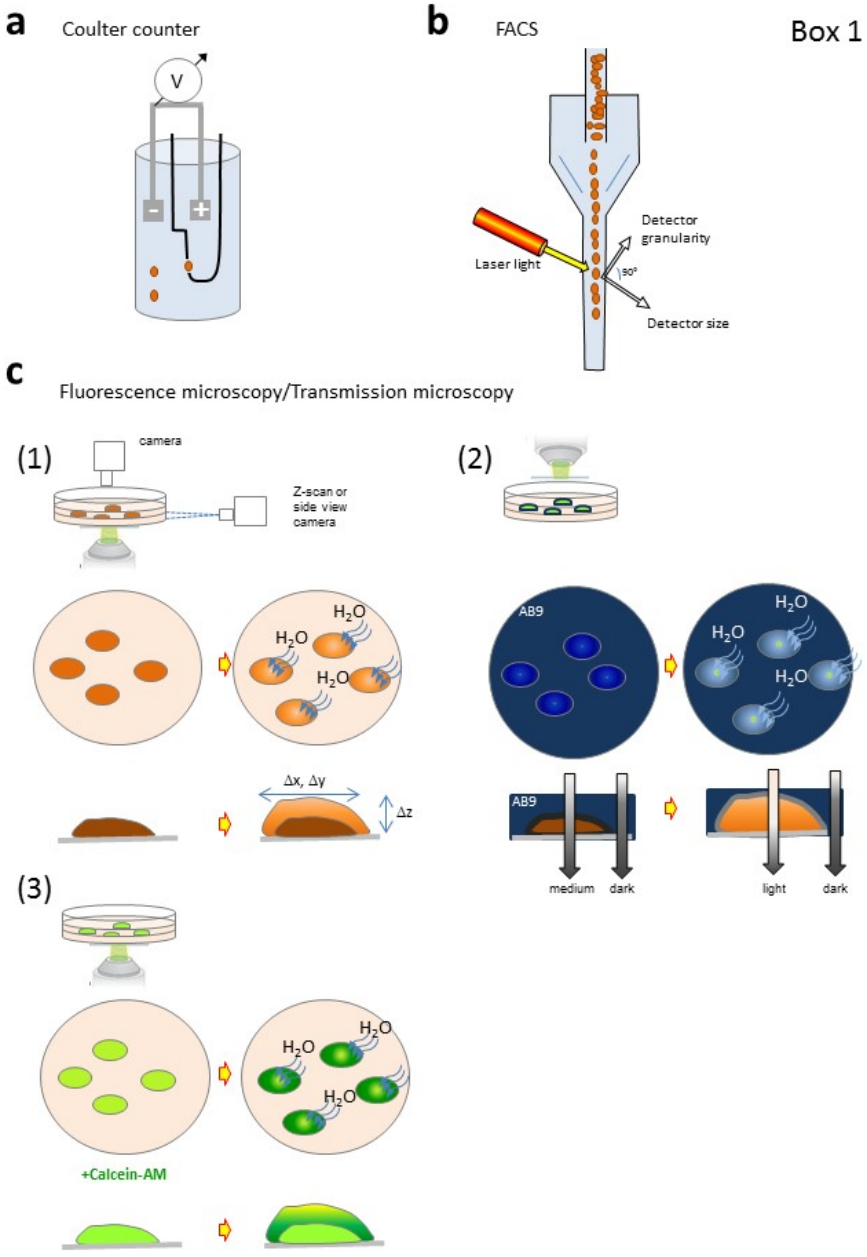
In format provided by Jentsch, T. J. (MAY 2016)

Supplementary Information to Jentsch: *VRACs and other ion channels and transporters in the regulation of cell volume and beyond*

Supplementary Box 1: Methods to measure cell volume



Several methods can be used to measure cell volume. These include **a** | Coulter-counter like instruments in which individual cells flow between two electrodes. The change in resistance between these electrodes is a measure of cell size. **b** | Fluorescence activated cell sorters in which droplets containing individual cells pass a laser beam. Fluorescence signals or light scattering can be used to determine cell size. Both (a) and (b) only work with cells in suspension, which is problematic if e.g. trypsin has to be used to detach adherent cells from their growth substrate. Both methods can yield statistical information on the distribution of volumes across a large number of cells, but do not allow to determine the volume regulation of individual cells. However, if e.g. cells are exposed at a given time to hypotonic medium, and then sequentially enter the analyzer over a time course of several minutes, volume changes of an ensemble of cells can be measured. **c**| Microscopical techniques can be used to investigate cell volume regulation of individual adherent cells. (1) Cell volume can be determined by measuring the vertical and lateral dimensions of individual cells in microscopic images, using e.g. Z-scans in confocal microscopy or with a lateral camera[1]. Cell height can also be determined precisely using attached fluorescent microbeads[2]. Another technique[3] (2) uses a perfusion chamber that is only slightly deeper than the cells and a dye (like acid blue 9) that does not enter cells. Swelling of individual cells displaces the light-absorbing fluid at that position and results in increased intensity of transmitted light which is proportional to an increase in cell volume. (3) A widely used, but less quantitative method uses standard equipment or TIRF microscopy to measure changes in the fluorescence of the cell membrane-impermeable dye calcein (loaded into cells as AM ester) with cell volume changes[4]. Both (2) and (3) can be used to measure volume changes of many cells in parallel. There are also several other less used techniques like atomic force microscopy.

References:

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