Newsletter

Of the

New York Microscopical Society

1 Prospect Village Plaza
(66F Mt. Prospect Avenue)
Clifton, New Jersey 07013-1918
GPS: Latitude 40.8648N, Longitude 74.1540W

Summer 2012 | **N.Y.M.S. (973) 470-8733** | **Volume 6 (26) Number 6**

Summer 2012 Edition

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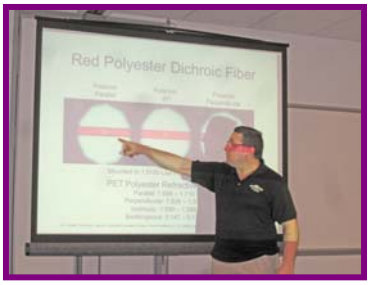
Microscope Day at John Jay, April 26, 2012

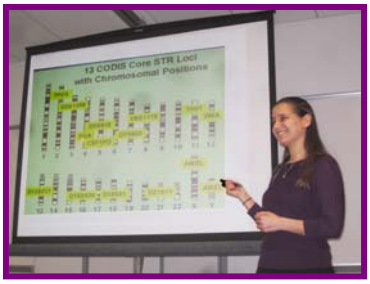
(Report on Page Three)







Save a Tree: Get The Extended Newsletter: By Email Only

Board of Managers

Diaczuk, Peter, pedicopete@earthlink.net; (212) 237-8896, ... Expy June 2013, ...President

Scott, John, nyconsnfdn@aol.com; ..... Expy June 2013, ...Vice President, Program Chair, Archivist

Pollinger, Mel, pollingmel@optonline.net; (201) 791-9826, ... Expy June 2013, ...Treasurer, Editor, Librarian

Klaus, Angela, Ph.D., klausang@shu.edu; ..... Expy June 2013, ...Secretary, Education Chair

O'Leary, Don, dkoleary@verizon.net; (201) 368-8849, ...Expy June 2013, ...Curator, Facilities Manager

Reffner, John A., Ph.D., jareffner@cs.com; (203) 348-8098, Expy June 2014,.....Awards Chair, Past President

McCann, Mary, mccanns@tiac.net; (617) 484-7865, ... Expy June 2015, ...Membership Chair

Huemmer, Craig, chuemmer@hotmail.com; ..... Expy June 2015, ...Board member

Mayer, Gary, mayer@co.somerset.nj.us; ..... Expy June 2014, ...Board member

Perlowitz, Seymour, perlowitzs@hotmail.com; ..... Expy June 2013, ...Board member

Reffner, John Jr., jrr1lp@gmail.com; (cell): (215) 527-1882, ... Expy June 2014, ...Board member

Scal, Roland, Ph.D., rscal@qcc.cuny.edu; (718) 631-6071, ...ExpyJune 2015, ...Webmaster

Dues and Addresses
Please remember to mail in your Dues to Mary McCann, Membership Chair (see this page for address).

Junior (under age 18) $10 Annually
Regular $30
Student (age 18 or above) $20 Annually
Supporting $60 Annually
Corporate (includes one advertisement in NYMS News) $175 Annually
Life $300 (payable within the year)
To avoid missing notices: Notify Mary McCann and Mel Pollinger if you have changed your address, phone or email.

Awards Given by the New York Microscopical Society
The New York microscopical Society takes great pleasure in recognizing and rewarding individuals who have contributed to either the activities of the society or to furthering microscopy. These awards are described in our website and in a pdf file for our email newsletter recipients. All members are eligible to nominate individuals for these various awards, and are encouraged to do so.
John A. Reffner, Awards Committee Chairperson

Awards Committee
Chair: John A. Reffner
Members
Jan Hinsch
Don O'Leary
Mel Pollinger


Mel Pollinger, Editor

To Order Your NYMS Lapel Pins
Send a check in the amount of $12.00 per pin to:
New York Microscopical Society
c/o Mel Pollinger, 18-04 Hillery Street, Fair Lawn, NJ 07410. To avoid shipping & handling charges, pins may be purchased directly at any NYMS meeting for $10.00.

The Mission of the New York Microscopical Society is the promotion of theoretical and applied microscopy and the promotion of education and interest in all phases of microscopy.

Alternate Meeting Notifications
Please note that due to time constraints in publishing, some meeting notices may be available by calling Mel Pollinger at 201-791-9826, or by visiting the NYMS website.

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Dues for 2012 is now due!

Buy and Read a Good Book on Microscopy.

From page 1

Report On Microscope Day 2012

by Peter Diaczuk

"The tenth annual Microscope Day" at John Jay took place on Thursday April 26th. The traditional event was well attended by John Jay College forensic science students. This year's event took place in the new addition to the College's Tenth Avenue building, which provided a pleasant atmosphere and change of scenery. Two microscope vendors also participated in the festivities, which allowed the attendees an opportunity to test drive some new microscopes and accessories. Jack Kolator from I Miller Precision Optical Instruments displayed Leica microscopes and Alex Lobozar from Micro-Optics displayed Motic microscopes. Both display tables were crowded during the breaks between the presentations and the microscope representatives were very accommodating".

Outstanding May 2012 talk by Dr. Stanislaus

"Examples of Contrast Enhancement in Human, Digital and Optical Imaging" Was an exciting and useful presentation. We have all seen examples of the various types of optical anomalies shown; Dr. Stanislaus not only explained the "whys," but also demonstrated the cures. Mel

In Memorium – Mitchell A. Sieminski

Mitchell A. Sieminski, a long-time Life member of New York Microscopical Society, passed away in the early morning of May 29, 2012. …continued

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Mr. Sieminski served as President (1970-1971) and also had held positions on the Board of Managers as Vice-president, Librarian and the Awards Committee. He was one of the most active members of the Society for many years. Later on in life he was unable to attend meetings, but as his surviving spouse, Ann writes, "He liked being a member of NYMS and enjoyed receiving the Newsletter even if he could not participate."

Our sincere and deepest condolences,
Mitchell's friends at NYMS

From information received from Ann Sieminki by letter and The New York Microscopical Society Centennial Yearbook 1877-1977. Picture (1970-71) from Yearbook.

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Junior NYMS Member, Jane Cohen Wins Scholastic Awards

Jane actually came home with a whole stack of certificates, a patch, a pin and the two trophies this week. Project Extra is a gifted program that Jane has been in since 4th grade (the first year she was eligible to participate). They take a challenging math test and Jane ranked second among all the Project Extra students in our district. (I understand that some schools officially submit students' scores to compete nationally, but since it's only administered to Project Extra kids in our district, which would be an unfair advantage, they don't submit the scores further).

She was a member of Mathletes which won her the "Highest Individual Score Math Olympiads" trophy for her elementary school. In the five 5-point tests administered this year, Jane earned 22 out of 25 possible points. (She also received a patch and gold pin in recognition of being in the top 50% and top 2% respectively.

She also received certificates in Spanish, chorus, math, recorder, Presidential Gold and a NYSSMA medal for singing.

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NYMS Welcomes Visitors

Although most NYMS events and meetings are held in Clifton, New Jersey on Sundays, the building may be opened for visitors at other times providing an appointment is made with Don O'Leary or Mel Pollinger at least two days prior to the desired appointment time. NYMS Headquarters at Clifton, NJ will be open by appointment only to members from 8:00pm to 10:00 pm most Tuesday evenings.

Those members wishing to visit must call Don O'Leary or Mel Pollinger to confirm. Don's cell-phone number is (201) 519-2176 or email: dkoleary@verizon.net. Mel's Home phone number is (201) 791-9826 or email: pollingmel@optonline.net

***From The Editor*… if you have email:**
Getting the newsletter by email means you receive an **extended pdf version** that cannot be sent by "snail mail." Even if you continue your USPS delivery of the newsletter, NYMS needs your email address for reporting priority events and special news. Being able to contact you by email means better communication between us■■ Mel

Dues for 2012 is now due!

Need to use a Microscope?

The various microscopes that are presently set up on the main floor of the New York Microscopical Society building in Clifton, N.J. are there for the use of its members.

Microscope Cleaning Kit

A complete set of tools and accessories to keep your microscope in optimum operating condition. The kit is put together by our Curator/Educational Chairman and available directly from NYMS for only $35.00 plus shipping & handling, or may be purchased at a meeting. Call or email Mel Pollinger or Don O'Leary for details (see page two for contact numbers).

Also: Slide boxes **100 capacity**, used: $5.00 while they last

Answer to Mystery Photo for May 2012

Drop of water on a pine needle:
Correctly guessed by Jay Holmes, Department of Education, American Museum of Natural History.

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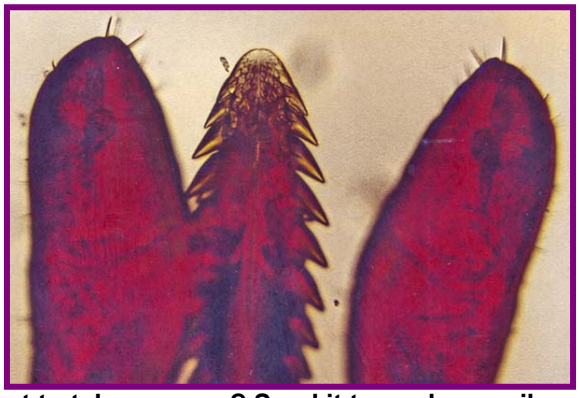
Mystery Photo for Summer 2012



Want to take a guess? Send it to me by email or call me: pollingmel@optonline.net, (201) 791-9826

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Additional Historical NYMS Supplements Email Newsletter recipients will also be getting copies of NYMS Newsletter pdf back-Issues from 2007. Copies of older newsletters will be sent as I convert them.

Got something you want to sell, trade or publish in the Newsletter and/or on the website? Write, call or send an email message to:
201-791-9826 or pollingmel@optonline.net (images ok)
or
Mel Pollinger, Editor
NYMS Newsletter
18-04 Hillery Street
Fair Lawn, NJ 07410

EASTERN ANALYTICAL SYMPOSIUM & EXPOSITION

Supporting Member

NYMS Newsletter Extended Section, Summer 2012

In This Section: Directions to NYMS

- Dues/Membership form
- NYMS Sale Items & Image
- Abstract on Stuttgart John Scott
- Insect SEMs – Dartmouth
- NYMS Outreach – John Scott
- NYMS Spring course images
- Inter-Micro notice
- Microscopic Vision Notice (out of State)
- Quartz Wedge scale – Mary McCann
- Vital Stains – R. Howey
- Scale factors – A curiosity
- Sales items/micro image

Directions to NYMS Headquarters

One Prospect Village Plaza
(66F Mount Prospect Avenue)
Clifton, NJ 07013

GPS: Intersection of Colfax & Mt. Prospect: Latitude 40.8656 N, Longitude 74.1531W,
GPS: Our building: Latitude 40.8648 N, Longitude 74.1540 W

From George Washington Bridge:
Take Interstate Route 80 west to Exit 57A, Route 19 South. Take Route 19 to Broad Street and continue two lights to Van Houten Avenue. Turn Left. Go to second light, Mount Prospect Avenue and turn left. Building 66F is on the left side , one and a half blocks from Van Houton.

From Lincoln Tunnel:
Follow exit road to NJ route three west. Continue to Bloomfield Avenue exit. Turn right to Circle and go three quarters to Allwood Road West. Mount Prospect Avenue is a few blocks on the right (a small street) Turn right and go to first light (Van Houton) continue. Building 66F is on the left side , one and a half blocks from Van Houton.

From North:
Take Garden state Parkway South to Route 46 Clifton Exit. On 46 Make second exit to Van Houton Ave. Continue to third light Mount Prospect Avenue and turn left. Building 66F is on the left side , one and a half blocks from Van Houton.

From Route 46 coming from west:
Take Broad Street Exit in Clifton and follow Directions above from GW Bridge.

From route 46 coming from East: Take Paulson Avenue Exit in Clifton and follow to Second light, Clifton Ave turn right. Go to next light, Colfax, turn left, go three blocks and turn right on Mount ProspectAve.. Building 66F is half block on right.

Public transportation from NY:
Take NJ Transit train from Penn Station to Secaucus Transfer Station. Change trains to Bergen Line to Clifton (call NJ Transit for schedules). From Clifton Station cross under tracks to first street and go left one block to Mount
Prospect Street, turn right and Building 66F is one half block on Right.

If you plan to come by bus or train, please copy the links below into your browser:
http://www.njtransit.com/sf/sf_servlet.srv?hdnPageAction=TripPlannerItineraryTo
http://www.njtransit.com/sf/sf_servlet.srv?hdnPageAction=BusSchedulesP2PTo
http://www.njtransit.com/sf/sf_servlet.srv?hdnPageAction=TrainTo

Dear NYMS Member,

Dues Are Due in January

NYMS Membership dues for 2012 are now payable. We are in the process of setting up a full program of speakers, courses, workshops and celebrations at our Clifton headquarters in 2012. NYMS values your support and participation.

Please make sure to include your current email address. Email communications are particularly useful for announcing any short-term program changes, and provide convenient means for sending supplementary materials. In addition email saves paper and postage - and saves you space. If you have a web site related to your microscopy interests please let us know – we'll add it to the roster.
And--Please include any of your Contact information that has changed in the last two years.

NYMS MEMBERSHIP CONTACT INFORMATION
Name:
Email address: (please print clearly) _____
Address for Newsletter? Email :__Y/N Home _____
Work _____
Microscopy Related Website _____

Address:
Telephone: Work_____ Home:_____

Microscopy interests:
I do Light ____Electron _____ Other (what?) _____ microscopy
I use microscopes at Work _____ Home_____
I use microscopes for Research _____ Teaching _____ QC _____ Hobby _____ other _____
Mostly I view specimens that are: Biological ___ Industrial _____ describe? _____
Or Other (what?)
I also enjoy viewing (what?)_____
In microscopy I am a Professional _____Amateur _____ Beginner_____

Are you interested in working on NYMS Committees? Awards ____ Membership ____ Education ____ Library ____ Finance ___ Curator ___ Program ____ Publications ____ History _____

Checks should be made out to NYMS. Updated contact information may be included with your check to the address below, or it may be sent by email to me at mccanns@tiac.net,
Mary McCann
Regular Membership: $30 per year. Supporting Membership: $60 per year. Life Membership is $300, payable within 1year Corporate Membership: $175
Junior Membership (18 or under): $10
Student Membership (over 18 & a student) is $20

Thank you for your response!

Mary McCann
NYMS Membership Chair
161 Claflin Street
Belmont MA 02478

A New Method for Conserving Weathered Surfaces of Copper and Bronze

JOHN SCOTT[1]

[1]New York Conservation Foundation, 261 Fifth Avenue, Rm 2000, New York, NY, 10016 United States of America nyconsnfdn@aol.com. Thanks to Alicia F. Boan, and to Jari Heinonen.

Conservators often need to mitigate unsightly patina on bronze or copper forms. Most conservation of weathered outdoor sculpture blocks up hues of weather patina by washing and waxing, or removes some metal during repatination. Textures change as we erode or reform metallic surfaces, and if corrosion products have psuedomorphically replaced metal surface, their removal is loss. With a new method developed using bioactive media which can be independently produced but are readily available, unsightly mottled greenish to black patinas give way to more pleasing reddish browns, with minimal or no loss of form.

The method in practice

1. Apply gel or immerse in liquid treatment medium, allow time for action (time based on pretests and observation). 2. Remove medium and rinse well. 3. Keep surface wet while removing softened matter. 4. Rinse well and allow to dry. 5. After treatment, apply coating(s) as needed. Greenish to black colors are replaced by reddish browns.

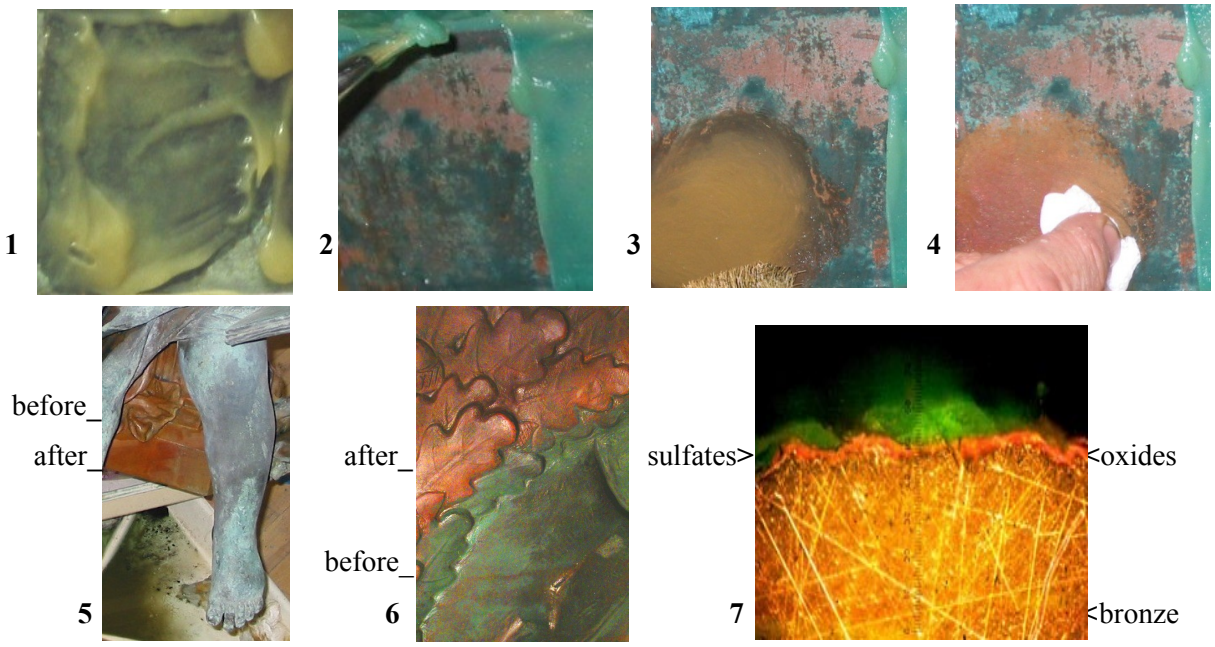


Fig. 1-4: Steps in treatment method. Photos: J. Heinonen & J. Scott, at NYCF, NYC, USA

Figs. 5, 6: Before/after, detail of figure, close detail of plaque. Photos: J. H. & J. S., at NYCF

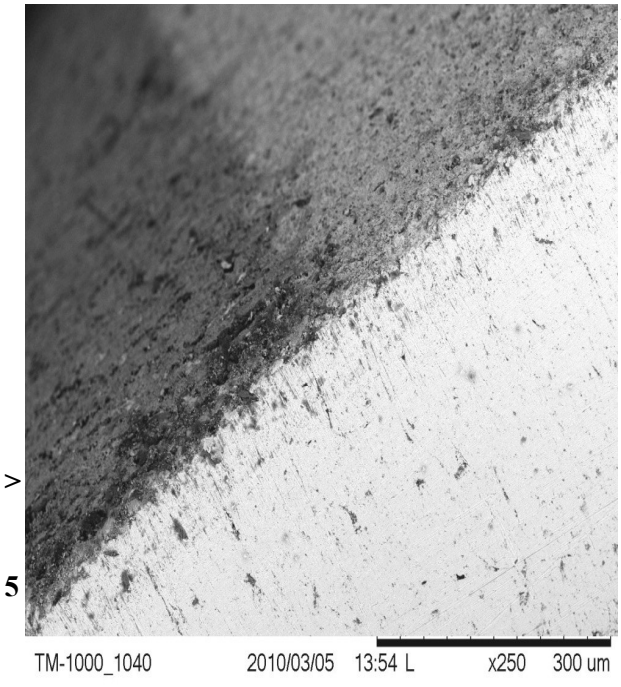
Fig. 7: Weather patina structure, magnified cross section. Photo: R. Lodge, Oberlin, OH, USA

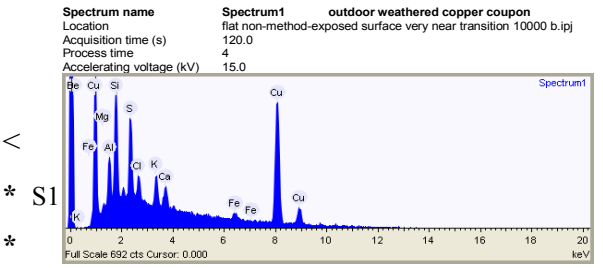
Weather patina on bronze and copper

Weather patina covering bronze and copper corroded over time in outdoor moisture and air, generally at first comprises only metal oxides such as cuprite (oxidized copper), in a dense reddish stratum directly integrated with underlying metal at an interpenetrating interface. Over long periods these patinas usually also comprise copper hydroxysulfates such as brochantite (reduced and hydrated copper), in a less dense greenish outer layer directly interfaced with the underlying oxides. Weather patinas always entrain diverse matter deposited from air. Many investigators over many years have studied outdoor corrosion of cultural heritage and other metal surfaces, often with some attention to patinas.

Experiment:

Hypothesis: Treatment removes greenish sulfates strata, exposes reddish oxides strata, without exposing metallic surface. Experimental: Our treatment method was applied to part of the surface of a coupon cut from weathered copper flashing, while adjacent surface was protected from treatment by waterproof adhesive tape masking, which was removed after treatment. Finally, the surface's resulting adjacent untreated and treated areas were imaged using SEM, and analyzed using EDS. Results support the hypothesis.

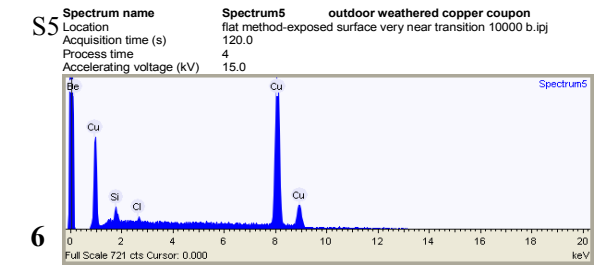




Quantification method All elements (normalised)

Summary results

Element	Weight %
Magnesium	1.4
Aluminum	5.2
Silicon	11.4
Sulfur	8.6
Chlorine	3.0
Potassium	3.9
Calcium	2.7
Iron	2.7
Copper	61.1



Quantification method All elements (normalised)

Summary results

Element	Weight %
Silicon	5.1
Chlorine	1.5
Copper	93.4

Fig. 5: Patina coupon surface: >untreated, <treated; edge: *CuO2&Cu*, Cu. Photo: J. Scott

Fig. 6: EDS spectra of Fig. 5 patina surface: S1 untreated, S5 treated. Spectra: J. Scott

Biochemical process

Literature and observation indicate a sulfates-oxidizing biochemistry enables our treatment, with treatment media adapted from biohydrometallurgy. In bioleaching and biomining, metals are recovered from leach solutions, after ore minerals have been digested via aqueous redox reactions catalyzed metabolically by bacteria such as *Acidithiobacillus ferrooxidans*.

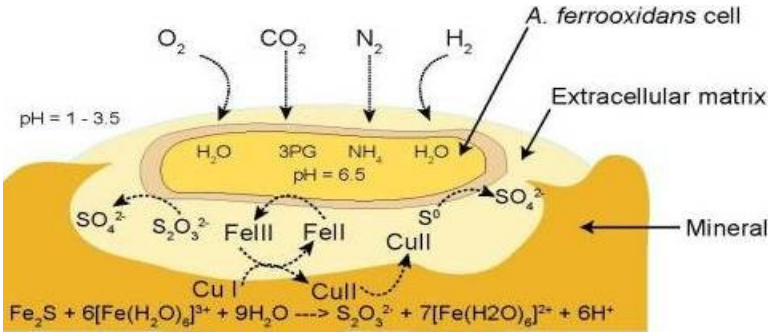


Fig. 6: *Acidithiobacillus ferrooxidans* catalysis of oxidation. Diagram: Valdes, et.al.

Biooxidation is already used in removing corrosion products from objects made from silver, copper, iron, aluminum and their alloys. Our biooxidative treatment works too quickly for the acidic treatment medium to erode the patina's oxides layer or underlying metal, and under acidic conditions *A. ferrooxidans*' respiration does not engage copper oxides.

Weather patina's layered structure, its layers' distinct compositions of reduced copper and oxidized copper, *A. Ferrooxidans*' oxidative respiration in aerobic acidic media, and conservator skill, are all key in this biooxidative treatment for outdoor patinas of copper and bronze. They allow us to soften and remove weather patina's outer sulfates layer, in order to exchange unsightly mottled greenish to black patinas for more pleasing reddish browns, with little or no alteration of texture.

References

Vernon, W. H. J., 'A laboratory study of the atmospheric corrosion of metals'. *Trans. Faraday Soc.* 27, 31, ff. (1931, ff.) and see Vernon and Whitby, *J. Inst. Metals* 42, 44 (1929, 1930)

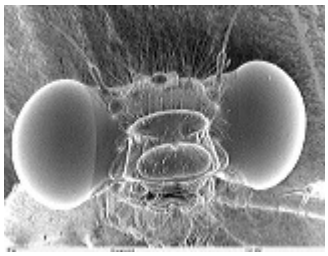
T.E. Graedel, et al, 'Copper Patina Formation'. *Corrosion Science* **27**:7 (1987)

Strandberg, H. (1997) *Perspectives on bronze sculpture conservation: modelling copper and bronze corrosion.* Göteborg: Göteborgs Universitet, AfOK

C. Chiavari, et al, 'Composition and electrochemical properties of natural patinas of outdoor bronze monuments', *Electrochim. Acta.* 52, 7760-69. (2007)

Valdés J, et.al., 'Acidithiobacillus ferrooxidans metabolism: from genome sequence to industrial applications'. BMC Genomics. 2008 9:597 http://www.ncbi.nlm.nih.gov/pubmed/19077236

Insect Part1 SEM



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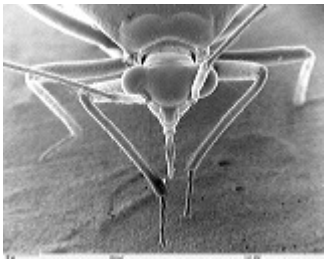

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417K

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465K

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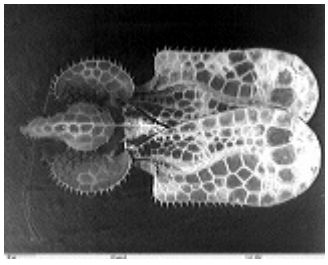

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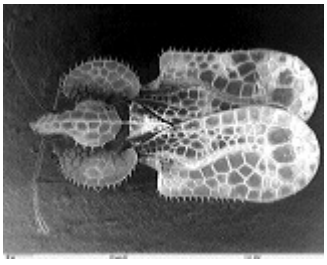

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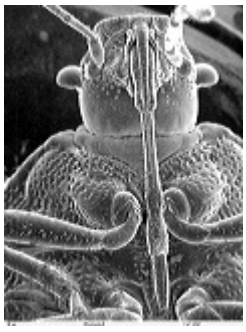

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422K

11Aphididae60
1560x1246
476K

NYMS Outreach Central Park images by John Scott 19May2012

NYMS's participation in Central Park's "On A Wing" program on Saturday was a hit, and that is largely thanks to the involvement of two volunteers new to this kind of outreach. They are Beatrice de Baere, Guy's daughter, and John Scott. John and his wife kindly stayed in NYC last weekend so he could be at Central Park by 10:30 a.m. Saturday to set up scopes with me in the NYMS area and help the many visitors who kept us busy until pack-up time at 3:00 p.m.. Beatrice arrived by bike at our designated site near the Belvedere Castle in time to work during the entire 3-hour program, then stayed on to help with the packing-up. They were STELLAR WORKERS! John even brought his camera and tripod and shot some photos, in response to Mel's plea for images to publish in the newsletter. Thanks to my urgent plea for additional NYMS volunteers, and to John Scott and Beatrice de Baere for getting involved, NYMS was spared the risk of not complying with a commitment to set up scopes in the Park that day if I couldn't come. The three of us had pleasant on-location cooperation from the two Parks officials who were in charge of the On A Wing program, which included two other invited event providers besides NYMS. The only compensation our Society requested was to be named in Central Park's online announcement describing the On A Wing Program's events. That happened. Guy and I ensured that adequate supplies of inspection scopes, charged-up compound microscopes, and slides & coverslips would be on hand. I brought along some feathers and winged insects for folks to view, so we wouldn't have to count on Park staff to supply natural specimens -- although this year they did. Guy had arranged with Tanja that all the scopes he and I had separately carried to the Park for the May 19 event would be stored in a locked cabinet until needed for the June 23d "Family Fishing Celebration." Tanja and I placed all 9 compound scopes (in their white plastic shipping containers plus plastic bags) and 3 little scopes (in the metal suitcase) and the rolled-up banner on two shelves of a metal cabinet that is kept locked, on the staff-only lower floor of Belvedere Castle.

NYMS Outreach Central Park images by John Scott 19May2012

NYMS Outreach Central Park images by John Scott 19May2012

NYMS Spring Microscope Use & Polarized Light Courses Spring 2012








NYMS Spring Microscope Use & Polarized Light Courses Spring 2012

Nicole Pizzini discusses the first use of artists' pigments at Inter/Micro 2011.

Download:

- Inter/Micro 2012 Attendee Registration Form

Inter/Micro 2012: July 9-13

Join us for Inter/Micro!

Inter/Micro is an internationally recognized conference that attracts microscopists from all areas of light and electron microscopy. Research presentations given during the first three days covered techniques and instrumentation, environmental and industrial microscopy, and forensic and chemical microscopy.

We welcome your questions and comments about Inter/Micro. Please contact:

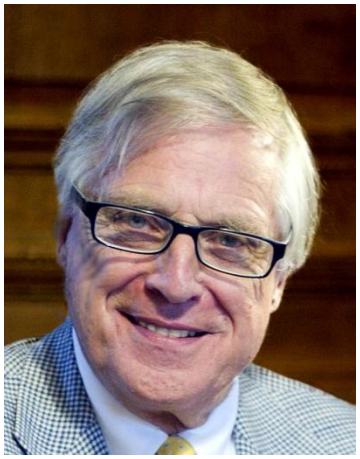
Inter/Micro
McCrone Research Institute
2820 S. Michigan Avenue
Chicago, IL 60616

phone: 312-842-7100
fax: 312-842-1078

intermicro@mcri.org

Sign up for our mailing list to receive information about Inter/Micro, our courses, publications, and other events

Gwinnett Technical College &
The Georgia Microscopical Society
Welcome guest speaker



"The world's leading expert"
Brian J. Ford

THE BIRTH OF MICROSCOPIC VISION

Over 350 years ago the first scientists began to look through tiny lenses at the microscopic world. Ever since, we have been perplexed at the remarkable observations they made - and nobody has known how they made their discoveries. Now, for the first time, we can see exactly what our ancestors observed, back in the 1600s - more than 100 years before the foundation of the United States! In a remarkable presentation, Brian J Ford will take us through those lenses dating from the birth of science, and show us the sights that they saw – and which led to today's world of cells, genetics and high-power magnification.

Brian J Ford is a leading research scientist and a specialist on the microscope. Search for microscope research on Google and he comes top of the list of almost 50 million sites worldwide. The author of more than 30 books (most on the microscope) and hundreds of research papers, Professor Ford first lectured in the USA over 40 years ago and now lectures all around the world. He is based at Cambridge University, England, and has hosted many TV programs in many countries. Professor Ford spoke on this topic at the prestigious Royal Society last year in London and his findings were reported in Nature:

http://www.nature.com/news/2011/110304/full/news.2011.116.html

Life Sciences Building
July 5th, 2012
7:00 pm
Building 900
Room 1109

7:00 pm:
Tour the facility and visit with the Georgia Microscopical Society

8:00 pm:
Presentation by Brian J. Ford

Please RSVP
Rich Brown
rbrown@mvainc.com
Jenny Hedges
jhedges@gwinnetttech.edu

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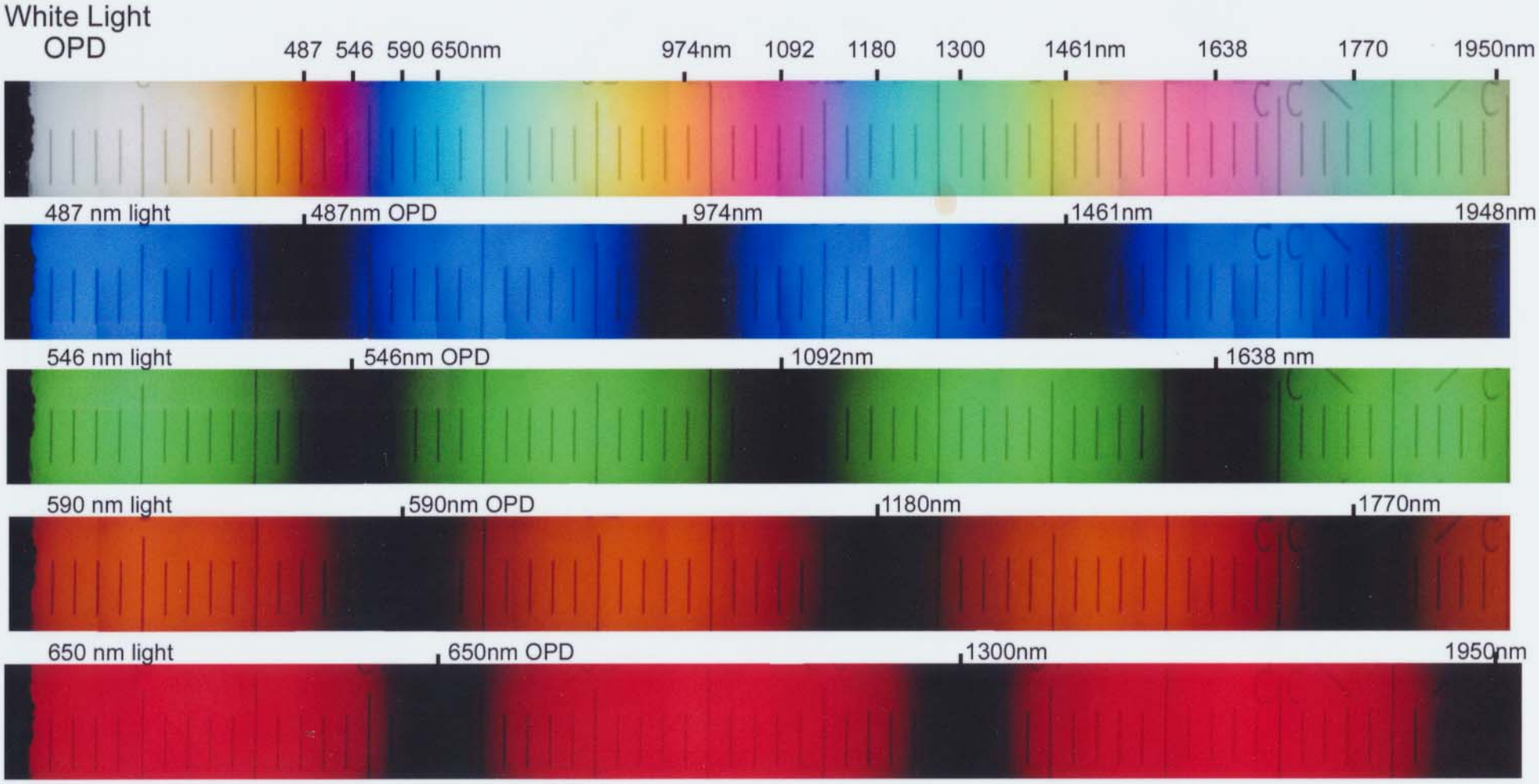

Vital Staining for Protozoa and Related Temporary Mounting Techniques

by Richard L. Howey, Wyoming, US

For the amateur microscopist, the use of vital stains can provide additional information about the cytological details of protozoa. Virtually all stains, even the socalled vital stains, are eventually toxic to the organisms. To achieve the desired effects, the stains must be used in extremely dilute solutions ranging from dilutions of 1:5,000 to 1:500,000. Although vital staining is not much used in modern microscopy, the older microscopists regarded this technique as a source of important information. They experimented with a wide variety of stains;most of which, it is worth remembering were developed for the textile industry. As a consequence of these investigations, a series of stains were identified as being effective as vital stains, and these, certainly are the ones to try out first, but it should not discourage one from trying out other stains which are not on the list. A very helpful list along with dilution and toxicity is provided by Mc Clung and I will include it here: From *McClung's Handbook of Microscopical Technique*, Paul B. Hoeber, Inc. New York, 1929, p. 395. This chapter on Protozoological Methods was written by D.H.
Wenrich.

Dyes	Minimum Concentration That Will Stain Paramecium	Toxicity: Per Cent dead in one hour
Bismarck brown	1 to 150,000	0
Methylene blue	1 to 100,000	5
Methylene green	1 to 37,500	5
Neutral red	1 to 150,000	3
Toluidine blue	1 to 105,000	5
Basic fuchsin	1 to 25,000	30
Safranin	1 to 9,000	30
Aniline yellow	1 to 5,500	0
Methyl violet	1 to 500,000	20
Janus green B	1 to 180,000	40

Wenrich comments on several other dyes that are useful for "intravitam" staining, among them Nile blue (1 to 30,000) and Rhodamine (1 to 20,000).
Some of the vital stains are rather general in their action and provide nice contrast for investigating cytological detail. Others show more specific detail as a consequence of their specific chemical properties. For example, Janus green B is a specific stain for mitochondria and neutral red, while providing general

cytological staining, is also a specific stain for "neutral red globules" mentioned by Gravé. [Gravé, Eric V., Discover the Invisible: A Naturalist's Guide to Using the Microscope, Prentice-Hall, 1984.] In addition to being a stain, neutral red is a pH indicator and will stain newly-formed food vacuoles a bright red. As the process of digestion proceeds, these vacuoles will take on a yellowish color indicating a shift to an alkaline pH as the digestion takes place.

While vital staining provides contrast, one should not ignore the possibilities of discovering additional detail by employing additional means of contrast, For example, in a previous note, I discussed the use of powdered carmine as a means of demonstrating food vacuoles in organisms, such as, *Paramecium*. I took some paramecia treated with carmine and looked at them with Nomarski differential interference contrast. The results of observing the effects of carmine with brightfield were very good, but with Nomarski, the results were truly spectacular.

Experimenting is imperative! Try brightfield, darkfield, oblique illumination, phase contrast, Rheinberg—in short, every type of optical contrast available to you. Another helpful technique, which does not actually involved staining, utilizes colored particles that are essentially inert and non-toxic to provide a colored background. For example, India ink can be used to provide a background "field" which can greatly facilitate the observation of the locomotion of certain protozoa. When one achieves a good concentration, the action of cilia, undulating membranes, and flagella become much clearer as the particles are moved around by the activity of these organelles. *Paramecium* and large flagellates are obvious candidates for experimentation, but try this technique also on some of the hypotrichs, such as, *Euplotes* and *Stylonychia*, as well as *Spirostomum*, *Blepharisma*, *Stentor*, and *Vorticella*. In fact, this technique is worth trying on any protozoan which produces strong currents in the surrounding water.

A further technique, which starts out with living organisms, but involves letting them dry up, uses stains which deposit themselves on the pellicle or surface membrane of the organisms, thus revealing the "sculpting" of the surface. This technique only works with protozoa which remain relatively intact during the drying process. The stain most widely used for this purpose is Nigrosin. One can get very nice results with *Paramecium* and *Stentor*. It is important to use samples from rich cultures, for some specimens will show considerable distortion and others will retain their basic features. I have also obtained good results with Toluidine blue. I am certain that will experimentation, one can discover other stains that also give good results.

There are some other techniques not related to "vital" staining, i.e., they involve killing the organisms, but they provide, as temporary preparations, information that can be very helpful in better understanding the morphology of certain types of protozoa. In identifying protozoa, it is often helpful to know the number and form of the nuclei. Slides containing numerous specimens of the organisms being

studied should be prepared. Then place a good-sized drop of methyl green-acetic or acetocarmine at one edge of the cover glass and allow it to gradually be dispersed under the cover glass. However, don't allow the preparation to dry. After a few minutes, the slides are ready to be examined. Methyl green-acetic can be prepared as a 2% solution with 1% concentrated acetic acid added to it. The proportions need not be exact. I have found that the solution works better after it has aged for some months and so, I always keep a stock of aged solution available. Aceto-carmine should be purchased from a supplier and used with caution as it contains 45% acetic acid. Some organisms do not tolerate this procedure well and they disassociate. However, if one has taken care to include on the slide only specimens of the organisms which you wish to examine, this is not always a complete disadvantage. This is fairly easily accomplished by placing a sample of the culture in a watch glass and then using a finely-drawn micro-pipet, transfer the desired organisms to a small drop of water on a clean slide. By having only one species on the slide, even if the cells disassociate (undergo lysis), one is guaranteed that whatever takes up the nuclear stains, did, indeed, belong to the organisms under investigation. Aceto-carmine stains nuclear material bright red and the Methyl green-acetic stains a vivid bluish-green. In general, the amateur should be content with identification at the genus level.

However some organisms are distinctive enough that with careful observation, species determination can be made. A *Stentor* with distinctive bluish-green pigment and a beaded nucleus is assuredly *Stentor coeruleus*. The pigment in this species, which is called stentorin, is dichroic. If one shifts the angle of the light appropriately, the organism has a lovely rose-colored tint. Knowing the form of the nucleus in *Spirostomum* can also help in narrowing the range of possible species, but other morphological characteristics must also be taken into account to get a definitive species determination. The use of Lugol's solution, an aqueous solution of iodine and potassium iodide, can also be very helpful in determining gross morphology. If one does not have access to Lugol's solution, the standard tincture of iodine sold for first aid kits can be substituted. However, since this is an alcoholic solution, try diluting it down with distilled water to minimize distortion. I have used Lugol's solution to make temporary preparations of *Lacrymaria olor*. A significant number of the specimens remain partially extended and few were killed quickly enough to leave the cytostome open. The cytostome in *Lacrymaria* is controlled by a group of proteinaceous rods called trichites. With luck, one may find a few specimens where the trichites are quite distinctive. Flagellates containing bodies which store starch also stain quite distinctively, since iodine is a specific stain for starch.

Certain fluorescent stains (or flurochromes) can be used to make either vital preparations or temporary fixed mounts. CAUTION: Some of the fluorochromes, in the powdered form or in concentrated solutions, are extremely toxic being both carcinogens and mutagens! Therefore, one must exercise great care in preparing solutions and avoid breathing any of the powder or allowing it to come in contact with the skin. Fortunately, the solutions are used in very great dilutions and some

biological supplies houses will provide widely-used flurochromes already in solution. Nonetheless, as with all potentially toxic materials, care must be exercised.

If one has access to a microscope with fluorescence or epi-fluorescence capabilities, there are two techniques which can provide spectacular results. A particularly useful fluorochrome is Acridine Orange. A few drops of a solution of 0.0001% can be introduced to a rich culture of paramecia. Allow this to sit for several days so that the paramecia slowly absorb a sufficient quantity of the stain. when examined using the techniques of fluorescence microscopy, the macronucleus and the food vacuoles of the paramecia fluoresce brilliant light green, cytoplasmic granules fluoresce orange and the so-called Neutral Red globules fluoresce yellow. As one can imagine, this produces a very striking image. An interesting side effect of the use of very dilute solutions of Acridine Orange is a fascinating phenomenon know as hormesis.

Hormesis involves the production of, at least, temporary benefits from a minute amount of a substance know to be toxic at higher levels. The phenomenon is fairly widely recognized, but has certainly not been widely, nor systematically studied in protists. With paramecia, the addition of tiny amounts of Acridine Orange seems to increase their vitality, their resistance to viral infections, and promote reproduction. Hormesis has been recognized for some time in human beings. For example, minute amounts of selenium are highly beneficial to the human diet, but too much can produce toxic reactions.

A second method involves using a somewhat more concentrated solution of Acridine Orange. Here, one can start with a 0.01% solution, but if this proves toxic too quickly, then experiment with dilutions. The object is to get the paramecia (or other protozoa) to absorb the stain fairly fast for a quick examination of nuclei under epifluorescence. Blue-violet fluorescence produces very good results with Acridine Orange. I use a 100 watt halogen light source for this purpose. However, Acridine Orange is an intensely photo-active stain and the cells will undergo lysis (disassociation) in a few seconds as the intense light activates the dye. Nonetheless, one can often get important information from such preparations, e.g., the number and form of the nuclei. To obtain preparations which can be observed for longer periods, I have modified this technique by first exposing the organisms to the Acridine Orange solutions for several minutes and then fixing them. The fixation prevents the disassociation of the organisms.

Even if one does not have access to a microscope equipped for fluorescence, one can, nonetheless, sometimes obtain interesting results using fluorochromes as one would use regular stains. Interesting preparations can also be made using either Delafield's or Ehrlich's hematoxylin. I make up a slide with a cover glass and place a drop of the hematoxylin at the edge of the cover glass and allow capillary action from evaporation to gradually pull the hematoxylin under

the cover glass. This method has a couple of nice advantages. As the stain slowly diffuses across the preparation, one gets differentiated staining. Some of the organisms, on the side where the stain is introduced, may be stained too darkly, but others, in the middle or on the opposite side, will achieve an excellent balance. Hematoxylin is an excellent stain for revealing both nuclei and cytoplasmic detail in protozoa. A further advantage is that both Delafield's and Ehrlich's solutions contain glycerine and so the preparations never completely dry out. I have had some slides that were still in good condition many weeks after I first made them.

Some staining techniques are highly sophisticated and complex, but the amateur, using simple techniques and possessing a willingness to experiment, can achieve interesting and informative results.

Comments to the author Richard Howey welcomed. Howey, Richard L
E-mail Address(es): tunicate@wyoming.com

NYMS safety note: As the author reports many stains are extremely toxic and some are both carcinogens and mutagens! The diluted stains are probably the safest form for the hobbyist and these are available commercially. The Material Safety Data Sheets for these chemicals should be provided by the suppliers, consulted and the appropriate precautions taken before use. In any event these chemicals are not suitable for youngsters.

Main Identity

From: "Richard Mishelof" <rmishelof@dcranch.com>
To: <Undisclosed-Recipient:;>
Sent: Saturday, May 26, 2012 4:56 PM
Subject: Scale factors - Big WOW factor (Thanks Hugh C)

Subject: Scale factors - Big WOW factor

This is an interesting comparative look at scale factors over the entire spectrum of size from less than nano to galactic - interesting perspectives.
Just slowly slide the bar AT THE BOTTOM to the left or right...or use your scroll wheel.
Click on the objects for explanation. The word awesome is usually over-used but not here.

Be sure to go BOTH ways on the sliding scale.

http://htwins.net/scale2/scale2.swf?bordercolor=white

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Photomicrograph by Mel Pollinger