Mini Review

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Insights in Cell Science – Towards a Quantitative Approach of Fluorescence Microscopy that Unravels Cellular Function

Ben Loos

Department of Physiological Sciences, Stellenbosch University, South Africa

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Corresponding author: Ben Loos

bloos@sun.ac.za

Distinguished University Professor, Department of Physiological Sciences, Stellenbosch University, South Africa.

Tel: +27218089196
Fax: +27218083145

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It is undoubtedly so, the time for fluorescence microscopy in cellular sciences has come, allowing the unraveling of the 'inside' of cells and their molecular itinererary in a novel and most precise manner. This is supported by the in 2014 awarded Noble price in chemistry, received for the development of the super-resolution fluorescence microscopy techniques photo-activation localization microscopy (PALM), stochastic optical reconstruction microscopy (STORM) and stimulated emission depletion (STED). These techniques provide for the first time a platform for sub-diffraction molecular imaging complementing the growing arsenal of fluorescence based techniques such as live cell imaging, photo-activation, fluorescence resonance energy transfer (FRET) or fluorescence recovery after photo-bleaching (FRAP). The challenge however remains, not to be drowning in a sea of image-data, that may be building up to terabytes of data, but to be able to extract meaningful and statistically sound data points, that are suitable to inform on cellular function and dysfunction [1]. More so, it is envisaged to expand our fluorescent toolbox in a manner that not only allows the utilization of fluorescence based image data in a most powerful manner, but also that may contribute towards enabling predictive and preventative medicine [2]. Here we comment on some of the recently developed and employed applications and trends, that enable to powerfully provide "Insights in cell science", both visually and quantitatively. In doing so, it is hoped to offer a perspective on current applications, trends and challenges that define our present research landscape of cellular imaging. This may assist in better and more carefully designed experimental settings that are most informative and well aligned with the powers of fluorescence microscopy technology.

Cellular decision making centers around maintaining homeostasis and cell viability. When the cell is unable to meet its metabolic demands, or is exposed to some form of cellular injury, cell death will be induced [3]. Although it is highly important to assess the overall contribution of cell death modalities, life cell imaging in combination with FRET-based caspase-sensors [4] allow for the quantification of the exact onset of cell death induction **(Figure 1A)** [5]. This enables to inform dynamically about cellular decision making, to trace the fate of individual cells and to assess the time span particular cells were able to maintain viability. Such an approach, based on long series of time lapses may reveal a more accurate understanding of cellular pathology and cell disease pathogenesis [6] compared to a static approach that merely quantifies parameters at few selected time points.

Integral part in this context is the availability of ATP, and its presence in regions of ATP consuming processes. The tubulin network **(Figure 1B)** and ATP dependent molecular motors such as kinesin and dynein [7] ensure the transport of mitochondria and autophagosomes to regions where either ATP is required (such as synaptic processes) or where fusion processes with other organelles (such as lysosomes in the perinuclear region) take place [8]. In the past, it has been a major challenge to visualize ATP, and usually luciferase-based methods have been employed to quantify overall ATP availability [9]. However, recently, the development of sensitive sensors, that are based on fluorescence resonance energy transfer (FRET) have been developed [10] and successfully employed to visualize and localize intracellular ATP hotspots **(Figure 2a).** Such an approach may not only allow to assess where intracellular ATP demand is highest or lowest and how such localization may change in disease or intervention, but it may also, through live cell imaging, enable the quantification of the duration a cell is able to maintain favorable ATP availability. This enhances greatly our understanding of metabolic processes and cellular decision making during metabolic adaptations or perturbations and cellular injury, such as in cancer or

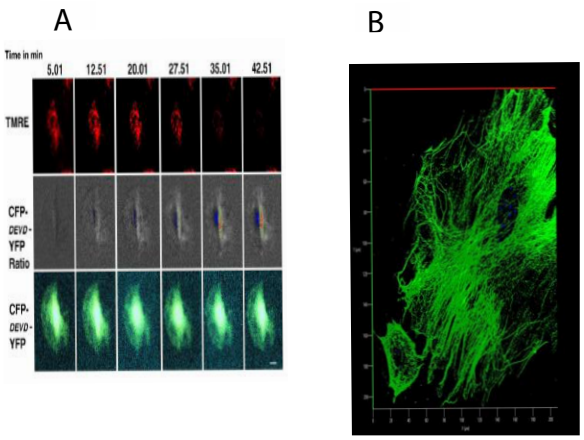

Figure 1 Apoptosis onset (1A) and the microtubule network (1B). Cells were transfected with a CFP-DEVD-YFP construct and loaded with tetramethylrhodamineethylester (TMRE) to monitor the onset of caspase-3 activation and mitochondrial depolarization. B: Acetylated tubulin allows for the assessment of tubulin network structural integrity. Image was acquired from z-planes and projected in maximum intensity. (From Loos et al., 2011).

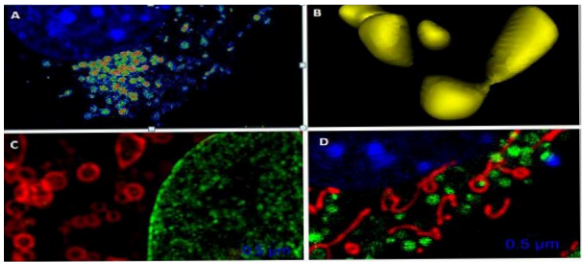

Figure 2 GT 1-7 neuronal cells indicating ATP (A) targeted to the mitochondria, using a FRET construct. Super-resolution structured illumination microscopy (SR-SIM) enables the visualization of labeled organelles down to 80 nm spatial x-y resolution. Fluorescent micrographs indicate lysosomes labeled for the lysosomal associated membrane protein-1 (B), mitochondria (C) and the interaction between GFP-LC3 labeled autophagosomes and mitochondria (D).

neurodegeneration. The precise labeling of selective proteins through either a transfection- or immuno-fluorescence based approach enables to visualize specific organelles of interest. The employment of super-resolution techniques, such as super-resolution structured illumination microscopy (SR-SIM) allows to do implement such approach at enhanced resolving power, in a three-dimensional space. This in turn enables to not only visualize organelles of interest, but, through optical z-stacking, to acquire complete cellular organelle pool sizes with their subsequent quantification. This is powerful, since, although higher resolution may be achieved through transmission electron microscopy (TEM), complete cell volume and organelle pool size quantification is enabled using a subdiffraction limit resolving power. In that manner, for example lysosomes **(Figure 2B)** can be visualized and their distribution expressed as complete lysosomal pool size *nA* [11]. SR-SIM also enables to assess the mitochondrial network at much greater detail **(Figure 2C),** allowing its quantification more precisely than using confocal-based images, by employing form factor analyses or aspect ratio approaches [12]. Together with techniques such as photoactivation-based mitochondrial fission and fusion dynamics [13], network properties can be assessed in their dynamic nature, which is more sensitive and by far more rapid in response compared to a change in protein expression levels. Here, fluorescence microscopy techniques compile a powerful tool box to understand mitochondria at their nexus in cell death and survival. If combined with pool size analyses of associated organelles (such as autophagosomes, **(Figure 2D)**, the dynamic behavior of interactions such as mitophagy or protein degradation through macroautophagy can be better understood and quantitatively described.

Advances in organic chemistry and molecular biology as well as material sciences have enabled the research community to choose from a wide range of classes of fluorescent probes for imaging in cell biology. Here, a combination of inherently binding fluorochromes, fluorescent proteins and an immuno-fluorescence approach allows the concomitant assessment of a number of parameters of interest **(Figure 3).** Such multi-colour imaging in turn enables the assessment of organelle and/or protein-protein interactions through colocalization analysis in a powerful manner, reporting on cell function and dysfunction. Multi-parameter imaging enables a better understanding of the relationship between the parts of interest in the cellular system. If performed in combination with super-resolution techniques, such intermolecular relationships can report on function and localization in a most precise manner [14].

The large amount of data that is being generated through

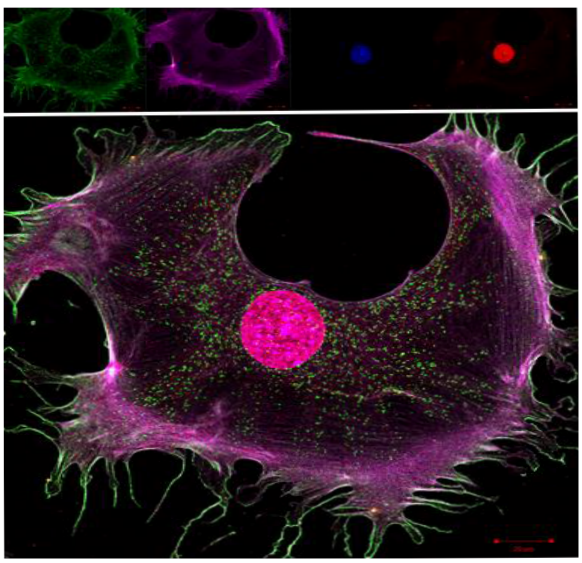

Figure 3 A combination of targeting methods and fluorescent probes. Mouse embryonic fibroblasts transfected with GFP-LC3 (green). After fixation cells were immune-labeled for mitochondrial DNA with a mouse monoclonal antibody (red) and counterstained using Alexa fluor 647 phalloidin (magenta) and Hoechst 33342 (blue). Images were acquired from z-planes and projected in maximum intensity. Scale bars: 20 µm.

fluorescence microscopy based techniques demands an integrative approach, in order to interpret distribution and intensities of fluorescent signal in a meaningful manner. This will become of even greater importance as we venture into PALM-3D and super-resolution time lapse acquisition techniques. Here, systems biology and computational modeling may become an even more crucial part in the future to integrate the vast amount of biological information, as quantification of molecular elements of a biological system is at the heart of this discipline [2]. This becomes particularly clear in current trends using fluorescence-based image data at a multi-scale level [15], i.e. from molecular density mapping based on PALM [16] to *in vivo* whole organ imaging based on light sheet microscopy [17]. With no doubt such an approach will enable us to map proteins in cells and tissues spatiotemporally like never before, building dynamic and most comprehensive organ systems based on real fluorescence microscopy derived data. When doing so, and when doing so quantitatively, we are likely one step closer towards enabling predictive or preventative medicine. Taken together, we can look forward to an exciting future, where insights in cell science will matter greatly and where the use of fluorescence microscopy techniques will play a fundamental and integral part.

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References

1 Giepmans BN, Adams SR, Ellisman MH, Tsien RY (2006) The fluorescent toolbox for assessing protein location and function. Science 312(5771): 217-224.

2 Hood L, Heath JR, Phelps ME, Lin B (2004) Systems biology and new technologies enable predictive and preventative medicine. Science 306(5696): 640-643.

3 Loos B, Lockshin R, Klionsky DJ, Engelbrecht AM and Zakheri Z (2013) On the variability of autophagy and cell death susceptibility. Autophagy 9(9): 1270-1285.

4 Tyas L, Brophy VA, Pope A, Rivett AJ and Tavaré M (2000) Rapid caspase-3 activation during apoptosis revealed using fluorescence-resonance energy transfer, EMBO Rep 1: 266-270.

5 Loos B, Genade S, Ellis B, Lochner A, Engelbrecht AM (2011) At the core of survival: Autophagy delays the onset of both apoptotic and necrotic cell death in a model of ischemic cell injury. Exp Cell Res 317 (10): 1437-53.

6 Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S (2004) Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. Nature 431(7010): 805-10.

7 Monastyrska I, Rieter E, Klionsky DJ (2009) Multiple roles of the cytoskeleton in autophagy. Biol Rev Camb Philos Soc 84: 431-448.

8 Rubinsztein DC, Ravikumar B, Acevedo-Arozena A (2005) Dyneins, autophagy, aggregation and neurodegeneration. Autophagy 1(3): 177-178.

9 Essmann F, Bantel H, Totzke G, Engels IH, Sinha B, Schulze-Osthoff K (2003) Staphylococcus aureus alpha-toxin-induced cell death: predominant necrosis despite apoptotic caspase activation, Cell Death Differ 10: 1260-1272.

10 Imamura H, Nhat KP, Togawa H, Saito K, Iino R, Kato-Yamada Y, Nagai T, Noji H (2009) Visualization of ATP levels inside single living cells with fluorescence resonance energy transfer-based genetically encoded indicators. Proc Natl Acad Sci U S A 106(37): 15651-15656.

11 Loos B, Du Toit A and Hofmeyr JH (2014) Defining and measuring autophagosome flux-concept and reality. Autophagy 10(11): 2087-2096.

12 Mortiboys H, Kelly Jean T, Koopman WJH, Klaffke S, Abou-Sleiman P, et al. (2008) Mitochondrial Function and Morphology Are Impaired in parkin -Mutant Fibroblasts. Annals of Neurology 64(5): 555-65.

13 Karbowski M, Arnoult D, Chen H, Chan DC, Smith CL, et al. (2004) Quantitation of Mitochondrial Dynamics by Photolabeling of Individual Organelles Shows That Mitochondrial Fusion Is Blocked during the Bax Activation Phase of Apoptosis'. The Journal of Cell Biology 164(4): 493-99.

14 Jovanovic K, Loos B, Da Costa B, Penny C, Weiss S (2014) High resolution imaging study of interactions between the 37kDA/67kDa Laminin receptor and APP, beta-secretase and gamma-secretase in Alzheimer's disease. PLoS ONE 9(6): e100373.

15 Martone ME, Gupta A, Ellisman MH (2004) e-Neuroscience: challenges and triumphs in integrating distributed data from molecules to brains. Nature Neuroscience 7: 467-472.

16 Betzig E, Patterson GH, Sougrat R, Lindwasser OW, Olenych S, et al. (2006) Imaging intracellular fluorescent proteins at nanometer resolution. Science 313(5793): 1642-5.

17 Pampaloni F, Ansari N and Stelzer EHK (2013) High-resolution deep imaging of live cellular spheroids with light-sheet-based fluorescence microscopy. Cell Tissue Res 352: 161-177.

