

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

Work performed May 2009 by:

Charissa de Bekker, Jerre van Veluw, University Utrecht, Netherlands

Rico Bongaarts, Union Biometrica, Geel, Belgium

Objective

The purpose of this experiment was to test the feasibility of using the COPAS™ PLUS instrument to analyze and sort micro-colonies of *Aspergillus niger* using size and fluorescence intensity measurements.

Introduction

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.

The COPAS PLUS instrument is 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

Union Biometrica

USA: Tel: +1 (508) 893-3115

Europe: Tel: +32-(0) 14-570628

<http://www.unionbio.com>

Fax: +1 (508) 893-8044

Fax: +32- (0) 14-570621

sales@unionbio.com



like-sized model organisms, large cells and cellular clusters. In this experiment we used the COPAS PLUS instrument to analyse 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.

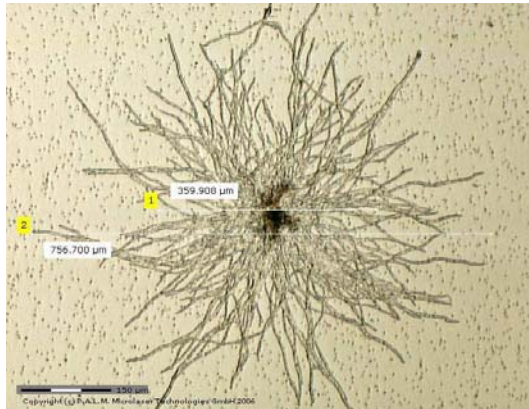


Figure 1a.
Aspergillus size measurement
using microscopic analysis.

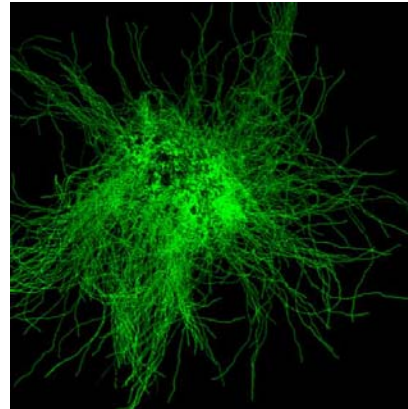


Figure 1b.
Aspergillus micro-colony showing
GFP labelling.

Materials and Method

Growth of *Aspergillus niger*

Liquid shaken cultures of the four *Aspergillus niger* strains were inoculated with 7×10^8 spores ml⁻¹ and grown at 30 °C for 16 h at 250 rpm in 1 L 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 micrometres.

Bead Size	TOF Mean
42	74
51	80
99	171
143	218
200	330

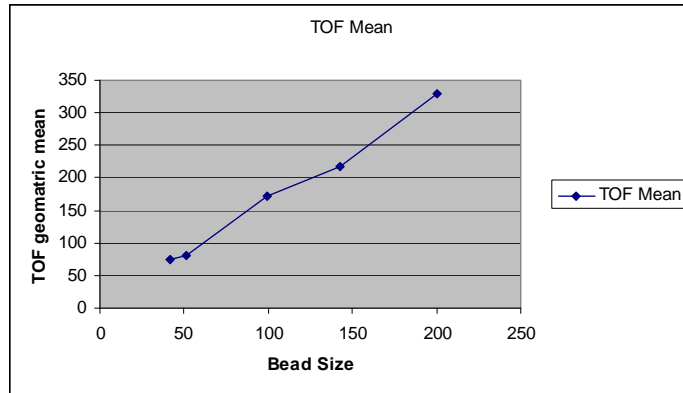


Figure 2. Standard curve to convert TOF values to micrometers.

Fluorescence intensity measurements:

In the test for fluorescence intensity we have been analysing 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.

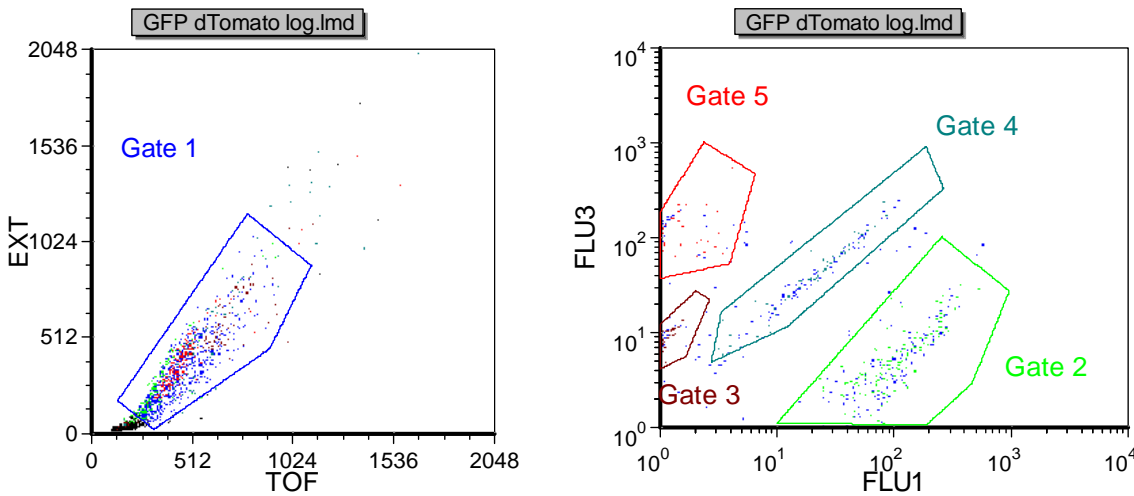


Figure 3. The left TOF and EXT dot plot is used for gating to eliminate small debris and large double pellets from the fluorescence analysis. The right dot plot shows a mixture of 4 different populations in the sample from Gate 1. Not labelled (Gate 3), GFP labelled (Gate 2) dTomato labelled (Gate 5)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

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.

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).

Conclusion

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.