MULTIPHOTON TOMOGRAPHY: A NEW IMAGING MODALITY FOR CORNEAL EVALUATION

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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

- 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

- **Epithelium**
  - Superficial cells
  - Wing cells
  - Basal cells

- **Interface**

- **Bowman’s Layer**

- **Stroma**

- **Descemet’s Membrane**

- **Endothelium**
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).

<table>
<thead>
<tr>
<th>E$\lambda$ [nm]</th>
<th>$\tau_m$ [ns]</th>
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</thead>
<tbody>
<tr>
<td><strong>Epithelium</strong></td>
<td></td>
</tr>
<tr>
<td>760 (NAD(P)H)</td>
<td>1.01 ± 0.09</td>
</tr>
<tr>
<td>850 (Flavins)</td>
<td>0.77 ± 0.16</td>
</tr>
<tr>
<td><strong>Stroma</strong></td>
<td></td>
</tr>
<tr>
<td>760</td>
<td>1.35 ± 0.12</td>
</tr>
<tr>
<td><strong>Endothelium</strong></td>
<td></td>
</tr>
<tr>
<td>760 (NAD(P)H)</td>
<td>1.01 ± 0.11</td>
</tr>
<tr>
<td>850 (Flavins)</td>
<td>0.81 ± 0.04</td>
</tr>
</tbody>
</table>

$\tau_m$ of NAD(P)H and flavins

Indirect measure of cells’ metabolism
RESULTS

CORNEAL STRUCTURAL ORGANIZATION

- SHG shows the structural organization of the stroma.

3D REPRESENTATION 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.

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