Microsc. Microanal. 8 (Suppl. 2), 2002
DOI 10.1017.S1431927602107173

Characterizing Visible Lipids Imaged by the Real Time Microscope in Fibroblasts from Patients with Lysosomal Storage Diseases

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A new light microscopic technology, the Real Time Microscope (RTM) provides the capability to detect cellular lipid membranes and lipid granules in cells without the use of stains. Standard imaging methods for cellular lipid droplets employ fluorescence staining of neutral lipids with nile red, and cholesterol with filipin. Do these lipid probes label the RTM-visible granules in fibroblasts?

The RTM-visible granules imaged in live cells cultured on slides (Figure 1a) and in cells that have undergone paraformaldehyde-fixation on slides (Figure 2a) are composed of high contrast, dark solid round granules (arrow). The image acquired in fluorescence and RTM modes (Figure 1b) identifies the high contrast RTM-visible granules as nile red labeled lipid droplets (yellow). Nile red fluorescence is relatively less in granules that have lower imaging contrast. By comparison, the image acquired in fluorescence and RTM modes of filipin stained cells (Figure 2b) shows the RTM-visible granules localizing to different regions than cholesterol-filipin inclusions (blue). The results demonstrate that the RTM-visible lipid granules observed in both the live and fixed cell consist primarily of neutral lipids.

How do the RTM-visible lipids behave in fibroblasts derived from individuals with lipid metabolic diseases? A fibroblast strain from a patient with Wolman disease had the highest nile red labeling compared to fibroblasts with other lipid disorders, or to healthy fibroblasts. Visible RTM granules with higher contrast and larger sizes labeled with more nile red. On the other hand, a GM1 gangliosidosis fibroblast strain that displayed fewer and smaller RTM-visible lipid granules had less nile red fluorescence. Interestingly, these cells had the highest filipin fluorescence when compared to the other diseased strains, or to healthy fibroblasts. By comparison, the greatest increase in filipin uptake, without change in RTM-visible lipid patterns was observed in fibroblasts derived from Niemann-Pick type C (NPC) patients cultured in lipoprotein deficient media.

Many studies have used either nile red, or filipin to analyze lipid content in cells. By imaging more than one type of lipids, this study demonstrated the variability and diversity of lipid expression both within one lipid disease and between lipid disorders. The Real Time Microscope was most effective in the imaging of cellular neutral lipid droplets, without stains, in live and fixed cells.

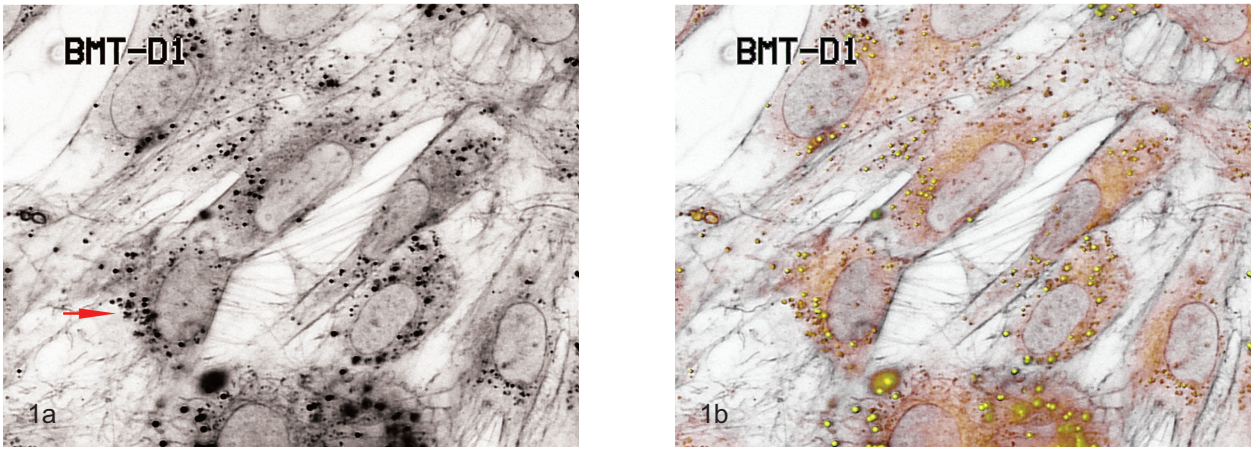

Figure 1. Fibroblasts grown on a slide, stained with 2 µM nile red for 5 min in cell culture conditions. Cytoplasmic granules in cells imaged by direct RTM mode (1a) are identical to the yellow nile red labeled neutral lipids visible in the RTM fluorescence mode observed in the overlay image, 1b. A filter cube ex 420-490 nm, em >515 nm was used to capture nile red fluorescence. BMT-D1 = 23 µm.

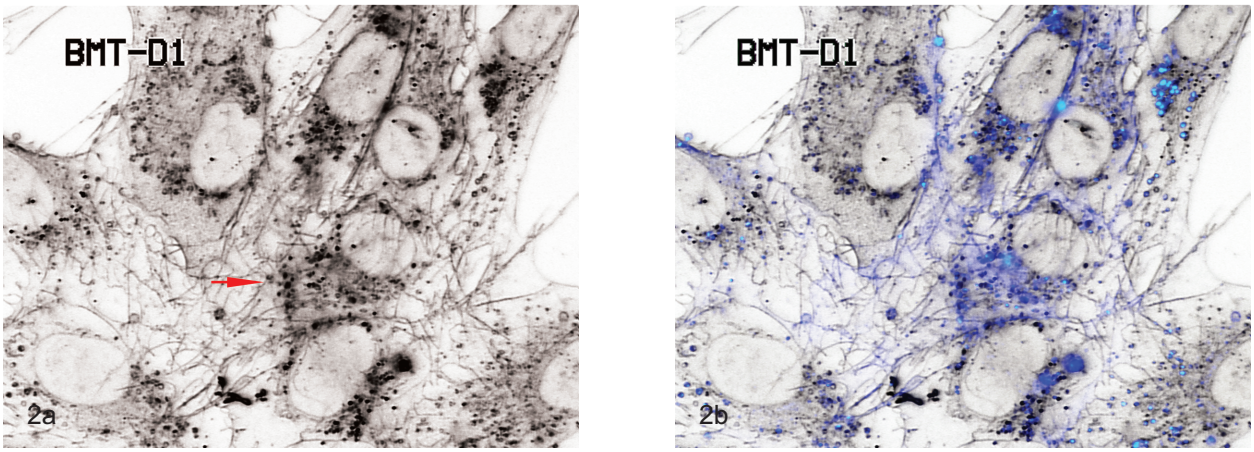

Figure 2. Fibroblasts grown on a slide, fixed with 3% paraformaldehyde and 0.2% glutaraldehyde, and stained with 0.005% filipin. Lipid granules imaged by direct RTM mode (2a) do not co-localize with the blue cholesterol-rich filipin labeled granules visible in the RTM fluorescence mode observed in the overlay image, 2b. A filter cube ex UV, em 340-380 nm was used to capture fluorescence of the cholesterol-filipin complex. BMT-D1 = 23 µm.

https://doi.org/10.1017/S1431927602107173 Published online by Cambridge University Press