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### Fluorescence multi-laser scanning microscopy of the cornea and ocular adnexa: a new era for functional confocal microscopy in ophthalmology

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#### ABSTRACT

**Purpose:** In vivo confocal microscopy (IVCM) is a routine investigation for the ocular surface in reference centres. It provides high resolution pseudo histology. Nevertheless its unique laser source and imaging principle (reflectance) only provide morphological information. Multi-laser and fluorescence laser scanning microscope add a supplementary dimension by allowing the use of fluorescent markers liable to provide specific functional data **Methods:** Ex vivo animal and human corneas, healthy volunteers and patients were successively examined using the multilaser vivascope 1500 (MAVIG GmbH, Germany) equipped with 3 lasers (488, 658, 785nm) and the corresponding emission (Em) filter sets. For each excitation (Ex) wavelength (?), 3 observation modes were available: reflexion (all ?), pure reflectance (?Ex = ?Em), fluorescence (3 specific band pass). Ex vivo, all corneal layers were analysed without preparation and after topical application of Fluoresceine (F) and Indocyanine green (ICG) and of numerous other molecules. Topical instillation and of intraveinuous injection of F and ICG were analysed in healthy volunteers and in patients

**Results:** Using reflexion and reflectance, the 3 Ex ? gave complementary structural informations with the highest resolution obtained at 488nm. Topical markers helped identify specific cell populations and intracellular structures. Intraveinuous ICG was inefficient whereas fluo provide highly contrasted conjunctival images

**Conclusion:** IVCM coupled with fluorescence opens a new era in the clinical imaging of the ocular surface and probably more largely in Ophthalmology. A new semeiology remains to be learned