SCIENTIFIC LITERATURE ON MOLECULAR BEACON DNA PROBES

MOLECULAR BEACONS ARE THE MOST SPECIFIC DNA PROBES AVAILABLE TODAY

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Simultaneous Detection of Pathogens in Clinical Samples from Patients with Community-Acquired Pneumonia by Real-Time PCR with Pathogen-Specific Molecular Beacon Probes

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In this study, real-time PCR with pathogen-specific molecular beacons (MB) and primers was evaluated for prediction of community-acquired pneumonia (CAP) causative agents, detecting six main CAP agents, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Legionella pneumophila*, and *Streptococcus pyogenes*, simultaneously. The PCR assay was evaluated for fresh clinical specimens from infants and children ($n = 389$) and from adults ($n = 40$). The MB probes and primers are both pathogen specific, namely, the *lytA* gene for *S. pneumoniae*, the *mip* gene for *L. pneumophila*, and 16S rRNA genes for the remaining four organisms. DNA extraction of clinical specimens was performed with a commercially available EXTRAGEN II kit, and amplification was performed with Stratagene Mx3000P. The limit of detection for these pathogens ranged from 2 copies to 18 copies. The whole process from DNA extraction to the analysis was finished in less than 2 h. The obtained sensitivity and specificity of this real-time PCR study relative to those of conventional cultures were as follows: 96.2% and 93.2% for *S. pneumoniae*, 95.8% and 95.4% for *H. influenzae*, 100% and 100% for *S. pyogenes*, and 100% and 95.4% for *M. pneumoniae*, respectively. The sensitivity and specificity for *M. pneumoniae* relative to those of a serologic assay were 90.2% and 97.9%, respectively. In six clinical samples of *C. pneumoniae*, the real-time PCR gave positive predictable values, and in those cases, elevation of the titer value was also observed. In conclusion, we demonstrated that a real-time PCR assay with pathogen-specific MB is useful in identifying CAP causative agents rapidly and in examining the clinical course of empirical chemotherapy in a timely manner, supporting conventional culture methods.

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A REVIEW

Molecular beacon: a multitask probe

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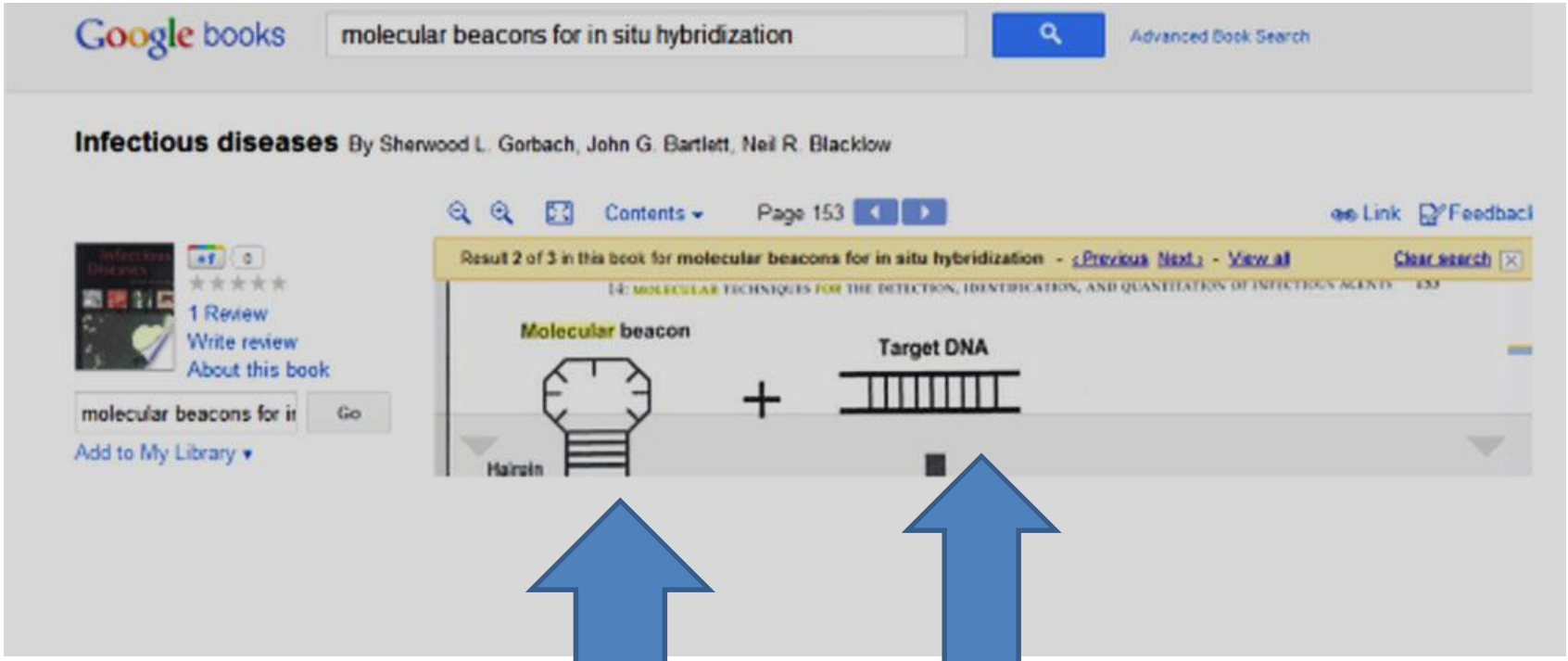
Molecular Beacons detect Pathogens (microbes)

Molecular Beacon DNA Probe Structure

A Short segment of SPECIFIC Single Strand DNA With a Fluorescent Label Attached at one End

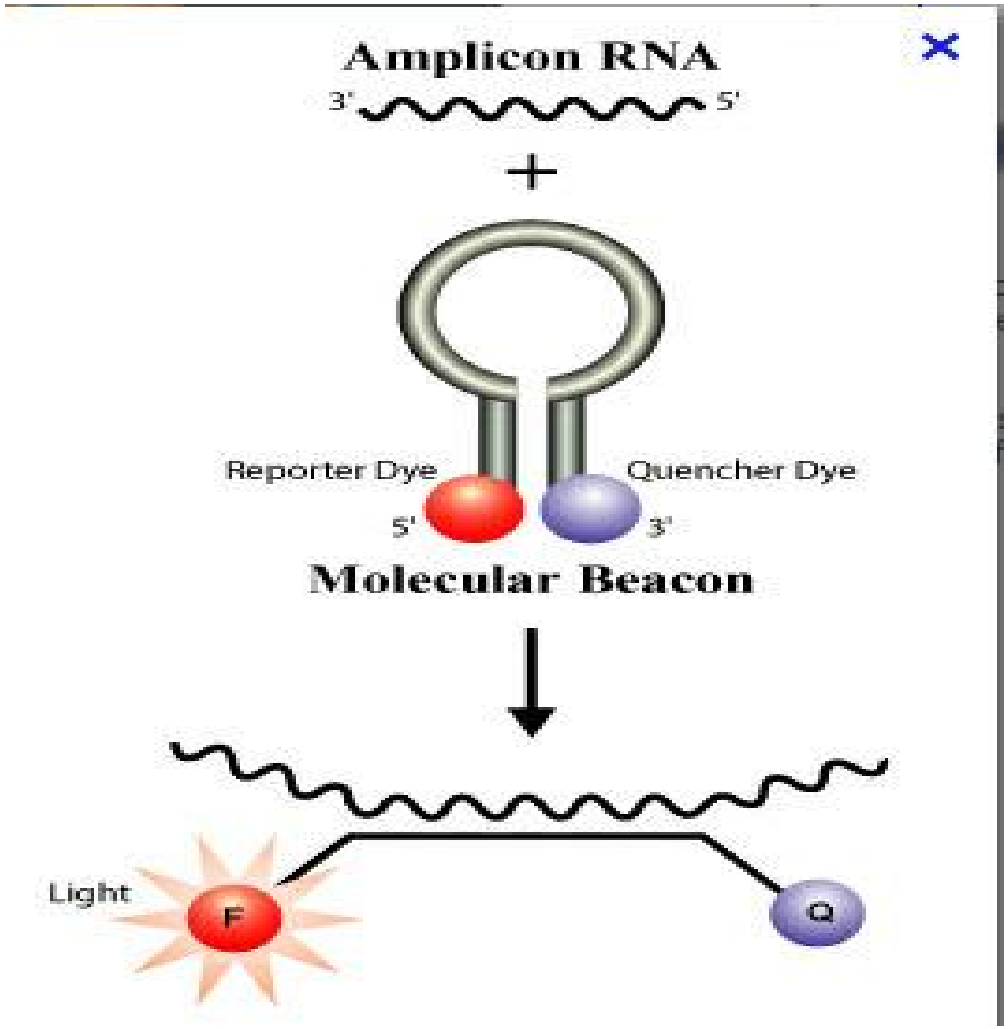
THE FLUORESCENT SIGNAL IS CHEMICALLY SUPPRESSED WHEN THE DNA *PROBE DOES NOT BIND TO ITS INTENDED TARGET DNA SEQUENCE*

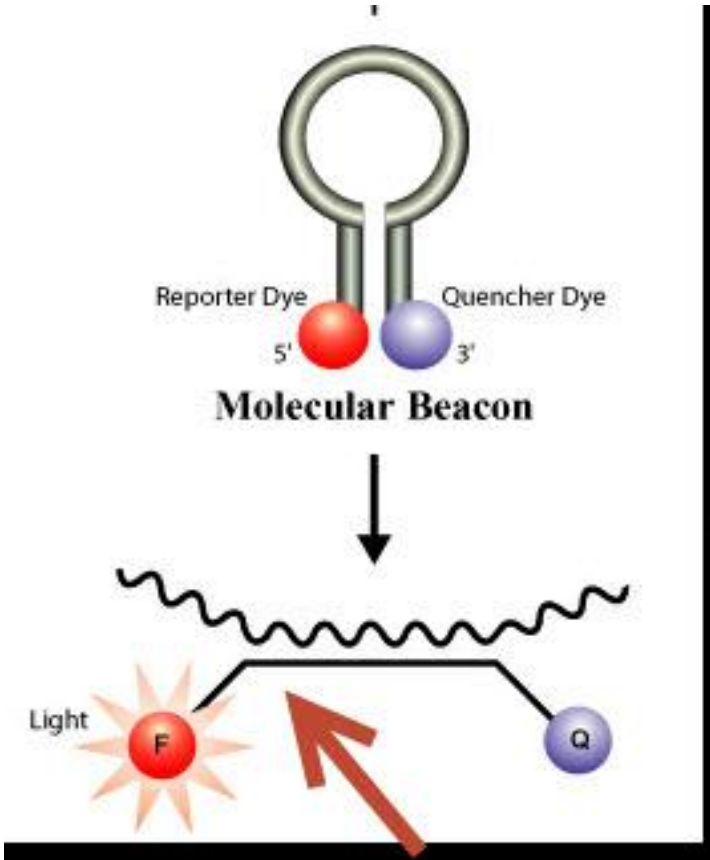
FLUORESCENCE IS EMITTED WHEN THE MOLECULAR BEACON BINDS TO ITS TARGET --DNA MATCH



Molecular Beacon In the CLOSED [non-fluorescing} shape

Specific TARGET DNA sequence


Amplicon RNA
3'
5'
+
Reporter Dye
Quencher Dye
5'
3'
Molecular Beacon
Light
F
Q



"CLOSED"
MOLECULAR BEACON
[NO FLUORESCENCE]

ALL dna Invisible

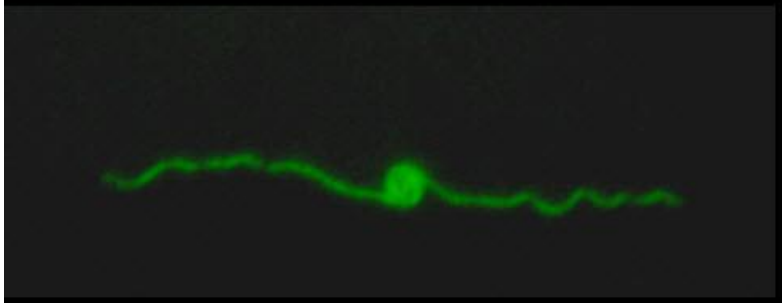
"open"
MOLECULAR
BEACON

FLUORESCENCE
IS
RELEASED
AND dna
BECOMES
VISIBLE

Positive Control studies
Does the Molecular Beacon DNA Probe Actually STAIN the Borrelia burgdorferi Spirochete

Borrelia Burgdorferi
Reference Strain - Control study
American type culture collection

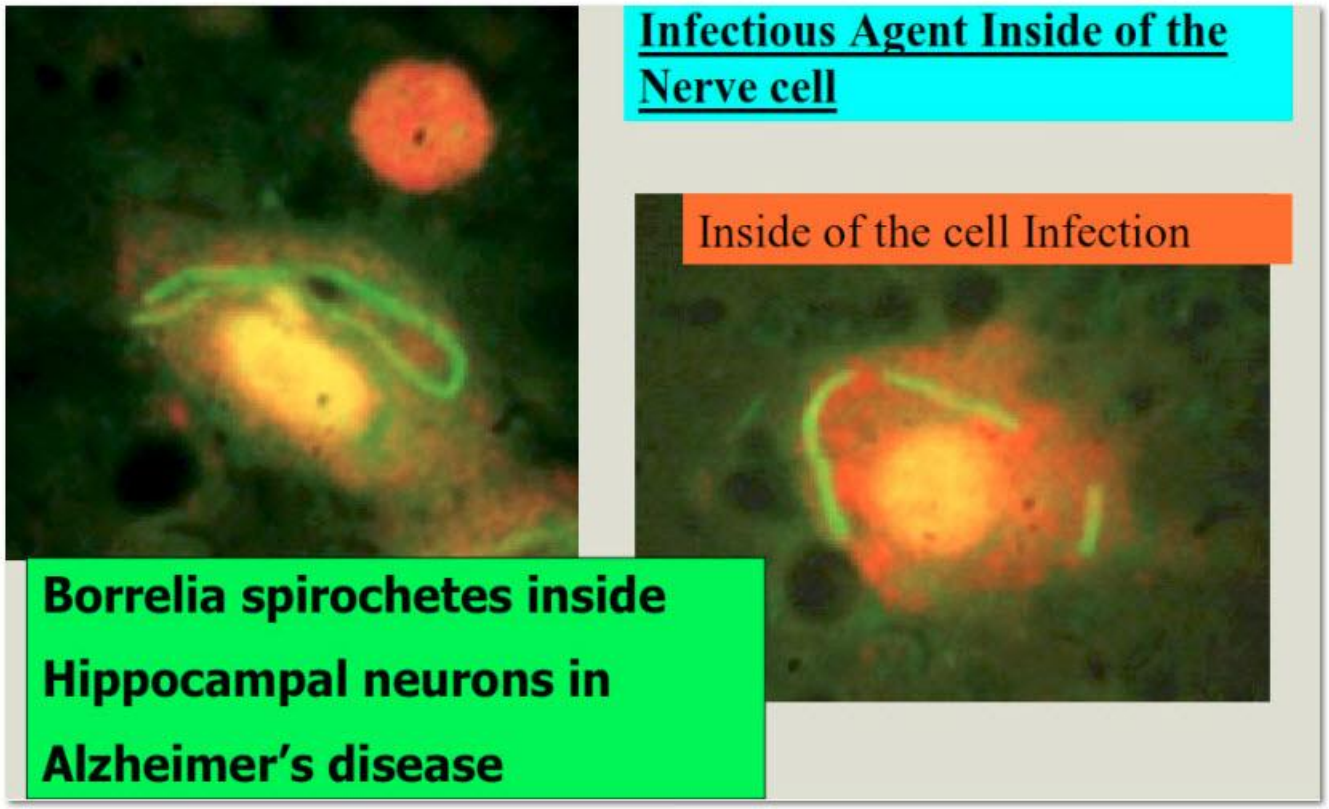
One PAIR of spirochetes
connected by a Round Body



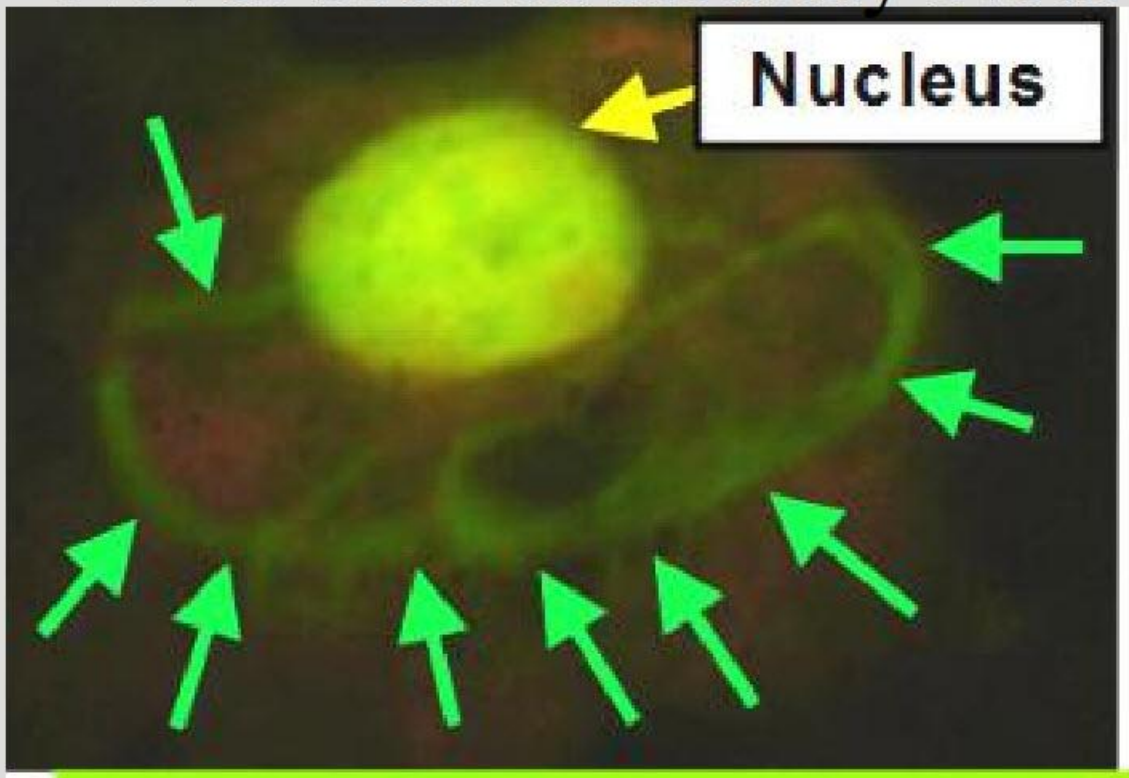
Molecular Beacon for DNa
Specific for Borrelia burgdorferi
DNA (BBO 147)

Research Funding
underwritten by
the Turn the Corner
Foundation
New York, New York

The Utility of The Molecular Beacon DNA probe To detect Individual Borrelia burgdorferi Spirochetes INSIDE of INDIVIDUAL NERVE Cells from Alzheimer's Disease

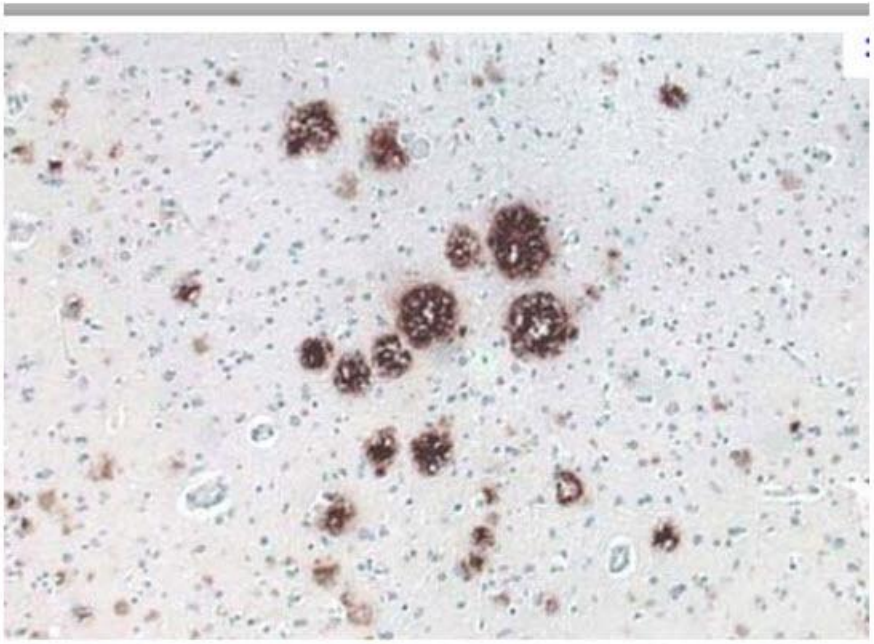

Infectious Agent Inside of the Nerve cell
Inside of the cell Infection
Borrelia spirochetes inside Hippocampal neurons in Alzheimer's disease

Single Strand DNA in Cytoplasm is Never seen in Healthy Neuron

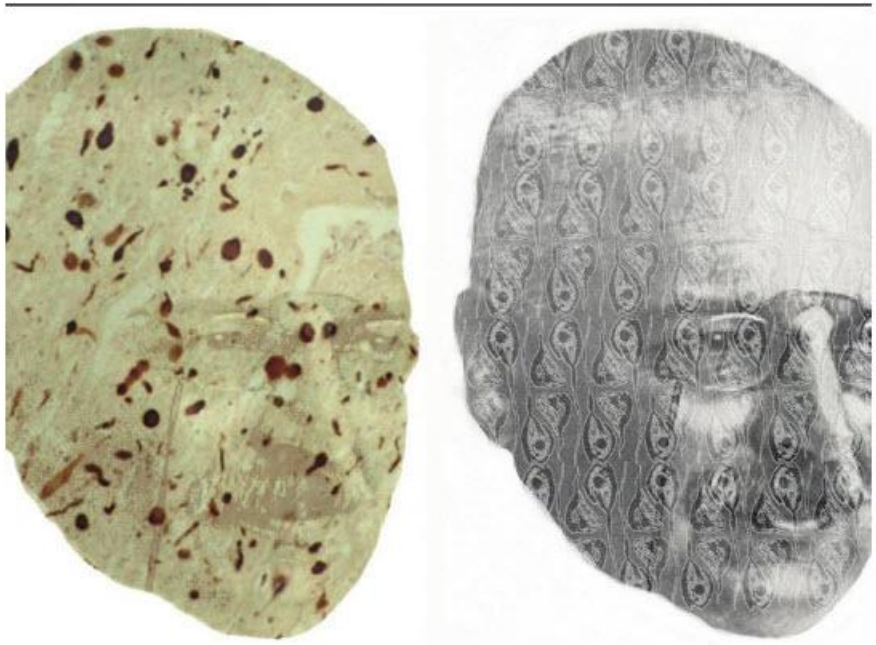


Spirochetal DNA
Green strands inside cell

The Plaques
Which typify the tissue
Damage in the brain
In Alzheimer's Disease



Alzheimer plaques - google



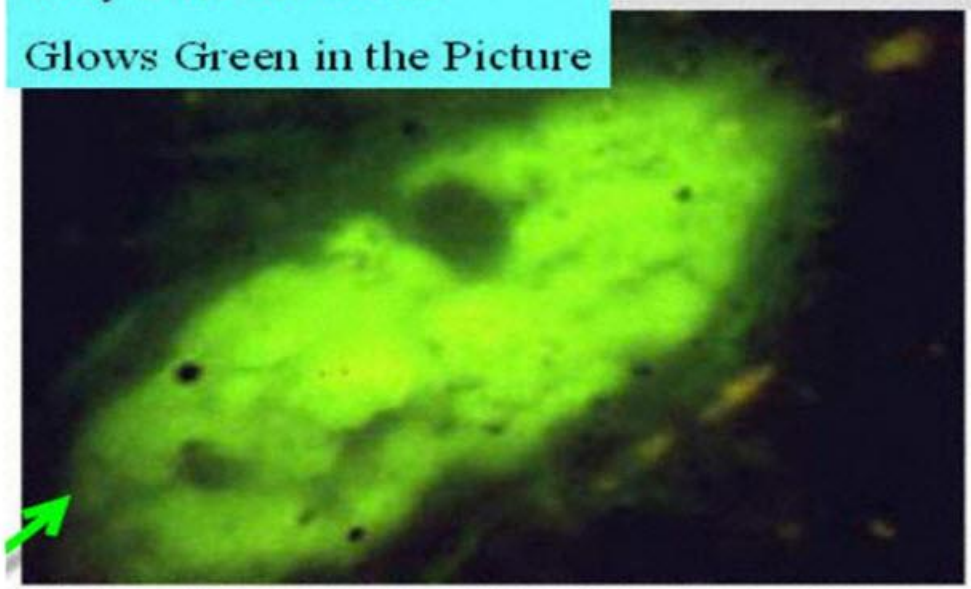
Dr Alois Alzheimer – with Morphing of Alzheimer plaques on his portrait

Utility of the Molecular Beacon DNA probe To detect The PLAQUES Of Alzheimer's Disease In Autopsy Brain tissue

DNA in Plaques

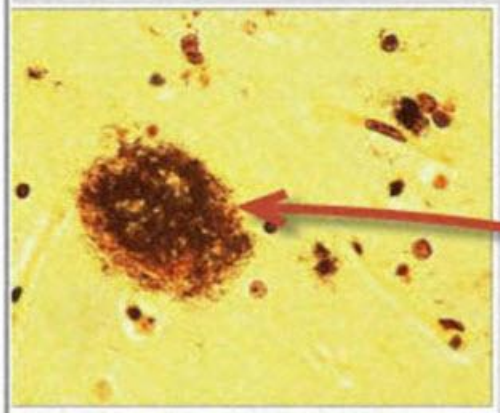
Only Borrelia DNA

Glows Green in the Picture



Borrelia burgdorferi **Flagellin DNA . In situ hybridization, Large Plaque**

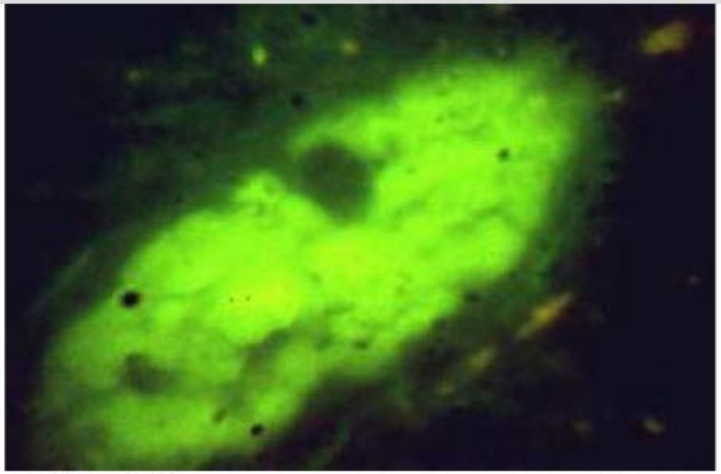
1000x original magnification



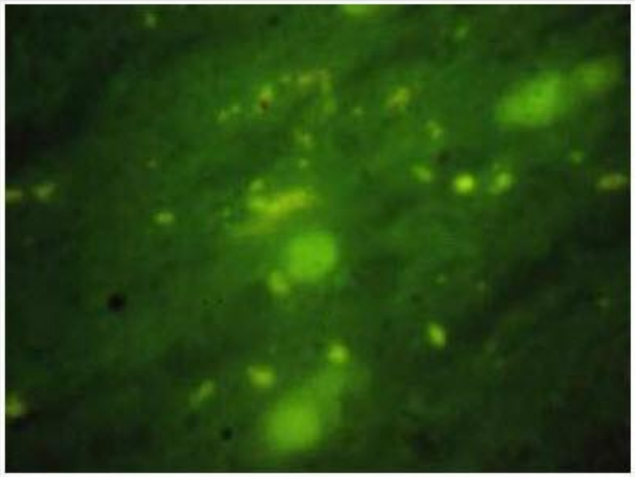
& Love: Neuropathology 2e © 2004 Elsevier Ltd.

slide////Image Right- Alzheimer plaque stained wit Bielschowsky Silver Stain (Brown arrow)

Mr Paul Christensen Alzheimer's At Autopsy 8 years after Spinal Fluid + for Borrelia burgdorferi at Stony Brook

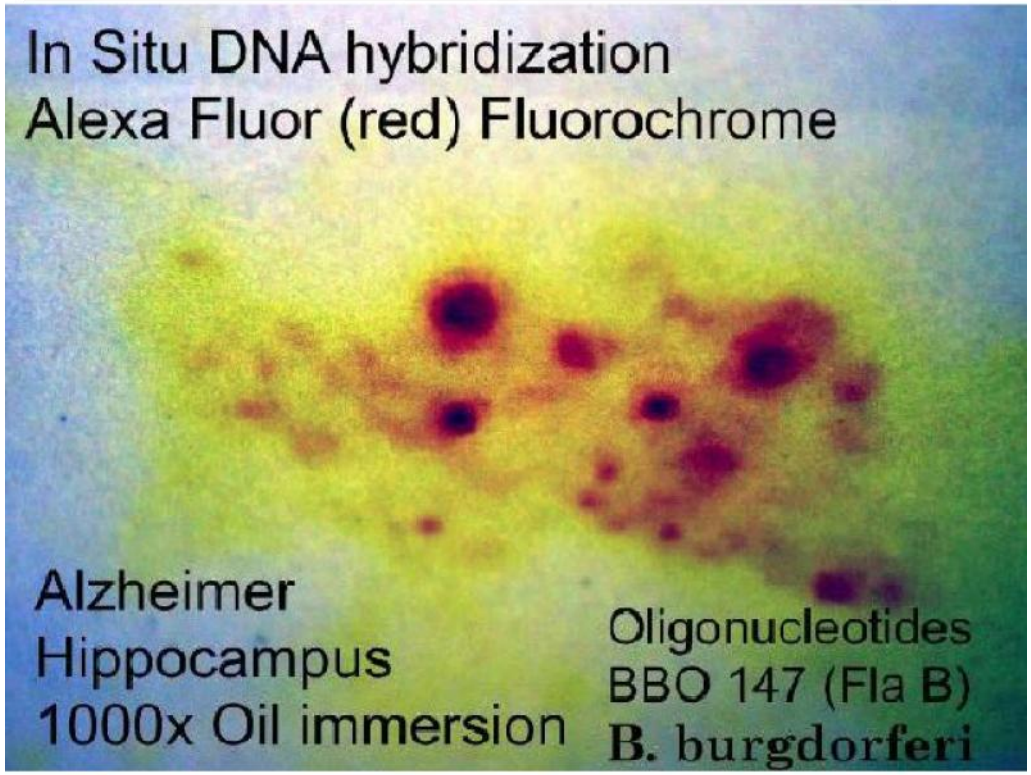


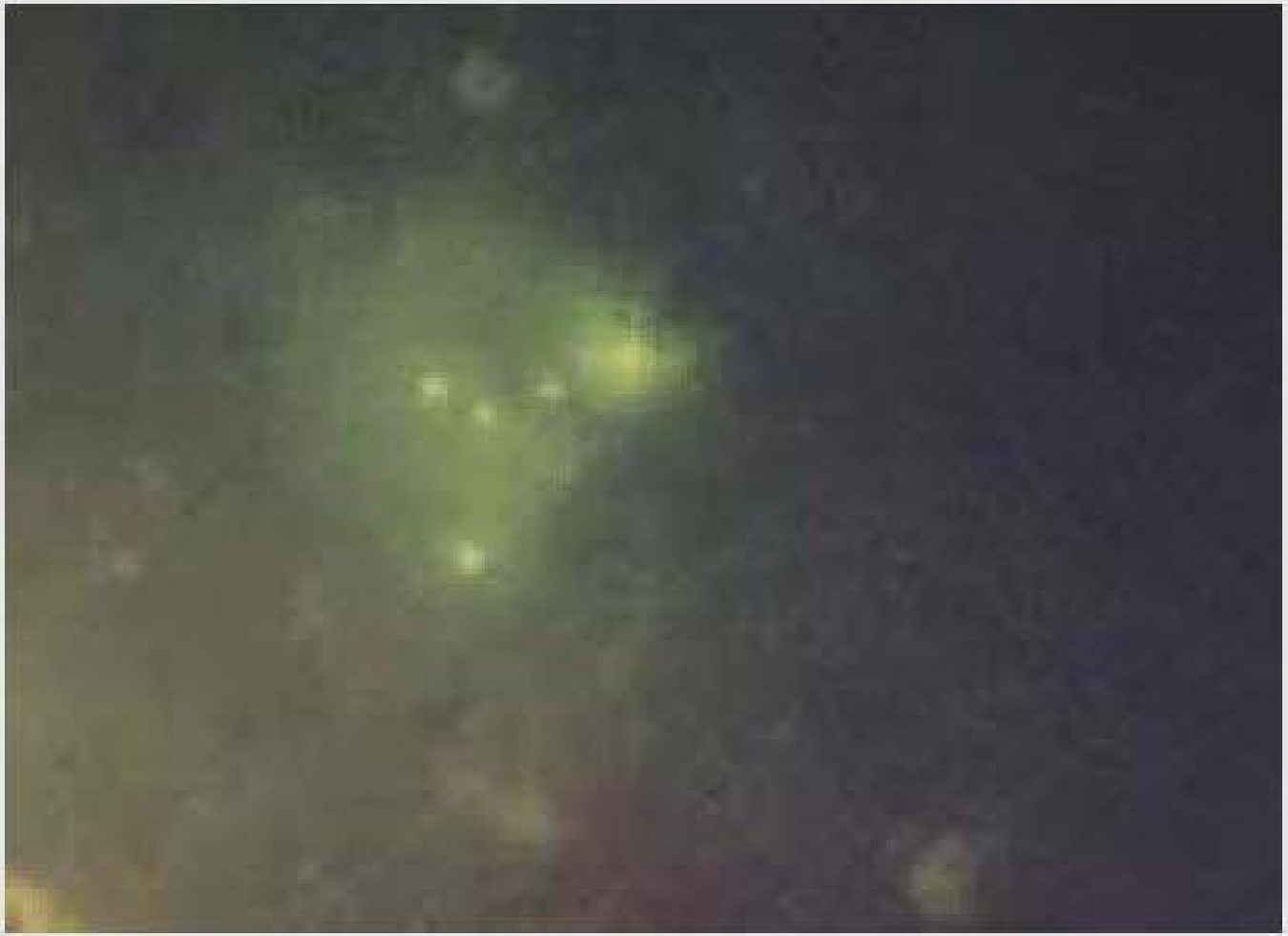
Borrelia burgdorferi **Flagellin DNA , In situ hybridization, Large Plaque**
1000x original magnification



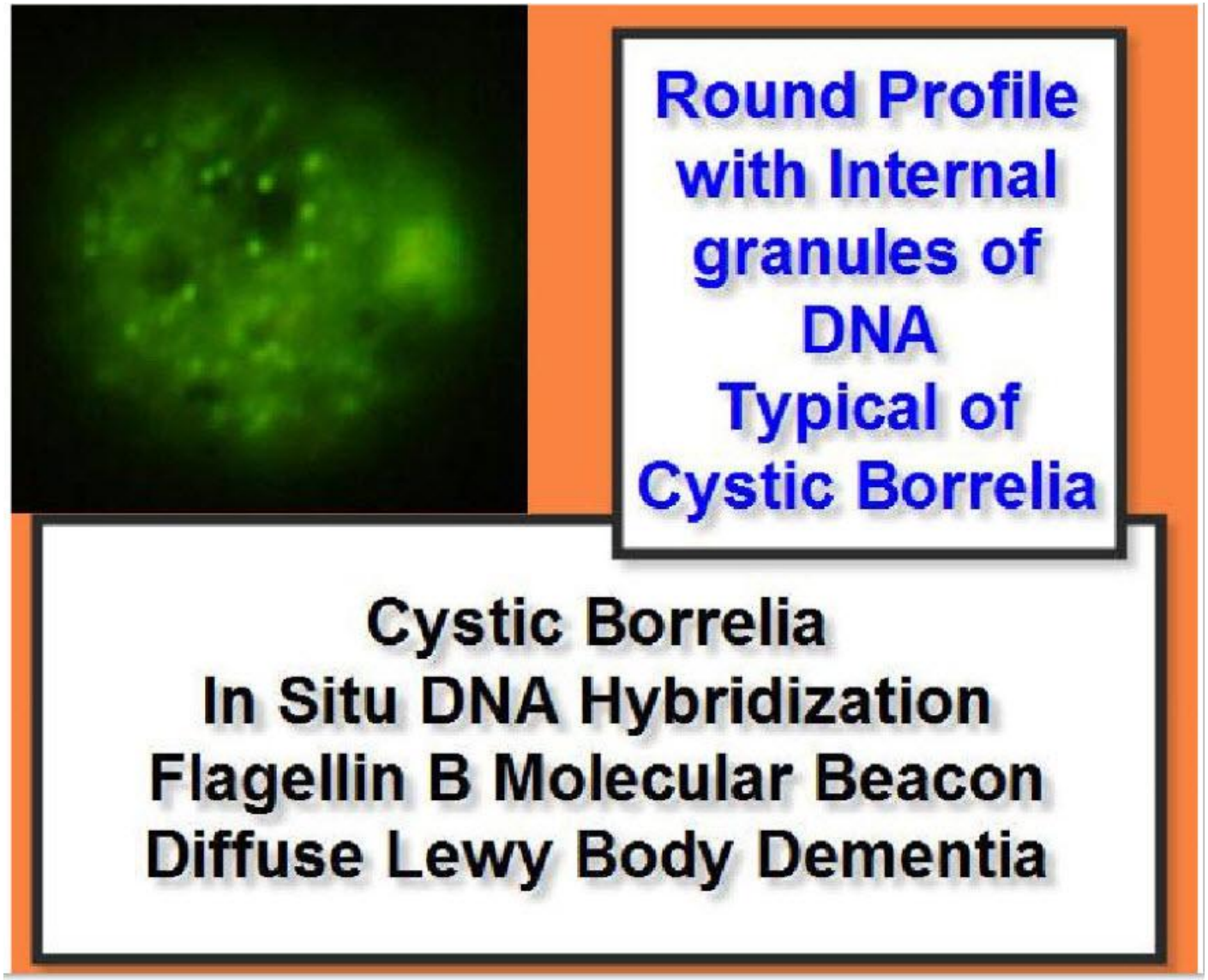
Borrelia burgdorferi **flagellin DNA in situ DNA hybridization, Alzheimer hippocampus 1000x magnification.**

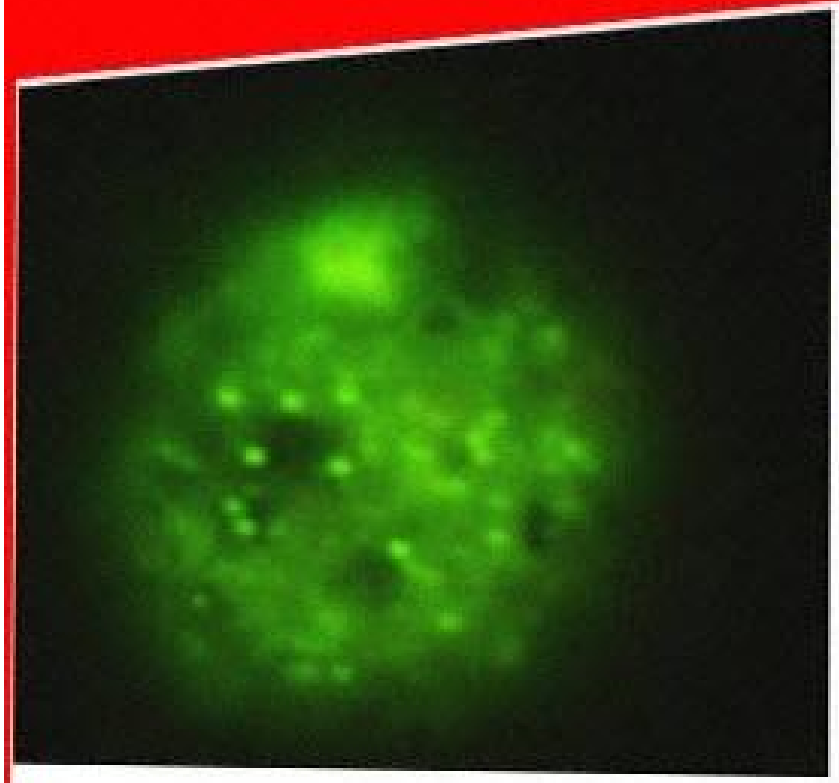
Utility of
Molecular Beacon Dna Probe
To Detect
The
Granulovacuolar Bodies
Which characterize
Alzheimer's Disease in
Autopsy Brain tissues


In Situ DNA hybridization
Alexa Fluor (red) Fluorochrome
Alzheimer
Hippocampus
1000x Oil immersion
Oligonucleotides
BBO 147 (Fla B)
B. burgdorferi



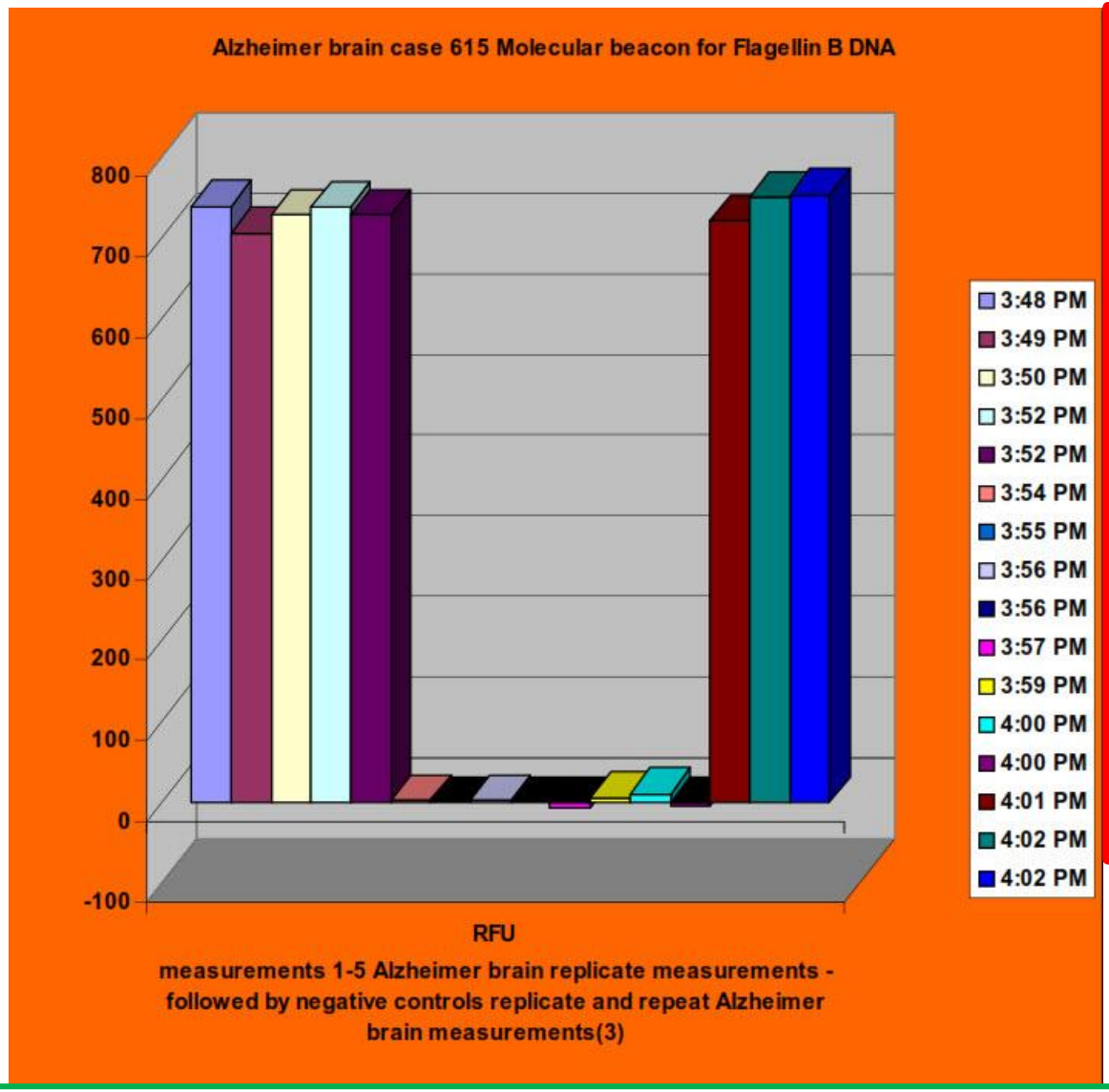
In situ DNA hybridization hippocampus tissue section from Alzheimer's disease showing dot like positive signals within the cytoplasm of nerve cells using flagellin DNA probe for open reading frame BBO 0147 of *Borrelia burgdorferi*, 1000x magnification


Round Profile with Internal granules of DNA Typical of Cystic Borrelia
Cystic Borrelia
In Situ DNA Hybridization
Flagellin B Molecular Beacon
Diffuse Lewy Body Dementia



Cystic Borrelia burgdorferi - Fresh Autopsy Brain imprint-
Stained with Molecular Beacon DNA probe-
specific for OSP BBO147 - Direct Hybridization

UTILITY OF MOLECULAR BEACON DNA PROBE TO DETECT THE DNA OF BORRELIA BURGDORFERI IN TISSUE DIGESTIONS FROM KNOWN ALZHEIMER'S DISEASE BRAIN TISSUES



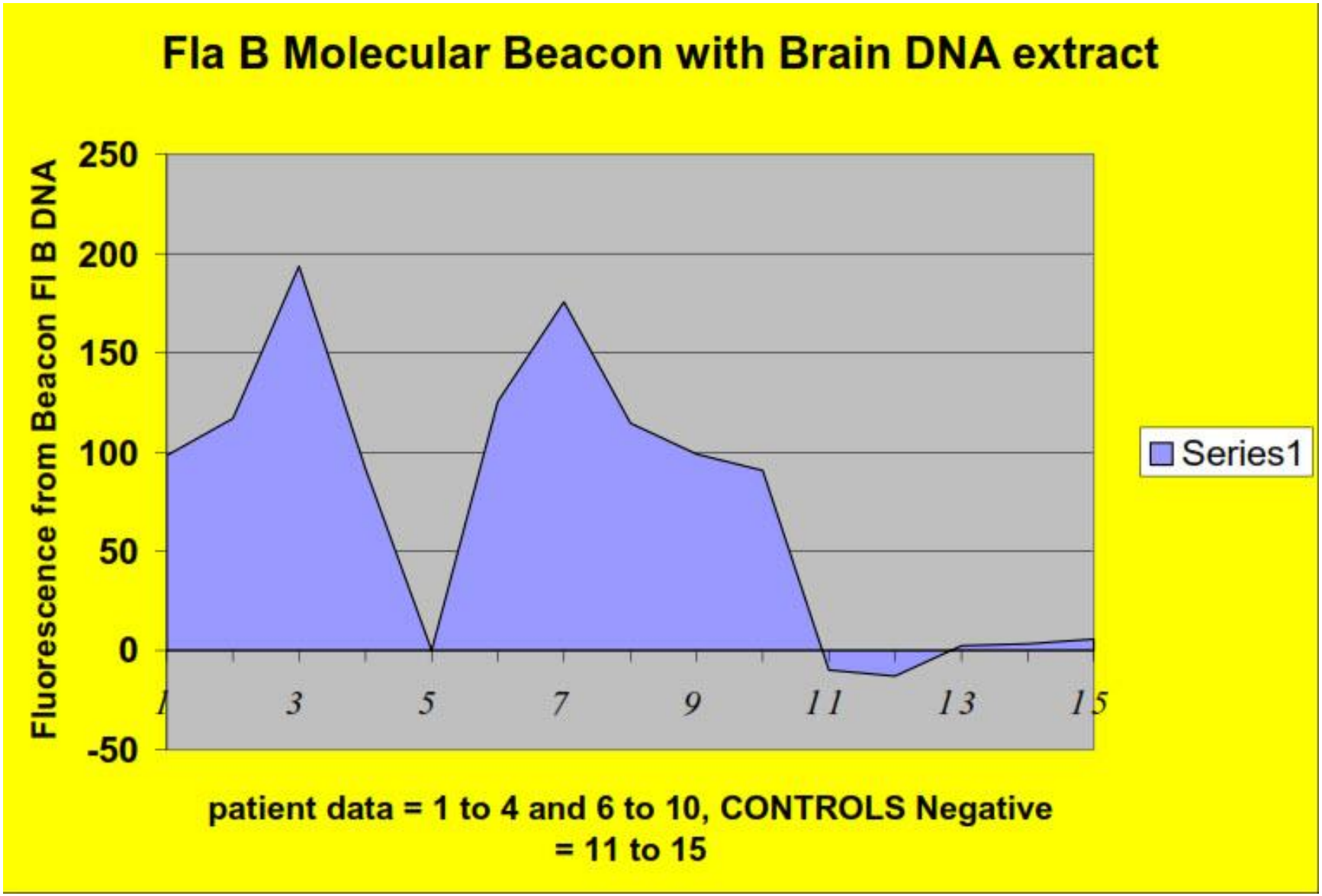
Fluorescence Signal Intensity From Brain tissue Digestions

Molecular Beacon DNA Probe (MacDonald) [BBO 147 of Borrelia burgdorferi genome]

Versus

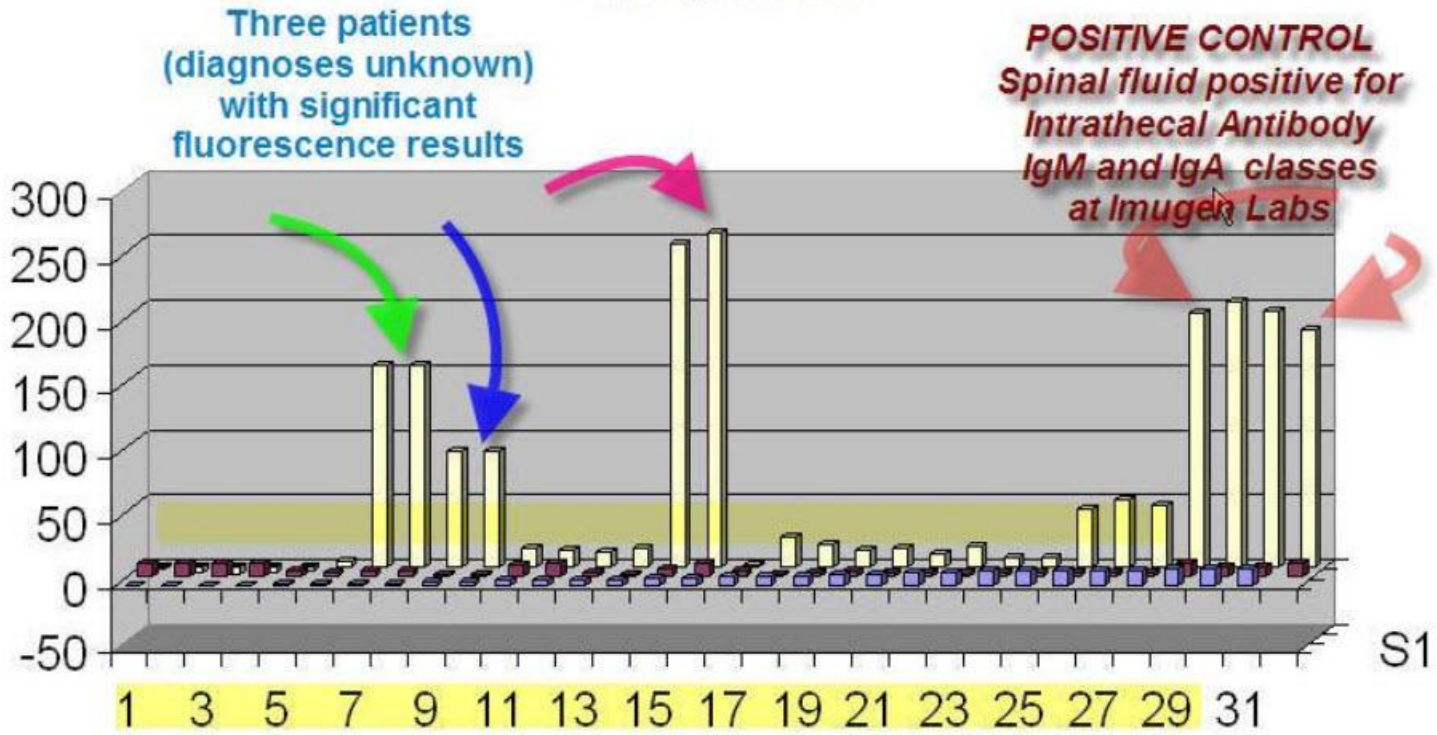
Brain tissue Negative For Alzheimer's [Negative Controls]

Harvard University Brain Bank – Alzheimer's disease – Case 615


Fla B Molecular Beacon with Brain DNA extract
Fluorescence from Beacon Fl B DNA
250
200
150
100
50
0
-50
1
3
5
7
9
11
13
15
Series1
patient data = 1 to 4 and 6 to 10, CONTROLS Negative
= 11 to 15

Utility of Molecular Beacon DNA probe To Detect the DNA Of Borrelia burgdorferi In Spinal fluid specimens

Molecular Beacon for Detection of DNA of Borrelia burgdorferi in Human spinal fluid specimens



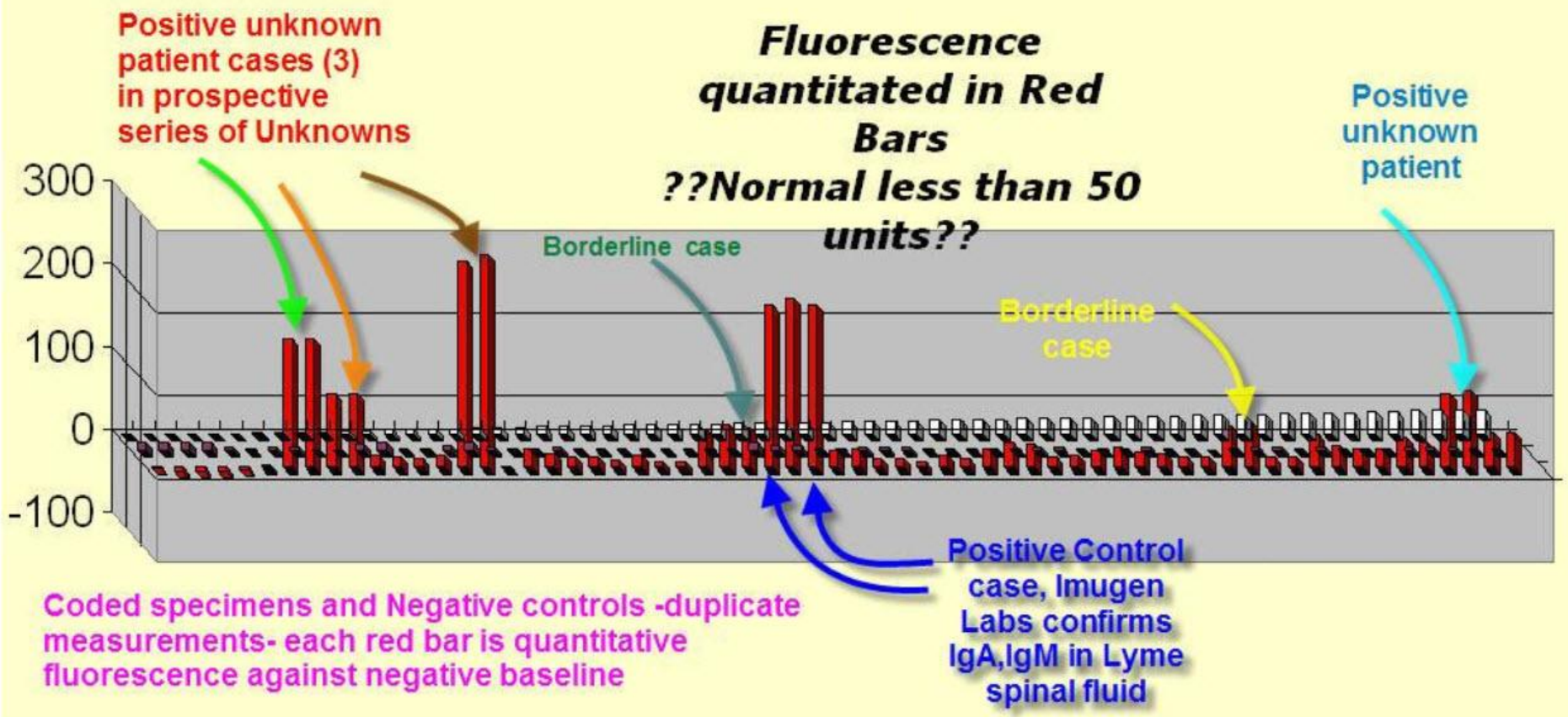
spinal fluid specimens (Coded Unknowns) from Year 2005

Each measurement of fluorescence done in duplicate

Bar graph of results of Fluorescence units

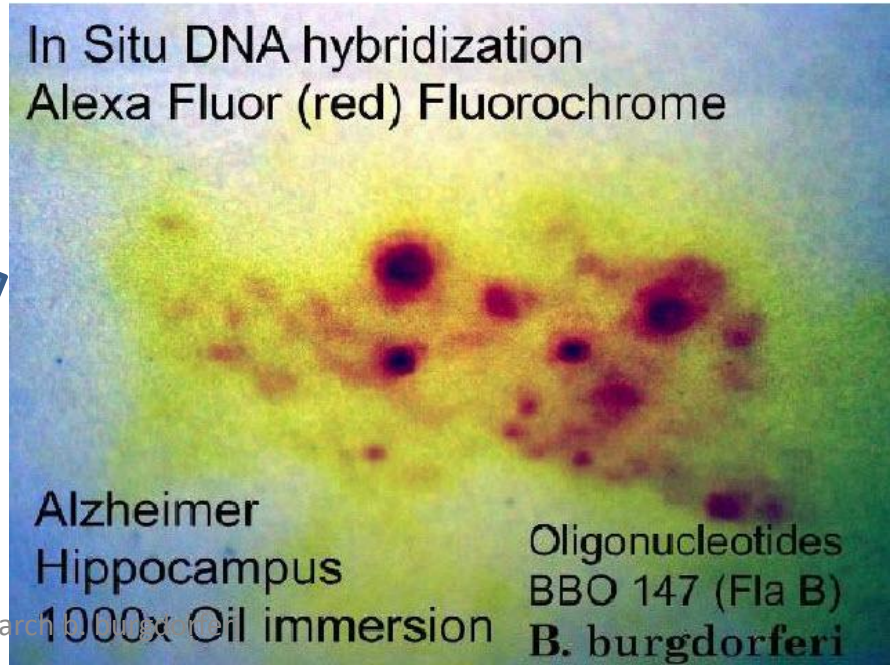
Results less that 50 units may be in so called Normal Range

Molecular Beacon for Detection of DNA of Borrelia burgdorferi in Human Spinal fliid

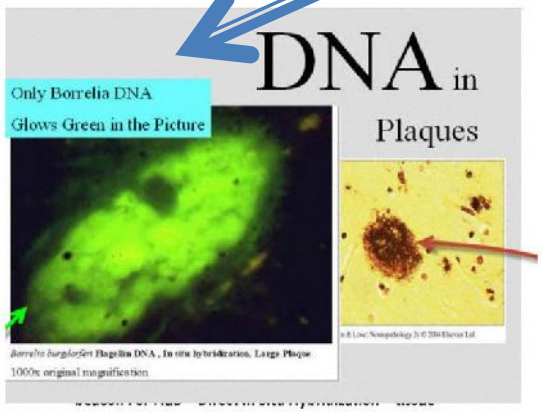


SO YOU CAN SEE FROM THE ALZHEIMER IMAGES

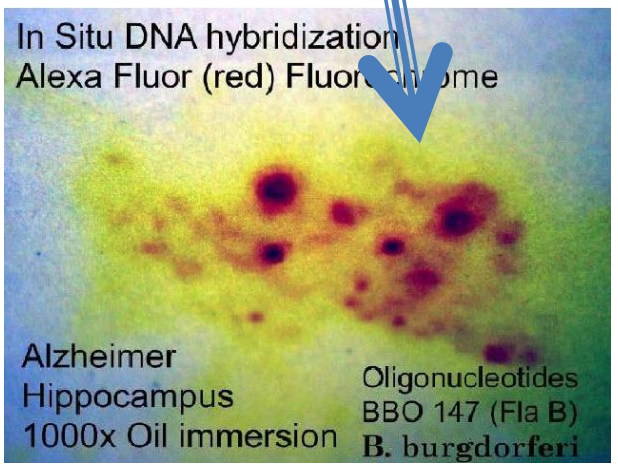
"Dots" mark areas of Tissue Injury



Molecular Beacons
Are "sent into the tissue"
To locate each and every
Area where the
Target Borrelia burgdorferi dna
Is Localized



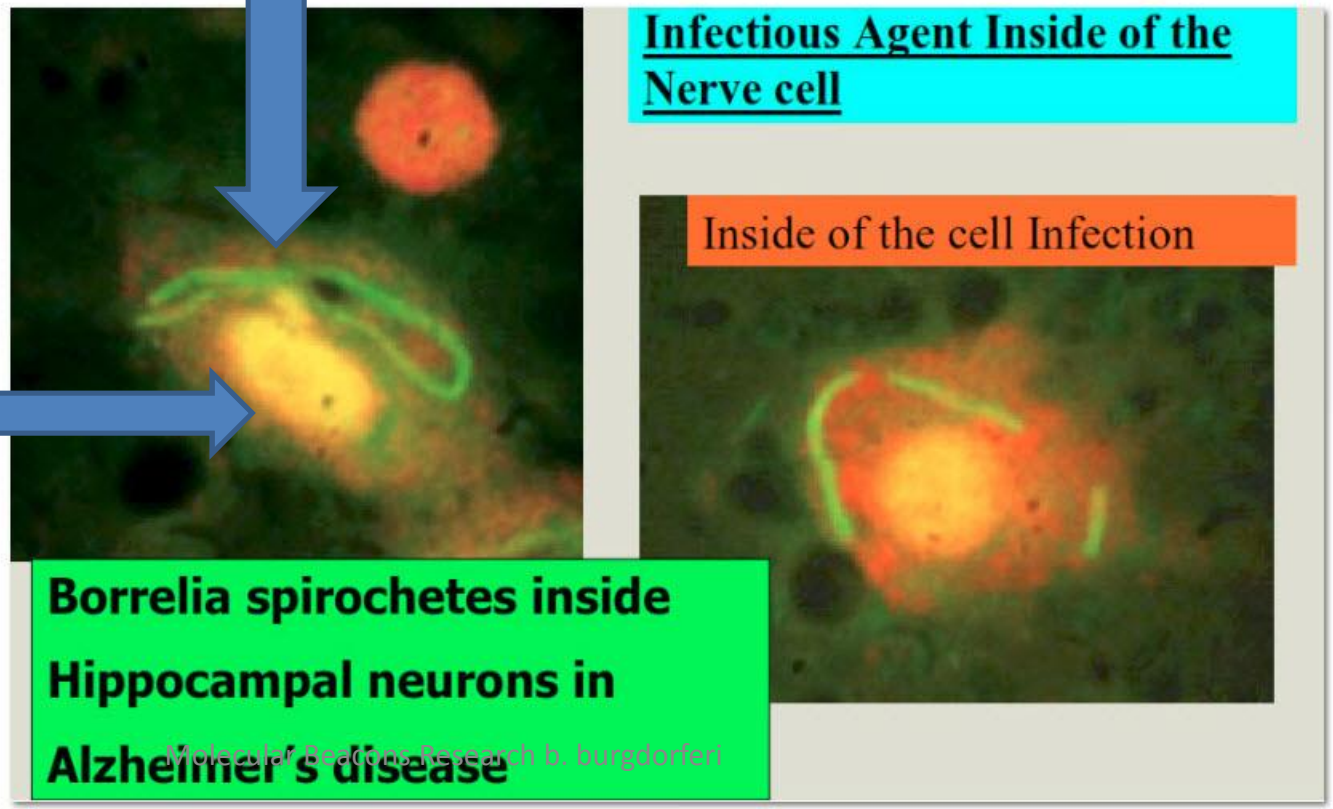
slide///Image Right- Alzheimer plaque stained wit
Bielschowsky Silver Stain (Brown arrow)



Utility of Molecular Beacons for Detection of Specific Borrelia burgdorferi DNA

Borrelia DNA Is Always in the *Cytoplasm* Of the NERVE Cell [GREEN]

Human DNA Is Always In the NUCLEUS Of the NERVE Cell [Yellow]

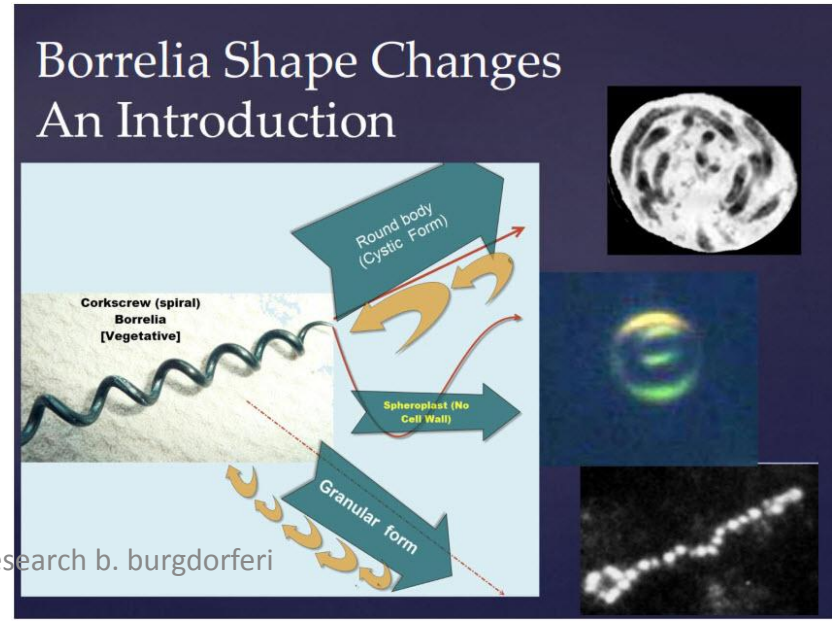


Ok..
So the textbooks
Tell us That:

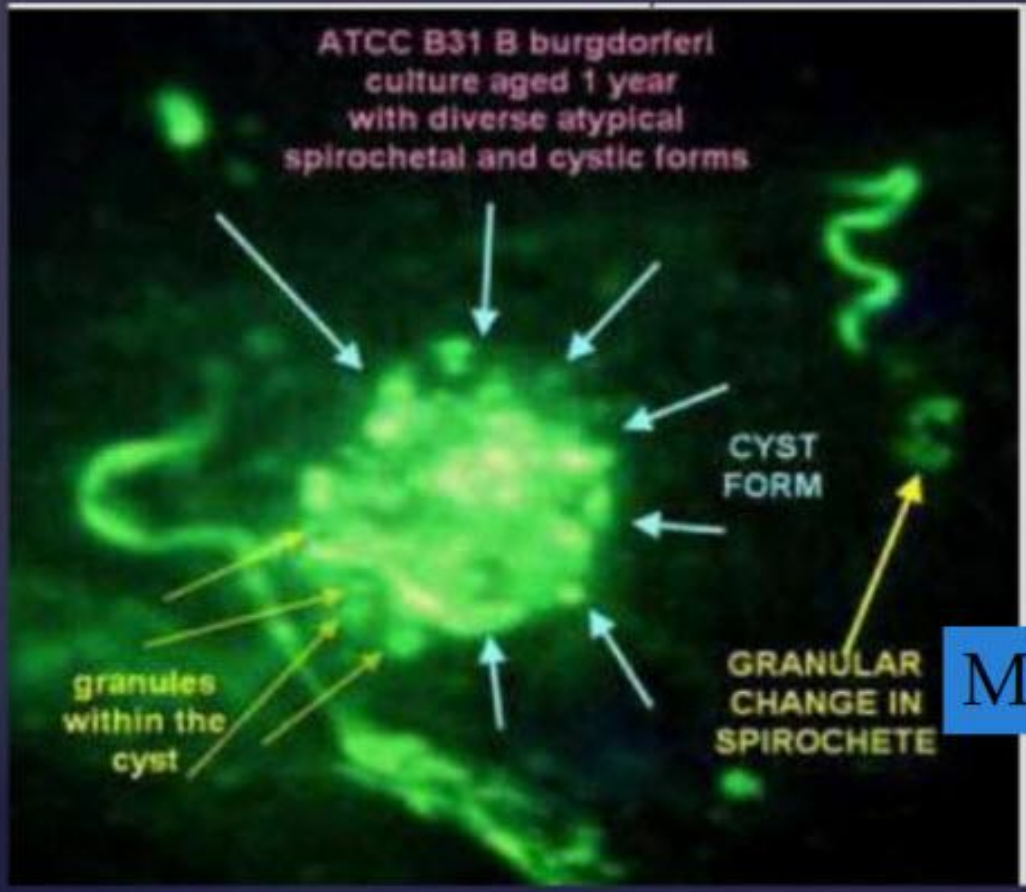
Spirochetes
Are supposed to
Corkscrew in Shape

So

How do We
Reconcile that
In Human disease
The borrelia burgdorferi
Spirochetes
Wind up as
Non-Spiral forms
Like
Dots (granular forms)
And Round Bodies (Cystic forms)???



Consequences of Granular elements in Borrelia Cysts



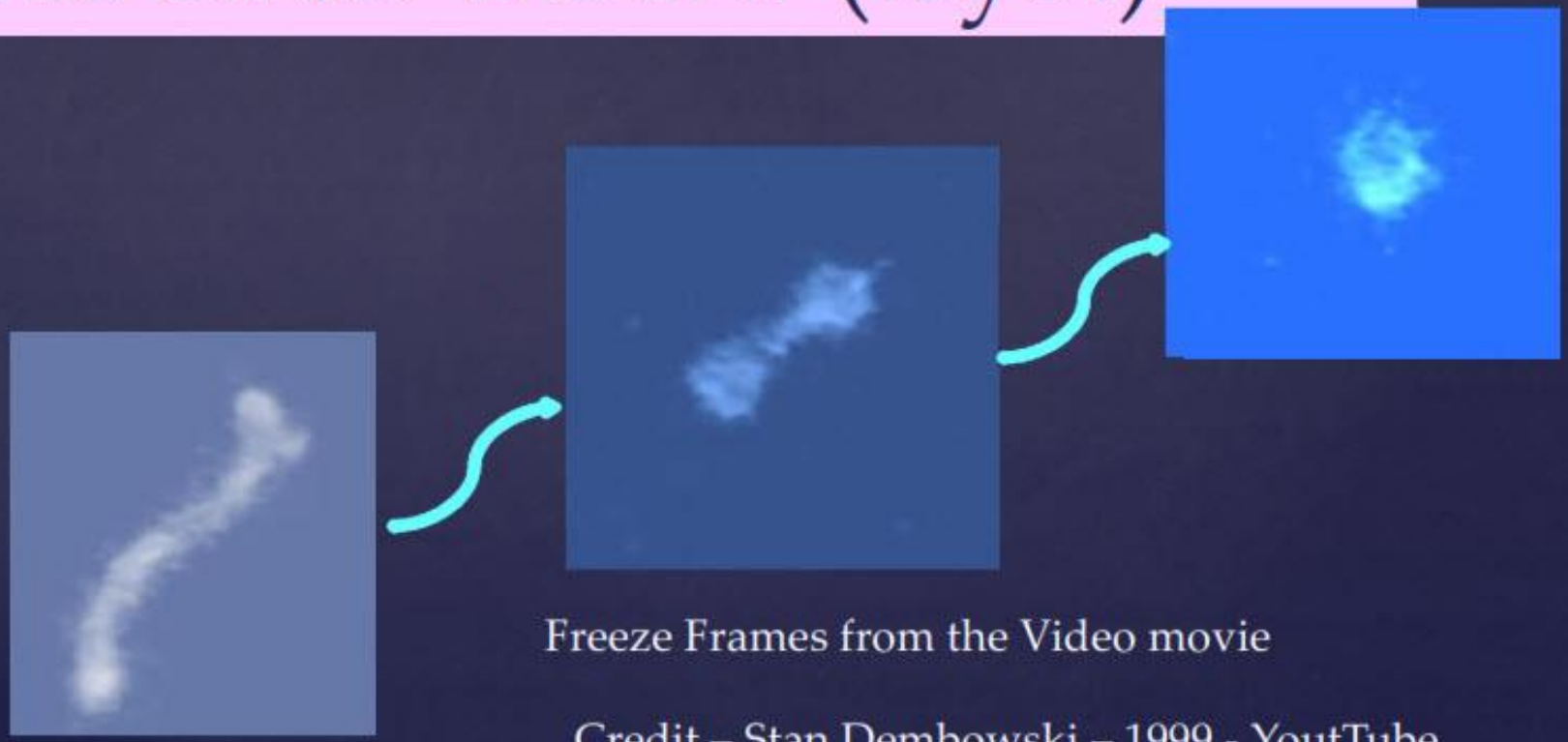
Cystic Borrelia burgodorferi with Abundant Granular elements

Photo Credit: Alan B. MacDonald MD 1988

Multi –GRANULAR Cyst

Spirochetes are expected to be Spiral (corkscrew) in shape according to Textbook teaching

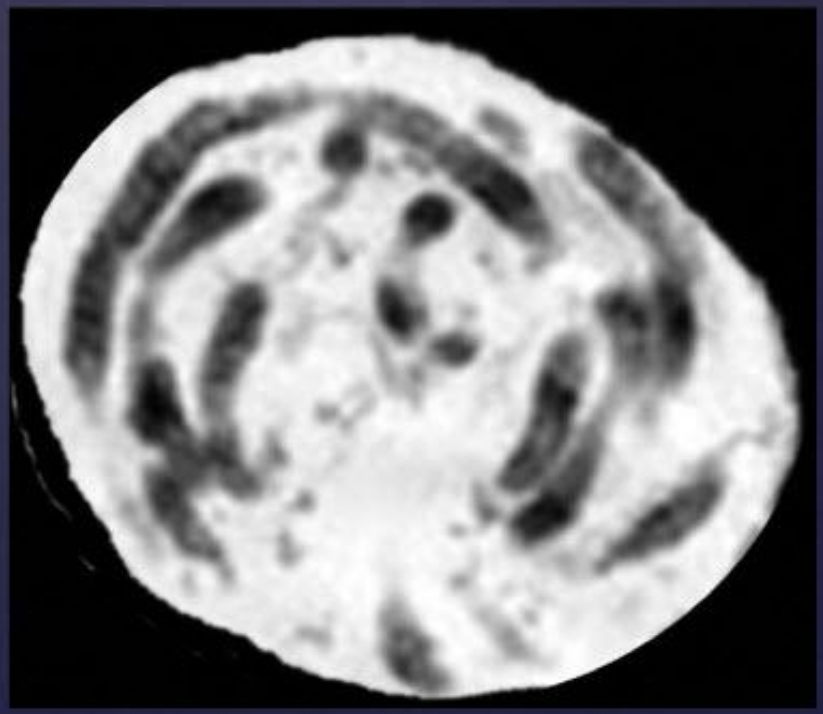
SPIRAL TRANSFORMS TO ROUND BODY (Cyst)

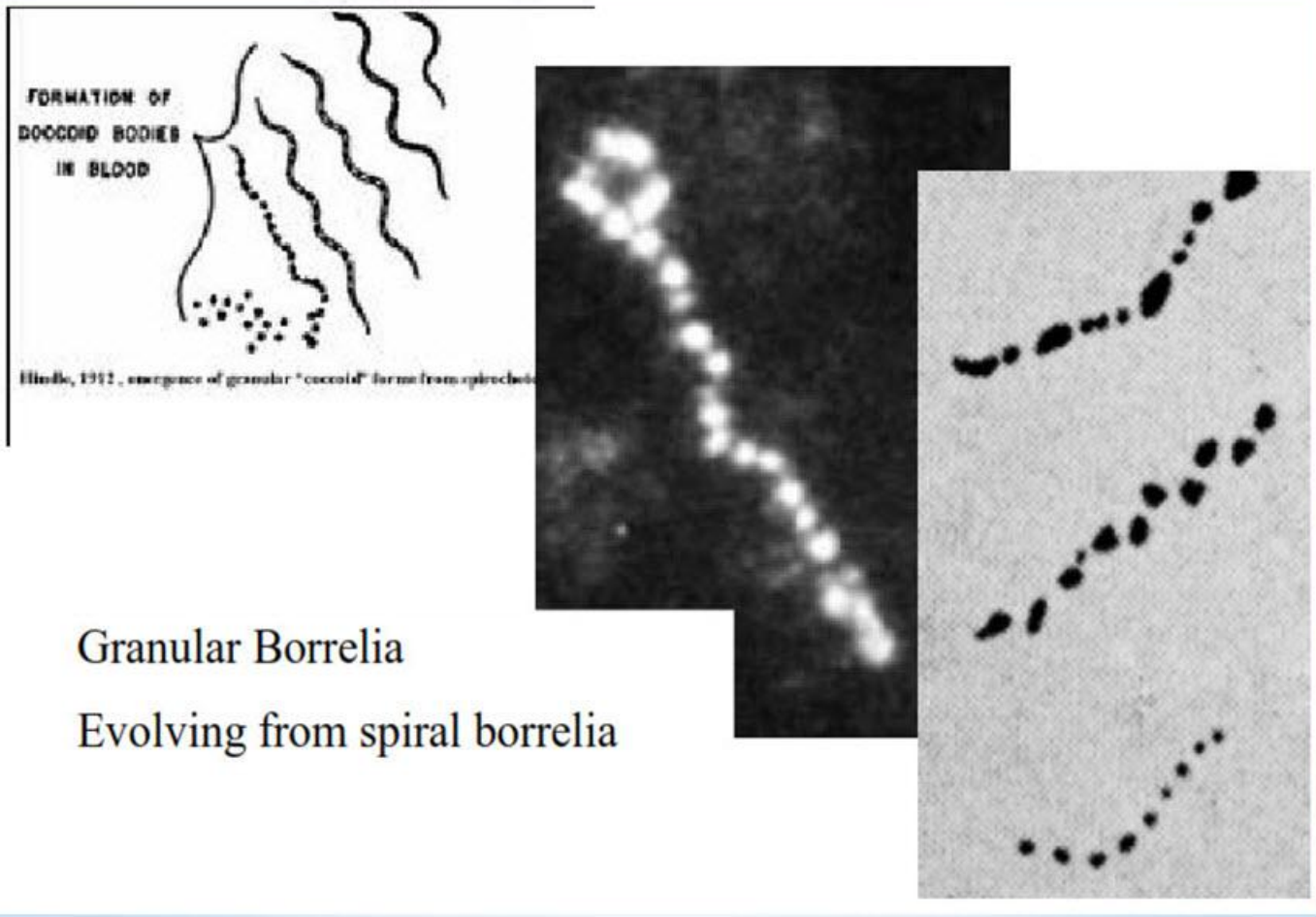


Freeze Frames from the Video movie

Credit – Stan Dembowski – 1999 - YoutTube

Completion of the Transformation All Round (Cystic) All the time

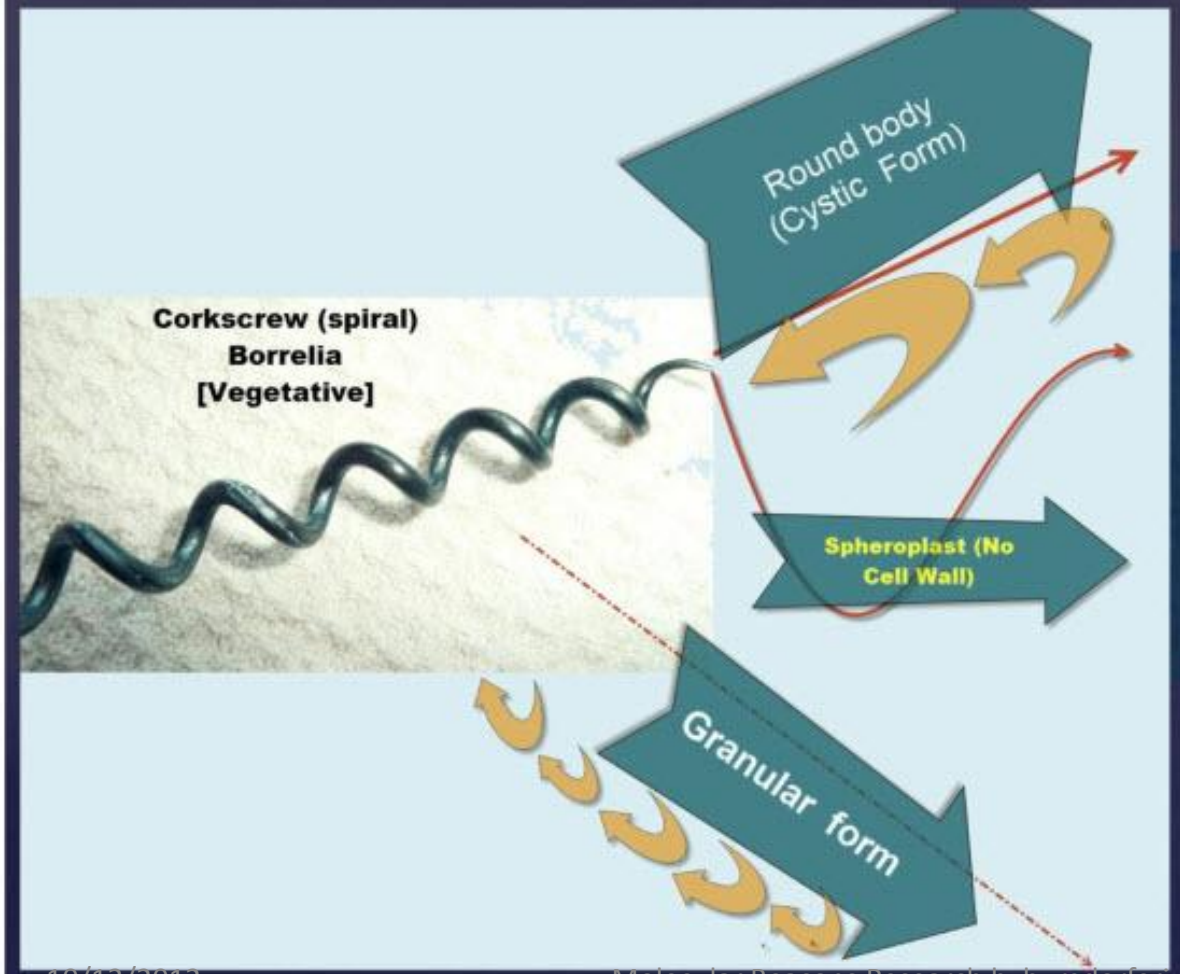


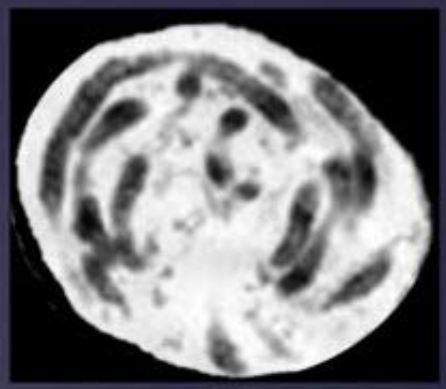


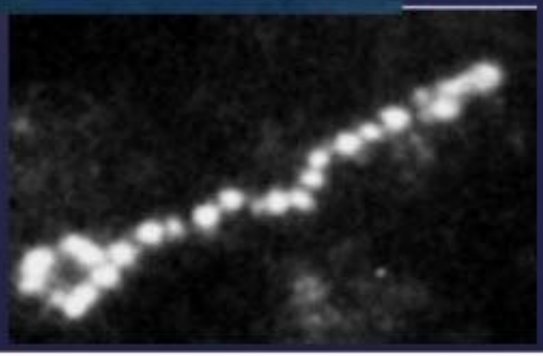
Granular Borrelia

Evolving from spiral borrelia

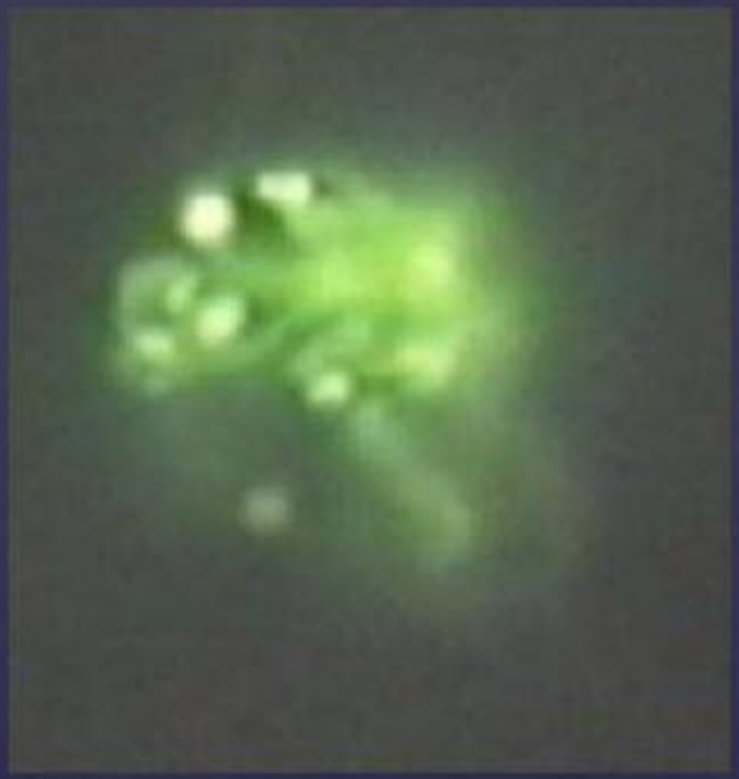
Borrelia Shape Changes An Introduction







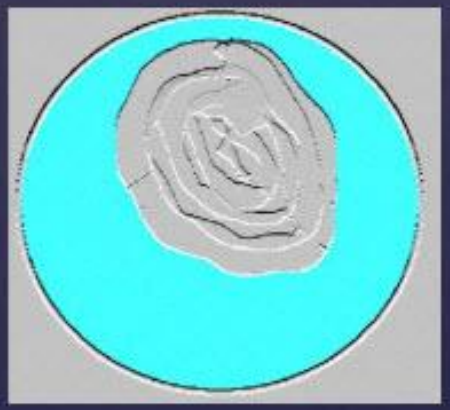
Borrelia Cysts Containing Liberated Flagellin Units



Alzheimer's Disease – Cystic Borrelia – Reactive with Murine Monoclonal Antibody H9724 (a Gift from Alan G. Barbour,MD

Photo credit : Alan B. MacDonald MD. Photograph date 1987

Round Bodies are established as part of the repertoire of spirochetes



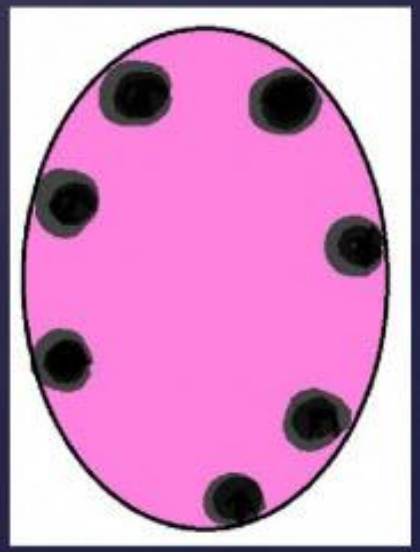
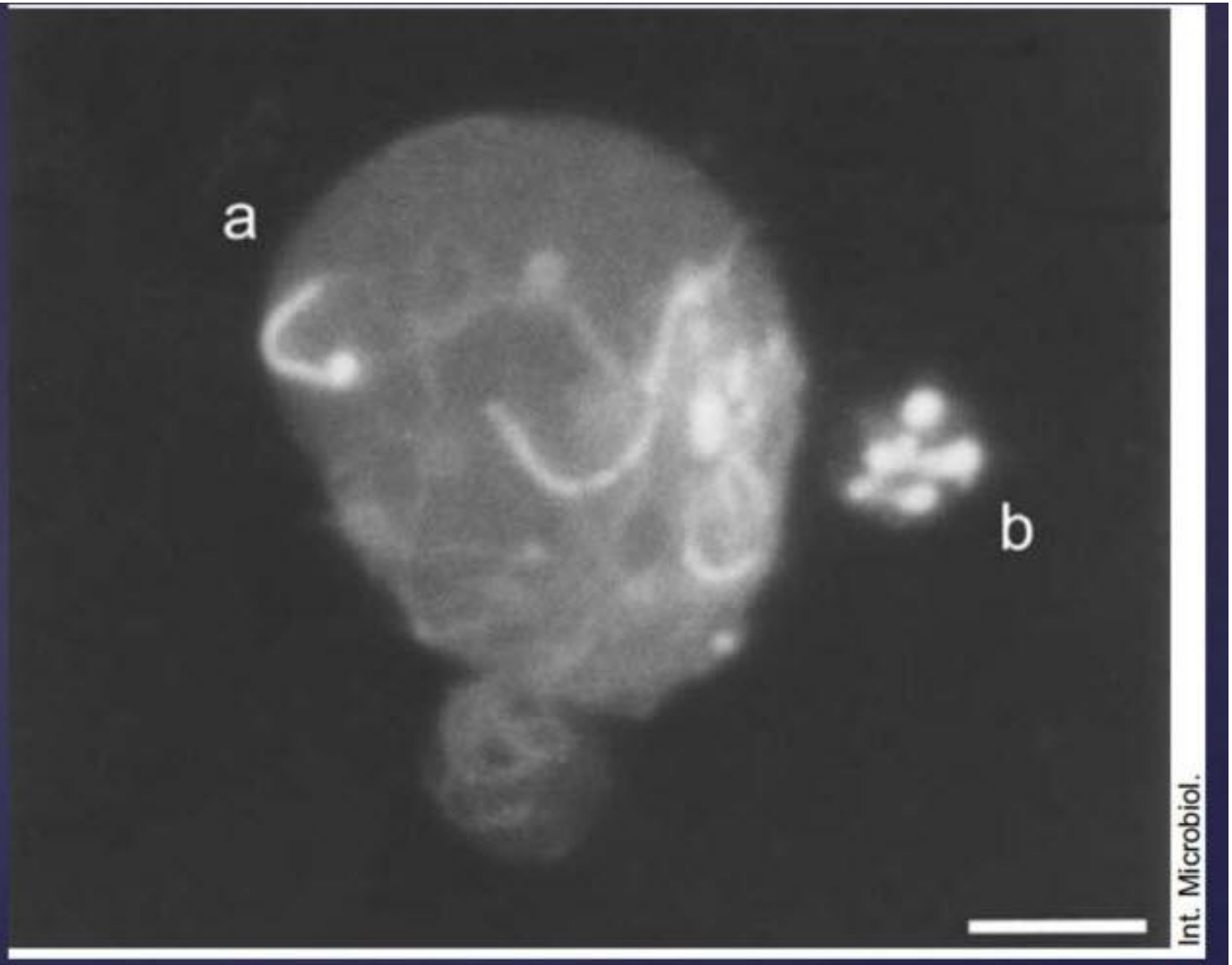


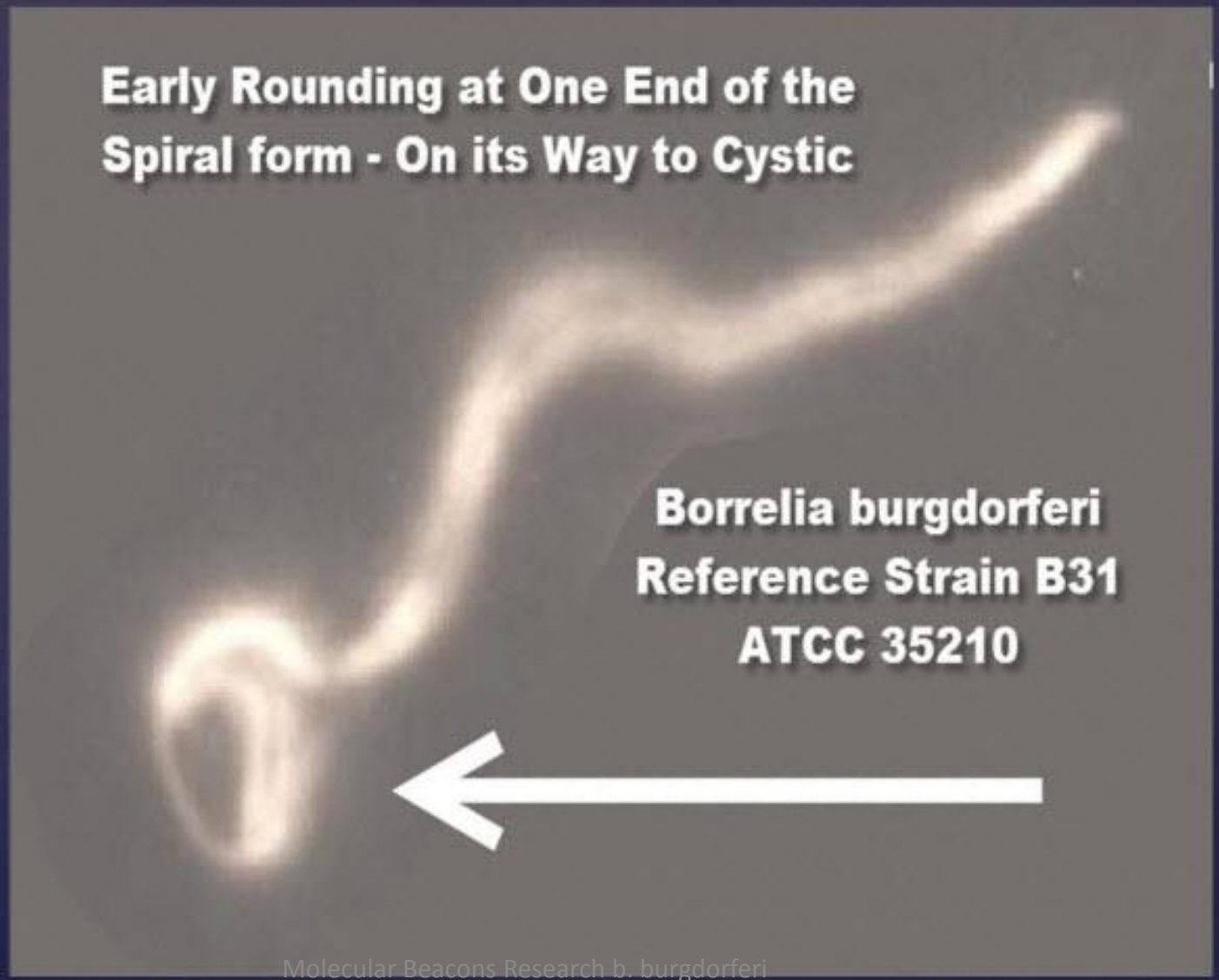
Photo Credit:

Oystein Brorson MD

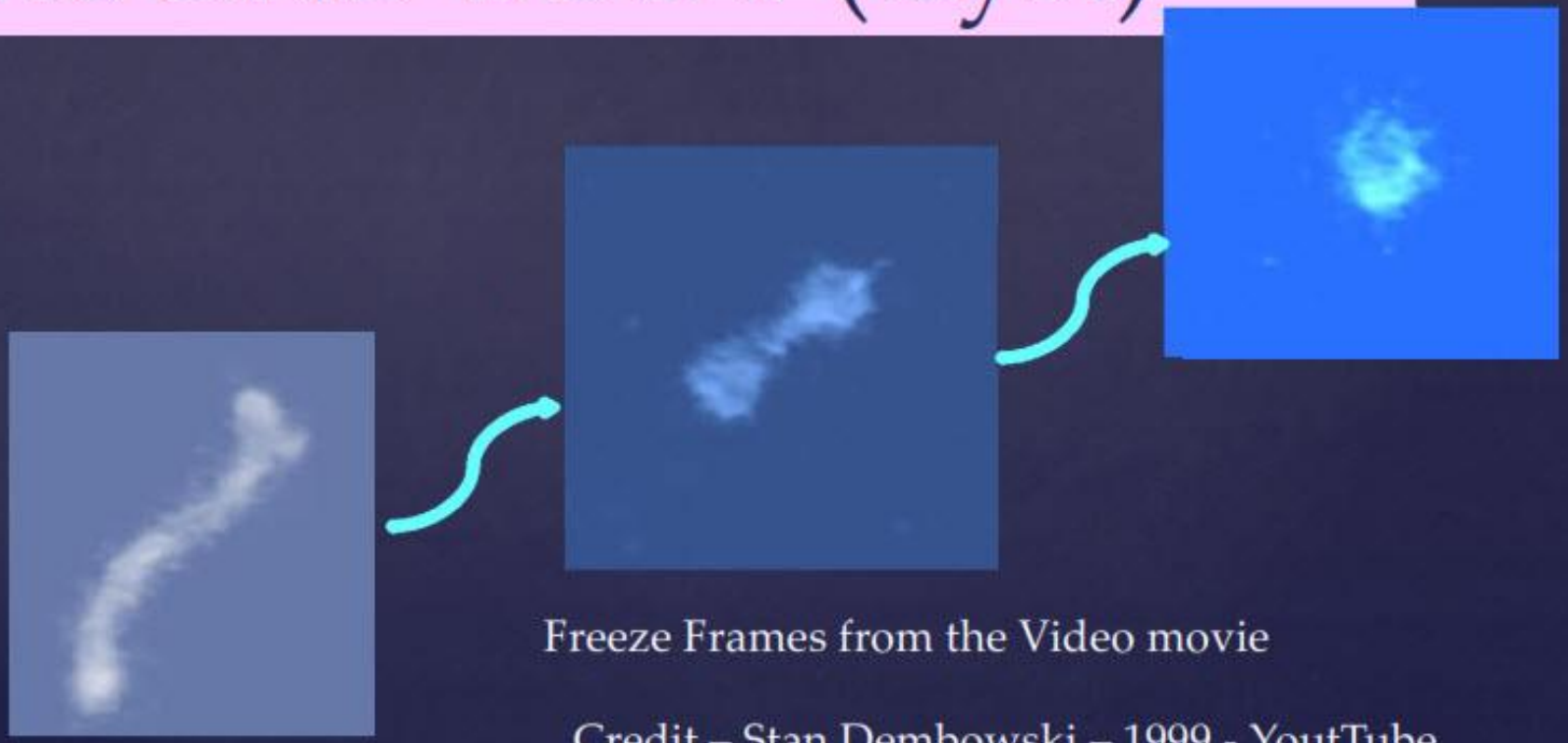


Cystic Borrelia burgdorferi (2) with
Internal content of Spirochetal forms [Large "a"}
and Smaller Cystic form with rounded granular bodies.

The Early Shape Change……



SPIRAL TRANSFORMS TO ROUND BODY (Cyst)

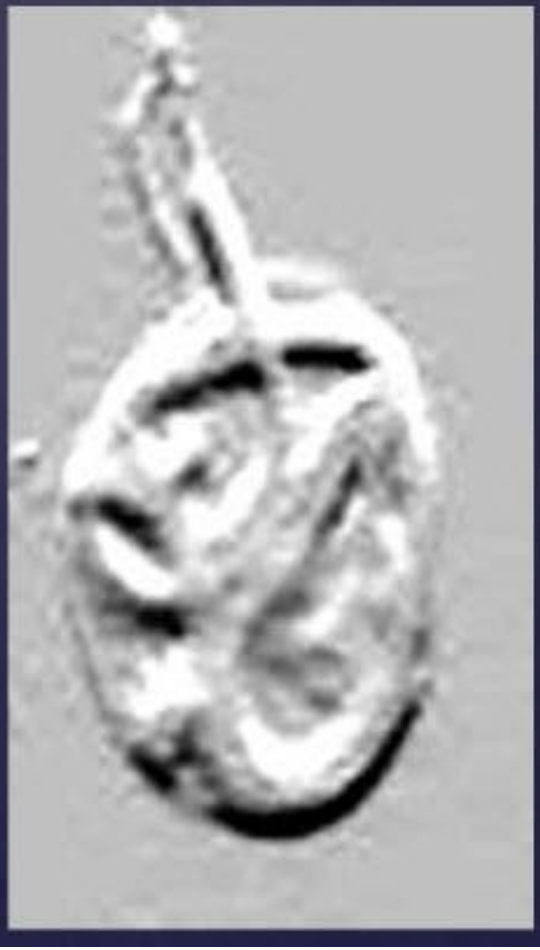


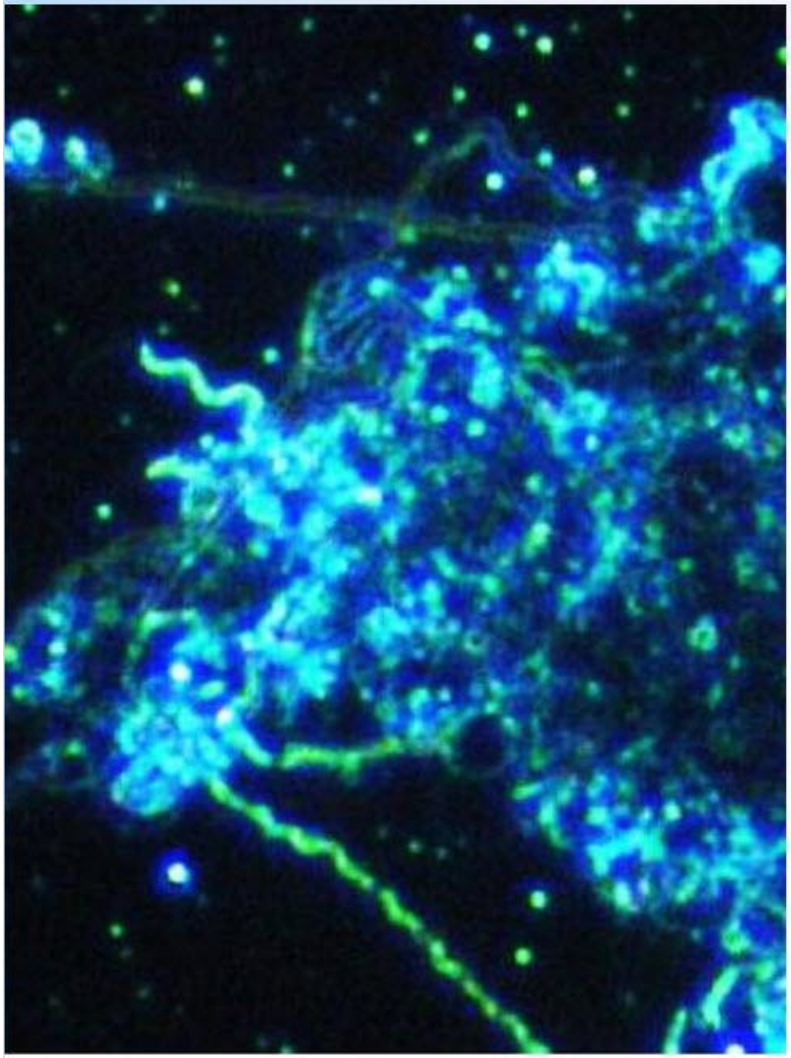
Freeze Frames from the Video movie

Credit – Stan Dembowski – 1999 - YoutTube

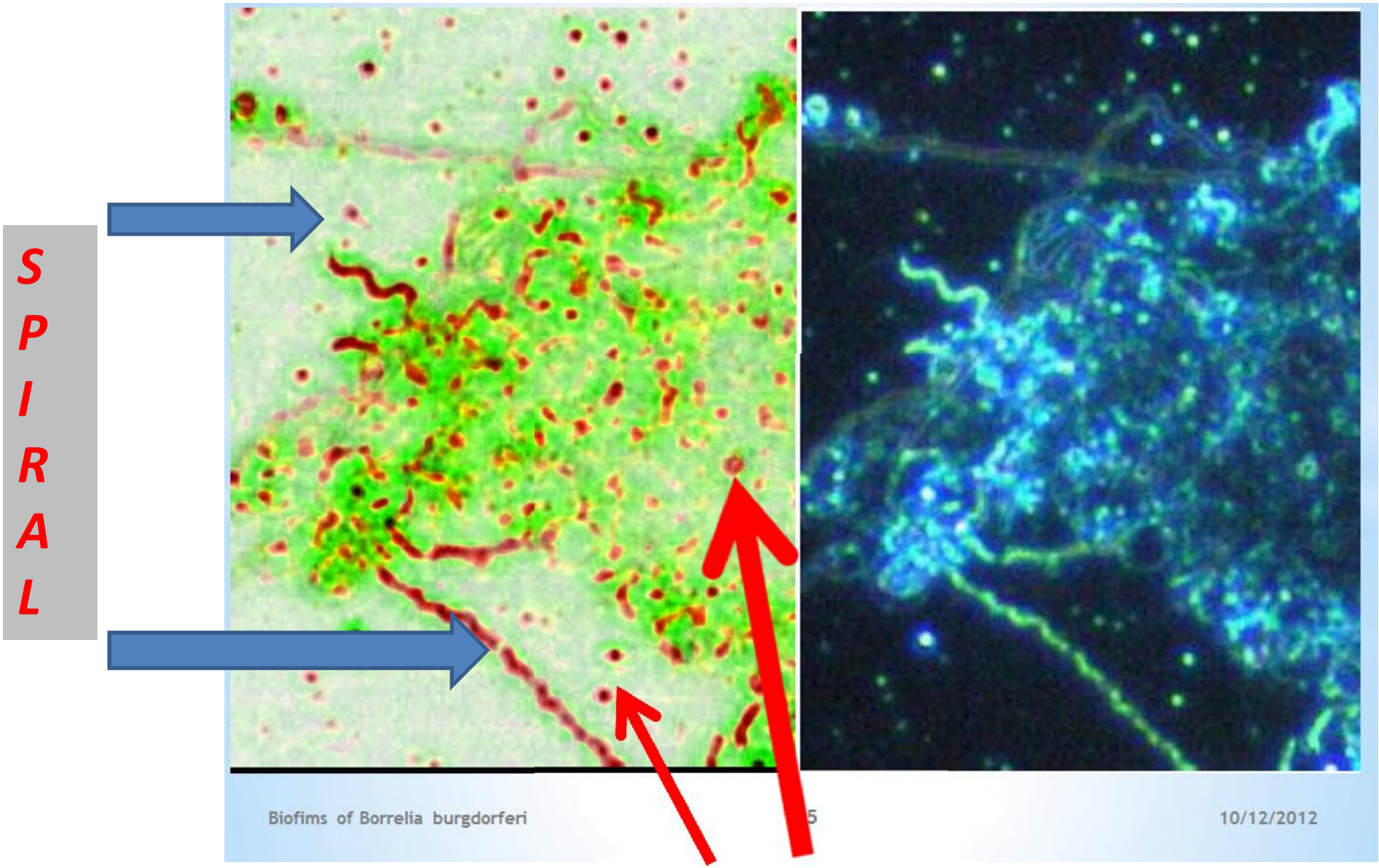
Alban et al Rhode Island

Cystic Borrelia Burgdorferi with protruding segments – **" tails"**

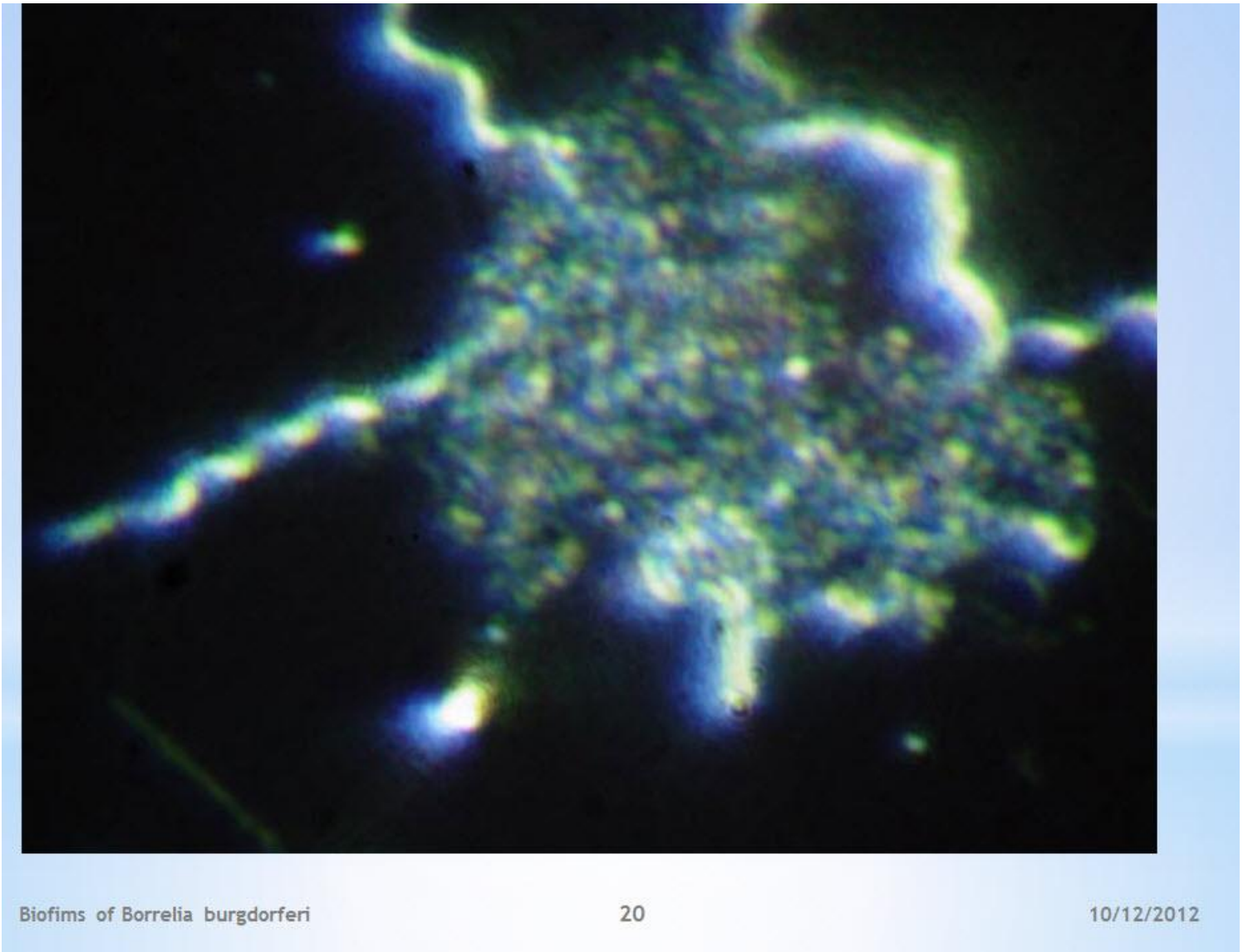




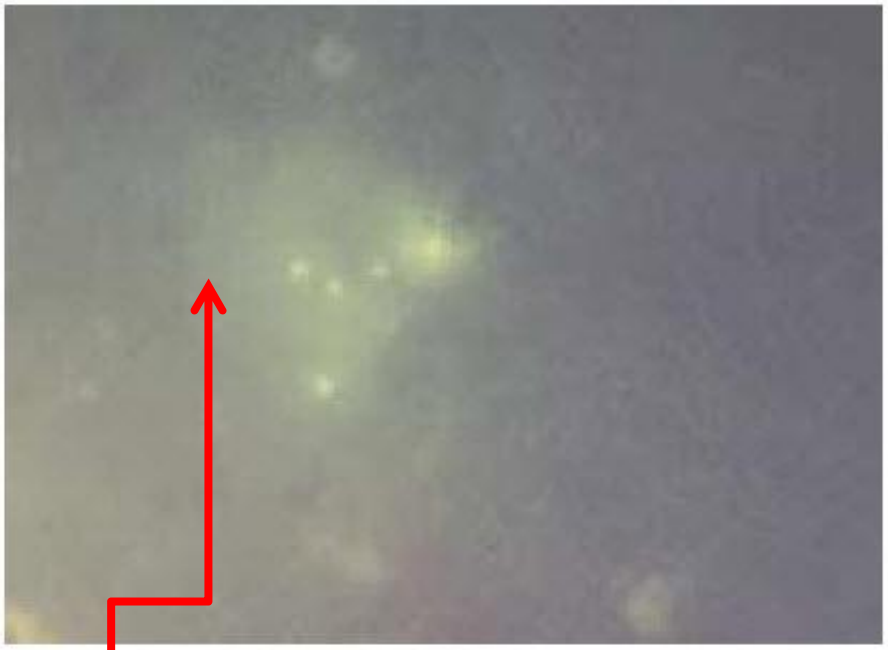
Biofilm Community Of Borrelia Burgdorferi Strain B31 Atcc 35210



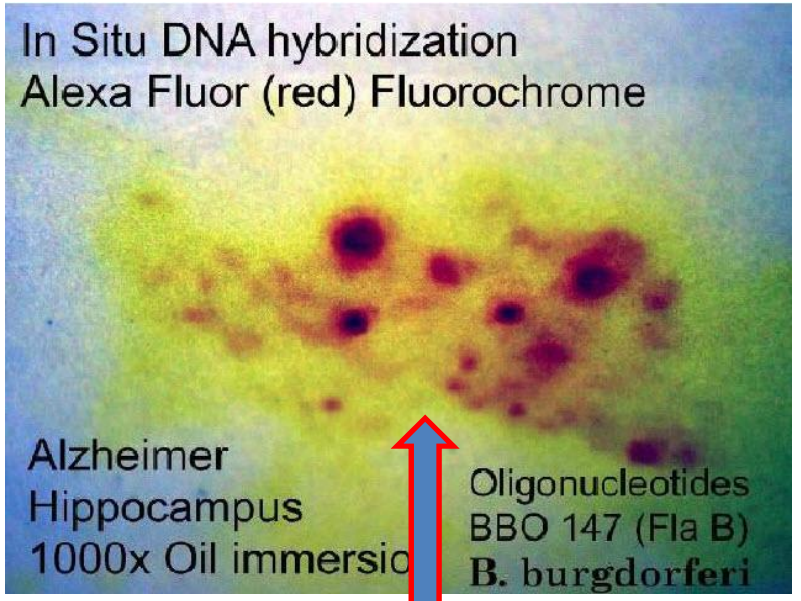
GRANULAR FORMS OF BORRELIA IN THE BIOFILM



GRANULAR FORM DOMINANT BIOFILM OF BORRELIA BURGDORFERI



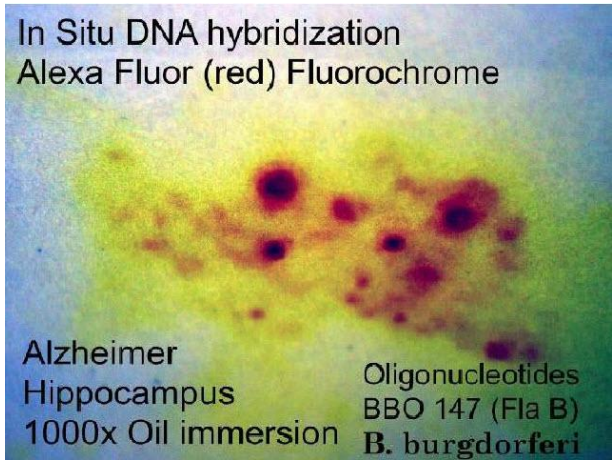
In situ DNA hybridization hippocampus tissue section from Alzheimer's disease showing dot like positive signals within the cytoplasm of nerve cells using flagellin DNA probe for open reading frame BBO 0147 of *Borrelia burgdorferi*, 1000x magnification



GRANULAR DEGENERATION IN ALZHEIMERS – ACTUALLY GRANULAR BORRELIA BY MOLECULAR BEACON ANALYSIS

RESEARCH Goals=

1. Use molecular beacons to prove that granular "degeneration" Is actually the granular form Of the borrelia spirochete Deposited inside of Alzheimer brain Neuron cells



Research goals=

2. To use molecular beacons In the analysis of Alzheimer's Autopsy brain tissue to prove That borrelia Burgdorferi DNA Is present in Digests of brain Tissue in high levels

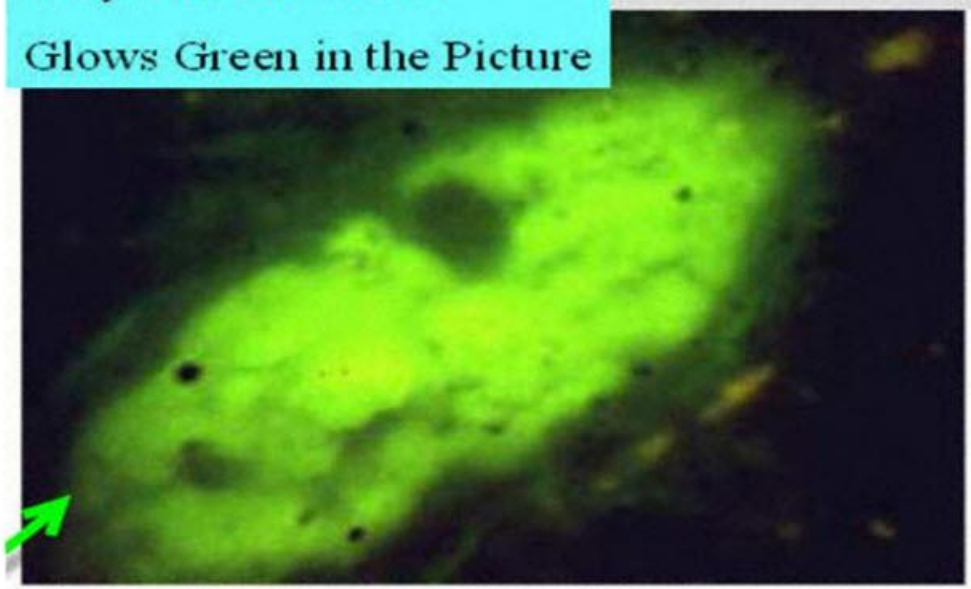
Research Goals=
3. To use Molecular beacons To image and photograph Borrelia burgdorferi Spirochetes INSIDE of Brain Neurons in Alzheimer's disease

Research Goals =
4. To Use Molecular beacons To demonstrate that the PLAQUES in Alzheimer's Disease Are composed of Biofilm Aggregates of Shape shifted borrelia burgdorferi: Granular, cystic, spiral, and cell wall Deficient shapes

DNA in Plaques

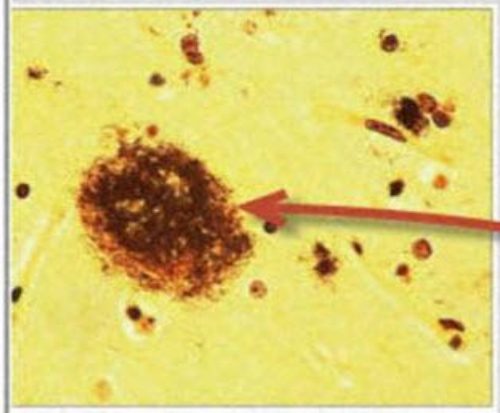
Only Borrelia DNA

Glows Green in the Picture



Borrelia burgdorferi **Flagellin DNA . In situ hybridization, Large Plaque**

1000x original magnification



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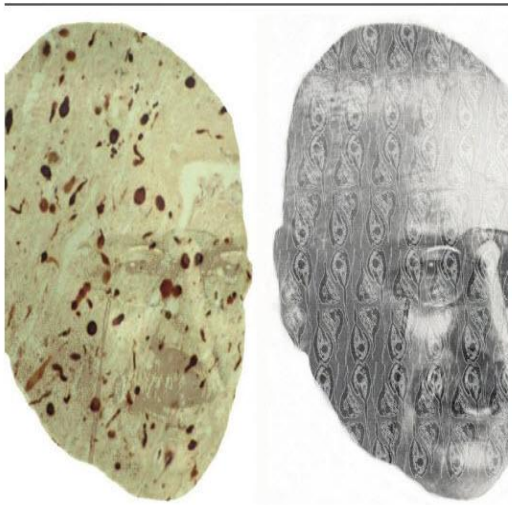
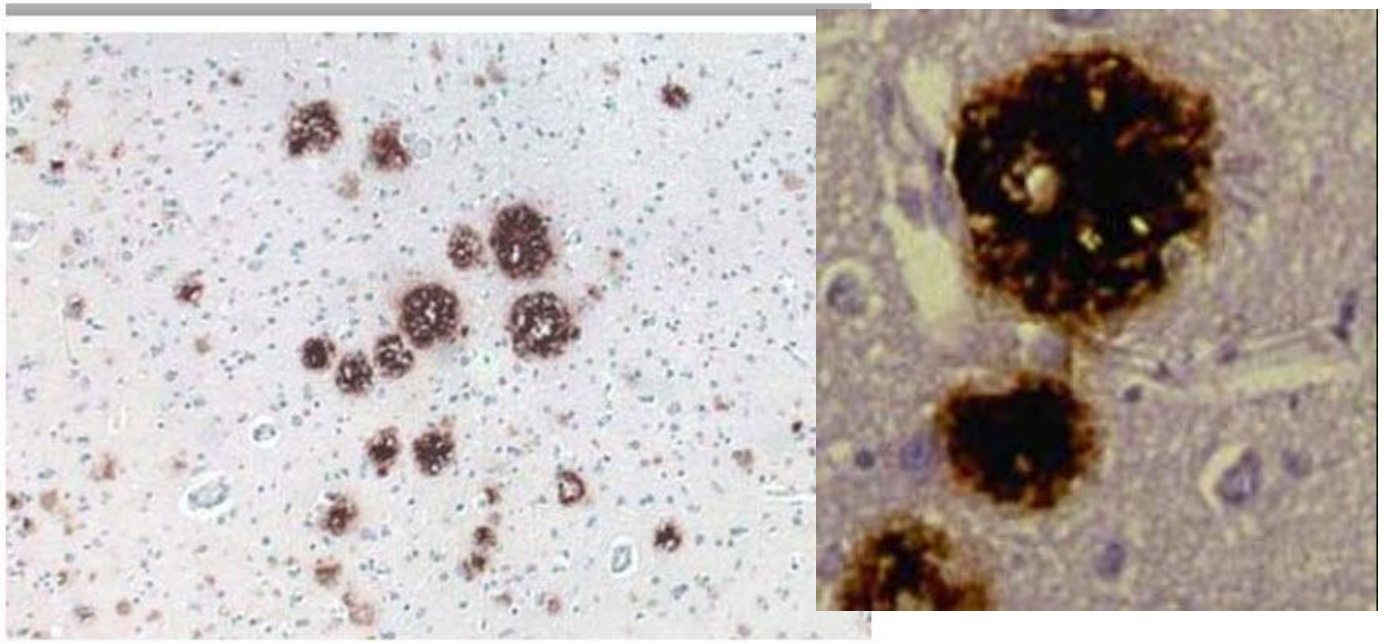
slide////Image Right- Alzheimer plaque stained wit Bielschowsky Silver Stain (Brown arrow)

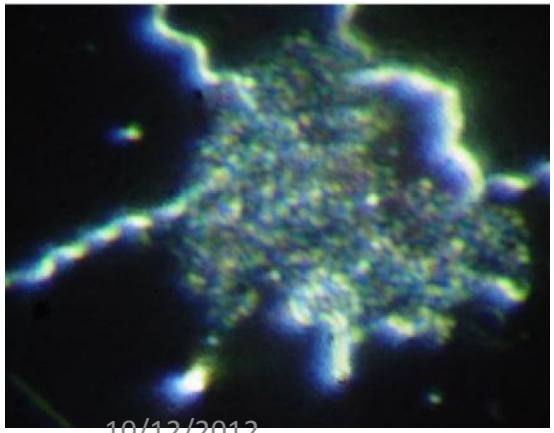


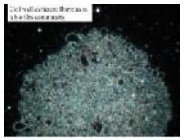
Dr Alois Alzheimer – with Morphing of Alzheimer plaques on his portrait

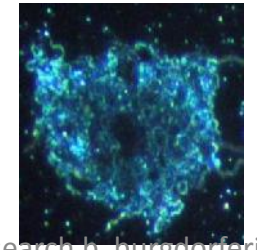


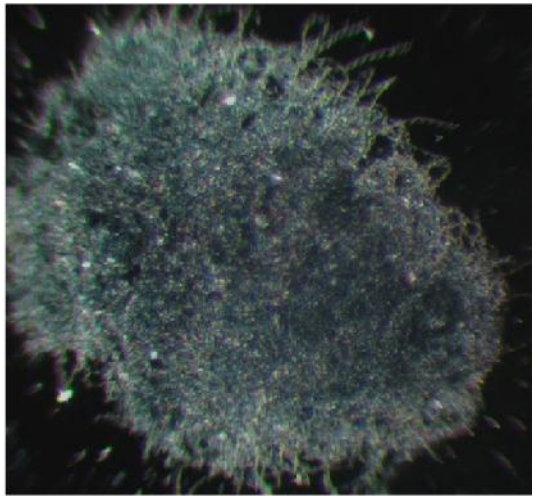
Alzheimer plaques - google

Borrelia Biofilm Units

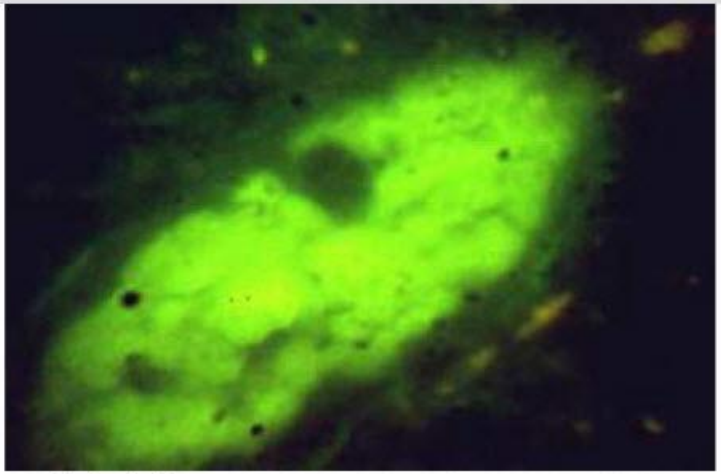




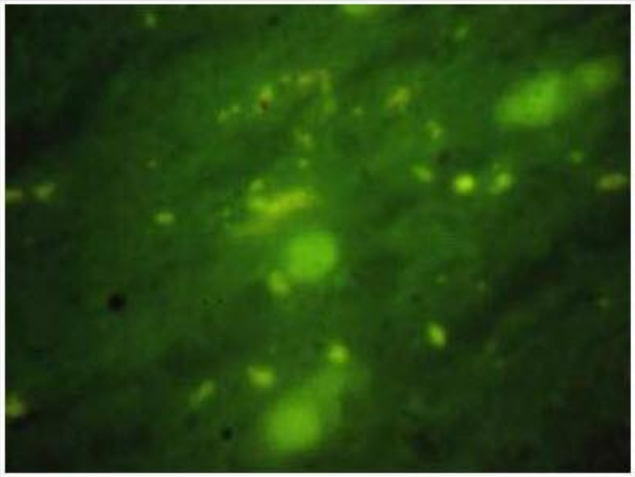




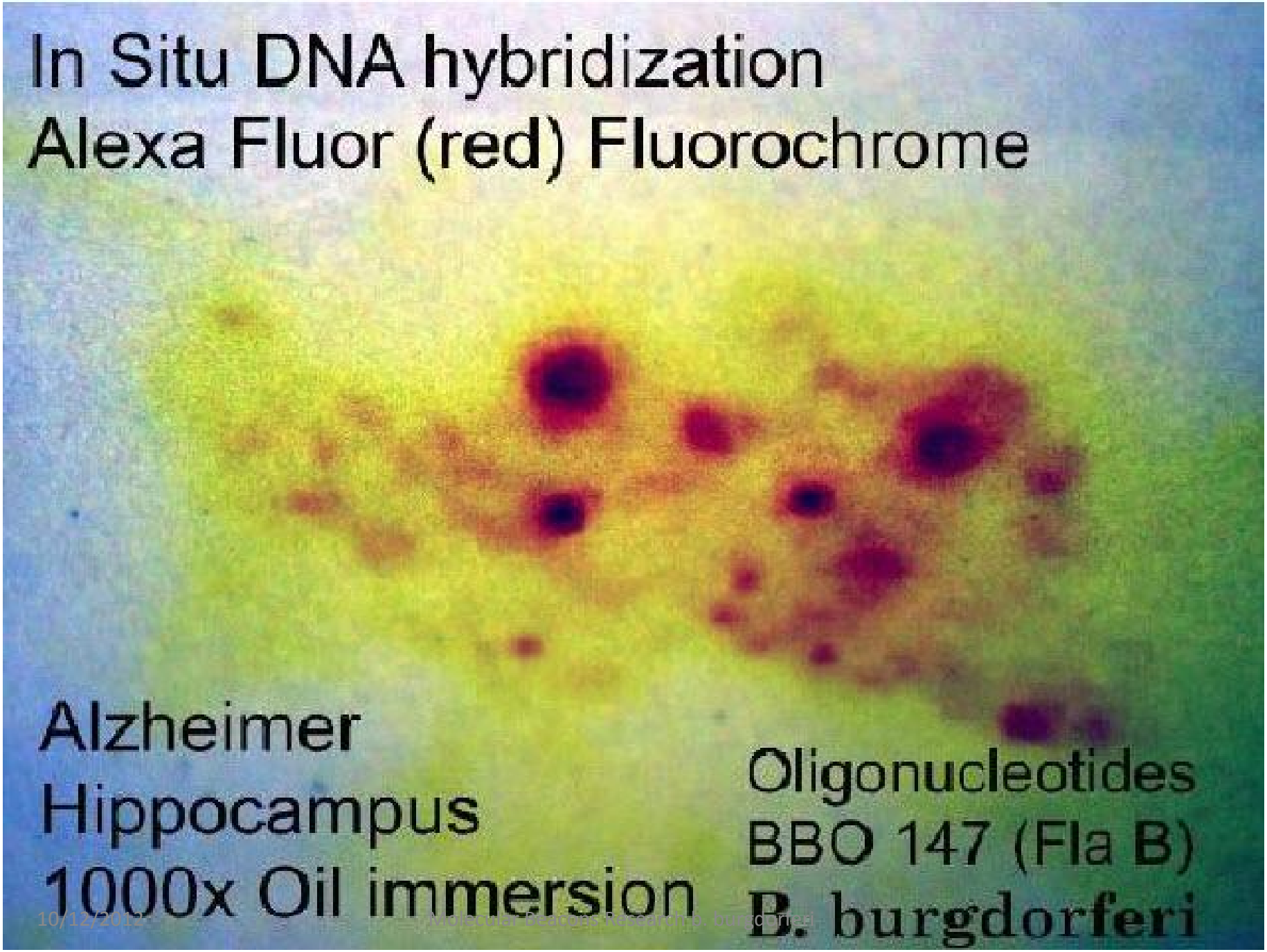
Mr Paul Christensen Alzheimer's At Autopsy 8 years after Spinal Fluid + for Borrelia burgdorferi at Stony Brook

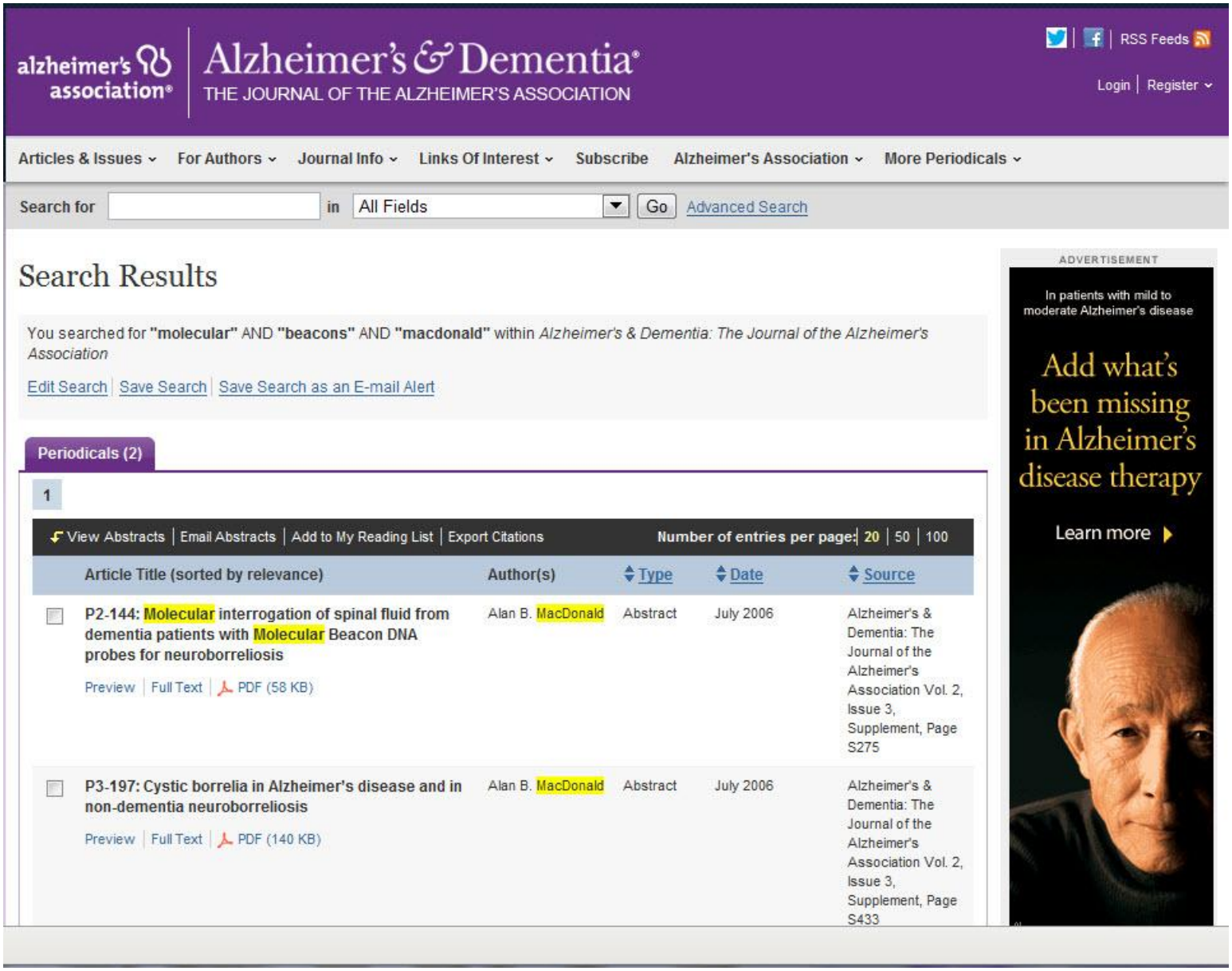


Borrelia burgdorferi **Flagellin DNA , In situ hybridization, Large Plaque**
1000x original magnification



Borrelia burgdorferi **flagellin DNA in situ DNA hybridization, Alzheimer hippocampus 1000x magnification.**


In Situ DNA hybridization
Alexa Fluor (red) Fluorochrome
Alzheimer
Hippocampus
1000x Oil immersion
Oligonucleotides
BBO 147 (Fla B)
B. burgdorferi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P2-144: Molecular interrogation of spinal fluid from dementia patients with Molecular Beacon DNA probes for neuroborreliosis
Alan B. MacDonald
Abstract
July 2006
Alzheimer's & Dementia: The Journal of the Alzheimer's Association Vol. 2, Issue 3, Supplement, Page S275
Preview | Full Text | PDF (58 KB)
P3-197: Cystic borrelia in Alzheimer's disease and in non-dementia neuroborreliosis
Alan B. MacDonald
Abstract
July 2006
Alzheimer's & Dementia: The Journal of the Alzheimer's Association Vol. 2, Issue 3, Supplement, Page S433
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Background: Accurate clinical diagnosis of AD is a challenge, especially when the disease is at its earliest stages. There are a few CSF biomarkers for AD but their sensitivity and specificity have limited clinical application. Therefore, identification of new and better biomarkers, especially those that could identify AD at its earliest stages will greatly aid us in AD prevention, diagnosis and treatment. **Objective(s):** To identify new CSF biomarkers for mild AD. **Methods:** We took a comparative proteomics approach by analyzing the proteomes of 12 CSF samples: 6 from subjects that have mild AD (CDR 1) and 6 from age-matched controls (CDR 0). A pooled sample was made that consisted of an aliquot from each of the 12 samples. After being depleted of high abundant proteins, these CSF samples were analyzed with 2D-DIGE. Each gel had a CSF sample from a subject that was CDR 0 and CDR1 as well as the pooled sample. A subset of protein spots were matched across all gels. MS/MS analyses were performed to identify spots that displayed differential abundance between the two CDR groups. The identified proteins include ones that have already been reported to be changed in AD CSF and/or implicated in AD pathogenesis (e.g. α1-antichymotrypsin (ACT), gelsolin) as well as a number of novel candidates. A few of these candidate biomarkers were selected for validation using ELISA. Their expression levels were first validated using the original 12 CSF samples and then with a much larger sample set that contained CDR 0 (n=55), 0.5 (n=20) and 1(n=17) CSF samples. The levels of two proteins were found to be significantly elevated in the AD vs. control group. Interestingly, their protein levels correlate with each other but do not correlate with that of CSF Aβ42, a biomarker for amyloid deposition. Validation studies on more candidates are underway. **Conclusions:** We have identified new CSF candidate biomarkers using novel proteomics approaches and also validated these candidates in a larger sample set. The orthogonal nature of the changes in some biomarker candidates to the existing AD biomarker Aβ42 suggests additional utility as part of a new protein biomarker panel.

P2-142 **DIAGNOSTIC PERFORMANCE OF A CSF-BIOMARKER PANEL IN 100 AUTOPSY-CONFIRMED DEMENTIA CASES AS ANALYZED WITH SINGLE AND MULTIPARAMETER TESTS**

Hugo M. Vanderstichele[1], S. Engelborghs[2], K. De Vreese[1], T. Van de Casteele[1], B. Van Everbroeck[3], P. Cras[3], J-J Martin[3], P. De Deyn[2], Eugeen Vanmechelen[1], *[1]INNOGENETICS, Zwijnaarde, Belgium; [2]Middelheim General Hospital, Antwerp, Belgium; [3]Institute Born-Bunge, University Antwerp, Antwerp, Belgium. Contact e-mail: hugovdr@innogenetics.be*

Background: An essential requirement for a good marker for Alzheimer's disease (AD) diagnosis is confirmation of its diagnostic accuracy in autopsy-confirmed patient samples. Studies with retrospectively collected cerebrospinal fluid (CSF) have shown that combined quantification of total tau, phosphorylated tau (P-tau_{181P}), and β-$amyloid_{(1-42)}$ ($Aβ_{1-42}$) can result in a diagnostic accuracy of more than 85%. **Objective:** To define the clinical performance of these biomarkers in autopsy-confirmed dementia subjects. **Methods:** A retrospective case-control study was set up consisting of subjects with clinically determined dementia after visiting a memory clinic (AD, n=72; NONAD dementia, n=25; healthy controls, n=100). For demented persons, post-mortem confirmation was available. The study was approved by the local ethics committee (CME Middelheim, Belgium). CSF levels of $Aβ_{1-42}$, total tau and P-tau_{181P} were determined with single-parameter[(1)] (INNOTEST®) and multiparameter (INNO-BIA ; xMap® technology) assays. The relationship between sensitivity and specificity was described for healthy controls versus dementia, and for AD versus NONAD using Receiver Operating Characteristic (ROC) curve analysis (MedCalc Program). Analysis with quantitative response variables were performed using general linear models assuming normal errors (SAS version 9.1). **Results:** No significant differences were observed for individual biomarkers between INNOTEST and INNO-BIA. With three biomarkers, an optimal differentiation between healthy controls and demented patients could be obtained for the INNO-BIA AlzBio3 assay using an algorithm with $Aβ_{1-42}$ and total tau, which was significantly ($P<0.01$) better than using the individual biomarkers. For differential diagnosis of AD and NONAD, best separation was obtained with an algorithm containing P-tau_{181P} and $Aβ_{1-42}$. [(1)]. S Engelborghs, K De Vreese, T Van de Casteele, H Vanderstichele, K Maertens, B.Van Everbroeck, P. Cras, JJ Martin, E Vanmechelen, PP De Deyn. Evaluation of a CSF-biomarker panel in autopsy-confirmed dementia (in preparation). **Conclusions:** The clinical performance of single and multiparameter testing of tau, P-tau_{181P}, and $Aβ_{1-42}$ was confirmed in autopsy-confirmed cases. New models useful in clinical practice were built showing that different sets of biomarkers predict the probability of disease with sensitivity, specificity and diagnostic accuracy systematically exceeding 80%.

P2-143 **QUANTITATION OF IN VIVO AMYLOID-BETA SYNTHESIS AND CLEARANCE RATES IN HUMANS USING STABLE ISOTOPE LABELING AND MASS SPECTROMETRY**

Randall J. Bateman, Ling Munsell, John C. Morris, Kevin Yarasheski, David M. Holtzman, *Washington University, St. Louis, MO, USA. Contact e-mail: batemanr@neuro.wustl.edu*

Background: The amyloid hypothesis suggests that amyloid-β (Aβ) plays an important role in causing Alzheimer's disease (AD). The central tenant of this hypothesis proposes that accumulation of amyloid-beta (Aβ), in toxic forms, leads to downstream events that culminate in dementia due to AD. **Objective(s):** In order to address the physiology of Aβ in humans, we developed a technique to quantify the synthesis and clearance rates of Aβ *in vivo* in humans. **Methods:** In healthy volunteers and in carriers of a genetic mutation that causes early-onset AD, Aβ was immuno-isolated from CSF, the amount of $^{13}C_6$-leucine incorporated into Aβ was quantified using mass spectrometry, and rates of synthesis and clearance were calculated. **Results/Conclusions:** The fractional synthesis rate of Aβ was one of the fastest measured production rates of a human protein. This technique may be used to study the pathophysiology of Aβ in AD patients and controls, and to determine differences in Aβ production and clearance rates in humans. It may also provide a biomarker of Aβ metabolism that can be used to monitor AD progression and the effects of novel therapeutic agents on Aβ synthesis or clearance.

P2-144 **MOLECULAR INTERROGATION OF SPINAL FLUID FROM DEMENTIA PATIENTS WITH MOLECULAR BEACON DNA PROBES FOR NEUROBORRELIOSIS**

Alan B. MacDonald, *St Catherine of Siena Medical Center, Smithtown, NY, USA. Contact e-mail: inmacdonald@yahoo.com*

Background: A prospective study of cerebrospinal fluid was undertaken to determine the incidence of DNA of Borrelia burgdorferi in a hospital population of patients with and without dementia using Molecular Beacon based Gene probes for the DNA of the spirochete. **Objective(s):** Detection of specific non-human DNA from a proven central nervous system bacterial pathogen (Borrelia burgdorferi), in an unselected population of inpatients in a community hosptial setting. **Methods:** Quantitative Fluorimetry analysis of positive DNA Hybridizations of Molecular Beacons specifically engineered to find Spirochetal DNA transcriptomes in human spinal fluid. **Conclusions:** Molecular Beacon technology offers a superior method for the detection of DNA of infectious organisms in human cerebrospinal fluid, when compared with Polymerase chain reaction methods. **Results:** Fifty spinal fluid specimens were analyzed with Molecular Beacons and with PCR primers designed to amplify similar target spirochetal transcriptomes. Superior detection of infectious DNA was realized in the Molecular Beacon testing DNA sequences.

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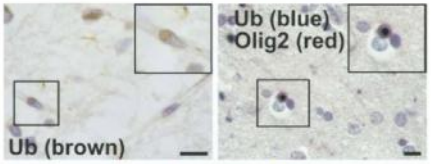
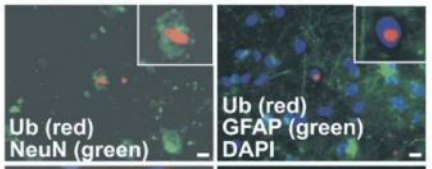
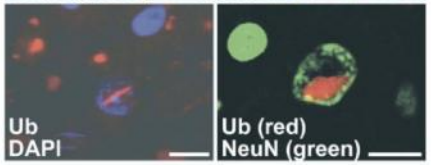
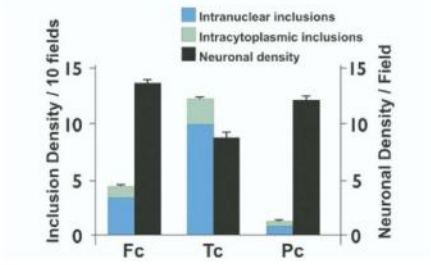
40 antibodies directed against, for instance, transcription factors, cell-specific antigens (p62, HLA-DR, GFAP, NeuN), heat-shock proteins (HSP), and cytoskeletal components. Stereologic point-counting techniques and Western blotting were used to quantify neuronal loss and soluble tau protein, respectively. **Results:** Clinically, 8 patients had FTLD. Behavioral problems and aphasia were an important finding and at least three patients suffered from parkinsonian features. No mutations were identified in MAPT, *APP*, *PS1*, *PS2*, and *PRNP*. We showed frontotemporal atrophy with filamentous Ub-positive intracellular inclusions in absence of tau pathology or any alterations in the levels of soluble tau. We characterized their cellular and subcellular localization and morphology. Ub-positive inclusions predominantly occurred within neurons (>97%), but were also observed within oligodendroglia (approx. 2%), microglia (<1%), but not within astroglia. Regarding the subcellular localization, the intranuclear inclusions (INI) were up to approx. 4 fold more frequent than the cytoplasmic inclusions, although the latter were more specific to neurons. The INIs frequently appeared spindle-shaped and 3-D confocal reconstructions identified flattened, leaf-like structures. Ultrastructurally, straight 10-18 nm diameter filaments constituted the spindle-shaped inclusions that occurred in close proximity to the nuclear membrane. Staining for HSP40, p62, and valosin/p97 was observed in only a minority of the inclusions. **Conclusion:** While the precise nature of the protein remains elusive, characterization of such familial FTLD-U patients would be helpful in identifying a common denominator in the pathogenesis of familial and the more prevalent sporadic FTLD-U.





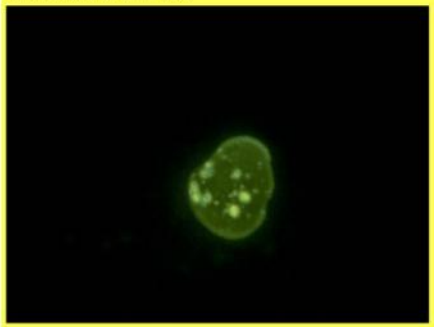




P3-197 **CYSTIC BORRELIA IN ALZHEIMER'S DISEASE AND IN NON-DEMENTIA NEUROBORRELIOSIS**

Alan B. MacDonald[1,2], *[1]Columbia University, Smithtown, NY, USA; [2]St Catherine of Siena Medical Center, Smithtown, NY, USA. Contact e-mail: inmacdonald@yahoo.com*

Background: A cystic form for *Borrelia burgdoferi* (Bb) was initially reported in 1988 in an autopsy study of Alzheimer's disease tissue obtained from Dr. George Glenner's brain bank. Cystic profiles were documented with silver stains, and with Murine Monoclonal antibodies to a Flagellin Epitope specific for Bb and *B. hermseli* [H9724 from Dr Alan Barbour]. **Objective(s):** Fresh frozen hippocampus tissues from Alzheimer's disease cases provided by the Harvard University McLean Hospital brain bank were cultured in BSK M medium to attempt to grow spirochetes in vitro. **Methods:** Triturated fresh hippocampus was cultured in vitro in sterile BSK M liquid media at 24 degrees C for one year. Darkfield microscopy examination and Acridine orange staining with epifluorescence microscopy was completed. Detection of specific Flagellin DNA sequences from ORF BBO147 using a Molecular Beacon DNA probe was used to measure incremental increases in Borrelia specific Flagellin DNA in cultures, as compared with the original fresh tissue retained uncultured DNA Extracts. **Results:** Cystic borrelia structures were recovered from in vitro cultures of fresh Alzheimer disease hippocampus tissue. Incremental increases in *Borrelia burgdorferi* flagellin B DNA were documented in cultured tissues. **Conclusions:** A subset of Alzheimer's disease is related to chronic Neuroborreliosis in the human host.



P3-198 **A CASE OF EARLY ONSET ALZHEIMER'S DISEASE WITH COTTON WOOL PLAQUES BUT WITHOUT SPASTIC PARAPARESIS**

Beata Sikorska[1], Pawel P. Liberski[1], Herbert Budka[2], *[1]Department of Molecular Pathology and Neuropathology, Medical University of Lodz, Lodz, Poland; [2]Institute of Neurology, Medical University of Vienna, Vienna, Austria. Contact e-mail: elmo@csk.am.lodz.pl*

Background: We report a case of 32-year-old man with myoclonus, rapidly progressive dementia, apraxia, ataxic gait and subtle right hemiparesis. The clinical and first pathological diagnosis was Creutzfeldt Jakob disease. **Methods:** A brain biopsy was obtained from the left temporoparietal area. Autopsy was performed one hour after death. Formalin-fixed, paraffin embedded tissues were used for routine and immunohistochemical microscopic stainings. Part of the autopsy material was fixed in 4% glutaraldehyde in cacodylate buffer for electron microscopy. **Results:** On

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This Research Proposal Is Submitted to The Board of the Tick Borne Disease Alliance

Alan B. MacDonald MD
Research Fellow
University of New Haven
Laboratory of Dry Eva Sapi
Lyme Disease Research Unit
West Haven, Conn