2:00 – 3:40pm  New Ways to Treat the Patient with Glaucoma
John Flanagan, Moderator
Steven Mansberger, Malik Kahook, David Sendrowski

Advances in imaging the anterior segment of the eye – Malik Kahook


Advances in Monitoring the Glaucoma Patient – David Sendrowski

This course will discuss new ways to treat the glaucoma patient. Included will be a discussion on advances in anterior segment imaging and surgery; the role that cataract surgery has in reducing intraocular pressure and how it may be utilized with new devices to reduce the IOP; and advances in structural and functional testing to allow improved monitoring of the patient with glaucomatous damage.

At the conclusion of this course, the attendee will understand:
• New advances in imaging the anterior segment of the eye.
• New surgeries to improve outflow from the eye.
• The amount of intraocular pressure reduction associated with cataract surgery.
• The ways that cataract surgery may be modified to further reduce intraocular pressure.
• New methods to detect structural change associated with glaucoma.
• New methods to detect functional progression associated with glaucoma.
Multi-Photon Microscopy in Ophthalmology:
Focus on the outflow system

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Financial Disclosures

- I have the following financial interests or relationships to disclose:
  - Alcon Laboratories Inc.  C,L,S
  - Allergan Inc. C,L,S
  - Genentech Inc. S
  - New World Medical P
  - Shape/Ophthamics O,P
  - ShapeTech O,P
  - The State of Colorado S
  - The United States Food and Drug Administration C
  - Calvita Medical O,P
  - Valeant C
  - Aerie C

University of Colorado Ophthalmic Imaging Team:

- David Ammar, PhD
- Tim Lei, PhD
- Omid Masihzadeh, PhD
- Malik Kahook, MD

The collaboration was born out of my clinical experiences with SOCT

Advantages of OCT Imaging

- Accurate (High resolution)
- Fast
- Ease of use
- Ability to detect disease

Limitations of OCT Imaging

- We are reaching limits of OCT resolution
- Structural without functional data
- Limited information on trabecular meshwork (TM)

As a clinician and surgeon, I still don’t have a reliable and objective way of measuring the state of the outflow system of the eye.

Loss of TM cells with age and disease

![Graph showing loss of TM cells with age and disease]

The Challenge of Imaging the Outflow System of the Eye

- Gonioscopy
  - Gross anatomy
  - Highly subjective
- Ultrasound
  - > 80 MHz probes needed
- Optical Coherence Tomography
  - Requires some degree of optical transparency
Optical coherence tomography (OCT) lacks the image contrast and resolution.

Non-Linear Microscopy: A look back over 4 years

Linear vs Non-Linear

Traditional confocal microscopy (optical microscopy) is based on linear absorption and emission processes. A single photon excites a fluorophore from the ground state to the excited state with emission of a single photon (red shifted).

TPM occurs when two optical photons of a specific energy simultaneously excite a fluorophore from the ground state to the excited state. (no pinhole needed)

SHG requires two photons to simultaneously excite a molecule which then scatters the combined energy as a single photon (asymmetric molecules).

Multiphoton Imaging

Non-Linear Microscopy: A look back over 4 years

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Multiphoton Imaging

Ti-Sapphire laser with pulse width of ~100 femtoseconds and a repetition rate of ~80 MHz.

Added Benefits:

- Longer wavelengths are scattered to a lesser degree
- Lower-energy photons are less likely to cause tissue damage

Several caveats to MPM:

- The technology is very expensive
- The hardware requires frequent TLC

Where to start?

Can we image the TM and improve upon current devices?

We started with an excised strip of tissue from a human eye.

First results very encouraging:

We have reason to believe that MPM can offer useful information

In Vitro: Going Back to Basics

TM has antioxidant capacity

- mN hydrogen peroxide ($H_2O_2$), believed to be constantly generated through a light-dependent mechanism
  - (Repine and Gorin, 1961; Price, 1969; Wielgus and Sarna, 2008)
- The anti-oxidant glutathione
  - NADPH, the factor responsible for glutathione regeneration
  - (Kahn et al., 1983; Padgaonkar et al., 1994)
NADPH: A co-factor for antioxidant enzymes

- Indirectly neutralizes peroxide through $\textbf{B}H\textbf{`}$ transfer.

\[ 2\cdot \text{NADPH} + 2\cdot \text{H}_2\text{O}_2 \rightarrow 2\cdot \text{NADP}^+ + 2\cdot \text{H}_2\text{O} \]

- NADPH, but not NADP$^+$ is fluorescent when excited at ~339 nm.

- High NADPH = High Antioxidant capabilities

Functional Imaging of TM Cells

- We can image TM cells in vitro
- We can quantify NADPH levels with minimal changes to a standard TPM system
- We can measure functional changes reproducibly and with clinically meaningful endpoints

The work so far has been on cells or excised tissue.

But our goal is to use this technology in the clinic and not just in the lab.

What next?

Whole Eye Imaging

There are of course significant differences between the mouse eye and the human eye.

Intact Mouse Eye

Intact Mouse Eye
The sclera was a formidable adversary.

If you can’t beat it, go around it!

Optical scattering reduced image resolution through the sclera.

Gonioscopic lens is used to break total internal reflection at the cornea.

Custom TPAF/SHG MPM gonioscopic microscope for eye imaging.

Detailed structure of the TM can be imaged with gonioscopic MPM.

Individual TM fiber bundles can be clearly observed.

TM images at different depths through MPM virtual sectioning.
Histology indicates no thermal damage in TM after MPM imaging.

Challenges with Gonioscopic Approach:
- Nonlinear magnification of commercial goniolens.
- Finding the targeted tissue (assist from OCT or white light imaging).
- In vivo microscopy → Movement/pulsation of the mouse eye.

Clear path forward for structural imaging, but what about functional?

Coherent anti-Stokes Raman Scattering (CARS)

Two photons simultaneously excite a lipid molecule, with the energy difference between the two photons equal to the vibrational energy of the molecule bond \( E_\Omega \). A probe photon then coherently interacts with the vibrational motion of the molecule to generate a release of the CARS photon.

What will CARS add?
- Study of lipid membranes and lipid vesicles
- The ability to discriminate between nuclear and cytoplasmic regions of cells
- Identifying organelles and investigating subcellular changes during physiological processes

In order to explore individual TM cell imaging:
Coherent Anti-stokes Raman Scattering (CARS) in a fresh excised TM tissue

Label Free Imaging of TM Cells

Next Steps
- We have combined OCT & MPM to enhance localization and decrease costs.
- In vivo studies of the mouse eye using the combined system
- Stay tuned!

Beyond the TM
References:


ADVANCES IN MONITORING THE GLAUCOMA PATIENT
David P. Sendrowski, O.D., FAAO
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Professor, UEC/MBKU

1. Glaucoma Progression
   - Requires ability to detect structural/functional change over time
   - Rate > normal aging (not that easy)
   - Most monitoring devices (OCT/VF) acquire/manage patient data
   - Three main devices: CSLO, SCP, SD-OCT
   - Earlier detection of progression results in treatment intervention/modification
   - Two main ways to identity progressive changes:
     a. Event-based analysis
     b. Trend-based analysis

2. Utilizing SD-OCT to detect disease progression
   - Normal patterns of RNFL loss
     o Expansion of pre-existing RNFL defects
     o Development of new RNF: defects
     o Deepening of pre-existing RNFL defects
   - Four most commonly used SD-OCT instruments
     o Spectralis OCT
     o Cirrus HD-OCT
     o RTVue-100
     o Topcon 3D-OCT 2000
   - RNFL thickness (circumpapillary profile)
     o Most common method of evaluation of structural damage

   a. Spectralis OCT provides a change report with event-based progression analysis of circumpapillary RNFL thickness profiles and trend-based progression analysis of average and sector RNFL thickness measurements. This analysis graphically shows the location and amount of significant changes in the RNFL thickness profile from baseline as a red area. The trend-based analysis plots the serial global and six sectoral maps over time to assess the rate of change using linear regression analysis and shows differences from baseline.

   b. Cirrus HD-OCT utilizes GPA to visualize RNFL changes and progression. It provides a visual display of the amount and location of significant change by comparing individual pixels of the follow-up images to the same pixels of the baseline image (event-based analysis). It also displays linear regression analysis of the average, superior and inferior RNFL thickness measurements (trend-based analysis).

   c. RTVue-100 does not provide automated comparative analysis of circumpapillary RNFL thickness profiles, but it offers a figure with overlapped baseline and follow-up RNFL thickness profiles for easy comparison. The device also offers the statistic Image Mapping module, which analyzes global RNFL thickness and six sectoral thicknesses.
Based on these measurements, it performs a trend-based analysis to create a regression line to show a rate of progress.

d. Topcon 3D-OCT 2000 provides a linear regression analysis of average, superior and inferior RNFL thickness and graphical comparison of baseline and follow-up RNFL thickness profiles. It also compares cup-disc ratio, cup volume and cup area between visits.

- Macular thickness maps
  - Evaluation of the macula can provide valuable information regarding glaucoma progression
  - Spectralis (total macular thickness) RTVue (GCC), Cirrus (ACC+IPL), Topcon GCC/"ganglion cell+inner plexiform layer"
  - Instruments do not provide localized automated progression analysis of the macula in localized fashion
  - Examiner can appreciate progressive thinning of the macular area by comparing serial macular thickness maps

- Take-home points
  - Integrating results of peripapillary RNFL and macular analysis provides one way to improve sensitivity/specificity to follow glaucoma progression
  - One should not use measured value from different instrument interchangeably
  - Age-related decline of particular importance in change analysis of RNFL/macular measurements (studies lacking in age-related decline)
  - OCT is "limited" to detect progression in advanced disease (RNFL-hits a bottom/cellar making progression hard to determine)
  - OCT best for early and moderate disease
  - Best to image early and often
  - GPA may overlook localized progression
  - Examiner must also rule-out "non-glaucomatous" damage in this patient population
  - SD-OCT imaging of the lamina cribosa holds great promise for utilized non-retinal ganglion cell strada in disease progress

3. Utilizing visual field in glaucoma progression (HFA)

A. VFI (Visual Field Index)
   - Concentrates on the central visual field
   - Less impacted by cataracts/pupil size
   - Excellent way to assess rate of change in functional loss
   - Reflective of ganglion cell damage
   - Trend analysis reflected in rate/plot/bar
   - May help with functional disability in glaucoma patients

B. GPA analysis (HFA)
   - Minimum of 5 exams over 3 years for linear regression results to be presented
   - Uses symbols of different shaded triangles to look for progress:
     1) Δ – deterioration from baseline p<5%
     2) Δ – deterioration at same location on 2 consecutive tests
     3) Δ – deterioration at same location. 3 consecutive tests
   - Octopus perimetry (Peri-trend)
1) Trend analysis based on global indices which has limitations on detecting focal change.

C. Pearls for detecting progression
   - Try to combine structure and function
     - Better progression detection than utilizing all technology alone
     - Newer technologies will provide software to help the clinician
   - Add in risk factors
     - Patients age, high IOP, pachymetry, stage of Dz
   - Collect meaningful data
     - VF (2x/yr), OCT (1x/yr), ONH photo (1x/yr)
   - Confirm progression by repeating test
I. Ocular Hypertensive Treatment Study Participants
A. Cataract surgery
B. Control group without cataract surgery

II. Data Analysis
A. Split point: study visit date when cataract surgery reported
B. IOP (preoperative and postoperative): mean IOP of the 3 visits prior or after cataract surgery
C. Control Group: one randomly selected eye from participants who had not undergone cataract surgery and met the same inclusion and exclusion criteria

III. IOP response
A. Postoperative IOP
   1. 16.5% drop in IOP with cataract surgery
   2. (23.9 + 3.2 SD) vs. 19.8 + 3.2 SD, p<0.001).
B. At 36 months, mean post-op IOP is still below pre-op IOP.
C. Trend for increasing IOP (Slope=0.05, p<.001).
D. IOP in Control Group changed slightly
   1. (23.8 + 3.6 SD vs. 23.4 +3.9 SD, p <.001).

IV. Associations for Postoperative IOP
A. Preop IOP* r=0.45, rsq=0.24, p=0.007
B. CCT r=0.07, p=0.71
C. Age r=-0.21, p=0.23
D. Gender r=0.13, p=0.46
E. Race r=-0.06, p=0.75

V. Summary
A. Higher Pre-op IOP was associated with higher Post-op IOP
B. Higher pre-op IOP was associated with a greater percentage reduction in IOP

VI. Why does Cataract Surgery Lower IOP?
A. Associated with anterior chamber depth and angle configuration (Shrivastava and Singh (Curr Opin 2010))
B. Narrower anterior chamber angles experience a greater decrease in IOP
C. Increased outflow facility (Meyer (Ophthalmology 1997) and Kee (BJO 2000))
D. Biologic (Interleukin, TNF-Wang, IOVS 2006)
E. Tension on zonule (Van Buskirk, 1981)