

* Structural Analysis of Biofilms of *Borrelia burgdorferi*

Report prepared by Alan B. MacDonald MD, FCAP, FASCP

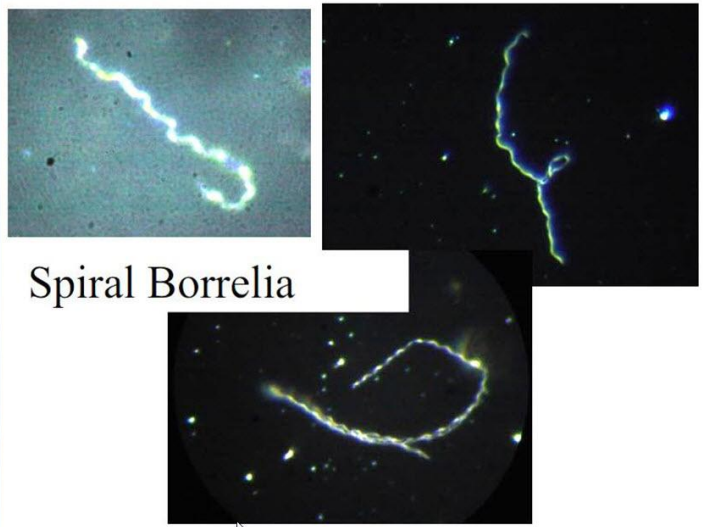
Lyme Disease Research Group
University of New Haven
Eva Sapi PhD, Director
University Research Scholar
Associate Professor
Departments of Biology Environmental Disease

We were all trained as planktonic MD's.
 So as Costerton points out, Planktonic trained scientists are like a duck out of water in the Biofilm universe. A consequence is that Planktonic trained reviewers (peer reviewers from academic Microbiology faculties) are not credentialed to review a Biofilm article.

The Image gallery, will hopefully "wake up" those who say or who believe the phrase "no spirals seen, no borrelia present".

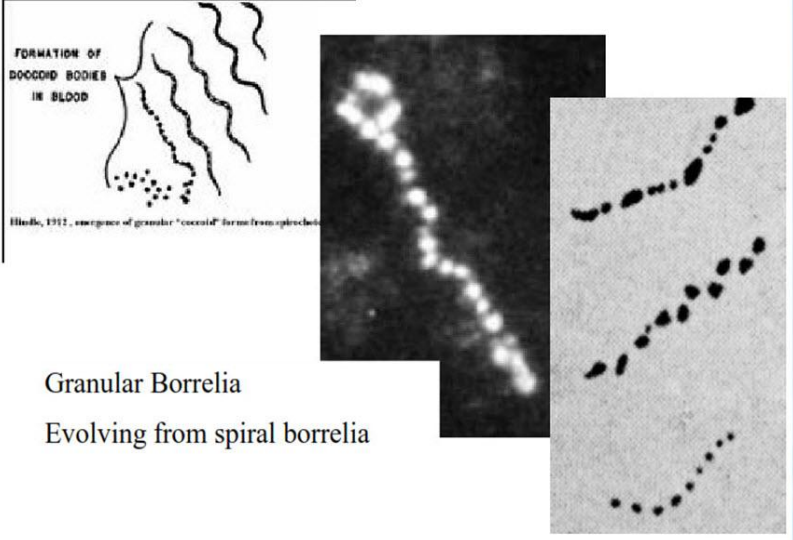
The Hyperspectral Analysis of biofilm communities {{in vitro}} from pure cultures of borrelia burgdorferi -- MOST of the biofilm communities of Bb have No visible spiral forms, especially in the center (deepest regions) of the borrelia biofilm.
 Granular forms are the leading community members in some Borrelia biofilms.
 Cystic Borrelia are the leading community members in some Biofilms of borrelia.
 Cell wall deficient members over-run all other borrelia forms in some borrelia biofilms.

So waiting to see a spiral form of borrelia in a biofilm community is like "Waiting For Godot".



Spiral Borrelia

Biofilms of Borrelia burgdorferi 16 10/22/2012

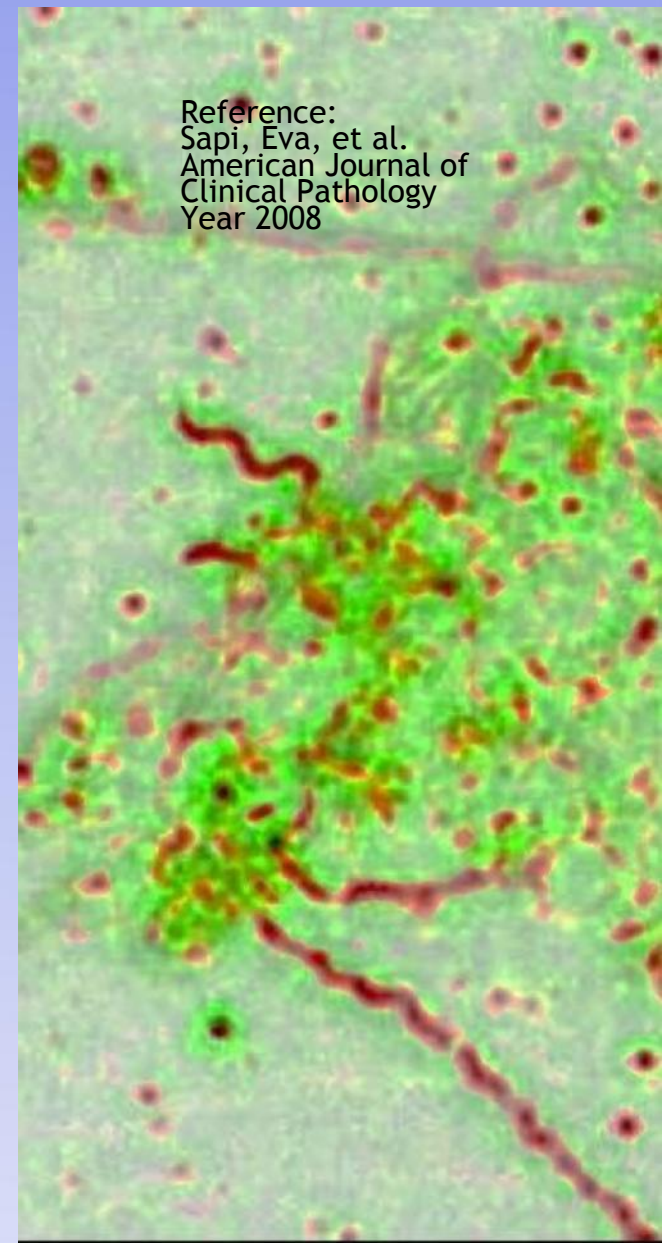


FORMATION OF DOCCOID BODIES IN BLOOD

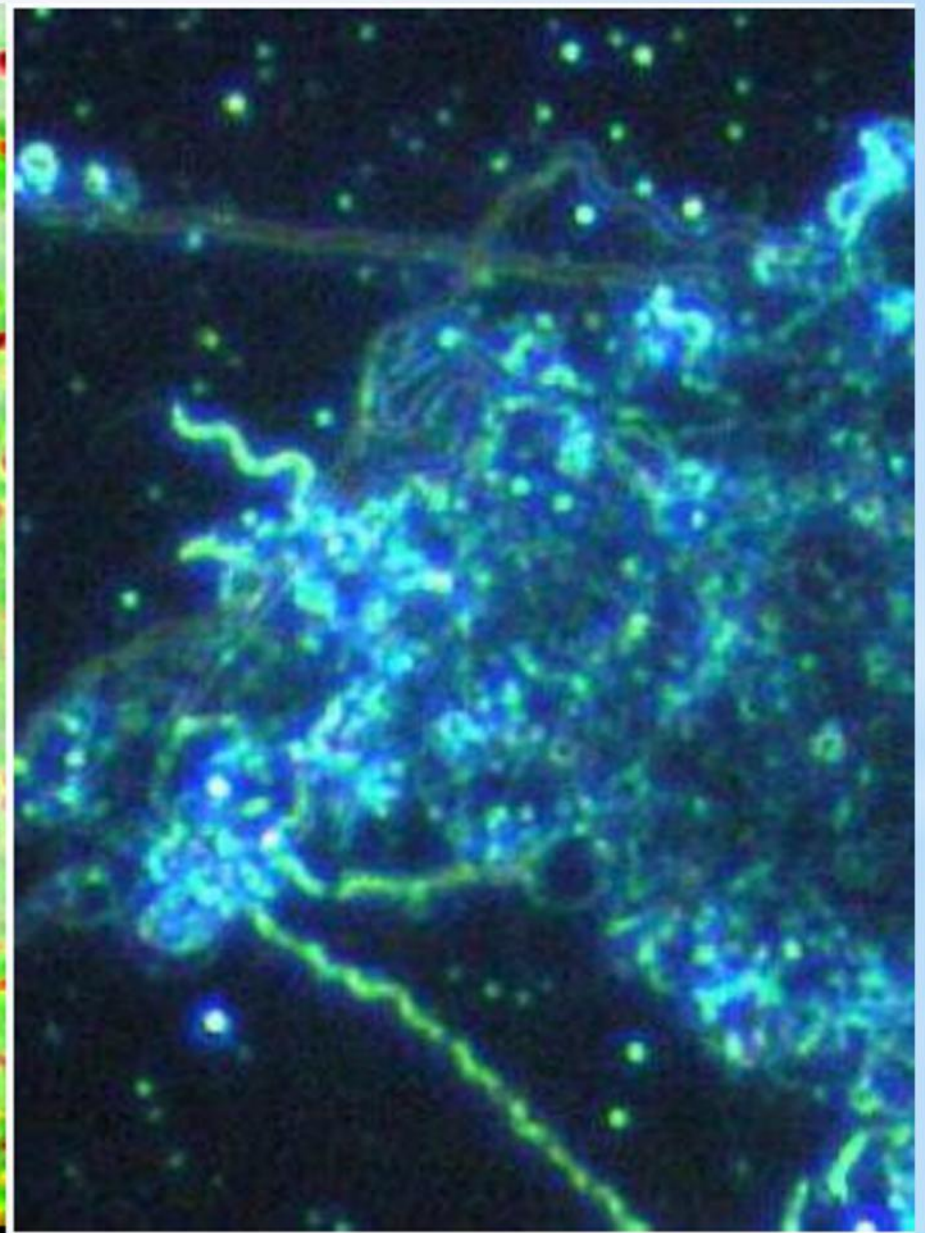
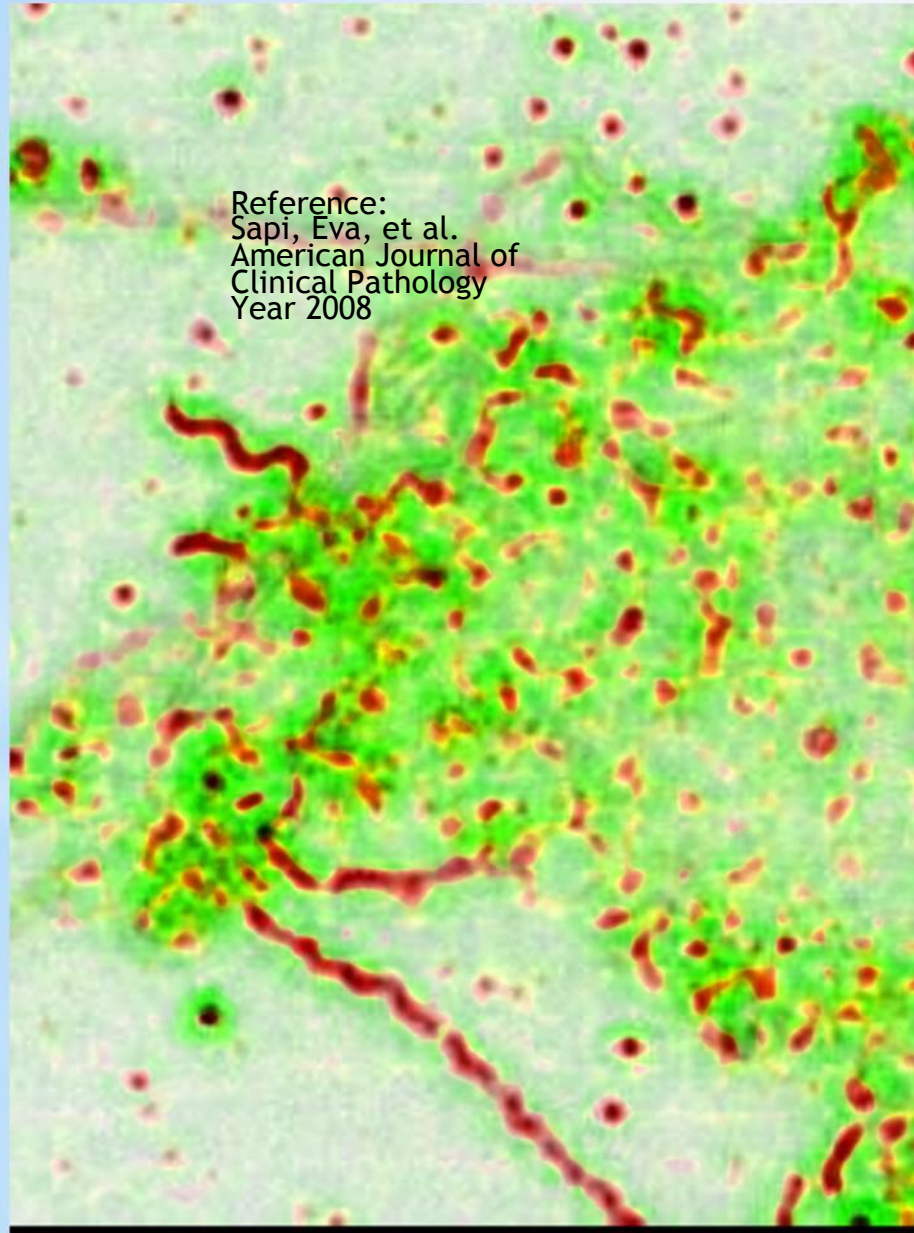
Hicks, 1912. emergence of granular "cocci" form from spirochetes

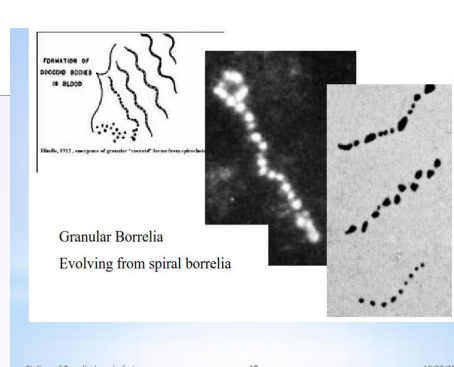
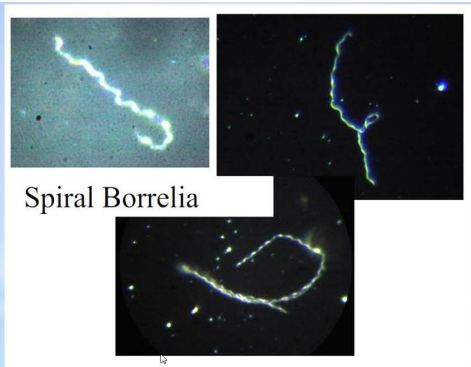
Granular Borrelia
 Evolving from spiral borrelia

Biofilms of Borrelia burgdorferi 18 10/22/2012



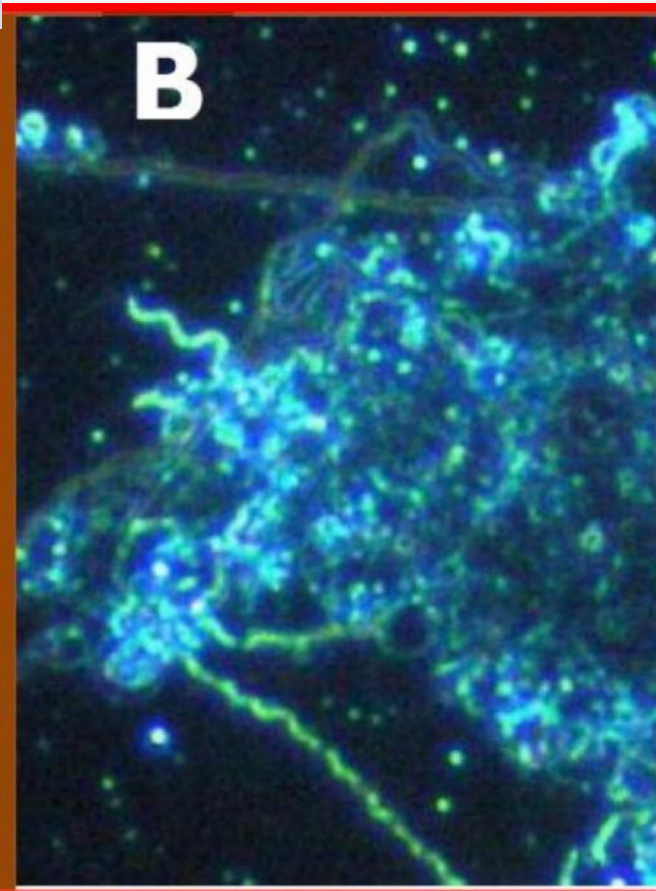
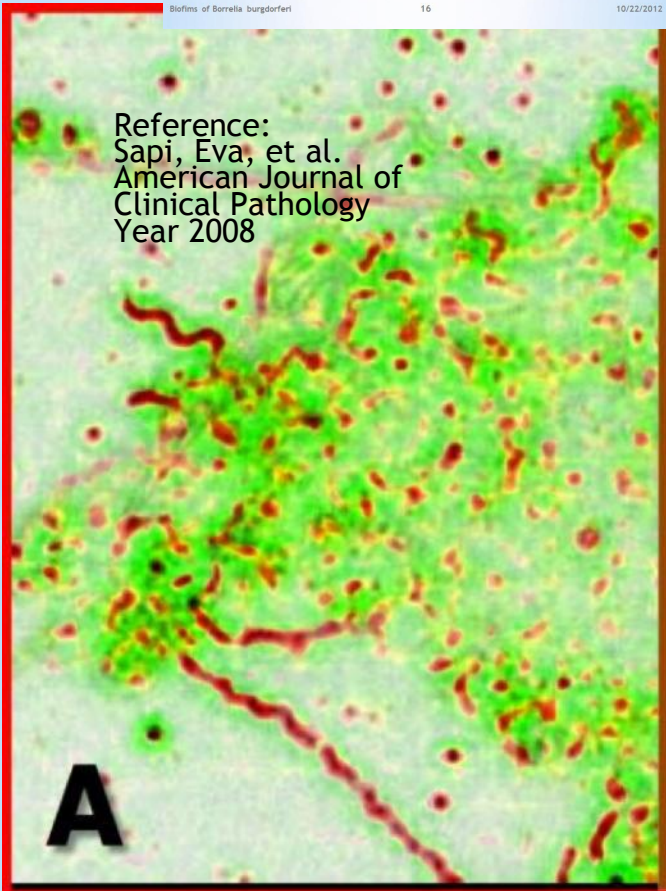
Reference: Reference:





Biofilms of Borrelia burgdorferi 16 10/22/2012

Biofilms of Borrelia burgdorferi 18 10/22/2012

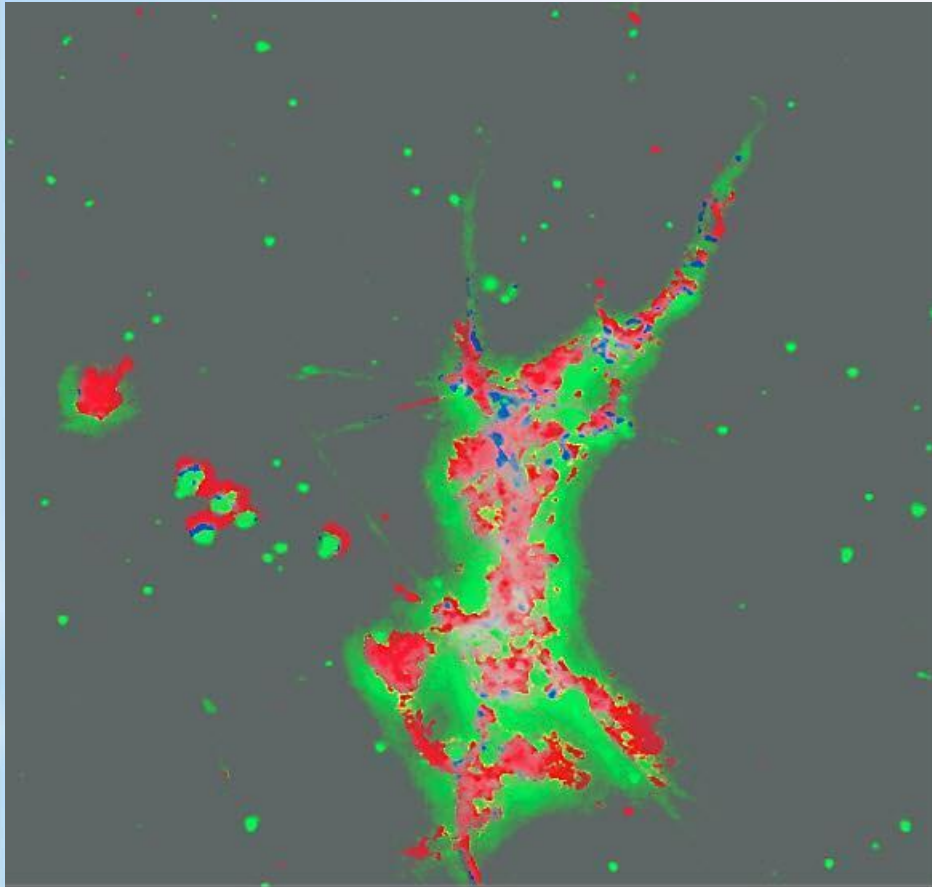


A Extracellular Matrix is Green
Spirochetes and Granular forms Red

**Biofilm of Borrelia burgdorferi ATCC35210
CytoViva Image with Spectral Analysis**

B No Spectral analysis of CytoViva Image

**Image by Alan B. MacDonald MD
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**Biofilm of Borrelia
burgdorferi strain B31
ATCC 35210**


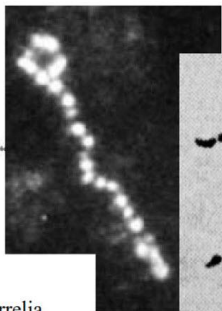
**Note: Biofilm units include spiral and granular forms (blue and red)
Extracellular Matrix investment (green)**



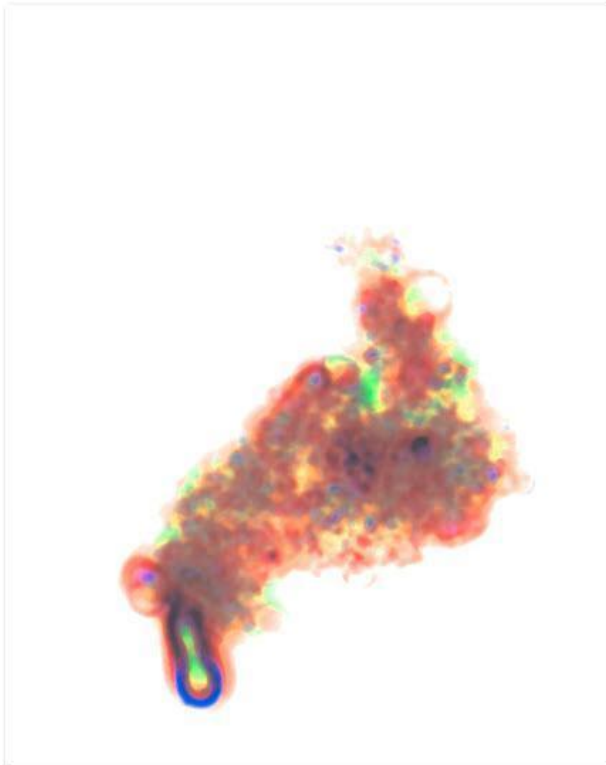
FORMATION OF DOCCOID BODIES IN BLOOD



Hilber, 1917, emergence of granular "cocci" from spirochetes



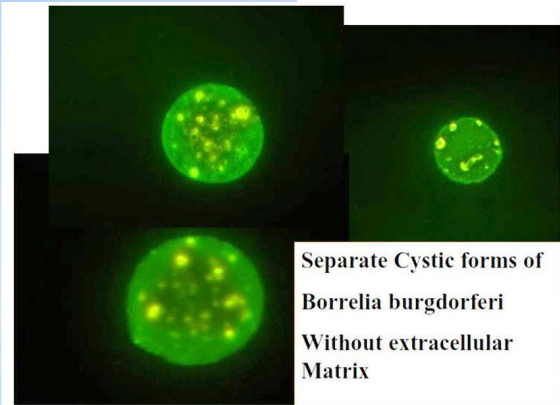
Granular Borrelia
Evolving from spiral borrelia



**Image by Alan B. MacDonald , MD
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**Biofilm of Borrelia
burgdorferi strain
B31 , ATCC 35210**

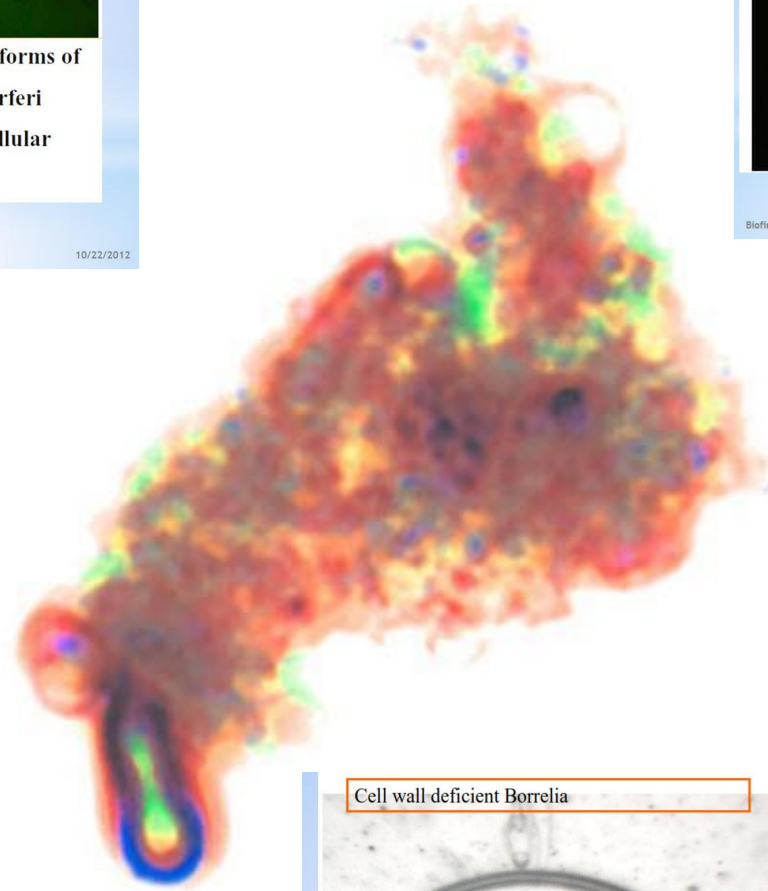
**Note: Cystic and spheroplast forms, Granular forms of
borrelia burgdorferi with Extracellular matrix investment
(E C Matrix stains vareigated Red and Green)**



Separate Cystic forms of
Borrelia burgdorferi
Without extracellular
Matrix



Separate Cystic forms of
Borrelia burgdorferi
Without extracellular
Matrix



Cell wall-deficient *Borrelia*





Separate Cystic forms of
Borrelia burgdorferi
Without extracellular
Matrix

Biofilms of *Borrelia burgdorferi*

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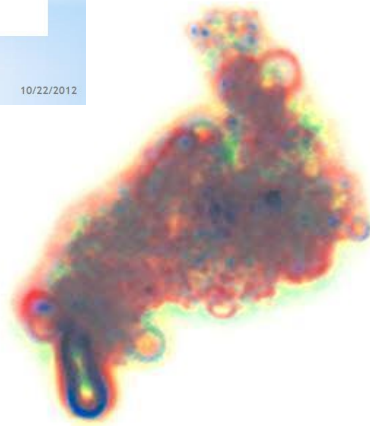
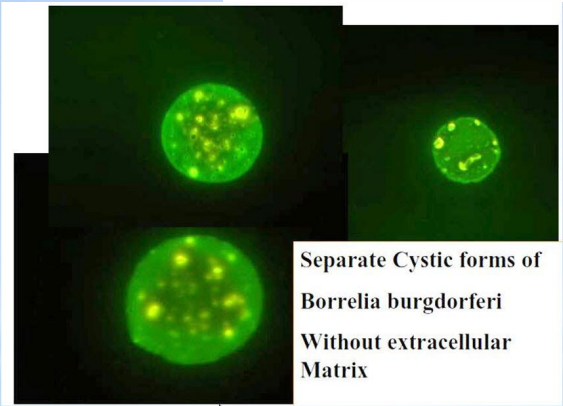


Image by Alan B. MacDonald MD
Copyright year 2006

**Biofilm of
borrelia
burgdorferi strain
B31
ATCC 35210**

**Note: Cystic forms and Spheroplast forms of *Borrelia burgdorferi* invested in an Extracellular matrix
Matrix stains red and green // Cysts and spheroplasts stain red**

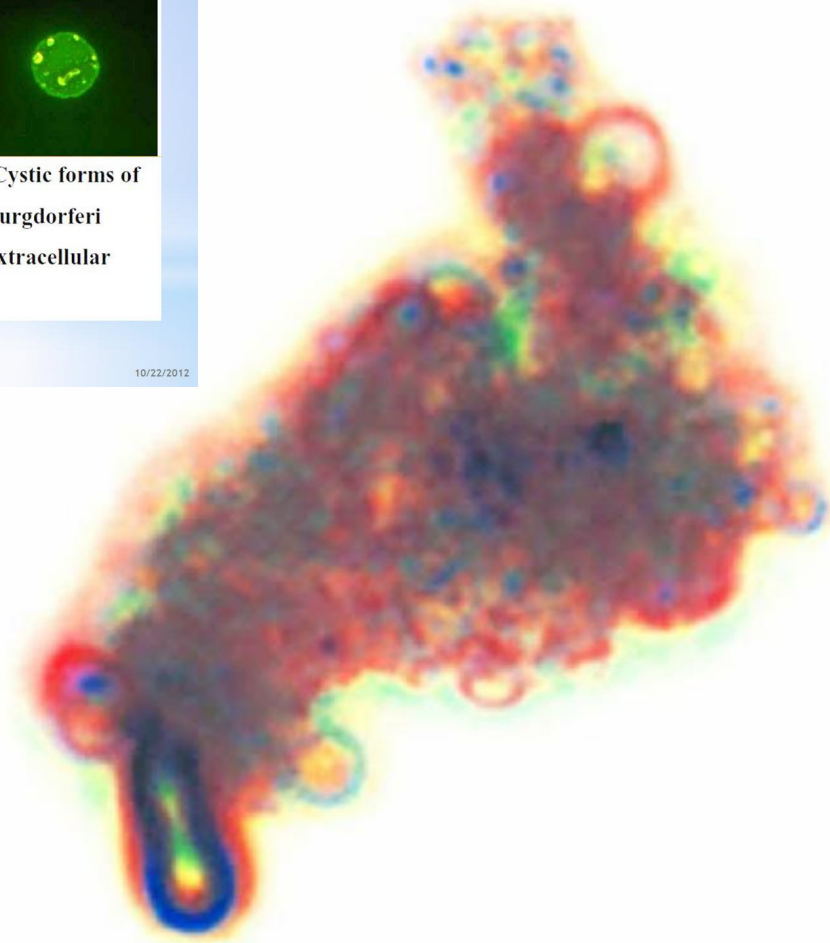


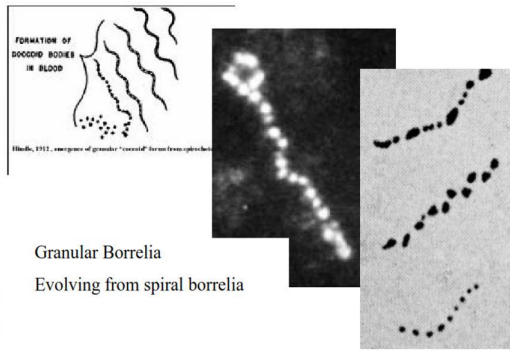
Separate Cystic forms of
Borrelia burgdorferi
Without extracellular
Matrix

Biofilms of *Borrelia burgdorferi*

17

10/22/2012

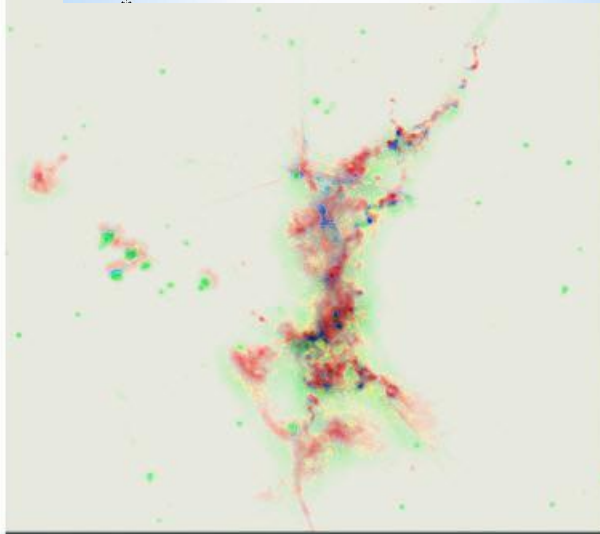




Biofilms of *Borrelia burgdorferi*

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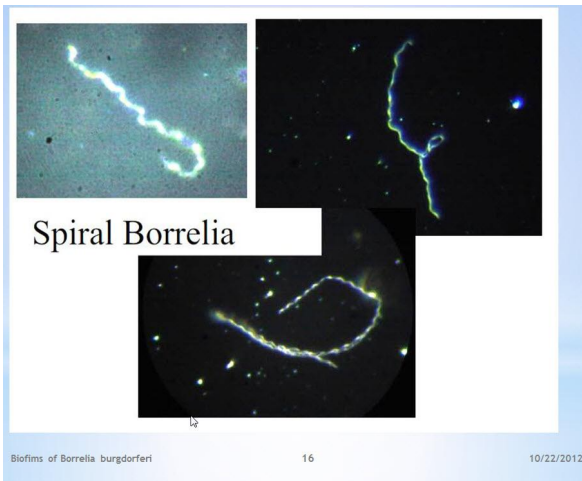
10/22/2012



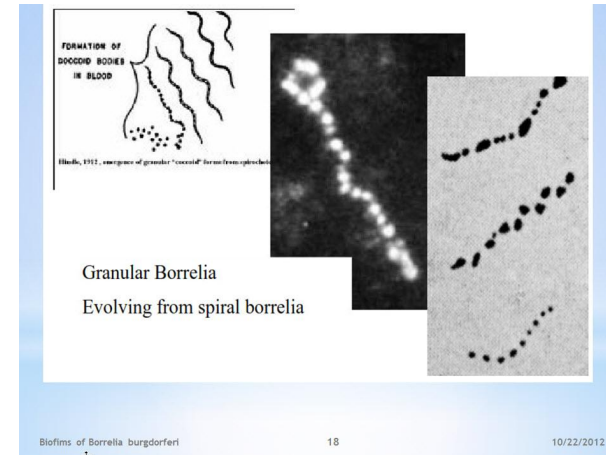
**Image by Alan B. MacDonald MD
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**Biofilm of
Borrelia
burgdorferi
1000x oil
immersion
CytoViva Image**

**Note: Diversity of forms in the biofilm community
material (slime layer) Spiral and granular spirochetal
forms red,blue //Matrix=Green**



Mixtures of Borrelia types maybe found in Borrelia biofilms

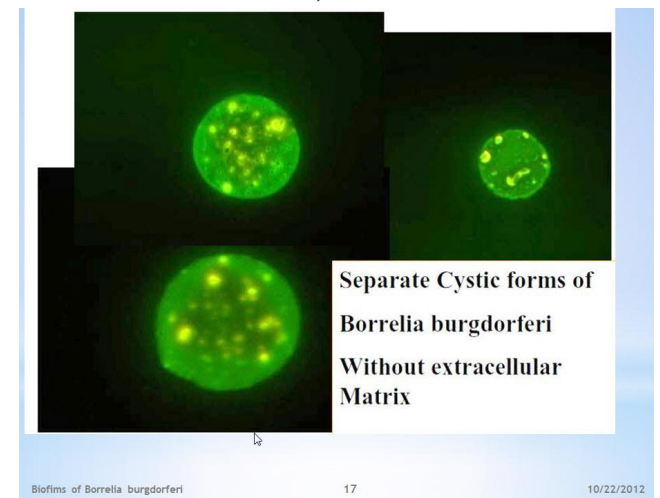
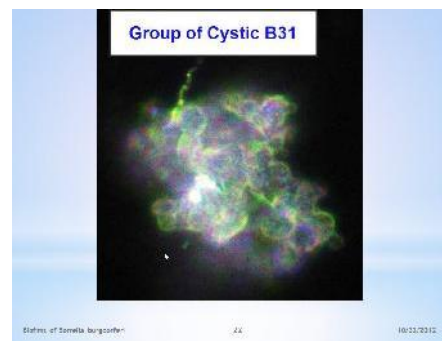
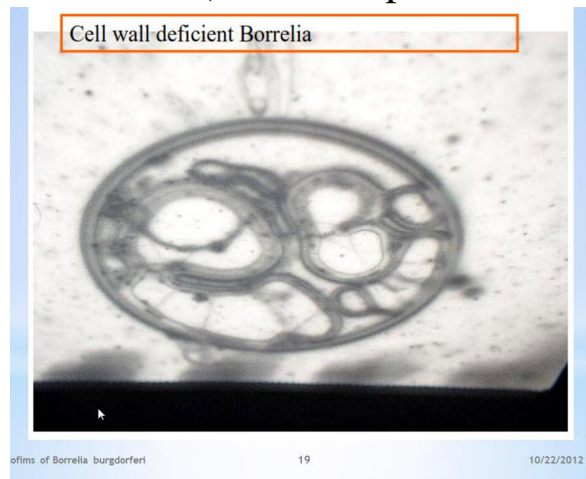


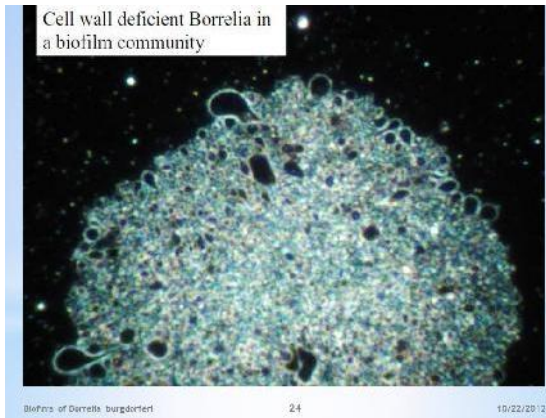
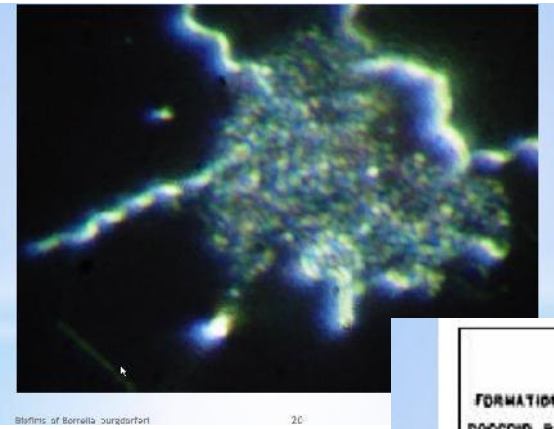
Some *Borrelia* biofilms may contain a majority of spiral *Borrelia*, while others may contain

A majority of granular or Cystic *Borrelia*

Biofilms may contain different species of pathogens

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



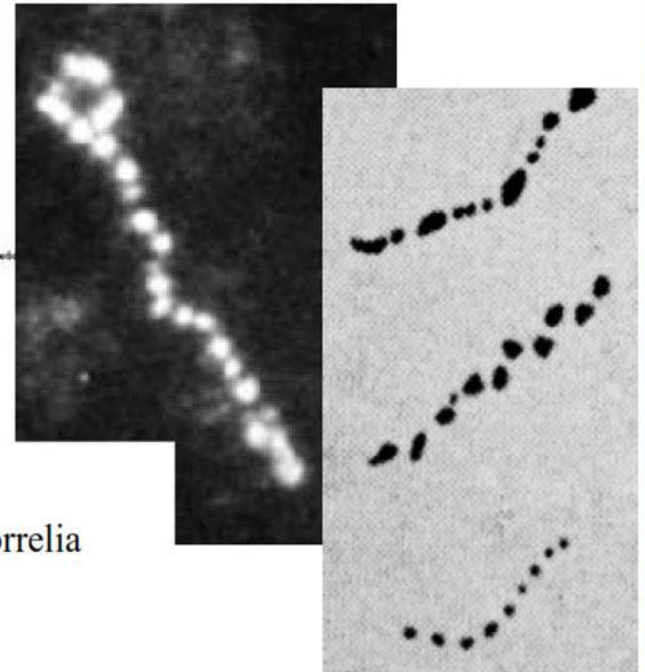


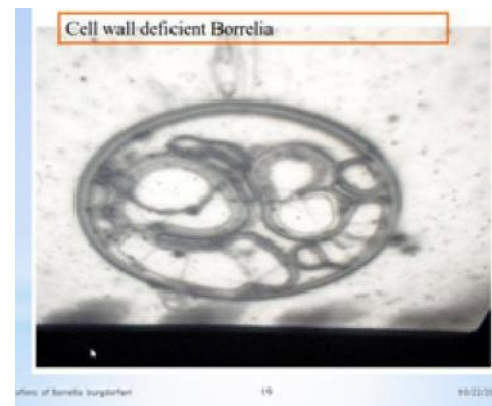
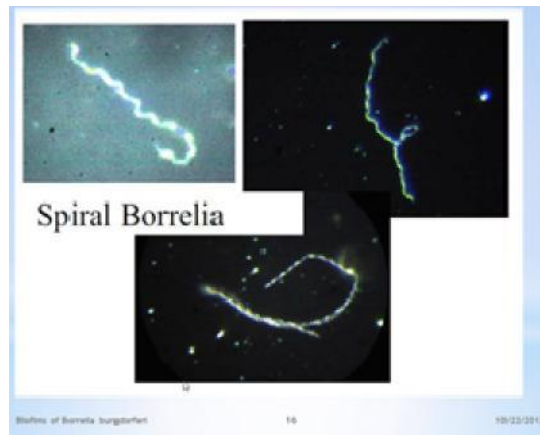
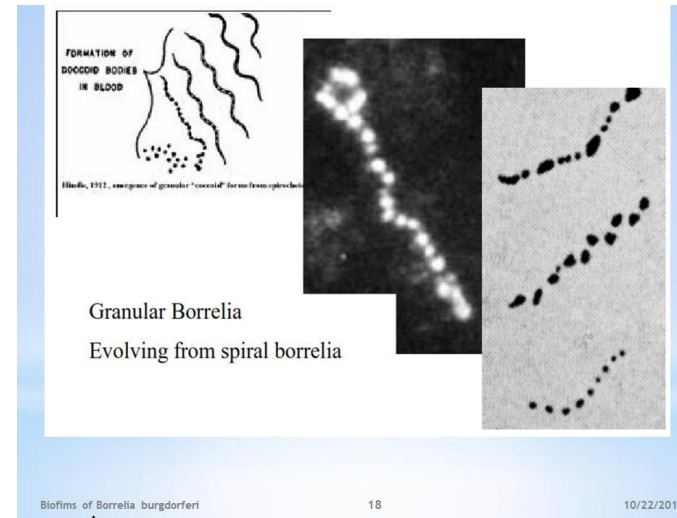
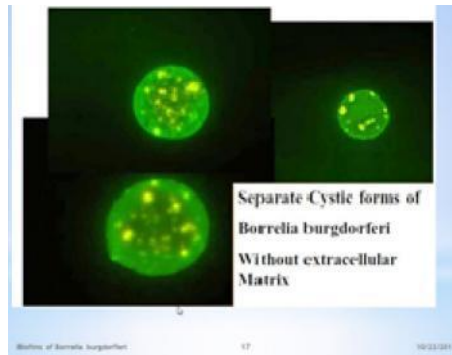
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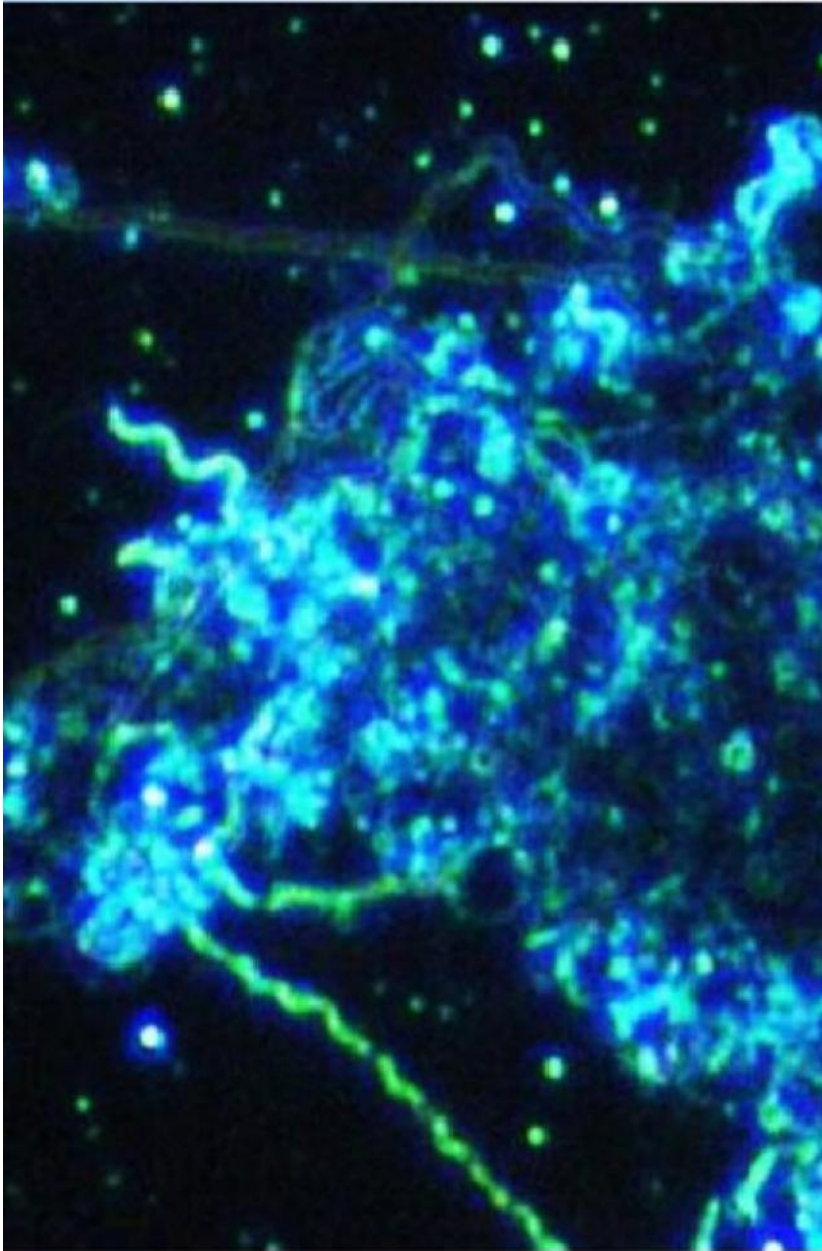
FORMATION OF DOCCOID BODIES IN BLOOD

Hinkle, 1912, emergence of granular "cocci" forms from spirochetes

Granular *Borrelia*
Evolving from spiral borrelia

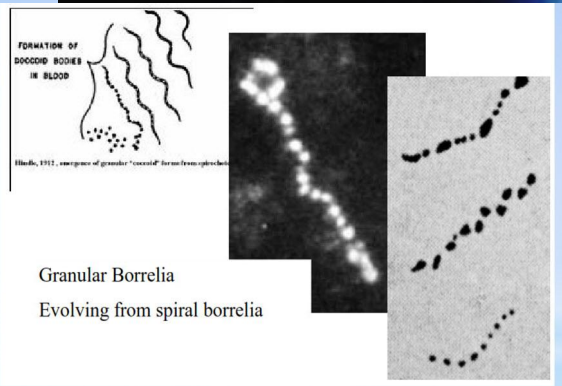






Biofilm Community Of *Borrelia Burgdorferi* Strain B31 Atcc 35210

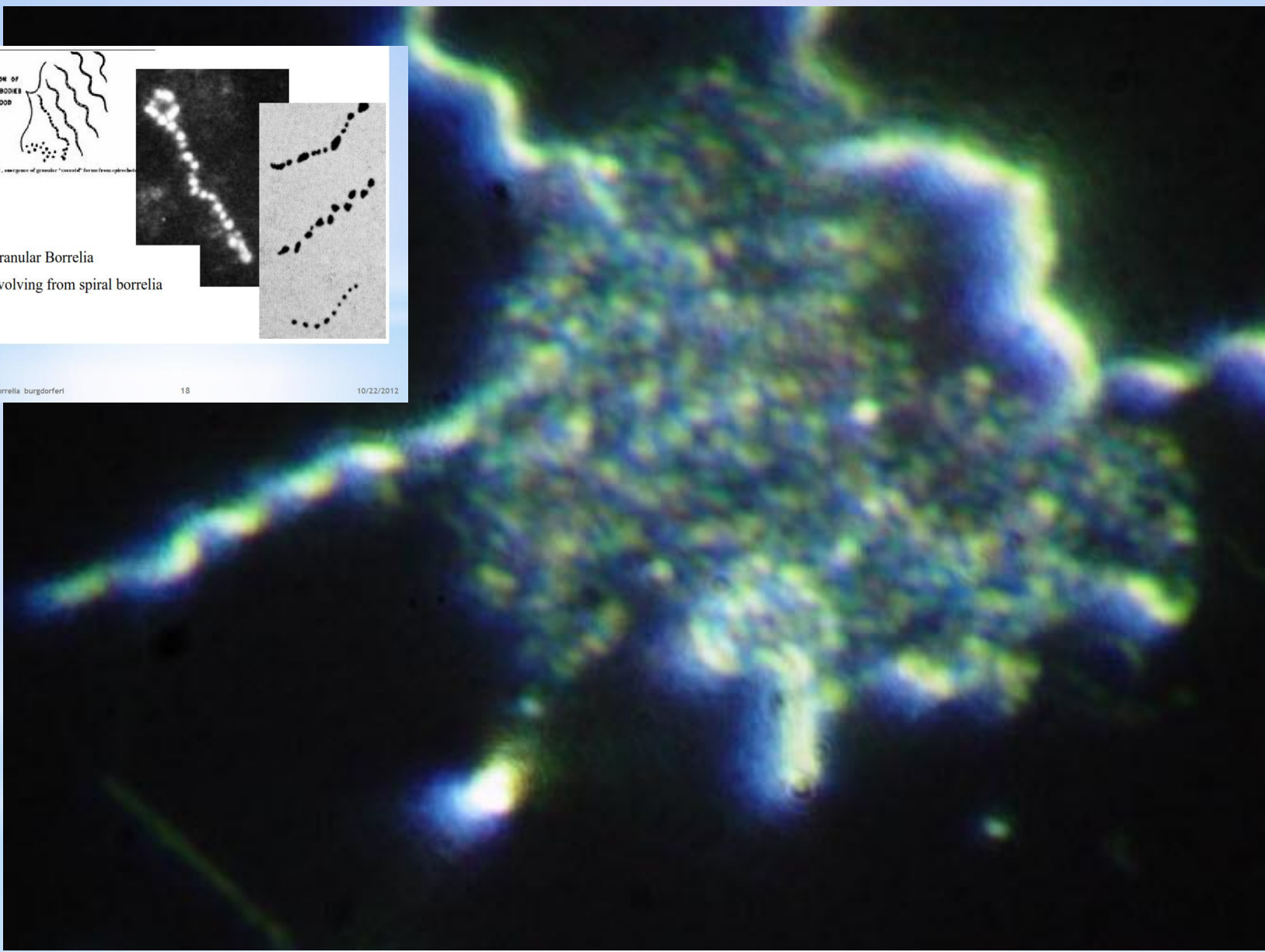
Reference:
Sapi, Eva, et al, American
Journal of Clinical Pathology,
2008, 129:988-90

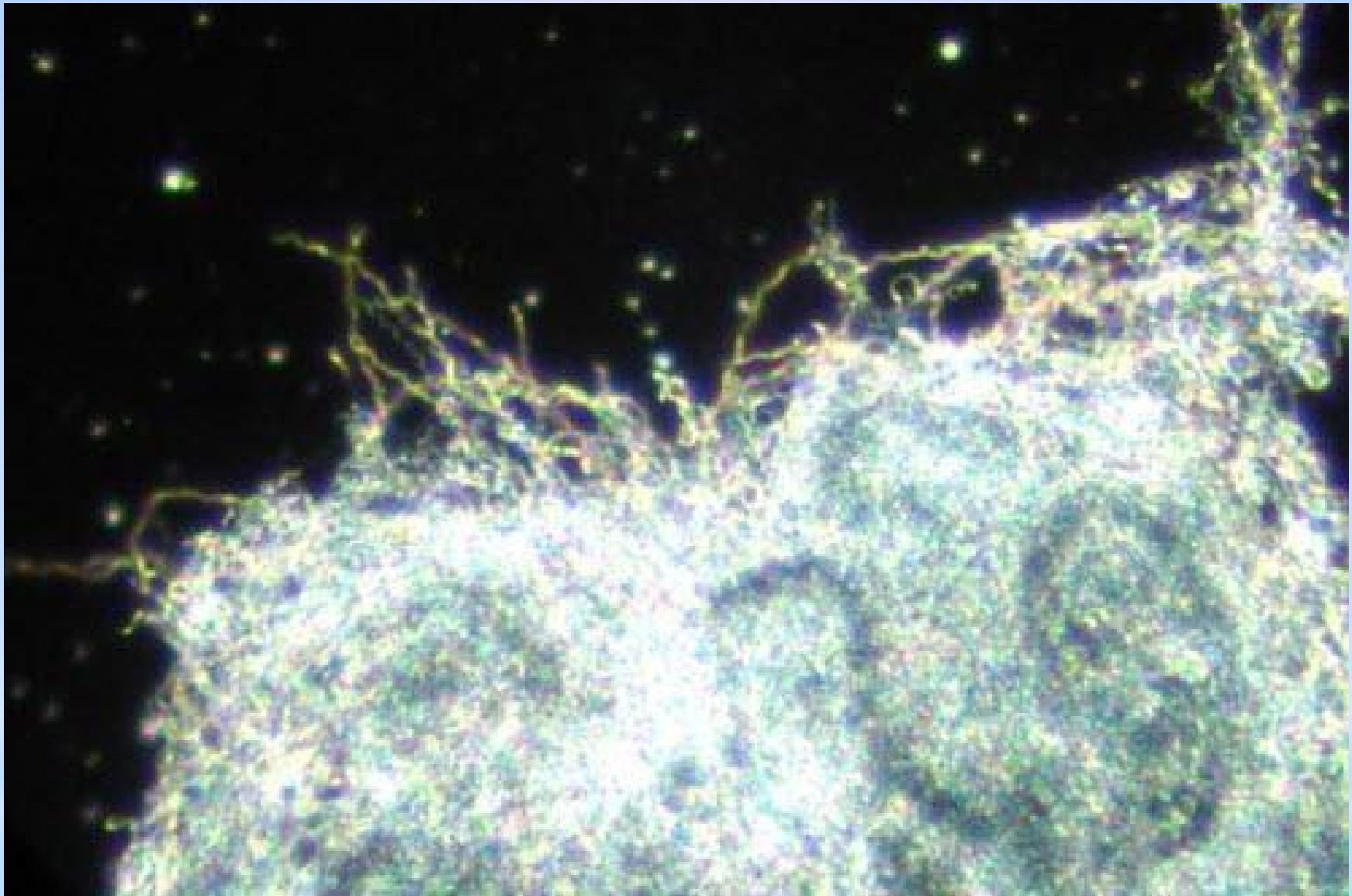


Biofilms of *Borrelia burgdorferi*

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Biofilms of *Borrelia burgdorferi*

WATER CHANNEL FORMATION - TYPICAL OF BIOFLMS - IN A BORRELIA BIOFILM COMMUNITY IN VITRO

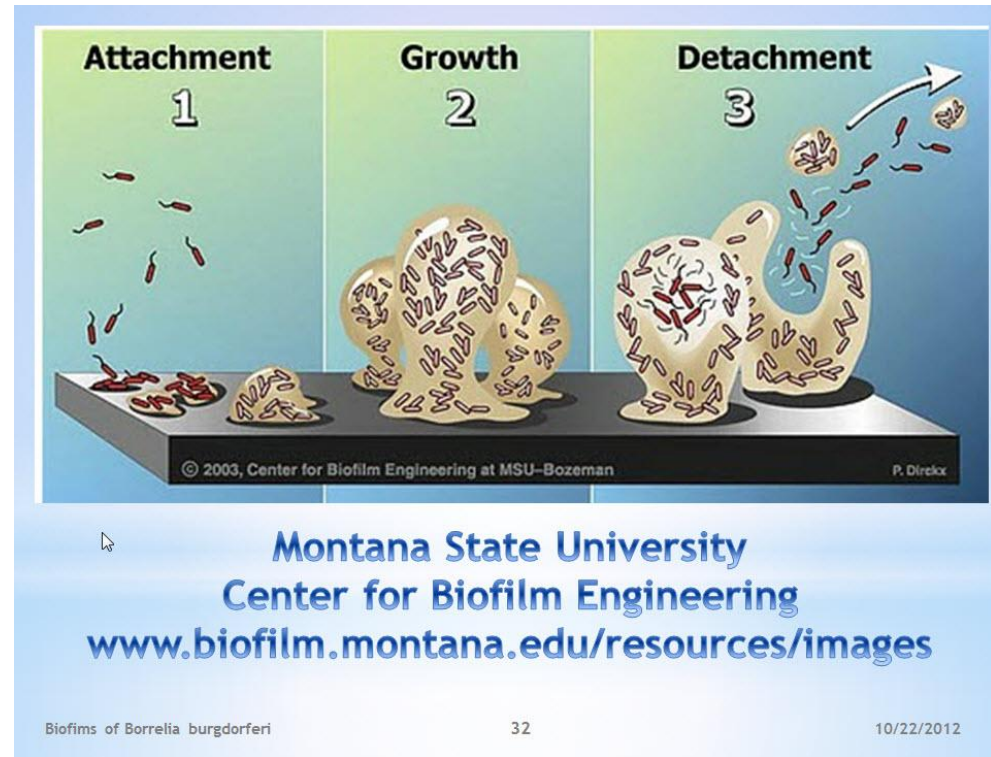
Water channel formation in a biofilm

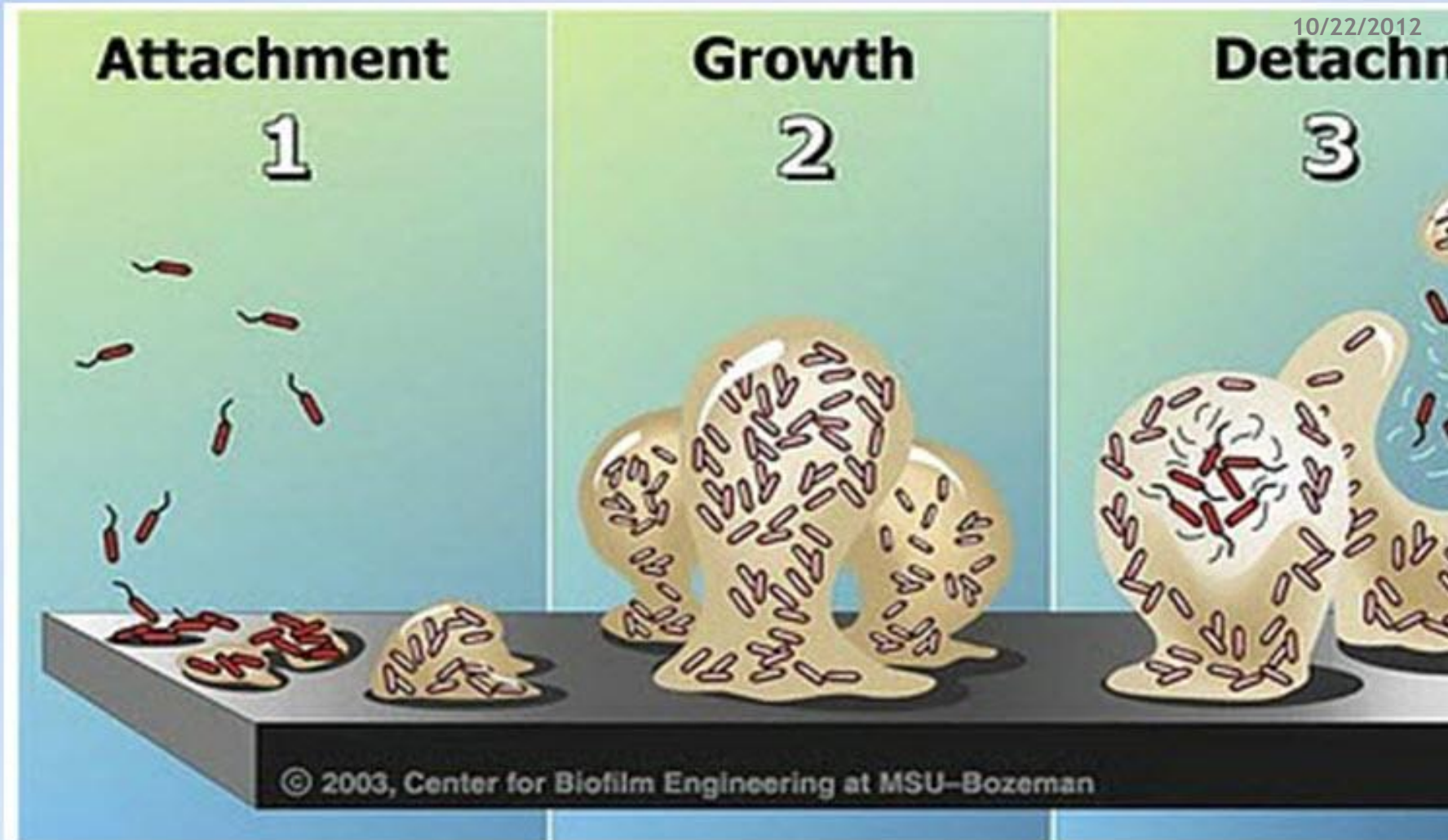
Biofilms of *Borrelia burgdorferi*

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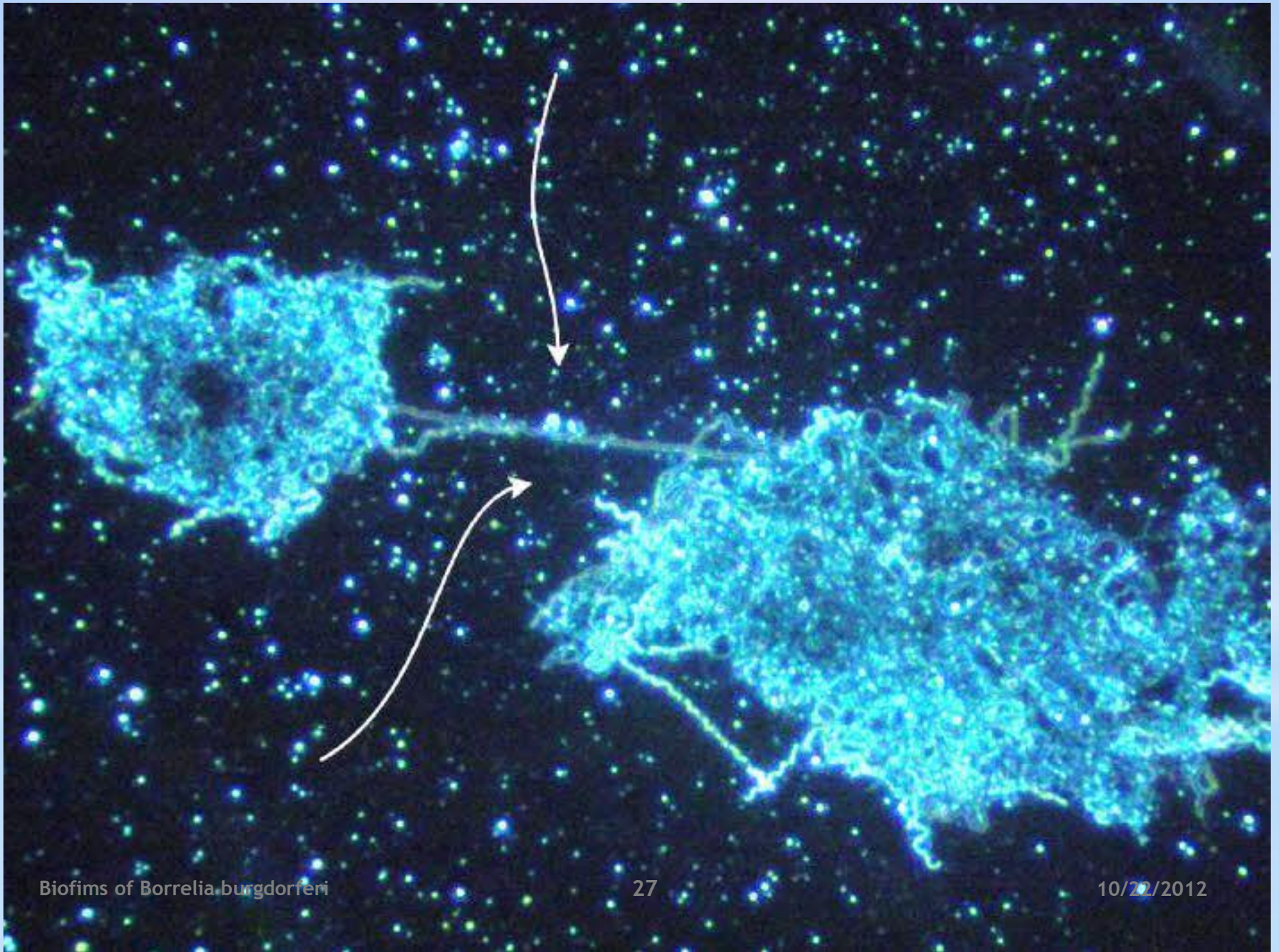
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MICROBES -
PLANKTONIC
MICROBES BECOME
INCORPORATED AND
ARE BIOCHEMICALLY
AND STRUCTURALLY
MODIFIED IN A
BIOFILM COMMUNITY





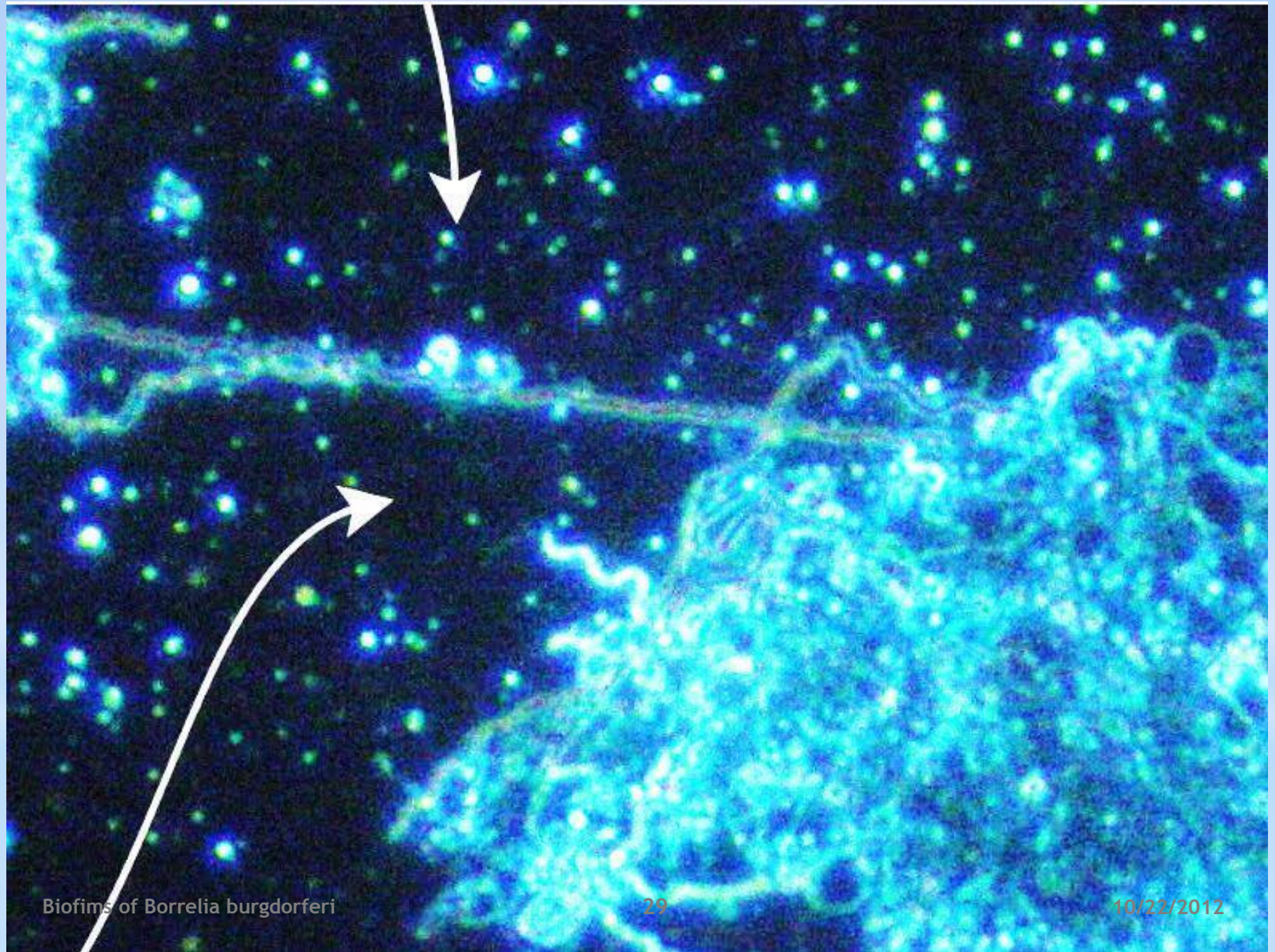
Montana State University
Center for Biofilm Engineering
www.biofilm.montana.edu/resources/



Biofilms of *Borrelia burgdorferi*

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Biofilms of *Borrelia burgdorferi*

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10/22/2012

Mixtures of Borrelia types maybe found in Borrelia biofilms

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A majority of granular or Cystic Borrelia

Biofilms may contain different species of pathogens

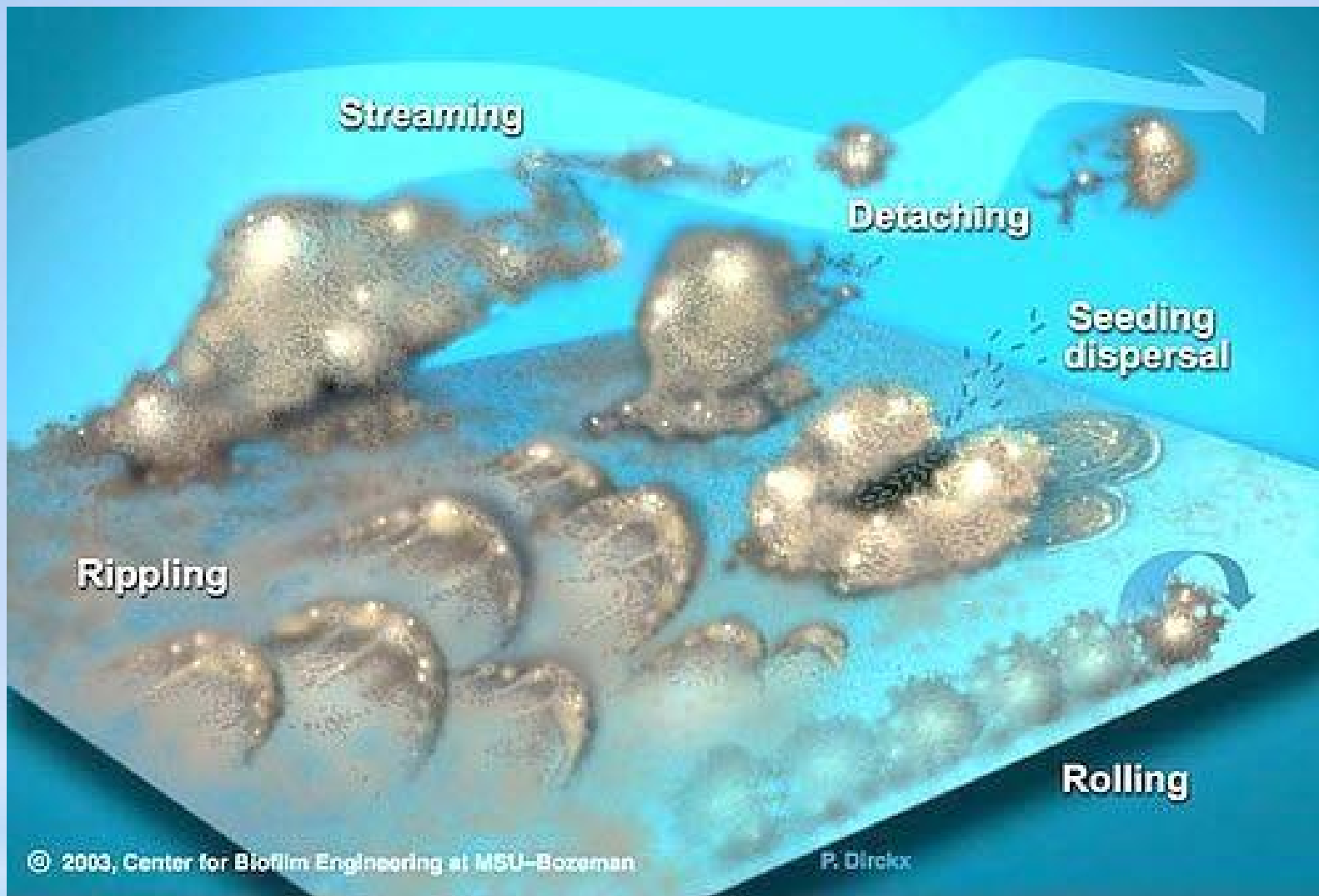
(For example Borrelia and Babesia, Or other multiorganism combinations)

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Montana State University Center for Biofilm Engineering

Link:

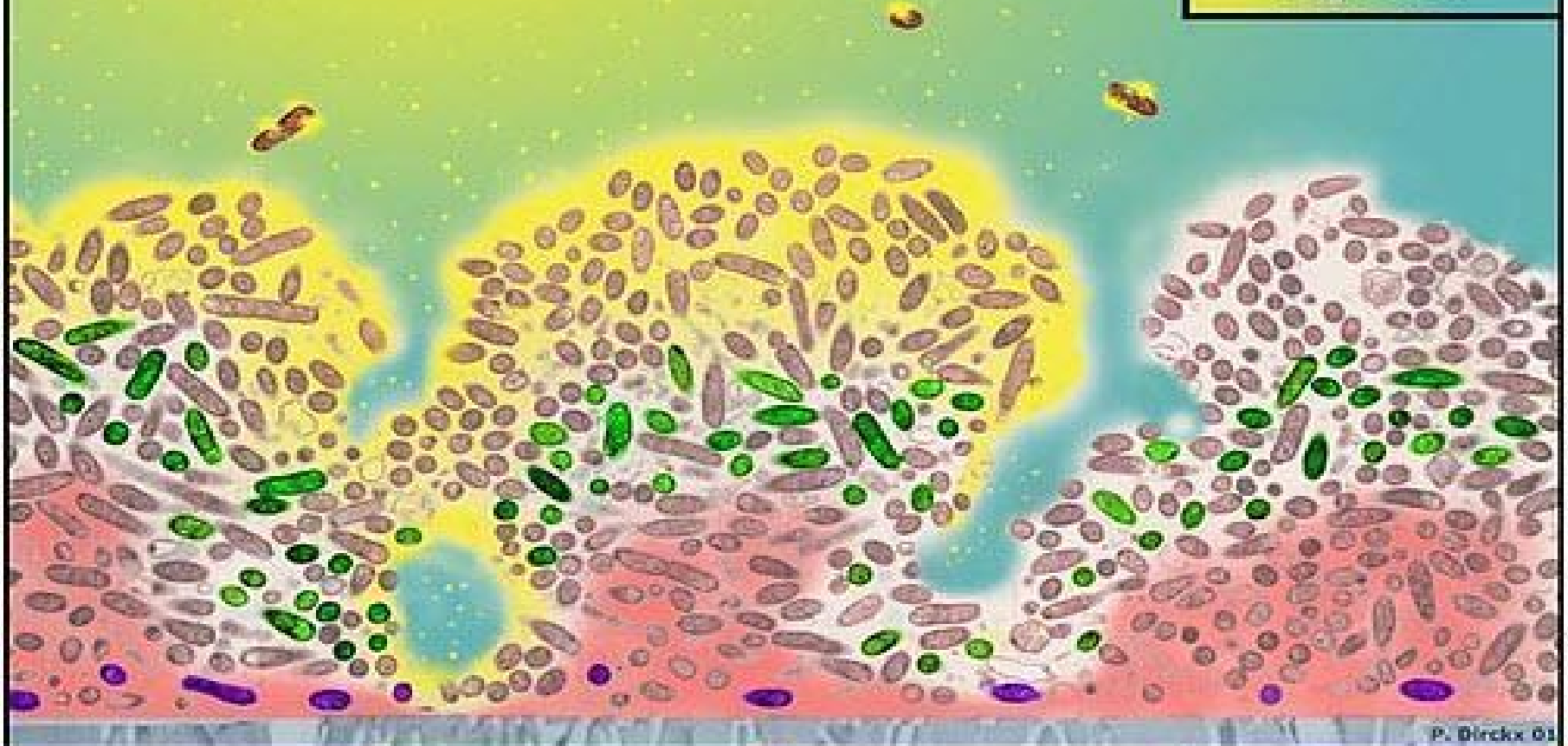
www.biofilm.montana.edu/resources/images



Montana State University Center
Center for biofilm Science and Engineering

Mechanisms of Biofilm Tolerance

Antimicrobial
Depletion



P. Dirckx 03

Slow
Penetration

Stress
Response

Altered
Microenvironment

Persisters

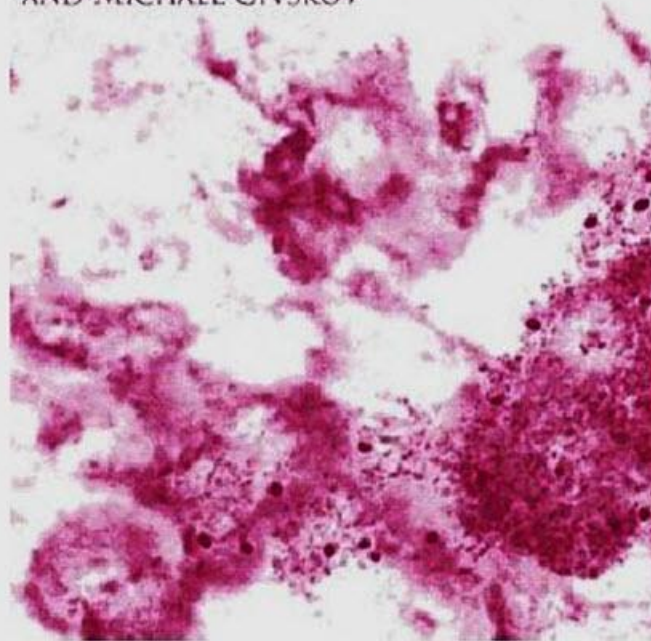
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Engineering
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Biofilm: *A community of microbes* enveloped in a protective Extracellular matrix

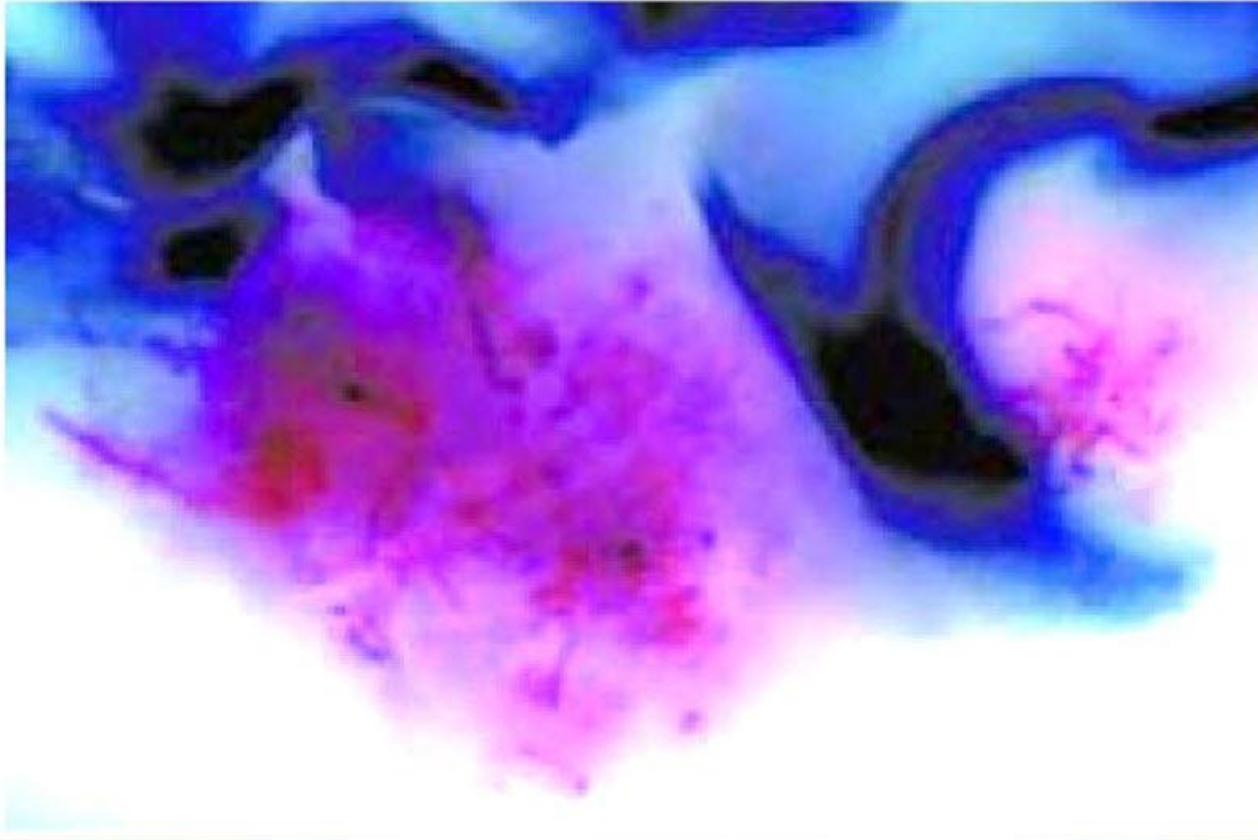
THE **BIOFILM** MODE OF LIFE
MECHANISMS AND ADAPTATIONS

EDITED BY STAFFAN KJELLEBERG
AND MICHAEL GIVSKOV



Dr K. Eisendle. BORRELIA LYMPHOCYTOMA

IMMUNOHISTOCHEMISTRY AJCP 2007,127:213-222



Biofilms of *Borrelia burgdorferi*

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TWO DISCRETE “GROUPINGS” OF
BORRELIA BURGdorFERI IN
HUMAN SKIN FROM A PATIENT
WITH BORRELIA LYMPHOCYTOMA:

SPECIALIZATION OF SHAPE IN A
MIXTURE OF GRANULAR FORMS
AND STRAIGHTENED FORMS AND
SPIRAL FORMS ALL CO-EXIST.

THE “REDDISH VEIL OF RED
STAINING “VEIL” SURROUNDING
THE UNITS IS ENTIRELY
CONSISTENT WITH
EXTRACELLULAR MATRIX

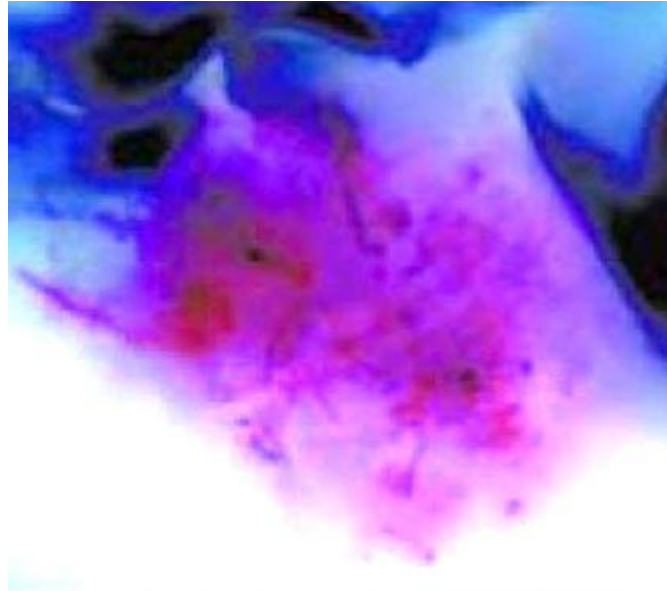
THEREFORE THESE ARE ENTIRELY
CONSISTENT WITH BIOFILM
COMMUNITIES

COMMENTS OF

ALAN B. MACDONALD, MD

Dr Klaus Eisendle. Et al, BORRELIA LYMPHOCYTOMA

IMMUNOHISTOCHEMISTRY Study (Focus Floating Microscopy- FFM) AJCP
2007 127:213-222



Granular forms Of borrelia

In a “colony” With a
“reddish veil”

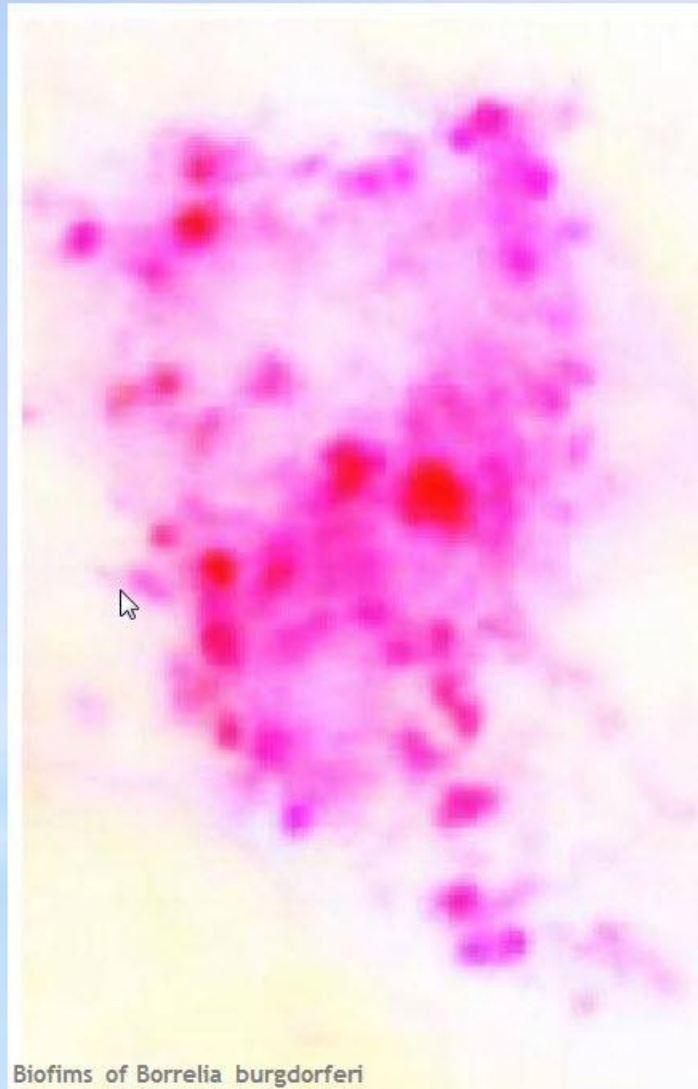
MacDonald’s comment:

The shape shifted
morphology indicates
specialization of Bb, and
the reddish veil supports
an Extracellular matrix

Therefore: a BIOFILM

This Image was obtained
by Dr. Klaus Eisendle MD
PhD using

Us Focus Floating
Microscopy (FFM)
Technique. The human
patient skin Was from
Acrodermatitis chronica
Atrophicans (ACA)

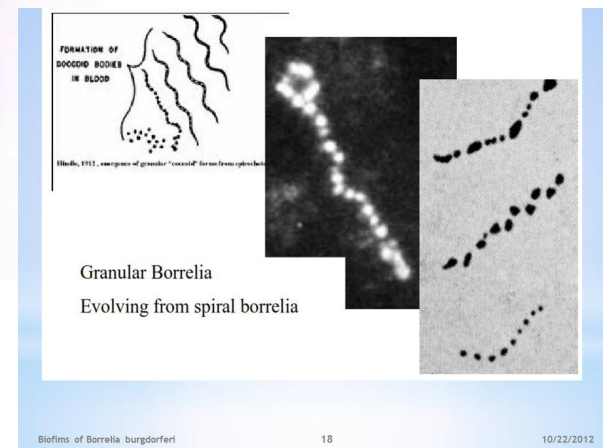


Dr K. Eisendle

Acrodermatitis Chronica
Atrophicans

Immunohistochemistry

“Granular forms of B
burgdorferi in a “colony”
With a “Reddish veil”



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.

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Unmatched optical
Resolution of Living
Complex Biological systems

*Funding for the Purchase
And ownership of
the CytoViVA Instrument
from
the following organizations
is Gratefully acknowledged

Time For Lyme Inc.

Lyme Disease Association(LDA)

End of Biofilms of *Borrelia burgdorferi* Slide Presentation

Prepared by Alan B. MacDonald ,MD,
FCAP,FASCP