Mass Cytometry

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- **Contacts**
  - Anne Sperling, Faculty Director, asperlin@medicine.bsd.uchicago.edu
    - Oversees core and initial project startup.
  - David Leclerc, Technical Director, dleclerc@bsd.uchicago.edu
    - Manages core facility. Please contact him for questions relating to billing.
  - Laura Johnston, Scientific Associate Director, ljohnston@bsd.uchicago.edu
    - Main contact for CyTOF projects. Please contact her about panel design, experiment design, troubleshooting, and data analysis. Can help you get reagents from the core facility.
  - Mike Olson, Lab Manager, molson@bsd.uchicago.edu
    - Main contact for running samples on Helios. Please contact him to schedule time on the helios and for any questions regarding running samples on the machine. Can help you get reagents from the core facility.
  - Bert Ladd, Research Specialist, rladd@bsd.uchicago.edu
    - Backup Helios operator. Can help you get reagents from the core facility.
- **Benefits**
  - Utilizes the atomic mass spectrum
    - Non-rare
    - Non-biological
    - Non-radioactive
  - Abundant tags of similar intensity
  - Discrete signals: minimal overlap
    - No compensation!
  - Signal Overlap is minimal and predictable (fewer controls to run)
  - Zero background from cells
    - Great for barrier tissues!
- **Reagents**
  - Fluidigm antibodies
    - anti-human, anti-mouse, and secondary metal-conjugated antibodies
    - Pre-designed panel kits
    - Metal labeling kits for custom antibody conjugations
  - Pd barcoding – multiplexing up to 20 samples simultaneously
  - Intercalator (Iridium) – identifies single cell events
  - Cisplatin – dead cell indicator
  - IdU – cell cycle similar to BrdU
  - Buffers
    - Maxpar or Rockland (preferred) PBS
    - Maxpar cell staining buffer
    - Maxpar fix/perm buffer
    - Maxpar water
  - EQ4 element calibration beads
- **Tips for sample preparation**
  - Titrate all reagents including Ir intercalator
  - Use Type I, Ultrapure water (18.2 MΩ) for all Helios related purposes including:
    - Making buffers and detergent solutions
    - Rinsing glassware
    - Carrier solution and diluent for beads and samples
  - Minimize barium in buffers (calcium, magnesium-free)
  - Completely wash out PBS to prevent salt clogs in nebulizer and sample tubing
  - Minimize debris and clumps
  - Count cells utilizing a method that visualizes debris, such as a hemocytometer
  - *Cell Concentration*
    - Staining: $< 3\times10^6$ per 100 µL staining volume

        - For sample acquisition (300 cells/sec target sampling rate)
            - $0.5x10^6$ /mL (0.030 mL/min introduction rate)
                - Concentration calculated on cells + particulates
    - *Sources of contamination*
        - Glassware and detergents
            - Use metal-free plastics and filter pipette tips
            - Clean glassware with Type I ultrapure water and wipe pipettes with 70% ethanol
        - Autoclaved items
        - Prolonged exposure to the air
            - Do not leave tubes and bottles uncapped
        - Ficoll ("paque" is sodium diatrizoate and contains iodine) and lymphoprep
            - CyTOF forums have suggested Ficoll-Paque PLUS and extra washing steps, but iodine contamination is an issue
        - Patients (exposure to contrast dyes, cisplatin chemotherapy)
- **Sample acquisition**
    - Liquid cell suspension 30 µL/min (fixed rate)
    - Bring samples in a smaller volume. Samples will be filtered and concentration will be adjusted prior to running
- **General Cell staining protocol**
    - Stain single cell suspension with cisplatin in PBS for 5 minutes
    - Quench with staining buffer and wash
    - Fc block in staining buffer for 10 minutes
    - Stain with surface antibodies for 30 minutes
    - Wash
    - Stain with Intercalator in fix/perm buffer overnight
    - Wash and resuspend in final volume of water with EQ4 element calibration beads

University of Chicago CyTOF Workflow

1. Meet with Anne and Laura to discuss project goals and determine if CyTOF is a good fit for your project
2. Panel Design with Laura and Kevin (Fluidigm)
    a. Current maximum is 37 markers
    b. Send Laura a list of markers
        i. Include protein/antibody name, NOT gene name
        ii. If known/preferred, please include clone of antibody
        iii. Information that can also be helpful for panel design, particularly for markers that are uncommon to immune panels:
            1. Expression level (high/medium/low/unknown)
            2. Cellular location/type (surface, cytoplasmic, nuclear, cytokine, phospho)
            3. Expression pattern on cells of interest (ex: regulatory T cells are CD3+CD4+CD25+Foxp3+ and classical monocytes are CD66b-CD14++CD16-)
    c. Laura and Kevin will assign metal tags and discuss with you
3. Titrate cisplatin and Ir-intercalator on cells of interest
    a. This will also identify if your sample prep contains any contaminants so we can adjust your protocol
    b. Can be done while panel is being designed
    c. Please schedule time with Laura to analyze your titration data and go over data quality.
4. Titrate antibodies
    a. Can titrate 10 at a time
5. Test all antibodies together on preferred tissue
    a. If barcoding samples, also test scaling up to the number of cells that will be in your combined barcoding sample
6. Run samples
7. Analyze Data