Human Intestinal Organoid Morphology Session

Lecture 3

Presenter
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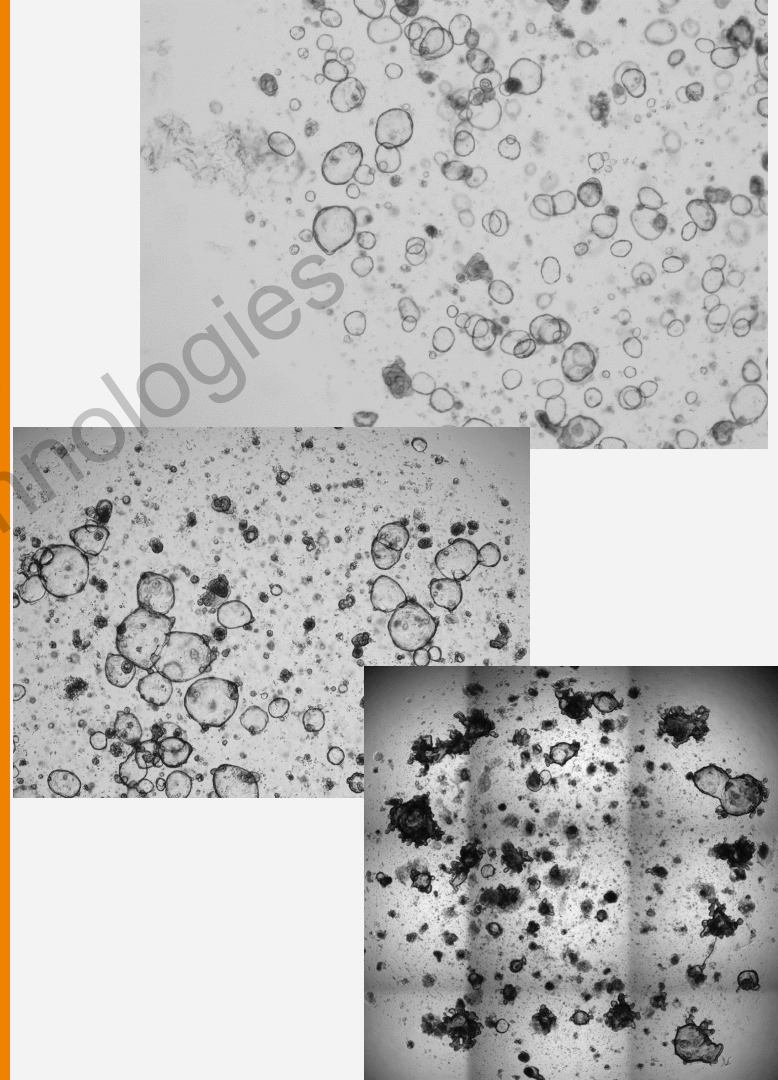

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Learning Objectives

After this session, you should be able to:

- Understand the range of organoid culture morphologies
- Be able to identify good vs bad culture morphologies for culture initiation and passaging

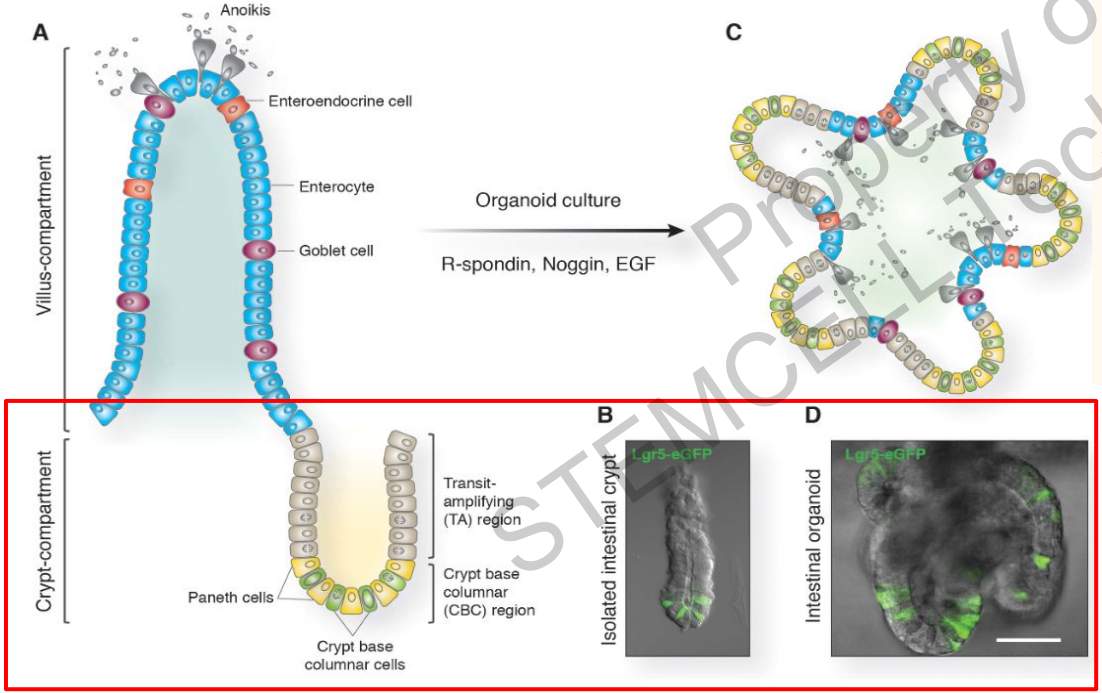
Outline

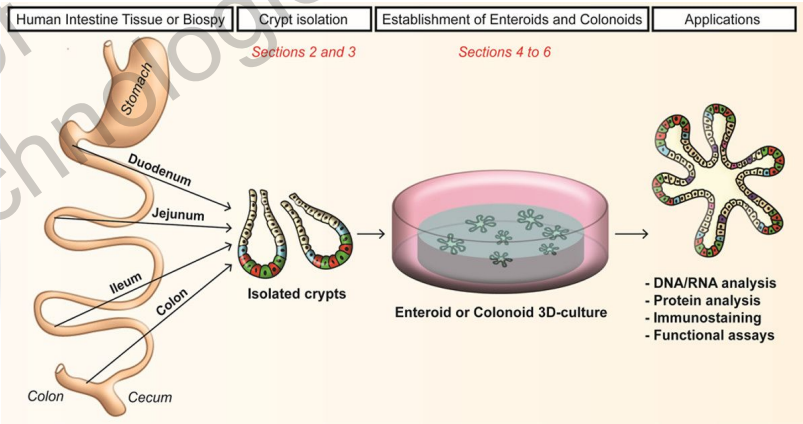
1. Morphological Assessment for Primary Initiation Cultures
2. Morphological Assessment of Human Intestinal Organoids (Passaging)

Section 1 Morphological Assessment for Initiation Cultures

Introduction to Crypt Isolation

- Crypt isolation protocol is designed to isolate crypts from the starting intestinal tissue.
  - If working with human biopsies, the process involves processing biopsies to isolate stem cell-containing crypts.
  - Biopsies must be fresh (24 hours); frozen samples have low viability and are not recommended.
  - This is the first step in intestinal organoid initiation workflow.



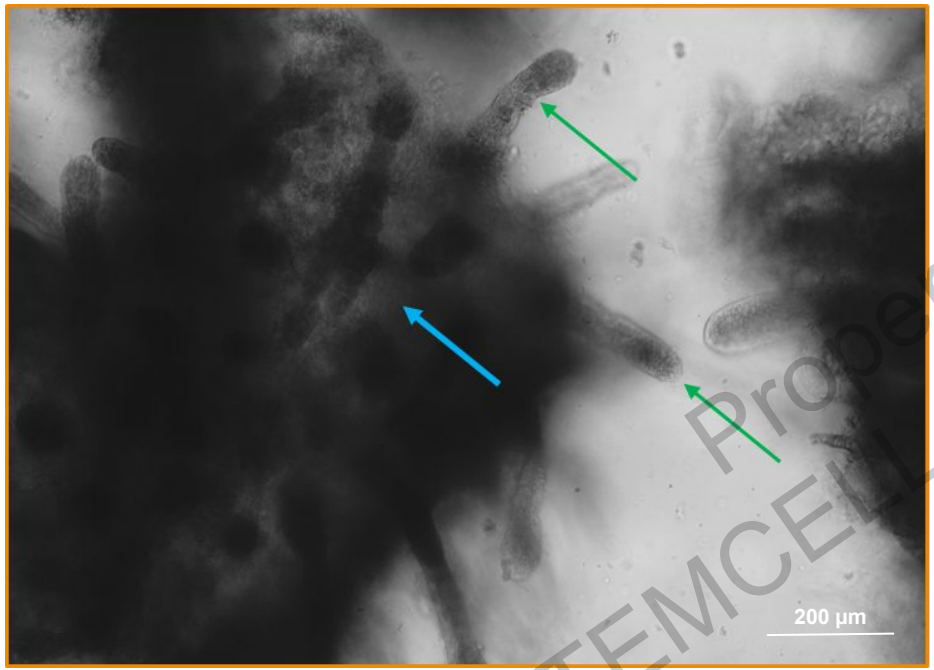


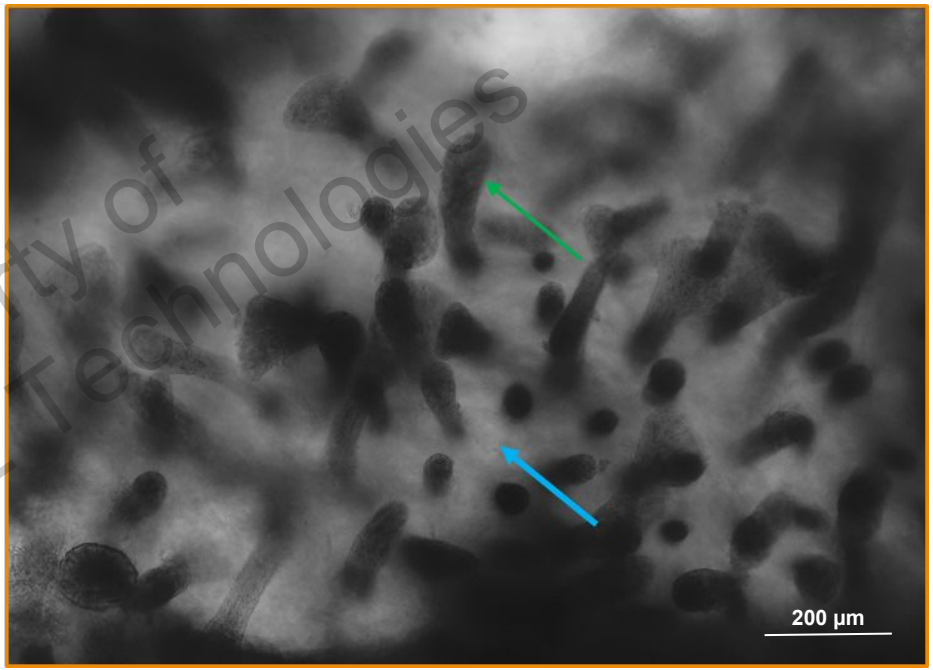
Mahe et al (2015), JoVE

Leushacke & Barker (2014), Gut

Crypt Identification: Colonic Tissue

Primary tissue from surgical samples post wash, prior to dissection/processing (4X magnification)

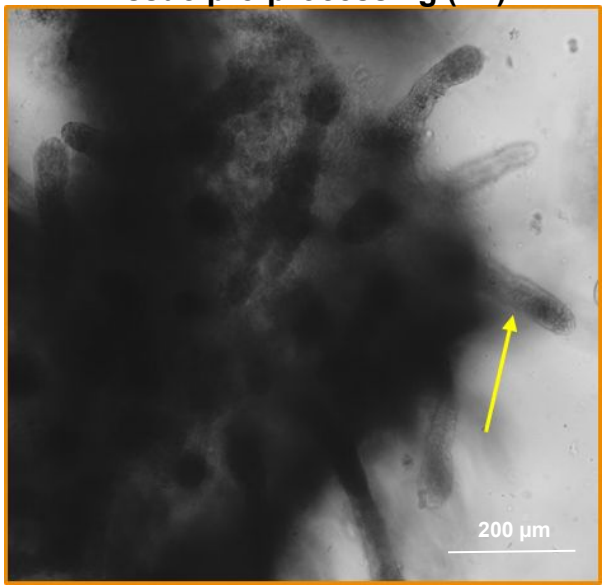




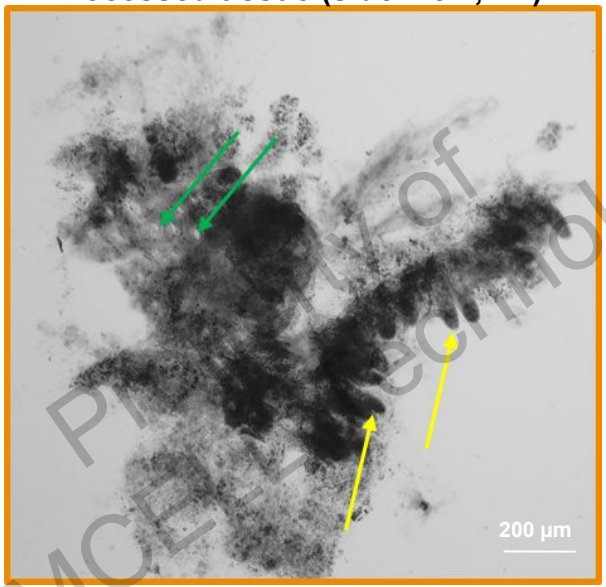
- Colonic crypts are visible in surgical samples prior to processing.
  - Green arrows indicate intact crypts
  - Blue arrows indicate the non-crypt epithelium and underlying layers

Crypt Identification: Colonic Tissue

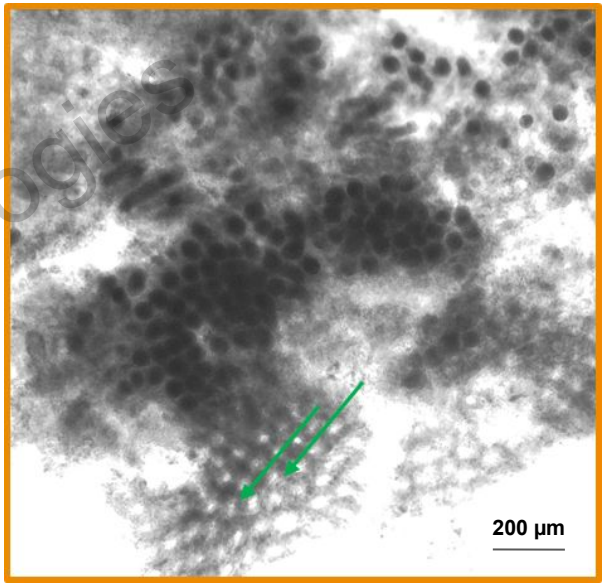
Tissue pre-processing (4X)



Processed tissue (side view; 2X)



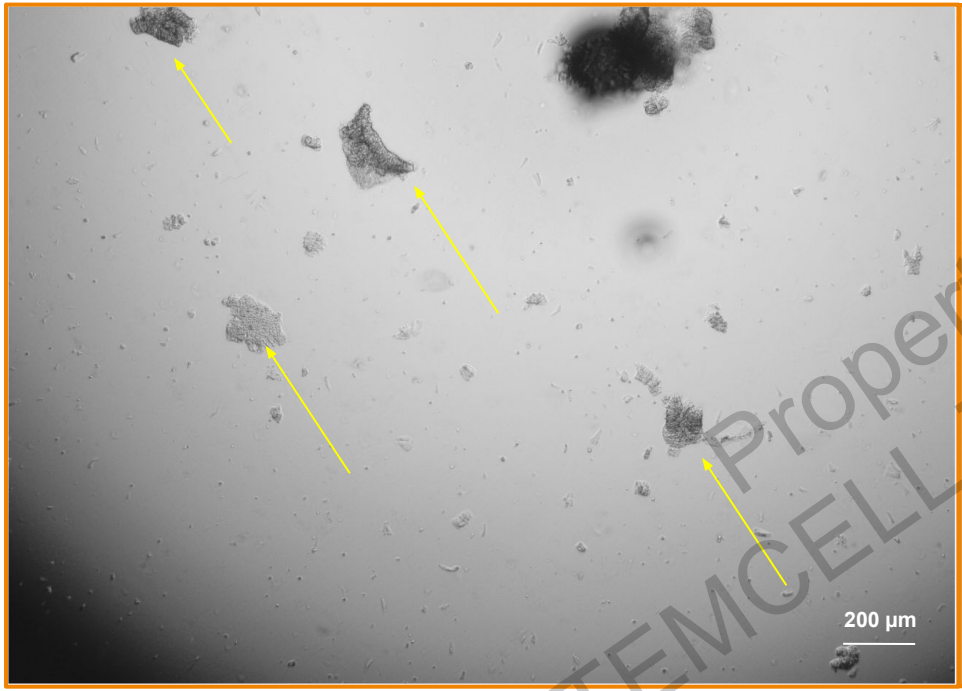
Processed tissue (top view; 2X)



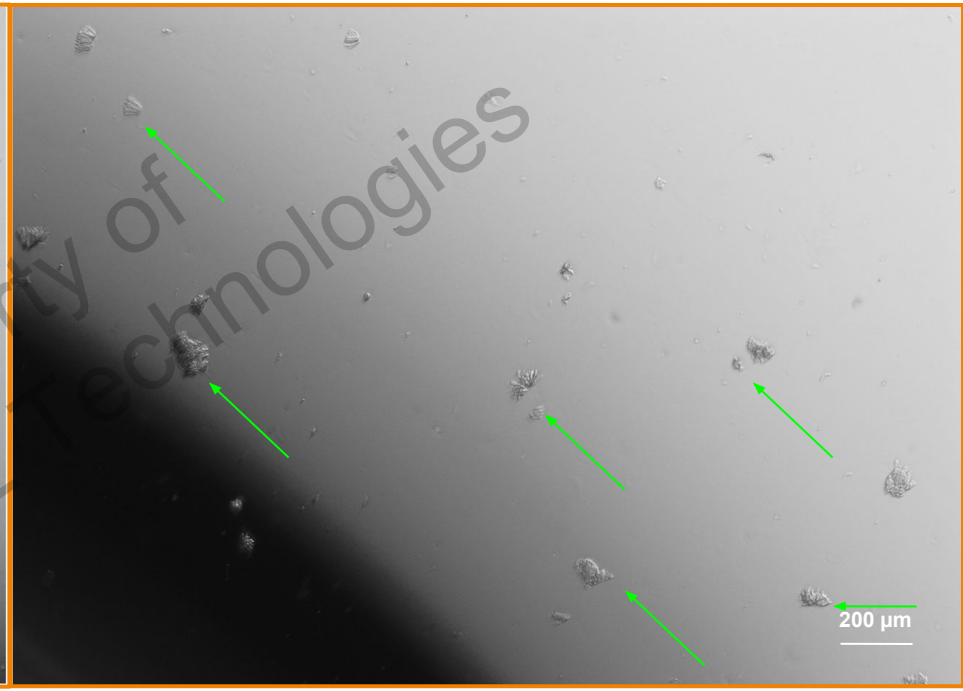
As tissue is dissociated and washed, crypts will be released from the tissue

- Green arrows indicate “holes” where crypts were attached but have been released as a result of processing
- Yellow arrows indicate crypts that have not been released from the surrounding tissue

Crypt Identification- Human Colonic Biopsy



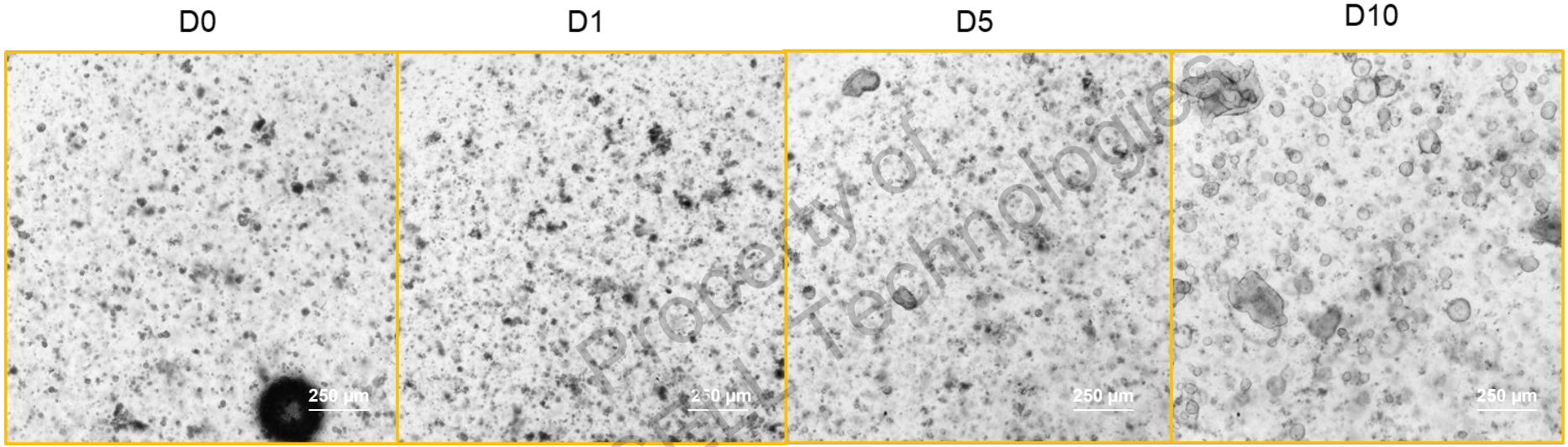
Before filtration



After filtration

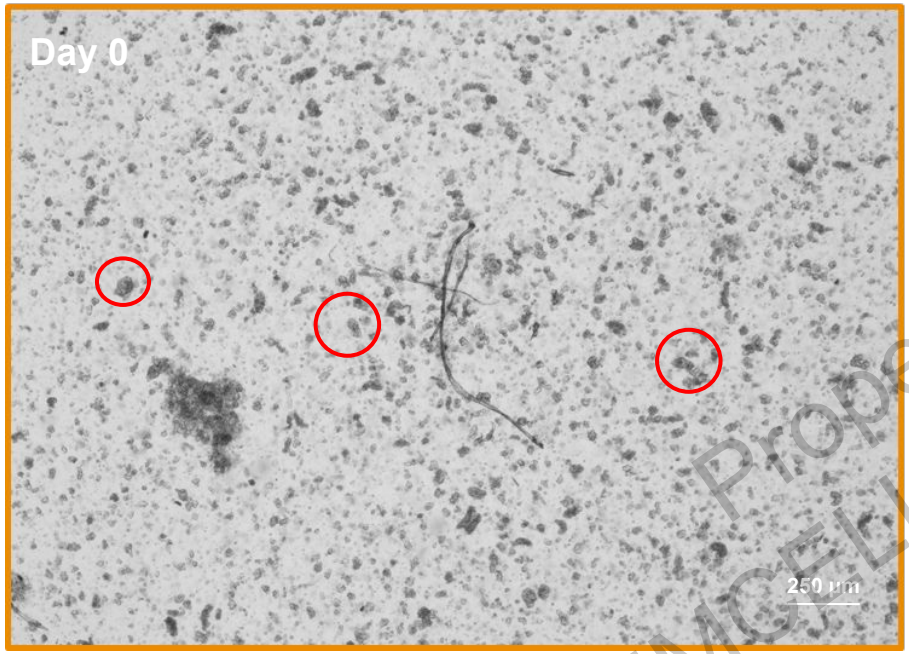
- Yellow arrows indicate fragments prior to filtration (larger in size); green arrow indicate pieces of crypt visible after filtration

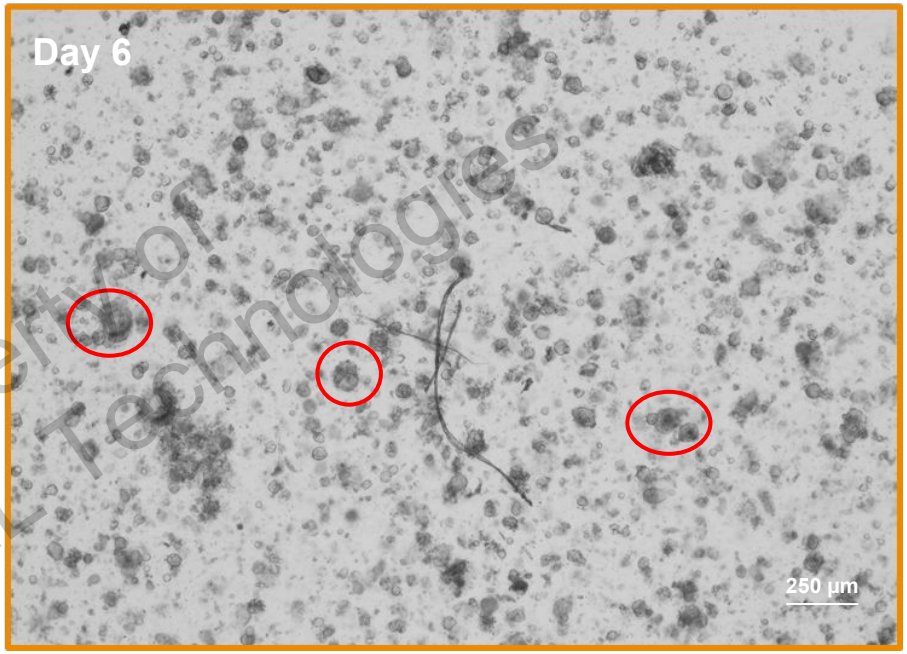
Human Biopsy Culture Initiation



- Growth of biopsy samples from Day 0 to Day 10 from primary culture
- Initiation culture have debris, small clumps of cells, and single cells
- It can be challenging to trace which fragment or single cell the organoids have arisen from

Human Biopsy Initiation Cultures





- At Day 0, most of the crypts are broken up
- Crypts are delicate and will break down in our primary culture protocol
- Organoids can form from crypt fragments (red circles) or single cells (Lgr5+)
- Add Rho Kinase inhibitor to your media during primary culture for the first few days (24 - 48 hours)
- Antibiotics can be added to initiation cultures, if desired

Section 2 Morphology Assessment of Human Intestinal Organoids During Maintenance and Passaging

Key Considerations for Human Organoid Cultures

Size: Larger organoids are better than smaller ones. Larger cystic or budded organoids will result in a higher yield of viable fragments than smaller, dark, collapsed, or overly-budded organoids.

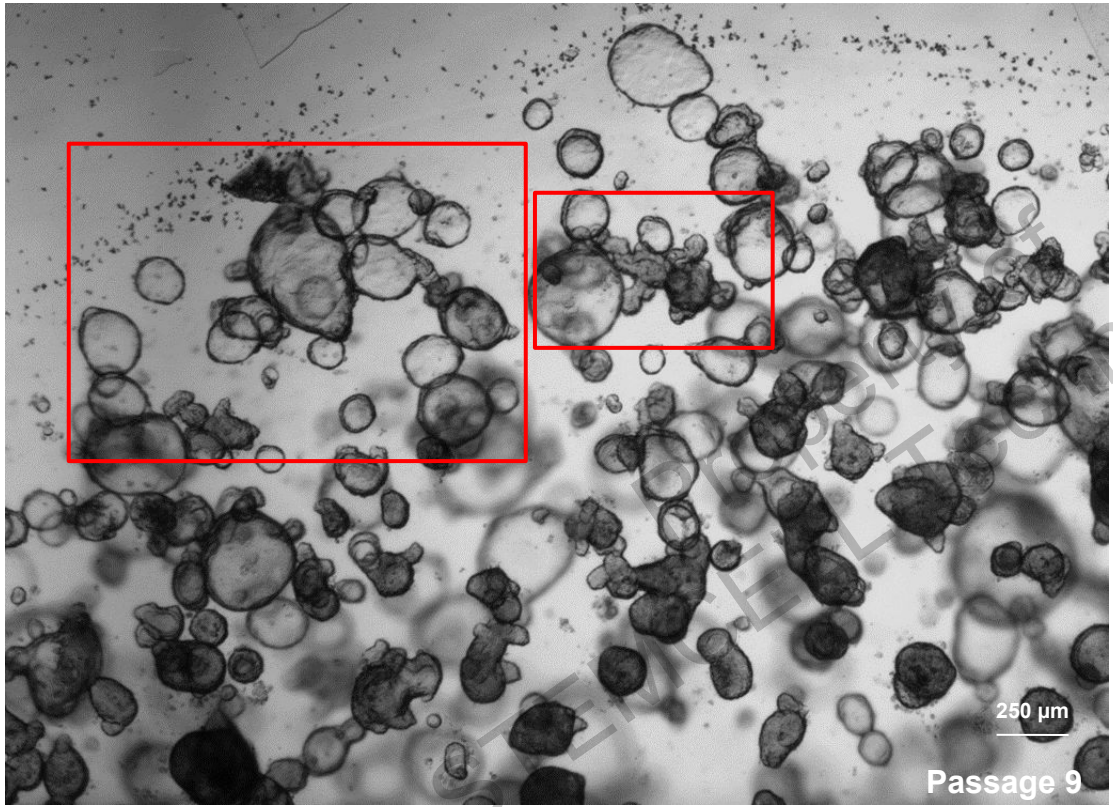
Density: Density can be too low or too high.

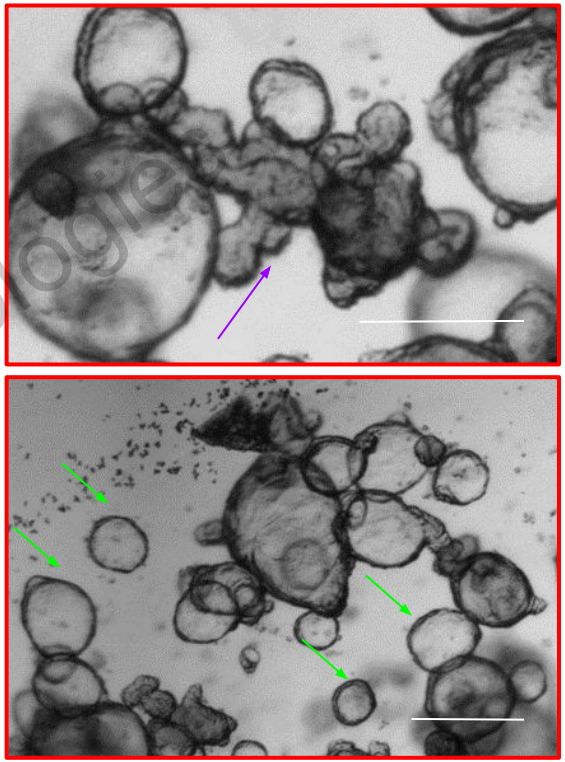
- Low densities can be difficult to passage and may require combining wells.
- Excessive densities will be evident as organoids closer to the center of the dome will begin to darken and collapse. Sizes will stay small.

Morphology: Will be cystic, budded, or a combination of both. Cystic organoids tend to be light in color, while budded organoids tend to be dark. Most organoids start highly budded at primary passages and become more cystic over time.

Donor: Significant variability can be seen between organoid donors and between passages. The morphology of human intestinal organoids are heterogeneous and highly variable.

Donor 1: Healthy Morphology with Cystic and Budded Organoids

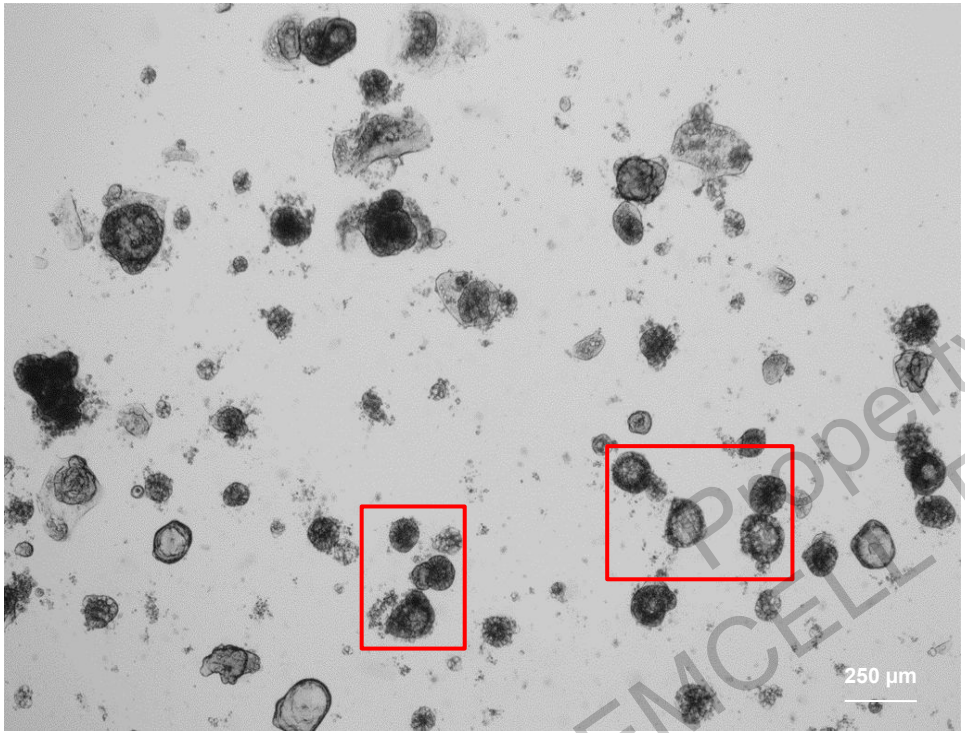


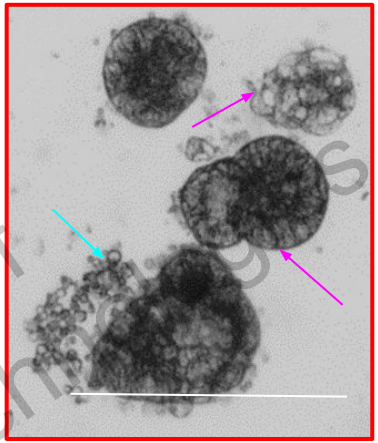


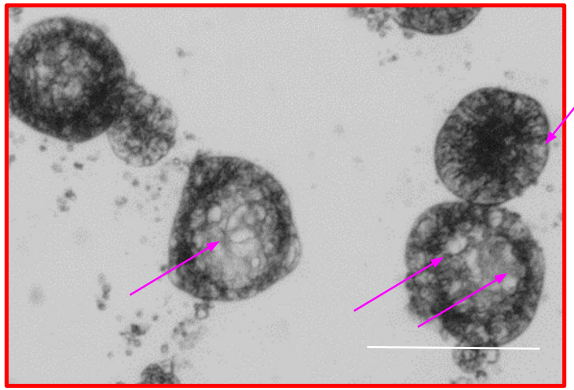
Images on the right are zoomed in of the red boxes of the larger image on the left.

- Heterogeneous population (left); some organoids are extensively budded (purple arrow), others are predominantly cystic (green arrows) (right)
- This culture is ready for passage and will passage well (based on culture density, and health)

Donor 2: Unhealthy Morphology with Dark/Differentiating Organoids

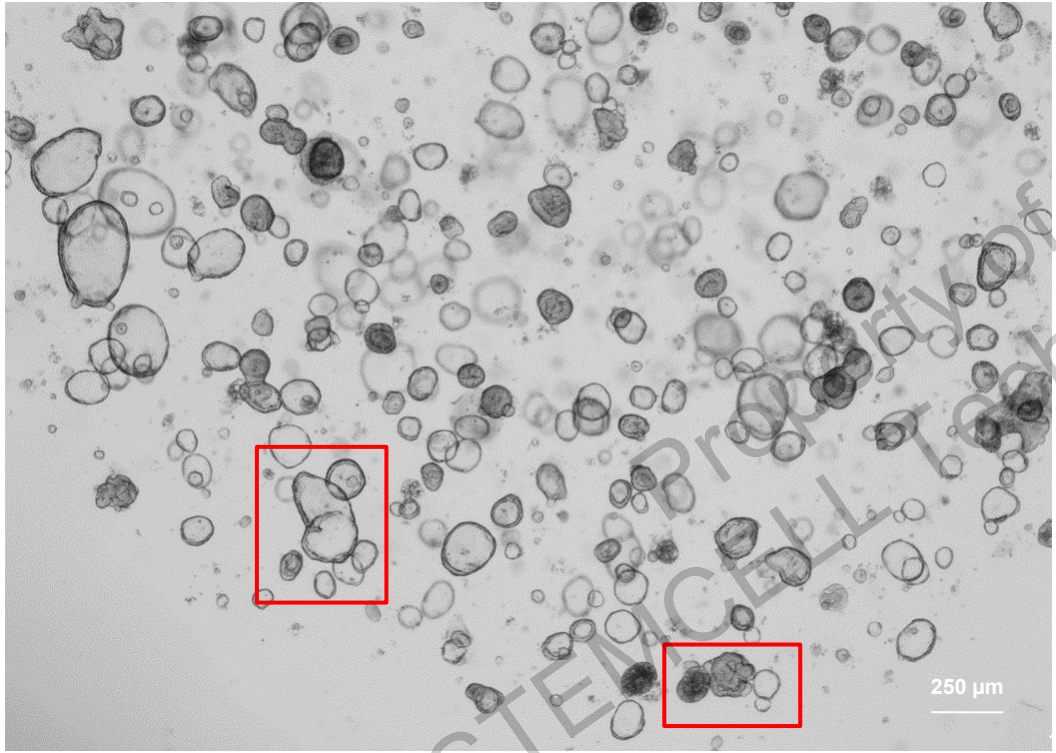


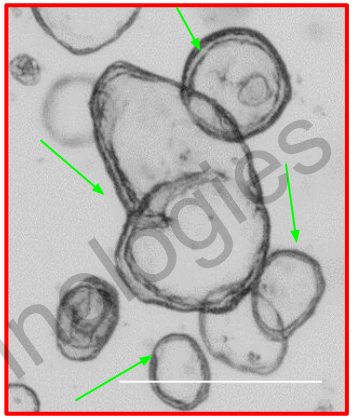


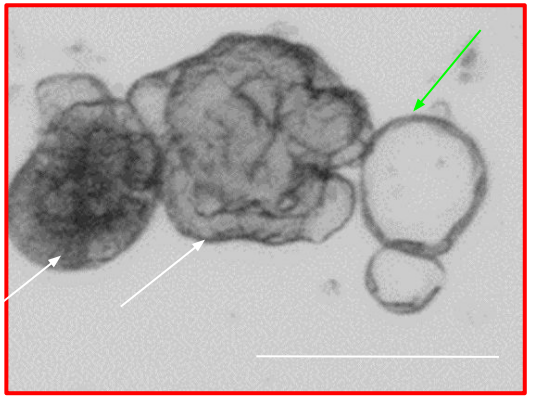


- Unhealthy culture; won't passage well
- Organoids are small with a thick dark epithelium
- White spaces in the epithelium; some of these are goblet cells (pink arrows) signifying differentiation
- Membrane blebbing/ cell shedding signifying death (blue arrow)

Donor 3: Healthy Morphology with Cystic Organoids

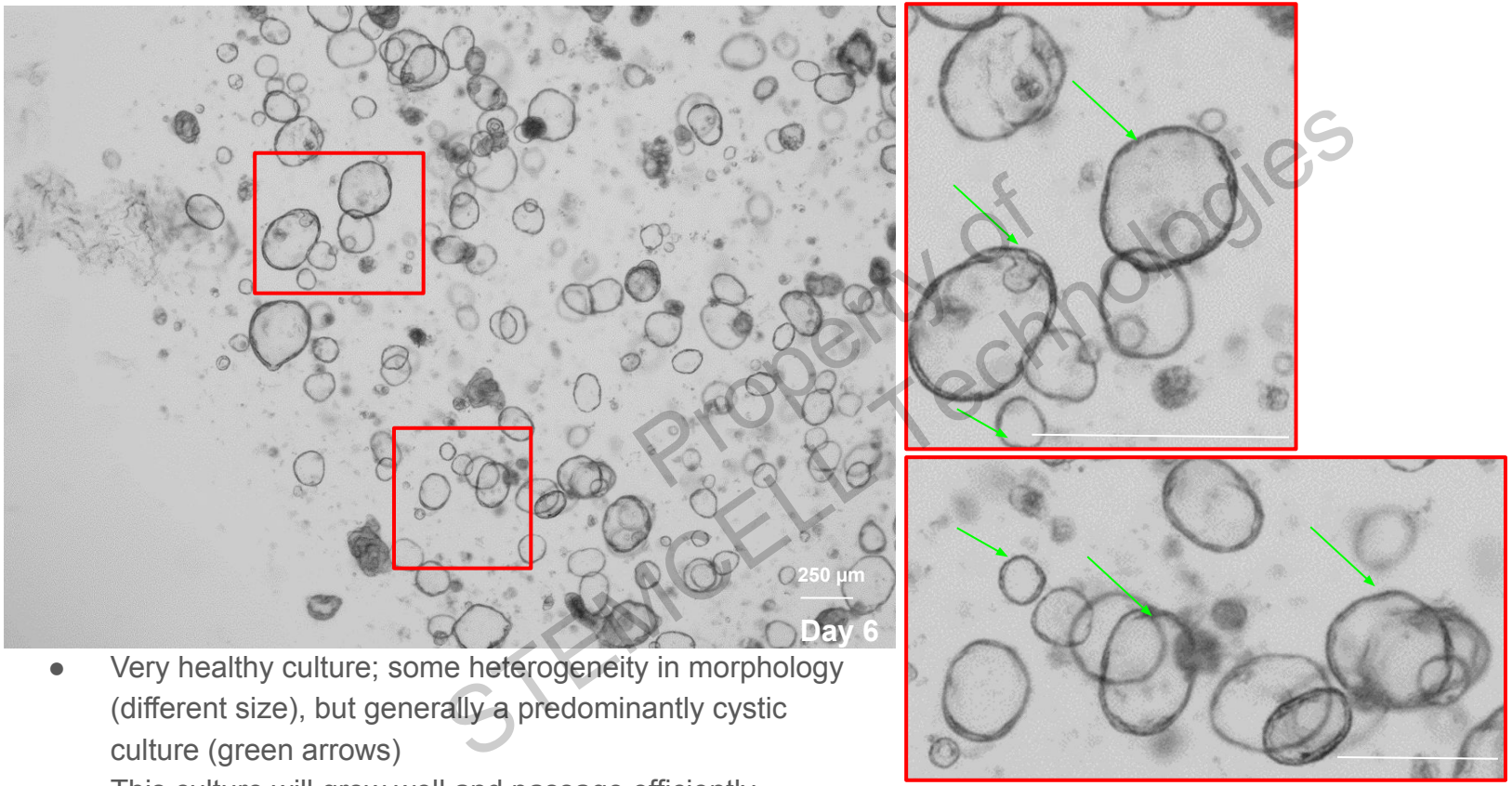






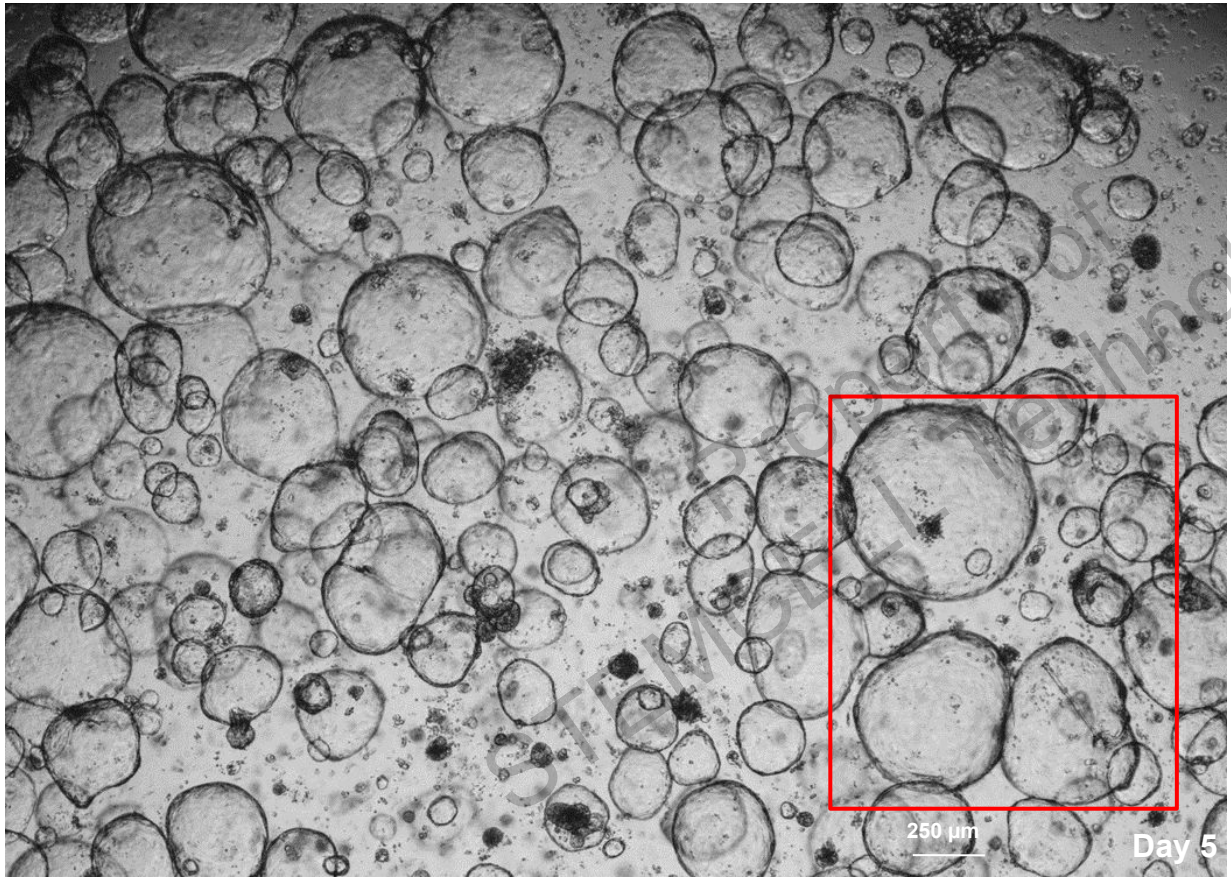
- A great Day 5 culture; not ready to passage yet
- Range in organoid sizes are due to fragment size differences upon plating
- Most organoids are cystic (green arrows) and expanding rapidly
- Some cystic organoids collapse and darken (white arrows)

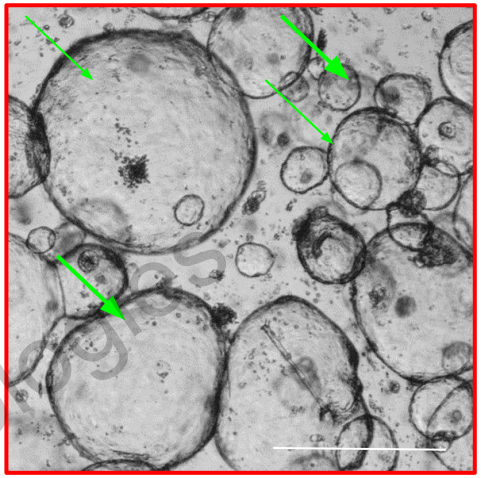
Donor 4: Healthy Morphology with Cystic Organoids



- Very healthy culture; some heterogeneity in morphology (different size), but generally a predominantly cystic culture (green arrows)
- This culture will grow well and passage efficiently
- This culture can be passaged on Day 7

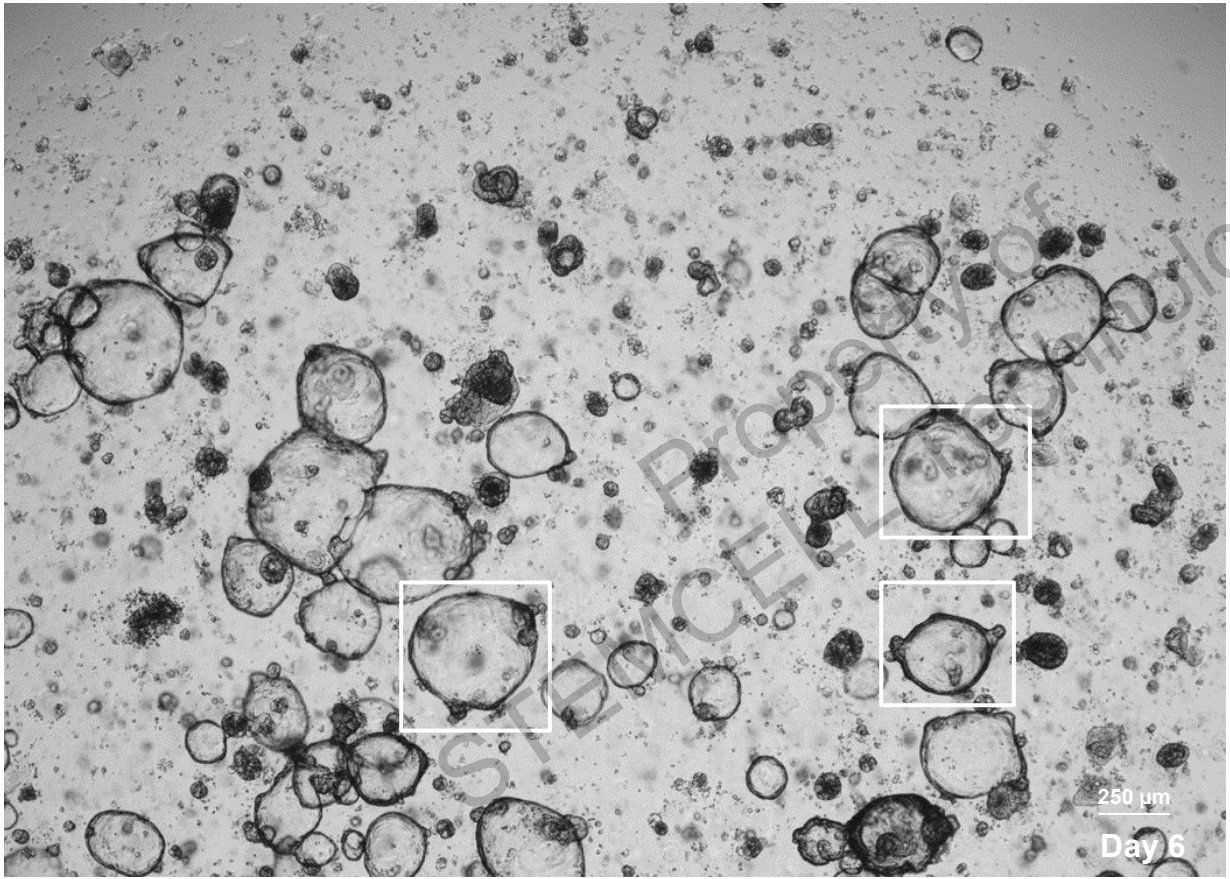
Donor 5: Healthy/Cystic Morphology





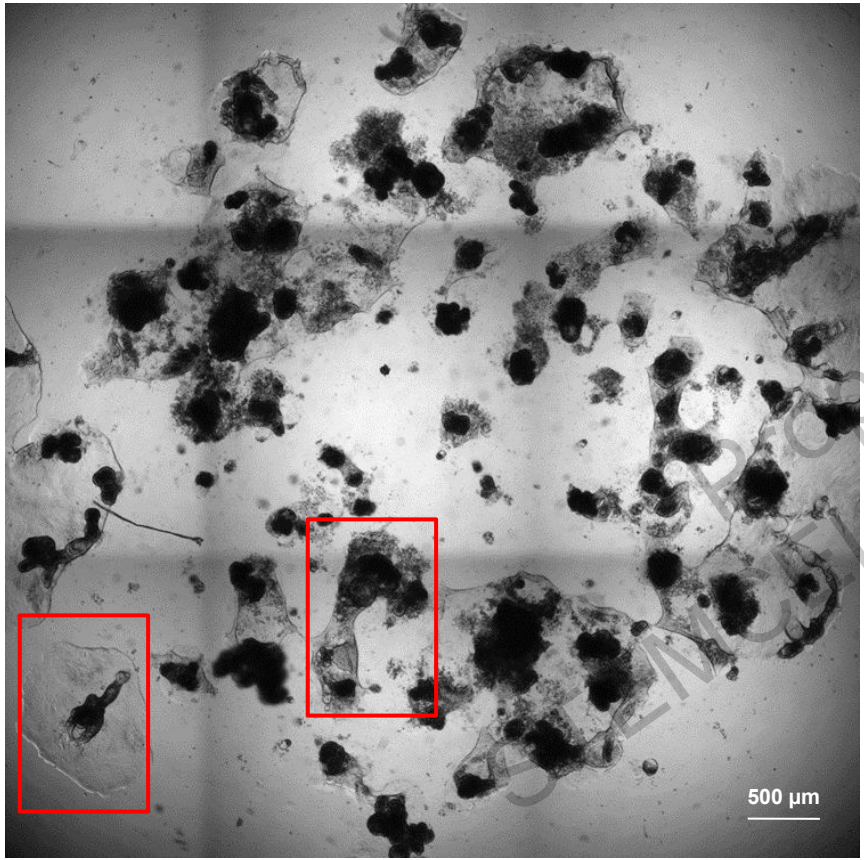
- Very healthy culture; some heterogeneity in morphology (size of organoids) but a predominantly cystic culture (green arrows)
- High density culture
- Titrate the split ratio to reduce culture density if desired
- This will passage well and can be passaged in a day or two

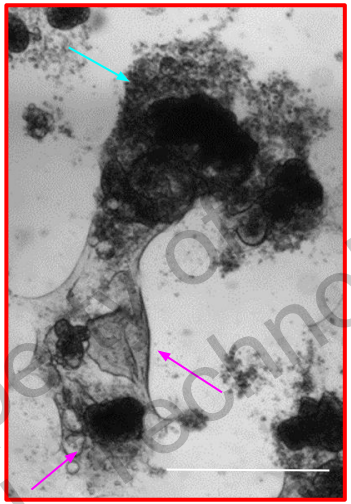
Donor 6: Healthy Morphology with Variable Size Cystic Organoids

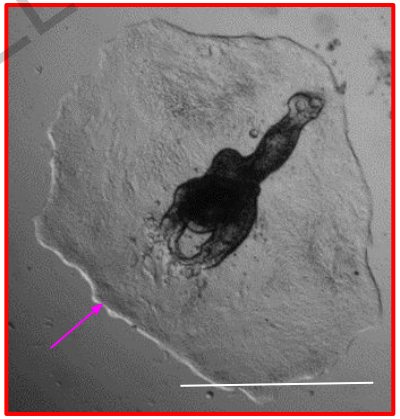


- Healthy culture; some heterogeneity in morphology (ranging from small cystic to large cystic organoids) but generally a predominantly cystic culture
- Cystic organoids with small buds are highlighted (white box)
- Culture can grow for a few more days as there is still room to grow
- This culture will passage well

Donor 7: Unhealthy Morphology with Extensive Differentiation

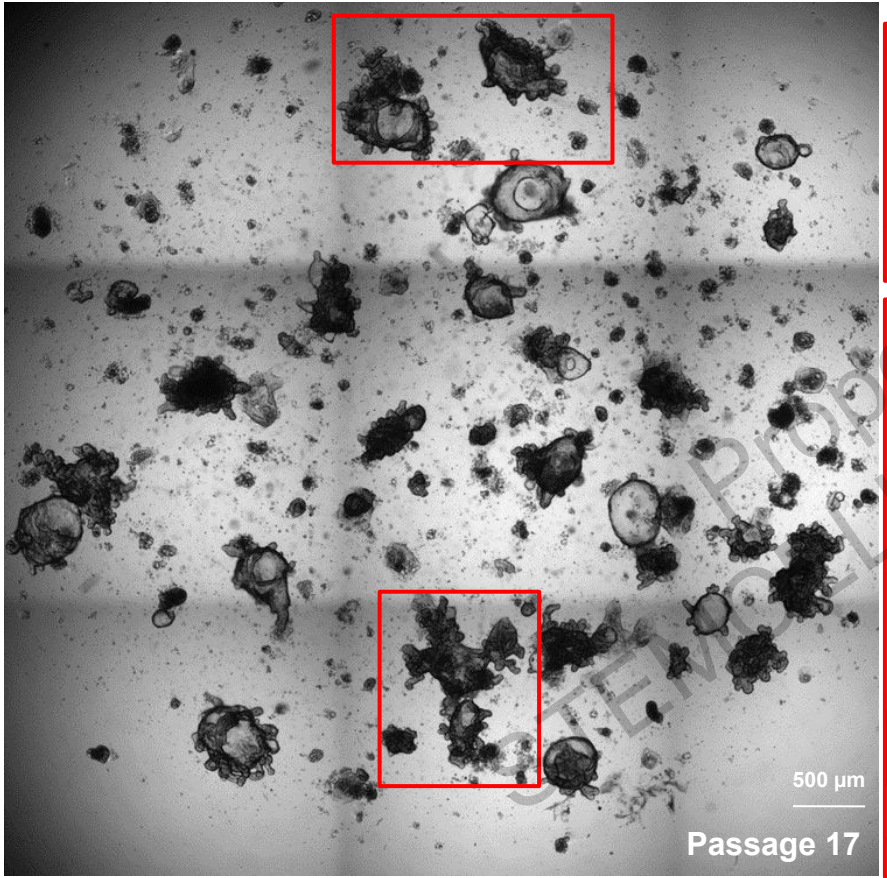


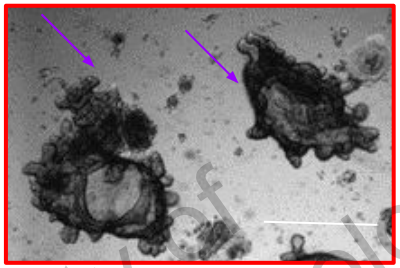


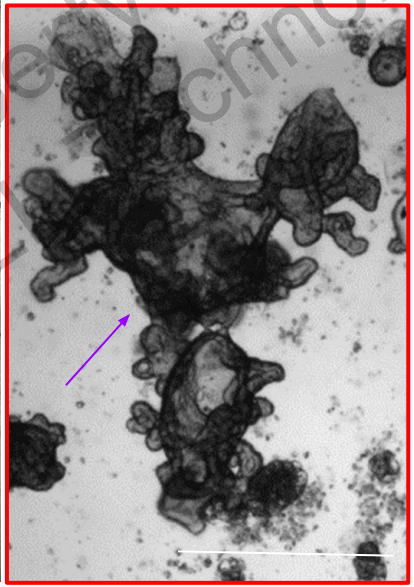


- This culture has extensive differentiation
- Many organoids have sunk to the bottom and flattened out (pink arrow)
- Dilute Matrigel or not using a pre-warmed tissue culture treated plate are potential causes of organoids coming in contact with the plate bottom
- Some organoids have a significant amount of shedding (blue arrows)
- This culture will not passage well, but could be rescued with few passages with low split ratios.

Donor 8: Healthy Morphology with Extensive Budding







- Excellent culture
- Despite undergoing 17 passages, there is extensive budding morphology (purple arrows)
- Due to the complexity and size of the organoids, this culture is ready to passage
- This culture will passage efficiently

Summary

- Human intestinal organoid culture can be initiated from crypts isolated form primary human biopsy or surgical resection samples.
    - Crypts break down really easily, once plated, you typically only see smaller crypt fragments.
- Initiation culture may take anywhere from 7-14 days to establish. General culture timelines are:
    - For primary cultures (P0), passage after 7 - 14 days.
    - For previously passaged organoids (P1+), passage every 7 - 10 days.
- Organoid morphology is highly variable from different donors, the split ratio at earlier passages can also be variable.
- Organoid cultures should be passaged before they start to become overgrown as indicated by the black, blebbing, dead organoids.