

## Quantification of light-promoted conidiation of *Neurospora crassa bd, al-2* using a video system and densitometry software

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Quantification of conidia from *Neurospora crassa* mycelia grown on solid media by turbidity assay or weighing of conidia is difficult. We demonstrate here measurement of the intensity of light-promoted conidiation bands of *Neurospora crassa* strain *albino-band (al-2, bd)* using a video image system and densitometry software.

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We have used *Neurospora crassa* strain *albino-band (al-2, bd)* derived from a cross done by S. Brody, University of California San Diego at La Jolla, USA, for the analyses of light-promoted conidiation (Klemm and Ninnemann 1978 Photochem. Photobiol. **28**:227-230; Ninnemann 1991 J. Photochem. Photobiol. **9**:189-199). The *band<sup>c</sup>* genotype restricts conidial production to circadian regulated time windows and the *al-2* mutation (phytoene synthase) eliminates the carotenoid-dependent shielding of blue light needed for light-promoted conidiation. Therefore, this strain is a good choice to test light-promoted photoconidiation. Since conidia could not be removed easily and quantitatively from mycelia grown on agar plates, a quantification by turbidity test or weighing of aerial hyphae is not practicable. Therefore, we used a video system and an image analysis computer program for quantification of band intensities.

For assays of light-promoted conidiation *Neurospora crassa bd, al-2* mycelium was grown from conidia that had been harvested and kept in water in the dark and were always handled under red safety light. The substrate was modified Vogel's medium (Vogel 1964 Amer. Naturalist **98**:435-446) with 1.2 % agar (w/v) and 50 mM NO<sub>3</sub> as the sole nitrogen source, complemented with 1 % glucose (w/v). Large Petri dishes (18.5 cm) were inoculated by placing a filter paper disc (5 mm), dipped into a solution of conidia (1-2 x 10<sup>8</sup> ml<sup>-1</sup>) near the margin. After 24 h at 30 C in the dark, the mycelial front had grown about one third across the dishes and the position of the front was marked under red safety light. After 2 h irradiation with white fluorescent light (5 W m<sup>-2</sup>), the plates were again incubated in the dark for 36 h. The Petri dishes were scanned with a greyscale video image processing system consisting of a charge coupled device video camera (756 x 581 pixel) with monochromatic monitor and control unit (S+L Gesellschaft für wissenschaftliche Geräte mbH, Heidelberg, Germany), a video printer (Sony UP-860), and a personal computer. Such video systems are often used for documentation of ethidium bromide-stained gels in molecular biology and are present in many laboratories. For intensifying the white-colored bands of aerial hyphae, the petri dishes were placed on a black cloth. The image was stored as a greyscale graphic file with 256 intensity levels. The picture was inverted to change the white colored bands of conidia to black greyscales.

For quantification the graphic file of the image was analyzed with the inexpensive densitometry program QuantiScan (Biosoft, Cambridge, UK). Any other program which can integrate intensities of a digitized image should also be appropriate.

[Figure 1](#) shows examples of the results from typical experiments. Plate A is a control experiment held in the dark, plates B,C and D show various intensities of light-promoted conidiation. On plate A there are only faint bands of conidia corresponding to the circadian rhythm of conidiation. On plates B, C and D, the central band of conidia, which was produced just after irradiation, is the most intense. Beside this central band (b), more or less intense bands resulting from circadian rhythm are located (a, c), especially well expressed in plate D.

For quantification, we integrated the intensities of greyscales in the x-direction in a constant region (indicated by a white frame) of 11.5 x 8.5 cm, not including regions near the margin of the petri dishes, where edge-induced conidia-production can occur. The graphs to the right of the images are the respective intensity plots spanning the integration frame. The background intensity is highest near the inoculation place, where mycelium has grown thickest, and drops continuously until it falls to the level of mycelium-free medium. The bands of conidia can easily be separated from this baseline by "Tangent Skim" procedures and from each other by "Split Peak" procedures.

For quantification, the b-bands, which are the first ones after illumination, were compared with each other. Subtracting the background level of plate A resulted in the following relative intensities: plate B: 916 (23.7 % of C), plate C: 3857, plate D: 3652 (94.7 % of C). After 36 h of growth after illumination - as in our case - the conidia bands were not so intense, that a shading effect occurs. However, after growth of several days, conidia bands would get so dense that they would shade themselves, and linearity would decrease. Our experiments were not extended beyond 36 h.

The intensity of the white color of distinct bands of conidial hyphae is correlated to the extend of conidia production. Therefore a video system and an image analysis computer program is suitable for quantification of band intensities. The linearity of the system depends on the thickness of the conidial layer, and is good, if the growth is analyzed within 36 h.

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[Figure 1](#). Analyses of digitized video camera images of light-promoted conidiation of *Neurospora crassa* *bd, al-2* with a densitometry computer program. A is a dark control, B-D are examples of light-promoted conidiation. The region of intensity integration is indicated by a white rectangle. Peak areas of the bands of conidia are indicated in the respective intensity plots right of the images, which show the integrated intensities (x-direction) against the y-direction.