EVALUATING INSTRUMENTS DESIGNED FOR RAPID, SHIPBOARD DETECTION OF LIVING MICROORGANISMS IN BALLAST WATER: AN INITIAL TEST OF COMPLIANCE TOOLS

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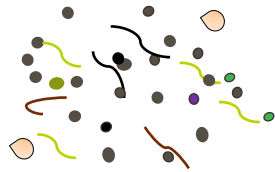[5]Moss Landing Marine Laboratories

[6]Buckley Environmental

Outline

Introduction: Discharge Limits
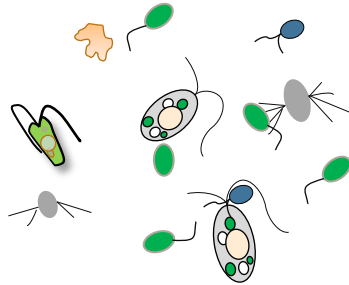
<10 μm   ≥10 and <50 μm   ≥50 μm
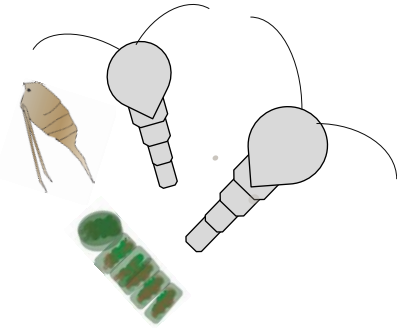
IMO and U.S.A. Limits*

V. cholerae: <1 cfu 100 mL^{-1}
E. coli: <250 cfu 100 mL^{-1}
Enterococci: <100 cfu 100 mL^{-1}

<10 living
organisms mL^{-1}

<10 living
organisms m^{-3}

cfu = colony-forming unit

*Specified in the International Maritime Organization (IMO) D-2 Ballast Water Performance Standard and in the U.S. Discharge Standard; limits only differ for *V. cholerae*, where IMO also limits concentrations to <1 cfu g^{-1} wet weight of zooplankton

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Characteristics of a Compliance Tool

- Applicable to a broad range of organisms

- Functional across a wide range of water types

- Rapid, simple to operate, easy to interpret

- Consistent and accurate

- Portable!

~8 m

M/V Indiana Harbor
Superior, WI
May 2012
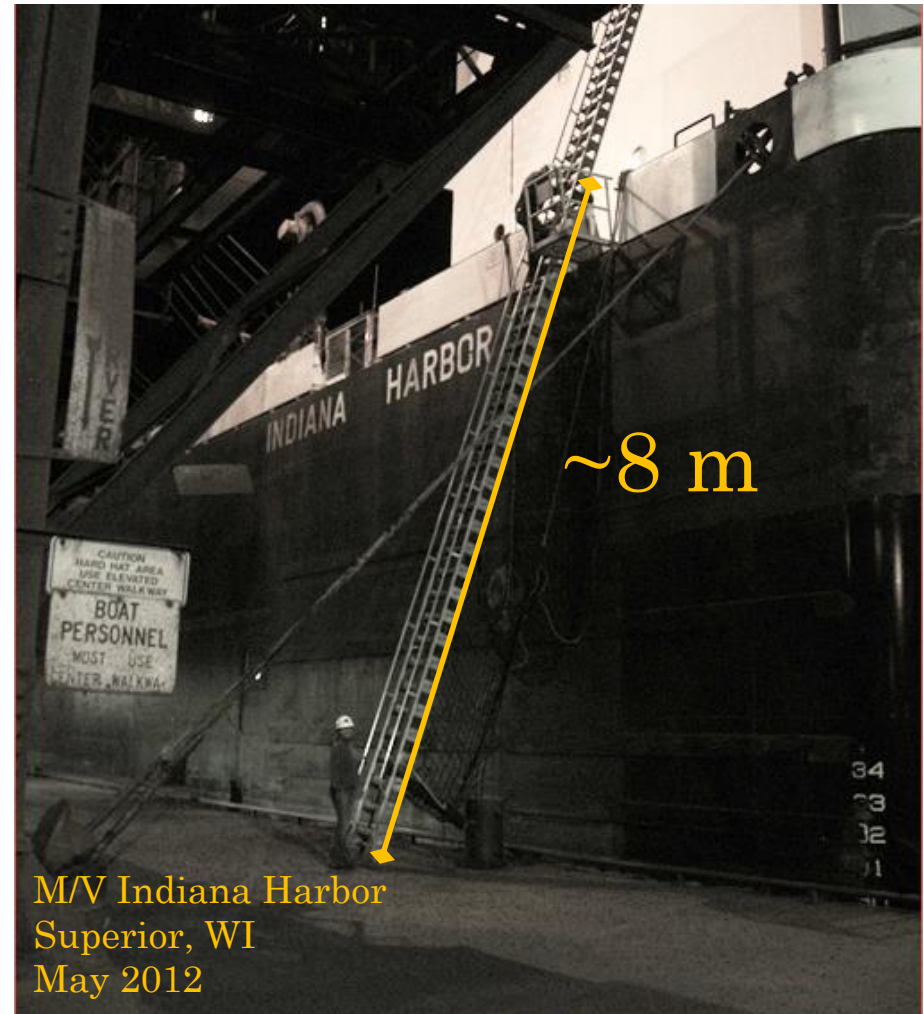
Targeting Organisms ≥10 and <50 μm

The ≥10 and <50 μm size class is ideal for compliance testing

- Organisms ≥10 and <50 μm
  - Sample volume ranges from milliliters to liters
  - Photosynthetic organisms are detectable by chlorophyll a fluorescence*
  - Living phytoplankton can be assessed by variable fluorescence

≥10 and <50 μm

- Organisms ≥50 μm
  - Large sample volumes are needed (e.g., ≥ 1 m^3)
  - Rapid sampling and analysis would be difficult

- Organisms <10 μm
  - Require targeted approaches (e.g., selective media) and complicated or lengthy assays
  - Also, absence of specific indicator organisms and pathogens is not indicative of ambient organisms
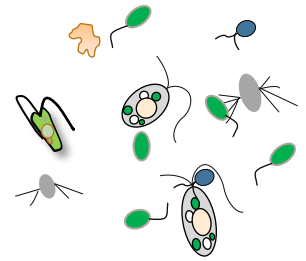
* Note: heterotrophic organisms are not detected with this approach
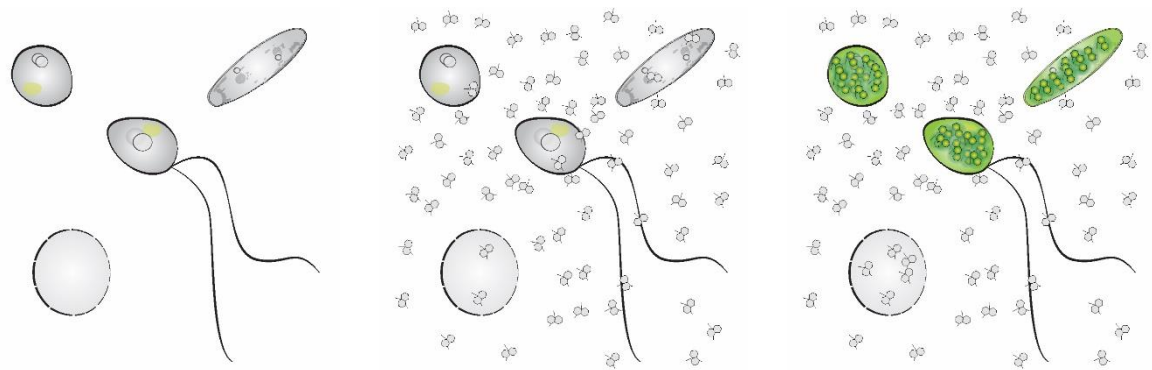
≥10 μm and <50 μm Method: Part 1

The Environmental Technology Verification Protocol (ETV)*
stipulates an approach based upon epifluorescence microscopy

Two fluorescent probes are introduced
into the sample
(and fluoresce within living cells):

- Chloromethylfluorescein diacetate (CMFDA)
- Fluorescein diacetate (FDA)

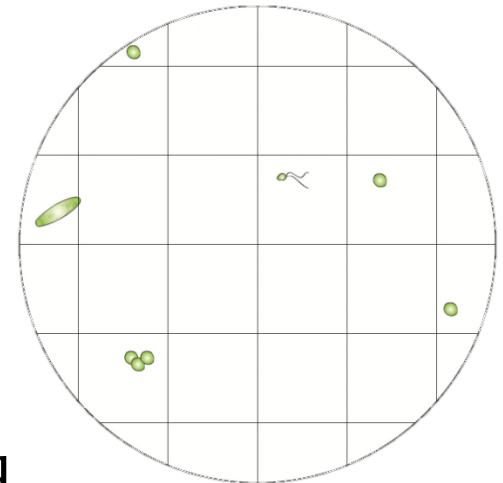*U.S. Environmental Protection Agency, 2010; the ETV is the U.S. protocol for
land-based verification testing of ballast water management systems

≥10 μm and <50 μm Method: Part 2
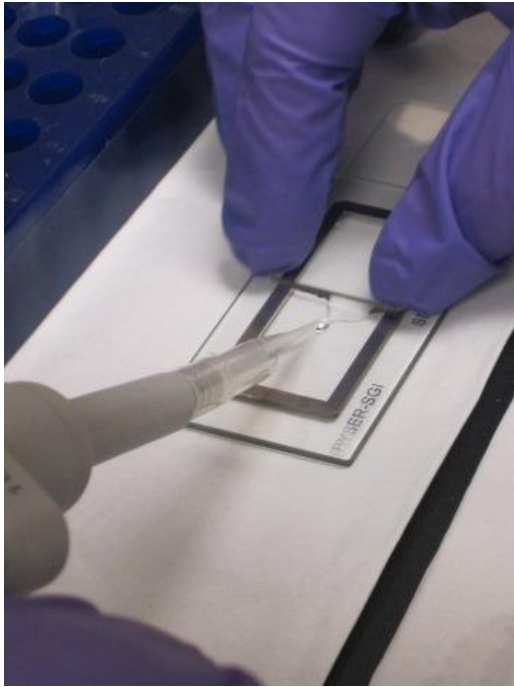
Manual microscopy:
Visual counts of
fluorescing or moving
(i.e., living) organisms

Reported value: Living organisms per mL
Directly comparable to discharge standard

Direct microscope counting:
likely not feasible for compliance analysis

A Framework for Validation*

1. Proof of Concept

   - Laboratory demonstrations
   - Pilot-scale experiments
   - Subject-matter-expert workshops

2. Verification and Validation

3. Feasibility and Selection

   - Rigorous experimental testing
   - Side-by-side comparisons with standard methods
   - Evaluation of measurement uncertainty

   - Consideration of data quality, but also safety, cost, and ease of use
   - Evaluation of the "Technology Readiness Level" (TRL)

*Drake et al. (2014) How many organisms are in ballast water discharge?
A framework for validating and selecting compliance monitoring tools.
Marine Pollution Bulletin 86: 122-128

Core Technology

The compliance tool must be capable of detecting *living* organisms

Current Approach: Variable fluorescence of chlorophyll a (Chl a)

- Chl a: Fluorescent biomolecule required for photosynthesis
- Microalgae: Contain Chl a and are ubiquitous in the ≥ 10 and <50 µm size range
- Chl a from living microalgae: Displays a unique response to variable illumination

Chl a:

In living cells

In solution

In dead cells
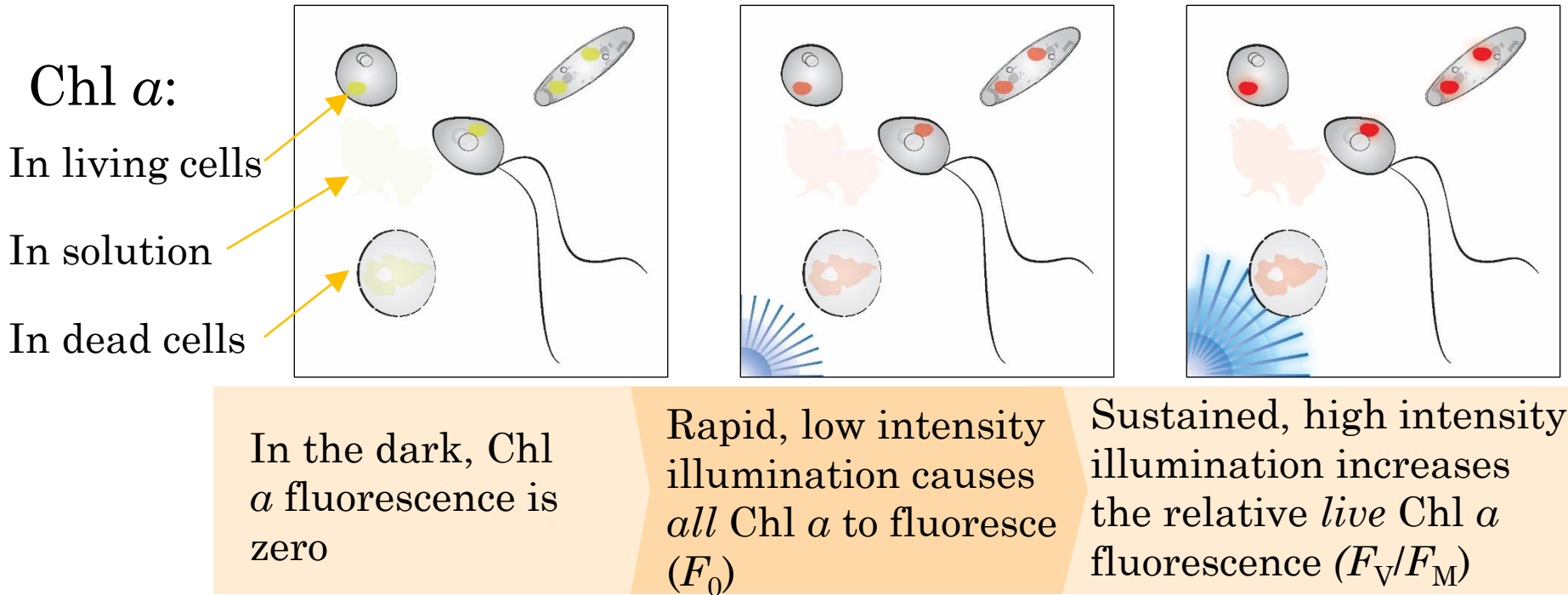
In the dark, Chl a fluorescence is zero

Rapid, low intensity illumination causes *all* Chl a to fluoresce (F_0)

Sustained, high intensity illumination increases the relative *live* Chl a fluorescence (F_V/F_M)

Variable fluorescence can rapidly estimate total concentrations of active microalgae

Testing and Analysis

- Initial Round of Testing (June – September, 2015)
  - Three fluorometry vendors participated
  - One set of laboratory-based experiments
  - Three sets of field-based experiments
  - Side-by-side comparison with the required method

Cultured microalgae used in laboratory-based experiments

Sampling ambient organisms from seawater in Key West, FL

Image: Gert Hansen
SCCAP K-1137
http://media.nordicmicroalgae.org

Prorocentrum micans

https://ncma.bigelow.org/

10 μm

Tetraselmis marina

Photo. M. First, NRL, Key West, June 2015

Testing, analysis, and reporting
follow the protocols of the
Alliance for Coastal Technologies (ACT)

Study Sites

Field trials were performed at three locations:
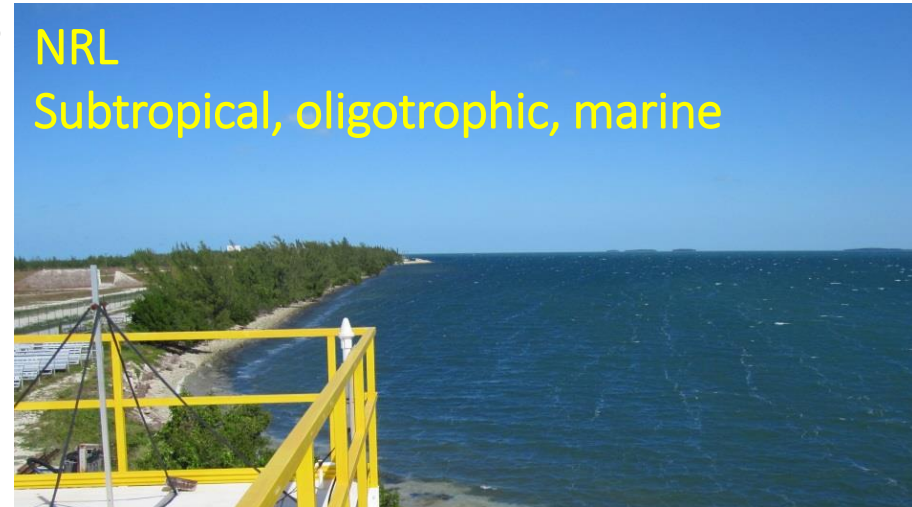
Naval Research Laboratory (NRL)
Key West, FL

Smithsonian Environmental Research Center (SERC)
Edgewater, MD

Great Ships Initiative (GSI)
Superior, WI

NRL
Subtropical, oligotrophic, marine

SERC
Temperate, eutrophic, brackish

GSI
Temperate, mesotrophic, fresh

Results: Tool 1

Risk Assessment

- ○ High (all replicates) *(red)*
- ○ High (some replicates) *(orange)*
- ○ Low (all replicates) *(green)*

F_V/F_M (No Units)

- ○ <0.2
- ◐ ≥0.2 and <0.4
- ● ≥0.4

Agreement with
microscopy
(n of 36 samples):

NRL:	27/36	(75%)
SERC:	21/36	(58%)
GSI:	26/36	(69%)
Overall: 74/108 (69%)		

Note: axes are log-scaled

Plot axis labels:

Variable Fluoro-metry, F_0 (No Units)

Symbols
- ○ NRL
- △ SERC
- □ GSI

F_0: Mean of 3 subsample readings

Discharge standard
10 cells mL⁻¹ →

Microscopy Concentration (Cells mL⁻¹)
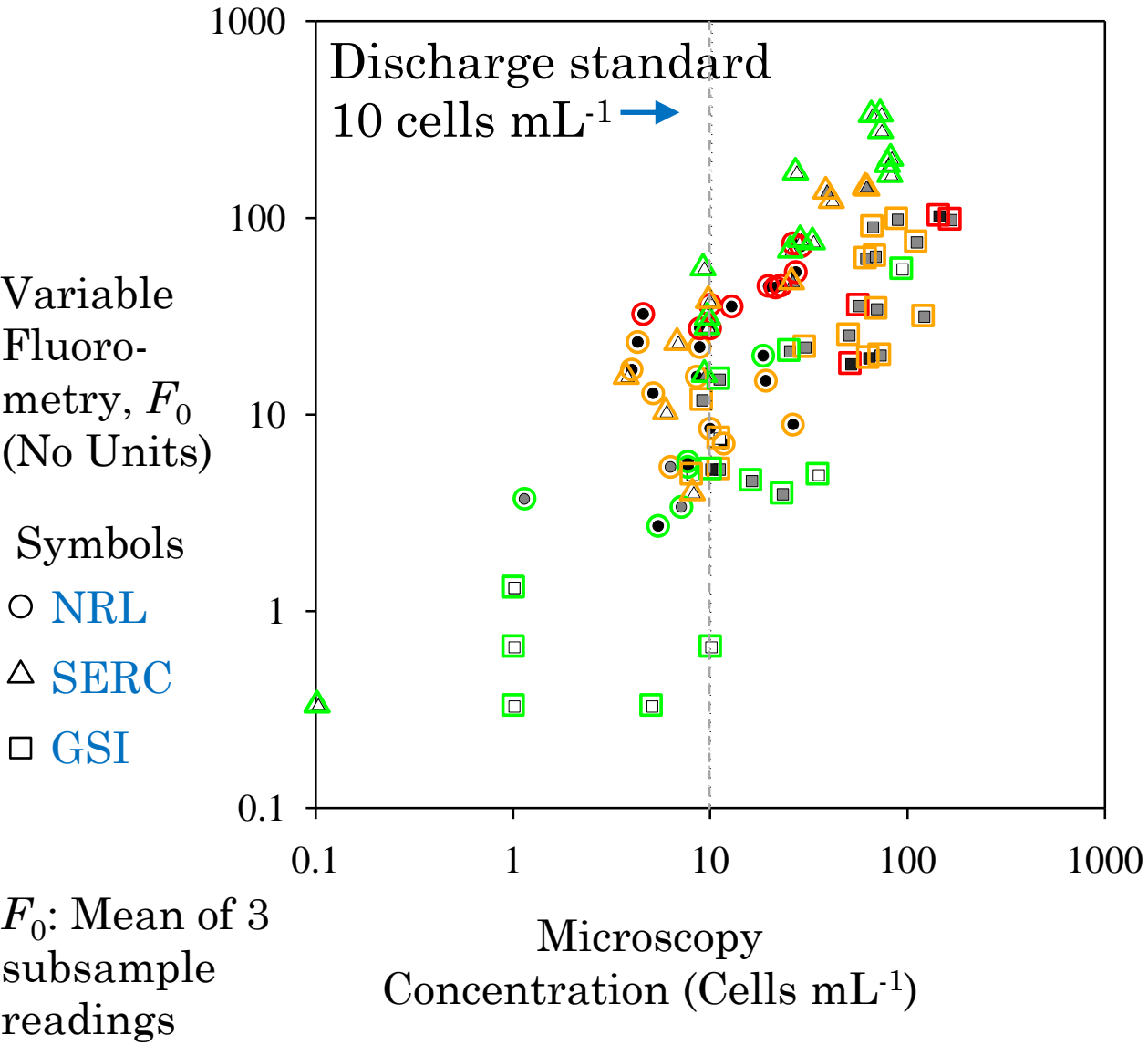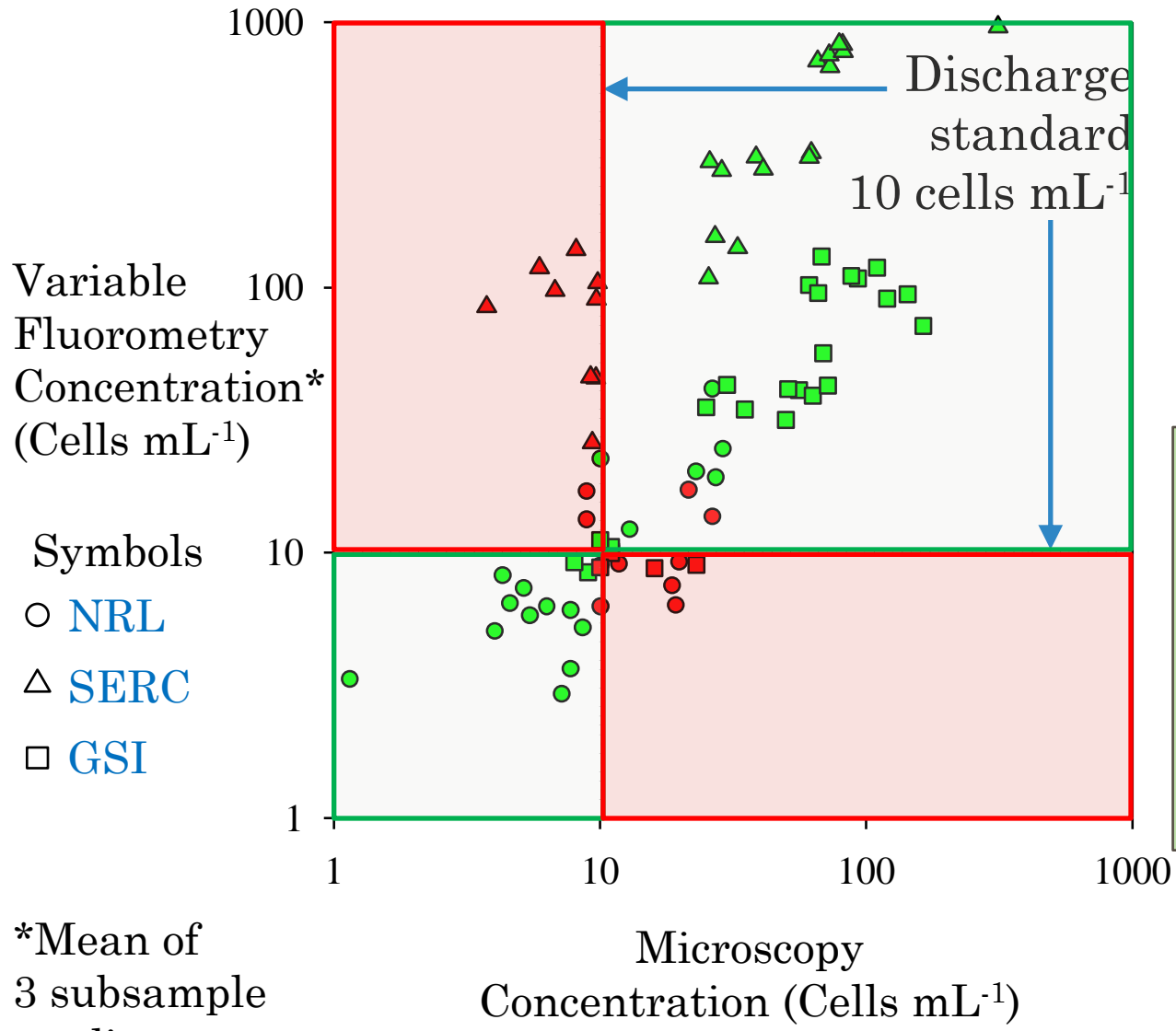
Results: Tool 2

Green regions → Methods agree
Red regions → Methods disagree

○ Methods agree
● Methods disagree

Discharge standard 10 cells mL^{-1}

Variable Fluorometry Concentration* (Cells mL^{-1})

Symbols
○ NRL
△ SERC
□ GSI

Microscopy Concentration (Cells mL^{-1})

Agreement with microscopy
(n of 36 samples):
NRL: 27/36   (75%)
SERC: 27/36   (75%)
GSI: 32/36   (89%)
Overall: 86/108 (80%)

*Mean of 3 subsample readings

Note: axes are log-scaled

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Results: Tool 3

Variable Fluoro-metry, F_0 (No Units)

Symbols
- ○ NRL
- △ SERC
- □ GSI
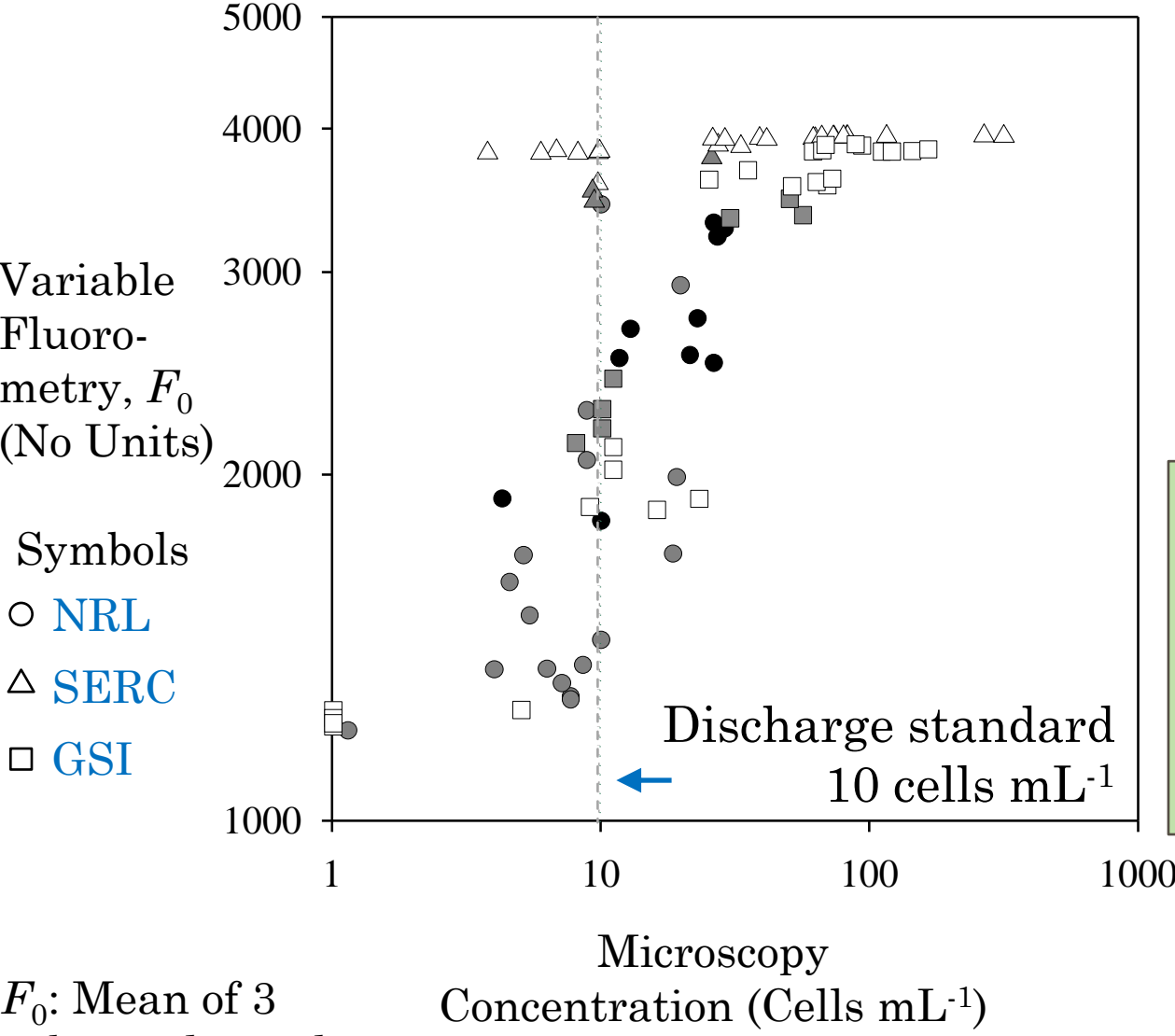
F_V/F_M*

- ○ <0.2
- ● ≥0.2 and <0.4
- ● ≥0.4

*No units

Discharge standard 10 cells mL^{-1}

Microscopy Concentration (Cells mL^{-1})

Tool 3 reports measurements of F_0 and F_V/F_M

Risk or exceedances not explicitly reported

F_0: Mean of 3 subsample readings

Note: axes are log-scaled

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Conclusions

- **Preliminary results** show **agreement** between the instruments and the microscope counts for the **majority of samples**
(when exceedances were reported)

- A new round of testing with different compliance tools will occur in 2016

- Statistical analysis of the entire data set is (including laboratory trials) is underway

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Additional Test Participants

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