Stepwise Improvement of a Biocrystallisation Assay for Examining Effects of Homeopathic Preparations Using Cress Seedlings

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Introduction

A major challenge in basic homeopathic potentisation research is the development of a well-defined bioassay that generates evidence for specific effects of homeopathic preparations. A preclinical assay that yielded very high evidence for specific effects of an ultra-molecular homeopathic dilution (Stannum metallicum 30x) was achieved by means of the biocrystallisation method. Cress (Lepidium sativum L.) germinated and grew in the homeopathic preparation. An extract from the seedlings was mixed with copper chloride ($CuCl_2.2H_2O$) and crystallized in a dish.

Computerized textural image analysis (classical geo-centric evaluation) yielded highly significant and reproducible effects for the treatment with Stannum metallicum 30x in 15 independent randomized and blinded experiments in two independent laboratories (Baumgartner et al., 2012).
Although the overall statistical significance over the 15 experimental days was high, only 4 individual experimental days showed a statistical significance whereas a tendency was apparent for 3 additional experimental days (*Cluster shade* ROI 0-100%). This rose the question whether the non-significant days were a consequence of the followed laboratory procedure for generating the cress biocrystallisation patterns, or due to a lack in the evaluation abilities of the applied image analysis

algorithm.

This report focusses on the combination of two topics:

1. **The applied image analysis algorithms:** in what way is the evaluation optimised when shifting from a simple rectangular evaluation approach (*classical algorithm*) to a more 'picture-mimicking' approach (i.e. an *angular and radial algorithm*).
2. **The types of applied ROIs** (Regions Of Interest): Image analysis is commonly performed on image sections; so-called ROIs, of the entire image. Two types of ROIs can be defined, according to their symmetry centre (geometric-centre based, and crystallization-(start) based ROIs), as shown in figure 1. The crystallization-start based ROI is of partical interest as it reflects the crystallization process from centre to periphery of the dish. The Baumgartner data-evaluation is based on a geometric-centre based ROI. The three evaluation approaches will be compared while applying both types of ROIs.

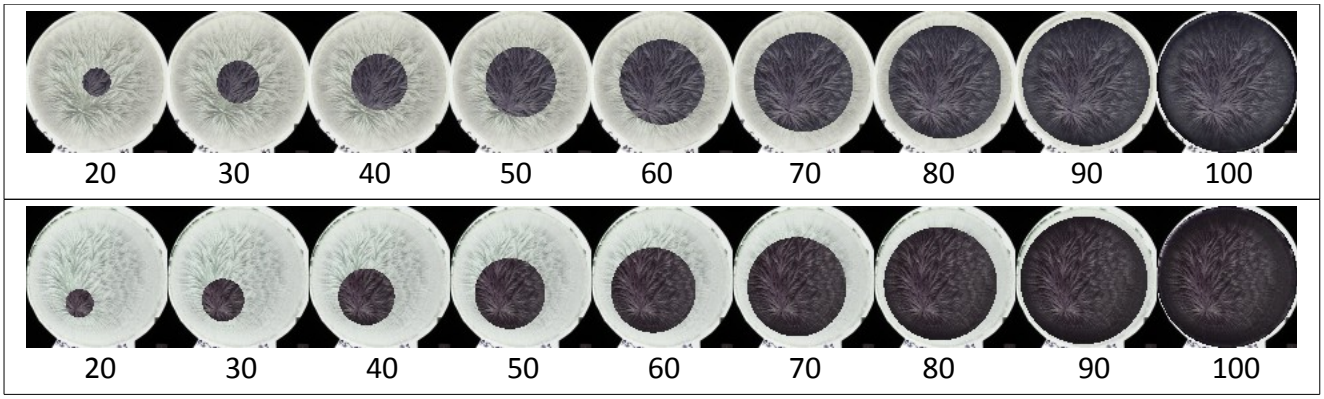

*Figure 1: From left to right. The darkened section is showing the **geometric-centre based ROI** (upper row) or the **crystallization-start based ROI** (lower row). The radius of the circle is in percentage of the total picture radius.*

In the following sections the results obtained with the two transformation algorithms (successively the angular and radial, applied on a geometric-centre versus a crystallization-start based ROI) applied to the Baumgartner 2012 data will be discussed in the light of the potential increase in the number of significant experimental days, and/or the reduction of the number of effect inversions. Investigating, as a first step, a more practical 'day to day use' of the cress-biocrystallization assay for homeopathic basic research.
As can be seen in figure 1, the ROI 0-100% yields similar sections for both the geometric-centre based as the crystallization-start based ROI (see figure 1). As the section-centres are used as starting points for the polar transformations, ROI 0-100% will give comparable results for both types of ROIs. Consequently the ROI 0-100% evaluation will be performed only for the geometric-centre evaluation.

Experimental setup according to Baumgartner et al. (2012)

Preparations of Stannum metallicum 30x and water 30x were investigated in randomized and coded ('blind') allocation in 15 independent experiments in total, thereof 7 at LBI (NL) and 8 at BRAD (DK). In each experiment, 6 cress extracts were examined in 6-fold glass plate replicates yielding 36 biocrystallograms (Figure 2) and in addition 7 biocrystallograms of a reference compound (freeze-dried wheat), thereby applying the crystallisation apparatus capacity of 43 biocrystallograms per day. This experimental design allowed controlling reproducibility as well as experimental stability at the same time.
Reproducibility could be examined between laboratories, between experiments (by comparing the

results as a function of individual experiments), and within experiments (by comparing the results as a function of the 3 internal replicates).
Experimental stability could be examined by incorporating the Systematic Negative Control approach (Baumgartner et al., 1998) in modified form, as one of the preparations was water 30X.

Computerised Textural Image Analysis

All biocrystallograms were scanned with a PowerLook III UMAX scanner in a transmitted light process. Dedicated software for pattern evaluation was used which connected each scanned image with the laboratory documentation information. All images were used for evaluation except 5 which exhibited technical errors (pre-defined crystallization disturbances due to leakage, etc.). The applied image analysis algorithm, which is described in Andersen et al. (1999), is based on textural features of the scanned images, resulting in a Grey-level co-occurrence matrix (GLCM). From this GLCM, 15 second order variables are calculated for different ROIs of the scanned image (Busscher et al., 2010).

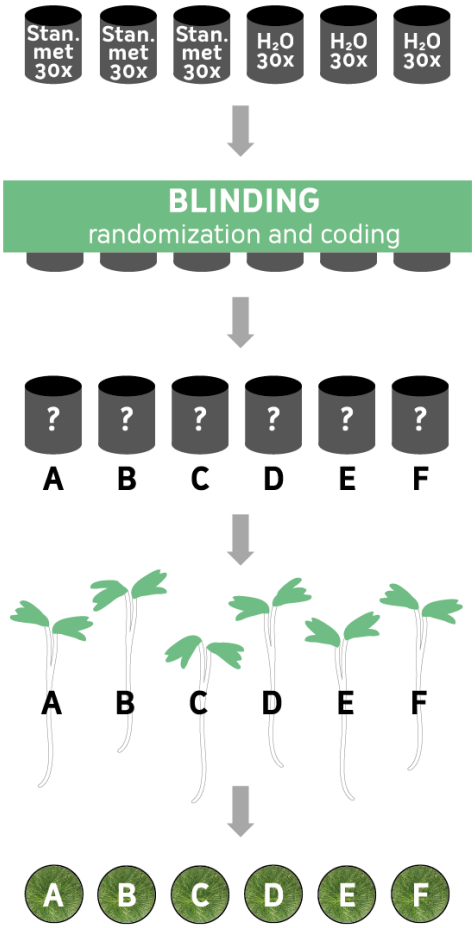

Figure 2: General design of the experiments. The homeopathic preparations Stannum metallicum 30x and water 30x prepared in triplicate were randomized and coded (A-F). The preparations were used to cultivate cress seedlings. After a 96h growth period an aqueous extract was prepared resulting in 6 biocrystallograms per treatment (A-F).

Analysis of Variance (ANOVA)

Texture analysis (TA) data was analysed by means of ANOVA F-tests with the independent parameters experimental day {1– 15} (N◦ 1–7 correspond to experiments 1–7 from LBI and N◦ 8–15 to experiments 1–8 from BRAD), treatment {1-2} (Stannum met. 30x, water 30x), and internal replicate

{1– 3} using the software Statistica 6.0 (StatSoft Inc., Tulsa, USA). Since the investigation was exploratory in nature, no corrections for multiple testing were applied in general.

Results Classical evaluation algorithm

Complexity reduction

Many of the 15 TA variables were correlated to each other at ROI 0-100%, meaning that most variables were not independent. Two main groups of variables can be distinguished which were closely correlated within the respective group, but not or only weakly correlated to those of the other group: (i) group I (*Cluster shade* and *Diagonal moment*, r = 0.96 within group), (ii) group II (all other TA variables with the exception of *Cluster prominence*, r > 0.6 within group). The variable *Cluster prominence* showed a moderate correlation to all but two other TA variables and takes an intermediate position (see Table 1).
This high correlation between multiple TA variables allows a considerable complexity reduction in the discussion of the results by focussing mainly on the two different groups of TA variables, although data for all 15 TA variables are shown.

Variable	Clust pr	Clust sh	Corr	Diag M	Diff en	Diff entr	Energy	Entr	Inertia	In Diff M.	Kappa	Max prob	Sum en	Sum entr	Sum var
Clust prom		0.56	0.6	-0.52	0.48	-0.56	0.39	-0.59	-0.6	0.45	0.45	0.2	-0	0.54	0.56
Clust shade	0.56		0.2	**-0.96**	0.04	-0.15	-0.01	-0.11	-0.2	-0.02	-0.02	-0	0.16	0.19	0.17
Corr	0.58	0.19		-0.23	**0.93**	**-0.98**	**0.89**	**-0.97**	**-1**	**0.9**	**0.89**	0.63	**-0.8**	**0.98**	**0.99**
Diag mom	-0.52	**-0.96**	-0.2		-0.11	0.19	-0.07	0.16	0.2	-0.08	-0.09	-0.12	-0.07	-0.23	-0.22
Diff ener	0.48	0.04	**0.9**	-0.11		**-0.98**	**0.99**	**-0.98**	**-0.9**	**0.97**	**0.96**	**0.74**	**-0.78**	**0.91**	**0.92**
Diff entr	-0.56	-0.15	**-1**	0.19	**-0.98**		**-0.96**	**0.99**	**1**	**-0.94**	**-0.93**	-0.69	**0.79**	**-0.96**	**-0.97**
Energy	0.39	-0.01	**0.9**	-0.07	**0.99**	**-0.96**		**-0.95**	**-0.9**	**0.96**	**0.95**	**0.78**	**-0.81**	**0.88**	**0.9**
Entr	-0.59	-0.11	**-1**	0.16	**-0.98**	**0.99**	**-0.95**		**1**	**-0.96**	**-0.95**	-0.69	**0.75**	**-0.93**	**-0.95**
Inertia	-0.59	-0.19	**-1**	0.23	**-0.93**	**0.98**	**-0.89**	**0.97**		**-0.9**	**-0.89**	-0.63	**0.79**	**-0.98**	**-0.99**
Inv Diff Mom	0.45	-0.02	**0.9**	-0.08	**0.97**	**-0.94**	**0.96**	**-0.96**	**-0.9**		**1**	**0.79**	**-0.77**	**0.87**	**0.89**
Kappa	0.45	-0.02	**0.9**	-0.09	**0.96**	**-0.93**	**0.95**	**-0.95**	**-0.9**	**1**		**0.79**	**-0.76**	**0.85**	**0.88**
Max prob	0.2	-0	0.6	-0.12	**0.74**	-0.69	**0.78**	-0.69	-0.6	**0.79**	**0.79**		-0.64	0.63	0.65
Sum ener	0.00	0.16	**-0.8**	-0.07	**-0.78**	**0.79**	**-0.81**	**0.75**	**0.8**	**-0.77**	**-0.76**	-0.64		**-0.83**	**-0.82**

*Table 1: **Correlation coefficient** between the 15 TA variables for the **classical evaluation algorithm** at ROI 0-100%. Strong correlations (r > 0.7) are shown in bold red font.*

Classical algorithm ROI 0-100%

TA data were analysed by full 3-way analysis of variance, performed on all 15 second-order TA variables, using experimental day, treatment, and internal replicate treatment as the three independent variables. Results are given in Table 2.

Group I TA variables yielded highly significant results for the potency treatment. Significant differences in absolute values for the two TA variables between the single experimental days were found (significant experimental day effect). Nevertheless, no significant interactions between experimental day and potency treatment were found (Table 1, Interaction 1-2), meaning that the potency effect was quite reproducible over the 15 independent experiments for the variables of group I.

The group I TA variables yielded *2* effect inversions. Fisher's LSD post hoc tests for variable *Cluster shade* using experimental day and treatment yielded significant or highly significant results for 4 out of the remaining 13 experimental days.

No significant differences (border-line significance for *Diagonal moment*) were observed between the three internal replicate treatments indicative of a stable experimental setup.

Variables of Group II yielded significant or highly significant results for the potency treatment. Similar to the group I variables, there was a significant experimental day effect. Furthermore, all except one (*Maximum probability*) variables yielded significant or highly significant interactions between experimental day and potency treatment (Table 2, Interaction 1-2), meaning that the difference between Stannum met. 30x and water 30x seemed to exhibit varying results for different experimental days. Variables yielded 5-7 effect inversions.
No highly significant differences were observed between the three internal replicate treatments. However, two TA variables showed a significant internal replicate effect pointing towards a weak systematic error.

Variable *Cluster prominence* yielded border-line significant results for the potency treatments.

Classical Geo ROI0-100

Parameters	Main effects (*P* value)			Effect interactions (*P* value)			
	(1) Exp. day	(2) Potency	(3) Replicate	1-2	1-3	2-3	1-2-3
Clust shade	**<0.0001**	**0.0004**	0.1296	0.3264	0.2145	0.4365	0.5069
Diag mom	**<0.0001**	**0.0002**	0.0508	0.2998	0.0943	0.6176	0.3498
Corr	**<0.0001**	**0.0039**	0.5375	**0.0001**	0.1567	0.0918	0.1896
Diff ener	**<0.0001**	**0.0076**	0.3948	**0.0040**	0.2444	0.1625	0.1077
Diff entr	**<0.0001**	**0.0058**	0.5062	**0.0009**	0.2248	0.1288	0.1591
Energy	**<0.0001**	**0.0055**	0.4246	**0.0143**	0.3051	0.1684	0.1437
Entropy	**<0.0001**	**0.0150**	0.2997	**0.0006**	0.1970	0.1947	0.1318
Inertia	**<0.0001**	**0.0041**	0.5262	**0.0001**	0.1567	0.0978	0.1934
Inv Diff Mom	**<0.0001**	**0.0024**	**0.0166**	**0.0001**	0.0893	0.2193	**0.0138**
Kappa	**<0.0001**	**0.0023**	**0.0112**	**<0.0001**	0.0923	0.2521	**0.0124**
Max prob	**<0.0001**	**0.0022**	0.0528	0.2930	0.1958	0.1342	0.0886
Sum ener	**<0.0001**	**0.0002**	0.4163	**0.0046**	0.0901	**0.0397**	0.3965
Sum entr	**<0.0001**	**0.0013**	0.7931	**0.0001**	0.1380	**0.0387**	0.1780
Sum var	**<0.0001**	**0.0029**	0.6470	**<0.0001**	0.1622	0.0507	0.1598
Clust prom	**<0.0001**	**0.0436**	0.2143	**0.0005**	0.1135	0.3126	0.1706

Table 2: ANOVA F-tests for all 15 TA variables (main effects and interactions). ***Classical evaluation algorithm ROI 0-100%****. Highly significant effects (P ≤ 0.01) are shown in bold red font; significant effects (P ≤ 0.05) are shown in bold blue font. Variables of group I are marked by light grey background colouring, variable Cluster prominence is marked by dark grey background colouring.*

Classical algorithm, geo-centre based ROIs 0-50%; 50-70%; 70-90% and 90-100%

In a subgroup analysis we aimed at identifying the spatial region of the biocrystallogram where the potency treatment effect manifests predominantly. We defined four different ROIs as circular segments of the entire biocrystallogram by the radius segments 0–50%, 50–70%, 70–90%, and 90–100%. Full 3-way analysis of variance was performed on all 15 second-order TA variables as above. Results for the potency treatment main effect and the interaction of potency treatment with experimental day are given in Table 3.
The signal in the group I TA variables manifests predominantly in the geometrical centre of the biocrystallogram (ROI 0-70%), with an emphasis on the outer region of the centre (ROI 50–70%). Whereas the group II TA variables and Cluster prominence seem to respond to two signal sources: (i) a reproducible signal (main effect) rather in the periphery of the biocrystallograms (70– 90%) and (ii)

a signal varying in time (interaction with experimental day) spread all over the biocrystallogram with emphasis on ROI 50–70%.

Classical geo segment

Parameters	Main effects (p-value)				Interaction effects			
	ROI 0-50%	ROI 50-70%	ROI 70-90%	ROI 90-100%	ROI 0-50%	ROI 50-70%	ROI 70-90%	ROI 90-100%
Clust shade	**0.0038**	**0.0002**	0.1552	0.1584	0.6700	0.9604	0.8339	0.9768
Diag mom	**0.0021**	**<0.0001**	0.1406	0.0764	0.4649	0.9091	0.8331	0.9135
Corr	0.6482	0.0938	**<0.0001**	**0.0131**	**0.0258**	**<0.0001**	**0.0185**	0.1023
Diff ener	0.6773	**0.0160**	**<0.0001**	**0.0131**	**0.0217**	**0.0001**	**0.0380**	**0.0419**
Diff entr	0.8923	**0.0460**	**<0.0001**	**0.0123**	**0.0185**	**<0.0001**	**0.0240**	0.0570
Energy	0.7202	**0.0170**	**<0.0001**	**0.0235**	**0.0292**	**0.0001**	**0.0330**	**0.0467**
Entropy	0.9313	0.0578	**<0.0001**	**0.0416**	**0.0207**	**<0.0001**	**0.0171**	**0.0351**
Inertia	0.6508	0.0939	**<0.0001**	**0.0145**	**0.0258**	**<0.0001**	**0.0186**	0.1158
Inv Diff Mom	0.3531	**0.0199**	**<0.0001**	**0.0069**	**0.0103**	**<0.0001**	**0.0379**	**0.0096**
Kappa	0.3137	**0.0339**	**<0.0001**	**0.0085**	**0.0120**	**<0.0001**	**0.0270**	**0.0083**
Max prob	0.4692	0.1979	**0.0140**	0.0653	0.1840	**0.0091**	0.3755	**0.0246**
Sum ener	0.1270	0.0550	**<0.0001**	**0.0008**	**0.0129**	**<0.0001**	0.0603	**0.0497**
Sum entr	0.5659	0.1241	**<0.0001**	**0.0026**	**0.0293**	**<0.0001**	**0.0289**	**0.0415**
Sum var	0.6193	0.0926	**<0.0001**	**0.0071**	**0.0260**	**<0.0001**	**0.0180**	**0.0487**
Clust prom	0.6344	0.3447	**<0.0001**	0.0651	0.1557	**0.0175**	**0.0085**	**0.0493**

*Table 3: ANOVA F-tests **Classical evaluation algorithm, geo-centre based ROIs** for the potency treatment (main effect and interaction with experimental day), analysing all 15 TA variables in four different ROIs. Highly significant effects (P < 0.01) are shown in bold red font; significant effects (P ≤ 0.05) are shown in bold blue font. Variables of group I are marked by light grey background colouring, variable Cluster prominence is marked by dark grey background colouring*

Classical algorithm, crystal-start based ROIs 0-50%; 50-70%; 70-90%, 90-100%

More or less identical to the geo-centre based ROI evaluation, the signal in the group I TA variables manifests predominantly in the geometrical centre of the biocrystallogram (ROI 0–70%, Table 4), with an emphasis on the outer region of the centre (ROI 50–70%). Whereas the group II TA variables and Cluster prominence seem to respond to two signal sources: (i) a reproducible signal (main effect) rather in the periphery of the biocrystallograms (70– 90%) and (ii) a signal varying in time (interaction with experimental day) spread all over the biocrystallogram with emphasis on ROI 50–70%.

Classical crystal-start segme

Parameters	Main effects (p-value)				Interaction effects			
	ROI 0-50%	ROI 50-70%	ROI 70-90%	ROI 90-100%	ROI 0-50%	ROI 50-70%	ROI 70-90%	ROI 90-100%
Clust shade	**0.0026**	**0.0034**	0.1261	0.1729	0.4762	0.4807	0.6414	0.9316
Diag mom	**0.0010**	**0.0002**	0.0938	0.0813	0.3679	0.6273	0.7958	0.8445
Corr	0.5589	0.2046	**<0.0001**	**0.0094**	**0.0299**	**<0.0001**	0.1465	0.1087
Diff ener	0.8576	**0.0182**	**<0.0001**	**0.0131**	**0.0283**	**0.0004**	0.1404	0.0550
Diff entr	0.7337	0.0987	**<0.0001**	**0.0101**	**0.0234**	**0.0001**	0.1398	0.0668
Energy	1.0000	**0.0236**	**<0.0001**	**0.0248**	**0.0489**	**0.0009**	0.1425	0.0613
Entropy	0.7941	0.1071	**<0.0001**	**0.0404**	**0.0285**	**0.0002**	0.1278	**0.0407**
Inertia	0.5604	0.2074	**<0.0001**	**0.0103**	**0.0296**	**<0.0001**	0.1415	0.1208
Inv Diff Mom	0.5139	**0.0029**	**<0.0001**	**0.0071**	**0.0117**	**<0.0001**	0.0789	**0.0149**
Kappa	0.6116	**0.0027**	**<0.0001**	**0.0082**	**0.0083**	**0.0001**	0.0844	**0.0111**
Max prob	0.5266	0.1590	**0.0001**	0.0591	0.1647	0.2105	0.2381	**0.0290**
Sum ener	0.0554	0.2013	**<0.0001**	**0.0007**	0.0563	**0.0004**	0.2371	0.1105
Sum entr	0.4883	0.2098	**<0.0001**	**0.0021**	**0.0344**	**0.0001**	0.2015	0.0588
Sum var	0.5413	0.1822	**<0.0001**	**0.0060**	**0.0331**	**<0.0001**	0.1887	0.0624
Clust prom	0.4815	0.2575	**<0.0001**	**0.0665**	0.0517	**0.0023**	0.0933	0.0514

*Table 4: ANOVA F-tests **Classical evaluation algorithm, crystal-start based ROIs** for the potency treatment (main effect and interaction with experimental day), analysing all 15 TA variables in four different ROIs. Highly significant effects (P ≤ 0.01) are shown in bold red font; significant effects (P ≤ 0.05) are shown in bold blue font. Variables of group I are marked by light grey background colouring, variable Cluster prominence is marked by dark grey background colouring.*

Results Angular transformation algorithm

Complexity reduction

The 15 TA variables calculated for the angular transformation GLCM at ROI 0-100% show a similar correlation as found for the classical transformation algorithm (Table 5), i.e. two main groups of variables that were closely correlated within the respective group, but not or only weakly correlated to those of the other group: (i) group I (*Cluster shade* and *Diagonal moment*, r = 0.91 within group), (ii) group II (all other TA variables with the exception of *Cluster prominence*, r > 0.67 within group).

Variable	Clust pr	Clust sh	Corr	Diag m	Diff en	Diff entr	Energy	Entr	Inertia	Inv Diff	Kappa	Max pro	Sum en	Sum entr	Sum var
Clust prom		0.36	0.37	-0.29	0.32	-0.34	0.26	-0.36	-0.32	0.31	0.34	0.16	-0.17	0.59	0.68
Clust shade	0.36		-0.13	**-0.91**	-0.15	0.13	-0.15	0.15	0.15	-0.2	-0.2	-0.15	0.28	-0.03	-0
Corr	0.37	-0.13		-0.04	**0.97**	**-0.99**	**0.96**	**-0.98**	**-1**	**0.97**	**0.96**	**0.82**	**-0.91**	**0.91**	**0.87**
Diag mom	-0.29	**-0.91**	-0.04		-0.02	0.04	-0.02	0.02	0.03	0.01	0.01	-0.04	-0.07	-0.11	-0.11
Diff ener	0.32	-0.15	**0.97**	-0.02		**-0.99**	**0.99**	**-0.99**	**-0.98**	**0.99**	**0.98**	**0.85**	**-0.88**	**0.86**	**0.82**
Diff entr	-0.34	0.13	**-0.99**	0.04	**-0.99**		**-0.98**	**0.99**	**0.99**	**-0.98**	**-0.97**	**-0.84**	**0.89**	**-0.88**	**-0.84**
Energy	0.26	-0.15	**0.96**	-0.02	**0.99**	**-0.98**		**-0.98**	**-0.97**	**0.98**	**0.97**	**0.86**	**-0.85**	**0.81**	**0.77**
Entr	-0.36	0.15	**-0.98**	0.02	**-0.99**	**0.99**	**-0.98**		**0.99**	**-0.98**	**-0.98**	**-0.83**	**0.86**	**-0.86**	**-0.83**
Inertia	-0.32	0.15	**-1**	0.03	**-0.98**	**0.99**	**-0.97**	**0.99**		**-0.97**	**-0.96**	**-0.82**	**0.9**	**-0.88**	**-0.83**
Inv Diff Mom	0.31	-0.2	**0.97**	0.01	**0.99**	**-0.98**	**0.98**	**-0.98**	**-0.97**		**1**	**0.88**	**-0.9**	**0.86**	**0.82**
Kappa	0.34	-0.2	**0.96**	0.01	**0.98**	**-0.97**	**0.97**	**-0.98**	**-0.96**	**1**		**0.88**	**-0.9**	**0.87**	**0.84**
Max prob	0.16	-0.15	**0.82**	-0.04	**0.85**	**-0.84**	**0.86**	**-0.83**	**-0.82**	**0.88**	**0.88**		**-0.78**	**0.71**	0.67
Sum ener	-0.17	0.28	**-0.91**	-0.07	**-0.88**	**0.89**	**-0.85**	**0.86**	**0.9**	**-0.9**	**-0.9**	**-0.78**		**-0.89**	**-0.83**
Sum entr	0.59	-0.03	**0.91**	-0.11	**0.86**	**-0.88**	**0.81**	**-0.86**	**-0.88**	**0.86**	**0.87**	**0.71**	**-0.89**		**0.99**
Sum var	0.68	-0	**0.87**	-0.11	**0.82**	**-0.84**	**0.77**	**-0.83**	**-0.83**	**0.82**	**0.84**	0.67	**-0.83**	**0.99**	

Table 5: Correlation coefficient between the 15 TA variables for the angular transformation algorithm at ROI 0-100%. Strong correlations (r > 0.7) are shown in bold red font.

Angular algorithm ROI 0-100%

Group I TA variables yielded similar to the classical algorithm variables highly significant results for the potency treatment, albeit at a 10-50 times lower significance level. Similarly, a significant experimental day effect was found without significant interactions between experimental day and potency treatment (Table 6, Interaction 1-2), pointing to a quite reproducible potency effect over the 15 independent experiments.
The group I TA variables yielded 4 effect inversions. Fisher's LSD post hoc tests for variable *Cluster shade* using experimental day and treatment yielded significant results for 1 out of the remaining 11 experimental days.
No significant differences were observed between the three internal replicate treatments indicative of a stable experimental setup.

Variables of Group II yielded similar to the classical algorithm variables highly significant results for the potency treatment, albeit at a 10-50 times lower significance level. Similar to the group I variables, there was a significant experimental day effect. Nevertheless, no significant interactions between experimental day and potency treatment were found for all but three TA variables (*Sum variance, Sum entropy, and Kappa;* Table 6, Interaction 1-2), pointing to a quite reproducible potency effect over the 15 independent experiments.
Variables yielded 3-6 effect inversions. No significant differences were observed between the three internal replicate treatments, indicative of a stable experimental setup.

Variable *Cluster prominence* yielded borderline significance for the potency treatments.

Angular Geo ROI0-100

Parameters	Main effects (*P* value)			Effect interactions (*P* value)			
	(1) Exp. day	(2) Potency	(3) Replicate	1-2	1-3	2-3	1-2-3
Clust shade	**<0.0001**	**0.0052**	0.2387	0.4722	0.1195	0.7081	0.7588
Diag mom	**<0.0001**	**0.0102**	0.1502	0.2089	0.3526	0.7716	0.5976
Corr	**<0.0001**	**0.0211**	0.3017	0.5191	0.2371	0.1139	0.2018
Diff ener	**<0.0001**	**0.0270**	0.3724	0.4605	0.4569	0.0524	0.4102
Diff entr	**<0.0001**	**0.0287**	0.3007	0.5709	0.4341	0.0751	0.3142
Energy	**<0.0001**	**0.0334**	0.3884	0.5763	0.4430	**0.0356**	0.5423
Entropy	**<0.0001**	0.0600	0.3002	0.3642	0.5157	0.0902	0.3928
Inertia	**<0.0001**	**0.0284**	0.2365	0.7010	0.3157	0.0878	0.2523
Inv Diff Mom	**<0.0001**	**0.0094**	0.7342	0.0743	0.2524	0.0911	0.2210
Kappa	**<0.0001**	**0.0088**	0.6934	**0.0366**	0.2405	0.1241	0.1883
Max prob	**<0.0001**	**0.0267**	0.6755	0.7016	0.4194	0.2088	0.0917
Sum ener	**<0.0001**	**0.0179**	0.3238	0.5683	0.1481	0.2419	0.2958
Sum entr	**<0.0001**	**0.0149**	0.6284	**0.0260**	0.0907	0.2480	0.2253
Sum var	**<0.0001**	**0.0250**	0.5580	**0.0071**	0.0708	0.3153	0.2404
Clust prom	**<0.0001**	0.0545	0.3398	**0.0018**	**0.0281**	0.7386	0.2662

Table 6: ANOVA F-tests for all 15 TA variables (main effects and interactions). ***Angular transformation algorithm ROI 0-100%.*** *Highly significant effects (P ≤ 0.01) are shown in bold red font; significant effects (P ≤ 0.05) are shown in bold blue font. Variables of group I are marked by light grey background colouring, variable Cluster prominence is marked by dark grey background colouring.*

Angular algorithm, geo-centre based ROIs 0-50%; 50-70%; 70-90% and 90-100%

Surprisingly no signal was observed for the group I TA variables (Table 7), which could be a

consequence of a reduced statistical power, or indicate that the signal is more dispersed over the biocrystallogram. The group II TA variables seem to respond to only a reproducible signal (main effect) in the periphery of the biocrystallograms, with an emphasis on ROI 70– 90%.

Angular geo segment

Parameters	Main effects (p-value)				Interaction effects			
	ROI 0-50%	ROI 50-70%	ROI 70-90%	ROI 90-100%	ROI 0-50%	ROI 50-70%	ROI 70-90%	ROI 90-100%
Clust shade	0.2830	0.2925	0.1854	0.8807	0.8276	0.8039	0.5958	0.8100
Diag mom	0.1690	0.0736	0.3854	0.1848	0.1772	0.7322	0.8726	0.6118
Corr	0.7556	0.8752	**0.0100**	**0.0404**	**0.0308**	0.6923	0.7508	0.8211
Diff ener	0.4030	0.2627	**0.0090**	**0.0447**	0.3569	0.9343	0.8454	0.2956
Diff entr	0.7667	0.6134	**0.0065**	**0.0328**	0.1484	0.9067	0.7950	0.4302
Energy	0.1242	0.0903	**0.0270**	0.0978	0.9283	0.7473	0.7307	0.1083
Entropy	0.2250	0.2219	**0.0179**	0.1647	0.4211	0.9984	0.6308	0.0959
Inertia	1.0000	0.8370	**0.0085**	**0.0300**	0.1550	0.8799	0.5064	0.7605
Inv Diff Mom	0.0920	**0.0259**	**0.0040**	**0.0342**	0.4407	0.8666	0.8700	0.0574
Kappa	0.0631	**0.0198**	**0.0038**	**0.0397**	0.2952	0.6297	0.8950	**0.0328**
Max prob	0.0597	0.1147	**0.0324**	0.5870	0.3907	0.1968	0.6586	0.5583
Sum ener	0.5333	0.2382	0.1932	0.1369	0.5191	0.6806	0.7651	0.7542
Sum entr	0.4624	0.3105	0.1831	0.1420	0.4498	0.8115	0.7808	0.7130
Sum var	0.7925	1.0000	0.0871	0.1081	0.2331	0.5341	0.9426	0.8603
Clust prom	0.9432	0.9596	0.0605	0.1346	0.0856	0.1318	0.9137	0.7969

*Table 7: ANOVA F-tests **Angular transformation algorithm, geo-centre based ROIs** for the potency treatment (main effect and interaction with experimental day), analysing all 15 TA variables in four different ROIs. Highly significant effects (P < 0.01) are shown in bold red font; significant effects (P ≤ 0.05) are shown in bold blue font. Variables of group I are marked by light grey background colouring, variable Cluster prominence is marked by dark grey background colouring.*

Angular algorithm, crystal-start based ROIs 0-50%; 50-70%; 70-90%, 90-100%

Different from the Angular geo-centre based ROI segment analysis, a highly significant signal was found for the group I TA variables which manifests predominantly in the outer geometrical centre of the biocrystallogram (ROI 50–70%). Whereas the group II TA variables and Cluster prominence seem to respond mainly to the periphery of the biocrystallograms (70– 90%).

Angular cryst-start segment

Parameters	Main effects (p-value)				Interaction effects			
	ROI 0-50%	ROI 50-70%	ROI 70-90%	ROI 90-100%	ROI 0-50%	ROI 50-70%	ROI 70-90%	ROI 90-100%
Clust shade	**0.0061**	**0.0024**	0.3619	0.1285	0.5808	0.2904	0.8319	0.8922
Diag mom	0.1394	**0.0120**	0.5564	0.0955	0.1992	0.3706	0.8004	0.7443
Corr	0.7095	0.6061	**0.0045**	**0.0189**	0.1106	0.6377	0.7759	0.5938
Diff ener	0.7683	0.3849	**0.0062**	**0.0321**	0.1157	0.5944	0.8590	0.2059
Diff entr	0.8432	0.6327	**0.0056**	**0.0216**	0.0651	0.5806	0.8736	0.3064
Energy	0.5536	0.3555	**0.0098**	0.0948	0.1129	0.5475	0.8487	0.1821
Entropy	0.9256	0.6989	**0.0096**	0.1098	0.0586	0.5353	0.8600	0.1354
Inertia	0.7402	0.6377	**0.0062**	**0.0210**	0.1048	0.6307	0.7021	0.6448
Inv Diff Mom	0.5196	0.1054	**0.0060**	**0.0400**	0.3212	0.4596	0.8012	**0.0342**
Kappa	0.6570	0.0790	**0.0067**	0.0524	0.4279	0.4638	0.8445	**0.0149**
Max prob	0.4679	0.2905	**0.0101**	0.1575	0.4035	0.8019	0.7686	**0.0137**
Sum ener	0.1388	0.4508	**0.0086**	**0.0070**	0.4168	0.1607	0.6743	0.4626
Sum entr	0.6740	0.4335	**0.0022**	**0.0082**	0.1251	0.0975	0.5505	0.1279
Sum var	0.5757	0.4588	**0.0022**	**0.0141**	0.1831	0.1200	0.5063	0.0566
Clust prom	0.6009	0.5145	**0.0016**	0.0546	0.0653	0.1164	0.2197	**0.0417**

*Table 8: ANOVA F-tests **Angular transformation algorithm, crystal-centre based ROIs** for the potency treatment (main effect and interaction with experimental day), analysing all 15 TA variables in four different ROIs. In bold red font P <0.01; significant effects (P ≤ 0.05) are shown in bold blue font. Variables of group I are marked by light grey background colouring, variable Cluster prominence is marked by dark grey background colouring.*

Results Radial transformation algorithm

Complexity reduction

The 15 TA variables calculated for the radial transformation GLCM at ROI 0-100% show a slightly different correlation as found for the classical transformation algorithm. Three main groups of variables can be distinguished which were closely correlated within the respective group, but not or only weakly correlated to those of the other group: (i) group I (*Cluster shade* and *Diagonal moment*, r = 0.87 within group), (ii) group II (all other TA variables with the exception of *Cluster prominence, Sum energy, Sum entropy,* and *Sum variance:* r > 0.86 within group), and (iii) group III (*Sum energy, Sum entropy,* and *Sum variance*: r > 0.73). The variable *Cluster prominence* showed a moderate correlation to all but three other TA variables and takes an intermediate position (see Table 9).

Variable	Clust pr	Clust sh	Corr	Diag m	Diff en	Diff entr	Energy	Entr	Inertia	Inv Diff	Kappa	Max pro	Sum en	Sum entr	Sum var
Clust prom		0.43	**0.75**	-0.37	**0.75**	**-0.74**	**0.71**	**-0.72**	**-0.73**	**0.76**	**0.75**	0.67	-0.15	**0.73**	**0.77**
Clust shade	0.43		0.15	**-0.87**	0.14	-0.15	0.14	-0.12	-0.14	0.12	0.11	0.17	0.05	0.26	0.24
Corr	**0.75**	0.15		-0.05	**0.96**	**-0.99**	**0.96**	**-0.98**	**-1**	**0.93**	**0.91**	**0.86**	-0.18	0.63	0.67
Diag mom	-0.37	**-0.87**	-0.05		-0.05	0.05	-0.05	0.02	0.04	-0.06	-0.06	-0.13	-0.08	-0.18	-0.17
Diff ener	**0.75**	0.14	**0.96**	-0.05		**-0.99**	**0.99**	**-0.99**	**-0.96**	**0.98**	**0.96**	**0.9**	-0.18	0.63	0.67
Diff entr	**-0.74**	-0.15	**-0.99**	0.05	**-0.99**		**-0.99**	**1**	**0.99**	**-0.96**	**-0.93**	**-0.89**	0.15	-0.61	-0.65
Energy	**0.71**	0.14	**0.96**	-0.05	**0.99**	**-0.99**		**-0.99**	**-0.96**	**0.96**	**0.93**	**0.91**	-0.09	0.55	0.6
Entr	**-0.72**	-0.12	**-0.98**	0.02	**-0.99**	**1**	**-0.99**		**0.99**	**-0.95**	**-0.92**	**-0.89**	0.1	-0.56	-0.61
Inertia	**-0.73**	-0.14	**-1**	0.04	**-0.96**	**0.99**	**-0.96**	**0.99**		**-0.92**	**-0.89**	**-0.86**	0.13	-0.58	-0.62
Inv Diff Mom	**0.76**	0.12	**0.93**	-0.06	**0.98**	**-0.96**	**0.96**	**-0.95**	**-0.92**		**1**	**0.9**	-0.31	**0.72**	**0.76**
Kappa	**0.75**	0.11	**0.91**	-0.06	**0.96**	**-0.93**	**0.93**	**-0.92**	**-0.89**	**1**		**0.89**	-0.37	**0.75**	**0.79**
Max prob	0.67	0.17	**0.86**	-0.13	**0.9**	**-0.89**	**0.91**	**-0.89**	**-0.86**	**0.9**	**0.89**		-0.11	0.54	0.58
Sum ener	-0.15	0.05	-0.18	-0.08	-0.18	0.15	-0.09	0.1	0.13	-0.31	-0.37	-0.11		**-0.76**	**-0.73**
Sum entr	**0.73**	0.26	0.63	-0.18	0.63	-0.61	0.55	-0.56	-0.58	**0.72**	**0.75**	0.54	**-0.76**		**0.99**
Sum var	**0.77**	0.24	0.67	-0.17	0.67	-0.65	0.6	-0.61	-0.62	**0.76**	**0.79**	0.58	**-0.73**	**0.99**	

Table 9: Correlation coefficient between the 15 TA variables for the radial transformation algorithm at ROI 0-100%. Strong correlations (r > 0.7) are shown in bold red font.

Radial algorithm ROI 0-100%

Group I TA variables yielded similar to the classical algorithm highly significant results for the potency treatment, albeit at a 2-4 times higher significance level. Similarly, a significant experimental day effect was found without significant interactions between experimental day and potency treatment (Table 10, Interaction 1-2), pointing to a reproducible potency effect over the 15 independent experiments. A borderline significant internal replicate effect was found, pointing towards a weak systematic error. Additional analysis revealed that this was not a consequence of a laboratory processing-order effect (data not shown).
The group I TA variables yielded 1 effect inversion. Fisher's LSD post hoc tests for variable *Cluster shade* using experimental day and treatment yielded significant or highly significant results for 3 out of the remaining 14 experimental days.

Variables of Group II showed contrary to the classical algorithm variables no effect for the potency treatment but maintained their highly significant interactions between experimental day and potency treatment (Table 10, Interaction 1-2). Similar to the group I variables, there was a significant experimental day effect. The group II TA variables yielded multiple effect inversions.

Variables of Group III yielded borderline significant to significant effects for the potency treatments together with highly significant interactions between experimental day and potency treatment (Table 10, Interaction 1-2). Thereby yielding a third signal.
The results from variable *Cluster prominence* closely resembled the results of the variables of group II.

Radial ROIgeo 0-100

Parameters	Main effects (*P* value)			Effect interactions (*P* value)			
	(1) Exp. day	(2) Potency	(3) Replicate	1-2	1-3	2-3	1-2-3
Clust shade	**<0.0001**	**0.0001**	**0.0309**	0.5109	0.0879	0.2292	0.5372
Diag mom	**<0.0001**	**0.0001**	**0.0284**	0.7243	0.0726	0.6141	0.3351
Corr	**<0.0001**	0.7069	0.0900	**0.0020**	0.5514	0.0757	0.5848
Diff ener	**<0.0001**	0.6148	0.0727	**0.0001**	0.4816	0.2301	0.3705
Diff entr	**<0.0001**	0.7420	0.0866	**0.0002**	0.5214	0.1507	0.5119
Energy	**<0.0001**	0.7398	0.0625	**0.0001**	0.5249	0.2552	0.4149
Entropy	**<0.0001**	0.8930	0.0772	**0.0004**	0.4641	0.1844	0.5163
Inertia	**<0.0001**	0.8190	0.0910	**0.0037**	0.5439	0.0759	0.6319
Inv Diff Mom	**<0.0001**	0.3456	**0.0100**	**<0.0001**	0.4424	0.1724	0.1458
Kappa	**<0.0001**	0.2744	**0.0081**	**<0.0001**	0.4080	0.1678	0.1106
Max prob	**<0.0001**	0.6094	**0.0033**	**0.0001**	0.7959	0.2210	0.4044
Sum ener	**<0.0001**	**0.0179**	0.5320	**0.0001**	0.4593	**0.0461**	0.5998
Sum entr	**<0.0001**	**0.0437**	0.2370	**<0.0001**	0.5140	0.0519	0.2902
Sum var	**<0.0001**	0.0756	0.2802	**<0.0001**	0.5097	0.0769	0.2529
Clust prom	**<0.0001**	0.1527	0.2832	**0.0001**	0.3369	0.2727	0.1927

Table 10: ANOVA F-tests for all 15 TA variables (main effects and interactions). ***Radial transformation algorithm ROI 0-100%.*** *Highly significant effects (P ≤ 0.01) are shown in bold red font; significant effects (P ≤ 0.05) are shown in bold blue font. Variables of group I and III are marked by light grey background colouring, variable Cluster prominence is marked by dark grey background colouring.*

Radial algorithm, geo-centre based ROIs 0-50%; 50-70%; 70-90% and 90-100%

Surprisingly of the group I TA variables only *Diagonal moment* showed a borderline significant effect (Table 11), this was also the case when larger biocrystallogram segments were analysed (50-100%;

data not shown). This could be a consequence of a reduced statistical power, or indicate that the signal source is dispersed over the biocrystallogram.
The group II TA variables seem to respond to two signal sources: (i) a moderately significant signal for a subset of the variables (*Energy, Inverse_diff._moment, Kappa,* and *Maximum_probability*) spread all over the biocrystallogram with exception of the outer periphery (ROI 90-100%), and (ii) a highly significant signal varying in time (interaction with experimental day) spread all over the biocrystallogram with emphasis on ROI 70-90%.
The group III TA variables and *Cluster_prominence* showed no significant signal.

Radial geo segment								
Parameters	**Main effects (p-value)**				**Interaction effects**			
	ROI 0-50%	**ROI 50-70%**	**ROI 70-90%**	**ROI 90-100%**	**ROI 0-50%**	**ROI 50-70%**	**ROI 70-90%**	**ROI 90-100%**
Clust shade	0.3476	0.2977	0.2127	0.8026	0.7276	0.7704	0.4218	0.5849
Diag mom	**0.0330**	0.0813	0.0928	0.2714	0.8525	0.9195	0.8865	0.8322
Corr	0.7746	0.9096	0.2507	0.8446	0.1613	**0.0406**	**0.0003**	**0.0025**
Diff ener	0.2815	0.3354	0.5052	0.8644	**0.0276**	**0.0053**	**<0.0001**	**0.0008**
Diff entr	0.6342	0.6395	0.6860	0.9199	**0.0424**	**0.0117**	**0.0001**	**0.0025**
Energy	**0.0406**	0.0605	0.2074	0.9826	0.4687	0.1454	**0.0084**	**0.0088**
Entropy	0.2467	0.2217	0.6069	0.7201	**0.0237**	**0.0282**	**0.0005**	**0.0089**
Inertia	0.9856	0.9107	0.7271	0.8296	0.0996	0.0500	**0.0032**	**0.0072**
Inv Diff Mom	**0.0435**	**0.0496**	**0.0426**	0.2424	**0.0227**	**0.0004**	**<0.0001**	**0.0012**
Kappa	**0.0318**	**0.0310**	**0.0114**	0.1177	**0.0319**	**0.0002**	**0.0001**	**0.0041**
Max prob	0.0630	**0.0495**	**0.0395**	0.2688	0.2894	0.0784	0.6427	0.9376
Sum ener	0.6398	0.1726	0.2496	0.2101	0.4984	0.6306	0.8607	0.6682
Sum entr	0.5566	0.3034	0.2928	0.2099	0.4453	0.8064	0.7642	0.6303
Sum var	0.6520	0.9251	0.0938	0.1631	0.2763	0.4150	0.9066	0.8211
Clust prom	0.8366	0.8877	0.0876	0.1574	0.1150	0.1045	0.7332	0.7650

*Table 11: ANOVA F-tests **Radial transformation algorithm** for the potency treatment (main effect and interaction with experimental day), analysing all 15 TA variables in four different ROIs. Highly significant effects (P < 0.01) are shown in bold red font; significant effects (P ≤ 0.05) are shown in bold blue font. Variables of group I and III are marked by light grey background colouring, variable Cluster prominence is marked by dark grey background colouring.*

Radial algorithm, crystal-centre based ROIs 0-50%; 50-70%; 70-90%, 90-100%

Different from the Radial geo-centre based ROI segment analysis, a highly significant signal was found for the group I TA variables which manifests predominantly in the geometric-centre of the biocrystallograms (ROI 0-50%).
Surprisingly no potency effect was observed for any of the group II TA variables, whereas a highly significant signal varying in time (interaction with experimental day) was found mainly in the outer region of the biocrystallograms, with an emphasis on ROI 70-90%.
The group III variables and *Cluster_prominence* seem to respond to two signal sources: (i) a moderate to high potency effect in the periphery (emphasis on ROI 70-90%), and (ii) a highly significant signal varying in time (interaction with experimental day) spread all over the biocrystallogram with emphasis on ROI 50-90%. Thereby confirming the third signal observed in the ROI 0-100% analysis.

Radial crystal-start segm

Parameters	Main effects				Interaction effects			
	ROI 0-50%	ROI 50-70%	ROI 70-90%	ROI 90-100%	ROI 0-50%	ROI 50-70%	ROI 70-90%	ROI 90-100%
Clust shade	**0.0007**	**0.0047**	0.1172	0.2616	0.4886	0.7878	0.5674	0.8436
Diag mom	**0.0001**	**0.0115**	0.0849	0.1773	0.5076	0.9220	0.1869	0.9284
Corr	0.9700	0.8961	0.3951	0.6782	0.3116	**0.0315**	**0.0002**	**0.0036**
Diff ener	0.5491	0.5151	0.5608	0.7911	0.1318	**0.0018**	**<0.0001**	**0.0012**
Diff entr	0.8209	0.7574	0.6254	0.9141	0.2050	**0.0080**	**<0.0001**	**0.0038**
Energy	0.4343	0.5359	0.9003	0.9420	0.1505	**0.0048**	**<0.0001**	**0.0015**
Entropy	0.8200	0.8077	0.8813	0.8557	0.2152	**0.0117**	**0.0001**	**0.0036**
Inertia	1.0000	0.9328	0.5984	0.9338	0.3261	0.0535	**0.0005**	**0.0086**
Inv Diff Mom	0.3510	0.2320	0.2902	0.2798	0.0616	**0.0002**	**<0.0001**	**0.0010**
Kappa	0.4010	0.2156	0.2305	0.2323	0.0550	**0.0002**	**<0.0001**	**0.0010**
Max prob	0.2483	0.5513	0.9382	0.3264	0.3372	0.1294	**0.0023**	**0.0047**
Sum ener	0.2334	0.4055	**0.0006**	**0.0368**	0.0863	**0.0002**	**0.0000**	**0.0024**
Sum entr	0.7451	0.4255	**0.0001**	**0.0239**	0.0824	**<0.0001**	**<0.0001**	**0.0006**
Sum var	0.7037	0.4447	**0.0001**	**0.0456**	0.0879	**<0.0001**	**<0.0001**	**0.0006**
Clust prom	0.6189	0.5372	**0.0003**	0.1239	0.1504	**0.0004**	**<0.0001**	**0.0021**

*Table 12: ANOVA F-tests **Radial transformation algorithm, crystal-start based ROIs** for the potency treatment (main effect and interaction with experimental day), analysing all 15 TA variables in four different ROIs. Highly significant effects (P < 0.01) are shown in bold red font. Variables of group I and III are marked by light grey background colouring, variable Cluster prominence is marked by dark grey background colouring.*

Conclusion and discussion

This report presents the results obtained with three different transformation algorithms (classical, angular and radial), applied to geometric-centre versus crystallization-start based ROIs for the Baumgartner 2012 data. The original Baumgartner publication applied a classical algorithm to geo-centre based ROIs, and observed significant effects of a homeopathic treatment with Stannum met 30x compared to water 30x on germinating cress seed over 15 independent experiments in two independent laboratories.

Two groups of correlated TA variables were identified (i.e. group I and II), which were closely correlated within the respective group, but not or only weakly correlated to those of the other group Two TA variables were regarded of primary interest for follow-up studies; *Cluster shade* and *Diagonal moment* (both variables of group I), of which the signal was found to manifest predominantly in the geometrical centre of the biocrystallogram (ROI 0–70%).

Although the overall statistical significance over the 15 experimental days was high, only 4 individual experimental days showed a statistical significance (LSD post hoc testing), and two effect inversions were observed over the 15 experimental days. This rose the question whether the non-significant days were a consequence of the followed laboratory procedure for generating the cress biocrystallisation patterns, or due to a lack in the evaluation abilities of the applied geo-centric classical transformation algorithm.

Geo-centre based classical evaluation versus polar-transformation

For the geo-centre based analyses (see Table 13 for a summary) the potency treatment is resolved best with the classical evaluation algorithm. This is mainly apparent in the analyses aimed at identifying the spatial region of the biocrystallogram where the potency treatment effect manifests

predominantly. The poor results for the two transformation routines could be a consequence of an incompatibility between the spatial onset of transformation versus evaluation. The transformations initiate at the crystal-centre (which are usually ex-centrically positioned), whereas the evaluation is geo-centre based.

Crystal-centre based classical evaluation versus polar-transformation

For the crystal-centre based analyses (see Table 14 for a summary) the potency treatment is resolved best with the radial transformation algorithm. Here three differently responding variable groups can be distinguished: Group I variables (*Cluster shade* and *Diagonal moment)* reveal a highly significant signal which manifests predominantly in the geometric-centre of the biocrystallograms (ROI 0-50%); variables of group II show only a time-modulated effect (interaction with experimental day) with an emphasis on ROI 70-90%; group III variables (*Sum energy, Sum entropy,* and *Sum variance)* reveal a moderate to high potency effect (emphasis on ROI 70-90%), and a highly significant interaction with experimental day with emphasis on ROI 50-90%.

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Geo-centre ROIs

Algorithm and Parameters	Main effects					Interaction effects				
	ROI 0-100%	ROI 0-50%	ROI 50-70%	ROI 70-90%	ROI 90-100%	ROI 0-100%	ROI 0-50%	ROI 50-70%	ROI 70-90%	ROI 90-100%
Classical Transf.										
Clust shade	**0.0004**	**0.0038**	**0.0002**	0.1552	0.1584	0.3264	0.6700	0.9604	0.8339	0.9768
Diag mom	**0.0002**	**0.0021**	**<0.0001**	0.1406	0.0764	0.2998	0.4649	0.9091	0.8331	0.9135
Inv Diff Mom	**0.0024**	0.3531	**0.0199**	**<0.0001**	**0.0069**	**0.0001**	**0.0103**	**<0.0001**	**0.0379**	**0.0096**
Kappa	**0.0023**	0.3137	**0.0339**	**<0.0001**	**0.0085**	**<0.0001**	**0.0120**	**<0.0001**	**0.0270**	**0.0083**
Sum entr	**0.0013**	0.5659	0.1241	**<0.0001**	**0.0026**	**0.0001**	**0.0293**	**<0.0001**	**0.0289**	**0.0415**
Sum var	**0.0029**	0.6193	0.0926	**<0.0001**	**0.0071**	**<0.0001**	**0.0260**	**<0.0001**	**0.0180**	**0.0487**
Angular Transf.										
Clust shade	**0.0052**	0.2830	0.2925	0.1854	0.8807	0.4722	0.8276	0.8039	0.5958	0.8100
Diag mom	**0.0102**	0.1690	0.0736	0.3854	0.1848	0.2089	0.1772	0.7322	0.8726	0.6118
Inv Diff Mom	**0.0094**	0.0920	**0.0259**	**0.0040**	**0.0342**	0.0743	0.4407	0.8666	0.8700	0.0574
Kappa	**0.0088**	0.0631	**0.0198**	**0.0038**	**0.0397**	**0.0366**	0.2952	0.6297	0.8950	**0.0328**
Sum entr	**0.0149**	0.4624	0.3105	0.1831	0.1420	**0.0260**	0.4498	0.8115	0.7808	0.7130
Sum var	**0.0250**	0.7925	1.0000	0.0871	0.1081	**0.0071**	0.2331	0.5341	0.9426	0.8603
Radial Transf.										
Clust shade	**0.0001**	0.3476	0.2977	0.2127	0.8026	0.5109	0.7276	0.7704	0.4218	0.5849
Diag mom	**0.0001**	**0.0330**	0.0813	0.0928	0.2714	0.7243	0.8525	0.9195	0.8865	0.8322
Inv Diff Mom	0.3456	**0.0435**	**0.0496**	**0.0426**	0.2424	**<0.0001**	**0.0227**	**0.0004**	**<0.0001**	**0.0012**
Kappa	0.2744	**0.0318**	**0.0310**	**0.0114**	0.1177	**<0.0001**	**0.0319**	**0.0002**	**0.0001**	**0.0041**
Sum entr	**0.0437**	0.5566	0.3034	0.2928	0.2099	**<0.0001**	0.4453	0.8064	0.7642	0.6303
Sum var	0.0756	0.6520	0.9251	0.0938	0.1631	**<0.0001**	0.2763	0.4150	0.9066	0.8211

*Table 13: Condensed ANOVA F-tests for **all transformation algorithms, geo-centre based ROIs** for the potency treatment (main effect and interaction with experimental day). Highly significant effects (P < 0.01) are shown in bold red font; significant effects (P ≤ 0.05) are shown in bold blue font. Representative variables of group I and III are marked by light grey background colouring, representative variables of group II have no background colouring.*

Crystal-start ROIs

Algorithm and Parameters	Main effects					Interaction effects				
	ROI 0-100%	ROI 0-50%	ROI 50-70%	ROI 70-90%	ROI 90-100%	ROI 0-100%	ROI 0-50%	ROI 50-70%	ROI 70-90%	ROI 90-100%
Classical Transf.										
Clust shade	**0.0004**	**0.0026**	**0.0034**	0.1261	0.1729	0.3264	0.4762	0.4807	0.6414	0.9316
Diag mom	**0.0002**	**0.0010**	**0.0002**	0.0938	0.0813	0.2998	0.3679	0.6273	0.7958	0.8445
Inv Diff Mom	**0.0024**	0.5139	**0.0029**	**<0.0001**	**0.0071**	**0.0001**	**0.0117**	**<0.0001**	0.0789	**0.0149**
Kappa	**0.0023**	0.6116	**0.0027**	**<0.0001**	**0.0082**	**<0.0001**	**0.0083**	**0.0001**	0.0844	**0.0111**
Sum entr	**0.0013**	0.4883	0.2098	**<0.0001**	**0.0021**	**0.0001**	**0.0344**	**0.0001**	0.2015	0.0588
Sum var	**0.0029**	0.5413	0.1822	**<0.0001**	**0.0060**	**<0.0001**	**0.0331**	**<0.0001**	0.1887	0.0624
Angular Transf.										
Clust shade	**0.0052**	**0.0061**	**0.0024**	0.3619	0.1285	0.4722	0.5808	0.2904	0.8319	0.8922
Diag mom	**0.0102**	0.1394	**0.0120**	0.5564	0.0955	0.2089	0.1992	0.3706	0.8004	0.7443
Inv Diff Mom	**0.0094**	0.5196	0.1054	**0.0060**	**0.0400**	0.0743	0.3212	0.4596	0.8012	**0.0342**
Kappa	**0.0088**	0.6570	0.0790	**0.0067**	0.0524	**0.0366**	0.4279	0.4638	0.8445	**0.0149**
Sum entr	**0.0149**	0.6740	0.4335	**0.0022**	**0.0082**	**0.0260**	0.1251	0.0975	0.5505	0.1279
Sum var	**0.0250**	0.5757	0.4588	**0.0022**	**0.0141**	**0.0071**	0.1831	0.1200	0.5063	0.0566
Radial Transf.										
Clust shade	**0.0001**	**0.0007**	**0.0047**	0.1172	0.2616	0.5109	0.4886	0.7878	0.5674	0.8436
Diag mom	**0.0001**	**0.0001**	**0.0115**	0.0849	0.1773	0.7243	0.5076	0.9220	0.1869	0.9284
Inv Diff Mom	0.3456	0.3510	0.2320	0.2902	0.2798	**<0.0001**	0.0616	**0.0002**	**<0.0001**	**0.0010**
Kappa	0.2744	0.4010	0.2156	0.2305	0.2323	**<0.0001**	0.0550	**0.0002**	**<0.0001**	**0.0010**
Sum entr	**0.0437**	0.7451	0.4255	**0.0001**	**0.0239**	**<0.0001**	0.0824	**<0.0001**	**<0.0001**	**0.0006**
Sum var	0.0756	0.7037	0.4447	**0.0001**	**0.0456**	**<0.0001**	0.0879	**<0.0001**	**<0.0001**	**0.0006**

*Table 14: Condensed ANOVA F-tests for **all transformation algorithms, crystal-start based ROIs** for the potency treatment (main effect and interaction with experimental day). Highly significant effects (P < 0.01) are shown in bold red font; significant effects (P ≤ 0.05) are shown in bold blue font. Representative variables of group I and III are marked by light grey background colouring, representative variables of group II have no background colouring.*