Confocal Imaging of Corneal Diseases

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Conventional vs. Confocal Microscopy

- Large region of the specimen is illuminated by the light source and condenser.
- In-focus and out-of-focus light is detected.

Advantages of In Vivo Confocal Microscopy (IVCM)

- Improved images.
  - In conventional light microscopes, reflections and light scattered from structures outside of the focal plane cause image degradation.
- Possible magnification up to 600x (slit lamp 40x).
- Rapidly evolving imaging and diagnostic tool.
  - Given insight into microstructural alterations in corneal diseases.
Disadvantages of In Vivo Confocal Microscopy (IVCM)

- Limited field of view.
- Image acquisition speed is critical because involuntary movements such as respiration or microsaccades cause image blurring.

Confocal Microscope Types

- **Tandem scanning** confocal microscope.
- **Slit-scanning** confocal microscope.
- **Laser scanning** confocal microscope.

Tandem Scanning Confocal Microscope (TSCM)

- No longer commercially available.
- Uses Nipkow disc technology (metal plate with a series of microscopic holes in a spiral).
- Whole specimen is scanned rapidly because pinholes provide multiple single spot illumination and because of fast disc rotation.

Slit-Scanning Confocal Microscope (SSCM)

- Confoscan series (Nidek)
- Uses vertical-slit apertures for illumination and observation of the field.
- Allows increased light throughput and reduces scanning time.
- Decreased illumination improves patient comfort.
Laser Scanning Confocal Microscope (LSCM)

- Rostock Corneal Module (Heidelberg)
- A coherent high intensity light source.
- The laser beam is scanned over a set of mirrors providing fast scanning.
- High-contrast, high quality images.

http://www.microscopyu.com/

Comparison of Confocal Microscopes

<table>
<thead>
<tr>
<th></th>
<th>TCM</th>
<th>SCOM node Continuum 4</th>
<th>LCM Heidelberg</th>
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<tbody>
<tr>
<td>Light source</td>
<td>Mercury lamp, 180W</td>
<td>Halogen lamp, 120W, 50W</td>
<td>Clr 1, 488 nm, 410 nm wavelength</td>
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<tr>
<td>Microscope lens</td>
<td>HeNe</td>
<td>HeNe</td>
<td>HeNe</td>
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<tr>
<td>Working distance</td>
<td>24 - 84</td>
<td>24 - 84</td>
<td>24 - 84</td>
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<tr>
<td>Numerical aperture</td>
<td>0.65 - 8</td>
<td>0.65 - 8</td>
<td>0.65 - 8</td>
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<tr>
<td>Field of view</td>
<td>475 - 250 mm</td>
<td>475 - 250 mm</td>
<td>475 - 250 mm</td>
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<tr>
<td>Lateral resolution</td>
<td>16 - 8.5 - 0.9 µm, 41</td>
<td>16 - 8.5 - 0.9 µm, 41</td>
<td>16 - 8.5 - 0.9 µm, 41</td>
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<tr>
<td>Z resolution</td>
<td>0.1 - 0.2 µm</td>
<td>0.1 - 0.2 µm</td>
<td>0.1 - 0.2 µm</td>
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<tr>
<td>Scan acquisition time</td>
<td>20 frames/sec</td>
<td>20 frames/sec</td>
<td>20 frames/sec</td>
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</table>

Corneal Histology

- Superficial cells
- Upper wing cells
- Low wing cells
- Basal cells
- Sub-basal nerve plexus
- Bowman’s membrane
- Anterior stroma
- Posterior stroma

Superficial Epithelial Cells

- Flat polygonal cells with bright central nuclei
**Intermediate (Wing) Epithelial Cells**

- Polygonal cells with bright cell borders.

**Basal Epithelial Cells**

- Smaller diameter cells: mosaic of dark cell bodies with light, narrow inter-cellular borders.

**Sub-Basal Nerve Plexus**

- Bright, well-defined linear structures with branches and anastomoses.

**Stromal Cells**

- Stromal keratocytes appear as hyper-reflective cell nuclei with poorly visualized cell processes.
Endothelial Cells

- Regular hexagonal cells with a honeycomb appearance.

Corneal Pathology

*Improved understanding of corneal microstructure:*
- Dry Eye
- Post-LASIK
- Keratoconus
- Contact lens wear
- Uveitis

*Improved corneal diagnostic tool:*
- Immune cells
- Iridocorneal endothelial (ICE) Syndrome
- Infections

*Improved understanding of corneal microstructure*

Normal Tear Film  Mild Dry Eye  Severe Dry Eye

IVCM studies have focused on the sub-basal nerve plexus:
- 2 studies showed decreased sub-basal nerve density
- 4 studies showed no difference
Post-LASIK Changes

- Reflective particles at flap interface
- Microfolds in Bowman’s layer
- Epithelial Ingrowth

- IVCM can be used to assess the amount of stromal haze and activated keratocytes.
- IVCM may be useful in determining the depth of epithelial ingrowth and planning surgical management.


Post-LASIK Changes

- Normal Sub-basal Nerve Plexus
- Post-LASIK Nerves

IVCM study showed no difference in the regeneration of the sub-basal nerve plexus between femtosecond laser created flaps and mechanical microkeratome created flaps.

Niederer RL, Prog Retin Eye Res. 2010

Keratoconus

- Normal Stroma
- Early Keratoconus
- Advanced Keratoconus

IVCM studies have focused on alterations in corneal structure in keratoconus:
- increase in cell area of epithelial cells
- lower keratocyte density
- lower sub-basal nerve fiber density

Haab’s Striae Case at the Proctor Foundation
**Contact Lens Wear**

*Changes seen with IVCM:*

- Twice the number of Langerhans' cells.
- No alteration in corneal nerve density.
- Stromal changes (microdot deposits) with long-term wear.
- Endothelial polymegathism is increased with long-term wear.

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**Dendritic (Langerhans') Cells**

- Langerhans' cells are antigen presenting cells that play a role in initiating the immune response.
- Located at the level of basal epithelium.

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**Uveitis Cases from the Proctor Foundation**

- Fine KP
- CMV Iritis
**Iridocorneal endothelial (ICE) Syndrome**
- Early promise in using in vivo confocal microscopy for diagnosis.
- The endothelium demonstrates epithelium-like transformation, with indistinct borders and prominent nuclei.

**Acanthamoeba Keratitis**
- Cysts appear as ovoid, hyper-reflective structures with the double wall sometimes apparent. Trophozoites have variable size and shape.

**Acanthamoeba Keratitis Case at the Proctor Foundation**
- Infection was confirmed by culture.

**Acanthamoeba Neuritis**
- Swollen corneal stromal nerves can be seen.
**Acanthamoeba Neuritis**

- Swollen corneal stromal nerves can be seen.

**Fungal Infections**

- Hyperreflective, branching or non-branching filaments
- Recent IVCM study demonstrated a sensitivity of 94% and a specificity of 74%.

**Fusarium Case at the Proctor Foundation**

**Future –**

Real-time, in vivo studies:

Chemically injured mouse cornea
Summary

• In vivo confocal microscopy offers **improved images** over conventional microscopy because of increased **resolution** and **magnification**.

• Rapidly evolving **imaging and diagnostic tool** that has given insight into structural alterations in corneal diseases.

References