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In vivo ocular imaging of the cornea of the normal female laboratory beagle using confocal microscopy

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Abstract

Objective—To obtain normative data for the normal laboratory beagle cornea using high-resolution in vivo confocal microscopy (IVCM).

Animals studied—Sixteen eyes of 8 healthy young female intact beagles.

Procedures—The central cornea was imaged using IVCM. Mixed effects linear regression was used for statistical analysis.

Results—IVCM allowed detailed visualization and quantification of epithelial cells (superficial epithelial cell diameter: 43.25 ± 6.64 μm, basal cell diameter: 4.43 ± 0.67 μm), and nerve fibers (subepithelial nerve fiber diameter: 2.38 ± 0.69 μm, anterior stromal nerve fiber diameter: 16.93 ± 4.55 μm). Keratocyte density (anterior stroma 993.38 ± 134.24 cells/mm^2, posterior stroma 789.38 ± 87.13 cells/mm^2) and endothelial cell density (2815.18 ± 212.59 cells/mm^2) were also measured.

Conclusion—High-resolution IVCM provides detailed non-invasive evaluation of the cornea in the normal laboratory beagle.

Keywords

Cornea; endothelial cell density; confocal microscopy; corneal nerves; canine; laboratory beagle

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INTRODUCTION

Anterior segment imaging is a rapidly developing field in ophthalmology. In vivo confocal microscopy (IVCM) is a noninvasive, real-time diagnostic modality, which provides high-resolution imaging of all corneal layers by using an optical imaging system where both the illumination (condenser) and observation (objective) systems are focused on a single focal point.(1) IVCM has been utilized for assessing normal morphologic features of the cornea in several species(2-5) and has led to significant enhancement of our understanding of the living healthy and diseased cornea. For example, IVCM has been proven to be an efficient tool to confirm the clinical diagnosis of fungal keratitis in humans, alpacas and horses and in corneal neuropathies associated with conditions such as dry eye and diabetes.(1, 6-10) IVCM allows for assessment of structures that would otherwise require histological examination and allows the examiner to evaluate and focus on cells in different depths of the cornea.(4, 11) For example, IVCM is currently the only diagnostic modality commercially available to evaluate corneal nerves *in vivo*. A limited number of IVCM studies of the canine anterior segment have been published.(4, 12, 13) However, none of these studies have focused on the laboratory beagle despite its wide use in the development of ophthalmic drugs and devices. Thus the purpose of this study was to report normative IVCM data for the laboratory beagle.

METHODS

All aspects of the study were approved by the Institutional Animal Care and Use Committees of the University of California-Davis and were performed according to the Association for Research in Vision and Ophthalmology resolution on the use of animals in research. Prior to study entry, all dogs underwent a complete physical examination and only systemically healthy dogs were included in the study. All dogs also received a detailed ophthalmic examination, including digital slit lamp biomicroscopy, indirect ophthalmoscopy, tear film break up time (TFBUT)(14), Schirmer tear test 1 (STT-1)(15), intraocular pressure (IOP)(16) and corneal sensitivity measurement using Cochet-Bonnet aesthesiometry(17-19). In addition, all dogs had their corneas stained with fluorescein and Rose Bengal and only dogs assessed as having normal eyes were used in the study. All baseline exams were done an hour or longer prior to imaging. None of the dogs had received topical eye medications prior to entry into the study. All IVCM measurements were obtained by the same person (DEC), highly skilled in the technique, to minimize variability.

Eight healthy female laboratory beagles (16 eyes) with a mean ± SD age of 0.52 ± 0.12 years and body weight of 6.83 ± 1.40 kg were included in the study. The dogs received dexmedetomidine (2.5-5.0 μg/kg) intramuscularly (IM) or intravenously (IV) as needed for sedation prior to imaging. Animals were placed in sternal recumbency for all imaging, and balanced salt solution (BSS; Akorn Inc., Buffalo Grove, IL, USA) solution was applied to the eyes as needed to prevent corneal desiccation. One drop of proparacaine 0.5% was instilled in both eyes prior to imaging for topical anesthesia. A Barraquer eyelid speculum was used to keep the eyes open on the sedated dogs as needed. Then, IVCM (ConfoScan 4; Nidek Technologies, Gamagori, Japan) with a 40x/0.75 objective lens was used to image the central cornea of each eye. An eye gel of 0.3% hypromellose/carbomer 980 (GenTeal® gel;

Novartis Ophthalmics, Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA) was placed on the tip of the objective lens as an optical coupling medium and the lens was manually advanced until the gel contacted the central surface of the cornea. Automatic, full scans were performed without autoalignment and 350 images/scan were collected for each eye. Three repeated measurements, located in the central cornea, were obtained for each structure. Superficial and basal epithelial and endothelial cells were identified by their previously described characteristic appearance in the dog.(4, 12, 13) Stromal images adjacent to epithelium and endothelium were utilized to identify keratocytes in the anterior and posterior stroma, respectively. Manual counts and cell area measurements were performed using the ConfoScan 4 NAVIS imaging software (Rev. 1.2, version 1.2.0, June 28th, 2006). Three images per location were analyzed, and these results were averaged. For anterior and posterior keratocyte cell density measurement the region of interest (ROI) was kept at 0.05mm^2 for each image and for the endothelial cell density counts the ROI was kept at 0.02 mm^2. For all cell counts, the cells touching the borderlines were counted only along the upper and right border. Those cells touching the left and lower border were omitted from analysis.

Statistics

Mixed effects linear regression was used to evaluate the main effects of eye (nested within individual), corneal location (nested within eye), replicates (nested within corneal location), and method of measurement, as well as their pairwise statistical interactions, on the outcome variables. Analyses were performed using Stata/IC 12.1 (StataCorp LP, College Station, TX, USA). All measurements were expressed as mean ± standard deviation. For all analyses, values of $P \leq 0.05$ were considered significant.

RESULTS

All dogs examined were normal on physical and ophthalmic examination, including an appropriate TFBUT (16.1 ± 4.82 s), STT-1 (21.3 ± 1.98 mm/min), IOP (18.3 ± 2.96 mmHg), and central corneal sensitivity as measured by Cochet-Bonnet aesthesiometry (2.05 ± 0.622 g/mm^2); all corneas were fluorescein and Rose Bengal stain negative.

High quality, high-resolution *in vivo* images of the canine cornea were obtained. Specifically, corneal superficial and basal epithelium (Figure 1 and 2) and stroma including nerves, keratocytes, and pre-Descemet's fibers (Figures 3-5), as well as endothelium (Figure 6) were successfully imaged and evaluated using IVCM.

There were no significant differences between replicates for all measurements obtained. Unless otherwise mentioned in the following, there were no significant differences between eyes for the measurements obtained ($P > 0.05$).

As measured by IVCM, superficial and basal epithelial cells possessed the largest and smallest cross sectional diameters (Table 1). Corneal nerve fiber bundles within the subepithelial plexus were much thinner in diameter in comparison to those posteriorly in the anterior stroma, at 2.38 ± 0.69 μm and 16.93 ± 4.55 μm, respectively. Keratocyte cell density was significantly greater ($P < 0.001$) while keratocyte nuclei were significantly

smaller ($P < 0.001$) in the anterior versus posterior stroma (Table 1). Pre-Descemet's stromal fibers were 2.14 ± 0.50 μm thick. Endothelial cells had the greatest density of all corneal cell types measured with a size intermediate between superficial and basal epithelial cells (Table 1). There was a significant difference ($P = 0.007$) between eyes in terms of anterior corneal stromal cell density at 1032.68 ± 163.43 cells/mm^2 and 954.09 ± 83.04 cells/mm^2 for the right and left eye, respectively. In addition the subepithelial nerve plexus thickness was significantly different ($P = 0.002$) between eyes at 2.16 ± 0.58 μm for the right eye and 2.60 ± 0.73 μm for the left eye.

DISCUSSION

This study demonstrated that high-quality IVCM images could be obtained for analysis from sedated laboratory beagles. We demonstrated that keratocyte density was significantly greater and keratocyte nuclei size was significantly smaller in the anterior stroma in comparison to the posterior stroma. This is consistent with previous reports in humans, rabbits, and dogs.(4, 5, 20) Endothelial cell density was similar (2815.18 ± 212.59 cells/mm^2) to previously reported values in young dogs measured by specular microscopy (approximately 2500 and 2816 ± 187 SD cells/mm^2, respectively).(21, 22) However, the endothelial cell densities reported in the present study were less than those reported in a study of dogs less than 1 year of age using a Heidelberg Retina Tomograph II unit with a Rostock Cornea Module (3641 ± 752 cells/mm^2).(4) This difference is likely due to difference in age and breeds (different breeds were included in the previously published study) though it can not be ruled out that differences in IVCM units used between studies contributed to differences in the data reported.(4)

In vivo confocal microscopy is the only commercially available diagnostic modality capable of evaluating corneal nerves *in vivo* and that can be used to evaluate nerves in neuropathies affecting the cornea.(8, 12, 13, 23) We were able to obtain high quality images of corneal nerves. The values and morphology were comparable to previous studies assessing canine corneal innervation,(4, 12, 24) as well as to normal humans.(25-28) We expect this imaging technique will be useful in evaluating canine models for corneal neuropathies associated with conditions such as KCS and diabetes mellitus.

In the present study, there was a significant difference between eyes in two measurements, including anterior stromal keratocyte density and subepithelial plexus thickness. These numerical differences were small and are unlikely to be clinically important. The pre-Descemet's stromal fibers found in this study were similar to those found in young dogs and cats in a previous study.(4) The authors of that study suggested that these fibers represent pre-elastic (i.e., oxytalan) fibers similar to those found in corneas of juvenile humans and kittens.(29, 30) The clinical significance of these distinct fibers is currently unknown. A limitation of this study is a small sample size using only young female subjects. Further normative studies of different dog populations are warranted.

In conclusion, we have generated normative data of the central cornea of the young, healthy, female laboratory beagle using IVCM. High-resolution IVCM enabled detailed noninvasive evaluation of in vivo canine cornea. This study provides normative values with which to

evaluate data obtained from dogs with spontaneous corneal diseases, induced models of anterior segment disease and in evaluation of ophthalmic therapeutics and devices.

Acknowledgments

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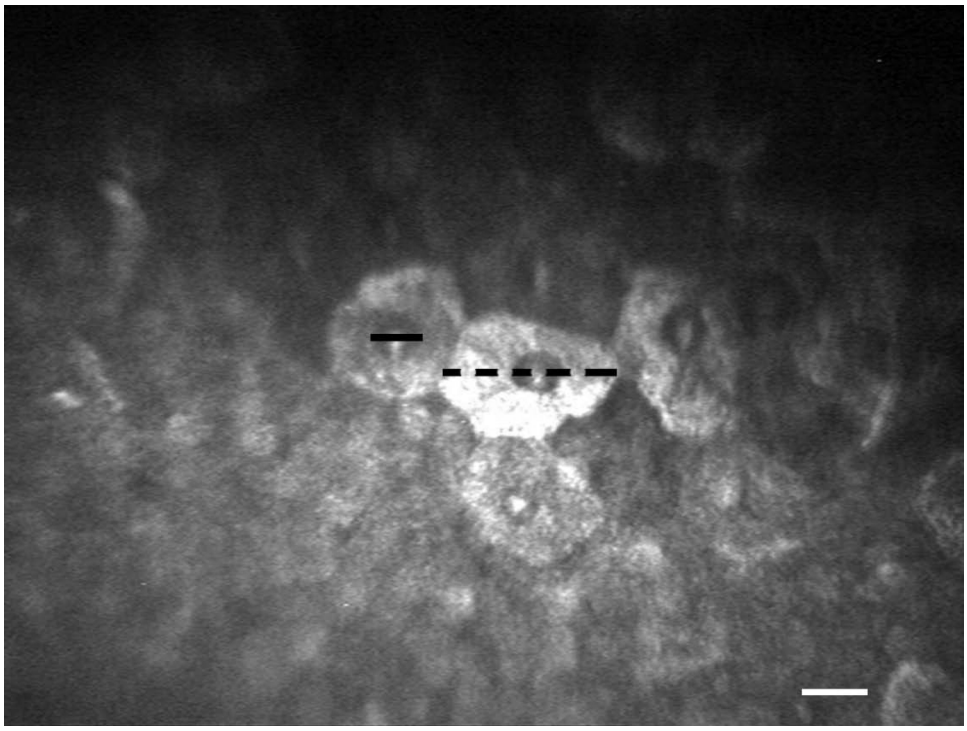

Figure 1.
IVCM of the cornea in a young healthy intact female beagle. Superficial epithelial cell diameter (black dotted line) and their nuclei diameter (black line) were measured. White scale bar = 20 μm.

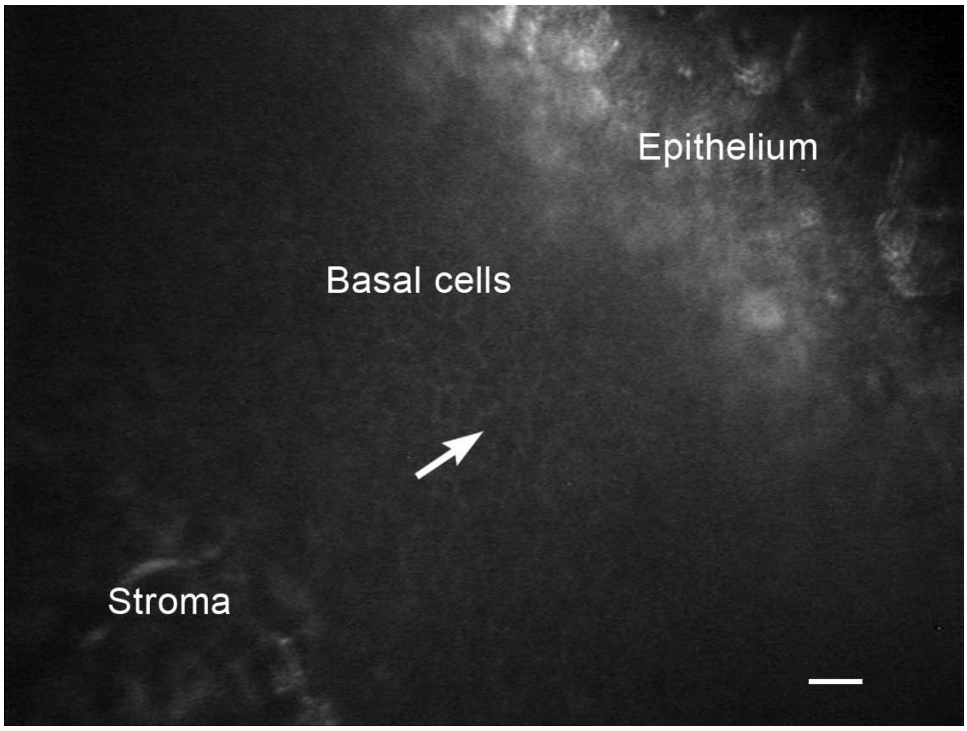

Figure 2.
IVCM of the cornea in a young healthy intact female beagle. The white arrow is pointing to a corneal basal cell. White scale bar = 20 μm.

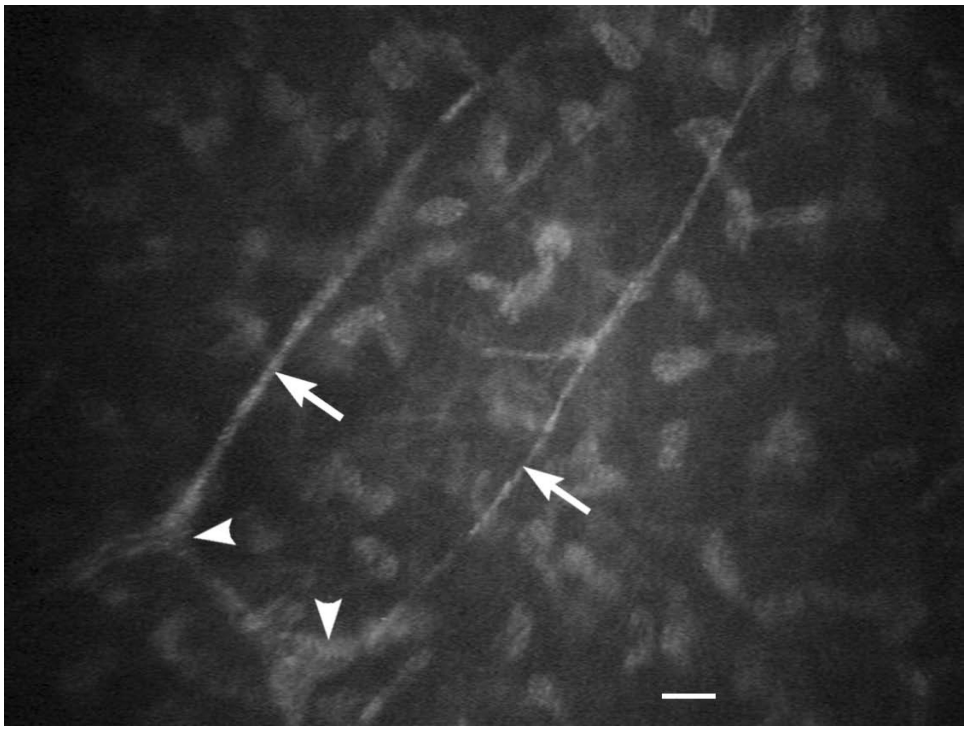

Figure 3.

IVCM of the subepithelial nerve plexus in young healthy intact female beagle (white arrowheads). The nerve fiber thickness of the subepithelial nerve plexus (white arrows) was measured. White scale bar = 20 μm.

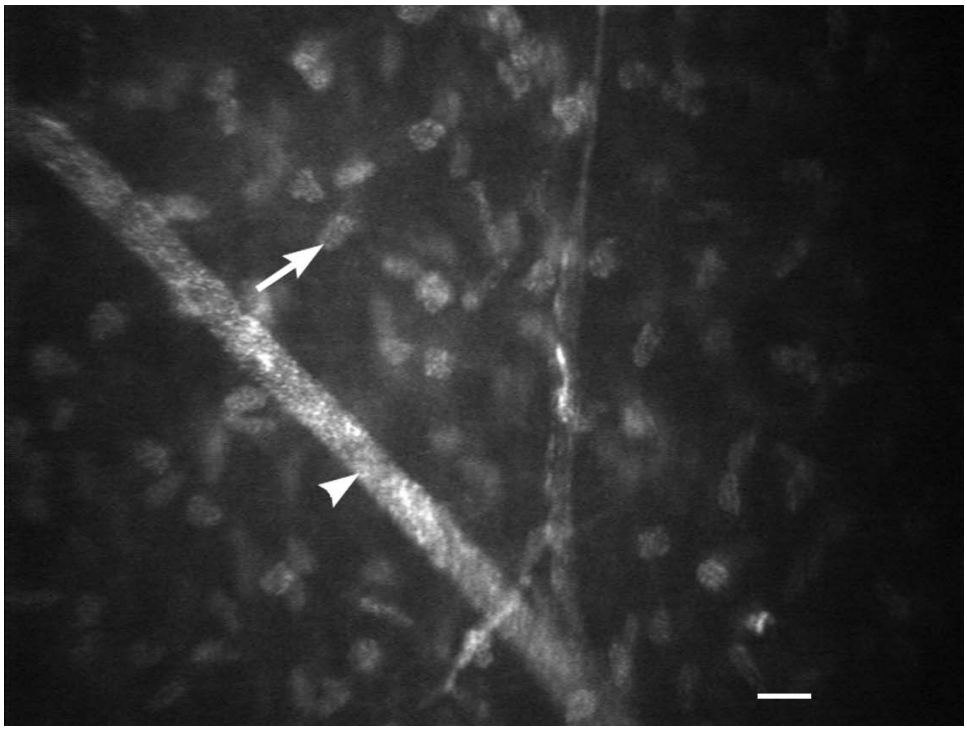

Figure 4.

IVCM of the anterior corneal stroma and nerve in a young intact female beagle. Density of keratocytes, keratocyte nucleus (white arrow) diameter and anterior stromal nerve (white arrowhead) thickness were measured. White scale bar = 20 μm.

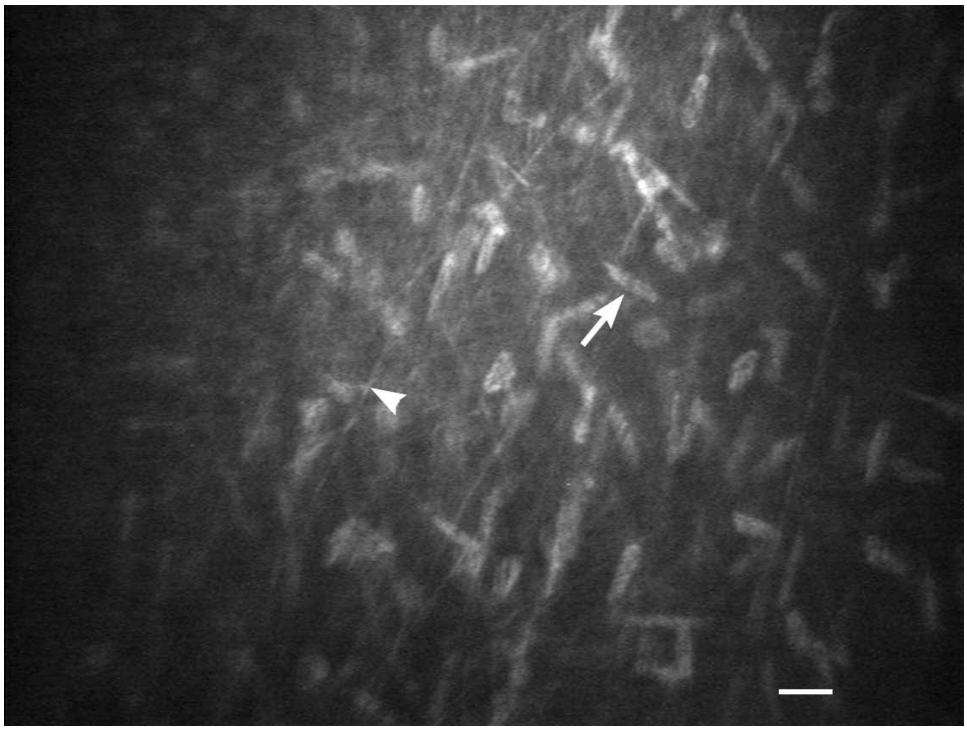

Figure 5.

IVCM of the posterior corneal stroma and pre-Descemet's fibers in a young intact female beagle. Density of keratocytes, keratocyte nucleus (white arrow) diameter, and pre-Descemet's fibers (white arrowhead) were measured. White scale bar = 20 μm.

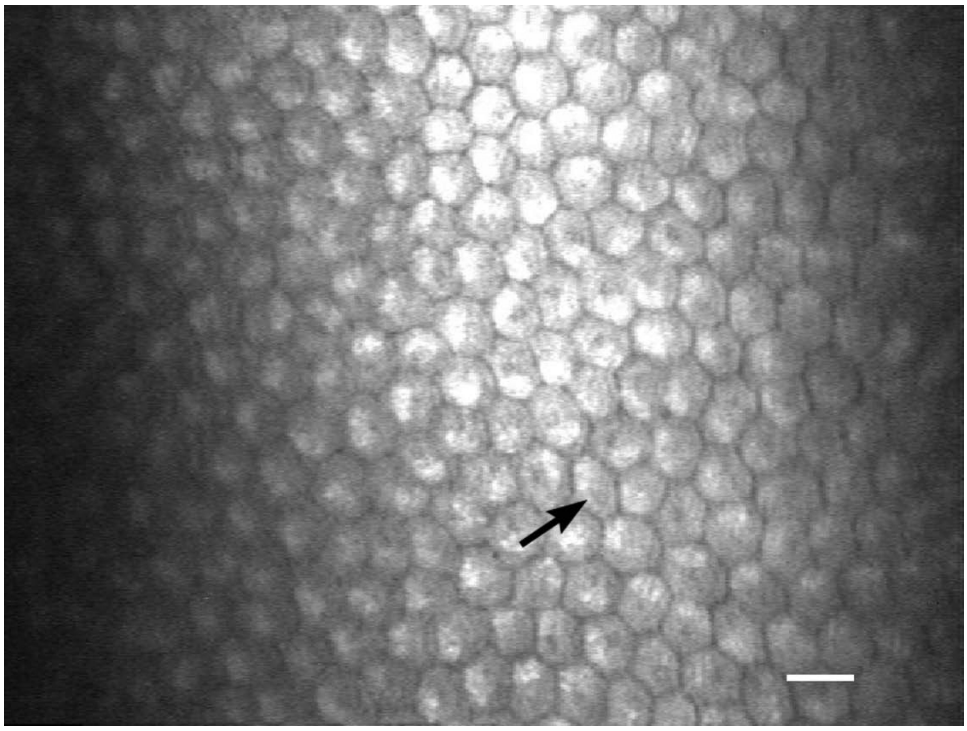

Figure 6.

IVCM of the corneal endothelium young healthy intact female beagle. Note the relatively uniform size (black arrow) and density between the endothelial cells. Endothelial cell density and diameter was measured. White scale bar = 20 μm.

Table 1

IVCM of the central cornea of healthy female laboratory beagles (Mean ± SD)

	Cell Density (cells/mm^2)	Cell Diameter (μm)	Nuclei Diameter (μm)	Thickness (μm)
Superficial epithelium	n/a	43.25 ± 6.64* (OD: 42.43 ± 5.61; OS: 44.07 ± 7.57)	14.19 ± 2.55* (OD: 14.14 ± 3.02; OS: 14.23 ± 2.03)	n/a
Basal epithelium	n/a	4.43 ± 0.67* (OD: 4.31 ± 0.56; OS: 4.55 ± 0.76)	n/a	n/a
Anterior stromal keratocytes	993.38 ± 134.24* (OD: 1032.68 ± 163.43; OS: 954.09 ± 83.04)‡	n/a	18.80 ± 3.11* (OD: 18.25 ± 3.16; OS: 19.35 ± 3.03)	n/a
Posterior stromal keratocytes	789.38 ± 87.13* (OD: 775.76 ± 82.61; OS: 803.00 ± 91.12)	n/a	27.21 ± 2.89†* (OD: 27.15 ± 2.82; OS: 27.26 ± 3.02)	n/a
Endothelium	2815.18 ± 212.59 (OD: 2780.58 ± 256.89; OS: 2849.78 ± 154.48)	20.53 ± 2.06* (OD: 20.94 ± 1.87; OS: 20.13 ± 2.19)	n/a	n/a
Subepithelial nerve plexus fibers	n/a	n/a	n/a	2.38 ± 0.69§,‡ (OD: 2.16 ± 0.58; OS: 2.60 ± 0.73)
Anterior stromal nerve fibers	n/a	n/a	n/a	16.93 ± 4.55§ (OD: 17.71 ± 4.58; OS: 16.16 ± 4.47)
Predescemet's fibers	n/a	n/a	n/a	2.14 ± 0.50 (OD: 2.24 ± 0.51; OS: 2.05 ± 0.49)

IVCM = In Vivo Confocal Microscopy

n/a = Not available (not measured)

OD = Oculus Dexter

OS = Oculus Sinister

*Statistically significant difference ($p \leq 0.05$)

†Statistically significant difference ($p \leq 0.05$)

§Statistically significant difference ($p \leq 0.05$)

‡Statistically significant difference between eyes ($p \leq 0.05$)