

Apogee Application Note:

DNA Content: Ploidy Analysis

Apogee flow cytometers give excellent population coefficients of variation (CVs). The CV is a function of

- particle variation (e.g. fluorescent labeling/staining variation)
- instrument noise and
- sample illumination quality (uniformity of laser illumination)

For mammalian or plant cells run on a high quality flow cytometer the CV is dominated by cell variation. All Apogee cytometers give excellent results (figure 1).

For cell cycle analysis of small cells with less DNA such as prokaryotes, noise contributes more significantly to CVs, so a high sensitivity (low noise compared to signal) cytometer such as the A40-MiniFCM model, provides CVs that are substantially better than normal flow cytometers (figure 2).

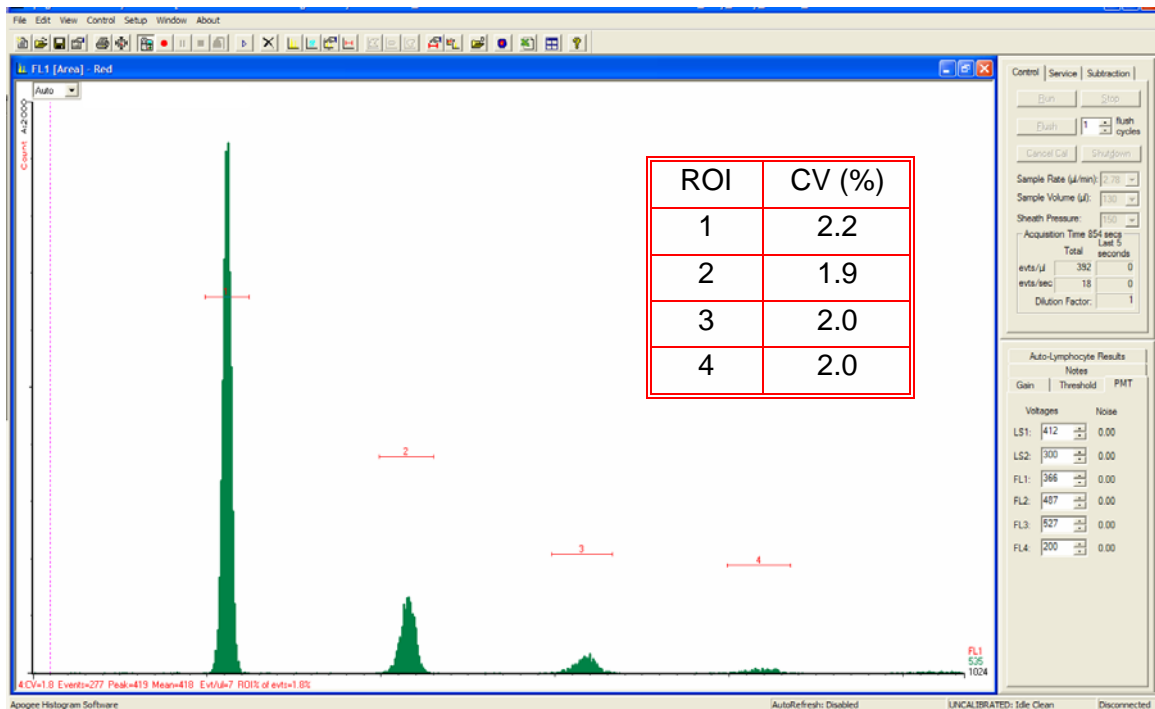


Fig 1: Chicken erythrocyte nuclei stained with propidium iodide

E. coli have far less DNA than chicken erythrocytes. Noise is therefore more significant and a high quality flow cytometer gives substantial benefit.

Figure 2 shows E. coli, treated to give populations of one and two chromosome cells. Their DNA was labeled with a mithramycin and ethidium bromide combination (green mithramycin emission excites the ethidium bromide).

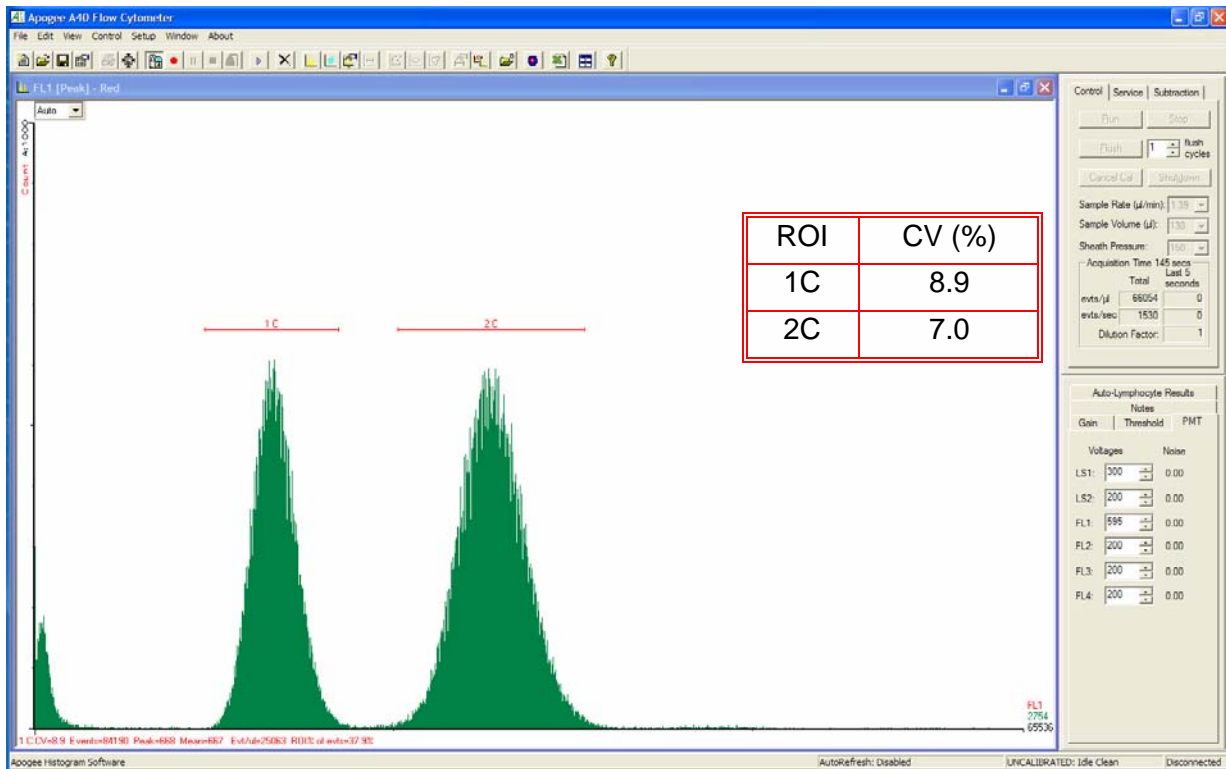


Fig 2: E. coli treated to give populations with 1 and 2 chromosomes

Figure 3 shows the 2n population of a citrus fruit. These nuclei are relatively large and bright so sample staining variation tends to dominate the CV. The sample preparation method and choice of buffer are critical in obtaining sufficient number and quality of nuclei (contact Apogee for method).

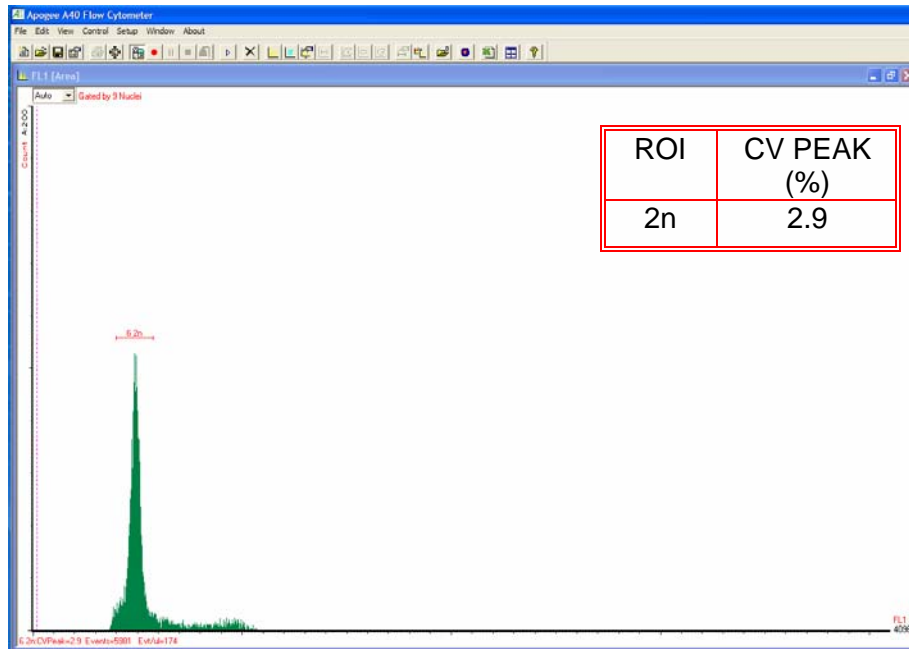


Fig 3: Clementine de Nules Ploidy Analysis (LB01 buffer, PI)

Figure 4 shows the 2n population of an aquatic fern. Again these nuclei are relatively large and bright so with a high quality flow cytometer, nuclei staining variation will usually dominate the CV.

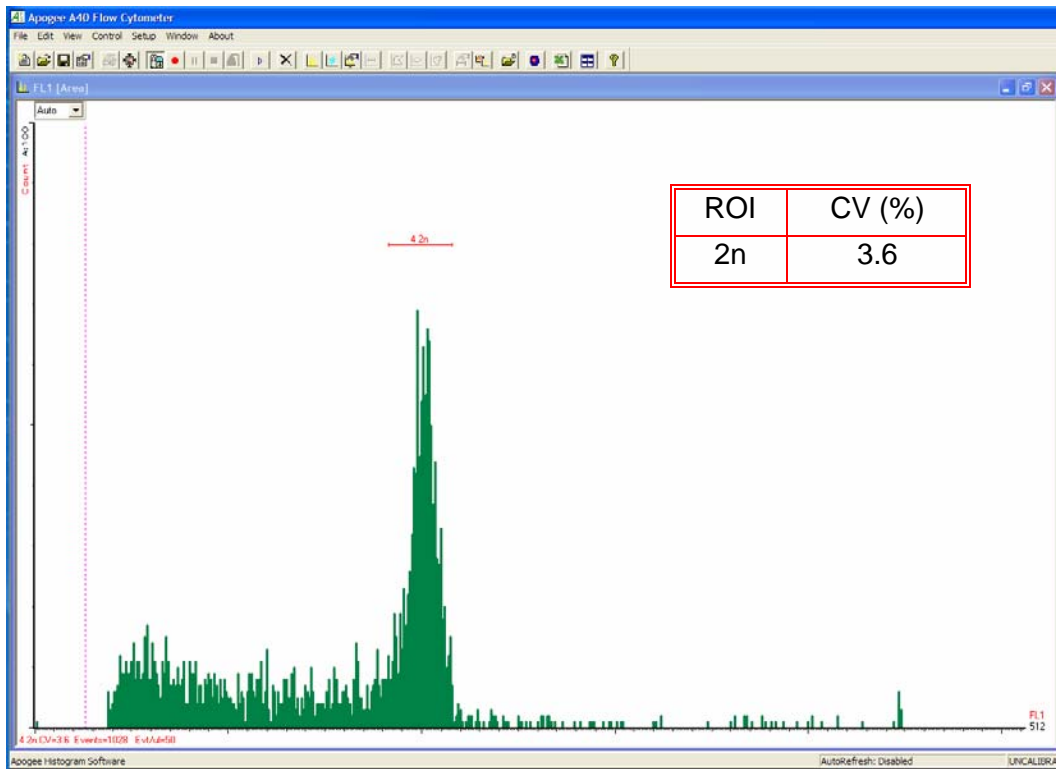


Fig 4: Marsilea, PI

SUMMARY

Apogee manufactures a range of flow cytometer models to meet different requirements. For routine work with mammalian cells or plant nuclei, our A40-Universal model with standard sensitivity (comparable to flow cytometers from the market leader) is ideal. Other users will find applications where only the high sensitivity A40-Microbiology model will give the quality of data they need.