Spectral algal fingerprinting and long sequencing in synthetic algal-microbial communities

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Abstract

Full-spectrum cytometry offers a novel technique in environmental research, enabling rapid, high-throughput analysis of single algal cells from different taxa by capturing entire emission spectra. In this work we demonstrate label-free identification and enumeration of phytoplankton, overcoming traditional methods limitations. We propose a strategy for monitoring synthetic algal communities based on integration of high-throughput spectral cytometry and long sequencing analysis.

Keywords: synthetic algal-microbial communities; full-spectrum cytometry; spectral flow cytometry; phytoplankton; imaging flow cytometry**;** long sequencing; nanopore-based sequencing

In recent decades, synthetic biology has made significant progress in building artificial microbial and algal communities[1]. However, the difficulty of engineering complex biological systems has been humbling. As biosystems evolve, they change and refine themselves, and the evolutionary process sets them apart from non-biological systems[2]. Community selection experiments are related to fundamental questions in ecology and evolution as well as applied biotechnological problems. Uni-algal systems are not stable, and bacterial contamination is likely if the algal community is maintained heterotrophically on a medium with a fixed source of carbon[3] or dead algae present. The algal-microbial systems development is often overdominated by one or two algae or bacterial part of the community, with some exceptions[4]. It is related to the absence of methods allowing the following dynamics of mixed algal-microbial systems.

Traditional methods for studying microbial and algal communities, such as microscopy and pigment analysis, have throughput, taxonomic resolution, and functional characterization constraints and were primarily designed to differentiate a specific taxonomic group or provide averaged integrated data at a population level[5-7]. Conventional flow cytometry struggles to distinguish cells from other particulate matter in the water matrix, and imaging flow cytometry is capable to differentiate only few fluorescent channels. Full-spectrum cytometry, on the other hand, has recently transformed the field by offering high-throughput, multidimensional analysis of algal cells based on their size, complexity, and spectral fingerprint[8-10].

By capturing the entire emission spectrum from each single cell, full-spectrum cytometry may provide insights into phytoplankton community composition, structure, dynamics[11-12], and responses to environmental stressors that are accompanied by shifts in community structure[13]. The spectral cytometry allows for the identification of highly autofluorescent cellular subpopulations by virtual filtering[11] or with autofluorescence finder[14]. The work presented herein demonstrates the proof-of-concept that contemporary spectral cytometry analysis has advanced to a level that enables good

identification and enumeration of different algae based on their autofluorescent pigment signatures in parallel with a long sequencing amplicon-based 16S rRNA analysis at the species level providing a powerful tool for advancing our understanding of phytoplankton ecology, synthetic biology, and evolution.

The first step in the analysis of complex communities requires construction of a library with unique spectral signatures for different phytoplankton groups. For this purpose, single phytoplankton cultures were recorded on ID7000 (Sony Biotechnology), and individual spectral signatures were extracted. (**Figure 1a**). Clear separation between the two *Microcystis* autofluorescent populations was observed across all lasers, except for 808 nm, given the optimal set of virtual filters (VFs) (**Figure 1b-d**). The same workflow was applied for the acquisition of spectral signatures of the remaining phytoplankton monocultures (**Figure 1e**), resulting in a spectral library used for further analysis.

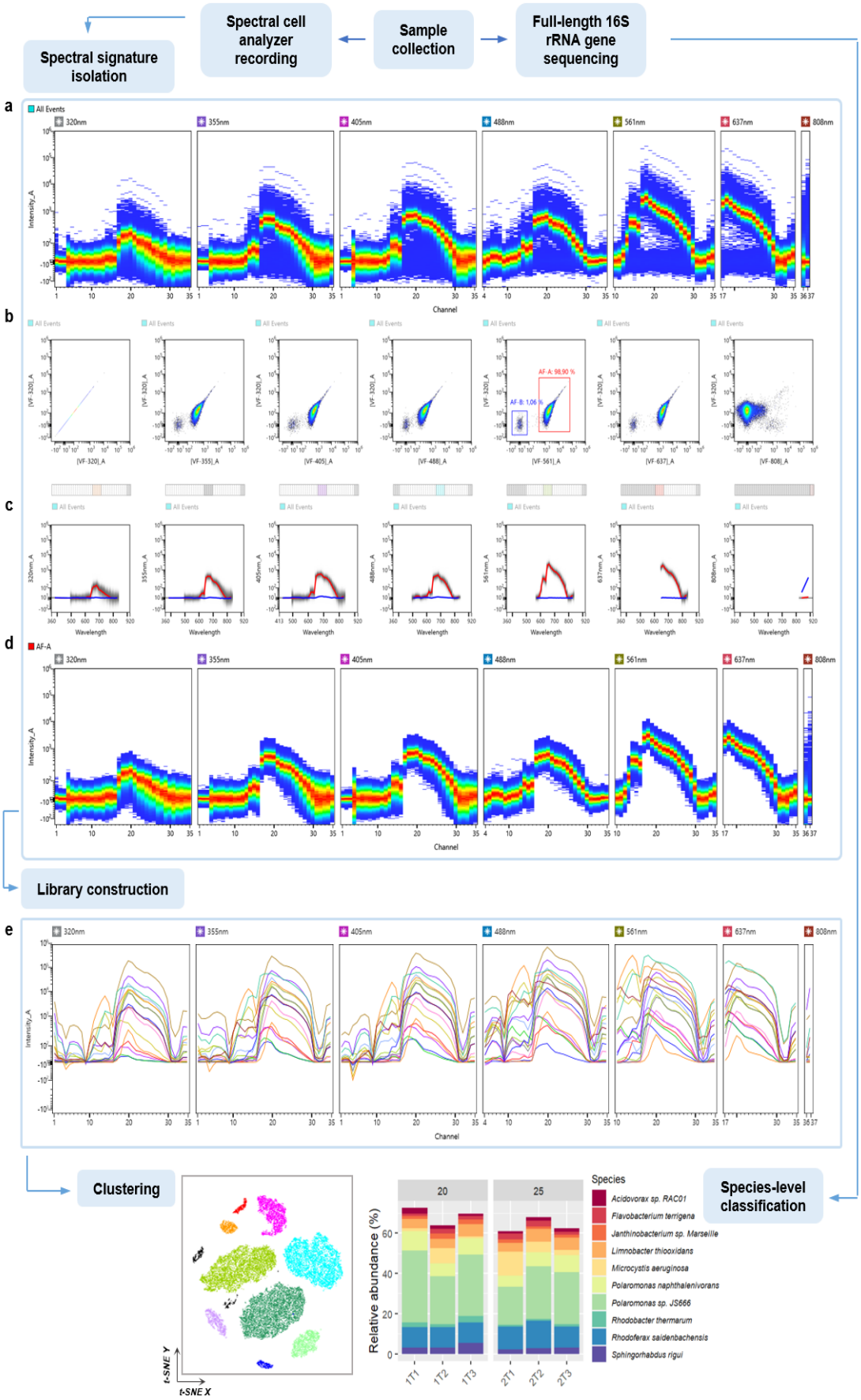

Spectral signature isolation
Spectral cell analyzer recording
Sample collection
Full-length 16S rRNA gene sequencing
a
b
c
d
Library construction
e
Clustering
Species-level classification
Relative abundance (%)
Species
Acidovorax sp. RAC01
Flavobacterium terrigena
Janthinobacterium sp. Marseille
Limnobacter thiooxidans
Microcystis aeruginosa
Polaromonas naphthalenivorans
Polaromonas sp. JS666
Rhodobacter thermarum
Rhodoferax saidenbachensis
Sphingorhabdus rigui
t-SNE Y
t-SNE X

Figure 1. Schematic overview of the proposed experimental workflow with MinION Mk1C-based sequencing platform (Oxford Nanopore) and ID7000. It includes: **(a)** recording and isolation of single spectral signatures with "Autofluorescence Finder" tool; **(b)** a set of optimal virtual filters for discrimination of autofluorescent populations with a set of optimal virtual filters (**AF-A** - *Microcystis* sp. and **AF-B** – debris); **(c)** respective emission spectra (red line – **AF-A**, blue line – **AF-B**); **(d)** *Microcystis*; **(e)** spectral signatures library corresponding to respective phytoplankton species, is then constructed, and utilized during subsequent analysis, including unsupervised clustering techniques (e.g., t-SNE) in parallel with species-level bacterial classification.

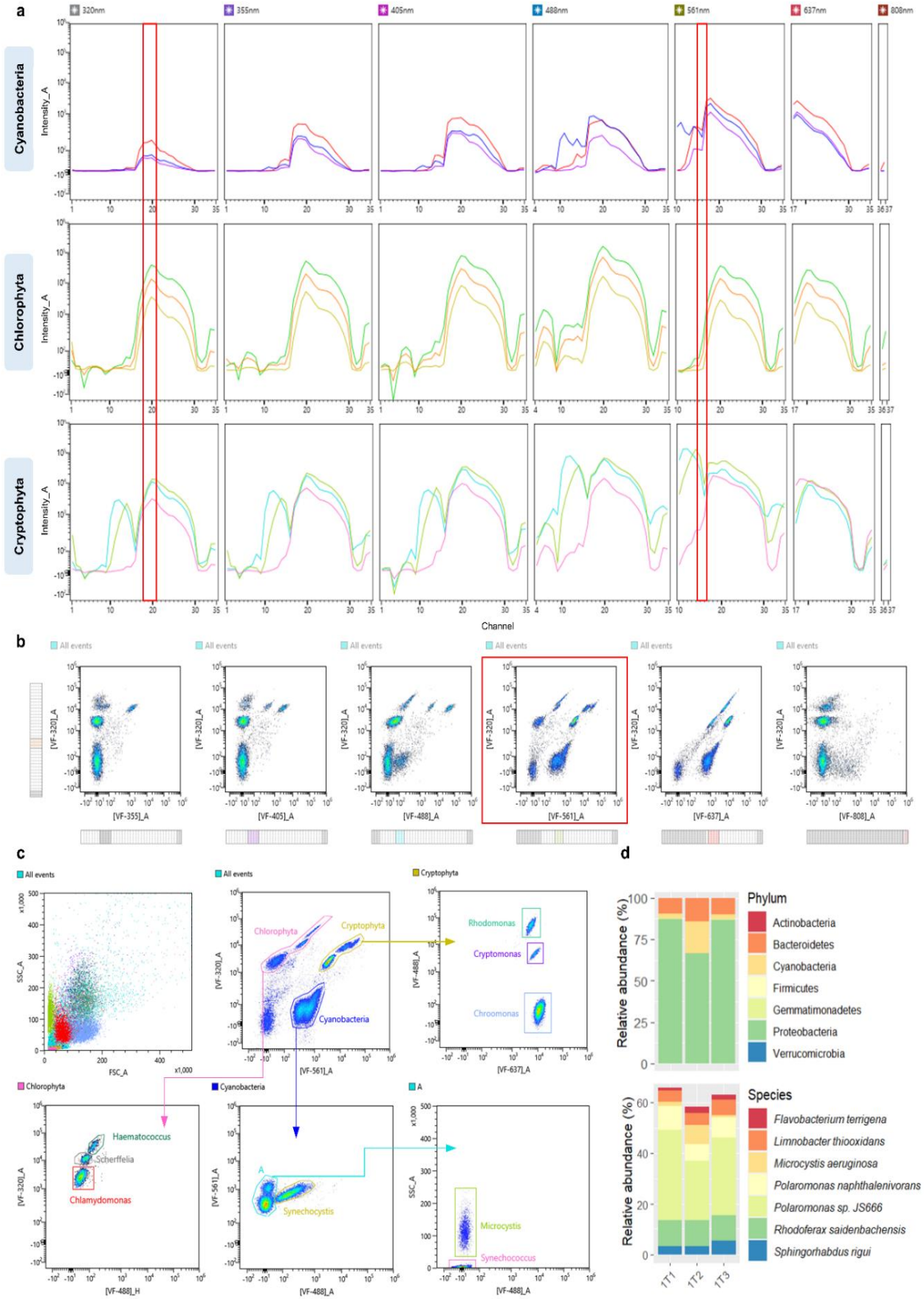
To discriminate multiple components in a suspension, a mixture of picoplanktonic cyanobacteria was used and recorded for further analysis (**Supplementary Figure 1**). In addition, components of the mix represented variable taxonomic groups at the Order level (*Microcystis* sp., belonging to Chroococcales), as well as members of the same Order (*Synechococcus* sp., *Synechocystis* sp., belonging to Synechococcales. The gating strategy is presented in **Suppl. Figure 1c**, where a combination of [VF-647]_A vs [VF-488]_A reveals two subpopulations, corresponding to *Synechocystis* sp. and population "A". Due to the similarity in the spectra of *Microcystis* sp., and *Synechococcus* sp. subpopulation "A" was then differentiated based on SSC values. Identified populations could not be discriminated based on FSC and SSC only (**Suppl. Figure 1d**). At this point, the maximum number of individual phytoplankton groups that could be discriminated reached 14 (**Supplementary Figure 2**).

Next, applicability of full-spectrum cytometry in the temporal monitoring of synthetic phytoplankton communities was assessed. Setting up an artificial mix experiment with 3 representatives of major phytoplankton groups – Cyanobacteria, Chlorophyta, and Cryptophyta (**Supplementary Table 1, Figure 3**), growth of which was monitored for the period of 9 days.

Triplicates were also analyzed in parallel on BD FACSAria (BD Biosciences, USA), ImageStreamX MarkII (Cytek, USA), and microbial community composition was assessed using Nanopore-based full-length 16S rRNA gene sequencing. Firstly, a library containing 9 individual spectral signatures covering each phytoplankton species within the mix was constructed (**Figure 2a).** As seen from the graph, regions unique for each of the groups are present, specifically between channels 10-15 (566-646 nm) of the spectra. This information was further utilized to define a set of optimal VF pairs to discriminate these populations in a complex mix. VFs were defined in a way that clustered subpopulations within the same group, while maintaining the difference between the groups. A set of filters [VF-320]_A vs [VF-561]_A (**Figure 2b**) was chosen as an optimal pair for the first step within the gating strategy (**Figure 2c**), where three groups are identified and discriminated from the rest of the unwanted events (background noise/cellular debris).

The next steps involved pairwise combinations of defined filters to discriminate the components of each of the subpopulations. As mentioned previously, due to the high similarity of spectra between *Microcystis* sp. and *Synechococcus* sp., SSC was used to differentiate these populations. A comparison of the data obtained on 3 instruments revealed the limitations of some of the instruments (**Supplementary Figure 4**). Opt-SNE dimensionality reduction algorithm was utilized to minimize "gating bias" and group data into clusters with similar characteristics in low-dimensional space. 3 datasets were compared: synthetic mix data obtained from the ID7000 instrument using a set of pre-defined VFs and a set of VFs matching the filters used on FACSAria, and lastly, data obtained from FACSAria. Both sets of filters utilized on the ID7000 resulted in a clear separation of all 9 subpopulations within the mix, while a single population of *Scherffelia* sp. was not possible to discriminate from the rest on the FACSAria instrument. These results demonstrate the higher flexibility a virtual filter system offers in the analysis of multi-component mixes. In addition to more precise population separation obtained with the ID7000 instrument, it was possible to track the

abundance of nearly all mix components, as demonstrated in **Supplementary Figure 5**. Overlay of temporal spectral signatures recorded for the period of 9 days for each mix member was used to assess the extent of signal intensity dispersion. As revealed through the coefficient of variation, regions most susceptible to differences in intensity generally included channels 1-13 (413.6-612.6 nm) and 30-34 (785.4-835.1 nm). High variation within these regions was also observed when comparing single spectral signatures between excitation lasers (**Supplementary Figure 6**).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[VF-355]_A
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[VF-488]_A
[VF-561]_A
[VF-637]_A
[VF-808]_A
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Cryptophyta
Cyanobacteria
Rhodomonas
Cryptomonas
Chroomonas
Haematococcus
Scherffelia
Chlamydomonas
Synechocystis
Microcystis
Synechococcus
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[VF-488]_A
[VF-561]_A
[VF-637]_A
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Relative abundance (%)
Phylum
Actinobacteria
Bacteroidetes
Cyanobacteria
Firmicutes
Gemmatimonadetes
Proteobacteria
Verrucomicrobia
Species
Flavobacterium terrigena
Limnobacter thiooxidans
Microcystis aeruginosa
Polaromonas naphthalenivorans
Polaromonas sp. JS666
Rhodoferax saidenbachensis
Sphingorhabdus rigui
1T1
1T2
1T3

154

Figure 2. Tracking temporal changes in synthetic community composition. **(a)** overlay plot of individual spectral signatures for each phytoplankton group representative (Cyanobacteria, Chlorophyta, and Cryptophyta); red regions indicate regions of interest used for setting an optimal pair of VFs; **(b)** "Autofluorescence Finder" tool with VFs set to regions of interest, demonstrating the best separation of subpopulations on [VF-320_A] VS [VF-561_A] plot; **(c)** The gating strategy, composed of sequential sub-gating steps, used to identify phytoplankton subpopulations within the synthetic community; **(d)** Microbial community composition in experimental triplicates at phylum and species level revealed through full-length 16S rRNA gene-based Nanopore sequencing.

In conclusion, by constructing spectral libraries for various phytoplankton groups and differentiating between algal taxa and debris based on autofluorescent pigment signatures and light scattering, spectral cytometry overcomes the limitations of traditional methods and provides detailed insights into community structure, dynamics, and responses to environmental stressors. The potential for parallel sequencing and associated microbial analysis at the multi-omics level is needed to fully elucidate the microbial functions occurring in artificial algal communities. This technology provides possibilities for a wide range of research applications, from addressing fundamental ecological and evolutionary questions to applied biology, thereby advancing the field of environmental research.

Methods

Sample preparation and instrument setup

Freshwater algal cultures (**Supplementary Table 1**) were obtained from The Culture Collection of Algae at the University of Göttingen (Germany) and Central Collection of Algal Cultures at the University of Duisburg-Essen (Germany). Cultures were maintained in Basal Medium (BM) at 21°C, under 12/12 L/D cycle in algae growth chamber AL-30L2 (Percival, USA). Samples were recorded in parallel on 3 instruments: 7 lasers ID7000 Spectral Cell Analyzer (Sony Biotechnology, USA), 5

lasers BD FACSAria (BD Biosciences, USA) and ImageStreamX MarkII (Amnis - Cytek, USA) equipped with 40X and 60X objectives. Samples prepared for cytometric analysis included mono- and mixed cultures and were recorded at spectral cytometer using a standard 24-tube rack. Each sample was placed in round-bottom 5mL tubes, with minimum volume of 250uL, and arranged in specified positions on the rack. Prior to data acquisition, instrument settings were adjusted and set in accordance with each sample (FSC and SSC gains depending on algal culture, threshold value – 11%, PMT voltage ranging from 40% to 70%). Samples were recorded at minimum flow rate (=1) and at least 50,000 events was set for data acquisition. Scatter plots were used for initial assessment of instrument settings in the "Preview" mode prior to recording.

Autofluorescence Finder Tool and Virtual Filters setup

The ID7000 software's Autofluorescence Finder Tool was used to analyze the algae samples, treating autofluorescence as an independent fluorescent parameter. This feature is crucial for the taxonomic identification of different algae. By incorporating excitation lasers ranging from 320nm to 808 nm, we included unique emission regions in the algal fluorescent signature, distinct from the Chl peak and highly variable among different algae. This setup allowed for the detection of individual autofluorescent signals for each of the recorded monoculture. Following the identification of the best separation between autofluorescent populations, subpopulations of interest were gated and examined for their spectral signatures. Each population demonstrated a unique autofluorescence spectrum. Additionally, the Autofluorescence Finder Tool facilitated the distinction between dead and living algae based on differences in autofluorescence.

Data analysis

Initial data analysis and population identification was performed using the ID7000 software. Identification of subpopulations within artificial mix involved consecutive gating steps using a combination of 2D plots with specified virtual filters for best separation. Furthermore, t-Distributed Stochastic Neighbor Embedding, otherwise known as t-SNE, was done using FCS Express (De Novo Software). A modified version of t-SNE - opt-SNE, a tool allowing to map high-dimensional cytometry data onto a two-dimension plot while conserving the original high-dimensional structure, was used to independently identify subpopulations within the artificial mix, minimizing gating bias. Obtained clusters were then used to compare data acquired on different cytometers.

Sequencing library preparation and bioinformatic analysis

Samples for Nanopore-based 16S rRNA sequencing were collected during the last day of the experiment and subsequent procedure consists of the following steps. Firstly, DNA was extracted using the DNEasy Power Water Kit (Qiagen, Germany) according to the manufacturer's instructions, with an additional heating lysis step. The full-length 16S gene was then amplified using the universal 16S primer pair 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). Library preparation followed the manufacturer's protocol (ONT, UK). The PCR reaction mixture included nuclease-free water, input DNA, LongAmp Hot Start Taq 2X Master Mix (New England Biolabs, USA), and the respective 16S barcode primer. PCR products were next purified using AMPure XP beads (Beckman Coulter, USA). In the final library preparation step, sequencing adapters were added to the barcoded samples. The sequencing run was performed on the MinION Mk1C device (Oxford Nanotechnologies, UK) for 40-46 hours. Raw signal data were stored in FAST5 files and underwent basecalling with the Guppy neural network-based basecaller integrated into the MinION Mk1C device. The resulting FASTQ files were processed using Python commands, beginning with read assessment and quality evaluation, followed by quality and read length filtering. Next steps involved adapter sequence removal and demultiplexing of reads into

their respective barcodes. The demultiplexed reads were then classified using the Emu taxonomic abundance estimator up to the species level (https://gitlab.com/treangenlab/emu)[15-16]. Lastly, the data were rarefied prior to further analysis.

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Authors contributions

A.M. designed the study, performed the experiments, analyzed the data. S.M. and N.F. contributed algal cells, in algal cells characterization. I.A.V. and N.S.B. initiated and supervised the project, and N.S.B. funded the project. A.M. and N.S.B. wrote the manuscript with contributions from all authors.

Data availability

Raw sequences obtained during the analysis were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under the BioProject ID PRJNA1131605. Spectral and cytometric data obtained during this study are available from the corresponding author on reasonable request.

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Competing interests

The authors declare no competing financial interests.

References

1. Niu, B. et al. PNAS 114, E2450-9 (2017).
2. Hartwell, L.H. et al. Nature 402, C47-52 (1999).
3. Andersen, R.A. Algal Culturing Techniques. Elsevier, Burlington, USA (2005).
4. Segev, E. et al. elife. 5, e17473 (2016).
5. Poryvkina, L. et al. J. Plankton Res. 16, 1315-27 (1994).
6. Liu, J.Y. et al. Appl. Spectroscopy Rev. 56, 171-92 (2021).
7. Swan, C.M. et al. Deep Sea Res. Part I: Oceanographic Res. Papers. 109, 137-56 (2016).
8. Futamura, K. et al. Cytometry Part A 87, 830-42 (2015).
9. Robinson, J.P. Biotechniques 72, 159-69 (2022).
10. Dott, T. et al. Cytometry Part A 105, 124-38 (2024).
11. Barteneva, N.S. et al. BioRxiv. 2019 Jan 10:516146 (2019).
12. Barteneva, N.S. et al. Using virtual filtering approach to discriminate microalgae by spectral flow cytometer. In: Spectral and Imaging Cytometry: Methods and Protocols, pp. 23-40. NY Springer (2023).
13. Jeppesen, E. et al. Freshwater Biol. 50, 1747-71 (2005).
14. Wanner, N. et al. Front. Bioengineer. 10, 827987 (2022).
15. Curry, K.D. et al. Nature Methods, 19, 845-53 (2022).
16. Len, P. et al. bioRxiv. 2023, 2023-12 (2023).

Supplementary material to "Spectral algal fingerprinting and long sequencing in synthetic algal-microbial communities"

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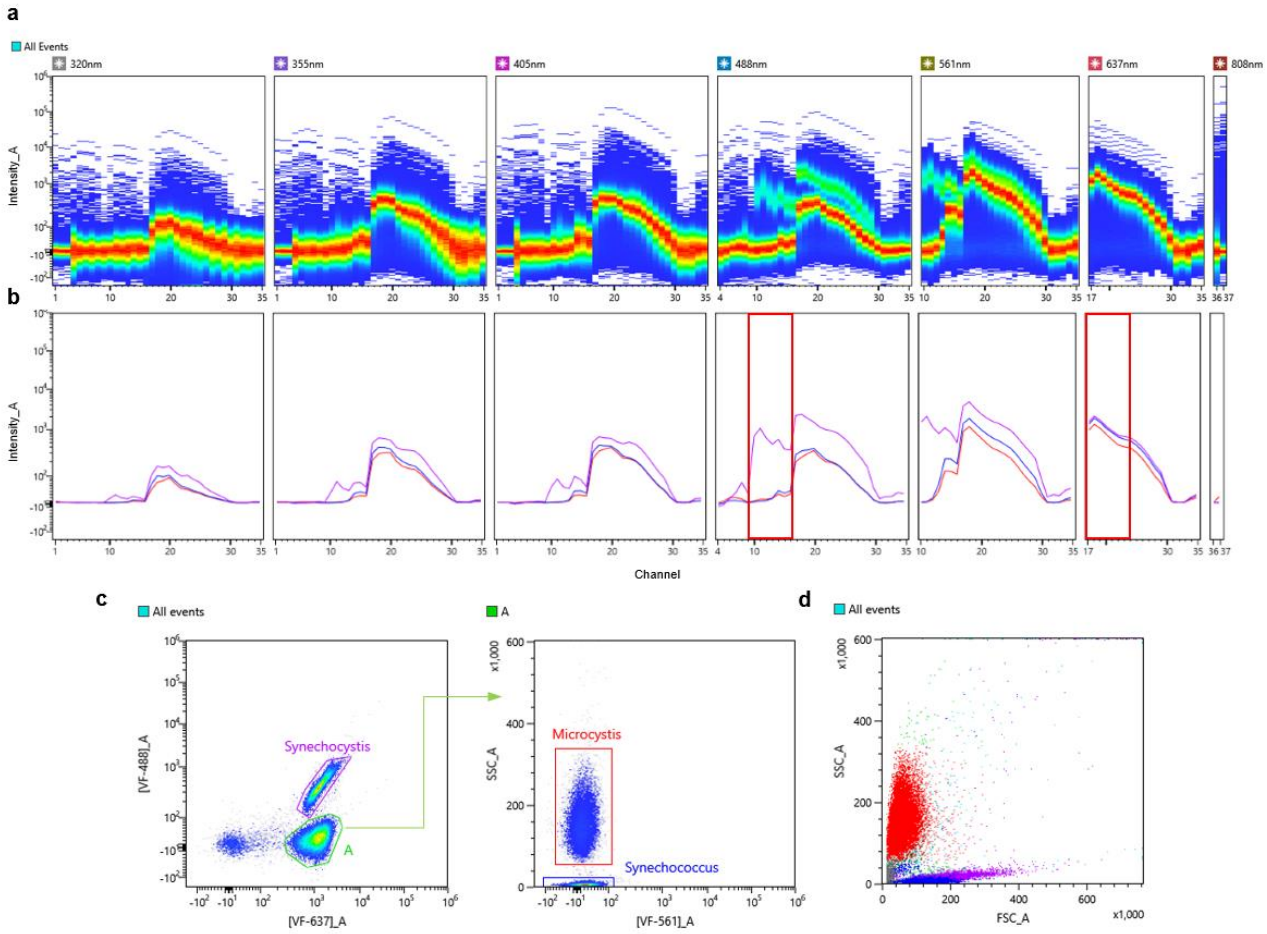
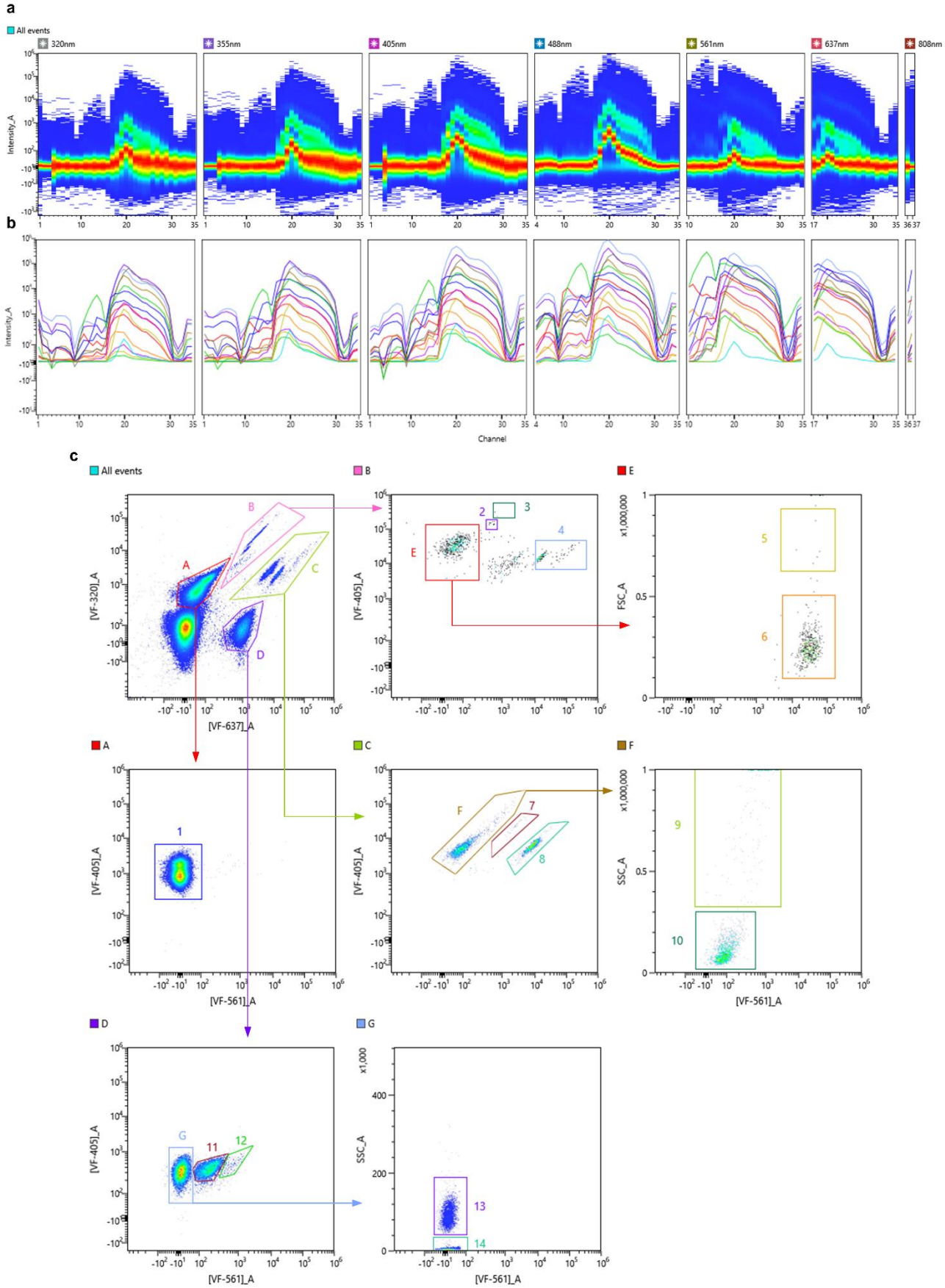
Supplementary Table 1. List of algal cultures used for recordings during artificial mix experiment and for library construction using ID7000 spectral cell analyzer

Phylum	Order	Species	Habitat	Strain number
Cyanobacteria	Nostocales	*Dolichospermum urugayensis*	Freshwater	2498
		Nodularia sphaerocarpa	Freshwater	50.79
	Gloeobacter	*Gloeobacter violaceus*	Freshwater	35.87
	Chroococcales	*Microcystis* sp.	Freshwater	CCAC 7146B
	Synechococcales	*Synechococcus* sp.	Freshwater	CCAC 2944B
		Synechocystis sp.	Freshwater	CCAC 4059B
Chlorophyta	Chlorodendrales	*Scherffelia dubia*	Freshwater	CCAC 1398 B
	Chlamydomonadales	*Chlamydomonas* sp.	Freshwater	CCAC 4527 B
		Haematococcus pluvialis	Freshwater	CCAC 2072B
	Chlorellalels	*Chlorella vulgaris*	Freshwater	211-12
	Sphaeropleales	*Acutodesmus obliquus*	Freshwater	22.81
Cryptophyta	Pyrenomonadales	*Chroomonas* sp.	Freshwater	CCAC 2614B
		Rhodomonas sp.	Freshwater	CCAC 1479B
	Cryptomonadales	*Cryptomonas* sp.	Freshwater	CCAC 2345B
Rhodophyta	Stylonematales	*Chroothece richteriana*	Marine	104.79
	Porphyridiales	*Porphyridium cruentum cf.*	Marine	CCAC 3771 B
Dinophyta	Peridiniales	*Peridinium* sp.	Freshwater	CCAC 1612 B
Euglenophyta	Euglenida	*Euglena sanguinea*	Freshwater	CCAC 3518 B
Bacillariophyta	Fragilariales	*Fragilaria* sp.	Freshwater	CCAC 5509 B



Supplementary Figure 1. Discrimination of Picocyanobacteria in a synthetic mix based on autofluorescence. **(a)** Ribbon plot demonstrating combined spectral signatures of 3 picocyanobacterial representatives across 7 excitation lasers: *Synechococcus* sp., *Synechocystis* sp., and *Microcystis* sp. **(b)** Overlay plot of individual spectral signatures for each picocyanobacterial representative (*Synechococcus* sp. - blue, *Synechocystis* sp. - violet, and *Microcystis* sp. - red); red regions indicate regions of interest, used for setting an optimal pair of VFs. **(c)** Dot plot of **[VF-637]**_A against **[VF-488]**_A with 2 autofluorescent populations identified: *Synechocystis* sp., population **A** was then plotted with **[VF-561]**_A against **SSC**_A to resolve two subpopulations *of Microcystis* sp. and *Synechococcus* sp. **(d)** Dot plot demonstrating relative position of each of the identified picocyanobacterial species on a FSC_A against SSC_A plot.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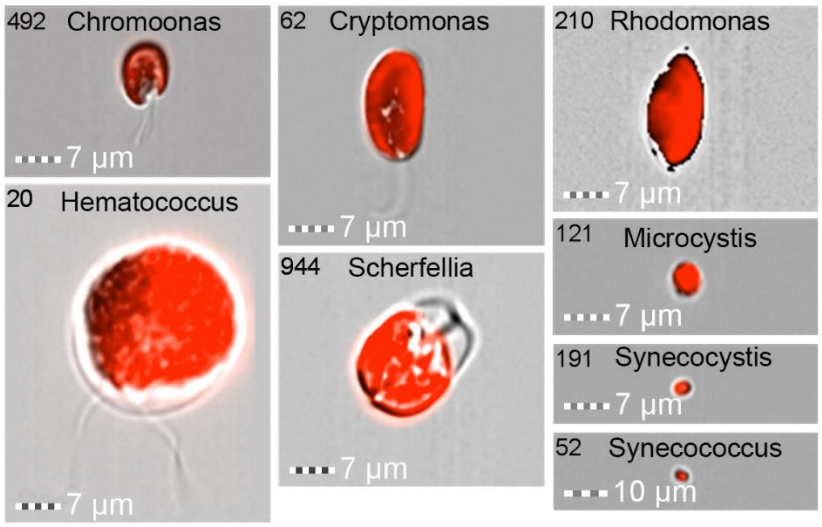
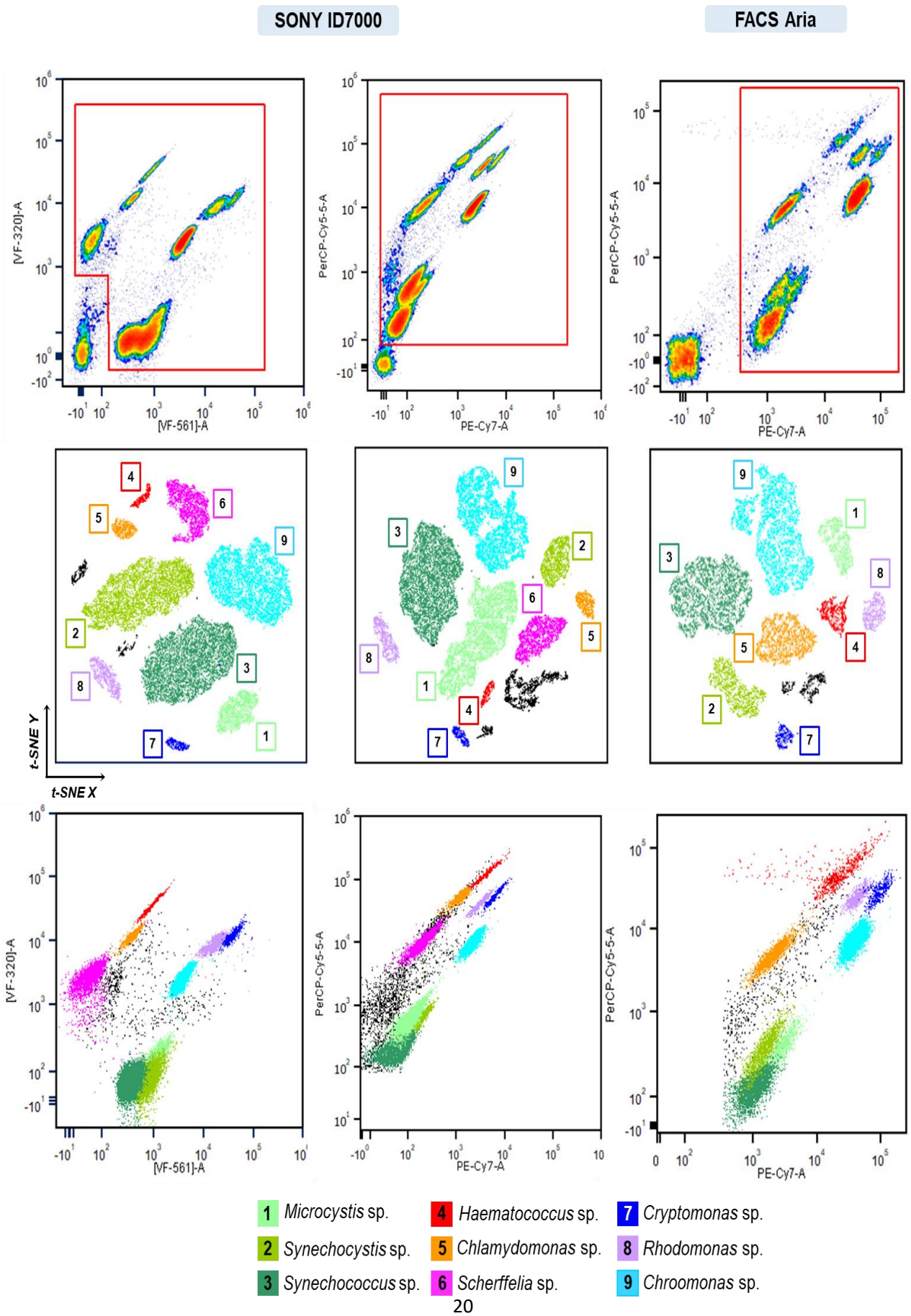
Supplementary Figure 2. Gating strategy for discrimination of 14 groups of phytoplankton species based on autofluorescence in a complex synthetic mix. **(a)** Ribbon plot demonstrating combined spectral signatures of 16 phytoplankton species across 7 excitation lasers. **(b)** Overlay plot of individual spectral signatures for each phytoplankton representative. (c) Gating strategy for discrimination of each subpopulation within the synthetic mix: 1 – Chlorophyta (*Chlorella vulgaris* and *Acutodesmus obliquus*), 2 – *Euglena sanguinea*, 3 – *Peridinium* sp., 4 – *Cryptomonas* sp., 5 – *Fragilaria* sp., 6 – *Haematococcus pluvialis*, 7 – *Chroothece richteriana*, 8 – *Porphyridium cruentum* cf., 9 – filamentous Cyanobacteria (*Dolichospermum urugayensis* and *Nodularia sphaerocarpa*), 10 – *Chroomonas* sp., 11 – *Synechocystis* sp., 12 – *Gloeobacter violaceus*, 13 – *Microcystis* sp., 14 – *Synechococcus* sp.



Supplementary Figure 3. The gallery of algal images taken with ImageStream MKII (Amnis-Cytek, USA) of phytoplankton species recorded during the artificial mix experiment.


SONY ID7000
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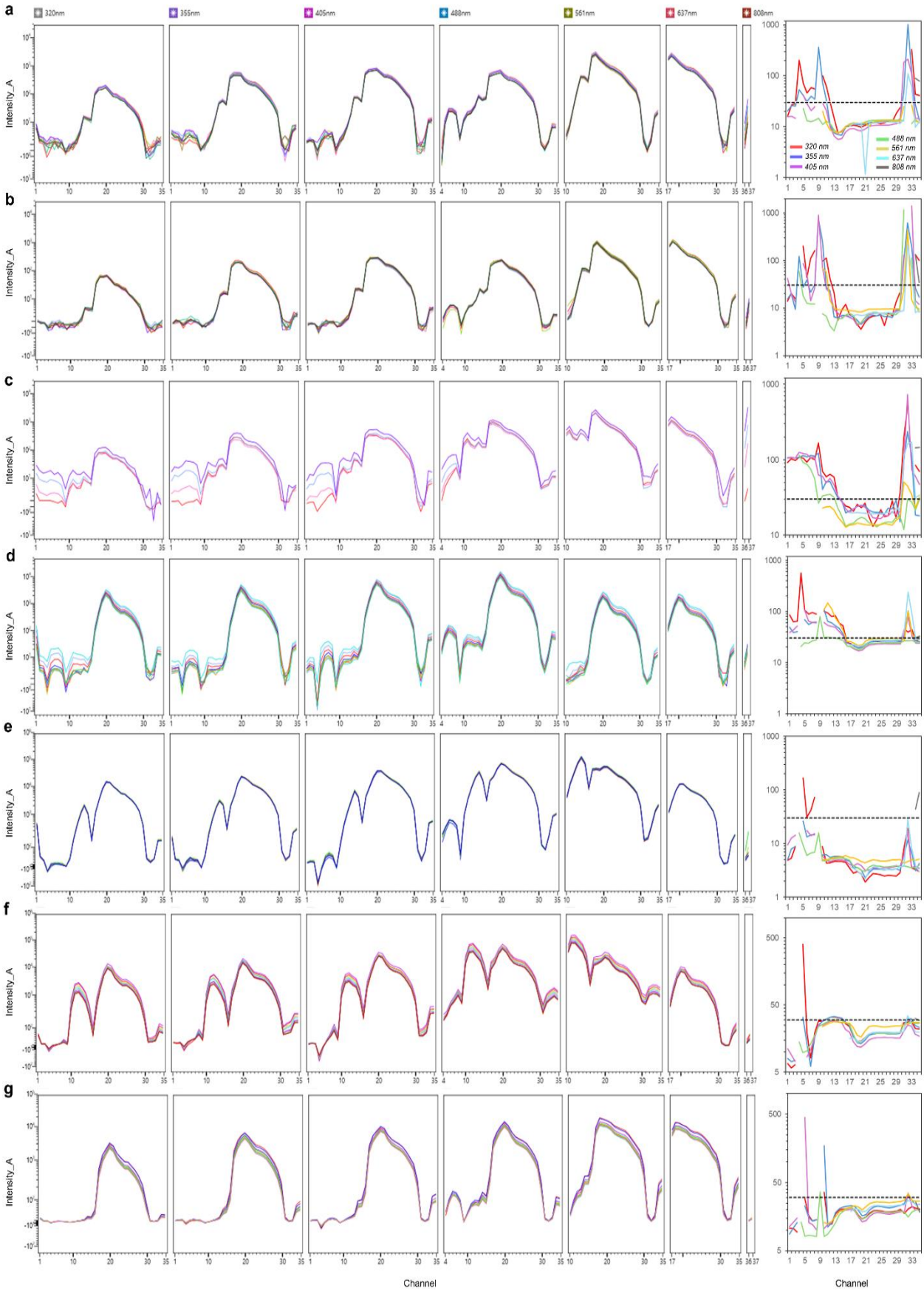
1 Microcystis sp.
2 Synechocystis sp.
3 Synechococcus sp.
4 Haematococcus sp.
5 Chlamydomonas sp.
6 Scherffelia sp.
7 Cryptomonas sp.
8 Rhodomonas sp.
9 Chroomonas sp.

Supplementary Figure 4. Identification of artificial mix subpopulations compared between SONY ID700 Spectral Cell Analyzer and BD FACSAria II. The first column represents data recorded on SONY ID7000 using a set of VFs best optimized for artificial mix discrimination. Opt-SNE demonstrates successful discrimination of the 9 subpopulations within the mix based on VF data. The second column represents data recorded on SONY ID7000 using a set of VFs matching optical filters used on BD FACSAria. Similarly, all 9 subpopulations were discriminated. Last column represents data recorded on BD FACSAria. Based on the optical configuration of the instrument, only 8 subpopulations were identified using opt-SNE.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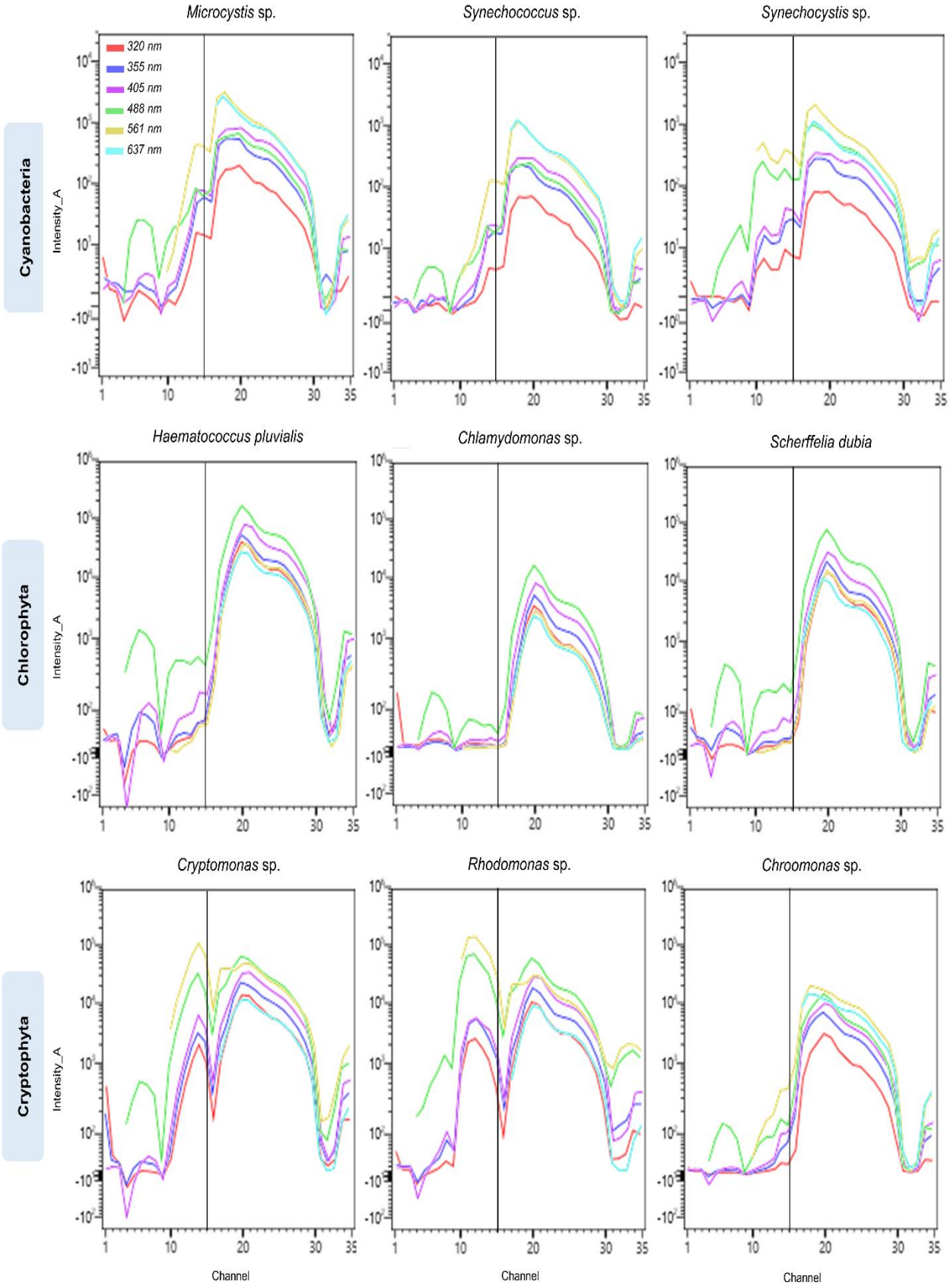
Supplementary Figure 5. Temporal overlay of spectral signatures recorded throughout the experiment. Individual spectral signatures for each member of the synthetic mix (**(a)** – *Microcystis* sp., **(b) -** *Synechococcus* sp., **(c)** – *Synechocystis* sp., **(d)** – *Chlamydomonas* sp., **(e) -** *Cryptomonas* sp., **(f)** – *Rhodomonas* sp., **(g)** – *Chroomonas* sp.) recorded over the period of 9 days overlayed to demonstrate temporal changes in signal intensity. To display signal dispersion, coefficient of variation (CV) was calculated and plotted across all detection channels for each of the excitation lasers. A threshold line at 30% was plotted for additional reference.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Chlamydomonas sp.
Scherffelia dubia
Chlorophyta
Cryptomonas sp.
Rhodomonas sp.
Chroomonas sp.
Cryptophyta
Intensity_A
Channel

353

Supplementary Figure 6. Overlay plots for each phytoplankton species within a synthetic mix across 7 excitation lasers to demonstrate the variability of emission signal for each excitation source. The first row demonstrates overlay plots for Cyanobacterial representatives, second – Chlorophyta, and third – Cryptophyta. The black line denotes cutoff at channel 15 (624 – 635.3 nm).