CytoSub = FacsCanto x 1.050
14. FacsCanto = CytoSub x 0.953

Except for PHB, which had a conversion factor of around 20% of the measured value, conversion factors based on NRS alone were close to 1 suggesting that the instrument measurements were very close. Following the same trend as the overall dataset, the FacsCanto measured more cells then the CytoSub for most of the NRSs apart from DAR and MAI. These two stations have consistently lower *Prochlorococcus* cell abundances than the other NRSs, therefore it would appear that further separation of data into taxonomic groupings, perhaps with an NRS overlay, would provide better resolution and more precise conversion factors.

4.1.3 DATA SEPARATION BY TAXONOMIC GROUPING

When data was separated by taxonomic grouping, there was less variation in correlation between the two instruments than when separated by NRS (Fig. 4), although correlations of taxonomic groupings generally fell somewhere in the middle of "NRS all data" correlations. *Synechococcus* data from all NRSs combined were most closely correlated ($R^2 = 0.82$), followed by all Picoeukaryotes ($R^2 = 0.79$) and all *Prochlorococcus* ($R^2 = 0.72$).

The following equations have been determined if data were to be converted from one instrument to another based on taxonomic grouping alone (all measurements in cells/mL):

Prochlorococcus

15. CytoSub = FacsCanto x 1.400
16. FacsCanto = CytoSub x 0.714

Synechococcus

17. CytoSub = FacsCanto x 1.046
18. FacsCanto = CytoSub x 0.956

Picoeukaryotes

19. CytoSub = FacsCanto x 0.728
20. FacsCanto = CytoSub x 1.374

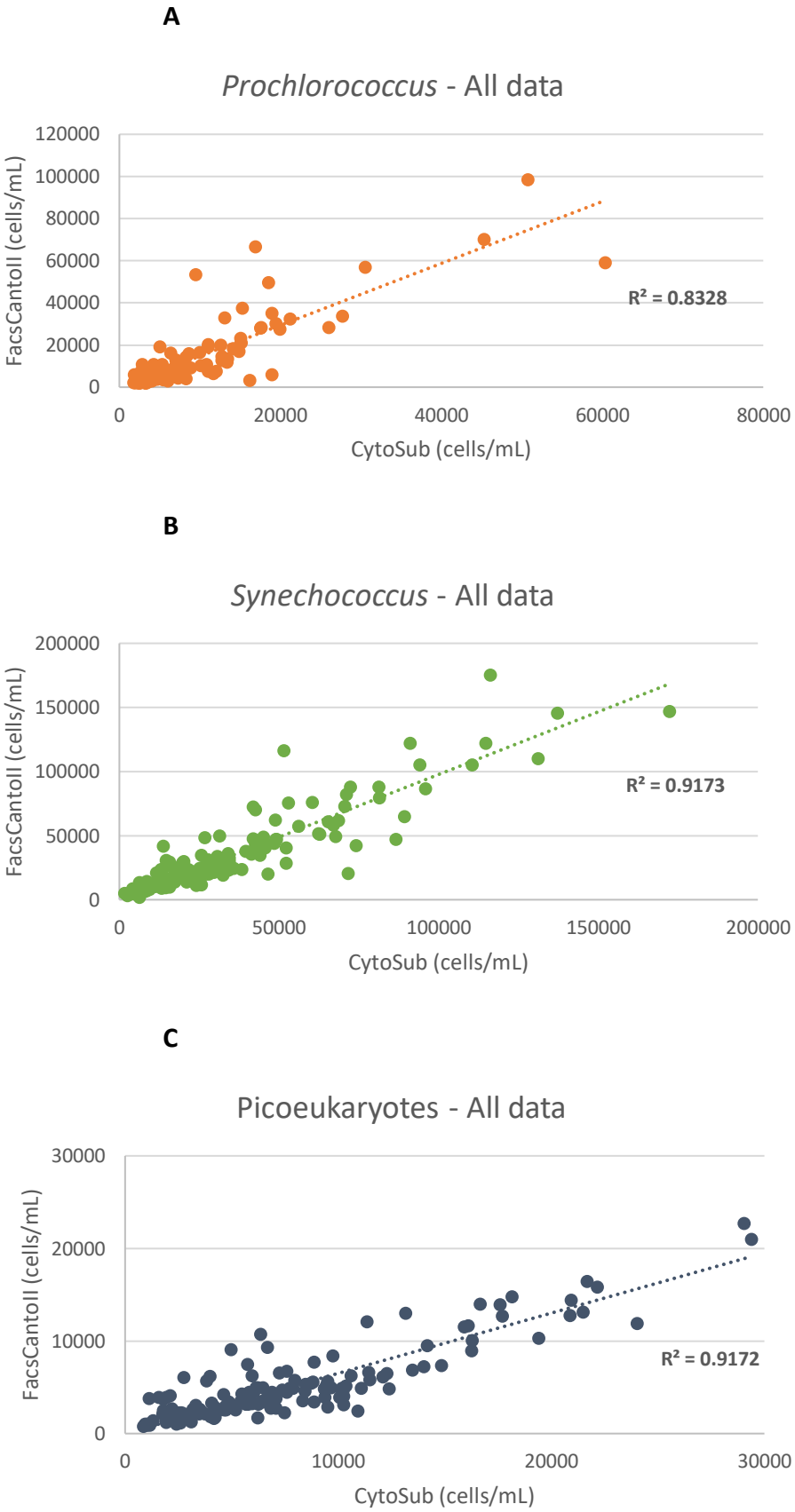

Figure 4 CytoSub versus FacsCanto flow cytometer measurements for each of the taxonomic groupings: A – *Prochlorococcus*, B – *Synechococcus*, and C – Picoeukaryotes. Data from all stations has been combined.

4.2 Comparisons by NRS and taxonomic group combined

When data was separated by the combination of NRS and taxonomic grouping, correlations between the two instruments become even more varied and distinct (Figs. 5 – 10).

4.2.1 MARIA ISLAND (MAI)

For MAI, *Synechococcus* data had an extremely good correlation (R^2 = 0.94), while Picoeukaryotes were slightly lower (R^2 = 0.90) and *Prochlorococcus* considerably lower (R^2 = 0.73).

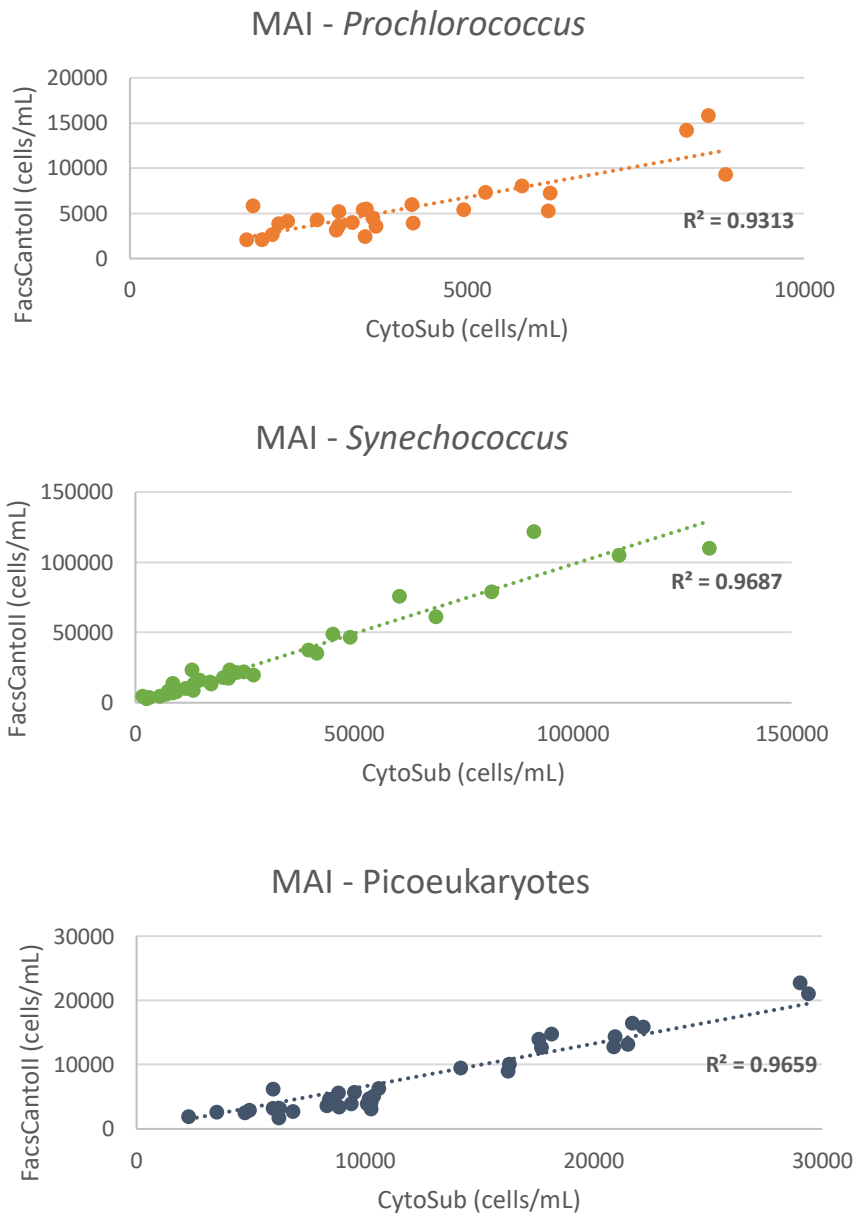

Figure 5 CytoSub versus FacsCanto flow cytometer measurements for MAI combined with the separate taxonomic groupings.

4.2.2 ROTTNEST ISLAND (ROT)

For ROT, the correlation for *Synechochoccus* was mid-range (R^2 = 0.65), while Picoeukaryotes and *Prochlorococcus* had poor correlations (R^2 = 0.41 and 0.26, respectively), with all values for these taxonomic groupings being considerably less than those for *Synechochoccus*.

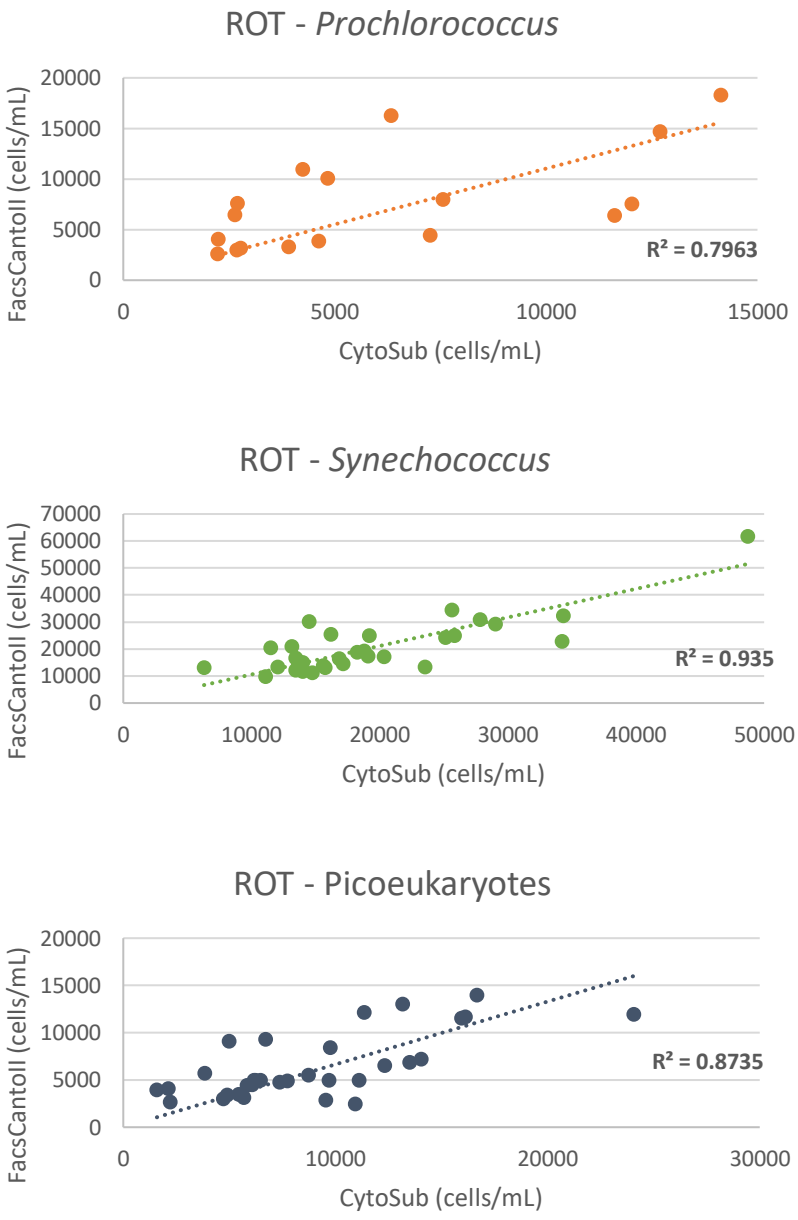

Figure 6 CytoSub versus FacsCanto flow cytometer measurements for ROT combined with the separate taxonomic groupings.

4.2.3 NORTH STRADBROKE ISLAND (NSI)

For NSI, unlike all other stations, *Synechochoccus* had the lowest correlation (R^2 = 0.40) of the taxonomic groupings. *Prochlorococcus* on the other hand had a relatively high correlation (R^2 = 0.67), despite all values being less than 10000 cells/mL, while Picoeukaryotes were still relatively high (R^2 = 0.58).

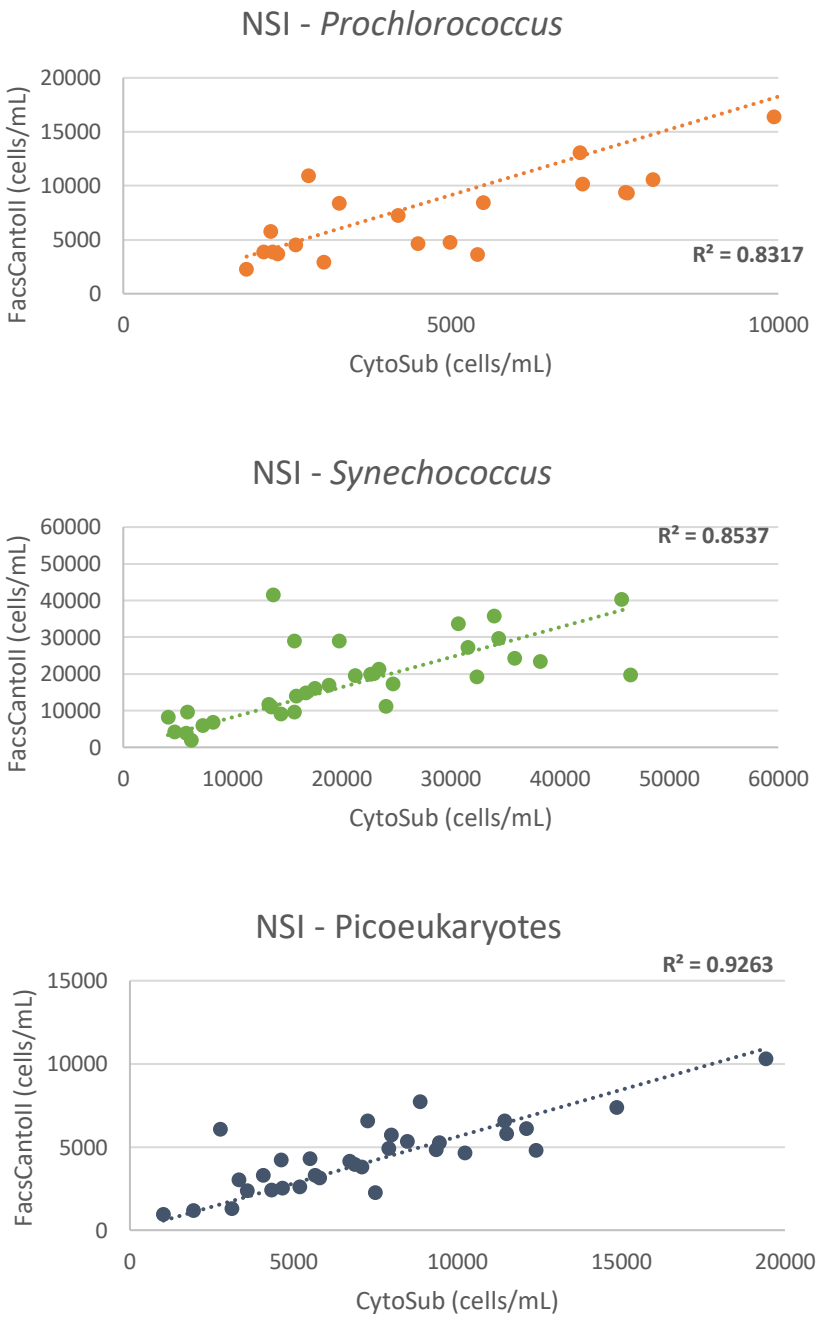

Figure 7 CytoSub versus FacsCanto flow cytometer measurements for NSI combined with the separate taxonomic groupings.

4.2.4 PORT HACKING BAY (PHB)

For PHB, *Prochlorococcus* and *Synechochoccus* both had high correlations with R^2-values of 0.89 and 0.74 respectively. Picoeukaryotes, with relatively few data points and all values under 10000 cells/mL, had no correlation.

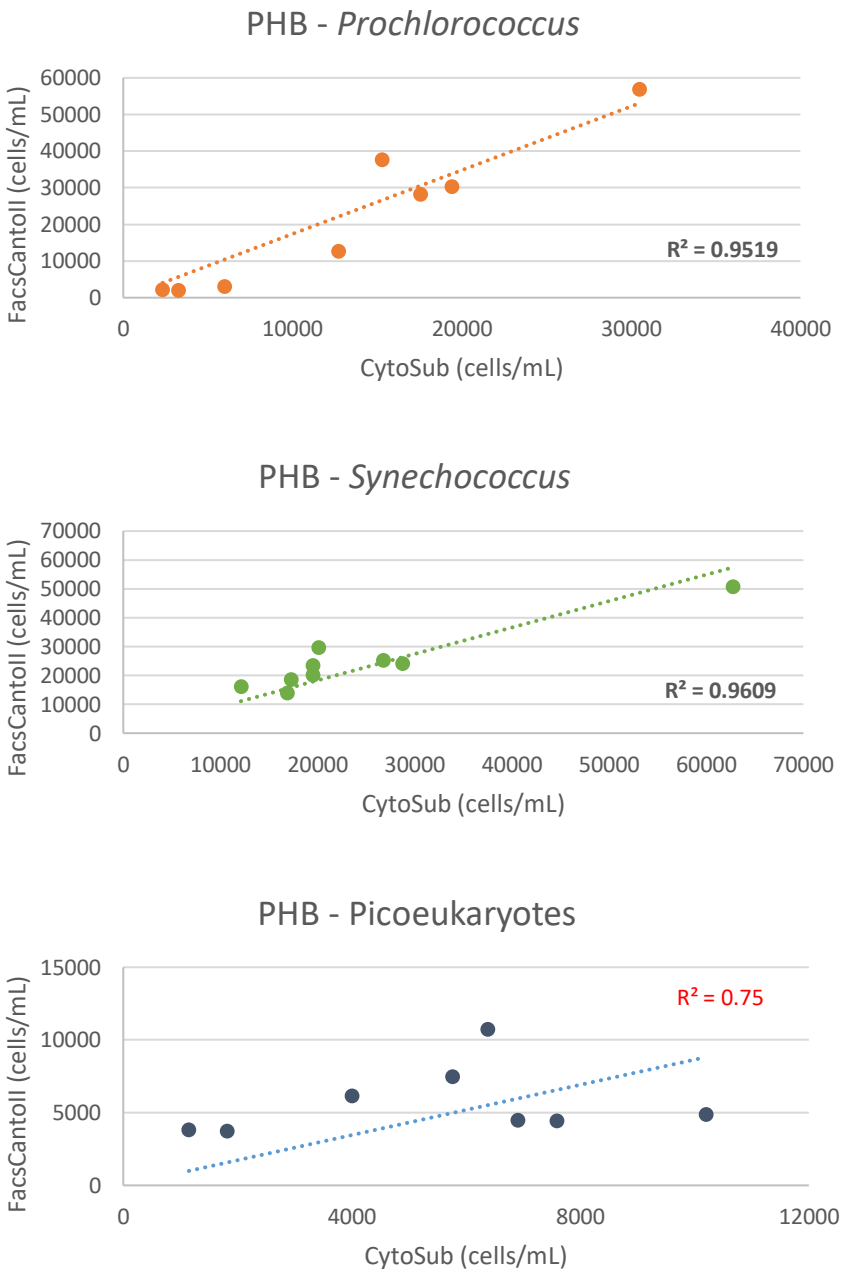

Figure 8 CytoSub versus FacsCanto flow cytometer measurements for PHB combined with the separate taxonomic groupings.

4.2.5 YONGALA (YON)

For YON, *Prochlorococcus* and *Synechococcus* had good correlations (R^2 = 0.73 and 0.61, respectively), a similar situation to PHB although not as convincing, while Picoeukaryotes had a relatively poor correlation (R^2 = 0.36). Only 8% of Picoeukaryote data points had values higher than 5000 cell/mL which was low relative to other NRSs occurring in more southern latitudes.

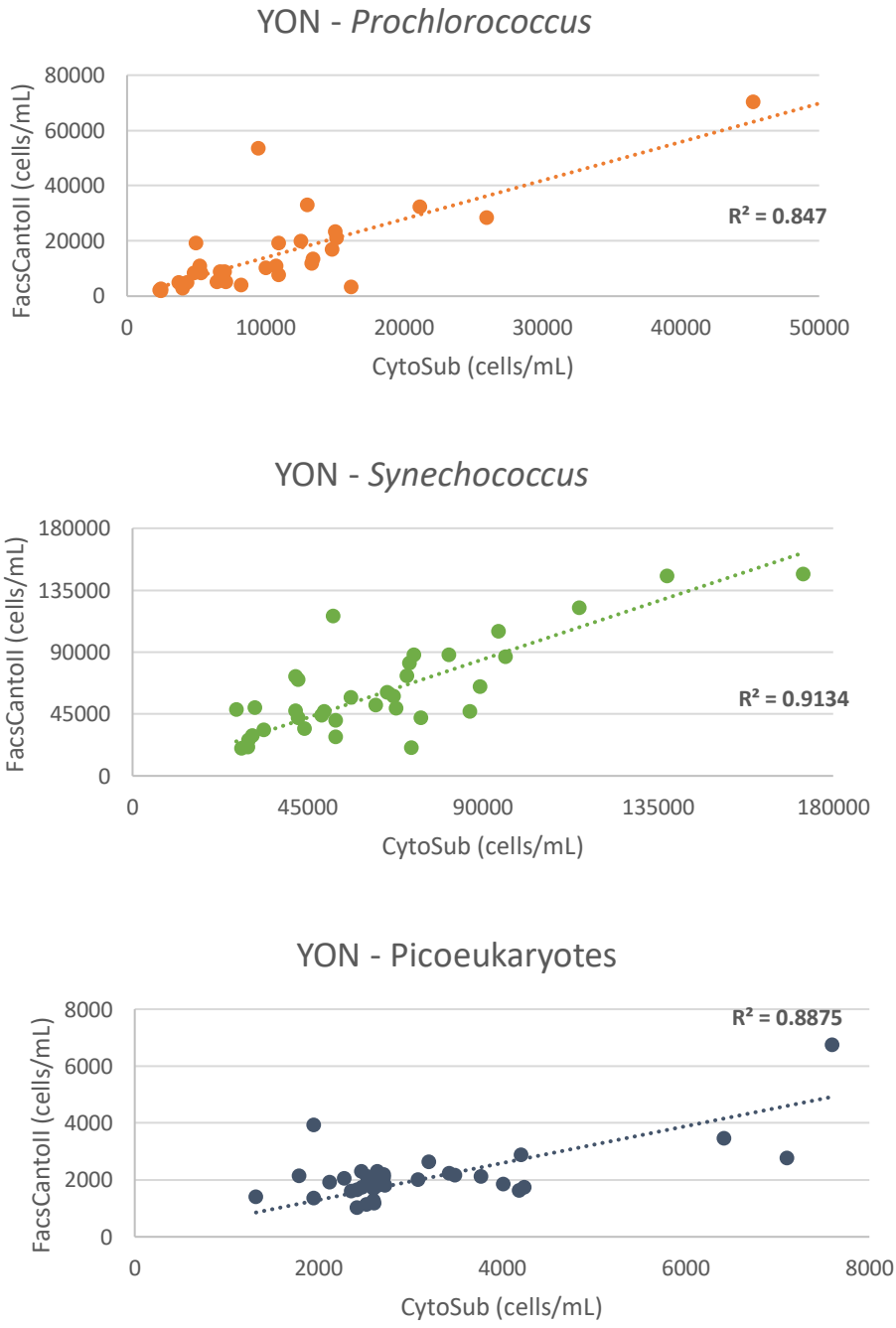

Figure 9 CytoSub versus FacsCanto flow cytometer measurements for YON combined with the separate taxonomic groupings.

4.2.6 DARWIN (DAR)

DAR had very few data points from which to draw a comparison and for *Prochlorococcus* no correlation was found. Nevertheless, *Synechococcus* had a high correlation (R^2 = 0.95) and the Picoeukaryote correlation was in the middle compared with other stations (R^2 = 0.50).

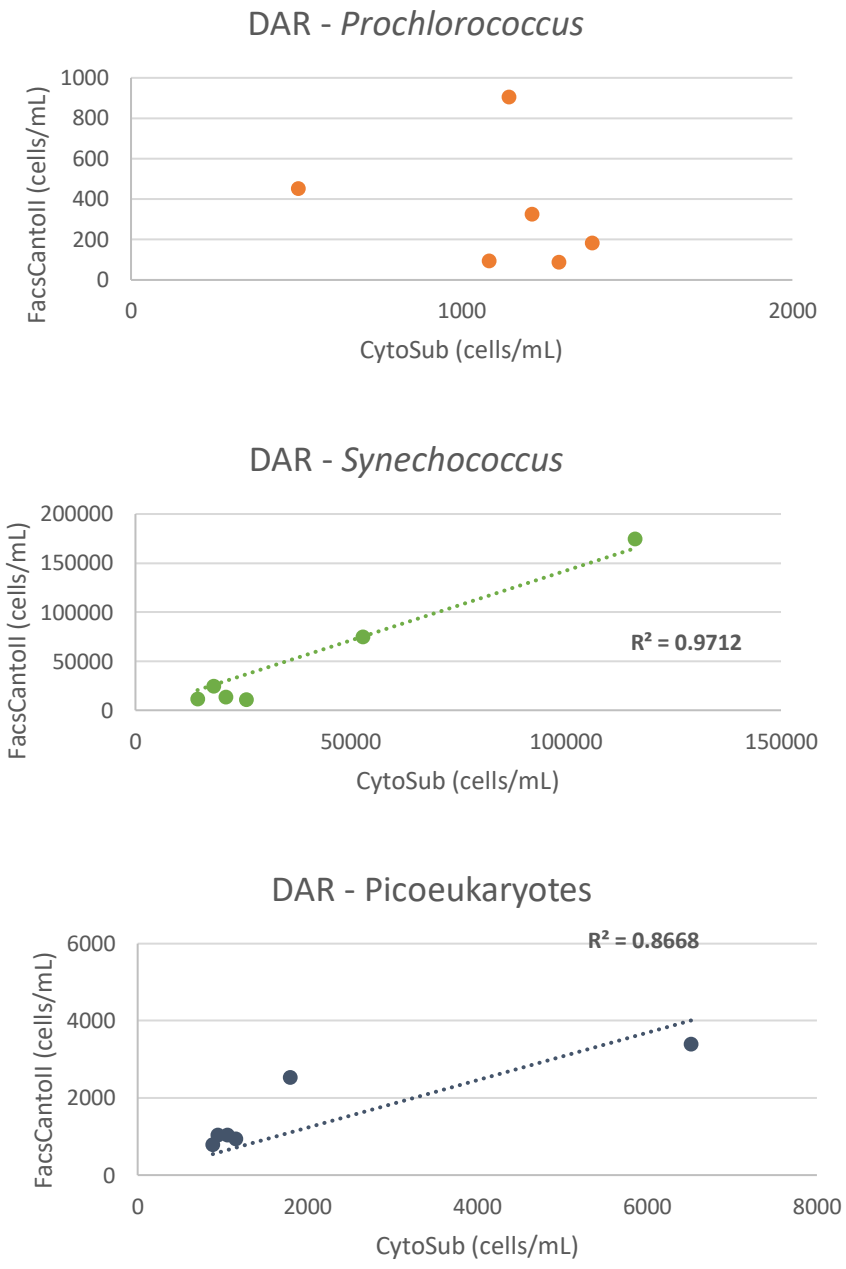

Figure 10 CytoSub versus FacsCanto flow cytometer measurements for DAR combined with the separate taxonomic groupings. With all *Prochlorococcus* measurements being under 1500 cells/mL no correlation was made (refer to Section 4.1.1).

4.3 Conversion factors

In determining any conversion factors that may be applied, several levels of data were interrogated: all NRSs combined; by NRS only; by taxonomic grouping only; and by NRS and taxonomic grouping. When all NRS data was combined or when data was separated by NRS, conversion factors were very small – less than 10% apart from Port Hacking Bay (PHB) which was just above 20% (Table 3). When data was separated by taxonomic grouping a distinct difference in the measured values was found between the two instruments and therefore the scale of conversion factor, from *ca.* 5% for *Synechococcus* to *ca.* 40% for *Prochlorococcus* and the Picoeukaryotes. Interestingly, the CytoSub underestimated *Prochlorococcus* relative to the FacsCanto whereas the FacsCanto underestimated the Picoeukaryotes relative to *Prochlorococcus* by a similar magnitude.

Table 3 Summary of conversion factors between CytoSub and FacsCanto flow cytometry measurements when IMOS data is combined or separated by NRS only or taxonomic grouping only.

Converting from: **Converting to:**	**CytoSub** **FacsCanto**	**FacsCanto** **CytoSub**
All NRS data combined	1.063	0.940
DAR	0.996	1.004
MAI	0.984	1.017
NSI	1.090	0.918
PHB	1.233	0.811
ROT	1.086	0.921
YON	1.050	0.953
Prochlorococcus	1.400	0.714
Synechococcus	1.046	0.956
Picoeukaryotes	0.728	1.374

Conversion factors calculated when data was separated by a combination of NRS and taxonomic grouping (Table 4) were similar to taxonomic grouping only conversion factors, with one or two exceptions per grouping: for *Prochlorococcus* and *Synechococcus* the exception was NSI whereas for the Picoeukaryotes the exception was MAI and Darwin DAR. The NSI + *Synechococcus* combination was the only example where one NRS had a conversion factor on the opposite side of 1 to all the other NRSs within that taxonomic grouping, i.e. for all NRSs except NSI, *Synechococcus* abundance was underestimated by the CytoSub relative to the FacsCanto.

Given the extra resolution provided by the combined “NRS + taxonomic grouping” data separation, both in terms of the relative abundances between instrument analyses as well as the strength of these data relationships, the combined approach appears to provide the greatest accuracy for conversion of data.

Table 4 Factors for converting between CytoSub and FacsCanto flow cytometry measurements.

FLOW CYTOMETER CONVERSION FACTORS (CFs) FOR IMOS DATA				
Converting:		CytoSub to FacsCanto II	FacsCanto II to CytoSub	R^2
Equation:		FacsCanto# = CytoSub# x CF	CytoSub# = FacsCanto# x CF	
Prochlorococcus*	DAR	1	1	-**
	KAI^	1.407	0.716	0.75
	MAI	1.312	0.735	0.73
	NSI	1.574	0.635	0.67
	PHB	1.332	0.751	0.89
	ROT	1.368	0.731	0.26
	YON	1.360	0.735	0.73
	ALL	**1.400**	**0.714**	**0.64**
Synechococcus	DAR	1.032	0.962	0.95
	KAI^	1.021	0.979	0.81
	MAI	1.065	0.939	0.94
	NSI	0.962	1.040	0.40
	PHB	1.060	0.943	0.74
	ROT	1.095	0.913	0.65
	YON	1.062	0.942	0.61
	ALL	**1.046**	**0.956**	**0.79**
Picoeukaryotes	DAR	0.959	1.042	0.50
	KAI^	0.795	1.317	0.65
	MAI	0.593	1.687	0.90
	NSI	0.666	1.501	0.58
	PHB	0.865	1.156	-0.90
	ROT	0.856	1.169	0.41
	YON	0.706	1.416	0.36
	ALL	**0.728**	**1.374**	**0.82**

*Conversion factors should only be applied to *Prochlorococcus* values above 1000 cells/mL

**All values were below 1000 cells/mL and should be taken as measured

^KAI conversion factor based on all-NRS average due to no data available for comparison

5 Conclusions

Despite the seemingly close relationship between the FacsCanto and CytoSub measurements of IMOS NRS picoplankton when data was either taken as a whole or by NRS only, the combined "NRS + taxonomic grouping" had greater resolution and the corresponding conversion factors are therefore recommended.

In general terms, when comparing CytoSub estimates to those of the FacsCanto, *Prochlorococcus* abundance was *ca.* 40% lower (range of 31 – 57%), *Synechococcus* abundance was *ca.* 5% (range of -4 – 10%) and Picoeukaryote abundance was *ca.* 38% higher (range of 4 – 69%). There are many possible contributing factors toward these differences, a general factor being that CytoSub samples were analysed in Tasmania while FacsCanto samples were analysed in Western Australia after first being sent to Tasmania from the NRSs, therefore involving an extra shipping step. Other contributing factors are relevant to specific stations and/or taxonomic groupings and then there are instrument-based factors.

If station-related factors are considered in isolation, then local differences in sample handling, climactic conditions, and water properties (turbidity, salinity, temperature, etc.) could contribute to sample condition and in turn the different signals detected by the respective instruments. If taxonomic grouping-related factors are considered in isolation then, again, water properties could affect the pigment concentration and/or profile of cells which in turn may affect fluorescence detection – this is especially the case for *Prochlorococcus* under high light conditions; differential uptake of glutaraldehyde and preservation of cell types that may occur at some NRSs and not others; and differential responses to freeze-thaw processes could be contributing factors. More instrument-specific contributing factors include: instrument noise affecting signal discrimination; flow cell and tubing sizes allowing more cells or different cell morphologies through the flow cell; flow rates and sample-sheath properties; laser, detector and/or optical differences; analytical software functionality and population gating; sample age at date of measurement; and sample storage. Yet, despite these many potential contributing factors, trends in detection between instruments showed considerable consistency as borne out in the data comparisons.

While the time period from which samples were considered in this instrument comparison spanned June 2013 to June 2017, the official switch from FacsCanto- to CytoSub-measured data occurred during 2014/2015 due to the timing and availability of samples from the various NRSs. Table 5 shows when this switch occurred for each NRS.

Table 5 Time period when flow cytometer was used for official IMOS measurements.

Station	FacsCanto measurements	CytoSub measurements
DAR	2009 – August 2014	September 2014 - present
MAI	2009 – January 2015	February 2015 - present
NSI	2009 – November 2014	December 2014 - present
PHB	2009 – June 2015	July 2015 - present
ROT	2009 – August 2015	September 2015 - present
YON	2009 – September 2014	October 2014 - present

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