·Clinical Research·

# Effect of 2% fluorescein on Scheimpflug central corneal thickness measurements

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Received: 2014-04-15 Accepted: 2014-12-12

## Abstract

• **AIM: To assess central corneal thickness (CCT) changes measured with Scheimpflug device following instillation of 2% fluorescein in normal subjects.**

• **METHODS: This was a prospective randomized study of 60 hospital volunteers. After baseline CCT measurements of both eyes of 40 subjects were obtained using Scheimpflug system, a drop of preservative-free 2% fluorescein, was instilled in one eye and in other eye, one drop of normal saline (control). Measurements were repeated after 1, 2, 5, 10, 20, 30, 40, 50 and 60min (continuous assessment group). Twenty subjects had baseline CCT taken, then fluorescein was instilled in one eye and measurements were taken at 1min. Ten eyes had saline rinse after 1min and 10 other eyes did not, measurements were repeated at 2min (eye rinse group).**

• **RESULTS: The mean baseline CCT for continuous assessment group was 546.2±32.1 μm (range, 489.0-606.0), control eyes was 546.6±30.7 μm (range, 489.0-602.0). At 1min after fluorescein instillation, CCT significantly increased by 37.0±34.0 μm ($P$<0.001), then decreased gradually, reaching baseline at 60min. CCT variations were not significant in control group ($P$>0.05). For eye rinse group, CCT mean differences between baseline and 2min were 18.2 μm (95 % CI: -54.7 to 18.3) with rinse and 26.5 μm (95% CI: -62.9 to 9.9) without rinse; paired sample tests were not significant ($P$>0.05).**

• **CONCLUSION: The presence of fluorescein increased CCT value to a clinically relevant level of 6.8%. Eye rinse did not significantly reduce the effect at 2min post fluorescein timepoint.**

• **KEYWORDS:** central corneal thickness; fluorescein; Scheimpflug device
**DOI:10.18240/ijo.2016.02.10**

Briggs S, Bin Moammar M. Effect of 2% fluorescein on Scheimpflug central corneal thickness measurements. *Int J Ophthalmol* 2016;9(2):239-242

## INTRODUCTION

Central corneal thickness (CCT) measurement by pachymetry is used clinically to detect corneal abnormalities. It is also used as an essential part of glaucoma examination and evaluation of patients undergoing refractive surgery. Literature review of various pachymetry methods (applying acoustic and optical technologies) show slight tendency to either underestimate or overestimate corneal thickness [1-4], even when correction factors are applied to some of these methods, the real thickness of the cornea remains to be determined. Inaccurate determination of corneal thickness could lead to complications in refractive surgery procedures, glaucoma and other ocular conditions that rely on corneal thickness data for diagnosis[2,5-7].

The Pentacam HR tomographer (Oculus, Wetzlar, Germany) with a rotating Scheimpflug camera is capable of imaging the cornea, anterior chamber, and lens, and is often used by refractive surgeons. Its accuracy and repeatability has been reported to be excellent [8-11]. This Scheimpflug device, in principle, measures corneal thickness from image created from backscattered light of the cornea that is based on the difference in refractive index of air ($n$=1) and the refractive index of the whole cornea ($n$=1.376), without including the tear layer [12]. On the contrary, Barkana *et al* [13] stated that optical technologies like the Scheimpflug device "conceivably may include the tear film in the measurement of corneal thickness, as the anterior reflecting surface is the air-tear film interface. The magnitude of this effect requires further studies." Inclusion of the tear film may not pose a measurement error as long as it remains clear and transparent. Tear film changes to yellow-green with instillation of fluorescein dye routinely used clinically for anterior segment examination. Study by Hirnschall *et al* [14] showed that the presence of fluorescein dye in the tear film created a more intense backscattered light that interfered with the Scheimpflug device measurements. Their result showed increased corneal thickness measurements immediately after instillation of a drop of 0.25% fluorescein dye with 0.5% proxymetacaine hydrochloride, a composition used for

measuring intraocular pressure with Goldmann applanation tonometry.

Extensive literature search on CCT measurements shows only two studies involved use of fluorescein with a Scheimpflug device: one on CCT, the other on tear film thickness [13-14]. Neither of these studies used 2% fluorescein which is commonly used clinically to assess corneal epithelial integrity. Its effect on CCT measurement with Scheimpflug device has not been determined.

This purpose of study is to investigate the effect of 2% fluorescein on CCT values measured with rotating Scheimpflug device on healthy subjects.

## SUBJECTS AND METHODS

**Subjects** In this randomized study, 40 subjects with healthy eyes were recruited (19 men and 21 women) with mean age of 33.8±8.3y. Written informed consent was obtained from each subject after explanation of the nature of the study. This study was conducted according to the tenets of Declaration of Helsinki and was approved by the institutional research ethics review board of the College of Applied Medical Sciences, and the management board of Security Forces Hospital. Irrespective of age or ethnicity, enrollment was based on the criteria of absence of ocular disease, contact lens wear, prior refractive surgery, dry eye related symptoms and other abnormalities that may interfere with measurement.

**Methods** CCT was measured using Pentacam HR tomographer (Oculus, Wetzlar, Germany) with a rotating Scheimpflug camera. It performs corneal pachymetry with a rotating Scheimpflug camera without direct contact with the cornea and can complete analyses of the entire cornea in 2s over a 180-degree rotation. Internal software (Pentacam® HR Basic software) automatically determines corneal thickness from three-dimensional reconstructed images of the anterior and posterior surface of the cornea. Subjects were appropriately positioned and were asked to focus on the fixation target. To reduce examiner-dependent variable, automatic release mode of the Scheimpflug device was set. This mode automatically determines the correct focus and alignment with the corneal apex, and then scans.

**Eye with continuous assessment** Baseline CCT measurement of 40 subjects were obtained and recorded as "0min". Thereafter, a drop of 2% fluorescein sodium preservative-free eye drops in unit dose applicators (Minims® , Chauvin Pharmaceuticals Ltd., UK) was placed inside the central part of the lower eyelids of one eye and the other eye instilled with one drop of sterile normal saline solution (minims) as control. CCT measurements were then recorded at 1, 2, 5, 10, 20, 30, 40, 50 and 60min after instillation. Two consecutive measurements of CCT were made in each eye of the 40 subjects for all time points and the average was recorded.

**Table 1 Mean CCT values measured with Scheimpflug device at time points after fluorescein and saline instillations for the continuous assessment eyes**

| Measurement time point (min) | Mean CCT in saline instilled eyes (μm) ±SD (range) | Mean CCT in flourescein instilled eyes (μm) ±SD (range) |
|---|---|---|
| 0 (baseline) | 546.6±30.7 (489.0-602.0) | 546.2±32.1 (489.0-606.0) |
| 1 | 546.5±30.8 (497.0-602.0) | 583.2±34.0 (520.0-645.0) |
| 2 | 546.5±30.7 (498.0-602.0) | 573.7±35.6 (514.0-643.0) |
| 5 | 546.3±30.5 (499.0-603.0) | 562.0±37.8 (500.0-639.0) |
| 10 | 546.7±30.8 (498.0-602.0) | 553.9±36.1 (495.0-634.0) |
| 20 | 546.8±30.8 (497.0-602.0) | 550.1±35.3 (491.0-620.0) |
| 30 | 546.6±30.8 (496.0-602.0) | 546.9±34.5 (490.0-605.0) |
| 40 | 546.6±30.8 (498.0-602.0) | 546.8±33.4 (483.0-605.0) |
| 50 | 546.6±30.7 (498.0-602.0) | 545.2±32.7 (481.0-605.0) |
| 60 | 546.6±30.7 (498.0-602.0) | 545.8±32.0 (489.0-605.0) |

**Eye with saline rinse** Twenty subjects were randomly taken from the group with continuous assessment group and measured again for baseline data on separate days. Ten subjects had 2% fluorescein was instilled in one eye and measurements were taken at 1min. Eye was immediately rinsed with 2 mL of normal saline and CCT measurements were repeated at 2min. The remaining ten subjects that did not have saline rinse had CCT measurements recorded at 1 and 2min.

**Statistical Analysis** Data for each measurement time point in the population sample are expressed as mean ±standard deviation (SD) and were analyzed with InStat software (Version 3, GraphPad Software, San Diego, CA, USA). Measurement time points were compared using one-way analysis of variance with Tukey multiple comparison.

## RESULTS

In the continuous assessment eyes, the mean CCT measured with rotating Scheimpflug device at three baseline measurement was 546.2±32.1 μm for 2% fluorescein eyes and the control eyes was 546.6±30.7 μm. The mean, SD and range for CCT according to measurement time points of 1, 2, 5, 10, 20, 30, 40, 50 and 60min after fluorescein and saline instillations are shown in Table 1. Control eyes did not show significant difference with multiple comparison of time points within the group ($P$>0.05) as illustrated in Figure 1.

Table 2 shows the mean difference in CCT values between 0min and other post fluorescein instillation time points for the continuous assessment eyes. The highest mean difference was between time point of 0min (baseline) and 1min and the lowest was between time point 0min and 60min. At 60min post fluorescein instillation, 100% of the eyes returned to baseline value, and approximately 50% of the eyes returned to baseline at 30min. However, mean difference between baseline CCT and time points after fluorescein instillation continued to show higher (negative) values and reached zero between 20 and 60min. In comparison between control and fluorescein instilled eye, significant differences were observed only at time points 1 and 2min ($P$<0.05).

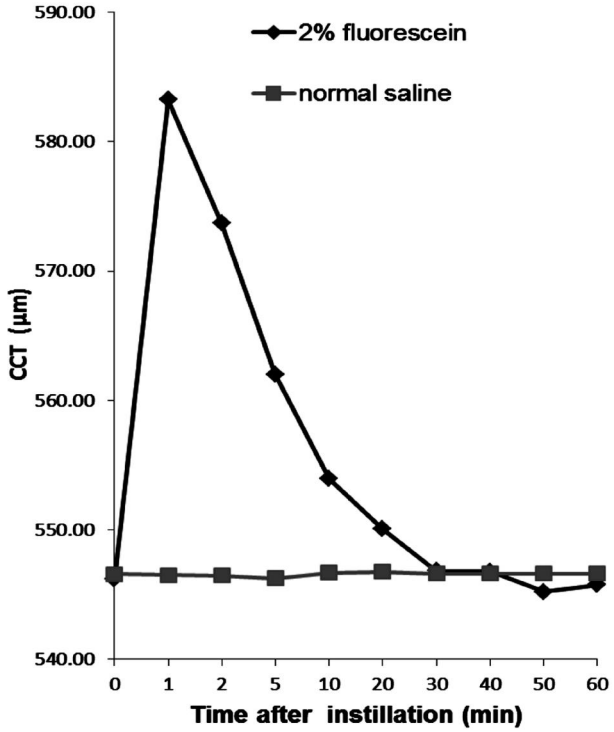


**Figure 1 Comparison of mean CCT between saline group and fluorescein group.**

**Table 2 Comparison of mean difference CCT values as measured with Scheimpflug device between baseline and fluorescein instillation time points for the continuous assessment eyes**

| Pairs | Mean difference (μm) | 95% CI (μm) | *P* |
|---|---|---|---|
| Baseline and 1min | -37.0 | -61.488 to -12.562 | <0.05 |
| Baseline and 2min | -27.5 | -51.938 to -3.012 | <0.05 |
| Baseline and 5min | -15.8 | -40.263 to 8.663 | >0.05 |
| Baseline and 10min | -7.7 | -32.188 to 16.738 | >0.05 |
| Baseline and 20min | -3.9 | -28.313 to 20.613 | >0.05 |
| Baseline and 30min | -0.7 | -25.088 to 23.838 | >0.05 |
| Baseline and 40min | -0.6 | -25.038 to 23.888 | >0.05 |
| Baseline and 50min | 1.0 | -23.463 to 25.463 | >0.05 |
| Baseline and 60min | 0.4 | -24.013 to 24.913 | >0.05 |

**Table 3 Mean CCT values as measured with Scheimpflug device at one time point after fluorescein instillation for eyes with and without saline rinse**

| Measurement time point (min) | Mean CCT μm (SD) | 95% CI (μm) |
|---|---|---|
| Eyes without rinse | | |
| Baseline | 544.90 (24.6) | 527.32 to 562.5 |
| 1 | 576.20 (28.2) | 556.05 to 596.4 |
| 2 | 571.40 (27.3) | 551.85 to 590.9 |
| Eyes with rinse | | |
| Baseline | 541.7 (28.0) | 521.7 to 561.7 |
| 1 | 578.50 (30.0) | 557.0 to 599.9 |
| 2 (after rinse) | 559.9 (27.1) | 540.5 to 579.3 |

In the eye rinse group, Table 3 shows data with and without saline rinse after fluorescein. There was no significant difference in CCT baseline value of eyes with rinse compared with the continuous assessment eyes. Instillation of fluorescein increased CCT value by 36.8 μm at 1min. After rinsing eyes with saline, CCT increase from baseline was 18.2 μm (50.5%) at 2min.

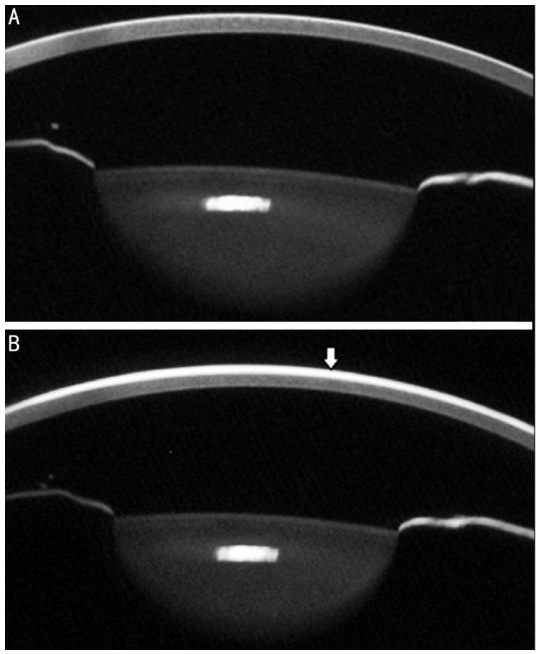


**Figure 2 Images of cornea from Scheimpflug device before and after fluorescein instillation** A: Scheimpflug image of a subject's cornea at baseline; B: Scheimpflug image of the same subject's cornea at 1min after fluorescein instillation. Fluorescein layer is indicated by the white arrow point.

## DISCUSSION

In this study, there was an increase from baseline measurement of CCT with rotating Scheimpflug device after a drop of 2% fluorescein was instilled into the eyes. Overall CCT difference of 25 μm (5%) with fluorescein instillation has been reported to be clinically relevant[14] and theoretically, compared to the baseline value, an increase in CCT was defined as a value greater than 5 μm [4]. The increase of 37.0 μm (6.3%) observed at 1min after fluorescein instillation may not be real anatomic tissue swelling but could be attributed to the mere physical presence of fluorescein thickening the tear film which the Scheimpflug imaging system detected as cornea (Figure 2A, 2B). It takes three hours of corneal hypoxia, induced by soft contact lens wear, to produce 12.1% increase in real total cornea thickness as measured by optical pachymetry[15].

Instillation of fluorescein resulted in higher CCT value due to effect of visualization of the now yellow-green tear film through the blue light of the Scheimpflug device. The tear film did not become thicker. Adaptation of this more intense backscattered light was used by Zhuang *et al* [16] as a novel method to measure tear film thickness. They found mean central tear film thickness in normal eyes to be 24.7±3.9 μm. From their study we can infer that the mean difference between baseline measurement and post fluorescein time points in our study could represent tear film thickness. CCT mean difference of 27.5 μm was obtained at 2min from baseline. They, however, did not state the exact time of measurement after fluorescein instillation, but stated it was

done after "after several blinks". CCT measurement with Scheimpflug device after fluorescein instillation might inadvertently include the tear layer thickness as real CCT measurement. This may result in overestimation of preoperative CCT measurement in refractive surgery which could result in complications. One such complication is corneal ectasia. It has been reported that preoperative thinner corneas of 521.0 μm developed more ectasia compared to corneas which were 546.5 μm [17]. Theoretically, it means CCT overestimation of 25.5 μm could result in refractive surgery complications. Our study identified CCT overestimation with the Scheimpflug device due to fluorescein presence to be in the range of 0.6 to 37.0 μm, depending on post fluorescein measurement time point. Six subjects in our study, who had baseline CCT below 521.0 μm, had CCT measurement values greater than 550 μm at 1min after instilling fluorescein. These would not have been detected as being at risk of corneal ectasia if the post fluorescein CCT value was the preoperative data recorded.

There is a pattern to the influence of fluorescein on CCT measurement values with the Scheimpflug device. There is a maximum increase at 1min of 37.00 μm followed by a continuous decrease, with eventual return to baseline between 20 and 60min (Figure 1, Table 2). Hirnschall *et al* [14] had similarly reported CCT increase of 46.6 μm after 1min of fluorescein instillation followed by return to baseline in some subjects at about 40min, with 3 cases where fluorescein layer was still present after 120min. All subjects in our study returned to baseline 60min after instilling fluorescein drop. This gradual decrease of CCT to baseline can be attributed to fluorescein decay caused by tear evaporation and drainage. Rinsing eye with saline solution right after the fluorescein measurement moderately reduced the backscatter of the tear film. We obtained a 50.5% decrease in rinsed eye at 2min measurement time point. Hirnschall *et al* [14] reported a 49.6% decrease but did not state the time of measurement. As the fluorescein diluted over time, the tear film becomes less and less visible until it becomes almost invisible again. The dynamics of this process remain to be accurately determined as it is often plagued with limitations in measurement techniques.

In conclusion, we have shown that instillation of 2% fluorescein affects the value of CCT measured with Scheimpflug device and the effect can last up to 60min. Rinsing the eye may moderately reduce the effect. These observations suggest avoiding fluorescein use prior to CCT measurement with a rotating Scheimpflug device.

**ACKNOWLEDGEMENTS**

**Foundation:** Supported by Research Centre, College of Applied Medical Sciences and the Deanship of Scientific Research at King Saud University.

**Conflicts of Interest: Briggs S,** None; **Bin Moammar M,** None.

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