LIONS VISIONGIFT

Validation of an eye bank slit-lamp examination process for DMEK preparations: A 3-year internal review

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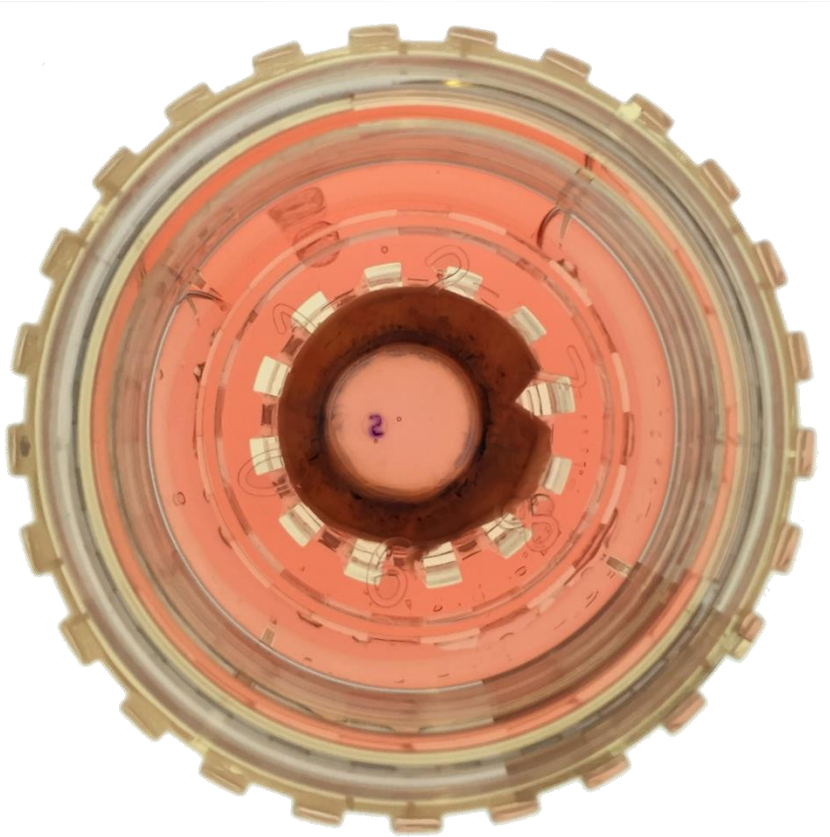

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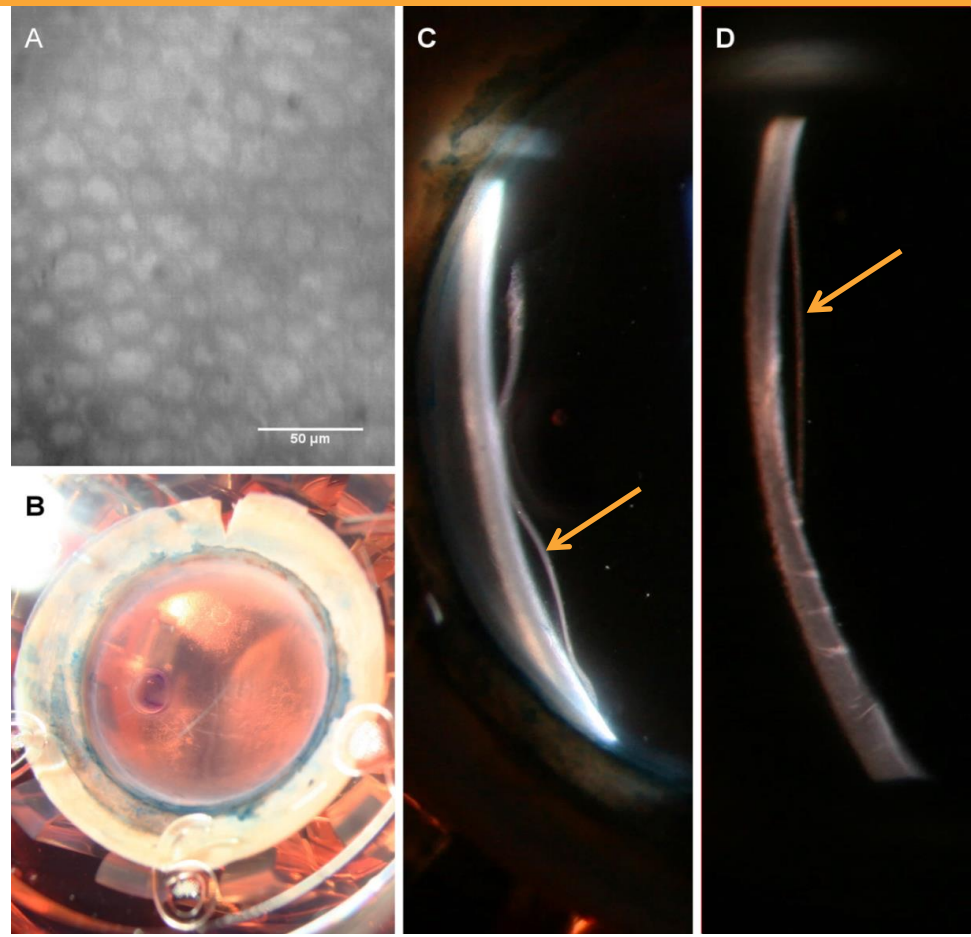
Background

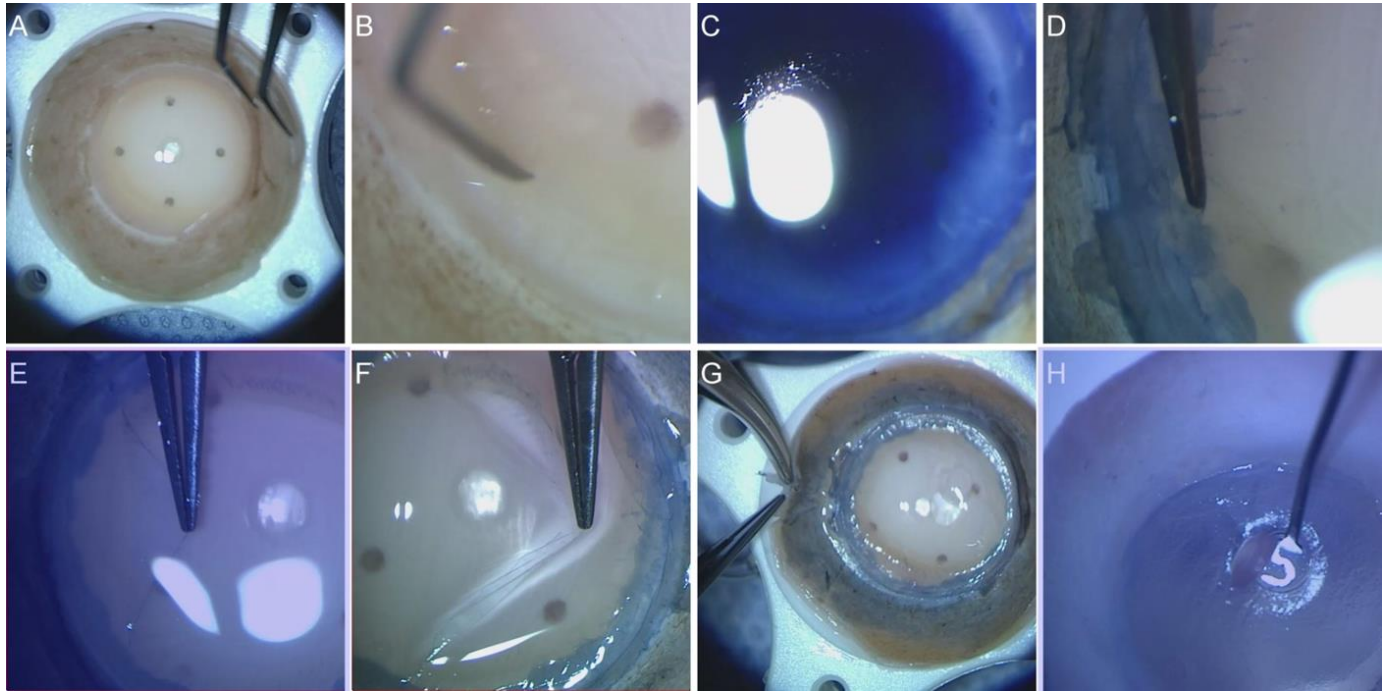
- Post-DMEK tissue preparation evaluation is an important quality control step in an eye bank setting. Ideally, this quality check will ensure that no excessive cell loss occurred during preparation of the delicate membrane
  - The prepared graft floats freely in the media
  - The free-floating graft poses challenges for accurate evaluation



Eye bank post-processing tissue quality assessment includes A, specular microscopy for determining cell density. B, C, D, Slit lamp evaluation using various illumination techniques. Arrows point to Descemet membrane which is separated from the stroma.

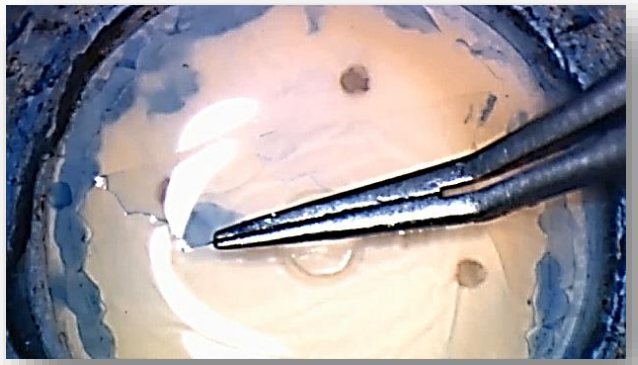
Purpose

The goal of this study is to validate our internal eye bank slit-lamp examination process for DMEK preparations and compare our results to data collected three years prior



General steps of eye bank preparation for DMEK. A, Tissue is placed on a suction block. B, The Descemet membrane (DM) is scored at the limbus. C, DM is stained with trypan blue. D,E,F, DM is peeled with forceps leaving a small area of attachment. G, The area of attachment (called the “hinge”) is denoted by a scleral resection. H, For surgeons who want an orientation mark, an “S” can be added to the stromal side of DM.

Methods: Peeling, Evaluation, Staining and Imaging



17 Corneas prepared with standard peeling method

1

Technician slit lamp evaluation

2

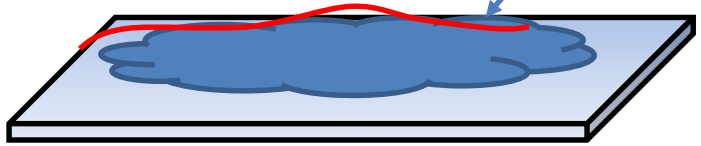
48 hours

Tissue Stained with Calcein AM

3

Graft placed on viscoelastic bed

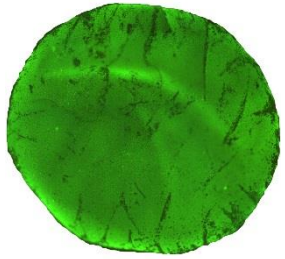
DMEK Graft



4

Imaged at 40X

5



15-20 images stitched into montage

6

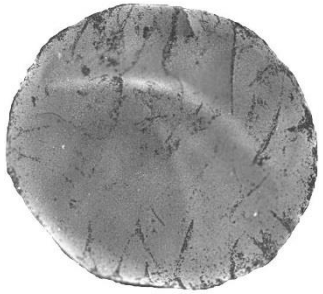
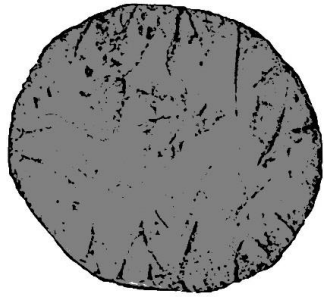


Image to grayscale

7



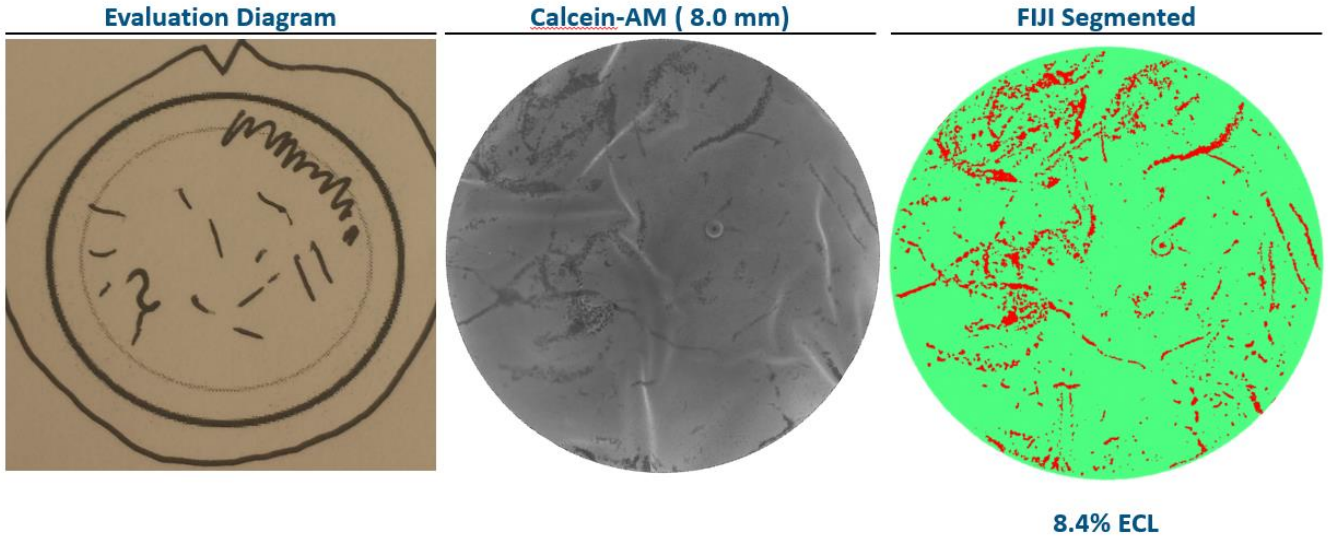
Segmented binary image

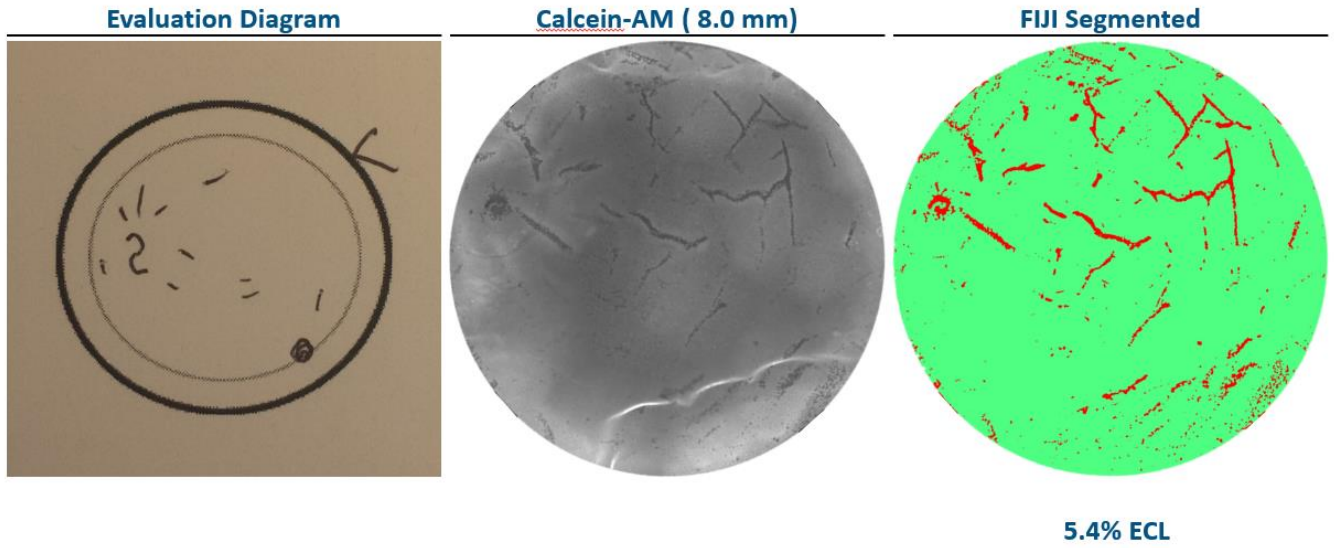
8

Cell Loss

12.1%

Results: Representative Samples





Evaluation diagram: The evaluation diagram is where technicians document any damage they see and rate the tissue as “pass” or “fail”

Calcein-AM: After technician evaluation, the graft is stained with vital dye and imaged as a way to validate the technician observations

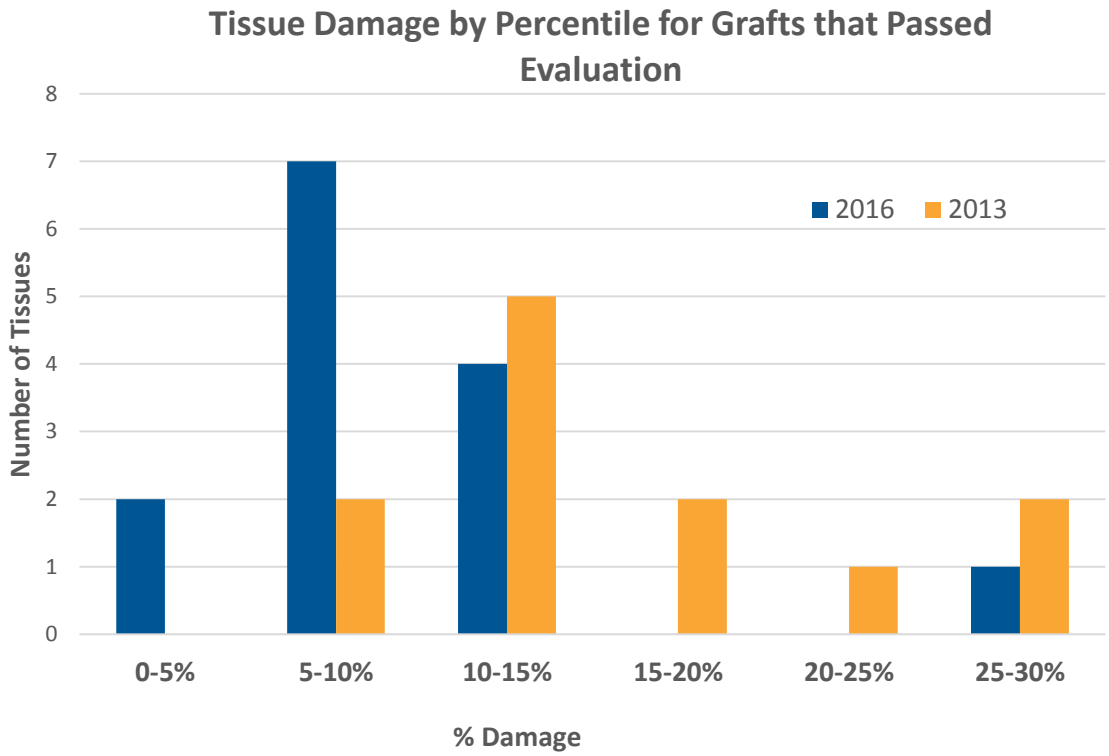
FIJI Segmentation: Trainable software is used to determine which shades of grayscale are damaged/absent cells versus living cells

Results

2016			2013		
Tissue Count	Tech Rating	% Cell Loss	Tissue Count	Tech Rating	% Cell Loss
1	Pass	4.9%	1	Pass	10.7%
2	Pass	13.7%	2	Pass	12.1%
3	Pass	8.4%	3	Pass	14.1%
4	Pass	5.6%	4	Pass	18.7%
5	Pass	5.2%	5	Pass	12.6%
6	Pass	5.4%	6	Pass	24.7%
7	Pass	6.0%	7	Pass	8.3%
8	Pass	5.8%	8	Pass	16.2%
9	Pass	12.2%	9	Pass	11.0%
10	Pass	3.7%	10	Pass	26.5%
11	Pass*	26.0%	11	Pass	9.6%
12	Pass	13.1%	12	Pass	29.4%
13	Pass	9.7%			
14	Pass	10.3%			
	Average:	9.3%		Average:	16.2%

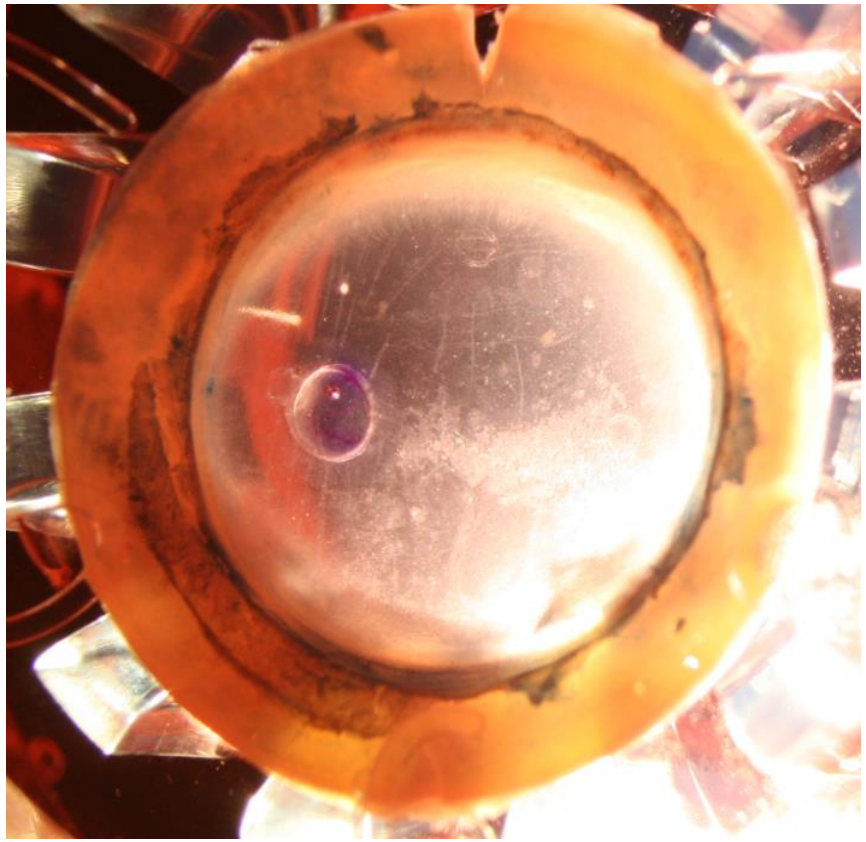
*Part of damage pattern consistent with slide transfer damage

2016			2013		
Tissue Count	Tech Rating	% Cell Loss	Tissue Count	Tech Rating	% Cell Loss
1	Fail	22.2%	1	Fail	18.9%
2	Fail	22.7%	2	Fail	24.0%
3	Fail	26.4%	3	Fail	50.3%
			4	Fail	26.6%



What have we learned in 3 years?

- Technicians can consistently rate the quality of DMEK prepared grafts
  - Grafts unsuitable for transplant based on a >25% cell loss standard were all excluded from the latest round of analysis except one outlier
- The amount of damage seen in DMEK prepared grafts has shifted down.
  - This could be due to improvements in DMEK preparation technique
  - Additionally, the laboratory staining protocol with a slide transfer could have improved as well

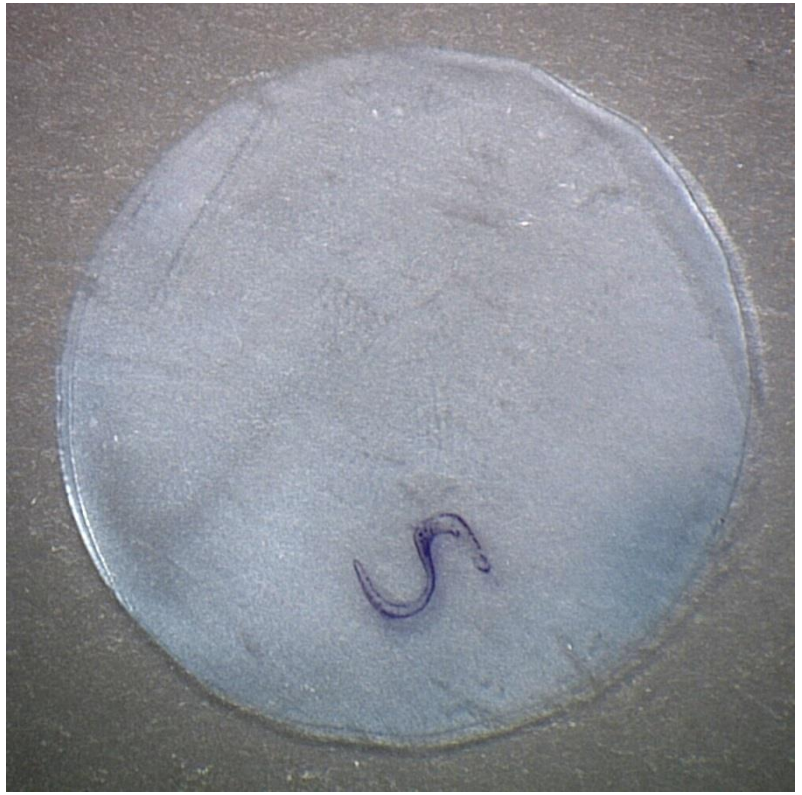


DMEK prepared cornea slit lamp image using low magnification retroillumination.

Study Limitations/Future Directions

- The transfer of the delicate DMEK graft to a slide for vital staining is very challenging and a potential cause for damage to the graft that is related to analysis alone and not due to the tissue processing

- Better ways to analyze DMEK prepared grafts may help optimize tissue preparation procedures



DMEK graft unscrolled on a glass slide in preparation for viability staining analysis