Long term mitochondrial live imaging without phototoxicity using RCM system

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Introduction

The main challenge for a biologist engaged in live cell imaging is phototoxicity. In order to avoid it, the total dose of illumination light on the sample should be minimized. In general, this is accomplished by reducing laser power or the exposure time per image, but it may result in a lower signal to noise ratio. Other methods are decreasing the number of pixels per frame, the number of frames in a z-stack or the number of images per unit of time, but they implicate the loss of spatial and temporal resolution.

Mitochondria are organelles that play a vital role in energy production and metabolism. Mitochondrial dysfunction is associated with an increasingly large number of human disorders[1] and live visualization of their dynamics and morphological changes is crucial. However, it can be challenging, due to their small size and their sensitivity to phototoxicity[2].

We performed long-term live imaging of the mitochondrial matrix using Re-Scan Confocal Microscopy (RCM)[3] and showed high-frequency live-cell imaging of mitochondria with high resolution and with no signs of phototoxicity.

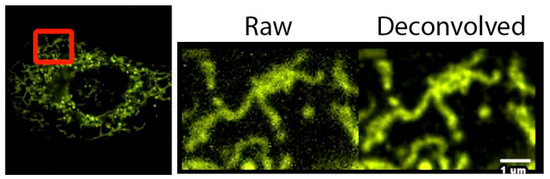


Figure 2. High resolution RCM images allowed visualization of individual mitochondria. Left panel shows one cell with a red square where images on the right locate.

Materials & Methods

Labeling of Mitochondria in HO1N1 cells with Mitochondria RFP through the Bacmam 2.0 expression system (Invitrogen™ C10601) was done according to the manufacturer's protocol. Cells were grown and imaged in a glass-bottom 8-well ibidi chamber.

Time-lapse experiments were performed with RCM1, equipped in a Nikon Ti2 microscope, Hamamatsu Orca Flash 4.0 V3 camera, Omicron 561nm 20mW laser and an Okolab incubator.

Images of 2048 x 2048 pixels were acquired every 10 sec during 44 hours (15840 frames in total) with CFI Plan Fluor 40x oil 1.3 NA objective. The laser power at the sample plane was 0.01 mW. Stage axial drift was corrected with PFS (Perfect Focus System).

Image processing was done in ImageJ. For ensemble analysis, all raw images were background substracted by batch processing, using the function image calculator. Average intensity per image was determined and normalized to the maximum intensity of the stack.

Deconvolution was performed with Microvolution software and the calculated PSF for RCM.

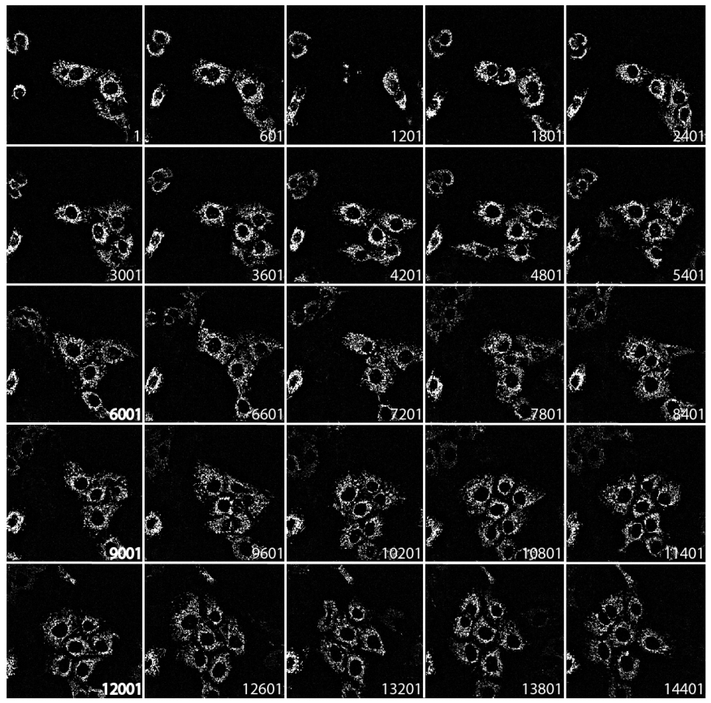


Figure 1. Compilation of time points observed during the 44 hours imaging experiment.
This compilation shows every 600th slice from the movie, appropriate to visualize macro events occurring during the 44h timeframe.

"RCM long term live-cell imaging keep cells happy & dividing, with no signs of damage"

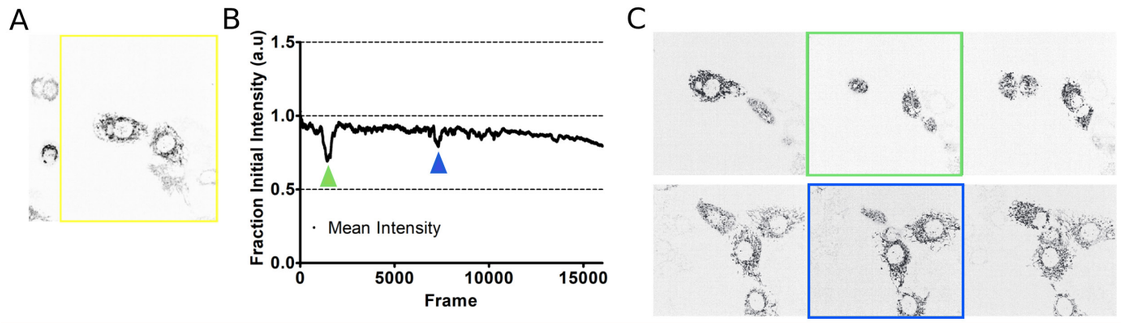


Figure 3. Analysis of bleaching during live cell imaging with RCM.
A. Yellow square represents the ROI selected for further analysis of fluorescence intensity. B. Graph representing the total average fluorescence intensity within the ROI across all the frames recorded. C. Frames showing the time points with cell divisions that correspond to the low-intensity peaks observed in B. Green and blue rectangles correspond with the negative peaks marked in the B with the arrowheads in the same color.

Results

Long term high resolution imaging with the RCM system did not lead to phototoxicity in HO1N1 cultured cells.

During the 44 hours of imaging experiment, cells in the field of view divided and moved in/out of the view and cell death events were not observed (Fig. 1A). All through the movie, we were able to spot cells dividing, indicating that RCM long term live-cell imaging did not lead to cell damage. In addition, mitochondrial dynamics and morphology did not show any apparent alteration due to phototoxicity.

Despite the small size of mitochondria (250-500nm), RCM images allowed to visualize individual mitochondria due to its high resolution and contrast (Fig. 2).

Minimal levels of bleaching after 44 hours of live cell imaging with RCM

In order to evaluate bleaching of the fluorophores during the experiment we selected a ROI in the image to exclude cells moving in/out of the field of view (Fig. 3A). The total average intensity within the selected ROI was measured across all frames (Fig. 3B)

As the cells divide, we analysed the change of the total average intensity per frame. We observed a total loss of less than 20% in fluorescence intensity after 44 hours of live cell imaging with RCM (Fig. 3B) indicating that the bleaching effect was very mild (note that the dye was diluted between daughter cells in each division).

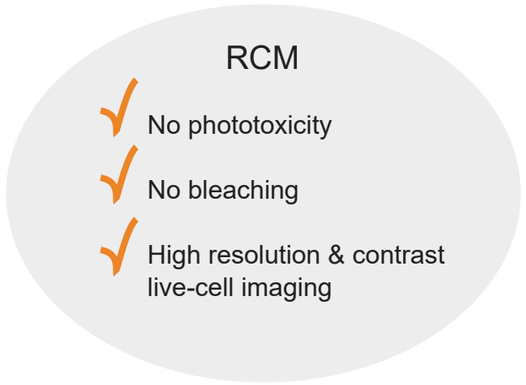
In the average intensity curve, sudden dips can be observed due to cells moving out of the focus prior to cell division (Fig. 3C).

Conclusions

The total average fluorescence intensity did not decline and cells kept dividing during the imaging experiment, demonstrating that RCM imaging conditions were gentle enough to not cause bleaching or phototoxicity.

The high sensitivity of the RCM system allowed to visualize the mitochondria with a high signal-to-noise ratio, even after 44h and several cell divisions. Moreover, RCM images had a high resolution which facilitated mitochondria visualization.

Overall, these data position RCM as an optimal confocal system to perform high resolution live cell imaging without photobleaching and phototoxicity facilitating the study of long term cell dynamics.



References

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[2] Mitra K, Lippincott-Schwartz J. Analysis of mitochondrial dynamics and functions using imaging approaches. Curr Protoc Cell Biol. 2010; DOI 10.1002/0471143030.cb0425s46.

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