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F. Debaste, A. Léonard, V. Halloin, B. Haut

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1 Microtomographic investigation of a yeast grain porous  
2 structure

3 F. Debaste<sup>\*,a</sup>, A. Léonard<sup>b</sup>, V. Halloin<sup>a</sup>, B. Haut<sup>a</sup>

4 <sup>a</sup>*Transfers, Interfaces and Processes, Université Libre de Bruxelles, 50, av. F.D. Roosevelt,*  
5 *C.P. 165/67, 1050 Bruxelles, Belgium*

6 <sup>b</sup>*Laboratory of Chemical Engineering, Department of Applied Chemistry, Université de*  
7 *Liege, FNRS. B6c, Sart Tilman, 4000 Liege, Belgium*

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8 **Abstract**

Drying behavior of baker's yeast depends on the internal structure of the grain. In this paper, investigation of the porous structure of a dry grain using X-ray microtomography is presented. The resulting data are treated to deduce pore network characteristics for drying modeling. The treatment algorithm is based on segmentation kriging and medial axis calculation by thinning. Complementary informations are also calculated using auto-correlation function. The complete procedure is first tested on a virtual solid and then applied to yeast. The results present a large pore size distribution and a high connectivity between the pores. Both those effects can partially be attributed to the choice of the algorithms to deduce the pore network.

9 *Key words:* Yeast, microtomography, porosity, porous network

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10 **1. Introduction**

11 Granular baker's yeast is industrially dried to reduce transport costs and to  
12 increase conservation time. This energy intensive process may cause viability  
13 loss of the product (Bayrock and Ingledew, 1997): drying conditions are crucial  
14 to achieve well inactivated yeast without causing the cells death (Volkov et al.,  
15 1990). Knowledge of the local conditions (temperature, air moisture content)  
16 inside and around the grains is therefore important to predict the yeast survival  
17 chances (Marechal et al., 1999). Thus, detailed modeling of the drying process

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\*Corresponding author, Tel: 00 32 2 650 67 56, Fax: 00 32 2 650 29 10  
Email address: [fdebaste@ulb.ac.be](mailto:fdebaste@ulb.ac.be) (F. Debaste)  
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18 would be a powerful tool to understand, control and optimize baker's yeast  
19 drying.

20 Most models applied to yeast drying hide the impact of the inner-grain struc-  
21 ture on the evaporation in desorption isotherms or in fitted diffusion coefficients  
22 that have a limited physical meaning. This approach neither allows correct un-  
23 derstanding of viability loss mechanisms nor offers a general explanation of the  
24 observed drying rate curves (Debaste et al., 2008).

25 Two modeling approaches have been tested recently to tackle those limita-  
26 tions. The first one, (Debaste et al., 2008), is based on the explicit expression  
27 of the limiting transport phenomena affecting drying considering two succes-  
28 sive steps. This model gives satisfactory results for process optimization but  
29 no insight is gained on viability. Indeed, the assumption of a known limit-  
30 ing phenomenon allows hiding the impact of the porous structure in physically  
31 based fitting parameters, offering only a rough description of the inner grain  
32 phenomena.

33 In a second step, porous network modeling of yeast drying has been investi-  
34 gated (Debaste and Halloin, 2009). Although offering encouraging and well fit-  
35 ting results, this model was based on drastic and unrealistic assumptions about  
36 geometry and pore network topology: two dimensional slices of the grain were  
37 simulated and pore network parameters were very roughly estimated, limiting  
38 the validity scope of the results.

39 The global aim of this work is to obtain a more detailed, three dimensional,  
40 pore network model of the drying of yeast. This paper is focused on the investi-  
41 gation of the porous structure and on the deduction of a porous network to be  
42 used to model the drying.

43 To characterize the porosity of *Saccharomyces cerevisiae*, a living organism,  
44 the use of classical intrusive pore size identification methods, such as mercury  
45 porosimetry, gives doubtful results. Although yeast is known to survive at very  
46 high pressure (Espinasse et al., 2007), little is known about its behavior in  
47 contact with high pressure mercury. Therefore X-ray microtomographic investi-  
48 gation of a yeast grain is realized. This non-intrusive technique has already been

49 shown to be an alternative to mercury porosimetry for foam materials shrinking  
50 under the pressure (Léonard et al., 2008b). The resulting three dimensional  
51 numeric representation of the grain is then used to deduce pore network para-  
52 meters.

53 X-ray microtomography has already been used to deduce information about  
54 the porous structure of food such as bread (Falcone et al., 2008; Babin et al.,  
55 2006), cellular foam food (Lim and Barigou, 2004; Trater et al., 2005), meat  
56 (Frisullo et al., 2009), dried bananas (Léonard et al., 2008a), apples and pears  
57 (Verboven et al., 2008). Except for the work of Verboven et al. (2008), who  
58 directly used the void space dimensions as a domain for solving numerically  
59 reaction-diffusion equations, focus was set on qualitative and simple quantitative  
60 datas. However, no complete networks parameters were deduced.

61 This last point has already been tested in geological science (Al-Raoush and  
62 Willson, 2005; Al-Raoush and Alshibli, 2006). In this paper, an application of  
63 similar algorithms to deduce detailed porous network in yeast grain is presented,  
64 highlighting the potential of this method for further detailed investigations.

## 65 **2. Materials and methods**

66 The applied image treatment procedure is similar to the one presented by  
67 Al-Raoush and Willson (2005). However, different algorithms are considered  
68 for the final network generation. The principle of the procedure is sketched  
69 on Figure 1. The original gray-level images (a) are segmented: every voxel is  
70 assigned to solid or void (Gonzalez and Woods, 1993). The medial axis, a one  
71 voxel thick assembly characteristic of all the paths present in the void network  
72 (Couprie et al., 2007), is then deduced (c). Finally, pore network properties  
73 are computed (d). The auto-correlation function and the tortuosity are also  
74 estimated.

75 This procedure, implemented in MATLAB 7.6, is tested on a computer gen-  
76 erated set of images and then applied to experimental images obtained through  
77 X-ray microtomographic investigation of a yeast grain. The different steps are  
78 detailed in the following subsections.

79 *2.1. Image segmentation*

80 The simplest segmentation procedure, further called the *threshold technique*,  
81 is based on one fixed threshold: every voxel whose gray level is darker than the  
82 threshold is assigned to fluid; the rest is assigned to solid.

83 This method is simple but highly sensitive to background noise on the im-  
84 ages (Oh and Lindquist, 1999). An alternative, less noise sensitive, is indicator  
85 kriging, further called the *kriging technique*, for which two thresholds are de-  
86 fined. Every voxel with a gray level lower than the lowest threshold is assigned  
87 to the solid and every voxel with a gray level higher than the highest threshold  
88 is put in the fluid. For intermediate values, indicator kriging, computation of  
89 the most probable value of a voxel based on the values of the surrounding vox-  
90 els, is used (Oh and Lindquist, 1999). This method has two drawbacks. First,  
91 when the size of the smallest objects to be found is only of a few voxels, the  
92 treatment might suppress physically relevant informations. Second, the kriging  
93 technique requires intensive calculations. For those reasons, the two techniques  
94 are compared in our application.

95 *2.2. Medial axis calculation*

96 The medial axis is calculated using the thinning algorithm presented by  
97 Palagyi and Kuba (1998). The principle of this algorithm is to modify iteratively  
98 the segmented image until the only fluid voxels left form the medial axis. At  
99 every iteration, every fluid voxel neighboring a solid voxel is considered. If the  
100 configuration of the types of its neighbors fits some specific configurations,  
101 called *masks*, the studied voxel is changed to solid. The key of this algorithm  
102 is the choice of the masks allowing to keep only the medial axis. The chosen  
103 algorithm and the corresponding set of masks are detailed by Palagyi and Kuba  
104 (1998).

105 *2.3. Porous network identification*

106 Different definitions of network properties are possible, depending on the goal  
107 of the network. In this paper, using the same definition as presented in Debaste  
108 and Halloin (2009), pores are defined as fluid volumes presenting no resistance to

109 flow. Capillary pressure, pressure drop and concentration gradient are assumed  
110 to be negligible in the pores. The pores are connected to each other by throats,  
111 that are zero volume objects where all the fluid flow resistance is concentrated :  
112 capillary pressure, pressure drop and concentration gradients in the throats are  
113 limiting the global mass transport within the network. The pores are therefore  
114 characterized by a volume and a connectivity (or pore coordination number) to a  
115 known number of throats. The throats are characterized by different parameters  
116 (global pressure drop coefficient, effective surface tension, diffusion length) to  
117 evaluate the transport phenomena limitation steps. Usually, those different  
118 parameters are correlated to the characteristic length and diameter of idealized  
119 throats (Metzger et al., 2007).

120 Each voxel of the medial axis which is at the intersection of multiple branches  
121 is defined as being a pore. At this stage, each pore corresponds to a single voxel.  
122 The connectivity of the different pores is evaluated by travelling on the different  
123 medial axis branches until reaching the other pores. While doing this, the  
124 distance from every voxel to the nearest solid voxel on the segmented image is  
125 calculated. The smallest value for a voxel of the medial axis between two pores  
126 is stored as being the radius of that throat.

127 As shown by Al-Raoush and Willson (2005), this pore definition leads to the  
128 definition of too many pores : multiple medial axis division occurs in a volume  
129 usually considered as a single pore. To solve this issue, pores are merged if the  
130 distance between them is smaller than distance between the pore and the nearest  
131 solid voxel. The new pore is located in the middle of the straight line between  
132 the two former pores. It inherits the connections of both previous pores, except  
133 the one linking the two former pores. All the pores are checked for merging one  
134 by one, iteratively, until no new merging happens.

135 The volume of each pore is then evaluated. Following the proposed network  
136 definition , the volume of the pore is limited by the pores walls and the connected  
137 throats. In the throat, the limit should be defined by the plan normal to the  
138 medial axis direction. However, due to the discretization of the medial axis,  
139 unsatisfactory volume division are achieved. Therefore, in this work, a simplified

140 evaluation is chosen, based on two iterative steps. First, all the voxels that are  
141 in the largest fully fluid sphere centered at a pore position are assigned to that  
142 pore. The pore merging step ensure that no overlapping occurs between pores.  
143 Second, every unassigned voxel neighboring an assigned voxel is assigned to the  
144 same pore. Unassigned voxels neighboring voxels assigned to different pores  
145 are assigned randomly to one of those pores. As those voxels correspond to  
146 a less than half a percent of the total number of voxels, their impact on the  
147 final volume distribution is considered to be negligible. The voxel assignment is  
148 iterated until all the fluid voxels are assigned to a pore. Counting the voxels of  
149 a pore gives its volume.

#### 150 *2.4. Auto-correlation function*

151 The auto-correlation function gives the probability of having two voxels,  
152 separated by a given distance, to be both in the fluid phase. It is correlated  
153 to informations on the structure such as the specific surface, the porosity, the  
154 typical size of a constitutive element of the solid and an estimate of the length  
155 to which porosity tends to reach its macroscopic value. This last value is useful  
156 to estimate the representative elementary volume (REV) to be used in porous  
157 media modeling following the continuous approach. Indeed, the REV can be  
158 defined as the volume over which an averaged property can be considered to  
159 be independent of the position (Bear, 1972). At the length scale of the REV  
160 for porosity, the autocorrelation function must be approximately constant. The  
161 mathematical expression of the autocorrelation function was taken from Berry-  
162 man and Blair (1986).

#### 163 *2.5. Tortuosity measurement*

164 Tortuosity is defined to take globally into account the increased fluid path  
165 length due to the presence of the solid. It is defined the ratio between the  
166 average effective length that fluid has to follow to connect two points in the  
167 fluid phase and the Euclidian distance separating those two points.

168 A direct computation of porosity is obtained by travelling on the medial  
169 axis and by counting the smallest number of voxels required to go from one

170 pore to another. However, in this case, this is not straightforward because the  
 171 uniqueness of the medial axis path between two directly connected pores is lost  
 172 at the pore merging step. Therefore, in this work, it is proposed to calculate  
 173 the porosity based on the product of two terms. The first one is the *throat*  
 174 *tortuosity*, calculated for two directly connected pores before the pore merging  
 175 step, and defined as the ratio between the medial axis length and the Euclidian  
 176 distance between those two pores. The second one is the *network tortuosity*  
 177 defined as the tortuosity between two non directly connected pores, considering  
 178 that the directly connected pores are joined by straight lines.

179 Network tortuosity is evaluated for couples of pores distant by one third  
 180 to one half of the sample dimension. For higher distances, considering a cubic  
 181 sample implies that some directions present more data than others, leading to  
 182 a bigger influence of those direction on a global averaged tortuosity. Inversely,  
 183 for pores that are too close, the deduced porosity represent a too local value.

#### 184 2.6. Virtual solid for validation

185 To validate the method, the complete algorithm is tested on a virtual sample  
 186 of known properties. The fluid phase is defined by the junction of regular  
 187 octahedrons of uniformly distributed size but centered on a regular cubic lattice.  
 188 The minimal and maximal value of the size distribution is chosen to ensure  
 189 that every octahedron is in contact with its six neighbors. Figure 2 presents  
 190 the visualisation of the void structure for 2x2x2 octahedrons already voxelized  
 191 (presented as small cubes). Figure 3 illustrates a slice in a structure of 10x10x10  
 192 octahedrons (white is void, black is solid).

193 For this solid, the throats have a square section with a side length  $d_{ia}$  given  
 194 by

$$d_{ia} = \left( \frac{\sqrt{2}}{2} \bar{d}_o \right) - L_o, \quad (1)$$

where  $\bar{d}_o$  is the average of the length of the border of the two connected octahedrons and  $L_o$  is the distance between the center of two successive octahedron.



Similarly, the volume of a pore, obtained by truncating the full octahedron, writes as

$$V_{pore} = \left( \frac{\sqrt{2}}{3} d_o^3 \right) - \frac{\sqrt{2}}{6} \sum_{lia} d_{lia}^3. \quad (2)$$

Exact throat tortuosity is 1 and the averaged isotropic network tortuosity is of 1.52. The generated samples contain 1000 pores with normally randomized sizes and distributed in 100x100x100 voxels. For a comparison with the experimental images obtained by microtomography, the voxel length is chosen to be 2.95  $\mu m$ , which is the calibrated voxel size for the yeast samples.

### 2.7. Noise addition to the virtual solid images

To test noise influence, present on experimental samples, a perturbed volume is computed based on the previously defined void space. A gray level in a range of 64 values, based on a normal random distribution, is given to every voxel. A different distribution is applied for fluid and for solid voxels. Both distributions have the same standard deviation but different averages. The solid distribution center is set on the 22nd gray level while the fluid is centered on the 43rd. Different noise standard deviation are tested going from 2.5 to 7.5 gray levels.

### 2.8. Microtomographic imaging of yeast grains

The analyzed *Saccharomices Cerevisiae* grains are industrially produced by Beldem (Andenne, Belgium) and then dried in a desiccator during one week at 25°C and 5 % of relative humidity. The microtomographic investigation was realized using a *Skyscan-1172 high-resolution desktop micro-CT system* (Skyscan, Kontich, Belgium). A dried yeast sample was placed vertically in a polystyrene holder, the latter being almost transparent to X-rays. The cone beam source operated at 60 kV and 167  $\mu A$ . The detector is 2D, 1048x2000 voxels, 16-bit X-ray camera. The distance source-object-camera was adjusted to produce images with a voxel size of 2.95  $\mu m$ . The rotation step was fixed at 0.4°. For each angular position, a radiograph of the sample, was recorded by a 2D camera. The Feldcamp back projection algorithm was used to reconstruct 2D images of the cross-sections of the sample from the radiographs. The corresponding yeast

221 3D image is obtained by stacking the 2D cross-sections. The obtained 3D image  
222 is 1024x1024x1024 voxels wide.

### 223 **3. Results**

#### 224 *3.1. Application of the algorithm to the virtual solid*

225 Ten solids, with no noise addition, were generated and tested with the full  
226 algorithm. No significant differences were observed between those ten solids.

227 The network deduction of the virtual solid leads to 1070 pores in average,  
228 instead of 1000. Figure 4 presents the coordination number of the different  
229 pores. Globally, predictions are satisfactory although toward lower values, a bias  
230 is observed. The merging algorithm leads to two error types that can explain  
231 the discrepancies. First, two pores that should be merged are not, leading to  
232 pores with lower coordination. Second, two pores that should not be merged  
233 are, leading to a highly coordinate pore.

234 As the total pore number is higher than theoretically, for this sample, the  
235 first error is slightly more present. Enhancing the resolution (number of voxel  
236 per pore) would lower both errors. For the virtual solid, only the computational  
237 power limits the resolution. For the yeast sample, measurement resolution is  
238 the limiting factor.

239 Figure 5 presents the throat size distribution. As the throat size is the  
240 smallest element to be identified, it is more affected by resolution limitation  
241 than other parameters. For the chosen resolution, only four different sizes can  
242 be distinguished. Indeed, the largest possible throat radius is only 8 time the  
243 estimated error on the radius measurement which is of around 2 voxels. The  
244 obtained size is globally underestimated. Tests with a higher resolution confirm  
245 the ability of the algorithm to reduce this error.

246 Figure 6 shows the pore volume distribution. Theoretically, as obtained from  
247 equation (2), the pore size should be, in the studied case, divided in four well  
248 marked peaks. However, although two of those peaks are predicted, these are  
249 included in a bell-shaped curve. The average value of the distribution is however

250 well predicted. The simplified algorithm proposed in this work is thus unable  
 251 to predict multimodal porosity.

252 The predicted throat tortuosity is 1.045, which is only slightly overevaluated.  
 253 The network tortuosity is estimated at 1.41, which corresponds to a 10 %  
 254 underestimation.

### 255 3.2. Impact of the segmentation algorithm

256 The virtual solid volume with different noises is submitted to the two seg-  
 257 mentation algorithms with various segmentation thresholds. Figure 7 presents  
 258 the percentage of wrongly attributed voxels for various threshold values with  
 259 different noises. For the kriging, where two thresholds have to be set, the ab-  
 260 scissa value corresponds to the lower threshold value. The higher threshold is  
 261 fixed seven grey levels higher. As expected, the number of errors are minimal for  
 262 both methods exactly at the average of the mean of the distributions. Except  
 263 when the noise is really small, the kriging leads to less errors than the one level  
 264 technique. However, kriging only leads to maximum 25% less errors than the  
 265 other method.

Bloc	1		2		3	
Method	Kriging	Thres.	Kriging	Thres.	Kriging	Thres.
Simple threshold		72		78		76
Lowest kriging threshold	71		75		73	
Highest kriging threshold	75		79		77	
Porosity	0.581	0.581	0.5805	0.579	0.5975	0.597
Specific surface ( $m^{-1}$ )	123000	123000	115600	115600	114100	114100
Mean coordination number	4.63	4.46	4.41	4.32	4.72	4.55
Median coordination number	4	4	4	4	4	4
Pores number	1536	1678	1582	1676	1518	1658
Throats number	7104	7496	6981	7242	7168	7545
Average pore volume ( $\mu m^3$ )	9200	8500	9400	8900	10100	9300
Throat tortuosity	1.33	1.31	1.30	1.30	1.37	1.36
Network tortuosity	1.53	1.53	1.55	1.54	1.61	1.58

Table 1: Comparison of yeast analysis results for 3 different samples.

### 266 3.3. Application to yeast

267 Figure 8 illustrates a reconstructed 2D slice obtained by X-ray microtomog-  
 268 raphy for a dry yeast grain.

269 Segmentation parameters and results for three regions of 100x100x100 voxels  
270 inside the yeast grain are summarized in Table 1. The segmentation threshold  
271 is unknown, it is chosen to achieve a global porosity around 0.6 which was  
272 estimated experimentally using a gravimetric method. Table 1 shows a fairly  
273 good reproductivity of the results. Details are therefore given only for one  
274 sample.

275 Figure 9 presents the coordination number of pores for the two segmenta-  
276 tion techniques. Both methods show similar results. A very large range of  
277 value is observed. Such distributions have been reported previously with sim-  
278 ilar algorithm applied to porous rocks (Baldwin et al., 1996; Al-Kharusi and  
279 Blunt, 2006). The large values of coordination numbers can be attributed to  
280 the definition given to a pore. Indeed, as every crossing of the medial axis is  
281 initially considered as a pore, every irregularity in the solid structure leads to  
282 the definition of a pore. The pore merging step reduces that effect, but leads to  
283 highly connected pores. Globally, pores with a coordination number superior  
284 to 8 represents less than 10% of the total and therefore only play a minor role.  
285 Pores having a coordination number of 2 are also present. These pores appears  
286 at the crossing of medial axis branches leading outside the sample. Such pores  
287 do not affect the connectivity of the network and are therefore generally sup-  
288 pressed. Their volume is added to the pore connected with the largest throat.  
289 The smaller throat is assigned to link the two pores connected to the suppressed  
290 one.

291 The throat diameter distribution is presented on Figure 10. No major dif-  
292 ference can be stressed out between the two segmentation techniques. Most  
293 observed throats have a diameter of maximum 3 voxels, which is estimated just  
294 above the error of the throat size evaluation. The largest throats are only 3  
295 times larger. Therefore, few informations can be deduced as the measurement  
296 resolution is limiting.

297 Similarly, the pore volume distribution is presented on Figure 11. As for the  
298 throats, a non negligible fraction of the pores have a size corresponding to the  
299 estimated precision of our algorithm. However, a very large size distribution

300 can be observed. As shown for the virtual solid, this distribution has to be  
301 interpreted with care as the methodology does not allow to predict multimodal  
302 porosity.

303 Also, the auto-correlation function predicts a porosity REV typical length  
304 scale of about  $100 \mu m$ .

#### 305 **4. Analysis and discussion**

306 The analysis of a limited region of  $100 \times 100 \times 100$  voxels allows the compu-  
307 tation of a set of average values characterizing the network of a yeast grain.  
308 The validity of using a reduced part of the grain is supported by both the re-  
309 productivity of the results for different regions of the grain and the fact that  
310 the autocorrelation function predicts a REV typical length three times smaller  
311 than the considered region. This value ensures that for common applications,  
312 where a grain is of a millimetric size, the continuous modeling approach can be  
313 used to describe the global behavior of the grain.

314 The choice of the segmentation technique seems to have only a limited impact  
315 on the deduced network and other averaged properties. Three main reasons can  
316 explain this :

- 317 1. the rest of the image treatment is not affected by the observed variations  
318 in segmentation,
- 319 2. the thresholds used were correctly estimated,
- 320 3. the noise on the samples is too high for the difference between the methods  
321 to be significant.

322 The average coordination number obtained is of the order of 4, which corre-  
323 sponds to the coordination number of the void space of a monodisperse dense  
324 sphere packing. This value is therefore acceptable. A few pores have an unreal-  
325 istically high coordination number, due to their deduction from a highly noise  
326 sensitive medial axis.

327 The throat size distribution is barely exploitable due to limitation in the  
328 resolution method. However, more careful treatment algorithm (based on the

329 whole throat section measurement rather than on their radius) could already  
330 offer better results using the same images. The obtained results only allows a  
331 rough description of the throats. This information is however the first to be  
332 available.

333 For the pore volume size distribution, also, a gross estimate has been ob-  
334 tained. Alternative algorithm for this step should be considered.

335 The results can be used as input parameters for different models. Except  
336 for the pore volume distribution, for which the results are not reliable, all the  
337 other parameter measurement present a net enhancement compared to the data  
338 previously used in Debaste and Halluin (2009).

339 The tortuosity and the approximate size of the REV are useful information  
340 to be used in continuous models, such as the one presented in Debaste et al.  
341 (2008). The total tortuosity obtained by this analysis is around 2, which is of  
342 an acceptable order of magnitude. However this value is lower than the value  
343 used in Debaste et al. (2008), where tortuosity, used as a fitting parameter, was  
344 evaluated around the value of 5, suggesting a need for further model refinement.

## 345 5. Conclusion

346 A general simplified method to deduce porous structure, based on existing  
347 algorithms, was presented, tested and applied to 3D images of granular yeast  
348 obtained by X-ray microtomography. This algorithm allows detailed investi-  
349 gation of the porous structure without altering the structure of the material,  
350 offering valuable information for inner grain drying modeling.

351 Pore and throat size distributions, connectivity, specific surface, tortuosity  
352 and REV size have all been determined. However, both algorithm approxi-  
353 mations and experimental resolution limitations limit the scope of the results.  
354 The obtained results can however be directly integrated in existing models for  
355 which simpler assumptions had been previously taken. Enhancement of the pre-  
356 sented algorithm could lead to more precise data, fully satisfactory for all the  
357 considered modeling approaches.

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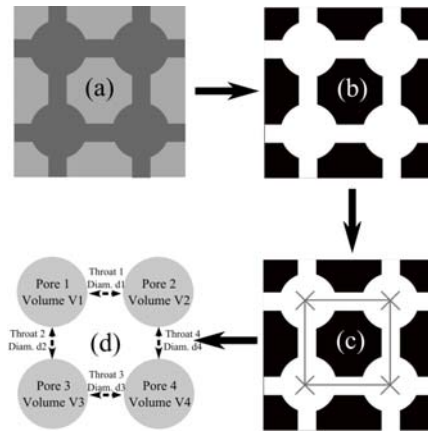


Figure 1: Different steps of image treatment to extract the network information.

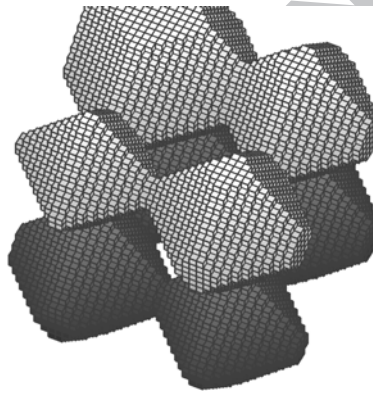


Figure 2: Virtual solid visualisation.

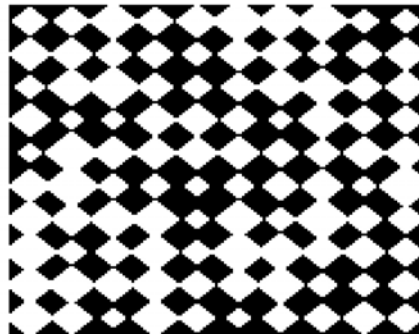


Figure 3: Virtual solid slice visualisation (void is in white, solid is in black).

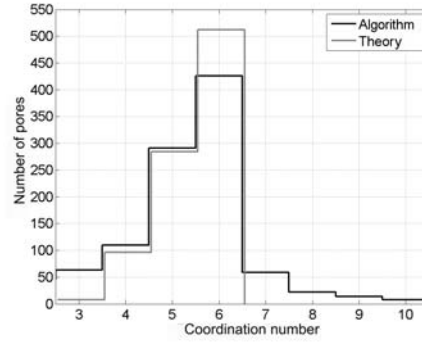


Figure 4: Predicted and theoretical coordination number of the virtual solid.

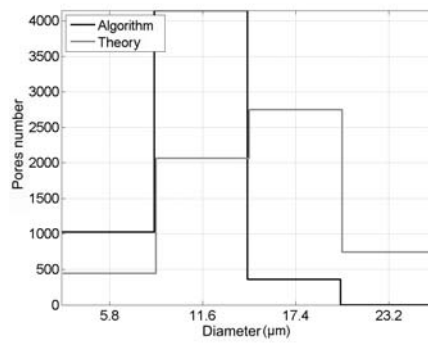


Figure 5: Predicted and theoretical throat diameter of the virtual solid.

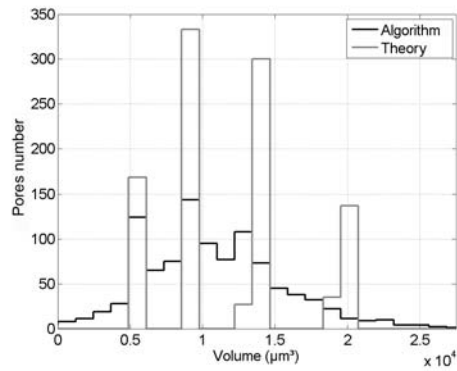


Figure 6: Predicted and theoretical pore volume distribution of the virtual solid.

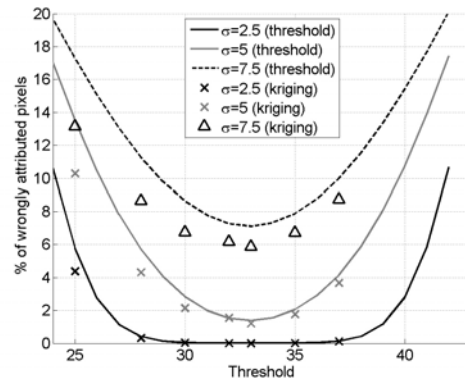


Figure 7: Fraction of wrongly attributed voxels using different segmentation techniques.

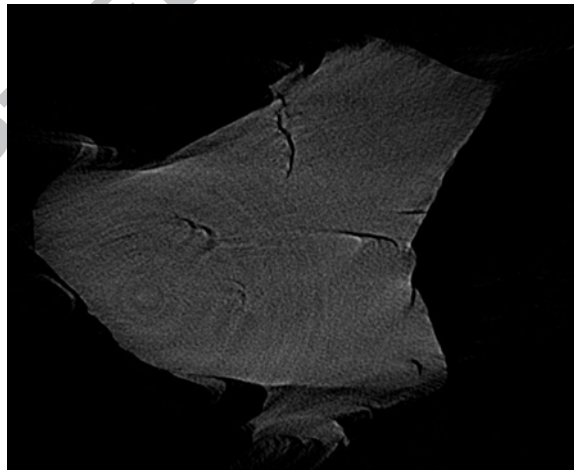


Figure 8: A reconstructed 2D yeast grain slice, obtained by microtomography.

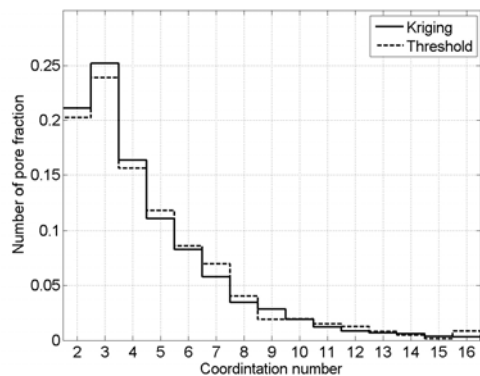


Figure 9: Coordination number for a yeast sample.

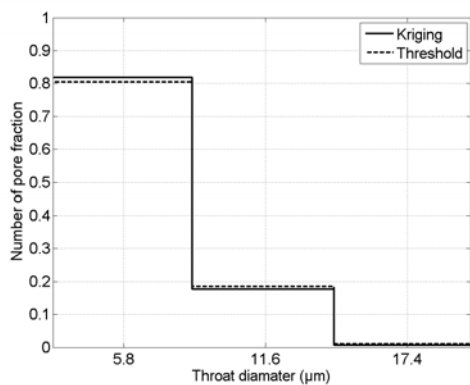


Figure 10: Throats diameters distribution for a yeast sample.

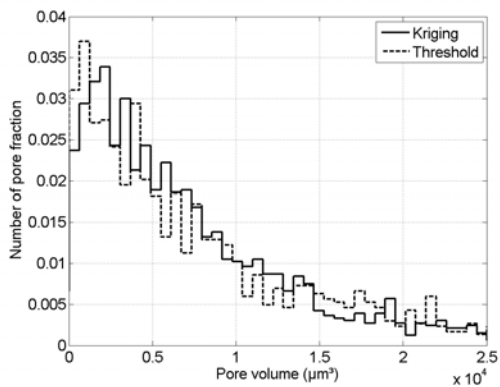


Figure 11: Pores volume distribution for a yeast sample.