



THE UNIVERSITY OF MICHIGAN

DEPARTMENT OF MOLECULAR, CELLULAR AND DEVELOPMENTAL BIOLOGY
COLLEGE OF LITERATURE, SCIENCE AND THE ARTS
1121 NATURAL SCIENCE BUILDING
830 N. UNIVERSITY AVENUE
ANN ARBOR, MICHIGAN 48109-1048
734 763-7142 FAX 734 647-0884
<http://www.mcdb.lsa.umich.edu/>

DR. LAURA BUTTITTA, PHD
3028B NATURAL SCIENCE BUILDING
OFFICE PHONE: 734-764-2802
EMAIL: buttitta@umich.edu

Dear Editors,

Please consider our submitted protocol for publication via Journal of Visualized Experiments. This protocol is a modification of the very popular and often cited protocol for cell cycle analysis of live *Drosophila* tissues via flow cytometry, used by Neufeld et al. (PMID 9657151) and developed by de la Cruz and Edgar (PMID 18641961).

Our modifications presented here incorporate a new violet DNA dye for DNA quantification that extends this useful technique to single-lab scale benchtop cytometers equipped with a violet 405 nM laser. We demonstrate this method using the new Attune Acoustic Focusing Cytometer, and provide step-by step details of sample preparation, staining and templates for cell cycle analysis of a variety of *Drosophila* tissues expressing fluorescent proteins with the violet dye and the Attune, including dissection, developmental staging and data analysis approaches for the understudied developmental stages of *Drosophila* metamorphosis.

We hope the growing *Drosophila* cell cycle community will find this technique a useful alternative to the existing protocols, which have thus far required larger, more expensive cytometers equipped with UV lasers, generally only found in places with institutional flow core support.

We suggest as reviewers, authors with demonstrated expertise in *Drosophila* growth and cell cycle analysis; Dr. Andrew Swan at the University of Windsor, Dr. Helena Richardson and Dr. Kieran Harvey at the Peter MacCallum Cancer Center, Dr. Tom Neufeld at the University of Minnesota and Dr. Robert Duronio at UNC Chapel Hill.

We do not wish to exclude any potential reviewers.
Thank you for your consideration and please contact me with any questions or concerns regarding this submission.

Sincerely,

Laura Buttitta PhD
Asst. Professor
University of Michigan, MCDB