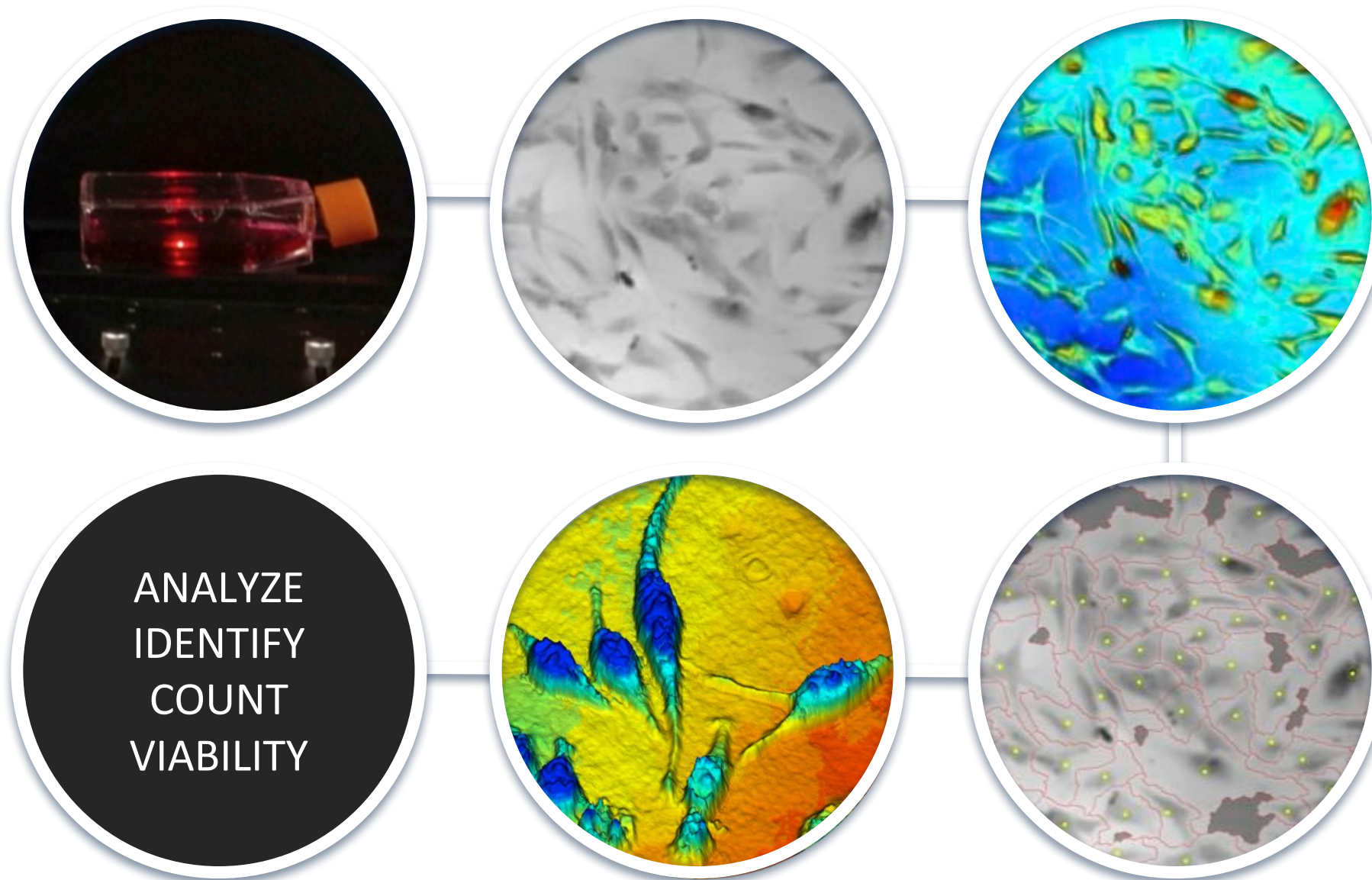


CELL COUNTING WORKSHOP NIST



Philip Mathuis April 10, 2017

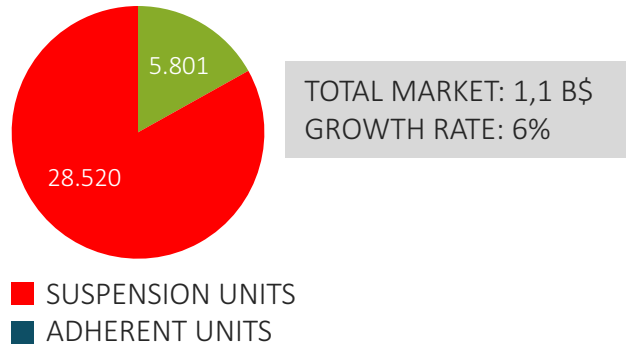
FROM IMAGE TO ACTION



STRICTLY CONFIDENTIAL

BACKGROUND

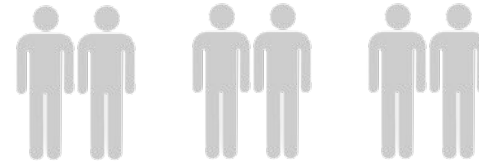
BEACHHEAD MARKET BIOPROCESSING



PEOPLE AND ECOSYSTEM

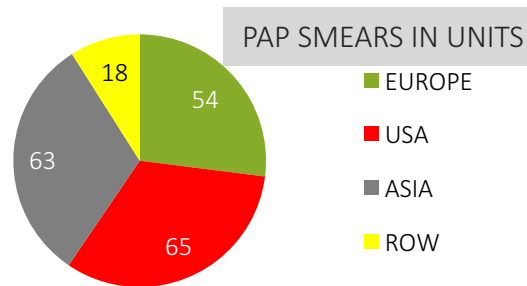


16 OVIZIO



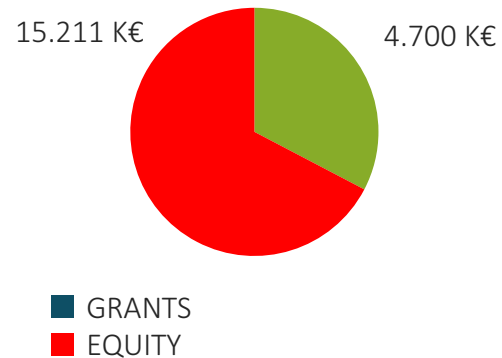
RESEARCH ECOSYSTEM

DIAGNOSTICS UPSIDE

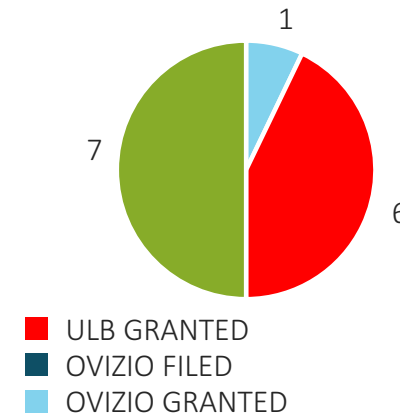


TOTAL MARKET: 3,46B\$
TOTAL # OF TESTS: 200 M
COST PER SMEAR: 18 – 30\$
GROWTH RATE: 4%

FUNDING



PATENTS



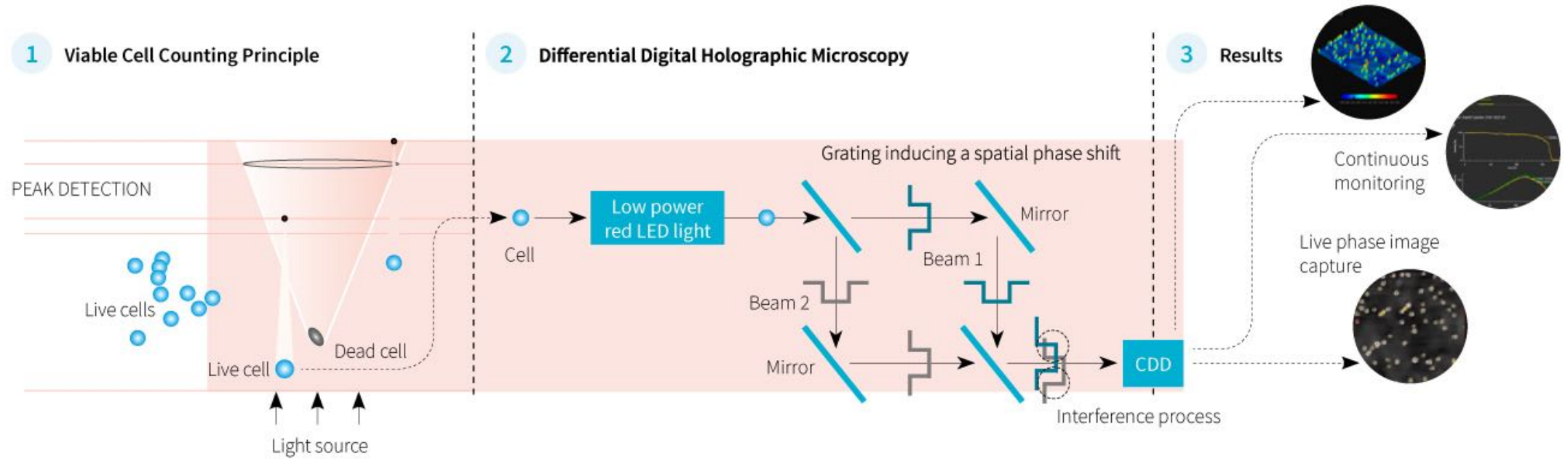
PARTNERS

APPLIKON
PALL LIFE SCIENCES

OTHER

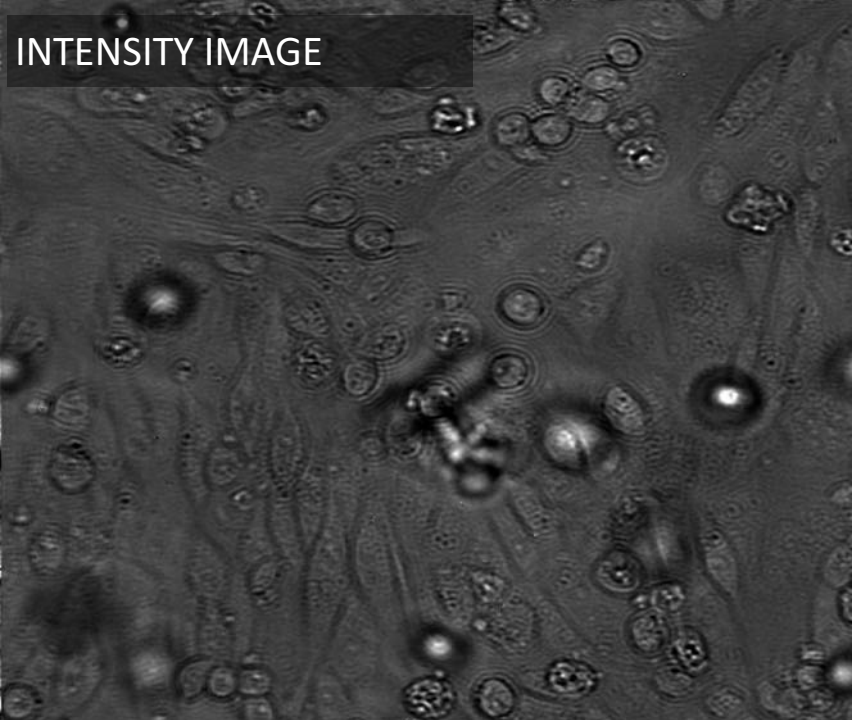
FOUNDED IN DEC 2009
SPIN-OFF ULB
ISO 13485 IN PROGRESS

DDHM technology

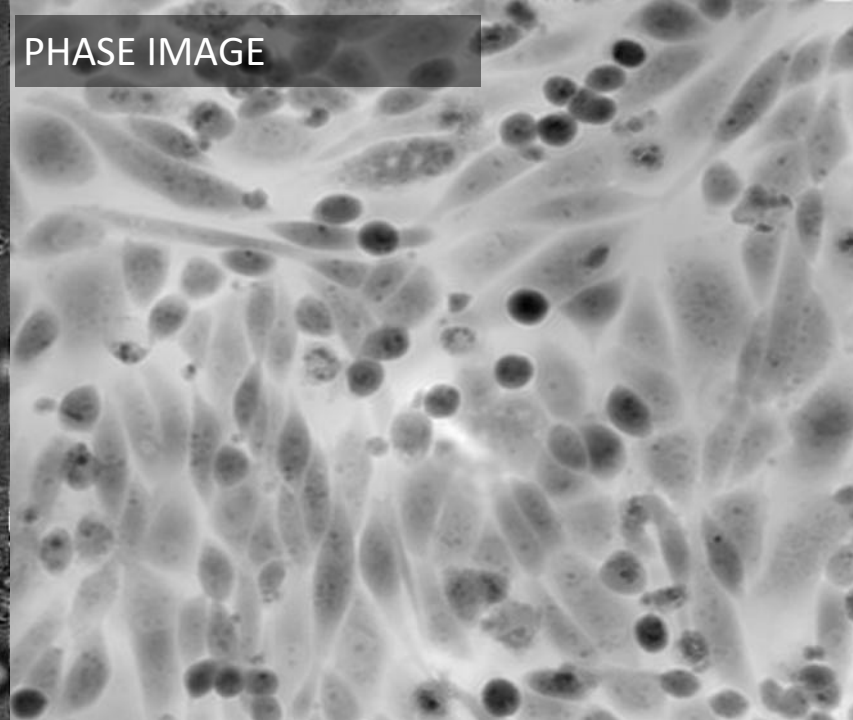


*Patented viability method
Invented by prof. Frank Dubois ULB (patent US 7,362,449 & ep1631788)*

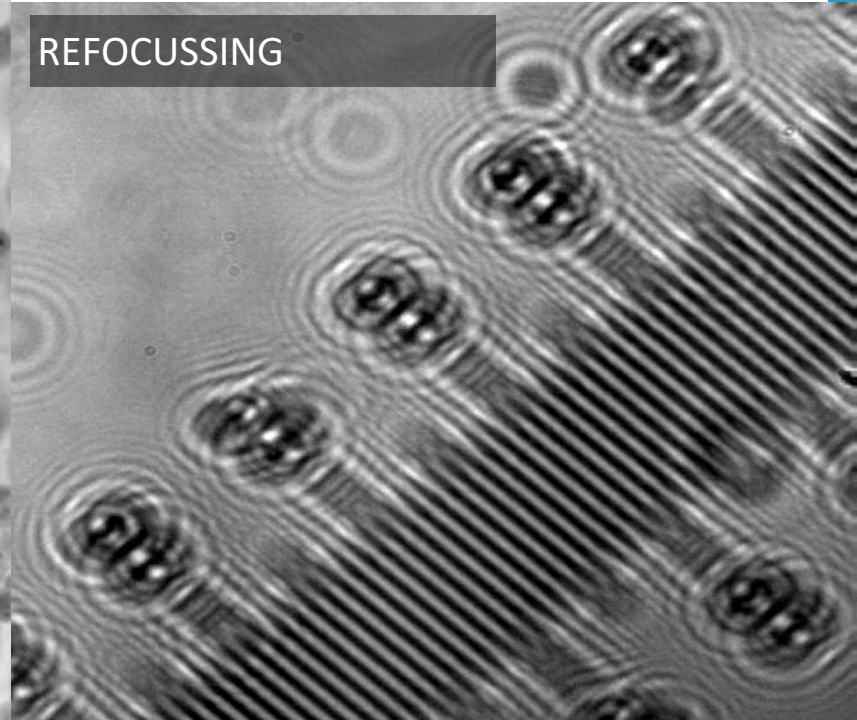
INTENSITY IMAGE



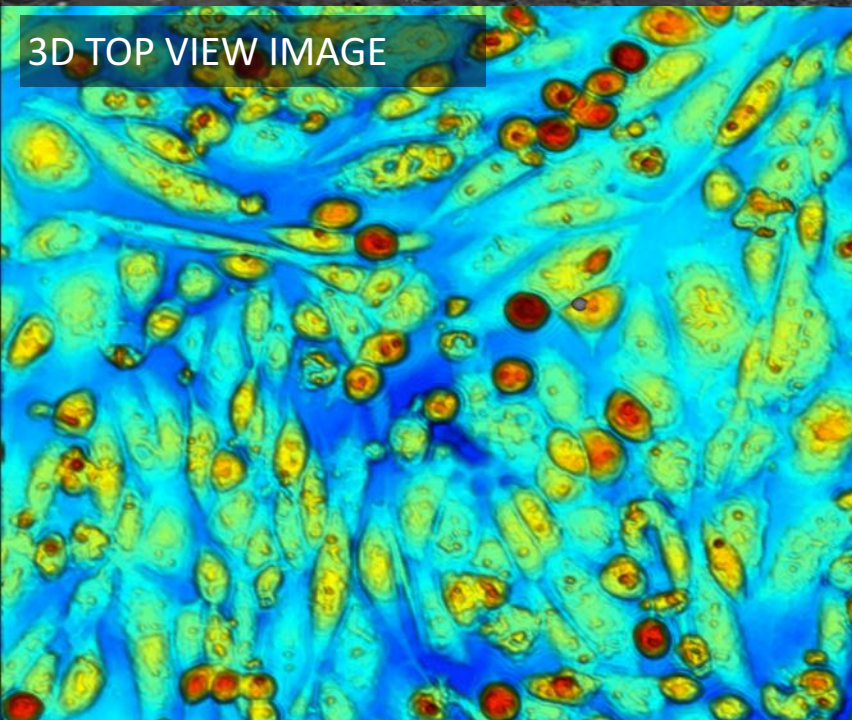
PHASE IMAGE



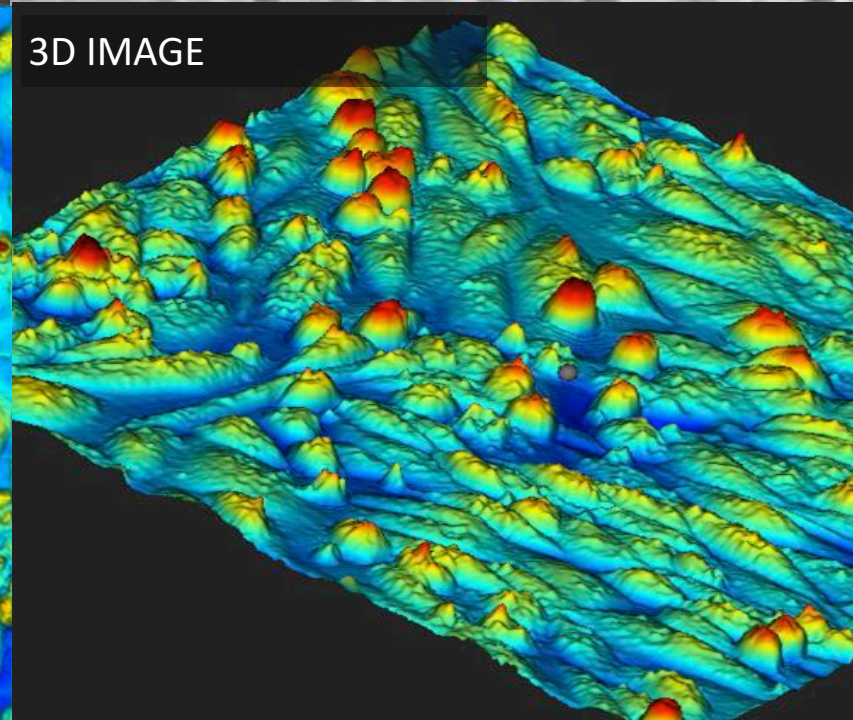
REFOCUSING



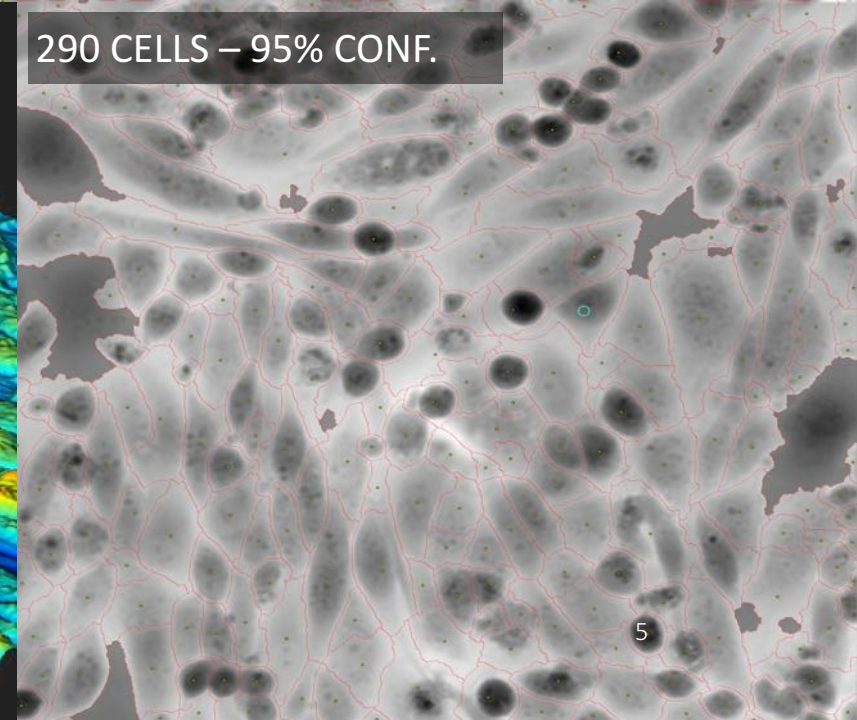
3D TOP VIEW IMAGE



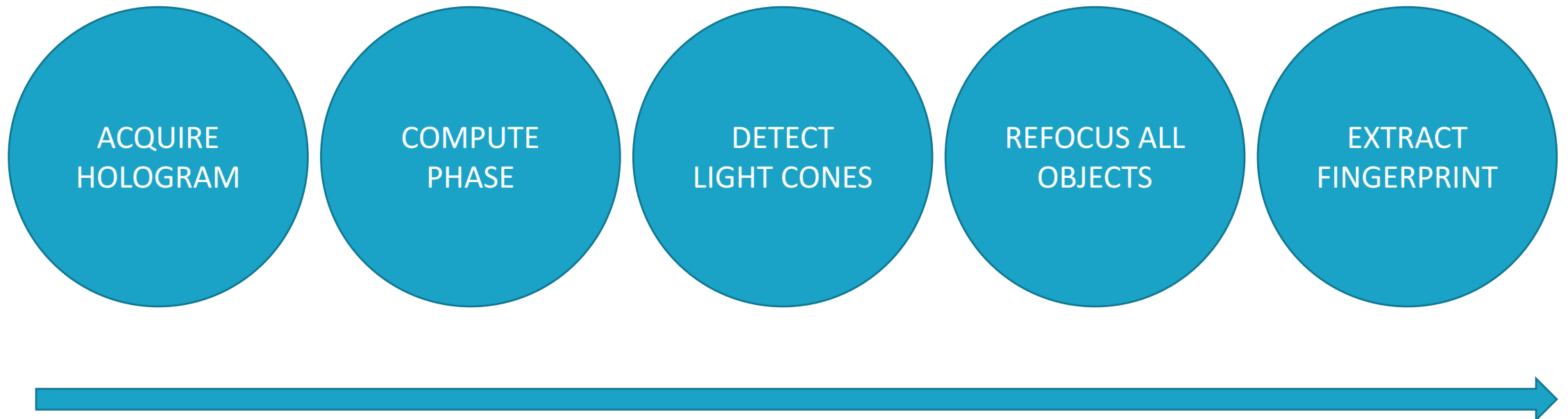
3D IMAGE



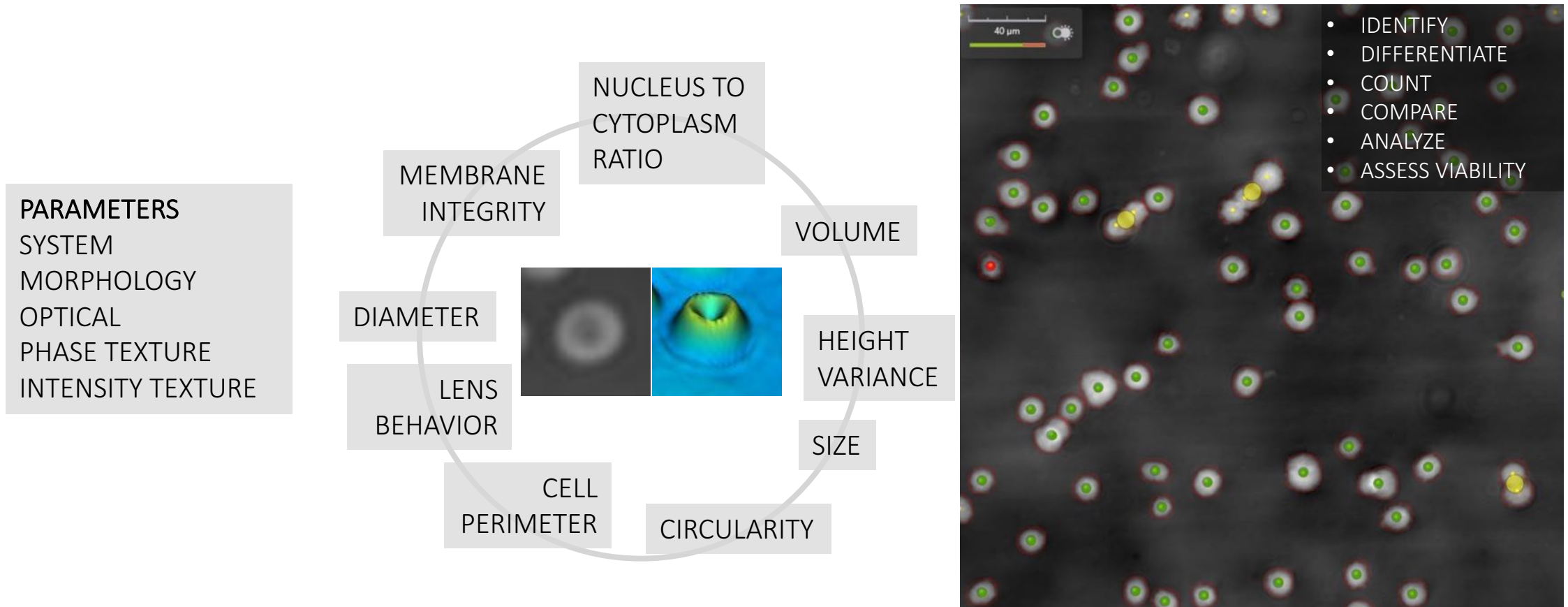
290 CELLS – 95% CONF.



HOW DO WE ANALYZE IMAGES



HOLOGRAPHIC FINGERPRINT FOR CELLS



74 PARAMETERS ARE RECORDED PER CELL – MACHINE LEARNING

MACHINE LEARNING METHODOLOGY

STEP 1 LEARNING SET CREATION



100 % LIVING CELL



100% DEAD CELLS

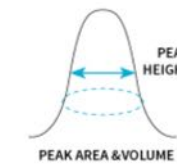
STEP 2 SYSTEM TRAINING

FEED SYSTEM WITH
KNOWN MIXTURES

FROM 100% TO 0%
IN STEPS OF 10

STEP 3 PREDICTION

Single cell analysis



CELL MORPHOLOGY	
DIAMETER	10,65 μm
PERIMETER	33,92 μm
CIRCULARITY	0,86
ASPECT RATIO	1,0998

Real-time measurements



Cell culture parameters



VIABLE CELL DENSITY
2,76*10⁶ cells/ml

BENEFITS OF AUTOMATION

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

20160105 DG CHO
BC20151215002 JBA

Sort
065h35 065h05 064h35 064h05

063h35 063h05 062h35 062h05

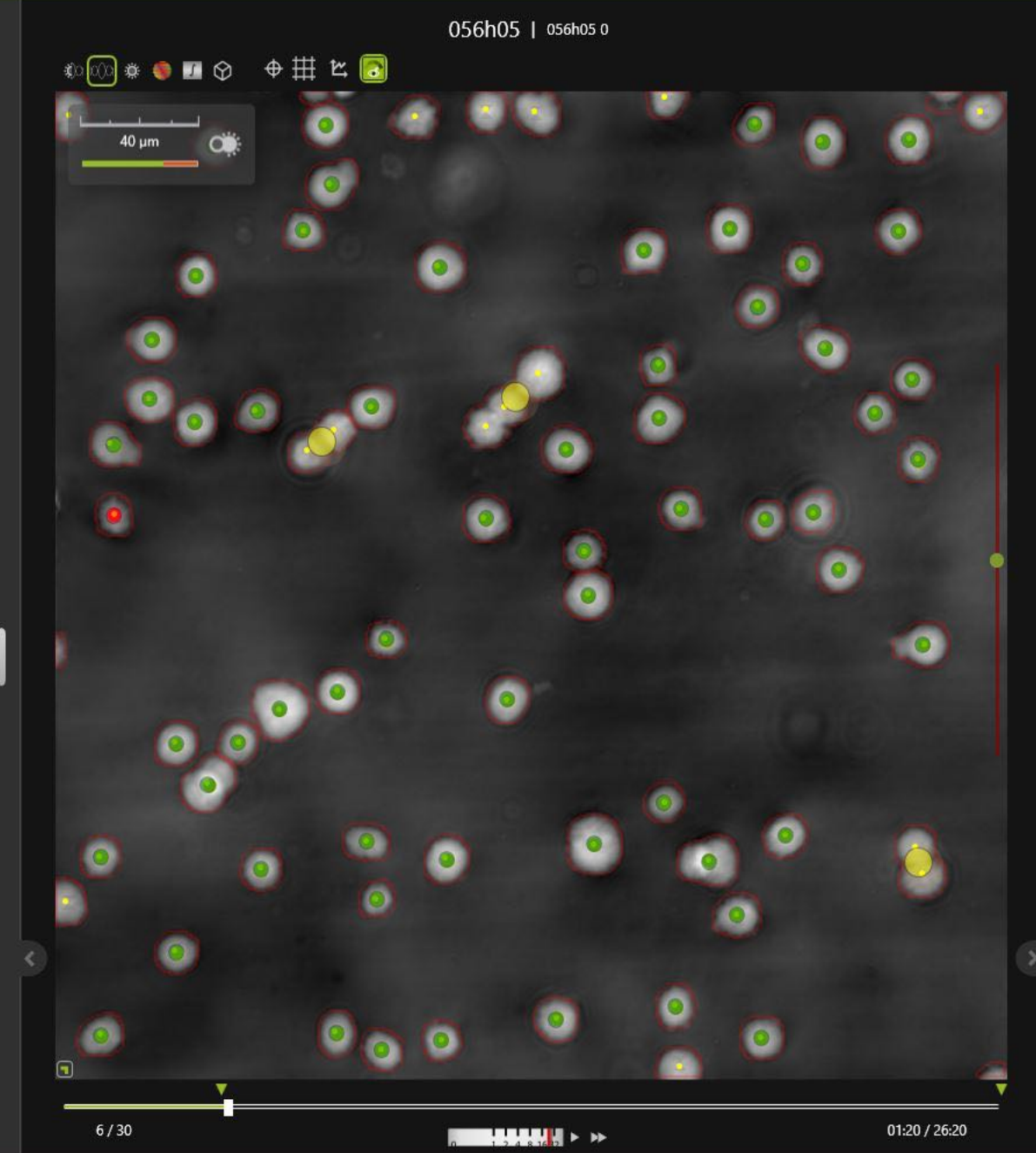
061h35 061h05 060h35 060h05

059h35 059h05 058h35 058h05

057h35 057h05 056h35 056h05

056h05

Creation Date 08/01/2016 02:13:33
 Height (px) 2048
 Width (px) 2048
 Description



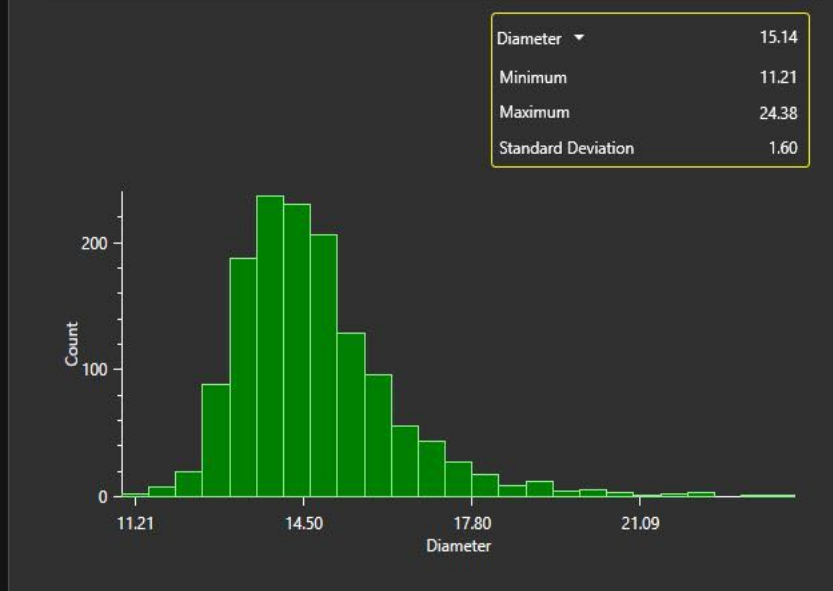
Channels Measures Browser Object detection

Viability 95.8%

Minimum 90.6%
 Maximum 100.0%

Viable Cell Density 4.36×10^6 cells/ml

Minimum 3.79×10^6 cells/ml
 Maximum 4.87×10^6 cells/ml



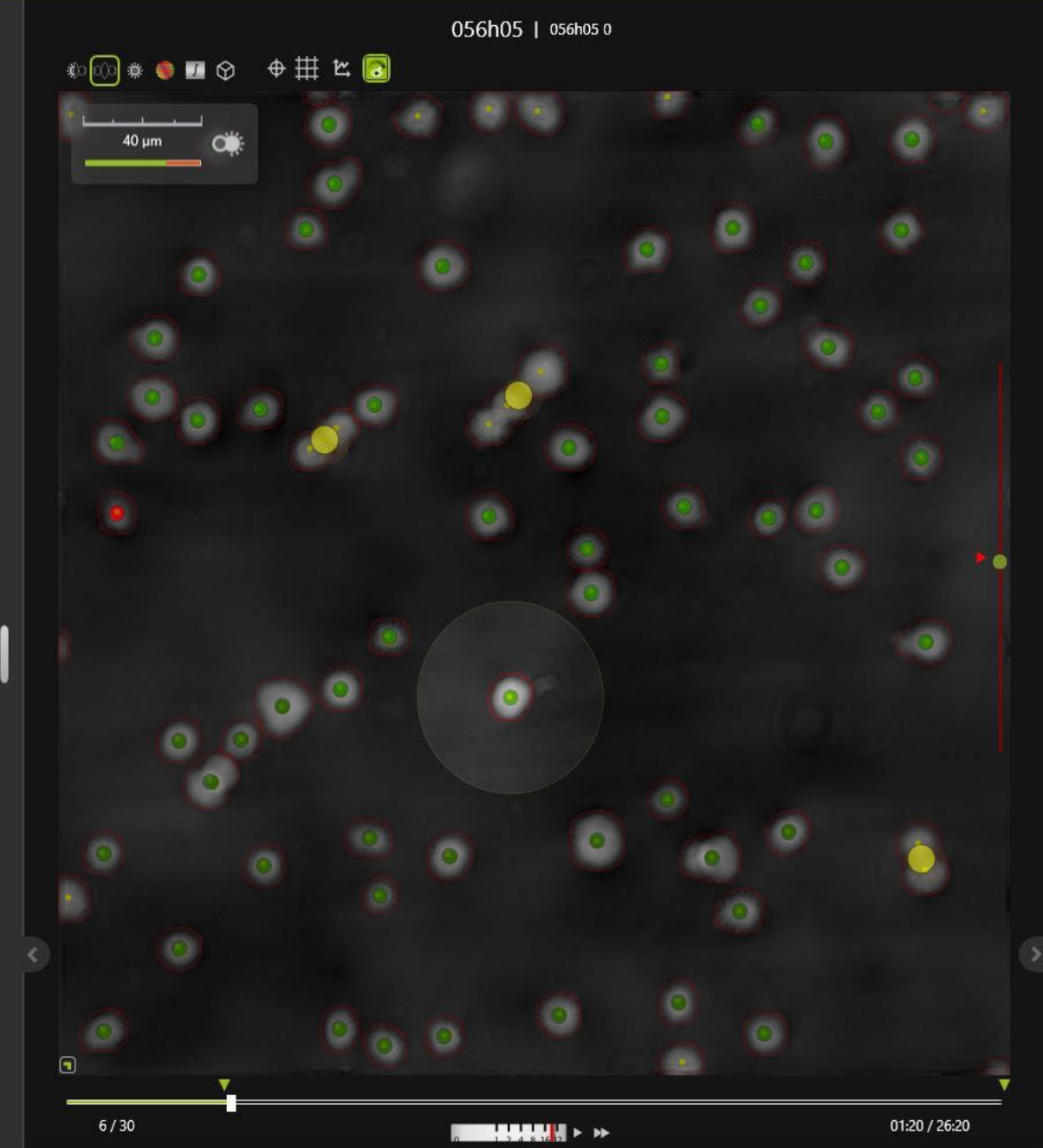
Advanced

Total Cell Density 4.55×10^6 cells/ml
 Total cell count 1388 cells in 25 images
 Viable cells 1330 cells
 Dead cells 58 cells
 Rejected events 307 events
 Aggregate Rate 8.7%

20160105 DG CHO
BC20151215002 JBA

Sort
065h35 065h05 064h35 064h05
063h35 063h05 062h35 062h05
061h35 061h05 060h35 060h05
059h35 059h05 058h35 058h05
057h35 057h05 056h35 056h05

056h05
 Creation Date 08/01/2016 02:13:33
 Height (px) 2048
 Width (px) 2048
 Description



Channels Measures **Browser** Object detection

Diameter

Circularity

Filter Ranking

Cell 19	0.98	☆
Cell 40	0.97	☆
Cell 26	0.97	☆
*Normalized Optical Height	0.0126	
*Normalized Peak Area	0.3685	
*Normalized Peak Height	0.1978	
*Normalized Radius Variance	0.0056	
Aggregate size	1.0000	
Aspect Ratio	1.2551	
Cell 54	0.97	☆
Cell 21	0.97	☆
Cell 31	0.97	☆
Cell 32	0.97	☆
Cell 57	0.97	☆
Cell 16	0.97	☆
Cell 61	0.96	☆

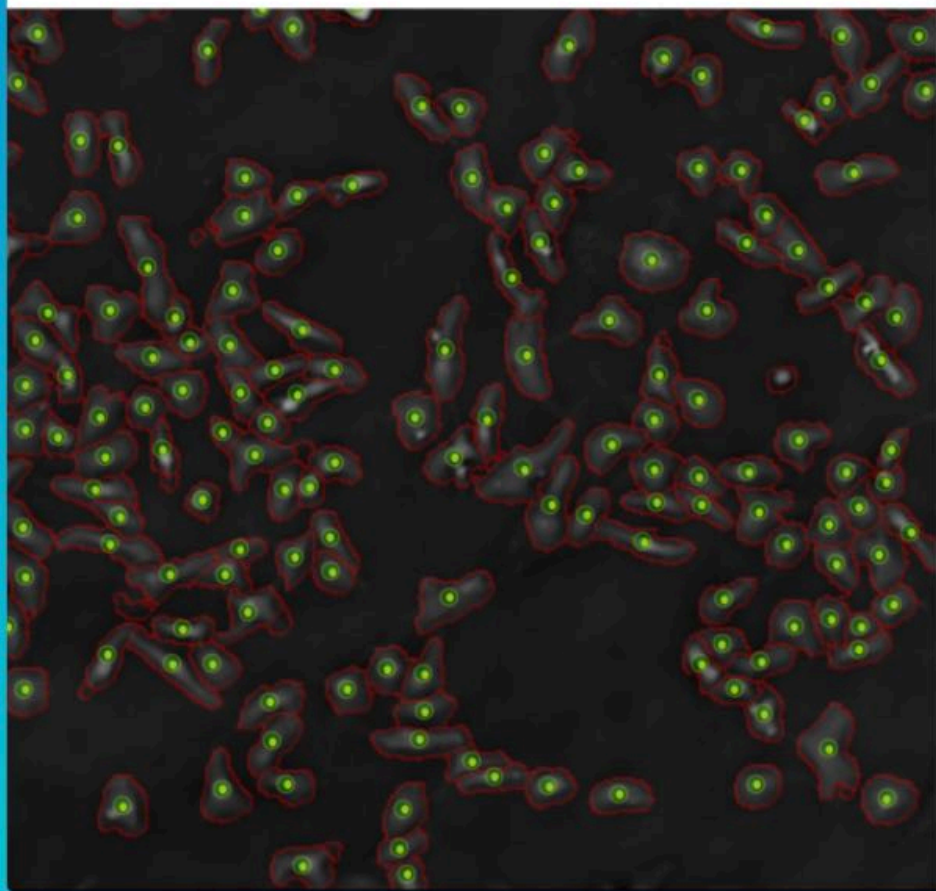
Capture 1216 Created: 11-05-2015 at 16:00

Measurement

Timelapse

Images Captured

1/4 Hologram



Viability

91.2%

Min: 90% Max: 92.4%

HIDE

Experiment 119

Started: 11-05-2015 at 16:00 Total Captures: 1256 Cell type: VERO Vessel: Corning T175

CONTINUOUS MONITORING

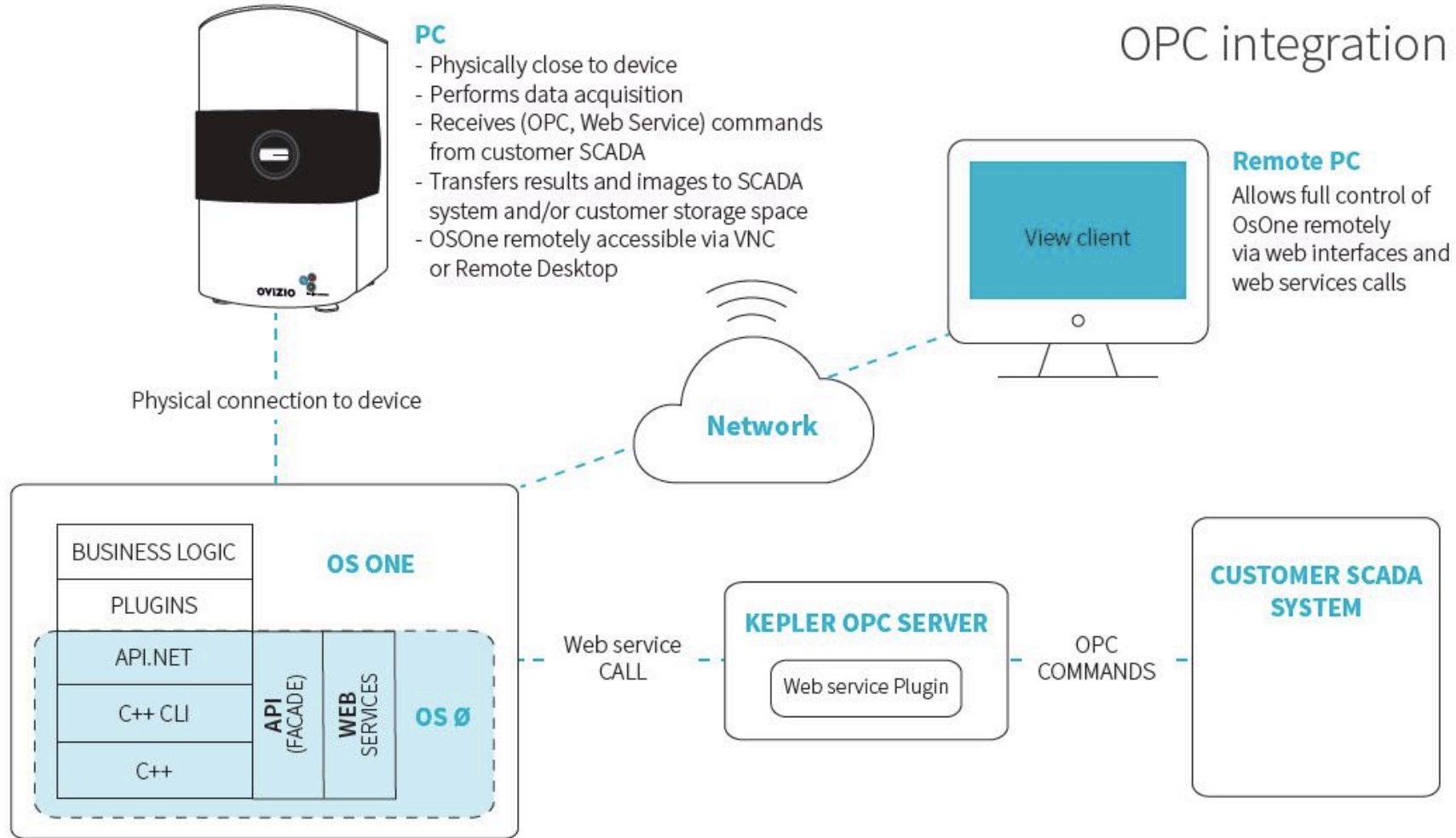


STATISTICAL RELEVANCE
ILINE F: 6000 IMAGES PER DAY

CELL COUNTER: 50-100 IMAGES PER SAMPLE

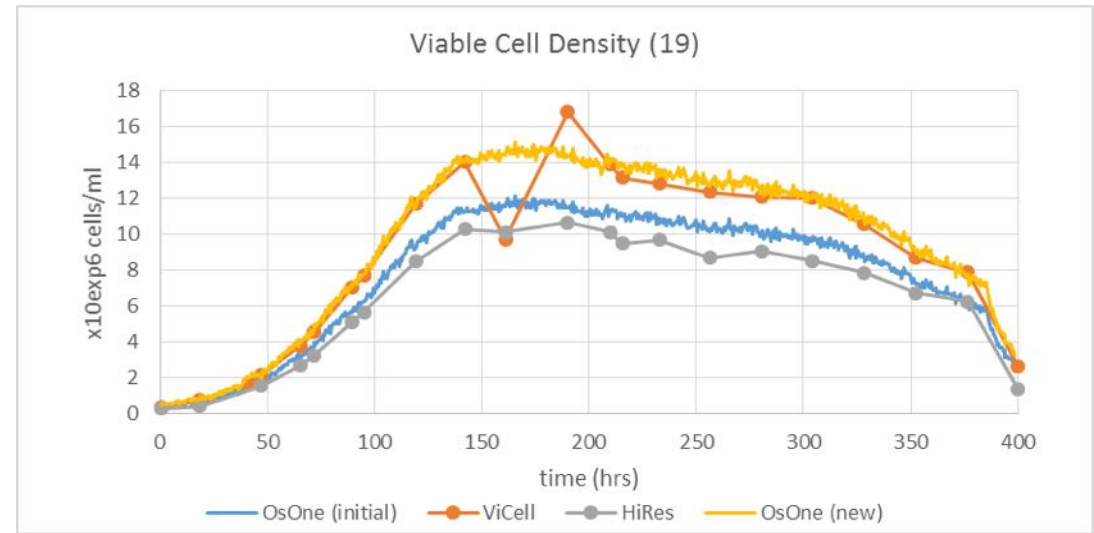
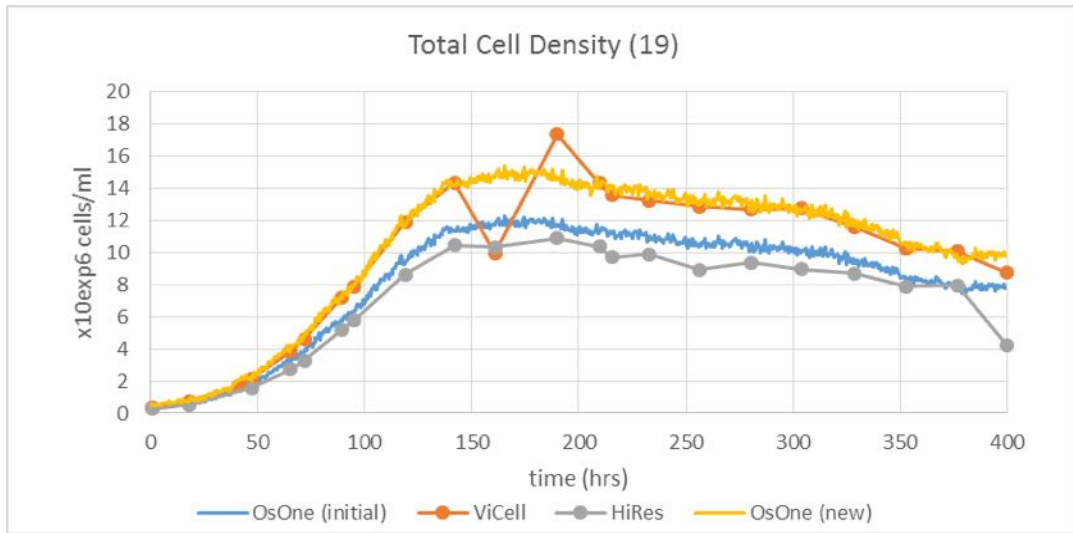
NO DILLUTION REQUIRED

OPC Integration

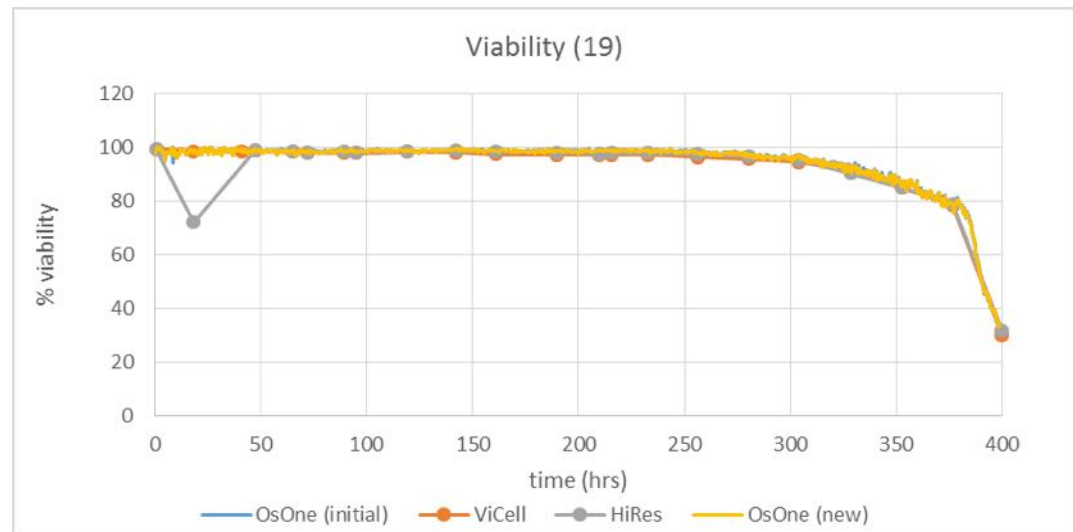


OPC integration

TECHNOLOGY COMPARED

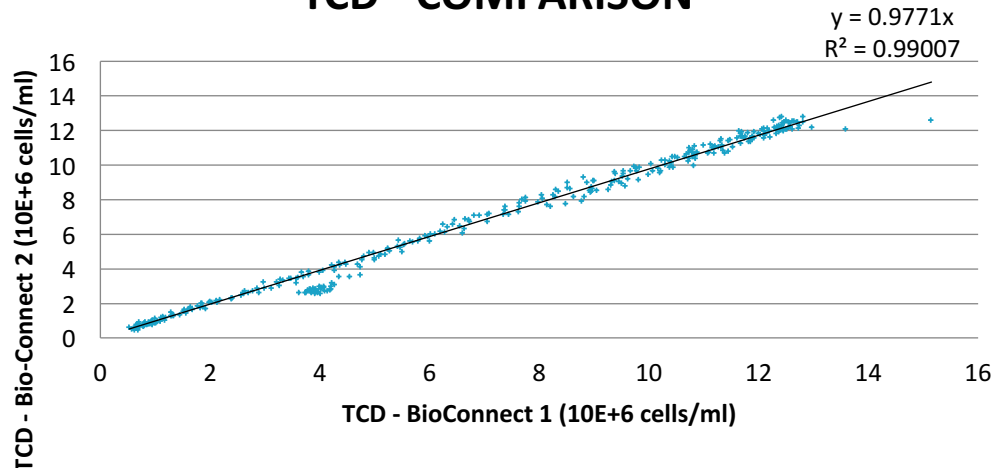


R= 0,968

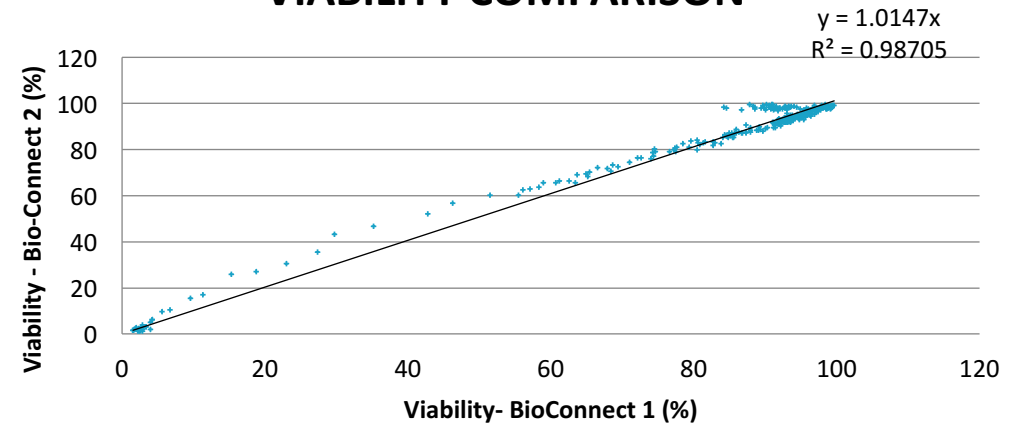


VARIABILITY-TCD

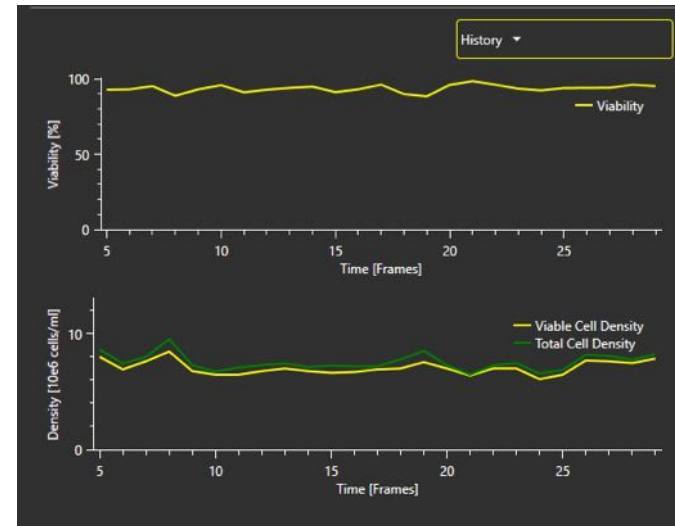
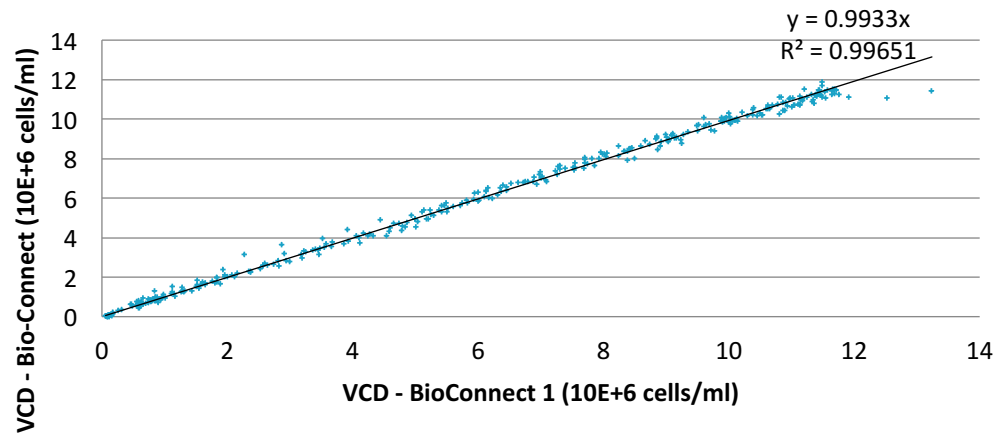
TCD - COMPARISON



VIABILITY COMPARISON

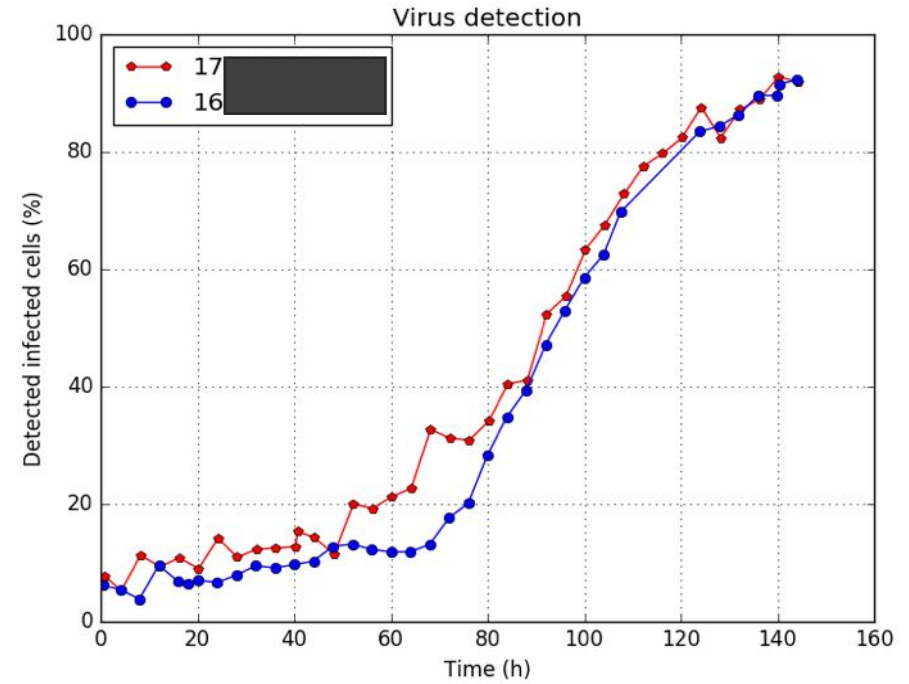
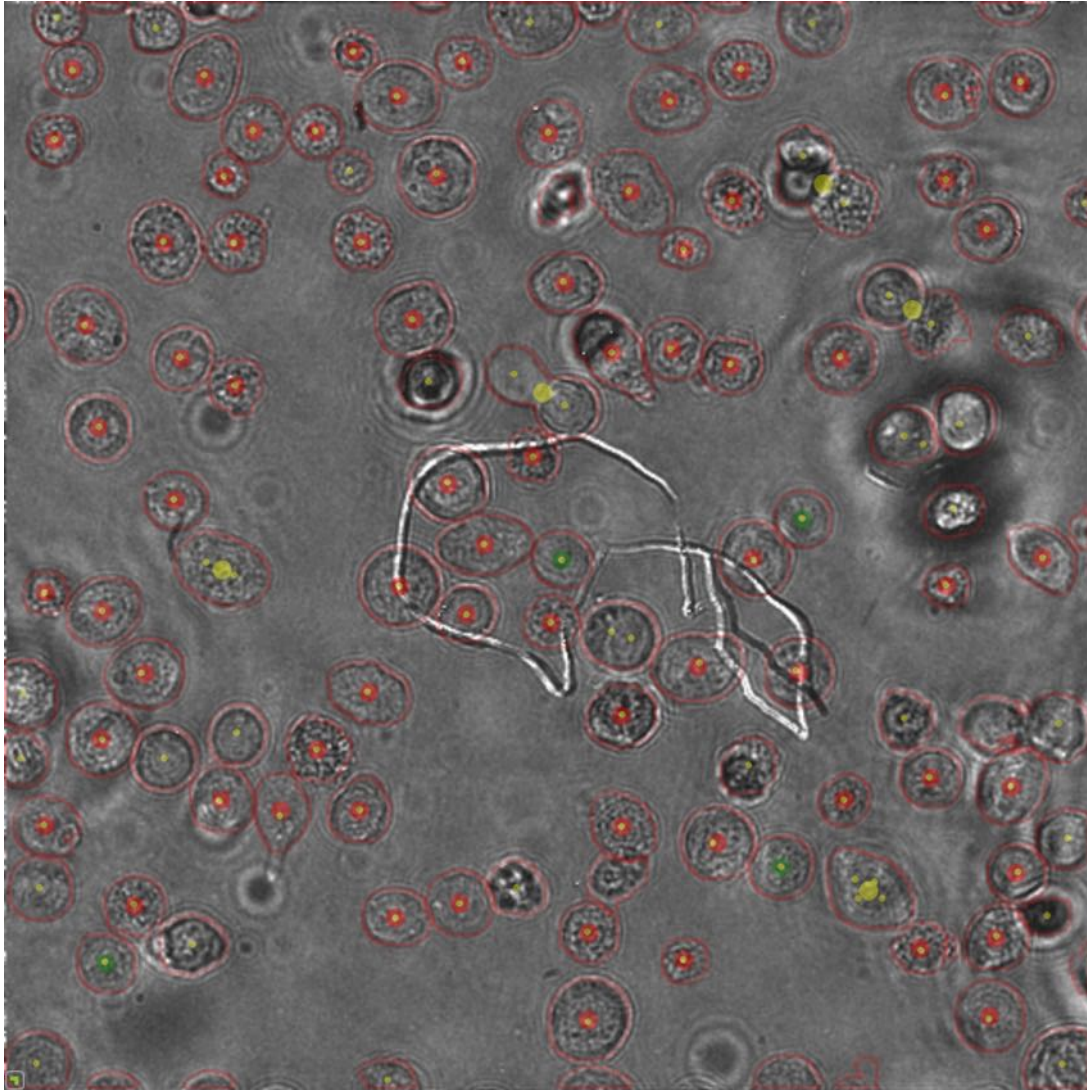


VCD COMPARISON

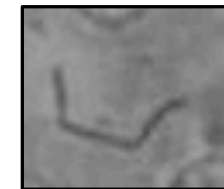
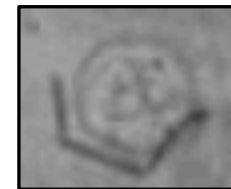


IN SAMPLE

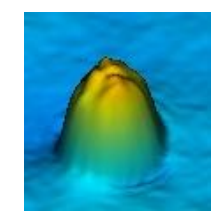
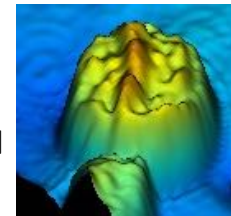
CASE STUDY: VIRAL LOAD MEASUREMENT



INFECTED
CELL



PROTEIN
EXPRESSION



NON-
INFECTED
CELL

CASE STUDY – DISCRIMINATE CELL TYPES

BACKGROUND

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

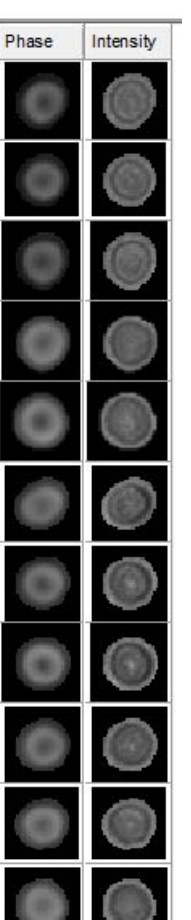
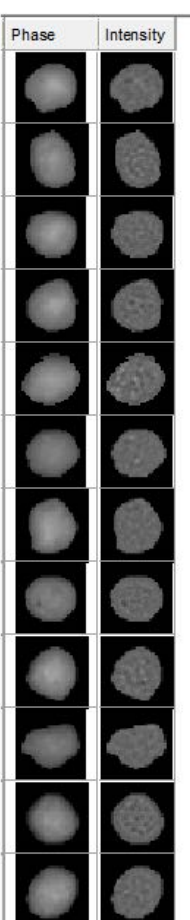
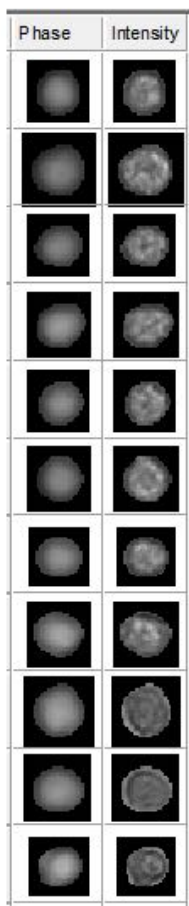
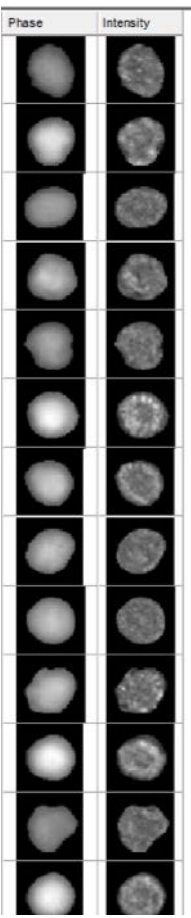
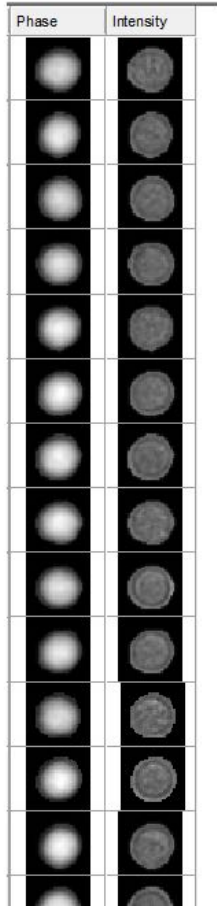
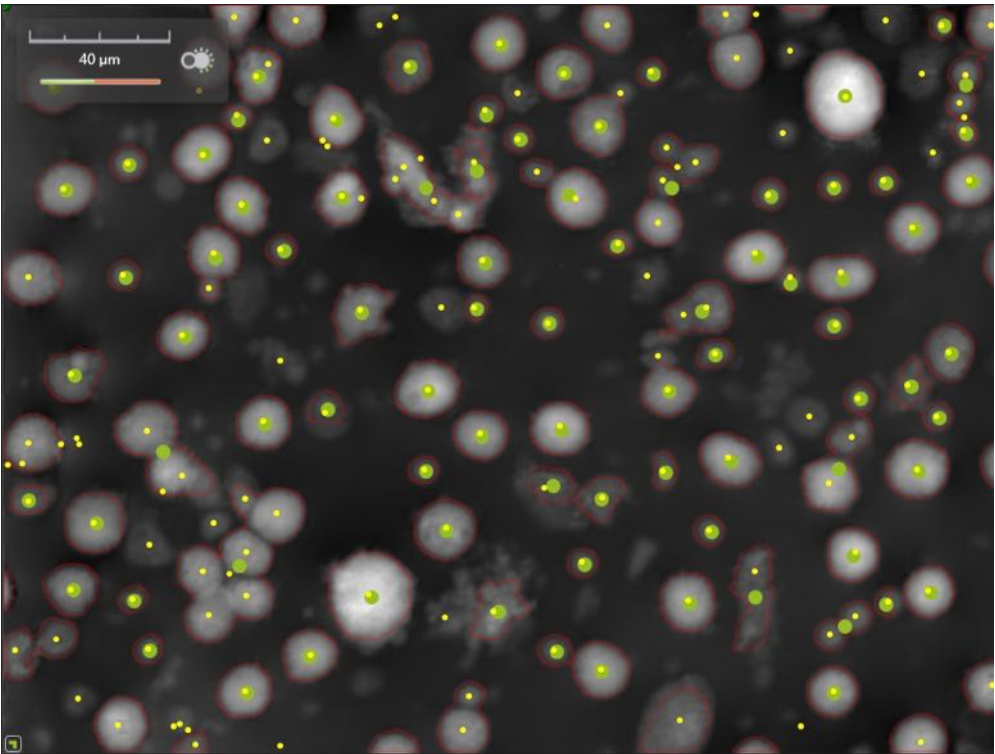
CHALLENGES

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

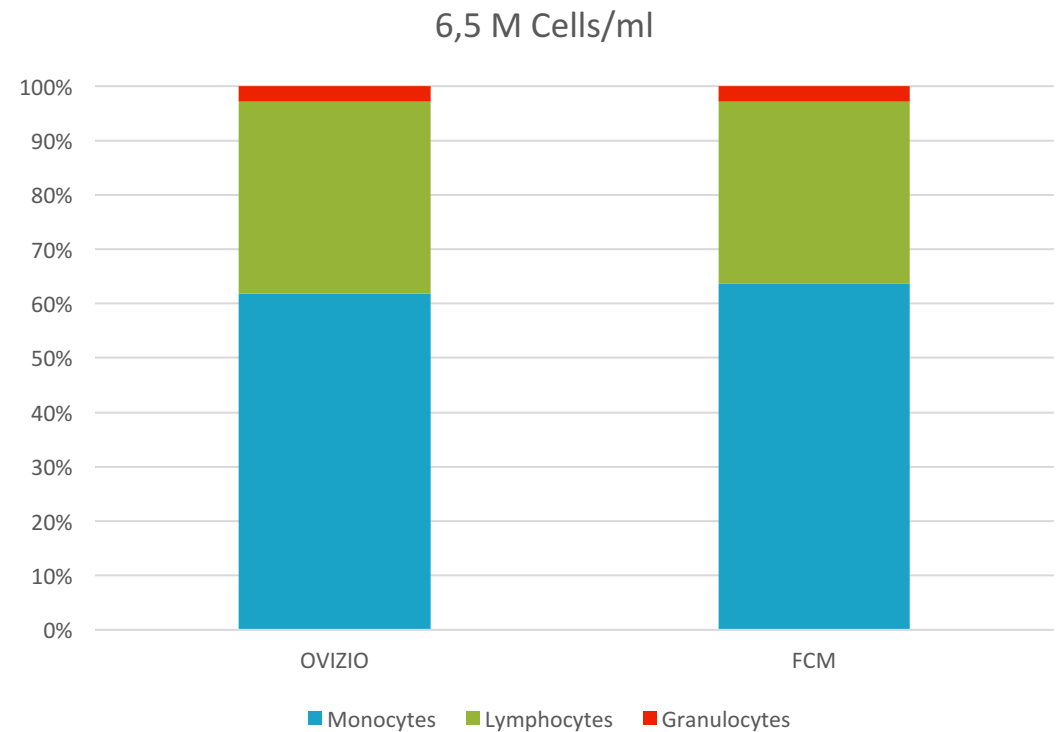
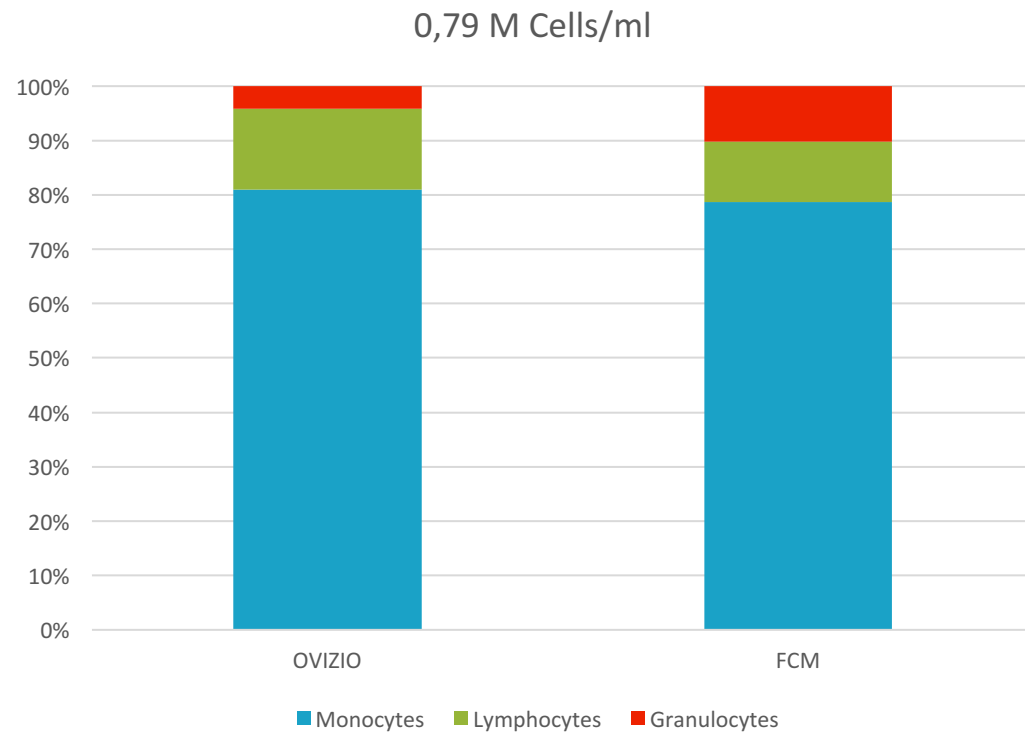
CRITICAL PARAMETERS HERE ARE TO MONITOR CELL CHARACTERISTICS

CELL THERAPY CLOSED MANUFACTURING SYSTEM DENDRIDIC CELLS

MONOCYTES MDC'S LYMPHOCYTES GRANULOCYTES RBC'S



CASE STUDY – RESULTS COUNT



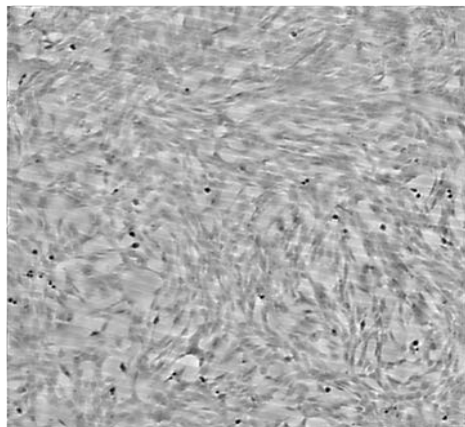
MULTI LAYER RECIPIENTS



MSC CELLS – XP10

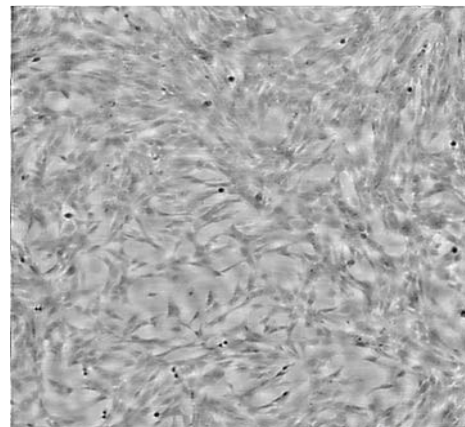
MULTIPLATE OBSERVATION

Plate 1



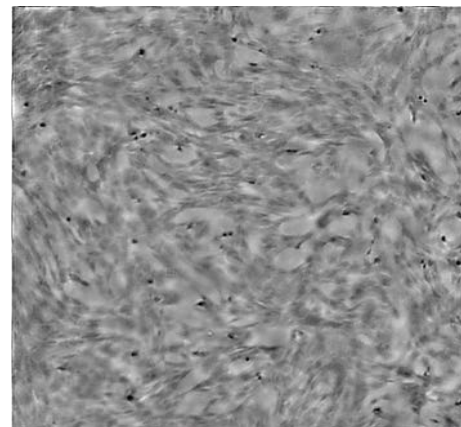
XP10_Day5_plate 1

Plate 3



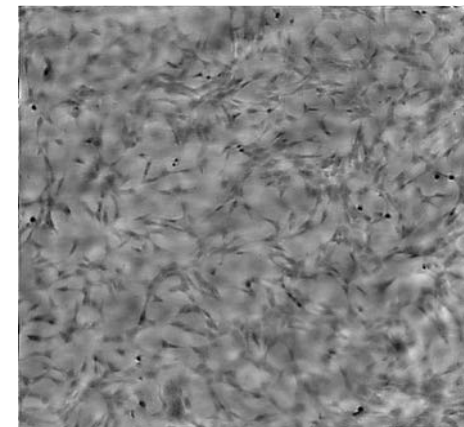
XP10_Day5_plate 3

Plate 6



XP10_Day5_plate 6

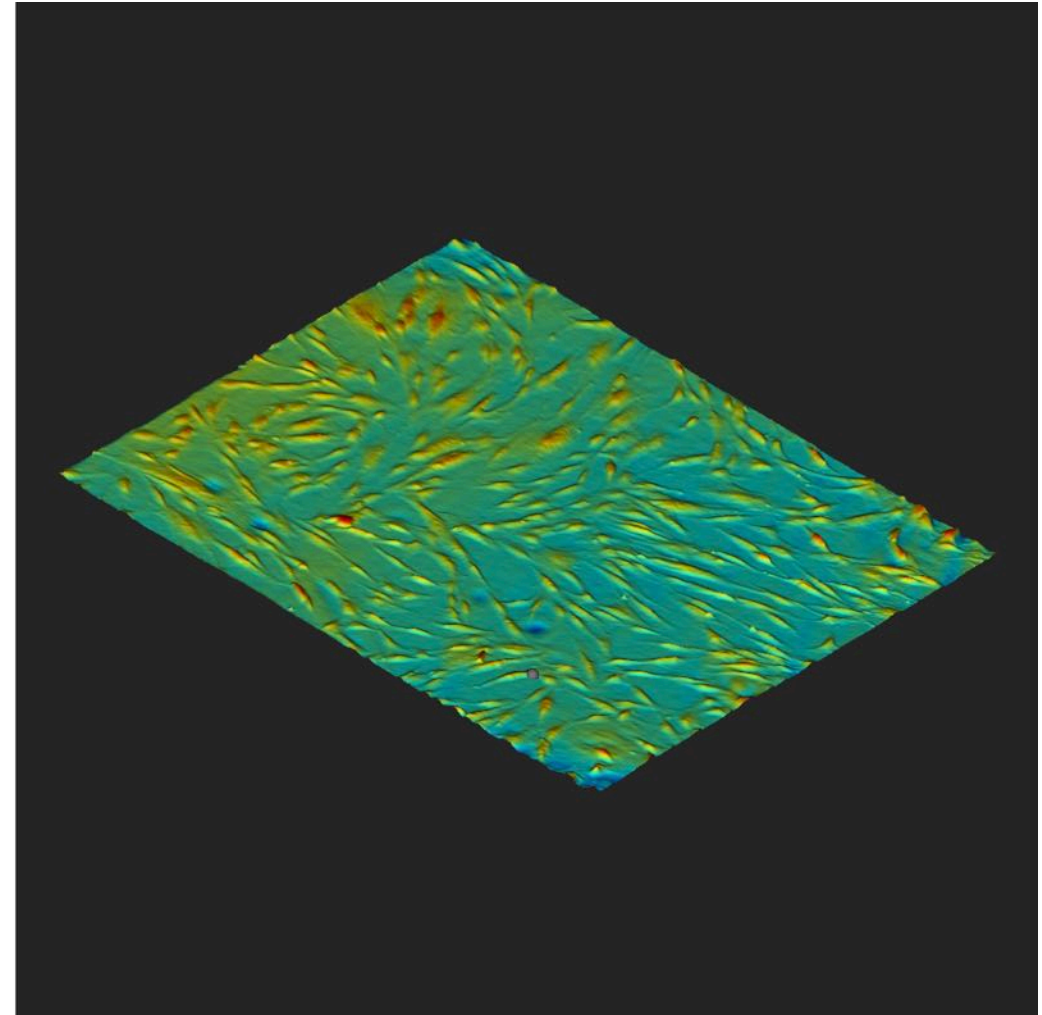
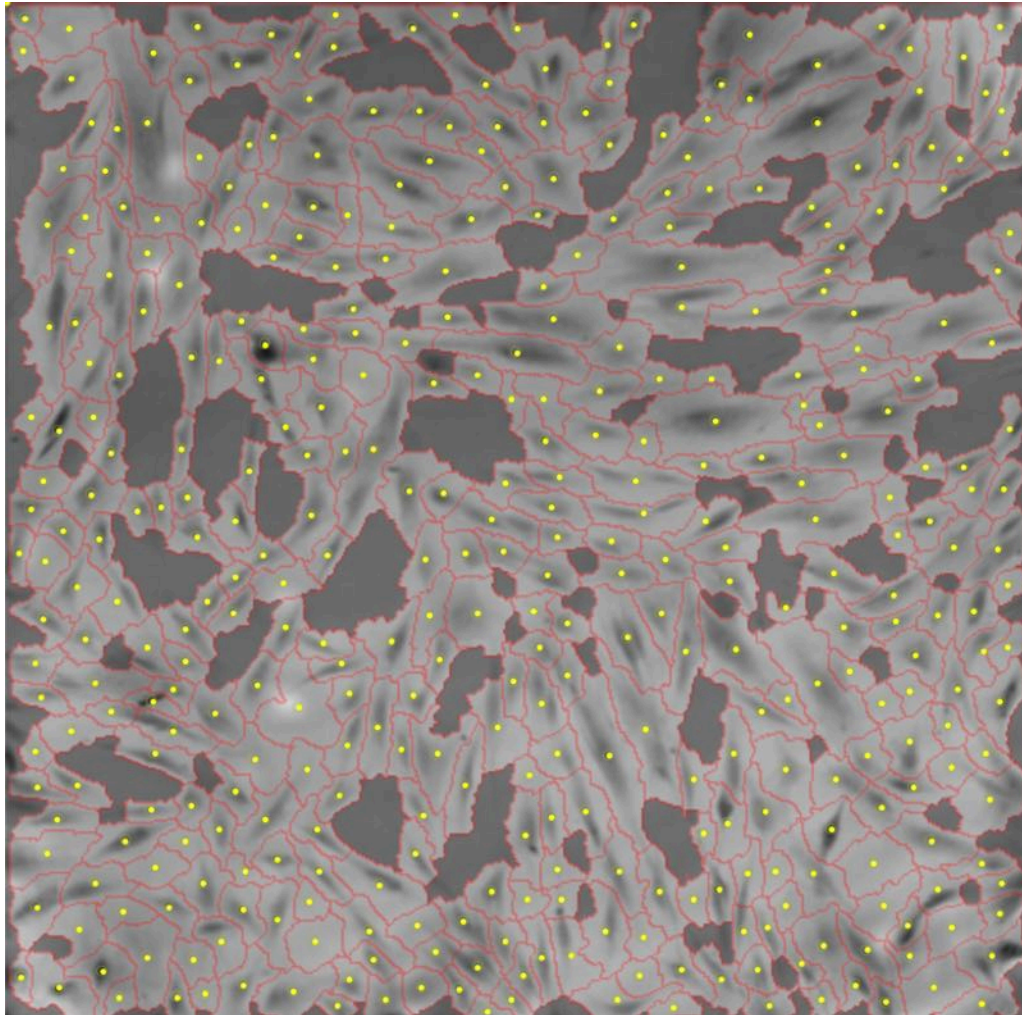
Plate 8



XP10_Day5_plate 8

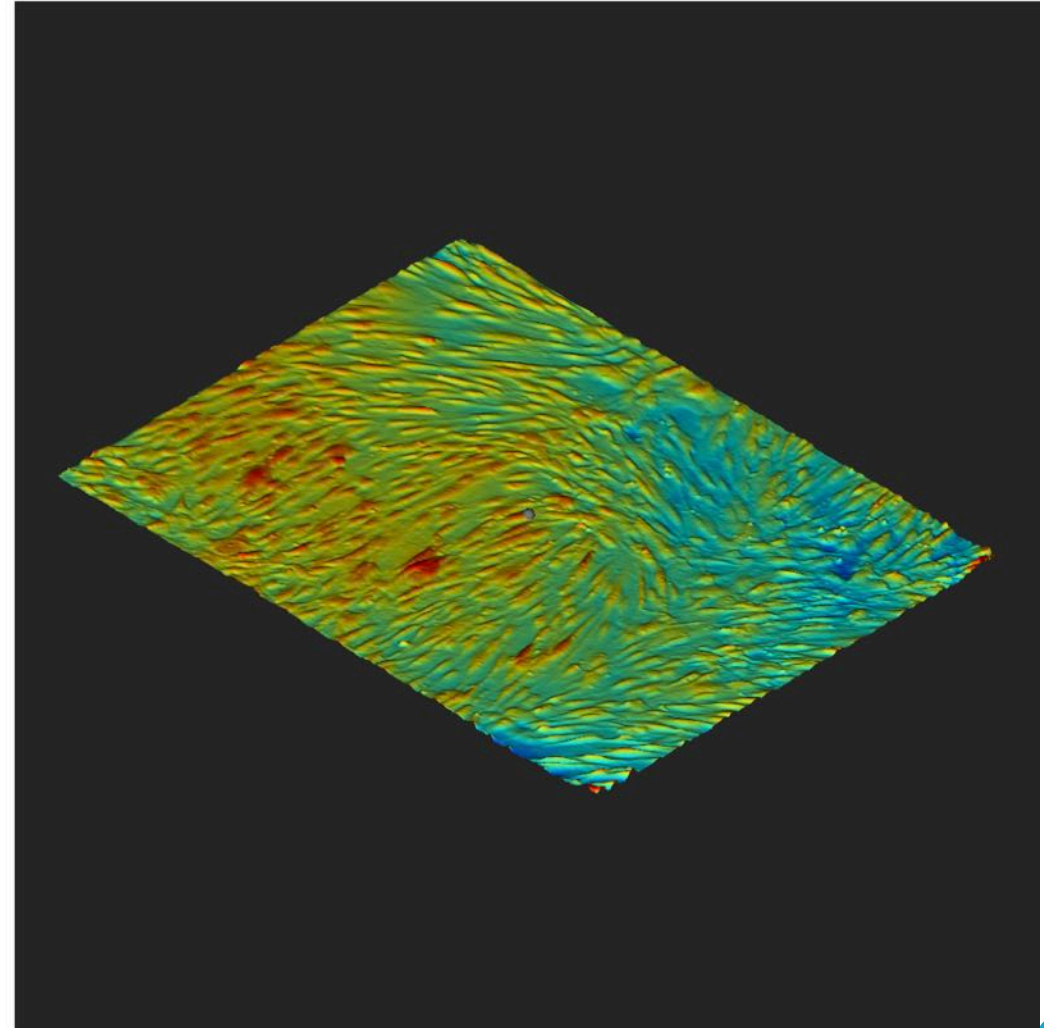
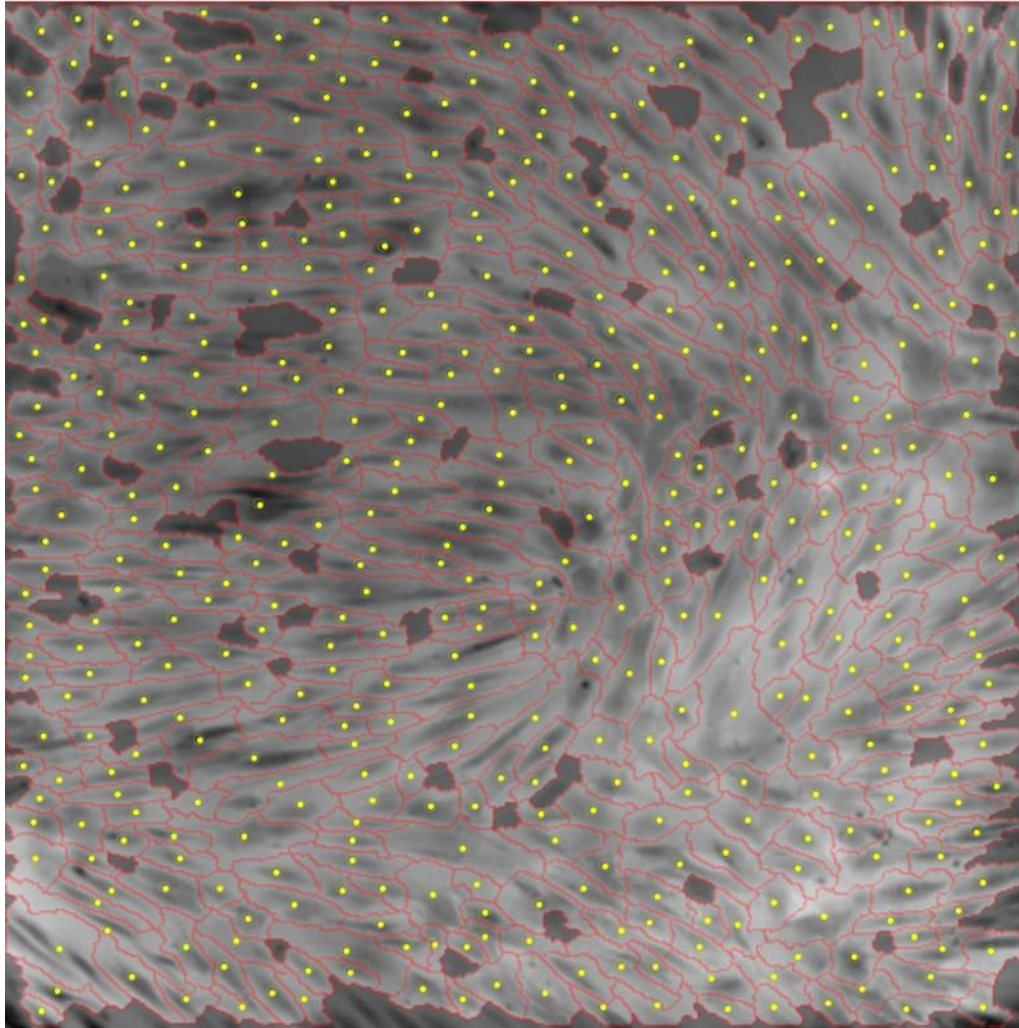
CLIENT TEST: AUTOMATED CONFLUENCY

20130205 Client MSC Stem Cells -70%

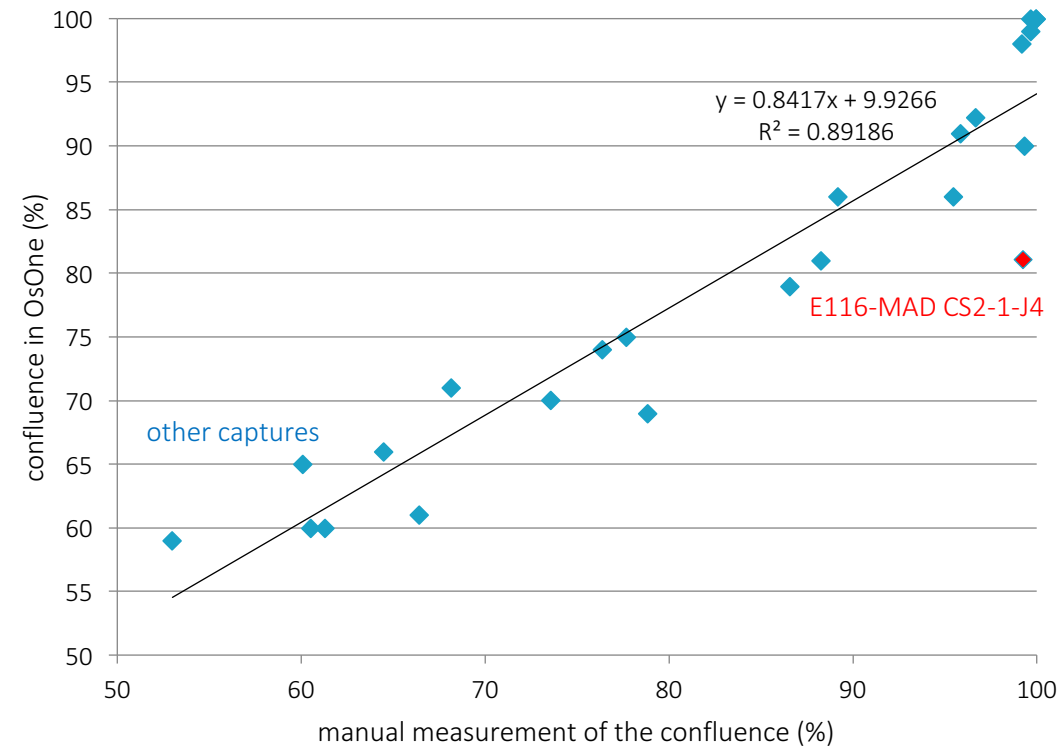


CLIENT TEST: AUTOMATED CONFLUENCY

20130205 Client MSC Stem Cells -90%



OVIZIO VS MANUAL MEASUREMENT



CONFLUENCE MEASUREMENTS MATCH THE ESTIMATIONS OF A HUMAN OPERATOR CLOSELY. CONFLUENCE IS THE PARAMETER OF MAIN IMPORTANCE AT THIS STAGE.



THANK YOU FOR YOUR ATTENTION!

We would love to continue the discussion with you...

IF YOU WANT TO TRY OUR SYSTEMS SEND MAIL TO:
info@ovizio.com