

MULTIPHOTON TOMOGRAPHY: A NEW IMAGING MODALITY FOR CORNEAL EVALUATION

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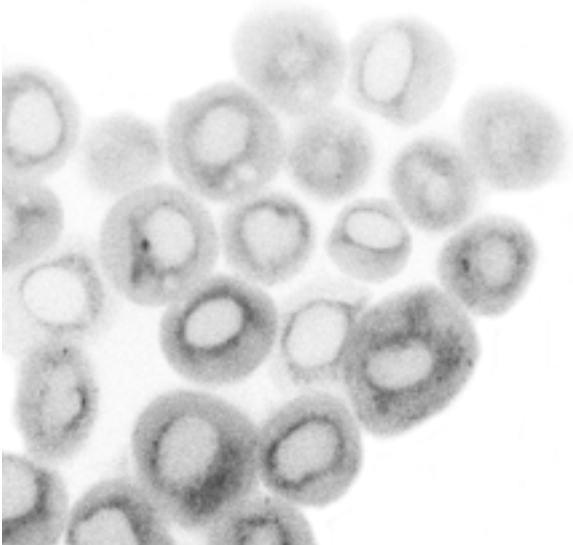
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PURPOSE

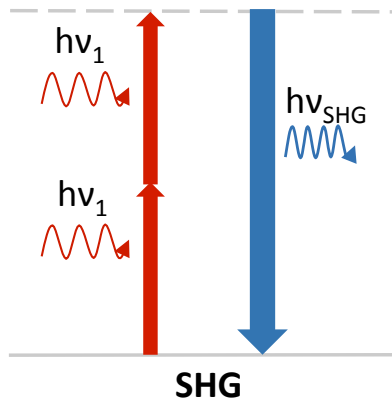
- To evaluate the corneal **cells metabolic state** and the **stroma structural organization** using **multiphoton tomography**.

INTRODUCTION

- The cornea is severely affected by dysfunctions and dystrophies → second major cause of blindness worldwide.
- A new diagnostic method capable of providing information on tissue metabolic state and structural organization is needed.

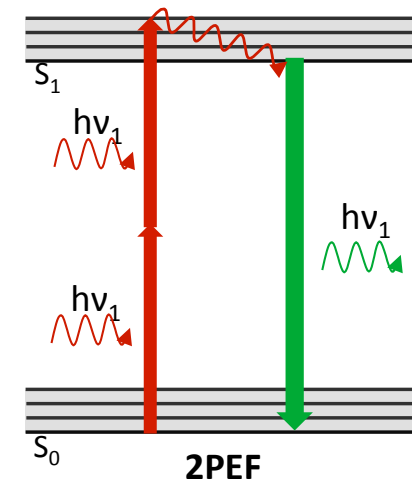
The determination of metabolic cofactors, NAD(P)H and flavins, **autofluorescence lifetime** provides information on the **cells' metabolism**.

- Independent of the molecular concentration
- Sensitive to the fluorophore microenvironment



Corneal stroma structural organization can be assessed using **second-harmonic generation (SHG)** imaging of the collagen fibrils.

- High-resolution and high-contrast imaging modality
- Photobleaching and photodamage are absent



METHODS

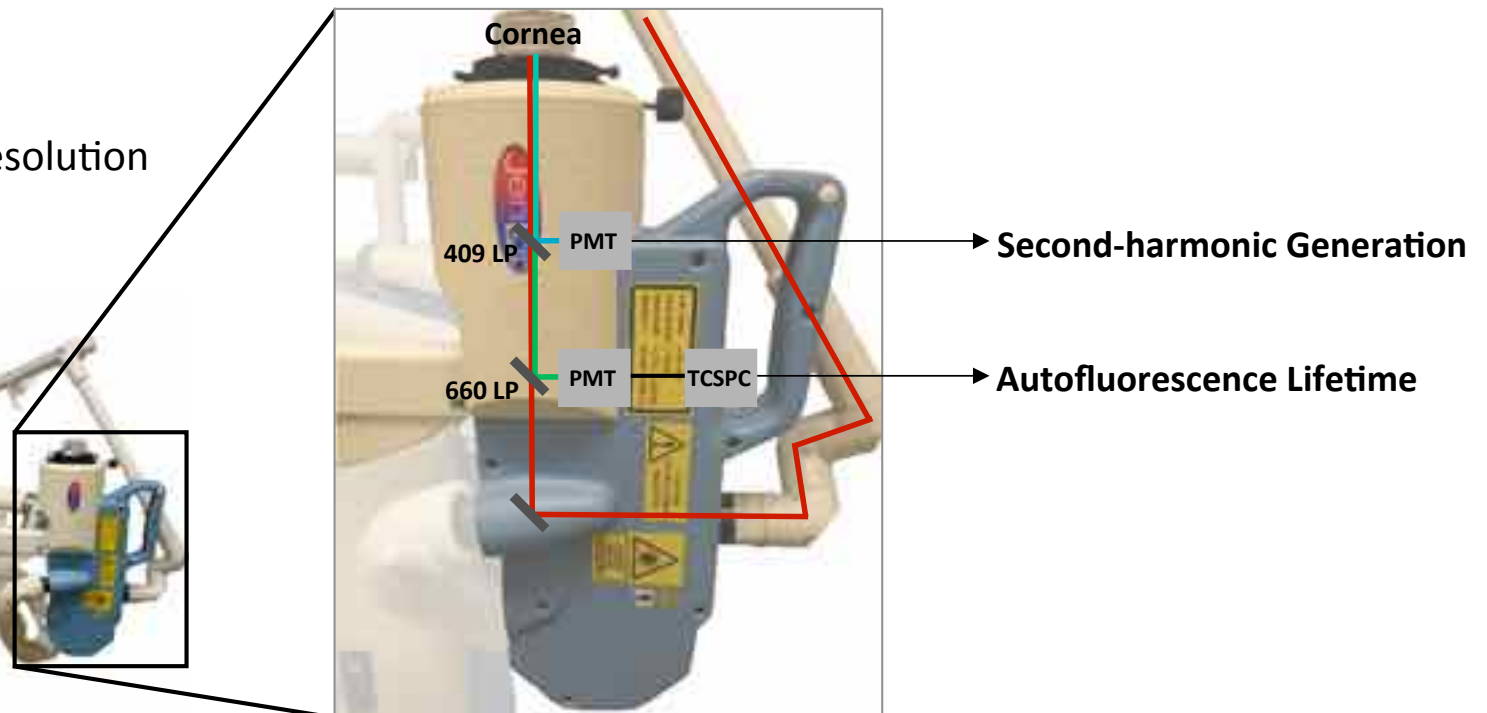
MPTFLEX

- The feasibility to characterize human corneas, unsuitable for transplantation, was assessed using the **clinical certified multiphoton tomograph – MPTflex.**

- Non-invasive
- Label-free
- Sub-cellular resolution



MPTflex
(JenLab GmbH, Jena, Germany)



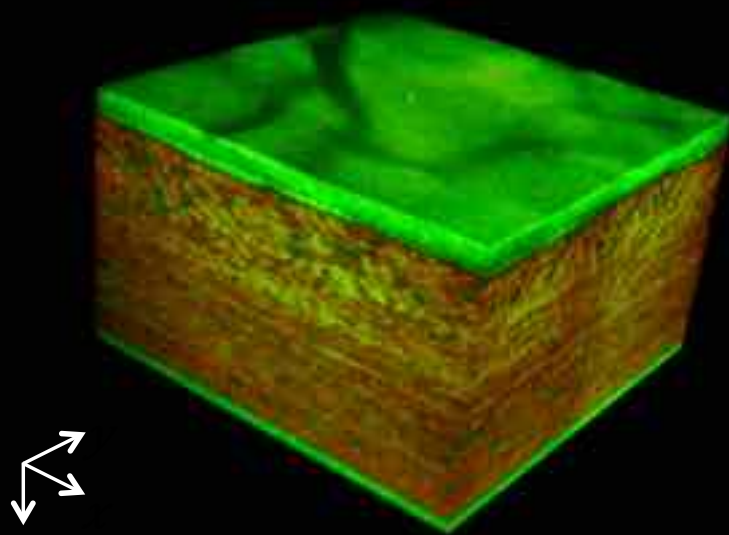
- 80 MHz NIR Ti:Sapphire 100 fs tunable laser
- Excitation wavelengths: **760 nm (NAD(P)H)**
850 nm (Flavins)

RESULTS

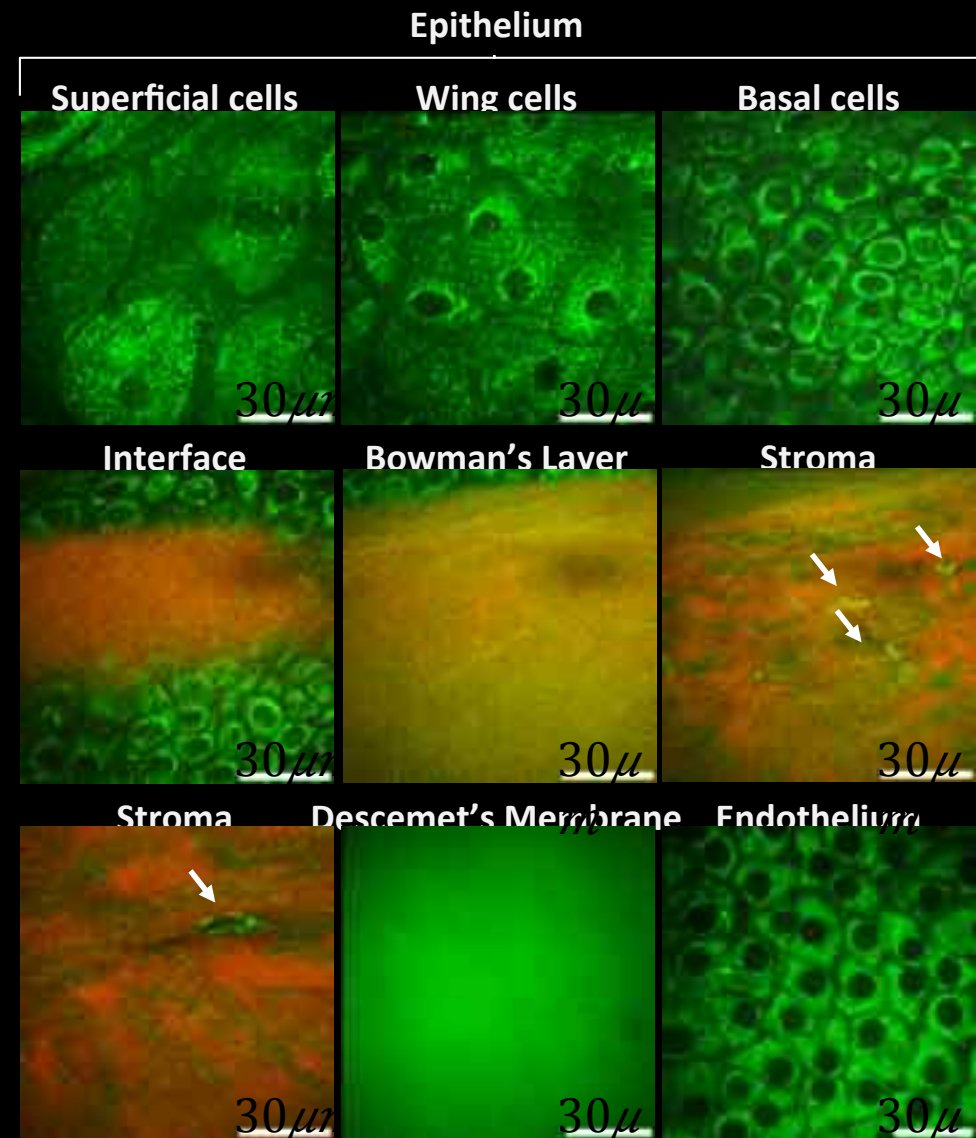
CORNEAL LAYERS

- Using the **autofluorescence intensity** of endogenous fluorophores and **SHG** all corneal layers can be identified.

3D REPRESENTATION OF THE CORNEA



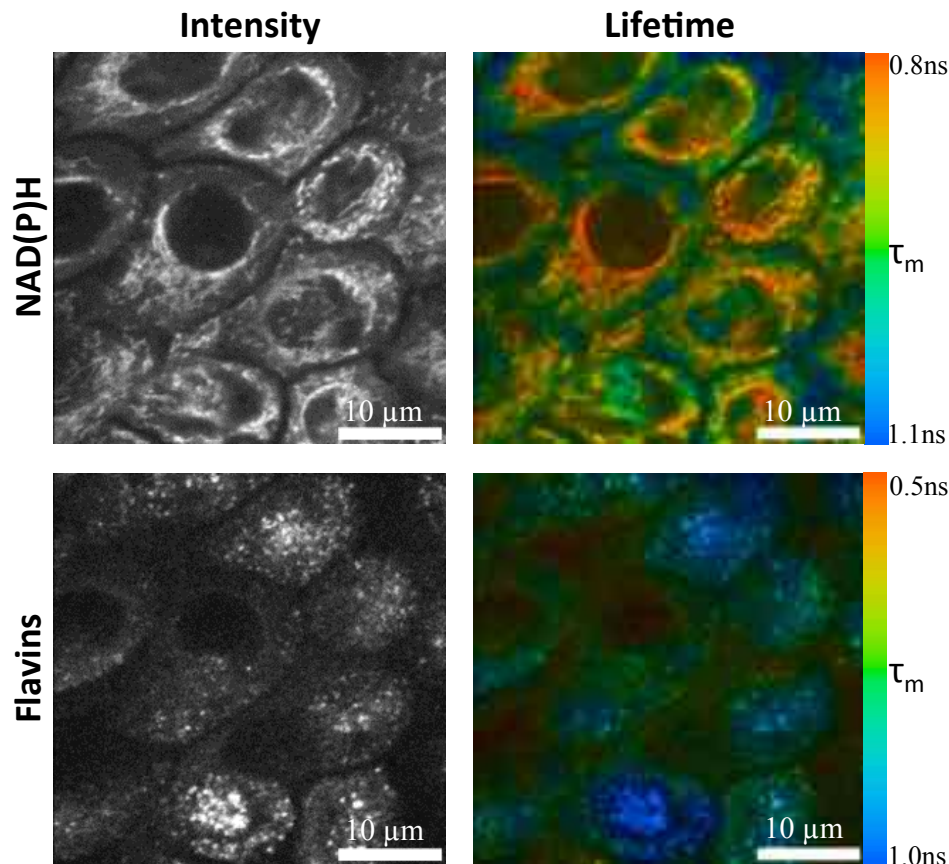
2PE AF AND SHG



RESULTS

CORNEAL METABOLISM

- Metabolic information can be retrieved from the autofluorescence of both metabolic cofactors: **NAD(P)H** (excitation at 760 nm) and **flavins** (excitation at 850 nm).



	$E \setminus \lambda$ [nm]	τ_m [ns]
Epithelium	760 (NAD(P)H)	1.01 ± 0.09
	850 (Flavins)	0.77 ± 0.16
Stroma	760	1.35 ± 0.12
Endothelium	760 (NAD(P)H)	1.01 ± 0.11
	850 (Flavins)	0.81 ± 0.04

τ_m of NAD(P)H and flavins

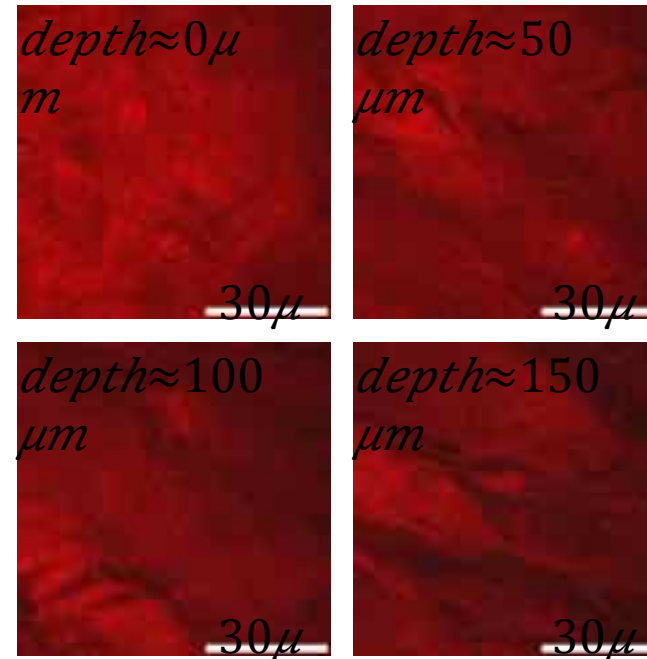
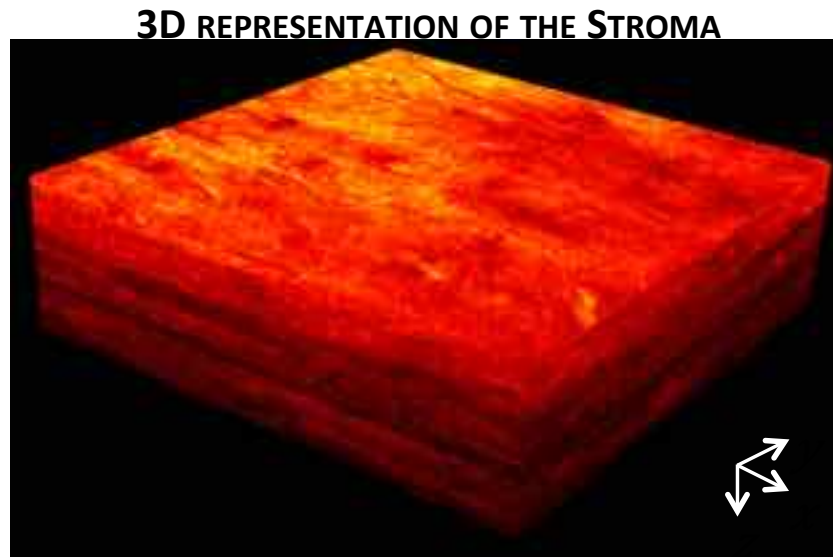


Indirect measure of cells' metabolism

RESULTS

CORNEAL STRUCTURAL ORGANIZATION

- SHG shows the structural organization of the stroma.



CONCLUSION

- Multiphoton tomography can be used to efficiently evaluate the human cornea morphology, metabolic state, and stroma structural organization.
- It may be used to diagnose pathologies and to evaluate the corneal status before transplantation or after clinical procedures such as corneal collagen crosslinking.

