Mucosal Flora in IBD

Alexander Swidsinski

Supported by Broad Medical Research Program

Mean± SD (x10^3 cfu/μL) of Mucosal Bacteria

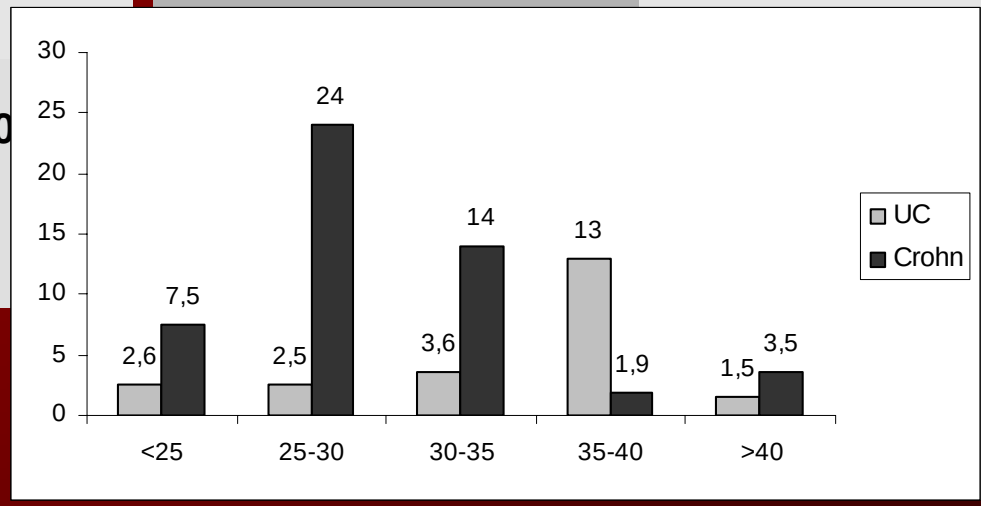
	Asymptomatic Controls (n=40)	Self-limiting Colitis (n=28)	Indeterminate Colitis (n=104)	UC (n=156)	CD (n=82)
Total anaerobes	**0.18±0.3**	**1.8±5.3** NS	**3.41±16** *P* < 0.08	**3.8±11** *P* < 0.01	**9.1±18** *P* < 0.001
Bacteroides	**0.02±0.05**	**0.26±0.6** NS	**0.64±2.1** *P* < 0.01	**1.4±9** *P*< 0.001	**3.1±5.5** *P* < 0.001
Total aerobes	**0.003±0.05**	**0.08±0.4** NS	**0.09±0.5** *P* < 0.005	**0.08±0.6** *P* <0.05	**0.14±0.8** *P* < 0.001
Enterobacteriaceae	**0.002±0.05**	**0.06±0.5** *P* < 0.06	**0.08±0.3** *P* < 0.005	**0.04±0.5** *P* <0.047	**0.090±0.8** *P* < 0.001

P as compared to controls

Mucosal bacteria (x10^3 cfu/μL) and clinical data

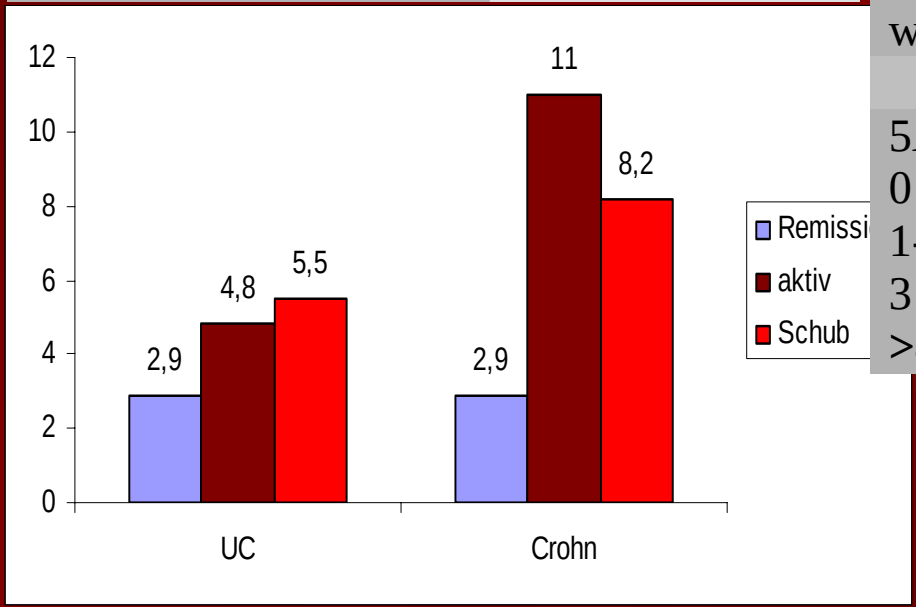
	UC (n=156)	CD (n=82)
All	3.8	9.1
patients age		
<25	2.0	2.6
25-30	1.7	9.1
30-35	4.5	11.9
35-40	3.4	10.8
40-45	3.2	4.3
45-50	6.9	5.5
>50	2.6	5.2
age at the time of manifestation		
<25	2.6	7.5
25-30	2.5	**24.0**
30-35	3.6	**14**
35-40	**13**	1.9
>40	1.5	3.5

	UC (n=156)	CD (n=82)
All	3.8	9.1
duration of disease in years		
<5	3.6	10
5-20	4.6	8.05
>20	2.5	1.9
f	4.2	**12.0**
m	2.8	**4.7**



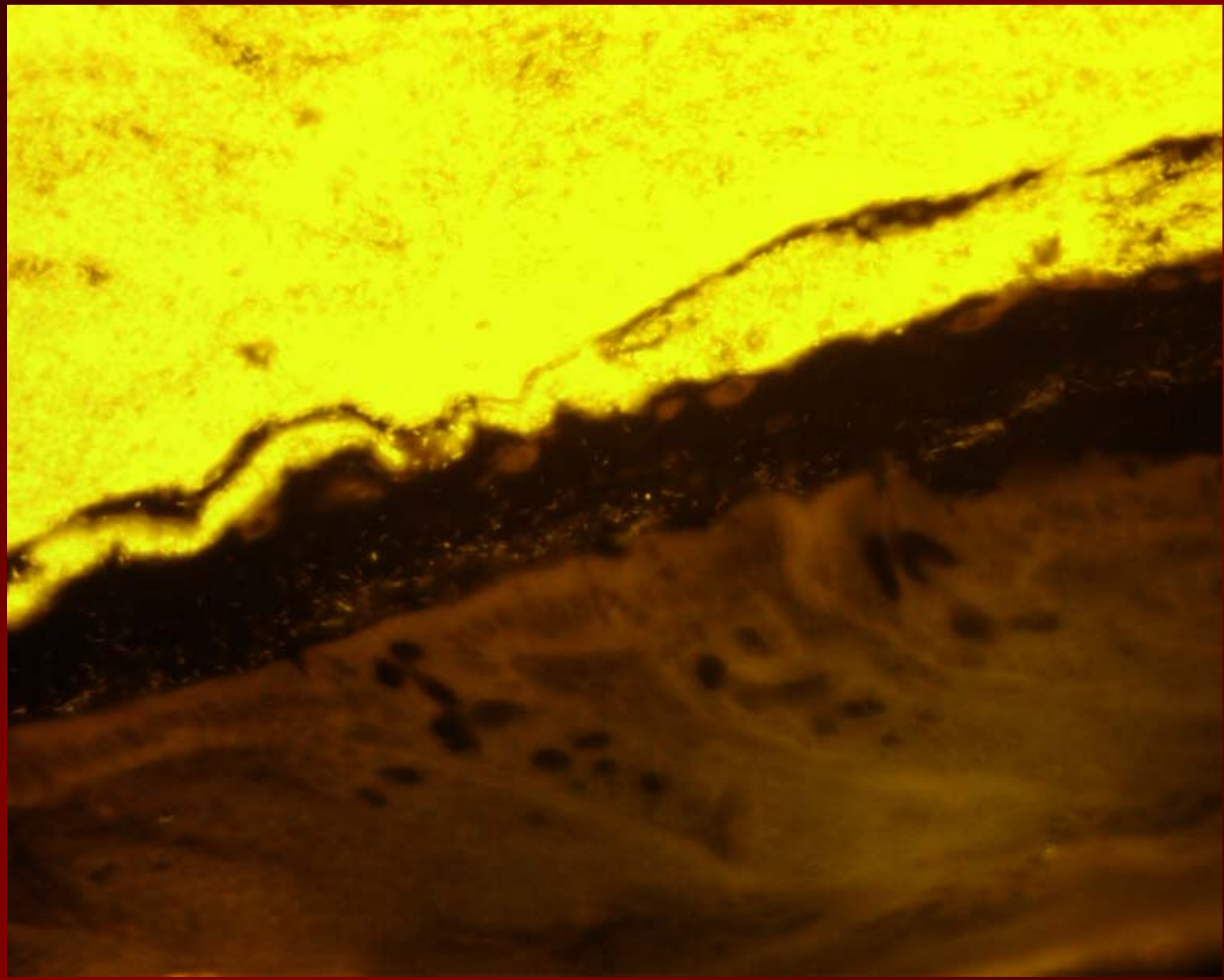
Mucosal bacteria, disease activity and therapy

	UC (n=156)	CD (n=82)
All	3.8	9.1
remission	2.9	**2.9**
activity	4.8	11.0
exacerbated	5.5	8.2
fistula		**20.1**
no		4.8
colonic surgery	2.8	9.0
without surgery	4.6	10.0

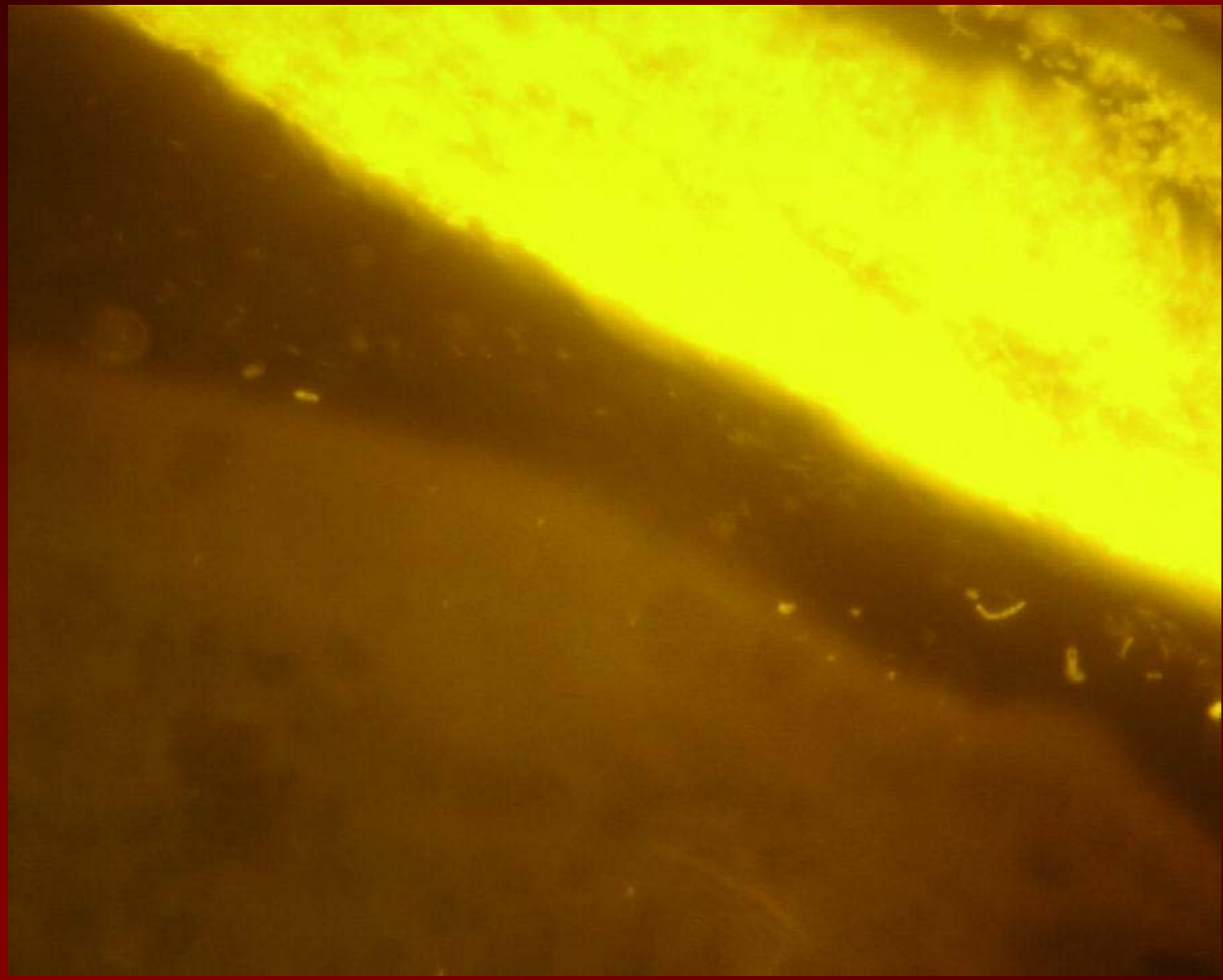


	UC (n=156)	CD (n=82)
All	3.8	9.1
no antibiotics in last 12 months	4.6	9.2
on antibiotics	**0.5**	**1.4**
after antibiotics (1-4 weeks)	5.9	27
no azathioprine	3.1	9.9
azathioprine	5.8	8.3
corticosteroids	**8.2**	12.0
without	3.7	8.7
5ASA in gramm		
0	4.2	**14.9**
1-2,5	3.7	6.5
3	2.7	3.5
>4	1.4	2.6

Multicellular bacteria forming stromatolith in Australian salt lakes



Mucus gap between the colonic wall and feces in healthy mouse



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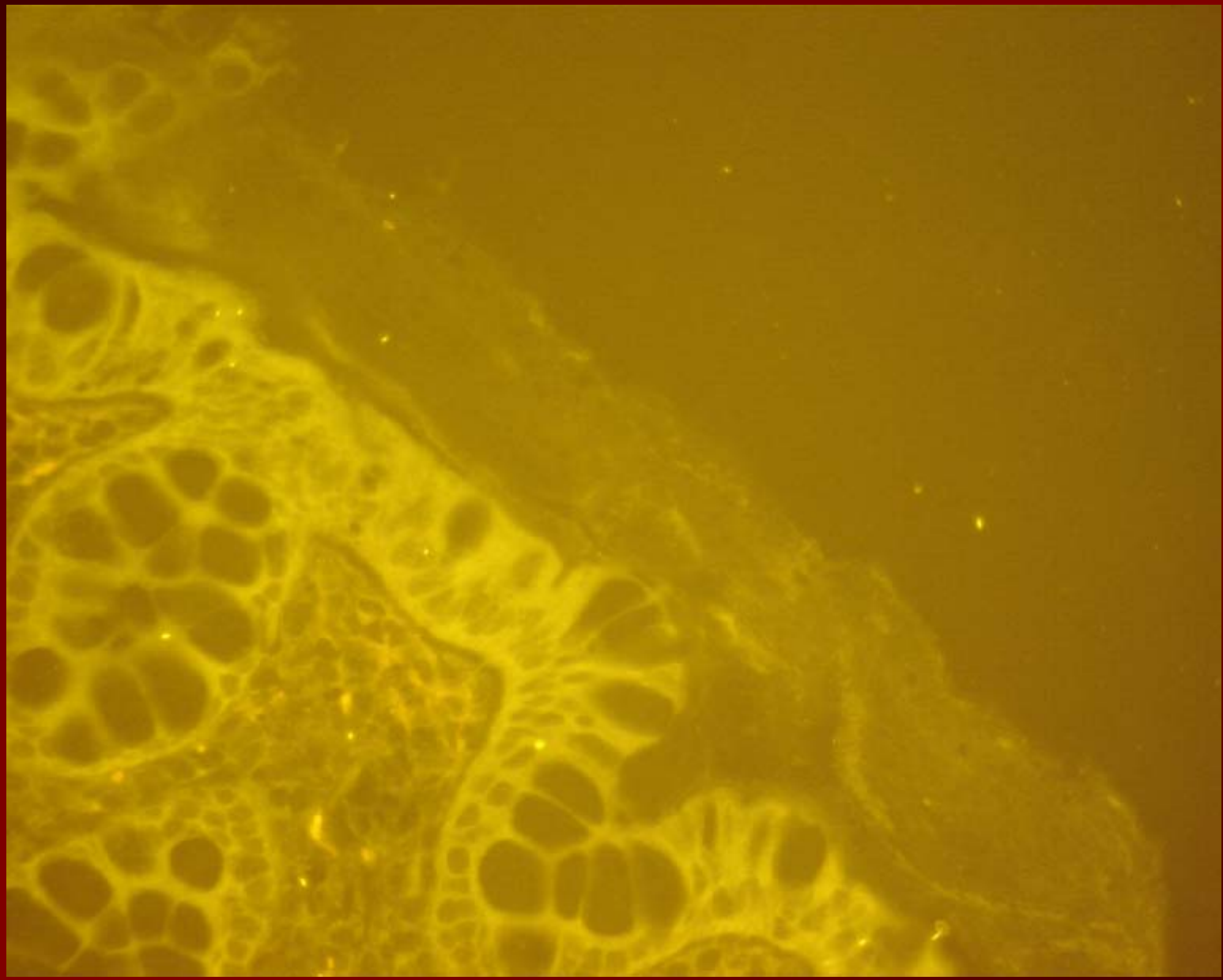
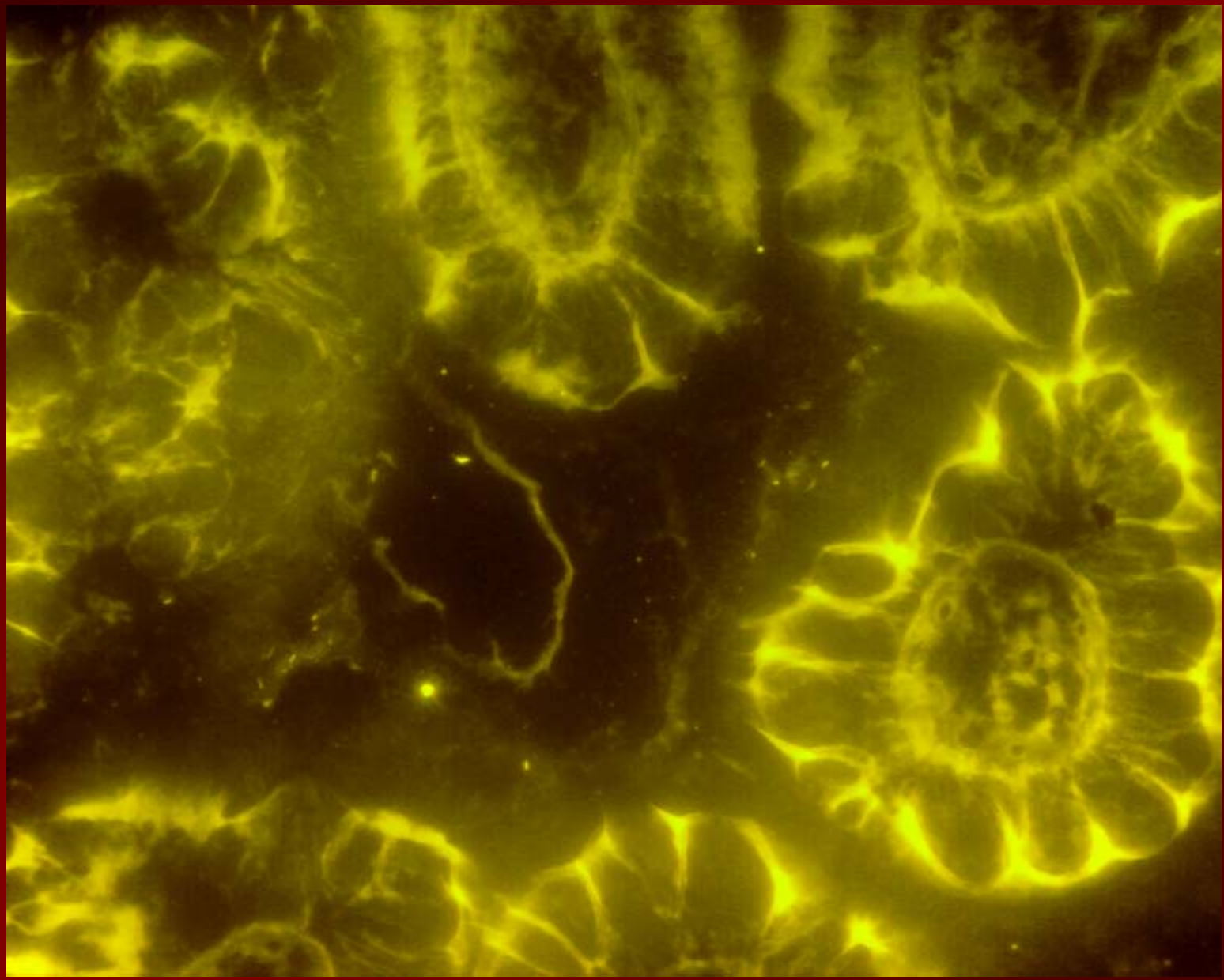
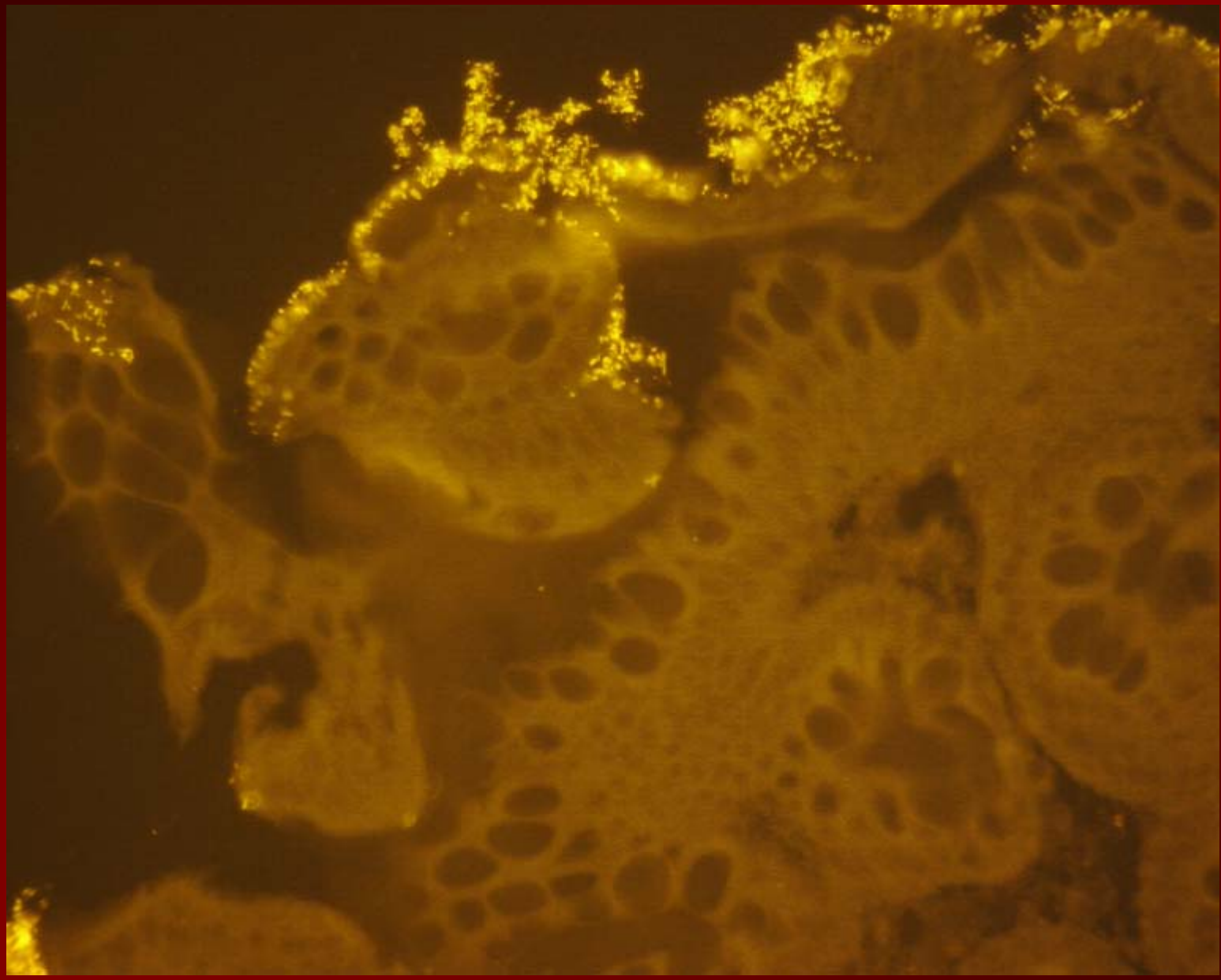


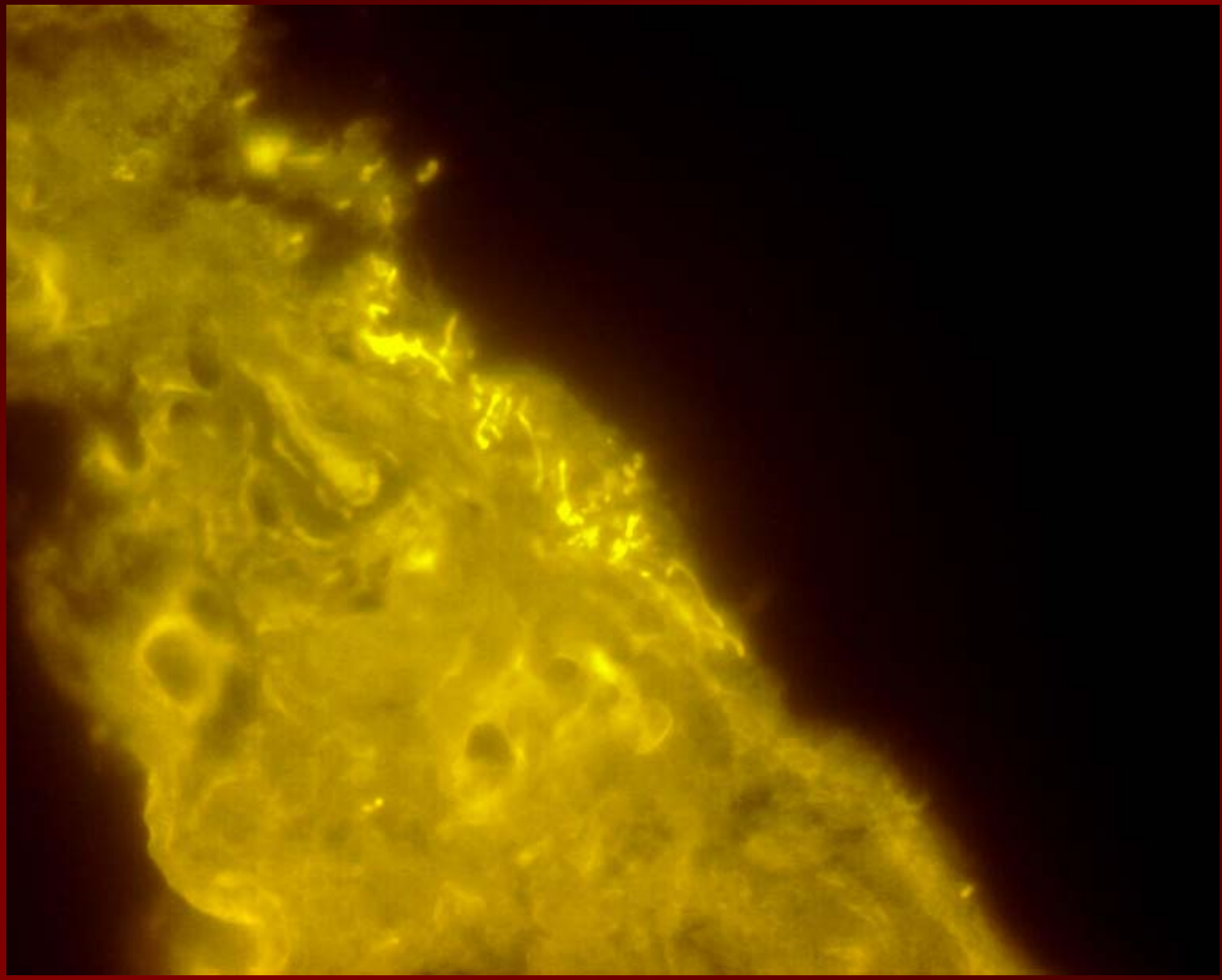
Human colonic wall covered with mucus omitting bacteria



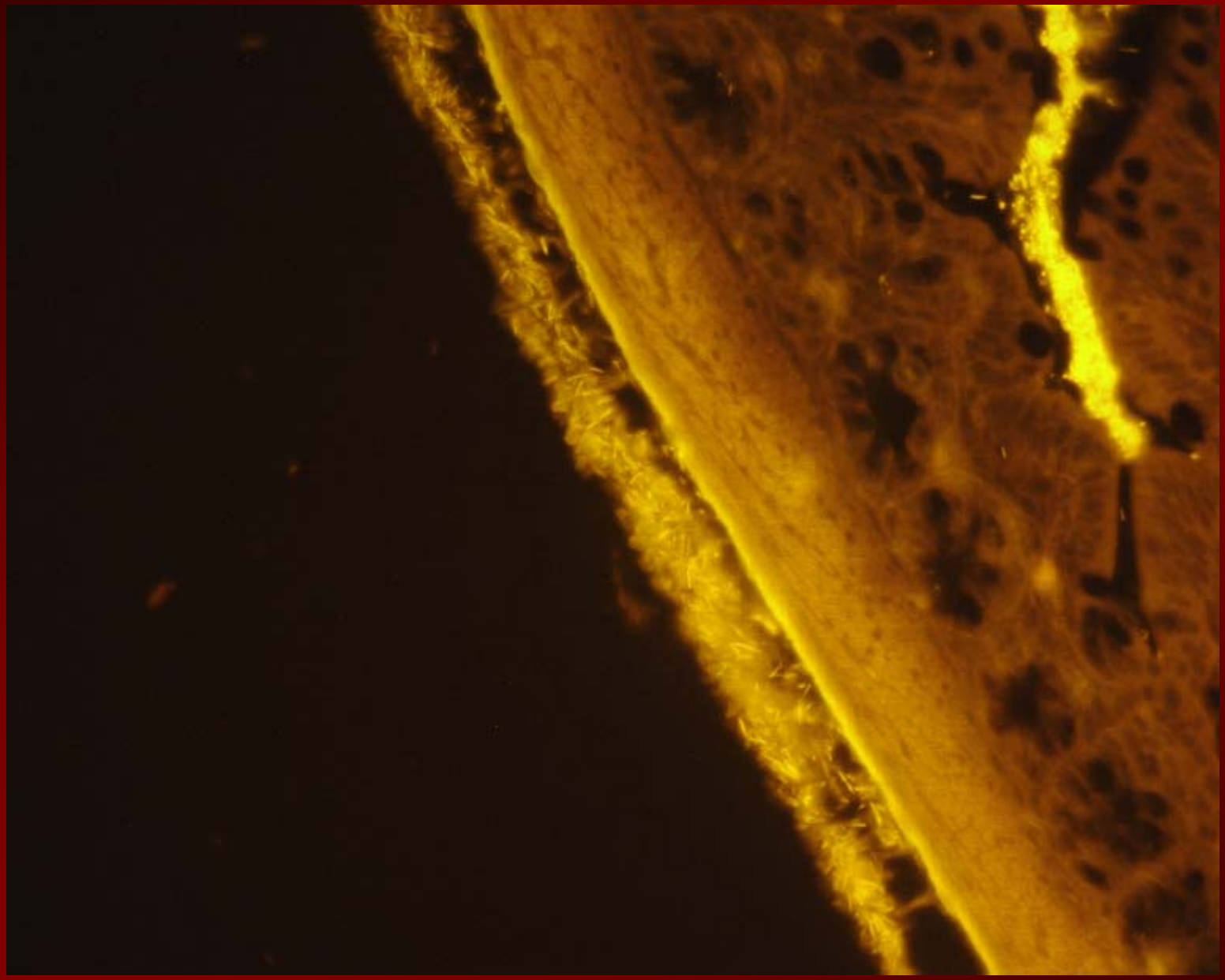
Human ileal wall covered with mucus omitting bacteria



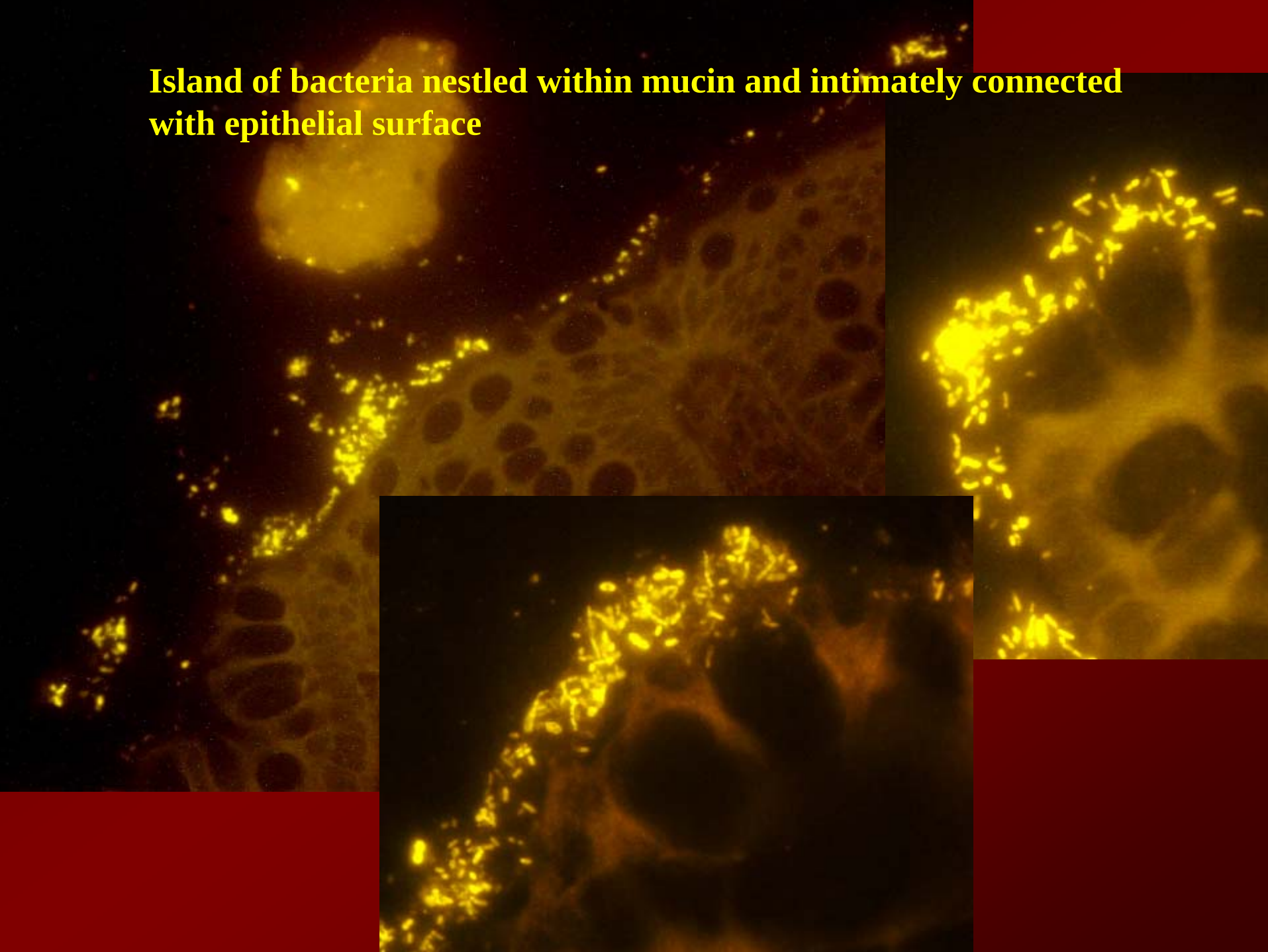
intact mucus is partially denuded, the biopsy is covered by bacteria

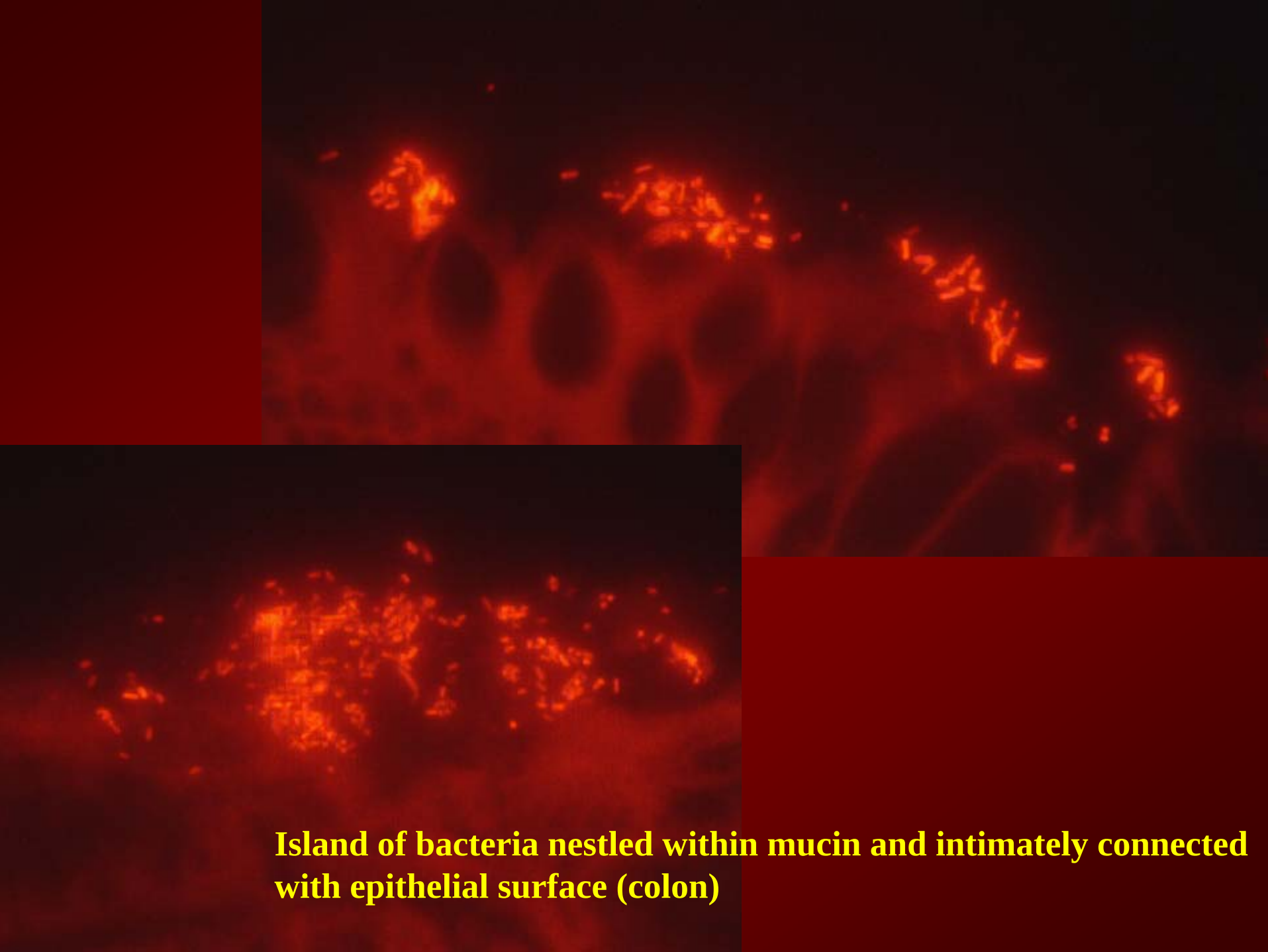


Fecal contamination on submucosal parts of the biopsy

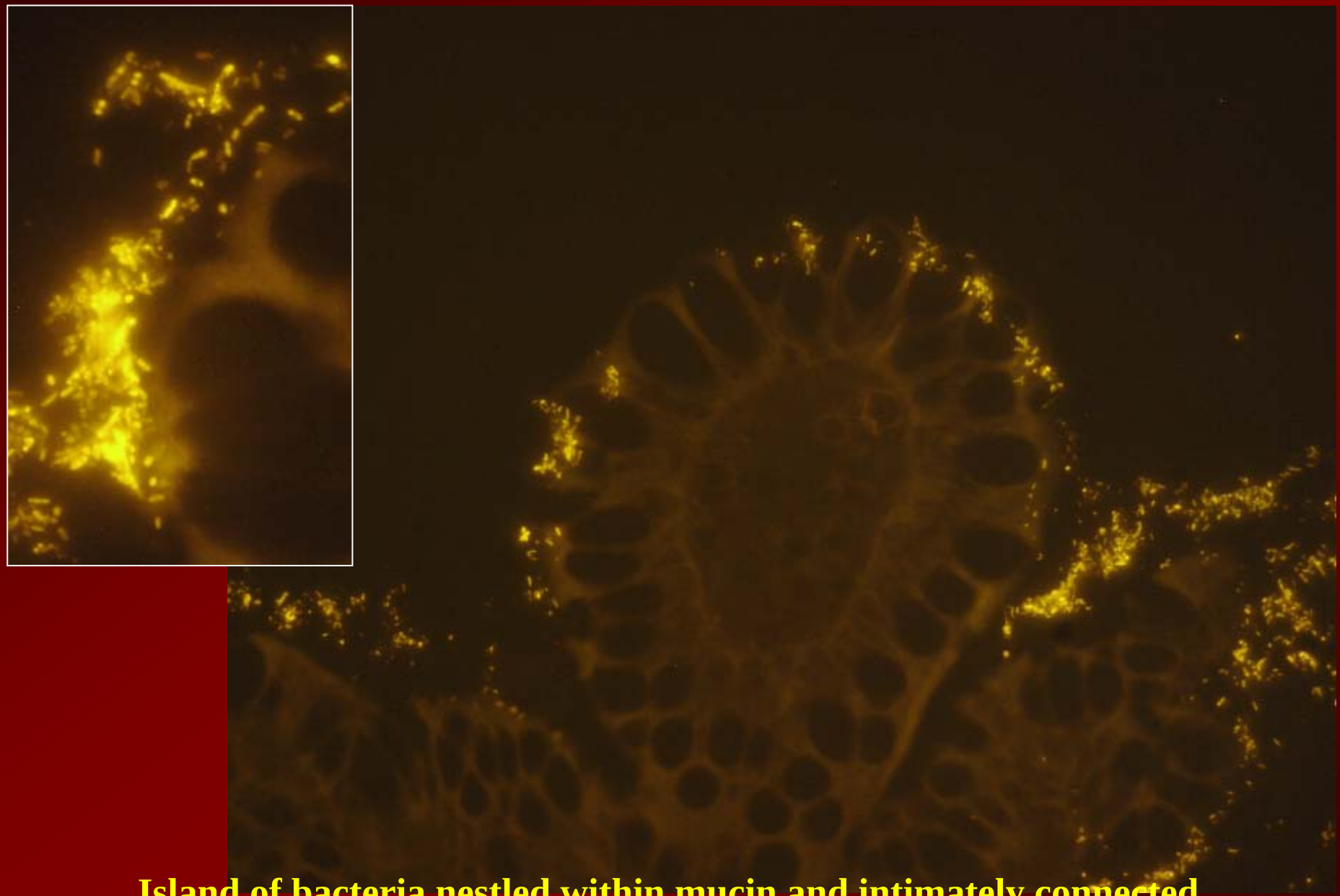


bacteria on the peritoneal side of the mouse intestine/ biases


Island of bacteria nestled within mucin and intimately connected with epithelial surface

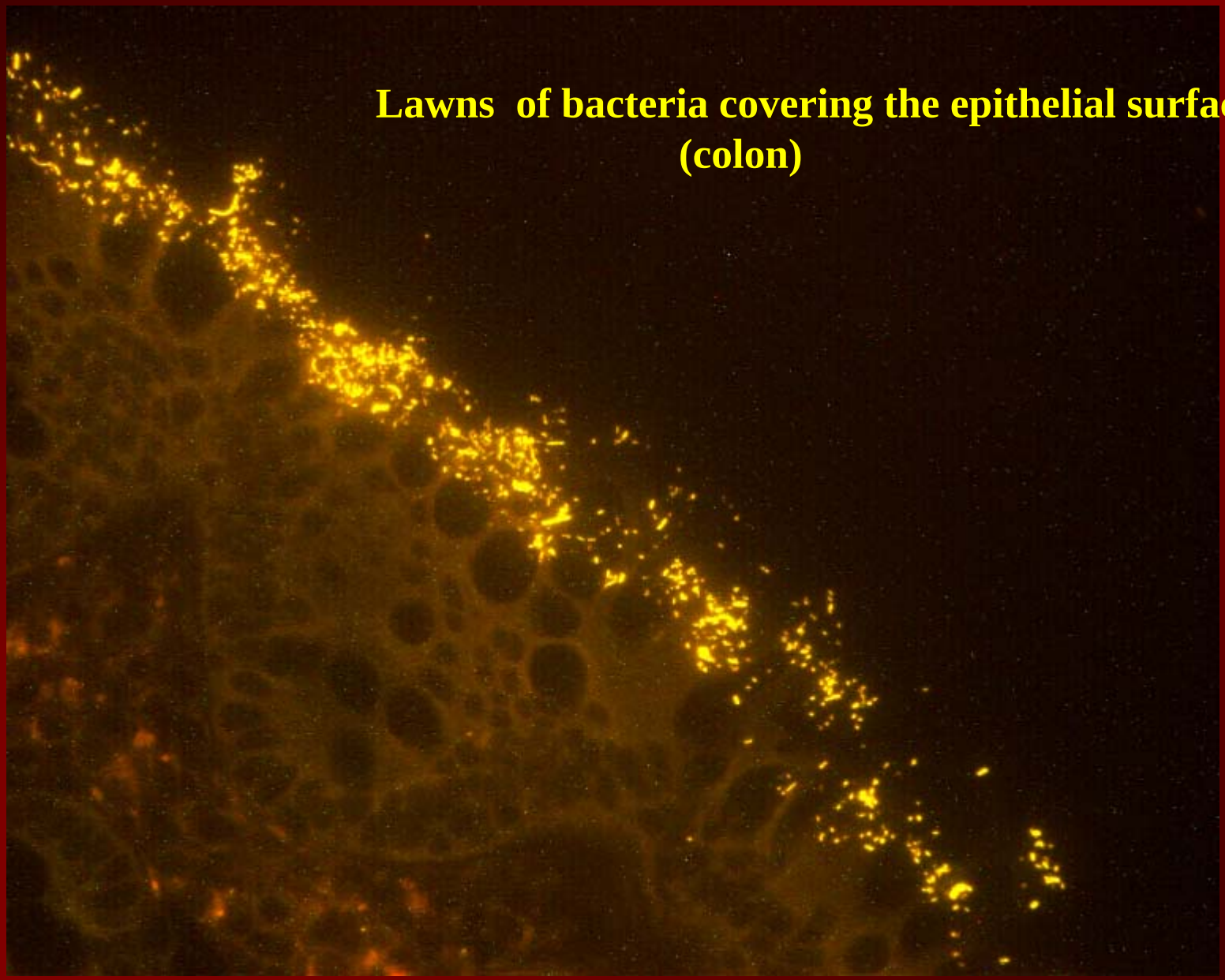


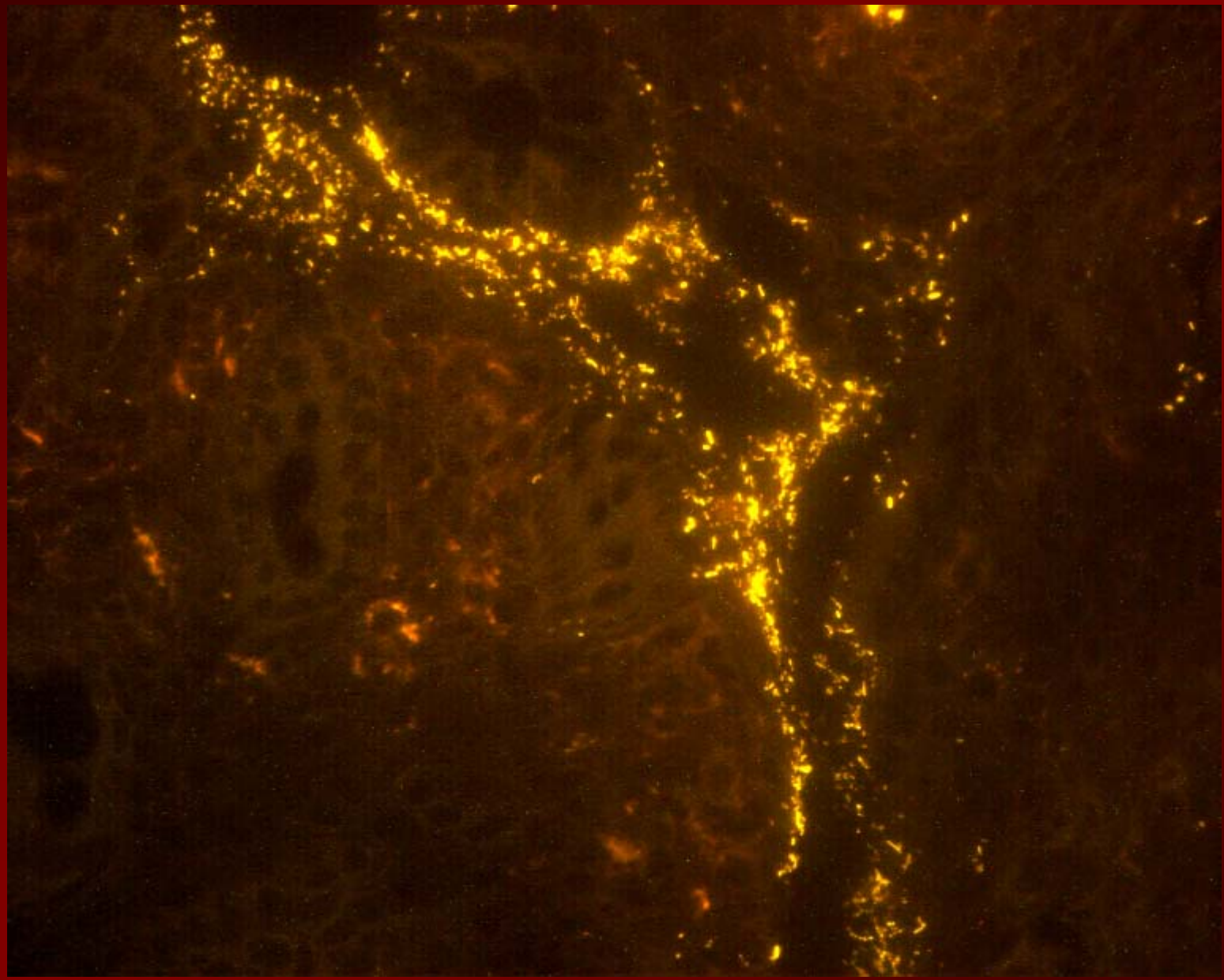
Island of bacteria nestled within mucin and intimately connected with epithelial surface (colon)



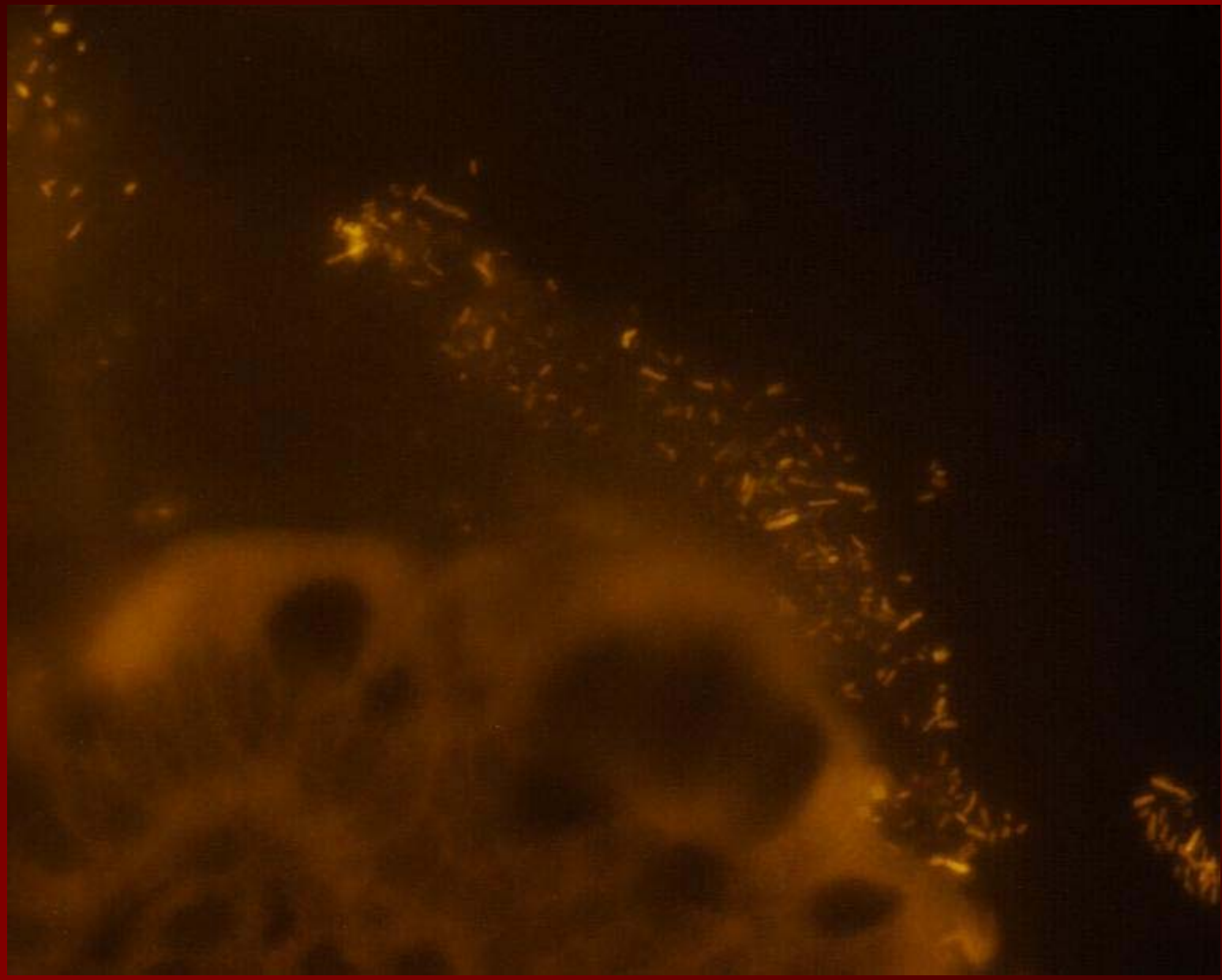
Island of bacteria nestled within mucin and intimately connected with epithelial surface (ileum)


Lawns of bacteria covering the epithelial surface

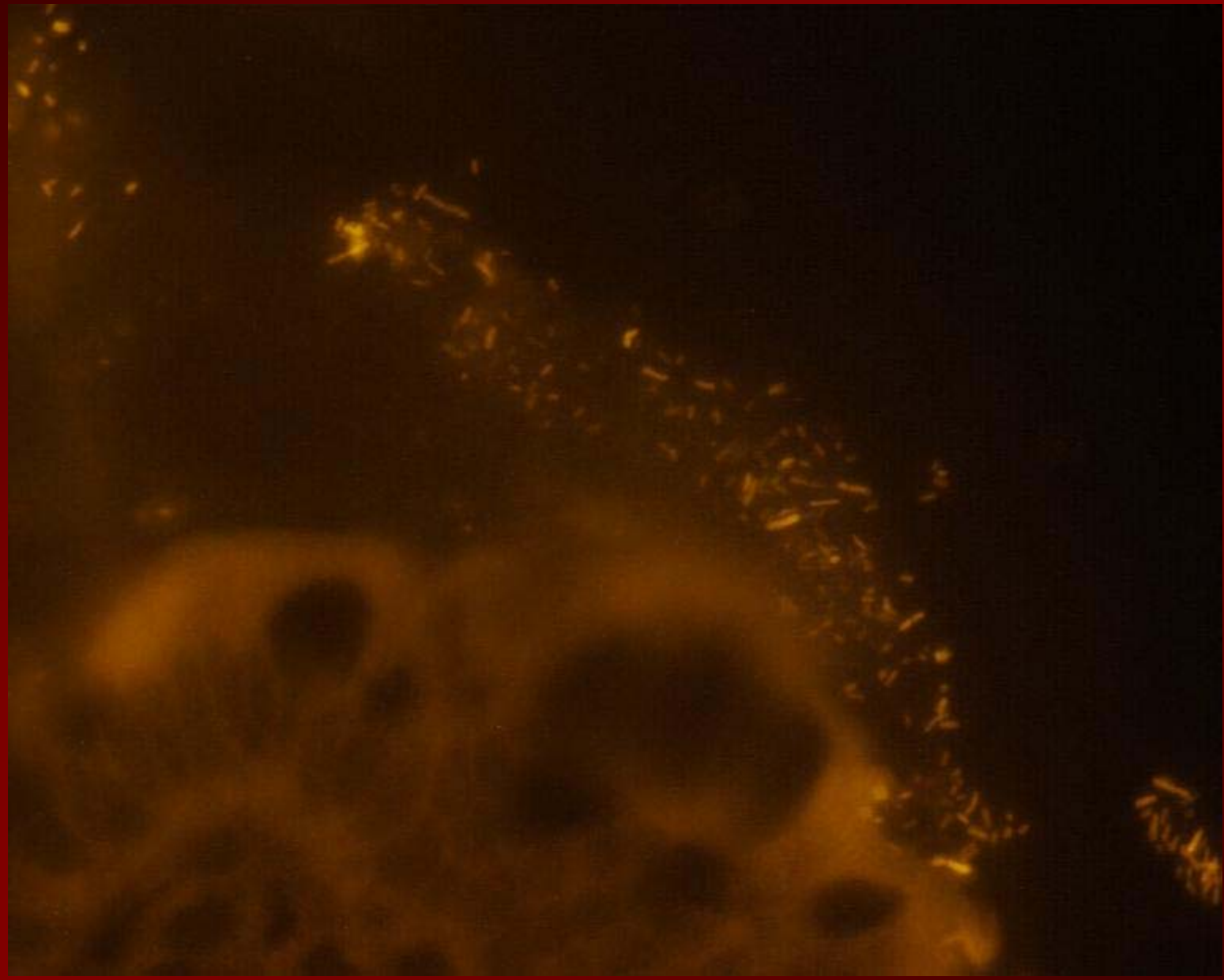

Lawns of bacteria covering the epithelial surface
(colon)

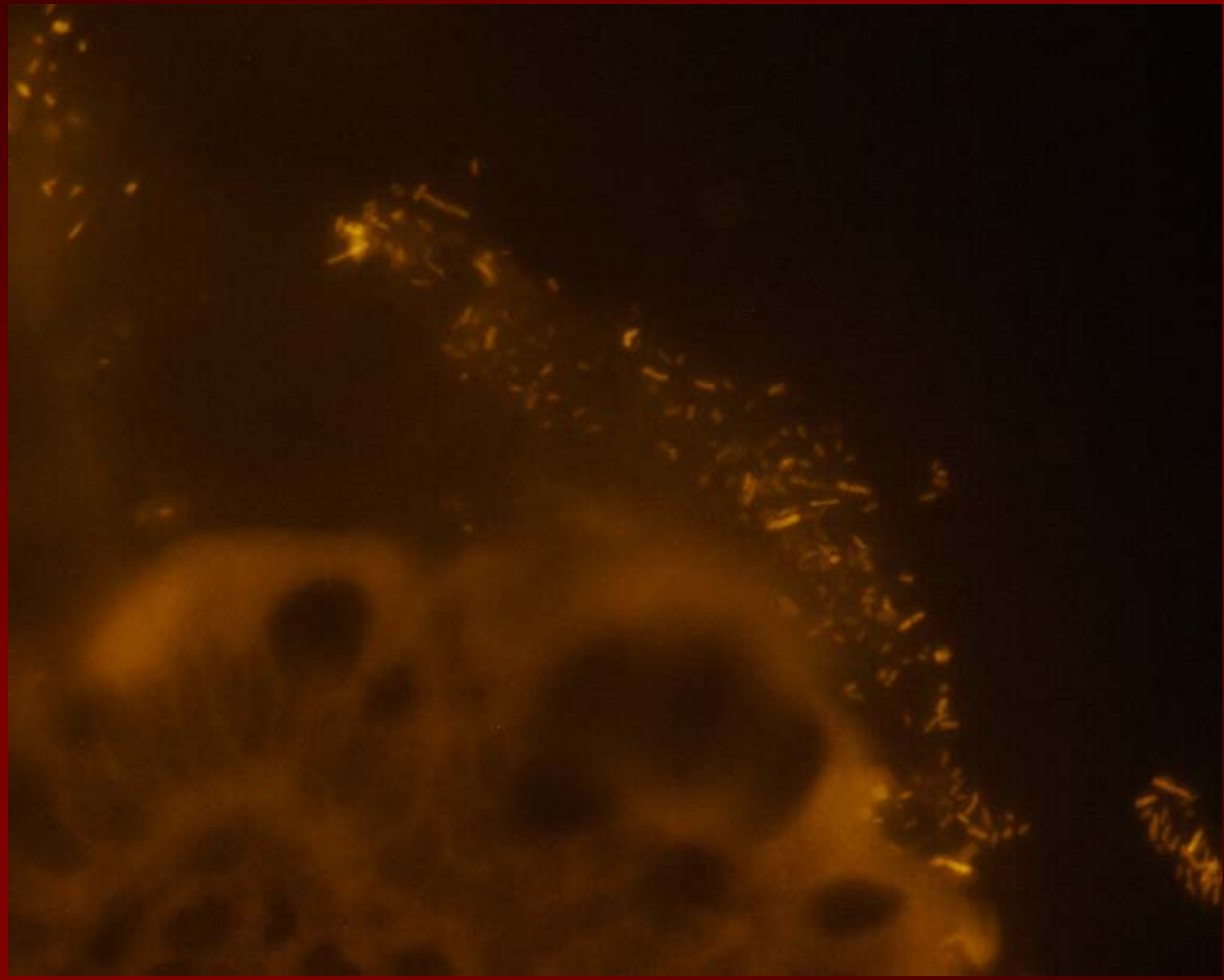


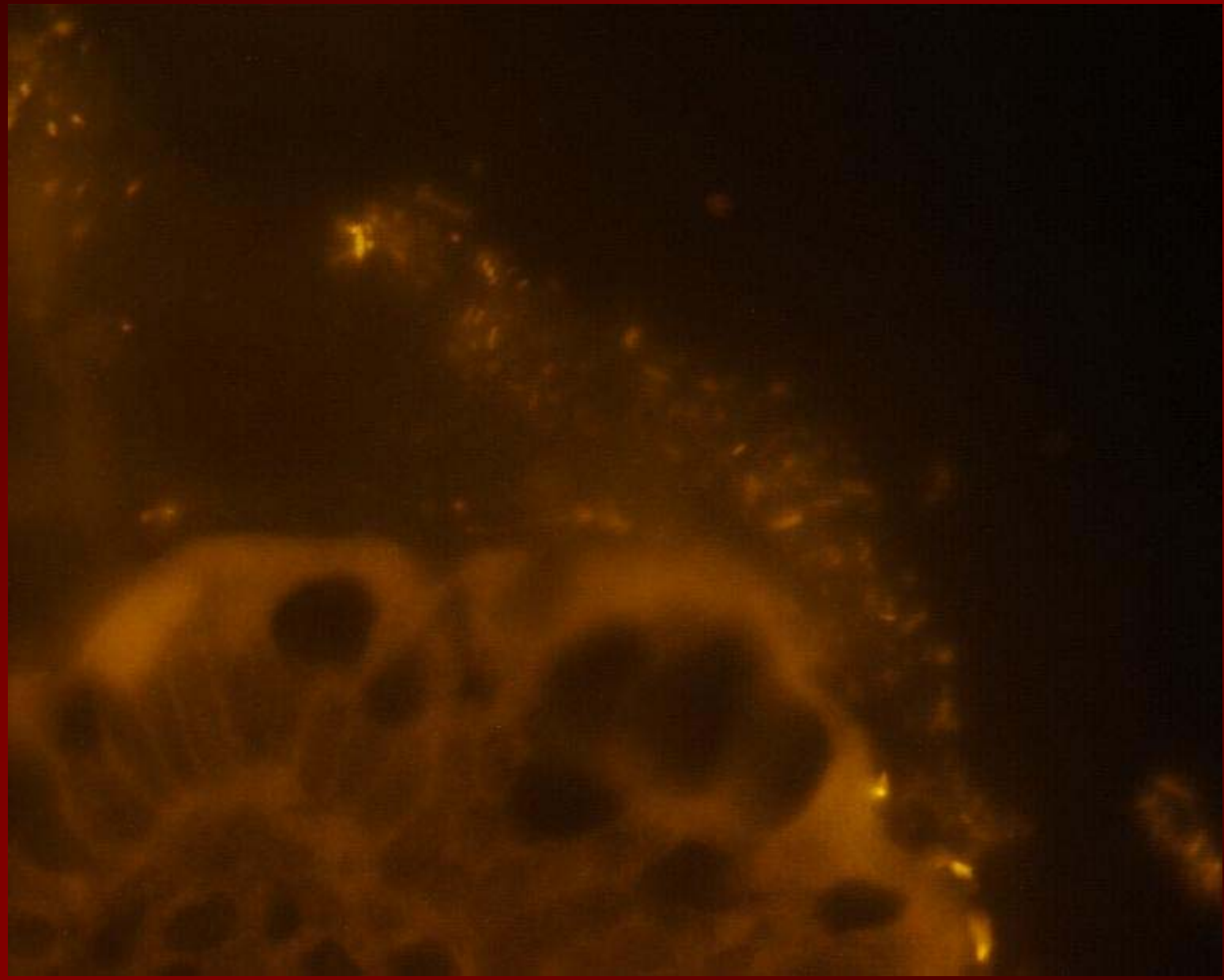
Lawns of bacteria covering the epithelial surface (ileum)

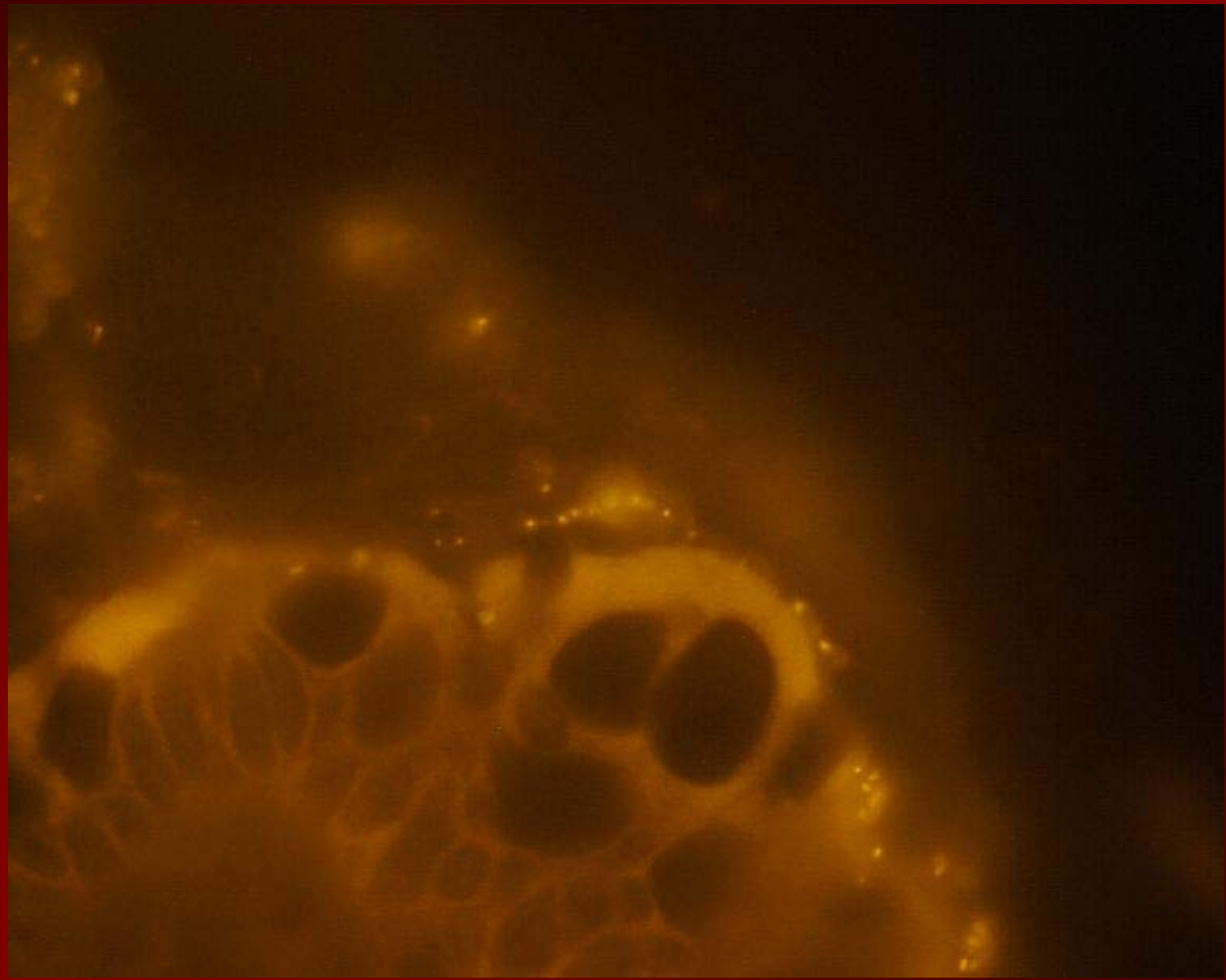


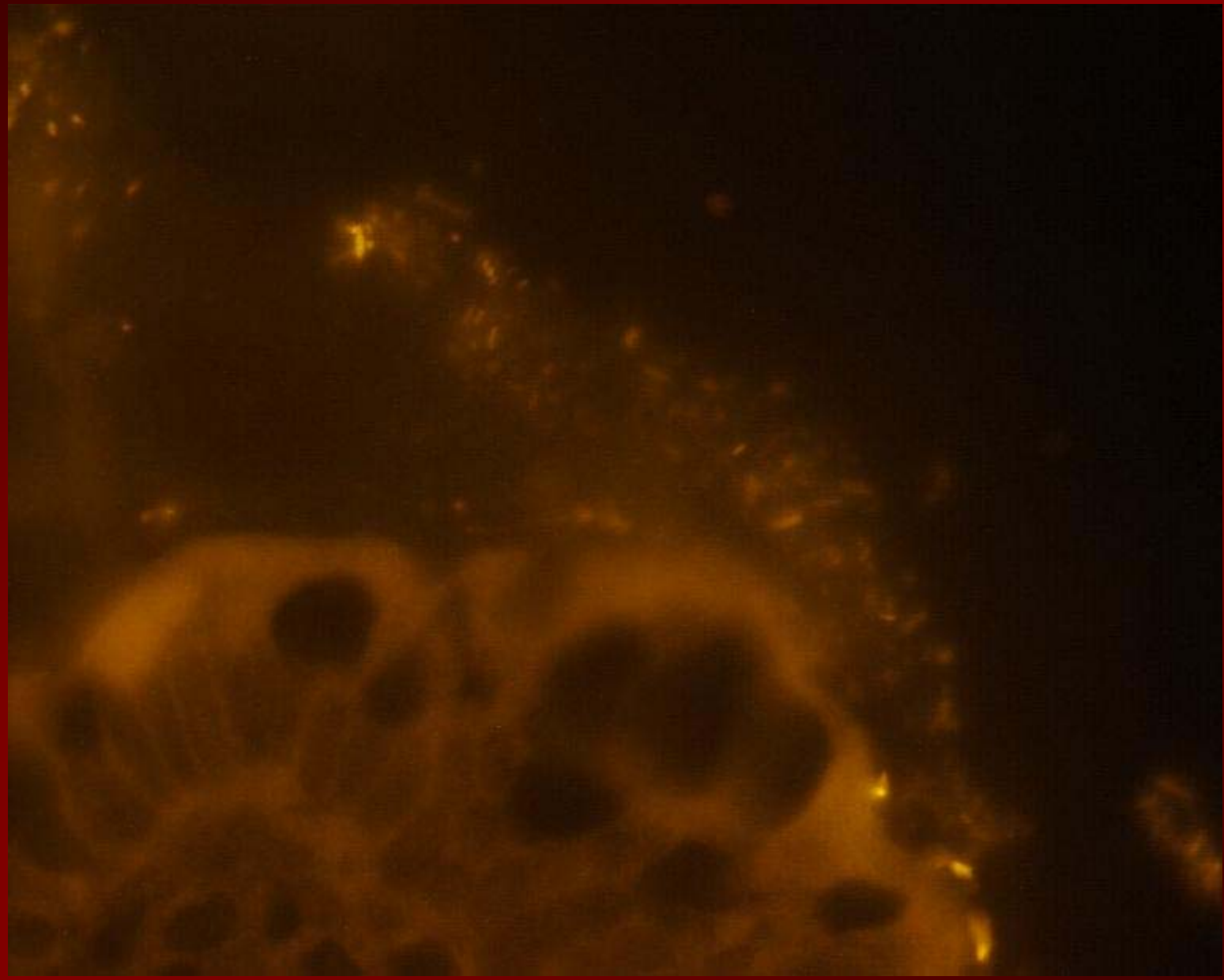
Differential focusing of bacterial lawns covering the epithelial surface

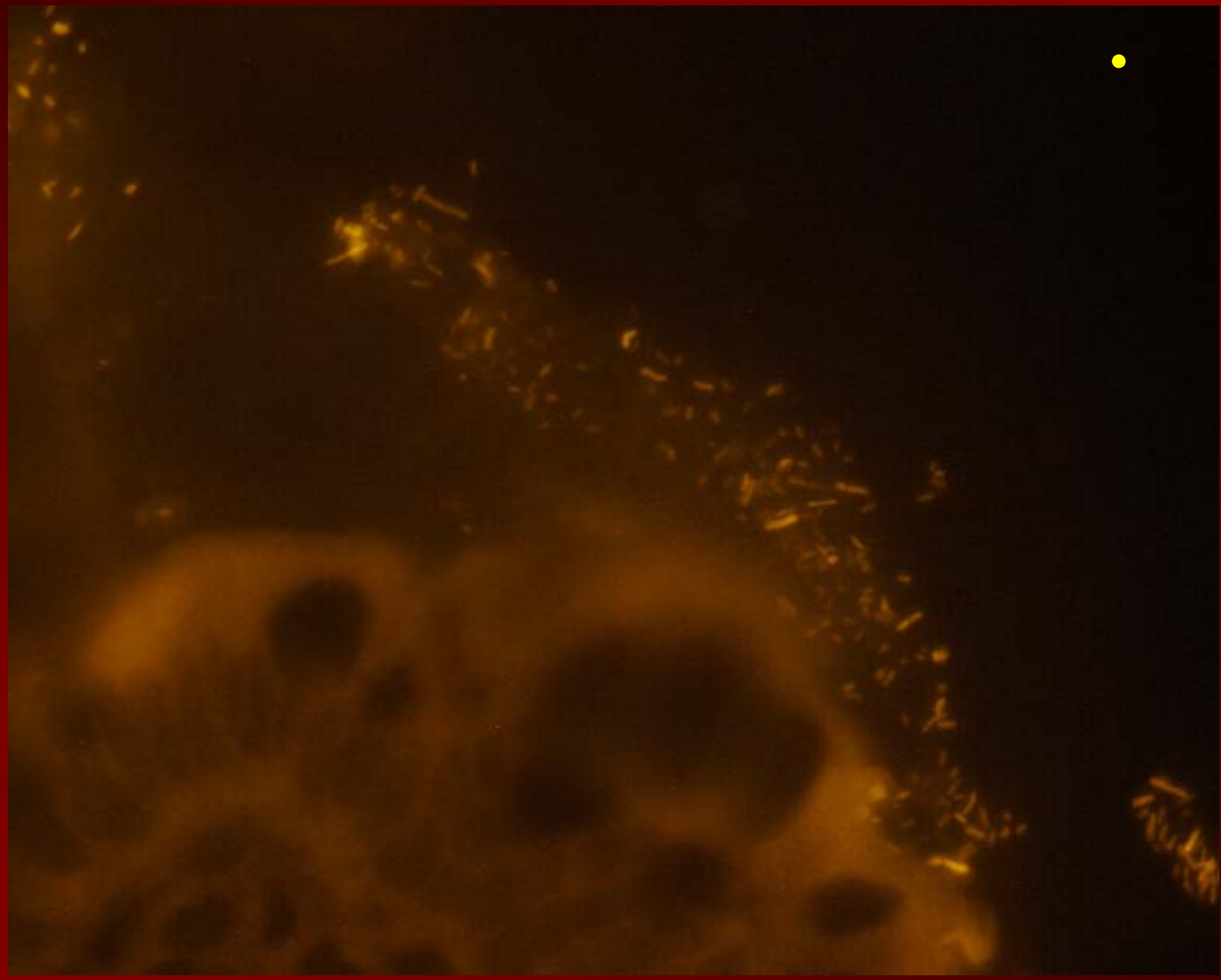


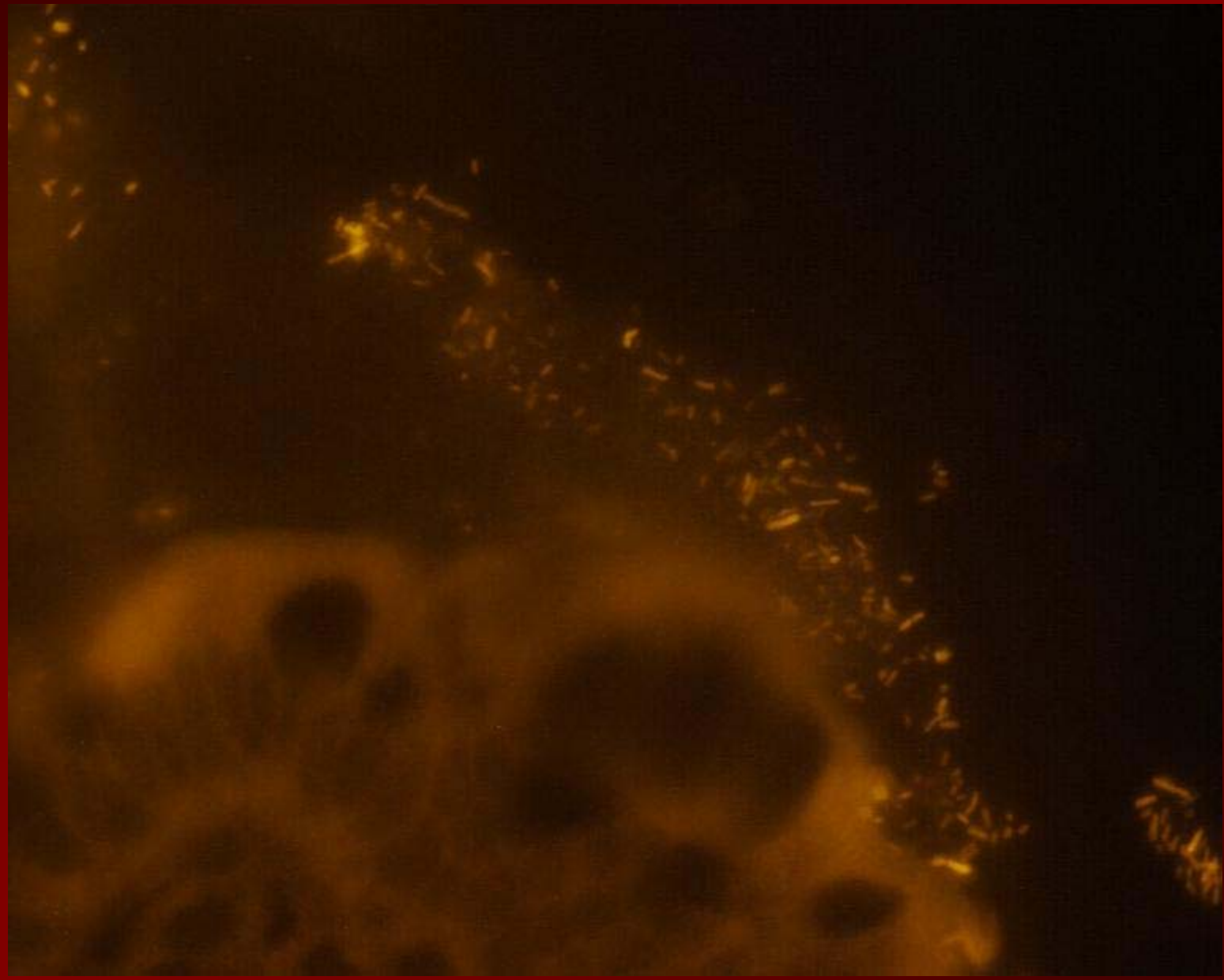


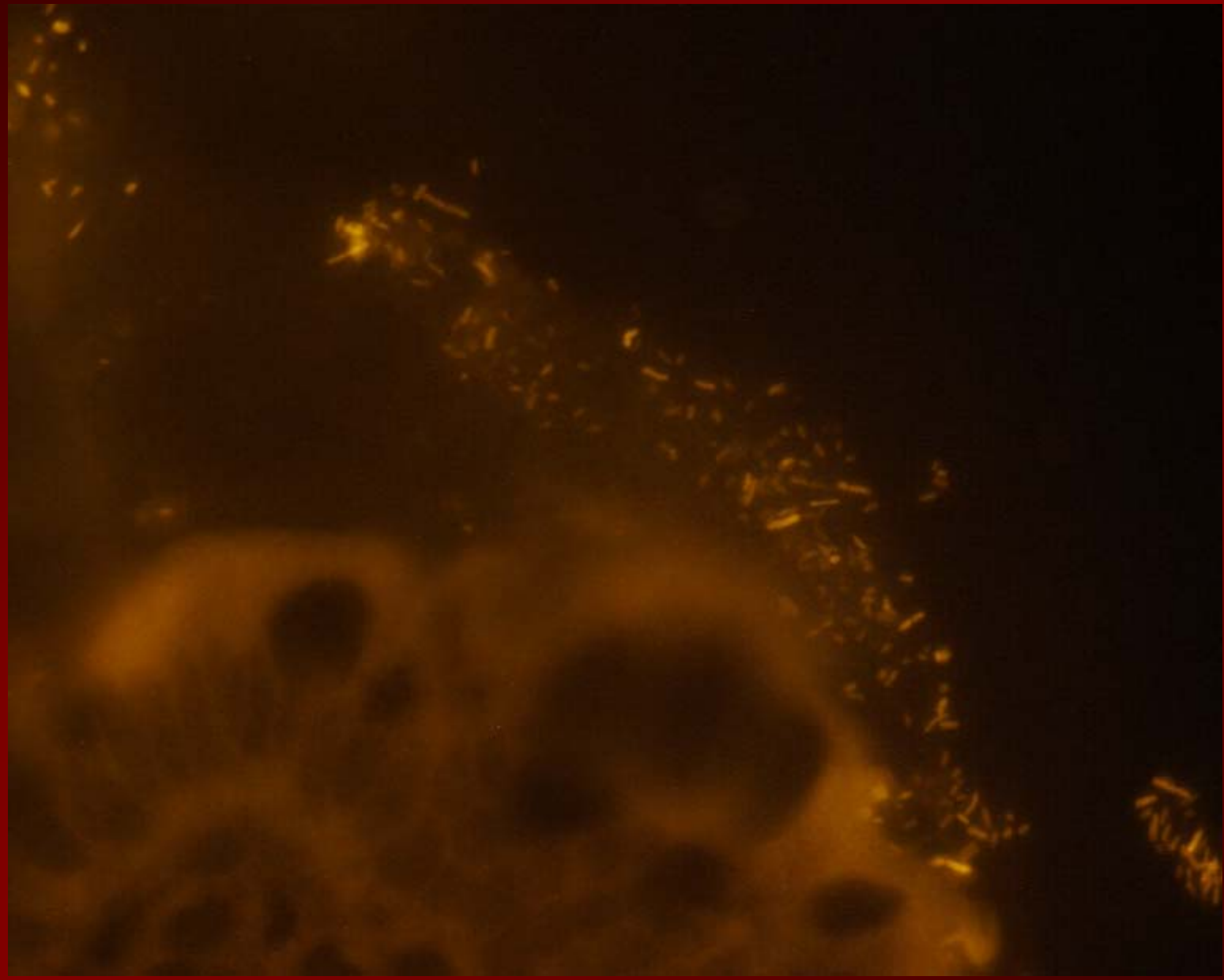


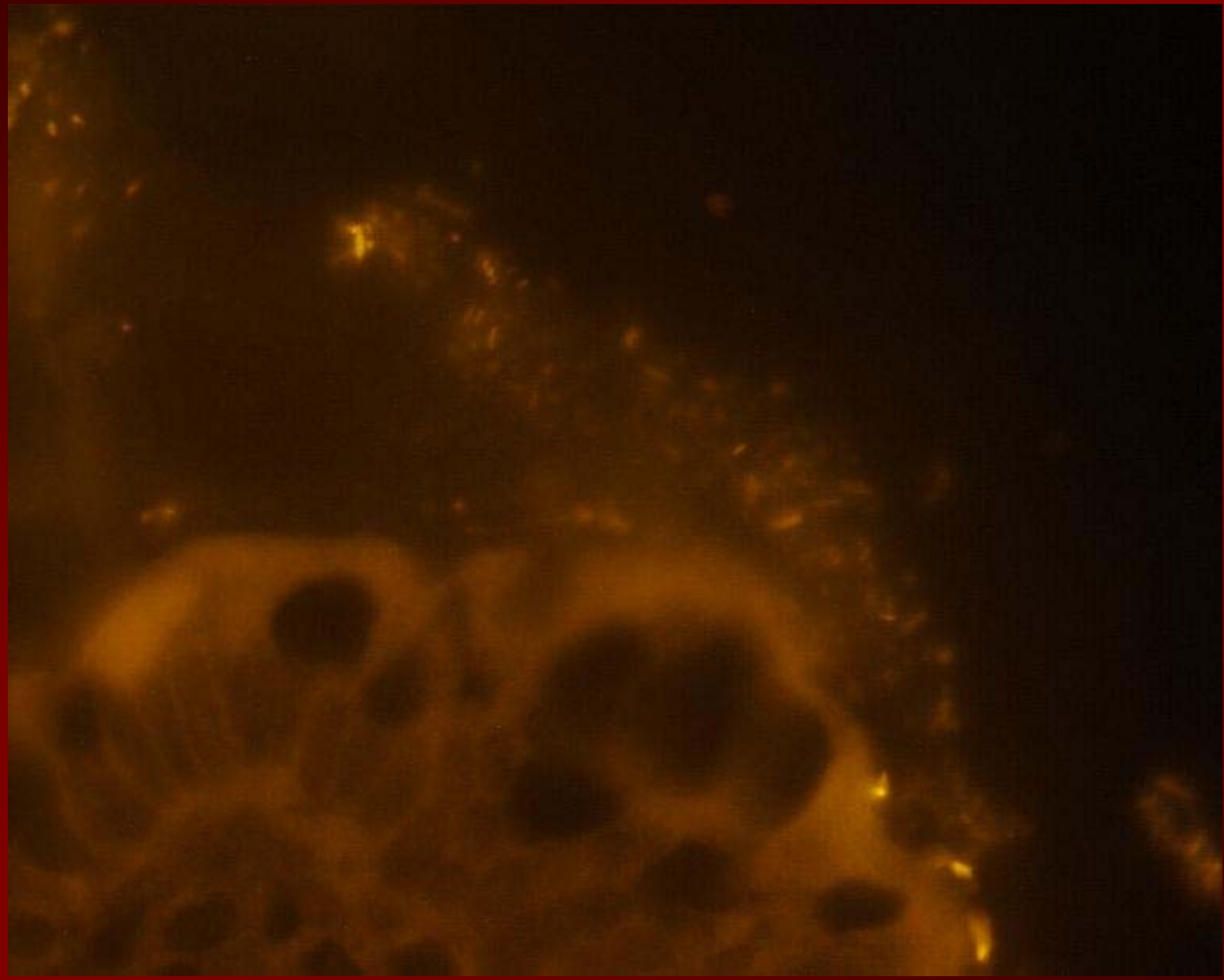


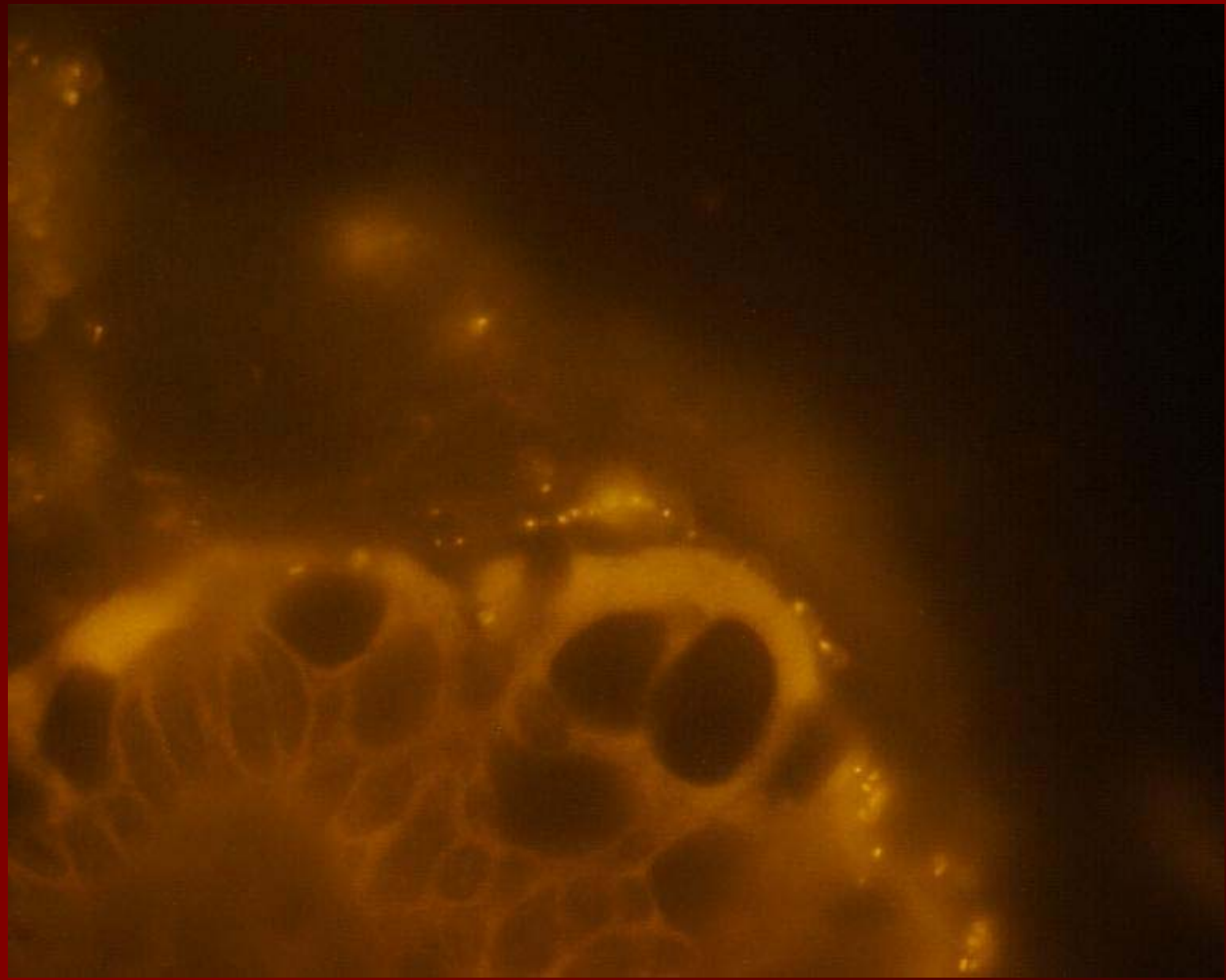


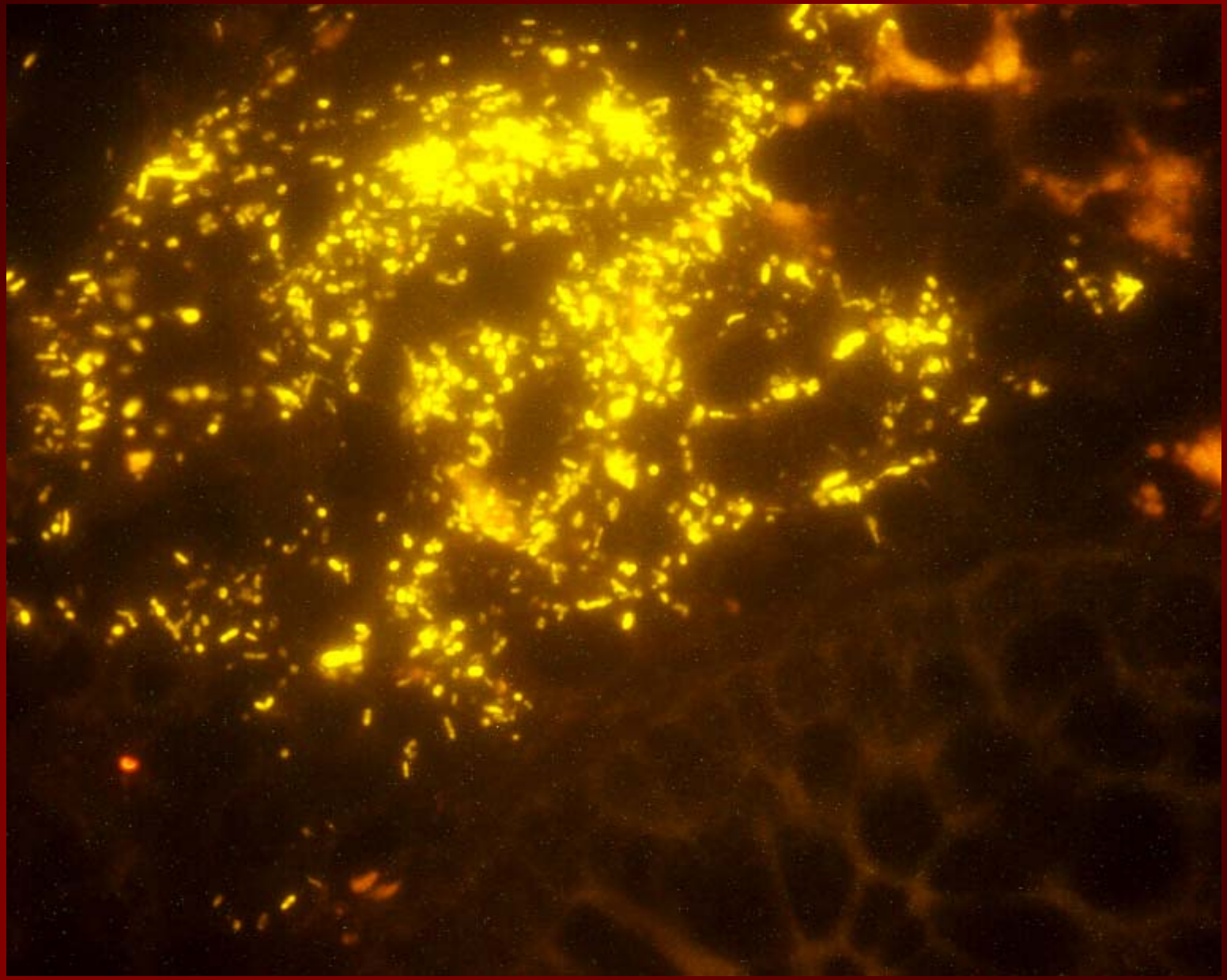


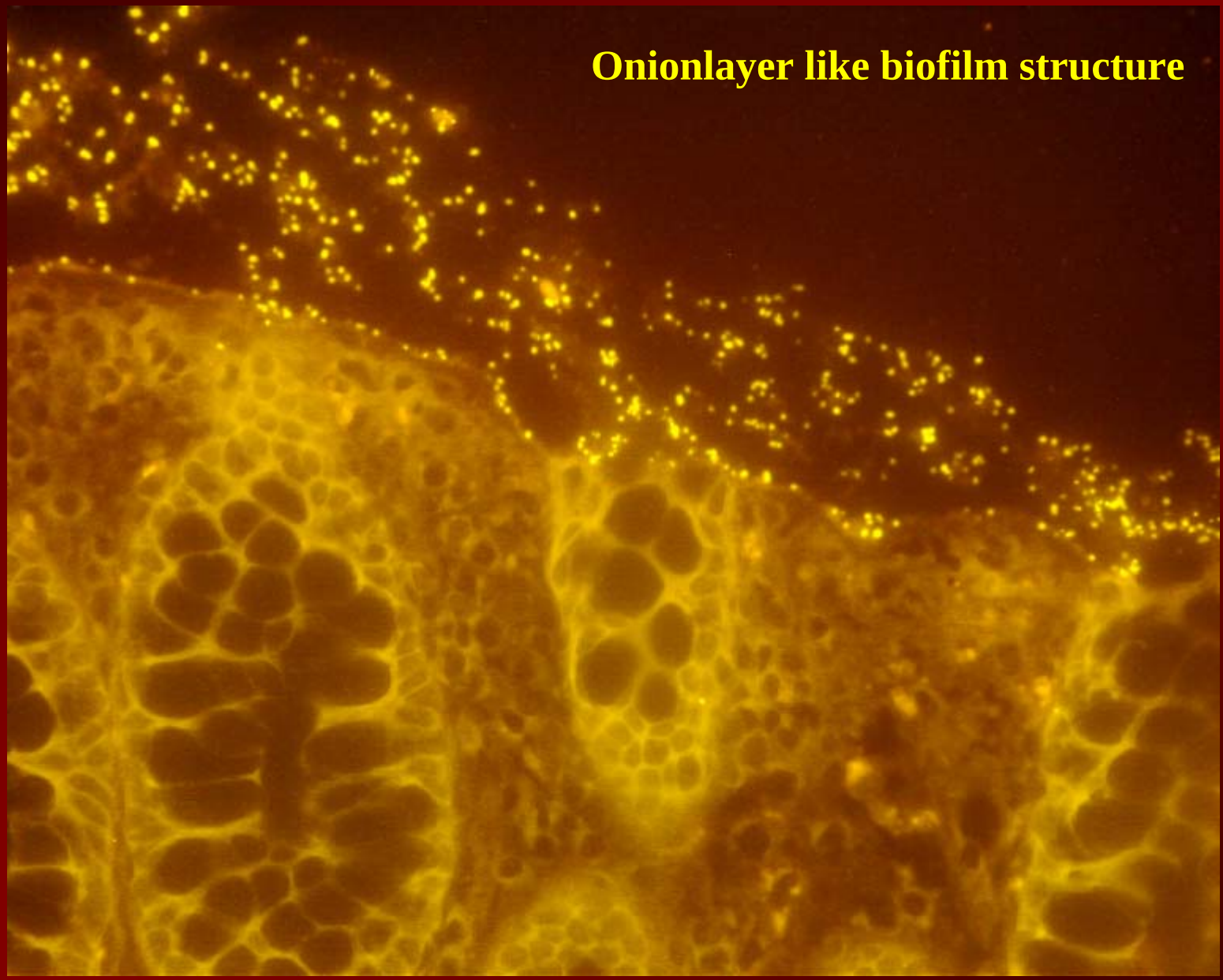











Onionlayer like biofilm structure

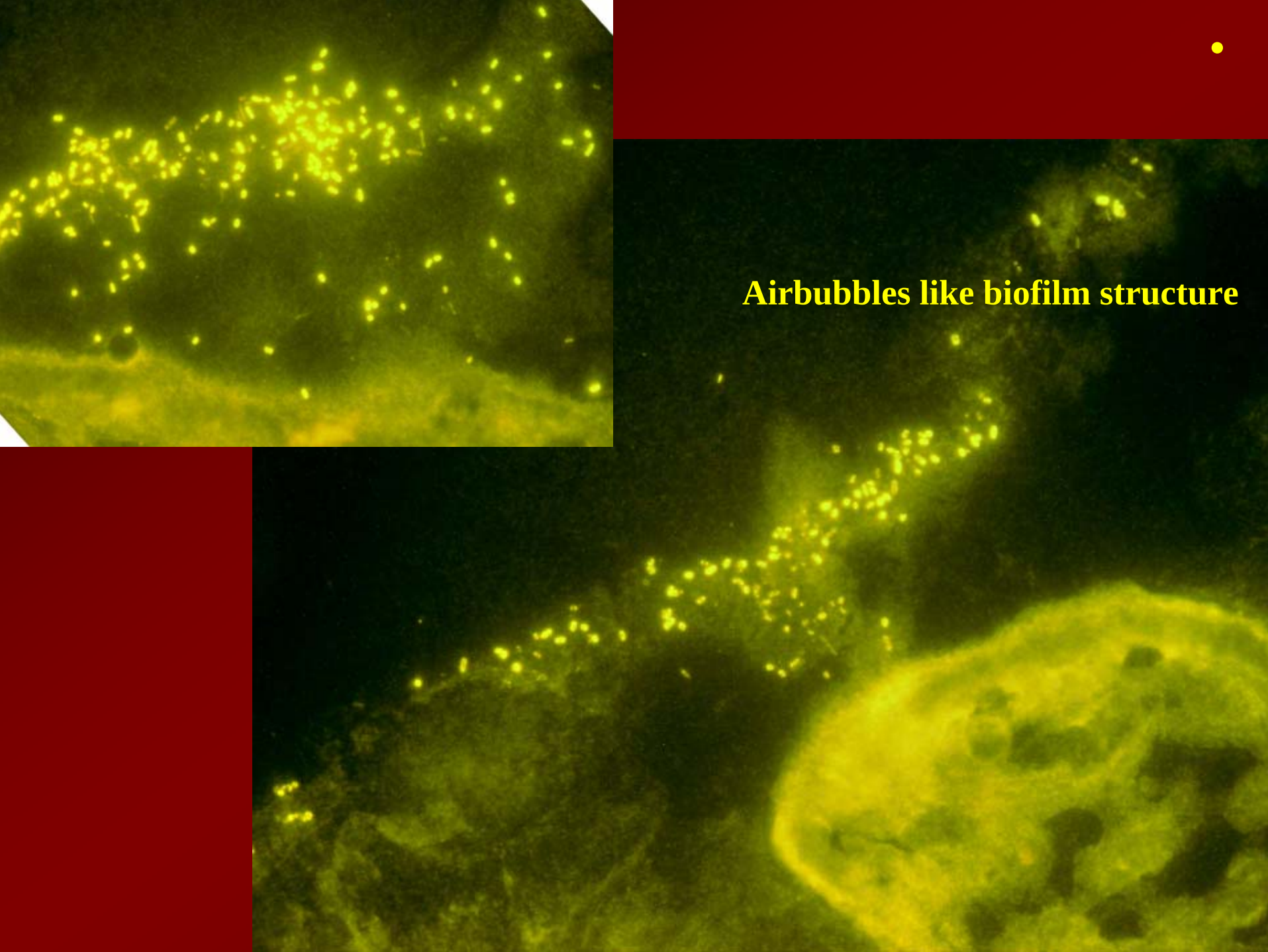
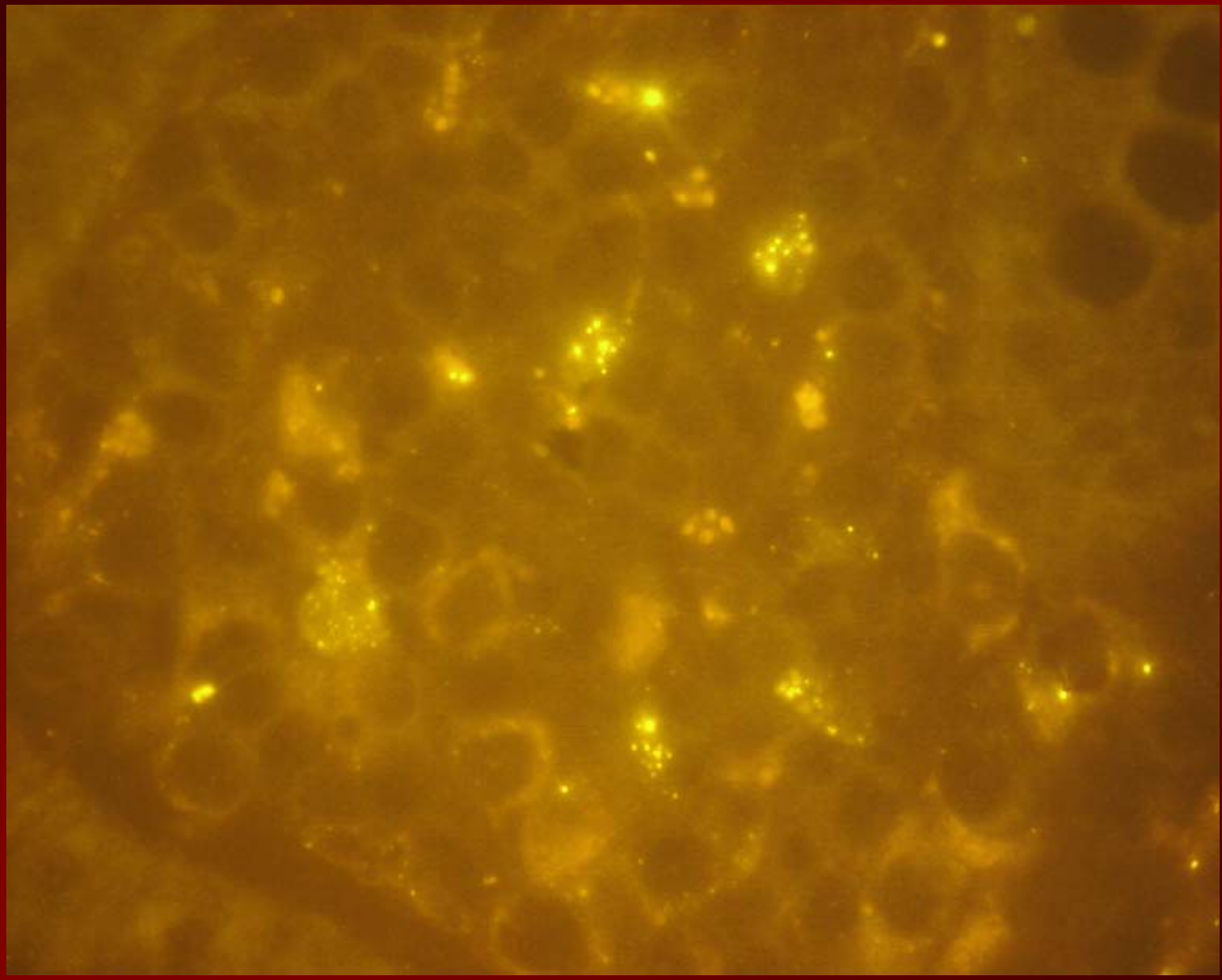
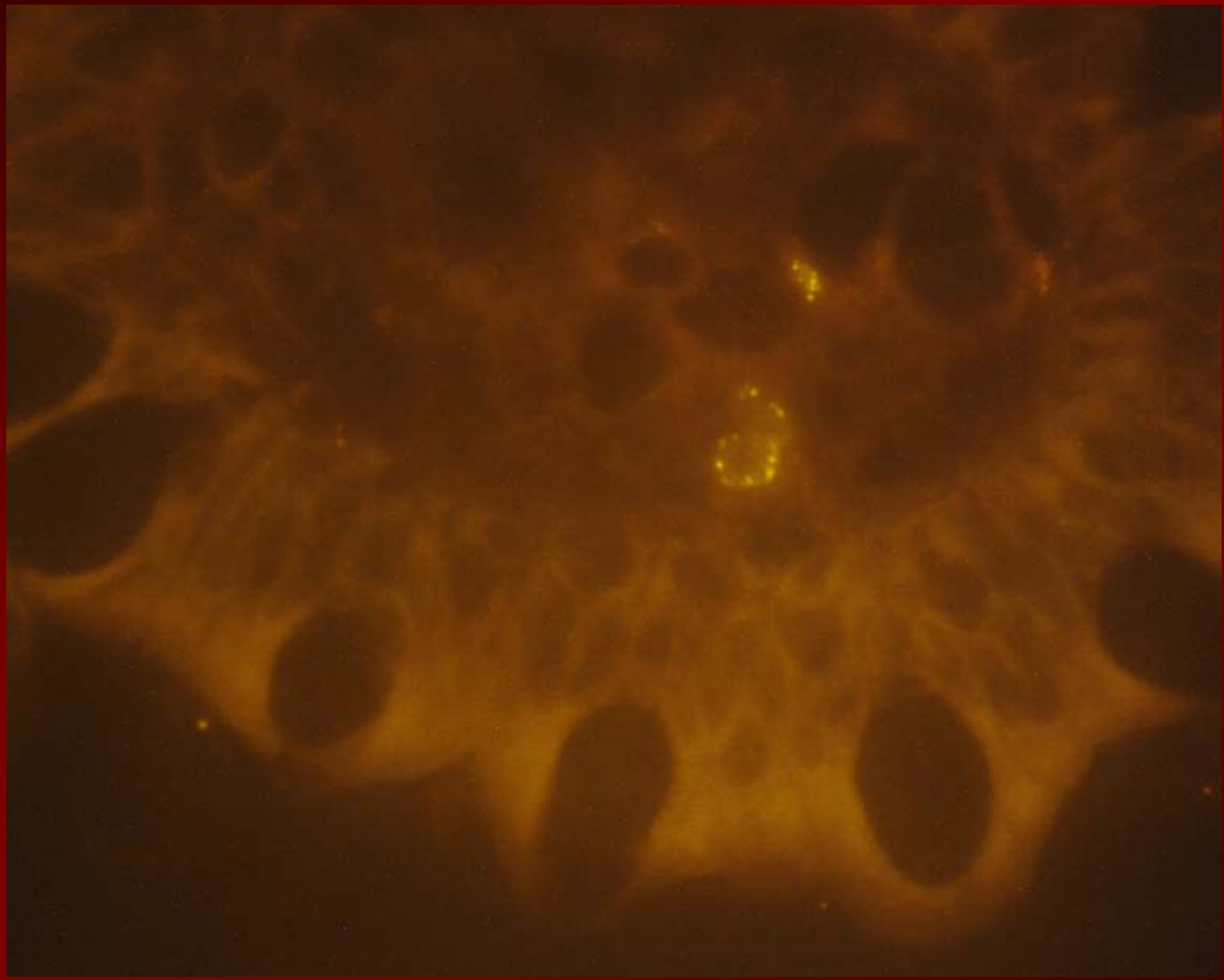

Airbubbles like biofilm structure

Table 1 FISH probes

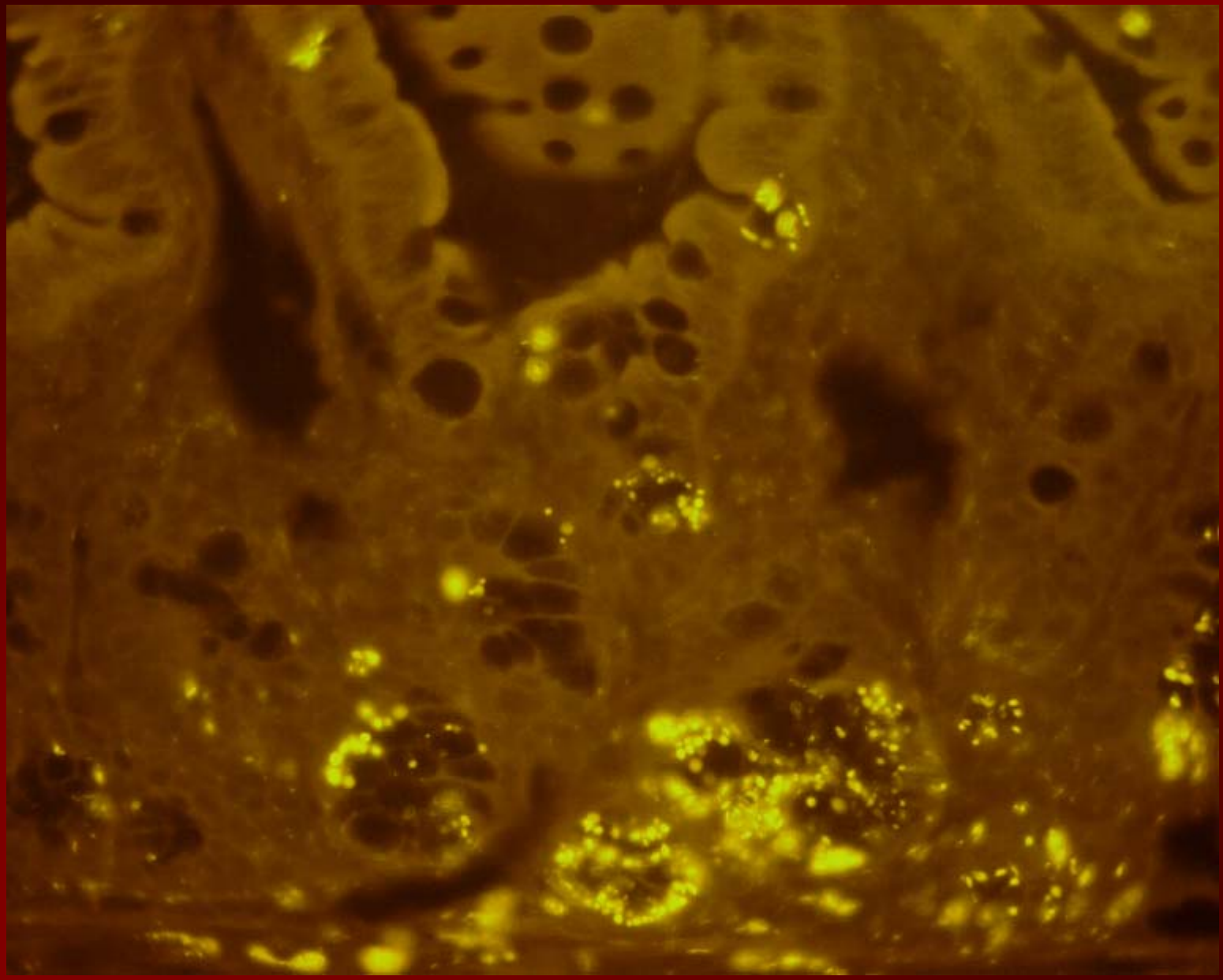
Name	Target
Eub338	virtually all *Bacteria*, Kingdom (*Eu)Bacteria*
Arch915	*Archaea*
Alf1b	Alpha group of *Proteobacteria: Rhodobacter, Acetobacter, Paracoccus,* some *Pseudomonas etc.*
Beta42a	Beta subclass of *Proteobacteria: Rhodocyclus, Bordetella, Neisseria,Thiobacillus, Alcaligenes* and other
Gam42a	gamma subclass of *Proteobacteria: Enterobacteriaceae, Proteus, Legionella,Azotobacter*
Ec1531	*Escherichia coli*
Srb385	sulfate reducing bacteria, the main component of the delta subclass of *Proteobacteria*
Hpy-1	*Helicobacter pylori* epsilon subclass of *Proteobacteria*
Arc1430	*Arcobacter* ssp. epsilon subclass of *Proteobacteria*
HGC	Gram positive bacteria with high G+C content
LGC	Gram positive bacteria with low G+C content
Sfb	*Segmented filamentous bacteria*
Erec482	*Clostridium coccoides - Eubacterium rectale* group
Chis150	*Clostridium histolyticum* group
Clit135	*C. lituseburense* group
Lab158	*Lactobacillus* and *Enterococcus* group
Strc493	*Streptococcus*
Ecyl	*Eubacterium biforme, Clostridium innocuum* and other
Phasco	*Acidaminococcus fermentans* and other
Veil	*Veillonella group*
Rbvo,Rfla	*Ruminococcus flavefaciens, Clostridium leptum*
Bif164	*Bifidobacterium*
Ato291	*Atopobium, Coriobacterium, Eggerthella* and *Collinsella spp*
CF319a	*Cytophaga-Flavobacterium* group
Bac303	*Bacteroides*/*Prevotella* group
Bfra602	*Bacteroides fragilis* group
Bdis656	*B. distasonis* group



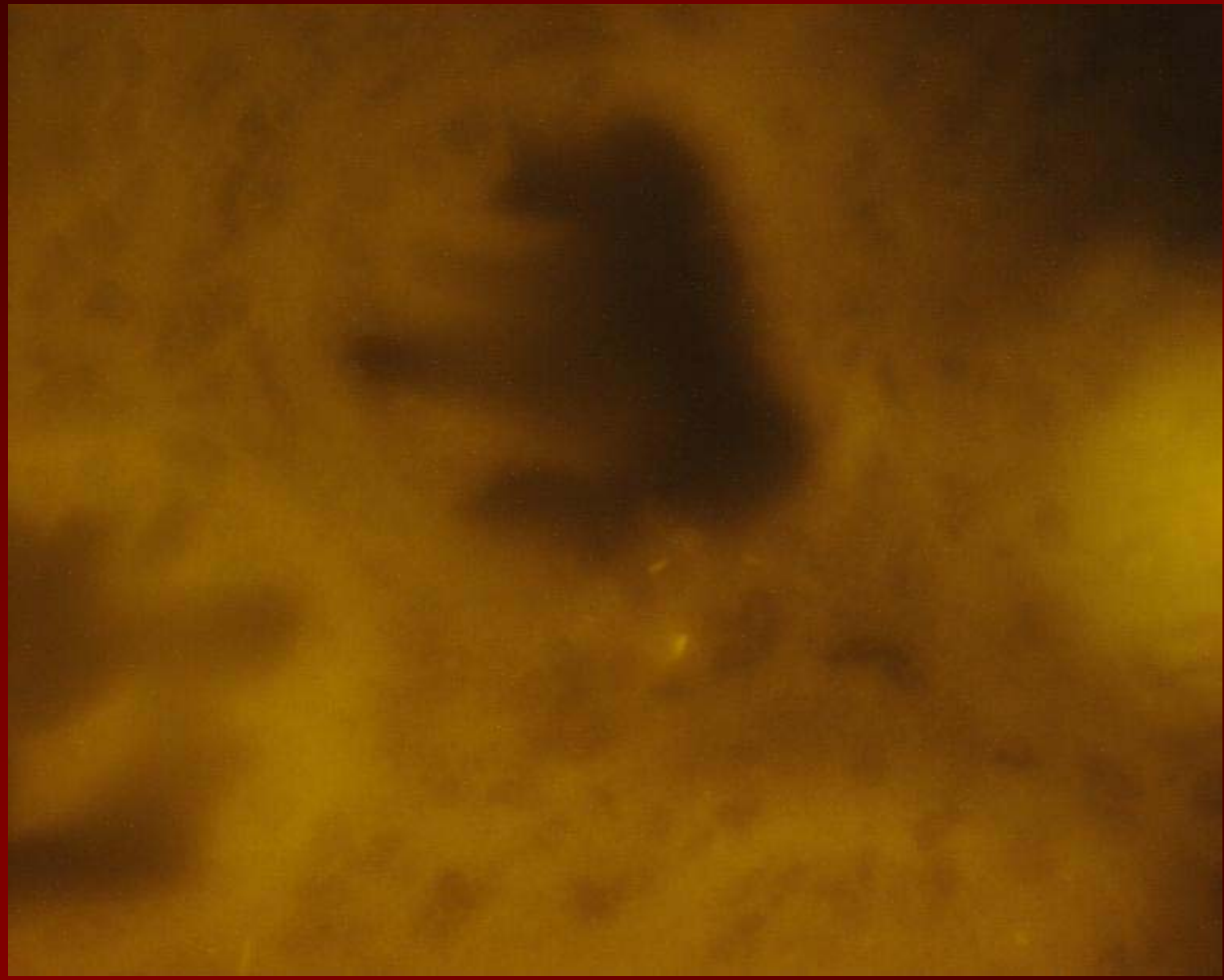
FISH signals simulating intracellular bacteria



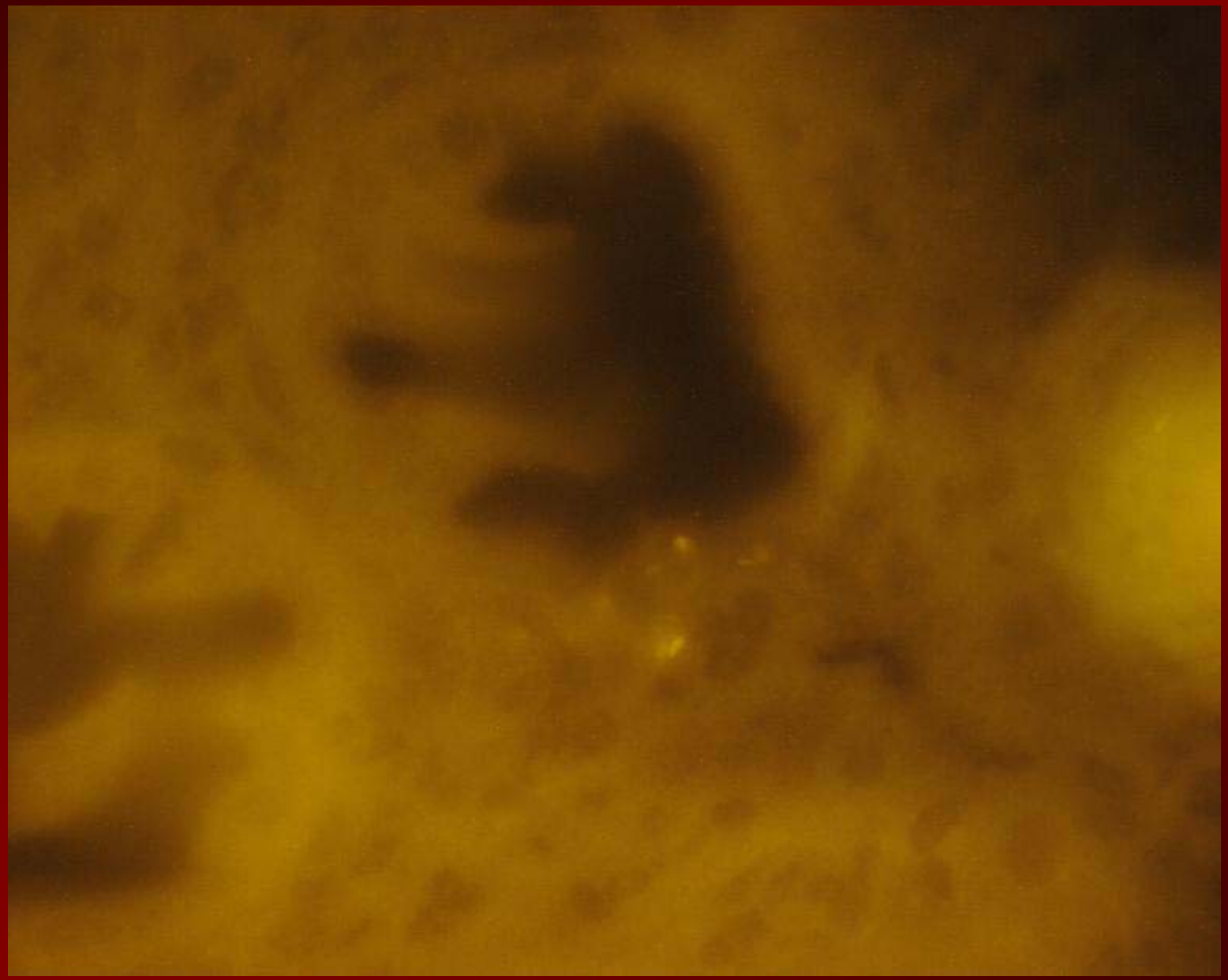
FISH signals simulating intracellular bacteria

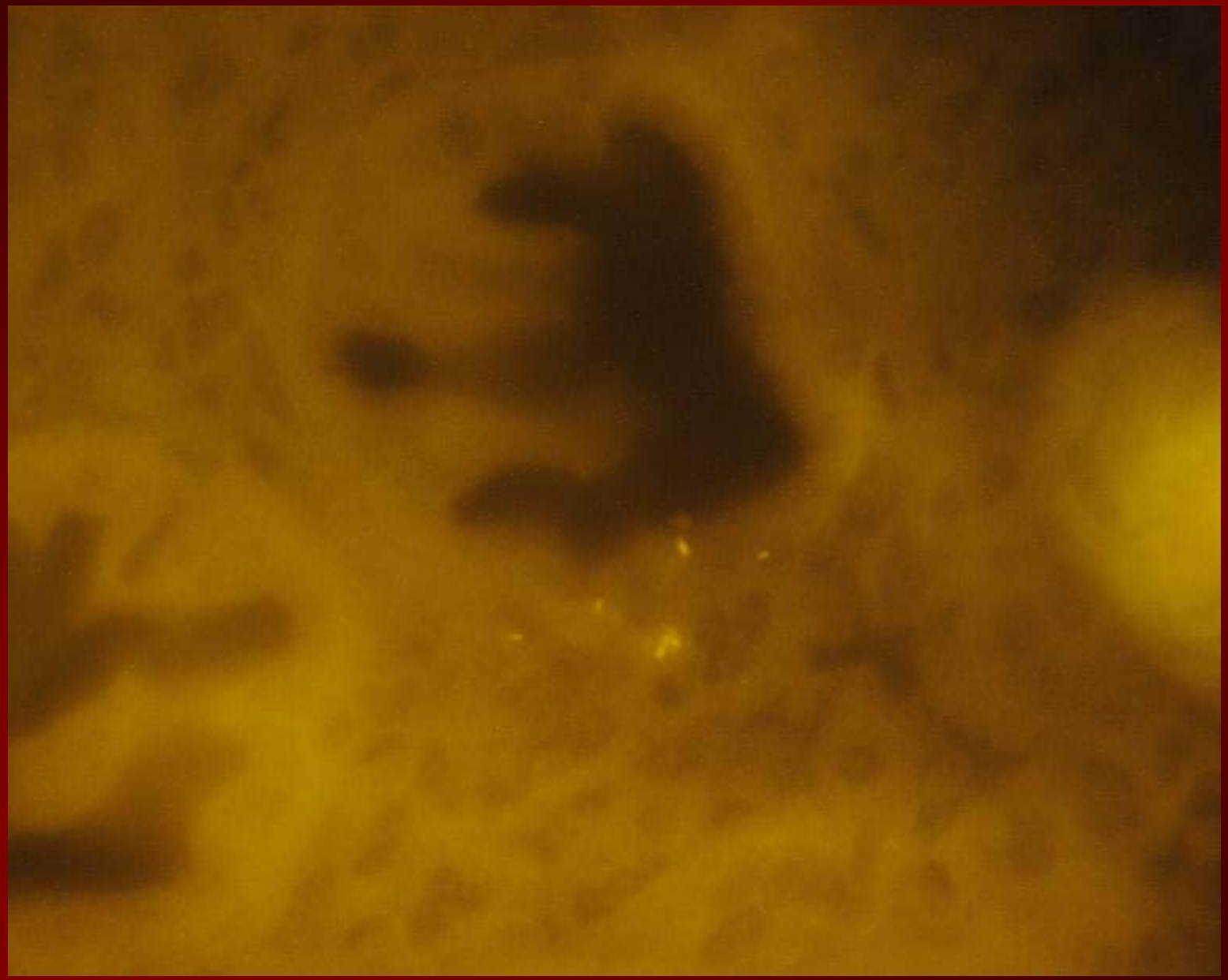


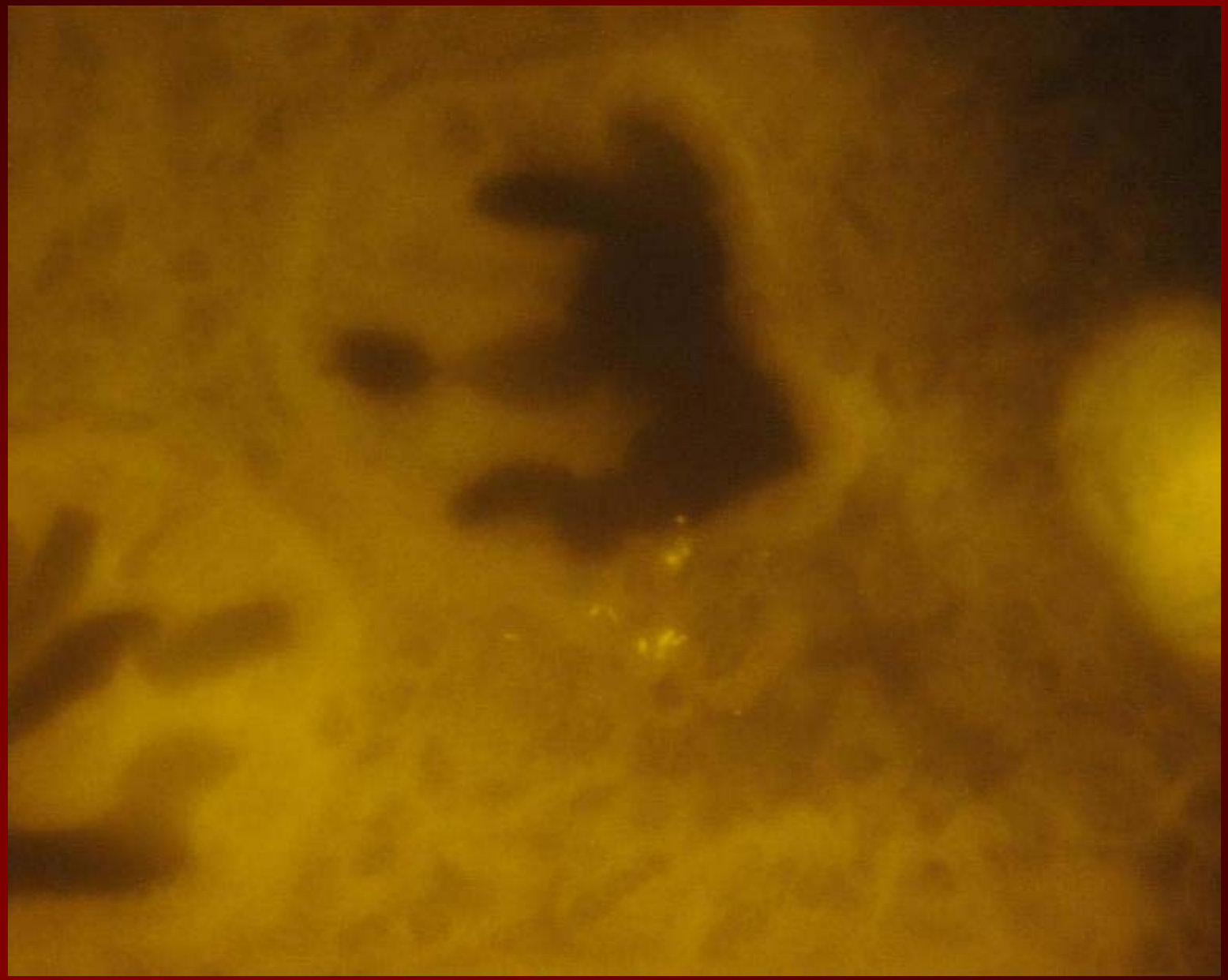
FISH signals simulating intracellular bacteria (SAMP mouse)

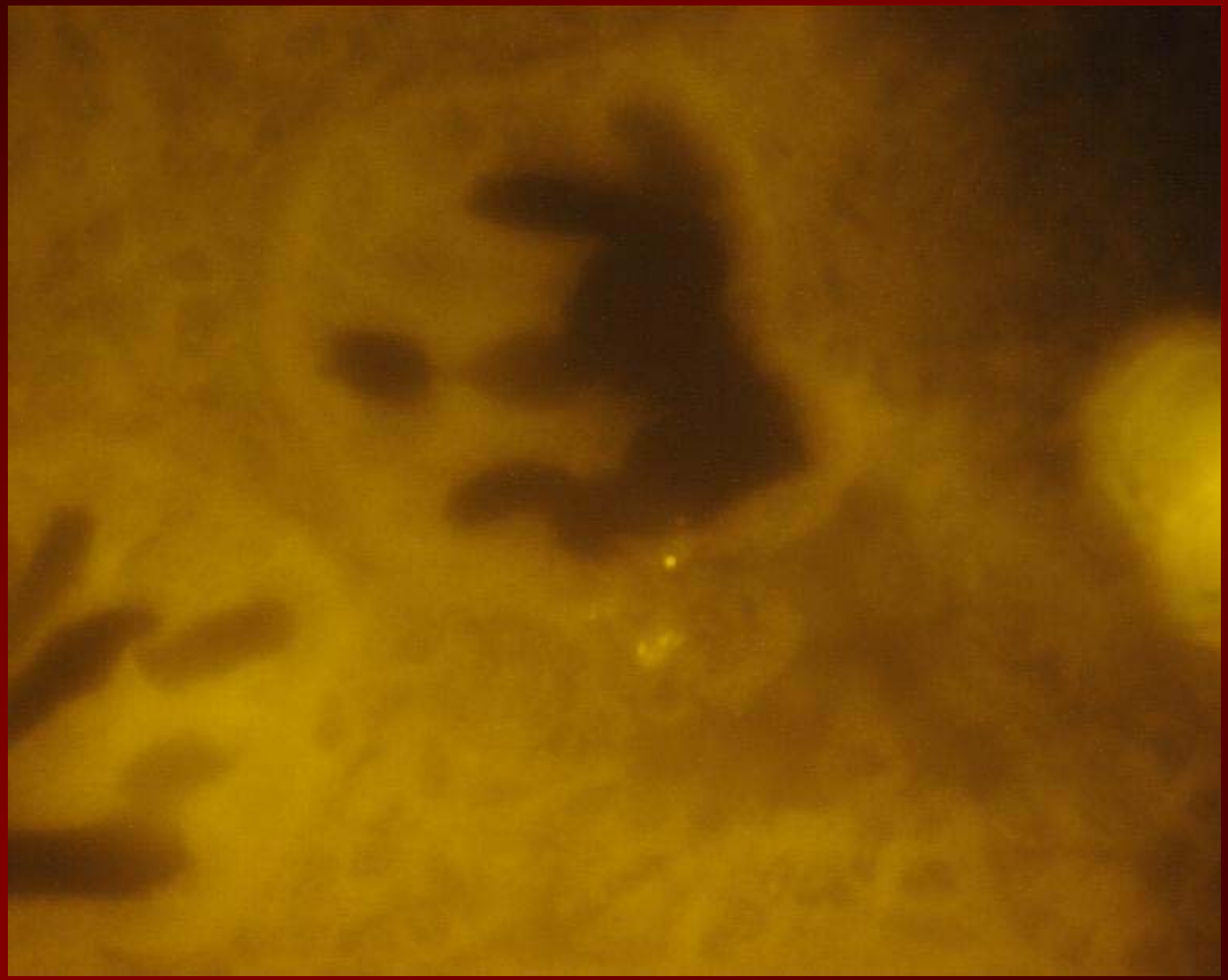


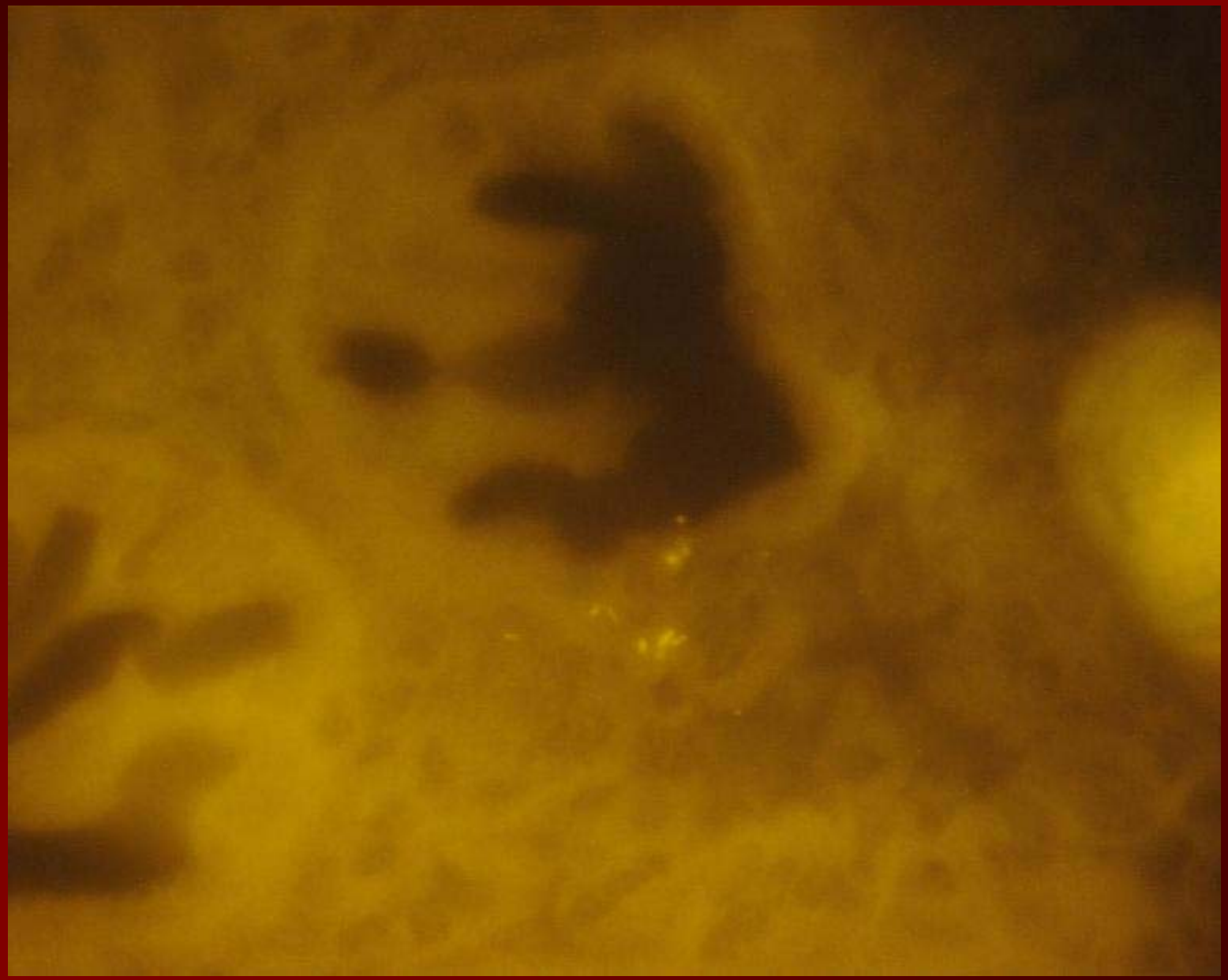
Intraepithelial bacteria located perinuclear at different levels

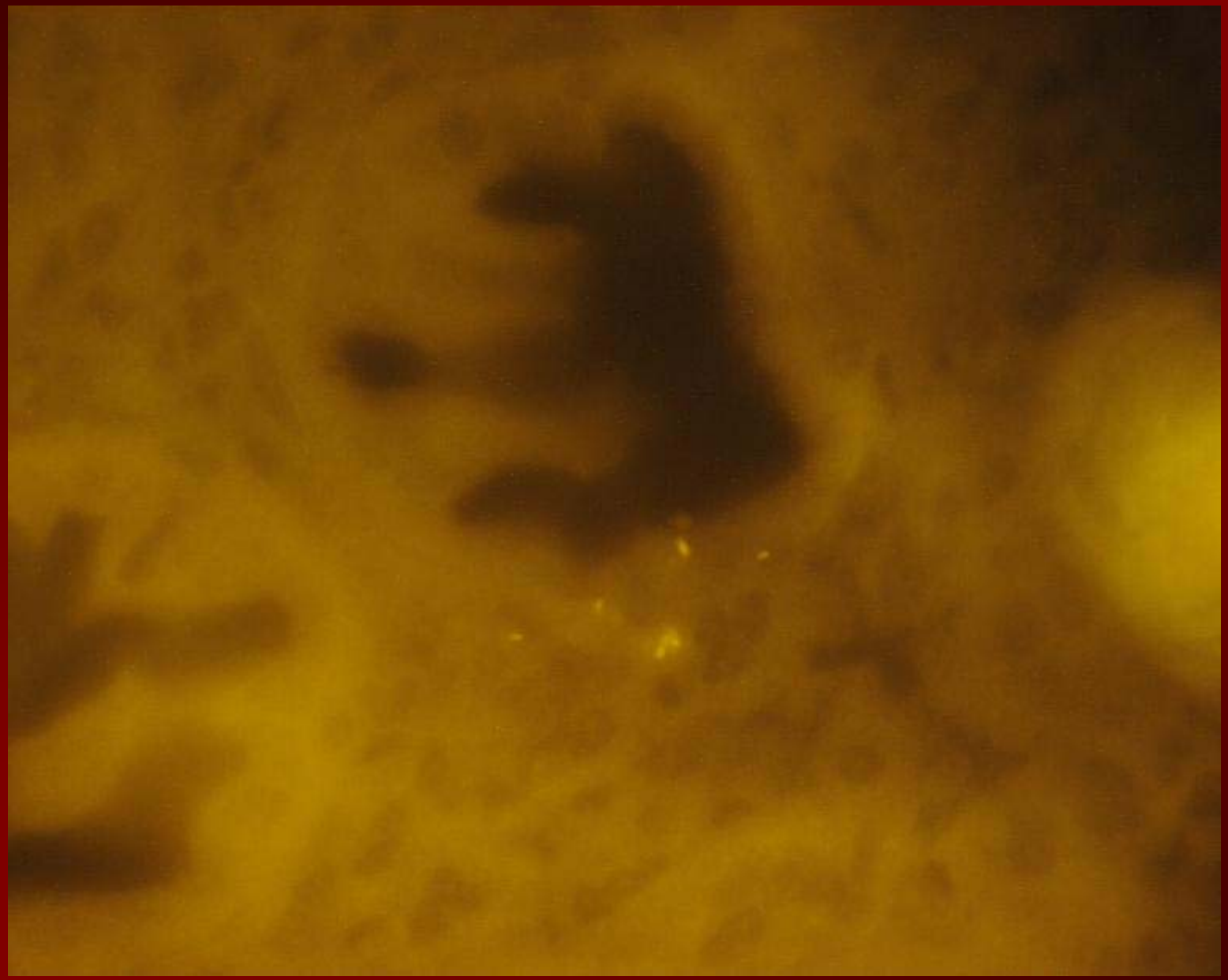


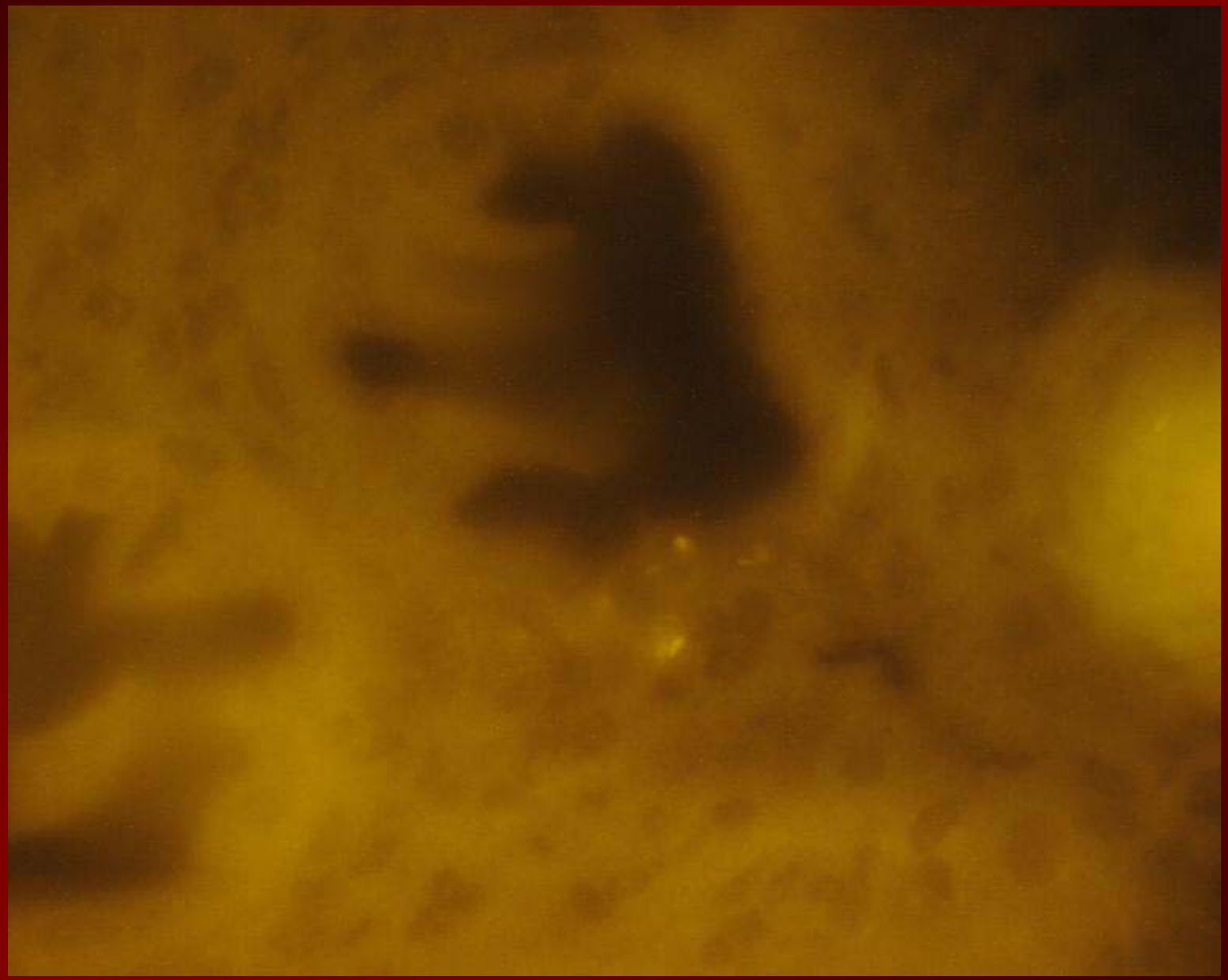


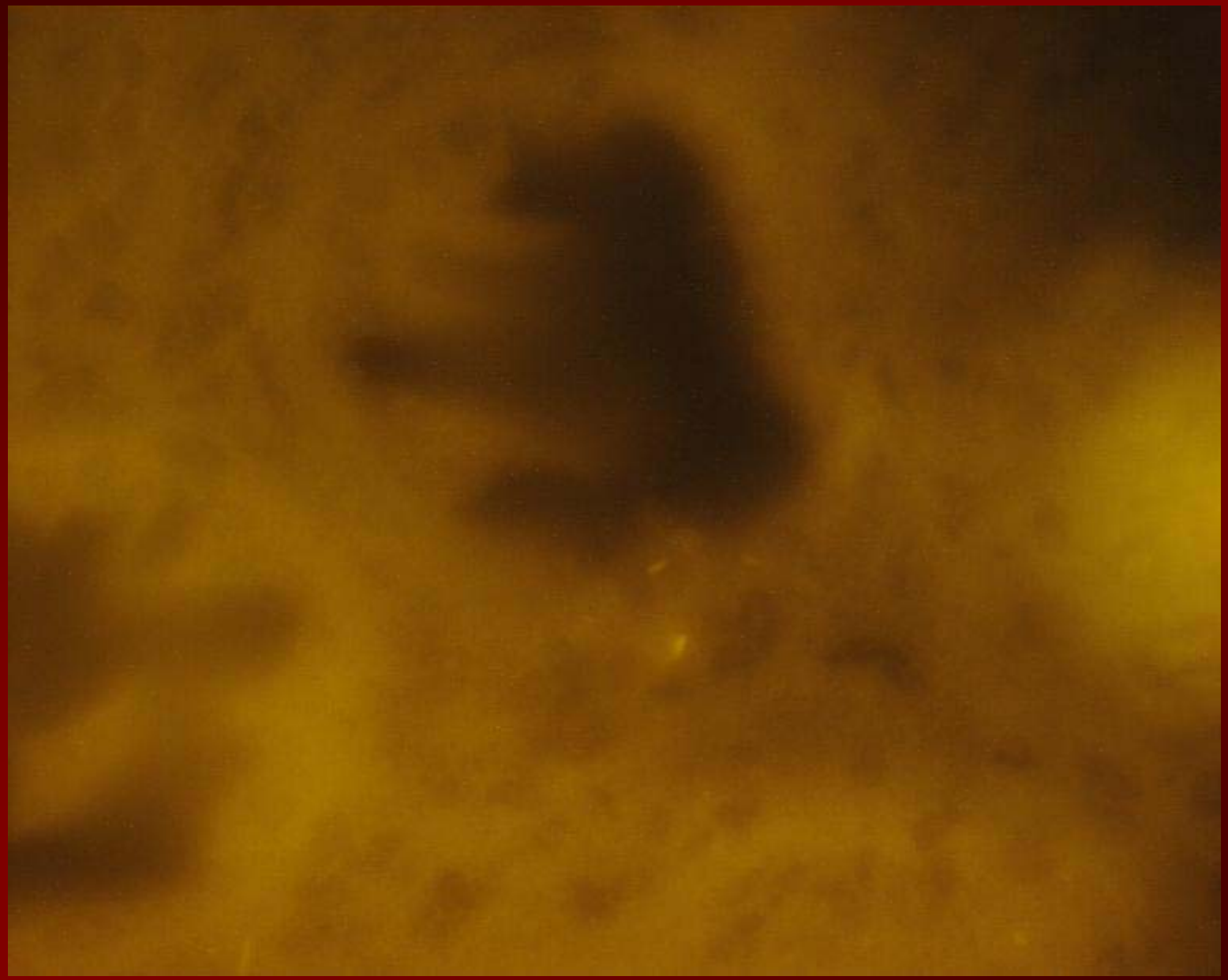


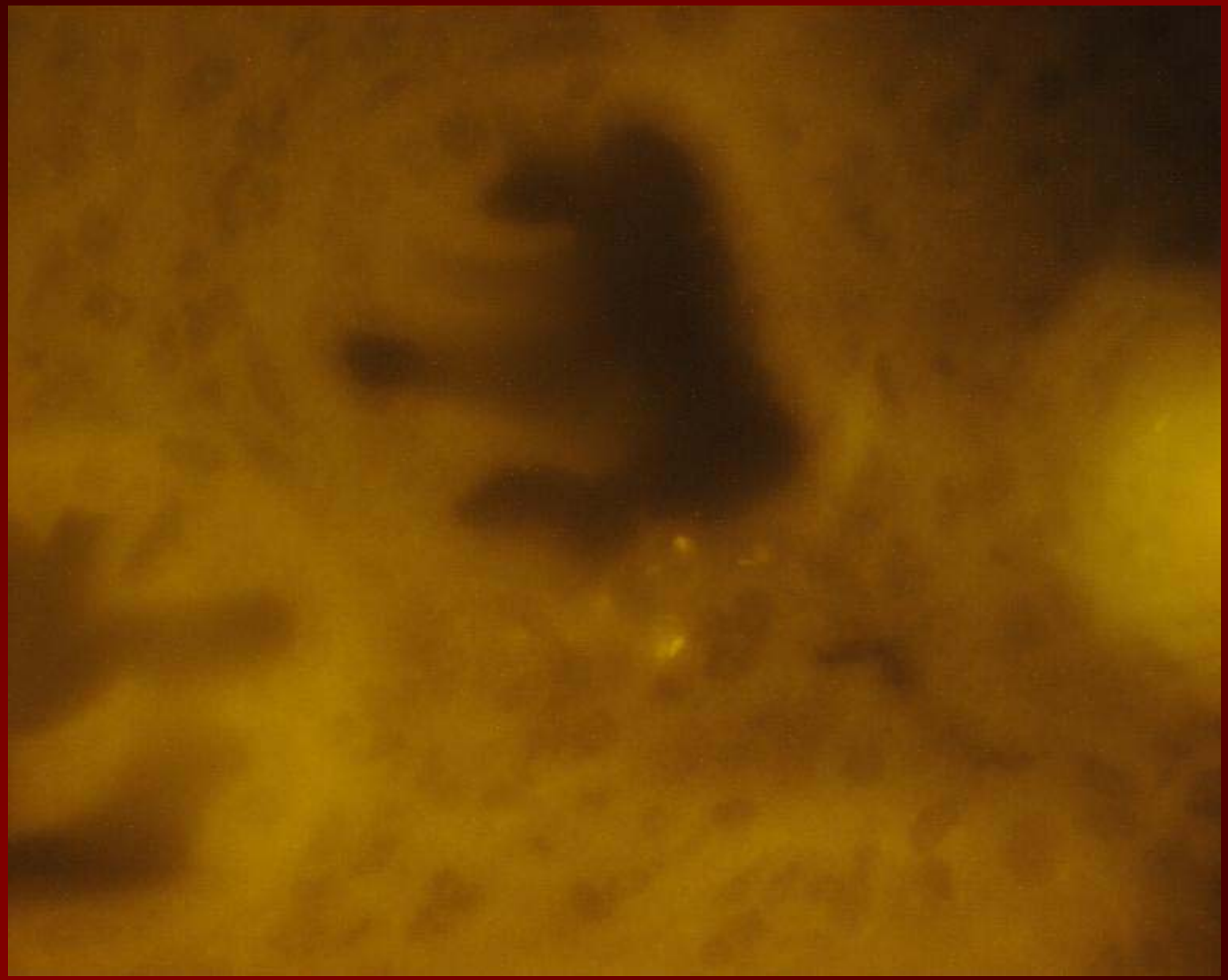


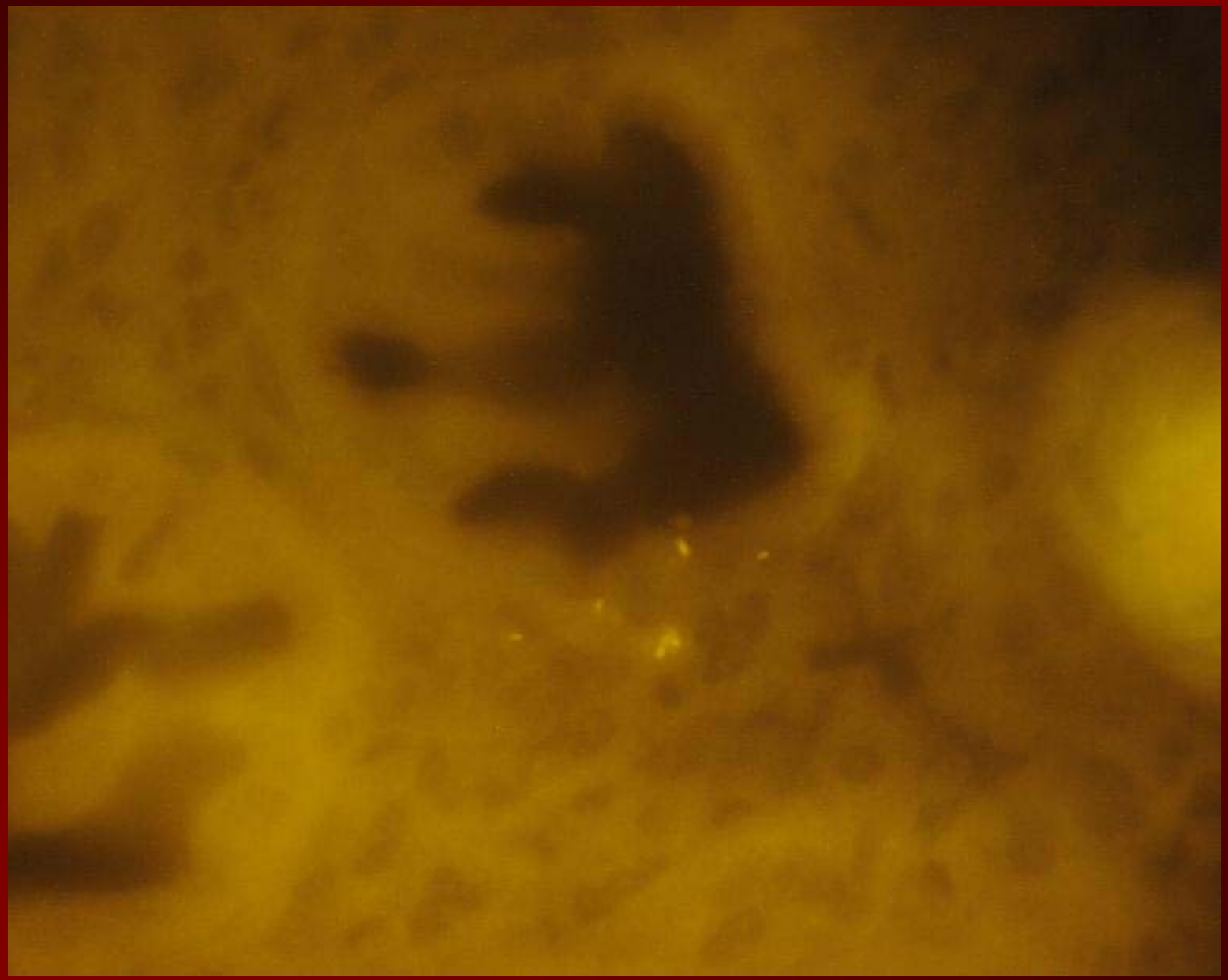


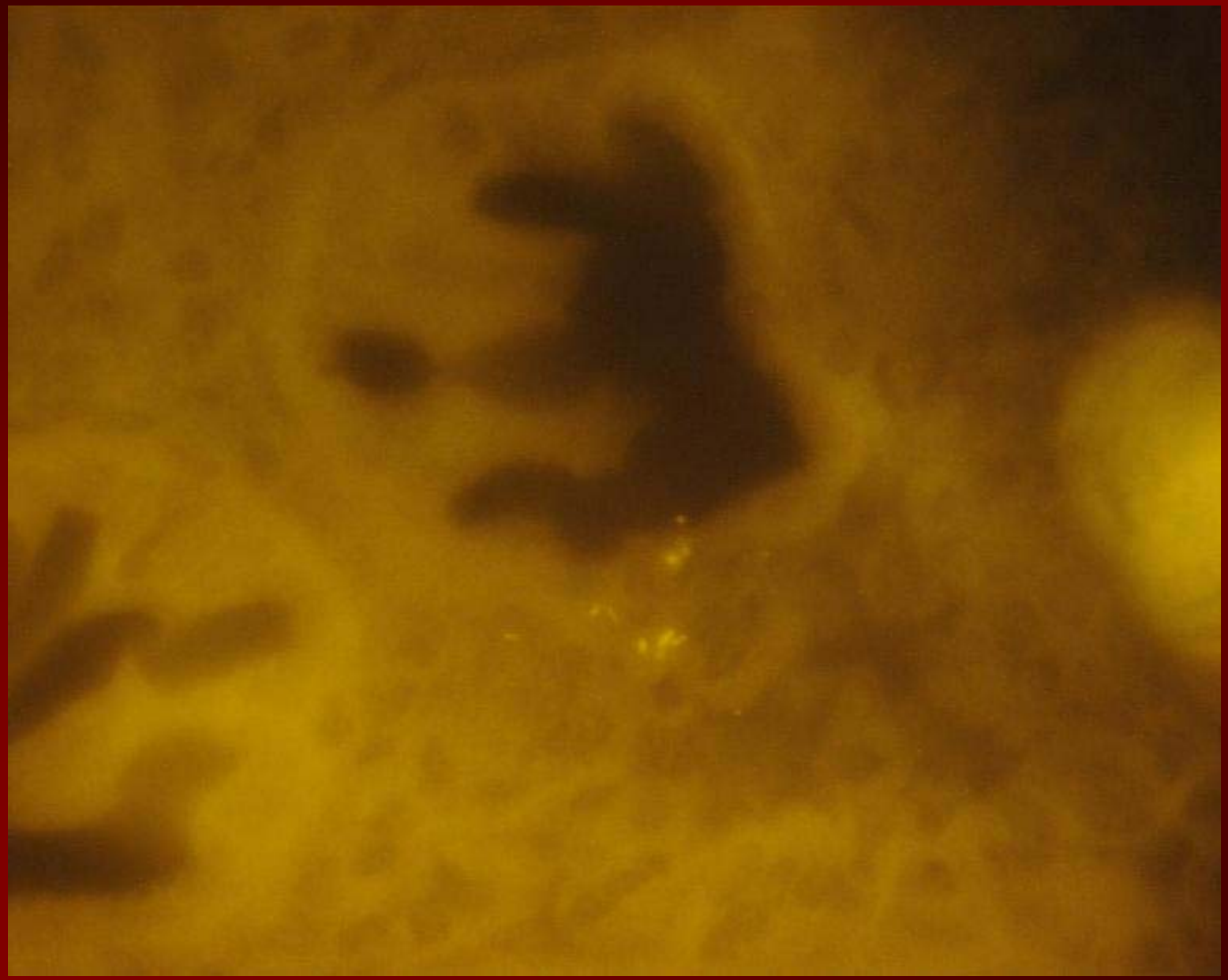


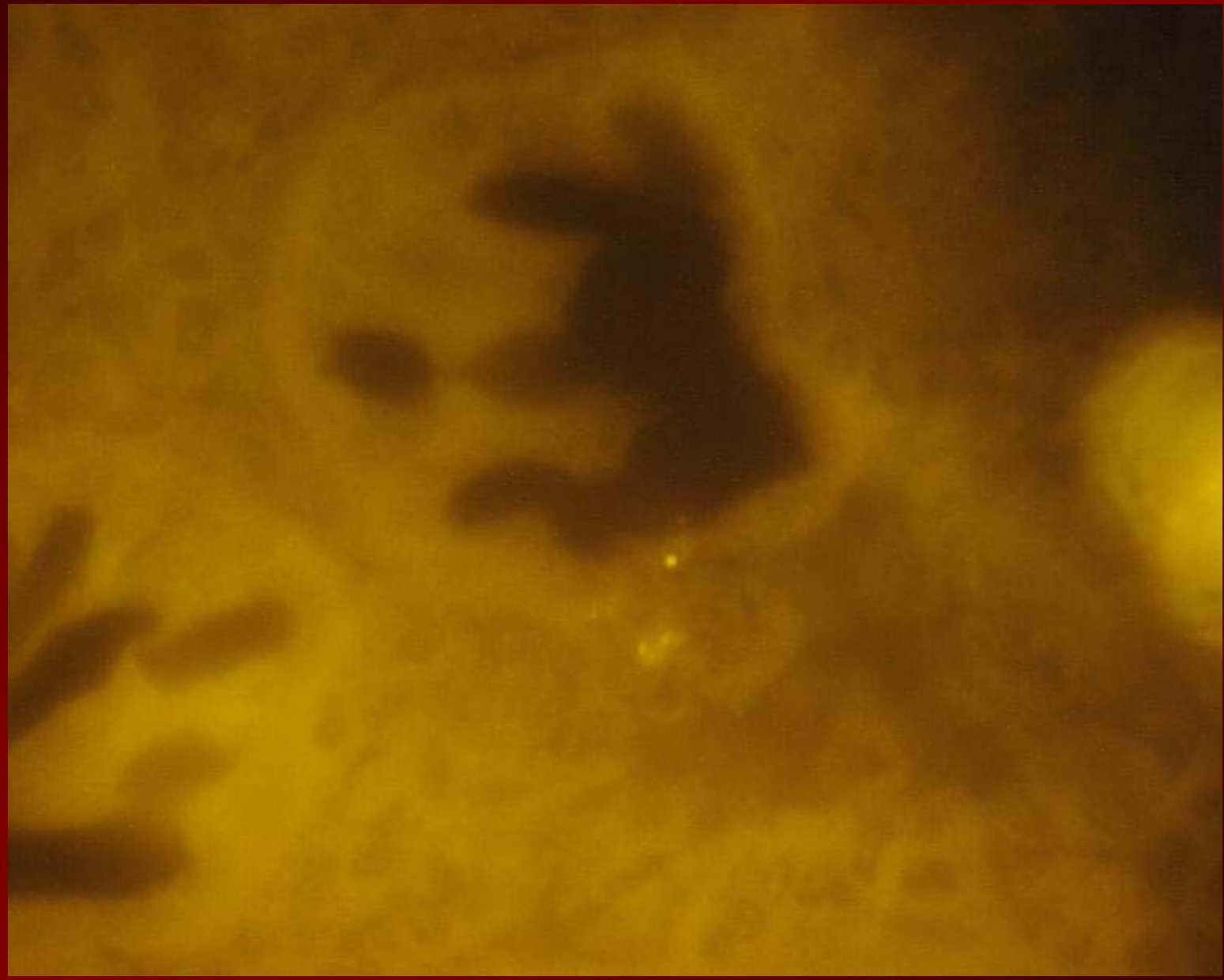












The mucosa of patients with IBD is covered with a complex spatially structured multispecies biofilm. This biofilm is organized in islands, patches and lawns of multispecies bacteria.

Biofilm patches and layers penetrate mucus leading to direct contact between fecal flora and epithelia. They are ideal for luminal antigens and toxins to reach the unshielded epithelial surface and to trigger cascades of host responses.

The peculiarities of individual immunity and genetic disposition may explain the rest.

We thank
Broad Medical Research Program
BMRP
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