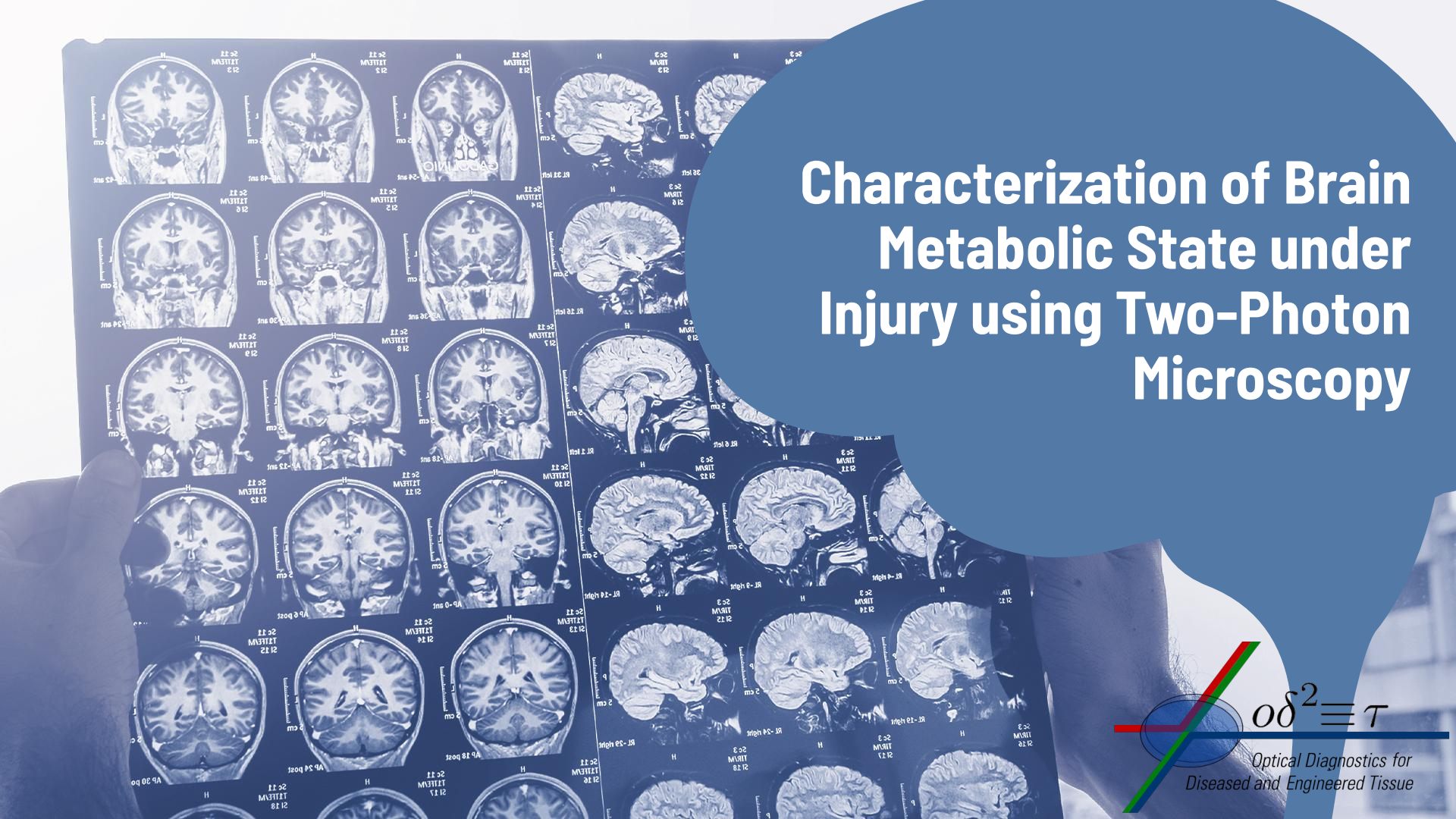
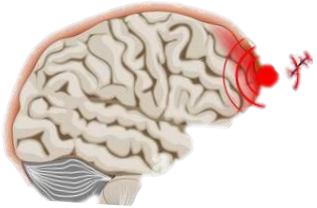


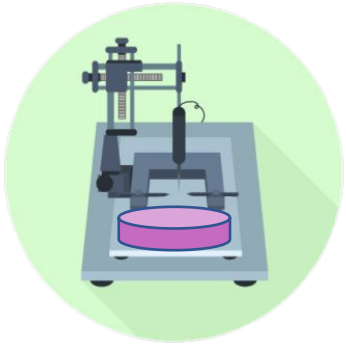
Characterization of Brain Metabolic State under Injury using Two-Photon Microscopy

Optical Diagnostics for Diseased and Engineered Tissue

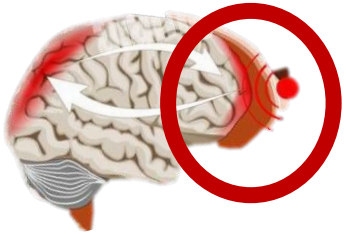
There are a lack of diagnostic and treatment solutions for traumatic brain injury

Impact





Progression



- Immediate stress response
- Long-term cell reprogramming
- Neurological deficits

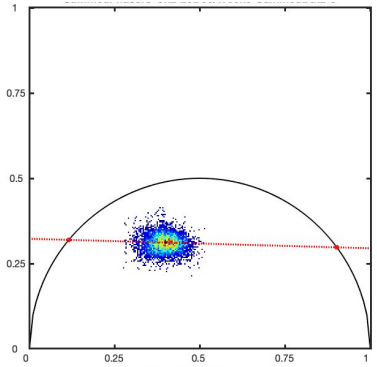
https://www.omtrial.com/dangerous-brain-injuries-that-dont-show-immediate-symptoms/

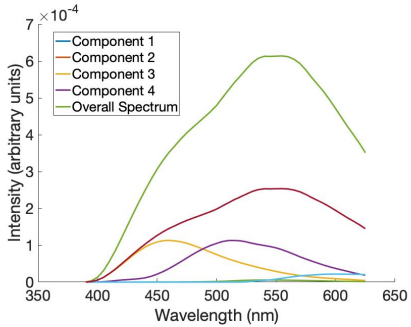
https://conductscience.com/controlled-cortical-impact-for-traumatic-brain-injury/

https://downloads.leica-microsystems.com/DIVE/Brochures/SP8_DIVE_Brochure_Web_E

Multimodal two-photon excited fluorescence is used to attain molecular specificity

- **TPEF** = 3D, high-resolution, label-free





- **Lifetime =** average time fluorophore is excited by light
- Specific to fluorophore identity and configuration

- **Spectrum =** how much a fluorophore responds to light of different energies
- Specific to fluorophore identity

Specific Aim: Optical Readouts of Targeted Secondary Injury

How do TPEF readout metrics differ between injury pathways?

- **Induction:** Controlled exposure to pathway activators
- **Assessment:** Glutathione (GSH/GSSG) assay, multimodal TPEF at 6h-24h, multimodal image analysis

Glutamate

Rationale: Primary excitatory neurotransmitter; TBI hallmark

Expected Results:

- Lower net glutathione
- Significantly different optical readouts from control **differing between neurons/astrocytes**

Lipopolysaccharide (LPS)

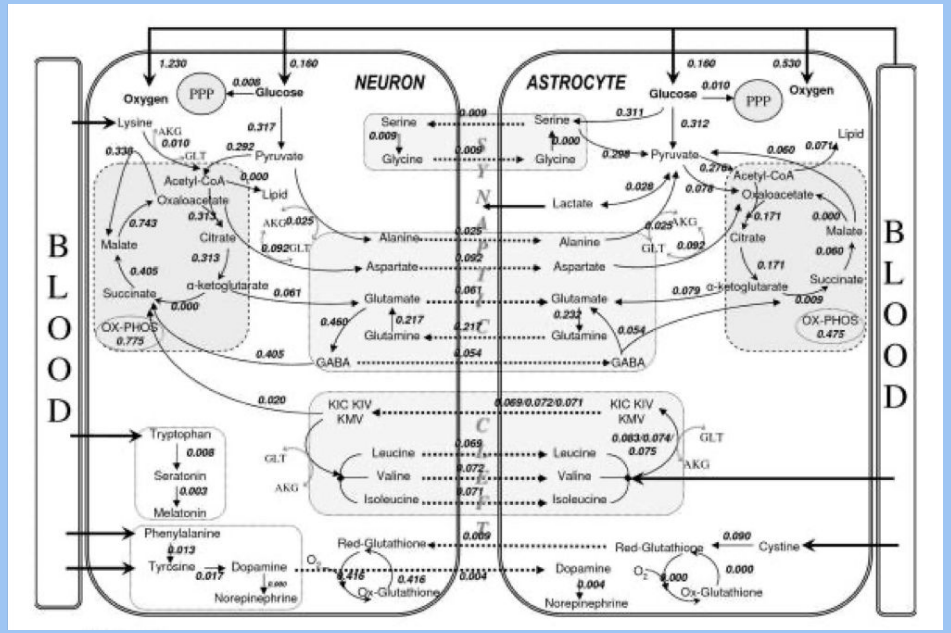
Rationale: Established method of inducing oxidative stress

Expected Results:

- Increased GSSG levels
- Optical readouts indicate **glycolytic metabolism** and **stress products**

Specific Aim: Metabolic Computation Model

TBI metabolite concentrations from biochemical assays and mass spectrometry



- **Differential equations** of **central metabolic pathways** between and within **neurons** and **astrocytes**
- Develop MATLAB framework based on published literature

Level of metabolic pathways activation under TBI conditions

Specific Aim: Correlate Metabolic Pathways to Optical Metrics

Hypothesis: Specific trends in optical readouts for each injury condition will be characteristic of an altered metabolic pathway

Rationale:

Characterization of optical metrics: quantify how optical metabolic ratios and fluorophore concentrations relate to metabolic shifts

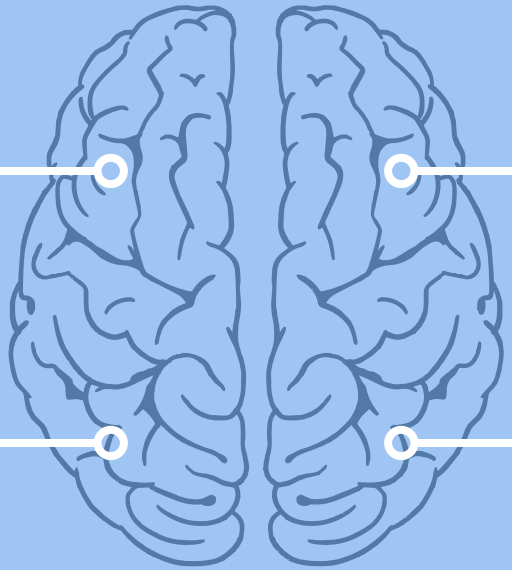
Novel platform : Non-invasive, depth-resolved, label-free method (TPEF) for TBI diagnosis

Methods: Linear regression across collected timepoints + validation based on 0.8 Pearson correlation

Next Steps

LPS Injury - In progress

Glutamate Injury



Refine computational model

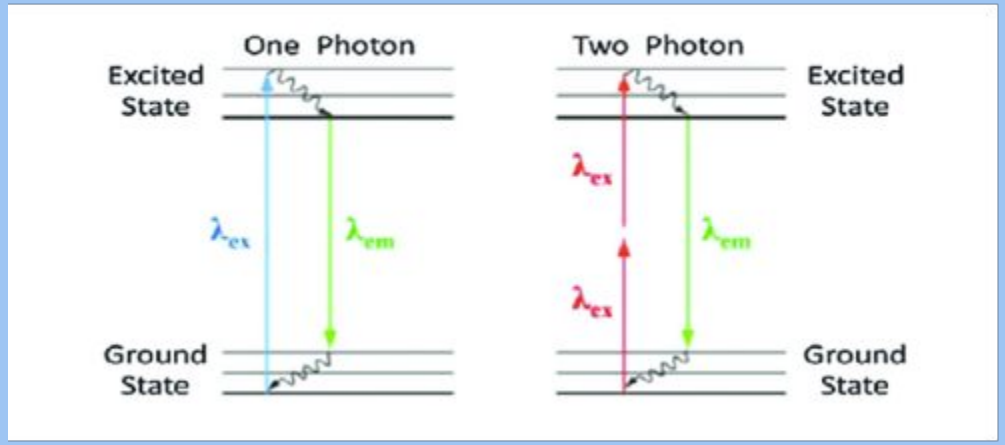
Correlation of optical readouts to injury pathways

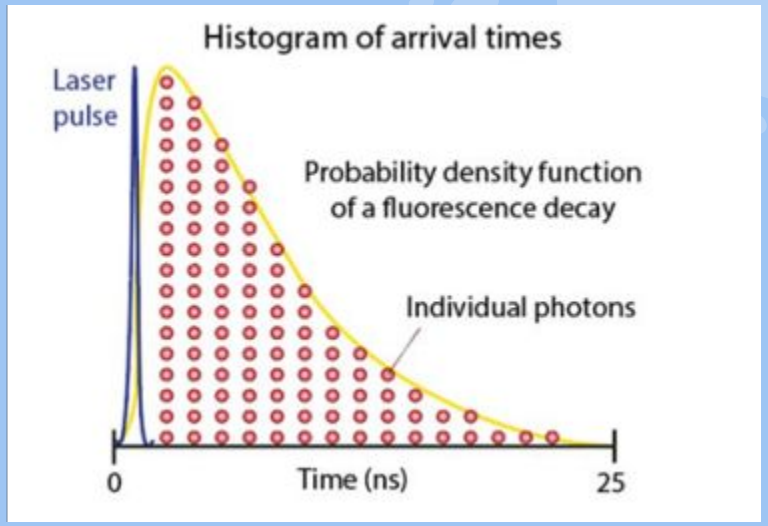
thank you!

Questions?

Supplemental Slides

Relevant Optical Metrics

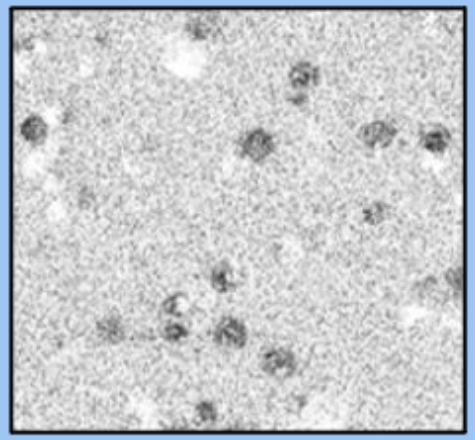




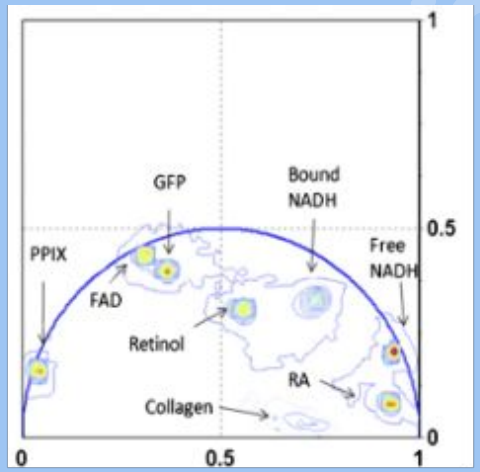
- Two-photon imaging = 3D, high-resolution, non-invasive imaging
  - Metabolic imaging

- FLIM = **F**luorescence **L**ifetime **Im**aging
  - Identify distinct fluorophores

Optical Readout Analysis Metrics



- Acquire images from excitation and emission wavelengths of NADH/FAD
- Obtain ratio of oxidative to glycolytic metabolism



- Acquire images of photon decay during fluorescence
- Obtain composition and binding state of fluorophores in imaged specimen

Project Timeline