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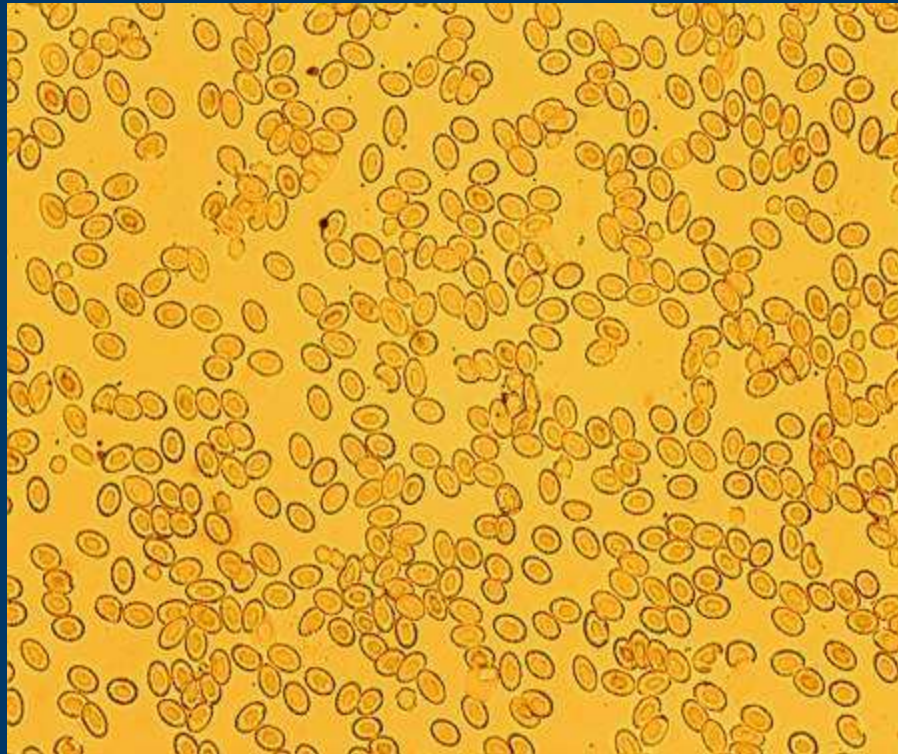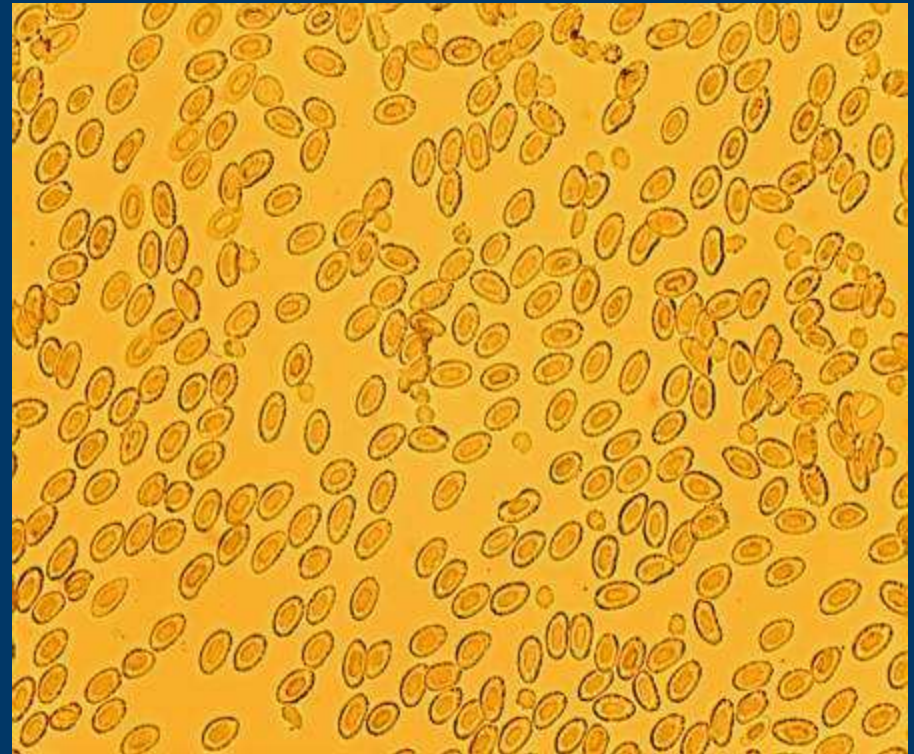
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A new method to confirm triploidy rates in salmonids

Diploid

Triploid

Outline

- Current FFSBC production and sampling

- Difference of Imaging software (ImageJ) and Flow Cytometry

- Example of imaging software sample analysis

FFSBC Production and Sampling

- Currently release 3 million triploid fish throughout the province of British Columbia

- Combination of hydrostatic pressure and heat shock since 1995

- Sampling to confirm triploid success at 0.05% of total release size

Imaging Software

Pro's

- Basic Lab equipment

- No fish transfer

- Timeline extended

- Cost less per sample

Con's

- New unknown process

- Sample Quality

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Imaging Software

• Blood smear

• Picture 200x magnification

• Upload pictures into software and run

• Output into datasheet

Flow Cytometry

Pro's

- Proven method

- Familiar with process

Con's

- Time sensitive

- Shipping fish

- More expensive

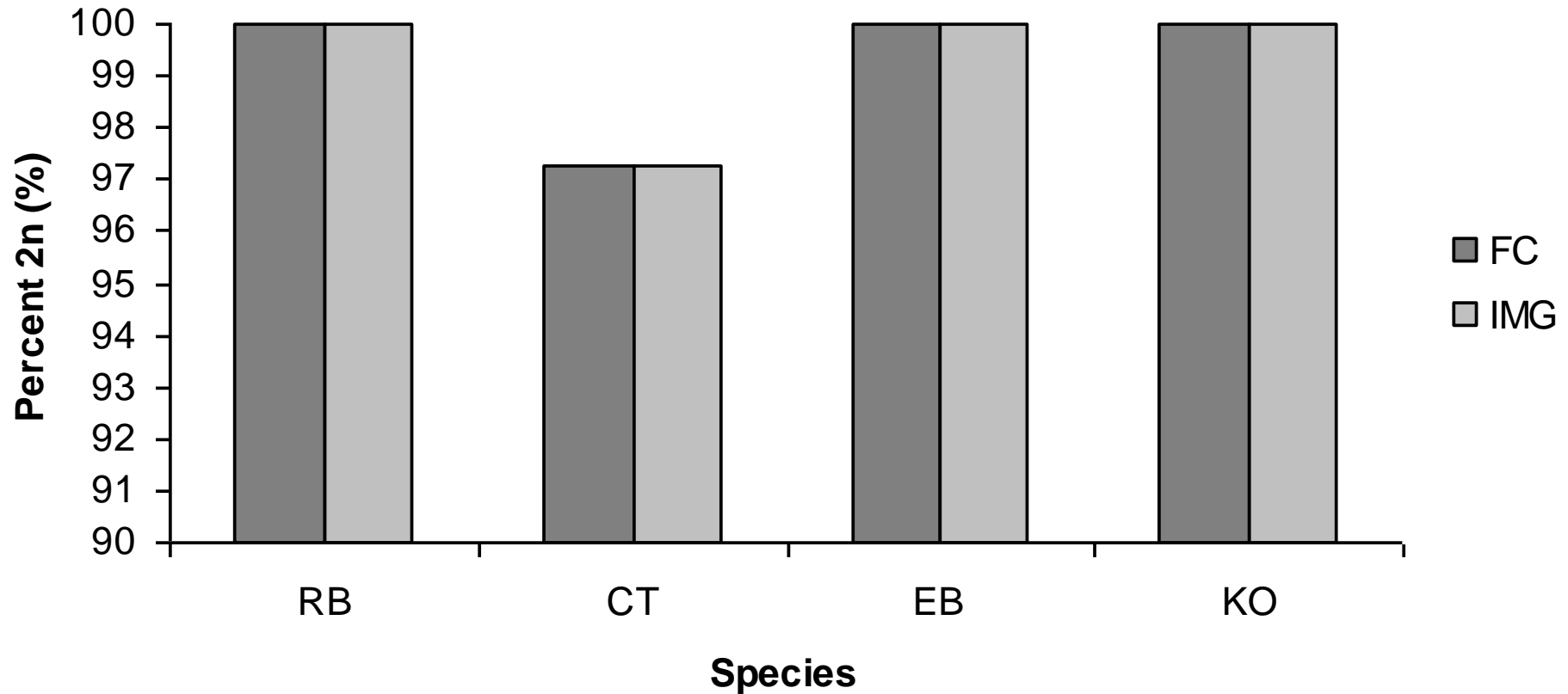- Chemical disposal

- Mutagen / Carcinogen

Flow Cytometry

- Hold fish onsite, lethal sample

- Apply Nuclear Stain, Preserve
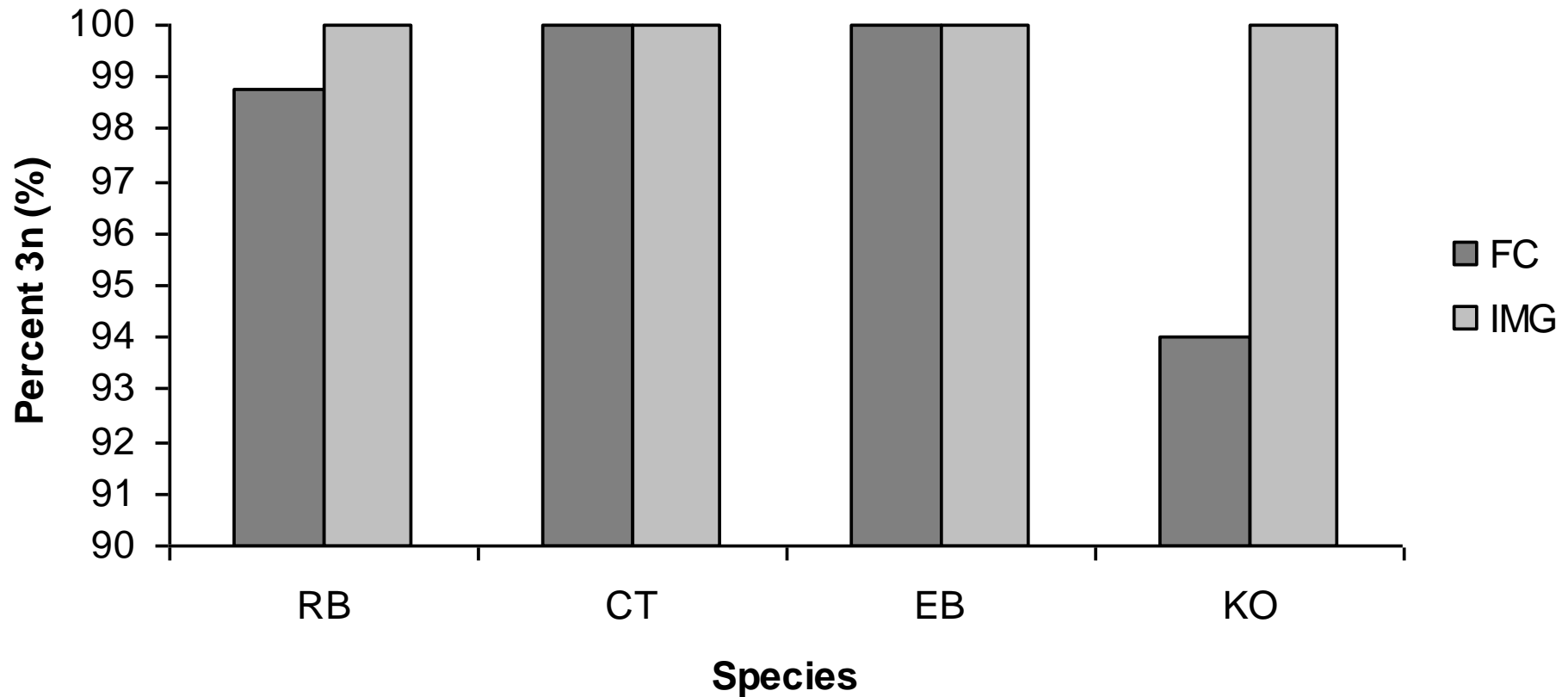
- Send to contractor

- Chemical disposal

Comparison of Methods

Comparison of Methods

Comparison of Methods

Imaging

0%

2%

10%

88%

Legend:
- ☐ Contract
- ☐ Transport
- ☐ Labour
- ☐ Equipment/Reagents

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Comparison of Methods

Flow Cytometry

- Contract — 54%
- Transport — 6%
- Labour — 32%
- Equipment/Reagents — 8%

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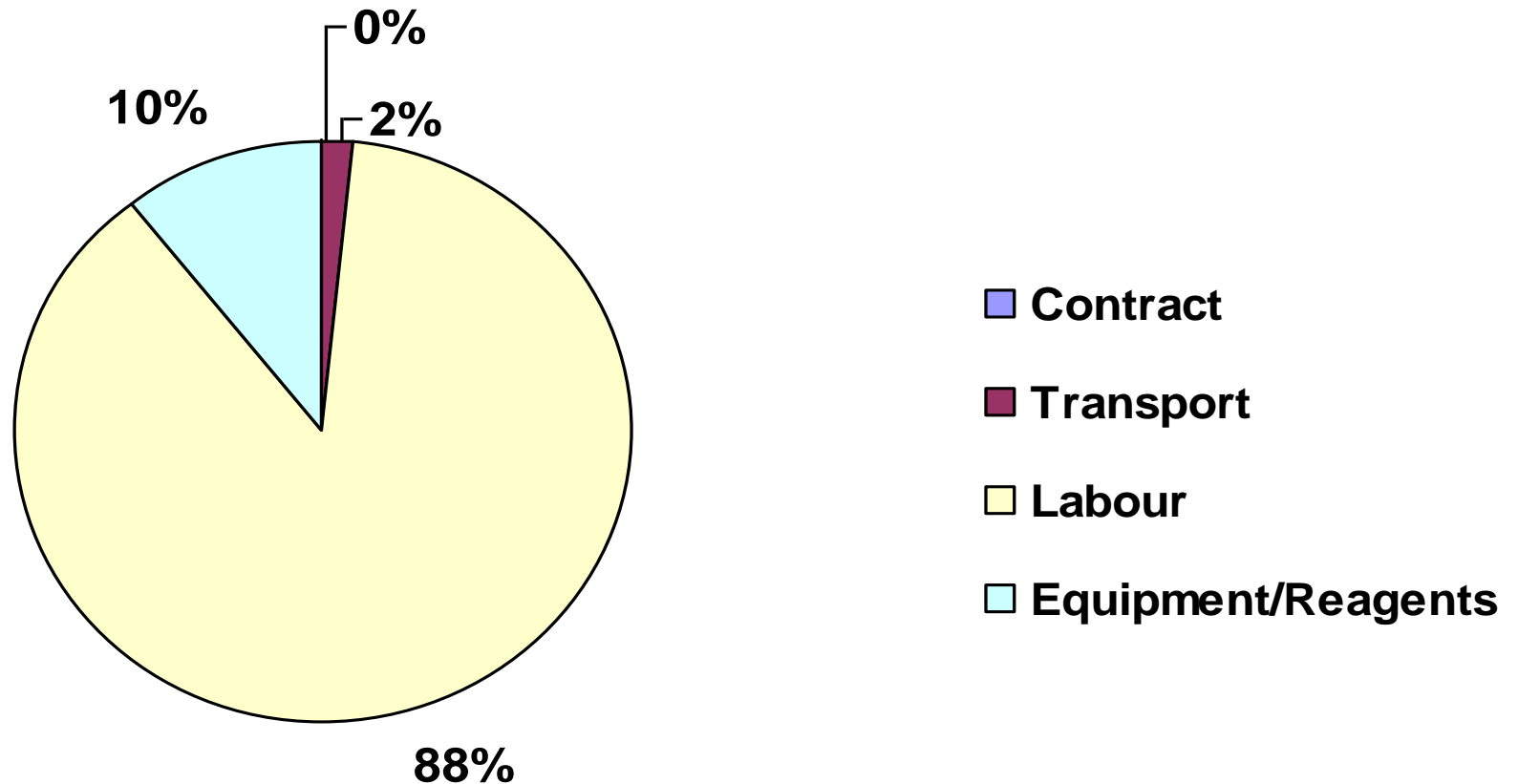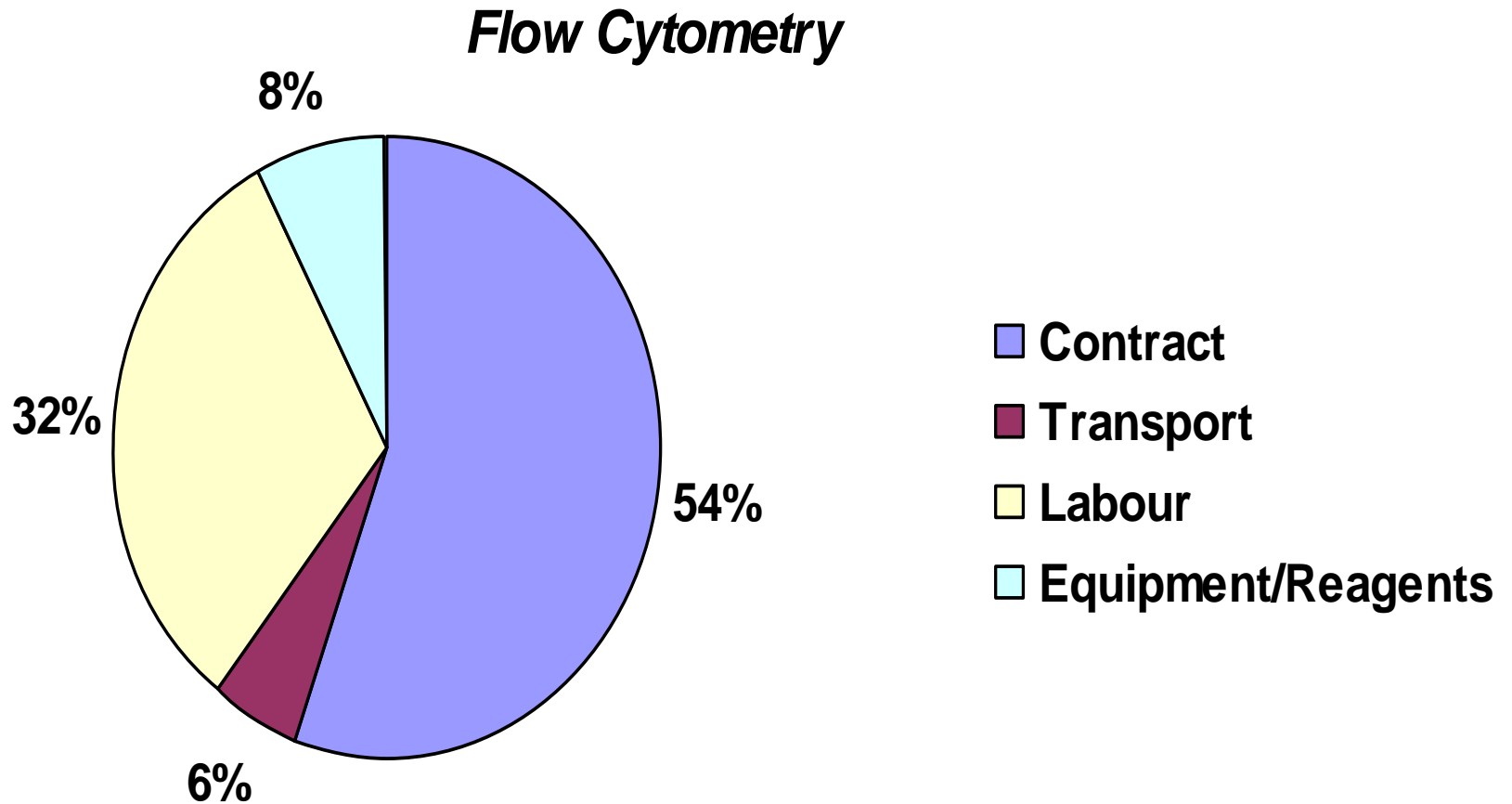
Costing

- Based on 1900 samples

- Imaging Cost $3.29 / sample

- Flow Cytometry $6.40 / sample

Imaging Software

- Parameters

- Manipulation, measurement

- Organizing output, testing
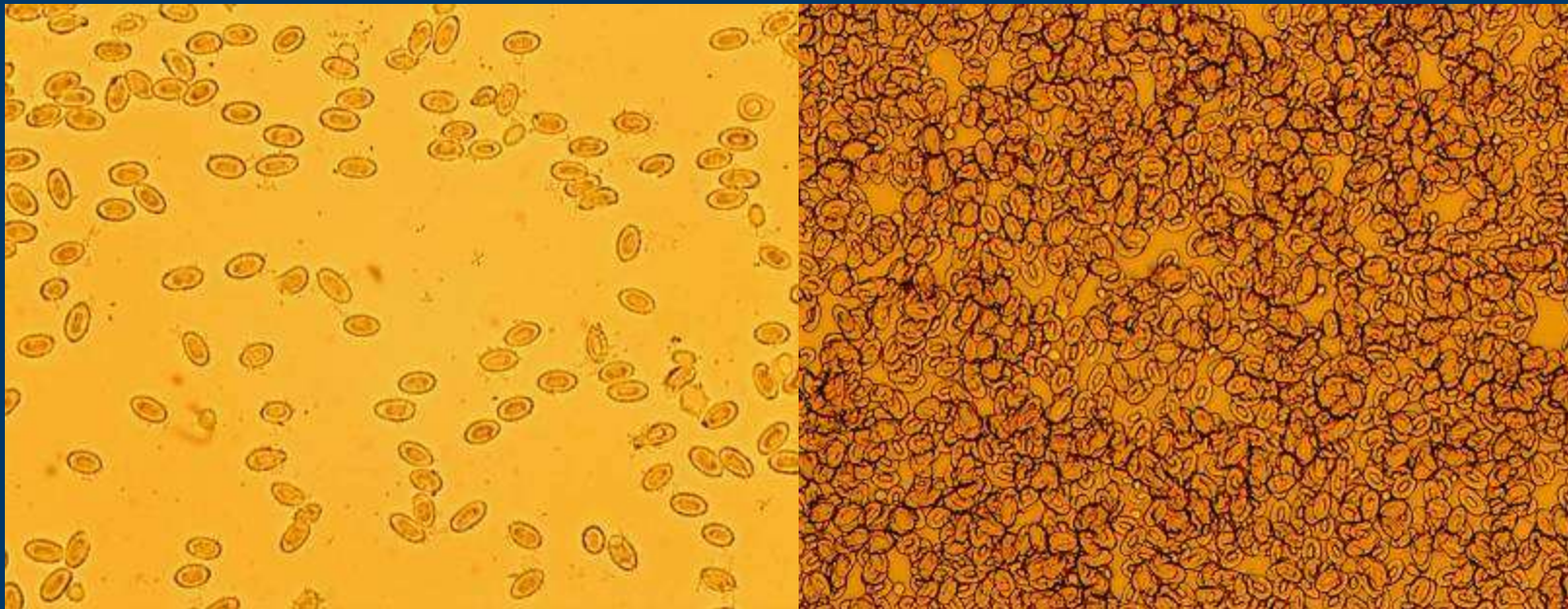
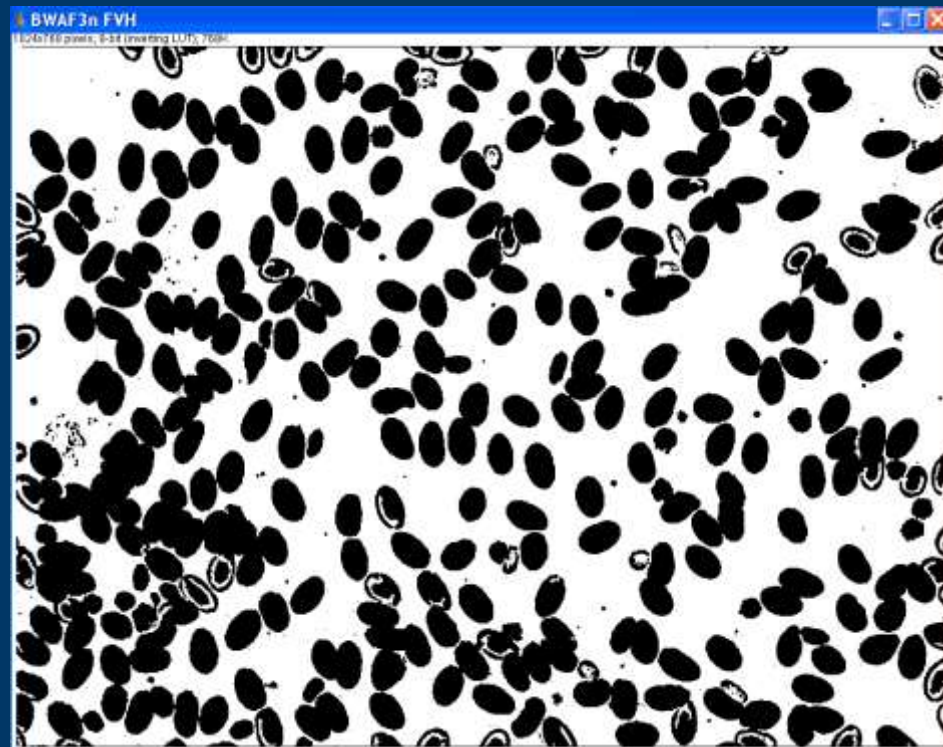# *Parameters*

- Cell area, major/minor axis, circularity

ImageJ Manipulation
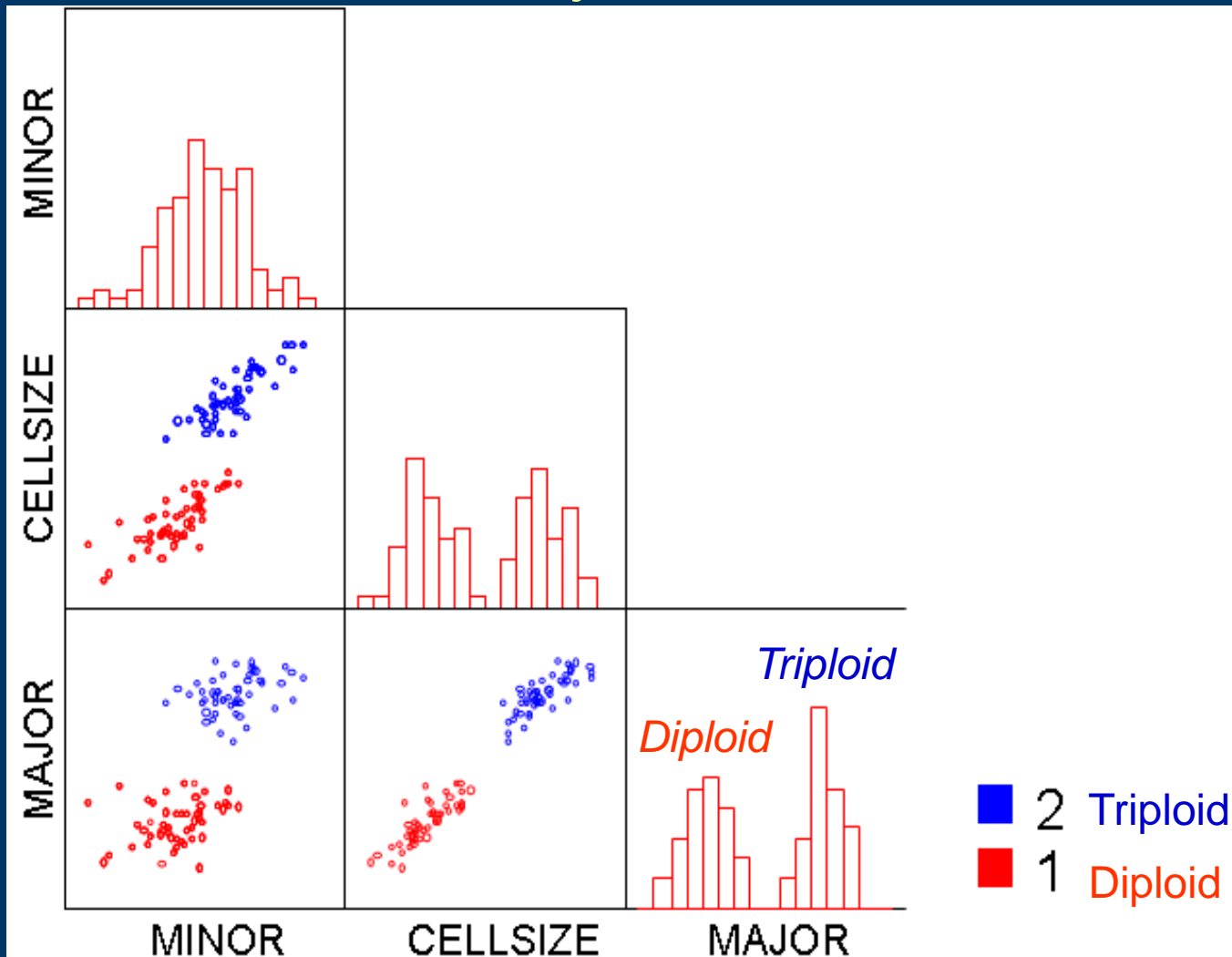
ImageJ Manipulation
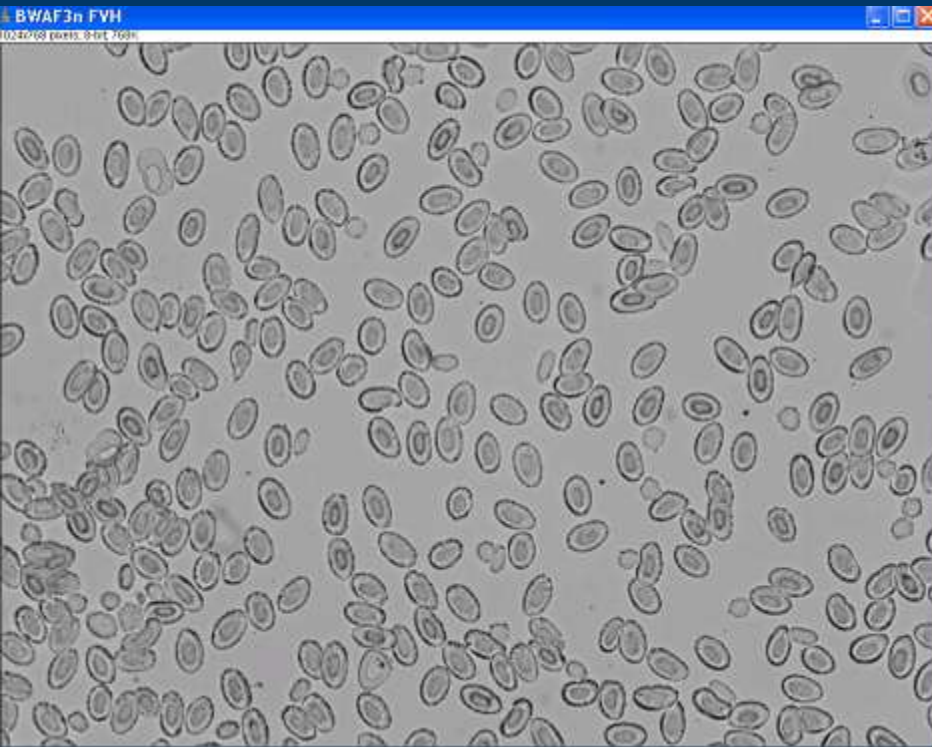
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ImageJ Manipulation

- Output into spreadsheet

- Sort by major axis identify outliers

- Check individual cell measurements

YEAR	CELLID	STRAIN$	AREA	MAJOR	MINOR	CIRC	FISHNO	PLOIDY$
2011	1	BW	187.282	19.064	12.508	0.846	1	3n
2011	2	BW	190.641	18.669	13.002	0.814	1	3n
2011	3	BW	191.313	19.418	12.545	0.82	1	3n
2011	4	BW	207.438	19.94	13.246	0.825	1	3n

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Analyzing

- Import data from both genotypes

- Sort by major axis identify outliers

- Categorizes the sample based on the major axis measurement, 2 cluster analysis

Analysis

- 2 cluster analysis using the long axis

Fish #		1		2		3	
		Freq	Percent	Freq	Percent	Freq	Percent
Cluster	1	0	0.00%	0	0.00%	0	0.00%
	2	56	100.00%	22	100.00%	83	100.00%
	Combined	56	100.00%	22	100.00%	83	100.00%

Flowchart

Blood sample

<20 measurements

>20 measurements

Nuclei Measurements

Software to classify

Error or 2n classification then use Nuclei measurement

2n/3n

2n/3n

Questions

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