

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

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Lions VisionGift

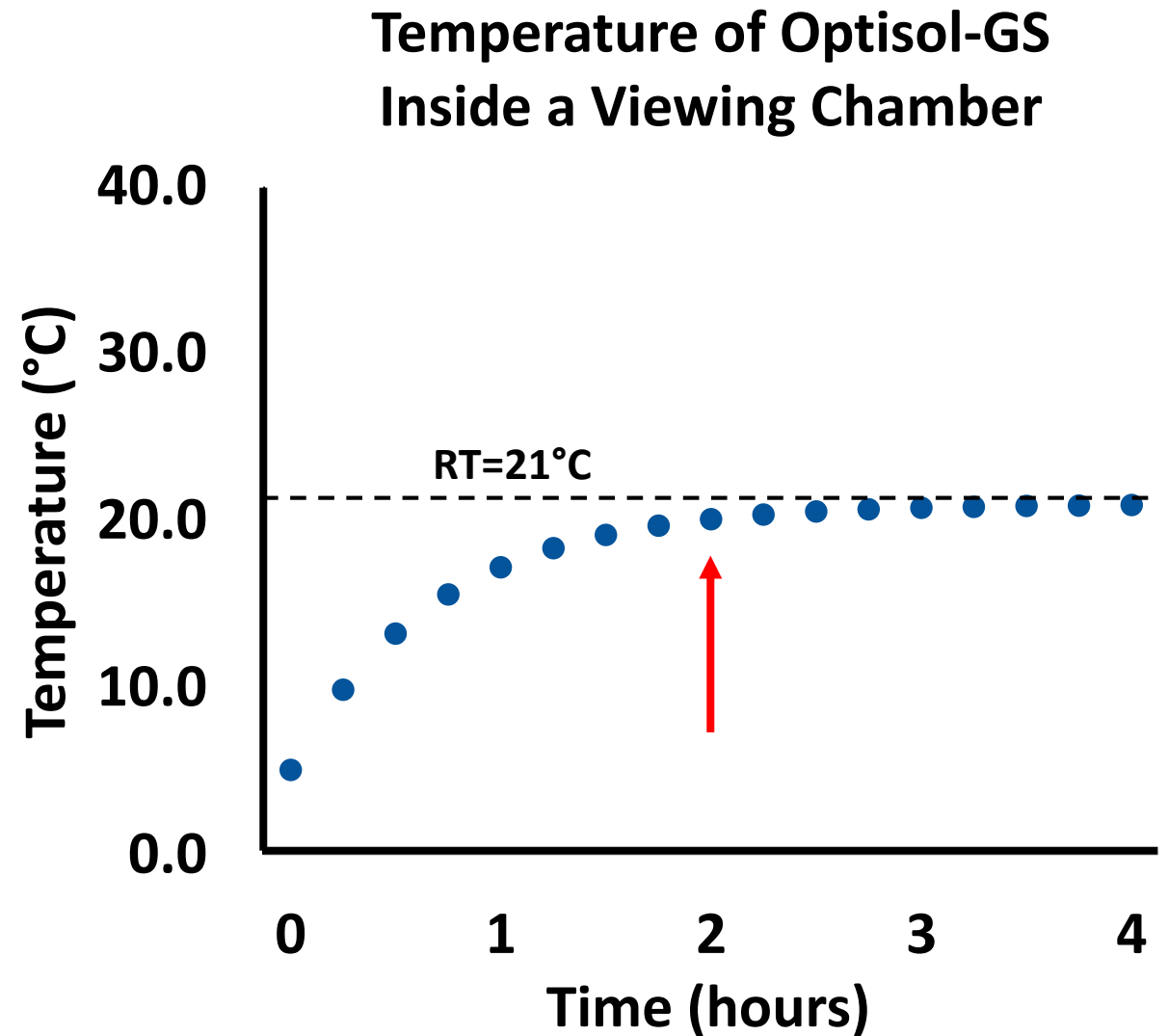
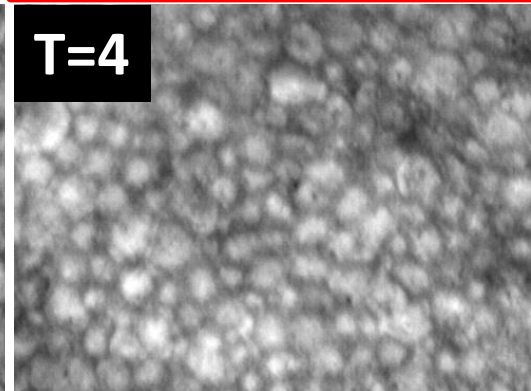
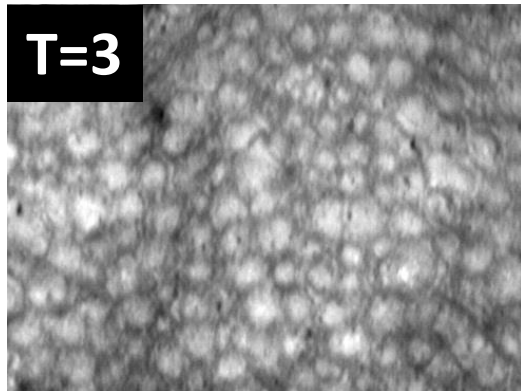
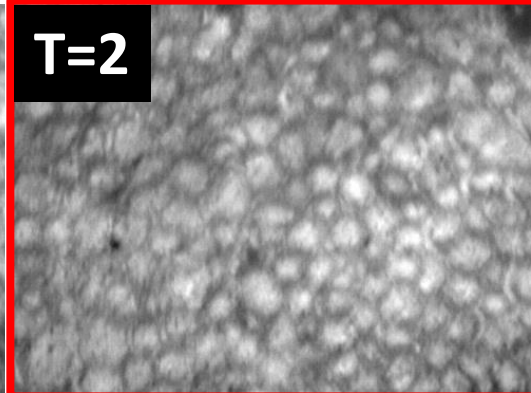
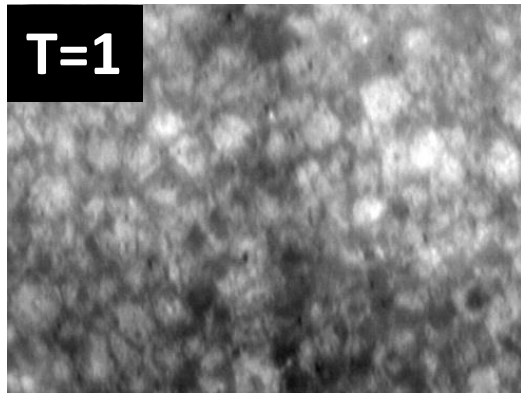
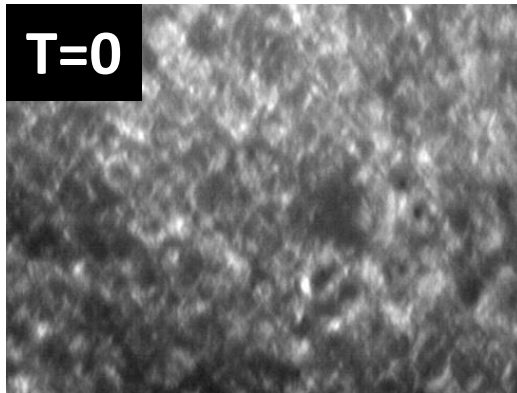
Bausch & Lomb (Unrestricted Research Grant)

Cornea Society

EBAA Fall Educational Symposium

October 14, 2016

It can take 2+ hrs at RT to get a decent specular image.



Not all corneas are 'ready' at the same time.

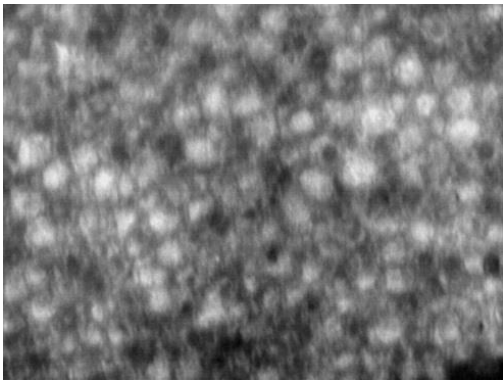
4 hours @ RT

Cornea #1



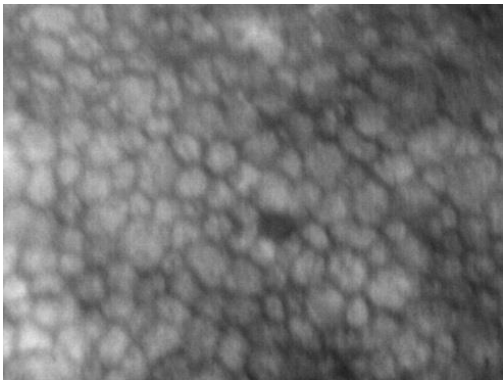
1. It can take 3-4 hours to get a specular image (sometimes corneas can be left out all day).

Cornea #2



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

Cornea #3

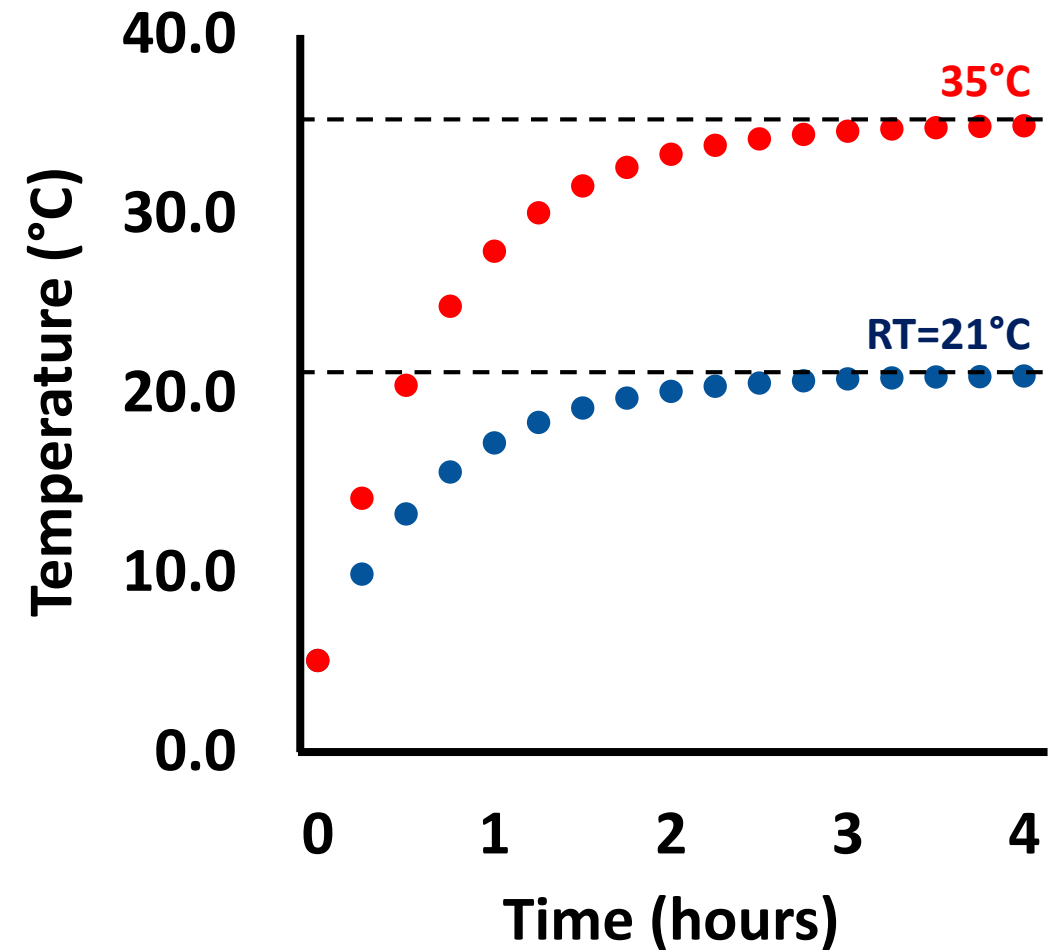


3. It can take several warming cycles before a specular image can be obtained.

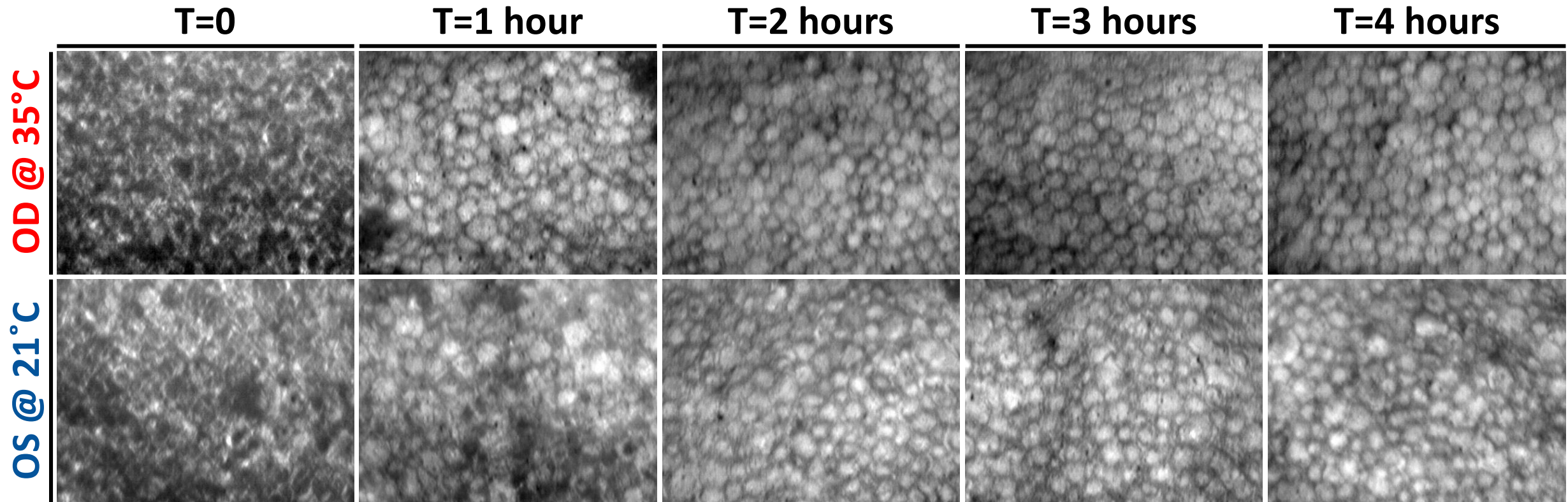
- Multiple warming cycles may increase risks of pathogen growth.

Can we improve the evaluation process?

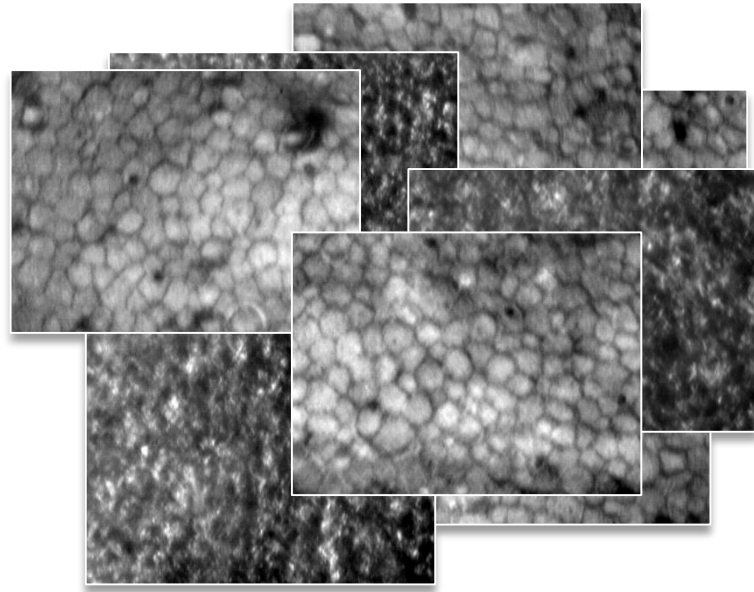
1. Find a consistent way to get specular images.
2. Reduce time tissue is unrefrigerated.
3. Make sure it's safe to do #1 and #2.



Comparing specular images over time at 35°C and RT.



Rating images for a quantifiable comparison.



363 Images

0 = Unanalyzable
1 = Poor
2 = Fair
3 = Good
4 = Excellent

2 Masked Readers



Image: Cliparts.co

Image Rating Module

Specular Rating Challenge

Show Baseline ☐ Open Report

Tissue ID: 2016-**** Search Remove Set Audit Set: 0472Incubator Mark Set Find Set

2016-1219.CNOS

☐ Unanalyzable
☐ Poor
☐ Fair
☐ Good
☐ Excellent

NOTE: If greater than 50% of the cells exhibit an inverted appearance, the rating should be dropped one level.

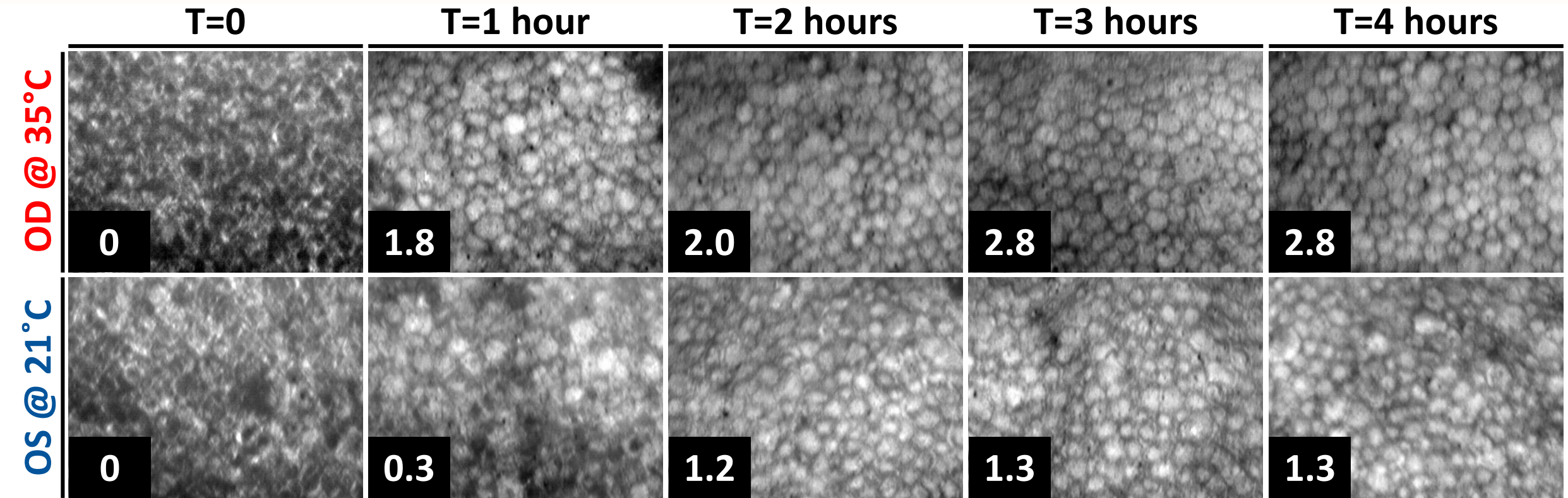
Audit Set: 0472Incubator Edit

2016-1137.CNOS

☐ Unanalyzable
☐ Poor
☐ Fair
☐ Good
☐ Excellent

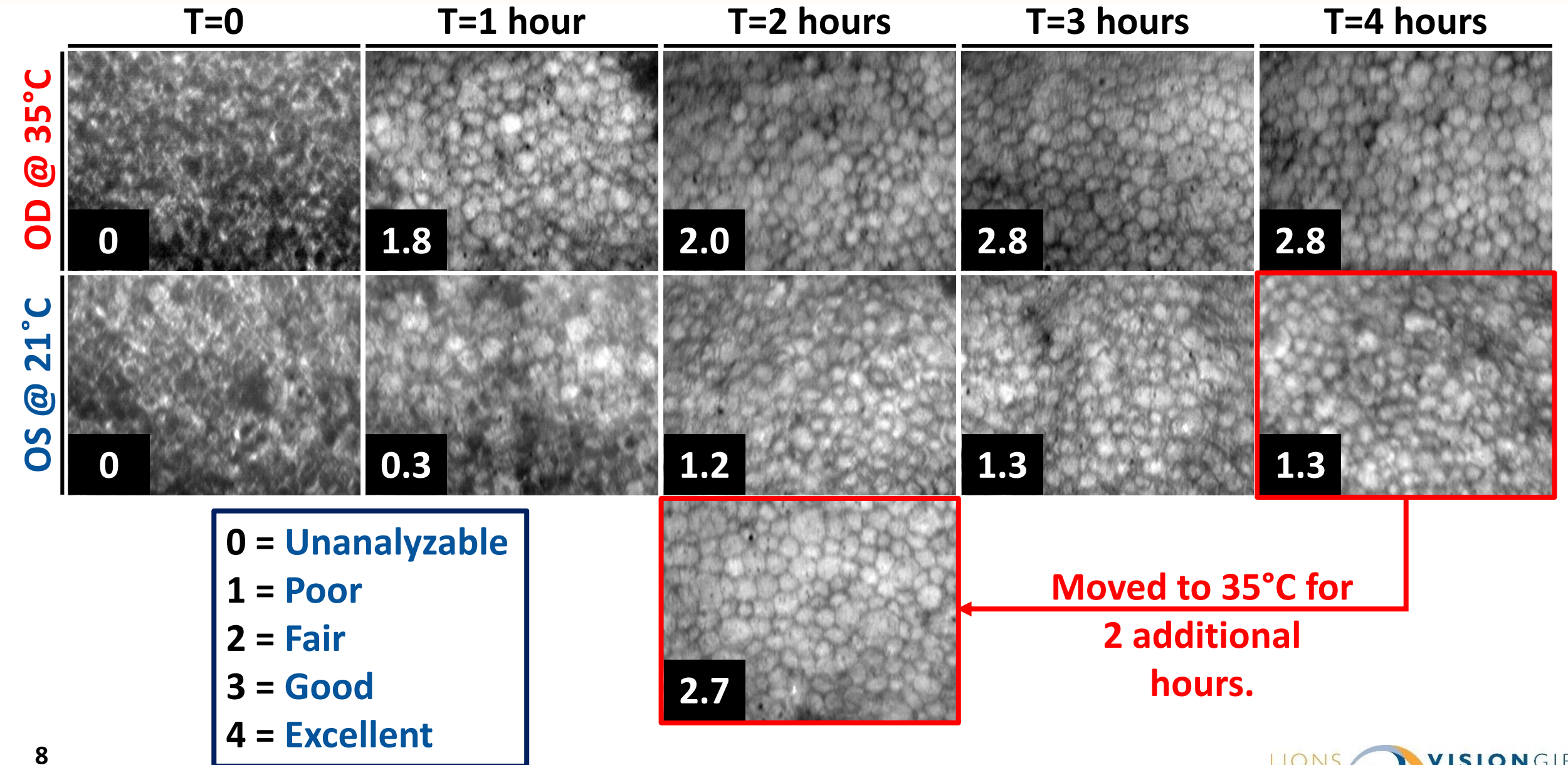
NOTE: If greater than 50% of the cells exhibit an inverted appearance, the rating should be dropped one level.

Improved specular images at higher temperatures.

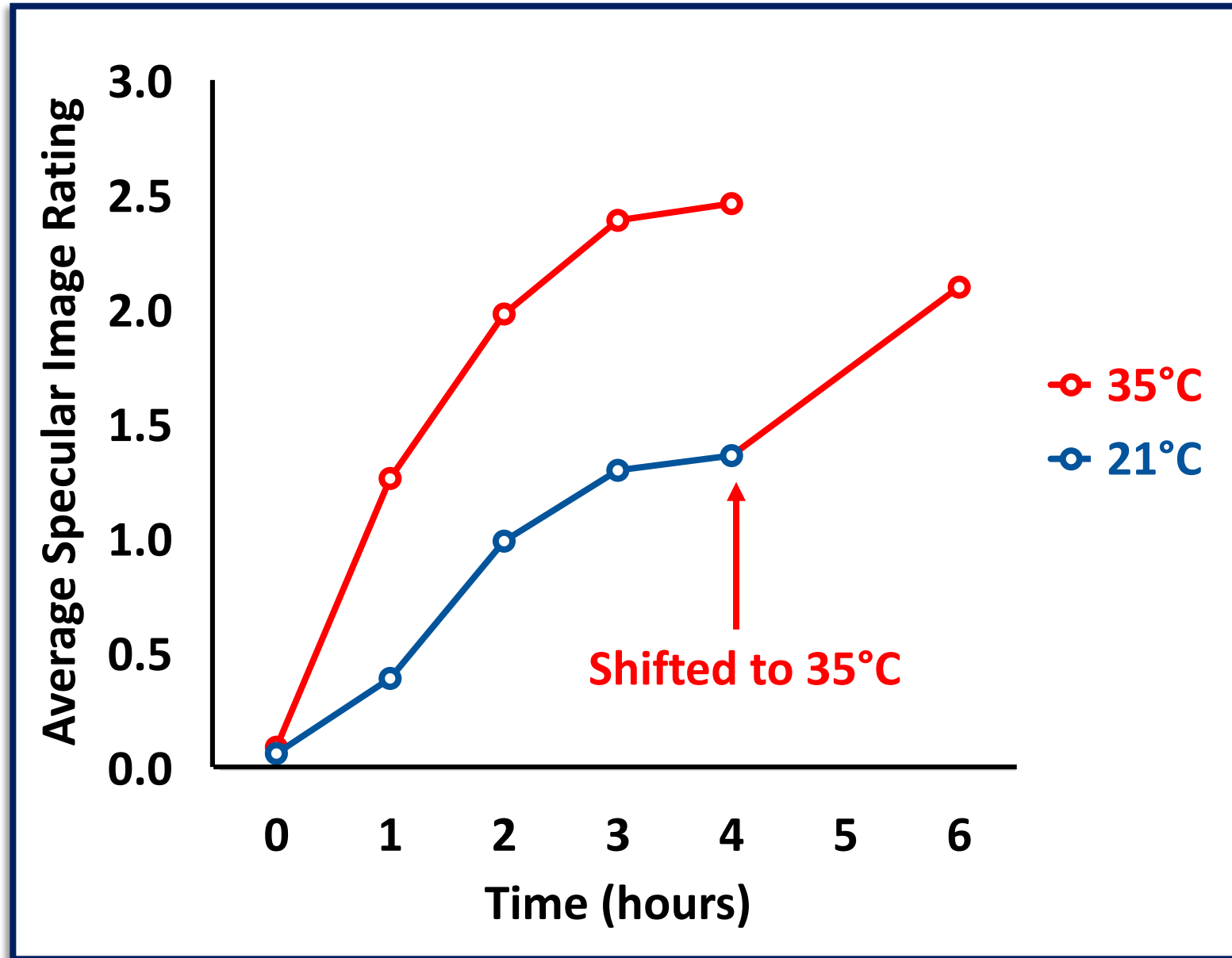


0 = Unanalyzable
1 = Poor
2 = Fair
3 = Good
4 = Excellent

Improved specular images at higher temperatures.



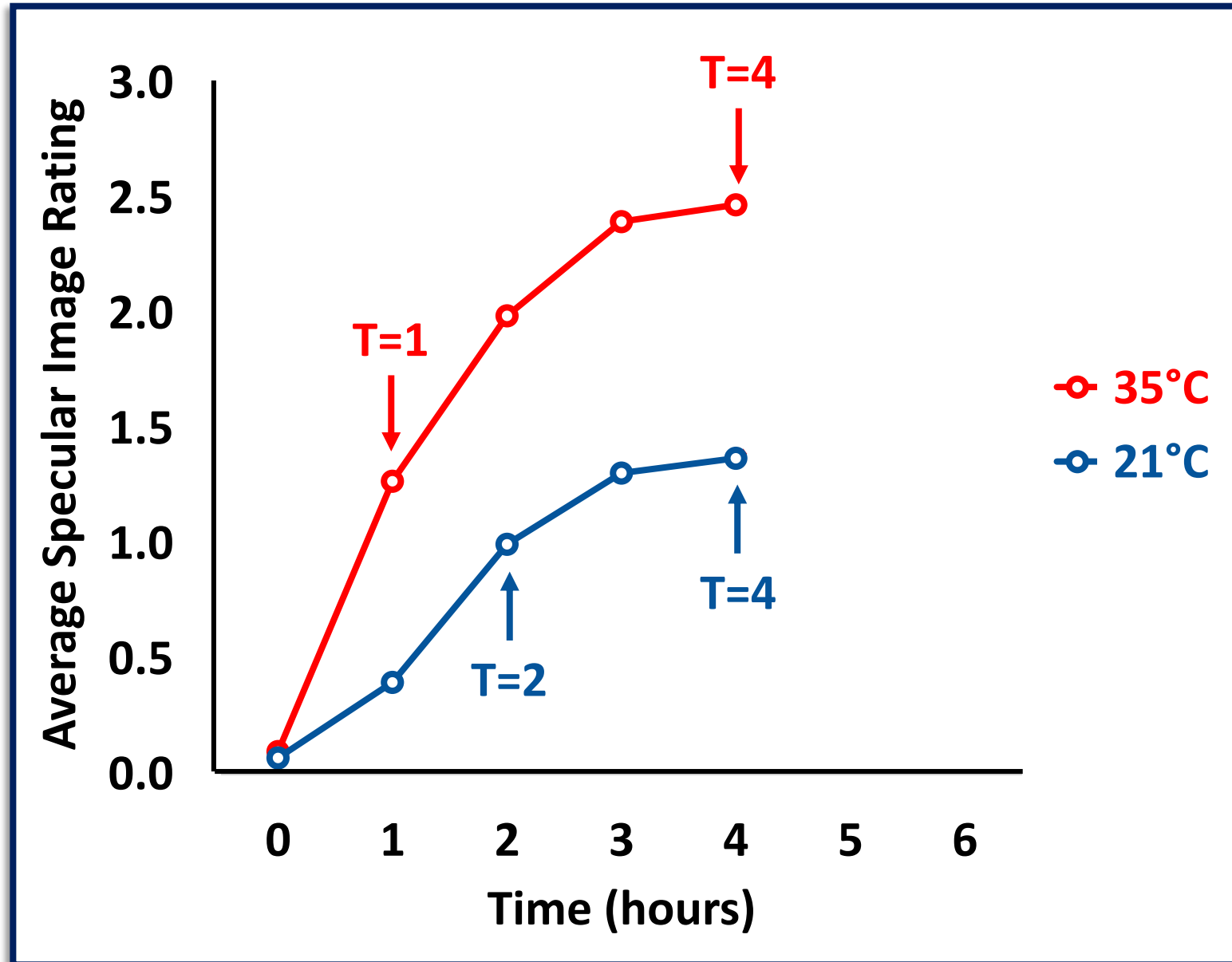
Improved image quality is consistent for the whole dataset.



N=11
cornea
pairs

33 images
per time
point per
condition.

Improved morphometric parameters at 35°C.



Trends over time:

35°C

ECD unchanged $p=0.76$

HEX increases $p=0.01$

CV decreases $p=0.04$

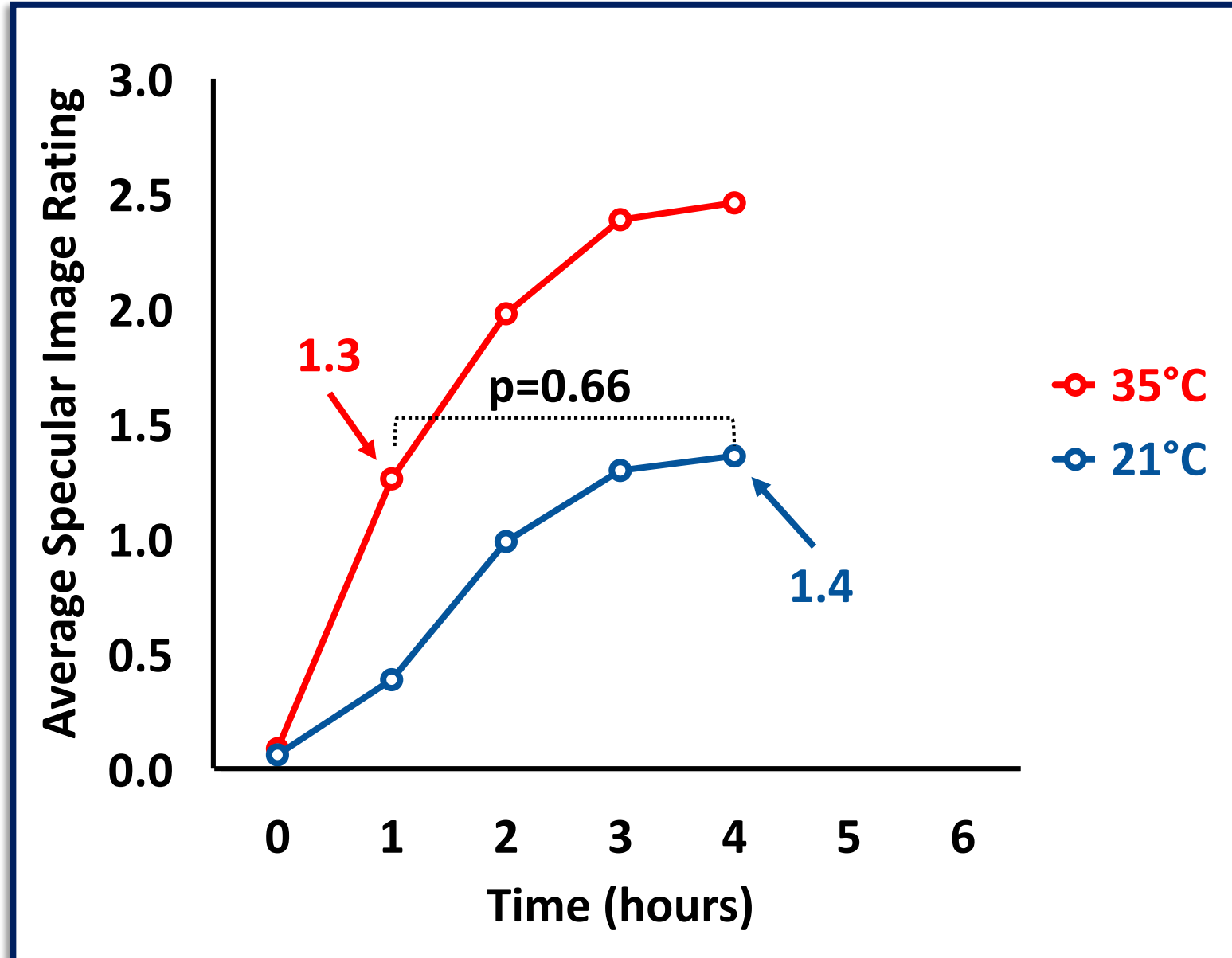
RT

ECD unchanged $p=0.82$

HEX unchanged $p=0.30$

CV unchanged $p=0.54$

Can we get better images in less time?

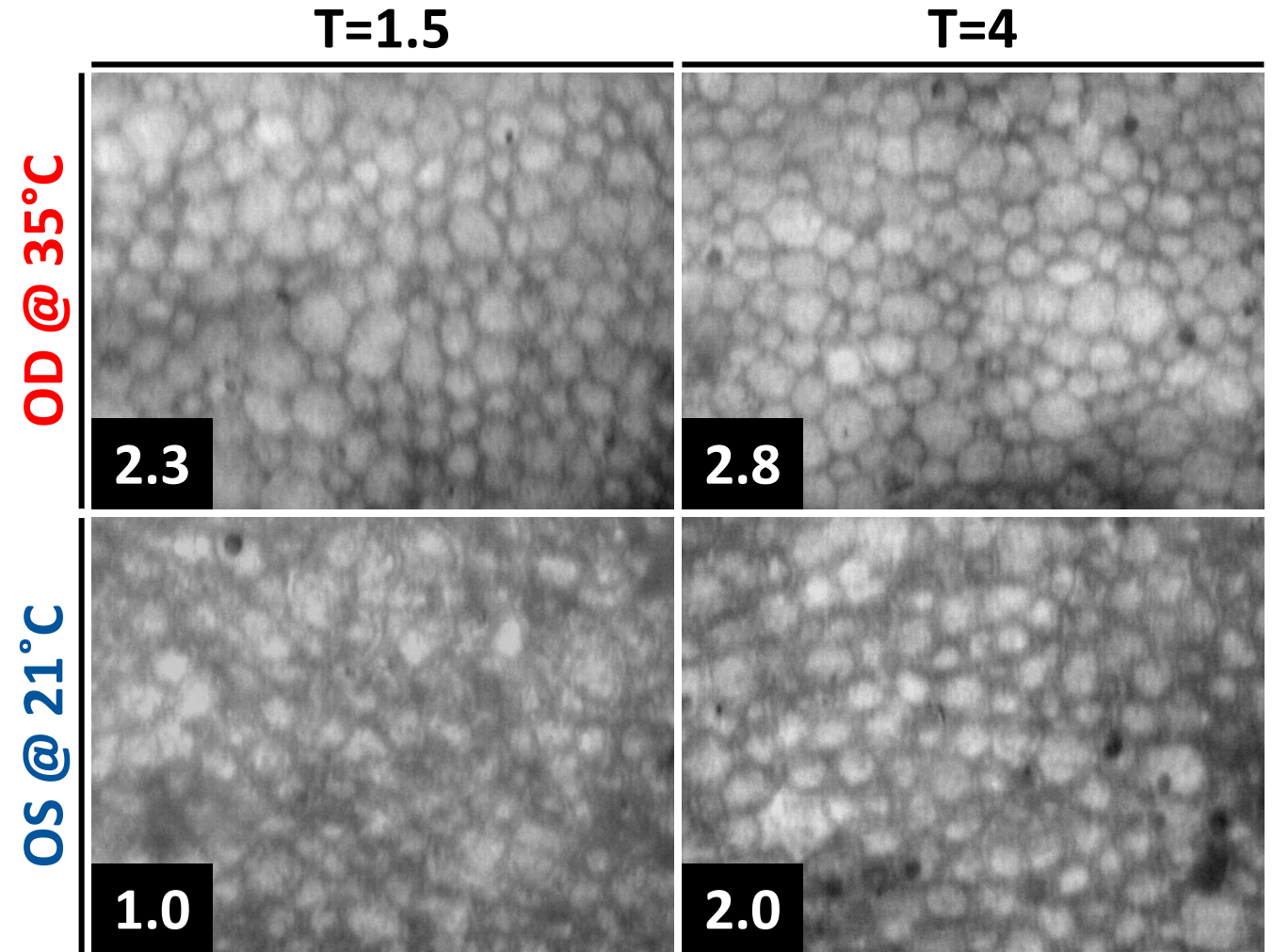
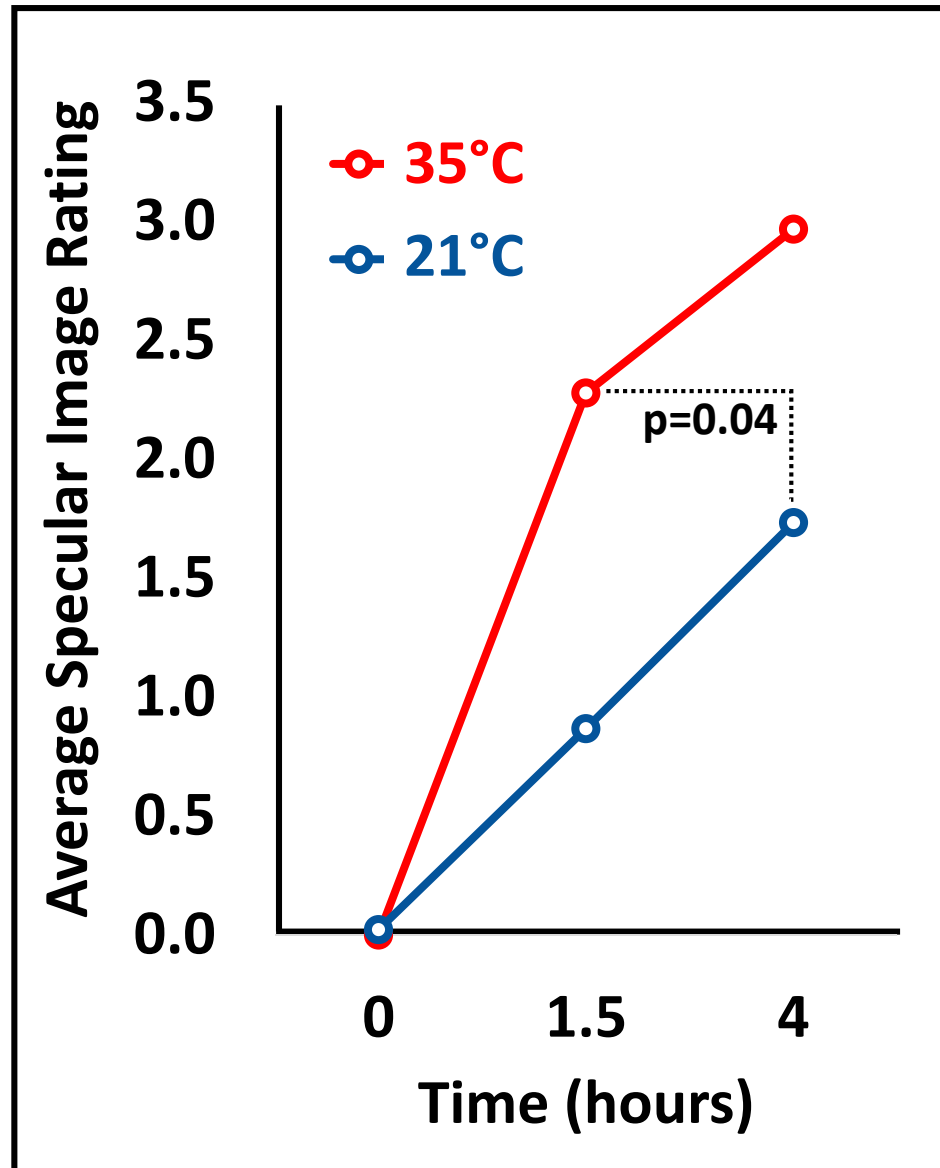


Comparable images:

1 hour at 35°C

4 hours at RT

Earlier evaluation = less time out of the refrigerator.



N=9 cornea pairs
27 images per time point per condition

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)

Calcein-AM (Day 14)

Fiji Segmentation

Incubation Regiment

Day 1: 4 hours **RT** or **35°C**

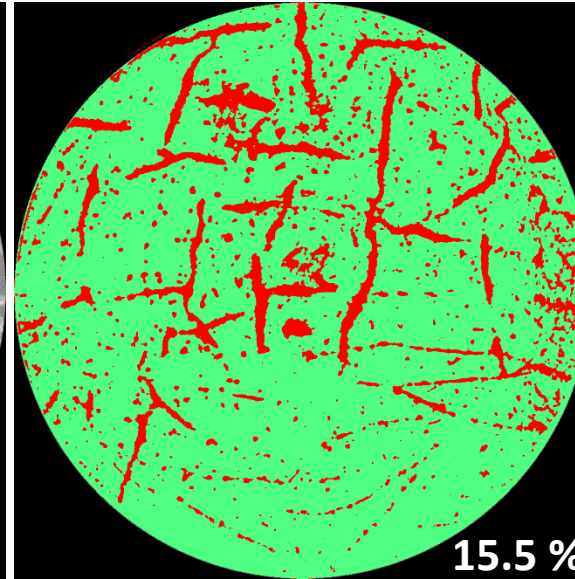
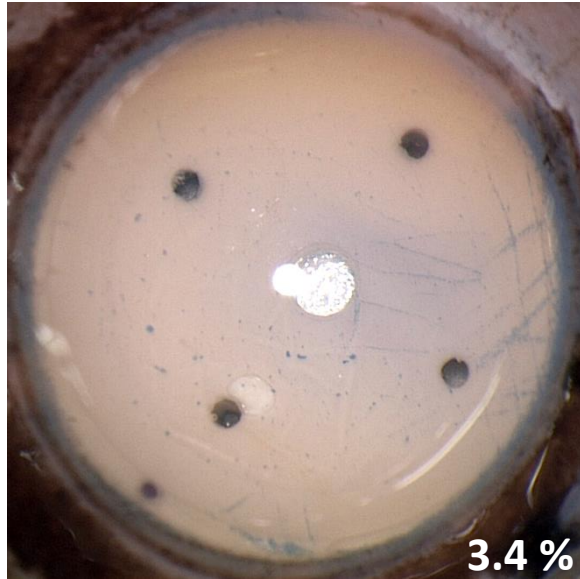
Cold
Storage

Day 3: 4 hours **RT** or **35°C**

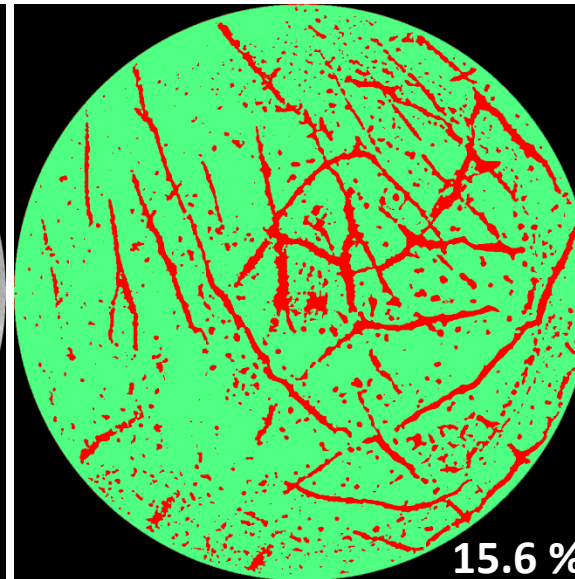
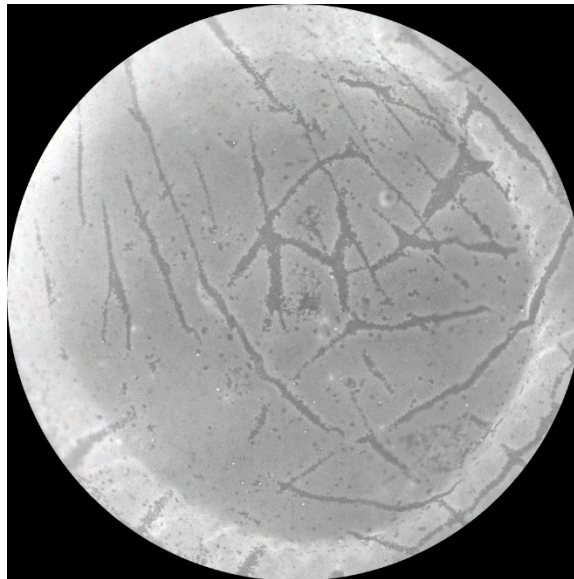
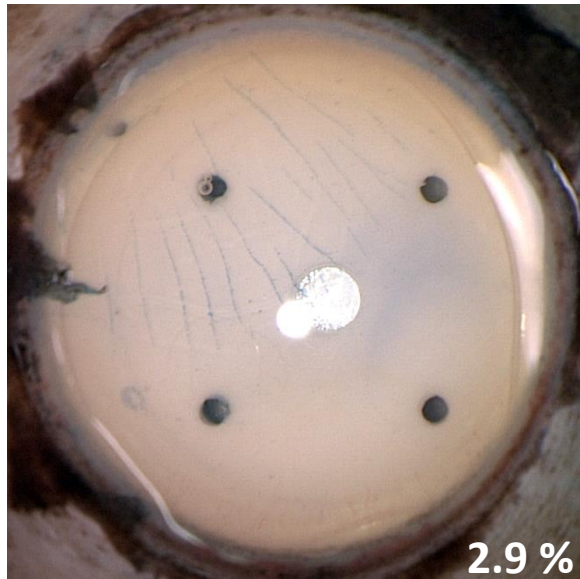
Cold
Storage

Day 14: Cell viability
analysis

Warm @ 35°C



Warm @ 21°C



No increase in endothelial cell loss at 35°C (n=9 pairs).

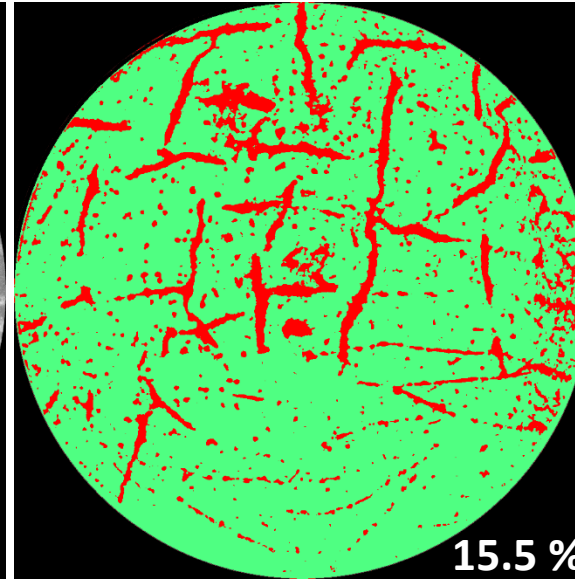
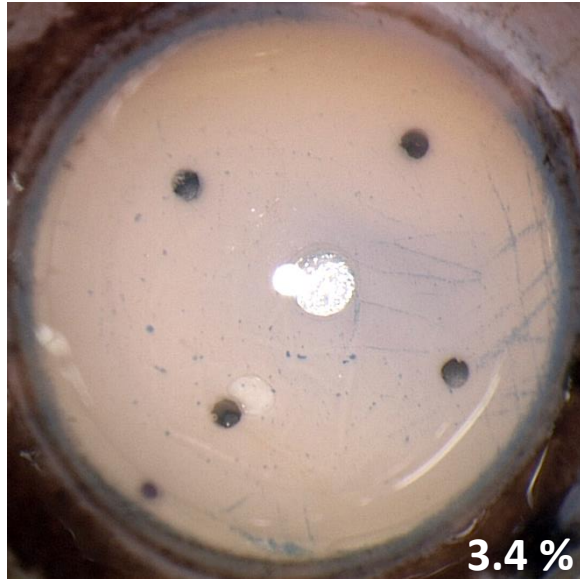
Trypan-blue (Day 1)

Calcein-AM (Day 14)

Fiji Segmentation

Looking at all 9 pairs

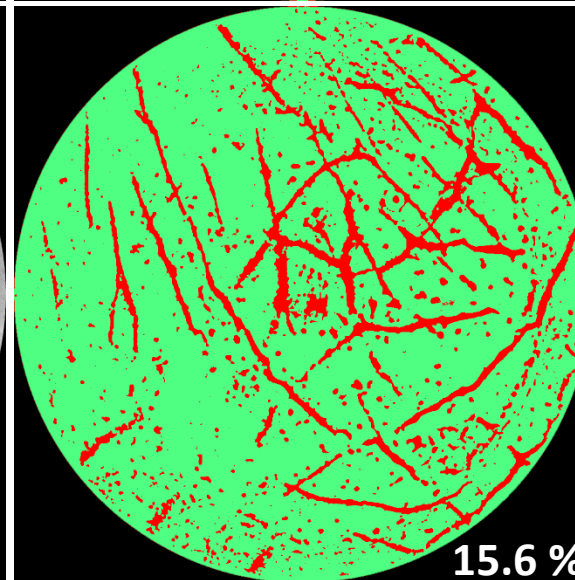
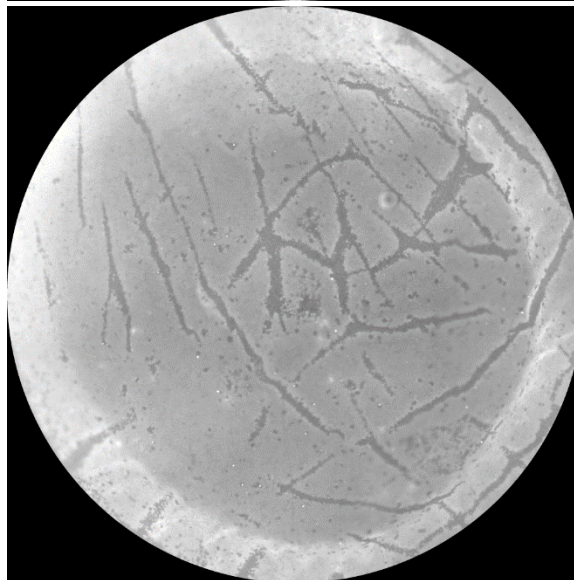
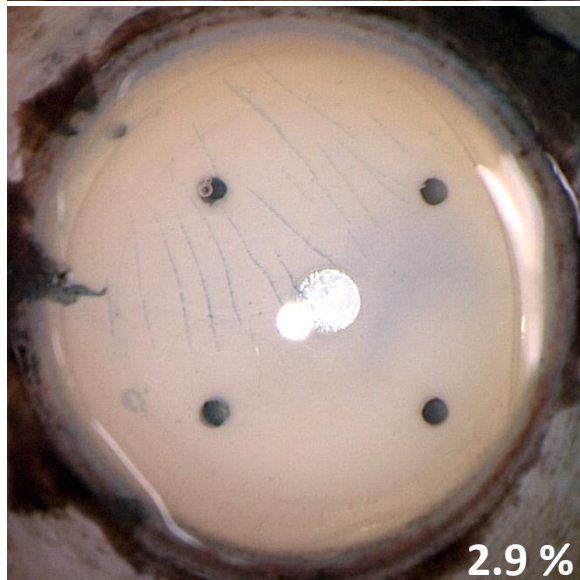
Warm @ 35°C



13.9 %

p=0.75

Warm @ 21°C



13.1 %

Examining potential pathogen growth at 35°C.

Optisol-GS Cultures

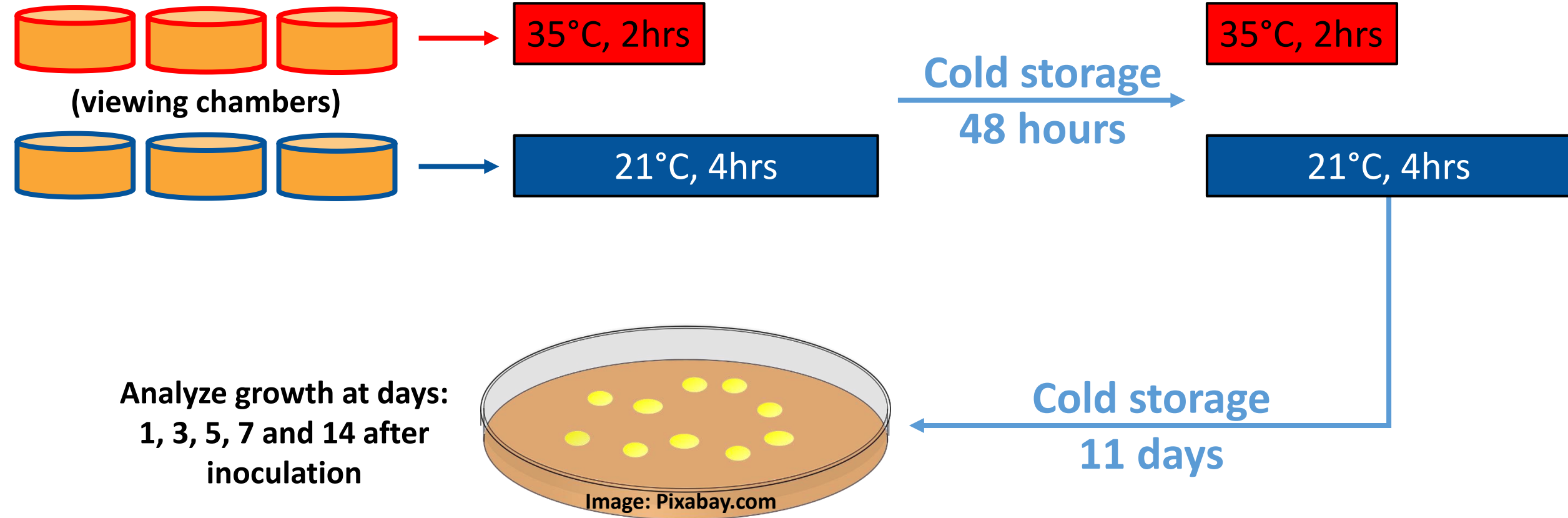
Inoculate + store
for 24 hours at 4°C

1st incubation

(Initial evaluation)

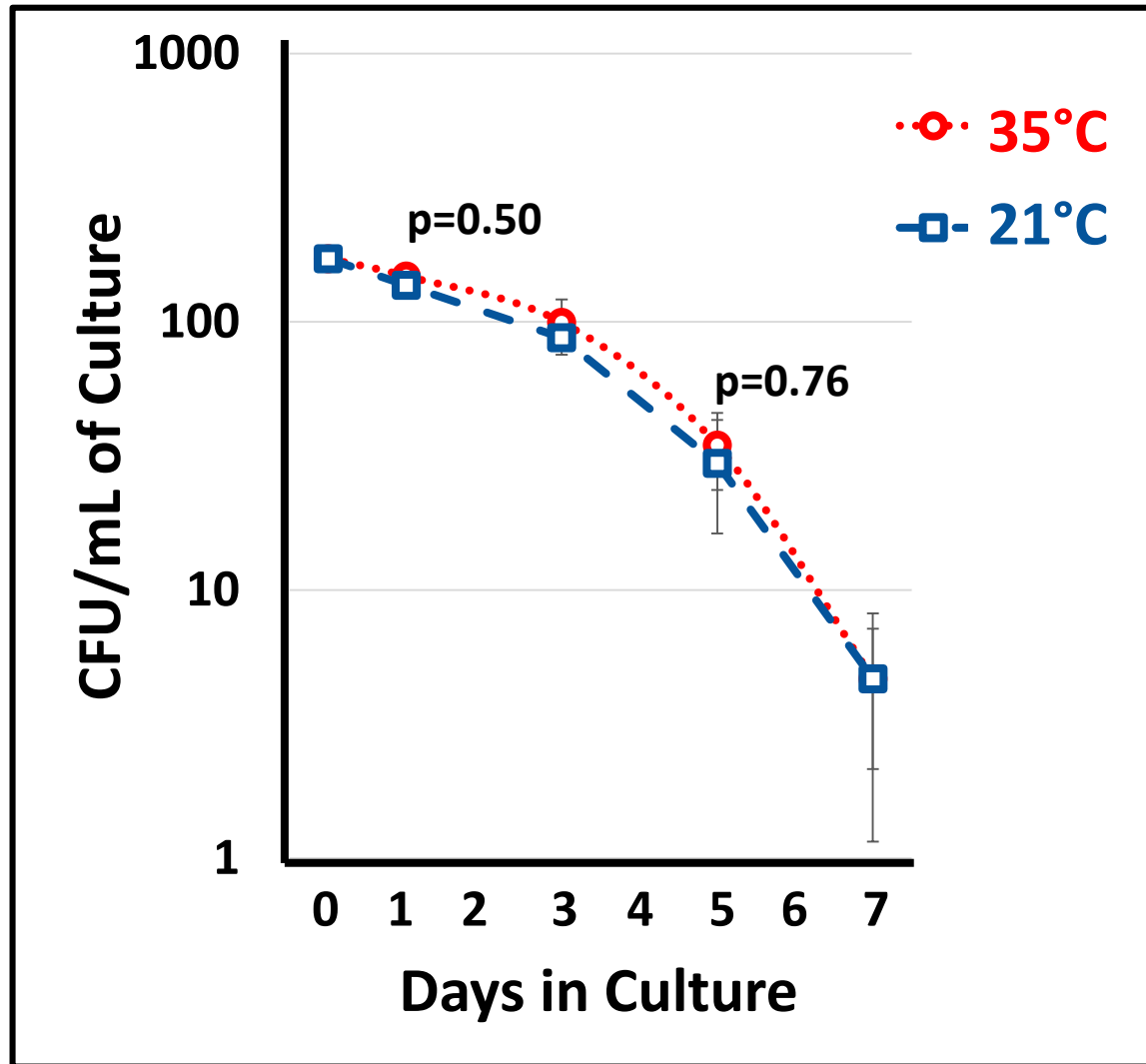
2nd incubation

(Post-processing
evaluation)

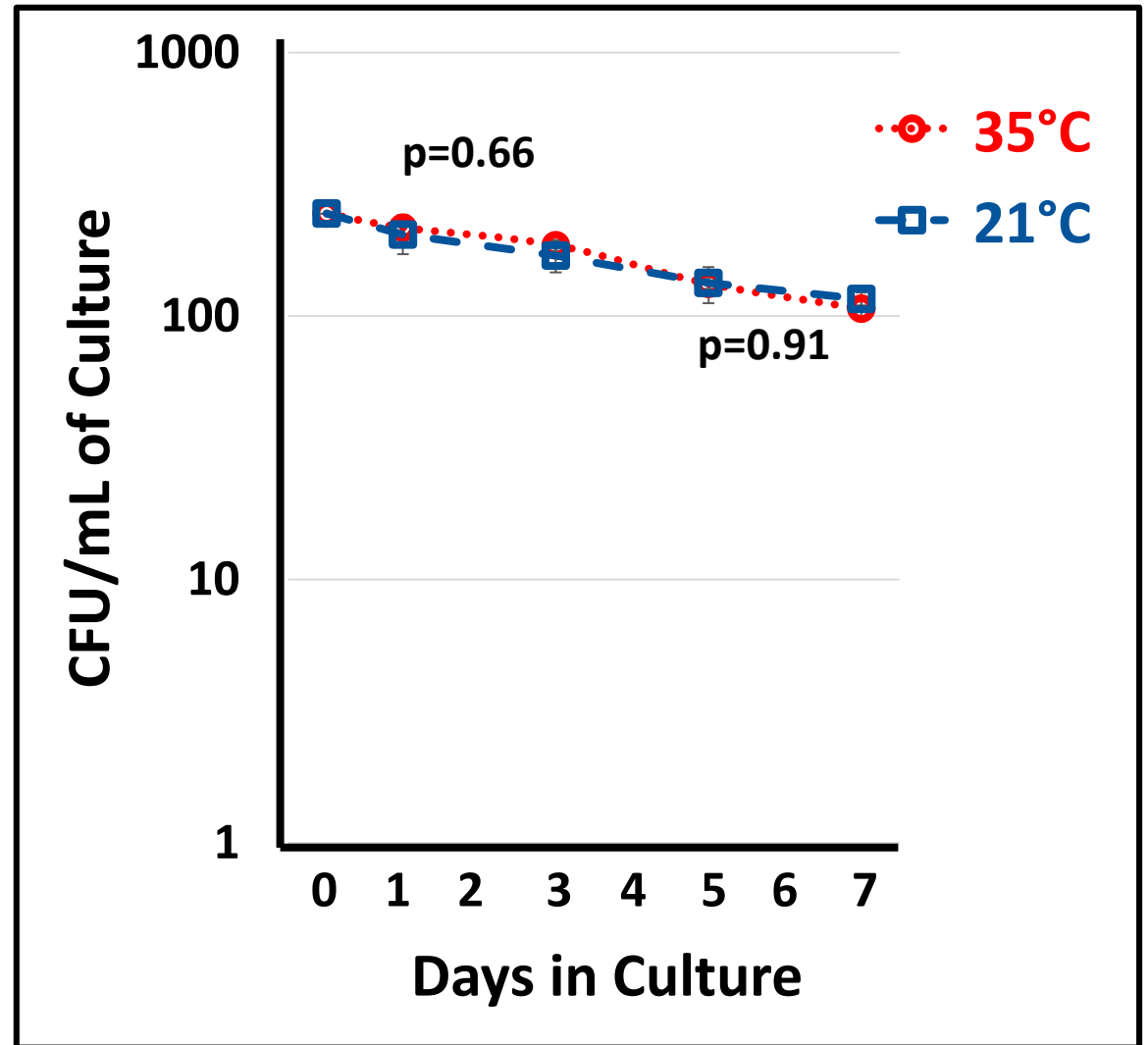


Fungal survival is similar between the two conditions.

Candida albicans

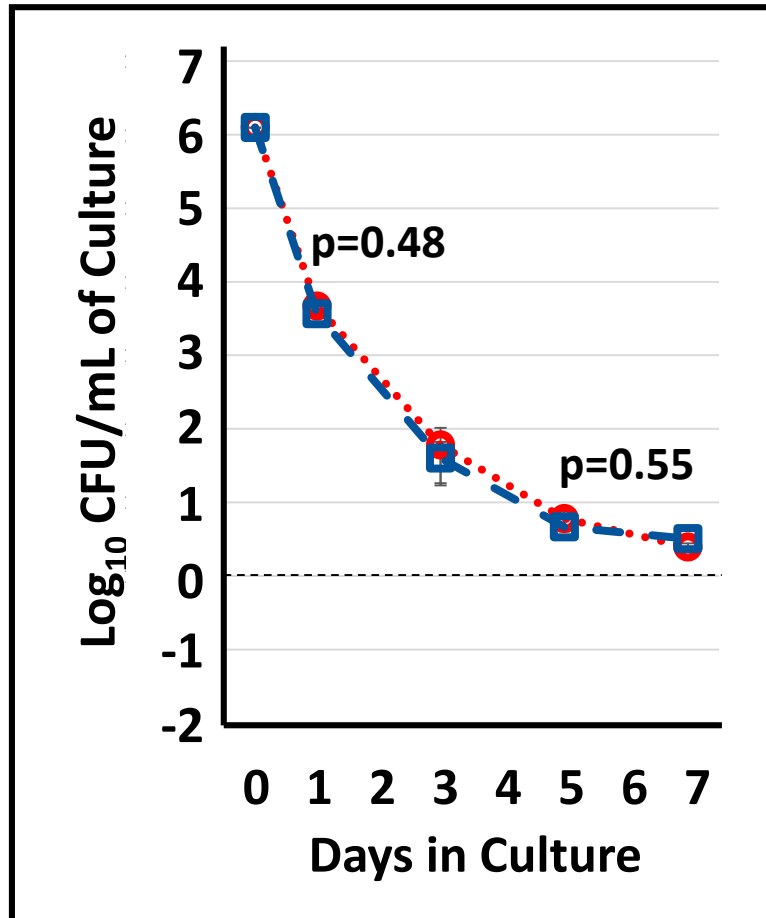


Candida glabrata



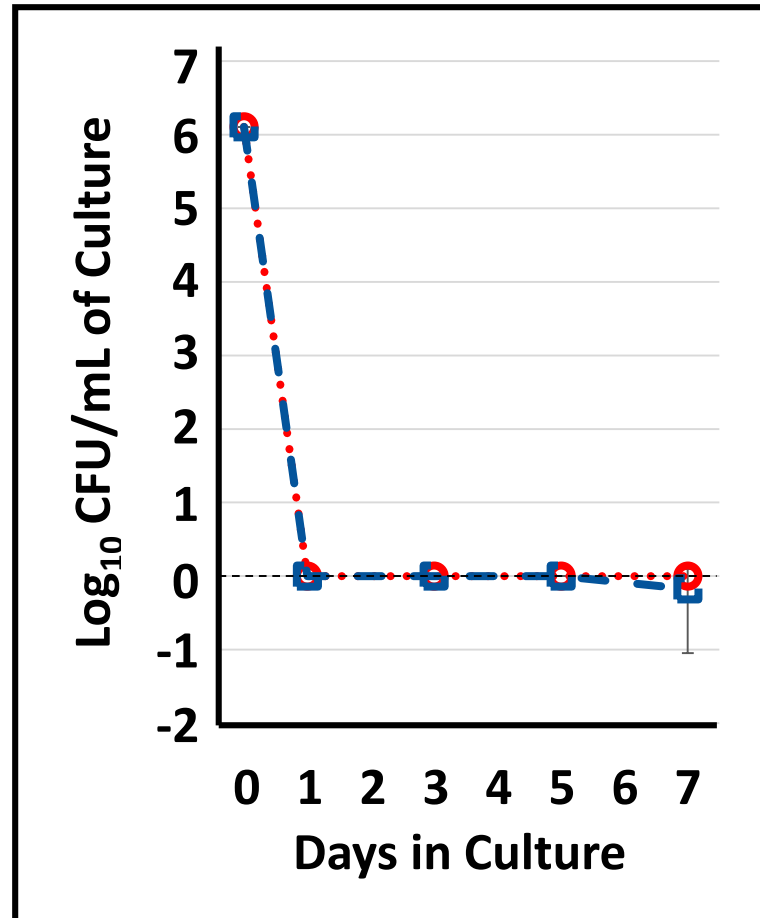
Bacterial survival is similar between the two conditions.

Staphylococcus aureus



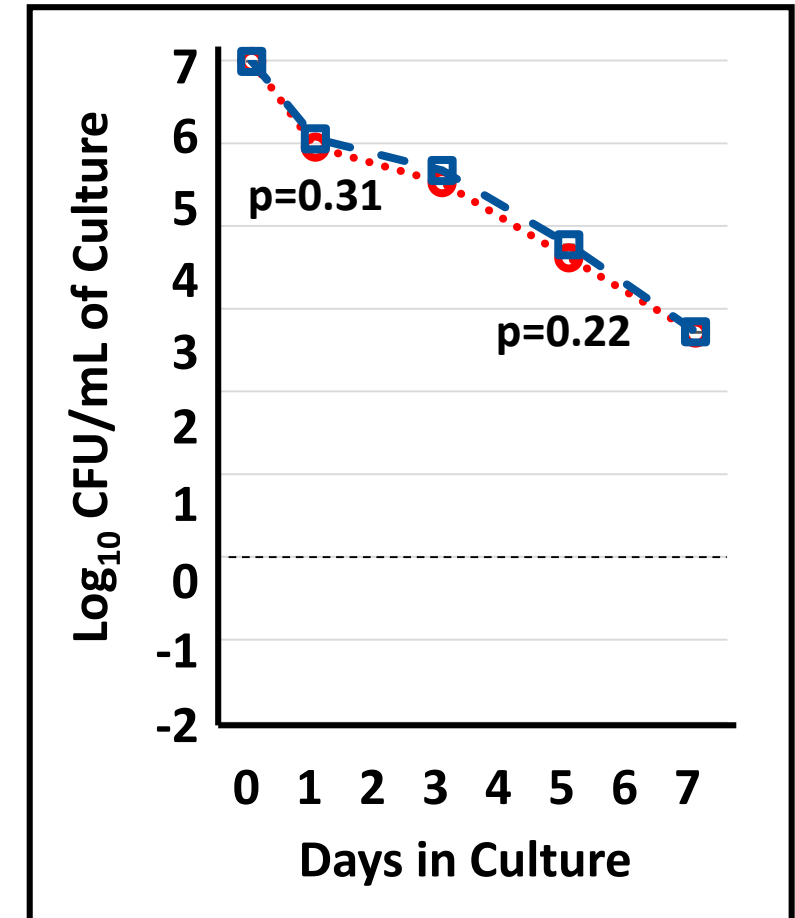
P-values @ Days 1 and 5

Pseudomonas aeruginosa



● 35°C
■ 21°C

Streptococcus pneumoniae



Gen/Strep resistant

Summary: Advantages of rapidly warming donor corneas.

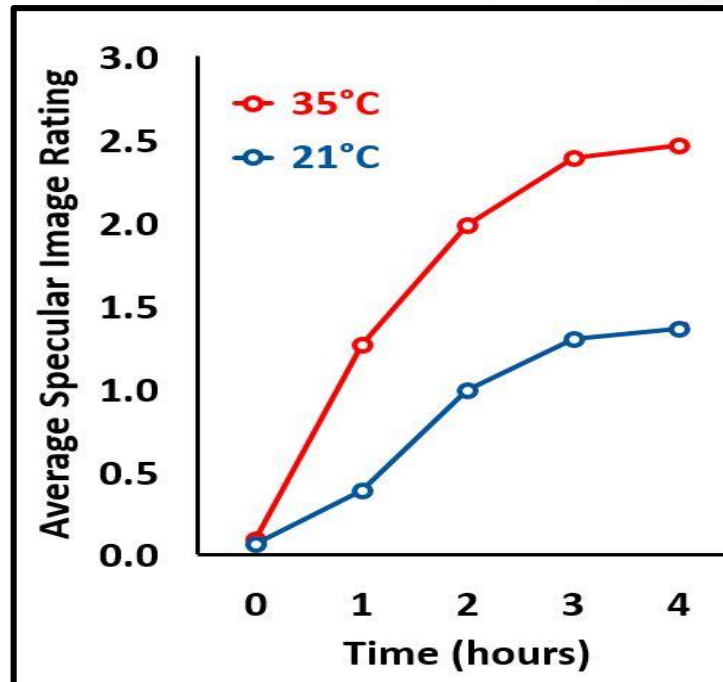
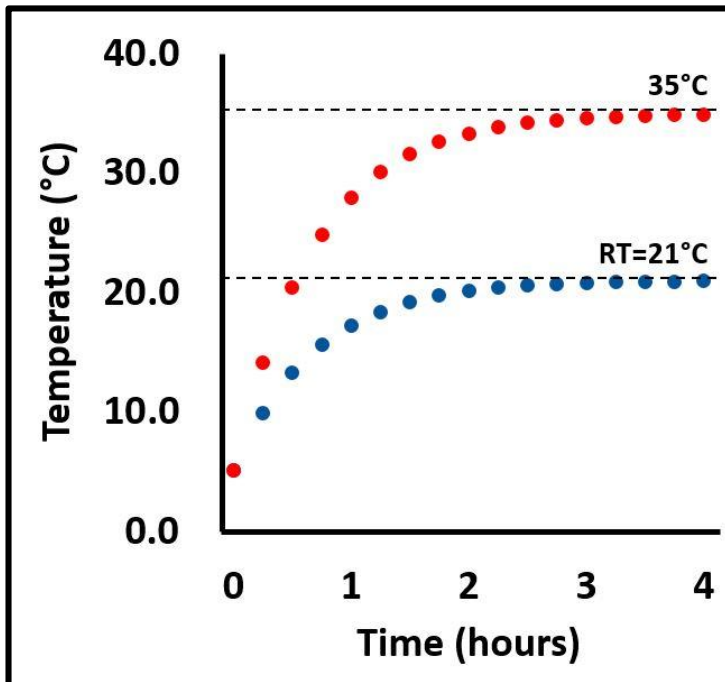
1. Consistently get better quality specular images.

- + 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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