Health Science Inquiry

# Imaging Glial Cells in Alzheimer's Disease:

## From Light Microscopy to Multiplexed Ion Beam Imaging

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## Introduction

Alzheimer's Disease (AD) is the leading cause of dementia worldwide, accounting for 60-70% of the nearly 50 million global cases of dementia in 2018 [1]. AD also significantly burdens Canadians, where the disease prevalence is 1,524 cases per 100,000 and incidence is 68 case per 100,000 [2]. The global societal costs of AD and dementia are estimated at $1 trillion dollars annually [1]. Furthermore, AD treatment options remain limited. All therapeutics targeted at decreasing production or increasing clearance of amyloid-$\beta$ plaques, the characteristic AD biomarker, have thus far been unsuccessful in phase III clinical trials [1]. With such a significant burden of disease and no effective treatments, it is clear that AD research must elucidate the complex mechanisms underlying clinical progression in order for the disease to be better prevented, managed, and treated.

Since the 1980's, researcher have understood AD through the amyloid cascade hypothesis (ACH). Specifically, the ACH posits that the clinical progression of AD is primarily caused by amyloid-$\beta$ plaque deposits, tau aggregation in neurofibrillary tangles, and subsequent neuron death [3]. While the original ACH remains widely accepted in the field, new research has highlighted the key role of glial cells, primarily microglia and astrocytes, in AD pathogenesis [3]. It is now thought that the intricate interactions between microglia, astrocytes, and neurons becomes initially disturbed in early stages of the disease. As toxic amyloid-beta and hyperphosphorylated tau protein accumulation continues, these glial cells polarize towards an inflammatory phenotype that further contributes to neuron death [3]. However, research aimed at understand the complex interactions between tauopathy and amyloid-$\beta$ accumulation, s glial cell phenotypes, neuroinflammation, and neuron death, has been slowed by single-plex imaging technologies and staining technologies. This paper will take a historical approach to the evolution of neuroimaging of glial cells in the context of AD, where the limitations of traditional light microscopy (LM) and immunohistochemical (IHC) techniques will be contrasted to the promising application of the novel imaging technology, High-Dimensional Multiplexed Ion Beam Imaging-Time of Flight(MIBI-TOF).

## Microscopy, Staining Techniques, and Glial Cell Imaging in AD

The timeline of major glial imaging discoveries in the context of AD can be seen in Figure 1. In 1846, Rudolf Virchow, considered the father of modern pathology, was the first to propose a new type of cell within the brain, which he termed 'neuroglia' [4]. Subsequently, many scientists continued to refine this notion of glial cells. Notably, the neuroscientists Santiago Ramón y Cajal used a chloride-sublimate staining technique to label glial fibrillary acidic protein (GFAP) expressed by astrocytes in 1913, and Rio Hortega described microglia in 1921 [4]. At approximately the same time as these two glial cell discoveries, neuropathologist Alois Alzheimer analyzed post-mortem brain tissue biospies from a patient presenting with symptoms of dementia and in 1907 he discovered what is now known as amyloid-$\beta$ plaque deposits and tau protein aggregations [5]. The cardinal tool of these scientists was LM combined with various staining techniques, and while they were able to make breakthrough discoveries, they were also limited by their imaging technologies which only permitted simultaneous visualization of one to two protein targets.

During the time that Alzheimer continued to research this new disease, the Carl Zeiss Company produced a novel microscopy technique known as fluorescence microscopy (FM), which uses ultraviolet light to excite fluorophores on target proteins to produce an image [6]. The principles of FM gave rise to IHC and immunofluorescence (IF) staining techniques. IHC and IF use antibody-antigen specificity to attach fluorophores to target antigens and cause light emission via enzymatic reactions or excitation, respectively. The advent of FM, as well as IHC and IF, have allowed for significant contributions to AD research. For instance, the involvement of microglia and astrocytes in AD pathogenesis was discovered using IHC microscopy [7] and the pathology of amyloid-$\beta$ plaques and neuron death was further elucidated via IF microscopy [8] (Figure 1).

Despite the progression of these various microscopy technologies, they are still limited by only being able to simultaneously visualize a small number of target genes or antigens. This is because the fluorophores and enzyme reporters that are used in current IHC protocols show spatial and spec-

tral overlap when analyzing multiple targets [9]. As new research has elucidated that AD pathogenesis consists of interactions between multiple cells and cellular phenotypes, the need for a technology able to image a large number of antigens while also maintaining the spatial localization of these interactions has become increasingly apparent.

## High-Dimensional Multiplexed Imaging and Potential Applications in AD

High-dimensional multiplexed imaging has the capacity to overlay multiple images with different biomarkers for simultaneous analysis and spatial localization. One example of this technology is MIBI-TOF (Figure 1). A recent study published in Cell by Keren *et al.* (2018) highlighted MIBI-TOF technology within the application of breast cancer pathobiology. This work built on a previous MIBI platform by adding TOF mass spectrometry, which allowed for increased channel multiplexing while decreasing times for data acquisition by 50-fold. With this improved technology, researchers simultaneously analyzed the sub-cellular expression and spatial arrangement of 36 proteins, effectively illuminating the tumor-immune microenvironment [9]. Researchers at Stanford have also used MIBI-TOF to analyze post-mortem brain samples of AD. In addition to validating previously defined AD pathologies, such as taupathothy, they were also able to analyze immune cell infiltration, cellular anatomy, and gene expression associated with AD [10]. This is an improvement to previous imaging techniques in AD research as MIBI-TOF can now simultaneously determine the tissue, immunological, and sub-cellular spatial localization of the human brain [10]. In both tumor pathology and AD imaging, MIBI-TOF is able to generate multiplexed imaging by overcoming the spectral and spatial overlap of fluorophores and enzyme reporters that confine IHC to a single-plexed analysis.

While MIBI-TOF has great potential, it is also limited in a number of ways. Firstly, there are a very few available units at this time as the technology has only recently become commercially available. Secondly, MIBI-TOF poses a high cost of reagents (*e.g.* metal-tagged antibodies), technical experiments, and quality control. Lastly, since MIBI-TOF generates highly dimensional data, researchers must have computational expertise to use the platform effectively [9]. As a result, MIBI-TOF is currently a much less accessible research tool compared to less expensive, simpler technologies such IHC staining visualized with LM.

## Future Directions and Conclusion

As new research has shown that AD pathogenesis is characterized by the complex interaction of multiple glial cells and their unique phenotypes, the advent of MIBI-TOF provides the capacity to elucidate these cellular interactions and provide insights into new biomarkers and potential therapeutic avenues. Indeed, MIBI-TOF technology has the potential to revolutionize neuroimaging in AD research. As this technology continues to be used throughout AD research, its true potential will be realized.

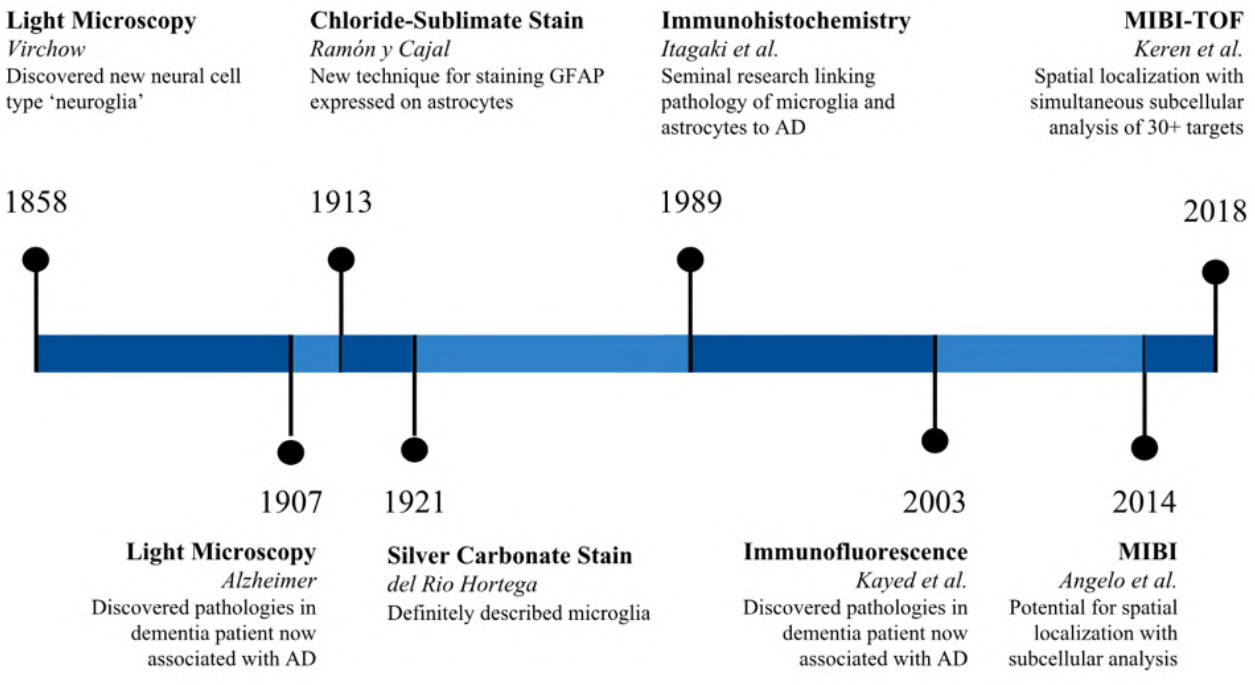


Figure 1: Historical timeline highlighting the various imaging technologies and staining techniques that have ultimately contributed to the current understanding of the role of glial cells in AD pathology. MIBI time-of-flight (TOF) is seen in the timeline near the present day as it has the potential to revolutionize neuroimaging in AD research.

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Dunavan Morris-Janzen will receive his Bachelor of Arts and Science (Honours) from McMaster University in April 2019. During his undergraduate degree, his research interests were broadly set in immunology. In his final year under the supervision of Dr. John Paul Oliveria, he focused on the role of glial cells in Alzheimer's Disease pathology and the potential of Mulitplexed Ion Beam Imaging technologies for studying these cells.

John Paul Oliveria received his PhD in 2017 and is currently an Adjunct Faculty Member at McMaster University in the Faculty of Health Sciences, Department of Medicine, where he supervises students completing projects and theses within the fields of allergy, immunology, and neurosciences. He is currently completing his post-doctoral fellowship at Stanford University where he is utilizing multiplexed ion beam imaging (MIBI) to unravel cellular and sub-cellular interactions in the brain to understand Alzheimer's disease. His research interests leverages novel technologies and high dimensional data to discover novel mechanisms in health and disease.