# Mucosal Flora in IBD

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Supported by Broad Medical Research Program

# Mean± SD (x$10^3$ cfu/μL) of Mucosal Bacteria

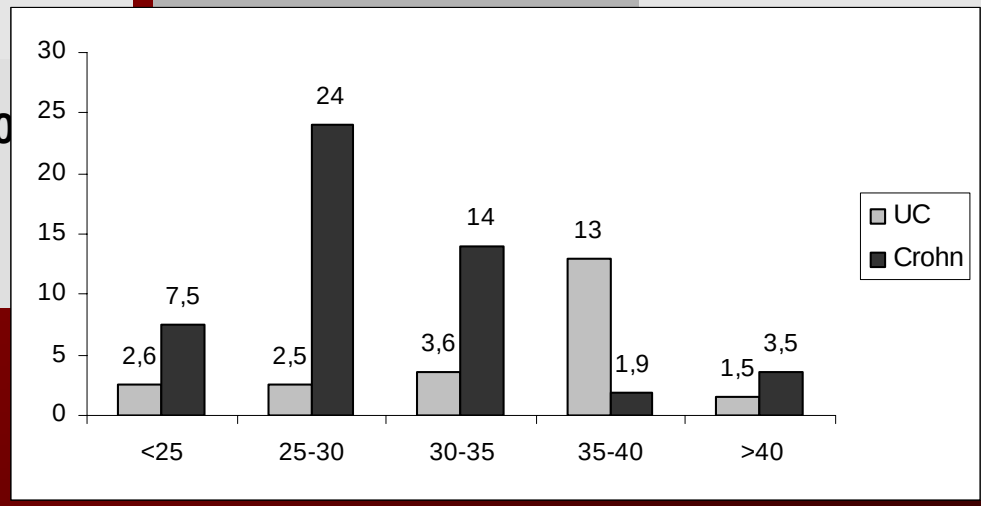
| | Asymptomatic Controls (n=40) | Self-limiting Colitis (n=28) | Indeterminate Colitis (n=104) | UC (n=156) | CD (n=82) |
|---|---|---|---|---|---|
| **Total anaerobes** | **0.18±0.3** | **1.8±5.3** NS | **3.41±16** *P* < 0.08 | **3.8±11** *P* < 0.01 | **9.1±18** *P* < 0.001 |
| ***Bacteroides*** | **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 (x$10^3$ cfu/μL) and clinical data

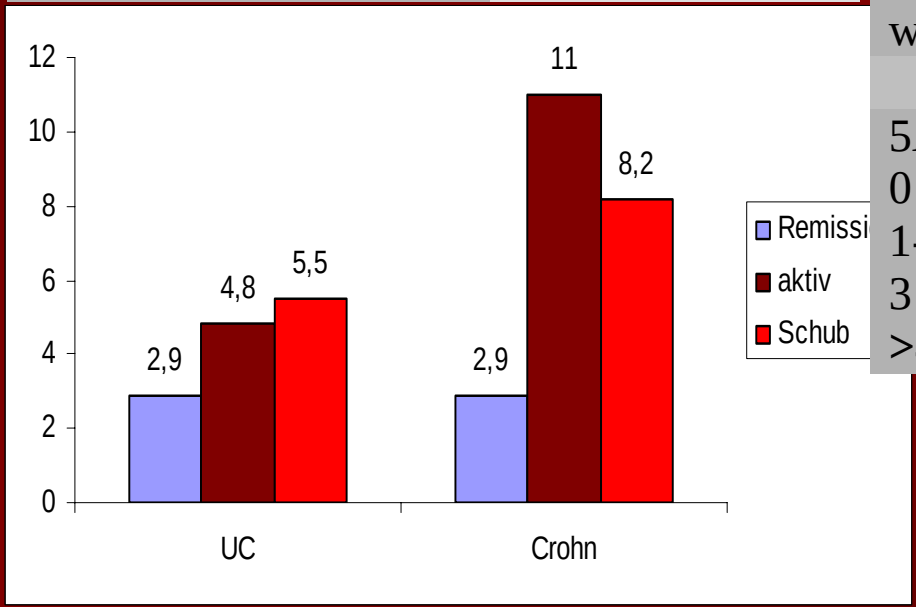
| | UC (n=156) | CD (n=82) |
|---|---|---|
| All | 3.8 | 9.1 |
| patients age | | |
| **<25** | 2.0 | 2.6 |
| **25-30** | 1.7 | 9.1 |
| **30-35** | 4.5 | 11.9 |
| **35-40** | 3.4 | 10.8 |
| **40-45** | 3.2 | 4.3 |
| **45-50** | 6.9 | 5.5 |
| **>50** | 2.6 | 5.2 |
| age at the time of manifestation |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

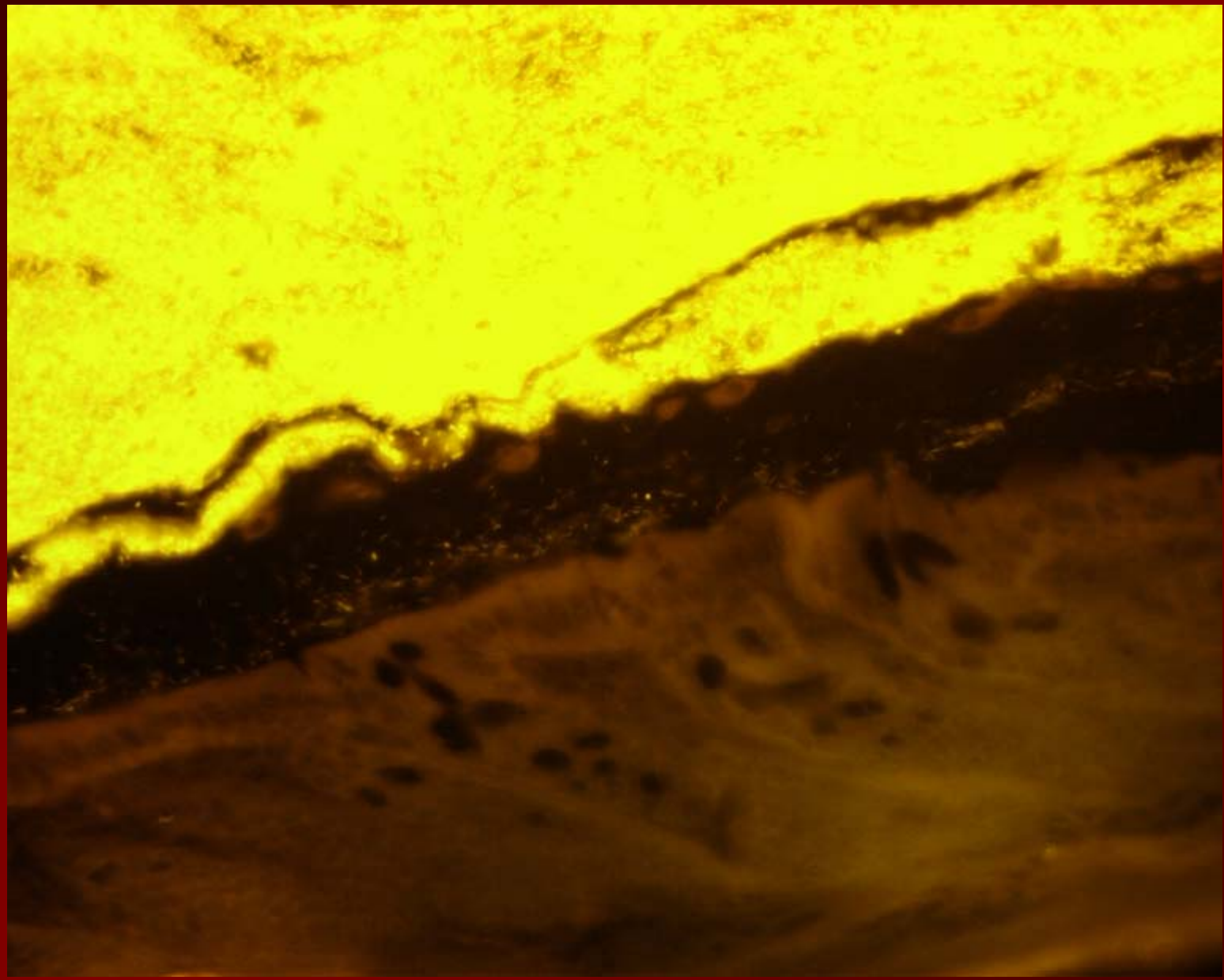



| | UC (n=156) | CD (n=82) |
|---|---|---|
| **All** | 3.8 | 9.1 |
| | | |
| no antibiotics in last 12 months | 4.6 | 9.2 |
| on antibiotics | **0.5** | **1.4** |
| after antibiotics (1-4 weeks) | 5.9 | 27 |
| | | |
| no azathioprine | 3.1 | 9.9 |
| azathioprine | 5.8 | 8.3 |
| | | |
| corticosteroids | **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 |

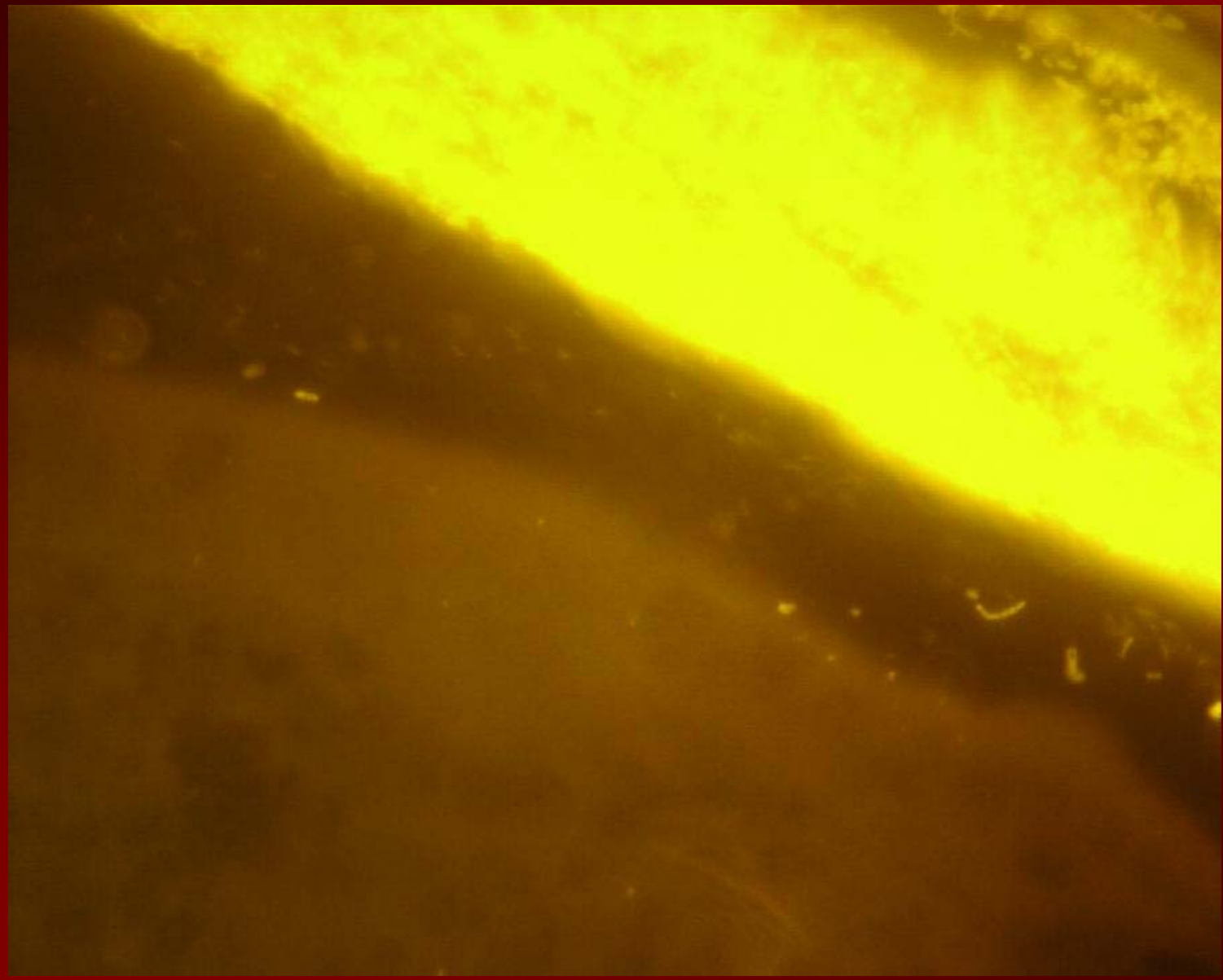
Multicellular bacteria forming stromatolith in Australian salt lakes

# FISH analysis of the mucosal biofilm

## using r-RNA targeted probes



**Mucus gap between the colonic wall and feces in healthy mouse**



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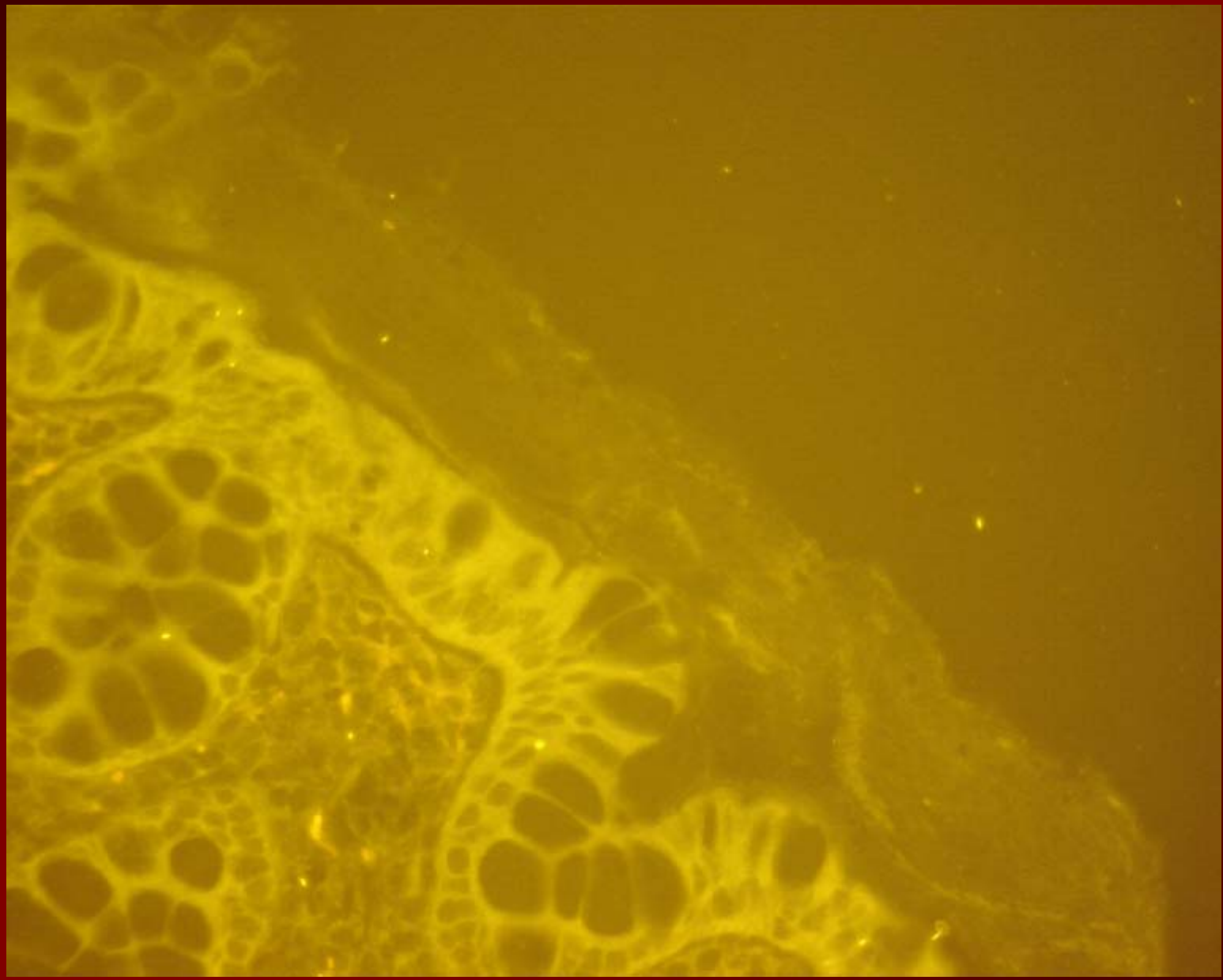
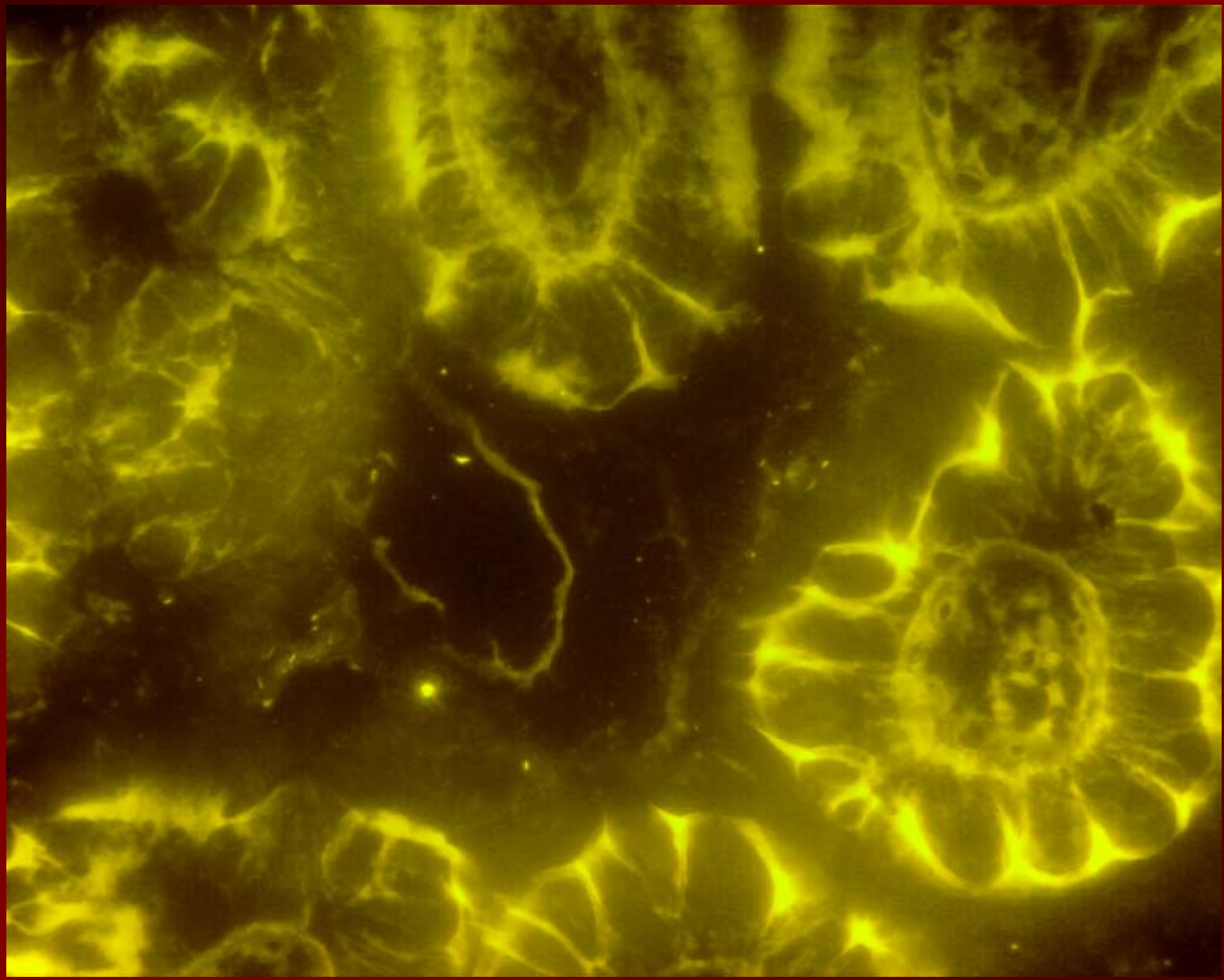
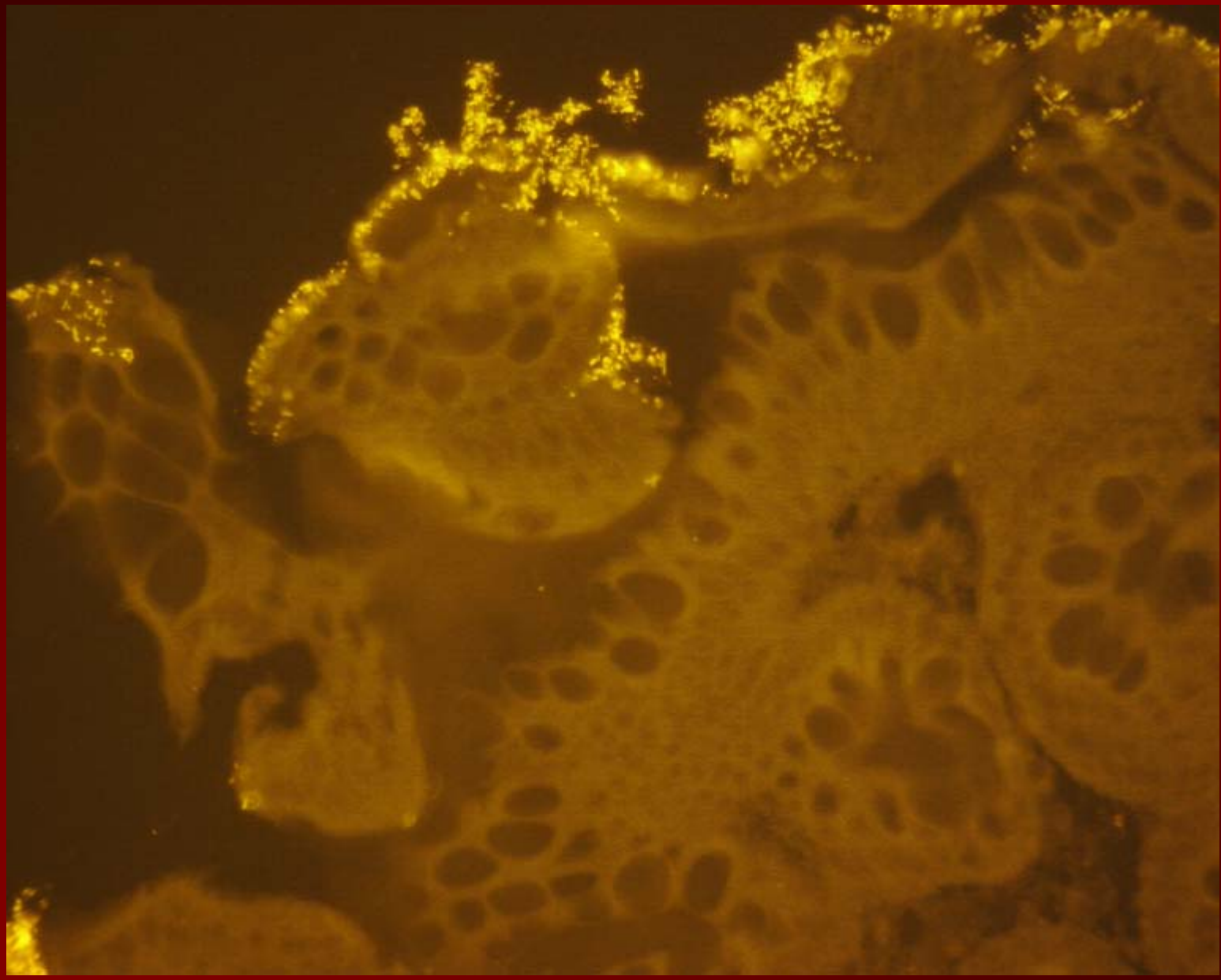


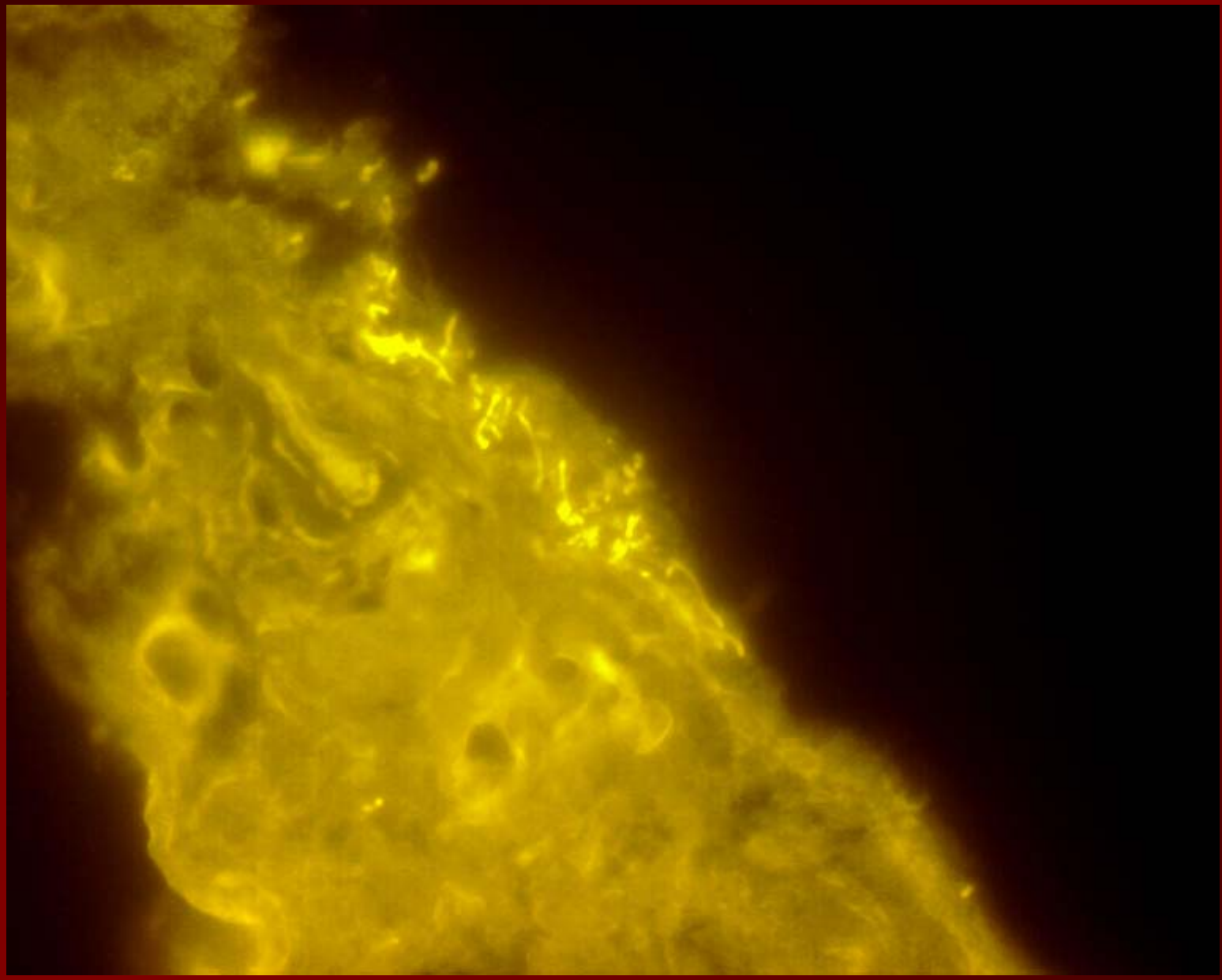
**Human colonic wall covered with mucus omitting bacteria**



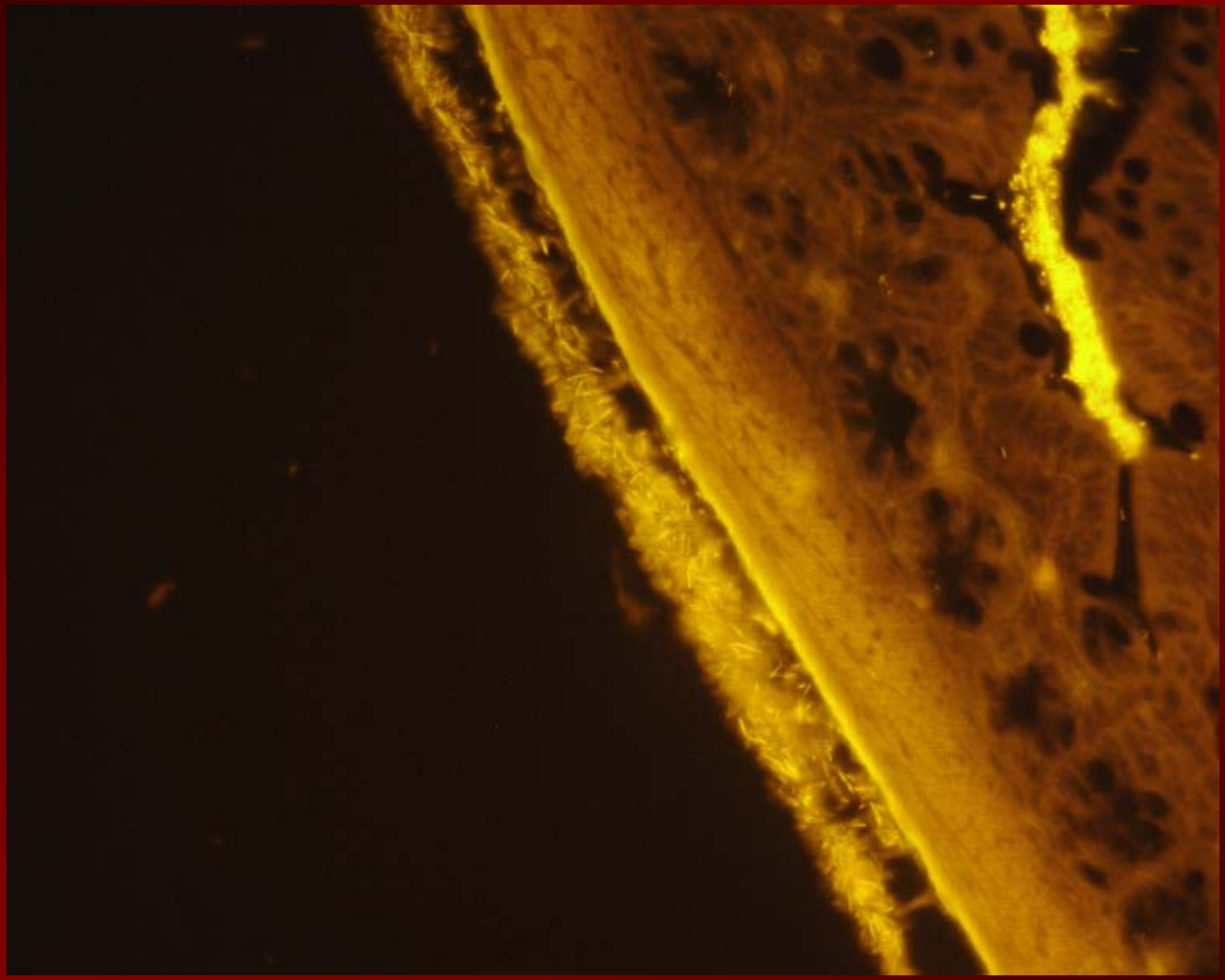
**Human ileal wall covered with mucus omitting bacteria**



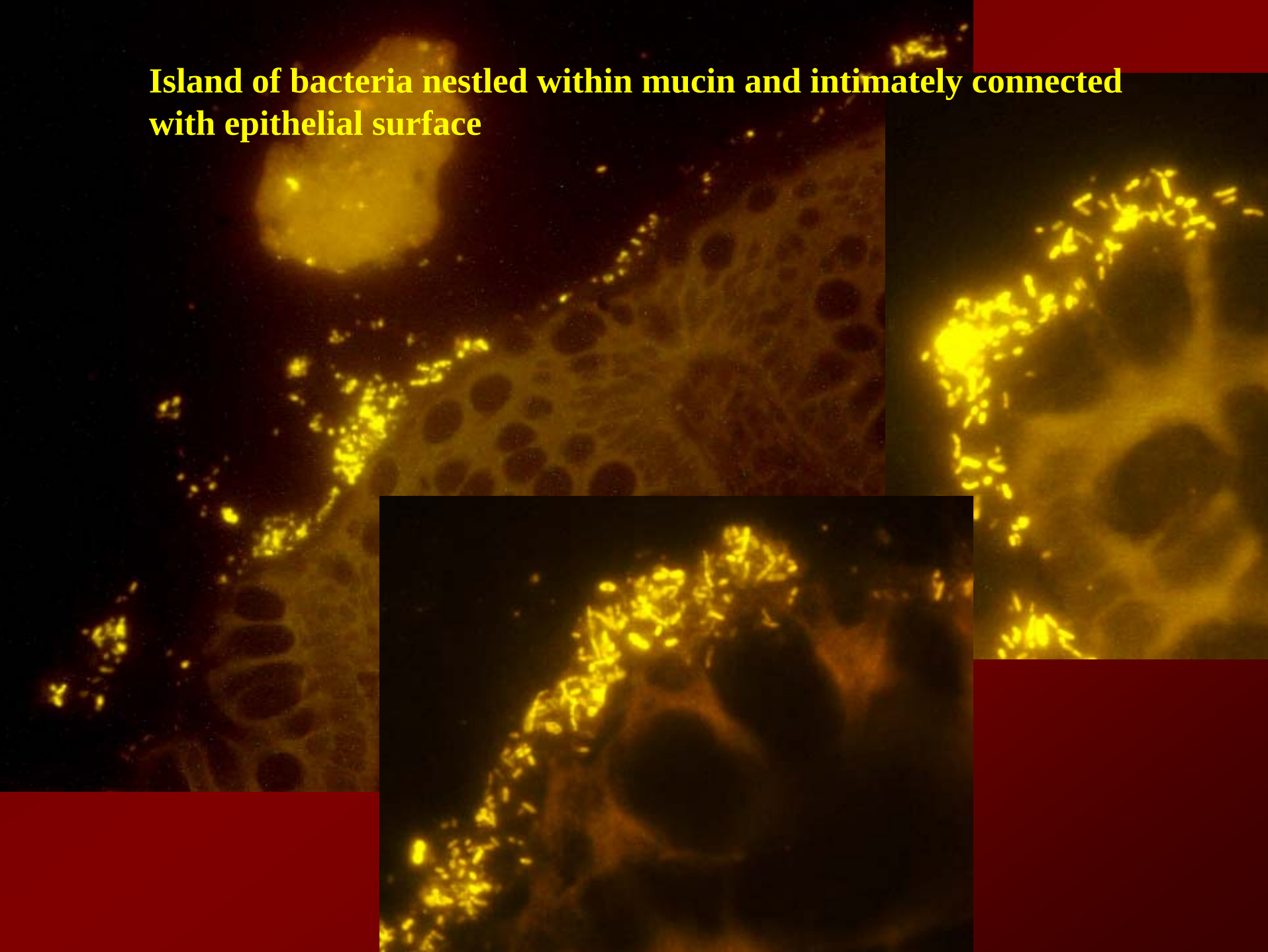
**intact mucus is partially denuded,  the biopsy is covered by bacteria**

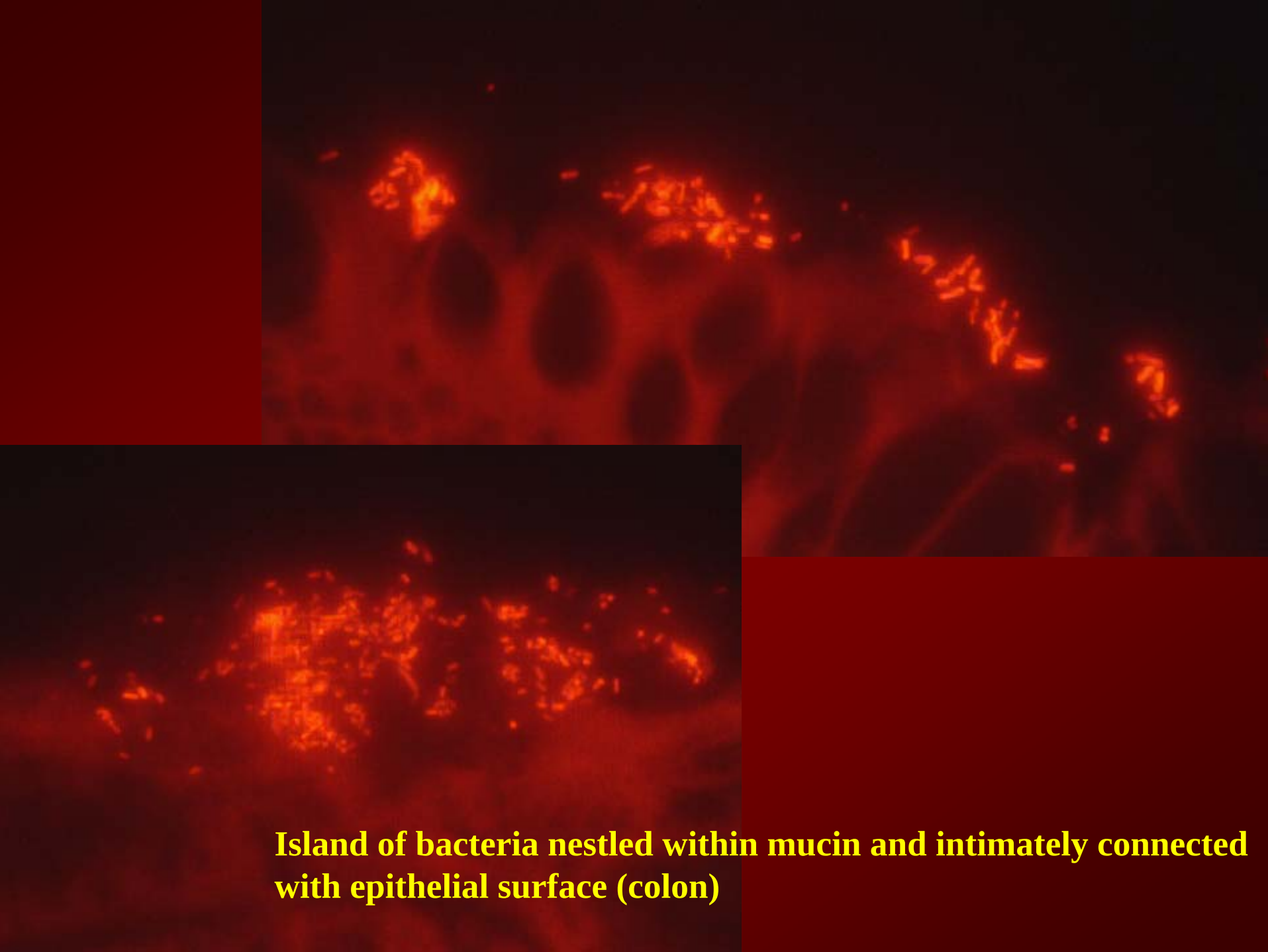


**Fecal contamination on submucosal parts of the biopsy**

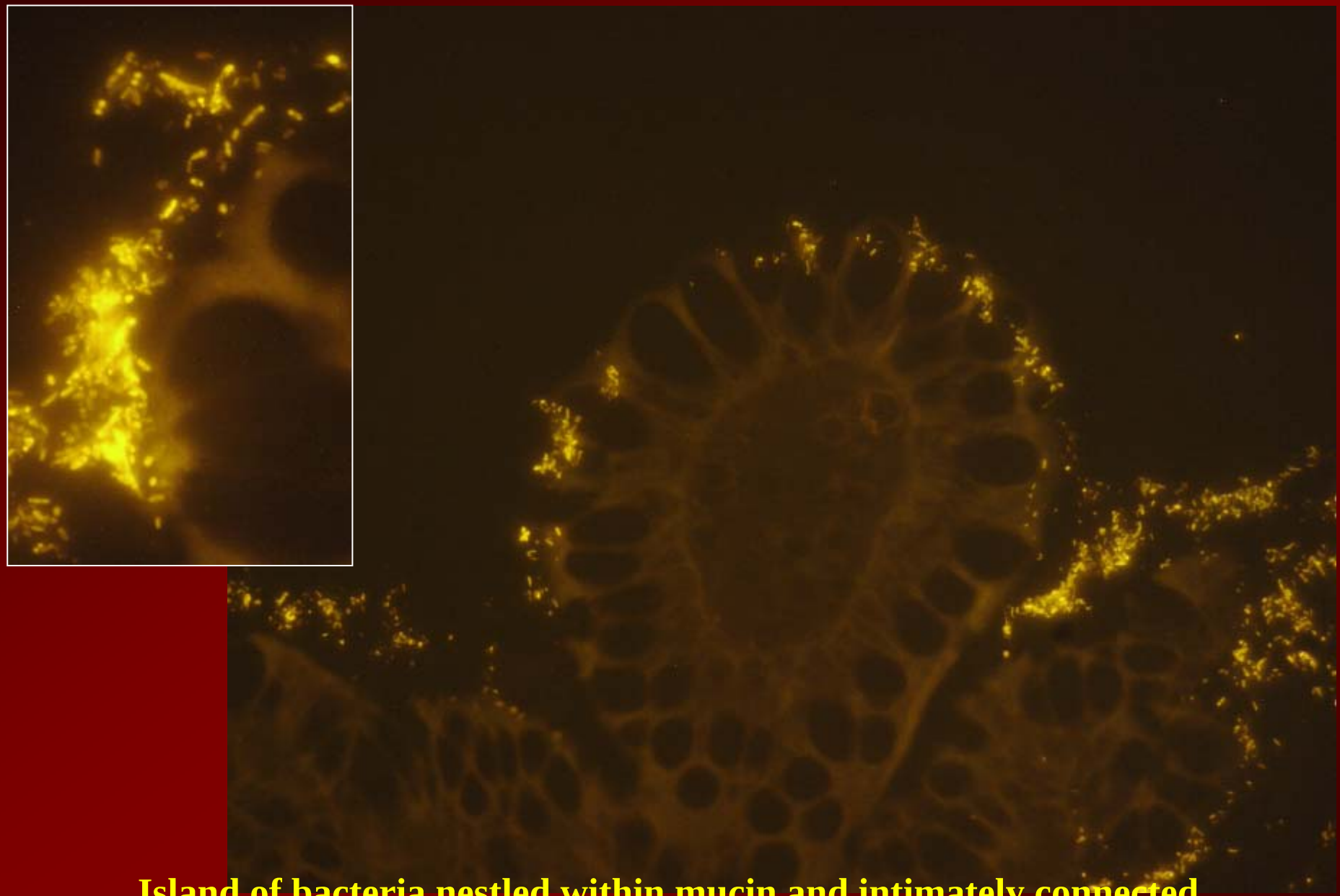


**bacteria on the peritoneal side of the mouse intestine/ biases**


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

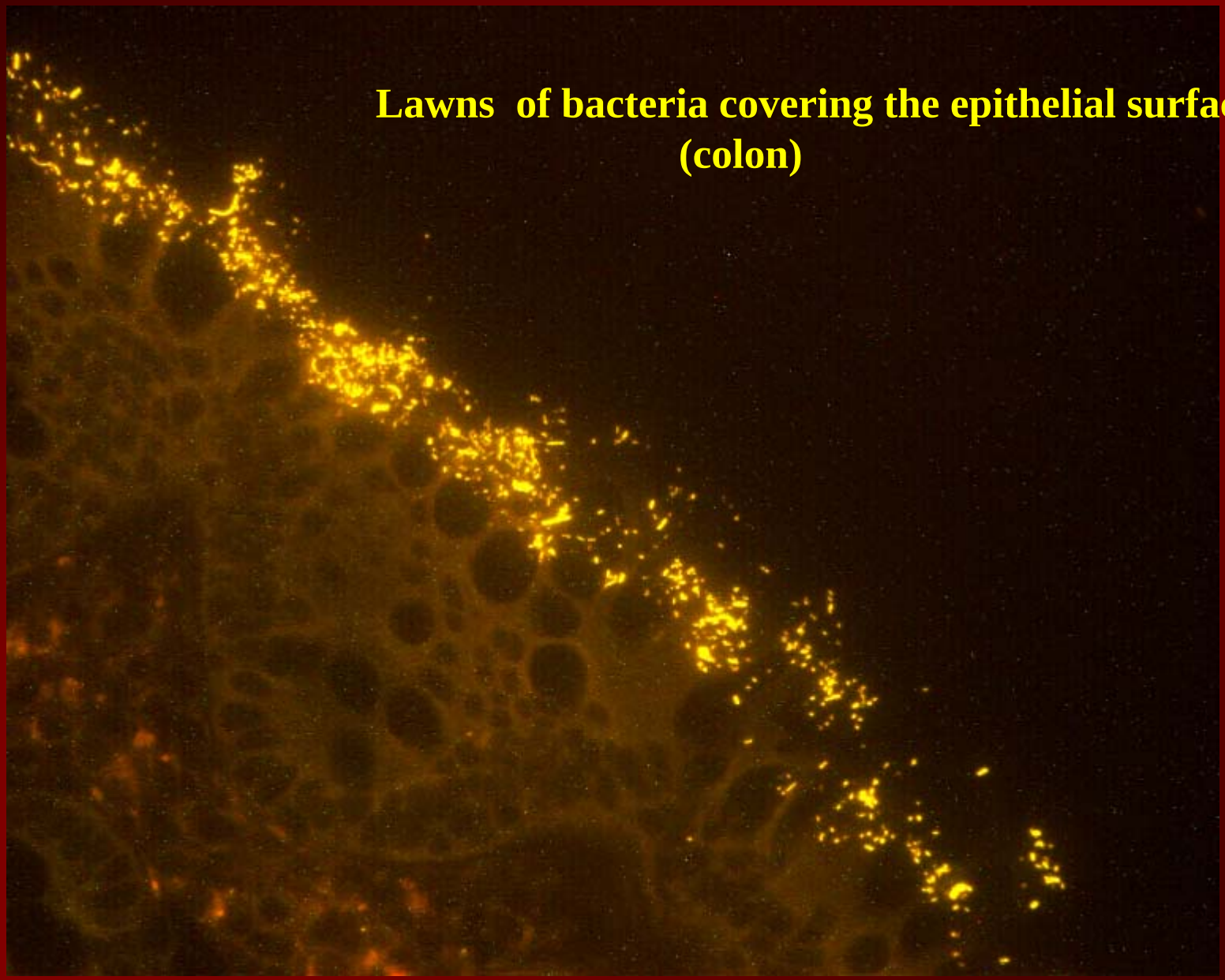


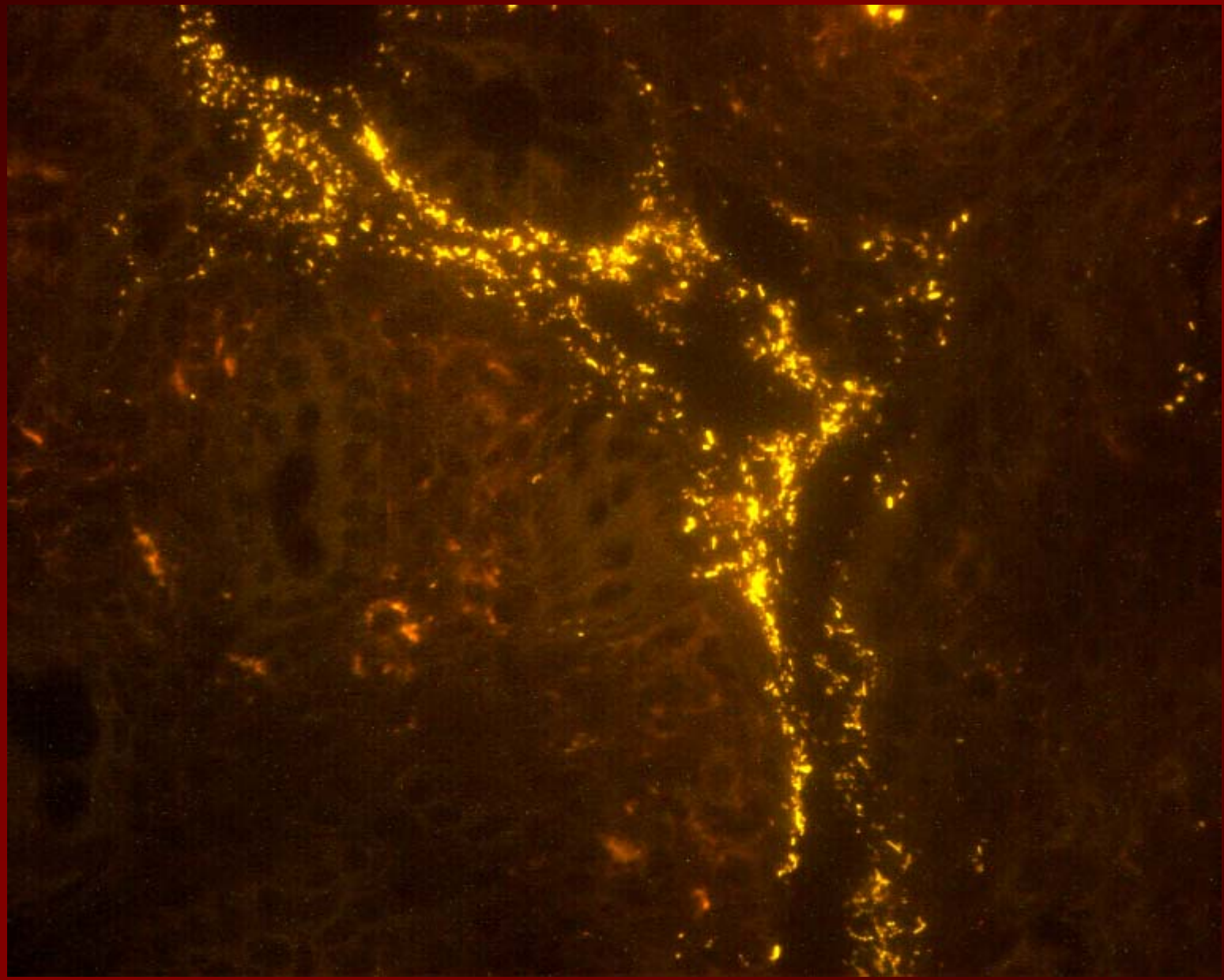
**Island of bacteria nestled within mucin and intimately connected with epithelial surface (colon)**



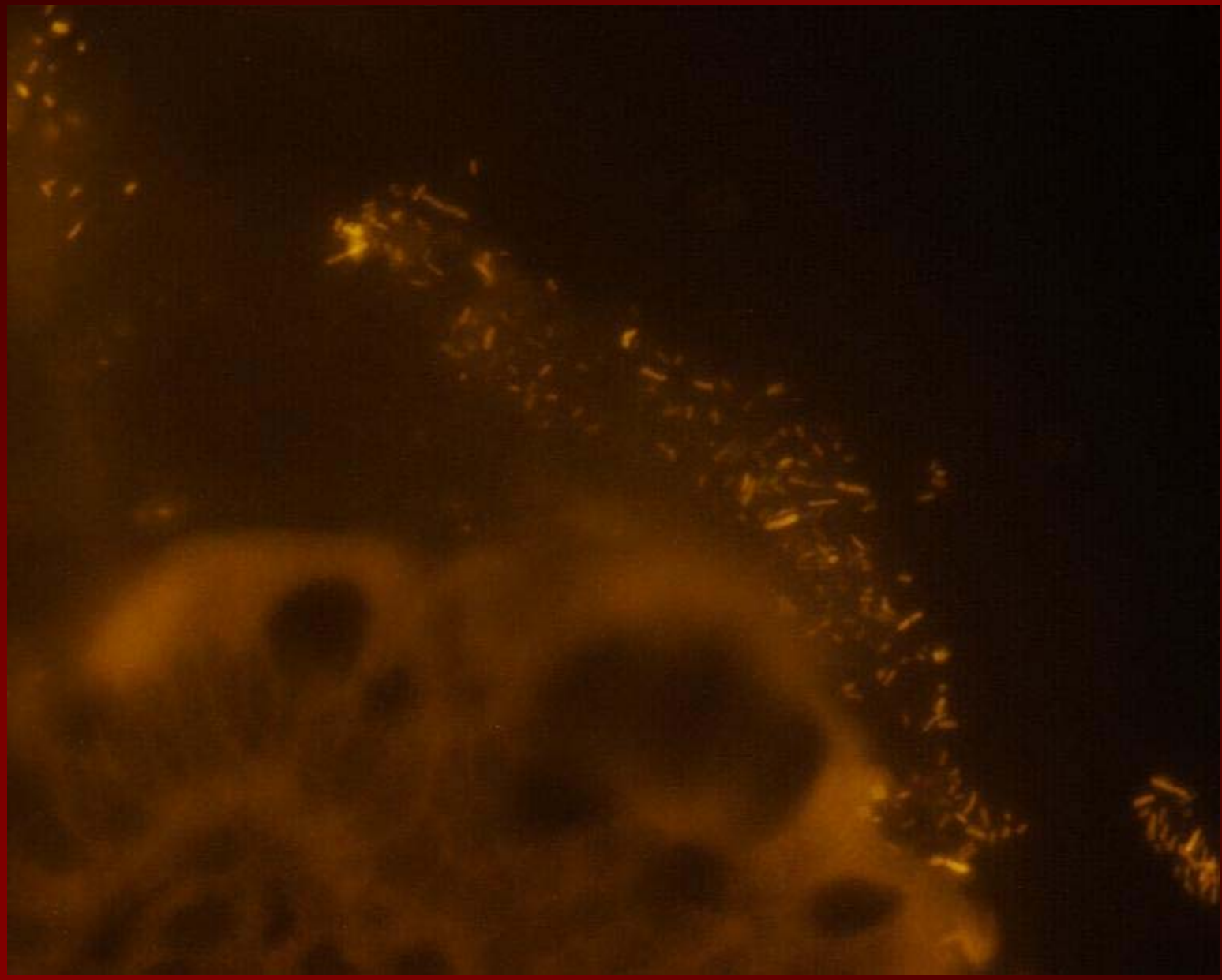
**Island of bacteria nestled within mucin and intimately connected with epithelial surface (ileum)**


Lawns of bacteria covering the epithelial surface

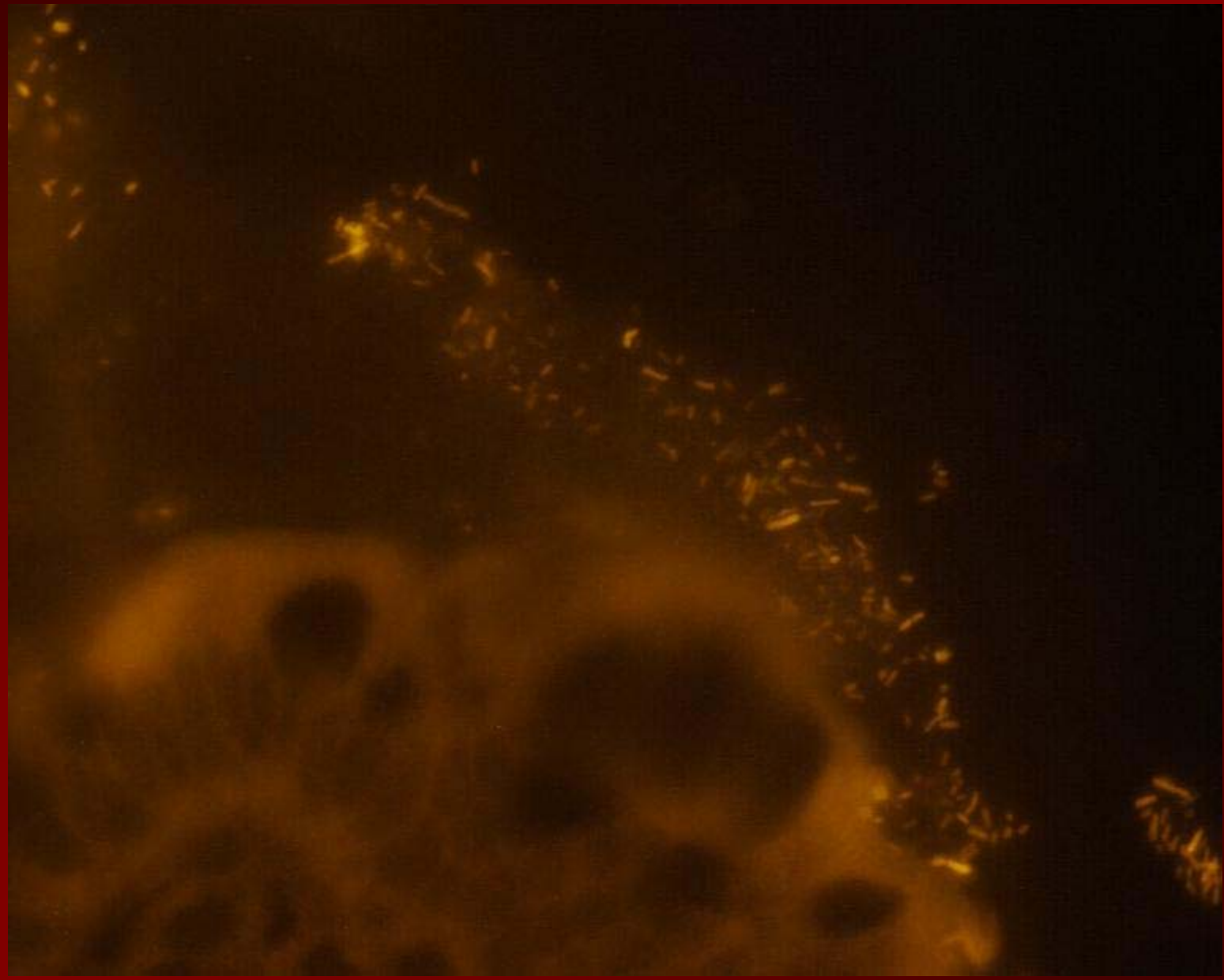

Lawns of bacteria covering the epithelial surface
(colon)

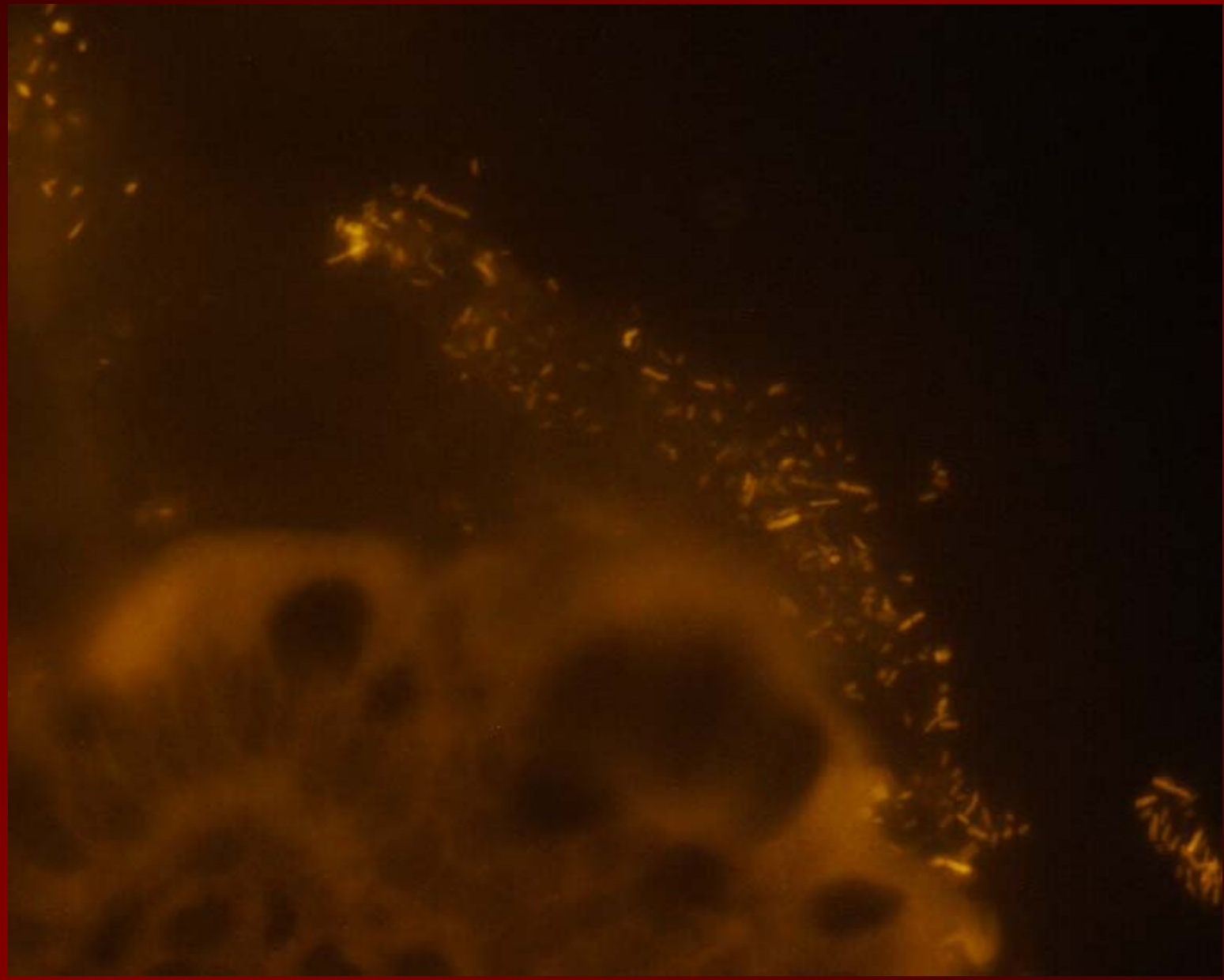


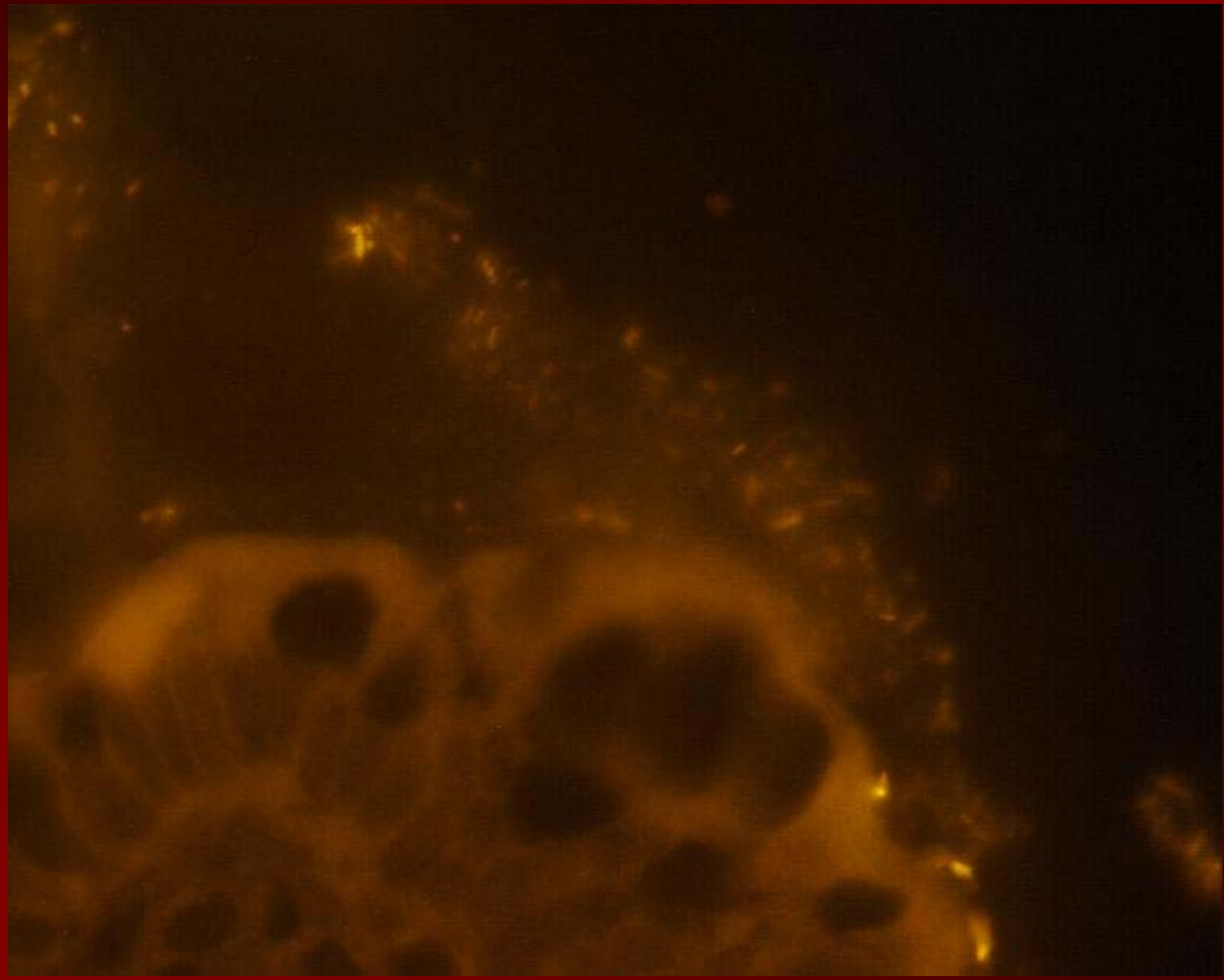
**Lawns of bacteria covering the epithelial surface (ileum)**

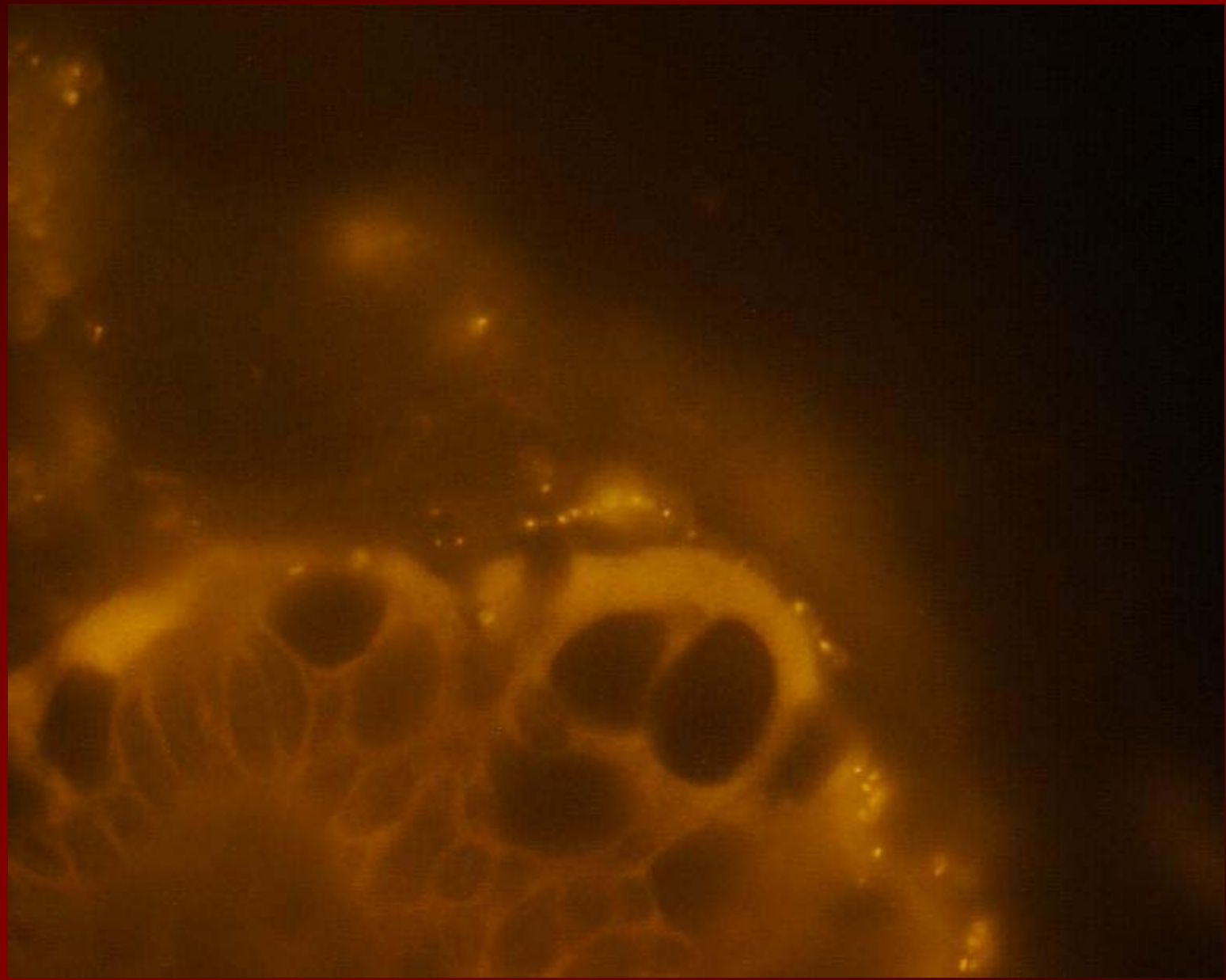


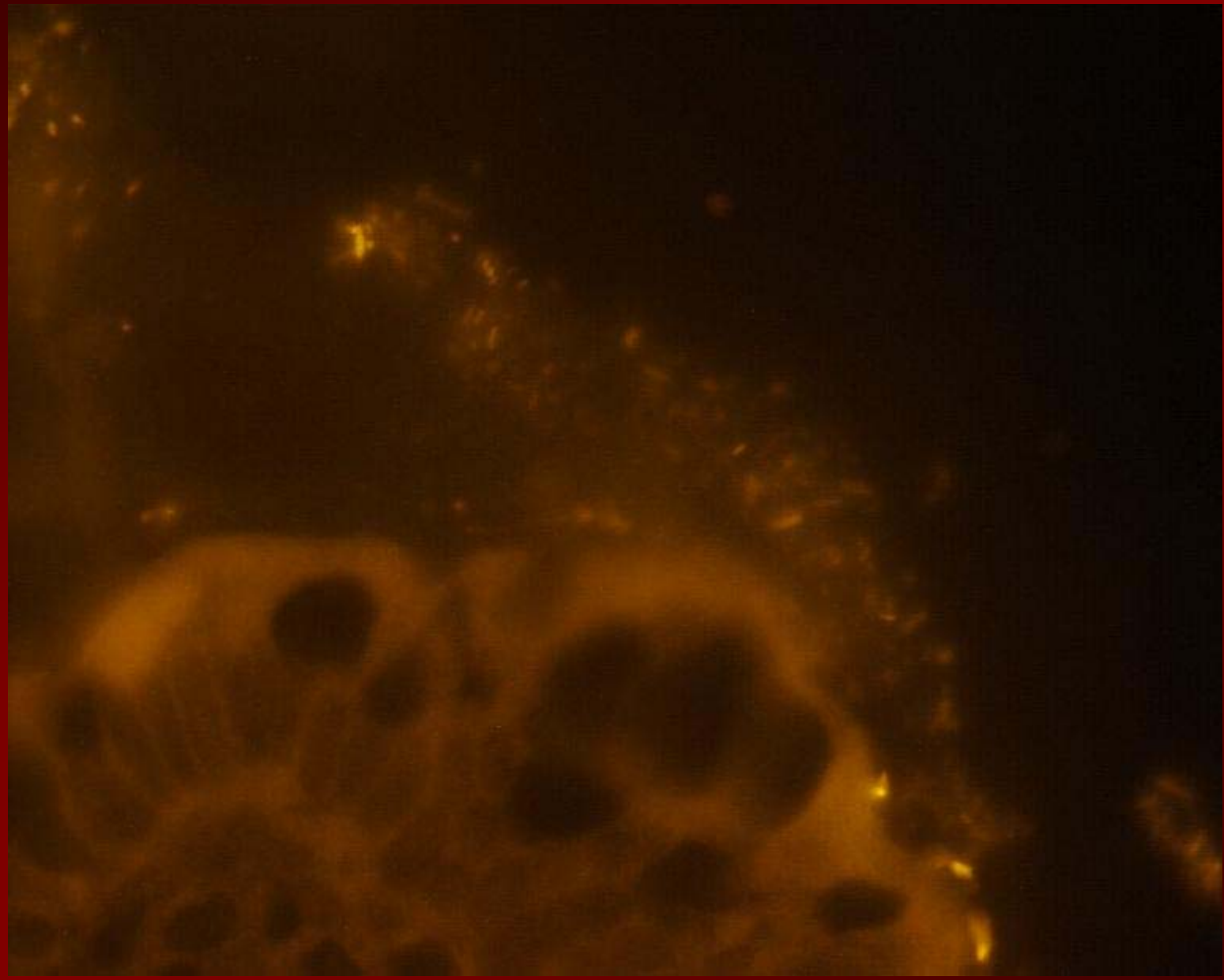
**Differential focusing of bacterial lawns covering the epithelial surface**

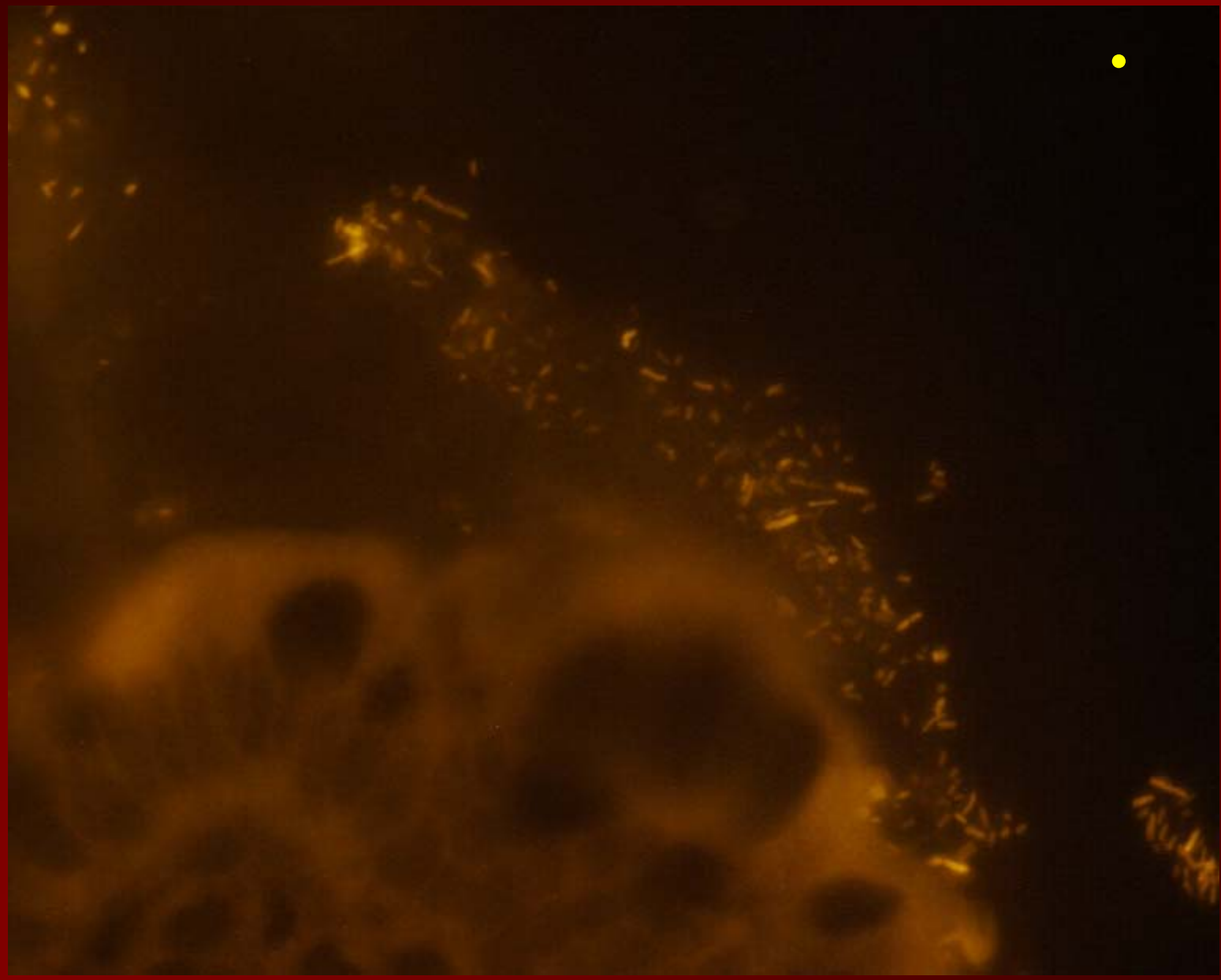


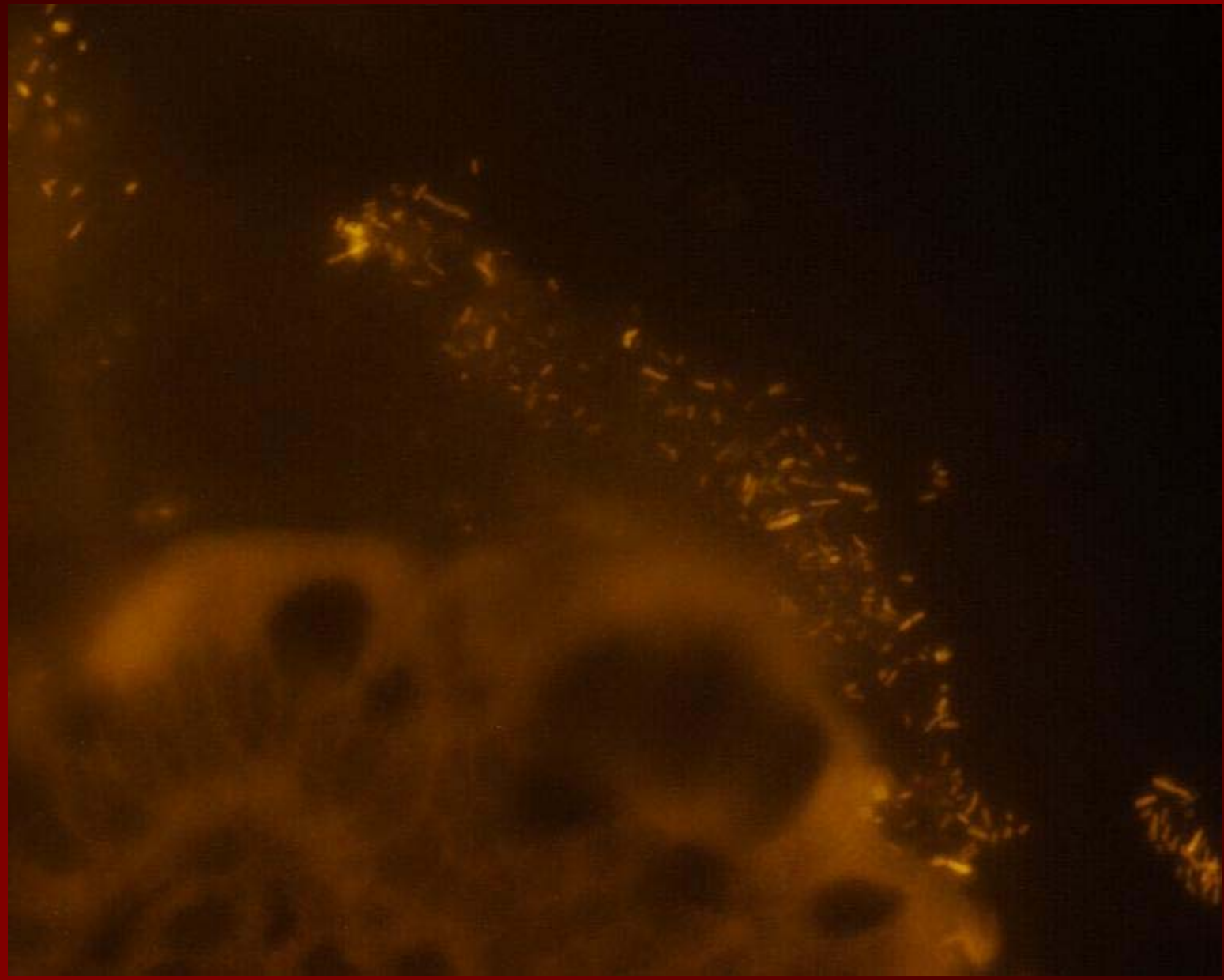


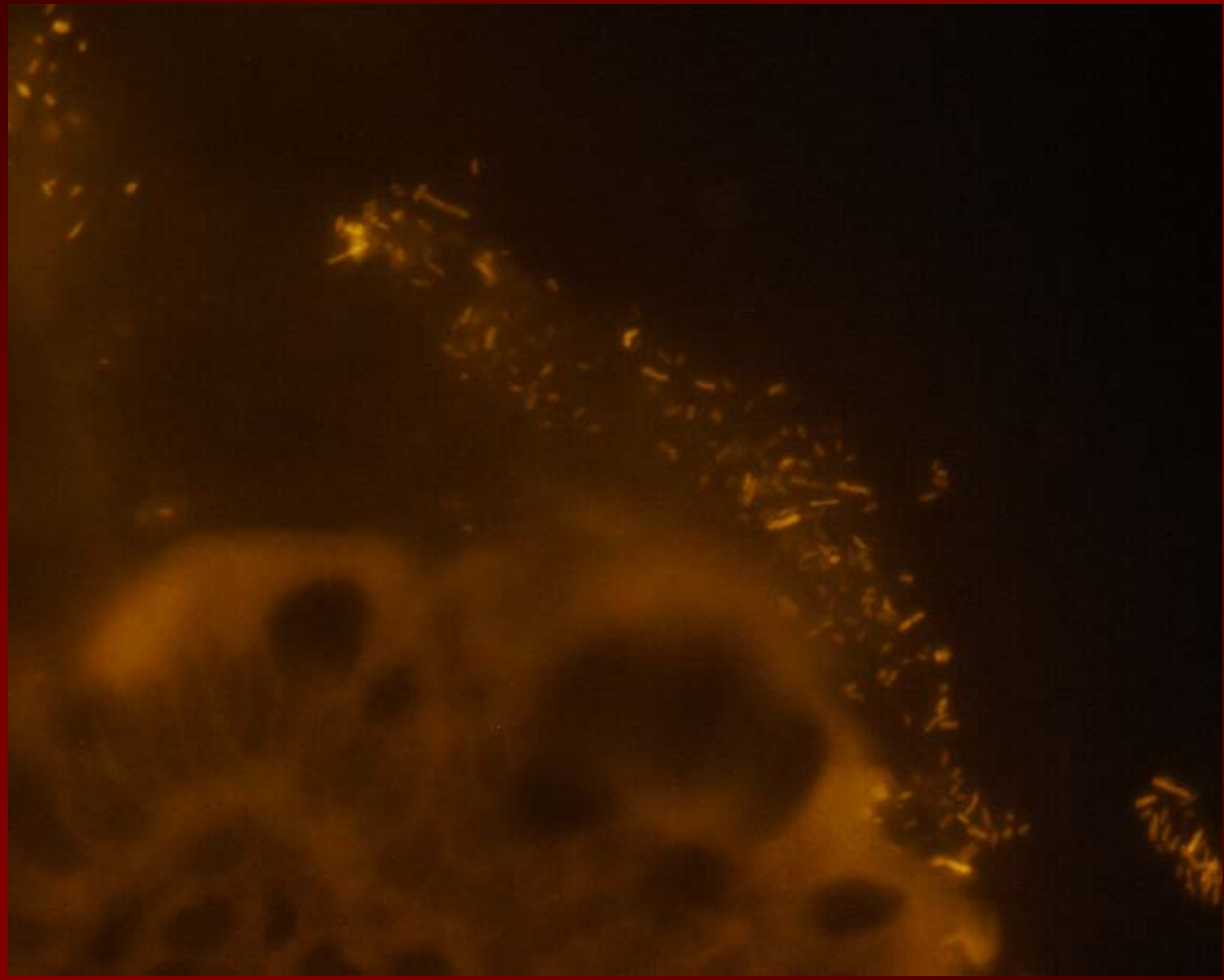


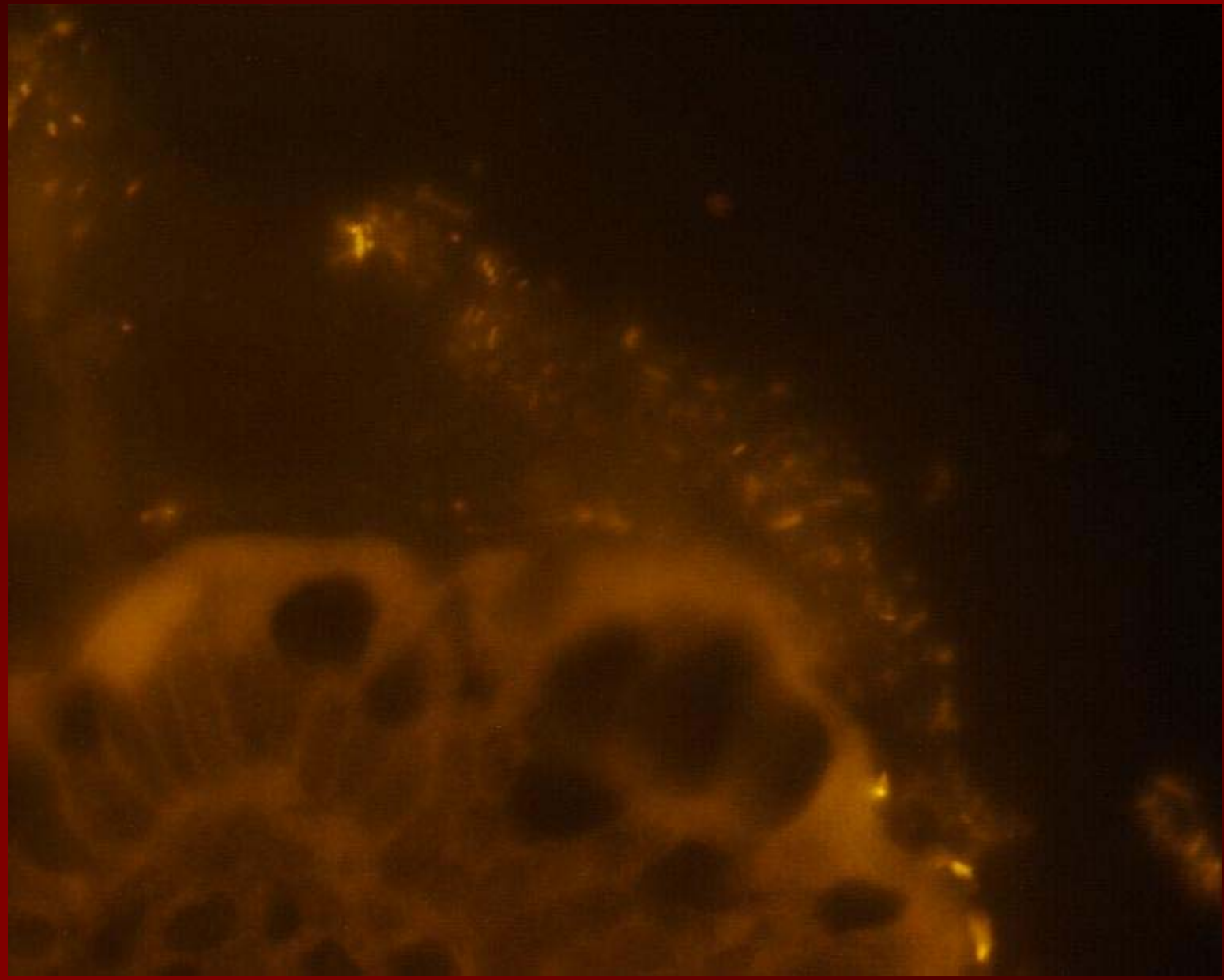


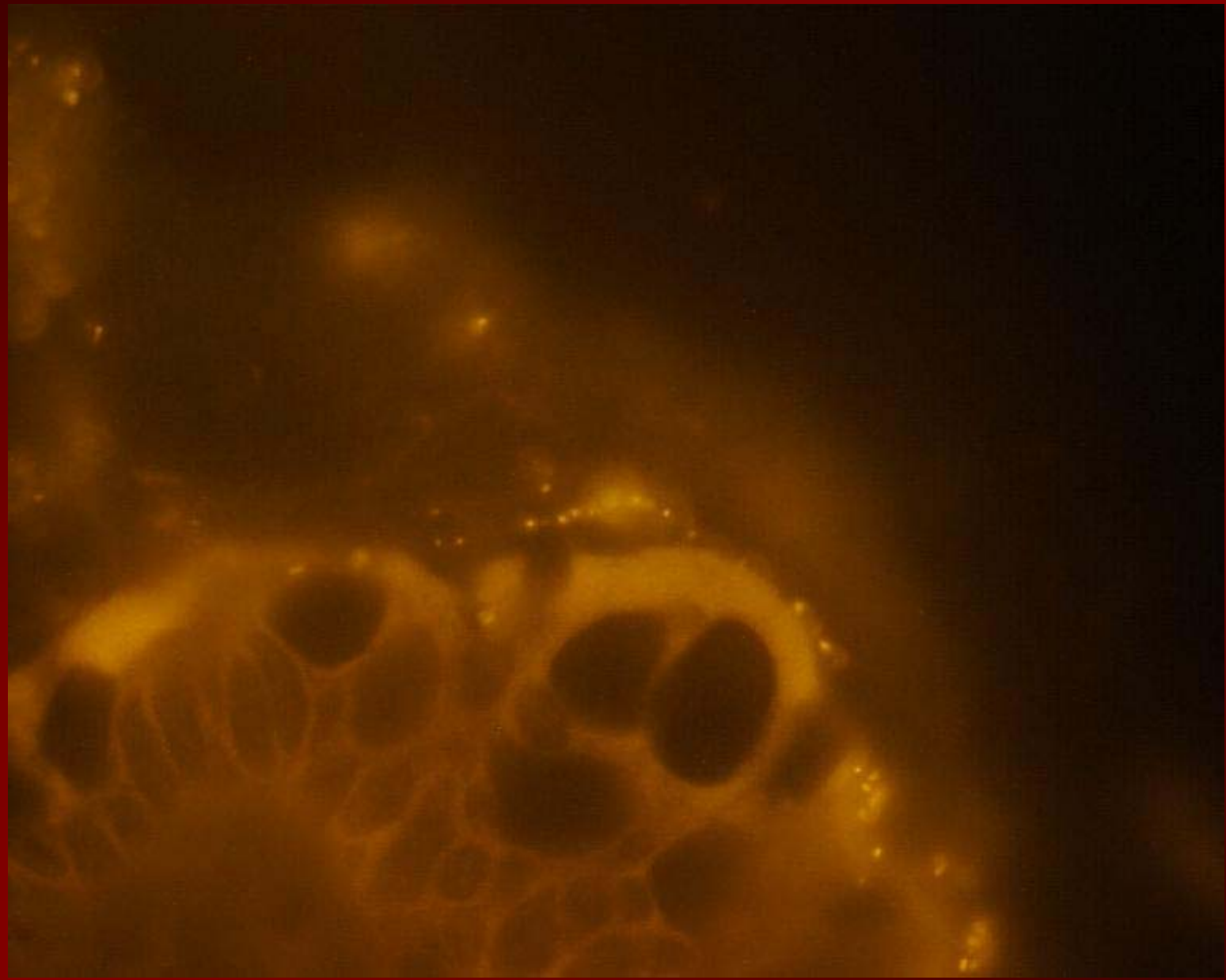


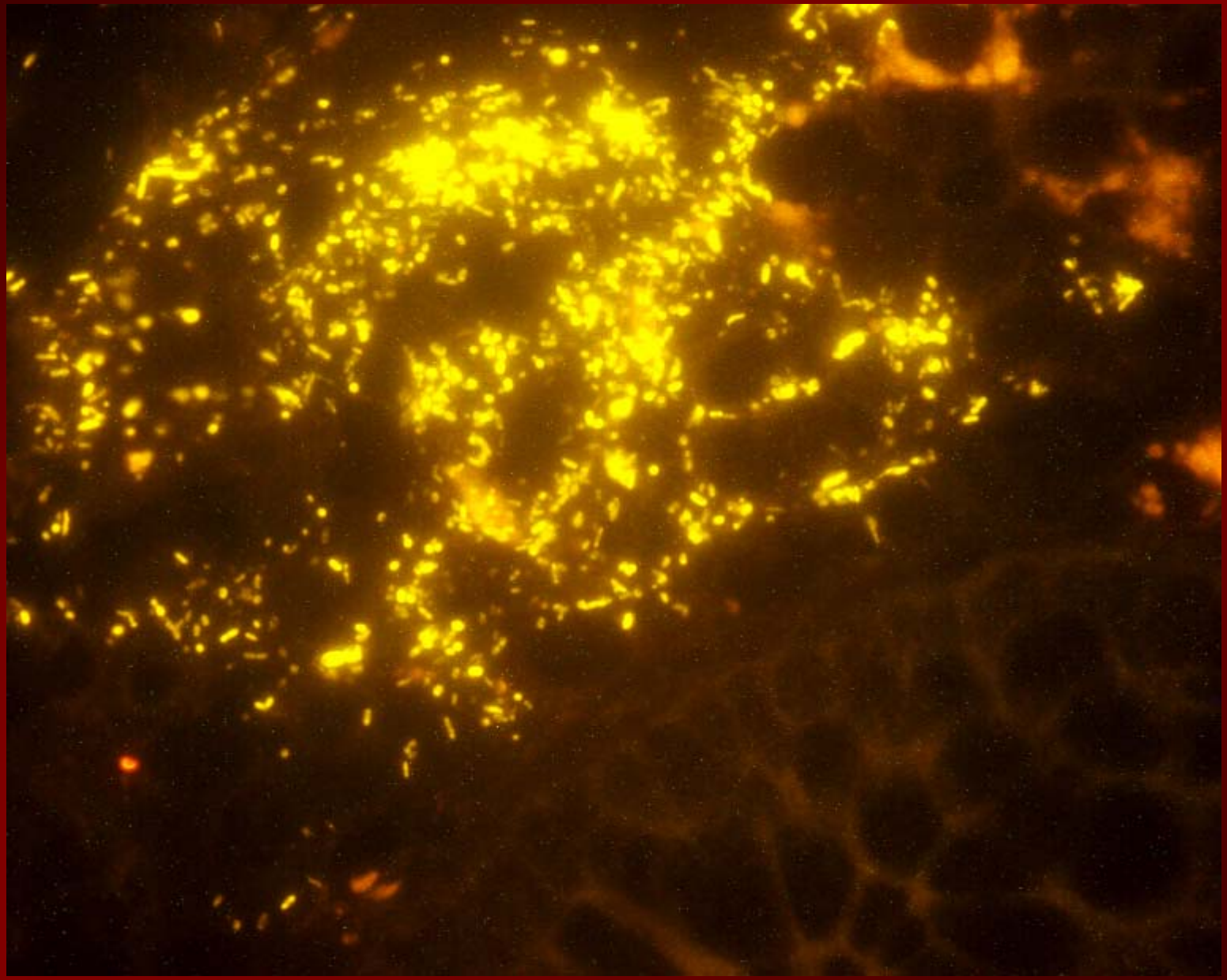












# Goblet cell empting

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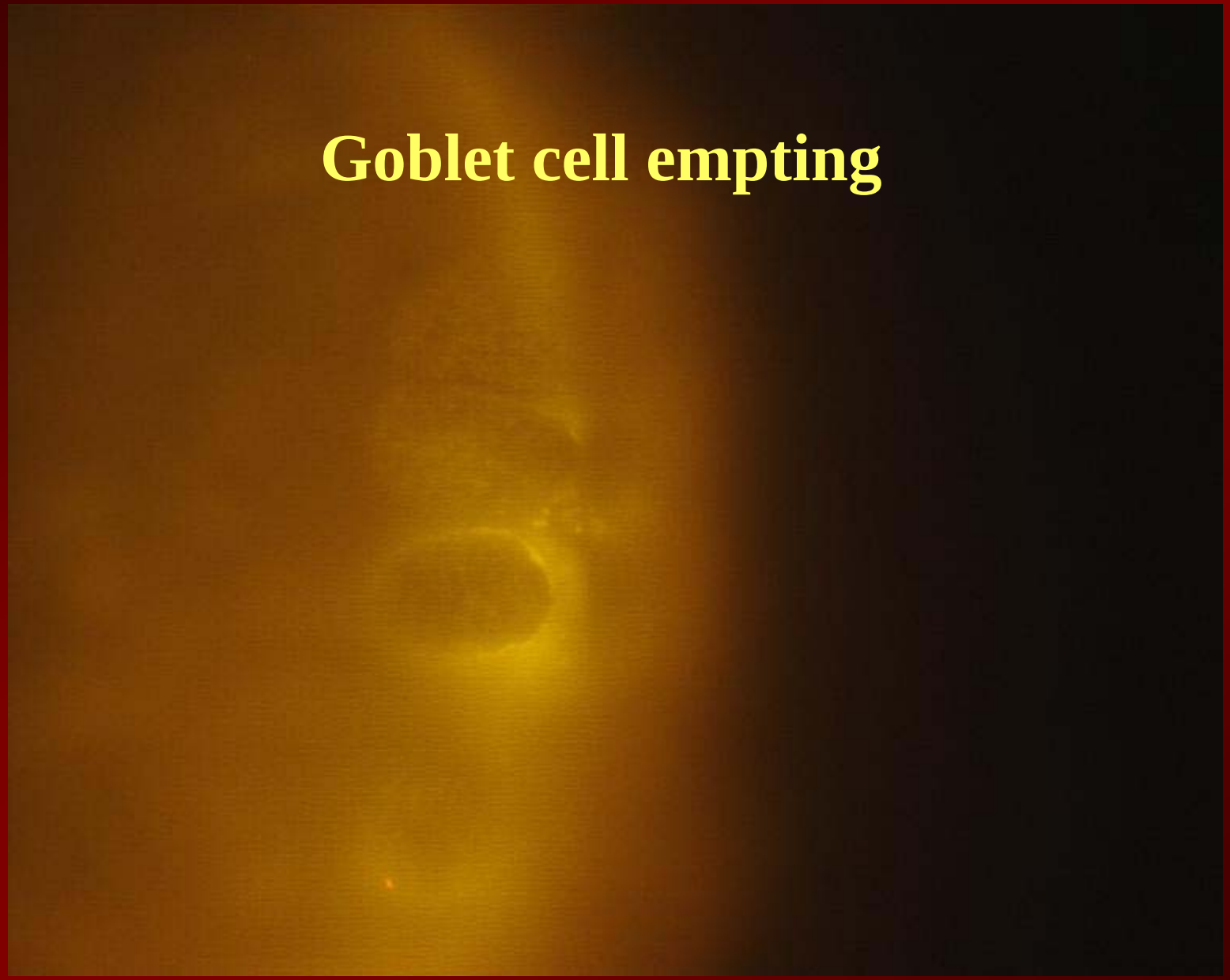

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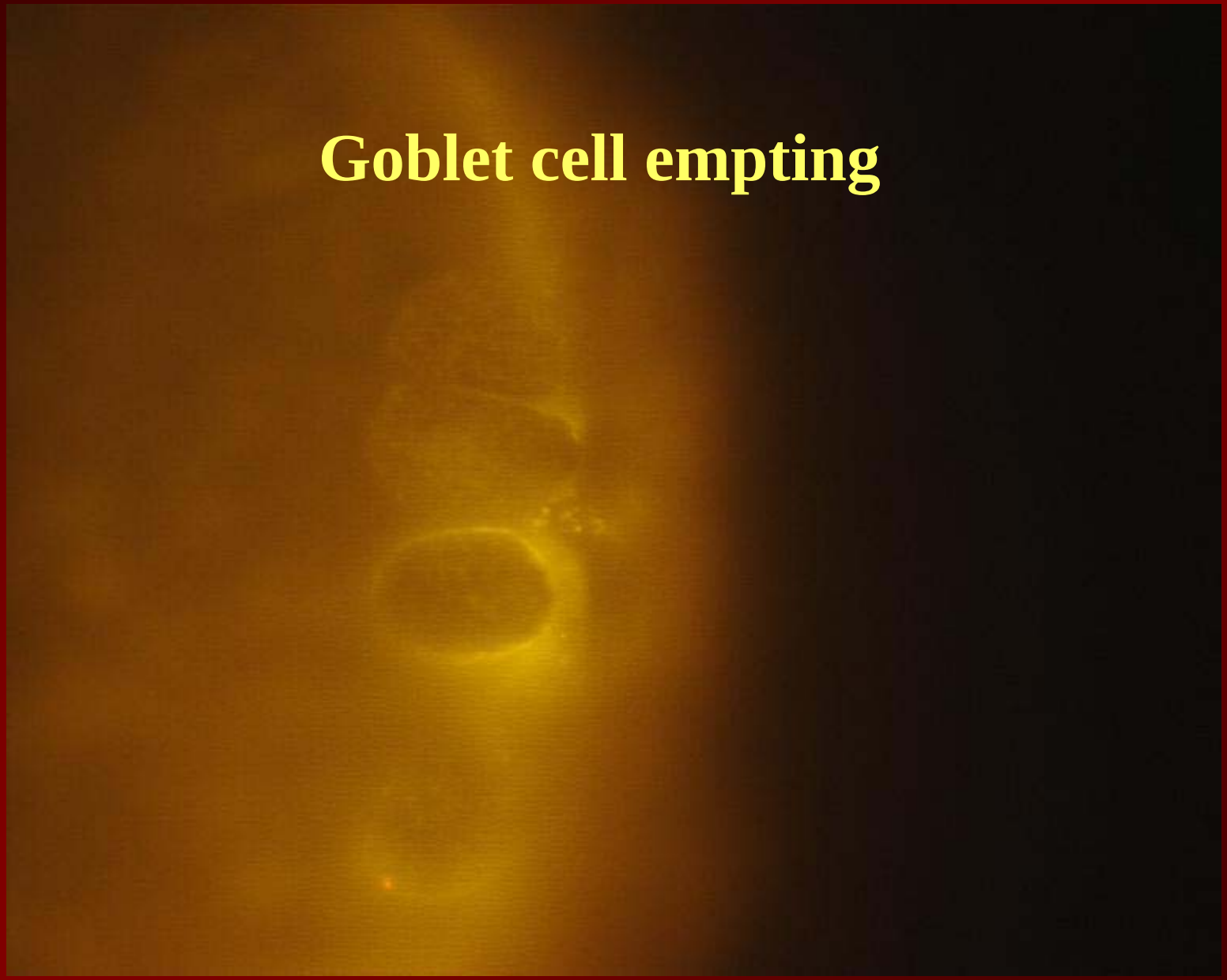

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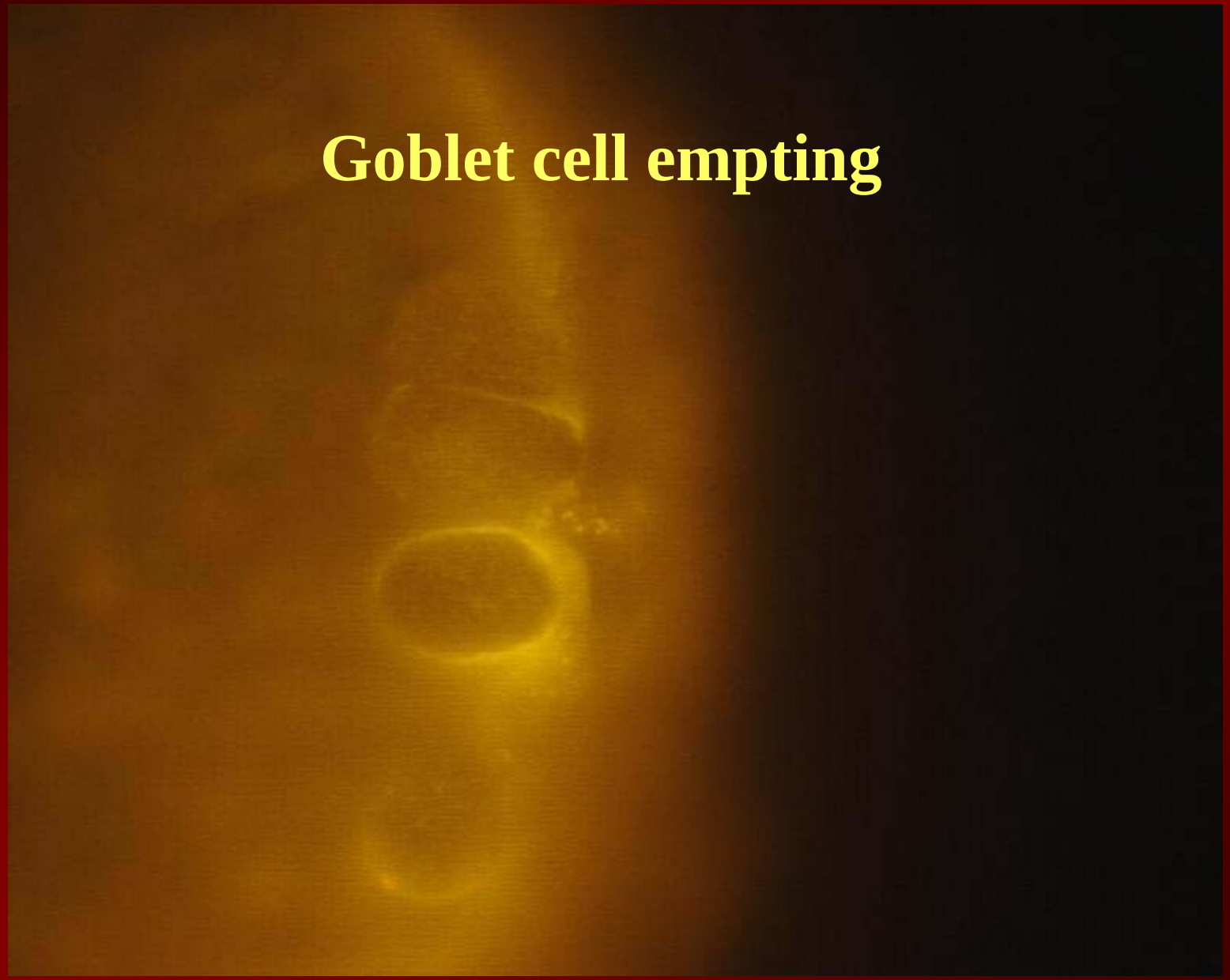

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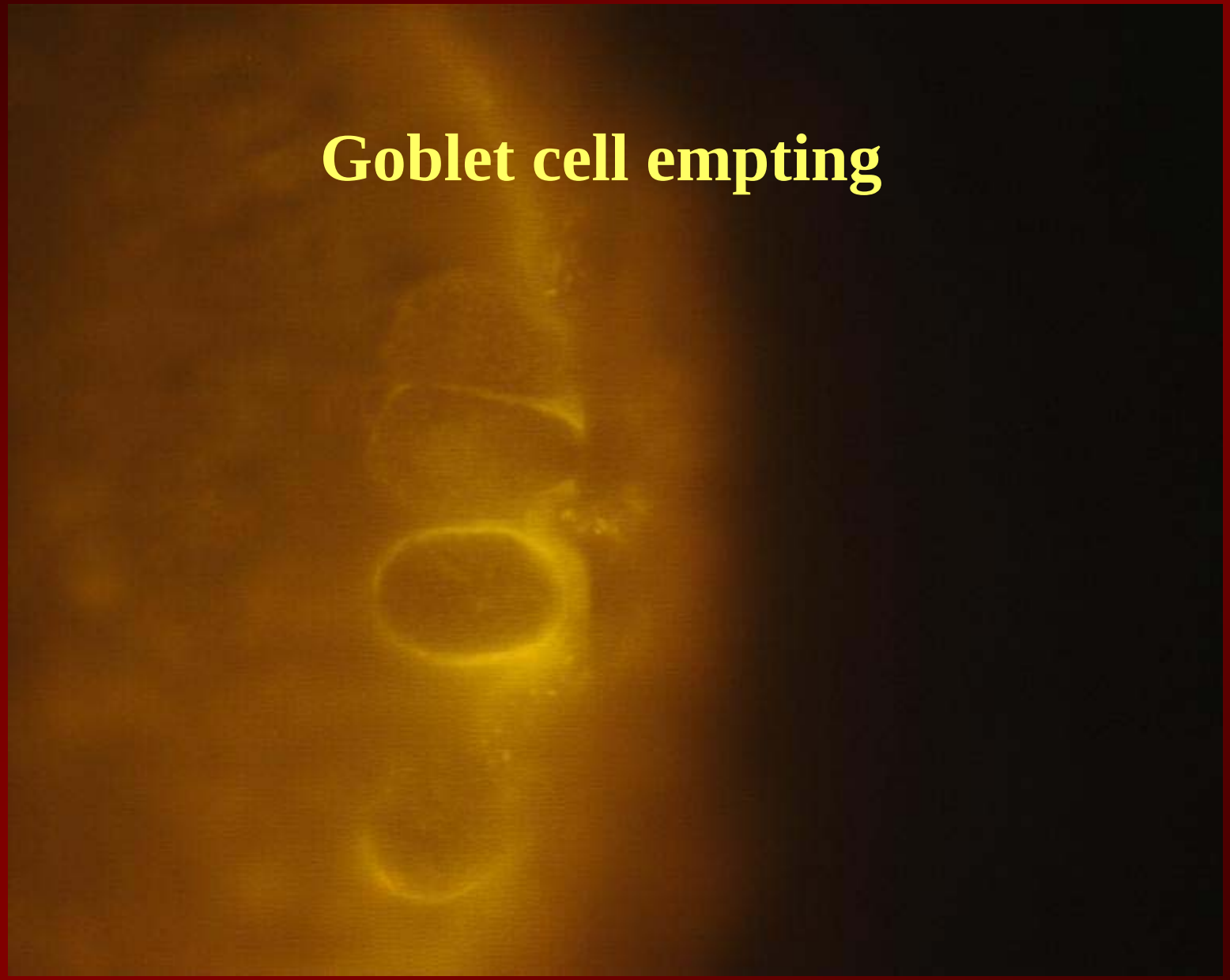

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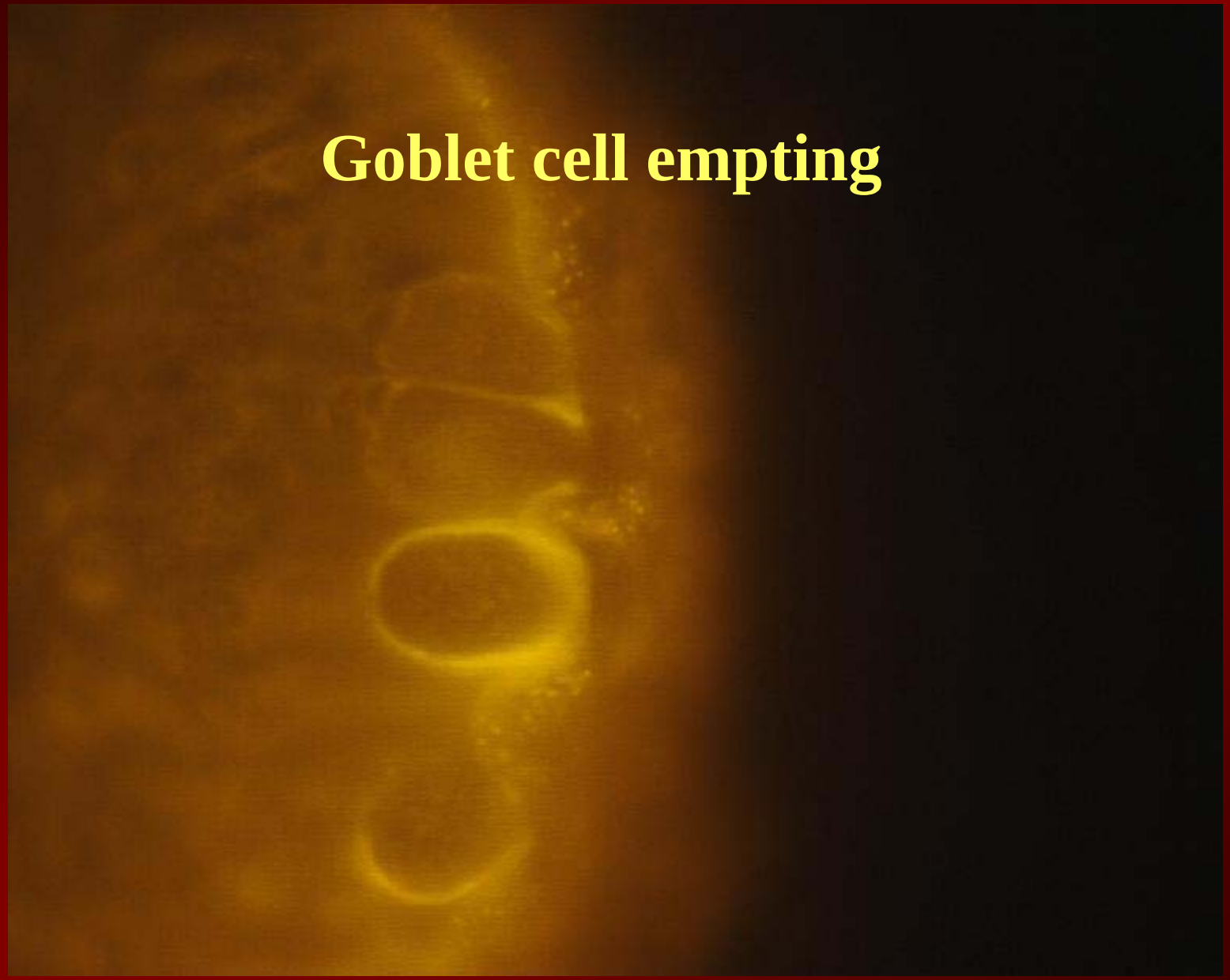

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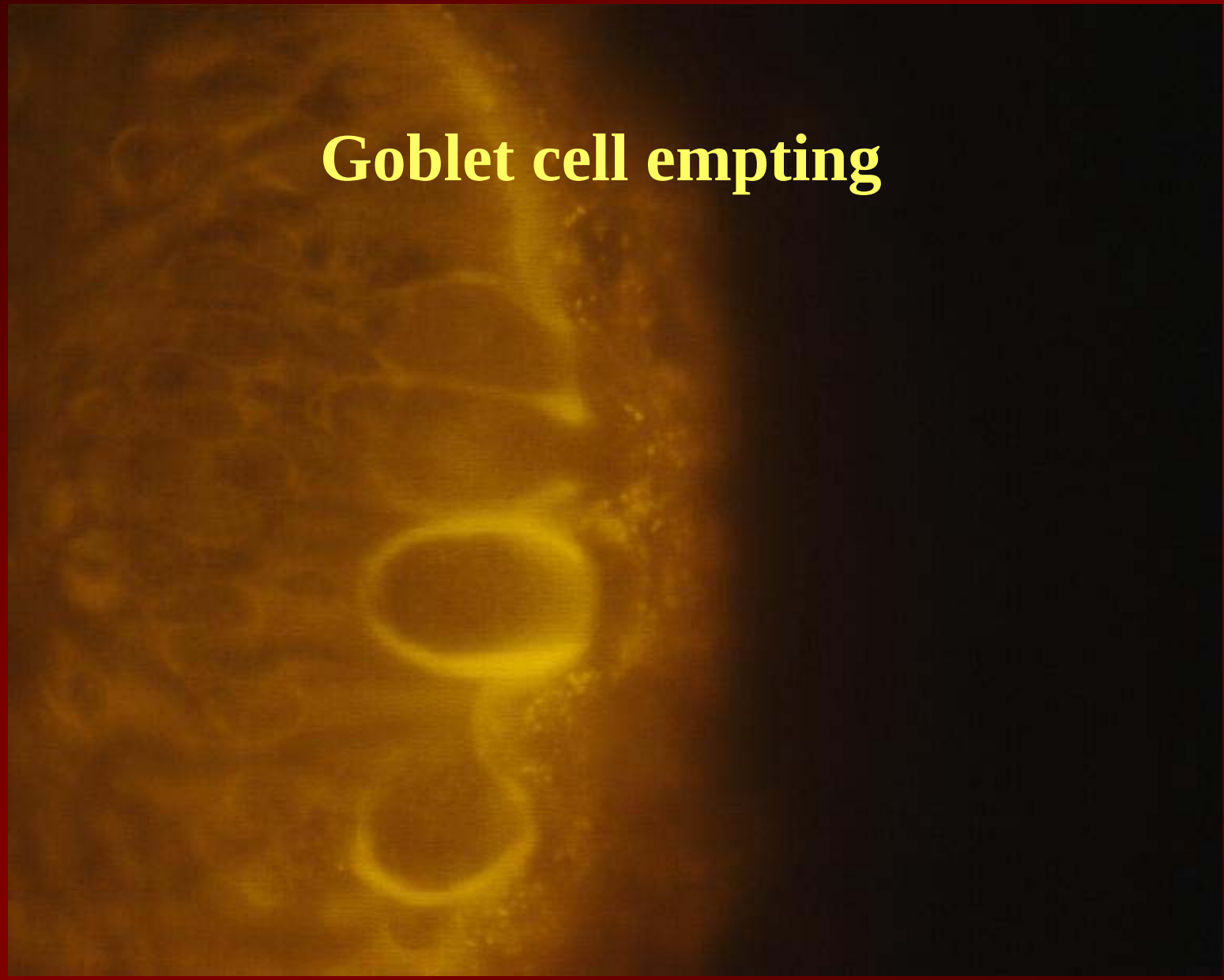

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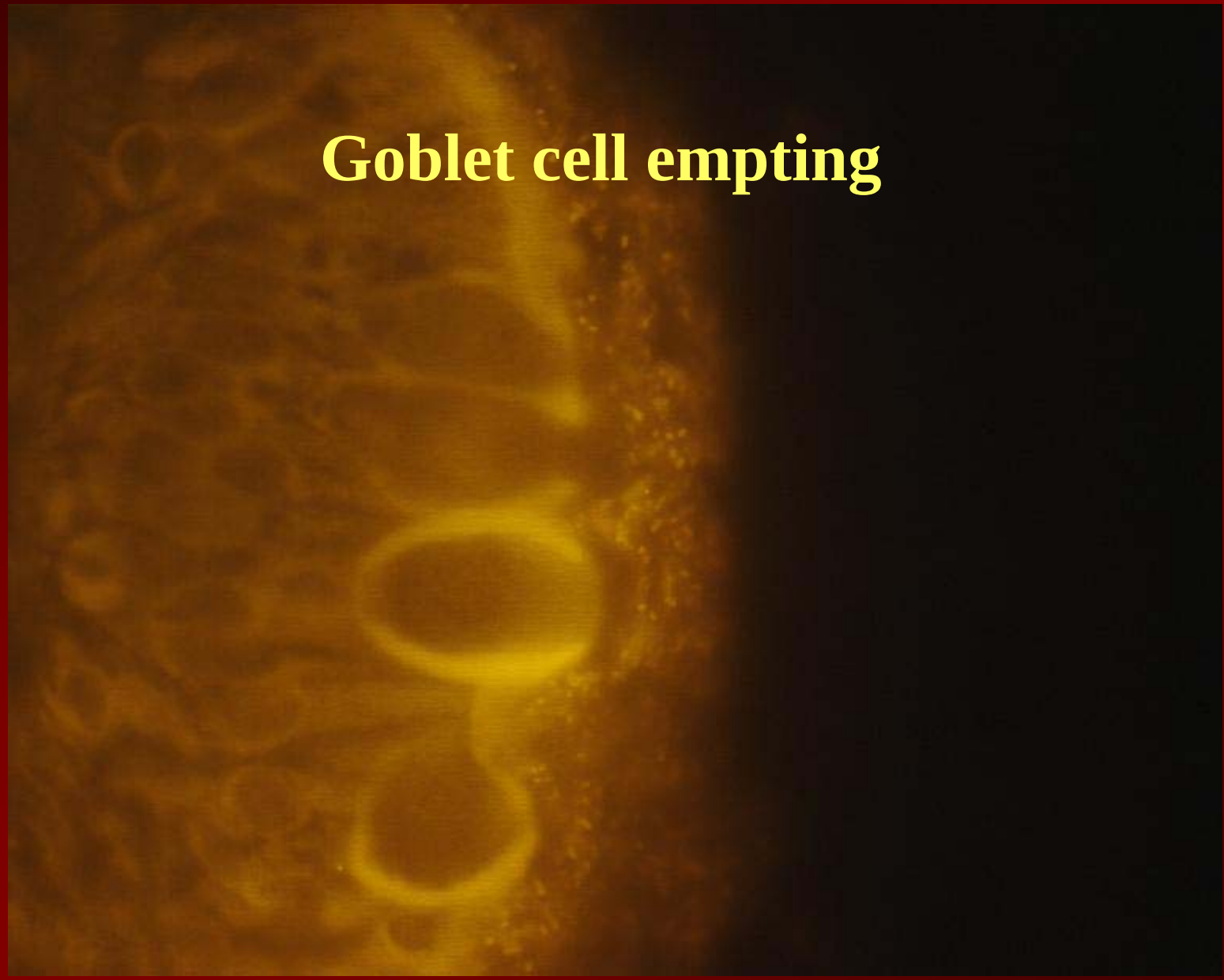

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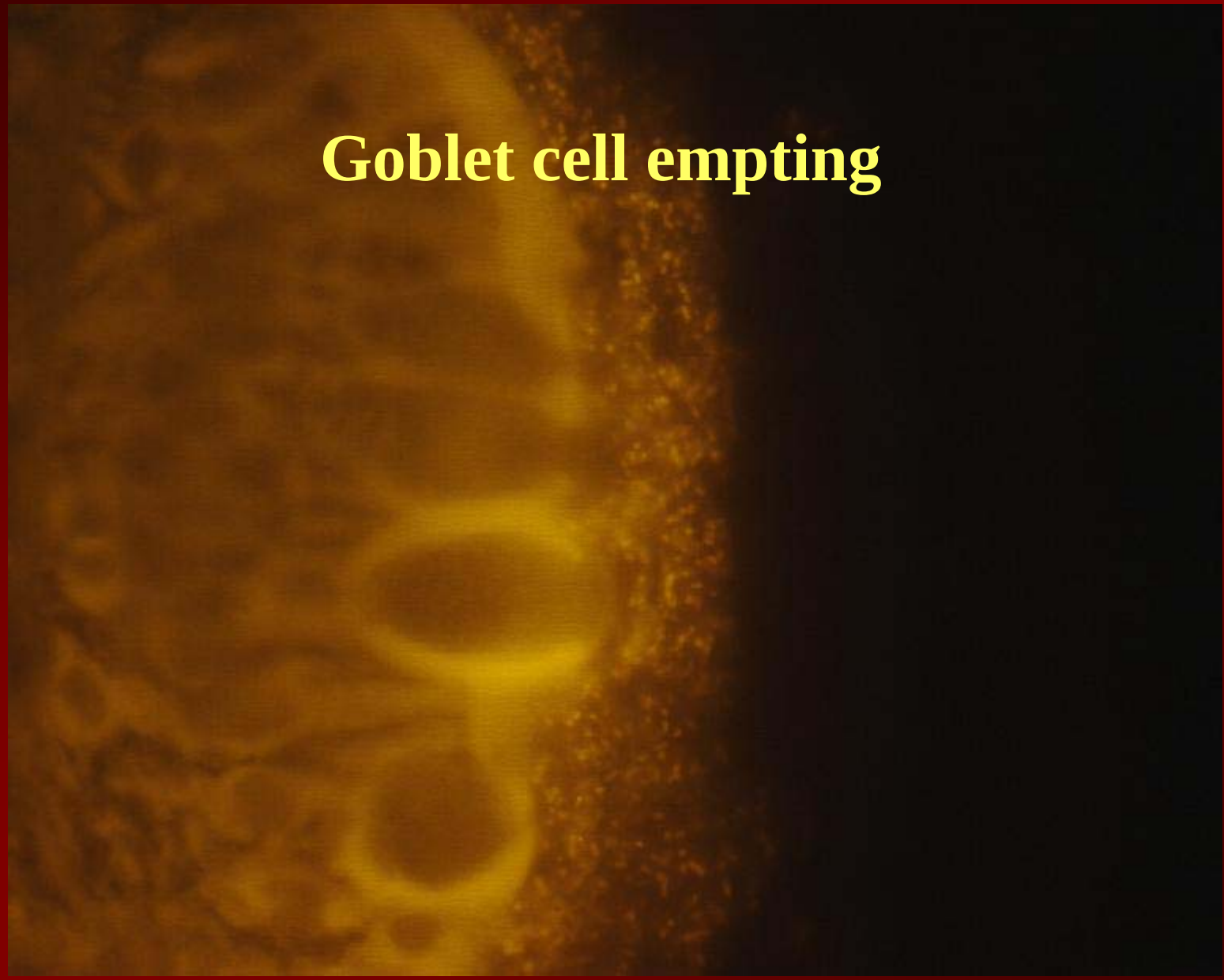

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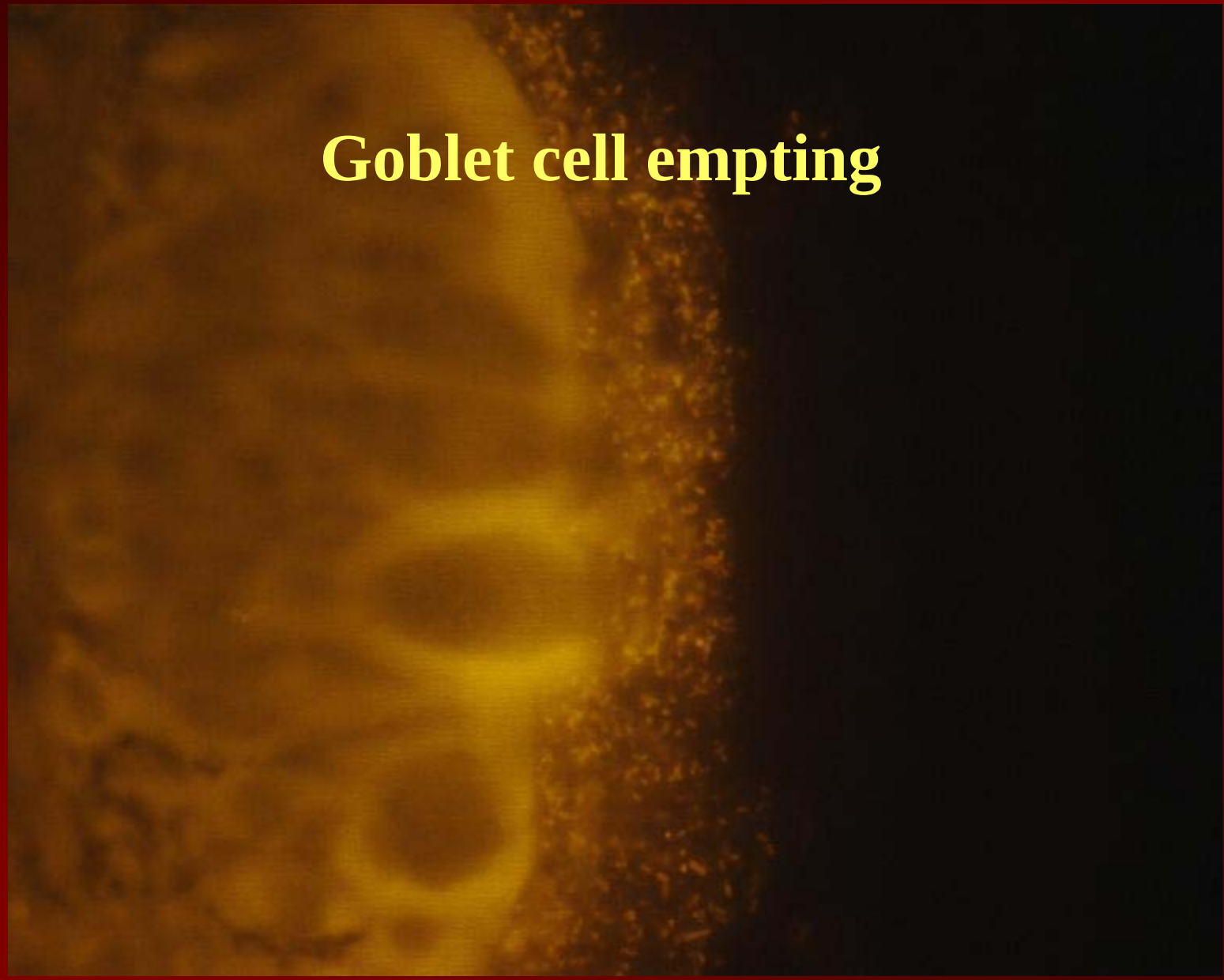

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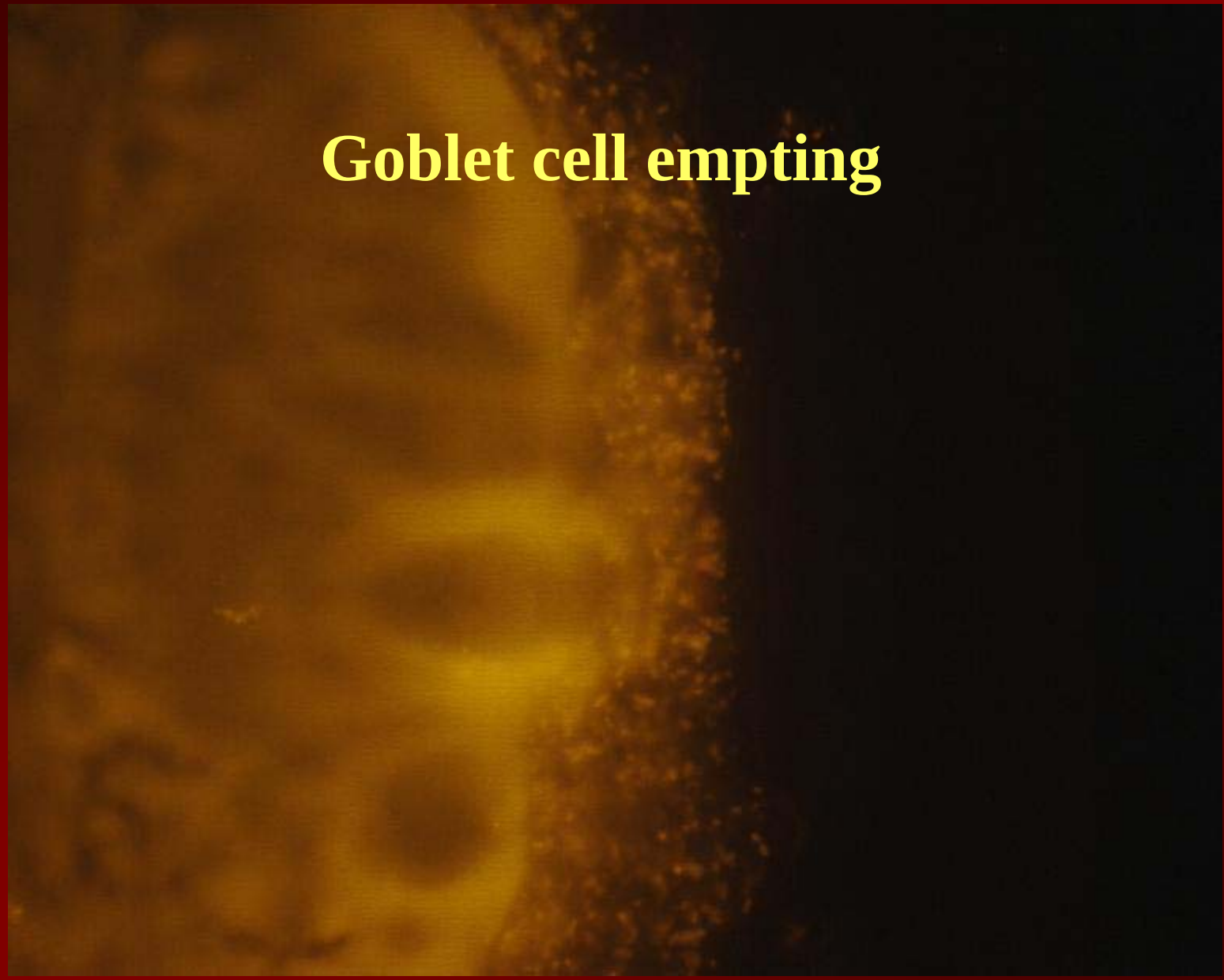
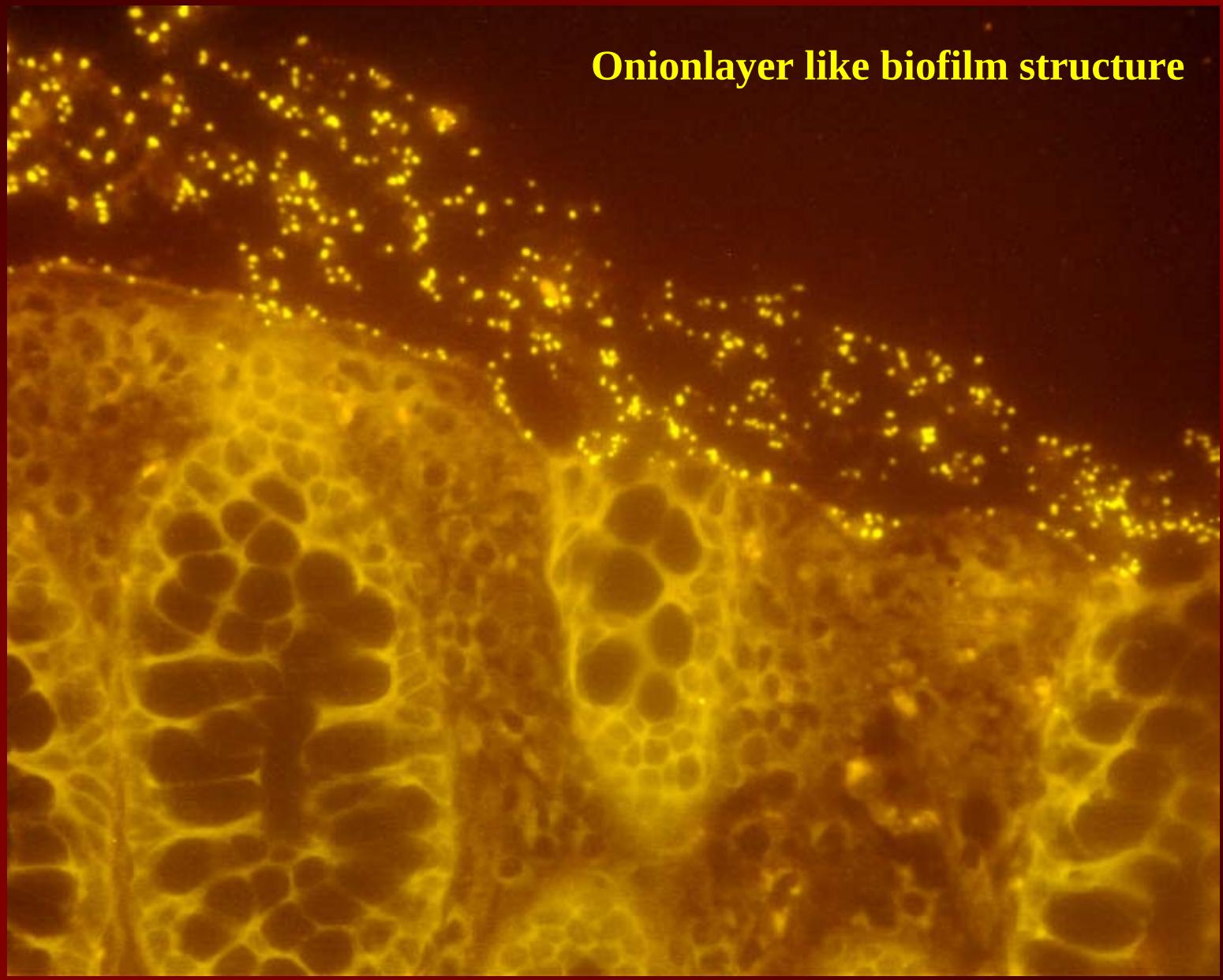



Onionlayer like biofilm structure

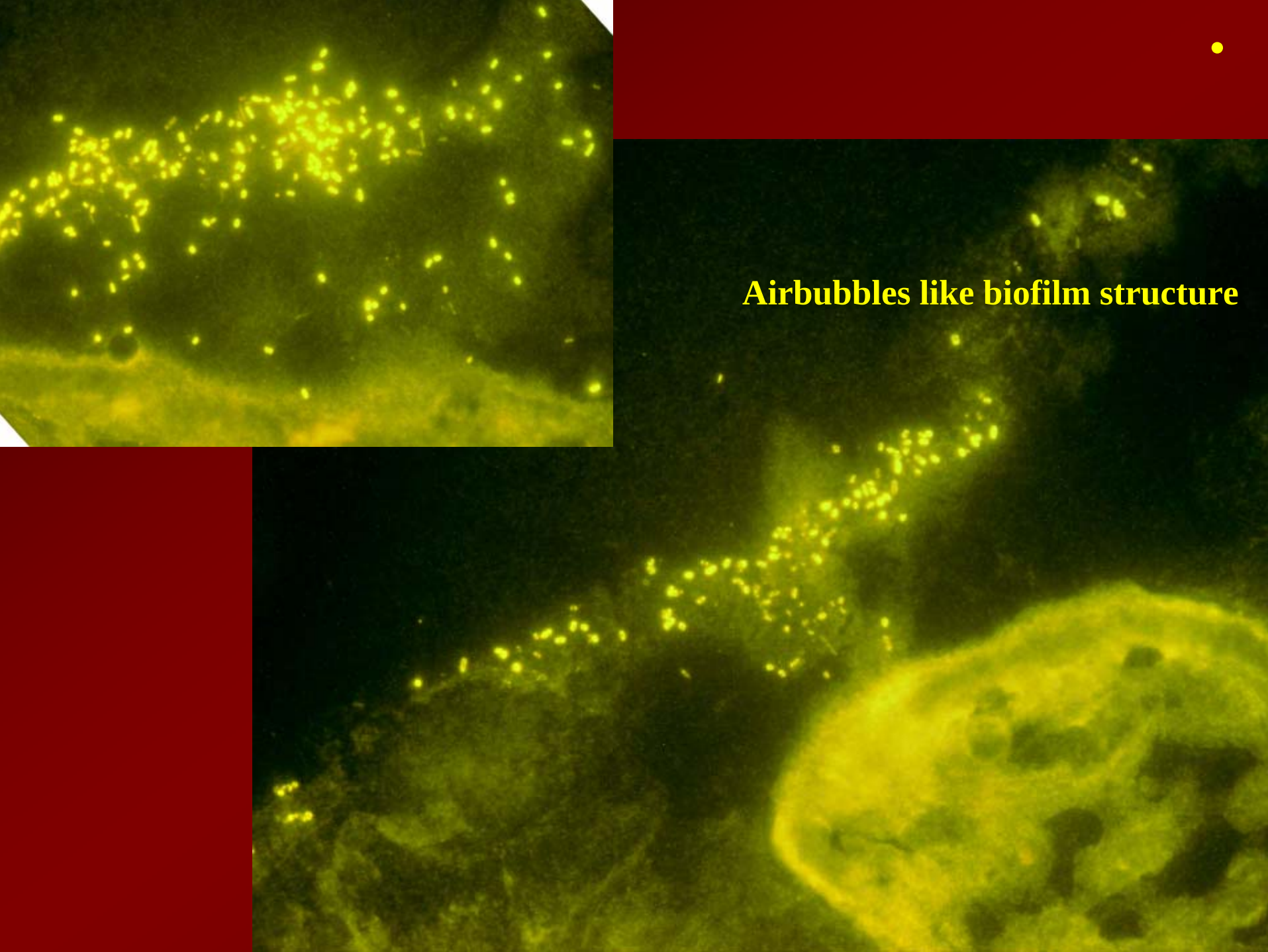
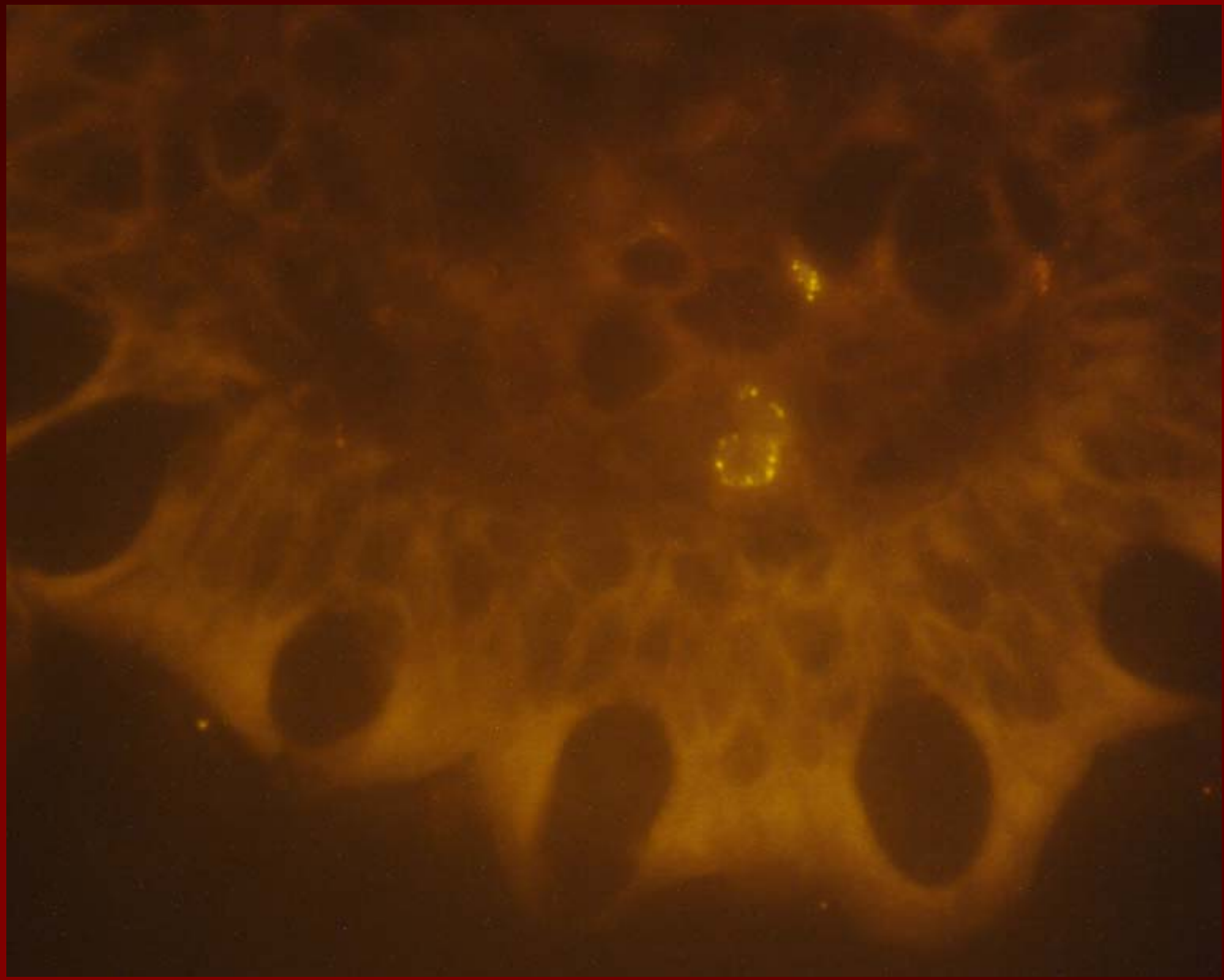
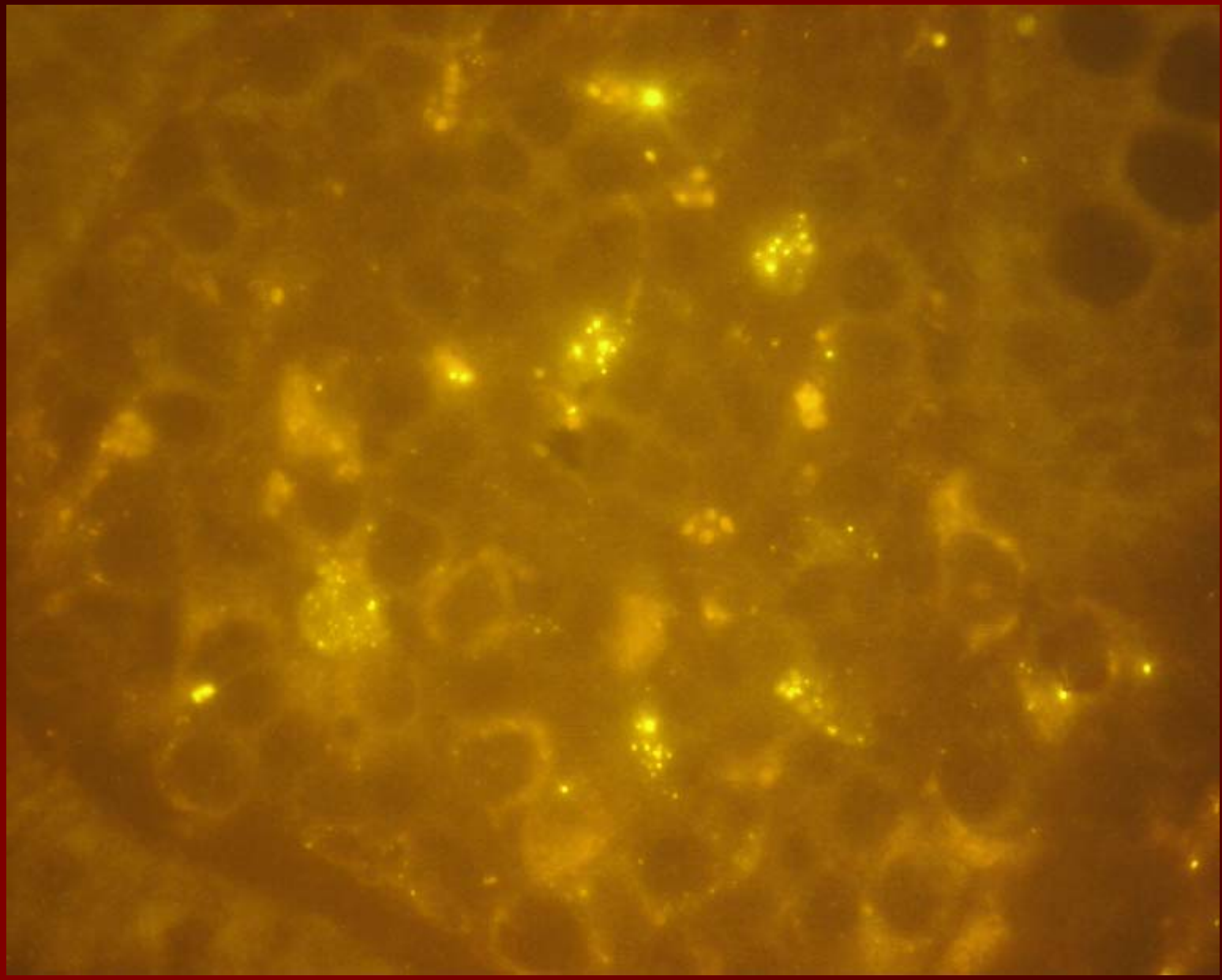

Airbubbles like biofilm structure

**Table 1 FISH probes**

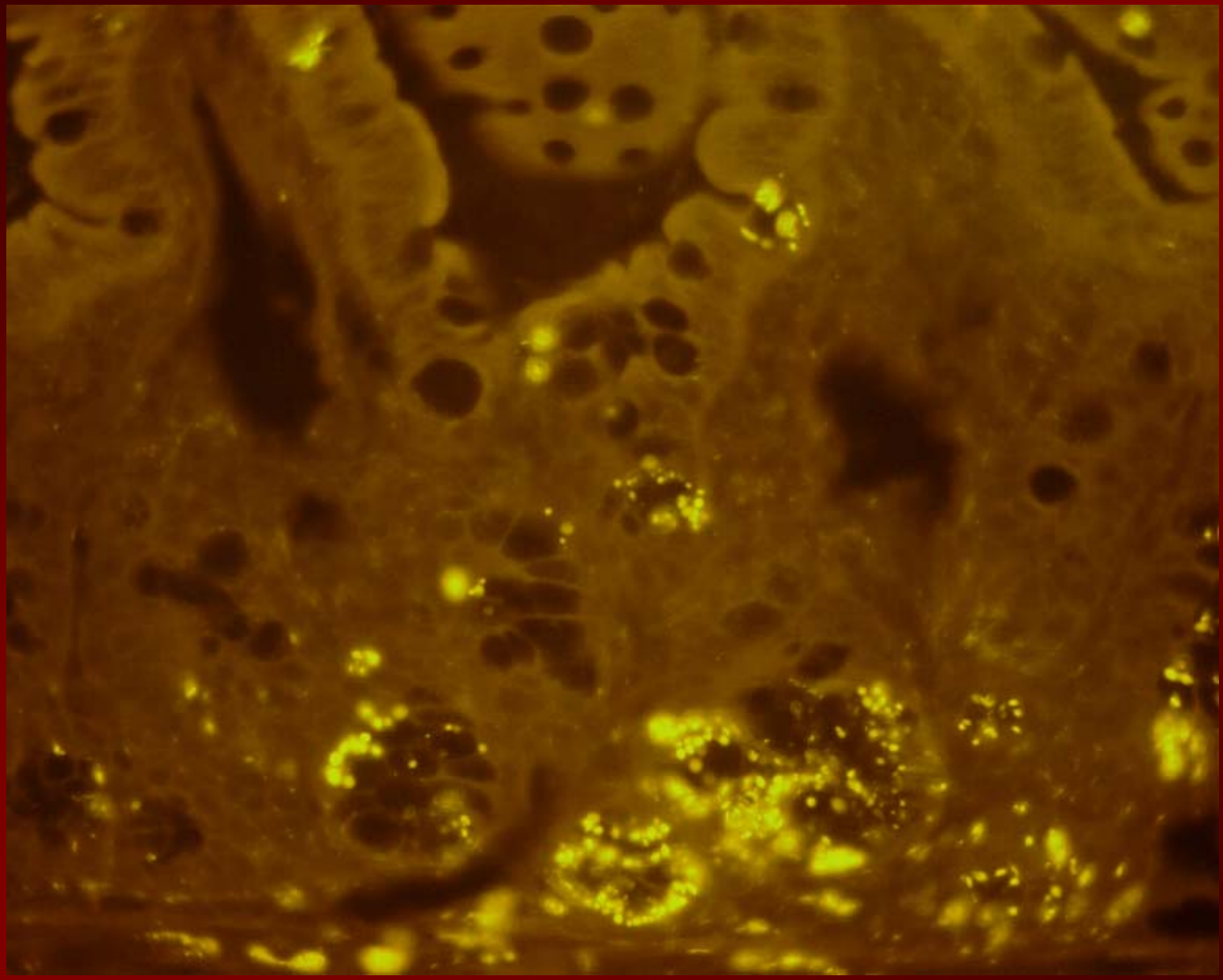
| Name | Target |
|---|---|
| Eub338 | virtually all *Bacteria*, Kingdom (*Eu)Bacteria* |
| Arch915 | *Archaea* |
| Alf1b | Alpha group of *Proteobacteria: Rhodobacter, Acetobacter, Paracoccus,* some *Pseudomonas etc.* |
| Beta42a | Beta subclass of *Proteobacteria: Rhodocyclus, Bordetella, Neisseria,Thiobacillus, 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 |



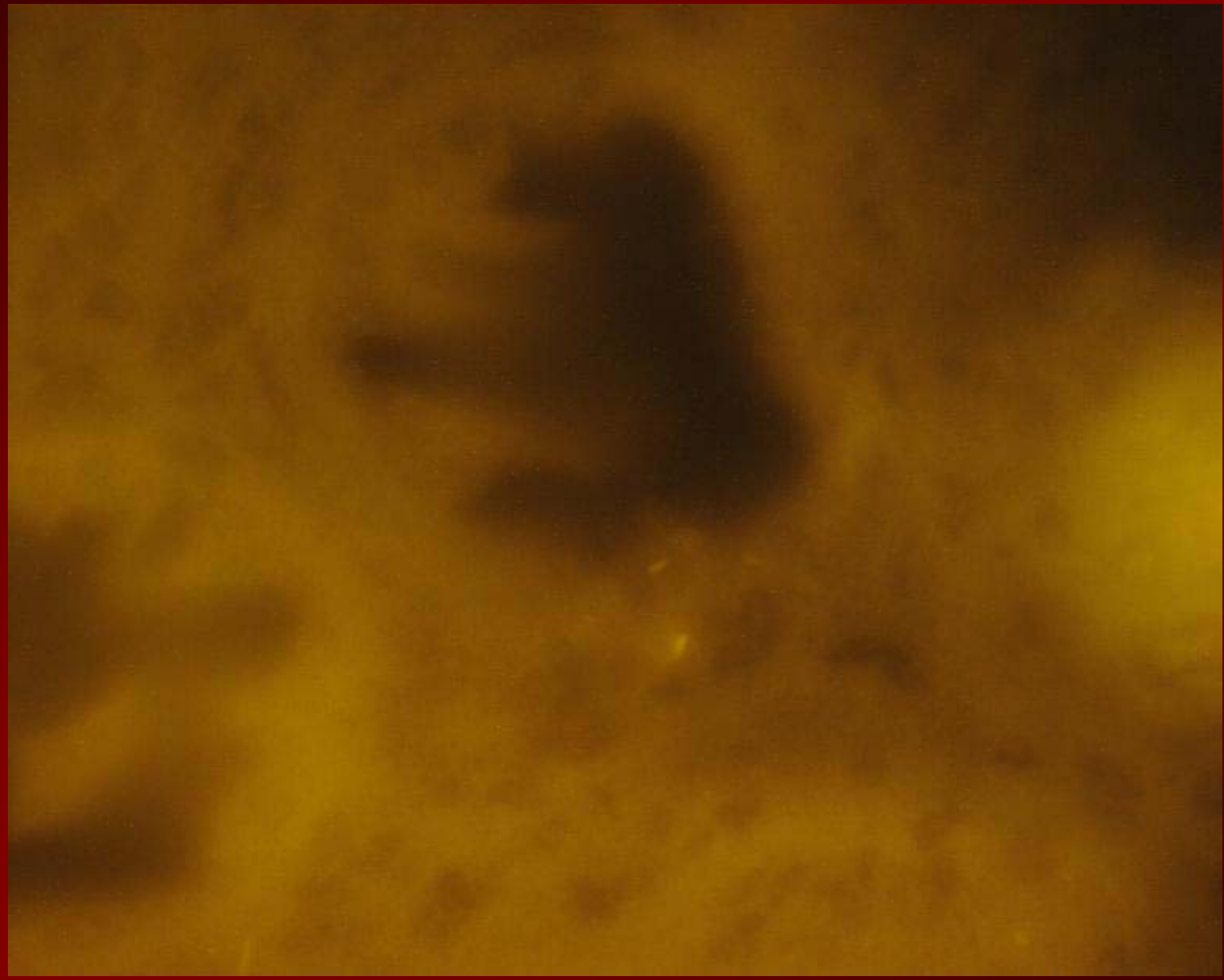
**FISH signals simulating intracellular bacteria**



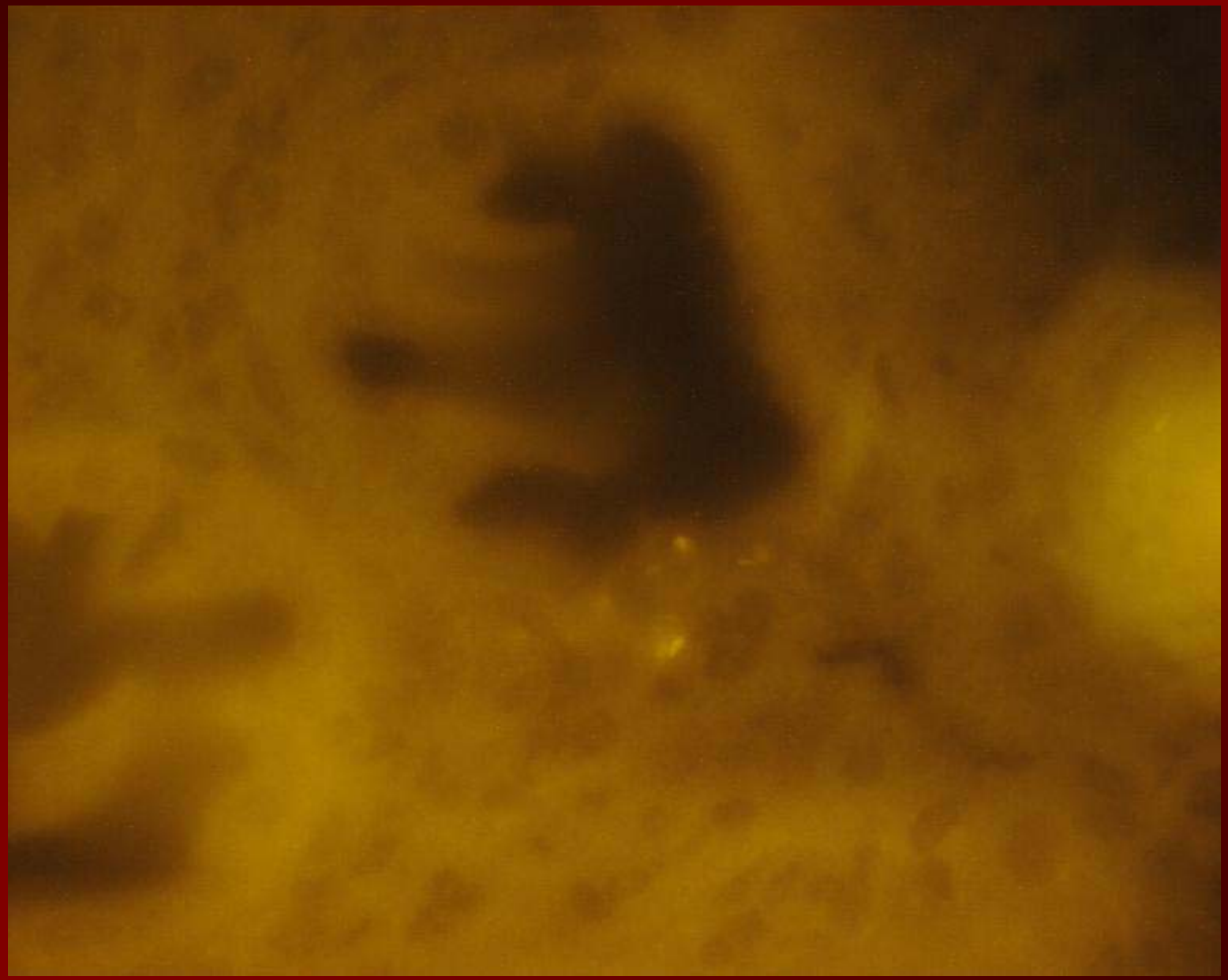
**FISH signals simulating intracellular bacteria**

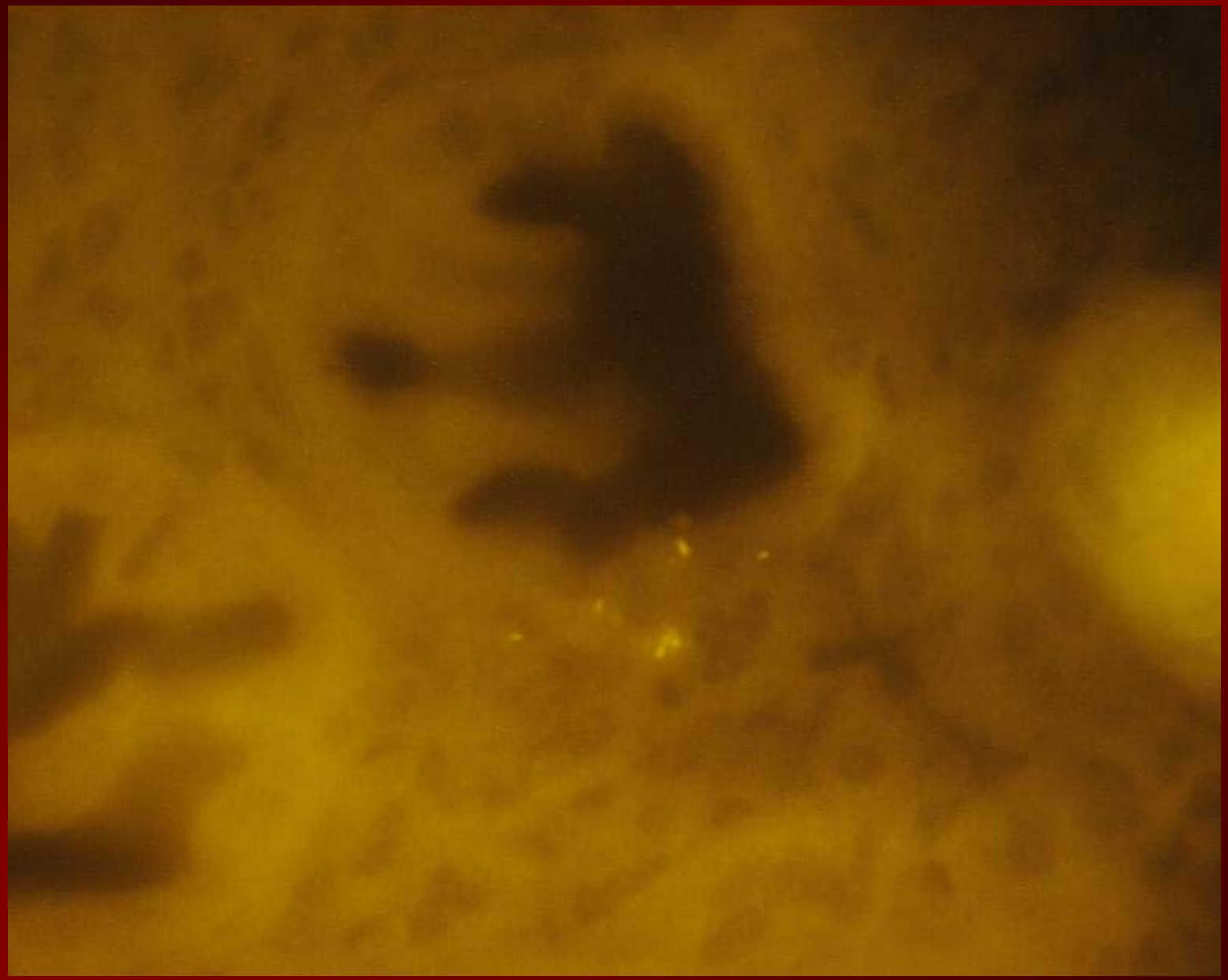


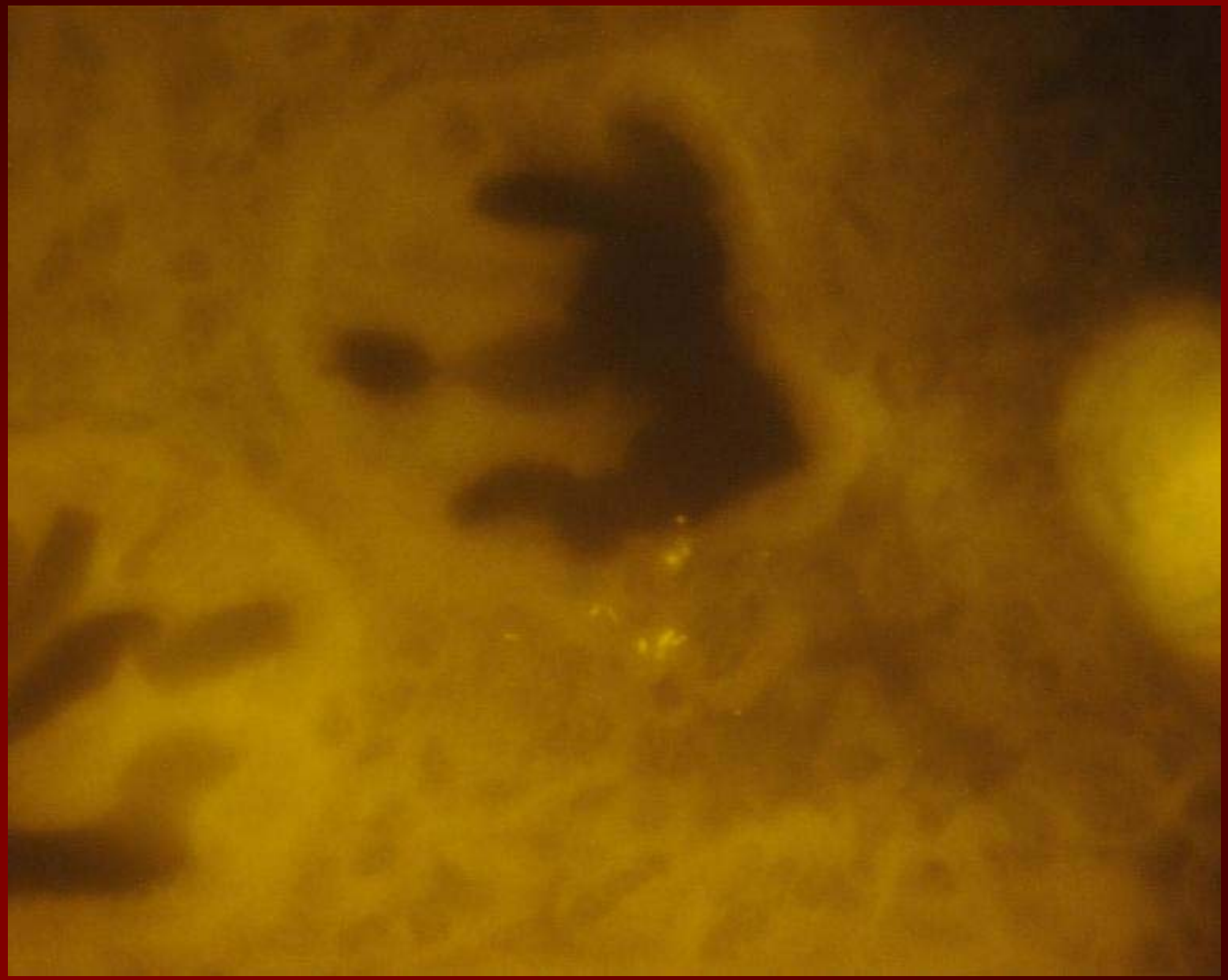
**FISH signals simulating intracellular bacteria (SAMP mouse)**

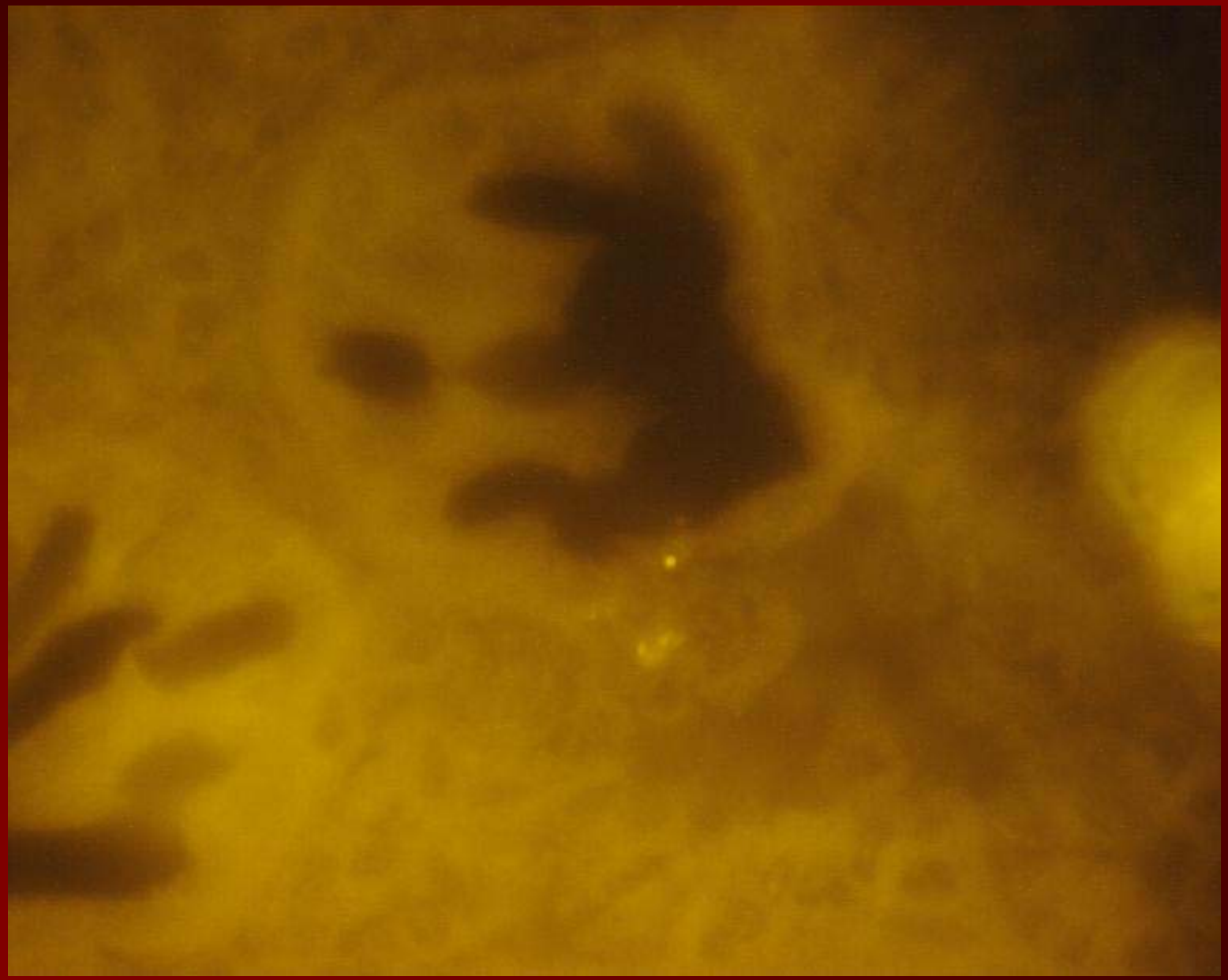


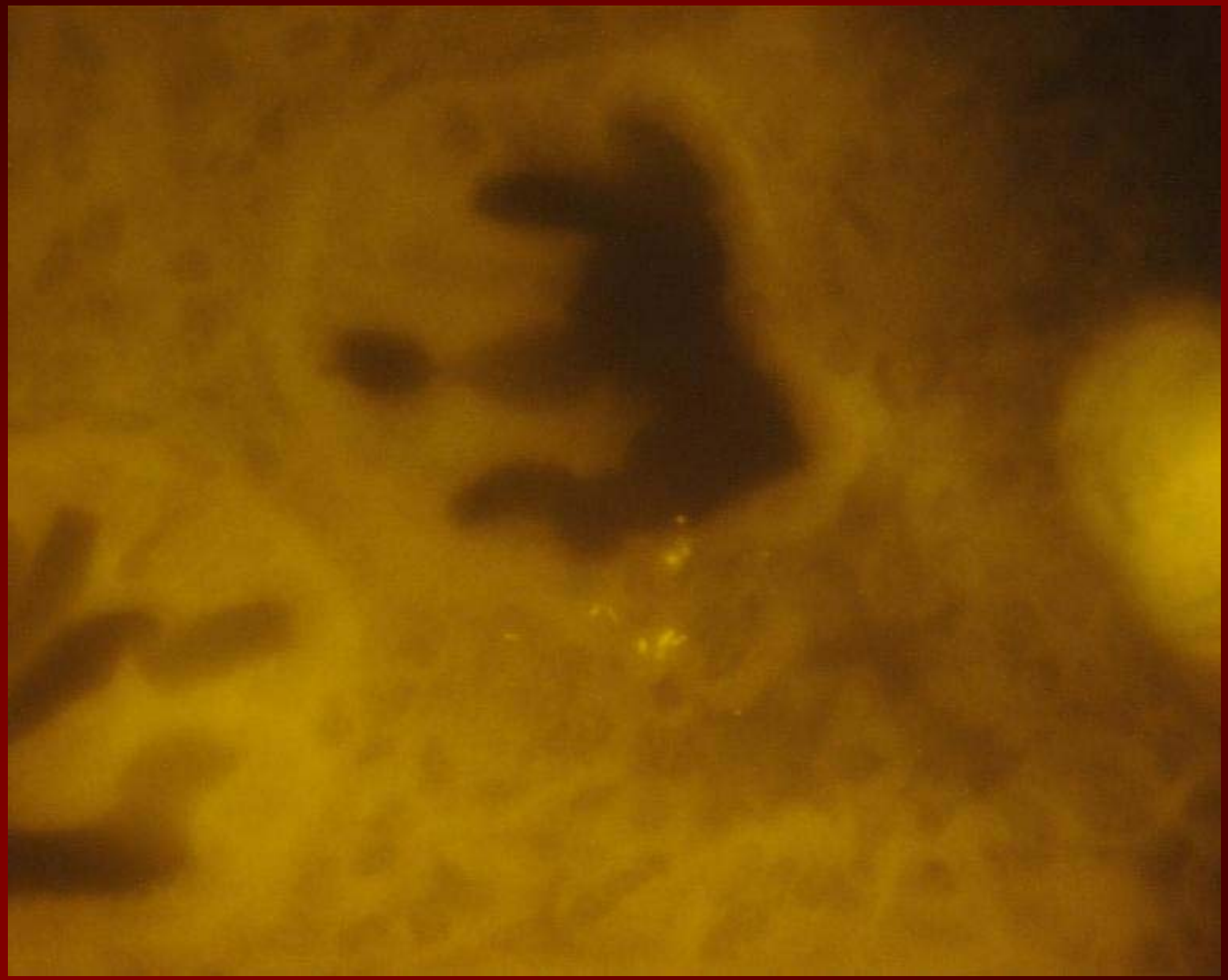
**Intraepithelial bacteria located perinuclear at different levels**

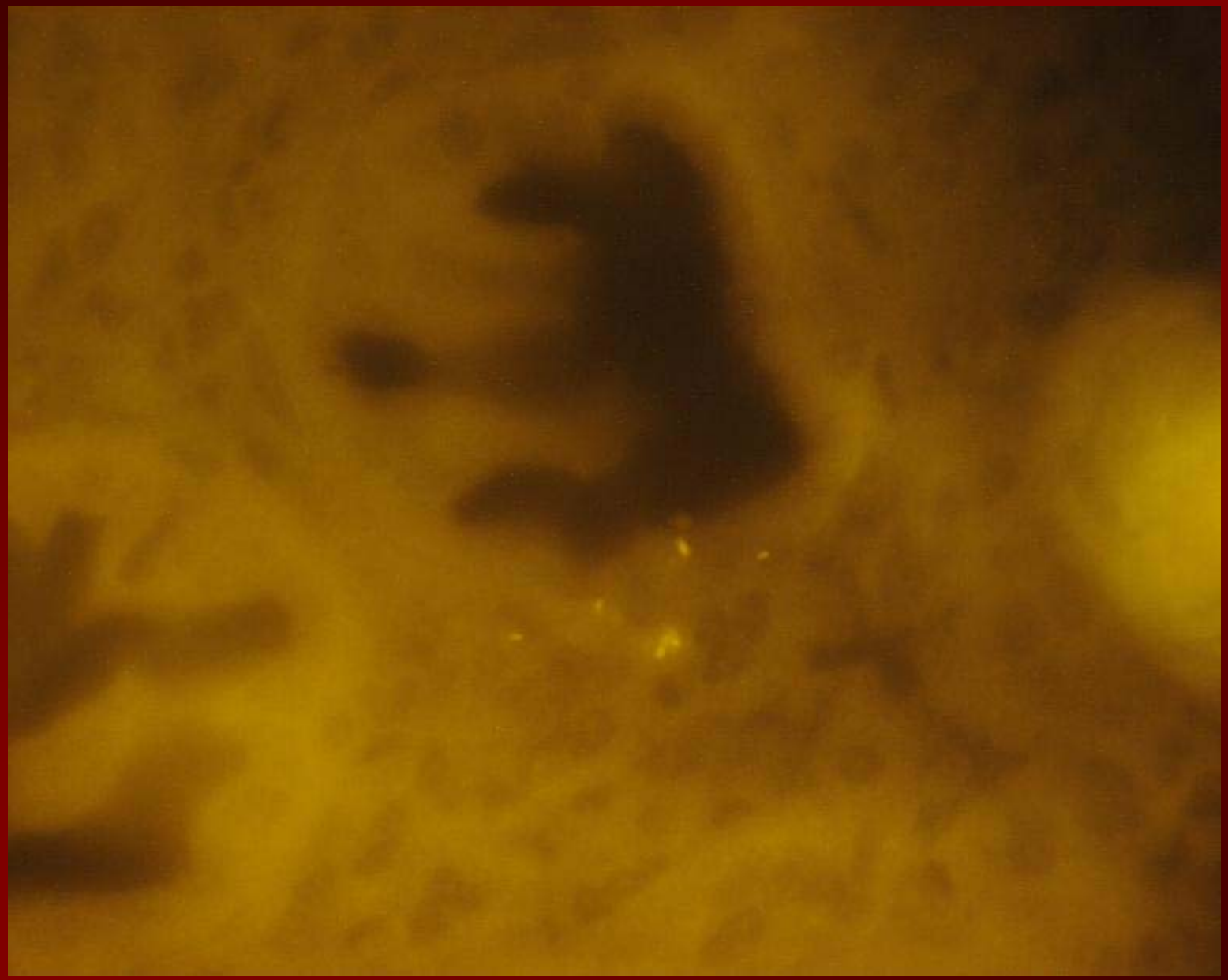


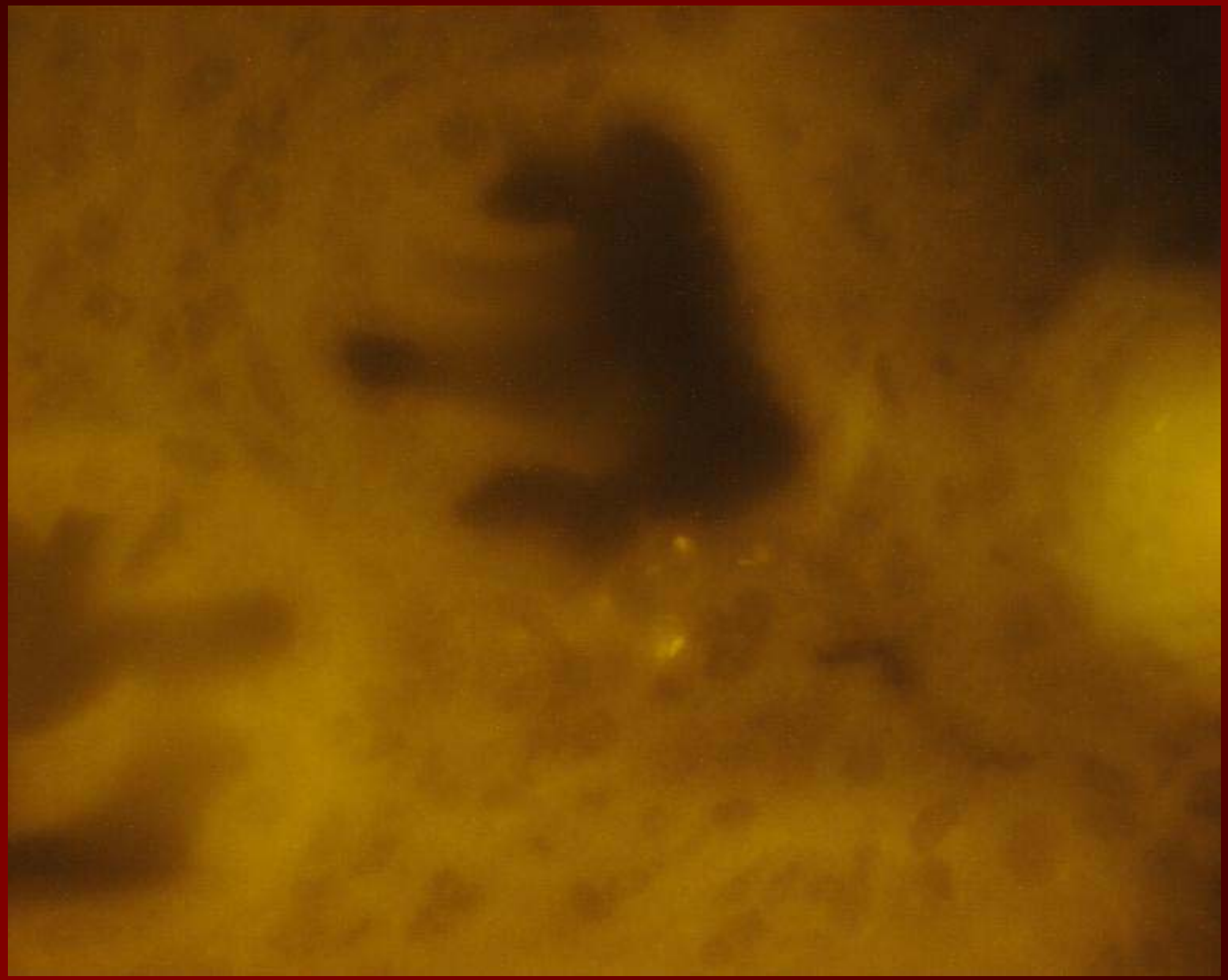


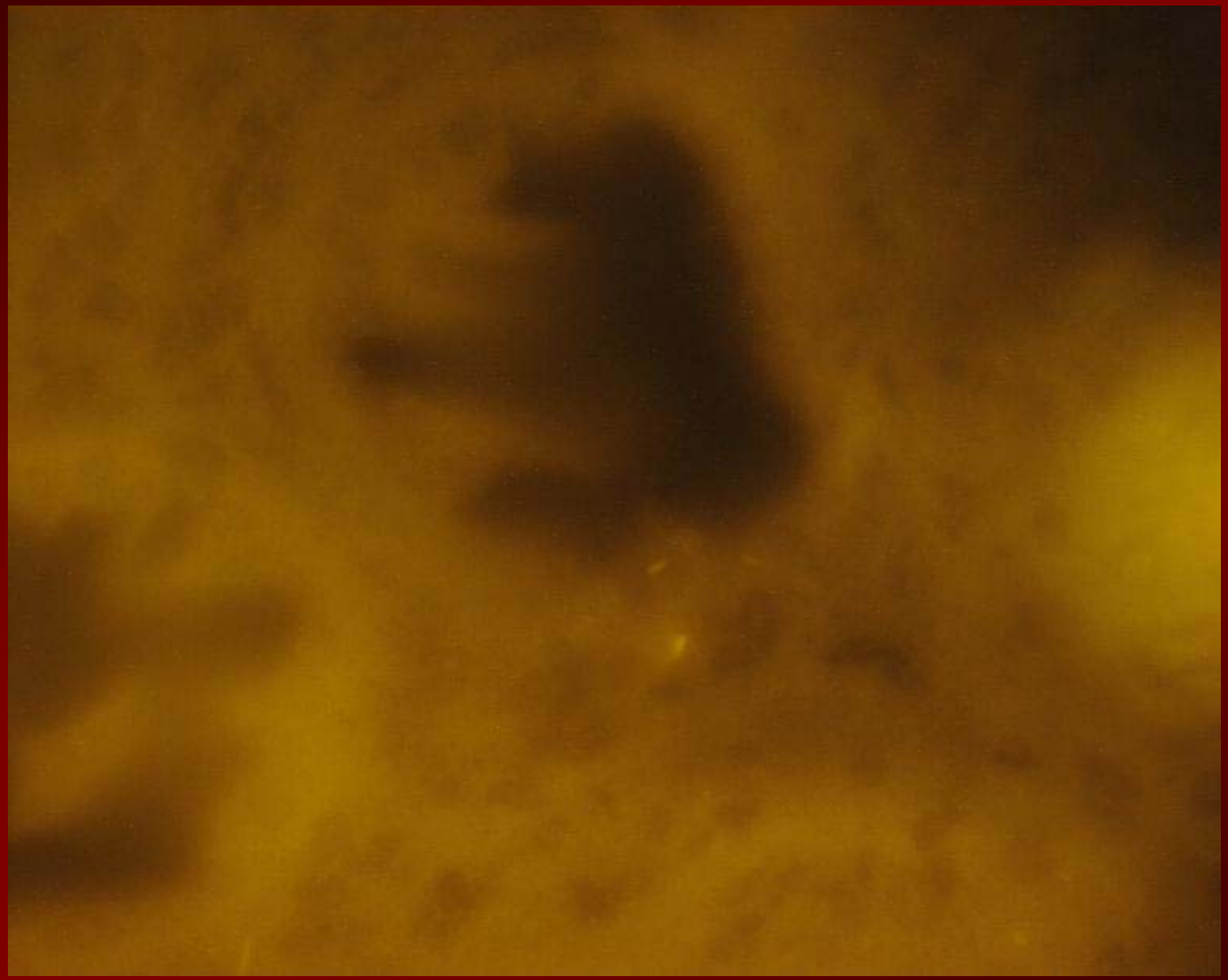


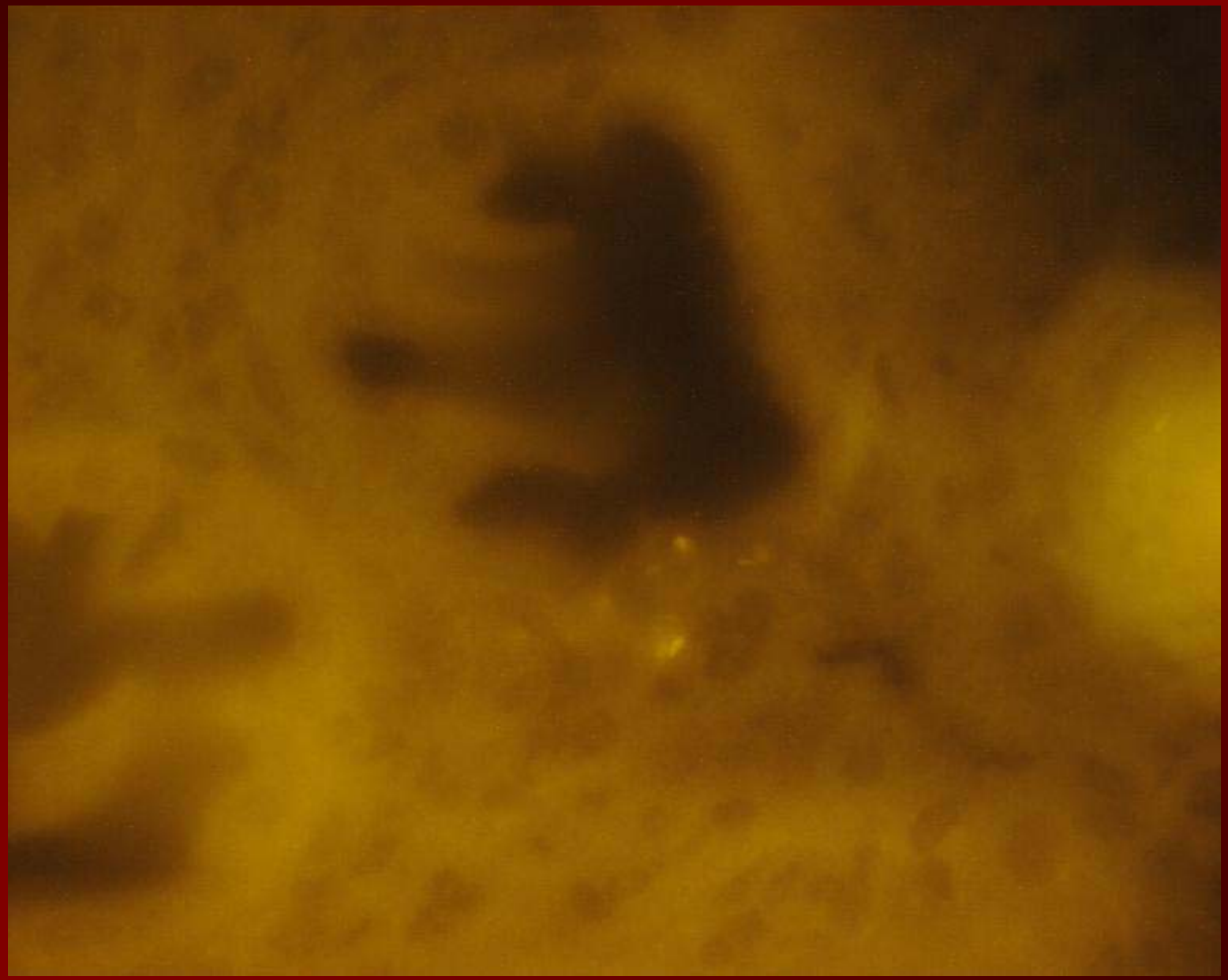


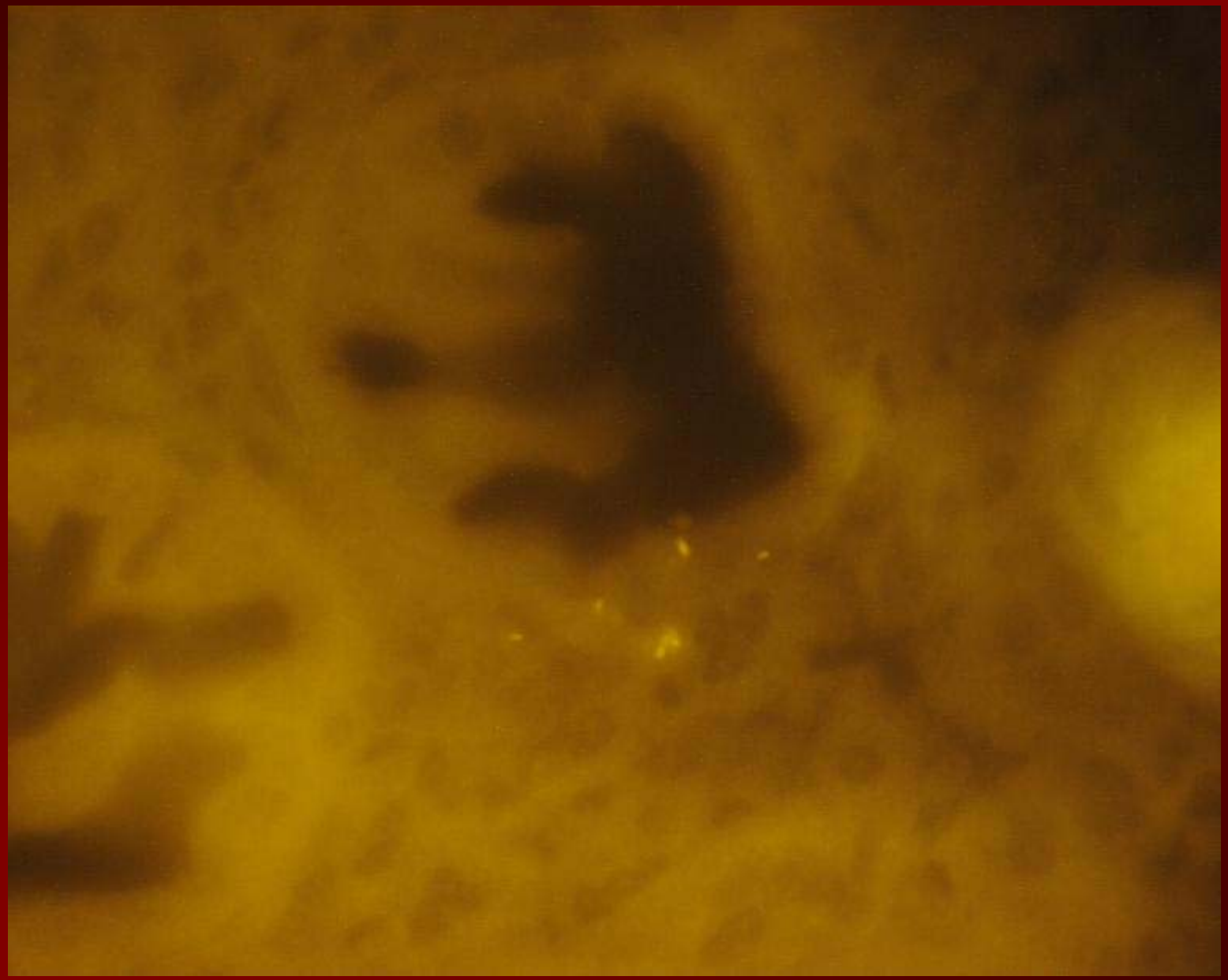


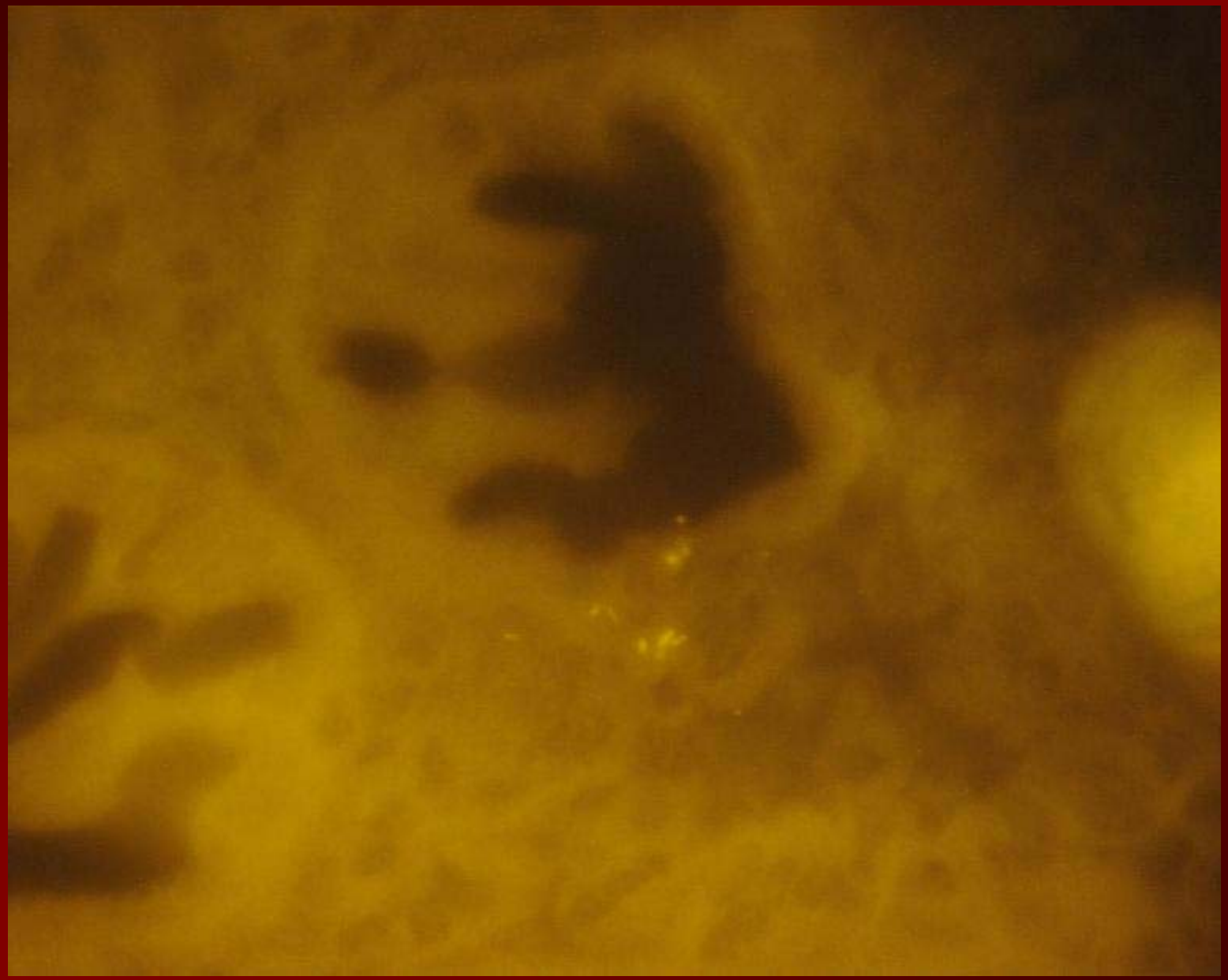


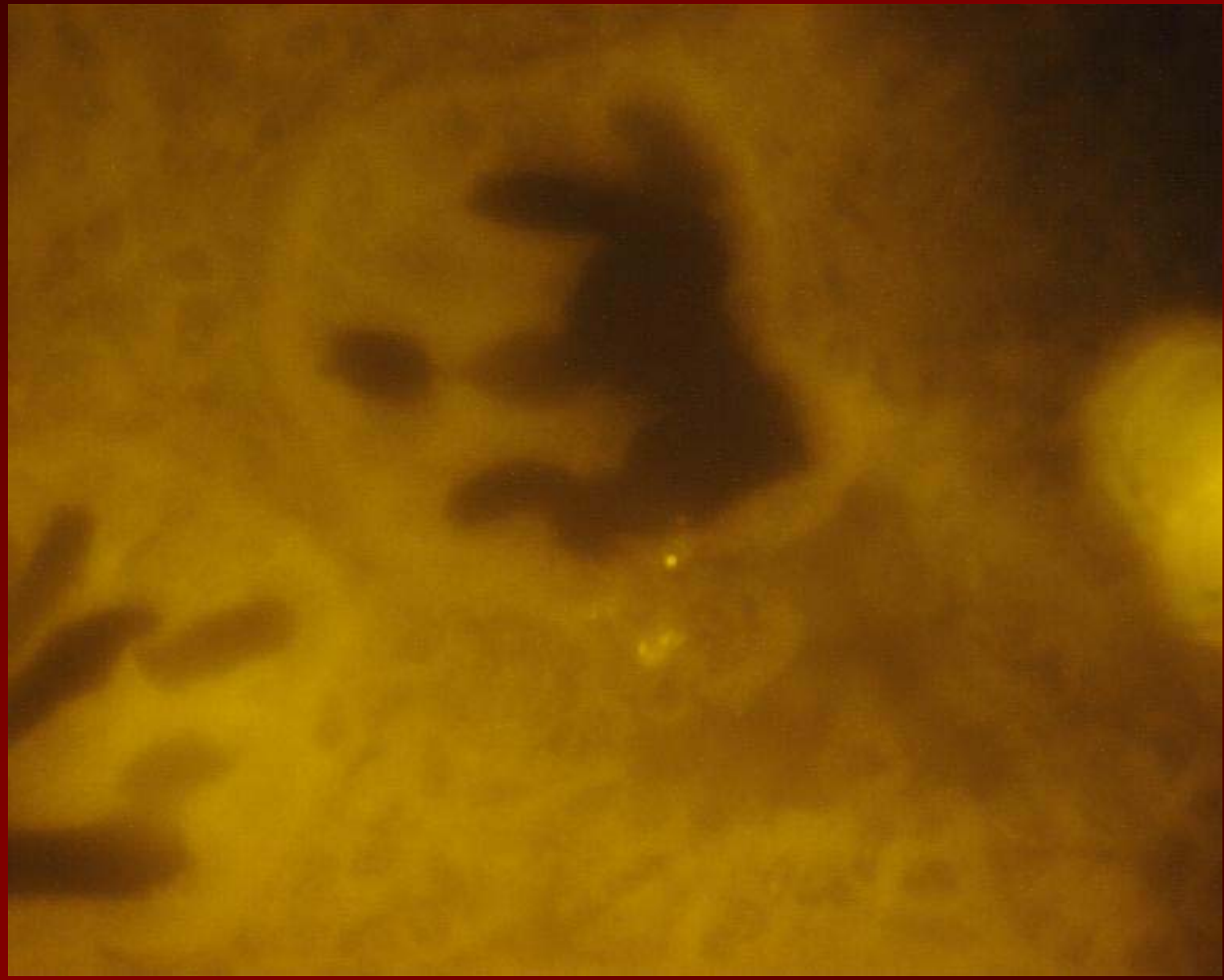












**The mucosa of patients with IBD is covered with a complex spatially structured multispecies biofilm. This biofilm is organized in islands, patches and lawns of multispecies bacteria.**

**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**
**for supporting this project**