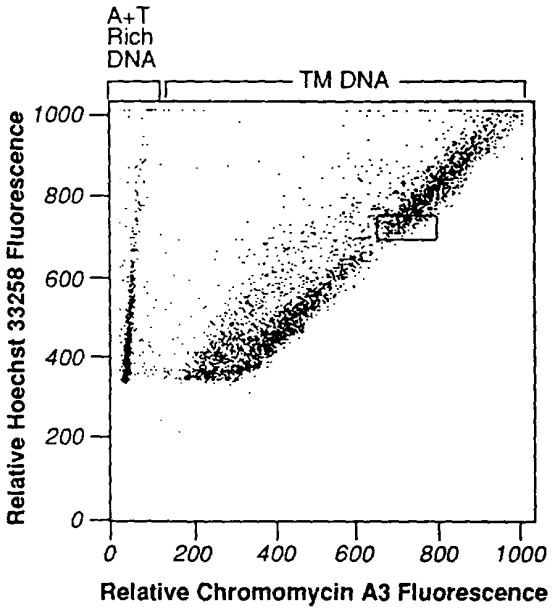
1. Explain what Koehler illumination is, and why it is necessary for optimal microscopy.

2. The figure at right is from the following paper:

Chen, D.F., P.J. Dale, J.S. Heslop-Harrison, J.W. Snape, W. Harwood, S. Bean, and P.M. Mullineaux. 1994. Stability of transgenes and presence of N^6 methyladenine DNA in transformed wheat cells. Plant J. 5:429-436.

The authors used bivariate karyotype flow analysis of cultured wheat cells. The particles along the 451 angle represent an A+T : G+C ratio of 0.545, which corresponds to the known ratio in wheat. The particles forming the steep line on the left hand side of the figure corresponded to very AT-rich DNA, which was eventually found to come from a mycoplasma contaminant of the wheat cells.

Use your knowledge of the way a flow cytometer works to explain how these results were obtained.



3. Answer the following:

A. Give a succinct description of how confocal microscopy operates.

B. Do the same for deconvolution based 3-D microscopy.

C. Finally, list one major advantage and one major disadvantage each for conventional light microscopy, for confocal microscopy, and for deconvolution based 3-D microscopy.

4. Describe the difference between a virtual image and a real image:

5. Provide a **SHORT** definition or description for each of the following. Do not exceed the space provided.

- **Dichroic mirror:**
- **Band pass filter**:
- **Photomultiplier tube:**
- **Stoke=s Law or Stoke=s Shift:**
- **Birefringement crystal prism:**
- **Analyzer and polarizer:**
- **Wollaston prism:**
- **Barrier and exciter filters:**
- **Pretreatment:**
- **Fixatives:**

6. The following is from:

Ollitrault, P., Vanel, F., Froelicher, Y. and Dambier, D. 2000. Creation of triploid citrus hybrids by electrofusion of haploid and diploid protoplasts. Acta Hort. (ISHS) 535:191-198. http://www.actahort.org/books/535/535_23.htm

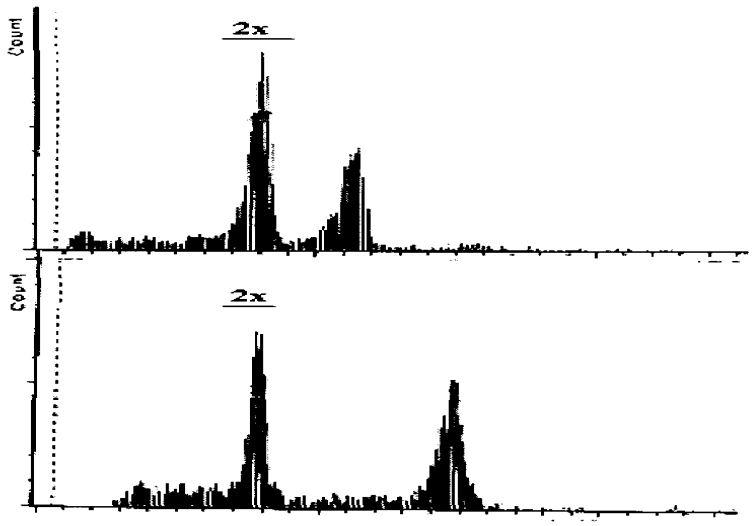
In this publication the authors were trying to obtain triploids through protoplast fusion of haploid and diploid cells. A second goal was to create new tetraploids from diploid-diploid protoplast fusion, for later use in triploid formation through tetraploid-diploid breeding.

A) Flow cytometry was used to analyze the regenerated plants obtained from protoplast fusion. In this case, which cell type should be evaluated?

B) List at least two properties of plant cells that need to be considered, as these properties make it difficult to use plant cells in flow cytometry?

C) Which type of dye should they have used in this case: Intercalating or base specific? Why?

D) Based on the following histogram would you consider this experiment successful or a failure? Explain.



8. Practical Exam –

Each student will come to the back of the lab one at a time with this page to answer the practical questions. These three questions are based on the three microscopes set up here.

Microscope 1:
This microscope has been adjusted so it is not possible to visualize the sample. Set up and Koëhler this microscope so that it can be viewed with the 20X objective. Once the microscope is set up, go to the coordinates: x=38.6, y=40.5. Observe the chromosome number in the diakinesis cell and fill in the blanks to designate ploidy and chromosome number in a somatic cell from the individual that produced the cell you are observing. Assume this organism is a diploid.

A. ____n = _____X = _____C = _____

B. You will also be graded on aspects of how the microscope was put into Koëhler.

Microscope 2:

C. This microscope has been set up for optimal viewing with the 20X objective but you may move the stage around and change focus. Were the cells on a slide taken from a monocot or a eudicot?

List distinguishing features of the cells/chromosomes that allow you to answer questions

D. 1)

E. 2)

9. The final question is a practicum:
You have fifteen (15) minutes to complete parts A-C. D can be completed upon return to your desk.

A) Go to the first microscope and perform Koehler illumination.

B) Then go the next scope, and go to the following coordinates: 31.2, 40.8

C) Identify the stage of division of the cells at these coordinates.

D) Then draw out all the stages of meiosis that happen before and after that cell, for an organism that is 2n = 2x = 4, with 1 pair of metacentrics and 1 pair of telocentrics. Use the back of this page.