Development of a Diagnostic Platform for Alzheimer's Disease Using Fluorescent Nanodiamond-Based Spin-Enhanced Lateral Flow Immunoassay

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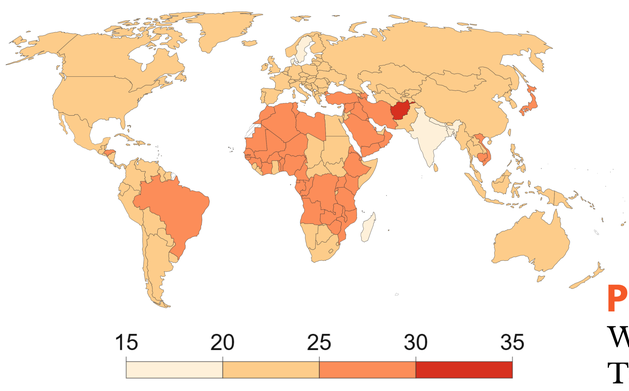
Abstract

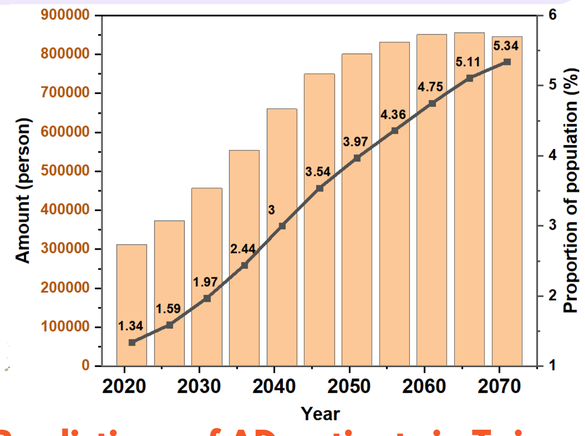
Alzheimer's disease (AD) is the most common cause of dementia; it is a degenerative neurological condition that leads to complete brain failure and death. Since Taiwan will become a super-aged population by 2026, developing efficient diagnosis is urgent to reduce the burden of caregivers and long-term care required for AD patients. Early detection is essential as there is no treatment for AD. We developed a spin-enhance lateral flow immunoassay (SELFIA) to accomplish background-free ultrasensitive detection by taking advantage of the spin character of the negatively charged nitrogen-vacancy center in the fluorescent nanodiamond (FND). By sandwich SELFIA, we could efficiently detect phospo-tau (p-tau) protein, an AD biomarker. The limit of detection (LOD) of the p-tau antigen using SELFIA is 10 pg/mL, which is among the lowest detection limits to date.

Background

Worldwide death rate from AD

Annual number of deaths from AD per 100,000 people.

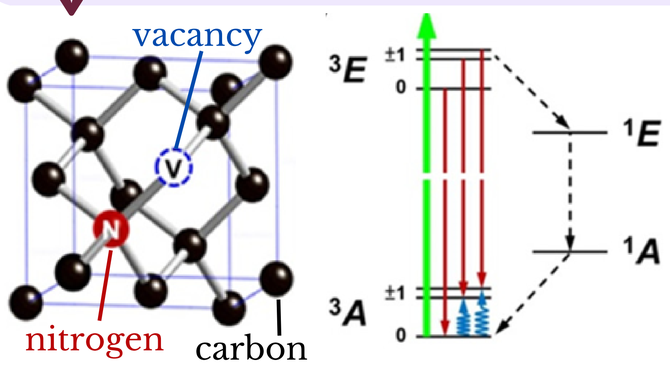




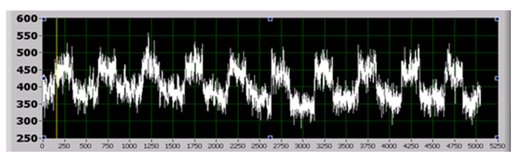
Predictions of AD patients in Taiwan

With >10,000 new cases added each year, Taiwan will have more than 620,000 people suffering from the disease by 2056.

Fluorescent Nanodiamond (FND)

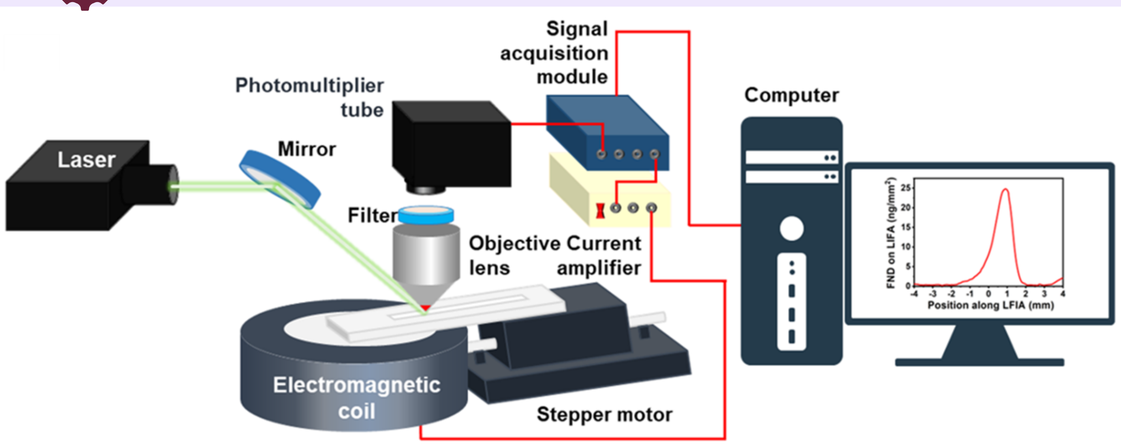


Magnetic modulation fluorescence can be an AC signal.



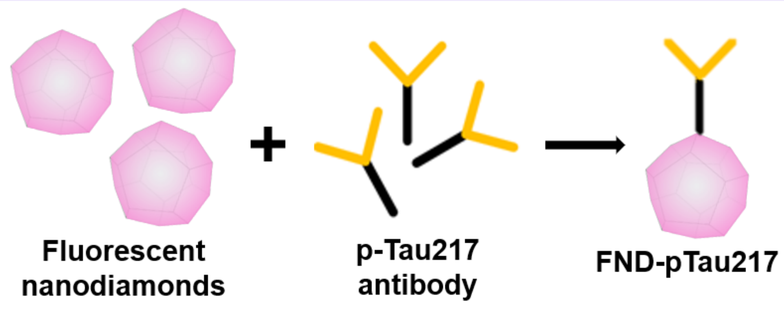
Structure and energy level diagram of the NV– center in diamond. The green, red, blue sinusoidal, and black dashed arrows denote optical excitation, fluorescence emission, microwave excitation, and intersystem crossing relaxation, respectively.

SELFIA Instrument Layout

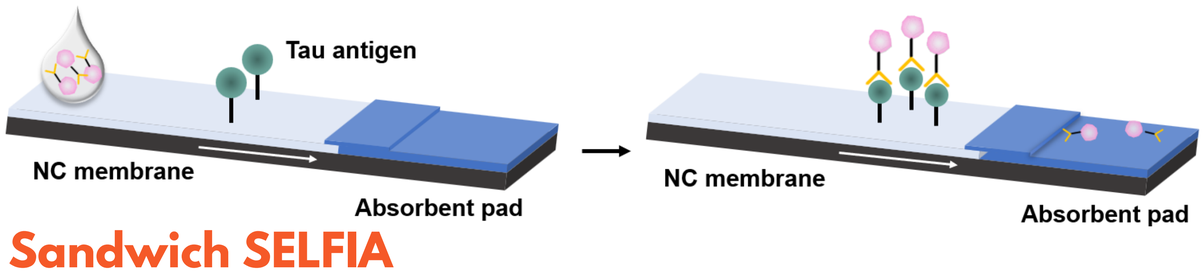


SELFIA Assay

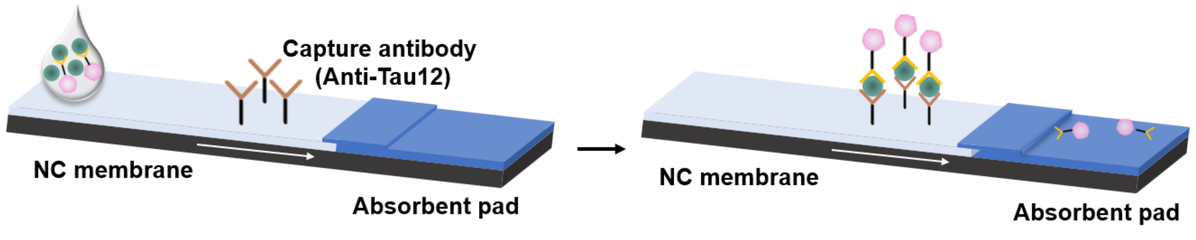
One step FND-antibody conjugation



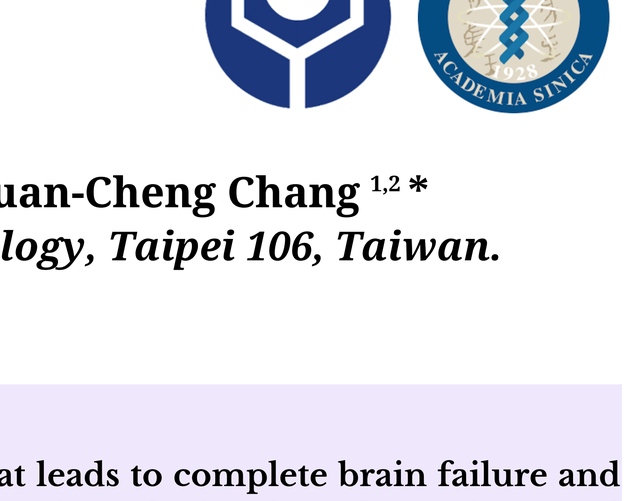
Direct SELFIA

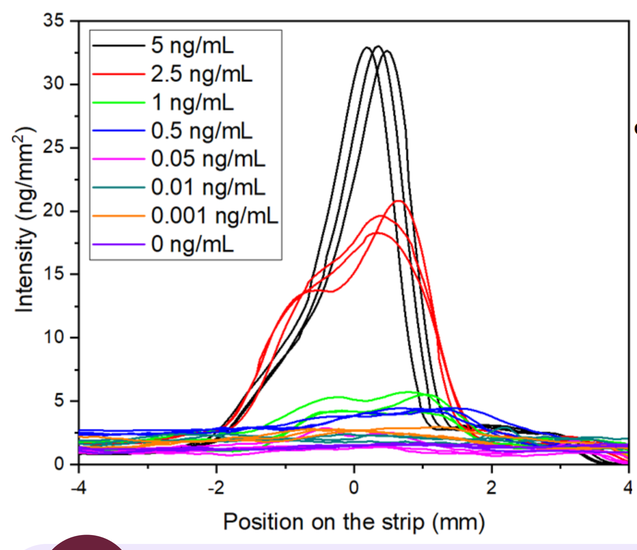


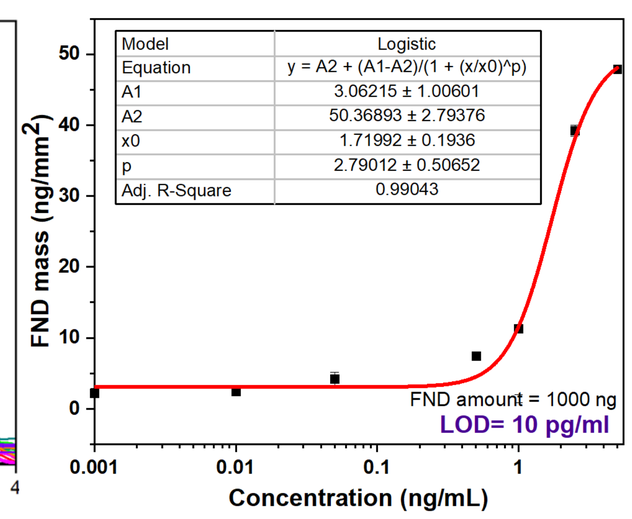
Sandwich SELFIA



Result

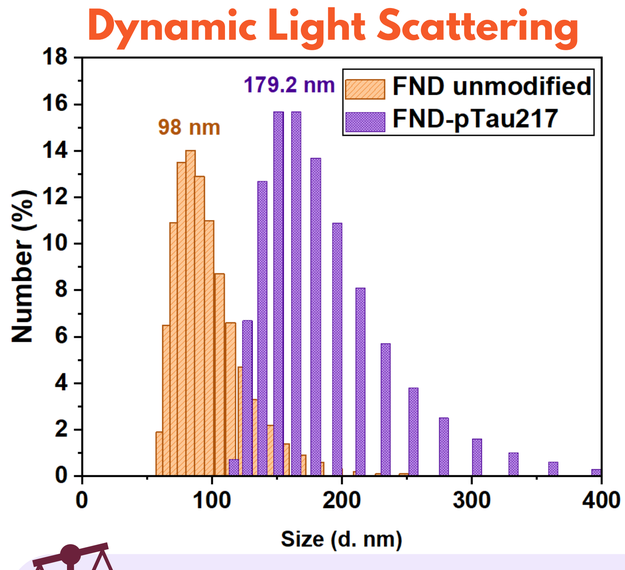




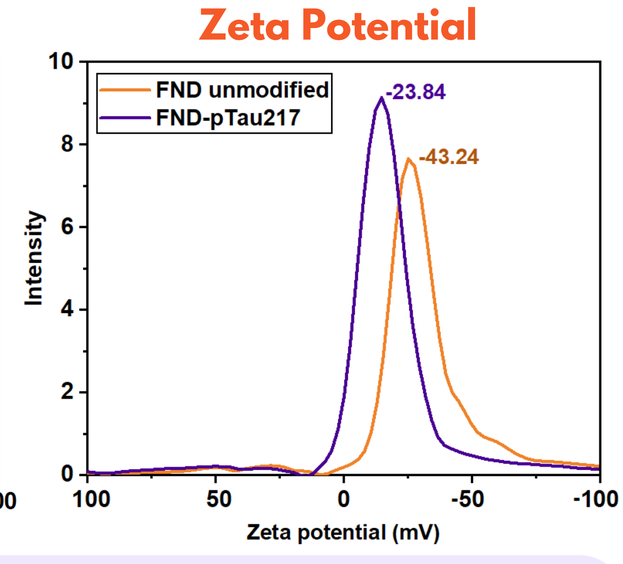


Characterization

Dynamic Light Scattering



Zeta Potential



Comparison

Property	ELISA	SELFIA (our study)
Detection Limit	10 pg/ml	10 pg/ml
Detection Time	~4h	~20 min
Operator	Certified operator	Simply trained operator
Preservation	Low	Very high
Marker	Enzyme, secondary antibody	FND

Conclusion

Taking advantage of the unique magneto-optical properties of NV^- centers in FND, the SELFIA utilizes electromagnetic fields to modulate FND's fluorescence signals to provide ultra-sensitive and specific results. The recombinant human tau protein at different concentrations were evaluated to determine the LOD compared to the tau protein ELISA kit. The distinct peaks of FND mass are observed with the LOD of 10 pg/mL, indicating the sensitive detection of the AD biomarker.

References

- Anal. Chem. 2021, 93, 18, 7140−7147
- Anal. Chem. 2022, 94, 51, 17819−17826
- Anal Chim Acta. 2022;1230:340389.