

# Applications

## AxioVision SFM

### Link between cell data and associated cell images

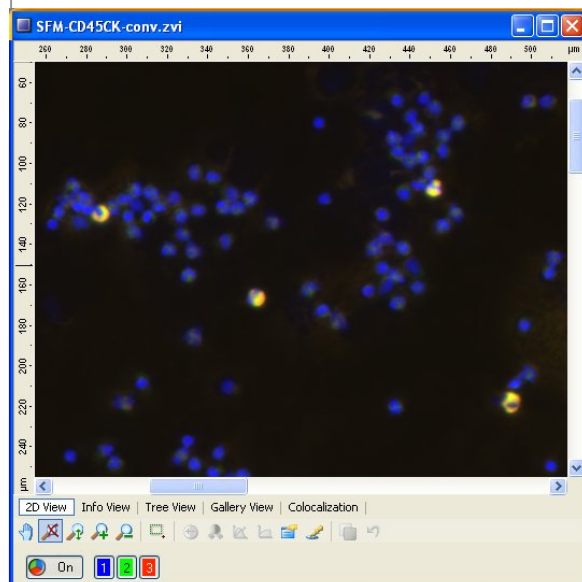
SFM (Scanning Fluorescence Microscopy) proved to be a reliable alternative method, providing results comparable to LSC (Laser Scan Cytometer) and FCM (Flow Cytometer). Slide based cytometry (SBC) proved to be more suitable for rare-cell detection than FCM. SFM with digital slides may prove an acceptable adaptation of conventional fluorescent microscopes in order to perform rare cell detection.

Based on the results of flow cytometry statistically sufficient cells from cytopsin specimens will be acquired automatically in multiple fluorescence channels (AxioVision-MosaiX) with a digital camera (AxioCam MRm) using an upright fluorescence microscope (Axio Imager). Using appropriate scripts these images will then be measured with

AxioVision image analysis software. The scripts may be adapted to all evaluation needs. AxioVision offers all currently available functions and parameters for image processing and image analysis. All single steps may be combined in any order to receive reasonable extraction parameters for the single cell analysis. The measurement results of all cells are finally integrated in a single data table.

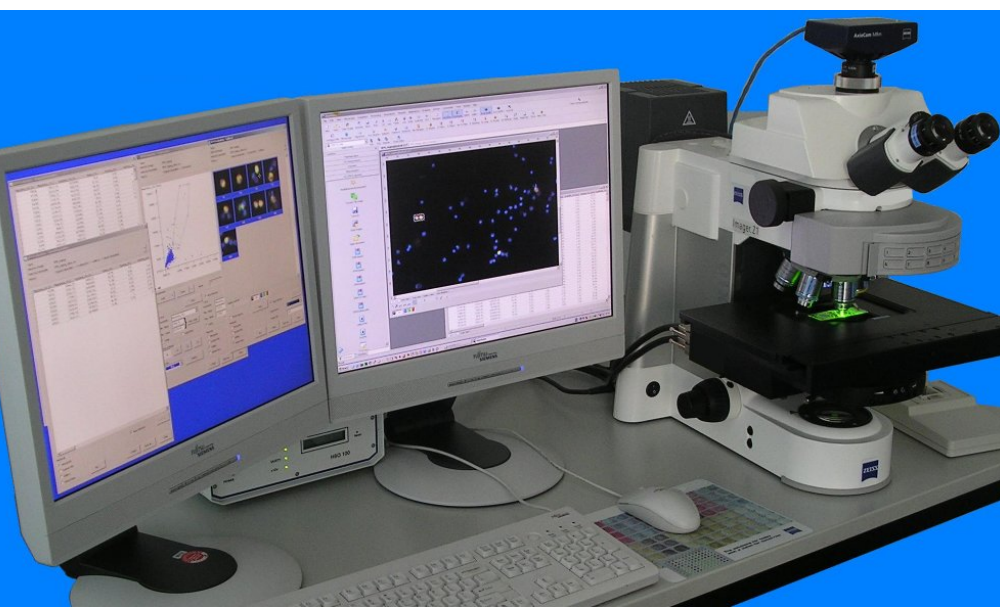
The AxioVision SFM module is finally used to link the data of the results table with the original multichannel image. From these data various distribution types may be created (histogram, scatter plot, gallery, gated data table), in order to isolate "rare event" cells as an image with the connected data

*AxioVision SFM system with Axio Imager, Piezo motor stage and AxioCam MRm*



*AxioVision-MosaiX-3-channel image extract):  
CD45-CK cells(CAPI, FITC, Texasred)*

- Acquisition of multichannel MosaiX images
- Measurements of geometric and densitometric cell data
- Coordinate measurements of single cells
- Histograms with gates
- Scatter plots with gates
- Special "rare events" gallery

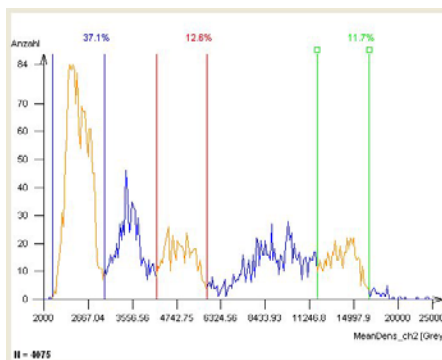


We make it visible.

# AxioVision SFM - Methods and Equipment

## SFM Methods

The results table for the AxioVision SFM evaluation holds all desired raw data and derived parameters for the further evaluation. Additionally the cell coordinates for the various channels and the window coordinates for each single cell may be found here. From this table various distributions may be generated in any sequence and for each available parameter.

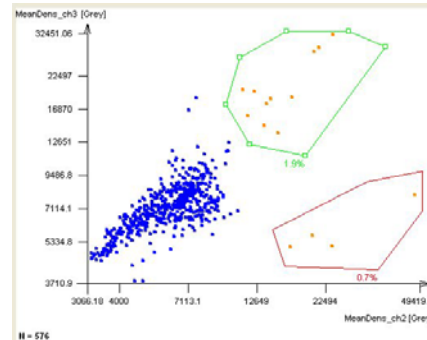


*SFM histogramm of rainbow beads with 8 populations.*

As an example in the histogram the nucleus area may be used to perform a size distribution and create a histogram. Using several gates the specific region of interest may be marked in the histogram. Further distributions may be generated for all cells lying inside these gates.

Using the scatter plot 2 parameters may be plotted against each other, as shown in the scatterplot. Again all values from all image channels may be used. In our example the cell regions have been created from all 3 channels. In the scatterplot the mean grey values of channel 2 (FITC-green) have been distributed

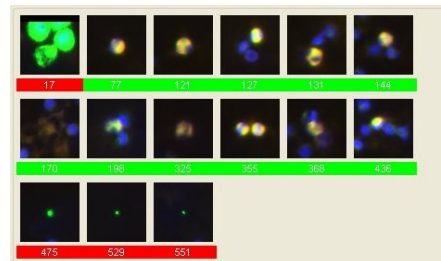
against the mean gray values of channel 3 (Texasred-red).



*SFM scatter plot CD45-CK cells (DAPI, FITC, Texas red)*

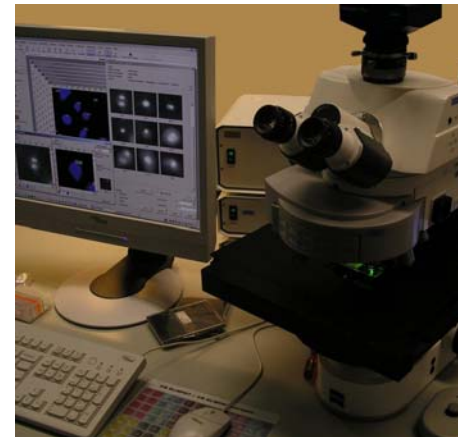
These „rare events“ surviving the gating processes may now be visualized in a gallery as cell images with adaptable window sizes and zoom factors (AV-SFM gallery).

The gallery also allows for further selection of specific cell images by a simple click to the image frames. The color bar below each cell corresponds to the color of the scatterplot gate.



*SFM gallery.*

A gated data table may be created from these final „rare events“. Using the object coordinates cells of interest may be relocated under the calibrated microscope for control.



*AxioVision SFM System*

## SFM Equipment

### A) Acquisition and evaluation station

- Microscope Axio Imager mot
  - FL-illumination
  - FL-filter sets
  - Motor stage with control
- AxioCam MRm camera
- Computer + 19" TFT monitor
- Software
  - MTB2004 microscope control
  - AxioVision Basis
  - Module Multichannel Fluorescence
  - Module MosaiX
  - Module Autofocus
  - Module Imaging Plus
  - Module Interactive Measurement
  - Module AutoMeasure Plus
  - Module Commander
  - Module SFM

### B) Additional Evaluation Station

- Computer + 19" TFT Monitor
- Software
  - AxioVision Basis
  - Module Imaging Plus
  - Module Interactive Measurement
  - Module AutoMeasure Plus
  - Module Commander
  - Module SFM

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