ND, which stands for nearest distances, is an ImageJ plugin that was developed to calculate the average size and distance between pores and their nearest neighbors in a porous scaffold by using images of the scaffold as input. The architecture of the porous scaffolds including pore size distribution and density dramatically affects their interaction with biological cells and mechanical properties of the scaffold [1–5]. Therefore, characterization of fabricated scaffolds using microscopy techniques and subsequent processing of the images is an essential step in correlating a specific cell-scaffold response with an effective scaffold design. Although ImageJ is taking center stage as a reliable open source and user-friendly tool for image processing in the biomedical field, image analysis is done either manually or by using propriety software associated with microscope companies or other commercial software whenever specialized plugins for ImageJ are not available. ND is an example of a specialized plugin that builds upon the built-in functionalities of ImageJ, and contributes to the collective efforts to enrich its library of available plugins and advances its versatility in image processing.

The main usage of the ND plugin is to analyze the wall thickness and average spacing of particles/pores/tubules located randomly in a sample. The plugin uses an image of the sample as input and outputs a result table listing the average distance of each particle from its neighbors, nearest neighbor distance, and the average wall thickness of its neighbors. The number of neighbors to be accounted for in calculating the averages is set by the user.

**Implementation/Architecture**

The plugin was written in Java, using the ImageJ built-in Java compiler.

**Installation**

To install the plugin, ImageJ must be installed first. ImageJ can be downloaded from http://rsbweb.nih.gov/ij. Variants like Fiji (http://fiji.sc/) may also be used. ND_.java file should be downloaded to the plugins folder of the ImageJ installation. The ND_.java file should be compiled for the first time using Plugins -> Compile and Run ....On restarting ImageJ, the plugin will appear under the menu Plugins. Alternatively, the already compiled file (ND_.class) can be downloaded and placed in the Plugin folder of ImageJ installation. Prior to running the ND plugin, an image should be opened first and the Results table must have records including centroid coordinates of the particles that need to be analyzed. (Centroid and Fit ellipse must be checked under Analyze -> Set Measurements). The user may run Analyze -> Analyze Particles built-in Plugin to generate a Results table needed by the ND Plugin. Messages will be displayed in case any of the above conditions are not met and users will be directed on how to proceed.

**Algorithm**

In a close packed configuration of particles/fibers having a circular cross section in 2D space there are 6 immediate neighbors surrounding each particle. In randomly packed scaffold systems, there are 12 immediate neighbors surrounding each particle. The ND plugin will analyze the wall thickness and average spacing of particles/pores/tubules located randomly in a sample. The plugin uses an image of the sample as input and outputs a result table listing the average distance of each particle from its neighbors, nearest neighbor distance, and the average wall thickness of its neighbors. The number of neighbors to be accounted for in calculating the averages is set by the user.
systems, coordination number depends on the visual perception and can be lower or higher. Estimation of particle spacing of a particle with its neighboring particles is performed as follows:

1. The centroid coordinates of each particle \((X,Y)\) is derived from the result table of the built-in Analyse Particles plugin.
2. A circle is fit on each particle with the center \((X,Y)\) and radius \(r\).
3. The spacing (wall thickness) between a pair of particles \((d)\) is calculated as:
   \[
   d = \sqrt{(Y_2 - Y_1)^2 + (X_2 - X_1)^2} - (r_1 + r_2)
   \]
4. The distances of each particle with all the other particles is stored in an array and sorted.
5. Depending on the coordination number of interest, average of the distances is calculated.
6. Results are shown in a new result table, which contain the distance of the closest neighbor to each particle, the average wall thickness, and the average distance of the closest neighbors.

**Quality Control**

Functional testing, load testing and end-to-end testing were carried out in Microsoft Windows 7-64 bit. The ND Plugin functions as expected and results were manually verified. The level of accuracy/precision of the software when compared to manual verification is solely dependent on the threshold settings and the parameters set in Analyze Particle built-in plugin before running the ND plugin. Therefore, the limiting factor in the accuracy/precision of the ND plugin is the accuracy/precision data generated in the results table. Therefore, with regard to values available in the results table, the ND plugin compared with manual calculation is completely accurate. One scenario that may lead to an inaccurate estimation of particle/pore distances might occur when particles/pores are too close to each other. In this situation a slight change in the values used for thresholding the image might lead to merging of two neighboring particles/pores.

**Example of Use**

*Measurement of tubule/fiber diameter and their spacing in tubular scaffolds or fiber reinforced composites*

The main usage of the ND plugin is to analyze the size and spacing of particles/pores/tubules located randomly in a sample. While the size of particles can be estimated using the built-in “Analyze Particle” plugin in ImageJ, the spacing between the features cannot be estimated. **Fig. 1** shows an example of a tubular scaffold used in tissue engineering, which was analyzed using ND plugin. ND provides this ability for the user to calculate the spacing between each particle/pore and its \(n\) closest neighbors with \(n\) being the coordination number of interest (**Fig. 2**).

Additionally, the histogram of the average distances of the closest neighbors provides a quantitative measure of how uniformly the feature of interest is spatially distributed. Initially, preliminary steps need to be taken for the plugin to do its function. These steps are as follows:

a) Open the image (File -> Open)

b) Threshold the image to make the feature of interest evident (Image -> Adjust -> Threshold)

c) Run the built-in Analyze Particles (Analyze -> Analyze Particles)

d) Run the ND plugin (Plugins -> ND)

e) The result table which outputs the average distance between tubules, the average wall thickness, and the distance of the closest neighbor distance for each tubule is shown in **Fig. 2**.
(2) Availability
Operating system
ImageJ runs and is used on different versions of Unix, Mac OS X, and Windows.

Programming Language
Java

Additional system requirements
None at runtime

Dependencies
At runtime, the plugin requires ImageJ to be running

List of contributors
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Archive
Code Repository Name
Github

Persistent Identifier
https://github.com/sedmorteza/ND/blob/master/ND_.java

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Morteza Haeri

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GNU General Public License version 3

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(3) Reuse Potential
Measurement of pore size distribution and fiber thickness in electro-spun fiber mats
Alternatively, in electro-spun fiber mats, ND can be used to estimate the average pore size as well as the average fiber size and distribution. In an electro-spun fiber mat, nano or micro sized fibers are stacked to form a mesh structure. The structure is highly porous with varying pore sizes. By using the procedure mentioned above to threshold images from electro-spun fiber mats and running the built-in Analyze Particle plugin, one can generate a result table containing the radii of pores. Running the

Fig. 2: ND plugin running. Input window to take the coordination number of interest to the user (top) and the generated result table listing the average distance of each particle from its neighbors, nearest neighbor distance, and the average wall thickness of its neighbors (bottom).
ND plugin subsequently will estimate the wall thickness between pores, which in this case will be the thickness of fibers forming a pore.

**Detection of co-localization**
A common measure used in biology is object-based co-localization (OBC). In OBC, the nearest neighbor distances (NND) from one point pattern to the other is plotted as a histogram and then a threshold distance is set (typically, the ‘diffraction-limited resolution’ of ~200 nm), so that all points with a NND less than the threshold is considered co-localized. ND plugin can be modified to provide this functionality. While the current code calculates the distances of all the neighboring particles from each particle, the ND plugin only uses the nearest neighbors up to maximum of 6, which corresponds to a close packed configuration into the subsequent averaging and display windows. However, the data required for OBC is stored in the matrix named “dist” in the code and can be used accordingly for other applications such as OBC.

**References**
1. Scott JB, Afshari M, Kotek R, Saul JM 2011 The promotion of axon extension in vitro using polymer-templated fibrin scaffolds. Biomaterials, 32(21): 4830–4839. DOI: http://dx.doi.org/10.1016/j.biomaterials.2011.03.037.
2. Martínez-Ramos C, Vallés-Lluch A, Verdugo JM, Ribelles JL, Barcia Albacar JA, Orts AB, Soria López JM, Pradas MM 2012 Channeled scaffolds implanted in adult rat brain. Journal of Biomedical Materials Research A, 100(12): 3276–3286. DOI: http://dx.doi.org/10.1002/jbm.a.34273.
3. Oh S, Bramer KS, Li YS, Teng D, Engler AJ, Chien S, Jin S 2009 Stem cell fate dictated solely by altered nanotube dimension. Proceedings of National Academy of Sciences, 106(7): 2130–2135. DOI: http://dx.doi.org/10.1073/pnas.0813200106.
4. Karageorgiou V, Kaplan D 2005 Porosity of 3D bio-material scaffolds and osteogenesis. Biomaterials, 26(27): 5474–91. DOI: http://dx.doi.org/10.1016/j.biomaterials.2005.02.002.
5. Peng-Yuan W, Lauren RC, Helmut T, Wei-Bor T, Nicolas HV 2013 High-throughput characterisation of osteogenic differentiation of human mesenchymal stem cells using pore size gradients on porous alumina. Biomaterials Science, 1(9): 924–932. DOI: http://dx.doi.org/10.1039/C3BM60026B

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