

Mucosal Flora in IBD



Charité

Alexander Swidsinski

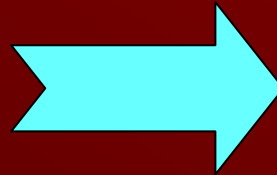
1. Wash

2. Wash

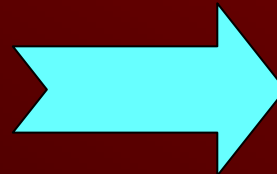
3. Wash

4. Wash

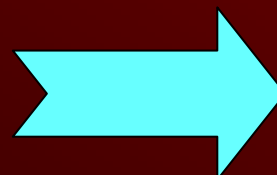
Hypotonic lysis



Superficial bacteria

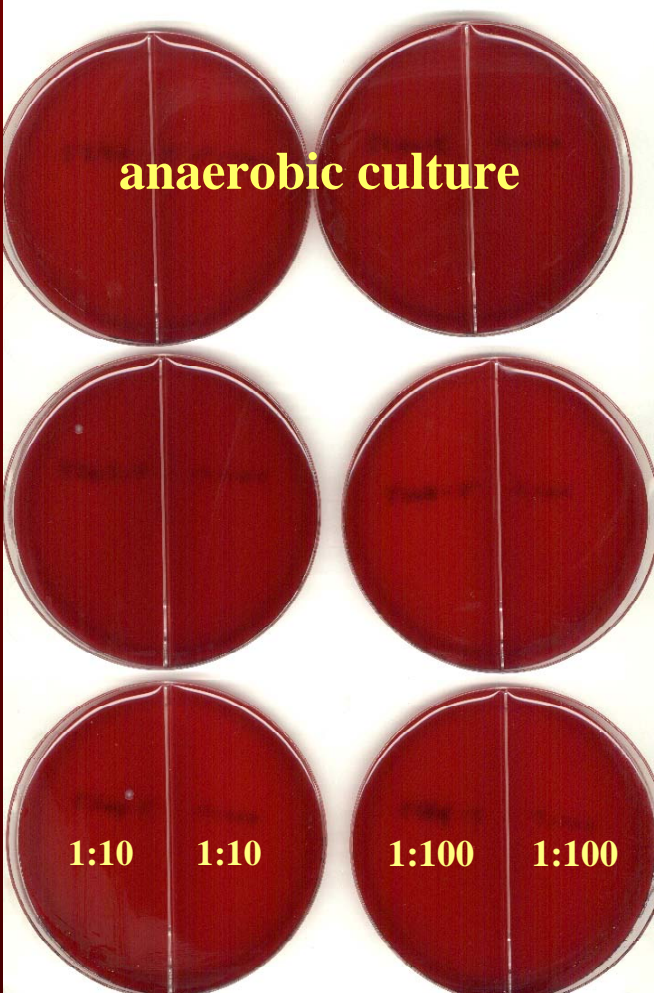
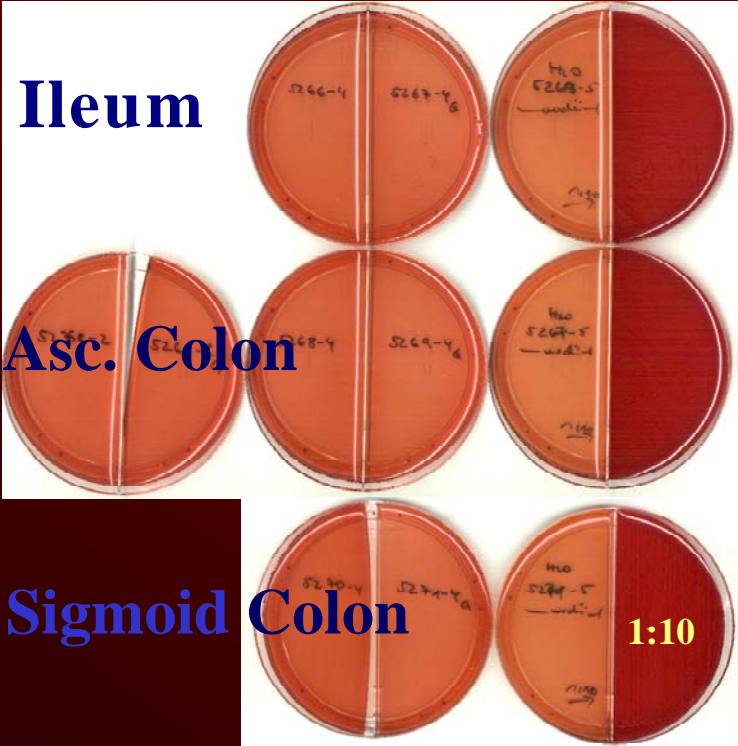


Mucosal bacteria



Mucosal Flora in Asymptomatic Controls

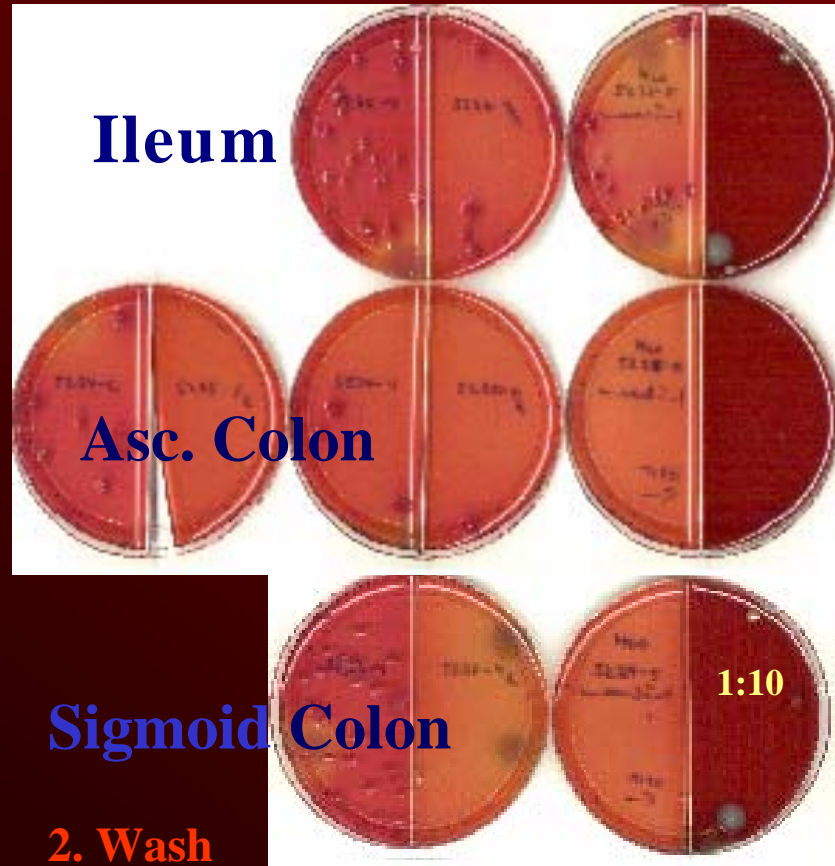
aerobic culture



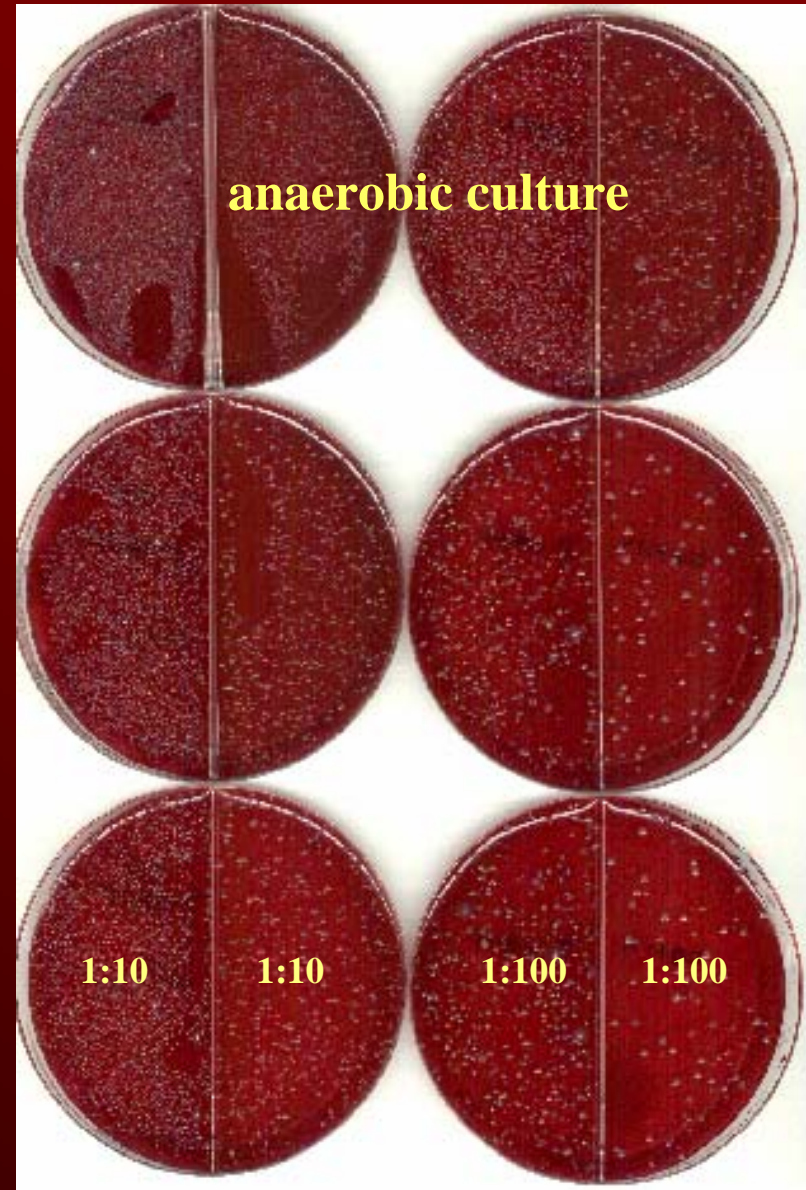
PCR negative

CD

aerobic culture



anaerobic culture



4. Wash

H. Lysis

PCR: 10^8 cfu/ μ L

Occurrence of Cultured Mucosal Bacteria in IBD Patients

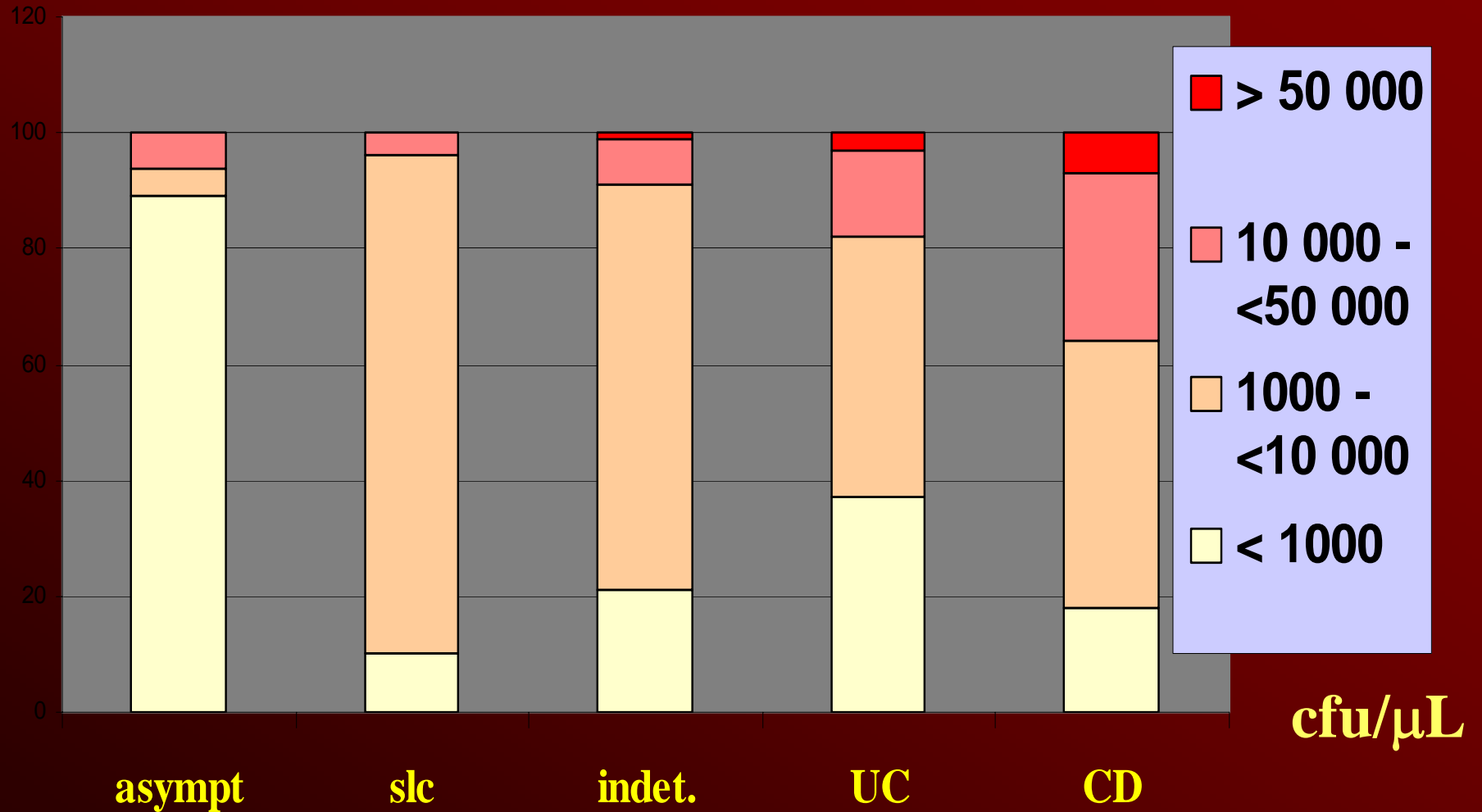
	Percent of patients with isolate of	Frequency of isolates from biopsies (mean/ maximum)
<i>Bacteroides</i>	95	56 (100)
<i>Collinsella aero.</i>	80	16 (92)
<i>Clostridia</i>	62	9 (35)
<i>Bifidobacteria</i>	34	6 (87)
<i>Peptostreptococci</i>	32	3 (24)
<i>Eubacteria</i>	23	3 (28)
<i>Propionibacteria</i>	15	<1 (9)
<i>Enterobacteriaceae</i>	82	4 (100)
other	90	3 (89)

Mean± SD (x10³ cfu/μL) of Mucosal Bacteria

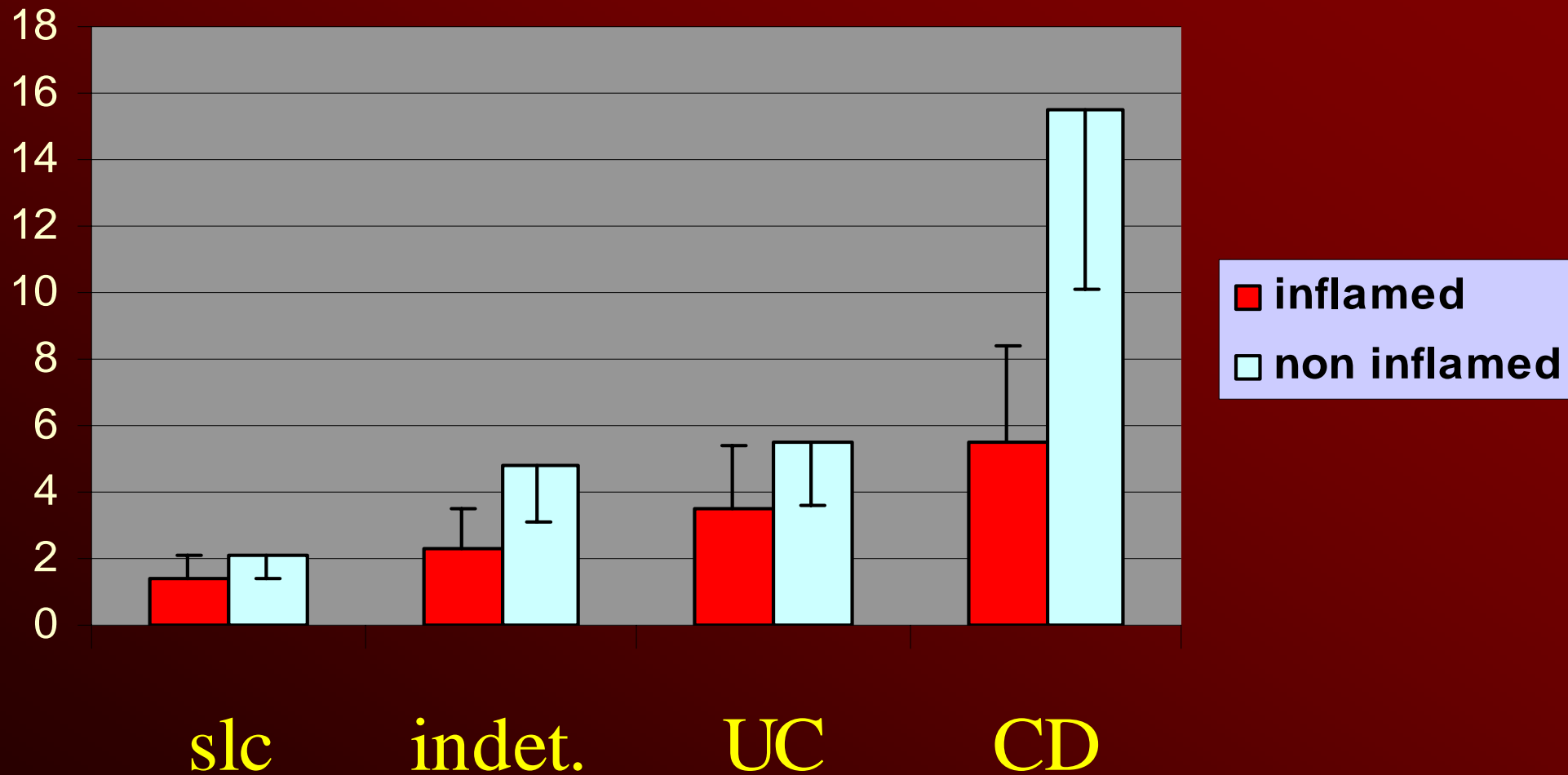
	Asymptomatic Controls (n=40)	Self-limiting Colitis (n=28)	Indeterminate Colitis (n=104)	UC (n=156)	CD (n=82)
Total anaerobes	0.18±0.3	1.8±5.3 NS	3.41±16 <i>P</i> < 0.08	3.8±11 <i>P</i> < 0.01	9.1±18 <i>P</i> < 0.001
<i>Bacteroides</i>	0.02±0.05	0.26±0.6 NS	0.64±2.1 <i>P</i> < 0.01	1.4±9 <i>P</i> < 0.001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

P as compared to controls

Percent of Patients with Concentrations of Mucosal Bacteria of



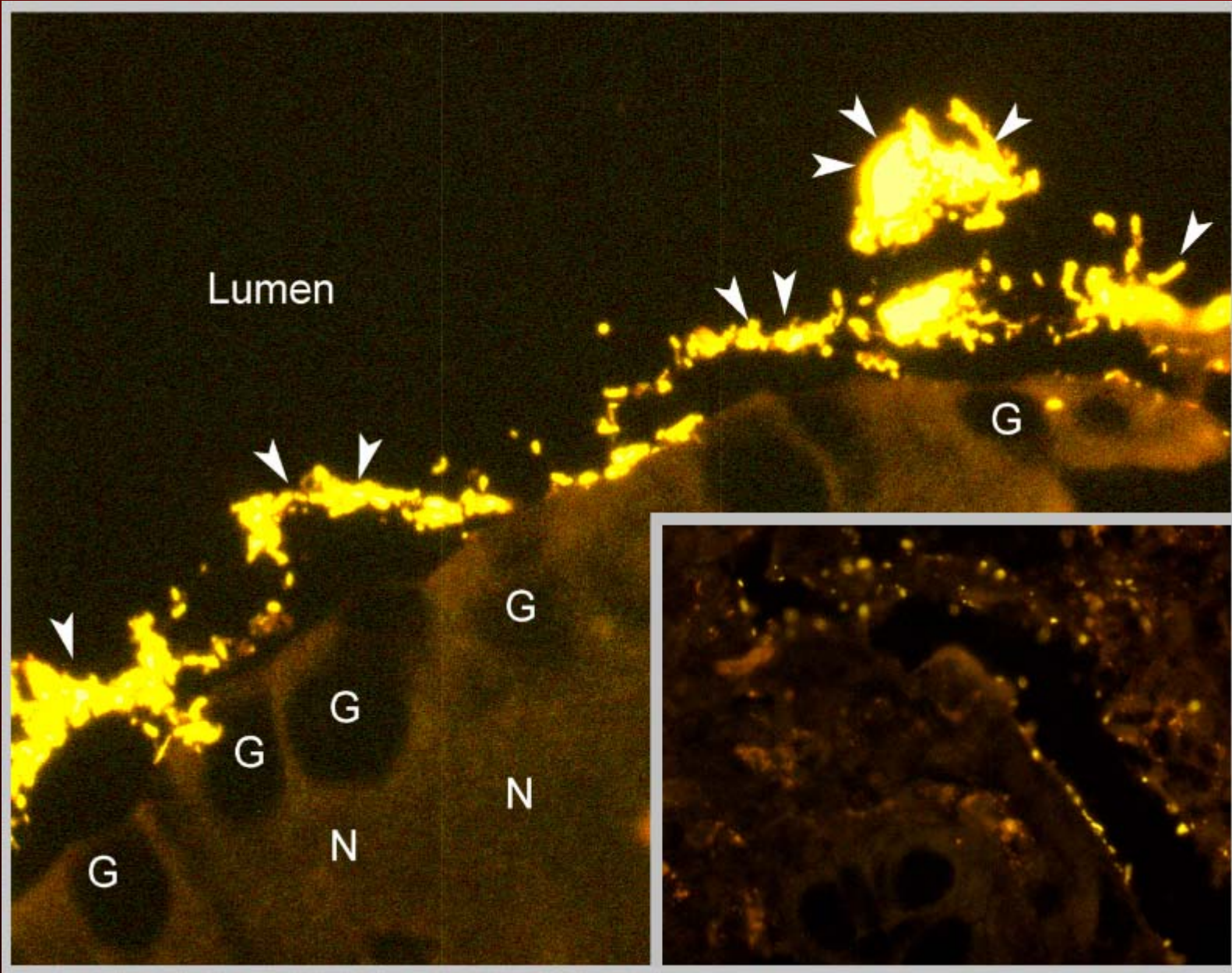
Mean $\times 10^3$ cfu/ μ L Mucosal Bacteria in Inflamed and Non-Inflamed Mucosa



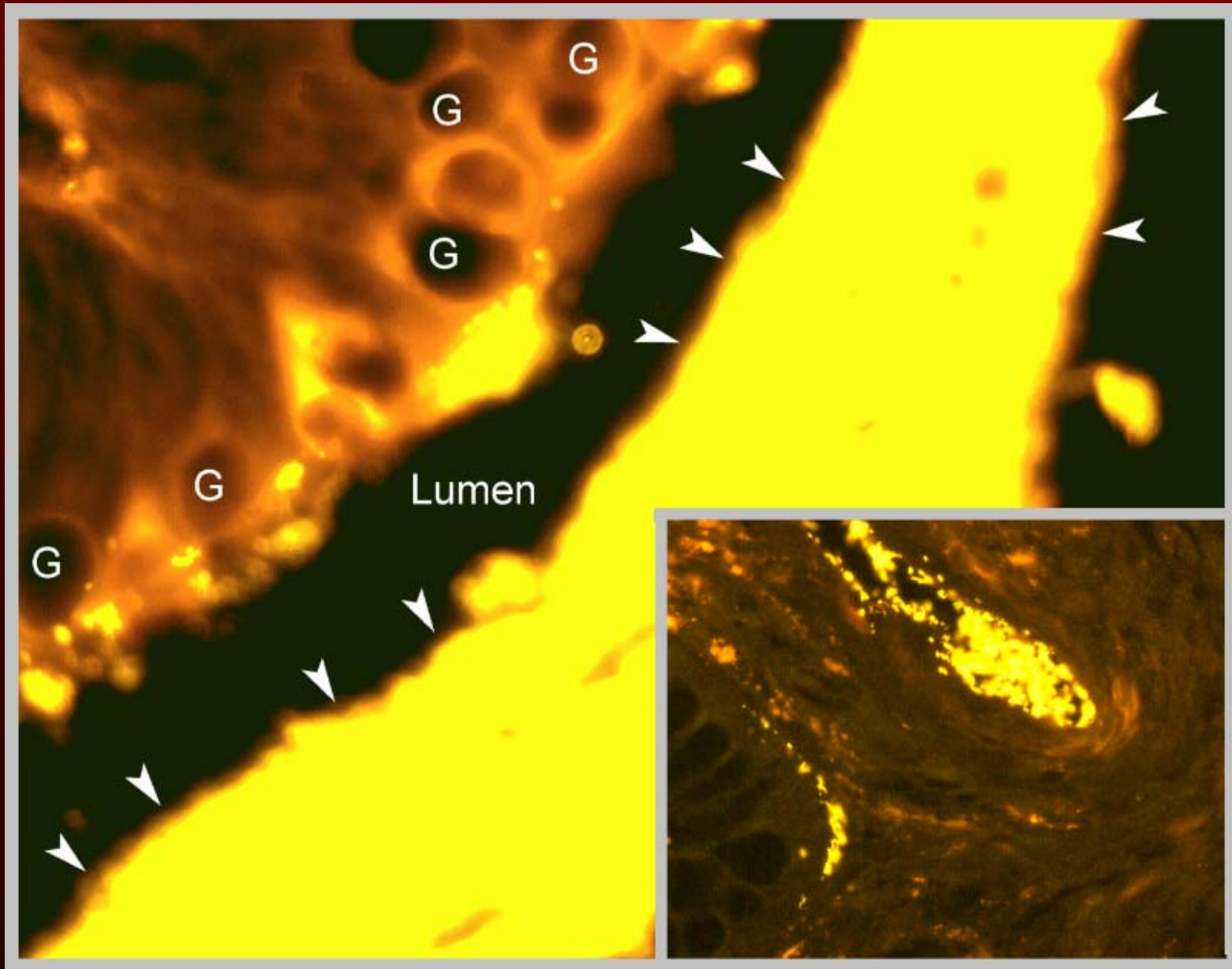
Percentage of Patients with Positive PCR (> 1000 cfu/μL) and the Predominance of Sequences of a Single Bacterial Group

	Asymptomatic controls (n=20)	Self-limiting Colitis (n=18)	Indeterminate colitis (n= 17)	UC (n=14)	CD (n= 10)
Mucosal bacteria (positive PCR)	10%	83%	71%	71%	80%
<i>E.coli</i>	5%	27%	29%	21%	20%
<i>Bacteroides</i>	0%	11%	12%	16%	30%
Mix of sequences*	5%	33%	29%	34%	30%
Other (<i>Yersinia</i> and <i>Campylobacter</i>)	0%	11%	0%	0%	0%

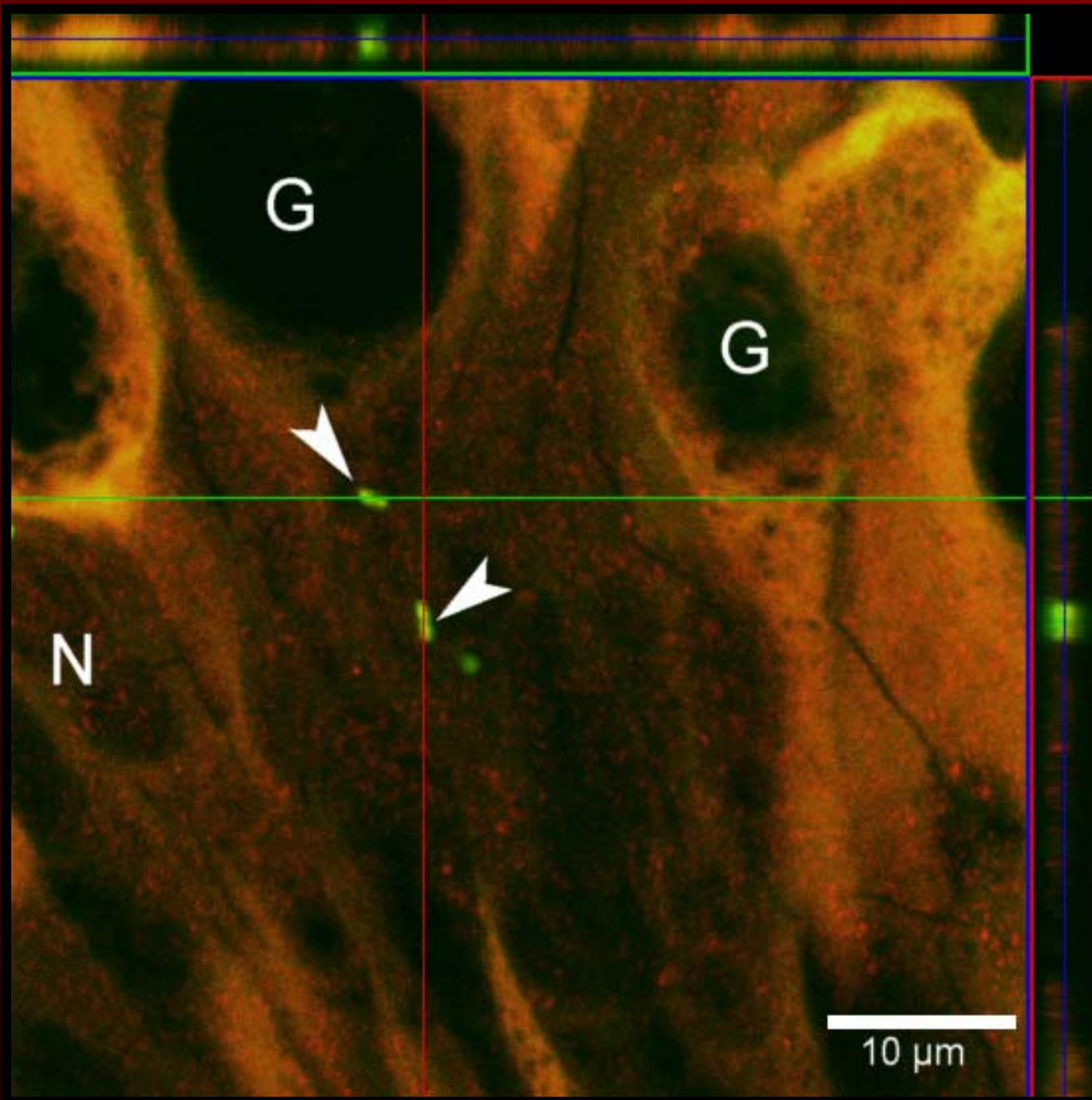
* High diversity of sequences, without any sequence appearing in more than 5% of the analyzed clones



Fluorescence in-situ hybridization



Fluorescence in-situ hybridization



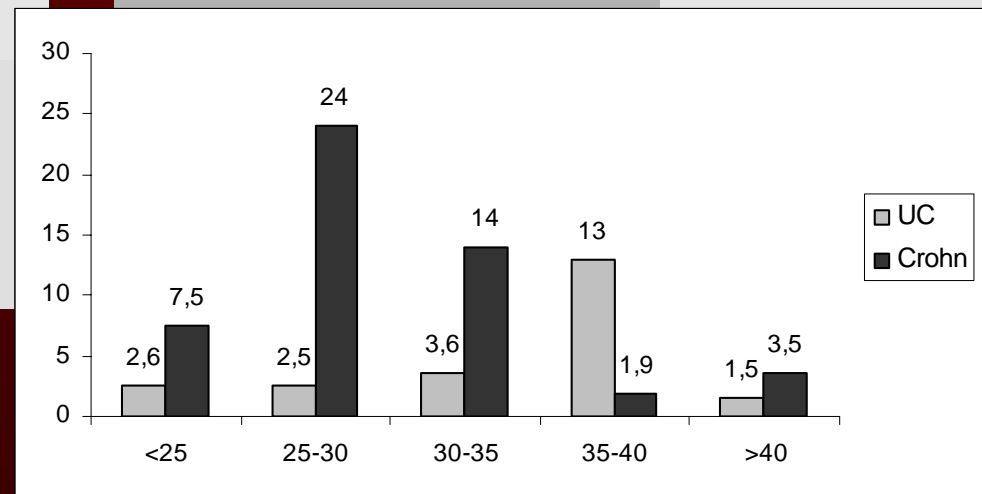
**Intracellular
bacteria (*arrows*)
visualized with
Bacteroides
specific probe**

**G= goblet cell
N= nucleus**

Mean \pm SD ($\times 10^3$ cfu/ μ L) of mucosal bacteria and clinical data

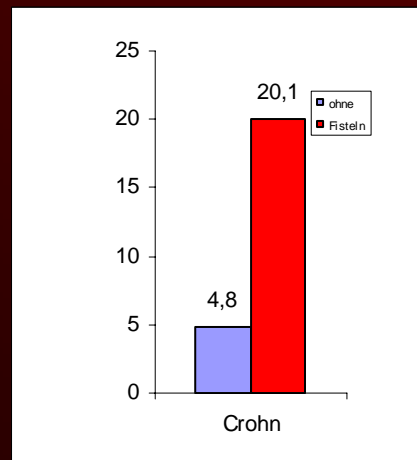
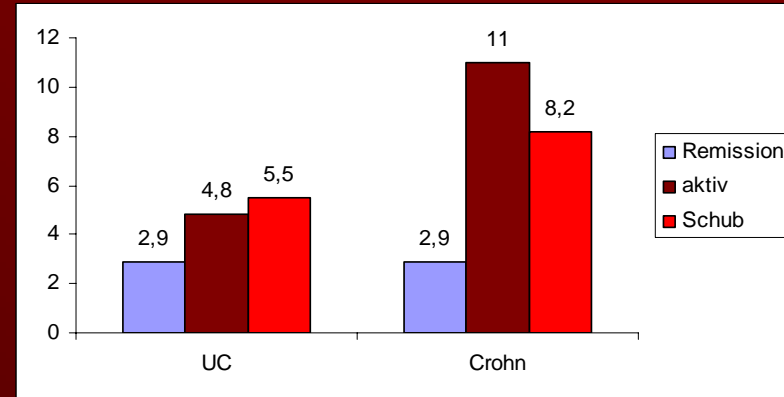
	UC (n=156)	CD (n=82)
All	3.8	9.1
patients age		
<25	2.0	2.6
25-30	1.7	9.1
30-35	4.5	11.9
35-40	3.4	10.8
40-45	3.2	4.3
45-50	6.9	5.5
>50	2.6	5.2
age at the time of manifestation		
<25	2.6	7.5
25-30	2.5	24.0
30-35	3.6	14
35-40	13	1.9
>40	1.5	3.5

	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



Mean ± SD (x10³ cfu/μL) of mucosal bacteria, disease activity and therapy

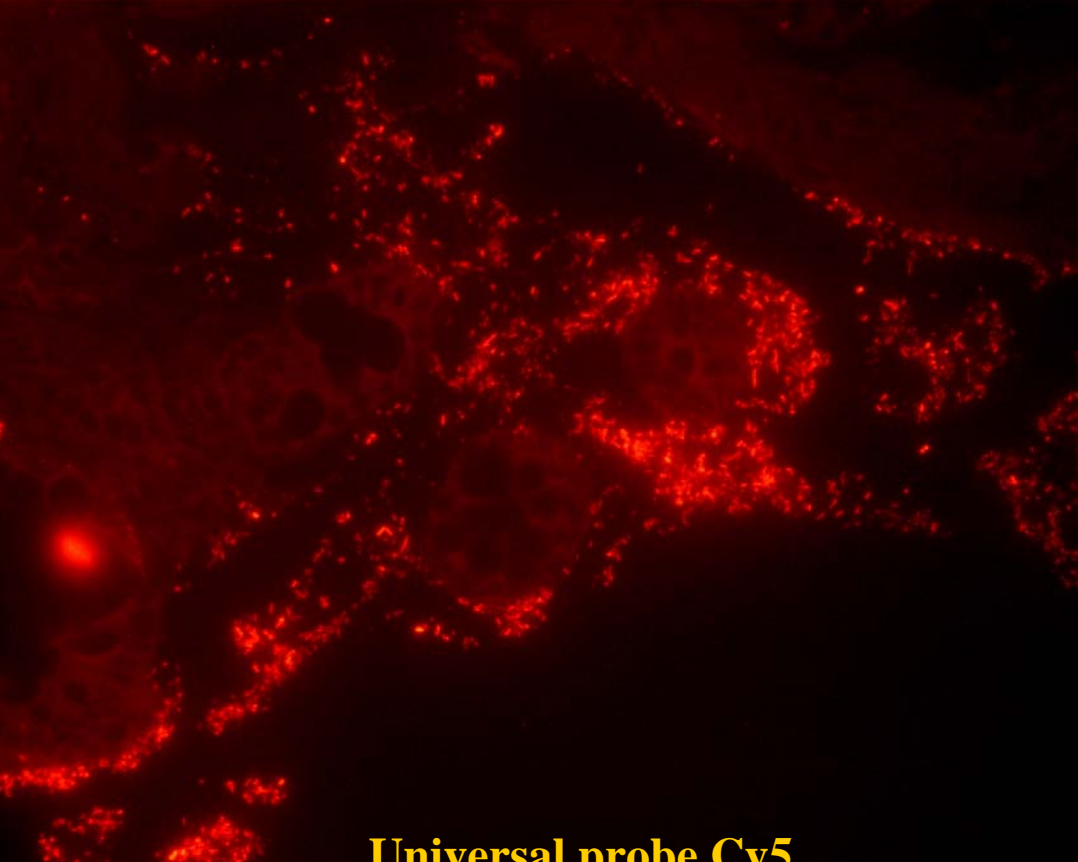
	UC (n=156)	CD (n=82)
All	3.8	9.1
remission	2.9	2.9
activity	4.8	11.0
exacerbated	5.5	8.2
fistula		20.1
no		4.8
colonic surgery	2.8	9.0
without surgery	4.6	10.0



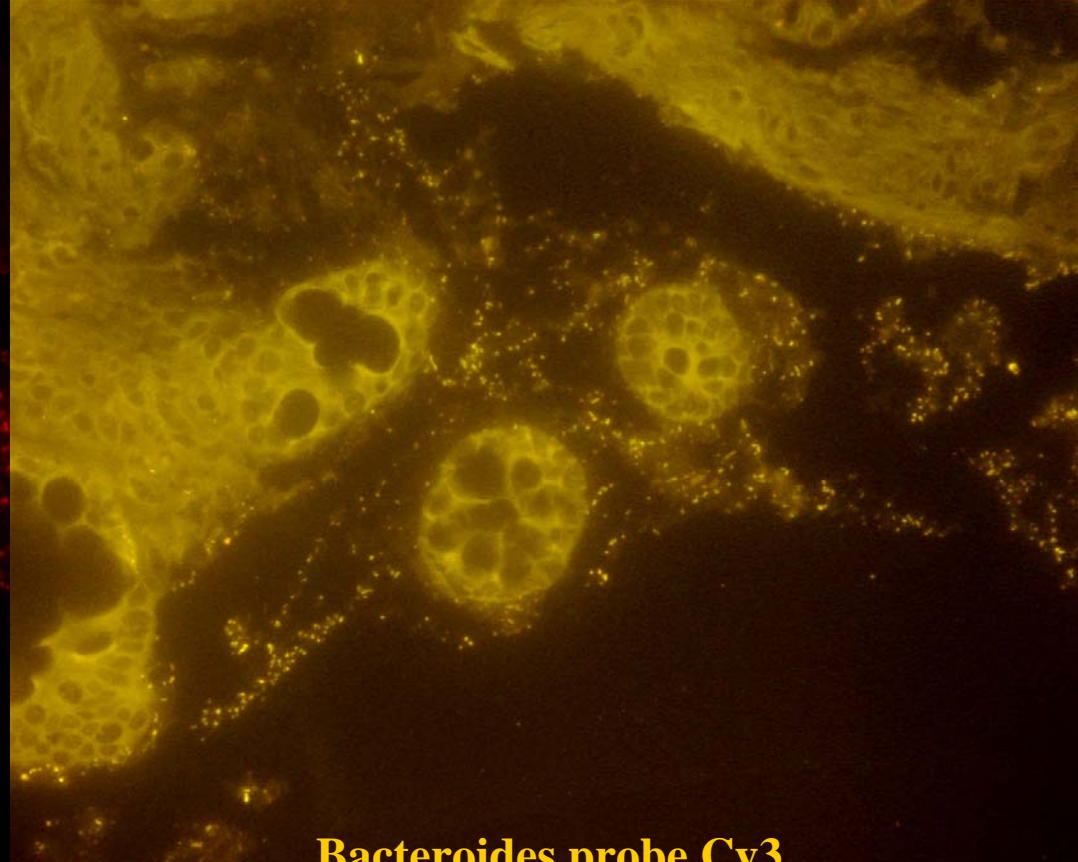
	UC (n=156)	CD (n=82)
All	3.8	9.1
no antibiotics in last 12 months	4.6	9.2
on antibiotics	0.5	1.4
after antibiotics (1-4 weeks)	5.9	27
no azathioprine	3.1	9.9
azathioprine	5.8	8.3
corticosteroids	8.2	12.0
without	3.7	8.7



Multicellular bacteria forming stromatolith
in Australian salt lakes



Universal probe Cy5



Bacteroides probe Cy3

