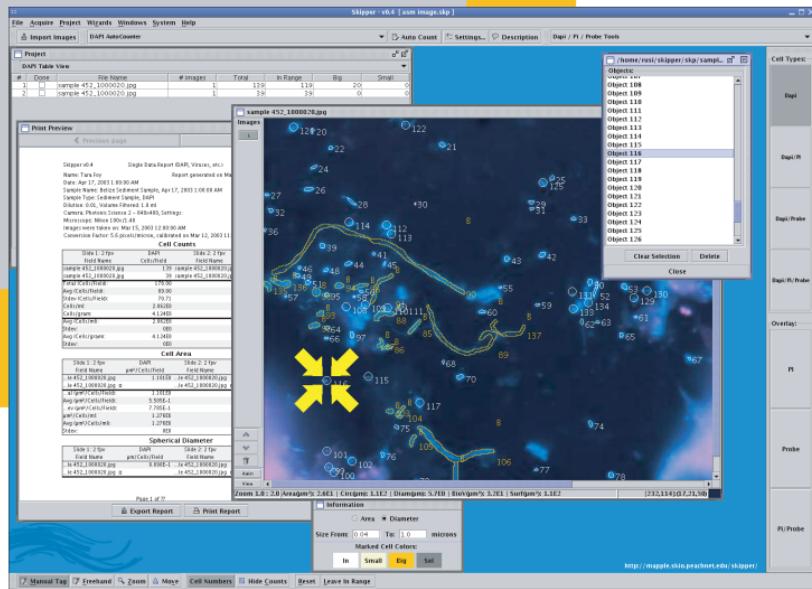




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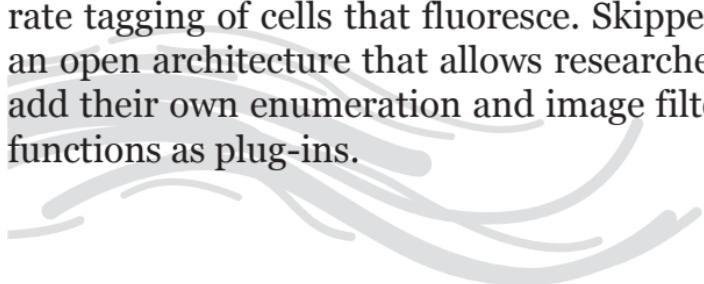
Software for Image Analysis



Skipper (version 0.5)

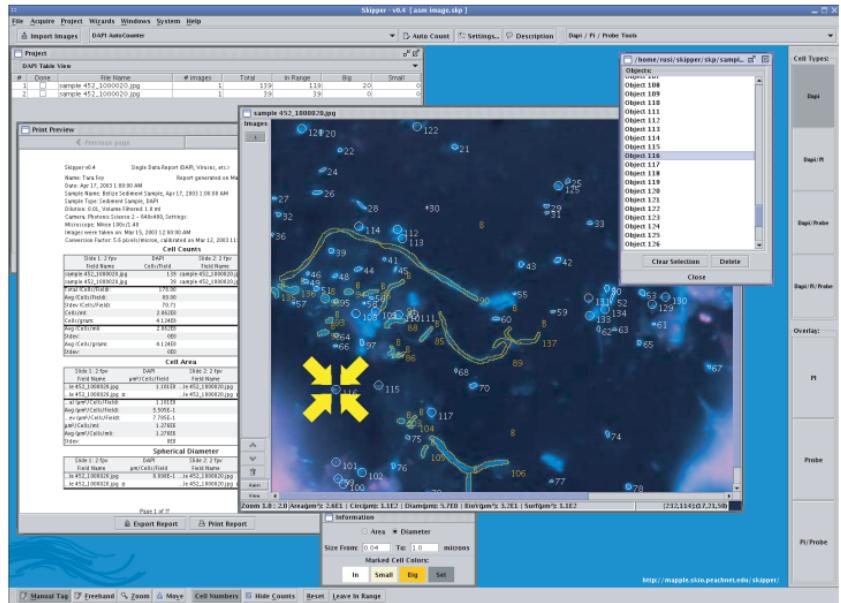
Introduction

Skipper is a comprehensive Java-based image processing package that was primarily developed for scientific research, allowing researchers to automatically categorize, measure, and enumerate tiny objects such as bacteria and other aquatic organisms. The software has multiple algorithms implemented for the automatic enumeration of bacteria, plankton, and other organisms, which makes the processing of multiple images fast and removes the aspect of human bias in counting. They work equally well for images (photographs) derived from astronomy, biology, geology, physics, and other fields of research. Various image processing functions, such as Gaussian blur, contrast manipulation, spatial convolutions with user-defined convolution masks, erosion, sharpening, mean and median filtering, edge detection, and others are also included and can be used to enhance the image quality. The program allows researchers to organize their images into a single project and produce printable reports that contain useful statistics about the obtained data. Multiple images can be overlaid and the data compared automatically, ensuring accurate tagging of cells that fluoresce. Skipper has an open architecture that allows researchers to add their own enumeration and image filtering functions as plug-ins.



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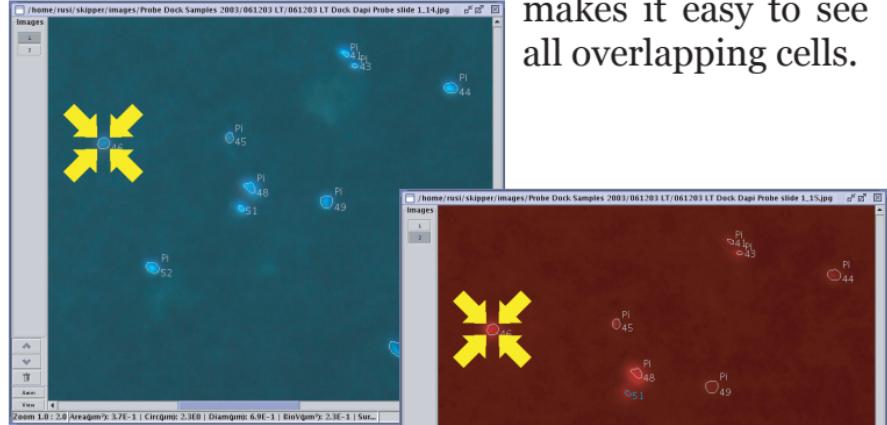
Features



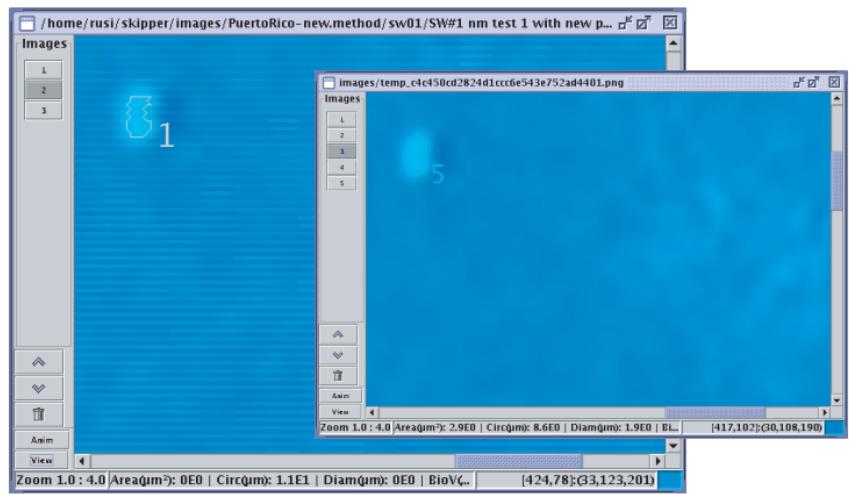
- Automatically or manually enumerate aquatic bacteria, viruses, plankton cells, bacteria in sediments, and other objects of interest.
 - Automatically or manually compare and group objects.
 - Calculate area, bio-volume, surface area, spherical diameter, and other object statistics.
 - Generate reports that include image and sample information, measurement data, text, and statistical information.
 - Perform manually tedious or complex tasks automatically, such as object enumeration.
 - Capture images independently.
 - Use built-in filters to clean images which make image recognition more accurate.
 - Write your own autocounters and filters as plug-ins.

Layers & Filters

Skipper supports layering of images for quick and easy comparison between two or more images. This makes it easy to see all overlapping cells.



It also has built-in filters that are used to remove unwanted noise and enhance countable cells. Some of these filters include Gaussian blur, spatial convolutions, mean and median filtering, sharpen, and various edge detectors. It is easy to combine any number of filters to enhance the images and produce more accurate results.



Camera noise (such as horizontal lines) can inhibit image processing software to perform optimally. By applying various filters, the image is corrected and Skipper is able to automatically detect all objects.

Data & Reports

Your data is easily organized and saved into Skipper projects. These projects contain all useful information pertaining to your images including cell counts and statistics.

#	Done	File Name	# Images	Total DAPI	PI/Probe Overlay	In Range	Big	Small
1	<input checked="" type="checkbox"/>	E.coli Cy3 probe test slide 1_00.jpg	2	25	25	25	0	0
2	<input checked="" type="checkbox"/>	E.coli Cy3 probe test slide 1_02.jpg	2	23	23	23	0	0
3	<input checked="" type="checkbox"/>	E.coli Cy3 probe test slide 1_04.jpg	2	15	15	15	0	0
4	<input checked="" type="checkbox"/>	E.coli Cy3 probe test slide 1_06.jpg	2	39	37	39	0	0
5	<input checked="" type="checkbox"/>	E.coli Cy3 probe test slide 1_08.jpg	2	26	24	26	0	0
6	<input checked="" type="checkbox"/>	E.coli Cy3 probe test slide 1_10.jpg	2	22	22	22	0	0
7	<input checked="" type="checkbox"/>	E.coli Cy3 probe test slide 1_12.jpg	2	10	10	10	0	0
8	<input checked="" type="checkbox"/>	E.coli Cy3 probe test slide 1_14.jpg	2	24	24	24	0	0
9	<input checked="" type="checkbox"/>	E.coli Cy3 probe test slide 1_16.jpg	2	24	24	24	0	0
10	<input checked="" type="checkbox"/>	E.coli Cy3 probe test slide 1_18.jpg	2	20	19	20	0	0
11	<input checked="" type="checkbox"/>	E.coli Cy3 probe test slide 2_20.jpg	2	12	12	12	0	0
12	<input checked="" type="checkbox"/>	E.coli Cy3 probe test slide 2_22.jpg	2	17	17	17	0	0
13	<input checked="" type="checkbox"/>	E.coli Cy3 probe test slide 2_24.jpg	2	17	13	17	0	0
14	<input checked="" type="checkbox"/>	E.coli Cy3 probe test slide 2_26.jpg	2	30	29	30	0	0
15	<input checked="" type="checkbox"/>	E.coli Cy3 probe test slide 2_28.jpg	2	5	5	5	0	0
16	<input checked="" type="checkbox"/>	E.coli Cy3 probe test slide 2_30.jpg	2	8	8	8	0	0
17	<input checked="" type="checkbox"/>	E.coli Cy3 probe test slide 2_32.jpg	2	6	6	6	0	0
18	<input checked="" type="checkbox"/>	E.coli Cy3 probe test slide 2_34.jpg	2	24	23	24	0	0
19	<input checked="" type="checkbox"/>	E.coli Cy3 probe test slide 2_36.jpg	2	19	18	19	0	0
20	<input checked="" type="checkbox"/>	E.coli Cy3 probe test slide 2_38.jpg	2	11	10	11	0	0

Open Selected Images Auto Combine Combine Show Image Preview

The data and cell statistics can be quickly viewed in the project table, or it can be presented in a detailed printable report.

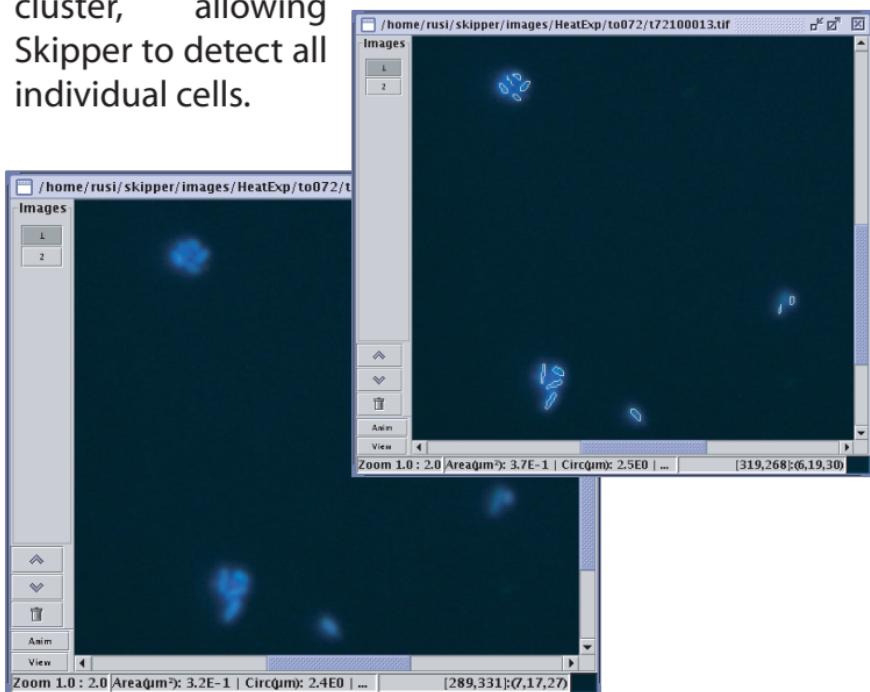
Skipper v0.5pre1 Multiple Data Report (DAPI/PI, DAPI/Probe, etc.) 4:52:29 PM
Name: Daphne Report generated on Mar 17, 2004 4:52:29 PM
Date: Jun 29, 2003 11:00:00 PM
Sample Name: E.coli Cy3 probe test, Jun 29, 2003 11:00:00 PM
Sample Type: Culture, DAPI / Probe
Dilution: 0.0010, Volume Filtered: 1.0 ml
Camera: Retiga 1300 - 1280x1024, Settings:
Microscope: Olympus 100x/1.35
Images were taken on: Jun 29, 2003 11:00:00 PM
Conversion Factor: 15.0 pixels/μm, calibrated on Feb 20, 2003 10:30:00 AM

Slide 1: 10 fpv Field Name	DAPI Cells/Field	PI Cells/Field	Slide 2: 10 fpv Field Name	DAPI Cells/Field	PI Cells/Field
...e test slide 1_00.jpg	25	25	...e test slide 2_20.jpg	12	12
...e test slide 1_02.jpg	23	23	...e test slide 2_22.jpg	17	17
...e test slide 1_04.jpg	15	15	...e test slide 2_24.jpg	17	13
...e test slide 1_06.jpg	39	37	...e test slide 2_26.jpg	30	29
...e test slide 1_08.jpg	26	24	...e test slide 2_28.jpg	5	5
...e test slide 1_10.jpg	22	22	...e test slide 2_30.jpg	8	8
...e test slide 1_12.jpg	10	10	...e test slide 2_32.jpg	6	6
...e test slide 1_14.jpg	24	24	...e test slide 2_34.jpg	24	23
...e test slide 1_16.jpg	24	24	...e test slide 2_36.jpg	19	18
...e test slide 1_18.jpg	20	19	...e test slide 2_38.jpg	11	10
Total (Cells/Field):	228.00	223.00		149.00	141.00
Avg (Cells/Field):	22.80	22.30		14.90	14.10
StdDev (Cells/Field):	7.55	7.06		8.06	7.69
Cells/μm ² :	8.884E3			5.806E3	
Percent PI:		97.81			94.63
Avg (Cells/μm ²):	7.345E3				
StdDev:	2.176E3				
Avg PI %:	96.22				
StdDev PI %:	2.249E0				

Skipper (version 0.5)

Example

In this example we have a set of images that contain clusters of bacteria cells. Running an automated counter without enhancing the images will cause each cluster to be counted as a single cell. However, we want to count each individual cell. We use a combination of filters to separate the cells within each cluster, allowing Skipper to detect all individual cells.



The set of filters used in the above example includes a Marr-Hildreth convolution filter, grayscale filter, and a threshold filter.

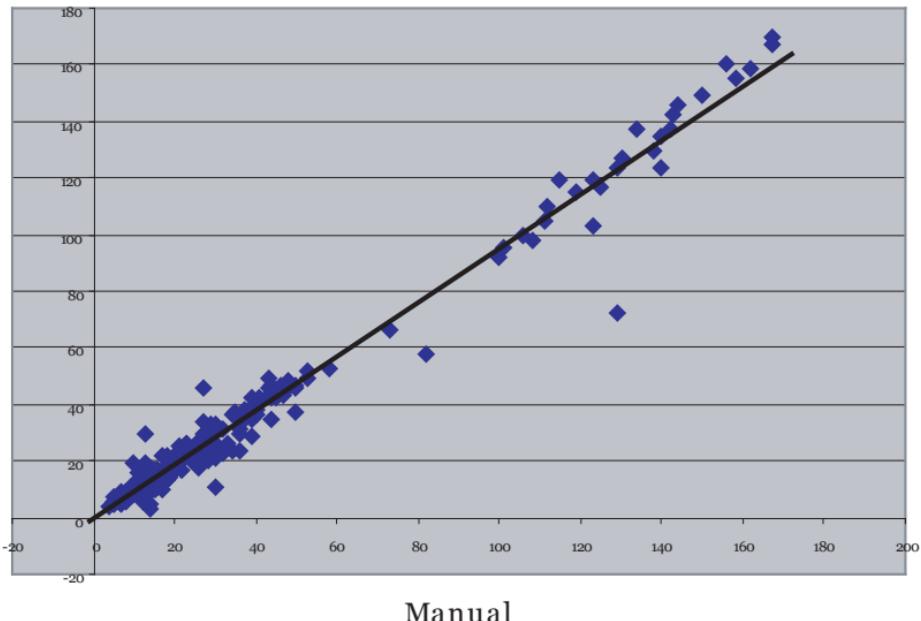
How accurate is Skipper?

The next page shows a comparison between automatic Skipper counts and manual counts conducted by an expert microbiologist. About 260 fluorescently stained bacteria images were analyzed and presented in the first graph. Another set of 260 images of molecularly probed cells were also analyzed and graphed. The regression lines show that Skipper's counts are indistinguishable from manual counts.

Results

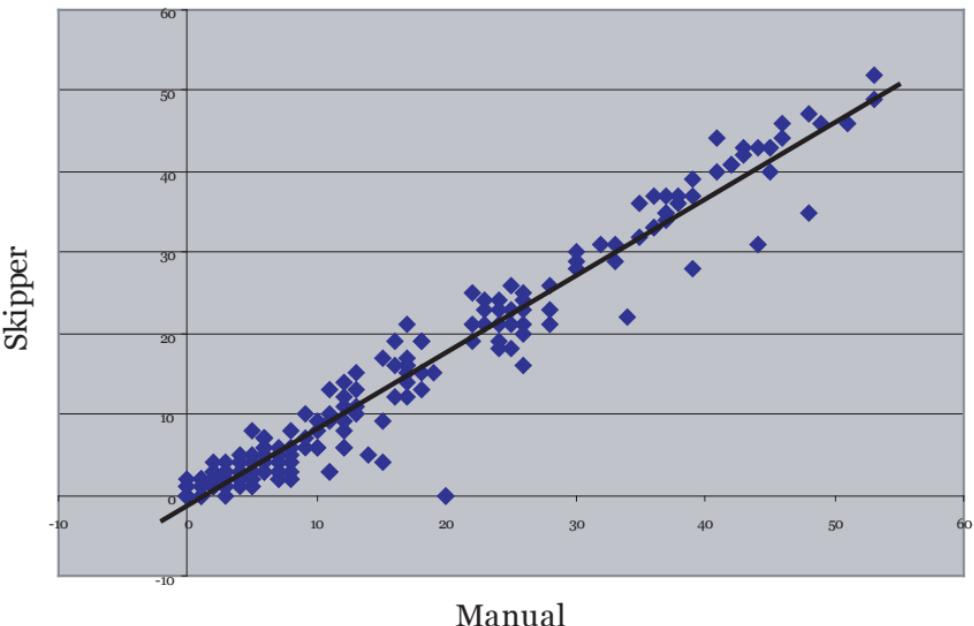
Fluorescently Stained Bacteria
Skipper vs. Manual

$$y = 0.95x - 0.11$$
$$R^2 = 0.97$$



Molecularly Probed Bacteria
Skipper vs. Manual

$$y = 0.95x - 1.12$$
$$R^2 = 0.95$$



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Home Page: www.skio.peachnet.edu/research/skipper/

Documentation: www.skio.peachnet.edu/research/skipper/docs/



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