



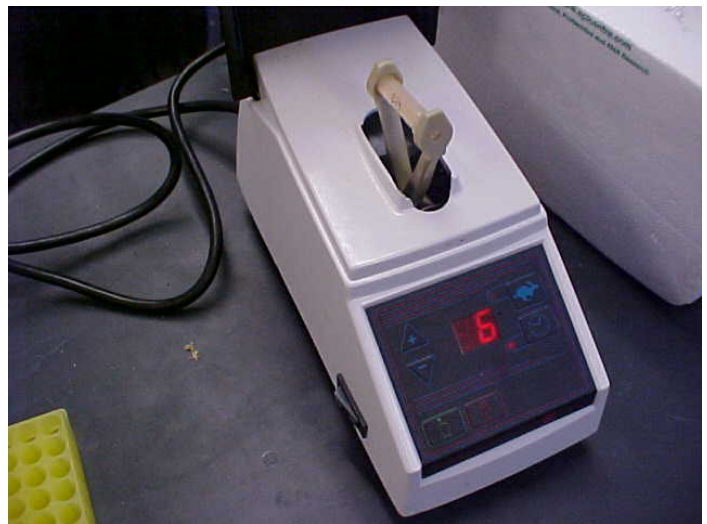
GERMS for Science Educators - July 2005 Yellowstone Lab Data by Curt Ralston



IN-LAB ACTIVITIES - PIGMENTS AND MICROSCOPY ***IMPERIAL GEYSER***

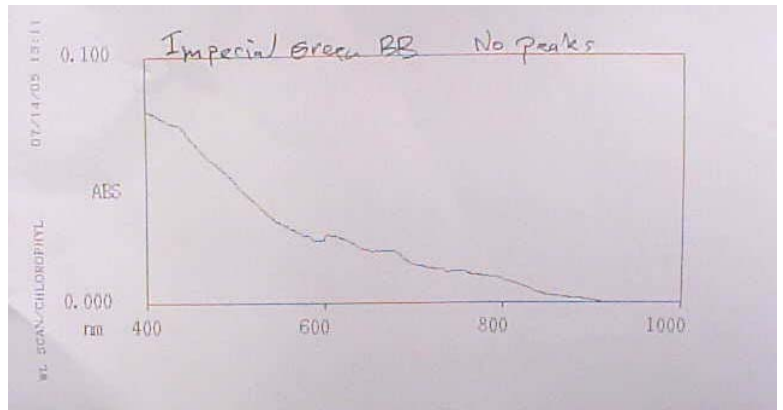
PIGMENT ANALYSIS - IN VIVO AND METHANOL EXTRACTION

General Preparation of all mat layer processing began with thawing collection and grinding mat with a disposable mortar and pestle with glucose-TRIS EDTA (GTE). 50 microliters of the suspended sample was set aside for microscopy. GTE was removed from 250 microliters of the suspension by spinning and methanol was added to remove pigments. 0.1 mm zirconium beads were added to 250 microliters of the suspension in a Bead-Beating (BB) tube. Samples were beat for one minute twice with a one minute rest in the ice bath. In vivo – bead beating of mat layers retains pigments with associated proteins. These proteins either modify the pigments or light transmission. Note that the absorption wavelengths are longer for the in vivo pigments. The procedure is useful to identify bacteria in mats.



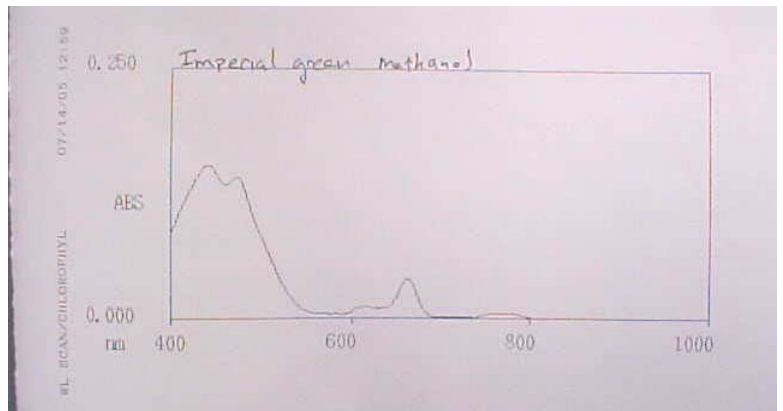
**In Vivo Extraction
Imperial Red Layer Pigments**

There were no major peaks which indicates an error in processing?



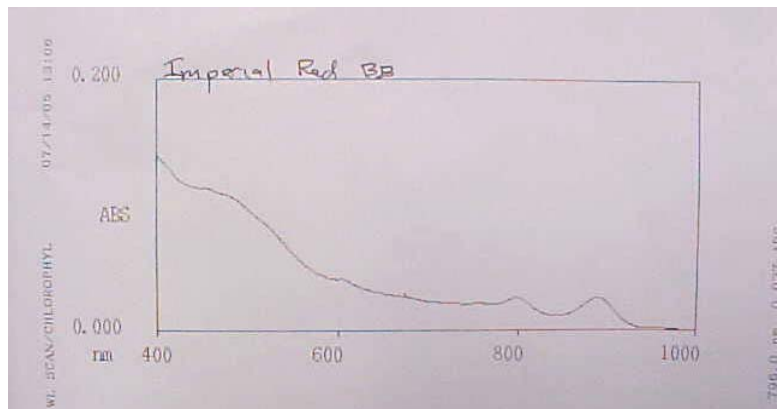
**Methanol Extraction
Imperial Green Layer Pigments**

<u>Absorption</u>	<u>Wavelength, nm</u>
0.153	768
0.040	662
0.141	473
0.153	440



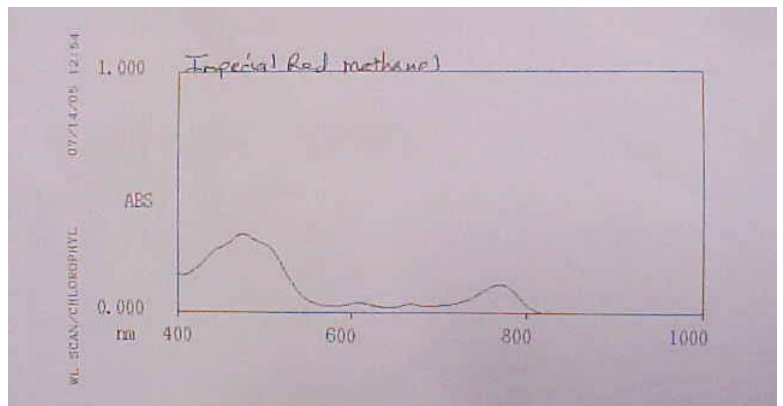
**In Vivo Extraction
Imperial Red Layer Pigments**

<u>Absorption</u>	<u>Wavelength, nm</u>
0.026	888.0
0.027	796.00

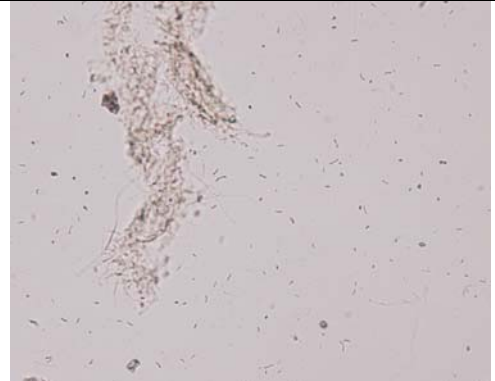
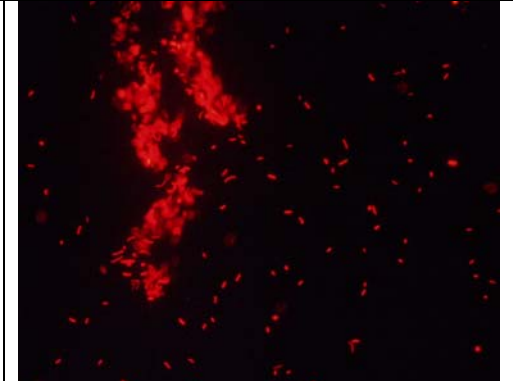
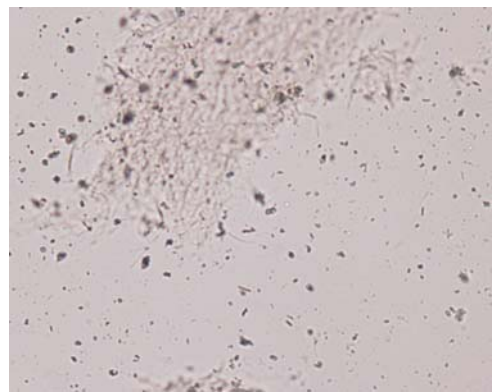
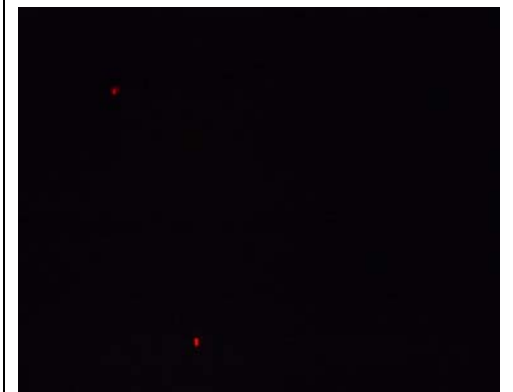


**Methanol Extraction
Imperial Red Layer Pigments**

<u>Absorption</u>	<u>Wavelength, nm</u>
0.116	768
0.037	667
0.042	606
0.327	476



MICROSCOPY AND FLUORESCENCE ANALYSIS

<p>Green layer after grinding with a disposable pestle. Slide = 20 microliter wet-mount. Rods, cocci, and filaments were identified. Fluorescence/right indicates cyanobacteria chlorophylls, green bacteriochlorophylls. This is evidence for an abundance of green bacteria.</p>		
<p>Red layer after grinding with a disposable pestle. Slide = 20 microliter wet-mount. Rods, cocci, and filaments were identified. Lack of any fluorescence/right indicates cyanobacteria not present or abundant in sample. This is evidence that bacteria with red bacteriochlorophylls are probably present.</p>		

COMPREHENSIVE ESSAYS

Which of the above methods or data would you find use for in your classroom and how/why?

It would be useful to develop a data set of photographs and spectra graphs for some common bacteria groups that students could analyze in general biology classes. This could be a web-based activity or with photograph and graph sets. Any activities would require background knowledge in bacteria and photosynthesis pigments. I can think of three areas where this would be an interesting activity: while investigating the bacteria kingdom, while investigating photosynthesis and metabolism, and while investigating tools of cell biology. For example, during a unit of kingdoms of life, students might investigate presence of bacteria groups in Ash Creek, our local stream. Teams could build and observe Windogradsky columns. Many students would obviously ask how do we know these are purple bacteria or green bacteria. This activity could provide actual science techniques to answer this question. Later, this information could be used to broaden investigations of photosynthesis to include microbes.

Which of the above methods or data would you find impossible to apply to your classroom and why?

Realistically we do not have access to this pigment analysis equipment or hot spring bacteria mats. I could envision a small student group visiting WOU and using this equipment, but not in our classroom setting for general biology. With classroom access to a spectrophotometer the methanol extraction techniques are simple. However purification of a sample would be difficult.

Summarize your findings using these methods – what kinds of phototrophs dominate each layer?

Fluorescence microscopy provided observations that **cyanobacteria** and **green bacteria** are dominant in the green mat layer because some cells and filaments were visible through the R and F filters with UV light. This technique wasn't practical to determine the presence or absence of the red

bacteriochlorophyll pigments associated with the purple bacteria. Pigment analysis with methanol supported presence of the carotenoid pigments of **cyanobacteria** with spectrophotometer absorption double peak in the 400-500 nm wavelength range. Also there was an absorption peak at 662 nm which indicates bacteriochlorophyll c found in green bacteria, especially **green nonsulfur bacteria** which absorbs at 667 nm.

In the red layer, fluorescence microscopy showed that most of the bacteria filaments and cells did not fluoresce through R or F filters. Cyanobacteria and green bacteria were not abundant or present. **Purple bacteria** may be present. In vivo pigment analysis shows absorption greater than 800 nm wavelength which must be Bchl a and b of **purple bacteria**. Methanol extraction of pigments confirms this with a major peak at 768 nm associated with bacteriochlorophyll a. Therefore, purple bacteria dominate the red layer but some green bacteria may be present.



I (Boomer) took the liberty of adding this great picture of Curt Geo-Caching at Old Faithful during a half-day of sightseeing.