**Staining *T. brucei* cells with MitoTracker Red**

**Updated 2/4/19**

1. Make MitoTracker medium (10 ml SDM-79 or HMI-9 + 0.5 μl of 1 mM MitoTracker stock). Pre-warm medium to appropriate temperature (this is critical!). If fixing BSF can also pre-chill PBS at this stage. [MitoTracker stock is made by resuspending 1 vial in 94.07 μl of sterile DMSO. Aliquot and store at -20 °C.]
2. Count cells.
3. Spin down 1E7 cells (1000 x g for 5 min).
4. Resuspend cells in pre-warmed MitoTracker medium to a final concentration of 1E7 cells/ml.
5. Incubate at 27 °C (PCF) or 37 °C (BSF) for 20 min.
6. Spin. Wash cells 1X in PBS. Resuspend cells in 1 ml PBS (+ 1% glucose for BSF).
7. Outline areas on charged slide with lipid pen.
8. Add 200 µl/area of washed cells to slide. Allow cells to adhere for 10 min in humid chamber.
9. Tilt excess liquid onto paper towel, then wash 1X briefly and 1X 5 min in PBS in Coplin jars. During 5 min wash take 4% paraformaldehyde in PBS out of fridge. For BSF, dilute 4% paraformaldehyde 1:1 with cold PBS (2% final).
10. Return slides to humid chamber. For PCF, add 200 µl per area of (cold) 4% paraformaldehyde. For BSF, add 200 µl per area of (cold) 2% paraformaldehyde. Incubate for 15 min.
11. Remove excess liquid. Wash 1X briefly and 2X 5 min in PBS (Coplin jars).
12. Prepare DAPI staining solution by diluting 1 µl of 2 mg/ml stock in 1 ml PBS to give 2 µg/ml final.
13. Return washed slides to humid chamber. Add 200 µl per area of DAPI solution. Incubate for 5 min.
14. Remove excess liquid. Wash 1X briefly and 1X 5 min in PBS (Coplin jars).
15. Mount with 10 µl per area of Vectashield. Seal edges of coverslip with nail polish.