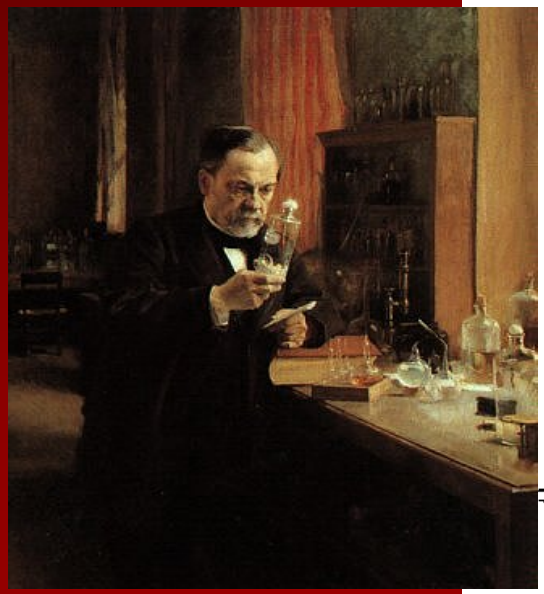
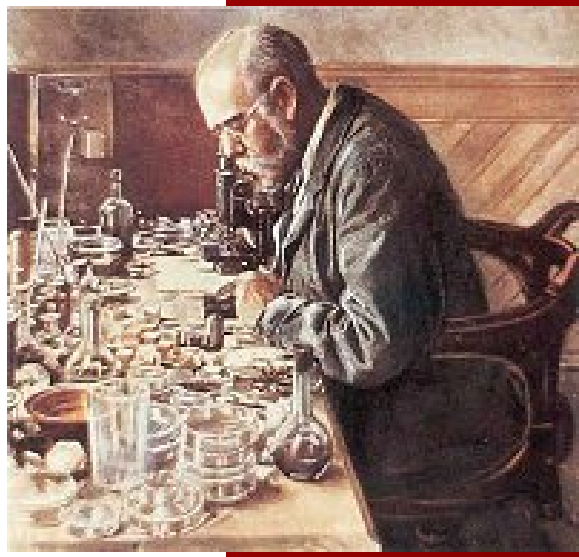
Mucosal Flora in IBD

Alexander Swidsinski

Supported by Broad Medical Research Program





Multicellular bacteria forming stromatolith in Australian salt lakes

FISH Analysis of mucosal Flora

Eub338
Alf1b
Beta42a
Gam42a
Ebac
Ec1531
Y16s-69
Srb385
Sgd
Hpy-1
Arc1430
HGC
LGC
Sfb
Erec
Lach
Ehal
Chis150
Clit135
Lab158
Strc493
Enc131
Efaec
Ato291
Cor653
Ecyl
Phasco
Veil
Rbro, Rfla
UroA, UroB
Ser1410
Bif164
CF319a
Bac303
Bfra602
Bdis656
Fprau
Dss658
Arch915

using r-RNA probes

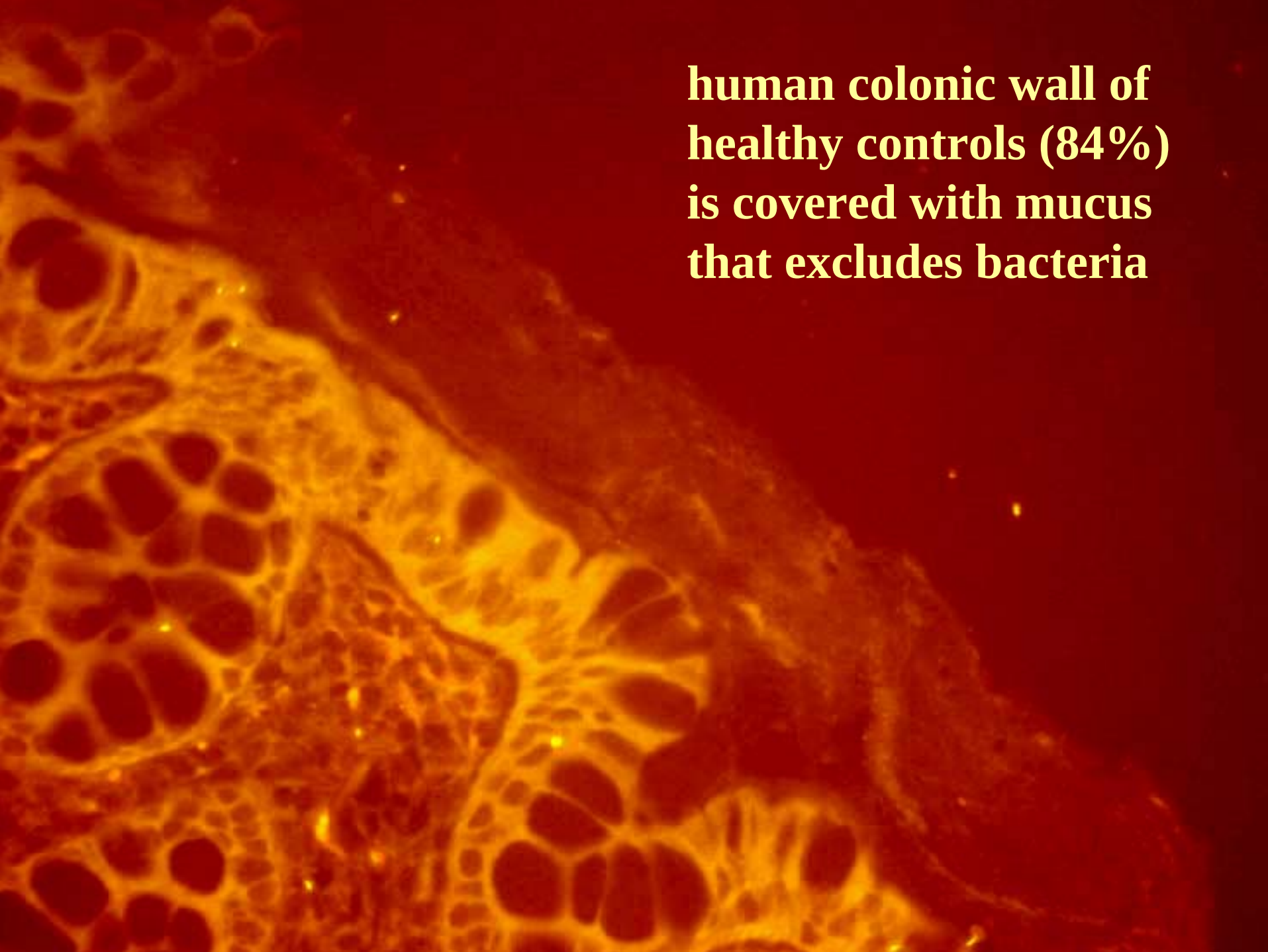
Analysis of mucosal biofilms using Fluorescence In-Situ Hybridization (FISH)

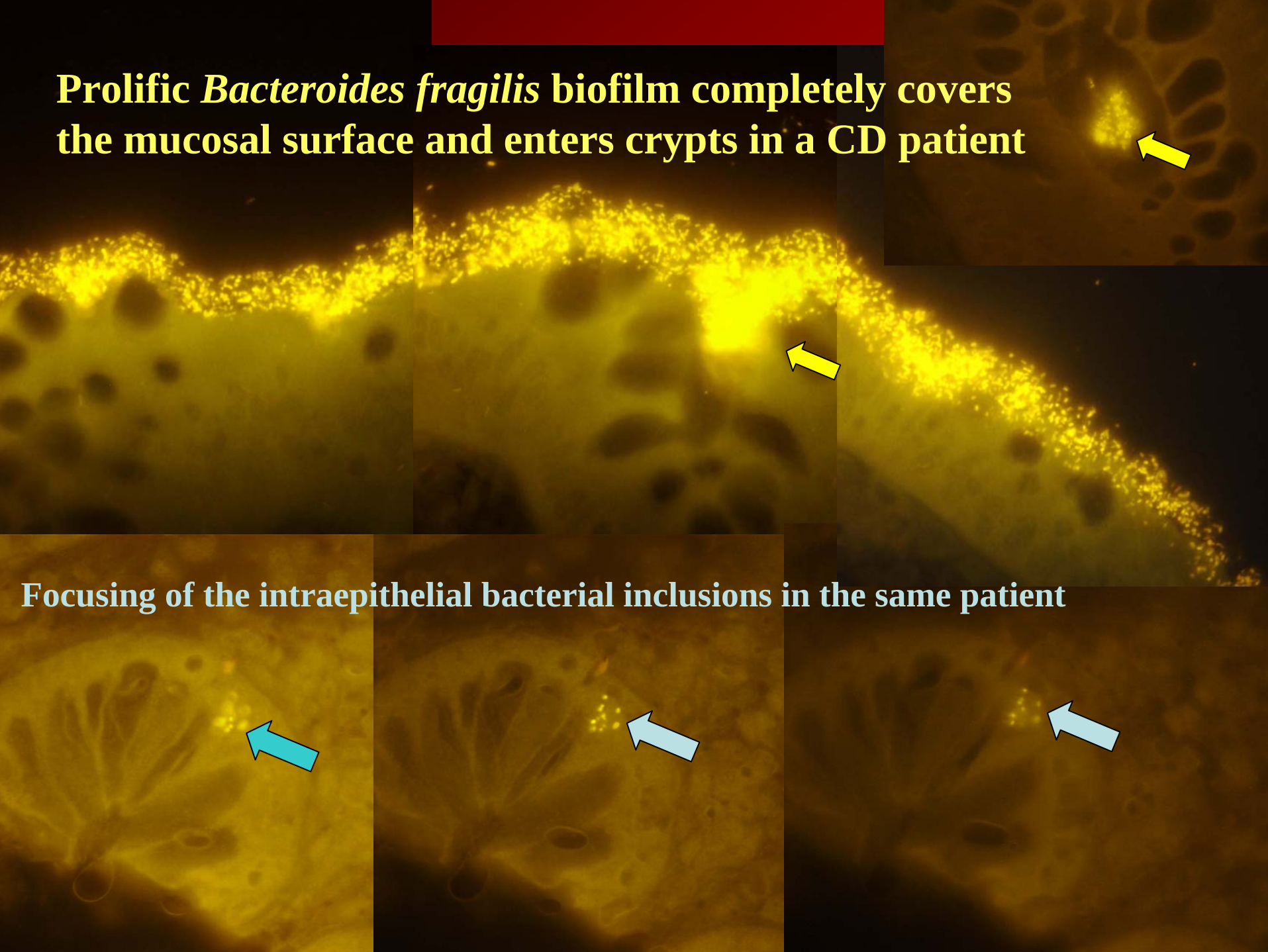
Adherent biofilms are the most prominent feature of IBD, which was not recognized due to lack of appropriate methods. Biofilms disappear after fixation in formalin – a main fixative in clinical pathology

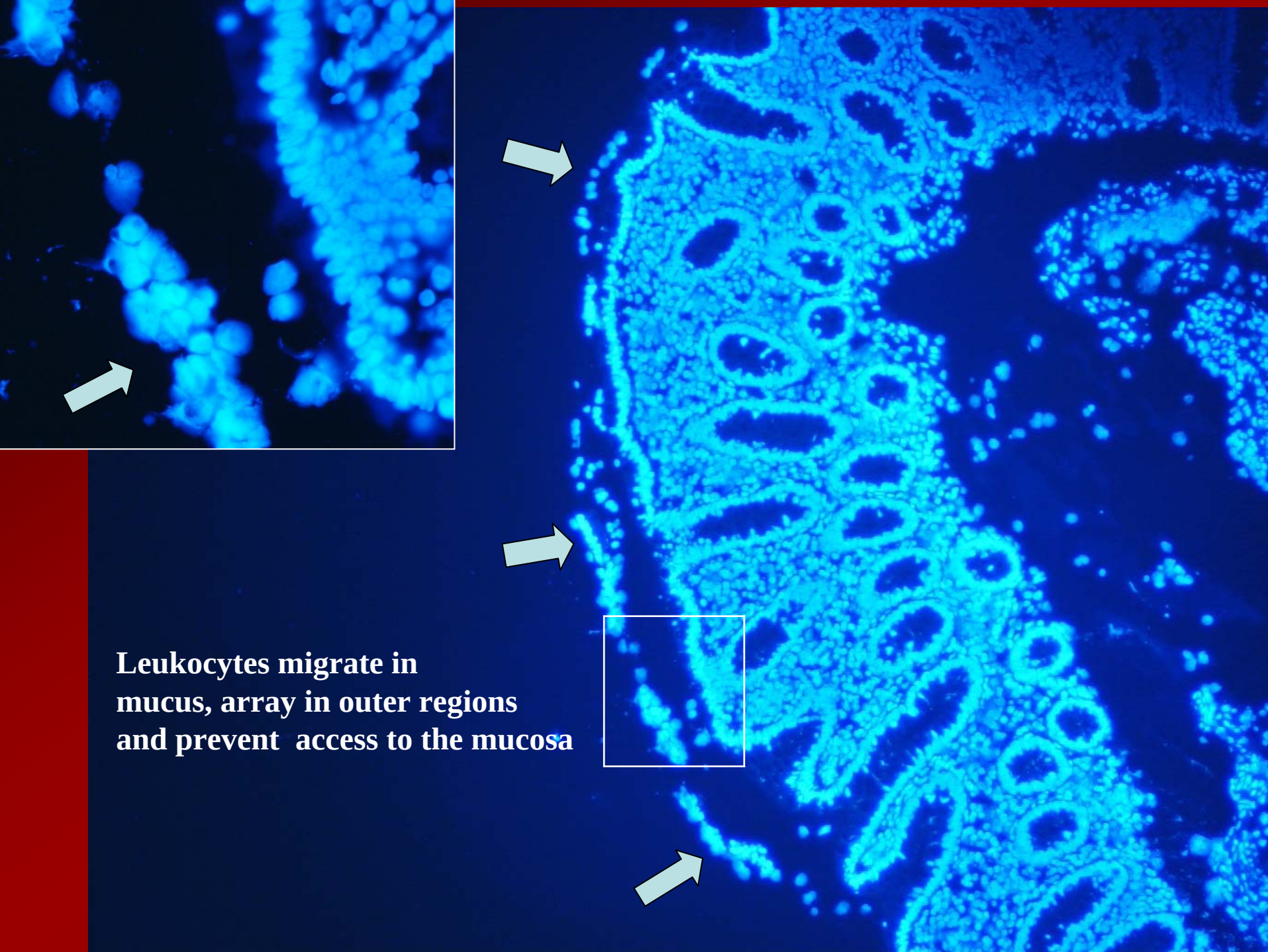
The same patient and the same location fixed either with

Carnoy or Formalin

Crohn´s Disease, DAPI stain

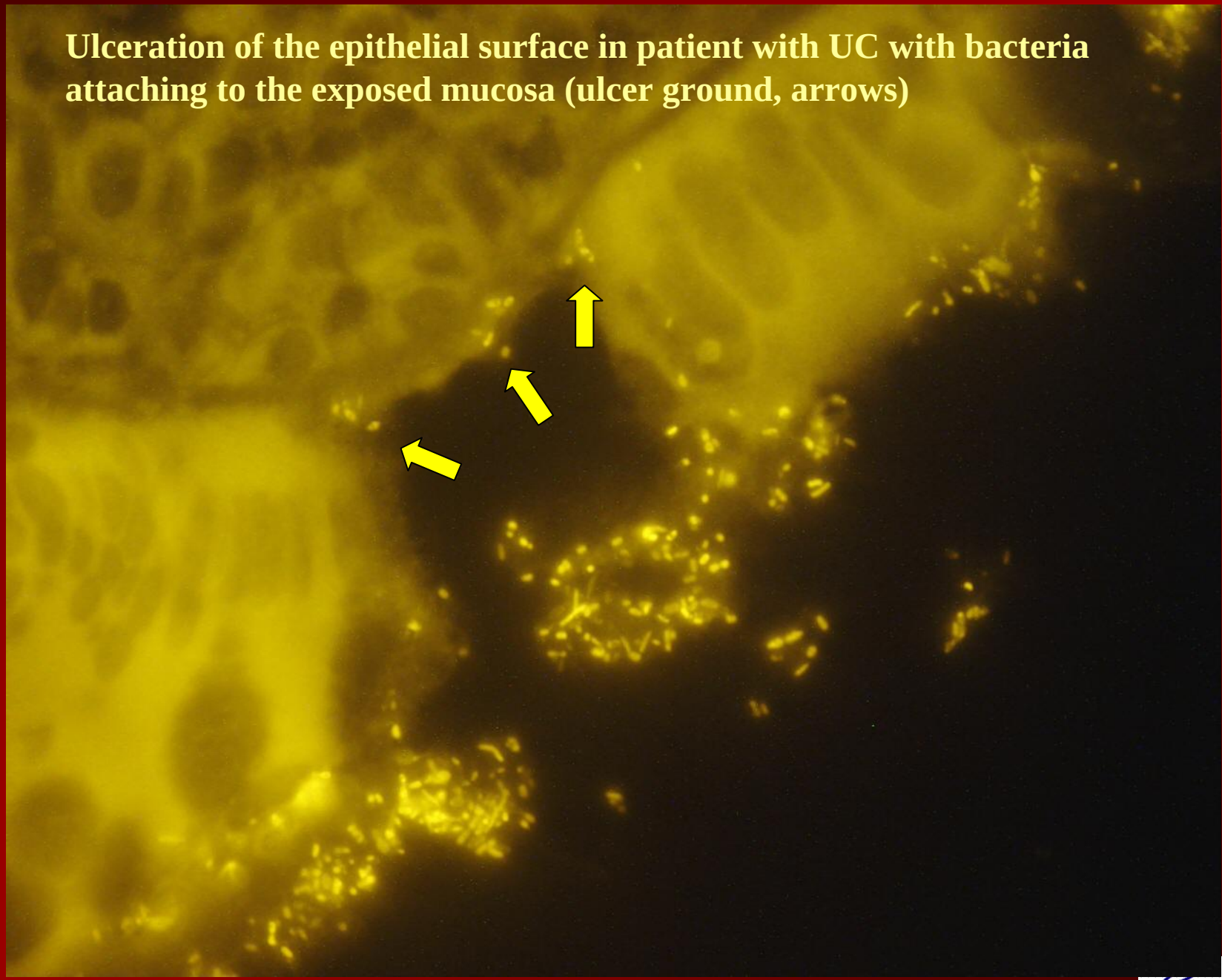

human colonic wall of
healthy controls (84%)
is covered with mucus
that excludes bacteria

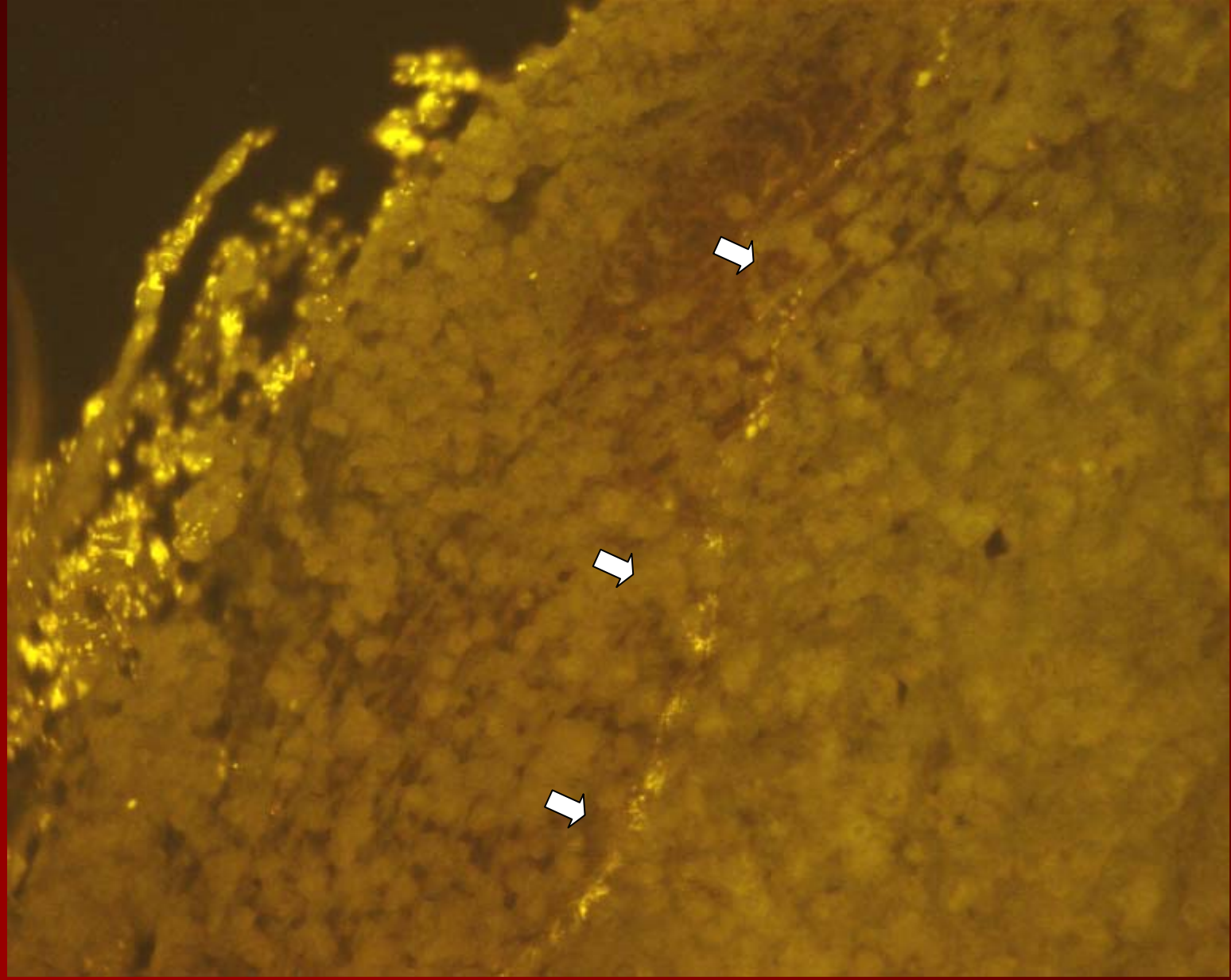

Prolific *Bacteroides fragilis* biofilm completely covers the mucosal surface and enters crypts in a CD patient
Focusing of the intraepithelial bacterial inclusions in the same patient



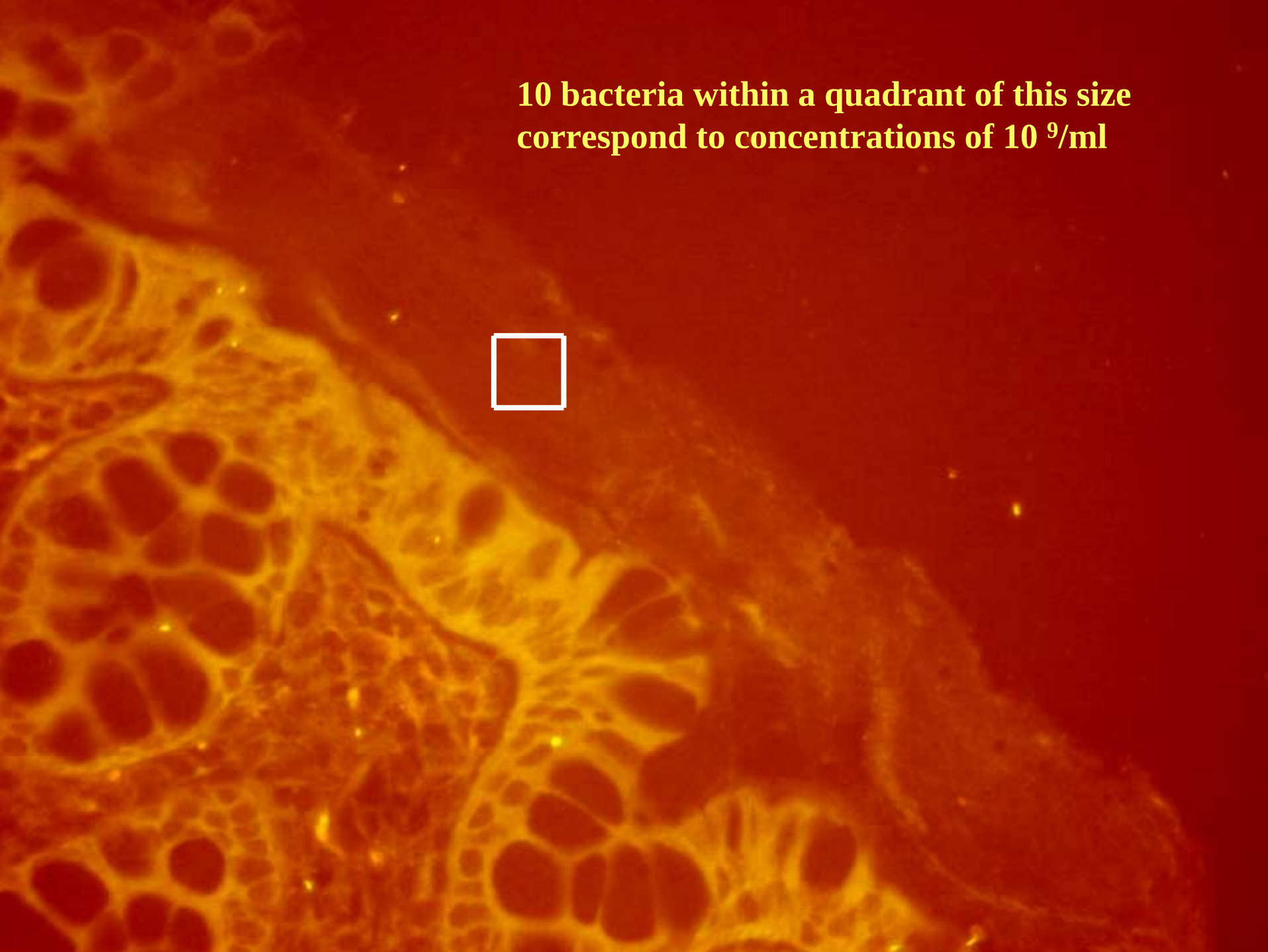
Leukocytes migrate in mucus, array in outer regions and prevent access to the mucosa

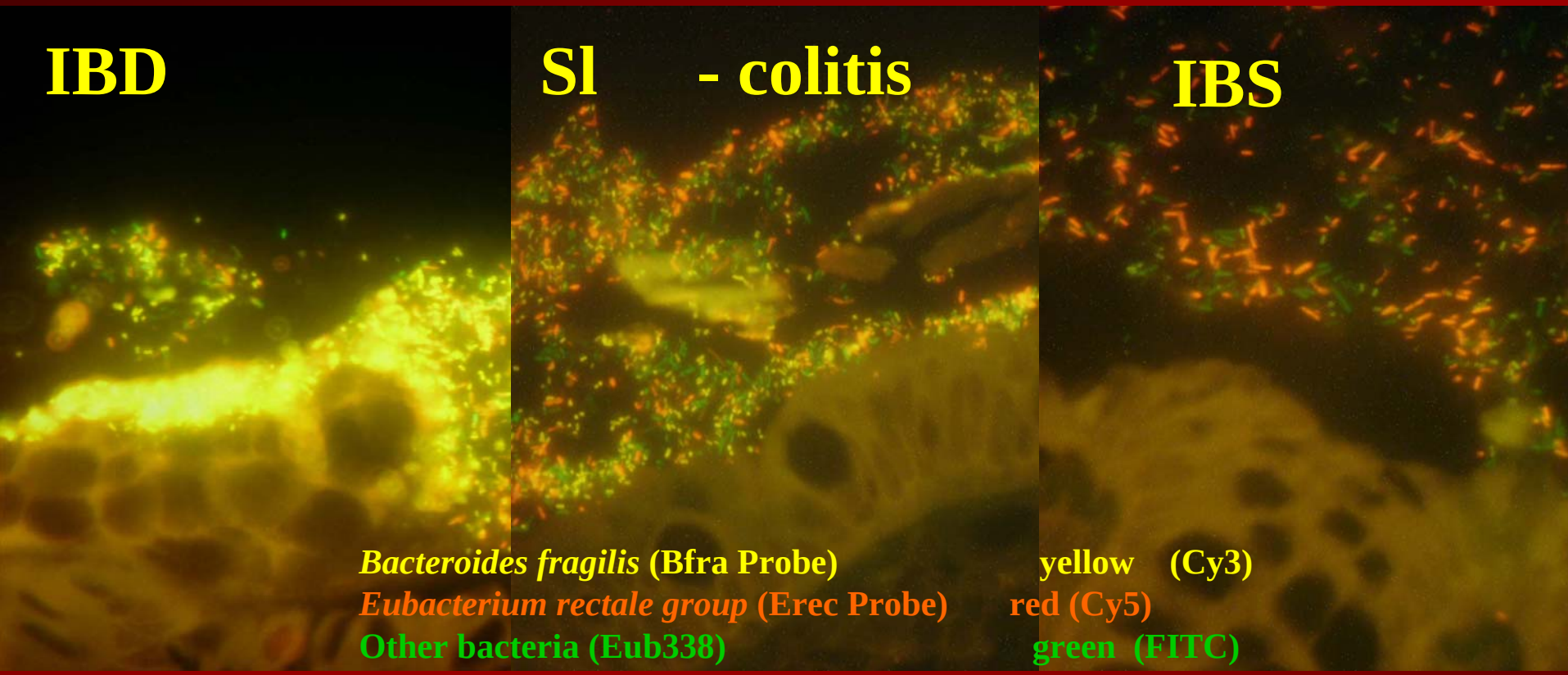
Ulceration of the epithelial surface in patient with UC with bacteria attaching to the exposed mucosa (ulcer ground, arrows)





***Bacteroides* infiltration of the intestinal wall, CD**

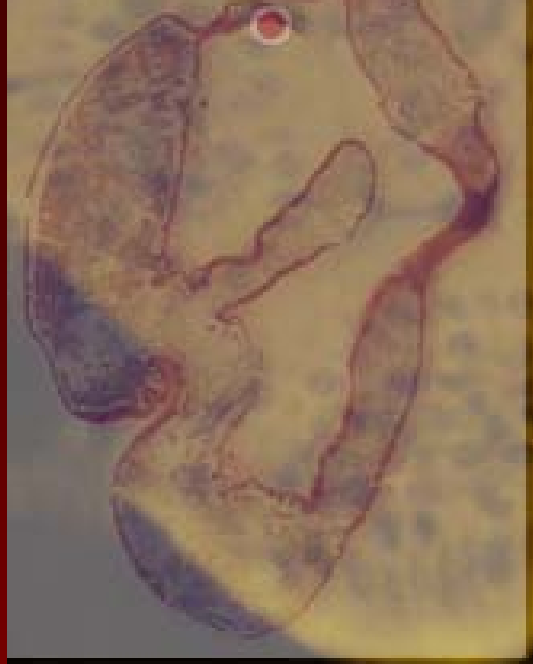

10 bacteria within a quadrant of this size
correspond to concentrations of 10 9/ml



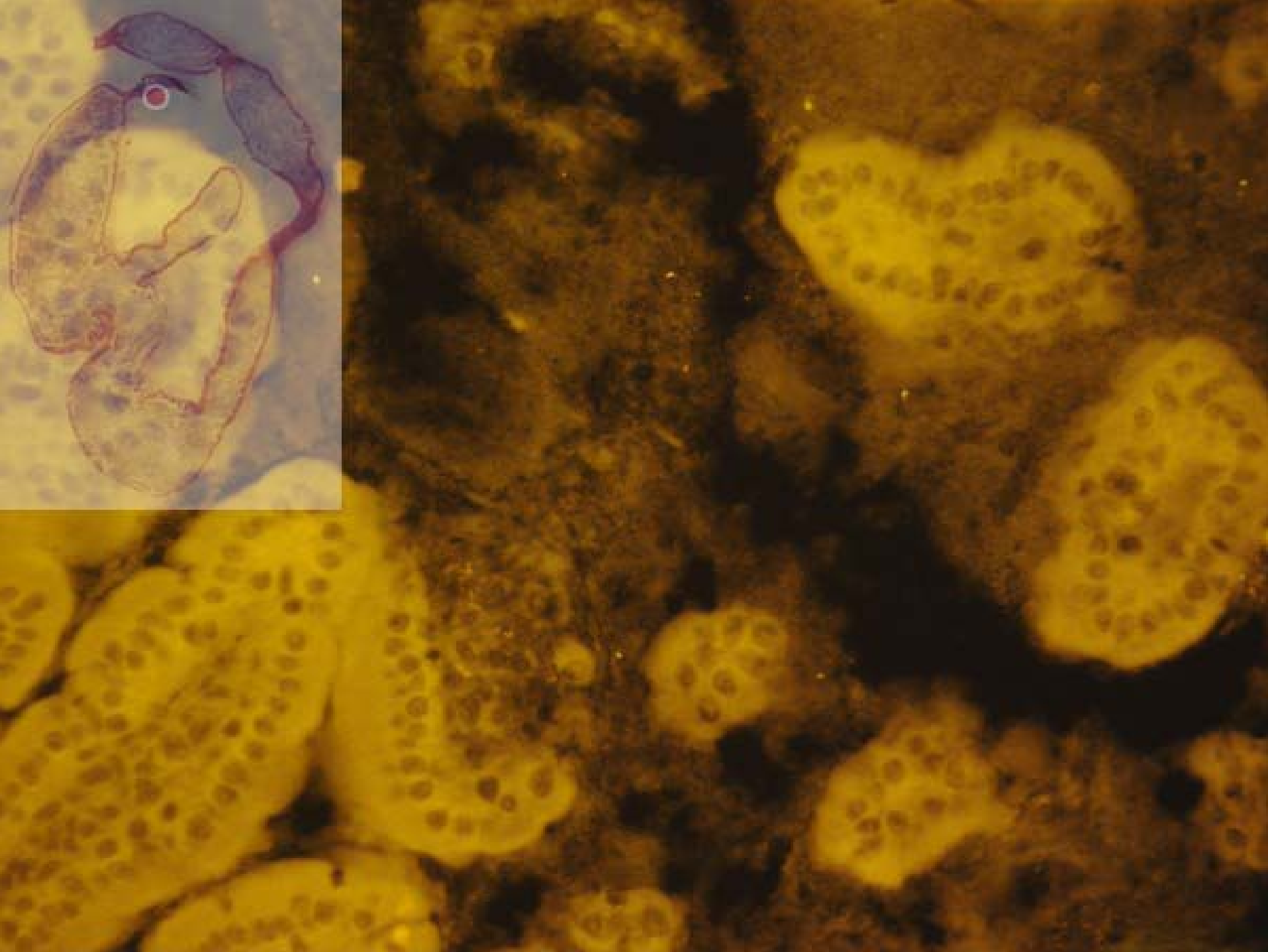
	CD 98%	UC 94%	Slc 78%	IBS 38%	Contr. 16%
Bfra	60%	30%	31%	14%	16%
Erec	10%	5%	18%	48%	32%

Percent of patients with 10^9 bacteria/ml

Percent of bacteria within biofilm



The number of bacteria in small intestine
of a healthy wild type mouse
is low



The bacterial concentrations
In the cecum of large intestine are
extreemely high

Bacteria (with an exception of *Bacteroides*)
are contacting the colonic wall and entering
crypts.

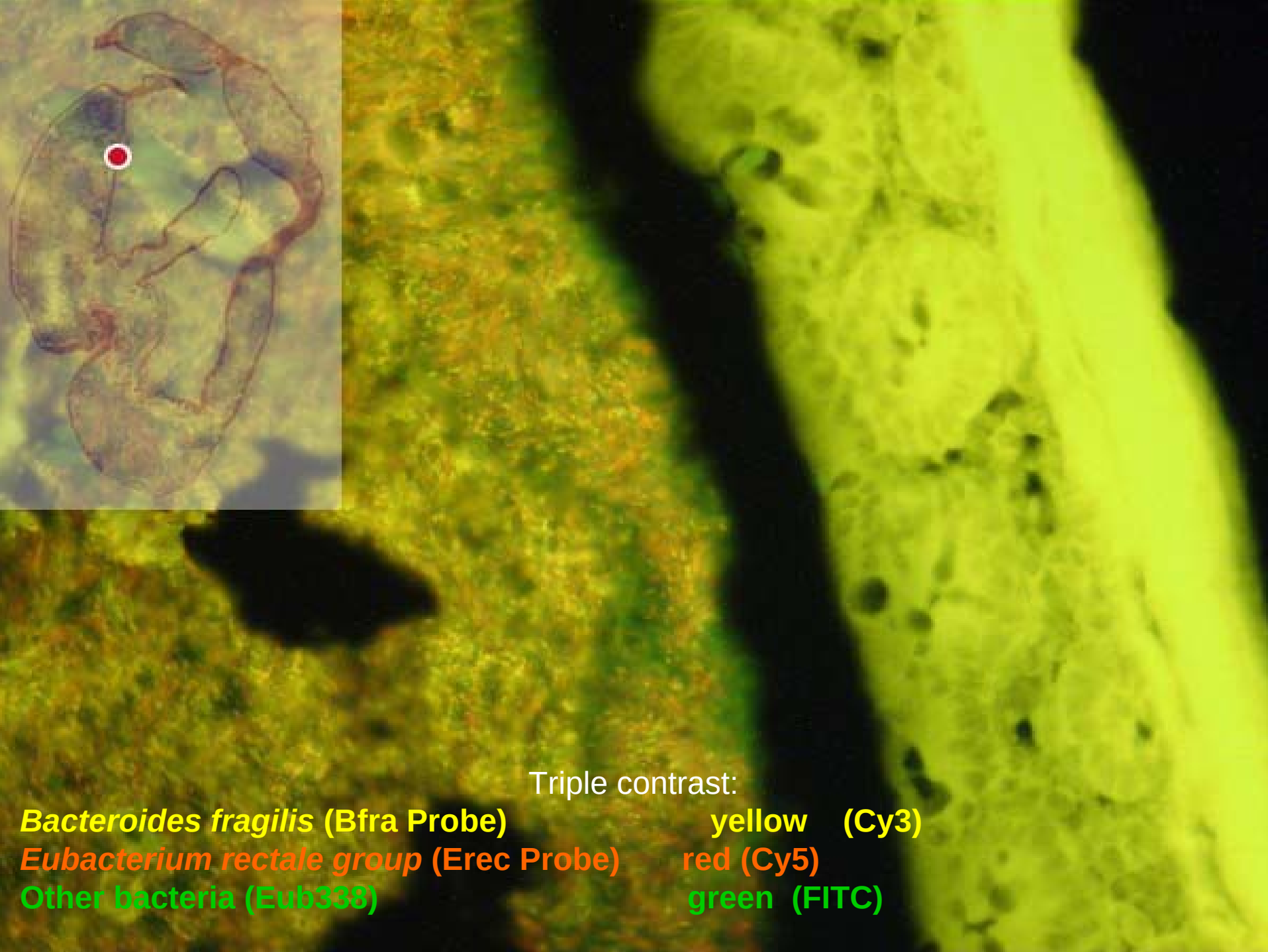
Bacteroides has no contact with the colonic wall

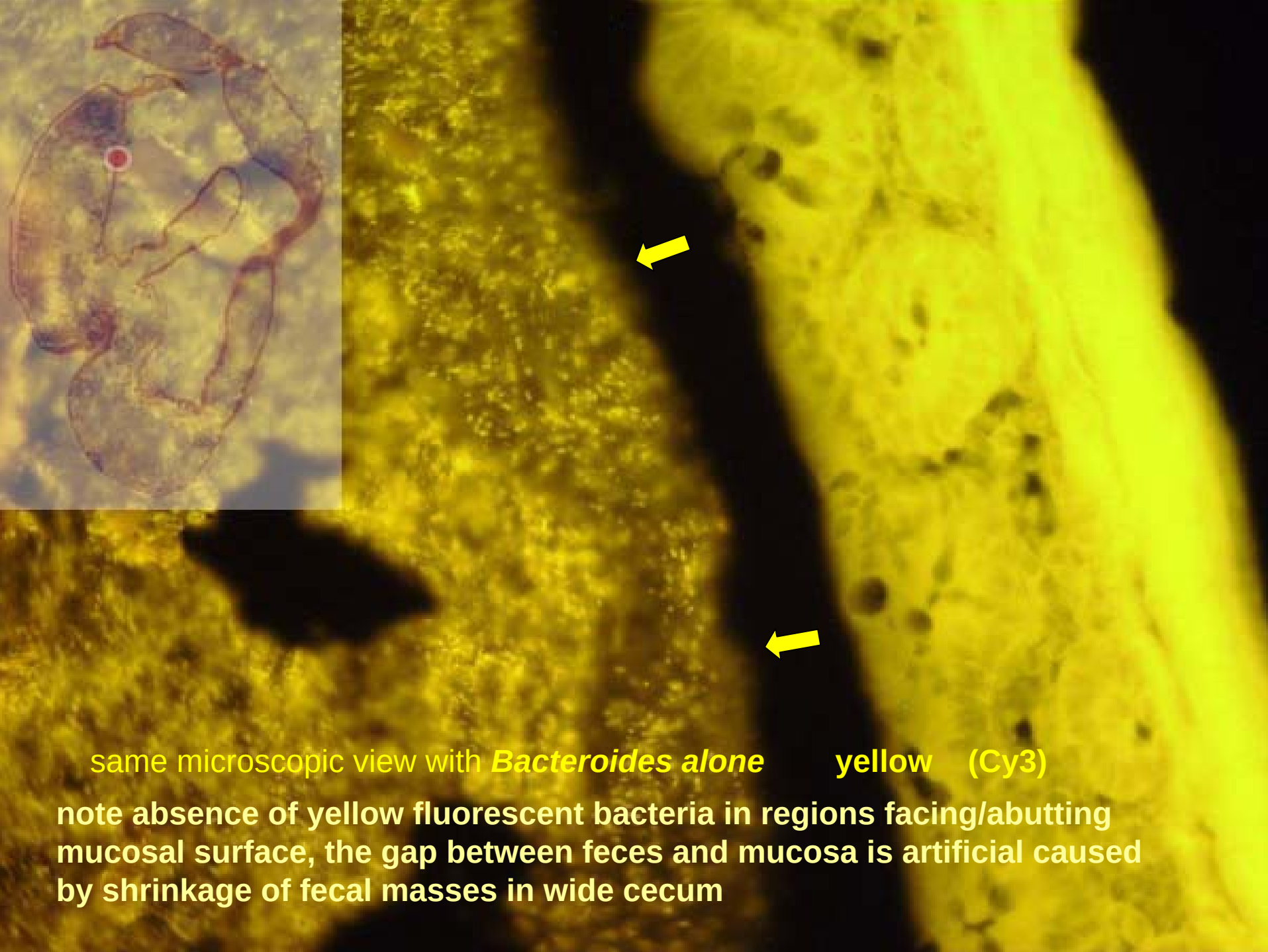
Triple contrast:

Bacteroides fragilis **(Bfra Probe)** **yellow (Cy3)**

Eubacterium rectale group **(Erec Probe)** **red (Cy5)**

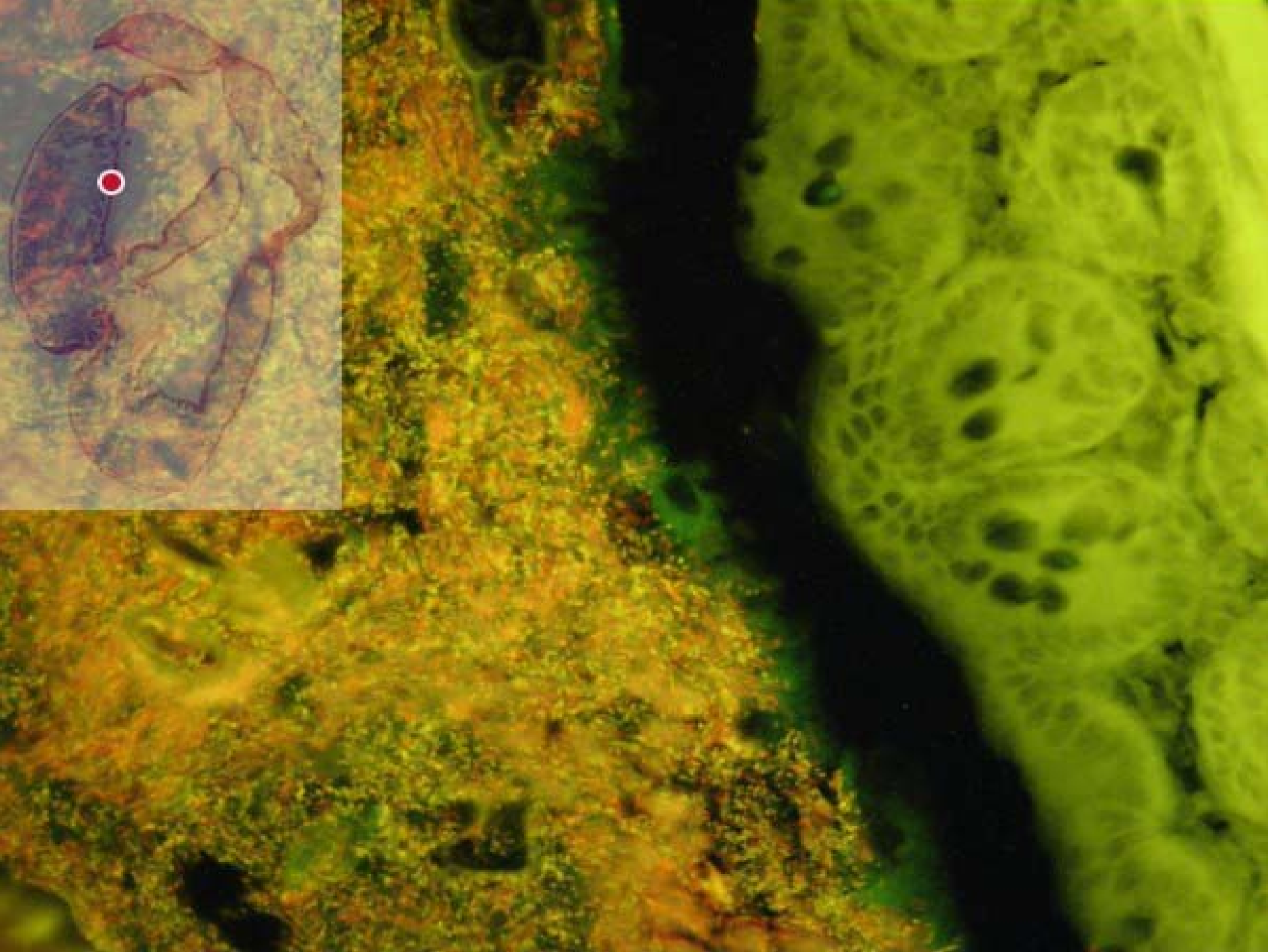
Other bacteria (Eub338) **green (FITC)**

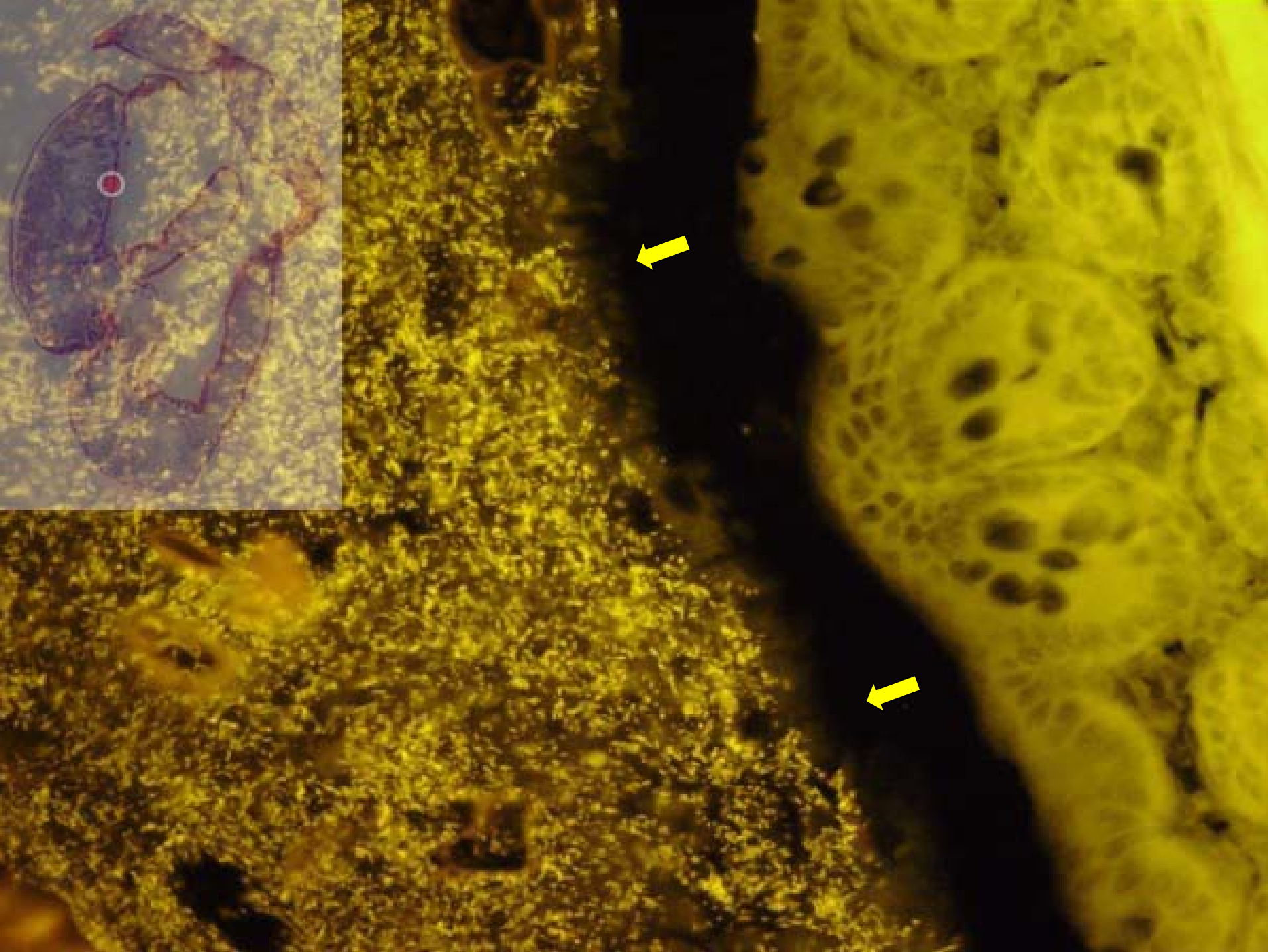

Triple contrast:
Bacteroides fragilis (Bfra Probe) yellow (Cy3)
Eubacterium rectale group (Erec Probe) red (Cy5)
Other bacteria (Eub338) green (FITC)

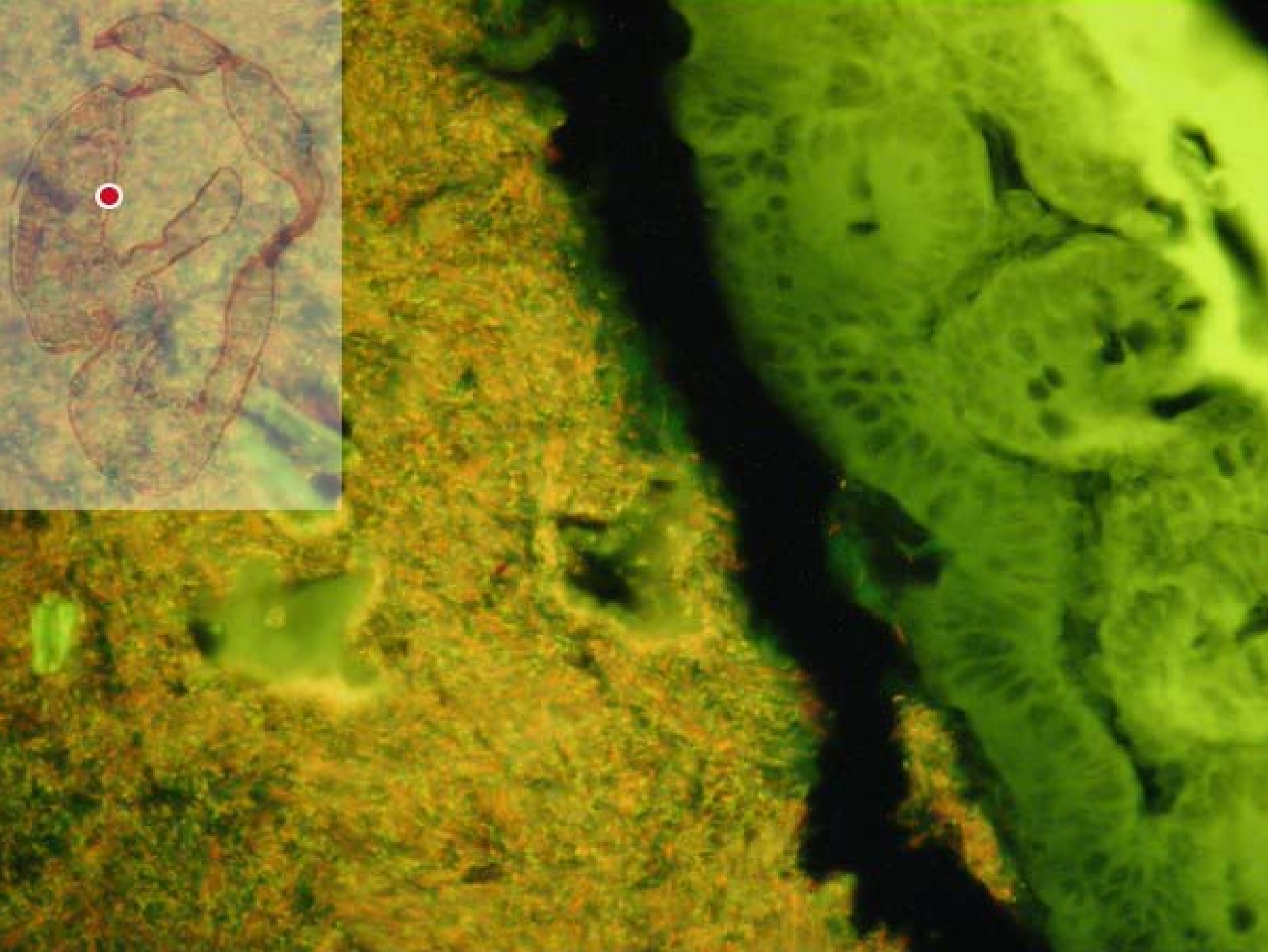


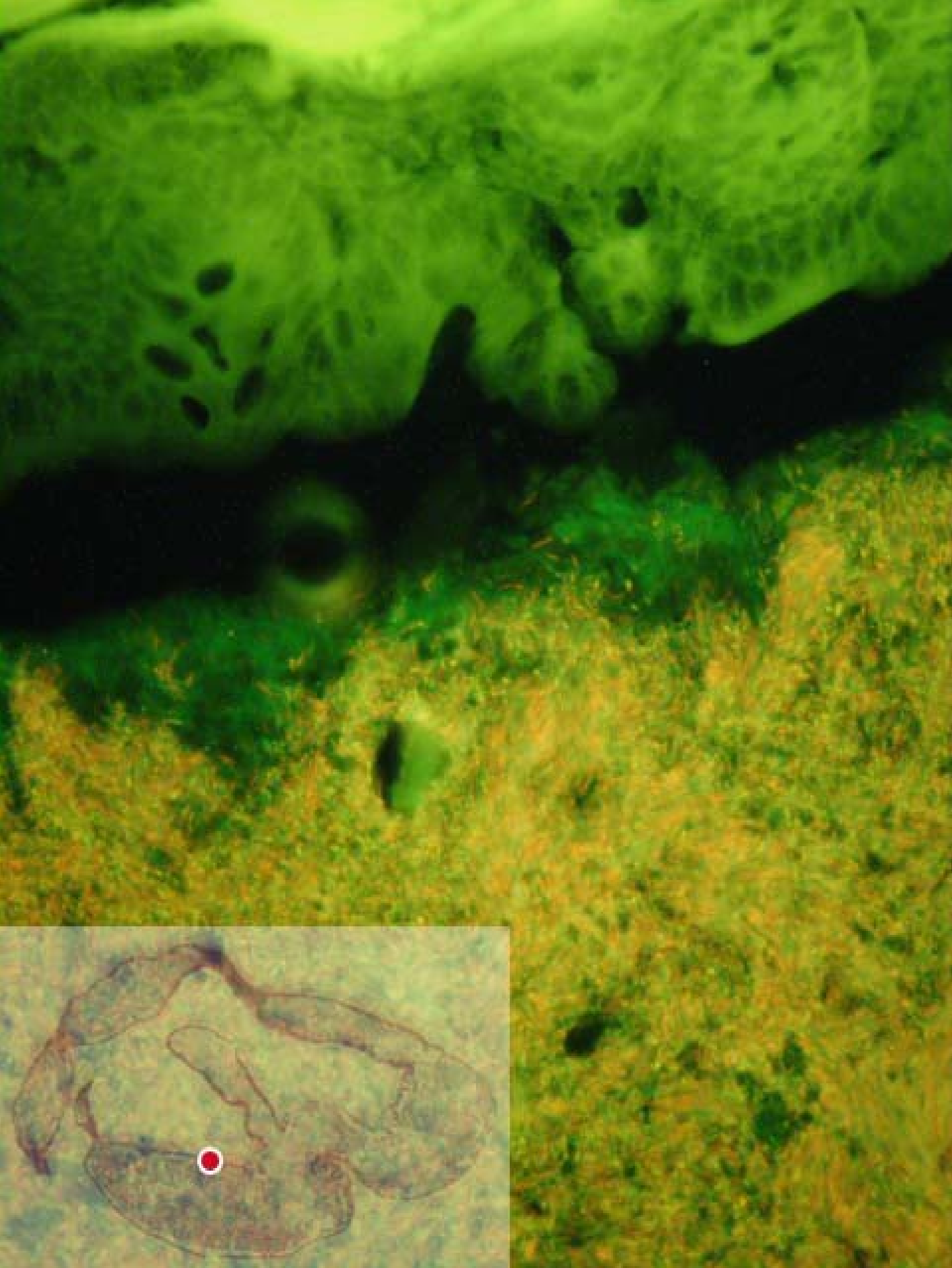
same microscopic view with ***Bacteroides alone*** **yellow (Cy3)**

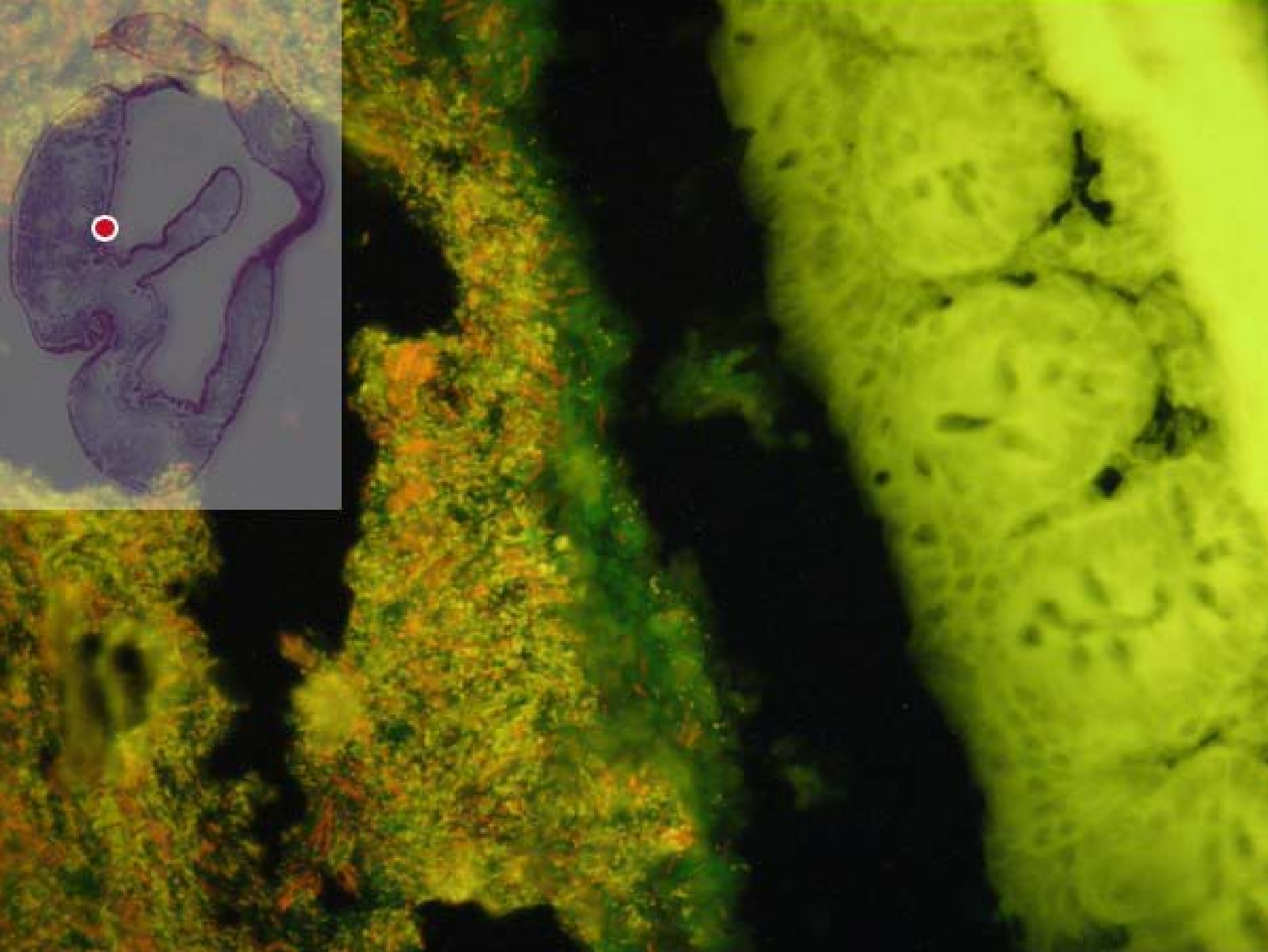
note absence of yellow fluorescent bacteria in regions facing/abutting mucosal surface, the gap between feces and mucosa is artificial caused by shrinkage of fecal masses in wide cecum

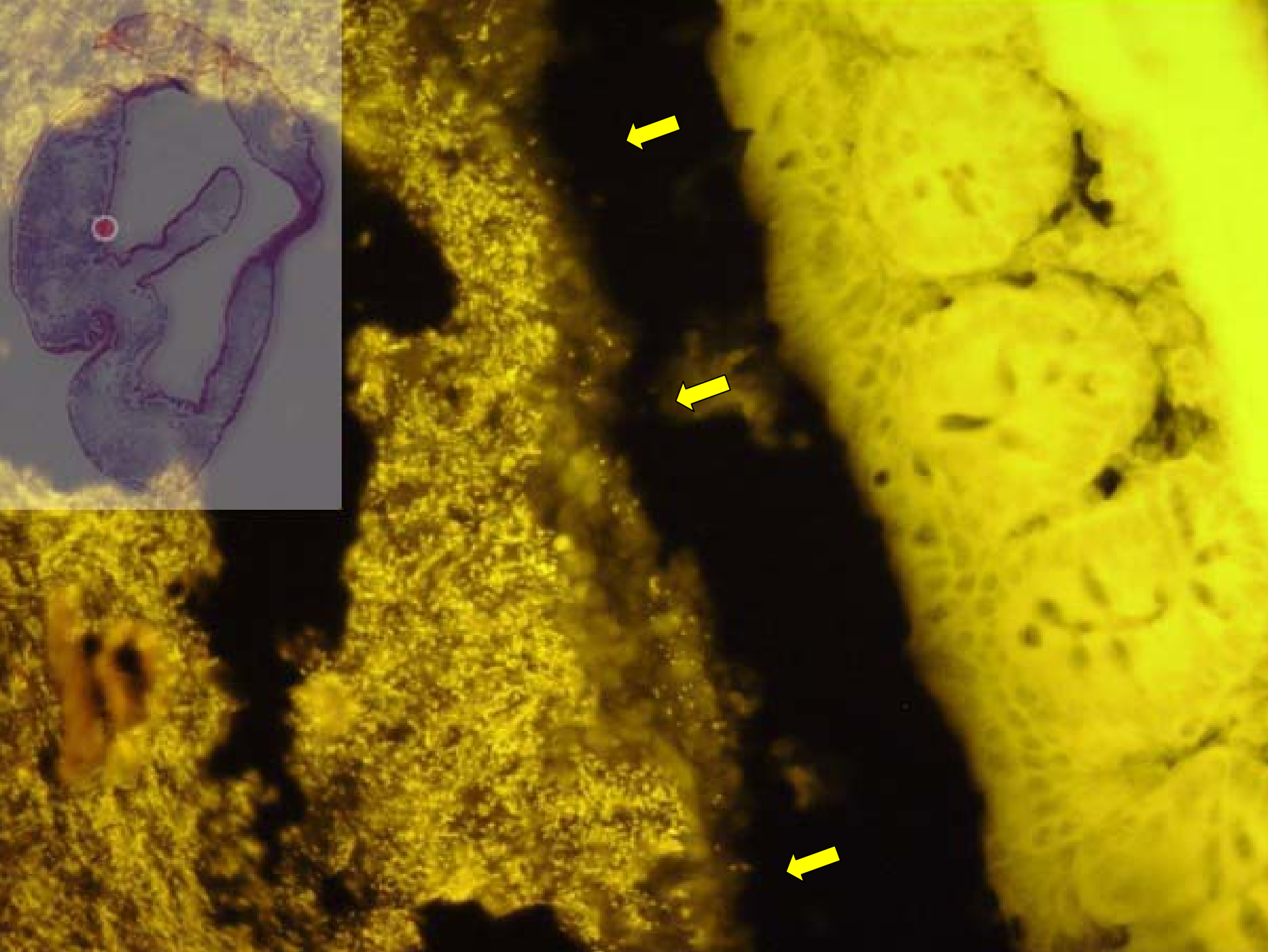


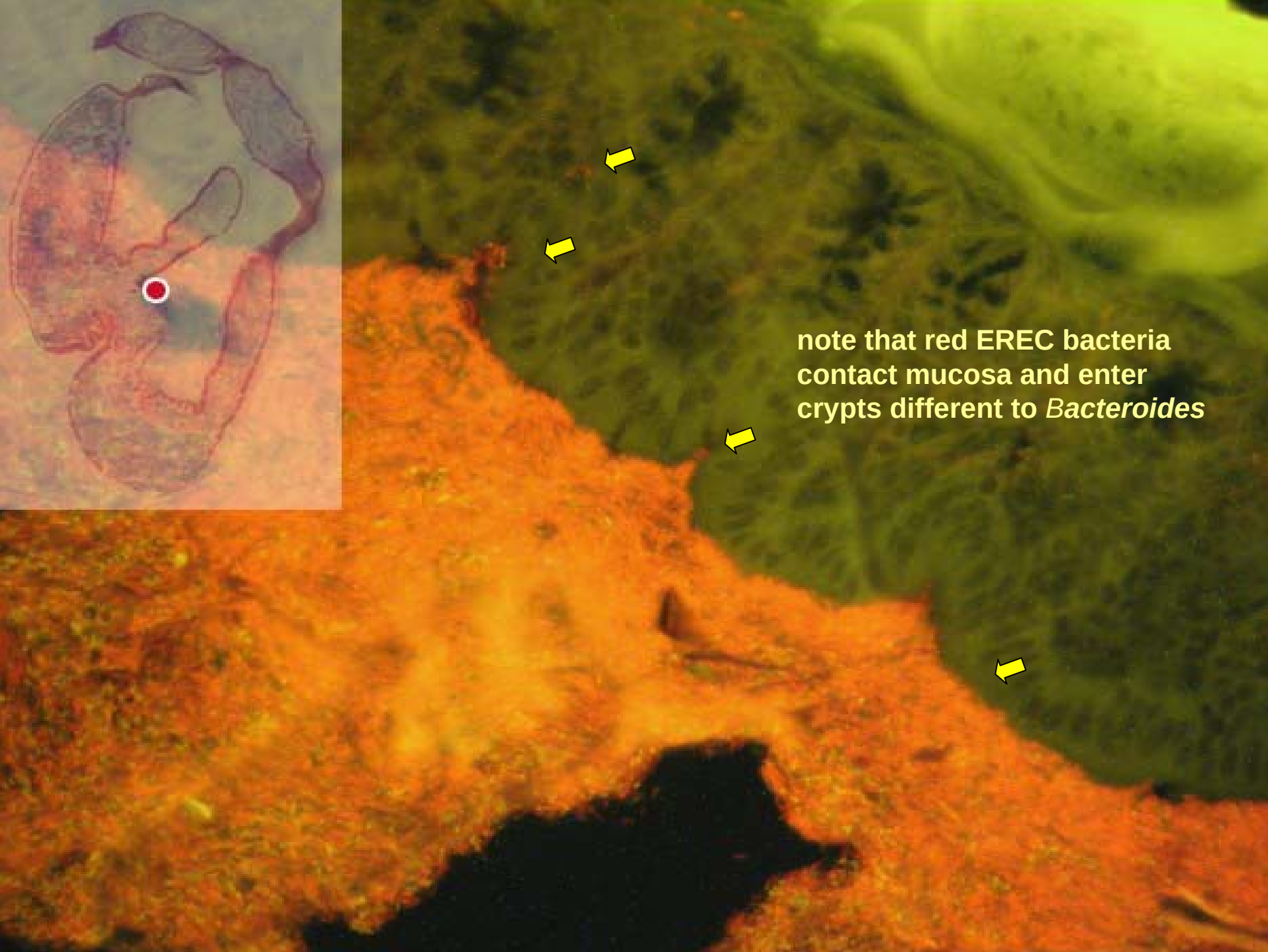


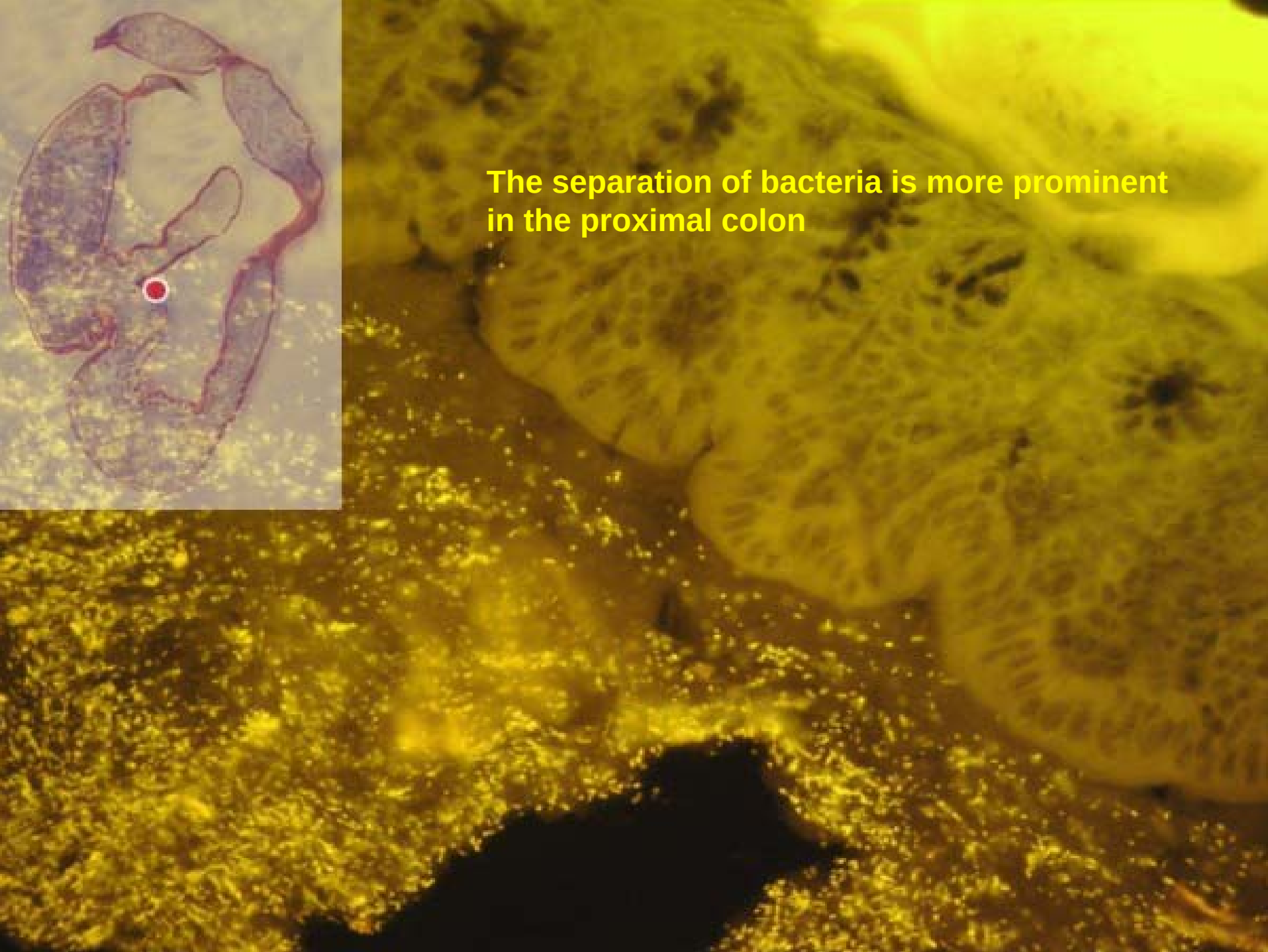


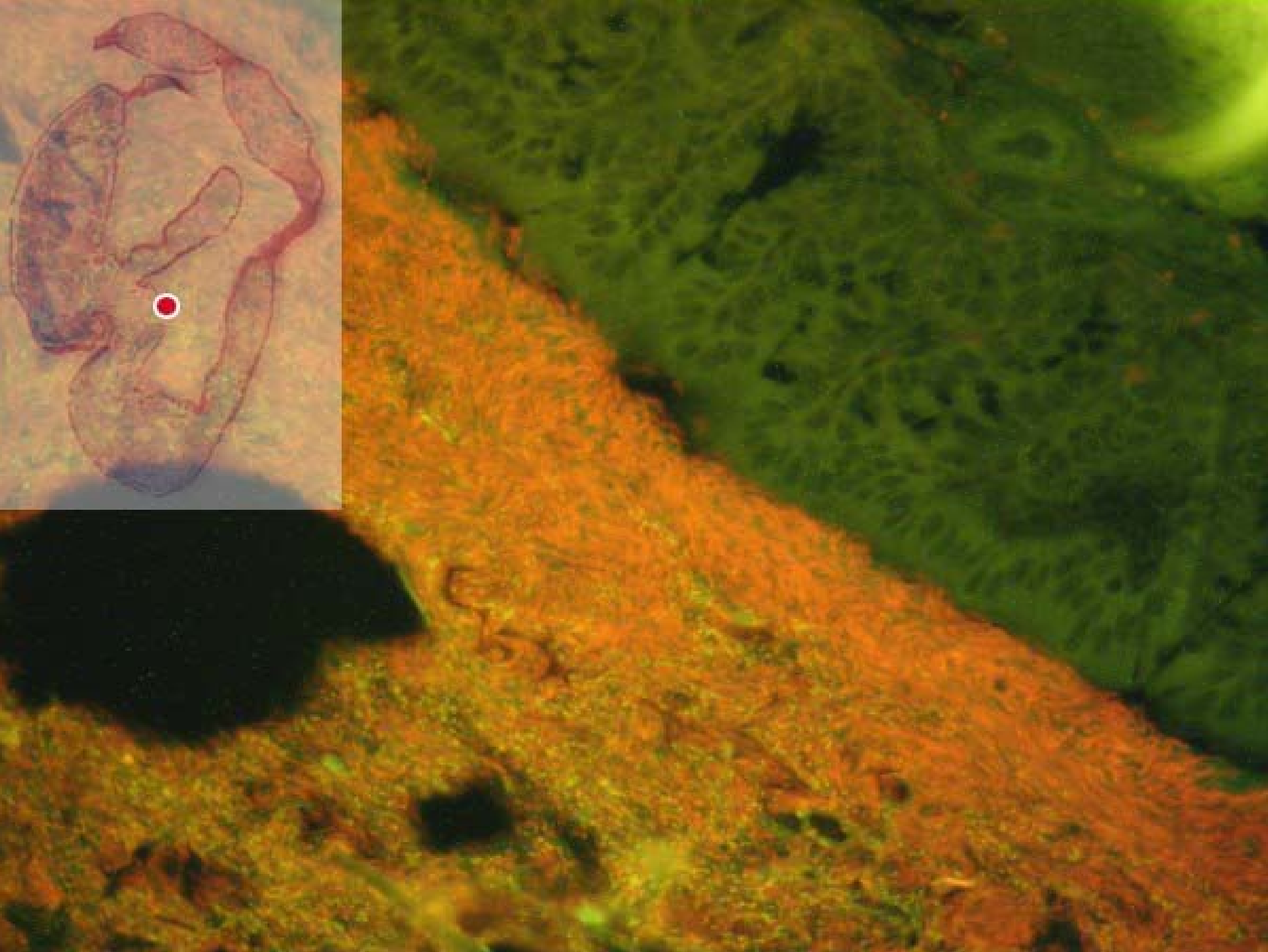


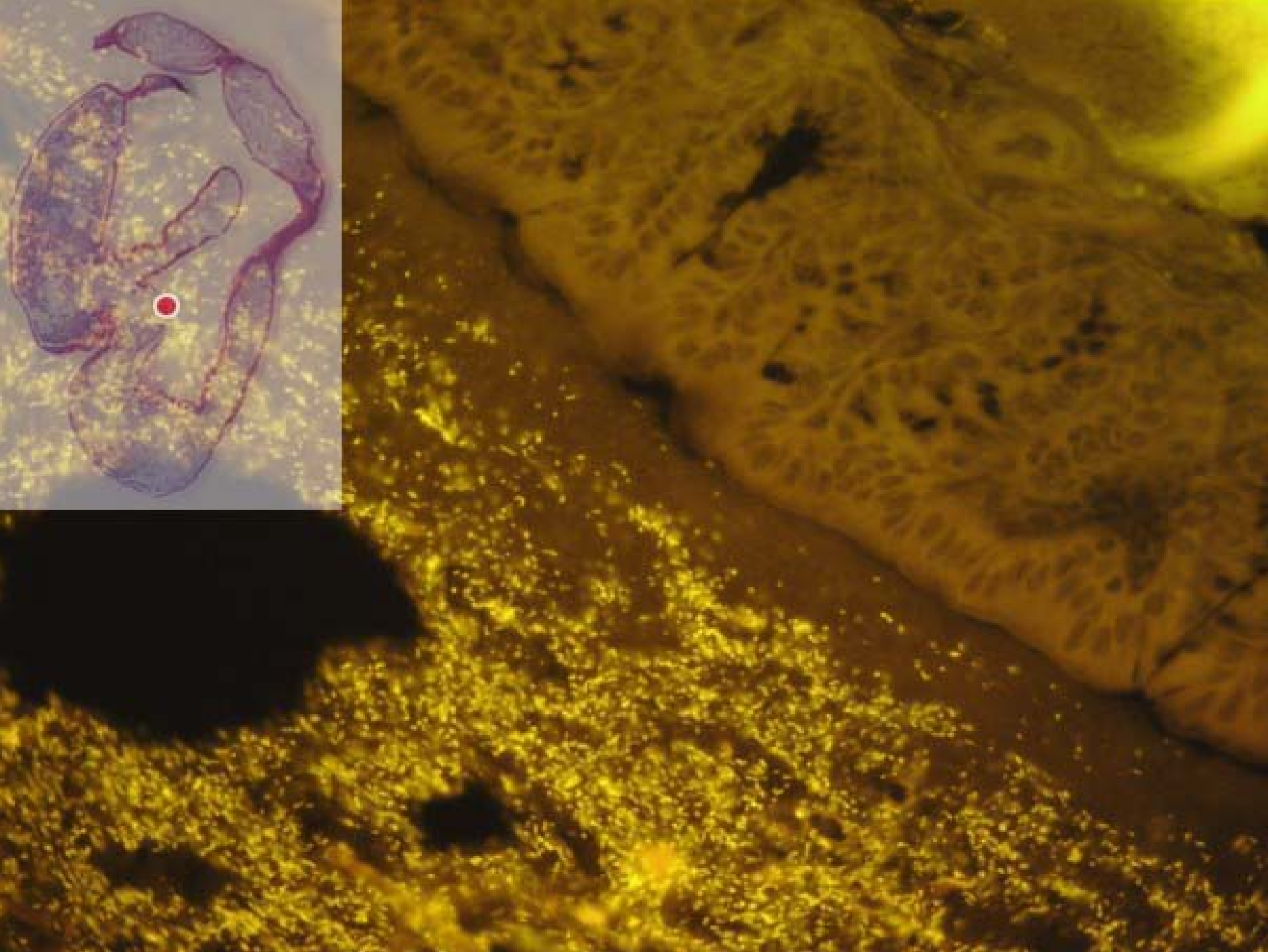


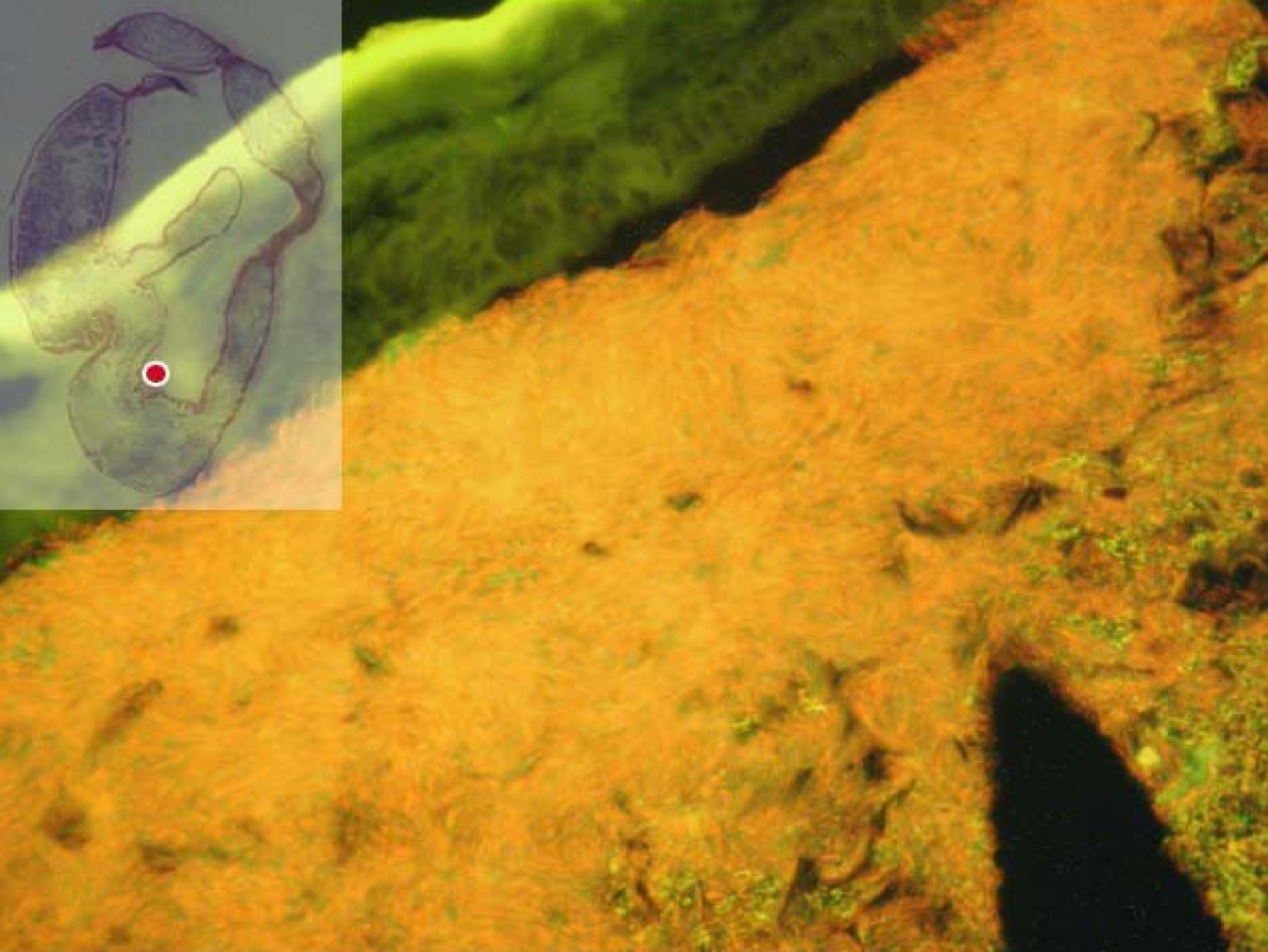


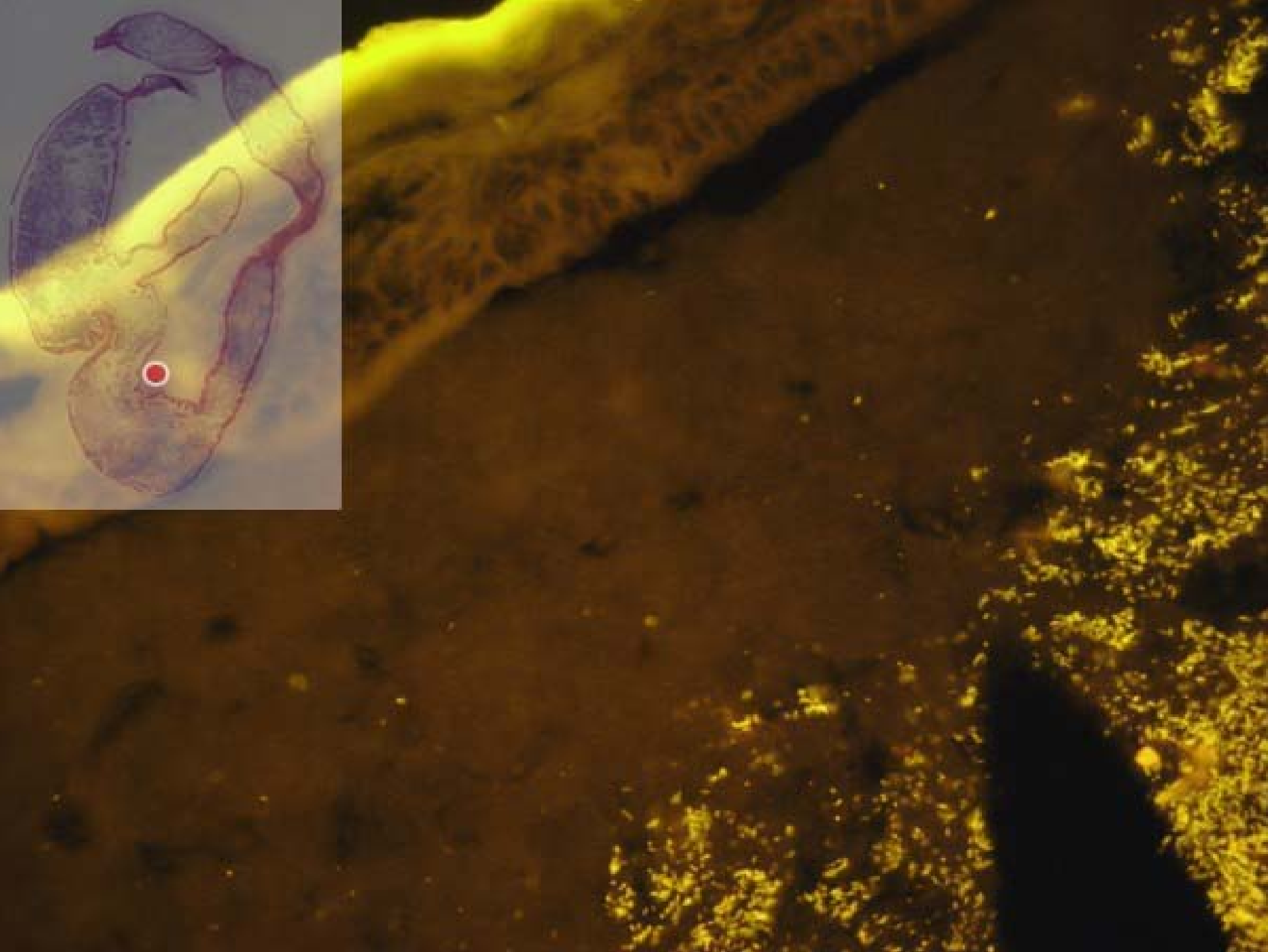

note that red EREC bacteria
contact mucosa and enter
crypts different to *Bacteroides*

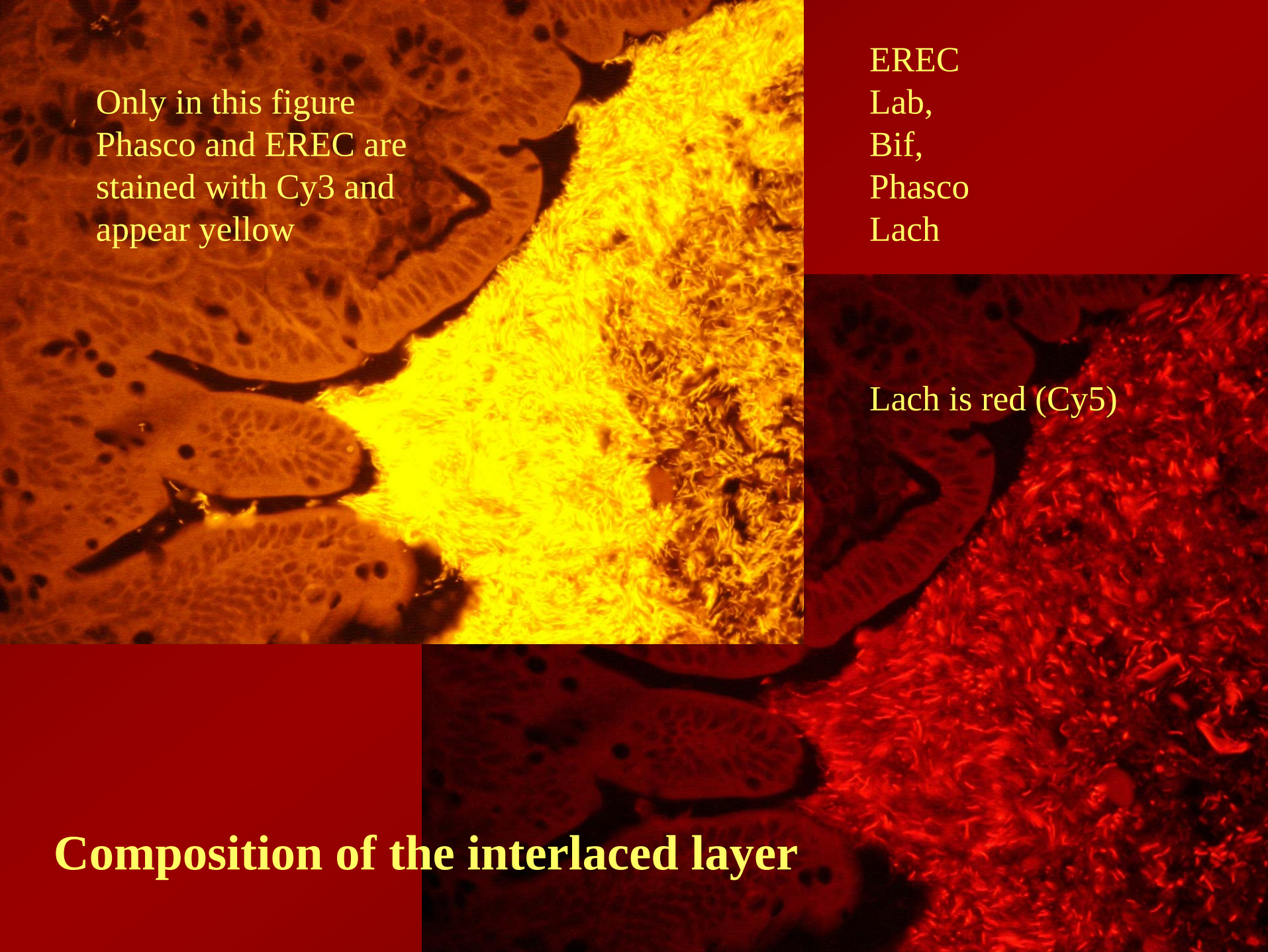

The separation of bacteria is more prominent
in the proximal colon





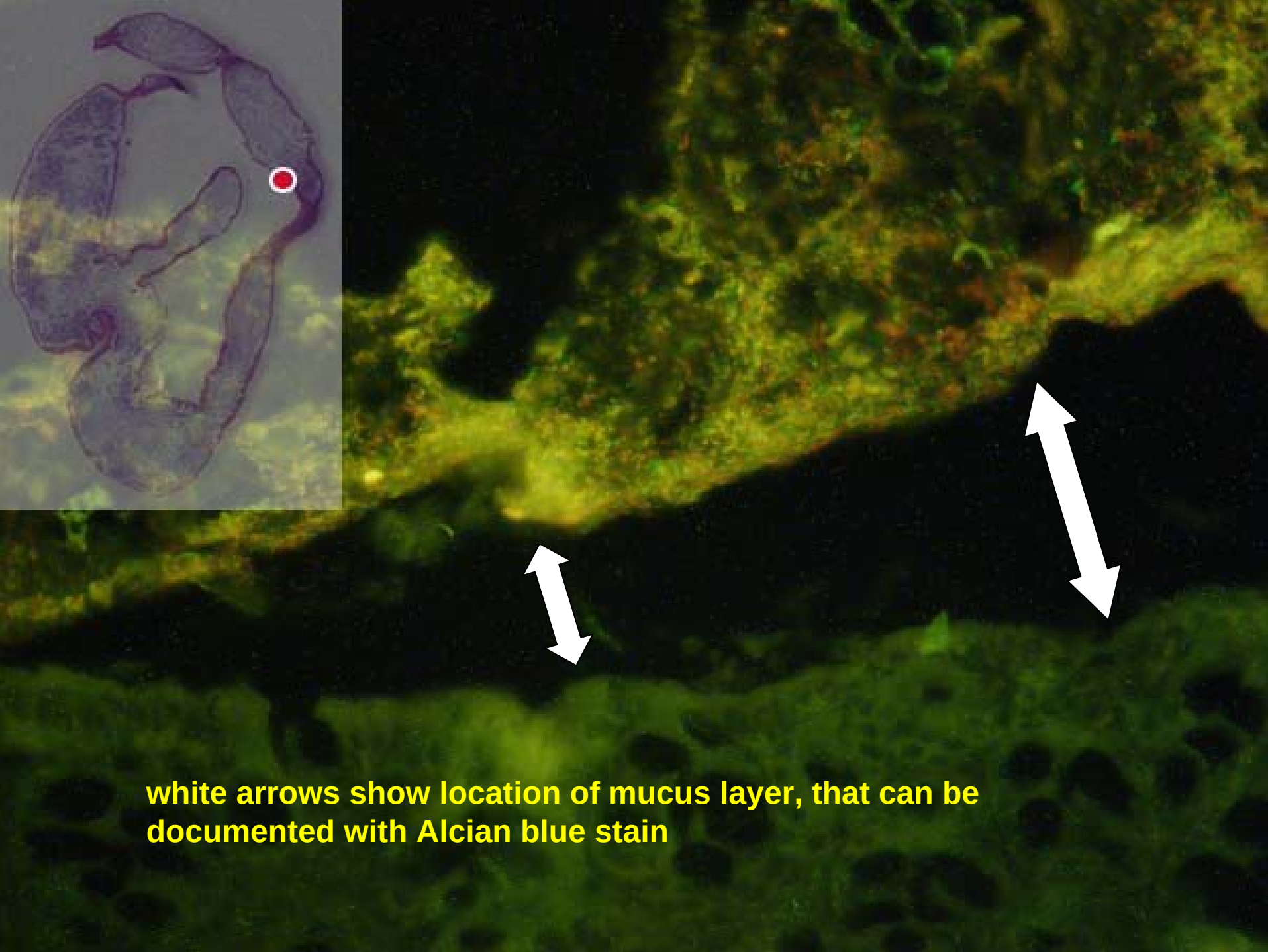


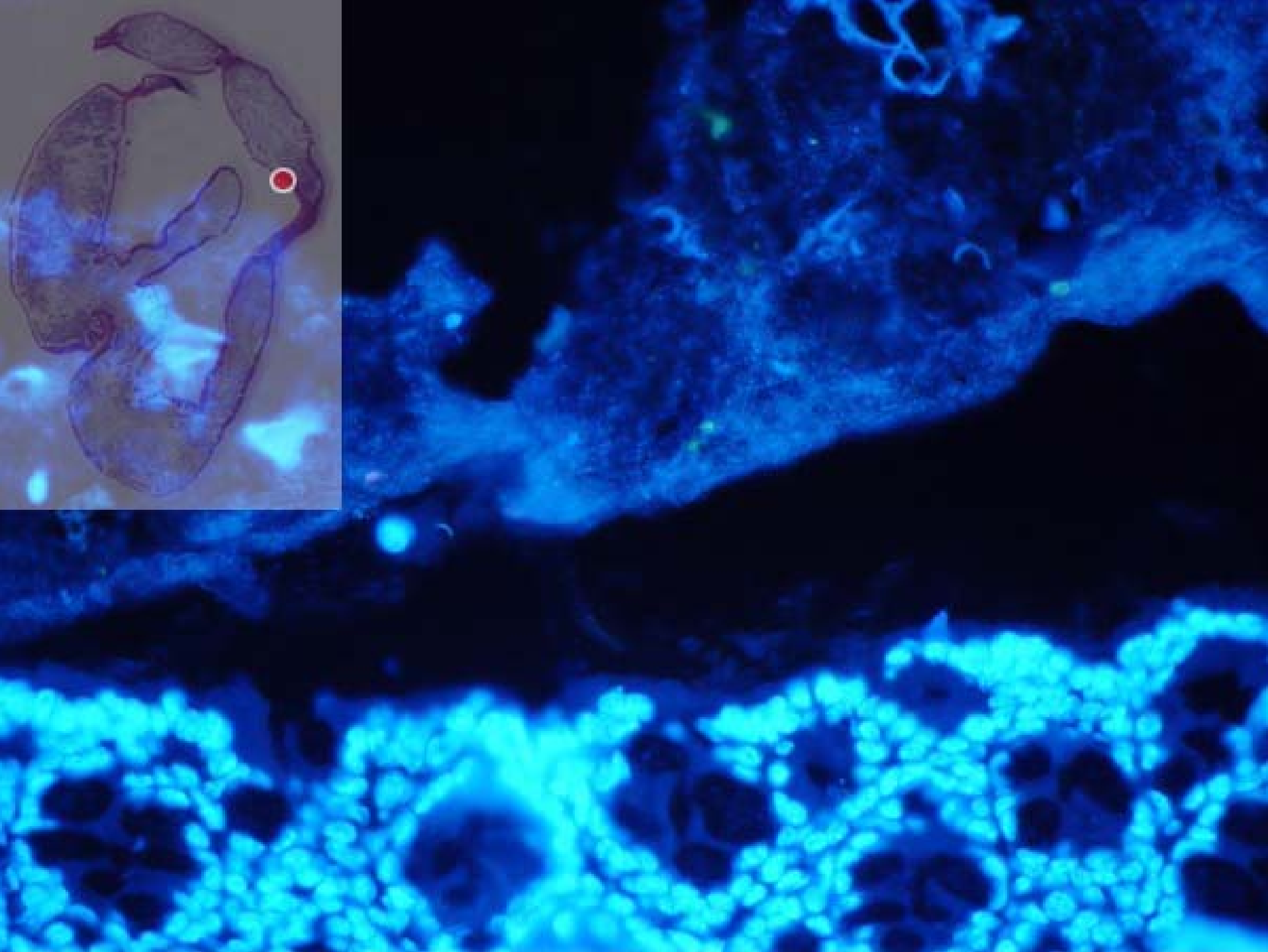


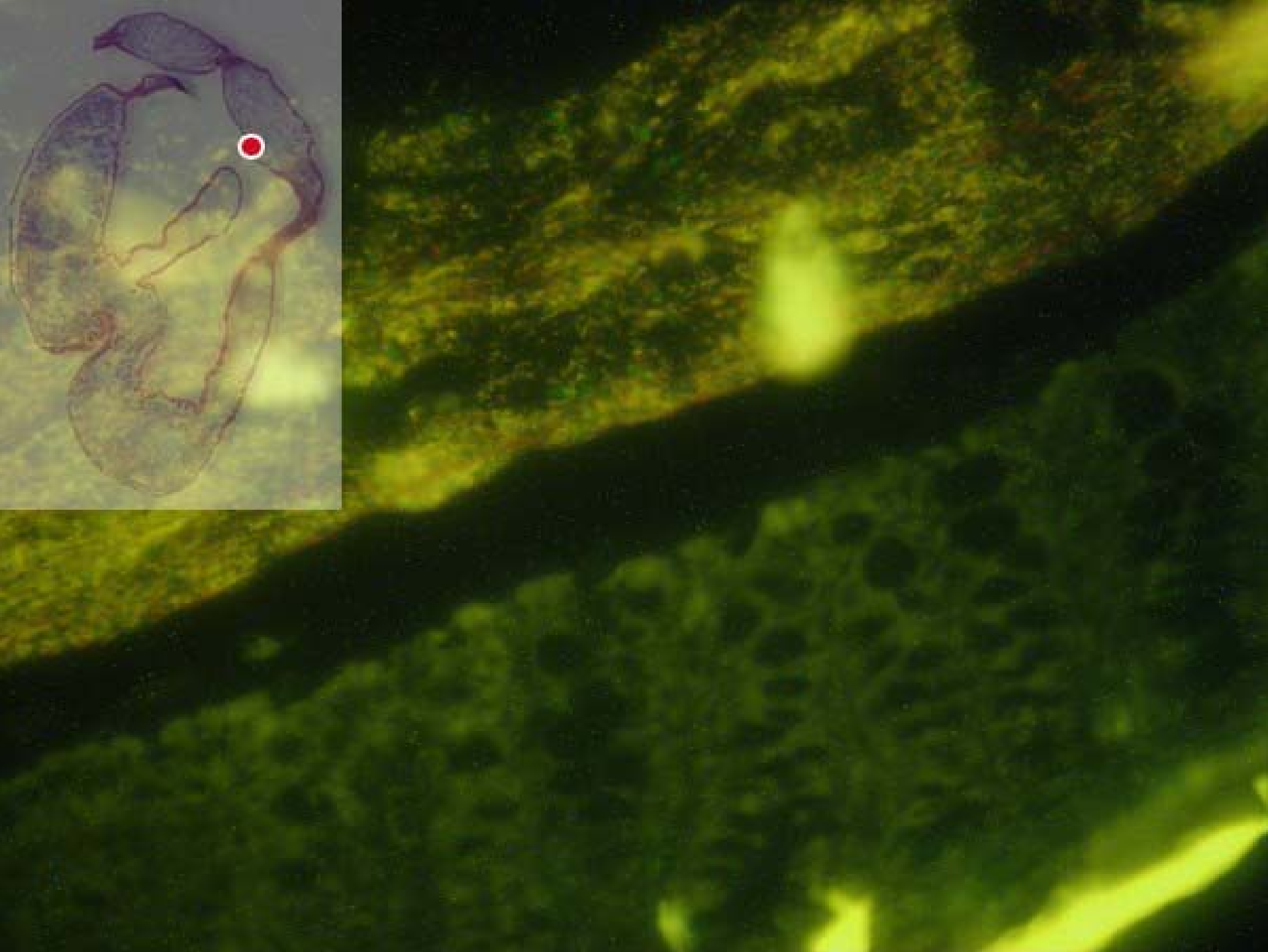


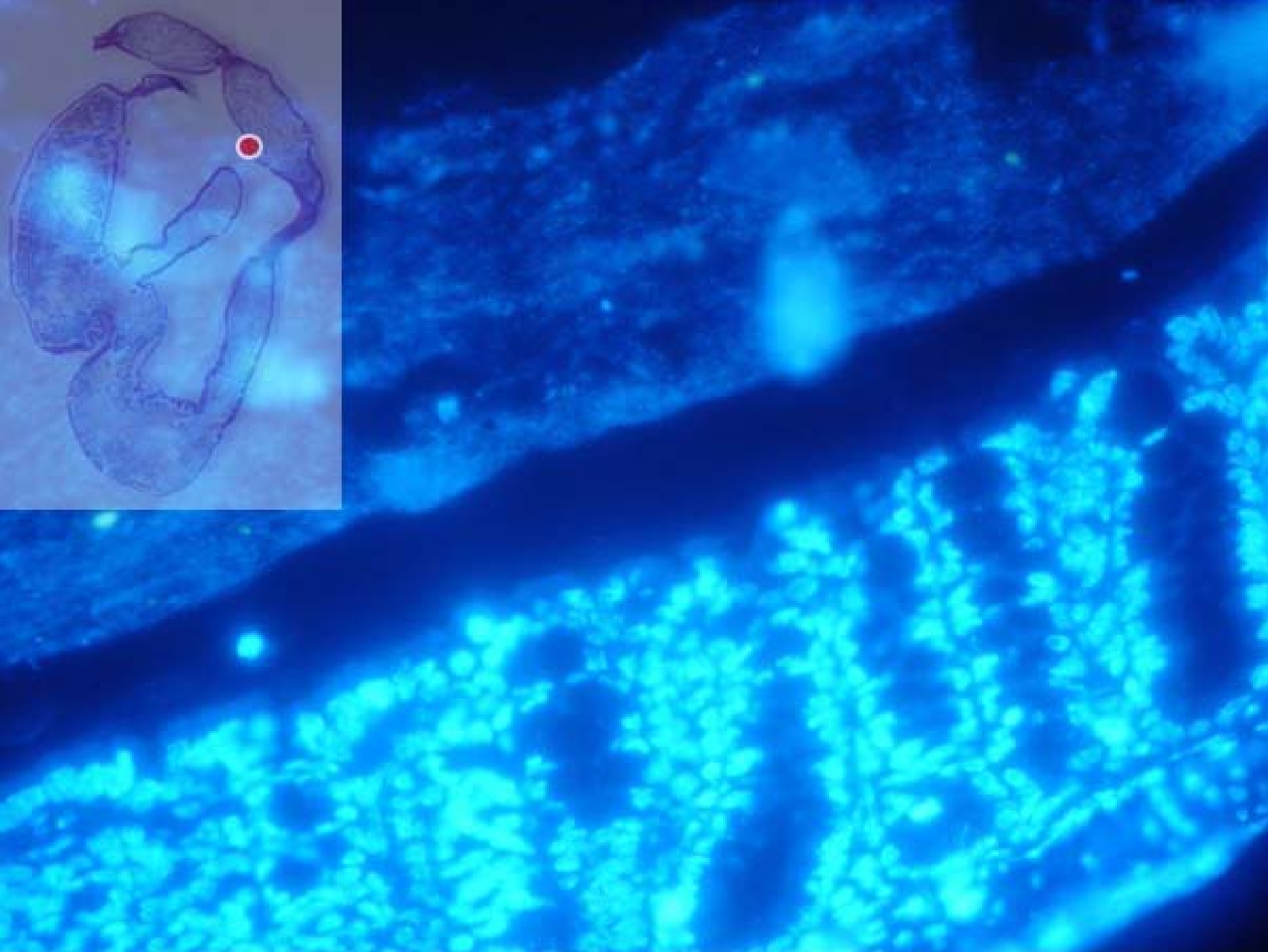
Composition of the interlaced layer

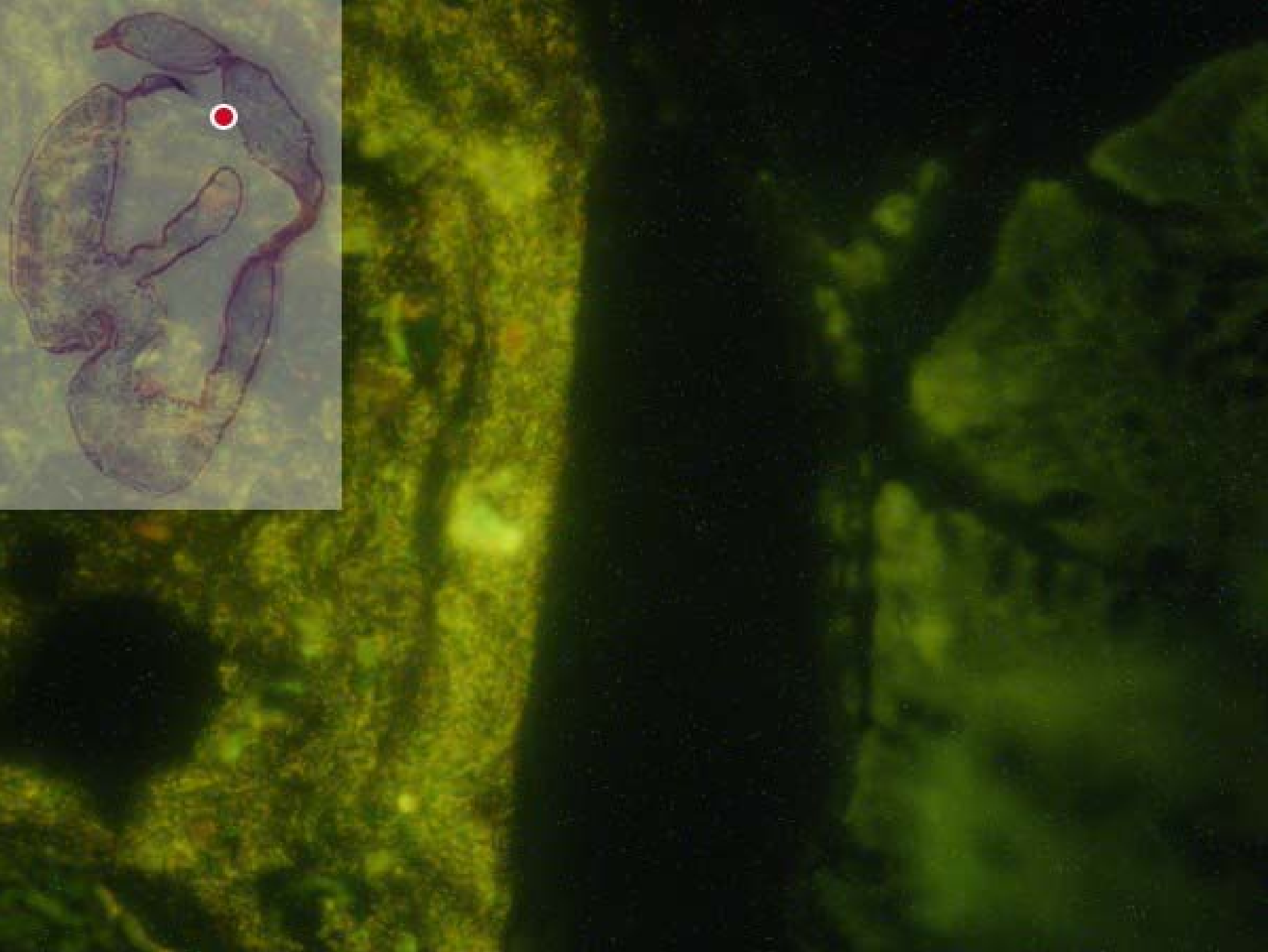
Short rods of *Bacteroides, Enterobacteriaceae, Clostridium difficile, Veillonella* groups have no contact with the colonic wall

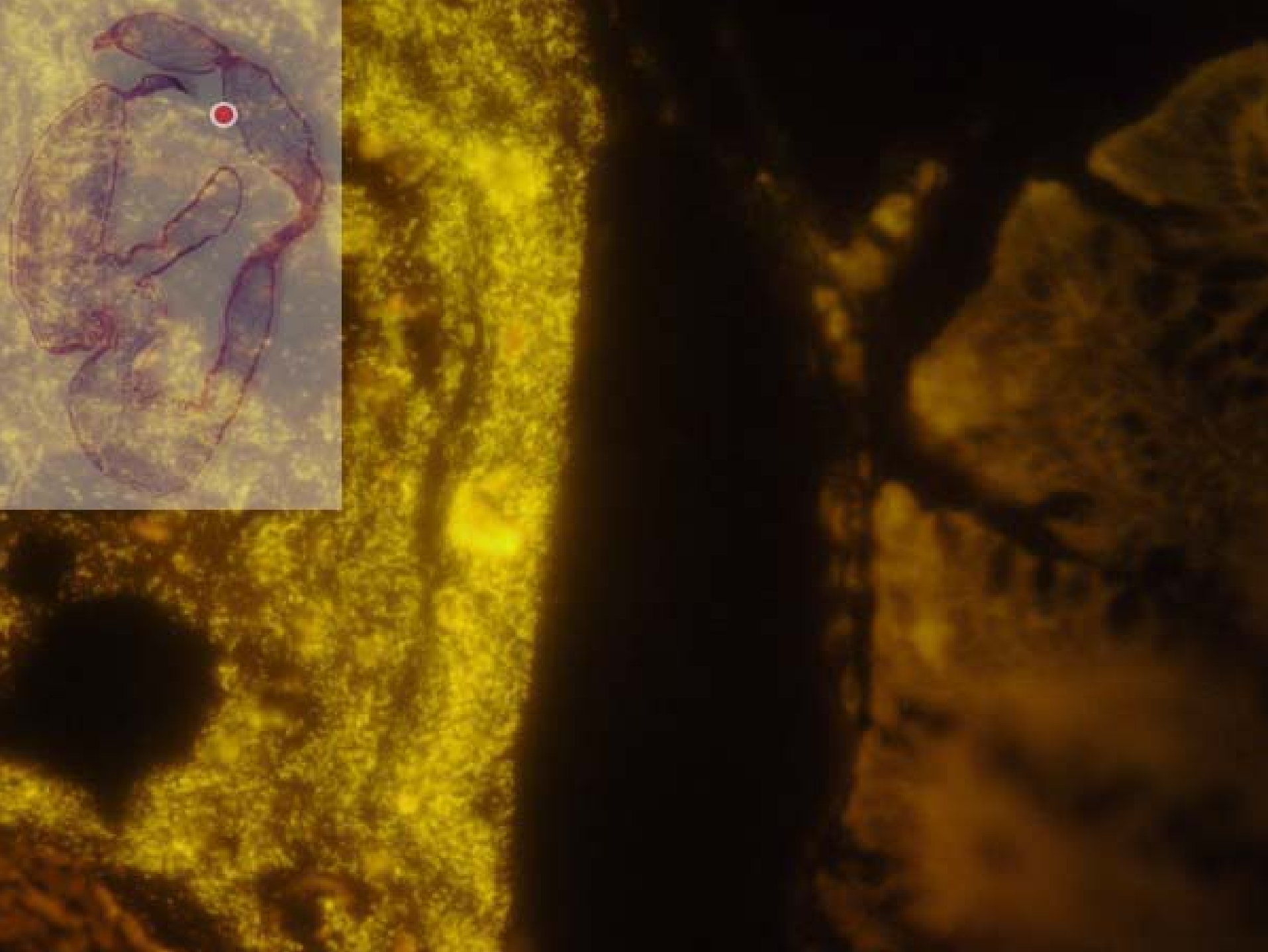

white arrows show location of mucus layer, that can be documented with Alcian blue stain

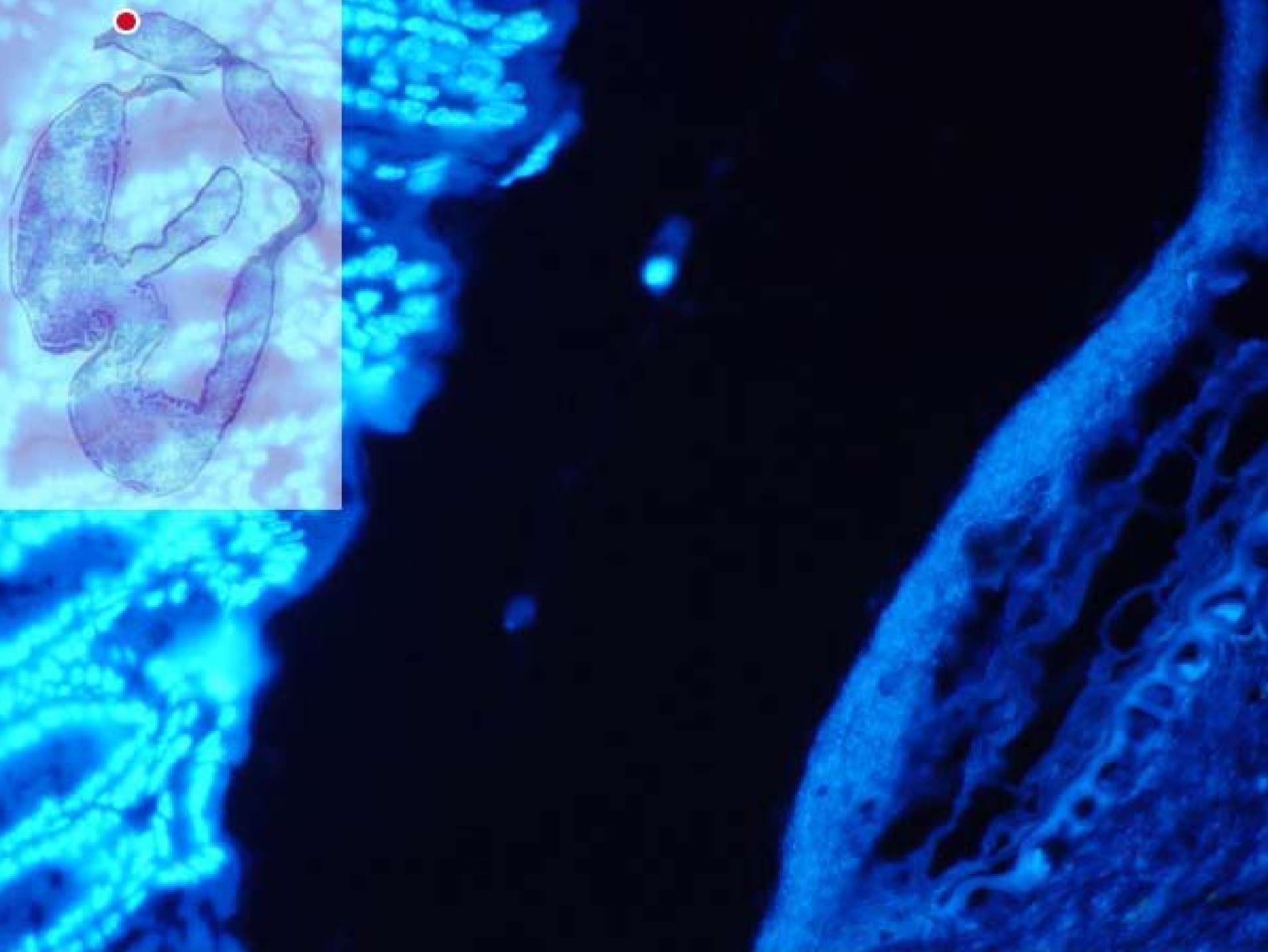


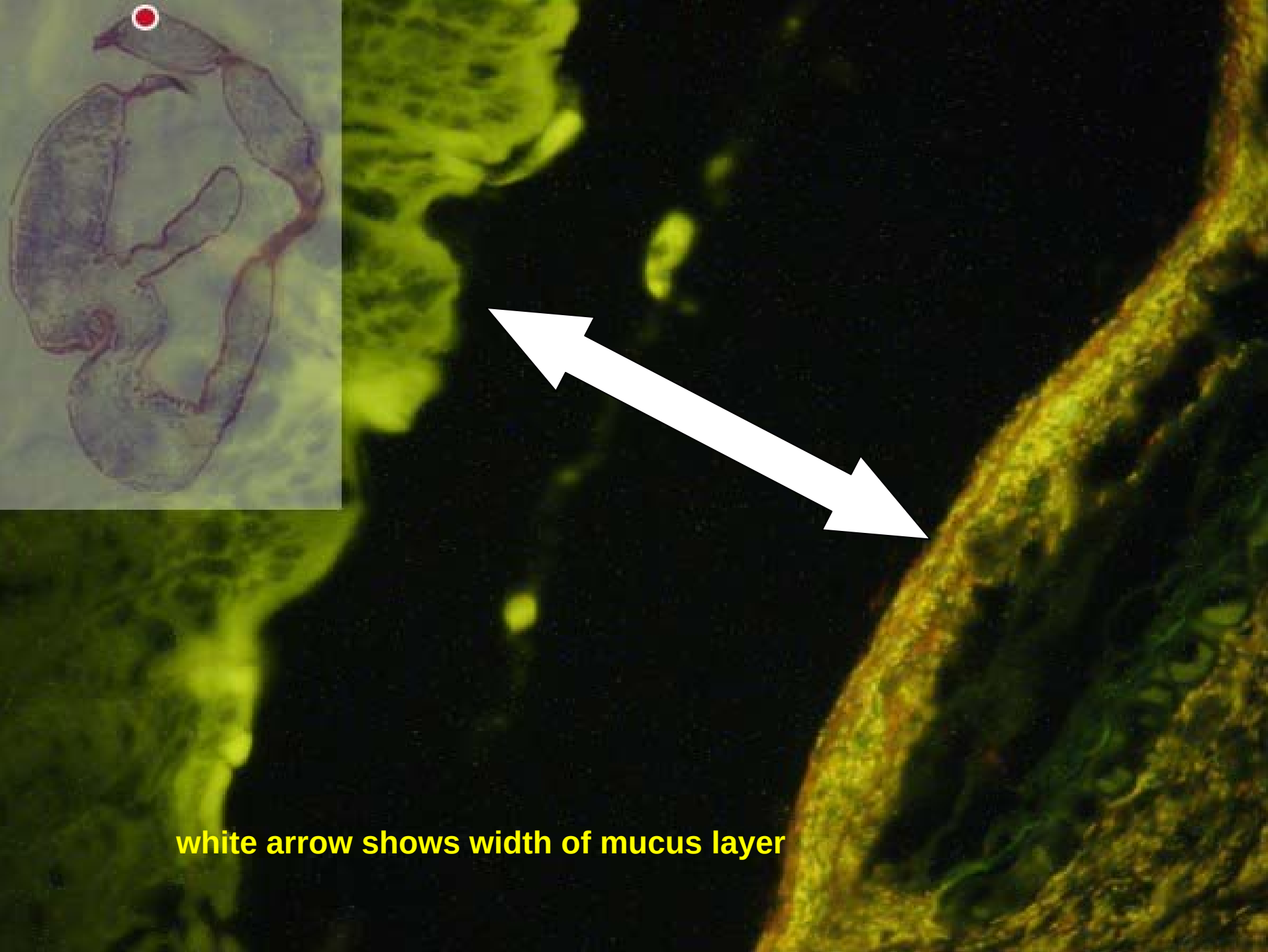




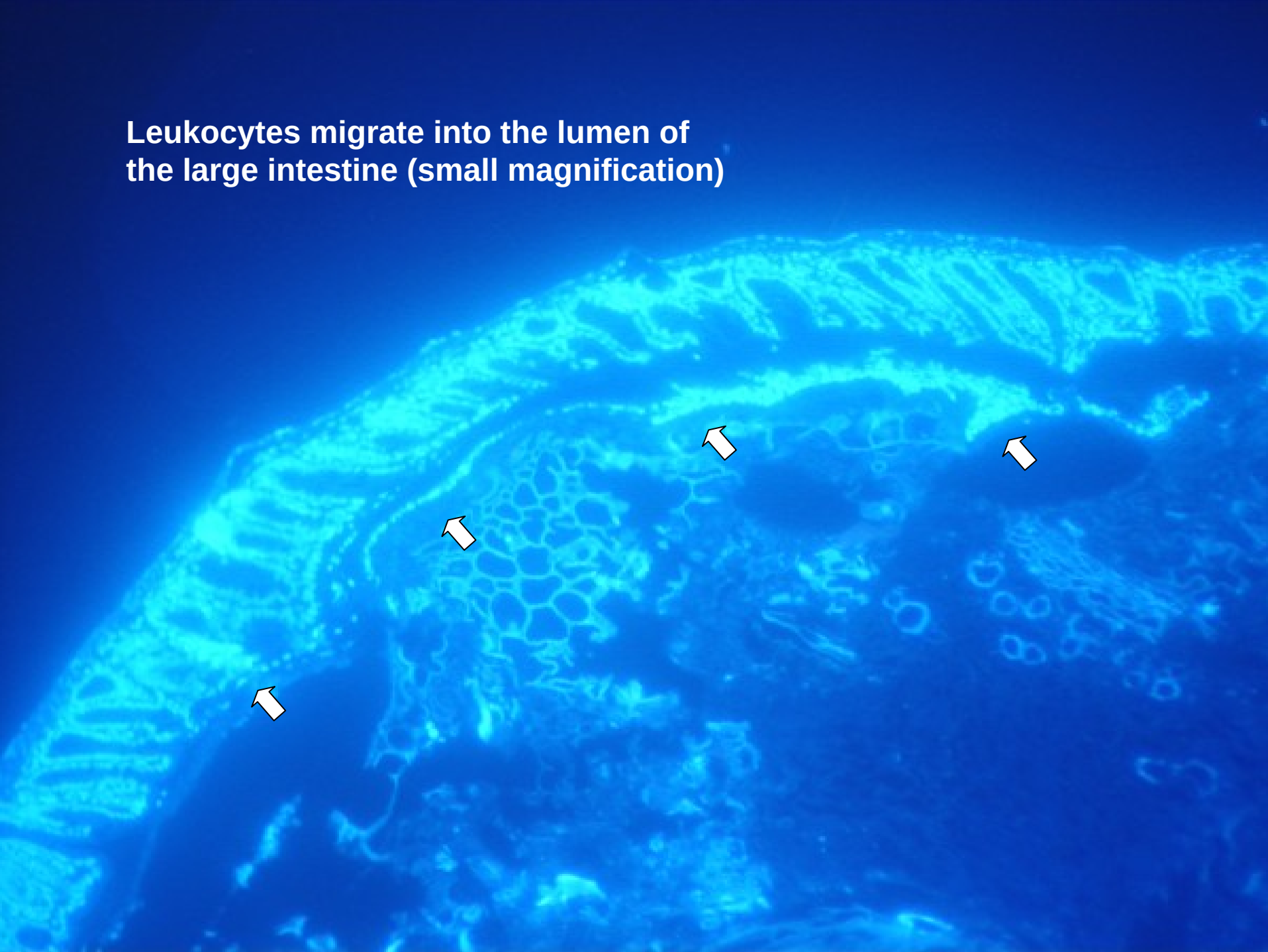


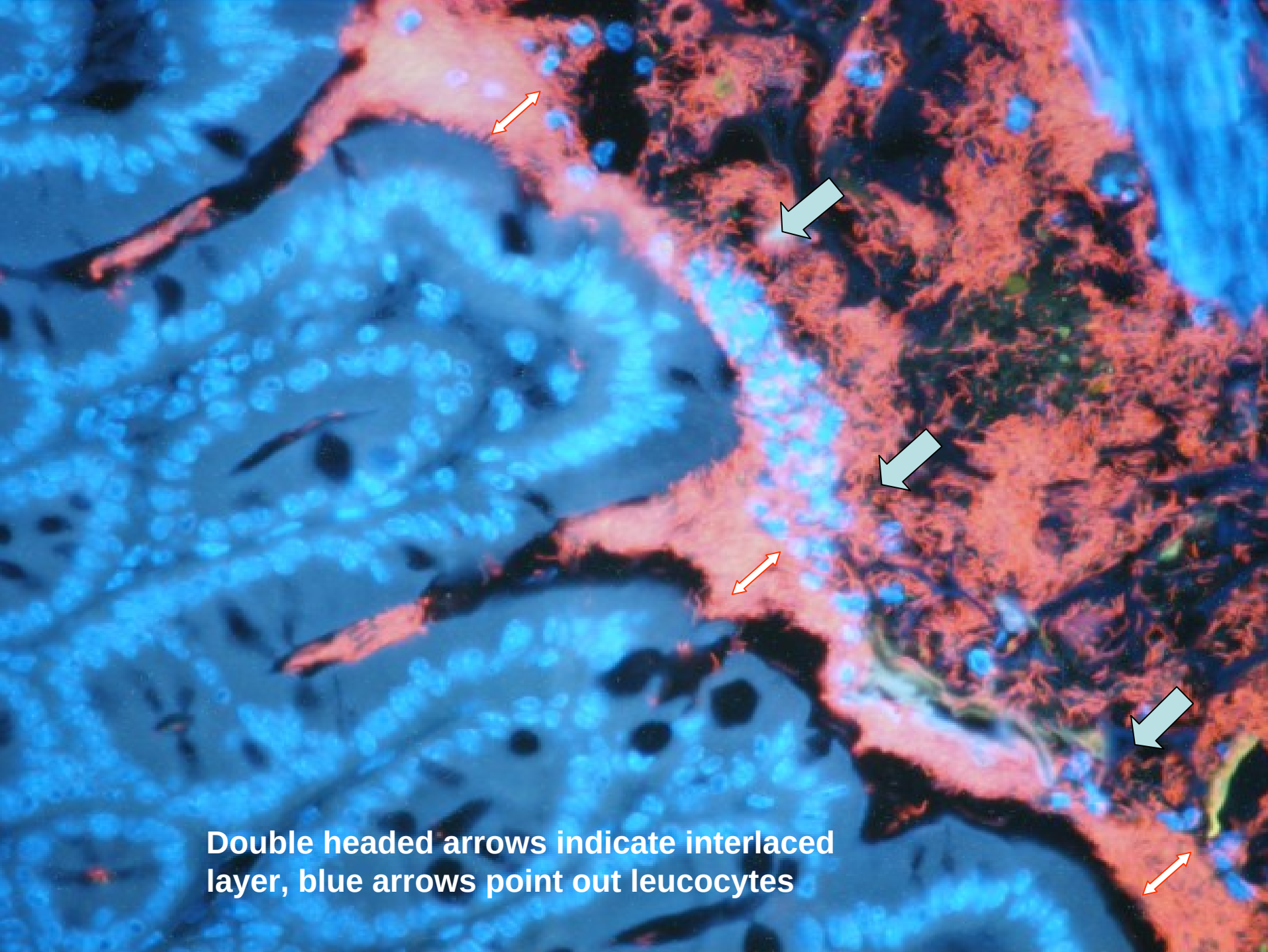


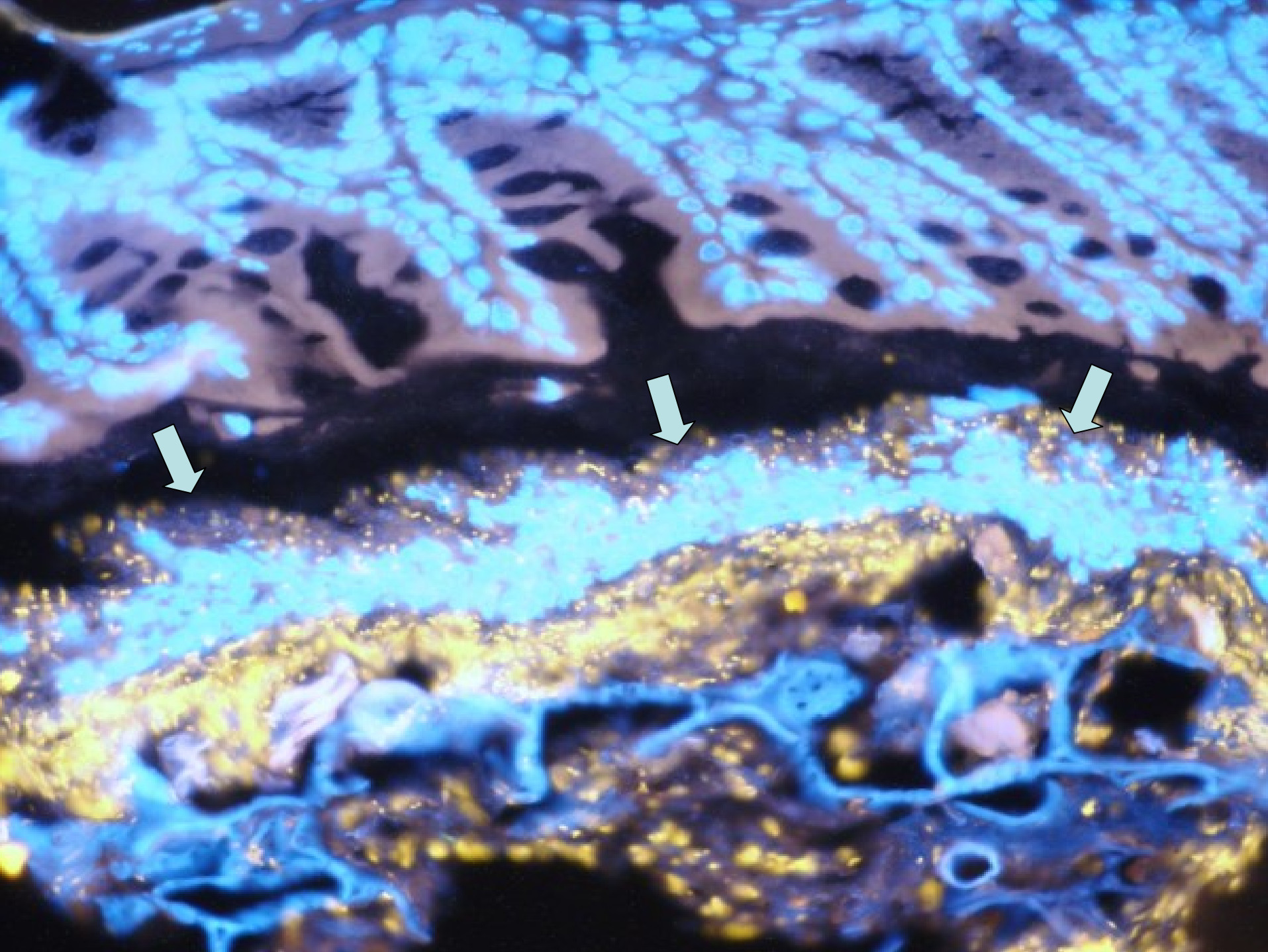


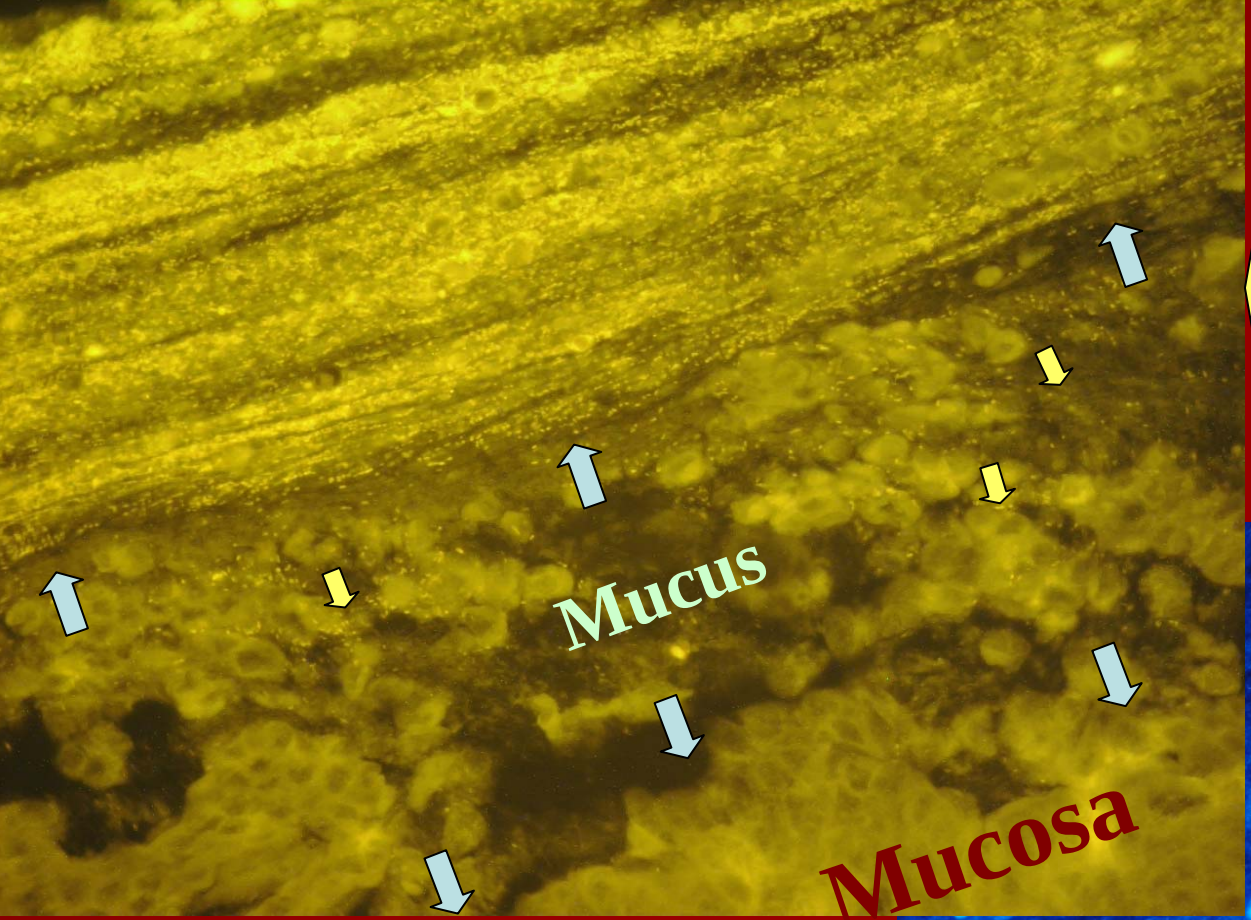

white arrow shows width of mucus layer

Leukocytes migrate into the lumen of the large intestine (small magnification)

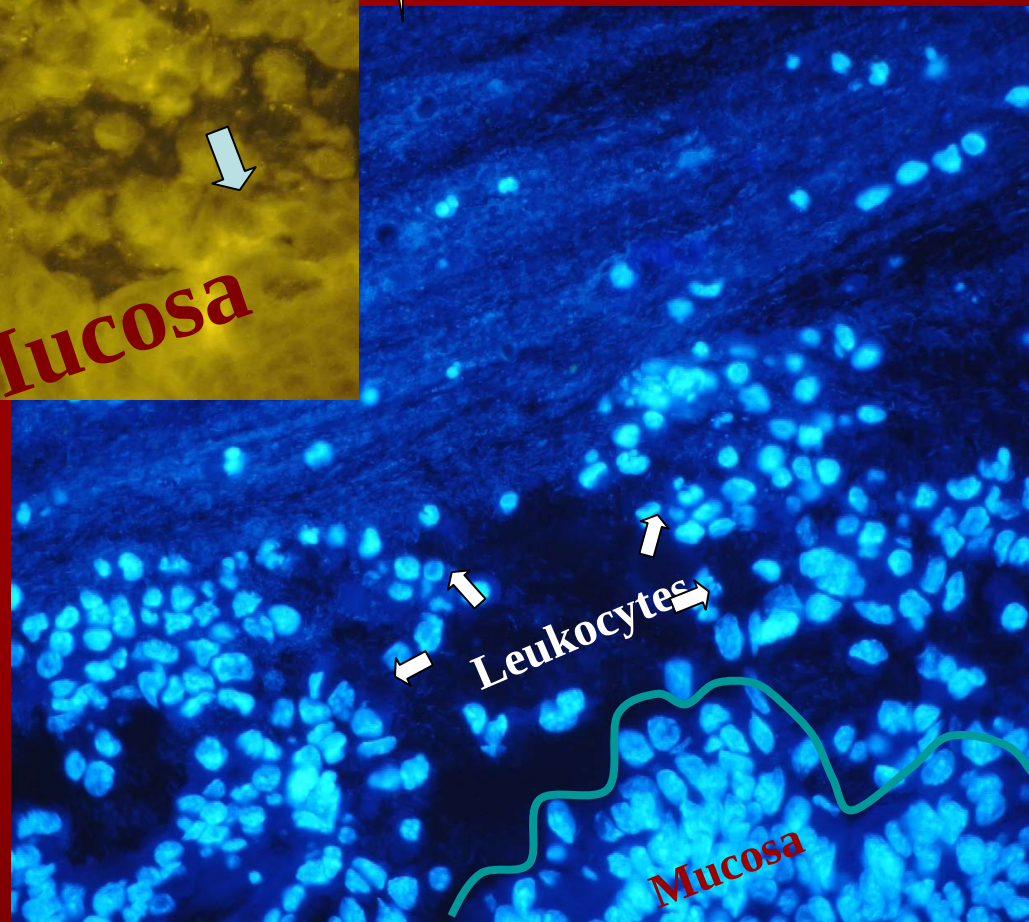



Double headed arrows indicate interlaced layer, blue arrows point out leucocytes

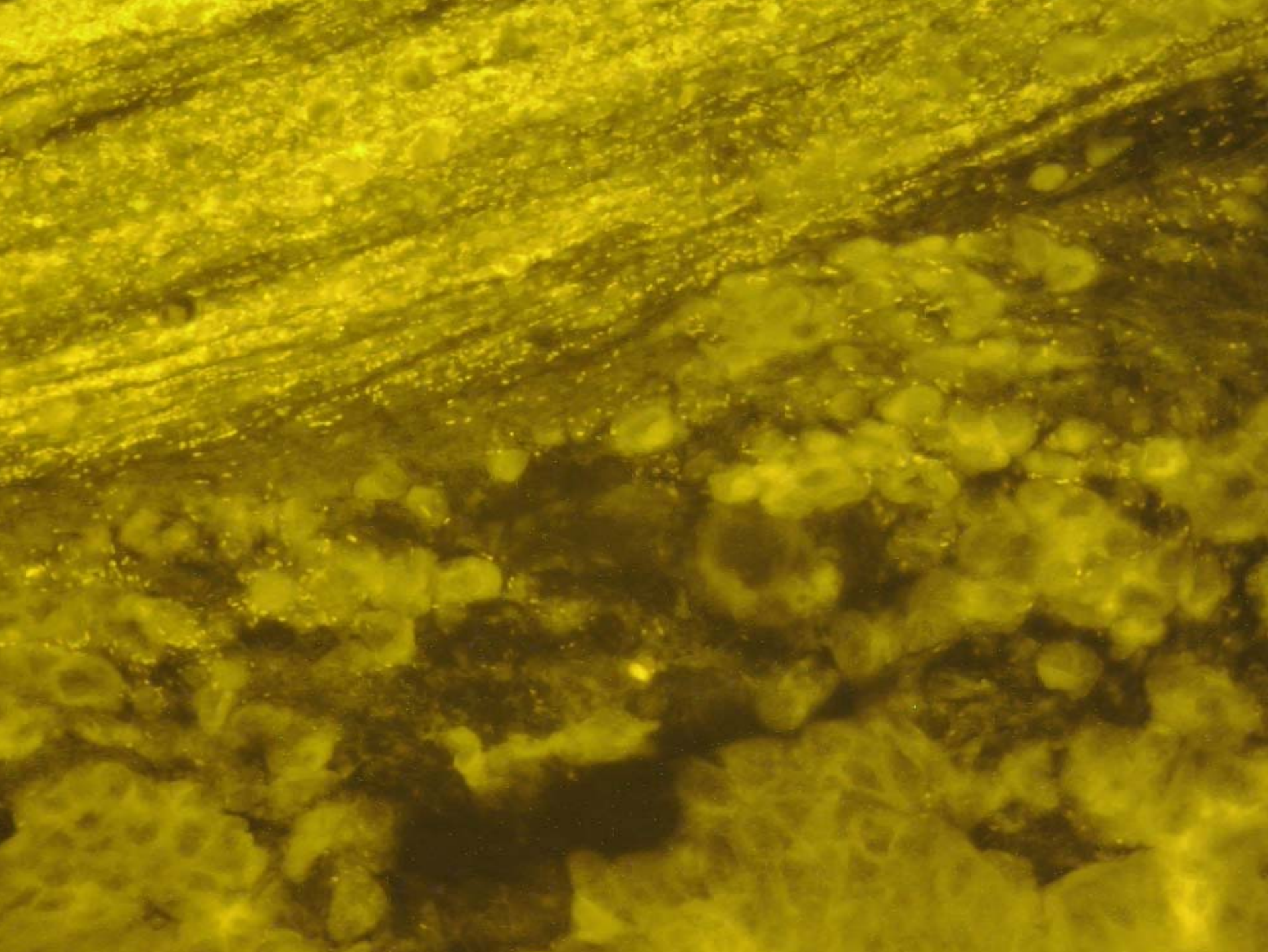


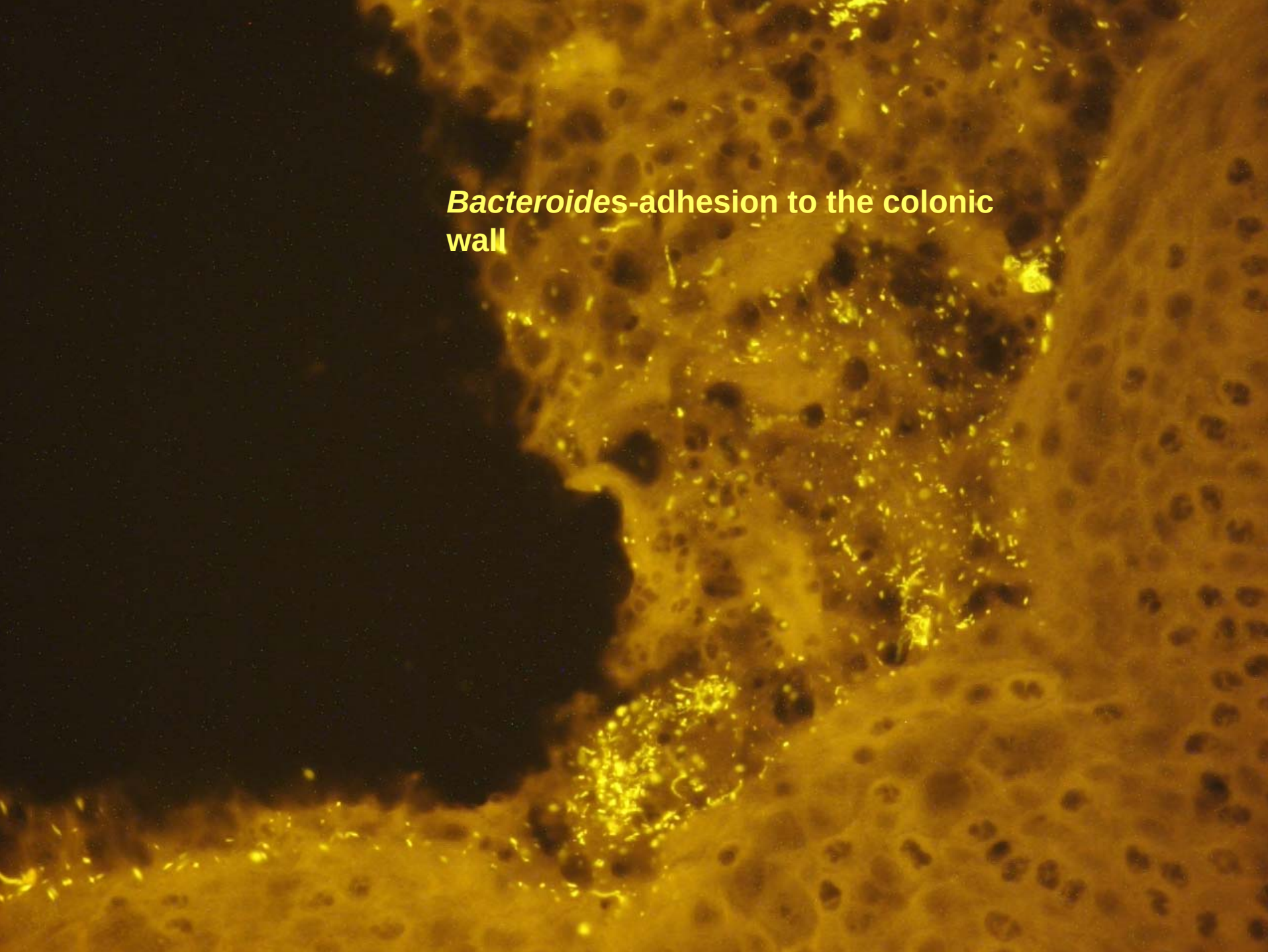


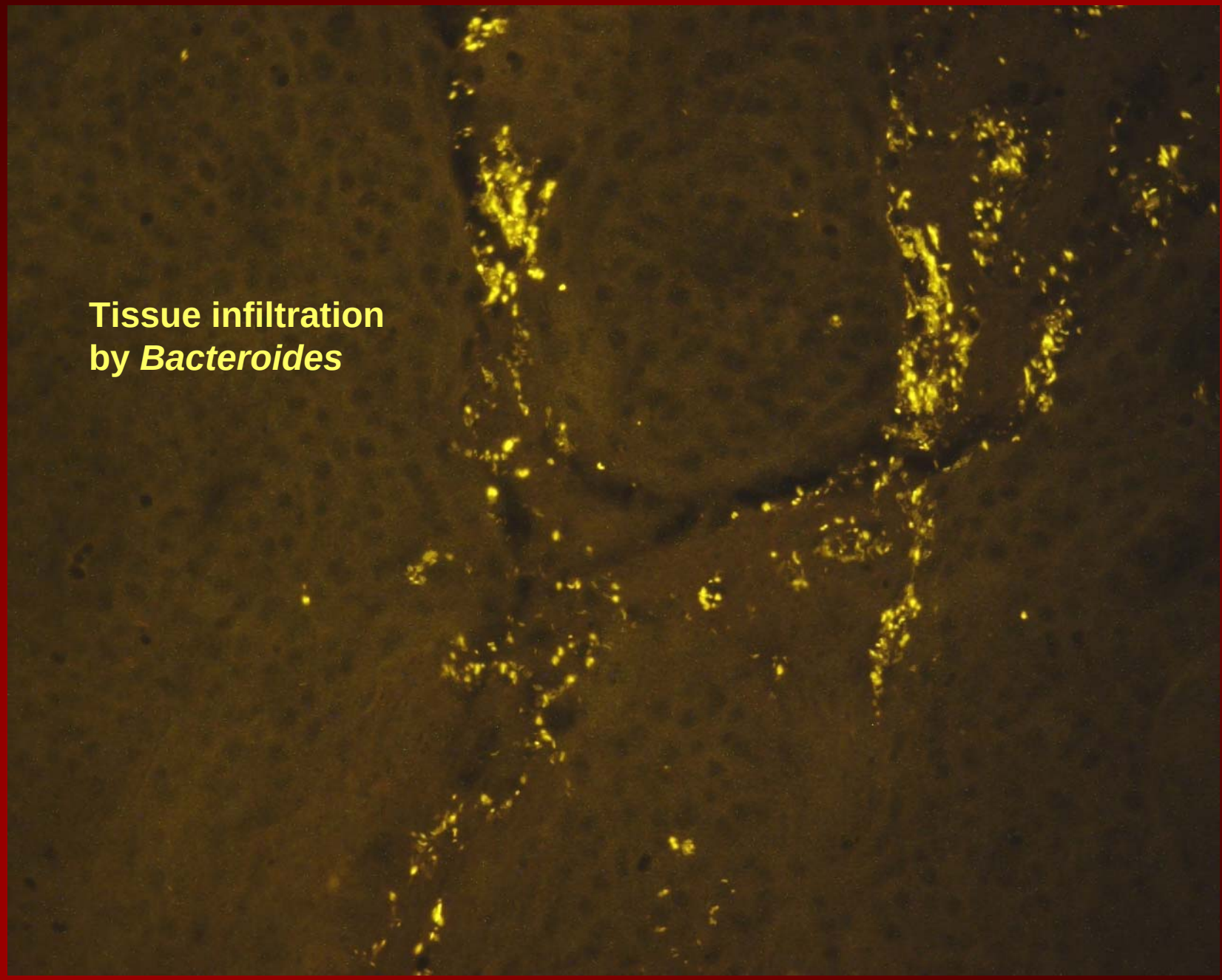
Bacteroides **crosses mucus**



The same microscopic field in DAPI shows leukocytes (large blue nuclei) migrating in mucus and hindering *Bacteroides* movement towards mucosa, normally only single leukocytes are present in mucus




Bacteroides-adhesion to the colonic wall

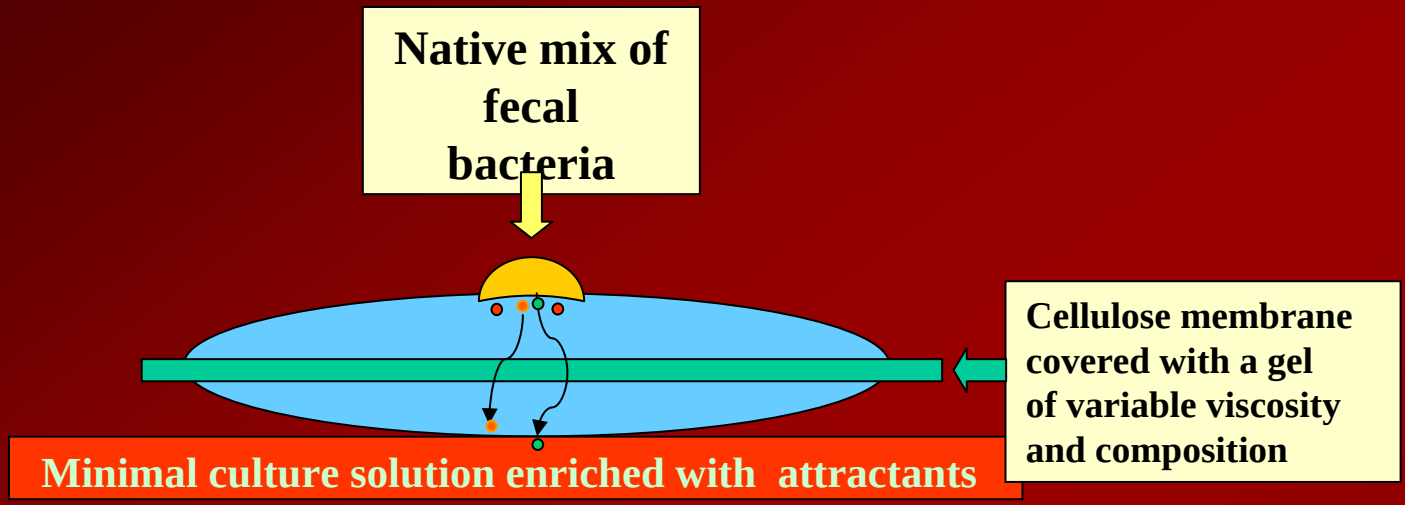

Tissue infiltration
by *Bacteroides*

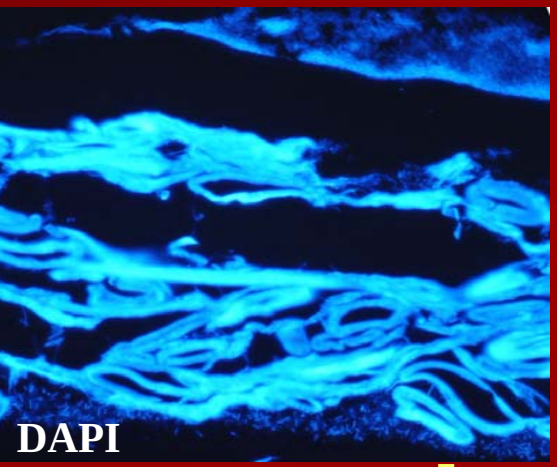
How can we explain differences in distribution of bacteria along the murine colonic wall?

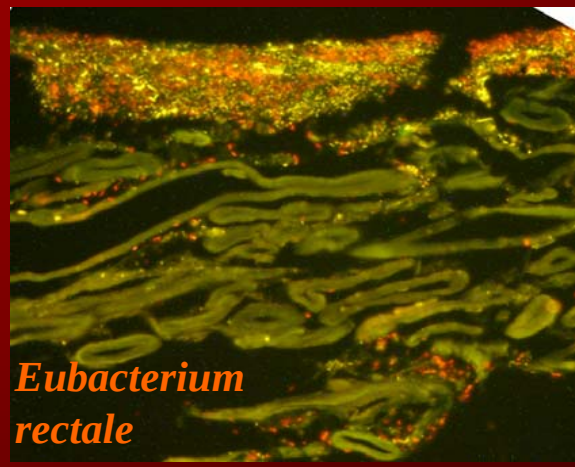
A full set of figures is available at

www.charite.de/arbmkl

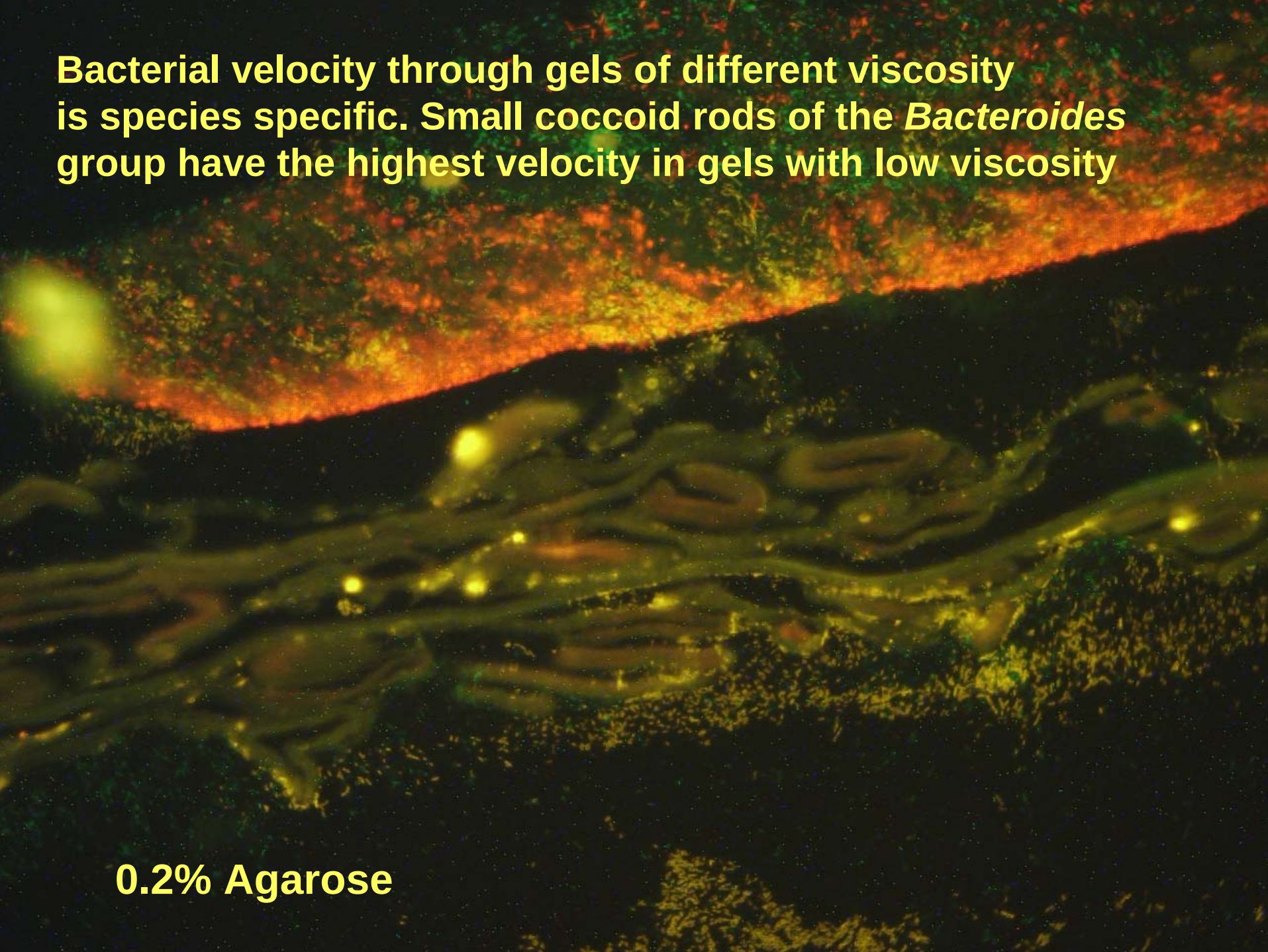
Mucus simulation in vitro

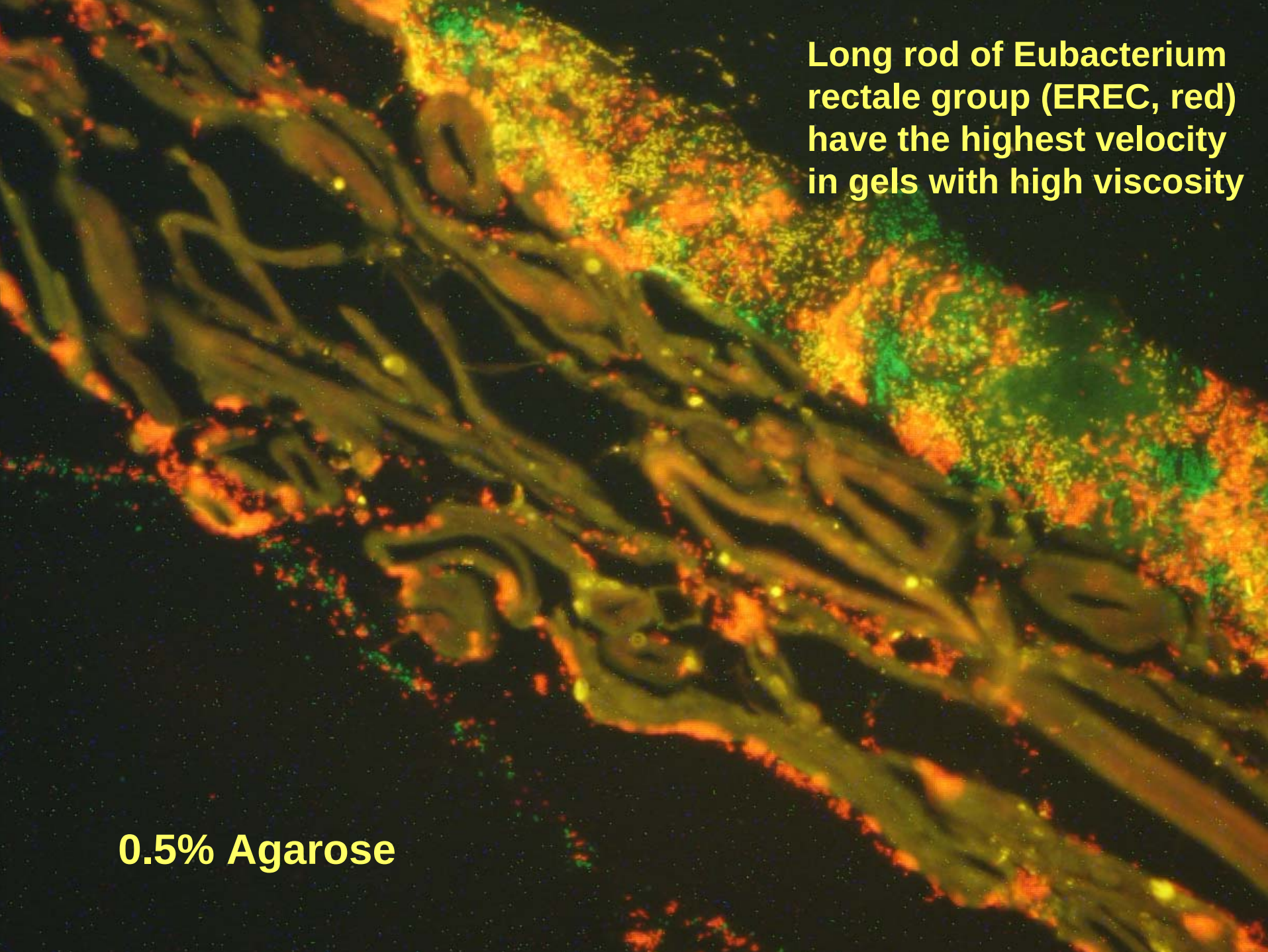




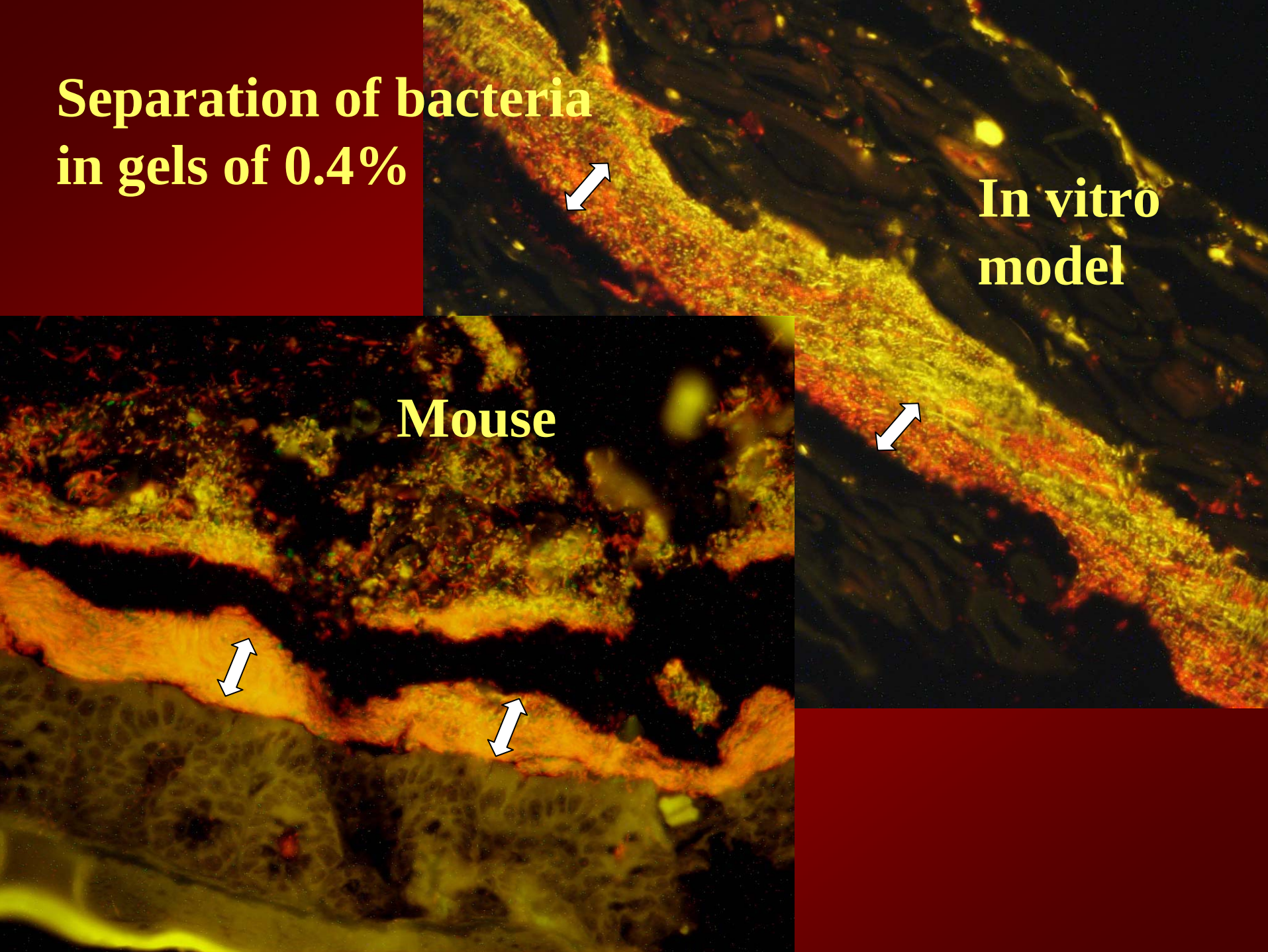


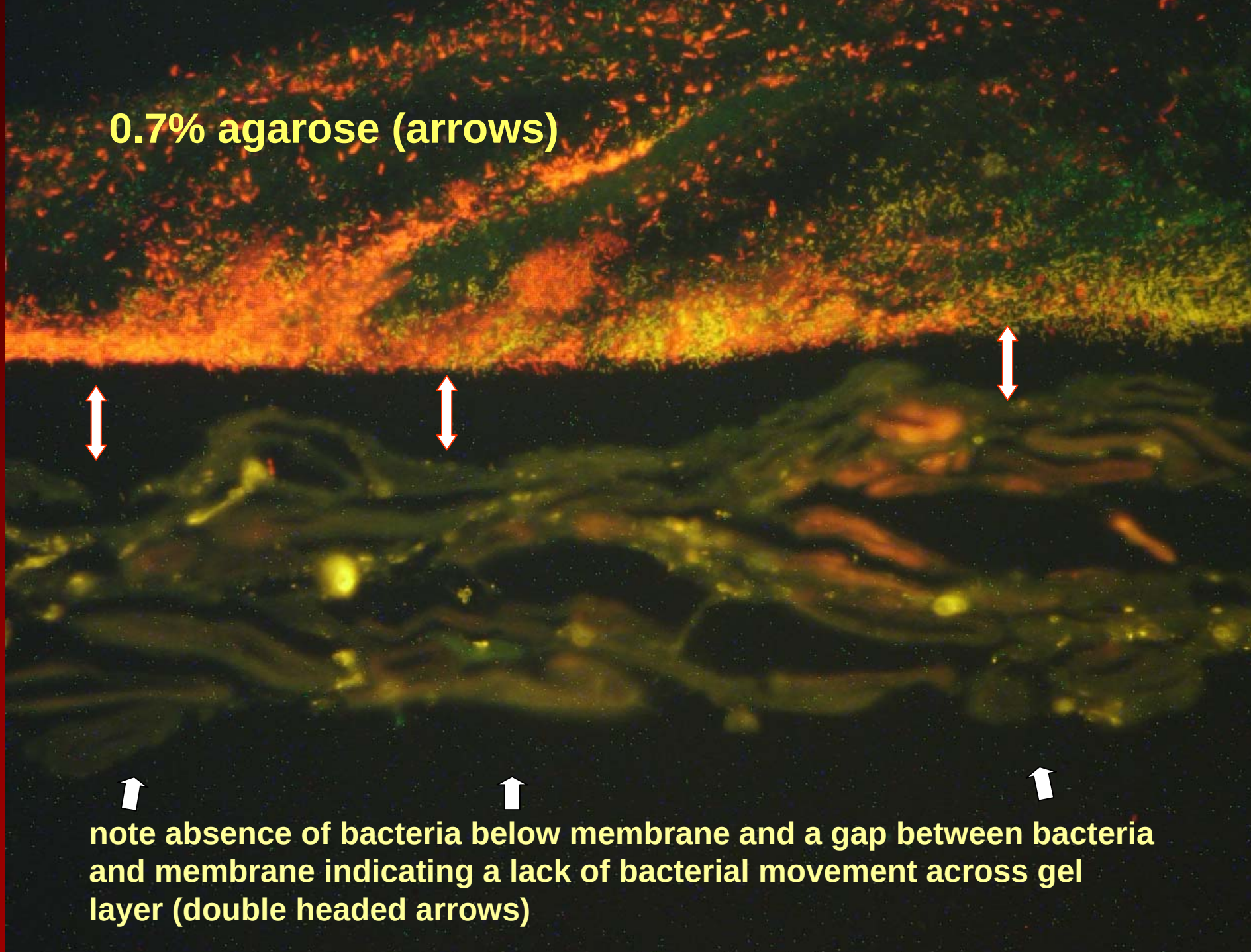
Examples of mobility


Bacterial velocity through gels of different viscosity
is species specific. Small coccoid rods of the *Bacteroides*
group have the highest velocity in gels with low viscosity
0.2% Agarose

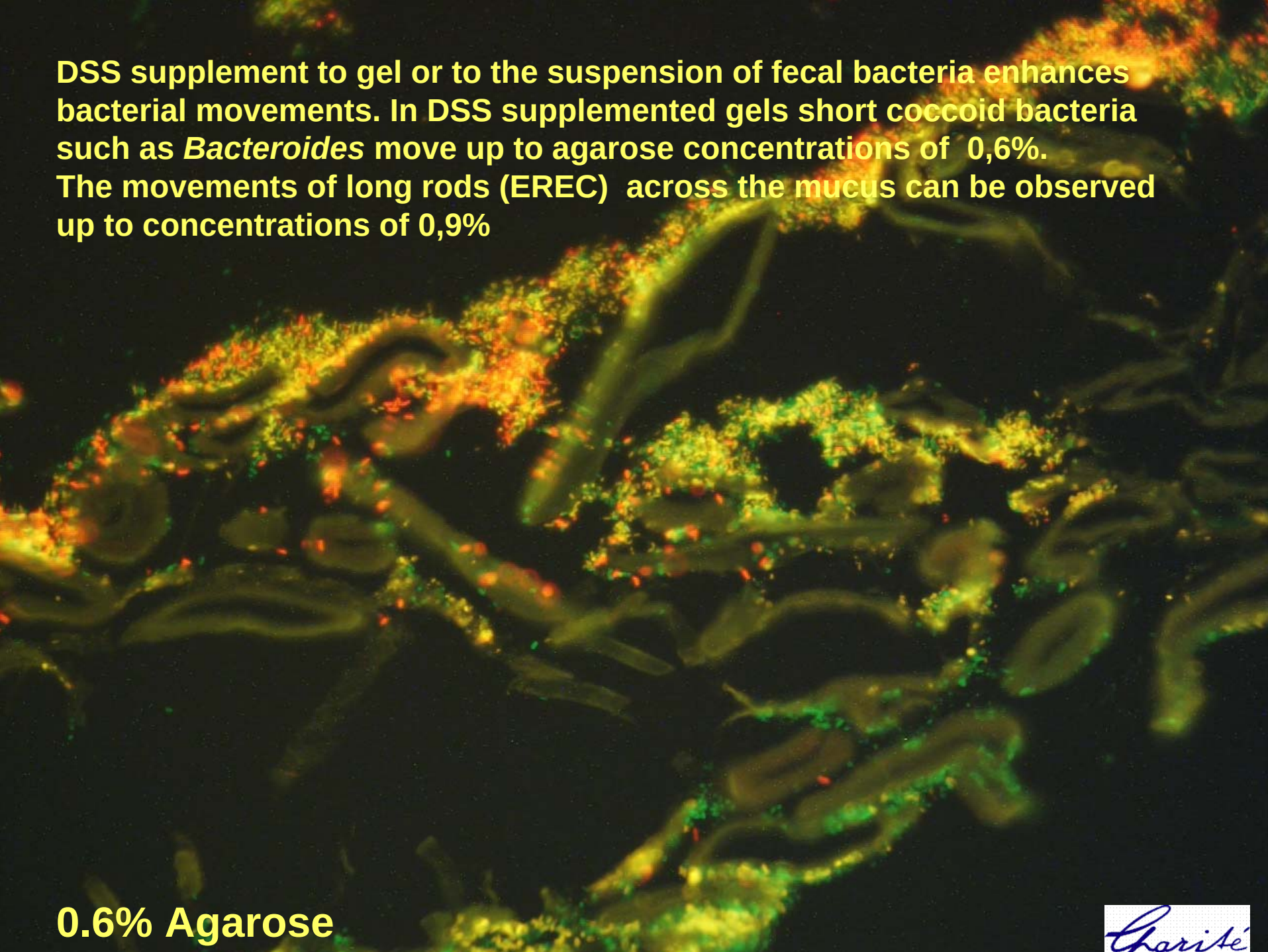

Long rod of Eubacterium rectale group (EREC, red) have the highest velocity in gels with high viscosity
0.5% Agarose

Separation of bacteria in gels of 0.4%



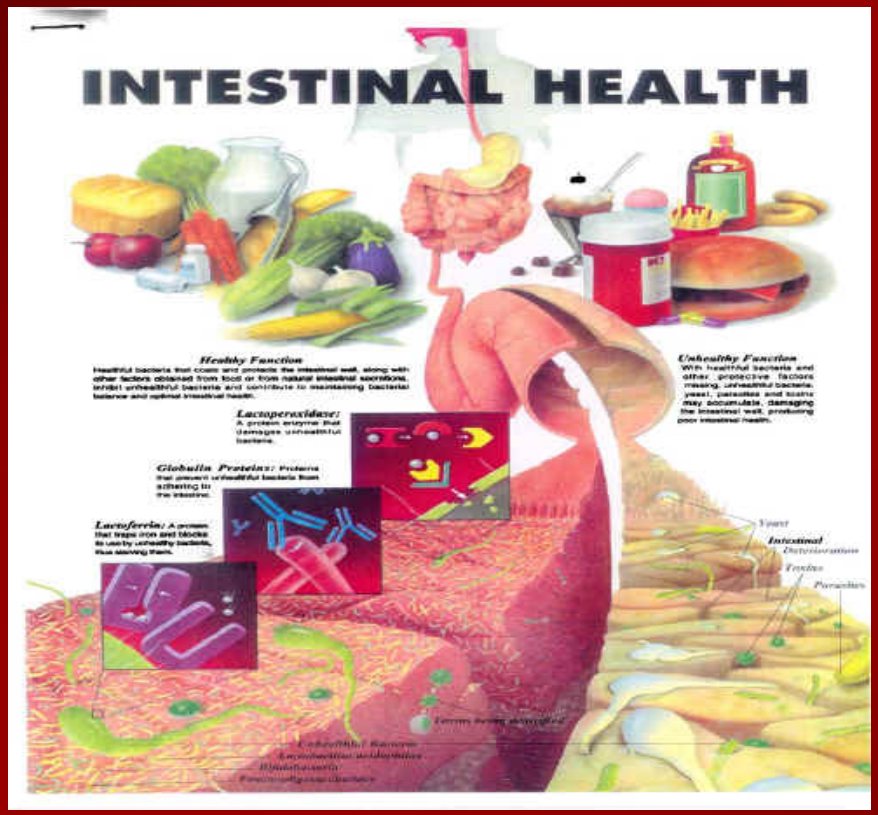


note absence of bacteria below membrane and a gap between bacteria and membrane indicating a lack of bacterial movement across gel layer (double headed arrows)


DSS supplement to gel or to the suspension of fecal bacteria enhances bacterial movements. In DSS supplemented gels short coccoid bacteria such as *Bacteroides* move up to agarose concentrations of 0,6%.
The movements of long rods (EREC) across the mucus can be observed up to concentrations of 0,9%
0.6% Agarose

Tolerance

normal
Flora



Inflammatory Response

Enteral
Pathogens

E. coli

Bacteroides

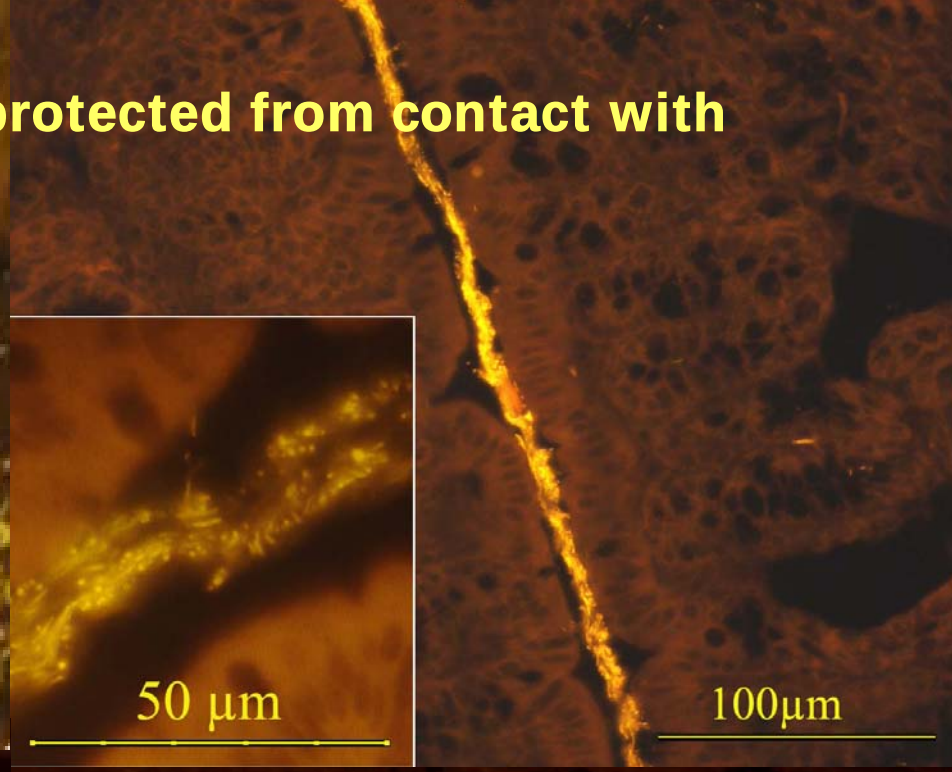
Clostridium difficile

Enterococci

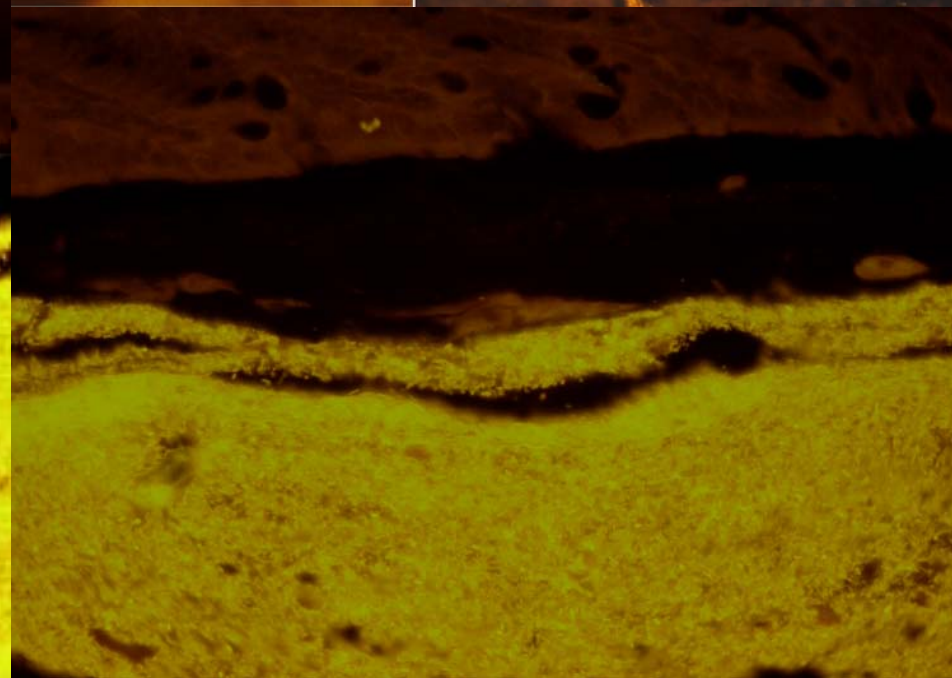
Salmonella

Shigella

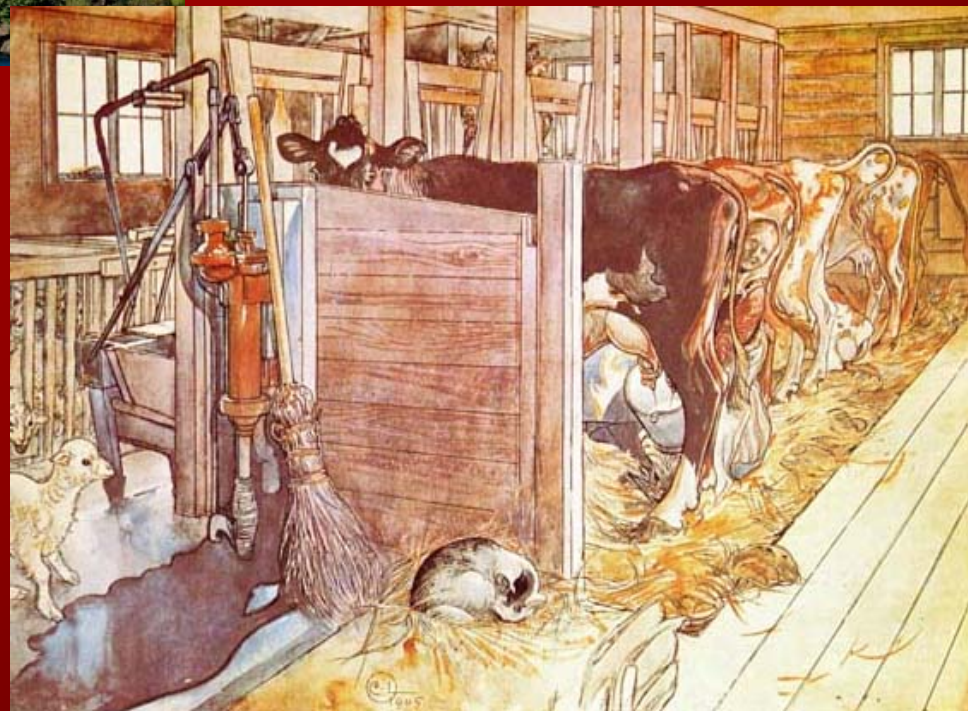
Intestinal mucosa is effectively protected from contact with pathogens through out the gut

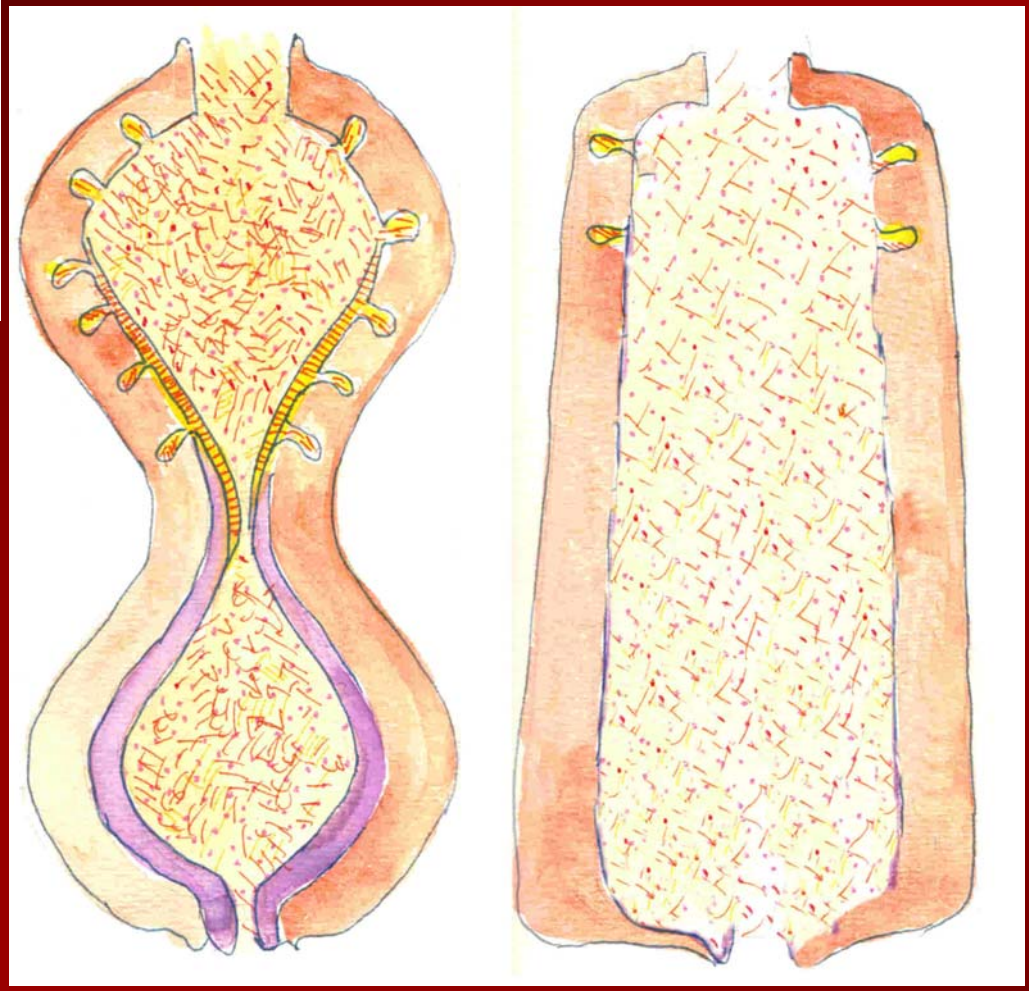


Viscosity of the mucus
Defensins
Antibodies
Interlaced layer or bacterial separation
Leukocyte-patrol within mucus layer



Hygiene hypothesis





Soaps and emulsifying substances make our environment clean. They may however have the same effect on the mucus of man as DSS on the mucus of mouse.

Factors affecting mucus barrier

Exogenic:

Detergents:

Bacterial virulence:

Glutens as natural emulsifiers need bacteria to be pathogenetic

Smoking

Endogenic:

Bile acids are normally fully resorbed in ileum but lead to diarrhea if arrive in large intestine

Defensins, Antibodies draining

Probiotics, Prebiotics,

Oligonucleotids Nucleinacidsderivates

Inflammatory response

Genetic

NOD 2 Mutation

EU admitted emulsifiers

E425, Konjak
E432 bis E436, Polysorbat
- E432, Polyoxyethylen-sorbitan-monolaurat (Polysorbat 20)
- E433, Polyoxyethylen-sorbitan-monooleat (Polysorbat 80)
- E434, Polyoxyethylen-sorbitan-monopalmitat (Polysorbat 40)
- E435, Polyoxyethylen-sorbitan-monostearat (Polysorbat 60)
- E436, Polyoxyethylen-sorbitan-tristearat (Polysorbat 65)

E440, Pektine, Amidiertes Pektin
E442, Ammoniumsalze von Phosphatidsäuren
E444, Saccharose-acetat-isobutyrat
E445, Glycerinester aus Wurzelharz/Kolophonester
E450 bis E452, Phosphate
E459, Beta-Cyclodextrin
E460 bis E469 Cellulose und Celluloseverbindungen
- E460, Cellulose, Mikrokristalline Cellulose, Cellulosepulver
- E461, Methylcellulose
- E463, Hydroxypropylcellulose
- E464, Hydroxypropylmethylcellulose
- E465, Ethylmethylcellulose
- E466, Carboxymethylcellulose, Natriumcarboxymethylcellulose
- E468, Vernetzte Natrium-Carboxymethylcellulose
- E469, Enzymatisch hydrolysierte-Carboxymethylcellulose

E470a und E470b, Salze von Speisefettsäuren
- E470a, Natrium-, Kalium- und Calciumsalze von Speisefettsäuren
- E470b, Magnesiumsalze von Speisefettsäuren

E471 bis E472f, Mono- und Diglyceride von Speisefettsäuren
- E471, Mono- und Diglyceride von Speisefettsäuren, Monoglycerid
- E472a, Essigsäureester von Mono- und Diglyceriden von Speisefettsäuren
- E472b, Milchsäureester von Mono- und Diglyceriden von Speisefettsäuren
- E472c, Citronensäureester von Mono- und Diglyceriden von Speisefettsäuren
- E472d, Weinsäureester von Mono- und Diglyceriden von Speisefettsäuren
- E472e, Mono- und Diacetylweinsäureester von Mono- und Diglyceriden von Speisefettsäuren
- E472f, Gemischte Essig- und Weinsäureester von Mono- und Diglyceriden von Speisefettsäuren

E473, Zuckerester von Speisefettsäuren
E474, Zuckerglyceride
E475, Polyglycerinester von Speisefettsäuren, Polyglycerinester
E476, Polyglycerin-Polyricinoleat
E477, Propylenglycolester von Speisefetten
E479, Thermooxidiertes Sojaöl mit Mono- und Diglyceriden von Speisefettsäuren
E481 bis E483, Natriumstearoyl-2-lactylat, Calciumstearoyl-2-lactylat, Stearyltartrat
E491bis E495, Stearin- und Palmitatverbindungen
E491 Sorbitanmonostearat

Conclusions:

The intestinal wall is protected from contact with potentially harmful bacterial groups such as *Bacteroides, Enterobacteriaceae, Enterococci, and Clostridium difficile, d*espite extremely high bacterial concentrations in colon.

A mucus barrier and not the epithelial cell layer is the first line of defense against a variety of enteral pathogens.

Inflammatory bowel disease is a polymicrobial infection that is characterized by a sustained broken mucus barrier with subsequent bacterial migration toward mucosa and proliferation of complex bacterial biofilms on the epithelial surface.

As long as the mucus barrier function is impaired, the inflammatory process cannot successfully clear bacteria from the mucosal surface and is harmful.

The rising incidence of IBD over the last century may result from changes in the types and numbers of bacteria within the intestine, growing bacterial burden, and disturbed mucus barrier function.

Further study of how viscosity, defensins, antibodies, antibiotics, probiotic bacteria, leukocytes, and other factors affect mucus barrier function will allow to identify new ways to prevent, treat ulcerative colitis and Crohn's disease.

Ulcerative colitis and Crohn's disease are curable