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Introduction

- Fluorescence lifetime imaging ophthalmoscopy (FLIO, Heidelberg Engineering, Figures 1 and 2), a novel ***in vivo retinal imaging biomarker,*** generates fluorescence decay lifetimes in 2 spectral channels corresponding to mitochondrial metabolic processes
- *Short Spectral Channel (SSC): 498 – 560 nm*
  - Corresponds to flavin adenine dinucleotide (FAD) and oxidative phosphorylation.
- *Long Spectral Channel (LSC): 560 – 720nm*
  - Corresponds to predominantly lipofuscin and lysosomal function
- Based upon animal experimental models and clinical data, mitochondrial dysfunction has a role in the pathophysiology of NMOSD

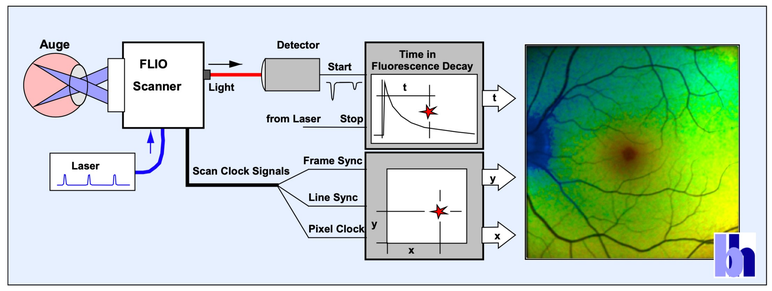

Figure 1: Diagram of FLIO and its output

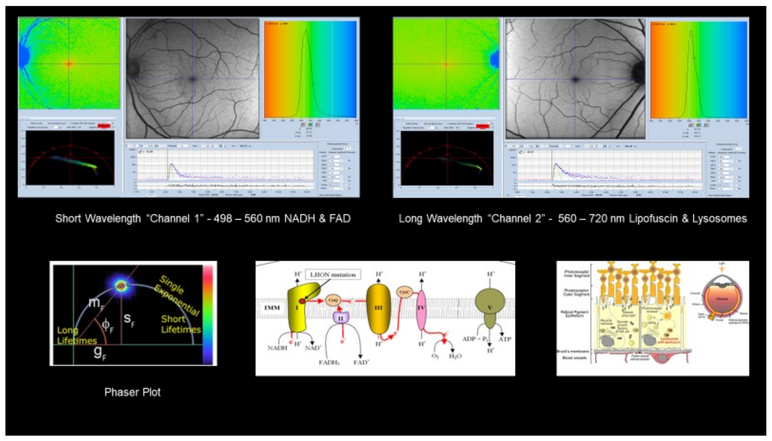

Figure 2: SSC and LSC output from FLIO shown (top). Phasor plot shown (bottom left) illustrating single lifetimes displayed on the semicircle compared to molecules (e.g complex tissues such as the retina) with multiple lifetimes appear inside the semicircle

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Methods

- Twenty-one individuals [42 eyes], 9 seropositive NMOSD (8 females, 1 male) and 12 controls underwent FLIO and OCT in both eyes
- FLIO imaging data were analyzed using SPCImage technology
- OCT imaging data were analyzed using Heyex 1 software
- Linear regression analysis performed for relationship between mean fluorescence decay time and GCL volume

Results

- FLIO demonstrated prolonged mean fluorescence decay times in the SSC of NMOSD patients
- NMOSD patients had higher mean channel wavelengths than controls for FLIO
- OCT showed differences in RNFL average, GCL volume, and GCL thickness
- Parameters displayed in Table 1
- Linear regression showed an inverse relationship (Figure 3)

Table 1: Statistically significant FLIO and OCT parameters comparing NMOSD patients with controls

Parameter	NMOSD (SD)	Control (SD)	P-value
Mean fluorescence decay time SSC (ps)	181.71 (59.07)	118.46 (76.15)	0.004
Mean channel wavelength SSC (nm)	368.98 (71.71)	275.16 (107.93)	0.002
RNFL average (μm)	80.5 (30.5)	96.2 (7.2)	0.046
GCL volume (mm^3)	0.86 (0.22)	1.07 (0.05)	0.0009
GCL thickness at 3mm nasal area (μm)	37.1 (14.1)	53.2 (3.1)	0.000245

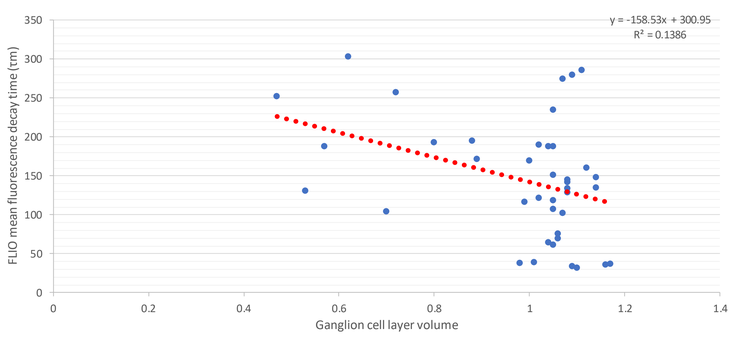

Figure 3: Regression analysis shows reduced GCL volume (GCL thinning in NMOSD) is associated with prolonged FLIO decay times, consistent with mitochondrial dysfunction localizing to the ganglion cell layer

Results

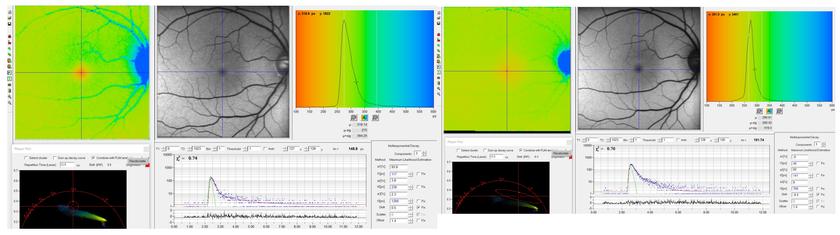

Figure 4: Normal SSC left, LSC right

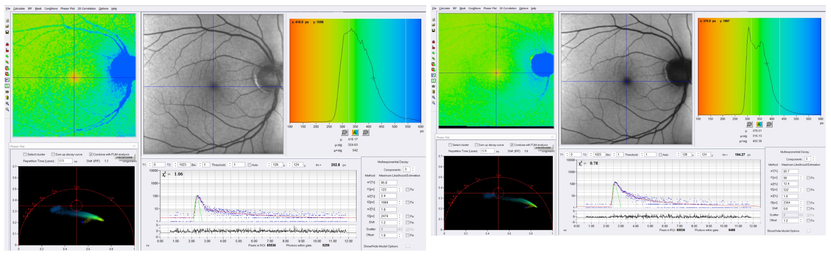

Figure 5: 30 year history NMOSD SSC left, LSC right

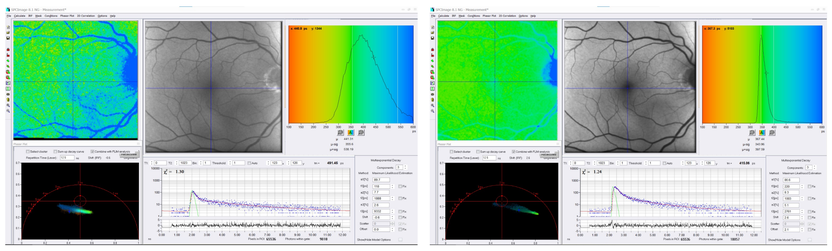

Figure 6: Acute onset NMOSD SSC left, LSC right

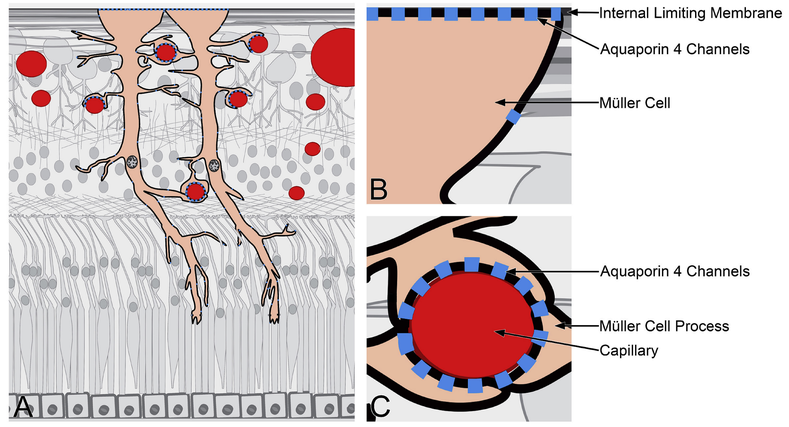

Figure 7: A) Müller cell (retinal astrocyte) location win the retina B) Footplates form the ILM of the retina with AQP4; C) Müller cell processes surround retinal vessels with high concentrations of AQP4 (*from Spaide, 2016*)

Conclusions

- FLIO and OCT together show both functional and structural deficits localizing to the GCL
- FLIO shows mitochondrial alterations in oxidative phosphorylation in NMOSD
- Since the GCL is solely supplied by the central retinal artery, these findings suggest a central retinal artery vasculitis contributes to permanent visual loss in NMOSD

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