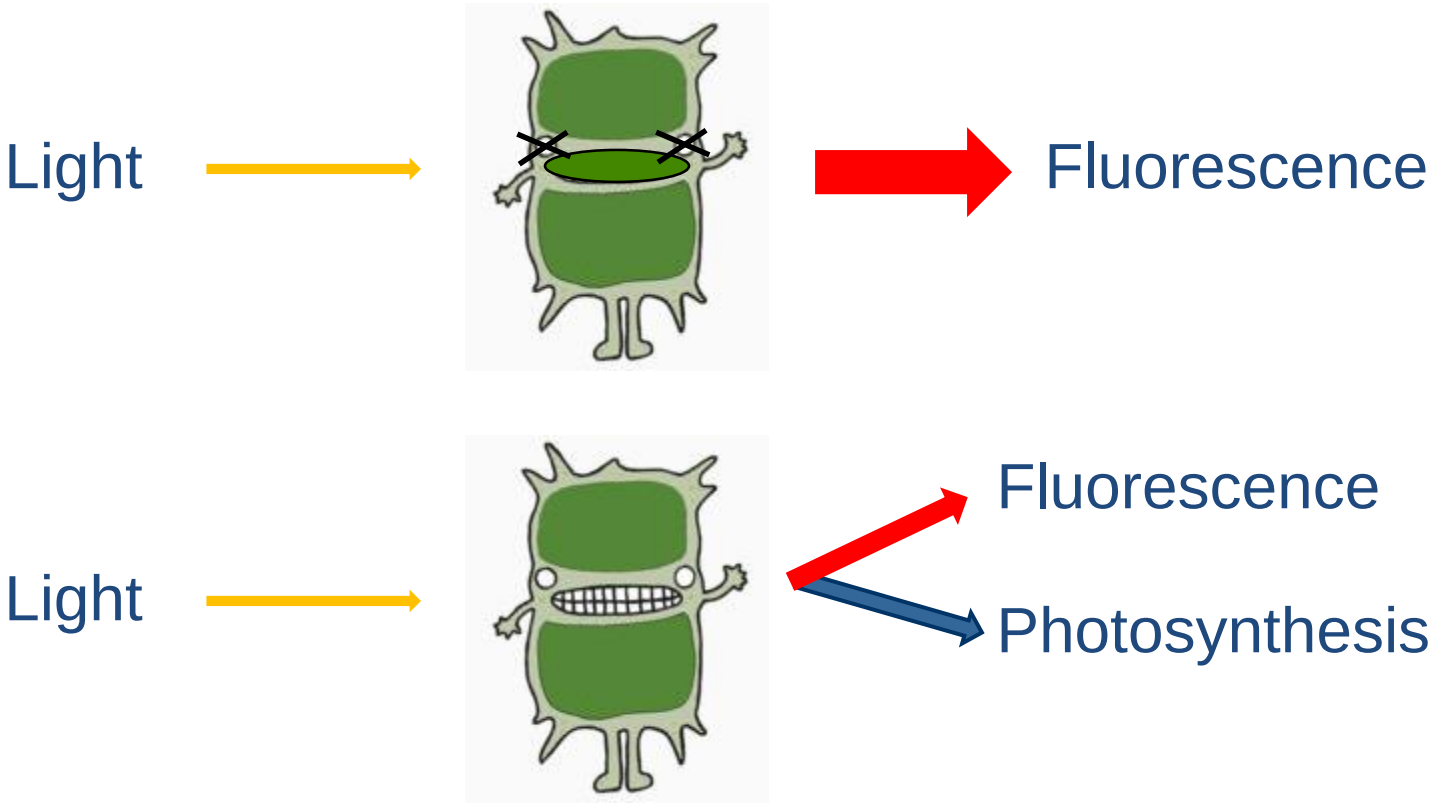
TURNER DESIGNS
Reliable Instruments for an Unreliable World

*Aqua*Flash: Handheld Active Fluorometer

Pam Mayerfeld
Vice President, Marketing & Sales
April 6, 2017

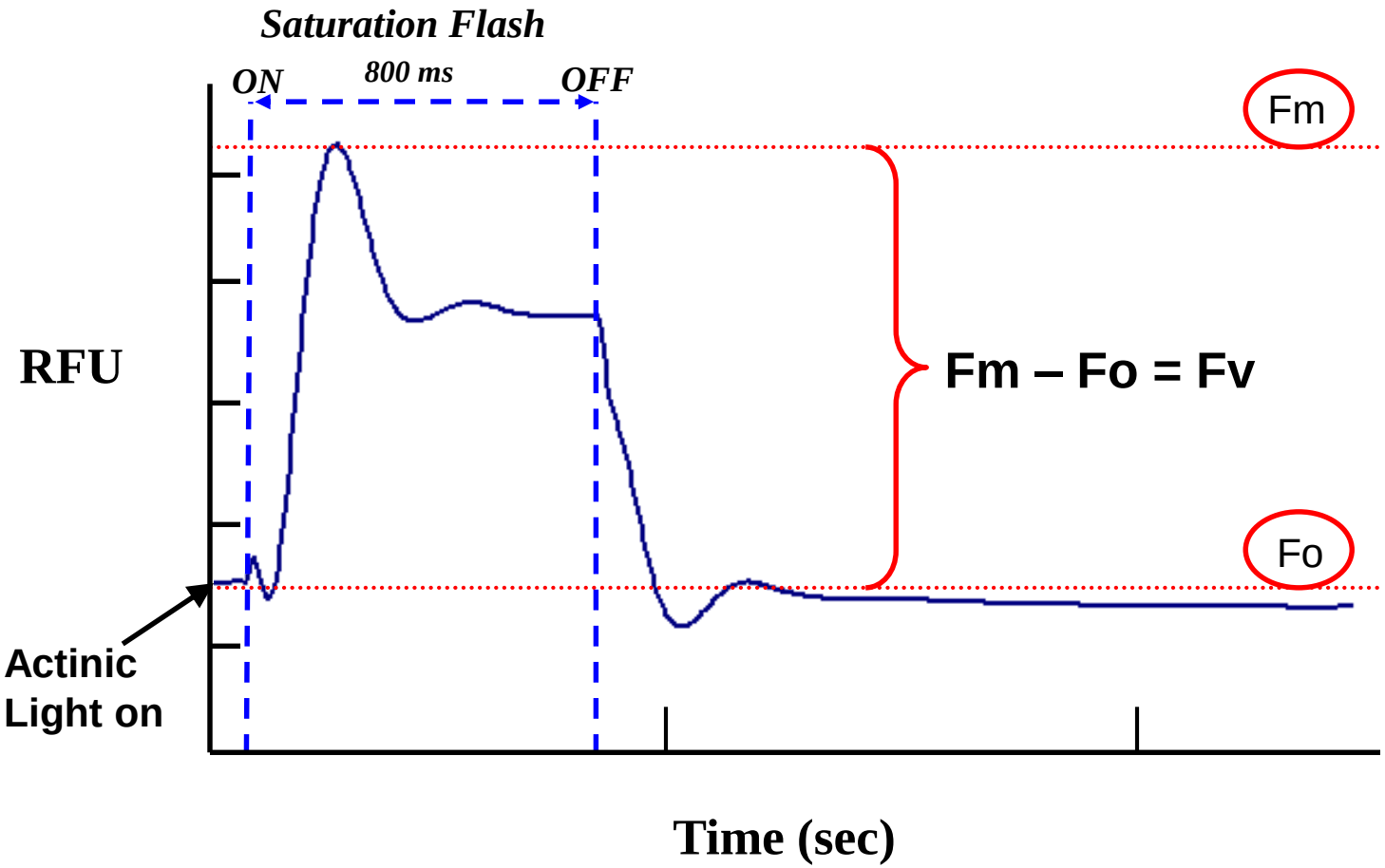
Active Fluorescence Review

Definition: Fluorescence from a physiologically active cell



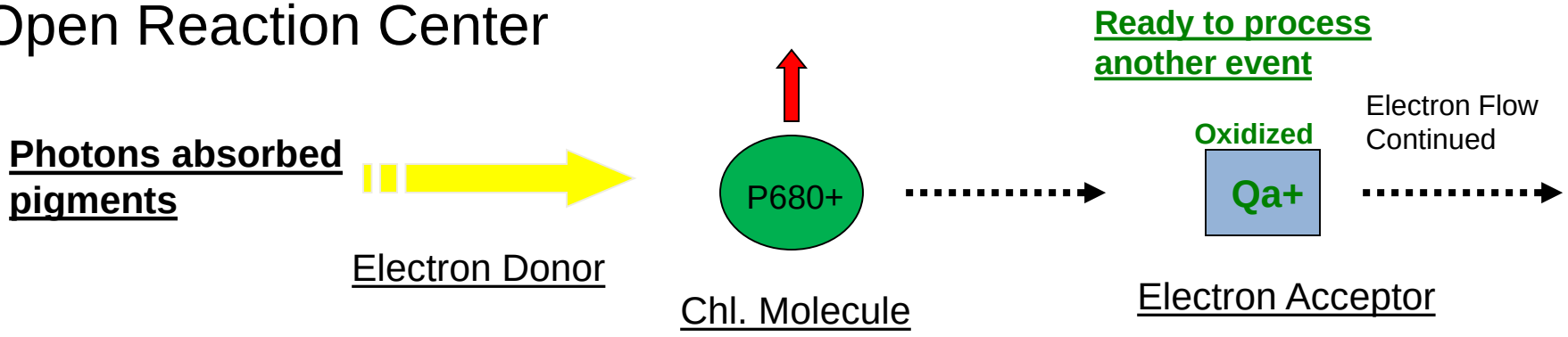
Active Fluorescence Review

An example of a typical response curve for algae



Active Fluorescence Review

Open Reaction Center



Active Fluorescence Review

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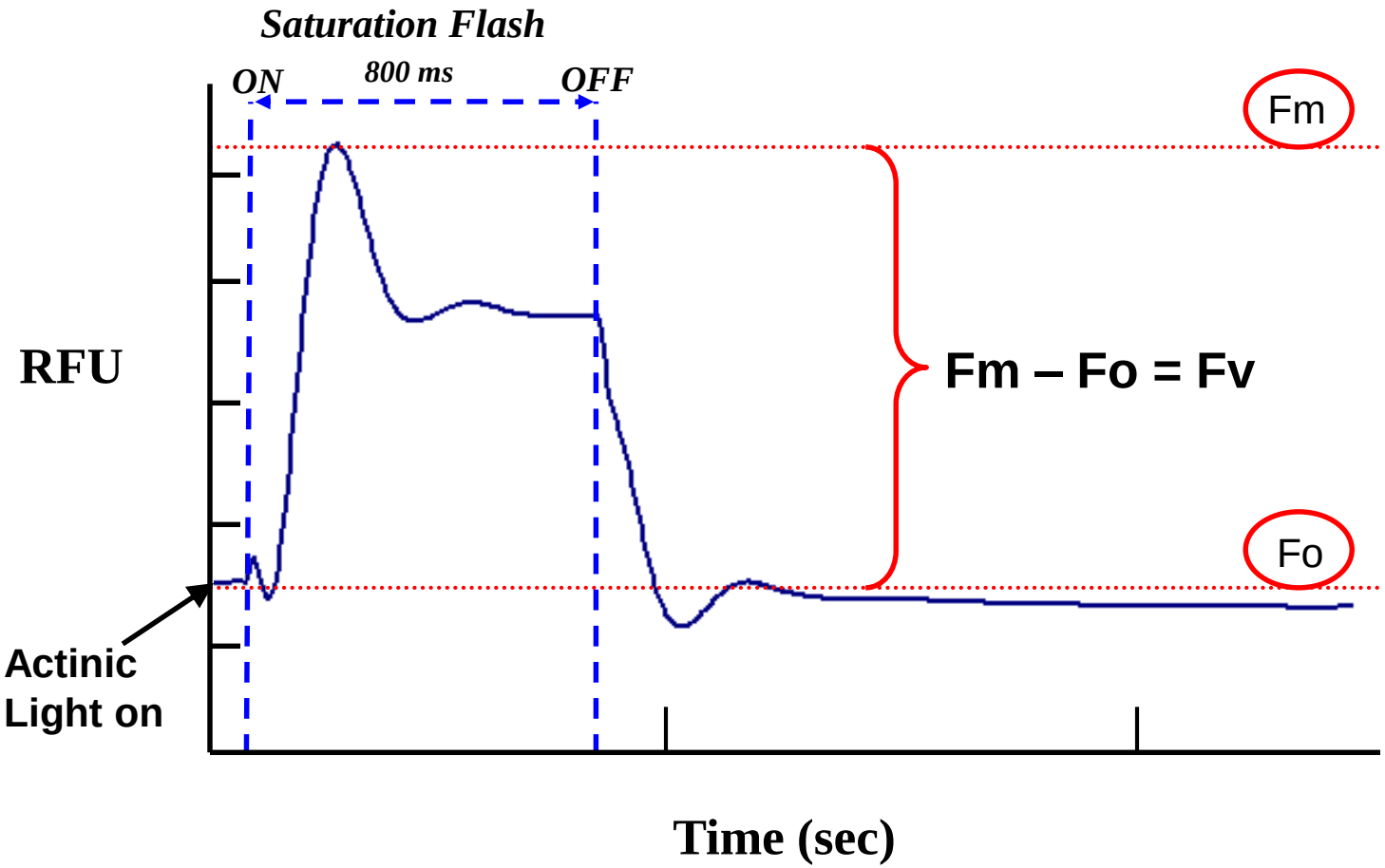

Active Fluorescence Review

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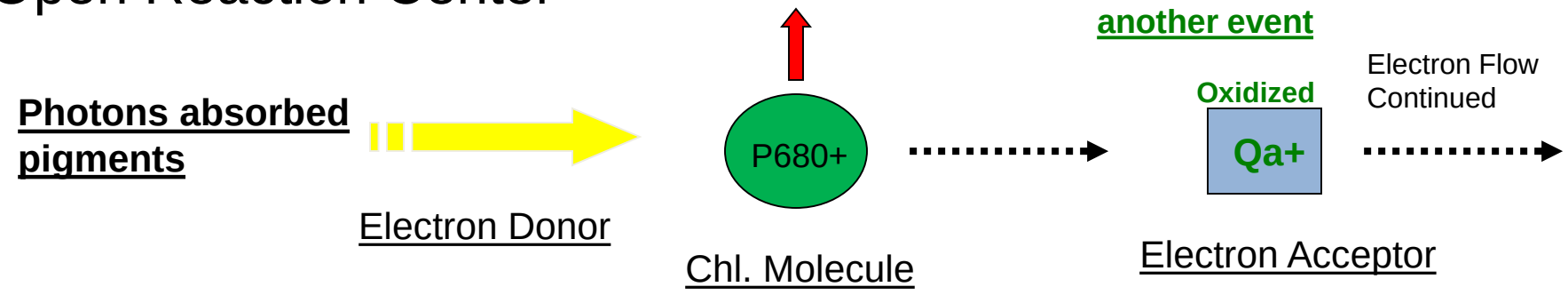
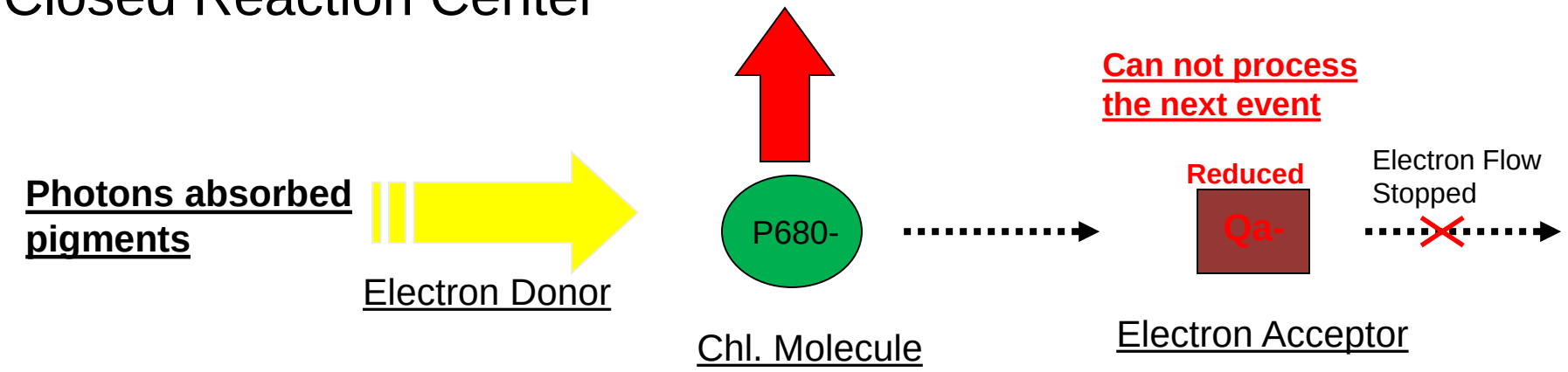


Closed Reaction Center



Active Fluorescence Review

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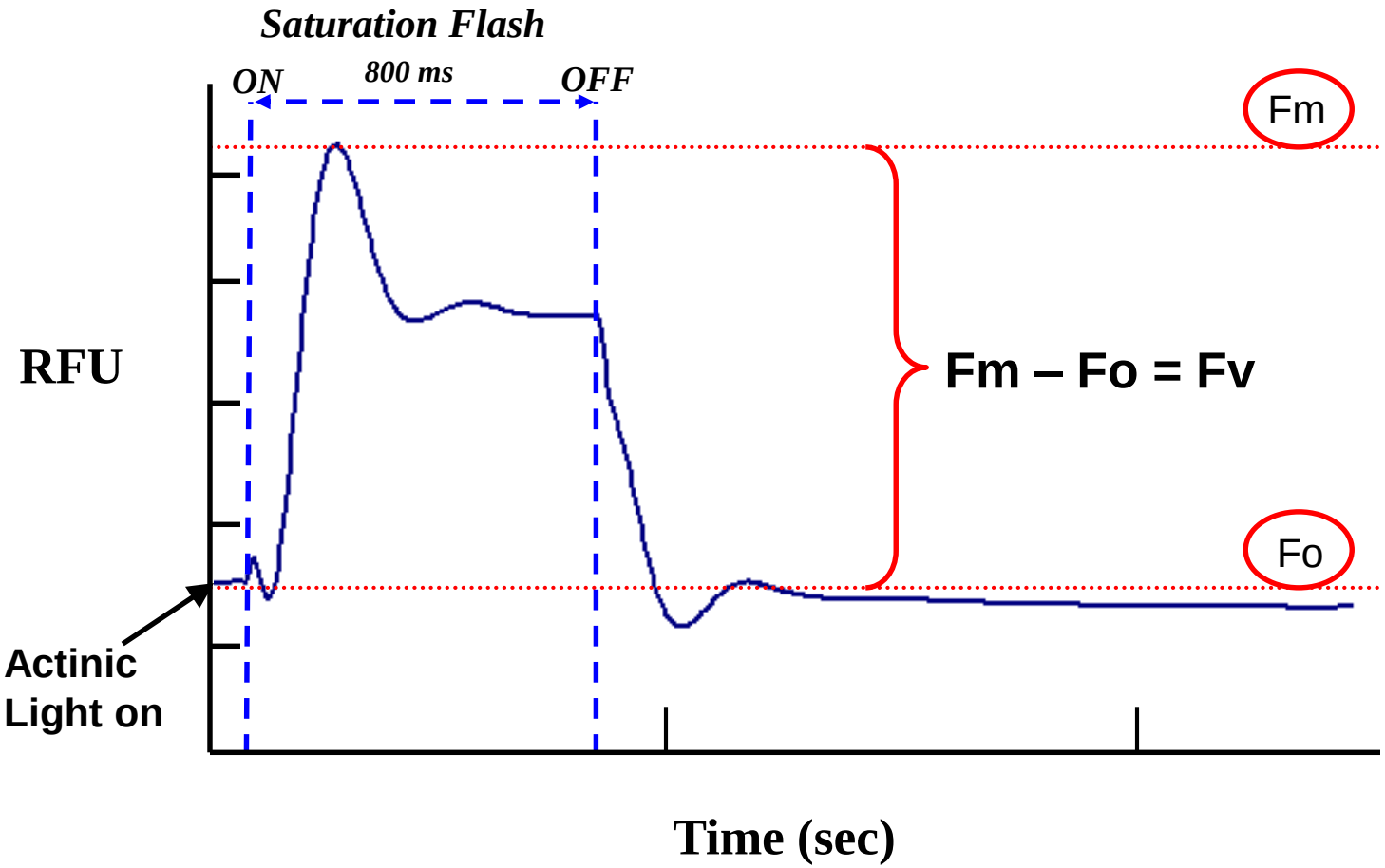
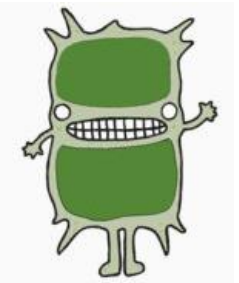


Active Fluorescence Review

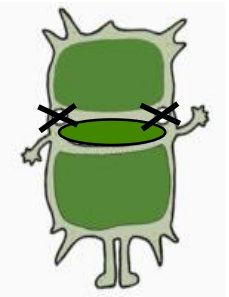
Healthy Phytoplankton

- Background fluorescence is low because their photosynthetic capabilities aren't hindered
  - Example, 100 RFU for background fluorescence (Fo)
  - (Fm) jumps to 200 RFU after saturation
    - (200 – 100)/200 = 0.50



Unhealthy Phytoplankton

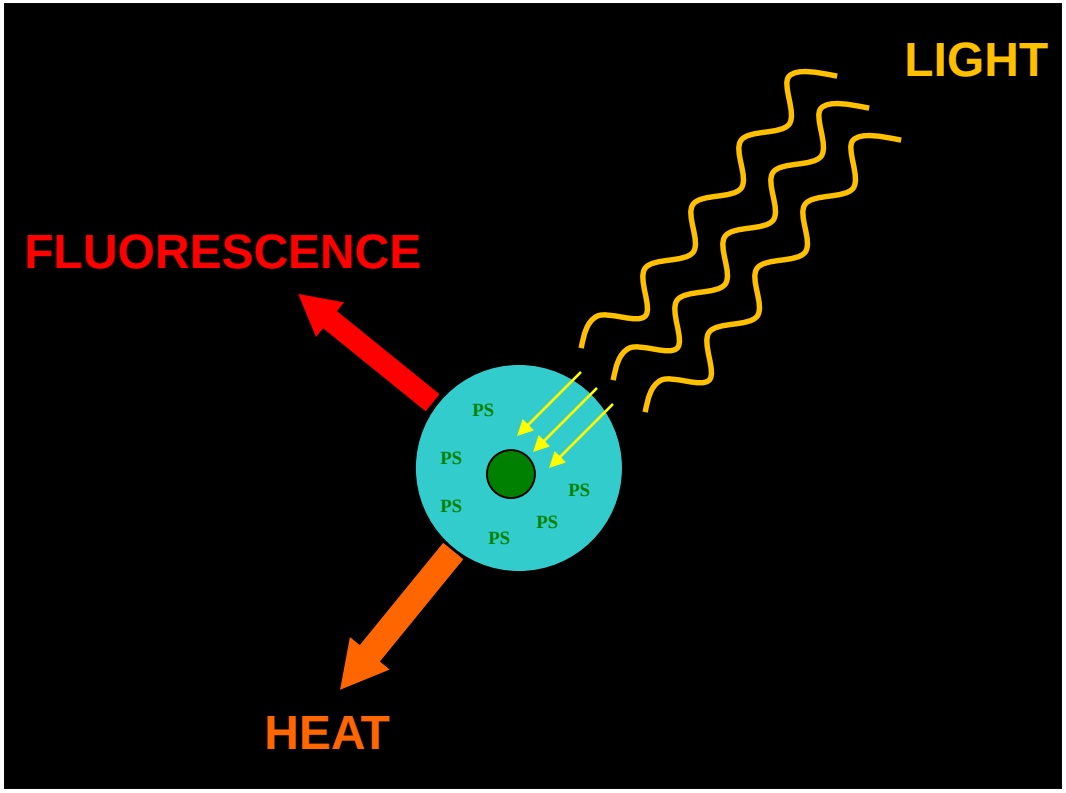
- Background fluorescence is high because their photosynthetic capabilities are hindered (not enough nutrients or too much light stress)
  - Example, 175 RFU for background fluorescence (Fo)
  - (Fm) jumps to 200 RFU after saturation
    - (200 – 175)/200 = 0.13



Active Fluorescence Review

There are 3 major pathway for light to take after being absorbed by the algal cell:

- Photosynthesis (PS)
- Fluorescence
- Heat



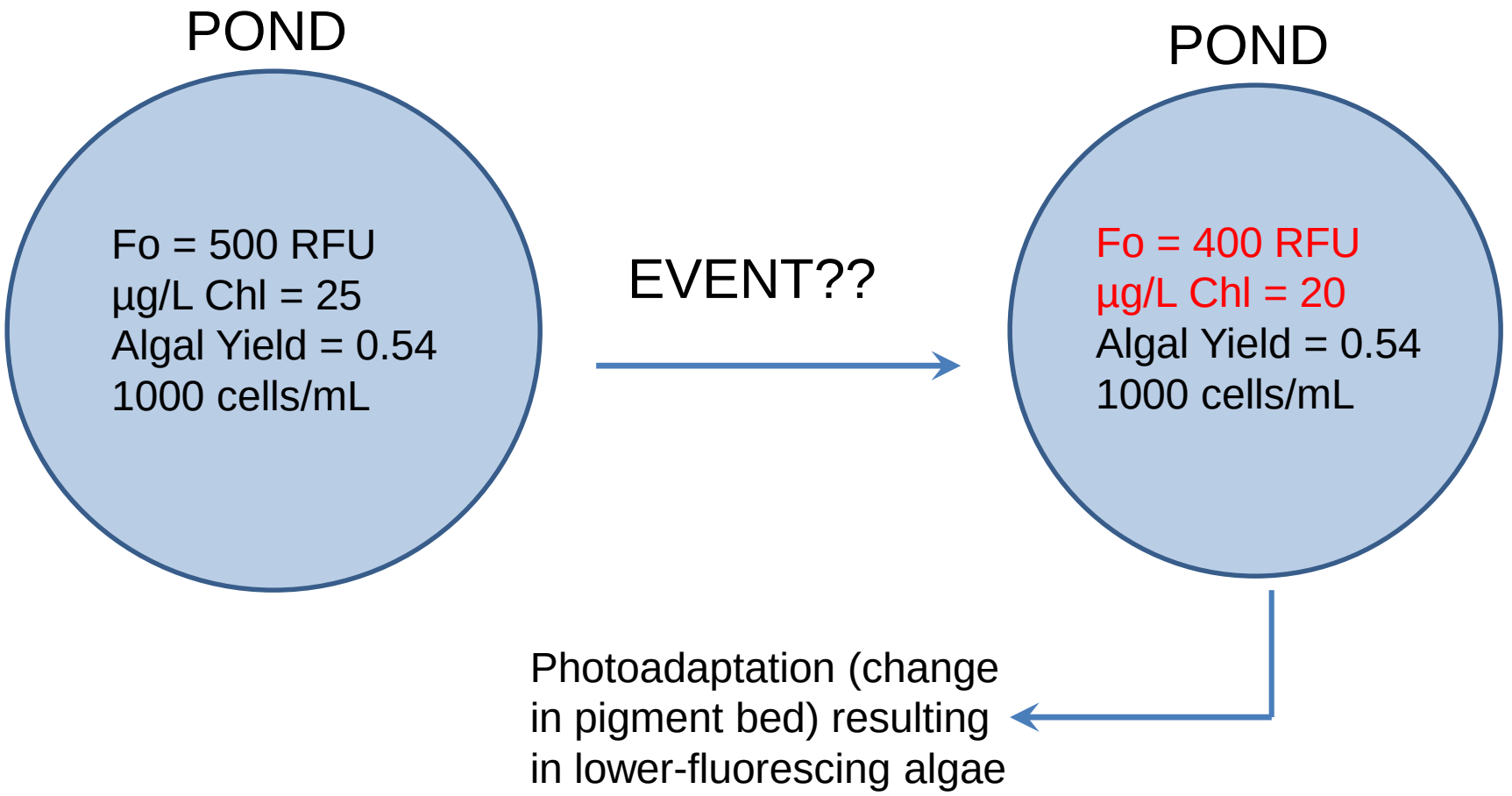
Fluorescence Variability

Factors that affect fluorescence response:

- Light history of the cells
- Algal phases
  - Exponential growth
  - Stationary
- Nutrient stress
- Presence of toxins
- Speciation

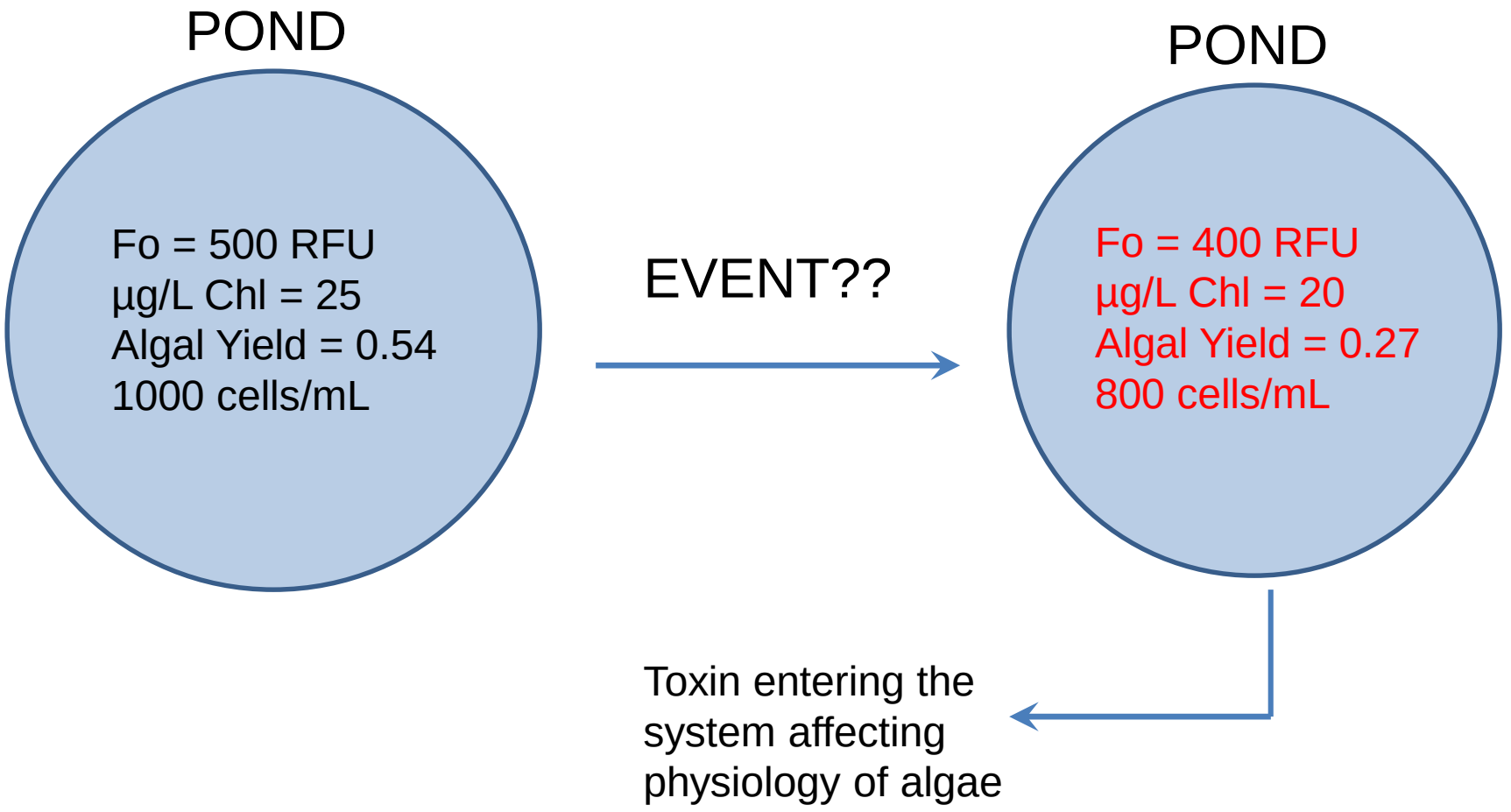
Fluorescence Variability

Yield is not affected by variability in fluorescence



Fluorescence Variability

Yield is a direct measure of the variability in fluorescence



Applications for Active Fluorescence

- Onset of algal blooms
  - Track changes in Yield
- Tracking of Toxins
  - Algal yields will drop if a toxin in system affects photosynthetic activity
- Nutrient Effects
  - Aquaculture monitoring
  - Eutrophication
- Estimate Algal Abundance
  - Use Fo to monitor or measure changes in algal abundance

Why Develop a Handheld Active Fluorometer?

- Easy to use, rugged
  - No software required
  - No set up or calibration required
- Self contained, quick
  - Use to study remote locations
  - Results in <15 seconds
- Provides multiple parameters enabling dual use
  - Algal monitoring
  - Algal assessment
- Low cost compared to other market instruments
  - Important measurement for an affordable price

*Aqua*Flash Data

- Chlorophyll µg/L
- Yield
- Time
- Sample Number
- Fo
- Fm
- Blank

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*Aqua*Flash Calibration

Why Calibrate

- Increases accuracy of chlorophyll estimates for specific environments

How to Calibrate

- Know the concentration of the sample used for the calibration
- Run the calibration procedure
  - This will convert RFU values to µg/L Chlorophyll estimates

When Calibrations Go Bad

- Can I get my factory cal back?
  - *Aqua*Flash Calibration Solution

Calibration Check

The Chk Std button is used to check whether the instrument's calibration has changed

"PASSED" – indicates the instrument's calibration has not changed and validates instrument stability

"FAILED" – indicates something wrong with the instrument such as an electronic, optical, or hardware failure. This would require users to call Turner Designs for support

*Aqua*Flash Specifications

Sensitivity:	0.3 µg/L of Chlorophyll
Linear Range:	0.3 – 100 µg/L
Linearity:	0.99R^2
Light Source:	LED
Detector:	Photodiode
Data Output:	ASCII
Data Capacity:	1,000 measurements
Temperature:	41-104°F; 5-40°C
Weight:	0.87 lbs. (0.4kg)
Size:	1.75” x 3.5” x 7.25” (4.45cm x 8.9cm x 18.4cm
Case:	IP 67 standard; dustproof/waterproof
Power:	4 AAA batteries (standard or rechargeable)

*Aqua*Flash limits were determined using *Tetraselmis sp.*; limits may vary for other algal species

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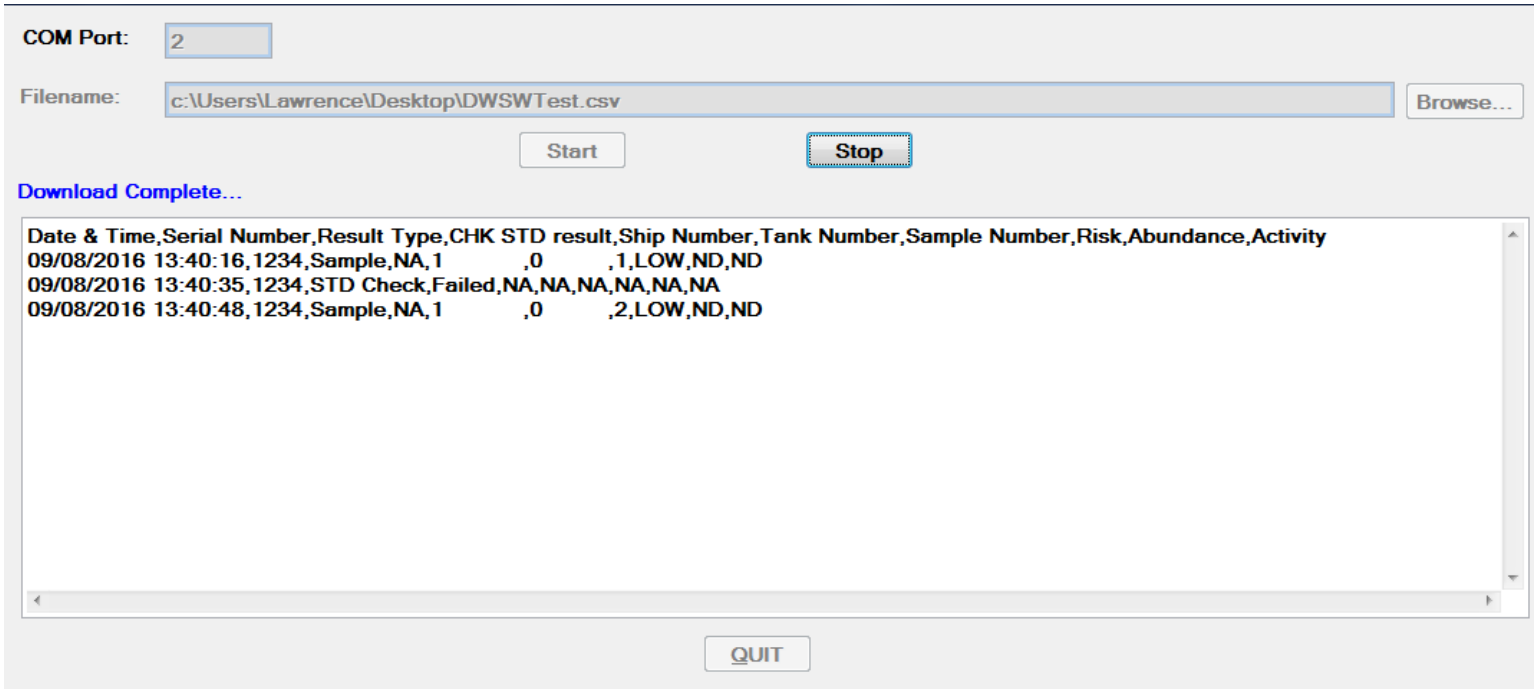
Easy Data Download

1. Set COM port
2. Click Browse to designate where to save file
3. Name file
4. Press Start
5. Select Data and Send Data from *Aqua*Flash
6. When download is complete press Stop
7. Data are saved as .txt file; can be opened with Excel



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Sample Data Sets

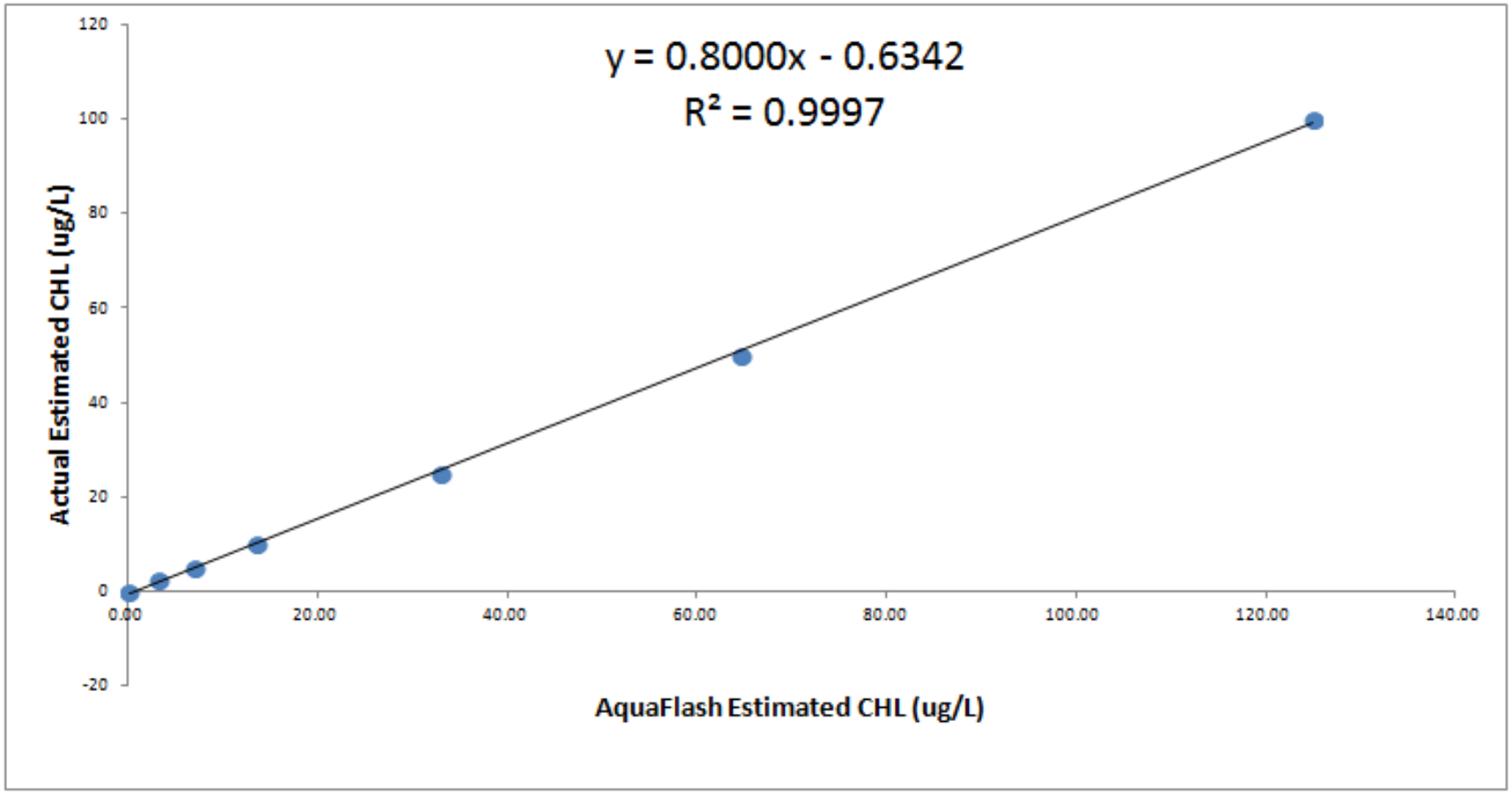
Alga used for these data was an old *Rhodomonas salina culture*

	0 ug/L		2.5 ug/L		5 ug/L		10 ug/L		25 ug/L		50 ug/L		100 ug/L	
	Chl	*Yield*	*Chl*	*Yield*	*Chl*	*Yield*	*Chl*	*Yield*	*Chl*	*Yield*	*Chl*	*Yield*	*Chl*	*Yield*
	0.23	0.41	2.82	0.42	7.26	0.36	12.1	0.35	32.93	0.31	64.62	0.38	118.35	0.34
	0.13	0.24	3.29	0.31	7.43	0.23	13.8	0.38	32.29	0.36	63.6	0.41	121.16	0.39
	-	-	3.38	0.38	6.51	0.39	13.34	0.38	33.31	0.36	68.49	0.31	125.84	0.38
	0.03	0.85	3.49	0.42	6.73	0.34	13.31	0.41	33.57	0.42	61.73	0.33	116.99	0.41
	0.07	0.63	3.13	0.25	6.78	0.38	14.25	0.38	33.05	0.4	64.02	0.38	129.2	0.34
	-	-	3.42	0.39	7.19	0.39	14.4	0.36	32.2	0.39			124.09	0.36
	-	-	2.65	0.32			13.27	0.36					124.09	0.39
	0.08	0.68	3.38	0.32									131.92	0.38
	-	-	3.68	0.38									122.77	0.39
													134.98	0.37
Average	0.11	0.56	3.25	0.35	6.98	0.35	13.50	0.37	32.89	0.37	64.49	0.36	124.94	0.38
Stdev	0.08	0.24	0.33	0.06	0.36	0.06	0.77	0.02	0.55	0.04	2.48	0.04	5.73	0.02
CV			10%	16%	5%	18%	6%	5%	2%	10%	4%	11%	5%	6%

Chl (µg/L)	Yield	CV
2.5	0.35	16 %
5	0.35	18 %
10	0.37	5 %
25	0.37	10 %
50	0.36	11 %
100	0.38	6 %

Old Rhodamonas Salina

Calculated MDL: 0.4 µg/L



Sample Data Sets

Dunaliella salina good culture used for testing

Chl (ug/L)	Yield	Stdev	CV
17.5	0.44	0.03	7%
8.8	0.45	0.04	8%
4.4	0.43	0.03	8%
2.2	0.51	0.01	3%
1.1	0.42	0.08	18%
0.6	0.46	0.03	6%
0.3	0.51	0.09	18%

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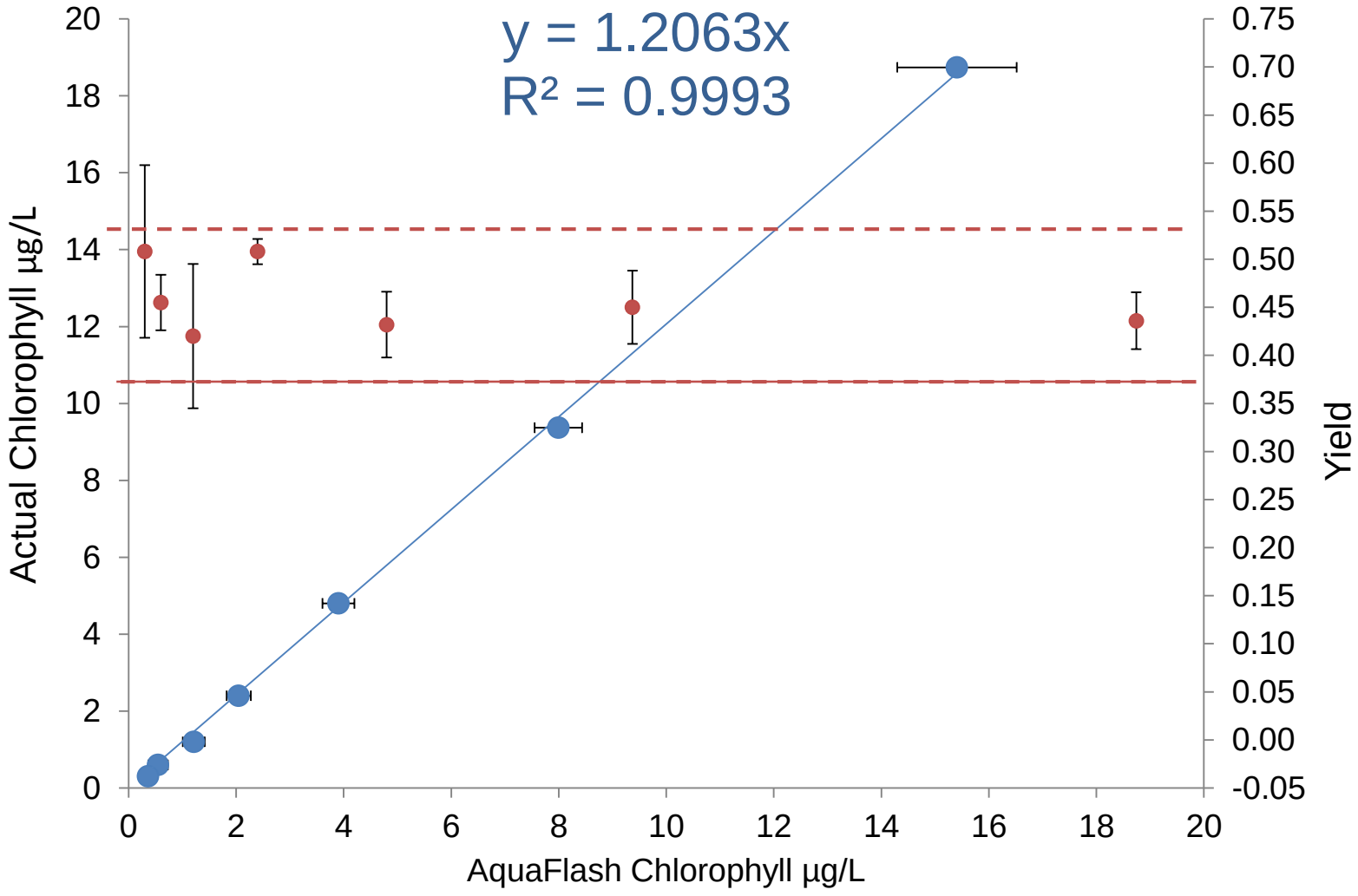
Fresh Dunaliella Salina



Sample Handling for Obtaining Good Data Sets

- Minimum of 3 replicates per sample
  - Don’t scan the same sample twice
- Make sure sample is well mixed
  - Settling or separation in sample source
- Keep cuvette clean
  - Wear gloves
  - Fingerprints on glass cuvette can cause variability
  - Excessive use of Kim Wipes
- Cross contamination
  - Minimum of 3 rinses after each sample
  - New pipet tips; don’t be sloppy

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Sample Considerations for Obtaining Good Sample Sets

- This measurement is qualitative, even if unit is calibrated
- Most of the variability is sample related
  - Could be as much as 30%
  - Actual instrument variability is +/- 2%
- Negligible effect from suspended sediments
  - Blue excitation, red emission
  - If concentration is too high problems with light blocking
- Significant effect from dissolved organics
  - Can be corrected using filtration
    - Simple subtraction

How to use the *Aqua*Flash

Measurement Procedure

1. Power on the *Aqua*Flash
2. Pour sample water into a clean glass cuvette
3. Wipe the sides of the cuvette using Kim Wipes
4. Insert cuvette with sample into *Aqua*Flash
5. Close the sample compartment's lid
6. Press the READ button
7. Wait for measurement to end
8. Remove cuvette and discard sample

Thank you

Pam Mayerfeld
pam_mayerfeld@turnerdesigns.com
408-212-4048