Using AutoEV on the ONi Nanoimager

Question 1: What species is your sample isolated from?

- Human
- Other

Question 2: Are your EVs isolated from healthy or cancerous cells/tissues?

- Healthy
- Cancerous

Human

- Use the Biotinylated Assay Chips stored at -20°C
- Choose your capture method:
  - ☐ Phosphatidylserine[1]
  - ☐ CD81
  - ☐ CD63
  - ☐ CD9
  - ☐ TetTrio[2]
- Choose your detection method:
  - ☐ Tetraspanin Profiling: CD81-647, CD63-561, CD9-488
  - ☐ EV Profiling: TetTrio-561, PanEV-488[3]
- Follow the protocol here.
- Additional considerations:

[1]The PS capture is calcium dependent. Add the capture supplement to your sample at a dilution of 1:10

[2]The TetTrio reagents contain a 1:1:1 ratio of antibodies against CD81, CD63, and CD9.

[3]For EV Profiling, you can add a custom biomarker detection to the 647 channel.

Other – Healthy

- Use the Biotinylated Assay Chips stored at -20°C
- Choose your capture method: CD9
  - ☐ Phosphatidylserine[1]
- Choose your detection method:
  - ☐ Tetraspanin Profiling: CD81-647, CD63-561, CD9-488
  - ☐ EV Profiling: TetTrio-561, PanEV-488[3]
- Follow the protocol here.
- Additional considerations:
  - Generally, cancer-derived EVs contain enough PS for efficient capture.
  - If you notice low capture efficiencies (<300 clusters/field of view) consider trying the PLL capture method.

[1]The PS capture is calcium dependent. Add the capture supplement to your sample at a dilution of 1:10

[2]The TetTrio reagents contain a 1:1:1 ratio of antibodies against CD81, CD63, and CD9.

[3]For EV Profiling, you can add a custom biomarker detection to the 647 channel.

Other – Cancerous

- Use the Uncoated Assay Chips stored at RT
- Prepare the surface of the chip:
  1. Apply 10ul of 0.1% Poly-L-Lysine solution to each lane and incubate for 20 min.
  2. Wash each lane with 100uL of distilled H_2O
  3. Add 10uL of your EVs to each lane. Your EVs should be at a concentration of 10^8-10^{11} particles/mL for best results.
  4. Incubate the chip with your EVs for 75 min in a humid chamber.
  5. Proceed with Step 10 of this protocol (Tetraspanin Detection).
- Additional considerations:
  - Generic dyes including lipophilic and nucleic acid stains will interact with the PLL and generate high background. If you want to use these dyes, please stain your EVs prior to adding them to the chip.
  - This includes the PanEV included in the ONI EV Profiling Kits