The Effects of a Novel Sterilization Process on Donor Cornea Clarity

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Disclosures

- J. Holiman:
  - Allograft Select, LLC (P Pending)
- C. Stoeger
  - No disclosures

Disclosures not related to this study:

- Y. Li
  - Optovue, Inc. (F, P), Carl Zeiss Meditec, Inc. (P)
- D. Haung
  - Optovue, Inc. (F, I, C, P, R), Carl Zeiss Meditec, Inc. (P)
- C=Consultant, I=Invenstor, F=Financial Support, P=Patent, R=Recipient,

The Ultimate Goal of Storing Corneas

- In a perfect world we would be able to preserve corneal tissue indefinitely.

- We are not there yet

  (*But we **are** trying*)

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Important Factors to Consider When Storing Corneas

- Bioburden / Sterility
- Tissue Handling
  - Biomechanical properties
  - How well does it hold sutures?
- Convenience
  - Does it require reconstitution?
- Clarity

Long-Term Storage of Cornea (Past and Present)

- Liquid paraffin- (1908, Carrel)
- Glycerin- (1955, King JH)
- Cryopreservation

- Irradiation: recently developed for corneas
  - Gamma Irradiation: Previously developed
  - Electron Beam Irradiation (E-Beam irradiation) : Novel Sterilization Process for corneas.

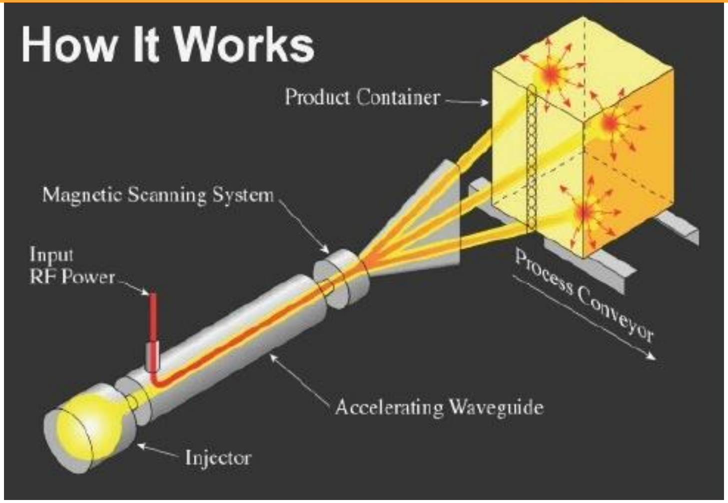
Purpose of this Study

- To quantify the effects of E-beam irradiation sterilization on the clarity of cornea tissue.

- Demonstrate the utility of darkfield biomicroscopy for evaluation of cornea clarity.

Electron Beam (E-Beam) Sterilization

- Accelerator creates a beam of electrons
  - Energized to near light speed
  - Magnet creates oscillations of beam
  - The electrons shower across conveyor belt as the tissue travels through.
  - Electrons and free radicals disrupt genetic material, rendering product sterile.



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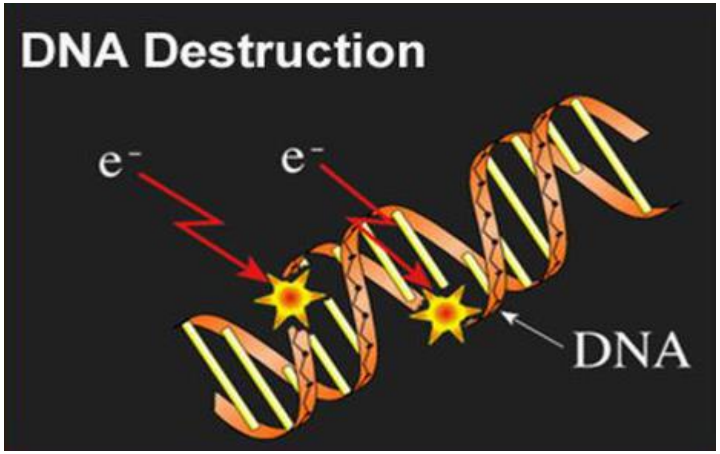

www.smebeam.com

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E-Beam vs. Gamma Irradiation

- Similarities
  - Fundamental mechanism of sterilization the same: ionizing radiation
  - Parametric release after processing
  - Utilize same dosimetry systems
  - Governed by same ISO standards
  - World-wide acceptability

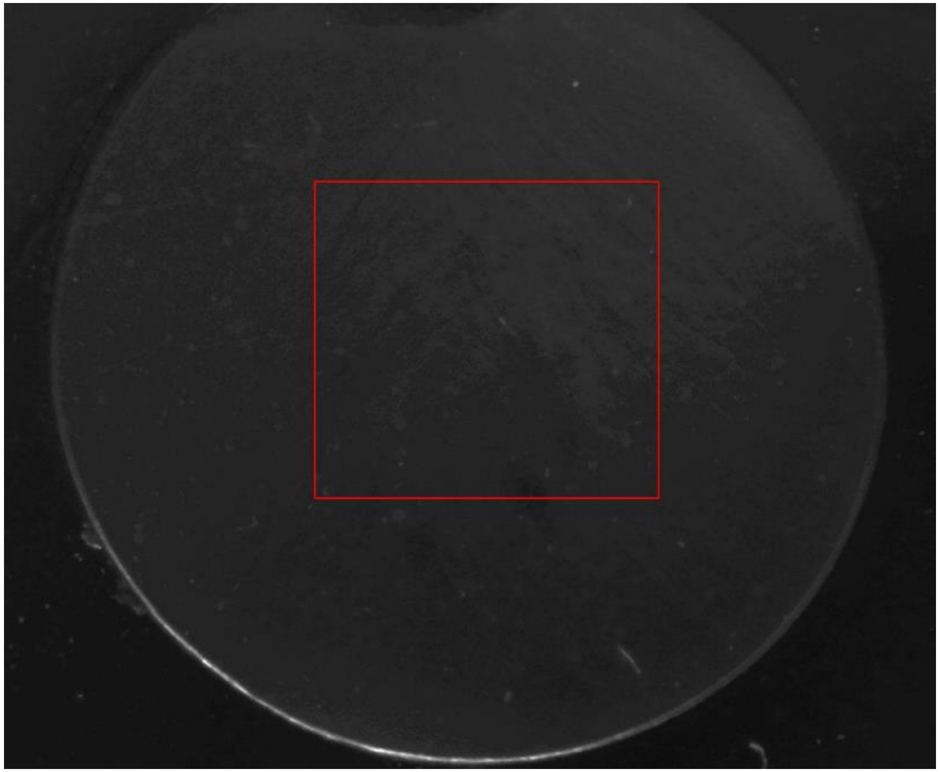
E-Beam Differences

Dose Rate and Penetration Depth

- E-beam has higher dose rate and lower penetration
- E-Beam typically requires less exposure time
- Lack of Cobalt 60 radioactivity

How does E-Beam Irradiation Affect Cornea Clarity?

- Can we use this sterile tissue for lamellar keratoplasty?



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How Clarity has been Measured

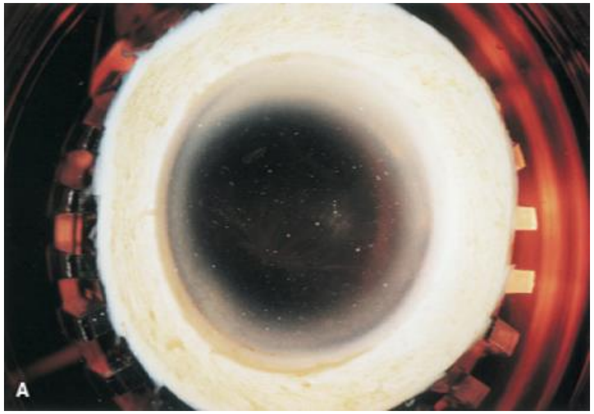
- **Spectrophotometry –**
  - glycerol preservation cornea (Li, 2012)
  - cryopreserved hAM (Ijiri, 2006)
  - bioengineered cornea (Ionescu, 2010)
- **Scatterometry –**
  - measurement of post-LASIK haze (McCally, 1993)
  - Gamma-irradiated corneas (Sikder, 2011)
- **Lux meter –**
  - fresh corneas comparing storage media (Parekh, 2014)

This Study Utilizes Darkfield for Assesing Cornea Clarity

Cornea 20(2): 210–213, 2001.

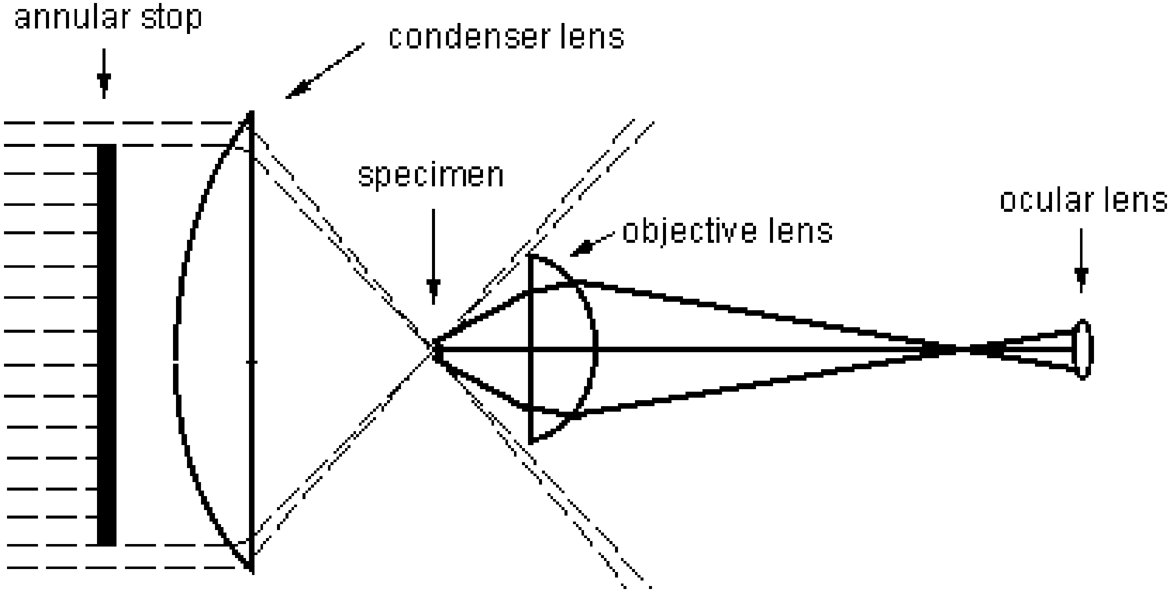
Darkfield Biomicrography of Eye Bank Donor Corneas

Lawrence M. Merin, R.B.P., F.I.M.I., F.O.P.S., F.B.C.A.,
Michael F. Brown, M.D., and Lindell L. Howdeshell, B.S., C.E.B.T.



This method can easily be performed 'In House'

Darkfield Light Path



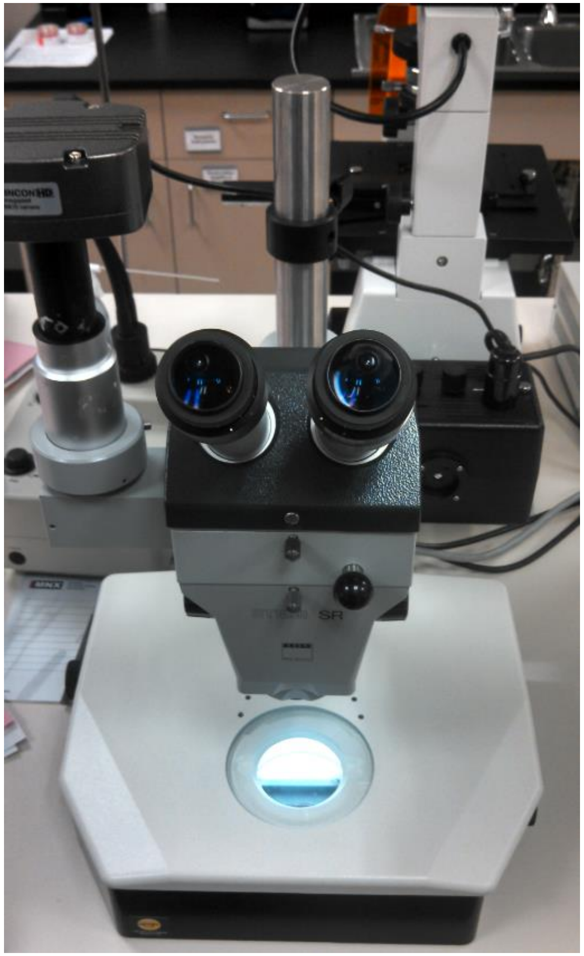
http://www.gonda.ucla.edu/bri_core/darkfld.htm

Darkfield Imaging System

Rincon HD

Zeiss STEMI SR
Dissecting Microscope

TLB 4000 Light Base
SPOT Imaging
Solutions



The Process

Remove Epi and Endo

Trephinate

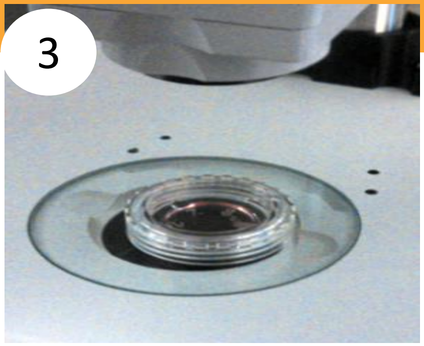
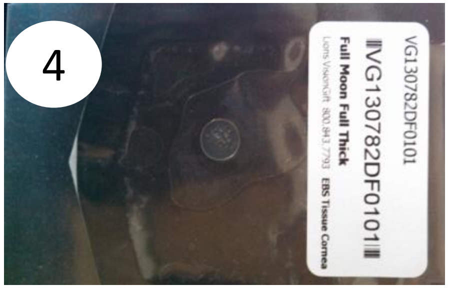
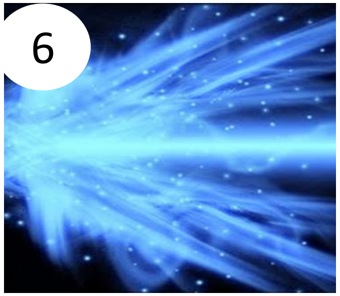


Image in Optisol

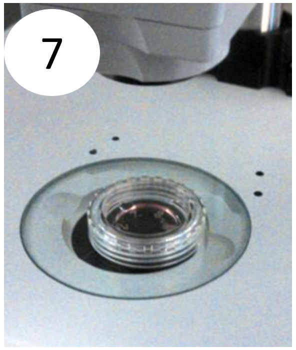


Package System

Freezing at -60°C



E-Beam Irradiated



Re-Image

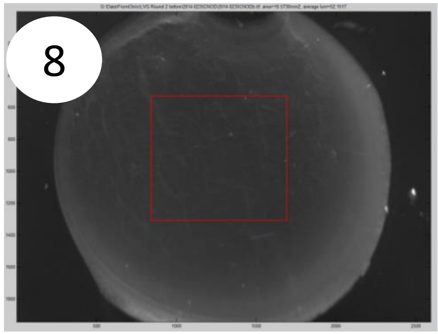


Image Comparisons

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Sample Images
Technique to minimize artifacts

Tissue in Air

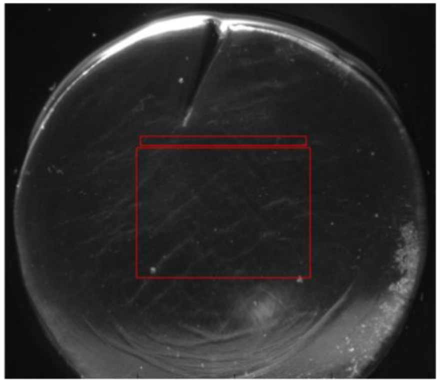

Fig. 1 Tissue #5 Before Treatment

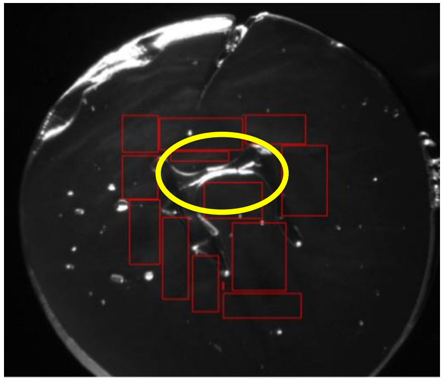

Fig. 2 Tissue #5 After Treatment

Tissue Submerged
(Removes Artifacts)

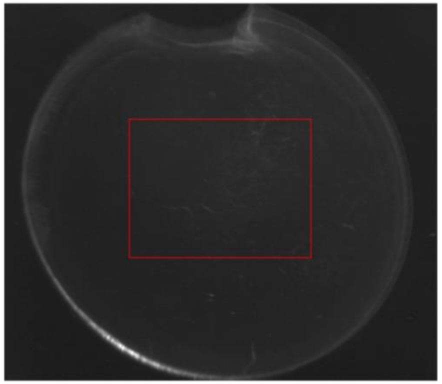

Fig 3 Tissue #18 Before Treatment
Submerged

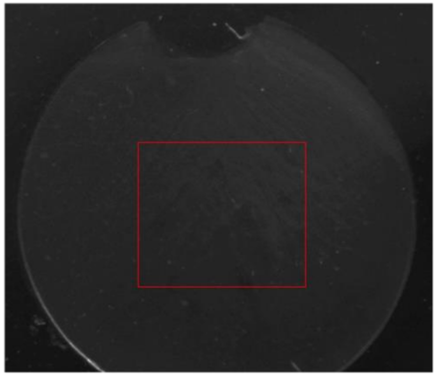

Fig 4 Tissue #18 After Treatment
Submerged

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Clarity Measurement

$$Clarity = 1 - \frac{Lum(ROI) - Lum(clear)}{Lum(opaque) - Lum(clear)}$$

Where *Lum(ROI):* average luminance of the region of interest;
Lum(clear): the average luminance of a clear glass slide;
Lum(opaque): the average luminance of an opaque object.

Therefore clarity of a clear glass slide = 1;
clarity of an opaque object = 0.

Clarity of the cornea tissue was measured

- Before treatment;
- After treatment.

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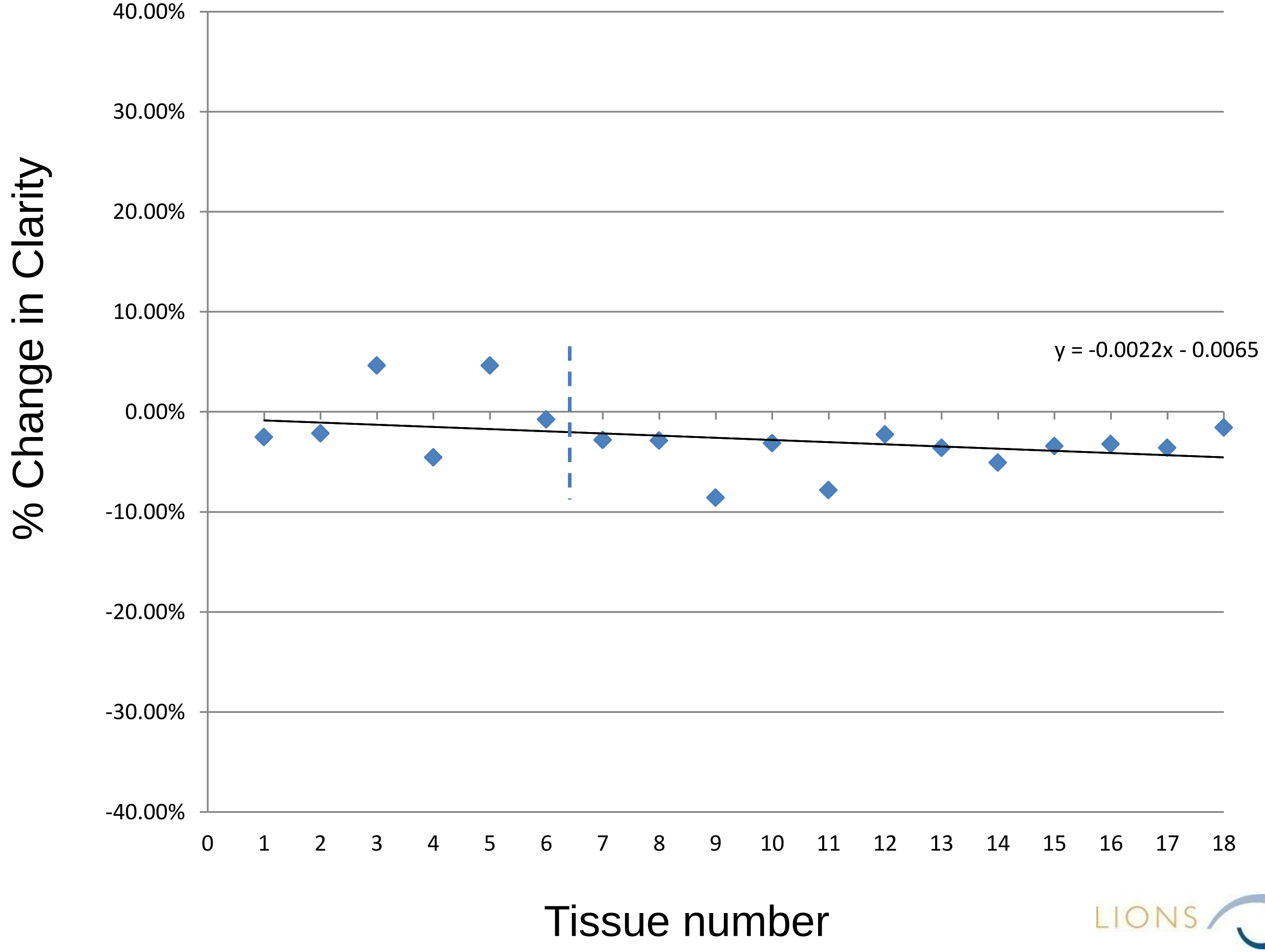
Data Summary

- Change in quantity of artifacts over study
  - Limitations in first n= 6
  - Improved image quality final n=12

	Average Clarity	Range
Fresh Tissue	92.4% ± 3.5 %	84.9% - 97.4%
Irradiated Tissue	89.7% ± 2.7%	85.6% - 93.9%
Average Difference	-2.7%	

P=0.009 with two-tailed paired t-test

Overview of Clarity Calculations



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Conclusions

- First study demonstrating the effects of e-beam irradiation on clarity of donor corneas
- Utilized darkfield microscopy to assess clarity
- Statistically significant small changes in clarity were observed in this study:
  - 2.7 % less clear on average

Thank you and Questions

- Family and Teachers

- Lions VisionGift