# Investigating Marine, Freshwater, and Soil Biodiversity with Large Particle Imaging Flow Cytometry

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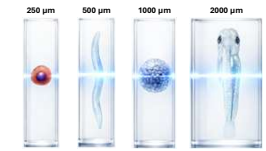
*Large Particle Analysis and Sorting*

## Introduction

Broadly speaking, there are three categories of scale in environmental surveys. At the small end, samples such as fungal spores, phytoplankton, and bacteria can be analyzed and sorted rapidly using traditional flow cytometers. On the other end of the scale are the large objects such as fish, butterflies, and birds. These are the types of samples that can be identified generally and sorted by hand and by eye (or with the aid of a dissection scope). Critically, it is the remaining middle ground, such as small insects, nematodes, Daphnia, that has the property of being too large for traditional flow cytometry and too small to be readily detected or handled with ease under a dissection scope. Owing to the lack of throughput for handling and identification, these sample types remain under explored.

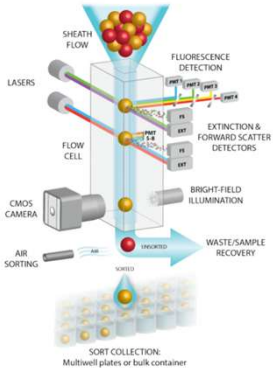
With large particle imaging flow cytometry however, this middle ground of organism sizes can be handled at high throughput speeds. Thousands upon thousands of organisms can then be processed, analyzed, imaged, and sorted, for use in novel species identification, population distribution counts, DNA barcoding, and sequencing.

## What is Large Particle Flow Cytometry?




**Large Particle Flow Cytometry:**

- Operates on the same principles as traditional flow cytometry
- Larger flow cells
  - 250µm, 500µm, 1000µm, 2000µm
- Lower sample pressures & shear forces
- Objects up to 1.5mm in cross sectional diameter




**Sorting**

An air diverter mechanism pushes the sheath/sample stream towards waste until a desired object is detected. Then, the air diverter shuts off and turns back on again. The resulting droplet which contains the object of interest falls into either a multi-well plate or a container.

**Multiparametric Data**

- Extinction (opacity)
- Forward & side scatter
- Time of Flight (length)
- Fluorescence and fluorescent intensity
- Live, real-time brightfield images

## Acknowledgements

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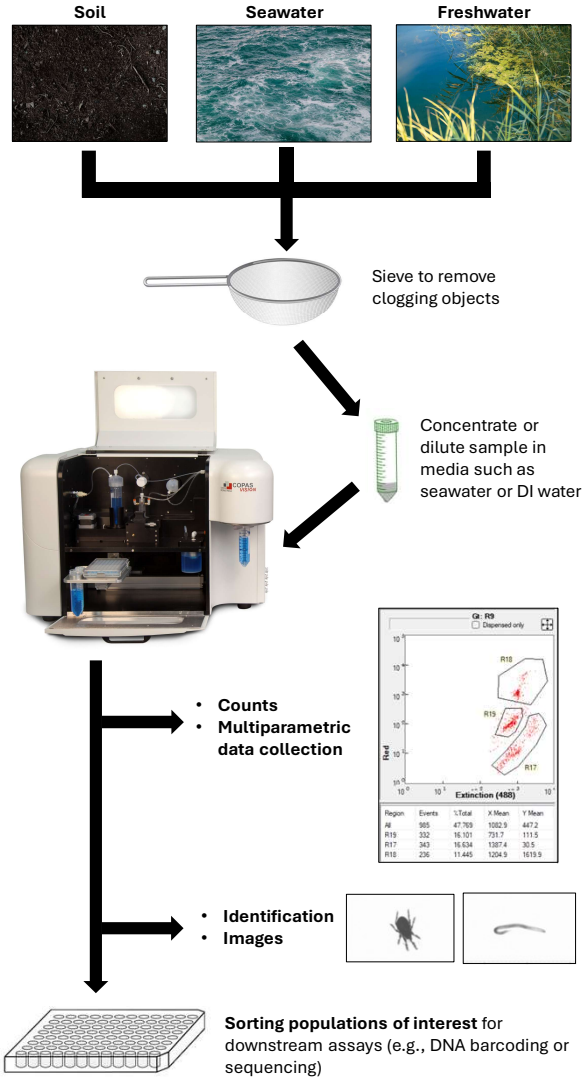
Soil samples provided by Dr. Camila Filgueiras & Dr. Denis Willett of the University of North Carolina, Asheville
Pondwater samples provided by Dr. Robert Cichewicz of the University of Michigan

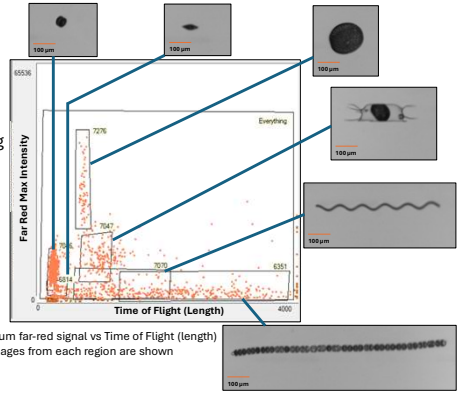
## Workflow




## Examples

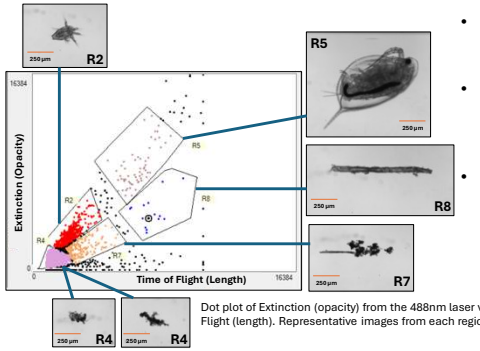
### Identifying Organisms in Seawater

- Strains of different organisms were cultured in artificial seawater and analyzed
- Groupings of different organisms were identified using far-red signal, length, and the collected images
- Organisms found in these groupings can be sorted for cryopreservation, microscopy, and molecular analysis




Dot plot of maximum far-red signal vs Time of Flight (length)
Representative images from each region are shown

### Identifying & Sorting Organisms from Pondwater

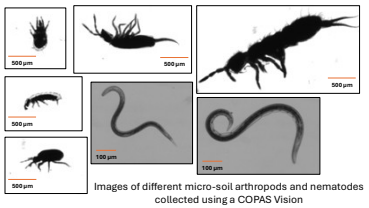



- Pondwater from a golf course runoff was collected and sieved to avoid clogging.
- Debris (regions R4, R7, & R8) and organisms of interest (regions R5 & R2) were identified using opacity and length
- Organisms were sorted, 1 per well, into multi-well plates for downstream assays such as proteomics and novel peptide discovery.

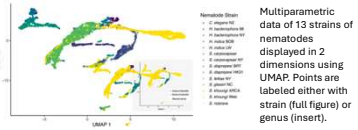
Dot plot of Extinction (opacity) from the 488nm laser vs Time of Flight (length). Representative images from each region are shown.

### Soil Organisms for Supervised Machine Learning

- Organisms from soil were processed using Berlese funnels and collected in ethanol for use with the 2000µm flow cell.
- Different nematode cultures were cultured and diluted in water for use with the 500µm flow cell.
- Both were used as training datasets for a supervised machine learning (ML) to automatically identify and count organisms within a given soil sample.
- Using the ML model, any unidentified organism could be marked within regions for sorting, DNA barcoding, and sequencing.



Images of different micro-soil arthropods and nematodes collected using a COPAS Vision



Multiparametric data of 13 strains of nematodes displayed in 2 dimensions using UMAP. Points are labeled either with strain (full figure) or genus (insert).

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