Maria Clara Zanellati  
**PILOT: Sharing Imaging Data**

Goal

Create a scalable approach for processing and sharing imaging data   
Draft:

* Pilot data "specifics"
  + **Cell line details**  
    Human hiPSCs (KOLF2.1 wt) were obtain from Dr. Michael Ward and were used to generate hiPSCs-PB-TO-hNGN2 cell line. All the cells were incubated at 37C, 5% CO2 and culture under feeder-free conditions in StemFlex medium (Gibco A3349401) on Vitronectin (Vitronectin (VTN-N) Recombinant Human Protein, Truncated, Gibco A14700). The cells were split using ReLeSR (STEMCELL Technologies) in absence of ROCK inhibitor.  
    hiPSCs-PB-TO-hNGN2 stable cell line were generated as previously described (PMID: 29924488). Briefly, KOLF2.1 wt were transfected with piggyBac plasmid carrying rrTA and Ngn2-Puro cassette (plasmids gifted from Dr. M.Ward). Transfected cells were selected for stable integration using Puromycin treatment and propagated as a non-clonal pool. Differentiation into cortical neurons. hiPSCs-PB-TO- hNGN2 were differentiated into cortical neurons as previously described (PMID: 29924488) with some modifications. In briefly hiPSCs were seeded in colonies (10-20 cells) at high confluency (50-60%) on Vitronectin coated plates. One day after seeding the medium was changed to Induction media [DMEM/F12 with HEPES (Gibco, 11330032); N2 supplement 100X (Gibco, 17502048); Non-essential amino acids (NEAA) 100X (Gibco, 11140050) and supplemented with Doxycycline at a final concentration of 1µM (Sigma, D9891)] and changed every day. After 2.5 days, the pre-induced hiPSCs, were split in Accutase (StemPro™ Accutase™ Cell Dissociation Reagent, Gibco A1110501) and seeded single cell (300,000) on Poly-L-Ornithine (Sigma, P3655; 10x stock: 50mg in 50ml Borate Buffer) and laminin 1µg/ml (Gibco, 23017015) coated plates (Thermo Scientific™ Nunc™ Lab-Tek™ II Chambered Coverglass, 2-well #12-565-336). The day of the seeding the media was changed in Cortical Neuron Culture medium (CM) [BrainPhys neuronal medium without Phenol-Red (STEMCELL Technologies, 05791); B27 supplement, 50X (Gibco, 17504044); BDNF (10µg/ml) in PBS containing 0.1% IgG and protease-free BSA (PeproTech 450-02); NT-3 (10µg/ml) in PBS containing 0.1% IgG and protease-free BSA (PeproTech 450-03); Laminin final con. 1µg/ml (Gibco 23017015)], supplemented with ROCK inhibitor (RevitaCell supplement 100X, Gibco A2644501). The day after the seeding the media was fully changed with CM medium without ROCK inhibitor and the i3Neurons were kept for 27 days prior transfection with half media changed at least once per week with freshly prepared CM medium.   
    Transfection, labeling and microscopy of i3Neurons: After 6, 13 and 20 days post-induction the i3Neurons were transfected as previously described (PMID: 15121163) with some modifications. The transfection mix was prepared in plain Neurobasal (Gibco 21103049) with 6µl of Lipofectamine2000 (Invitrogen, 11668019), and 6µg of total DNA divided as followed: 2µg of pEIF1a::Transposase (gifted by Dr. Michael Ward), and 4µg of Lysosome, Mitochondria, Peroxisomes, Golgi and ER plasmids (Twist Technologies). At the day of the transfection, the media of i3Neurons was changed to plain Neurobasal (without Glutamine) and let them stand at least 30minutes before adding the transfection mix. Next, half media was removed, and the transfection mix was added dropwise. After 2hrs of incubation the media was fully changed with CM medium, and added BODIPY665 80ng.  
    The day after the transfection (24hrs) the neurons were tested for vitality by NeuroFluo final conc. 0.20µM (STEMCELL Technologies, 01801) and NuclBlue (Invitrogen, R37605) staining. The images were taken with a Confocal Microscope Zeiss800 (Axio Observer.Z1/7) with a 63x Objective (Plan-Apochromat 63x/1.40 Oil DIC M27).
  + **Organelle labels**  
    Plasmids:  
    - Lysosomes (pEF1a::Lamp1::mTurquoise2)  
    - Mitochondria (pEF1a::cox8::eGFP)  
    - Peroxisomes (pEF1a::mOrange2::SKL)  
    - Golgi (pEF1a::Sit::OxVenus)  
    - Endoplasmic Reticulum (pEF1a::Sec61b::mApple)  
       
    LiveDrop:  
    - Lipid Droplet (BODIPY665) Far Red
  + **Dataset sampling:**   
      
    - undifferentiated: hiPSCs (30 zstacks, 30 time series)  
    - induced Neurons day7: iNday7 (29 zstacks, 25 time series)  
    - induced Neurons day14: iNday14 ( zstacks, 28 time series)  
    - induced Neurons day21: iNday21 (26 zstacks, 24 time series)  
      
    AIM: Z-stacks (30), timelapses (30)
  + **File naming** convention for data collection and CellP analysis  
    Date\_ImageType\_sampleName\_BiologicalReplicate\_NumbImage
  + **Data size, formats**  
      
    ZSTACKS hiPSCs: 14-25 slices  
    ZSTACKS iNeurons: 20-40 slices  
    - raw data (ZSTACKS: 700,000 KB, TIME SERIES: 3,688,652 KB)  
    - unmixed (ZSTACKS: 200,000 kB, TIME SERIES: 922,000KB)
  + meta-data format and nominal resolutions: x,y, z, t, channels  
    800x800 pixels, 32 spectral channels  
    Image size: (pixel) 768x768, (scaled) 61.34um x 61.34 um  
    Scaling per pixel: 0.08um x 0.08um x 0.51 um
  + **Inferred data ("150 parameters")**
    - size, count, adjacency, co-locality, etc

**Softwares**:

* **Processing zstacks 2D:**  
  Fiji or ZEN software: Image substacks  
  CellProfiler: Use TIFF. For denoise, segmentation and measurements   
  Rstudio: tidyverse and dplyr for clearing and normalizing data  
    
  CellP pipeline NAME  
  CellP-2D\_PART-1of3\_v1\_wNU\_05042022  
  CellP-2D\_PART-2of3\_v1\_wNU\_05042022  
  CellP-2D\_PART-3of3\_v1\_wNU\_05042022
* **Processing Timeseries:**CellProfiler: Use czi files. Denoise, segmentation and Tracking objects  
  CellProfiler Analyst + Rstudio  
    
  CellP ()