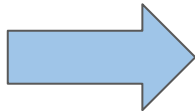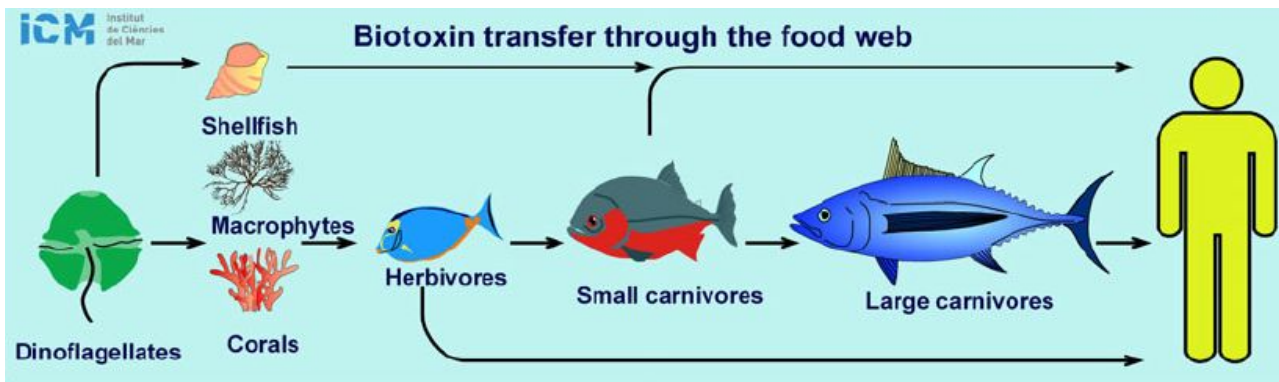# *High-Throughput Imaging Flow Cytometer for Real-Time Monitoring of Toxic Marine Algae*

Shounak Roy, Likai Wei, Vilius Vysniauskas

Former HHMI Students:
Kyrollos Yanny
Patrick Wolf

Mentors: Zoltan Gorocs, Miu Tamamitsu
PI: Aydogan Ozcan

Increased Neurotoxin Accumulation at Higher Levels of the Food Chain

Biotoxin transfer through the food web

https://www.researchgate.net/figure/284282288_fig2_Fig-2-Biotoxin-transfer-pathways-through-the-marine-food-web-to-humans-A

http://www.simplyrecipes.com/recipes/ingredient/shellfish/

Neurological Disorders and Death Result from Shellfish Poisoning
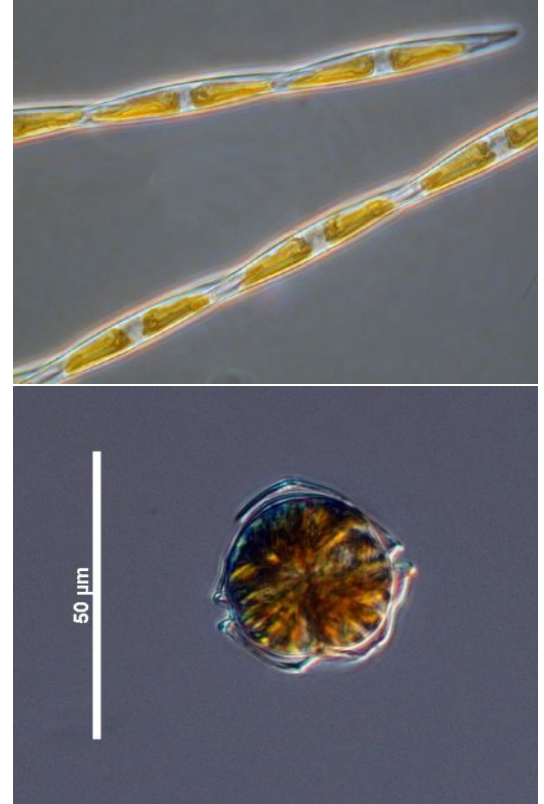
Amnesic Shellfish Poisoning (ASP)

Caused by: Pseudo-nitzschia (Diatom)

Symptoms: Confusion, disorientation, seizures, breathing difficulty, coma, **death**

Paralytic Shellfish Poisoning (PSP)

Caused by: Alexandrium (Dinoflagellate)

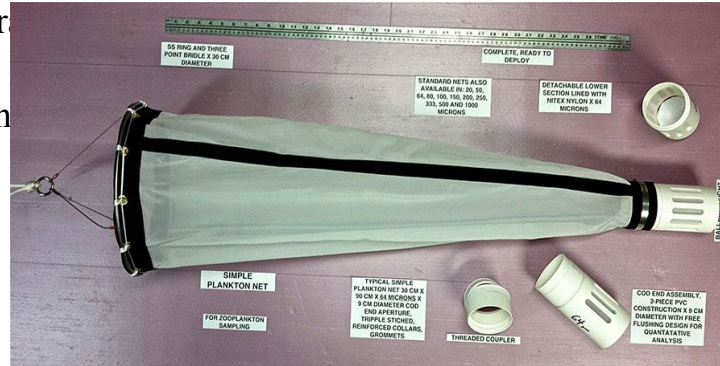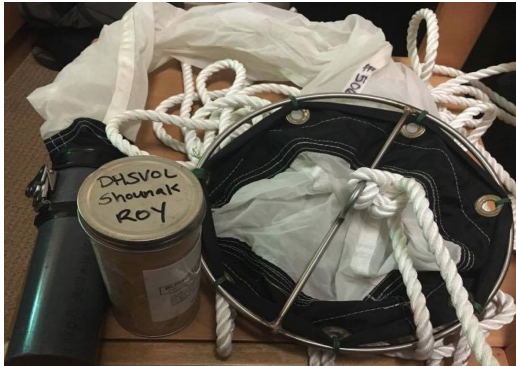Symptoms: Lack of muscular coordination, slurred speech, **death** by paralysis of breathing muscles

50 μm

Current Method for Phytoplankton Monitoring

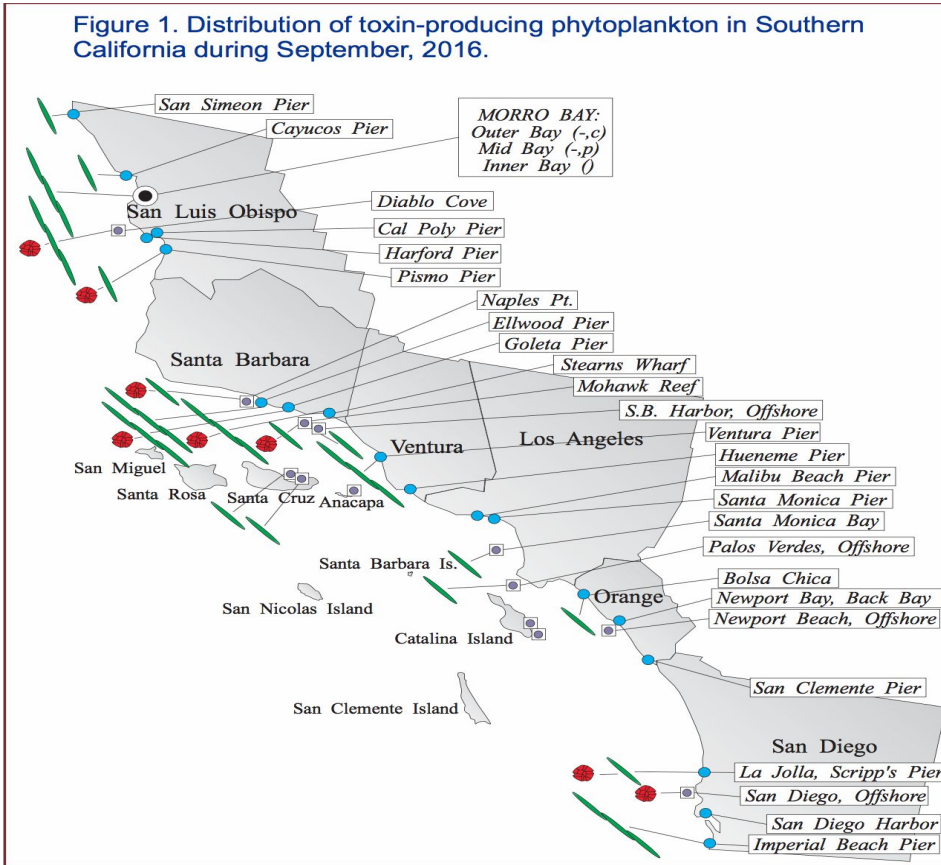Manually collecting seawater samples and observing it under a
light microscope

Limitations

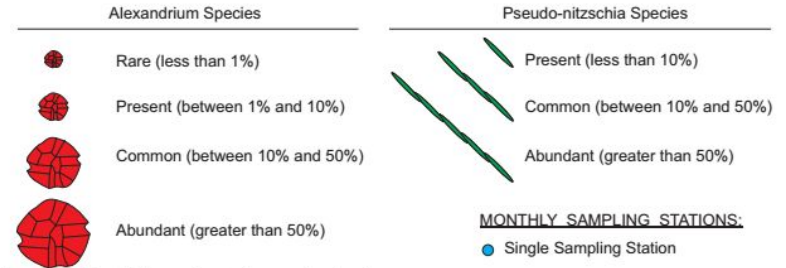Requires lots of manpower and a laboratory

Distribution of Toxic Phytoplankton in California

Figure 1. Distribution of toxin-producing phytoplankton in Southern California during September, 2016.

San Simeon Pier
Cayucos Pier

MORRO BAY:
Outer Bay (-,c)
Mid Bay (-,p)
Inner Bay ()

San Luis Obispo

Diablo Cove
Cal Poly Pier
Harford Pier
Pismo Pier

Santa Barbara

Naples Pt.
Ellwood Pier
Goleta Pier
Stearns Wharf
Mohawk Reef
S.B. Harbor, Offshore
Ventura Pier
Hueneme Pier
Malibu Beach Pier
Santa Monica Pier
Santa Monica Bay
Palos Verdes, Offshore

Ventura
Los Angeles

San Miguel
Santa Rosa
Santa Cruz
Anacapa

Santa Barbara Is.

San Nicolas Island

Bolsa Chica
Newport Bay, Back Bay
Newport Beach, Offshore

Orange

Catalina Island

San Clemente Pier

San Clemente Island

San Diego

La Jolla, Scripp's Pier
San Diego, Offshore
San Diego Harbor
Imperial Beach Pier

Relative Abundance of Known Toxin Producers

Alexandrium Species

- Rare (less than 1%)
- Present (between 1% and 10%)
- Common (between 10% and 50%)
- Abundant (greater than 50%)

Pseudo-nitzschia Species

- Present (less than 10%)
- Common (between 10% and 50%)
- Abundant (greater than 50%)

MONTHLY SAMPLING STATIONS:
- Single Sampling Station
- Multiple Sampling Stations
- Offshore Sampling Station

For areas with multiple sampling stations, species abundance at each station is represented as follows:
(a,p) = Abundance for Alexandrium and Pseudo-nitzschia.
e.g., (c,p) = common, present; (a,-) = abundant, not observed

TOXIC SHELLFISH

SHELLFISH FROM THIS AREA ARE UNSAFE TO EAT DUE TO PARALYTIC SHELLFISH TOXIN. DO NOT EAT CLAMS, OYSTERS, MUSSELS OR SCALLOPS.
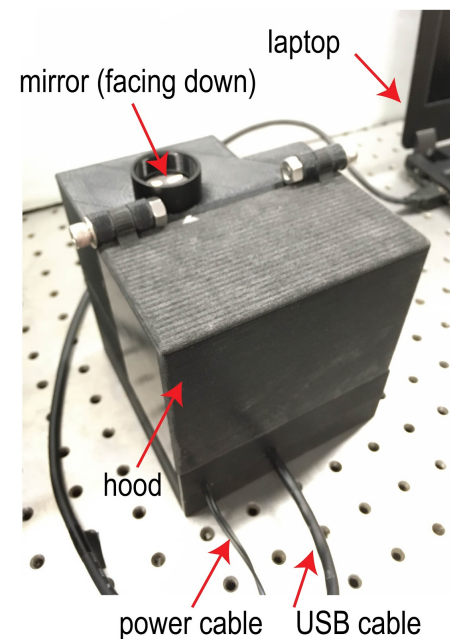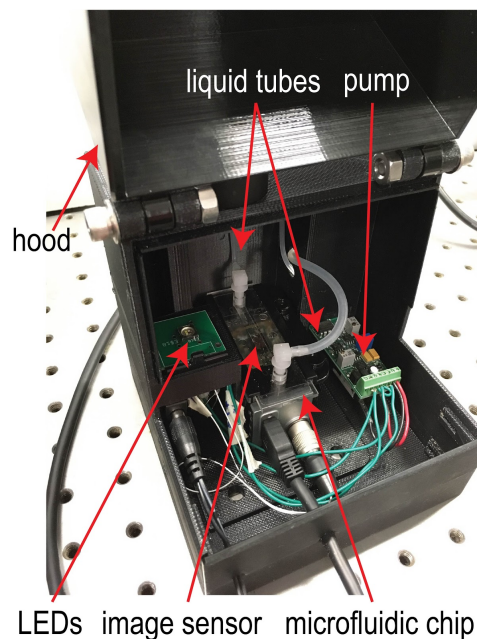
Holographic Imaging Flow Cytometer

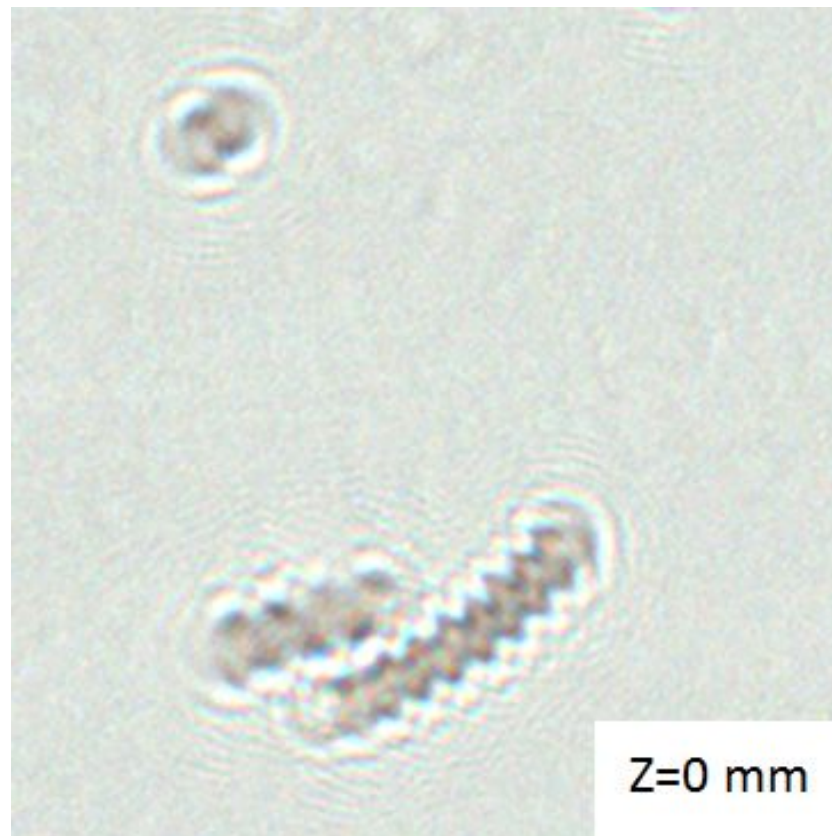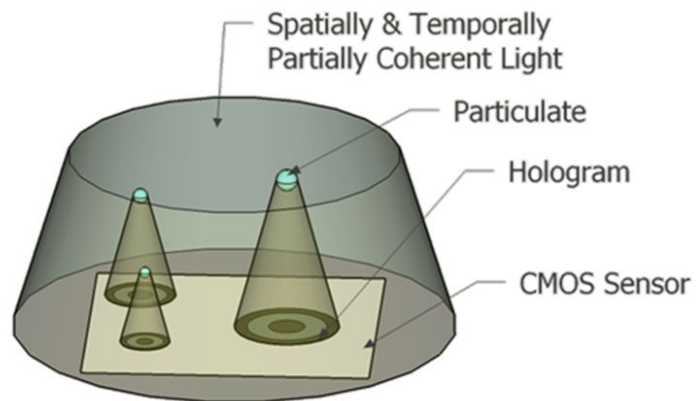Automated monitoring system using
  digital holography.

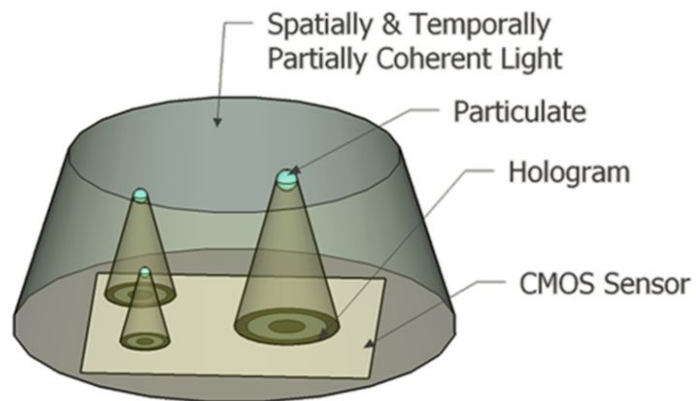High-throughput: 100 ml/hr at 3 fps
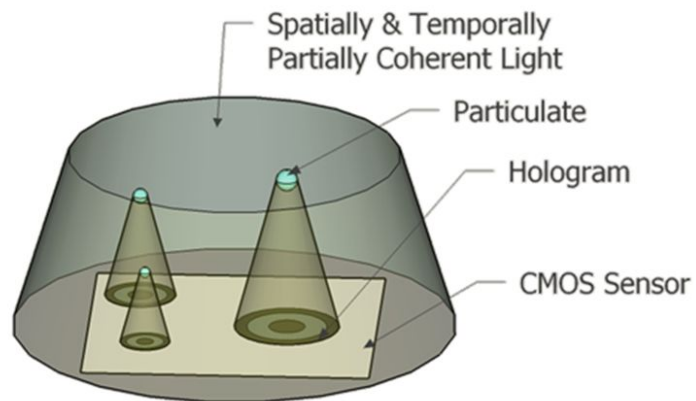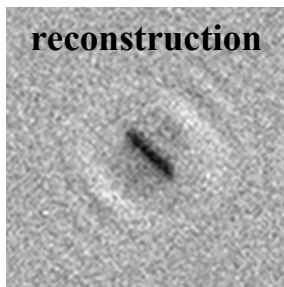
Field-portable

Real-time image analysis

Principle of Digital Holography

Principle of Digital Holography

Spatially & Temporally Partially Coherent Light

Particulate

Hologram

CMOS Sensor

Z=1.65 mm

Principle of Digital Holography

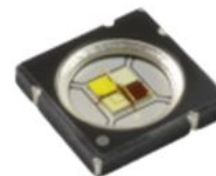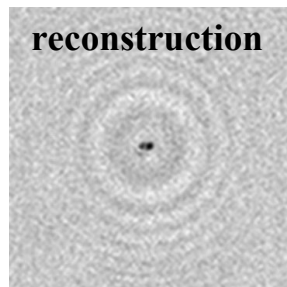Spatially & Temporally Partially Coherent Light

Particulate

Hologram

CMOS Sensor

Z=2 mm

Challenges of Motion Blur in Flow Monitoring

With motion blur

hologram

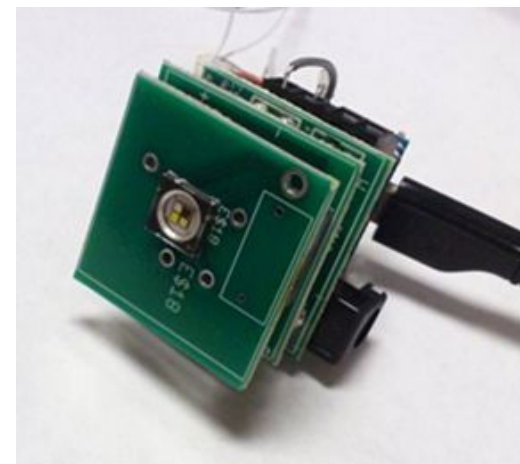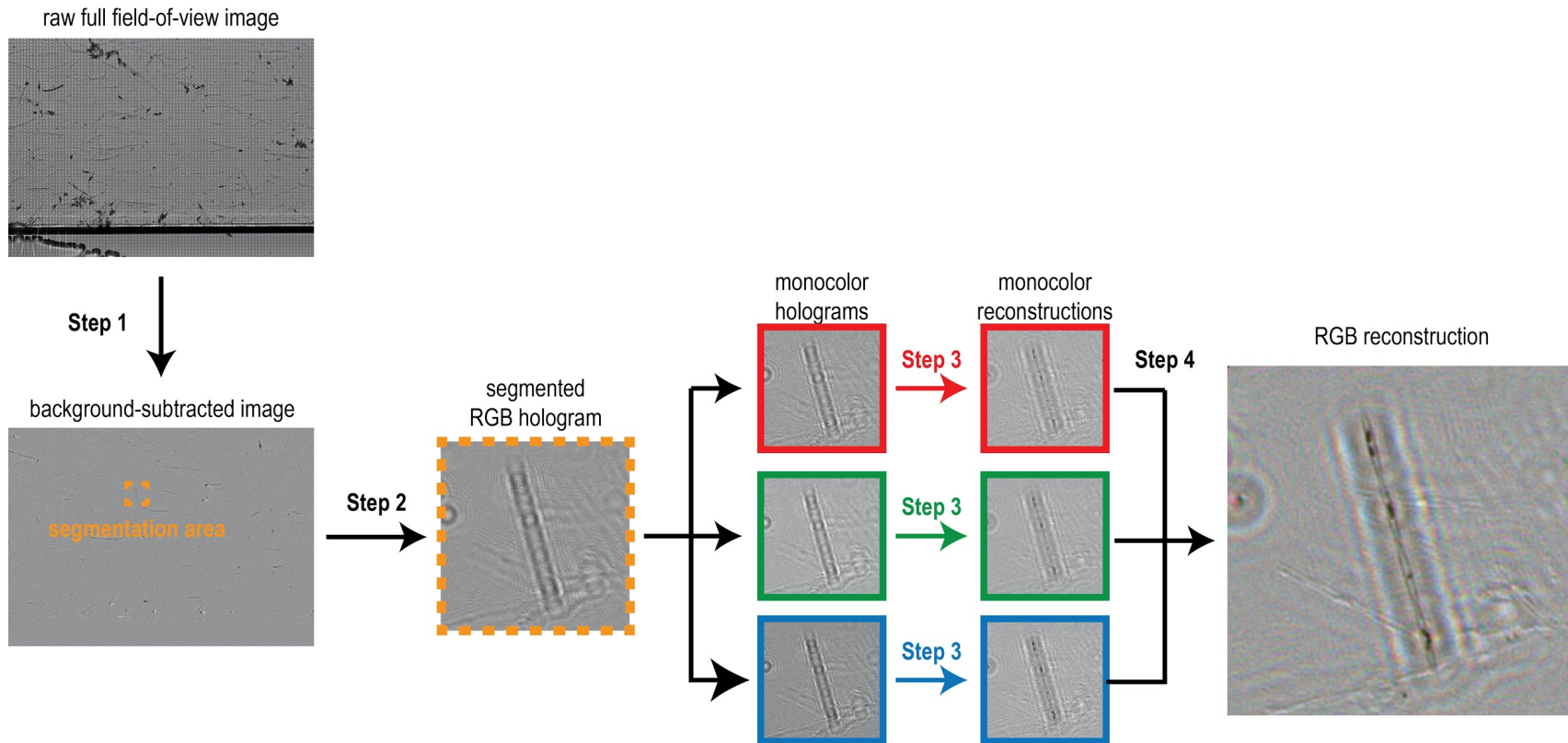Flow direction

reconstruction

Without motion blur

hologram

reconstruction
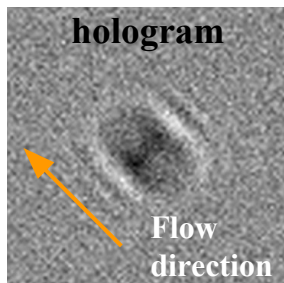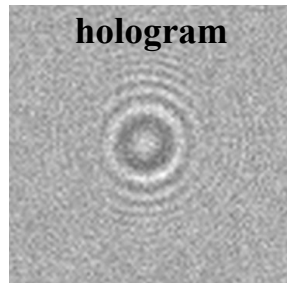
Red = 4.8 W
Green = 5.5 W
Blue = 4.3 W
Total = 14.6 W

Overview of Image Processing Algorithm

raw full field-of-view image

Step 1

background-subtracted image

segmentation area

Step 2

segmented RGB hologram

monocolor holograms

monocolor reconstructions

Step 3

Step 3

Step 3

Step 4

RGB reconstruction
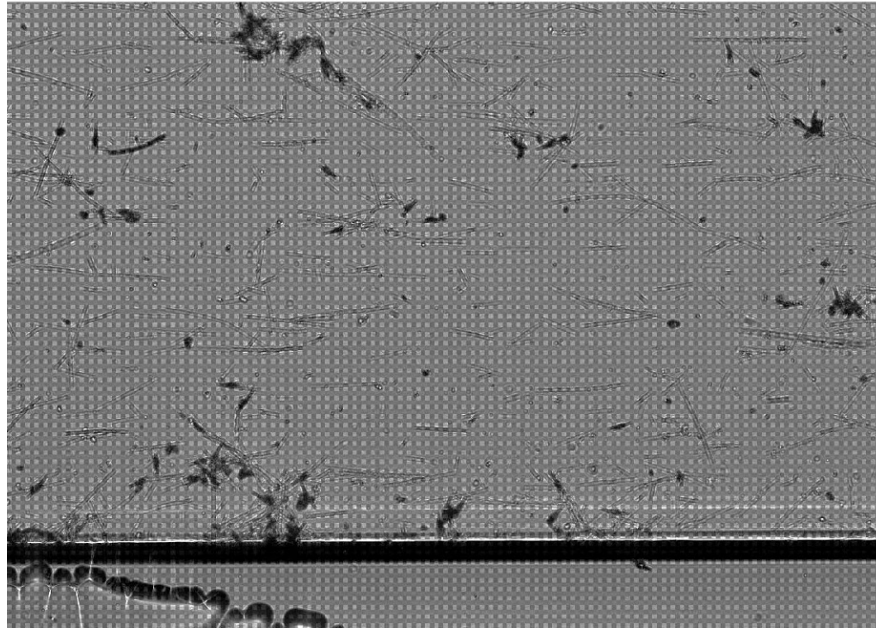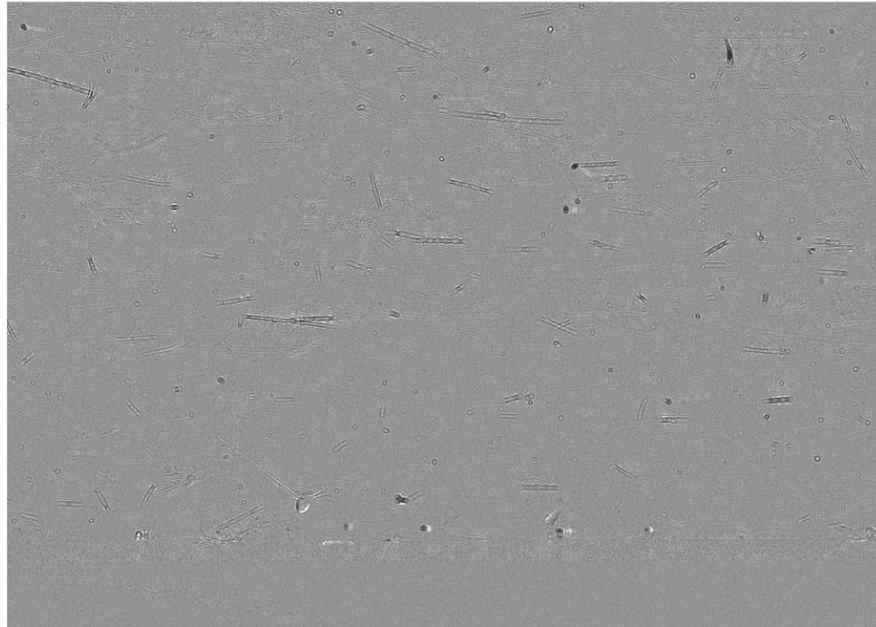
Background Subtraction Removes Static Objects from the FOV

raw full field-of-view image

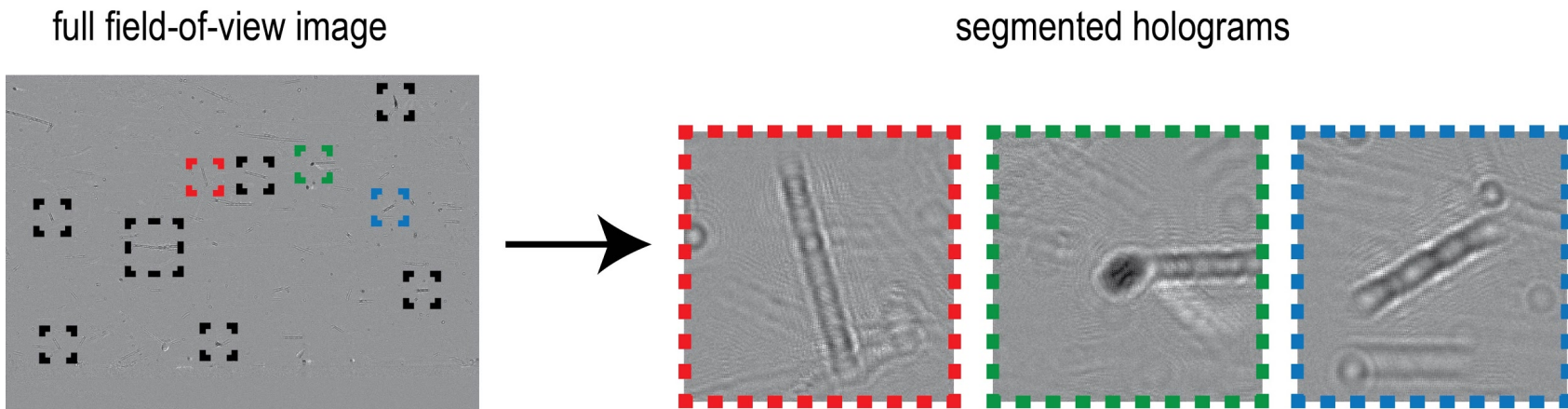# *Background Subtraction Removes Static Objects from the FOV*


background-subtracted image

Segmentation of Detected Objects in the Image

● Each hologram of the target objects in the field of view is detected and segmented
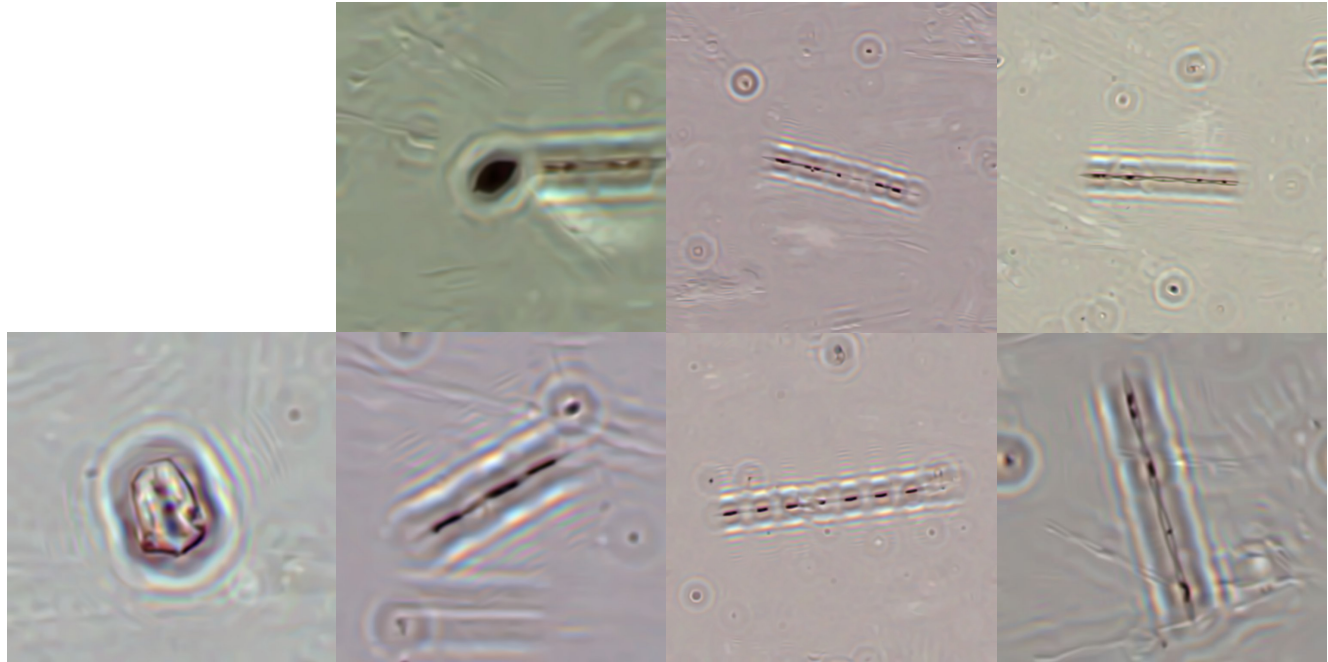
full field-of-view image

segmented holograms

Auto-focus & Reconstruction

- For each object, we use an algorithm to iteratively search for the correct focus
- In the end, we obtain in-focus images of each region of interest
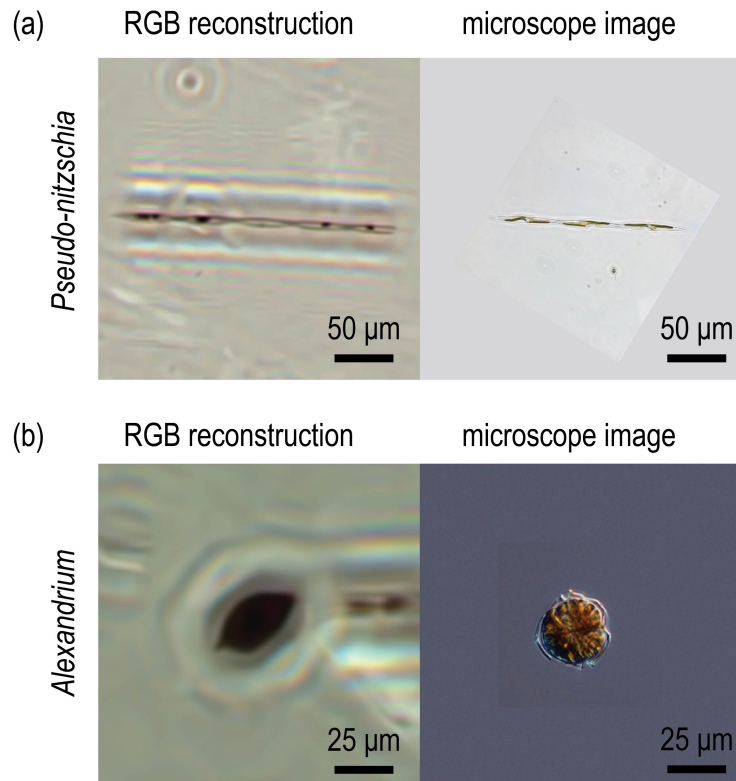
full field-of-view image

segmented holograms

810 µm   690 µm   620 µm
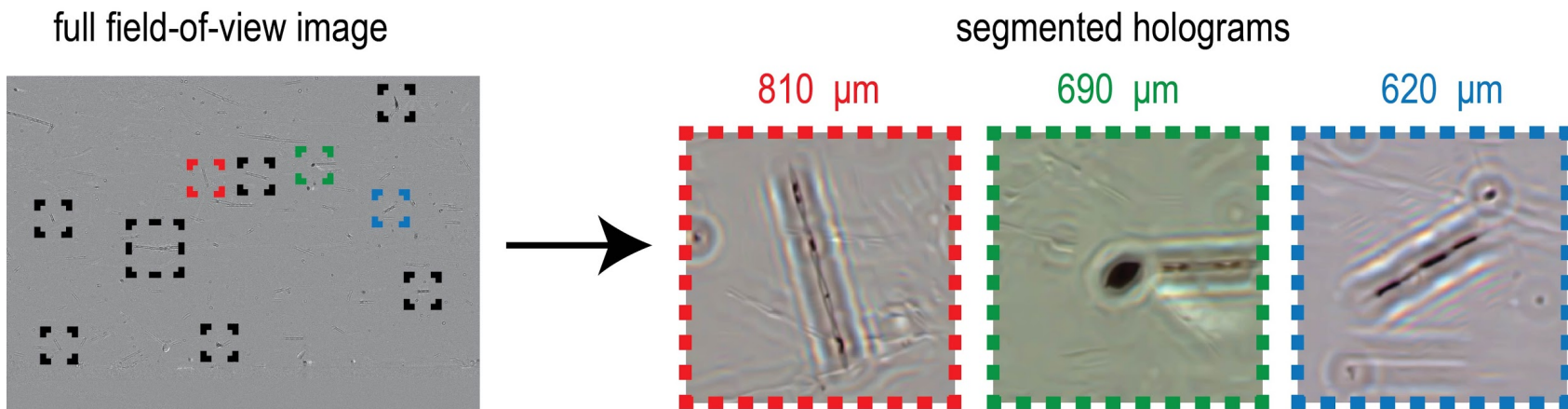
Display and Save Detected Objects

- Each reconstructed algae can be displayed for the user to see, and is saved for later viewing

On-site Testing of Algae Concentration

(a) RGB reconstruction   microscope image
Pseudo-nitzschia
50 µm   50 µm

(b) RGB reconstruction   microscope image
Alexandrium
25 µm   25 µm

Summary

- We are monitoring toxic algae concentration along the Los Angeles coastline

- We have developed a field portable imaging flow cytometer to provide real-time, on-site results

- Our system can image ~100 mL/hour of seawater and provide microscope image of every organism inside it

Thank you!