New Method for Sperm Evaluation by 3-Dimensional Laser Scanning Microscopy in Different Laboratory Animal Species

Klaus Weber¹, Alexander Waletzky², Diana Fendl³, Patricia Ordóñez⁴, Pradeep Takawale³, Felix Hein³, Wolfram Riedel³, Andres König⁴, Marc Kunze³, Anne-Laure Leoni³, Javier Rivera⁴, Roberto Quirici⁵, Ivano Romano⁶, Susanne Paepke², Yoshimasa Okazaki¹, and Jerry F. Hardisty⁶

Abstract
Sperm analysis is one of the end points in reproductive toxicology studies. Different methods for quantitative sperm analysis have been described. For qualitative morphological sperm analysis, either such techniques or smears of sperm and histological sperm staging are in use. Any of these methods provides morphological results on a light microscopy level. Laser scanning microscopy is a technique using a focused laser for scanning an object. The Olympus 3D Laser Scanning Microscope LEXT OLS4000 with optional possibilities of differential interference contrast provides a microscopic method for visualizing microasperities, which are far beyond the resolving power of a typical light or laser microscope. This technique was applied to sperm of mice, rats, rabbits, and cynomolgus monkeys at magnifications up to ×17 090. The obtained images are comparable to those of a scanning electron microscope under relatively low-power magnifications. Measurements on sperm parameters were taken by an integrated image analysis software tool. Abnormalities were easily detectable.