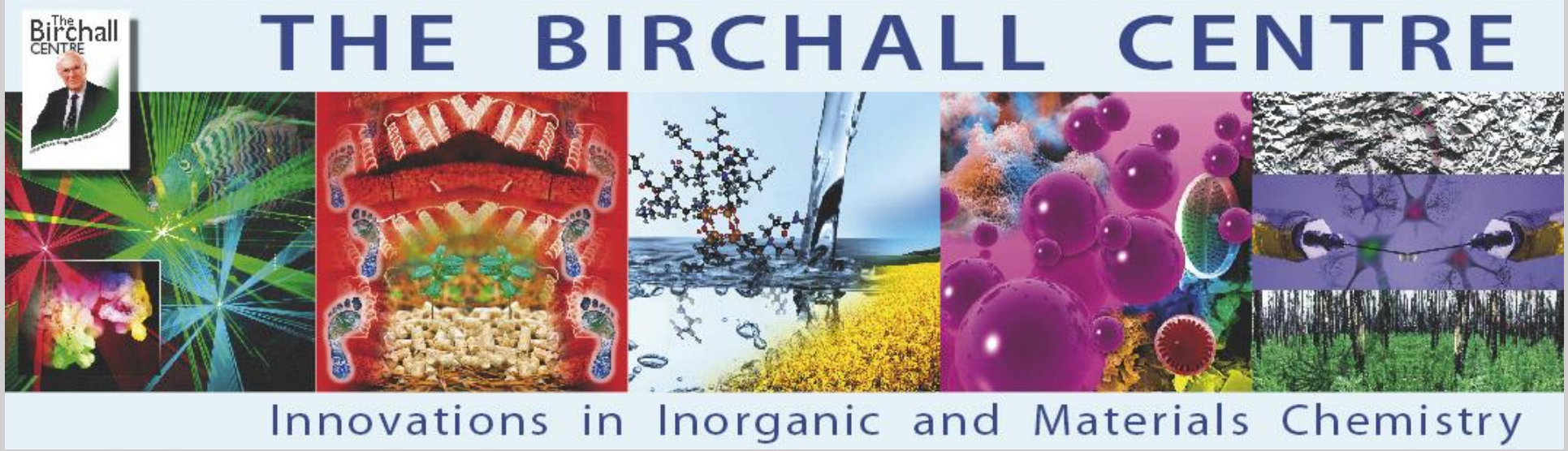

Imaging Aluminium in Human Brain Tissue

Christopher Exley PhD FRSB

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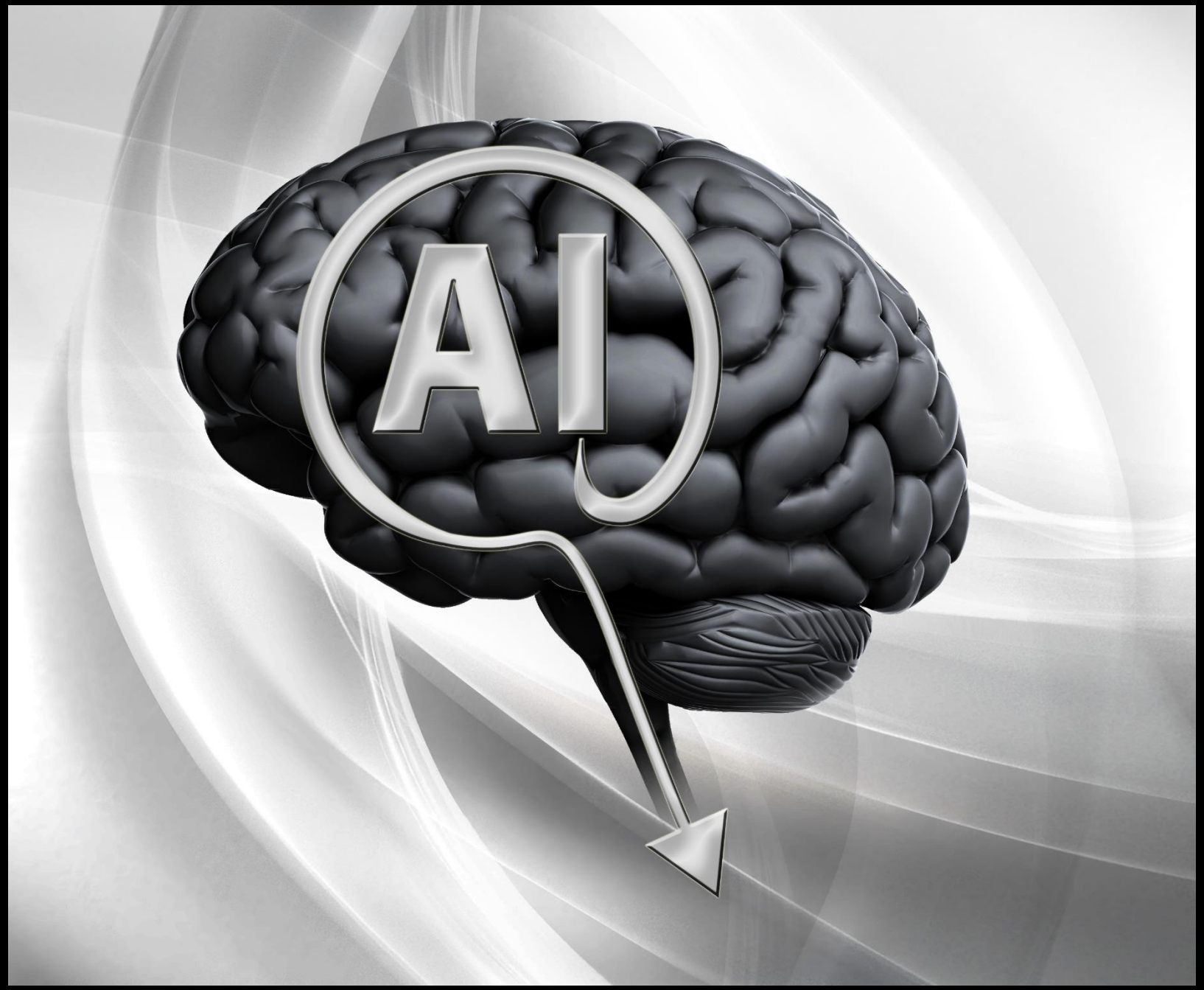
c.exley@keele.ac.uk

http://www.keele.ac.uk/aluminium/

Geneva 2019


AI

Aluminium in Human Brain Tissue

Our Published Data

Exley C & Esiri M (2006) Severe cerebral congophilic angiopathy coincident with increased brain aluminium in a resident of Camelford, Cornwall, UK. Journal of Neurology Neurosurgery and Psychiatry 77, 877-879.
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Mirza A, King A, Troakes C and Exley C (2016) The identification of aluminium in human brain tissue using lumogallion and fluorescence microscopy. Journal of Alzheimer's Disease 54, 1333-1338.
https://content.iospress.com/articles/journal-of-alzheimers-disease/jad160648

Mirza A, King A, Troakes C and Exley C (2017) Aluminium in brain tissue in familial Alzheimer's disease. Journal of Trace Elements in Medicine and Biology 40, 30-36.
https://www.sciencedirect.com/science/article/pii/S0946672X16303777

Published Research Continued

Mold M, Umar D, King A, Exley C (2018) Aluminium in brain tissue in autism. Journal of Trace Elements in Medicine and Biology 46, 76-82.
https://www.sciencedirect.com/science/article/pii/S0946672X17308763

Mold M, Chmielecka A, Rodriguez MRR, Thom F, Linhart C, King A, Exley C (2018) Aluminium in brain tissue in multiple sclerosis. International Journal of Environmental Research and Public Health 15, 1777.
https://www.mdpi.com/1660-4601/15/8/1777

Mold M, Cottle J, Exley C (2019) Aluminium in brain tissue in epilepsy: A case report from Camelford. International Journal of Environmental Research and Public Health 16, 2129.
https://www.mdpi.com/1660-4601/16/12/2129

So, there is aluminium in your brain BUT…what does it look like?

Journal of Alzheimer's Disease 54 (2016) 1333–1338
DOI 10.3233/JAD-160648
IOS Press

The Identification of Aluminum in Human Brain Tissue Using Lumogallion and Fluorescence Microscopy

Ambreen Mirza[a], Andrew King[b,c], Claire Troakes[c] and Christopher Exley[a,*]
[a]*The Birchall Centre, Lennard-Jones Laboratories, Keele University, Staffordshire, UK*
[b]*Department of Clinical Neuropathology, King's College Hospital, London, UK*
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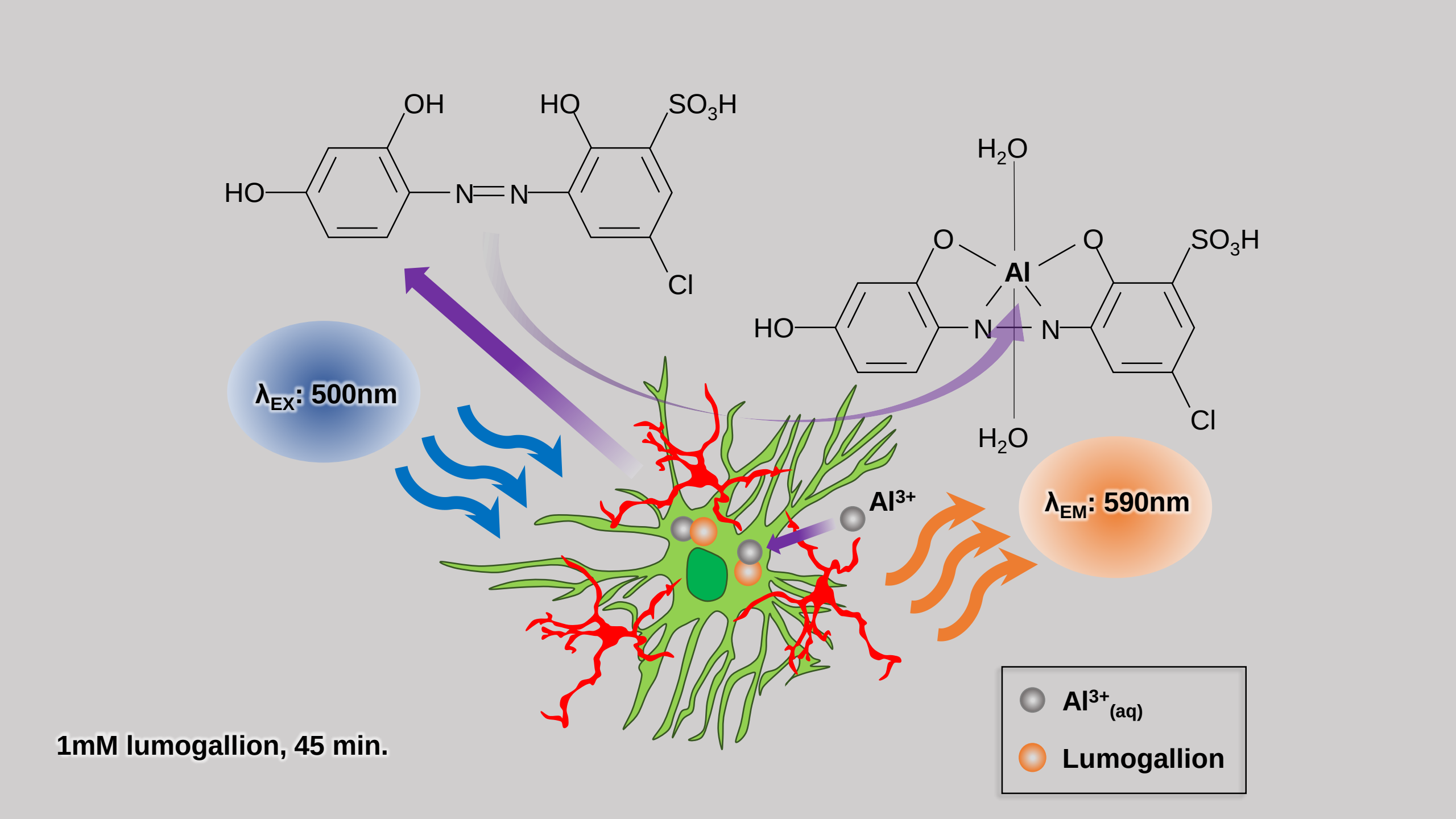
Accepted 4 July 2016

Abstract. Aluminum in human brain tissue is implicated in the etiologies of neurodegenerative diseases including Alzheimer's disease. While methods for the accurate and precise measurement of aluminum in human brain tissue are widely acknowledged, the same cannot be said for the visualization of aluminum. Herein we have used transversely-heated graphite furnace atomic absorption spectrometry to measure aluminum in the brain of a donor with Alzheimer's disease, and we have developed and validated fluorescence microscopy and the fluor lumogallion to show the presence of aluminum in the same tissue. Aluminum is observed as characteristic orange fluorescence that is neither reproduced by other metals nor explained by autofluorescence. This new and relatively simple method to visualize aluminum in human brain tissue should enable more rigorous testing of the aluminum hypothesis of Alzheimer's disease (and other neurological conditions) in the future.

Keywords: Aluminum, Alzheimer's disease, brain tissue, fluorescence microscopy, lumogallion, transversely heated graphite furnace atomic absorption spectrometry

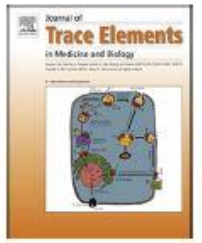

OH
HO
HO
SO3H
N
N
Cl
H2O
O
Al
O
SO3H
HO
N
N
Cl
H2O
λEX: 500nm
Al3+
λEM: 590nm
Al3+(aq)
Lumogallion
1mM lumogallion, 45 min.

Journal of Trace Elements in Medicine and Biology 46 (2018) 76–82

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb



Aluminium in brain tissue in autism

Matthew Mold[a], Dorcas Umar[b], Andrew King[c], Christopher Exley[a,*]

[a] The Birchall Centre, Lennard-Jones Laboratories, Keele University, Staffordshire, ST5 5BG, United Kingdom
[b] Life Sciences, Keele University, Staffordshire, ST5 5BG, United Kingdom
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ARTICLE INFO

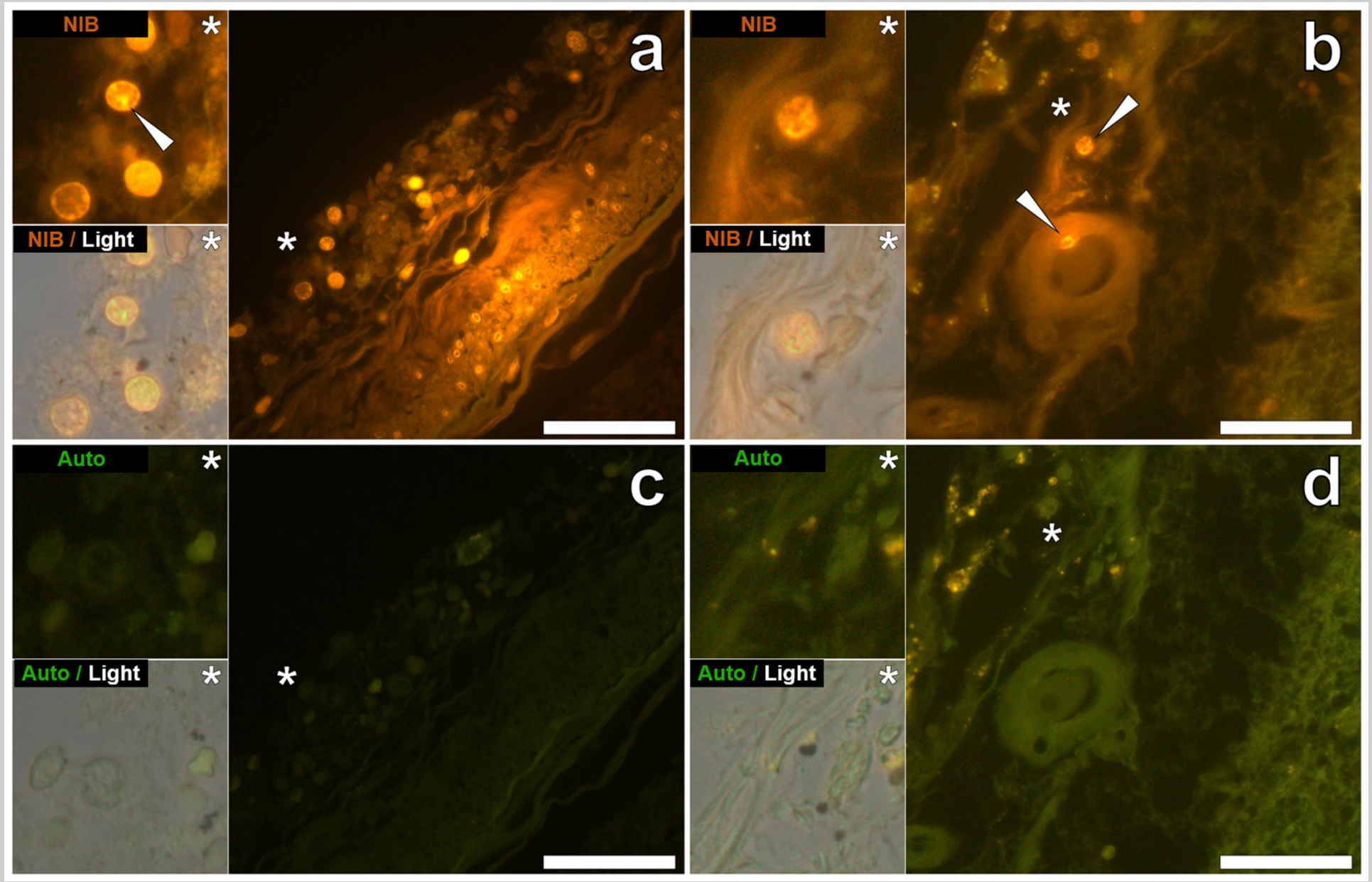
Keywords:
Human exposure to aluminium
Human brain tissue
Autism spectrum disorder
Transversely heated atomic absorption spectrometry
Aluminium-selective fluorescence microscopy

> 600,000 views on publishers' website.

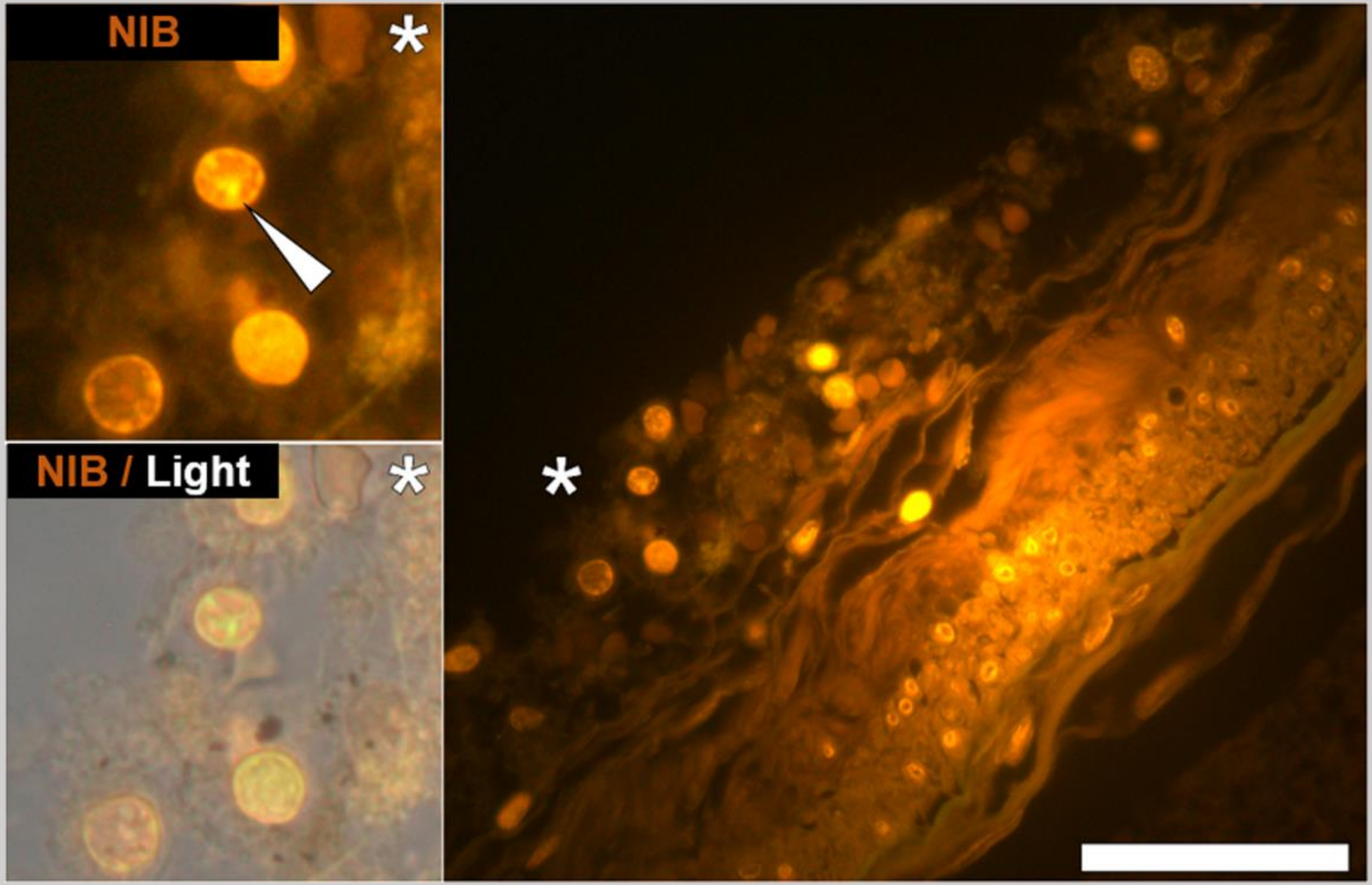
ABSTRACT

Autism spectrum disorder is a neurodevelopmental disorder of unknown aetiology. It is suggested to involve both genetic susceptibility and environmental factors including in the latter environmental toxins. Human exposure to the environmental toxin aluminium has been linked, if tentatively, to autism spectrum disorder. Herein we have used transversely heated graphite furnace atomic absorption spectrometry to measure, for the first time, the aluminium content of brain tissue from donors with a diagnosis of autism. We have also used an aluminium-selective fluor to identify aluminium in brain tissue using fluorescence microscopy. The aluminium content of brain tissue in autism was consistently high. The mean (standard deviation) aluminium content across all 5 individuals for each lobe were 3.82(5.42), 2.30(2.00), 2.79(4.05) and 3.82(5.17) μg/g dry wt. for the occipital, frontal, temporal and parietal lobes respectively. These are some of the highest values for aluminium in human brain tissue yet recorded and one has to question why, for example, the aluminium content of the occipital lobe of a 15 year old boy would be 8.74 (11.59) μg/g dry wt.? Aluminium-selective fluorescence microscopy was used to identify aluminium in brain tissue in 10 donors. While aluminium was imaged associated with neurones it appeared to be present intracellularly in microglia-like cells and other inflammatory non-neuronal cells in the meninges, vasculature, grey and white matter. The pre-eminence of intracellular aluminium associated with non-neuronal cells was a standout observation in autism brain tissue and may offer clues as to both the origin of the brain aluminium as well as a putative role in autism spectrum disorder.

- **A2: Hippocampus & frontal lobe, 50-year-old Male**

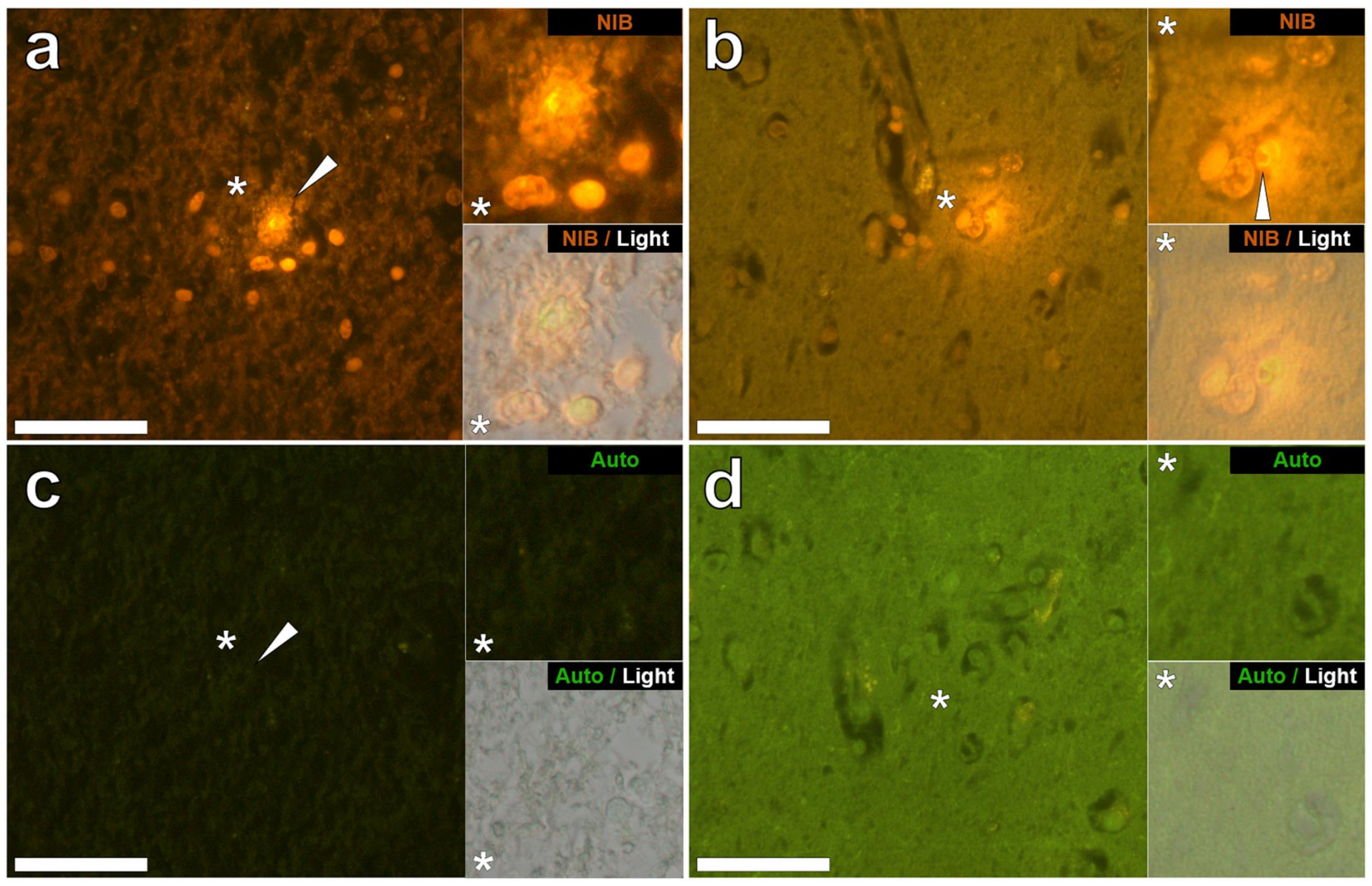


Mononuclear inflammatory cells (lymphocytes) in **leptomeningeal** membranes in the hippocampus (**a & c**) and frontal lobe (**b & d**) of a 50-year-old male donor with autism.



Aluminium in leptomeningeal membranes (50, M)

- **A4: Hippocampus & parietal lobe, 15-year-old Male**



Intracellular aluminium in **glia** in the hippocampus (**a & c**) and a **neuronal** cell in the parietal lobe (**b & d**) of a 15-year-old male donor, diagnosed with autism.

International Journal of
Environmental Research and Public Health

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Article

Aluminium in Brain Tissue in Multiple Sclerosis

Matthew Mold [1], Agata Chmielecka [2], Maria Raquel Ramirez Rodriguez [1], Femia Thom [2], Caroline Linhart [3], Andrew King [4] and Christopher Exley [1,*]

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2 Life Sciences, The Huxley Building, Keele University, Staffordshire ST5 5BG, UK; aggychmi@gmail.com (A.C.); femiathom@hotmail.com (F.T.)
3 Department of Medical Statistics, Informatics and Health Economics, Medical University of Innsbruck, A-6020 Innsbruck, Austria; Linhart.Caroline@i-med.ac.at
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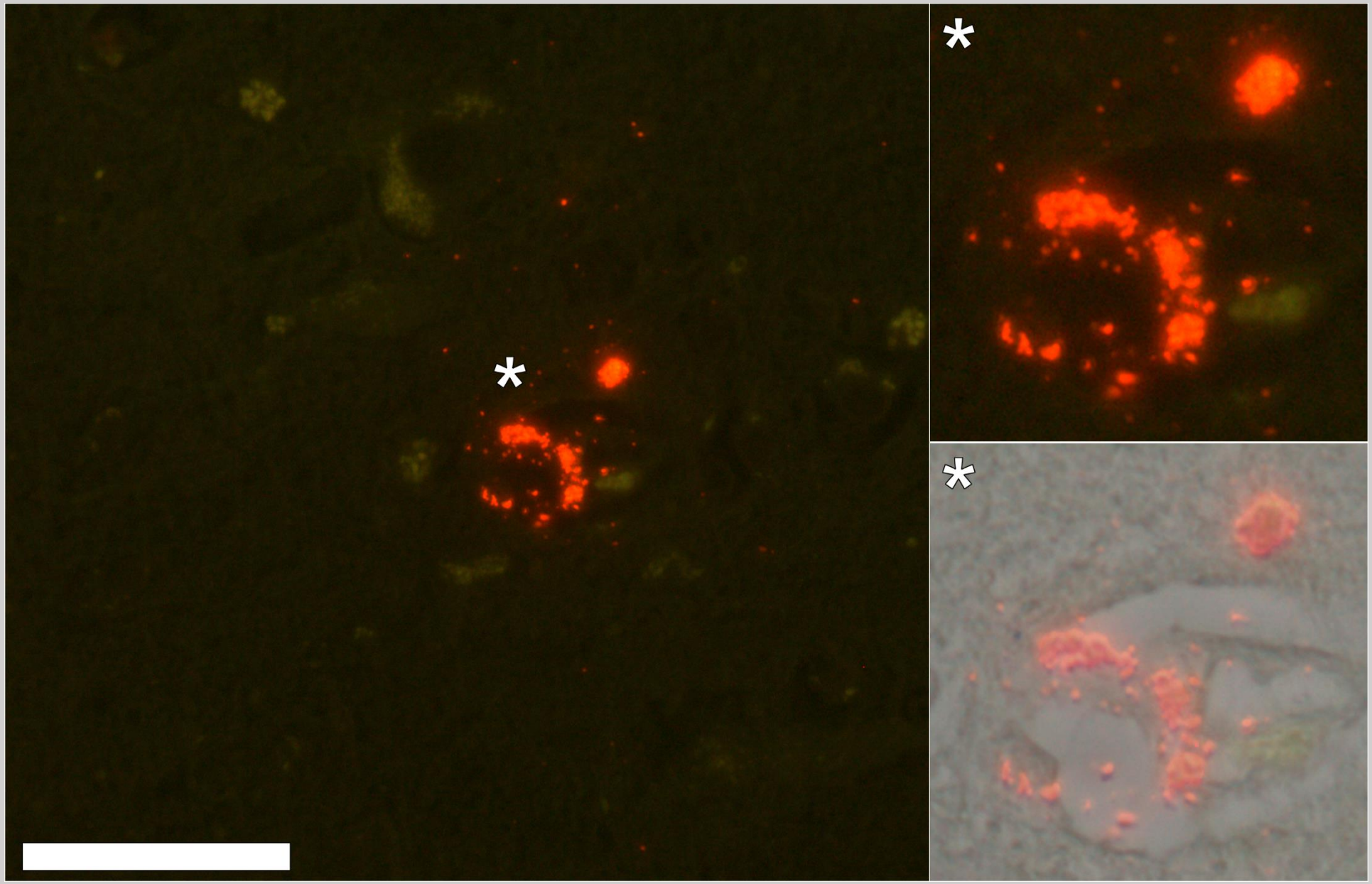
Received: 25 July 2018; Accepted: 15 August 2018; Published: 18 August 2018

check for updates

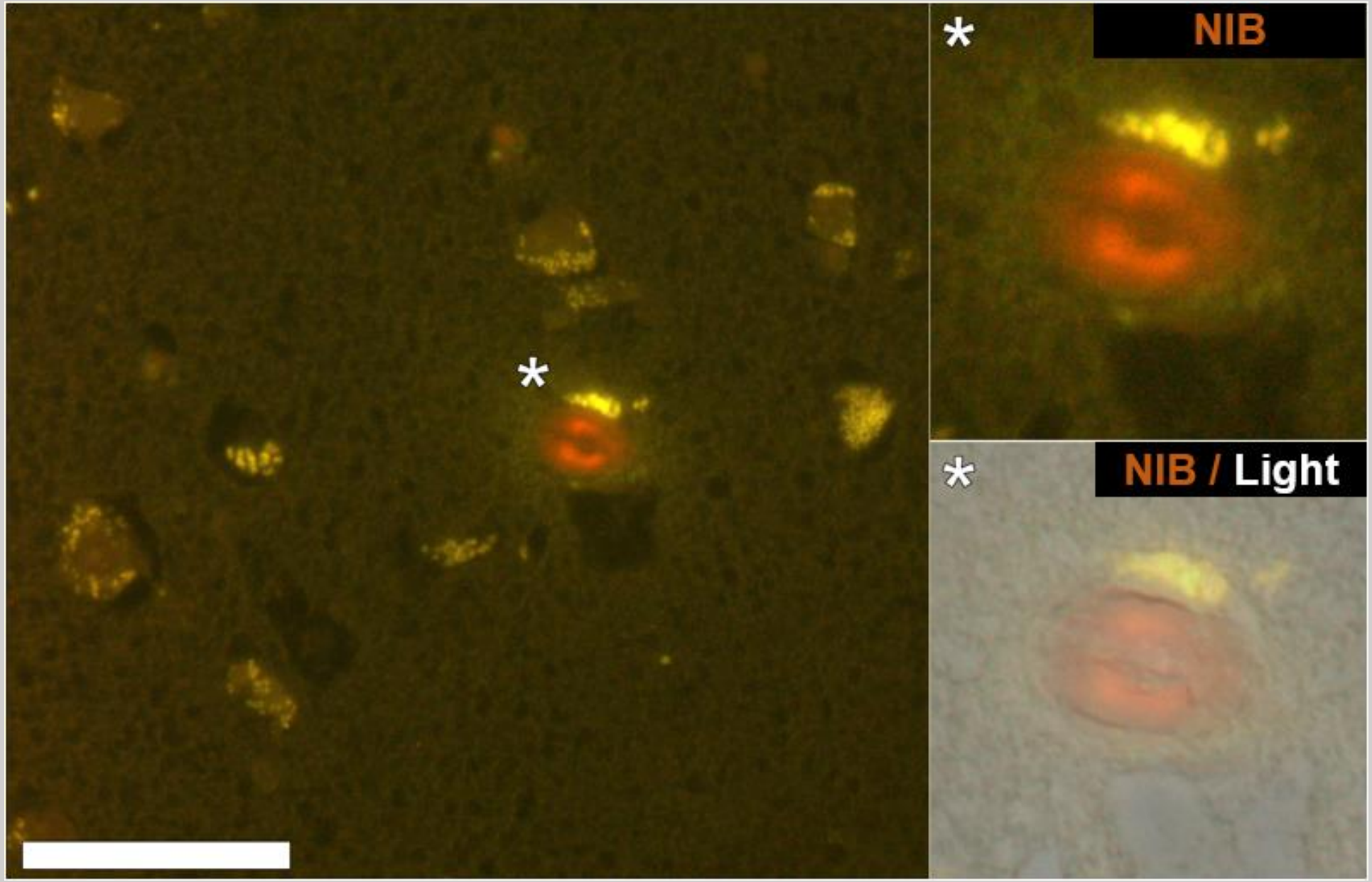
Abstract: Multiple sclerosis (MS) is a devastating and debilitating neurodegenerative disease of unknown cause. A consensus suggests the involvement of both genetic and environmental factors of which the latter may involve human exposure to aluminium. There are no data on the content and distribution of aluminium in human brain tissue in MS. The aluminium content of brain tissue from 14 donors with a diagnosis of MS was determined by transversely heated graphite furnace atomic absorption spectrometry. The location of aluminium in the brain tissue of two donors was investigated by aluminium-specific fluorescence microscopy. The aluminium content of brain tissue in MS was universally high with many tissues bearing concentrations in excess of 10 μg/g dry wt. (10 ppm) and some exceeding 50 ppm. There were no statistically significant relationships between brain lobes, donor age or donor gender. Aluminium-specific fluorescence successfully identified aluminium in brain tissue in both intracellular and extracellular locations. The association of aluminium with corpora amylacea suggests a role for aluminium in neurodegeneration in MS.

Keywords: multiple sclerosis; human exposure to aluminium; human brain tissue; TH GFAAS; aluminium-specific fluorescence

Mold et al., 2018. IJERPH. 15(8): 1777.



Extracellular aluminium (56, M)



Aluminium in corpora amylacea (48, F)

International Journal of
Environmental Research and Public Health

MDPI

Case Report

Intracellular Aluminium in Inflammatory and Glial Cells in Cerebral Amyloid Angiopathy: A Case Report

Matthew Mold [1], Jason Cottle [2], Andrew King [3] and Christopher Exley [1,*]

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2 School of Medicine, David Weatherly Building, Keele University, Staffordshire ST5 5BG, UK; jasoncottlee@gmail.com
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* Correspondence: c.exley@keele.ac.uk

Received: 19 March 2019; Accepted: 18 April 2019; Published: 24 April 2019

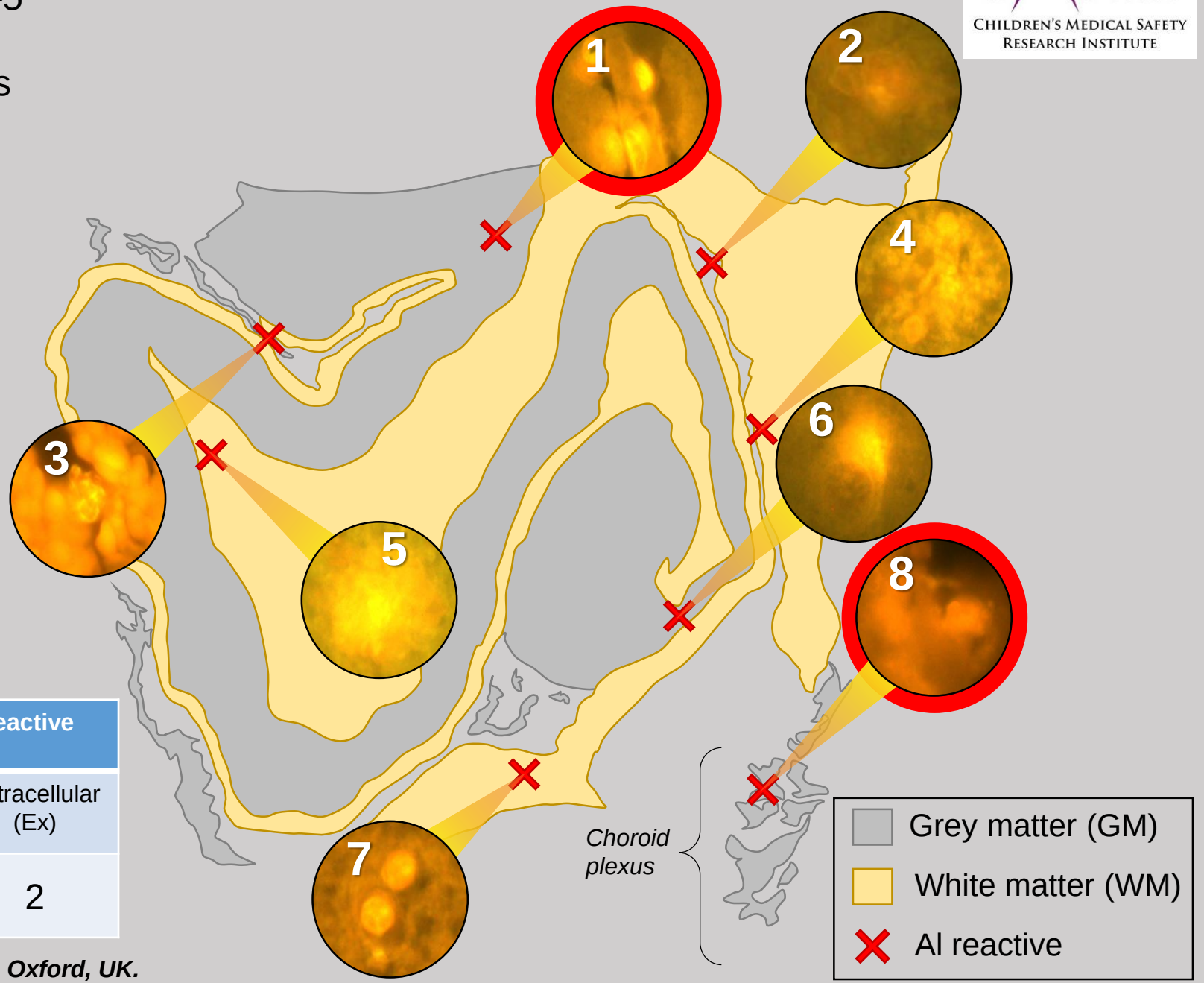
Abstract: (1) Introduction: In 2006, we reported on very high levels of aluminium in brain tissue in an unusual case of cerebral amyloid angiopathy (CAA). The individual concerned had been exposed to extremely high levels of aluminium in their potable water due to a notorious pollution incident in Camelford, Cornwall, in the United Kingdom. The recent development of aluminium-specific fluorescence microscopy has now allowed for the location of aluminium in this brain to be identified. (2) Case Summary: We used aluminium-specific fluorescence microscopy in parallel with Congo red staining and polarised light to identify the location of aluminium and amyloid in brain tissue from an individual who had died from a rare and unusual case of CAA. Aluminium was almost exclusively intracellular and predominantly in inflammatory and glial cells including microglia, astrocytes, lymphocytes and cells lining the choroid plexus. Complementary staining with Congo red demonstrated that aluminium and amyloid were not co-located in these tissues. (3) Discussion: The observation of predominantly intracellular aluminium in these tissues was novel and something similar has only previously been observed in cases of autism. The results suggest a strong inflammatory component in this case and support a role for aluminium in this rare and unusual case of CAA.

Keywords: cerebral amyloid angiopathy; brain aluminium; pro-inflammatory cells; human exposure to aluminium; Camelford in Cornwall

Patient ID:
NP040/2004-5
Region:
Hippocampus

CASE STUDY 1

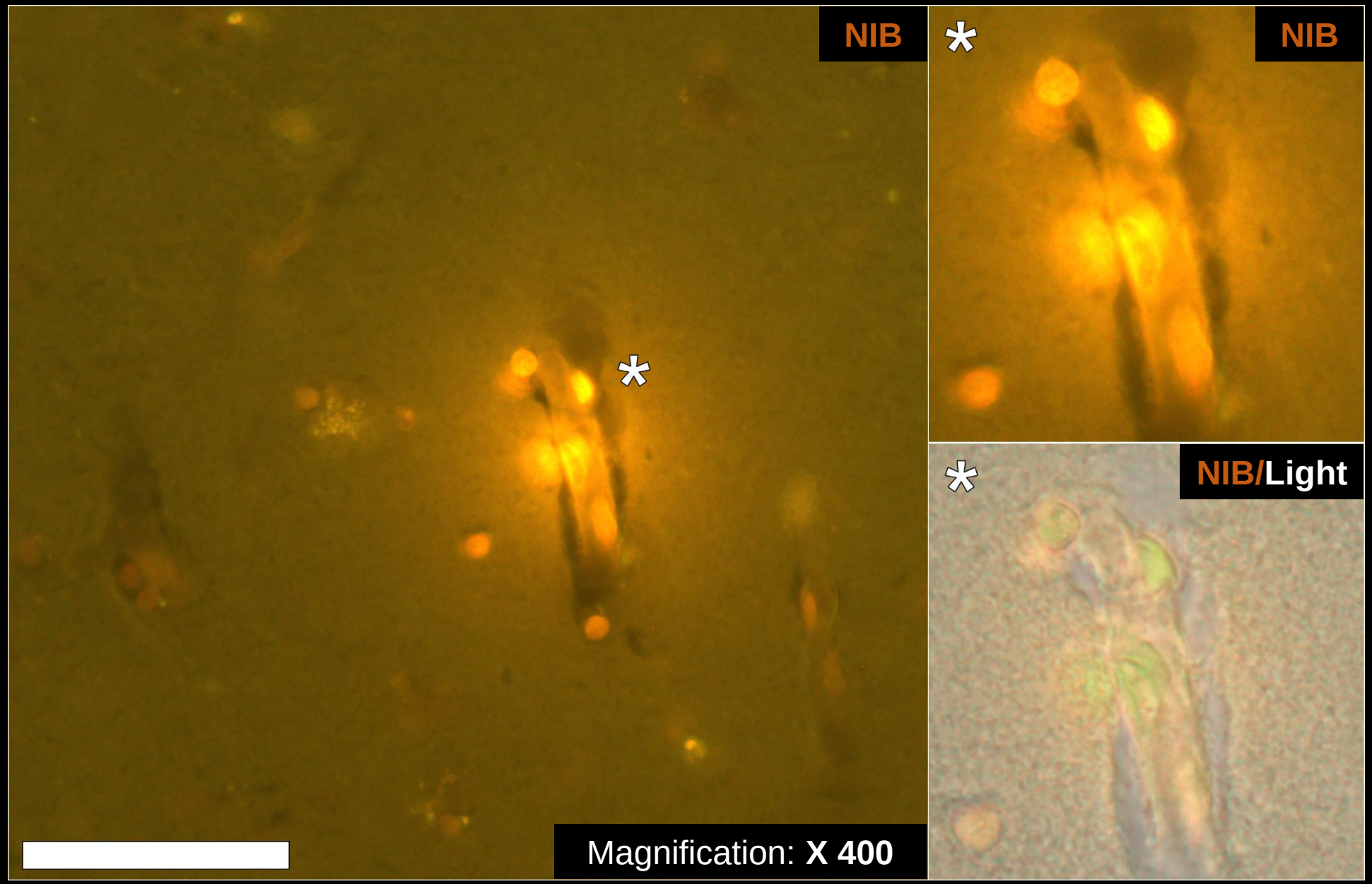
CMSRI
Children's Medical Safety Research Institute



Number of AI reactive regions	
Intracellular (In)	Extracellular (Ex)
6	2

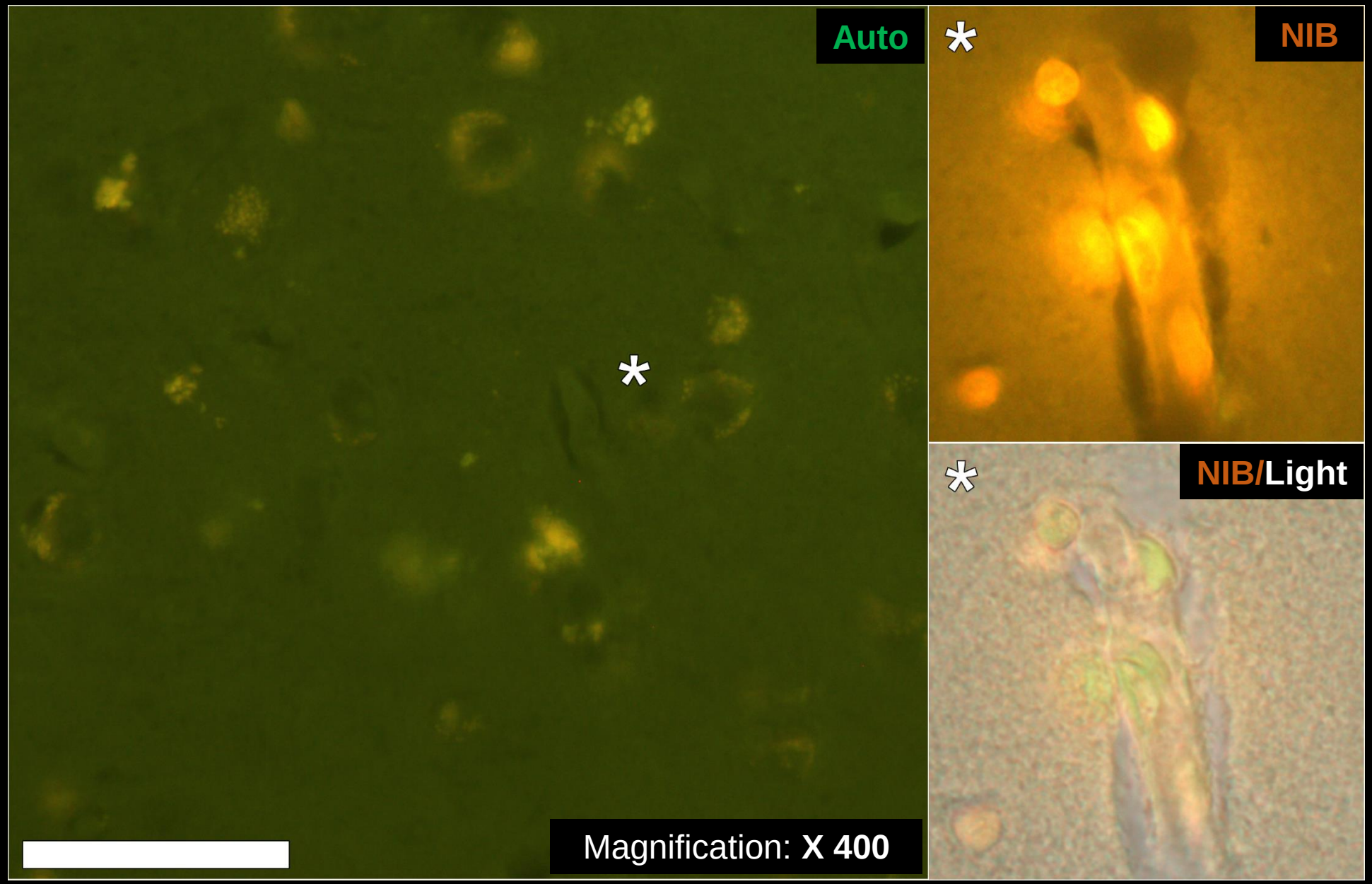
Oxford Brain Bank, Oxford, UK.

- **Hippocampus**



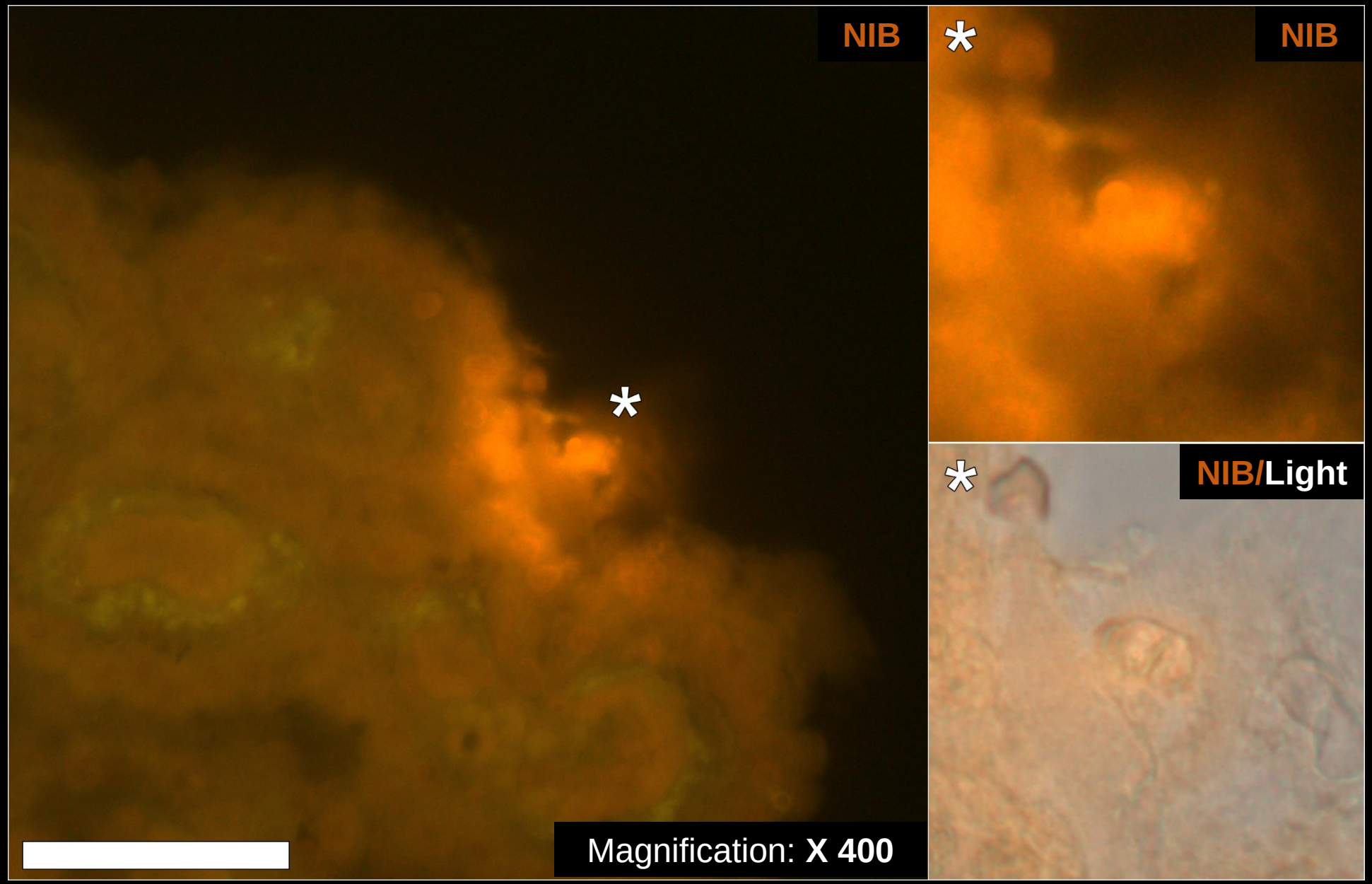
Intracellular aluminium in the vessel wall. (Region: #1)

- **Hippocampus**



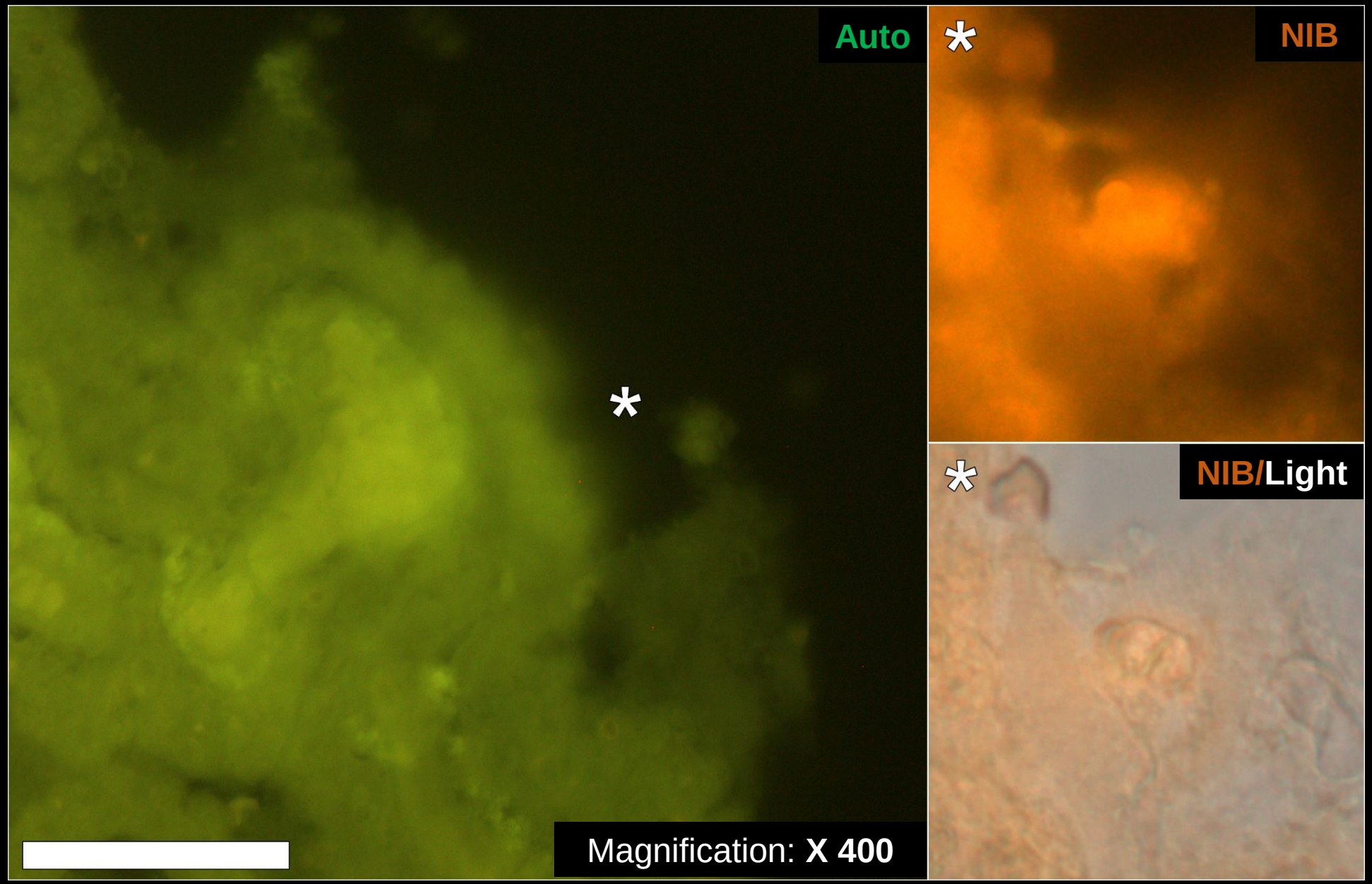
Intracellular aluminium in the vessel wall. (Region: #1)

- **Hippocampus**



Epithelial cells lining the choroid plexus. (Region: #8)

- **Hippocampus**



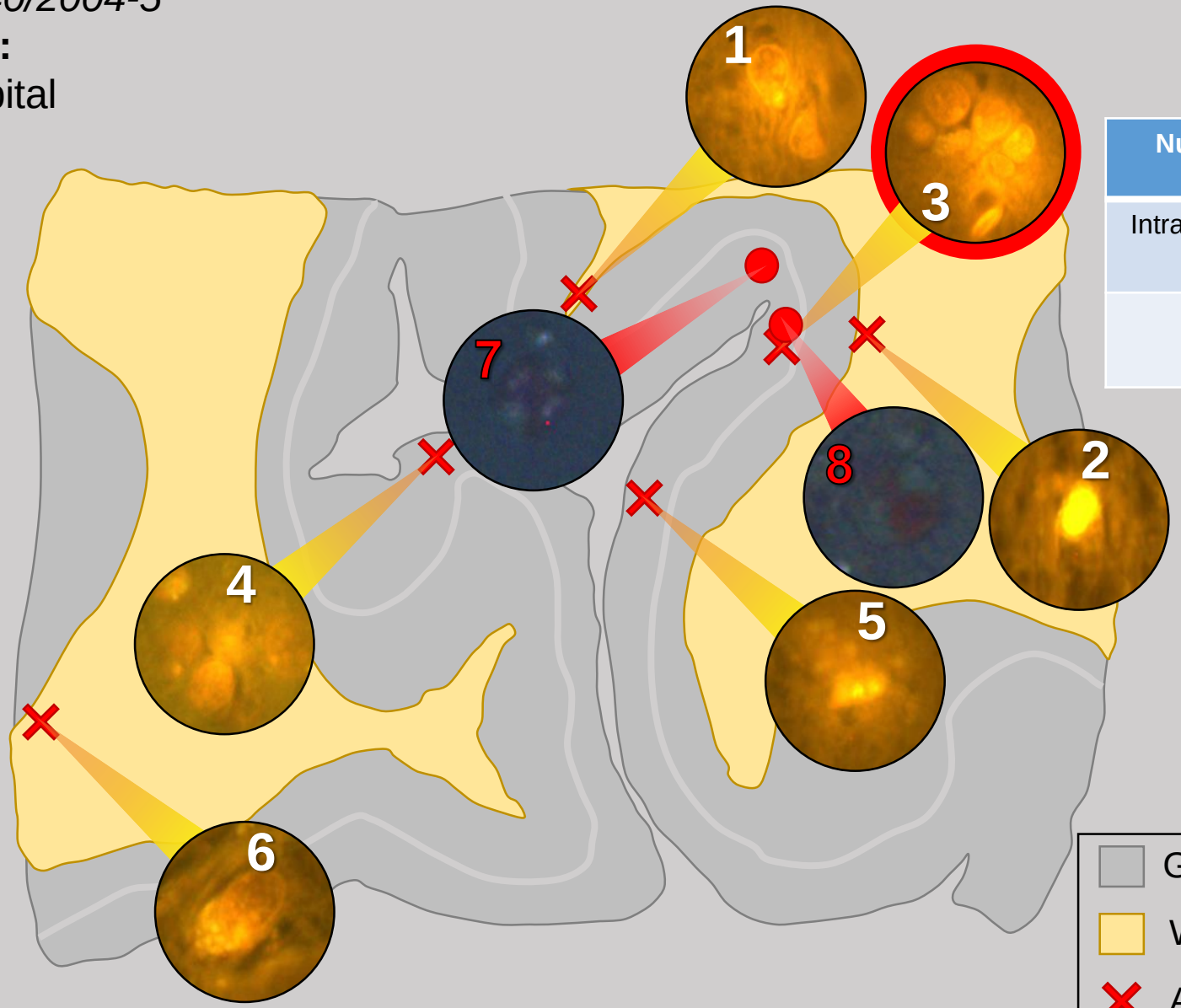
Epithelial cells lining the choroid plexus. (Region: #8)

Patient ID:
NP040/2004-5
Lobe:
Occipital

CASE STUDY 1

CMSRI
Children's Medical Safety Research Institute

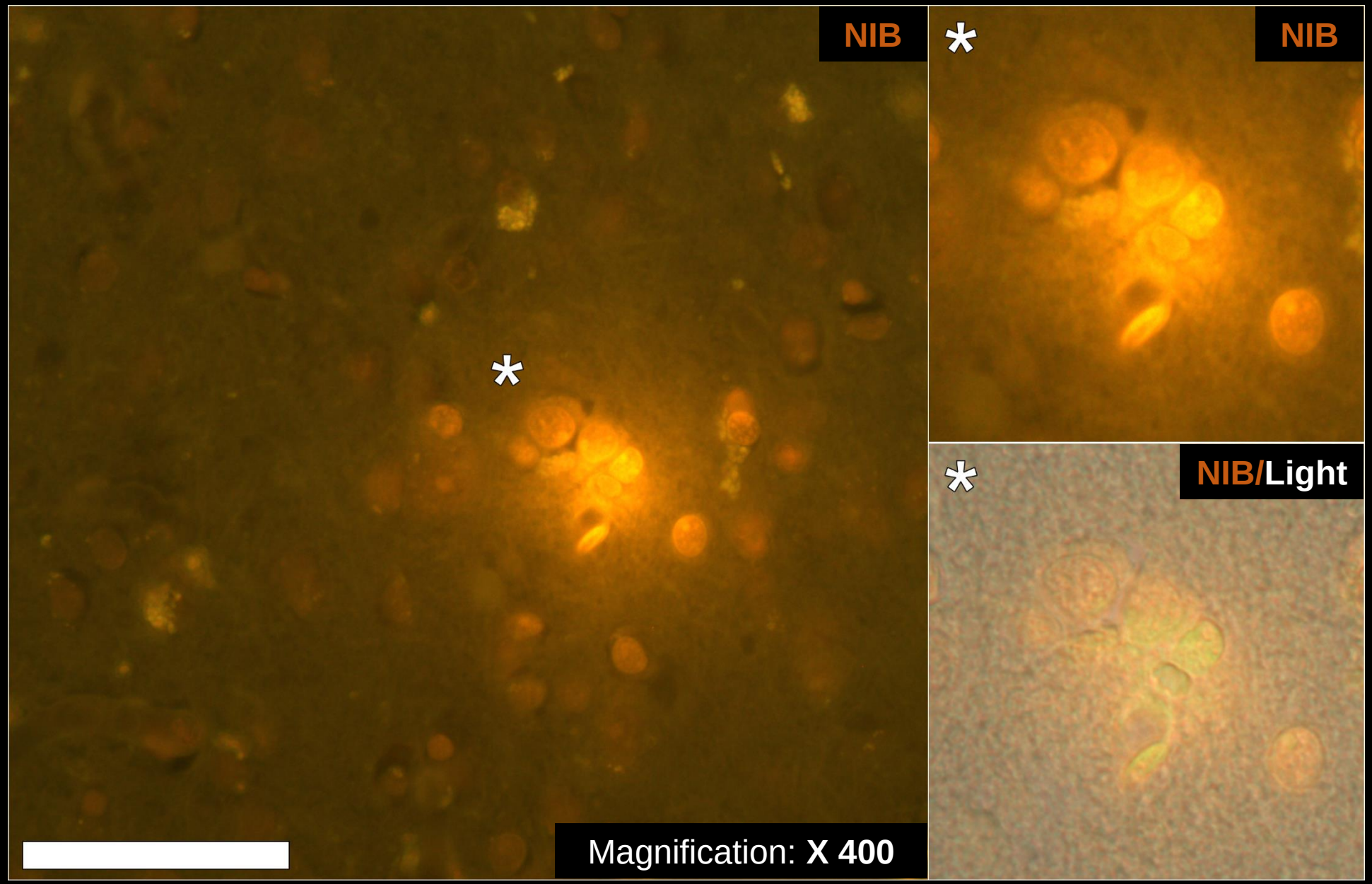
Number of AI reactive regions	
Intracellular (In)	Extracellular (Ex)
6	0



Grey matter (GM)
White matter (WM)
AI reactive
CR positive

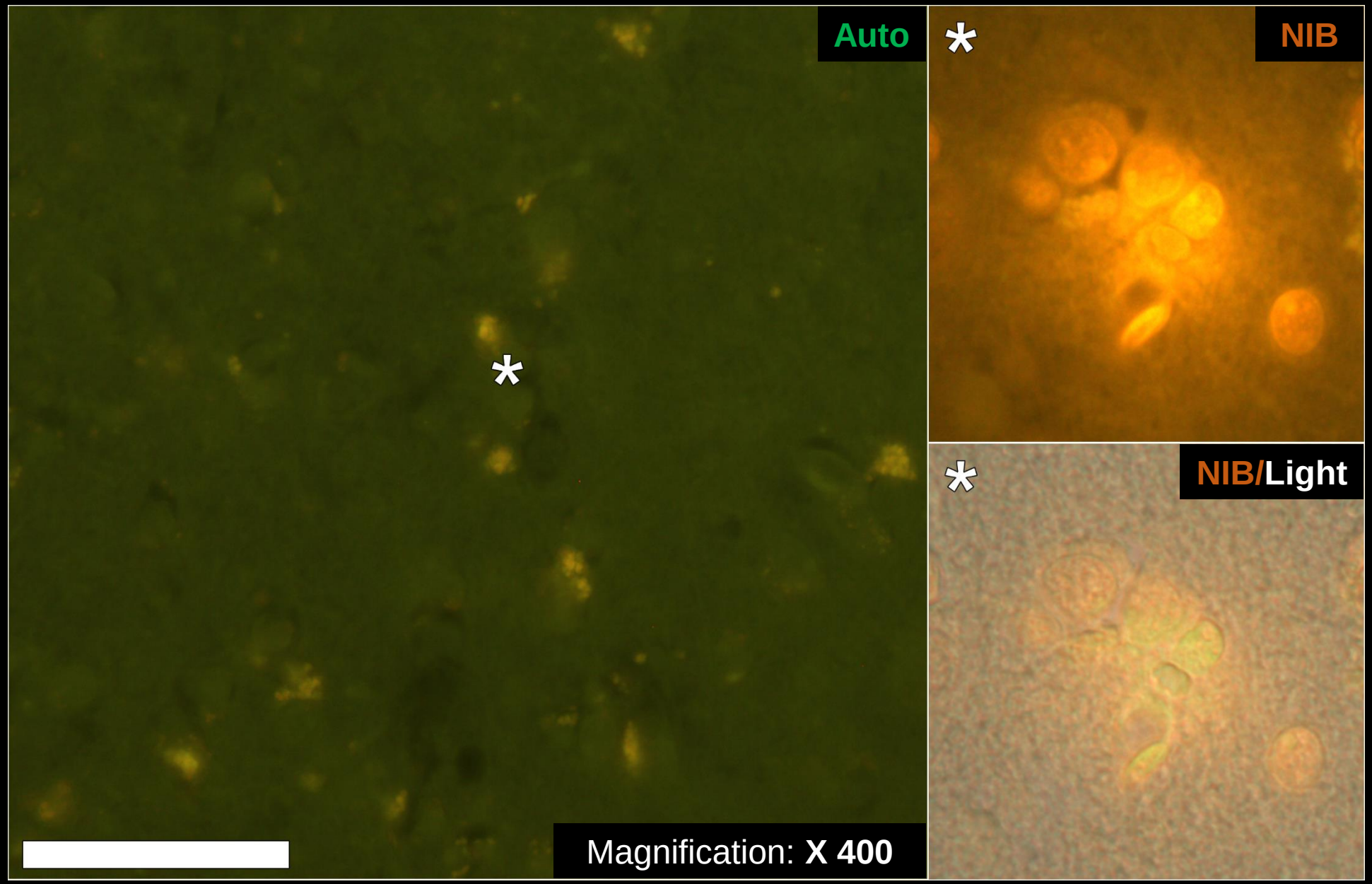
Oxford Brain Bank, Oxford, UK.

- **Occipital cortex**



Aluminium in astrocytes & microglial cells. (Region: #3)

- **Occipital cortex**

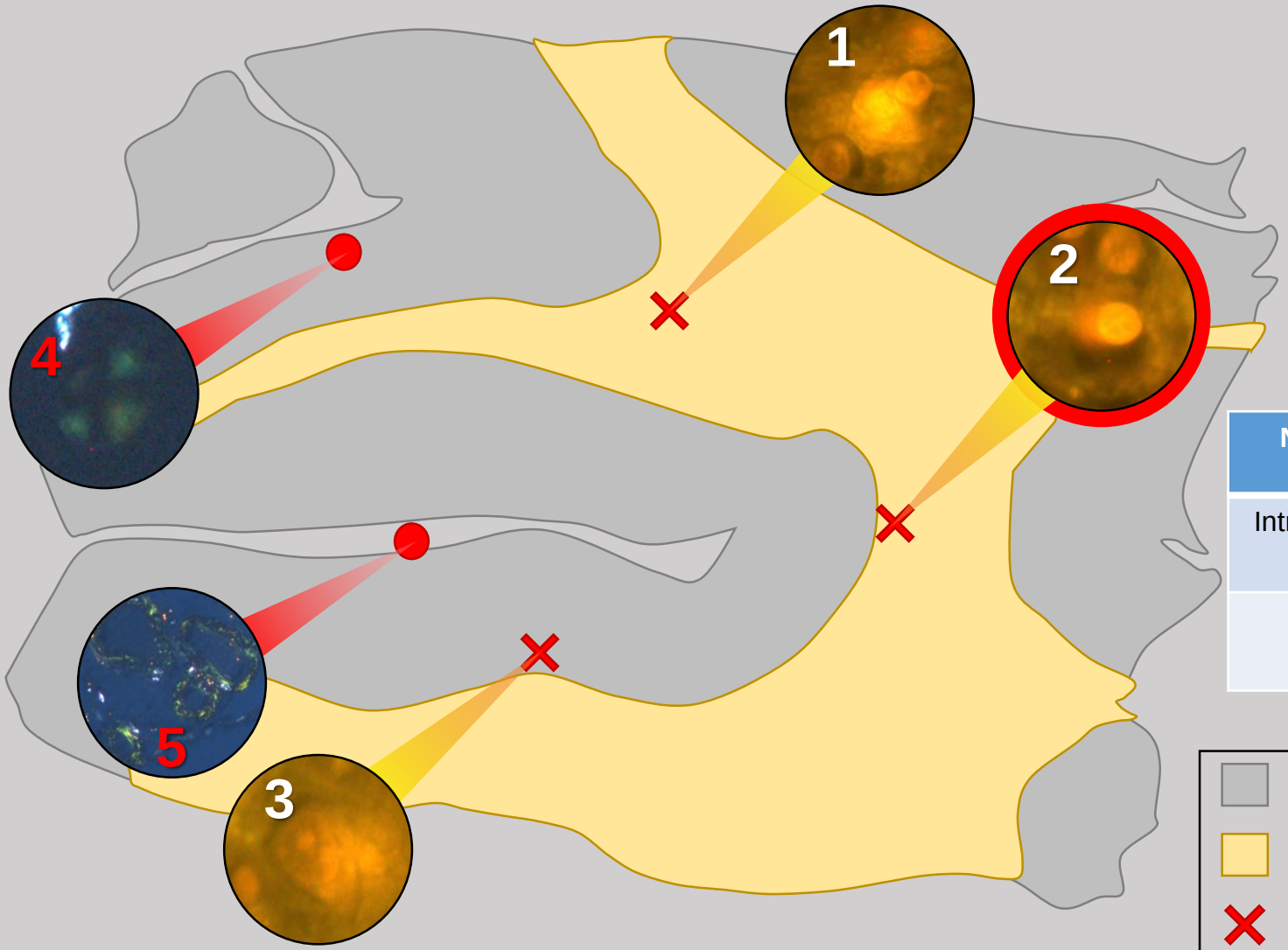


Aluminium in astrocytes & microglial cells. (Region: #3)

Patient ID:
NP040/2004-5
Lobe:
Parietal

CASE STUDY 1

CMSRI
Children's Medical Safety Research Institute

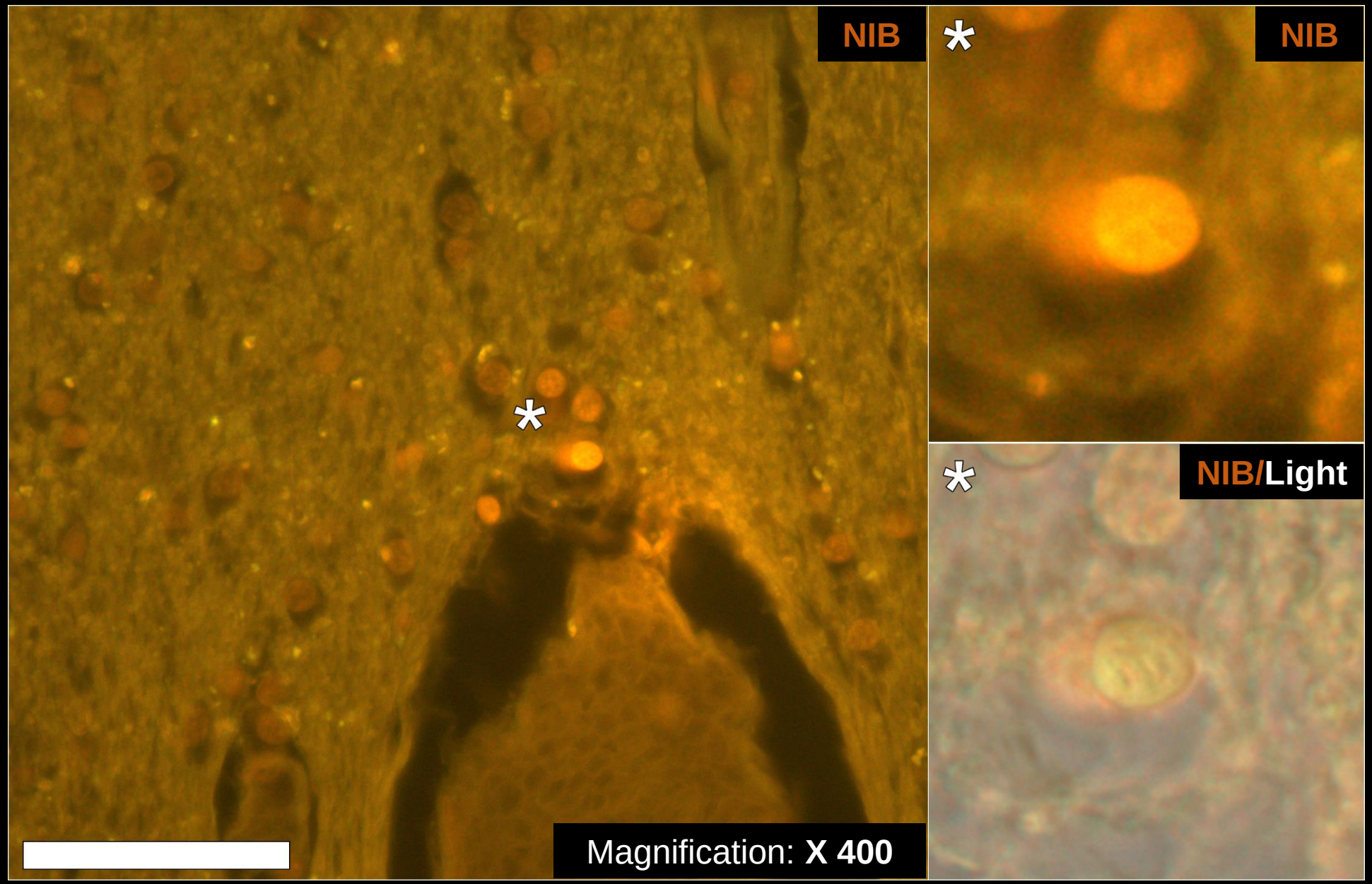


Number of AI reactive regions	
Intracellular (In)	Extracellular (Ex)
3	0

- Grey matter (GM)
- White matter (WM)
- AI reactive
- CR positive

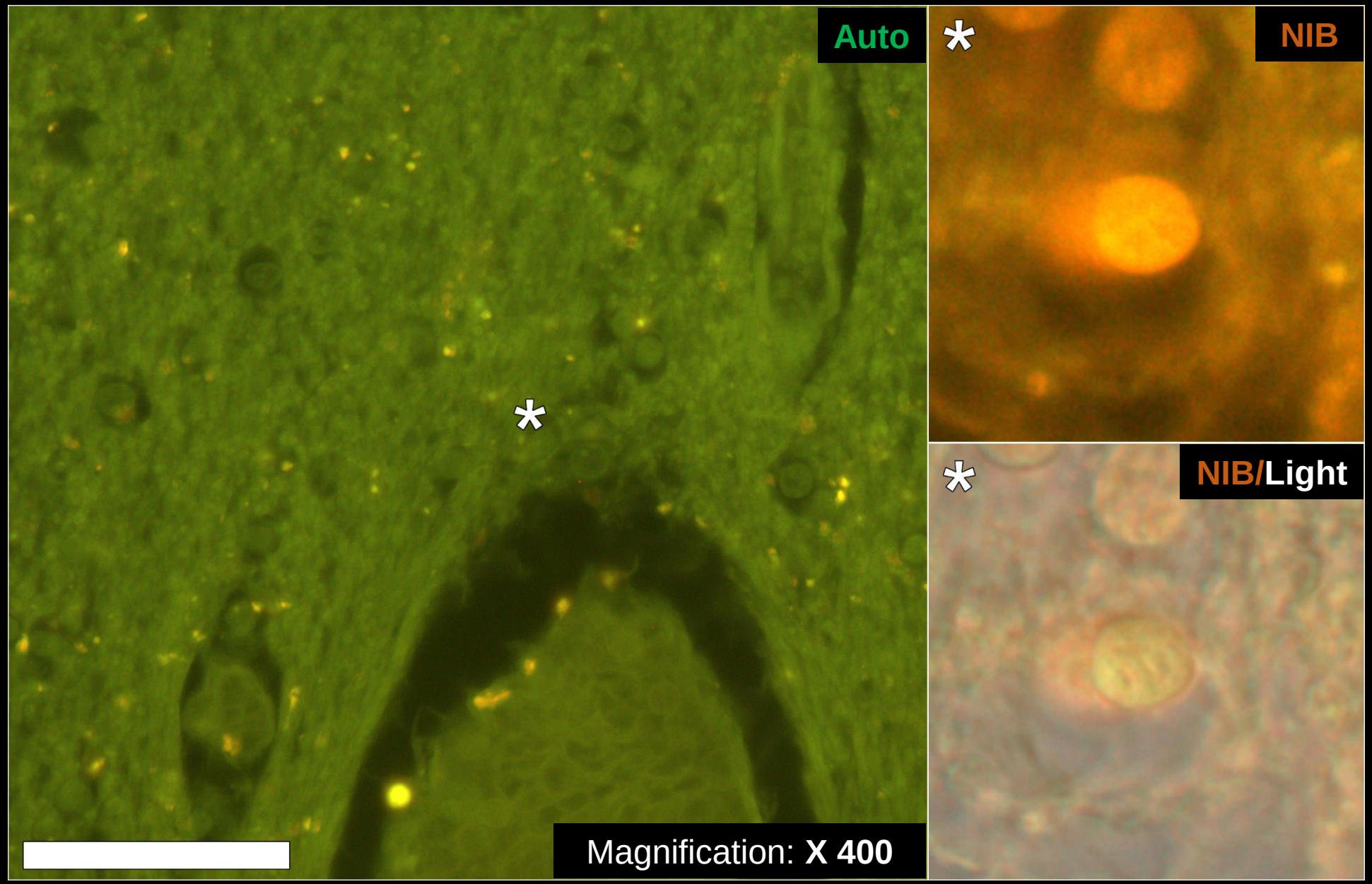
Oxford Brain Bank, Oxford, UK.

- **Parietal lobe**



Aluminium in glial cells. (Region: #2)

- **Parietal lobe**

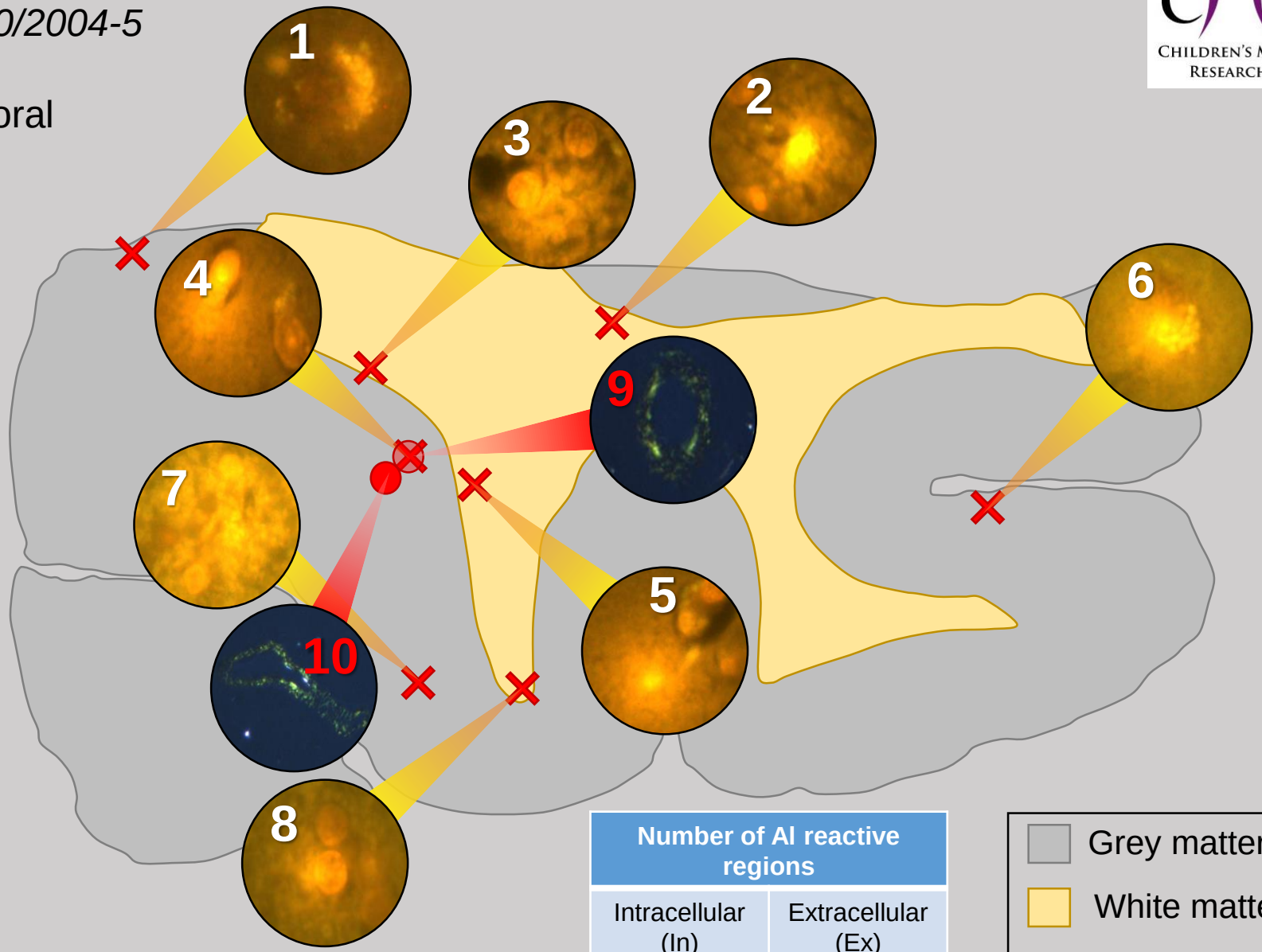


Aluminium in glial cells. (Region: #2)

Patient ID:
NP040/2004-5
Lobe:
Temporal

CASE STUDY 1

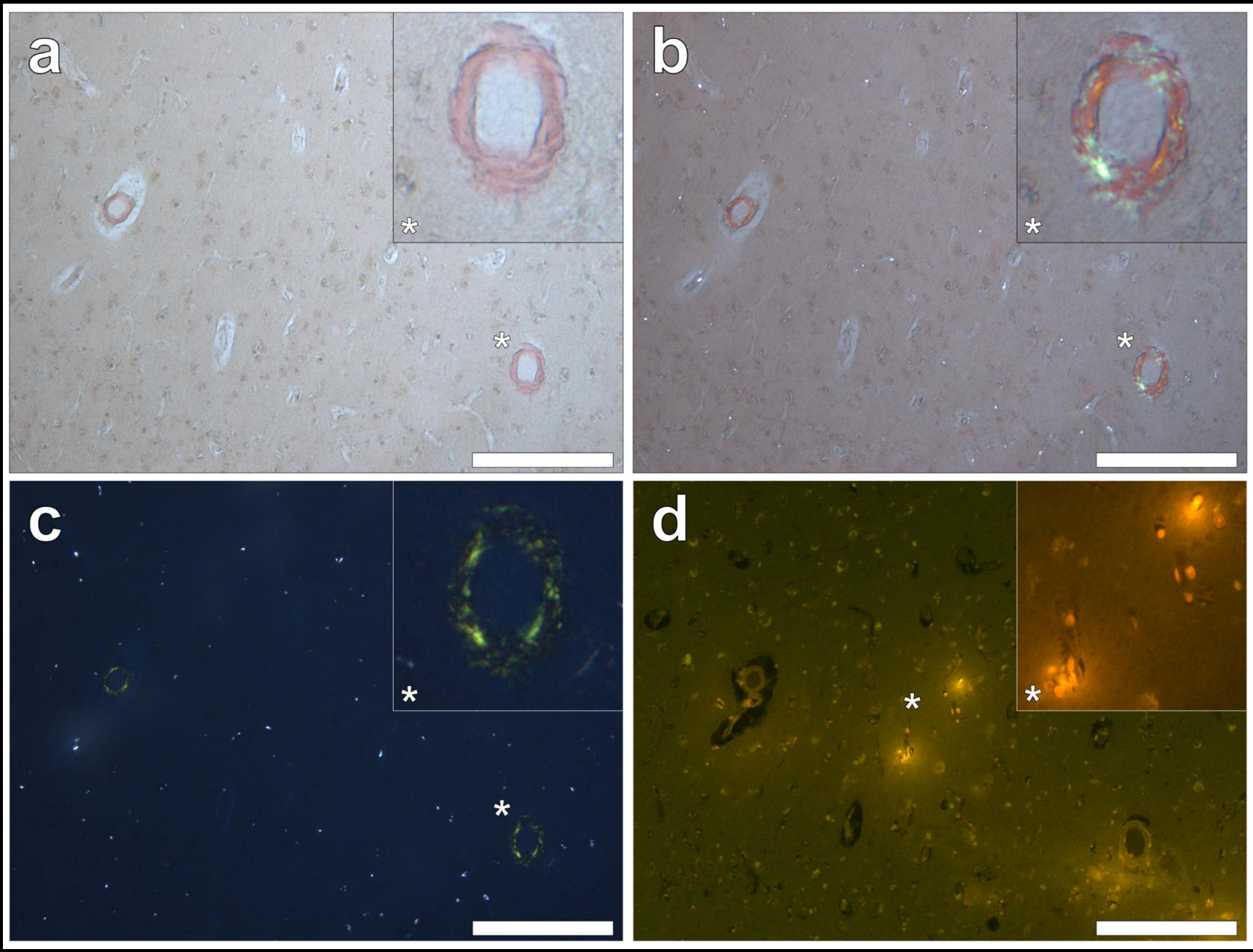
CMSRI
Children's Medical Safety Research Institute



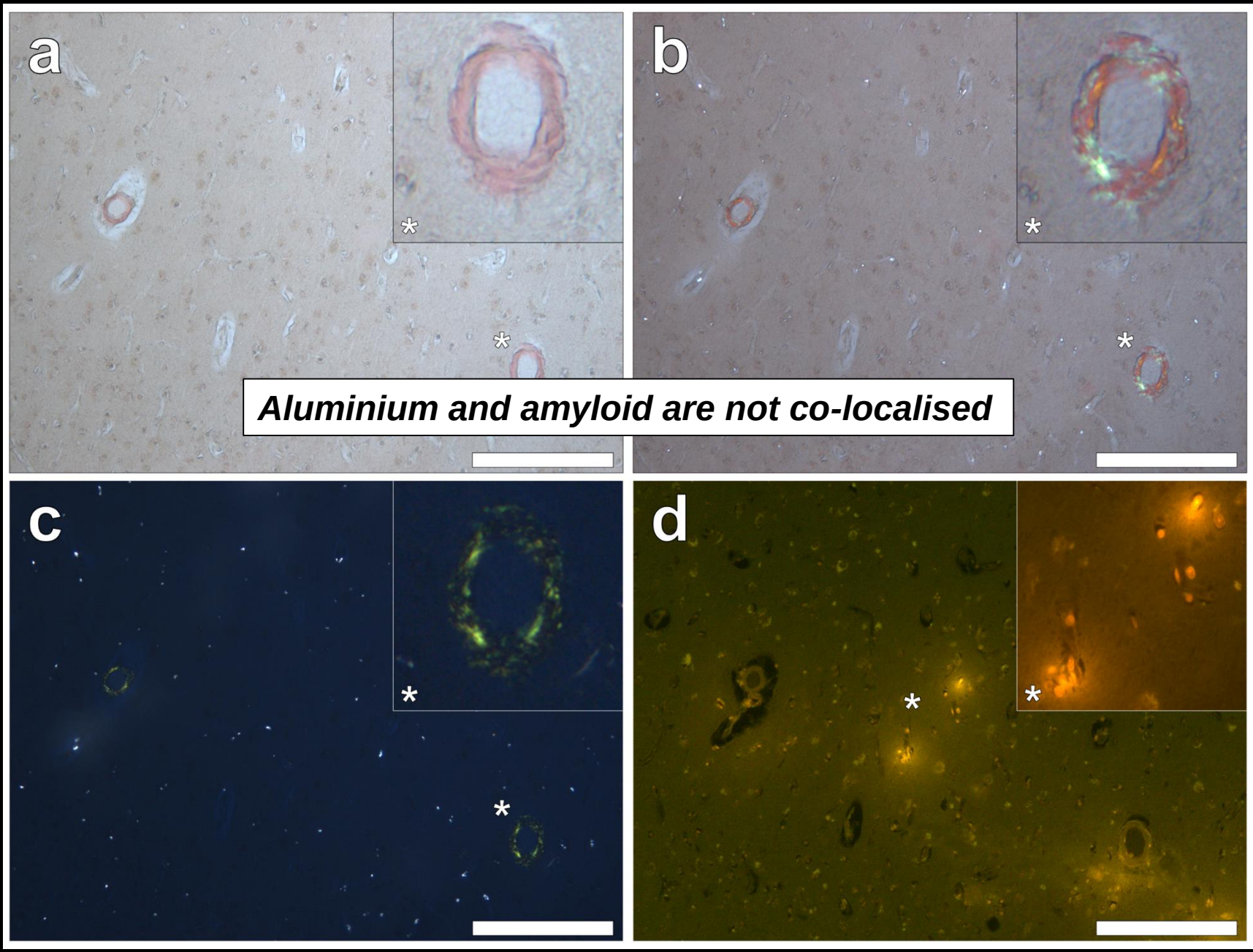
Number of AI reactive regions	
Intracellular (In)	Extracellular (Ex)
6	2

- Grey matter (GM)
- White matter (WM)
- AI reactive
- CR positive

Oxford Brain Bank, Oxford, UK.



a: Congo red, light, (b): ½ polarised, (c): polarised, (d): lumogallion staining.



a: Congo red, light, (b): ½ polarised, (c): polarised, (d): lumogallion staining.

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Environmental Research and Public Health

MDPI

Case Report

Aluminium in Brain Tissue in Epilepsy: A Case Report from Camelford

Matthew Mold [1], Jason Cottle [2] and Christopher Exley [1,*]

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[2] School of Medicine, David Weatherly Building, Keele University, Staffordshire ST5 5BG, UK; jasoncottlee@gmail.com
* Correspondence: c.exley@keele.ac.uk

Received: 2 May 2019; Accepted: 14 June 2019; Published: 16 June 2019

Abstract: (1) Introduction: Human exposure to aluminium is a burgeoning problem. In 1988, the population of the Cornish town of Camelford was exposed to exceedingly high levels of aluminium in their potable water supply. Herein we provide evidence that aluminium played a role in the death of a Camelford resident following development of late-onset epilepsy. (2) Case summary: We have measured the aluminium content of brain tissue in this individual and demonstrated significant accumulations of aluminium in the hippocampus (4.35 (2.80) μg/g dry wt.) and the occipital lobe (2.22 (2.23) μg/g dry wt., mean, SD, $n = 5$), the latter being associated with abnormal calcifications. Aluminium-specific fluorescence microscopy confirmed the presence of aluminium in both of these tissues and made the consistent observation of aluminium-loaded glial cells in close proximity to aluminium-rich cell/neuronal debris. These observations support an inflammatory component in this case of late-onset epilepsy. Congo red failed to identify any amyloid deposits in any tissue while thioflavin S showed extensive extracellular and intracellular tau pathologies. (3) Discussion: We present the first data showing aluminium in brain tissue in epilepsy and suggest, in light of complementary evidence from scientific literature, the first evidence that aluminium played a role in the advent of this case of late-onset adult epilepsy.

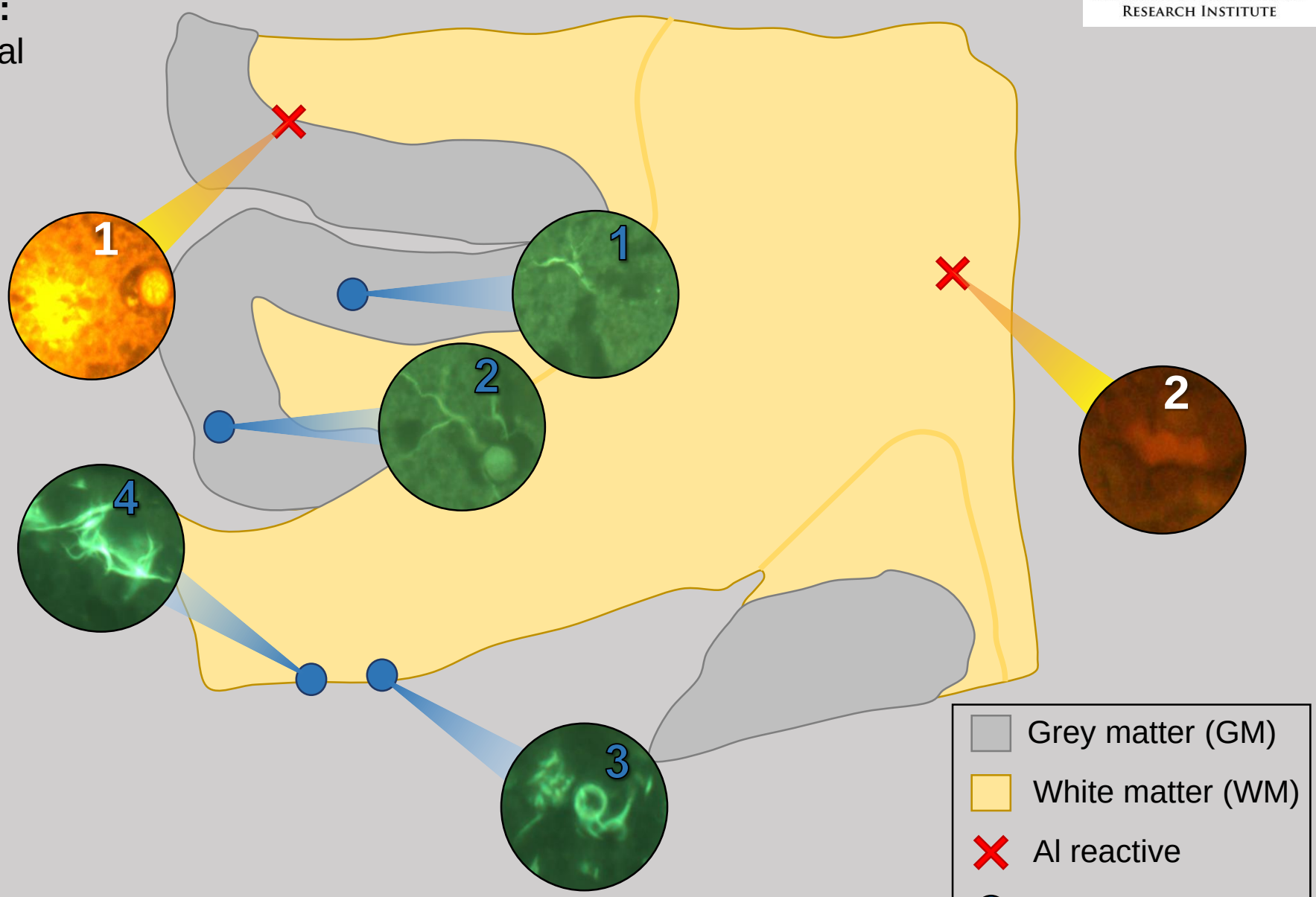
Keywords: aluminium in brain tissue; epilepsy; aluminium-specific fluorescence; occipital calcifications; tau pathologies; Camelford in Cornwall

Mold et al., 2019. IJERPH. 16(12): 2129.

Patient ID:
PB1286-10
Lobe:
Frontal

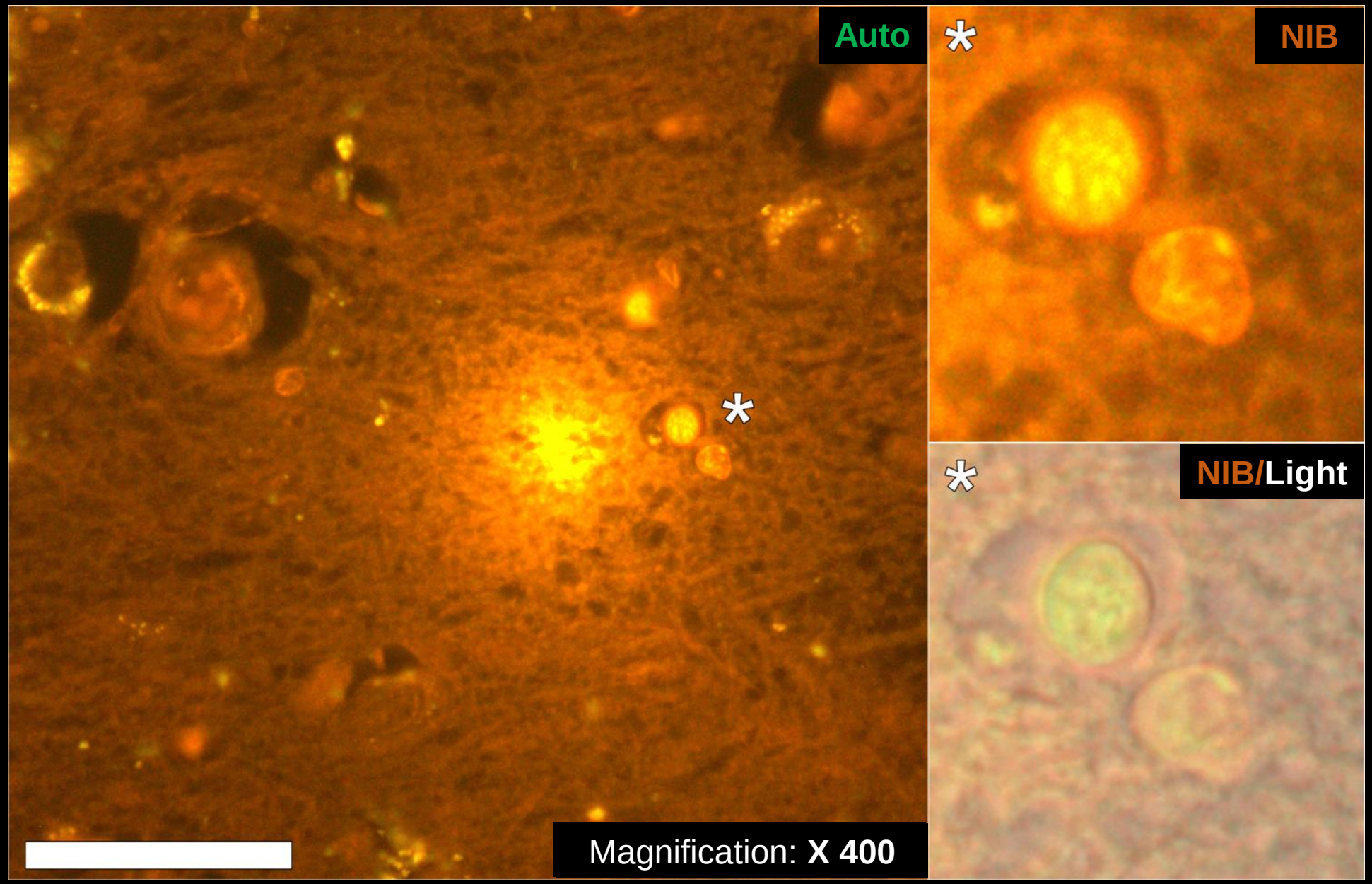
CASE STUDY 2

CMSRI
Children's Medical Safety Research Institute



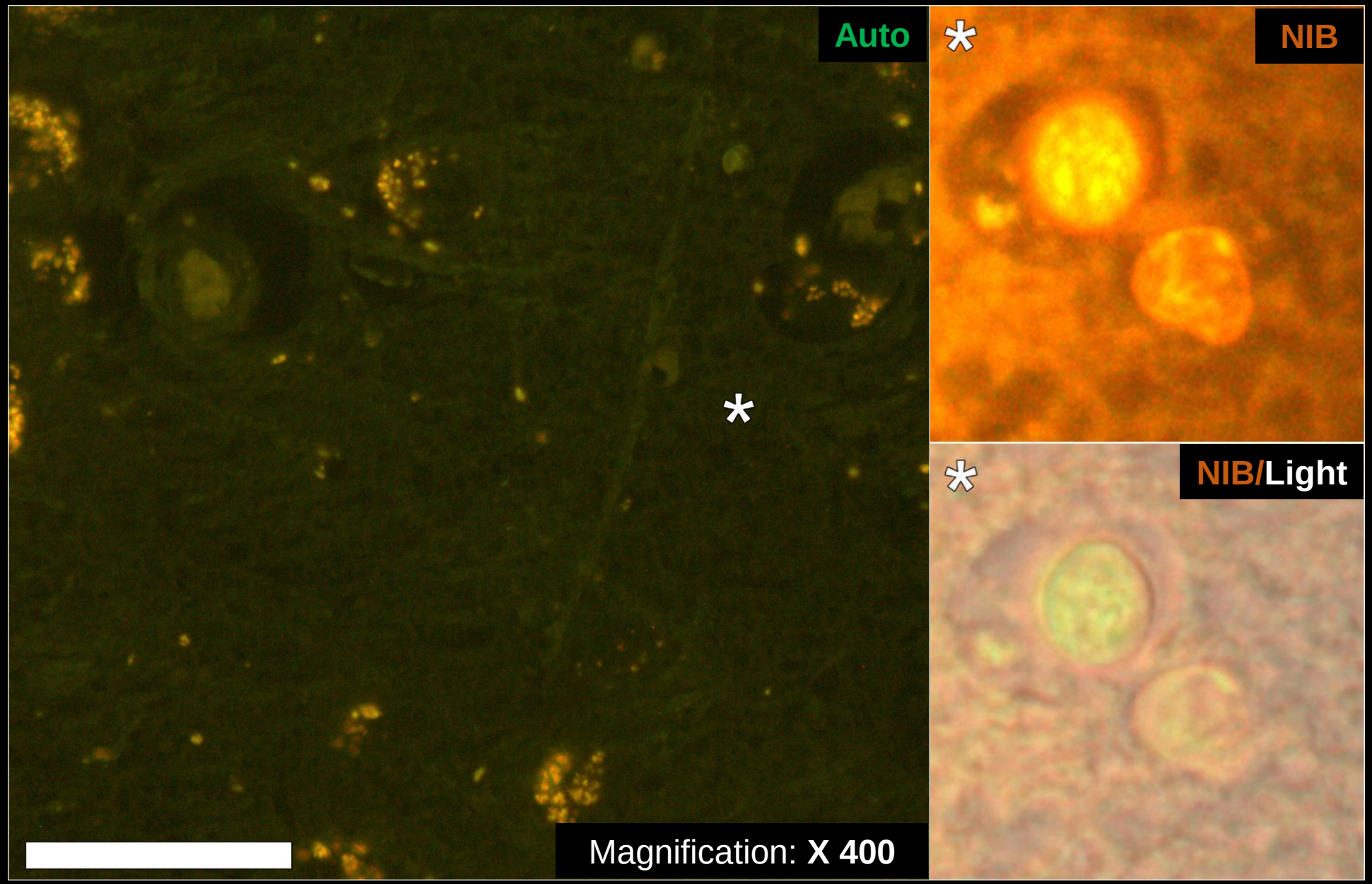
NHS, Plymouth, UK.

- **Frontal cortex**

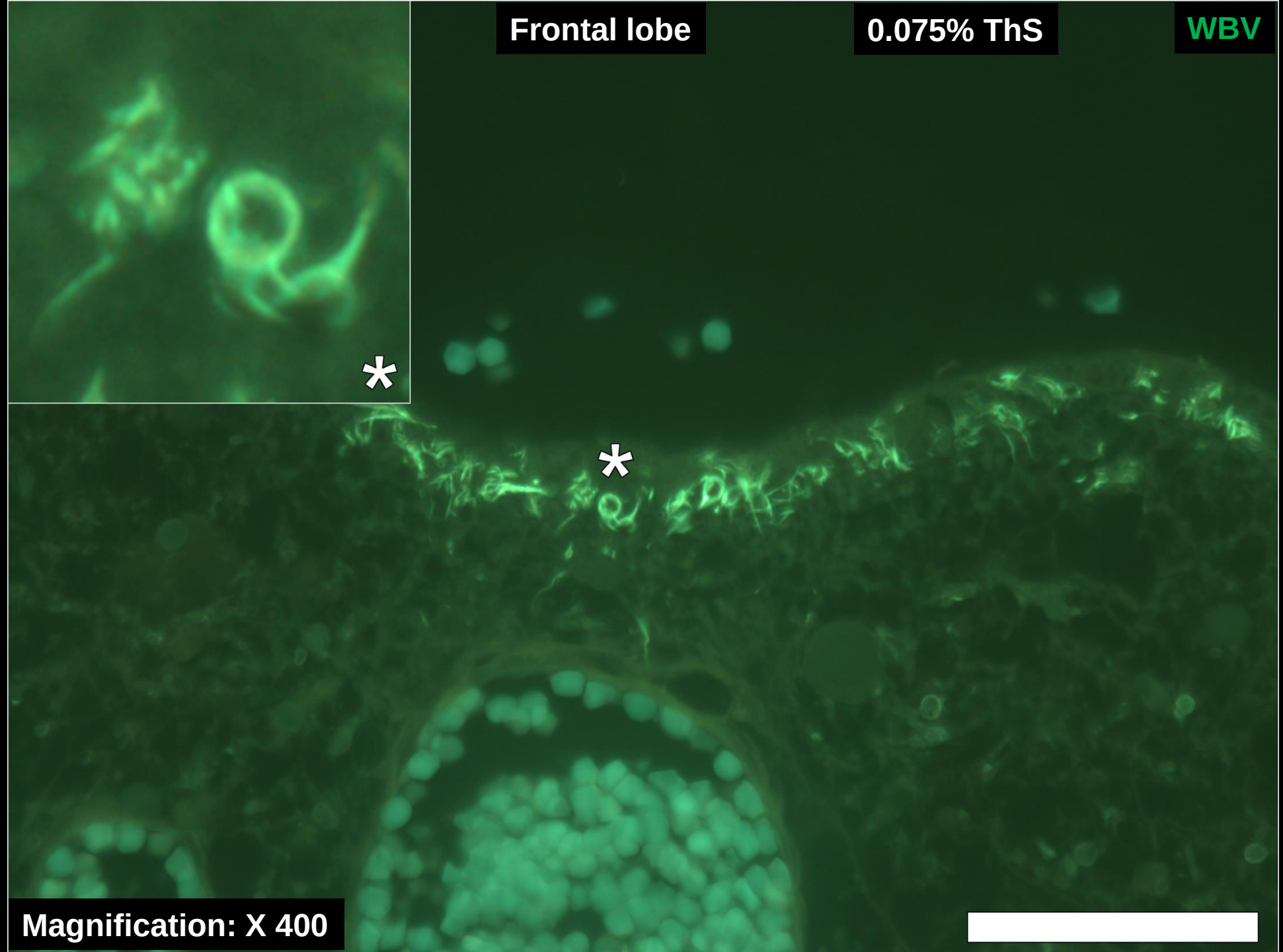


. Intracellular and extracellular aluminium (Region: #1)

- **Frontal cortex**

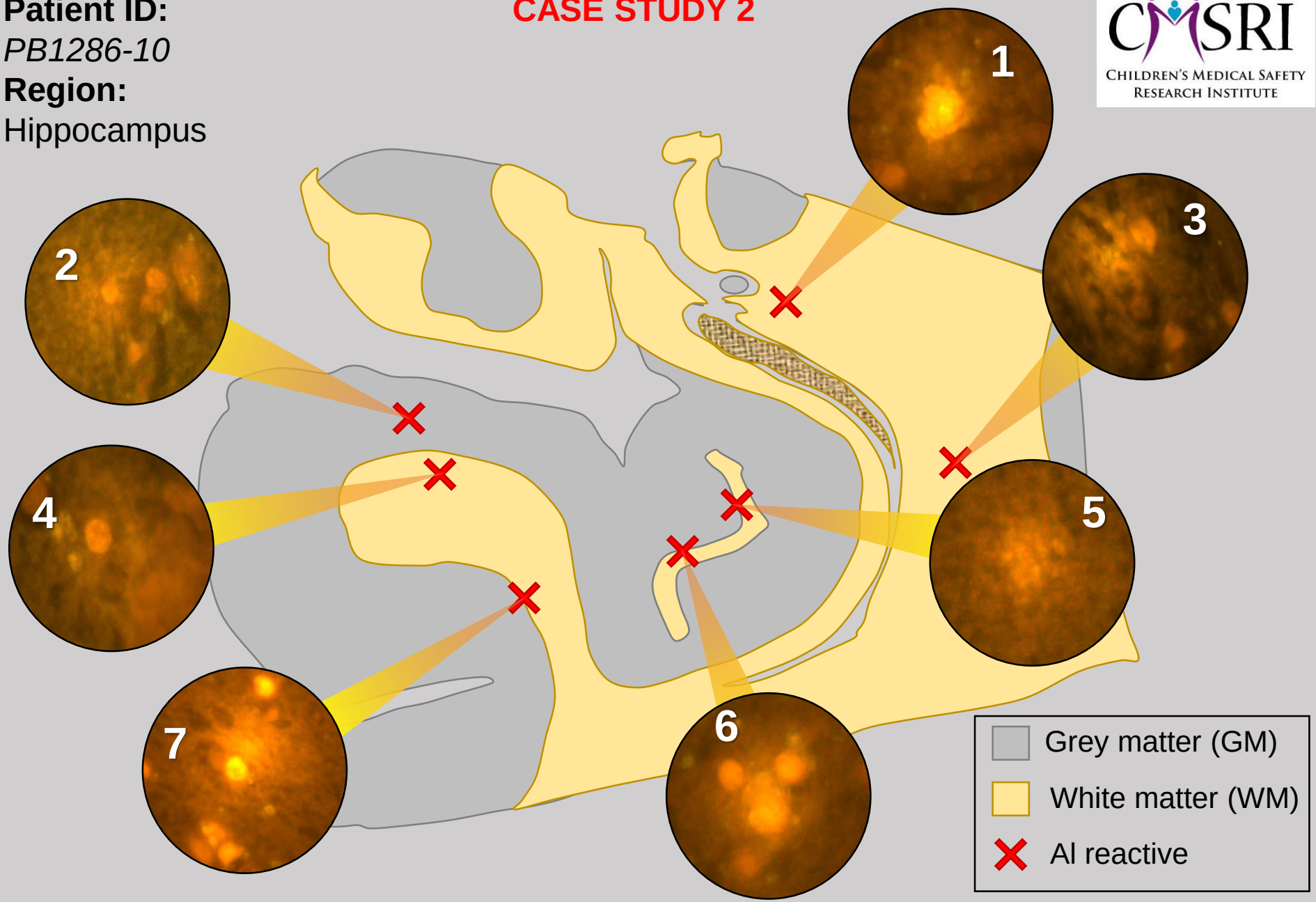


. Intracellular and extracellular aluminium (Region: #1)


Frontal lobe
0.075% ThS
WBV
Magnification: X 400

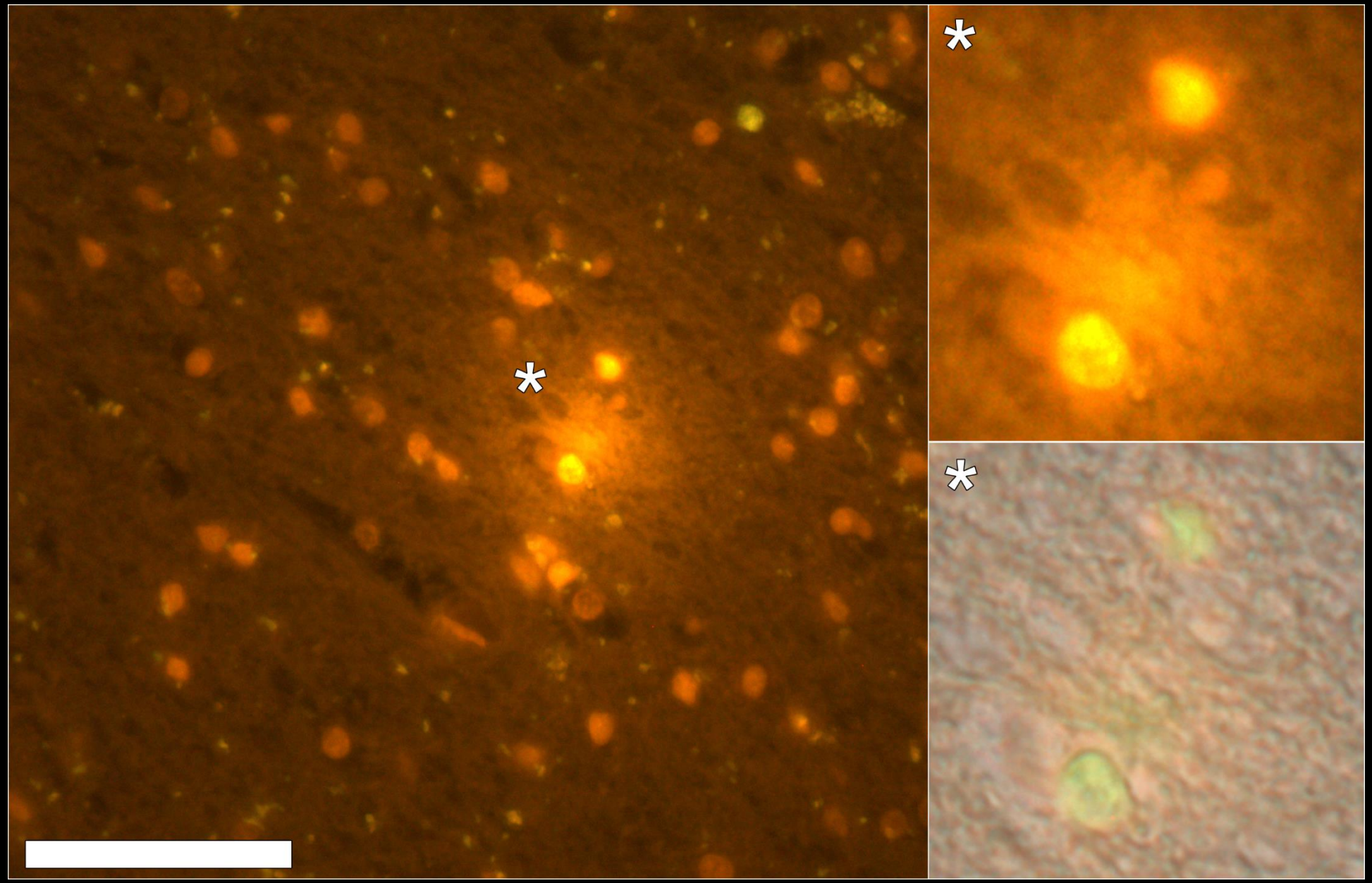
Patient ID:
PB1286-10
Region:
Hippocampus

CASE STUDY 2



NHS, Plymouth, UK.

Region 7, 60-year-old Male: Epilepsy

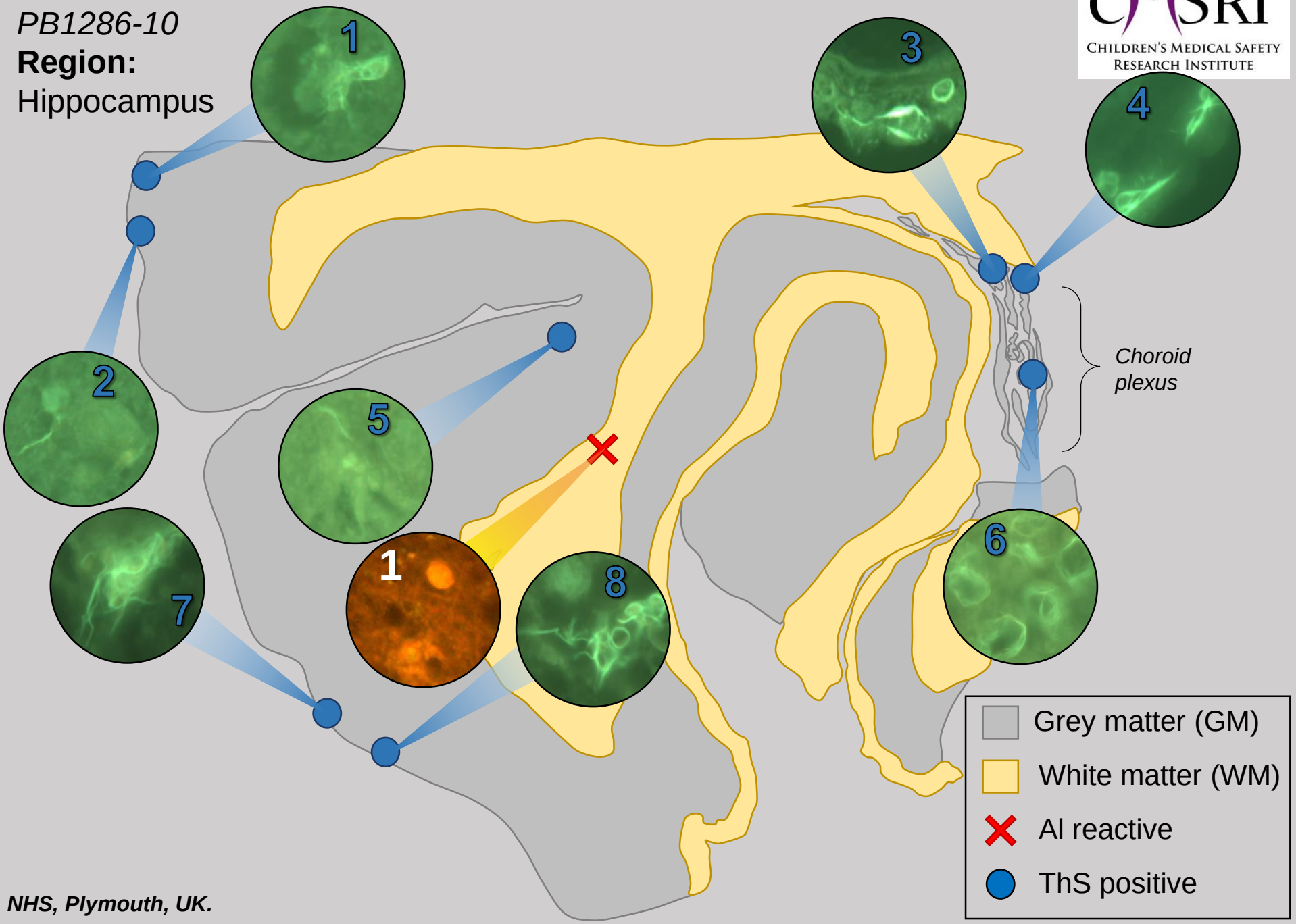


Intracellular aluminium in **glial cells** in the parahippocampal gyrus.

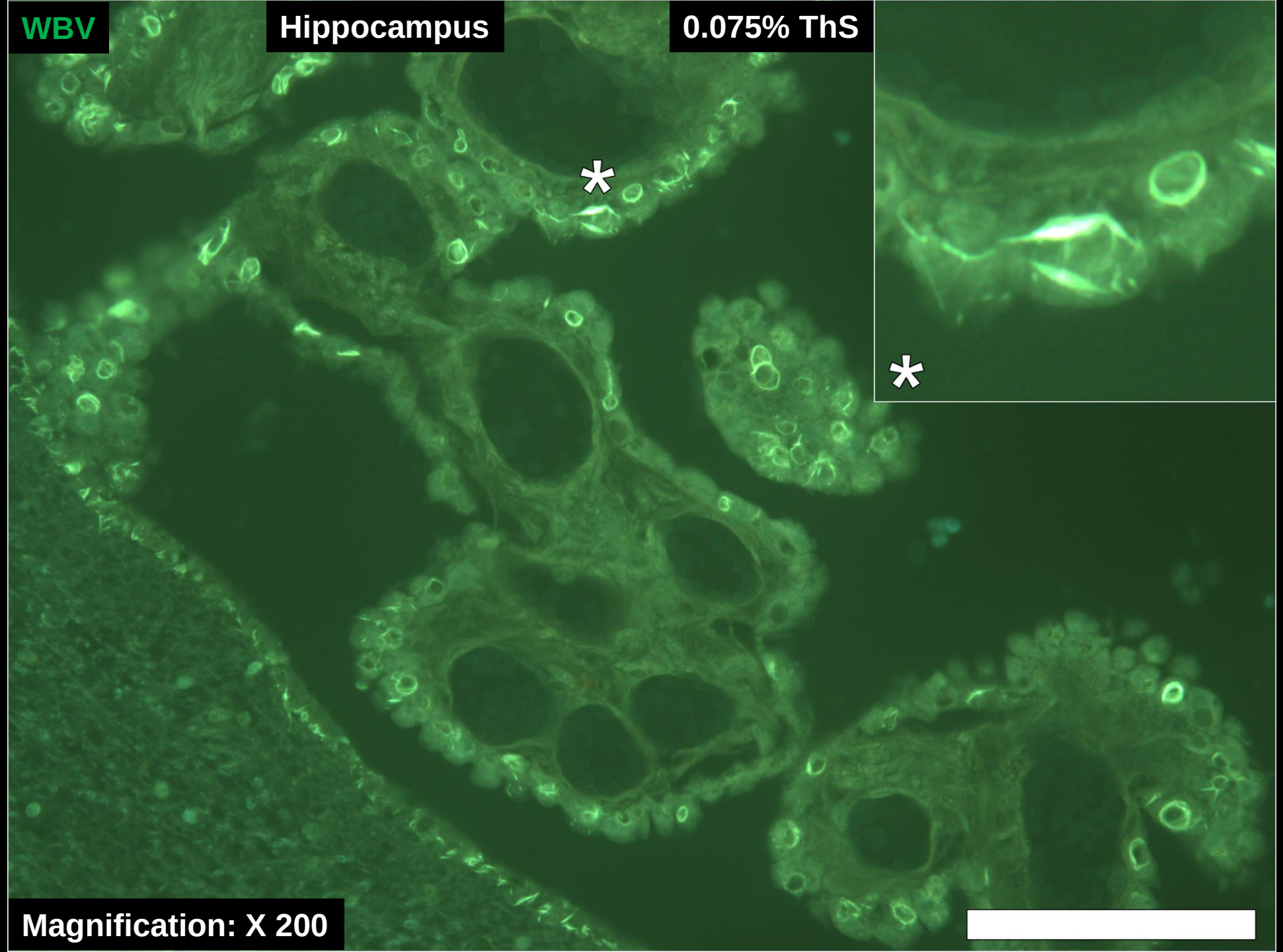
Patient ID:
PB1286-10
Region:
Hippocampus

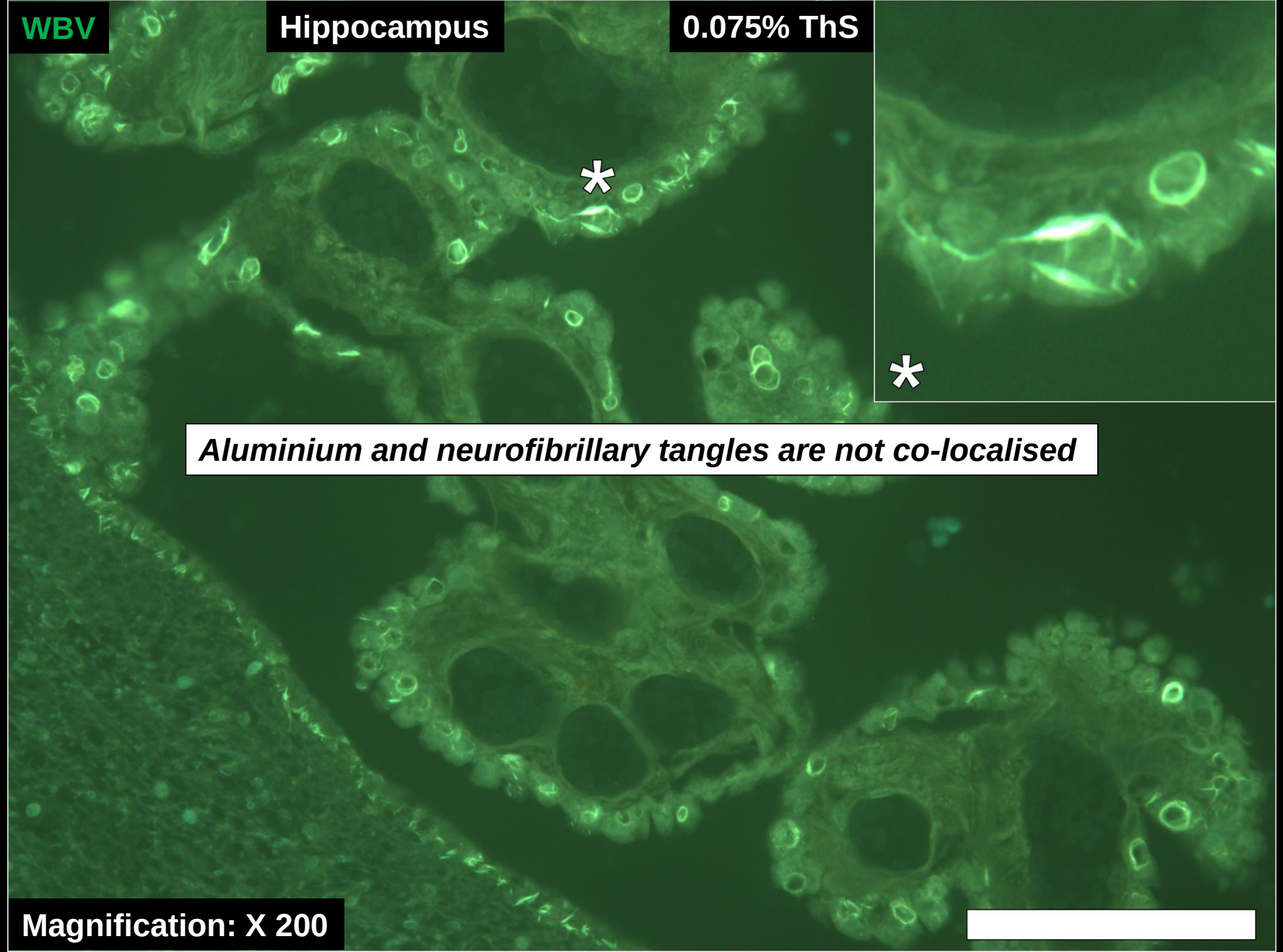
CASE STUDY 2

CMSRI
Children's Medical Safety Research Institute



NHS, Plymouth, UK.

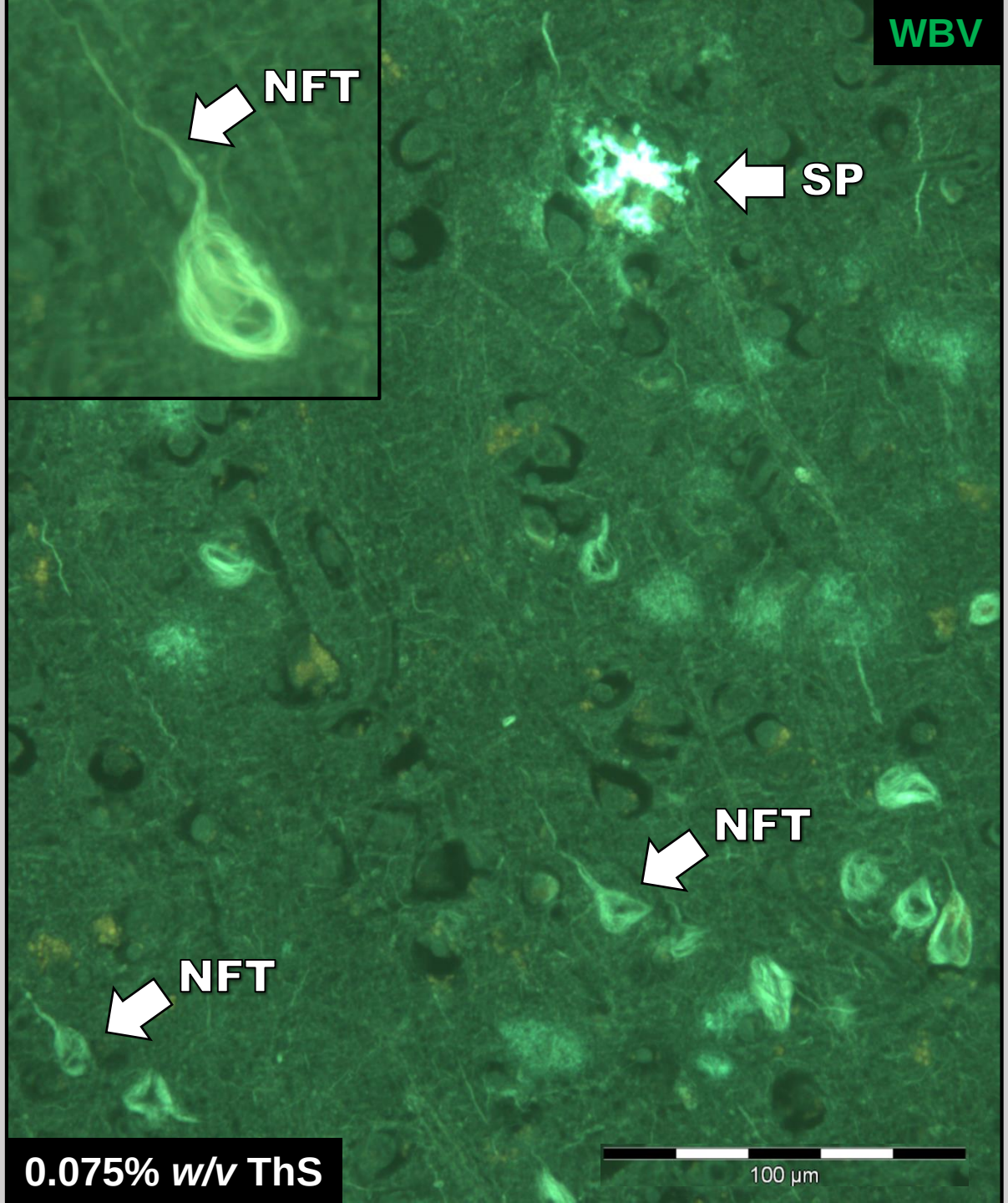

WBV
Hippocampus
0.075% ThS
Magnification: X 200


WBV
Hippocampus
0.075% ThS
*
*
Aluminium and neurofibrillary tangles are not co-localised
Magnification: X 200

CURRENT RESEARCH

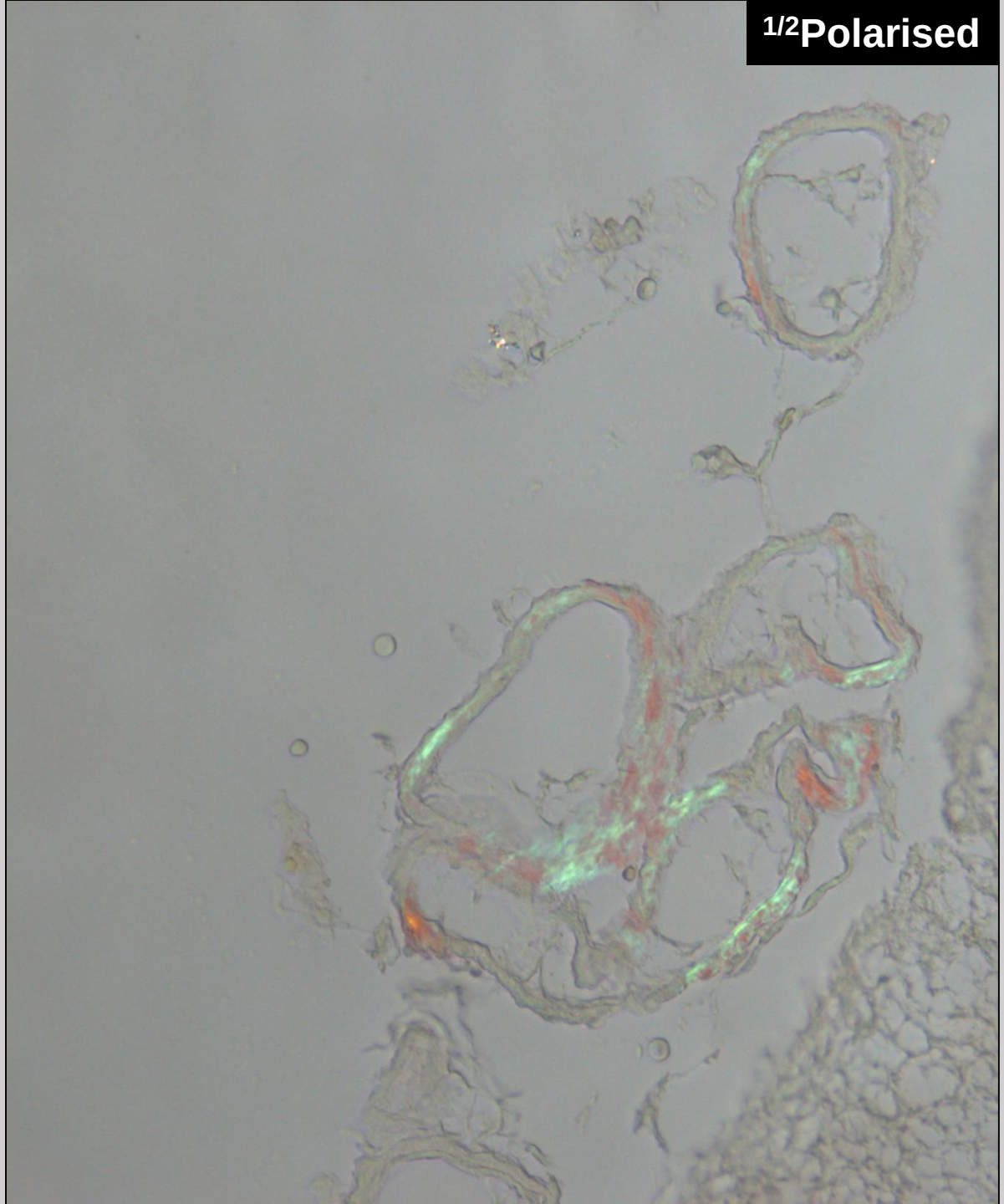
Colombian donor tissues:

Familial Alzheimer's disease (PSEN1 E280A)



Thioflavin S Staining

- Colombian donor presenting with *PSEN1 E280A* mutation.
- Early onset / familial Alzheimer's disease (fAD).
- Temporal cortex.
- **Thioflavin S** (ThS) staining reveals senile plaques (**SP**) and neurofibrillary tangles (**NFT**).

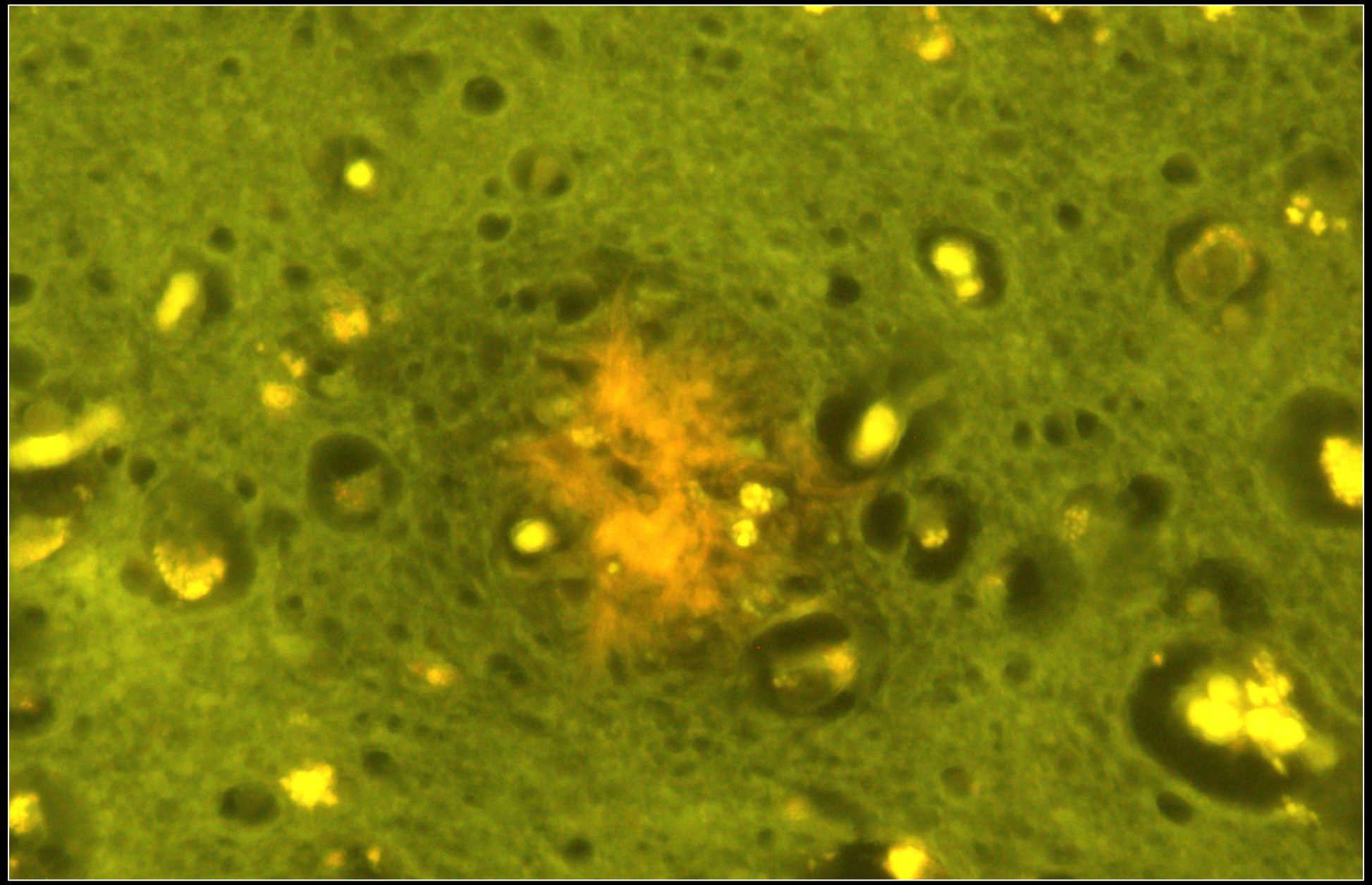


Congo Red

- Positive amyloid staining revealing Congophilic amyloid angiopathy (**CAA**).

- **Frontal lobe**

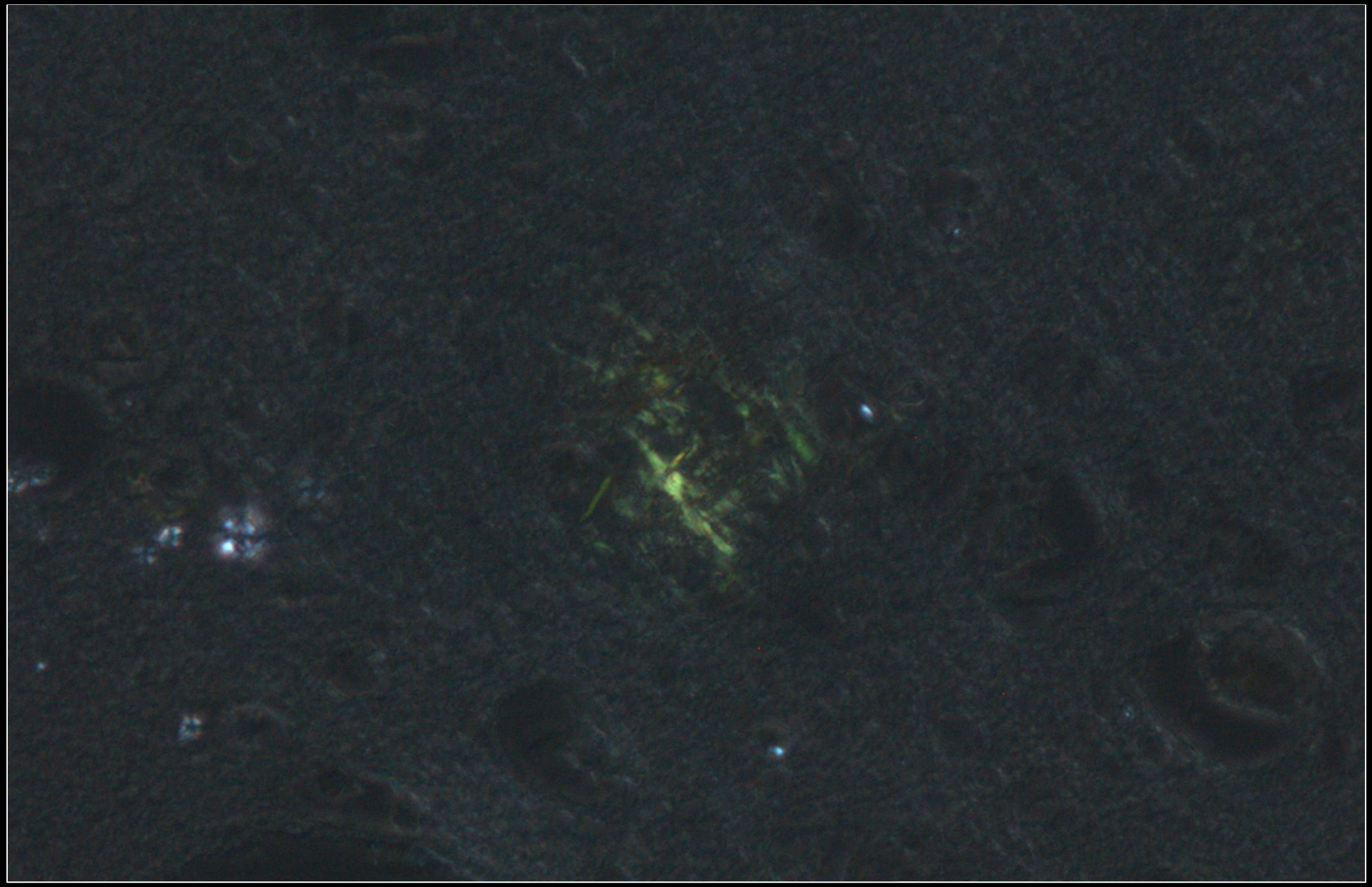
NIB / Lumo



Cortical senile plaque.

- **Frontal lobe**

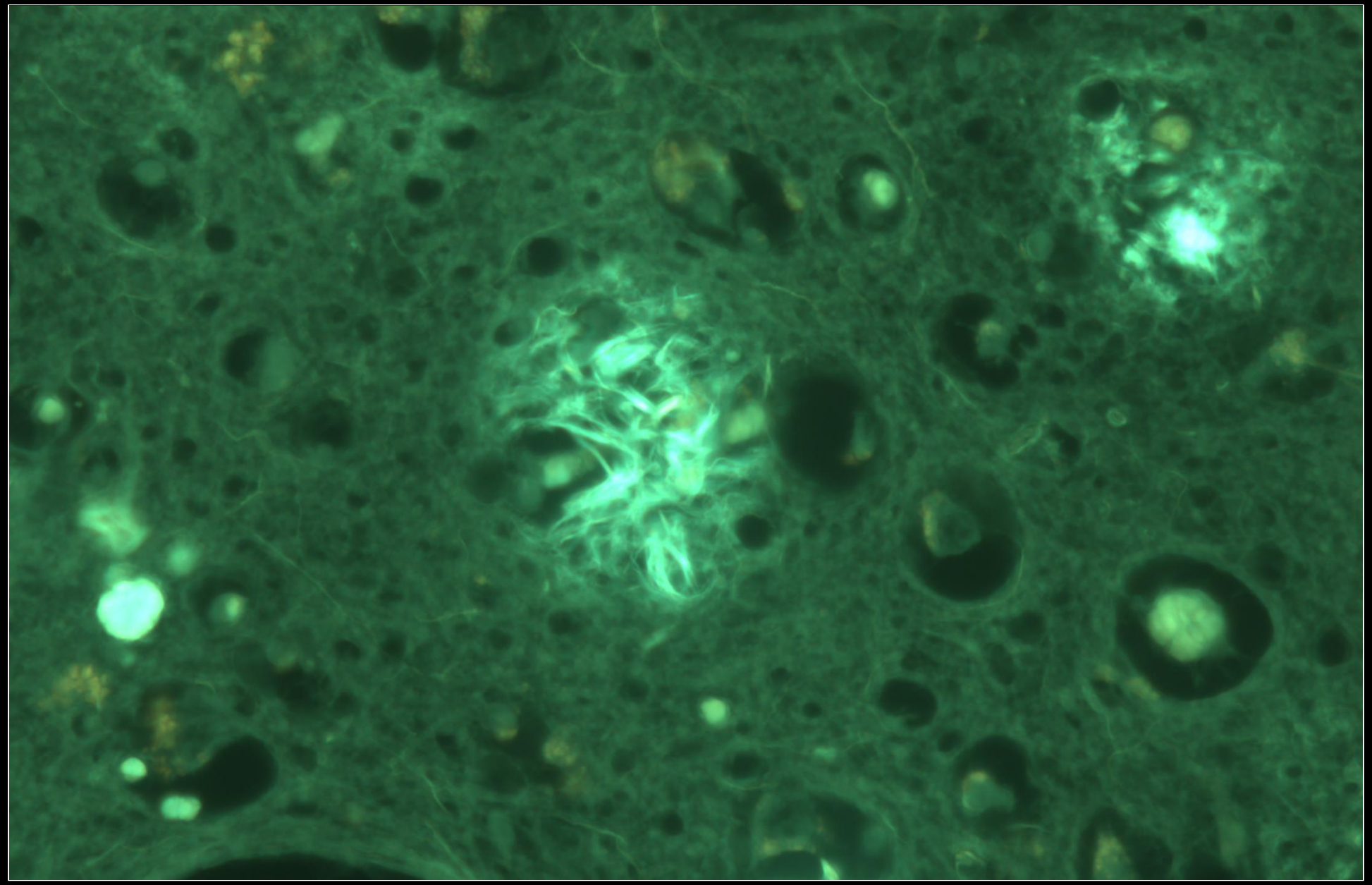
Polarised



Cortical senile plaque.

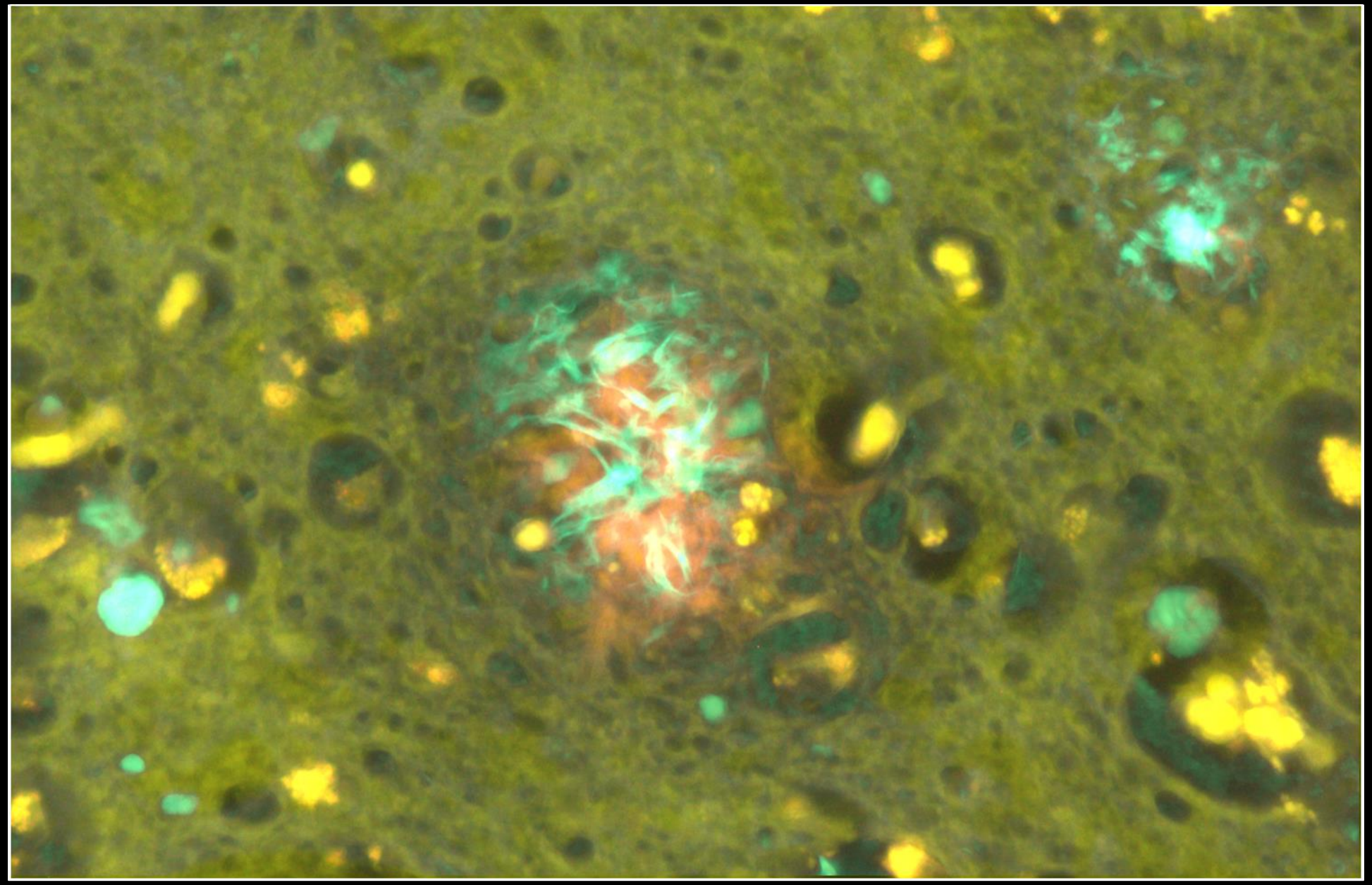
- **Frontal lobe**

WBV / ThS



Cortical senile plaque.

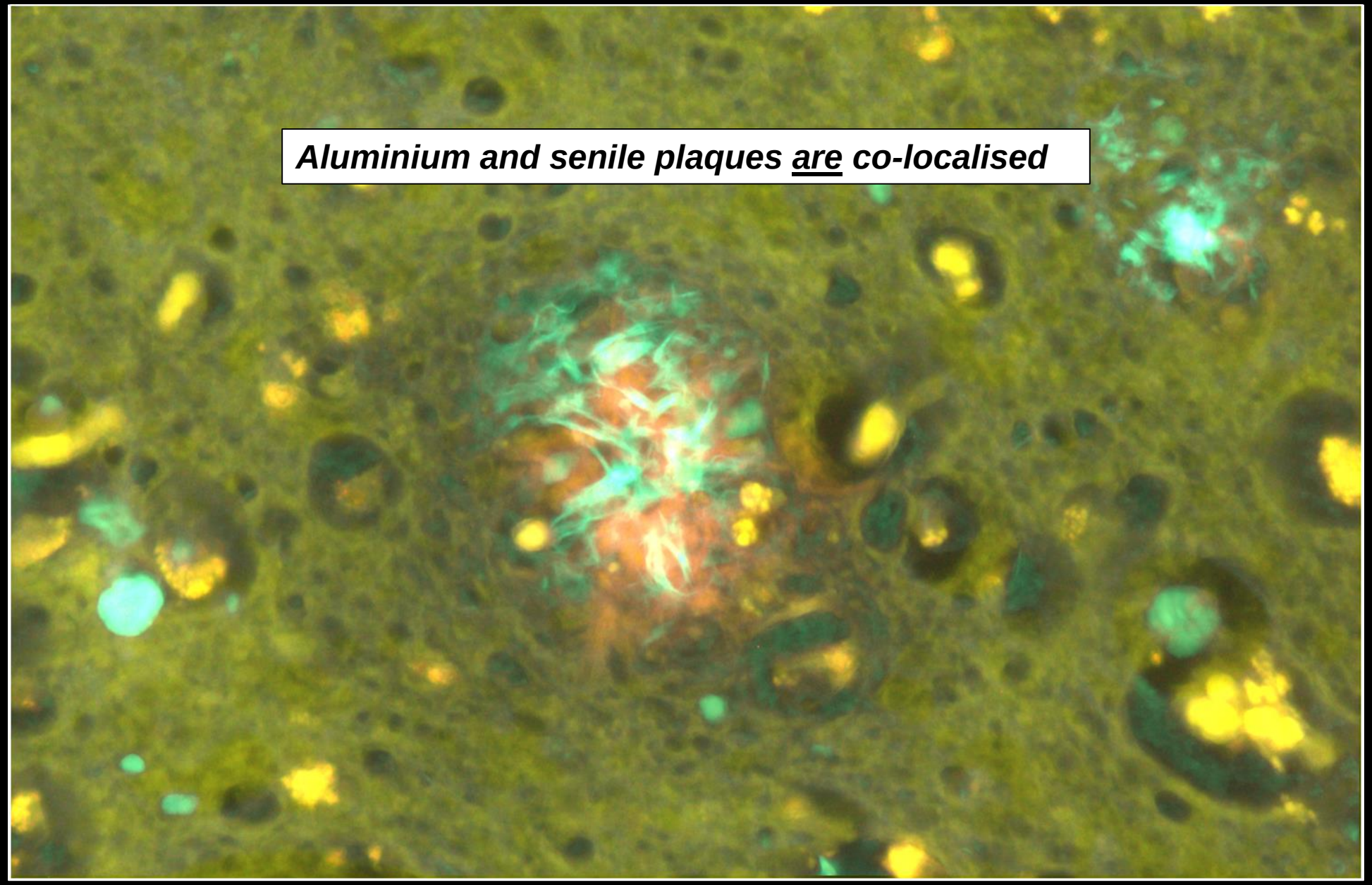
- **Frontal lobe** NIB / Lumo Polarised WBV / ThS



Cortical senile plaque.

- **Frontal lobe**

NIB / Lumo Polarised WBV / ThS



Cortical senile plaque.