

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

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University of New Haven

Lyme Disease Symposium

New Haven, Conn

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# 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

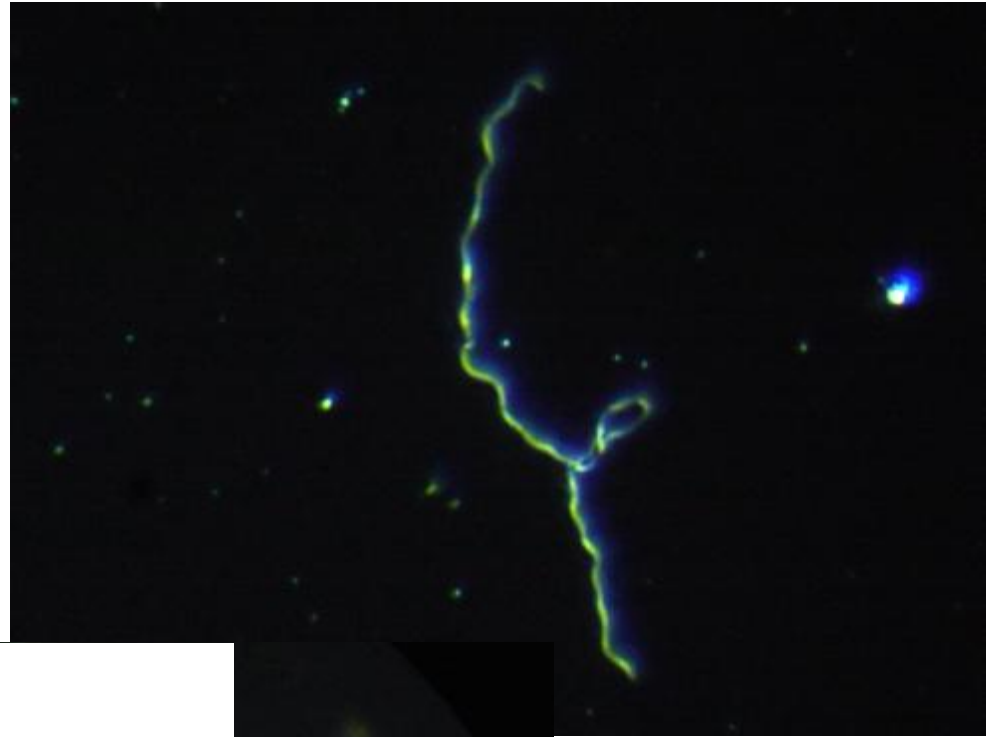
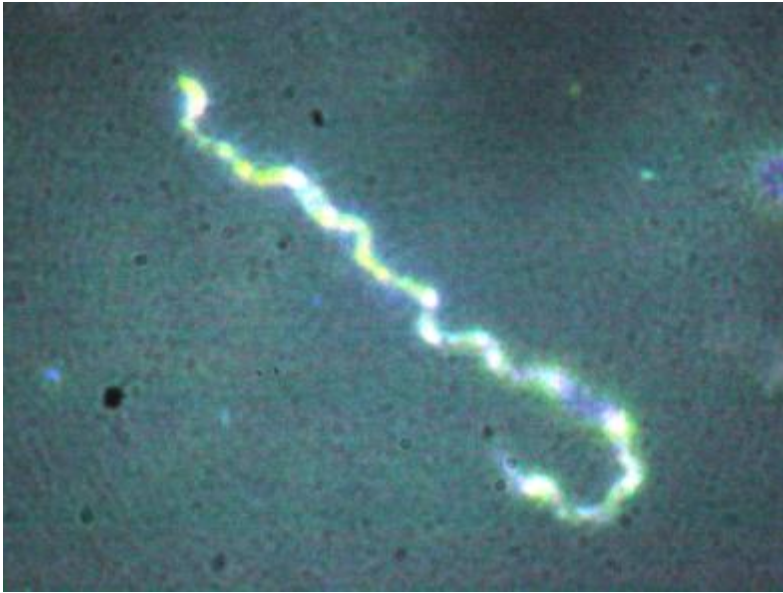
# Mixtures of Borrelia types may be found in Borrelia biofilms

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

A majority of granular or Cystic Borrelia

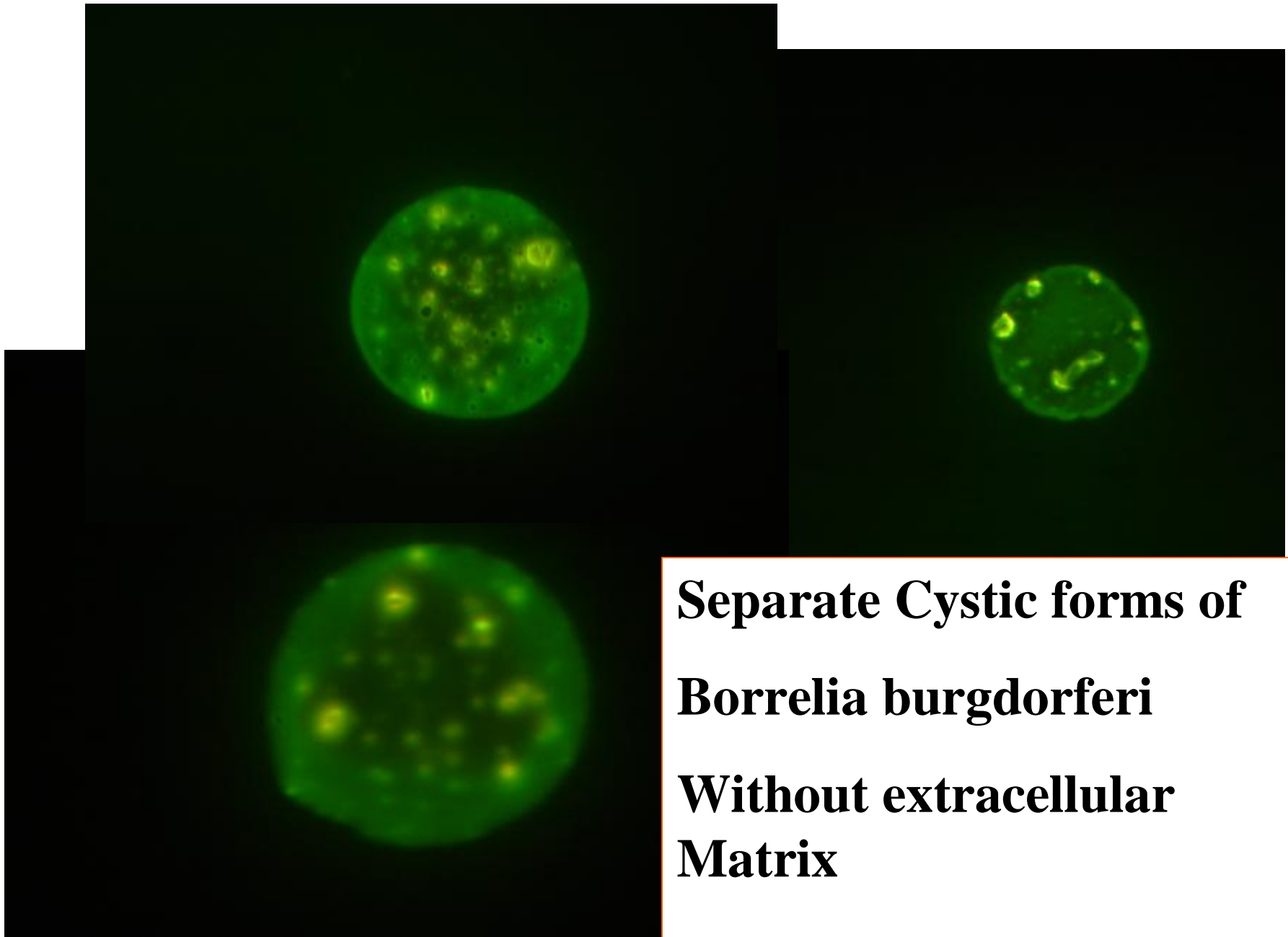
Biofilms may contain different species of pathogens

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

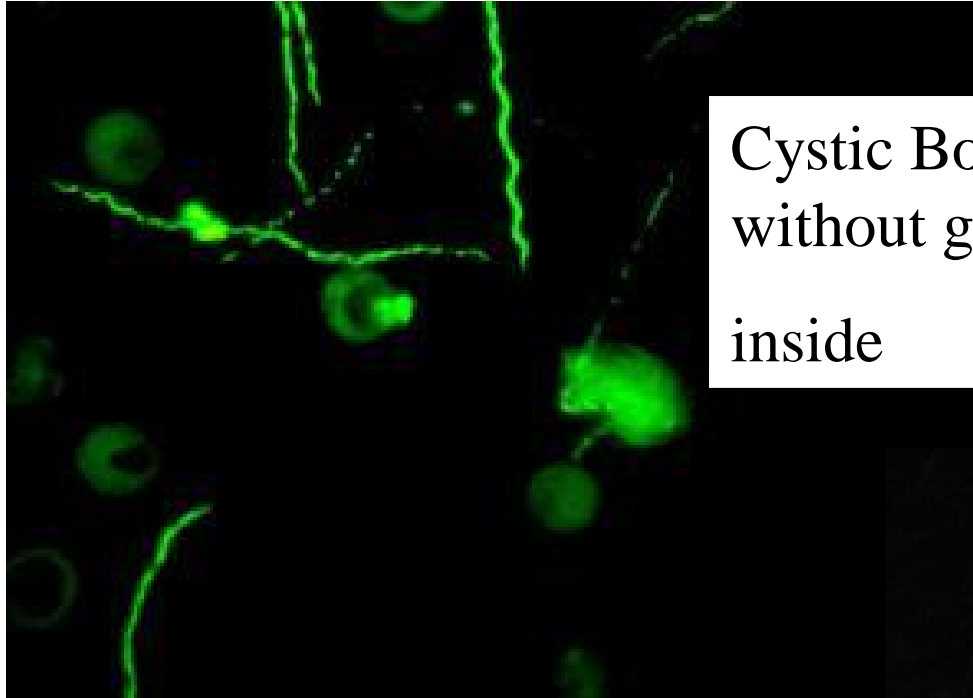


Spiral Borrelia



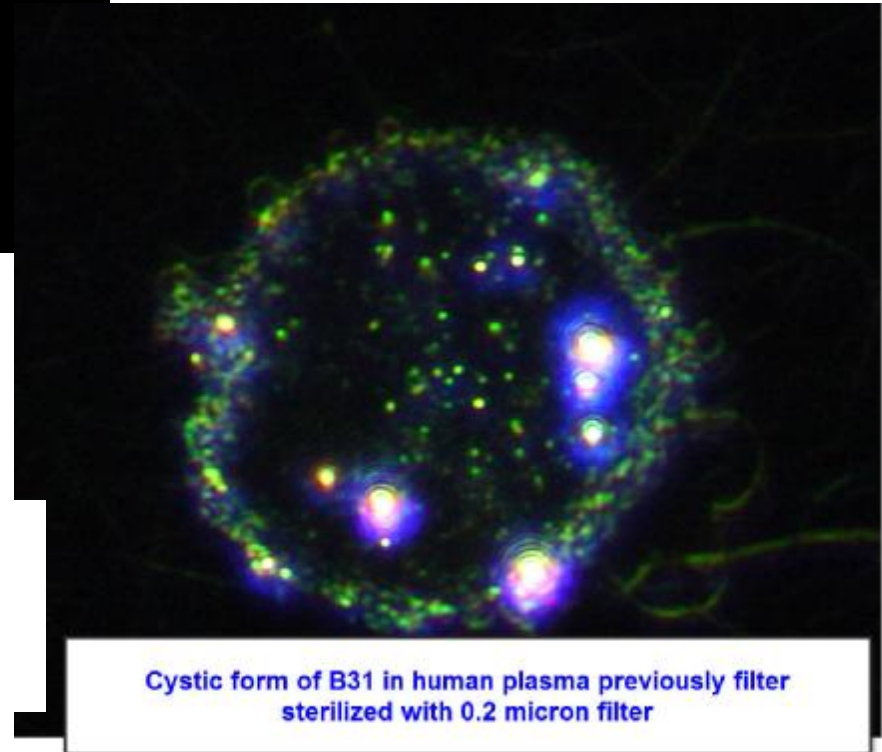


**Separate Cystic forms of  
Borrelia burgdorferi  
Without extracellular  
Matrix**



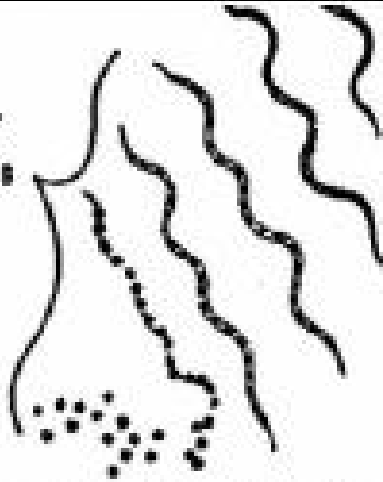
Cystic Borrelia  
without granules  
inside

Cystic Borrelia with  
Granules inside

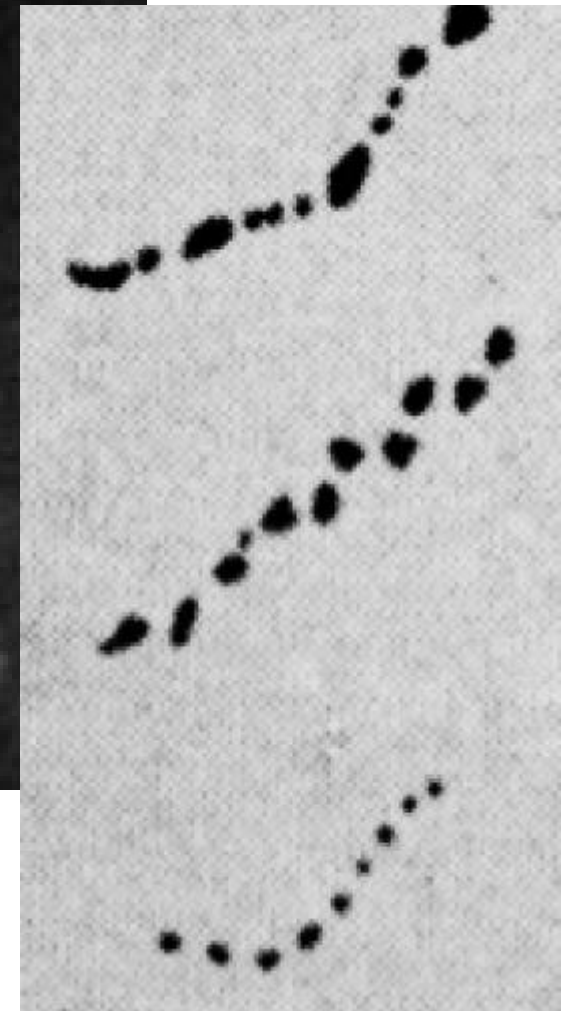
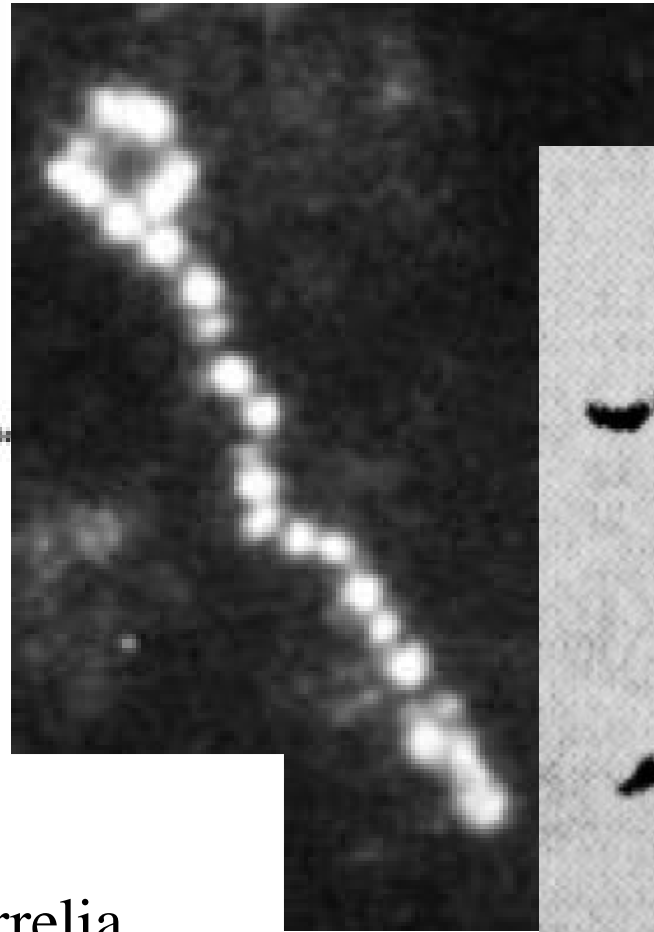


Cystic form of B31 in human plasma previously filter  
sterilized with 0.2 micron filter

FORMATION OF  
BOCCARDI BODIES  
IN BLOOD



Hindle, 1912, emergence of granular "boccardi" forms from spirachela



Granular Borrelia

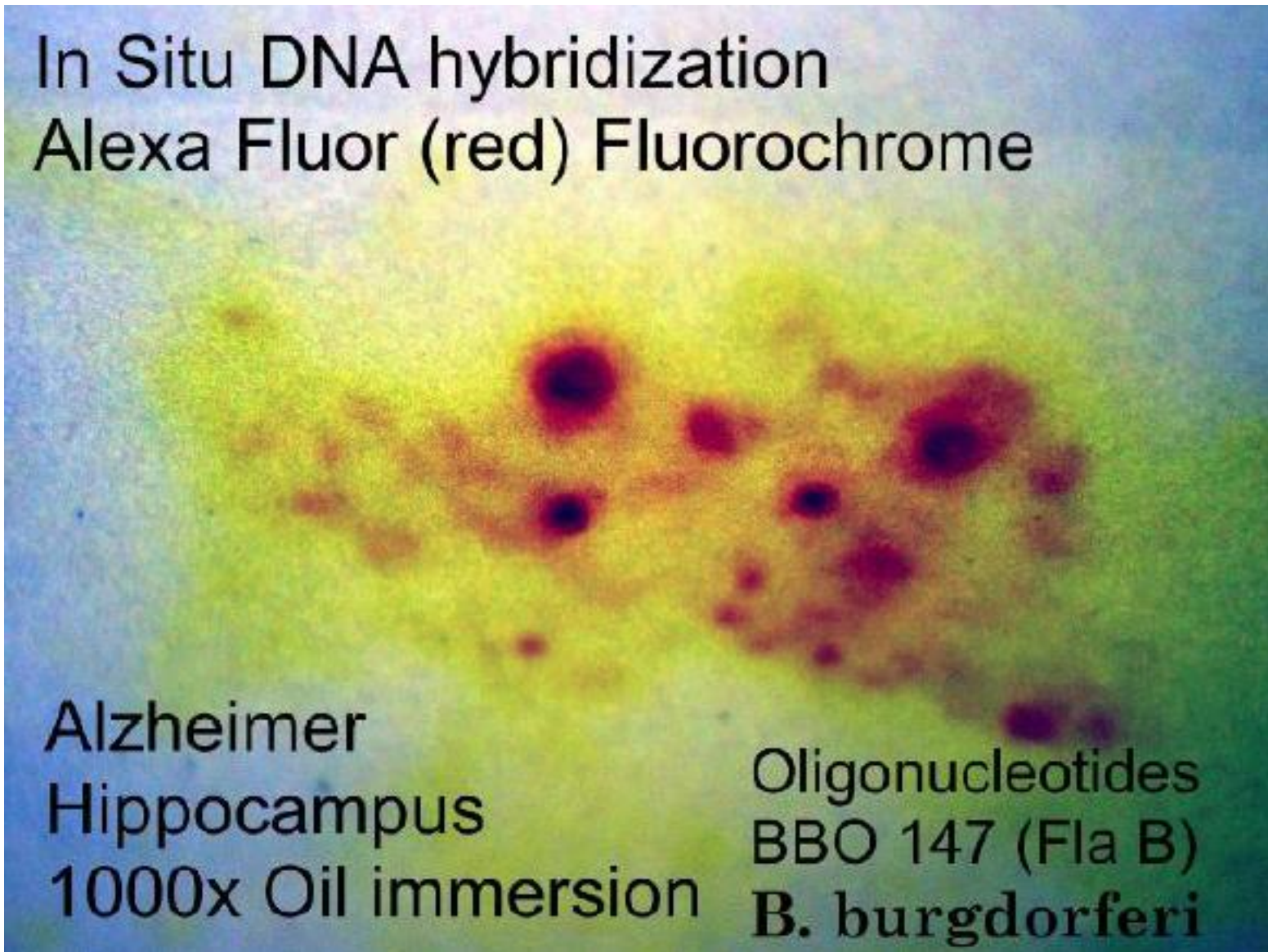
Evolving from spiral borrelia



In Situ DNA hybridization  
Alexa Fluor (red) Fluorochrome

Alzheimer  
Hippocampus  
1000x Oil immersion

Oligonucleotides  
BBO 147 (Fla B)  
**B. burgdorferi**



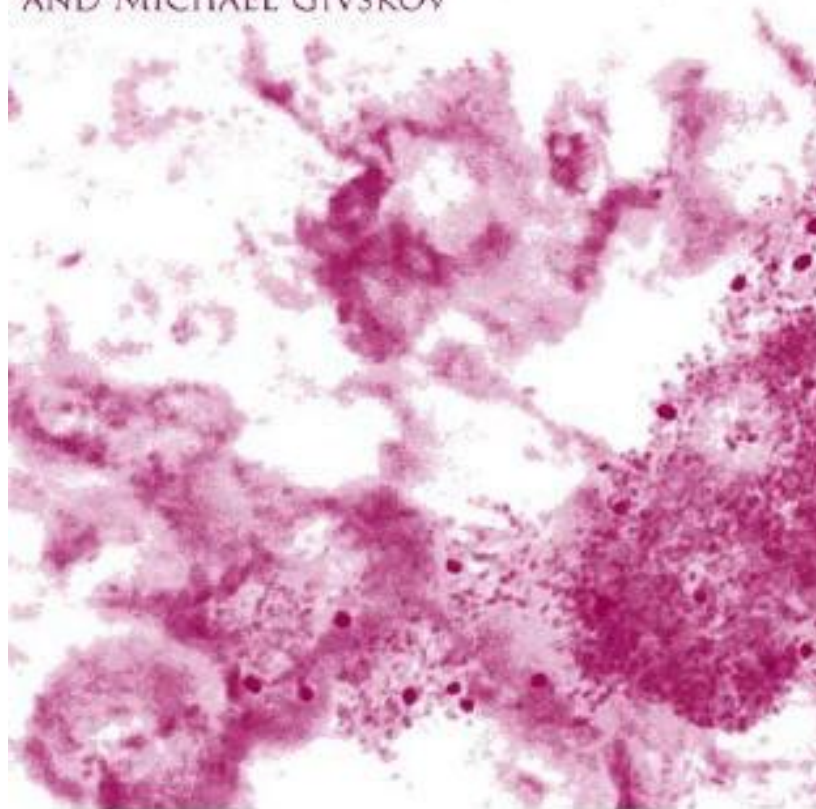
Cell wall deficient Borrelia



# Biofilm: A community of microbes enveloped in a protective Extracellular matrix

THE **BIOFILM** MODE OF LIFE  
MECHANISMS AND ADAPTATIONS

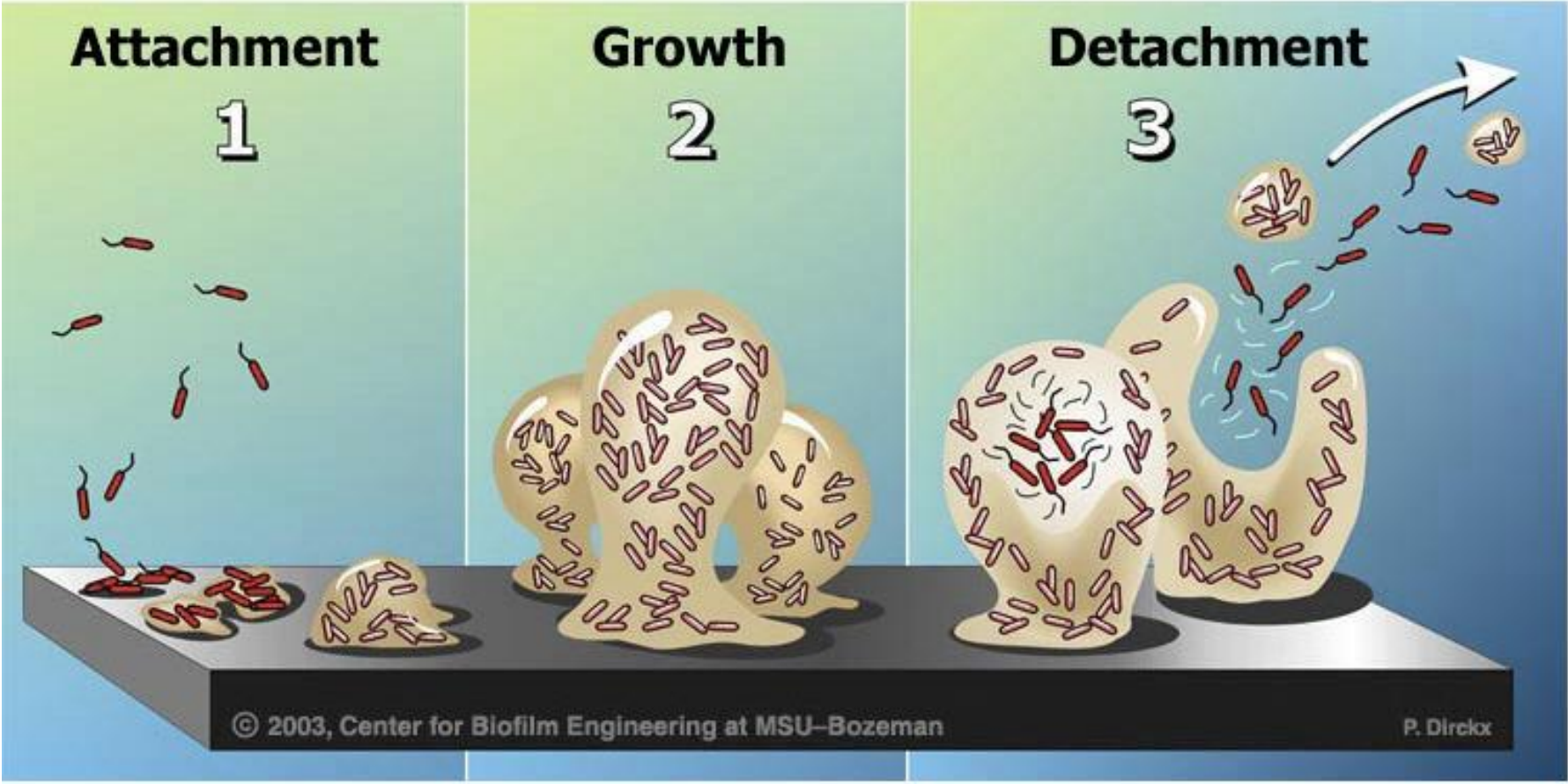
EDITED BY STAFFAN KJELLEBERG  
AND MICHAEL GIVSKOV



“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.

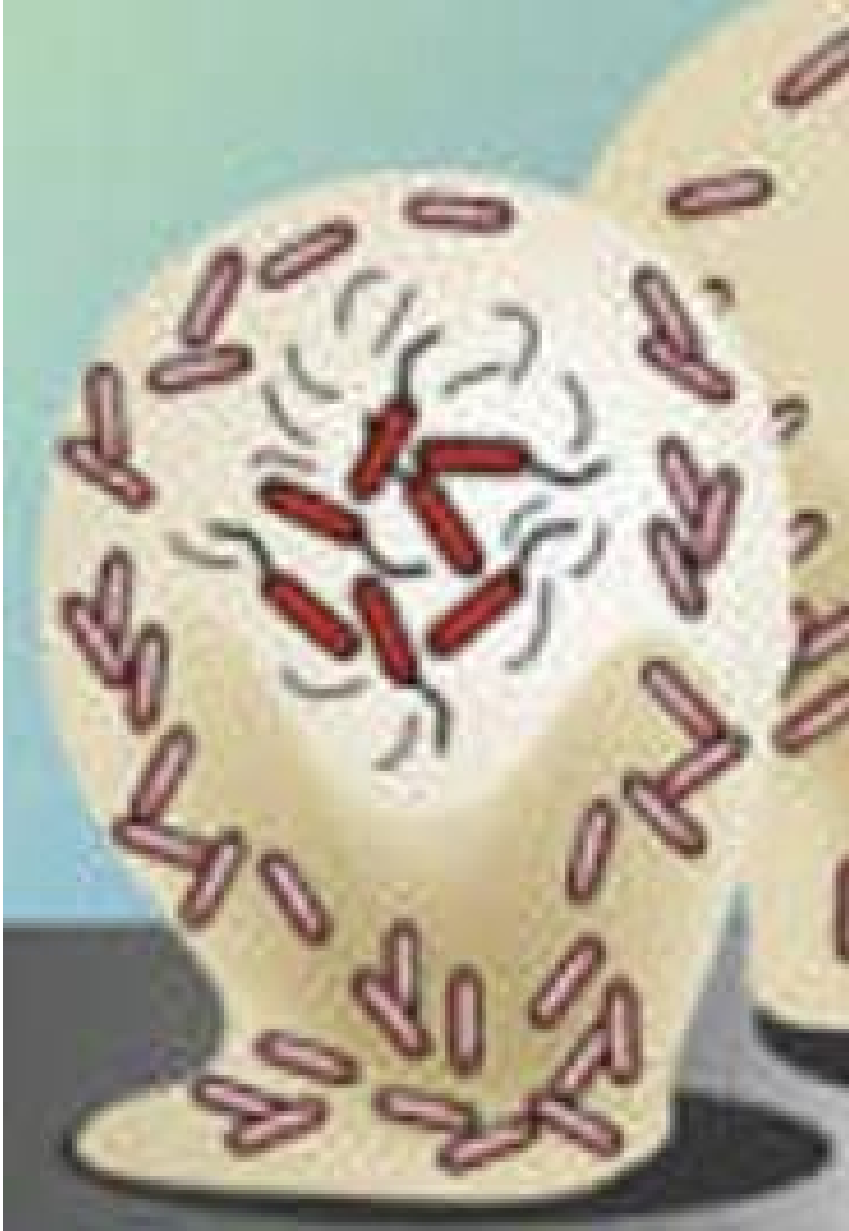




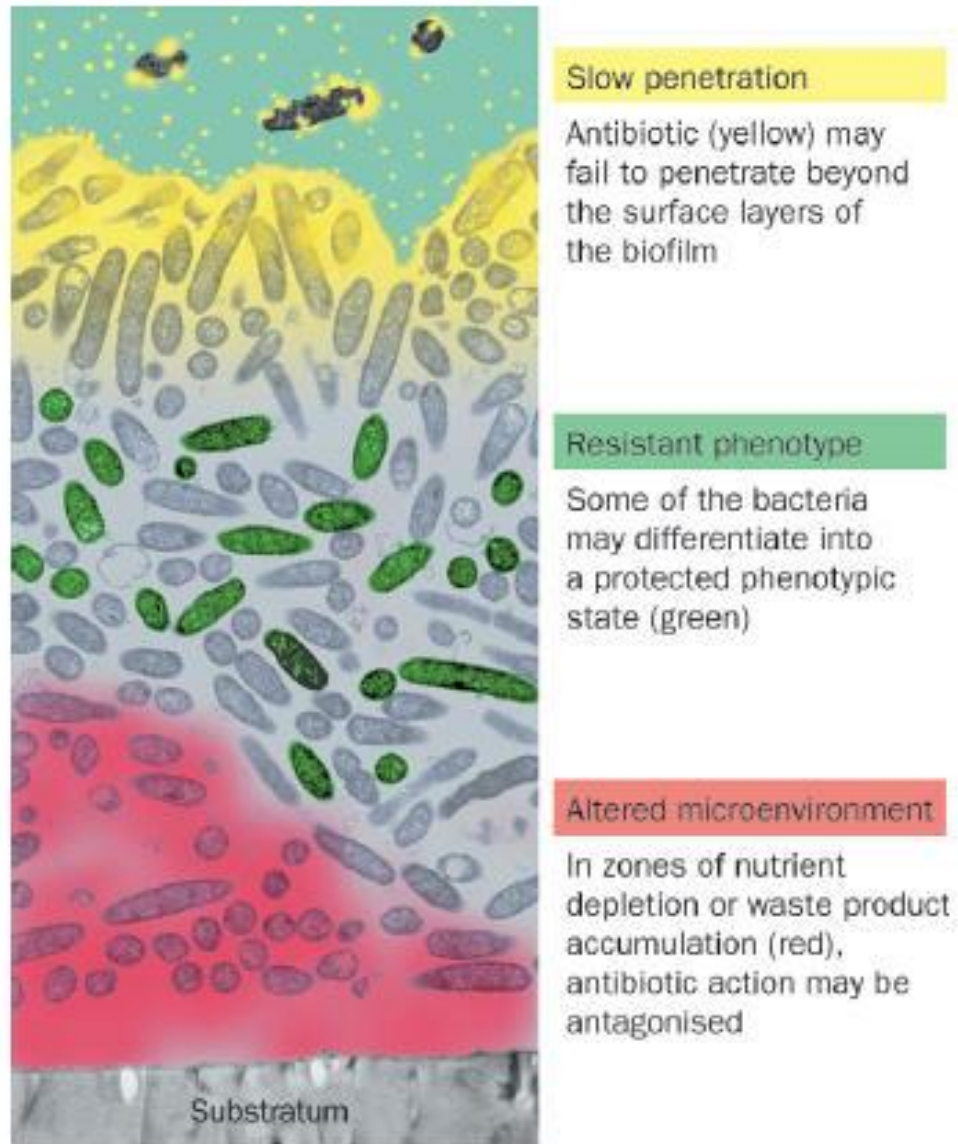












**Figure 2: Three hypotheses for mechanisms of antibiotic resistance in biofilms**

**Review**

## Antibiotic resistance of bacteria in biofilms

*Phillip 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

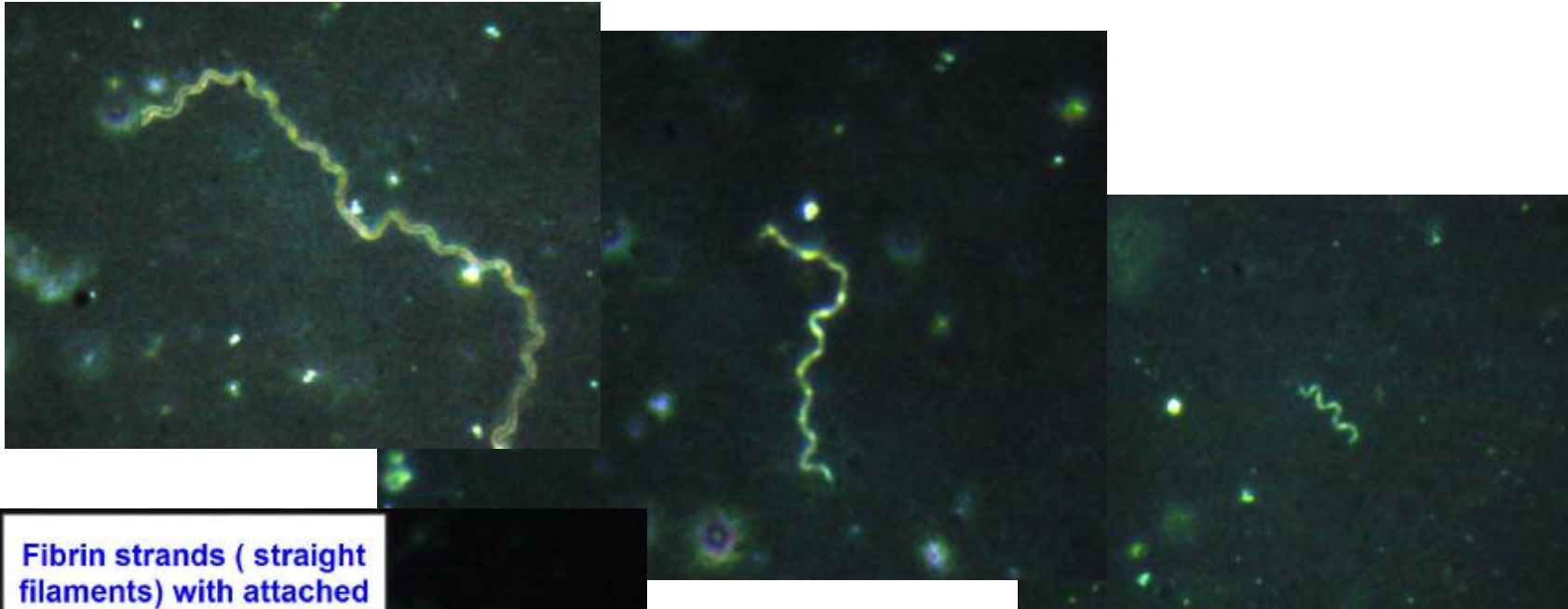
Failure of Antibiotic to penetrate the Biofilm

Differentiation of Bacteria within the Biofilm -

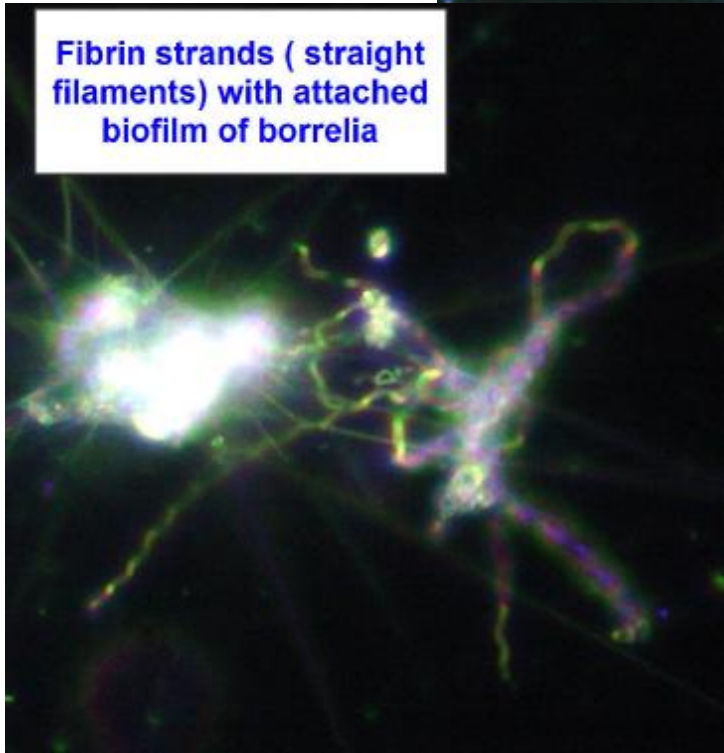
Dormant State and Altered Genetics

Bacterial Heterogeneity in Biofilms

Accumulation of Molecules in the biofilm  
which antagonize the Antibiotic action

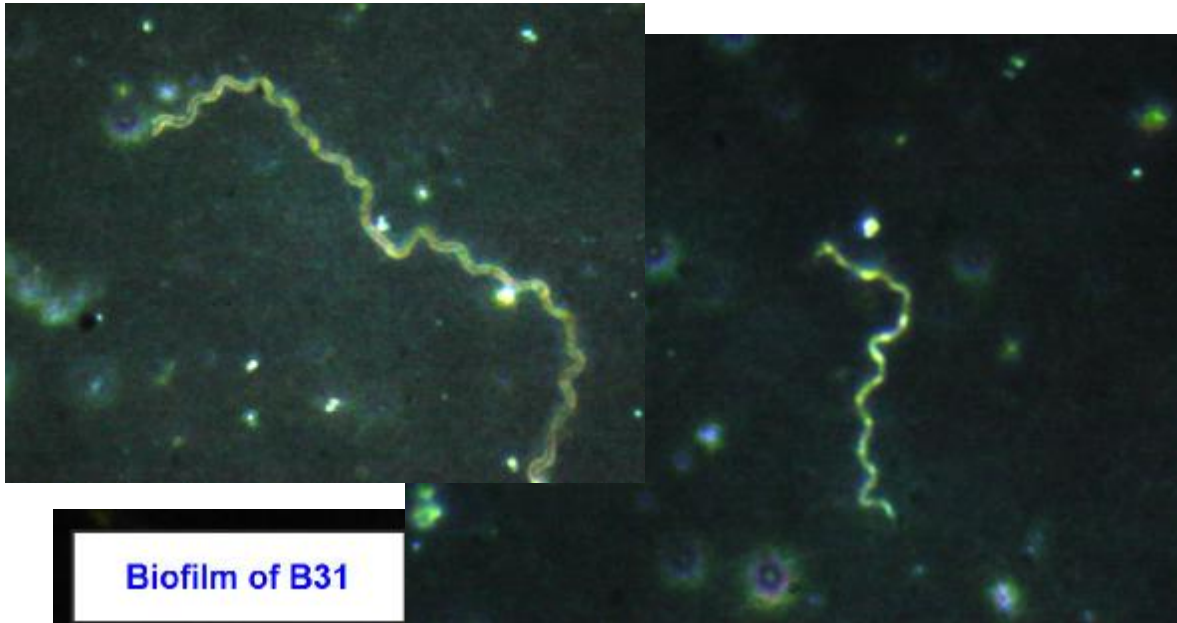


Fibrin strands ( straight filaments) with attached biofilm of borrelia

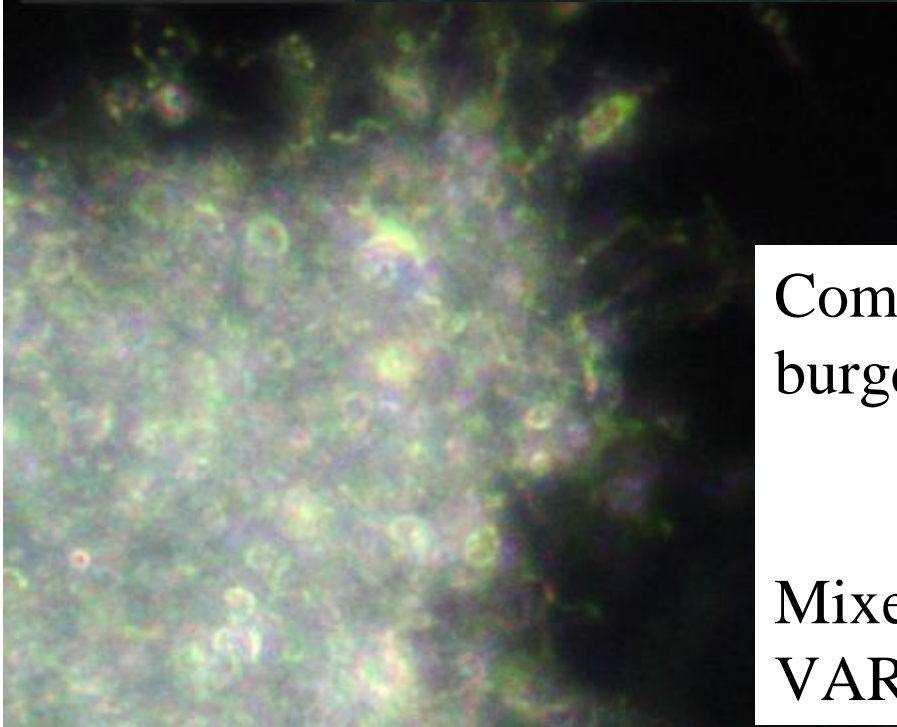
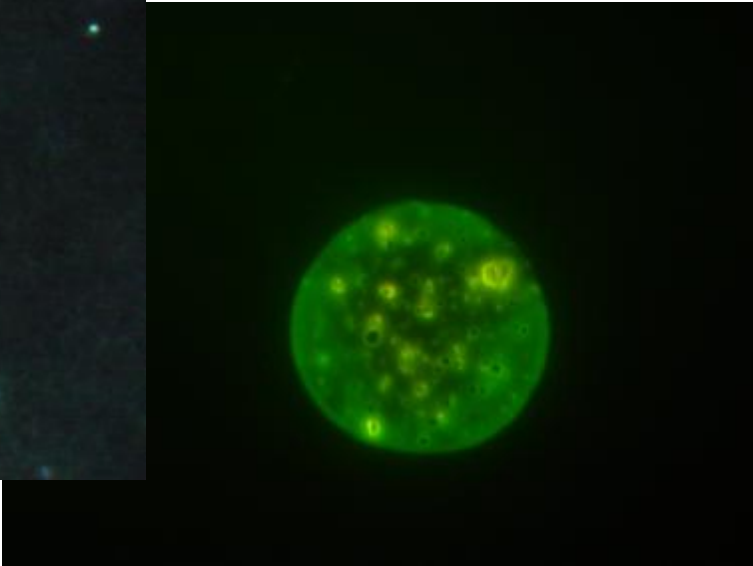


Communities of pure *Borrelia burgdorferi* ( corkscrew/ spiral)

Spiral Biofilm VARIANT

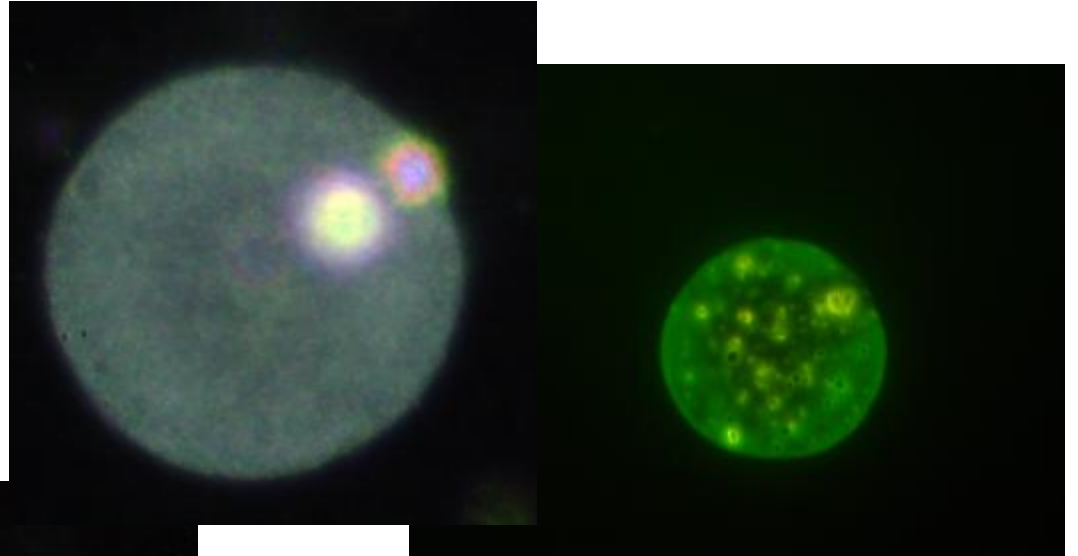
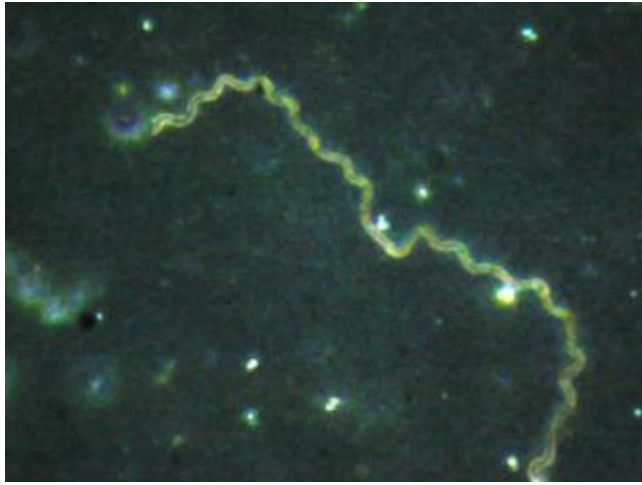


Biofilm of B31

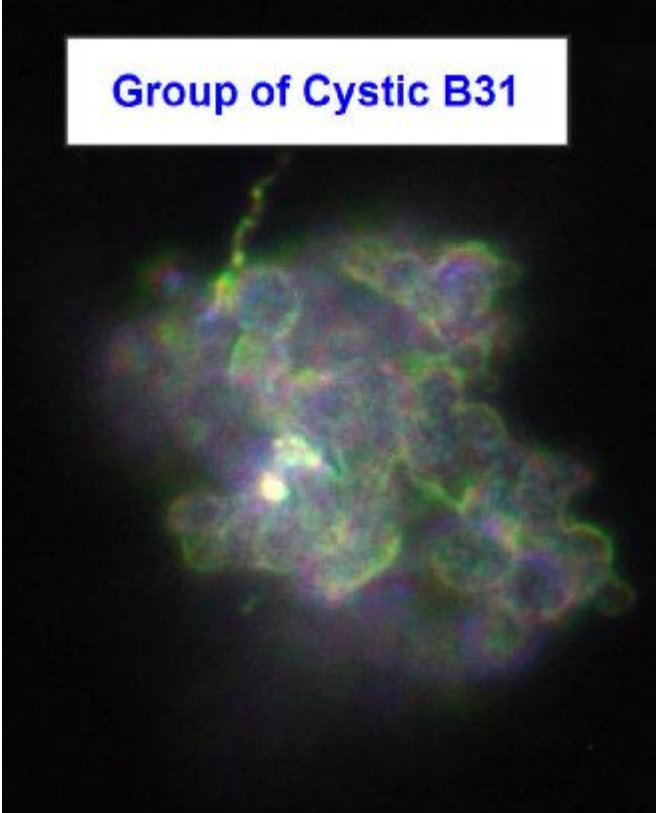


Communities of pure *Borrelia burgdorferi*

Mixed Cystic and Spiral  
**VARIANT**



Group of Cystic B31



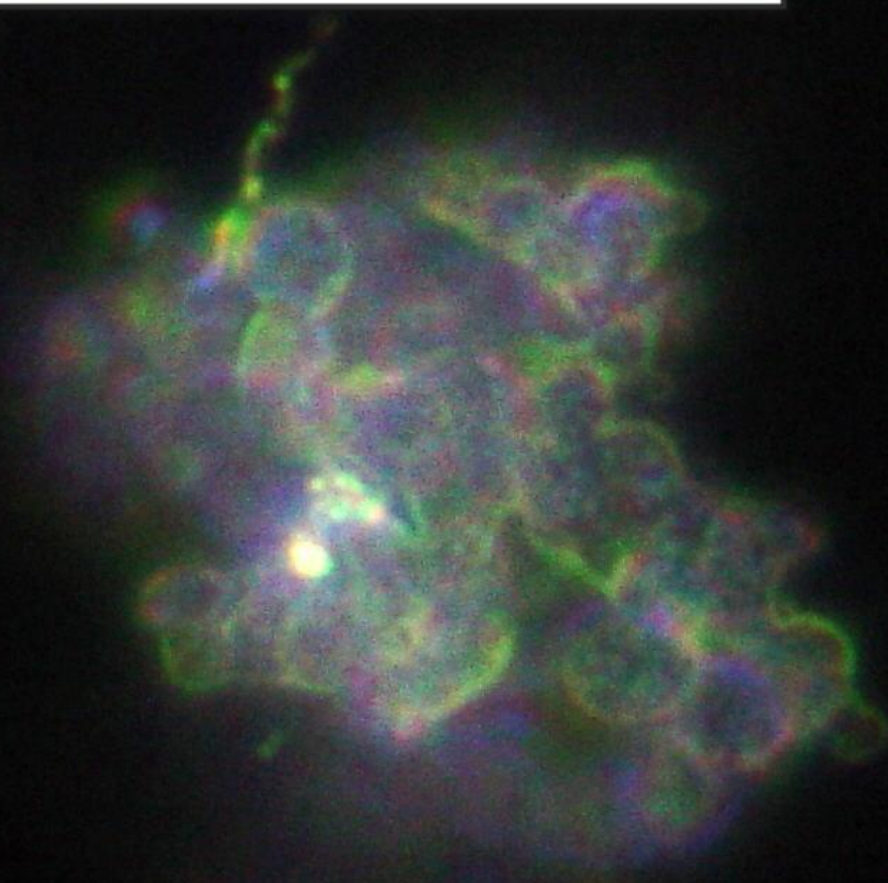
Communities of Pure *Borrelia burgdorferi*

Biofilm composed of Cystic forms

Cystic Biofilm VARIANT

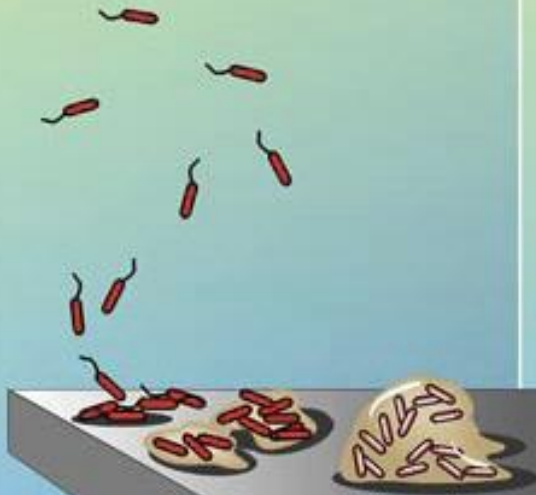


## Group of Cystic B31



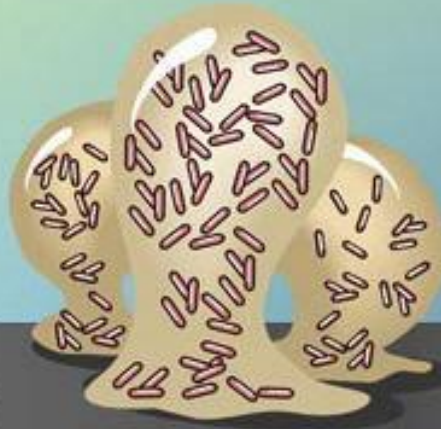
# Attachment

1



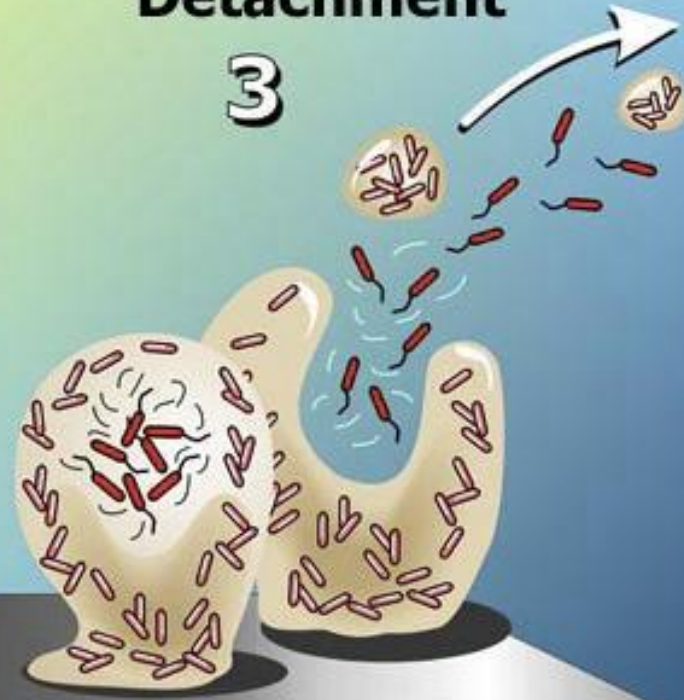
# Growth

2

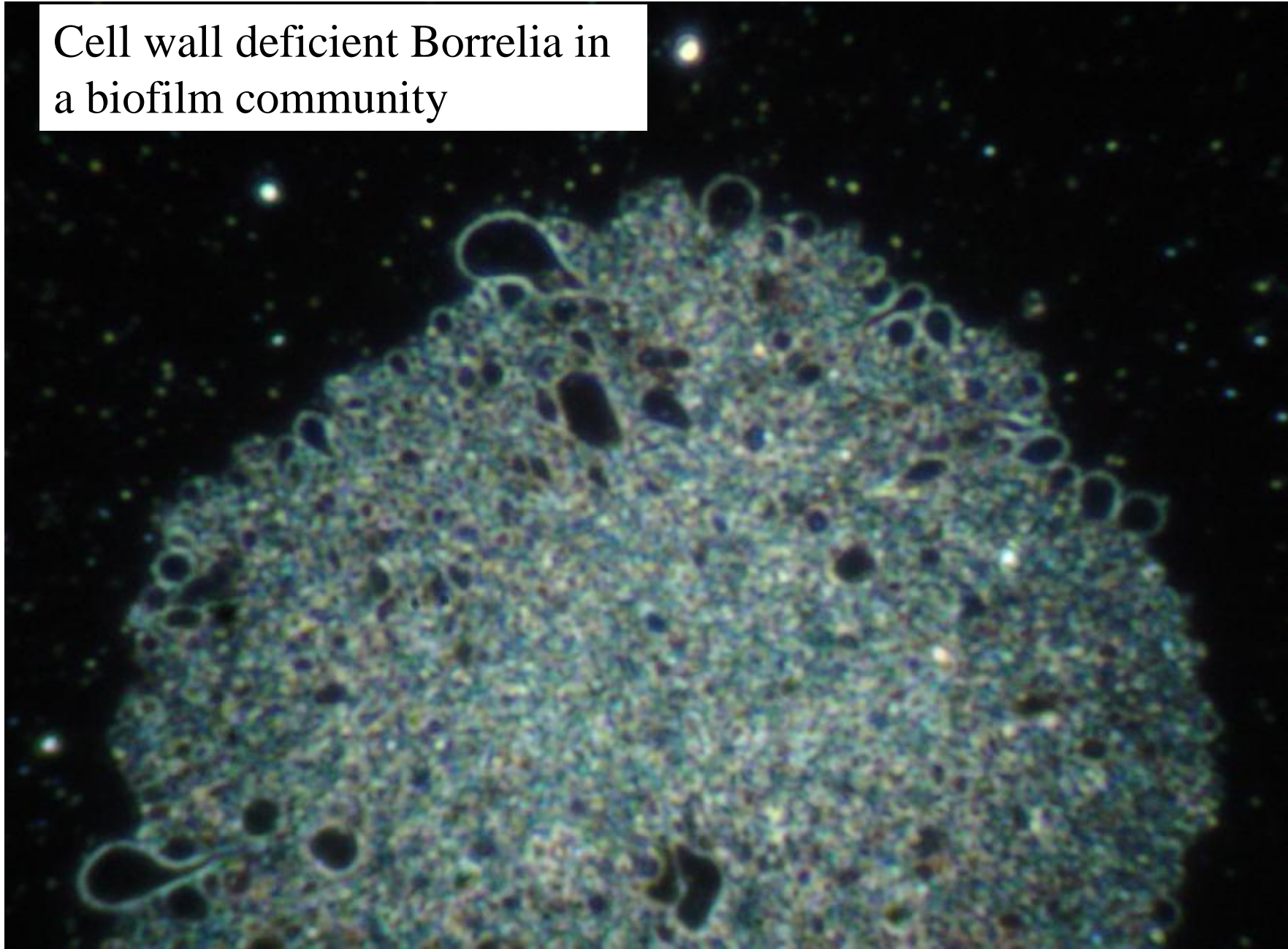


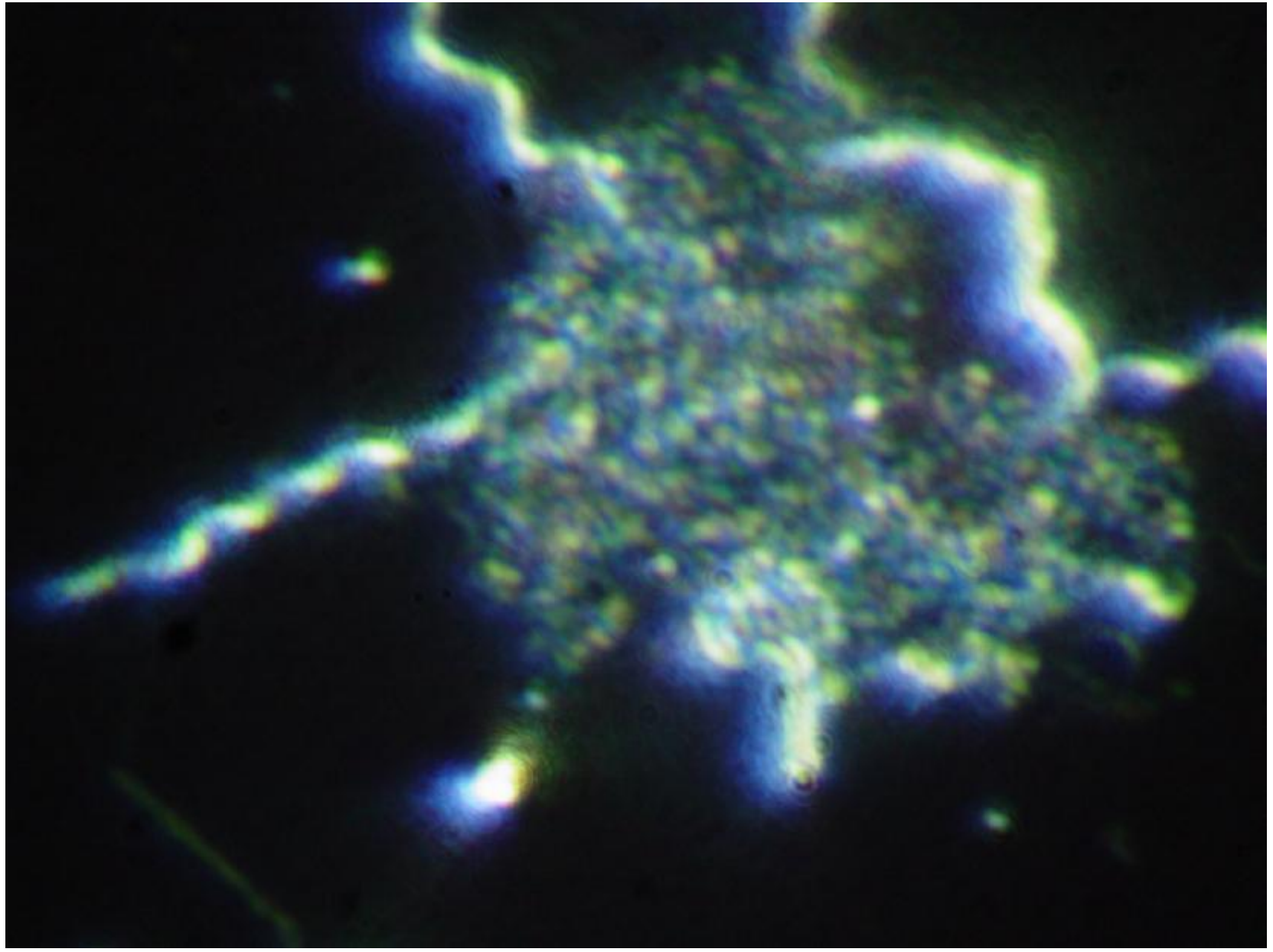
# Detachment

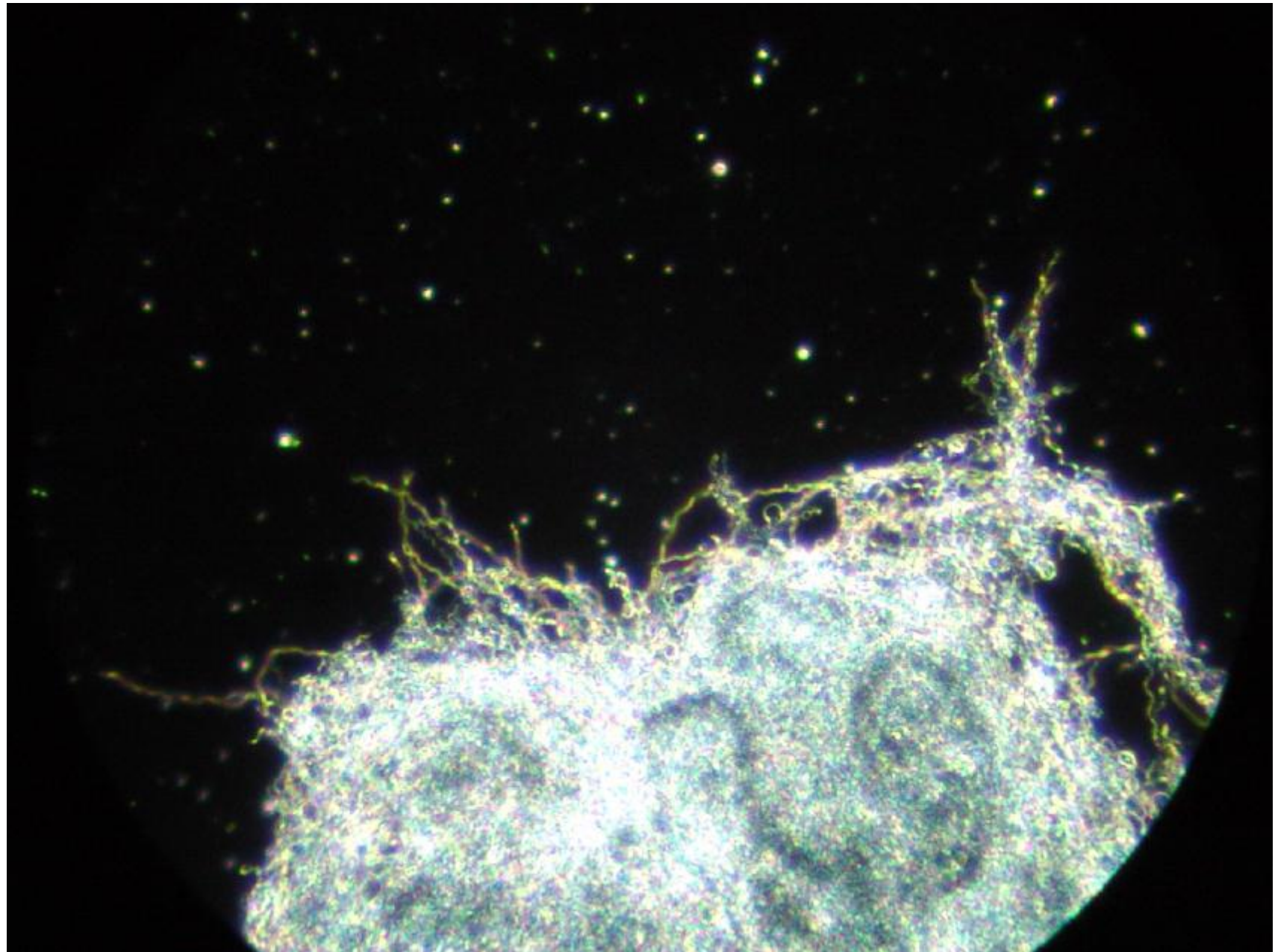
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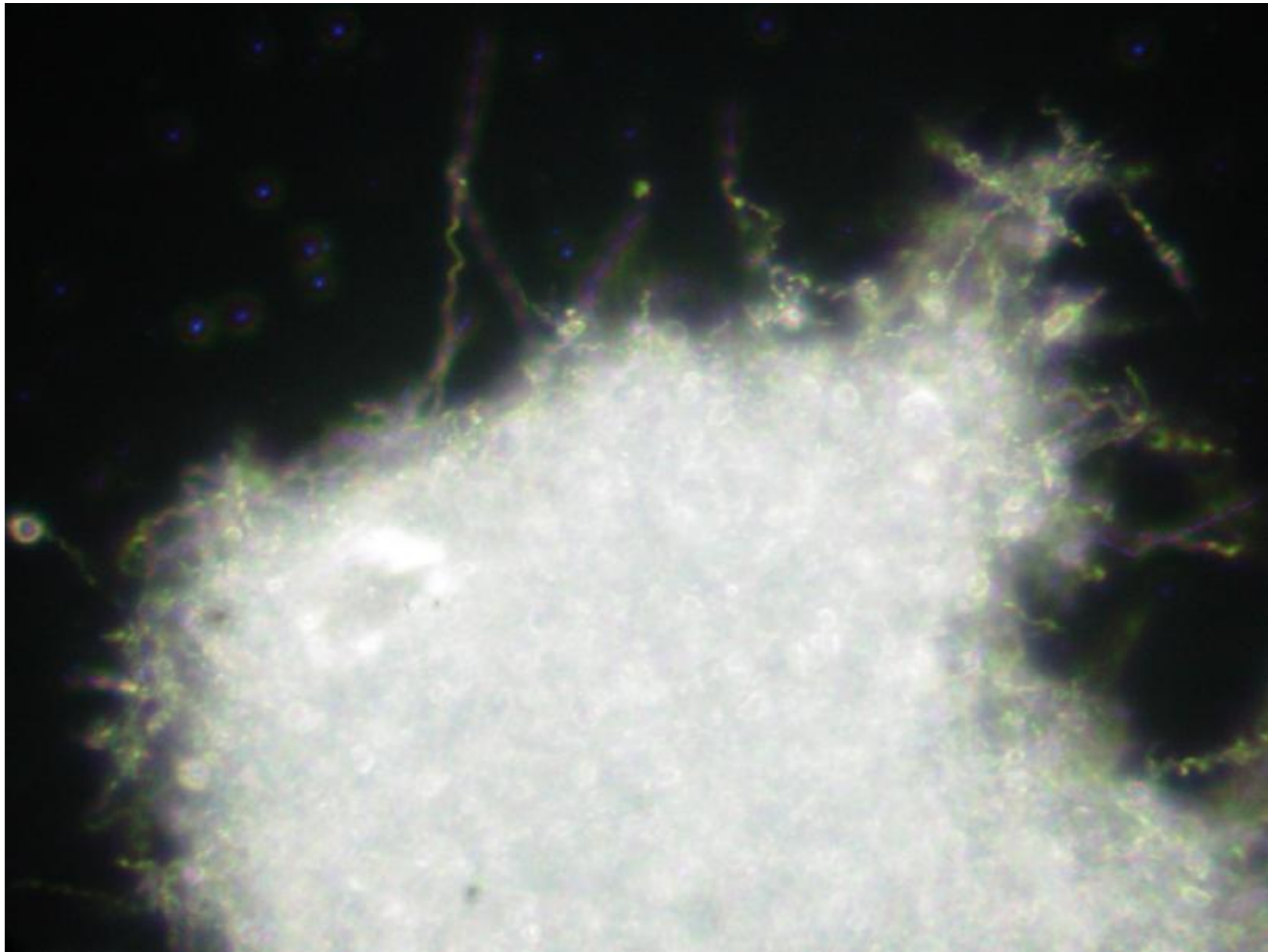


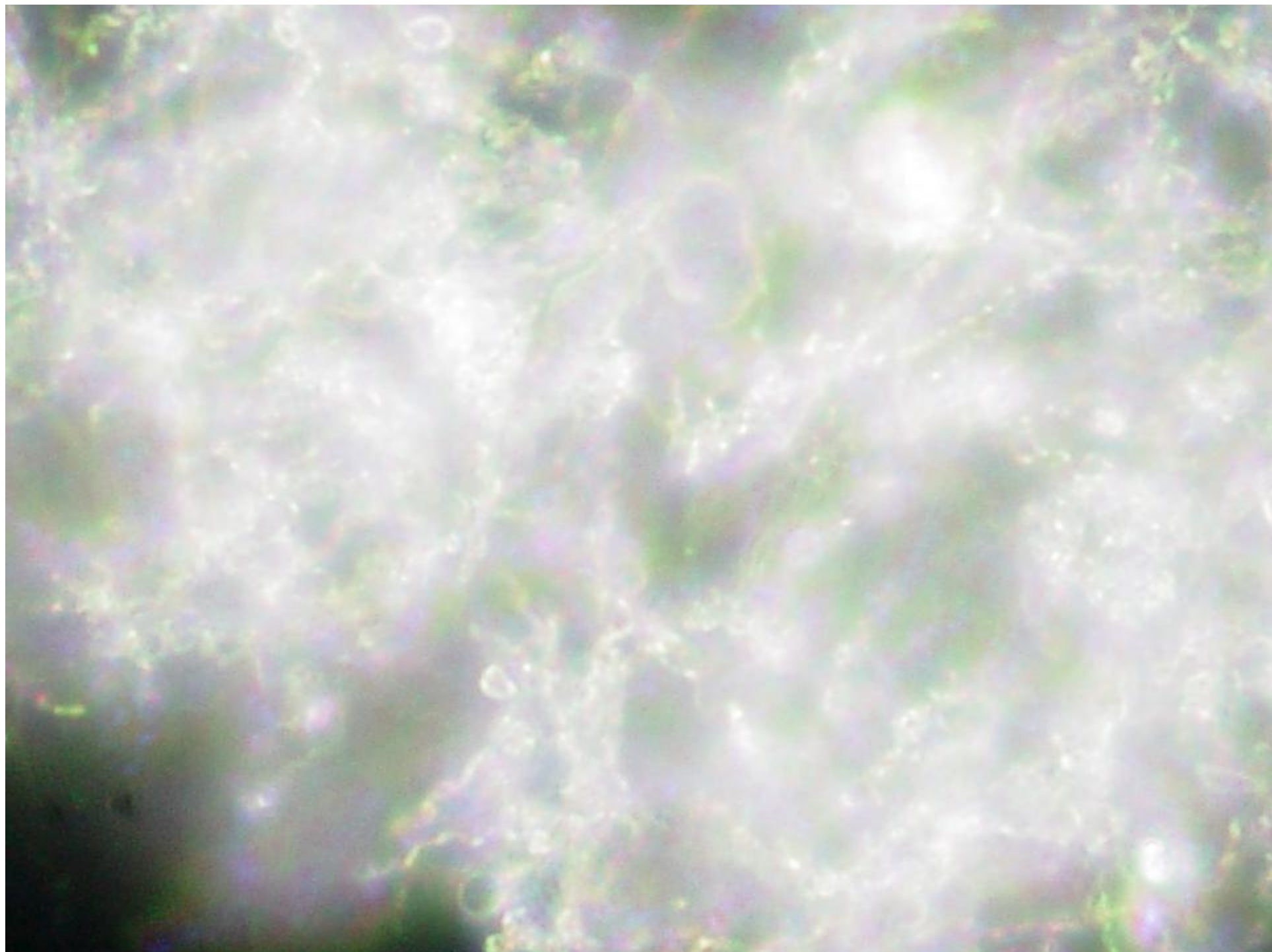
Cell wall deficient *Borrelia* in  
a biofilm community

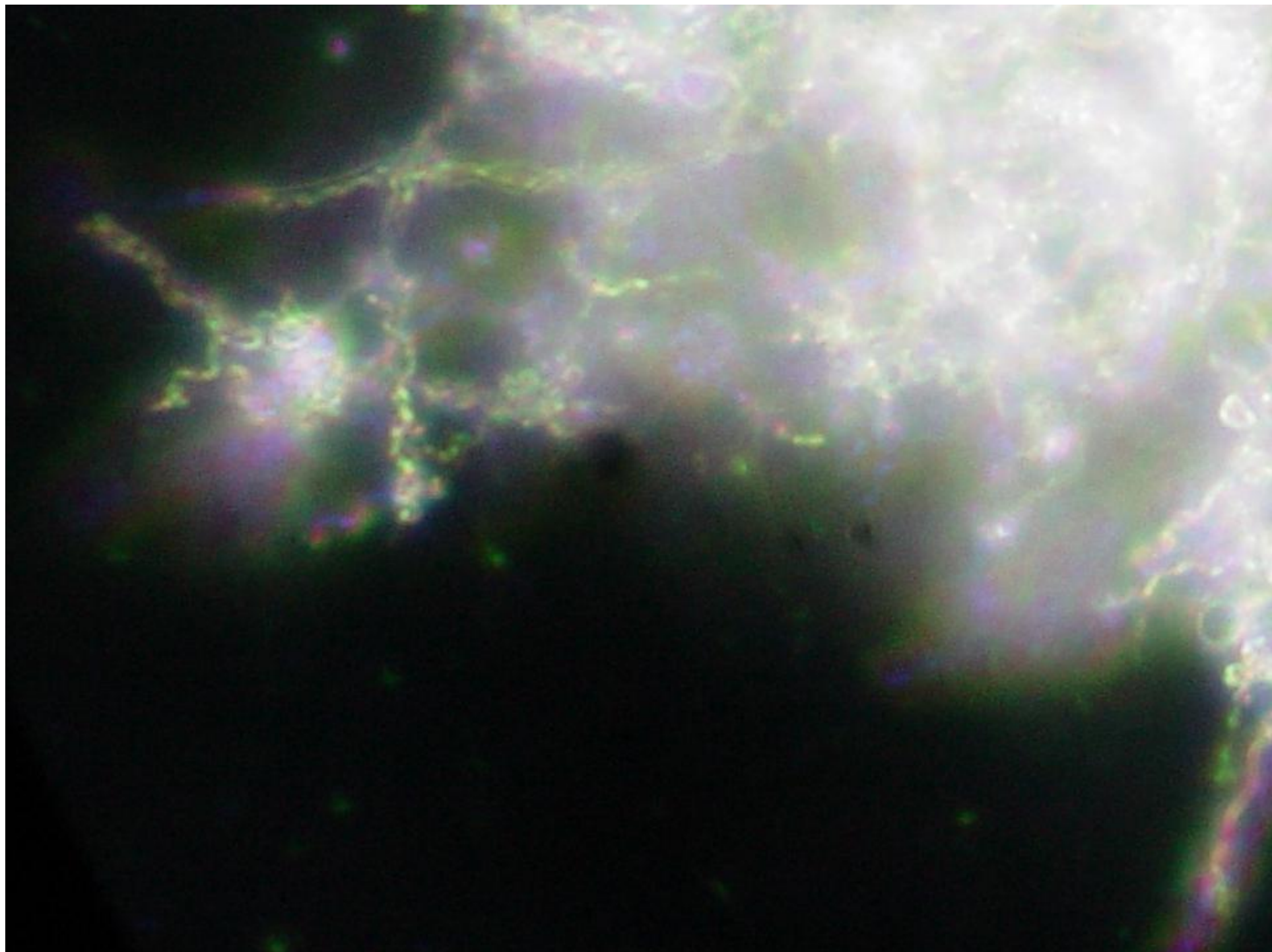




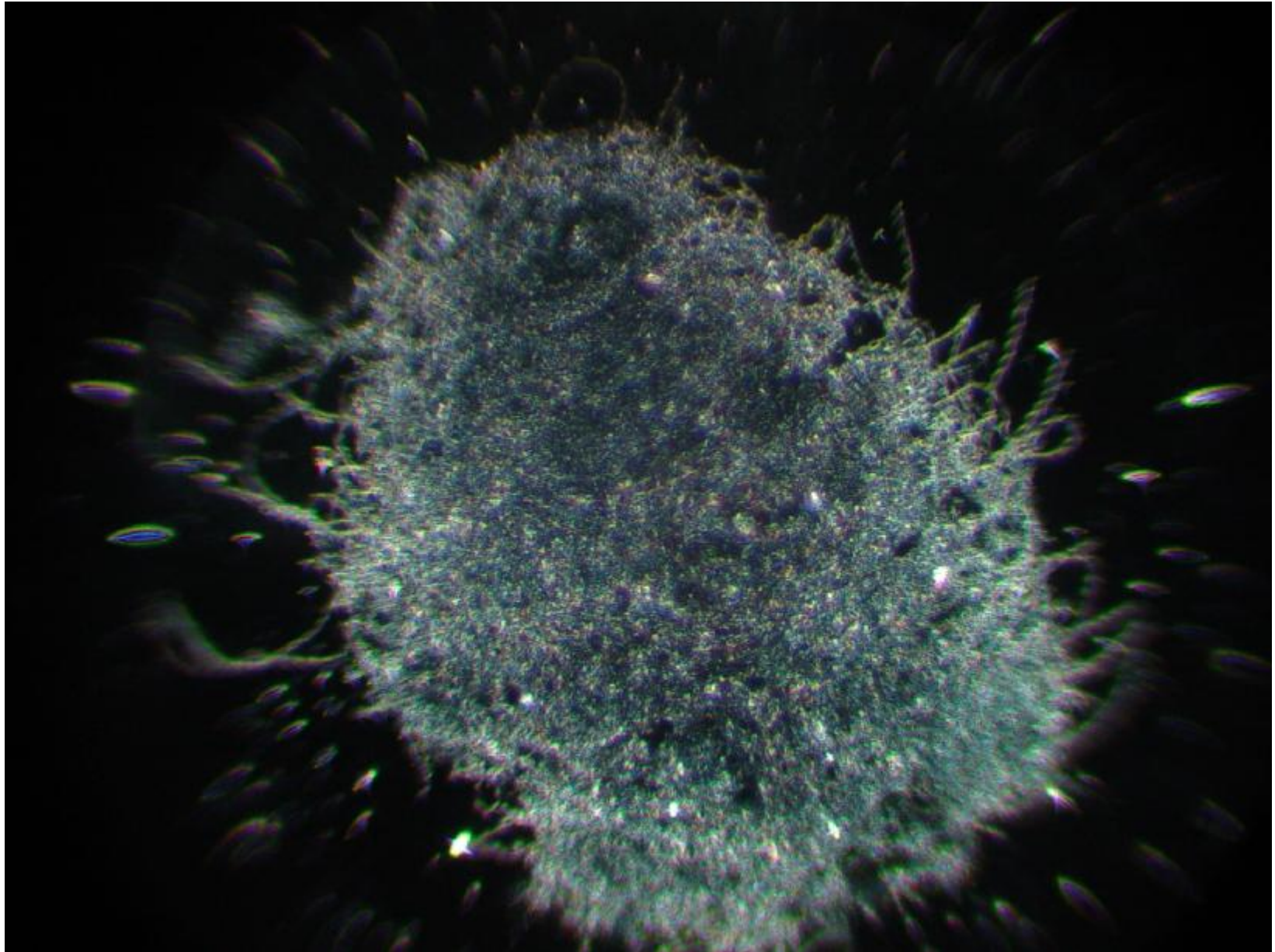


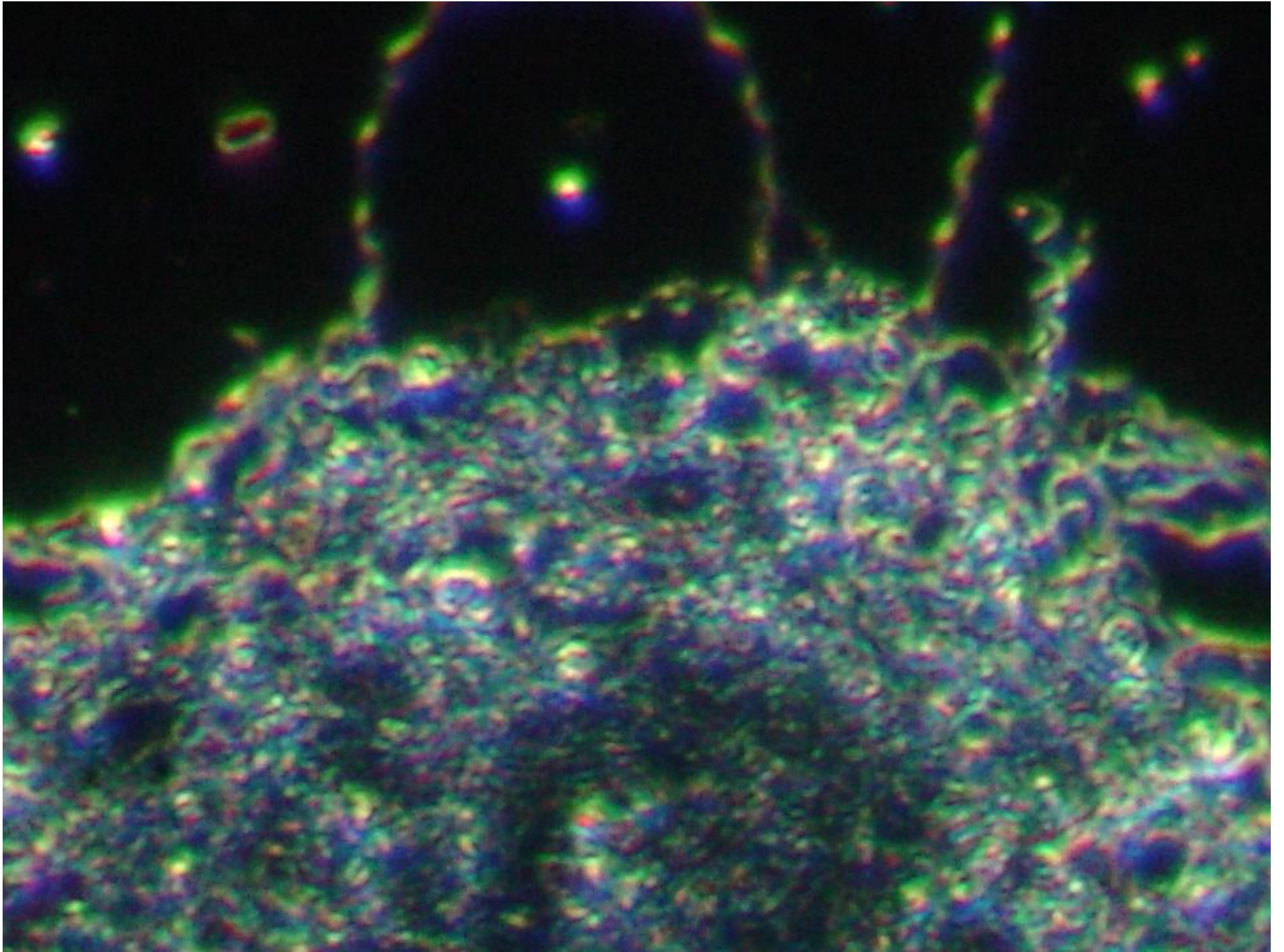


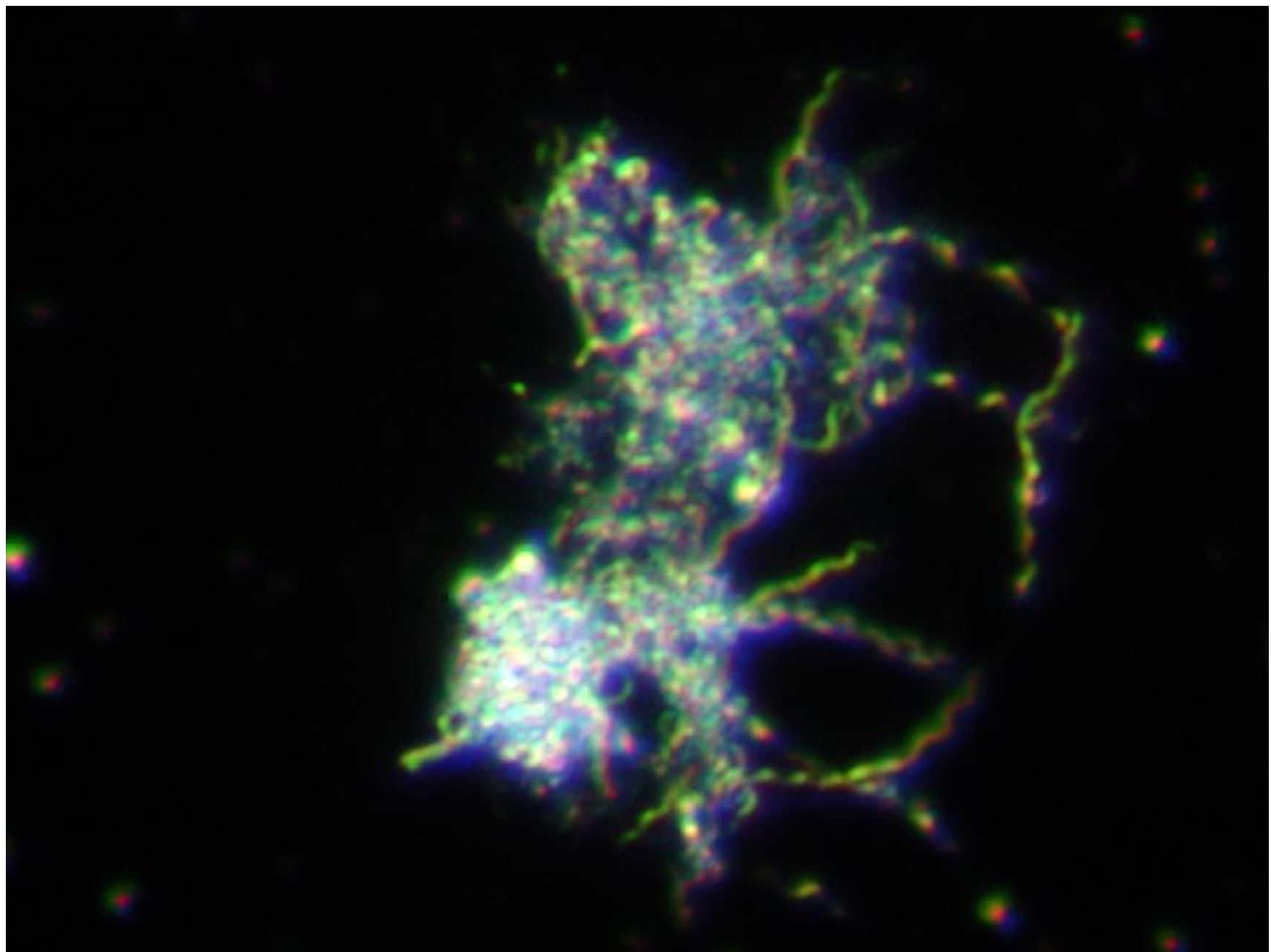


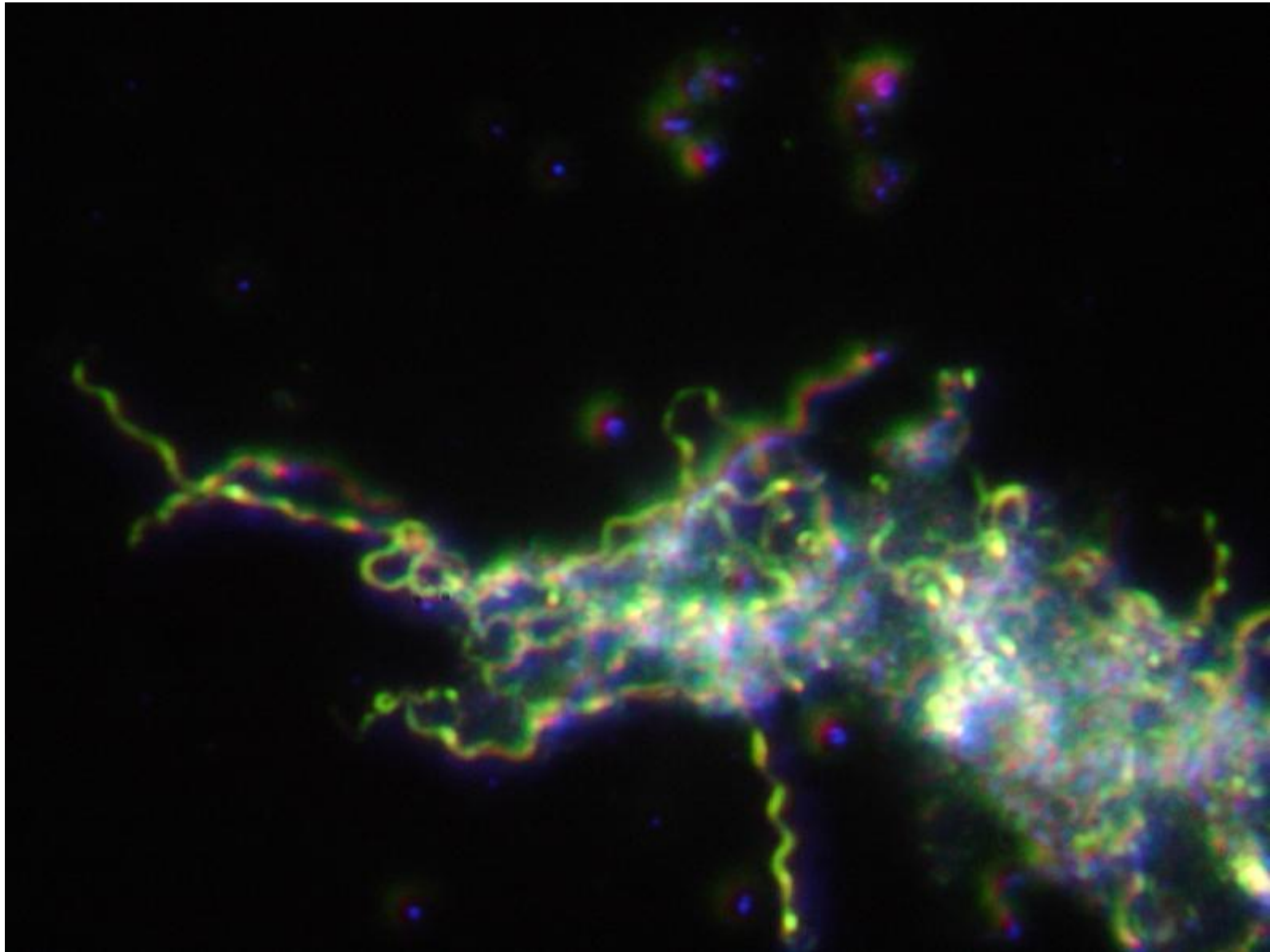


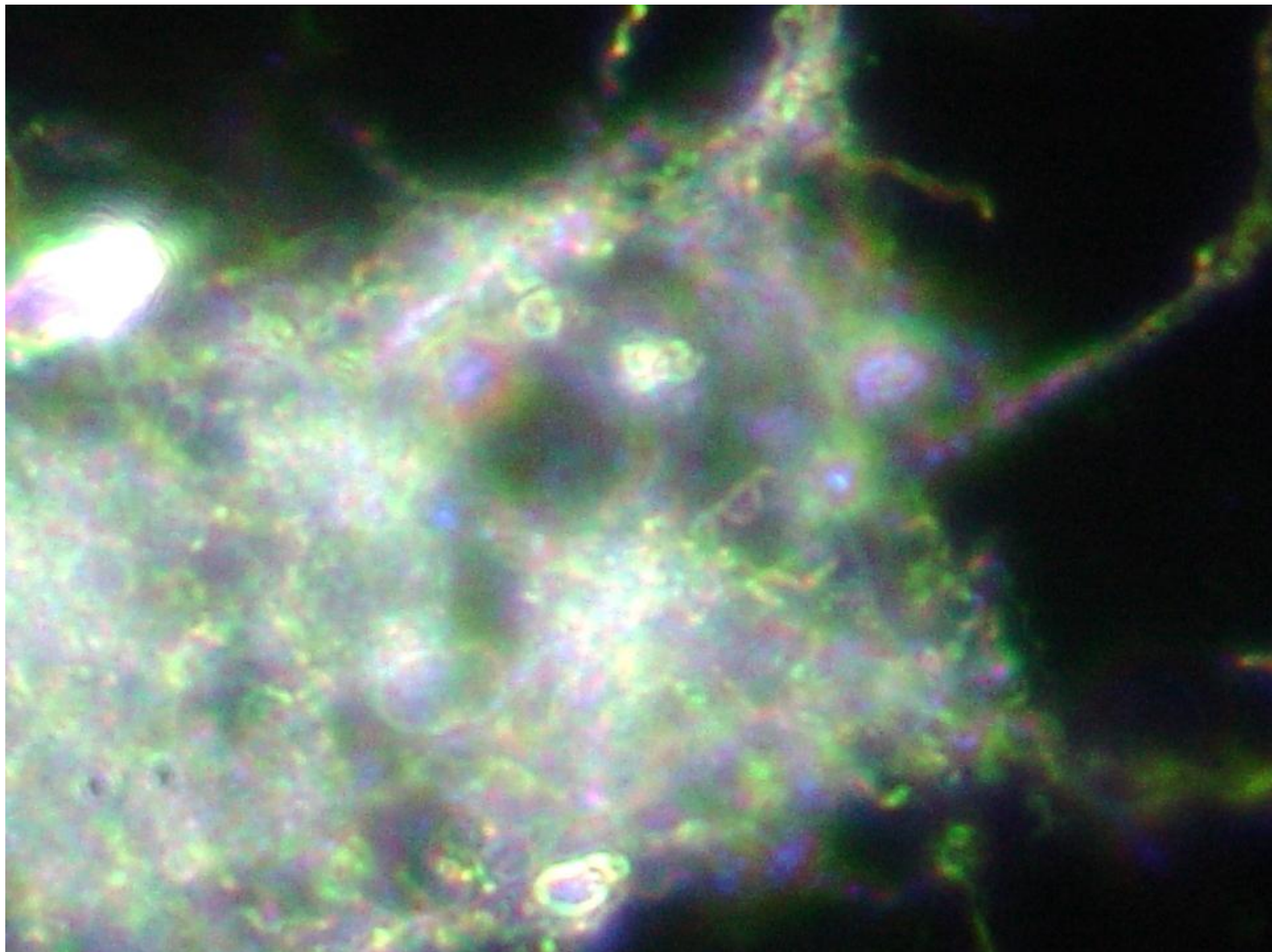


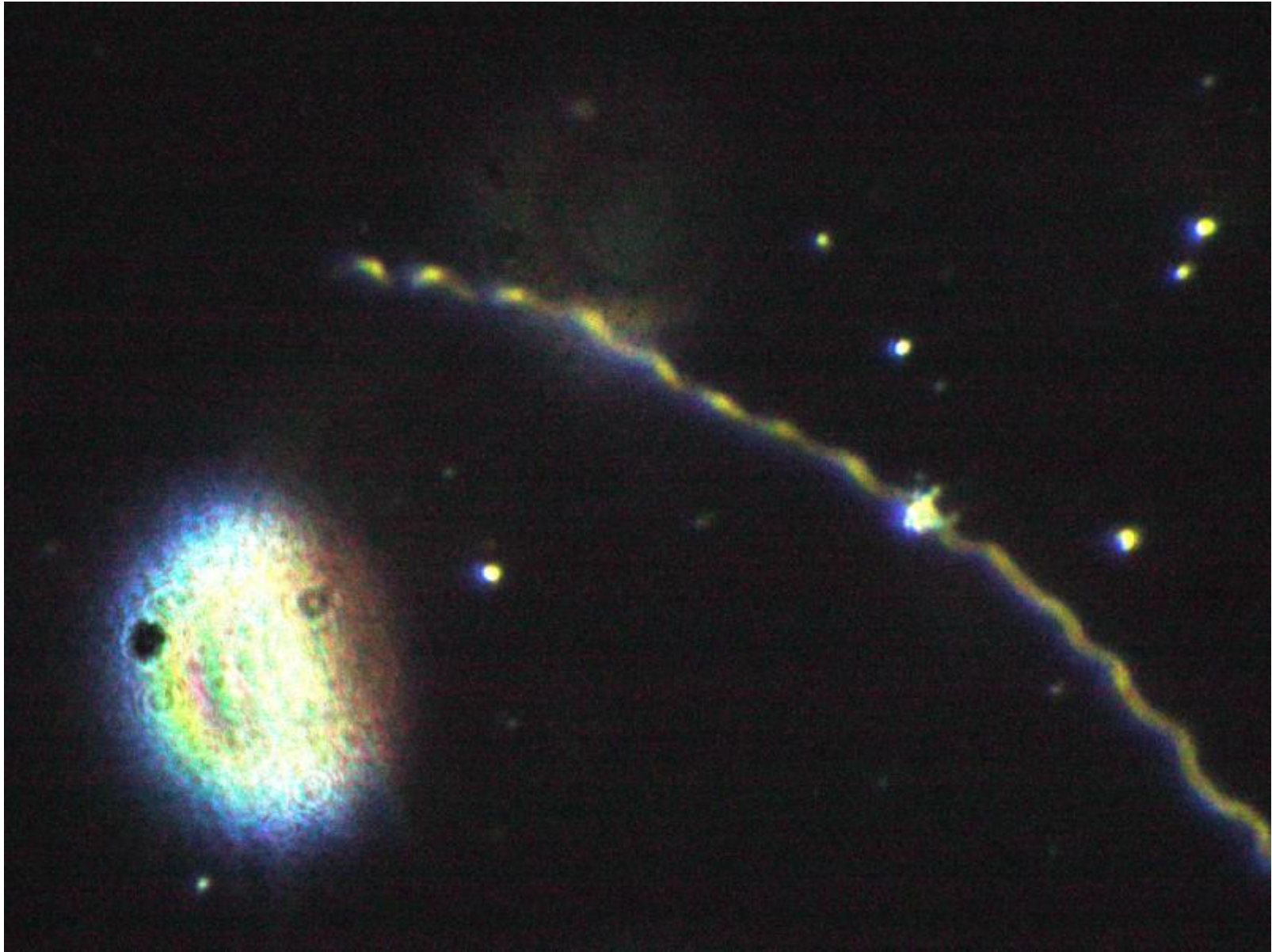


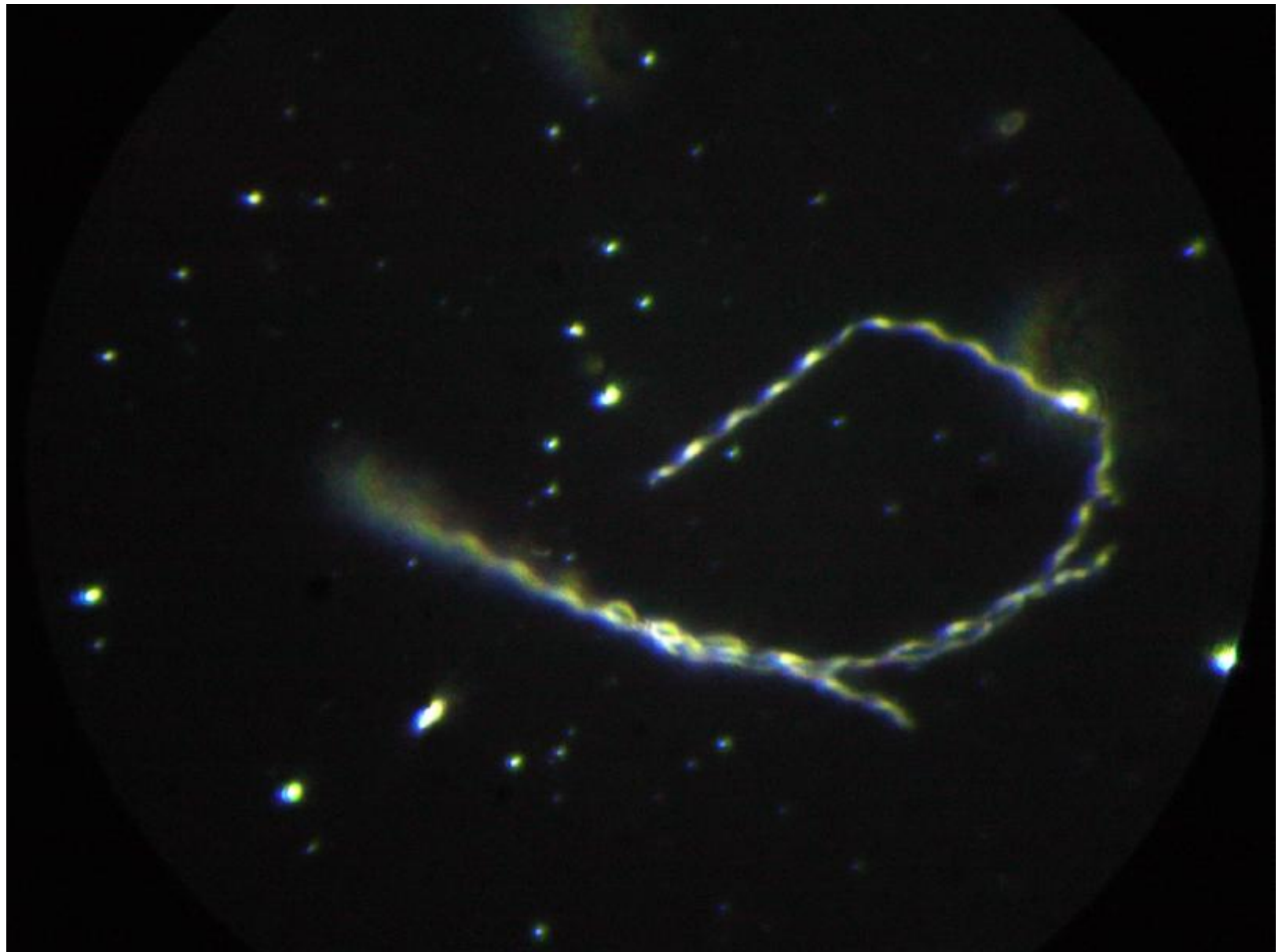


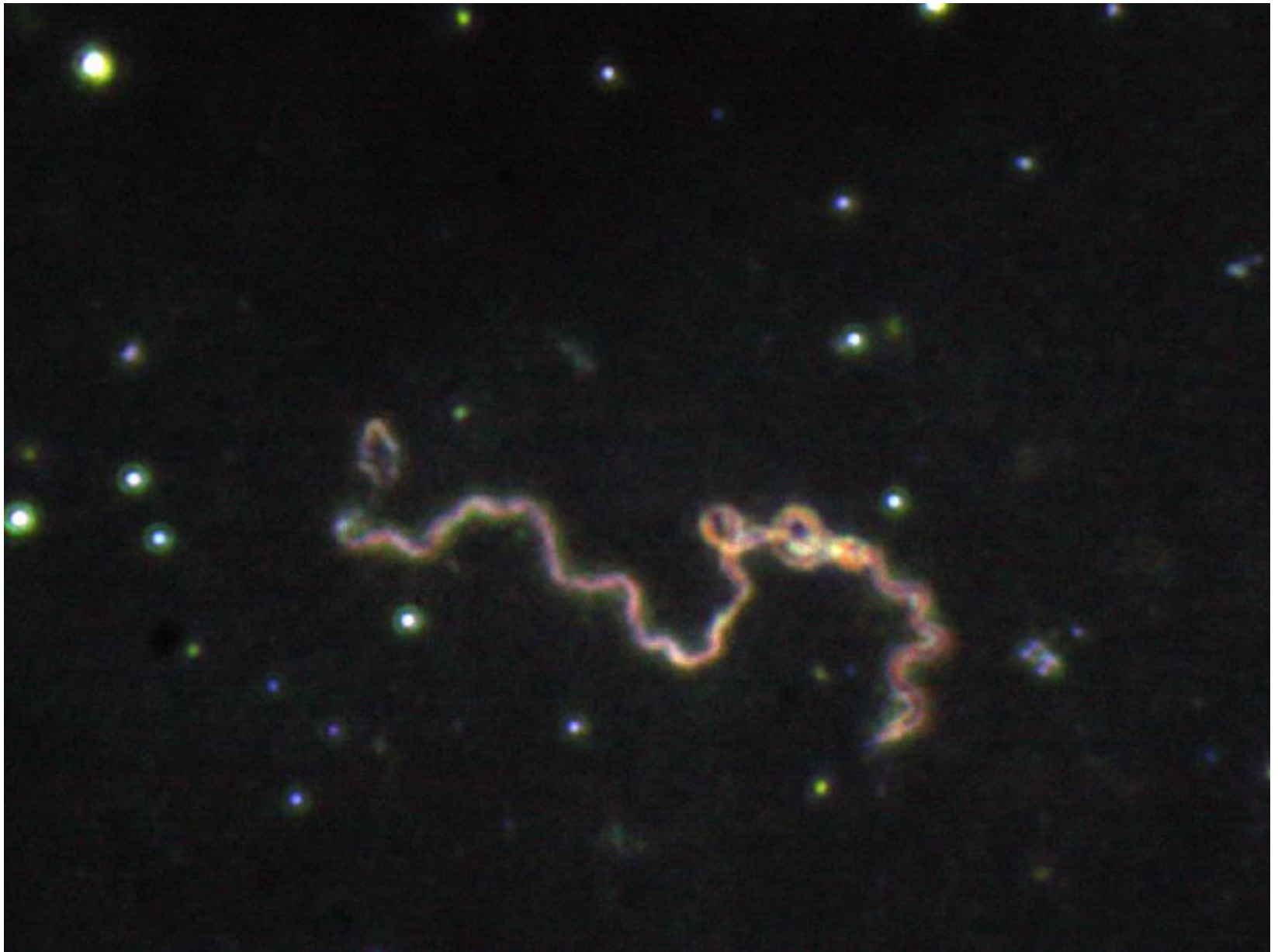




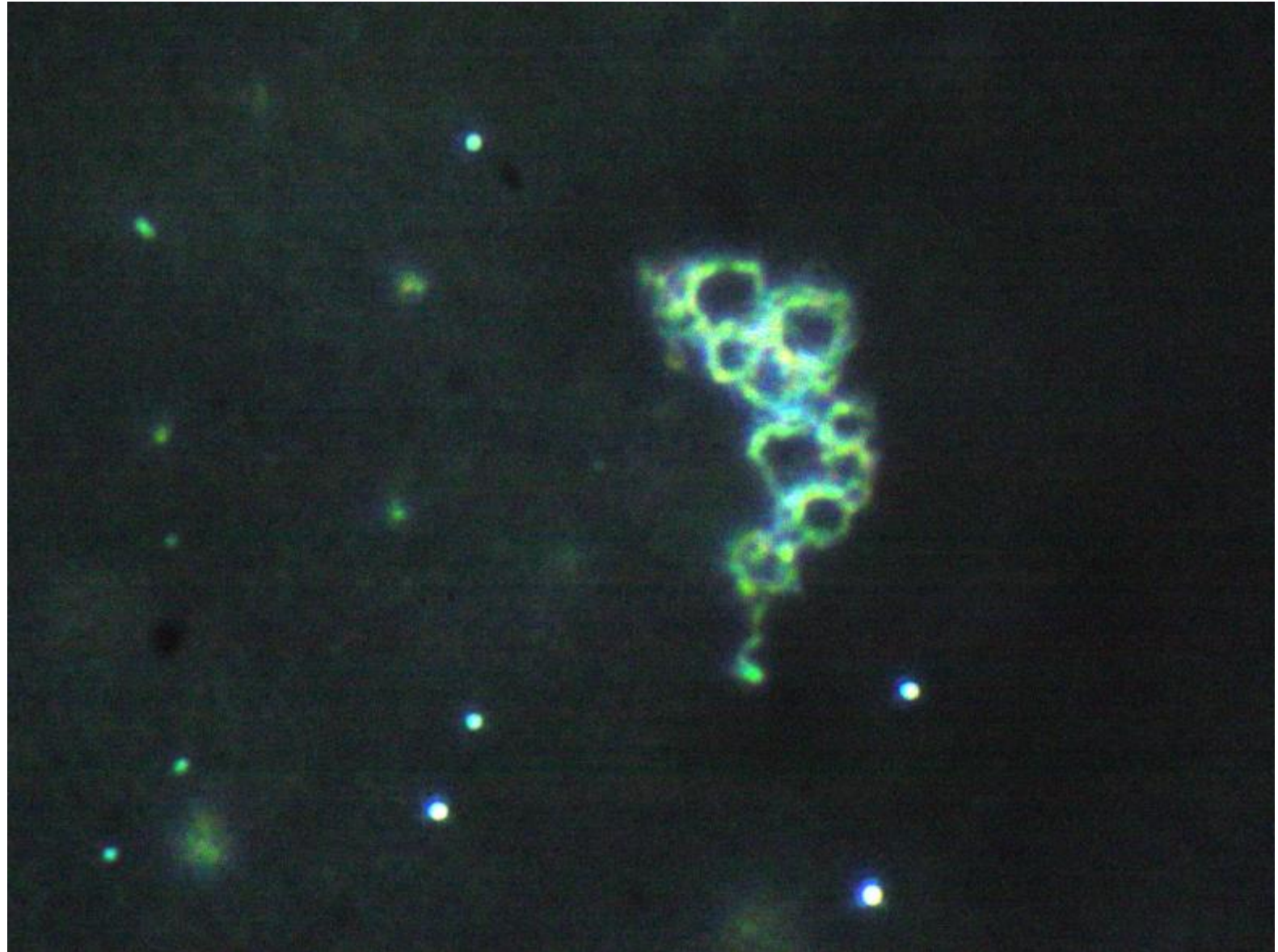


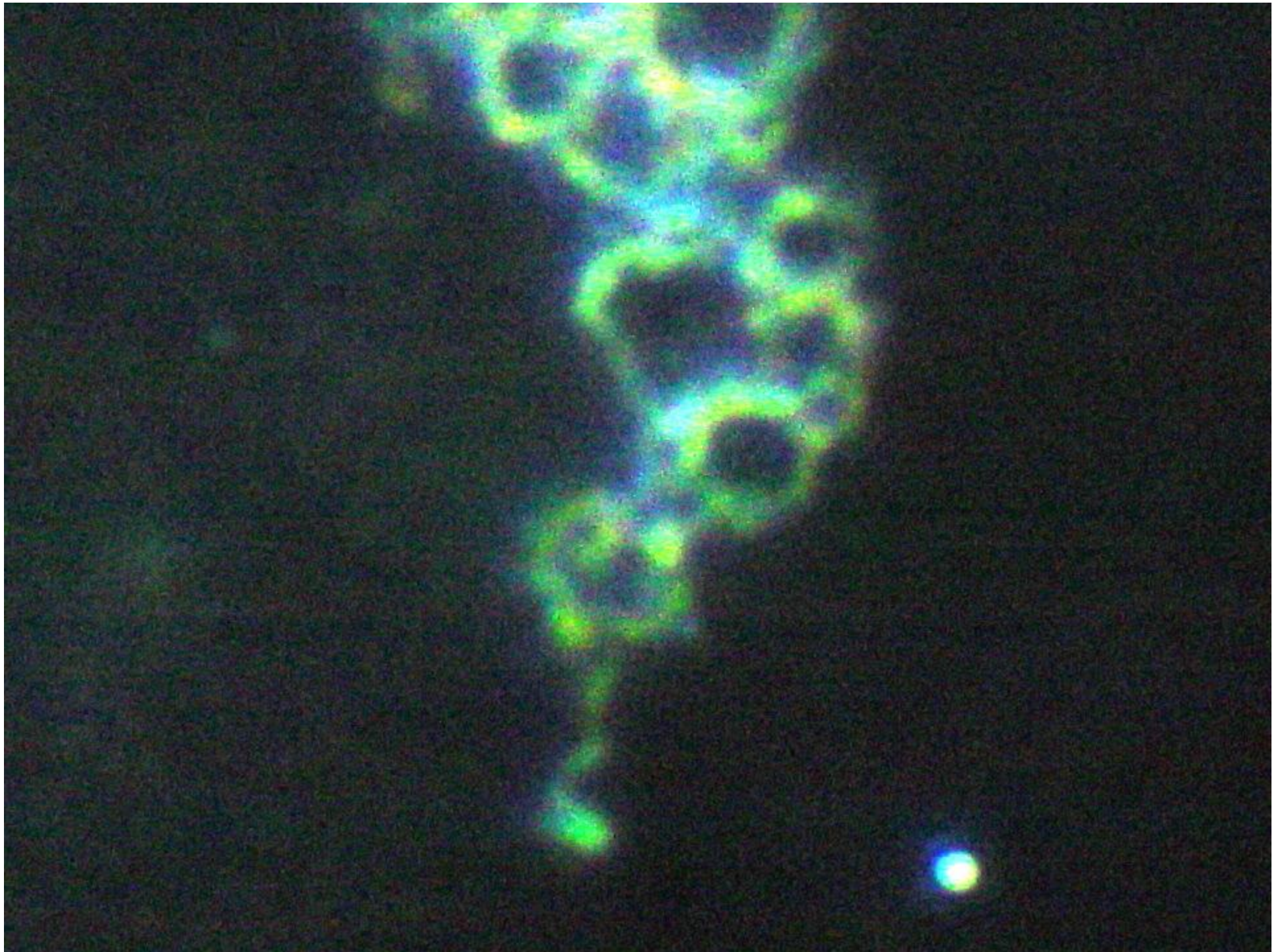


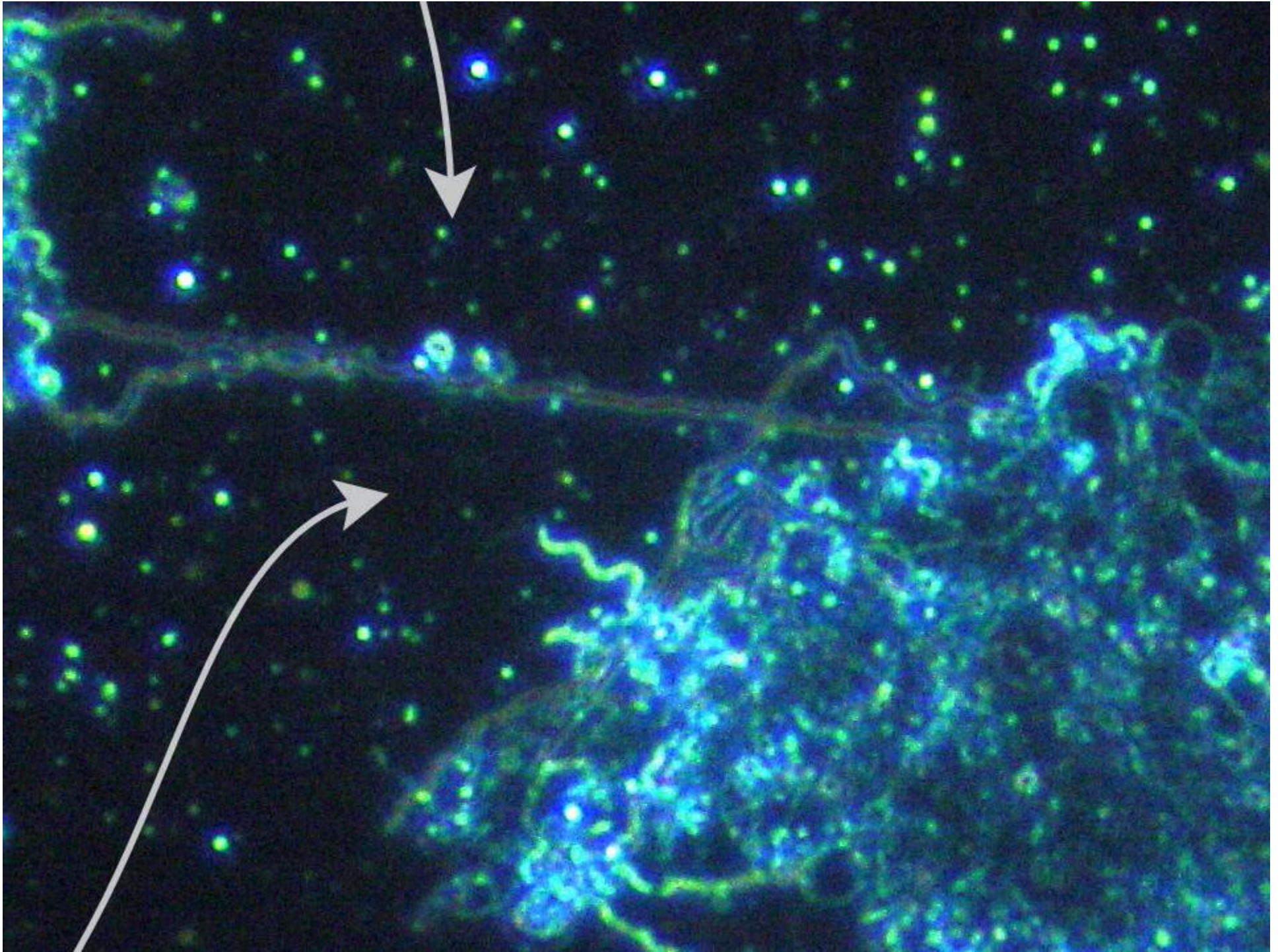


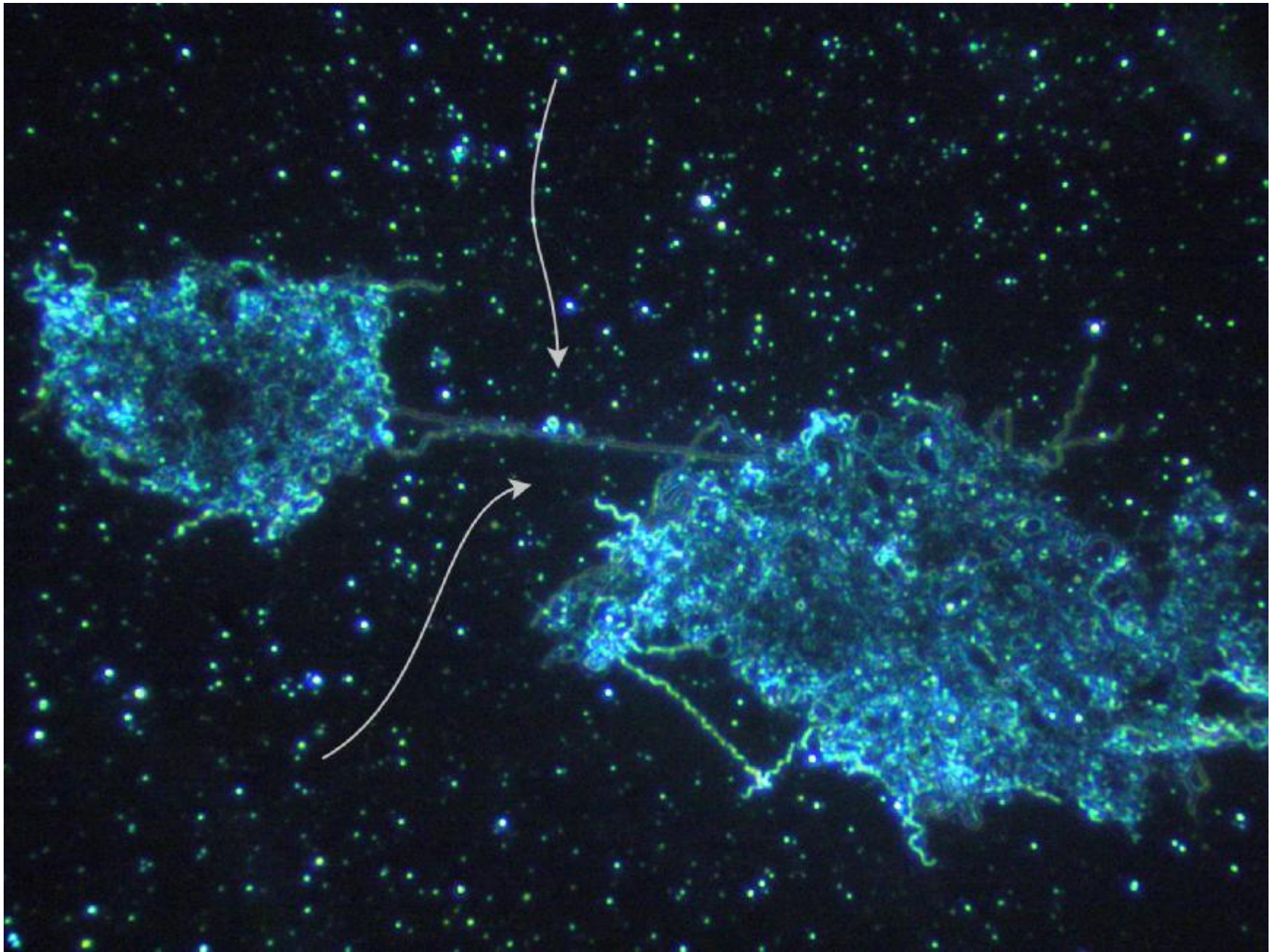




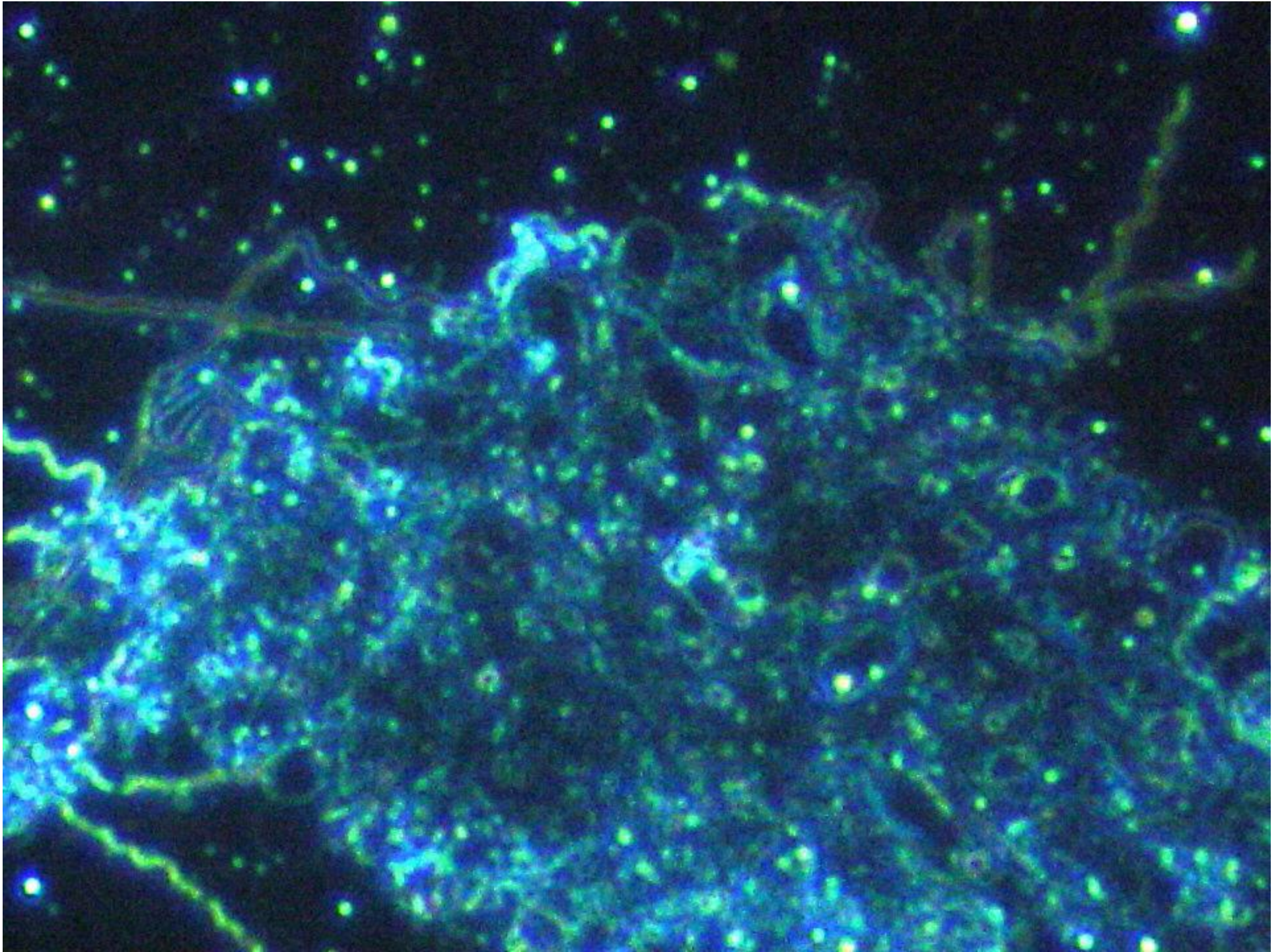






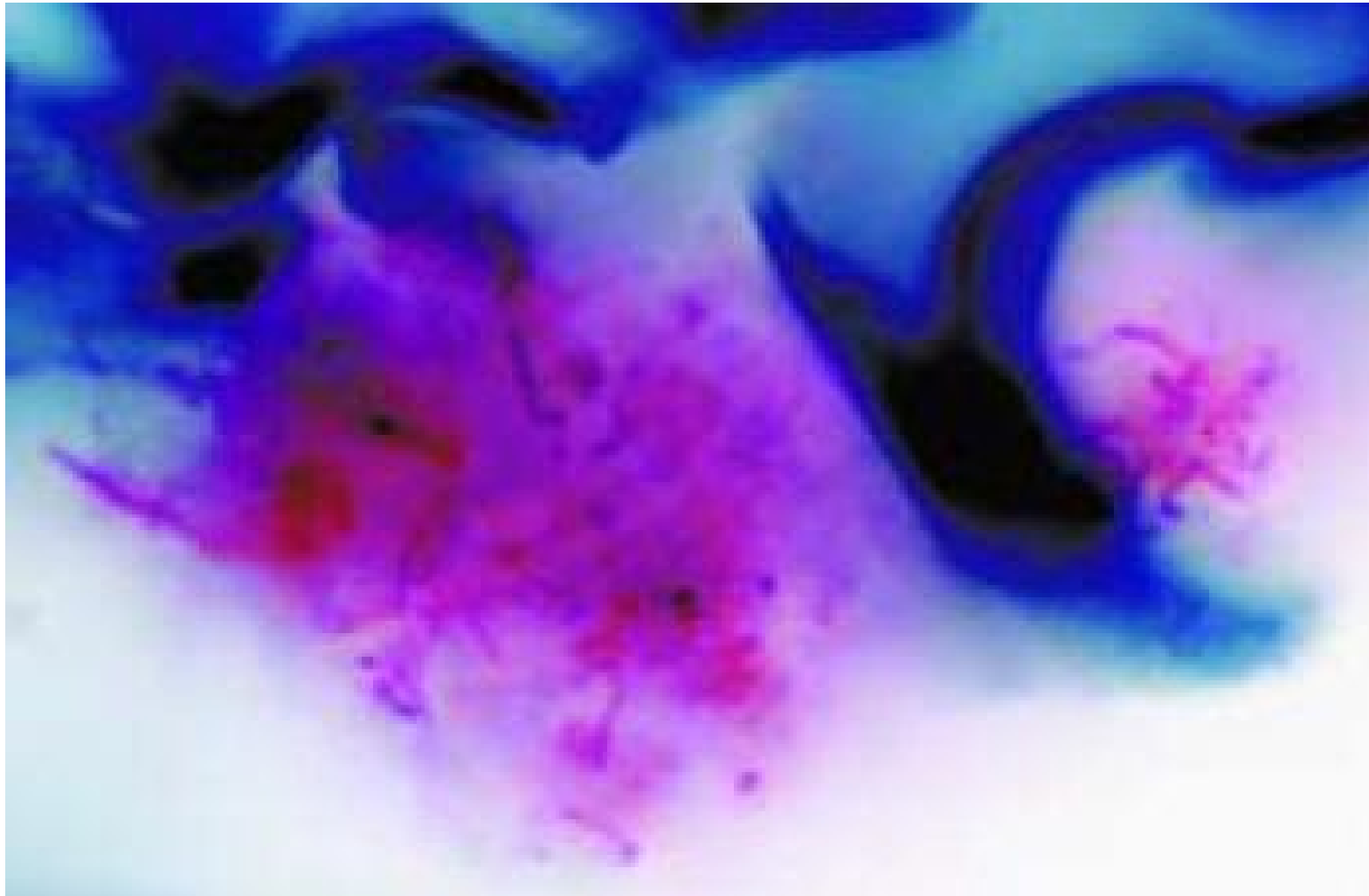


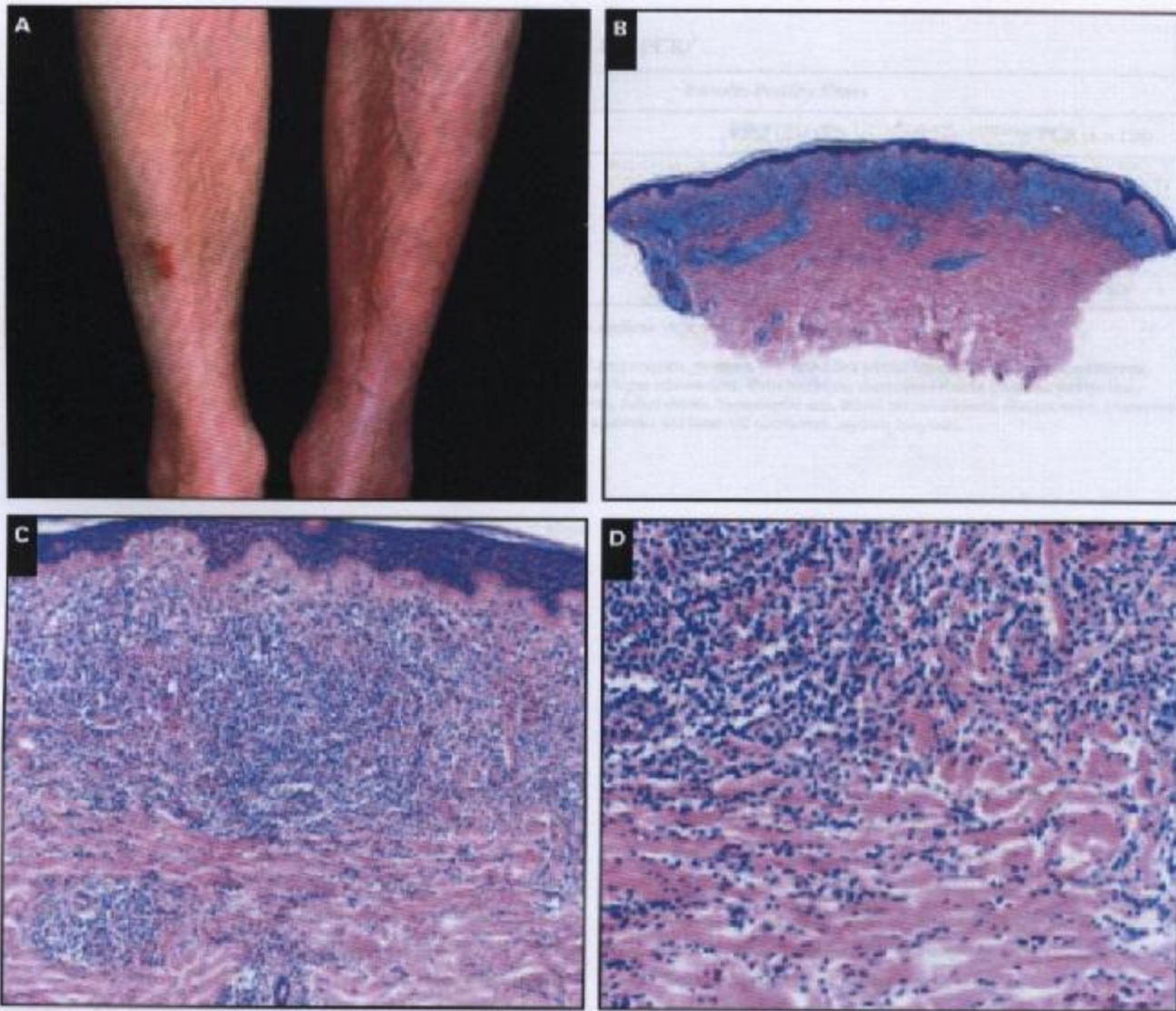




Dr K. Eisendle. BORRELIA LYMPHOCYTOMA

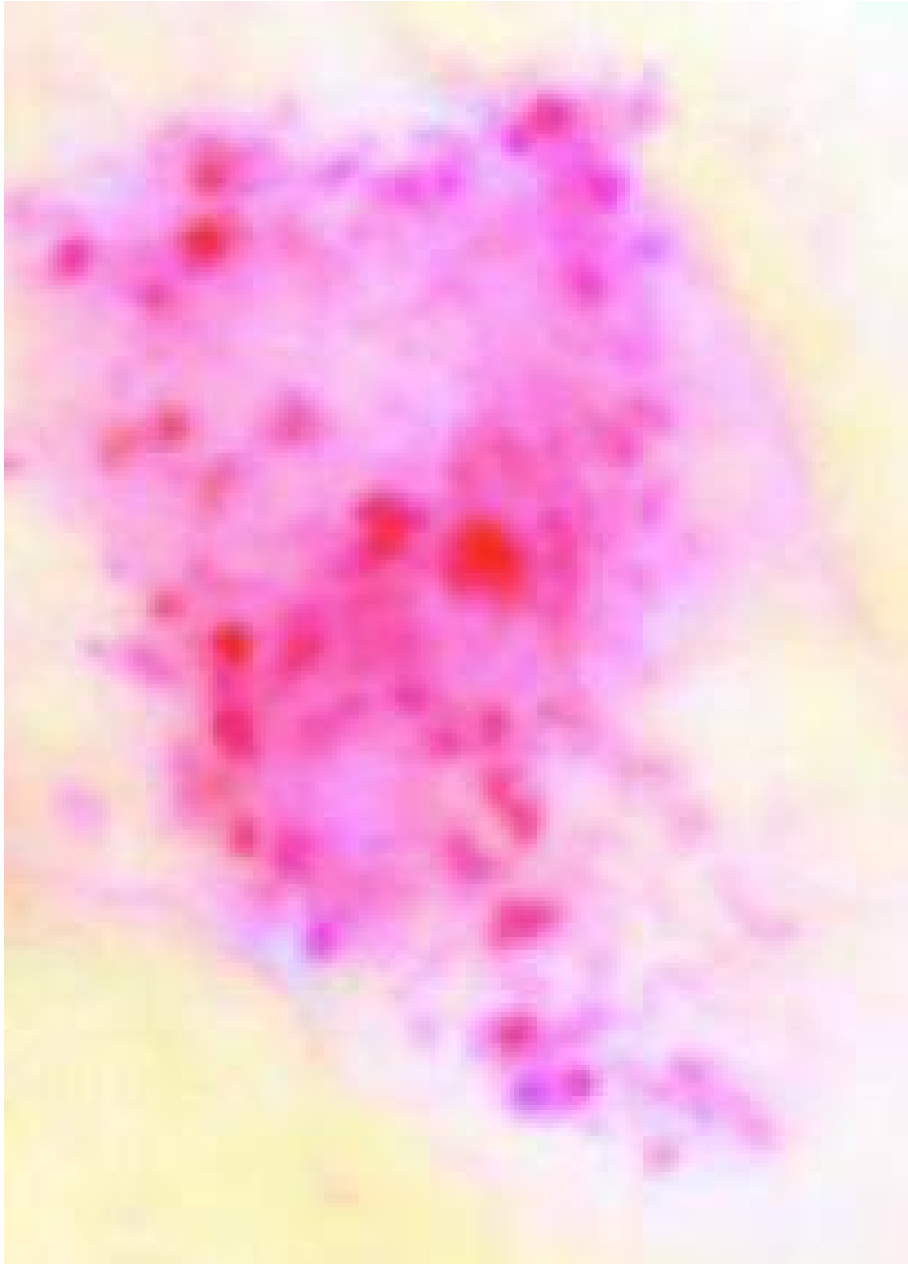
IMMUNOHISTOCHEMISTRY AJCP 2007,127:213-222





**Image 31** **A**, Acrodermatitis chronica atrophicans of left leg characterized by ill-defined, hyperpigmented, and atrophic patch (note prominent veins). **B**, Histologic examination (H&E,  $\times 10$ ) reveals a dense lichenoid and middermal perivascular infiltrate with hints of follicle formation (**C**, H&E,  $\times 100$ ) composed of lymphocytes, some plasma cells, and an increase of fibroblasts between fibrosclerotic collagen bundles (**D**, H&E,  $\times 200$ ).





Dr K. Eisendle

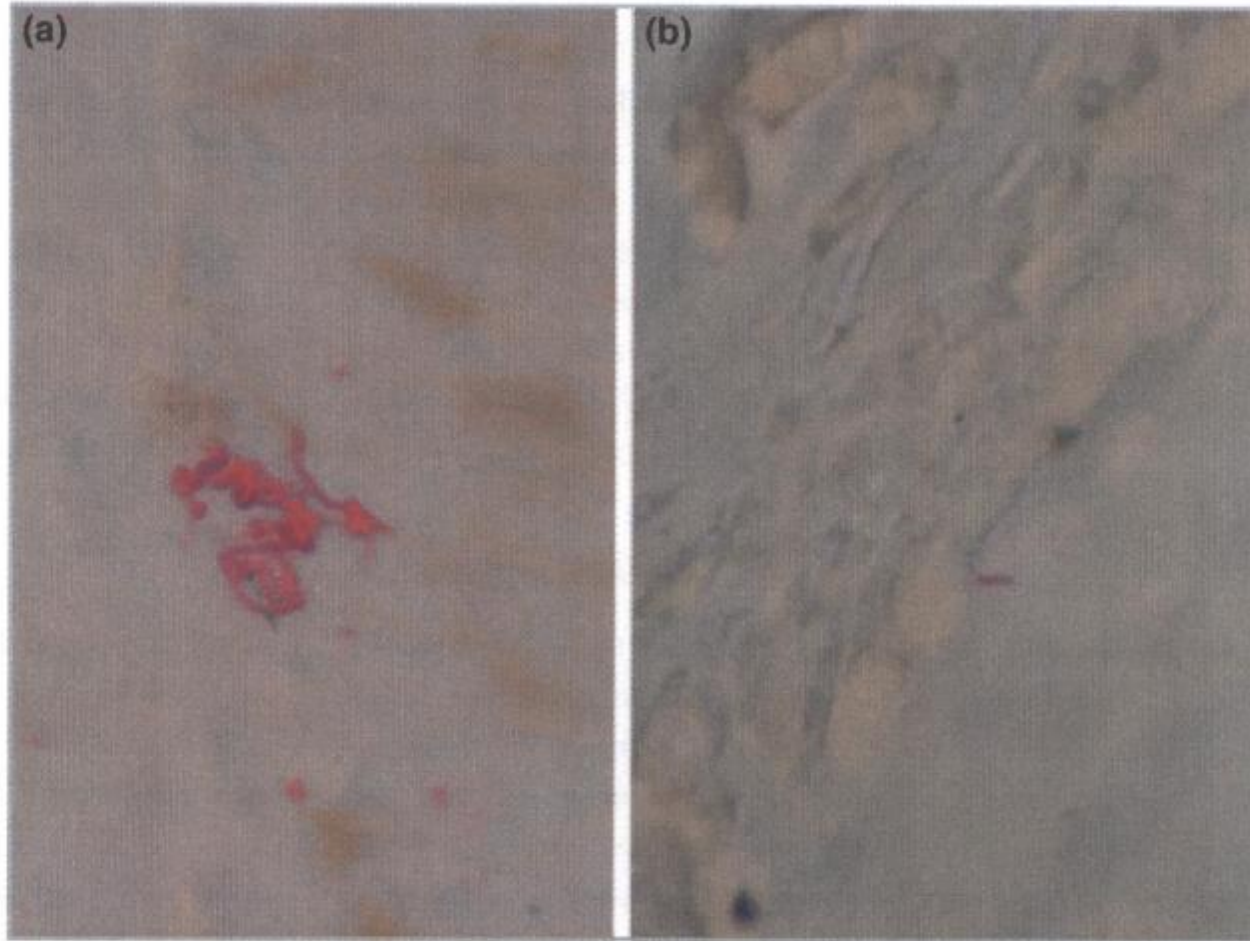
Acrodermatitis Chronica  
Atrophicans

Immunohistochemistry

“Granular forms of B  
burgdorferi in a “colony”

With a “Reddish veil”

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*

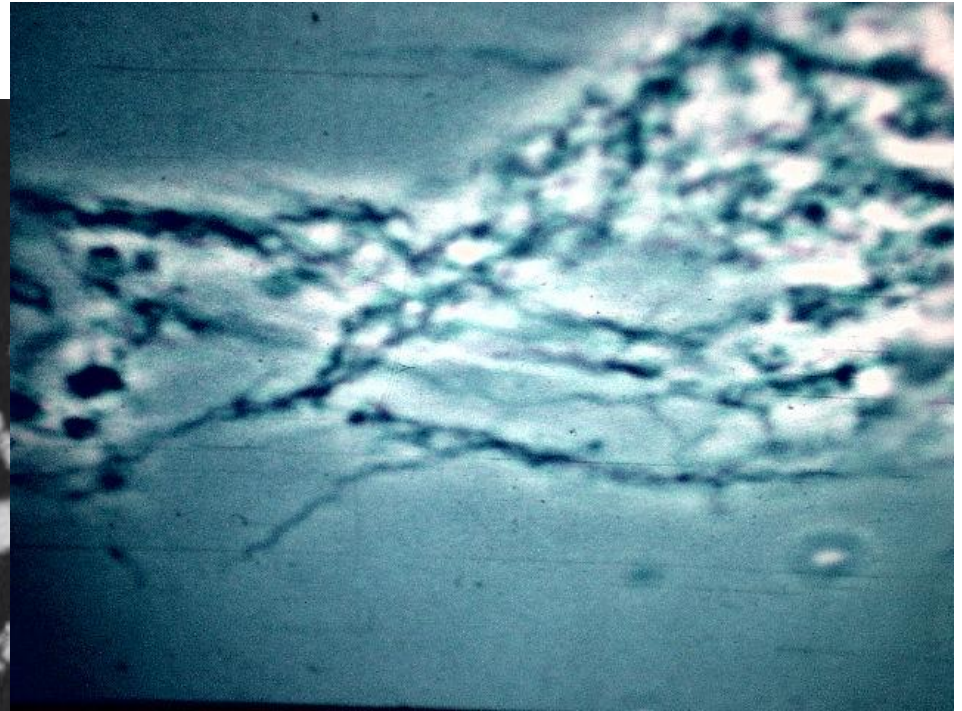
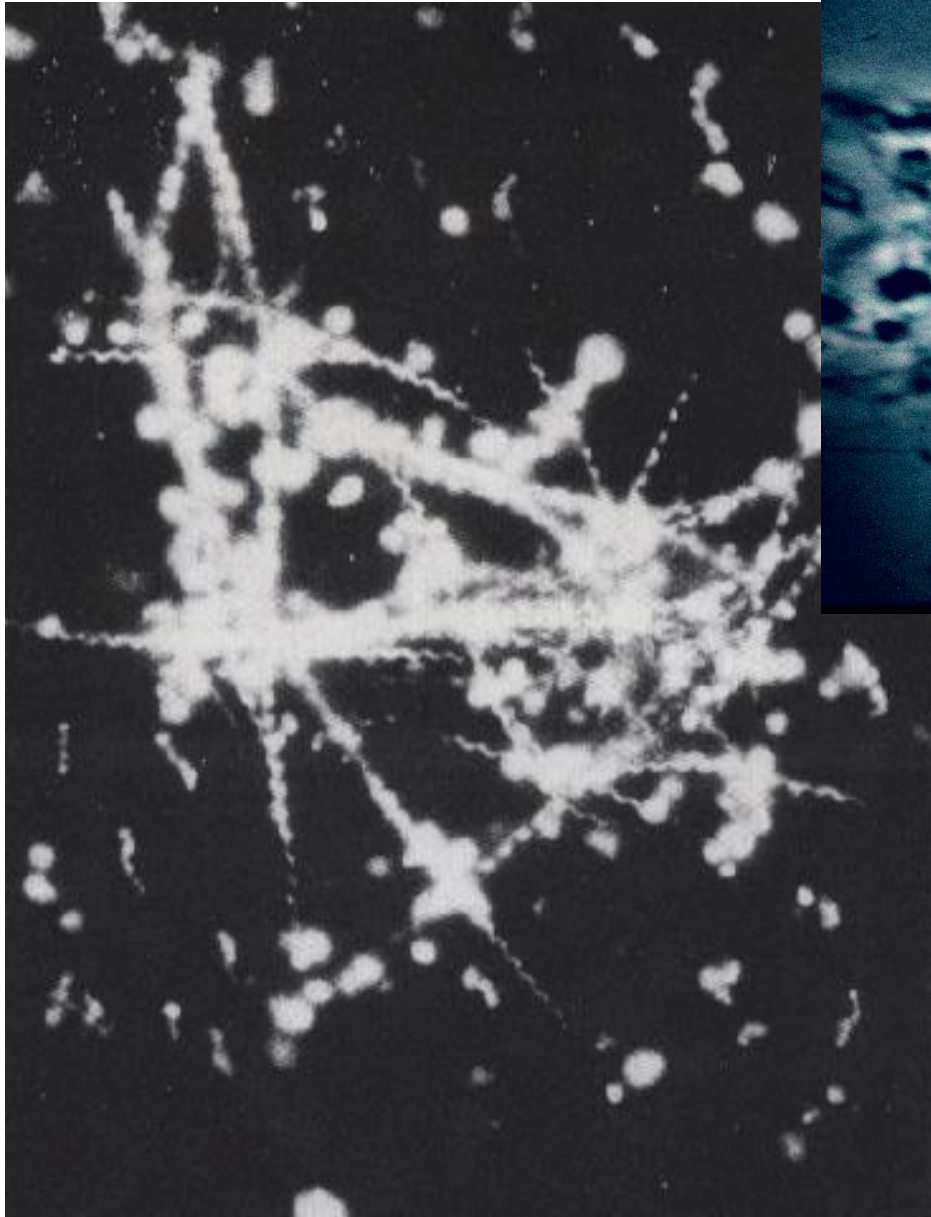
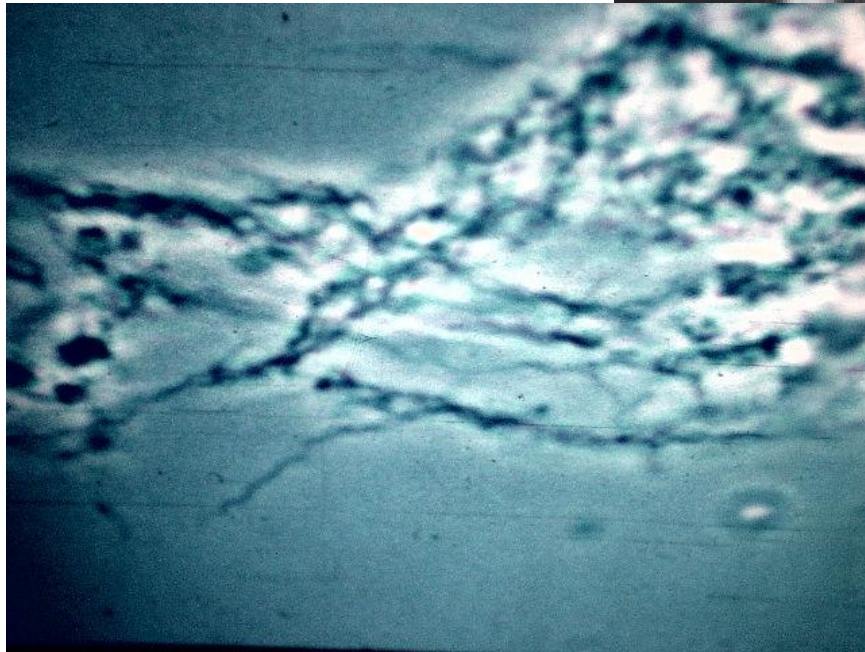
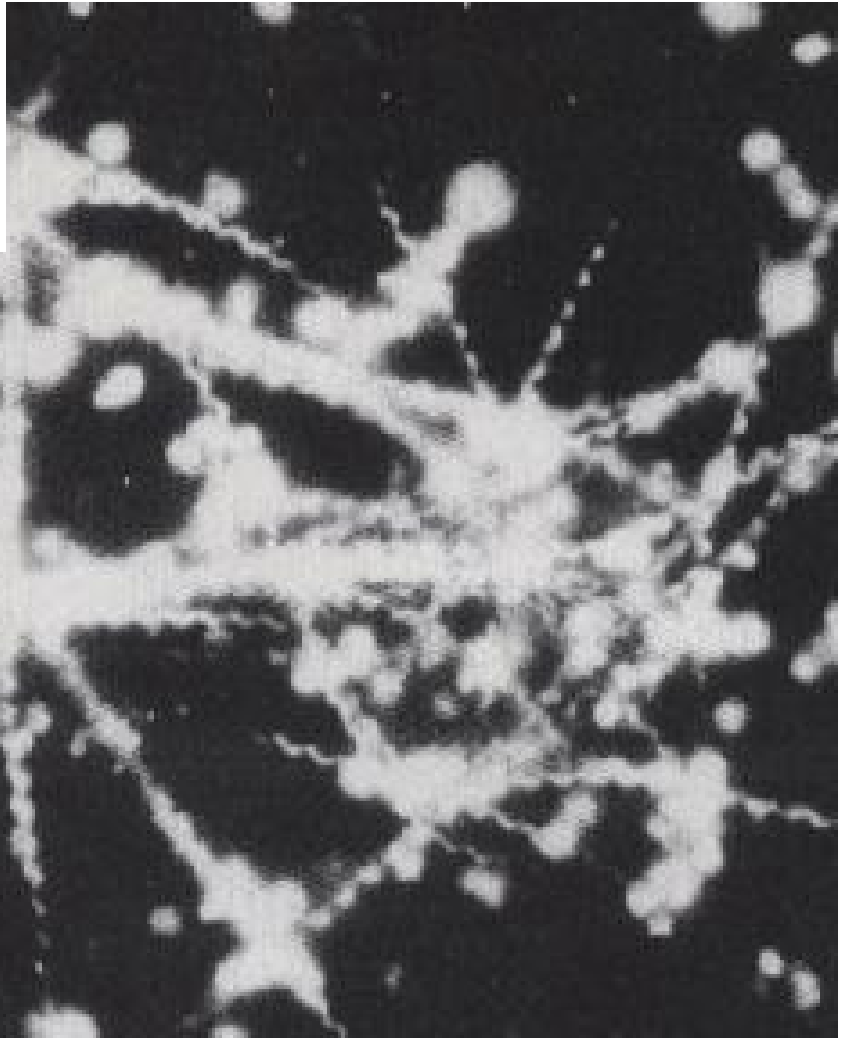


Image from 1981

Image from 1987

Human Brain Culture  
demonstrating a Biofilm of  
*Borrelia burgdorferi*

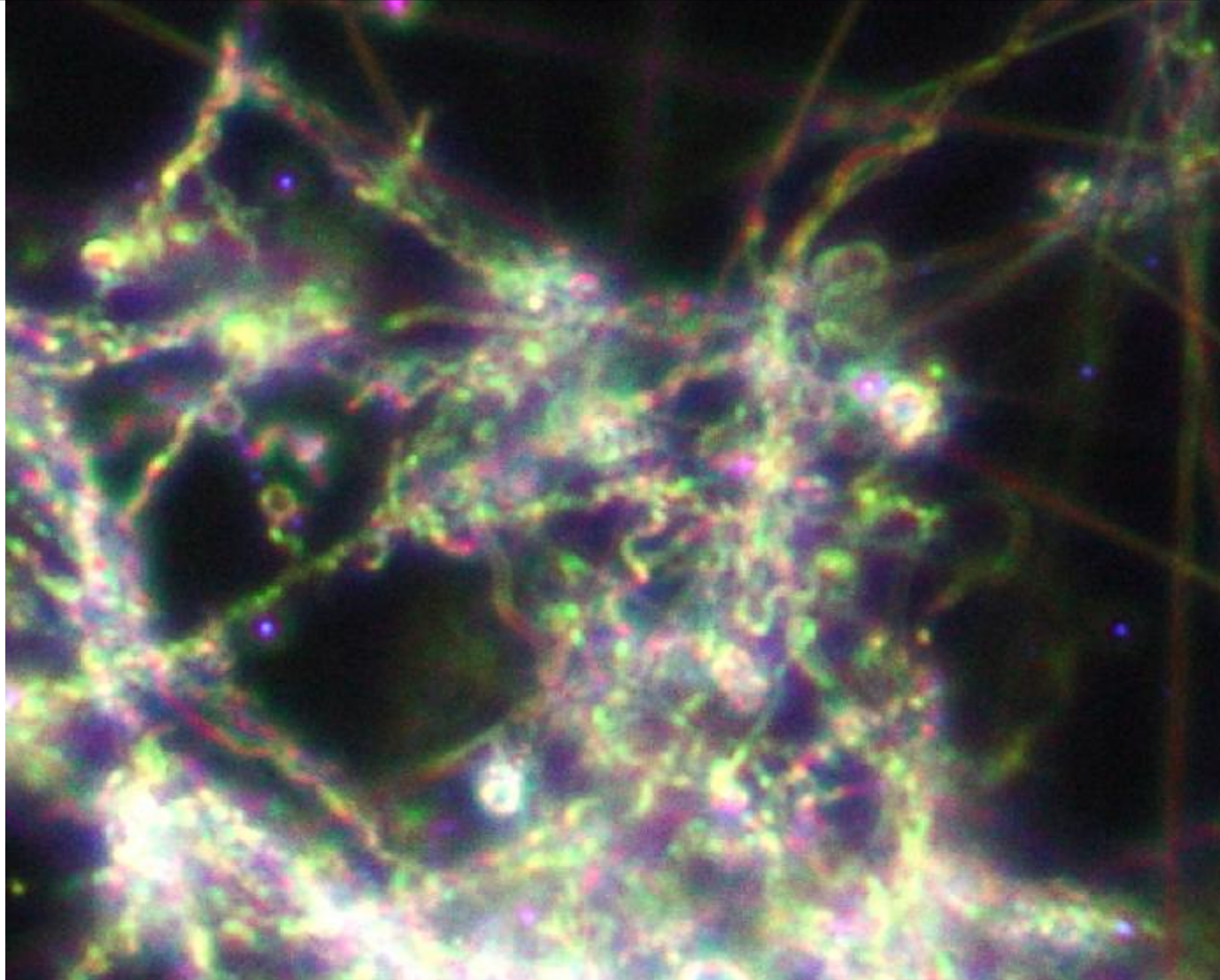
Year 1987

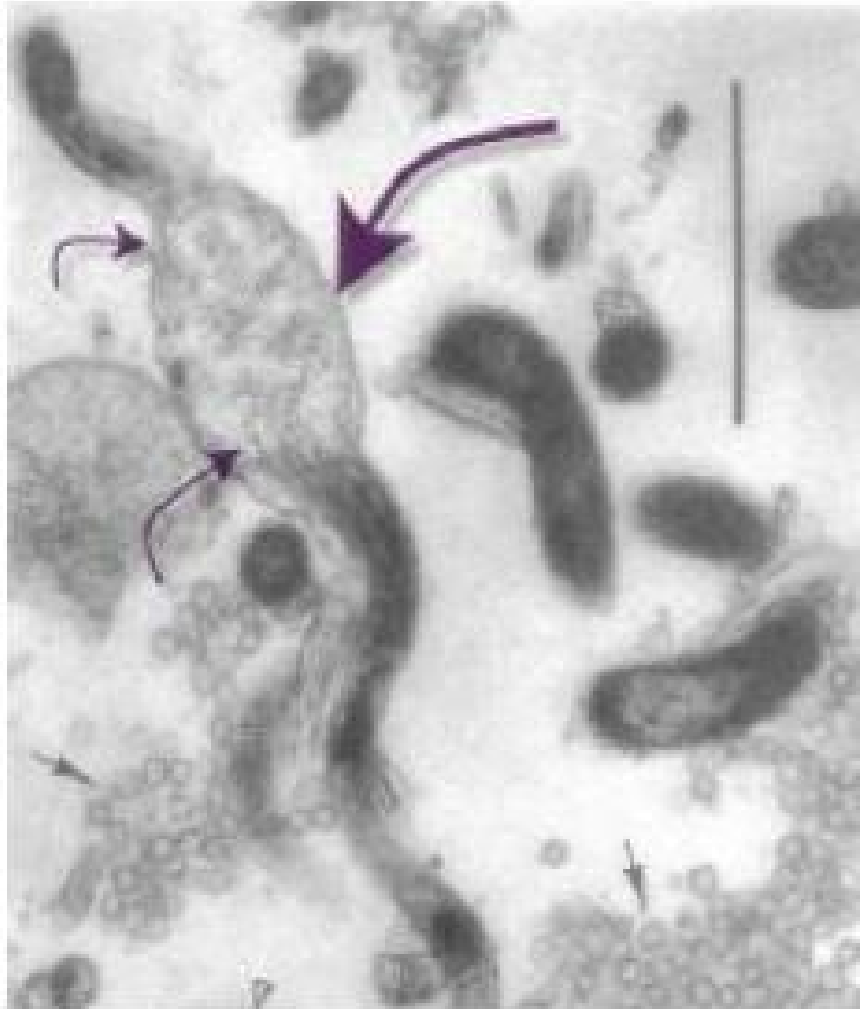


Tick gut Culture showing *Borrelia burgdorferi* in a Biofilm Unit

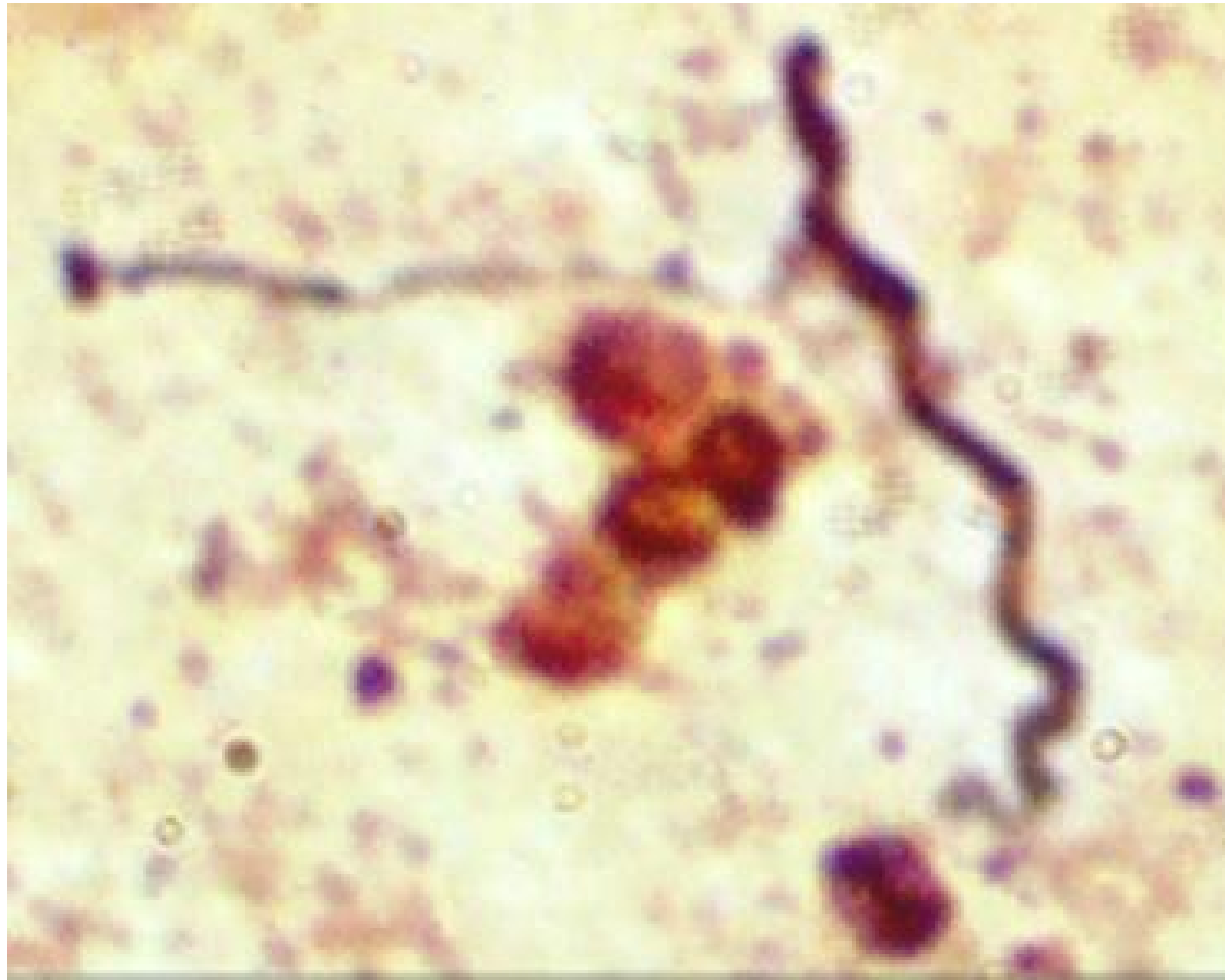
Year 1981

For comparison – *Borrelia burgdorferi* biofilm 2008





**Borrelia  
hermsii  
in transit to  
spherical  
form  
after  
penicillin  
treatment**



# The In Transit concept For Borrelia biofilms

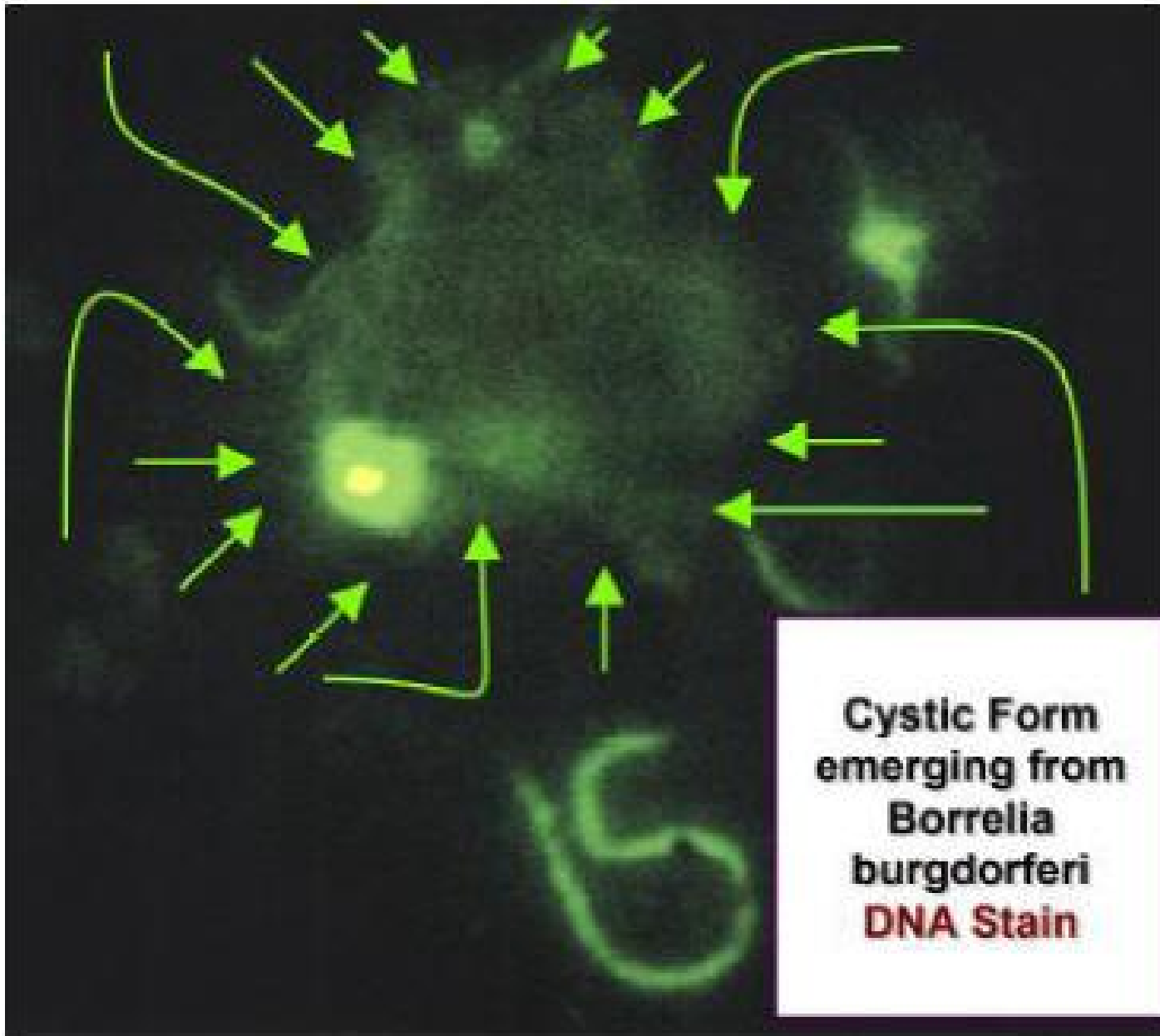
Contribution of Borrelia DNA to the formation  
of Extracellular Matrix in Borrelia biofilms





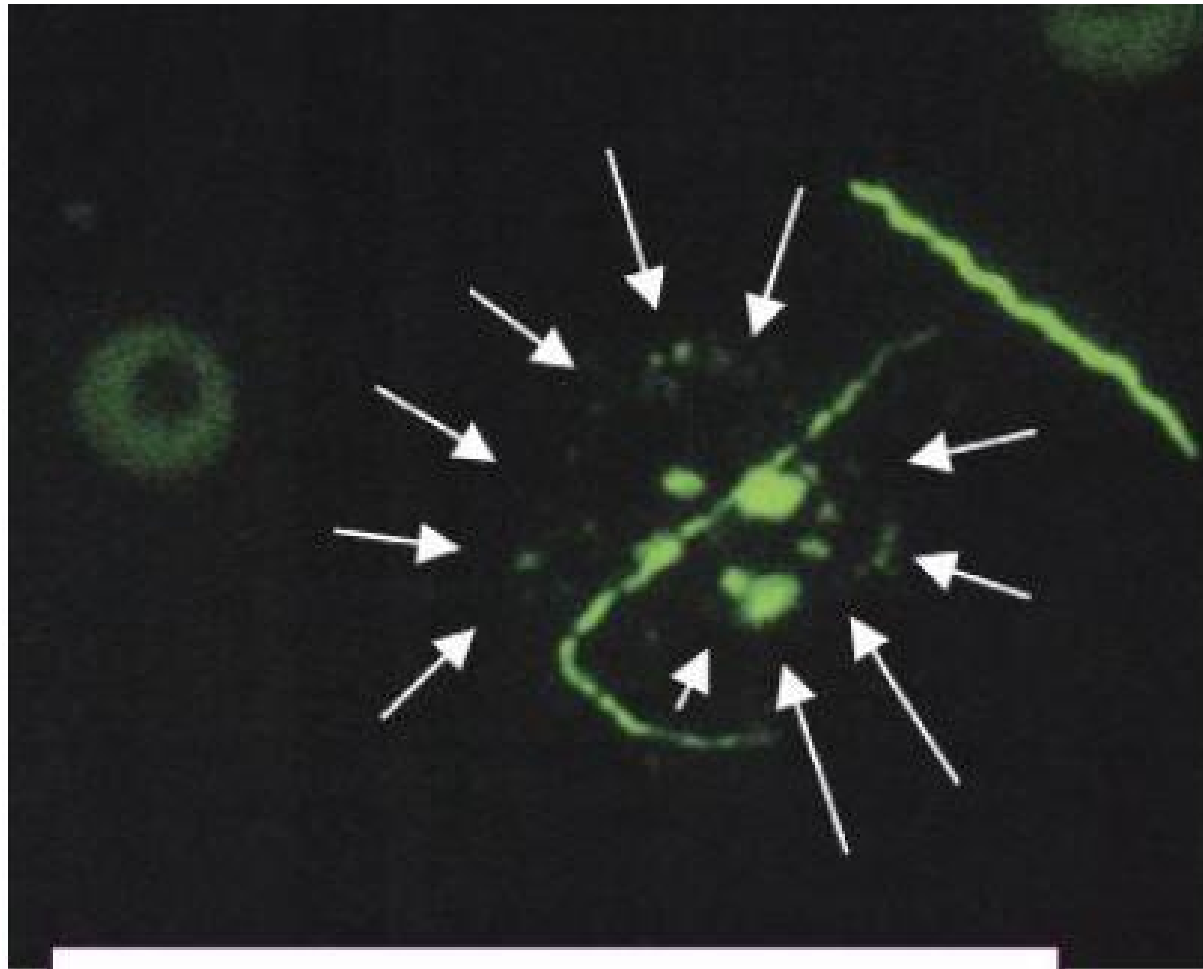
**Figure 1 B. Hermsii with loss of cell wall and developing spheroid form**

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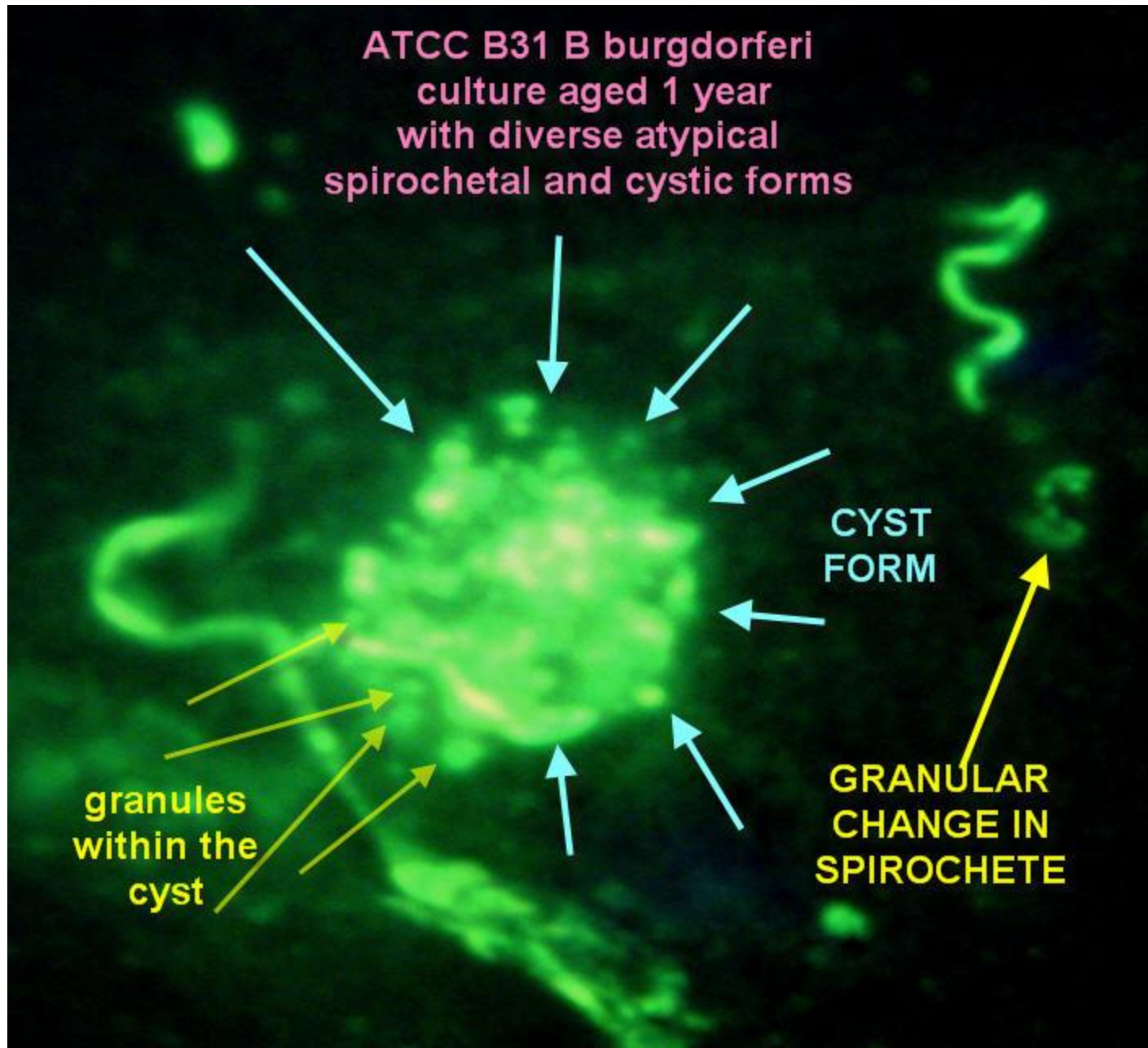
Emerging  
Cystic Form  
attached to  
corkscrew shaped  
Borrelia  
Burgdorferi  
**RED ARROW**  
**SHOWS FILAMENT**  
**FORM**  
**INSIDE OF**  
**CYST**  
**CURVED GREEN**  
**ARROWS**  
**SHOW CYST**  
**PERIMETER**  
**DNA STAIN**





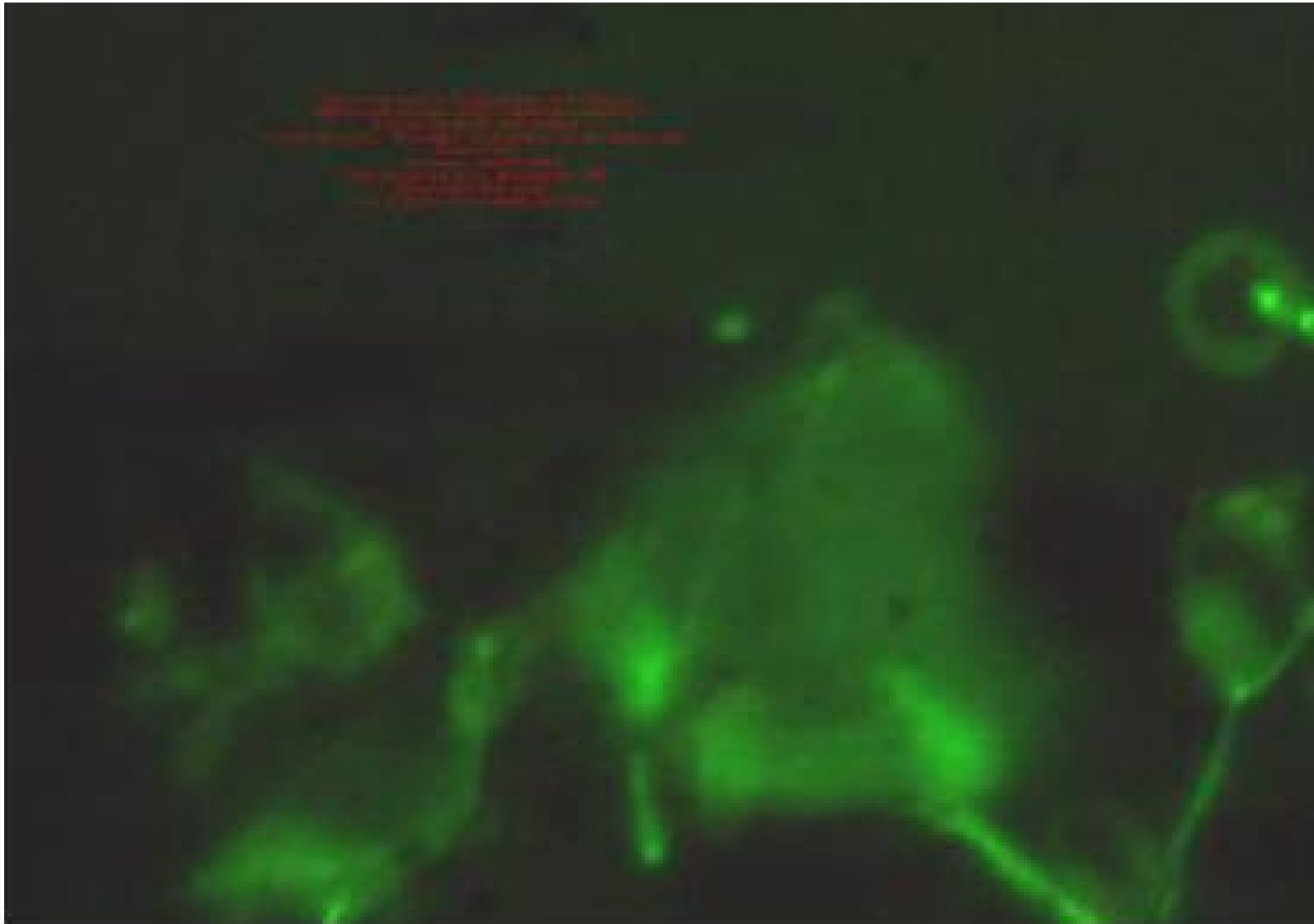
**Emerging Cyst form of Borrelia Burgdorferi  
( see rounded area of dots)  
White arrows show boundaries of the  
emerging cystic form containing granular  
elements**

ATCC B31 *B. burgdorferi*  
culture aged 1 year  
with diverse atypical  
spirochetal and cystic forms





**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**

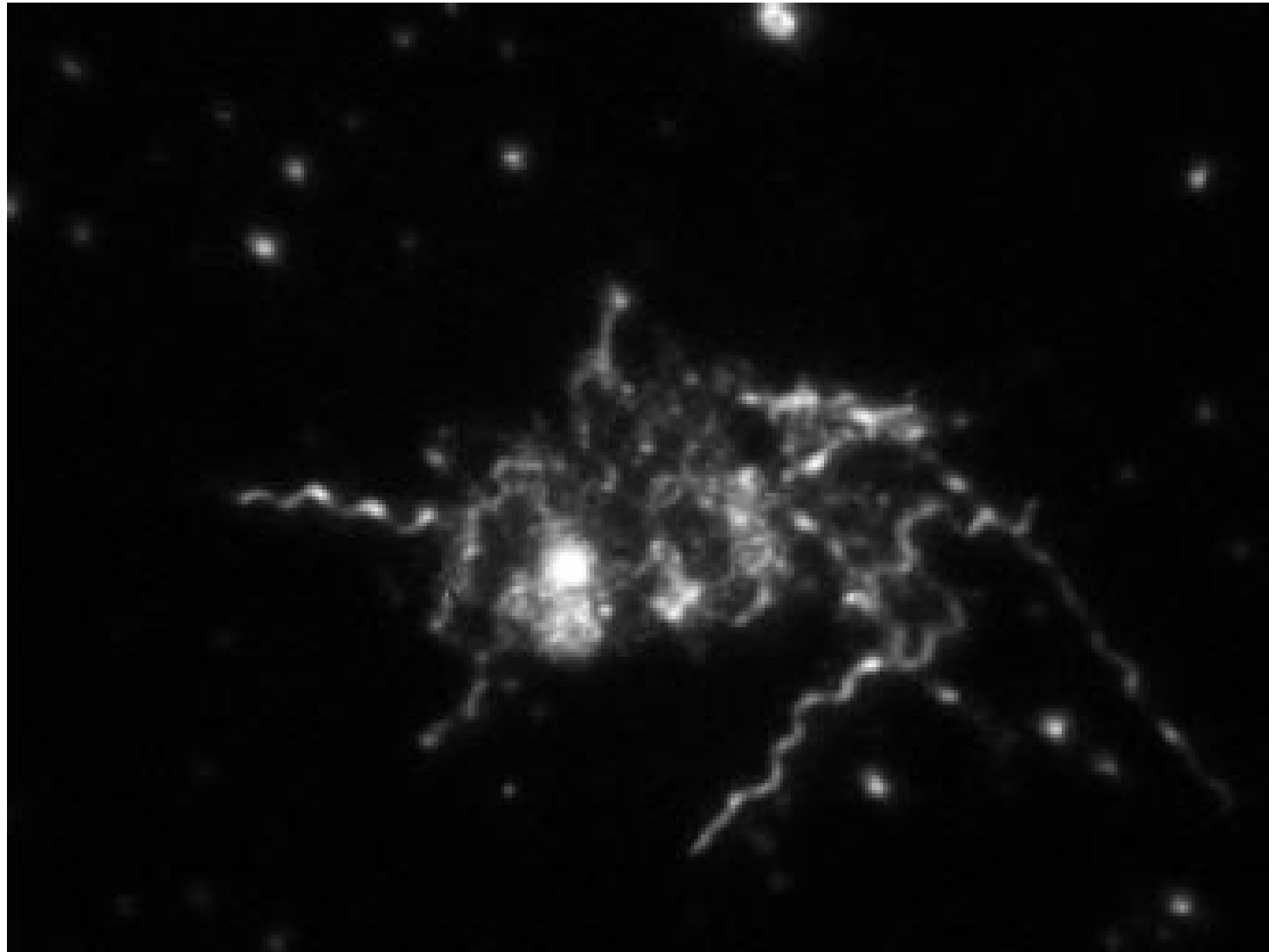


**Figure 7 - In Transit form of *Borrelia burgdorferi* with externalized cellular elements**





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

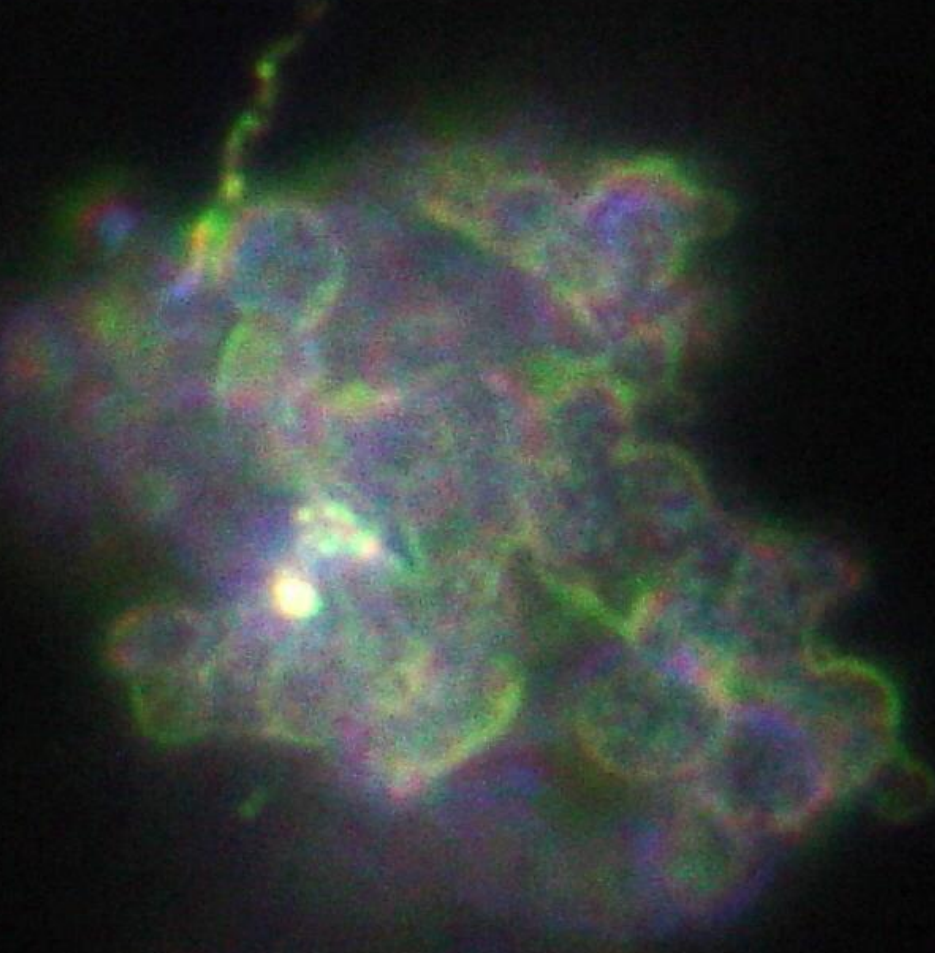


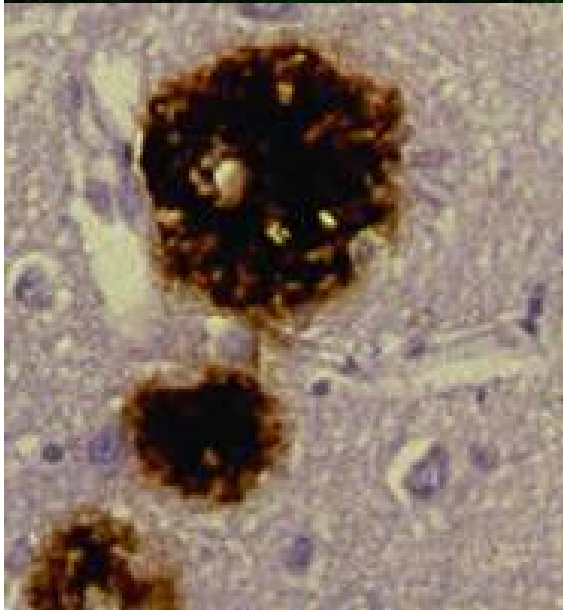
**Figure 8 Early Biofilm of *Borrelia burgdorferi*.**



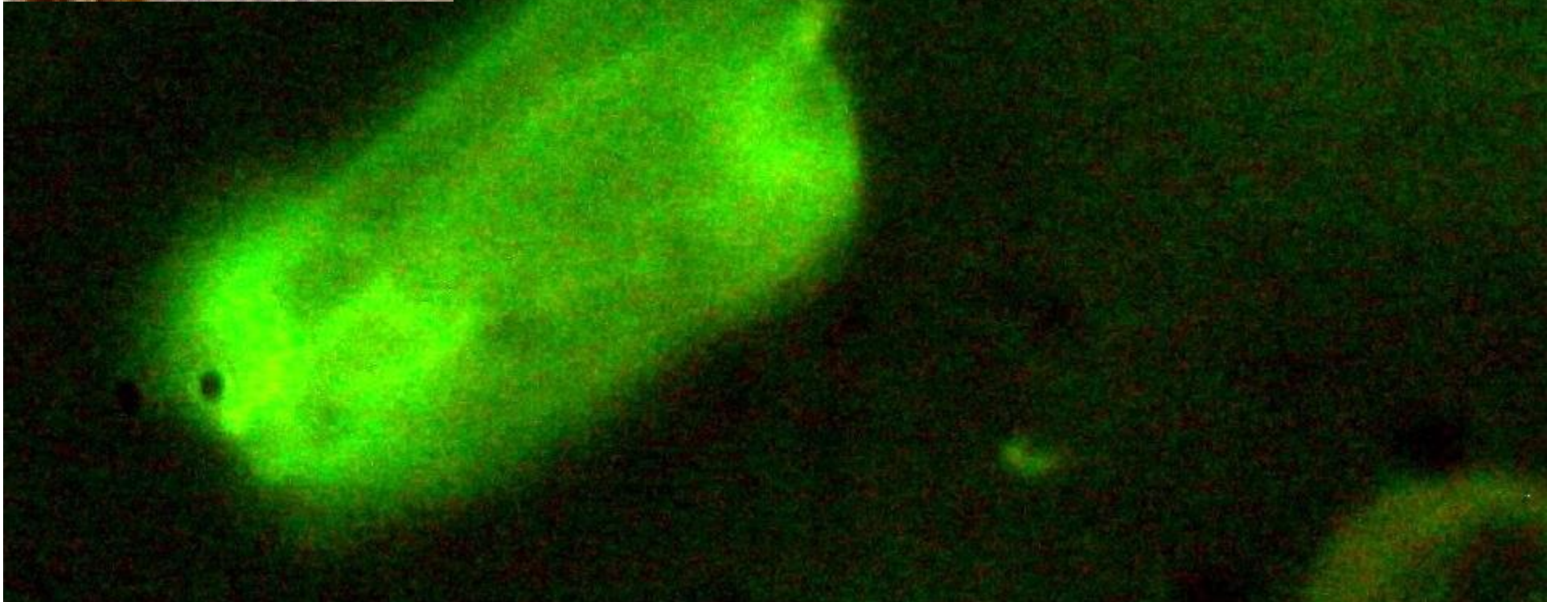


## Group of Cystic B31





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



## 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.

Borrelia biofilm works in progress

--Quorum sensing in Biofilms

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



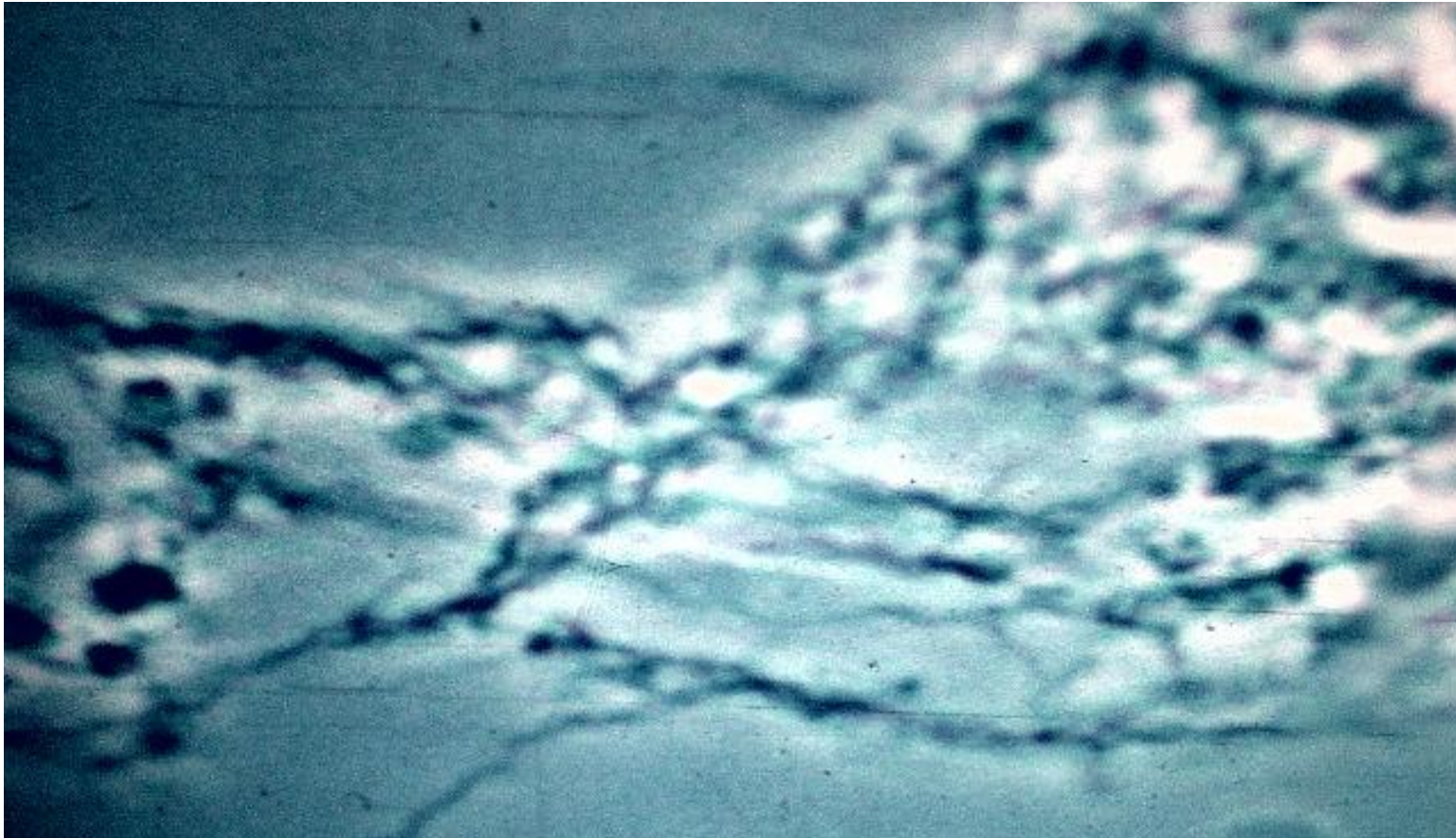
Thank you for your Kind attention.

This research was made possible from  
support from:

Turn The Corner Foundation

The Lyme Disease Association

Time for Lyme Foundation



Original Isolate of *Borrelia burgdorferi* , 1981

Image from the Yale Journal of Biology and Medicine