Evaluation of cardiomyocytes mitochondrial membrane potential (ΔΨm) and mitochondrial fragmentation after low level laser irradiation.

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Introduction and objectives

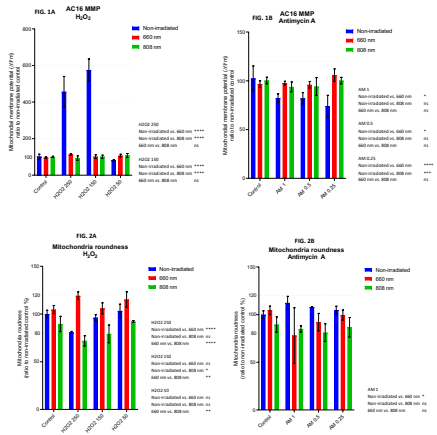
Introduction: Photobiomodulation (PBM) performed with lasers and LEDs, seems to have a wide range of applications in medicine. Despite all the studies performed in this field, the exact mechanism of action on human body is still unknown. Therefore, experimental studies are still necessary in this field. **Objectives:** To evaluate the effect of PBM over cardiac cells under mitochondrial stress conditions.

Metodology

Human adult ventricular cardiomyocytes cells AC16 were treated with 250, 150 or 50 µM of H2O2 for 2 hours or 1, 0.5 or 0.25 µM of antimycin A for 24h. Cells were irradiated with diode laser with the following parameters λ= 660 nm and λ=808 nm for 29 seconds, output power of 69mW, 2J, 50J/cm2 and fluence rate 1.7W/cm2 and incubated for 24h. Cells were stained using different methods for mitochondrial evaluation. Mitochondrial membrane potential (MMP) was evaluated in live cells by fluorescent microscopy. Mitochondrial ΔΨ was measured only in stained regions. MMP uncoupler was used as control. Fragmentation was evaluated by mitochondrial's number and roundness. Images were analyzed and data are reported as ratio to non-irradiated group.

Results and conclusion

AC16 cells greatly increase MMP after 48 hours when exposed to H2O2 at 250 µm (456.5±83.5 p<0.0001) and 150 µm (575.1±60.6 p<0.0001) compared to control (102.5±12.7). Cells subjected to H2O2 followed by irradiation displayed MMP levels similar to the non-irradiated group (660 nm: 124.5±3.9; 808 nm: 110.2±14.4). MMP was reduced in cells treated with 1, 0.5 and 0.25 µM antimycin A (-20.23±4.35; -20.37±5.7; -28.40±10.9, p<0.01) and irradiation maintained MMP levels equivalent to control. In addition, we identified changes in the number of mitochondria and their roundness when cells were treated with 250 µM of H2O2 or 1 µM of antimycin A and irradiated. **Conclusions:** Preliminary data indicate that PBM exhibit a protective effect in cardiac cells under mitochondrial stress.



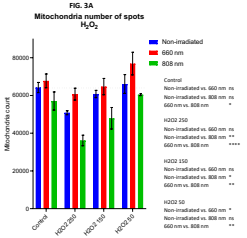


Fig 1: A) MMP H2O2 at 250 µm (p<0.0001) and 150 µm (p<0001) B)compared to control .B) MMP in cells treated with 1, 0.5 and 0.25 µM antimycin A (p<0.01) . Fig 2A Mit. Roundness cells with 250 µM of H2O2 or 2B) Cells- 1 µM of antimycin A and irradiated FIG 3) number of mitochondria-spots- treated with 250 µM of H2O2 or B) 1 µM of antimycin A (p<0.01).

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FIG. 1A

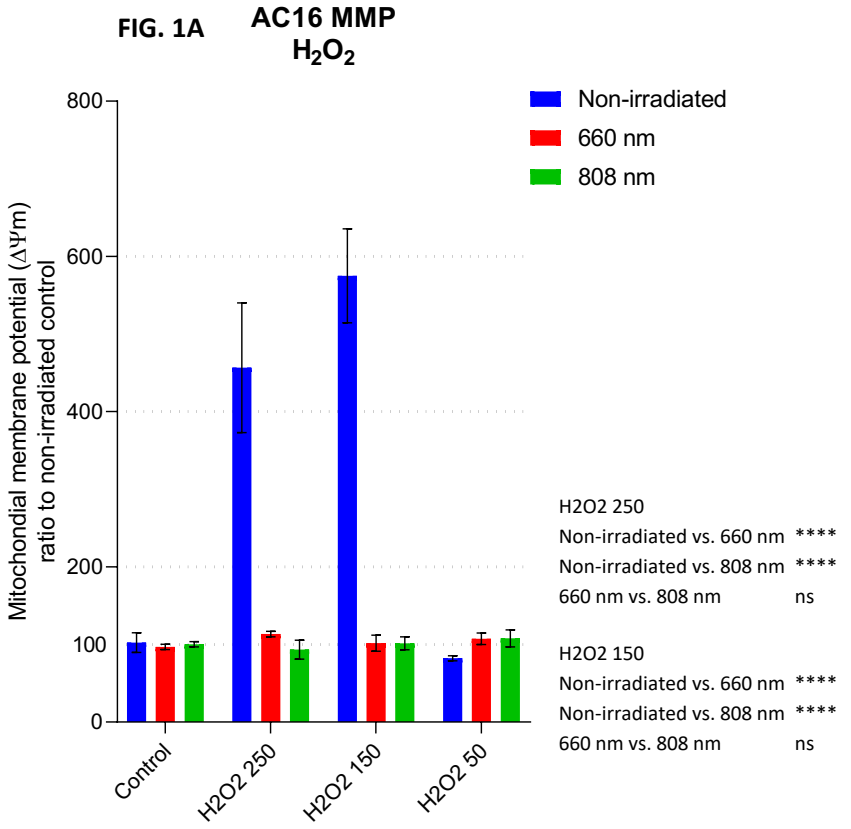
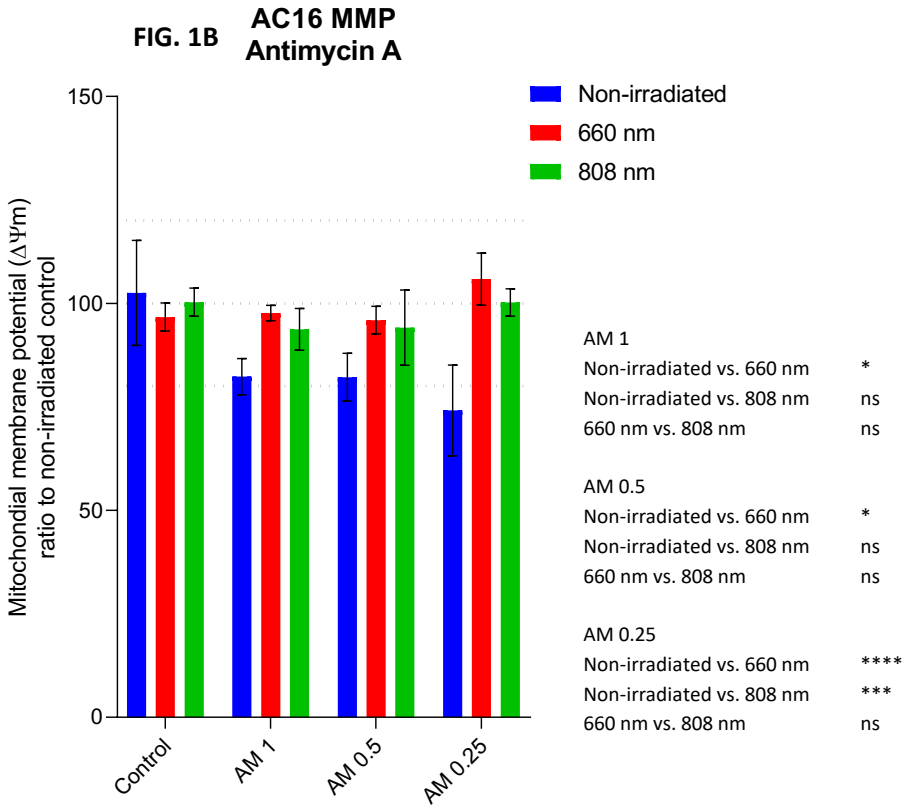
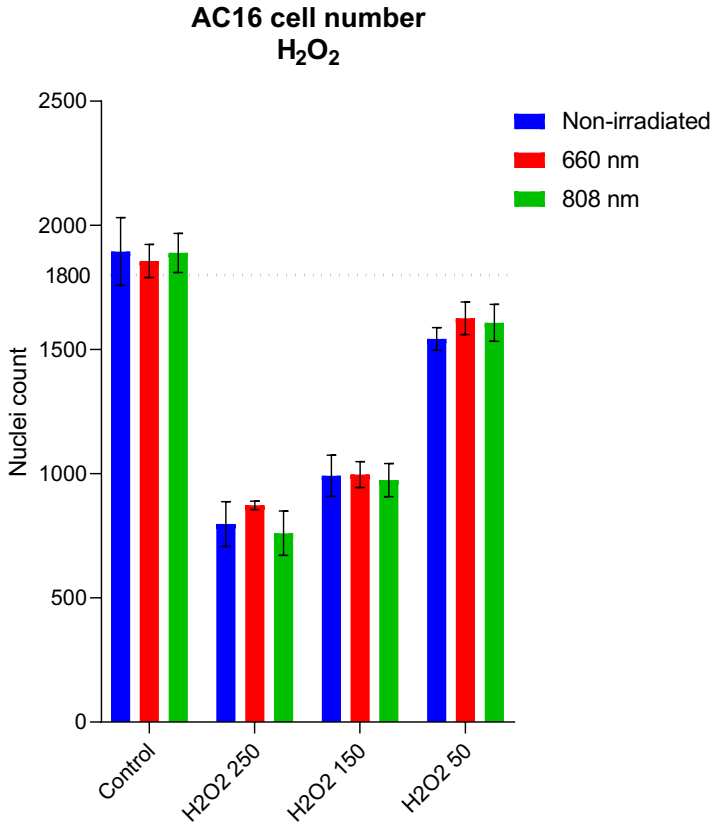

AC16 MMP
H2O2
Non-irradiated
660 nm
808 nm
Mitochondial membrane potential (ΔΨm)
ratio to non-irradiated control
800
600
400
200
100
0
Control
H2O2 250
H2O2 150
H2O2 50
H2O2 250
Non-irradiated vs. 660 nm ****
Non-irradiated vs. 808 nm ****
660 nm vs. 808 nm ns
H2O2 150
Non-irradiated vs. 660 nm ****
Non-irradiated vs. 808 nm ****
660 nm vs. 808 nm ns

FIG. 1B


AC16 MMP
Antimycin A
Non-irradiated
660 nm
808 nm
Mitochondial membrane potential (ΔΨm)
ratio to non-irradiated control
150
100
50
0
Control
AM 1
AM 0.5
AM 0.25
AM 1
Non-irradiated vs. 660 nm *
Non-irradiated vs. 808 nm ns
660 nm vs. 808 nm ns
AM 0.5
Non-irradiated vs. 660 nm *
Non-irradiated vs. 808 nm ns
660 nm vs. 808 nm ns
AM 0.25
Non-irradiated vs. 660 nm ****
Non-irradiated vs. 808 nm ***
660 nm vs. 808 nm ns


AC16 cell number
H2O2
Non-irradiated
660 nm
808 nm
Nuclei count
2500
2000
1800
1500
1000
500
0
Control
H2O2 250
H2O2 150
H2O2 50

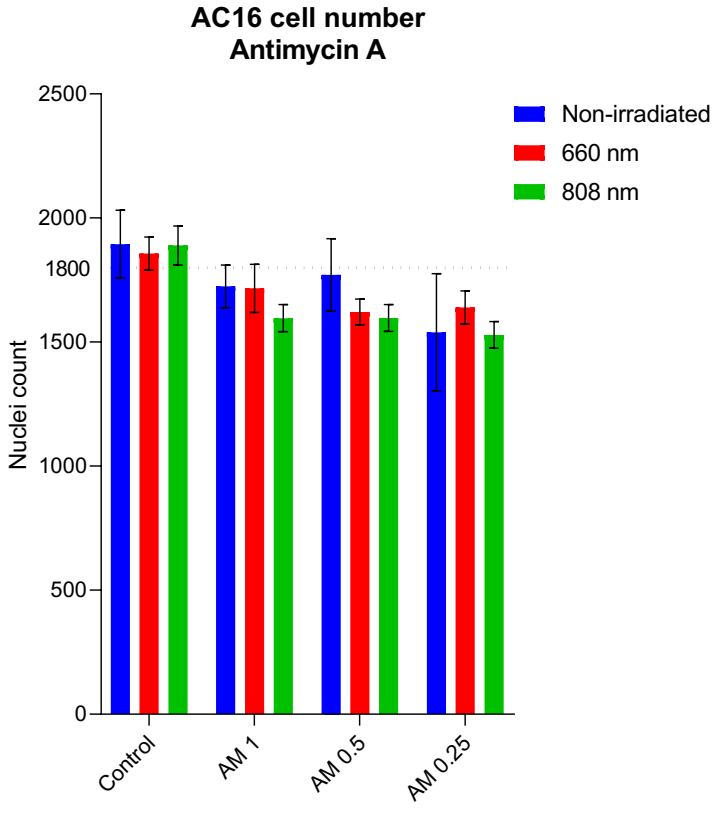
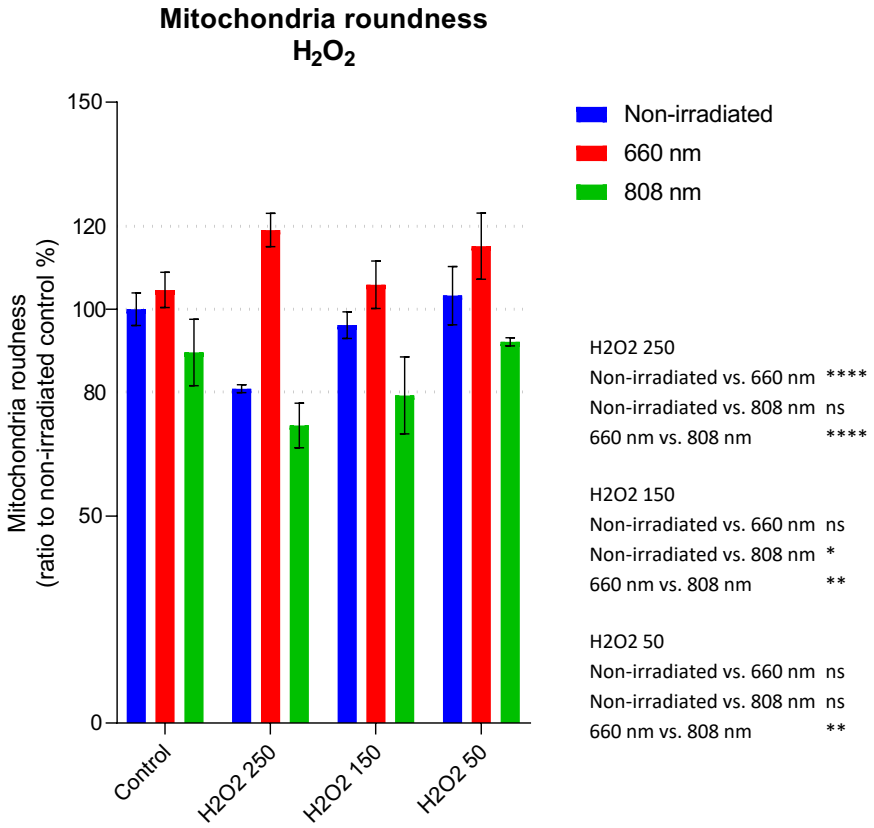

AC16 cell number
Antimycin A
Non-irradiated
660 nm
808 nm
Nuclei count
2500
2000
1800
1500
1000
500
0
Control
AM 1
AM 0.5
AM 0.25

FIG. 2A

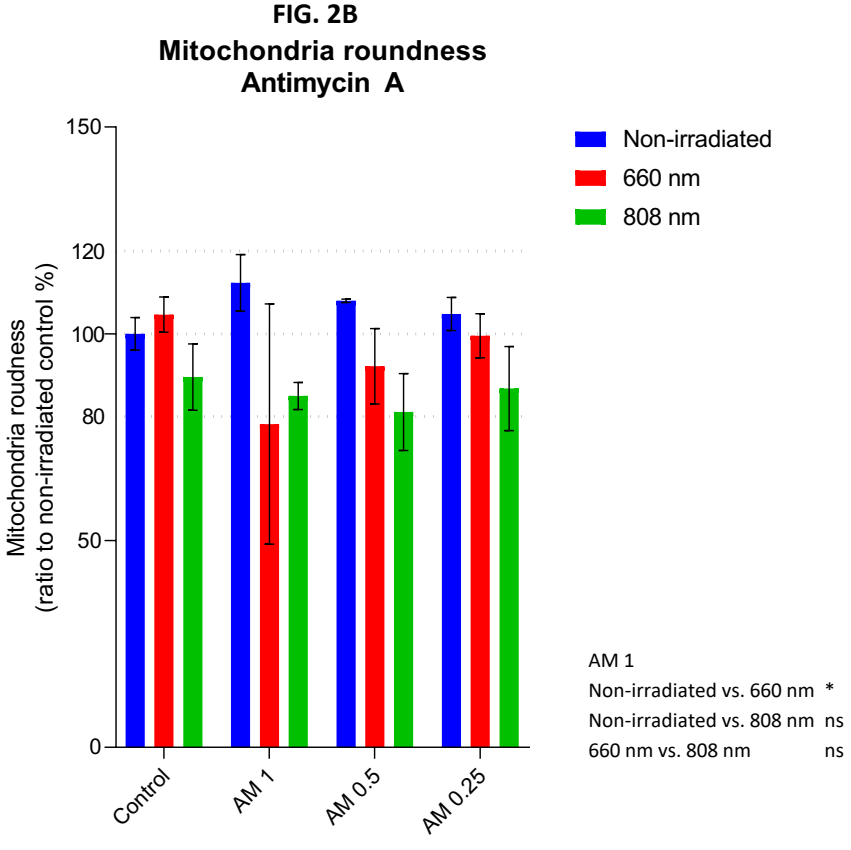


H2O2 250
Non-irradiated vs. 660 nm ****
Non-irradiated vs. 808 nm ns
660 nm vs. 808 nm ****

H2O2 150
Non-irradiated vs. 660 nm ns
Non-irradiated vs. 808 nm *
660 nm vs. 808 nm **

H2O2 50
Non-irradiated vs. 660 nm ns
Non-irradiated vs. 808 nm ns
660 nm vs. 808 nm **

FIG. 2B

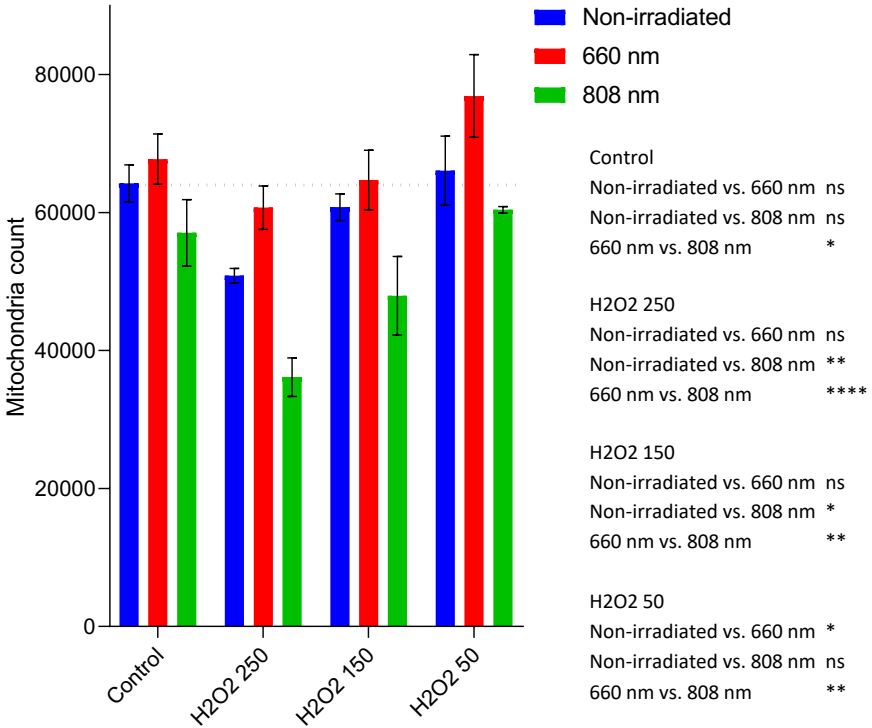


AM 1
Non-irradiated vs. 660 nm *
Non-irradiated vs. 808 nm ns
660 nm vs. 808 nm ns

FIG. 3A

Mitochondria number of spots

H_2O_2



Control
Non-irradiated vs. 660 nm ns
Non-irradiated vs. 808 nm ns
660 nm vs. 808 nm *

H2O2 250
Non-irradiated vs. 660 nm ns
Non-irradiated vs. 808 nm **
660 nm vs. 808 nm ****

H2O2 150
Non-irradiated vs. 660 nm ns
Non-irradiated vs. 808 nm *
660 nm vs. 808 nm **

H2O2 50
Non-irradiated vs. 660 nm *
Non-irradiated vs. 808 nm ns
660 nm vs. 808 nm **

FIG. 3A

Mitochondria number of spots

Antimycin A

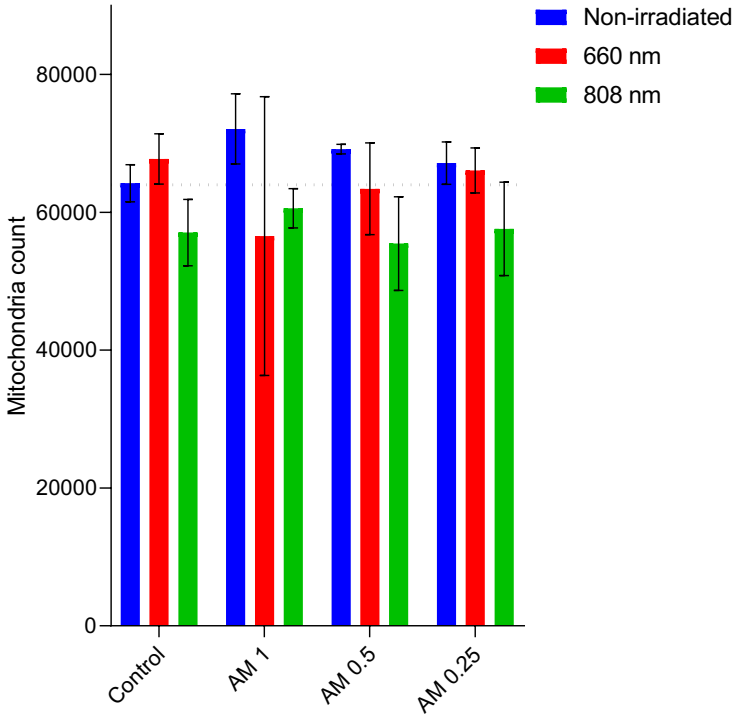
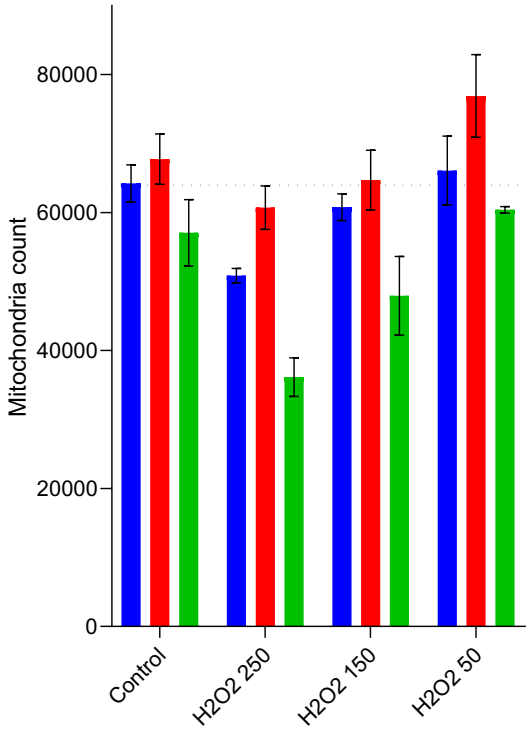


FIG. 3A

Mitochondria number of spots

H_2O_2



Control
Non-irradiated vs. 660 nm ns
Non-irradiated vs. 808 nm ns
660 nm vs. 808 nm *

H2O2 250
Non-irradiated vs. 660 nm ns
Non-irradiated vs. 808 nm **
660 nm vs. 808 nm ****

H2O2 150
Non-irradiated vs. 660 nm ns
Non-irradiated vs. 808 nm *
660 nm vs. 808 nm **

H2O2 50
Non-irradiated vs. 660 nm *
Non-irradiated vs. 808 nm ns
660 nm vs. 808 nm **

Fig.1 MMP

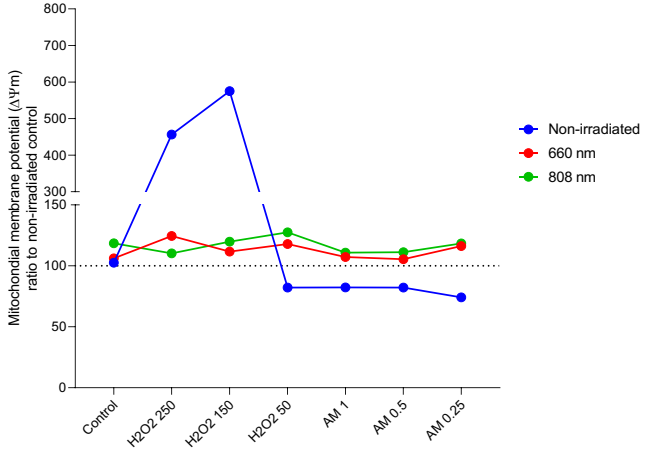


Fig.2 Cell number

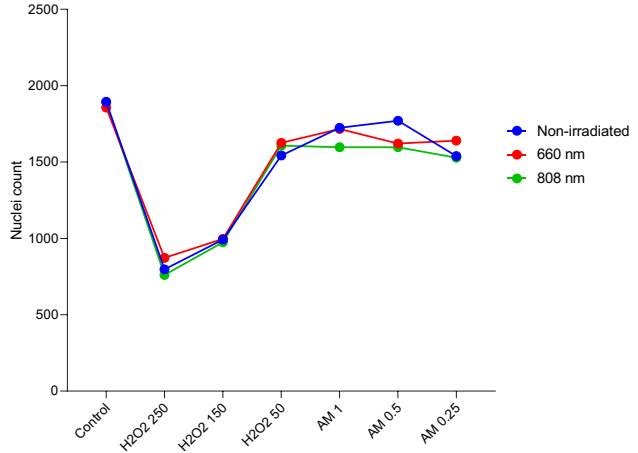


Fig.3 Mitochondria roundness

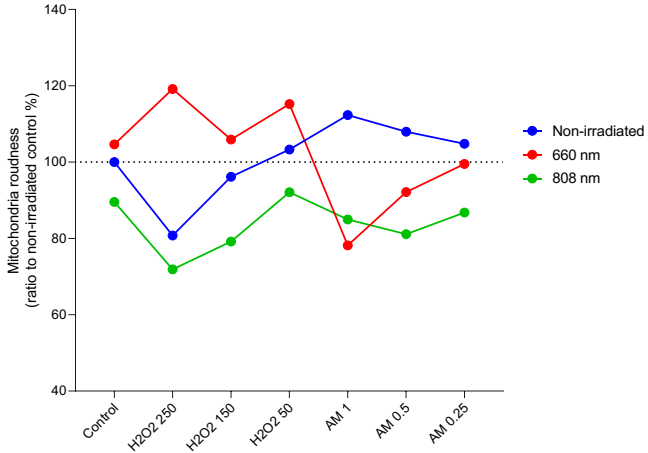


Fig.4 Mitochondria number of spots