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A Biomedical Application Software for Measuring Particles Sizes in Electro-Microscopic Images

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Abstract. **Nowadays, technological development in the field of microscopy provides an opportunity for obtaining medical images with high resolution. Therefore, more advanced image processing and analysis techniques are required for processing these images. A wide range of programs are available for scientific research. However, the key is to find an open-source application that provides more precise quantitative measurements. In scientific research, open-source software proves valuable due to its ease of modification and redistribution capabilities. The software development process continues to attract the interest of researchers in all fields, particularly in computer science field in order to implement new image processing algorithms. In this work, a new biomedical application software is presented. In this software, image processing techniques were used to separate particles from the background of microscope images. Furthermore, the software gives the researcher the ability to choose and measure nanoparticle elements size in the image. Finally, the proposed software could become a promising tool in the laboratory research compared to other used software.**

Keywords: **microscopy, Java software, image processing, nanoparticles, SEM.**

Introduction

The introduction of digital-imaging tools in the world of science, revealed the need to use image processing techniques to analyze image data (such as biomedical image data). The goal is to use numerical methods to improve quantitative results, and accelerate repetitive routines because the numerical findings are statistically more convincing than qualitative observations. Nowadays, there is a great interest in the image processing field. Therefore, numerous specialized techniques have been developed specifically for application in biomedical images [1]. The processing of biomedical images is a branch of computer vision science. As a result, the field of computer vision encounters various challenges, including but not limited to low-light conditions and optical properties that need to be addressed. The development in biomedical image processing methods made it possible to confirm the research results by measuring them in a strict statistical way to quantitatively support their studies.

Several studies were applied to the area of biomedical image processing [2, 3]. Furthermore, there are also many books that deal with issues in image processing [4], digital microscopy and digital imaging in optical microscopy [5]. Biomedical image processing tools became one of the most popular instruments for the applications of image processing algorithms [7–9].

In this study, a new application software was introduced in order to compute the size of nanoparticles by processing microscopic images. Furthermore, the proposed software provides the ability to freely choose an area of interest to be measured. This paper is organized as follows: section 2 includes the materials and theory of the proposed software. The execution and Results are provided in Section 3. Finally, conclusion is presented in section 4.

Materials and method

SEM Imagery

Scanning electron microscopy (SEM) is an optical-based system for generating high resolution images [10], which could be used to collect information about the surface's features and structures. When an area of interest is illuminated by the imager (with a high energy beam of electrons), the electrons interact with the sample. Therefore, secondary electrons, backscattered electrons and characteristic X-rays are produced. SEM creates images that could be magnified between 10 and 1 000 000 times [11]. This imager is considered as a key tool for scientific research. It is used for monitoring metals, alloys and ceramics, as well as polymers and biological materials [12]. Figure 1 presents the SEM schematic diagram.

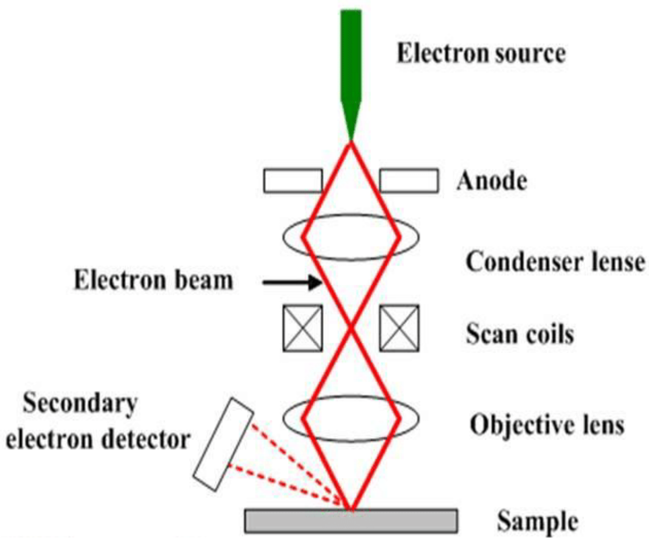

Fig. 1. The schematic diagram of scanning electron microscopy

ImageJ

ImageJ is a software that was developed using java language. The initial release of this software was made available through a collaboration between the National Institutes of Health and the Laboratory for Optical and Computational Instrumentation (LOCI, University of Wisconsin) [13]. ImageJ has been designed with an open architecture, allowing for extensibility through Java plugins and recordable macros. The software facilitates development through its built-in editor and Java compiler. Hence, users have the capability to customize the ImageJ software to tackle diverse image processing and analysis challenges. This includes applications like three-dimensional live-cell imaging, radiological image processing, and automated haematology, among others. Therefore, these features of ImageJ made it a popular tool for studding image processing applications.

Particle

Particles refer to small, localized objects that possess certain physical or chemical attributes, including shape, volume, or density. These particles exhibit a range of sizes and quantities, spanning from subatomic particles like electrons, to microscopic particles such as atoms and molecules, to macroscopic particles like powders and granular materials. Figure 2 shows an example of SEM images of nanoparticles. Particles serve as building blocks for scientific models that represent larger objects based on their density. These models can range from studying human movement in a crowd to examining the motion of celestial bodies [14]. The term «particle» has a broad definition that varies across scientific disciplines, encompassing any entity composed of particles, thus referred to as particulate.

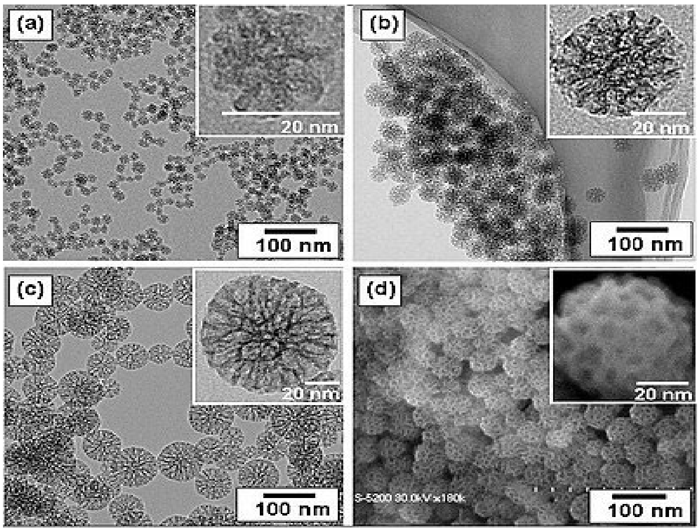

Fig. 2. An example of SEM images of nanoparticles

THE EXECUTION AND RESULTS

The block diagram of the proposed application software is shown in Figure 3.

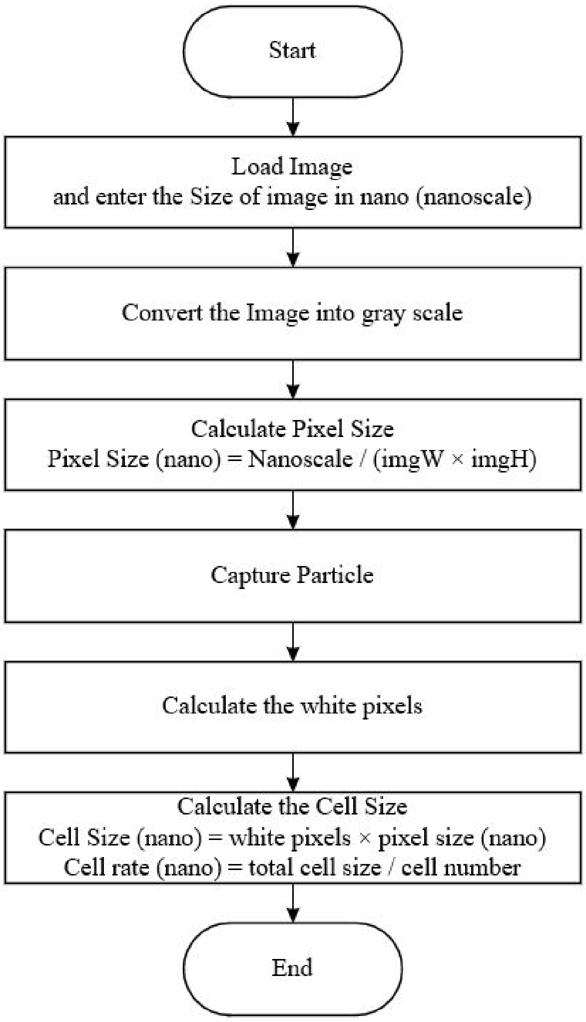

Fig. 3. Flowchart of the proposed application software

The user interface consists of five main action buttons that are aligned in the left side of the interface as shown in Figure 4.

Fig. 4. User interface

First step, the microscope image is loaded using the load image button, where a popup window will appear to help search and load the desired file. If the input image is colored, the program provides a function for converting the colored image to a gray-scale image because it is more efficient to determine the edges of the particles in the image. Once the desired image is loaded, the user can convert the image to a gray-scale image by using the convert button as shown in Figure 5. Weighted method was used in order to convert the colored image to gray-scale as shown in the code below:

```
for (int i = 0; i < img. get Height (); i++) {
for (int j = 0; j < img. get Width (); j++) {
Color c = new Color (result.getRGB(j, i));
int red = (int) (c.getRed() * 0.299);
int green = (int) (c.getGreen() * 0.587);
int blue = (int) (c.getBlue() * 0.114);
int graycolor=red + green + blue;
Color newColor = new Color (graycolor, graycolor, graycolor);
img.setRGB(j, i, newColor.getRGB());
```

Our proposed software provides an option to freely highlight an area of particles to be examined as shown in Figure 6.

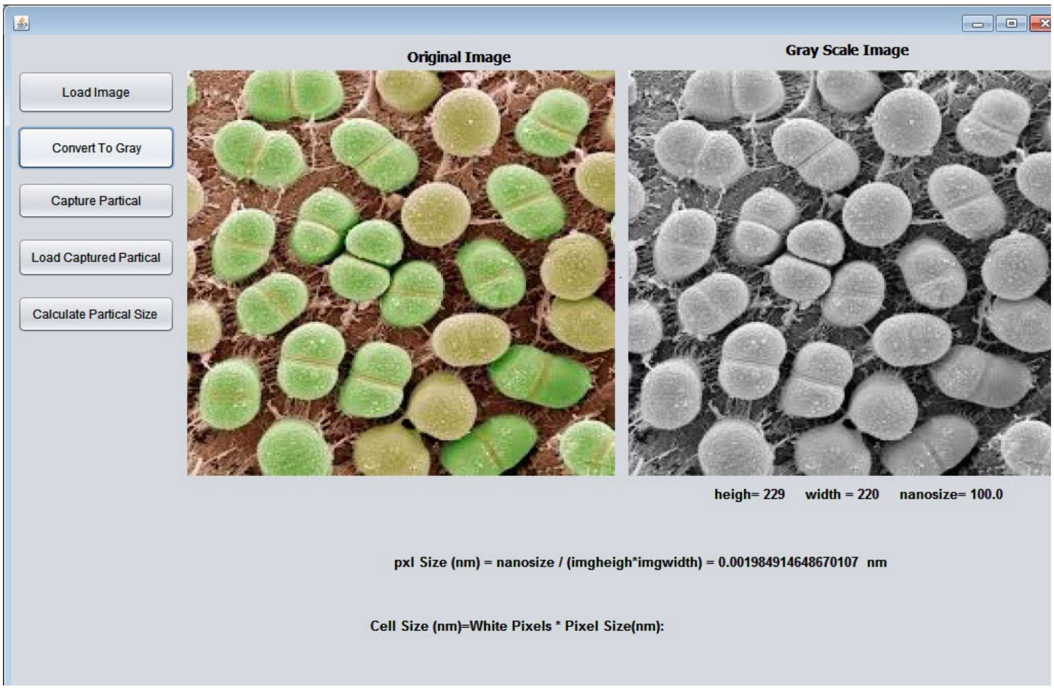

Fig. 5. Converting image to gray-scale image

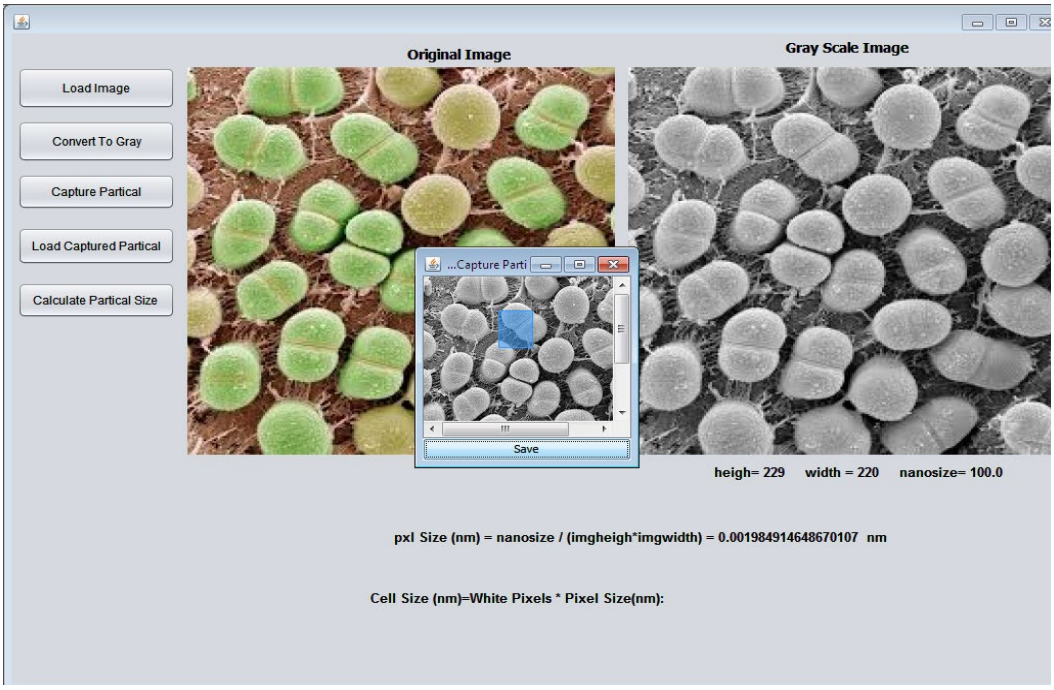

Fig. 6. Choosing an area of particles

After capturing the particle, the white pixels are calculated, and measures are given in nanometers as shown in Figure 7. Furthermore, Table 1 presents the results of 10 measures of different areas in the same image. The results show that our proposed software provides high accuracy results.

The system performs the following steps in order to calculate the nanoparticles size of microscopy images:

1. Calculate the pixel size in nanometers as follows:

If X = No. of pixels in Y (Y is nanometers in scale bar), then size of 1 pixel (in nanometers): Z = Y / X.

2. Calculate the black/white pixels in the particle under investigation.

3. Find the particle's size as follows:

Particle's size (nano) = pixel's size Z×N (No. of pixels).

4. Find the Average particle's size in the image as follow:

Average particle's size (nano) = total No. of pixels in all measured particles / No. of particles measured.

Table 1

Measures of 10 different areas in the same image

Number of area	Particle size, nm
1	0,0132
2	0,0131
3	0,0140
4	0,0131
5	0,0133
6	0,0134
7	0,0132
8	0,0133
9	0,0135
10	0,0142

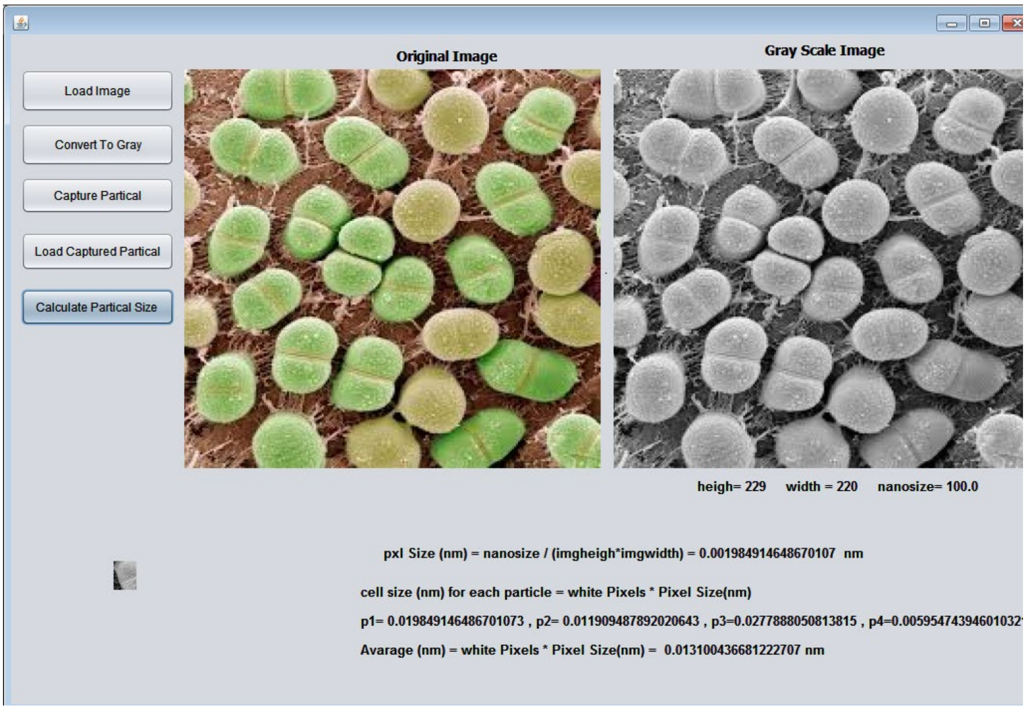

Fig. 7. Measuring nanoparticles size

Furthermore, it is worth mentioning that a pre-processing step was applied to the gray-scale image to remove any noise. Therefore, a non-linear filter (median filter) was used to improve the results of image processing. In fact, the median filter is an appropriate filter for such situations because it preserves edges and removes noise from the image.

Conclusions

From what has been discussed above, the proposed software provides more freedom in choosing the particles to be examined, collecting sizes of many selected particles from one image or many different images. Furthermore, the program is very simple and easy to use where any researcher can use it with ease and without necessarily having a computer specialist nearby to do the job. The user can examine and calculate as many particles as needed and is not limited to a fixed number of particles as in other available software.

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Биомедицинское прикладное программное обеспечение для измерения размеров частиц на электронных микроскопических изображениях

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Аннотация. **В настоящее время технологическое развитие в области микроскопии предоставляет возможность получения медицинских изображений с высоким разрешением. Поэтому для обработки этих изображений требуются более совершенные методы обработки и анализа изображений. Для проведения научных исследований имеется широкий спектр программ. Однако ключом является поиск приложения с открытым исходным кодом, которое обеспечивает более точные количественные измерения. В научных исследованиях программное обеспечение с открытым исходным кодом оказывается ценным благодаря простоте его модификации и возможностей перераспределения. Процесс разработки программного обеспечения продолжает привлекать внимание исследователей во всех областях, особенно в области информатики, в целях реализации новых алгоритмов обработки изображений. В работе представлено новое прикладное программное обеспечение для биомедицинских исследований, в котором методы обработки изображений использовались для отделения частиц от фона изображений микроскопа. Кроме того, программное обеспечение дает исследователю возможность выбирать и измерять размер элементов наночастиц в изображении. И наконец, предлагаемое программное обеспечение может стать перспективным инструментом лабораторных исследований по сравнению с другим используемым программным обеспечением.**

Ключевые слова: **микроскопия, программное обеспечение Java, обработка изображений, наночастицы, SEM.**

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