Identification of black carbon in the brain using Raman microscopy

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INTRODUCTION

- Particulate Matter with aerodynamic diameter < 2.5um ($PM_{2.5}$) is a leading cause of global morbidity and mortality.
- $PM_{2.5}$ linked to central nervous system (CNS) disease, neuroinflammation and dementia.
- Ultrafine particles may directly enter CNS through olfactory bulb vs systemic circulation
- RAMAN microscopy detects chemical signatures of molecules.

RESEARCH QUESTIONS

1. Are black carbon particulates detectable in a quantifiable manner using RAMAN microscopy techniques?
2. Can $PM_{2.5}$ be found in human brain tissues and correlated to modeled exposure data?

METHODS

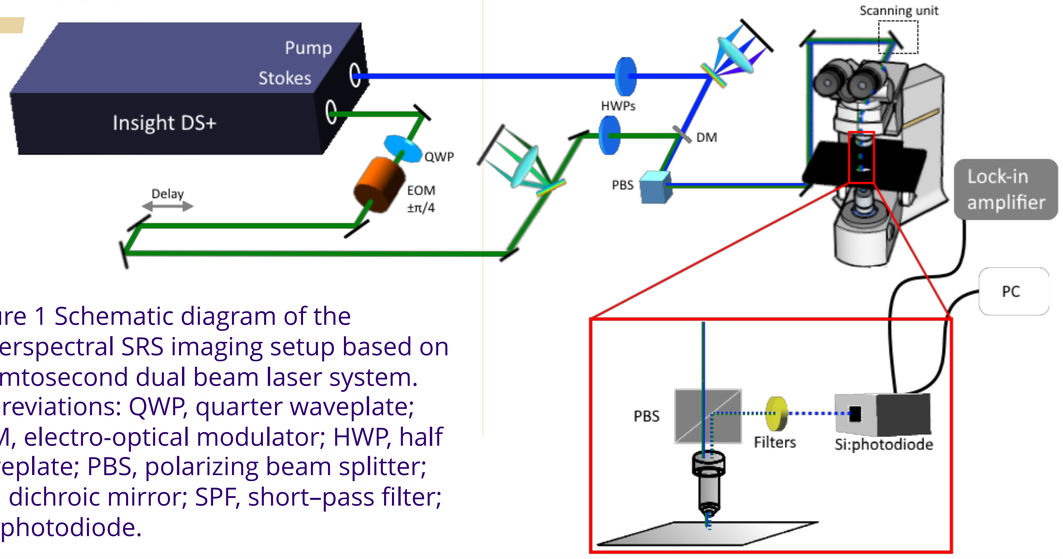

Figure 1 Schematic diagram of the hyperspectral SRS imaging setup based on a femtosecond dual beam laser system. Abbreviations: QWP, quarter waveplate; EOM, electro-optical modulator; HWP, half waveplate; PBS, polarizing beam splitter; DM, dichroic mirror; SPF, short–pass filter; PD, photodiode.

Study Population

Brain Biopsies of 20 subjects with high estimated pollution exposure from Adult Changes in Thought (ACT) study.

- Prospective Cohort of elderly (>65 yo) in Puget Sound area investigating changes in cognition.
- Subset of the cohort autopsied for direct neural tissue investigation and correlation to neuropathology.
- Specimens from olfactory bulb, frontal lobe, cerebellum.

Validation Steps

- Diesel soot imaged directly.
- Murine Alveolar Macrophages incubated with 0, 1, 5 or 30 ug/mL media of diesel soot for 24hrs.
- Murine Brain tissue from subjects perinatally exposed to diesel exhaust of 300ug/m^3 for 6hrs/d, 5d/wk x 6wks and naïve cohorts.

RAMAN imaging

Insight DS+ broadband femtosecond dual beam laser system was used for hyperspectral SRS imaging. (Fig. 1)

Ambient air pollution Exposure Model[1]

Spatiotemporal model incorporating data from regulatory monitors substituted with intensive community monitoring during prior research campaigns and low cost sensors from 1978-2019. Average 10 year exposure to ambient PM2.5 estimated at residential home address. The model has a cross validated R^2 (R^2_{CV}) of 0.87 with regulatory monitoring and 0.78 with low cost monitors.

Image Analysis

- Alveolar macrophages: BC fluorescence threshold determined and confirmed using FIJI. Relative intensity and concentration within the FOV to be correlated with linear regression.
- Brain specimens: Same thresholding analysis and correlation to estimated exposures obtained for the human subjects in the modelling study.

PRELIMINARY RESULTS

- Diesel soot: Fluorescence spectra between 1300 and 1600 cm^{-1} corresponding to similar published studies
- Murine Alveolar Macrophage data correlation analysis pending additional confirmation of imaging parameters.
- Murine brain tissues: Inconclusive identification of particulate matter with initial scans of the olfactory bulb regions.

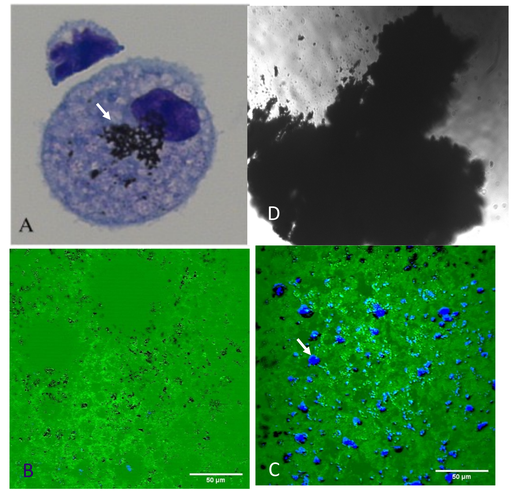

Figure 2: A) H&E stained alveolar macrophage with intracellular black carbon. B) Colorized fluorescence and SRS images of unexposed (B) and 30ug/mL media diesel soot exposed (C) murine macrophages. White arrows pointing to suspected black carbon particle aggregates. D) Brightfield image of diesel soot alone.

NEXT STEPS

- Finalize murine brain tissue analysis
- Completion of human specimen imaging.
- Image analysis to quantify particle concentrations.
- Correlation analysis to exposure estimates.

LIMITATIONS

Resolution of 2um reduces concentration estimates

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References:
1. R.M. Shaffer etal. (2021) J Alz Disease 79.pg 1761-73