Chlorins: A Novel Family of Violet Laser-Excitable Red to Far-Red Fluorophores for Polychromatic Flow Cytometry

J. Bruce Pitner[1], Rosemary B. Evans-Storms[1], Duane A. Olsen[1], Masahiko Taniguchi[2], Jonathan S. Lindsey[2]

[1]R&D, NIRvana Sciences, Durham, NC (USA), [2]Chemistry, North Carolina State University, Raleigh, NC (USA)

INTRODUCTION

Cytometric panels of 10-18 colors are becoming routine. The newest instruments are capable of detecting over 30 parameters, but the spectral overlap of currently available dyes hinders the full exploitation of their capabilities. We present a selection of synthetic chlorins, a class of dyes that possess ultra-narrow emissions with minimal spectral overlap. These violet laser-excitable dyes could increase the number of channels that can be used to more fully utilize the latest instrumentation.

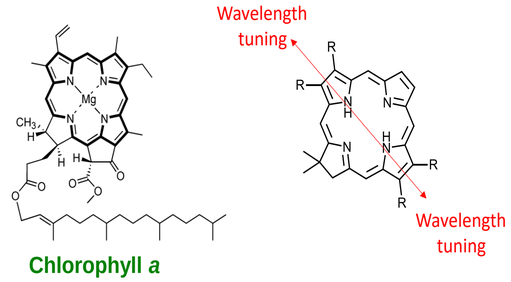


Figure 1. Chlorophyll a and synthetic chlorin core structures

Chlorins are distinguished from porphyrins by having a reduced pyrrole bond in their core tetrapyrrole system. Our synthetic chlorins feature emission wavelengths “tuned” through placement of auxochromes at the beta-pyrrole positions and a dimethyl group at the reduced pyrrole for enhanced stability[1] (Figure 1).

The effective width of each chlorin’s peak emission (full width-half max, (FWHM) is typically ≤ 20 nm. Thus, emissions from these chlorins can be compressed into a much smaller spectral range than those from currently available violet laser dyes.

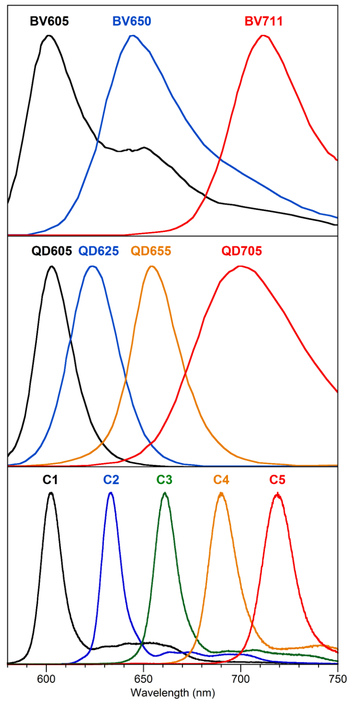


Figure 2. Emission spectra for polymer-based BD Horizon Brilliant™ Violet dyes, Life Technologies’ Qdot® semiconductor labels, and NIRvana Chlorin dyes C1 – C5.

METHODS and RESULTS

Five chlorins (C1-C5) that possess emission peaks from 600-720 nm were synthesized de novo. These share significant excitation band overlap at 405 nm (Figure 3). The peak emission wavelengths of each chlorin are shown in Table 1.

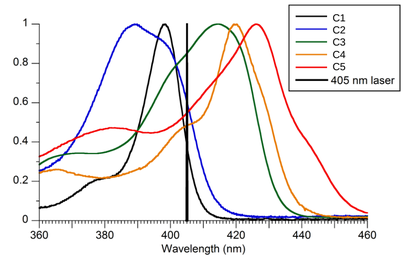
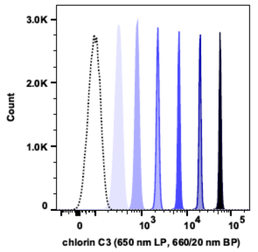


Figure 3. Absorption spectra for chlorins C1-C5

Polystyrene beads of approximately 5.4 µm diameter were doped with a dilution series (half-log) of chlorin C3. These beads were analyzed on a BD LSRII SORP and data further analyzed with FlowJo software. Signal was detected over two logs of dye concentration with good linearity of signal (Figure 4).

Figure 4. Compiled histogram of a dilution series of C3 with a non-doped negative control. All data sets for Figures 4 and 5 were gated on single bead populations.



Polystyrene beads were subsequently doped with equal concentrations of chlorins C1–C5. These samples were analyzed on a BD LSRFortessa flow cytometer using the long pass (LP) and bandpass (BP) filter sets specified in Table 1. A compensation matrix was generated by running individual chlorin beads (Table 2). Beads with C1-C5 plus a negative control were mixed and dot plots for the compensated data were generated (Figure 5).

Chlorin:	C1	C2	C3	C4	C5
λ_{max} (nm)	602.6	633.4	661.0	689.6	719.0
Violet (405 nm) Channel	E	D	C	B	A
LP filter (nm)	570	615	650	670	710
BP filter (nm)	610/20	630/22	660/20	690/20	730/45

Table 1. λ_{max} of chlorins C1-C5 and optical configuration for analysis using the 405 nm laser of the BD LSRII SORP or LSRFortessa. All flow cytometry experiments were performed at the University of North Carolina (UNC) at Chapel Hill Flow Cytometry Core Facility. Filters were obtained from BD and Chroma.

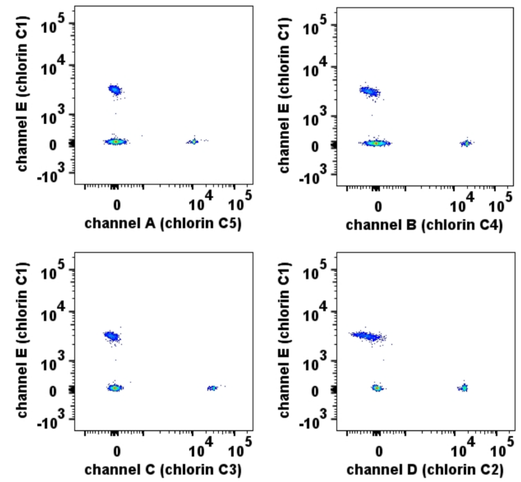


Figure 5. Dot plots of compensated data from chlorins C1-C5

Channels

Chlorins	C1	C2	C3	C4	C5
C1	100	67.19	42.20	44.54	27.64
C2	0.58	100	8.51	12.21	3.46
C3	0.12	0.72	100	16.59	13.44
C4	0.33	0.44	5.41	100	15.93
C5	0.05	0.62	0.91	4.03	100

Table 2. Compensation matrix for chlorins C1-C5 generated by FlowJo from data collected on the BD LSRFortessa.

CONCLUSIONS

We demonstrate that a palette of five violet-excitable chlorins with narrow emissions can be effectively separated using conventional flow cytometer optics and commercially available filter sets. The ultra-narrow emissions and minimal spectral overlap of these dyes, when combined with other available violet dyes, could soon enable detection of eight or more colors from the violet laser.

REFERENCES

1. J. S. Lindsey, “De Novo Synthesis of Gem-Dialkyl Chlorophyll Analogues for Probing and Emulating Our Green World”, Chem. Rev., 2015, 115, 6534–6620

ACKNOWLEDGEMENTS

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