

# Vortex-like vs. turbulent mixing of a Viscum album preparation affects crystalline structures formed in dried droplets

Maria Olga Kokornaczyk

[maria.kokornaczyk@unibe.ch](mailto:maria.kokornaczyk@unibe.ch)

University of Bern

Carlos Acuña

National Polytechnic Institute

Alfonso Mier y Terán<sup>3</sup>

National Polytechnic Institute

Mario Castelan

National Polytechnic Institute

Stephan Baumgartner

University of Bern

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## Article

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# Abstract

The droplet evaporation method could provide a fairly inexpensive tool for testing the effects of flow in liquids as an alternative to the methods used today.

production of microparticles and microbubbles.

We applied turbulent mixing vs. laminar flow induced by a vortex vs. diffusion based mixing during the production of *Viscum album quercus L.*  $10^{-3}$  following the guidelines for manufacturing homeopathic preparations. The so obtained differently mixed variants of the preparation were analyzed by means of the droplet evaporation method. The crystalline structures formed in dried droplets were photographed and analyzed by means of a computer supported image analysis and deep-learning.

Computer supported evaluation and deep-learning revealed that the patterns of turbulent succussed variant are characterized by smaller complexity, whereas those obtained from the vortex mixed sample by greater complexity vs. the diffusion-based mixed control variant.

## Background

Investigating the impact of succussion upon a pharmaceutical preparation, detecting the succussion's modality, or proving its occurrence represent challenging tasks for both research and quality control of medicinal products.

Interestingly, different branches of medicine have different views on the importance of succussing of medicinal solutions: In the allopathic medicine even an accidental agitation of a preparation is avoided, as it is considered a risk factor for reducing the therapeutic properties of the solution by altering the proteins structure or increase of oxidation processes [1–3]; In contrast, in homeopathy, but also in anthroposophical pharmaceuticals, that use the potentization procedure (i.e. subsequent dilutions and succussions performed until reaching the desired potency), agitation represents an integral part of the production protocol that determines the therapeutical properties of the preparation [4–6] rather of reducing them.

The succussion itself performed during the potentization may be characterized by its (i) modality, (i.e. type of movement used to induce motion into the solution), (ii) duration (i.e. time of succussing or quantity of movements performed during one potentization step) and (iii) performance (i.e. handmade vs. machine made vs. sonication) [6].

Among the different producers of homeopathic and anthroposophic preparations the applied succussion techniques vary greatly for what regards the above mentioned characteristics. Generally, producers following the Hahnemannian guidelines perform turbulent agitation by hitting with the flask against an elastic surface, that is performed by hand or machine [7]; whereas producers of anthroposophical products induce rather an ordered motion (i.e. vortex- or lemniscate-like flow), or an alteration of ordered and turbulent motion (i.e. ordered vortex-like flow that stops suddenly, causing a short turbulence, and starts almost immediately again or a sequence of vortexes turned into left and right with a turbulence when changing direction) [8]. Further, it can be generalized that low potencies (mostly used in anthroposophic medicine) are potentized rather by ordered motions and high and ultra-high potencies (mostly applied in classical homeopathy) by turbulent flow.

In a series of previous experimentations it could be demonstrated that self-assembled patterns formed in droplets in course of their drying represent a suitable tool to investigate low dilutions. As output parameters characteristics of the self-assembled structures such as grey level distribution, texture and fractal features were used. The parameters showed to be substance specific and so differentiated between dilutions prepared from different substances until 4x [9], sensitive

towards the impact of vertical vigorous shaking [5], as also towards interactions occurring among the components of complex preparations [10].

In the present study we applied the droplet evaporation method to obtain patterns from a *Viscum album quercus* L.  $10^{-3}$  dilution produced following the guidelines for manufacturing homeopathic preparations [7] by applying three different mixing modalities between the dilution steps: (i) turbulent vertical machine-made succussions (variant T), (ii) laminar flow induced by a handmade vortex (variant L) and (iii) diffusion-based mixing (unsuccussed control, variant D).

## Results

### Visual pattern assessment

The droplet residues of all here tested differently mixed *Viscum album quercus* L.  $10^{-3}$  variants contained in their central parts dendritic, fractal structures formed, most probably, in course of diffusion limited aggregation of particles taking place during droplet evaporation (Fig. 1). The central structures were bright and besides them there were no other forms visible inside the droplet residues. In most patterns the structures showed a distinct crystallization center, or a central-area, out of which the longest and thickest first-order ramifications started their run towards the structure outer region. There were also ramifications of higher orders, that were shorter and thinner. The branches seemed bushy, covered by needles. In most cases the variant T (mixed by inducing the turbulent flow; Fig. 1a) formed smaller and lesser complex central structures then the variants L and D (mixed by means of laminar flow and diffusion-based mixing, respectively). The difference between the later two variants was visually hard to access (Fig 1b, c).

### Computerized pattern evaluation

The results of the computerized pattern evaluation are shown in Table 1. All analyzed pattern evaluation parameters describing the structures grey level distribution, texture, and fractality were able to significantly differentiate between the variant T and the other two variants; whereas four parameters, *mass fractal dimension*, *fractal dimension of structures with highest value r2*, *ascending second moment* and *inverse difference moment* were able to differentiate significantly between all three variants; in all these cases the largest difference was observed between the variants T and L; whereas, variant D showed smaller differences to either variant L and T.

The systematic control experiments detected no significances between the control groups for six out of ten (6/10) analyzed parameters or detected significances with p-values that were much smaller from those of the corresponding main experiments for 4/10 parameters. Thus it can be assumed that the experimental system was reasonable stable and the differences observed in the main experiments were due to the applied mixing procedures. In the main experiments, the factor day (i.e. influence of the experimentation day) was strongly significant for all 10 parameters; the interaction between day and mixing procedure was also significant however with the much smaller p-value than that of the factor mixing procedure.

Table 1: On the left side: Mean values of analyzed pattern evaluation parameters for the three differently mixed *Viscum album quercus* L. 3x variants (T: turbulent, L: laminar, and D: diffusion based) analyzed in the main experiments and three control groups analyzed in the systematic control experiments. On the right side: results of the 2-way analysis of variance with independent factors mixing procedure (Mixing) and experimentation day (Day). Different letters following the mean values indicate significant differences at  $p < 0.05$ .

| Parameter         | Mixing | Main experiments |            | SCE |            |            | Main experiments |           | SCE   |           |
|-------------------|--------|------------------|------------|-----|------------|------------|------------------|-----------|-------|-----------|
|                   |        | n                | Mean       | n   | Mean       |            | F                | p         | F     | p         |
| LCFD              | T      | 202              | 0.85 (b)   | 181 | 0.74 (a)   | Mixing     | 55.9             | 0.0000*** | 1.37  | 0.2557 ns |
|                   | L      | 193              | 1.21 (a)   | 179 | 0.82 (a)   | Day        | 24.06            | 0.0000*** | 19.26 | 0.0000*** |
|                   | D      | 204              | 1.17 (a)   | 182 | 0.81 (a)   | mixing*day | 3.8              | 0.0002*** | 2.99  | 0.0027**  |
| MFD               | T      | 202              | -1.54 (a)  | 181 | -1.45 (ab) | Mixing     | 33.59            | 0.0000*** | 4.72  | 0.0093**  |
|                   | L      | 193              | -1.69 (c)  | 179 | -1.47 (b)  | Day        | 20.87            | 0.0000*** | 32.38 | 0.0000*** |
|                   | D      | 204              | -1.65 (b)  | 182 | -1.4 (a)   | mixing*day | 5.82             | 0.0000*** | 4.45  | 0.0000*** |
| D with highest r2 | T      | 202              | 1.24 (c)   | 181 | 1.08 (a)   | Mixing     | 42.44            | 0.0000*** | 2.46  | 0.0862 ns |
|                   | L      | 193              | 1.58 (a)   | 179 | 1.18 (a)   | Day        | 14.08            | 0.0000*** | 27.7  | 0.0000*** |
|                   | D      | 204              | 1.5 (b)    | 182 | 1.13 (a)   | mixing*day | 3.51             | 0.0006*** | 3.01  | 0.08626** |
| LAC               | T      | 202              | 0.37 (a)   | 181 | 0.36 (a)   | Mixing     | 26.39            | 0.0000*** | 0.35  | 0.7035 ns |
|                   | L      | 193              | 0.28 (b)   | 179 | 0.36 (a)   | Day        | 17.42            | 0.0000*** | 5.66  | 0.0002*** |
|                   | D      | 204              | 0.27 (b)   | 182 | 0.34 (a)   | mixing*day | 6.11             | 0.0000*** | 1.32  | 0.2305 ns |
| GLD               | T      | 206              | 11.84 (b)  | 198 | 11.74 (a)  | Mixing     | 37.95            | 0.0000*** | 2.95  | 0.0532 ns |
|                   | L      | 196              | 16.18 (a)  | 197 | 13.08 (a)  | Day        | 41.96            | 0.0000*** | 25.06 | 0.0000*** |
|                   | D      | 204              | 16.46 (a)  | 198 | 11.64 (a)  | mixing*day | 3.24             | 0.0013**  | 3.43  | 0.0007*** |
| ASM               | T      | 206              | 0.021 (a)  | 198 | 0.022 (b)  | Mixing     | 16.98            | 0.0000*** | 5.02  | 0.0069**  |
|                   | L      | 196              | 0.016 (c)  | 197 | 0.021 (b)  | Day        | 84.67            | 0.0000*** | 56.66 | 0.0000*** |
|                   | D      | 204              | 0.018 (b)  | 198 | 0.024 (a)  | mixing*day | 6.59             | 0.0000*** | 2.07  | 0.0368*   |
| Contrast          | T      | 206              | 518.27 (b) | 198 | 547.68 (a) | Mixing     | 32.47            | 0.0000*** | 2.26  | 0.1053 ns |
|                   | L      | 196              | 752.57 (a) | 197 | 637.62 (a) | Day        | 36.8             | 0.0000*** | 21.05 | 0.0000*** |
|                   | D      | 204              | 810.62 (a) | 198 | 557.88 (a) | mixing*day | 5.71             | 0.0000*** | 4.71  | 0.0000*** |

|                |                    |          |     |               |     |               |                   |        |           |       |           |
|----------------|--------------------|----------|-----|---------------|-----|---------------|-------------------|--------|-----------|-------|-----------|
|                | <b>Correlation</b> | <b>T</b> | 206 | 0.0015<br>(a) | 198 | 0.0023<br>(a) | <b>Mixing</b>     | 19.27  | 0.0000*** | 0.24  | 0.7824 ns |
|                |                    | <b>L</b> | 196 | 0.0010<br>(b) | 197 | 0.0021<br>(a) | <b>Day</b>        | 11.08  | 0.0000*** | 37.55 | 0.0000*** |
|                |                    | <b>D</b> | 204 | 0.0008<br>(b) | 198 | 0.0023<br>(a) | <b>mixing*day</b> | 4.59   | 0.0000*** | 1.23  | 0.2786 ns |
| <b>IDM</b>     |                    | <b>T</b> | 206 | 0.38<br>(a)   | 198 | 0.40<br>(b)   | <b>Mixing</b>     | 22.36  | 0.0000*** | 4.78  | 0.0087**  |
|                |                    | <b>L</b> | 196 | 0.35<br>(c)   | 197 | 0.39<br>(b)   | <b>Day</b>        | 119.01 | 0.0000*** | 42.98 | 0.0000*** |
|                |                    | <b>D</b> | 204 | 0.36<br>(b)   | 198 | 0.41<br>(a)   | <b>mixing*day</b> | 4.99   | 0.0000*** | 1.81  | 0.0728 ns |
| <b>Entropy</b> |                    | <b>T</b> | 206 | 5.64<br>(b)   | 198 | 5.49<br>(ab)  | <b>Mixing</b>     | 31.23  | 0.0000*** | 3.69  | 0.0256*   |
|                |                    | <b>L</b> | 196 | 6.17<br>(a)   | 197 | 5.57<br>(a)   | <b>Day</b>        | 42.14  | 0.0000*** | 43.95 | 0.0000*** |
|                |                    | <b>D</b> | 204 | 6.06<br>(a)   | 198 | 5.34<br>(b)   | <b>mixing*day</b> | 4.05   | 0.0000*** | 2.48  | 0.0118*   |

## Deep learning based pattern evaluation

The results of the supervised and unsupervised pattern evaluation based on deep-learning are described in detail elsewhere [11, 12].

Both the supervised and unsupervised approach revealed that the patterns obtained from dried droplets of the turbulently mixed variant T had a less fractal composition in comparison to the diffusion-based mixed control variant D, whereas the patterns obtained from variant L, mixed by means of the laminar flow, had a more fractal composition when compared to the control variant (Fig 2-4).

Figure 5 depicts the results of the fully automated pattern classification by means of the unsupervised pattern evaluation approach based on deep-learning. The patterns obtained from the *Viscum album quercus* 3x preparation could be correctly classified into the applied mixing procedures for the turbulently mixed variant for 72% of patterns, for the variant mixed by means of the laminar flow for 60% of patterns, and for the diffusion-based mixed control variant for 33% of patterns. The control variant was most frequently confounded with the turbulently mixed variant (43% of cases).

## Discussion

The results of the present study indicate that the mixing modality applied during the production of the *Viscum album quercus* L.  $10^{-3}$  preparation influences the complexity of patterns formed in drying droplets. A comparison of three differently mixed variants of the preparation shows that turbulent flow (variant T) reduced the patterns fractality, whereas laminar flow (variant L) increased the patterns fractality vs. an unmixed, diffusion-based mixed sample (variant D).

In previous studies the droplet evaporation method (DEM) was already proposed as a tool to test the influence of the number of vertical succussion strokes performed during mixing of dilutions [4, 5], here we demonstrate that not only the succussion number but also the mixing modality has an impact on the DEM patterns.

As shown by other authors, flow modality influences the molecules in suspension. Laminar flow causes the alignment of especially long molecules [13, 14], whereas turbulent flow increases coagulation, particle formation and oxidation of proteins and causes a rather disordered and chaotic positioning of molecules in suspension [14]. In various works the influence of mixing upon the suspension of particle upon crystallization processes has been predicted [15] and measured [16].

We may hypothesize at this point that in the here presented experimentation the laminar mixing applied following three decimal dilution steps of the *Viscum album quercus* L.  $10^{-3}$  dilution contributed to a greater order of molecules in suspension that in turn led to the emergence of more complex patterns; whereas turbulent vertical succussions caused agglomeration of molecules and particle and microbubbles formation that disturbed rather the pattern formation process and led to decreased form complexity. Further, it can be hypothesized that the changes induced by mixing were rather stable in order to not being alternated or cancelled by the pipetting of the differently mixed variants for droplet deposition on substrates.

Patterns resulting from the droplet evaporation method (DEM) as well as other methods based on evaporation-induced pattern formation are often evaluated only through visual assessment, which can be subjective and lead to biased results [17, 18]. Deep-learning based evaluation of patterns formed in dried solution droplets has proven effective in various models [19–24] and enables fast and objective image classification. We show here that DEM images obtained from a *Viscum album quercus* L.  $10^{-3}$  solution mixed using turbulent or laminar flow show differences from a diffusion-based mixed control when subjected to semi- and fully-automated deep-learning pattern classification [11, 12]. In our study all applied pattern evaluation approaches used (i.e. the visual, computer-assisted and deep-learning based approach) led to the same conclusions regarding the impact of different mixing methodologies. The use of deep learning for DEM pattern evaluation enables the rapid evaluation and comparison of large image databases and can significantly support the development of further DEM applications [25–28].

## Materials and methods

### Workflow

Three variants of *Viscum album quercus* L 3x (i.e. third decimal dilution prepared in decimal dilution steps, each followed by a succussion) were prepared by the application of either: (i) turbulent succussions made by a machine and performed for 2.5 min (variant T), (ii) laminar flow induced by a series of handmade vortexes for 2.5 min (variant L), or (iii) diffusion-based mixing (variant D). Variants T, L and D, were blinded and analyzed in a series of five droplet evaporation method (DEM) experiments gaining in total 606 patterns. The patterns were photographed by use of a darkfield microscope in a magnification 100×; the so obtained image database of 606 patterns was analyzed by means of the software ImageJ for grey level distribution texture and fractality. The differences between the image evaluation parameters between the variants were accessed in a statistical analysis. The experimental system stability was controlled by performing five systematic control experiments following the same experimental set-up as the corresponding main experiments but using only the variant T (insignificant results indicate a stable experimental system); the systematic control experiments gained in total 593 patterns. Additionally, the database from five main experiments was shared with the Pattern Analysis Lab at CINVESTAV, Mexico and analyzed by supervised and unsupervised deep learning algorithms (the results of deep-learning based pattern evaluation are published elsewhere [11, 12], here we shortly present the main outcomes only).

### *Viscum album quercus* L.

The use of plants in the present study followed the international institutional guidelines. *Viscum album Quercus* tincture was prepared by ISCADOR AG (Arlesheim, Switzerland). The plants were harvested from *Quercus robur* growing in natural habitats in Switzerland (owned by ISCADOR AG) and were identified by Mirio Grazi (Society for Cancer Research, Arlesheim, Switzerland). A Voucher specimen (C.H. Quaresma 18.329) was deposited at the Herbarium of the Faculdade de Formação de Professores, Universidade Estadual do Rio de Janeiro, Brazil [29-31].

## Potentization

As dilution medium ultrapure water containing 2g-5/ml NaCl and 2g-5/ml KCl was used. The salt addition served for enhancing the pattern forming capacities. First dilution was in ratio 1:20. Into each of three 50ml capacity Erlenmeyer flasks 1 ml of the *Viscum album quercus* extract and 19 ml of the dilution medium were put. The content of first flask was gently stirred with a glass stirrer, without inducing any kind of vortex or formation of foam; the flask was then closed with a tap and left for 15 min (variant D, diffusion based mixing). The two other flasks were closed with a tap; one was succussed on a succussion machine performing vertical vigorous strokes for 2.5 min (variant T, turbulent mixing); the other was mixed by hand by repeatedly turning the flask in a circular and vigorous movement upside-down in order to create a vortex and letting it to settle (variant L, laminar flow). The potentization to 2x and 3x was performed in dilution ratios 1:10 and in Erlenmeyer flasks with the capacity of 250ml filled up to 100ml. The amount of the solution in the flasks was experimentally set for optimal vortex formation.

## Droplet evaporation method

For each of the ten main and systematic control experiments 12 microscope slides (76 × 26 mm, pre-cleaned, cut edges; Thermo Scientific, Gerhard Menzel B.V. & Co. KG, Braunschweig, Germany) were used. The slides were degreased by washing them with a dishwasher liquid, thoroughly rinsed under hot tap water following 4 consecutive purified water baths. Cleaned slides were wiped dry with a laboratory wiper (KIMTECH science, Kimberly-Clark Professional, Roswell, Canada) just before droplet deposition. In the main experiments 3 µl droplets of variants T, L and D were deposited on four slides each in two parallel rows, 7 droplets per row, by the use of a micro-pipette of 2-20 µl capacity (Eppendorf Research Plus, Eppendorf, Hamburg, Germany). For the systematic control experiments all 12 slides were covered with droplets of variant T. Droplet desiccation took place in an incubator (KBF 720, cooled incubator with controlled humidity system, WTB Binder Labortechnik GmbH, Tuttingen, Germany) with an inner plexi-glass-chamber covered with a semi-permeable cover and placed on a vibration absorbing basis. The 12 slides with droplets were placed in the inner-chamber, in 4 rows of 3 slides each following a quasi-randomization design (i.e. the tested T, L and D variants were randomized within each row securing so their uniform distribution within the chamber) and left at 26 °C and 44%RH until dry.

## Acquisition of patterns

The dried droplet residues were examined and photographed in darkfield in 100-fold magnification 100× by use of an optical microscope (Zeiss Lab.A1; Carl Zeiss Microscopy GmbH, Jena, Germany) with an attached camera (Moticam 5.0 MP; CMOS; Motic Electric Group Co., Ltd, Xiamen, China). The photos have been taken in order to contain the dendritic structure emerged inside the droplet residues. Residues showing disturbed pattern formation due to presence of contaminating particles or edge effects on the slide were not considered. The images were saved in jpeg-format in a resolution of 2592 × 1944 pixel. The five main experiments yielded in total 606 images (206, 196 and 204 for the variants T, L and D, respectively) and the five systematic control experiments yielded in total 593 images (198, 197 and 198 of the control groups of variant T, treated as control-T, control-L and control-D, respectively).

# Computerized pattern evaluation

Image analysis was carried out with the software ImageJ (v. 1.50b) [32] with installed plug-ins GLCM Texture [33] and Frac-Lac. All images were subjected to a background extraction (sliding paraboloid with rolling ball radius set at 50 pixels) ensuring uniform background throughout the image database and reducing glares. The images were then analyzed (i) by means of the ImageJ tool “measure” for the grey-level distribution, (ii) after conversion into 8-bit type and by applying the GLCM algorithm with distances between pixel pairs set to 4 pixels and angles of 90°, for their texture (parameters ascending second moment, contrast, correlation, inverse difference moment, and entropy), and (iii) after size reduction to 500 × 375 pixels, and conversion into binary, by means of Frac-Lac’s DLC tool with odd sizes scaling method and size limits for the grid caliber of 4 to 40 pixels, for parameters describing their local connected fractal dimension (box count fractal dimension, mass fractal dimension, fractal dimension of structures with highest r2, and lacunarity). Due to errors occurring in the image background after conversion to binary some images were excluded from the fractal analysis (for the main experiments 4 images of variant T, and 1 of variant L; for systematic control experiments 14 images of variant control-D, 17 of variant control-M and 18 of variant control-V).

## Statistical analysis

The data of the computerized image evaluation were transferred to excel and analyzed by means of a two-way analysis of variance (CoStat, v. 6.311) (CoHort Software, Monterey, USA) at alpha = 0.05 with independent factors mixing method and day. The interaction between the factors was considered for assessing the reproducibility within experiments performed on different days. Data distribution was checked visually; slight deviations from normality were irrelevant due to the central limit theorem. The global significance was determined by means of F-tests; whereas, pairwise mean comparison was run two-tailed with the protected Fisher’s least significant difference test (pairwise comparisons were evaluated only if the global F-test was significant at  $p < 0.05$ ). This procedure ensures a good safeguard against type I and II errors, and thus balances well between false-positive and false-negative conclusions [34].

## Deep-learning based pattern evaluation

Supervised and unsupervised deep-learning based methodologies for the evaluation of the DEM pattern database of the main experiments were developed for the purpose of this project; the protocols of these methodologies and obtained results are described in detail elsewhere [11, 12]. In short: Images were subjected to a background subtraction and converted to binary. From each image patches were automatically selected in order to have the whole area covered with uniform structures and not contain any background, obtaining 705 patches from each mixing procedure of size 128 × 128 pixels. In the semi-supervised approach, based on their textures [35], the patches were assigned to three pre-defined clusters (more fractal, medium fractal, and less fractal). In the unsupervised approach the number of clusters was chosen automatically by the elbow method under a hierarchical clustering framework [36] used to group the patterns obtained through the network.

## Declarations

## Conflict of interest

Authors declare no conflict of interest

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## Author Contribution

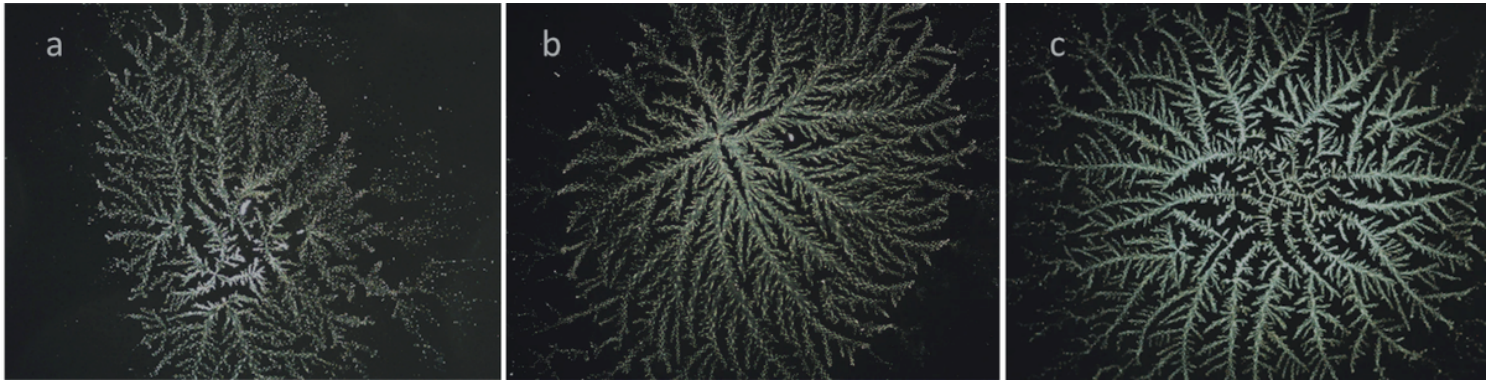
M.O.K. and S.B. designed the experimentation, M.O.K. performed the DEM analysis and statistics. C.A., A.M-T. and M.C. designed the deep learning algorithms and run the pattern classification based on AI. M.O.K. and C.A. wrote the manuscript, all authors reviewed it.

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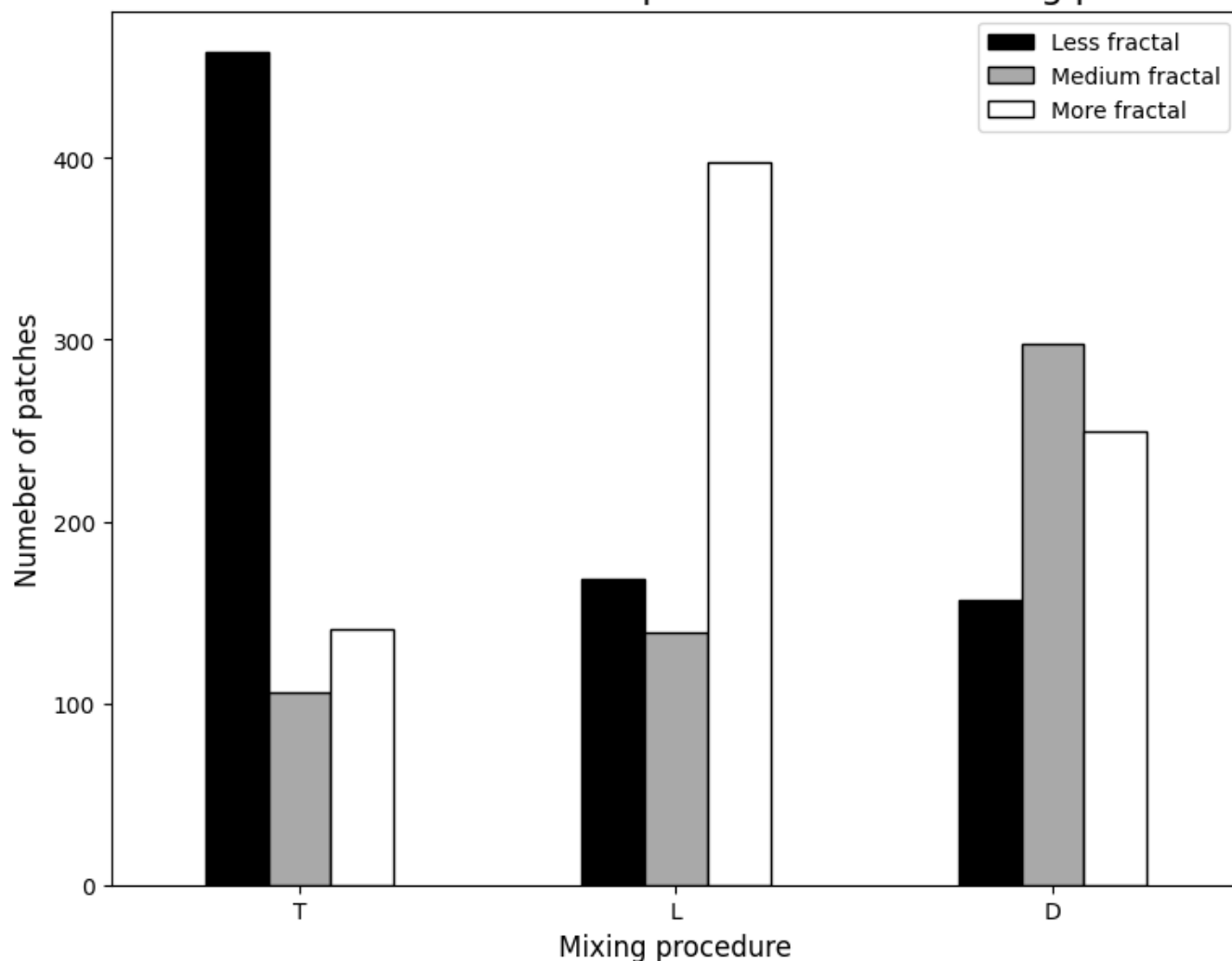
## Figures



**Figure 1**

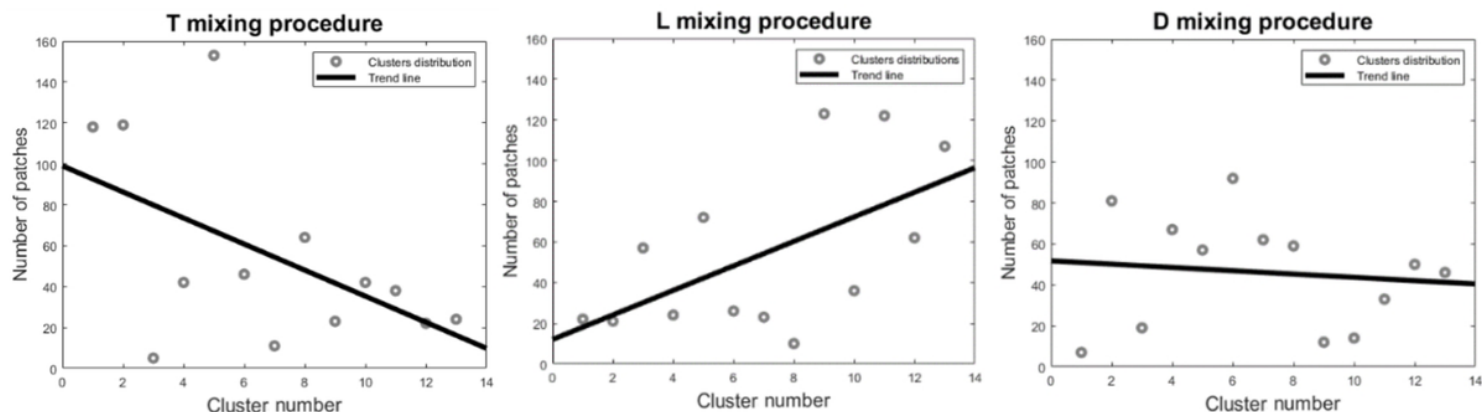
Examples of central structures formed inside dried droplets of *Viscum album quercus L.* 10<sup>-3</sup> variants prepared by different mixing techniques: machine-made turbulent succussions (a), laminar flow induced by handmade vortex (b), and diffusion based mixing (c). Images with local connected fractal dimension equal or similar to that of the variant's mean are presented. Photographs were taken in darkfield and magnification 100x.

## Fractal distribution of the 705 patches of each mixing procedure



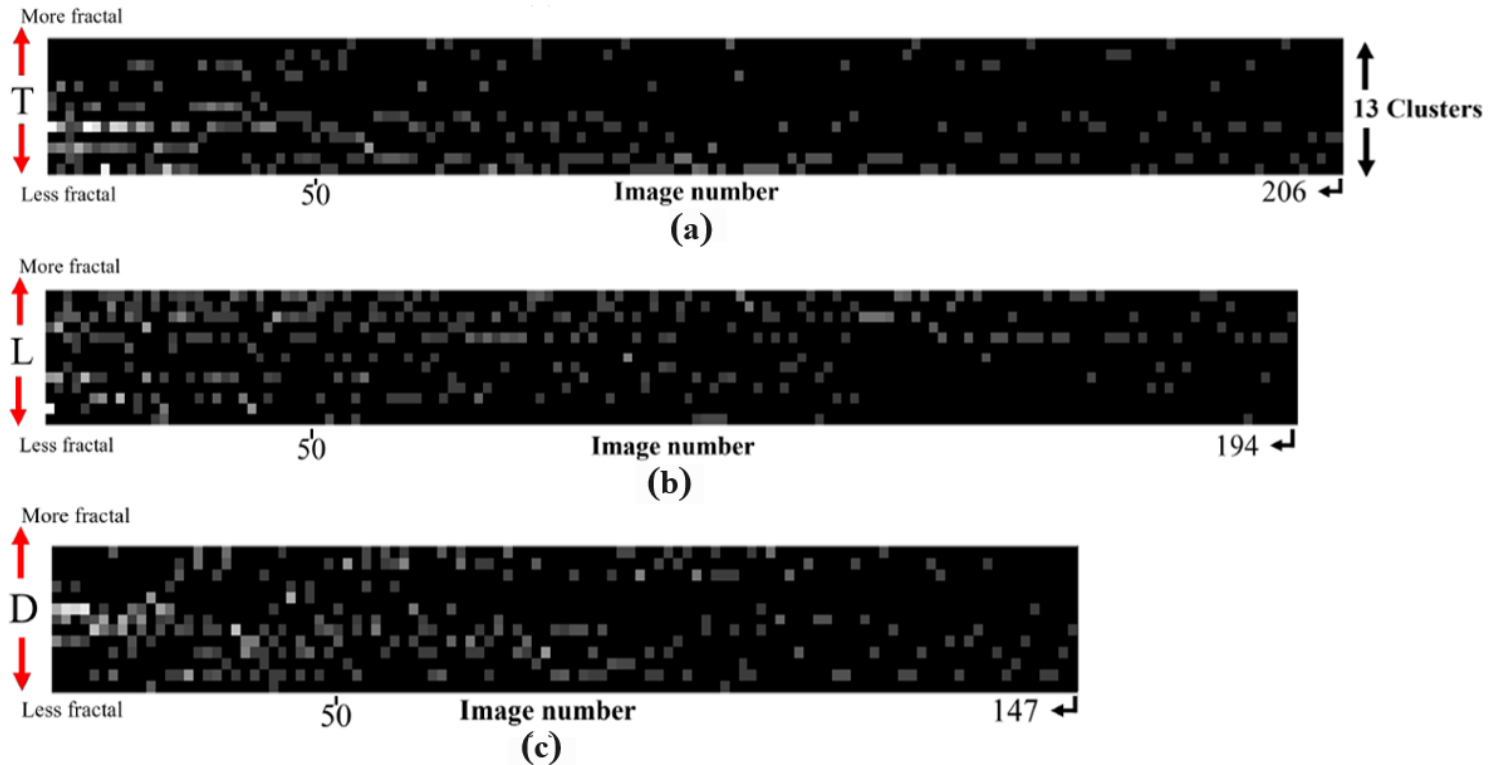
**Figure 2**

Distribution of image-patches grouped after applying semisupervised deep learning approach, for categories less fractal, medium fractal, and more fractal found in the patterns from dried droplets of *Viscum album Quercus 3x* produced with different mixing procedures: diffusion based mixing (D), turbulent vertical succussions (T), and laminar flow induced by handmade vortex (L). The patches obtained from the L mixing procedure show the highest fractal composition, having 56.60 % of the patches in the “more fractal” category. The patches obtained from the T mixing procedure exhibit the lowest fractal composition, having 65.2 % of the patches in the “less fractal” category.



**Figure 3**

Distribution of image-patches grouped after applying unsupervised deep learning approach, for categories less fractal (closer to 0 along the x-axis), medium fractal (closer to 7 along the x-axis), and more fractal (closer to 13 along the x-axis) found in the patterns from dried droplets of *Viscum album Quercus* 3x produced with different mixing procedures: D - diffusion based mixing (middle), T - turbulent vertical succussions (left), L - laminar flow induced by handmade vortex (right). The 13 groups obtained from the unsupervised approach emphasize the fractal tendency already exhibited in Figure 2, for the different mixing methods.



**Figure 4**

Patch distribution at image level for images obtained from unsupervised deep learning applied on dried droplets of *Viscum album Quercus* 3x produced by means of diffusion-based mixing (variant D) (a), turbulent mixing (variant T) (b), and mixing by means of laminar flow induced by a handmade vortex (variant L) (c). The bias toward more and less fractal behavior of the dried droplet is visible for the laminar and turbulent modalities, while the diffusion modality presents both biases.

## Confusion matrix

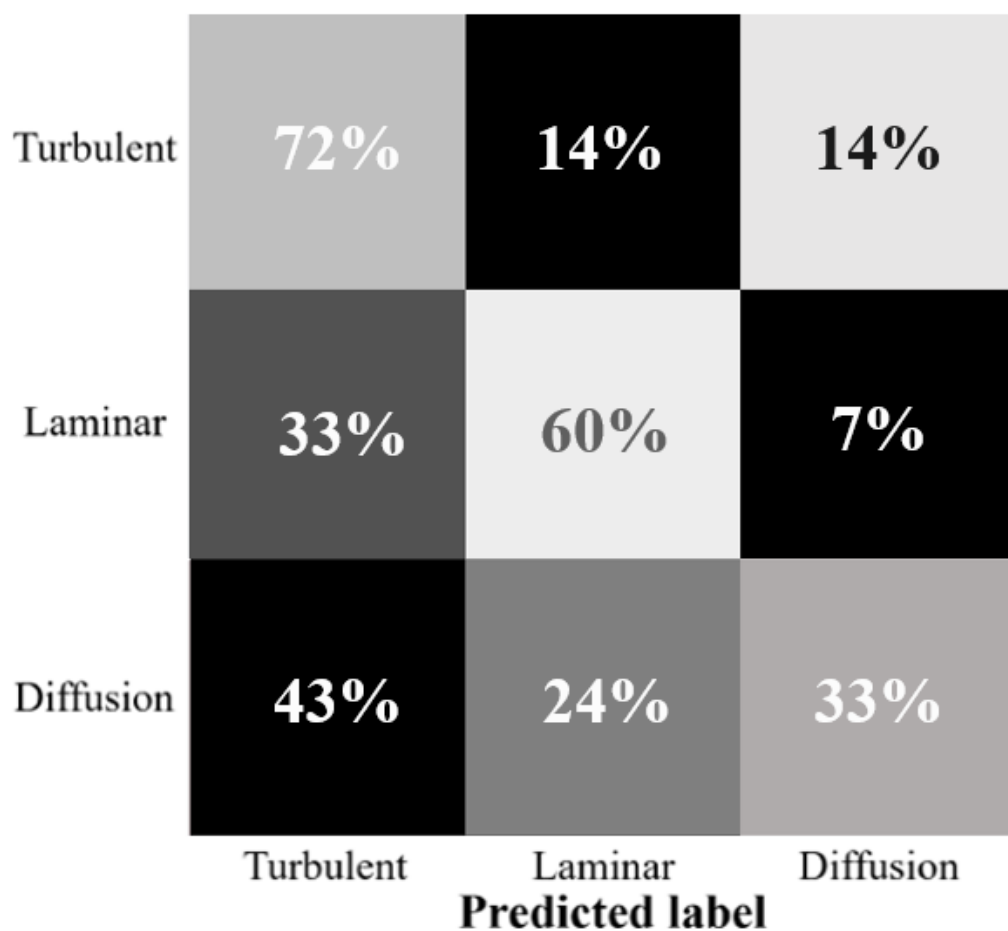


Figure 5

Confusion matrix. Results of the classification task using support vector machine on images characterized as feature vectors obtained from unsupervised deep learning applied on dried droplets of *Viscum album quercus* 3x preparation produced by means of the diffusion based mixing, turbulent succussing, or laminar flow. These results provide a more profound insights on the separability of the different mixing procedures.