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In vivo multiphoton imaging of a filamentous fungus *Phycomyces blakesleeanus*: the effect of small ambient temperature increase on mitochondrial morphology and lipid droplets density

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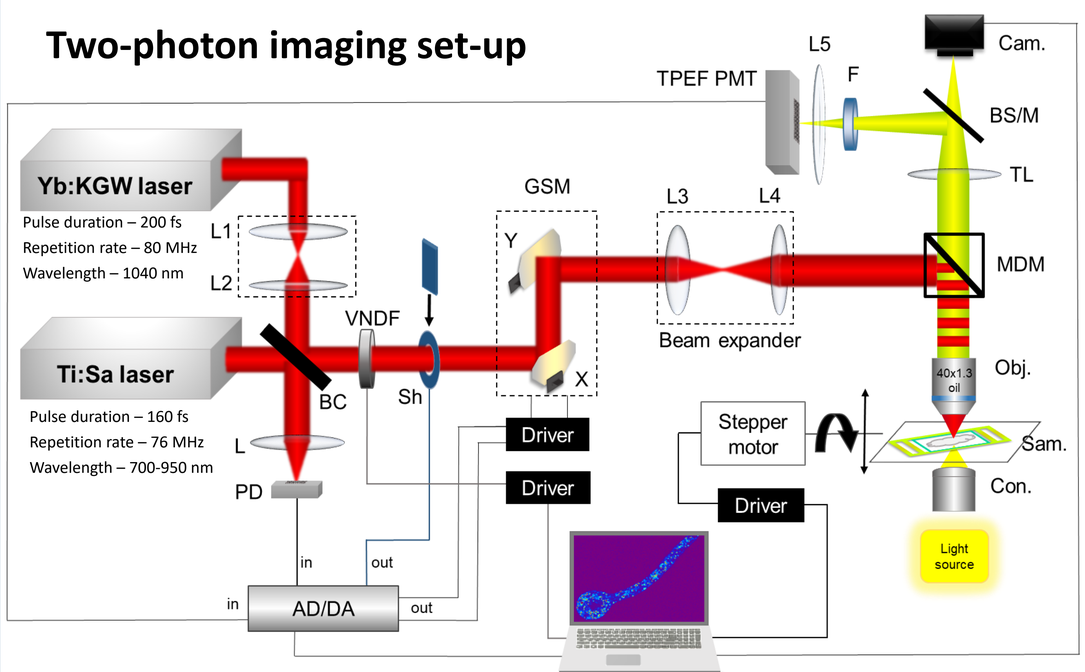
Introduction

Mitochondrial function, and consequently cellular metabolic status and fitness of a cell, is tightly linked to the dynamic changes of mitochondrial morphology, including mitochondrial fusion, fission and mitophagy [1]. Lipid droplets (LDs) can be in close contact with mitochondria, and accumulate autophagy or mitophagy generated material during the reparatory processes [2]. The effect of increased ambient temperature on mitochondrial morphology and LDs density in living cells of the filamentous fungus *Phycomyces blakesleeanus* was investigated.

For ***in vivo*** imaging of mitochondria and lipid droplets **two-photon excitation fluorescence (TPEF)** microscopy was used.

Two-photon imaging set-up



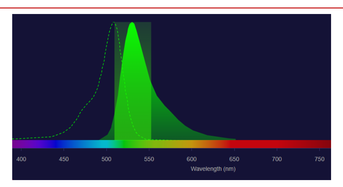
Schematic drawing of NLSM setup. , L1 and L2 – lenses of 1:1 beam expander for recollimation, BC – beam combiner, VNDF – motorized variable neutral density filter, Sh – shutter, GSM - galvanometer-scanning mirrors, L3 and L4 - lenses of 1:3.75 beam expander, MDM - main dichroic mirror (cut-off 700 nm), Con.- aspheric condenser lens, TL - tube lens, BS/M - beam splitter or mirror toggle, F - VIS filter 400–700 nm + bandpass interference filter 530/43 (for Rh123) or 570LP (NR), L5 - focusing lens, TPEF PMT - photomultiplier tube for TPEF signal, L – lens, PD – photodiode, AD/DA - acquisition card.

Advantages:

- *High contrast images*
- *3D imaging in high resolution*
- *Reduced photodamage and photobleaching of the sample using IR ultrafast pulsed lasers*
  → *possibility of extended in vivo imaging*

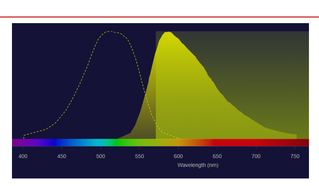
Vital dyes

Two-photon exc. (800 nm) of Rhodamine 123



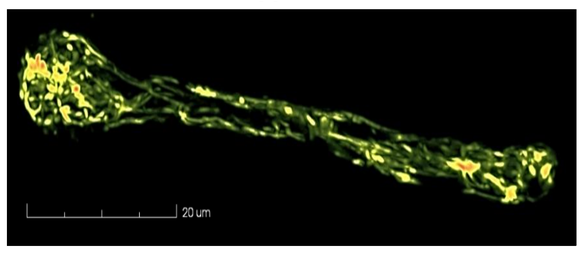
Rhodamine 123 stains active mitochondria in living cells. Dye entry depends on the mitochondrial membrane potential.

Two-photon exc. (1040 nm) of Nile Red dye

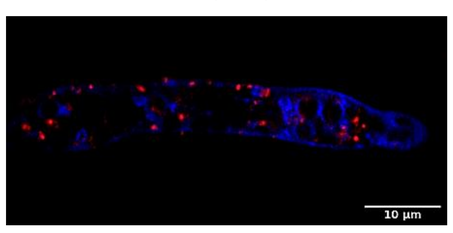


Nile Red stains cellular lipid droplets

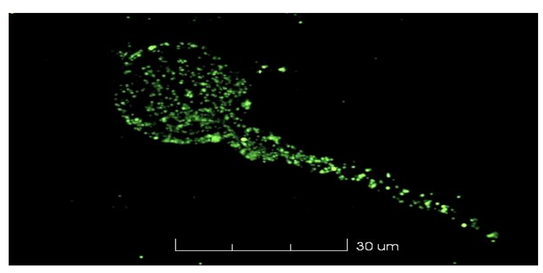
TPEF imaging of *Phycomyces blakesleeanus* live hyphae



3D model (21 slices/images, 0.9 um apart along the z axis) of **tubular mitochondria** (22ºC) in live hypha

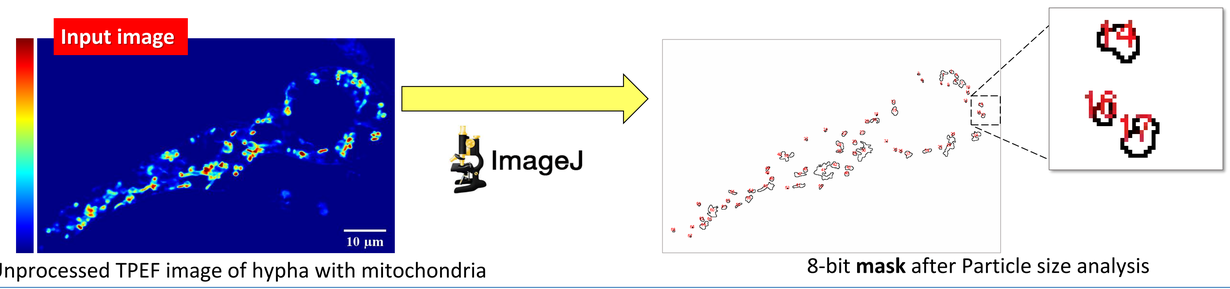


Merged 2D image of **Rh123** (blue signal) and **NR** (red signal) in same hypha



3D model (15 slices/images, 0.9 um apart along the z axis) of **lipid droplets** (25ºC) in live hypha

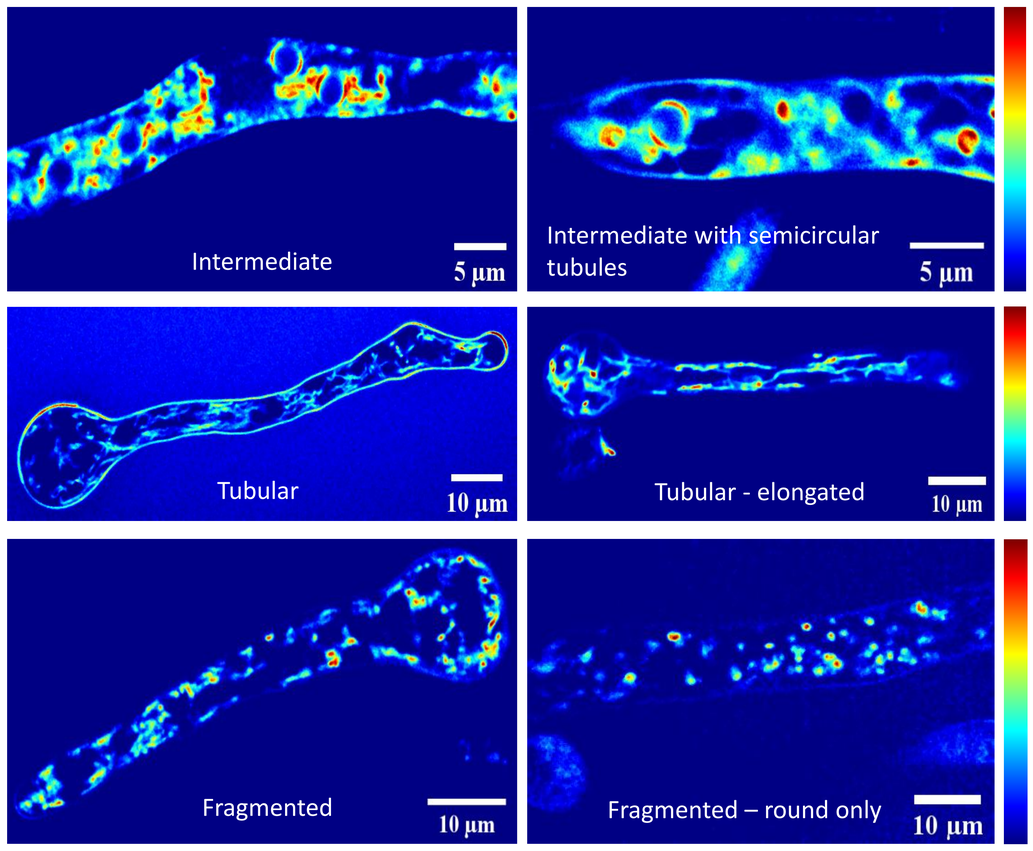
TPEF images analysis for surface area calculation by *Particle Size Analysys* method



Unprocessed TPEF image of hypha with mitochondria

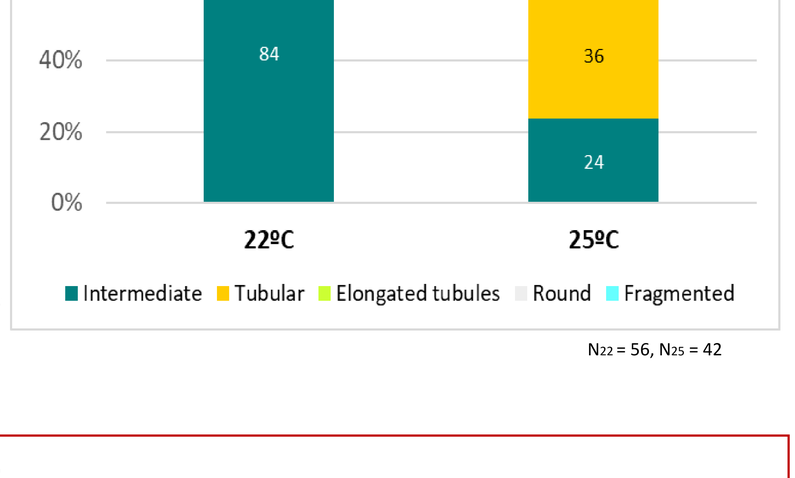
8-bit **mask** after Particle size analysis

TPEF images of mitochondrial morphology in *Phycomyces blakesleeanus* hyphae



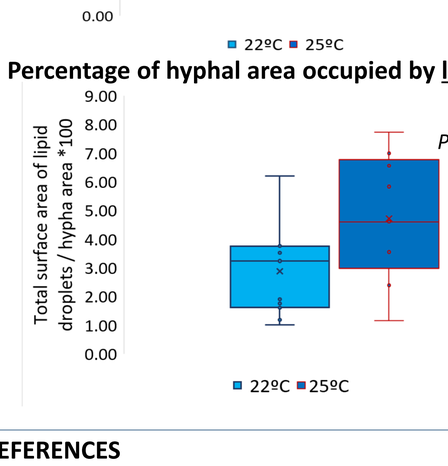
All images were stained with 5 µM Rh123. Color intensity bar for the TPEF signal: dark blue – lowest TPEF signal, dark red – highest TPEF signal. The average laser power in the sample plane - 4-5 mW at 800 nm.

The effect of increased temperature on the abundance of mitochondrial morphology types defined per individual cell

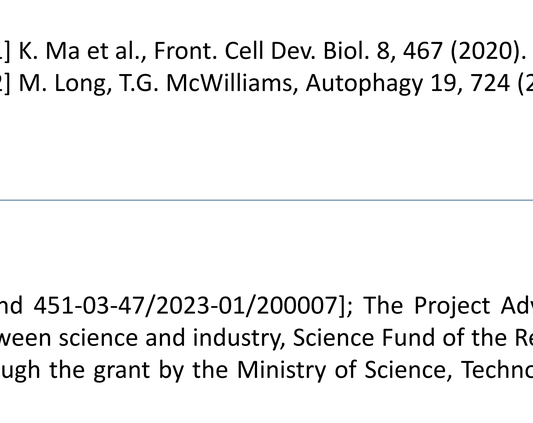


$N_{22} = 56$, $N_{25} = 42$

Percentage of hyphal area occupied by mitochondria



Percentage of hyphal area occupied by lipid droplets



Conclusions

Changes in mitochondrial morphology were induced by a small temperature change.

- An increase of 3°C had a dramatic effect on mitochondrial morphology, inducing the appearance of a predominantly tubular morphology.
- The total area percentage of mitochondria showed an increasing trend when grown at 25°C.
- Increasing the ambient temperature to 25°C induced a statistically significant increase in the percentage of hyphal area occupied by LDs from 2.9 ± 1.6 to 4.7 ± 2.2.

The observed response to the small temperature increase points to the physiological adaptation of hyphal metabolism.

REFERENCES

[1] K. Ma et al., Front. Cell Dev. Biol. 8, 467 (2020).
[2] M. Long, T.G. McWilliams, Autophagy 19, 724 (2023).

Acknowledgment

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