Analytical Techniques in Freshwater Sciences
FRSHWTR-514
3 credit hours

Instructors: Dr. Laodong Guo & Dr. Ryan Newton

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Office: School of Freshwater Sciences, Room 3009 (Guo), Room 3041 (Newton), and Room 1053 (Anderson, Analytical laboratory manager)

Meeting Time: Wed (12:00 - 2:40 pm for lecture/labs)
Location: 1st floor classroom 1080, School of Freshwater Sciences, 600 East Greenfield Ave., Milwaukee WI 53204

Prerequisites: Graduate Standing or permission of instructors. For Undergraduates: Chemistry (Chem 104 or equivalent), Biology (Bio Sci 152 or equivalent), or permission of instructors.

2. Course Description
This course focuses on two groups of techniques that are used to study aquatic systems: a) modern analytical/geochemical techniques (2 credit hours) and b) nucleic acids/genomics principles and methods (1 credit hour).

This course includes standard methods for the characterization and examination of aquatic environments via samples collected from water, sediment, and biota. Both lectures and hands-on laboratory experiments will be offered to cover chemical principles and analytical procedures and applications, as well as nucleic acids/genomics principles and methods. Upon completion of the course, students should be able to: Research relevant literature and develop robust experimental procedures; Maintain a laboratory book and document experimental methods; Understand the utility of and operate analytical equipment/instruments commonly used in environmental/geochemical/nucleic acid/genomic based procedures; Understand the origin of data generated from chemical and biological experiments and discuss critically the procedures and outcomes that can be obtained with the data generated.

Module 1: Analytical and geochemical techniques. Topics to be covered include:

1) Introduction, lab safety and basic lab skills (pipetting/balance/dilution/calibration etc)
2) Quality Assurance/Quality Control (QA/QC), uncertainties, significant figures, errors and error propagation
3) Methods for basic water and environmental parameters such as dissolved organic carbon (DOC), nutrients: Nitrogen (N), Phosphorus (P), and Silicon (Si), major anions
(e.g., HCO₃, Cl, SO₄) by Ionic Chromatography, and major cations (e.g., Na, K, Ca, Mg) by Atomic Absorption

4) Methods for total organic carbon (TOC) and optical properties of water samples including chromophoric dissolved organic matter (CDOM); and derived properties including absorption coefficient, spectral slope, and specific UV absorbance (SUVA).

5) Instrumentation for the measurements of nutrients, major ions, metals, stable isotopes, organic pollutants, aquatic colloids and nanoparticles, and other chemical species:
   • Autoanalyzer and Ionic chromatography for selected nutrient species (e.g., NO₂, NO₃, PO₄, Cl, SO₄, etc.)
   • Atomic absorption for selected major ions (Na, K, Ca, Mg)
   • Inductively coupled plasma-mass spectrometry (ICP-MS) for selected trace metals (e.g., Cu, Pb, Zn, Cd)
   • Picarro water isotopes analyzer for d²H and d¹⁸O for tracking hydrological cycle
   • Delta-V isotopic ratio-mass spectrometry (IR-MS) for carbon and nitrogen isotopic composition (d¹³C and d¹⁵N)
   • Zetasizer/nanosizer for the characterization of aquatic colloids and nanoparticles.
   • Additional instruments such as gas chromatography-mass spectrometry (GC-MS) for the analysis of persistent organic pollutants (POPs) such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), Liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS) for emerging organic contaminants. Some of the instruments will be used for demonstration only.

Module 2: Nucleic Acid/Genomic Analysis. Topics to be covered include:

1) Introduction to basic lab techniques
   a. Safety related to molecular methods
   b. Pipetting
   c. Basic lab etiquette & procedures for minimizing contamination

2) Preparation for sample collection
   a. Sterilization & disinfection techniques
   b. In the field considerations for sample collection
   c. The organisms, understanding what you are collecting & why this matters to sampling choices
   d. In the field storage options
   e. Filtering setup/choices & variations
   f. Environmental DNA (eDNA) collection

3) Nucleic Acids
   a. What are they? Structure and function in cells
   b. How nucleic acids are used to interrogate biology
   c. How to extract nucleic acids, lab procedures – basis of various steps in the procedure - phenol/chloroform & kit based extractions & how they work
d. Cell disruption, Deoxyribonucleic acid/Ribonucleic acid (DNA/RNA) stabilization

e. Microorganisms and common extraction methods

f. Eukaryotes and common extraction methods

4) Polymerase Chain Reaction (PCR), what it is and how it works
   a. Basic PCR components
   b. Hybridization theory & primer design
   c. Visualizing nucleic acids: gel electrophoresis, fluorescent based detection
   d. Quantifying nucleic acids, Spectrophotometer vs. Fluorescent methods
   e. Quantitative PCR (qPCR) and Droplet digital (ddPCR), theory and application

5) Genomic Sequencing Methods
   a. Available Technologies (Illumina, PacBio, Sanger, Nanopore) – how they work and what information is produced
   b. Applications of Genomic technologies – genome data production, microbial community composition, RNA-seq / transcriptome, meta-omics
   c. Comparative analysis using genomic information – the basis of basic local alignment search tool (BLAST)

Graduate and upper level undergraduate students from freshwater sciences, biological/environmental sciences and geosciences will benefit from this course. The course is offered to students, both within and external to the School of Freshwater Sciences, who are interested in gaining knowledge of modern analytical techniques and instrumentation.

List of Required Reading:
Below is a list of articles that students will be expected to read and discuss over the semester. Readings will be assigned each week according to the learning objectives and material covered during that time. Also, students may be required to view a number of online videos that illustrate the techniques presented in the readings listed below. Note: this list is subject to change as new materials become available.

Weeks 1-3:
   • Clesceri et al (1998), Part 1010 Introduction

Week 5-10:

Week 11:
**Week 12:**
- Kennedy et al., 2014. The impact of different DNA extraction kits and laboratories upon the assessment of human gut microbiota composition by 16S rRNA gene sequencing PLOS ONE 9:e88982.
- Promega Technical Note on DNA purification.
- Rubin et al., 2013. Investigating the impact of storage conditions on microbial community composition in soil samples. PLOS ONE 8:e70460.

**Week 13:**
- Promega Technical Guide to Nucleic Acid Amplification
- The beginner’s guide to real-time PCR
- BioLine. One-step vs. two-step real-time RT PCR.

**Week 14:**
- Illumina Inc. 2016. An introduction to next-generation sequencing technology.

**Week 15:**
- Thermo Fisher – Agarose Gel Electrophoresis Tips & Tricks

**3. Course Objectives**
This course is designed to provide students with the theoretical basis for and hands on experience in using analytical techniques and genomic methods as applied to aquatic systems. Students will gain an understanding of the principles, procedures, instrumentation and applications of these methods. The knowledge and skills gained from this course should provide a broad range of approaches that can be applied to student research projects or in the workplace.
Students will

- Become familiar with lab and sampling equipment preparation and use in chemistry-based analytical techniques and in nucleic acid based analyses
- Become familiar with theoretical and practical aspects of analytical and basic nucleic acid laboratory procedures that lead to genomic data generation
- Understand how analytical and genomic methods can be applied to address aquatic environmental questions
- Understand the type of data produced from various analytical and genomic analyses and the insights and limitations of these data types
- Develop collaborative skills in a laboratory setting

Workload

This is a 3 credit-hour course consisting of both lectures and laboratory components. Students should expect to devote at least 2 hours of work for each 1 h lecture/lab. This time commitment will include:

- In-class time and labs (approximately 3 hours per week, 45 hours);
- Reading assignments & study of assigned materials (45 hours)
- Completion of lab assignments and lab reports (20 hours)
- Preparation for research projects (20 hours)
- Preparation of presentations (20 hours)

4. Recommended Books (There is no assigned textbook)

References for analytical chemistry:


References for genomics

Students may find the following textbooks useful for the nucleic acid/genomics module:


Internet resources

- Science Direct (http://www.sciencedirect.com/)
- Web of science (http://apps.isiknowledge.com/)
- UWM Libraries Online Resources
- Google Scholar (https://scholar.google.com/)
5. Course Grading

Regular assignments include researching and reading about the analytical equipment prior to each lab and completing lab reports from in class work. Each student will give 3 presentations related to the analytical equipment in the course (2 from the geochemical section and 1 from the molecular/genomics section). Students will also write 1 mini-review regarding genomics techniques and applications. More details about specific topics will be relayed in class.

Grades will be determined based on the following formula.

**Grading (for graduate students)**
- Participation (5%)
- Homework/Lab reports: (7 reports - 50%)
- Oral Presentation and Discussion Leader (3 presentations - 30%)
- Genomics Research Project Mini-Review (15%, Grad student only)

**Grading (for undergraduate students)**
- Participation (10%)
- Homework/Lab reports: (7 reports - 60%)
- Oral Presentation and Discussion Leader (3 presentations - 30%)

Genomics Research Project Mini-Review (extra credit – up to 10%)

The grading scale is:

<table>
<thead>
<tr>
<th>Grade</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>A</td>
<td>91-100%</td>
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<tr>
<td>B</td>
<td>81-90%</td>
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<tr>
<td>C</td>
<td>71-80%</td>
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<tr>
<td>D</td>
<td>61-70%</td>
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**Assignment Details**

**Participation (5-10%)**
We expect all students to be active participants in this class. This is primarily a hands-on class experience, so you will get the most out of it by contributing actively to discussions and participating in the use of equipment.

All students are expected to attend all class periods. Absences must be cleared with the instructors in advance. All absences will result in a loss of points from the participation grade. Unexcused absences will result in the loss of 25% from the participation grade.

**Homework and Lab reports (50-60%):**
Lab reports will include interpretation of calibration data, running of unknowns and interpretation of results, and possible comparisons among sample types. Each major piece
of equipment will have an associated lab report (six reports in the geochemical section and 1 report in the molecular/genomics section). Lab reports should include principles of the specific instrument, major components, analytical procedures, instrument calibration, data produced, and discussion. These reports should also include an evaluation of how the equipment and resulting data could be used in freshwater research and/or regulatory applications. In some cases problem sets may be include in lab reports. These problem sets may pertain to data from the laboratory experiments, lecture content, and reading assignments.

Reading and assignment materials will be handed out by e-mails or posted on D2L website.

Assignments/reports should be turned in at the beginning of class on the assigned due date.

**Oral Presentation and Discussion Leader (30%):**
Each student will give three presentations (powerpoint or other media-based style) on assigned instruments. Specific topics will be chosen by students in the first two weeks of class. Each presentation must cover the major principles of the instrument, the major components of the instrument and how they work together, and a detailed description of basic procedures to run the instrument, including: reagents needed, calibration parameters, and typical settings. The presentation should also include relevant research papers using the technique, especially those from the aquatic sciences. We encourage presenters to involve the class in discussion related to the topics being presented. Presentations should be sent to the instructors and classmates prior to class or handouts should be included with the presentations.

**Genomics Research Project Mini-review (grad-only 15%, undergrad extra credit):**
Graduate students are required to write a “mini-review” style paper on a specific genomics research technique. On the first class of the genomics section, we will discuss the specifics of this review in more detail and cover the potential topics. In brief, this mini-review will be based on a specific genomics analysis sequencing and analysis technique. The review should describe the basic technique, explain how it used, describe what types of data are produced and how these are used in aquatic sciences, include the relevant literature, and describe the newest developments in its use or procedural modifications. Students are encouraged to choose topics that are relevant to their own research or career goals.

Mini-reviews are due during Finals Week – Wednesday May 17 at 11:59 PM.

**Deadlines**
- All assignments are due at the beginning of the class on the due date. Late submissions will result in a grade deduction.

**Safety:**
This course requires laboratory work. An initial safety introduction with material specific to the lab and classroom will be performed prior to the laboratory portions of the class. Goggles and lab coat may be needed for some labs.
Other Class Policies

- There is no extra credit available for this course.
- Attendance is expected and mandatory. Arrival on time is required.
- There is no make-up for analytical experiments/laboratory. Lab work requires your flexibility in scheduling.
- Return all equipment and materials to their proper location and clean your space of debris and refuse.
- Please discuss any special circumstances with your instructor.

Academic Misconduct:
All UWM students are required to adhere to University standards of student conduct especially with regard to academic honesty and plagiarism. Plagiarizing is prohibited. Cheating, plagiarism, and other misconduct will result in severe penalty, as per University of Wisconsin System Chapter 1: 

University Policies
The Secretary of the University web site contains the university policies.  
http://www4.uwm.edu/secu/SyllabusLinks.pdf

1. Students with disabilities. Special accommodations are provided to meet learning and testing needs in a timely manner.  
http://www4.uwm.edu/sac/

2. Religious observances. Accommodations for absences due to religious observance. 
http://www4.uwm.edu/secu/docs/other/S1.5.htm

3. Students called to active military duty. Accommodations for absences due to call-up of reserves to active military duty. 
http://www4.uwm.edu/current_students/military_call_up.cfm

4. Incompletes. A notation of "incomplete" may be given in lieu of a final grade to a student who has carried a subject successfully until the end of a semester but who, because of illness or other unusual and substantiated cause beyond the student’s control, has been unable to take or complete the final examination or to complete some limited amount of term work. http://www4.uwm.edu/secu/docs/other/S31.pdf

5. Discriminatory conduct (such as sexual harassment). Discriminatory conduct will not be tolerated by the University. It poisons the work and learning environment of the University and threatens the careers, educational experience, and well-being of students, faculty, and staff. http://www4.uwm.edu/secu/docs/other/S47.pdf
6. **Academic misconduct.** Cheating on exams or plagiarism are violations of the academic honor code and carry severe sanctions, including failing a course or even suspension or dismissal from the University. [http://www4.uwm.edu/osl/dean/conduct.cfm](http://www4.uwm.edu/osl/dean/conduct.cfm)

7. **Complaint procedures.** Students may direct complaints to the head of the academic unit or department in which the complaint occurs. If the complaint allegedly violates a specific university policy, it may be directed to the head of the department or academic unit in which the complaint occurred or to the appropriate university office responsible for enforcing the policy. [http://www4.uwm.edu/secu/docs/other/S49.7.htm](http://www4.uwm.edu/secu/docs/other/S49.7.htm)

8. **Grade appeal procedures.** A student may appeal a grade on the grounds that it is based on a capricious or arbitrary decision of the course instructor. Such an appeal shall follow the established procedures adopted by the department, college, or school in which the course resides or in the case of graduate students, the Graduate School. These procedures are available in writing from the respective department chairperson or the Academic Dean of the College/School. [http://www4.uwm.edu/secu/docs/other/S28.htm](http://www4.uwm.edu/secu/docs/other/S28.htm)

9. **Other.** The final exam requirement, the final exam date requirement, etc. [http://www4.uwm.edu/secu/docs/other/S22.htm](http://www4.uwm.edu/secu/docs/other/S22.htm)
### Tentative Schedule for FRSHWTR-514 (Instrument used between Week 4-10 will depend on total number of students)

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<thead>
<tr>
<th>Week</th>
<th>Date</th>
<th>Topic</th>
<th>Note</th>
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<tbody>
<tr>
<td>1</td>
<td>Jan 24</td>
<td>Overview of class and syllabus</td>
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<td>Lab safety</td>
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<td>Assignment for projects/instrument</td>
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<td></td>
<td></td>
<td><strong>Geochemical Instrumentation Section</strong></td>
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<tr>
<td>2</td>
<td>Jan-31</td>
<td>Introduction and Instrumentation (visit labs)</td>
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<tr>
<td></td>
<td></td>
<td>Pipetting/balance/dilution/calibration</td>
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<tr>
<td>3</td>
<td>Feb 7</td>
<td>QA/QC (also significant figures)</td>
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<tr>
<td></td>
<td></td>
<td>Errors and propagation</td>
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<tr>
<td>4</td>
<td>Feb 14</td>
<td>Field sampling for water, SPM, biota, sediment</td>
<td>Field sampling</td>
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<td></td>
<td></td>
<td>Sample processing</td>
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<tr>
<td>5</td>
<td>Feb 21</td>
<td>Nutrients (N, P, and Si)</td>
<td>Instrument 1&amp;2</td>
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<td></td>
<td></td>
<td>Auto analyzer/Ion Chromatography (IC)</td>
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<tr>
<td>6</td>
<td>Feb-28</td>
<td>Major ions – cations</td>
<td>Instrument 3&amp;4</td>
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<td></td>
<td></td>
<td>Atomic Absorption (AA)/ICP-MS (demo only)</td>
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<tr>
<td>7</td>
<td>Mar 7</td>
<td>Total Carbon and Nitrogen Analyzer</td>
<td>Instrument 5</td>
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<td></td>
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<td>Shimadzu TOC analyzer/DIC and TDN</td>
<td>3 parameters</td>
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<tr>
<td>8</td>
<td>Mar 14</td>
<td>UV-vis spectroscopy</td>
<td>Instrument 6</td>
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<td>Colored dissolved organic matter (CDOM) and others</td>
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<td></td>
<td>Mar 21</td>
<td><strong>No Class Spring Break</strong></td>
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<tr>
<td>9</td>
<td>Mar 28</td>
<td>Elemental (C, N, O, S) Analyzer/IR-MS</td>
<td>Instrument 7&amp;8</td>
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<td>Elemental analyzer/IR-MS (demo)</td>
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<td>10</td>
<td>Apr 4</td>
<td>Student Presentation</td>
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<td>Water isotopes, Isotopic ratio-Mass Spectrometry</td>
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<td>ICP-MS, LC-MS, GC-MS (demonstration only)</td>
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<td></td>
<td></td>
<td><strong>Molecular/Genomic Instrumentation Section</strong></td>
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<tr>
<td>11</td>
<td>Apr 11</td>
<td>Intro to Nucleic Acids/Genomic Methods</td>
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<td>Basic molecular lab techniques &amp; sample collection</td>
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<tr>
<td>12</td>
<td>Apr 18</td>
<td>Nucleic Acids I</td>
<td>Instrument 9</td>
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<td>DNA/RNA extractions and QA/QC</td>
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<tr>
<td>13</td>
<td>Apr 25</td>
<td>Nucleic Acids II &amp; PCR</td>
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<td>Hybridization principles, PCR/qPCR</td>
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<tr>
<td>14</td>
<td>May 2</td>
<td>Genomic Methods I</td>
<td>Instrument 10</td>
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<td></td>
<td></td>
<td>Finish PCR topics &amp; Intro to Genomic Information</td>
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<td>15</td>
<td>May 9</td>
<td>Genomic Methods II</td>
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<td><strong>Sequence data comparison &amp; Available genomic analysis tools and basic operations</strong></td>
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Total contact hours:
3 credits = 45 hours of lecture and labs