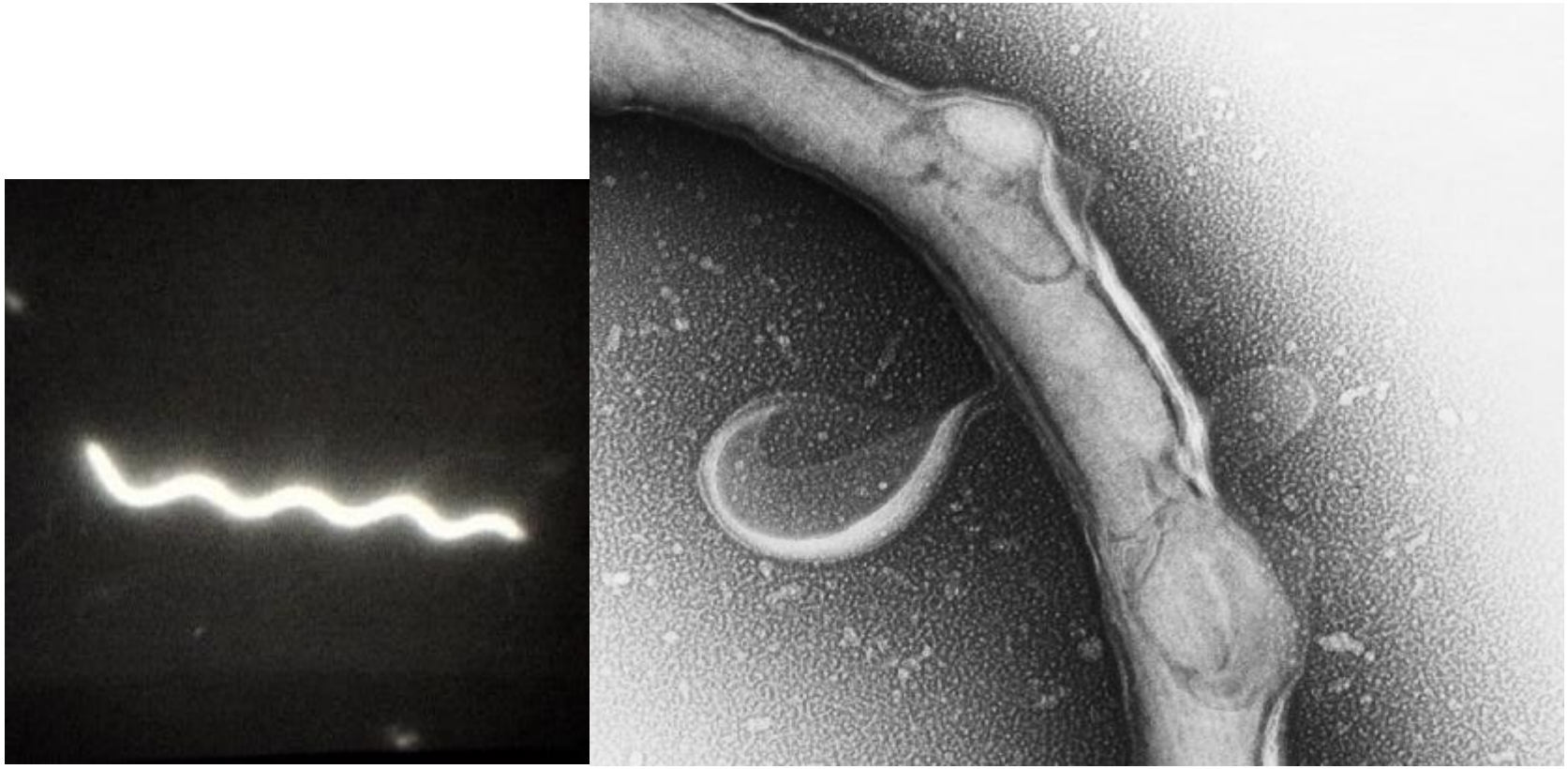
“The majority of bacteria exist in nature attached to a substratum”

MacEachran, D.P. O’Toole, G.A.

The Biofilm Mode of Life, 2007 p23.



Biofilms of *Borrelia burgdorferi* And Clinical Implications for Chronic Borreliosis

Alan B. MacDonald, MD,

July 7, 2008

University of New Haven

Lyme Disease Symposium

New Haven, Conn

Clinical Implications of Biofilms of Borrelia burgdorferi

Biofilms of Borrelia burgdorferi in human tissue provide microscopic proof of persistence of spirochetes in cases of chronic Lyme borreliosis.

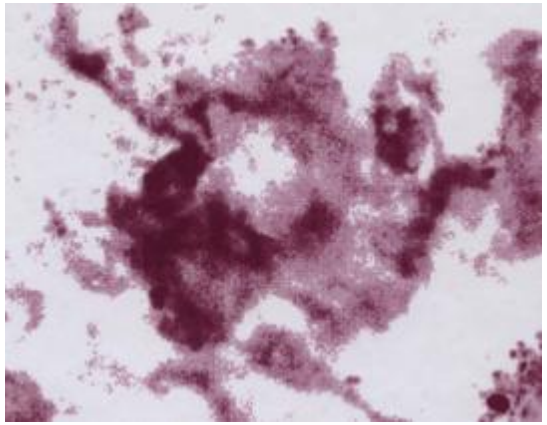
Biofilms of borrelia,by definition, explain Persistence of infection after antibiotic therapy and recurrence of disease symptoms in chronic Lyme borreliosis.

Dr Eva Sapi

The first to recognize that Borrelia burgdorferi

Could exist in Biofilm Communities

Common shared properties in “mature “Biofilms



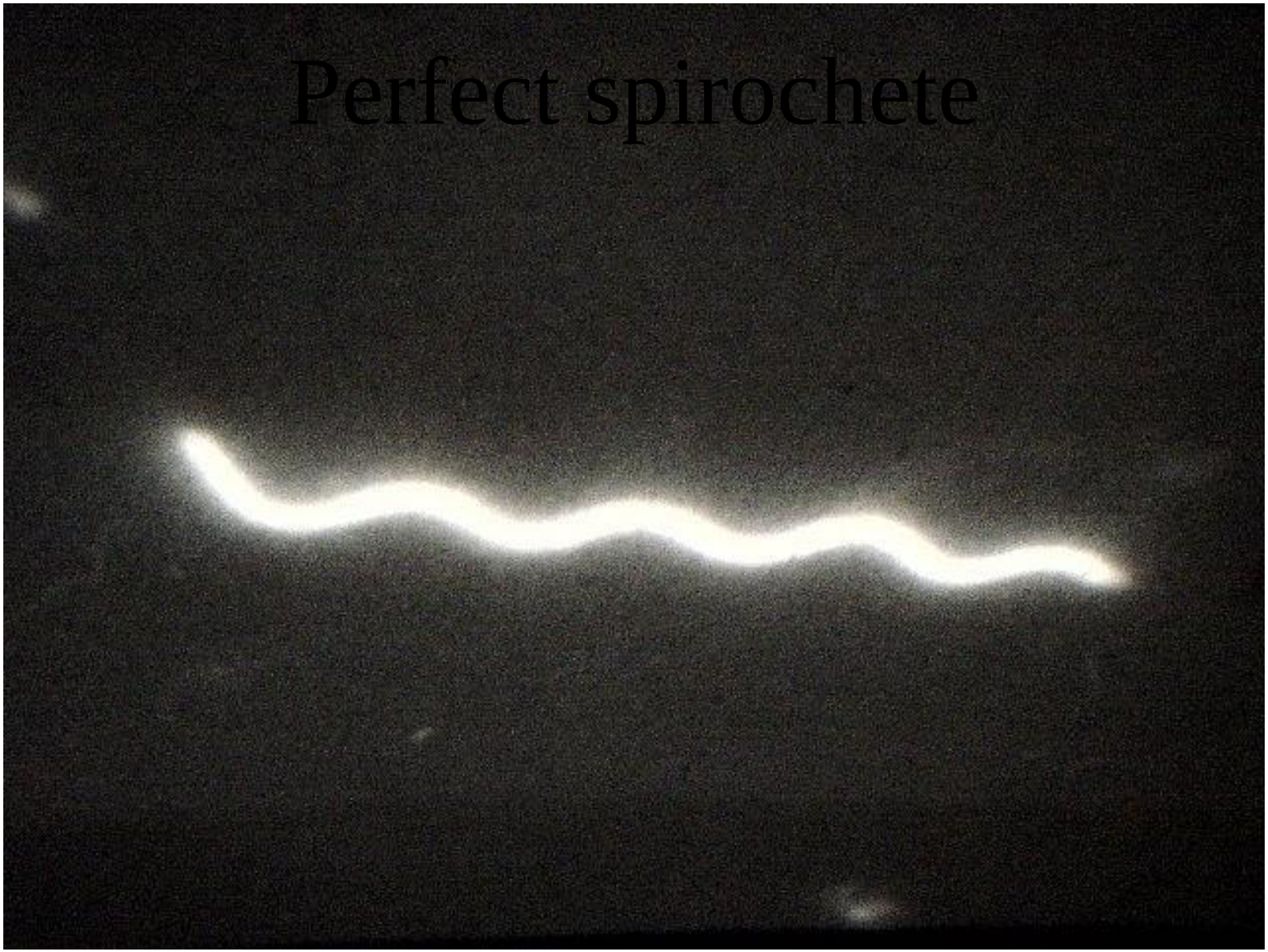
“The microcolony structure observed in established Mature biofilms is strikingly similar across mono-and Multispecies biofilms, across different habitats, as well as for Different organismal levels”

Kjelleberg, S., and Givskov, M.

The Biofilm mode of Life, 2007, page 5.

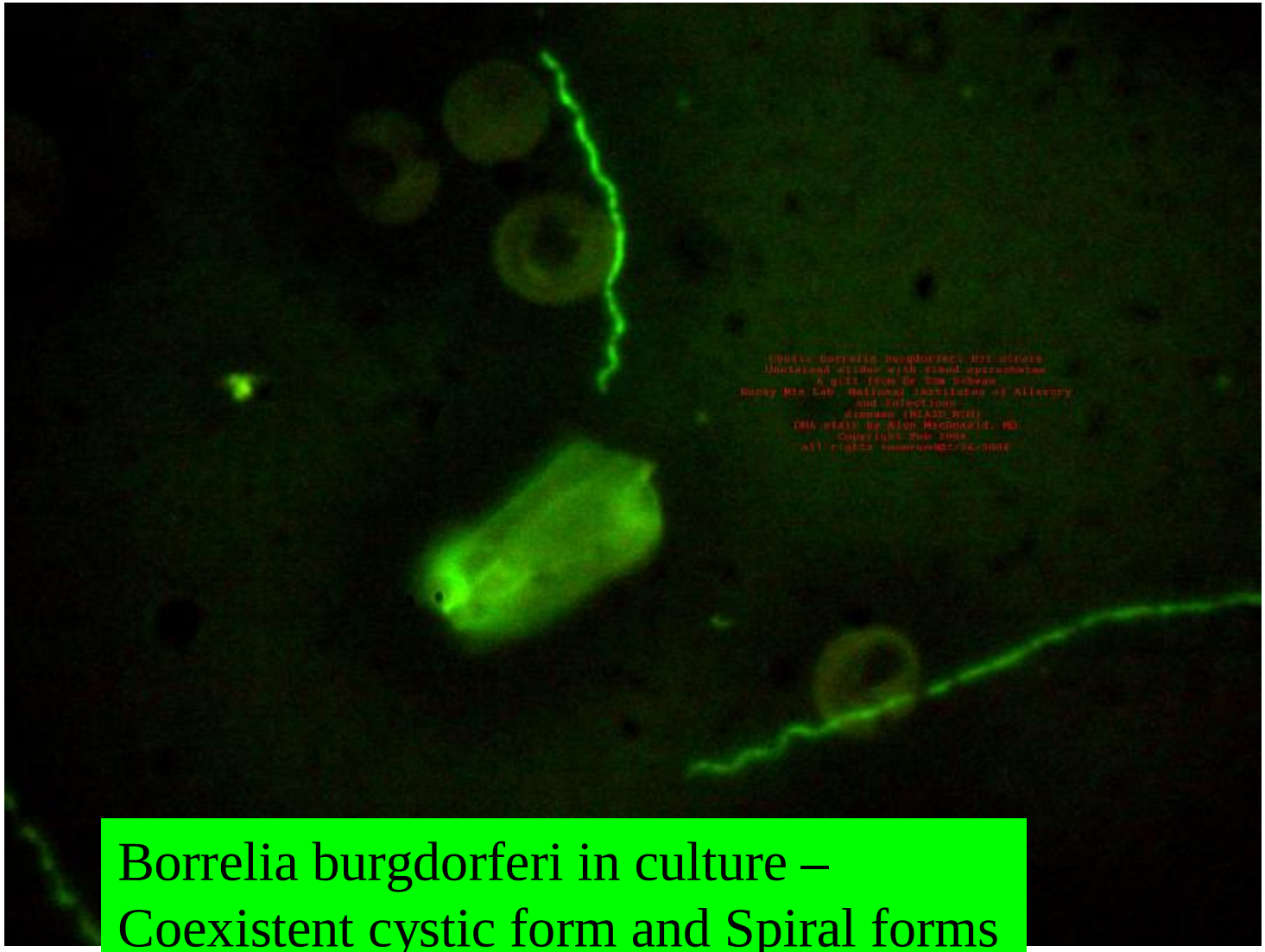
Copyright notice

Perfect spirochete



Routes to the formation of Biofilms

Multiple, Parallel pathways to Biofilm Formation



Borrelia burgdorferi in culture –
Coexistent cystic form and Spiral forms

A vocabulary of words and images

Borrelia of the ***Spiral*** type

Borrelia of the ***Cystic*** type

Borrelia of the ***Granular*** type

Borrelia of the ***Cell wall deficient*** type

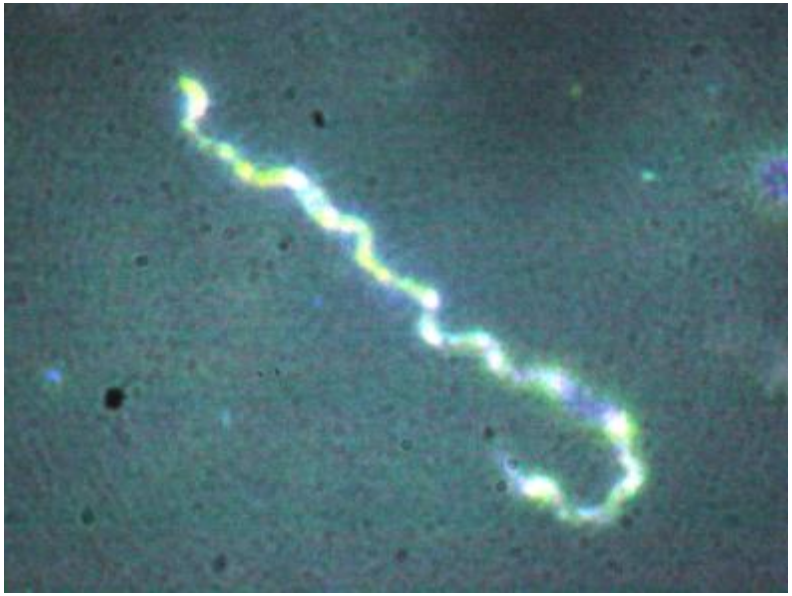
Mixtures of Borrelia types may be found in Borrelia biofilms

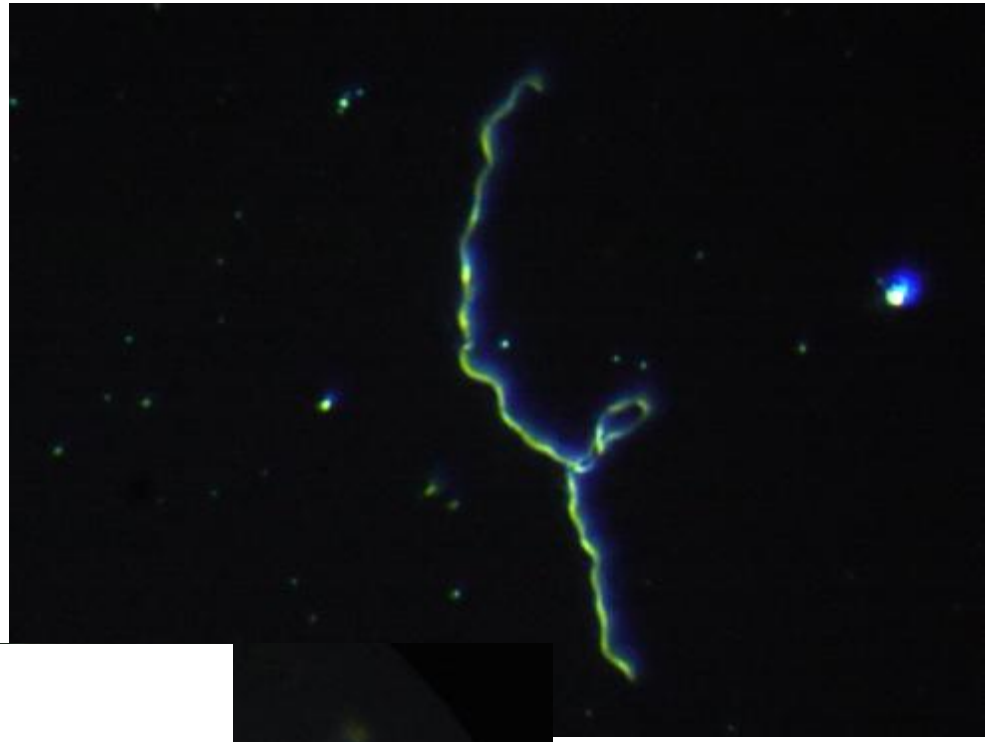
Some Borrelia biofilms may contain a majority of spiral Borrelia, while others may contain

A majority of granular or Cystic Borrelia

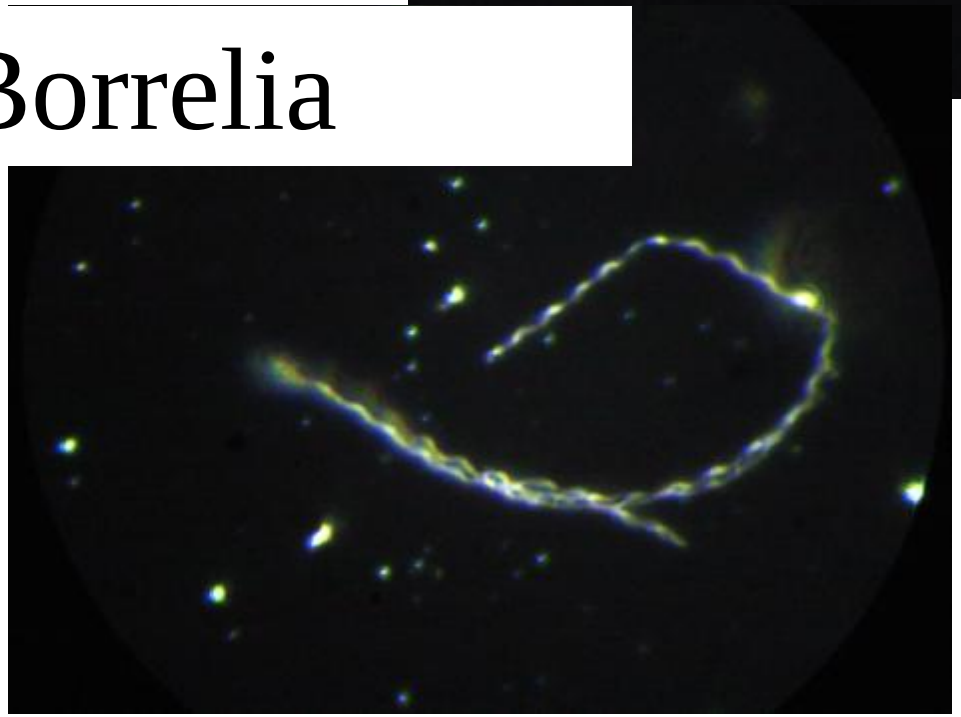
Biofilms may contain different species of pathogens

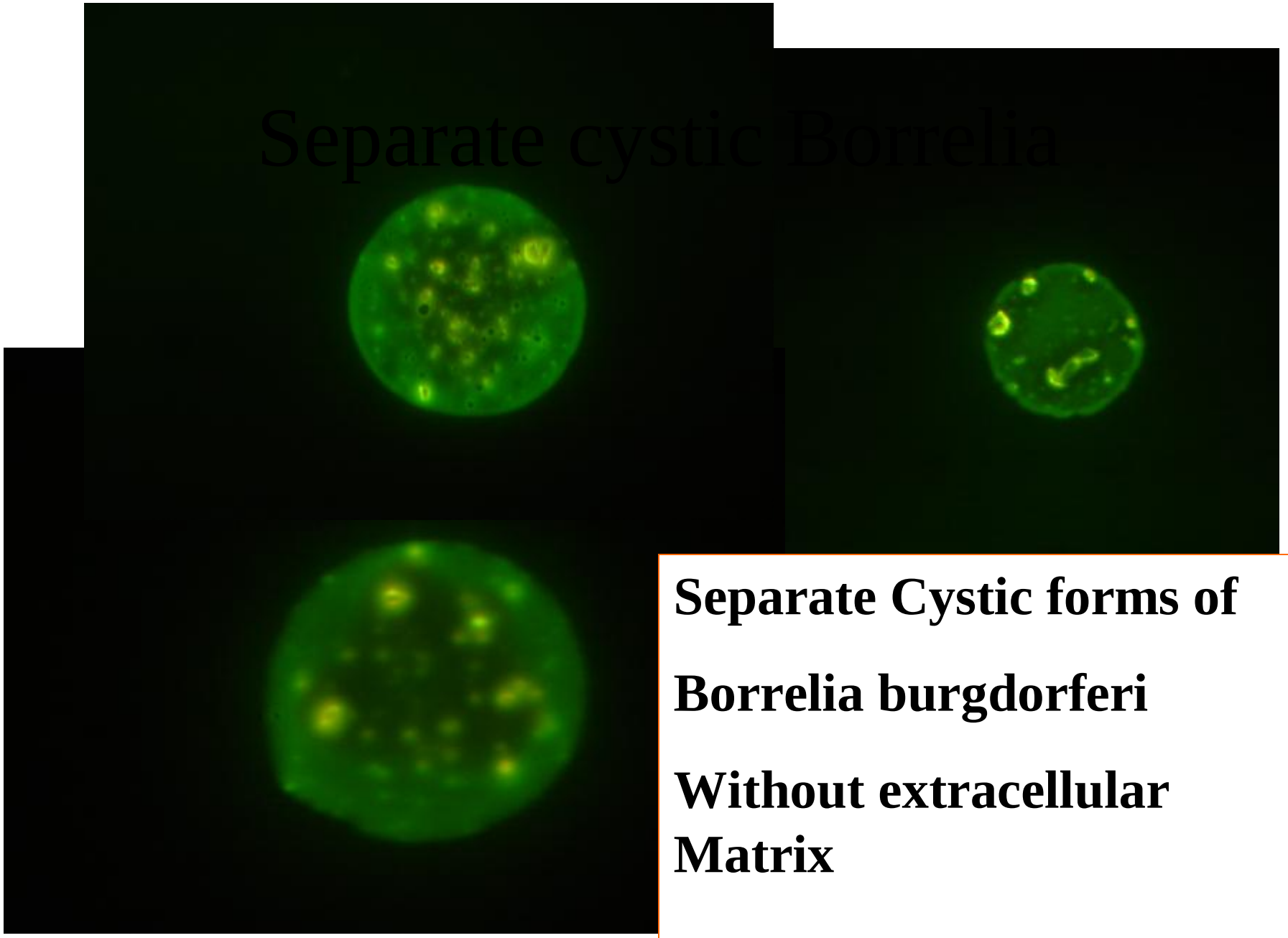
(For example Borrelia and Babesia, Or other multiorganism combinations)





Spiral Borrelia

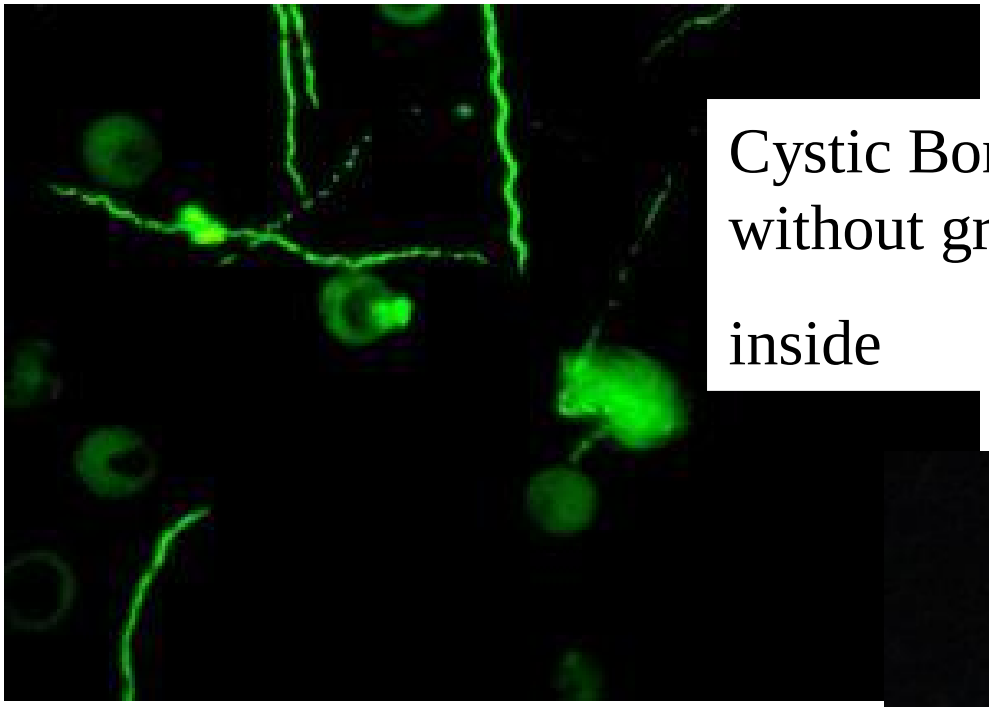




Separate Cystic forms of

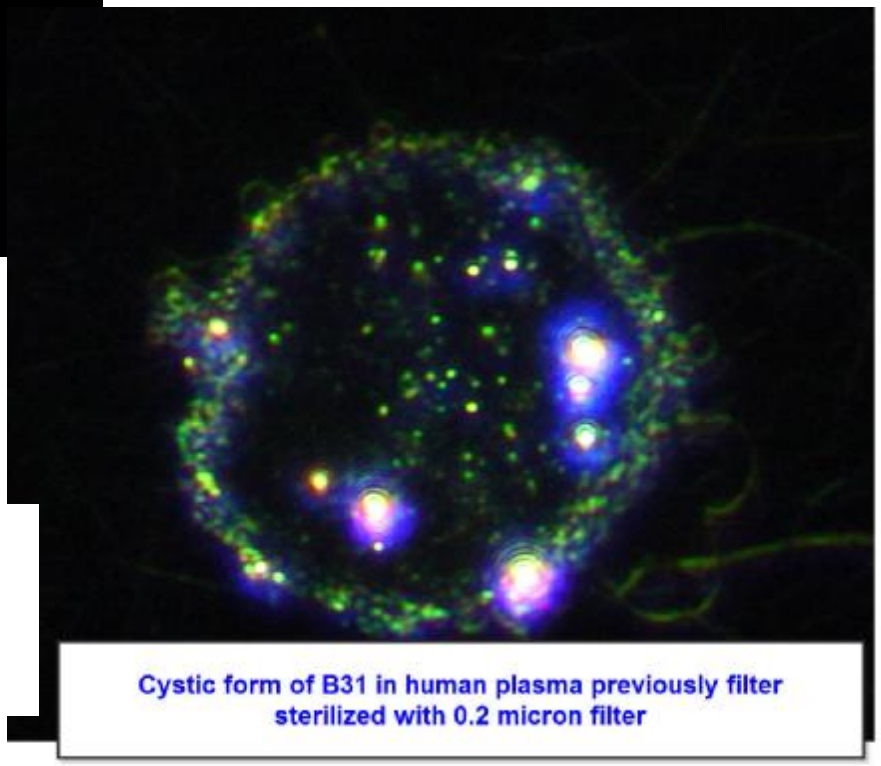
Borrelia burgdorferi

Without extracellular Matrix



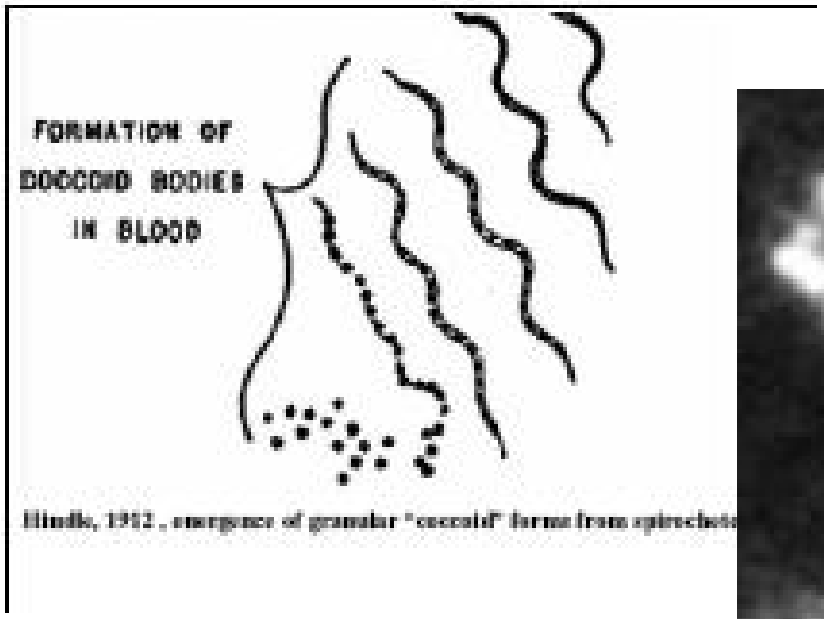
Cystic Borrelia
without granules

inside

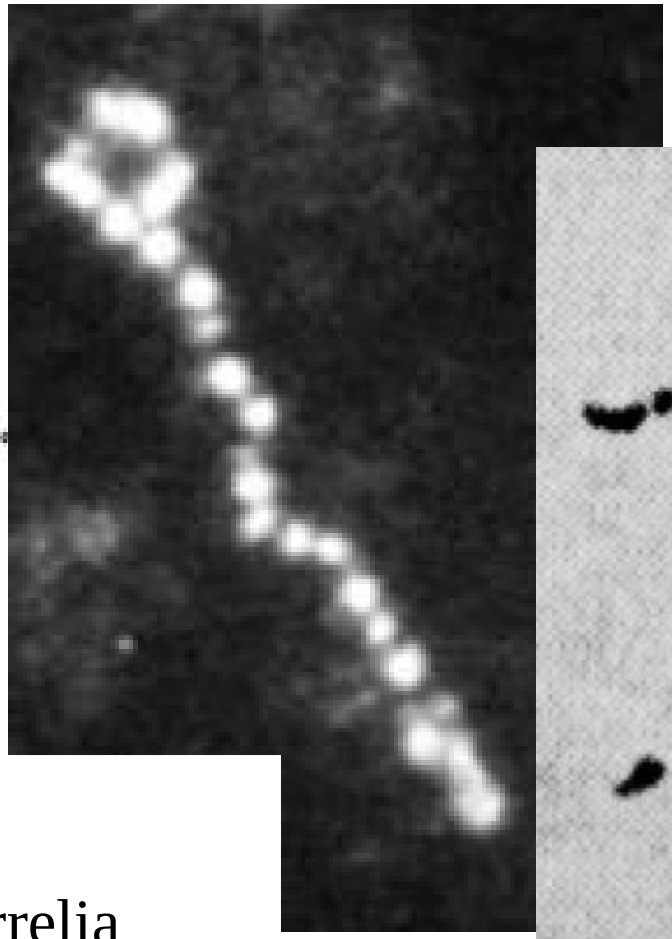


Cystic Borrelia with

Granules inside

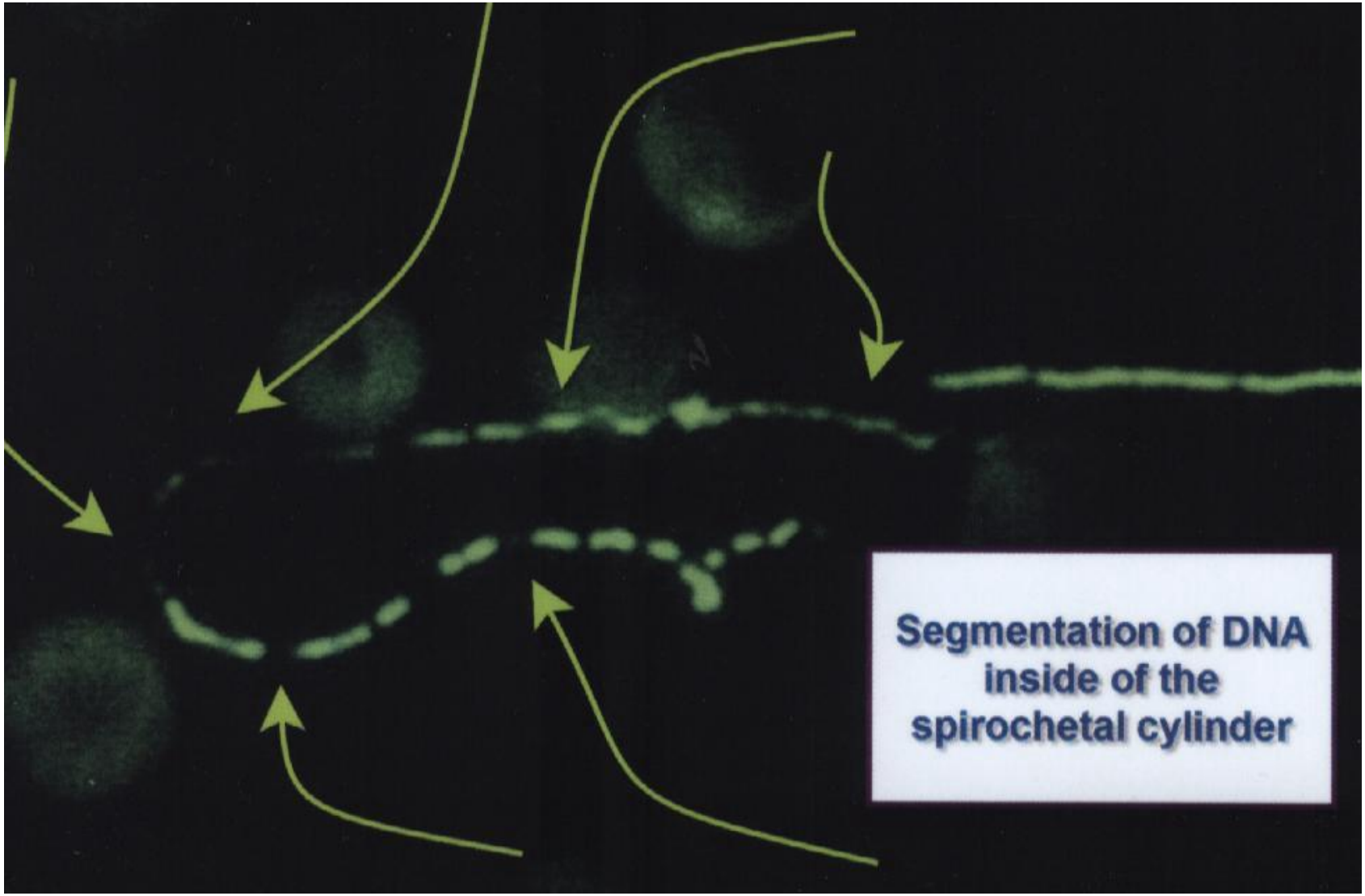


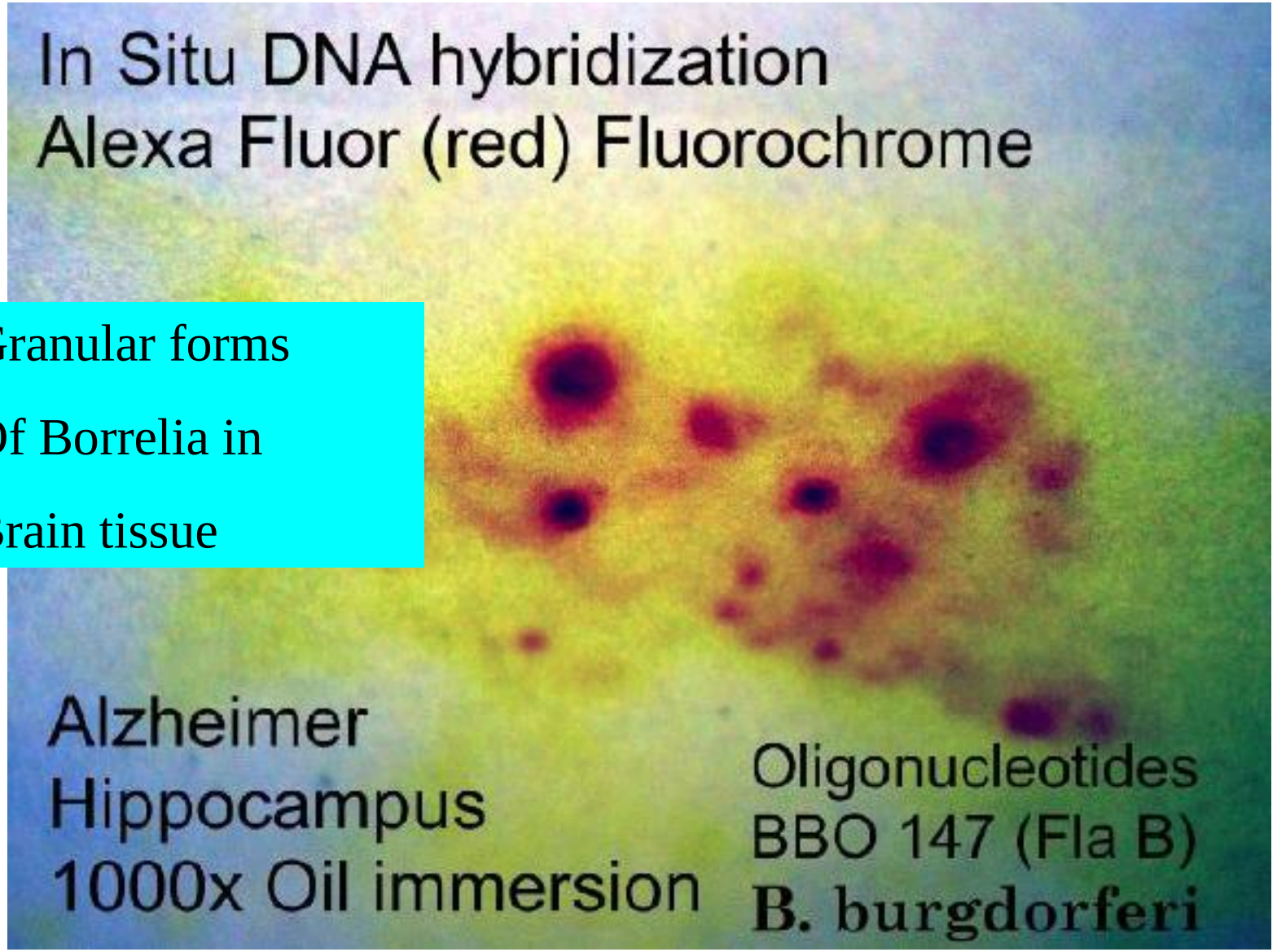
Hinds, 1912., emergence of granular "coccoid" forms from spirochete



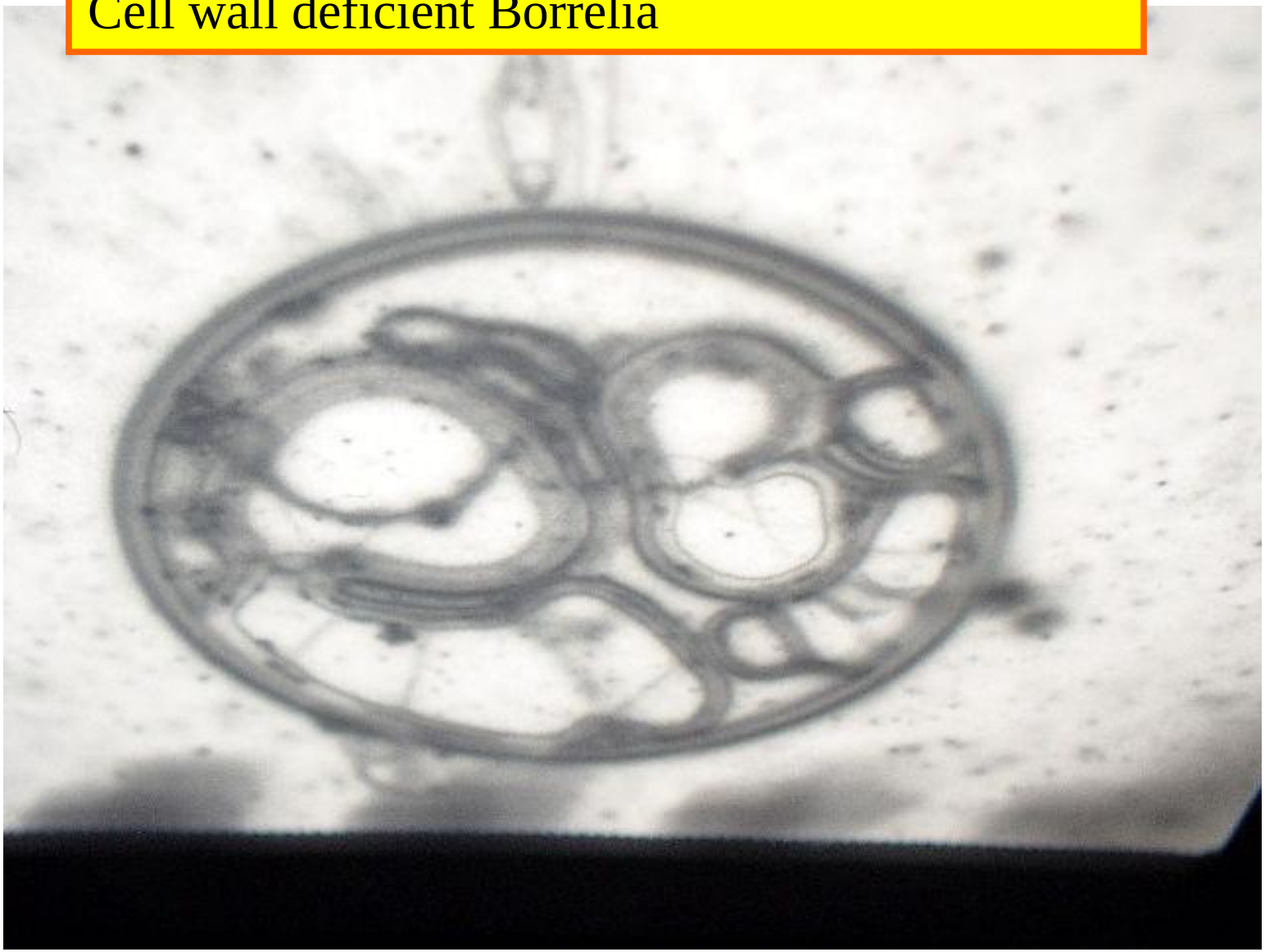
Granular Borrelia

Evolving from spiral borrelia

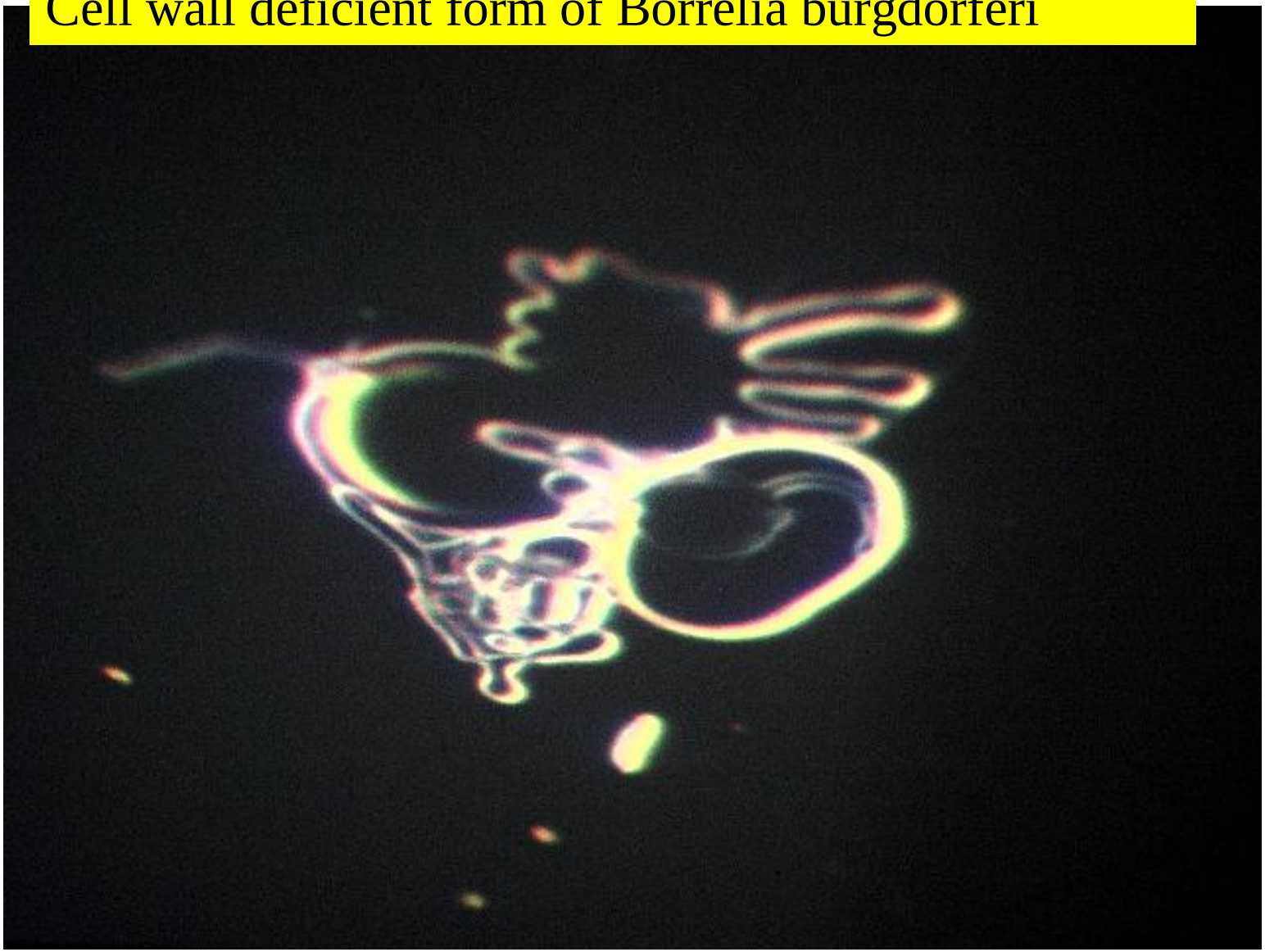

Segmentation of DNA
inside of the
spirochetal cylinder


In Situ DNA hybridization
Alexa Fluor (red) Fluorochrome
Granular forms
Of Borrelia in
Brain tissue
Alzheimer
Hippocampus
1000x Oil immersion
Oligonucleotides
BBO 147 (Fla B)
B. burgdorferi

Cell wall deficient Borrelia



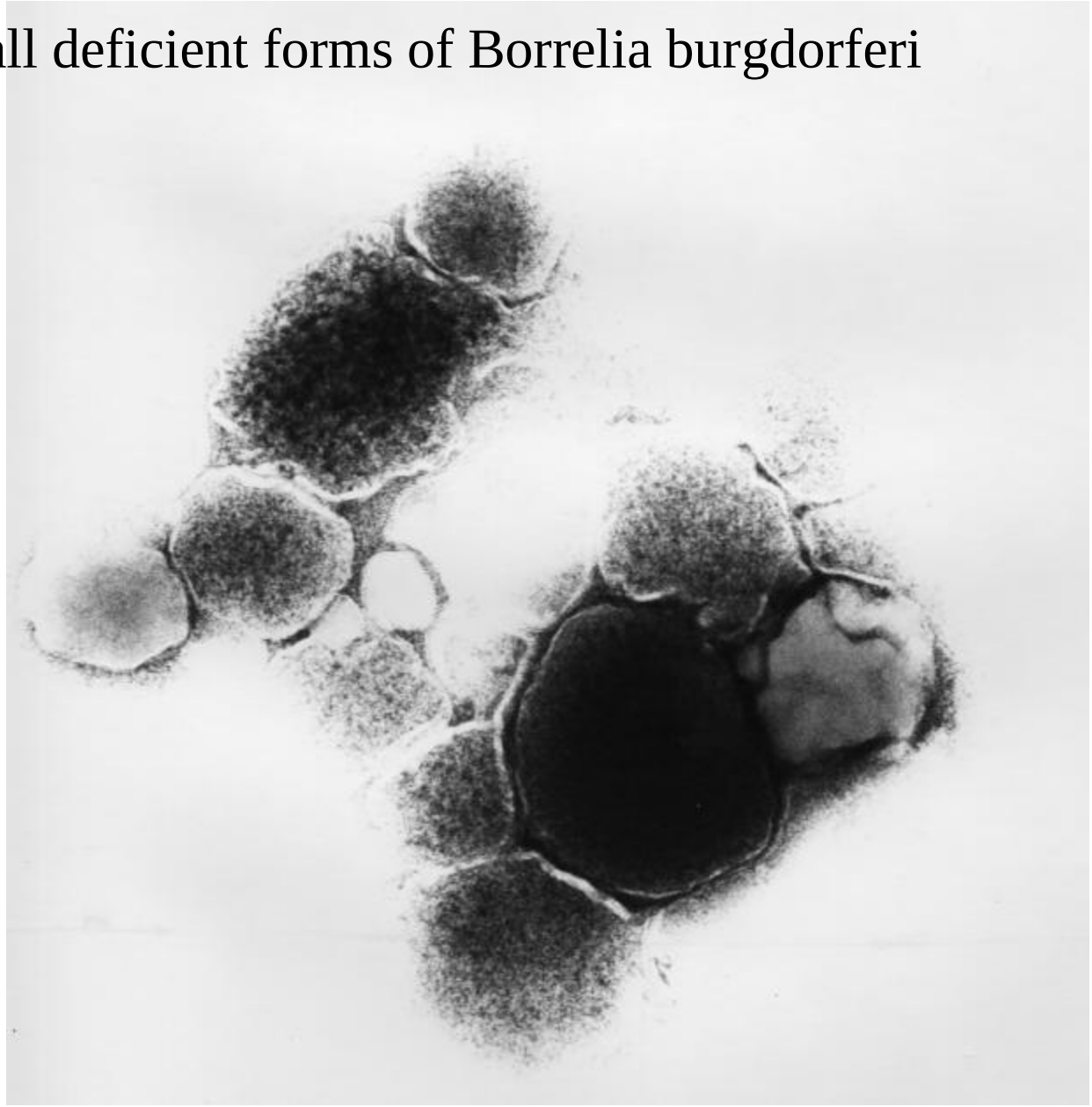
Cell wall deficient form of Borrelia burgdorferi



Membrane material separating from Borrelia burgdorferi

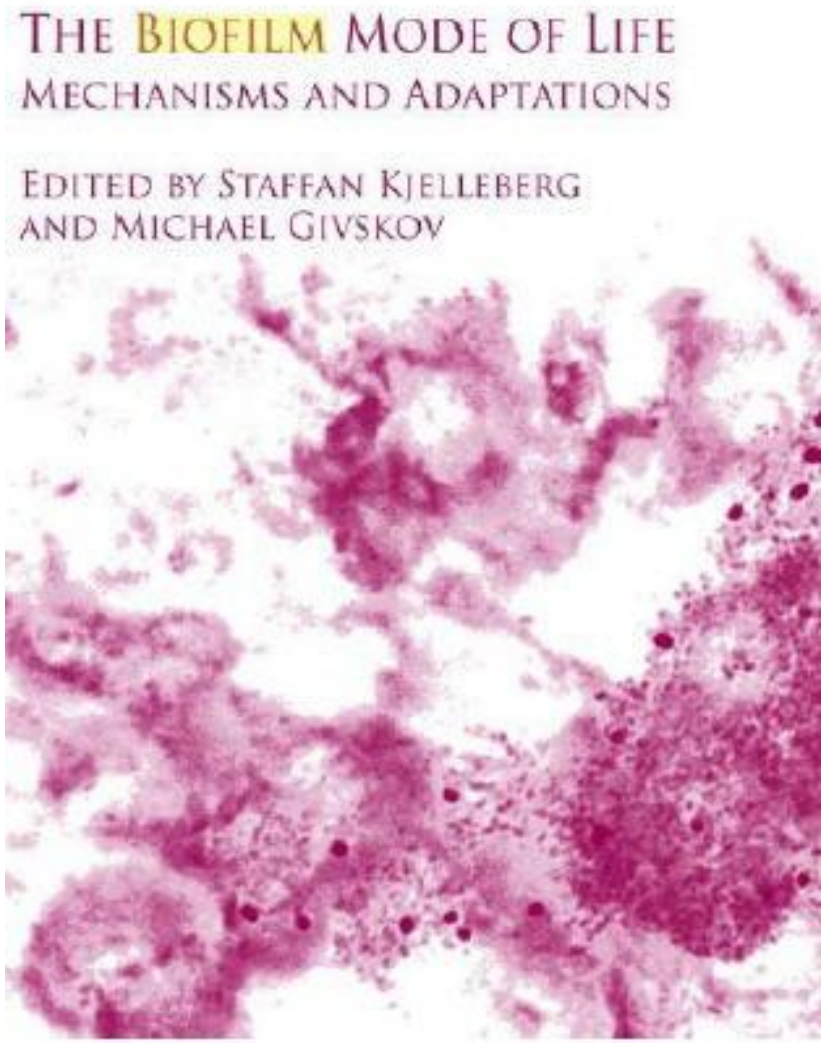
Membrane material separating from Borrelia burgdorferi

Cell wall deficient forms of Borrelia burgdorferi



Biofilm : A *community of microbes*

enveloped in a protective Extracellular matrix



“Biofilm” is the Extracellular material which holds the communities of Bacteria together in a sessile community”

The biofilm composition is often mucopolysaccharide material.

Some biofilms (Pseudomonas species) are composed of Extracellular DNA.

Other biofilms may incorporate Flagellae,Fimbriae,Pili into the biofilm

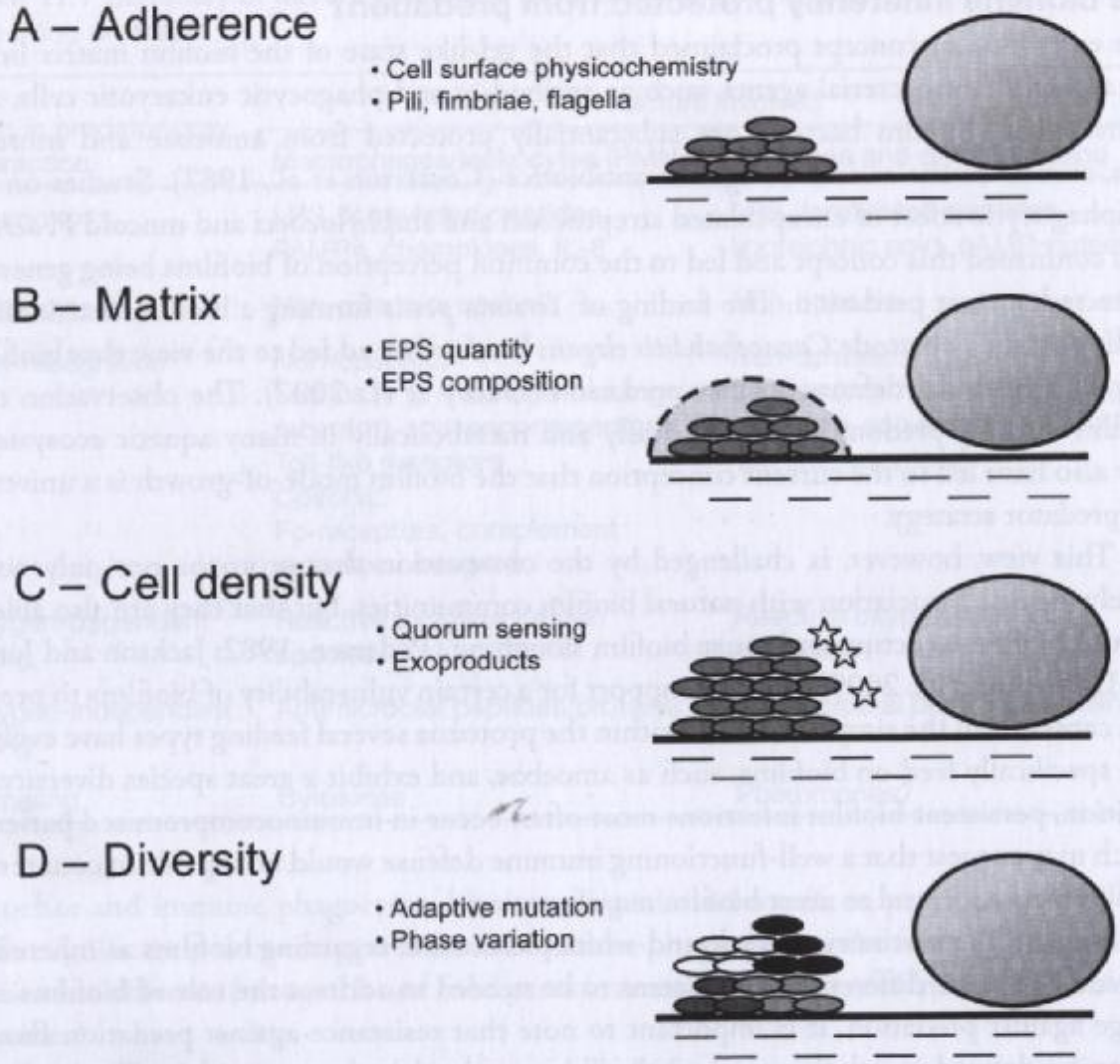
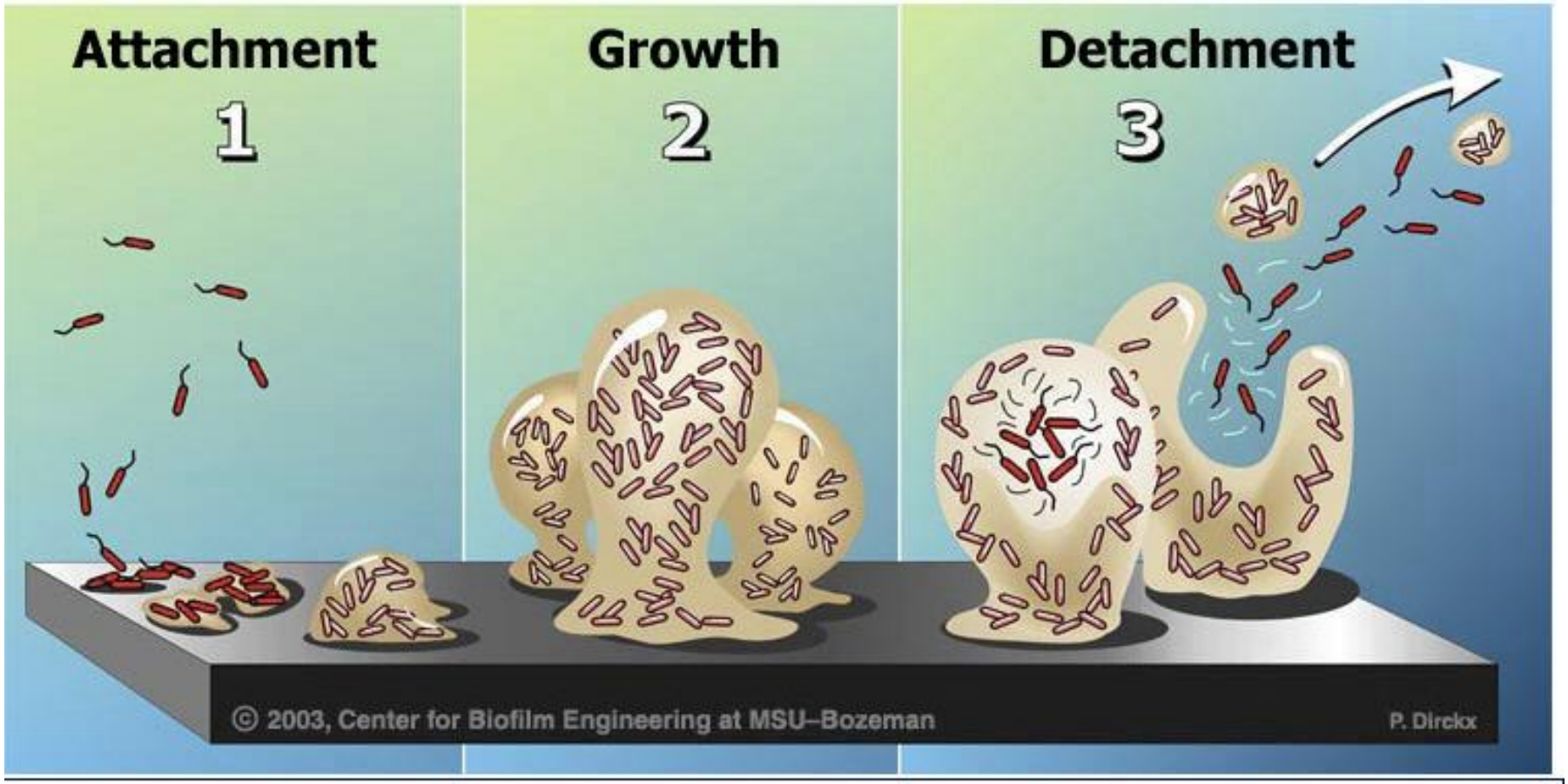


Figure 11.1 Biofilm-specific traits and their role in antipredator resistance.


Attachment
1
Growth
2
Detachment
3
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P. Dirckx

Planktonic microbes

Motility

Provided

By Flagellae

Attachment to surface

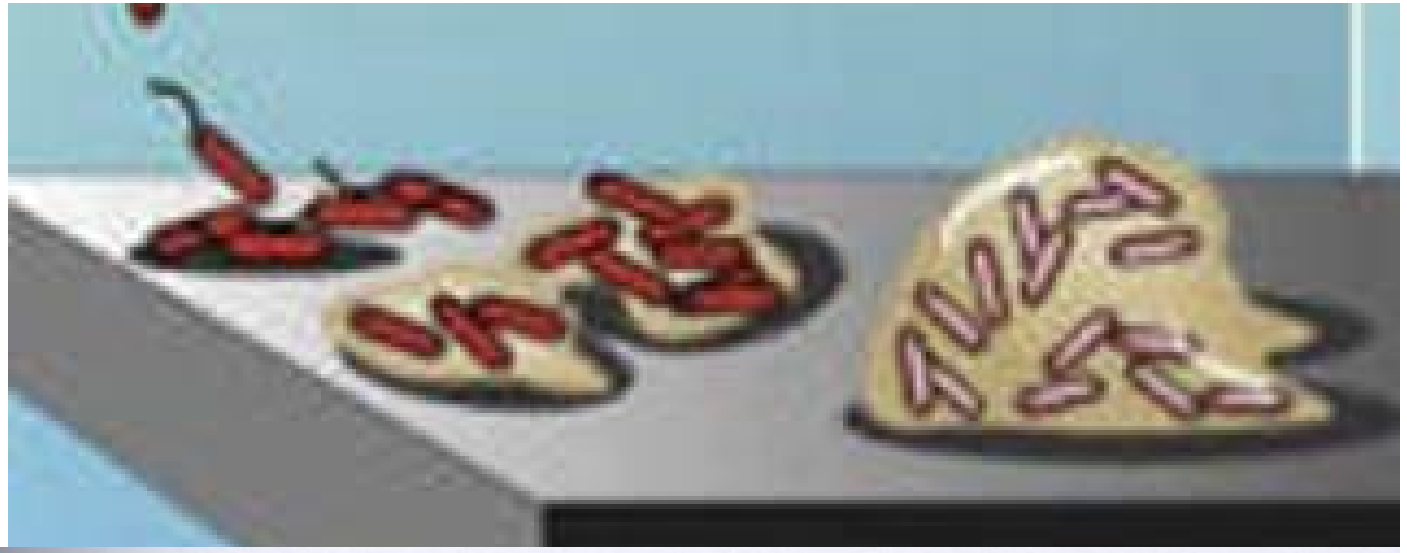
Provided by Flagellae

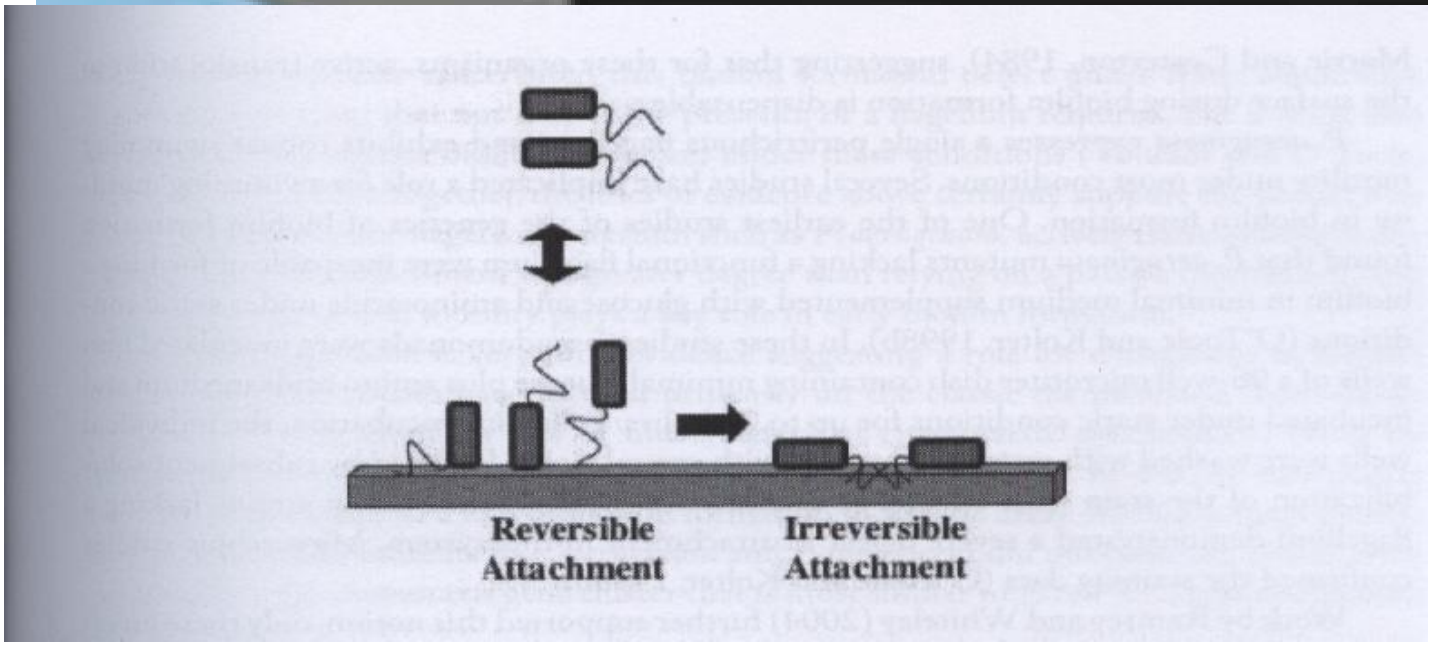
Two functions of Flagellae:

Propulsion

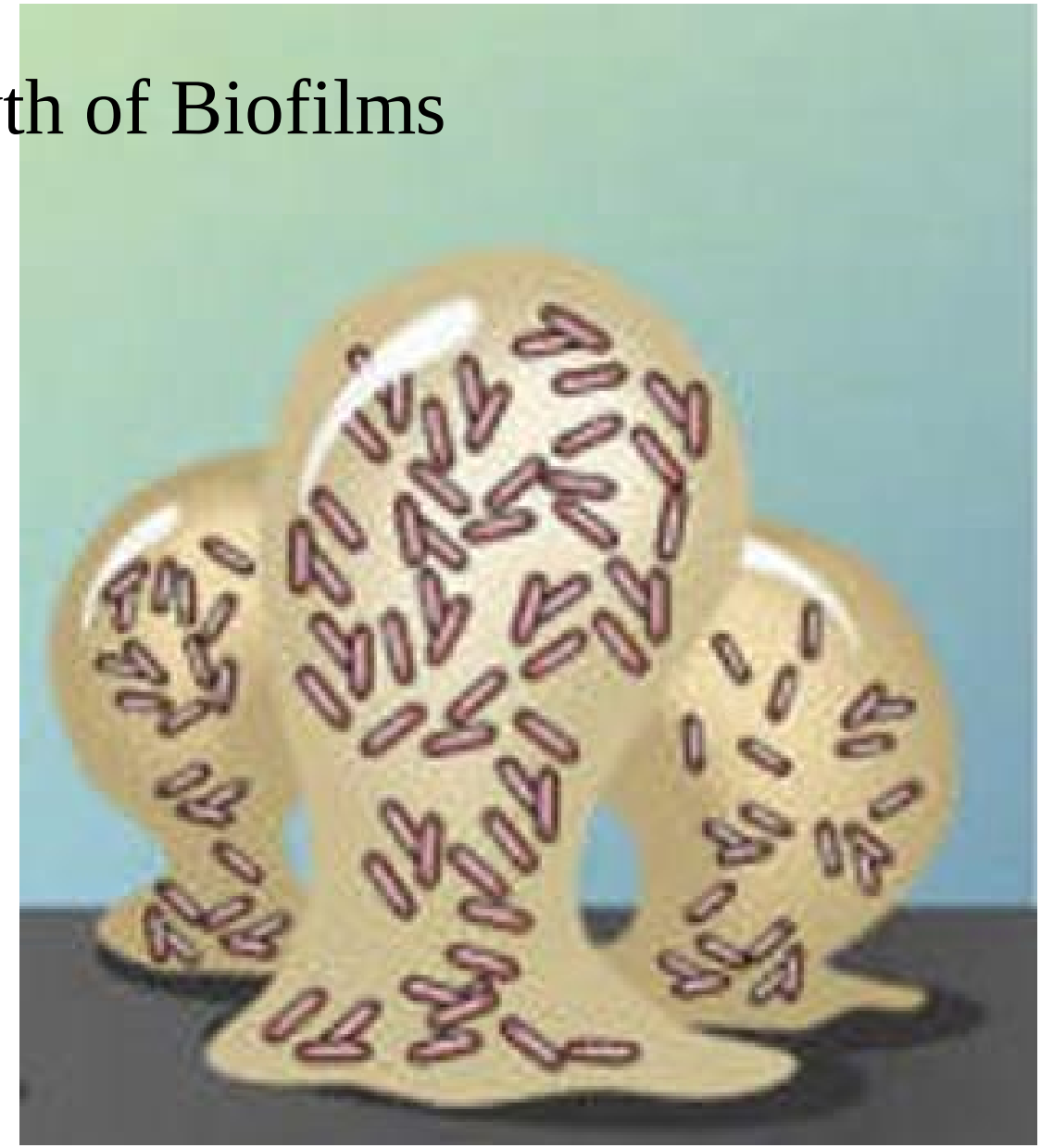
Adhesion to surface

Attachment of early biofilm – Reversible and Irreversible

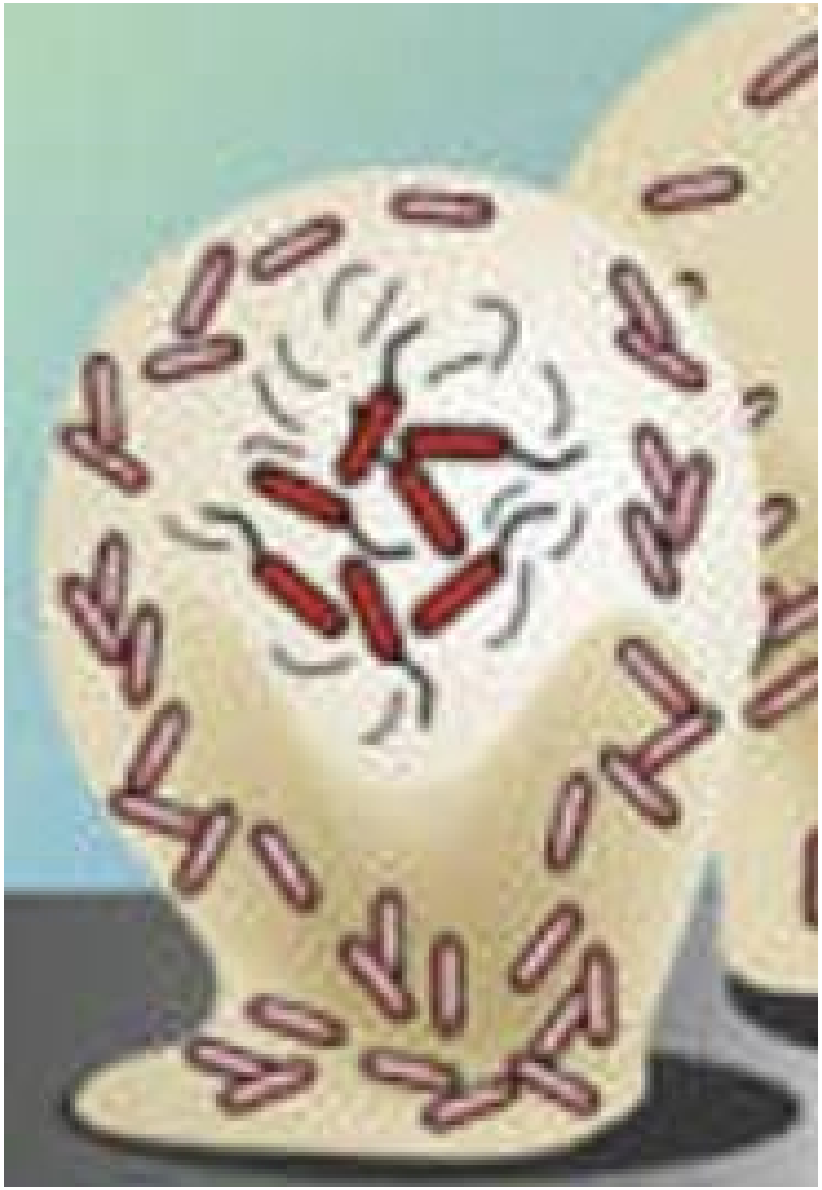




Growth of Biofilms



Regeneration of Planktonic microbes within the biofilm




Detachment
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Regional

ASPECTS

Zonation

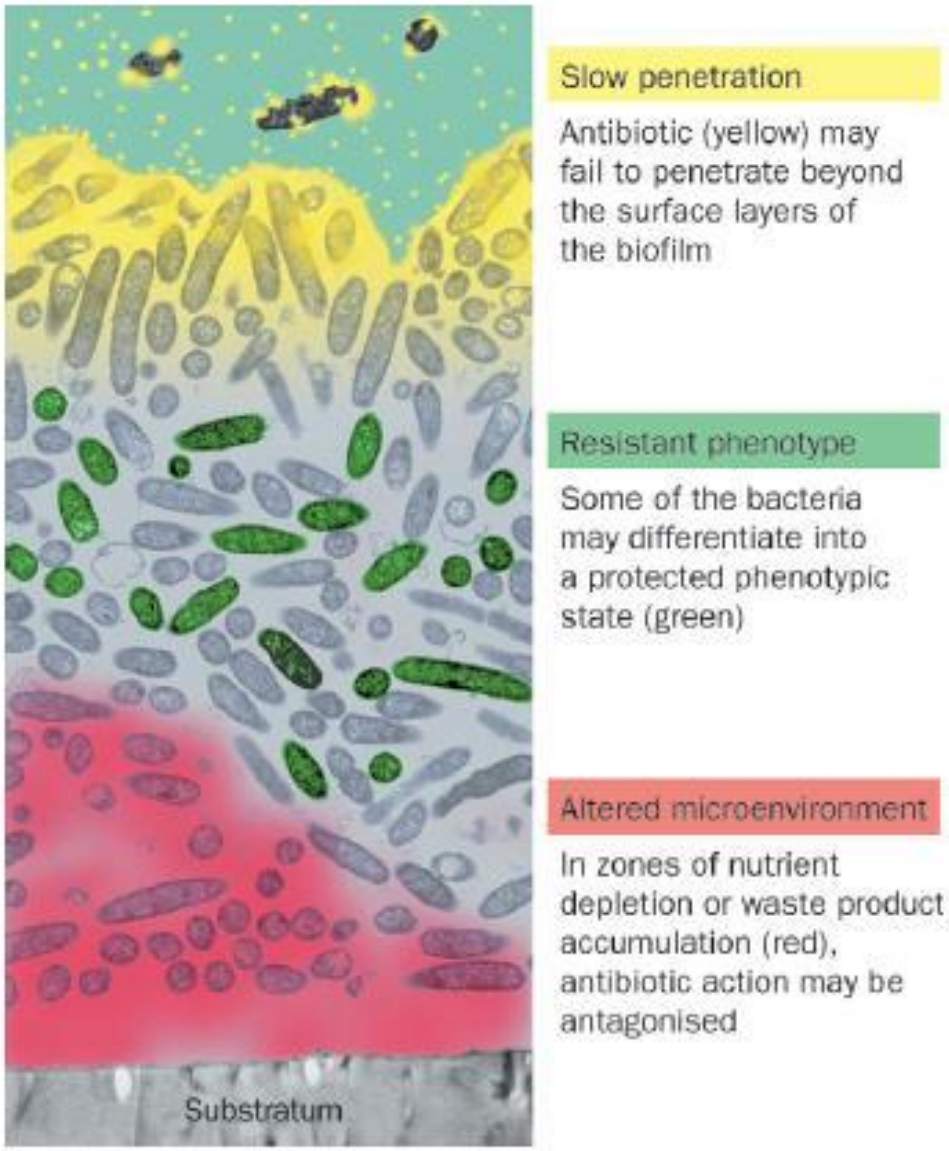


Figure 2: **Three hypotheses for mechanisms of antibiotic resistance in biofilms**

Review

Antibiotic resistance of bacteria in biofilms

Philip S Stewart, J William Costerton

Bacteria that adhere to implanted medical devices or damaged tissue can encase themselves in a hydrated matrix of polysaccharide and protein, and form a slimy layer known as a biofilm. Antibiotic resistance of bacteria in the biofilm mode of growth contributes to the chronicity of infections such as those associated with implanted medical devices. The mechanisms of resistance in biofilms are different from the now familiar plasmids, transposons, and mutations that confer innate resistance to individual bacterial cells. In biofilms, resistance seems to depend on multicellular strategies. We summarise the features of biofilm infections, review emerging mechanisms of resistance, and discuss potential therapies.

Bacteria that adhere to implanted medical devices or

As an example of sequelae of biofilms, let us consider

Altered MicroEnvironment in Biofilms and Antibiotic Resistance

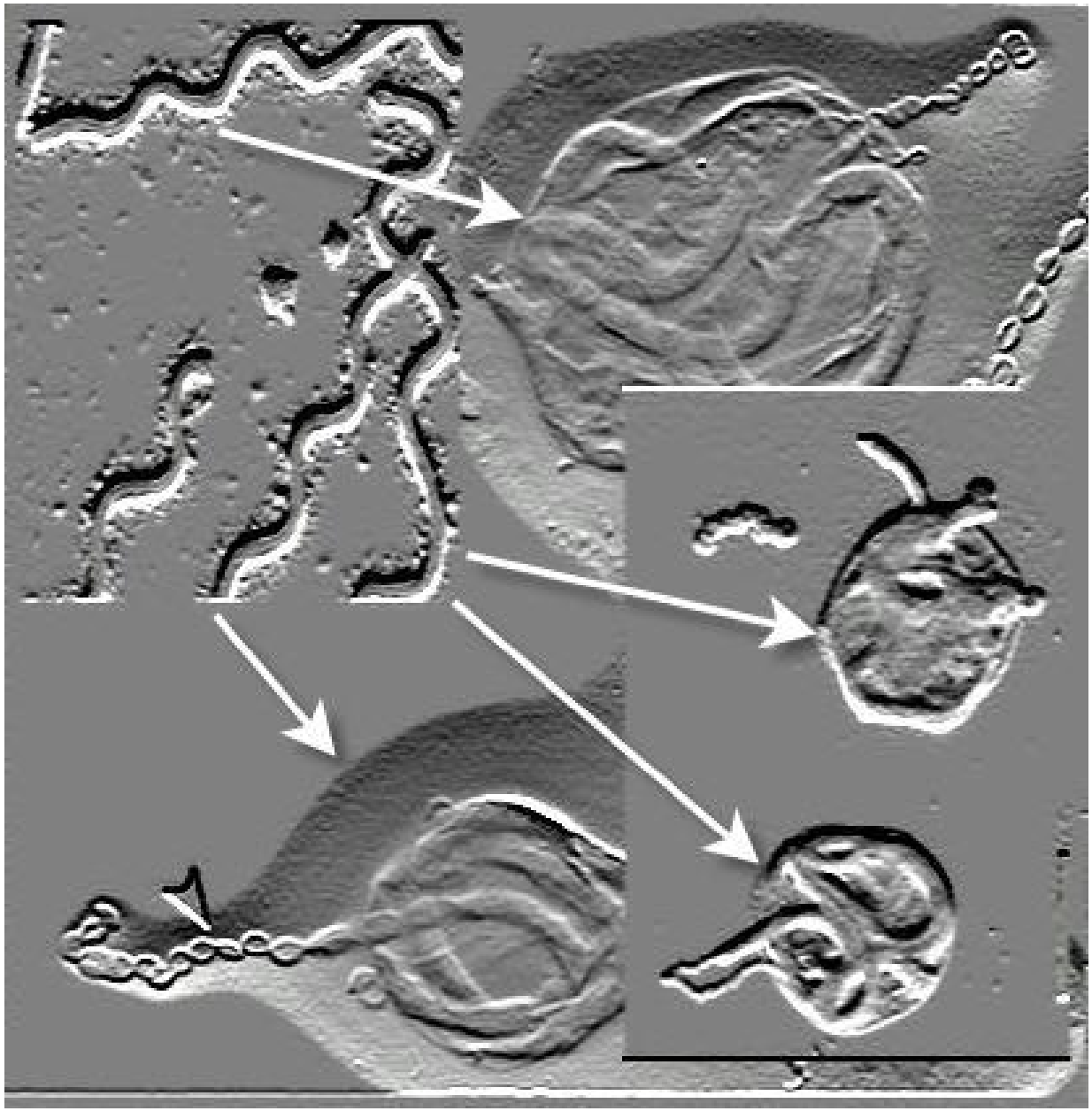
Failure of Antibiotic to ***penetrate*** the Biofilm

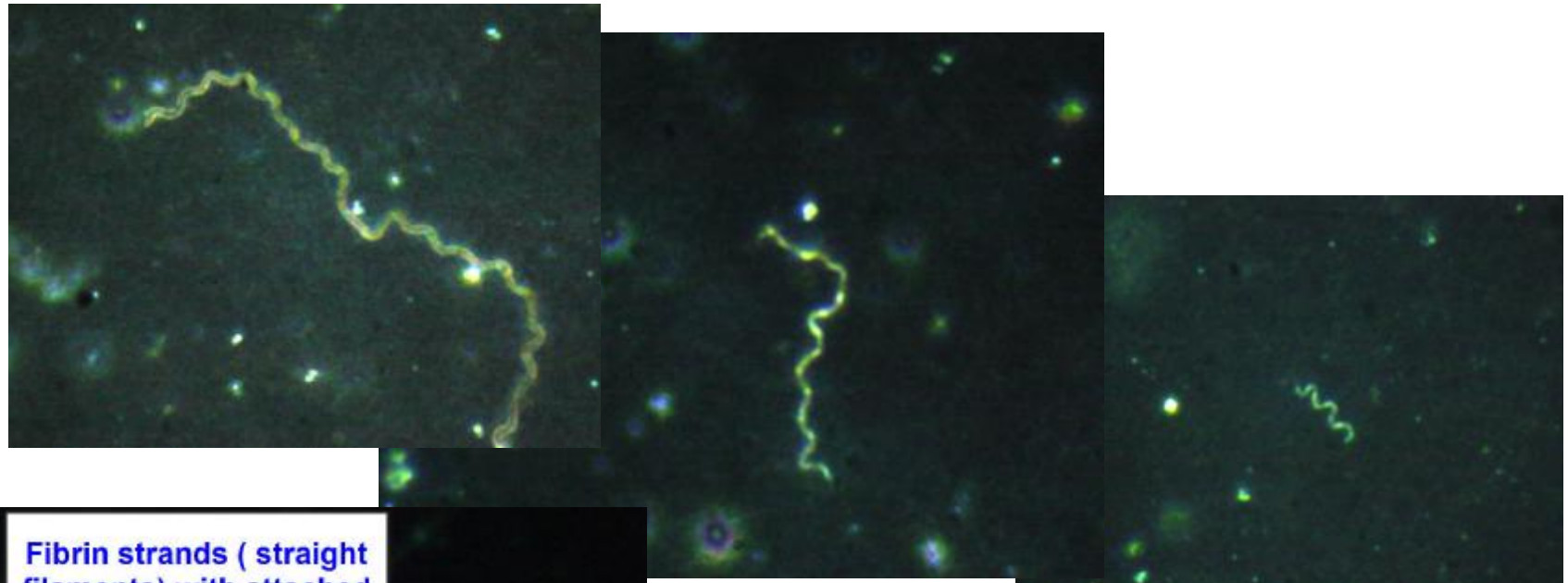
Differentiation of Bacteria within the Biofilm -

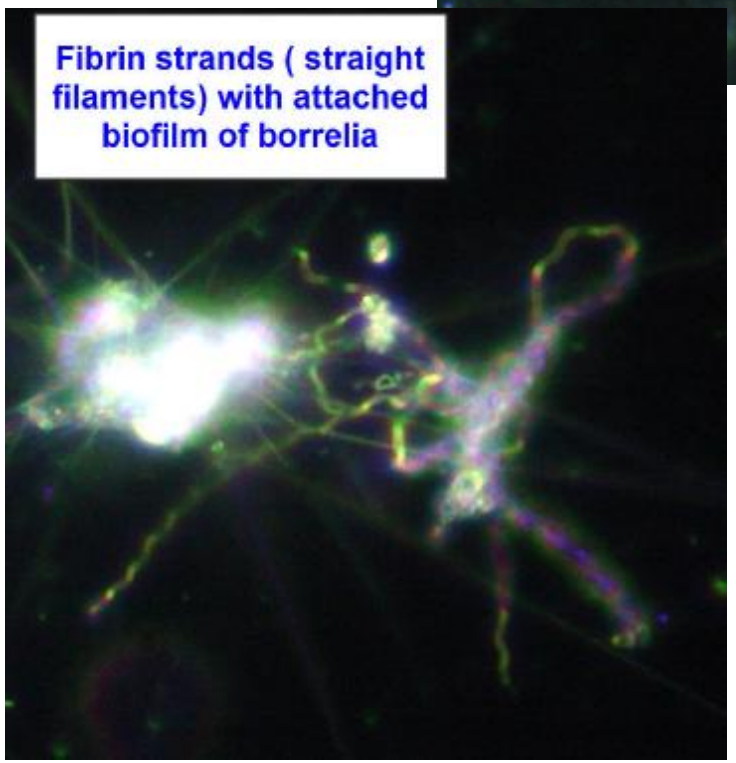
Dormant State and Altered Genetics

Bacterial Heterogeneity in Biofilms

Accumulation of Molecules in the biofilm
which ***antagonize the Antibiotic action***

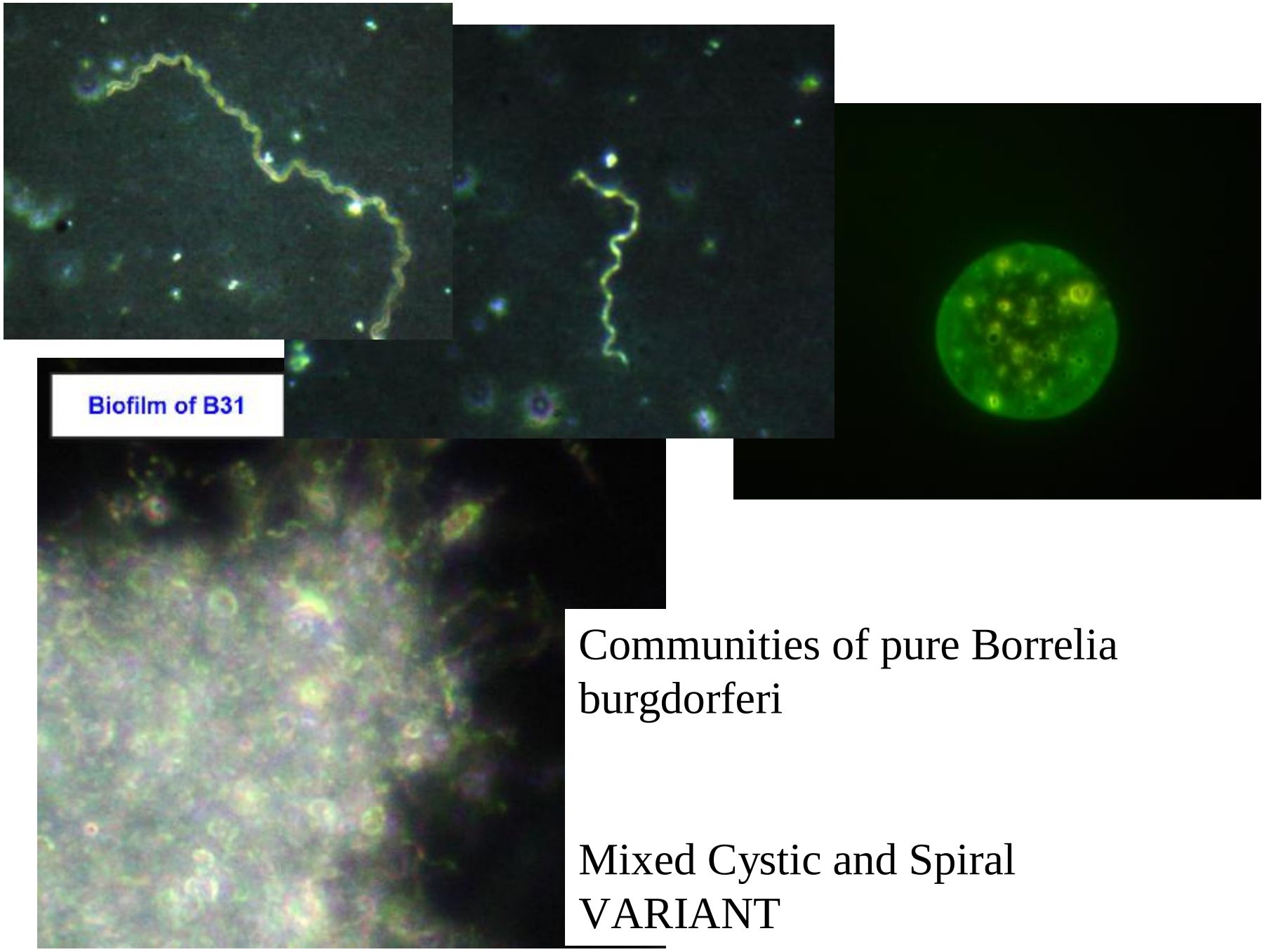






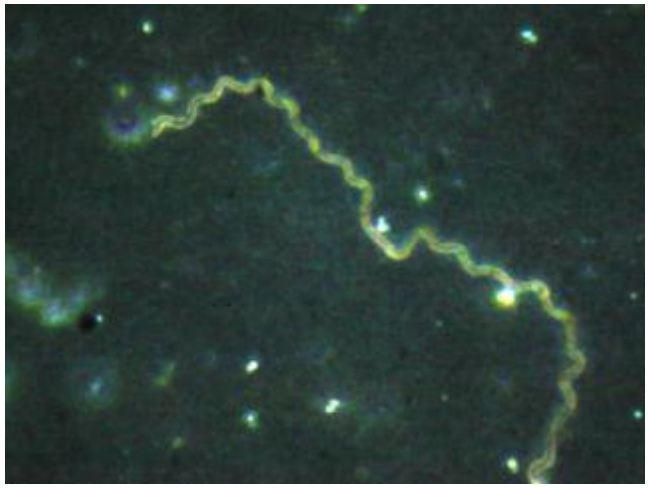
Communities of pure Borrelia burgdorferi (corkscrew/ spiral)

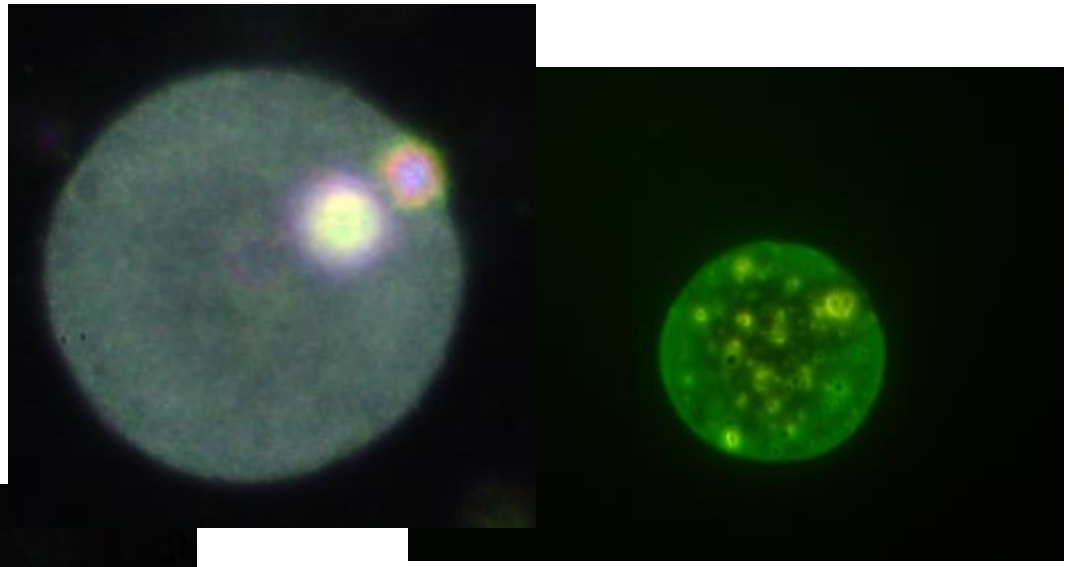
Spiral Biofilm VARIANT

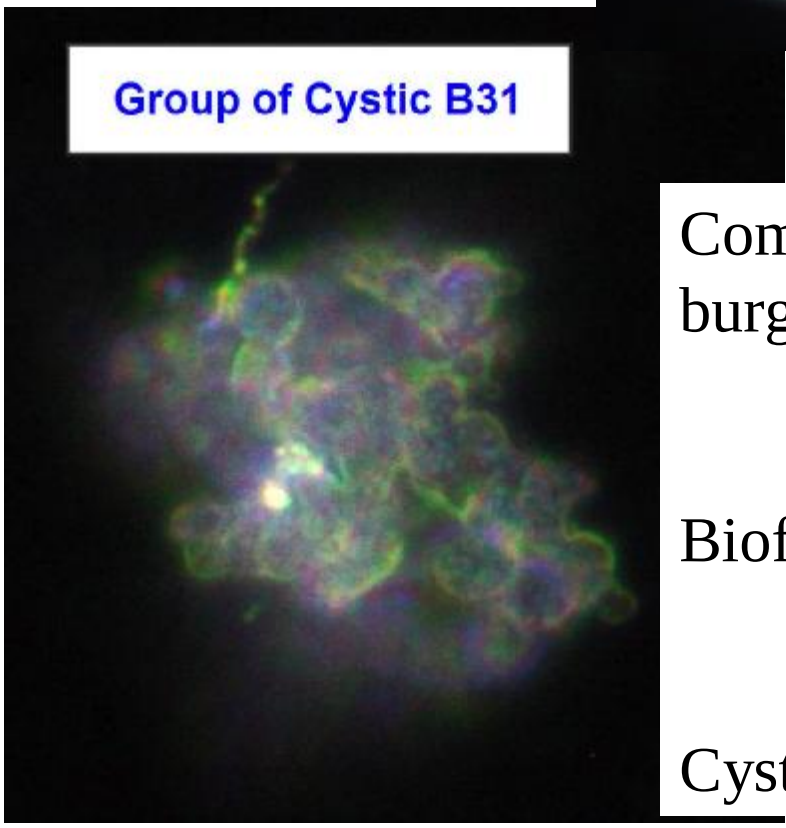


Communities of pure Borrelia burgdorferi

Mixed Cystic and Spiral VARIANT



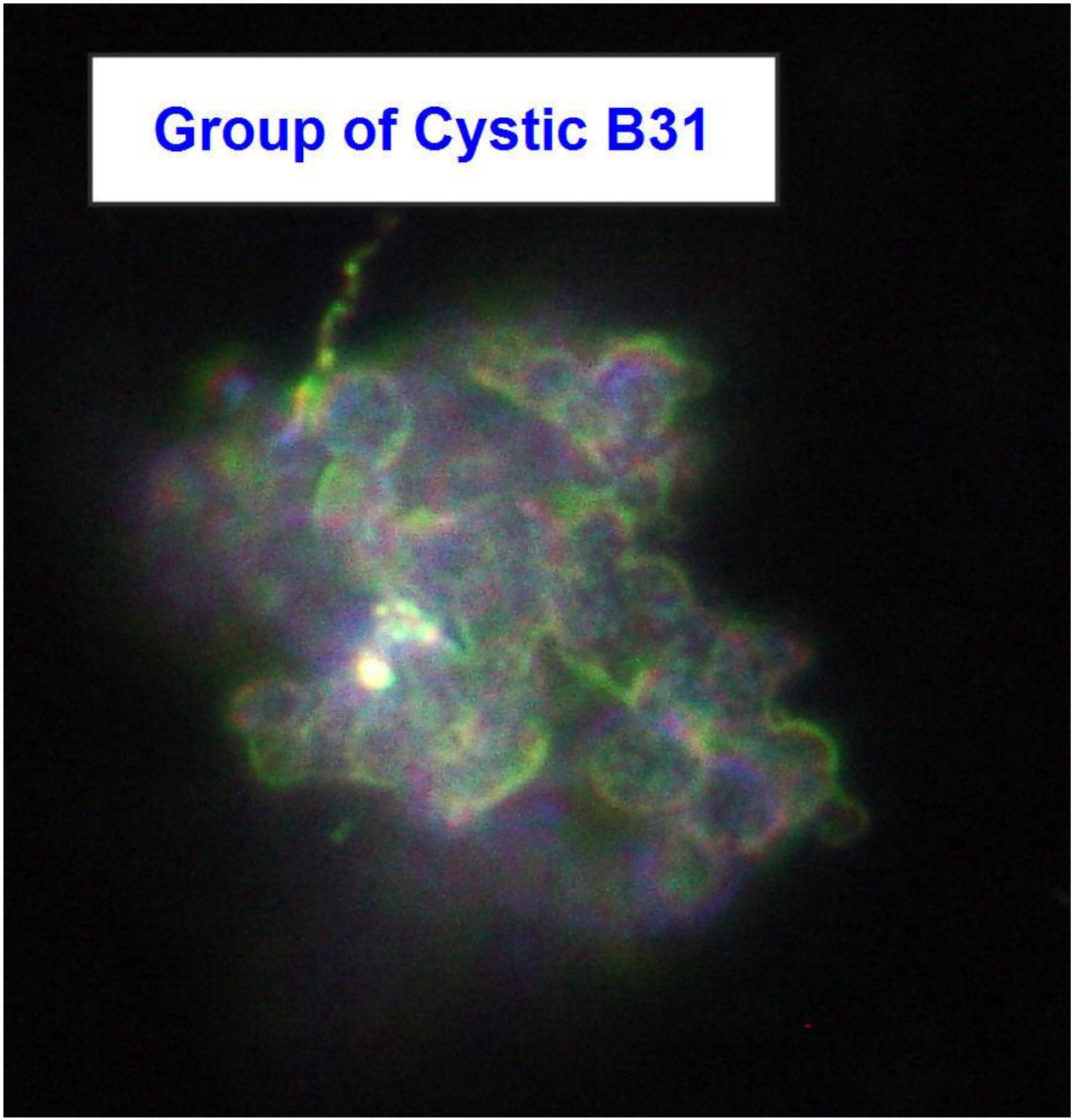


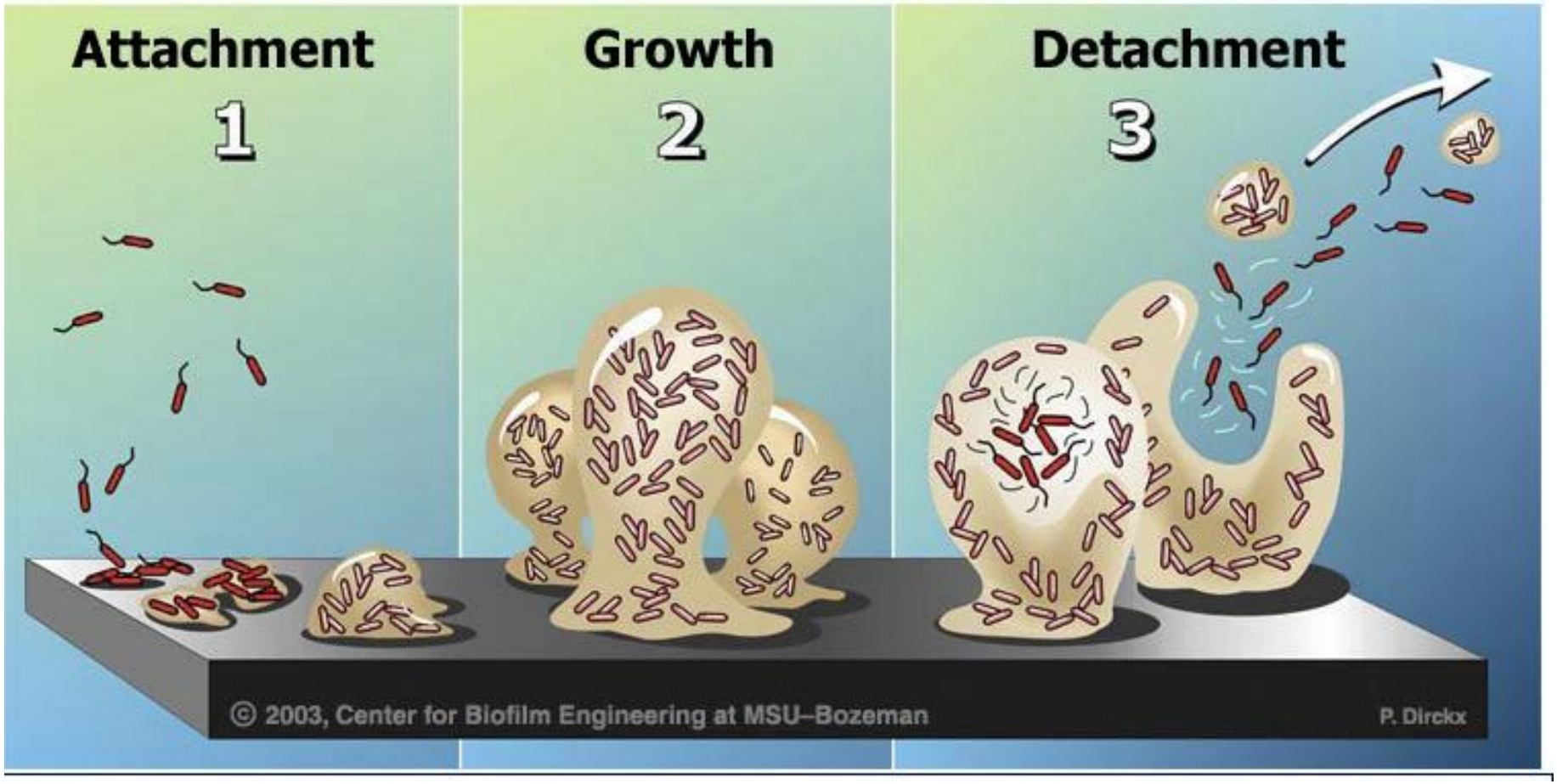


Communities of Pure Borrelia burgdorferi

Biofilm composed of Cystic forms

Cystic Biofilm VARIANT


Group of Cystic B31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Growth
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P. Dirckx

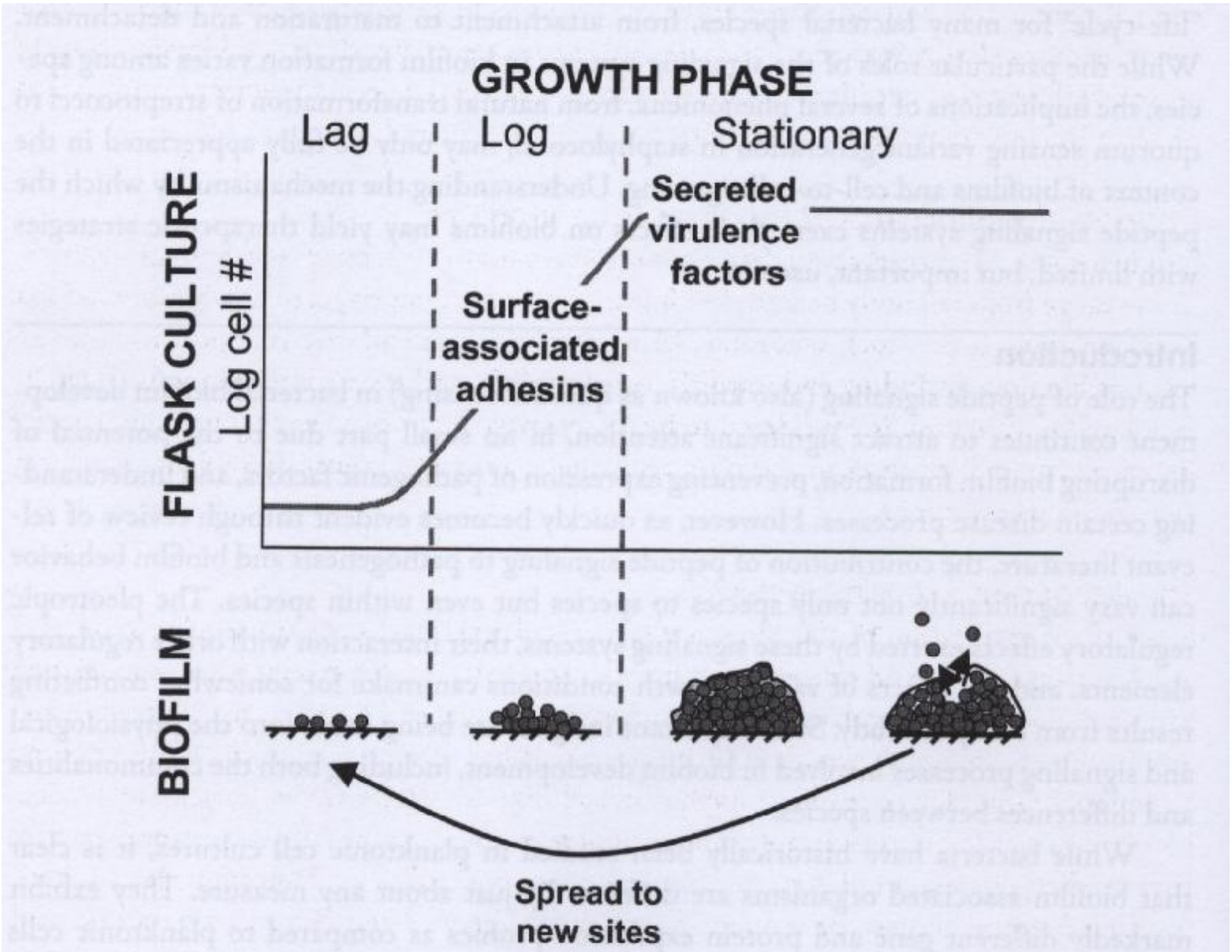
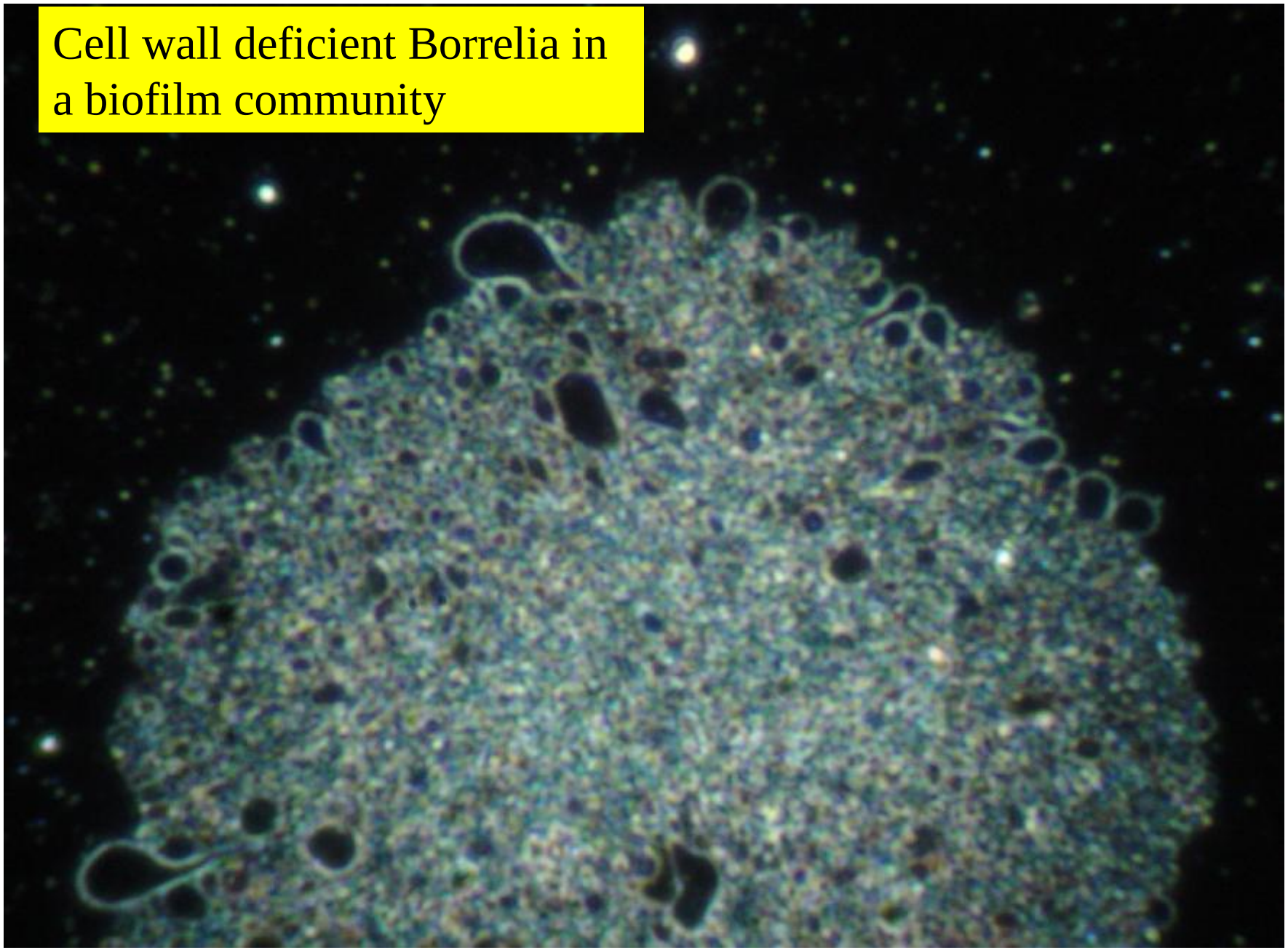
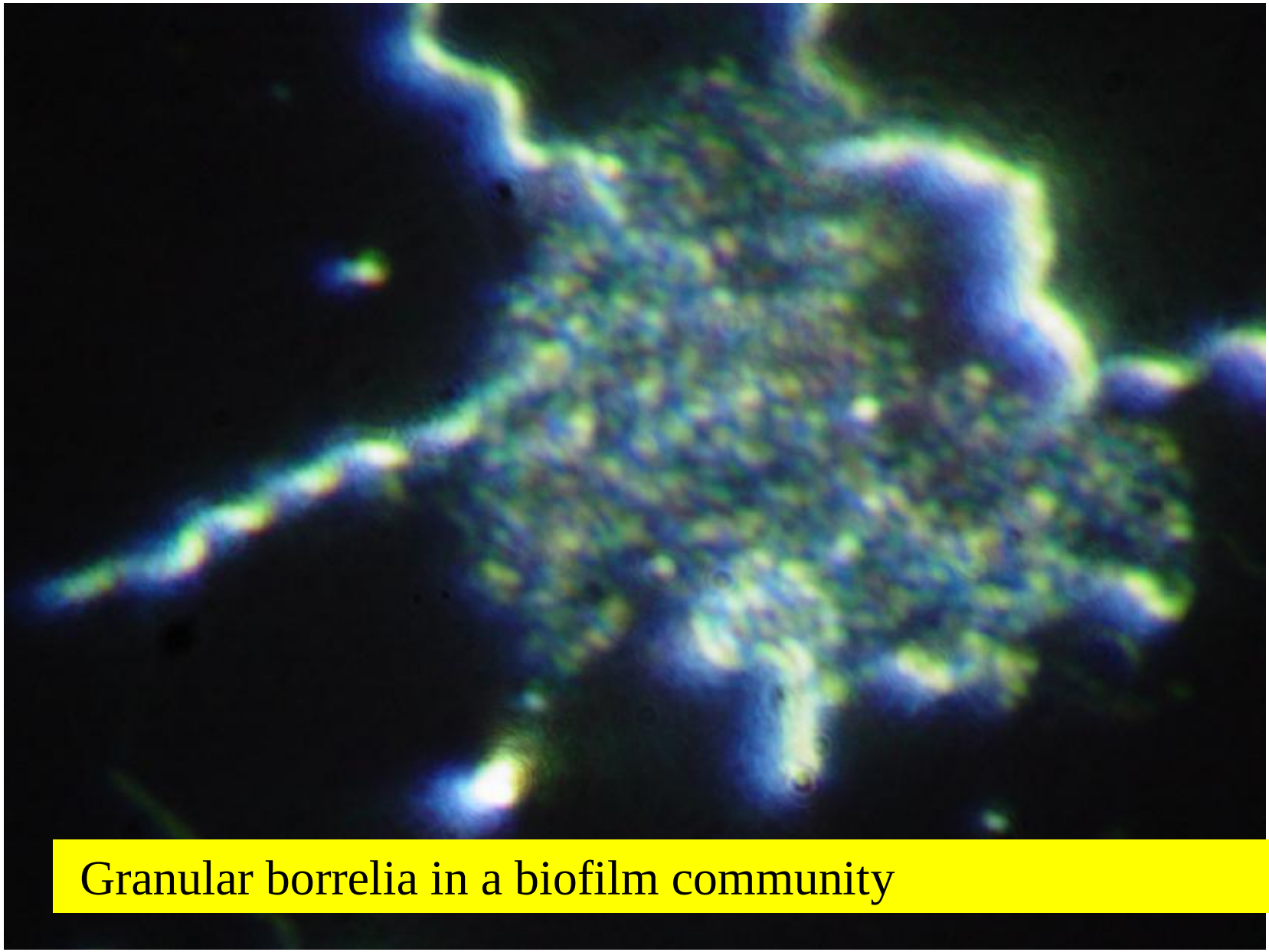
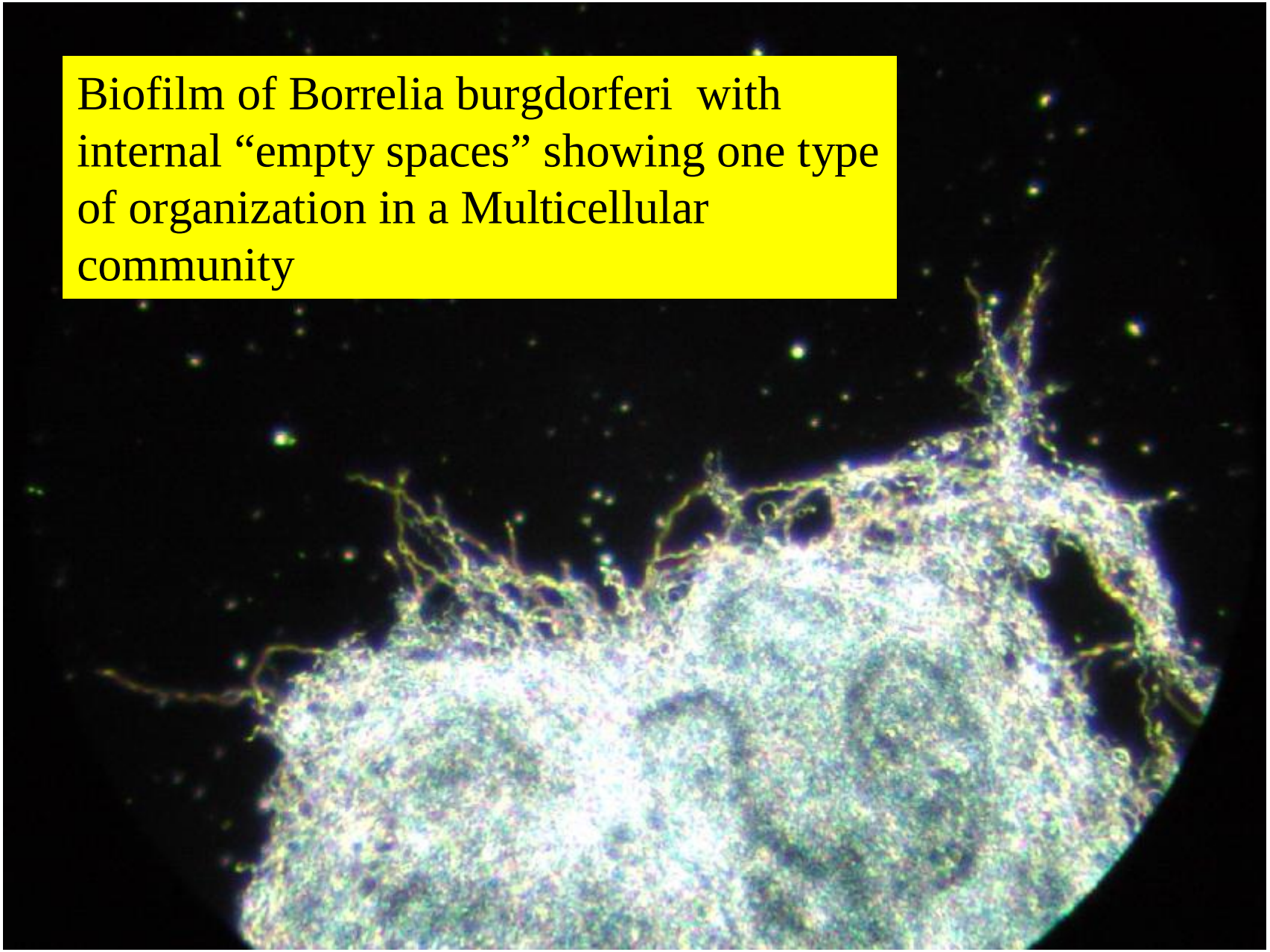


Figure 8.1 Model of staphylococcal virulence gene expression *in vitro* and *in vivo*. Expression

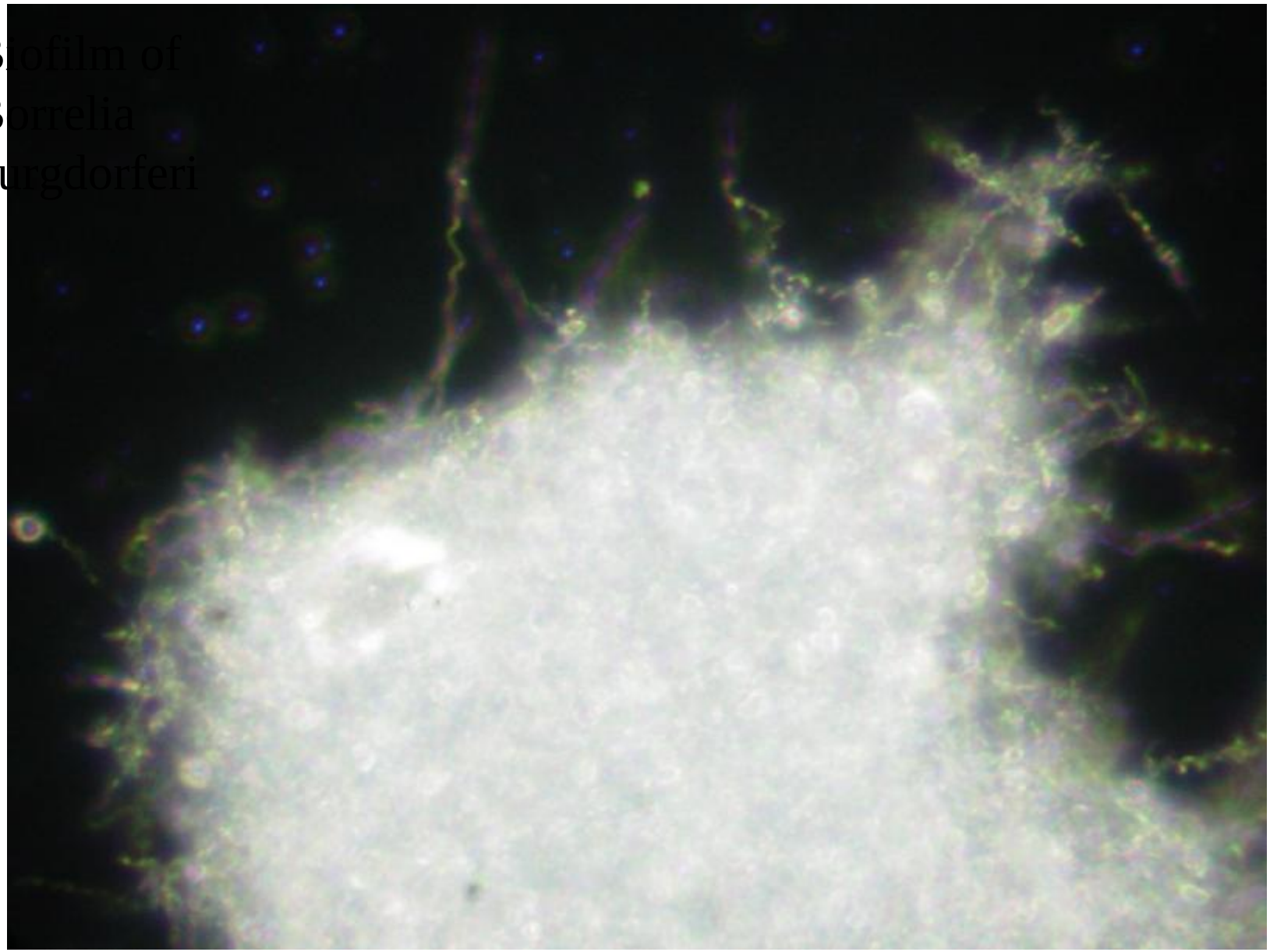

Cell wall deficient Borrelia in
a biofilm community

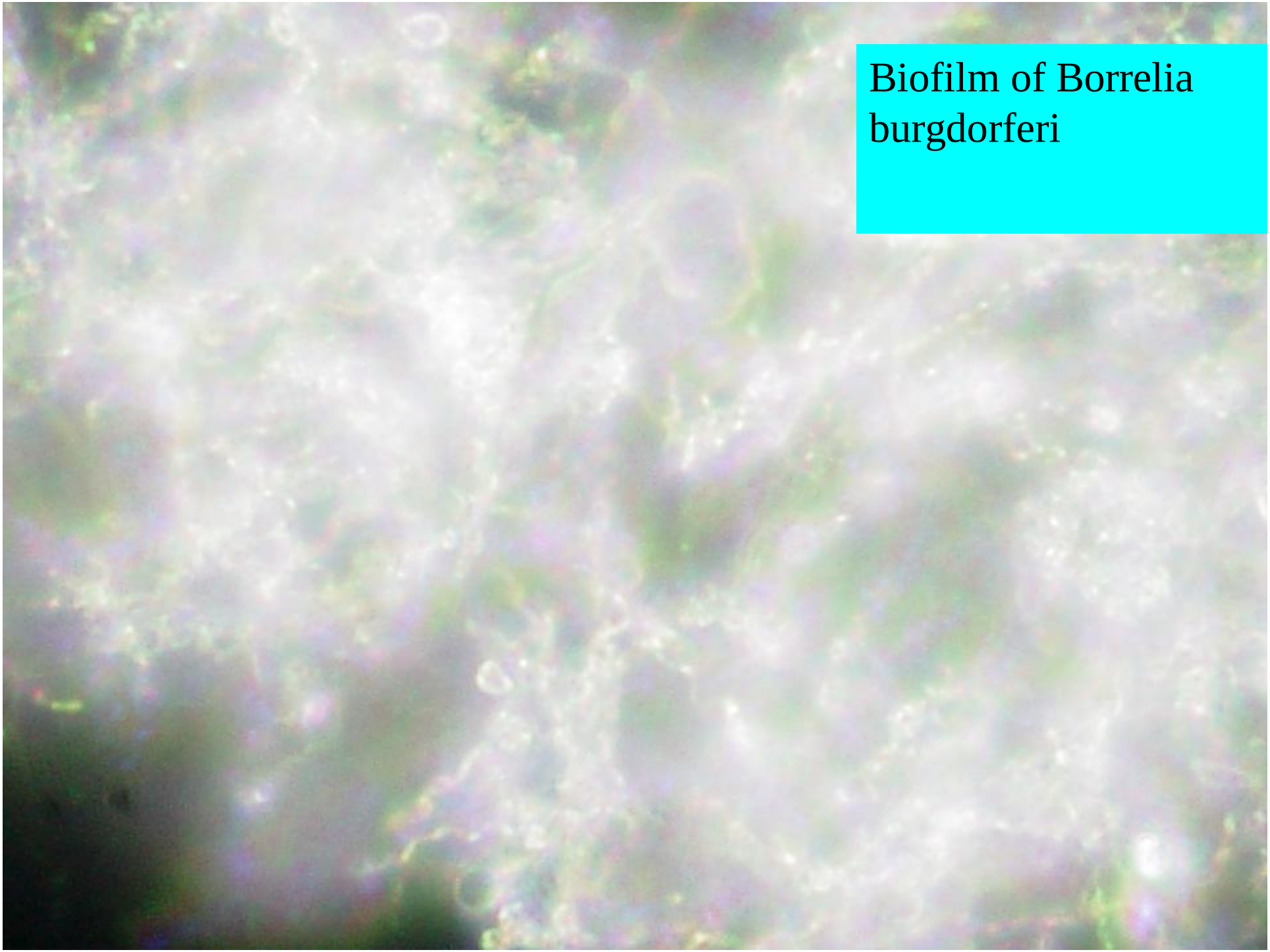


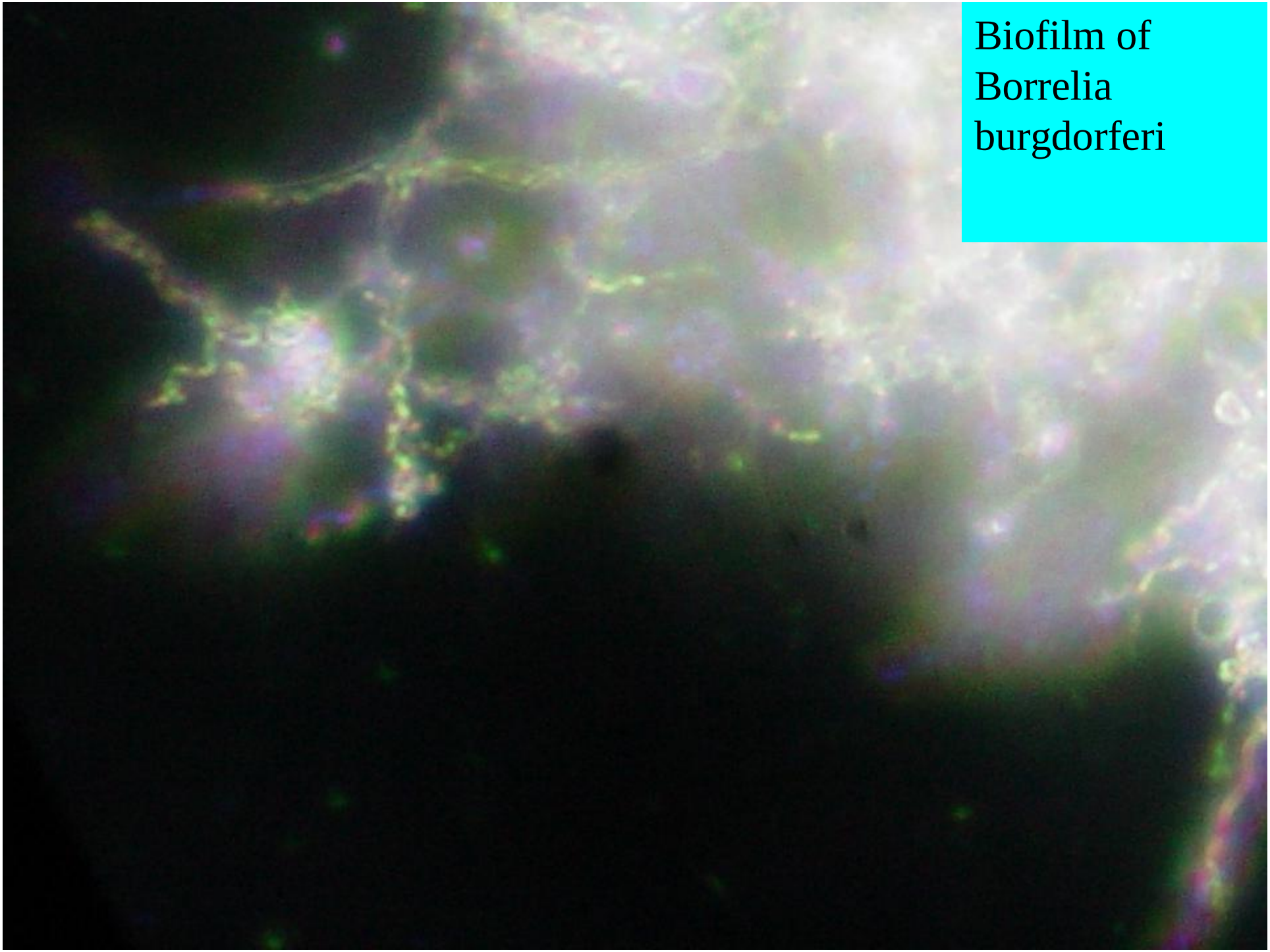
Granular borrelia in a biofilm community

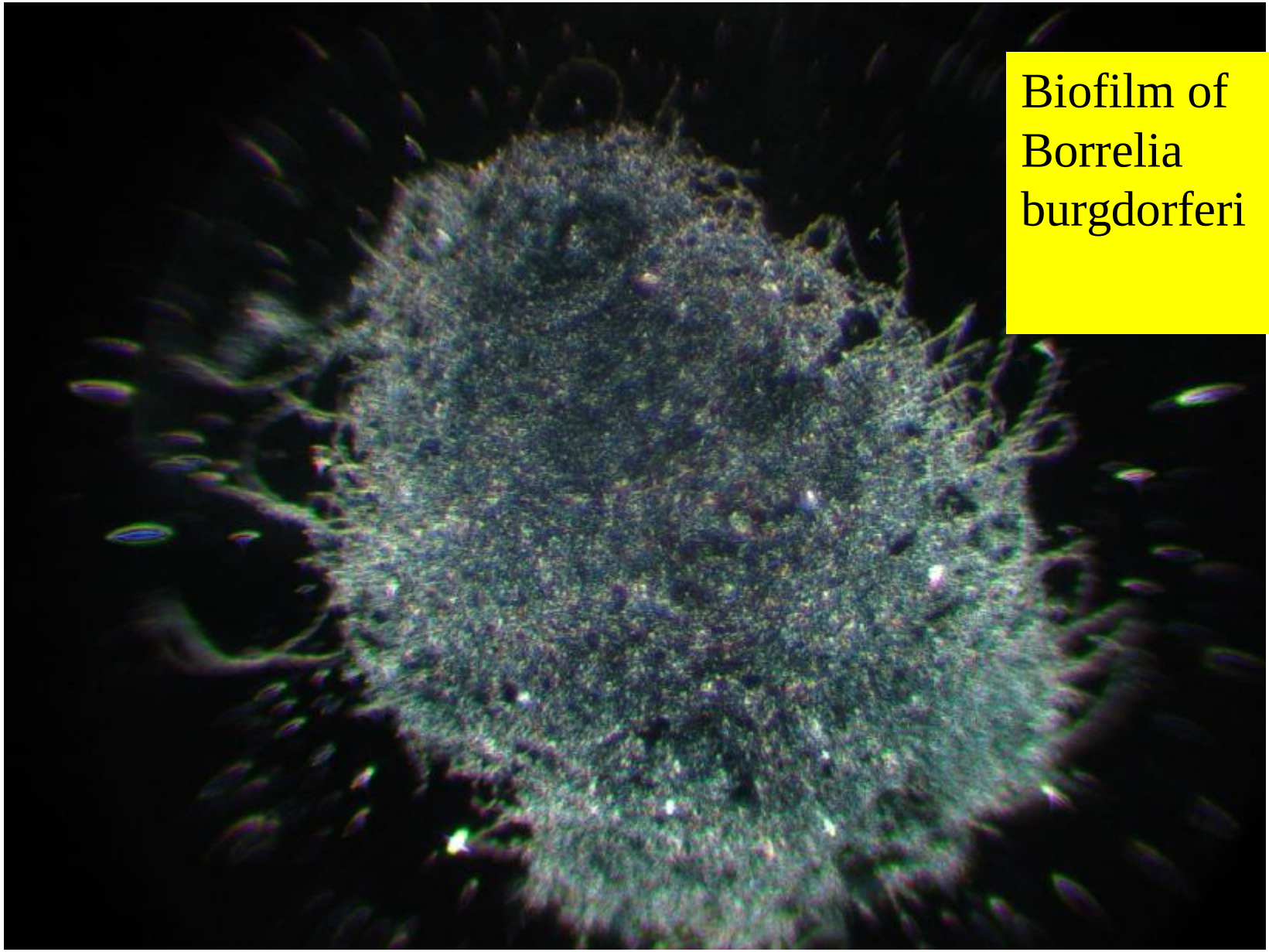

Biofilm of Borrelia burgdorferi with internal “empty spaces” showing one type of organization in a Multicellular community

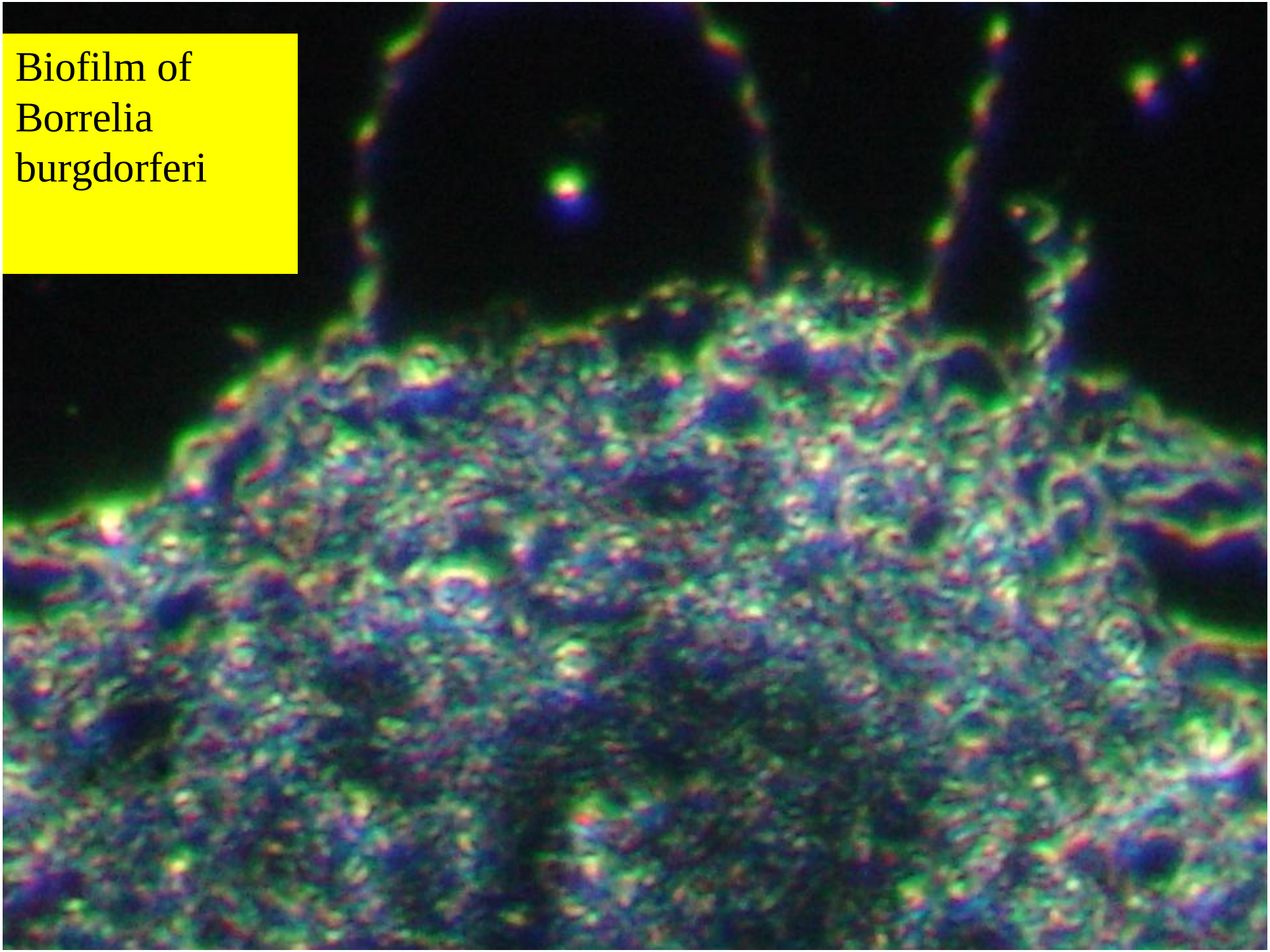
Biofilm of Borrelia burgdorferi

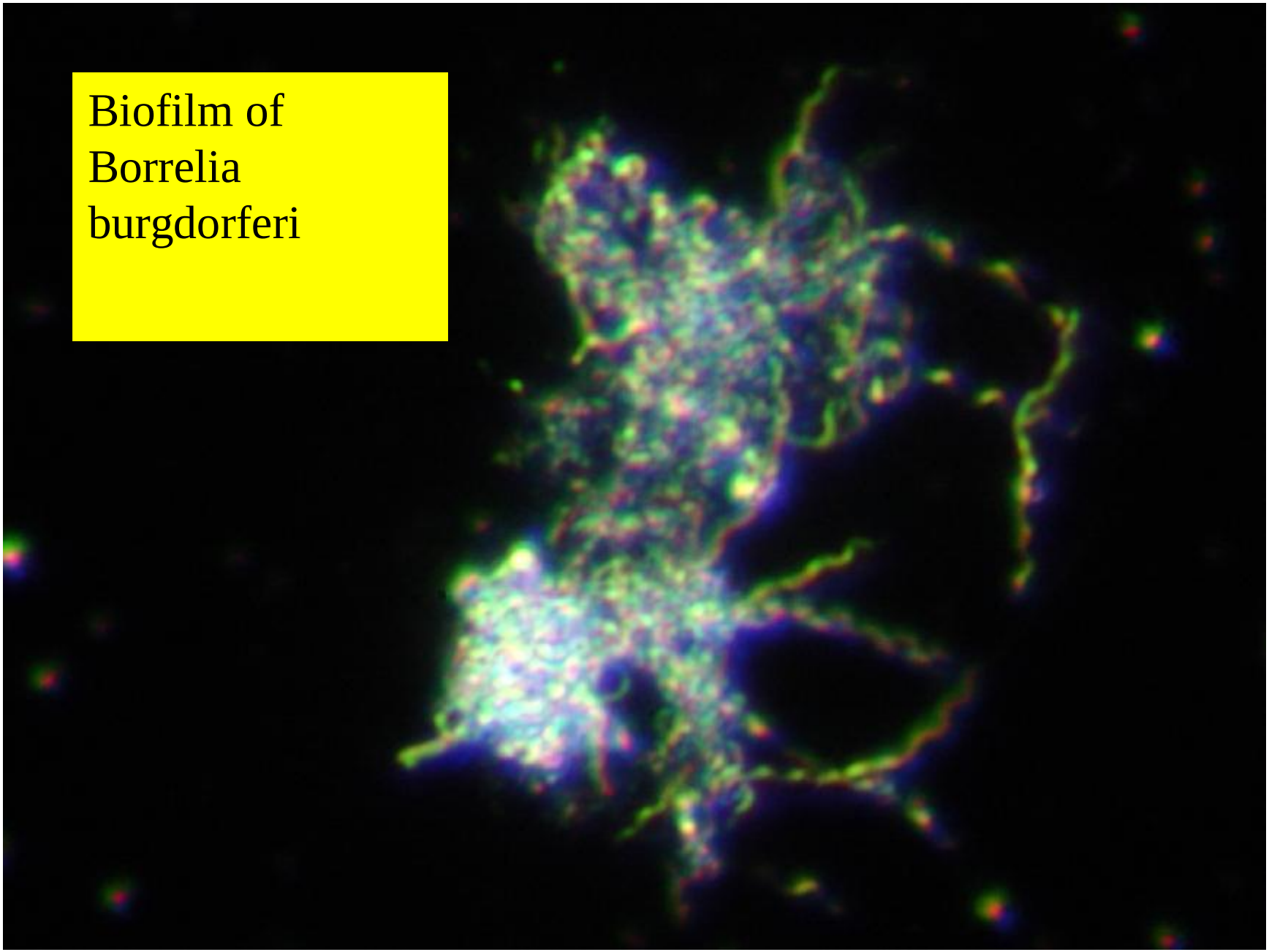


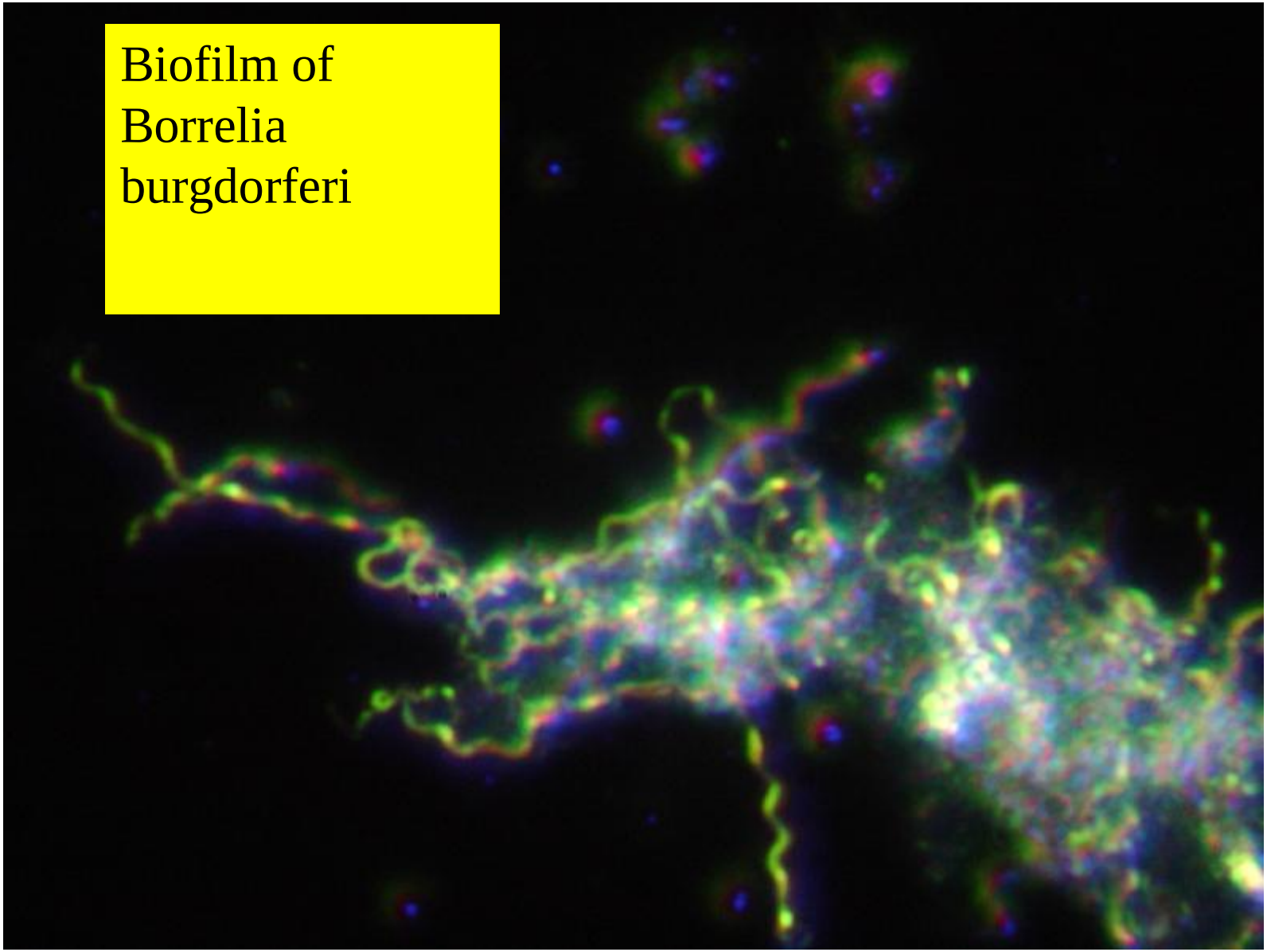

Biofilm of Borrelia burgdorferi

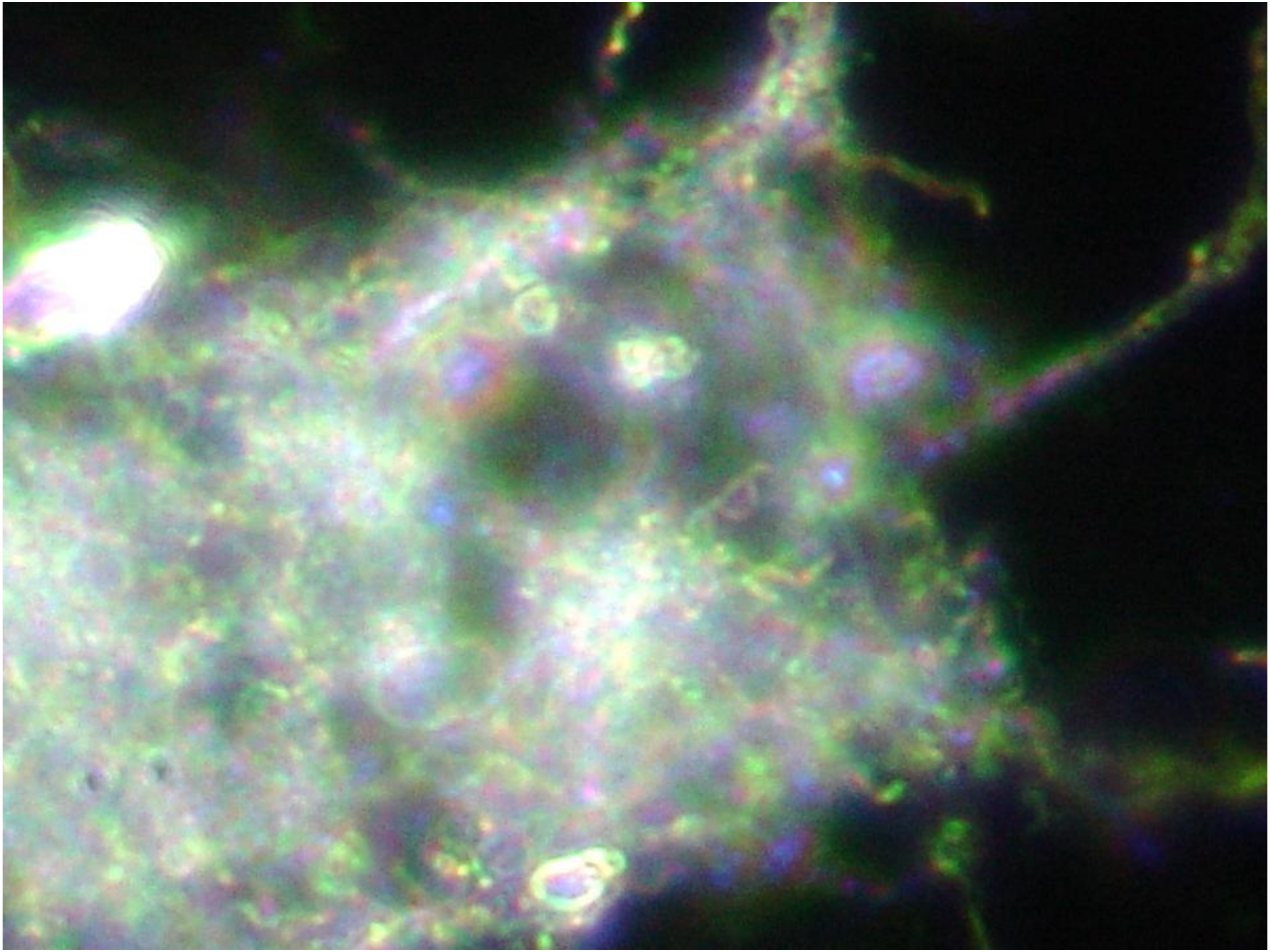

Biofilm of
Borrelia
burgdorferi


Biofilm of
Borrelia
burgdorferi


Biofilm of
Borrelia
burgdorferi

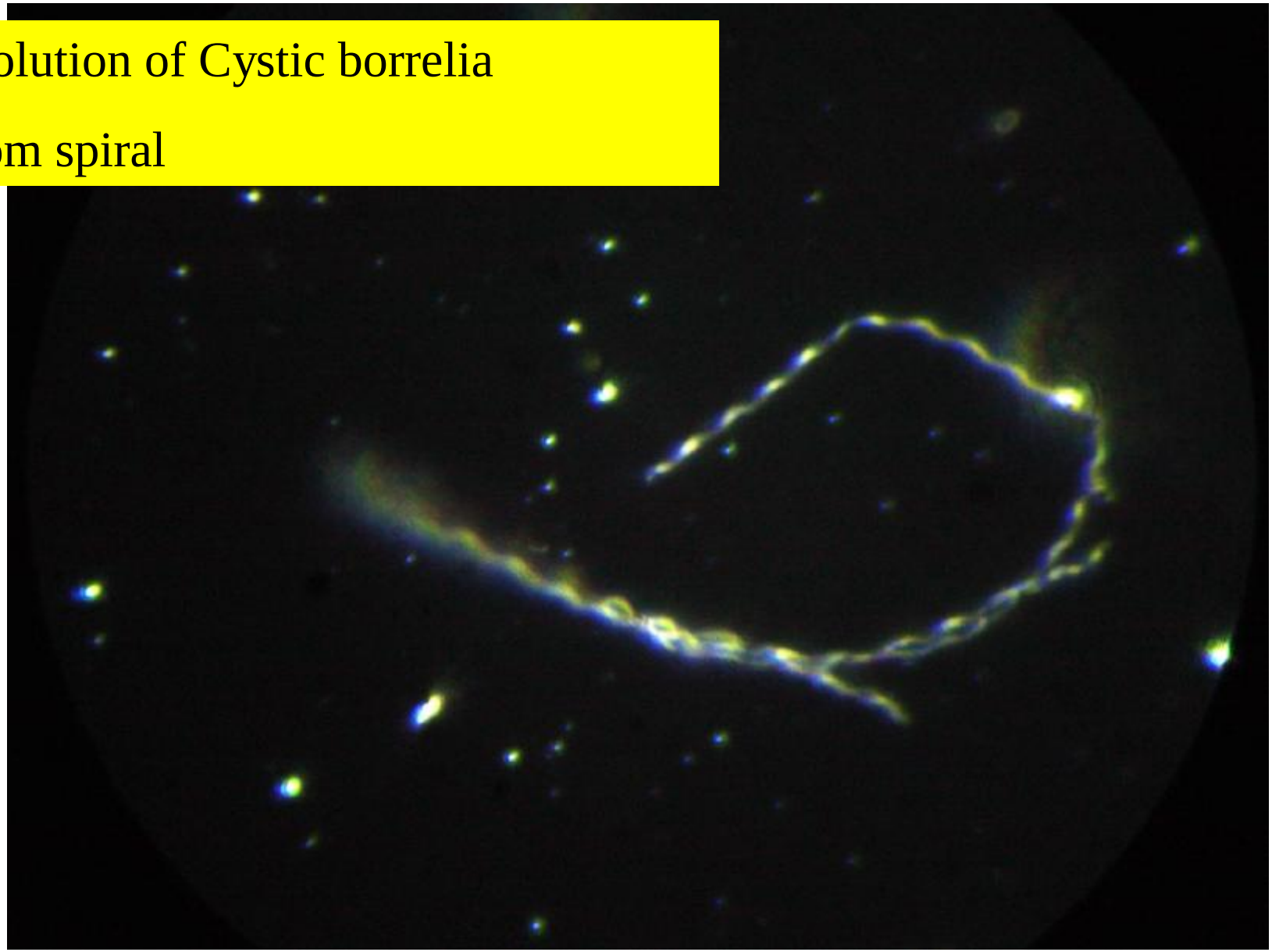

Biofilm of
Borrelia
burgdorferi


Biofilm of
Borrelia
burgdorferi



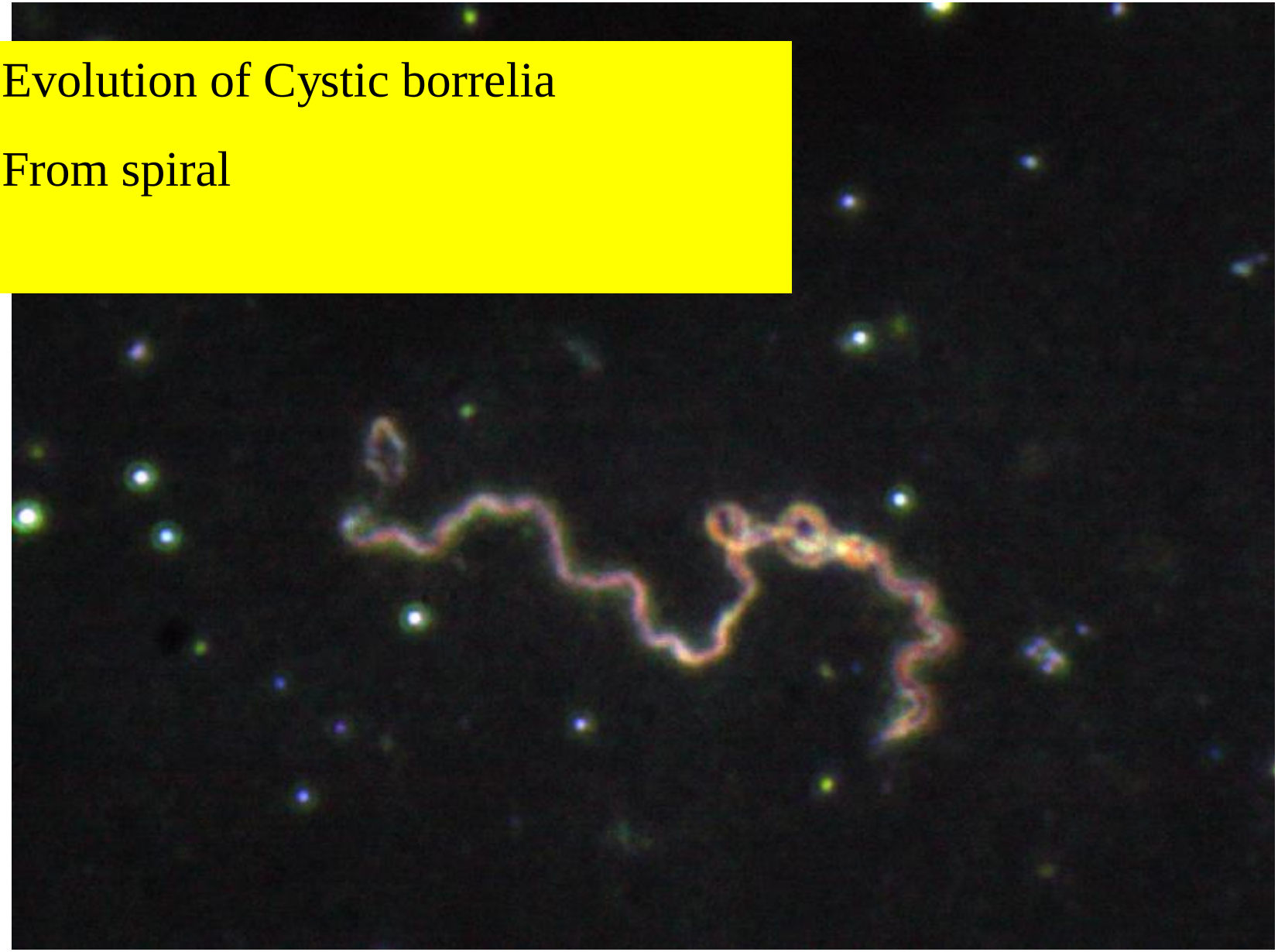
Evolution of Cystic borrelia

From spiral



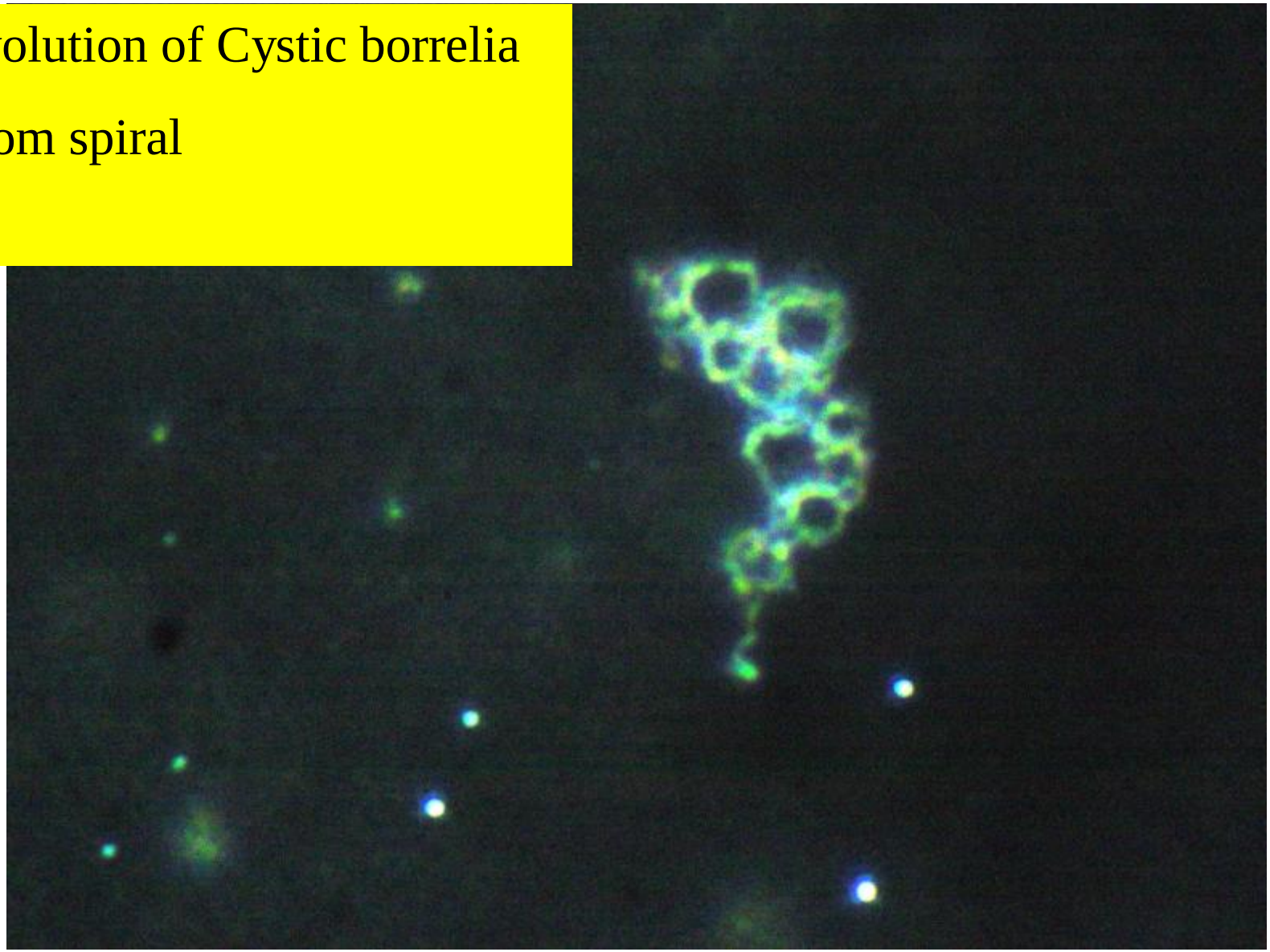
Evolution of Cystic borrelia

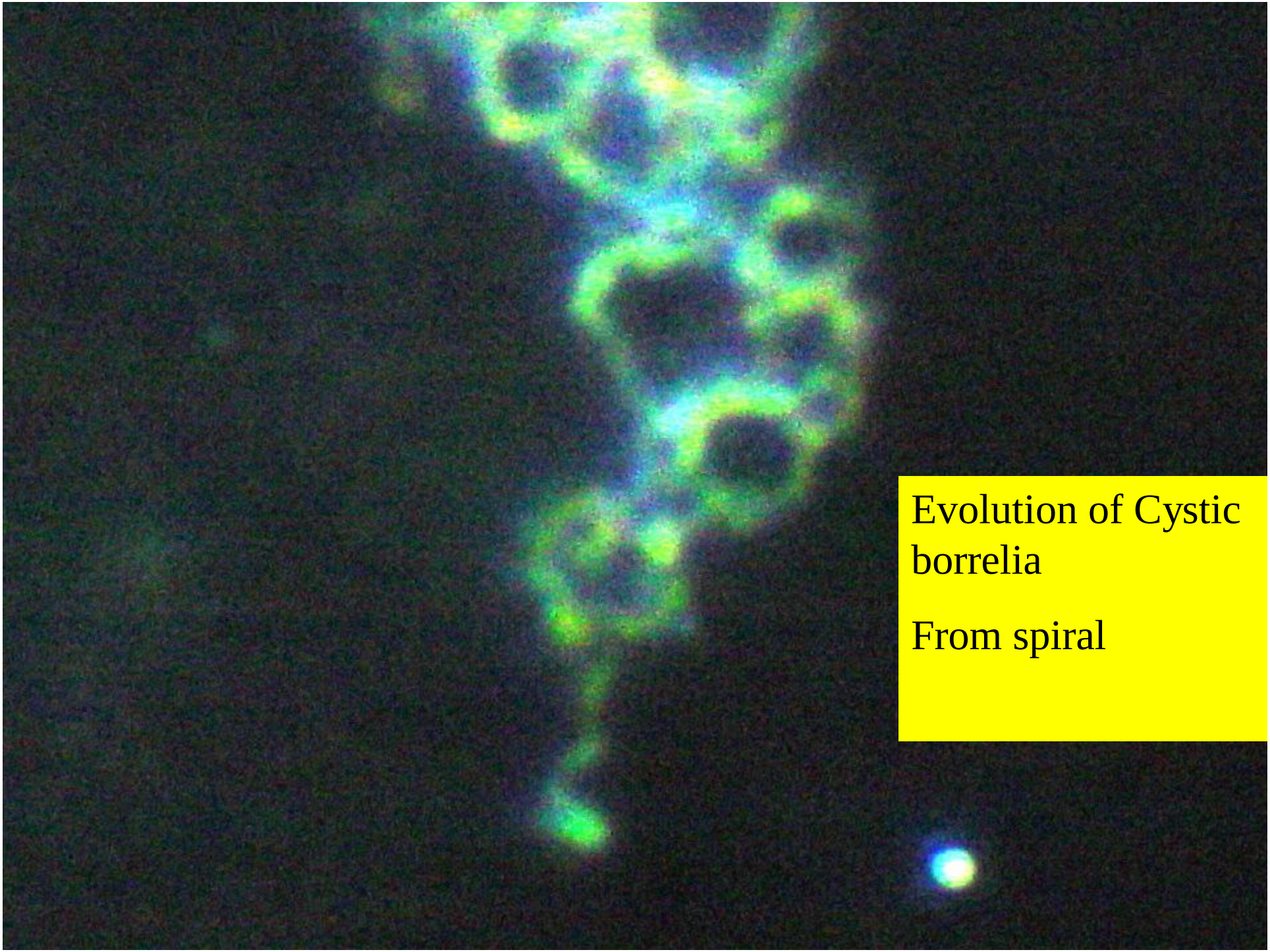
From spiral

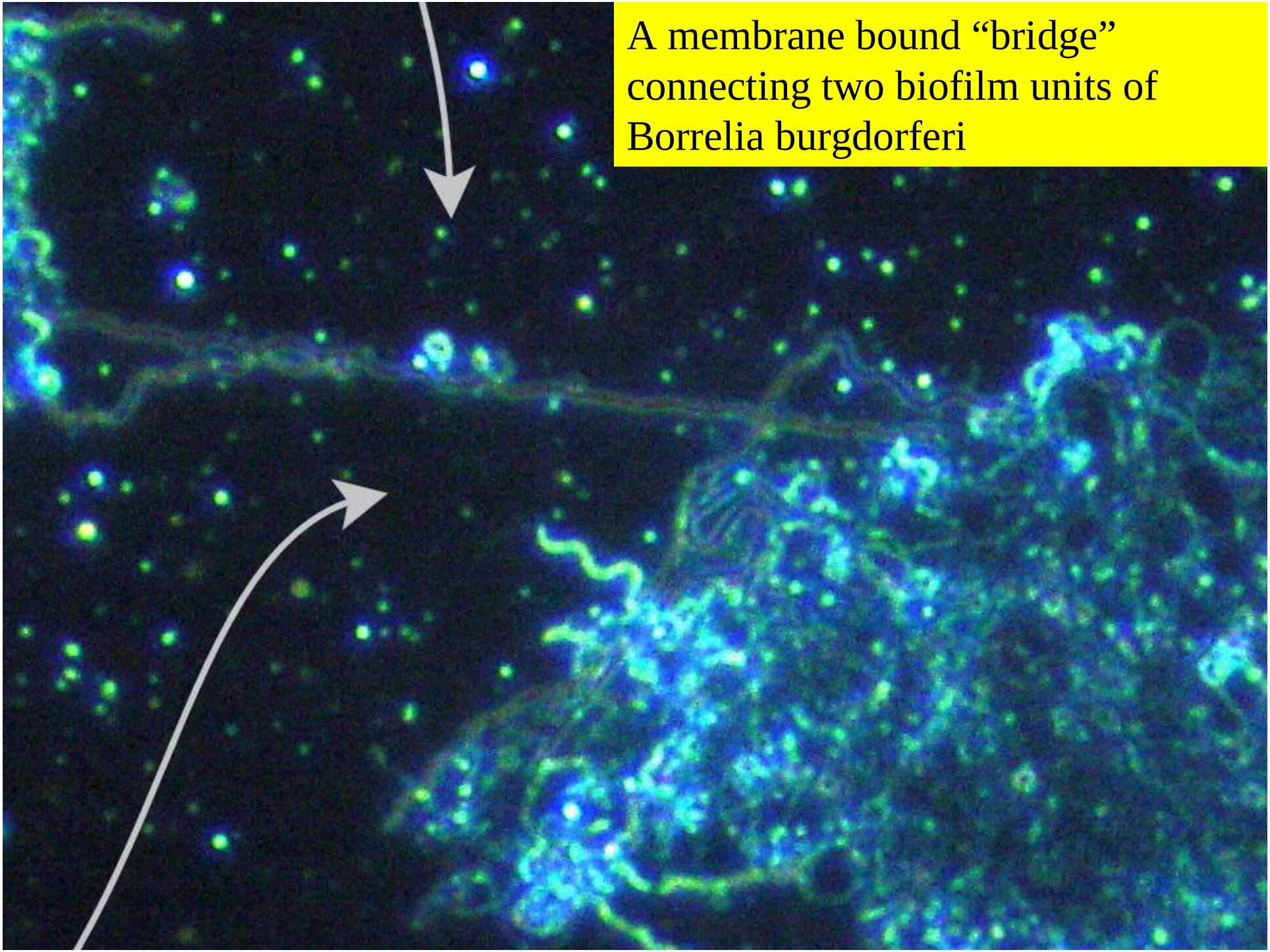


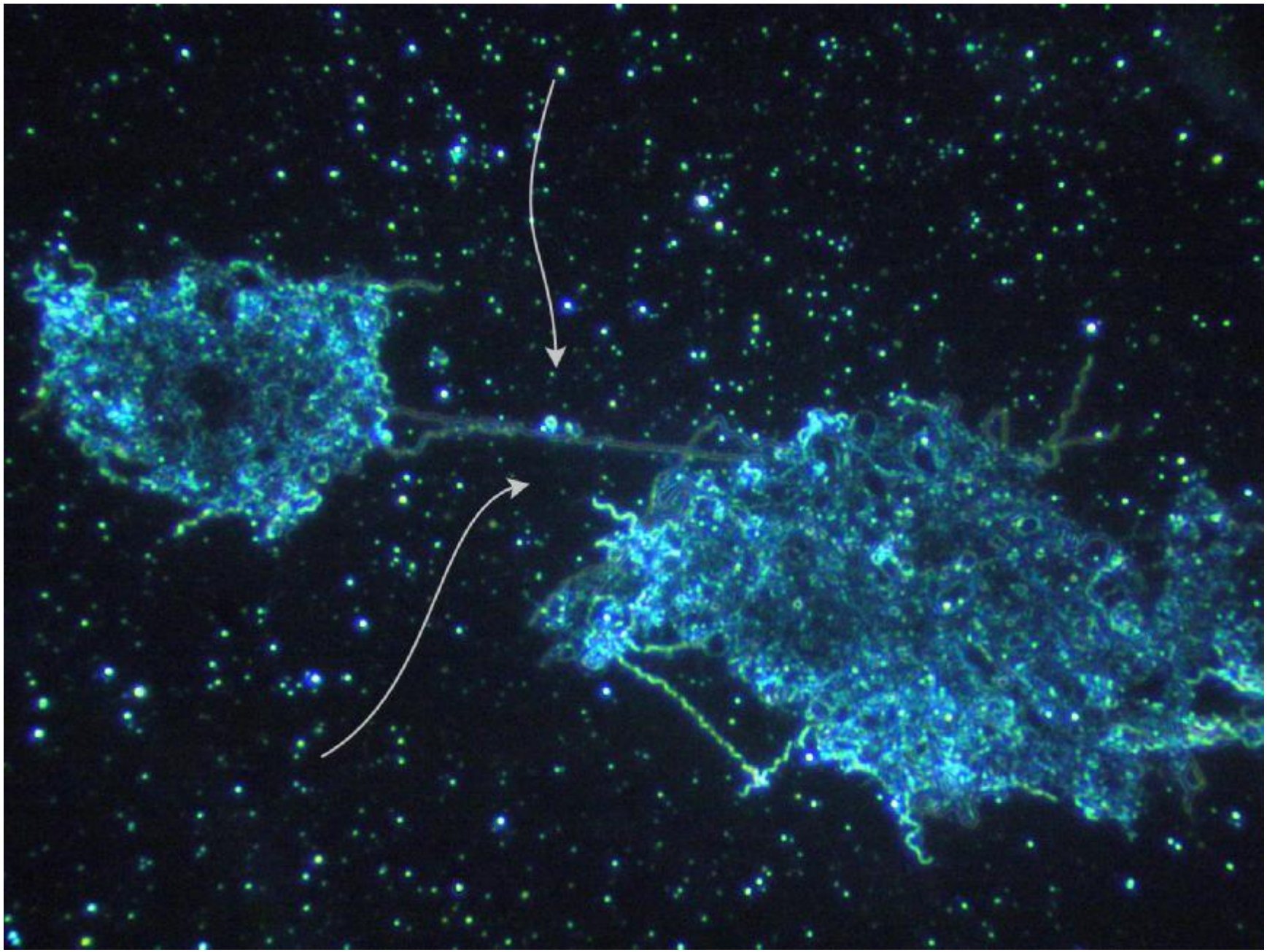
Evolution of Cystic borrelia

From spiral




Evolution of Cystic borrelia
From spiral


A membrane bound “bridge” connecting two biofilm units of Borrelia burgdorferi



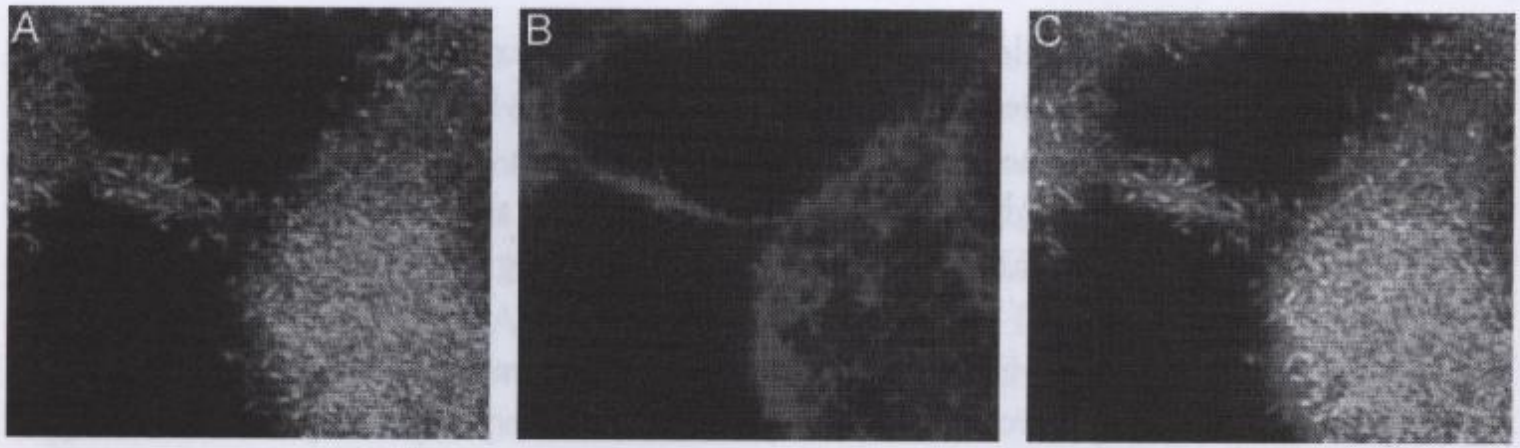
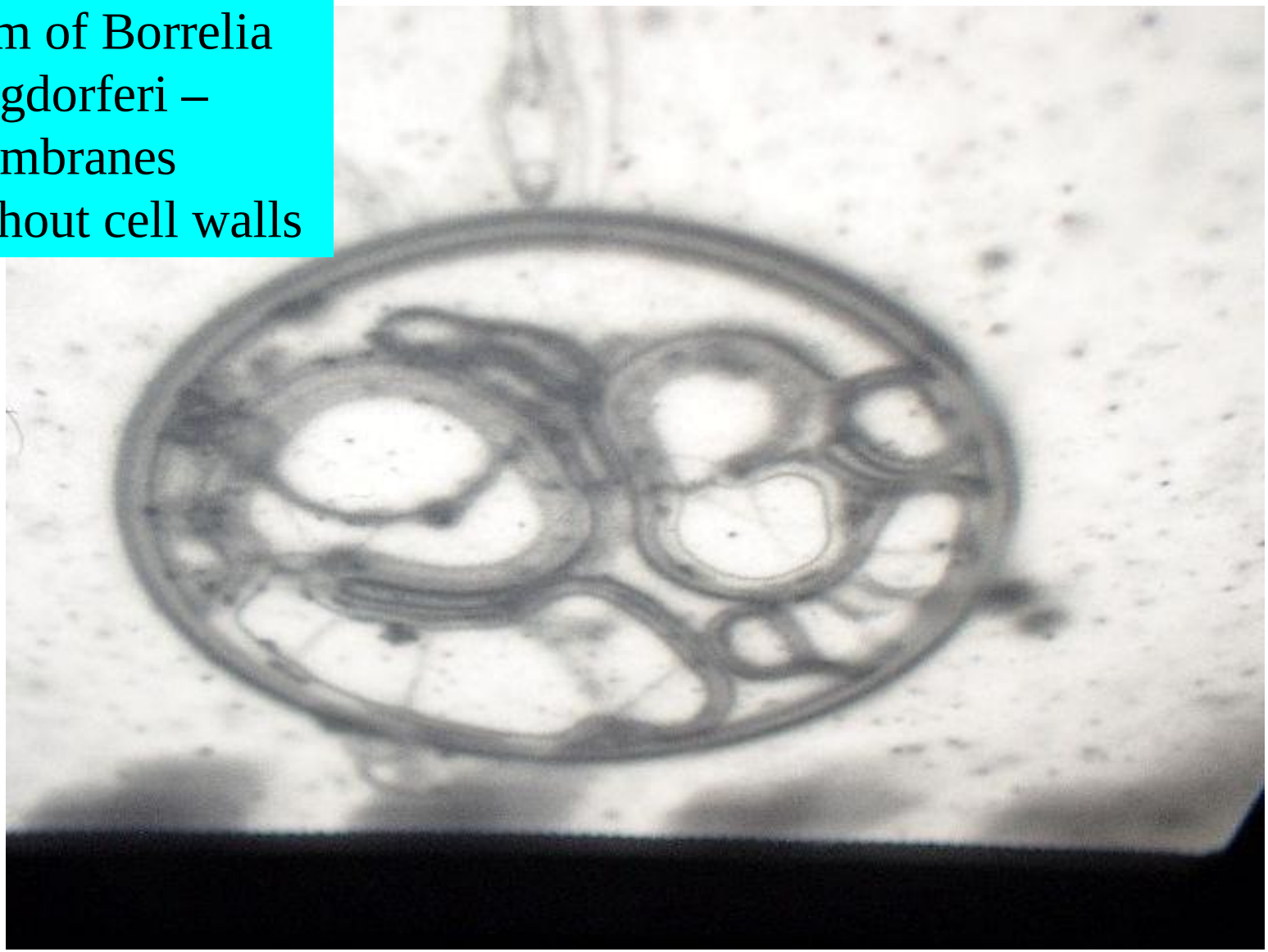
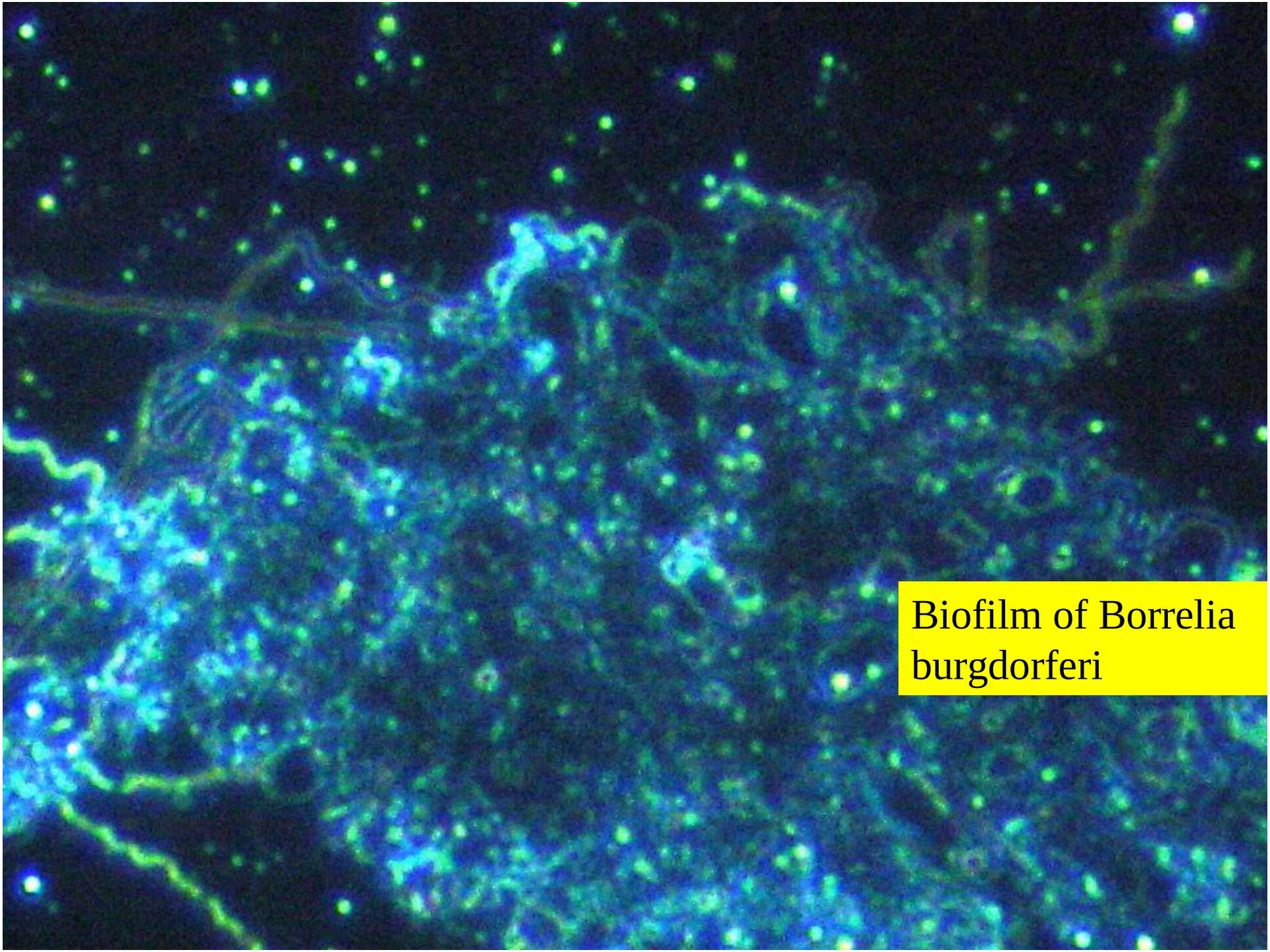


Figure 4.3 Horizontal confocal laser scanning microscope sections in a 2-day-old DDAO-stained biofilm formed by Gfp-tagged *P. aeruginosa* PAO1. The images show the fluorescent bacteria (A), the fluorescent extracellular DNA (B), and an overlay of the two (C). Reproduced from Mol. Microbiol. 59:1114–1128 with permission from Blackwell Publishing.

same frequencies as when transformation was done with an equivalent amount of purified

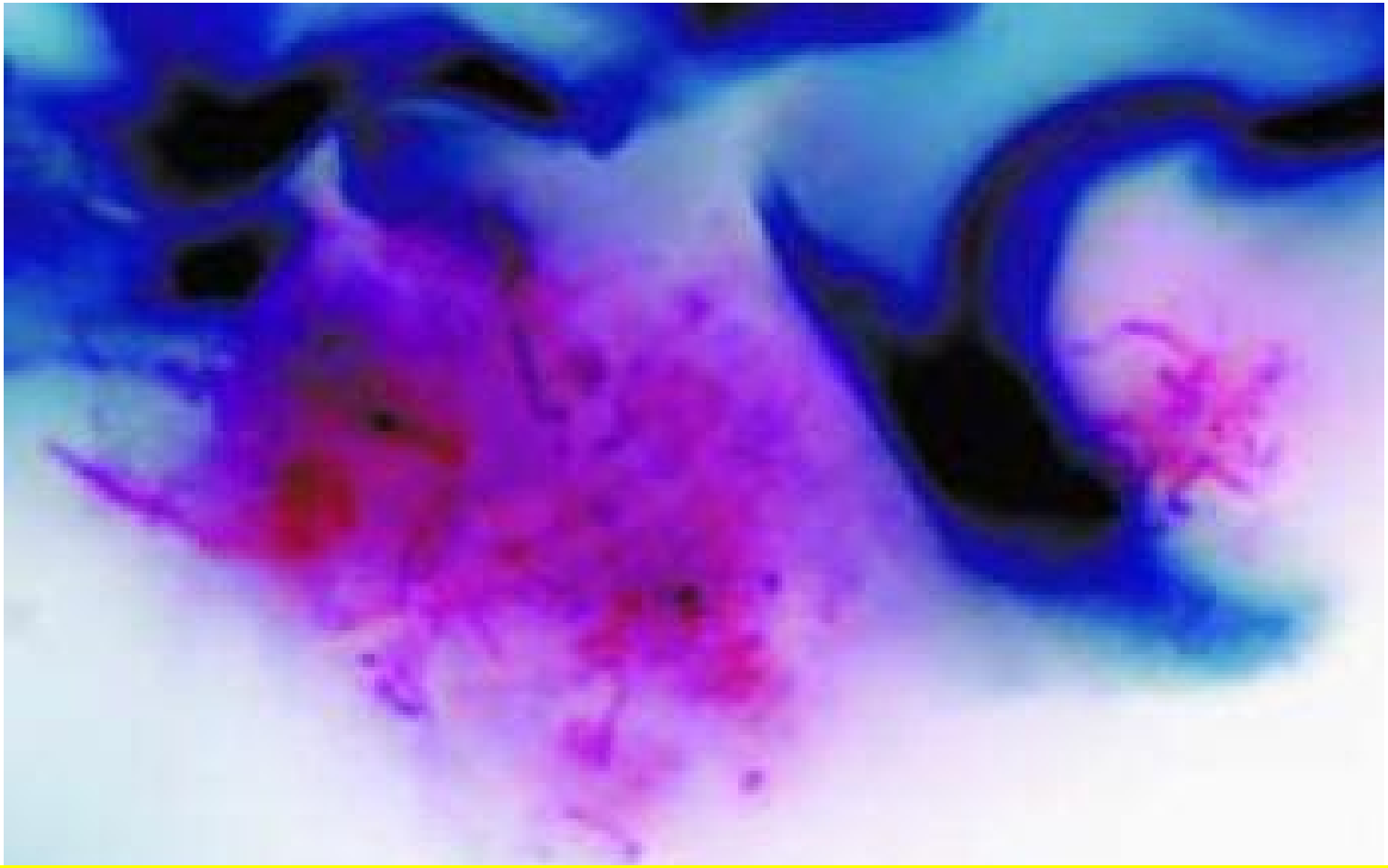
Cell wall deficient form of Borrelia burgdorferi – Membranes without cell walls




Biofilm of Borrelia burgdorferi

Dr K. Eisendle. BORRELIA LYMPHOCYTOMA

IMMUNOHISTOCHEMISTRY AJCP 2007,127:213-222



Two groupings of Borrelia burgdorferi in Skin – Biofilms

Dr K. Eisendle.

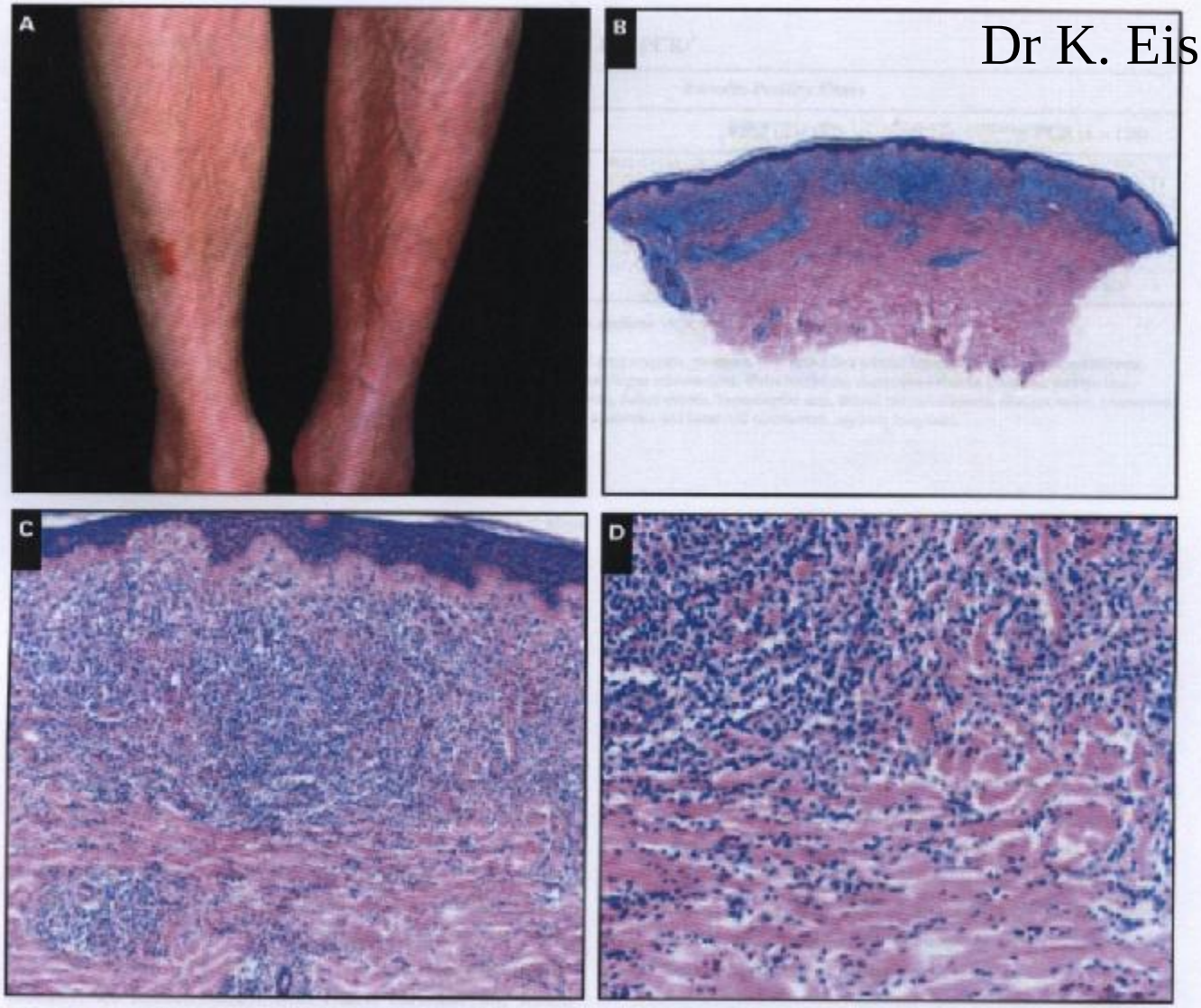
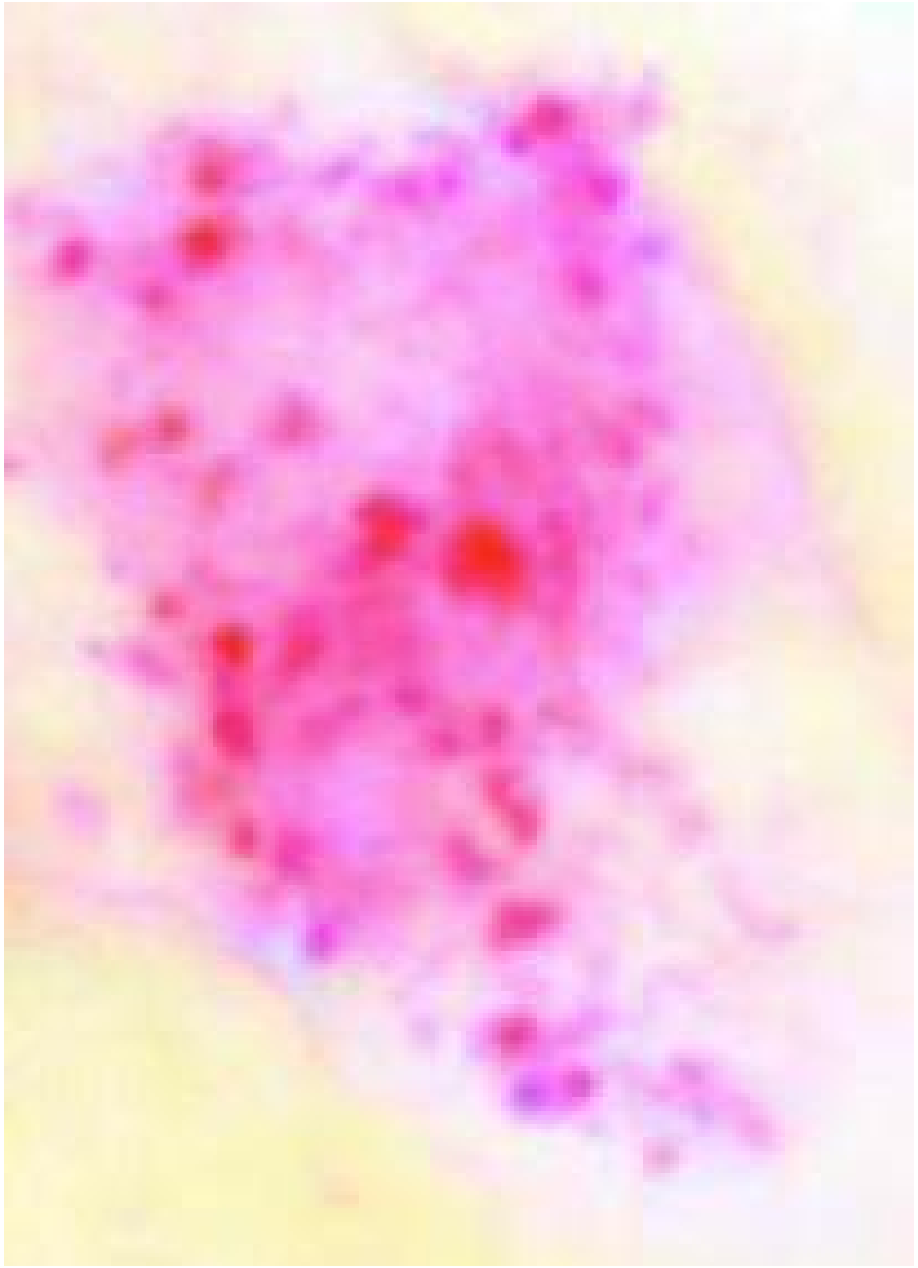


Image 3 **A**, Acrodermatitis chronica atrophicans of left leg characterized by ill-defined, hyperpigmented, and atrophic patch (note prominent veins). **B**, Histologic examination (H&E, ×10) reveals a dense lichenoid and middermal perivascular infiltrate with hints of follicle formation (**C**, H&E, ×100) composed of lymphocytes, some plasma cells, and an increase of fibroblasts between fibrosclerotic collagen bundles (**D**, H&E, ×200).



Dr K. Eisendle

Acrodermatitis Chronica Atrophicans

Immunohistochemistry

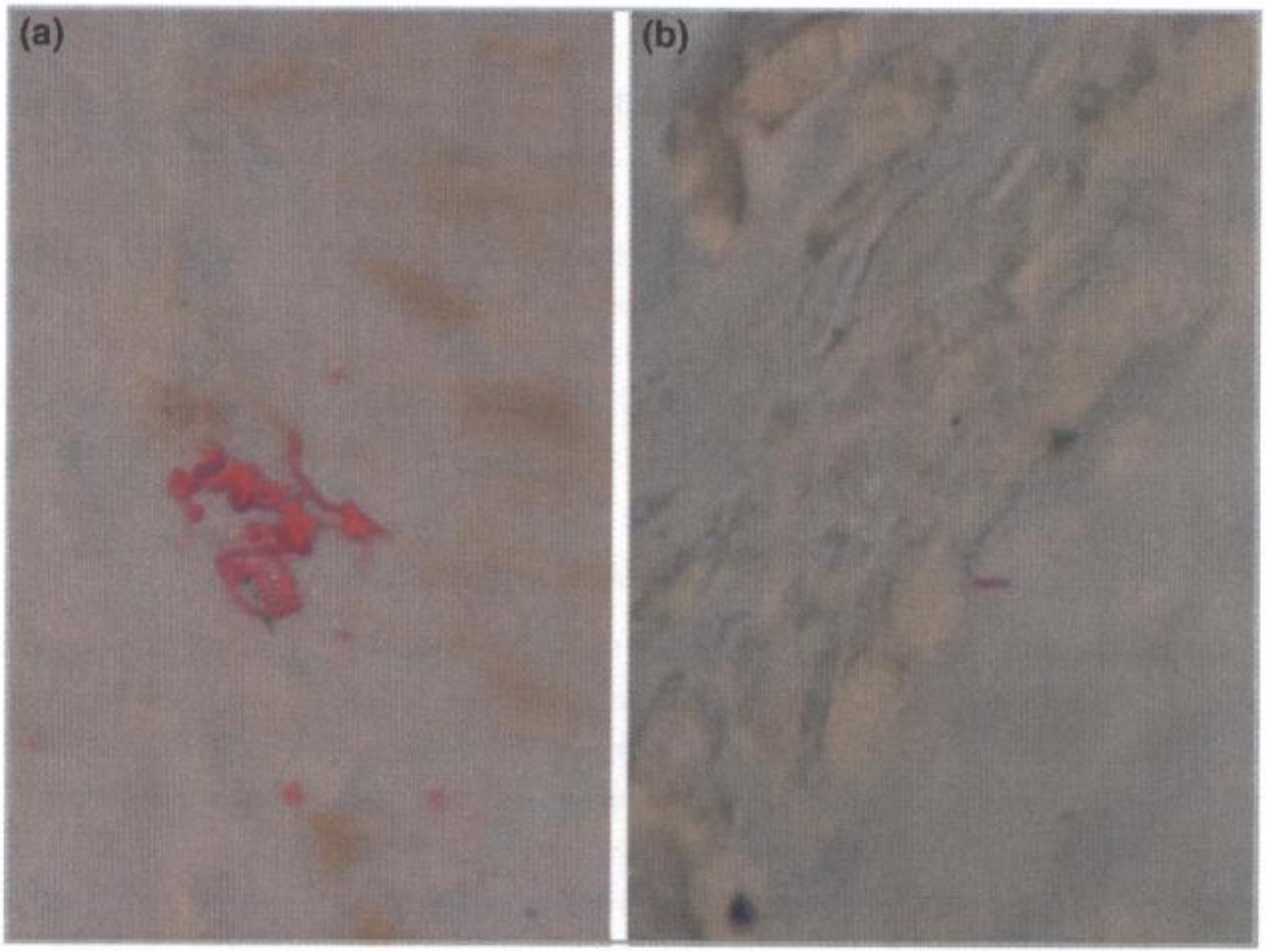
“Granular forms of B burgdorferi in a “colony”

With a “Reddish veil”

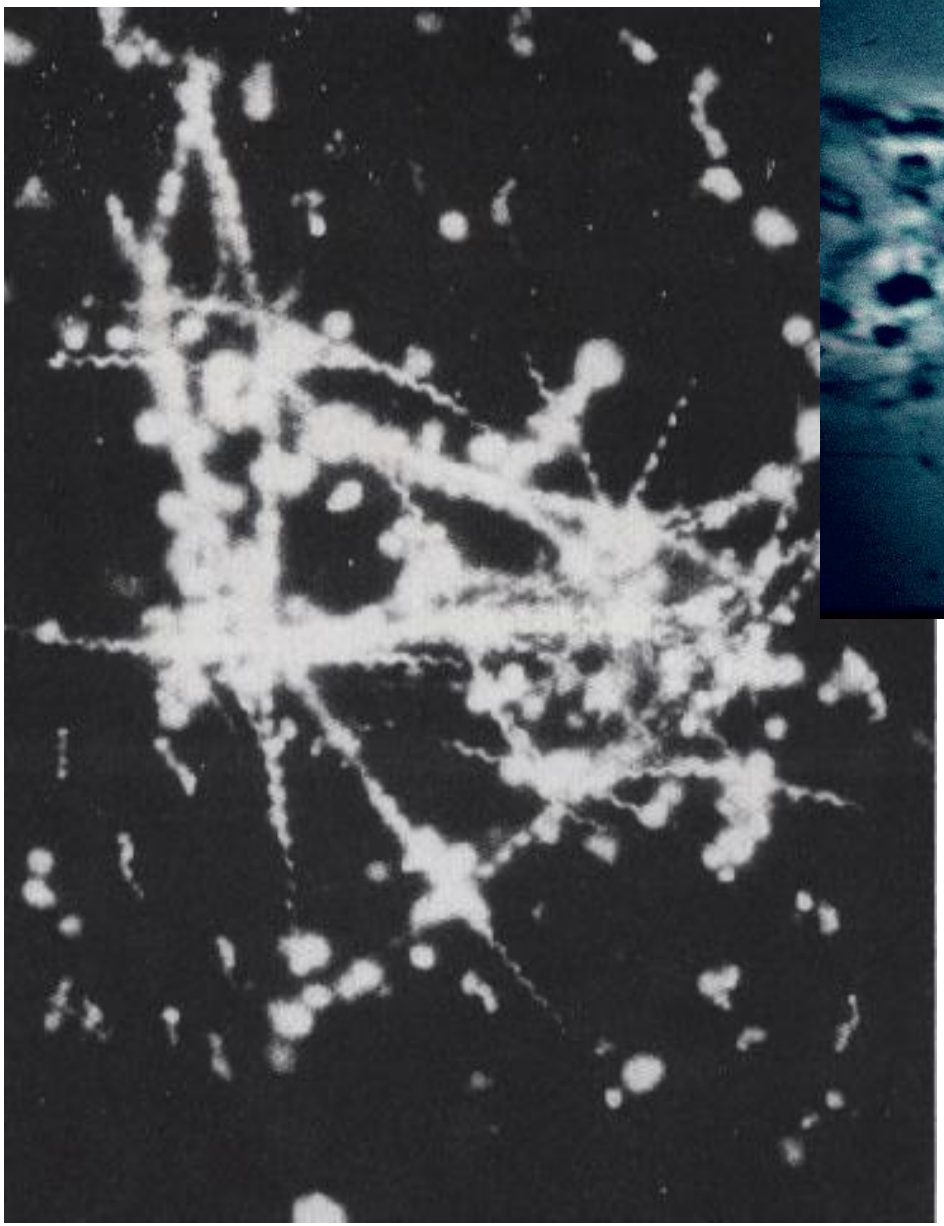
A colony of granular Borrelia burgdorferi

- Reclassified as a Biofilm unit in Skin of ACA

Eisendle et al, “ Morphea” a manifestation of infection with Borrelia species”, British J Dermatology 2007, 157:1189-1198



Morphea – with biofilm-like “clump” of Borrelia



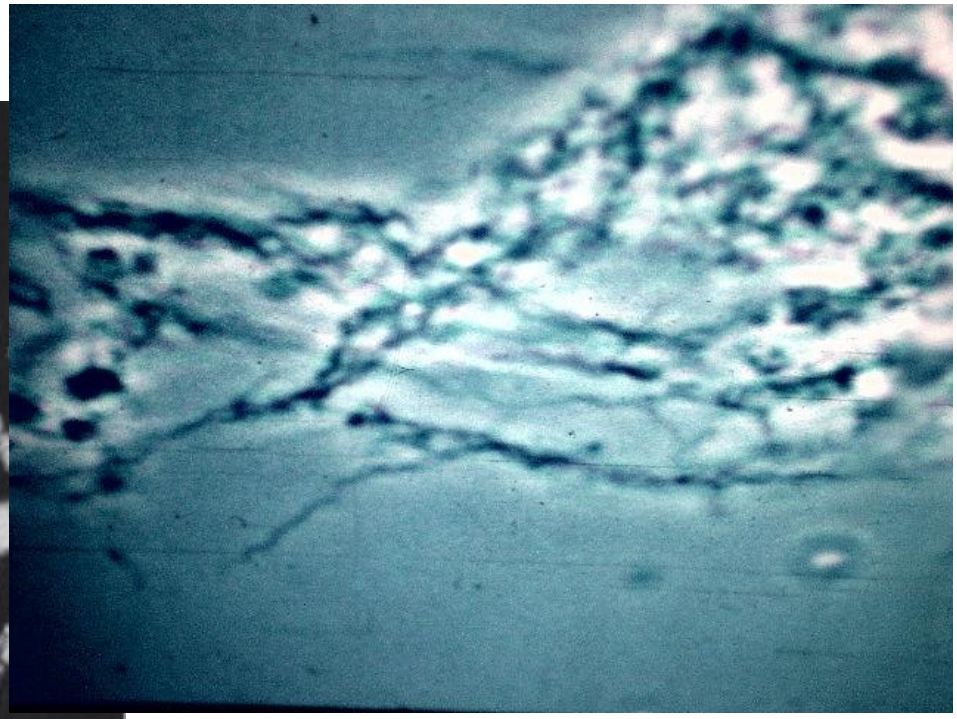


Image from 1981-

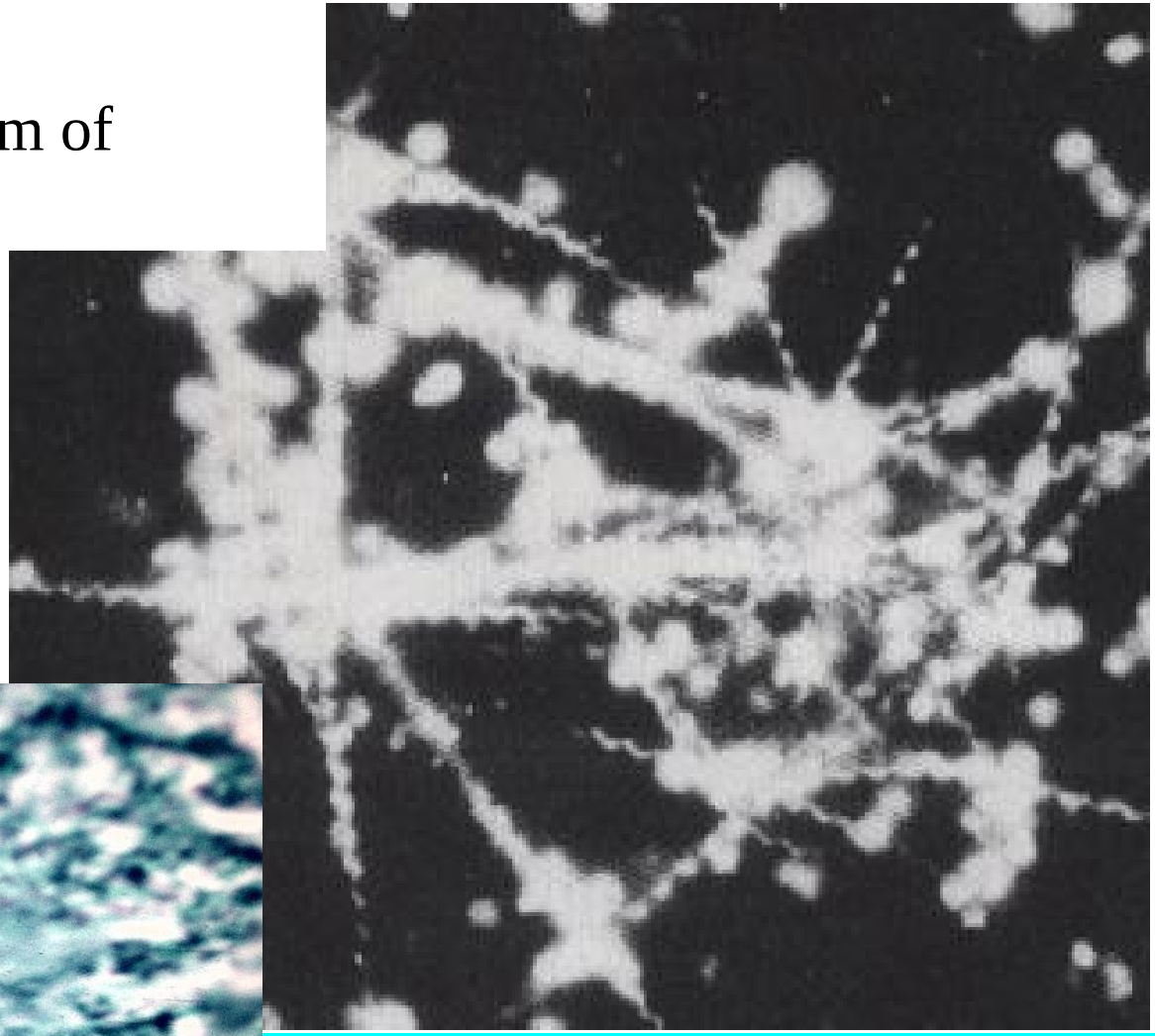
What is the source?

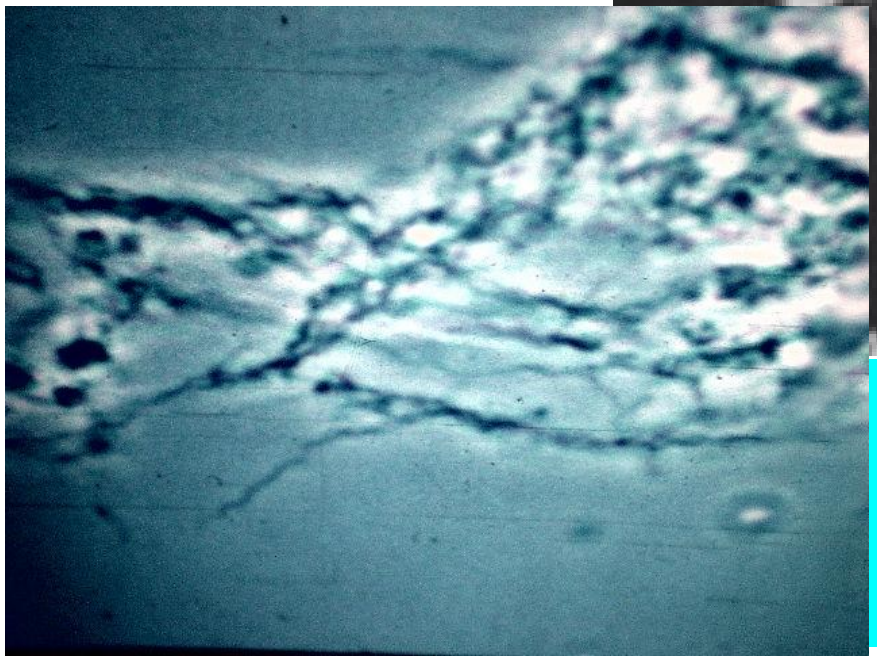
Image from 1987-

What is the source?

Human Brain Culture demonstrating a Biofilm of Borrelia burgdorferi

Year 1987

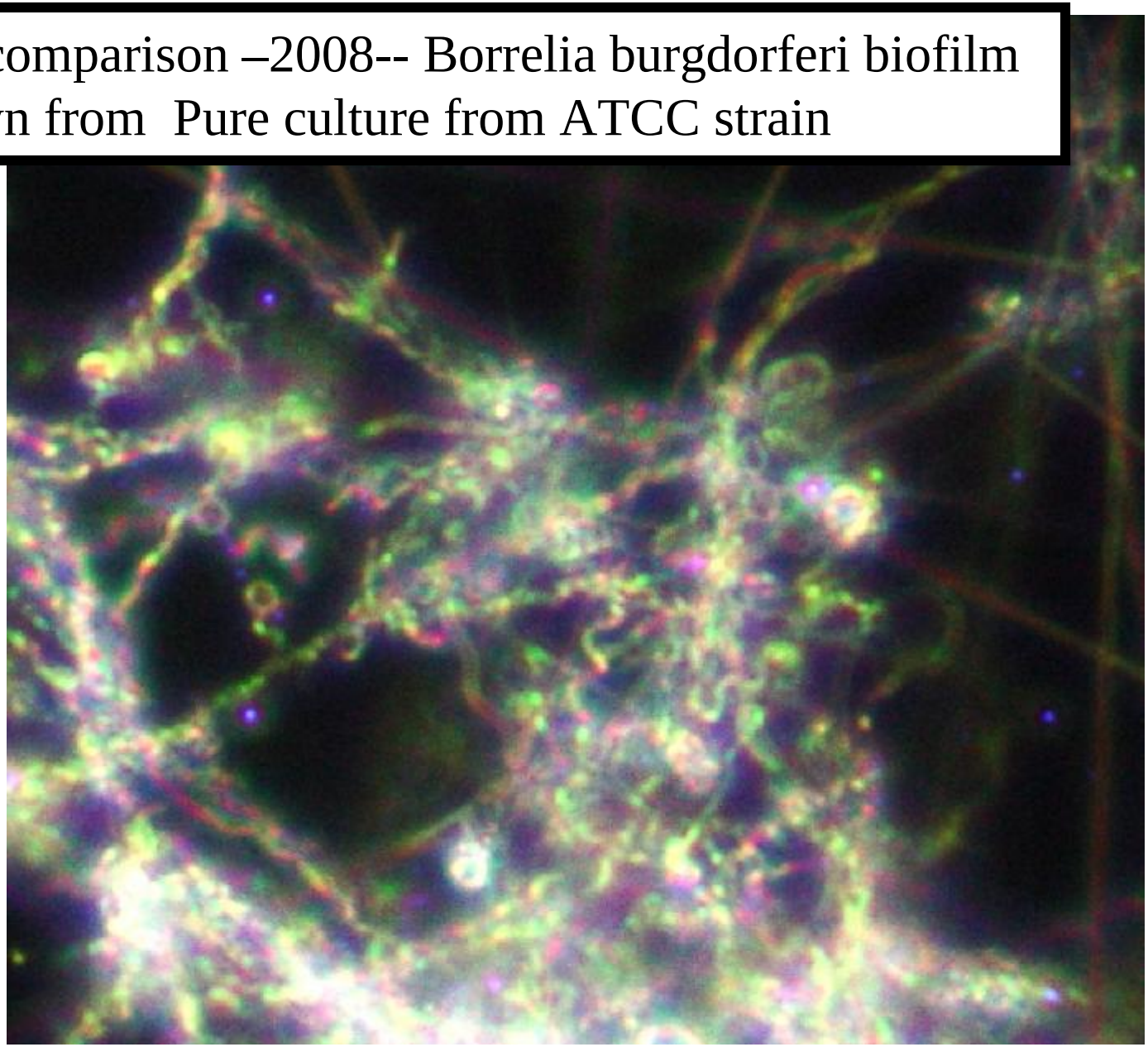


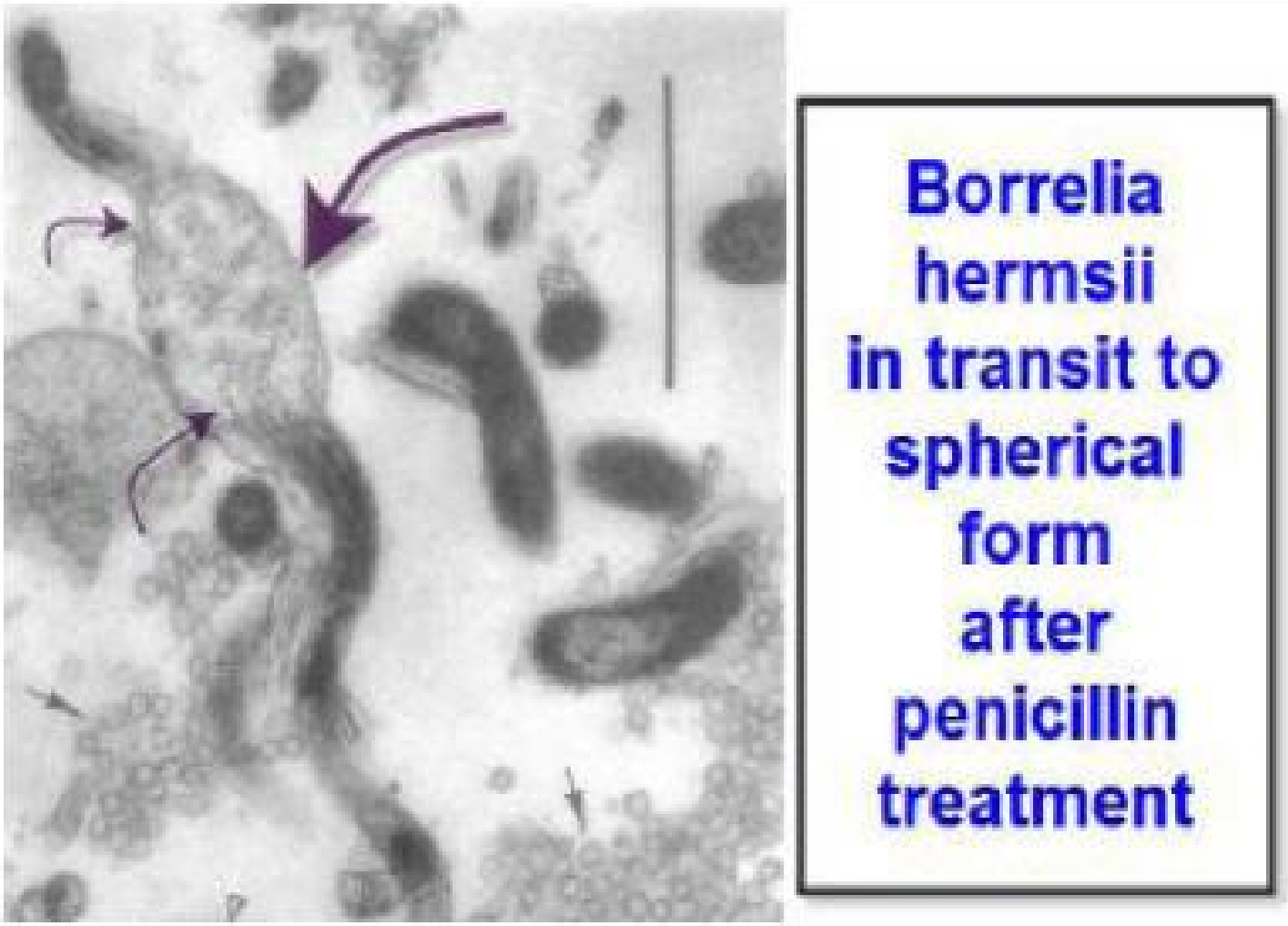


Tick gut Culture showing Borrelia burgdorferi in a Biofilm Unit

Year 1981

For comparison –2008-- Borrelia burgdorferi biofilm grown from Pure culture from ATCC strain





Formation of Cystic and Cell wall deficient Spherical forms is initiated by Localized LOSS of Cell Wall

Cystic and spiral Borrelia burgdorferi in Aged pure culture

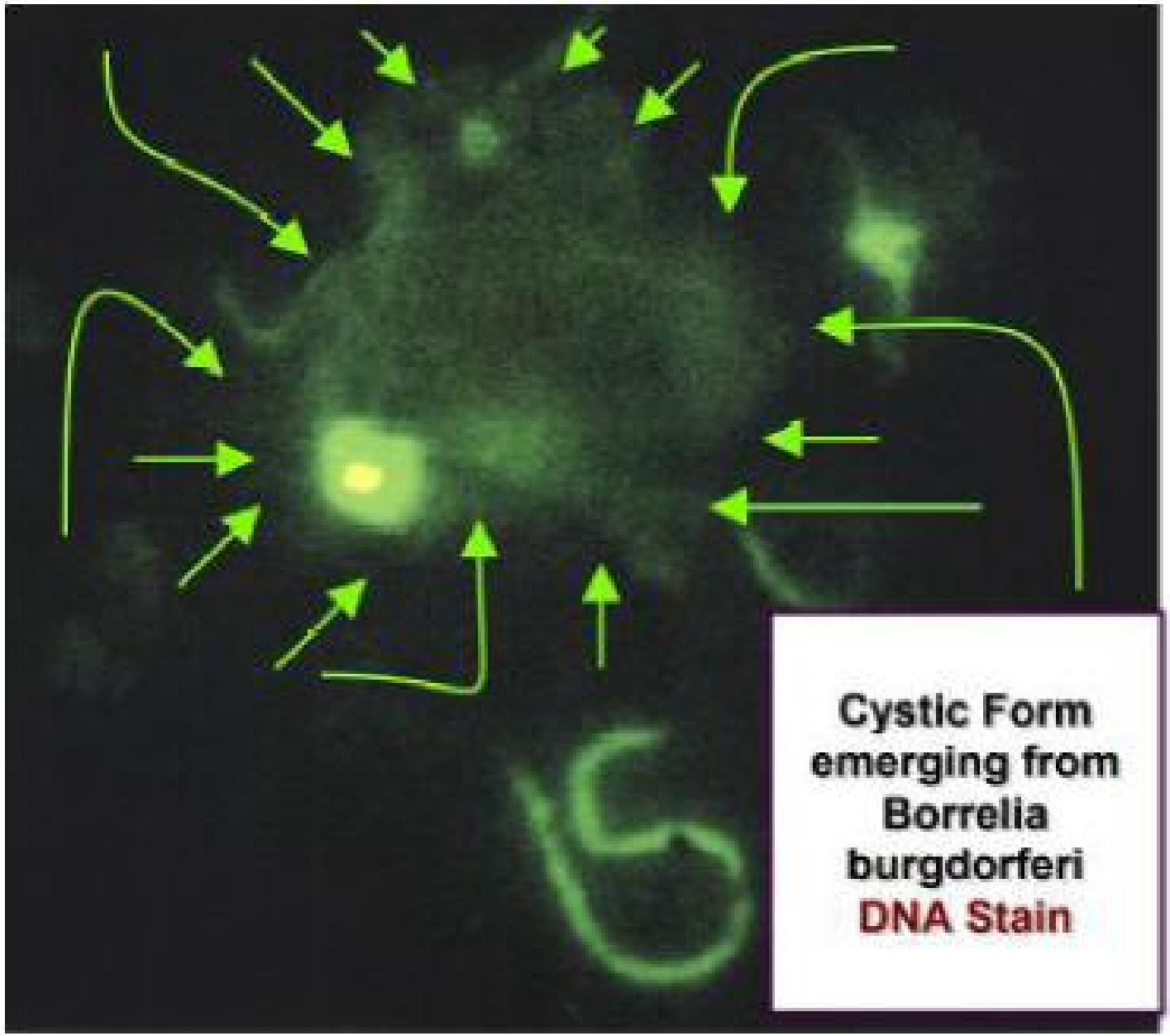
The In Transit concept

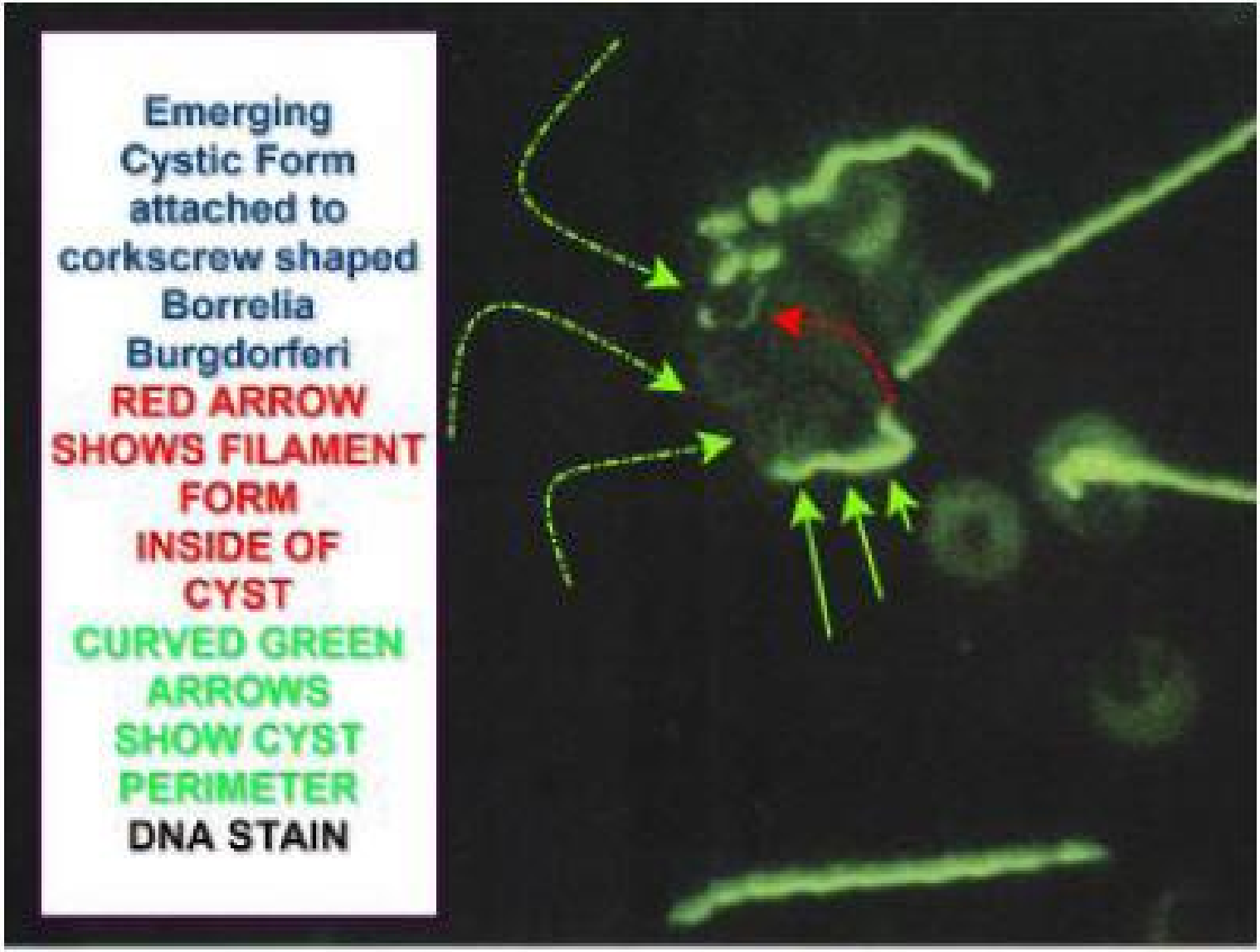
For Borrelia biofilms

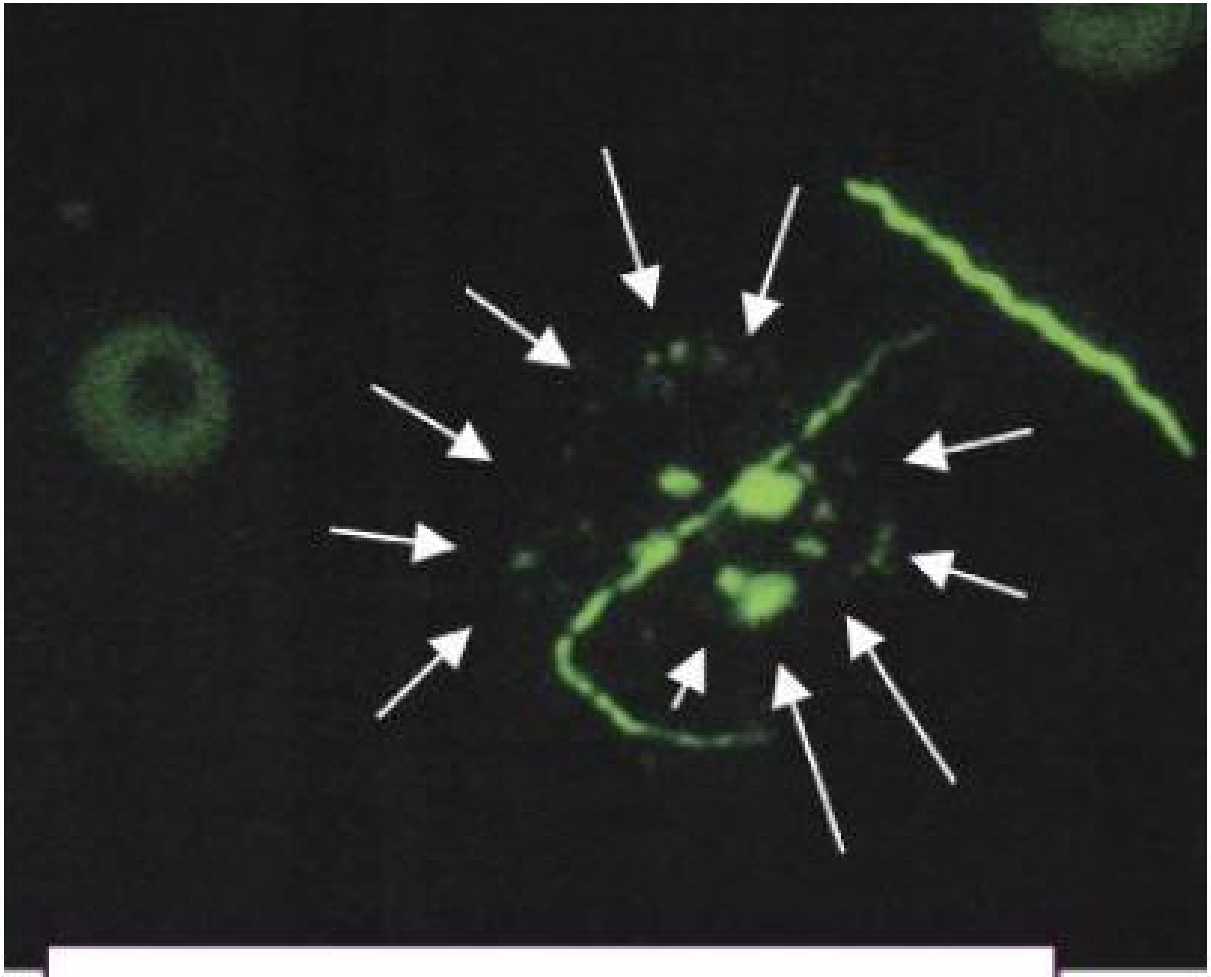
Contribution of Borrelia DNA to the formation

of Extracellular Matrix in Borrelia biofilms

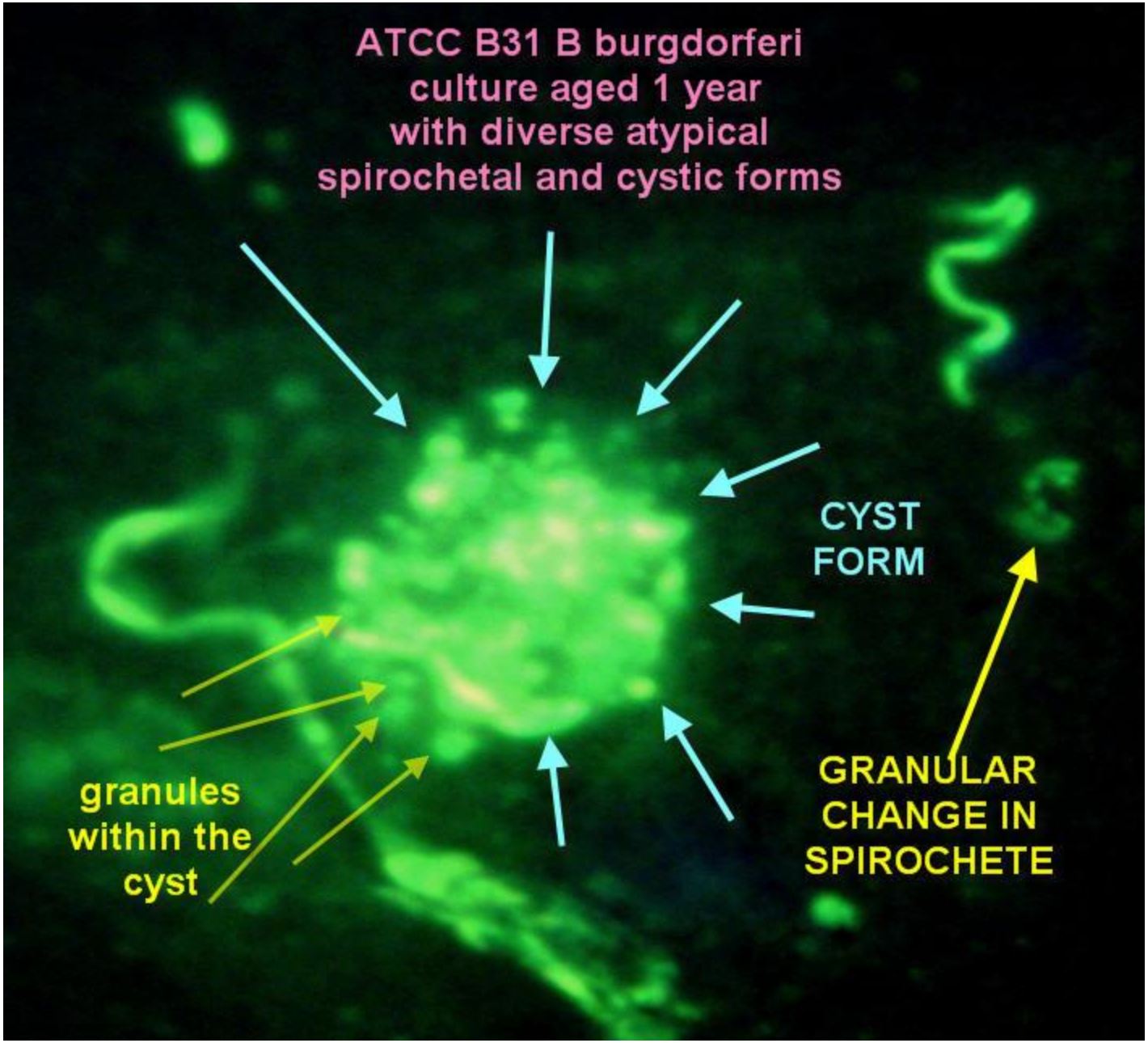
Figure 1 B. Hermsii with loss of cell wall and developing spheroid form


Cystic Form
emerging from
Borrelia
burgdorferi
DNA Stain


Emerging
Cystic Form
attached to
corkscrew shaped
Borrelia
Burgdorferi
RED ARROW
SHOWS FILAMENT
FORM
INSIDE OF
CYST
CURVED GREEN
ARROWS
SHOW CYST
PERIMETER
DNA STAIN



Emerging Cyst form of Borrelia Burgdorferi
(see rounded area of dots)
White arrows show boundaries of the emerging cystic form containing granular elements


ATCC B31 B burgdorferi
culture aged 1 year
with diverse atypical
spirochetal and cystic forms
CYST
FORM
granules
within the
cyst
GRANULAR
CHANGE IN
SPIROCHETE

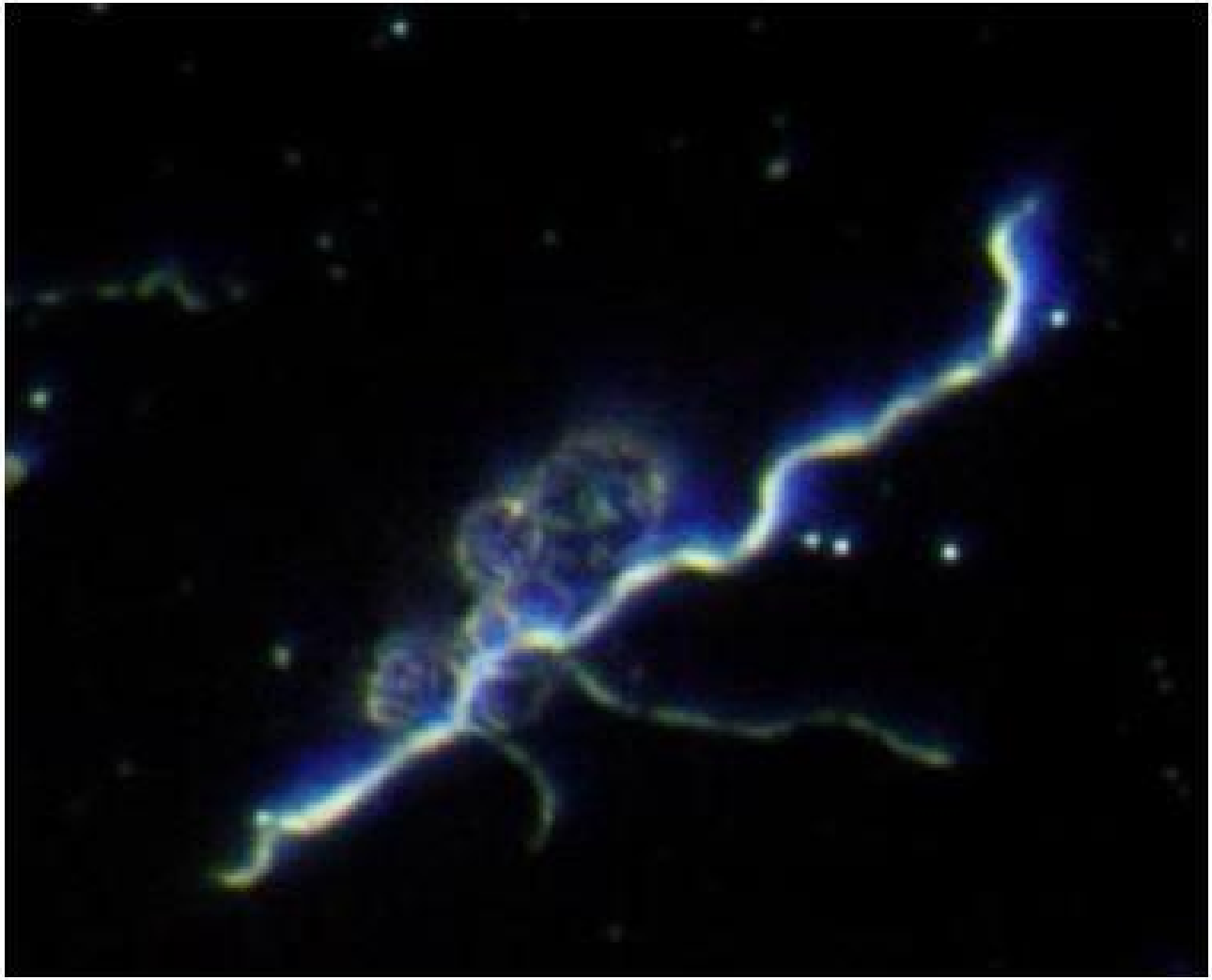


Figure 3 - "In transit" form of Borrelia burgdorferi. Note the "herniations of rounded cellular material not bound by the confines of the rigid cell wall of the spirochete

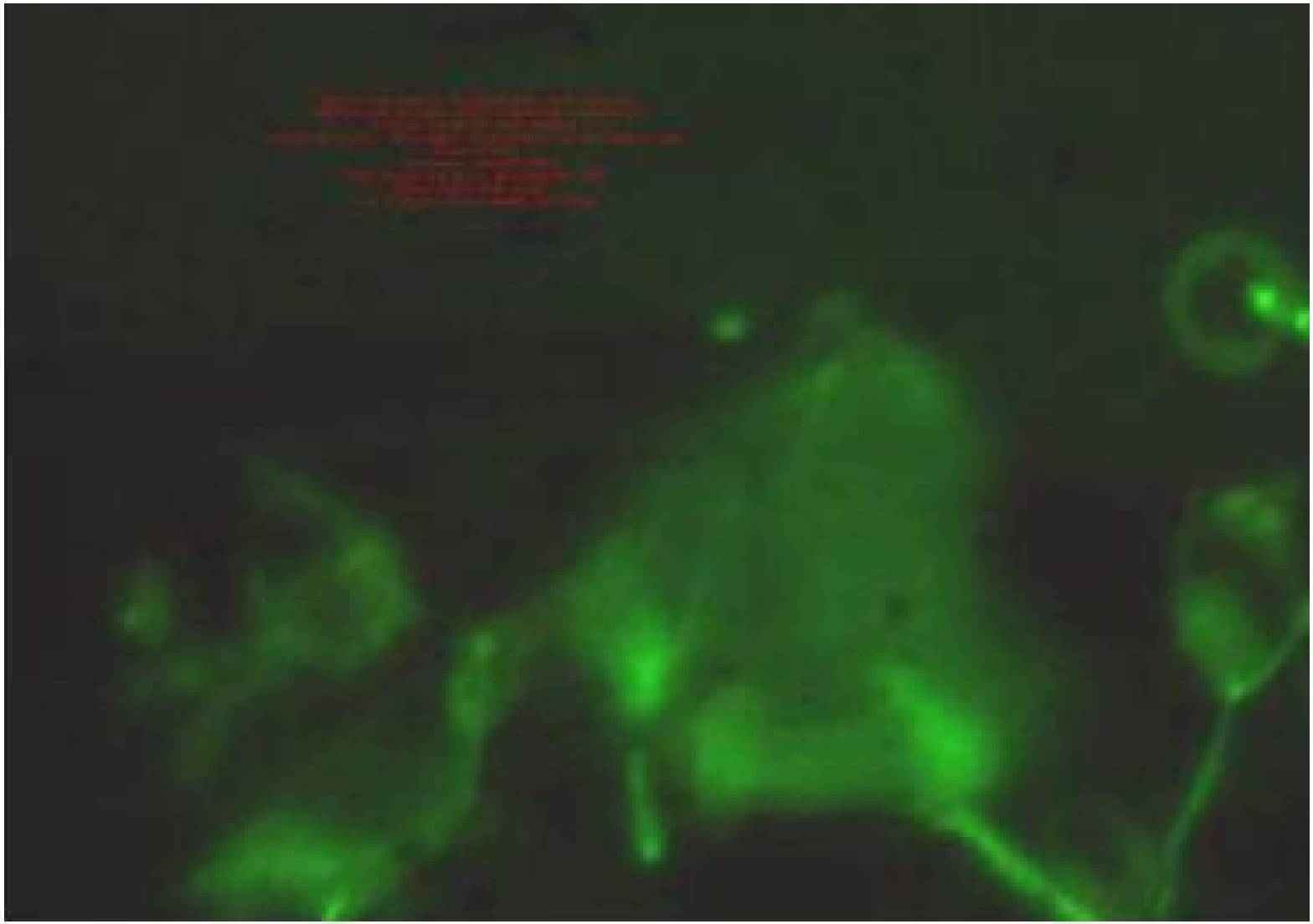


Figure 5 - "In transit " form of Borrelia burgdorferi with "blush" of External DNA

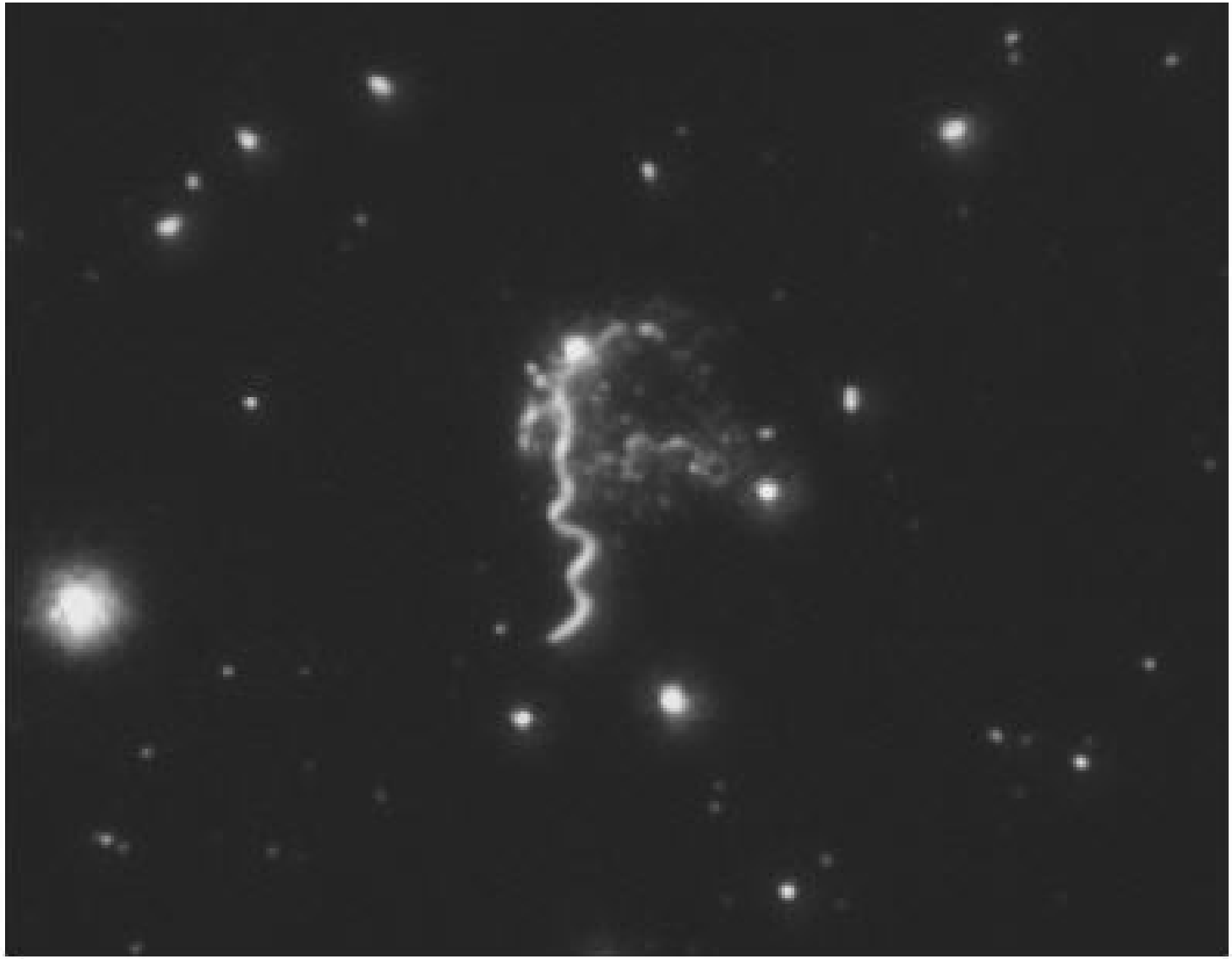


Figure 7 - In Transit form of Borrelia burgdorferi with externalized cellular elements

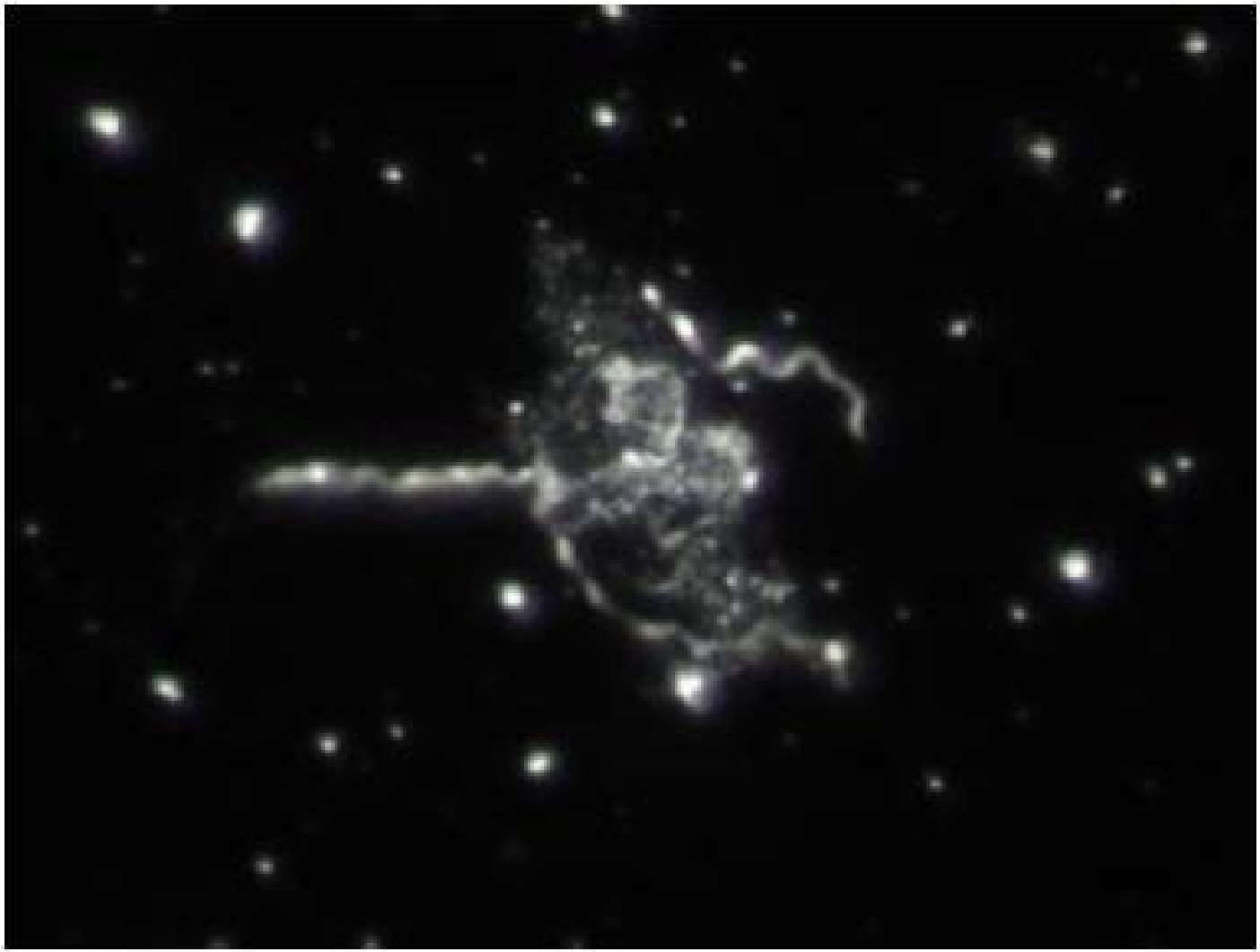


Figure 6 externalized cellular constituents Early biofilm form of Borrelia Burgdorferi . Note coalescence of externalized cellular constituents

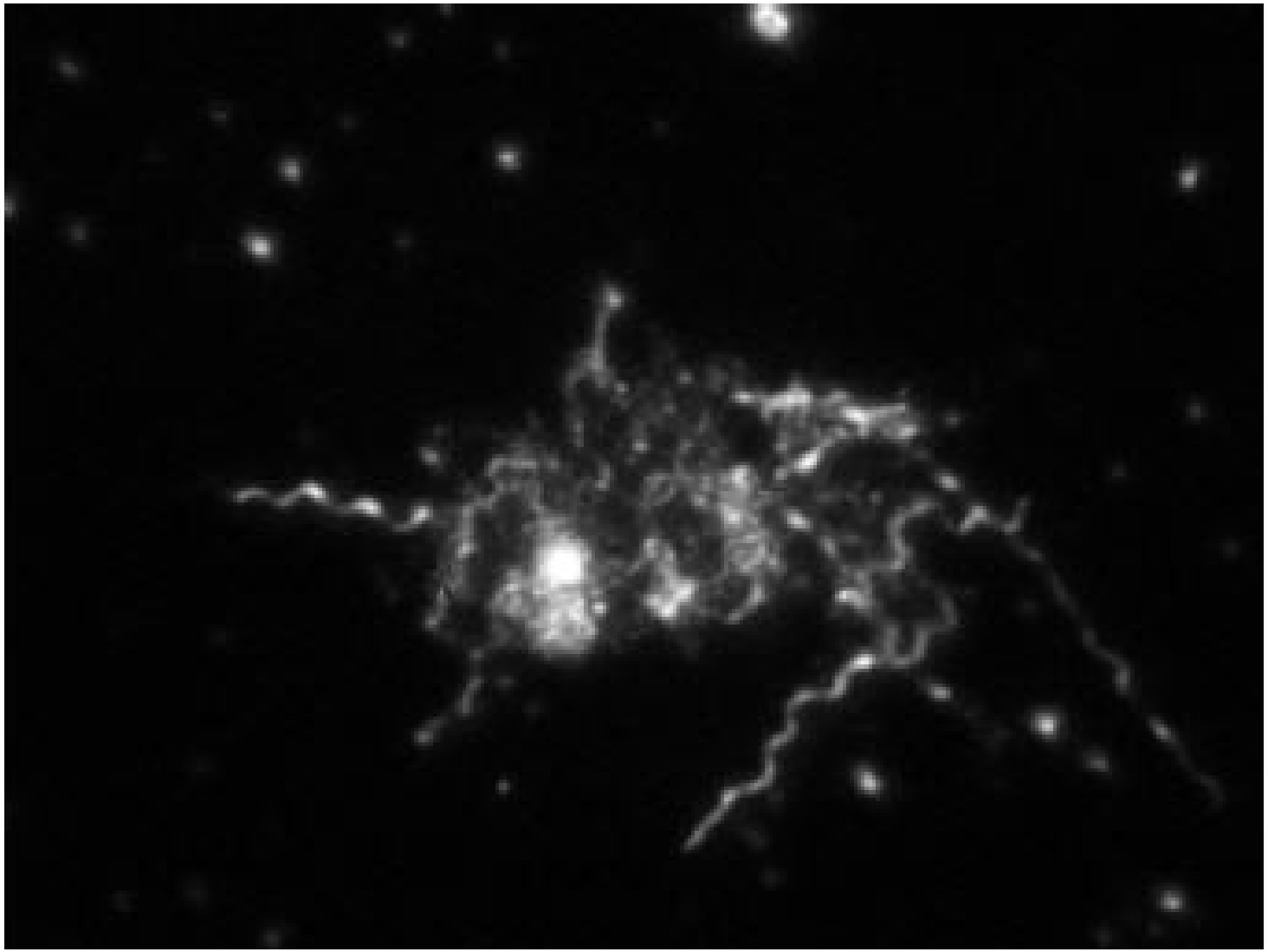


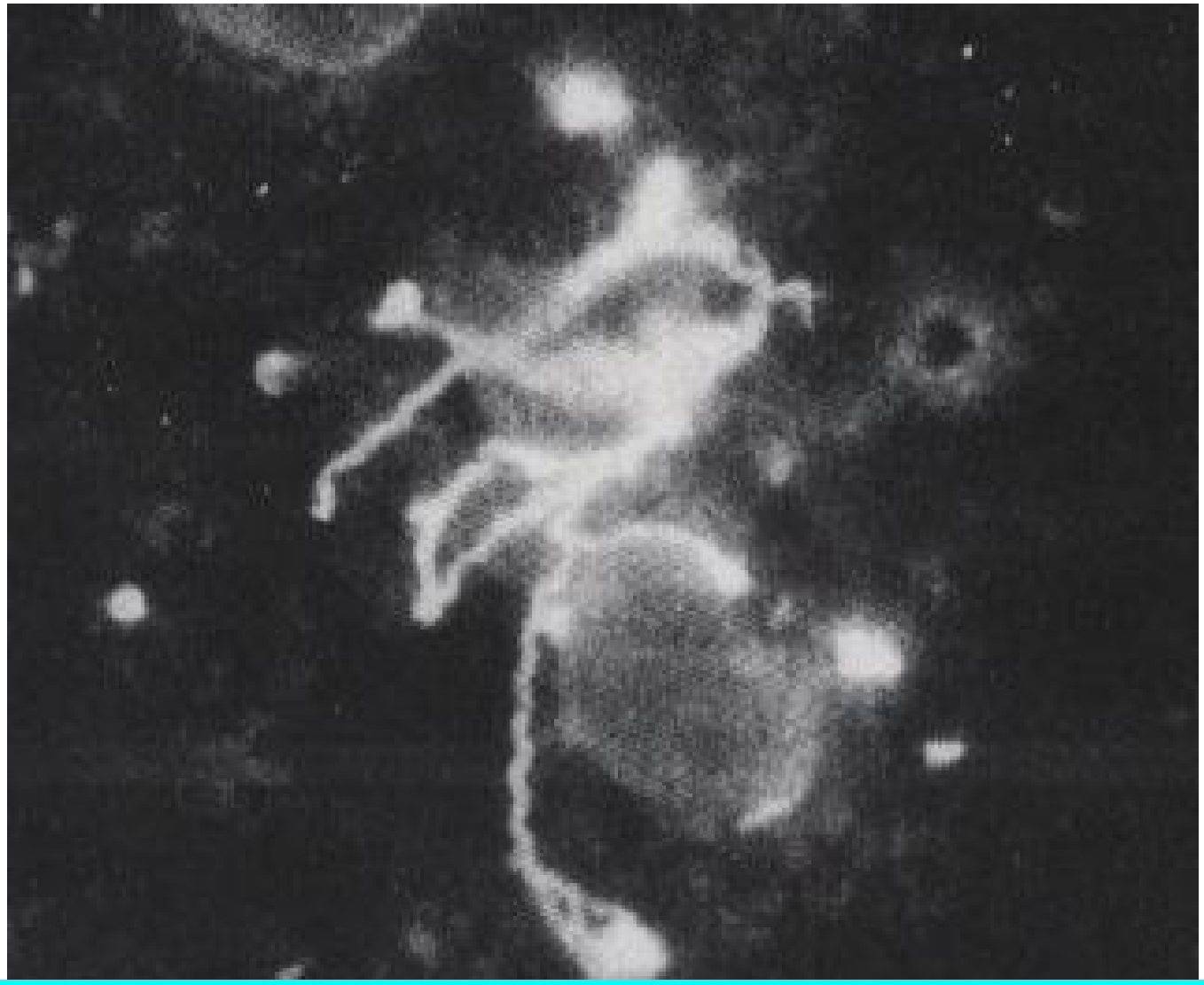
Figure 8 Early Biofilm of Borrelia burgdorferi.

Dr Klaus Eisendle - American Journal of Clinical Pathology

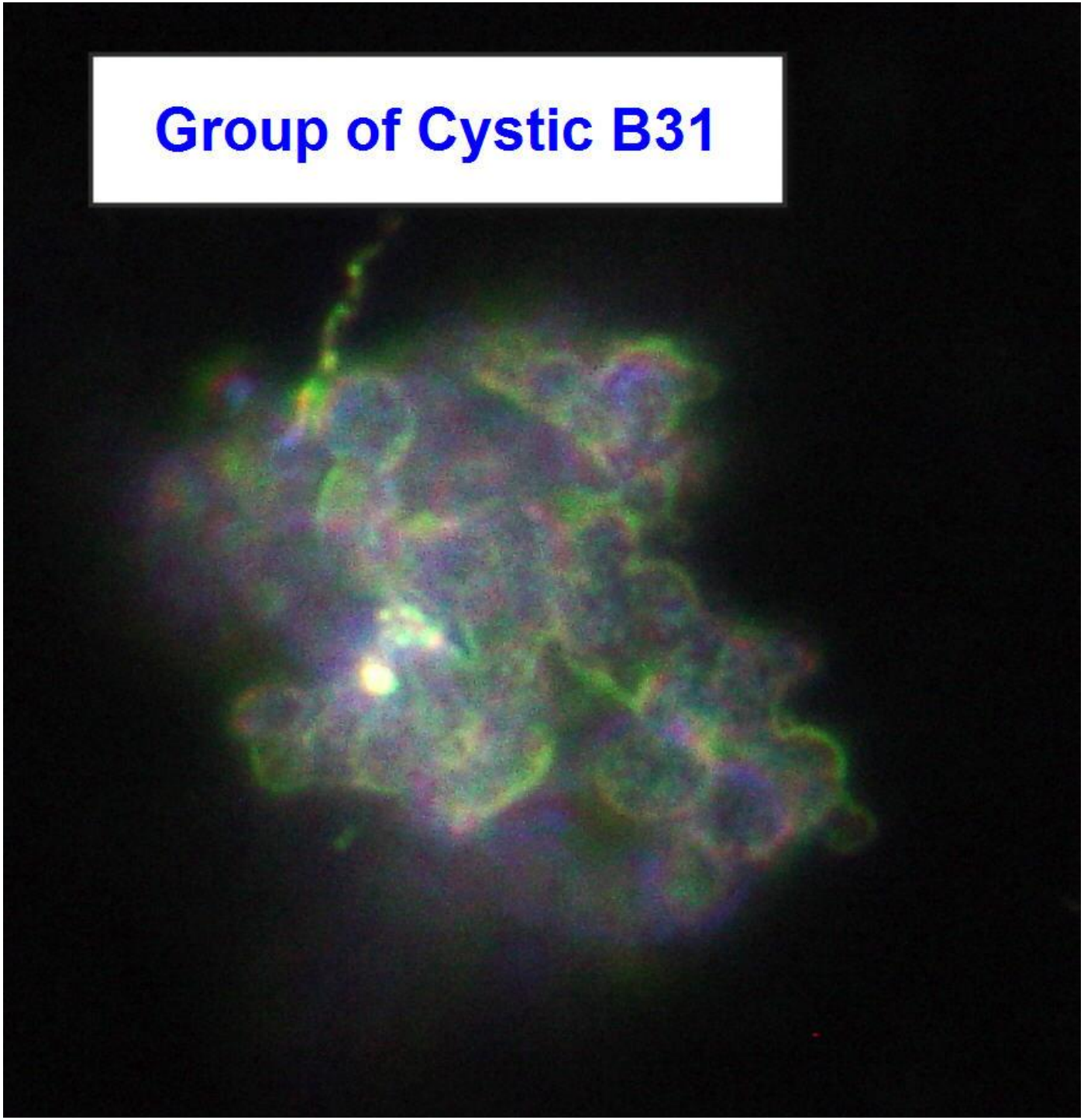
2007

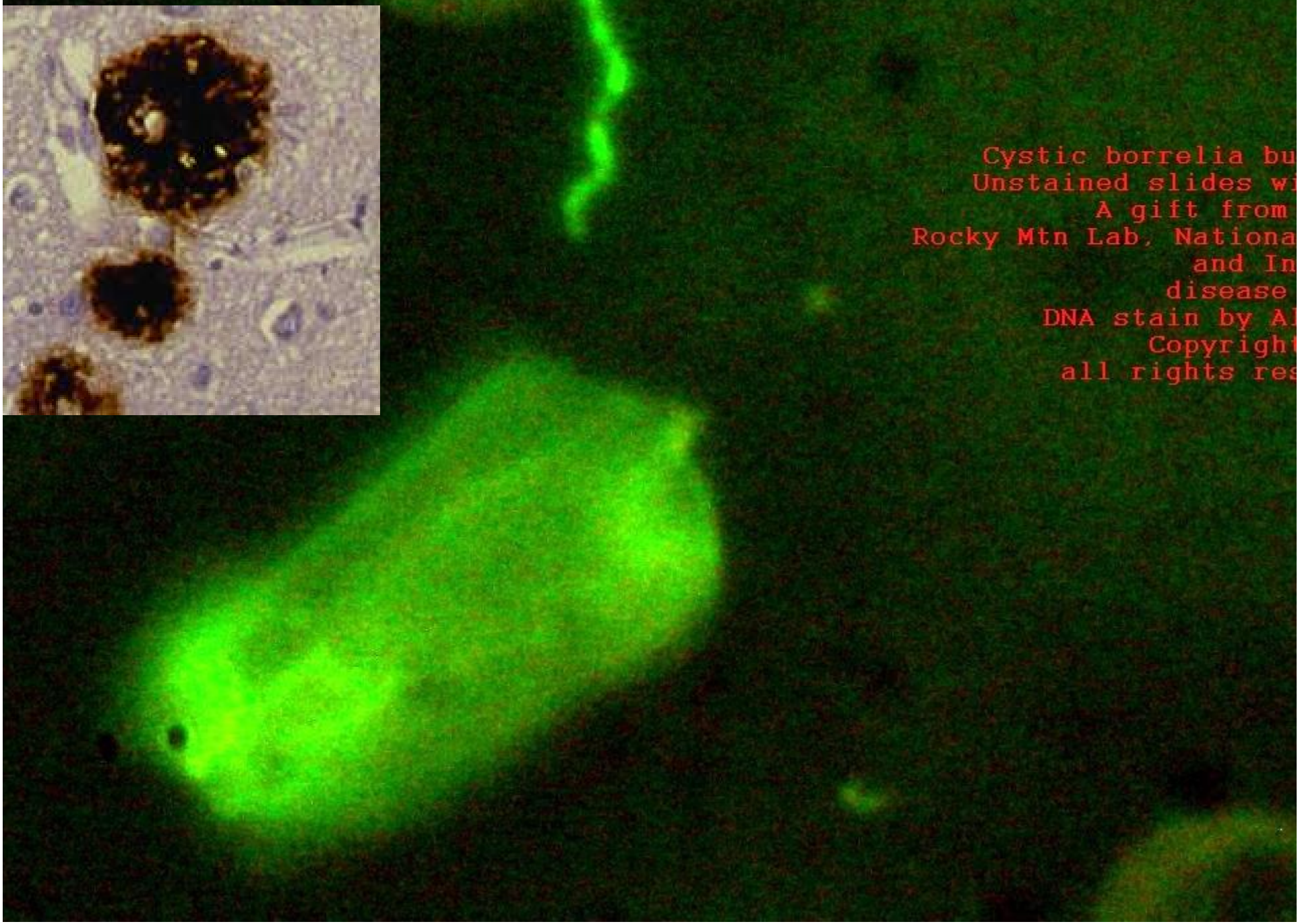
Vol 127 :213-222

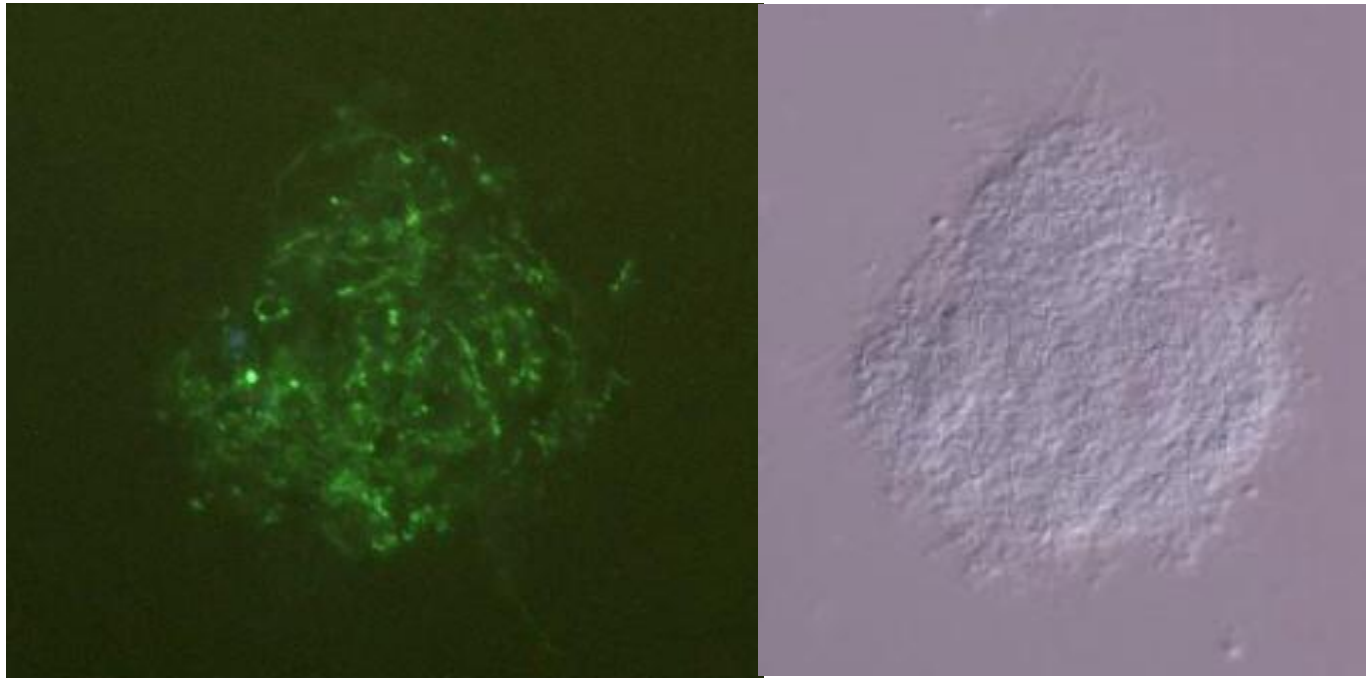
Paired Borrelia in ACA skin with adjacent red blush staining ?? In Transit biofilm form ??



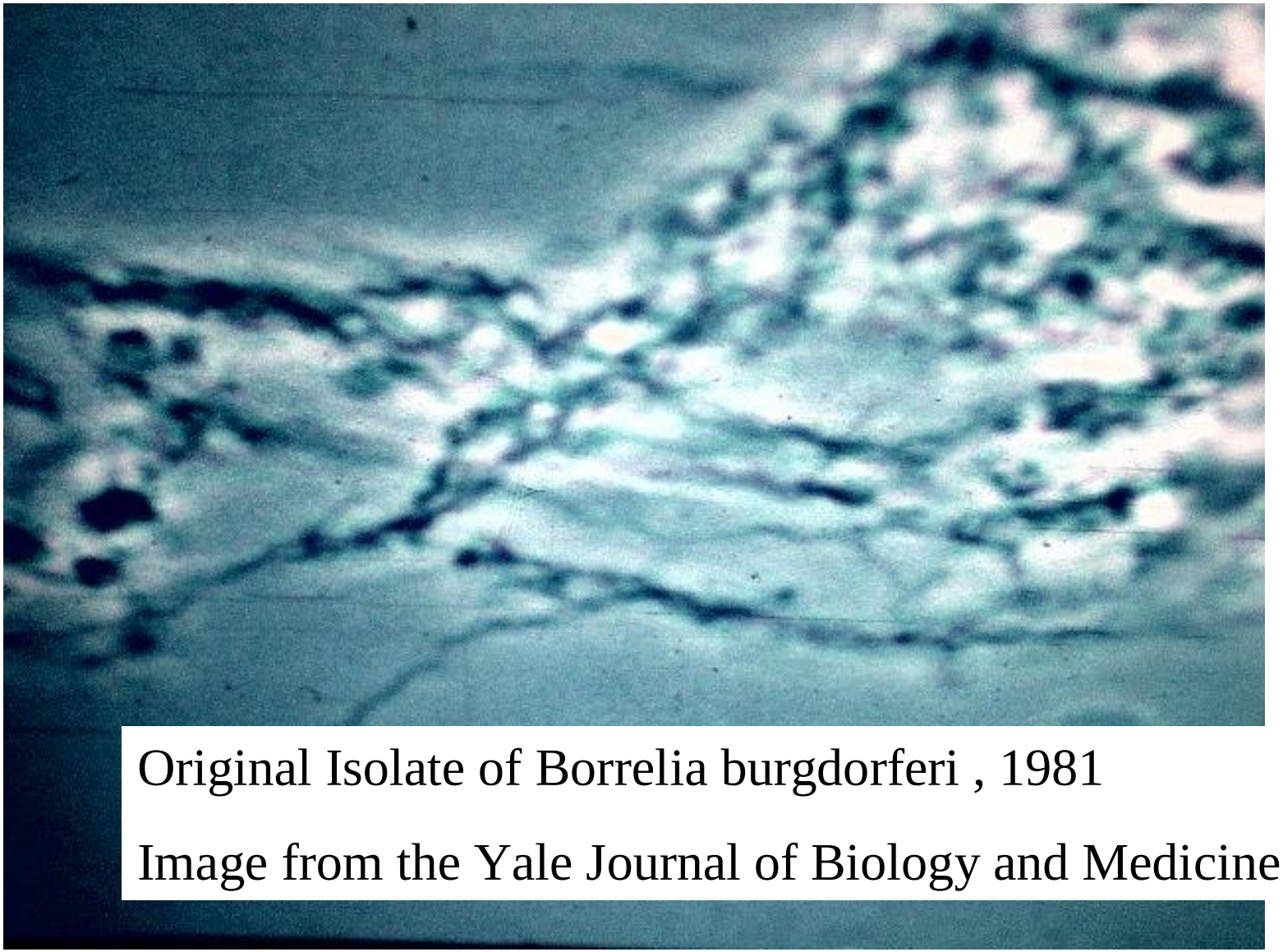
Alzheimer’s disease – Frontal lobe Cortex – Imprint cytology showing a group of Borrelia with adjacent


Group of Cystic B31


Cystic borrelia bu
Unstained slides wi
A gift from
Rocky Mtn Lab, Nationa
and In
disease
DNA stain by Al
Copyright
all rights res



DNA distribution in biofilm of Borrelia burgdorferi



Original Isolate of Borrelia burgdorferi , 1981

Image from the Yale Journal of Biology and Medicine

Biofilms as primitive Multicellular systems

Micro Colony formation

Differentiation of Microbes within the biofilm

Dispersal from biofilm colonies

- Microfilm “units”
- Planktonic “units”

Nitric oxide – Signal for differentiation

and Dispersal from biofilms

Signal transmission within Biofilms

Cell to cell communication

Cyclic diGMP (2nd messenger) [GGDEF/EAL SYSTEM]

Nitric Oxide

Peptide signaling

“melting” phenomenon – formation of ***Syncytial Units***

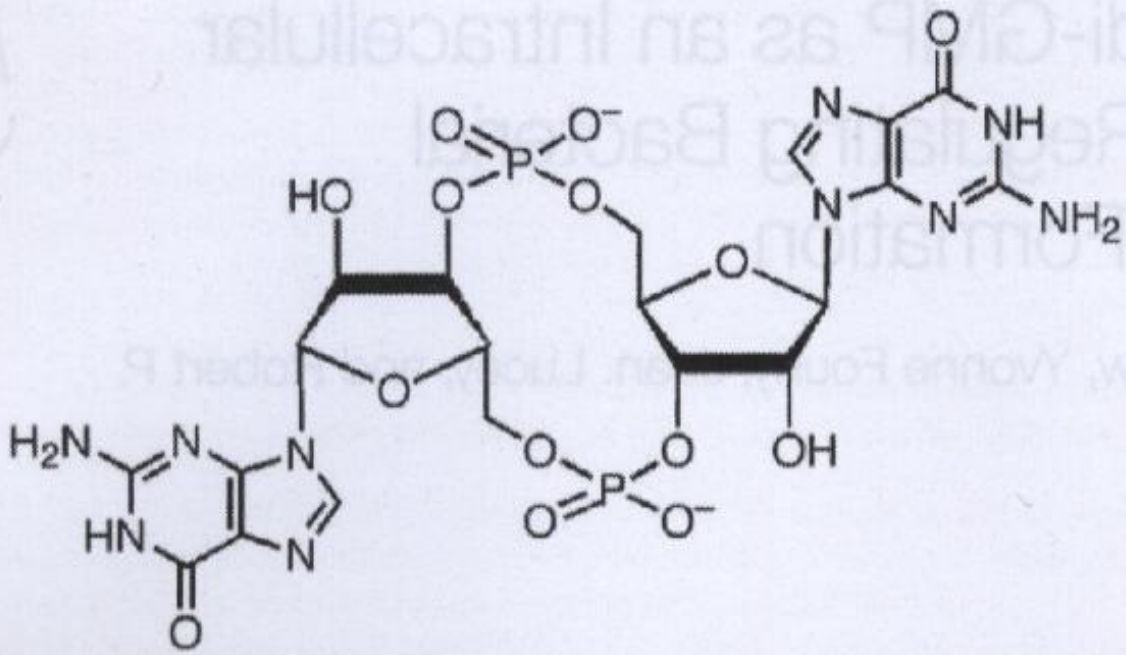


Figure 5.1 Structure of the second messenger bis-(3′-5′)-cyclic di-guanosine monophosphate (cyclic di-GMP).

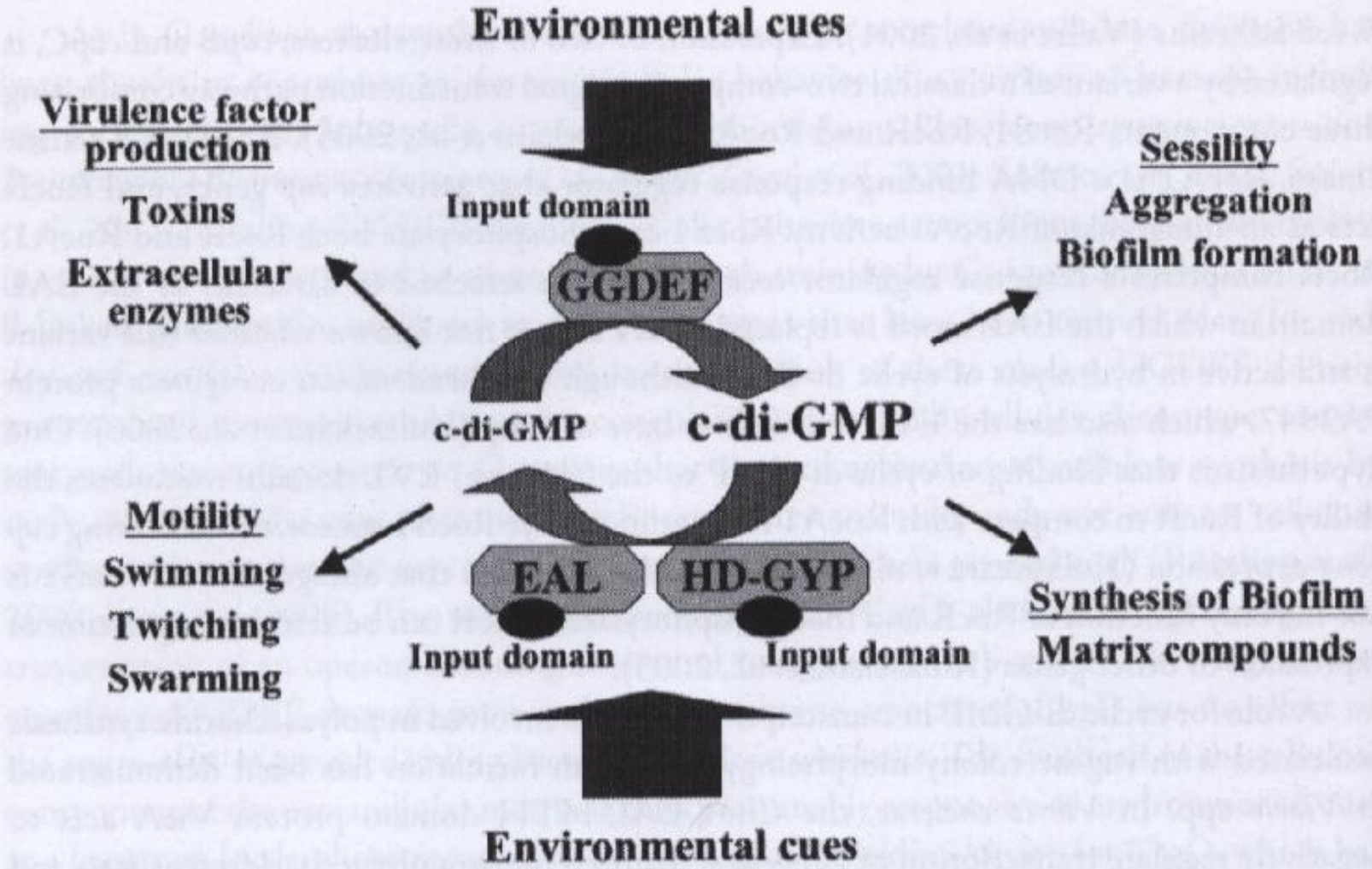
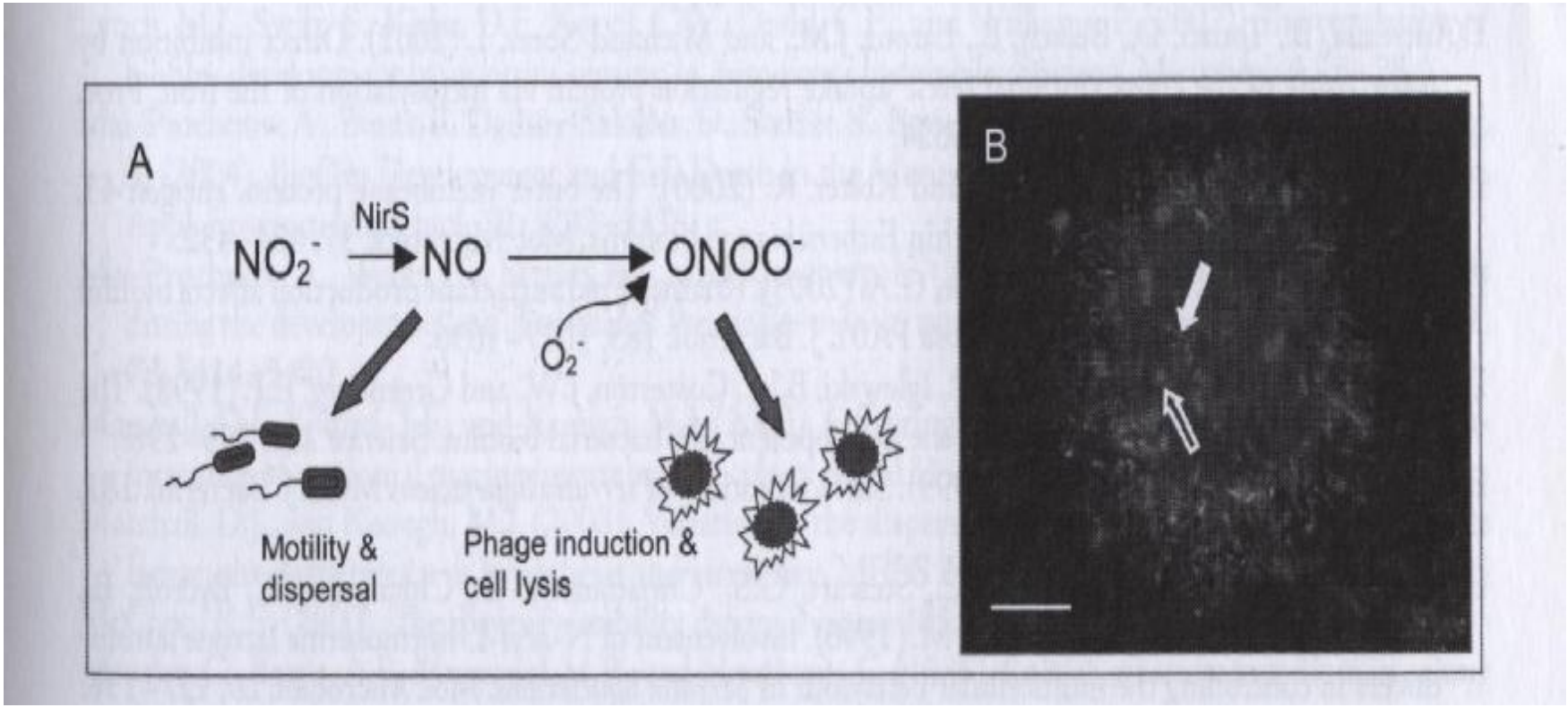


Figure 5.3 Cyclic di-GMP as a second messenger links the perception of environmental


A
NirS
NO_2^-
NO
ONOO$^-$
O_2^-
Motility &
dispersal
Phage induction &
cell lysis
B

Viable but NonCultivatible Microbes

Strains of Borrelia burgdorferi and other borrelia species

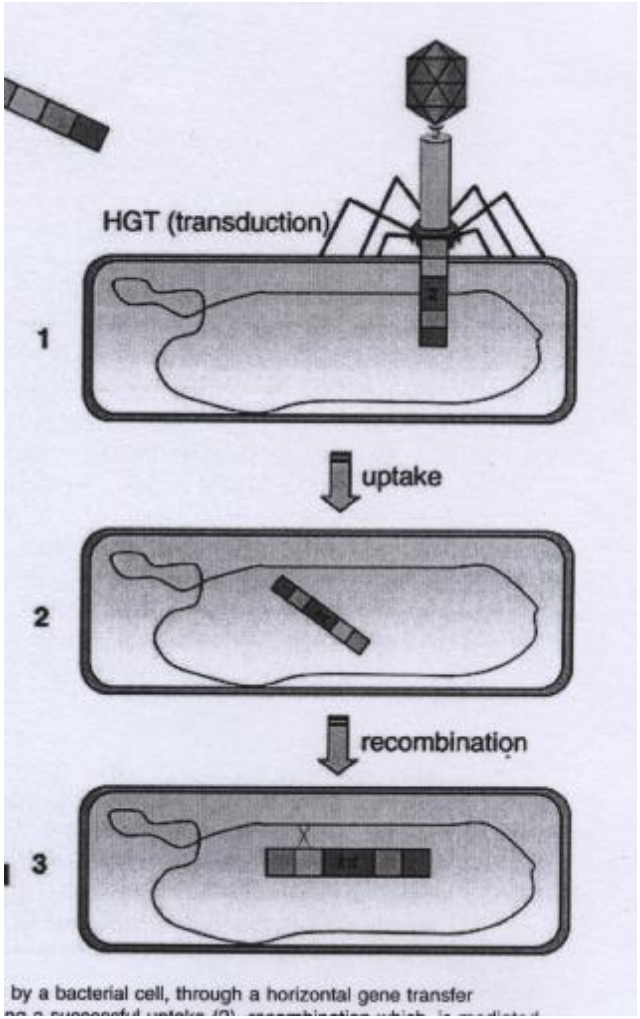
Difficult to grow in Laboratory

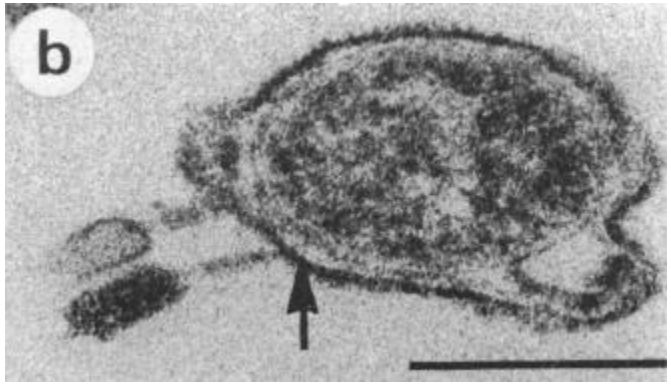
Viable in the human host

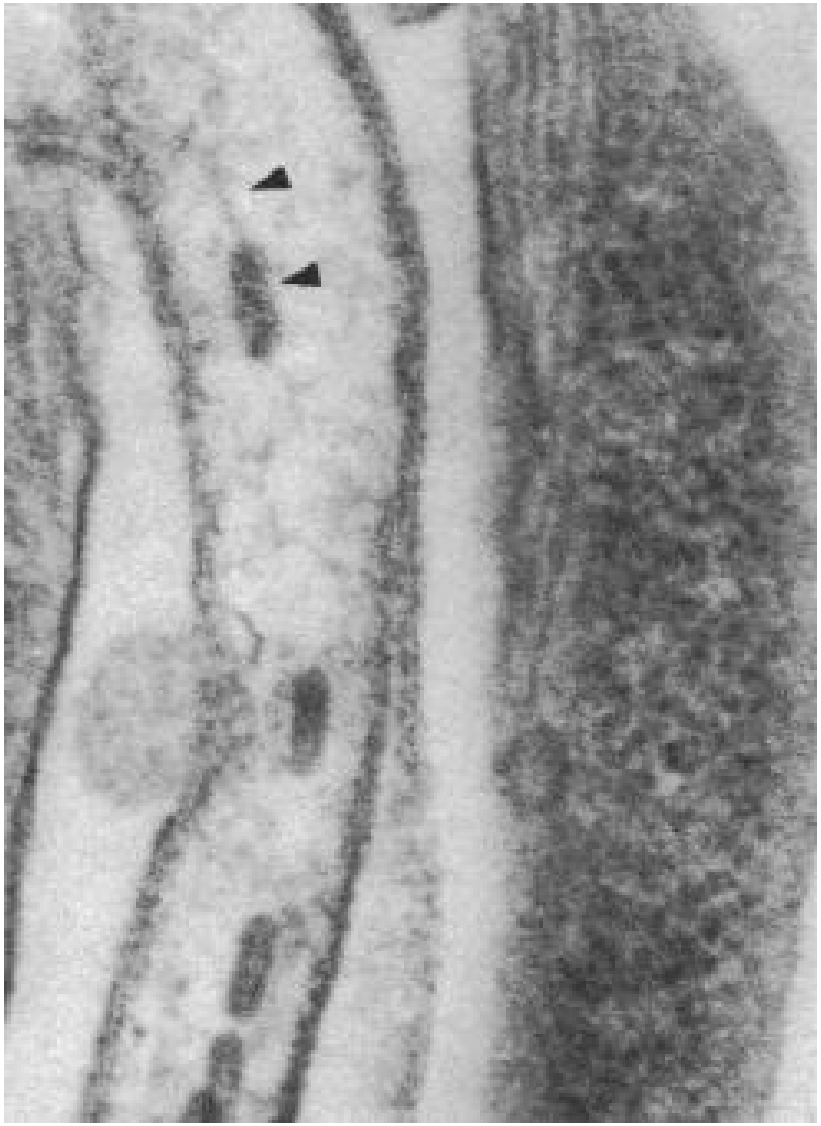
Stationary Phase

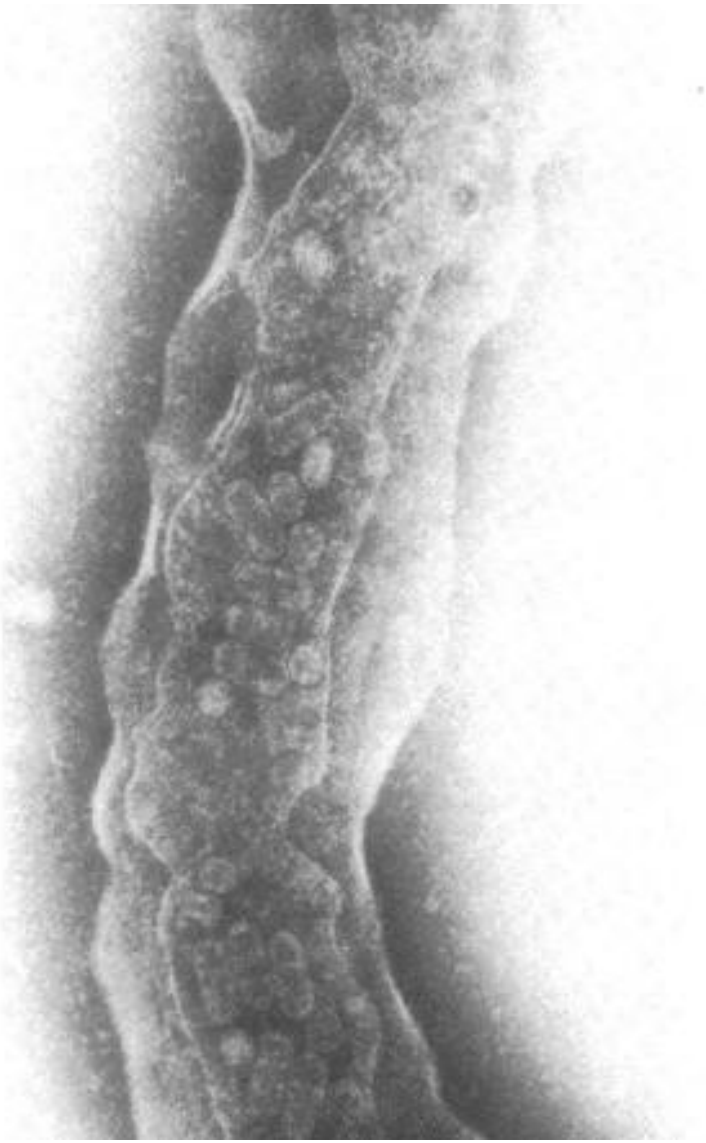
Not killed by antibiotics

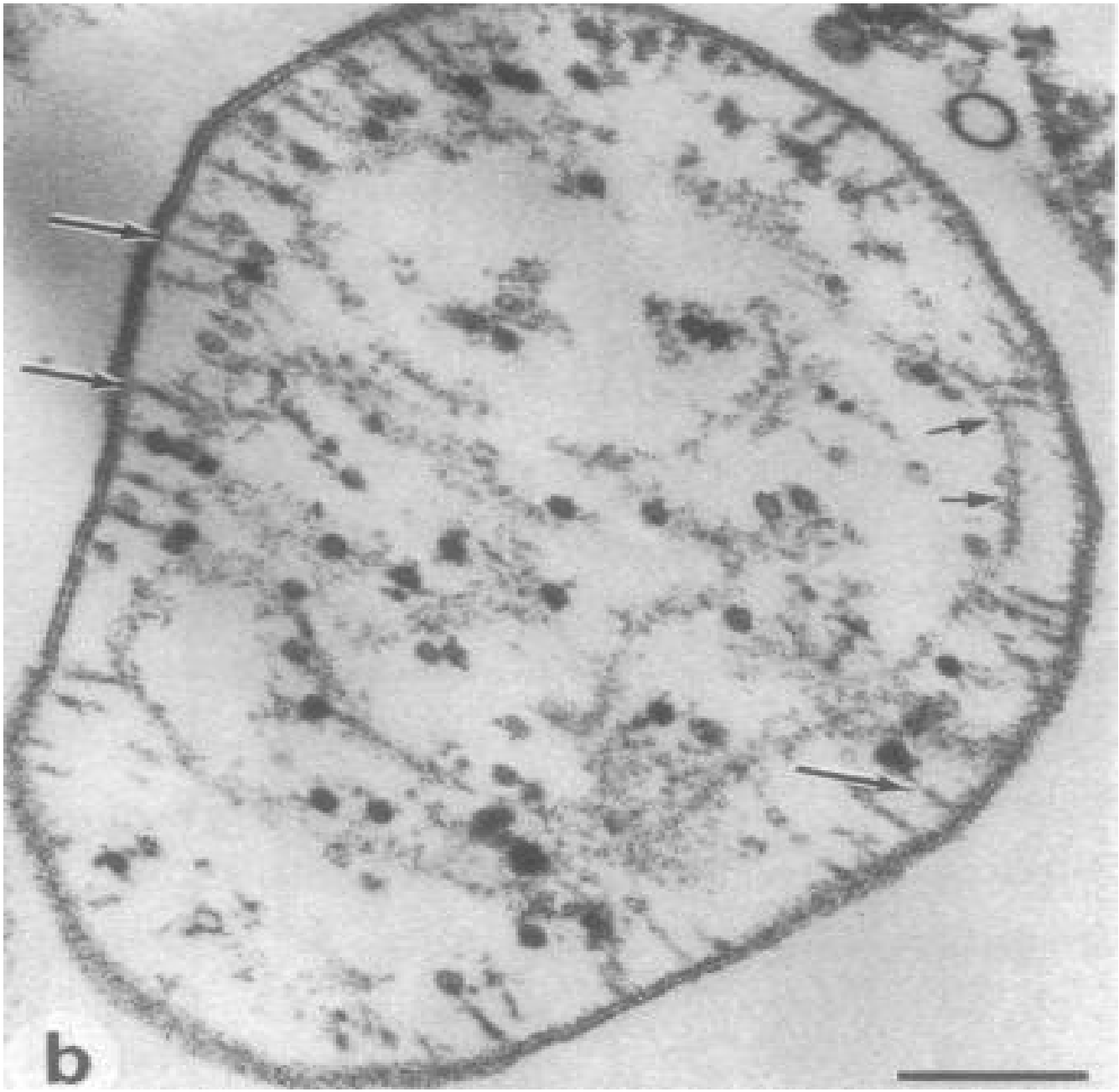
Bacteriophage Activities within Biofilms










b

The Biofilm Matrix

Components

- Extracellular Polysaccharides
- Lipoproteins
- Peptidogylcans
- Extracellular DNA (Pseudomonas model)

Multispecies Biofilms –

Examples from other Species

Complex Systems of Microbes and Protozoans

Survival benefits

Exchange of DNA between Species?

Multicellular -Biofilm - Variations under the microscope

Flocks

Granules

Rounded shaped units (microcolonies)

Mushroom shaped units

Filamentous biofilms

Loose biofilm aggregates

Life Cycle Concept

For Microbes

Biofilm Life cycles

Planktonic Life cycles

Two components

Predators of Biofilms ??

Protozoans ?

Phagocytes ??

Bacteriophages??

Other bacteria??

Attachment Considerations in Biofilm

Specific Adhesive proteins – bind to surfaces

Cell to Cell cohesion by Cell Binding proteins

Carbon sources at the site of attachment

Presence of mucin at site of attachment

Competition with other bacteria at attachment

Resistance to Shear Forces

Up Regulation and down Regulation of genes

Future Research in Borrelia biofilms

Mutations and Horizontal Gene Transfer in Biofilms

Transcriptome analysis in Biofilms **–**

Current constraints

Comparative Analysis of Events in Biofilm Life Cycles across bacterial species

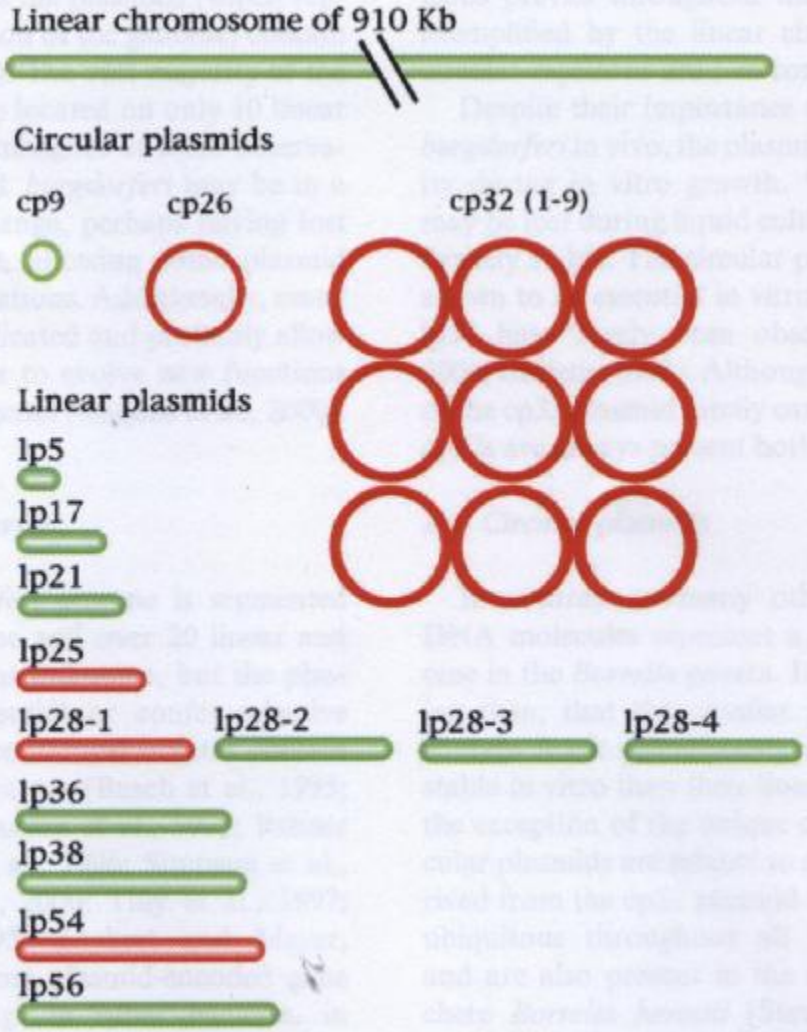


Fig. 2. The segmented genome of *B. burgdorferi*. Linear plasmids are abbreviated lp and circular plasmids cp, the number represents the approximate size of the plasmid in kilobase pairs. Evidence supports that plasmids shown in red are required for infectivity or persistence in the tick or vertebrate hosts. Sizes are not drawn to scale.

Borrelia biofilm works in progress

--Quorum sensing in Biofilms

AHL model for QS in Gram Negative bacteria

---Viable but non- cultivatable Borrelia in Biolfilm communities

Persister forms of bacteria

Non dividing forms

Slow to divide forms

Quorum Sensing- Chemical messenger molecules produced by a single bacterium are different (quantitatively) from those produced by a population of bacteria in a biofilm.

Quorum Sensing Blockers

Can we identify the Genetic underpinnings of Quorum Sensing chemical species in Borrelia and utilize these in treatment of Chronic infections?

[Examples - furanones, patulin, penicillic acid, garlic extract – as natural QS blockers in biofilm via downregulation of genes in pathogenic bacteria]

Clinical Implications of Biofilms of Borrelia burgdorferi

Biofilms of Borrelia burgdorferi in human tissue provide microscopic proof of persistence of spirochetes in cases of chronic Lyme borreliosis.

Biofilms of borrelia,by definition, explain Persistence of infection after antibiotic therapy and recurrence of disease symptoms in chronic Lyme borreliosis.

Summation: Biofilms of Borrelia burgdorferi

1. Biofilms of Borrelia are indispensable elements for species survival in hostile environments.
2. Biofilms of borrelia provide protection to the microbes which live inside of the matrix
3. DNA of Borrelia (externalized) constitutes a ??portion of the borrelia biofilm matrix?
4. Exchange of genomic material occurs between the borrelia in the biofilm.
5. Morphologic diversity of borrelia within biofilms (cyst, granular, L form, and spiral forms) is evident.

Biofilms and Chronic Infections

Randall D. Wolcott, MD
Garth D. Ehrlich, PhD

THE PREVAILING PARADIGM OF INFECTIOUS DISEASE IS based on the work of Koch and colleagues, who more than 150 years ago isolated individual strains of bacteria and developed the pure culture method that is still used today. That work enlightened medicine by firmly establishing the germ theory of transmissible diseases and demonstrated that diseases like dysentery, tuberculosis, and anthrax are caused by microbiological agents.[1] Hence, the field of microbiology developed around Koch's methods with clinical microbiologists working overwhelmingly with pure log-phase cultures in nutrient-rich media because this approach provided such a powerful tool for the study of acute epidemic bacterial diseases. However, this approach that examines only planktonic bacteria (free-floating, single cell phenotype) may have limited development of a more thorough understanding of microbial processes. In most natural environments and in chronic bacterial infections, the planktonic phenotype generally exists only transiently, and usually as a minor population.

Emerging evidence describes bacterial populations as predominantly polymicrobial, sessile, community-based aggregations embedded in a self-secreted matrix that provides numerous advantages for persistence in the face of environmental and host challenges. Therefore, biofilms and the existence of a complex bacterial life cycle provide a new perspective through which to view infectious diseases. Much of the support for this perspective has come about through the application of new detection and visualization methods that have provided evidence for the theory that chronic infections are fundamentally different than acute infections, and that different interventional approaches are necessary to treat these biofilm infections more efficiently.

What Is a Biofilm?

A biofilm is a thin layer of microorganisms that adhere to the surface of an organic or inorganic structure, together with their secreted polymers. Biofilms are the predominant phenotype of nearly all bacteria in their natural habitat, whether pathogenic or environmental. The biofilm provides a bulwark against environmental stressors and can include organisms from multiple kingdoms as in the case of mixed bacterial-fungal biofilms. Thirty years ago, Costerton et al[2] was the first to examine the attributes of biofilms, examining the extracellular polymeric substances (EPS) that holds these community bac-

Author Affiliations: Medical Biofilm Research Institute, Lubbock, Texas (Dr Wolcott); and Center for Genomic Sciences, Allegheny Singer Research Institute, Allegheny General Hospital, and Departments of Microbiology and Immunology, and Otolaryngology–Head and Neck Surgery, Drexel College of Medicine, Allegheny Campus, Pittsburgh, Pennsylvania (Dr Ehrlich).
Corresponding Author: Randall D. Wolcott, MD, Medical Biofilm Research Institute, 2002 Oxford Ave, Lubbock, TX 79410 (randy@randallwolcott.com).

Thank you for your Kind attention.

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Turn The Corner Foundation

The Lyme Disease Association

Time for Lyme Inc.