Supplemental material: On the measurement and simulation of influences on the acoustic contrast factor of biological cells

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1 Data analysis of the experimental data

The low metastatic parental cell line SaOs-2 was chosen as the baseline. This baseline was not only used to compare effects of treatments on the ACF and to analyse the difference between the metastatic potential and its influence on the ACF, but was used to calibrate the setup and create the workflow for the experiments. The calibration of the setup and the creation of the workflow included preliminary a data analysis on the robustness of the data. This was in part comprised of a check for normality for the two cell lines and two different conditions and a comparison of the outcome between different observers. All data was analysed with R 4.2.1 in R studio.

1.1 Data normality

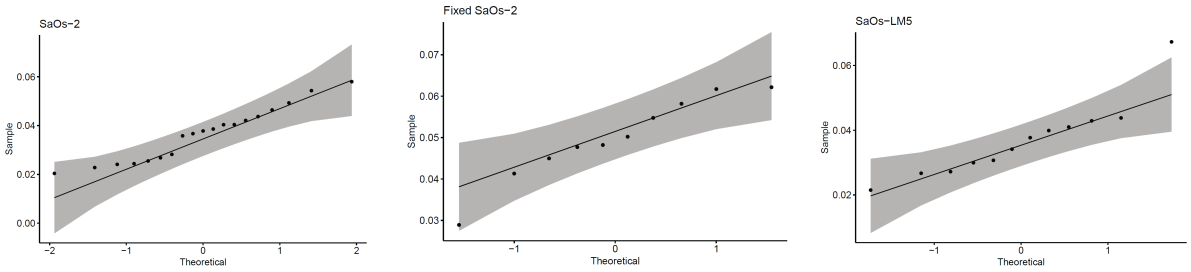
The data sets of the SaOs-2, fixed SaOs-2 and LM5 cells were tested for normality with a Shapiro test. If the p-value of the Shaprio test is greater than 0.05, then the data is considered to be normally distributed. The data was found to be normal as seen in table S1 and the QQ-plots in Fig. S1.

Cell type	p-value
SaOs-2	0.4052
Fixed SaOs-2	0.5708
LM5	0.09502

Table S1: The ACF data of the SaOs-2, fixed SaOs-2 and LM5 cells was tested for normality. If the p-value is greater than 0.05 the data is considered normal, which is the case for all cell lines and conditions presented here.

1.2 Data comparison between observers

Furthermore, the experiments to measure the ACF for the SaOs-2 cells were repeated by two observers (C.H. and D.B.) to test the inter-observer variability. The measurements do not seem to be observer dependent as seen in table S2 and Fig. S2.



(a) QQ-plot for SaOs-2 cells. (b) QQ-plot for fixed SaOs-2 cells. (c) QQ-plot for LM5 cells.

Figure S1: QQ-plots for the SaOs-2, fixed SaOs-2 and LM5 cells indicating normality of the data sets.

Observer	Mean Φ / ACF [1]
D.B.	0.0366
C.H.	0.0372

Table S2: Comparison of the data of two observers where the data did not show a large variation between the observers C.H. and D.B. for the baseline SaOs-2 cells, especially as the data was usually rounded to 3 decimal points which would result in both values for the ACF being 0.037.

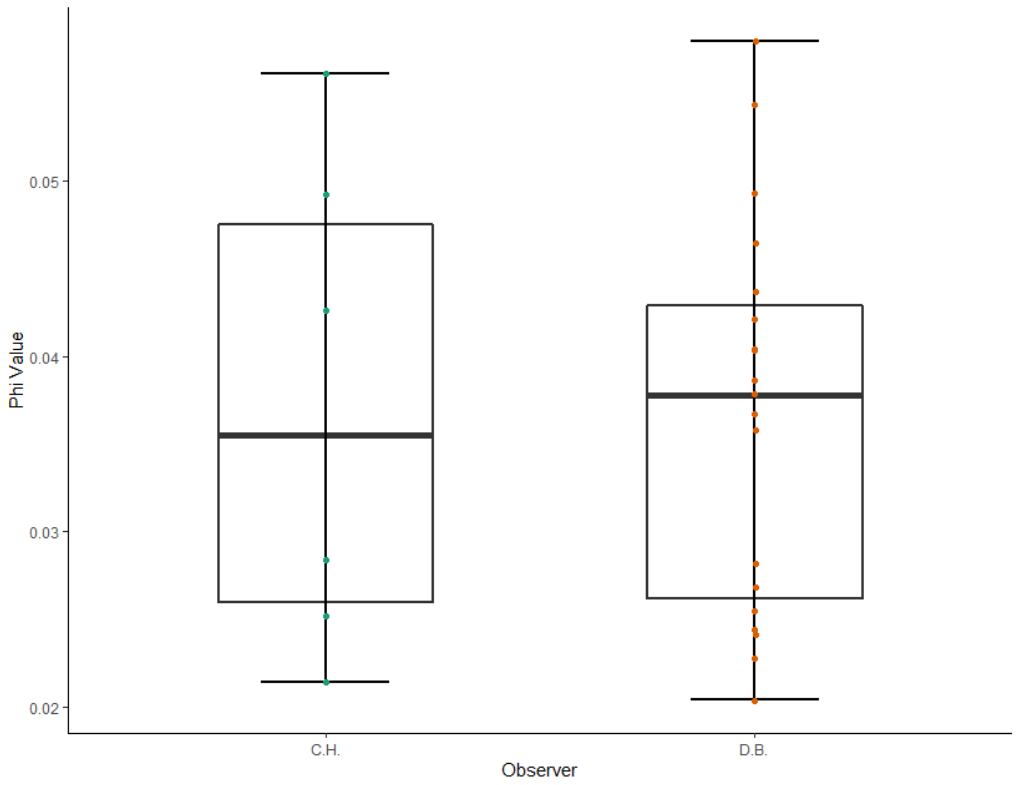


Figure S2: Comparison of the data of two observers where the data did not show a large variation between the observers C.H. ($n = 6$) and D.B. ($n = 19$) for the baseline SaOs-2 cells.