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CellCelector™ Application Note

Isolation of sperm cells

CellCelector is protected by US patent 9,822,331 B2

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The availability of single sperm cells for the analyses of the genetic fingerprint (Fig. 1) may be of special importance, e.g. to clarify questions in terms of family relationships (paternity test) as well as for the forensic medicine to uncover sexual offences. Moreover the isolation of vital single sperm cells can be necessary for in-vitro fertilization processes.

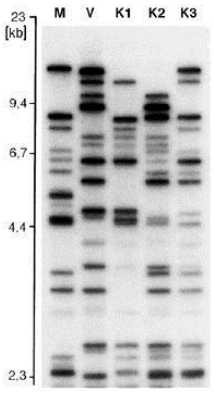

Fig. 1: Example of a genetic fingerprint comparing the genetic pattern of mother (M) and three kids (K1, K2 and K3) with that of the potential father. In this case the fatherhood of the potential father to all thee kids was proofed clearly („spektrumdirekt.de - Vaterschaftstest).

This article describes the isolation of single sperm cells using the CellCelector system which was performed in the laboratories of the forensic department of the French police. The CellCelector system is composed of an inverted microscope (with CCD-cam, motorized stage, several fluorescence channels), a highly precise robot arm and a deck tray for holding picking tools, liquids and destination plates (Fig. 2). Via a precise syringe pump and very small glass capillaries (20 µm) the robot arm is able to aspirate extremely low volumes (less then 10nl) allowing the precise and gentle aspiration of single cells from different origin.

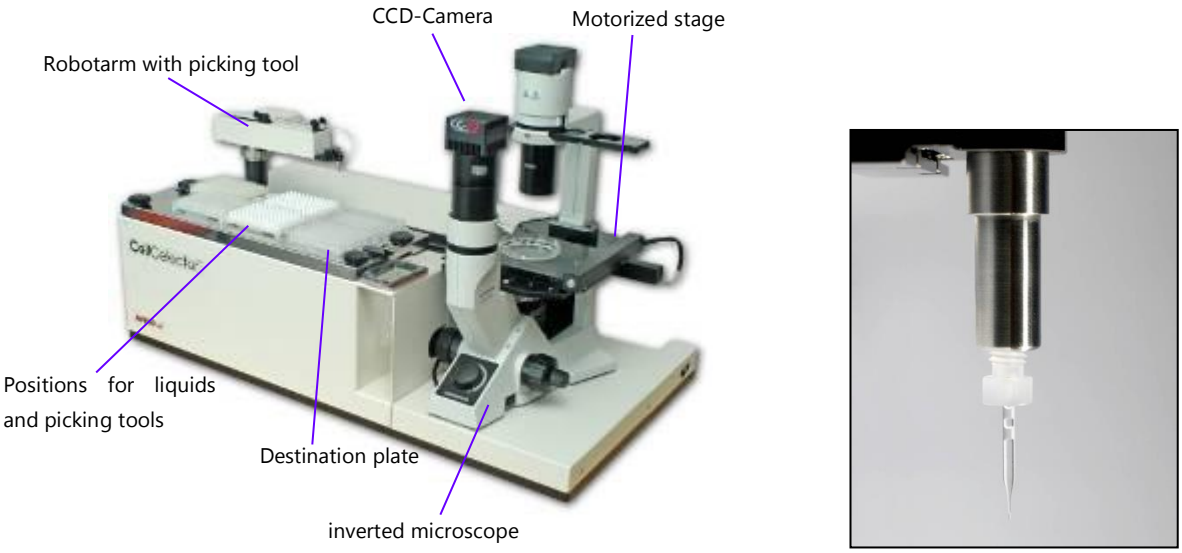

Fig. 2: Cell Celector™ - total view (left); detail view from the picking tool to isolate single cells (right)

Single sperm cells where observed and isolated in bright field illumination using a 20x-Objective and an exposure time of 21 ms. The size of the sperm cells were determined on about 5 x 33 µm using some special software features for measuring objects microscopically (Fig. 3)

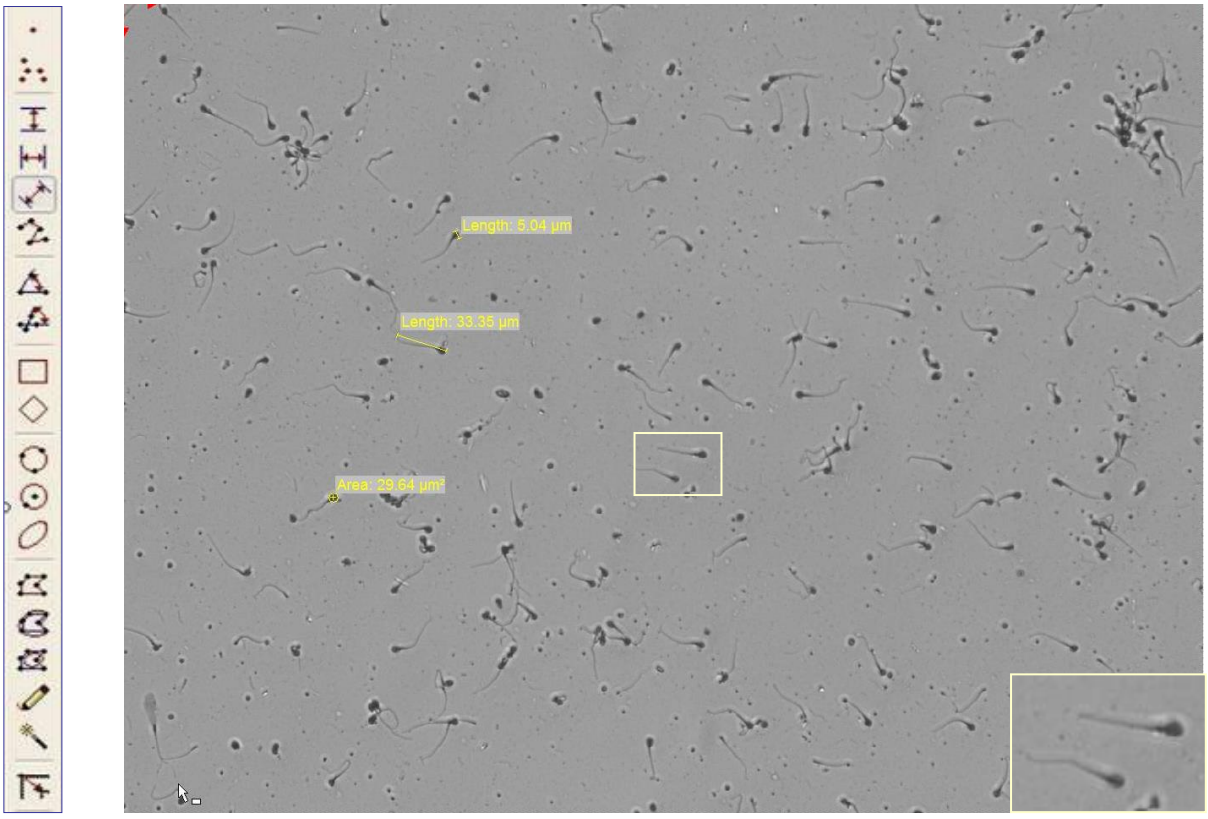

Fig. 3: Example to observe single sperm cells in bright field illumination mode (big image). For a better view the rectangular marked area is shown enlarged (bottom right). Measurement tool bar with a number of features to measure objects (left)

Detection of single sperm cells within a sample

The detection of single sperm cells may be done either in manual mode by mouse click on the life image screen or automatically using a set of adjusted detection parameter (diameter, Area, elongation etc. …) and use it for scanning the sample automatically. The basic detection is done by setting a range of grey values. Target cells can be separated from neighbour cells and from the background. Depending on setting the grey value thresholds either complete single sperms are detected or just the head of the sperm cells (Fig. 4)

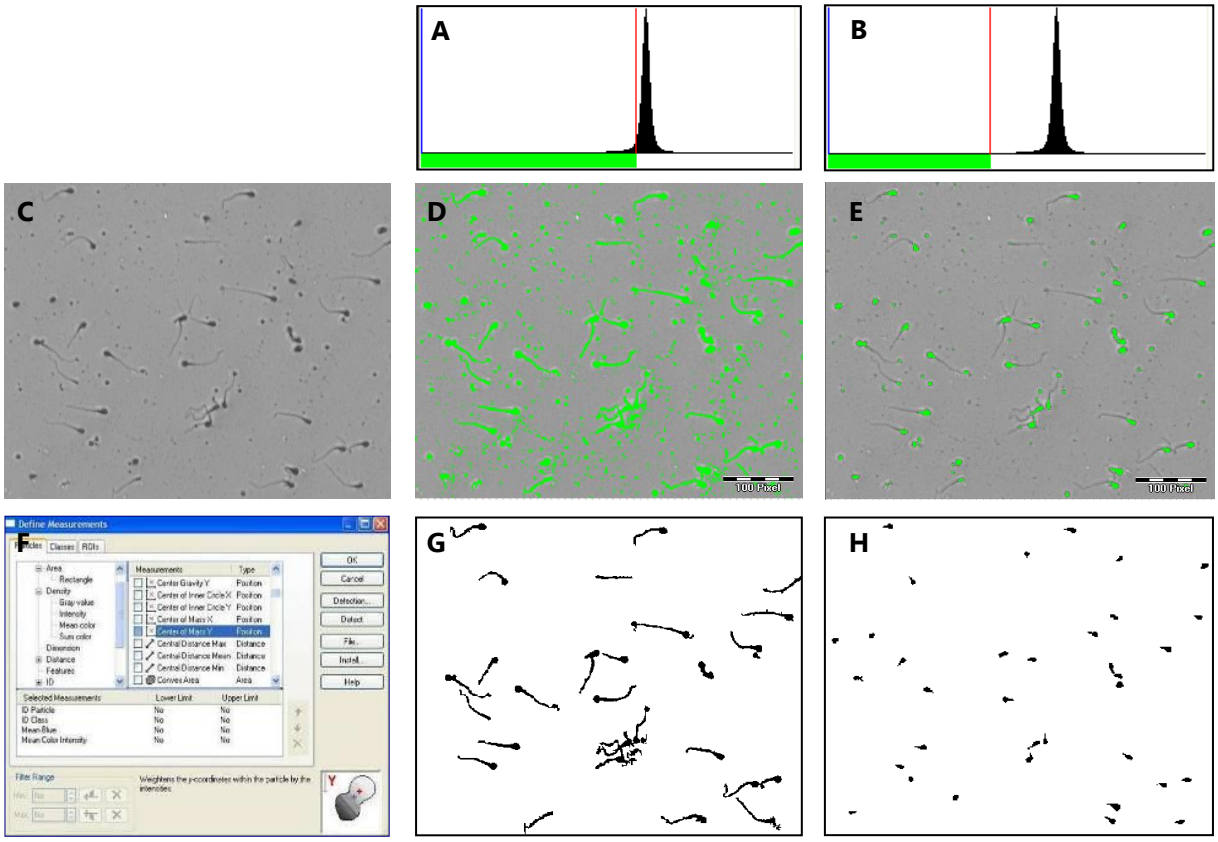

Fig. 4: Set up of parameters to detect automatically single human sperm cells

Different ranges of grey values (A & B) allows the detection (marked in green) of different parts of the sperms (D & E) based on the reference image (C). Additional parameter as diameter, area, elongation … (F), can improve the scanning result even more (G & H).

Isolation of single sperm cells

Borosilicate glass capillaries with an inner diameter of 30 µm were used to isolate single sperm cells out of a mixture. By developing an aspiration pressure right above the cell using a volume of 60 nl liquid the cell was detached from the bottom and soaked with the liquid into the glass capillary. The isolated sperm cells were transferred into single wells of 2x9well Ibidi-slides. Each single cell isolation is documented by images taken before and after the picking process (Fig. 5) which are saved in databases.

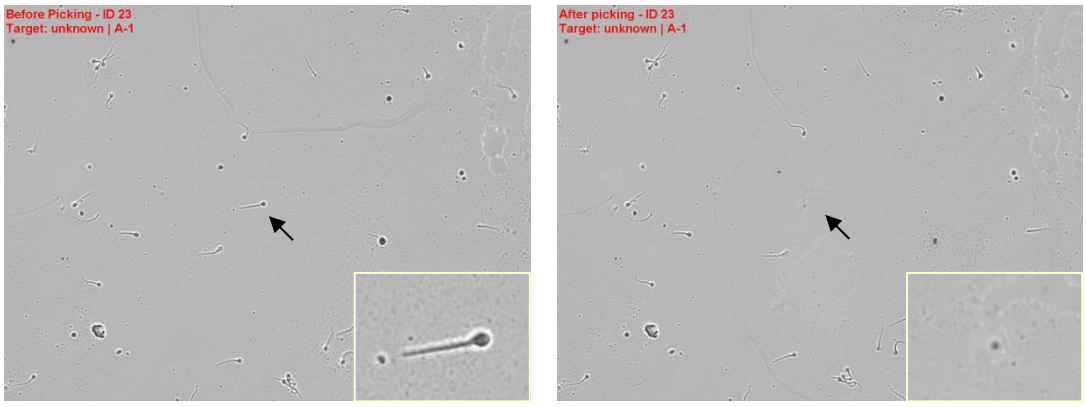

Fig. 5: Microscopic view of a sperm cell: Images were taken right before (left) and right after (right) the isolation of a single sperm cell.

Summary

This study clearly shows the possibility to detect and to isolate single cells from a sample by using the CellCelector system. The unique combination of highly precise robot technology with inverted microscopy allows automating the detection as well as the isolation of single sperm cells using glass capillaries. Time consuming manual work may be replaced by standardized conditions using the CellCelector system.

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ALS Automated Lab Solutions headquarters in Jena, Germany

ALS Automated Lab Solutions GmbH is located in Jena, a dynamic city famous for microscopy and material science. ALS is a specialist for the development of innovative technological solutions for cell biology research and molecular biology. ALS lifts cell culture to a new level of choice and control on the leading edge in cell biology, cell therapy research, regenerative medicine and drug discovery. With automation and standardization of laborious manual procedures, ALS supports science and research for more efficiency and the creation of new methods for the science of tomorrow.

ALS Automated Lab Solutions GmbH is partner of:

Please do not hesitate to contact us for further information:

Jens Eberhardt

ALS Automated Lab Solutions GmbH
Otto-Eppenstein-Str. 30
07745 Jena
Germany

Phone: +49 (0) 3641 4820-0
Fax: +49 (0) 3641 4820-11
E-Mail: info@als-jena.com