Abiotic Formation of Particles from the DOM Pool in Kaneohe Bay Water

Chanteal Craft, C-MORE Scholar Trainee (Fall 2008)

C-MORE: Center for Microbial Oceanography Research and Education

What is C-MORE?

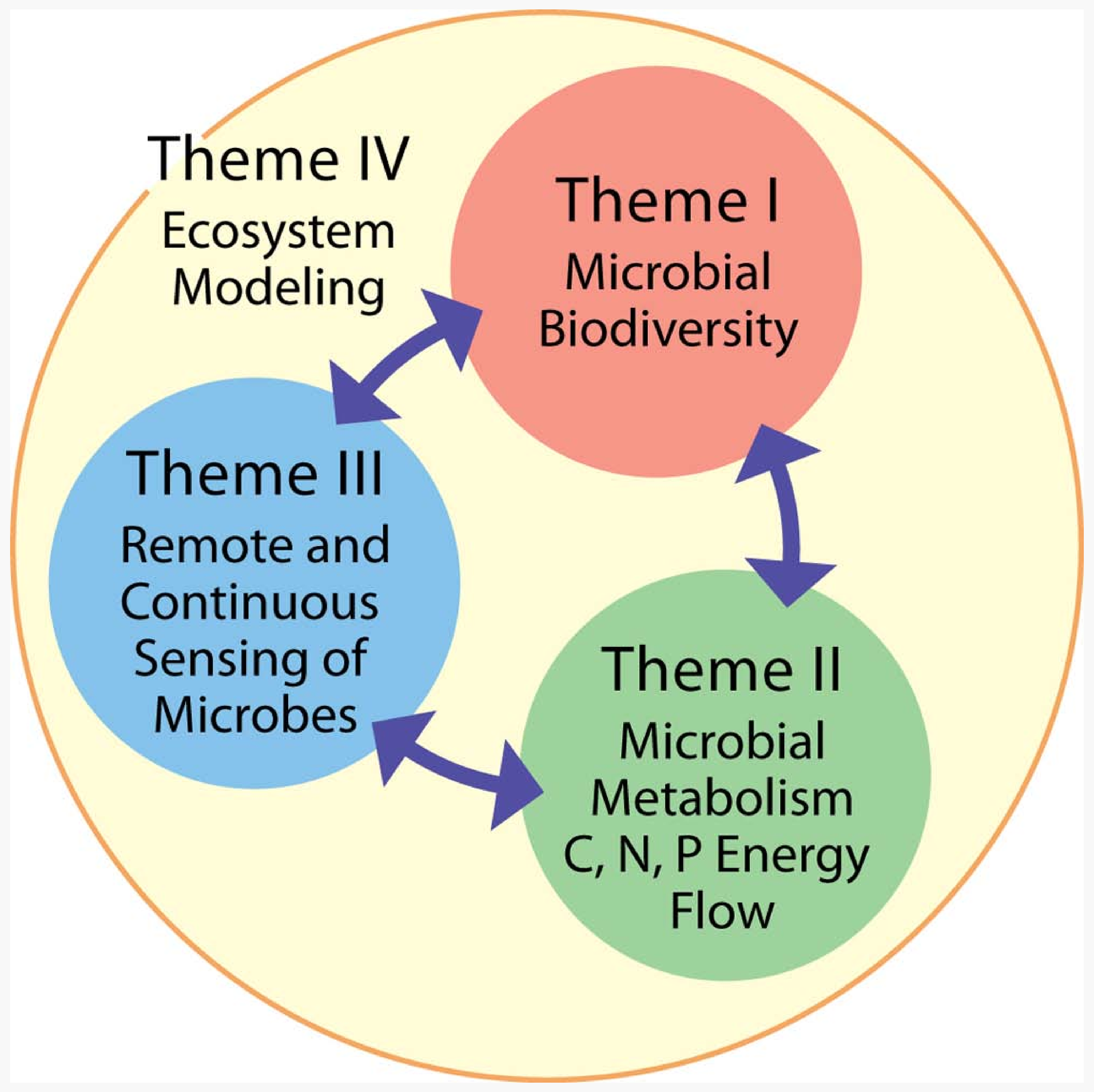
C-MORE is a National Science Foundation (NSF) sponsored science and technology center. Founded in 2006 it brings scientists, undergraduate students on research scholarships, and educators together through research and educational programs to further understand the biological and ecological diversity of marine micro- organisms.

What is Microbial Oceanography?

Microbial Oceanography studies the role of microorganisms in the structure and function of marine ecosystems . Microbes, very small organisms less than 100 micrometers (µm), are the most abundant organisms on Earth.

What are C-MORE's main research focuses?

C-MORE focuses its research on four main themes; Microbial biodiversity, microbial metabolism, remote and continuous sensing or microbes an ecosystem modeling.

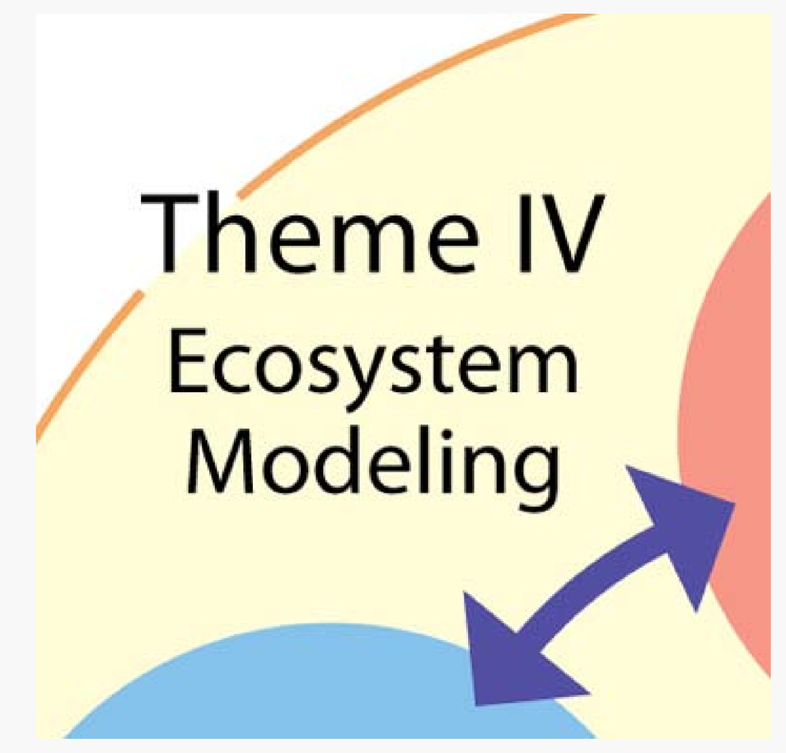


Picture Credit: http://cmore.soest.hawaii.edu

What is C-MORE's Scholars Program?

The Scholar's program is an undergraduate research scholarship comprised of 3 levels: fellowships, internships, and trainee. Trainee's are typically freshman or sophomores who learn basic research skills, scientific concepts and techniques while working under a mentor.

Trainee Research: Theme IV



Project Overview:

The possible formation of particles abiotically (without a biological intermediate) was tested by collecting surface water from Kaneohe Bay, Hawaii and pre-filtered to a nominal pore size of 0.2um. Pre-filtered water was then poisoned (0.02%w/v Sodium Azide) before it was incubated in 50L glass carboys for 76 days. Sub-samples were collected at 5 different time-points to observe any changes in particles >0.2um size.

Trainee Overview:

" The Abiotic Formation of Particles from the DOM pool in Kaneohe Bay Water" was designed and researched by Courtney Daniels, a masters student at the University of Hawaii at Manoa. After samples were collected Chanteal Craft, a Fall 2008 C-MORE trainee, aided the project by collecting images using an Epifluorescent microscope, processing data using the computer software programs "Image J" and assisted in analyzing data results using the program MATLAB. As a C-MORE trainee, Chanteal assisted the ongoing research project to learn scientific concepts and techniques. Before aiding in the project relevant background information was first researched.

Background Information

• **Classic & Microbial Food Web:**
The Classic Food Web links tropic relationships of a community. Primary Producers create their own energy . Photosynthetic organisms, the lowest level of the food chain, make organic material from inorganic nutrients. A loss of organic material occurs by exudation and excretion as the organic materials are passed to higher trophic levels. But what is consuming these degraded consumers and the organic material produced by photosynthetic organisms?

The "Microbial Food Web" accounts for the consumption of organic material. Microbes consume organic material as an energy source.

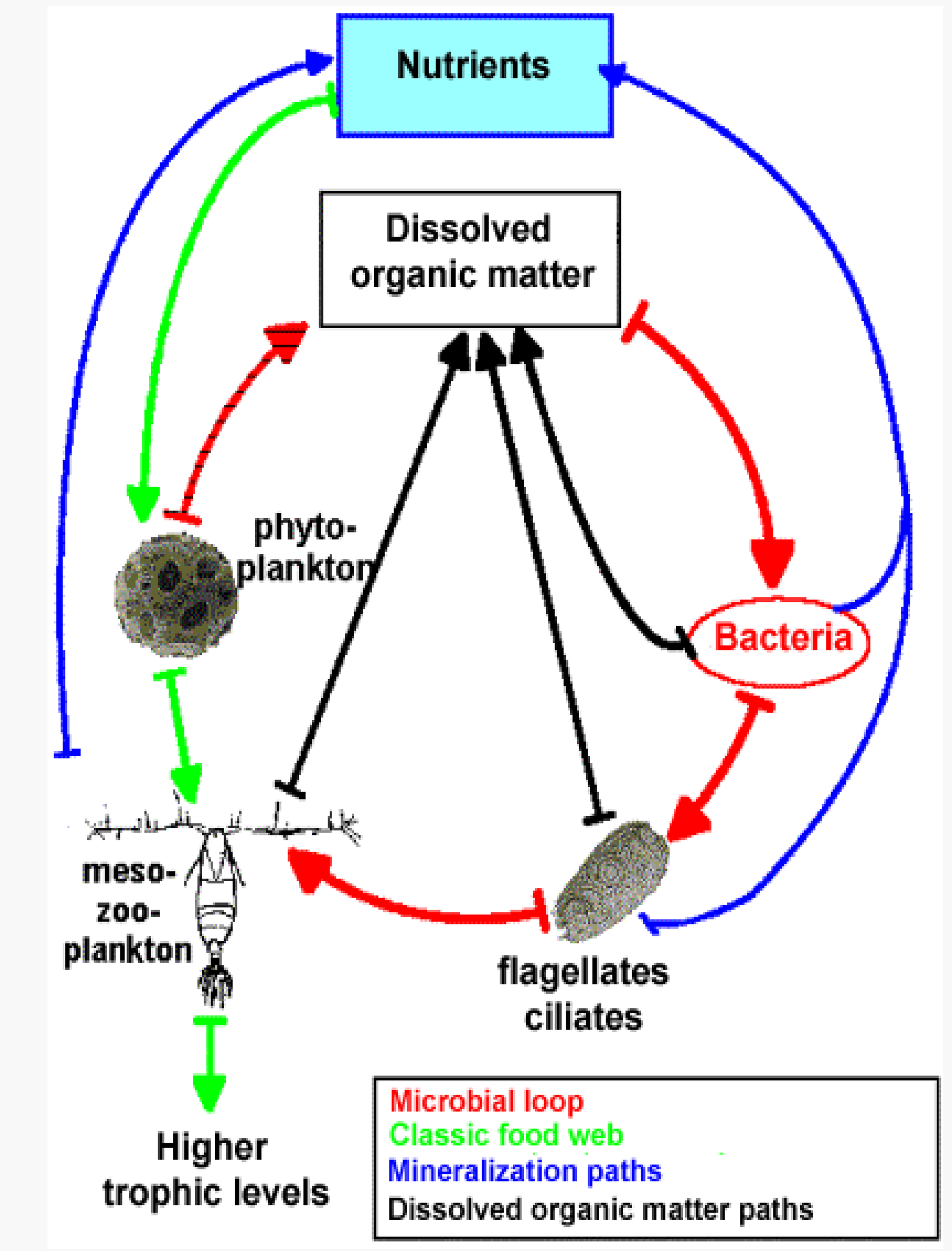


Photo Credit: http://www.com.univ-mrs.fr/IRD/atollpol/commatoll/images/ukbactp1.gif

• **Detridal DOM:**
Dissolved Organic Material (DOM) are sub-micron (10^7 - 10^{10} particles ml^{-1}) waste particles, including colloids, produced by phytoplankton and bacteria. Dissolved organic matter is considered dissolved when it can pass through a glass fiber filter (under .2µm). Colloidal organic matter (size 0.001 – 1µm) are high-molecular weight (HMW) small DOM particles. Dissolved organic carbon (DOC) consists of the "colloidal fraction" and submicron particles, however differences in chemical behavior of dissolved and colloidal organic matter have been observed. Heterotrophic bacteria consume most DOC in the water column. This carbon consumption is approximately 50% of marine primary production.

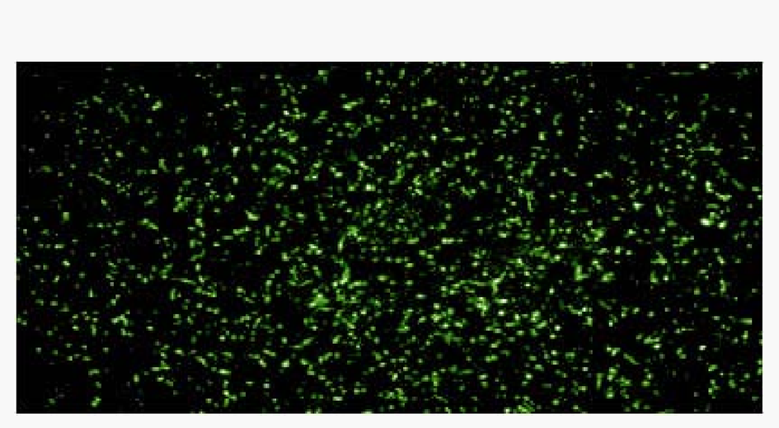
Sample Processing: Epifluorescent Microscopy

Purpose:

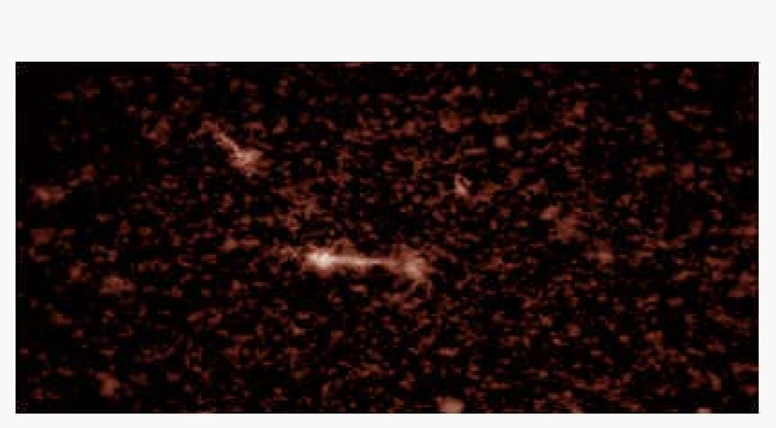
The Epifluorescent microscope emits images not of visible light but of light that has fluoresced from the sample specimens. Excitation filters (330-380nm, 530-650nm, 450-490nm) allow only specific wavelengths to pass and reach the fluorescence specimen. Any amount of light reflecting off of the specimen into the microscope objective is filtered out by corresponding emission filters (420nm, 590-650nm and 515nm respectively). Using this microscope images or hand counts of particles can be collected. Multiple images in this project were taken from each sample as most samples contained high volumes of particles which were uncountable by hand. Images were taken to be processed by computer software.

Microscope Details: Nikon Eclipse E400 Epifluorescent Microscope features an ergonomic head, transmitted light 6V 30W halogen illuminator, black mechanical stage Includes 4x, 10x, 40x, 100x(oil) objectives on five-place revolving nose piece. Connected to a Nikon super high pressure mercury lamp power supply and a SPOT RT monochrome camera.

Dyes: Dyes were chosen based on their emission fluorescence. Fluorescent dyes absorb light at certain wavelengths then emit their fluorescence energy at a higher wavelength.

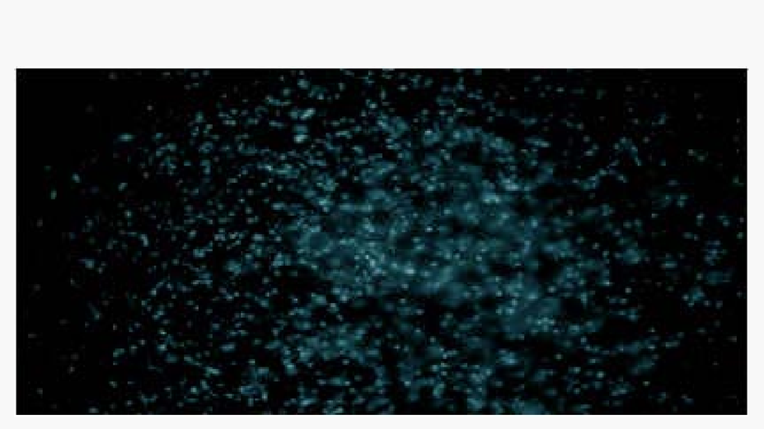


Pico Green (PG)

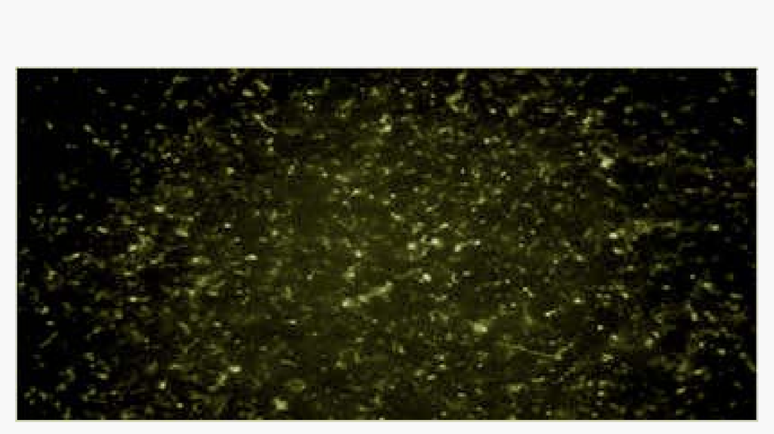


Concanavalin A (CON A)€

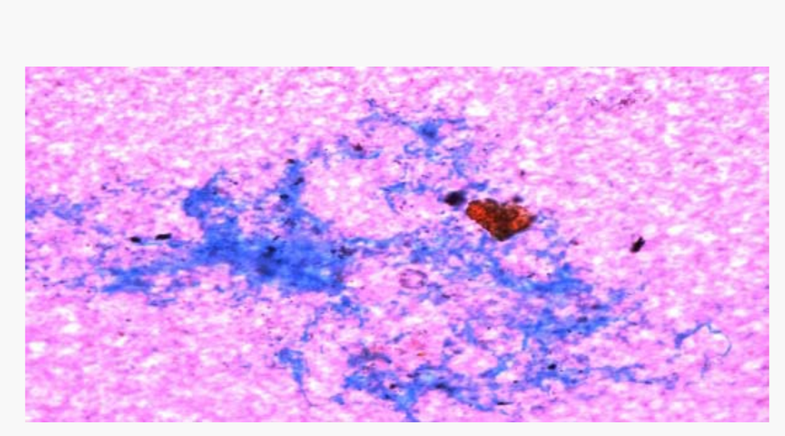
Nile Red (NR)



Propidium Iodide (PI)



5-(4, 6-dichlorotriazinyl)aminofluorescein (DTAF)



Alcian Blue (TEP)

Data Collection using software analysis:

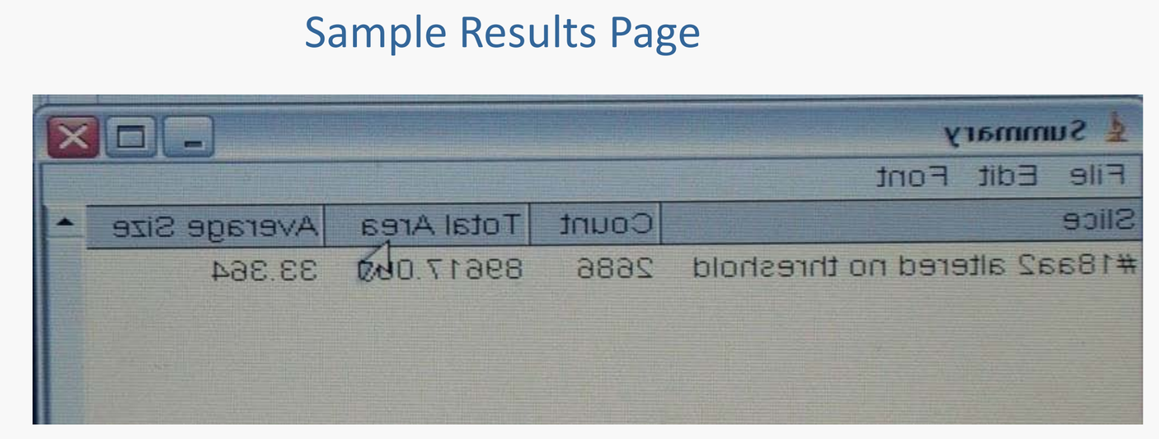
Purpose:

Images taken from the Epifluorescent microscope were processed using the computer program "Image J." In this experiment Image J was used to analyze particles as most samples collected had a high number of particles which could not be hand counted. Particles were analyzed for fluorescence to provide particle counts, average particle size, total image area, and average area fraction for each image.

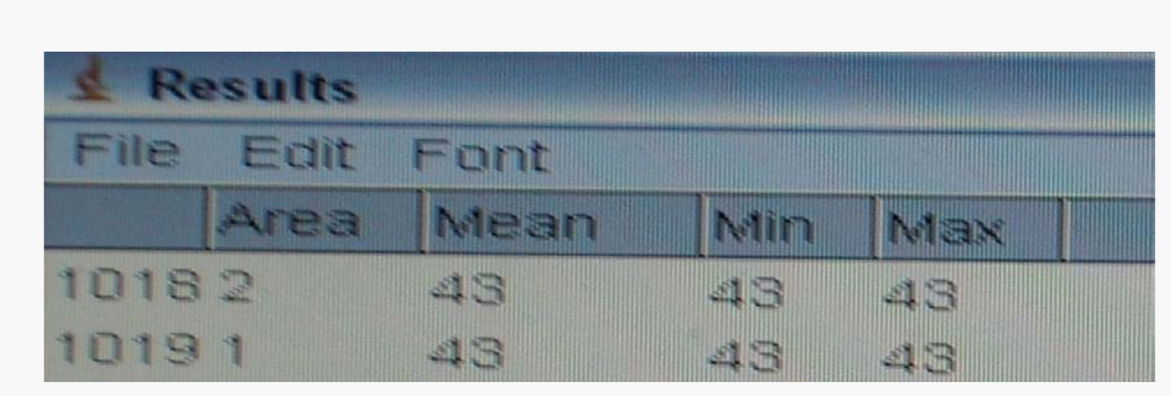
Program specifics:

Image J is a free, downloadable program which can highlight and analyze color contrasts in images, analyze data using area, mean, and standard deviation along with editing and stacking multiple images.

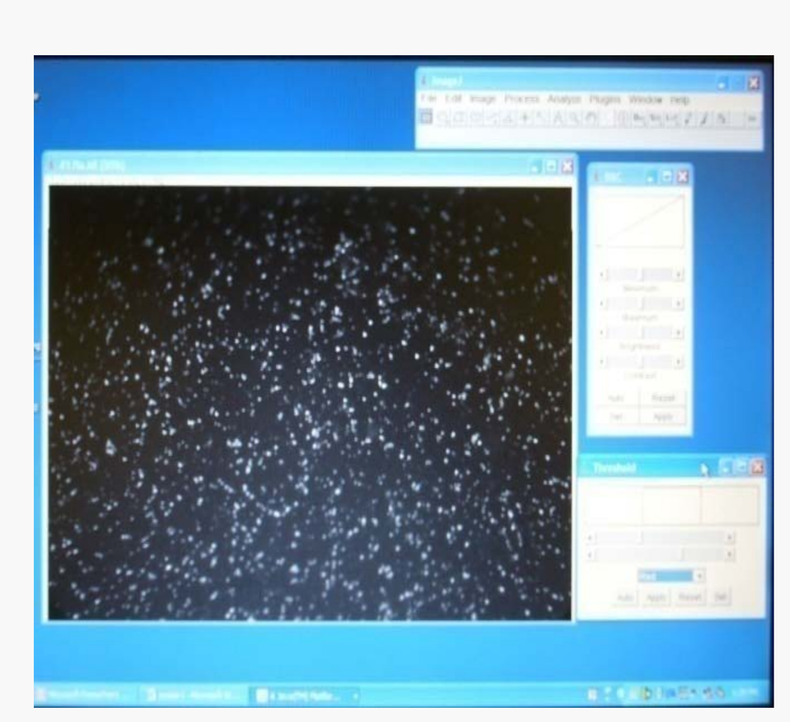
Sample Results Page



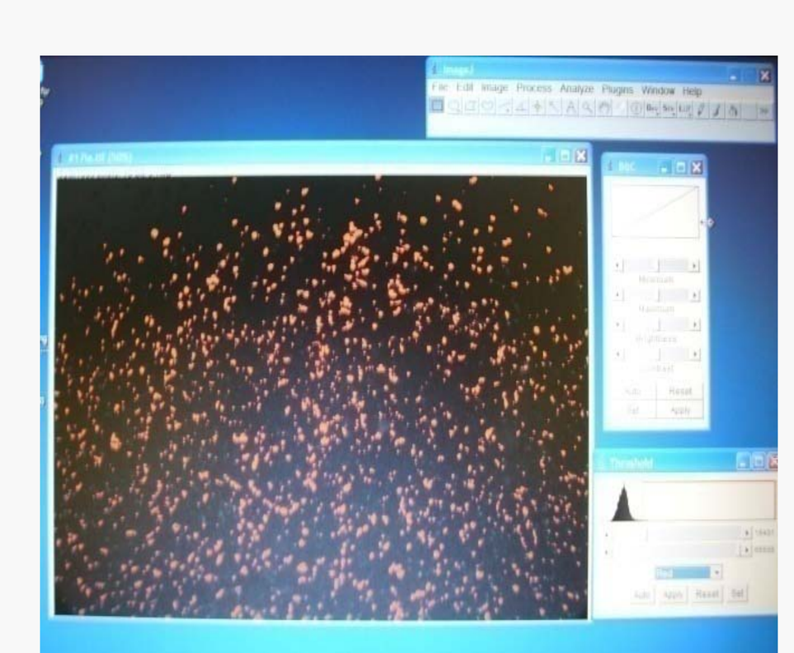
Sample Summary Page



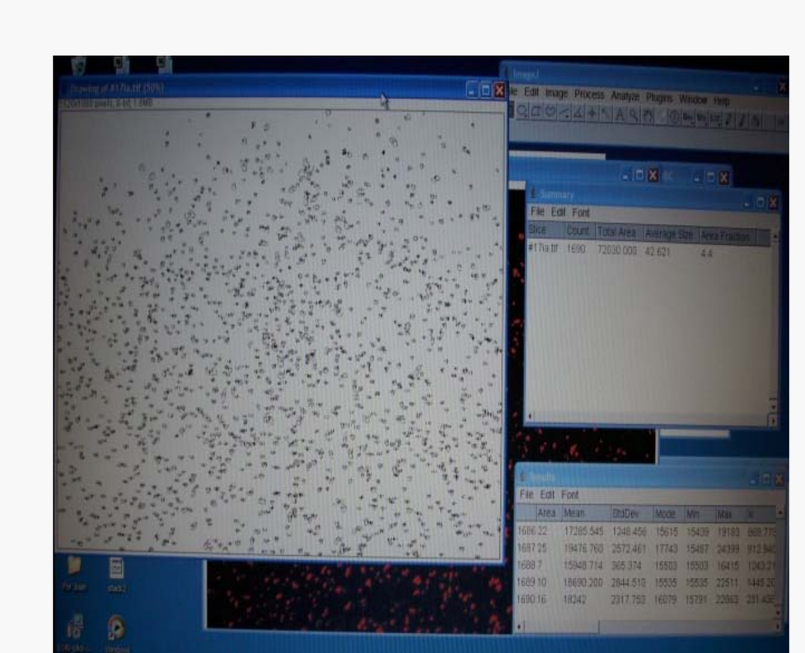
Process:



1) Images were altered by adjusting brightness and contrast, filling un-highlighted particles, and stacking triplicates of images.



2) Altered images were then isolated (red highlight) using the threshold module. Highlighted particles using threshold will be included in software analysis, while the background would be ignored.



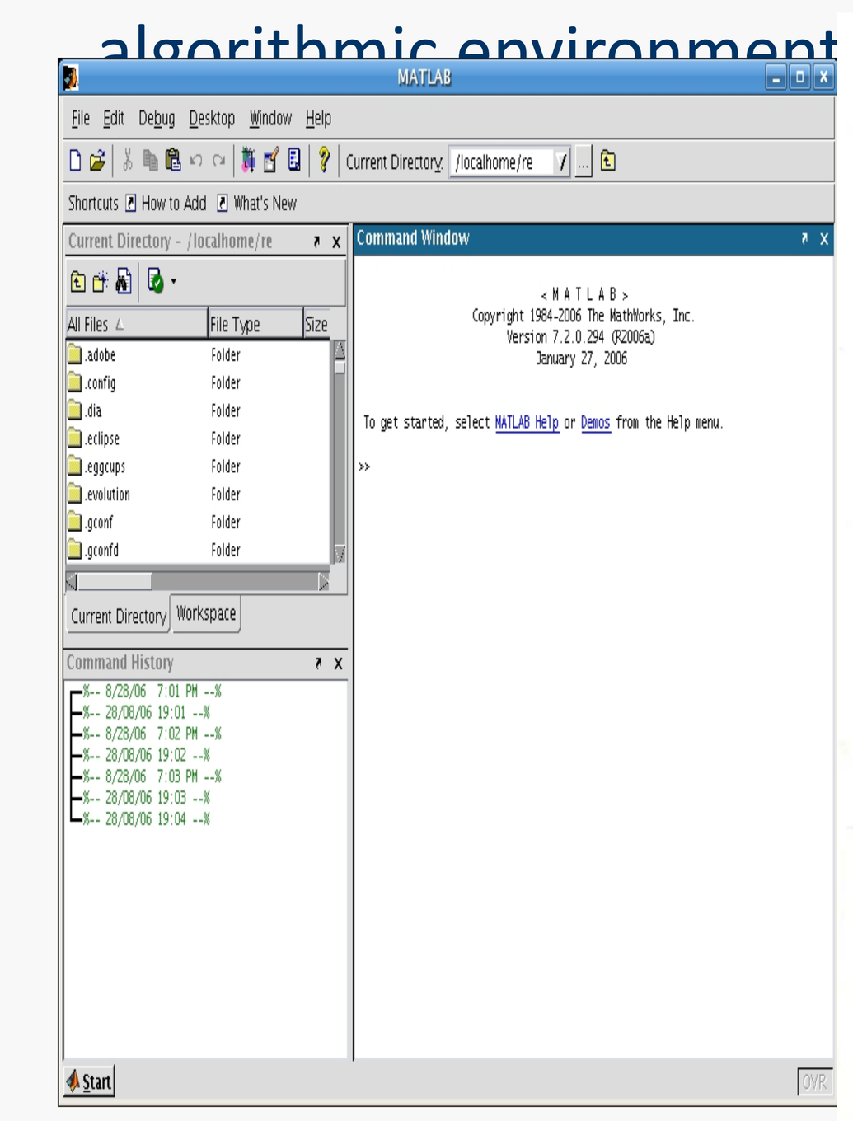
3) Highlighted particles were analyzed using "analyze particles." Image of outlined counted particles, results page, and analysis summary were given. Data given was compiled in excel to give workable data.

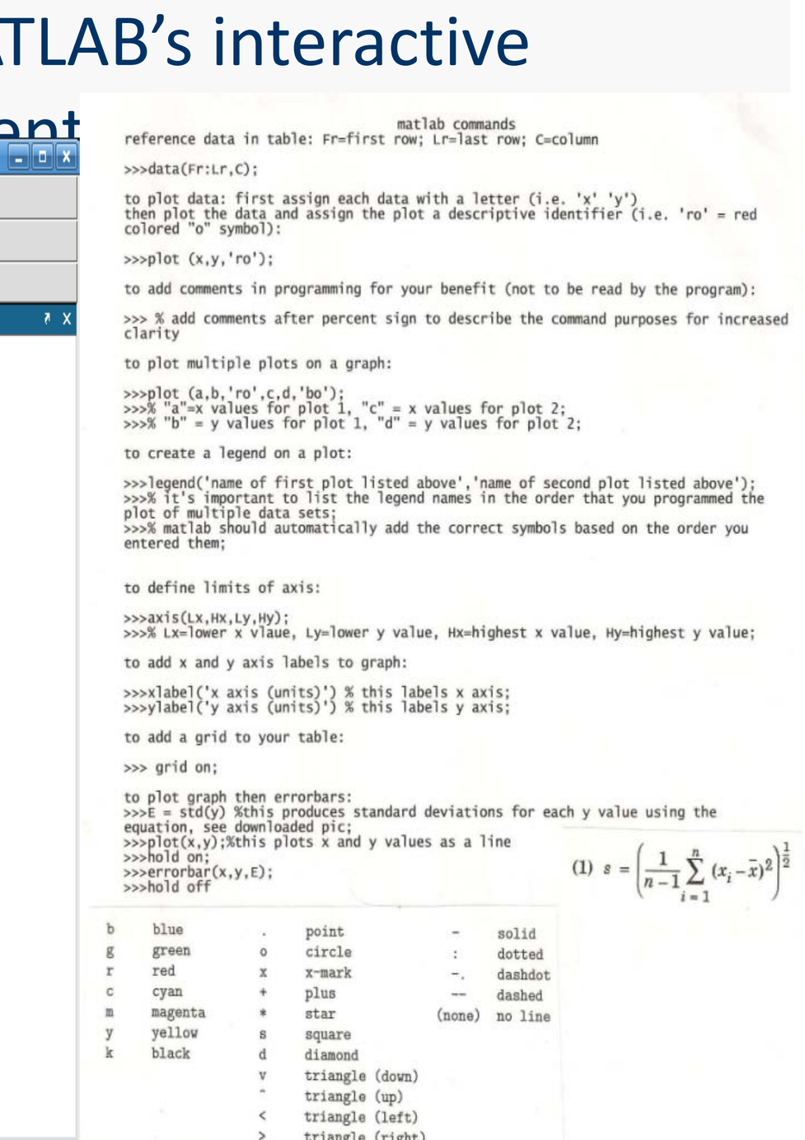
Data analysis using software program:

Purpose:

Individual sample summary data collected from "Image J" were compiled in excel data sheets sorted by dye. Data was analyzed using MATLAB. MATLAB commands were developed, such as standard deviation and mean. MATLAB commands were developed and used to graph results to draw conclusions for project.

Program specifics: MATLAB is high level technical computing system used for signal and image processing. MATLAB's interactive algorithmic environment





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matlab commands
reference data in table: Fr=first row; Lr=last row; C=column
>>>data(Fr:Lr,C);
to plot data: first assign each data with a letter (i.e. 'x' 'y') then plot the data and assign the plot a descriptive identifier (i.e. 'ro' = red colored "o" symbol):
>>>plot (x,y,'ro');
to add comments in programming for your benefit (not to be read by the program):
>>> % add comments after percent sign to describe the command purposes for increased clarity
to plot multiple plots on a graph:
>>>plot (a,b,'ro',c,d,'bo');
>>>% "a"=x values for plot 1, "c" = x values for plot 2;
>>>% "b" = y values for plot 1, "d" = y values for plot 2;
to create a legend on a plot:
>>>legend('name of first plot listed above','name of second plot listed above');
>>>% it's important to list the legend names in the order that you programmed the plot of multiple data sets;
>>>% matlab should automatically add the correct symbols based on the order you entered them;
to define limits of axis:
>>>axis(Lx,Hx,Ly,Hy);
>>>% Lx=lower x vlaue, Ly=lower y value, Hx=highest x value, Hy=highest y value;
to add x and y axis labels to graph:
>>>xlabel('x axis (units)') % this labels x axis;
>>>ylabel('y axis (units)') % this labels y axis;
to add a grid to your table:
>>> grid on;
to plot graph then errorbars:
>>>E = std(y) %this produces standard deviations for each y value using the equation, see downloaded pic;
>>>plot(x,y);%this plots x and y values as a line
>>>hold on;
>>>errorbar(x,y,E);
>>>hold off
```

$$(1)\quad s = \left(\frac{1}{n-1}\sum_{i=1}^{n}(x_i-\bar{x})^2\right)^{\frac{1}{2}}$$

```
b  blue     .  point              -     solid
g  green    o  circle             :     dotted
r  red      x  x-mark             -.    dashdot
c  cyan     +  plus               --    dashed
m  magenta  *  star             (none)  no line
y  yellow   s  square
k  black    d  diamond
            v  triangle (down)
            ^  triangle (up)
            <  triangle (left)
            >  triangle (right)
            p  pentagram
            h  hexagram
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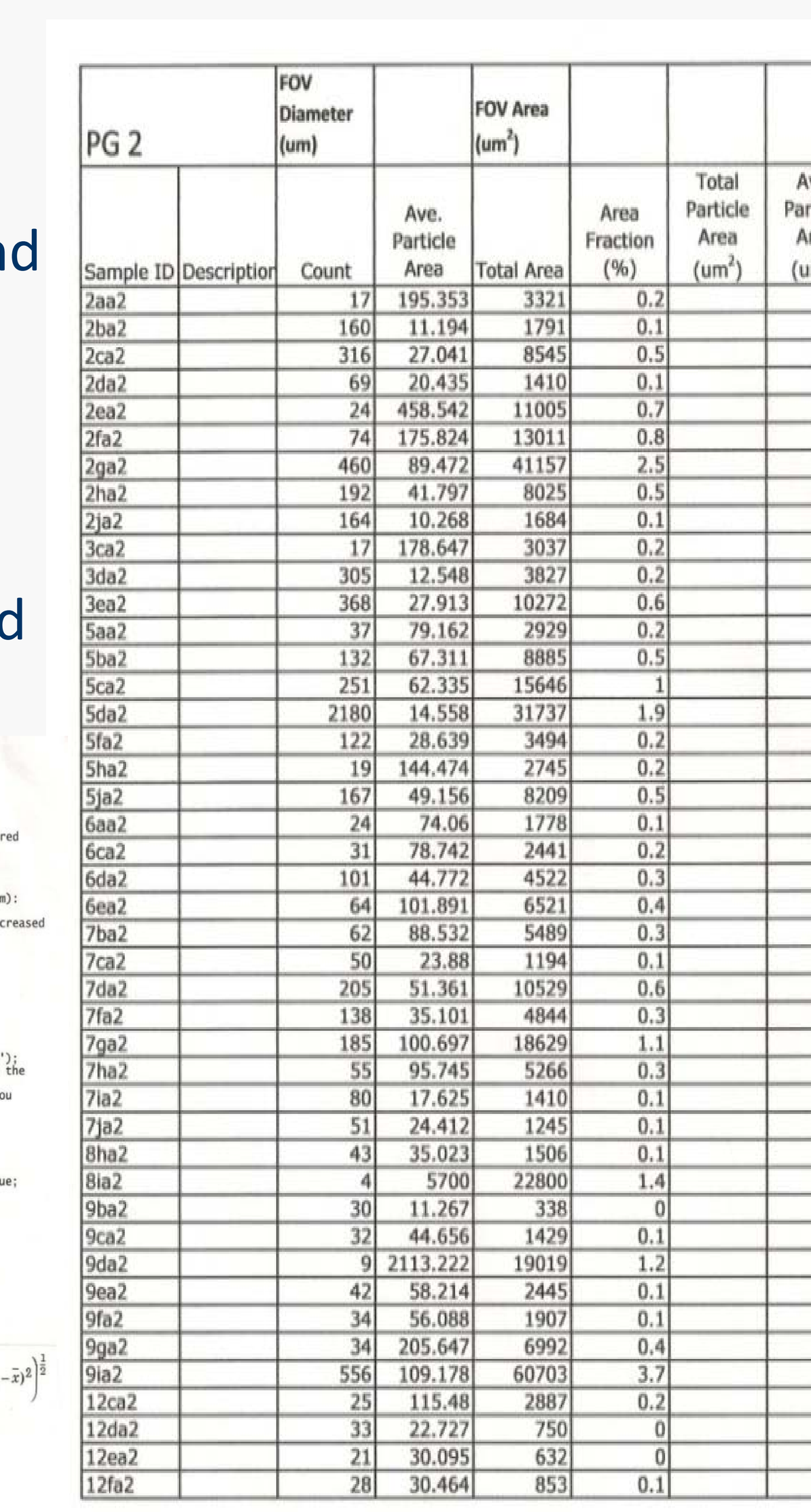

PG 2		FOV Diameter (um)		FOV Area (um²)			
Sample ID	Description	Count	Ave. Particle Area	Total Area	Area Fraction (%)	Total Particle Area (um²)	Ave. Particle Area (um²)
2aa2		17	195.353	3321	0.2		
2ba2		160	11.194	1791	0.1		
2ca2		316	27.041	8545	0.5		
2da2		69	20.435	1410	0.1		
2ea2		24	458.542	11005	0.7		
2fa2		74	175.824	13011	0.8		
2ga2		460	89.472	41157	2.5		
2ha2		192	41.797	8025	0.5		
2ja2		164	10.268	1684	0.1		
3ca2		17	178.647	3037	0.2		
3da2		305	12.548	3827	0.2		
3ea2		368	27.913	10272	0.6		
5aa2		37	79.162	2929	0.2		
5ba2		132	67.311	8885	0.5		
5ca2		251	62.335	15646	1		
5da2		2180	14.558	31737	1.9		
5fa2		122	28.639	3494	0.2		
5ha2		19	144.474	2745	0.2		
5ja2		167	49.156	8209	0.5		
6aa2		24	74.06	1778	0.1		
6ca2		31	78.742	2441	0.2		
6da2		101	44.772	4522	0.3		
6ea2		64	101.891	6521	0.4		
7ba2		62	88.532	5489	0.3		
7ca2		50	23.88	1194	0.1		
7da2		205	51.361	10529	0.6		
7fa2		138	35.101	4844	0.3		
7ga2		185	100.697	18629	1.1		
7ha2		55	95.745	5266	0.3		
7ia2		80	17.625	1410	0.1		
7ja2		51	24.412	1245	0.1		
8ha2		43	35.023	1506	0.1		
8ia2		4	5700	22800	1.4		
9ba2		30	11.267	338	0		
9ca2		32	44.656	1429	0.1		
9da2		9	2113.222	19019	1.2		
9ea2		42	58.214	2445	0.1		
9fa2		34	56.088	1907	0.1		
9ga2		34	205.647	6992	0.4		
9ia2		556	109.178	60703	3.7		
12ca2		25	115.48	2887	0.2		
12da2		33	22.727	750	0		
12ea2		21	30.095	632	0		
12fa2		28	30.464	853	0.1		

Figure 1: Sample MATLAB screen. Current directory imports outside data sources (excel) which MATLAB uses for code sources. Command history shows recent code. Command window is active code commands.

Figure 2: Sample excel data sheet. MATLAB code takes information directly from downloaded excel worksheets as code for algorithmic functions.

Figure 3: Sample MATLAB code commands. MATLAB recognizes abbreviations associated with algorithmic equations or symbols. Abbreviations can be used to graph data. Code records keep code commands for later use.

(Chanteal Craft would like to acknowledge her C-MORE scholars mentor, Ms. Courtney Daniels, and the C-MORE program for funding and providing this scientific research position)