

# Using the FlowCAM for Plankton Studies in San Francisco Estuary

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# What is a FlowCAM?

Digital imaging flow cytometer

Computer and visual spreadsheet program

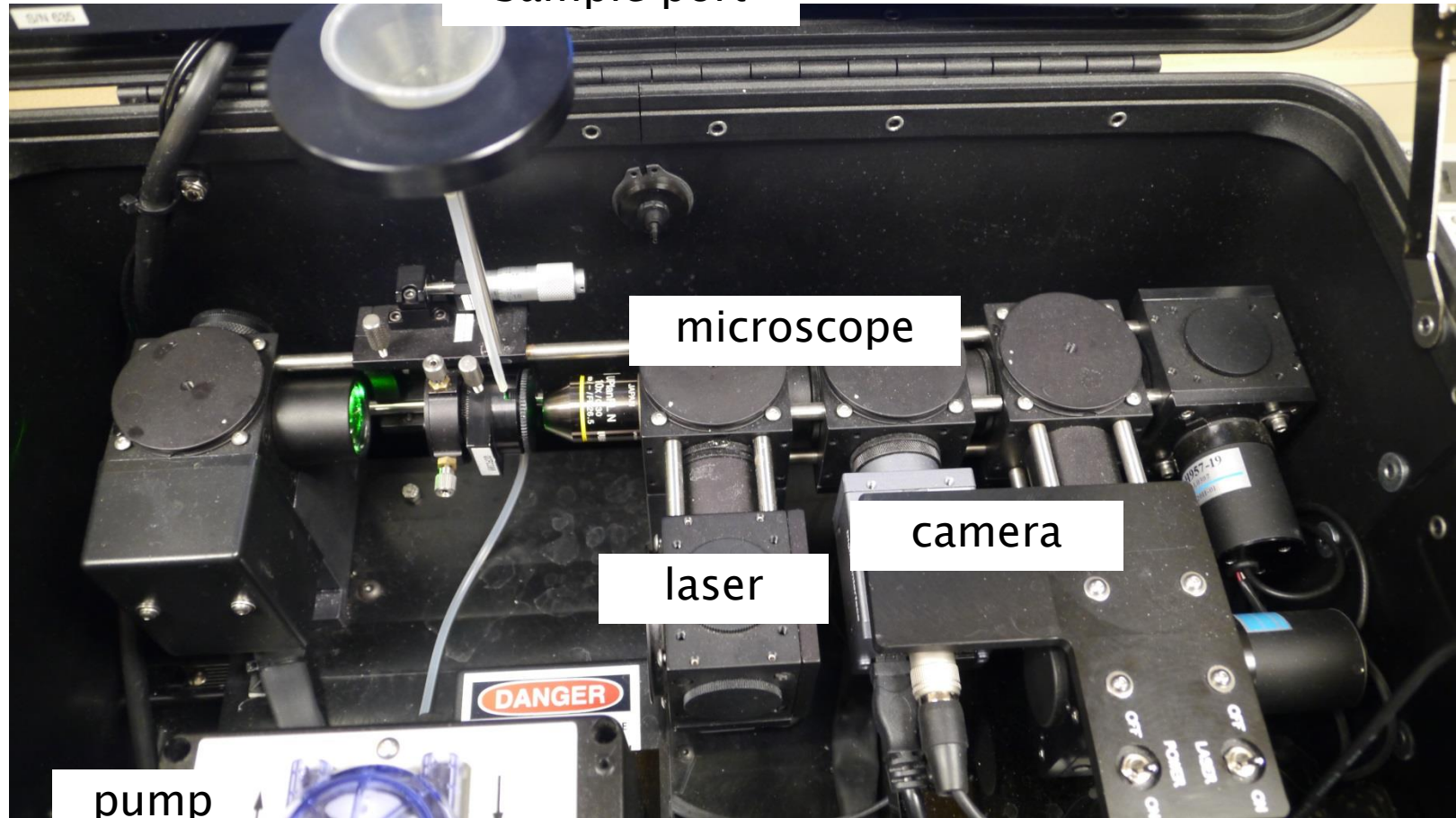
Microscope, flow cytometer, Laser fluorescence probe, and digital camera

Fluid Imaging Technologies, Inc.



Portable model

Sample port



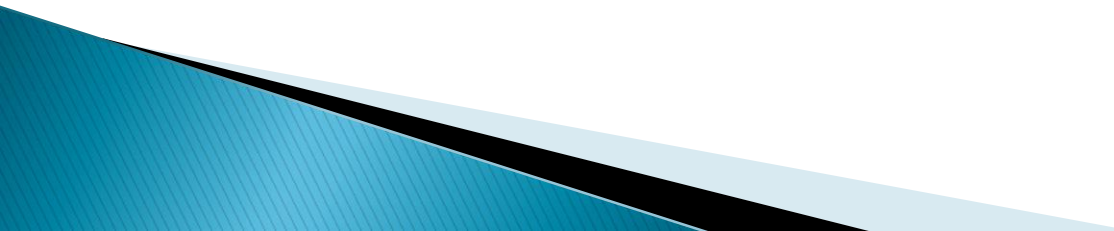
pump

laser

microscope

camera

# What does it do?

- ▶ Takes pictures of objects in the water sample
  - ▶ measures the dimension, volume and number of objects
  - ▶ Tracks the volume of the sample
  - ▶ Automatically classifies objects into groups
  - ▶ Computes abundance, volume and size of each object and group per unit volume
  - ▶ Exports data into EXCEL spreadsheet
- 

# How does it work?



Watch Video at:  
[http://info.fluidimaging.com/how-the-flowcam-works?&\\_\\_hssc=110599322.1.1403720266392&\\_\\_hstc=110599322.17cce891349722a43a8f7e2e62a035b0.1380558602317.1402957562364.1403720266392.11&hsCtaTracking=b628da99-aaf9-4d13-9b0e-7a1e1588694e%7Cb16800a4-f88a-424f-9d26-febee481b1fb](http://info.fluidimaging.com/how-the-flowcam-works?&__hssc=110599322.1.1403720266392&__hstc=110599322.17cce891349722a43a8f7e2e62a035b0.1380558602317.1402957562364.1403720266392.11&hsCtaTracking=b628da99-aaf9-4d13-9b0e-7a1e1588694e%7Cb16800a4-f88a-424f-9d26-febee481b1fb)

# How do we use the FlowCAM at California Department of Water Resources ?



# Field sampling live phytoplankton

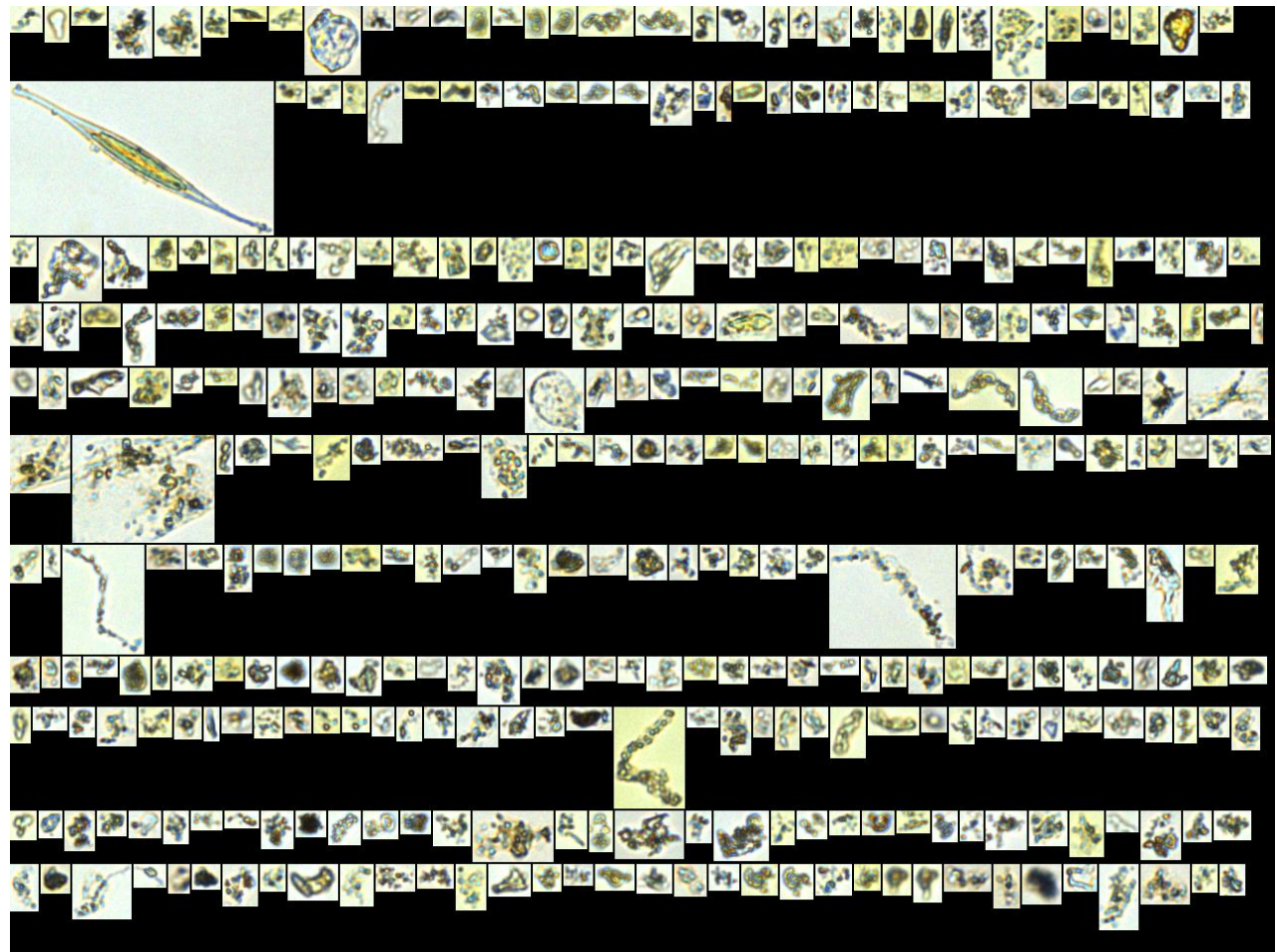




# Delta phytoplankton



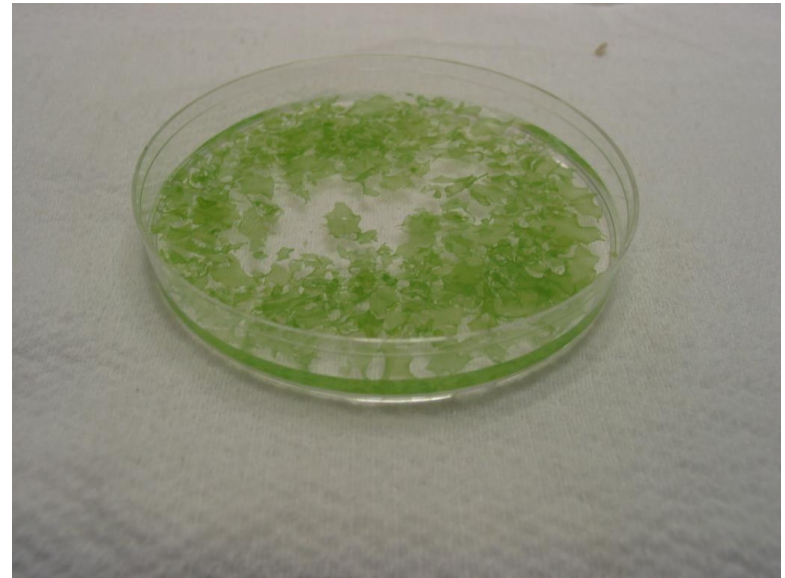
# Laboratory analysis – preserved phytoplankton



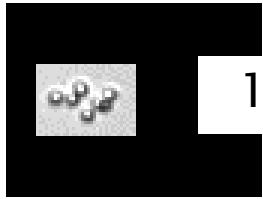
# Laboratory analysis preserved *Microcystis* samples



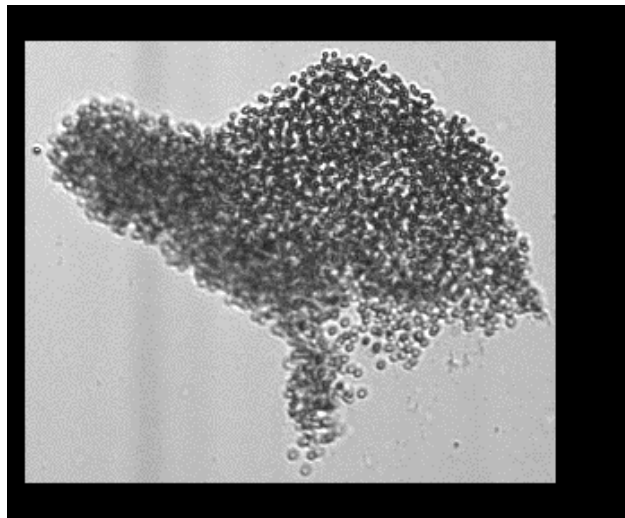
Size range 6 to 50,000  $\mu\text{m}$



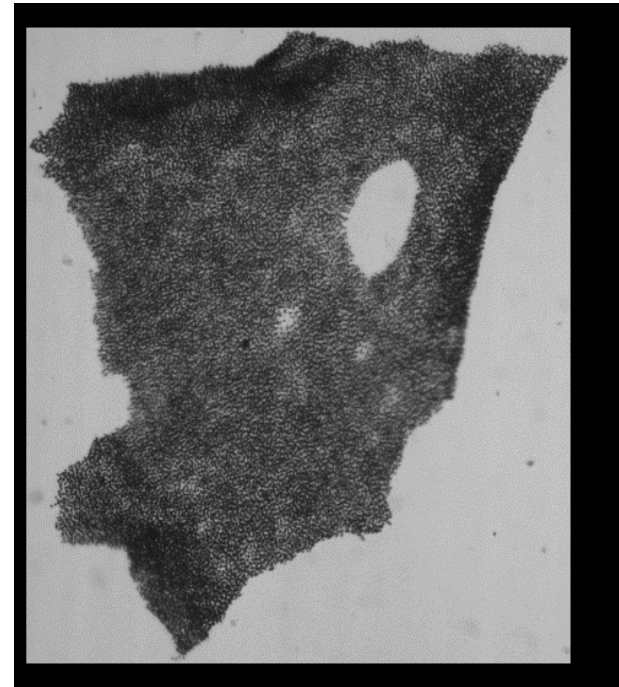
# *Microcystis* colonies



15–35  $\mu\text{m}$  size range  
10X

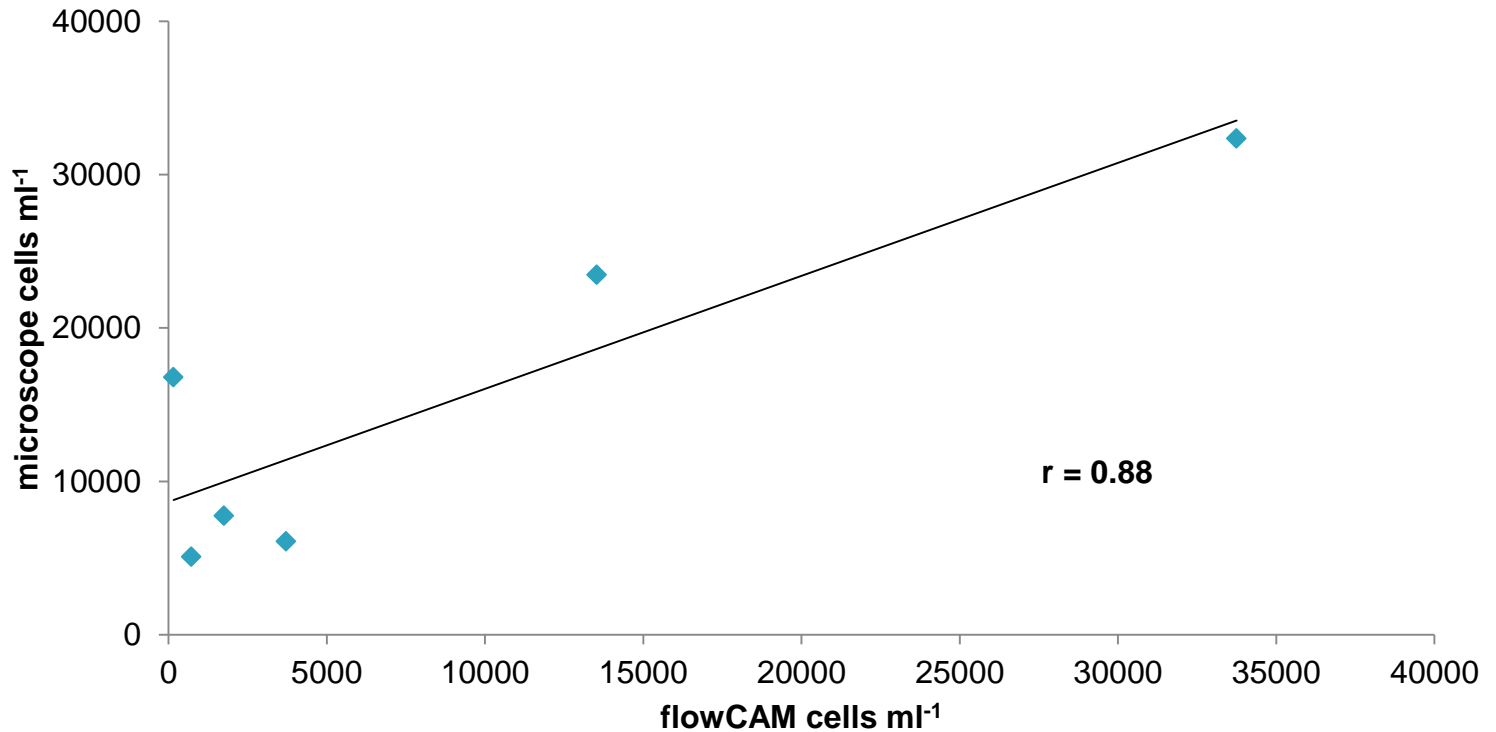


36–300  $\mu\text{m}$  size range  
10X



> 301  $\mu\text{m}$  size range  
2X

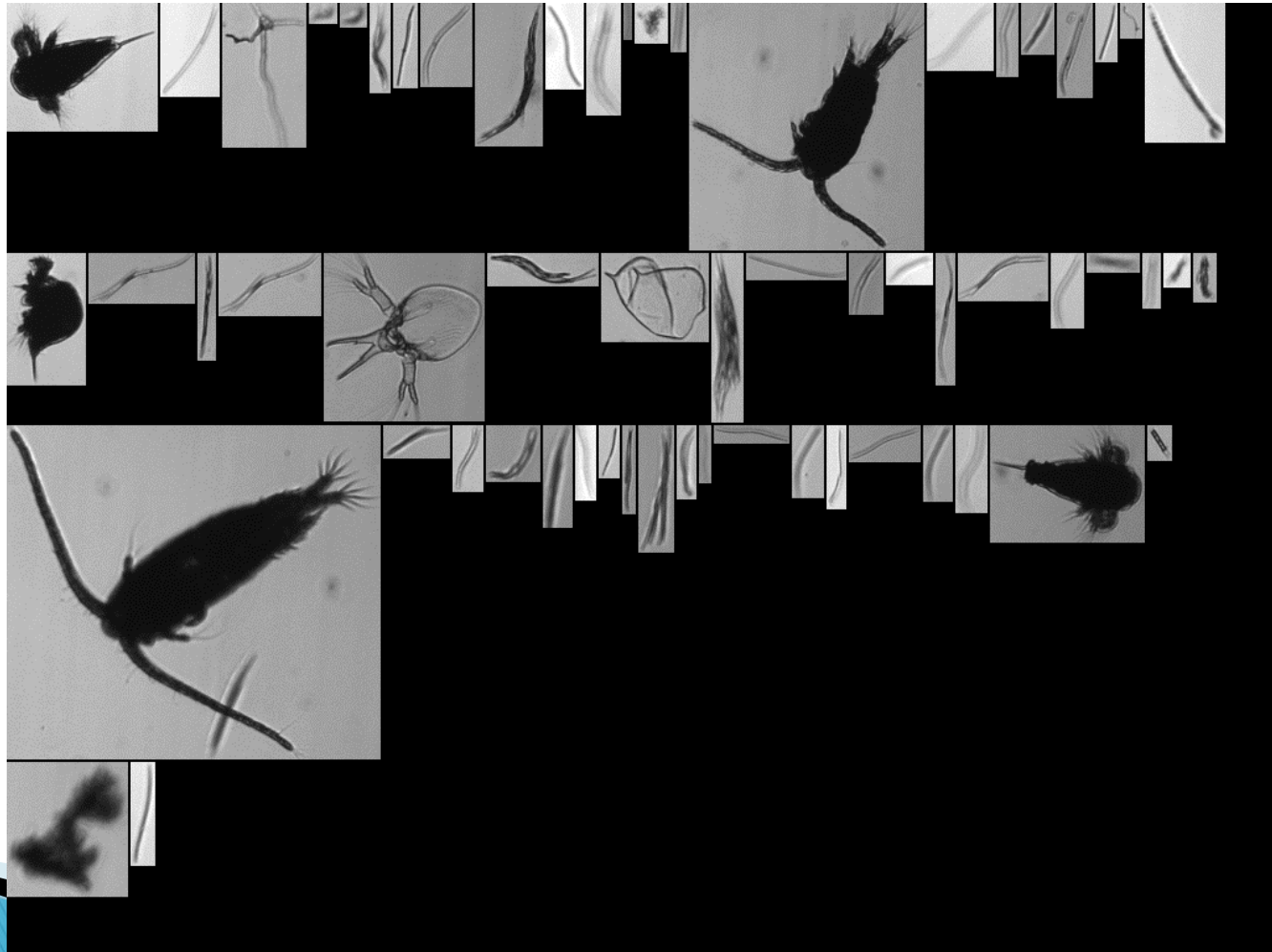
# Microscope versus FlowCAM



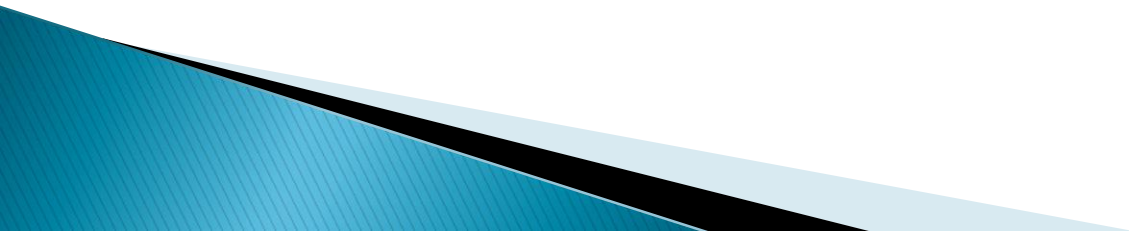
# Laboratory analysis preserved Zooplankton



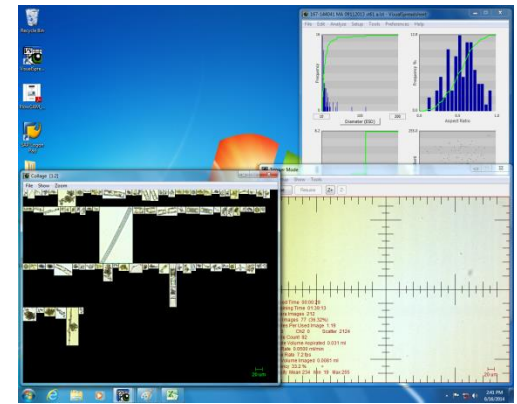
- ▶ Lab analysis



# Analysis



# Sample processing



Watch Video at:  
[http://info.fluidimaging.com/video-automate-particle-classification?&\\_\\_hssc=110599322.3.1403720266392&\\_\\_hstc=110599322.17cce891349722a43a8f7e2e62a035b0.1380558602317.1402957562364.1403720266392.11&hsCtaTracking=7f4f898d-cf6f-4a56-98c1-9f036451f051%7Cedd32377-b96f-44d1-bade-fc2a9a6e0e4d](http://info.fluidimaging.com/video-automate-particle-classification?&__hssc=110599322.3.1403720266392&__hstc=110599322.17cce891349722a43a8f7e2e62a035b0.1380558602317.1402957562364.1403720266392.11&hsCtaTracking=7f4f898d-cf6f-4a56-98c1-9f036451f051%7Cedd32377-b96f-44d1-bade-fc2a9a6e0e4d)



# Summary data

Count	14126 of 50000	Start Time	2008-09-24 07:50:25			
Particles / ml	NA	End Time	2008-09-24 09:10:10			
Summary Stats	Filters	Cumulative Stats	Context Summary			
Summary Stats	Mean	Min	Max	StdDev	% CV	D50
Aspect Ratio	0.72	0.14	0.97	0.13	17.39	0.74
Diameter (ESD)	58.52	30.00	99.96	15.59	26.64	57.31
Edge Gradient	0.00	0.00	0.00	0.00	0.00	0.00
Length	66.80	31.54	138.36	18.55	27.77	64.87
Sigma Intensity	72.08	3.20	99.12	15.14	21.01	74.60
100% D <sub>50</sub>	47.04	0.00	05.00	14.07	20.42	47.00
Objective 10X, Autolmage						

# Object data

The image shows a screenshot of a Microsoft Excel spreadsheet titled "data\_export - Microsoft Excel". The spreadsheet contains a table with 38 rows of data. The columns are labeled as follows:

Particle ID	Area (ABD	Aspect Ra	Calibratio	Calibratio	Camera	Capture X	Capture Y	Ch1 Area	Ch1 Peak	Ch1 Width	Ch2 Area	Ch2 Peak	Ch2 Width	Ch2/Ch1 R	Circle Fit	Compactn	Convex	Pe	Date	Dis
1	42.29	0.48	0.5575	1	1	891	614	0	0.75	0	0	0.75	0	1	0.35	4.33	41.15	#####		
2	45.31	0.41	0.5575	1	1	357	598	0	0.75	0	0	0.75	0	1	0.18	7.89	60.96	#####		
3	38.15	0.38	0.5575	1	1	846	928	0	0.75	0	0	0.75	0	1	0.22	5.24	44.67	#####		
4	103.89	0.78	0.5575	1	1	972	635	0	0.75	0	0	0.75	0	1	0.66	2.19	47.72	#####		
5	88.69	0.43	0.5575	1	1	504	91	0	0.75	0	0	0.75	0	1	0.39	2.32	50.24	#####		
6	898.03	0.62	0.5575	1	1	261	543	0	0.75	0	0	0.75	0	1	0.13	6.03	150.32	#####		
7	151.91	0.84	0.5575	1	1	226	597	0	0.75	0	0	0.75	0	1	0.88	1.32	50.81	#####		
8	60.06	0.28	0.5575	1	1	581	818	0	0.75	0	0	0.75	0	1	0.27	2.11	44.47	#####		
9	49.54	0.59	0.5575	1	1	1	36	0	0.75	0	0	0.75	0	1	0.49	2.11	37.44	#####		
10	78.44	0.83	0.5575	1	1	443	628	0	0.75	0	0	0.75	0	1	0.8	1.93	40.93	#####		
11	652	0.28	0.5575	1	1	780	629	0	0.75	0	0	0.75	0	1	0	13.45	152.41	#####		
12	74.23	0.8	0.5575	1	1	1203	408	0	0.75	0	0	0.75	0	1	0.71	1.78	40.46	#####		
13	42.52	0.53	0.5575	1	1	227	623	0	0.75	0	0	0.75	0	1	0.39	2.58	37.32	#####		
14	67.59	0.63	0.5575	1	1	1014	305	0	0.75	0	0	0.75	0	1	0.58	1.55	39.8	#####		
15	174.14	0.39	0.5575	1	1	361	555	0	0.75	0	0	0.75	0	1	0.12	11.14	75.39	#####		
16	172.81	0.39	0.5575	1	1	384	612	0	0.75	0	0	0.75	0	1	0.12	8.45	78.02	#####		
17	63.69	0.63	0.5575	1	1	592	603	0	0.75	0	0	0.75	0	1	0.68	1.39	37.2	#####		
18	63.2	0.7	0.5575	1	1	761	632	0	0.75	0	0	0.75	0	1	0.65	2.77	38.85	#####		
19	121.36	0.77	0.5575	1	1	621	626	0	0.75	0	0	0.75	0	1	0.52	4.09	52.74	#####		
20	88.44	0.49	0.5575	1	1	170	79	0	0.75	0	0	0.75	0	1	0.55	1.98	46.22	#####		
21	93.23	0.52	0.5575	1	1	86	615	0	0.75	0	0	0.75	0	1	0.58	1.87	45.64	#####		
22	90.96	0.91	0.5575	1	1	543	201	0	0.75	0	0	0.75	0	1	0.9	1.25	40.7	#####		
23	82.93	0.56	0.5575	1	1	1	122	0	0.75	0	0	0.75	0	1	0.47	2.3	45.68	#####		
24	94.75	0.6	0.5575	1	1	80	533	0	0.75	0	0	0.75	0	1	0.36	5.06	53.02	#####		
25	74.48	0.42	0.5575	1	1	1040	626	0	0.75	0	0	0.75	0	1	0.39	2.15	46.33	#####		
26	88.19	0.61	0.5575	1	1	990	624	0	0.75	0	0	0.75	0	1	0.6	4.8	47	#####		
27	67.35	0.63	0.5575	1	1	1032	272	0	0.75	0	0	0.75	0	1	0.57	2.87	42.83	#####		
28	183.47	0.75	0.5575	1	1	1	477	0	0.75	0	0	0.75	0	1	0.49	4.54	70.29	#####		
29	87.69	0.28	0.5575	1	1	279	604	0	0.75	0	0	0.75	0	1	0.12	3.26	59.73	#####		
30	61.75	0.55	0.5575	1	1	825	891	0	0.75	0	0	0.75	0	1	0.4	4.54	47.2	#####		
31	133.57	0.77	0.5575	1	1	559	3	0	0.75	0	0	0.75	0	1	0.66	2.52	53.41	#####		
32	149.54	0.7	0.5575	1	1	460	627	0	0.75	0	0	0.75	0	1	0.43	4.08	60.37	#####		
33	109.78	0.27	0.5575	1	1	572	10	0	0.75	0	0	0.75	0	1	0.13	3.17	62.3	#####		
34	118.01	0.38	0.5575	1	1	435	870	0	0.75	0	0	0.75	0	1	0.16	5.51	62.91	#####		
35	201.69	0.2	0.5575	1	1	387	627	0	0.75	0	0	0.75	0	1	0	9.21	102.79	#####		
36	58.61	0.69	0.5575	1	1	407	263	0	0.75	0	0	0.75	0	1	0.6	2.12	38.43	#####		
37	59.81	0.66	0.5575	1	1	307	881	0	0.75	0	0	0.75	0	1	0.57	2.09	39.01	#####		

**How do we use these data?**



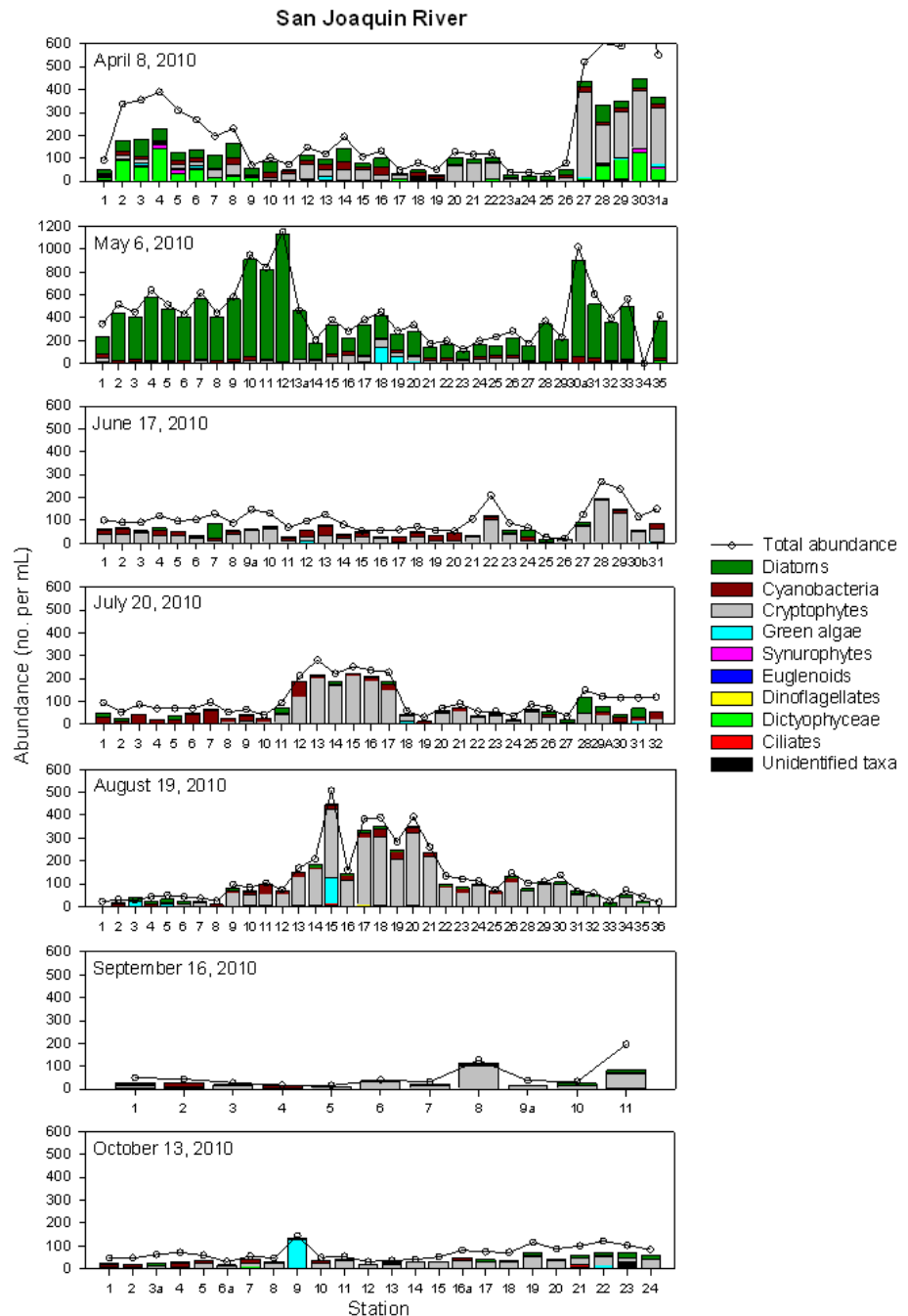
# High frequency studies



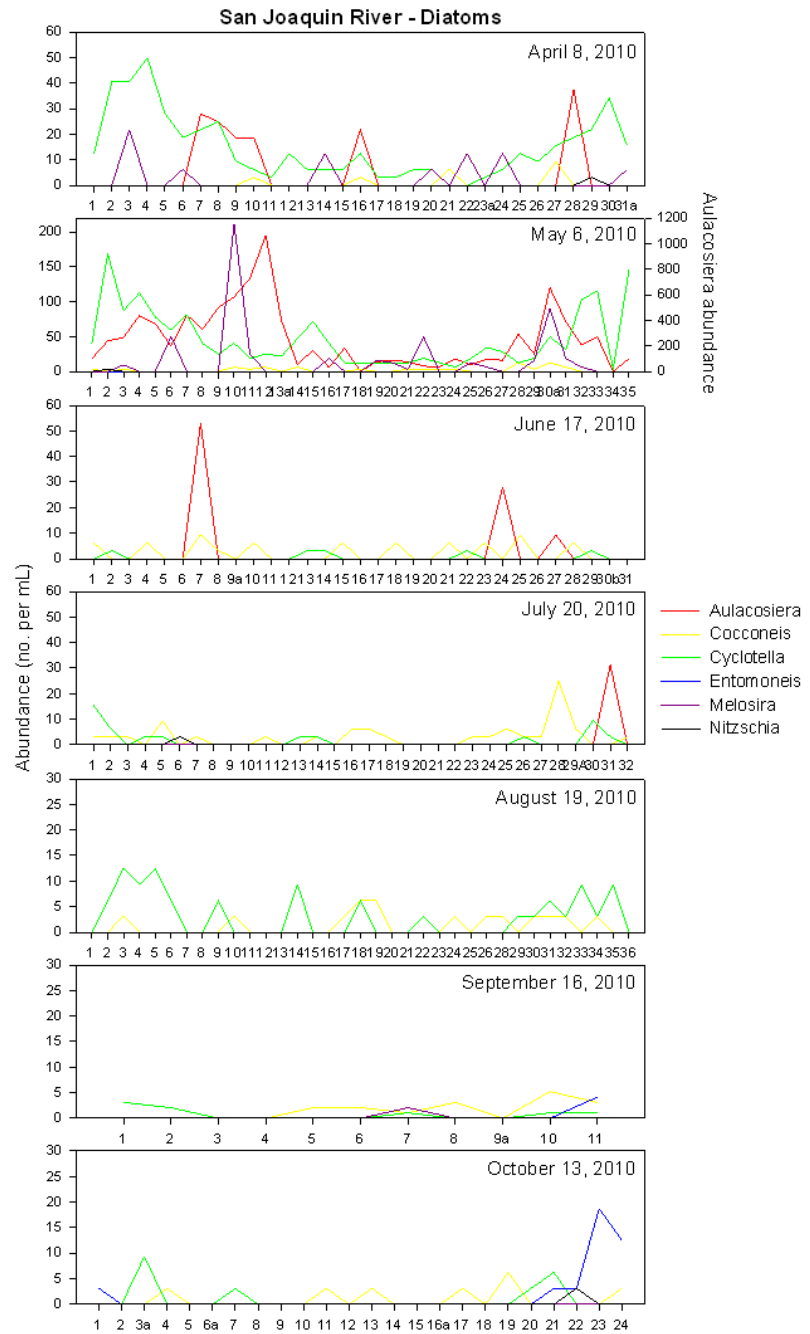
San Francisco Bay-Delta



# Abundance

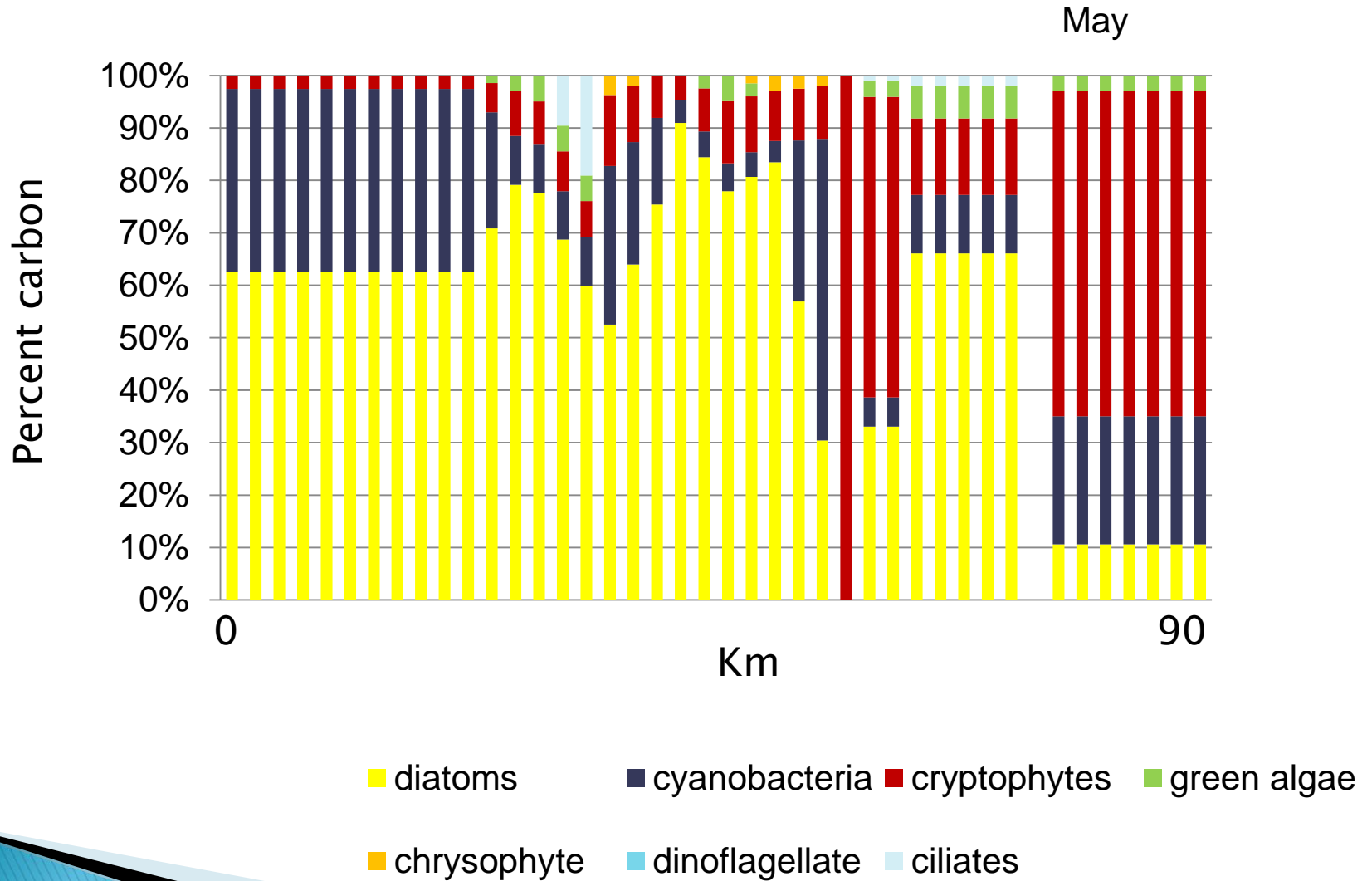


# Genera

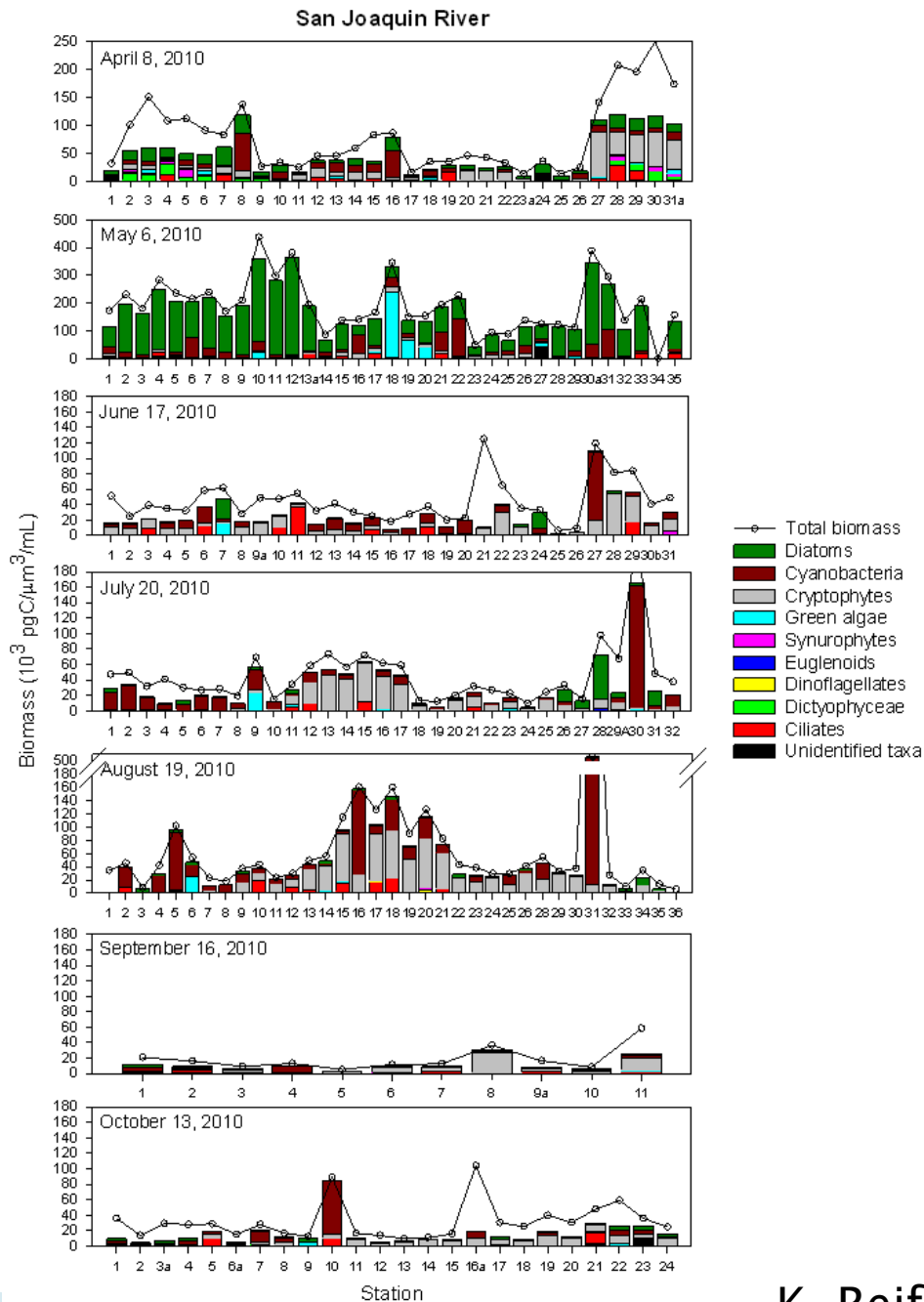


station

# Community composition

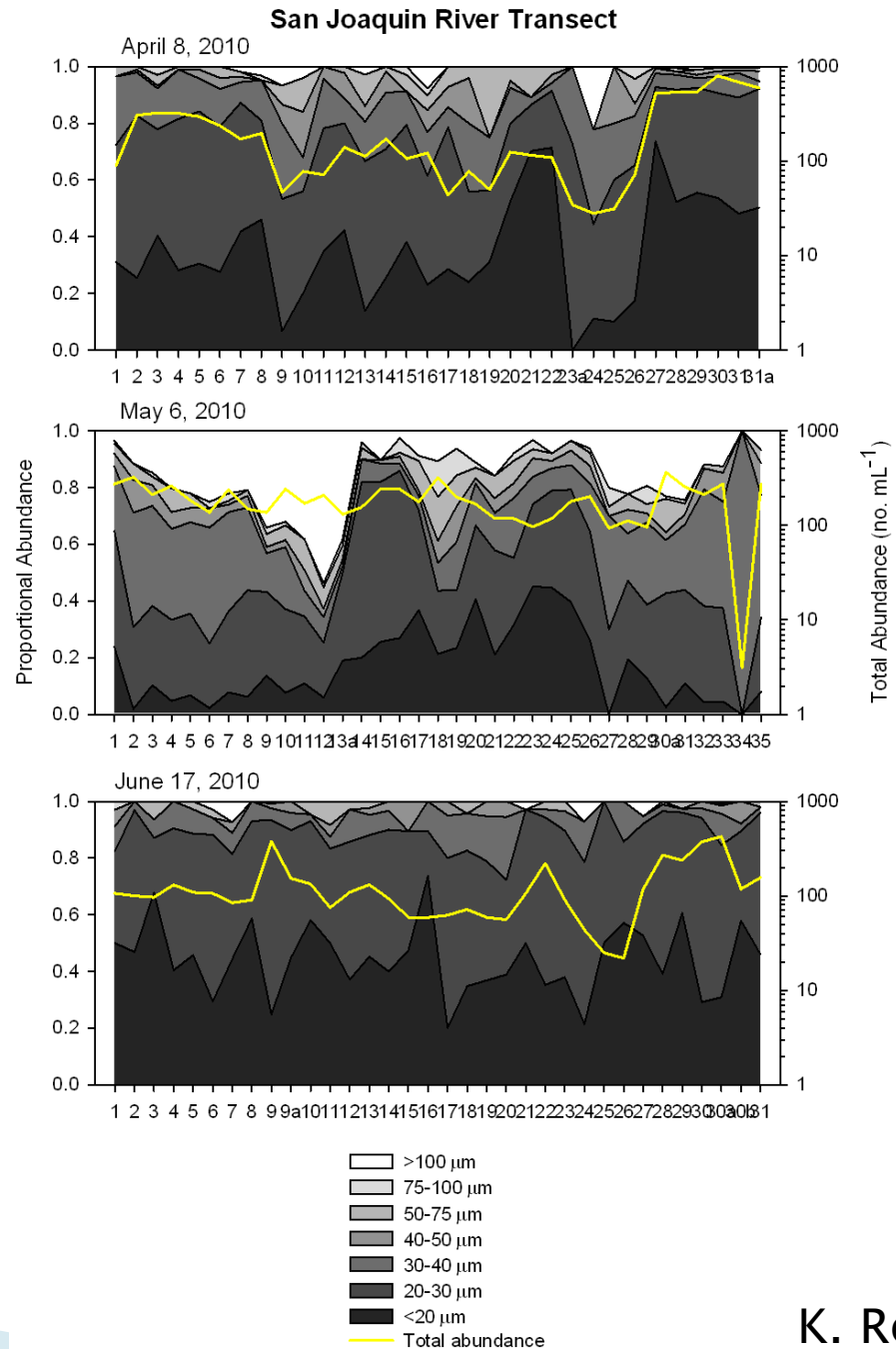


# Biomass

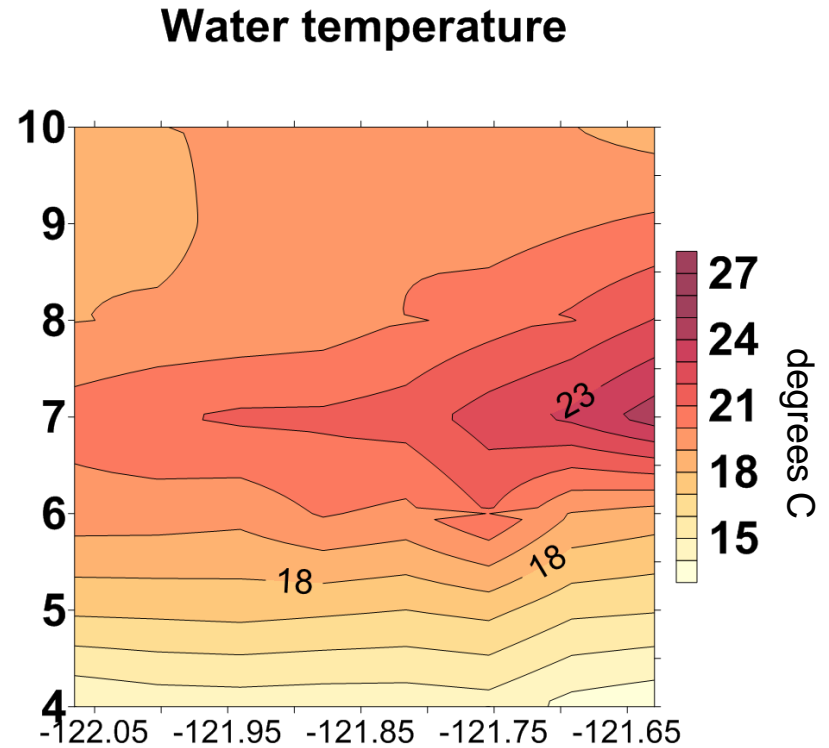
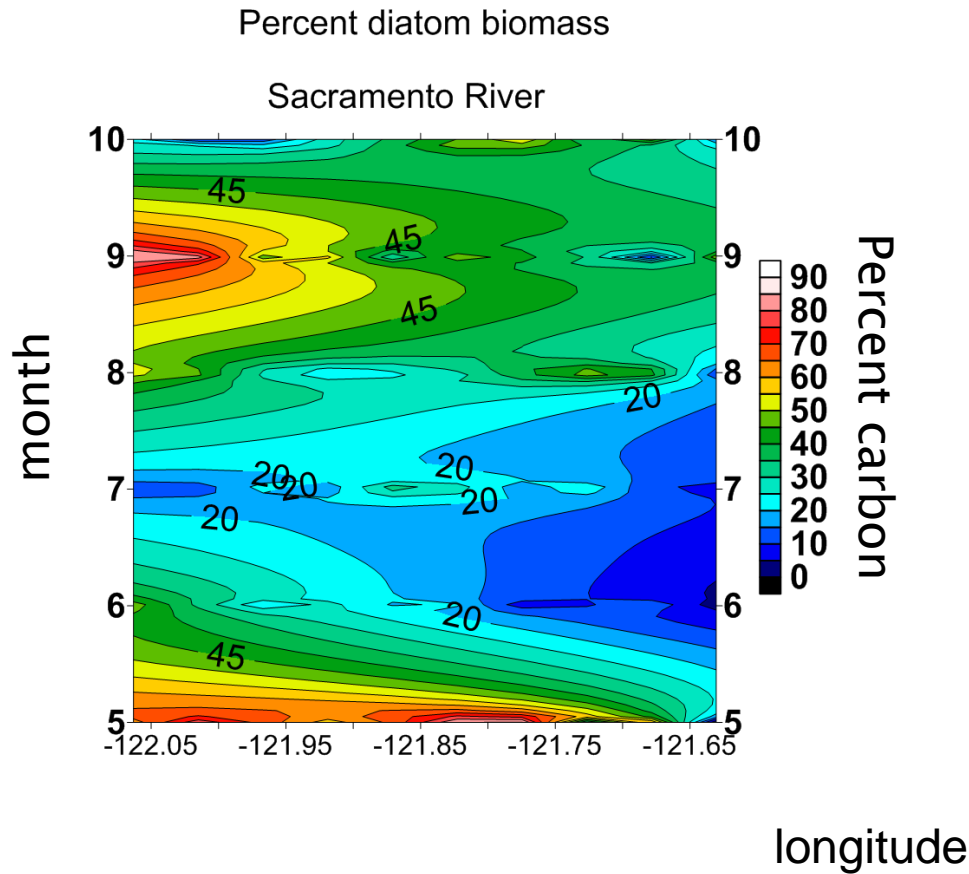




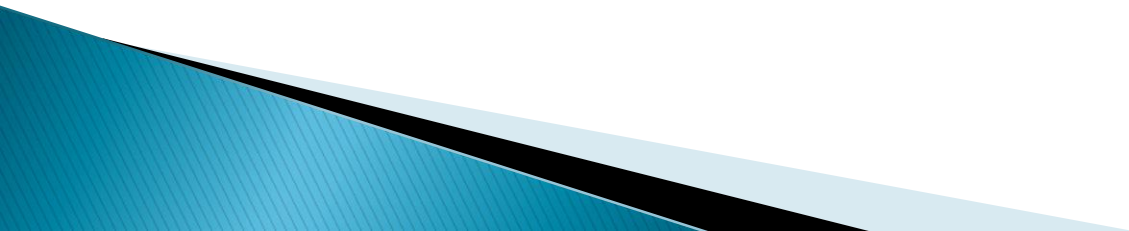
# Size structure



# Biological and Environmental gradients

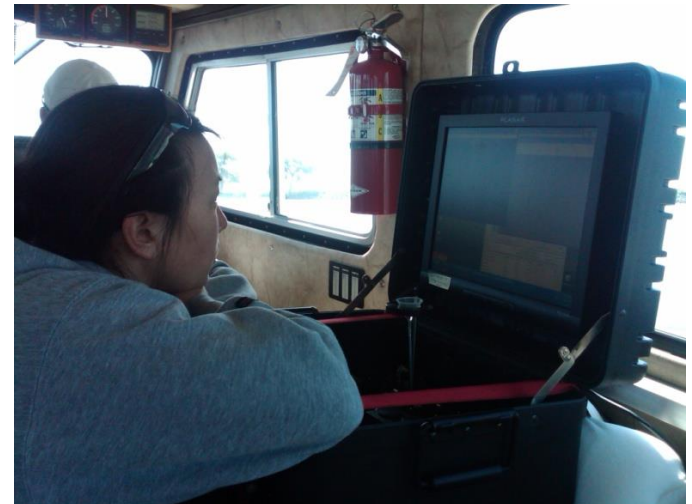


# Evaluation

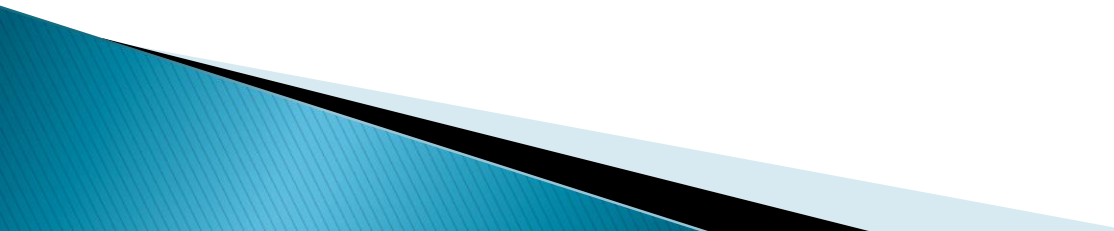


# FlowCAM advantages

- ▶ Nondestructive
- ▶ Handles wide range of object sizes
- ▶ Simple to operate
- ▶ Easier to use than microscope for identification
- ▶ Reliable
- ▶ Fast processing time compared with microscope
- ▶ Automatic sorting
- ▶ Good precision
- ▶ No problem with turbidity
- ▶ Clear images
- ▶ Permanent record
- ▶ Record updates
- ▶ EXCEL spreadsheet record
- ▶ Inexpensive per sample



# FlowCAM disadvantages

- ▶ Relatively high initial cost
  - ▶ Decreased resolution below 10  $\mu\text{m}$
  - ▶ Preserved phytoplankton samples have high number of detritus objects
  - ▶ Species identification can be limited
  - ▶ Field use must be in controlled environment
  - ▶ Requires dedicated staff time
- 

# Recommendation

## ❖ Phytoplankton

- Best for live phytoplankton collection
- Good separation into taxa
- Expertise needed to develop libraries and check identification
- Fast and easy sample processing

## ❖ *Microcystis*

- Best way to analyze colony biomass
- Little expertise needed
- Fast and easy sample processing

## ❖ Zooplankton

- Good separation into taxa
- Little expertise needed
- Fast and easy sample processing

# Summary

FlowCAM is fast, easy and cost effective way to quantify phytoplankton, *Microcystis* and zooplankton into genera or taxa, but requires dedicated staff

More information:



[www.fluidimaging.com](http://www.fluidimaging.com)

# Questions ?



[Peggy.Lehman@water.ca.gov](mailto:Peggy.Lehman@water.ca.gov)



# Information page

- ▶ Video 1:

- ▶ [http://info.fluidimaging.com/how-the-flowcam-works?&\\_\\_hssc=110599322.1.1403720266392&\\_\\_hstc=110599322.17cce891349722a43a8f7e2e62a035b0.1380558602317.1402957562364.1403720266392.11&hsCtaTracking=b628da99-aaf9-4d13-9b0e-7a1e1588694e%7Cb16800a4-f88a-424f-9d26-febee481b1fb](http://info.fluidimaging.com/how-the-flowcam-works?&__hssc=110599322.1.1403720266392&__hstc=110599322.17cce891349722a43a8f7e2e62a035b0.1380558602317.1402957562364.1403720266392.11&hsCtaTracking=b628da99-aaf9-4d13-9b0e-7a1e1588694e%7Cb16800a4-f88a-424f-9d26-febee481b1fb)

- ▶ Video 2:

- ▶ [http://info.fluidimaging.com/video-automate-particle-classification?&\\_\\_hssc=110599322.3.1403720266392&\\_\\_hstc=110599322.17cce891349722a43a8f7e2e62a035b0.1380558602317.1402957562364.1403720266392.11&hsCtaTracking=7f4f898d-cf6f-4a56-98c1-9f036451f051%7Cedd32377-b96f-44d1-bade-fc2a9a6e0e4d](http://info.fluidimaging.com/video-automate-particle-classification?&__hssc=110599322.3.1403720266392&__hstc=110599322.17cce891349722a43a8f7e2e62a035b0.1380558602317.1402957562364.1403720266392.11&hsCtaTracking=7f4f898d-cf6f-4a56-98c1-9f036451f051%7Cedd32377-b96f-44d1-bade-fc2a9a6e0e4d)