Institute of Biocybernetics & Biomedical Engineering Polish Academy of Sciences

The MeMoExplorer™

An Advanced Membrane Morphology Software release 18.11.30.1

WARNING THE DOCUMENT IS NOT UP TO DATE THE WORK IS IN PROGRESS WILL BE COMPLETED SOON

User Guide

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About the program

MeMoExplorer software is designed to analyze microscopic images of different types of membranes.

Its main task is the full automation of the membrane image processing process and the precise morphometric measurements of pores present in the membranes for objective estimation of the size distribution and data preparation for any statistical analysis that can be performed using external tools (e.g. Excel). Computer analysis of the membranes - and more specifically, their images obtained from the electron microscope - is intended to evaluate the morphological parameters of the porous microstructure and the reproducibility of obtaining these parameters in a complex membrane production process.

Particular attention when designing the application we turned to the ease and intuitive operation of the program for the end user (technician, biochemist).

MeMoExplorer is an original work from start to finish. It is a result of the cooperation of several of our laboratories. It is dedicated for the Microsoft Windows 10 operating system. The lowest level of software uses one of the best (and fastest) low-level image processing tool - *Intel Open Computer Vision Library* (OpenCV). It is written in the most advanced and effective programming C/C++ language, using two development environments: Microsoft Visual Studio (Image Processing) and Embarcadero (Borland) RAD Studio (Graphical User Interface).

Program installation and starting

Using *MeMoExplorer* requires that you install it on your computer. The application installer is available as a file called *instMeMoExplorer.exe*, which you can get by contacting the appropriate IBIB PAN (ibib.waw.pl) laboratory authorized to distribute the program, or faster and easier, by downloading it directly from the internet site *memo.somee.com* (temporary server for testing, then it will be probably one of the IBBE PAS servers).

If you already have this file, just run it and follow the on-screen instructions. During the installation, accept proposed (default) settings.

When the installation procedure is complete, look for the *MeMoExplorer* icon on the screen. This will be a shortcut to run the installed application, now and in the future. Remember or change the location of the icon according to your preferences.



Double click the icon to start *MeMoExplorer*.

The MeMoExplorerTM An Advanced Membrane Morphology Software

Laboratory of Fundamentals of Computer-Aided Image Diagnostics Laboratory of Semipermeable Membranes and Bioreactors

Institute of Biocybernetics and Biomedical Engineering Polish Academy of Sciences

Click here or go to Help menu for a User Guide

Pay attention to the possibility of displaying the program manual now, which may be especially important for an inexperienced operator who runs the program for the first time. See line *Click here or go to Help menu ...*

Every time you start the program it is very important to check whether the server in the network contains update of the software. For this click *Try update* button.



Two situations are possible now. First, there is no software update on the server. Then you will see a message as shown in the image below.



And the second situation - update is available. In this case, the newer files will automatically be downloaded from the server. You will see the *Downloading* ... message and the progress indicator on the *Try update* button.



When downloading of all the necessary items is finished, you will be asked for final approval for the update. If you agree click *Finalize* ...



Working panel - Work

Switch to the *Work* tabsheet.



You are now in the application main working panel. Most of the work with the program is done right here. On the left, the analyzed images are displayed. On the right there is a task control console.



The functions of the first three keys are exactly like their names indicate and do not require clarification. *Single Run* (after loading the image with *Load*) starts the analysis of a single image. The one that has been loaded and is currently being displayed. *Batch Run* starts automatic analysis of all images in the current directory.

The directory from which the current image was loaded. So, to execute **Batch Run** first load any image file from selected directory.

Also, notice the panel containing some pattern images - *Predefined Reference Patterns*.



These are arbitrarily chosen patterns of different types of membranes. One of the elements of any image analysis is its classification into a group represented by a pattern whose characteristics are closest to the case in question. The term "closest" means the similarity of histograms describing the distribution of pore sizes.

Single image analysis – Single Run

Load an image. Let it be the *Sample.tif* file from the *C:\MeMoExplorer* root directory (as in the image below). Then click the *Single Run* button.





Pores are automatically extracted and their size measured. In addition, a comparison of the current image with all reference patterns and the classification to the most similar group is performed (green backlight).



Pore size distributions in selected ranges are also displayed.





Detailed measuring data is **APPENDED** to several files located in the **OutFiles** subdirectory:

MeMoAreaFreq.txt – histogram of absolute pore quantity in fixed 8 ranges *MeMoAreaFreq%.txt* - histogram of relative pore quantity in fixed 8 ranges *MeMoAreaSum.txt* – histogram of absolute total area in ranges *MeMoAreaSum%.txt* – histogram of relative total area in ranges

If you are going to analyse data in Excel, click the **Data for Excel** button and all of the above text files (txt) will be translated into Excel (xls) files:

MeMoAreaFreq.xls MeMoAreaFreq%.xls

MeMoAreaSum.xls

MeMoAreaSum%.xls

Example content of data files:

MeMoAreaFreq%.txt

Bin1 Bin2 Bin3 Bin4 Bin5 Bin6 Bin7 Bin8 Image Class

14.25 15.86 19.35 24.73 3.76 4.3 8.6 9.15 D:_PROJECTS\Embarcadero\MeMoExplorer\Win32\Release\Sample.tif 05 16.75 19.8 14.72 19.8 1.02 4.57 6.09 17.25 D:_PROJECTS\Embarcadero\MeMoExplorer\Win32\Release\RefPatterns\Pattern01.tif 01 15.35 11.62 14.94 28.63 2.07 4.56 8.71 14.12 D:_PROJECTS\Embarcadero\MeMoExplorer\Win32\Release\RefPatterns\Pattern03.tif 02 15.71 18.91 21.15 22.76 3.53 3.53 2.24 12.17 D:_PROJECTS\Embarcadero\MeMoExplorer\Win32\Release\RefPatterns\Pattern03.tif 03 12.18 7.56 16.81 22.27 3.36 8.82 7.98 21.02 D:_PROJECTS\Embarcadero\MeMoExplorer\Win32\Release\RefPatterns\Pattern05.tif 05 8.92 7.04 13.62 27.7 7.04 4.69 6.1 24.89 D:_PROJECTS\Embarcadero\MeMoExplorer\Win32\Release\RefPatterns\Pattern06.tif 06 5.18 13.47 15.03 26.94 3.11 7.77 9.84 18.66 D:_PROJECTS\Embarcadero\MeMoExplorer\Win32\Release\RefPatterns\Pattern07.tif 07 11.11 14.4 13.17 20.99 2.47 4.94 11.11 21.81 D:_PROJECTS\Embarcadero\MeMoExplorer\Win32\Release\RefPatterns\Pattern09.tif 09 10.84 14.46 16.87 25.3 1.2 7.23 7.83 16.27 D:_PROJECTS\Embarcadero\MeMoExplorer\Win32\Release\RefPatterns\Pattern09.tif 09

MeMoAreaFreq%.xls

| N | Microso | oft Excel | - MeM | oAreaFr | eq%.xl: | s | | | | |
|----|--------------|-----------------------|-----------------------|----------------|-----------------|---------------|----------------|---------------|---|-------|
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| 1 | Bin1 | Bin2 | Bin3 | Bin4 | Bin5 | Bin6 | Bin7 | Bin8 | Image | Class |
| 2 | 14,25 | 15,86 | 19,35 | 24,73 | 3,76 | 4,3 | 8,6 | 9,15 | Sample.tif | 5 |
| 3 | 16,75 | 19,8 | 14,72 | 19,8 | 1,02 | 4,57 | 6,09 | 17,25 | D:_PROJECTS\Embarcadero\MeMoExplorer\Win32\Release\RefPatterns\Pattern01.tif | 1 |
| 4 | 15,35 | 11,62 | 14,94 | 28,63 | 2,07 | 4,56 | 8,71 | 14,12 | D:_PROJECTS\Embarcadero\MeMoExplorer\Win32\Release\RefPatterns\Pattern02.tif | 2 |
| 5 | 15,71 | 18,91 | 21,15 | 22,76 | 3,53 | 3,53 | 2,24 | 12,17 | D:_PROJECTS\Embarcadero\MeMoExplorer\Win32\Release\RefPatterns\Pattern03.tif | 3 |
| 6 | 12,18 | 7,56 | 16,81 | 22,27 | 3,36 | 8,82 | 7,98 | 21,02 | D:_PROJECTS\Embarcadero\MeMoExplorer\Win32\Release\RefPatterns\Pattern04.tif | 4 |
| 7 | 14,25 | 15,86 | 19,35 | 24,73 | 3,76 | 4,3 | 8,6 | 9,15 | D:_PROJECTS\Embarcadero\MeMoExplorer\Win32\Release\RefPatterns\Pattern05.tif | 5 |
| 8 | 8,92 | 7,04 | 13,62 | 27,7 | 7,04 | 4,69 | 6,1 | 24,89 | D:_PROJECTS\Embarcadero\MeMoExplorer\Win32\Release\RefPatterns\Pattern06.tif | 6 |
| 9 | 5,18 | 13,47 | 15,03 | 26,94 | 3,11 | 7,77 | 9,84 | 18,66 | D:_PROJECTS\Embarcadero\MeMoExplorer\Win32\Release\RefPatterns\Pattern07.tif | 7 |
| 10 | 11,11 | 14,4 | 13,17 | 20,99 | 2,47 | 4,94 | 11,11 | 21,81 | D:_PROJECTS\Embarcadero\MeMoExplorer\Win32\Release\RefPatterns\Pattern08.tif | 8 |
| 11 | 8,2 | 9,29 | 15,85 | 23,5 | 4,92 | 7,1 | 8,2 | 22,94 | D:_PROJECTS\Embarcadero\MeMoExplorer\Win32\Release\RefPatterns\Pattern09.tif | 9 |
| 12 | 10,84 | 14,46 | 16,87 | 25,3 | 1,2 | 7,23 | 7,83 | 16,27 | D:_PROJECTS\Embarcadero\MeMoExplorer\Win32\Release\RefPatterns\Pattern10.tif | 10 |
| 13 | | | | | | | | | | |

Remember!

The results of each image analysis, initiated either by the *Single* or *Batch Run*, are APPENDED to the resulting output files. Copy files or rename them at the right moment if you want to have separated data from different tests.

Automatic analysis of image series – Batch Run

Load any image from the directory whose content you want to analyze. Let it be now the *RefPatterns* subdirectory, located in the main application directory, C:\MeMoExplorer\RefPatterns (as in the image below). Then click the *BatchRun* button.



Image processing and parameter measurements are exactly the same as for a single image, only the image files in the catalog are loaded and processed one after another automatically. For batch operation, there is no display of pore size distribution for each image being analyzed. As in the case of single image testing, the measurement results of a series of images from a selected directory are APPENDED to the existing data files:

MeMoAreaFreq.txt – histogram of absolute pore quantity in fixed 8 ranges
 MeMoAreaFreq%.txt - histogram of relative pore quantity in fixed 8 ranges
 MeMoAreaSum.txt – histogram of absolute total area in ranges
 MeMoAreaSum%.txt – histogram of relative total area in ranges

Report generator

If you check the *Generate PDF report after each run* box, a report / summary will be generated after analyzing every single image. Display will use the default PDF viewer. It is recommended to install Adobe Acrobat Reader for this purpose.



The report generated after the analysis of the series of images will have a different form, as shown below.

| 🔁 MeMo | BatchReport_ | 2017 <mark>-07-06_</mark> 07-56-28.pc | lf - Adobe Acrob | oat Reader DC | | | | | | | | |
|--|--|---------------------------------------|------------------|---------------|---------|-----------|-----------|----------|-----------|-----------|--|--|
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| | MeMo Explorer(tm) Report Generator Copyright (c) 2017 IB&BE PAS All rights reserve | | | | | | | | | | | |
| | C:\MeMoExplorer\RefPatterns | | | | | | | | | | | |
| | The list of all both was seened in a way from the coloring folder. | | | | | | | | | | | |
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| | #) | Image file r | ame | Classifica | tion re | sult (clo | sest rete | rence pa | ttern ide | entifier) | | |
| | 1) | Pattern01.t | f | | | | 01 | | | | | |
| | 2) | Pattern02.t | f | | | | 02 | | | | | |
| | 3) | Pattern03.t | f | | | | 03 | | | | | |
| | 4) | Pattern04.t | f | | | | 04 | | | | | |
| | 5) | Pattern05.t | f | | | | 05 | | | | | |
| | 6) | Pattern06.t | f | | | | 06 | | | | | |
| | 7) | Pattern07.t | f | | | | 07 | | | | | |
| | 8) | Pattern08.t | f | | | | 08 | | | | | |
| | 9) | Pattern09.t | f | | | | 09 | | | | | |
| | 10) | Pattern10.t | f | | | | 10 | | | | | |
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The report specifies the location and name of the generated PDF, the location of the directory which content was cumulatively analyzed, and the list of files (images) that were present in the selected directory. In addition, for each image the result of its classification is given. This is the identifier (number) of the pattern to which the image is most similar (in the sense of pore size distribution).

Details of configuring working session - Settings

Go to the Settings tab.

| File H | MeMoExplorer™ elp | Build 17.10.24.0 | Copyright © 2017 IB&BE PA | S All rights reserved | |
|--------|----------------------|------------------|---------------------------|-----------------------|--|
| | Welcome | | Settings | Work | |
| | | | | | |
| | | | | | |
| | Basic Set | tings A | dvanced Settings | | |
| | | | | | |

The settings are divided into two groups: *Basic* and *Advanced*. Now select the *Basic Settings* tab.

It is very important at the beginning to select the proper image type. Otherwise processing results may be not be accurate. Just click the image type you want to investigate.





< First, select (click) input image type >



Because one of the main tasks of the program is to analyze pore size distributions, and the results of the analysis are described for the convenience of the user in μ^2 , an appropriate calibration (*Object size calibration*) is required. It is responsibility of the application user to specify the correct recalculation factor to change μm to pixels and vice versa.

| Object size calibration | |
|---------------------------------|---|
| Microscope magnification = 300 | • |
| Microscope magnification = 300 | ^ |
| Microscope magnification = 400 | |
| Microscope magnification = 500 | |
| Microscope magnification = 600 | |
| Microscope magnification = 700 | |
| Microscope magnification = 800 | |
| Microscope magnification = 900 | |
| Microscope magnification = 1000 | ~ |



One of the settings in the basic group decides how to deal with objects (pores) at the edges of the image, so that they are not fully visible (*Border objects*).



Objects smaller than the bottom value of the last histogram bar (default $300\mu^2$) should be removed from the analysis always because it is not possible to determine their actual size (*Remove small*).

The last bar of the histogram contains objects larger than the user-defined upper limit (default $300\mu^2$). So, if a large object is not visible in its entirety but its fragment has a surface greater than predefined limit it should not be removed because it passes naturally to the last histogram bar. **Remove large** does not apply to these cases and this should be precisely understood. But, is there any upper boundary of the object size? The **Remove large** option allows the user to make a decision on this. This is the situation shown in the image below.



It seems obvious that the dark area to the right should not be treated as a pore. It is not an object that should be taken into account. It is only the effect of incorrect positioning of the membrane in the microscope and the membrane does not occupy the entire surface of the image (but it should, we should not capture such images). Unfortunately, it happens and checking the **Remove large** box removes those false objects that are larger than a fixed value from the set of extracted ones. This value is defined in μ^2 in the **MeMoExplorer.ini** file, the **TooLargeThr** parameter in the **BinThrMicro** section.

```
•••
[Work]
Report=1
[Files]
LastLoad=Sample.tif
[BinThrMicro]
bthr1=3
bthr2=8
bthr3=20
bthr4=80
bthr5=100
bthr6=150
bthr7=300
TooLargeThr=76953
[SizeCalib]
1000u2=2560
[Border]
RemoveSmall=1
RemoveLarge=1
```

...

And the last setting in the basic group is the configuration of the image classification procedure, that is, how to calculate similarity to reference patterns. You choose here which histogram bars (object size ranges) are to be taken into account when calculating this similarity.

Select the entire histogram for calculating similarities to patterns and classification.



Select bars 2, 3, 5 and 8 to calculate similarities to patterns and classification.



20

Now, select the *Advanced Settings* tab.

... and do not change anything if you are not sure what these settings mean.

Optimize for object size allows you to tailor processing methods to a specific type of membrane, and more precisely, configure processing algorithms to best suit the pores of the indicated size. **Medium** is best for most images.



Object appearance allows you to optimize image processing and object extraction depending on your preferred pore appearance. The essence of the problem is shown below.



Extracting objects with option Uniform dark gray levels



Extracting objects with option *Floating gray leves*

All problems, questions and suggestions regarding extensions or improvements of the program send directly to the person responsible for developing the project software: *darek@ibib.waw.pl*