Analysis, imaging and sorting of germinated fungal spores on the COPAS VISION Flow Cytometer

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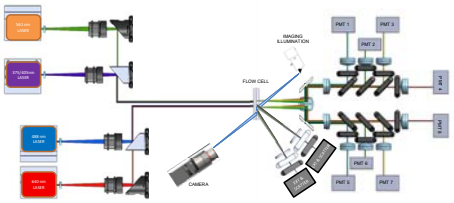
UNION BIOMETRICA
Large Particle Flow Cytometry

Introduction

Union Biometrica developed instrumentation for large-particle flow cytometry that can capture images in flow. Adding imaging capability to flow cytometry greatly enhances the phenotyping of samples by providing morphological and spatial information of the sample constituents not collected by conventional flow cytometers. Traditional measurements of size, optical density, and (auto-)fluorescence, as well as Profiler data, are also collected, and these measurements are used for making sorting/dispensing decisions. The collected images and flow cytometry measurements are synchronized so that objects dispensed to wells of multiwell plates can be traced back to their corresponding image. The COPAS VISION technology platform is designed for large particles, making it ideally suitable for small model organisms, large single cells, and cell clusters. Our data from the COPAS VISION shows proof-of-principle support for increased level of phenotyping of these types of samples, including germinated fungal spores which is presented here.

COPAS VISION

COPAS *VISION* analyzes and sorts (germinated) fungal spores in a continuous flow stream at high rate (up to 100 events/sec), gently and without harming the sample. The device measures object size (TOF), optical density (EXT), side-scatter and up to 8 fluorescent markers.

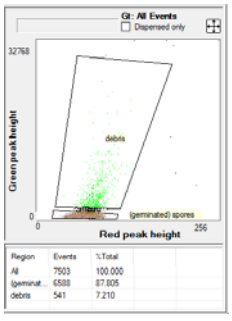


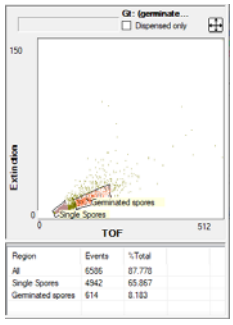
- Up to 4 excitation lasers
- 2 banks of 4 PMT's
- Brightfield imaging with high speed camera

The sorting is accomplished with the use of a pneumatic device located below the flow cell. Furthermore, the fluid pressure through the flow cell is significantly lower than in conventional flow cytometers, thus providing gentle sorting conditions.

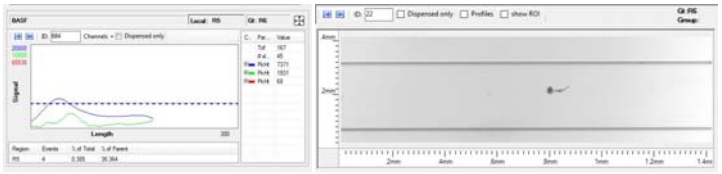
Flow Cytometry data

A population of *Phakopsora pachyrhizi* ungerminated and germinated spores were analyzed and dispensed on the COPAS VISION to illustrate the possibilities for fungal genetics researchers. The instrument runs on FlowPilot software. Parameters like TOF (~size), EXT (~optical density) and (auto-)fluorescence are collected and displayed in histograms and/or dual parameter dot plots. Individual objects can be selected using histogram or dot plot regions and combined gating. Here a TOF vs EXT dot plot with (germinated) spores was plotted and gated based on their red and green autofluorescence properties.





The Profiler option shows the position of fluorescence expression in every individual (germinated) spore along its axis. Fluorescence positioning can be visualized using Profiler graphs and sorting decisions can be made based on the location, intensity or number of peaks. In addition to the profiles an image of every (germinated) spore will be taken, in flow, as it passes through the flow cell.



Individual Profile of a germinated spore (Left) and its corresponding image (Right)

The images are particularly useful for object identification (what is in a particular region, with additional information from the Profiles), morphology determination (post data collection analysis, grayscale morphological characteristics) and quality control.

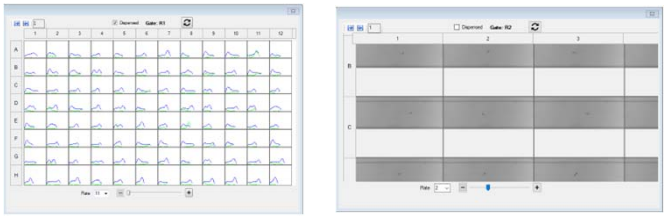
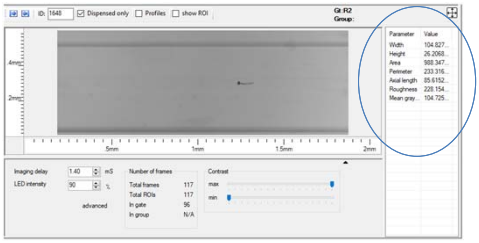


Plate view Feature of dispensed germinated spores by well (Profiles and Images)

Image analysis software determines parameters like length of mycelia and area



The images captured by COPAS VISION can be analyzed subsequently (post-data collection) and allow determination of width, height, area, perimeter, mycelia length and roughness of the object.

An autosampler (LP Sampler) can be connected to the COPAS VISION for sampling from multiwell plates

The LP Sampler was connected to the COPAS VISION in a pilot compound screening to determine the effect of the compound on germination. Based on the percentage germinated spores in the gate region compounds were selected that have an inhibitory effect on germination. Data not displayed.

Conclusions

COPAS VISION, our newest large particle flow cytometer, adds brightfield imaging to our large particle sorting capabilities. It provides automated high throughput analysis and sorting of fungal spores, pollen, seeds, small model organisms, cell clusters, bead-based libraries and other sample types that are too large or too fragile for traditional flow cytometers. COPAS VISION has expanded on these capabilities in several ways, most noteworthy is the ability to capture images of the sample objects. The images that are captured by the COPAS VISION enhances the phenotyping of samples by providing morphological and spatial information. Combining fluorescence profiles with imaging greatly enhances its interpretation, while the image analysis allows for new parameters like accurate length, area, width and height.