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MEASURING THE SIZE OF PORES BY THE SEGMENTATION OF IMAGES FROM SCANNING ELECTRON MICROSCOPY

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Abstract: Segmentation is an image processing method used for partitioning an image into multiple sets of pixels, which are defined as its "super-pixels". Here, we are proposing a method based on segmentation, for determining the super-pixels corresponding to the pores resulting from freeze-drying of a pharmaceutical solution. The sizes of these pores, evidenced by the Scanning Electron Microscopy (SEM), are estimated through the areas of the super-pixels of the segmented image. These sizes can be used to estimate the resistance to mass transfer and hence optimise the cycle of production.

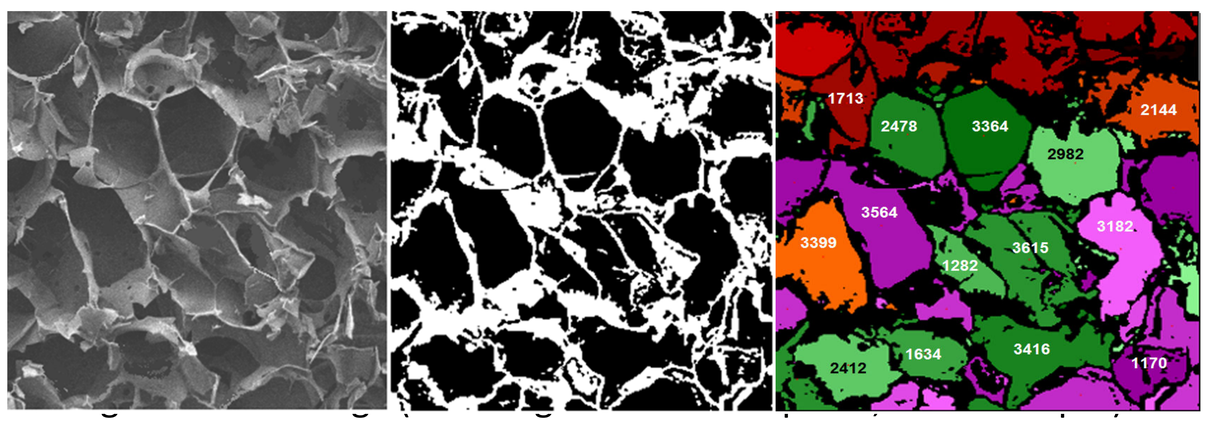
Keywords: freeze-drying, pore sizing, image segmentation, image processing, pharmaceutical solutions

Materials and Methods

To illustrate the approach, let us start using a **lyophilized sample produced from an aqueous solution of sucrose, having 5% w/w as solid content**. The solution was freeze-dried as discussed in [1]. In the Fig.1 (left), we can see a **SEM image of the sample**. To **estimate the size of the pores**, the approach we follow is that of segmenting the image.

To obtain the **segmentation**, the SEM image, such as that given in the left panel of Fig.1, must be pre-processed to enhance brightness and contrast. After, some further filtering is necessary: in this case, the image was smoothed by a Gaussian filter.

Then, the image is mapped into a **binary black and white map** (Fig.1, middle), which is segmented through a **thresholding method** [2]. **The result of the segmentation is the partitioning of the SEM image into sets of super-pixels**, which are represented in the Fig.1 (right), by the **colored domains**. Each super-pixel is characterized by a label, by means of which we can easily evaluate **the area (in pixels) covered by the domain**.



The **area of the super-pixel is a measure of the observed cross-section of the considered pore**. Then, the segmentation gives us a set of data reporting the areas of the several cross-sections given by the image. As in the example given above, the cross-section of pores is 2600 ± 400 (in pixels), where we have estimated the uncertainty by means of the standard deviation of the chosen sample. That is, **we obtain a cross-section of (8000 ± 1200) µm², corresponding to a radius of (50 ± 8) µm**.

[1] Pisano R., Barresi A.A., Capozzi L.C., Novajra G., Oddone I., and Vitale-Brovarone C., 2017, Characterization of the mass transfer of lyophilized products based on X-ray micro-computed tomography images. Drying Technology 35(8), 933-938. [DOI: 10.1080/07373937.2016.1222540] [online 11Nov 2016]

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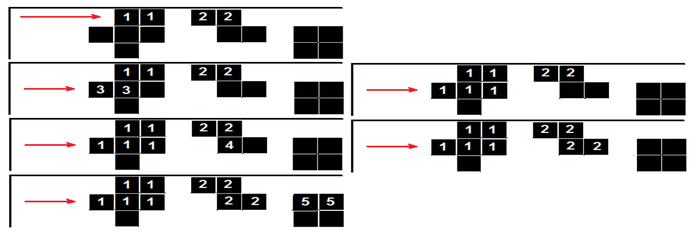
Image segmentation

An image segmentation is a process of partitioning the image into multiple sets of pixels, defined as super-pixels, in order to have a representation which is simpler than the original one and more useful to the following desired analyses [3]. Segmentation is often used in many applications of image processing [4-5]: **several methods exist, mainly based on the use of binary (black and white) images** [6].

The images that we are here considering have **grey-tone pixels**. In the segmentation of the images, we move on with **a method based on the thresholding of the brightness map, turning the image into a black and white one**. Once we have obtained the binary image, we have at our disposal a matrix of pixels containing black and white domains. Starting from the left/upper corner of this matrix, we move following rows and columns of the matrix. We focus on black pixels and characterize each of them by a sequential integer number *k*, which is acting as a label of the single pixel. Some of these labels will be the labels identifying the domains (see Figure 2), as it is discussed in detail in the article.

Fig. 2.

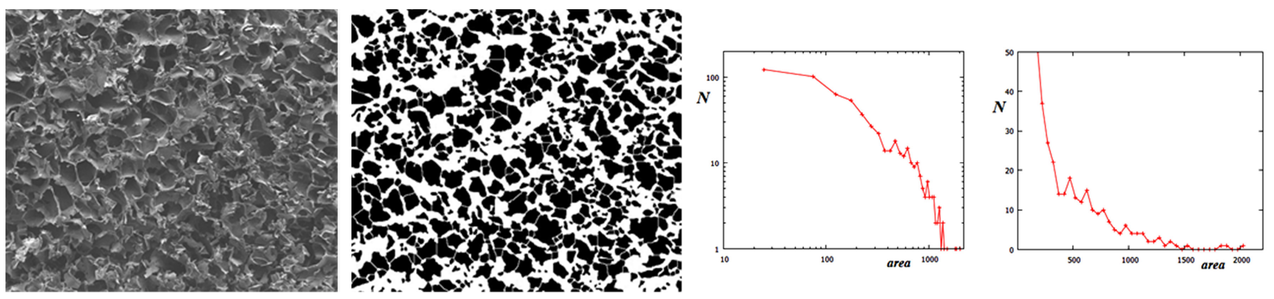


[4] Pham, D.L., Xu, C., Prince, J.L., Current methods in medical image segmentation, Ann. Rev. Biomed. Eng., 2, (2000), 315-337.

[5] Forghani, M., Forouzanfar, M., Teshnehlab, M., Parameter optimization of improved fuzzy c-means clustering algorithm for brain MR image segmentation, Eng. Appl. Artif. Intel., 23(2), (2010), 160-168.

[6] Schladitz, K., Quantitative micro-CT. J. Microsc., 243(2), (2011), 111-117.

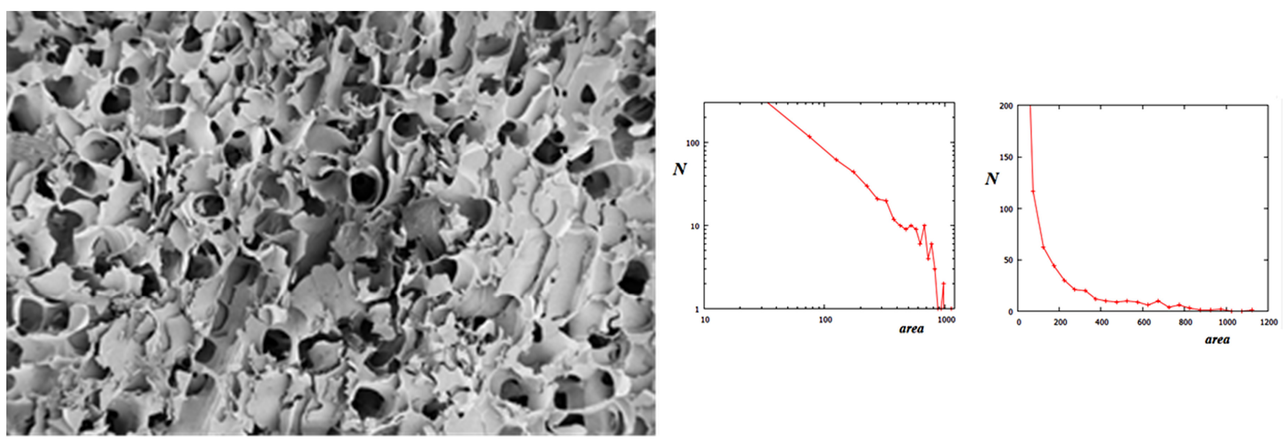
Results on a SEM image of a sucrose cake



Left: SEM image of a cross-section of the sucrose cake, and the binary image (600x511 pixels , 1785x1520 µm). Right: Distribution of the super-pixels, by counting them according to their area (in pixels) within intervals spaced of 50 pixels.

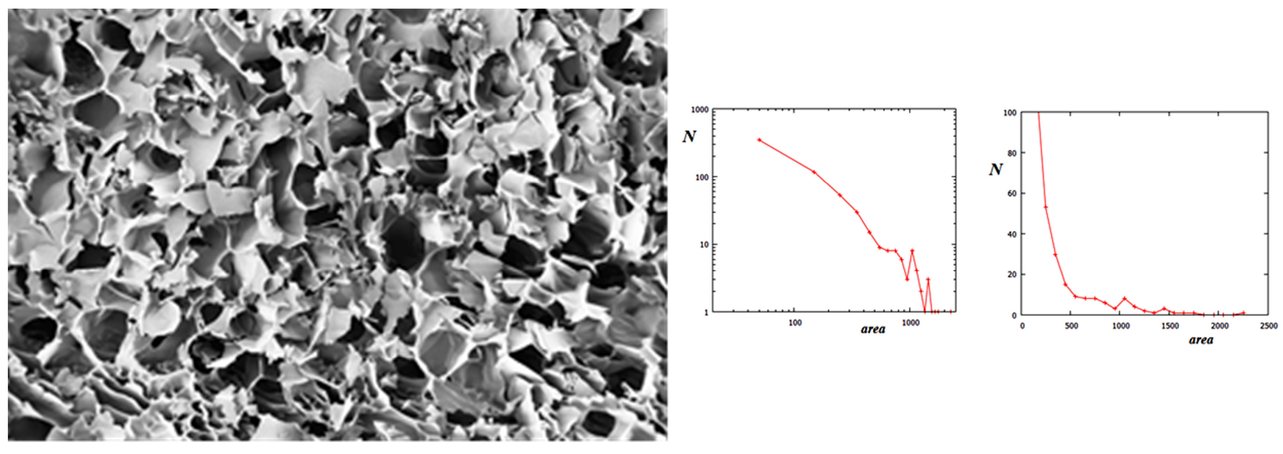
As we have segmented the image, it is possible to deduce the area of each pore, because it is the area of the corresponding super-pixel. We can plot the distribution of the super-pixels, and consequently the occurrences *N* of them, for given areas of the pores within intervals spaced of 50 pixels. We deduce the pores have areas comprised between 500 and 1500 pixels. **Using the scale provided by SEM instrument, we have areas comprised between 4400 µm² and 13000 µm².**

Results from other cakes: Section of a 5% dextran cake



Left: SEM image of a cross-section of a 5% dextran cake. The image is 600 x 397 pixels (1875 x 1240 µm). Right: Distribution of the super-pixels, by counting them according to their area (in pixels) within intervals spaced of 50 pixels. The cross-sections of the pores have an area comprised between 200 and 800 pixels, that is, between 1950 µm² and 7800 µm². It corresponds to radii from about 25 µm to 50 µm.

Cake 1% mannitol + 4% dextran



Left: SEM image of a cross-section of a 1% mannitol + 4% dextran cake. The image is 600 x 400 pixels (1875 x 1250 µm). Right: Distribution of the super-pixels, by counting them according to their area (in pixels) within intervals spaced of 50 pixels. Let us note that, in this case, we have a peak about 1200 pixels. This corresponds to a cross-section of 11700 µm², that is, to a radius of about 60 µm.