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Digitalization of a Wide Field Contact Specular Microscope

G. Hor [a,*], Y. Gavet [b], A. Bernard [a], C. Urrea [c], P. Gain [a,c], G. Thuret [a,c,d]

[a] *Corneal Graft Biology, Engineering and Imaging Laboratory, EA2521, Federative Institute of Research in Sciences and Health Engineering, Faculty of Medicine, Jean Monnet University, St-Etienne, France*
[b] *Ecole Nationale Supérieure des Mines de Saint-Etienne, France*
[c] *Ophthalmology Department, University Hospital, Saint-Étienne, France*
[d] *Institut Universitaire de France, Paris, France*

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Abstract

Objectives: Non-contact specular microscopes (SM) have a very small field of observation (usually 0.1 mm^2). This constitutes a major limitation when accurate and extended assessment of the corneal endothelium is needed, for instance during clinical trials or difficult diagnosis. Previous contact SM had the advantage of a wide field thanks to the use of an applanating cone but none of the old commercial devices passed the stage of digitalization. This paper aims at upgrading a wide field contact SM with a digital camera and dedicated software in order to combine wide field with the advantages of non-contact SM.

Materials and methods: The digital device was composed of an acquisition unit consisting in a contact Konan Specular SP5500 equipped with a digital camera Canon EOS 6D and a Linux-based computer. Image processing uses segmentation algorithms (Gestalt theory and watershed with markers).

Results: The device acquired wide field (0.5 mm^2) highly contrasted images. The software provided cell boundaries and statistics: endothelial cell density, coefficient of variation of cell area and percentage of hexagonality. Contact SM images significantly increased the sampling of endothelial cells and consequently the reliability of endothelial quality analysis.

Conclusion: The digital contact SM is not destined to compete with commercial non-contact SM but is an exceptional device for reference centers. It will allow obtaining accurate and reproducible endothelial qualification for specific studies.

Keywords: Specular microscope; Contact; Digital; Image processing; Wide field; Corneal endothelium

1. Introduction

1.1. Corneal endothelial cells

Corneal endothelial cells (ECs) play a major role in vision. They form the posterior-most layer of the cornea, at the interface between cornea and the liquid, called aqueous humor, that fills the anterior chamber of the eye. They keep the cornea transparent by pumping stromal water in order to maintain a specific hydration rate. As, in humans, they do not divide, the 400 000 ECs present in each cornea at birth survive until death. In healthy corneas, their mortality rate is indeed extremely low, and their population slowly decreases at a rate of only 0.6% per year. During normal aging, they never spontaneously reach the minimal threshold under which they are to few to maintain the cornea transparent. It is unfortunately not the case in several frequent diseases that are responsible for premature ECS death, resulting in irreversible loss of corneal transparency and blindness. An accurate analysis of the quality of the endothelial

* Corresponding author at: Laboratoire BiiGC, EA 2521, Faculté de Médecine, 10 Chemin de la Marandière, 42270 Saint-Priest en Jarez, France (permanent address).
E-mail addresses: g.hor@univ-st-etienne.fr (G. Hor), gavet@emse.fr (Y. Gavet), aurelien.bernard@univ-st-etienne.fr (A. Bernard), caroline.urrea1@gmail.com (C. Urrea), philippe.gain@univ-st-etienne.fr (P. Gain), gilles.thuret@univ-st-etienne.fr (G. Thuret).

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layer is therefore necessary to establish the diagnosis of these diseases at an early stage, follow their evolution and choose the best therapeutic options. In patients with transparent corneas, ECs are observed with a specular microscope (SM, see below).

Eye banking is another domain where an accurate determination of the endothelial quality conditions the fate of each donated cornea. One of the roles of eye banks is to verify that stored corneas bear an healthy endothelium able to ensure corneal transparency for years in the future recipient. There is a threshold of endothelial cell density (ECD in cells/mm^2) under which a cornea is considered unsuitable for corneal graft. Usually of 2000 cells/mm^2 for penetrating keratoplasty (replacement of the full thickness of the cornea) and 2400 cells/mm^2 for endothelial keratoplasty (selective replacement of only the posterior part of the cornea) this threshold is responsible for the elimination of 20 to 30% of the donated corneas. Cell morphometry (regularity in cell size called polymegethism, and regularity in cell shape called polymorphism) is an additional parameter of quality. Two types of microscopes can be used to visualize ECs of stored corneas, depending on the storage method: a SM for corneas stored at 4° C for 5 to 10 days (the most frequent, worldwide) or a transmitted light microscope (bright field or phase contrast) for corneas stored at 31° C for a maximum of 35 days. Stored corneas are characterized by a more heterogeneous endothelium than in living patients, because the mortality rate of ECs is highly increased (600 times faster) resulting in local variations in ECD. A reliable cell count therefore requires to observe the largest possible field and count hundreds of ECs. This is unfortunately not the case in most of the eye banks that only count 50 to 100 ECs (less than 0.03% of the total), because microscopy techniques, most of the time not revisited since the 1970s, do not allow to do more.

In this work we rehabilitated a contact-SM devoted to the analysis of wide fields of the corneal endothelium, by adding a digital image acquisition and a new image analysis software.

1.2. Specular microscopes

In 1968, David Maurice introduced the first specular microscope [1]. With this imaging technique, the reflexion of light at the interface between the endothelial layer and the aqueous humor (2–5% of the incoming light) allows to visualize ECs boundaries that slightly modify the light reflexion. In case of abnormal endothelial structures like excrescents in the membrane underlying ECs (lesions seen in a frequent primary endothelial dystrophy called cornea Guttata) or inflammatory deposits onto the ECs, typical dark or bright images appear. First generations SMs, called contact SMs, used a transparent complex truncated cone shape objective put in contact with the patient cornea after local anaesthesia with eye drops. This cone slightly applanated the cornea, allowing observation of a wide flattened area of the endothelium. During the seventies, contact SMs were commercialized for clinician [2,3]. They were coupled with a film camera. Photographs comprised a calibrated grid overlay in order to ease the manual cell counting. Acquisition of good quality images required a learning curve, and, for patients contact with the cornea could be sometimes uncomfortable. Nevertheless, sterilization of the applanation cone between patients was binding. During the 1980s, the development of non-contact SM, by ophthalmology companies, was seen as an important progress because they eliminated the potential contamination risk and were also less dependent of the observers' skill. It was also possible to capture digital images and treat them with image analysis software. Modified version of non-contact SMs were also soon adopted by eye banks because they allowed observation through the walls of the storage bottle or viewing chambers. All these important improvements were nevertheless made at the expense of a dramatic decrease of the size of the field of observation. All non-contact SM have a very small field of observation (typically 0.1 mm^2 covering only 0.1% of the endothelial area). This small area is sufficient for the routine verification of normal corneas but constitute a major limitation each time that an accurate and reproducible endothelial cell count is required. For clinicians, several endothelial diseases are characterized by a heterogeneous distribution of endothelial lesions over the whole surface of the endothelium, and by progressive worsening with time. This is the case for the Fuchs corneal endothelial dystrophy (FCED), the most frequent endothelial disease that is a primary dystrophy characterized by premature ECs death and abnormal accumulation of proteins under the cells, forming excrescents and thickening of Descemet membrane. Small field non-contact SM provide a too narrow window on the endothelium to allow satisfying staging of cases necessary to establish the prognosis. Wide field contact SM constitutes an excellent device for the diagnosis and follow-up of FCED. This is also particularly important in fundamental, translational and clinical research works during the development of new therapeutics for endothelial diseases. In these works, a reliable quantification of the endothelial parameters (ECD and morphometry) is necessary. The ECD is often the main criterion used in experimental or clinical studies to calculate the number of experiments to perform or the number of patients to include. An accurate ECD is mandatory to obtain reliable data on the efficiency and safety of new drugs, new devices, new graft storage or preparation techniques. All these specific circumstances constitute excellent applications for the old wide field contact SMs. Nevertheless, as none of them survived the era of non-contact SM, they were abandoned before being adapted with digital image acquisition and treatment. Consequently, we upgraded a contact-SM in order to obtain an unprecedented wide field digital SM devoted to research.

2. Material and methods

2.1. Building the digital device

We used a Konan Iconan SP5500 contact SM (Konan, Tokyo, Japan), which was the reference device at the peak of contact-SMs. It was equipped with a lenses assembly constituting an ×40 optic zoom. Images were originally acquired by a full frame 35 mm camera film, that we replaced by a Canon EOS 6D digital camera with a full frame, 20.2 megapixel CMOS sensor (Canon, Tokyo, Japan). A specific mount was