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# Characterization of the mucus lining a co-culture model of Caco-2/HT29-MTX cells

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## Introduction

### Context

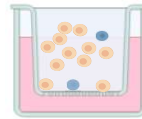
- Co-cultures integrating enterocyte-like cells (Caco-2) and goblet cells (HT29-MTX), are promising models of a functional epithelial barrier <sup>1</sup>.
- It is expected that such cultures are lined by a mucus layer but the spatial characteristics of this layer is poorly described.

### Objectives

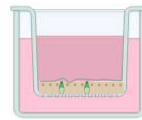
- To visualize mucins in a co-culture of Caco-2/HT29-MTX
- To set up a method of mucus characterization based on image analysis

## Materials and methods

Caco2 + HT29-MTX (9:1) seeding density 2.10<sup>5</sup> cell/cm<sup>2</sup>



Incubation at 5% CO<sub>2</sub> / 37°C 21 days



Differentiated monolayer: fixation with 4% PFA

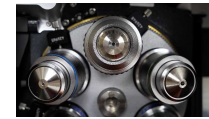
Open-source software ilastik <sup>2</sup>



classification of pixels into three categories (corresponding to bright staining mucin clusters, diffuse mucin staining, background)



Ten 3-D images (80 stacked images on average) from 2 independent wells



Photograph: Jordane Ossemond (STLO)

Confocal microscopy Zeiss-LSM 880

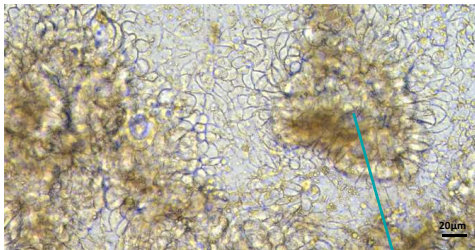


Staining:

F-actin with phalloidin-rhodamine → cytoskeleton  
Sialic acid with WGA-Alexa 488 → mucins

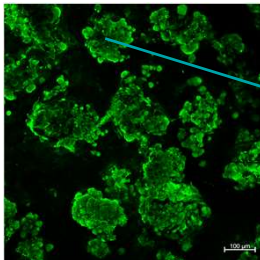
## Results

### 1- Uneven distribution of the mucus



Observation of cultures by optical microscopy (X20)

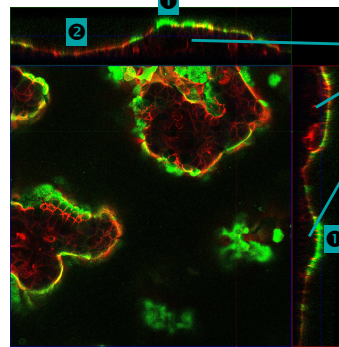
Cells are confluent and they form a monolayer but some cells are grouped and lined with a blurry material



Mucins are abundantly expressed as clusters

Observation of cultures after staining of sialic acid

### 2- A typical topography



Observation of one stack of a 3-D reconstituted image

The culture is not planar. Domes (visible on z-views) are present. Height up to 135 µm.

Mucins are present mainly at the apical sides of domes (see 1 bright staining; sites of secretion) but also between domes (see 2 diffuse staining: secreted mucins spreading over the culture, loose mucus)

### 3- Development of an image analysis workflow to characterize the mucus structure

Machine learning: annotation of pixels representative of bright /diffuse staining on a subset of images, followed by automatic classification on all images.

Extraction of quantitative data

	Dense mucin clusters	Loose mucus
Average thickness	6.9 µm	21.2 µm
Average volume	183000 µm <sup>3</sup>	7502000 µm <sup>3</sup>

On-going: segmentation and classification of cells

## Conclusions

- Staining of sialic acid and confocal microscopy enable three-dimensional visualization of the mucus layer
- The mucus on the co-culture model is heterogenous in density
- The protocol of image analysis is suitable to extract quantitative data on the mucus layer (NB: lower thickness than *in vivo* data)

These methodological developments open the way to studying the impact of digested food constituents or digested foods on the intestinal mucus

### References:

- (1) Béduneau et al. 2014. A Tunable Caco-2/HT29-MTX Co-Culture Model Mimicking Variable Permeabilities of the Human Intestine Obtained by an Original Seeding Procedure. Eur J Pharm Biopharm 87 (2), 290-298.  
(2) Berg et al. 2019. A. Ilastik: Interactive Machine Learning for (Bio)Image Analysis. Nat Methods 16 (12), 1226-1232.