OVIZIO IMAGING SYSTEMS

PAT CAN BE EASY.
YOU JUST NEED SMART SOLUTIONS.

A3P BIOPRODUCTION CONGRESS

Philip Mathuis,

Brussels, May 25 2016

QUALITY IS BUILT INTO THE PRODUCT

PROCESS ANALYTICAL TECHNOLOGY

PAT

A system for **designing**, **analyzing**, and **controlling** manufacturing through timely measurements of critical **quality** and performance attributes of raw and in-process materials and processes, with the goal of ensuring **final product quality**.

?

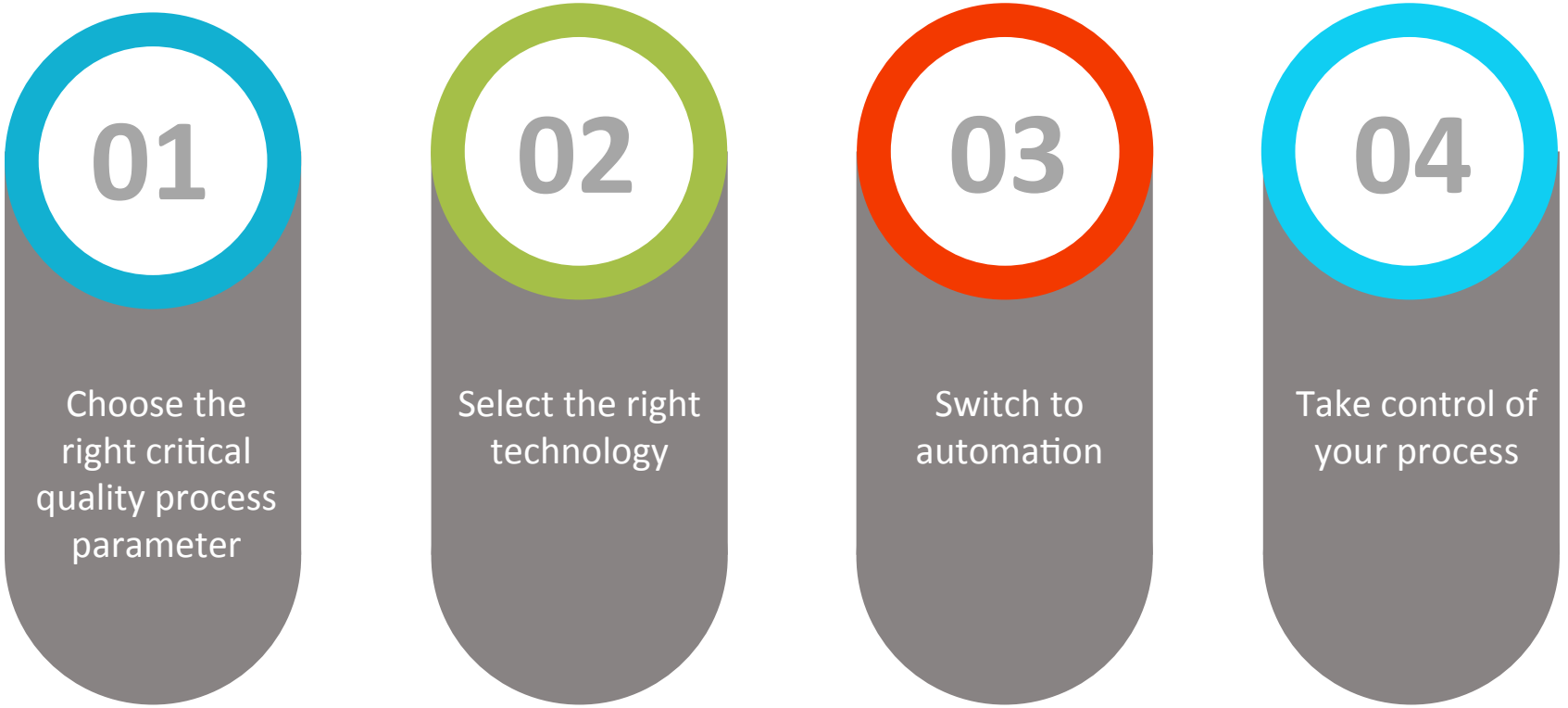
OVIZIO IMAGING SYSTEMS

WHY IMPLEMENTING PAT

- Product quality, low variability
- Prevent rejects & reprocessing
- Real-time release
- Time & cost reduction

REALITY IS…

IMPLEMENTING PAT IN 4 STEPS

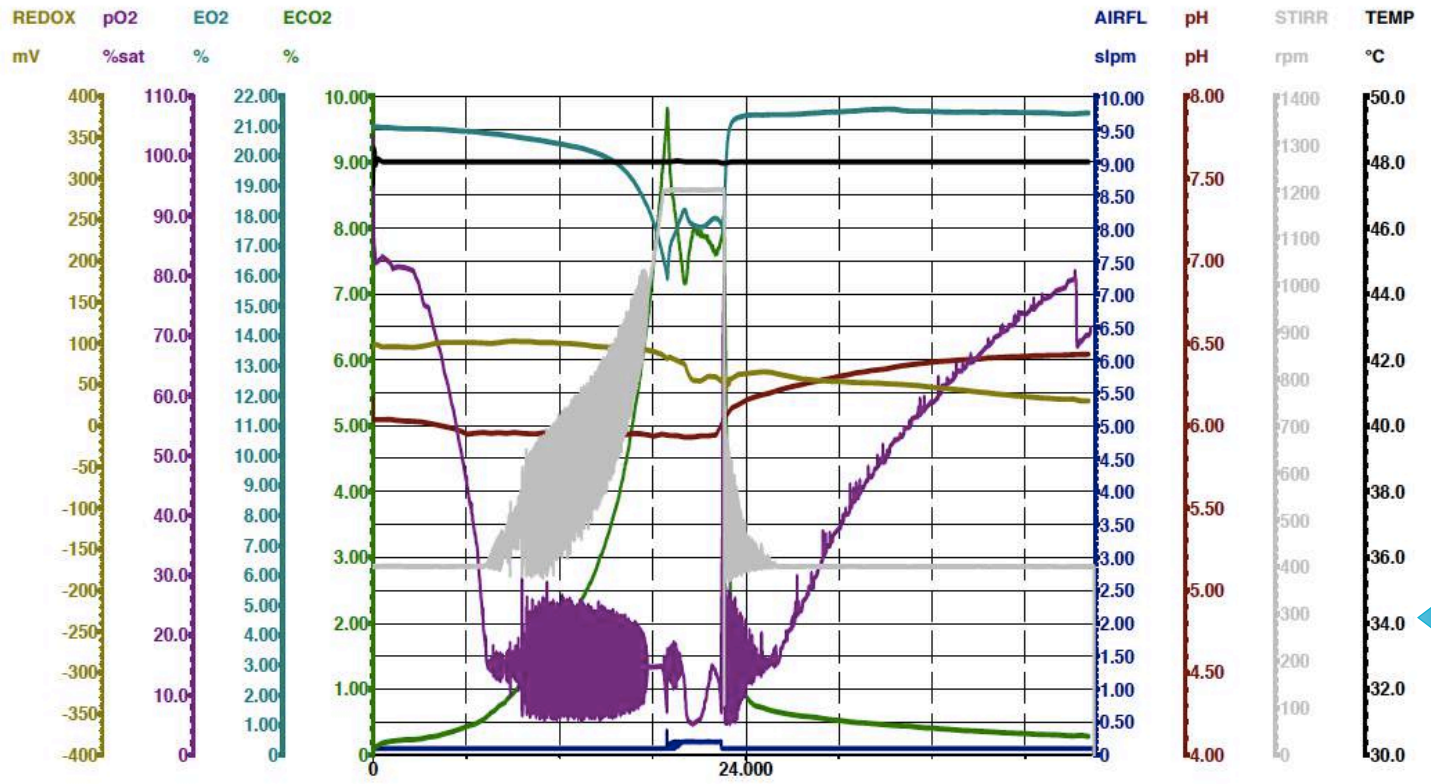


OVIZIO IMAGING SYSTEMS

STEP 1 – CHOOSE THE RIGHT CRITICAL QUALITY PROCESS PARAMETER

THE “PROBLEM” IS VARIABILITY, W. Edwards Deming’s

CELL CULTURE MONITORING



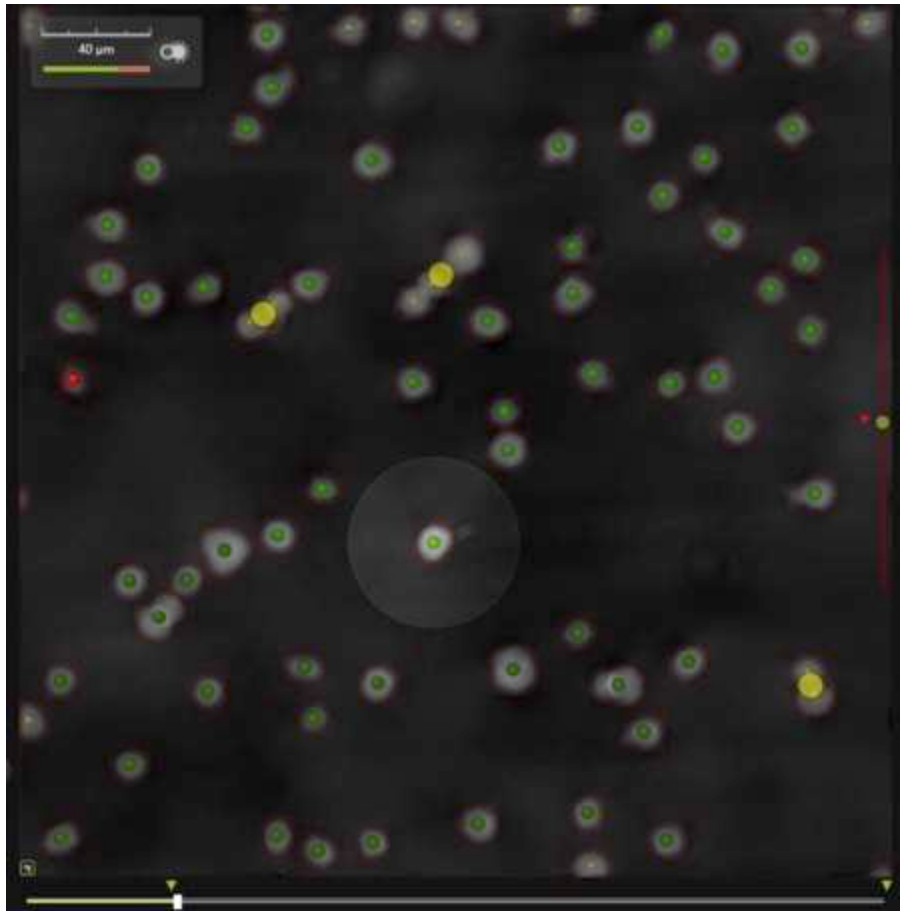
ROUTINE MEASURES

- pH
- Dissolved Oxygen
- Temperature
- Pressure
- Metabolites
- Cell density

Classical sensors measure a product parameter shown to directly affect the product

OVIZIO IMAGING SYSTEMS

SINGLE CELL MONITORING



CELL BEHAVIOR

- Culture health status
- Quantify volumetric productivity
- Specific production rate
- Feed/Harvest time
- Document the process

Looking at the cells helps anticipate the variability in product quality

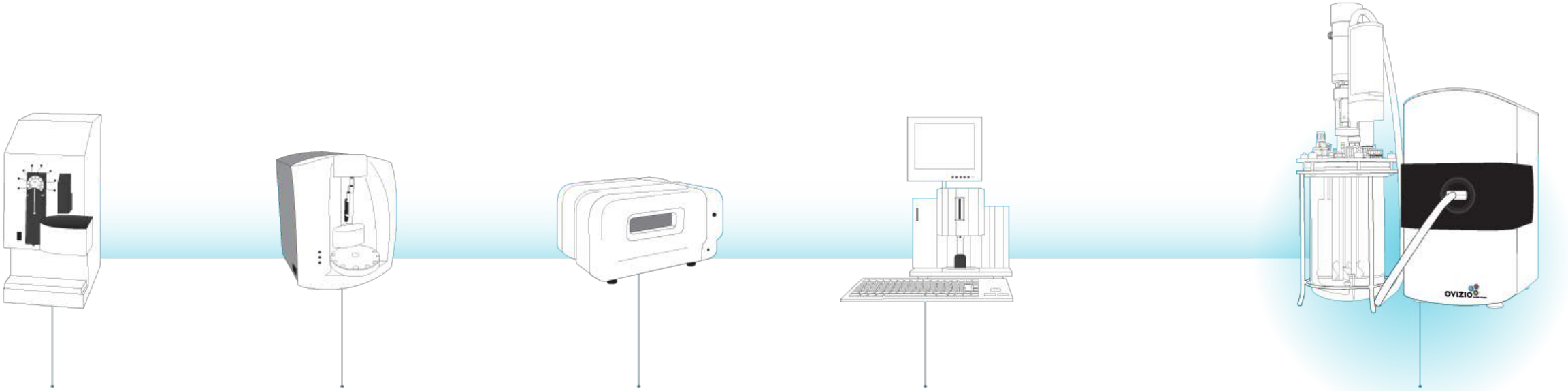
OVIZIO IMAGING SYSTEMS

OVIZIO IMAGING SYSTEMS

STEP 2 – SELECT THE RIGHT TECHNOLOGY

SMART CELL CULTURE MONITORING

THE ESTABLISHED METHODS



2000
Trypan blue

2004
Automated Trypan blue

2005
Fluorescence illumination

2008
Coulter counter

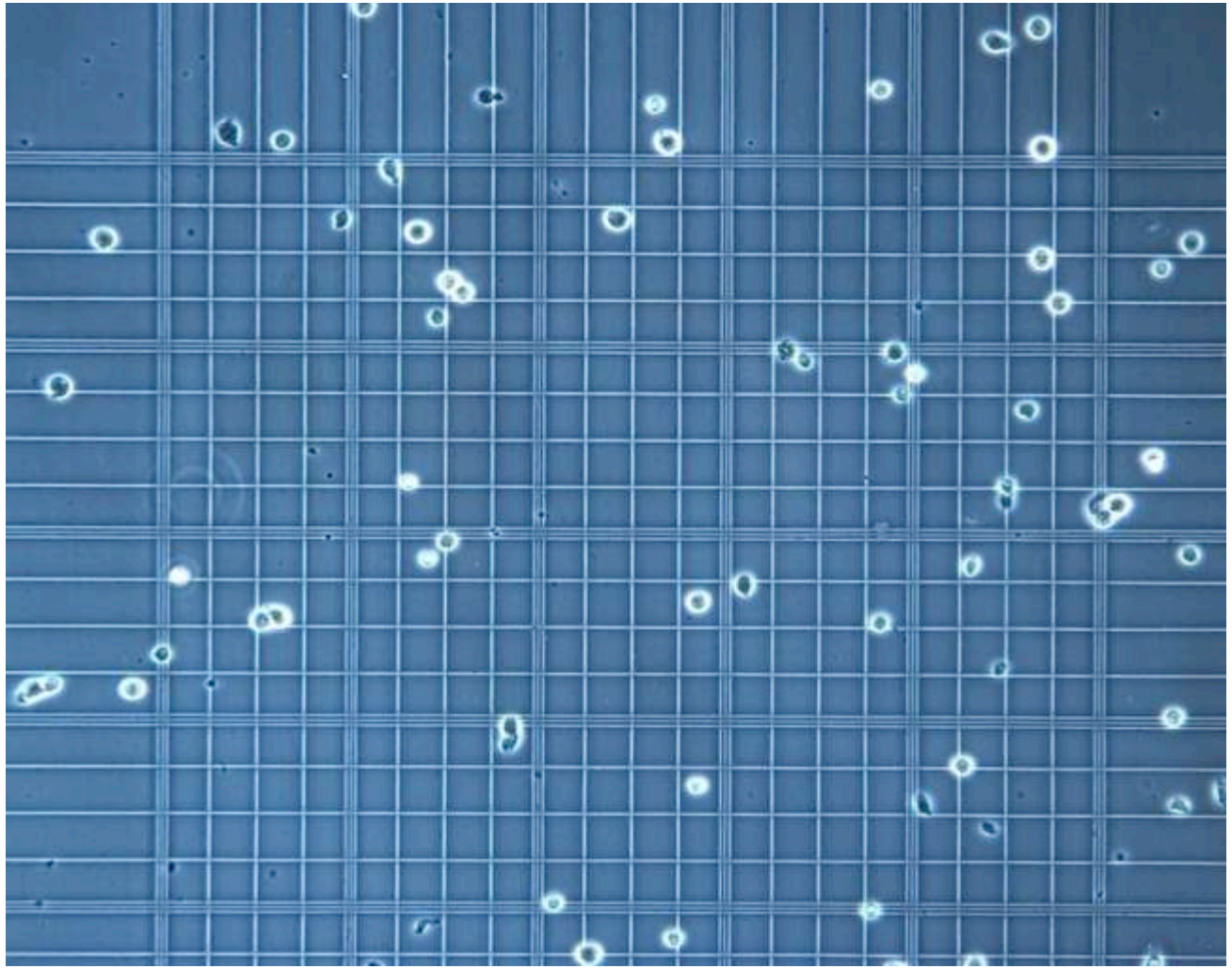
2015
In-line microscopy

THE **TRYPAN BLUE DYE** EXCLUSION METHOD

The standard method to monitor and document cell concentration in biotech research and production processes.

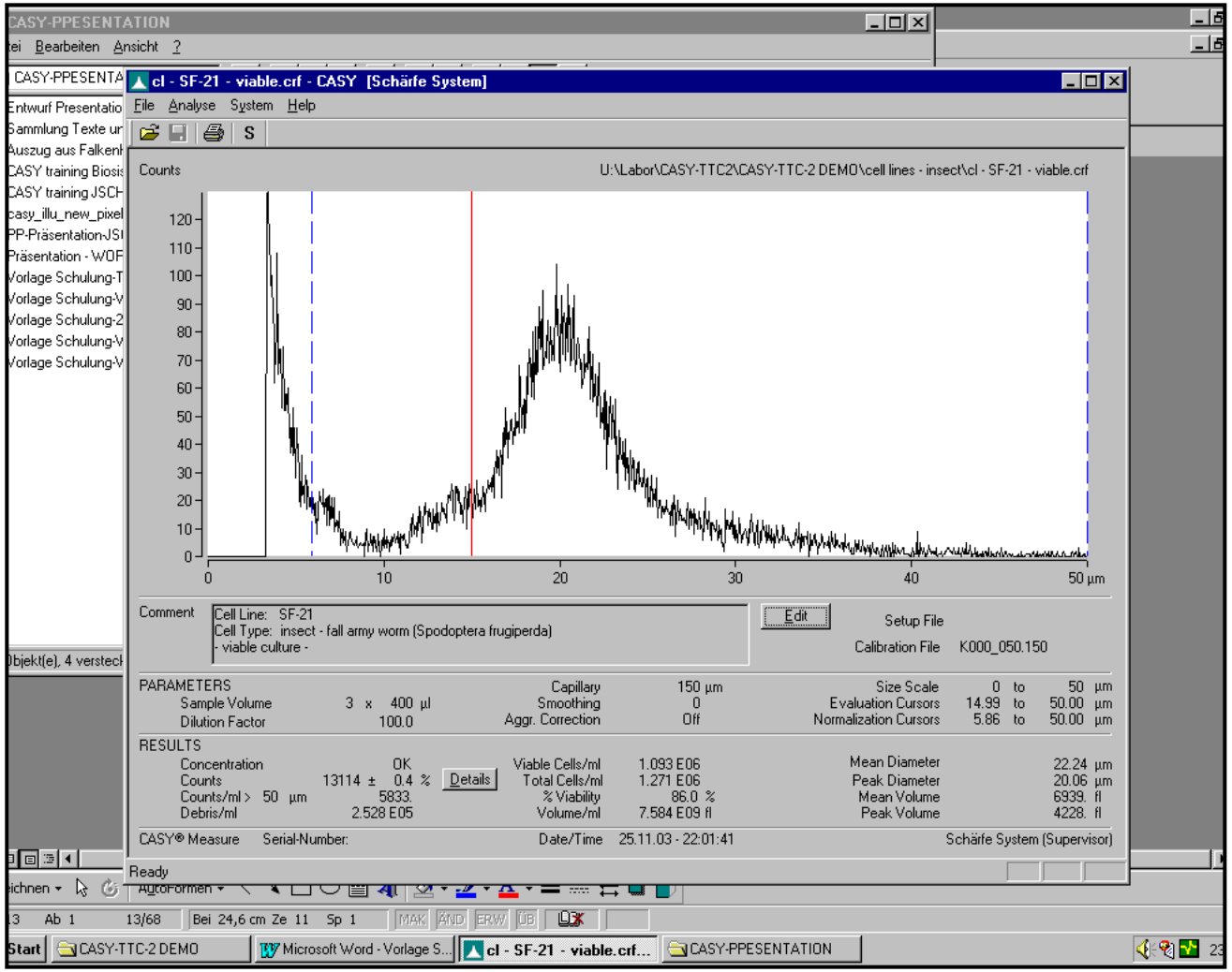
- Subjective
- User-dependent
- Low precision
- Poor accuracy

HOW MANY VIABLE AND DEAD CELLS DO YOU COUNT?



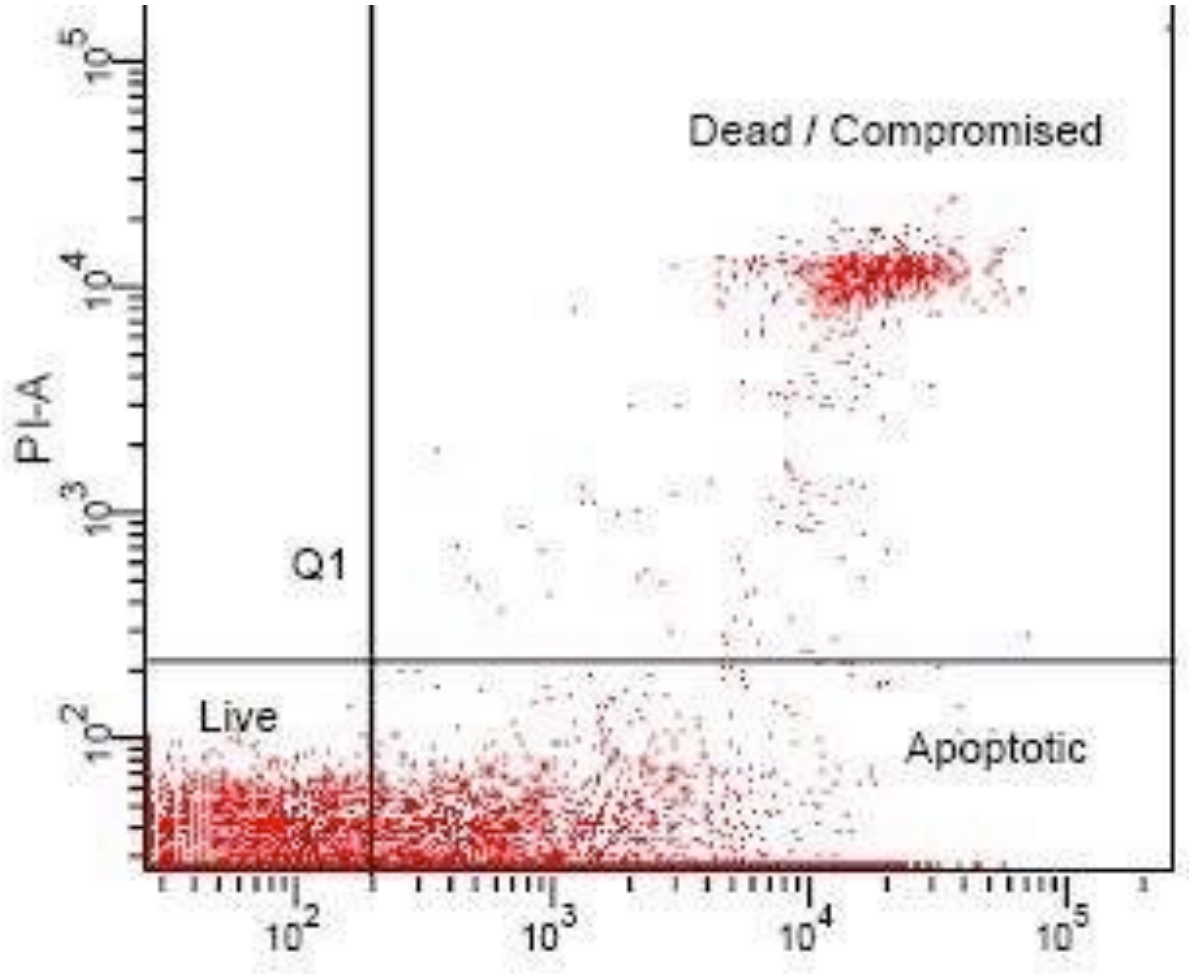
AUTOMATED CELL COUNTING – **CELL COULTER COUNTER**

- No defined separation between dead & viable cells
- User needs to interpret data to get numbers
- Results are user-settings dependent
- Dilution required >10m cells/ml
- Poor accuracy and reproducibility



AUTOMATED CELL COUNTING - **FACS**

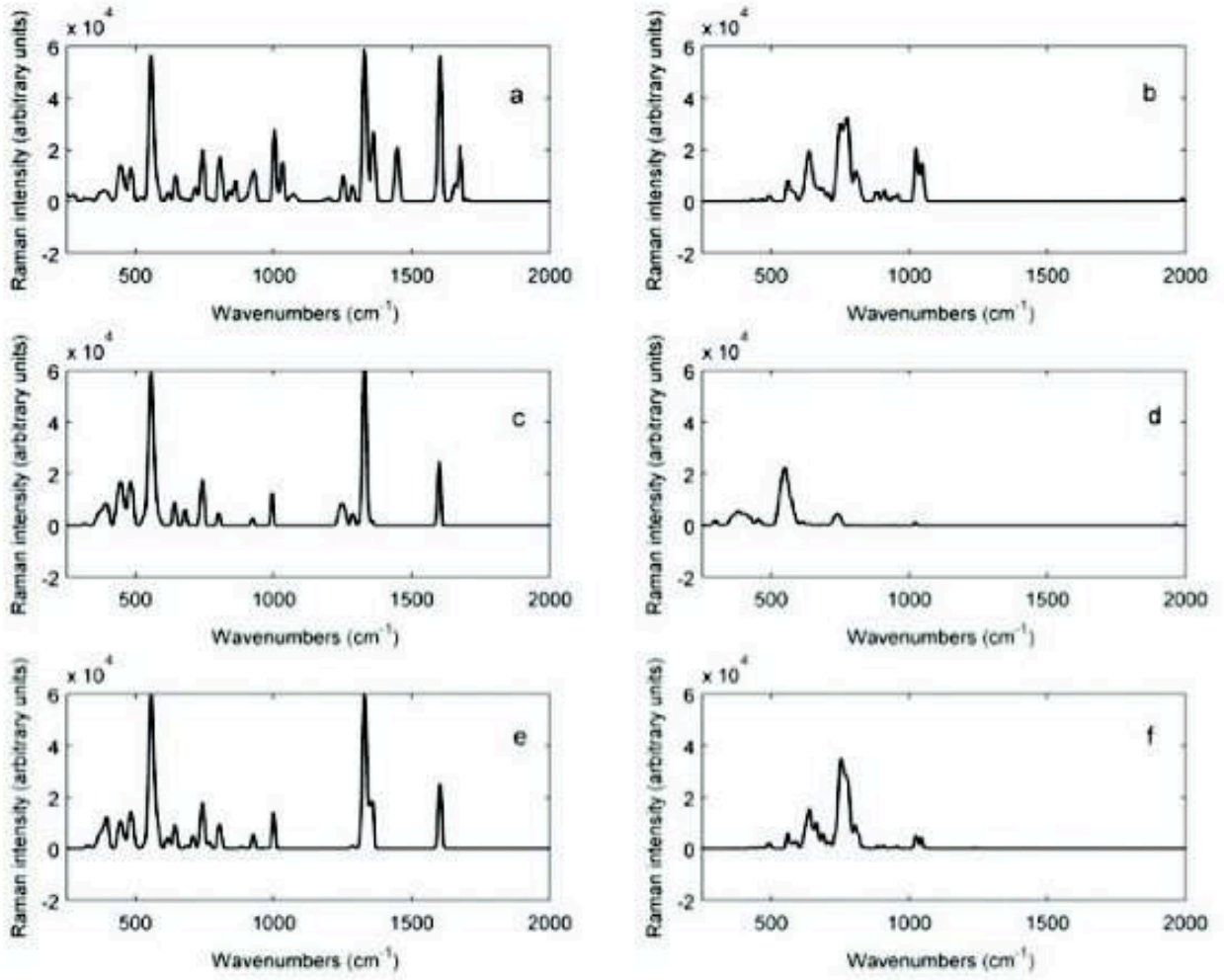
- No defined separation of dead, apoptotic and viable cells.
- User needs to interpret data to get numbers.
- Definition of different areas gives different results.



IN SITU MONITORING

Some in-situ spectroscopy techniques have been developed to monitor the status of the culture looking mostly at the metabolites generated by cells.

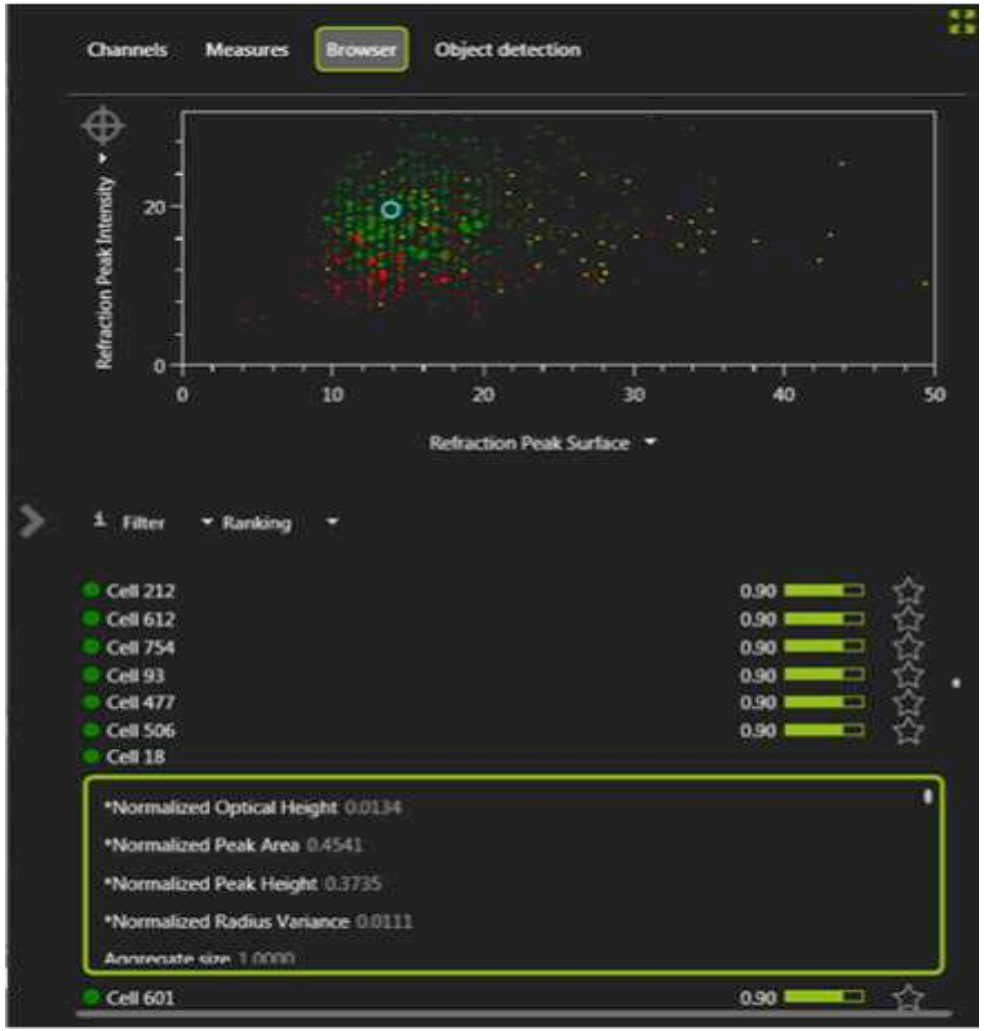
- Not always reliable measures due to complex interactions caused by cell debris and bubbles.
- Only viable cell density



SMART
MONITORING

Measure and track data per single cell

- Anticipate cell health status
- Detect cell debris
- Differentiate dead and living cells
- Statistical analysis on the cell population to define and control critical process parameters



STEP 3 - SWITCH TO AUTOMATION

IMPLEMENT PAT IN YOUR PROCESS

MANUAL CELL COUNTING STEPS

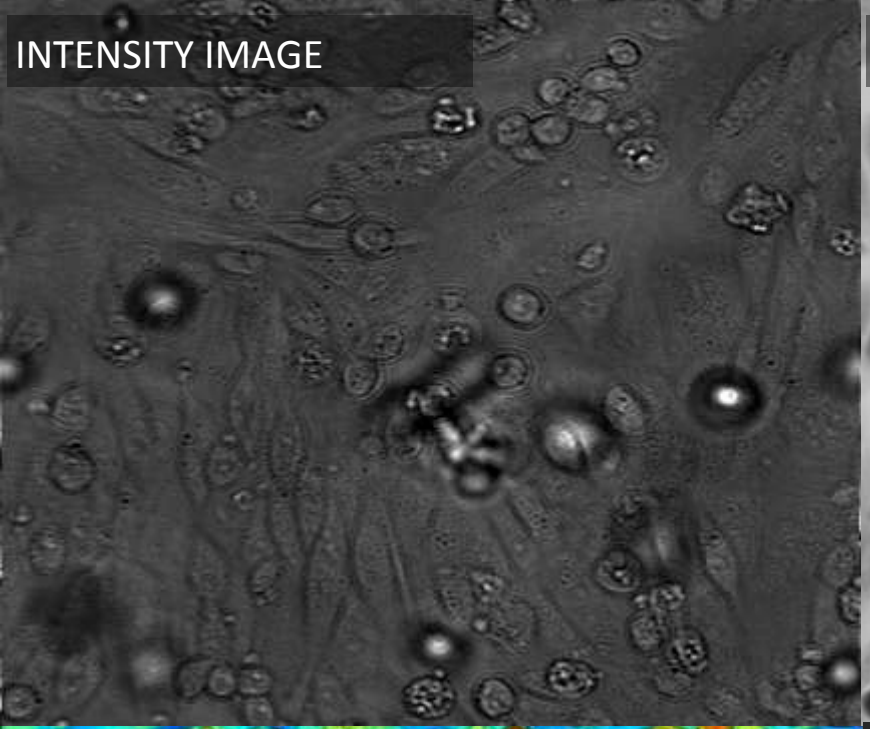
SAMPLE	PREPARE	COUNT
DETACH OR SAMPLE	DILUTE – SUSPEND – MIX – ADD TRYPAN BLUE – GENTLY MIX – WAIT 5 MINUTES – WASH CHAMBER – WASH COVER SLIDE – APPLY CELLS TO CHAMBER – WAIT 1-2 MINUTES → **10 STEPS**	COUNT UNDER MICROSCOPE – COMPUTE CELL COUNT – COMPUTE % VIABLE CELLS

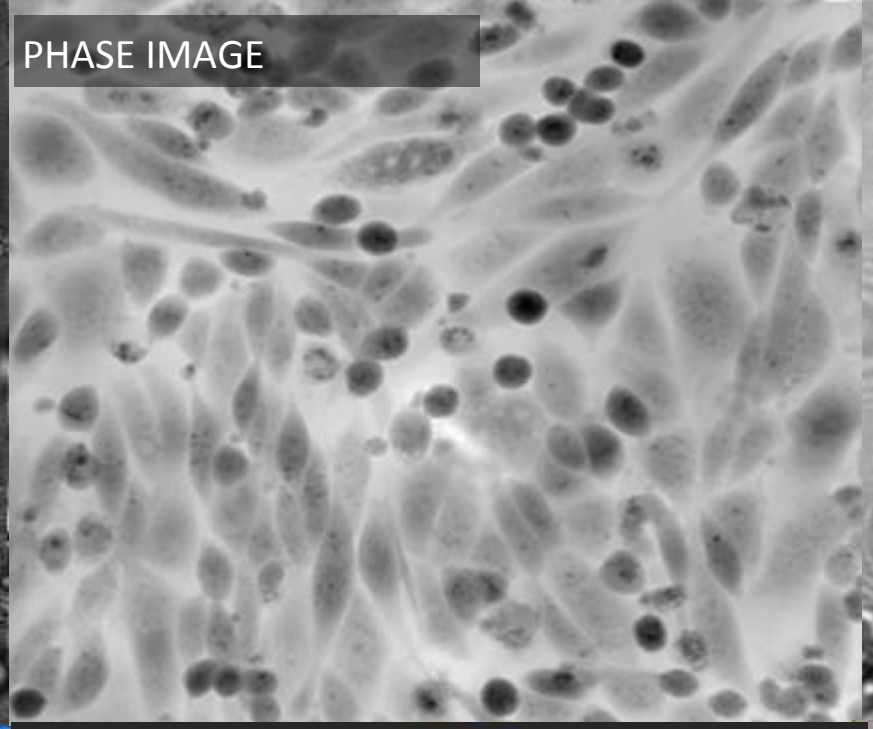
AUTOMATIC CELL COUNTING STEPS

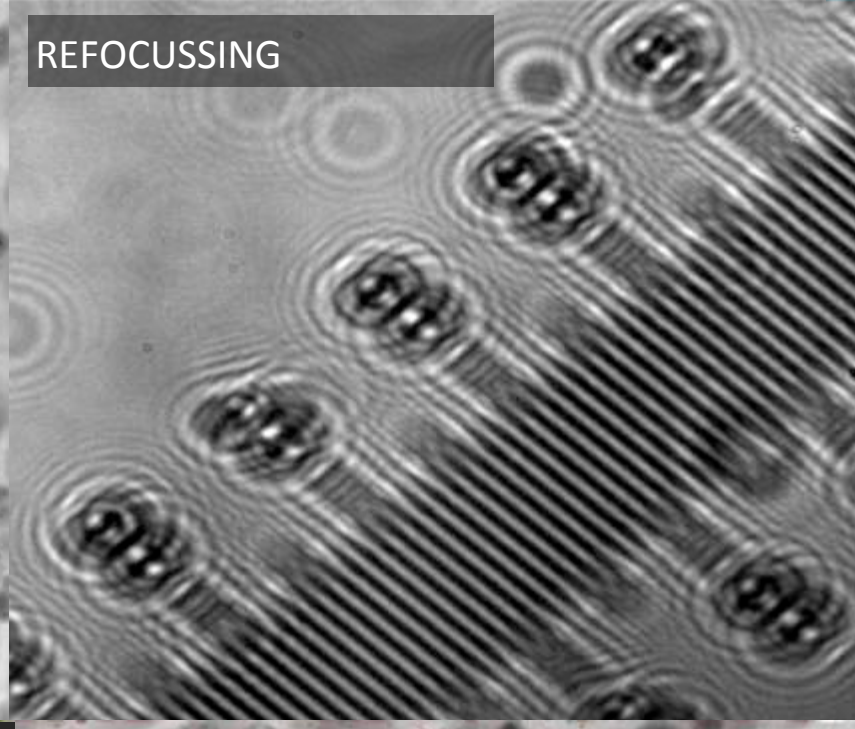
SAMPLE	PREPARE	COUNT
	DILUTE – SUSPEND – ADD TO SAMPLE VIAL – ADD REAGENTS → **4 STEPS**	INSERT VIAL IN COUNTER – START ANALYSES – RESULTS ON SCREEN

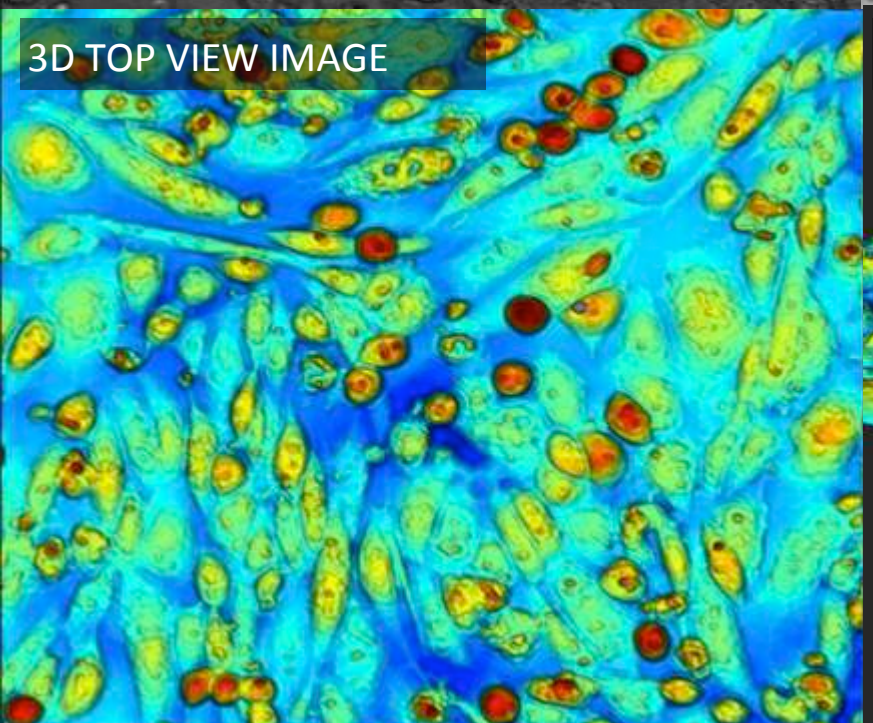
OVIZIO CELL COUNTING STEPS

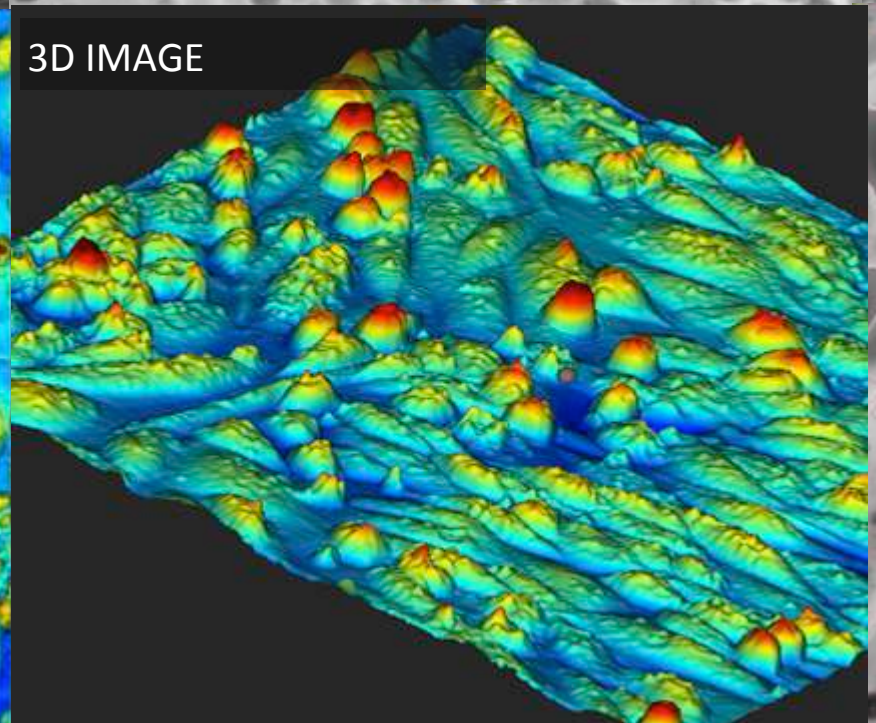
SAMPLE	PREPARE	COUNT
		IN LINE OR PLACE RECIPIENT ON STAGE – START ANALYSES – RESULTS ON SCREEN

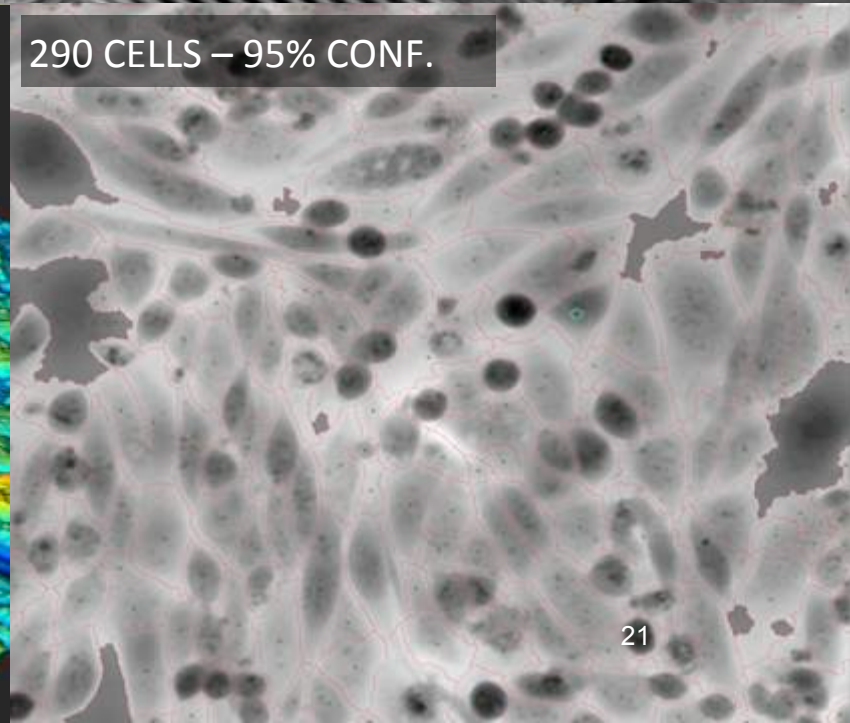

INTENSITY IMAGE


PHASE IMAGE

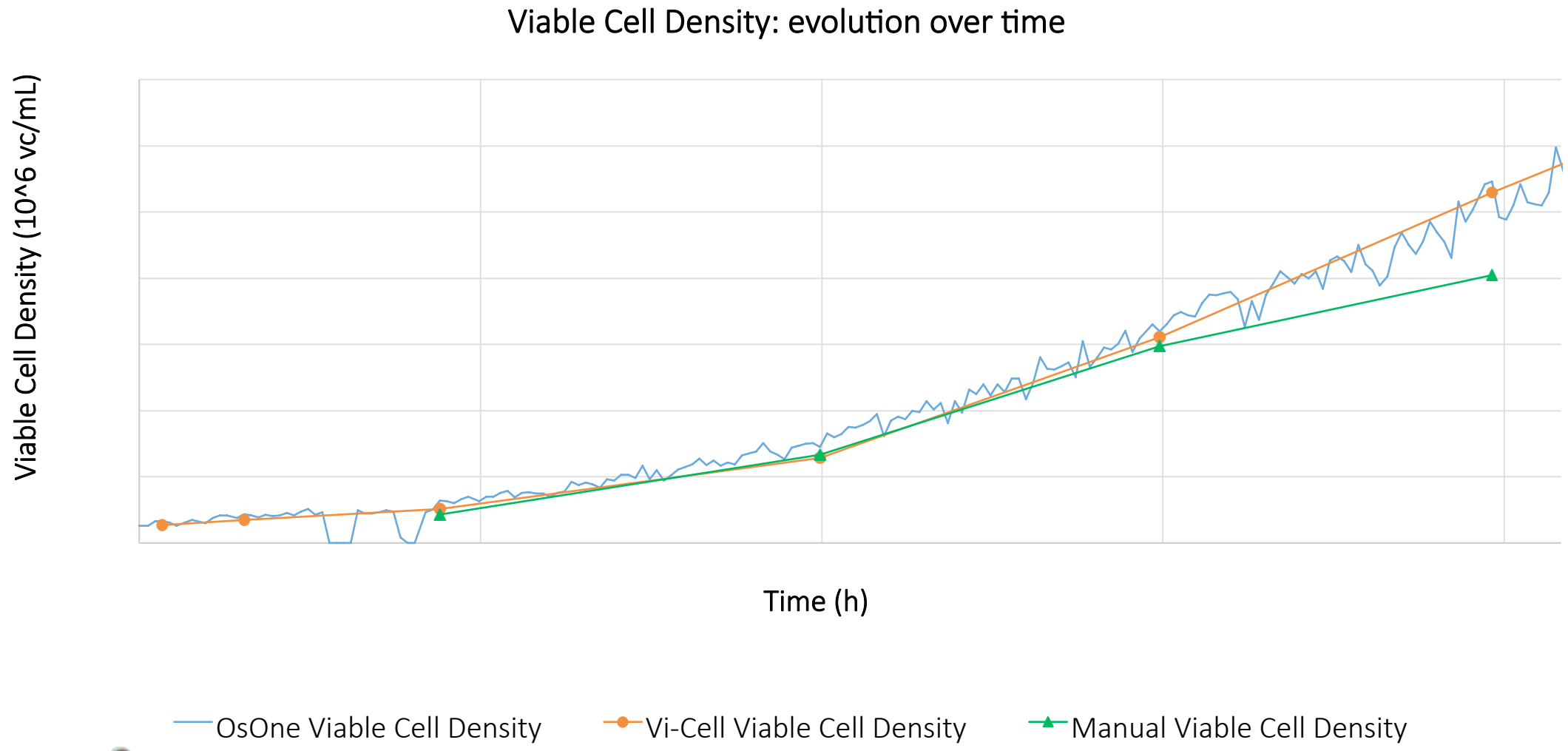

REFOCUSSING


3D TOP VIEW IMAGE


3D IMAGE

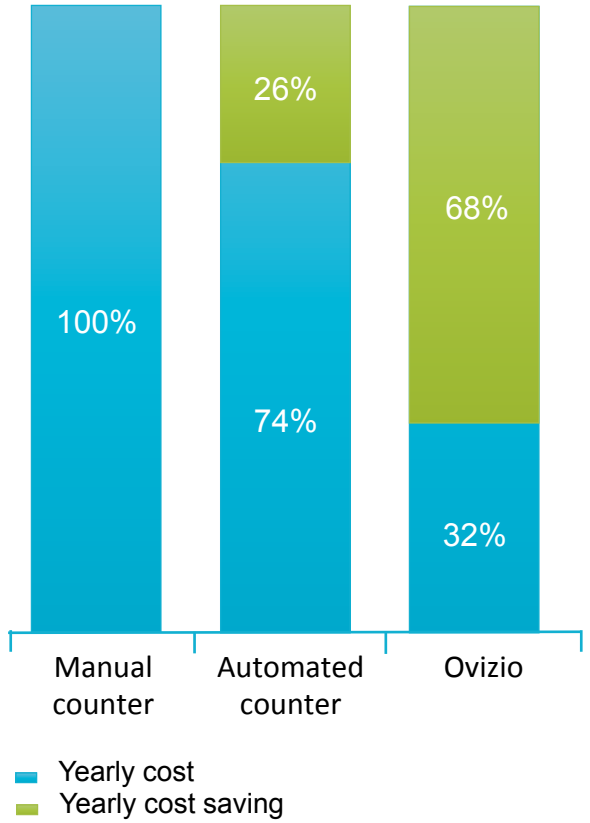

290 CELLS – 95% CONF.

A ROBUST PAT TOOL - COMPARABLE TO ROUTINE OFF LINE LAB RESULTS



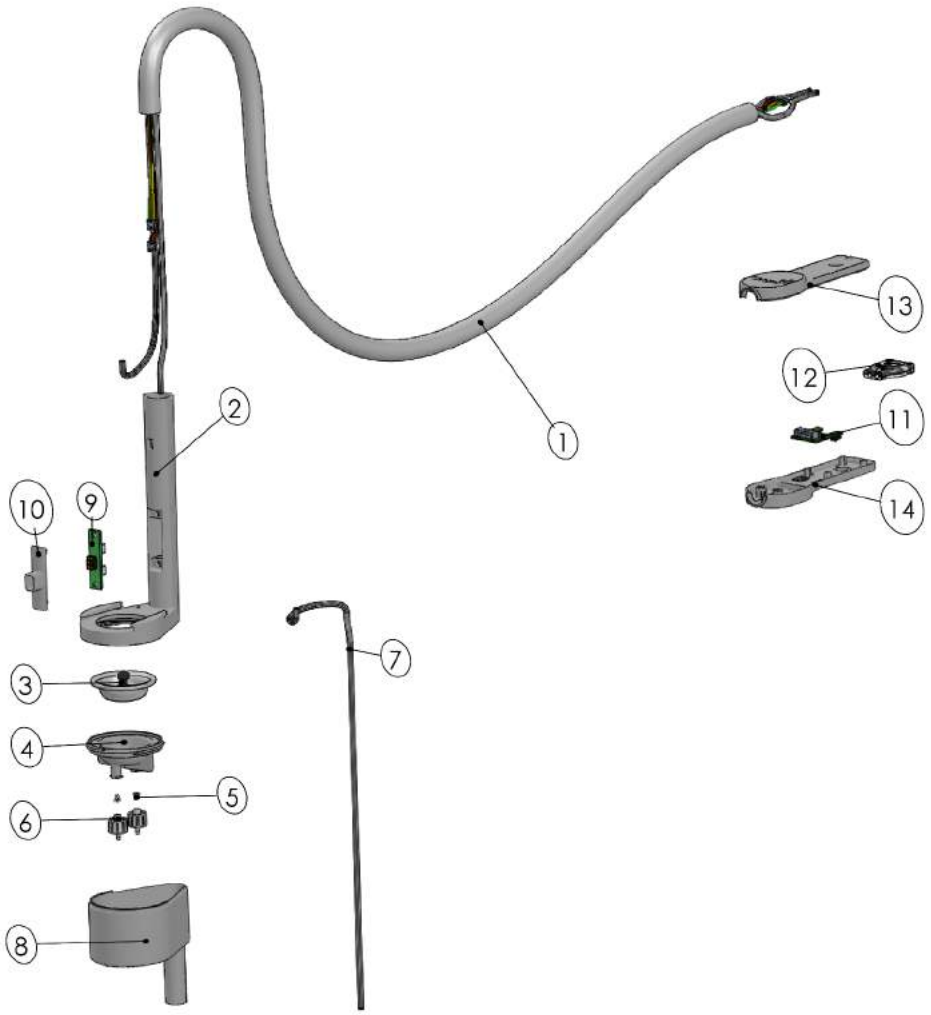
VALUE OF SMART MONITORING

- INCREASED CONTROL
- TIME GAIN AND TRACEABILITY OF RESULTS
- INCREASED REPRODUCIBILITY
- DRASTIC REDUCTION OF MANUAL OPERATIONS
- REDUCED INVESTMENT AND FTE COST - Fast ROI



BASE ASSUMPTIONS

- 200 WORKING DAYS
- 15 SAMPLES PER DAY
- 46 K€ COST OF LABOR PER YEAR
- 20% OVERHEAD
- 5 YEAR AMORTIZATION



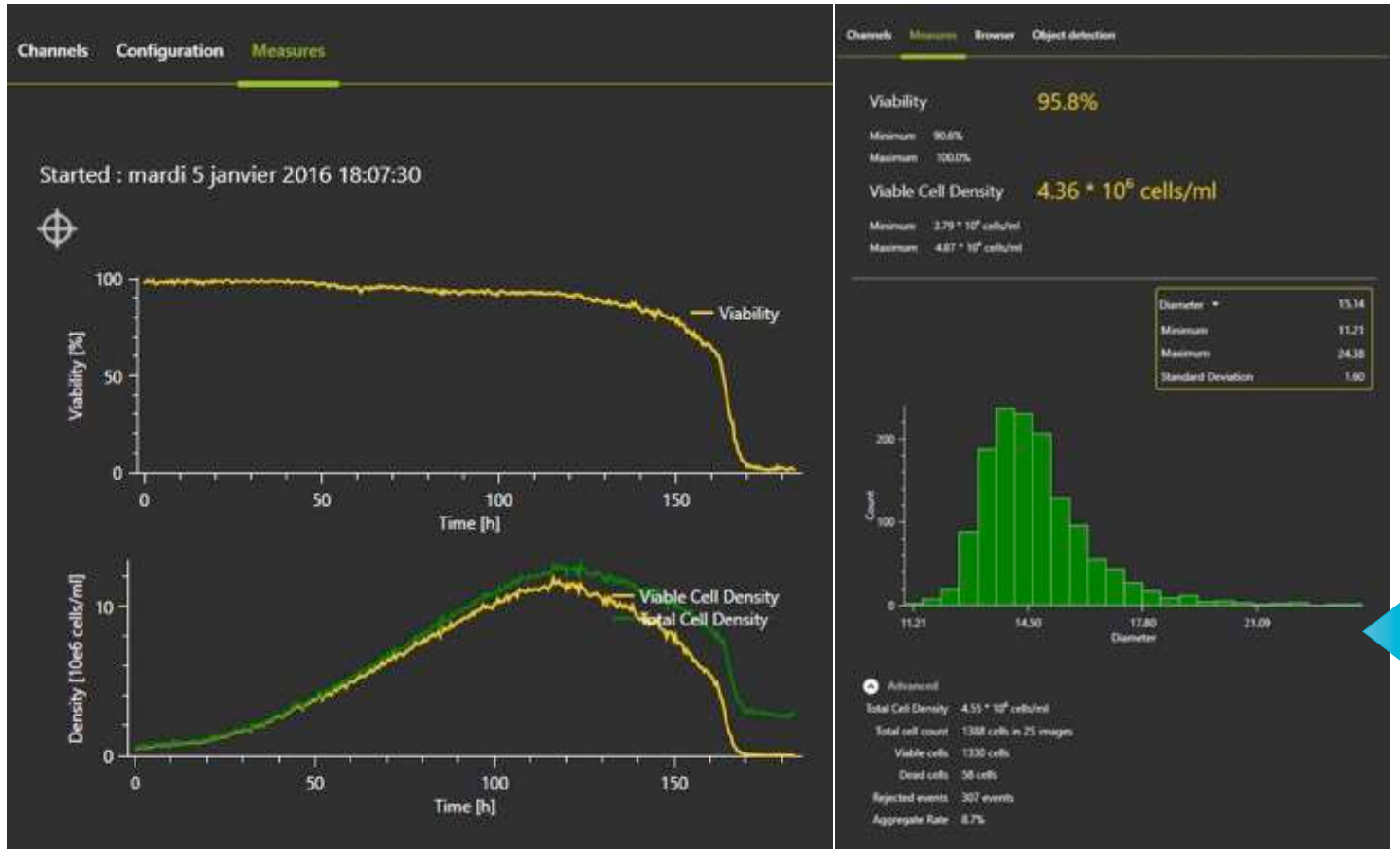
STEP 4 - TAKE CONTROL OF YOUR PROCESS

CONTINUOUS MONITORING AND CLOSED LOOP CONTROL OF YOUR PROCESS

CONTINUOUS MONITORING

For the whole experiment

By time point



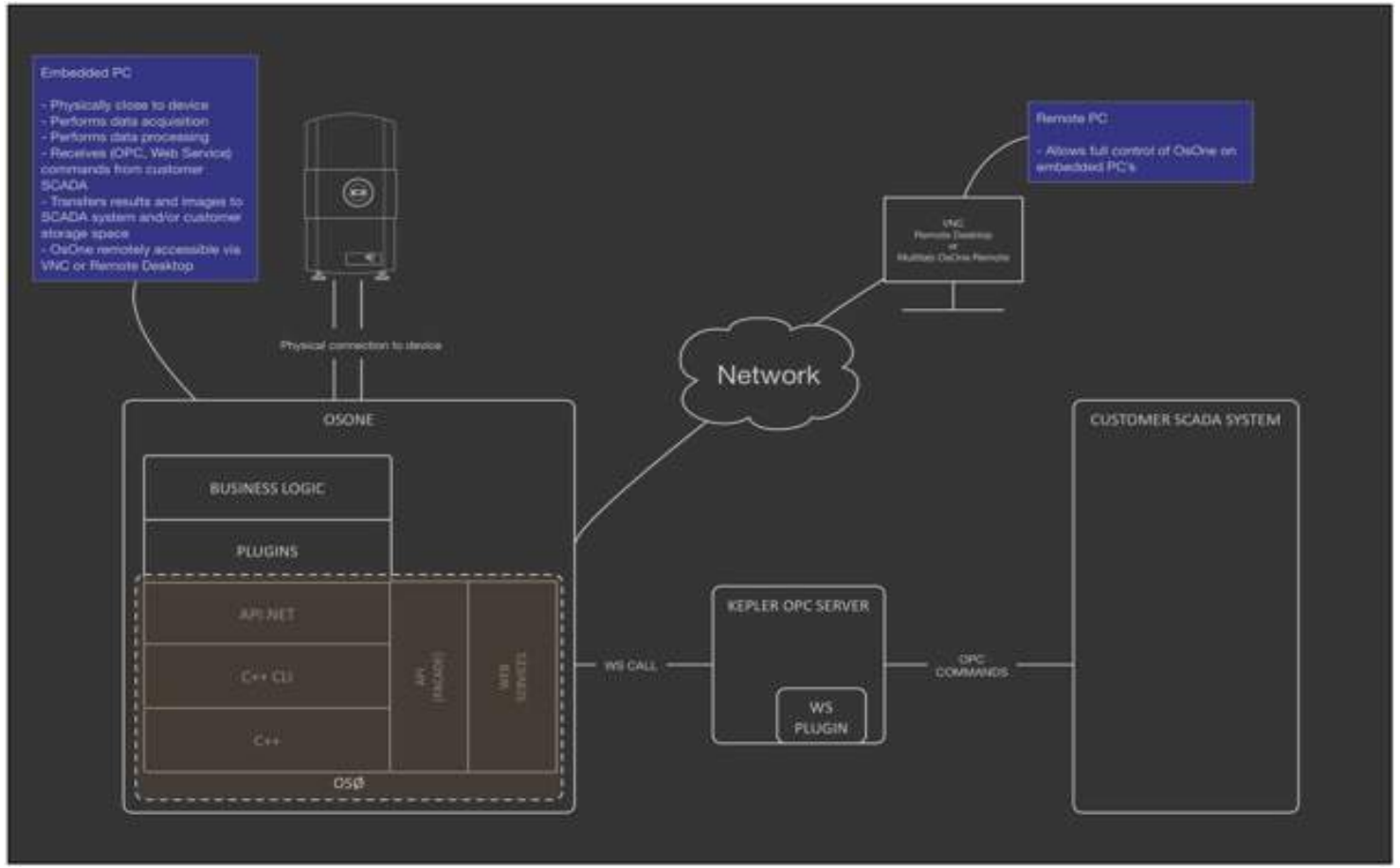
CLOSED LOOP CONTROL OF THE BIOPROCESS

- Retro-control
- Feeding time
- Harvest time

The process performance could be improved by direct adaptation of the culture parameters.

OVIZIO IMAGING SYSTEMS

OPC INTEGRATION

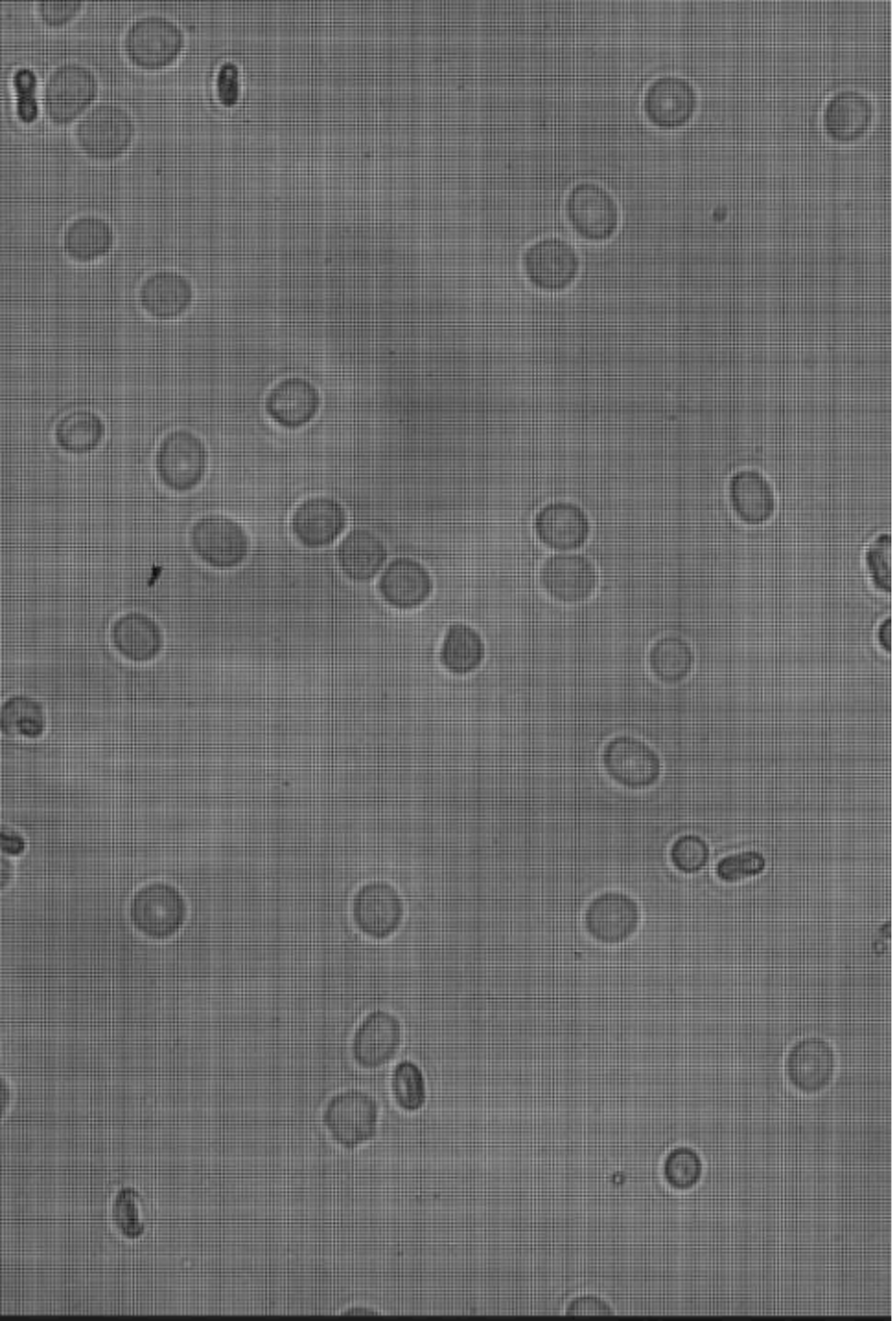


ADVANCED PROCESS CONTROL

In development:

- Central control server collecting data from several iLine-F simultaneously
- API (Application Programming Interface) to integrate into other Processes Information Management Systems

OVIZIO IMAGING SYSTEMS



OVIZIO IMAGING SYSTEMS

CASE STUDY

CLOSED MANUFACTURING SYSTEM

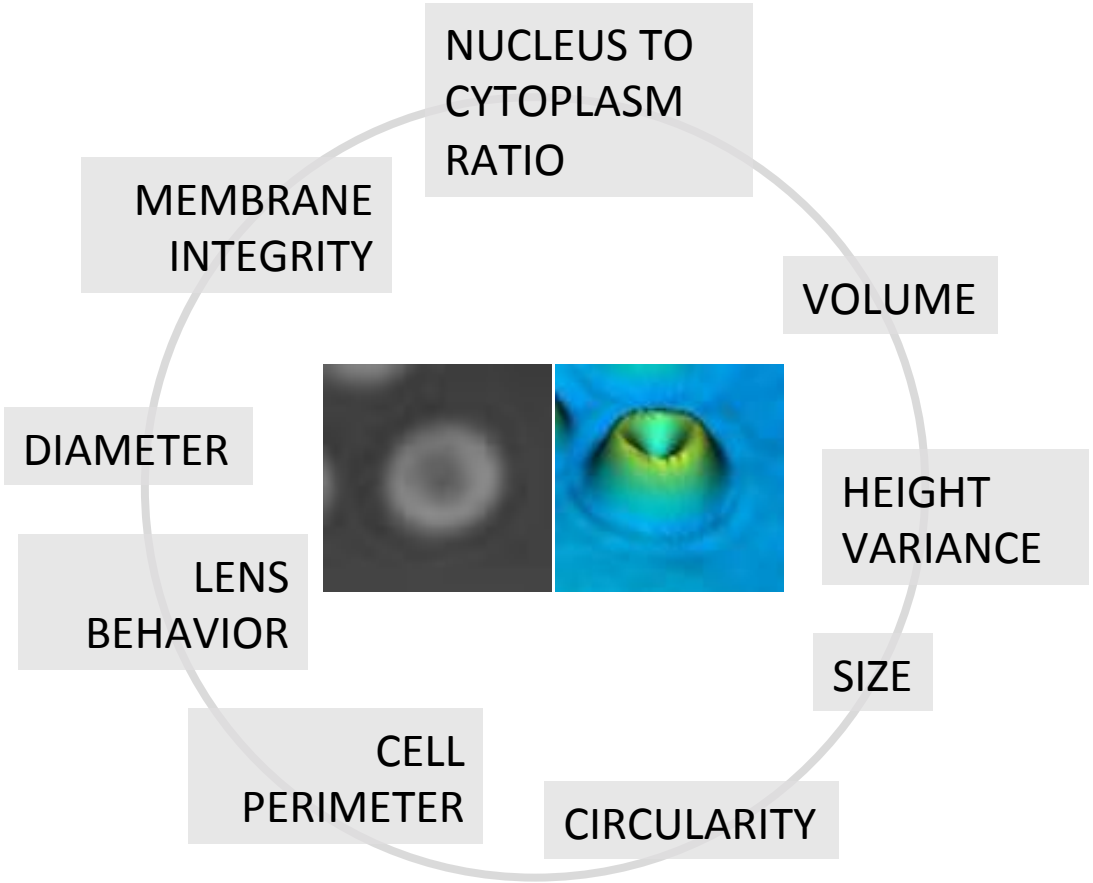
CASE STUDY – DISCRIMINATE CELL TYPES

- US CUSTOMER
- IMMUNOTHERAPY
- ENHANCED DENDRITIC CELLS
- 6 – DAY PROCESS

- IN-PROCESS COUNTING OF VIABLE CELL DENSITY OF:
  - RED BLOOD CELLS
  - GRANULOCYTES
  - LYMPHOCYTES
  - DENDRITIC CELLS
- NO LOSS OF SAMPLE VOLUME

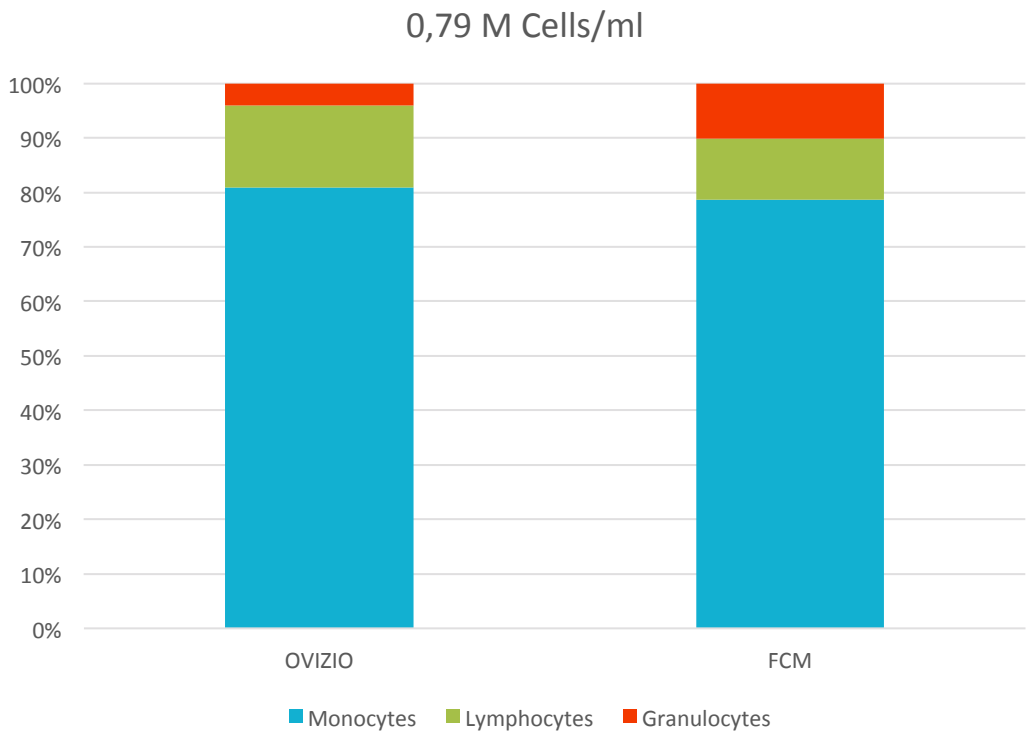
CRITICAL PARAMETERS HERE ARE TO MONITOR CELL CHARACTERISTICS

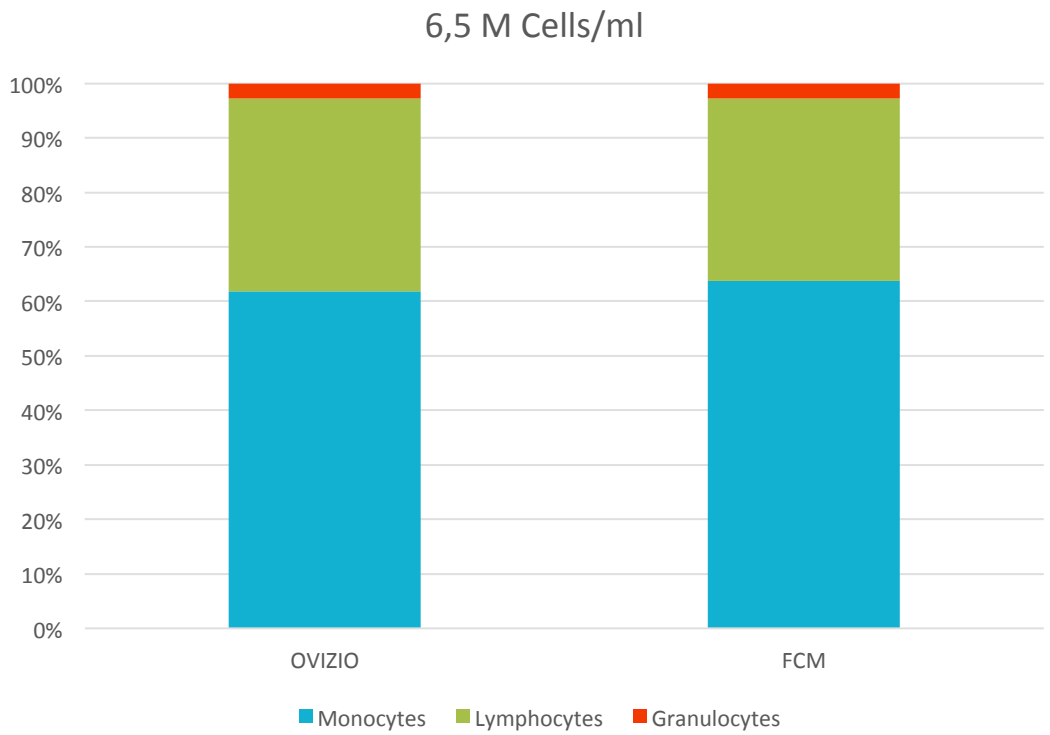
HOLOGRAPHIC FINGERPRINT SHAZAM™ FOR CELLS



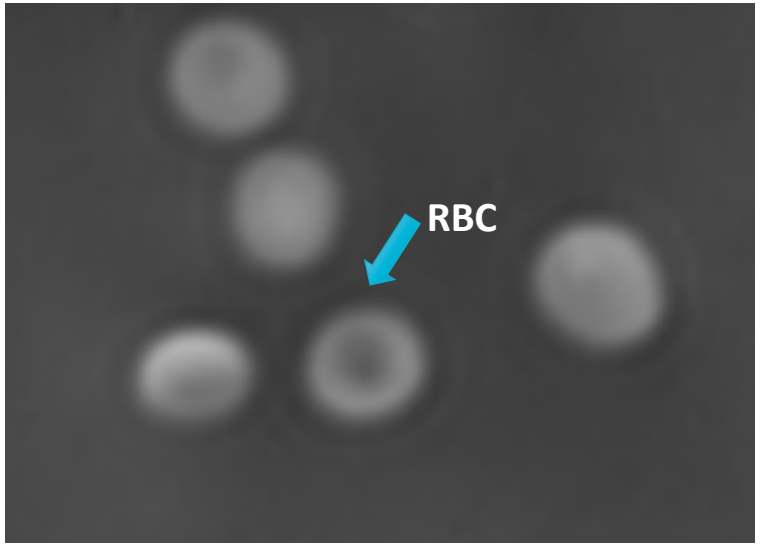
59 PARAMETERS ARE RECORD PER CELL – MACHINE LEARNING

CASE STUDY - RESULTS

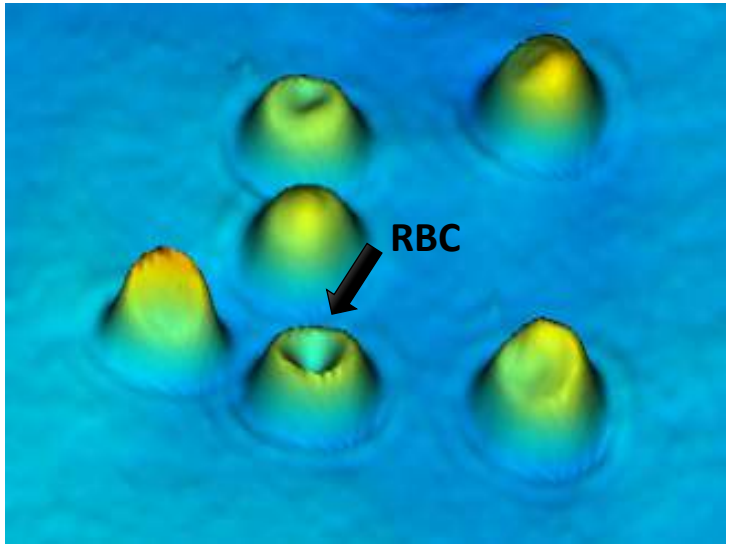




CASE STUDY - CONCLUSION



Phase image - Red Blood Cell



3D View of the phase image
"Donut" shape of Red Blood Cell

RESULTS

- Cell types differentiation
- Viable cell counting
- Quality Control

Ovizio's technology can be used in a closed manufacturing systems

OVIZIO IMAGING SYSTEMS

OVIZIO IMAGING SYSTEMS

CONCLUSION

PAT can be easy with Smart solutions.

- Simplify measurements
- Automate analysis
- Facilitate electronic batch record

IF YOU WANT TO TRY THE SYSTEM SEND MAIL TO:
info@ovizio.com