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Automated analysis of pore structures in biomaterials

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Quantitative assessment of pore size and morphology is crucial in biomaterials design and evaluation, particularly hydrogels and scaffolds used in tissue engineering and drug delivery. In recent years, a growing number of studies have proposed or adopted automated image analysis tools to evaluate pore characteristics; however, the absence of standardised protocols, validation criteria, and consistent reporting practices has limited reproducibility and cross-study comparability. This perspective, for the first time, examines recent trends in automated pore size analysis in biomaterials research, highlighting commonly used algorithms, their implementation in image-based workflows, and their ability to resolve pore geometries in disordered materials. We discuss the influence of imaging dimension, resolution, algorithm assumptions, and image pre-processing on outcomes and highlight common challenges such as over-segmentation, user bias, and the misidentification of irregularly shaped pores. By drawing on selected examples from the literature, we illustrate both the strengths and limitations of current approaches and emphasise the need for transparent, standardised methodologies in the field.

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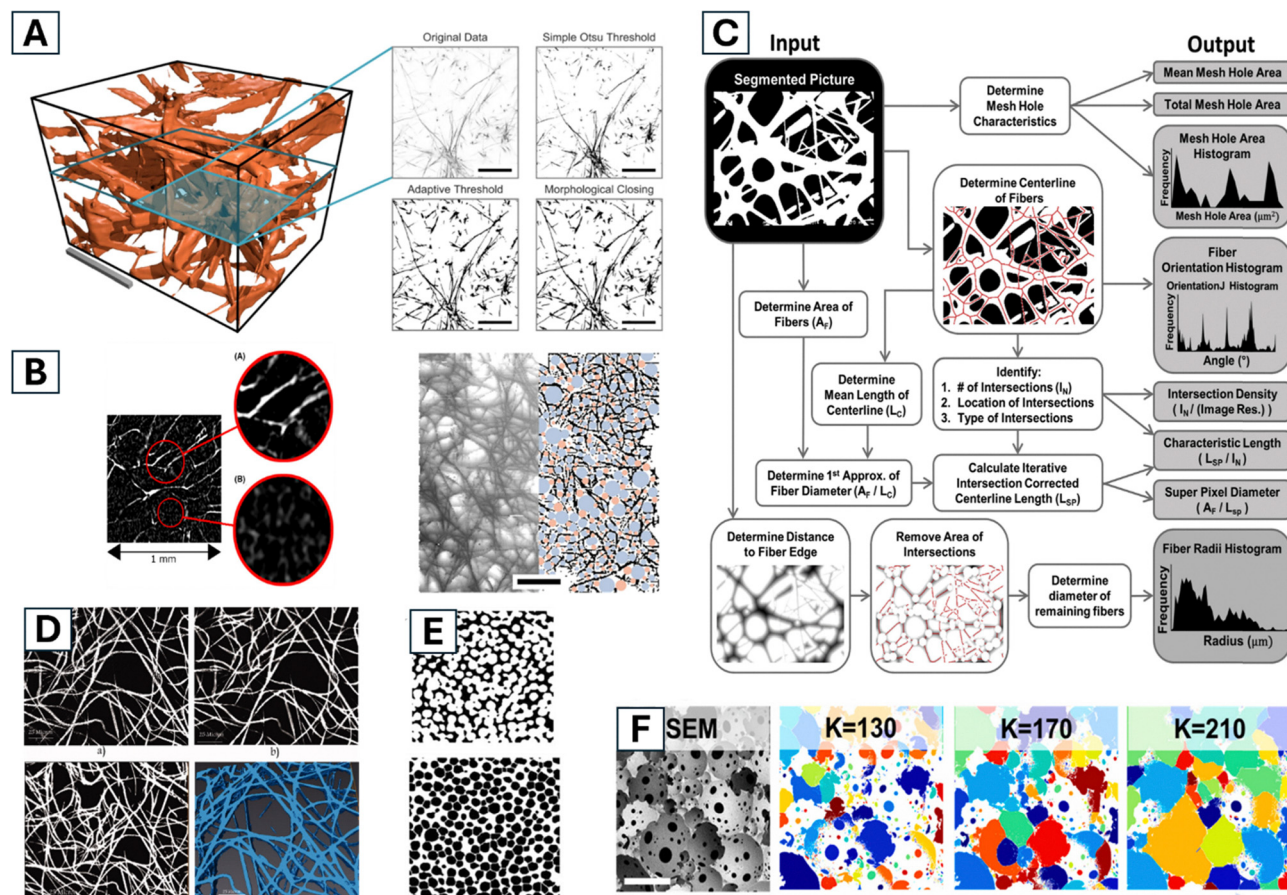


Fig. 2 Examples of segmentation methods. All images have been reproduced with permission. (A) 3D collagen network binarisation method. Left: Representative image cube of a 5(6)-carboxytetramethylrhodamine *N*-succinimidylester (TAMRA)-labelled collagen scaffold. Right: A representative 2D slice of original image data, Otsu's thresholding, adaptive local thresholding of a denoised and the final segmentation result.¹⁶ (B) An unthresholded, undespeckled section of a micro-CT image of a collagen scaffold, demonstrating the noise which can be present in this type of data. Top right: Shows collagen in white, with the noise manually removed. Black represents void. Bottom right: Shows the isolated noise, seen as light grey specks.⁵⁶ (C) Overview of how the DiameterJ algorithm analyses fiber diameter and other scaffold properties.⁵⁷ (D) Top left: CLSM images of nanomicrofiber scaffolds. Top right: Filtered with median 3D. Bottom left: Binarised. Bottom right: 3D reconstructed with Avizo Fire software.⁴³ (E) Top: Binarised image of a PVA scaffold. Bottom: Separation of pores using the watershed algorithm.⁴⁵ (F) Effect of pixel intensity threshold, K , on pore identification and resulting effect on pore size of scaffolds.³

corrections with machine learning-assisted 3D reconstruction, are emerging as promising solutions.⁵²

As we focus on direct 2D and 3D imaging techniques, the accuracy of pore quantification relies on automated image processing techniques that extract meaningful information from images. This section discusses the segmentation process (to isolate pores from the surrounding material) in Tables 1, 2 and Box 1, and pore analysis techniques (to quantify pore characteristics) in Box 2 and Table 3. Given the complexity of porous materials, different approaches, ranging from thresholding-based methods to machine learning-driven segmentation,¹⁰ have been developed to improve accuracy and reproducibility in pore characterisation.¹ It is important to note that the suitability of algorithms will vary as a function of the considered biomaterial and imaging modality. Depending on available equipment, signal-to-noise ratio and contrast, separation of background and specimen and image resolution will vary, changing the task difficulty, accuracy and consequently the choice of segmentation and analysis approach.^{47,54,55}

3.2 Segmentation: extracting pores from images

Segmentation is the first step in automated pore analysis, where the material and pores are distinguished as separate entities. Examples of segmentation methods used in the literature are given in Fig. 2. The choice of segmentation technique directly impacts the quality of extracted pore features and should be as close to 'ground-truth' as possible. Due to the range of imaging modalities and factors such as background lighting and noise, the choice of segmentation technique is imperative to effectively visualise pores. To evaluate segmentation success, an objective 'ground-truth' comparator is required. However, at the moment, 'ground-truth' is frequently established through manual measurement and inter-rater reliability which introduces bias and is subject to fatigue effects for large sample sizes.^{45,56} Due to the associated effort, reliance on automated segmentation and analysis approaches without 'ground-truth' validation is increasing.⁴⁶ Automated methods, including methods discussed below in Box 1, offer standardised, reproducible segmentation but may still misclassify



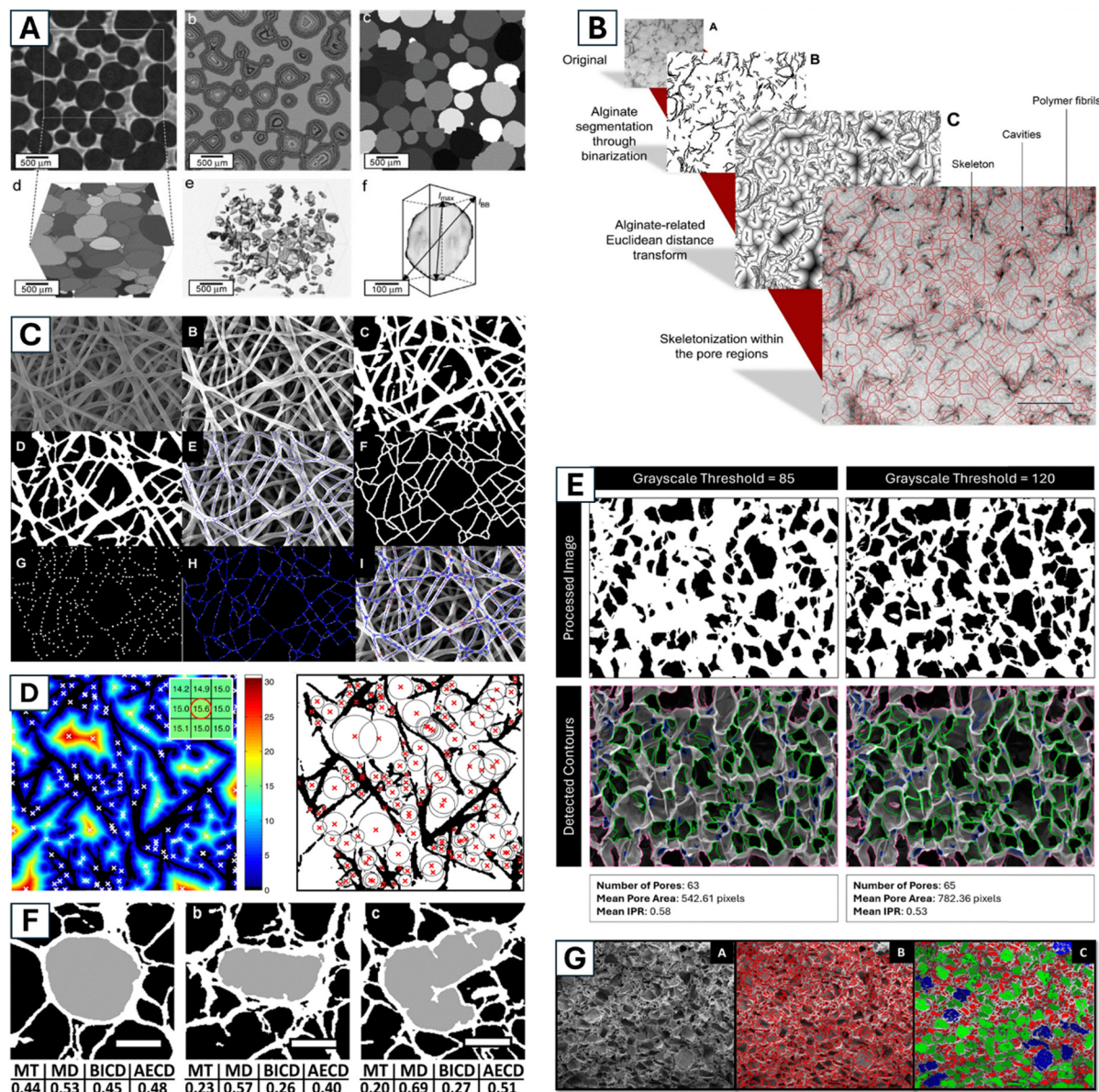


Fig. 3 Examples of automated pore size determination methods. All images have been reproduced with permission. (A) Quantification of 3D pore networks of sol-gel derived bioactive glass foams from micro-CT data: (a) 2D slice of raw data, (b) 2D slice showing application of the dilation algorithm, (c) 2D representation of pores derived from the watershed algorithm, (d) 3D image of identified pores, (e) 3D image of the interconnects obtained from the top down algorithm, and (f) demonstration of the bounding box method of measuring the interconnect length.¹⁹ (B) Illustration of pore size determination of alginate hydrogel microcapsules using TEM images. Image A depicts a hydrogel as it is typically observed using transmission electron microscopy. Image B shows the results of the image segmentation after binarisation. Image C shows the result of a Euclidean distance transformation. Image D gives an overlay of the pore region image skeleton (red lines) with the original image. Image skeletons are one-pixel wide center axes. They are defined via the set of inner pore pixels. The set is defined via local distance maxima with respect to alginate segments.³⁸ (C) Fiber network diameter determination of scaffolds (opposite of pore size determination, using similar algorithms). Top left: Starting SEM image. Top center: Image histogram equalisation followed by 3 by 3 median filtering. Top right: Local thresholding through Otsu method. Middle left: Thinning, smoothing and removal of isolated pixel areas through a cascade of different morphological operators. Middle center: Skeletonization. Middle right-bottom left: Binary filters for Delaunay network refinement. Bottom center: Modified Delaunay network associated to the real fiber network. Bottom right: Final network and fiber diameters detected.²⁵ (D) Simplified bubble analysis of the pore space of a random biopolymer network in two steps. Left: First, the Euclidean distance map (EDM) of the fluid space of the network structure is computed (shades of gray (colours online) indicate the distance of each fluid pixel to the nearest fiber pixel). (black) Fibers. Second, the local maxima of the EDM (white crosses) determine the centres of all 2D bubbles. To avoid bubbles of similar size in close proximity, the EDM was smoothed with a 5×5 Gaussian kernel with a sigma of one before the local maxima were determined. (inset) A local maximum of the EDM (red circle) is a pixel whose eight neighbours all have smaller values. Right: Resulting 2D bubbles (black circles) fit into the pore zones of the fiber structure (black). (red crosses) Bubble centers.¹⁰² (E) Comparison of detected pores with cutoff values of 85 vs. 120 in chitosan-gelatin cryogels using PoreVision software. Red outlines are pores outside the analysis boundary, blue outlines are pores removed for being too small (most likely dust, cracks, or folds), and green outlines are identified pores.²⁰ (F) Illustration of varying results provided by micro-CT 2D pore size analysis of collagen-based scaffolds. Pores (in gray) of 3 differing shapes (left, centre, right) were evaluated by means of 4 micro-CT 2D parameters (MT – mean thickness, MD – major diameter, BICD – biggest inner circle diameter, AECD – area-equivalent circle diameter) and their values are presented in panels below the images (in mm). The results tend to differ with increasing shape irregularity.⁴⁶ (G) Colour map and pixel intensity based measurement of macro pore size distribution in a polymeric scaffold.²¹



Table 1 Examples of preprocessing steps in segmentation

Pre-processing modality	Purpose	Software for implementation
Edge detection	Allows the separation of touching objects without specifying a subjective threshold. ⁵²	ImageJ/MatLab/OpenCV
Contrast maximisation	Maximises the contrast between pores and walls or pores and the background to ease segmentation and analysis. ²¹	ImageJ/MatLab/OpenCV
Despeckling	Despeckling sweep removes all but the largest objects in a space. Despeckling sieve removes objects below a certain area or volume. Adaptable for 2D or 3D space. ⁵⁷	ImageJ
Morphological operations	Operations that can analyse and modify image shapes and structures. Can also be implemented for noise removal and feature extraction. ^{18,19,21,24,45,52,54}	Image J/MatLab/Quanfima python package ²⁴
Gaussian blur	A technique to smooth an image by averaging pixel values within a Gaussian window, effectively reducing noise. ^{58,59}	ImageJ/MatLab/OpenCV/ Scikit – image package for python
Anisotropic diffusion filtering	Reduces noise while preserving edges by allowing diffusion to occur primarily in homogenous regions. ⁶⁰	ImageJ/MatLab/MedPy Python Package
Mean/median filtering	Reduces noise by replacing each pixel's value with the mean or median value of the surrounding pixels. ^{12,19,25}	ImageJ/MatLab/SciPy Python Package/OpenCV

Table 2 Examples of some readily available segmentation software

Software	Built-in segmentation
SPIP ^{82–84}	Intensity-based thresholding.
PoreSpy ^{85–88}	Watershed segmentation, deep-learning for pixel-wise classification.
Insight toolkit (ITK) ^{80,89}	Otsu and binary thresholding, watershed segmentation.
Thermo Fisher porometric software ⁹⁰	Gradient-based and marker-controlled watershed methods for segmentation refinement.
MIPAR ^{42,91}	Multiple threshold, watershed and deep learning-based methods.
Mathematica ^{38,92,93}	Adaptive and histogram threshold, various watershed analyses.
ImageJ based software	Global and local thresholding, and watershed segmentation. Compatible with segmentation plugins including BoneJ and MorphoLibJ.

Table 3 Examples of commonly used pore-analysis software

Software	Built-in analysis
SPIP ^{82–84}	(1) Pore size distribution
MIPAR ^{42,91}	(2) Interconnectivity, and transport pathways
Sigma Scan Pro 5 ^{96,104}	(3) Quantification using geometric fitting methods
Materialise Mimics ⁶⁵	(4) Skeletonisation
CTAn (Bruker) ^{46,105,106}	(5) Voxel-based analysis
Quanfima ²⁴	
Image-Pro Plus ^{107–110}	
PoreSpy ^{79–82}	Enhances pore network characterisation with skeletonisation-based metrics.
GeoDict ⁴⁴	Covering radius transform (CRT) and micro-CT-based pore size distribution (PSD) reconstruction.
Amira (TGS, San Diego, CA) ^{19,111}	Skeletonisation.
ImageJ Plugin DiameterJ ^{24,41,56}	Measure porosity of fibre networks.
ImageJ Plugins ND ¹¹²	Measure porosity of scaffolds.
ImageJ Plugins BoneJ ⁹⁹	Measure porosity of bone structures.
PoreVision ²⁰	Pore size analysis including measurement, distribution and range. Morphological analysis.

features, over-segment noise or under-segment adjacent pores (Fig. 2). While these methods are thus more time effective and objective than manual approaches, their lack of validation presents a new risk of bias.²⁷ Hybrid approaches, combining



automated segmentation with manual refinement, could be a balanced approach, however, would require pre-processing steps such as contrast enhancement and noise reduction (as outlined in Table 1) to improve the accuracy of pore boundary detection. Table 1 details several preprocessing steps to improve the efficacy of image-based segmentation and analysis.

In Box 1, we outline a range of (semi-)automated segmentation approaches. Thresholding is the simplest and most widely used segmentation technique, where pixels are classified as either pore or material, based on intensity values (Fig. 2). Thresholding-based segmentation is computationally efficient

but may struggle with detecting intricate pore networks, particularly in fibrous or highly porous materials.⁴³ Additionally, it may yield variable results due to user-defined parameters and a lack of benchmarking.^{20,27} Machine learning (ML) techniques, both shallow learning (*e.g.*, support vector machines, decision trees) and deep learning (*e.g.*, convolutional neural networks (CNN), U-Net models), can be used for pixel-wise classification in pore segmentation.^{5,20,39,61–63} However, machine learning is data-hungry, thus requiring larger sample sizes and manual 'ground-truth' labelling which is labour intensive and may introduce user-bias into the segmentation process.

Box 1: Segmentation approaches

✦ Thresholding-based approaches

- Global thresholding: a single threshold is applied across the entire image, making it suitable for materials with uniform contrast but less effective for heterogeneous structures.^{1,3,8,21,38,43,47,52,56,64–67} These techniques are also more sensitive to noise.⁶⁸ Some examples of global thresholding methods are given below, more methods and an in-depth discussion on thresholding could be found through Rajagopalan *et al.*²⁷
 - Otsu's global thresholding: selects an optimal segmentation threshold by maximising inter-class variance. This method is effective for materials with bimodal intensity distributions, where pores and solid structures have distinct grayscale values.^{19,67,69}
 - Entropy-based thresholding: the foreground and background are treated as separate sources and the optimal threshold is chosen at the maximum of the sum of the two class entropies. This is useful for images with complex histograms.^{24,27,45}
 - Histogram thresholding: analysing the concavity points of the image's histogram's convex hull. Any valley may be considered as a potential threshold, with the deepest concavities being favoured.^{27,70}
- Local (adaptive) thresholding: the threshold varies across different regions, allowing segmentation in images, in which contrast changes due to uneven illumination or varying material densities. This is useful for heterogeneous structures.^{16,20,25,54,71} Several local thresholding techniques exist as outlined by Rajagopalan *et al.*²⁷
- Contrast enhancement with Fourier transform: Fourier-based contrast enhancement has been integrated to improve edge detection in biomaterial samples, particularly in scaffold imaging.⁵
- Manual thresholding in ImageJ: adjusting the threshold manually to match original image features, as seen in some studies.^{1,72–76} This technique can introduce various problems associated with room lighting, fatigue of the operator and limited grey-scale shade perception.²⁷
- MATLAB-optimised thresholding:
 - Region-growing and edge-detection algorithms: allows for adaptive thresholding, enhancing pore segmentation in fibrous and highly interconnected structures.^{52,77}
 - Gradient filters: improve thresholding accuracy by refining intensity distributions, minimising over-segmentation errors.^{78,79}
- Watershed-based segmentation: a region-based segmentation technique that treats an image as a topographic surface, where intensity variations correspond to elevations. It is useful for segmenting complex pore structures in porous materials by identifying watershed lines that separate adjacent regions. Following initial segmentation, the watershed algorithm refines pore boundaries by treating intensity gradients as a height map. By simulating the flooding of an image, it effectively separates adjacent pores that may have been grouped together in thresholding-based segmentation. While this method enhances segmentation accuracy, it may require post-processing to address over-segmentation.^{18,19,43,45,48,66,80} MATLAB-based implementations of the watershed algorithm further improve pore segmentation accuracy through gradient-based seed point refinement.⁴³
 - Gradient-based watershed: uses the gradient magnitude image to detect high-intensity ridges that define the segmented boundaries.²⁴
 - Marker-controlled watershed: introduces predefined seed points to prevent over-segmentation, which is beneficial for fibrous or highly interconnected porous materials.^{20,43,66}
 - 3D watershed for pore connectivity: applied in voxel-based 3D reconstructions to quantify pore interconnectivity in porous scaffolds.^{18,19,43,48,66,80,81}

✦ Machine learning-based pixel classification

- Shallow ML models: shallow learners struggle to classify raw image input and depend on efficient preprocessing and predefined, representative characteristics such as intensity and edges.²⁷
- Deep learning models: deep learning models have demonstrated enhanced performance in segmenting irregular and disordered pore architectures, particularly in hydrogels and scaffolds.^{10,62,63} CNNs and U-Net architectures learn hierarchical representations from large datasets, allowing robust segmentation of complex pore structures.^{5,20} These models are capable of automatic feature selection and extraction, enhancing contrast in low-resolution porous material and removing the need for manual tuning.⁶² Furthermore, advanced machine learning techniques improve segmentation across multiple imaging modalities, making them adaptable to various pore architectures.⁶¹ One problem with deep learning models is their data hungry nature, requiring large, annotated datasets for successful training and prevention of overfitting.
 - CNN-based approaches: the convolutional nature of CNN's enables multi-scale feature extraction, which improves segmentation accuracy, enhances pore boundary detection and reduces errors in low-contrast regions.⁶³ The hierarchical nature of CNNs refines pore morphology, effectively distinguishing pores from material phases. Pretrained CNN models, such as VGG16 and ResNet, further accelerate adaptation by reducing the need for large, manually annotated training sets, while transfer learning enhances model generalisability across different biomaterial types, ensuring consistent segmentation accuracy.^{61,62}
 - U-Net architecture approaches: provide pixel-wise segmentation, making them particularly effective for highly interconnected porous biomaterial. Their encoder-decoder structure allows for detailed feature extraction, while skip connections preserve fine-grained pore structures, significantly improving segmentation accuracy. Furthermore, data augmentation techniques enhance model robustness, compensating for limited training datasets and increasing adaptability across various biomaterial imaging conditions.^{5,61}



Software given in Table 2 include modalities for different segmentation techniques. It is important to note that many studies utilise in-house, tailor-made code to conduct segmentation, that best befits their requirements.

Both threshold and machine learning-based approaches offer unique advantages to image segmentation with neither demonstrating consistently better performance albeit machine learning offers faster computation time. The choice of segmentation technique directly influences measures of porosity and pore structures, and the careful validation of image-based pore data remains crucial.^{45,64,94}

3.3 Pore analysis: techniques for quantification

Once segmentation is complete, various pore analysis techniques are employed to quantify structural parameters such as pore size distribution, shape, and connectivity. Below, in Box 2, we examine some commonly used automated approaches of quantifying pore size. In addition, Table 3 provides an overview of some pore-analysis software packages and their respective capabilities with regard to pore size determination. While some software maybe more commonly used, others may be at an emerging level. The outcome of analysis may differ depending on software choice (Fig. 3).

Box 2: Pore analysis approaches

★ Basic metrics from segmentation

- Pore-to-material ratio: the fraction of pore area relative to material area, expressed as a decimal or percentage. This is calculated using tools such as ImageJ or Fiji, which is useful for assessing porosity, however, lacks structural context.^{24,64,67,77,95,96}
- Pore count: the total number of detected pores; limited when pores are irregular or overlapping. Suited for homogenous structures.^{5,21}
- Pore area distribution: captures variability in pore sizes; however, segmentation errors can skew results.^{6,21,45,59,67,77}

★ Advanced pore analysis techniques

Pixel-based approaches

- Exponential decay fit to pore pixel spacing distribution: quantifies the spatial arrangement of pores by fitting an exponential decay model to the pore-pixel spacing distribution.²⁷ This approach helps distinguish between homogeneous (evenly distributed pores) and heterogeneous (randomly distributed or clustered pores) porous networks.
- Fourier transform analysis: used to detect periodicity in pore spacing, Fourier analysis can reveal structural anisotropy within porous scaffolds and hydrogels.^{36,71,77,97}
- Skeletonisation-based analysis: by reducing pore structures to their one-dimensional (1D) medial axis, this approach allows for measuring pore branching, interconnectivity, tortuosity and transport pathways in porous biomaterials scaffold.^{19,38,56,98}

Geometric transform-based methods

- Maximum covering radius transform: the CRT method determines the largest inscribed circle within each pore, quantifying local pore size variations across a sample.^{46,55,66,99,100}
- Morphological opening and closing transformations: these operations refine pore boundaries by removing small artifacts and enhancing true pore structures, aiding in more accurate segmentation.^{1,16,18,19,47,54,65,66} For instance, connected pores can be separated using morphological dilation followed by erosion—dilation shrinks the pores by expanding scaffold boundaries, and erosion then restores pore size while maintaining separation.¹
- Voxel-based pore size distribution reconstruction: 3D voxel reconstruction has been used to quantify PSDs, for improved interconnectivity analysis.^{4,38,56,66,67}
- Fourier-based feature extraction for pore shape: fourier-based shape analysis has been integrated into voxel-based reconstructions to improve pore morphology quantification.⁵

Pore fitting methods

- Sphere/circle fitting: pore fitting methods are based on fitting a circle or a sphere to a pore, based on criteria such as major diameter (MD, major diameter of analysed pore), mean thickness (MT, based on circle-fitting algorithm similar to sphere-fitting method), biggest inner circle diameter (BICD, diameter of the biggest circle that fits the pore) and area-equivalent circle diameter (AECED, diameter of the circle with an equivalent area to that of the pore).^{12,45,46,48,65–67,97,99,101} Ideally well suited for circular pores.¹
- Bubble analysis: a geometric method that mimics fluid dynamics in fibrous networks and is based on the largest possible circle inside a pore that touches three surrounding fibers,^{16,17,46,53} however, according to Fischer *et al.* may not fully account for the residual fluid volume.¹⁶ In an article-response dynamic, Molteni *et al.* proposed an algorithm that randomly seeds a pore and expands its boundary until it tangentially contacts three fibers,¹⁷ for which Münster & Fabry developed a simplified approach using Euclidean distance mapping to detect local maxima, identifying the largest pore regions.¹⁰² However, Molteni *et al.* argued that this method lacks filtering and requires additional post-processing to remove overlapping pores and those that do not satisfy the three-fiber contact criterion.¹⁰³ Applicable to fibrous networks such as bundled F-actin, fibrin, cytoskeletal filament networks, given individual fibers can be resolved.⁵³
- Destroy and rebuild method for PSD analysis:

This micro-CT-based technique reconstructs 3D pore networks from 2D slice data, offering an alternative to direct segmentation-based methods. ImageJ's particle analyser (PA) function extracts mass centre coordinates (Xc, Yc) and pore section areas for each binarised slice. An algorithm groups sections by spatial proximity, assuming pores maintain a spherical shape. Edge-affected pores are removed, and equivalent volume and radius are calculated for each pore to determine the PSD.^{5,45,56} In addition, MATLAB-based PSD reconstruction methods have been applied to improve accuracy in volume estimations of irregularly shaped pores.⁷⁷

4. Summary, recommendations and future directions

Automated pore size analysis in biomaterials remains a field of active development, with several challenges and opportunities ahead. A persistent issue is the control of measurement error,

which can arise at multiple stages of the workflow, from image acquisition to segmentation and feature extraction, ultimately affecting data reliability.

Imaging quality—including resolution, magnification, and field of view—strongly influences the accuracy of segmentation and measurement methods. Poor image quality or insufficient



Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this perspective.

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