## Q Exactive Plus Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Fisher Scientific)

The Q Exactive Plus Orbitrap is coupled to an autosampler and nanoAcquity UPLC system. This instrument features high ion currents because of an S-lens, and fast high-energy collision-induced dissociation (HCD) peptide fragmentation because of parallel filling and detection modes. The image current from the detector is processed by an "enhanced Fourier Transformation" algorithm, enabling high mass spectrometric resolution (up to 140,000 FWHM). With almost instantaneous isolation and fragmentation, the instrument can sequence 10 peptides per second. This instrument is also capable of proteomics approach termed parallel reaction monitoring (PRM), which is a targeted proteomics strategy wherein all product ions of a peptide are simultaneously co-detected under conditions that offer high resolution and high mass accuracy.

## **Q Exactive HF-X Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific)**

The Q Exactive HF-X mass spectrometer harbors a high-field (HF) Orbitrap mass analyzer and an Advanced Quadrupole Technology (AQT) resulting in higher resolution (240,000 at m/z 200) and scan speed (up to 40 Hz). A High Capacity Transfer Tube (HCTT) and electrodynamic ion funnel also contributes towards the improved sensitivity of the instrument. This mass spectrometer enables fast identification and analysis of peptides, label-free and TMT quantitation. An EASY-nLC<sup>™</sup> 1200 system is connected with the mass spectrometer for peptide separation.

## **Orbitrap Fusion Tribrid Mass Spectrometer (Thermo Fisher Scientific)**

The fusion is an Orbitrap hybrid mass spectrometer and is coupled to an UltiMate 3000 RSLCnano system. The Fusion is equipped with a mass filter, a collision cell, a high-field Orbitrap analyzer, and, finally, a dual cell linear ion trap analyzer (Q-OT-qIT). This system offers high MS/MS acquisition speed of 20 Hz and detects up to 19 peptides sequences within a single second of operation. The Fusion also has resolution in excess of 450,000 allowing for separation of isobaric interferences.

# Orbitrap Fusion Tribrid Mass Spectrometer with Electron Transfer Dissociation (ETD) (Thermo Fisher Scientific)

The fusion is an Orbitrap hybrid mass spectrometer, which is coupled to an UltiMate 3000 RSLCnano system. The Fusion is equipped with a mass filter, a collision cell, a high-field Orbitrap analyzer, and, finally, a dual cell linear ion trap analyzer (Q-OT-qIT). This system offers high MS/MS acquisition speed of 20 Hz and detects up to 19 peptides sequences within a single second of operation. The Fusion also has resolution in excess of 450,000 allowing for separation of isobaric interferences. This Fusion is also equipped with Electron Transfer Dissociation (ETD), which is an orthogonal fragmentation technique to HCD and collision induced dissociation (CID), which will enhance peptide identification and localization of labile post-translational modifications (PTMs), such as glycosylation and phosphorylation.

# Orbitrap Fusion Lumos Mass Spectrometer with Electron Transfer Dissociation (ETD) (Thermo Fisher Scientific)

The Fusion Lumos mass spectrometer has three mass analyzers— quadrupole, orbitrap, and linear ion trap as in the fusion tribrid system. The instrument houses an upgraded ion optics (High Capacity Transfer Tube and Electrodynamic Ion Funnel), an Advanced Quadrupole Technology (AQT), and a new ETD-HD feature. The MS/MS acquisition rates of Lumos is up to 20 Hz for both Orbitrap and linear ion trap measurements. Ultra-highfield Orbitrap mass analyzer increases the resolving power up to 500,000 FWHM at m/z 200. Synchronous Precursor Selection (SPS) feature significantly augments the number of peptides and proteins identified and improves quantitative accuracy in TMT experiments. This feature is also available in other Fusion instruments. Lumos offers several fragmentation modes, like CID, HCD, ETD, and EThcD, that are helpful for PTM analysis. Lumos is connected to an EASY-nLC<sup>™</sup> 1200 system for UHPLC.

## UltiMate 3000 RSLCnano (Thermo Fisher Scientific)

UltiMate 3000 RSLCnano system can be operated with nano-, capillary-, and micro-flow options. ProFlow technology in this system improves nano flow rate control resulting in high retention time precision that is crucial for LC-MS based proteomics methodology. The pressure capacity of this UHPLC is up to 860 bar (12473 psi).

# nanoAcquity UPLC (Waters Corporation)

The nanoACQUITY UltraPerformance LC System is designed for nano-scale, capillary, and narrow-bore separations to attain the highest chromatographic resolution, sensitivity, and reproducibility. Both qualitative and

quantitative projects benefit from the added pressure capacity (up to 10, 000 psi); sensitivity is increased several folds with the use of sub-2 µm chromatographic resins and retention time reproducibility enables label-free quantitative approaches.

## EASY-nLC 1200 System (Thermo Fisher Scientific)

EASY-nLC 1200 System is a capillary UHPLC system that allows for a maximum backpressure up to 1200 bar (17,404 psi). This higher pressure allowance increases the peak capacity resulting in better peptide separation and increased throughput.

#### Liquid Handling Station (LHS)

The BRAND Liquid Handling Station handles routine pipetting tasks (e.g., BCA assay, protein digestion, aliquoting, etc.) at high speed and with the highest precision.

#### Off-line Liquid Chromatography systems (Agilent Technologies)

An Agilent 1100 series system comprising of a degasser, binary pump, autosampler, UV-detector and a fraction collector is available for offline fractionation of samples. The system is capable to handling all current fractionation methods including high-pH reverse phase, SCX and ERLIC.

#### Computing

The Emory Integrated Proteomics Core (EIPC) has multiple search engine platforms including Sequest, Mascot, and Byonics. A Sage-N Sorcerer2 IDA is located in-house and currently runs SorcererSequest and Scaffold. The Mascot, Proteome Discoverer 2.0, and Byonic platforms are licensed on a computing cluster in collaboration with the Emory Integrated Computational Core (EICC, <u>http://www.cores.emory.edu/eicc/</u>). Core facility personnel have access to high-end workstations capable of processing both raw and post-analyzed data.

**Shared equipment** also includes gel imagers for chemiluminescence and fluorescence, equipment for protein, a LiCor fluorescent system for quantitative immunoblotting. Fluorescence and standard microplate readers are available for protein quantification. High-speed and ultra-centrifuges are available as core equipment. There are sliding microtomes, standard and dual beam spectrophotometers, cryostats, pH meters, balances, oven, refrigerators, hot plates, stirrers, -70° C freezers, and other standard equipment.