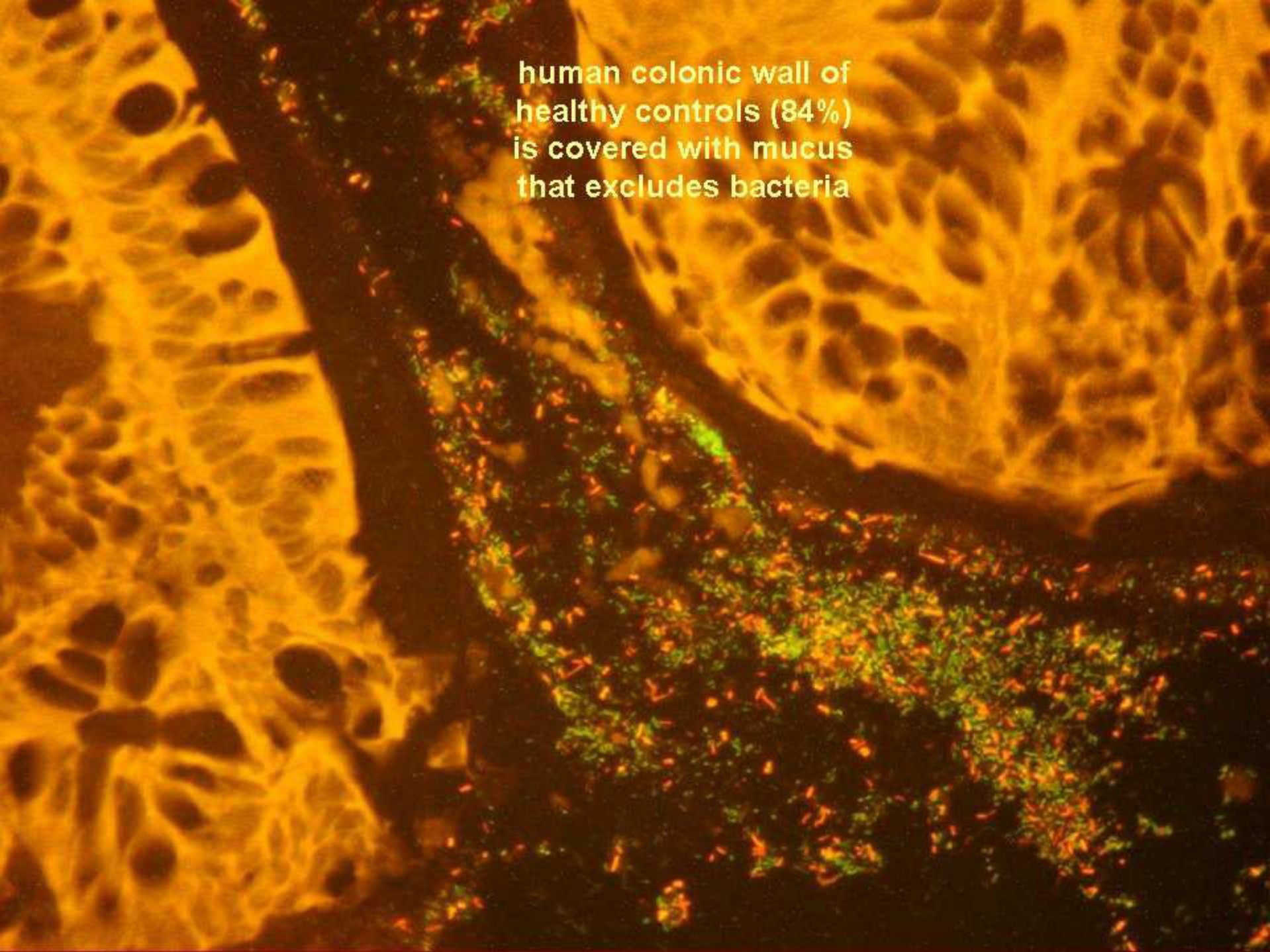
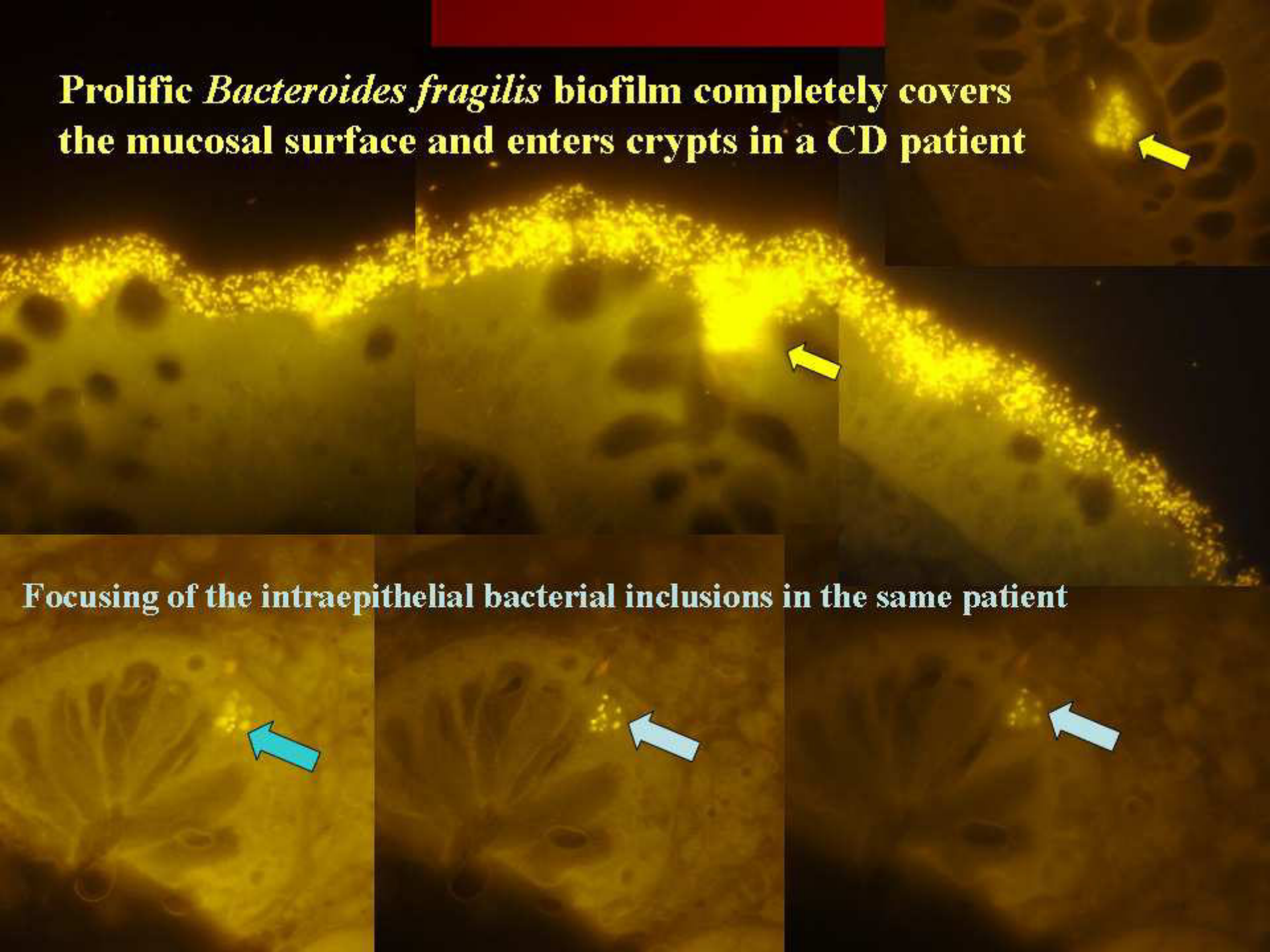
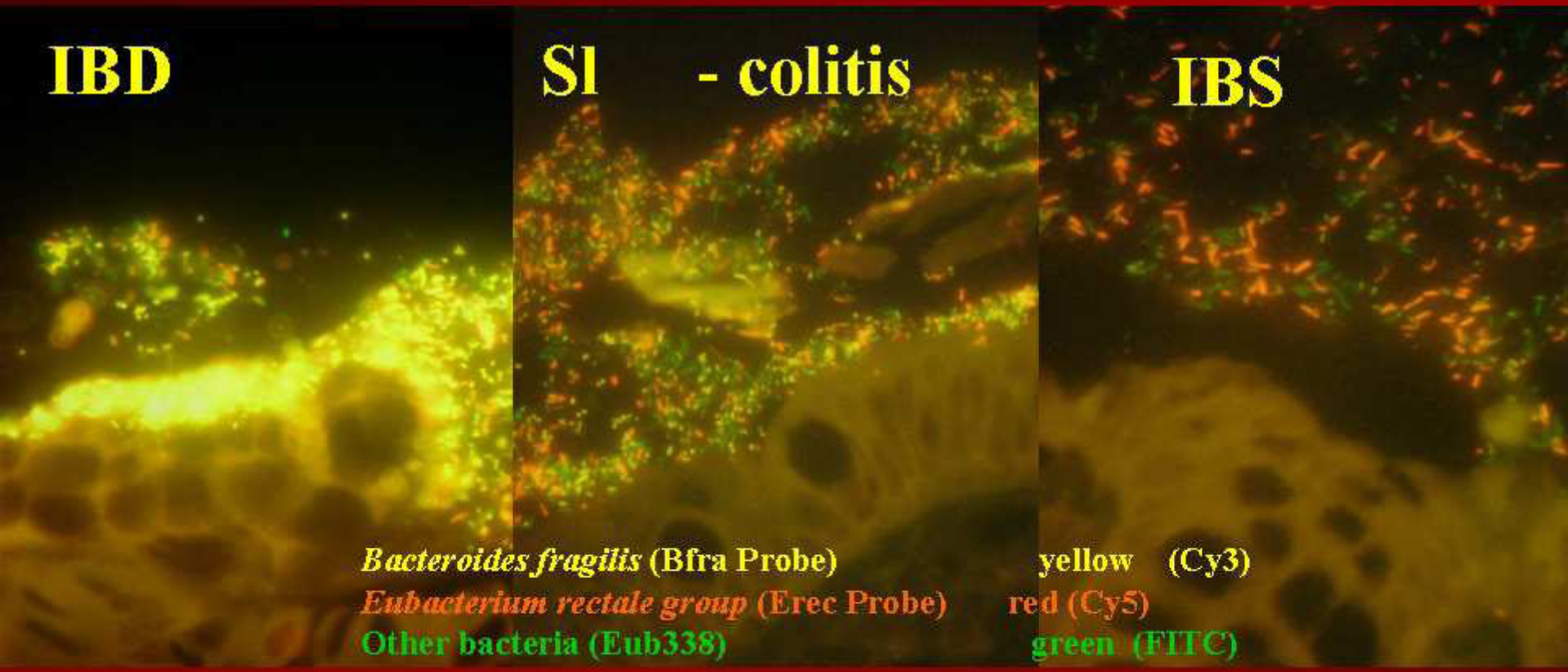
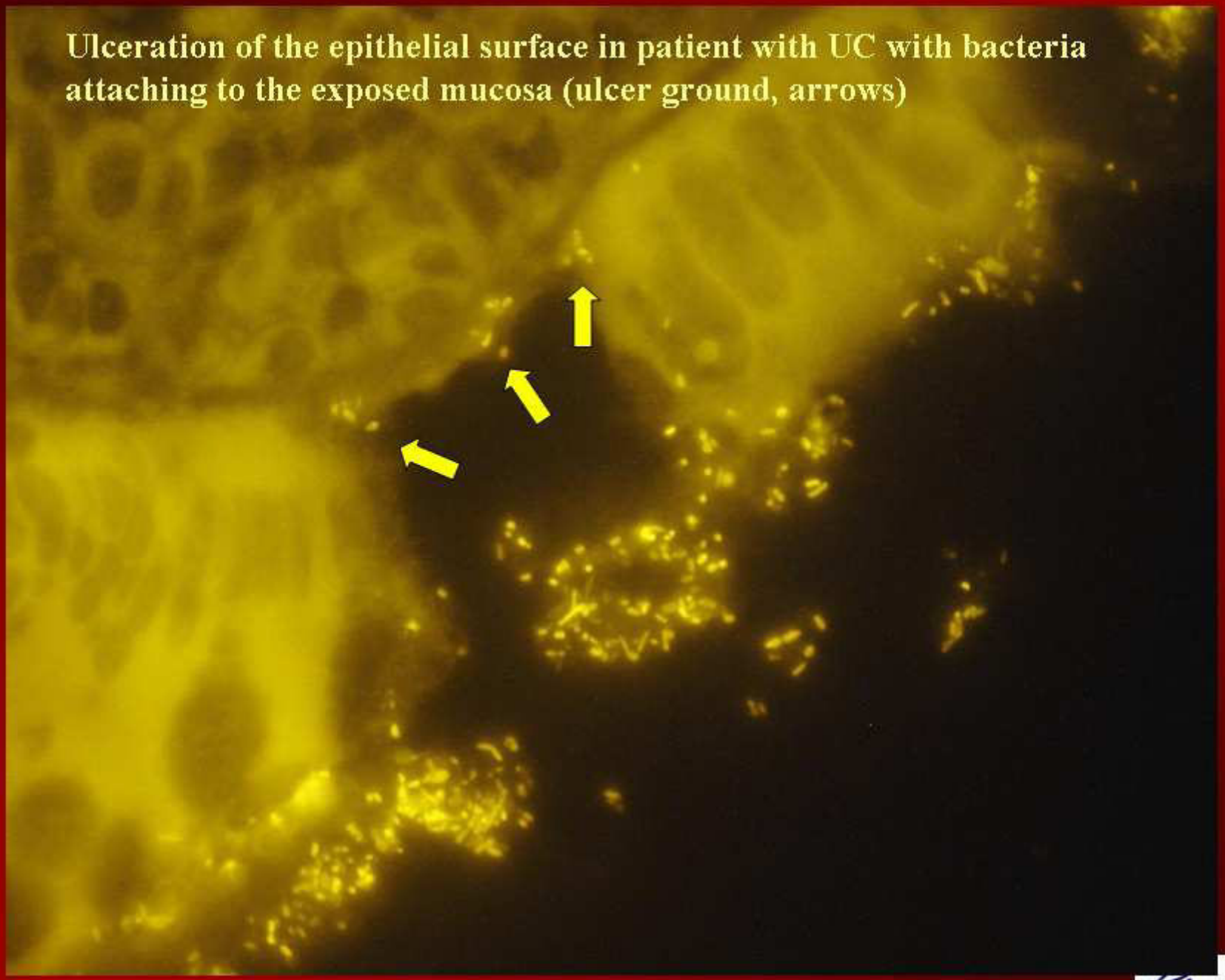
Multicellular bacteria forming stromatolith in Australian salt lakes

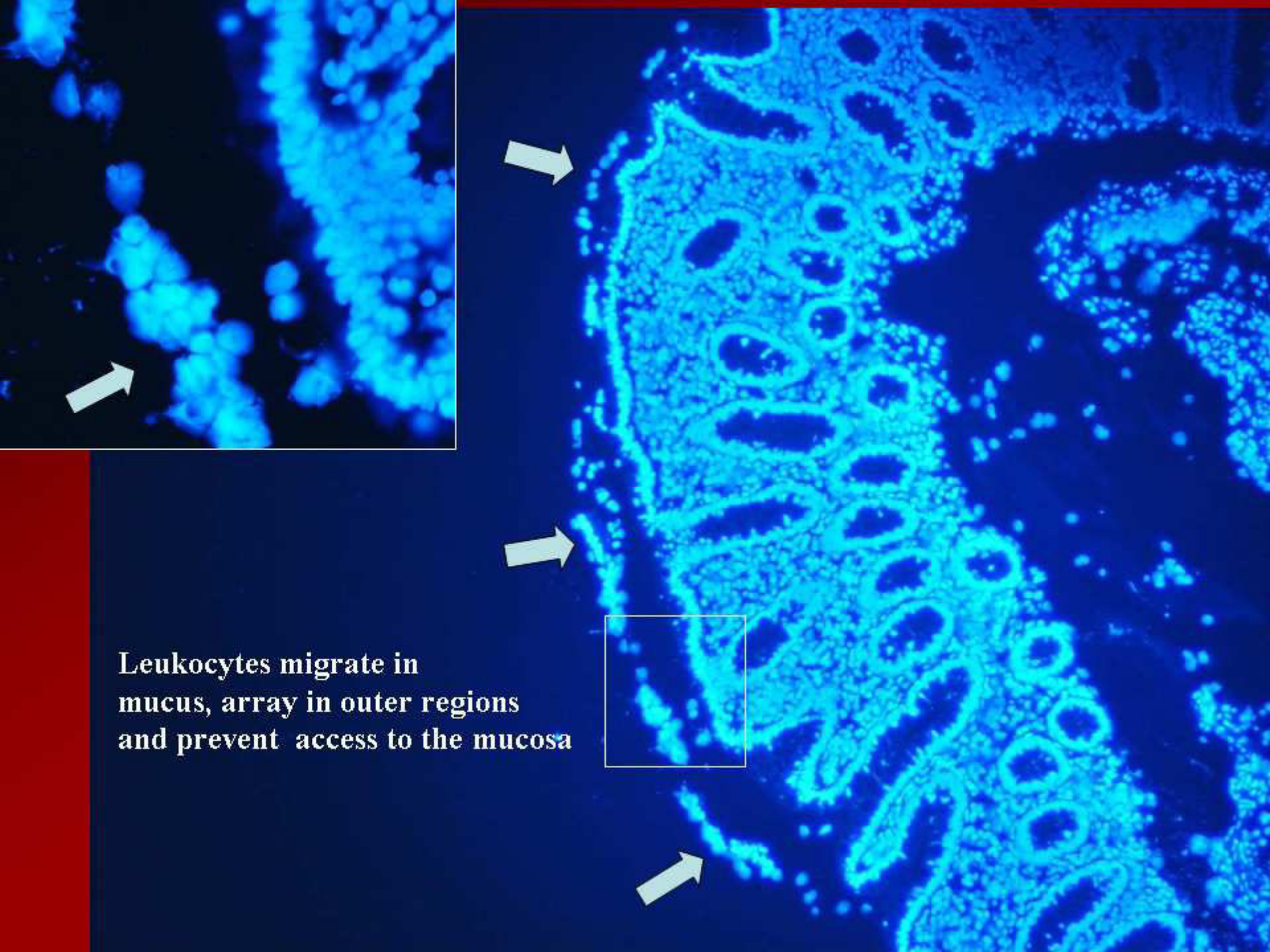

human colonic wall of
healthy controls (84%)
is covered with mucus
that excludes bacteria


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

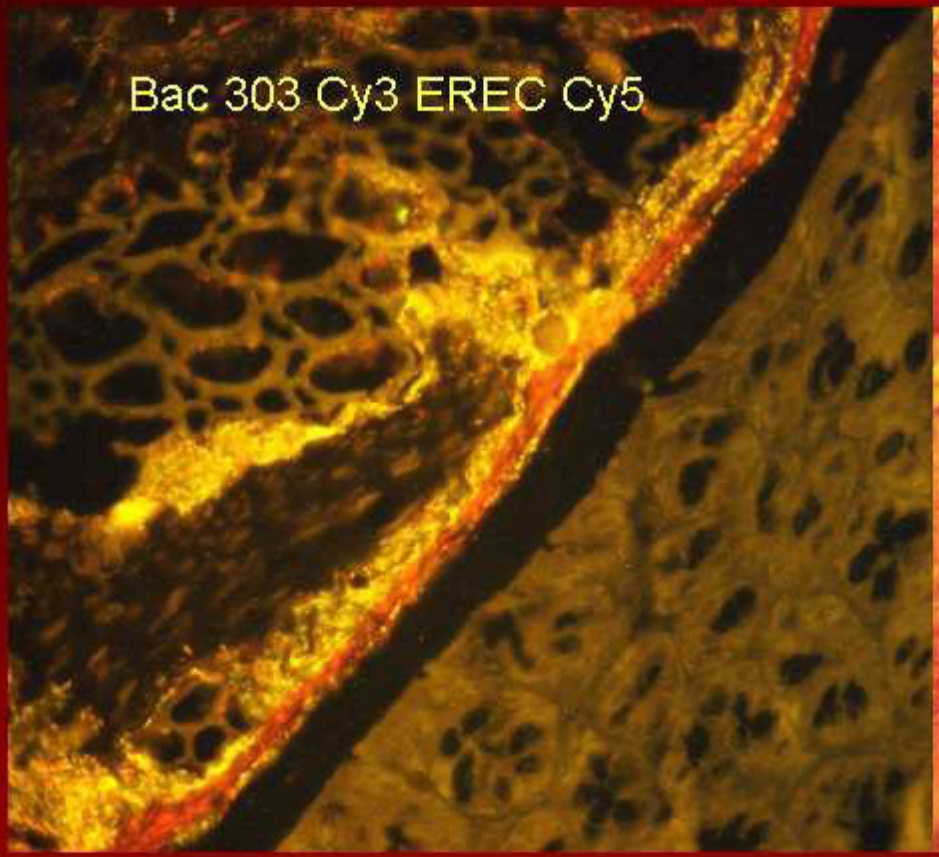


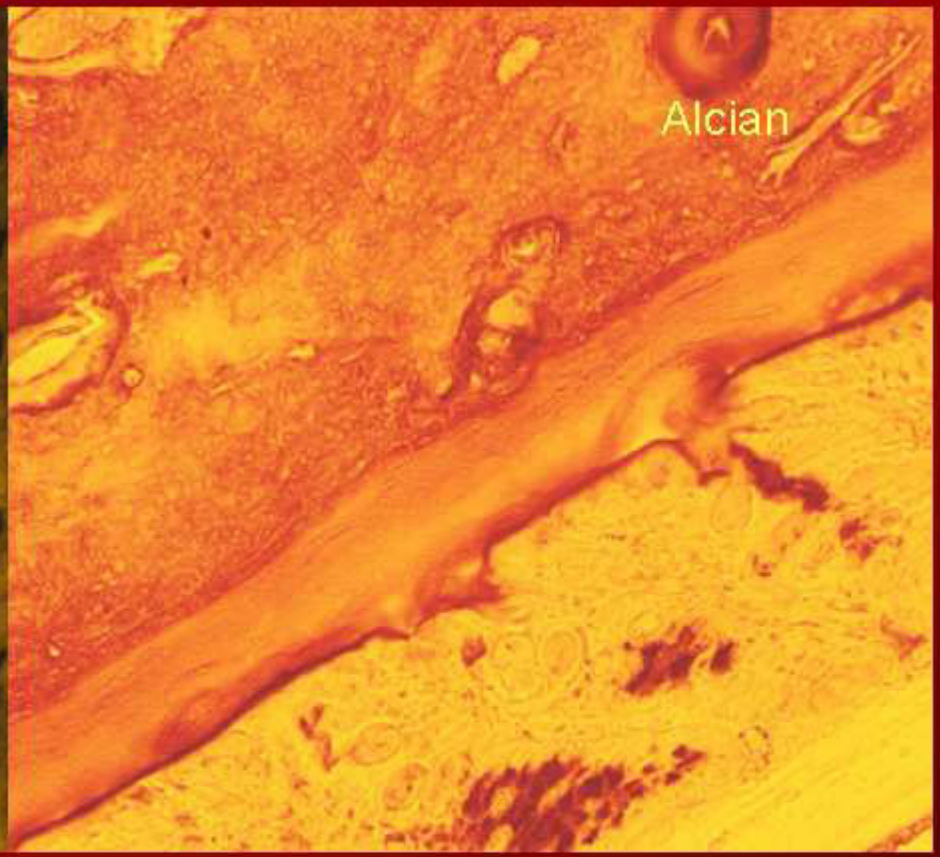
Percent of patients with 10^9 bacteria/ml		CD 98%	UC 94%	SIc 78%	IBS 38%	Contr. 16%
Percent of bacteria within biofilm	Bfra	60%	30%	31%	14%	16%
	Erec	10%	5%	18%	48%	32%


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

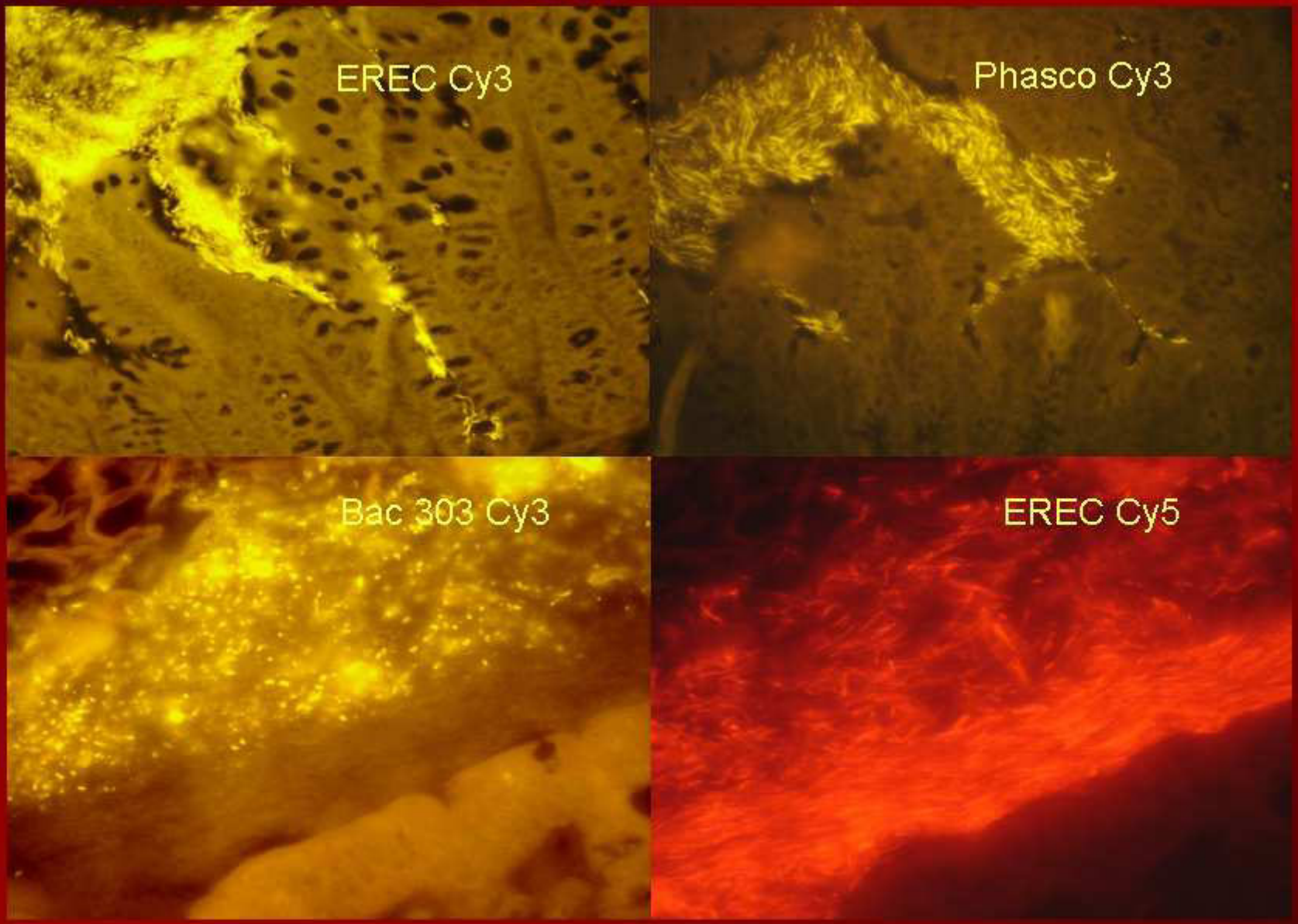

Leukocytes migrate in
mucus, array in outer regions
and prevent access to the mucosa

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

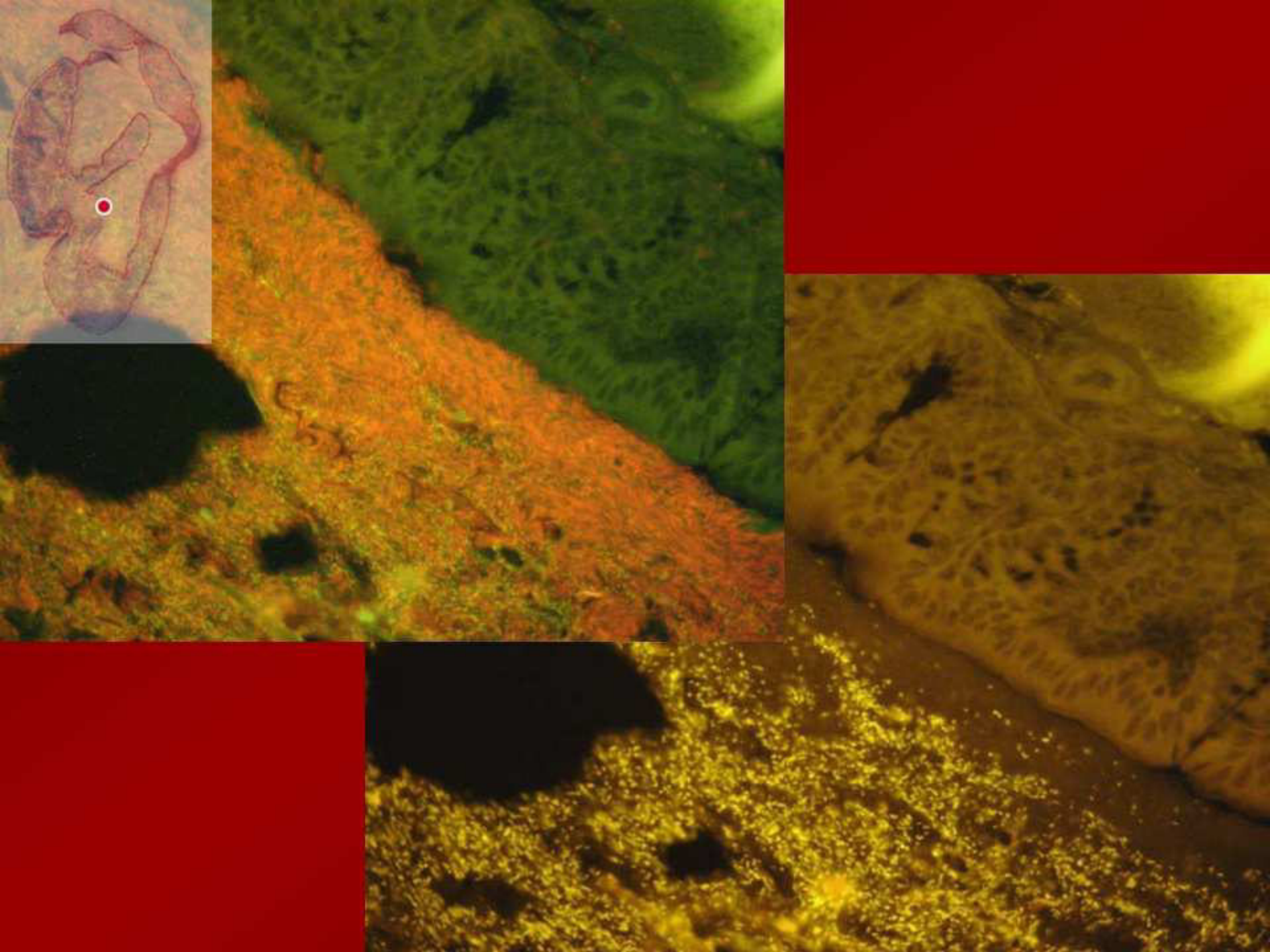




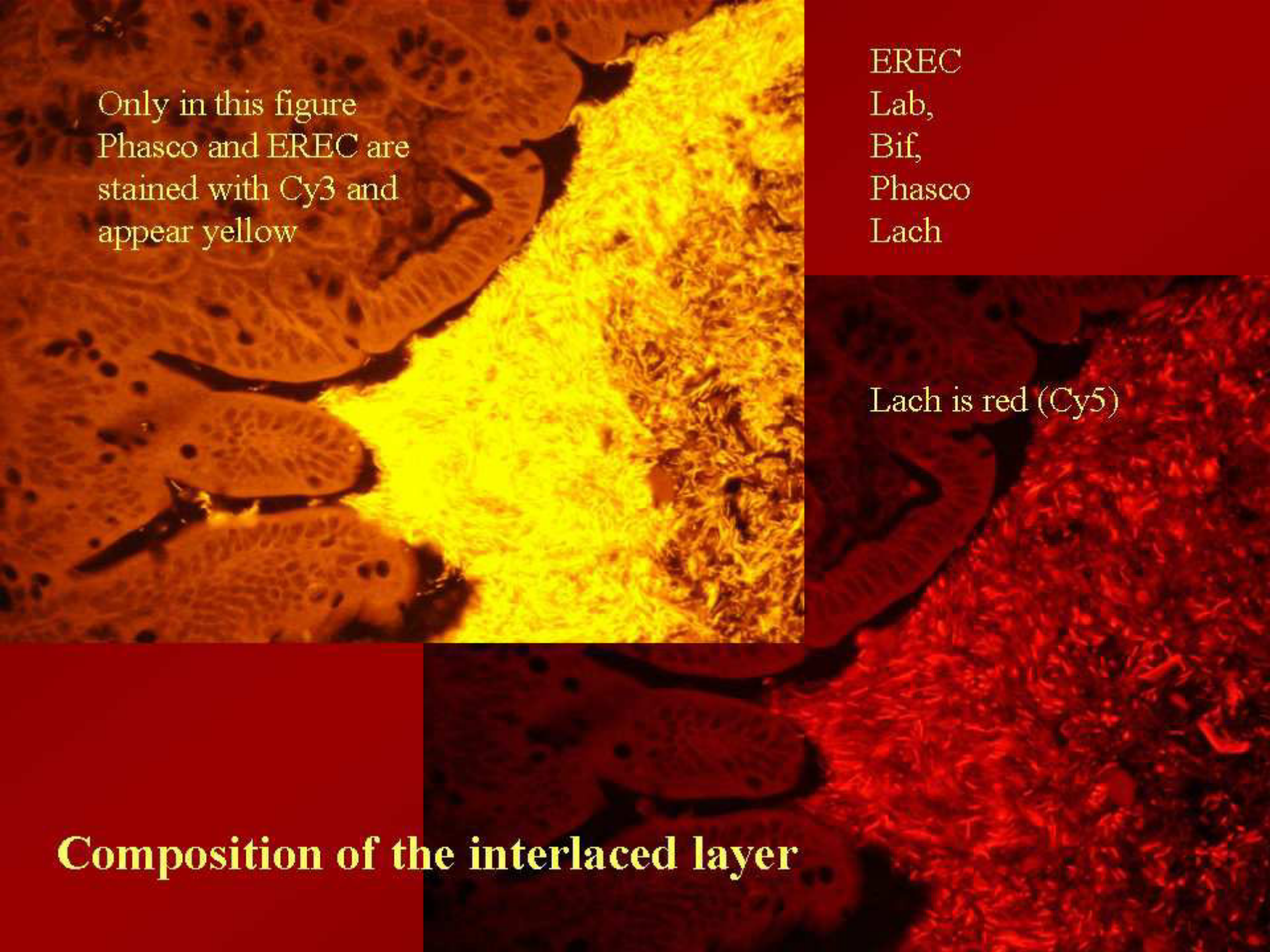
The mucus completely separates mucosa from feces in distal colon of mice similar to the situation in man



The separation of bacteria in the proximal colon of mice is selective, EREC enters crypts, *Bacteroides has no contact with colonic wall*




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

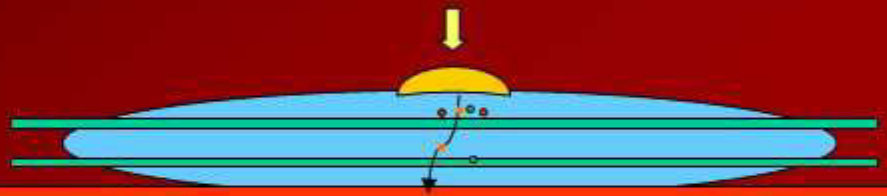


Composition of the interlaced layer

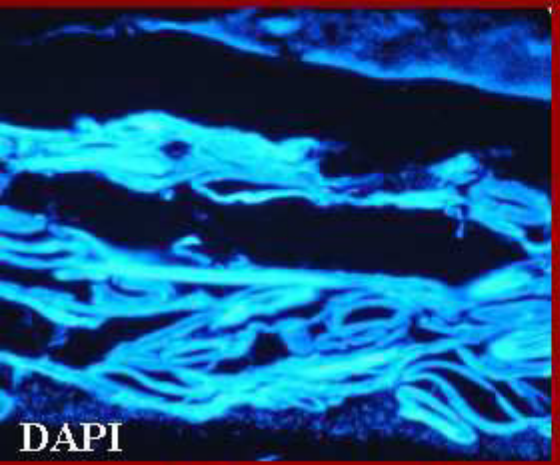
Mucus simulation in vitro

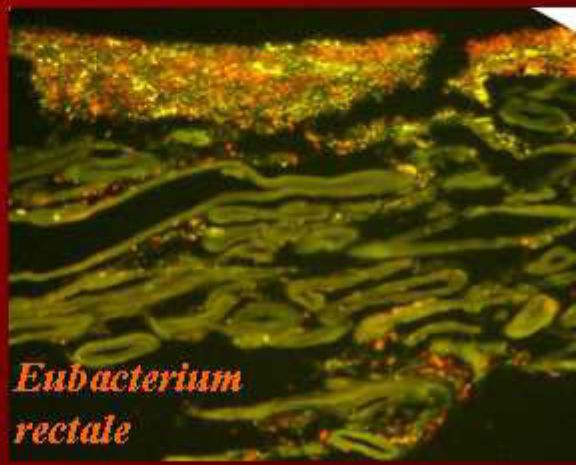
Native mix of fecal bacteria

Two layers of cellulose covered with a LB-agarose gel of variable viscosity

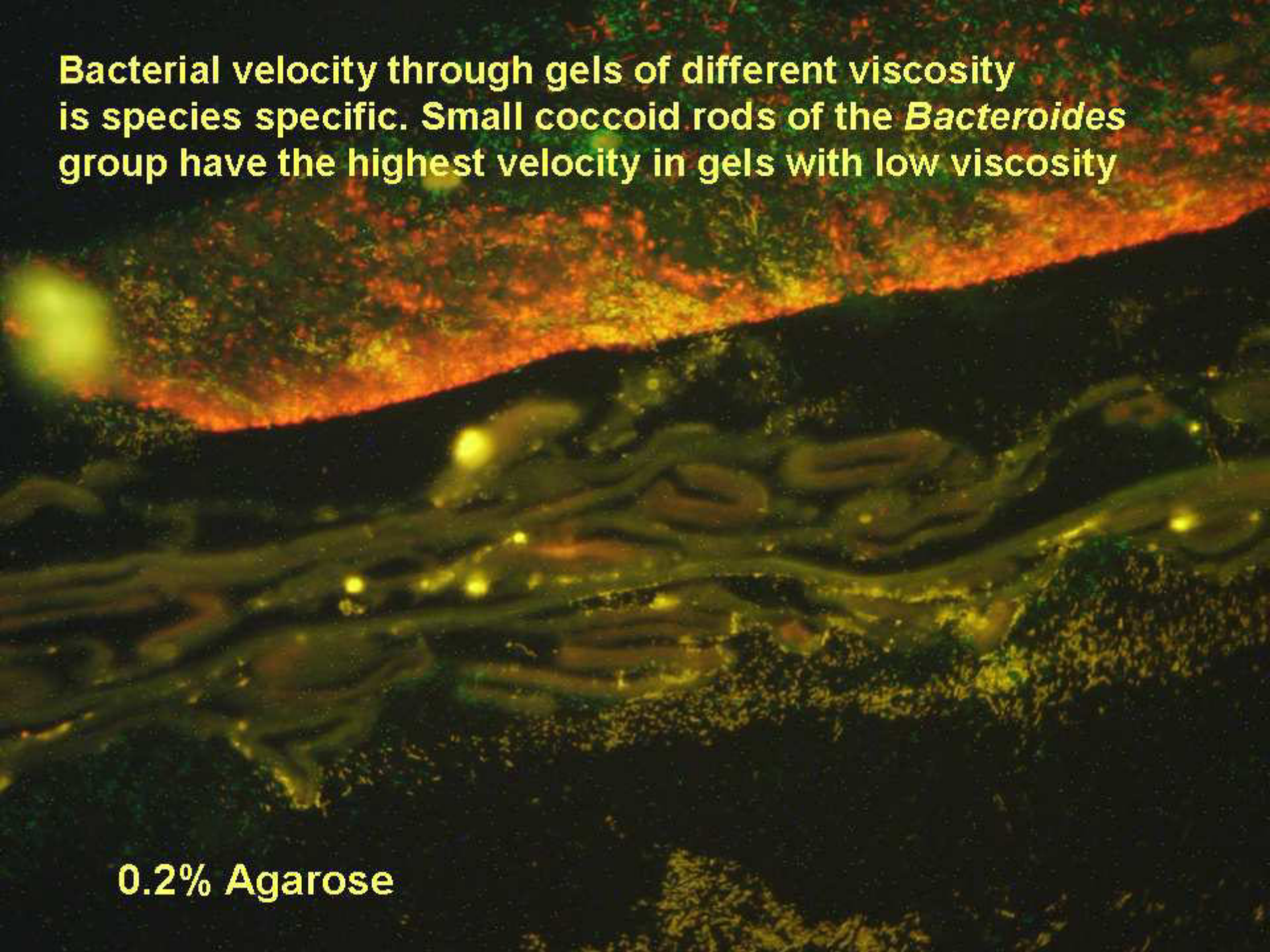


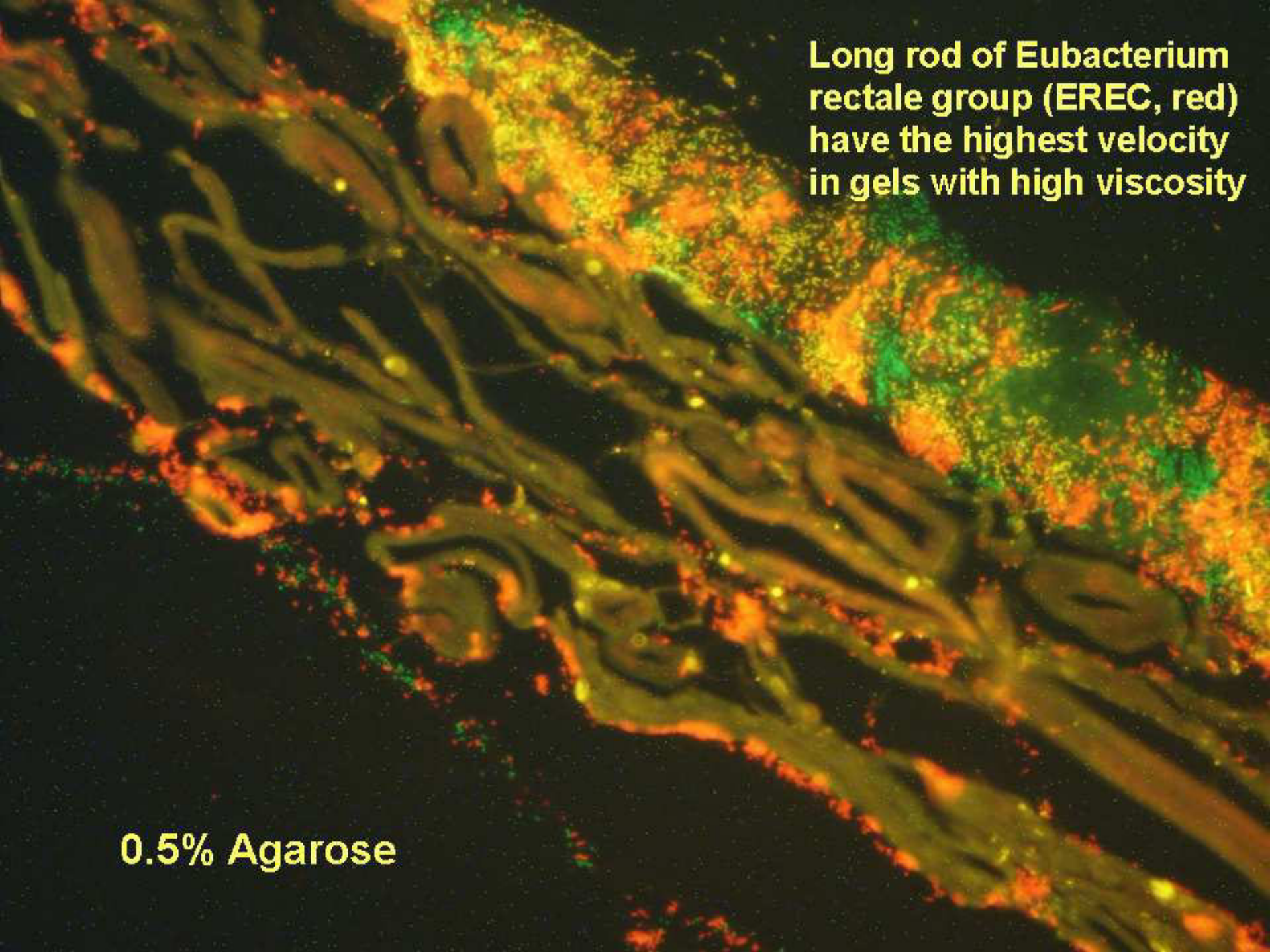
Blood agar plate





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

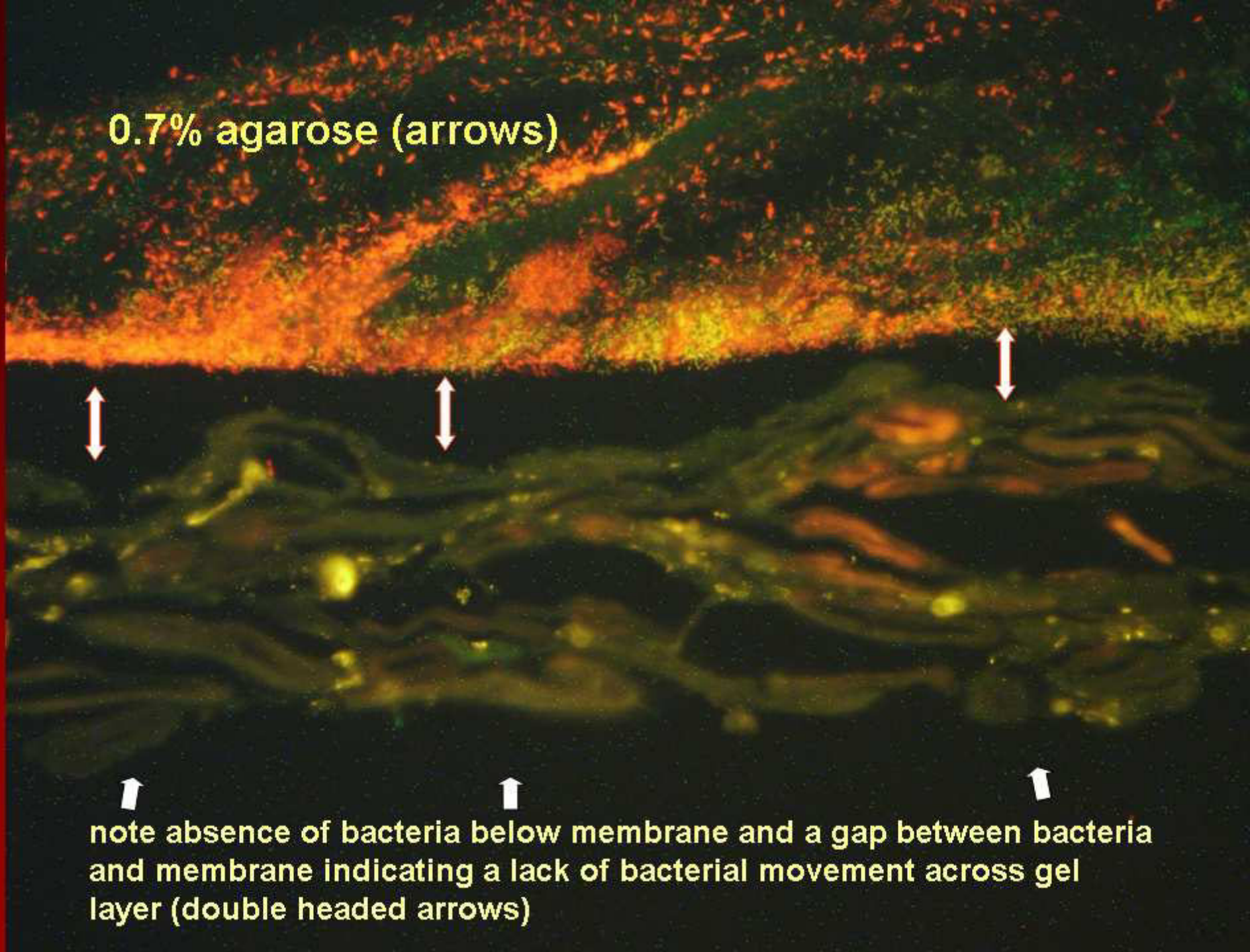
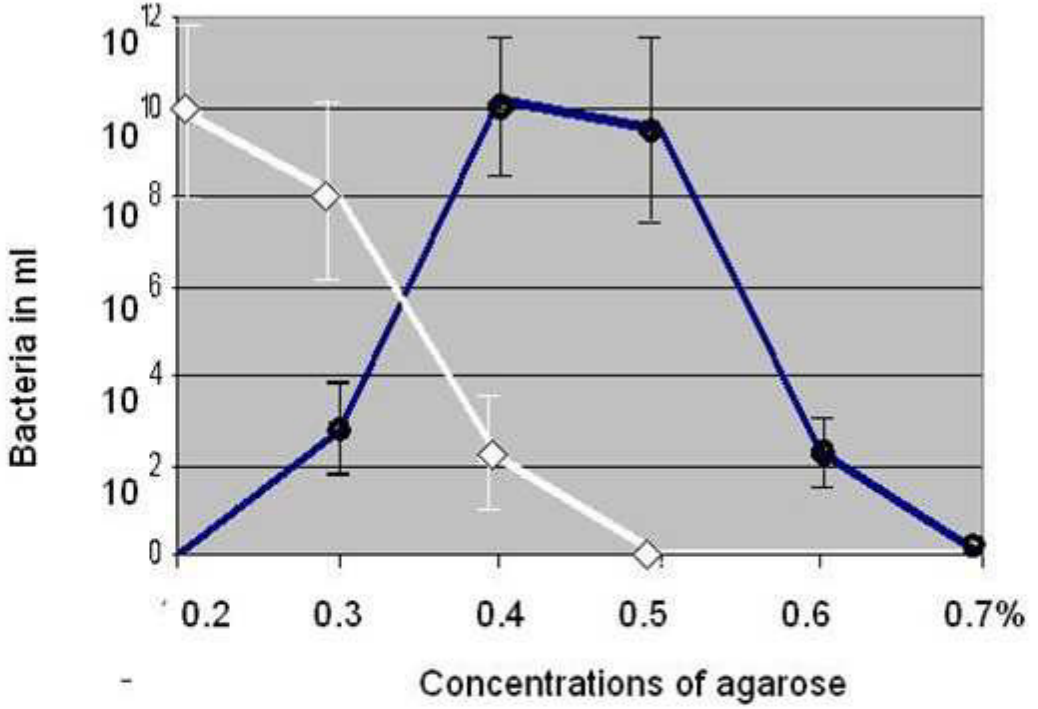
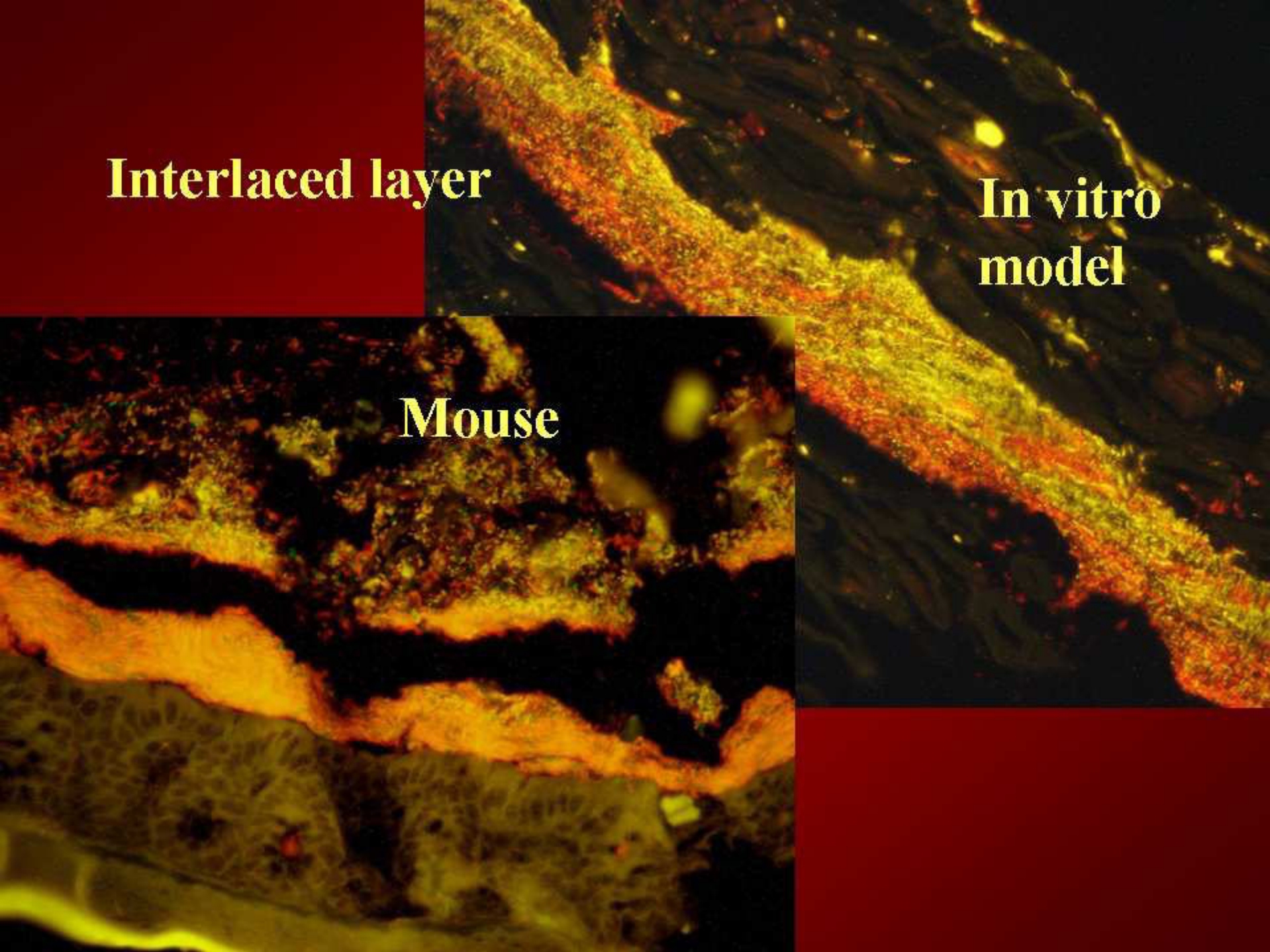
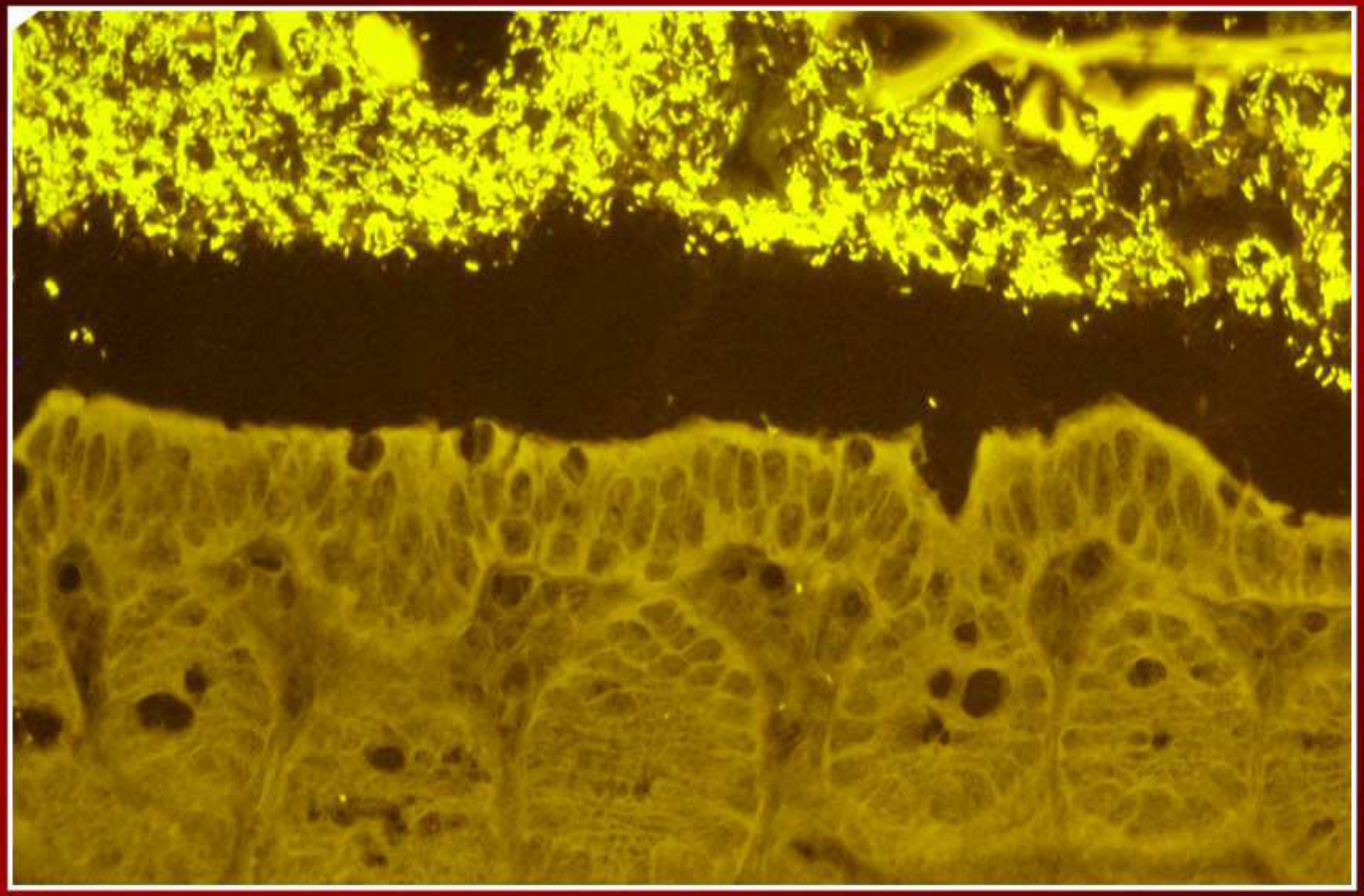

0.7% agarose (arrows)
note absence of bacteria below membrane and a gap between bacteria and membrane indicating a lack of bacterial movement across gel layer (double headed arrows)

Figure 2

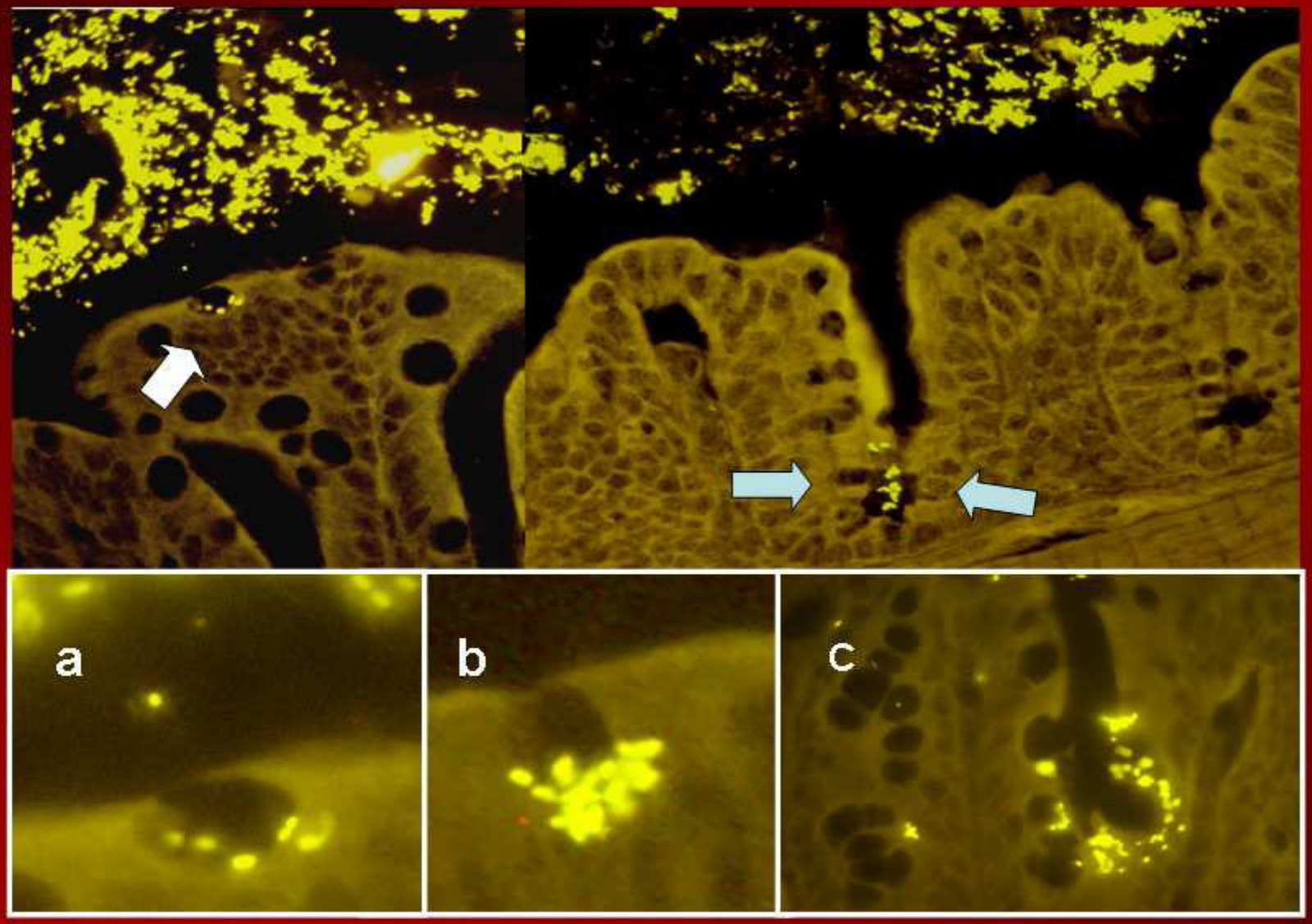


The viscosity-dependent changes in the concentrations
of bacteria moving across *LB-agarose* after 20 hours of anaerobic growth.

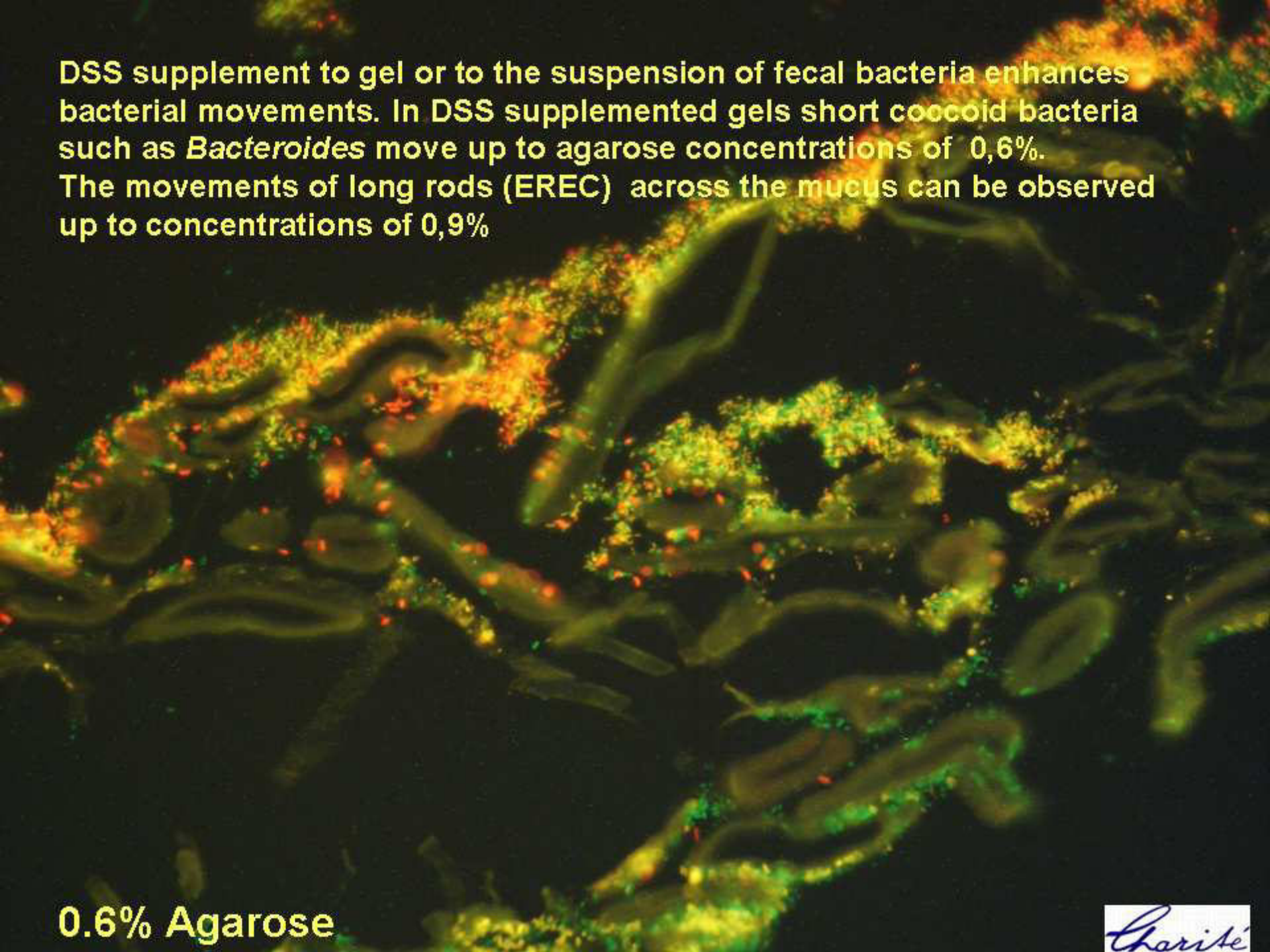

Interlaced layer
In vitro model
Mouse

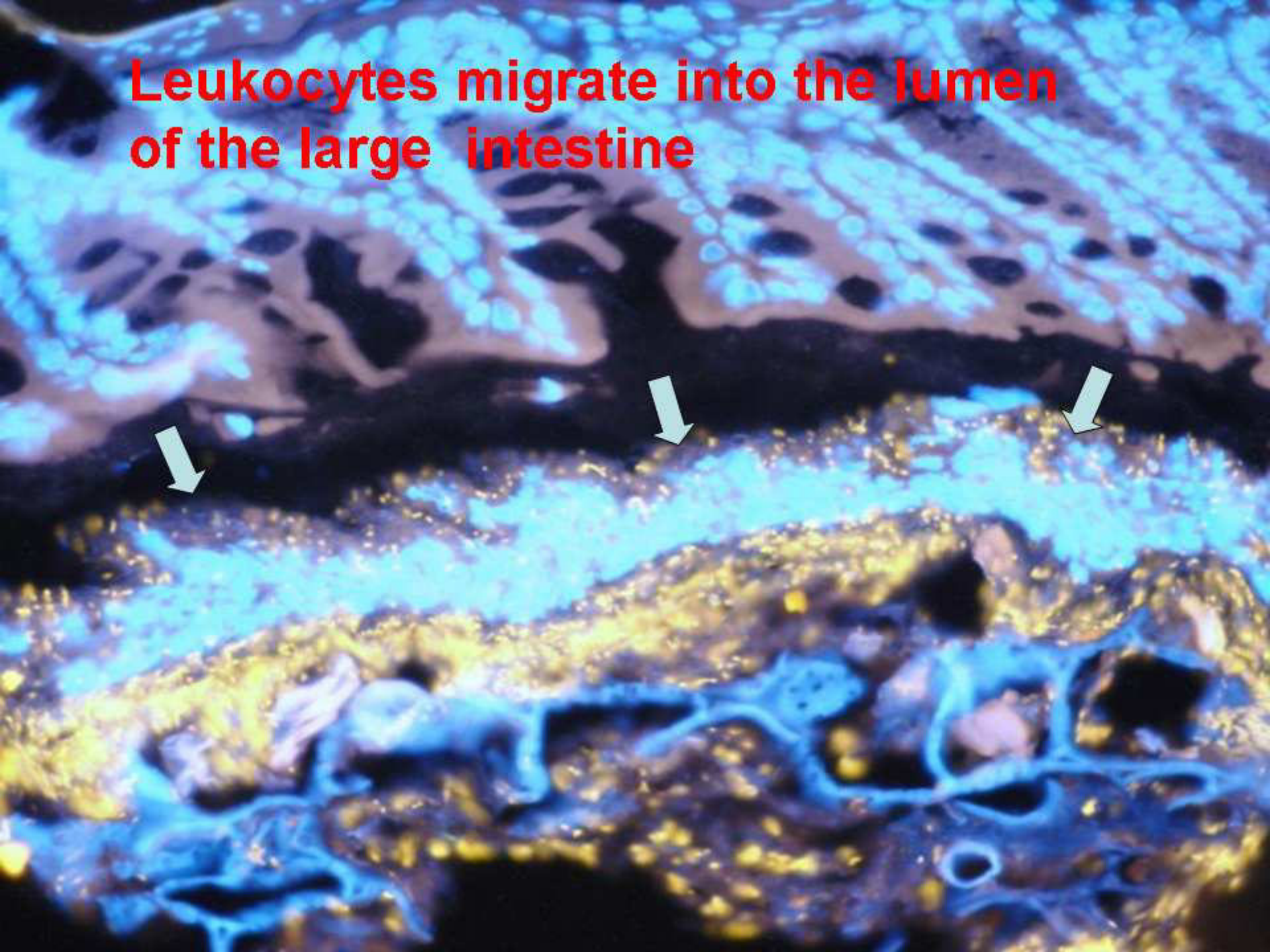


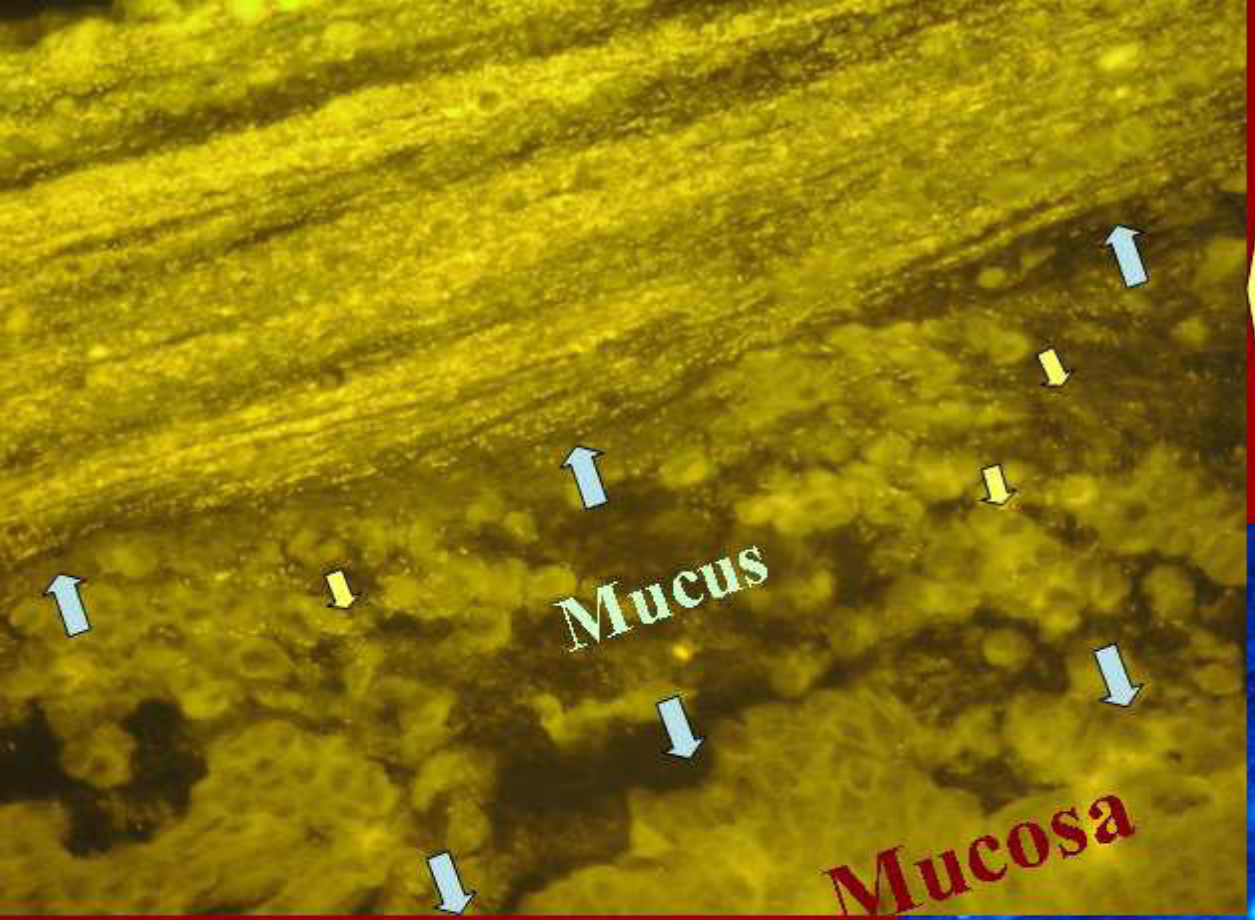
- Distal colon of mice mono-associated with *Enterobacter cloacae*



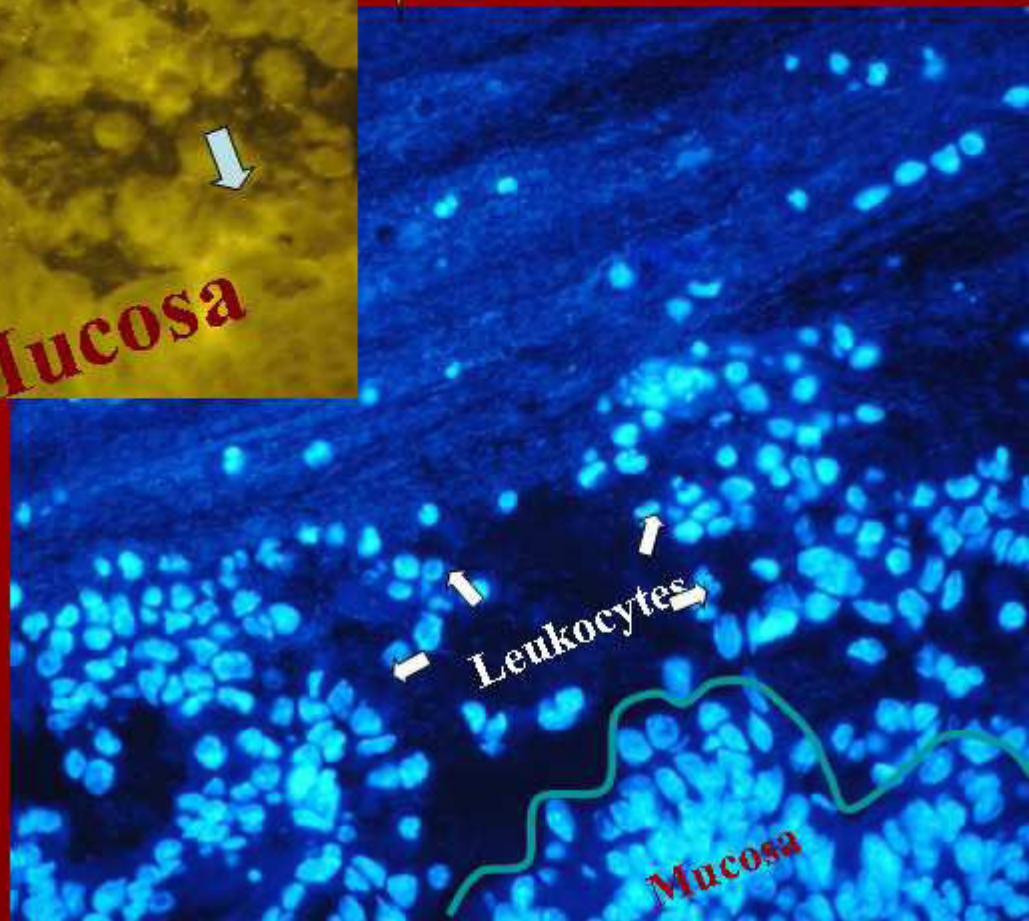
Proximal colon of mice mono-associated with *Enterobacter cloacae*


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

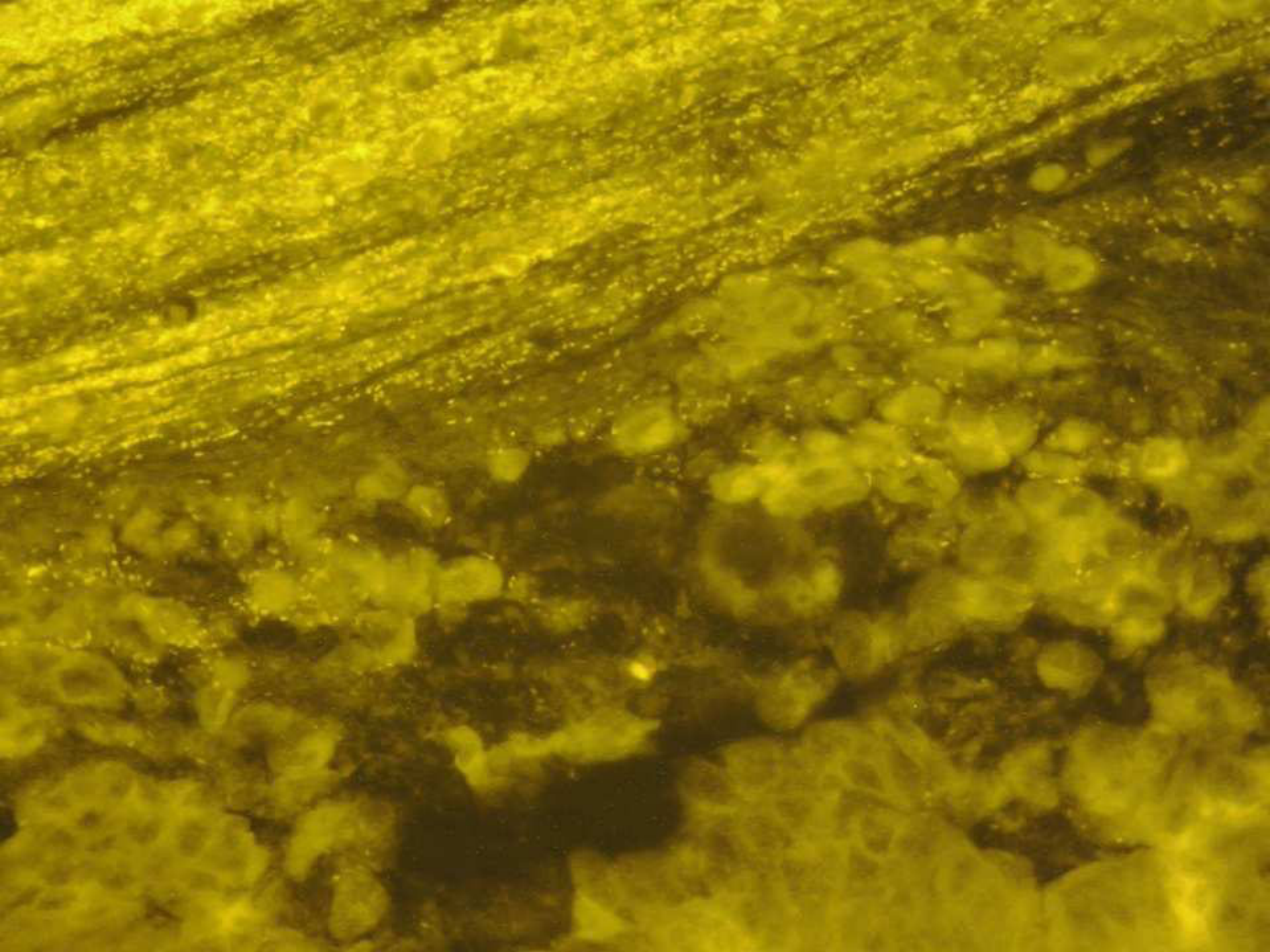

Leukocytes migrate into the lumen
of the large intestine



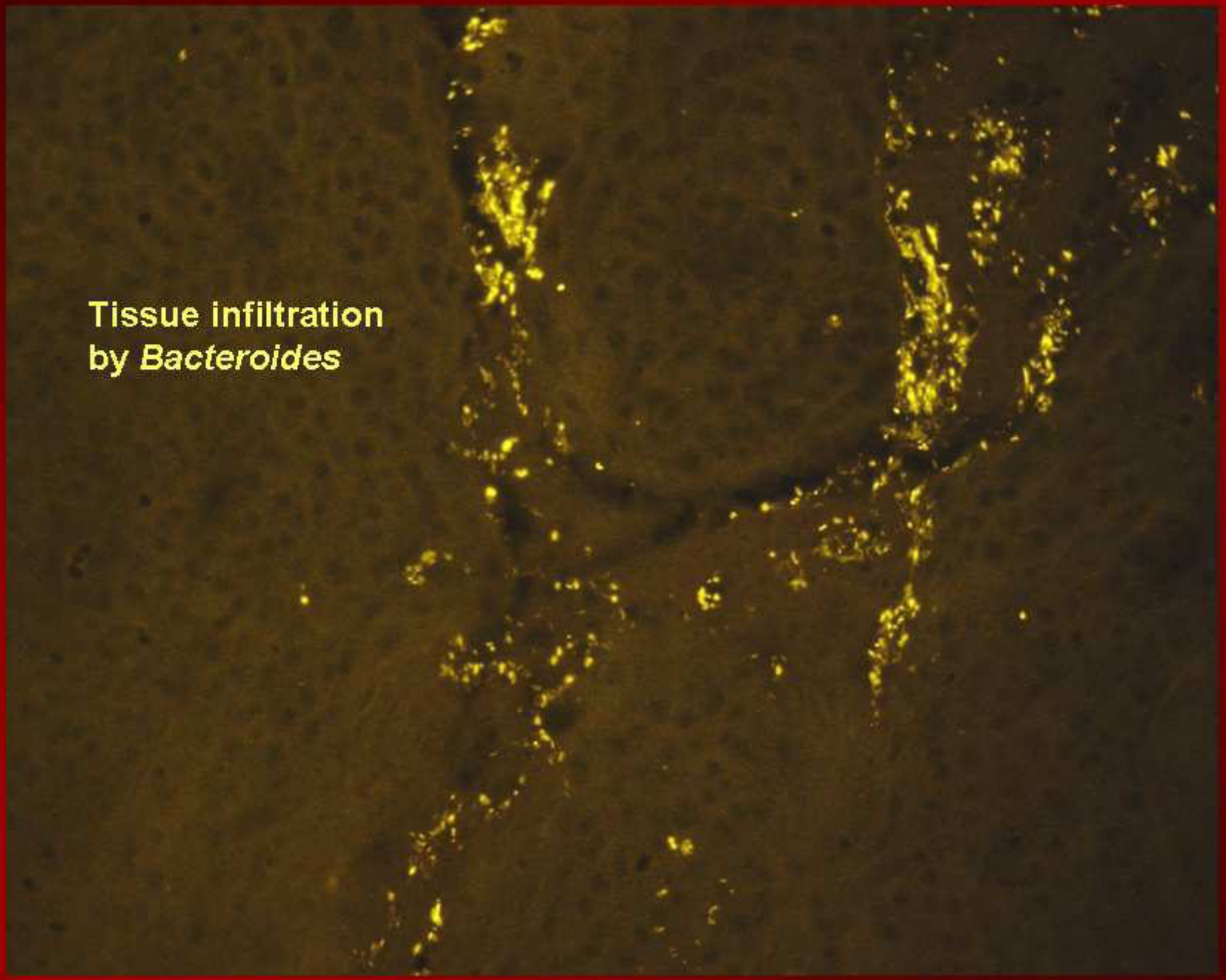
Bacteroides crosses mucus



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

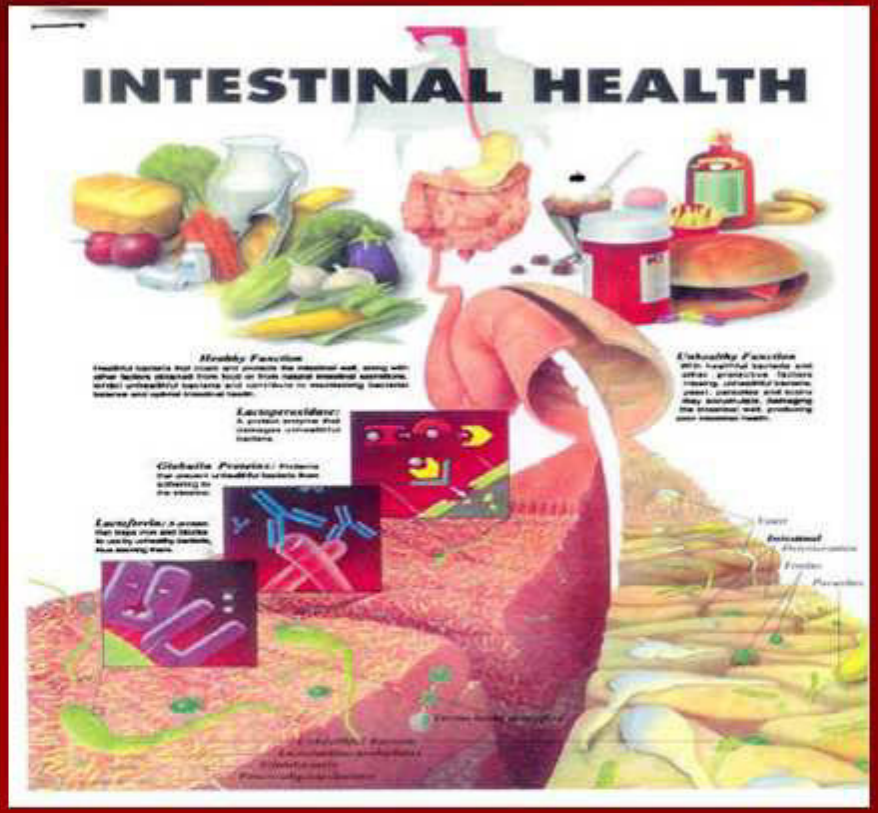



Bacteroides-adhesion to the colonic wall


Tissue infiltration
by *Bacteroides*

Tolerance

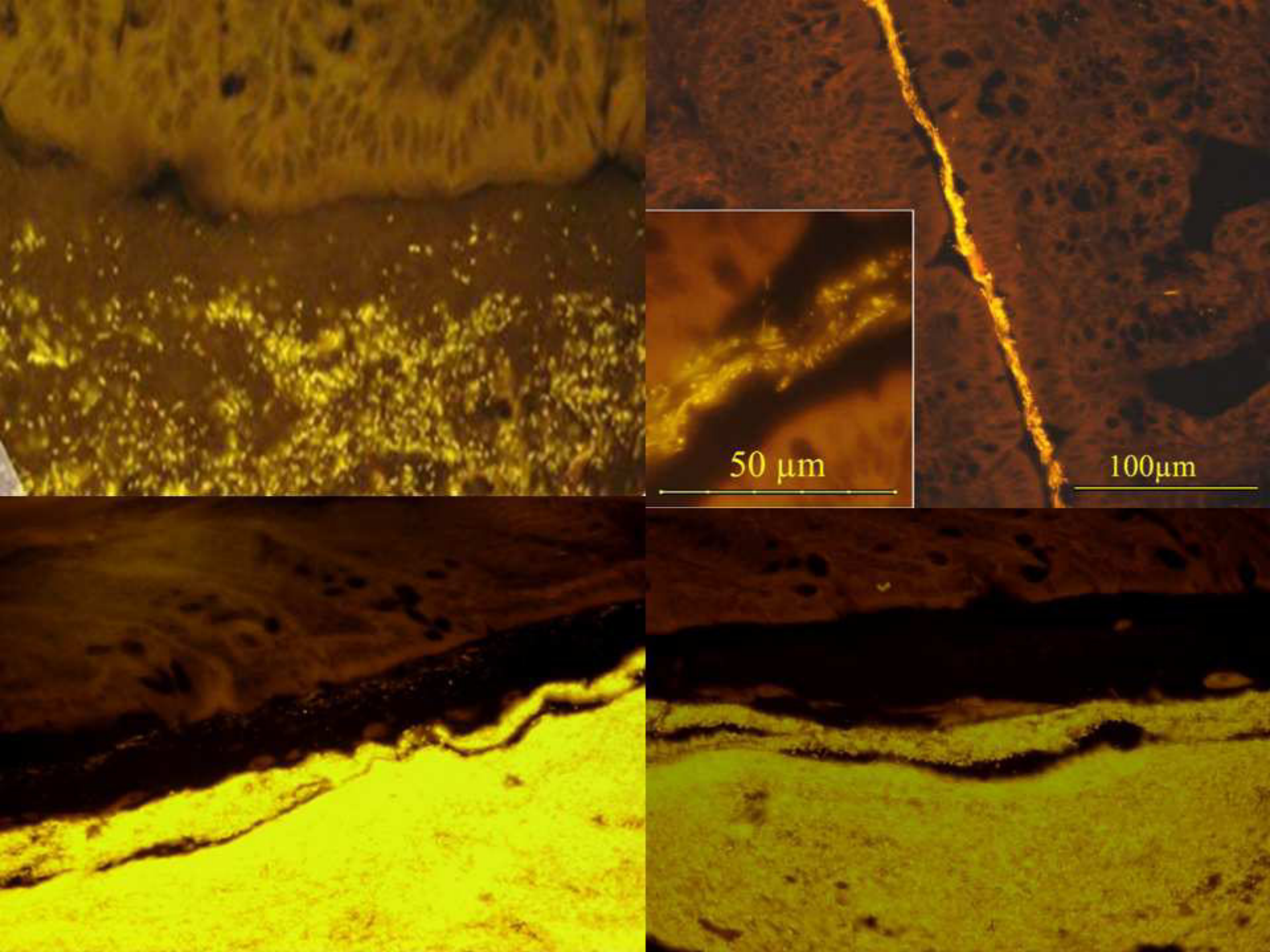
normal
Flora



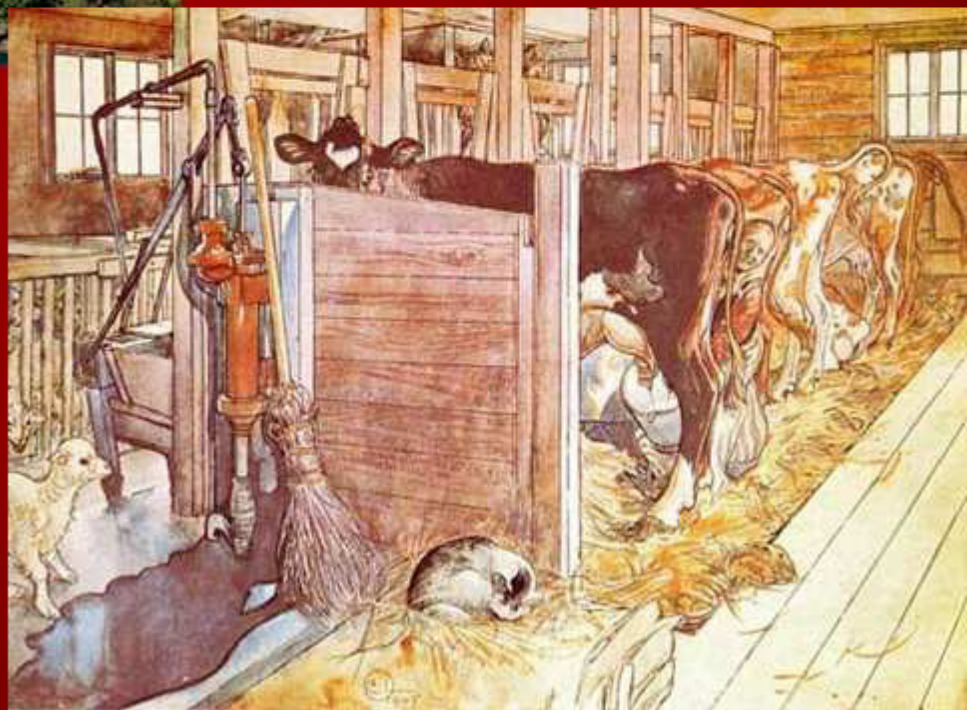
Inflammatory Response

Enteral
Pathogens

E. coli
Bacteroides
Clostridium difficile
Enterococci
Salmonella
Shigella


50 µm
100µm

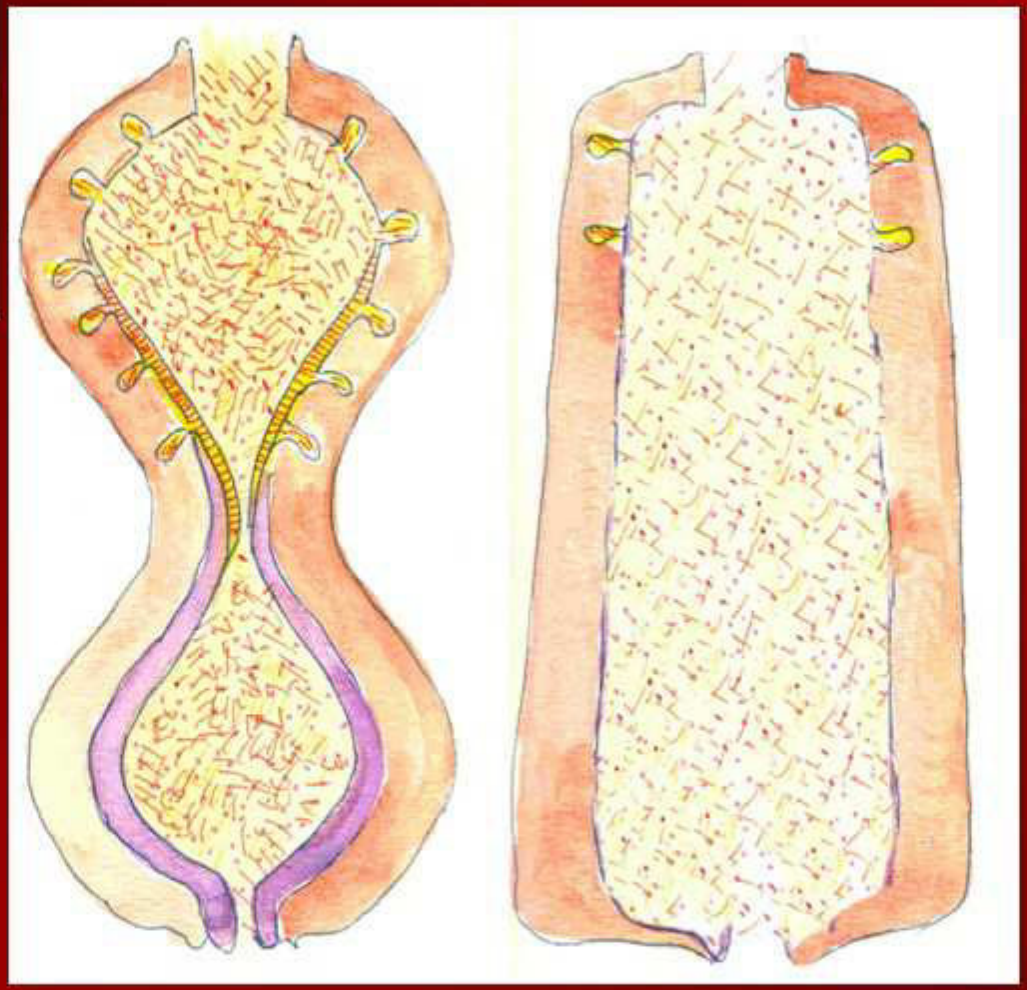
Hygiene hypothesis



Kochen mit dem WOK

Indien, **Mini Auberginen,**
Südafrika, **Mini Zucchini,**
Peru, **Mini Spargel grün oder**
Kenia, **Kaiserschoten**
Kl. I, 100 g = 1.00, je 200-g-Packung

1.99



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

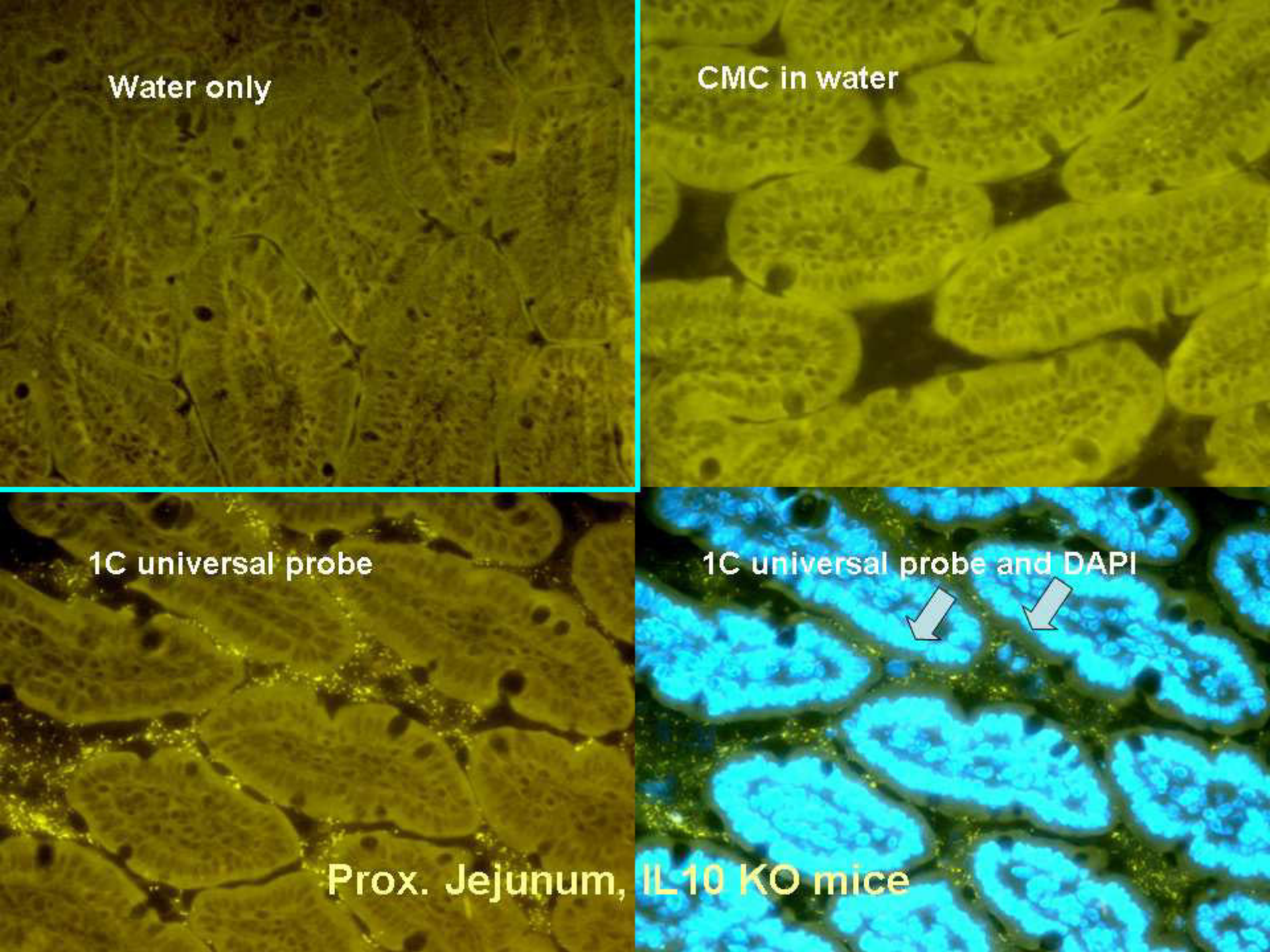
E471 bis E472f, Mono- und Diglyceride von Speisefettsäuren
- E471, Mono- und Diglyceride von Speisefettsäuren, Monoglycerid
- E472a, Essigsäureester von Mono- und Diglyceriden von Speisefettsäuren
- E472b, Milchsäureester von Mono- und Diglyceriden von Speisefettsäuren
- E472c, Citronensäureester von 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

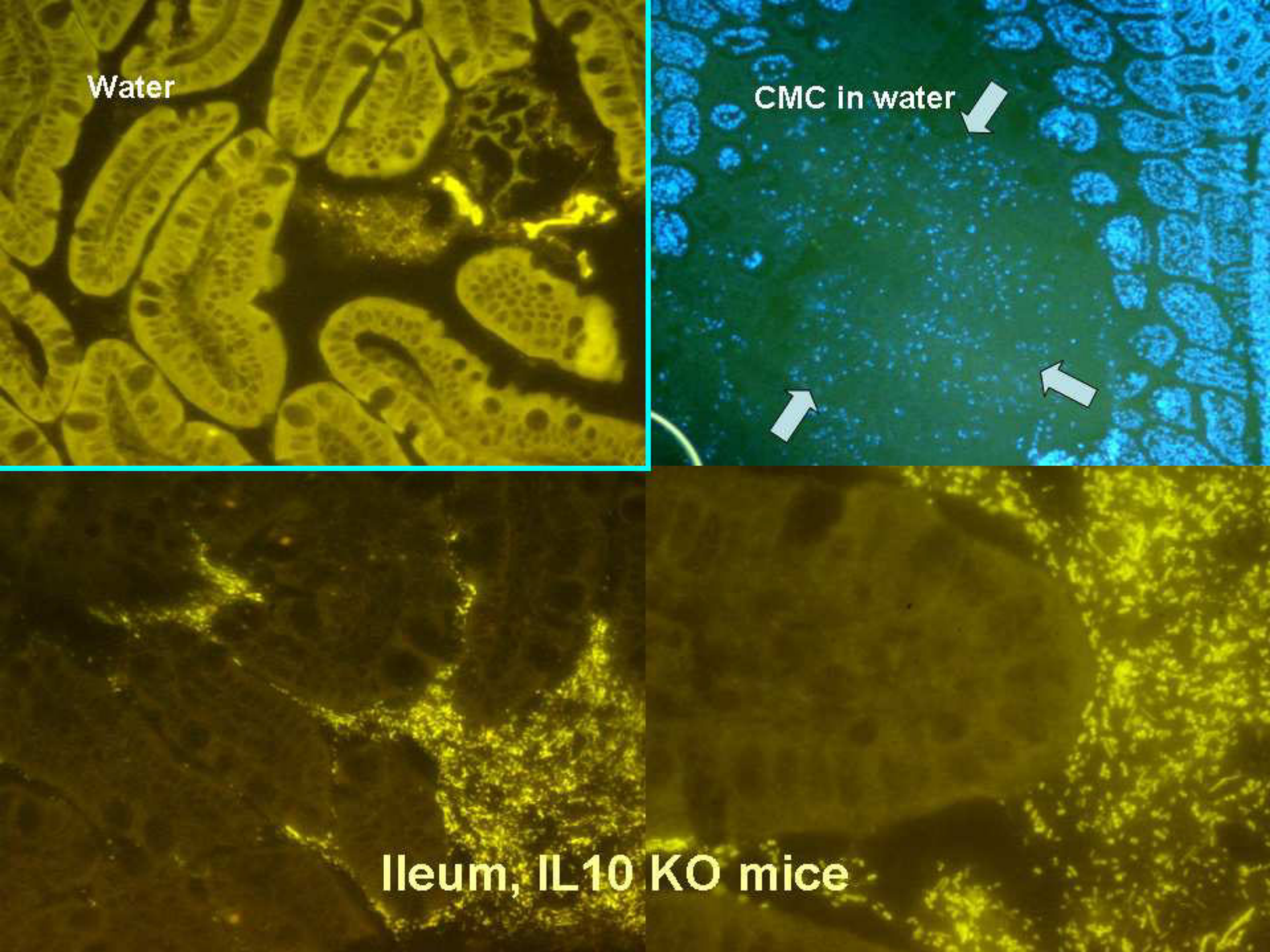
E473, Zuckerester von Speisefettsäuren
E474, Zuckerglyceride
E475, Polyglycerinester von Speisefettsäuren, Polyglycerinester
E476, Polyglycerin-Polyricinoleat
E477, Propylenglycolester von Speisefetten
E479, Thermooxidiertes Sojaöl mit Mono- und Diglyceriden von Speisefettsäuren
E481 bis E483, Natriumstearoyl-2-lactylat,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

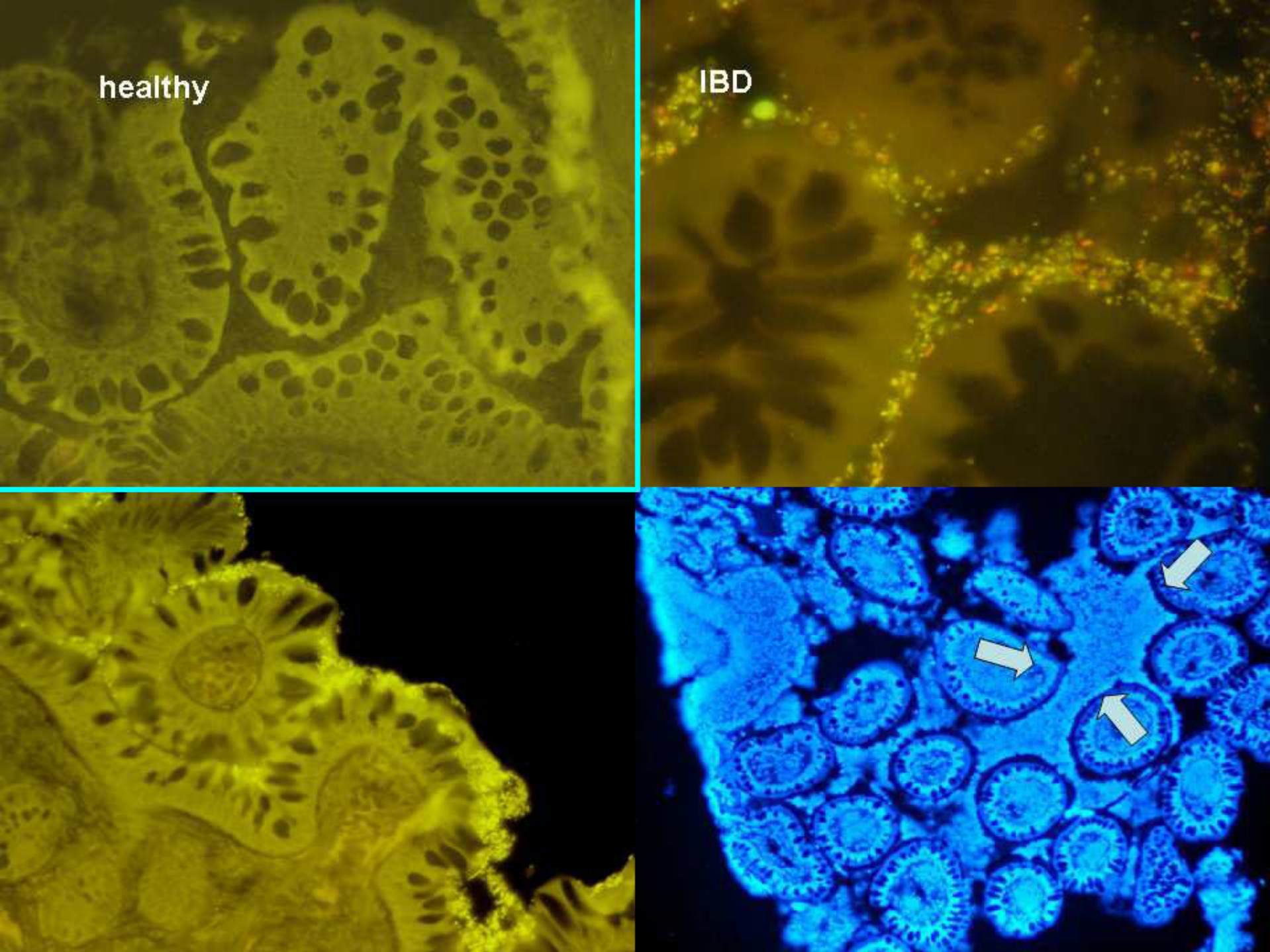
CMC

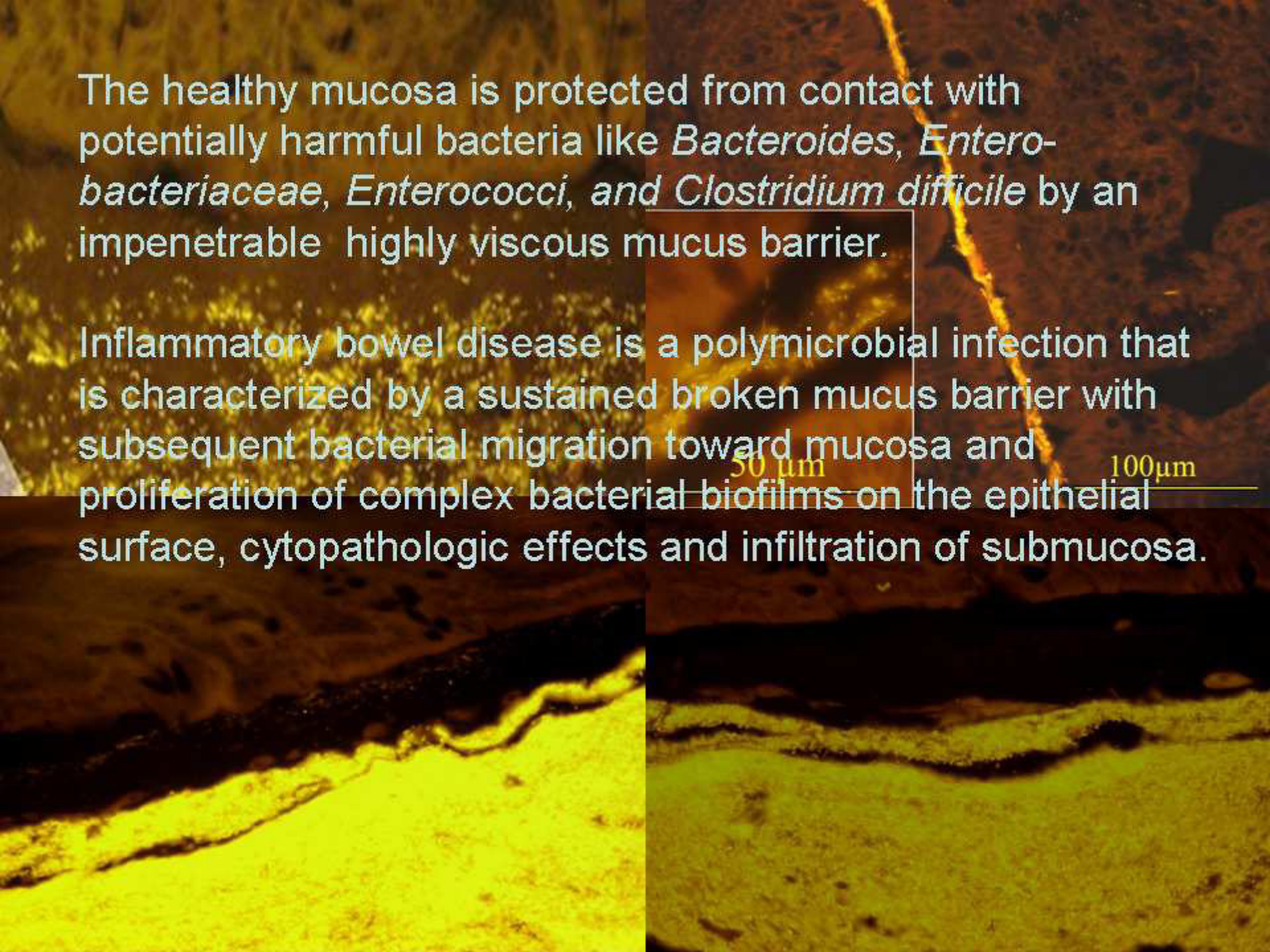
Carboxymethylcellulose

In IL 10 KO mice


Water only
CMC in water
1C universal probe
1C universal probe and DAPI
Prox. Jejunum, IL10 KO mice


Water
CMC in water
Ileum, IL10 KO mice


healthy
IBD



The healthy mucosa is protected from contact with potentially harmful bacteria like *Bacteroides, Entero-bacteriaceae, Enterococci, and Clostridium difficile* by an impenetrable highly viscous mucus barrier.

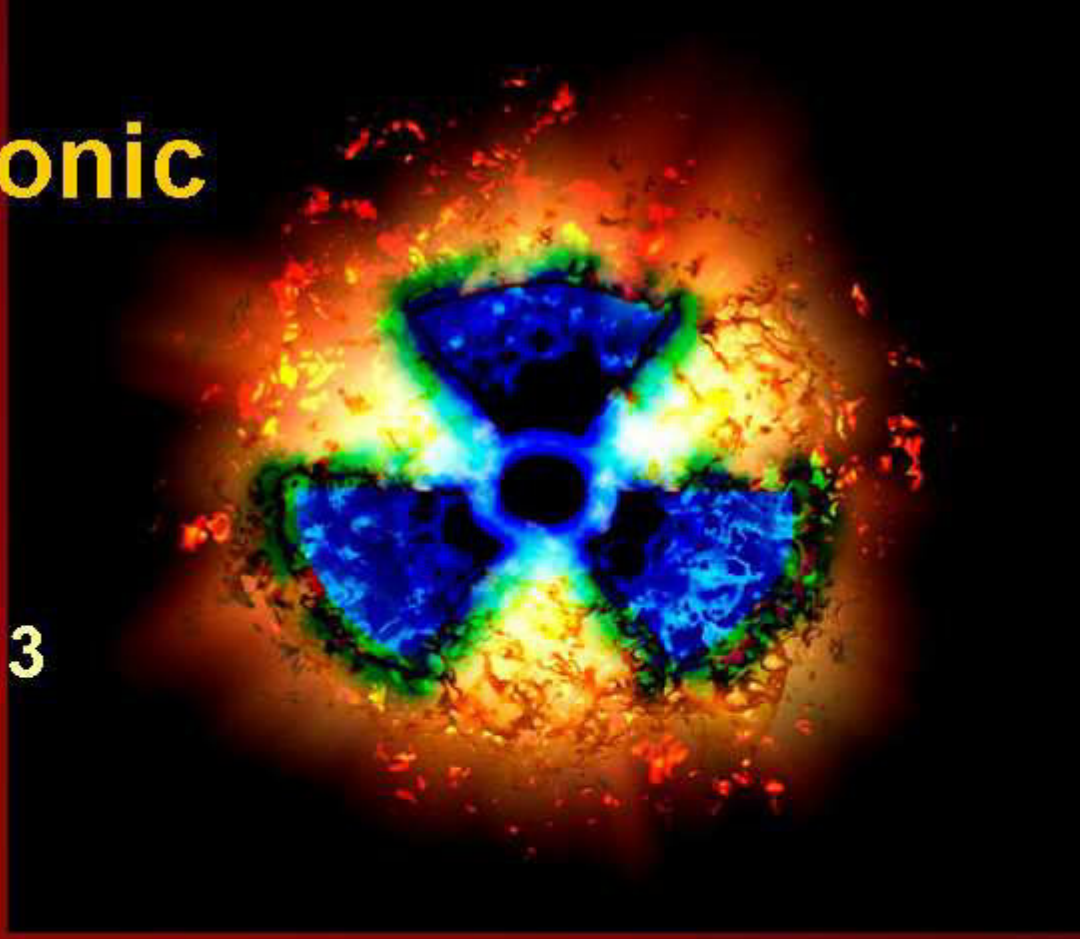
Inflammatory bowel disease is a polymicrobial infection that is characterized by a sustained broken mucus barrier with subsequent bacterial migration toward mucosa and proliferation of complex bacterial biofilms on the epithelial surface, cytopathologic effects and infiltration of submucosa.

Possible ways to remodel the mucus barrier

- Selective control of mucus secretion and dehydration
  (analoga of cortisol)
  Induction of a higher differentiation of epithelial cells, which leads to switch from mainly secretary to adsorptive function
  (analoga of anti TNF suppressing apoptosis, MTX, Azathioprin?)
- Suppression of adherent bacterial biofilms
  (effects of 5-ASA)
- Reduction of the burden of detergents and emulsifiers in our foods
- (Colestyramin, Ursofalk)
- Eradication of occasional pathogens comprising mucus barrier
  like Entero-adhesive E.coli, Fusobaterium nucleatum, Serpulina
  (antibiotics, probiotics?)
- Simulation of innate immunity
  (GM CSF, Interferon, probiotics?)
- Regulation of CNS (Amitriptylin) and local neuronal control (Imodium)

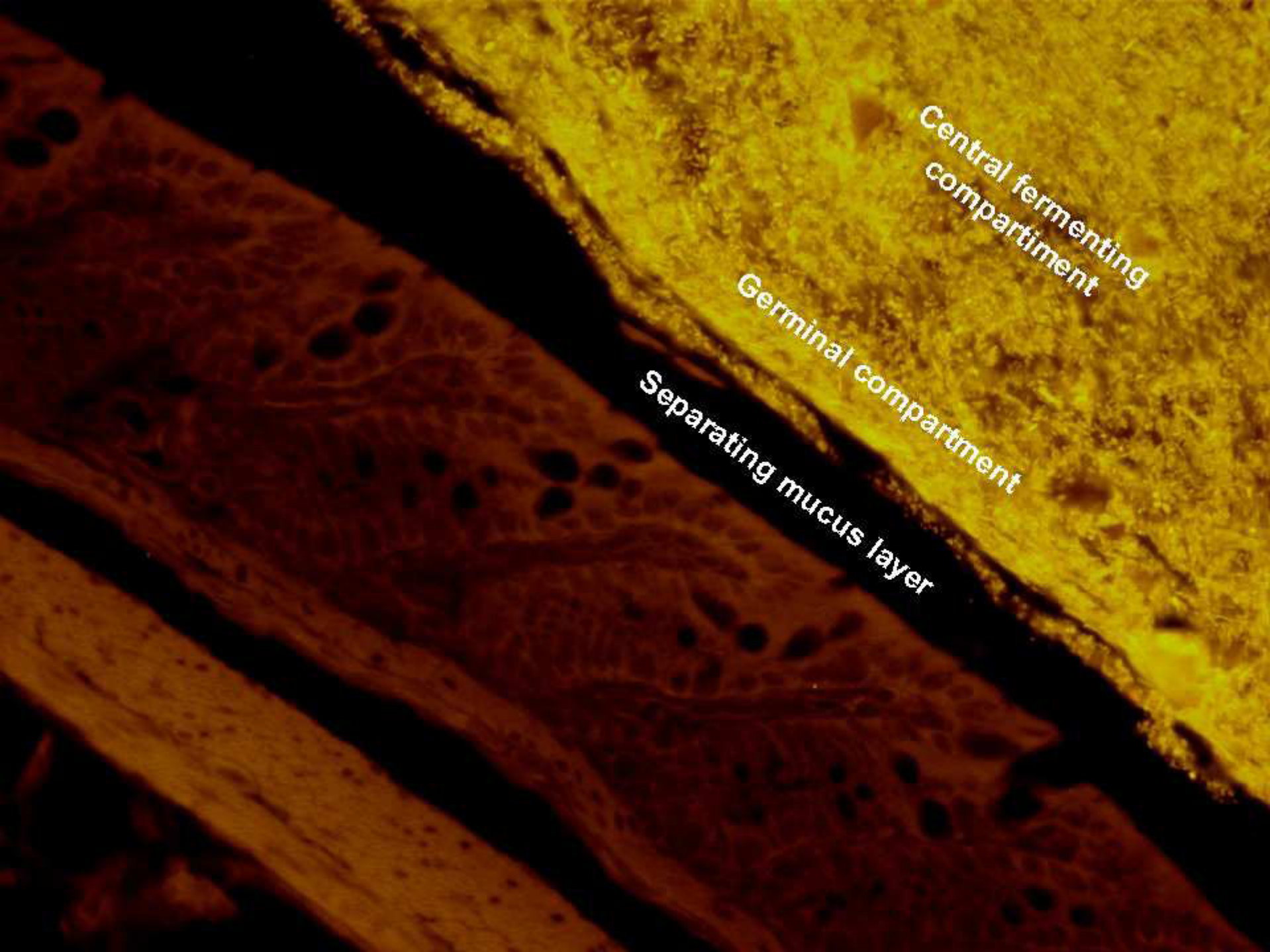
- Structure-functional compartment analysis of colonic microbiota

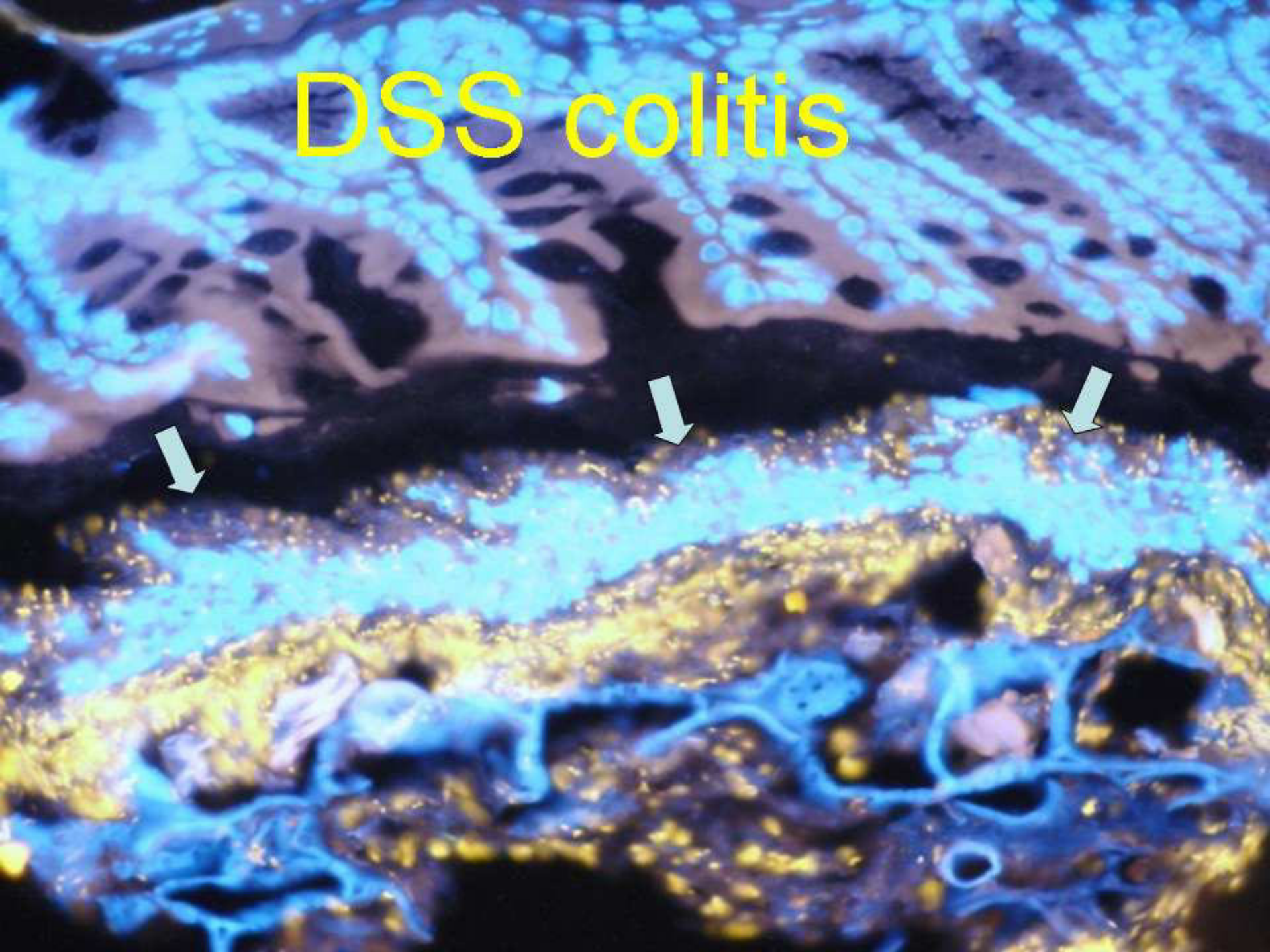
Hazards of colonic bioreaktor

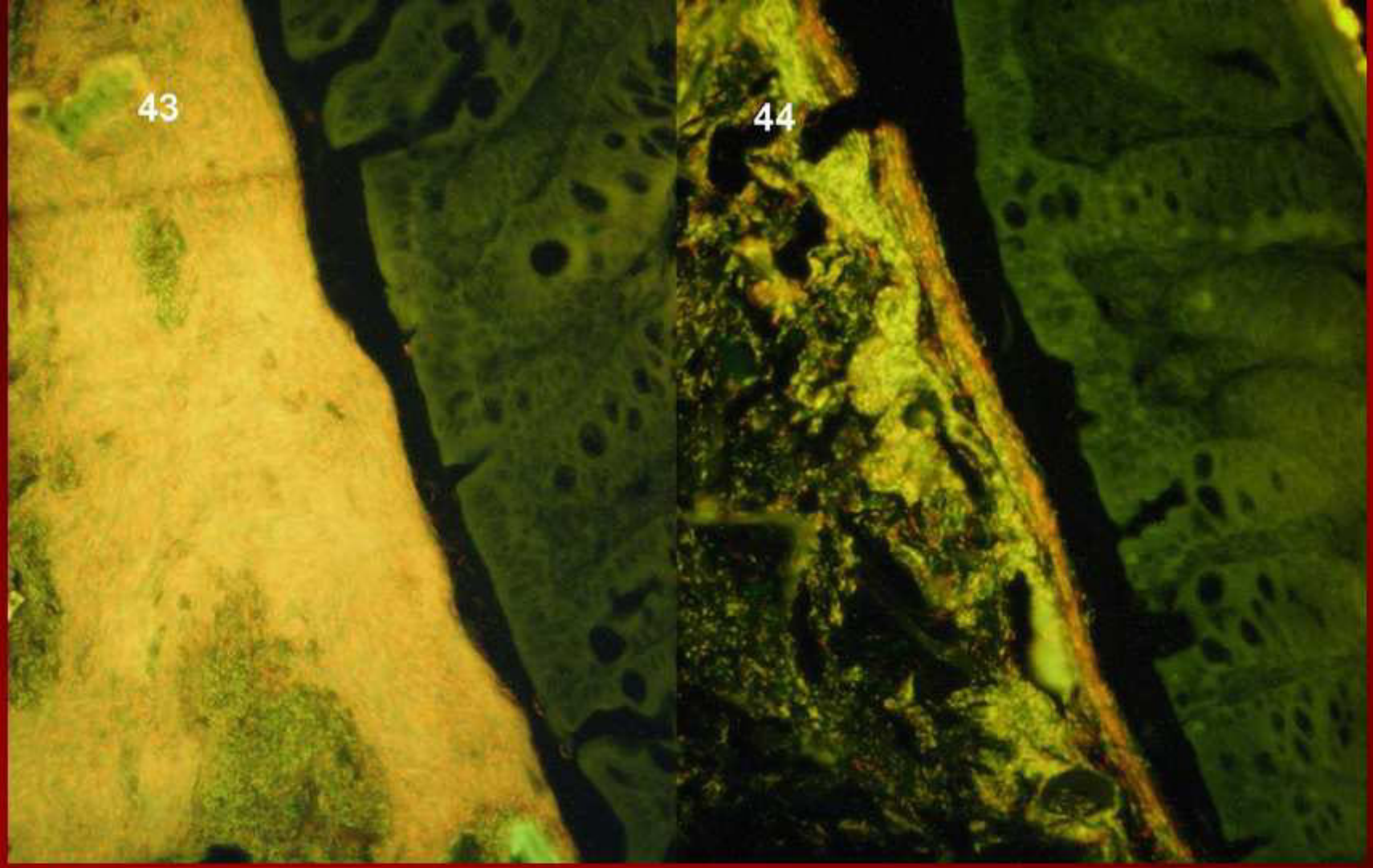


all bacteria 10^{13}

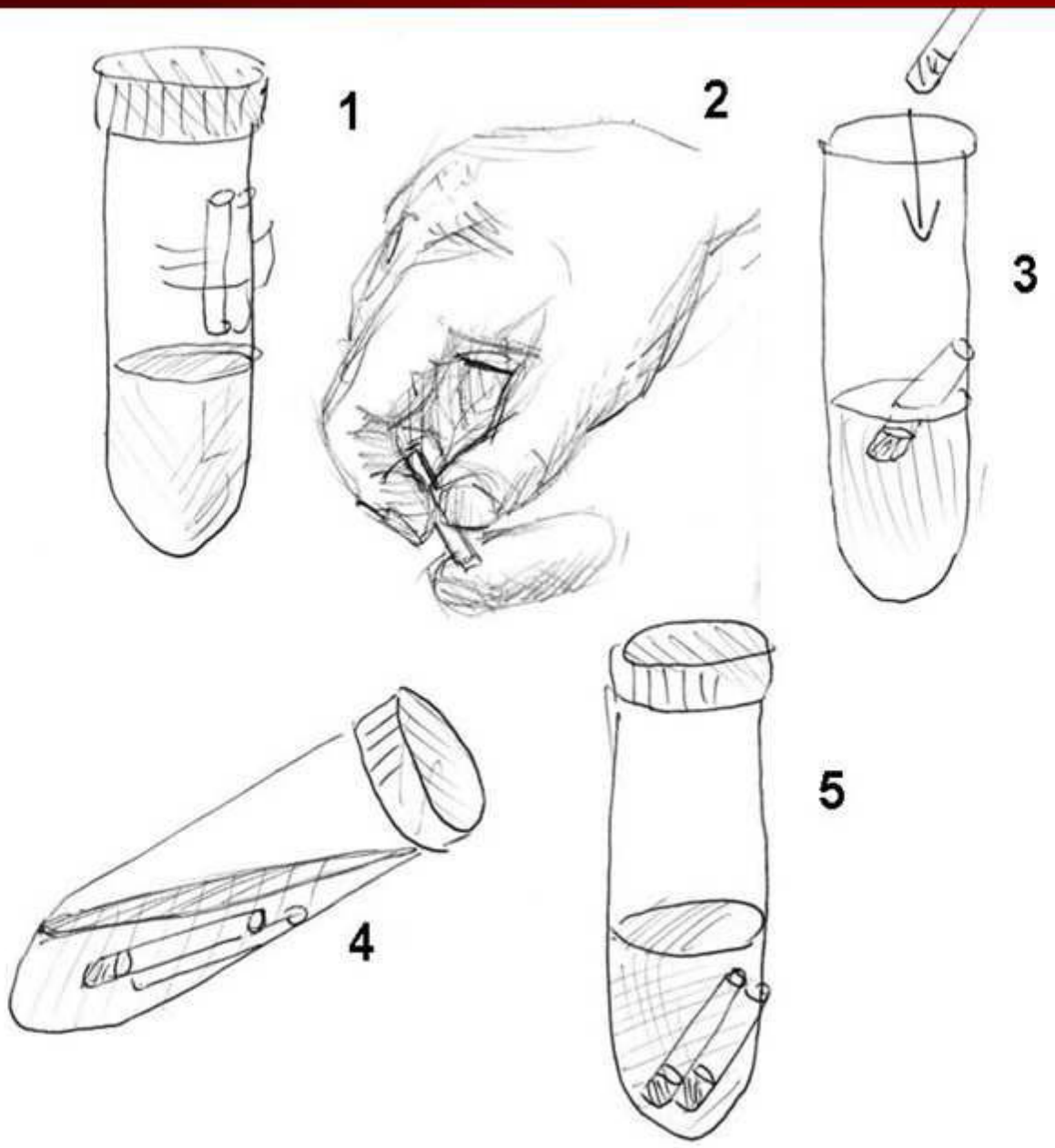
- **>10^{10}/Gr.**
- Clostridium perfringens (gas gangrene)
- Enterococci (Endocarditis)
- Bacteroides (Abscess)
- E.coli (Sepsis)
- 1/5 has Clostridium botulinum !!!

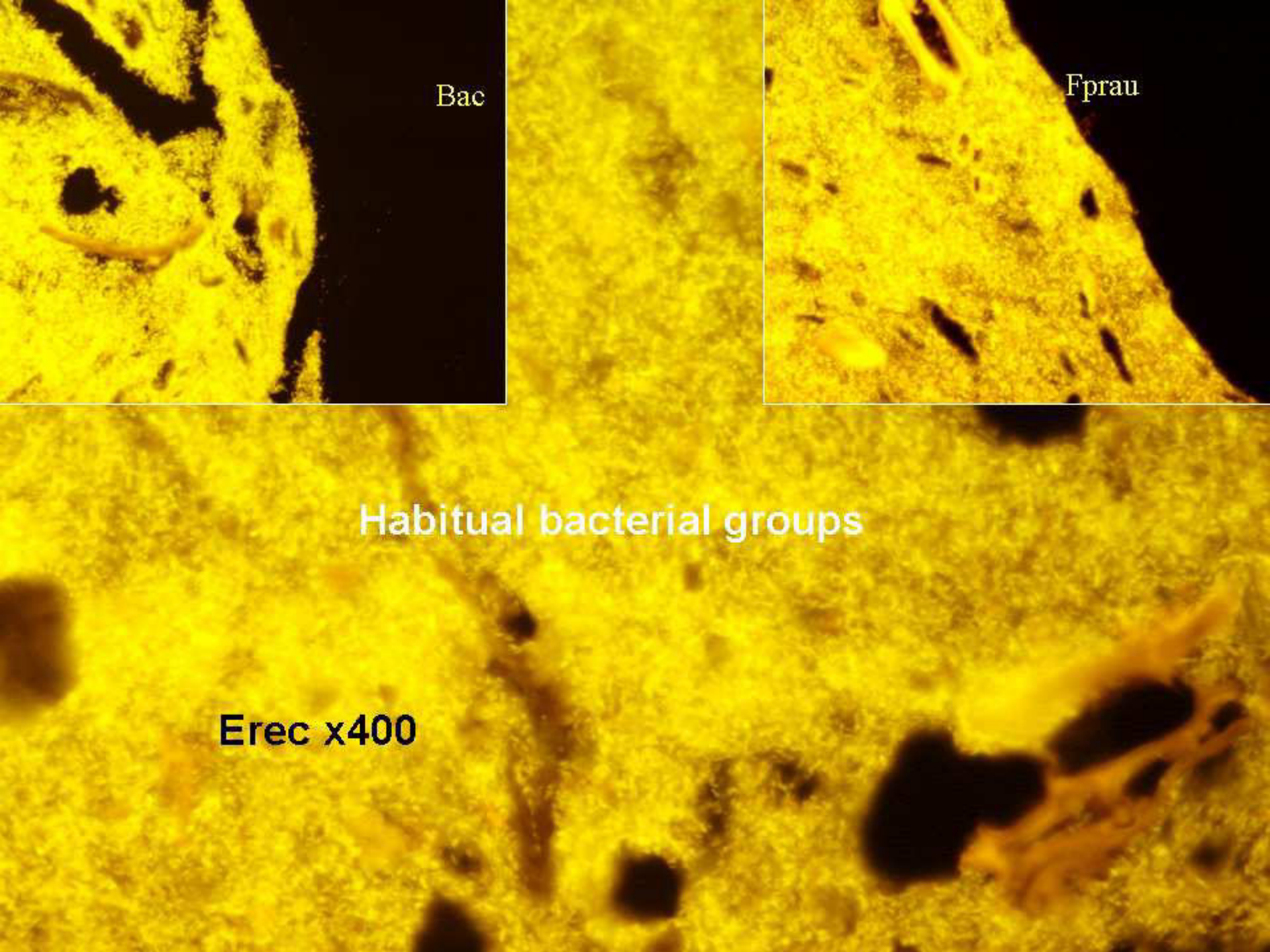

Central fermenting compartiment
Germinal compartment
Separating mucus layer

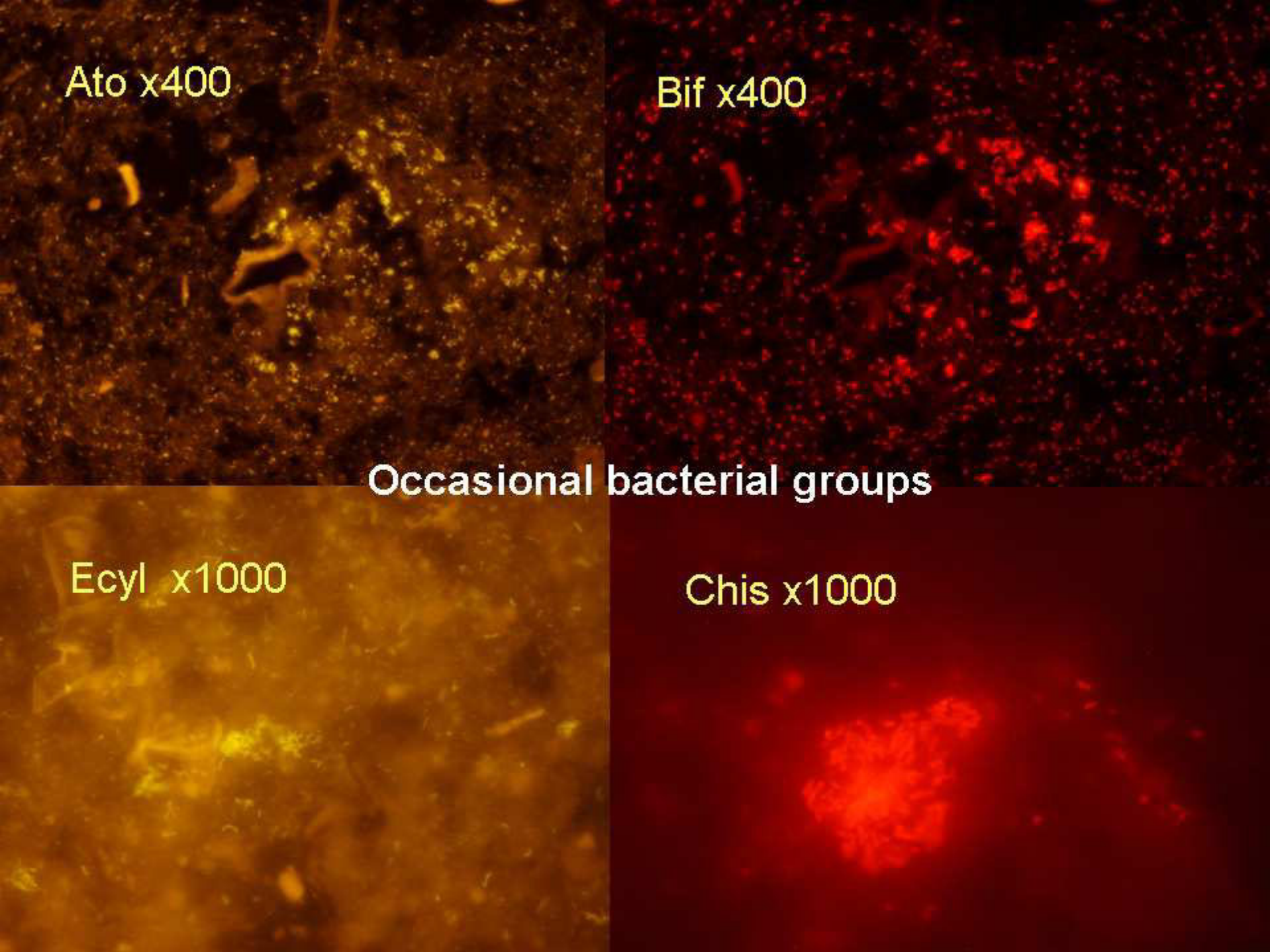

DSS colitis



- Colonic bacteria in the healthy wild-type mouse are diffusely distributed and have similar high concentrations at the center of feces and in the “germinal” zone.
- Bacteria are suppressed in a 28 week-old mouse with IL-10 deficiency, especially at the center of feces. The germinal zone is not involved.


1
2
3
4
5

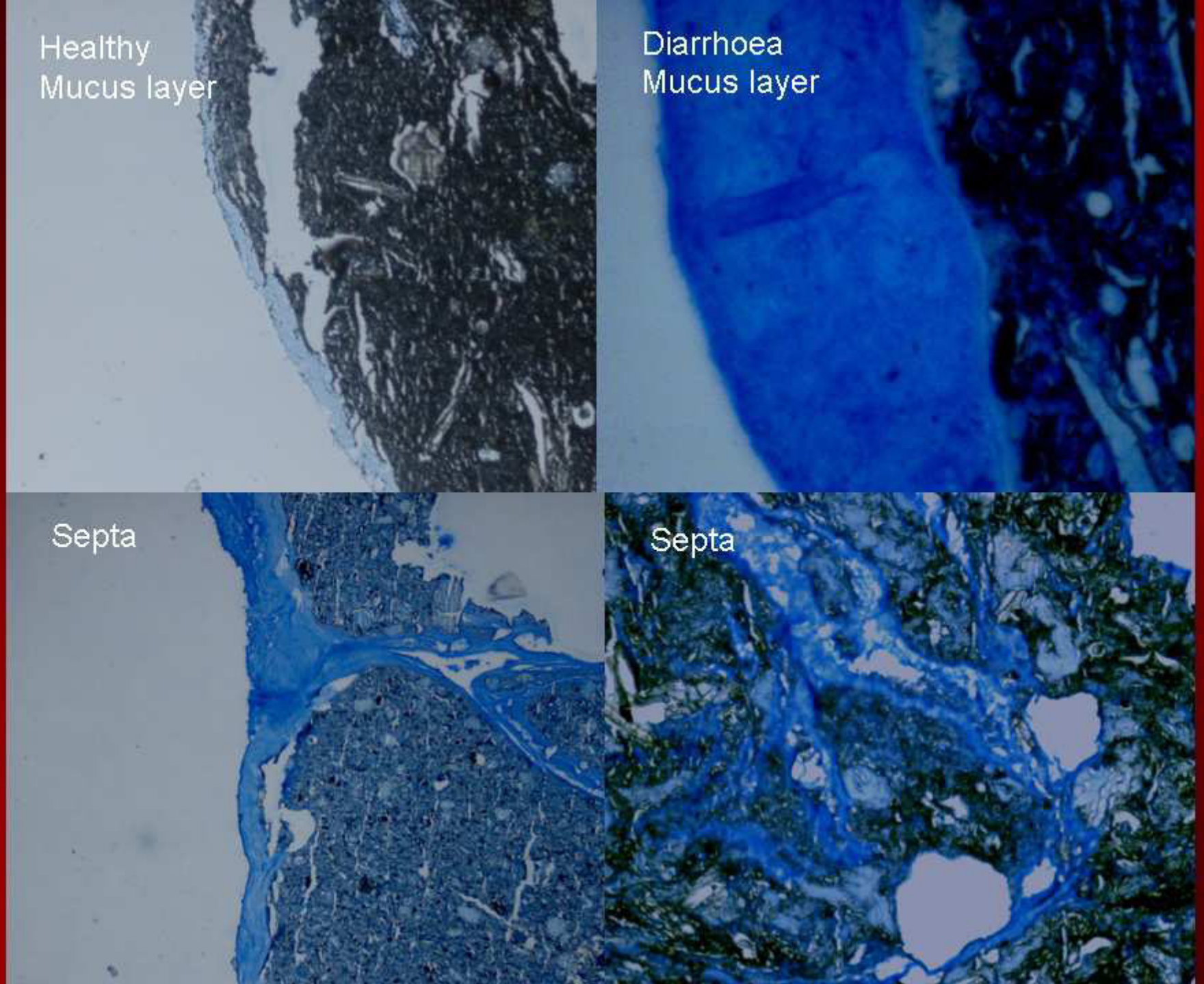

Bac
Fprau
Habitual bacterial groups
Erec x400


Ato x400
Bif x400
Occasional bacterial groups
Ecyl x1000
Chis x1000

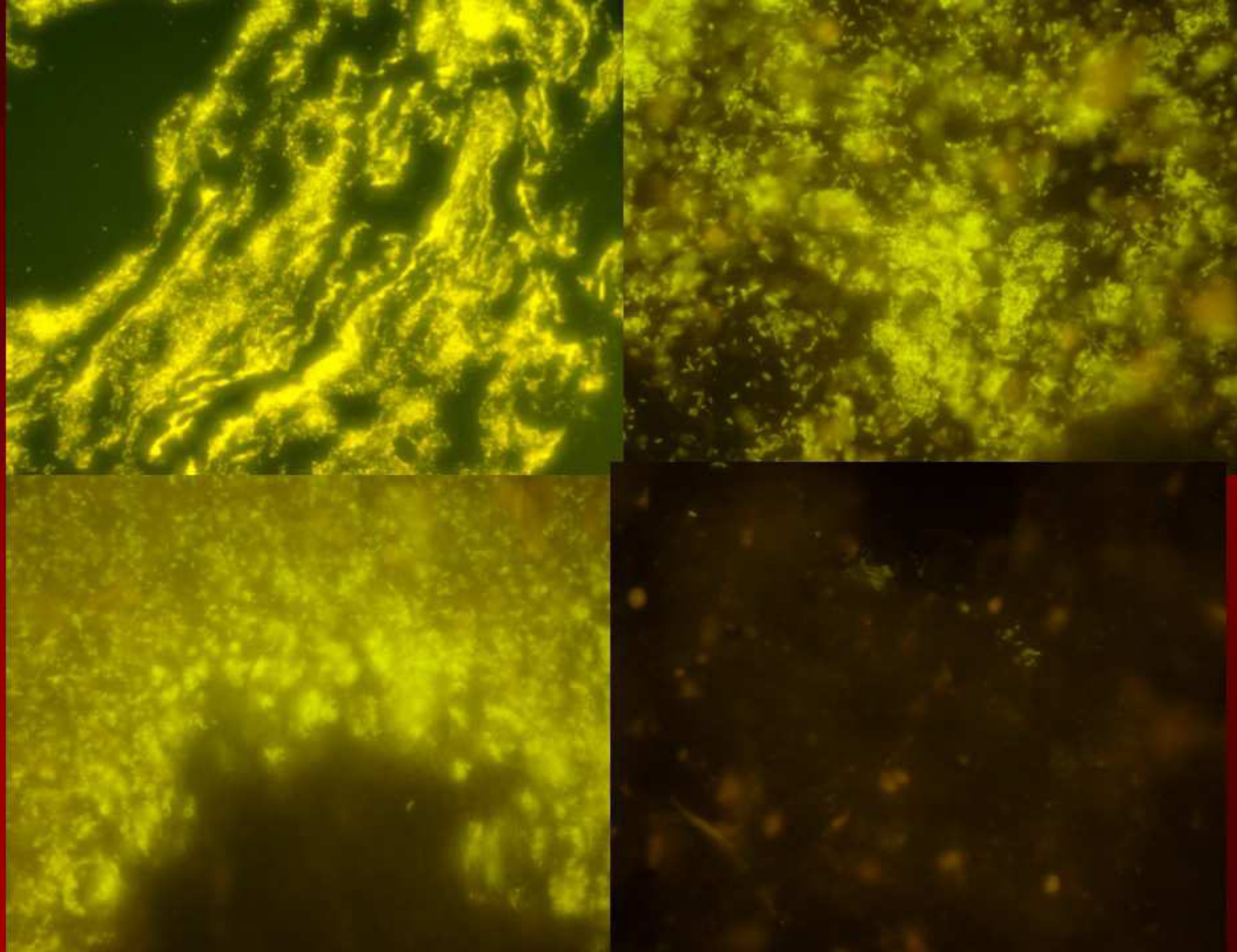
- Protection
- Purging
- Decontamination
- Restocking

- **Protection**

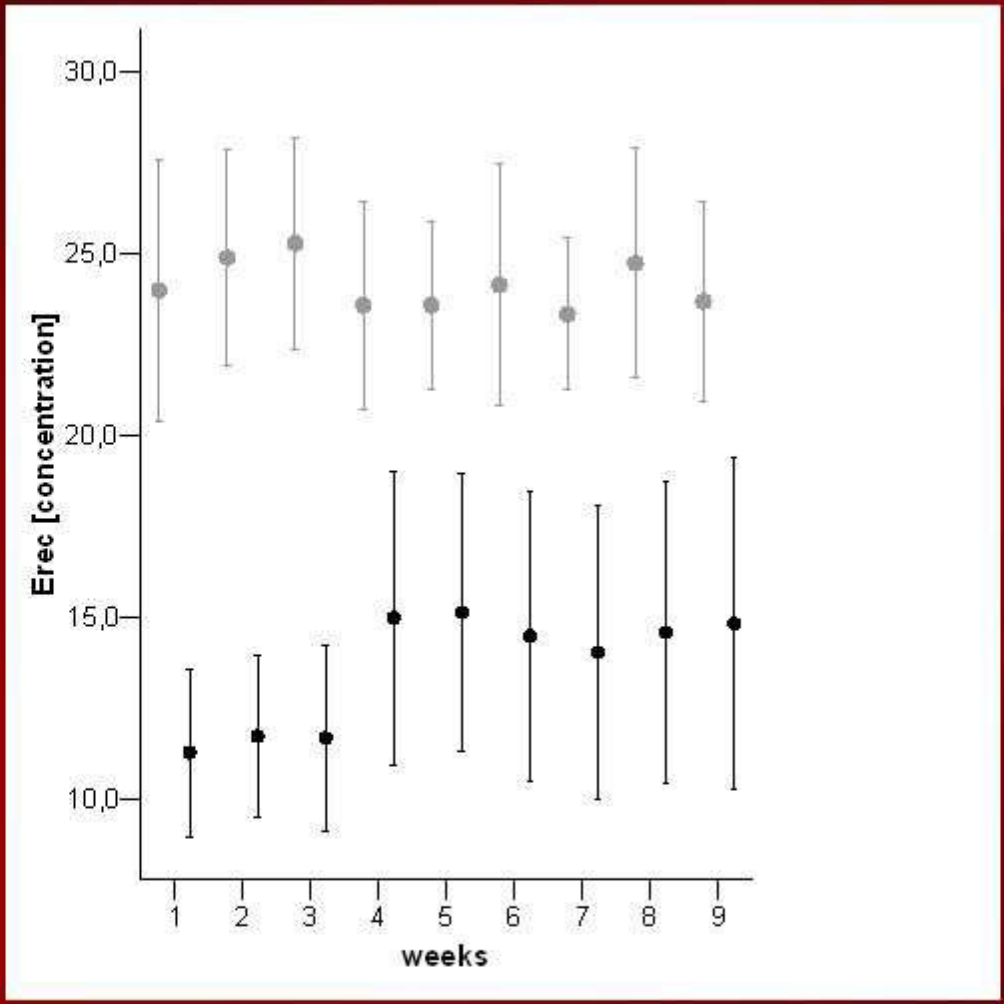
- Mucus thickening,
- Flatulence,
- colic

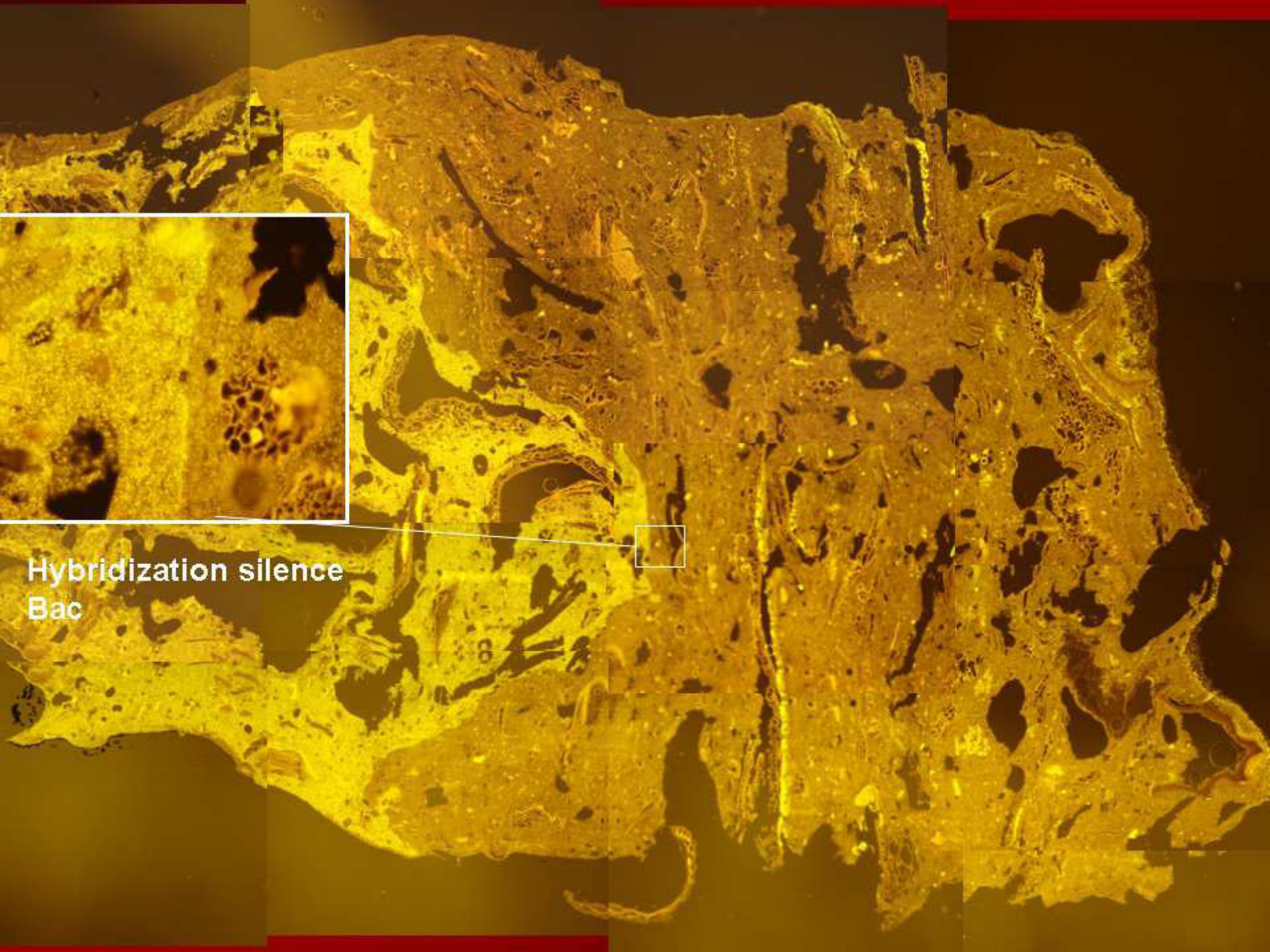

Healthy
Mucus layer
Diarrhoea
Mucus layer
Septa
Septa

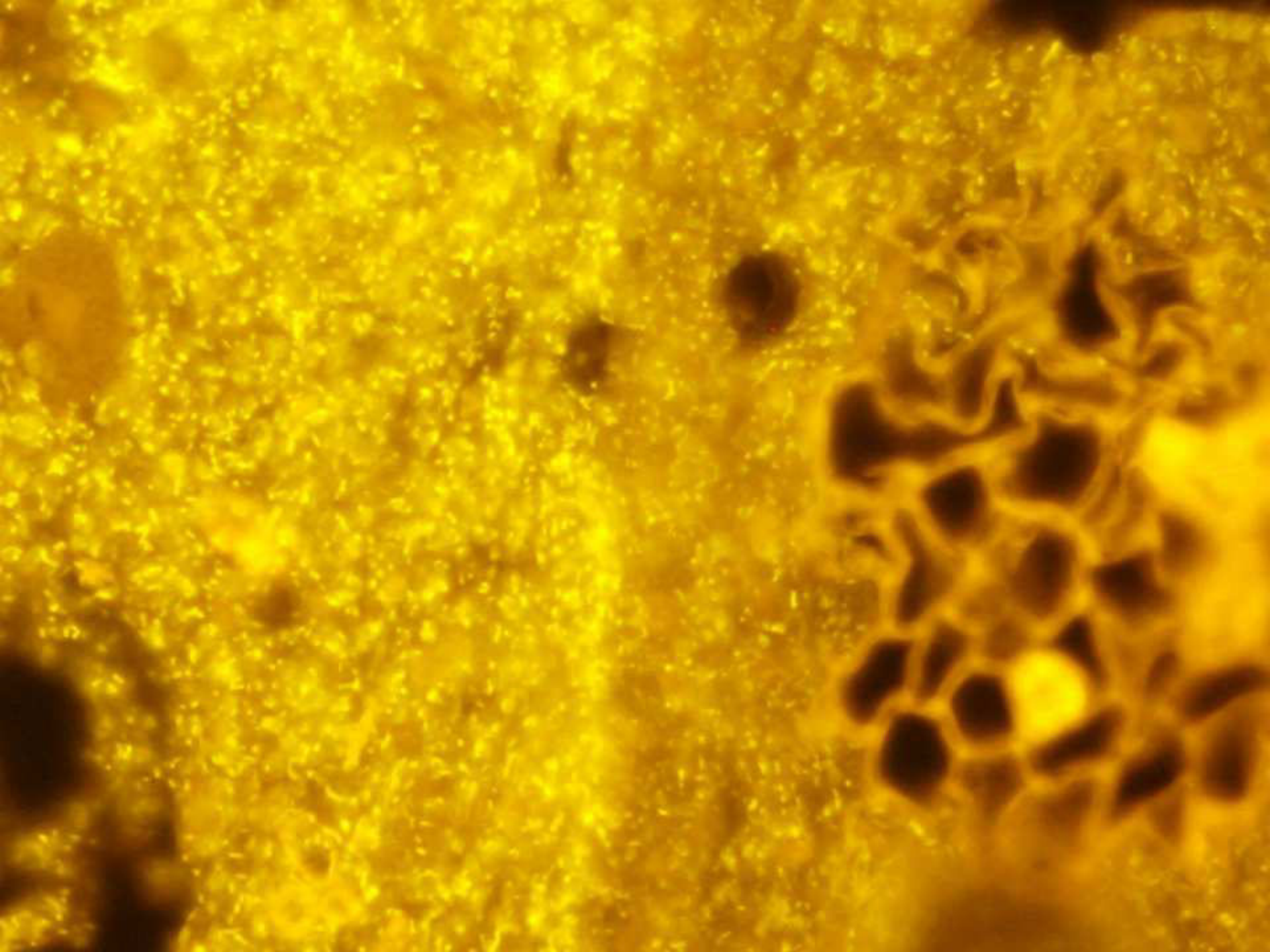
- Purging
- (destructuring of the habitual bacterial groups)



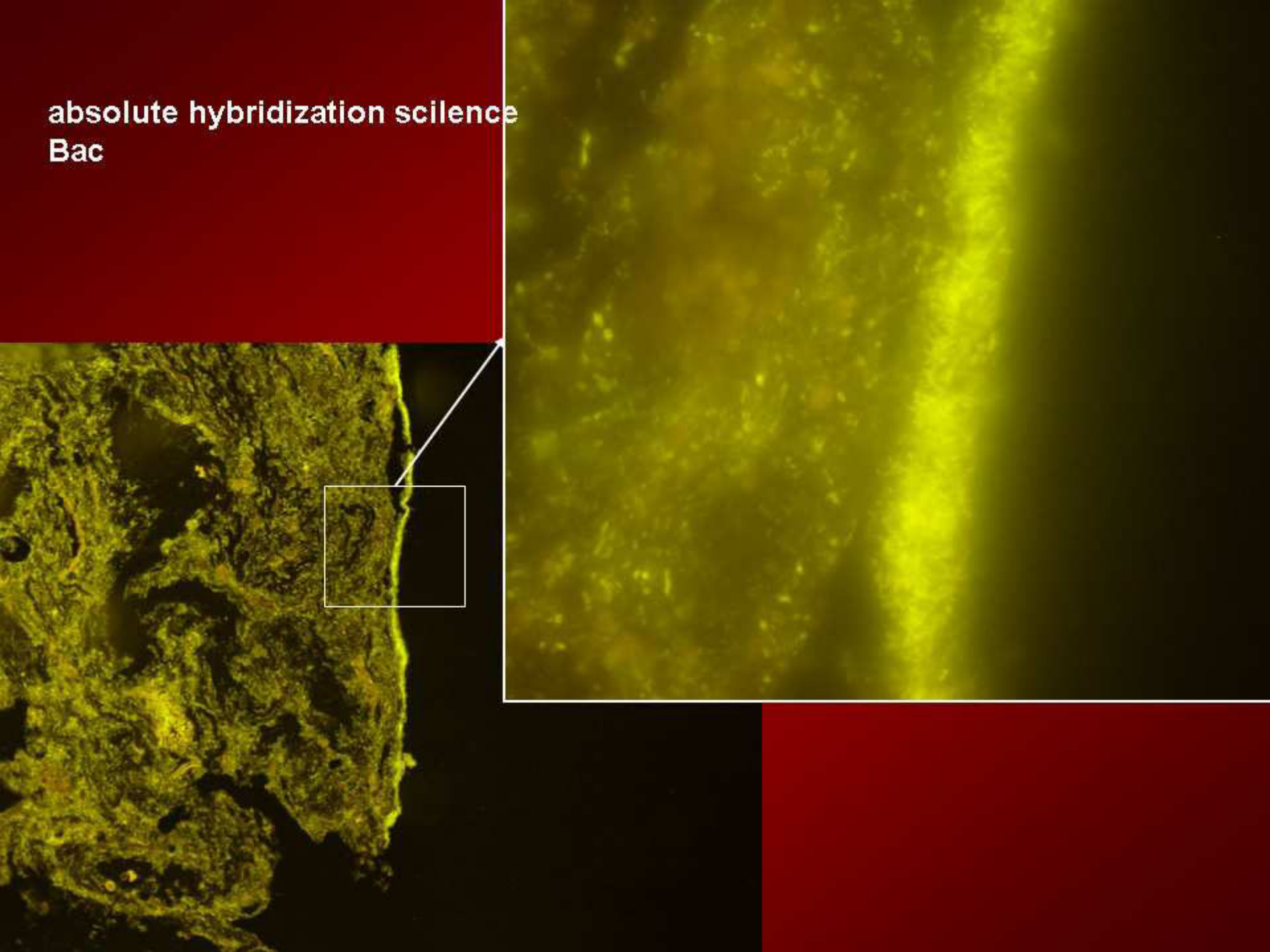
- **Decontamination**
- (Hybridization silence or supression of habitual bacterial groups)

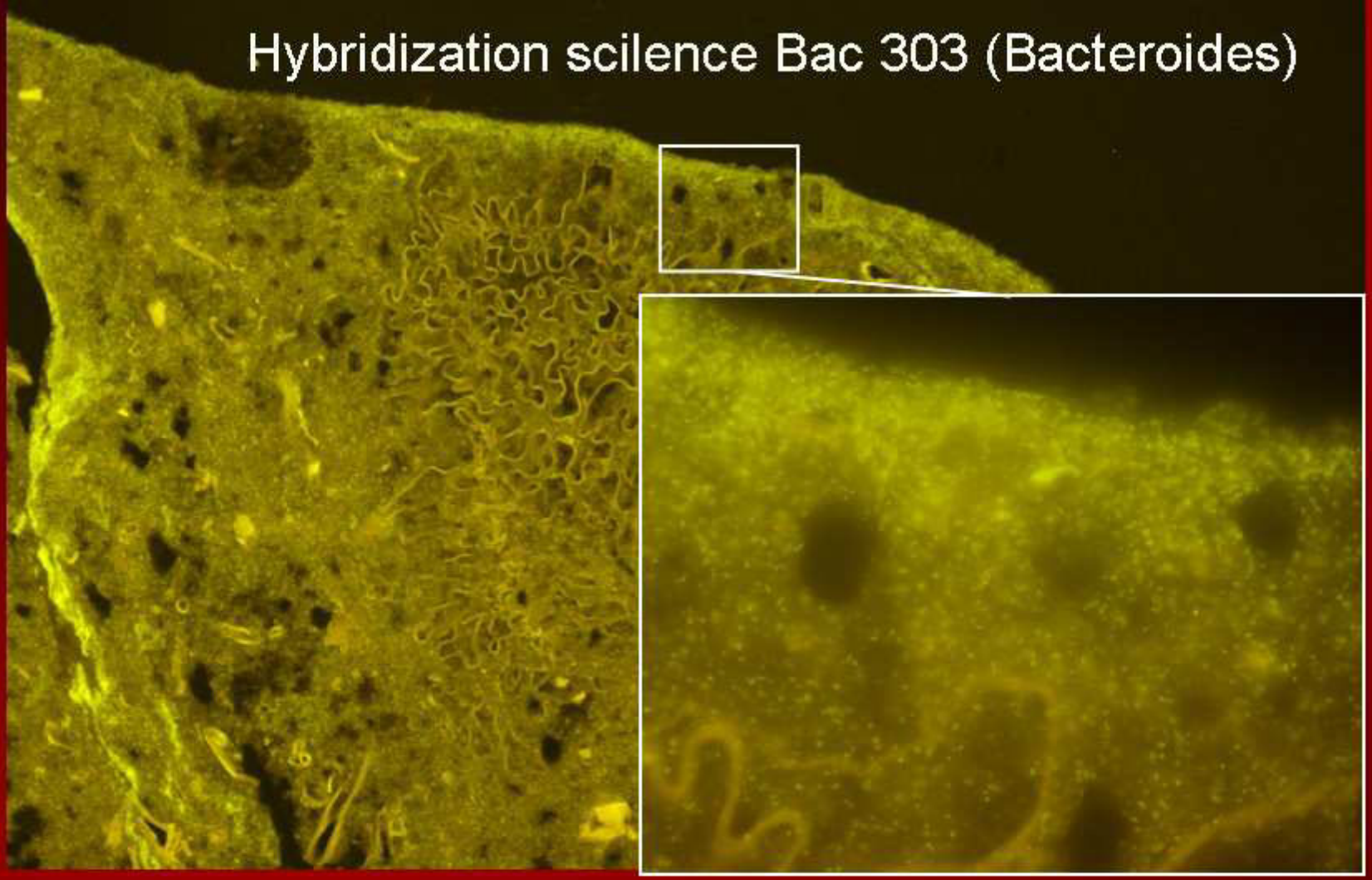

30,0
25,0
20,0
15,0
10,0
Erec [concentration]
1
2
3
4
5
6
7
8
9
weeks

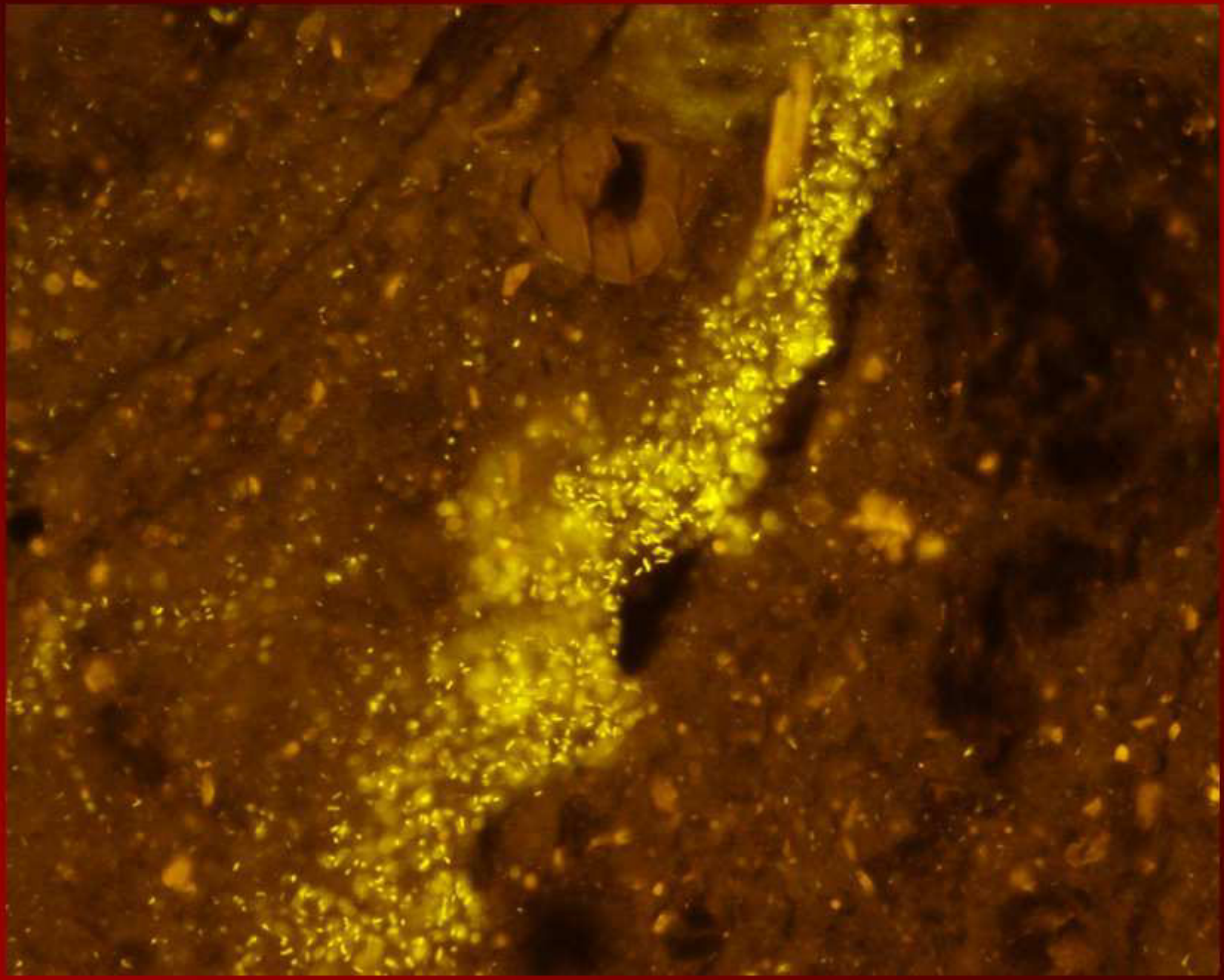

Hybridization silence
Bac



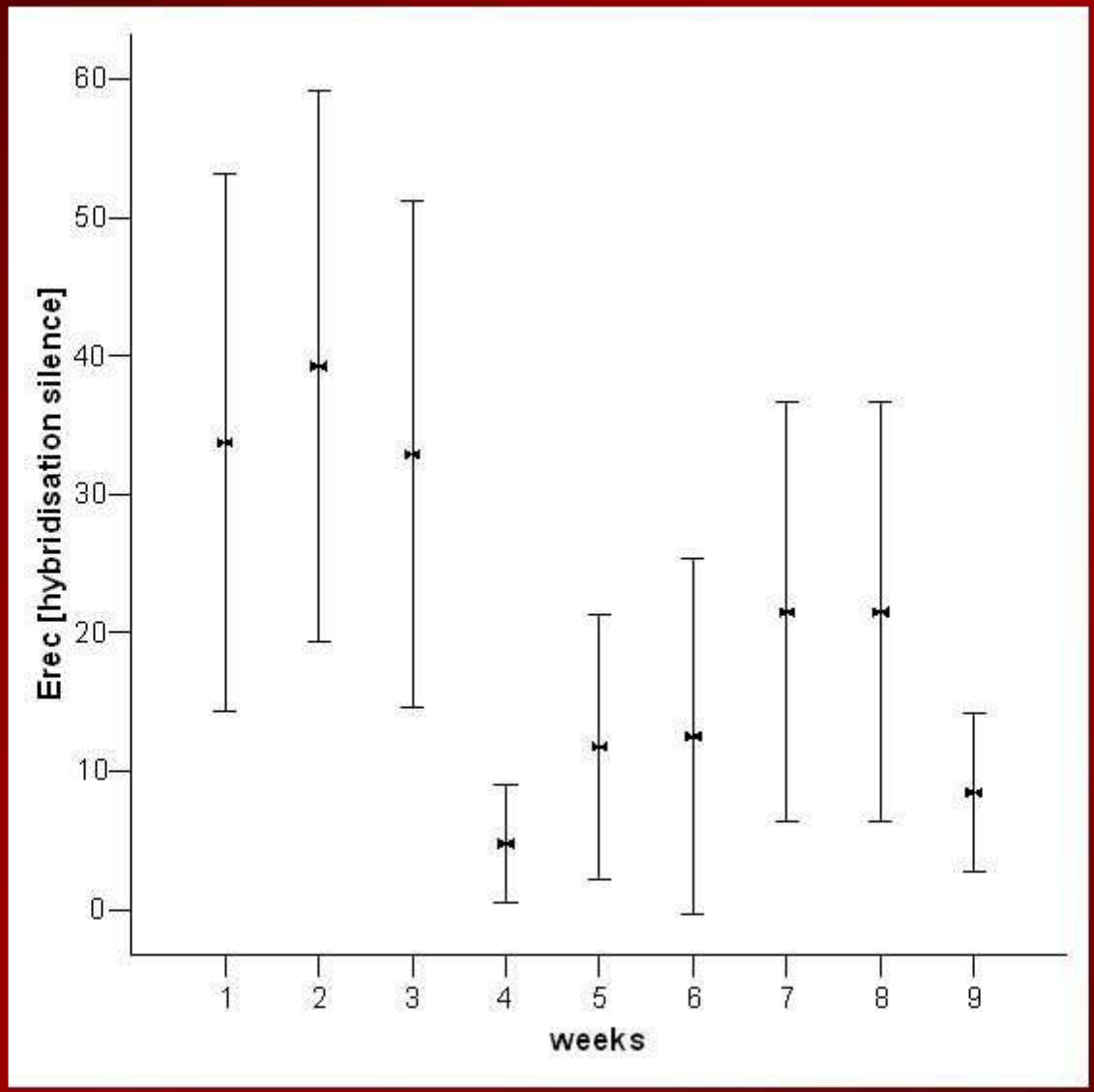
absolute hybridization scilence
Bac



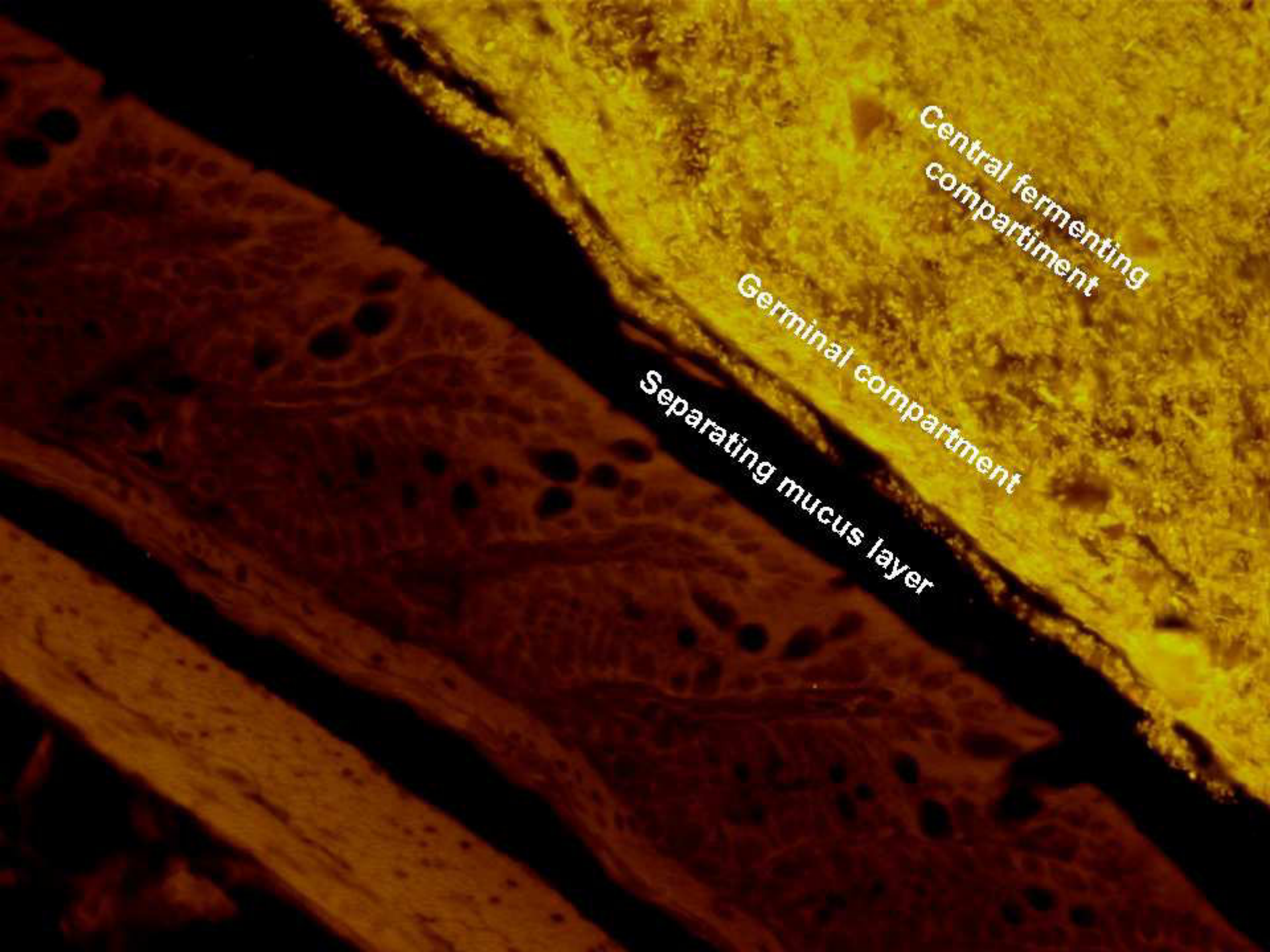

Hybridization scilence Bac 303 (Bacteroides)

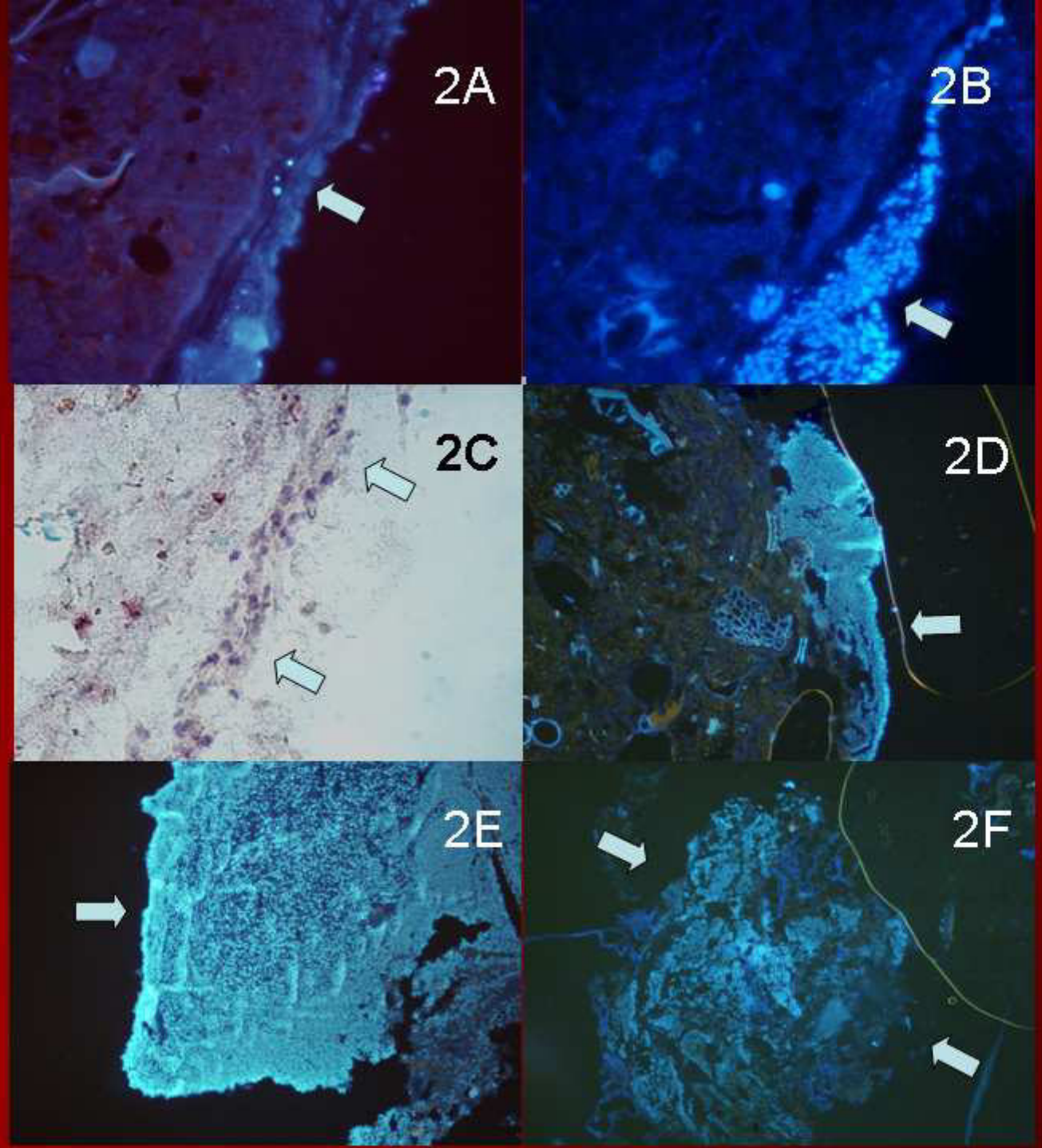


- Restocking


Erec [hybridisation silence]
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
weeks

IBD

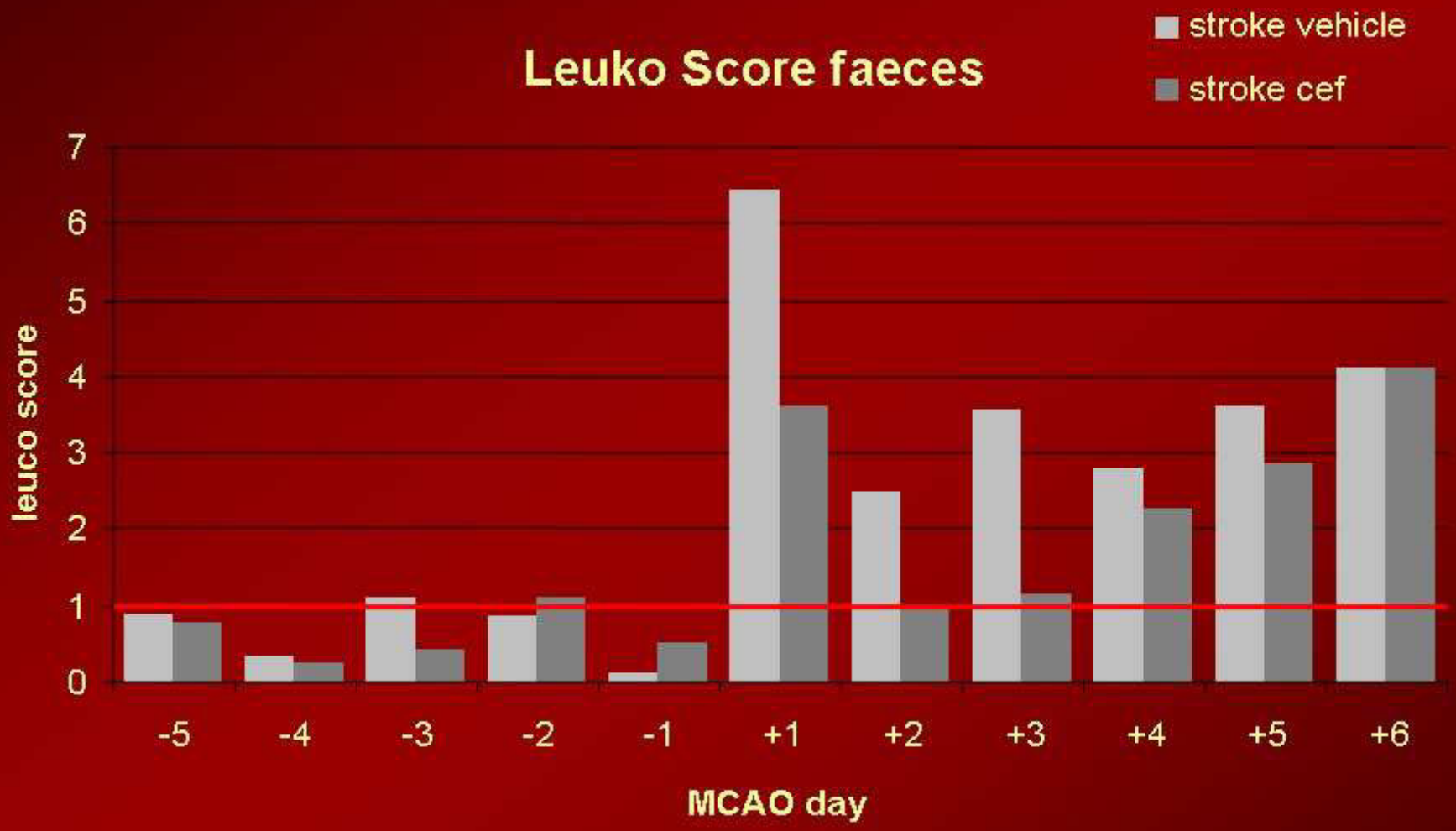

Central fermenting compartiment
Germinal compartment
Separating mucus layer

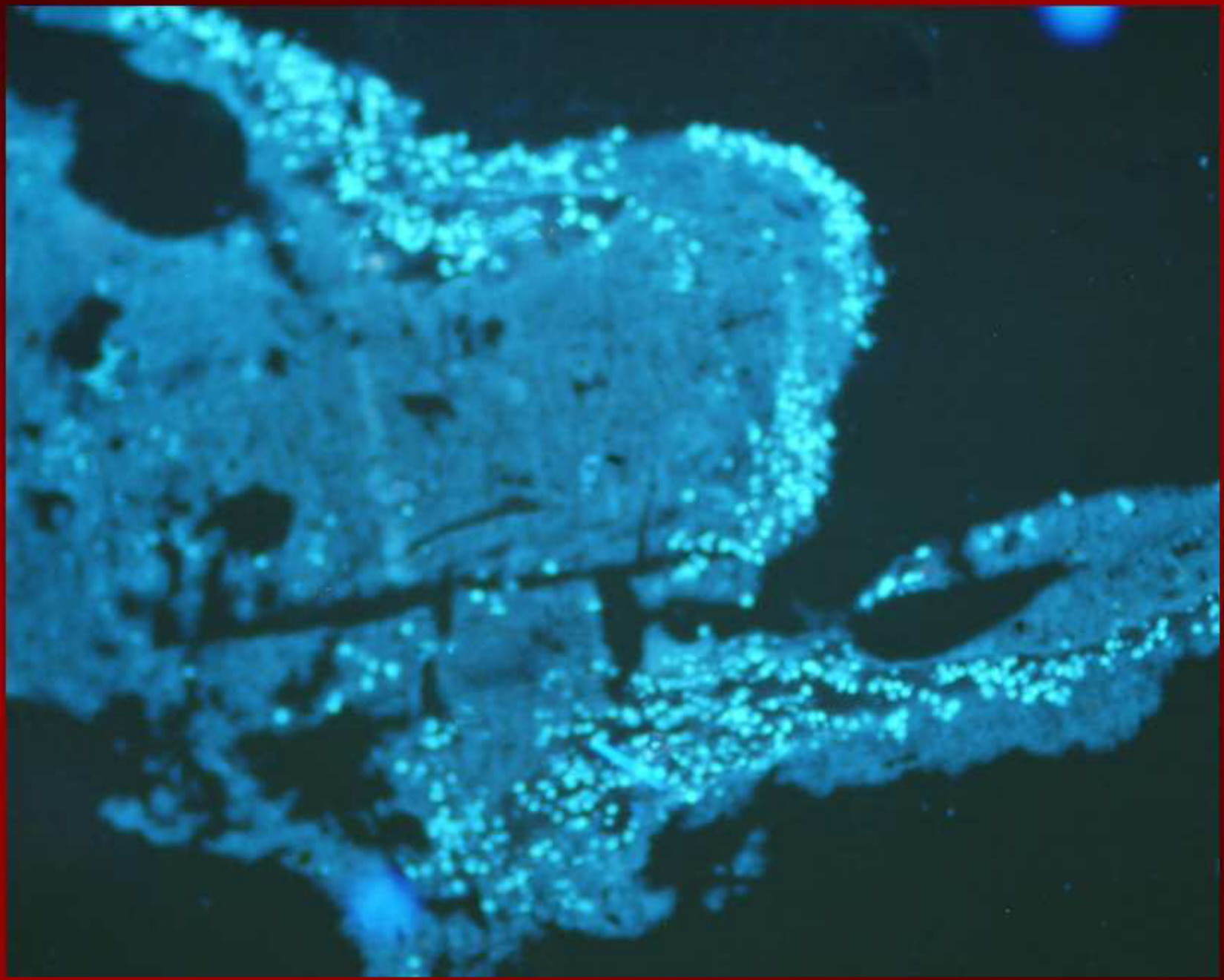

2A
2B
2C
2D
2E
2F

	Ulcerative colitis				Crohn				IC	CD celiac	Slc	Healthy	other
	CAI >9	3≤ CAI ≤9	Rem. ≤1 year	Rem >1 year	CDAI >300	150≤ CDAI ≤300	Rem ≤1 year	Rem >1 year					
85 82	N=27	N=32	N=14	N=32	N=19	N=23	N=18	N=22	N=17	N=12	N=9	N=32	N=165
Fprau <1 Leuko≤30 **Crohnsease pattern**		3%	7%	8%	**84%**	**74%**	**50%**	**18%**	18%	58%			
Fprau >1 Leuko>30 **UC pattern**	**93%**	**69%**	**50%**	**14%**	11%	9%	17%	10%	29%				
Fprau <1 Leuko >30 **Intermediate IBD pattern**	8%	7%	14%	4%	5%	9%			24%				
Leuko≤30 Fprau>1 **Non-inflammatory pattern**	**0**	21%	29%	78%	**0**	9%	33%	72%	29%	42%	**100%**	**100%**	**100%**

Stroke


Ratten Kolon nach dem Abbinden der A cerebri media

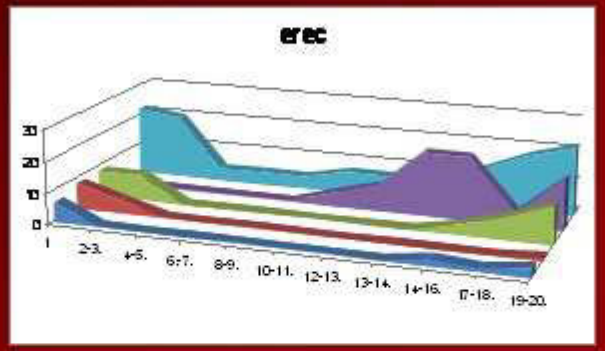

Leuko Score faeces
stroke vehicle
stroke cef
leuco score
0
1
2
3
4
5
6
7
-5
-4
-3
-2
-1
+1
+2
+3
+4
+5
+6
MCAO day

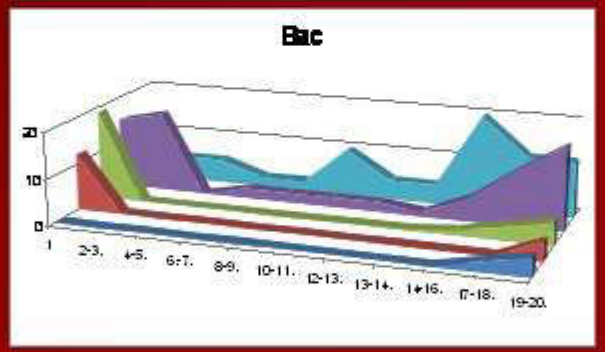


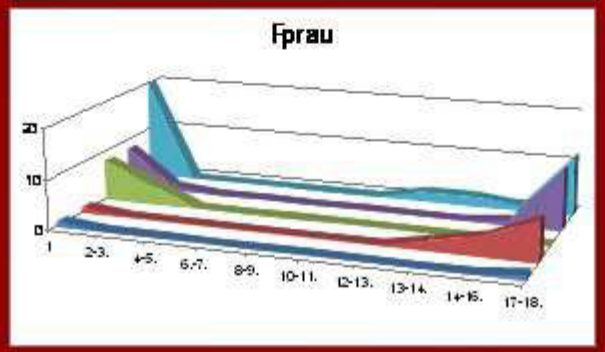
uc

Stroke, day 2

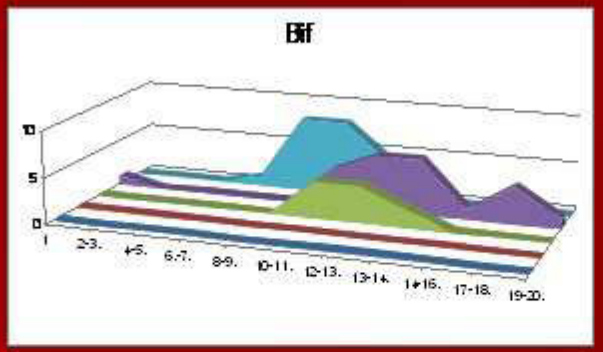
Habitual Bacteria

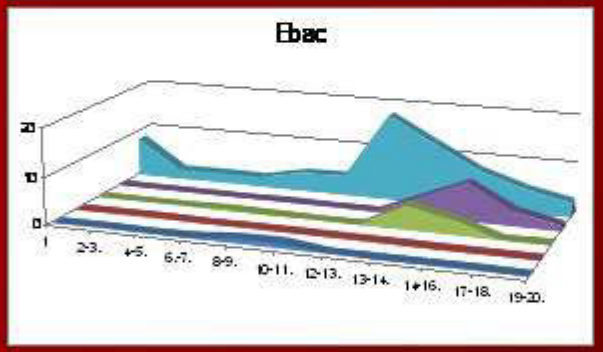


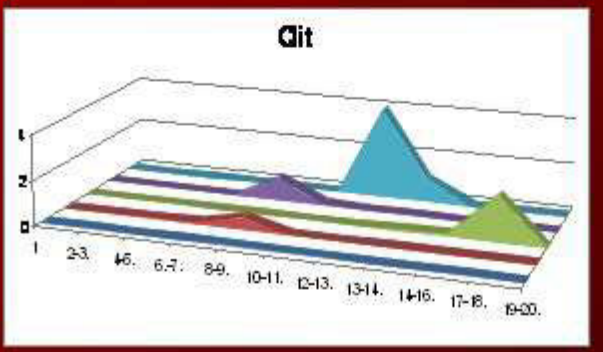




Occational Bacteria







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Spatial organisazation of intestinal microbiota

English www.charite.de/arbmkl Arbeiten aus der Medizinischen Klinik der Charite'

Kontakt

Molekulargenetisches Labor für polymikrobielle Infektionen und bakterielle Biofilme

Darm Publikationen	Galle Publikationen	HNO Publikationen	Haut Seite in Arbeit
	Übersichts-Arbeiten	Uro-genital Publikationen	Veterinär
Zusätzliche Informationen Darm von Innen	Patientenseite	 FISH-Methode Seite in Arbeit	Nachdenkliches

Die ärztliche Tätigkeit am Krankenbett, im Labor, Lehre, Forschung, Gesellschaft und Kultur lässt sich nicht in den engen Rahmen einer wissenschaftlichen Publikation unterbringen. So geht eine Fülle an wertvollem Material verloren. Die vorliegende Homepage soll nach und nach Beiträge zugänglich machen, die wegen ihrer Größe oder Form nicht publiziert worden sind. A. Swidsinski

Publikationen
Präsentationen
Projekte
Nachdenkliches
Zusätzliche Informationen

Patientenseite

Appendizitis
CED
Darmkrebs
Tonsillitis
Vaginose
Harnwegsinfektionen
Schlaganfall
Hp Gastritis
NET/Karzinoid
Rheuma

Possible areas of cooperation:

Influence of probiotics, foods and foods ingredients on function of colonic bioreactor: increasing output of main fermentative habitual bacterial groups (Roseburia, Fecalibacterium prausnitzii, Bacteroides), maintaining stability of individual biodiversity, reduction in concentrations of substitutive bacterial groups, positive effects on clinical symptoms

- IBS
- IBD
- Celiac disease
- Bacterial overgrowth

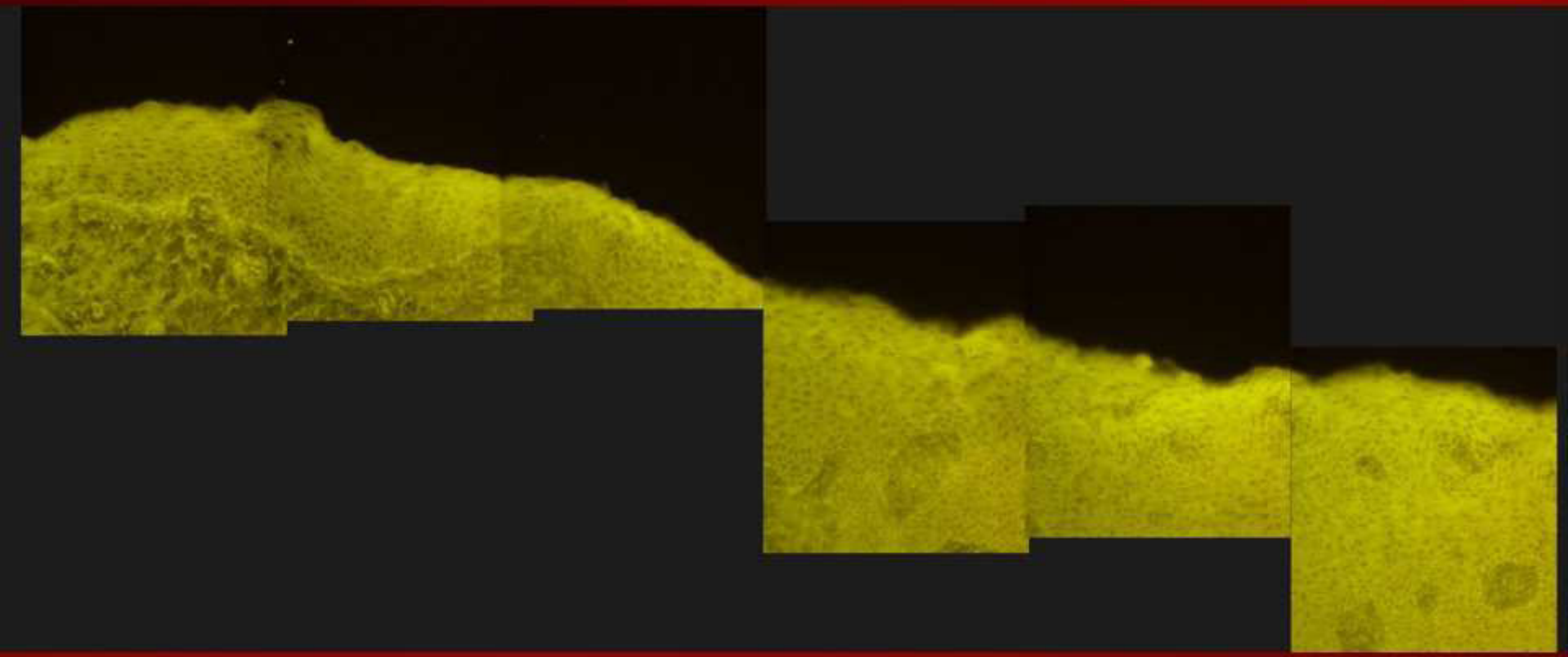
Reprogramming of colonic microbiota (real option to fecal transplantation)

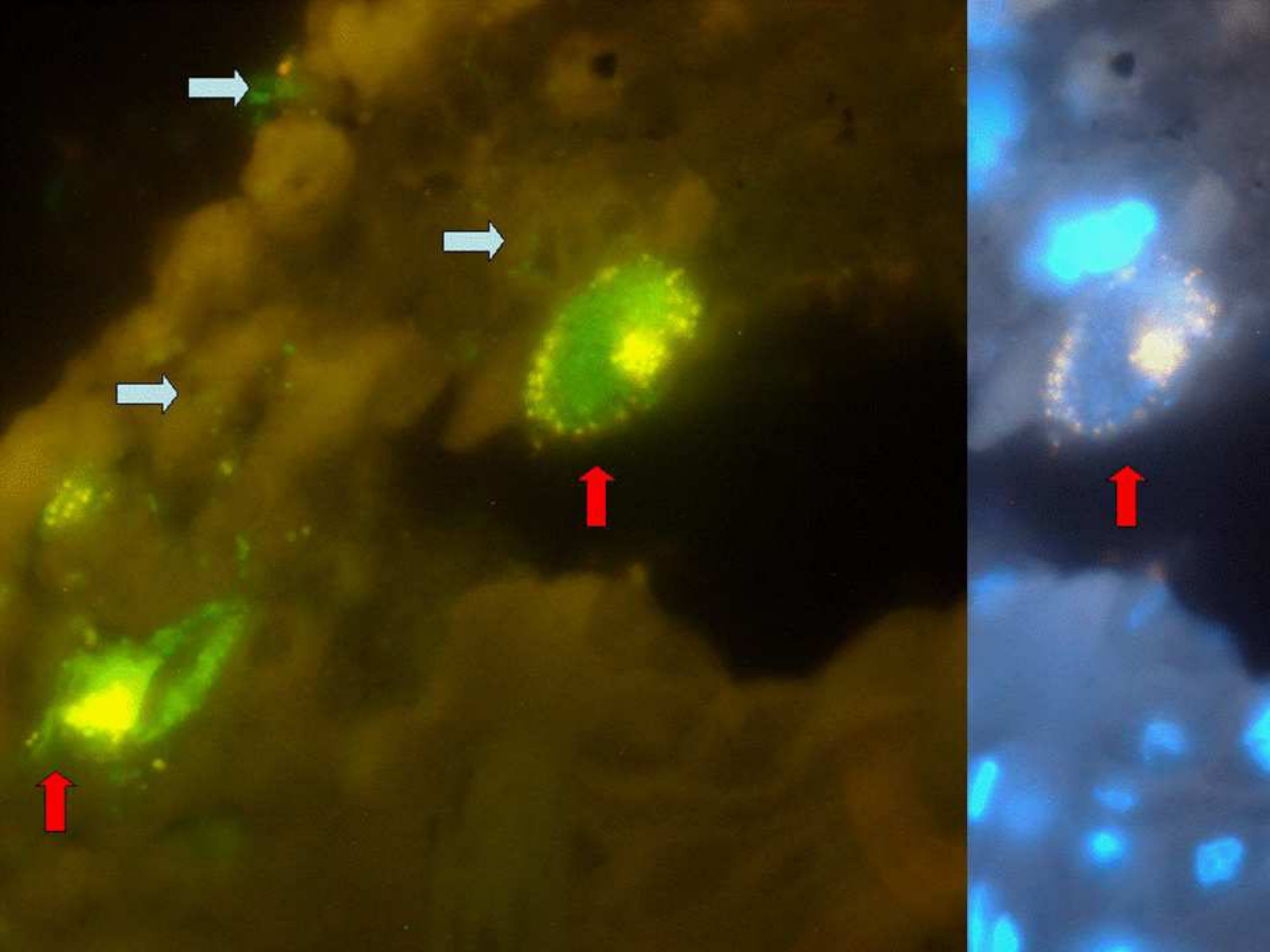
- NASH
- Obesity
- Chronic fatigue (burn out) syndrome

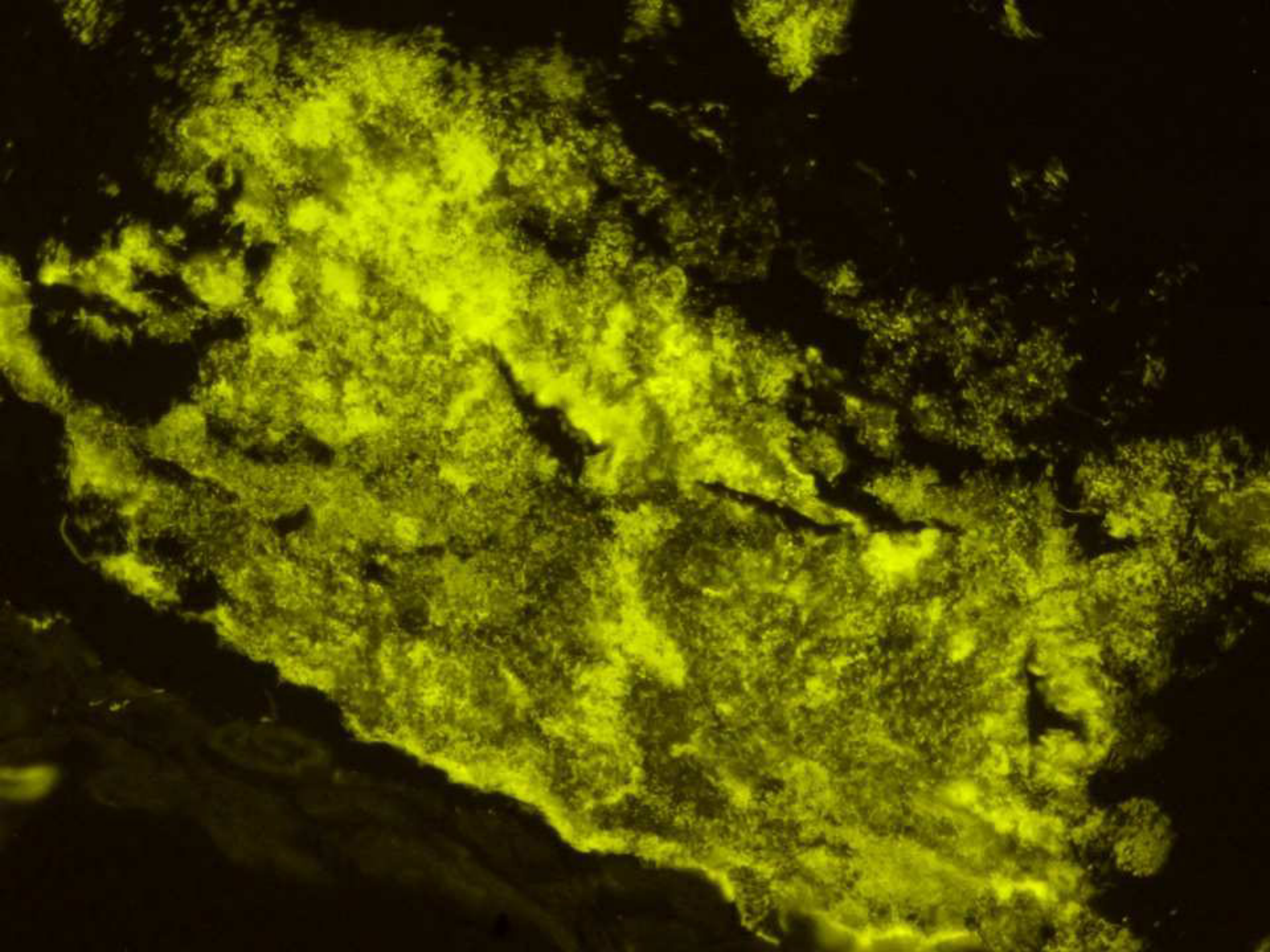
Effects of probiotics or prebiotics on mouth health

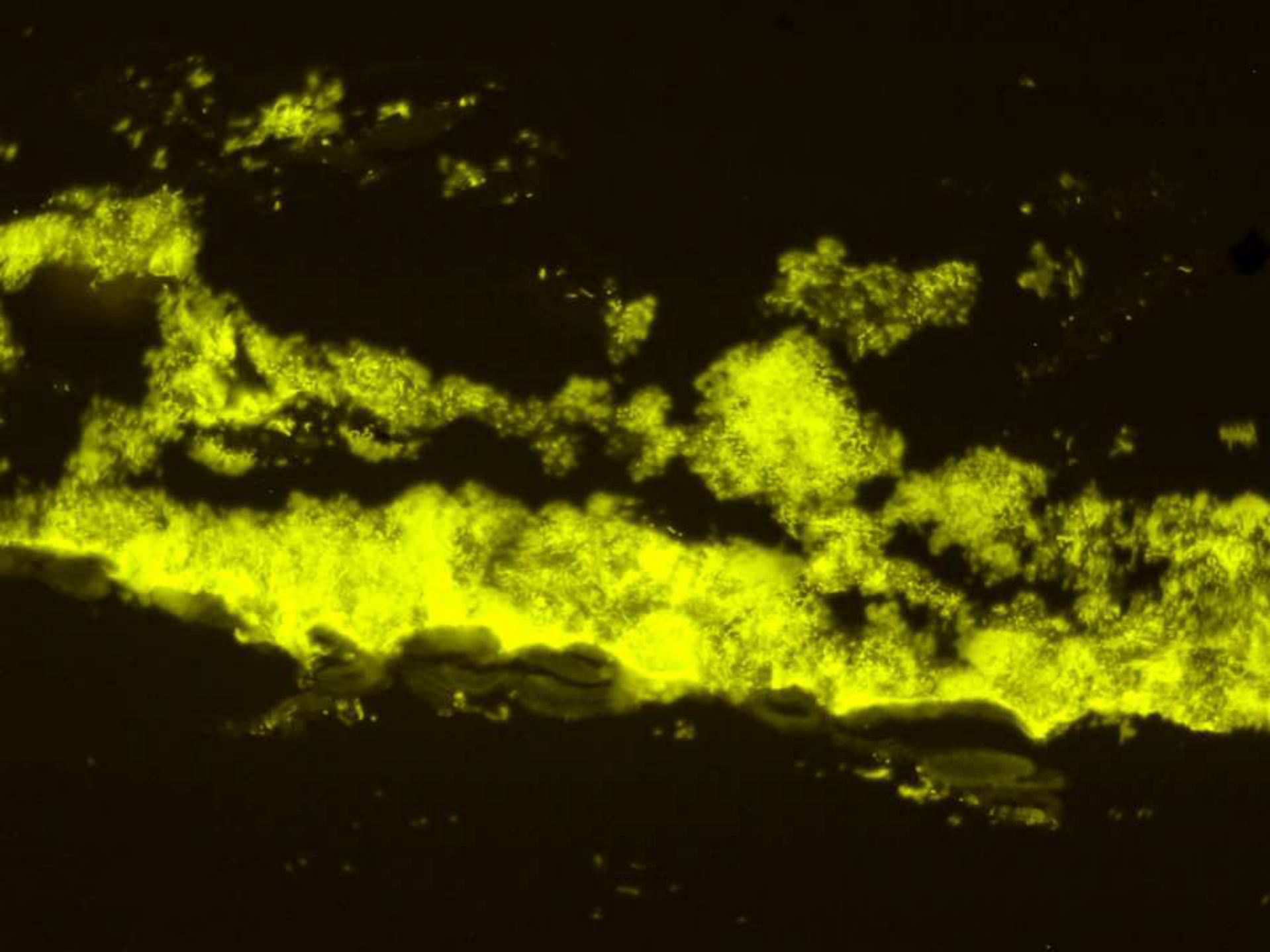
- Halitosis
- Gingivitis

Mund

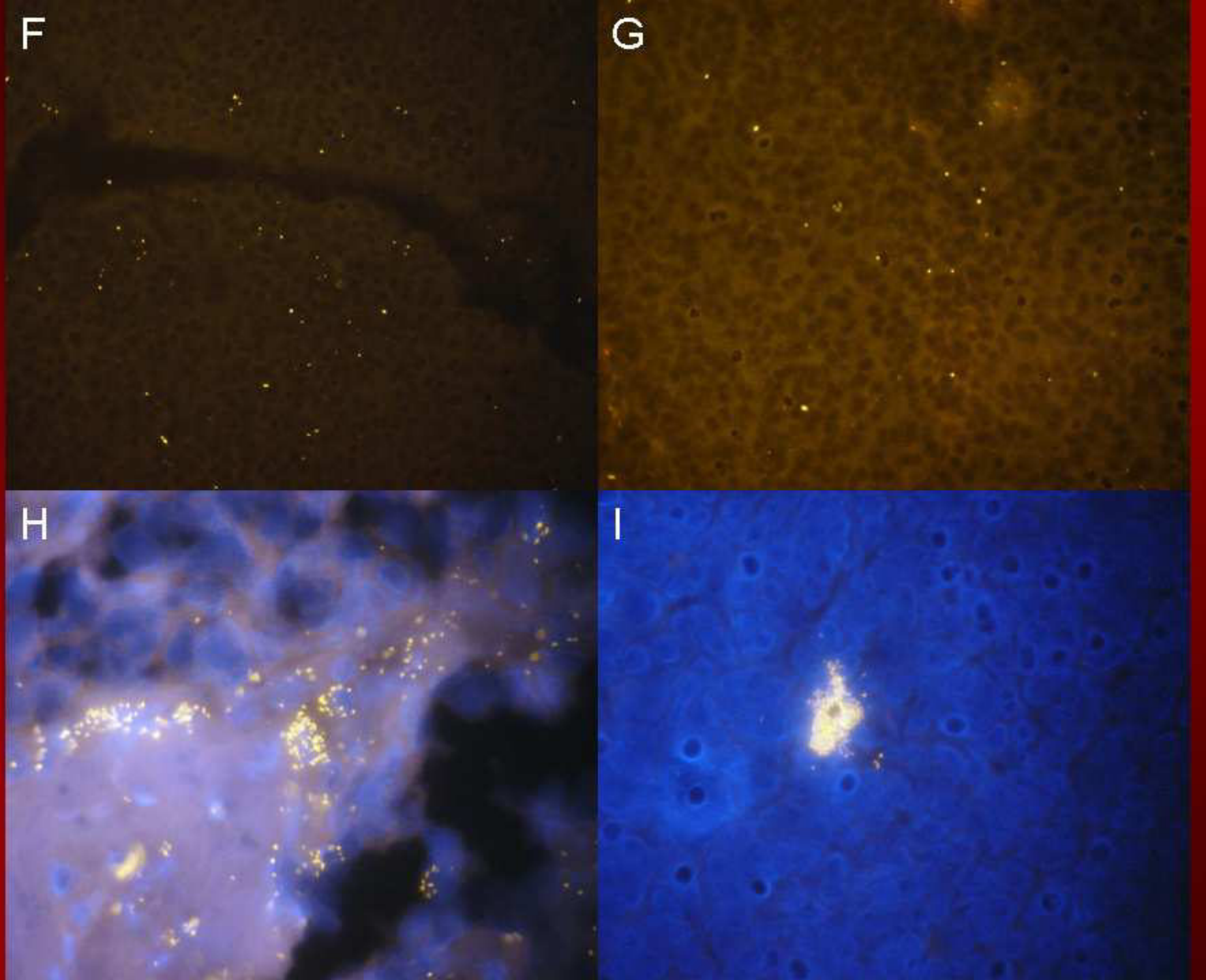


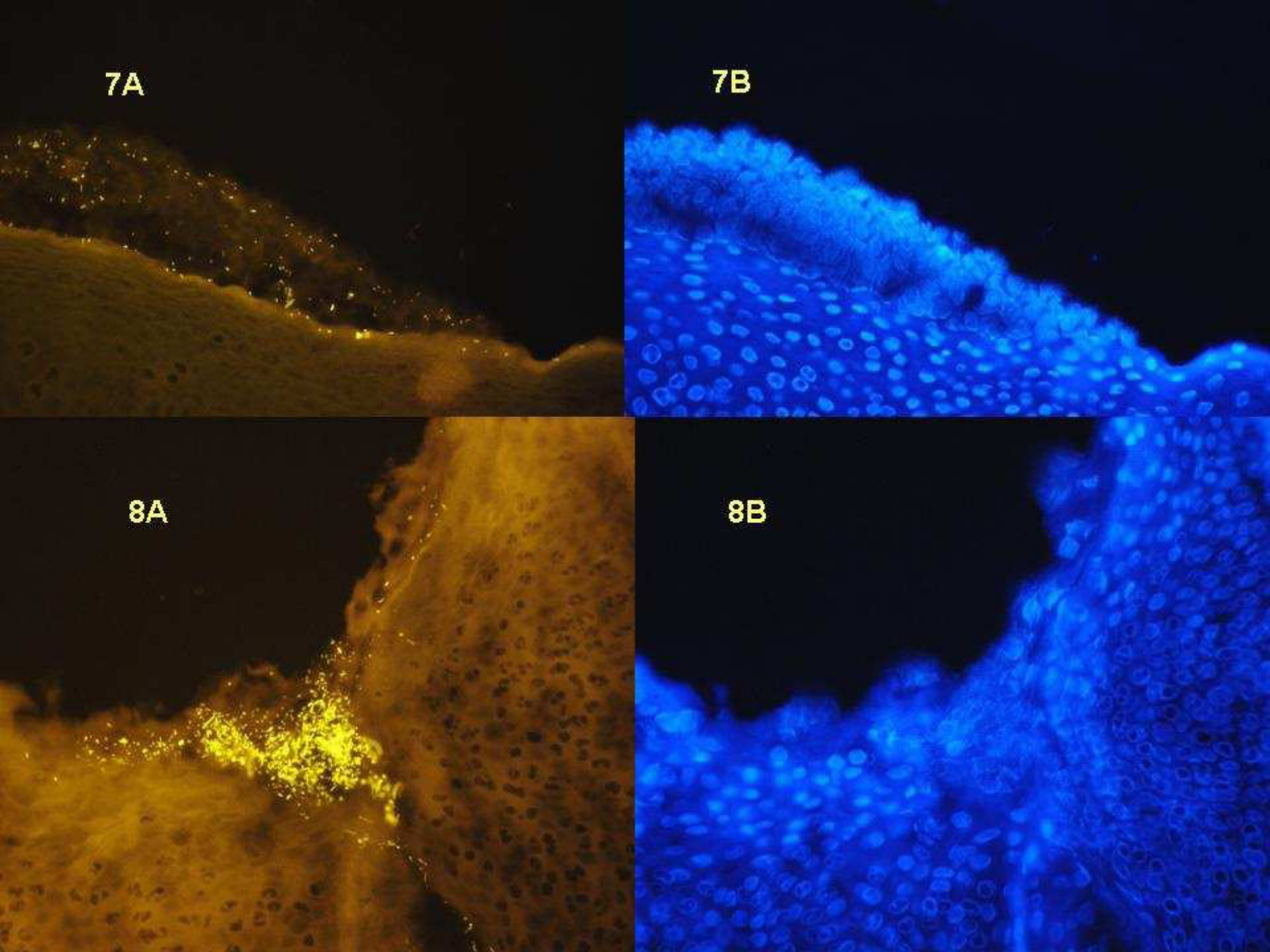


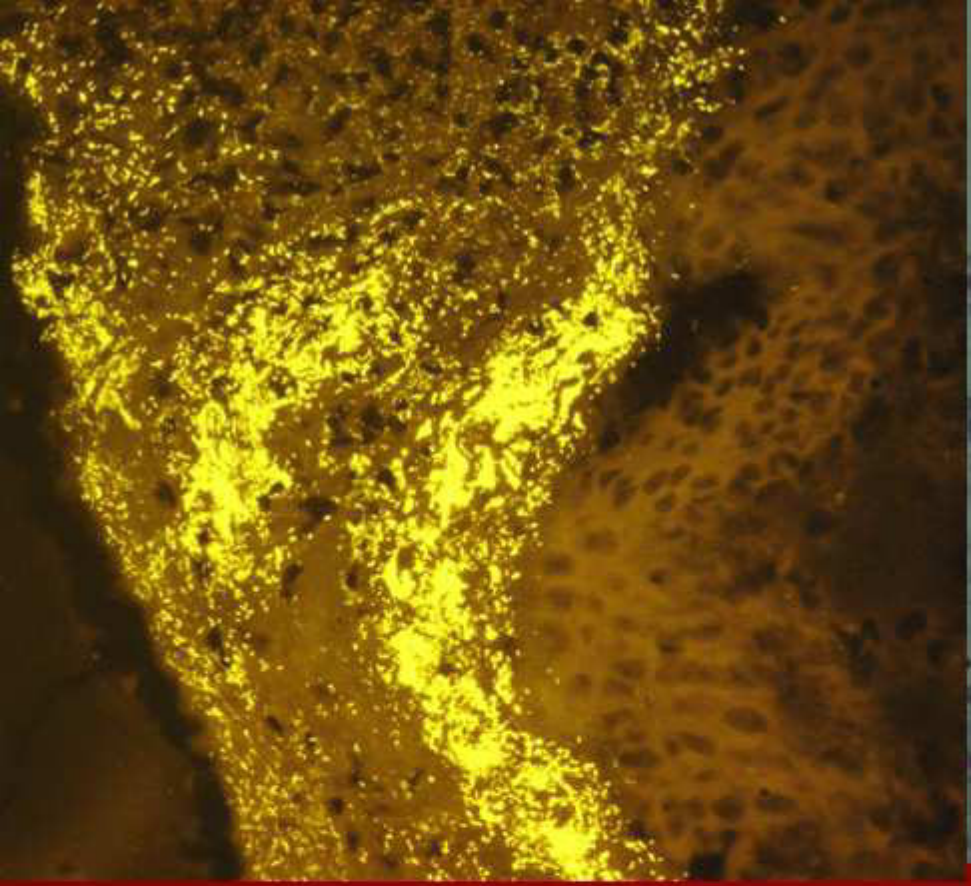


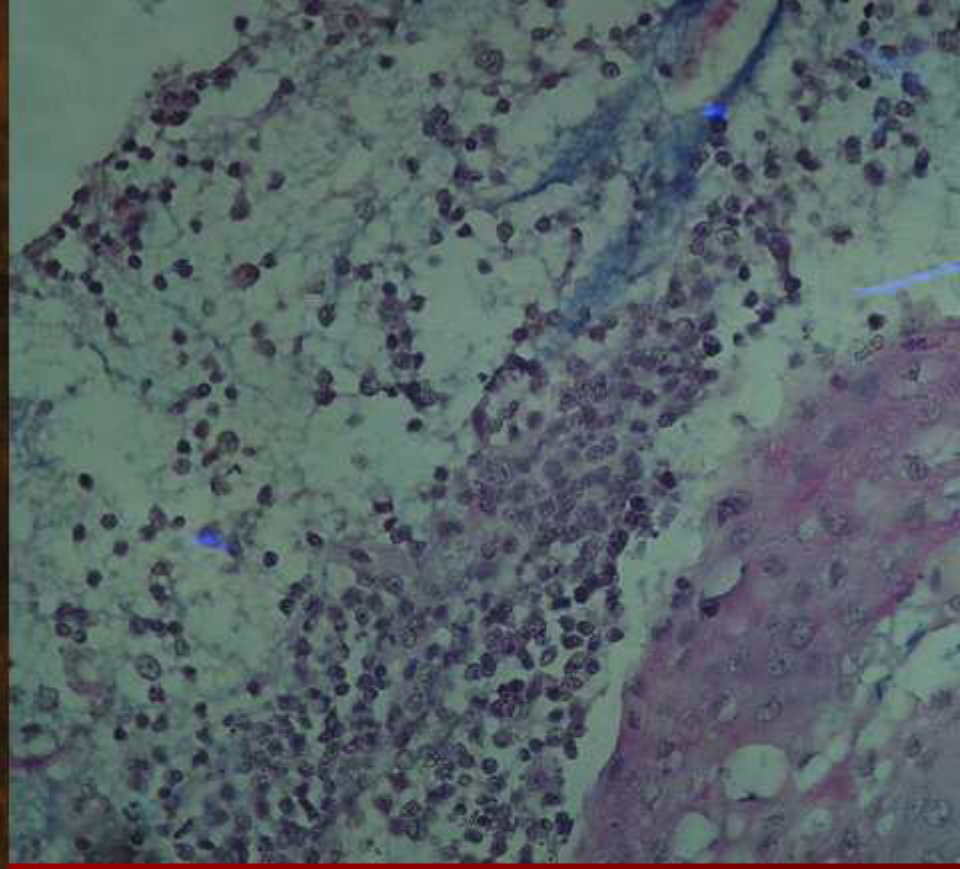


HNO

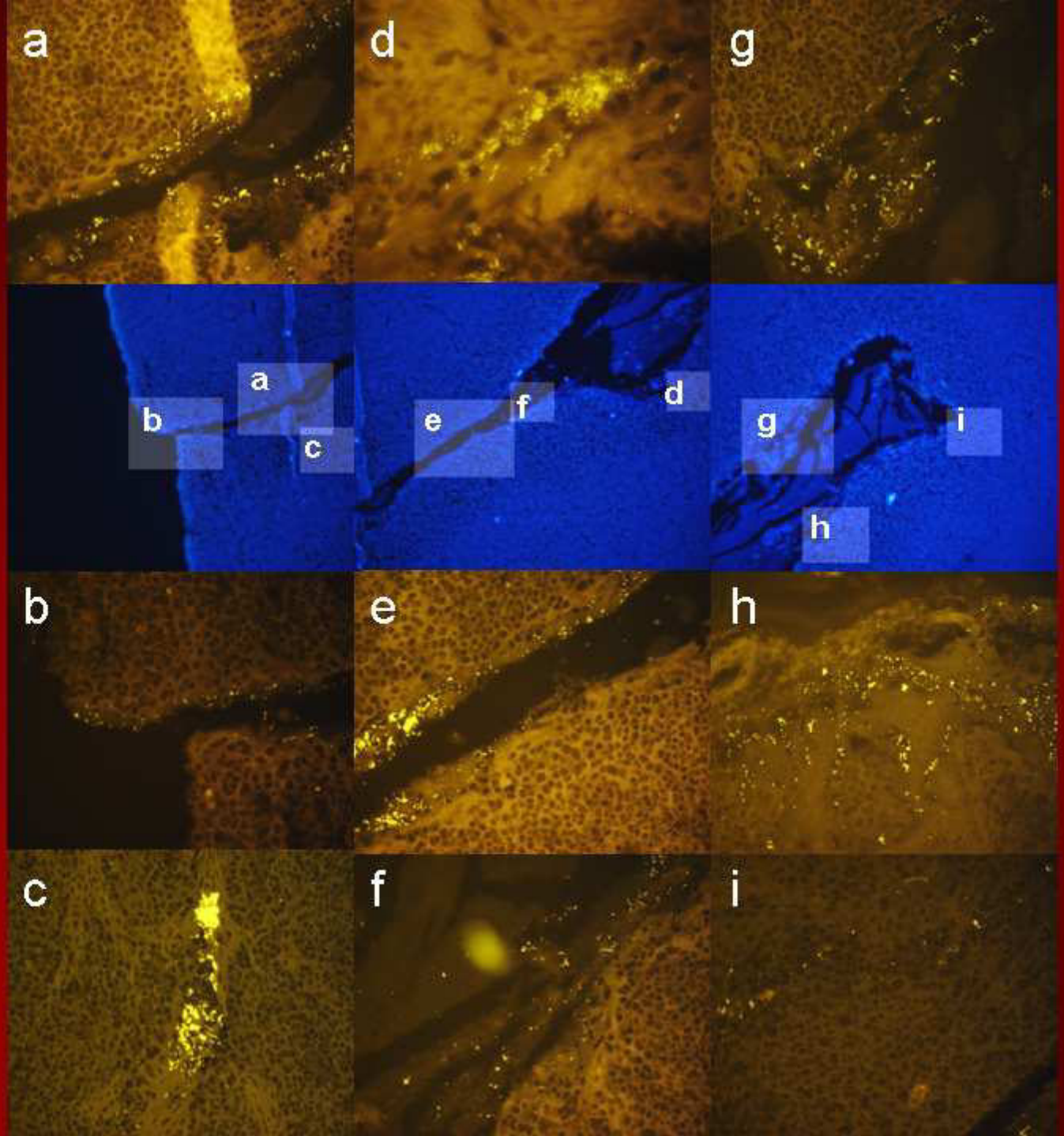

F
G
H
I


7A
7B
8A
8B





E


a
d
g
a
b
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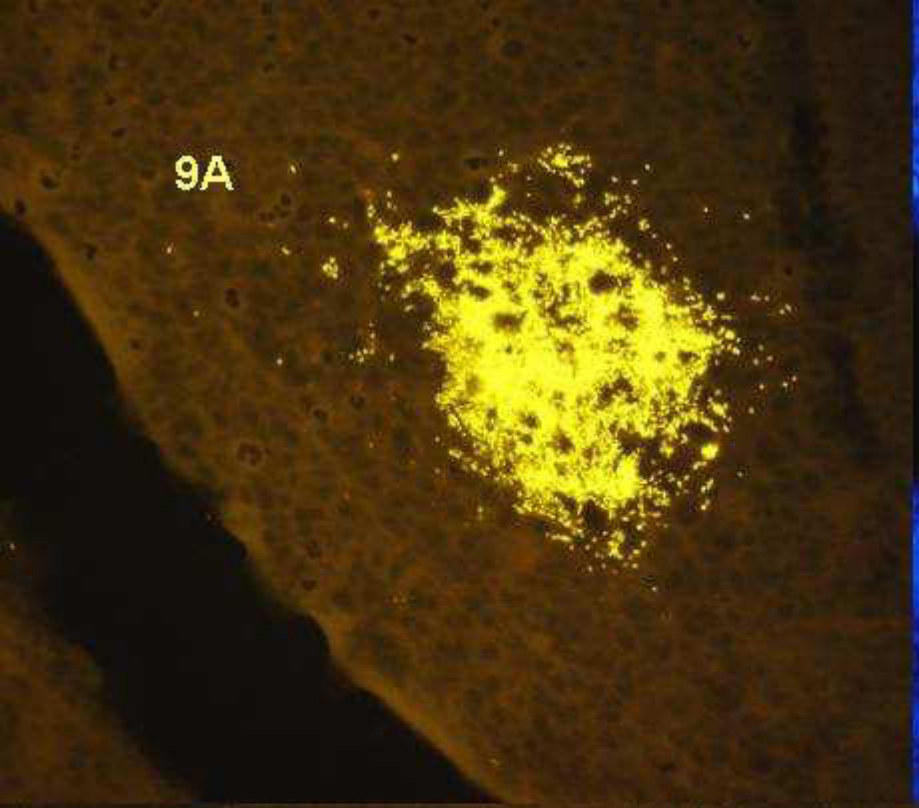
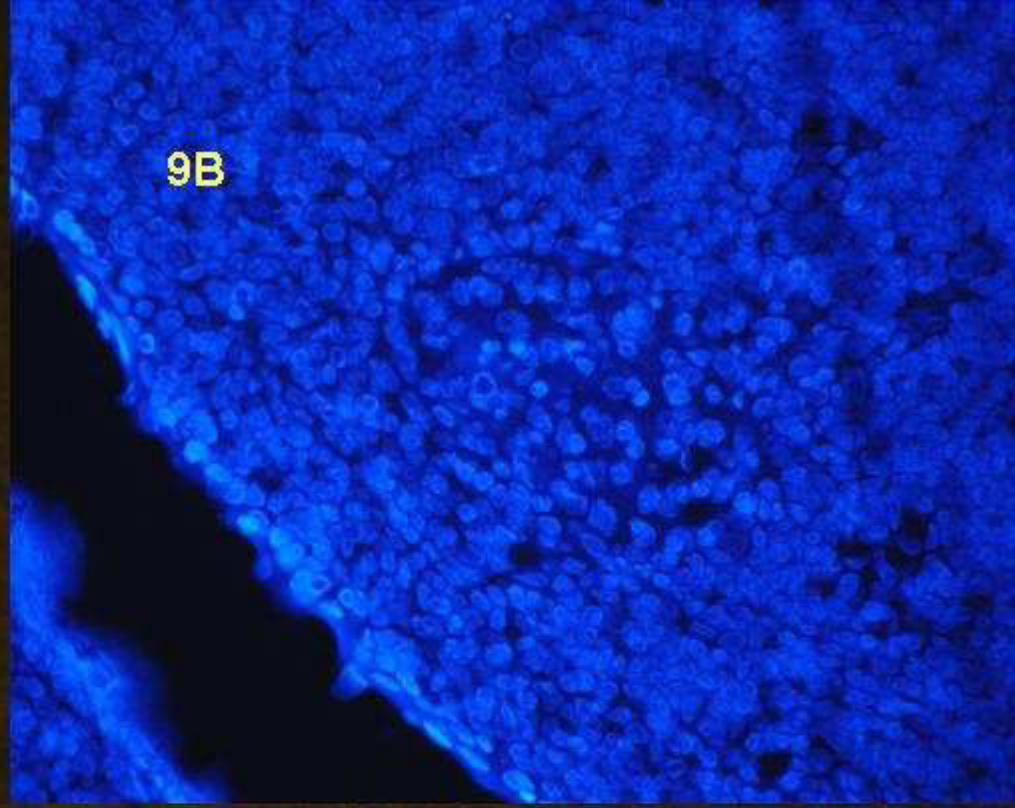
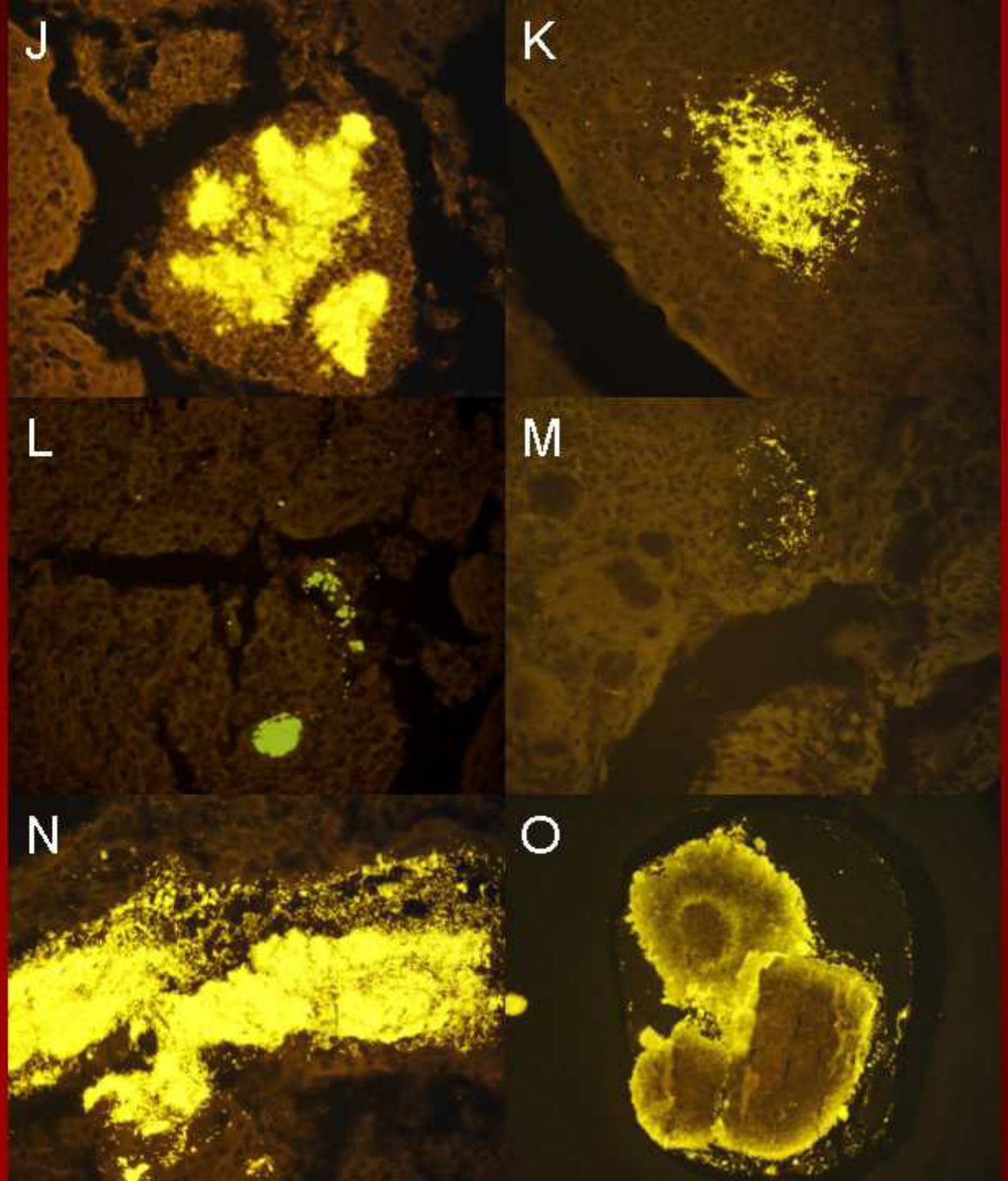
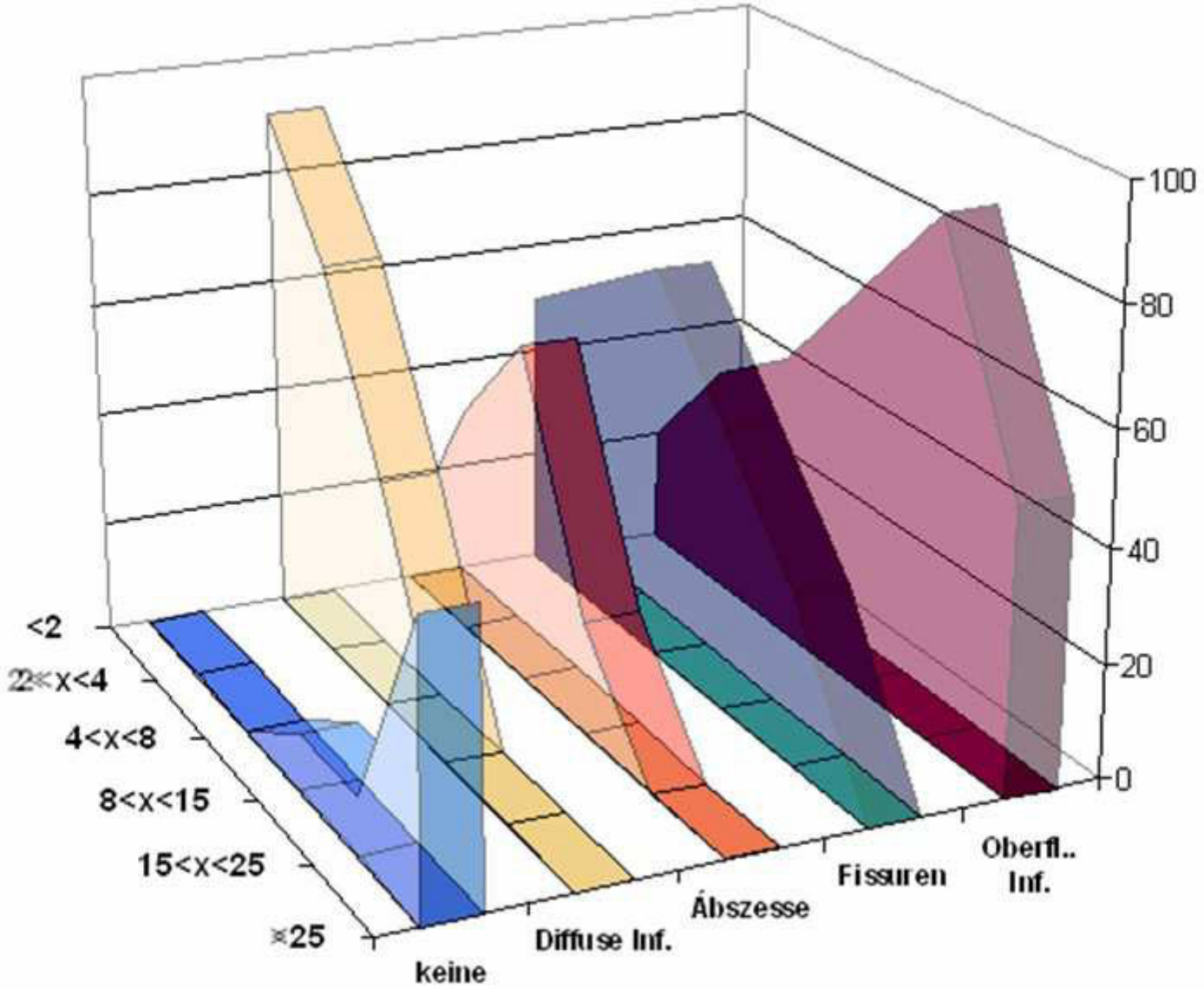

9A


9B


10


J
K
L
M
N
O


100
80
60
40
20
0
<2
2<x<4
4<x<8
8<x<15
15<x<25
x>25
keine
Diffuse Inf.
Abszesse
Fissuren
Oberfl.. Inf.

Occurrence of different bacterial groups within local tonsillar lesions such as fissures and diffuse infiltrates

Superficial Infiltration and Fissures*	%	Diffuse Infiltration*	%
Fusobacteria spp. (Fuso)	36	*Firmicutes* (LGC)	74
Pseudomonas (Ps, Pseaer A, Pseaer B)	34	*Streptococcus* (Strc493)	74
Beta-Proteobacteria inclusive. *Neisseria* (Bet42a)	33	*Haemophilus influenzae* (Haeinf)	66
Burkholderia (Burcep, Burkho)	30	*Actinobacteria* (HGC)	50
Lactobacillus and *Enterococcus* (Lab)	24	*Bacteroides/Prevotella* (Bac303)	39
Veillonella group inclusive *Veillonella parvula* (Veil,Vepa)	23	*Cytophaga-Flavobacteria* (CF319)	34
Clostridium coccoides – E. rectale (Erec)	20	*Streptococcus pyogenes* (Strpyo)	11
Staphylococcus aureus (Staaur)	11	*Atopobium* and others (Ato291)	6
Prevotella intermedia (Prin)	10		
Ruminococcus bromii, R. flavefaciens (Rbro, Rfla)	7 6		
Coriobacterium group (Cor653)	6		
Listeria,Brochothrix (Lis637,1255)	4		

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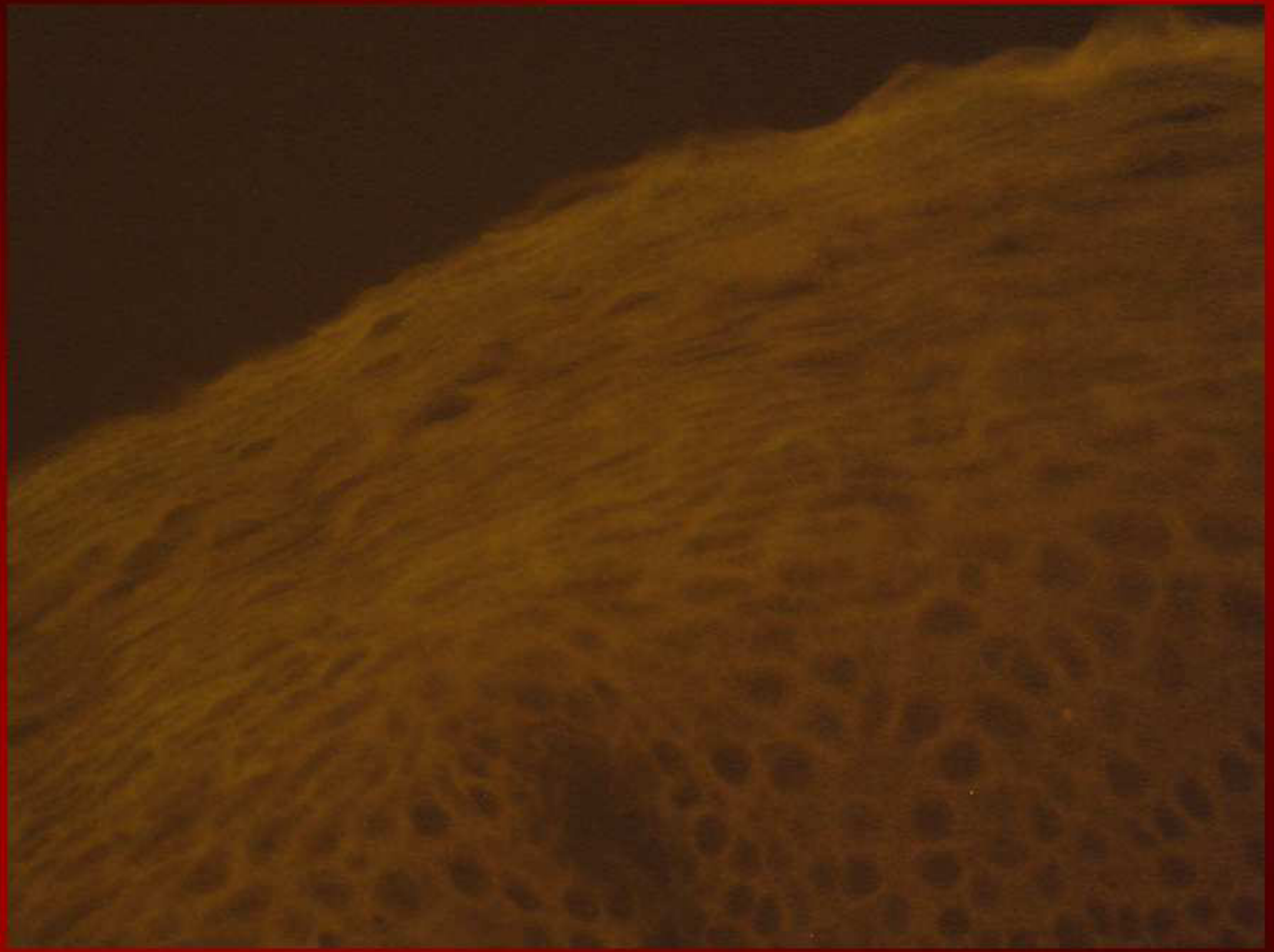
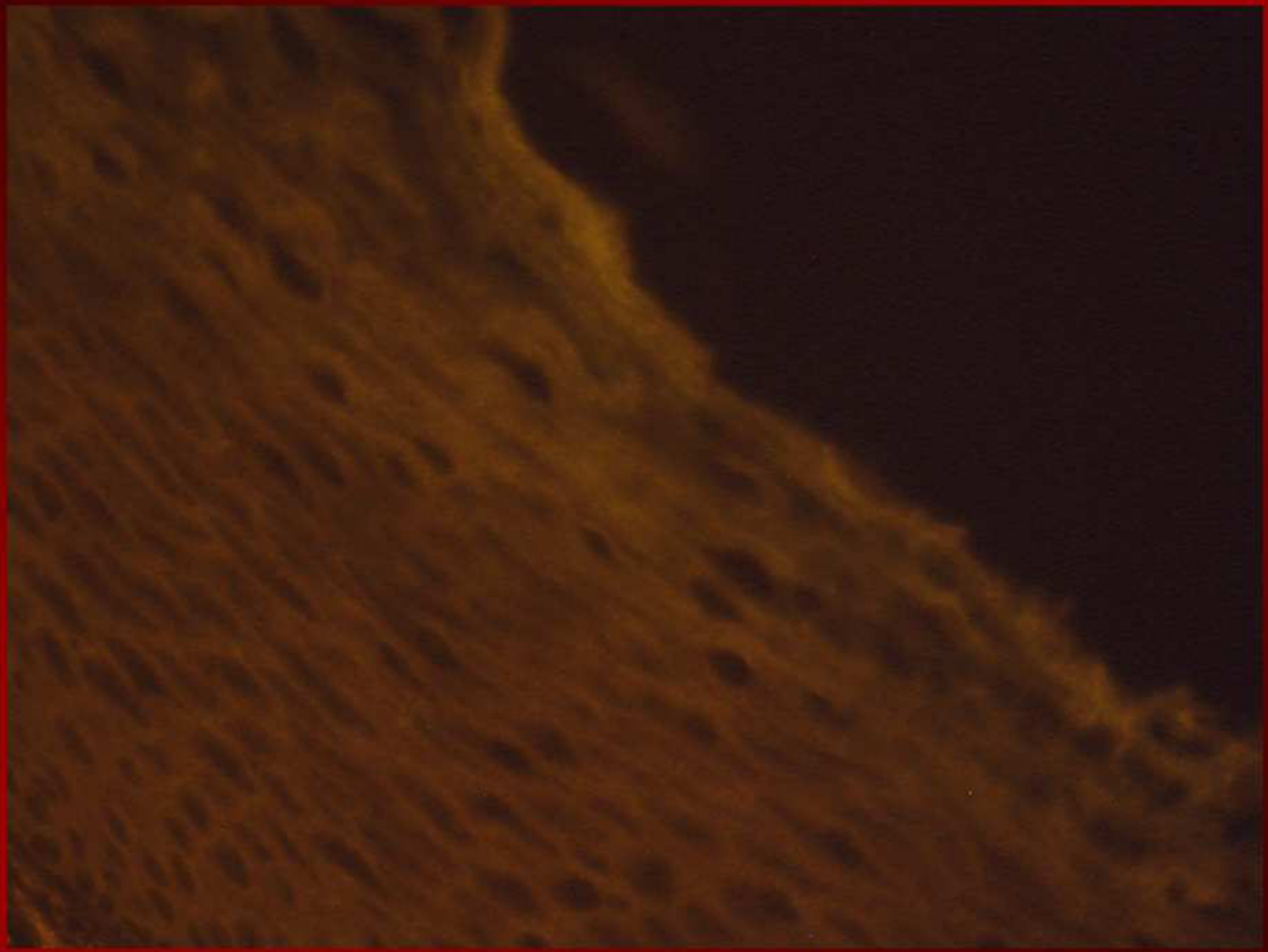
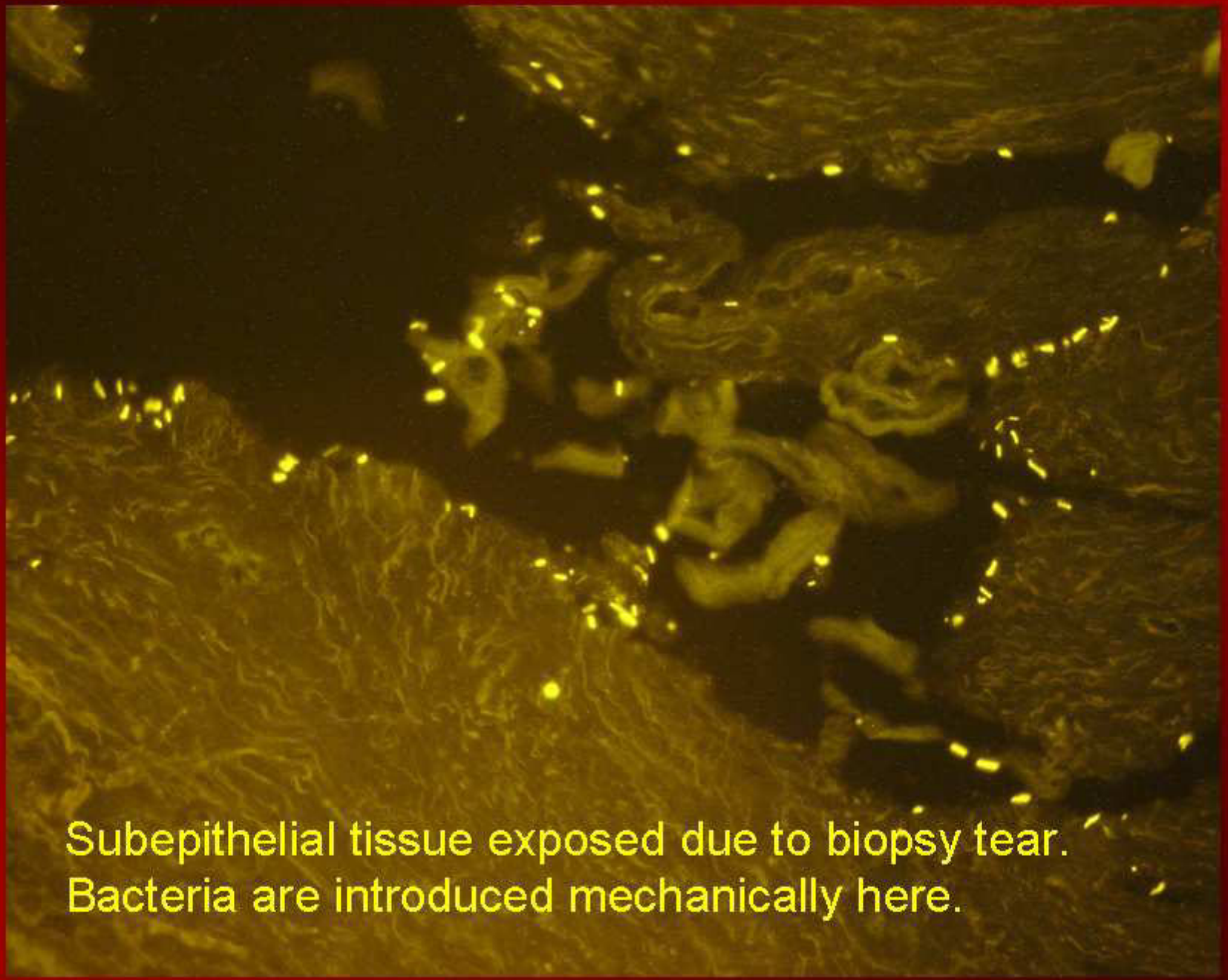
Spatial organisazation of intestinal microbiota

BV

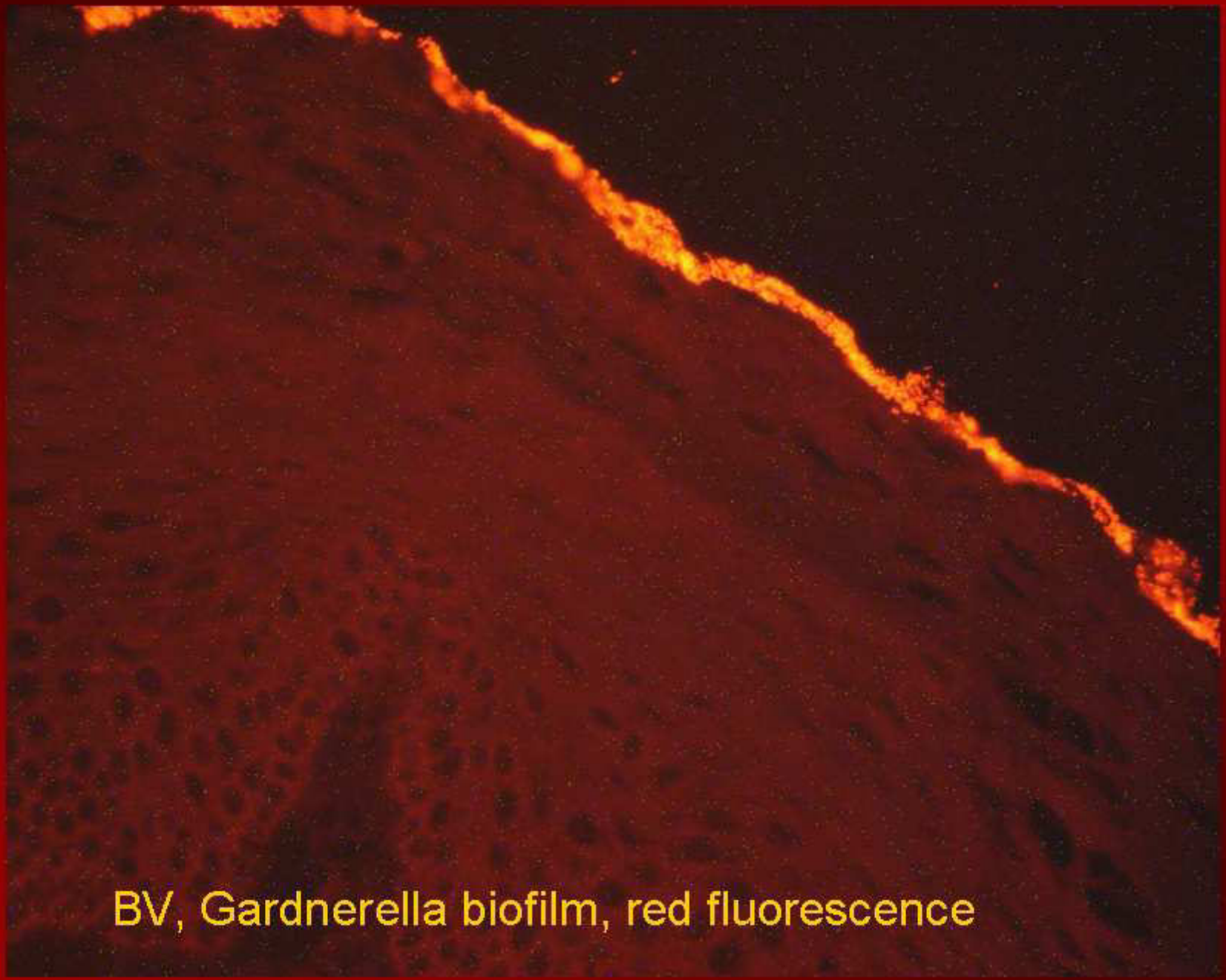

Healthy vaginal epithelium, no bacteria detectable

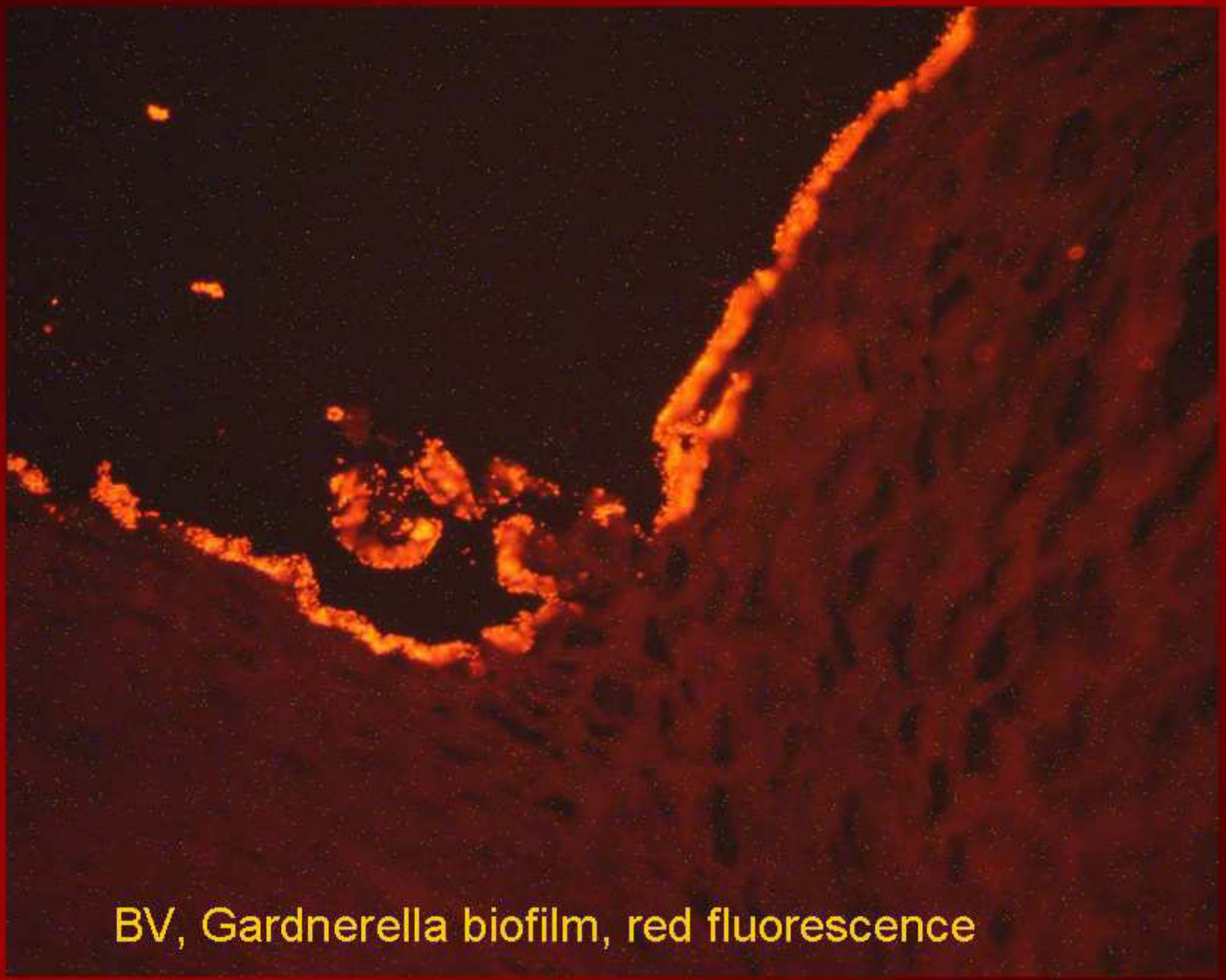


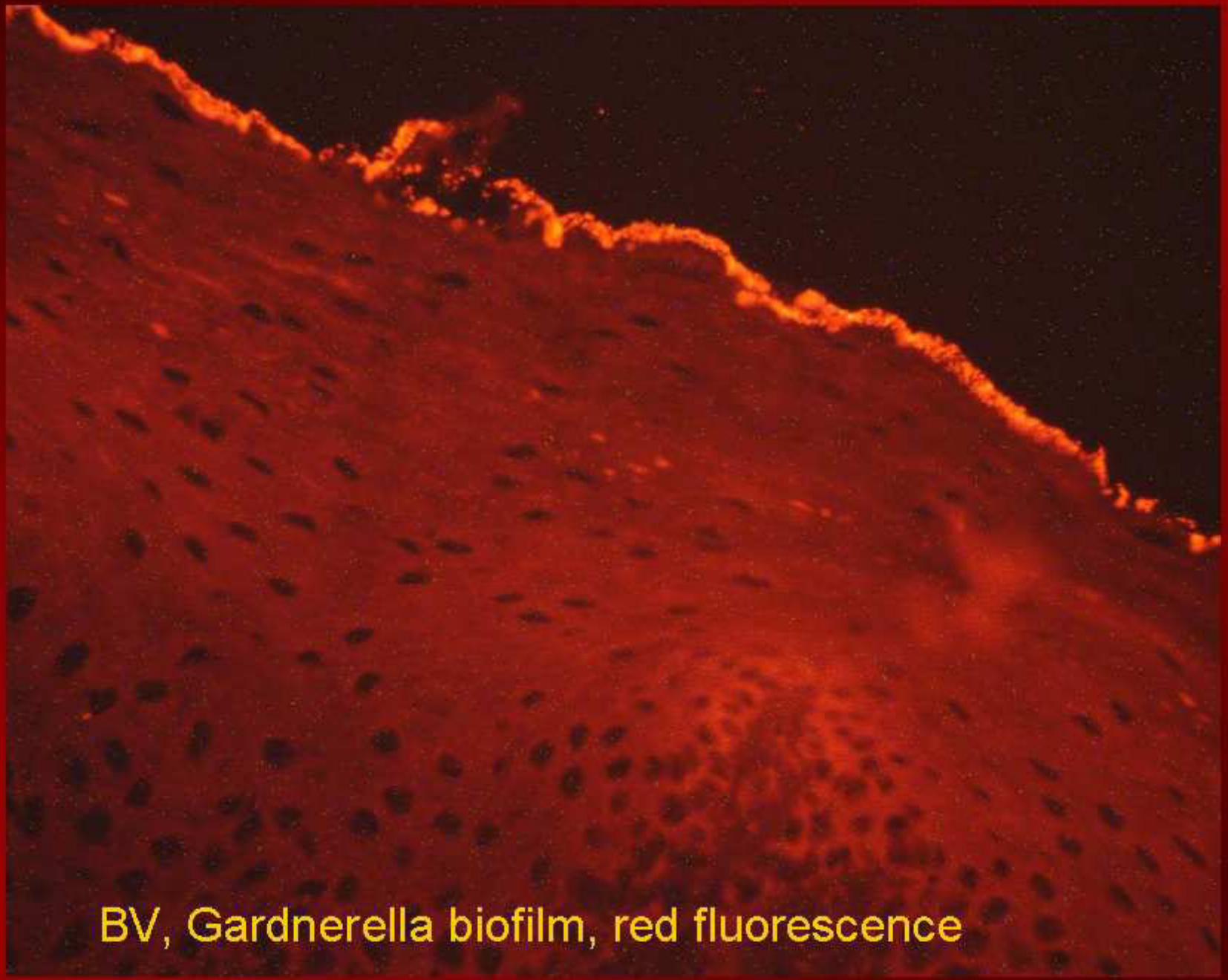



Subepithelial tissue exposed due to biopsy tear.
Bacteria are introduced mechanically here.


Subepithelial tissue exposed due to biopsy tear

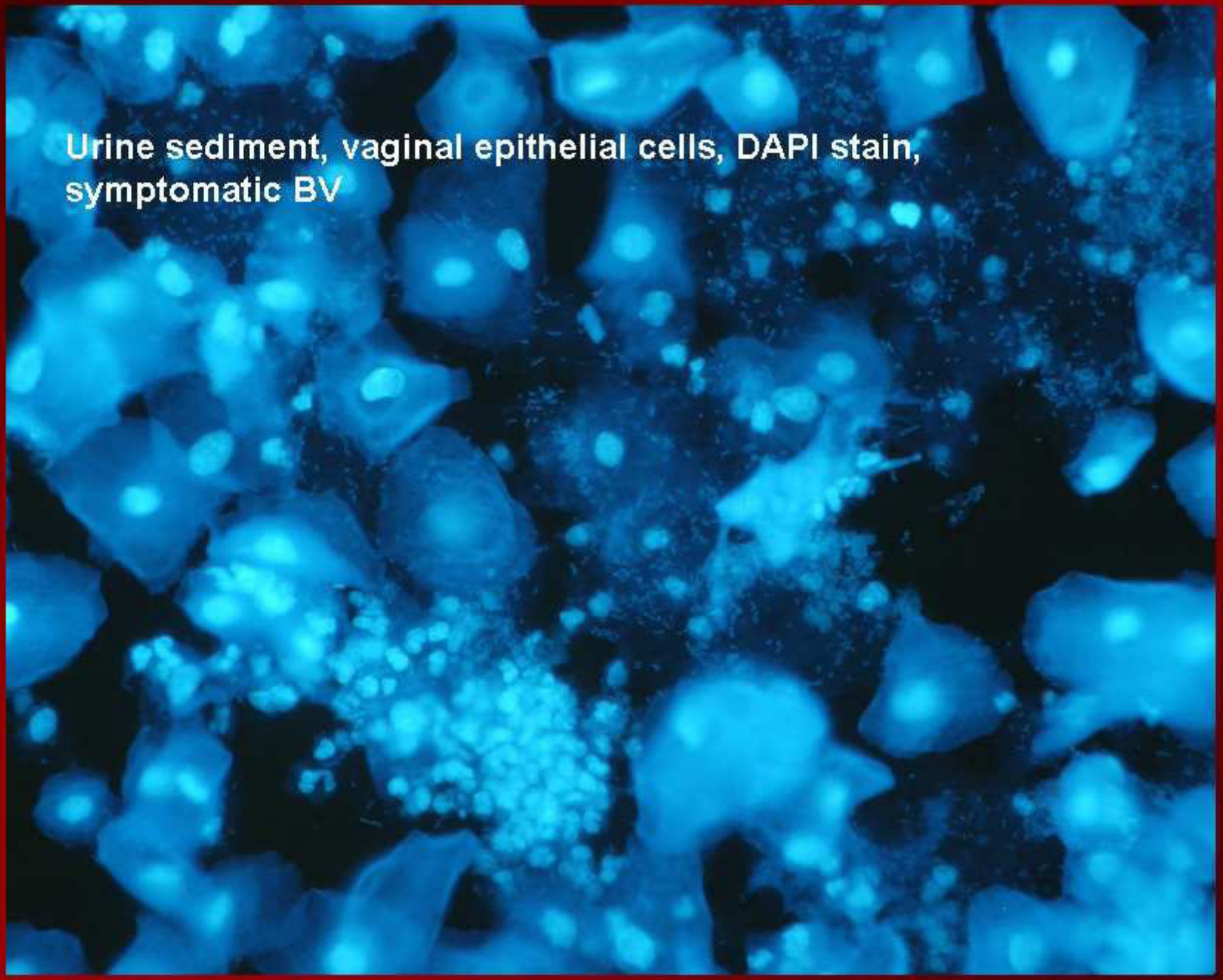

BV, Gardnerella biofilm, red fluorescence

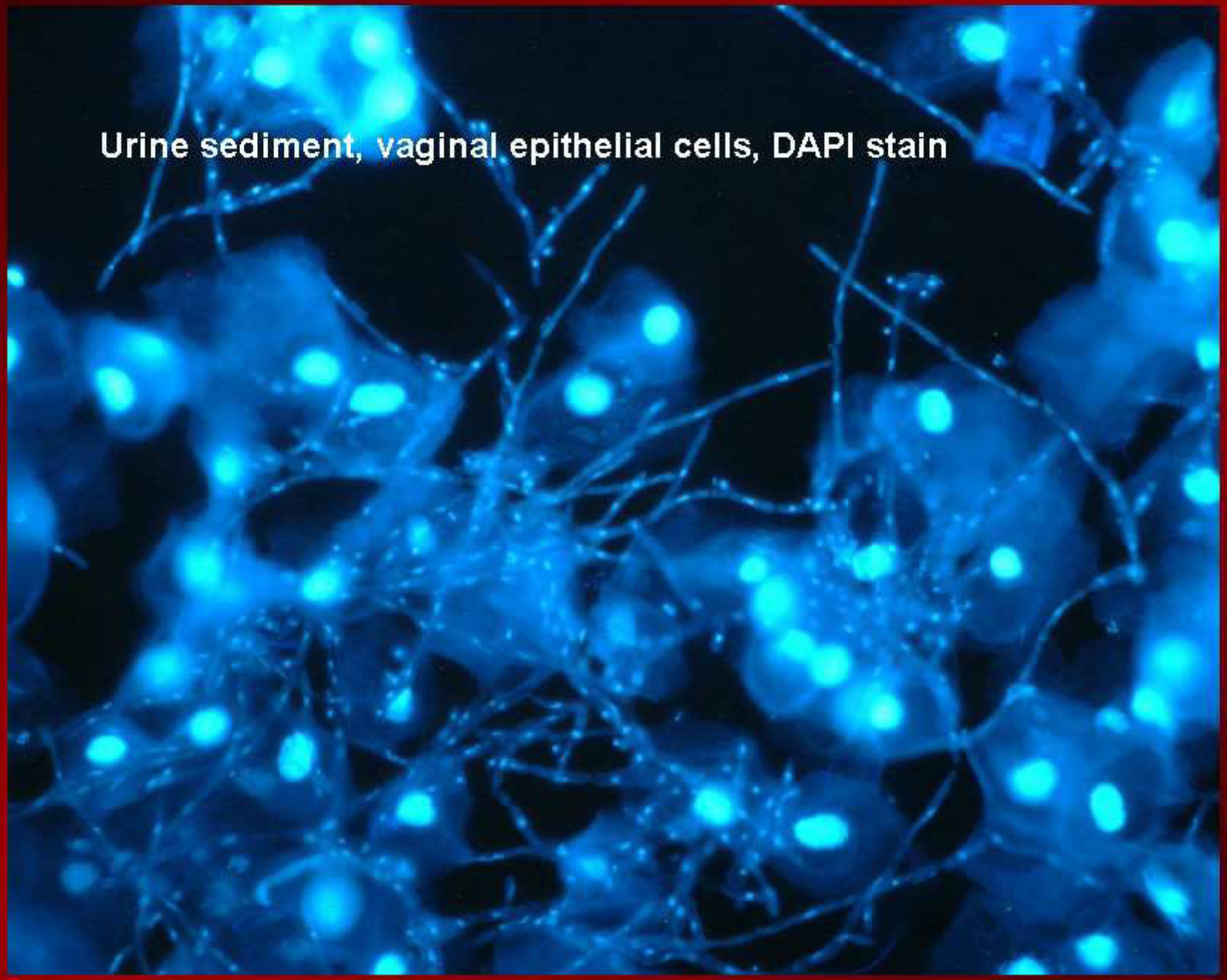

BV, Gardnerella biofilm, red fluorescence

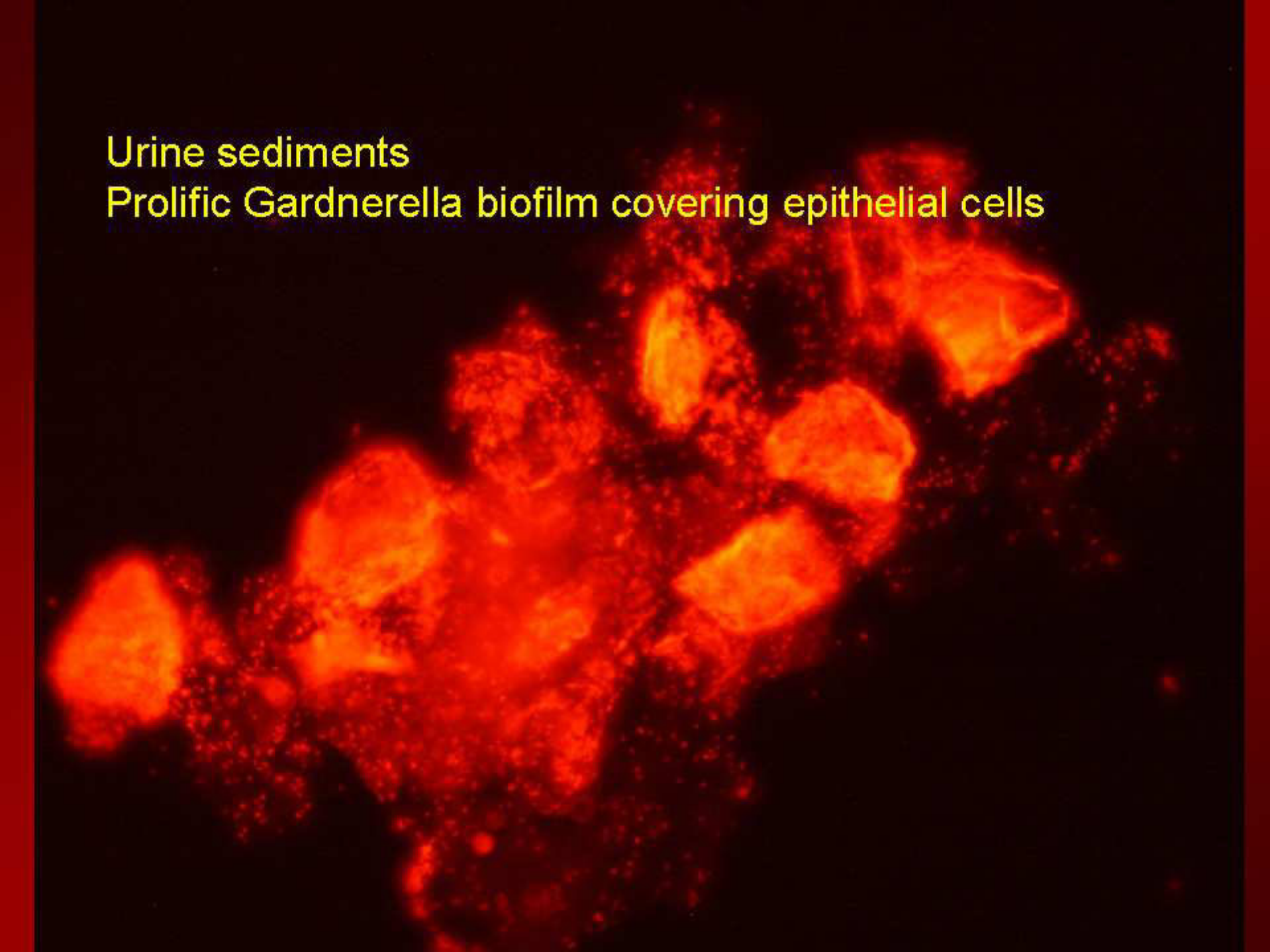

BV, Gardnerella biofilm, red fluorescence


Abundant Lactobacilli, yellow fluorescence
within Gardnerella biofilm

		Controls		BV	
		Max. Concentration ml	Occurence	Max. Konzêntration ml	Vorkommen
		10^9	**40%**	**10^{11}**	**100%**
Gardnerella	**(Gard 5)**	10^7	10%	**10^{11}**	**100%**
Atopobium	(Ato)	10^7	8%	10^{10}	60%
Lactobacillus	(Lab)	**10^9**	**40%**	10^9	80%
Coriobacterium	(Cor)	10^7	5%	10^8	17%
Enterobacteriaceae	(Ebac)	0	0	10^6	10%
Bacteroides	(Bac)	0	0	10^6	5%
Veilonella	(Veil)	0	0	10^6	8%
Cytophaga-Flavobacteria	(CF)	0	0	10^6	10%
Clostridien	(Clit, Chis, Erec)	0	0	10^6	3%
Fusobacterien	(Fus)	0	0	10^6	5%

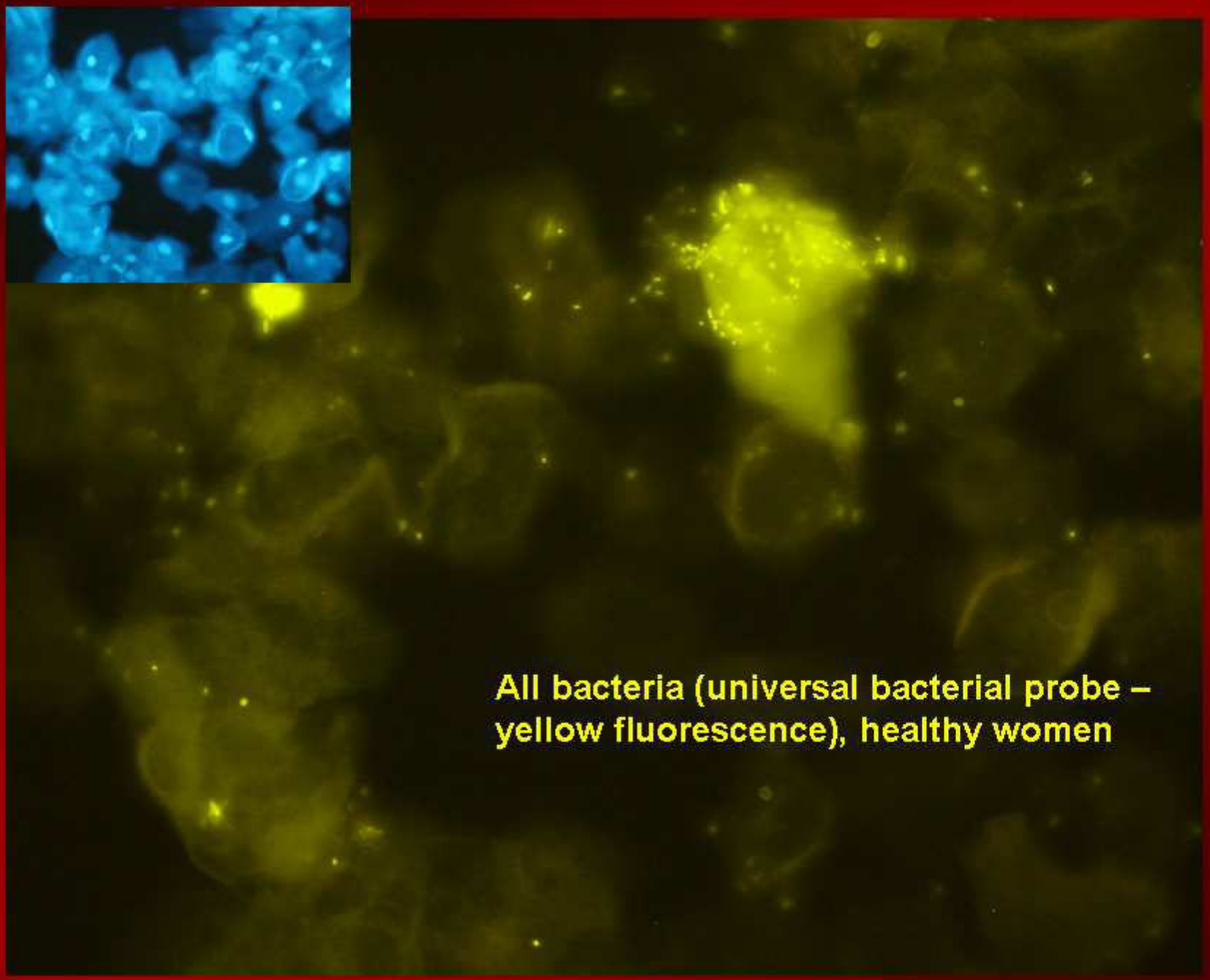

Urine sediment, vaginal epithelial cells, DAPI stain,
symptomatic BV

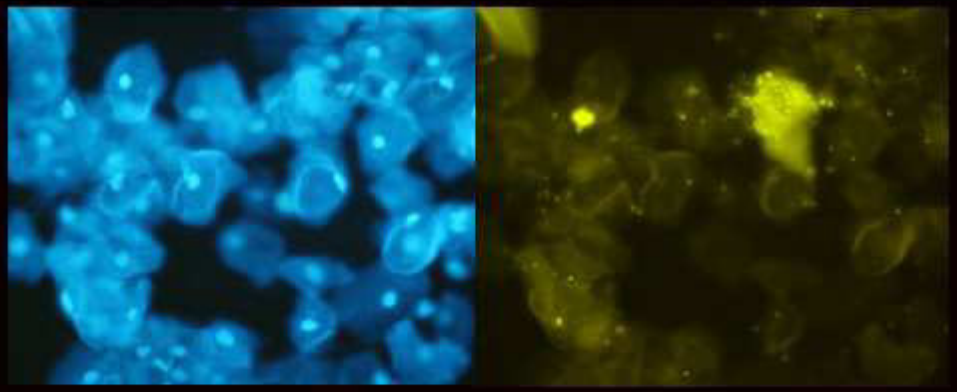

Urine sediment, vaginal epithelial cells, DAPI stain


Urine sediments
Prolific Gardnerella biofilm covering epithelial cells

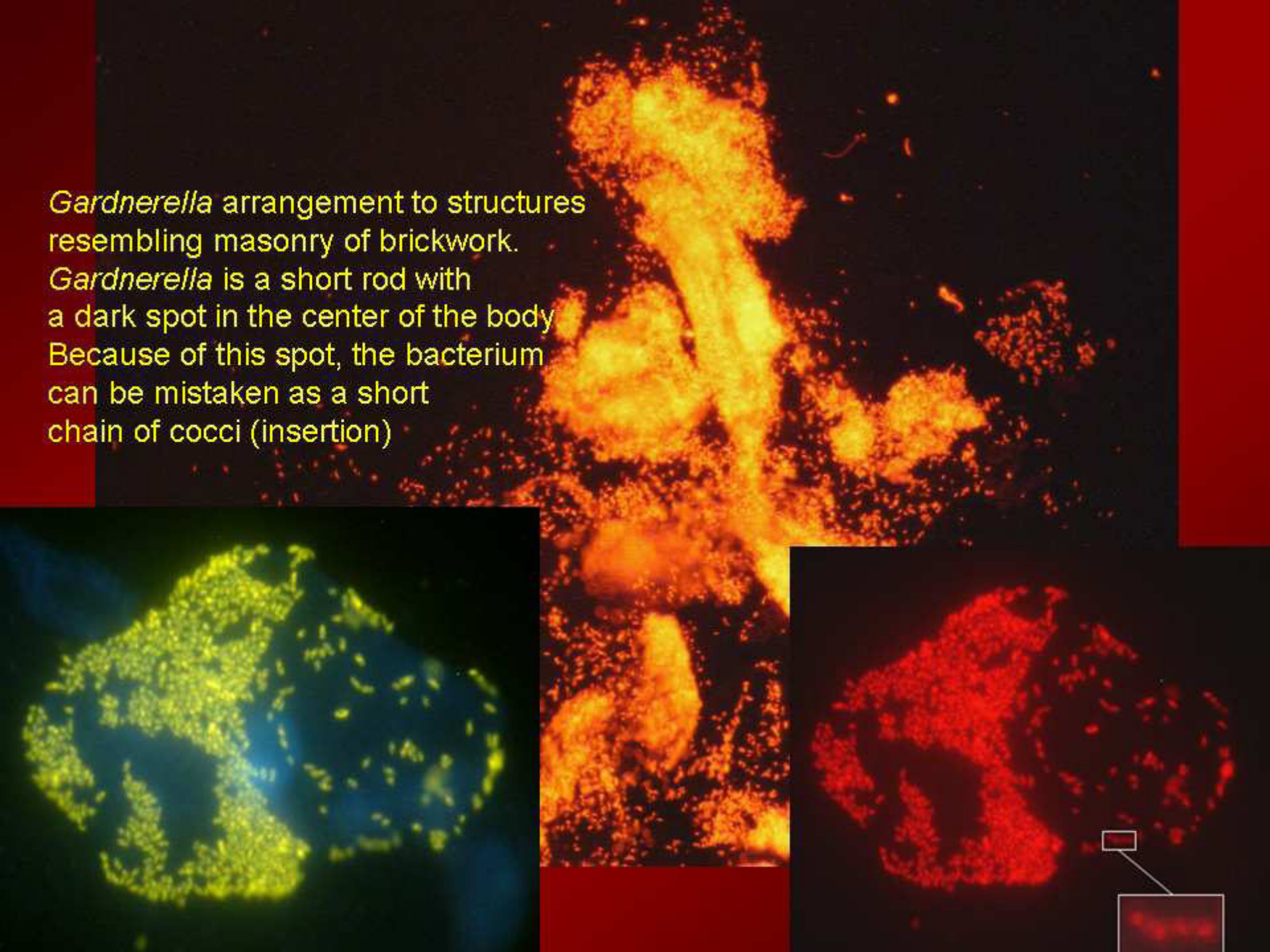
Multicolor FISH. prolific Gardnerella biofilm (red fluorescence)
is associated with high concentrations of Lactobacilli (orange fluorescence),
symptomatic BV

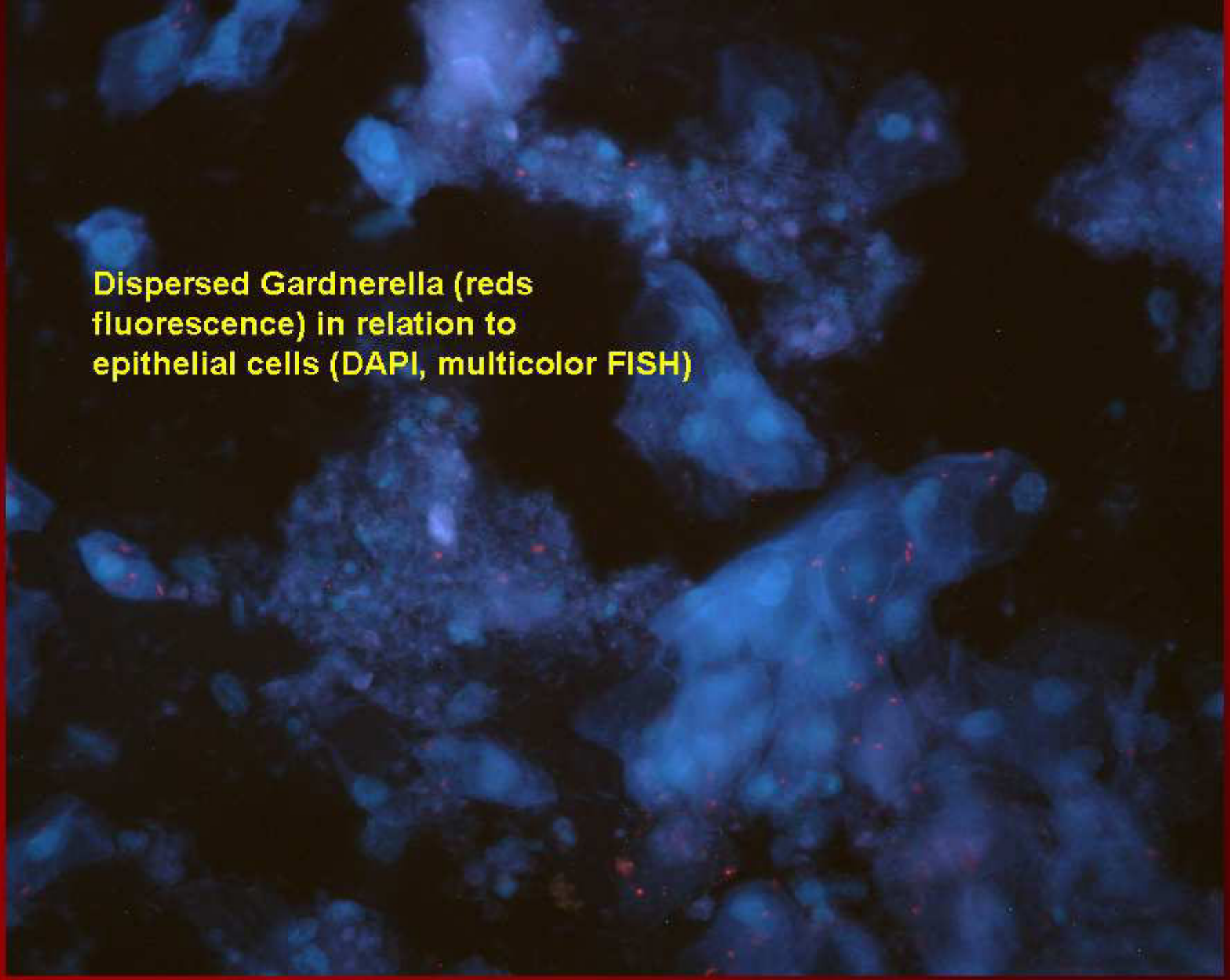

Urine sediment, vaginal epithelial cells,
DAPI stain,
healthy women

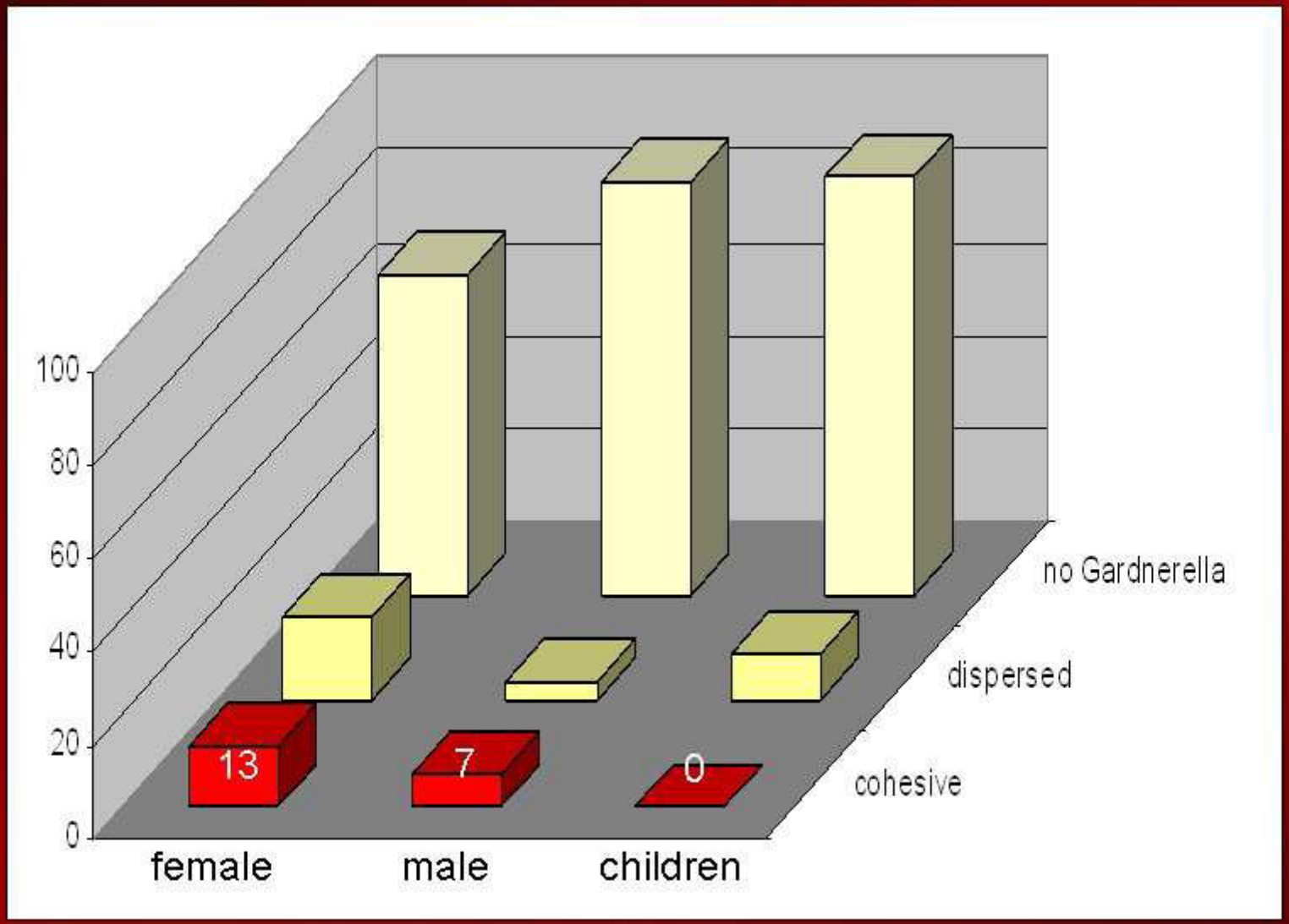

All bacteria (universal bacterial probe – yellow fluorescence), healthy women



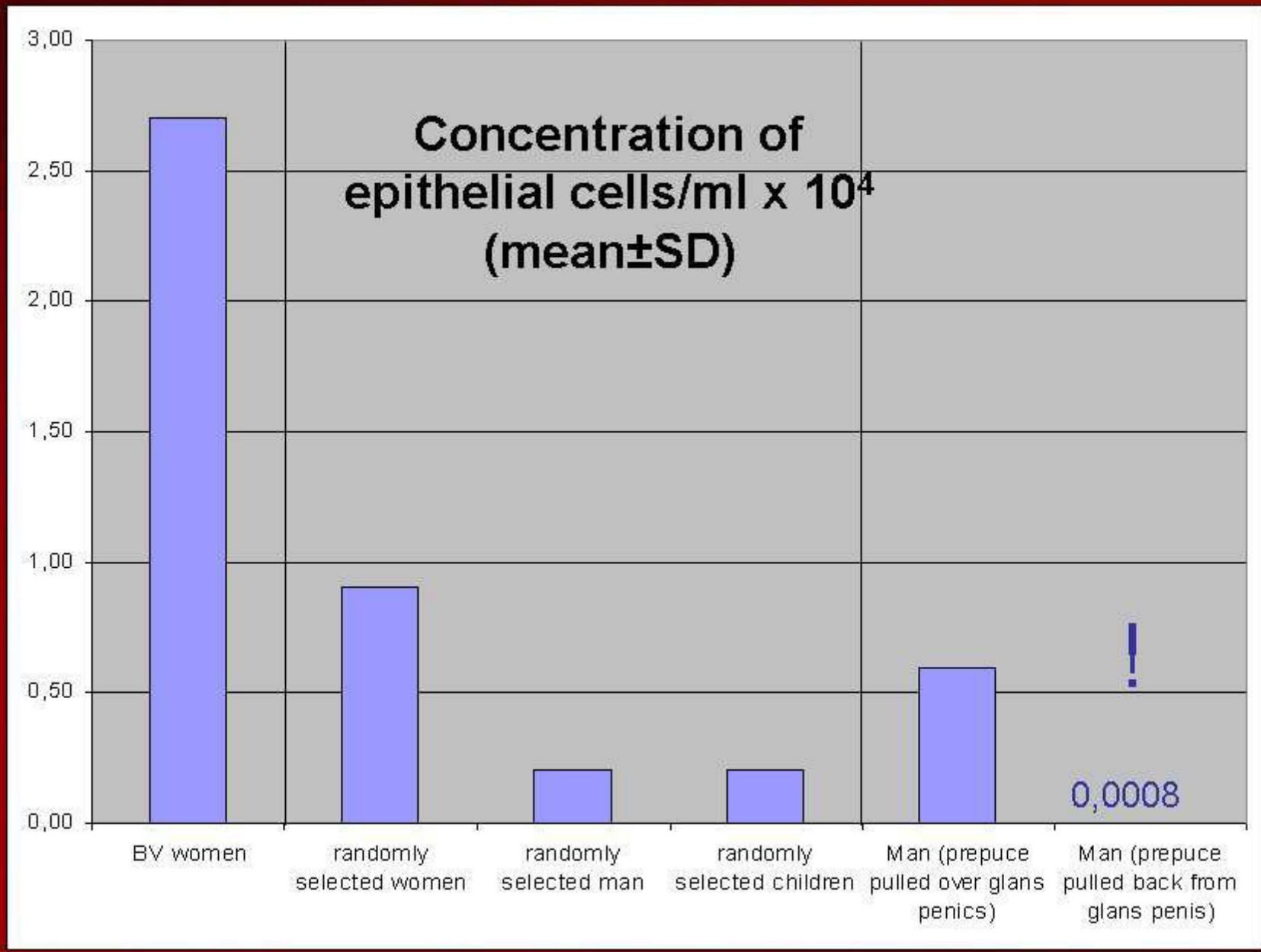
Dispersed Gardnerella (reds fluorescence) in relation to
epithelial cells (DAPI, left insertion)
and other bacteria (Eub 338, right insertion)

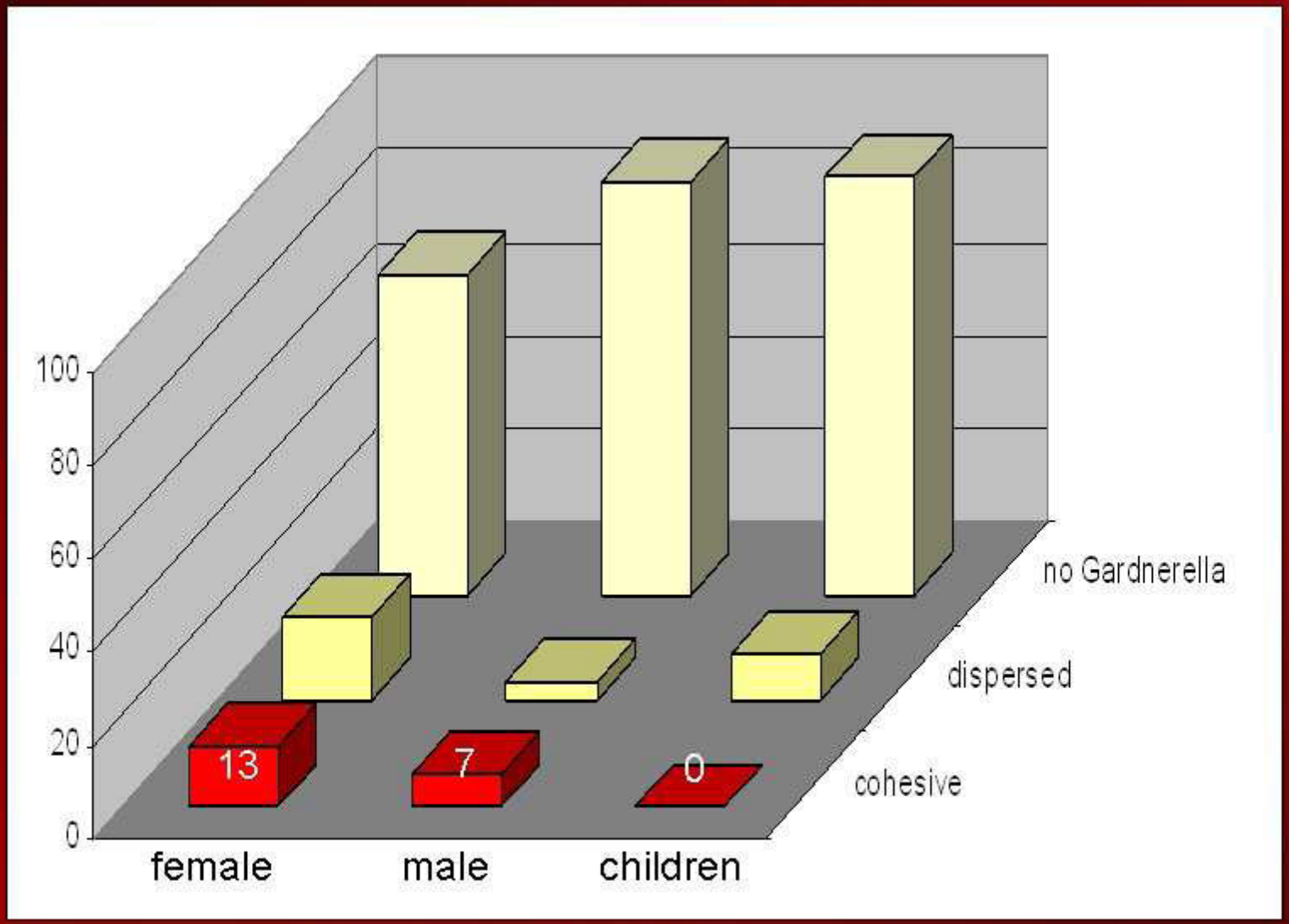

Gardnerella arrangement to structures resembling masonry of brickwork.
Gardnerella is a short rod with a dark spot in the center of the body
Because of this spot, the bacterium can be mistaken as a short chain of cocci (insertion)


Dispersed Gardnerella (reds
fluorescence) in relation to
epithelial cells (DAPI, multicolor FISH)

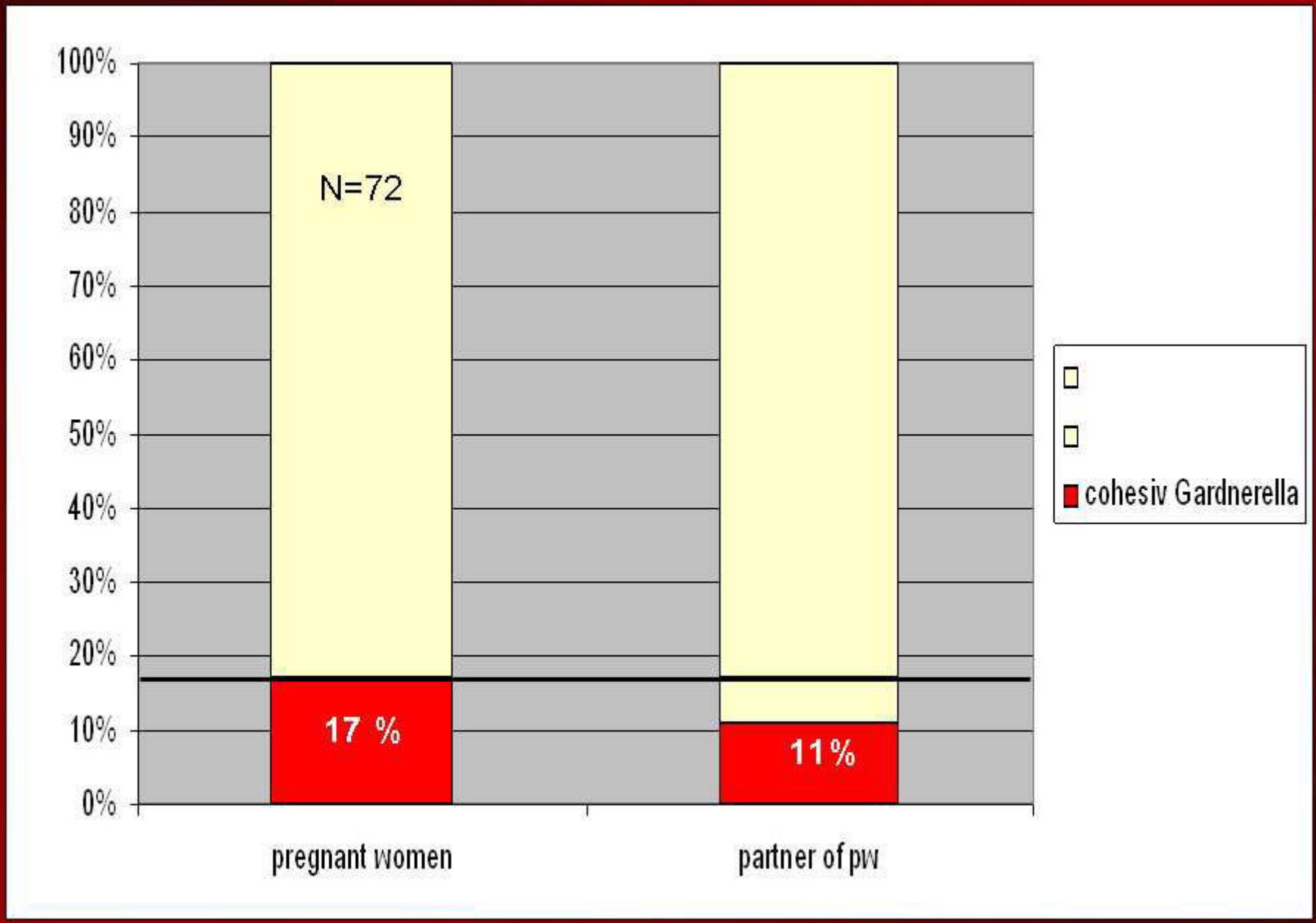


Randomly selected patients hospitalized for reasons other than BV

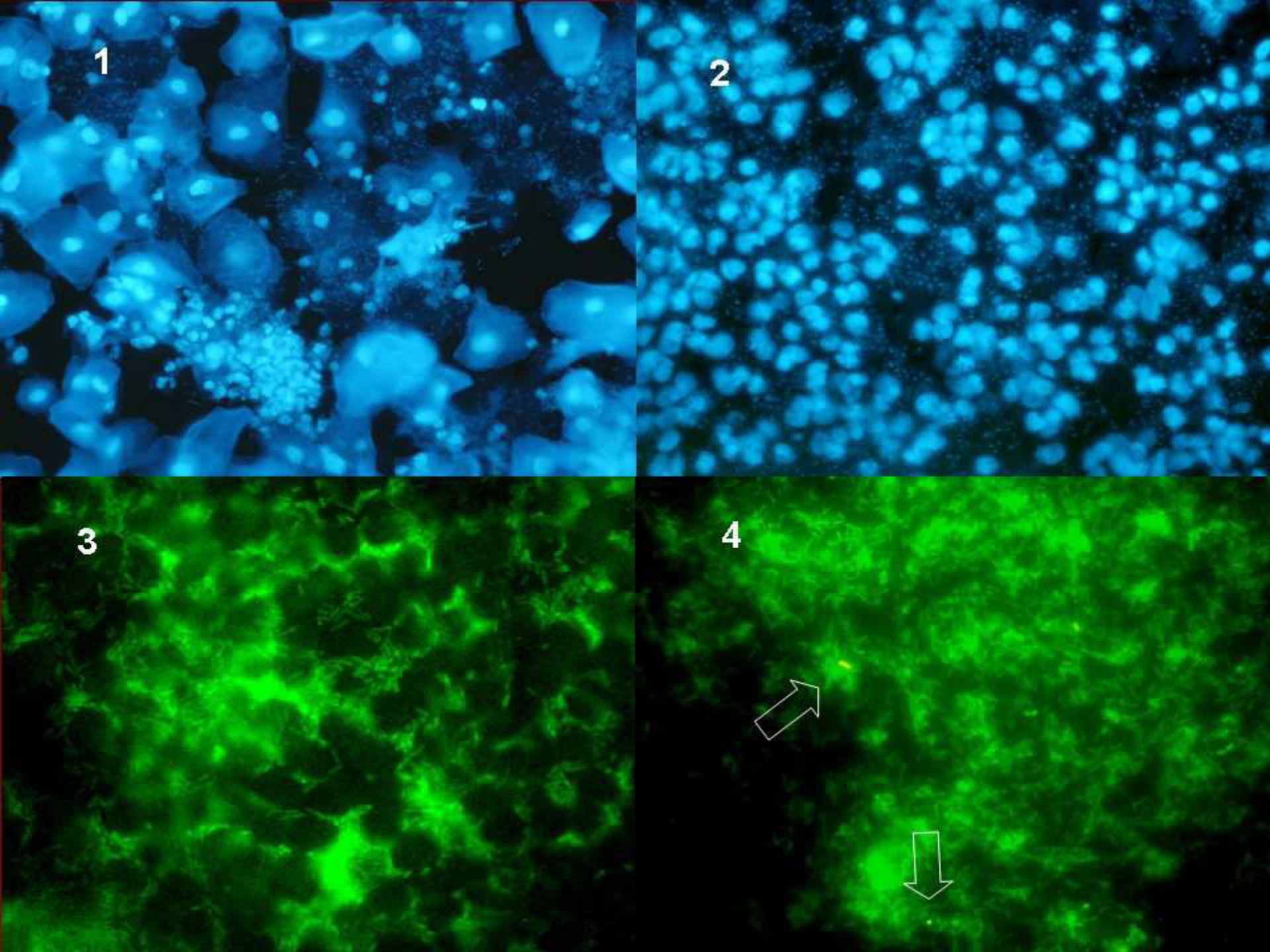

Concentration of epithelial cells/ml x 10⁴ (mean±SD)
3,00
2,50
2,00
1,50
1,00
0,50
0,00
!
0,0008
BV women
randomly selected women
randomly selected man
randomly selected children
Man (prepuce pulled over glans penics)
Man (prepuce pulled back from glans penis)



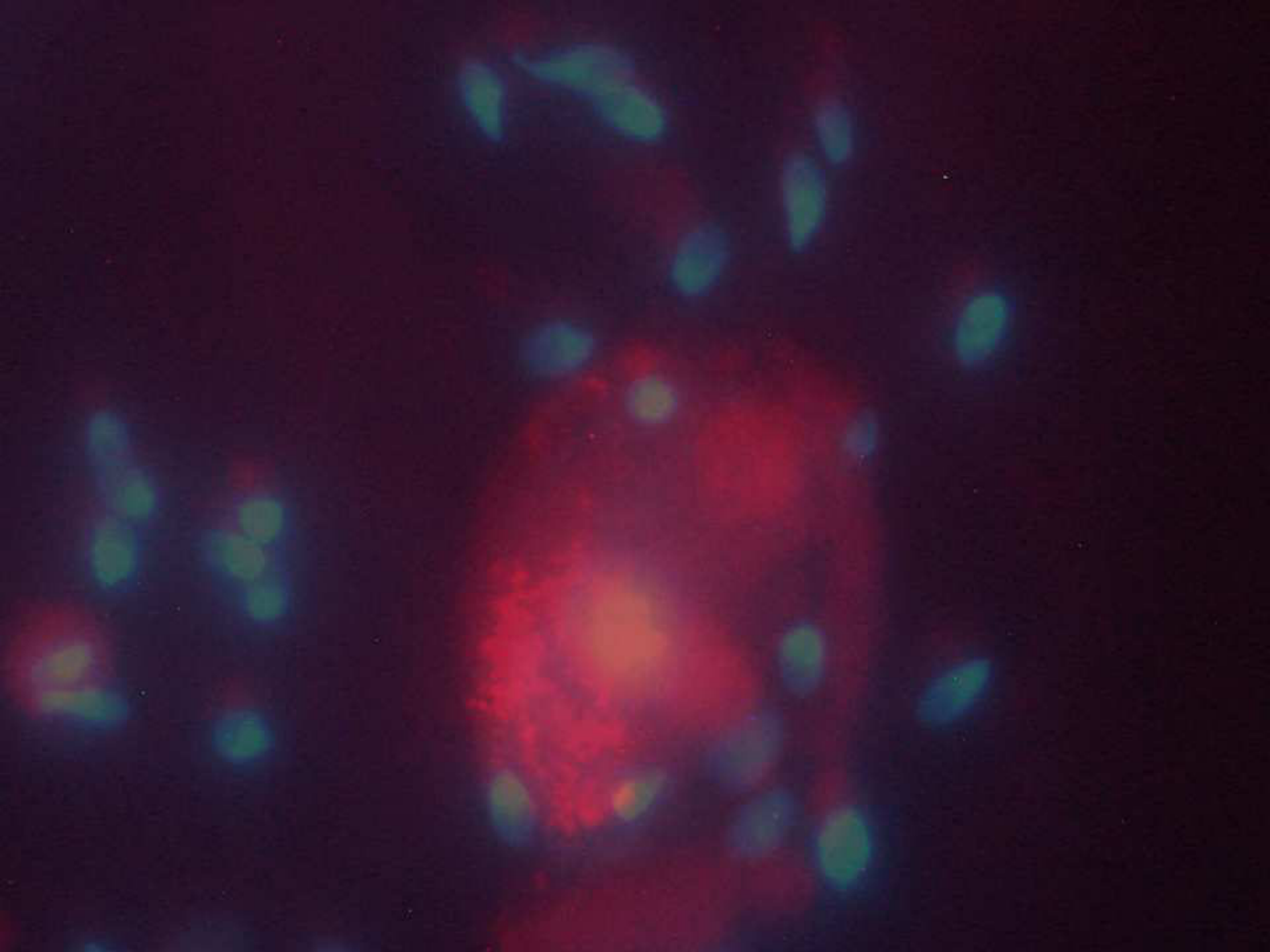
Randomly selected patients hospitalized for reasons other than BV


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
N=72
17 %
11%
pregnant women
partner of pw
cohesiv Gardnerella

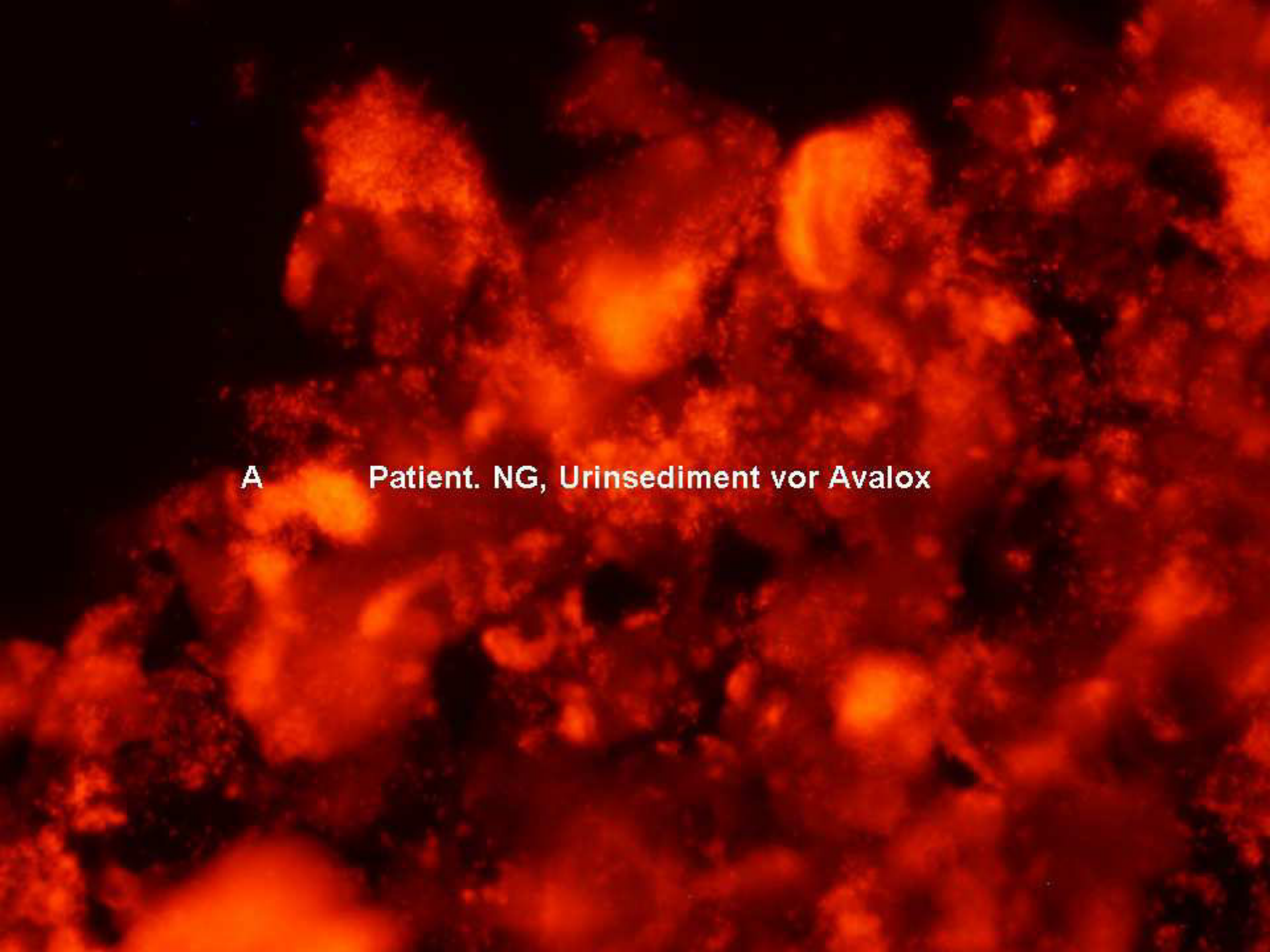
Gardnerella biofilm is a specific disease entity which involves both genders and is sexually transmitted. The condition should be named Gardnerellosis and the bacterium *Gardnerella genitalis.*

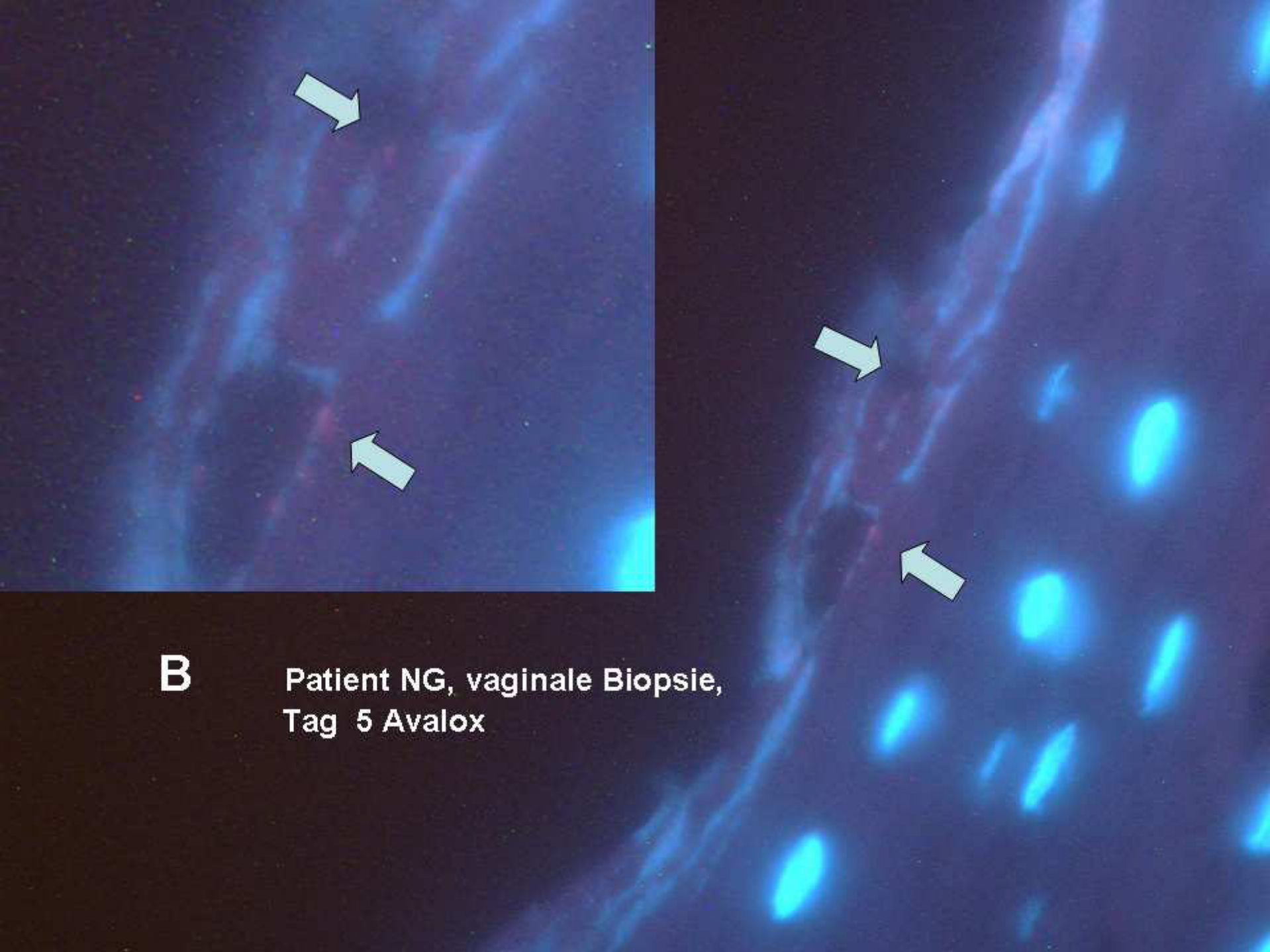

1
2
3
4

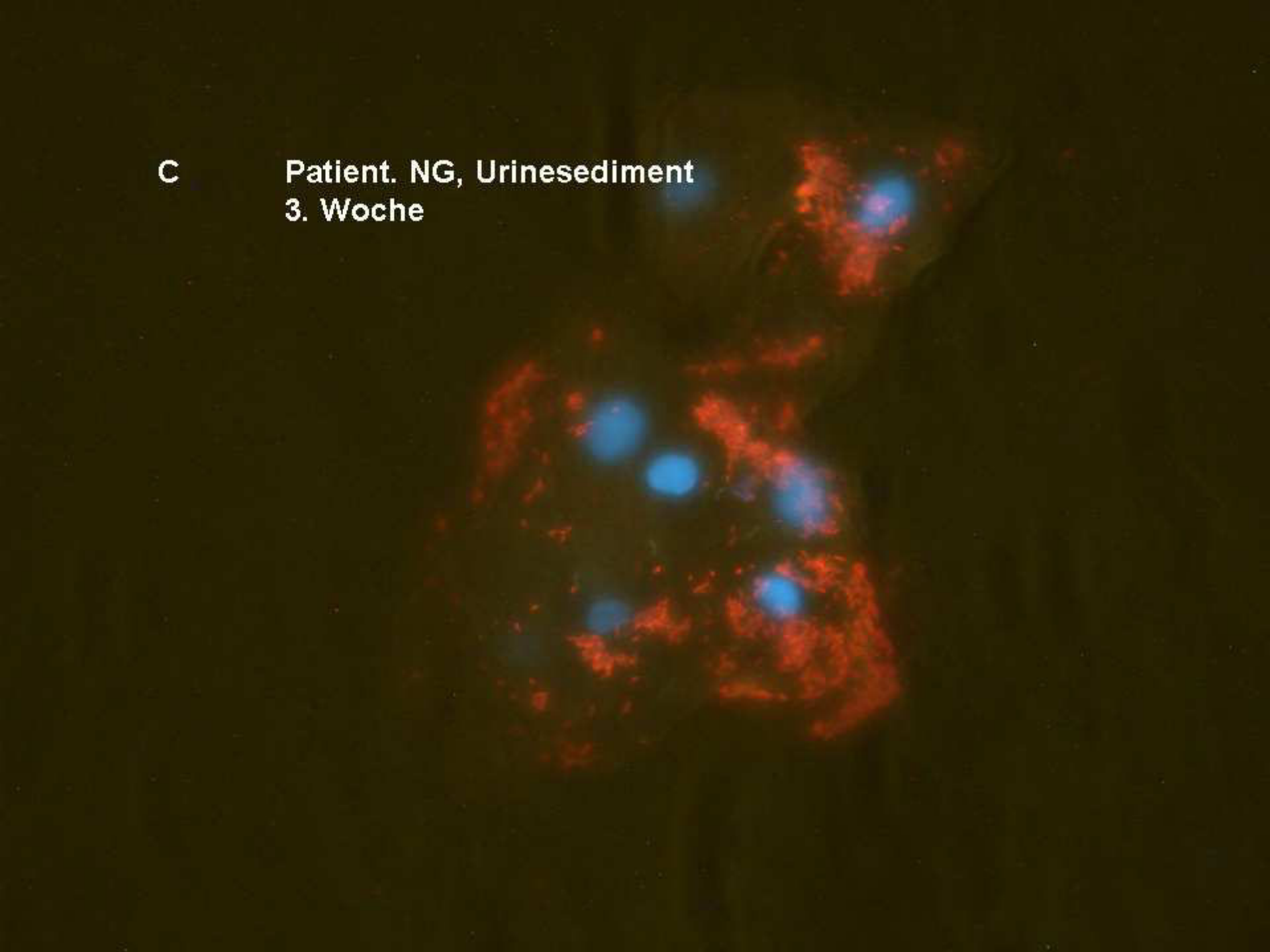
Fertilisierung



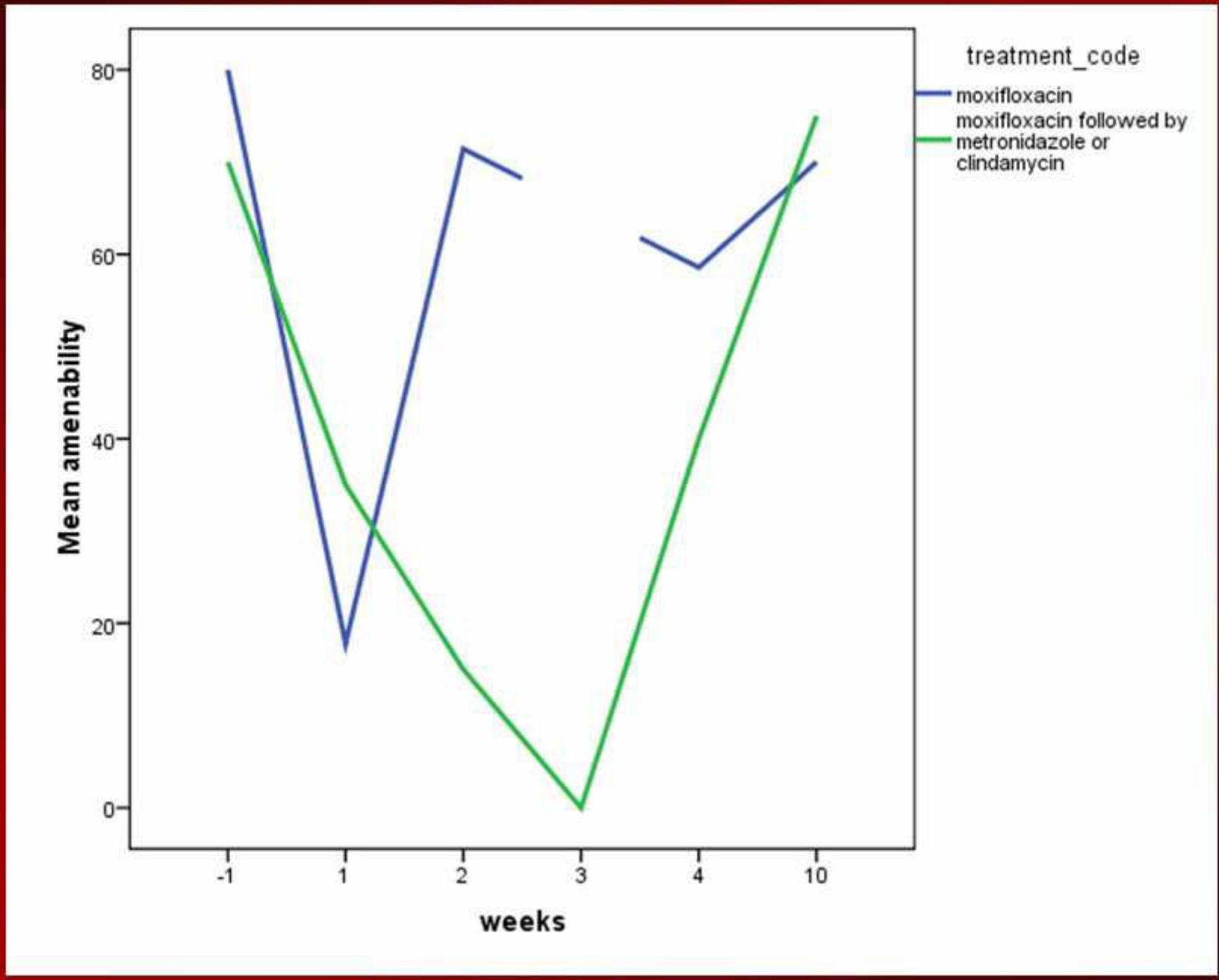
Avalox

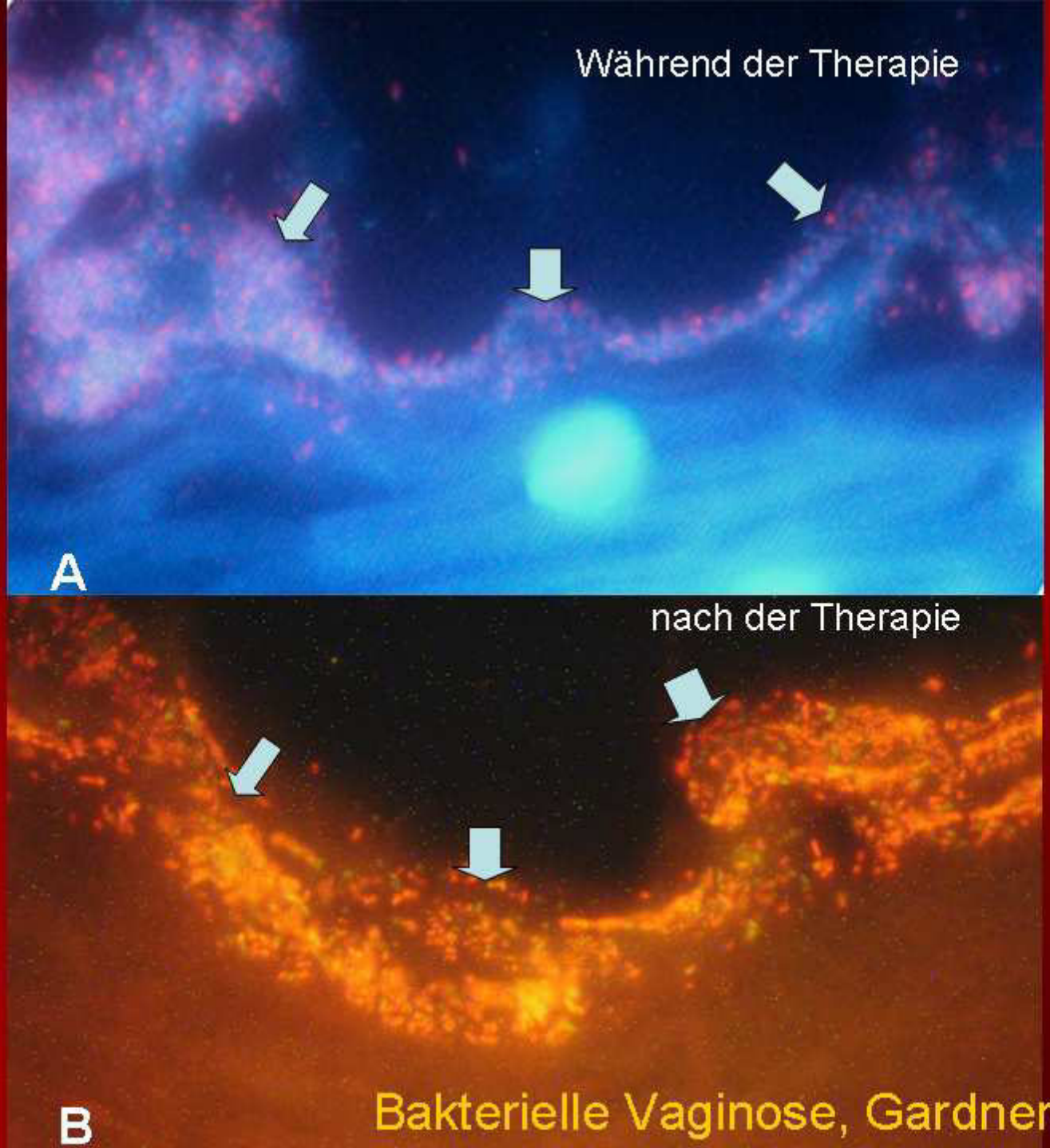

A Patient. NG, Urinsediment vor Avalox


B
Patient NG, vaginale Biopsie,
Tag 5 Avalox


C
Patient. NG, Urinesediment
3. Woche

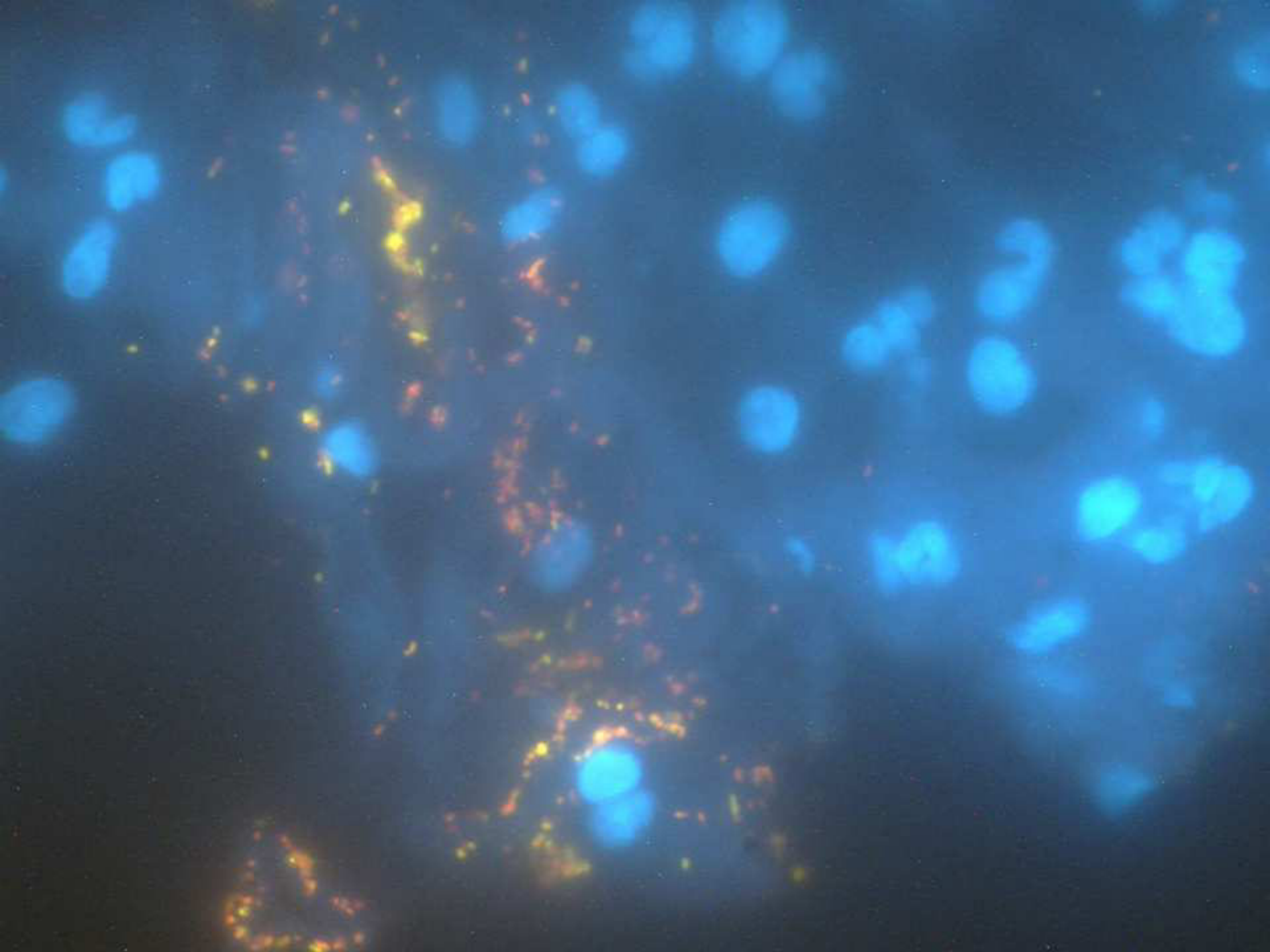

D
Patient NG, vaginale Biopsie,
12. Woche

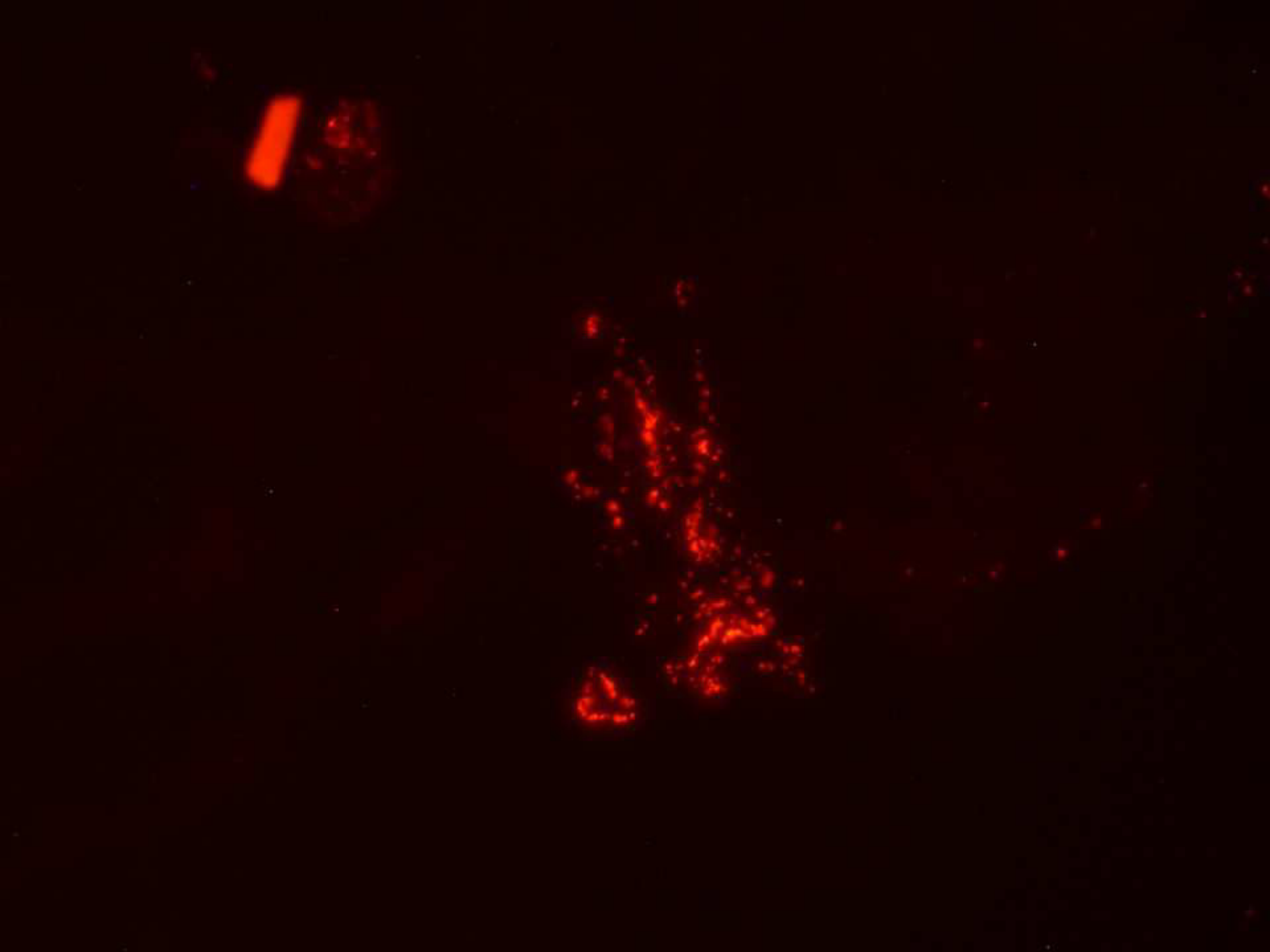

treatment_code
moxifloxacin
moxifloxacin followed by metronidazole or clindamycin
Mean amenability
80
60
40
20
0
-1
1
2
3
4
10
weeks



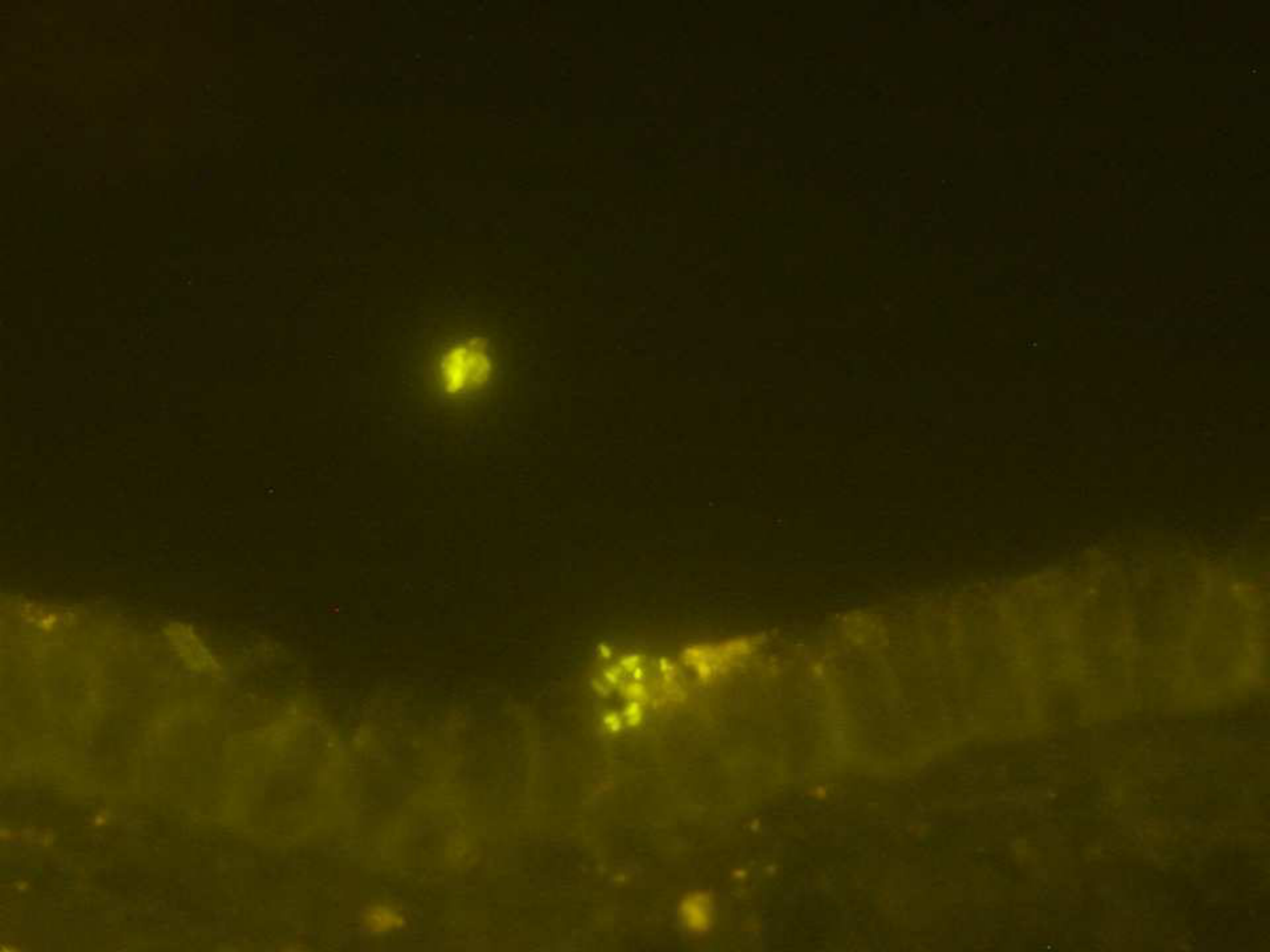
Bakterielle Vaginose, Gardnerella biofilm

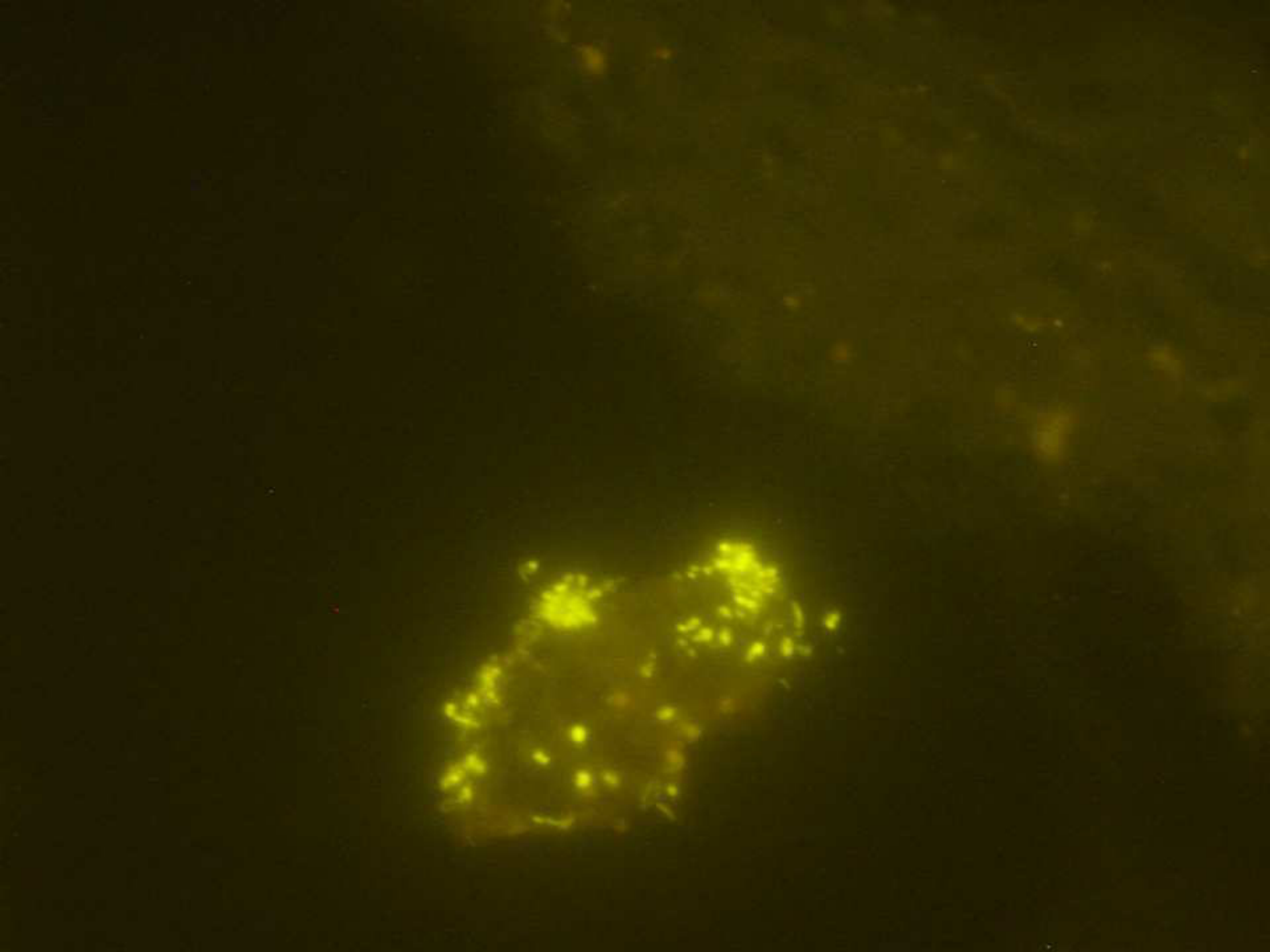
Uterus



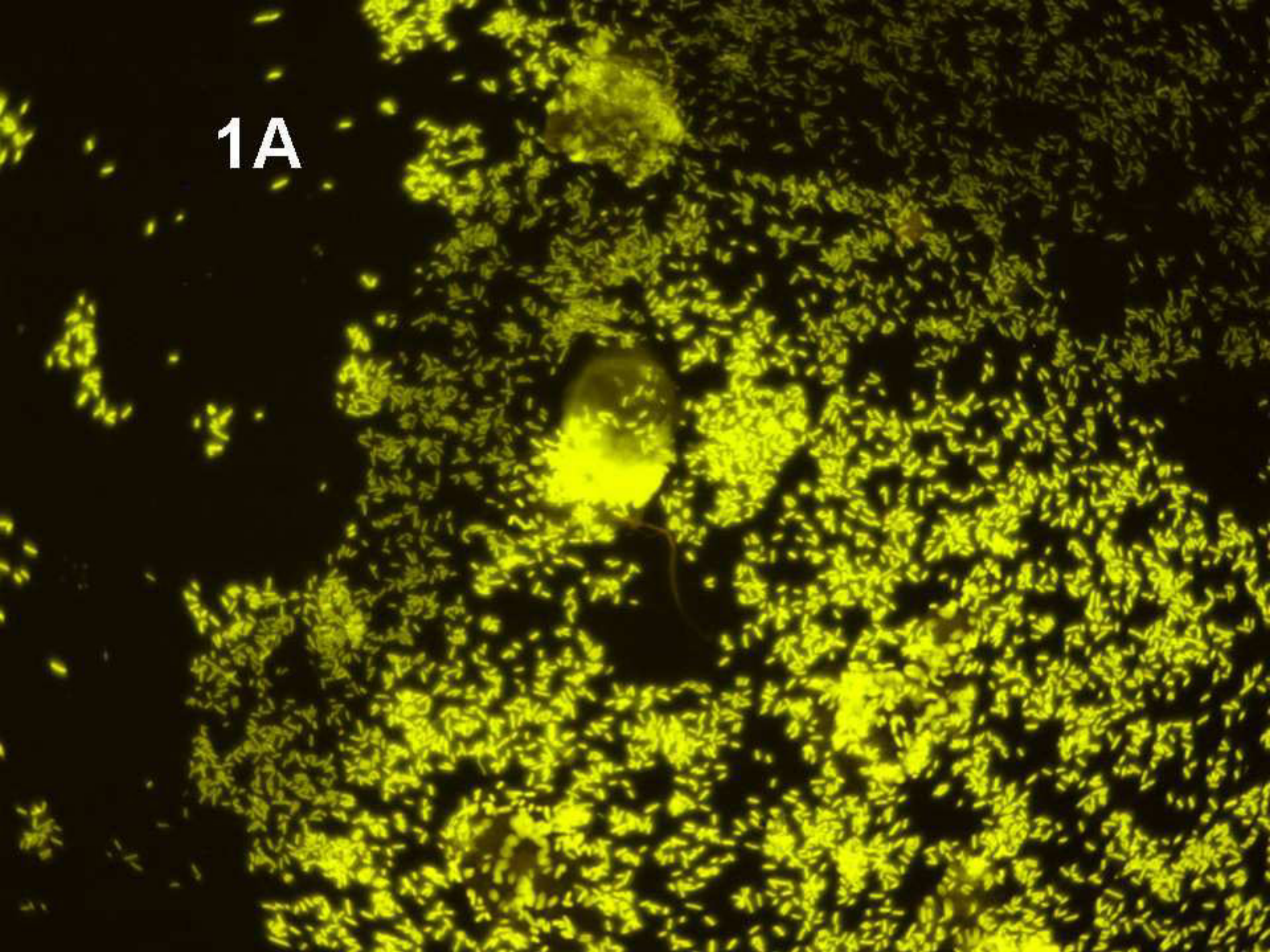


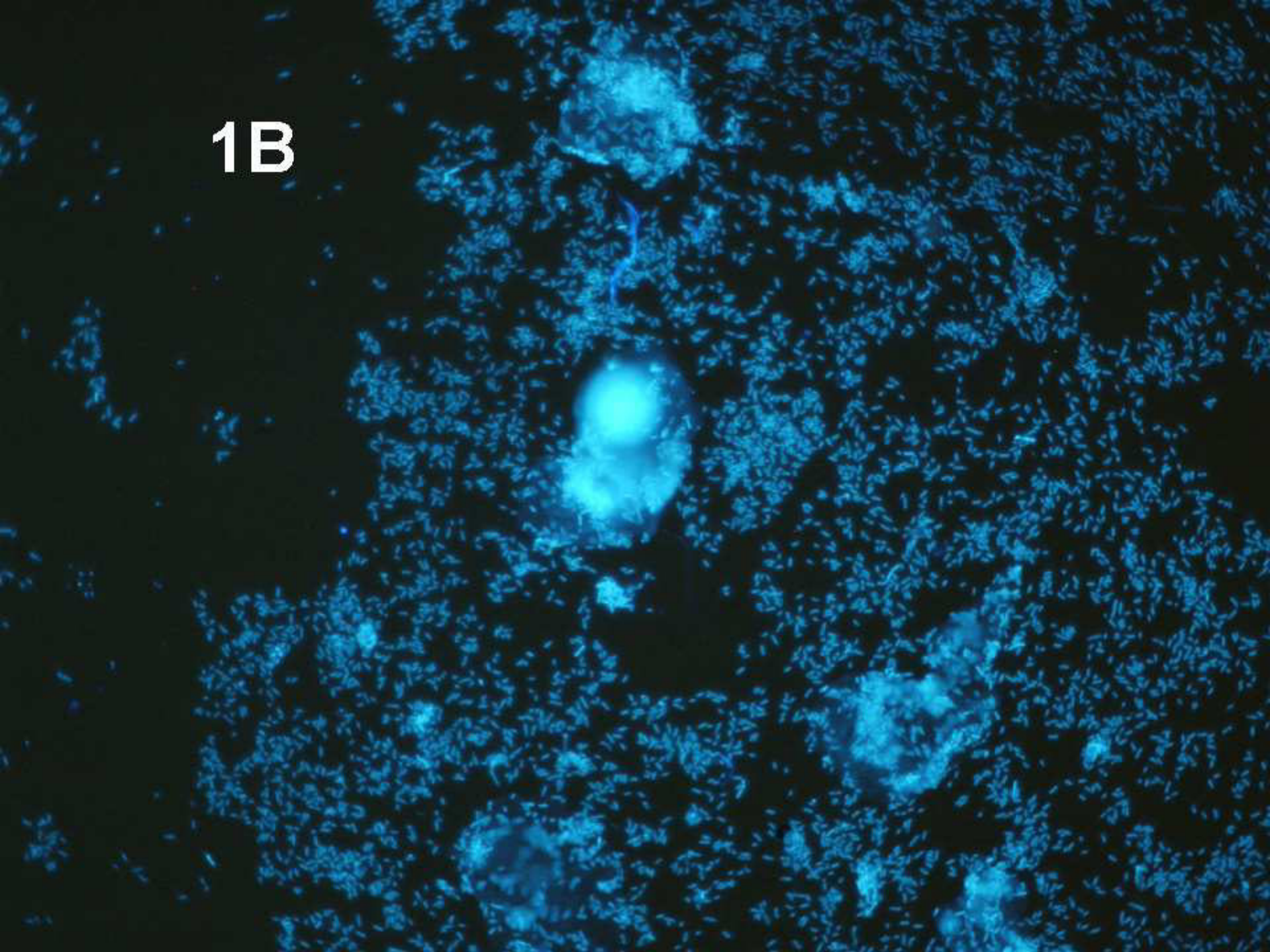
Tube

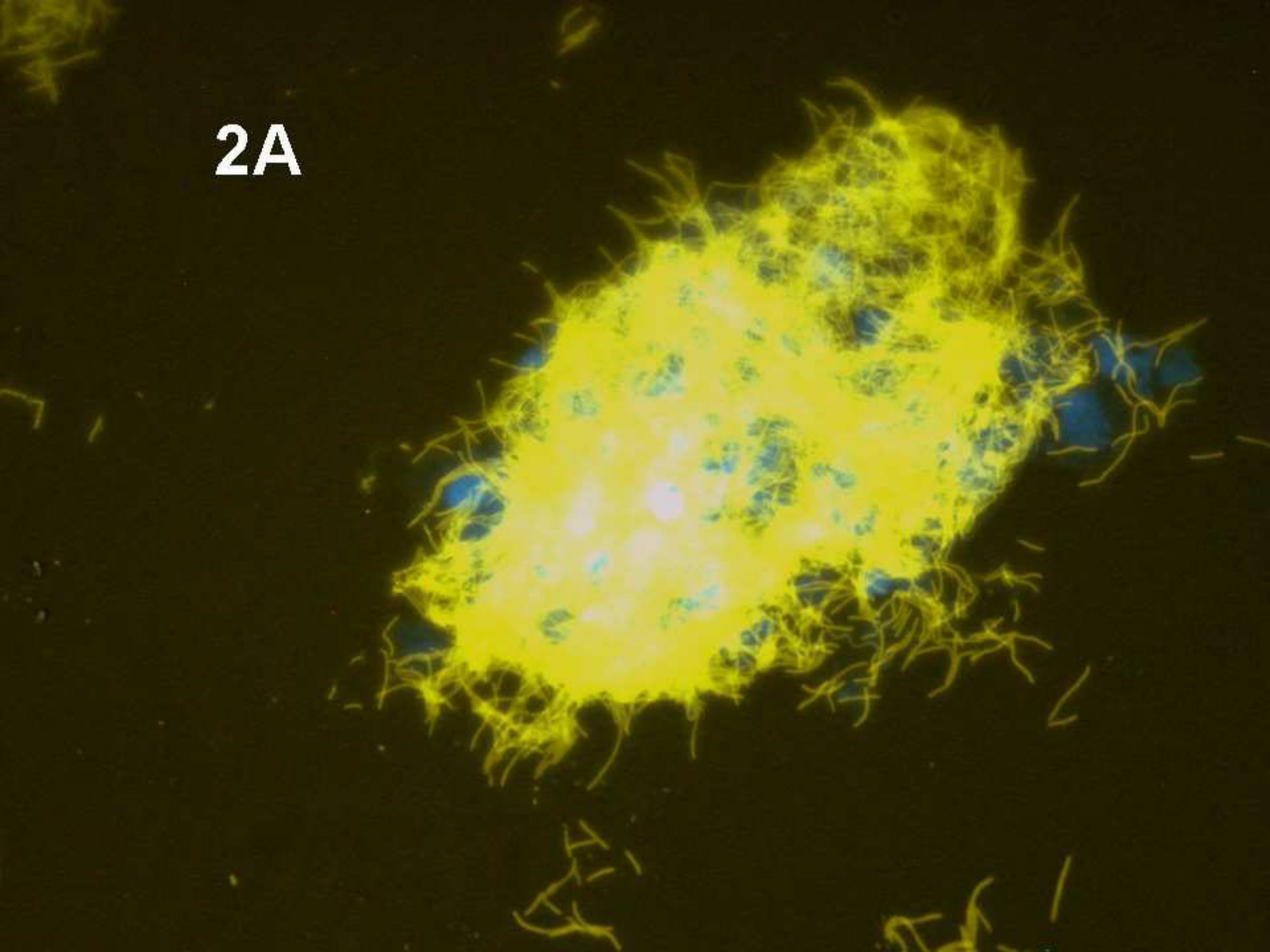


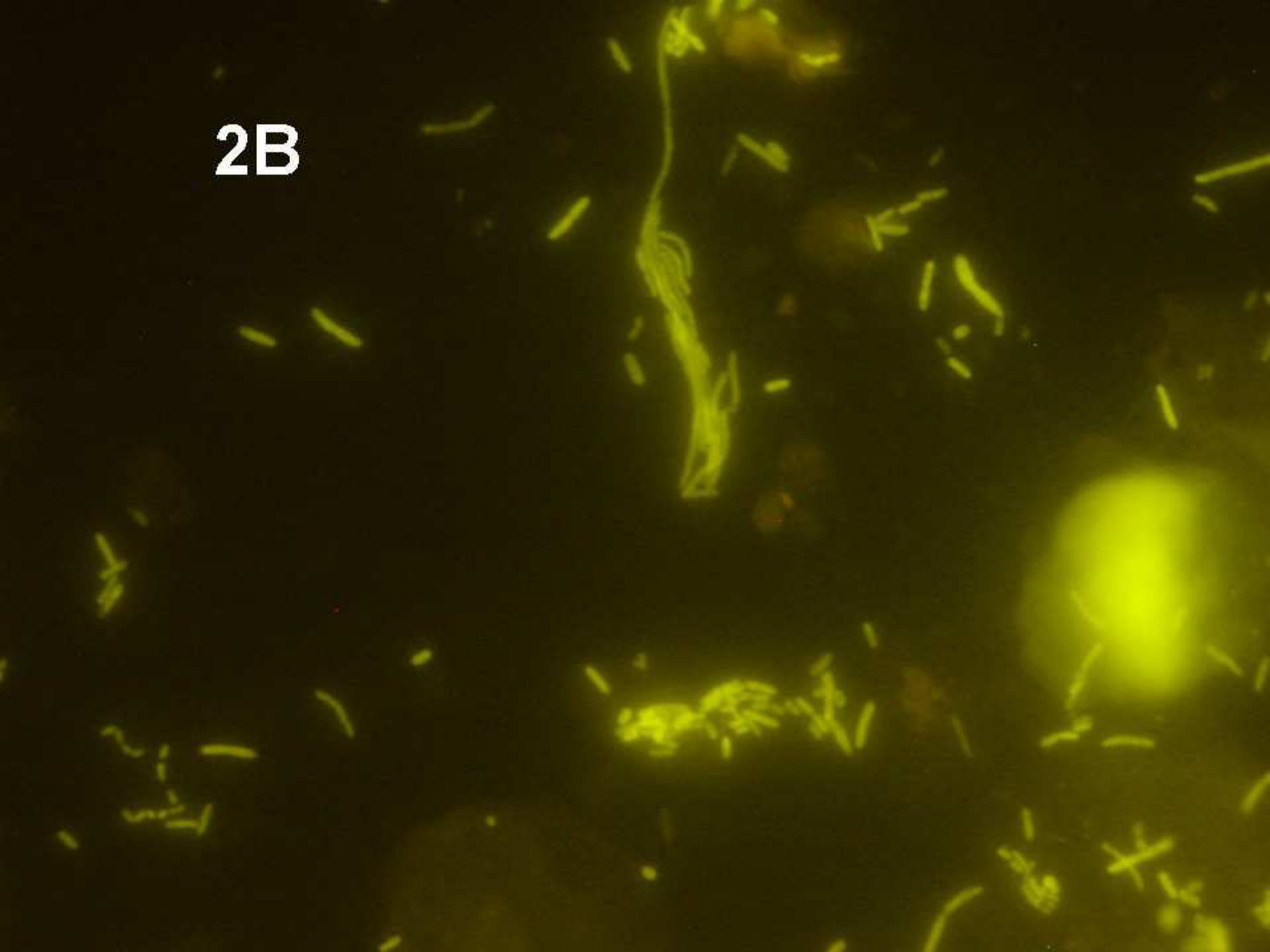


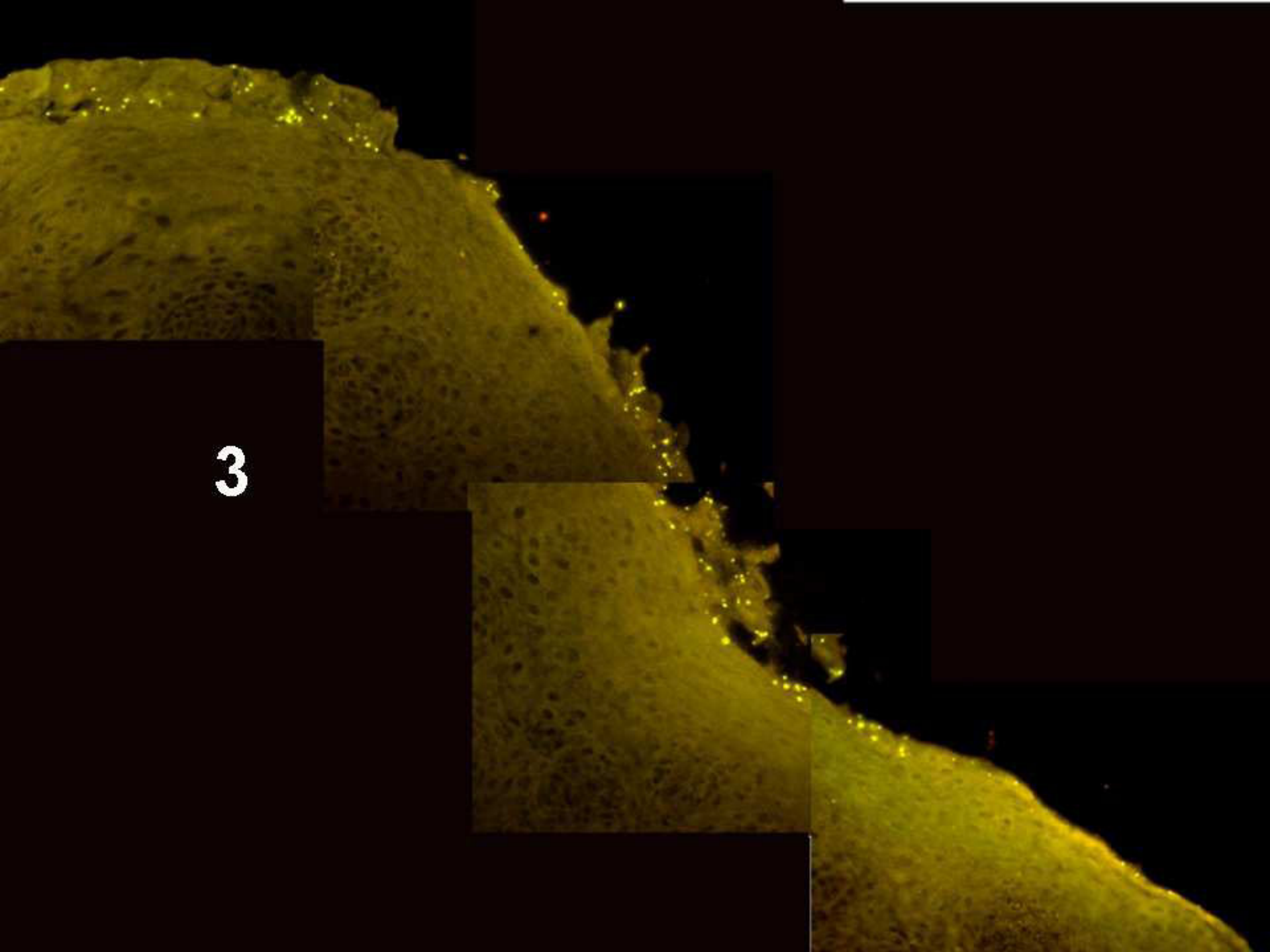
Cystitis

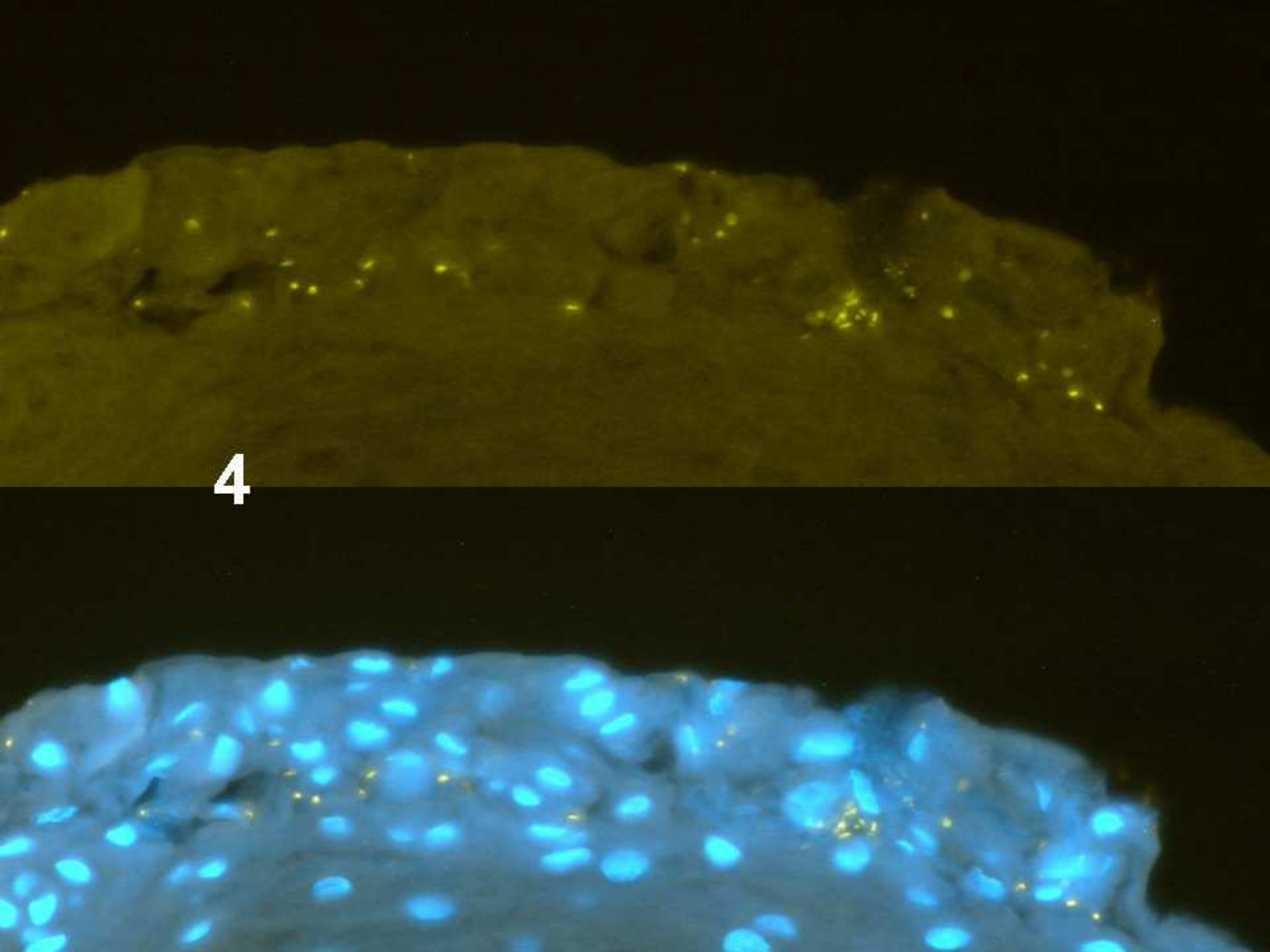

1A


1B


2A


2B


3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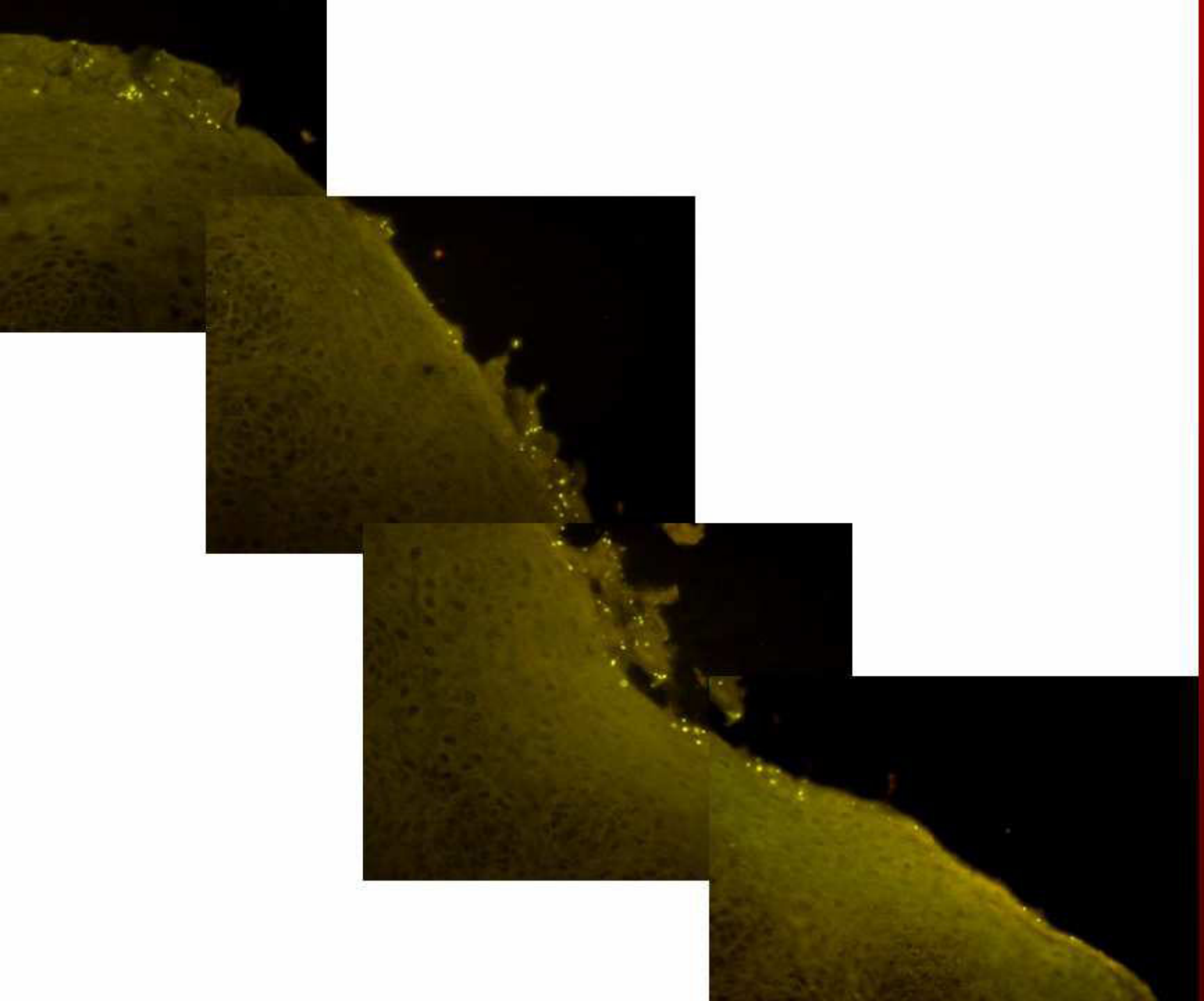

Haut