Analysis and sorting of Aspergillus niger pellets based on size and fluorescence intensity measurements

Objective

The purpose of this experiment was to test the feasibility of using the COPAS Plus (1000um flow channel) instrument to analyze and sort micro-colonies of *Aspergillus niger* using size and fluorescence intensity measurements.

Introduction

The COPAS family of research instrumentation comprises a collection of large particle cytometers utilizing at least 1 excitation laser and up to 8 channels of fluorescence collection. Unique to COPAS instrumentation is the Profiling feature which graphically plots the fluorescence intensity changes along the length of the object as it passes through the laser(s). Large objects up to 1.5mm in diameter can be analyzed for physical and fluorescence characteristics and gently dispensed into a multi-well plate or other collection container for further investigation or reuse. The COPAS Vision also has equipped a camera to take an image of the object inside the flow channel. This image accompanies the cytometry data and can be analyzed using Union Biometrica's FlowPilot software or other image analysis tools. In this experiment, the COPAS PLUS utilizing a 1000 micron flow channel was used to gently handle the analysis and sorting of *Apergillus niger* samples.

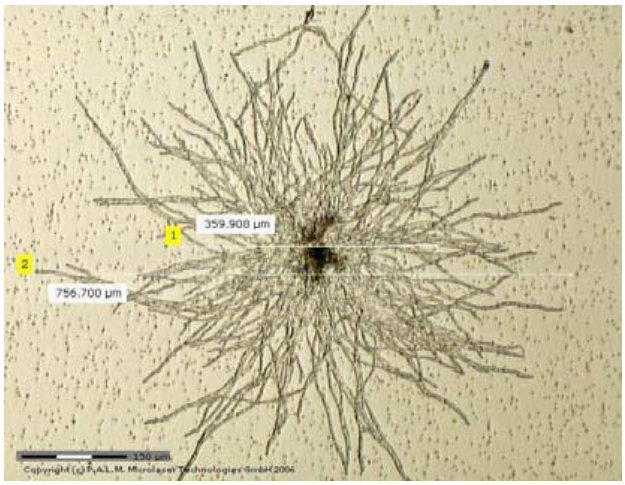

Figure 1a. *Aspergillus size measurement using microscopic analysis.*

Germination of spores of the filamentous fungus *Aspergillus niger* results in the formation of hyphae that grow at their apices and that branch sub-apically. As a result, a colony is formed that consists of a network of interconnected cells. *Aspergillus niger* forms centimeter scale macro-colonies on solid media, whereas (sub)millimeter micro-colonies are formed within a liquid shaken culture. Using the COPAS PLUS instrument we recently showed that micro-colonies of Aspergillus niger are heterogenic with respect to size distribution and gene expression (de Bekker et al., submitted). Here we mixed micro-colonies of four Aspergillus niger strains. These strains consisted of a wild-type strain and recombinant strains expressing GFP, dTomato, or both reporters from inducible promoters.

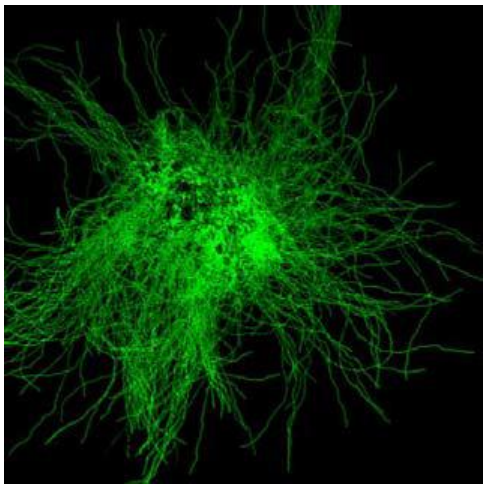

Figure 1b. *Aspergillus micro-colony showing GFP labelling.*

The COPAS PLUS instrument was able to analyze and sort large objects (30-700 microns) on the basis of the physical characteristics of size, density, and fluorescence signals. Objects are passed axially, one by one, through the focus of a laser beam. Relative size is determined by the time of flight (TOF) measurement. The optical density of the object is determined by the extinction (EXT) measurement. Objects can be dispensed into microtiter plates or stationary receptacles. Using a unique, pneumatic sorting mechanism, the COPAS is gentle enough to sort and dispense live organisms without affecting the viability. Applications for the PLUS include Drosophila, Arabidopsis and other like-sized model organisms, large cells and cellular clusters. In this experiment we used the COPAS PLUS instrument to analyze and sort individual Aspergillus pellets by size (TOF), optical density (EXT), green fluorescence (GFP) and red fluorescence (dTomato). The instrument was equipped with 488 nm and 561 nm solid state lasers for excitation.

Methods

Growth of Apergillus niger

Liquid shaken cultures of the four *Aspergillus niger* strains were inoculated with $7x10^8$ spores ml^{-1} and grown at 30 °C for 16 h at 250 rpm in 1L Erlenmeyer flasks with 250 ml transformation medium (TM) using 50 mM maltose or 50 mM xylose as a carbon source. Micro-colonies were harvested by filtration over a Büchner funnel with nylon gauze and fixed with 4% formaldehyde in PBS.

Size Measurements:

Aspergillus micro-colonies from liquid cultures of each of the four strains were suspended in water, mixed, and added to the sample cup. TOF measurements for the COPAS PLUS indicate numbers from 1 to 2048 data points. Using different size beads, a calibration curve was established to relate the numbers of size to micrometers.

Bead size (um)	TOF mean
42	74
51	80
99	171
143	218
200	330

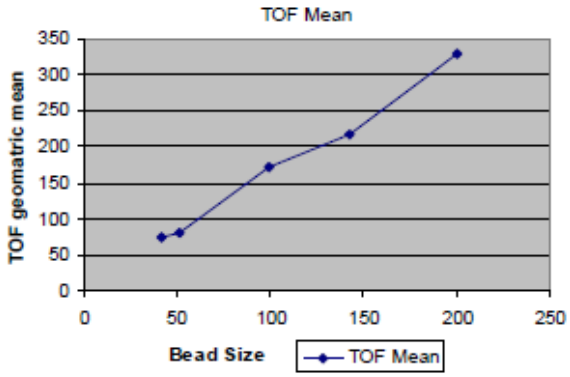

Figure 2. *Standard curve to convert TOF values to micrometers.*

Fluorescence intensity measurements:

In the test for fluorescence intensity, we have been analyzing GFP and dTomato expression in micro-colonies. Fluorescence intensity can be correlated to size measurements. The values for the fluorescence intensity of a pellet are stored in data files using values from 1-256.

Results

Mixed micro-colonies expressing either or not GFP and/or dTomato were sorted on the basis of size and fluorescence using the COPAS PLUS. The majority of the micro-colonies showed a TOF of 300-1000 (area indicated by Gate 1). Material with a lower and higher TOF mainly represented small debris and entangled micro-colonies, respectively. The micro-colonies with a TOF between 300 and 1000 had a diameter ranging from 340 to 460 µm. Microscopic examination revealed that these diameters represented the compact core of the micro-colony.

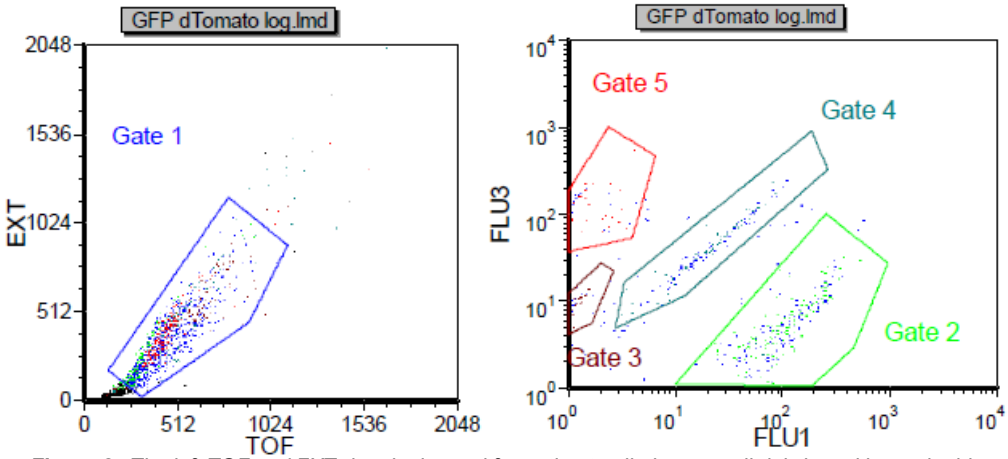

Figure 3. *The left TOF and EXT dot plot is used for gating to eliminate small debris and large double pellets from the fluorescence analysis. The right dot plot shows a mixture of 4 different populations in the sample from Gate 1. Not labelled (Gate 3), GFP labelled (Gate 2) dTomato labelled (Gate 5) and double (GFP and dTomato) labelled (Gate 4).*

The micro-colonies of the different stains were separated by sorting on the basis of GFP and dTomato fluorescence. Wild-type colonies had low green and red auto fluorescence (Gate 3). These colonies could be clearly distinguished from the colonies expressing GFP (Gate 2), dTomato (Gate 5) and GFP and dTomato (Gate 4).

Conclusions

The COPAS PLUS instrument can be used to sort micro-colonies of fungi on the basis of size and fluorescence. This enables us to study the genetic basis of heterogeneity in liquid shaken cultures.

Work performed in 2009, QTN reissued 2024:
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