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Re-scan Confocal Microscopy of ESCRT-mediated lysosome repair

Stefan G. Stanciu[1], Iustin Floroiu[1], Radu Hristu[1], Efstathios Fiorentis[1], Maja Radulovic[2], Camilla Raiborg[2], Harald A. Stenmark[2]

[1] *Center for Microscopy-Microanalysis and Information Processing, Politehnica University of Bucharest, Bucharest, 060042 Romania*
[2] *Department of Molecular Cell Biology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway.*

E-mail: stefan.g.stanciu@upb.ro

Super-resolution microscopy techniques, capable of circumventing the resolution limit imposed by diffraction, are extremely useful for resolving important properties of eucaryotic and procaryotic cells that are not accessible to conventional microscopies[1]. While the resolution advantages of some of these techniques come at the cost of high laser beam power, or very specific contrast agents[2], Re-scan Confocal Microscopy (RCM)[3] has emerged over the past few years as a valuable tool to investigate biological specimens at sub-diffraction resolution using ultra-low beam power, and any type of fluorophores. In addition to a standard Confocal Laser Scanning Microscope (CLSM), where the beam is scanned on the sample by means of a system of mirrors, the RCM incorporates a second system of mirrors whose role is to scan the light emitted by the sample upon laser excitation on the surface of a CCD/s-CMOS image sensor. If the angular amplitude of the camera scanner (re-scanner) is double the angular amplitude of the sample scanner, a resolution advantage of $\sqrt{2}$ over a conventional CLSM is achieved. With additional deconvolution resolutions down to 120nm under 488nm excitation can be achieved. Conventional RCMs are designed for fluorescence imaging, but recent efforts showed that the Re-scan concept can also be exploited for achieving optical super-resolution based on non-linear effects not requiring contrast-agents, such as Second Harmonic Generation[4].

In this work we discuss our results on RCM imaging of the lysosome repair process. Lysosomes are specialized organelles with many important roles, including the degradation of macromolecules, pathogen killing, and cell signaling, but their damage, which can occur due to pathogens, amphiphilic drugs, or other membrane-disrupting agents, impose serious threat to cell viability[5]. Severely damaged lysosomes are removed by lysophagy, an autophagic pathway that sequesters and degrades damaged lysosomes[6]. Lysosomes with milder damage are repaired by the endosomal sorting complex required for transport (ESCRT) machinery[7]. In the current work we place additional attention to this subject, discussing how RCMs resolution advantages are useful to investigate subtle morpho-structural aspects of cells linked to the lysosome repair process.

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1. Sigal, Y. M.; Zhou, R.; Zhuang, X., Visualizing and discovering cellular structures with super-resolution microscopy. *Science* **2018,** *361* (6405) 880-887.
2. Schermelleh, L.; Ferrand, A.; Huser, T.; Eggeling, C.; Sauer, M.; Biehlmaier, O.; Drummen, G. P., Super-resolution microscopy demystified *Nature cell biology* **2019,** *21* (1), 72.
3. De Luca, G. M.; Breedijk, R. M.; Brandt, R. A.; Zeelenberg, C. H.; de Jong, B. E.; Timmermans, W.; Azar, L. N.; Hoebe, R. A.; Stallinga, S.; Manders, E. M., Re-scan confocal microscopy: scanning twice for better resolution. *Biomedical optics express* **2013,** *4* (11), 2644-2656.
4. Stanciu, S. G.; Hristu, R.; Tranca, D. E.; Stanciu, G. A.; Manders, H.; Cherian, A.; Tark-Dame, M.; Manders, E. M. In *Higher-harmonic generation microscopy beyond the diffraction barrier based on re-scan strategies for optical data acquisition (HARMOPLUS)*, Proc. ATTRACT-Final Conf.—Igniting Deep Tech Revol., 2020; pp 1-5.
5. Papadopoulos, C.; Meyer, H., Detection and clearance of damaged lysosomes by the endo-lysosomal damage response and lysophagy. *Curren Biology* **2017,** *27* (24), R1330-R1341.
6. Hung, Y.-H.; Chen, L. M.-W.; Yang, J.-Y.; Yang, W. Y., Spatiotemporally controlled induction of autophagy-mediated lysosome turnover *Nature communications* **2013,** *4* (1), 1-7.
7. Radulovic, M.; Schink, K. O.; Wenzel, E. M.; Nähse, V.; Bongiovanni, A.; Lafont, F.; Stenmark, H., ESCRT-mediated lysosome repair precedes lysophagy and promotes cell survival. *The EMBO journal* **2018,** *37* (21), e99753.