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ImageJ (Fiji) as a free, useful tool for medical researchers and students; A review article

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Abstract: Digital image processing is increasingly being used in a variety of industries, including food processing, medical science, particle technology, cement, and powder manufacturing. Medical image processing is a discipline in medical science that involves the use of technology to take images of the human body's interior in the least invasive way possible. As medical and biological sciences advance, imaging has become a more important discipline. One of the most useful programs is ImageJ, a public-domain Java image processing program inspired by NIH Image for the Macintosh. In this study, we demonstrated some, but not all, applications of ImageJ in the medical field and for medical and biological students that can be easily implemented in their institutions. One of these applications was bacterial cell counting, in which a microscopic image of gram-stained bacterial cells was captured using a student's smartphone, treated with ImageJ, and the bacterial cells were easily counted automatically using ImageJ. The second application of ImageJ in this review was to calculate the antimicrobial zone of inhibition. We calculated the percentage of the inhibition zone for three different amoxicillin antibiotic brands using very simple steps. The third application of ImageJ was to analyze a CT scan brain images, and we were able to define the hemorrhage location. Finally, we demonstrated that this free software can estimate protein-protein colocalization. This technique is useful in many cell biological and physiological studies to demonstrate the relationship between pairs of biomolecules. In another example of co-localization, the researchers confirmed the SyGCaM2-mCherry sensor's presynaptic

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localization to hippocampal synapses, where it was co-localized with a bassoon (a presynaptic protein) in the *stratum radiatum* of area CA1. In general, ImageJ is a very useful, free program that can be used easily by specialized people and the beginner medical students.

Keywords: ImageJ, Fiji, Digital image processing, bacterial cell counting, protein-protein colocalization.

Introduction

The field of medical image processing uses technology to take images of the human body with the least amount of invasiveness possible. Consequently, imaging has become an important field of research as a result of developments in the biological and medical sciences. Although there are many image processing programs available, most of them lack flexibility and do not support complex image editing. ImageJ is one of the programs that could make this field easier and more engaging. Wayne Rasband, who retired from the National Institute of Mental Health's Research Services Branch in 2010, created the widely used public domain Java image processing and analysis tool ImageJ at the National Institutes of Health and the Laboratory for Optical and Computational Instrumentation (LOCI, University of Wisconsin). Rasband is continues to develop the software[1, 2]. ImageJ was designed with an open architecture that provides extensibility via Java plugins and recordable macros [3]. Users are completely free to run, copy, distribute, research, alter, and enhance the software because its source code is publicly available. To put it simply, it enables users to group imaging operations into macros, which are text files that are simple to create, modify, and debug [4]. A Java compiler and the built-in editor of ImageJ can be used to create custom acquisition, analysis, and processing plugins [5]. Numerous image processing and analysis issues can be resolved with user-written plugins, ranging from radiological image processing[6], to three-dimensional live-cell imaging [7], multiple imaging system data comparisons [8] and for automated hematology systems [9]. ImageJ is a well-liked platform for teaching image processing because of its plugin architecture and integrated development environment [4].

As imagej is constantly updated, ImageJ2 has come as a result, where ImageJ2 is a complete rewrite of ImageJ tailored for multidimensional image data, focusing on the field of scientific imaging. Its primary objective is to extend the capabilities of ImageJ beyond the constraints of the original application, enabling support for the

upcoming generation of multidimensional scientific imaging. To maintain backwards compatibility, ImageJ2 has been constructed to integrate seamlessly with the current ImageJ user interface. This enables users to continue utilizing ImageJ in their usual manner while also gaining the option to transition to more potent new features as necessary [10].

Later, the Fiji project has been developed on the foundation of ImageJ2 for a considerable time, so you might already be acquainted with some of the functionalities of ImageJ2-some features, like the Updater and Launcher, were initially created as part of Fiji [11]. It is an open-source image processing package, was developed by Johannes Schindelin and others. It is an update of ImageJ, featuring an integrated updating system [11] . The Stable release was released on March 7, 2011 (official release, plugins are continuously updated). Fiji's main purpose is to provide an ImageJ distribution with a large number of bundled plugins. Fiji includes an integrated updating system and aims to provide users with a consistent menu structure, extensive documentation in the form of detailed algorithm descriptions and tutorials, and the ability to avoid having to install multiple components from different sources. There are numerous ImageJ plugins with a wide range of applications and quality. [12]. As a result, Fiji attracts an increasing number of active users. While Fiji was originally designed for neuroscientists (and continues to be so) [13], it accumulated enough functionality to attract scientists from a variety of fields, such as parasitology,[14] cell biology, [15] genetics, life sciences in general, material science, etc. Fiji is most popular in the life sciences community, where the 3D Viewer [16] helps visualizing data obtained through light microscopy, and for which Fiji provides segmentation, registration, and other advanced image processing algorithms [11, 17, 18]. ImageJ is still being developed on a daily basis, and its users are growing. The most recent update was (2.9.0 / September 14, 2022) [19].

Some examples of ImageJ applications

In this part, we will show some but not all applications of ImageJ in medical field that can be very useful for medical and biological researchers and students.

Bacterial cells counting

ImageJ program was applied to an images that obtained from gram stained bacterial smear. The manual counting of gram stained bacteria examined under a microscope

becomes very difficult especially, when a large number of particles exist in a microscopic field. The small size of these organisms (0.5–2 μm) usually makes manual counting difficult as numbers of organisms increase. Here we have applied ImageJ to counting of gram stained bacteria[20]. Gram stained bacteria images were captured by a smartphone (Samsung X7) and then transferred to the pc and treated using ImageJ. The method for enumeration of gram stain using ImageJ required the image file to be converted from RGB color to 8-bit grayscale. Automated counting of the bacterial particles uses threshold algorithms to discriminate the features of interest from background. To set the counting threshold following opening the selected image, the following commands (Image → Adjust → Threshold → select algorithm to be applied → Apply were used and the image converted to a binary image by selecting Process → Binary → Make binary. bacterial particles were counted using the commands Analyze → Analyze Particles, with the upper and lower limits for the particle size set at 0–infinity, selected to 'Show outlines' and checked box to 'Summarize'. Each counted particle was outlined and numbered in a new window[21].

Figure 1 showed a microscopic image of the bacterial cells captured using a student's smartphone where the collected image was treated using ImageJ and bacterial cells was counted using ImageJ. The total number of bacterial particles was 332 bacterial cells. This method is less expensive and less laborious than other methods and is more rapid and reproducible than counting using manual microscopy methods. Therefore we suggest the application of the ImageJ program as an alternative method to manual quantification of bacterial cells [21].

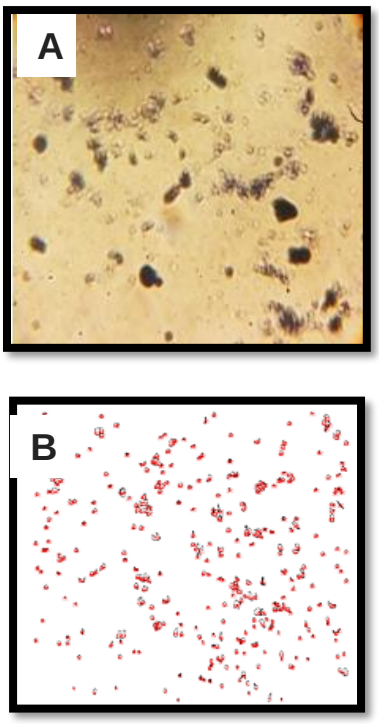
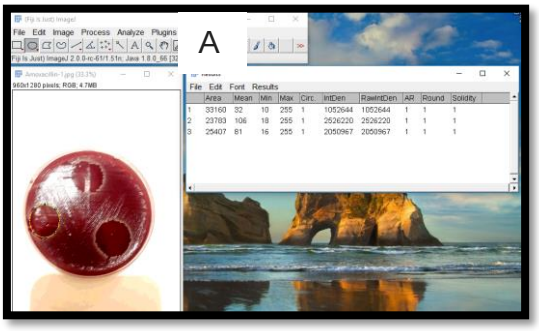


Figure 1. Bacterial cell counting using ImageJ. A. Microscopic image of the bacterial cells captured using a student's smartphone. B. Image in A is treated using ImageJ and bacterial cells was counted using ImageJ. The image sourced from [21].

Calculation of the antimicrobial zone of inhibition using imageJ

ImageJ can be used to determine the antimicrobial zone of inhibition. The disk-diffusion agar method tests, also known as the Kirby-Bauer test, determine the antibiotic sensitivity of bacteria. Antibiotic discs are used to determine the extent to which antibiotics affect bacteria. In this test, antibiotic-containing discs are placed on an agar plate with bacteria and allowed to incubate. If an antibiotic prevents or kills the bacteria, there will be an area around the wafer where the bacteria have not grown sufficiently to be visible. [22]. This is called a zone of inhibition.

The method in brief, an agar plate is first spread with bacteria, then a drop (0.2 ml) of antibiotics are added. The bacteria is allowed to grow on the agar media, and then observed. The amount of space around each antibiotic plate indicates how lethal the antibiotic is to the bacteria in question. Highly effective antibiotics will result in a wide ring of no bacterial growth, whereas ineffective antibiotics will cause no change in the surrounding bacterial concentration at all. Intermediate antibiotics' effectiveness can be assessed using their zone of inhibition. This method is used to identify the most effective antibiotic to use against a new or drug-resistant pathogen. In here, we have compared the efficacy of amoxicillin syrup (250 mg) manufactured at three different companies from three different countries (U.K, Egypt and Tunisia). The zones of inhibition of the three antibiotics were captured using a smartphone and the images were treated using the following command: (ImageJ → Oval tool → Analyze → Measure → repeat the same process for each inhibition zone) and then the collected data were transferred to an excel file and the bar charts was generated as seen in figures 2. The bar chat in figure shows the percentage of the inhibition zone of the three-different amoxicillin antibiotic, calculated using ImageJ.



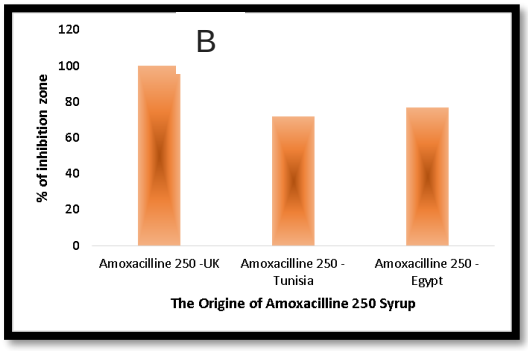


Figure 2. A-Using ImageJ to calculate the zones of inhibition by the three-different brands of amoxicillin antibiotic. B-Bar chart shows the percentage of the inhibition zone of the three-different brands of amoxicillin antibiotic, calculated using ImageJ (the experiment is repeated only one time just for the demonstration).

Analyzing CT scan images using ImageJ

Image-processing software for radiology applications is in high demand, thanks to advances in both image-acquisition and image-analysis techniques. The utility of existing image-processing software is frequently limited by its cost, lack of flexibility, and/or specific hardware requirements. Many existing packages, in particular, cannot directly use images formatted in accordance with the DICOM (Digital Imaging and Communication in Medicine) standard specifications. The ability to read DICOM images, combined with the recent addition of a macro language for ImageJ, has made it possible to develop low-cost image-processing applications on this platform[(6)]. An example of imaging techniques that can generate DICOM images is Diagnostic computed tomography (CT). In brief, CT is a relatively simple, non-invasive technique used for diagnostic and surveillance purposes. CT imaging scans the body like slices of bread, producing cross-sectional images or "slices." [(23, 24)].

In this part, we have tried to analyze DICOM images collected from a patient suffering from cerebral hemorrhage, using diagnostic computed tomography (CT) technique (Alexion, Canon Medical Systems Corporation's. Japan) located in radiology department in Msallata central hospital (Msallata City; Libya) (figure 3).

Figure 3. Alexion, a multi-slice CT scanner from TOSHIBA, located in radiology department of Msallata central hospital.

The collected images were transferred using USB storage device to a PC were the ImageJ software is installed. The target images were opened and treated using the following command (ImageJ → image → lookup tables → fire); this command used to show the extent

and the distribution of the hemorrhage where the yellow color is.

Figure 4 showed that the hemorrhage is located in the parietal lobe of the cerebral cortex. In addition to this act we could calculate the hemorrhage size using a free hand tool and analyze tool, we could also generate a 3D image for this hemorrhage.

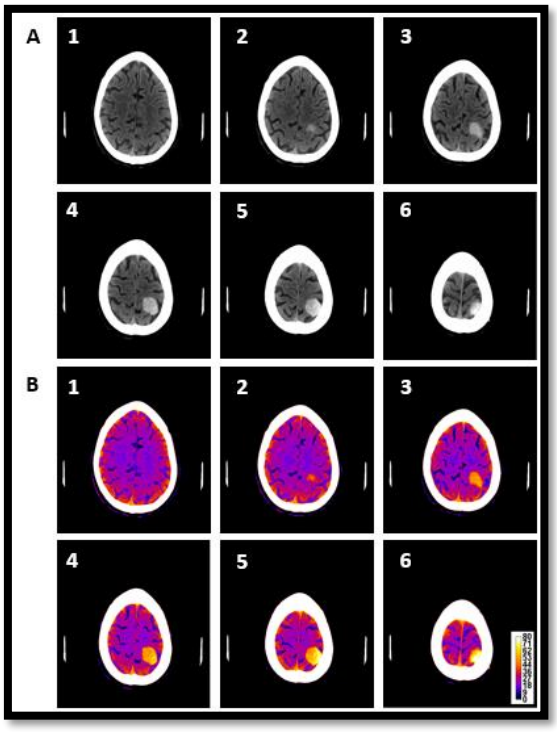


Figure .خطأ! لا يوجد نص من النمط المعين في المستند4. CT scan brain images treated with ImageJ software, collected from a patient suffering from cerebral haemorrhage; yellow coloured areas show the extent of the cerebral haemorrhage. A. images from 1A to 6A show row images collected from CT scan device, these images show the extent and the location of the cerebral hemorrhage. B. images from 1B to 6 B show the same images in A treated using ImageJ software.

Using ImageJ to measure protein Co-localization

In fluorescence microscopy, co-localization is the observation of the spatial overlap between two (or more) different fluorescent labels, each with a different emission wavelength, to determine whether the different "targets" are located in the same area of the cell or very close to one another. This technique is important in many cell biological and physiological studies when demonstrating the relationship between pairs of biomolecules[25]. Another key advantage of ImageJ is its abilities in co-localization analysis. Given that no single co-localization quantification technique is appropriate for all circumstances, ImageJ's large suite of co-localization plugins provides options for this additional functionality. Since users are not restricted to a specific approach, the most appropriate co-localization technique from the toolbox can be chosen. Among others, plugins are available to perform qualitative overlays [and convert them from Red-Green-Blue (RGB) to the color-blind-friendly Magenta-Green-Blue], generate Pearson's coefficients, generate Mander's coefficients. However, as with all analysis tools, they can be misused, and researchers are encouraged to understand these analyses before selecting and using one. The open-source nature of ImageJ also allows the development of novel co-localization routines. Intensity correlation analysis by Li

and colleagues was refined only after implementation in ImageJ enabled an improved rate of analysis [1, 26] . In this part we will mention only one example of the application of ImageJ in immunohistochemistry and co-localization studies which is Fluorescent Cells.

From imaging library of ImageJ we can open an image called Fluorescent Cells as shown in figure 5-A. This image is a bovine pulmonary artery endothelial cells stained using immunohistochemistry technique where the Blue color represents the nucleus stained with DAPI, Green color is for Tubulin stained with Bodipy FL goat anti-mouse IgG and the Red colour is for F-Actin stained with Texas Red X-Phalloidin. Figure 5-B shows that ImageJ opens this image as a 3 different channel, 8-bit composite image, this image can be split into 3 different images; red green and blue. This image shows the location of each individual protein in the cell where the Blue color is for nucleus, the green color is for Tubulin and the Red color is for F-Actin, as we mentioned befor.

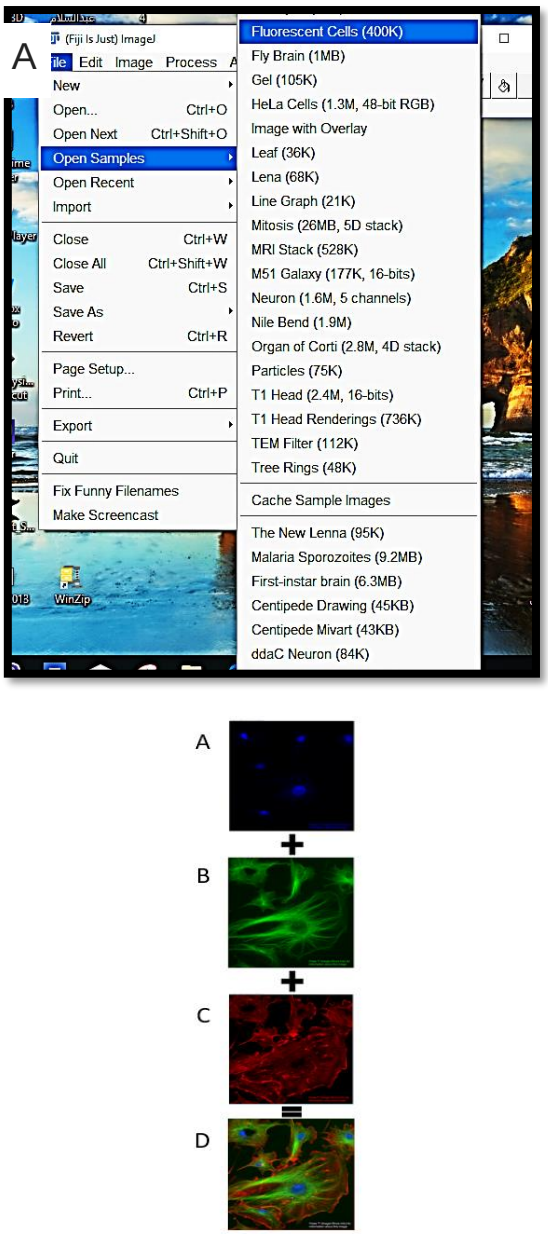


Figure 5.A-a screen shot shows the process of opening sample image (Fluorescent Cells) from the imaging library of ImageJ. B-Fluorescent Cell From imaging library of ImageJ where split into 3 different color channels. The cell is a bovine pulmonary artery endothelial cell; 1. shows nucleus stained with DAPI (blue). 2. shows Tubulin stained with Bodipy FL goat anti-mouse IgG (green). 3. shows F-Actin stained with Texas Red X-Phalloidin (red). 4. shows a merged image of the three different cell components. The image sourced from (http://imagej.nih.gov/ij/images/FluorescentCells.zip)

To gain a better understanding of this topic, see the study Immunohistochemical Localization of SyGCaMP2-mCherry (a presynaptic calcium sensor) to Presynaptic Terminals. Professor Nick Hartell and his colleagues tackled the challenge by developing a special strain of mice that expresses a calcium-sensing fluorescent protein within the pre-synaptic terminals of their hippocampus [27-29]. To confirm that SyGCaMP2-mCherry was expressed in presynaptic boutons, a series of double labelling experiments were performed that combined anti-mCherry and anti-bassoon labeling. ImageJ lets you highlight the co-localized points in two 8-bit images (or stacks). The two images (or stacks) will be affected by an RGB image's two Red and Green channels. Co-localized points are displayed in white by default. The plugin first creates an 8-bit image containing only the colocalized points (image available by validating Colocalized points 8-bit), and then combines the three 8-bit images to produce an RGB image. Two points are considered colocalized if their respective intensities are strictly greater than the thresholds of their channels (which are 50 by default: Threshold channel 1 (0-255). and if their ratio (of intensity) is strictly higher than the ratio setting value (which is 50% by defect: ratio (0-100%).

(https://imagej.nih.gov/ij/plugins/colocalization.html)

Conclusion

In medical science field, medical image processing is a discipline that involves the use of technology to take images of the inside of the human being in a way that is as non-invasive as possible .Hence with the advancements in medical and biological sciences, imaging has become an increasingly important discipline. One of the very useful softwares is ImageJ which is a public domain Java image processing program inspired by NIH Image for the Macintosh. It can display, edit, analyze, process, save and print 8-bit, 16-bit and 32-bit images. It can calculate area and pixel value statistics of user-defined selections. It can measure distances and

angles. It can create density histograms and line profile plots. It supports standard image processing functions such as contrast manipulation, sharpening, smoothing, edge detection and median filtering. It does geometric transformations such as scaling, rotation and flips. Image can be zoomed up to 32:1 and down to 1:32. All analysis and processing functions are available at any magnification factor.

The program supports any number of windows (images) simultaneously, limited only by available memory. Spatial calibration is available to provide real world dimensional measurements in units such as millimeters. ImageJ was designed with an open architecture that provides extensibility via Java plugins. Custom acquisition, analysis and processing plugins can be developed using ImageJ's built in editor and Java compiler. User-written plugins make it possible to solve almost any image processing or analysis problem. (https://imagej.nih.gov/ij/docs/intro.html).

In this paper, we have shown some but not all applications of ImageJ in medical field and for medical and biological students that can be easily applied in their institutes. One of those applications was a bacterial cells counting where a microscopic image of the gram stained bacterial cells captured using a student's smartphone, treated using ImageJ and the bacterial cells was easily automatically counted using ImageJ. This software-based methodology is cheaper, more standardized and better reproducible than a manual-based microscopy methods. Therefore, we suggest the application of the ImageJ program as an alternative method to manual quantification of bacterial cells. The second application of ImageJ in this research was the calculation of the antimicrobial zone of inhibition. By very simple steps, we were able to calculate the percentage of the inhibition zone of the three-different brands of amoxicillin antibiotic. The amoxicillin manufactured at the UK had a larger zone of inhibition than the others did which means that it is more effective than the others are. The third application of ImageJ was to analyze a CT scan brain images, where we were able to define the hemorrhage location; in addition to ImageJ ability to calculate hemorrhage size and its ability to generate 3D images of the same hemorrhage. Finally, we have demonstrated the ability of this free software to estimate protein-protein Co-localization. This technique is important to many cell biological and physiological studies during the demonstration of a relationship between pairs of bio-molecules [25]. In the second example of co-localization; the researchers

confirmed a presynaptic localization of the SyGCaM2-mCherry sensor to hippocampal synapses, where, the sensor was co-localized to a bassoon (a presynaptic protein) in the *stratum radiatum* of area CA1. In general, ImageJ is a very useful, free program that can be used easily by specialized people and the beginner medical students.

It is worth noting that software applications used for diagnostic purposes in a clinical setting are considered medical devices and are generally subject to regulatory approval from authorities such as the Food and Drug Administration (in the United States), requiring rigorous verification and validation procedures [6].

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