Live Cell Cycle Analysis of dissected *Drosophila* tissues using VybrantDyeCycle Violet on the Attune Cytometer

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Dissect desired tissues with proper controls (for Gal4/UAS experiments this means a control with GFP alone. For RNAi this means a control cross to white RNAi. For Flp/FRT this means a control cross generating wild-type clones.)

** Also always include a wild-type control of 10-20 proliferating L3 wings, to set your G1 and G2 peak regions for each run.

For pupal wings or eyes use at least 10 pupae (20 wings) per sample. 15-20 pupae are optimal. Try not to spend more than 45 min. dissecting each sample, as the tissues will degrade if left in the dish longer.

Using a glass Pasteur pipet, (previously coated with fatbody to keep discs from sticking), transfer discs to FACs tube containing 0.5 mL of Live FACS solution. Try to transfer as little solution from the dissection dish as possible.

Place FACS tubes in eppendorf mixer set to 23oC at speed of 500rpm.

Allow cells to dissociate on rocker for 70-90min. (70-90min. is sufficient for larval tissues and pupal eyes. 90min-2 hours is generally needed for pupal wings, due to the pupa cuticle).
Gently vortex (on setting 5 for 5 sec.) to aid in dissociation. Do not vortex hard or you will generate dead cells and debris.

Run cells on the Attune cytometer using high sensitivity at a flow rate of 100uL/min.

Live tissue FACs solution:
For 10mL:

9mL 10X Trypsin –EDTA solution (Sigma)
1mL 10 PBS (makes final concentration 1X)
5uL Dye Cycle Violet **

0.5mL per tube.

~10-20 discs per tube
** We find that a final concentration of 0.5µl/mL DyeCycle Violet provides sufficient DNA staining without toxicity to *Drosophila* cells observed at higher concentrations.