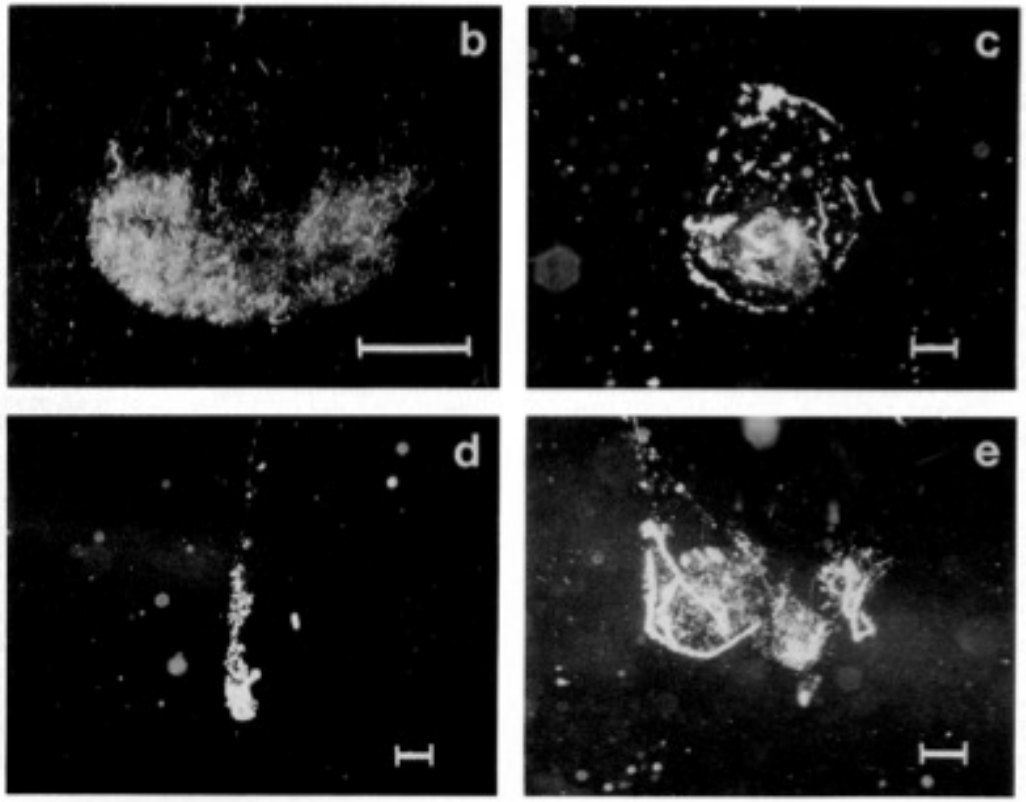
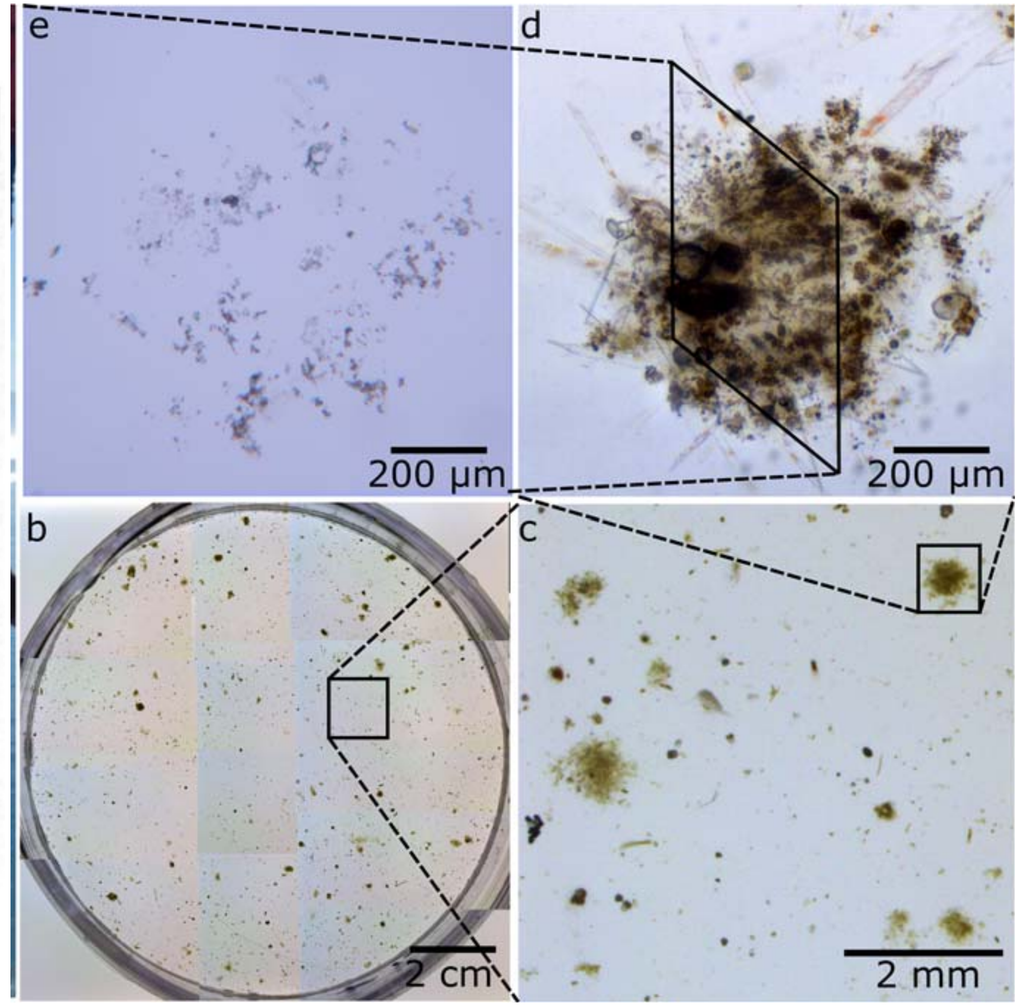
Particle imaging

Meg Estapa, Ocean Optics Course 2021



Alldredge and Gotschalk, 1988 (panel "b" scale bar = 1 cm, others = 1 mm)



Flintrop et al. 2018

Why particle imaging?

(i.e., What information content do you gain? What processes are captured?)

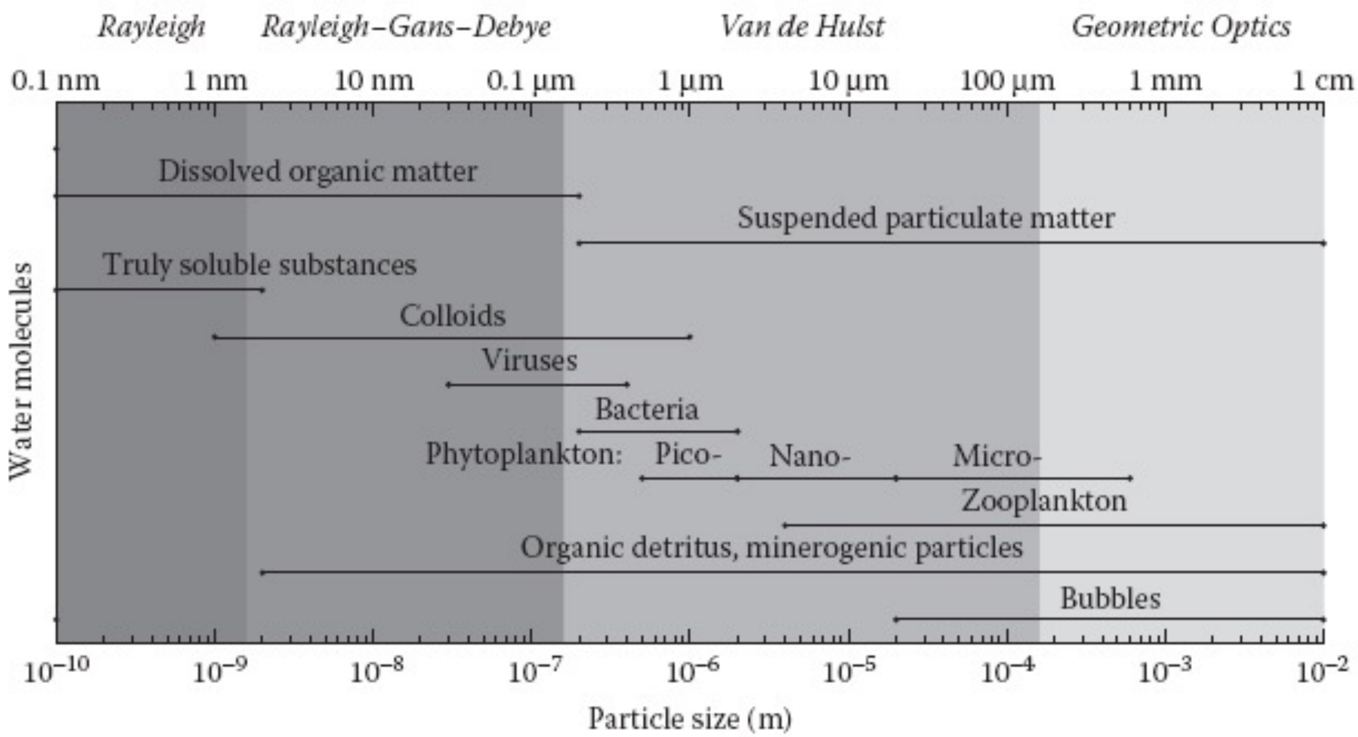
Emphasis today on

- Particles ~100 um and larger (mostly)
- In situ techniques (mostly)
- Digital systems

not remote sensing images! (those will come later...)

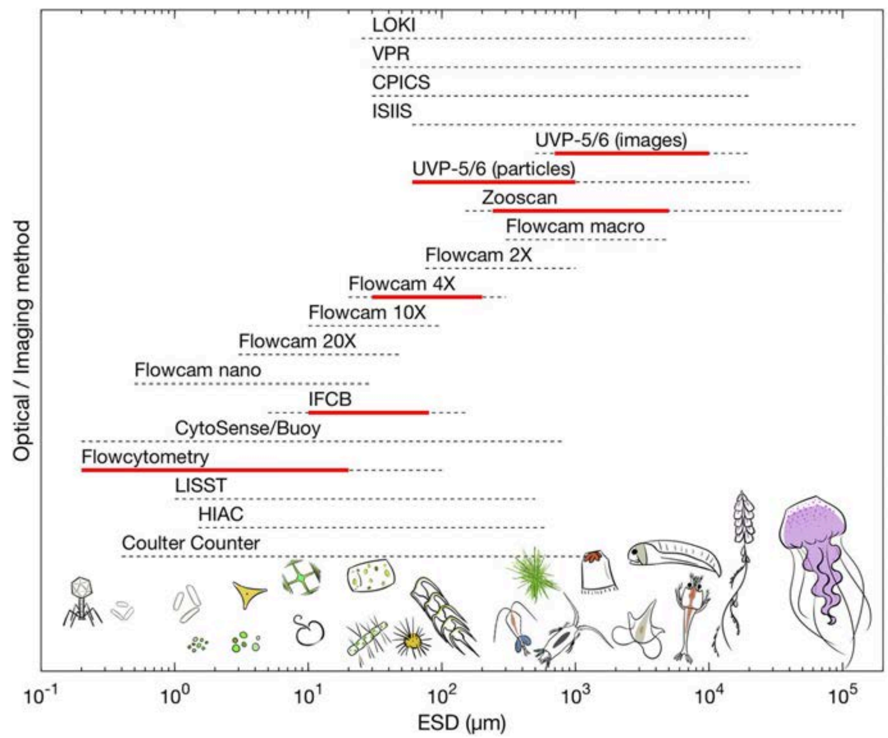
Overview

- Theory
- Instrumentation examples (major types, emphasis on systems in wide use)
- Particle detection & classification



Particle size ranges, optical modeling regimes, and particle sizing/imaging instruments

Upper: Clavano et al. 2007; lower: Lombard et al. 2019



Optical resolution of an imaging system

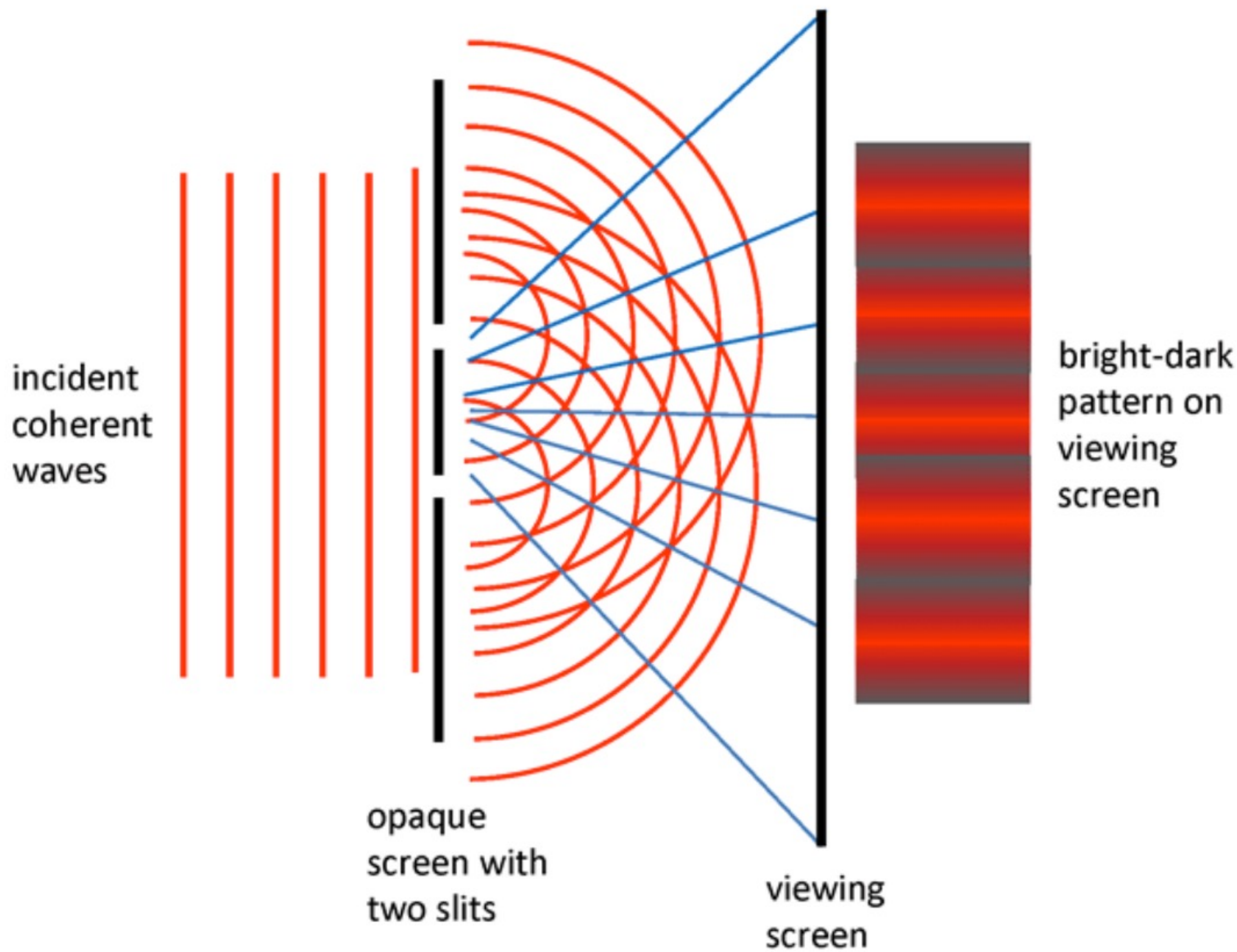


Figure 1.1, Ocean Optics Web Book (Mobley et al.)

Optical resolution of an imaging system

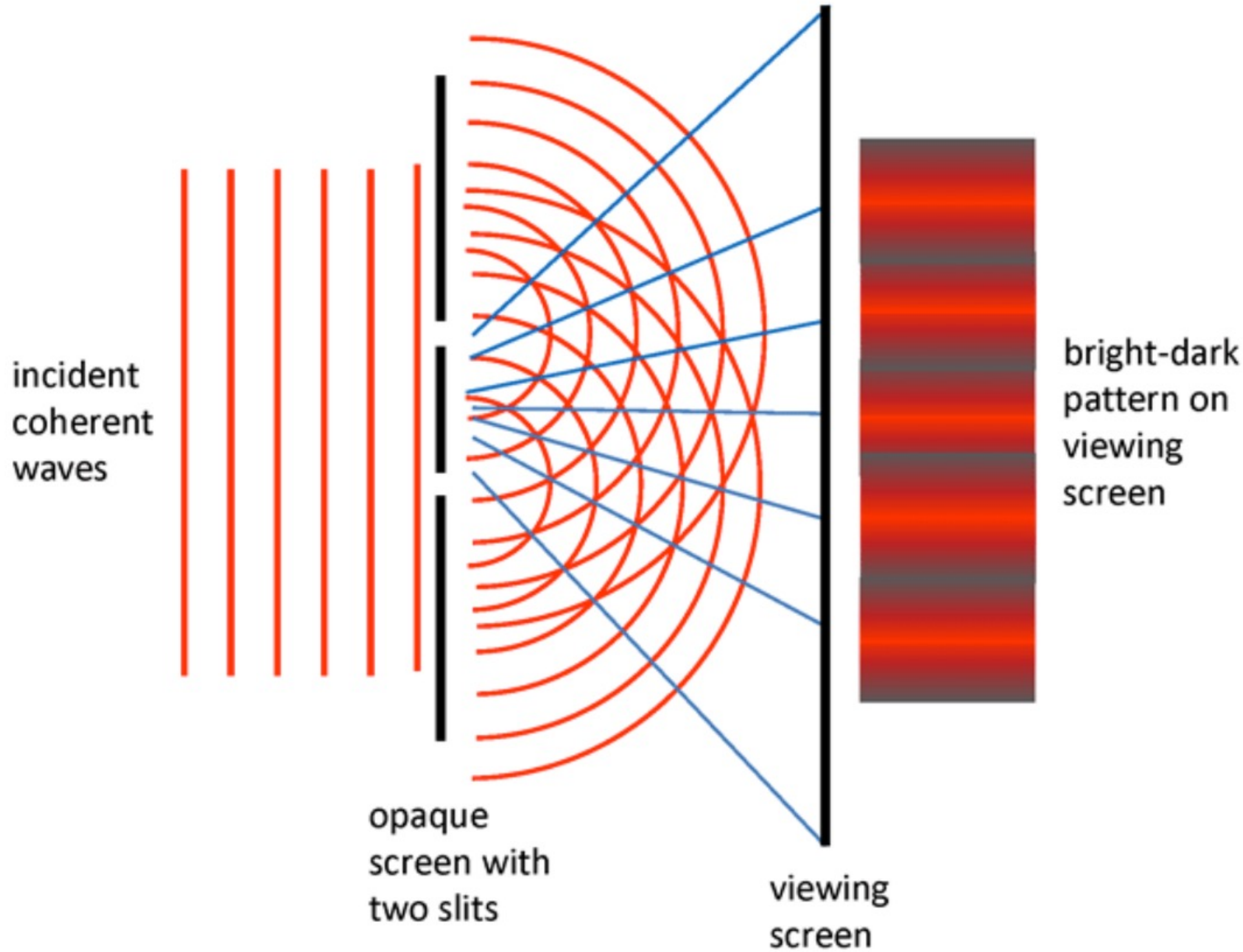
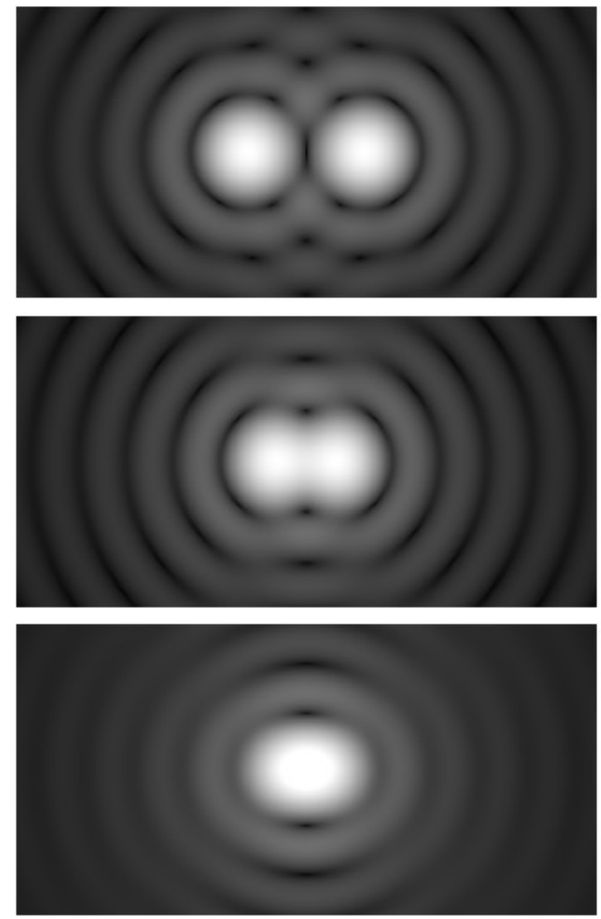


Figure 1.1, Ocean Optics Web Book (Mobley et al.)



Wikimedia Commons

Optical resolution of an imaging system

- Rayleigh criterion: Diffraction-limited horizontal resolution (*r*) of an imaging system

$$r = \frac{1.22\,\lambda}{NA} \quad \text{where} \quad \begin{array}{ll} NA = n\ \sin\alpha & \text{if objective lens only} \\ NA = n\ \sin(2\alpha) & \text{if objective + condenser lenses} \end{array}$$

(NA = numerical aperture)

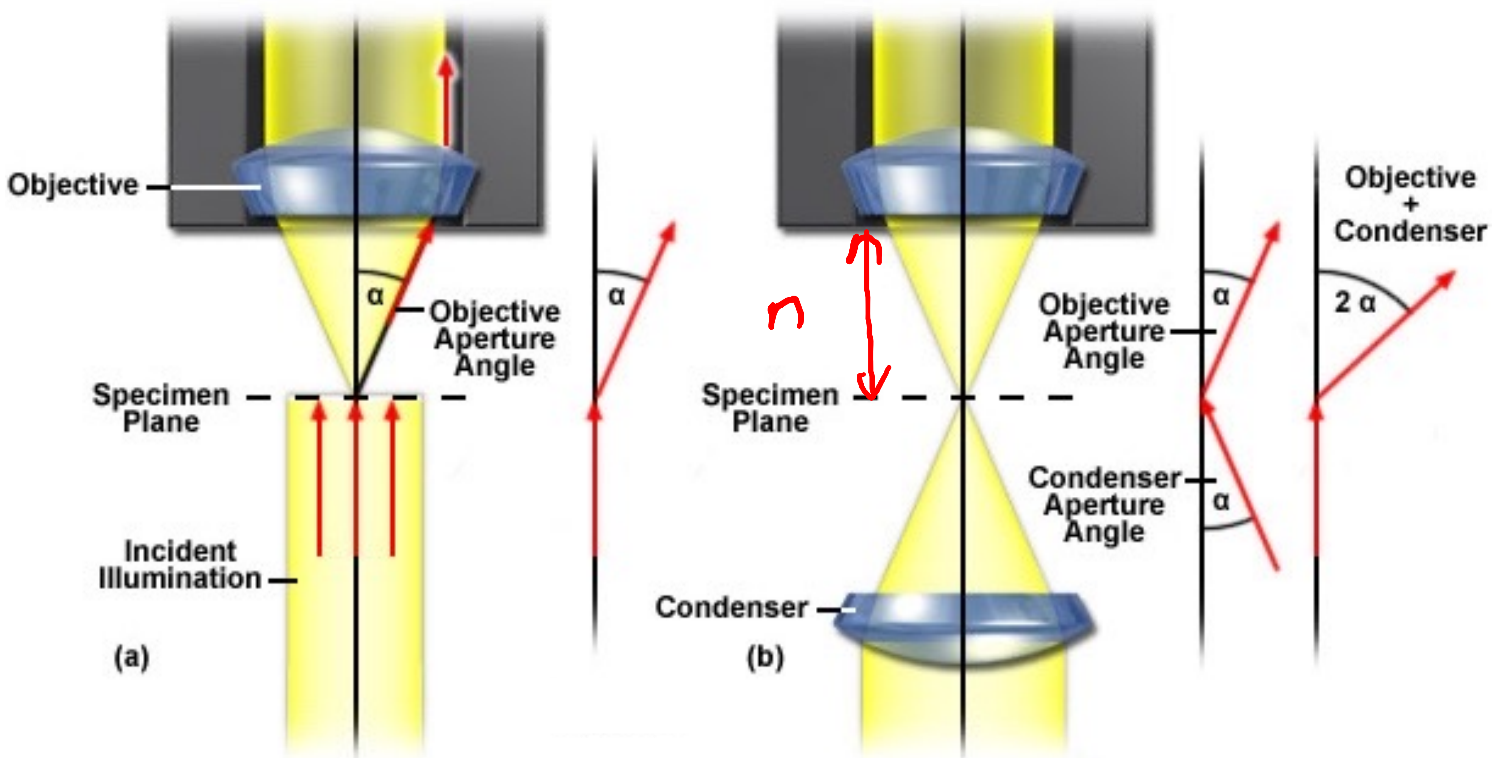


figure: http://zeiss-campus.magnet.fsu.edu/articles/basics/resolution.html

Optical resolution of an imaging system

- Rayleigh criterion: Diffraction-limited horizontal resolution (r) of an imaging system

$$r = \frac{1.22\,\lambda}{NA} \quad \text{where} \quad \begin{array}{ll} NA = n\ \sin\alpha & \text{if objective lens only} \\ NA = n\ \sin(2\alpha) & \text{if objective + condenser lenses} \end{array}$$

- However *depth of field* varies in proportion to $1/(NA)^2$

higher NA

lower NA

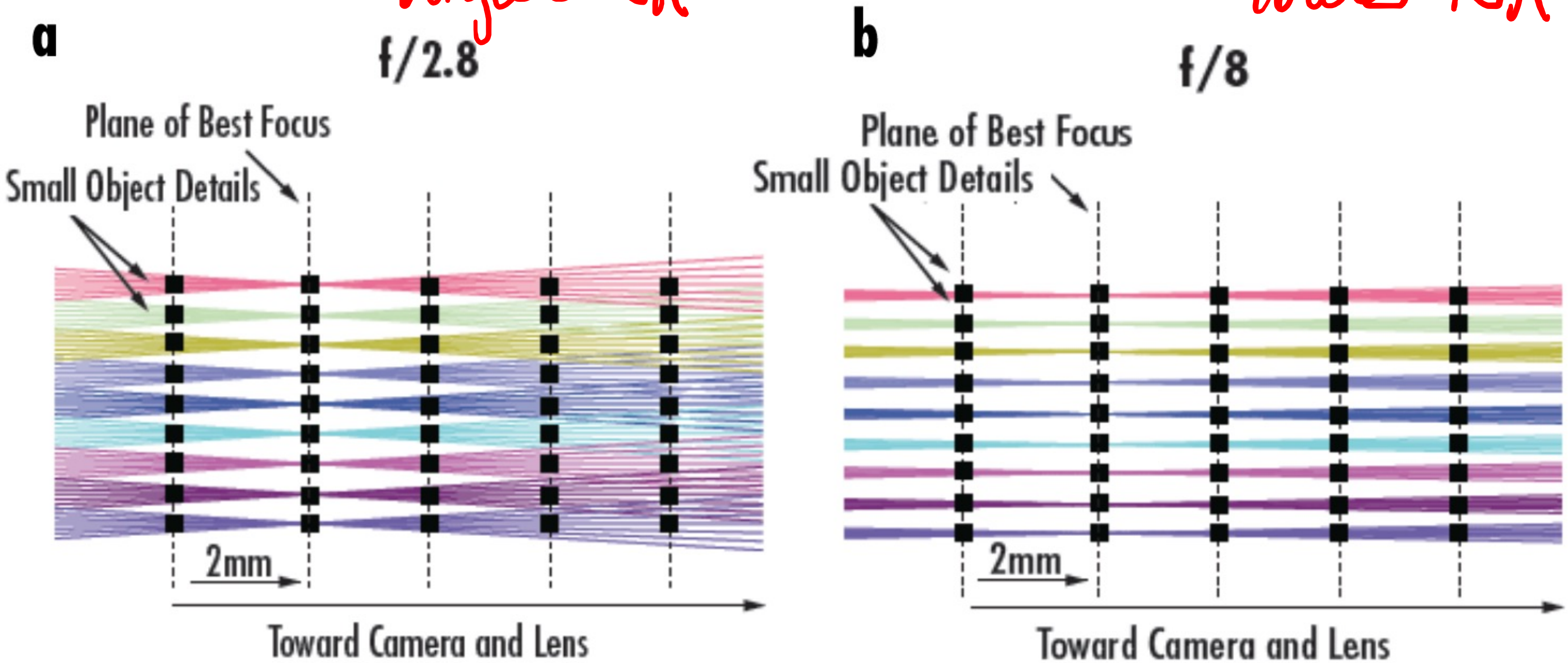
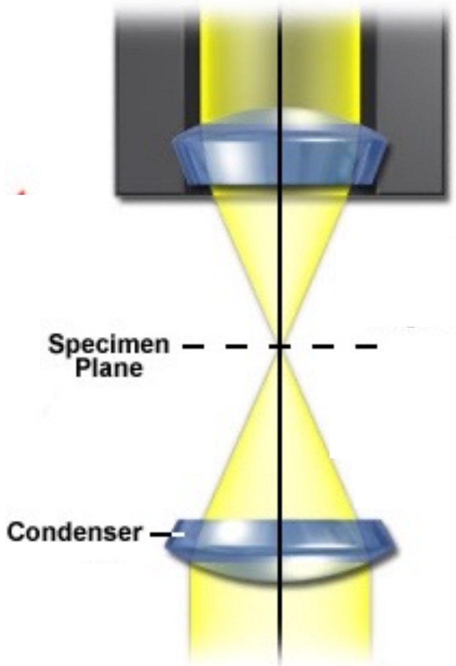


figure: https://www.edmundoptics.com/knowledge-center/application-notes/imaging/depth-of-field-and-depth-of-focus/

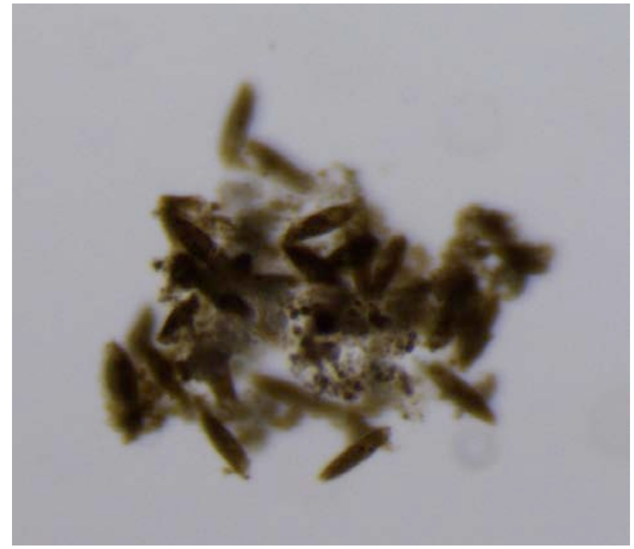
Sampling density of an imaging system

- Ideally want sampling density / camera resolution (pixels per physical length) to match optical resolution
- Nyquist sampling theorem: sampling frequency should be at least 2x the highest-frequency features in the specimen



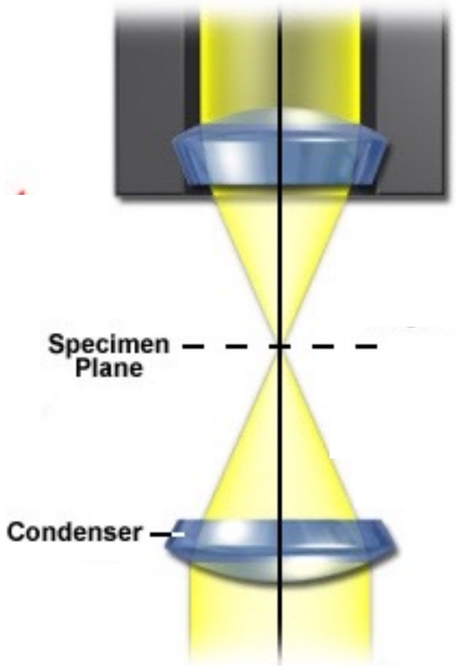
Illumination types (by analogy to microscopy)

- Brightfield (transmitted-light) microscopy
- Imaging Flowcytobot
- Flowcam



aggregate images: C. Durkin, *unpublished*

Illumination types (by analogy to microscopy)



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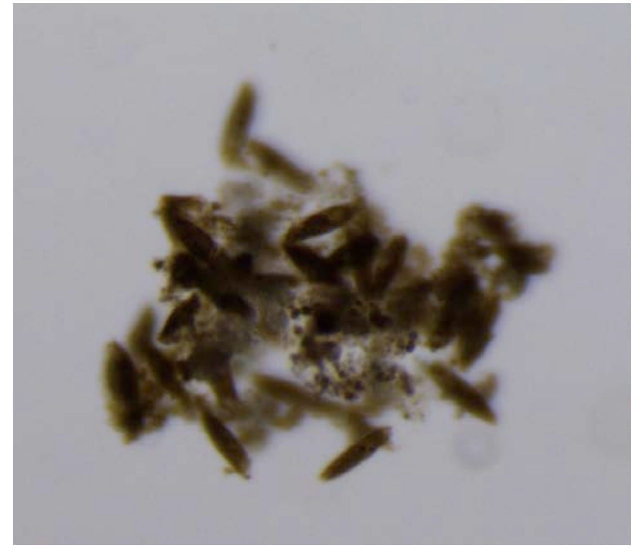

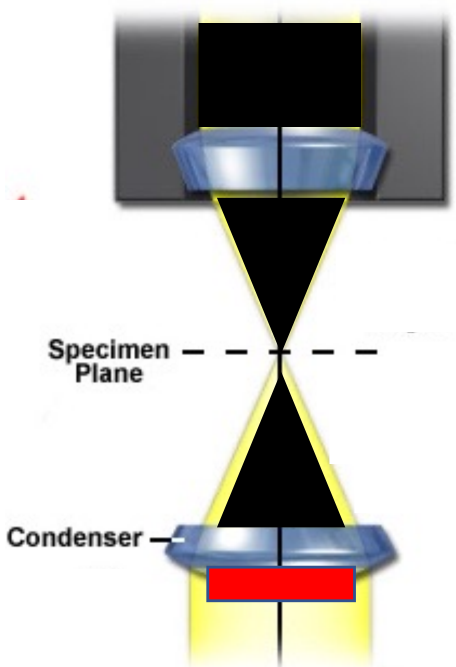

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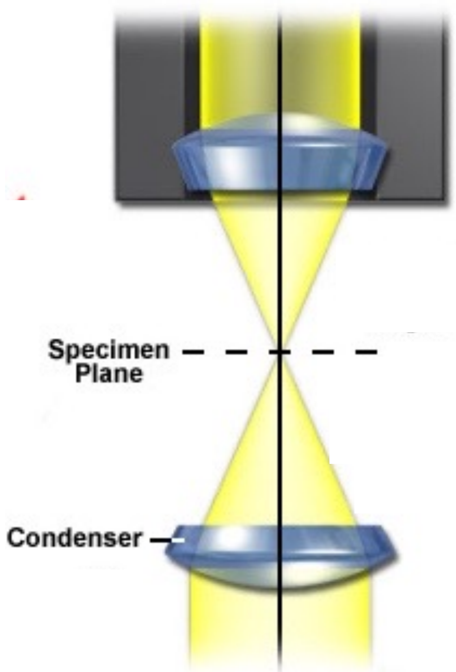

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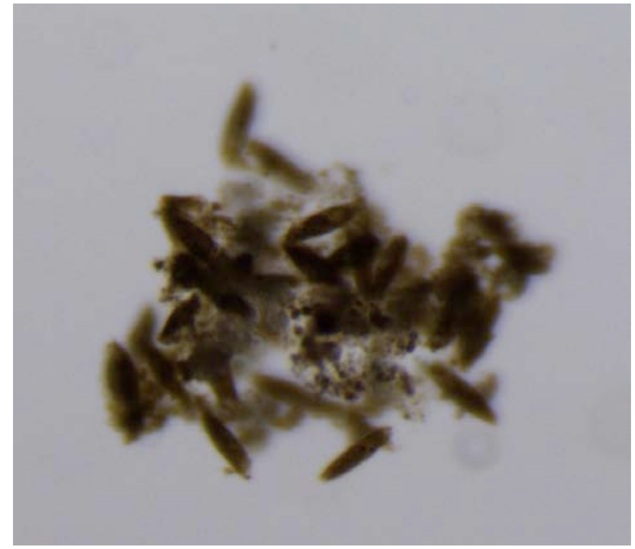
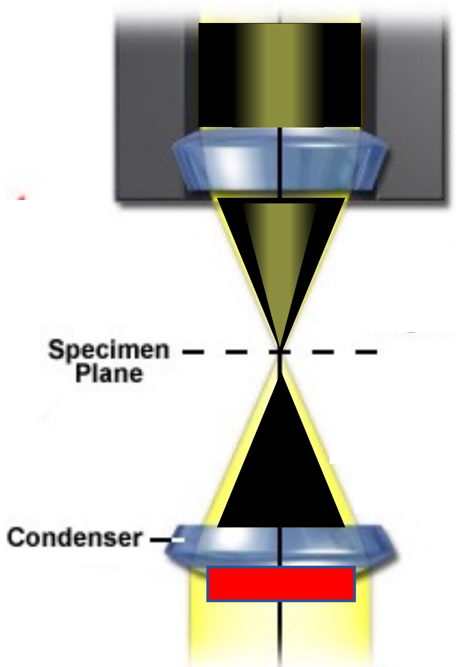
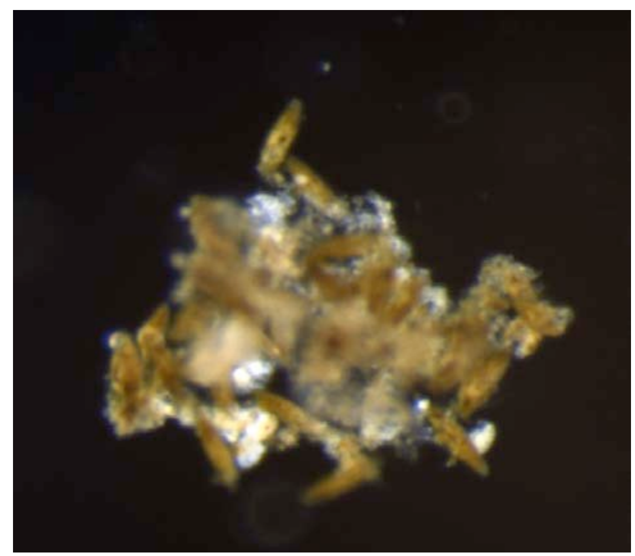


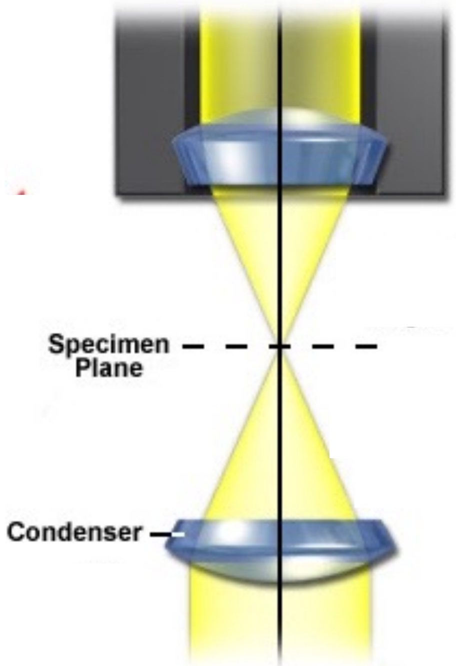


- Darkfield (scattered-light) microscopy
- Underwater Vision Profiler

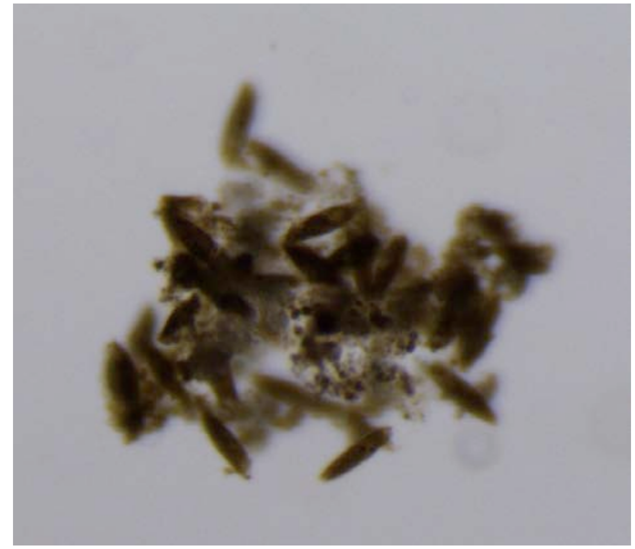


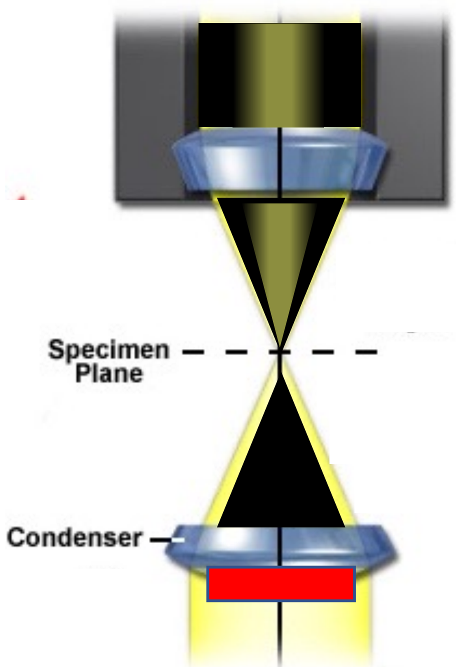
aggregate images: C. Durkin, *unpublished*

Illumination types (by analogy to microscopy)



- Brightfield (transmitted-light) microscopy
- Imaging Flowcytobot
- Flowcam





- Darkfield (scattered-light) microscopy
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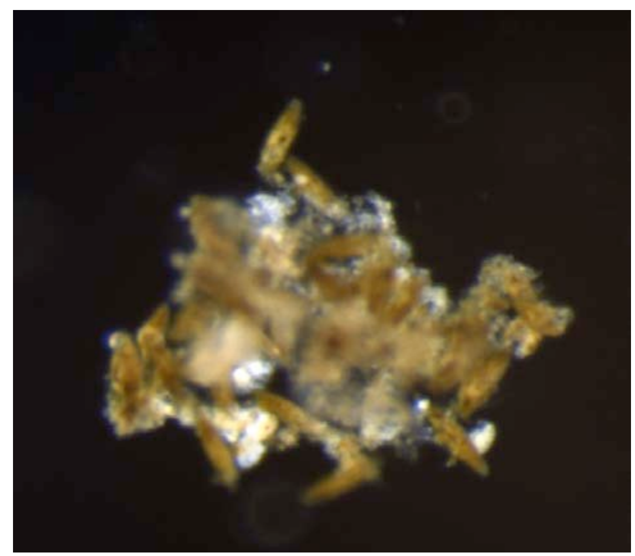
Also holography, line-scanning cameras...



aggregate images: C. Durkin, *unpublished*

Overview

✓Theory

- Instrumentation examples (major types, emphasis on systems in wide use)
- Particle detection & classification
- Challenges

Imaging Flowcytobot (Olson & Sosik, 2007)

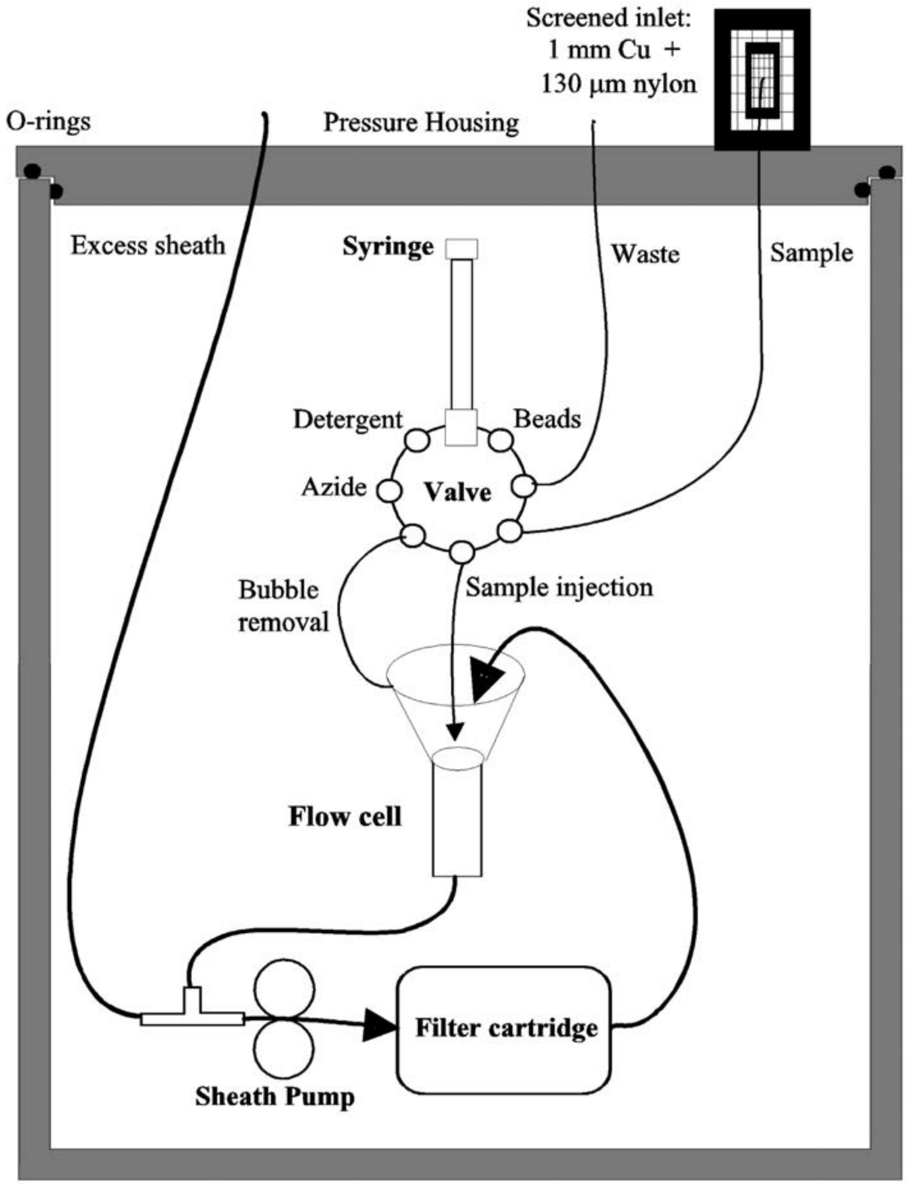


Fig. 2. Schema of fluidics system of Imaging FlowCytobot.

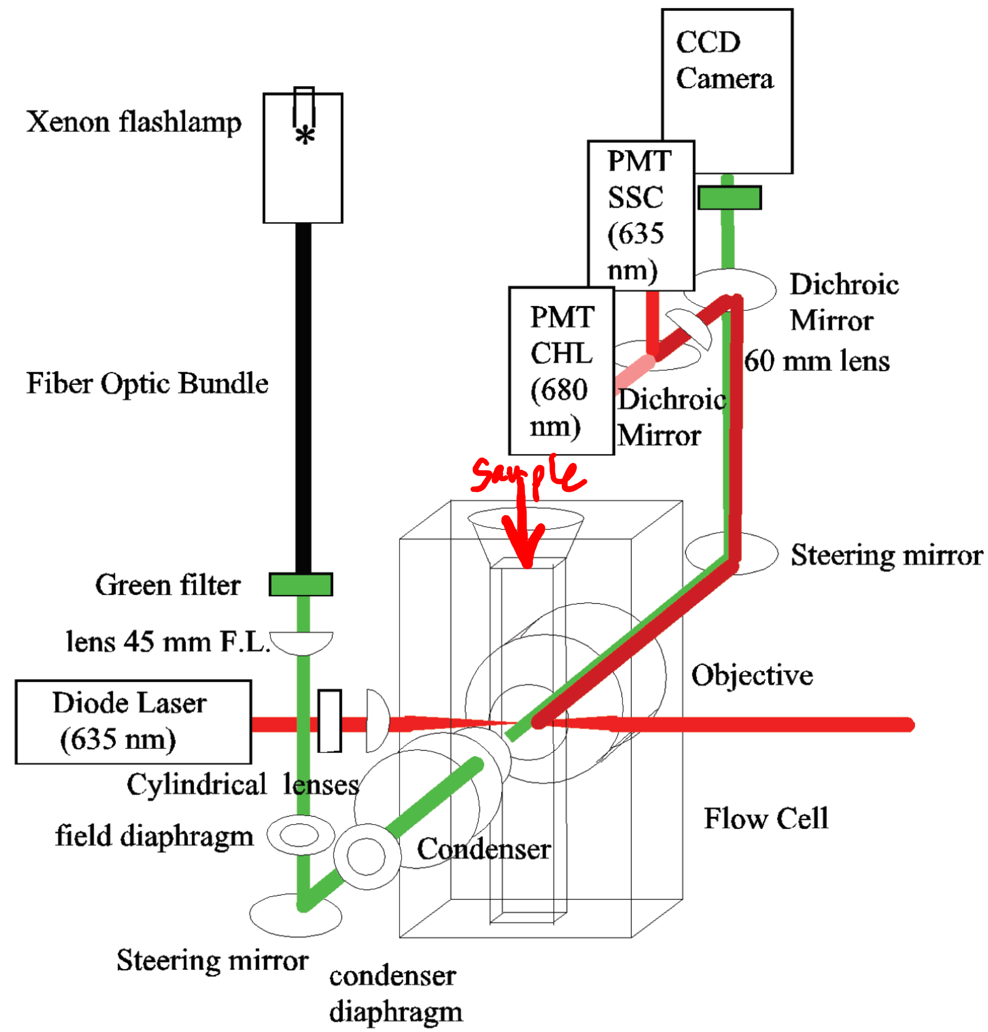
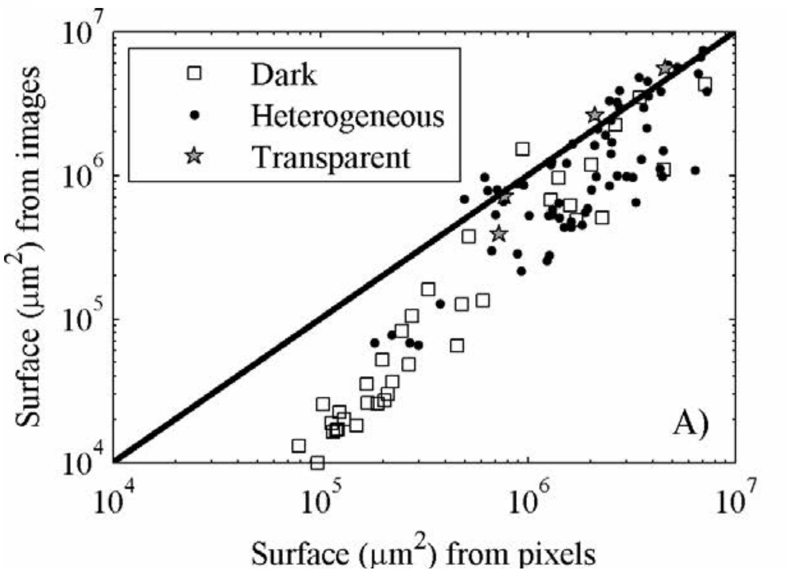


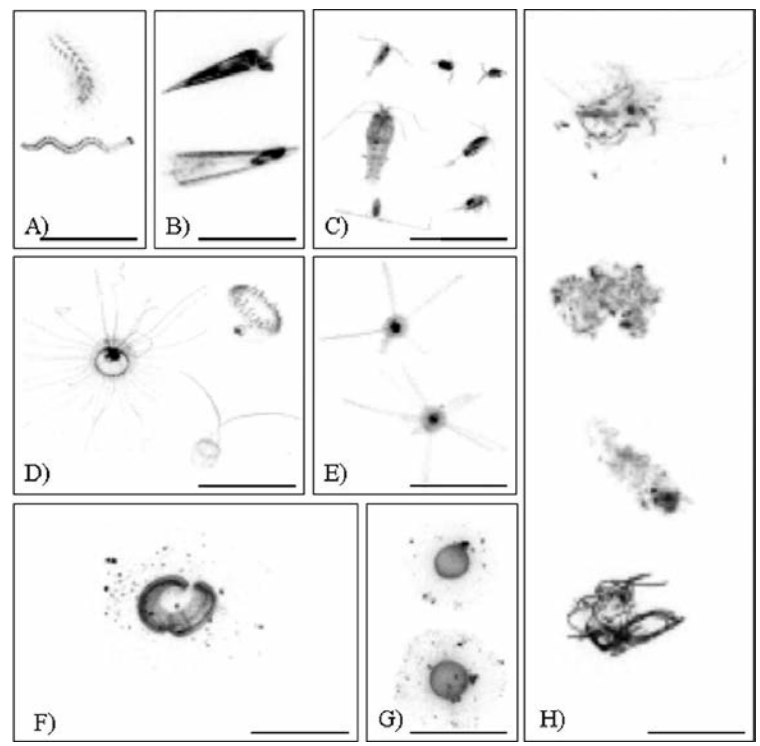
Fig. 3. Schema of optical layout of Imaging FlowCytobot.

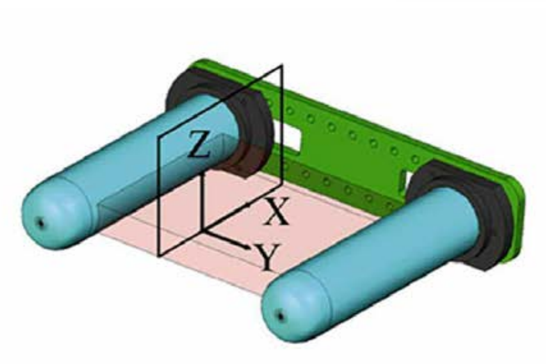
Underwater Vision Profiler 5 (Picheral et al., 2010)

- Sidescattered light detection
- Empirical imaged volume and pixel size calibrations required
- Particles below ~100 um not well-resolved
- Large (~1 L) sample volume/image



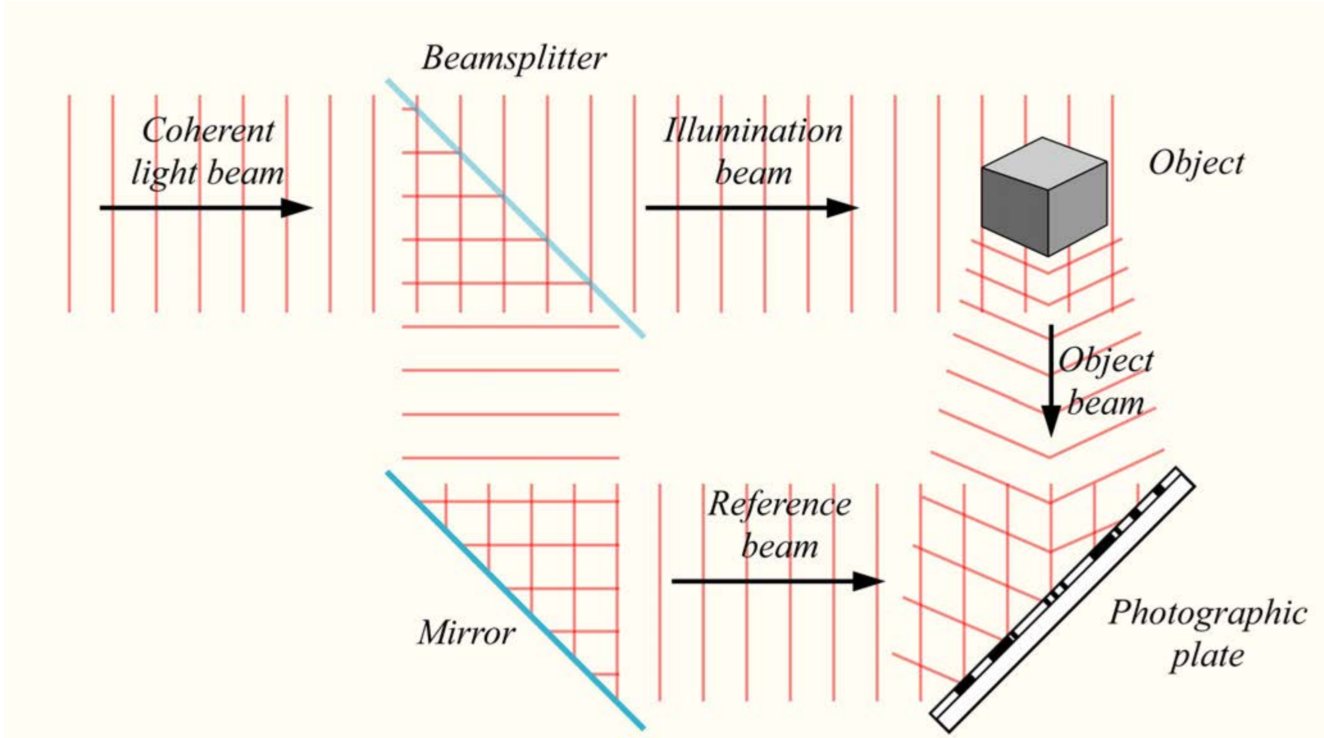
Thumbnails below:
scalebars = 5 mm





Holographic imaging - basics

- Sample volume illuminated by coherent, monochromatic light source
- Interference between diffraction pattern and the original, unscattered beam is recorded
- Computational reconstruction provides 3D image of particle size, shape, orientation



https://commons.wikimedia.org/w/index.php?curid=18103931

Off-axis holography

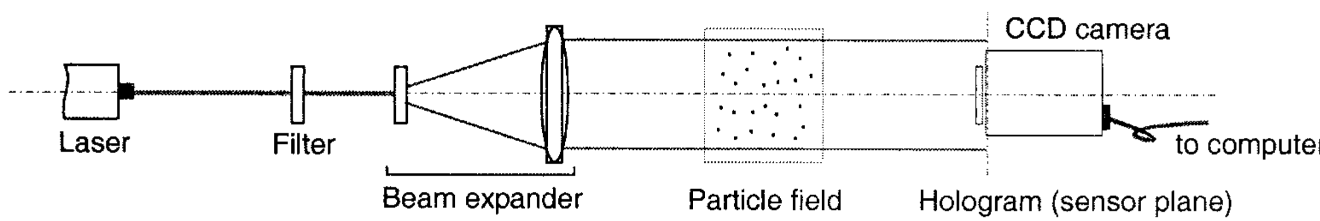
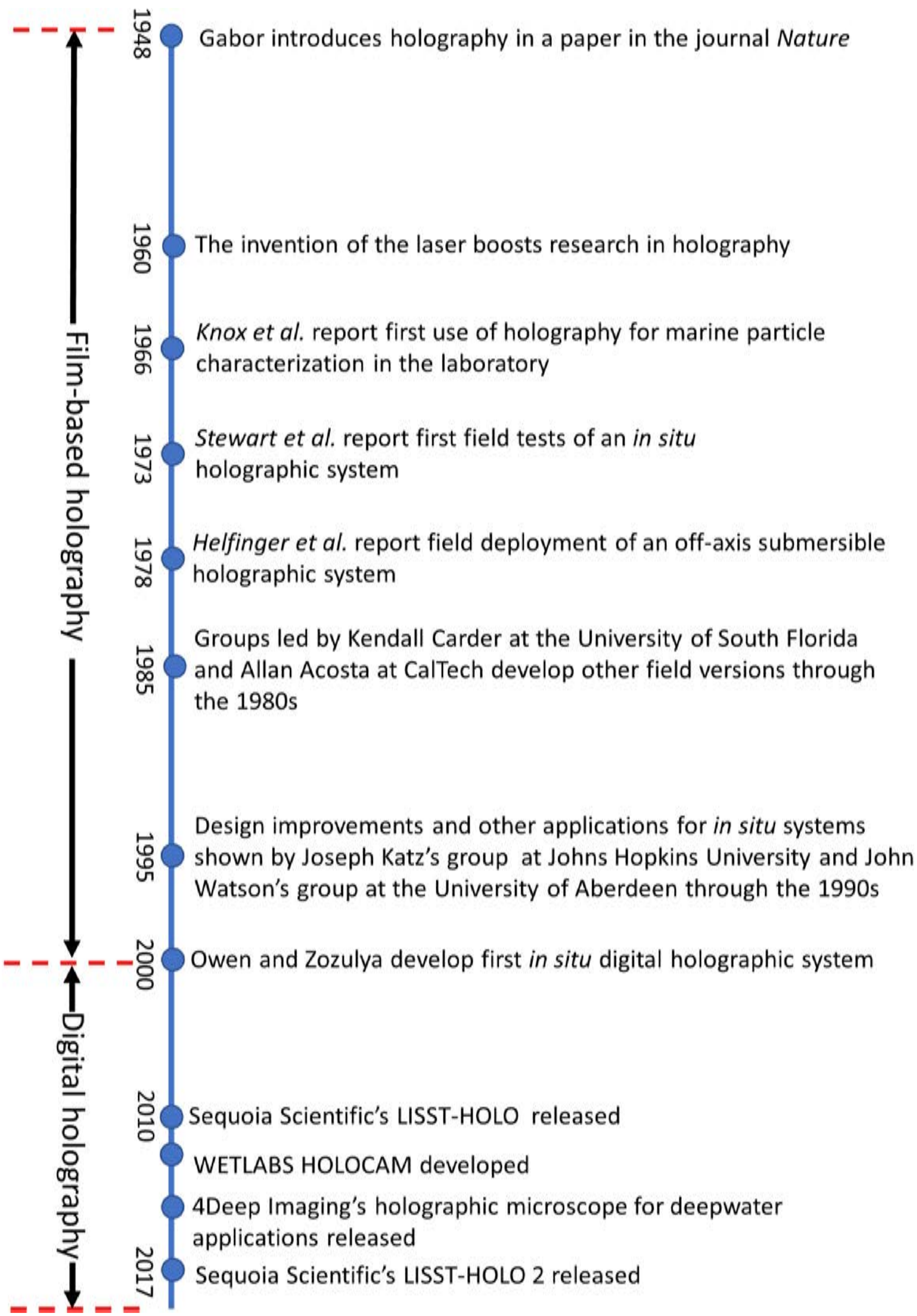


Fig. 1. Typical setup of digital holographic recording of a particle field based on in-line holography.

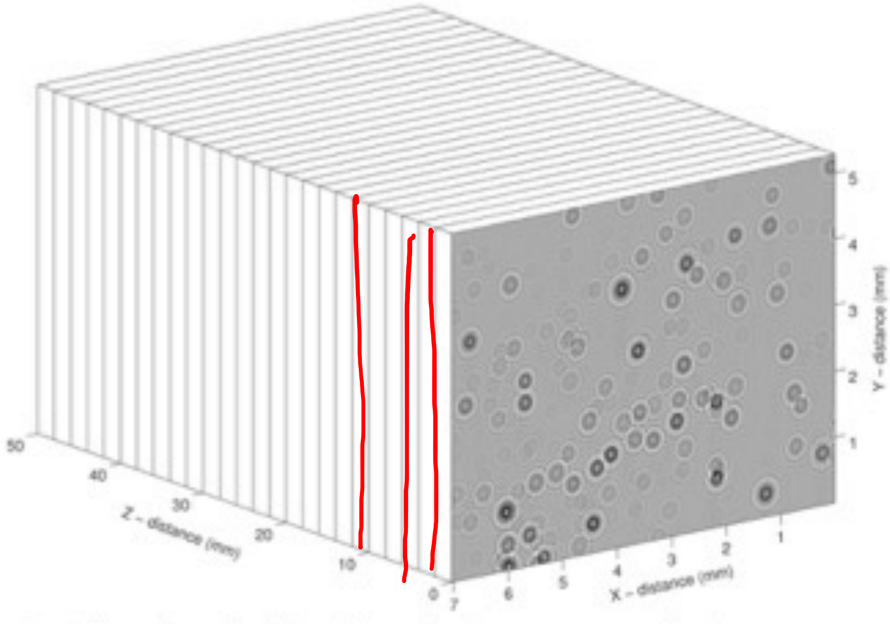
In-line holography; Pan and Meng 2003

Holographic imaging - basics



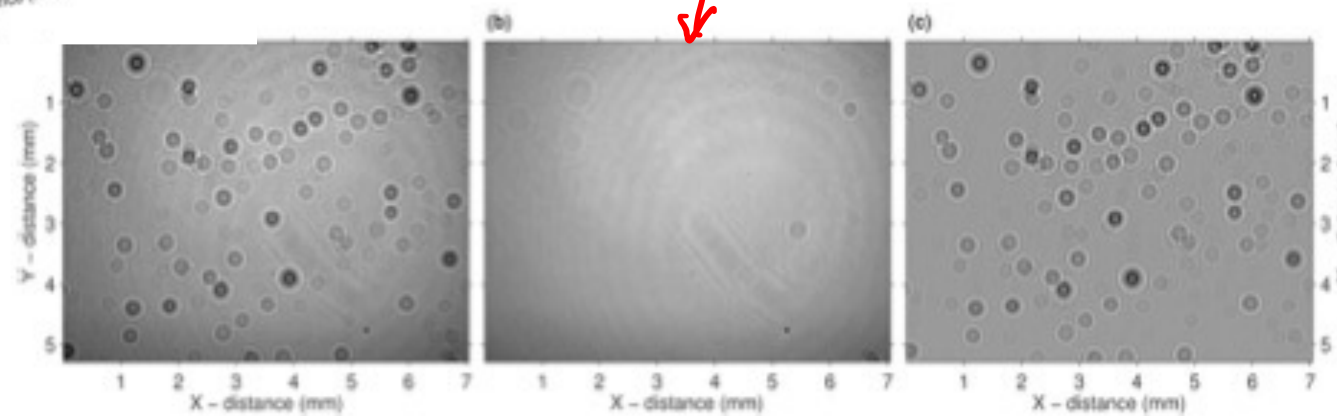
Nayak et al., 2021

Holographic image reconstruction (example)



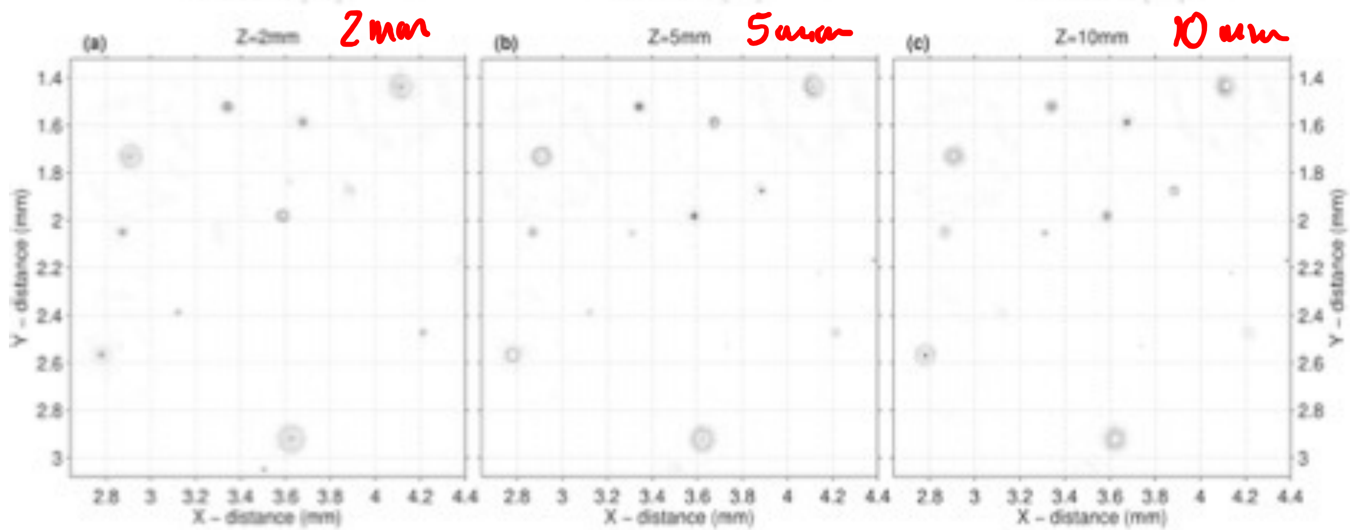
- Sample volume geometry (z axis is the optical axis)

background



- Background = average along z axis (or over some time interval)

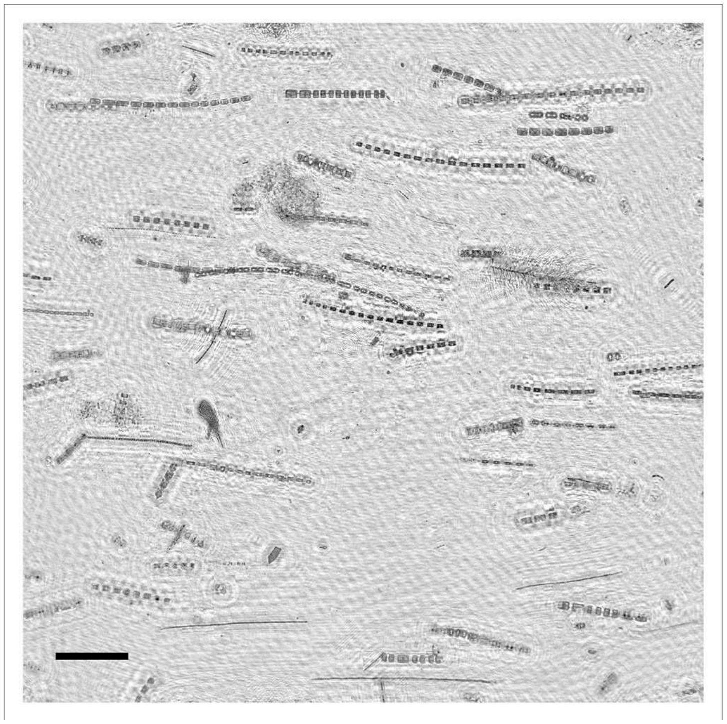
- Identify in-focus z-coordinate of particles and create composite 2D image

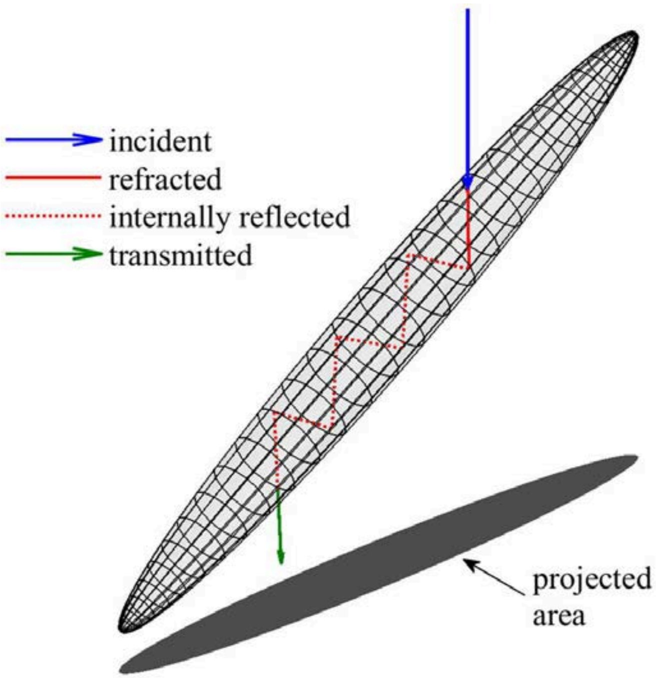


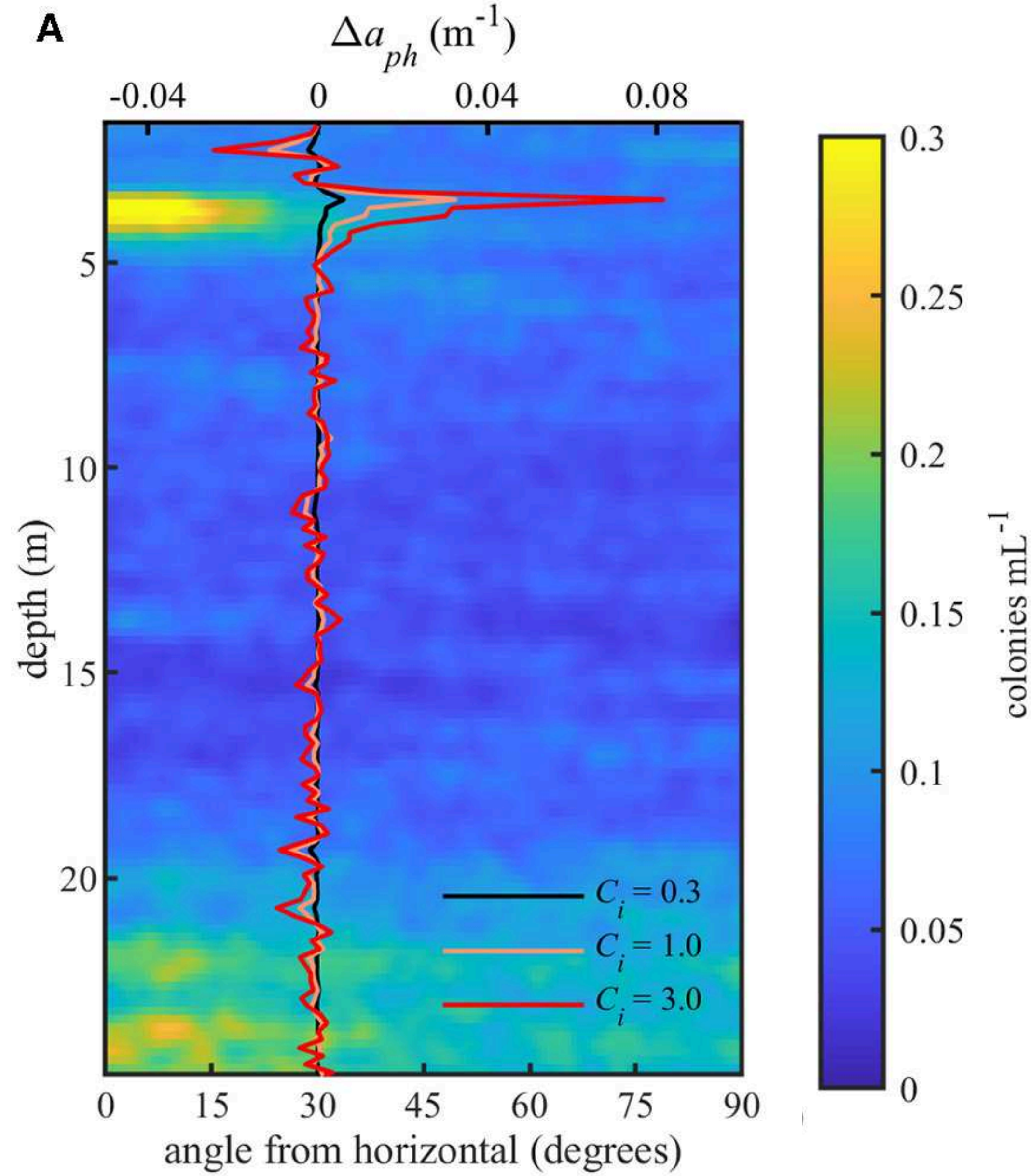
Figures: Davies et al., 2015

Does orientation matter?

- In situ digital holography
- *D. brightwellii* diatom colonies with preferential orientation increased a_ϕ by 4.5-24.5%.





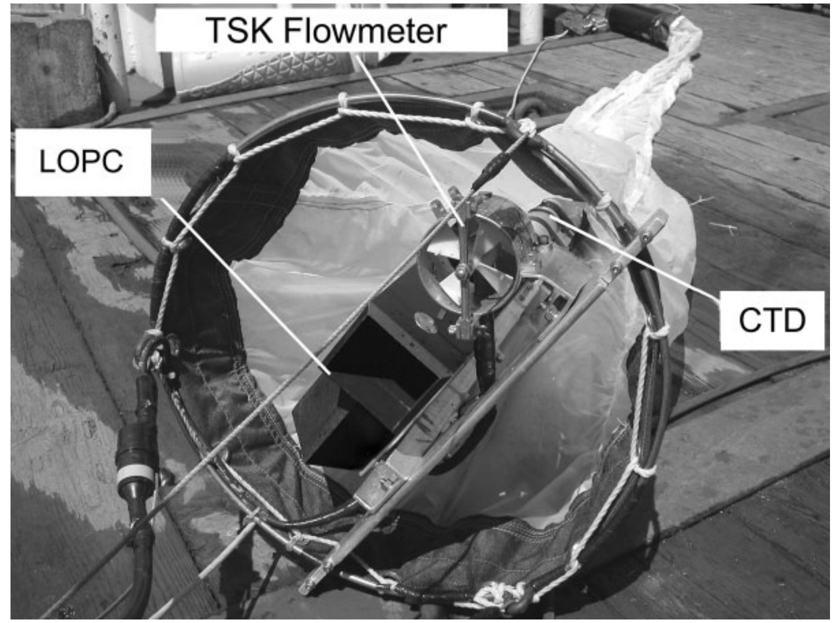


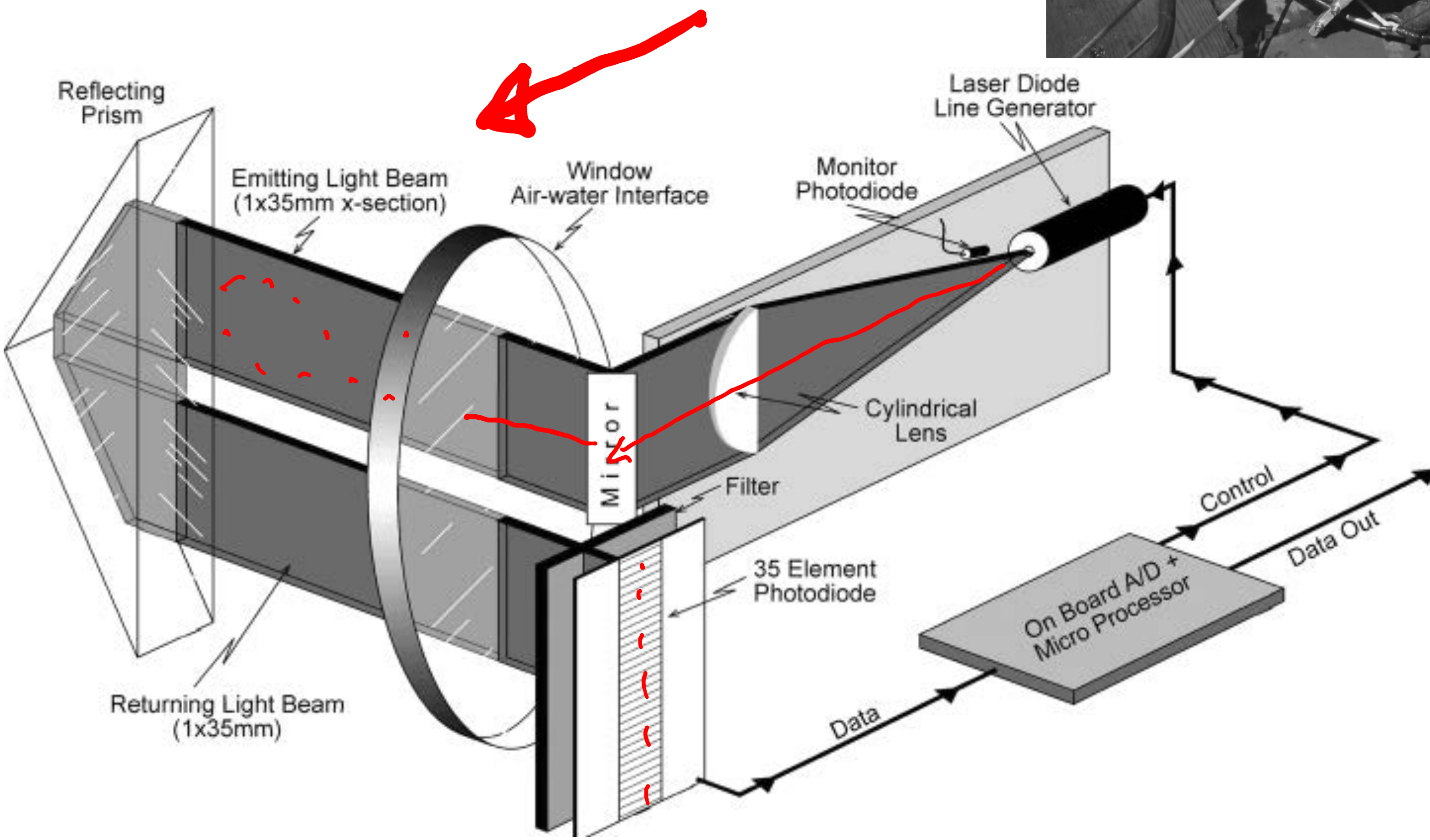
McFarland, et al. 2020

Line-scanning (shadowgraph) cameras

Laser Optical Plankton Counter (LOPC, Hermann et al. 2004)

- Simple optics (linear diode array detector)
- Relatively large depth of field/sampling volume
- Limited particle image detail





Line-scanning (shadowgraph) cameras

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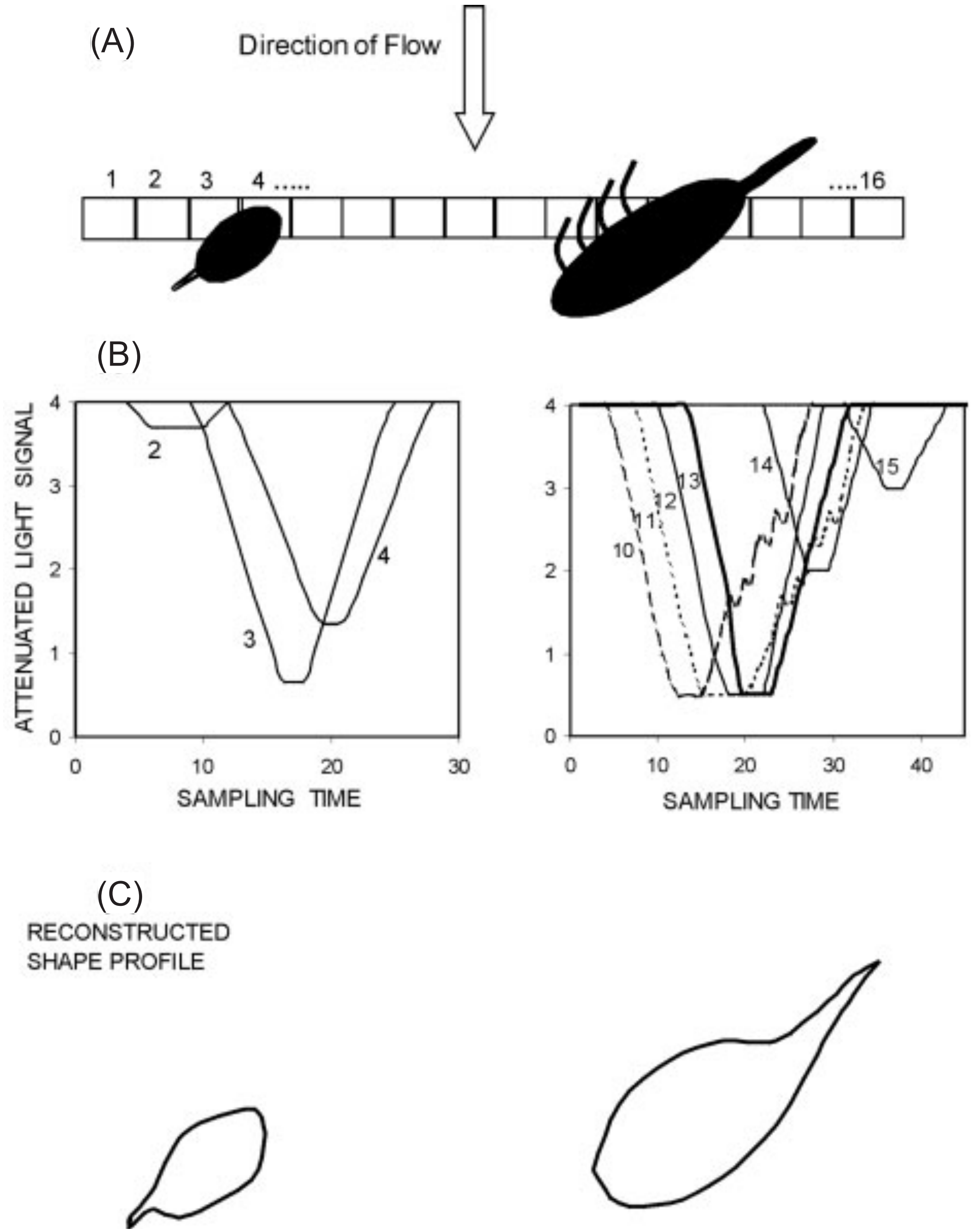


Line-scanning (shadowgraph) cameras

In Situ Icthyoplankton Imaging System (ISIIS; Cowen and Guigand, 2008)

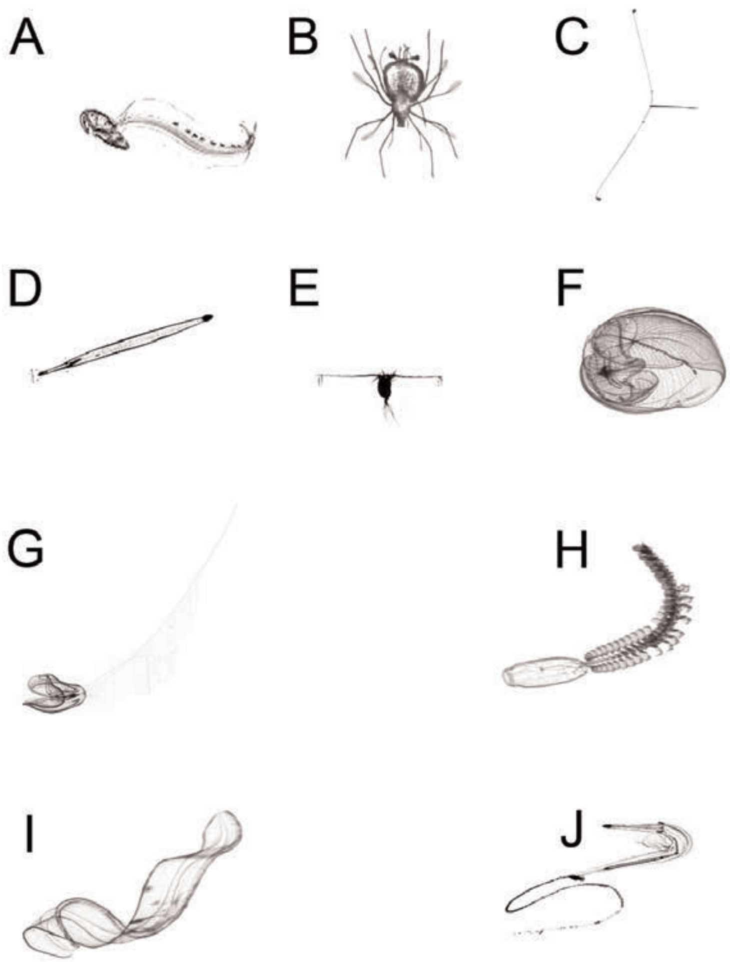


Fig. 4. In situ invertebrate zooplankters. 0–40 m depth, Florida current. Selected images of invertebrate plankton captured via *ISIIS*. Organisms are not scaled to each other in this composite image; sizes range from a few millimeters to several centimeters. A. Larvacean (*Oikopleura sp.*). B. Scyllarid lobster larva. C. Unidentified larval crustacean (?). D. Chaetognath. E. Copepod with eggs. F. Ctenophore. G. Ctenophore with feeding tentacles extended. H. Aggregate phase Thaliacean salp with reproductive buds. I. Ctenophore (*Velamen sp.*). J. Pterotracheid heteropod.

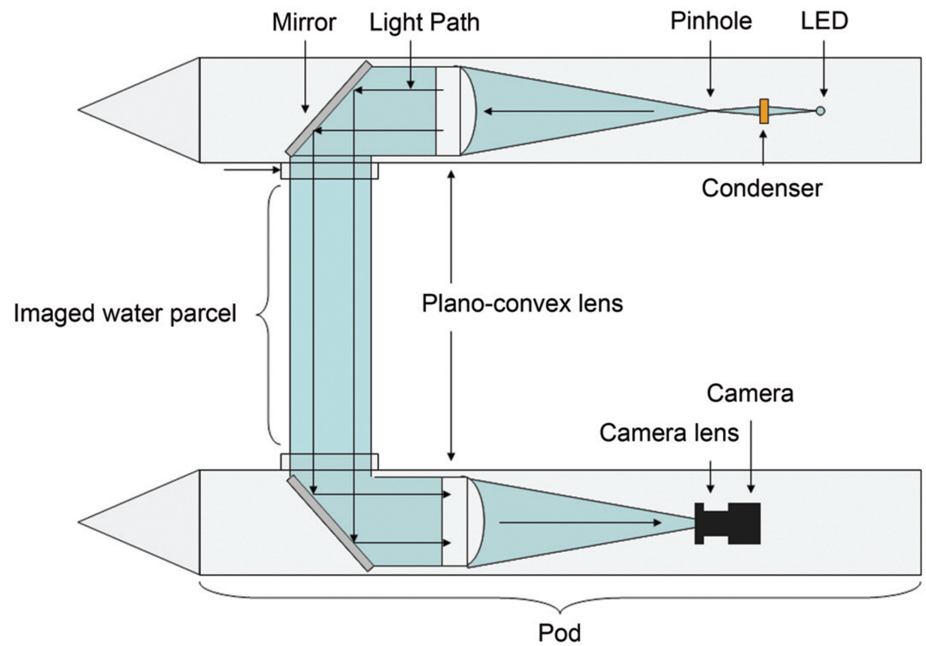


Fig. 1. Light scheme using shadowgraph technique. Light passes through plano-convex lens, thereby establishing a collimated light beam. The advantages of this approach over other lighting techniques include high depth of field (20+ cm), telecentric image (magnification level not affected by distance from object to the lens), and very sharp outlines of organisms and internal structures (facilitates automated recognition).

- Very large sampling volume (70 L/s at 2.5 m/s tow speed)
- Ability to observe large, fragile organisms *in situ*

Overview

- ✓ Theory
- ✓ Instrumentation examples (major types, emphasis on systems in wide use)
- Particle detection & classification
- Challenges

Particle detection and classification

1. Find the particles

2. Measure and identify the particles

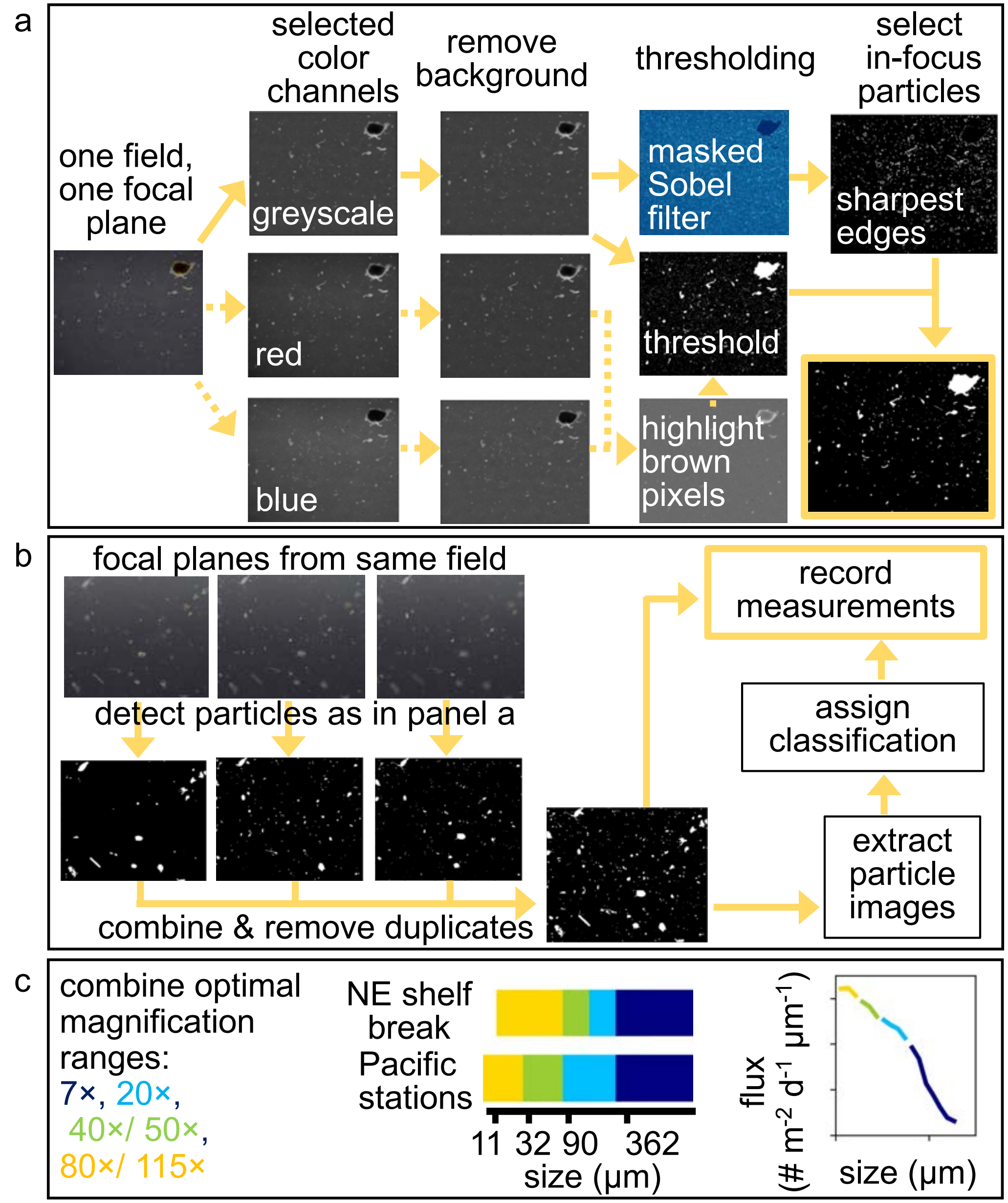
3. Interpret the data

Particle detection and classification

1. Find the particles

2. Measure and identify the particles

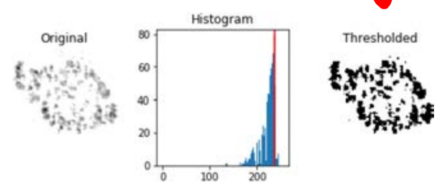
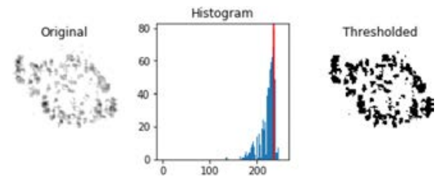
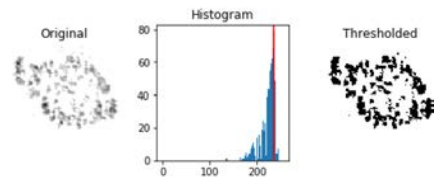
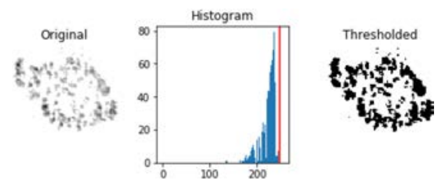
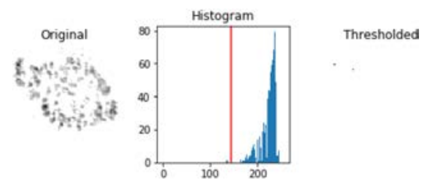
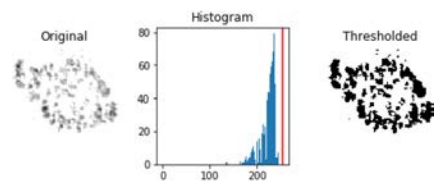
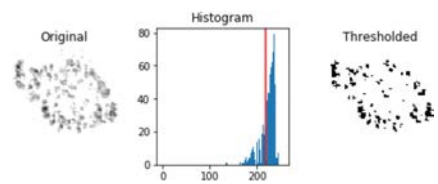
3. Interpret the data



Figure, Durkin et al., *in revision*

Segmentation: differentiating between background and particle

TABLE 1 | Threshold algorithms.

Type	Name	Example	Description and reference
Threshold (Reference)	Otsu		Finds threshold that minimizes the intra-class variance Ref: Otsu, 1979
Threshold	Isodata		Takes an initial threshold and averages the pixels below and above the threshold. The averages of these two values are calculated. Threshold is incremented and the process is repeated until the threshold is larger than the composite average Ref: Ridler and Calvard, 1978
Threshold	Li		Implements minimum cross entropy Ref: Li and Tam, 1998
Threshold	Mean		Uses the mean of gray levels as the threshold Ref: Glasbey, 1993
Threshold	Minimum		Assumes bimodal histogrammes Ref: Prewitt and Mendelsohn, 1966
Threshold	Triangle		Uses a geometric method assuming a maximum peak (mode) near one end of the histogram and searches toward the other end Ref: Zack et al., 1977
Threshold	Yen		Uses two criteria: the discrepancy between the thresholded and original image, and the number of bits required to represent the thresholded image Ref: Yen et al., 1995

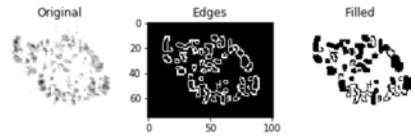
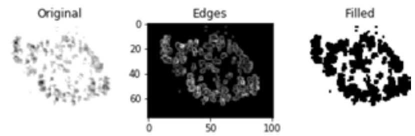
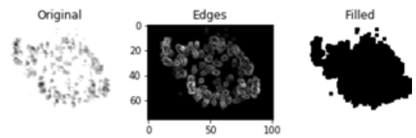
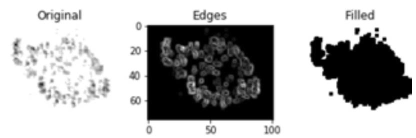
Threshold algorithms

Giering et al, 2020a

Segmentation: differentiating between background and particle

Table, continued...
Edge detection algorithms

TABLE 1 | Threshold algorithms.

Type	Name	Example	Description and reference
Edge	Canny		First smoothes the image using Gaussian convolution and then highlights regions with high first spatial derivatives (edges) using a 2D gradient operator similar to Roberts Ref: Canny, 1986
Edge	Roberts		Performs 2D spatial gradient measurements by passing two 2 × 2 convolution masks along the image Ref: Roberts, 1963
Edge	Scharr		Variation of Sobel algorithm Ref: Scharr, 2000
Edge	Sobel		Performs 2D spatial gradient measurements by performing convolution between two 3 × 3 kernels and the image Ref: Sobel and Feldman, 1973

Giering et al, 2020a

Segmentation: differentiating between background and particle

Table, continued... Edge detection algorithms

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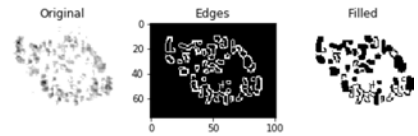
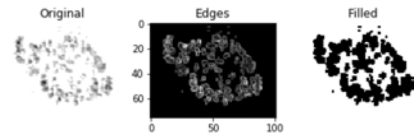
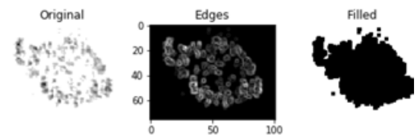
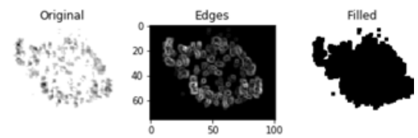
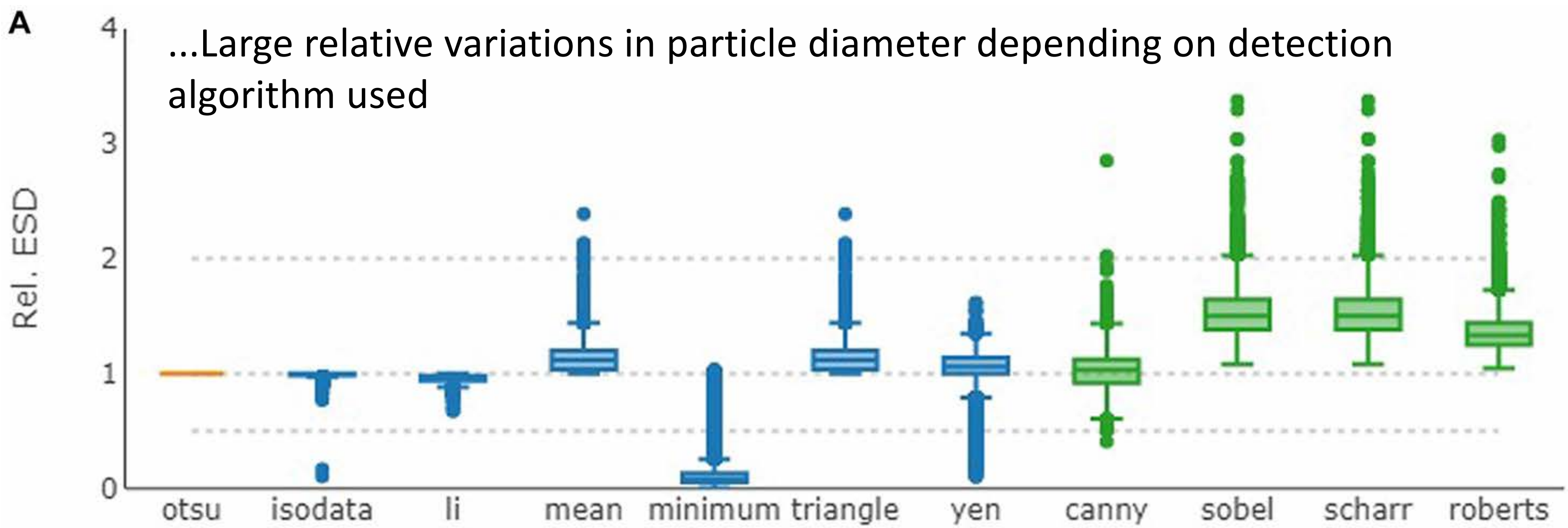
Type	Name	Example	Description and reference
Edge	Canny		First smoothes the image using Gaussian convolution and then highlights regions with high first spatial derivatives (edges) using a 2D gradient operator similar to Roberts Ref: Canny, 1986
Edge	Roberts		Performs 2D spatial gradient measurements by passing two 2 × 2 convolution masks along the image Ref: Roberts, 1963
Edge	Scharr		Variation of Sobel algorithm Ref: Scharr, 2000
Edge	Sobel		Performs 2D spatial gradient measurements by performing convolution between two 3 × 3 kernels and the image Ref: Sobel and Feldman, 1973

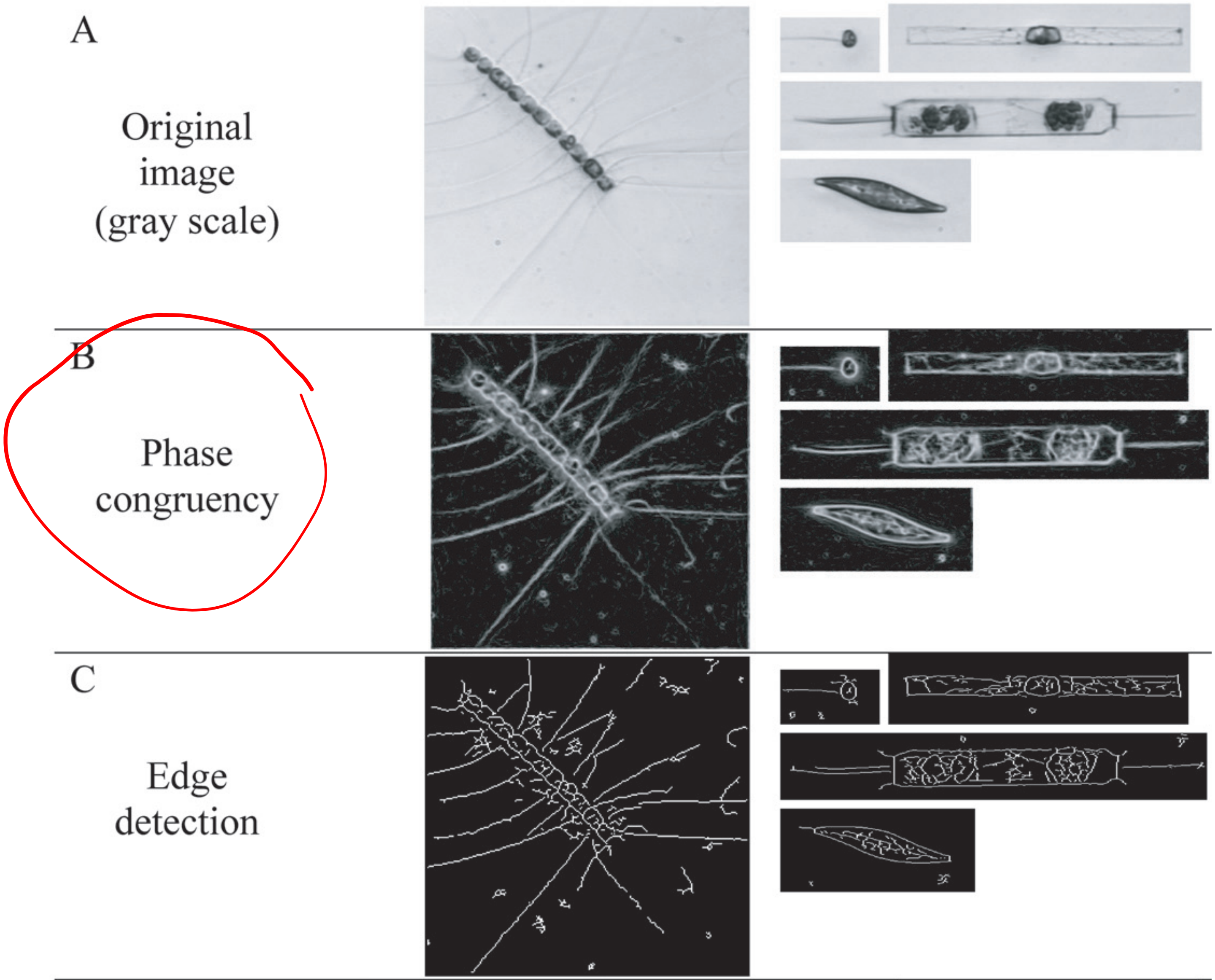
...Large relative variations in particle diameter depending on detection algorithm used



Giering et al, 2020a

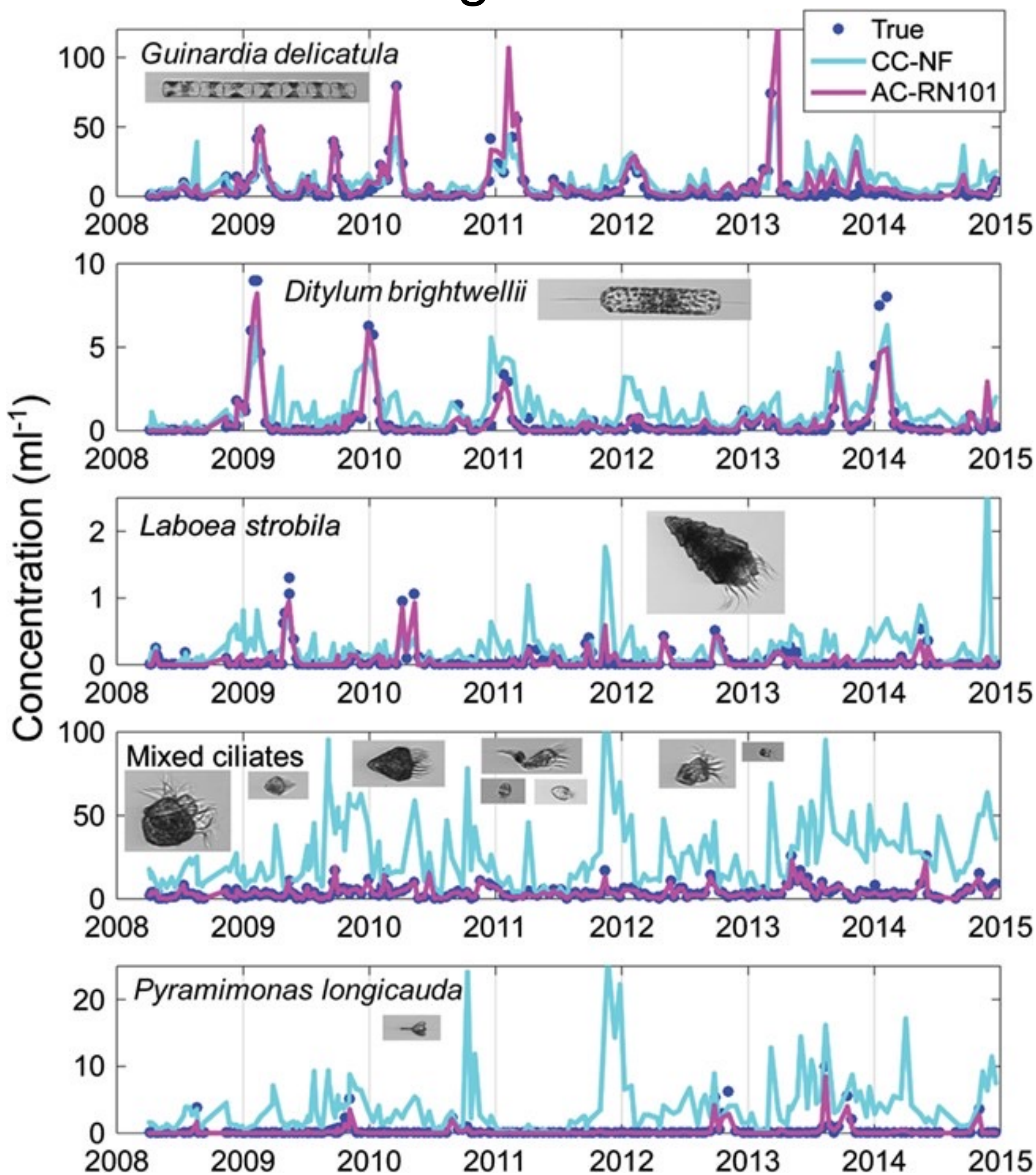
Segmentation: differentiating between background and particle

IFCB analysis – phase congruency calculation step (although computationally intensive) improves performance of threshold-based edge detection



Sosik and Olson, 2007

Particle identification – machine learning



Gonzàlez et al., 2019

Particle identification – machine learning

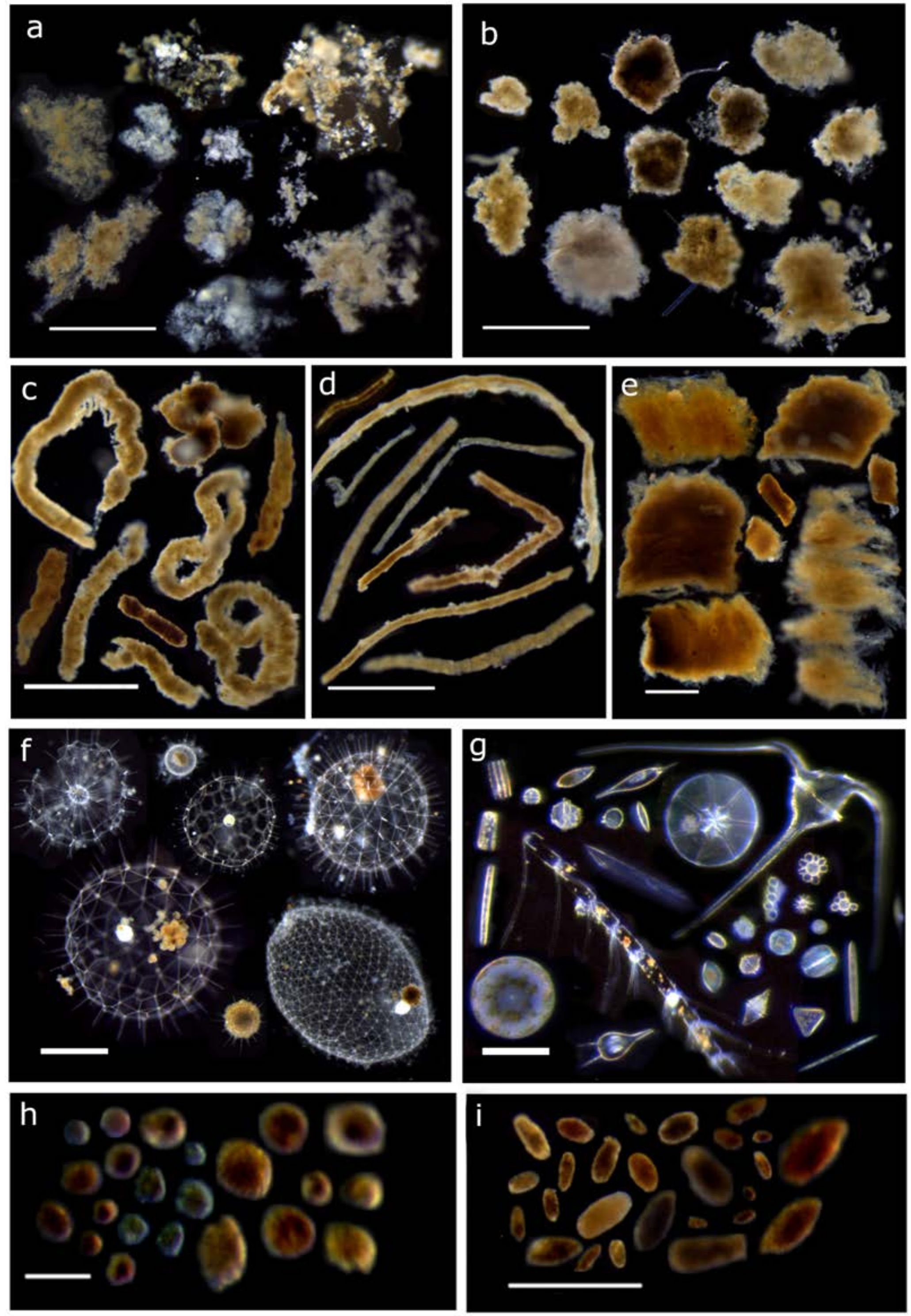
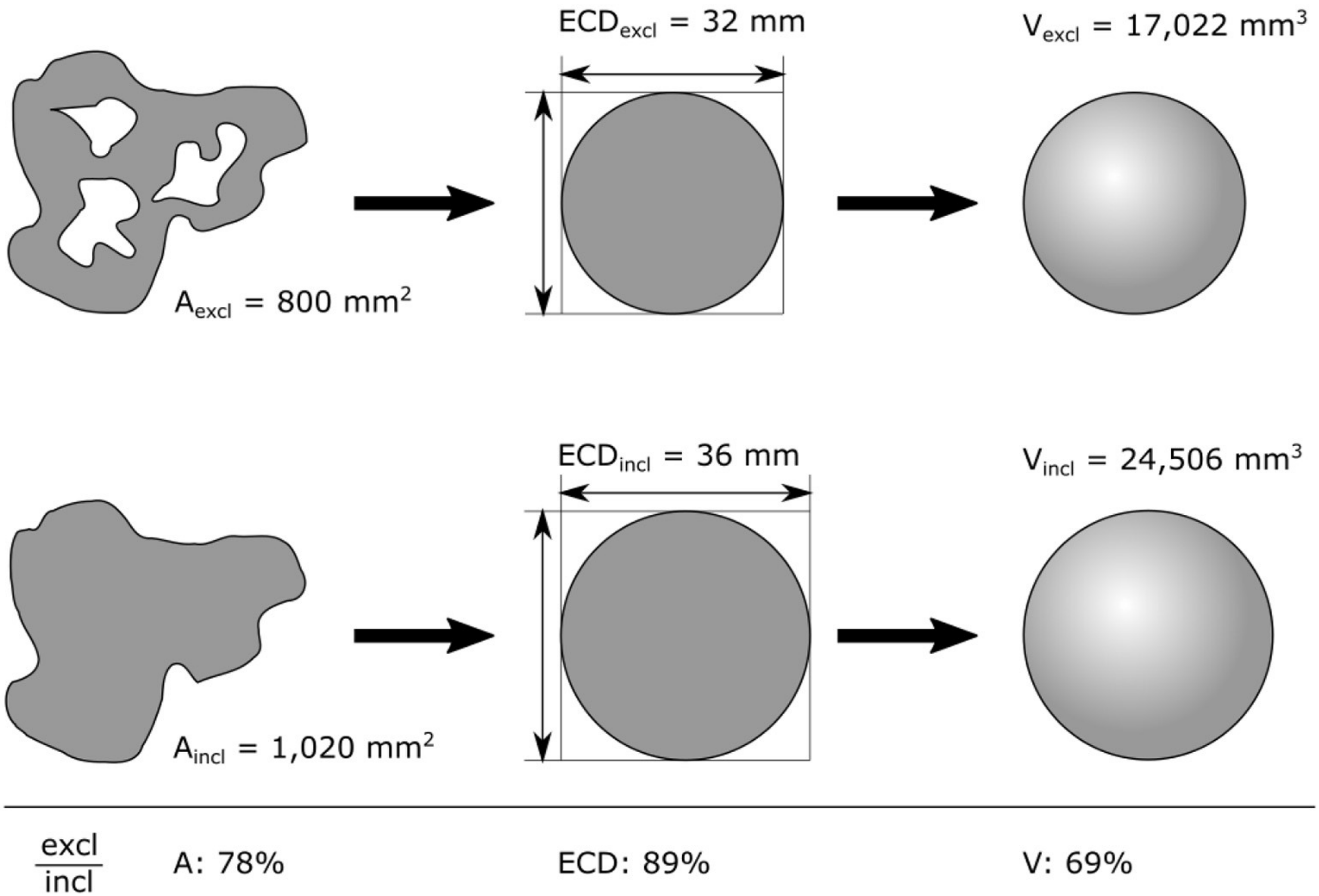


Figure: Classified particles collected in sediment traps

What if classes are not distinct from one another?

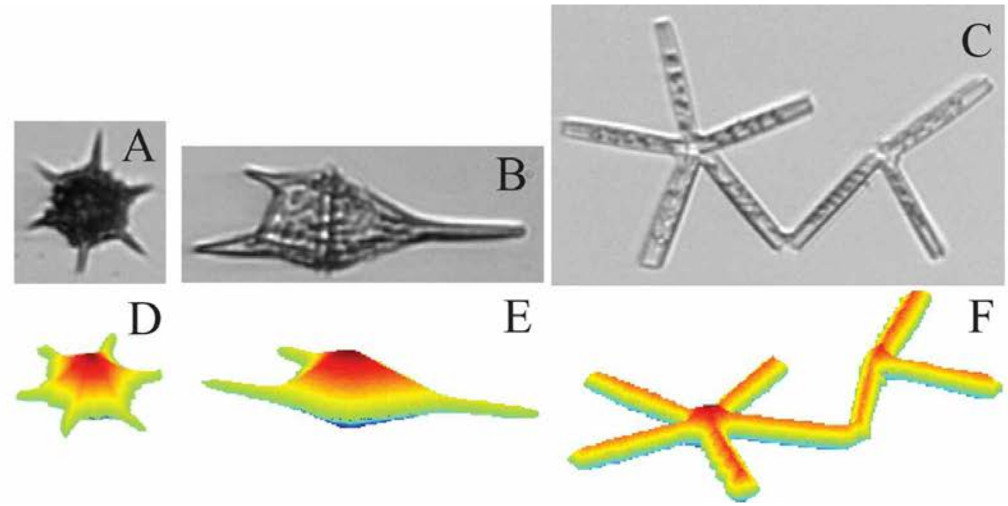
Durkin et al., *in revision*

Computing particle volume (or carbon) from image area



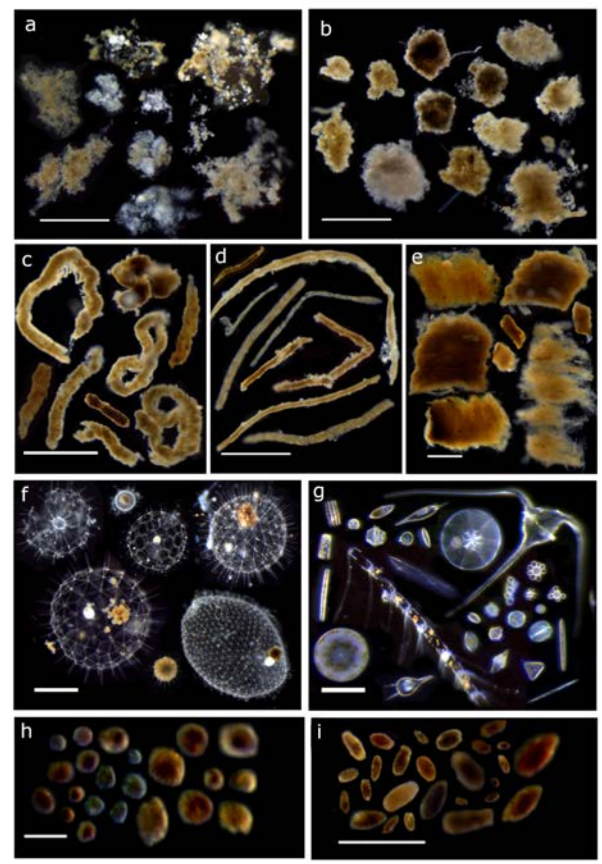
Giering et al., 2020b

Computing particle volume (or carbon) from image area



Moberg and Sosik, 2012

Classification	Shape	width	length	Volume	$C = A \times V^B$ (Equation 2)		
					A	B	ref
aggregate	sphere	$w = ESD$	$l = ESD$	$V = \frac{4}{3} \times \pi \times (\frac{ESD}{2})^3$	0.1×10^{-9}	0.8	1
dense detritus	sphere	$w = ESD$	$l = ESD$	$V = \frac{4}{3} * \pi \times (\frac{ESD}{2})^3$	0.1×10^{-9}	0.83	1
large loose fecal pellet	cylinder	$w = \frac{553 \times ESD}{ESD + 996}$	$l = \frac{\pi \times (\frac{ESD}{2})^2}{w}$	$V = l \times \pi \times (\frac{w}{2})^2$	0.1×10^{-9}	0.83	1
long fecal pellet	cylinder	$w = \frac{187 \times ESD}{ESD + 424}$	$l = \frac{\pi \times (\frac{ESD}{2})^2}{w}$	$V = l \times \pi \times (\frac{w}{2})^2$	0.1×10^{-9}	1	1
short fecal pellet	ellipsoid	$w = 0.54 \times ESD$	$l = \frac{ESD^2}{w}$	$V = \frac{4}{3} \times \frac{l}{2} \times \pi \times (\frac{w}{2})^2$	0.1×10^{-9}	1	1
mini pellet	sphere	$w = ESD$	$l = ESD$	$V = \frac{4}{3} \times \pi \times (\frac{ESD}{2})^3$	0.1×10^{-9}	1	1
salp fecal pellet	cuboid	$w = 0.63 \times ESD$	$l = \frac{\pi \times (\frac{ESD}{2})^2}{w}$	$V = l \times w \times \frac{w}{4}$	0.04×10^{-9}	1	2,3
rhizaria	sphere	$w = ESD$	$l = ESD$	$V = \frac{4}{3} \times \pi \times (\frac{ESD}{2})^3$	0.004×10^{-9}	0.939	4,5
phytoplankton	sphere	$w = ESD$	$l = ESD$	$V = \frac{4}{3} \times \pi \times (\frac{ESD}{2})^3$	0.288×10^{-9}	0.811	4



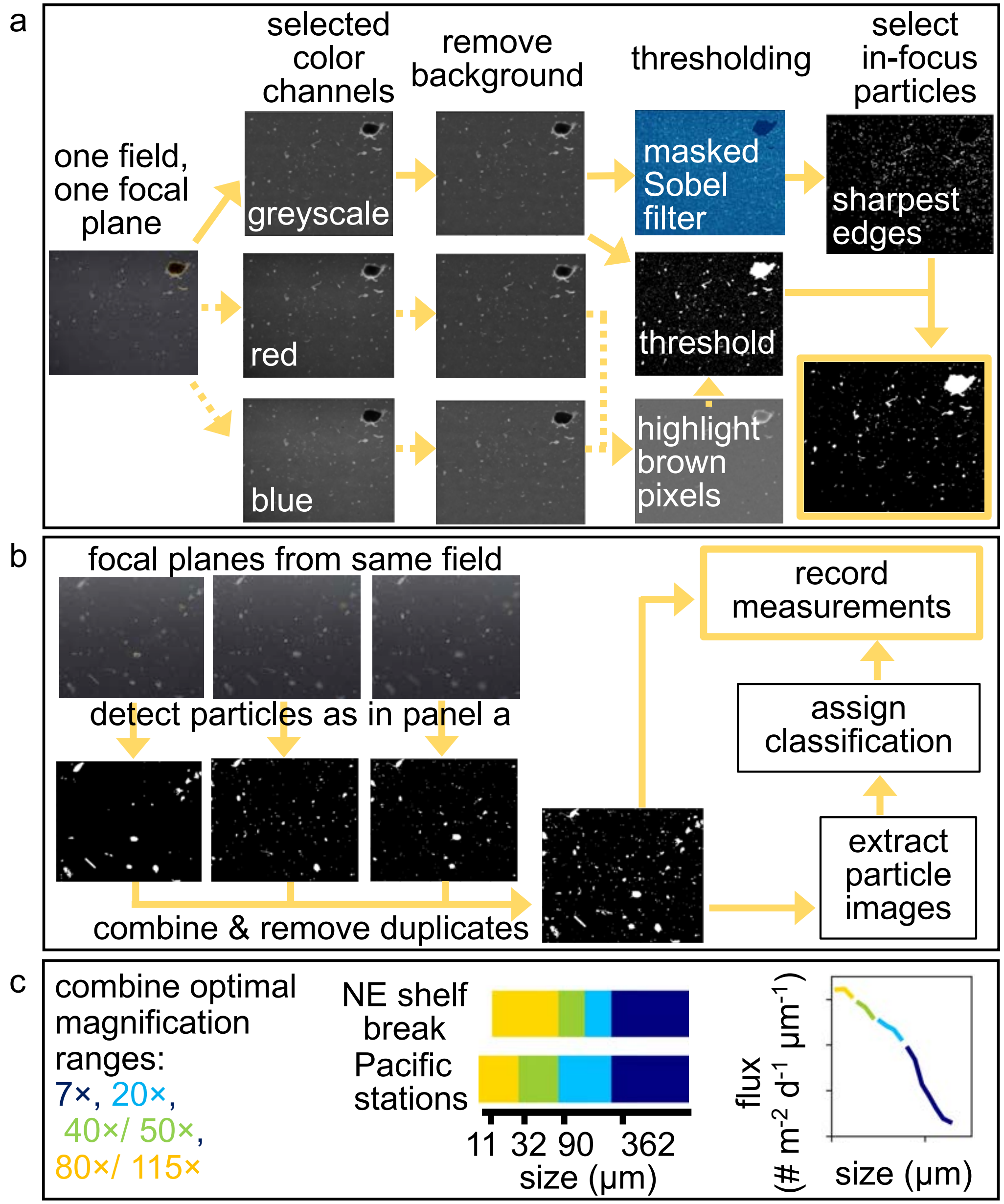
Durkin et al., *in revision*

Particle detection and classification

1. Find the particles

2. Measure and identify the particles

3. Interpret the data



Figure, Durkin et al., *in revision*

Take-homes

- Imaging techniques provide important information about particle processes, validation for ocean color models
- Needs for the future: standards; shared details of image analysis methods; classification tools
- Collaboration across groups, funding sources, international community