Views of the Laboratory of Dr. J. William Louda; An annotated tour:

The laboratory is located in room 123 of the Science and Engineering Building on the north side of the main (see finger on Figure 1 below) campus (Boca Raton) of Florida Atlantic University.

Note the proximity to the Atlantic Ocean, Lake Okeechobee, the Everglades, Florida Bay and the Gulf of Mexico. Recently, we have initiated a working relationship with the Cape Eleuthera Institute and Island School on the beautiful island of Eleuthera in the Bahamas. A great place for marine, hypersaline and climate change studies.

Fig. 1: Satellite view of southern Florida (ex. SFWMD) and Bahamas Map

Fig. 2: Entrance to Louda Lab.

Shown here in Figure 2 is the entrance to the main lab. Notices to upcoming environmental, limnological and oceanographic conferences, seminars and the-like are also posted here.
**Fig. 3:** Inside the door, *welcome to the lab.* Wee bit of a galley for student use. NOTE: eating, food and drink NOT allowed past the office areas shown below in Figure 4.

**Fig. 4:** Office area for 5 students, plus 1 of 3 PC stations. Note; Lots of reference texts. Literature searches (SUS links, SciFinder etc.), structure drawing (ChemDraw) and other software are available on these.

**Fig. 5:** Fluorescence spectrometers. Larger one to the left is a Perkin-Elmer LS-50B spectrofluorimeter / luminescence spectrometer. Smaller unit to the right is the Turner Designs TD-700 fluorometer. Both are used for chlorophyll and phycobiliprotein investigations.
The laboratory has 2 large fume (exhaust) hoods. The one shown below (Figure 6) is used primarily for ‘wet chemistry’. That is, we routinely need to generate known compounds for comparison the myriad of unknown carotenoids and chlorophyll-derivatives encountered in our studies, both environmental and geochemical. Thus, this is where partial syntheses and numerous derivatizations (reductions, oxidations {not all “planned”!}, esterifications, deesterifications, etc.) occur.

![Wet chemistry fume hood.](image)

**Fig. 6:** Wet chemistry fume hood.

The second fume hood (Fig. 7) in the lab is reserved for processing samples (extractions). In this way we separate the synthetic / hemisynthetic and analytical functions of the lab. A small amount of a prepared standard pigment could adversely affect the results of an environmental or culture analysis. In addition, it is highly important that all extractions are performed in the fume hood as on component of of extraction cocktail is dimethylformamide, a know liver toxin. Gloves and goggles are mandatory as well!

![Analytical fume hood area used for extractions.](image)

**Fig. 7:** Analytical fume hood area used for extractions. In the lower right is the (aqueous) sample filtration area. The grey cabinet stores field equipment.
The area to the right in Figure 8 (below) is utilized for water analyses. This includes macro-nutrients (N as ammonia, nitrite, nitrate; P as SRP {Soluble Reactive Phosphorous} and acid-hydrolyzable P, both as ortho-phosphate, certain micro-nutrients (iron, silicate, sulfur {sulfate}*) and selected bulk parameters (COD {Chemical Oxygen Demand}). Sulfate is also important in providing an oxidant as waters become suboxic to anoxic. As the HACH DR-5000 has certain lower limits of detection, samples with very low amounts of nutrients are analyzed by classic ‘micro-cuvette’ methods.

Also shown here (Fig.8) are three of the 5 High-Performance Liquid Chromatograph (HPLC) –systems four with full-spectrum (190-900nm) photodiode array (PDA) detectors (also called diode array detectors; DAD). The HPLC systems form the heart of a pigment analysis laboratory.

Fig. 8: Instrumentation at the back of the lab. HACH DR-5000 (nutrients, COD, etc.) to the left and three HPLC systems to the right. (1) ThermoSeparations Autosystem with Waters Mdl. 490E dual programmable wavelength detector. (2&3) Identical HPLC-PDA Systems comprised of ThermoSeparations Mdl. 4100 quaternary pumps and Waters Mdl. 990 PDAs.

The HPLC-PDA systems use an octyldecylsilane (ODS, C-18) column for complete pigment analyses or an octyl (C-8) column for the separation of the divinyl analogs of chlorophylls-a and –b, so important in the identification / quantification of members of the Prochlorophyta. The Waters 990 systems are seen below (Fig.9) as well.

Both of these systems provide full spectral (190 – 800nm, 330-800nm typical) data acquisition. It is the 2-dimensional data (retention time plus absorption spectrum) which provides the basis for the identification of the chlorophylls, chlorophyll derivatives and carotenoids that we analyze.

Standardization (QA/QC) of such systems requires a great number of known pigments. Our laboratory has accumulated many knowns about 18 of which could be obtained by purchase or as gifts. Numerous others (>100) were generated by partial syntheses and derivations (See Louda et al. 2002: Org. Geochem. Vol. 33, pp. 1635 – 1653 in vita), a process which continues.
Fig. 11: View of the matching quaternary Waters 990 HPLC-PDA systems.

Analyses of related samples and increased sample throughput is achieved using the SpectraPhysics ‘SpectraSystem’ automated HPLC system. Here (Fig.12) an autosampler with a Peltier device for chilling samples, a quaternary HPLC pump and dual (upgraded since photo was taken) wavelength monitoring system allow for the analysis of larger sample suites. This system runs the UNESCO developed separation scheme (see Jeffrey et al., 1997. Phytoplankton Pigments in Oceanography. UNESCO Publishing, Paris, 661pp.) and PeakSimple in Windows for data acquisition.

Fig. 12: The SpectraPhysics ‘SpectraSystem’ automated HPLC.
The other 2 full spectral HPLC-PDA systems are shown at the right in Figure 12 and are matching Waters 996 systems. These use ThermoSeparations Mdl. P4000 quaternary HPLC pumps for ternary gradient plus storage solvent control.

On the left in Figure 12 is the Perkin-Elmer Lambda-2 UV/Vis spectrophotometer. UV/Vis forms the initial analysis of any pigment extract. Additionally, we use UV/Vis to adjust injectate volumes prior to HPLC in order to not overload the analytical capability of our columns. Pigment quantitation relies on the Beer-Lambert relationship \( A = \varepsilon l c \), where \( A \) = absorptivity \( \) (in AU = absorbance Units), \( \varepsilon \) = the extinction coefficient of a particular pigment, \( l \) = the path length through the sample \( \) (1 cm usually), and \( c \) = the concentration of the pigment.

**Fig. 12:** From the left side, PE Lambda-2 UV/Vis spectrophotometer and 2 matching Waters 996 HPLC-PDA systems.

Pigments are ‘pigments’ to man’s eyes because the absorb light in certain wavelengths and we see what is not absorbed. Thus, as seen below in Figure 13, we work in very dim yellow light to ensure against unwanted photo-oxidation and potential isomerizations. Light, heat and temperature – only 1 allowed at any time!

**Fig. 13:** Photograph of the lab under “working with pigments” conditions.
As just stated above, we work in very dim YELLOW light. Why yellow? Glad you asked, I actually have an answer for you. Below, in the top half of Figure 14, are the spectra of chlorophylls-a and -b and a 'generalized' carotenoid. The bottom half of this figure is an ‘action spectrum’ for algal photosynthesis. As the black bar indicates, there is a minimum in light absorption in the yellow range (also why we buy “bug lights” to work on the front porch at night, especially in the South). The phycobiliproteins do however absorb in this area but are more stable in this regard.

![Figure 14: Why we work with chlorophylls and carotenoids under yellow light.](image)

![Figure 15: Central aisle.](image)  ![Figure 16: Turbidity meter, centrifuges, ovens.](image)

In the center of the lab (Figure 15), we find (Figure 16) a Hach 2100 turbidity meter, various centrifuges, ovens and a muffle furnace for AFDW etc.
Also in the central aisle, we find (Figure 17) the two Ace-Glass Michael-Miller (low pressure: P < 200 psig) LP-HPLC systems which we use for purification of larger (10 - 100 μg) batches of pigments. We use a micro-cuvette in digital Spectronic-20 colorimeters interfaced to Peak-Simple software for data acquisition. On the other side of the aisle (Figure 18) is the Coulter Counter (right side of picture) used for counting single celled phytoplankton. As in the ‘olde’ days, we rely on microscopic exams with a hemocytometer and/or graduated ocular for enumerating (‘guesstimating’) filamentous forms. Here (left side of picture) are the Nikon compound microscope, a Bausch & Lomb dissection microscope and a Nikon fluorescence microscope.

**Fig. 17:** Low Pressure HPLC systems.  
**Fig. 18:** Microscopes and Coulter (cell) counter.

Down the hall (SE-130) is a shared lab facility. The part that we occupy houses our 3 programmable incubators for culturing microalgae. Figures 19 and 20 are views of the cell culture area. The inverted microscope is used for examining cultures, especially those in flat culture bottles.

**Fig. 19:** Incubators and inverted microscope.  
**Fig. 20:** Algae under culture.
Additional, low light in this case, incubations are shown in Figure 21. Figure 22 is the refrigerated Sorvall RC-5C *Plus* centrifuge in which we use 15, 50, and 500 mL centrifuge tubes/bottles for harvesting bulk microalgae when filtration on Whatman GF/F filters is not adequate.

**Fig. 21:** Low light incubation setup.  **Fig. 22:** Sorval RC-5C-*Plus* refrigerated centrifuge.

In addition to instrumentation/facilities directly in the Organic Geochemistry labs, the Department maintains a variety of common ‘shared’ instrumentation also available for use on our projects.

As part of our MS/NMR core facilities, we have two (2) Gas Chromatograph–Mass Spectrometers (GC-MS) as seen in Figure 23. Figure 24 shows the Thermo-Finnigan LCQ-Deca, used in ESI and APCI ionization modes. The LCQ-Deca operates either in “infusion” mode or, much more interesting for pigment studies, in HPLC-PDA-MS mode with an autosampler. That is, Samples can be separated using a quaternary HPLC pump, eluates ran through the PDA and MS in sequence. Each peak then is characterized by (1) retention time, (2) UV/Vis spectrum and (3) mass spectrum.

**Fig. 23:** Perkin-Elmer (left) and Hewlett-Packard GC-MS instrumentation.  **Fig. 24:** Thermo-Finnigan LCQ-Deca HPLC-PDA-MS.
The main portion of the MS/NMR core facility contains two nuclear magnetic resonance spectrometers (NMR). These are the 400MHz (Figure 25) and 500 MHz (Figure 26) Varian instruments shown below. In my studies, these are used only to verify structures or hemisynthetic standard pigments and to more fully characterize novel (unknown) pigments from natural samples.

We have two (2) identical ABI Voyager DE-STR MALDI-TOF* mass spectrometers (Figure 27). These are used to obtain parent molecular weights of pigments. As a matrix, we usually use elemental sulfur (S₈) / carbon disulfide in order to not have matrix interference with the pigments. The ABI Q-Star (Figure 28) rounds out our mass spectrometry lab. This instrument operates in many modes, produces high resolution MS/MS data for structural verifications.

Also extremely handy in pigment studies are the Fourier Transform Infrared (FTIR: Figure 29) and circular dicroism (CD: Figure 30) spectrometers. Both are of JASCO manufacture and are recent departmental purchases.
I hope that you have enjoyed the pictorial tour of my lab. I will be very glad to give personal guided tours and to discuss joint research projects including, most importantly to the University’s mission, those with prospective graduate students.

Always remember – read slowly or this could happen!

Cheers,

Dr. J. William Louda
(Sept. 2010)