

Apogee Application Note:

DNA Content & Ploidy Analysis

Apogee flow cytometers give excellent sensitivity and resolution. The coefficient of variation (CV) of a population is a function of:

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

Apogee's new A50 models (A50-Micro & A50-Universal) give enhanced CVs compared to the earlier A40 generation, especially at high sample flow rates. Contact Apogee for application dependent instrument configuration advice.

Calibration beads can be used to evaluate the instrument alignment, but for many samples (e.g. plant cell nuclei) analyzed on a high quality flow cytometer, the CV of the DNA content histogram is dominated by nucleic acid staining variation.

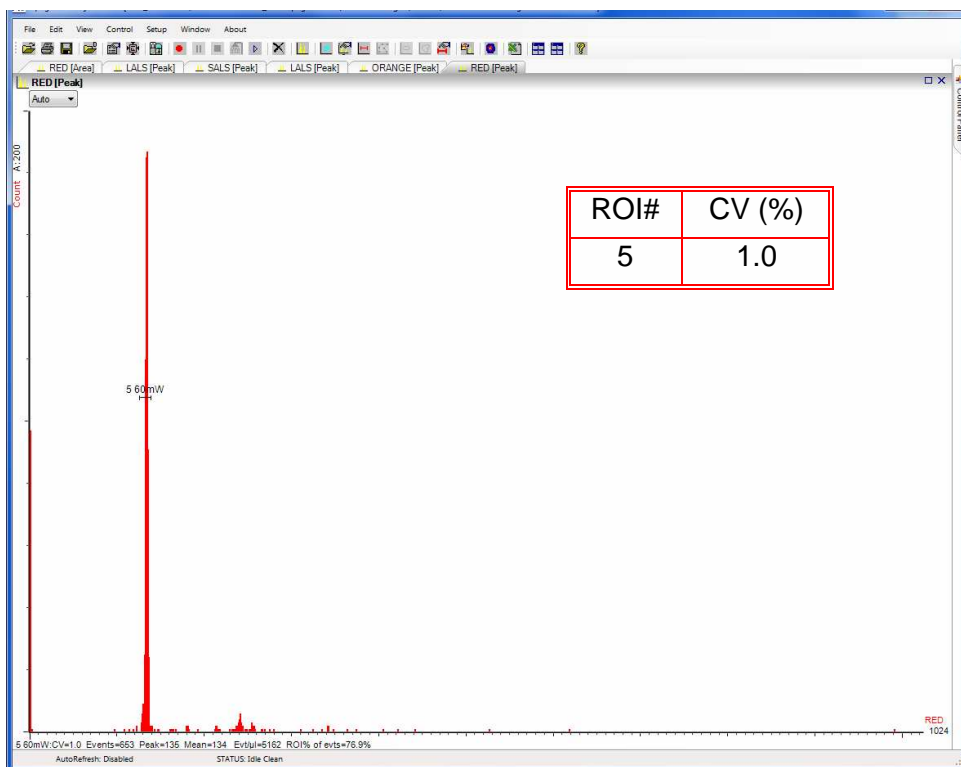


Fig 1: Red Fluorescence from 2µm Calibration Beads

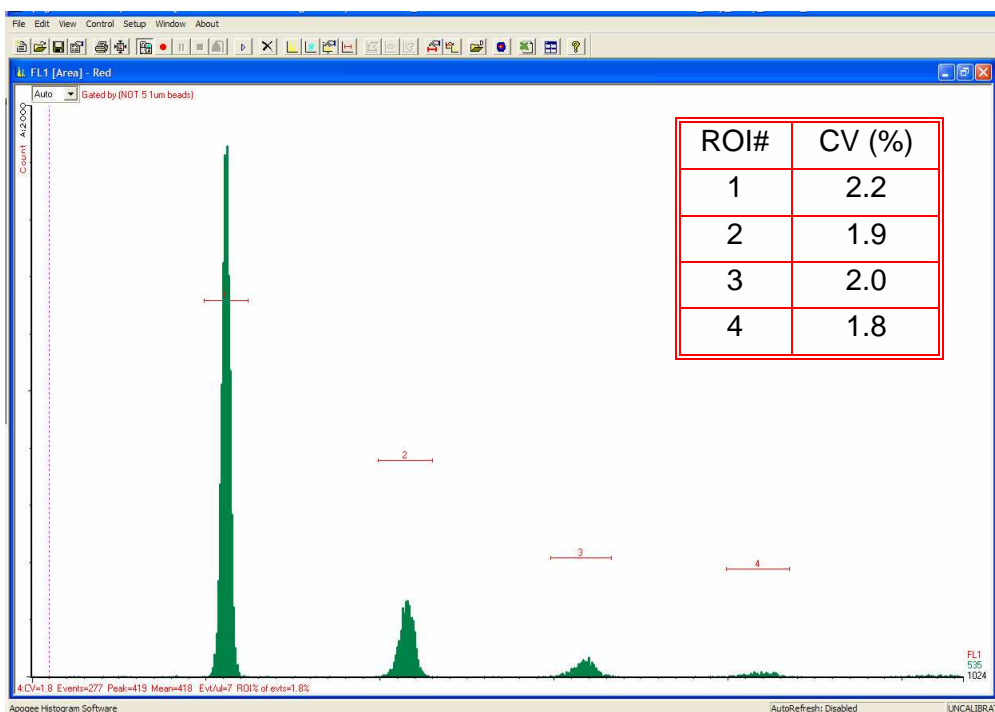


Fig 2: Chicken erythrocyte nuclei stained with propidium iodide (data from older generation A40-Micro model)

For cell cycle analysis of small cells with less DNA such as prokaryotes, optical noise contributes more significantly to the cell population's CV. In these situations a high sensitivity flow cytometer such as the A50-Micro model can provide CVs substantially better than a conventional flow cytometer (figure 3).

For example, E. coli contain far less DNA than chicken erythrocytes, so instrument noise is more significant and a high quality flow cytometer gives substantial benefit (although the CVs are still typically higher for prokaryote samples).

Figure 2 shows E. coli, treated to give populations of one and two chromosome cells. In this case the DNA was labeled with mithramycin and ethidium bromide.

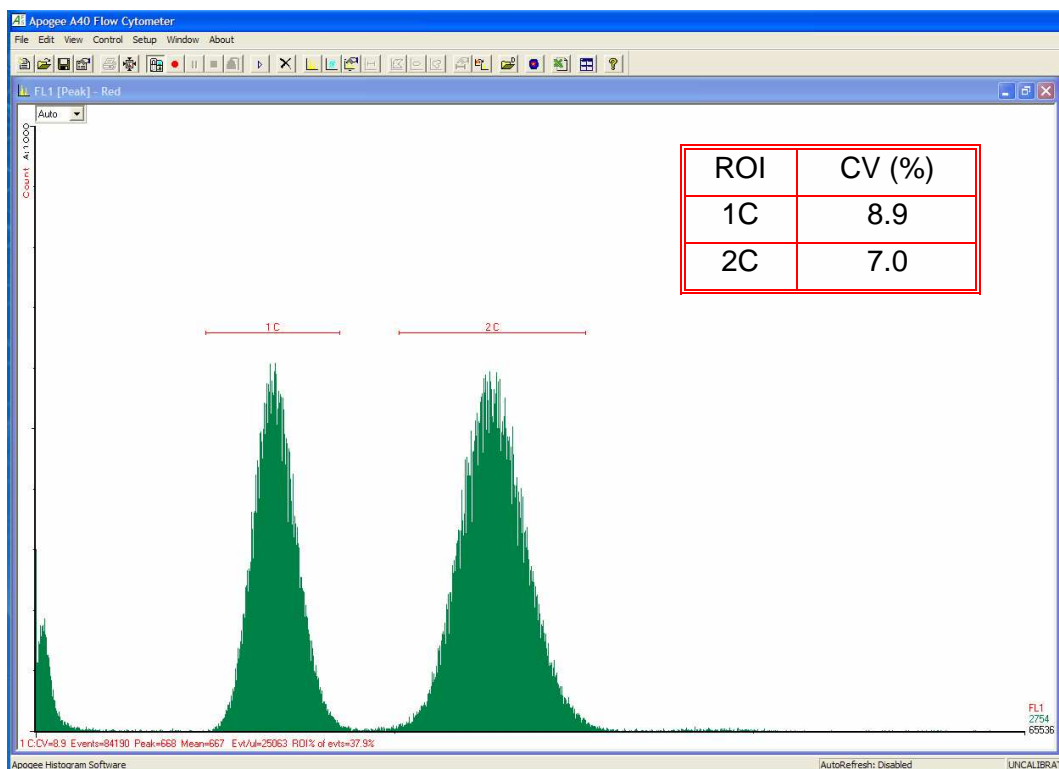


Fig 3: E. coli treated to halt growth at 1C and 2C

Plant Cell DNA Content Analysis

The analysis of plant cell nuclei (chemically extracted from cell) is particularly sensitive to the sample preparation method. Most methods involve finely chopping the leaf with a sharp blade in preparation for chemical extraction of the nuclei. It takes time to prepare a sample of sufficient concentration, but flow cytometry is the ideal method for evaluating plant ploidy.

Note that Apogee flow cytometers offer light scatter as well as fluorescence measurements. The light scatter data is often used to eliminate unwanted debris. Furthermore pulse height and integral are recorded for every parameter so the user can choose the optimum configuration depending on the size of particle being studied (e.g. for large particles the pulse integral will give the best linearity).

We present data below from a range of plants. The data is typical of what the user can expect on a routine basis rather than best possible scenarios. In all the below plant nuclei samples, sample quality dominates the CV. The sample preparation method and choice of buffer are critical in obtaining sufficient number and quality of nuclei.

Figure 4 shows the 2n population of a citrus fruit.

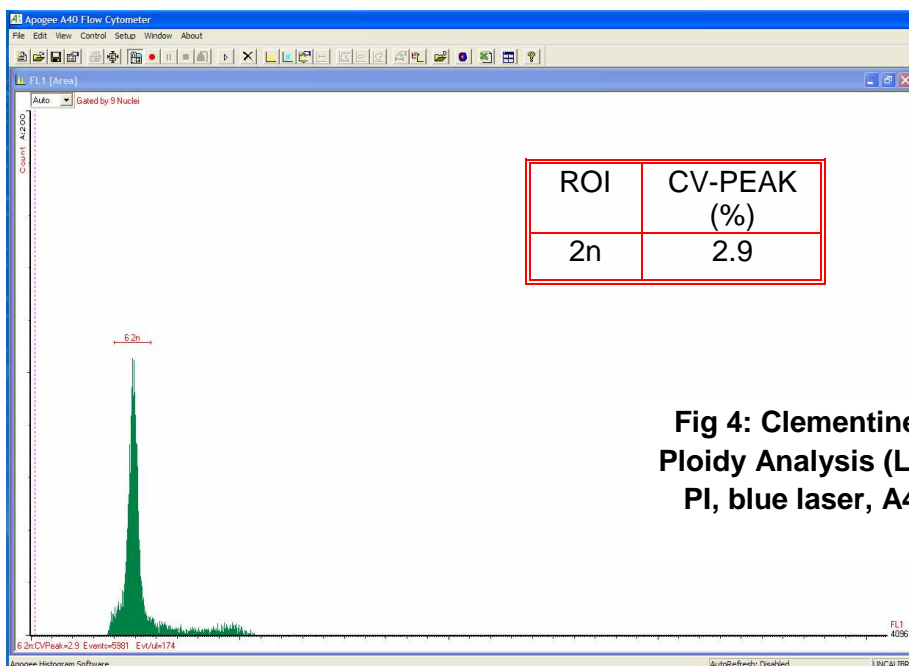


Fig 4: Clementine de Nules Ploidy Analysis (LB01 buffer, PI, blue laser, A40 model)

Figure 5 shows the 2n population of an aquatic fern (Marsilea).

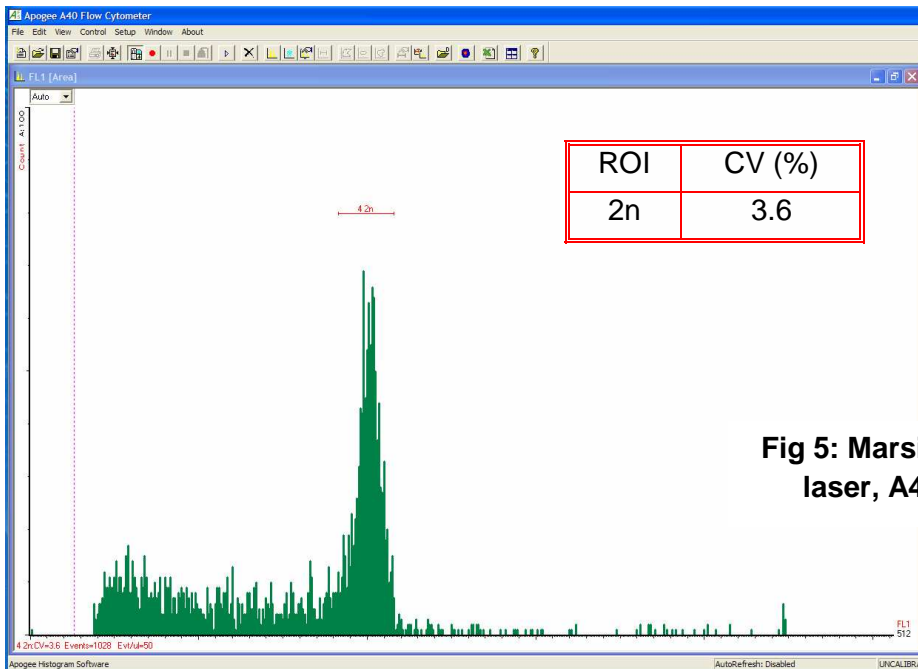


Fig 5: Marsilea (PI, blue laser, A40 model)

Figure 6 shows a sample of Cyperaceae nuclei stained with DAPI (375nm laser). Figure 7 shows Arabidopsis stained with propidium iodide. These nuclei are relatively large and bright so with a high quality flow cytometer, sample quality dominates the CV.

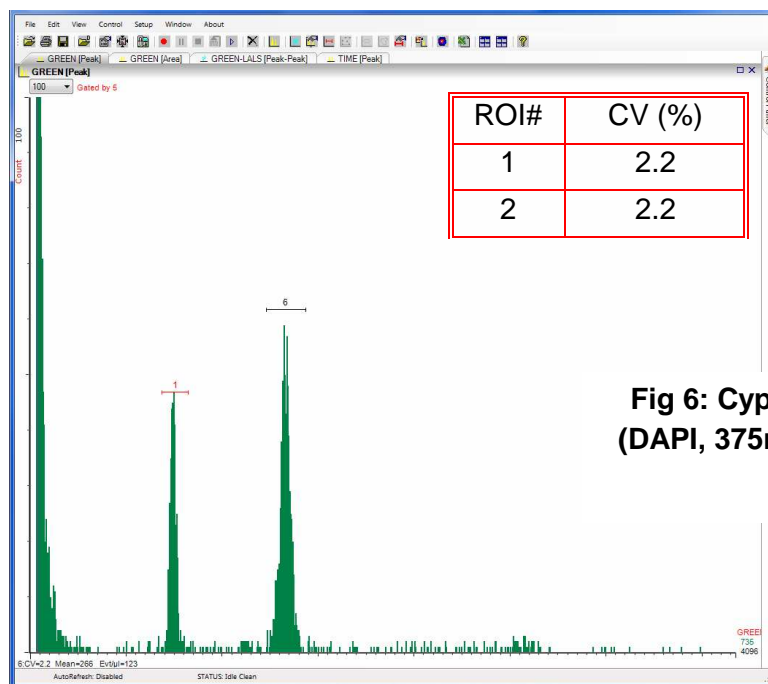
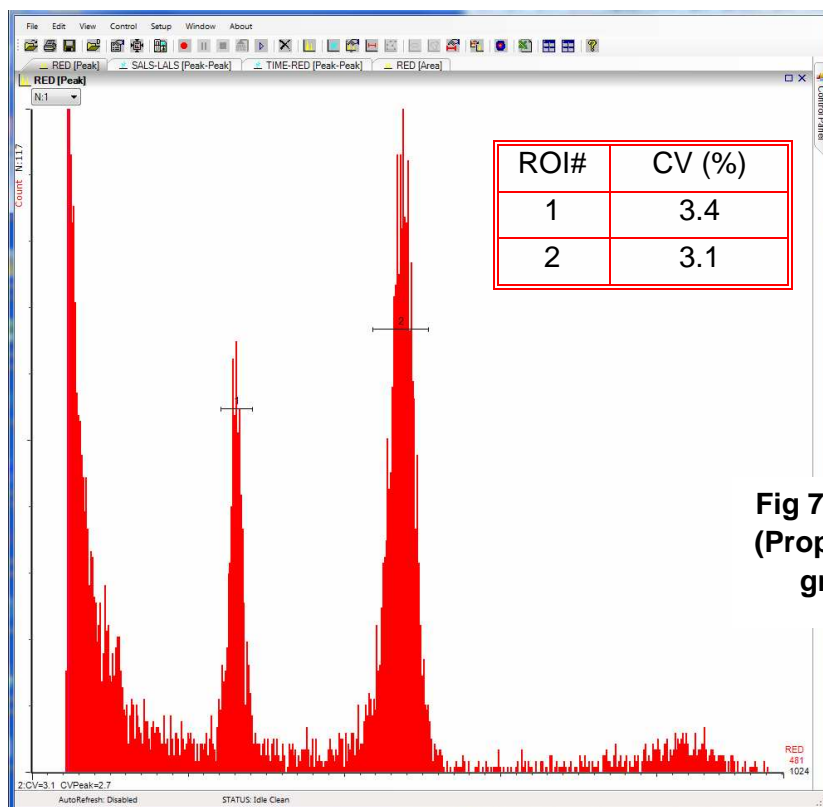


Fig 6: Cyperaceae (DAPI, 375nm laser)



**Fig 7: Arabidopsis
 (Propidium iodide,
 green laser)**

DATA ANALYSIS

Apogee's standard software offers most common flow cytometry data presentation features and the files generated are FCS compatible so that third party software can be used e.g. FlowJo software (www.flowjo.com).

SUMMARY

Apogee manufactures a range of flow cytometers to meet different requirements. For routine work with mammalian cells or plant cell nuclei, our A50-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 A50-Micro model gives the quality of data they need.

REFERENCES

Jaroslav Dolez'el, Johann Greilhuber & Jan Suda , "Estimation of nuclear DNA content in plants using flow cytometry", NATURE PROTOCOLS, VOL.2, NO.9, 2007, p.2233