Warming tissue rapidly increases quality, efficiency and safety when assessing donor tissue.

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It can take 2+ hrs at RT to get a decent specular image.
Not all corneas are ‘ready’ at the same time.

1. It can take 3-4 hours to get a specular image (sometimes corneas can be left out all day).

2. Eye bank technicians need to check on corneas often.

3. It can take several warming cycles before a specular image can be obtained.
   - Multiple warming cycles may increase risks of pathogen growth.
Can we improve the evaluation process?

1. Find a consistent way to get specular images.

2. Reduce time tissue is unrefrigerated.

3. Make sure it’s safe to do #1 and #2.
Comparing specular images over time at 35°C and RT.

<table>
<thead>
<tr>
<th>OD @ 35°C</th>
<th>T=0</th>
<th>T=1 hour</th>
<th>T=2 hours</th>
<th>T=3 hours</th>
<th>T=4 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS @ 21°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Rating images for a quantifiable comparison.

363 Images

2 Masked Readers

0 = Unanalyzable
1 = Poor
2 = Fair
3 = Good
4 = Excellent
**Improved specular images at higher temperatures.**

<table>
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<tbody>
<tr>
<td>OD @ 35°C</td>
<td>0</td>
<td>1.8</td>
<td>2.0</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>OD @ 21°C</td>
<td>0</td>
<td>0.3</td>
<td>1.2</td>
<td>1.3</td>
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0 = Unanalyzable  
1 = Poor  
2 = Fair  
3 = Good  
4 = Excellent
Improved specular images at higher temperatures.

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<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
</tr>
</tbody>
</table>

- **OD @ 35°C**
  - T=0: Unanalyzable
  - T=1 hour: Poor
  - T=2 hours: Fair
  - T=3 hours: Good
  - T=4 hours: Excellent

- **OD @ 21°C**
  - T=0: Unanalyzable
  - T=1 hour: Poor
  - T=2 hours: Fair
  - T=3 hours: Good

Moved to 35°C for 2 additional hours.
Improved image quality is consistent for the whole dataset.

- **N=11** cornea pairs
- **33 images** per time point per condition.

![Graph showing Average Specular Image Rating over time with 35°C and 21°C conditions, with a shift to 35°C highlighted.](image-url)
Improved morphometric parameters at 35°C.

Trends over time:

- **35°C**
  - ECD unchanged, p=0.76
  - HEX **increases**, p=0.01
  - CV **decreases**, p=0.04

- **RT**
  - ECD unchanged, p=0.82
  - HEX unchanged, p=0.30
  - CV unchanged, p=0.54
Can we get better images in less time?

Comparable images:

1 hour at 35°C

4 hours at RT
Earlier evaluation = less time out of the refrigerator.

- **Average Specular Image Rating**
  - **Time (hours)**: 0, 1.5, 4
  - **Conditions**: 35°C, 21°C
  - **p-value**: 0.04
  - **N=9 cornea pairs**
  - **27 images per time point per condition**

- **Images**
  - **OD @ 35°C**
    - T=1.5: 2.3
    - T=4: 2.8
  - **OS @ 21°C**
    - T=1.5: 1.0
    - T=4: 2.0
We get better images...

1. Is rapid warming safe for endothelial cells?

2. Can we raise the temperature without promoting more pathogen growth?

...but is it safe?
No increase in endothelial cell loss at 35°C (n=9 pairs).

- **Trypan-blue (Day 1)**
  - Warm @ 35°C: 3.4%
  - Warm @ 21°C: 2.9%

- **Calcein-AM (Day 14)**
  - Warm @ 35°C: 15.5%
  - Warm @ 21°C: 15.6%

- **Fiji Segmentation**
  - Warm @ 35°C: 15.5%
  - Warm @ 21°C: 15.6%

**Incubation Regiment**

- **Day 1:** 4 hours RT or 35°C
  - Cold Storage

- **Day 3:** 4 hours RT or 35°C
  - Cold Storage

- **Day 14:** Cell viability analysis
No increase in endothelial cell loss at 35°C (n=9 pairs).

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<th>Calcein-AM (Day 14)</th>
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<tr>
<td>Warm @ 35°C</td>
<td>3.4 %</td>
<td>15.5 %</td>
<td>13.9 %</td>
<td>p=0.75</td>
</tr>
<tr>
<td>Warm @ 21°C</td>
<td>2.9 %</td>
<td>15.6 %</td>
<td>13.1 %</td>
<td></td>
</tr>
</tbody>
</table>

Looking at all 9 pairs:

- 13.9% (p=0.75)
Examining potential pathogen growth at 35°C.

Optisol-GS Cultures

Inoculate + store for 24 hours at 4°C

1\textsuperscript{st} incubation

(Initial evaluation)

35°C, 2hrs

21°C, 4hrs

2\textsuperscript{nd} incubation

(Post-processing evaluation)

35°C, 2hrs

21°C, 4hrs

Cold storage 48 hours

Cold storage 11 days

Analyze growth at days: 1, 3, 5, 7 and 14 after inoculation
Fungal survival is similar between the two conditions.

**Candida albicans**
- 35°C: p=0.50
- 21°C: p=0.76

**Candida glabrata**
- 35°C: p=0.66
- 21°C: p=0.91

**P-values @ Days 1 and 5**
Bacterial survival is similar between the two conditions.

*Staphylococcus aureus*

*Pseudomonas aeruginosa*

*Streptococcus pneumoniae*

P-values @ Days 1 and 5

- 35°C
- 21°C

Gen/Strep resistant
Summary: Advantages of rapidly warming donor corneas.

1. Consistently get better quality specular images.
   - Better images lead to more accurate information for surgeons.
   - Increases efficiency in eye bank processes.

2. Reduces time tissue is not refrigerated.
   - Reducing risk of pathogen growth due to multiple warming cycles.

3. No difference in pathogen growth with shorter incubation at 35°C.
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