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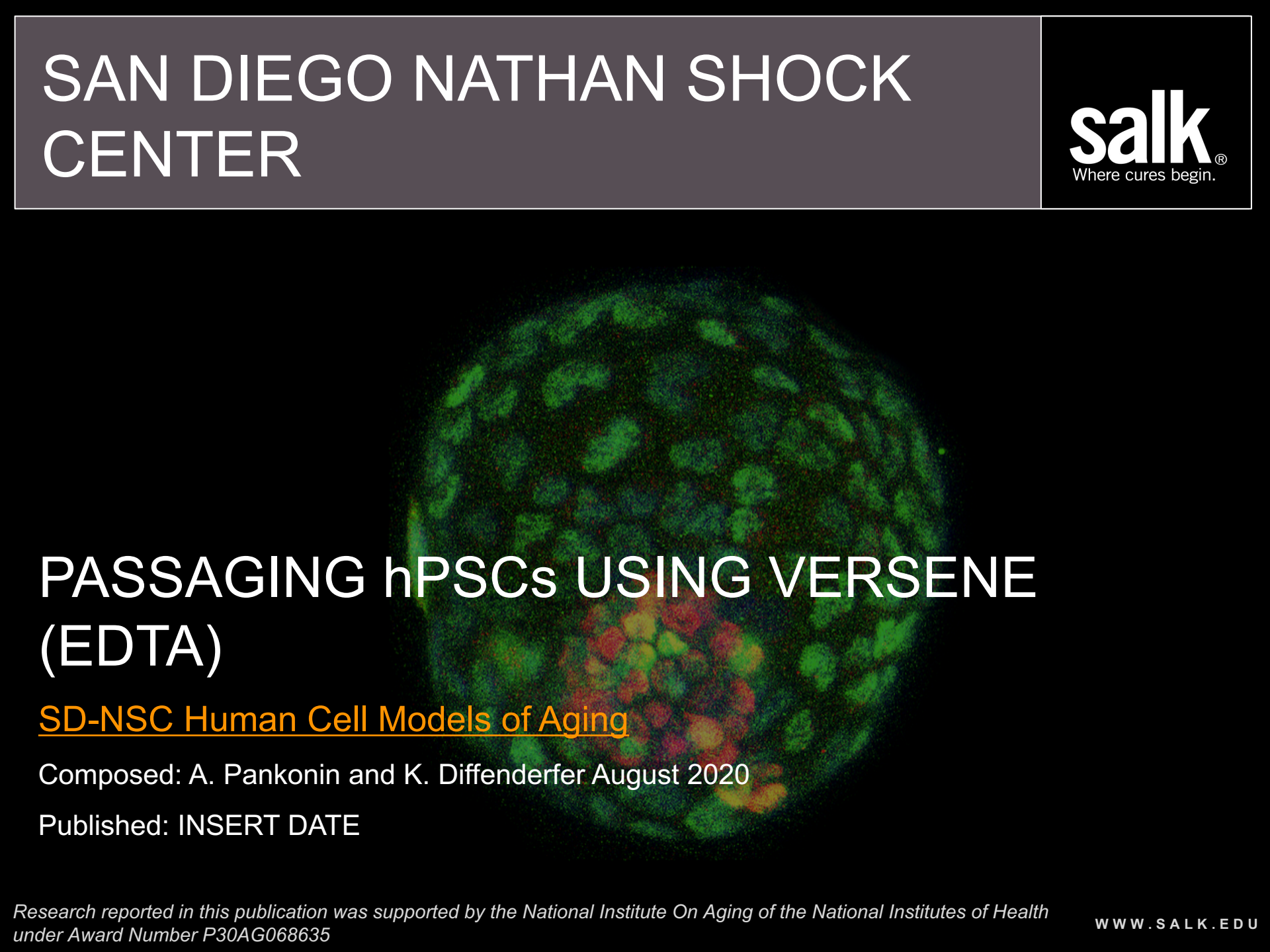

# PASSAGING hPSCs USING VERSENE (EDTA)

SD-NSC Human Cell Models of Aging

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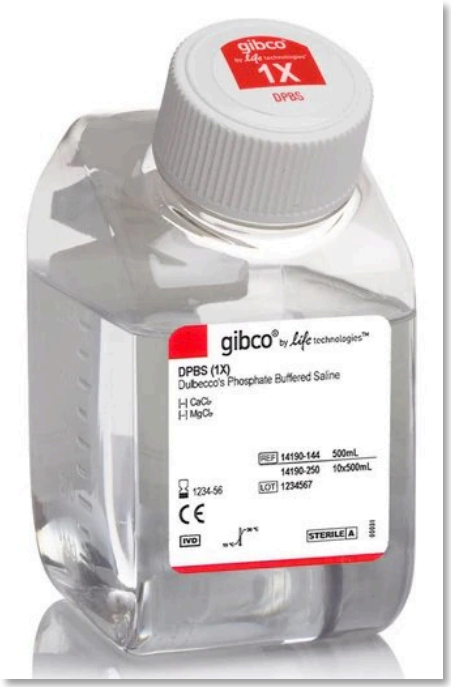
Published: INSERT DATE

*Research reported in this publication was supported by the National Institute On Aging of the National Institutes of Health under Award Number P30AG068635*

# Versene EDTA passaging is ideal for routine cell maintenance

- Versene EDTA is a gentle non-enzymatic cell dissociation reagent suitable for routine passaging of hES and hiPS cells.
- This enzyme-free protocol does not require centrifugation or scraping - reducing cell stress and promoting maximum cell survival.
- Due to increased survival rate, more aggressive passage ratios can be obtained.
- EDTA dissociates colonies into small consistently sized aggregates which helps achieve even plating and predicable cell expansion after passaging.

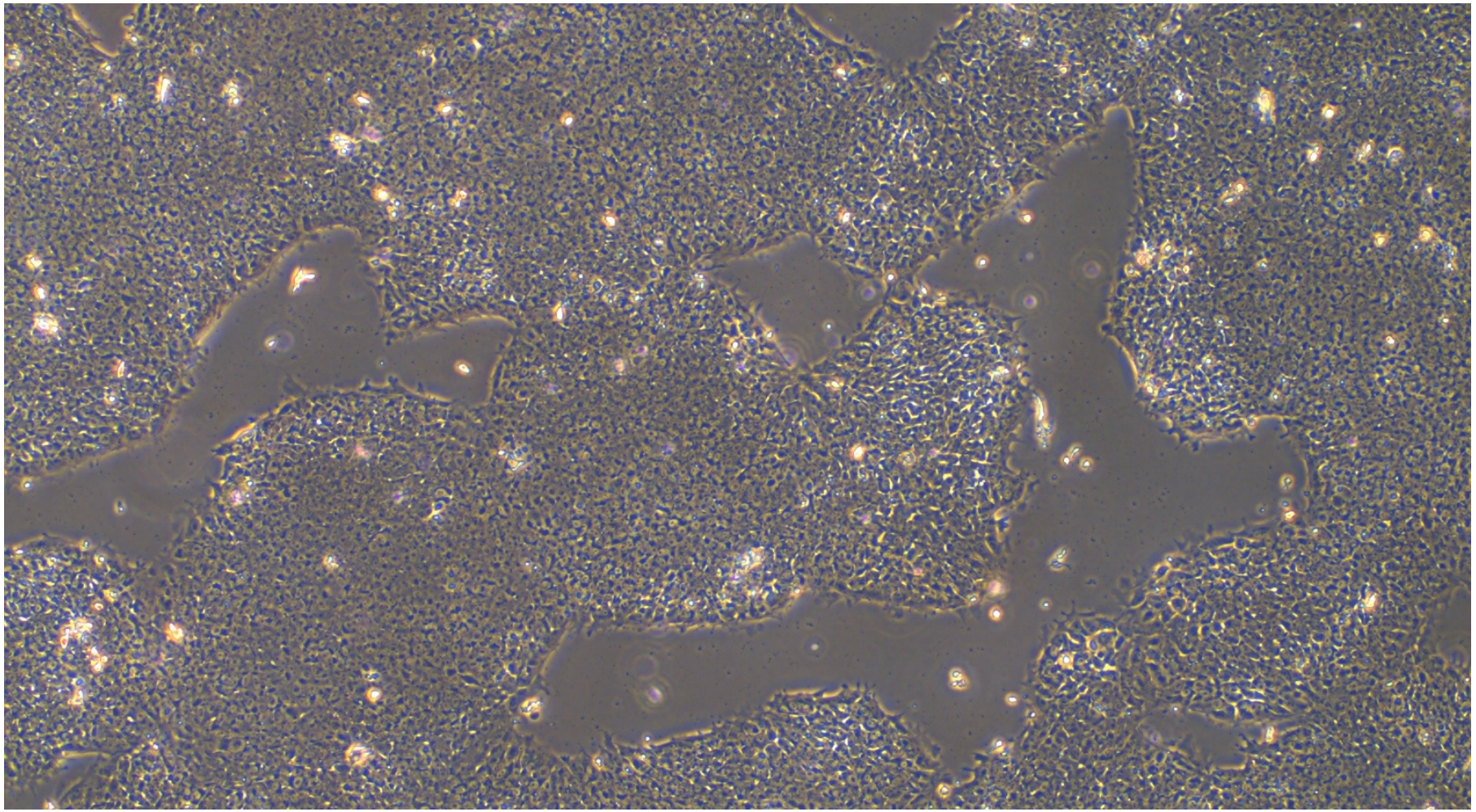
# Gather your materials



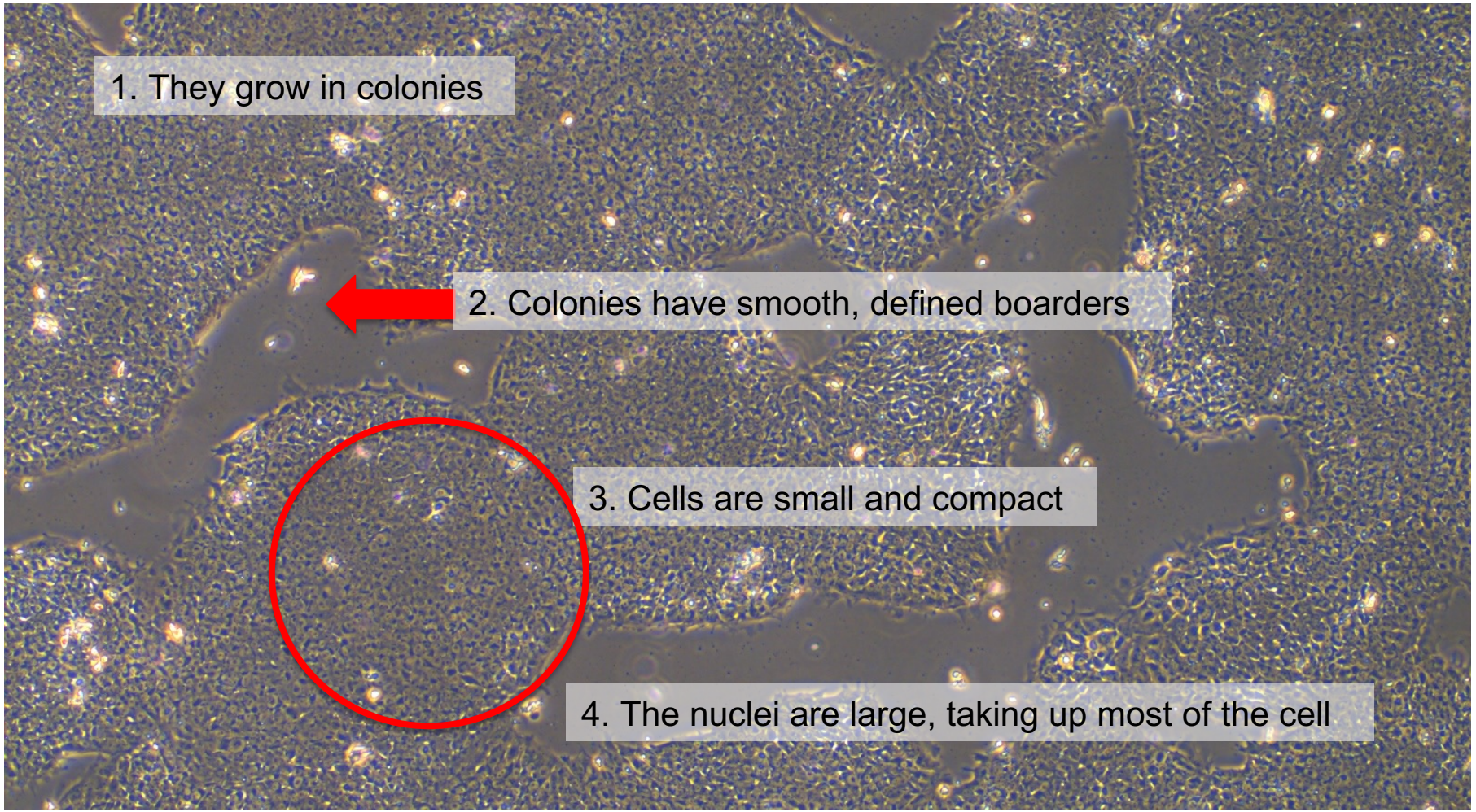


- Human pluripotent stem cells (70-80% confluent)
- TeSR media
- Versene EDTA (0.02%)
- PBS -/- (do NOT use +/+!)
- 5mL glass pipettes
- Matrigel coated 6 well plate

# hPSCs are passaged at approximately 70% confluence

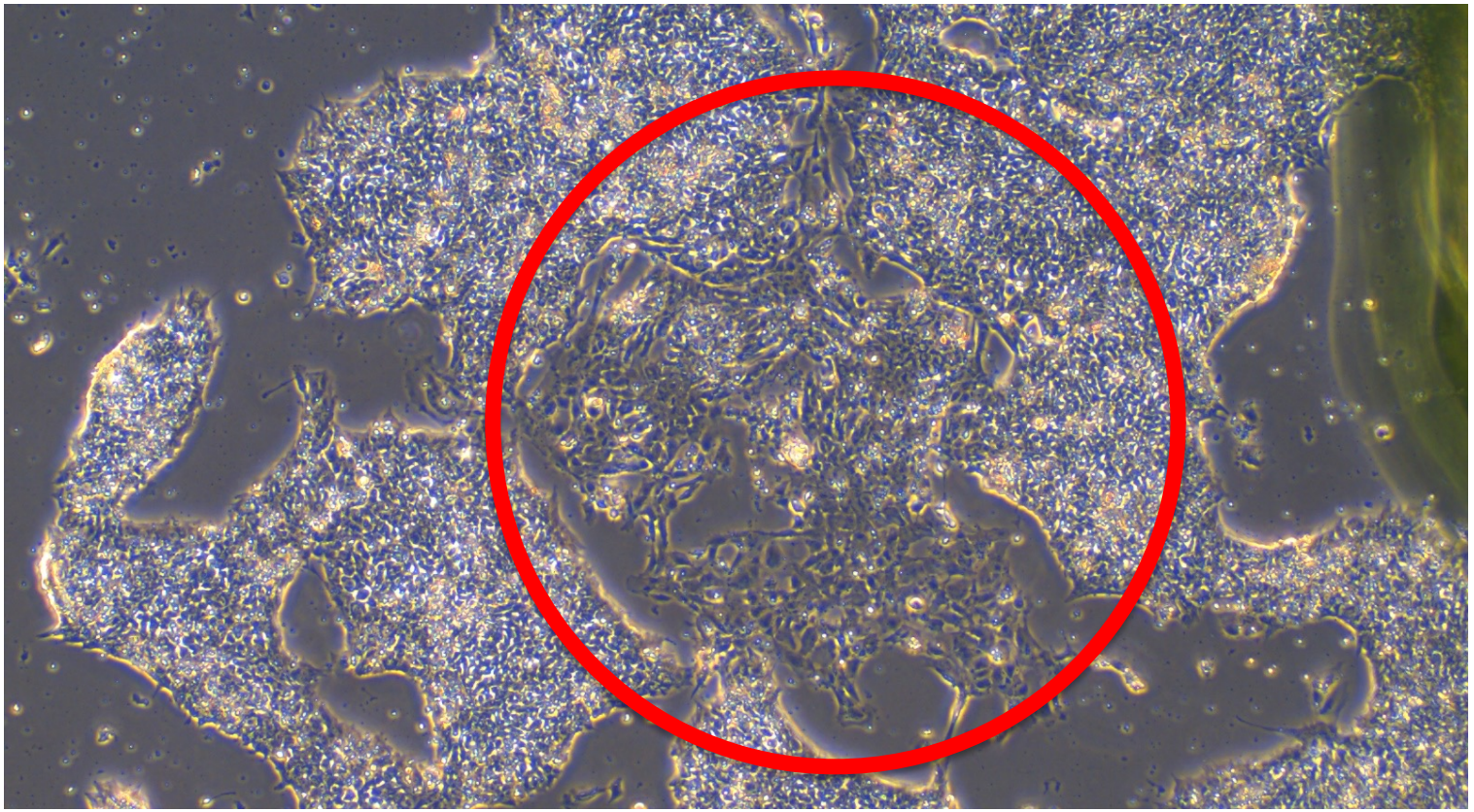


# Healthy hPSCs have 4 key characteristics



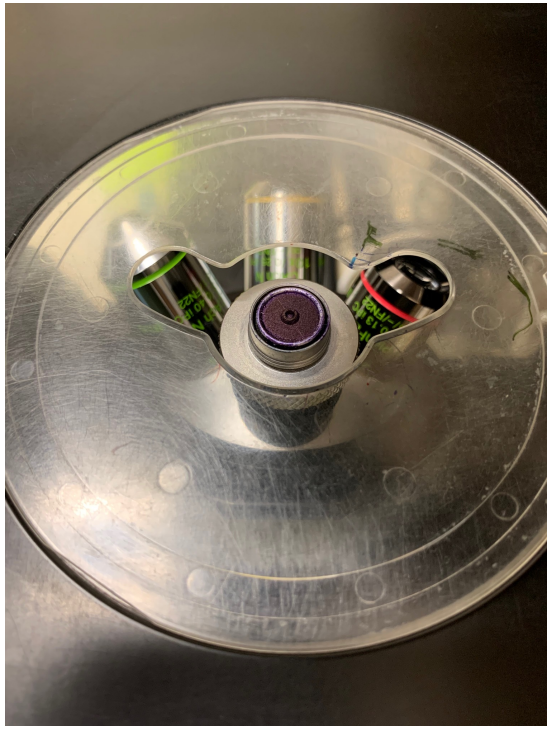
# Identify and mark spontaneous differentiation

Identifying areas of spontaneous differentiation before passaging is a critical step. Scan the well for clusters of cells that do NOT look like hPSCs (hint: if the 4 characteristics described above do not apply – its differentiation!)



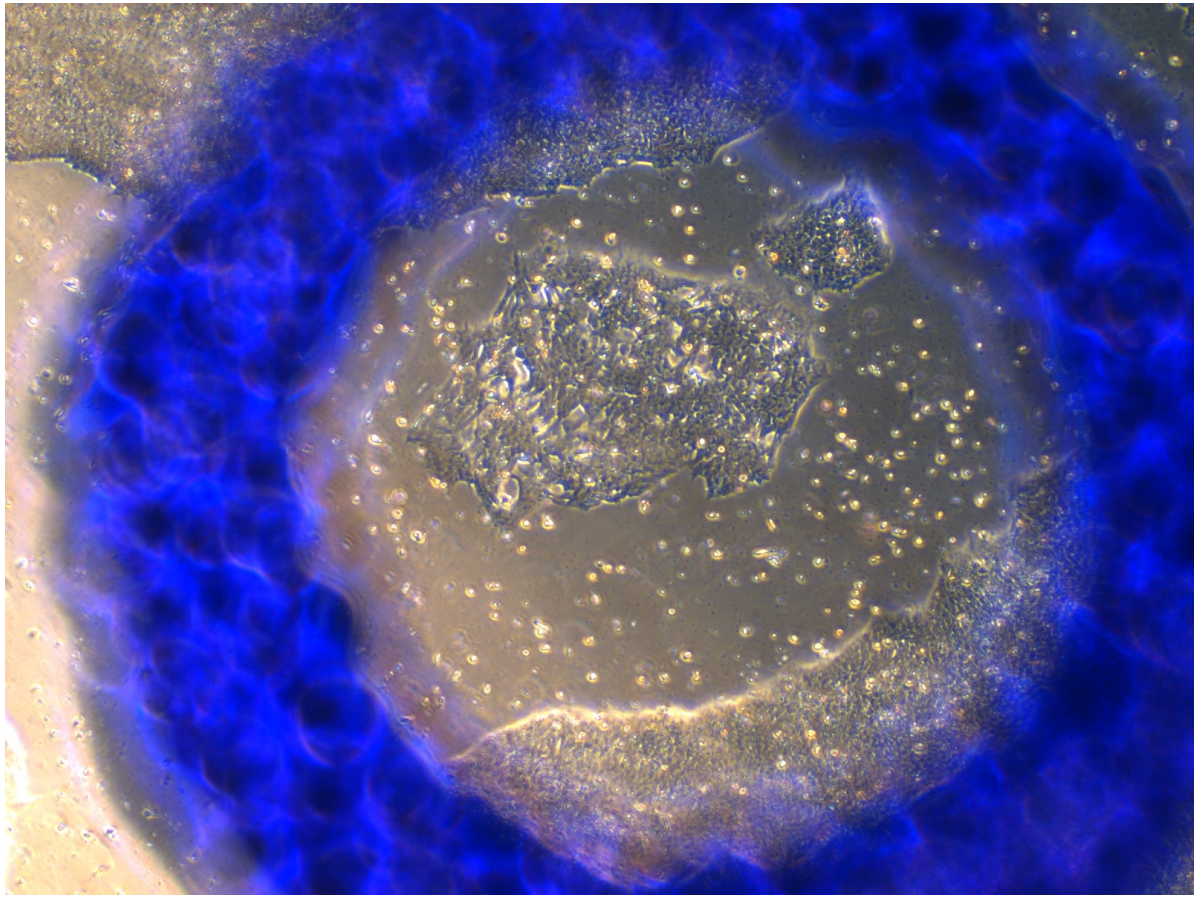
# Identify and mark spontaneous differentiation

Our microscopes have spring loaded marking pens mounted to one of the objective positions. To use the marking pen center the area of interest in the middle of the field of view (4x objective), rotate the marking pen into position, and lift the outer metal ring (see red arrow below).



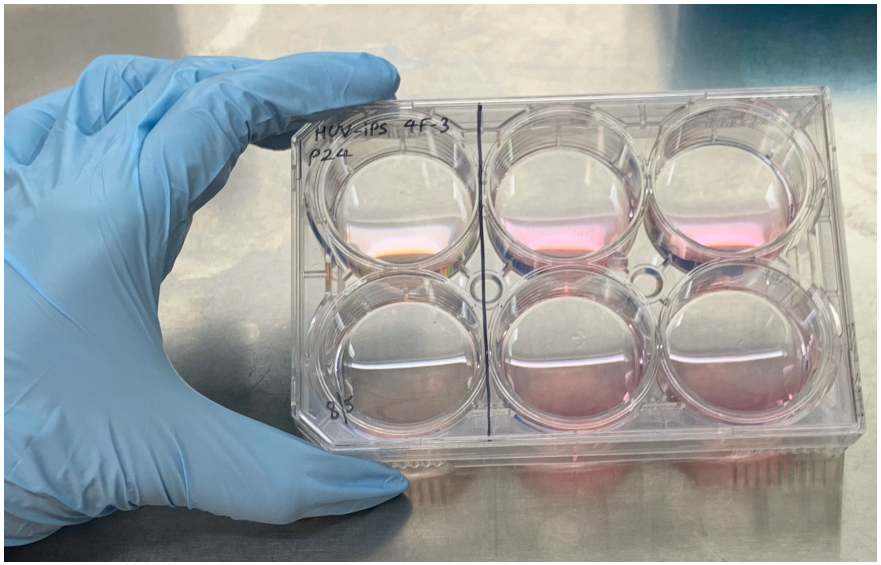
# Identify and mark spontaneous differentiation

A purple/blue circle will be left on the bottom of the plate to easily identify the marked area when in the hood.



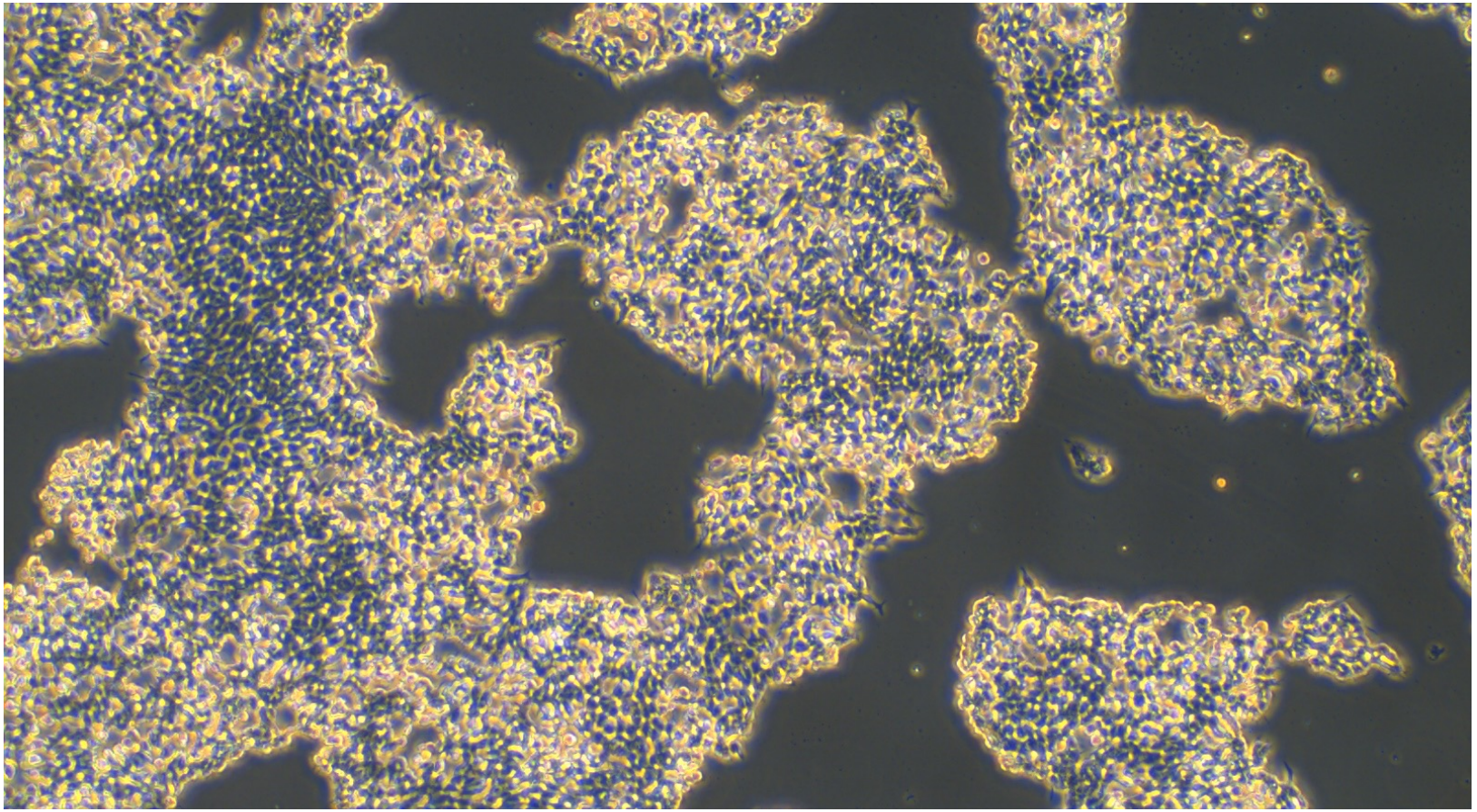
# Aspirate areas of differentiation and add EDTA

- Tilting the plate at a 45° angle, aspirate the marked differentiation
- Wash with 1 mL of PBS -/-
- Add 1 mL EDTA to the well
- Incubate at 37C for 6-9 minutes



# After incubation, check the cells under a microscope

Cells should appear shiny and separated but still attached to the surface. In some cases, slightly curled edges will be visible around the colonies.

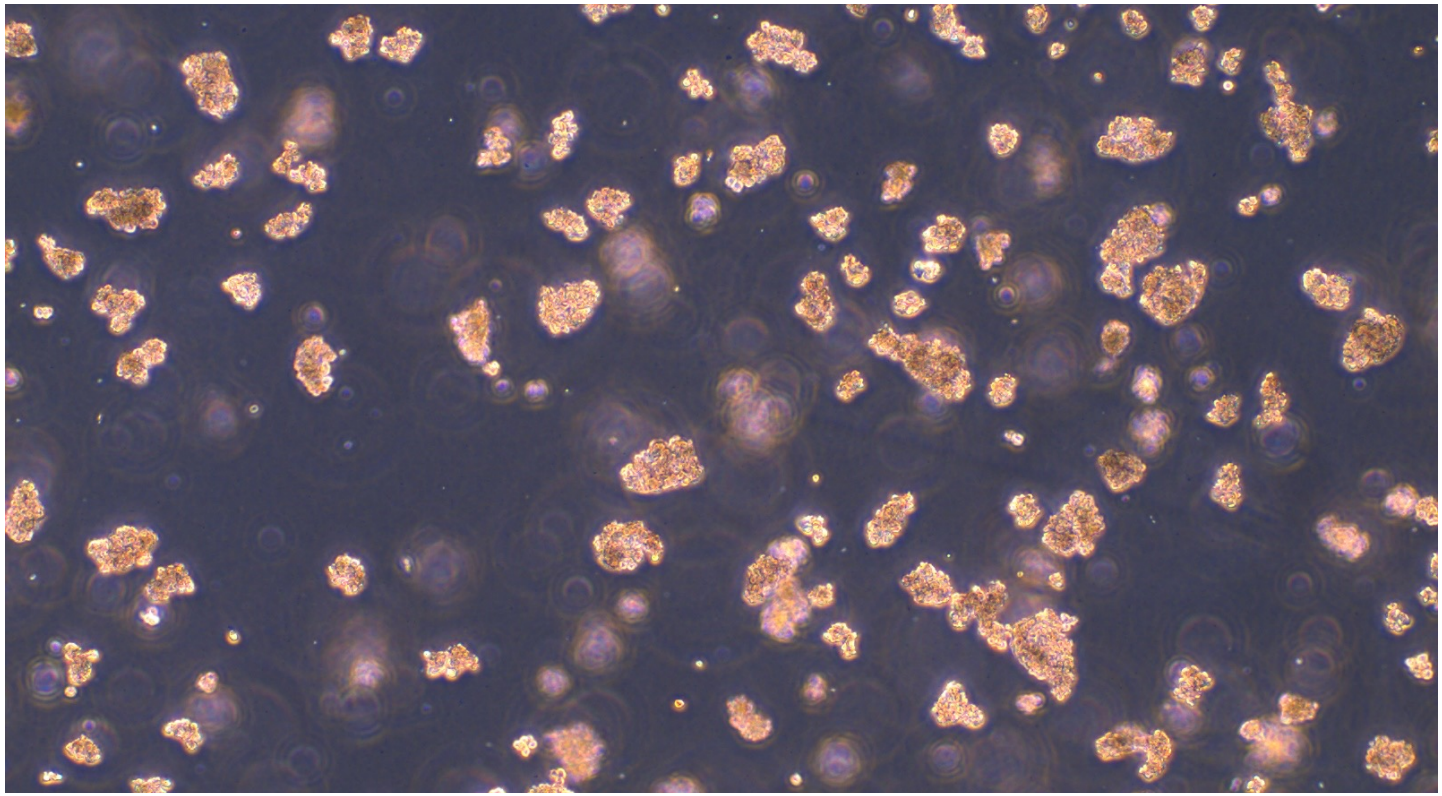


# Replate cells onto a fresh matrigel-coated plate

- Aspirate the EDTA without disturbing the cell layer
- The split ratio for EDTA passaging is usually between 1:8 and 1:12. Higher ratios are possible if aggressive expansion is needed.
- Collect the needed volume of TeSR into a 5mL glass pipette and remove cells from the growth surface with aggressive pipetting (avoid bubbles).
- If cells remain attached after pipetting gently scrape the growth surface with the 5mL glass pipette (this should NOT be required – EDTA incubation time should be increased if scraping becomes routine).
- Mix the cell suspension and seed 0.5-0.25mL to new wells.

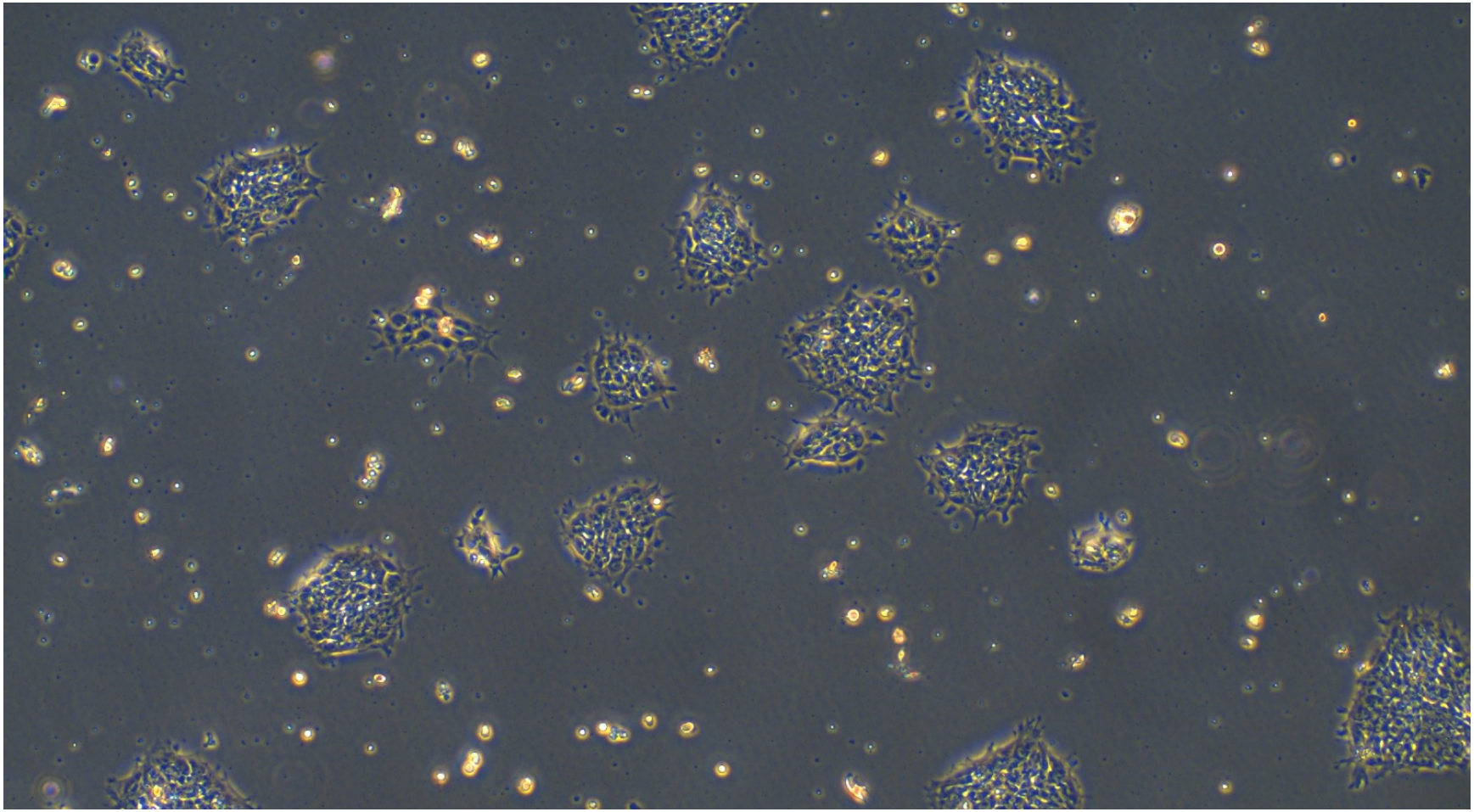
# Look at aggregates under a microscope

- Aggregates should be small and relatively consistent in size
- Gently rock the plate up/down and left/right after placing on the incubator shelf



*Note: Aggregates passaged with EDTA will sit quickly. If passaging multiple lines do the rocking motion in the hood after seeding each set of cells.*

# Check colonies the day after and feed everyday



There will be debris - this is normal. Small colonies often have spikey borders.