

*Supplementary material for the article: Pais, D; Galrão, P; Kryzhanska, A; Barbau, J; Isidro, I; Alves, P. Holographic imaging of insect cell cultures: online non-invasive monitoring of adeno associated virus production and cell concentration, Processes 2020, 6.*

1 **Table S1** - Estimates, corresponding standard error, t-ratio and logworth / p-value for all the models shown in  
 2 Figures 1 and 2. For leave one batch out (LOBO) models, the batch used for model calibration is indicated  
 3 between brackets. Logworth is defined as  $-\log_{10}(p\text{-value})$ .

Model	Parameter	Estimate	Std Error	t-Ratio	LogWorth	p-value
Extracellular AAV titer - 3CV	Intensity Average Intensity	-2.5E+11	1.7E+10	-14.4	12.6	2.5E-13
	Phase Skewness	3.9E+09	2.9E+08	13.6	12.0	9.7E-13
	Peak Height Normalized	-3.3E+10	2.5E+09	-12.9	11.6	2.8E-12
	Optical Height Maximum	4.5E+09	3.7E+08	12.3	11.2	7.0E-12
	Intensity Average Contrast	2.8E+11	2.4E+10	12.0	10.9	1.3E-11
	Intensity Average Entropy	-4.1E+10	3.6E+09	-11.4	10.4	3.7E-11
	Hu Moment 6	-1.7E+15	4.0E+14	-4.3	3.6	2.4E-04
	Hu Moment 5	3.9E+16	1.0E+16	3.8	3.1	8.7E-04
	Intensity Geometric Mean	1.2E+10	4.3E+09	2.7	1.9	1.1E-02
	Center of Mass Vertical Position	3.4E+07	1.5E+07	2.2	1.5	3.5E-02
	Intercept	2.0E+11	1.4E+10	14.2		
Specific AAV titer - 3CV	Peak Height	-3.2E+03	1.5E+02	-21.4	14.0	9.2E-15
	Optical Volume	3.2E+03	1.7E+02	19.4	13.3	5.3E-14
	Peak Area Normalized	-5.0E+05	3.4E+04	-14.7	11.1	8.1E-12
	Cell Area	-6.2E+02	4.8E+01	-13.1	10.2	5.8E-11
	Best Depth	2.8E+03	4.8E+02	5.9	4.9	1.1E-05
	Hu Moment 5	1.7E+11	3.3E+10	5.2	4.2	5.6E-05
	Intensity Average Intensity	4.3E+05	8.6E+04	5.0	4.1	8.4E-05
	Phase Skewness	9.4E+03	2.0E+03	4.7	3.8	1.6E-04
	Hu Moment 6	-6.4E+09	1.5E+09	-4.2	3.3	4.7E-04
	Intensity Maximum	-3.5E+04	9.1E+03	-3.8	2.9	1.3E-03
Viable cell concentration - 3CV	Phase Average Uniformity	-5.0E+05	1.6E+05	-3.2	2.3	5.0E-03
	Hu Moment 4	-2.5E+08	1.1E+08	-2.3	1.5	3.1E-02
	Intercept	9.1E+04	3.5E+04	2.6		
	Intensity Geometric Mean	-6.4E+07	4.0E+06	-16.2	15.7	2.2E-16
	Intensity Correlation	5.2E+07	6.3E+06	8.2	8.4	3.7E-09
	Phase Average Uniformity	-1.6E+08	2.5E+07	-6.3	6.2	5.8E-07
Viability - 3CV	Optical Height Maximum	-8.9E+05	3.4E+05	-2.6	1.9	1.4E-02
	Optical Height Minimum	-9.2E+06	4.0E+06	-2.3	1.6	2.8E-02
	Intercept	9.0E+06	6.2E+06	1.5		
	Peak Height Normalized	1.7E+02	1.3E+01	13.4	13.2	5.7E-14
	Phase Skewness	-1.4E+01	1.1E+00	-12.8	12.7	1.8E-13
	Optical Height Maximum	-2.5E+01	2.7E+00	-9.3	9.5	3.0E-10
	Hu Moment 3	8.9E+04	1.6E+04	5.7	5.4	3.8E-06

**Table S1 (cont.)**

Model	Term	Estimate	Std Error	t-Ratio	LogWorth	p-value
Viability - 3CV	Intercept	-4.2E+02	1.4E+02	-3.0		
	Intensity Geometric Mean	-6.3E+07	7.7E+06	-8.2	4.4	3.7E-05
	Intensity Correlation	6.2E+07	2.6E+07	2.4	1.4	4.1E-02
	Phase Average Uniformity	-1.3E+08	5.9E+07	-2.2	1.3	5.6E-02
	Optical Height Minimum	-1.6E+07	3.7E+07	-0.4	0.2	6.8E-01
	Optical Height Maximum	-6.7E+04	2.0E+06	0.0	0.0	9.7E-01
VCC LOBO (growth)	Intercept	-2.9E+06	3.0E+07	-0.1		
	Phase Average Uniformity	1.0E+08	4.6E+07	2.2	1.4	4.4E-02
	Optical Height Maximum	7.1E+05	5.0E+05	1.4	0.7	1.8E-01
	Intensity Geometric Mean	1.1E+07	9.2E+06	1.2	0.6	2.6E-01
	Intensity Correlation	6.1E+06	6.1E+06	1.0	0.5	3.4E-01
	Optical Height					
VCC LOBO (infected)	Maximum*Intensity Geometric Mean	9.7E+06	1.0E+07	0.9	0.4	3.7E-01
	Intensity Geometric Mean*	-2.1E+08	2.5E+08	-0.8	0.4	4.3E-01
	Intensity Geometric Mean					
	Phase Average Uniformity *	7.1E+08	1.1E+09	0.6	0.3	5.4E-01
	Intensity Correlation					
	Intercept	-1.3E+07	6.3E+06	-2.0		
Viability LOBO (growth)	Intensity Average Intensity	1.6E+03	1.1E+02	15.0	6.4	3.9E-07
	Peak Height Normalized	8.3E+01	8.8E+00	9.4	4.9	1.4E-05
	Aspect Ratio	7.3E+01	2.4E+01	3.1	1.8	1.6E-02
	Circularity	4.6E+02	1.7E+02	2.7	1.6	2.6E-02
	Peak Area	4.9E-01	2.1E-01	2.3	1.3	4.8E-02
	Intercept	-1.3E+03	1.6E+02	-7.7		
Viability LOBO (infected)	Peak Height	2.1E+00	2.3E-01	9.2	5.5	3.3E-06
	Circularity	-7.7E+02	1.1E+02	-6.7	4.3	5.3E-05
	Optical Height Maximum	-2.8E+01	4.3E+00	-6.4	4.1	7.6E-05
	Aspect Ratio	-6.8E+01	1.1E+01	-6.3	4.1	8.8E-05
	Hu Moment 4	2.9E+05	6.3E+04	4.6	3.0	1.1E-03
	Phase Correlation	5.3E+02	1.2E+02	4.5	2.9	1.1E-03
Viability LOBO (infected)	Optical Height Minimum	7.7E+01	1.8E+01	4.2	2.7	1.8E-03
	Best Depth	3.7E+00	9.0E-01	4.1	2.6	2.3E-03
	Peak Area Normalized	1.4E+02	3.6E+01	3.7	2.4	3.9E-03
	Intensity Correlation	1.6E+02	4.7E+01	3.3	2.1	7.6E-03
	Phase Skewness	-5.8E+00	2.0E+00	-2.9	1.8	1.5E-02
	Intensity Average Entropy	2.5E+01	1.0E+01	2.4	1.4	3.9E-02
	Intercept	3.0E+02	8.2E+01	3.6		

5   **Table S2** - RMSE for calibration and validation models, scaled for the variable range, using the 3-fold cross-  
 6 validation strategy. The dataset was divided in 3 partitions. 2 partitions were used for model calibration, which  
 7 was used for prediction of the reference data for the third partition. This process was repeated for the 3 partitions.

Model	Partition for testing	R <sup>2</sup>	RMSE calibration	Normalized RMSE calibration	Q <sup>2</sup>	RMSE validation	Normalized RMSE validation	Number of model datapoints	Number of model parameters
Extracellular AAV titer	1	0.98	4.5E+08	3.2	0.95	8.8E+08	6.2	23	10
	2	0.99	3.9E+08	2.7	0.98	7.4E+08	5.2	24	
	3	0.99	2.8E+08	2.0	0.98	4.9E+08	3.4	23	
Specific AAV titer	1	1.00	7.5E+02	1.5	1.00	1.0E+03	2.1	22	12
	2	0.99	1.3E+03	2.6	0.98	2.1E+03	4.4	23	
	3	1.00	7.2E+02	1.5	0.98	2.2E+03	4.5	20	
Viable cell concentration	1	0.97	4.5E+05	4.5	0.92	7.6E+05	7.7	23	5
	2	0.94	7.2E+05	7.3	0.93	7.7E+05	7.8	26	
	3	0.98	4.4E+05	4.5	0.94	7.0E+05	7.1	23	
Viability	1	0.99	2.6E+00	3.7	0.98	3.6E+00	5.2	24	7
	2	0.99	1.9E+00	2.8	0.99	2.1E+00	3.1	26	
	3	0.98	2.1E+00	3.1	0.98	2.6E+00	3.7	24	

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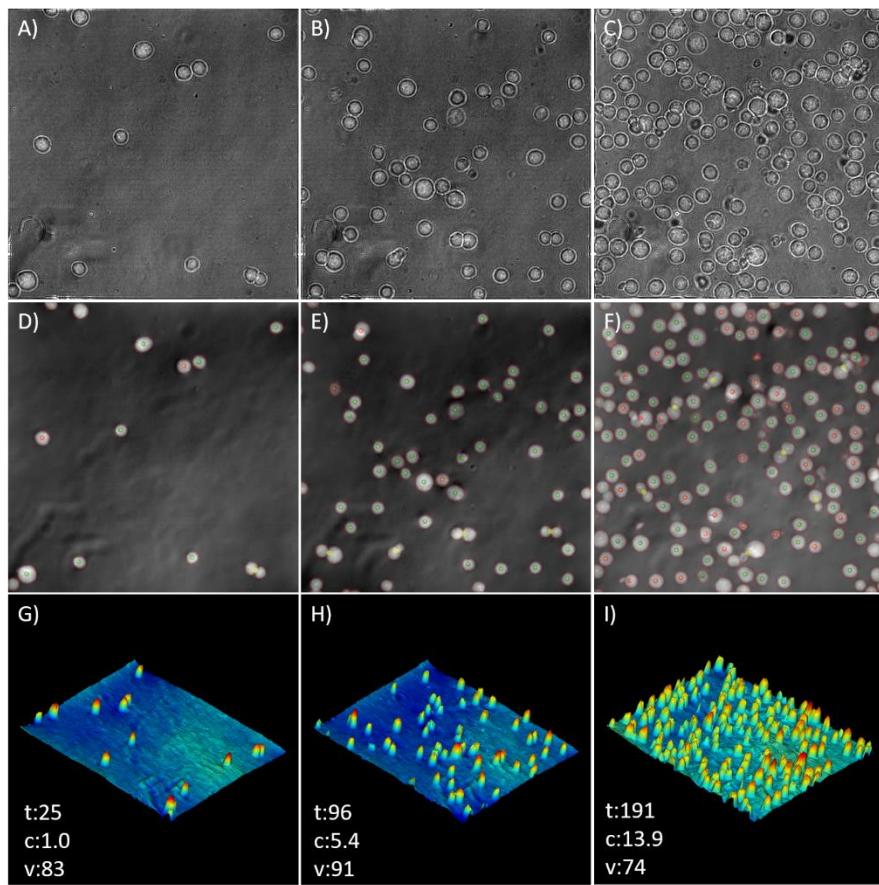
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10           **Table S3** – Quality characteristics overview for the models presented in Figure 1 and 2.  $R^2$  and  $Q^2$  are  
 11           the correlation coefficients of calibration and validation, respectively. Also shown are the root mean  
 12           squared errors (RMSE) for calibration and validation and the RMSE scaled by the variable range.

Variable	Validation Method	$R^2$	RMSE calibration	Normalized RMSE calibration (%)	$Q^2$	RMSE validation	Normalized RMSE validation (%)
Viability	LOBO	1.00	$6.8 \times 10^{-1}$	1.0	0.74	$1.1 \times 10^1$	15.8
Viable cell concentration	LOBO	0.97	$4.7 \times 10^5$	4.8	-0.69	$3.6 \times 10^6$	36
Viability	3CV	0.99	2.2	3.2	0.98	2.9	4.1
Viable cell concentration	3CV	0.96	$5.4 \times 10^5$	5.4	0.93	$7.1 \times 10^5$	7.2
Intracellular specific AAV titer	3CV	1.00	$9.1 \times 10^2$	1.9	0.99	$1.9 \times 10^3$	3.8
Extracellular AAV titer	3CV	0.99	$3.8 \times 10^8$	2.7	0.97	$7.2 \times 10^8$	5.0

LOBO – leave one batch out; 3CV – 3-fold cross-validation;  $R^2$  –correlation coefficient of calibration; RMSE - Root Mean Squared Error;  $Q^2$  –correlation coefficient of validation.

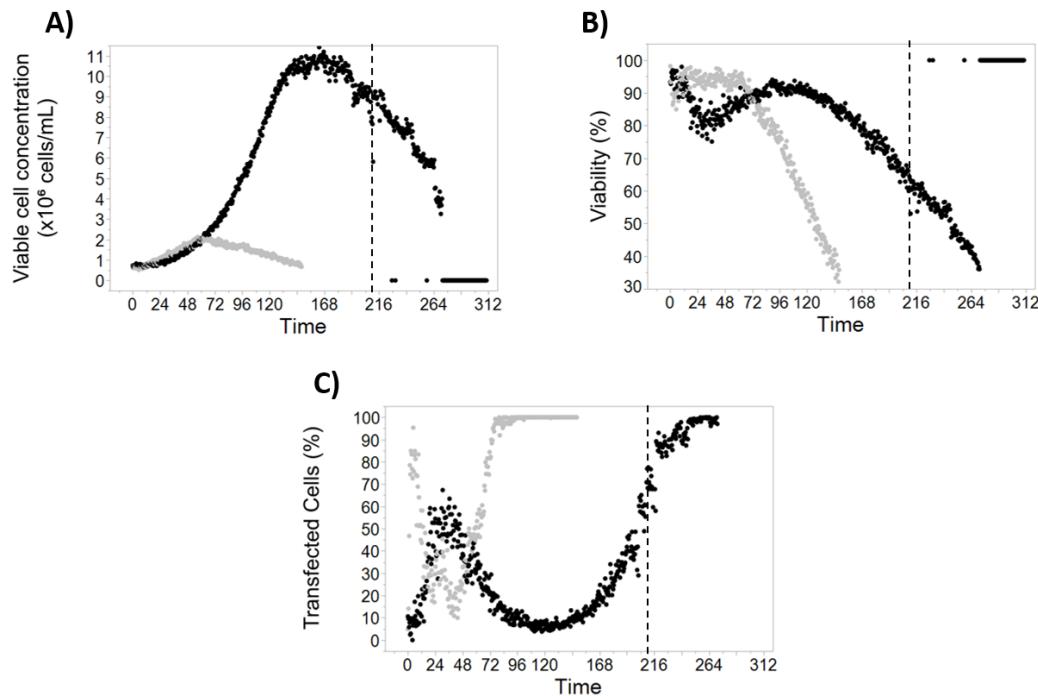
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15 **Figure S1** - Overview of the cell culture evolution profile over time, as captured by OsOne software. Phase  
16 images are presented in top row; middle row shows OsOne identification of each cell, with indication of the cell  
17 viability by a coloured circle in the middle of each cell: green - viable cells, red - non-viable, yellow - cell cluster;  
18 the corresponding calculated holograms are shown in bottom row. Each column represents different image types  
19 from the same culture timepoint, indicated in the bottom of the column. Abbreviations: t – culture time (h);  
20 c - viable cell concentration ( $10^6$ cells/mL); v: viability (%).

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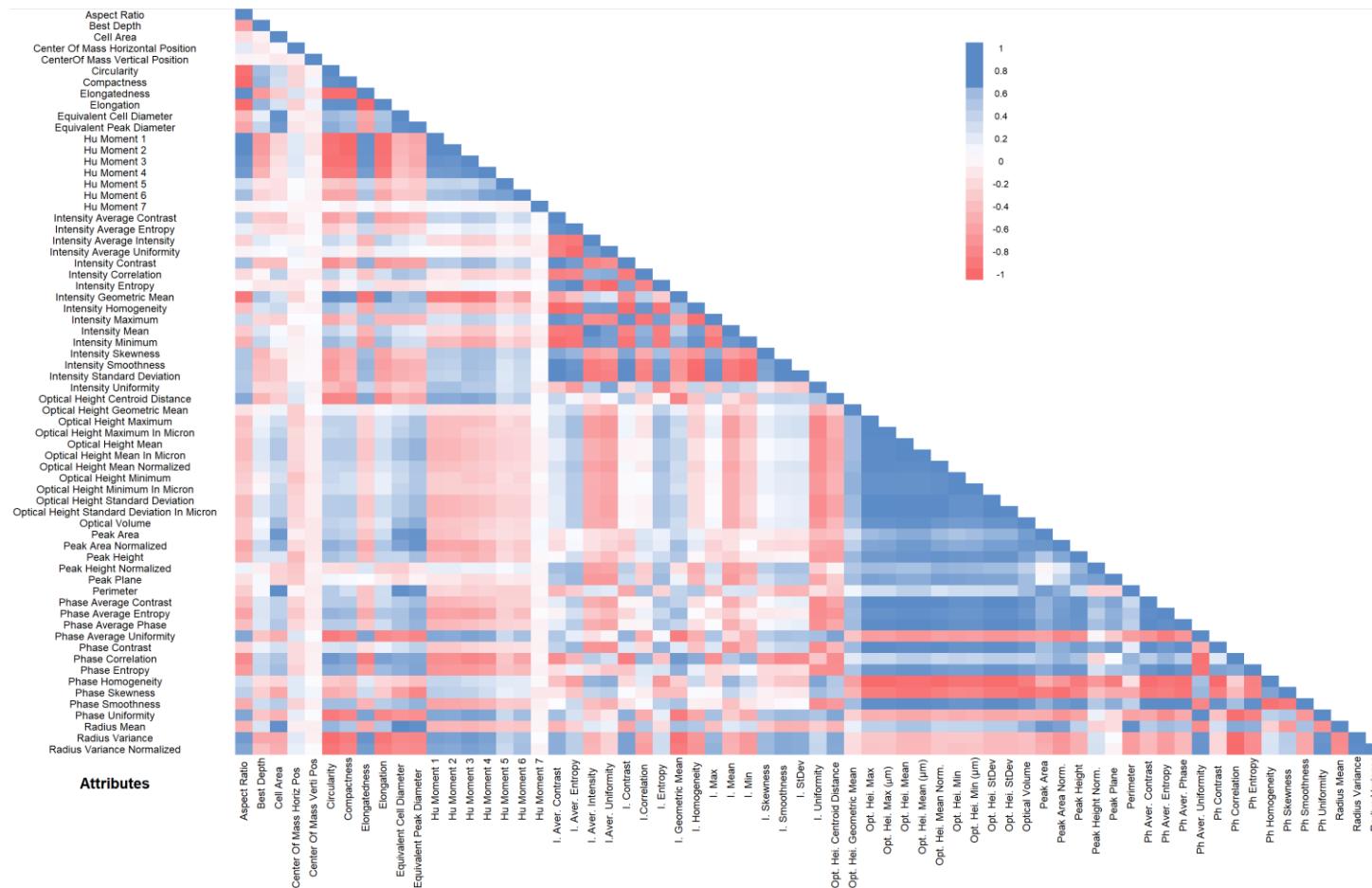


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23 **Figure S2** - Evolution of the predicted process variables using Ovizio proprietary models. Growth batch is  
24 represented in black, while infection batch is colored in grey. A) Viable cell concentration. B)  
25 Percentage of transfected cells. Dashed line indicates the time when controller setpoints were changed, with  
26 noticeable alterations in the iLine F profiles.

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Supplementary material for the article: Pais, D; Galrão, P; Kryzhanska, A; Barbau, J; Isidro, I; Alves, P. Holographic imaging of insect cell cultures: online non-invasive monitoring of adeno associated virus production and cell concentration, Processes 2020, 6.



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29 **Figure S3** - Pearson correlation coefficients for all attributes. A heatmap representation is shown, with blue colors indicating positive correlation and red colors indicating negative correlations.  
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