USE OF THE KONAN NONCON-ROBO SPECULAR MICROSCOPE IN CLINICAL RESEARCH

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Purpose

- Understand variability issues with specular microscopy that may bias results
Objectives

- Provide examples of good and poor photography
- Illustrate variability in specular microscopy photography and analysis
- Illustrate variability within a single image
What is a Good Image?

- Distinct cells
- Can identify at least 150 cells
- Cells can be grouped in a uniform area
- What may be good for clinical purposes may not be good research
Things to Consider That Affect Quality of Image

- Dry eye
- Contact lens use
- Wrong Specular Manual Settings
- Keratoconus
- Patient Compliance
- Age
- Training, experience of photographer
Poor Quality Images
Poor Quality Images Continued...
Poor Quality Images Continued...
Conditions that Potentially Increase Variability

- Guttata (Fuch’s dystrophy)
- Polymegethism/Pleomorphism
- Injury
- Low Cell density (Huge cells)
Guttata (Fuch’s dystrophy)
Capturing the Best Image Possible

- Make sure Pt is comfortable
- Instruct Pt to blink
- Instruct Pt not to move and to open eyes wide
- Instruct Pt to focus on the green light
- Be patient
- Use Manual setting to improve quality when cornea is unusually thicker than normal
Things to Consider When Analyzing Images

- Locate the best and most representative area
  - Number of cells
  - Quality of Cells
    - No shadows
    - Disease
  - Use area with the fewest distortions
    - Blurring
    - Washed-out images
    - Shadows
Locating the Best Analysis Area (Sample Images)
Dotting Cells

- Dot all Cells at the Center
  - Remain accurate and consistent throughout
- Dot 150 cells
- Grouping is important
Where to Group the Analysis?
What is Wrong With This Analysis?

- Analysis is not representative
- Introducing Bias
- Not likely to repeat
- Not enough cells counted
Grouping Details

- Easy
- Clear
- No shadows
- Dot 150 cells

Normal

- Need Good rep.
- Take more time
- Dot > 150

Polymegathism
Grouping your Analysis

- Correct Grouping
  - Concentric
  - Even
  - Uniform

- Incorrect Grouping
  - linear
  - uneven
  - Winding
Cell Grouping - Guttata

- Group only in one area
To Analyze the Cells:

- You need to be able to visualize cells
- Find a pattern
- Identifying
  - Cells vs
  - Damage vs
  - Shadows
Where an Image is Analyzed Can Create Variability
Examples of Variability

CD = 2873
SD = 170
CV = 48
6A = 53

CD = 2976
SD = 113
CV = 33
6A = 53

$\Delta CD = 103$
(4%)
Examples of Variability Within Readers

\[ \Delta CD = 173 \]
\[ (7\%) \]
Examples of Variability Between Readers

Analysis
Repeated 4x

#1 - 2631
#2 - 2557
#3 - 2531
#4 - 2570
#5 - 2624

Range 2531 - 2631
## Consequences of Under or Over Counting

<table>
<thead>
<tr>
<th>Analysis</th>
<th>N</th>
<th>CD (Cells/mm$^2$) Mean</th>
<th>SD</th>
<th>$p$-value</th>
<th>$\Delta$ CD (Cells)</th>
<th>%</th>
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Study by Nauman Rashid
Precision of 36 Robo corneal endothelial specular images of each eye (OD, OS) taken on 18 different days and analyzed with the Robo software.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Cell Density</th>
<th>Precision</th>
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</thead>
<tbody>
<tr>
<td>OD</td>
<td>18</td>
<td>2545 ± 45 cells/mm²</td>
<td>(1.7%)</td>
</tr>
<tr>
<td>OS</td>
<td>18</td>
<td>2600 ± 41 cells/mm²</td>
<td>(1.5%)</td>
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</table>

(From AJO 125:465-471, 1998 LASIK Paper)
Age Dependent Cell Density Variation Within 3 Different Corneal Regions

- Central: $y = -18x + 3454$
- Paracentral: $y = -14x + 3622$
- Limbal: $y = -13x + 4310$

Cell Density (cells/mm²) vs. Age (Years)

- Central
- Paracentral
- Limbal
Sources of Variability
Summary

- Difficult to return to same location (1 mm = ± 56 cells/mm² - 2.0%)
- Poor image quality (minimal # of analyzable cells = 100)
- Technician error (Training/consistency)
- Reader analysis (Training/consistency)
- Equipment calibration/alignment