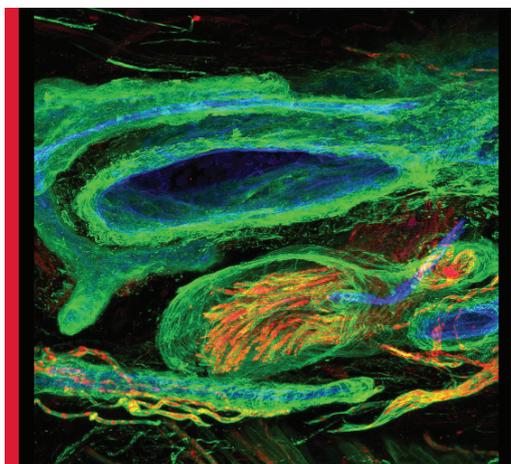


# Nebraska Center for Biotechnology Newsletter



*Immunofluorescence confocal 3D image from a triple-labeled mouse skin section*

## Director's Message

This will be my sixth year as the Director of the Nebraska Center for Biotechnology. During the past six years the Center has focused on improving customer service and upgrading all of our instrumentation. We have added a full service Proteomics and Metabolomics core and a Flow Cytometry core. In the past year new instruments have been added to the Flow Cytometry and Microscopy core as described in this newsletter. Those two cores are now working more closely together which has benefited faculty members who may use the flow cytometry core first and then move to microscopy to visualize cells with particular characteristics. We have also established a satellite flow cytometry instrument in the Beadle Center.

Covid 19 has brought about a huge amount of upheaval and change to our world. The Biotech Center went through a period of shutdown, but is now open and fully operational. Each core has taken precautions to limit their exposure to Covid while they work to serve faculty, staff and student research needs. Please contact the core Directors and/or consult the core websites for specific information on how to initiate projects or to have samples analyzed.

We continue to teach a one unit course in the spring semester to introduce students to our capabilities in the hope that as their research projects progress, they will make better use of the cores. Students who successfully complete the course also receive a \$500 voucher to use at any of the five cores in the Center. This voucher is designed to give students time to interact with and use the cores, which is something that we cannot easily do during class.

The mission of the Nebraska Center for Biotechnology is to assist faculty, students and staff in research. Please let us know about ideas you have for improving our services and providing new instruments. We look forward to 2020 being another productive year of scientific discovery in which we are able to enhance your research efforts!

## IN THIS ISSUE

- Director's Message
- Microscopy Updates
- Flow Cytometry News
- PMF - What's New?
- PMF Personnel Spotlight
- Bioinformatics Bulletin

### Nebraska Center for Biotechnology

DANIEL SCHACHTMAN, DIRECTOR

N300 Beadle Center

1901 Vine Street

Lincoln NE 68588-0665

SOPHIE ALVAREZ, [PROTEOMICS & METABOLOMICS](#)

DIRK ANDERSON, [FLOW CYTOMETRY](#)

TOM CLEMENTE, [PLANT TRANSFORMATION](#)

JEAN-JACK RIETHOVEN, [BIOINFORMATICS](#)

YOU ZHOU, [MICROSCOPY](#)



## MICROSCOPY UPDATES

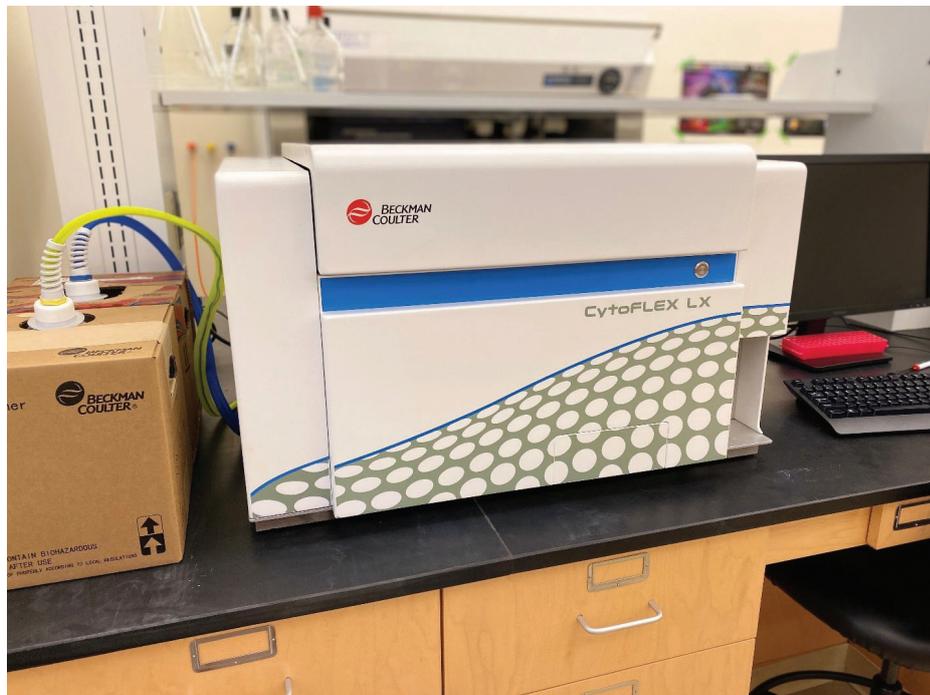
### New instruments for Microscopy Core

The [Microscopy Core Research Facility](#) has purchased a new cryo-sample preparation and initial evaluation package, which includes a Leica Grid Freeze Plunger with viewing station (Leica GP2 shown in picture at right) and a Gatan Elsa Cryo-Transfer Holder (Cryo-holder) with temperature controller and pumping system. The core also added an EMS dual glow (positive and negative) discharger (also shown in the picture) for the cryo-grid sample preparation. The installation was just completed. The core plans to provide cryoEM sample preparation and initial evaluation service using the newly installed system in spring semester 2020.

# FLOW CYTOMETRY

## Flow Cytometry Service Center Introduces New Analyzer and Expands Footprint

The Flow Cytometry Service Center has recently installed a new state of the art analyzer to the core facility. A Beckman Coulter CytoFLEX LX system complete with four lasers (405, 488, 561, and 638nm) and 16 fluorescence detectors was installed in the facility over the summer. This powerful system is a strong addition to the lab, and allows users to visualize numerous sample types including primary and cultured cells, algae, yeast, bacteria, beads, and is even designed to be sensitive enough to visualize extracellular vesicles. The new Avalanche Photo Diode (APD) technology dramatically increases the sensitivity of the fluorescence channels, allowing for better resolution of fluorescent probes. The system was purchased with the capability to collect samples out of a number of vessels, including a variety of tubes or 96 well plates.



Between the updated detectors, automated sampling option, the intuitive data analysis software (CytExpert), and its ability to be easily upgraded in the future, the CytoFLEX LX is going to be a wonderful system both now and for years to come.

Another huge benefit of the installation of this new system, is that it allowed the Flow Cytometry Service Center to be able to expand its footprint to City Campus. During October, the Cytek DxP10 system (four lasers, 10 fluorescence detectors) was transferred to the Beadle Center and is available for immediate use.

To request training on either the new CytoFLEX LX, or the Cytek DxP10, please contact Dirk Anderson, Flow Cytometry Service Center Director, [dirk.anderson@unl.edu](mailto:dirk.anderson@unl.edu) 402-472-3129.

## PMF -WHAT'S NEW?

The Proteomics and Metabolomics Facility (PMF) will soon be celebrating five years of operation since its start back in October 2015. The PMF team has expanded and now counts, in addition to the Director Dr. Alvarez and the Assistant Director Dr. Naldrett, a lab manager, Anne Fischer, and two research technicians, Lori Loucks (who joined last August – see personnel spotlight to learn more about Lori!) and Felicia Phares, who recently moved from a temporary to a permanent position. With the expansion of personnel, all the analytical equipment is now set up in the adjoining rooms E156 and E118 with wet bench space available for sample preparation to give easy access for self-service users.

The facility recently implemented a new online [Sample Submission Form](#), via the platform Smartsheets, to streamline the submission process and assist with project management. All submissions must be done through that form. Users should also refer to the new [PMF Policies and Procedures Manual](#), which provides valuable user information.



Follow us on our new twitter page [@PMF\\_UNLBiotech](#) to stay up to date on what is happening in the lab!

## PMF TRAINING

Training is offered all year round for several of the facility's self-service analytical platforms. This includes the Agilent UPLC Infinity II which is equipped with three detectors (fluorescence, diode array, and evaporative light scattering) for use in combination or alone; the Agilent single quadrupole GCMS which offers three different types of injection (liquid, headspace, and SPME [solid phase microextraction]), ideal for volatile compounds; and the Sciex QTRAP 6500+ with SelexION+ LC-MS system coupled to a Shimadzu UPLC Nexera X2 for customized specific and sensitive targeted assays. Workshops on proteomics and metabolomics are also offered once a year. Please see the [Special Events](#) page for current offerings or e-mail us to be put on our mailing list. Please also see our [website](#) for instrument specific policies applicable to self-service users.



## PMF PERSONNEL SPOTLIGHT:

### Lori Loucks

I have been a Research Technician with the Proteomics and Metabolomics Facility since August 2019. I completed my studies as a nontraditional student, receiving my B.S. in Biochemistry in 2009 from the University of Nebraska at Lincoln. My education has provided me with an excellent background for the skills I have gained in the different laboratories in which I have worked. After graduation I became a Histotechnician for a pathology laboratory where I prepared surgical specimens for analysis using

multiple staining methods, including immunohistochemistry. I also worked as a Quality Control Chemist and Chemistry Laboratory Supervisor for a company that manufactures enzymes and proteins. This laboratory tested raw and processed material for enzyme concentrations of protease, amylase, and lipase using USP and BP monographs. In addition to learning valuable techniques at the bench, my experience has allowed me to develop a high degree of attention to detail and the ability to think critically. I enjoy these aspects of my current position, as well as the opportunity to continually learn new skills.

## NEW SERVICES IN PROTEOMICS AND METABOLOMICS!

The facility regularly adds new services. The most recent additions are:

1. Complete workflows for tandem proteomics and phosphoproteomics studies using label-free or TMT-labeling approaches. A quote must be requested as cost is based on individual experimental design.
2. Several new targeted metabolomics assays have been optimized: a flavonoid assay which includes a total of 30 different compounds including phenolic acids, isoflavone, flavonone, flavonol, and sugar-conjugated flavonoids; a carotenoid assay for any type of plant-based sample which includes lutein, zeaxanthin, violaxanthin, and antheraxanthin; and a THC/THCA assay for cannabinoids in hemp.
3. Our phytohormone assay has been expanded further to now quantify a total of 29 different compounds in one assay, it includes ABA, SA, JA, JA-Ile, OPDA, DIMBOA, IAA and 4 IAA conjugates, c/t-zeatin, c/t-zeatin-riboside, 3 strigolactones, and 11 gibberellins.
4. LC-MS untargeted metabolomics using our high-resolution mass spectrometer is also offered and is custom-based on the metabolite coverage required.

The full list of services is available at <https://biotech.unl.edu/proteomics-and-metabolomics>.



# BIOINFORMATICS BULLETIN

## METAGENOMICS

The core is now offering routine analysis of genetic material from microbiome community samples obtained from the environment (e.g., soil, waste, lake, or sea water) and individuals (e.g., gut or skin). Three types of sample types can be processed: amplicons (16S/18S/ITS/ITS2), whole transcriptome (RNA), and whole genome (DNA). Each type answers different questions about the biology: while amplicon sequencing and analysis can tell us about the biodiversity and abundance of Bacteria, Archaea, and Fungi (mainly), it does not elucidate what is currently transpiring on a biochemical level (based on transcription) or the potential of the community, except for the latter by inferring this by currently sparse whole genome databases. Whole transcriptome analysis of a community gives us insight into which transcripts and biochemical pathways are currently active, and if there are differences between sample groups. Whole genome analysis is more geared towards the potential of a microbiome as genes might not be expressed at all. Both transcriptome and whole genome sequencing and analysis will capture other organisms as well, such as protists or higher Eukaryotes.

Routine services:

1. For amplicon analysis: quality control, alpha and beta diversity, PCoA/MDS plots, absolute and relative abundance plots of any taxonomic level, CCA plots if measured additional data is present (e.g., geophysical or -chemical properties of soil). Custom additions are inferred potential biochemical capabilities via known complete genomes.
2. For transcriptome analysis: quality control, meta-transcriptome assembly and annotation, differential transcript analysis between groups or conditions, GO enrichment analysis (of known transcripts), heatmaps.
3. For whole genome analysis: quality control, meta-genome assembly and annotation, NCBI submission-ready genome files, abundance plot for the genomes based on their taxa.

Additional plots, analyses, and integration of the three data streams above can be quoted as well upon discussion. We are capable of processing tens to thousands of samples with samples being defined as a unique combination of factors (e.g., genotype, condition, growth stage, etc.) and each sample can (should) include several to many replicates. For up to 50 samples the fee is \$81/sample; any sample after that is \$55/sample.

## TRANSFER SERVER AT 10 GB/SEC

The Core often transfers large datasets (TBs) to the Holland Computing Center (HCC) and back, which previously could take several days to a week to complete. We have invested in a transfer server that is connected to 10Gb/s fiber optic direct connection to HCC and can copy data up to approximately 1GB/s or 980MB/s. This is ten times faster than normal copper wired desktops or servers, and even more when comparing to wireless laptops.

The server has 30TB of fast SSD caching space available. It is not meant for storing data longer term but a means to get data sets on, transferred to HCC fast, and off. Any research group in Beadle can contact [bcrf-support@unl.edu](mailto:bcrf-support@unl.edu) if they want to take advantage of this (no charge).



CENTER FOR BIOTECHNOLOGY

