*Research & Perspectives*

# ENVIRONMENTAL INVESTIGATIONS OF PLANTS EXHIBITING DISEASE

***Rhododendron leaves: observations of forms, fluorescence properties & transfection considerations***

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The widespread deterioration of vegetative health has been a growing concern of this citizen researcher in connection with atmospheric geoengineering activities. Observation reveals leaves of many tree/plant species exhibiting signs of disease that include holes and burn-like symptoms as shown on pg 2, figure 2. This report, however, focuses only on leaves taken from the same rhododendron plant which also presents a more rare finding of raised spherical forms ("bumps"). These bumps are visible to the naked eye and can be easily felt by running fingers over the leaf surface pg 3, figure 3. This report combines examination of two different rhododendron leaf samples with focus on the raised spherical formations and findings therein. The specimens were examined using a Barlow modified compound microscope; the procedure is outlined in materials/methods on pg 16. Further investigation was carried out using a chemical digestion technique—outlined on pg 12.

From leaf sample #1 a distorted insect-like form was observed protruding from a raised spherical form as shown pg 3, figure 4. Subsequent investigation revealed fiber/filament forms as well. The initial observation was that the insect-like form looked to be out of the ordinary as specimen was not well formed. Subsequent inspection and research revealed more anomalies when comparing the observed form to typical spider photographs, nesting and reproductive cycles. Please see also http://en.wikipedia.org/wiki/Spider

These observations and comparisons bring into question the methods of transfection, which have been a major research thrust. Transfection is the deliberate introduction of nucleic acids into cells. Surfactants are an integral part of modern day chemistry/bio chemistry. A general definition of surfactants is any molecule with a polar head (hydrophilic) and non-polar tail (hydrophobic). As such, surfactants can span natural, synthetic and bio-mimic molecules. It is advances in cationic surfactants that play a major role in transfection efficiency attributable to DNA having a net negative charge. [1-3,5]. There have also been reports of insects (and other forms such as fibers and crystals) emerging from human skin. While insects erupting from skin and plants might seem like something from a Sci-Fi movie there is, in fact, a scientific explanation if one considers transfection and related chemical compounds (cationic surfactants). The mechanism of surfactant interaction with cell membranes (fusion/disruption) provides a logical explanation bridging both widespread cellular membrane disruption as well as the potential for transfection.

There is a large amount of literature on the subjects of surfactants, transfection and transgenic plants. Agrobacterium, along with surfactants, are involved in gene transfer processes for plants; this includes surfactants such as Silwet L-77 used in the floral dip method [4]. Surfactants are typically considered adjuvants, yet it is often more accurate to categorize them as active ingredients. Case in point, in research conducted by Monsanto it was revealed that; *"The application sites of Ultra and Roundup demonstrated a well demarcated zone of injury showing extensive rupturing of cell membrane in both epidermal and mesophyll cells. Studies using blank formulations without glysphosate confirmed that tissue damage was by the surfactant formulants"*. ..Feng et al 1998 [5]. Further recommended research terms include: *transfection strategies, surfactant mediated transfection*, surfactant phytotoxicity, Agrobacterium/T-DNA[6,7] and transgenic plants.

Bioengineering and chemical engineering activities are intertwined disciplines. Comprehending the role of surfactants in transfection techniques provides a bridge to understanding unnatural manifestations while also explaining wider spread damage to plant and human cells. Simply put, surfactants break down natural barriers. In addition to bringing the subject of transfection into greater discussion a point of this report is the fluorescence characteristics observed in plant leaf damage sites. While chlorophyll does fluoresce[9-11] an observation is made that leaf damage sites displays more intense fluorescence clusters than healthy sections. An underlying question is to investigate if the fluorescence is related to transfection labeling.

The persistent observations of many concerned citizens of aerosol dispersions by aircraft (atmospheric geoengineering) are specifically called into question. Despite denial from officials, "the evidence on the ground" reveals these activities are indeed real and having negative consequences to life on this planet. It should be noted that there is a documented precedent of military release of fluorescent particles in what is referred to as Operation Large Area Coverage in which fluorescent zinc cadmium sulfide particles were released from aircraft operated by US Army Chemical Corp. This information is declassified and available to the public: https://en.wikipedia.org/wiki/Operation_LAC

Hopefully there will be a wider awakening that such operations and data-gathering exercises are not benign and such molecular markers can be harmful. It is the hope of this researcher that the precautionary principle will be exercised more vigorously for the health of this planet. It is understood that exploration and pushing boundaries are part of human nature. However, what might seem like rational design today might very well be deemed irrational tomorrow. Scientific history is littered with stories of old medical modalities that have since been determined to be toxic. History has a way of repeating. Industry and institutional researchers should not fall into the trap of thinking scientific matters are beyond the comprehension or interest of the lay person. Nor should the public at large be so naïve as to think research is always conducted with the best interest of the planet or its citizens. One needs to look no further than the widespread nuclear weapons testing that have taken place [8]. Society has been so busy trying to understand and exploit nature, but it seems that it is really science that needs to be tamed.

Figure 1

Figure 2

Figure 1 Rhododendron plant from which all samples were collected. Figure 2 displaying burn and rust discoloration. This condition is noted across many other plant and tree species. Molecules which promote photo-induced oxidation or photo "bleaching" are in particular question. Careful observation reveals that apparent sites of photo bleaching start as small (dots) and spread into larger areas.

**Figure 3**

Figure 3 - Leaf sample #1 displaying raised spheres being investigated.

**Figure 4**

Figure 4 is the underside of the leaf shown above in Figure 3. Shown directly above in the upper middle of the photo is an insect-like form emerging from within the leaf surface. It is partially camouflaged with leaf underside. The insect-like form was apparently motionless and not well formed. A first impression was that this was out-of-the norm; further research revealed that this occurrence is not in keeping with spider reproduction which involves surface nests and laying thousands of eggs, or that baby spiders are miniature versions of their adult form. Subsequent microscopic inspection are shared on pages 3-4. If spiders gene expression in plants seems farfetched consider the following paper from Nature in 2001: http://www.ncbi.nlm.nih.gov/pubmed/11385464

## Production of spider silk proteins in tobacco and potato

Jürgen Scheller[1], Karl-Heinz Gührs[2], Frank Grosse[2], and Udo Conrad[1]

*[1]Institut für Pflanzengenetik und Kulturpflanzenforschung Gatersleben, Corrensstra®e 3, D-06466 Gatersleben, Germany. [2]Institut für Molekulare Biotechnologie e. V.,*
*PF 100 813, D-07708 Jena, Germany.*Corresponding author (conradu@ipk-gatersleben.de).*

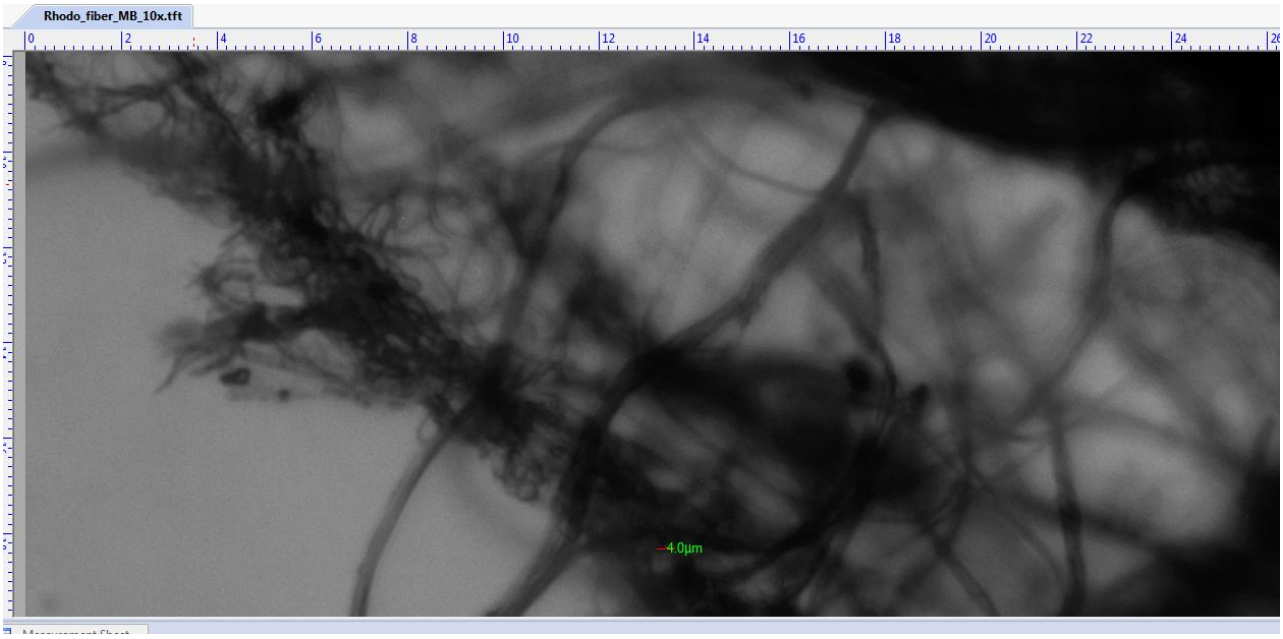



**Figure 5**

Fibers taken from the site of insect-like form emerging form spherical structures. Comparisons to spider web filament is necessary both in terms of visual appearance and also chemical characteristics. However similarities in size and texture would not rule out the involvement of potential bio-mimic spider silk. Replication of spider silk has long been a goal of scientists and has received government funding. See also: http://en.wikipedia.org/wiki/Spidroin, http://en.wikipedia.org/wiki/BioSteel

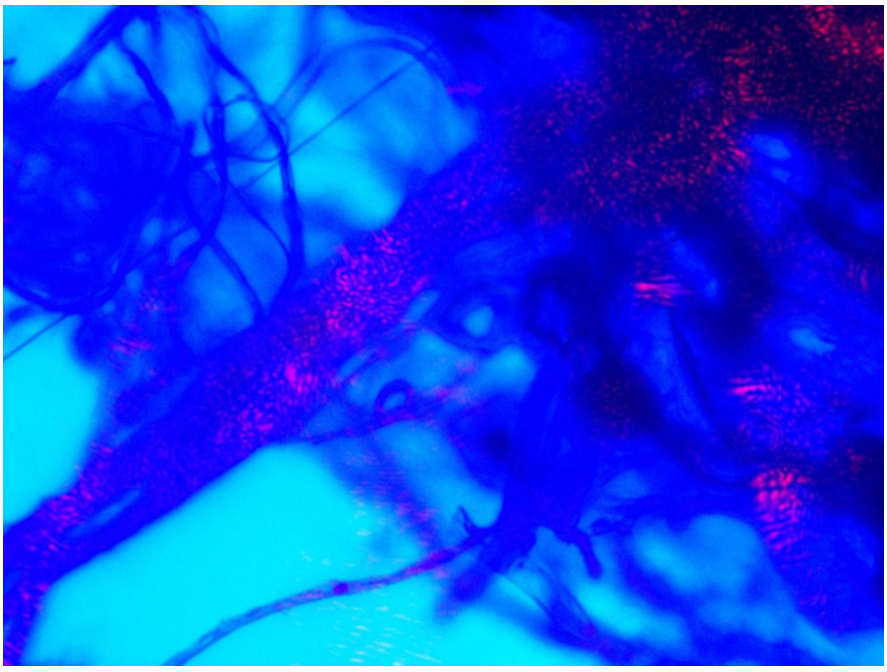


**Figure 6**

The above micrograph is of the fiber forms near the insect/spherical cluster. The slide was prepared with methylene blue and viewed using bottom illumination as well as epi-fluorescent technique using 395nm LED black light.

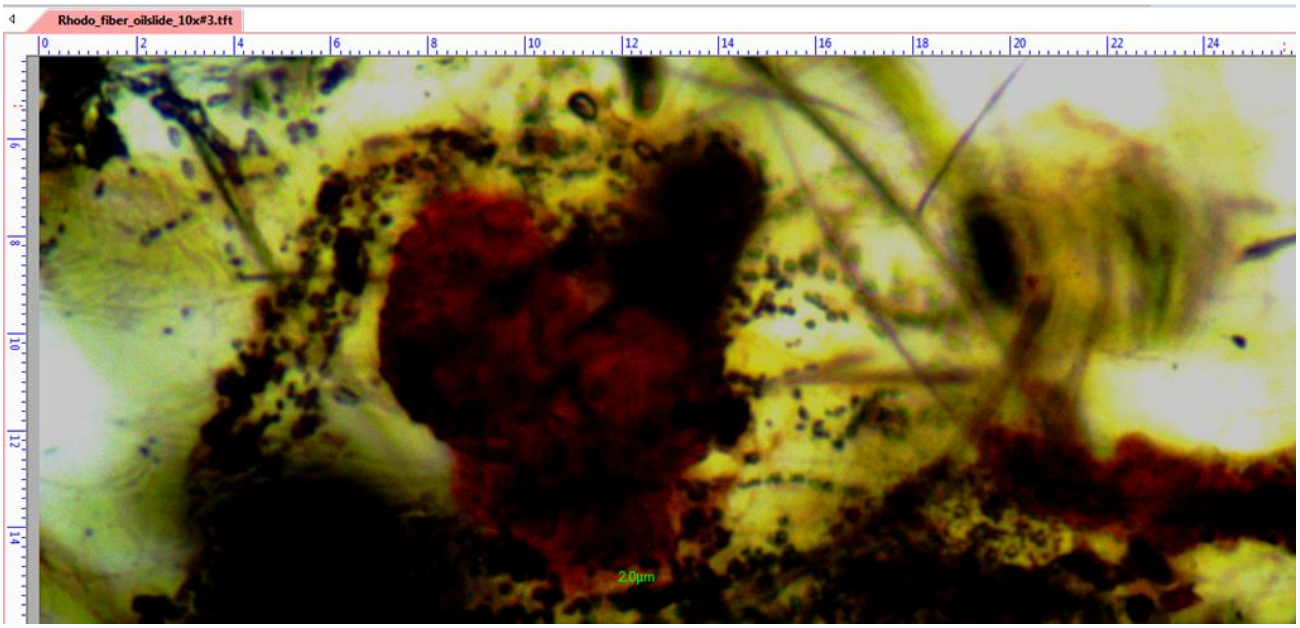



**Figure 7**

The above micrograph is of the site near insect-like form. It shows rust colored aggregates along with fiber and spherical forms. The sample was placed in immersion oil on glass slide and cover slip. Based on reports from other investigators immersion looks to be a useful medium to suspend suspect samples in.

## RHODODENDRON LEAF SPECIMEN #2

The following images are of leaf sample #2 shown in figure 8 below. This leaf displayed the same raised spheres but there were no visible insect-like forms emerging from the leaf. However the leaf was obviously not healthy and did have some anomalous forms that fluoresced upon inspection using 395 nm LED light:

Figure 8

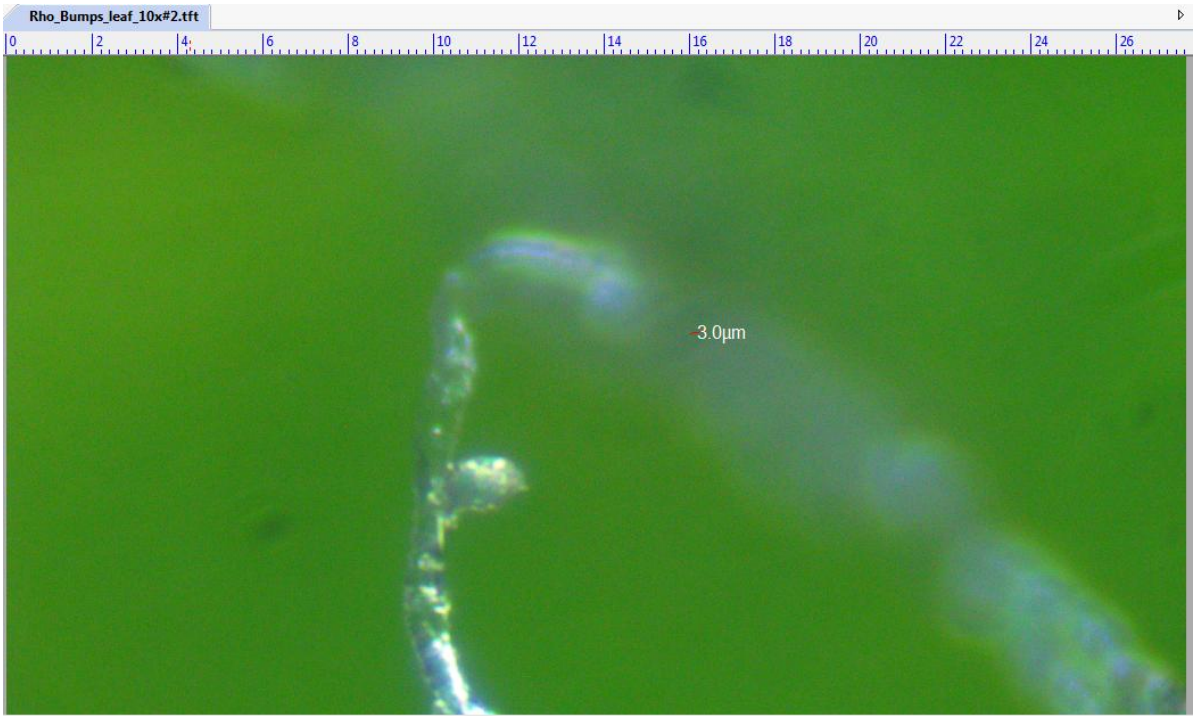



Figure 9

Translucent fiber was observed emerging from leaf surface.

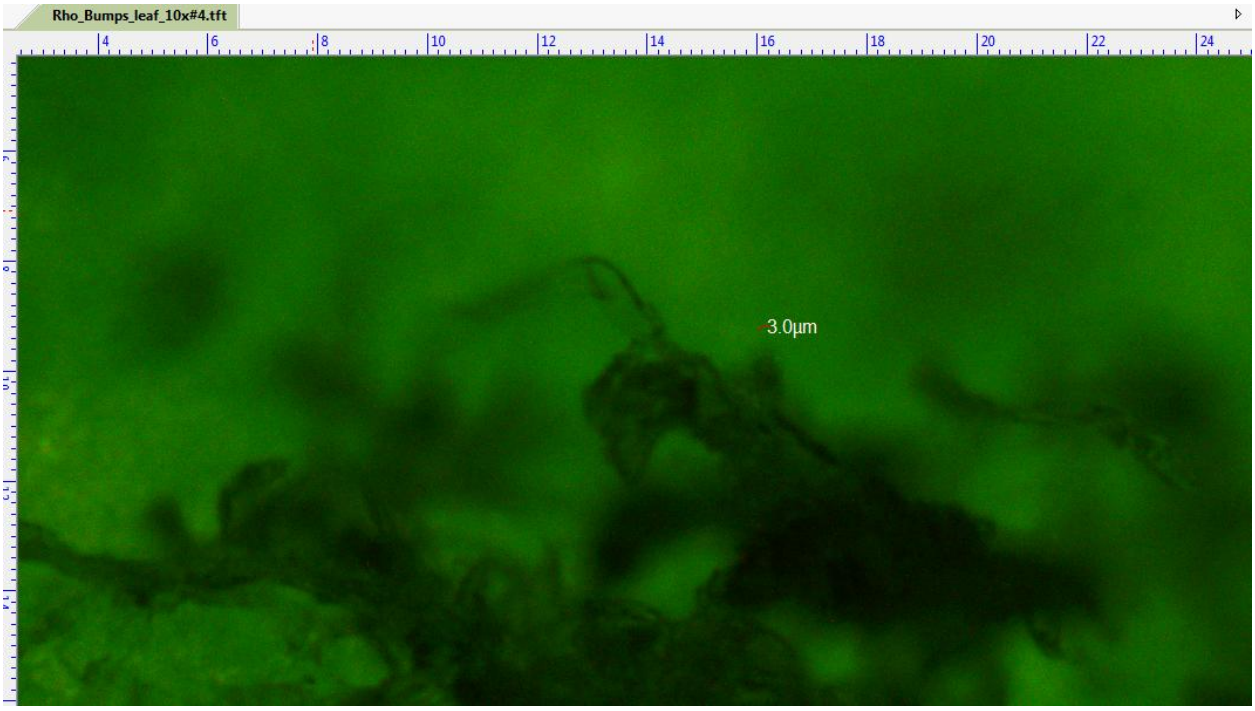



**Figure 10**

Various fiber and ribbon- like forms could be observed at the raised spherical sites. Figure 11 below is of this same form using epifluorescence technique. The anomalous dark areas above fluoresce blue and pink below:

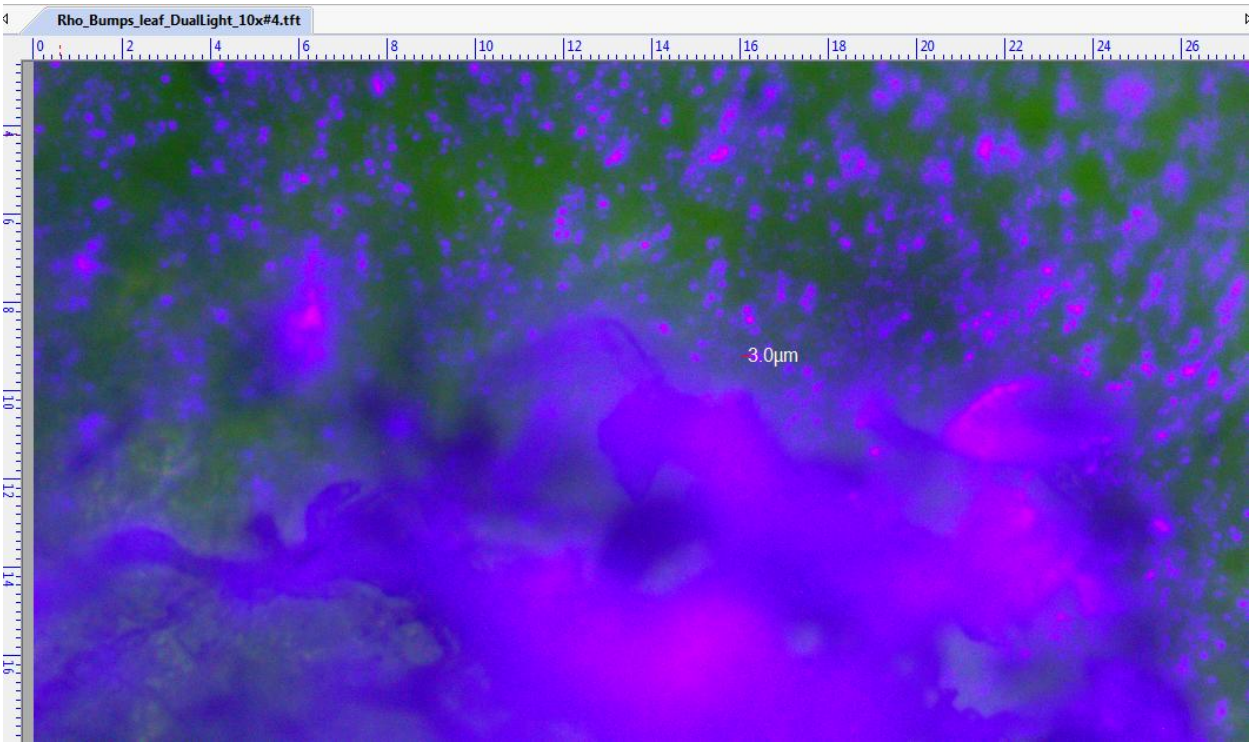



**Figure 11**

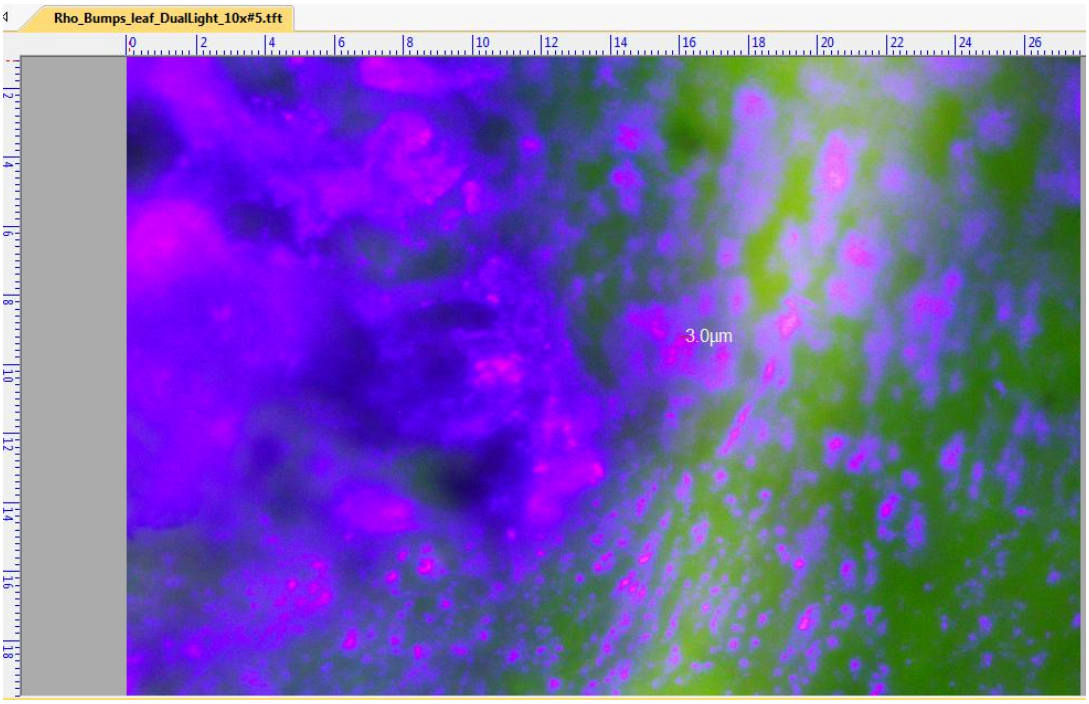



**Figure 12**

The above image is of heavily damaged section next to healthier sections. It has been a repeated observation that fluorescence is much more intense in the areas of damage and also in various formations such as aggregates and fibers.

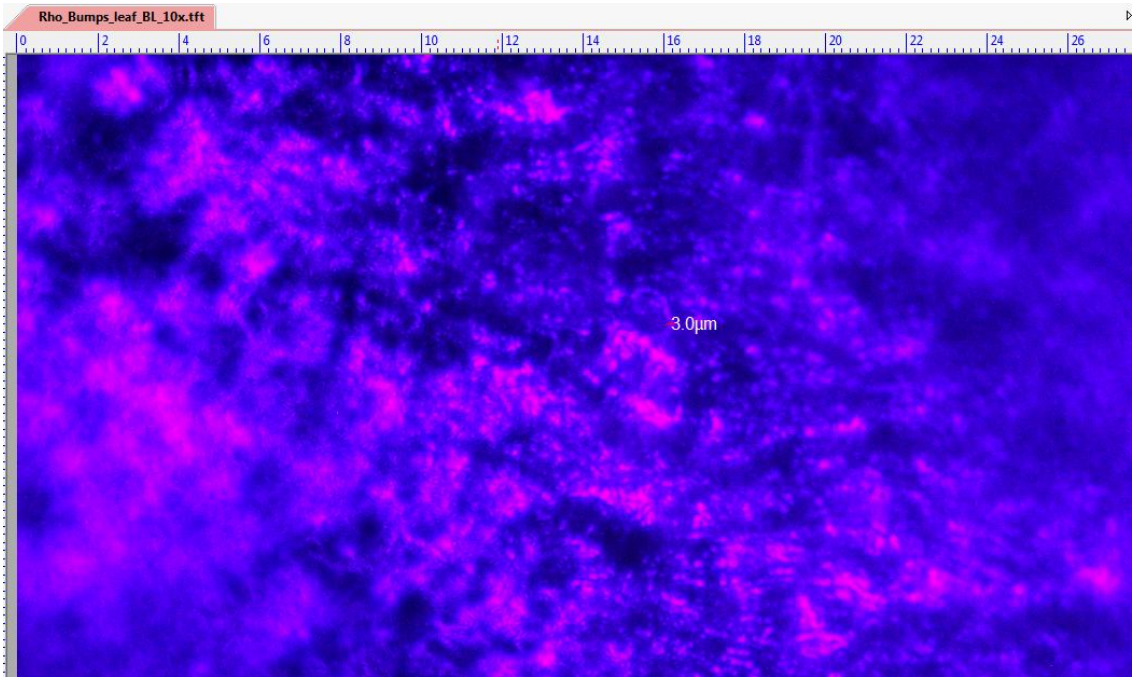



**Figure 13**

The above image is captured using 395nm black light only on area of damage

The following micrographic images are of the raised spherical forms which were crushed and contents smeared onto glass slide. They were examined with 395nm LED black light and laser lights

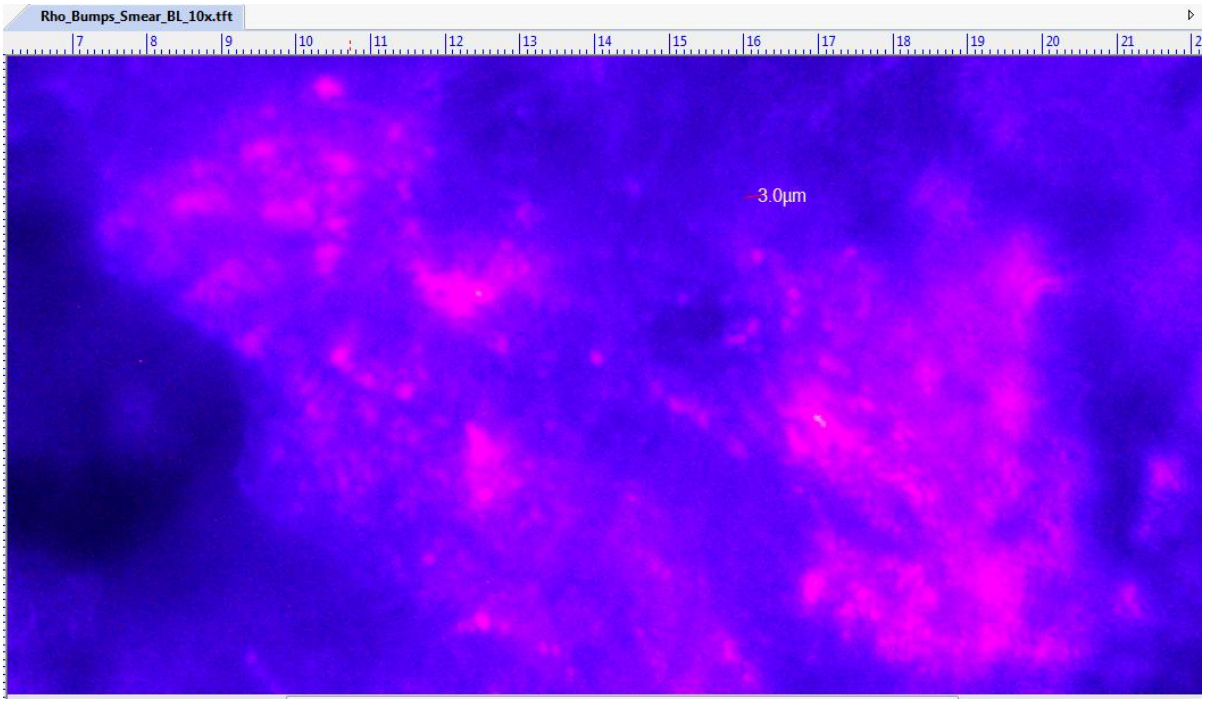



Figure 14

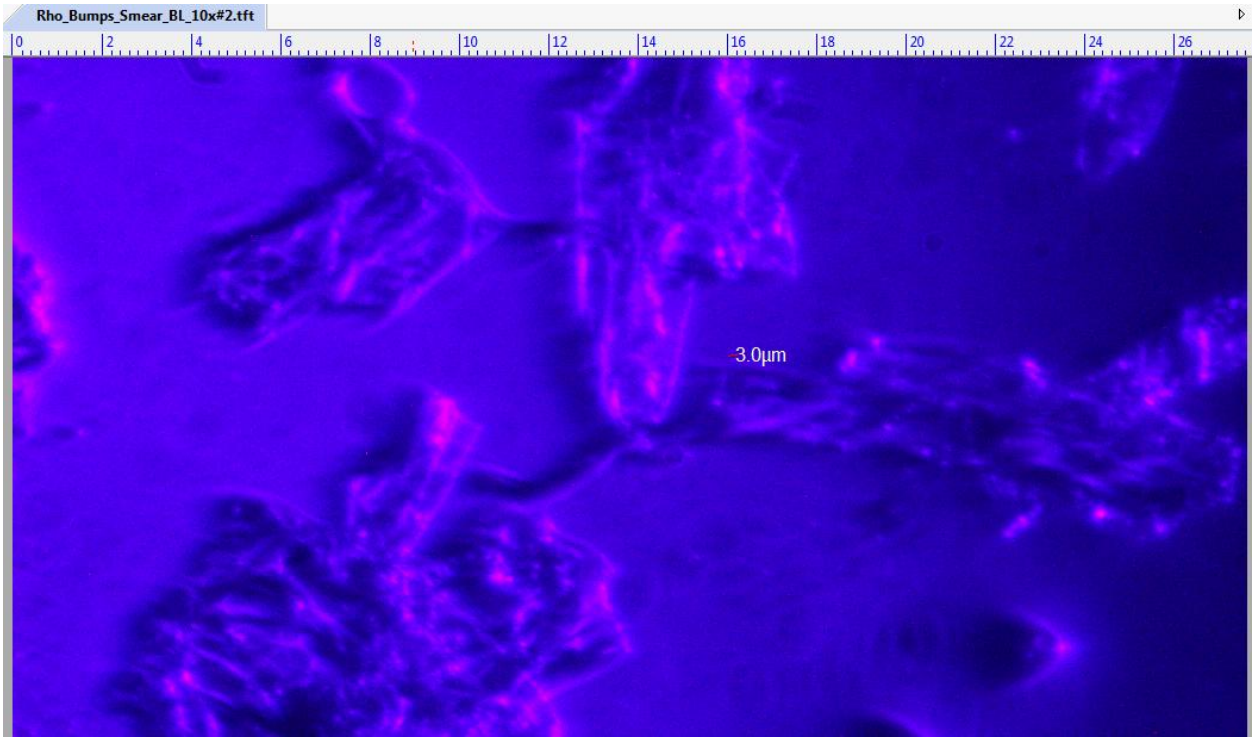



Figure 15

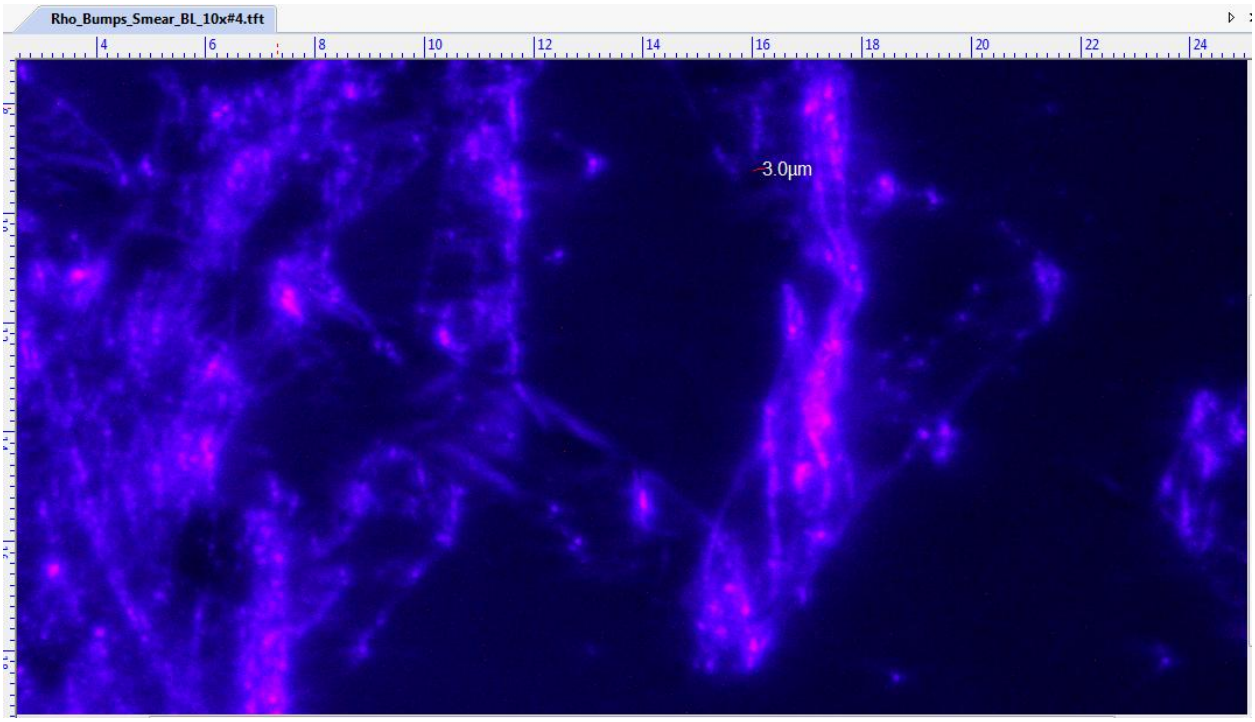



Figure 16

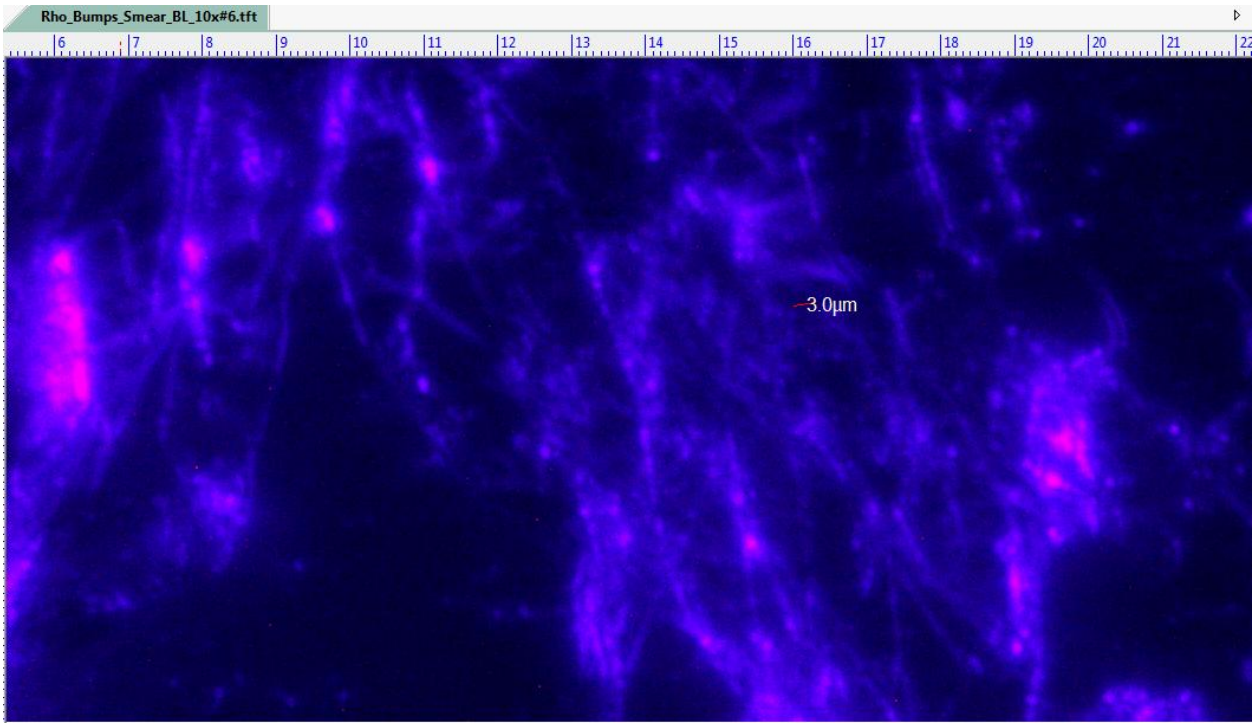



Figure 17

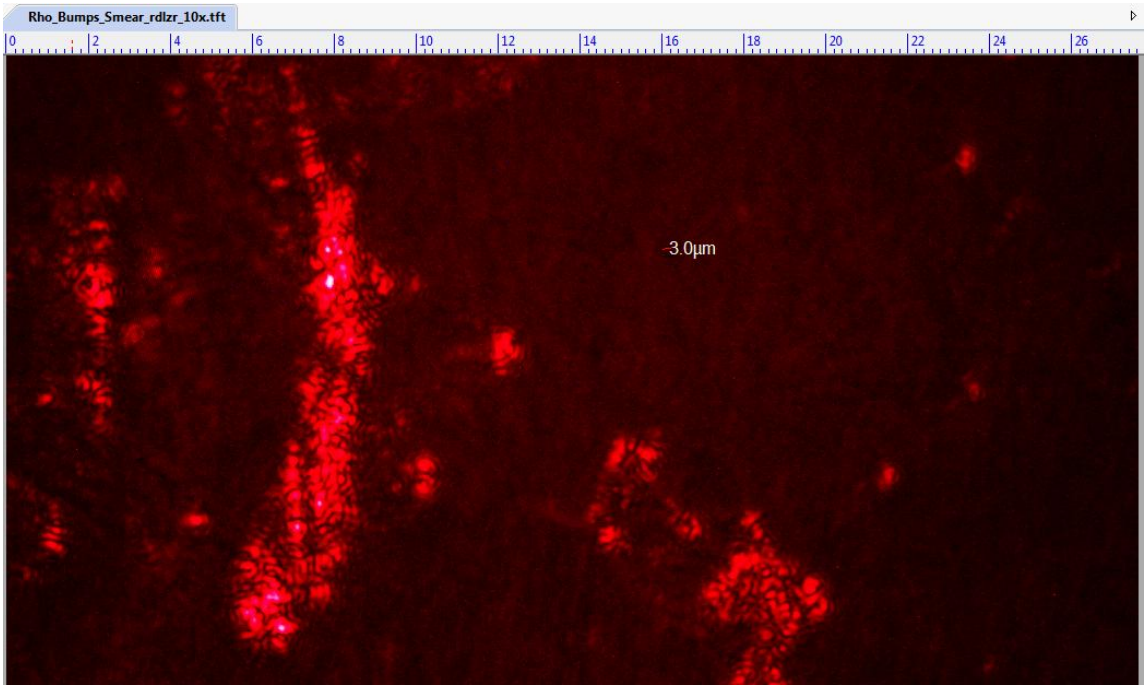



**Figure 18**

The same spherical contents smeared on a glass slide and viewed while shining red 650 nm laser. Fig. 18 below is the same except illuminated with green laser - 530 nm

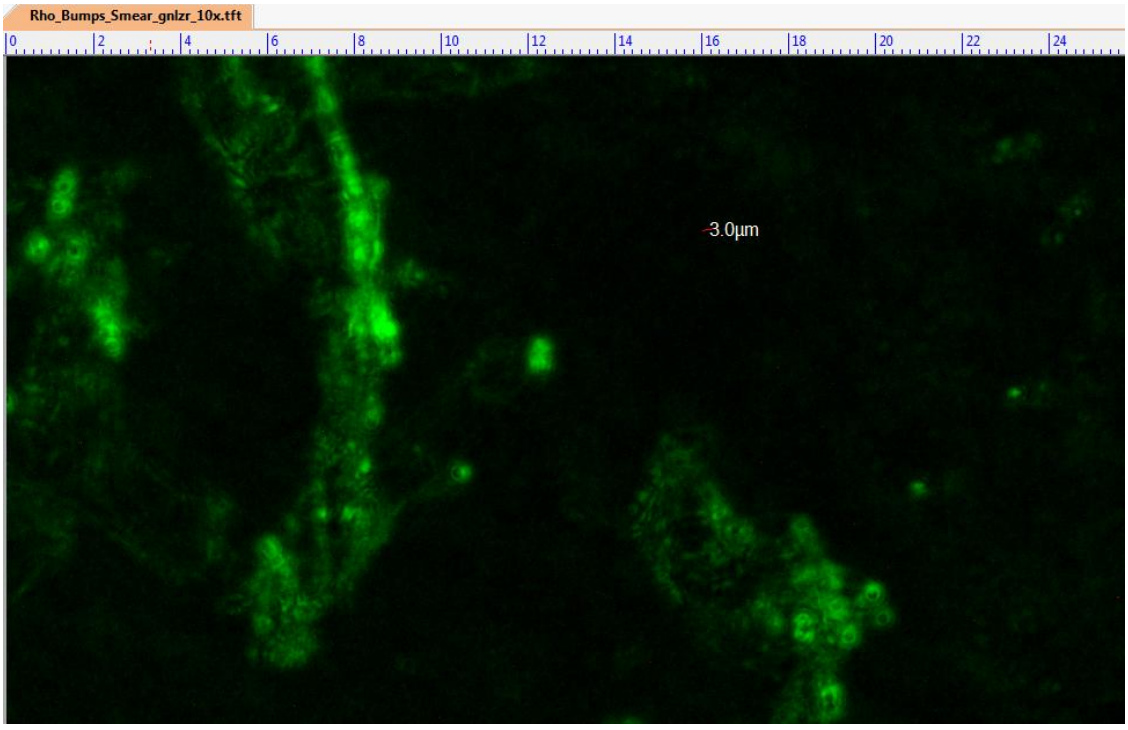



**Figure 19**

## CHEMICAL DIGESTION AND MICROSCOPIC OBSERVATION OF LEAF #2

Figure 20

1 gram of the heaviest concentration of the raised forms was removed, cut into small pieces and digested in a solution of 100ml of filtered water and 1tbs of food grade NaOH. The solution was heated to a boil and placed on magnetic stir plate overnight with a starting pH of 13. pH was measured with pH paper and also confirmed with digital temp compensated meter (Hana pHep 98128).

The above solution was left stirring for 12 hours after which a single drop was taken from the rust color solution and placed between glass slide and cover slip, then inspected under the microscope with UV 395nm black light shown on pg. 13. What was observed were spherical forms that assembled to form rod like structures as shown on the following pages. Initial scan under conventional bottom illumination did not reveal in detail what was evident upon "black light" inspection. Also observed were larger aggregation clusters with dimensions of several micrometers. The forms exhibited a fluorescent glow that was apparently more intense pink at sites of aggregation clusters.

Figure 21

A Hanna magnetic stir plate with large + shape Teflon stirrer was used. The EMF generated exceed 100mili gauss at 6" distance. This was the upper limit of the Trifield meter. Electromagnetic fields are important considerations as possible influence and variables.

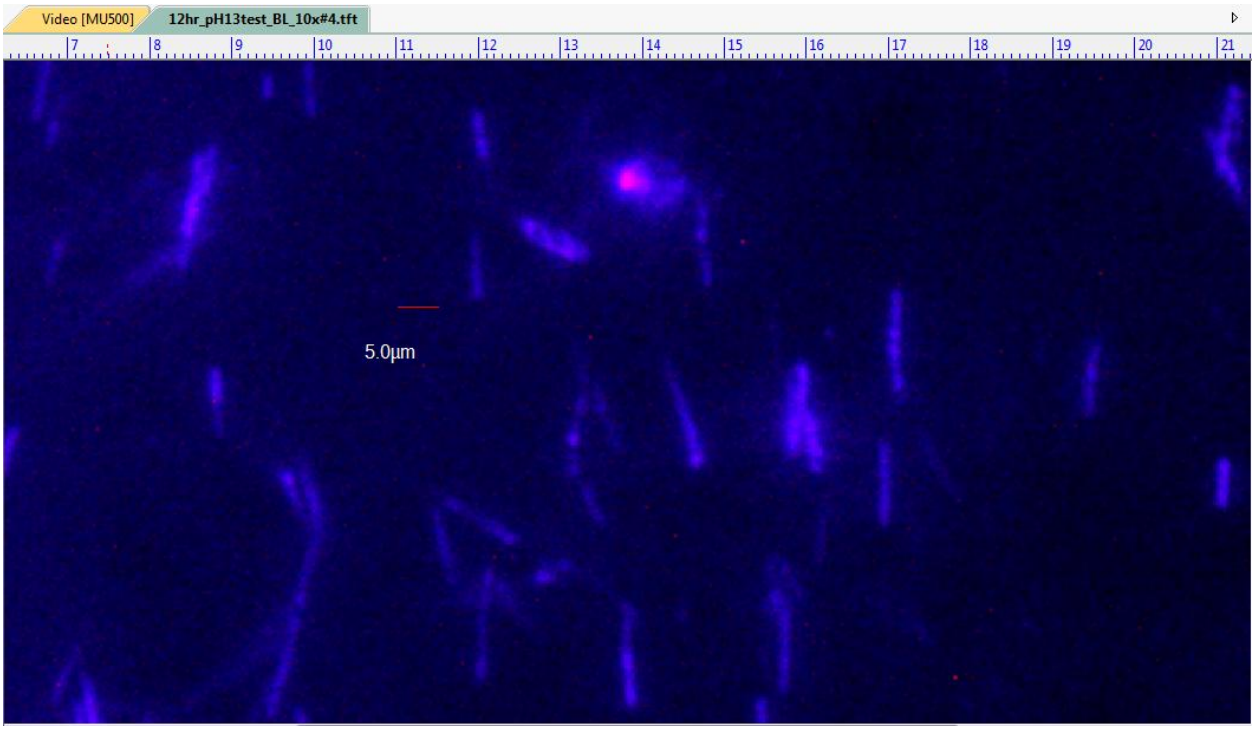



Figure 22

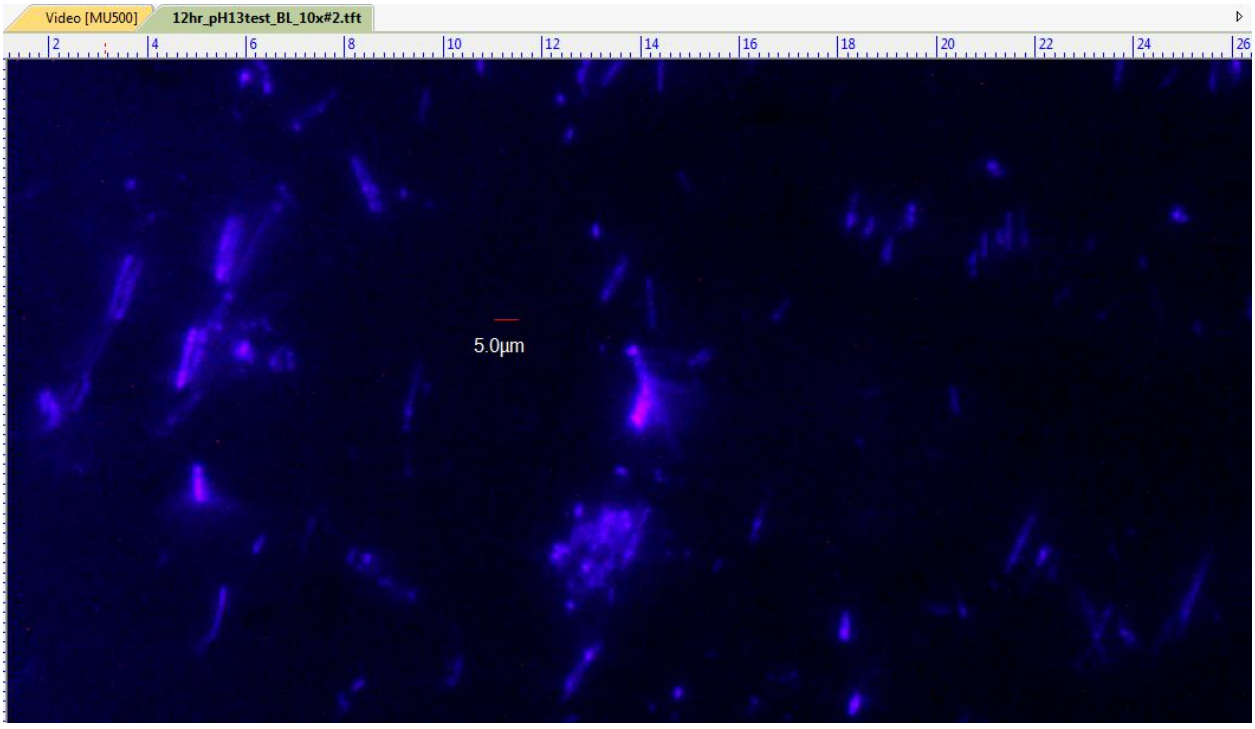



Figure 23

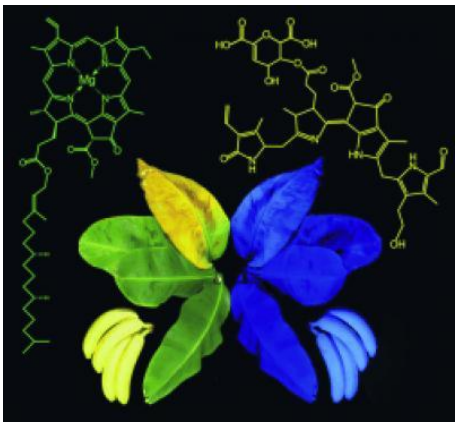
## ADDITIONAL READING

The following literature is of interest as it relates to absorption and emissions. The molecular structure of porphyrins is at the core of hemoglobin and chlorophyll are of prime interest.

The "Structures of Chlorophyll Catabolites..." article is interest as it notes the fluorescence of the breakdown of chlorophyll. It should be noted that a preliminary investigation of organic ripe banana did not reveal visible fluorescence. This may be worthy of further investigation and comparisons. However, a main point is that the breakdown of chlorophyll apparently fluoresces under UV. Ripening of a banana would be a natural event, however disturbances in plant leaf presented is indicative of disease.

### Structures of Chlorophyll Catabolites in Bananas (Musa acuminata) Reveal a Split Path of Chlorophyll Breakdown in a Ripening Fruit

Moser, S., Müller, T., Holzinger, A., Lütz, C. and Kräutler, B. (2012), Structures of Chlorophyll Catabolites in Bananas (*Musa acuminata)* Reveal a Split Path of Chlorophyll Breakdown in a Ripening Fruit. Chem. Eur. J., 18: 10873–10885. doi: 10.1002/chem.201201023



Source: http://arrowthroughthesun.blogspot.com/2008_10_01_archive.html

### Fluorescent Nanoparticles Based on Self-Assembled $\pi$-Conjugated Systems

1. Adrien Kaeser and
2. Albertus P. H. J. Schenning*

Article first published online: 9 JUN 2010

http://www.researchgate.net/publication/44661307_Fluorescent_nanoparticles_based_on_self-assembled_pi-conjugated_systems/file/79e4150294ca3a2f96.pdf

DOI: 10.1002/adma.201000427

**Keywords:**

- fluorescent materials; self-assembly; organic nanoparticles; imaging; biolabels; sensors

**Abstract**

$\pi$-Conjugated molecules are interesting components to prepare fluorescent nanoparticles. From the use of polymer chains that form small aggregates in water to the self-assembly of small chromophoric segments into highly ordered structures, the preparation of these materials allows to develop systems with applications as sensors or bio labels. The potential functionalization of the nanoparticles can lead to specific probing. This progress report describes the recent advances in the preparation of such emittive organic nanoparticles.

## REFERENCES AND ADDITIONAL RESEARCH LINKS:


**1 - Cationic lipids, lipoplexes and intracellular delivery of genes**

Luc Wasungu, Dick Hoekstra

Department of Cell Biology, Section Membrane Cell Biology, University of Groningen, University Medical Center Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands

Received 1 June 2006, Accepted 21 June 2006, Available online 28 June 2006

**2- Evidence of Interlipidic Ion-Pairing in Anion-Induced DNA Release from Cationic Amphiphile−DNA Complexes. Mechanistic Implications in Transfection‡**

Santanu Bhattacharya * and Subhrangsu S. Mandal

Department of Organic Chemistry, Indian Institute of Science, Bangalore 560012, India

*Biochemistry*, 1998, 37 (21), pp 7764–7777

**DOI:** 10.1021/bi971772j Publication Date (Web): May 8, 1998 Copyright © 1998 American Chemical Society

**3 - Dimerizable Cationic Detergents with a Low cmc Condense Plasmid DNA into Nanometric Particles and Transfect Cells in Culture**

Emmanuel Dauty , Jean-Serge Remy , Thomas Blessing , and Jean-Paul Behr *

Contribution from the Laboratoire de Chimie Génétique associé CNRS/Université Louis Pasteur de Strasbourg, Faculté de Pharmacie BP 24, 67401 Illkirch, France

*J. Am. Chem. Soc.*, 2001, 123 (38), pp 9227–9234, **DOI:** 10.1021/ja015867r

Publication Date (Web): August 31, 2001, Copyright © 2001 American Chemical Society

**4- Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana**

The Plant Journal (1998) **16**(6), 735–743

**Steven J. Clough and Andrew F. Bent***

Department of Crop Sciences, University of Illinois at Urbana-Champaign, 1201 W. Gregory Dr, Urbana, IL 61801, USA

**5 - Analysis of Surfactant leaf damage using microscopy and it's relation to glyphosate or deuterium oxide uptake in Velvet Leaf**

**(Abutilon theophrasti)**

Paul CC Feng[1], Jan S Ryerse[2], Claude R Jones[1] and Douglas Sammons[1]

[1]Monsanto Co, [2] St. Louis University Health Sciences Center

6. http://en.wikipedia.org/wiki/Transfection
7. http://en.wikipedia.org/wiki/Transfer_DNA
8. http://en.wikipedia.org/wiki/Nuclear_weapons_testing
9. http://en.wikipedia.org/wiki/Chlorophyll_fluorescence
10. http://biologywiki.apps01.yorku.ca/index.php?title=Main_Page/BIOL_4160/Fluorescence_and_Reaction_Centers
11. http://www.botanik.kit.edu/molbio/agbuschmannenglish.php


Non referenced links:

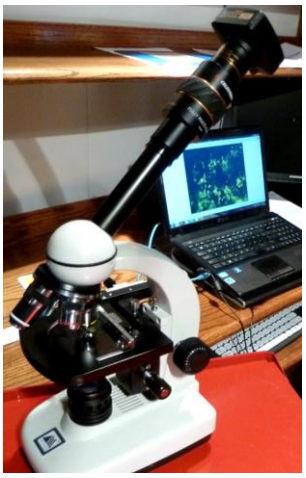
- http://www.google.com/patents/US20130212739
- http://download.bioon.com.cn/view/upload/month_0912/20091216_80b95769b0b4cd38f2dcQgmdkjvcXRwR.attach.pdf
- http://onlinelibrary.wiley.com/doi/10.1046/j.1365-313x.1998.00343.x/full
- http://sciencestage.com/uploads/text/d5W2dzSBuitoEOoI5kxo.pdf
- http://www.momentive.com/Products/home.aspx?id=21471
- http://www.entsoc.org/PDF/Pubs/Periodicals/JEE/JEETOCS/PDF/ec020000180p.pdf

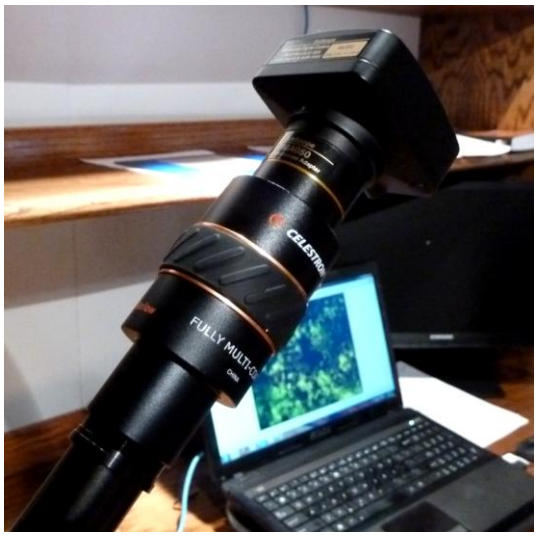
## MATERIALS & METHODS

It is hoped that many citizen and academic researchers will engage in this research. The methods used are illustrated in the photo's below. Improvements to equipment can be made and further information on similar techniques can be found in the following links:

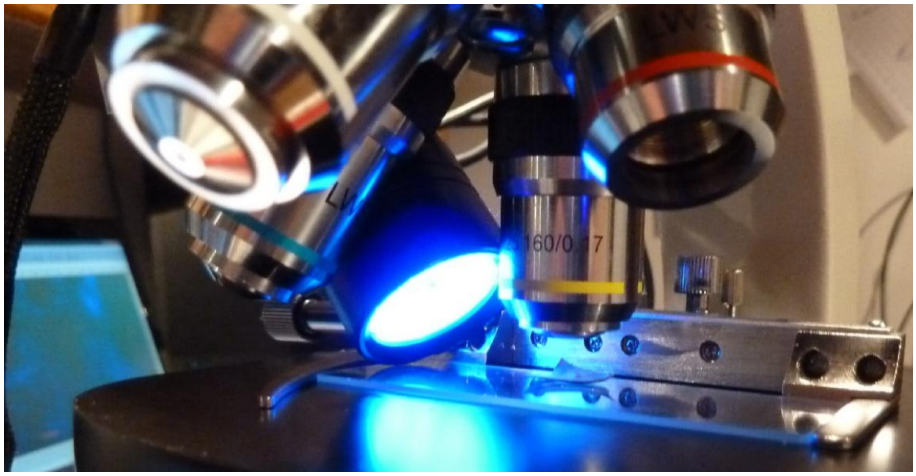
https://en.wikipedia.org/wiki/Fluorescence_microscope

http://www.microscopyu.com/articles/fluorescence/index.html






Equipment used is "entry level" representing approx $650 total investment (excluding computer). Equipment is LW Scientific Observer microscope, Celestron 3x Barlow lens, fitted with 5 mpx AmScope MU500 Camera and software. Use of 30.5mm adapter allows coupling of the Barlow bushing to the camera. In this case the regular eyepiece was cut which allowed insertion into tube. This technique is not a perfect solution as only allows partial threading into Barlow filter threads which are typically 1.125" x 42 threads per inch on most 1.25" Barlow lenses. Alternative configuration would be mounting Barlow to photo port or C-mount on trinocular models.



395 nM UV Ultra Violet 21 LED Blacklight Flashlight, by LEDwholesalers 7305UV395

# Research & Spirit:

"Natural Science"  Music/lyrics:  Rush

When the ebbing tide retreats
Along the rocky shoreline
It leaves a trail of tidal pools
In a short lived galaxy
Each microcosmic planet
A complete society

A simple kind of mirror
To reflect upon our own
All the busy little creatures
Chasing out their destinies
Living in their pools
They soon forget about the sea

Wheels within wheels in a spiral array
A pattern so grand and complex
Time after time we lose sight of the way
Our causes can't see their effects

A quantum leap forward in time and in space
The universe learned to expand
The mess and the magic, triumphant and tragic
A mechanized world out of hand

Computerized clinic for superior cynics
Who dance to a synthetic band
In their own image, their world is fashioned
No wonder they don't understand

Wheels within wheels in a spiral array
A pattern so grand and complex
Time after time we lose sight of the way
Our causes can't see their effects

Science, like nature must also be tamed
With a view towards its preservation
Given the same state of integrity
It will surely serve us well

Art as expression not as market campaigns
Will still capture our imaginations
Given the same state of integrity
It will surely help us along

The most endangered species, the honest man
Will still survive annihilation
Forming a world, state of integrity
Sensitive, open and strong

Wave after wave will flow with the tide
And bury the world as it does
Tide after tide will flow and recede
Leaving life to go on as it was