*Supplemental Data for:*
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New Observations on the Integrity, Structure, and Physiology of Flesh Cells from Fully Ripened Grape Berry.
Am. J. Enol. Vitic. 62:279-284. doi:10.5344/ajev.2011.10126.

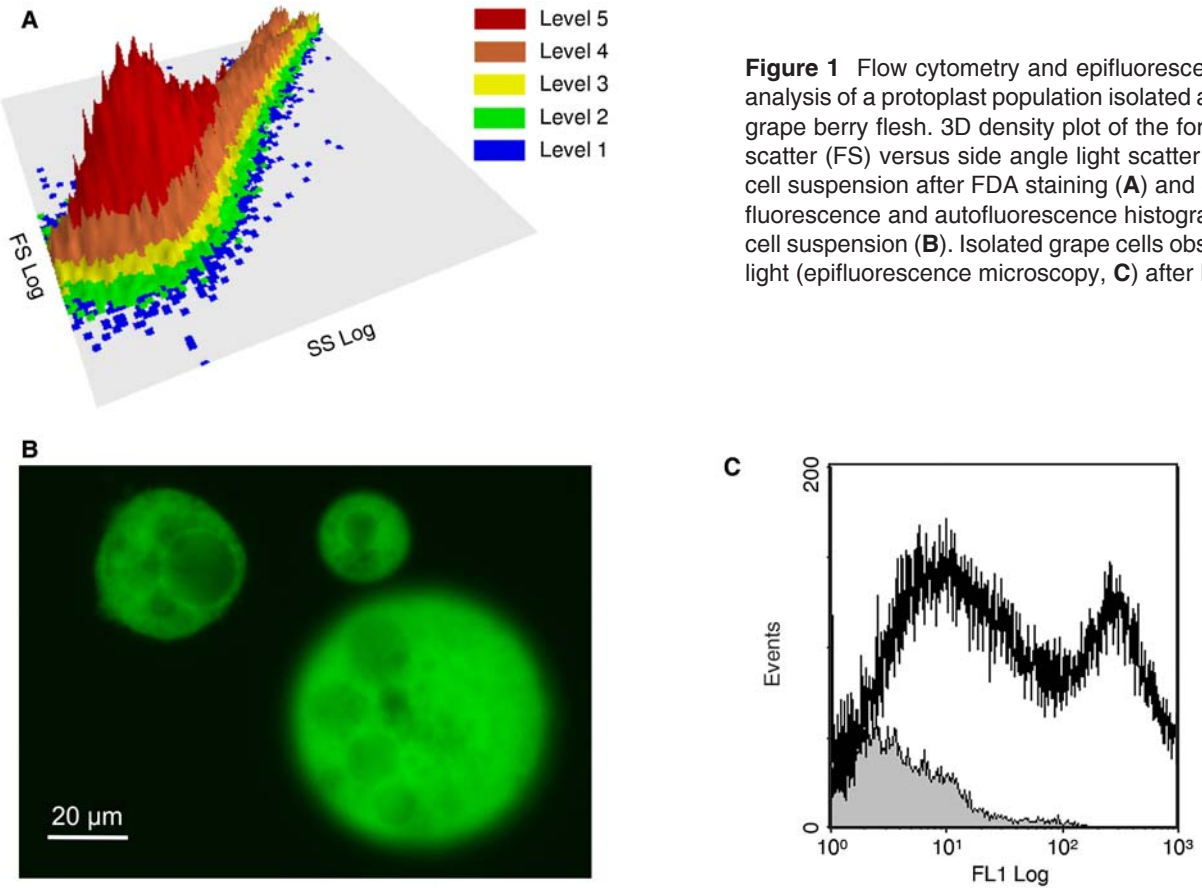


**Figure 1** Flow cytometry and epifluorescence microscopy analysis of a protoplast population isolated and purified from grape berry flesh. 3D density plot of the forward angle light scatter (FS) versus side angle light scatter (SS) of a grape cell suspension after FDA staining (**A**) and overlay of green fluorescence and autofluorescence histograms of the same cell suspension (**B**). Isolated grape cells observed under UV light (epifluorescence microscopy, **C**) after FDA staining.

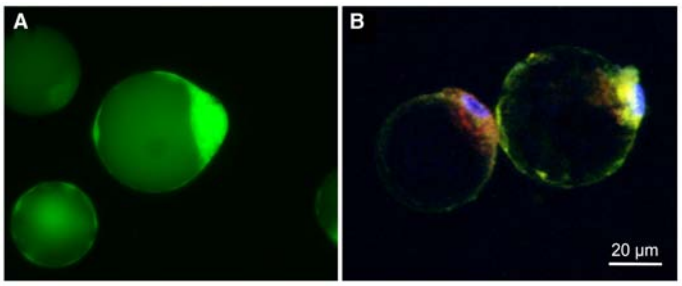


**Figure 2** Protoplasts from actively dividing Cabernet Sauvignon berry cells observed by both epifluorescence and confocal microscopy. Cells were stained with FDA (**A**) and with Hoechst (blue, nucleus), MitoTracker Red (red, mitochondria), and FM1-43 (green, plasma membrane) (**B**).

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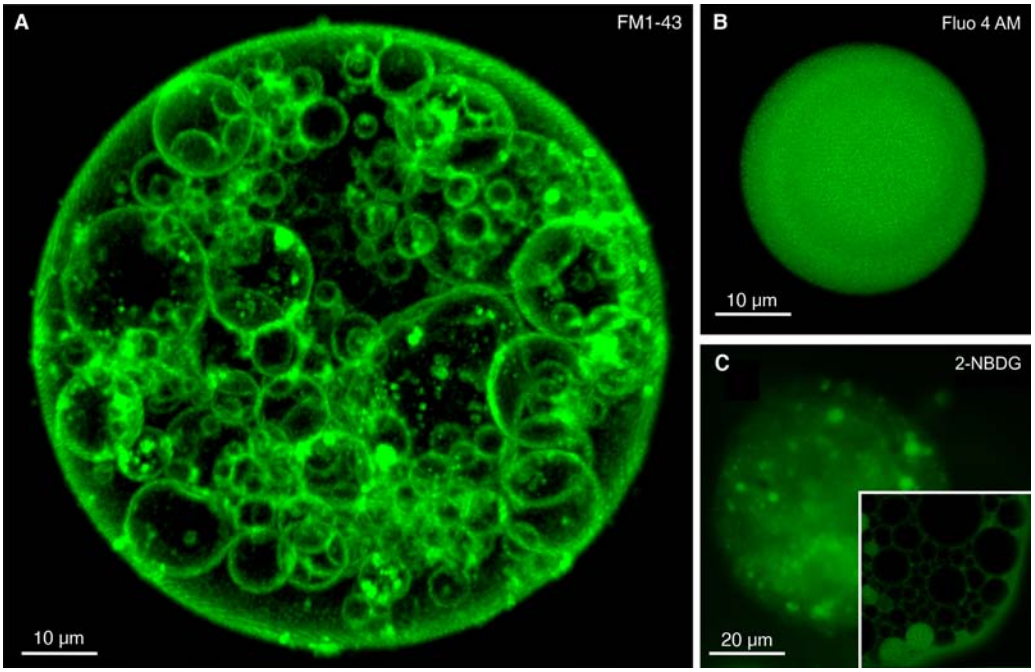


**Figure 3** Mesocarp cells from fully ripened berry observed under the confocal microscope. Protoplasts were imaged by confocal laser scanning after being immersed overnight with the styryl dye FM1-43. Maximum Z projection of 20 sections covering ~30 µm (**A**). Intact vacuoles imaged after staining with the calcium fluorescent probe Fluo-4 AM (**B**). Plasma membrane integrity assessed under the fluorescence microscope with the fluorescent glucose analogue 2-NBDG (16-hr incubation) (**C**). Inset in C: single section of protoplast loaded with 2-NBDG observed by confocal microscopy 16 hr after incubation.

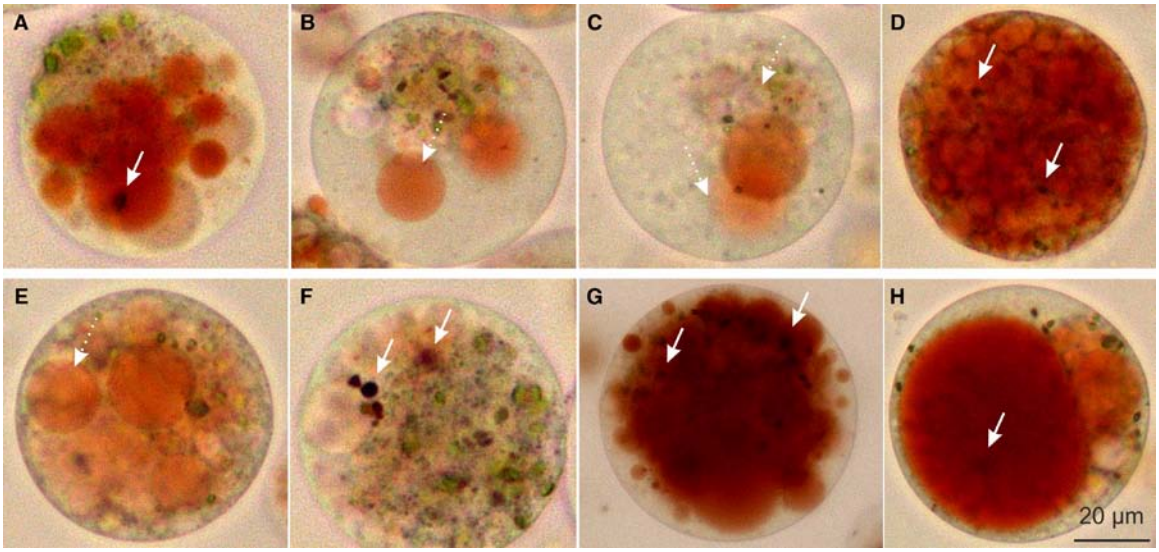


**Figure 4** Diversity in size, number, and vacuole sap composition/pH of berry-derived protoplasts as assessed after staining with the lipophilic phenazine dye Neutral Red. Vacuoles with precipitates (full cell saps; solid arrows, **A**, **D**, **F**, **G**, **H**) and without precipitates (empty cell saps; dashed arrows, **B**, **C**, **E**).