MicroNews

San Francisco Microscopical Society Volume 6, # 3 September 2011

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INSIDE

SEPTEMBER 14TH PROGRAM IN SAN FRANCISCO AT THE RANDALL MUSEUM 7:30 pm

Multiphoton fluorescence microscopy is a powerful research tool that combines the advanced optical techniques of laser scanning microscopy with long wavelength multiphoton fluorescence excitation. See the images from Molecular Expressions, Exploring the World of Optics and Microscopy, illustrates the complexity of modern optical microscopes and the techniques used to elicit information from biological materials, particularly cell

Parts of a Multiphoton Exitation Flourescence Microscope

Inverted microscope

Photomultiplier Detector System

X-Y Scanning Unit

Ti: Sapphire Mode-Locked Laser System

Pulsed Laser Control

Imaging and Data Analysis Workstation Computer

Monitor

My Adventures Building a Two Photon Microscope

by Dr. Paul Herzmark

The speaker for out September 14, 2011 meeting at the Randall Museum is Dr. Paul Herzmark, Imaging Specialist at the Center for Host-Pathogen Studies, at UC Berkeley.

This program project studies the interplay between pathogens and the immune system using mouse and several intracellular pathogens as model experimental systems. The

experimental approaches take advantage of 2-photon imaging to track pathogens and immune cells within tissues in real-time, and transgenic/knockout mice to explore the role of specific molecule and cells in immune responses. The investigators in this proposal are an interactive group of immunologists/microbiologists, who are actively investigating host-pathogen interactions.

Beyond the Very Small

Among the tasks that microscopist enjoy, is measuring the visible specimens that optical or electron microscopes reveal. There are, in our universe distances that are so extreme that we are not able to measure them accurately but refining the known measurements continues to be one of the passions of physicists. Particles comprising the atom, invisible under the light microscope, have recently been measured to a new level of accuracy that stretches our ability to encompass the meaning of such numbers.

Most of us can manage to visualize the units of everyday life. Yards and miles, meters and millimeters give us no pause even when used to describe dimensions. The circumference of the earth is 25,000 miles and the speedometer of an older car reads 75,000 miles, the equivalent of three times the circumference. We are comfortable and familiar with such figures because they fit our experience. But what are we to make of the following description from the October issue of the Scientific American?

> …"researchers were able to estimate the proton's radius to be 0.84184 femtometer (one quadrillionth of a meter). This figure is smaller than all previous measurements made, which range between 0,8768 and 0.897 femtometer."*

Knowing the radius hardly matters since the proton, one of the particles of an atom, is in turn made up of three smaller elementary particles called quarks. Proton used to be thought of as impenetrable but orbiting particles such as electrons or muons often pass through protons. So protons, like most of the volume of an atom, are mostly empty space. In the words of Gertrude Stein, there is not there there.

To illustrate the size relationships between a hydrogen atoms and its proton, the author, Davide Castelvecchi, suggests that if the hydrogen atom were the size of a football field, the proton would be the size of an ant. While the ratio may bring a response of "awesome" it does not help in getting a better understanding of the femtometer scale. Human cell diameter measurements average 1 x 10^{-5} meters. Femtometers are $1X10^{-15}$ meters. They are not quite in the range of my sensory perception!

We live in an age where the scale by which we measure vast or minute length, vast cost of oil spills or other catastrophes, or frequency of atomic fission in a nuclear reactor, far exceeds our understanding of the numbers used. We have spent a trillion dollars on the two wars we are fighting since 1961. We have no measure by which a 'trillion' becomes meaningful. Is it any wonder that our politicians are unable to solve the ma-

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BOOK NOTES by Peter Werner, President, SFMS

I shared this with the Merritt Microscopy Group, and think this will be of interest to some of you: There is an unbelievable deal on a recent textbook, *Bioimaging: Current Concepts in Light & Electron Microscopy*. By Robert W. Robertson. ISBN -9780763738747.

I've seen this recently and its very good, and makes an excellent text for all semesters of the microscopy program at Merritt College.

There are several brand-new under-$25 copies and of this $100+ hardcover book available.5-6 copies are available for under $25 and several more for $25-$30. Get 'em while they're hot. I have snapped one up already. Previews and info here: http://www.amazon.com/reader/0763738743

http://www.jblearning.com/catalog/9780763738747/

http://search.barnesandnoble.com/used/product.asp?EAN=2695705013387

http://www.amazon.com/gp/offer-listing/0763738743/

http://www.bestwebbuys.com/Bioimaging-Current-Concepts-in-Light-and-Electron-Microscopy-ISBN-9780763738747

On a personal note, I see that the co-author is Robert W. Robertson. Robertson is a mycologist specializing in fungal cell biology who I've met at the Fungal Genetics Conference at Asilomar. He's one of the most talented and knowledgeable people I've come across in the area of imaging fungal cells

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How to Make and Use a Simple Microspectroscopic Eyepiece

by John Gustav Delly, Scientific Advisor *Monday, July 07, 2003*

From: MODERN MICROSCOPY JOURNAL

Here's a fascinating and highly useful accessory for your microscope, which you can make for less than one U.S. dollar. The simple microspectroscopic eyepiece described is suitable for most qualitative work, and even some semi-quantitative analyses. Let me first tell you how to make your own microspectroscopic eyepiece, and then I'll tell you how to use it and experiment with it. I first wrote about this device in 1966 (1), when inexpensive, acetate plastic diffraction grating replicas having about 13,400 lines per inch first became commonly available. Since that time, holographic diffraction grating replicas have become available at very reasonable cost, allowing for improvements in performance, with no increase in cost.

Shortly thereafter, as fate would have it, I was hired by the defense for a case in which the accused was charged with the kidnap, rape, and murder of a teen-aged girl in one of our northern states. There was a great deal of physical evidence in the case, but the relevant sample concerned a small, reddish-brown deposit taken by the State from the dashboard of the accused's truck; he is said to have taken the girl into his truck after first striking the back of the motorscooter she was riding on, knocking her off. The reddish-brown deposit was thought to be blood. The State was reluctant to conduct tests for typing because of the small amount present. The defense attorney said that he needed to know if the reddish-brown deposit was blood or not; if it was, the accused would have to account for it; if it was not, precious time could be devoted to the many other items of physical evidence. The defense attorney asked me if I could tell if the spot was blood just by looking at it with my microscope. I said "yes," and the next day, above the protests of the Crime Lab personnel and warnings that I could not conduct any chemical or other destructive tests, and with my repeated assurances that I was only going to ***look*** at the material, I was allowed to put the sample on the stage of my portable microscope. I inserted the microspectroscopic eyepiece, which, of course, you could not tell from any other eyepiece from the outside, and tilted my mirror to the overhead laboratory lights, which were fluorescent. Having now my marker wavelengths from the superimposed mercury lines, I looked for the absorption bands around 400-450 nm, 535-550 nm, 575-585 nm, 730+ nm, and did not see them. I thanked them, returned the sample, and left. I told the attorney I did not know what the reddish-brown deposit was, but it was not blood. The State never introduced the deposit into evidence.

It was a source of enormous personal satisfaction to have been able to use my simple microspectroscopic eyepiece, which cost about 25¢ to make, to help make a decision on such an important question. As a matter of curiosity, I searched the then-current textbooks of criminalistics, and never did find this method of blood detection mentioned. I guess it got lost somewhere over the last 140 years.

The microspectroscopic examination of blood is, however, thoroughly discussed in at least one book on medical jurisprudence and toxicology; the twelfth edition of Glaister and Rentoul's Medical Jurisprudence and Toxicology (15) contains a complete description of the method together with reference absorption spectra for blood.

Anyway, for a few dollars, and an hour or two of your time, I guarantee you will learn something about filters and colored solid and liquid specimens that you didn't know before - and have fun doing it - by constructing and using your simple microspectroscopic eyepiece.

Beyond the Very Small, cont.

(Continued from page 2) Hancock

jority of the fiscal problems of our society?

Numerical literacy, sometimes referred to as numeracy, is not only having a facility with numbers, it also requires an understanding of the significance of those values derived from experimentation or direct measurement. In microscopy, the thrill of seeing something that normally is invisible to the unaided eye makes us aware that we are passing through a border of normal perception. But how far beyond this border have we advanced? In light microscopy the product of the eye piece and objective magnification gives us a rough value such as 100x or 400x. Accurate measurements are possible but understanding their significance in the scale of everyday experience is much more challenging. Bacteria are small but visible with a good microscope. We do not perceive them in every day events although they are present in numbers that far exceed our imagination.

The Editor, HS

* Scientific American, October 2010, *Advances* ,pp 24.

The Natural History of Microbial Communities in Variable Extreme Environments

By Wayne Lanier, Ph.D.

We are prisoners of our history. Until quite recently, the historical paradigm of microbiology dictated laboratory study with pure cultures, either of disease-causing microbes or model organisms of genetics and physiology. Studying the natural history of microbial communities in extreme and variable habitats does not fit into that paradigm. Here, I define a new paradigm to inform such study.

By *microbe* I include all organisms best studied by microscopy. Besides the various Prokaryotes, this includes many small Eukaryotes, such as diatoms [Fig. 01], Protista [Fig. 02], fungi, algae [Fig. 03], and aquatic larvae.

> **NOTE**: These Figures are displayed on the Author's Flickr Photostream in the Collection entitled **The Natural History of Microbial Communities in Variable Extreme Environments**. Enter the URL [http://www.flickr.com/photos/w_lanier/collections/] and click on the title to view the figures. Clicking on individual Figure images will show enlarged versions. Use your computer "back" button to return to the Collection after viewing enlarged figures.

A *Microbial Community* is a stable association of microbes in a well-defined environment. Typically, one or two organisms dominate the community. The most common community-formers are genera of the filamentous Cyanobacteria [Figure 04]. A community is not a "pure" or *axenic* culture and it is not a uniform suspension of free-living cells. *Axenic* culture of many important members of such a community is either impossible, or very difficult. The dynamics of a Microbial Community are rarely as simple as growth in a culture flask or on a Petri plate. Typically, the growth form is a Microbial Mat, either on the bottom of the pond or floating [Fig. 05].

From laboratory studies we take the concept of a culture "life cycle" and the kinetics of microbial growth. Within an actual pond community, an individual microbial member may undergo the classic culture sequence of "lag phase" – "log phase" – "stationary phase". Cells of an individual microbial species may also exhibit a "bell-curve" growth response to ranging environmental variables.

The key paradigm for understanding Microbial Community dynamics in a natural environment is to follow the community changes in response to widely changing physical and chemical variables, particularly in extreme environments. These variables include changing concentration of various minerals, particularly salts [Fig. 06]; range of temperature; range of pH; range of light intensity; and, liquid/solid interface. Each member of the community will have its unique growth curve [Fig. 07]. When the ranging variable intersects the maximum growth point in such a growth curve, that organism has the potential of becoming a dominant member of the community; and, through its association with other microorganisms, shaping the Microbial Community. *The goal of this paradigm is a predictive model of Microbial Community composition and dynamics over the variable range*.

In a typical application of this paradigm, consider the "WEEP" study site in the Don Edwards San Francisco Bay National Wildlife Refuge. Several years have been devoted to studying the changing

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Microbial Communities in this linear pond averaging 1-meter wide and ~300-meters long. The WEEP is located on the trail leading north out of the Alviso Marina, immediately west of the Rail Road track berm and ~10-meters east of Pond A15, where the salinity exceeds 110-PPT. Immediately east of the Rail Road track berm is a ditch about 1-meter wide, which runs south from Coyote Creek to New Chicago Marsh. The ditch salinity ranges between 5-PPT and 15-PPT. Both Pond A15 and the ditch are slightly higher than the WEEP, so there is a hydraulic head of about 1-meter between Pond A15 and the WEEP; and, a hydraulic head of about 15-cm between the ditch and the WEEP [Fig. 08].

Seepage from the ditch underneath the Rail Road berm and into the WEEP appears to be constant, except during the time when a gate-valve upstream is temporarily closed and the height of water flowing in the ditch drops. Seepage from Pond A15 to the WEEP is much more variable, depending on the maintenance pumping of Pond A15 and rain catchment.

The net result of the interplay between these two seepages and evaporation of the very shallow WEEP is a seasonal change in the salinity [Fig. 09].

In February of 2008, when the WEEP salinity was 52-PPT, the Microbial Community was mixed, but dominated by Cyanobacteria, mostly of the genus *Lingbya* [Fig. 10].

Later in March, when the salinity had risen, we were startled to see the Marine Euglena dominate the Microbial Community [Fig. 02]. This organism has been, otherwise, quite rare in the salt marsh.

Predictively, the May Microbial Community is almost exclusively composed of the Archea *Halobacter* and dinoflagellate *Dunaliellia* [Fig. 11], both of which tolerate the very high salinity of 250-PPT.

This uniformity of prediction does not appear to hold at lower salinities, particularly at a salinity of around 50-PPT. The function which determines the community composition in this range of salinity is apparently chaotic in the mathematical sense. That is, the outcome is so sensitive to tiny variations in the initial conditions as to be presently non-predictive.

For example, later in August, when the salinity was again around 50-PPT, the Microbial Community was dominated by the diatom *Cylindrotheca* [Fig. 12] instead of Cyanobacteria.

This endeavor promises so many puzzles to unravel that I can imagine simple field research and measurements being productive for some time. Much more detailed observations are needed to build better models of Microbial Community dynamics. Tools to aid in rapid and reliable Community taxonomy are also needed. Domesticating some of these microbial species would enable better understanding of the interaction between physiology and environment. More importantly: Willing microscopists are needed.

I really hope this idea of Web Figures works for all the readers. I tried to write the article so it would make some sense to readers disinclined or unable to use the Internet. Photomicrographs almost demand color to be clear, and print on paper is comfortable to read but very expensive to include color separations. Wayne Lanier

(Continued from page 2)

Optics Demystified
by Stan Gibilisco

The other night I went by Moe's Books in Berkeley and came across a very interesting book I hadn't seen before:. It is a very thorough introduction to optics "for the rest of us" who sometimes have trouble understanding optical concepts out of a physics textbook, where complicated concepts are laid out too briefly and heavily couched in the language of mathematical physics. Optics Demystified instead spreads these concepts out over a leisurely 400 pages, and takes time to explain the concepts before laying out any mathematical formulas. There is math throughout the book, but it is mainly at the level of simple algebra or occasionally trigonometry. The book is laid out workbook style with practice questions and problems and a quiz at the end of each chapter. It is essentially a mini-course in optics.
Since a refresher in optics is exactly what I've been wanting recently, I snapped up the copy and am reading/working through it now.

Here's a list of discount sources of this book:
http://www.bestwebbuys.com/Optics-Demystified-ISBN-9780071494496?isrc=b-search

Here's the Amazon page, which has a preview of a small part of the book:
http://www.amazon.com/Optics-Demystified-Stan-Gibilisco/dp/0071494499/ref=sr_1_1?s=books&ie=UTF8&qid=1310859101&sr=1-1

Enjoy!,
Peter July 16, 2011

OTHER BOOKS OF INTEREST

BY Henry Schott

Cell and Microbe

Cell and Microbe Science Fair Projects Using Microscopes, Mold, and More, by Kenneth C. Rainis, Enslow Publishers, Inc. 2005, 112 pp with appendices and index.
Appendix A: The Microbe Identification Guide, Separates bacteria, Microfungi and Protists. Then the key suggests 12 bacterial groups students may see. Microfungi are categorized as basidiomycetetes, molds, yeasts, deuteromycetes and zygomycetes. Six fungi you may see are listed. The key to the protists is slightly longer ending in 26 organisms that students may see.
Appendix B: Microscopy and Image Processing provides a few suggestions including Image J developed by the National Institute of Health. A few other image processing programs are listed.
Appendix C: Science Supply Companies lists 12 sources of materials including several biological supply houses.

Using the Microscope, A Guide for Naturalists, 1984 Dover Pub.1991 by Gravé, Eric V.:

Ten Chapters, Resources, References, Glossary, Notes and Index. 193 pp

Samples: Chapter 3 Special Methods of Illumination includes Rheinberg, Polarization, Incident, Modulation Contrast, Phase Contrast Interference and Fluorescence

Clearly out of date in some material such as photomicrography, the description of various animals and plants that can be observed under the microscope make this a good source of information for amateurs..

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Replica of one of Leeuwenhoek's microscope.

CELLPHONE AND MICROSCOPE VIDEO: UK ANIMATORS USE CELLPHONE AND MICROSCOPE TO FILM SMALLEST STOP-MOTION ANIMATION EVER

By Rebecca Boyle Posted 09.20.2010

Using a Nokia N8 smartphone and a CellScope, the team behind the Wallace & Gromit series has made the world's smallest stop-motion animation film.

Follow 0.35-inch-tall Dot as she runs through an obstacle course made of British currency, rides a bumblebee and stitches her way out of trouble. The music is catchy too.

Animators at the UK studio Aardman used a 3D printer to make 50 different versions of Dot, because she is too small to manipulate or bend like they would other stop-motion animation characters. The figurine's tiny features stretched the limit of the printer — any smaller and it would be hard to make distinct limbs. Each one was hand-painted by artists looking through a microscope.

Directors Ed Patterson and Will Studd attached a **CellScope** (winner of a PopSci Best of What's New award in 2008) to a Nokia N8 12-megapixel camera to **film** Dot's struggle in her microscopic world. They said Nokia commissioned them to make the film in celebration of CellScope's potential to improve medicine in the developing world.

CellScope is the brainchild of Daniel Fletcher, a bioengineer at the University of California-Berkeley, who combined a cell phone camera with a 50x magnification microscope.

To see the Cell Scope: http://europe.nokia.com/find-products/nseries#

Wow! What a Day! BUG DAY

I wanted to send a very big THANK YOU to all of you who joined us again this year and those who came for the first time for BUG DAY 2011! 987 people came to see all your bugs, wares and you. Bug Day remains the most popular family event day of the year at the Randall in very large part due to your wonderful contributions.

From viewing bugs under the microscope and watching beekeepers work the bees, to touching live giant bugs and making lip balm, to racing in the Insect Olympics and dancing to the music of the Honey Tones, Bug Day could not happen without each and every one of you. Teaching people to understand and appreciate all that insects do for us is our reason for Bug Day. I appreciate all of your enthusiasm, expertise and willingness to give us a whole day with you. The Randall Museum is honored to have such wonderful volunteers!

Thank you very much. And hopefully, we'll see you next year so that we can do it all over again!

Nancy Ellis

ONLY 126 YEARS AGO– SFMS WAS ALIVE, ACTIVE AND GOING STRONG

San Francisco had burned down several times and had been rebuilt. The San Francisco Bulletin was sharing news with the New York Times who published such news as what occurred at the last meeting of the San Francisco Microscopical Society.

F.L. Howard had picked up a Nudibranch, a sea slug, near the Pacific Mail docks. Nudibranchs have no shell and in some cases are beautifully colored with naked gills protruding from their backs. They have " six tree-like branchiae or tentacles, nearly as long as the body itself, with waving, feathery, palmate branches, tipped with coral and covered along their sides with downy filaments" reported the New York Times on November 20, 1885. Since these sea slugs are quite small, often only one half to an inch in length, they are best seen in their natural environment. HS

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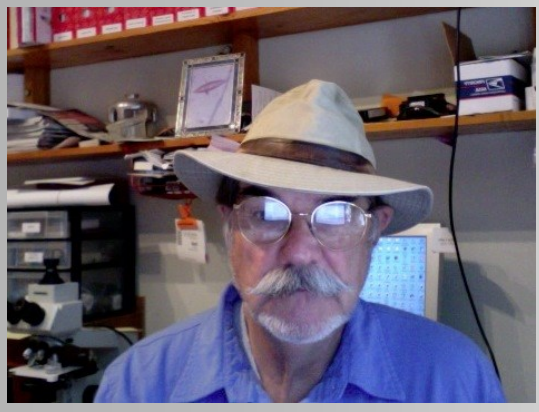

Wayne Lanier, Ph.D., Author of our main article/

Magnification not needed

Child & parent look at bugs at SFMS's display on Bug Day at the Randall Museum.

SFMS Bug Day Display table: R. Griffin, P. Werner, M. Chan & M. Kan on far right.