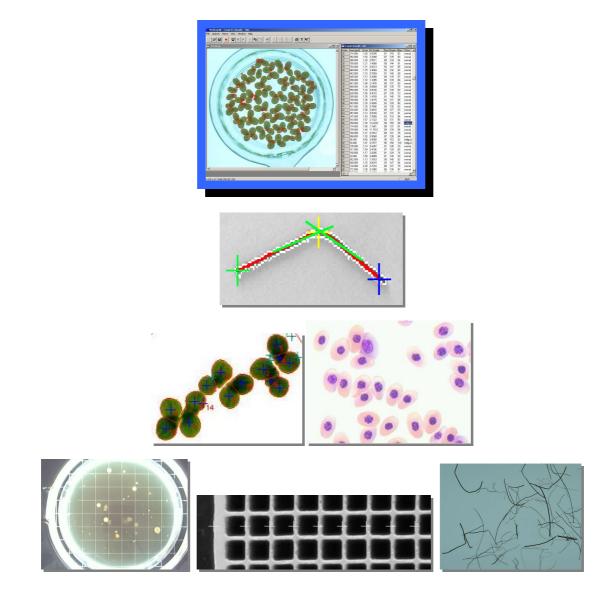


medeaLAB Count & Classify Image Analysis System *Tutorial*







is a product of



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Thank you for your interest in our medeaLAB system!

This tutorial will help you to get a first impression of the practical use and easy handling of the medeaLAB imaging system.

1. Program installation

Important:

Administrator rights are essential to install the software.

To install the medeaLAB Software put the installation CD into your drive. The medeaLAB setup starts automatically, otherwise start the installation by double clicking on the file *setup.msi* in the CD root directory. Choose "Typical" setup and follow the instructions.

2. Basic information

2.1. Registration

When starting medeaLAB for the first time a message "medeaLAB is not registered" appears. You may continue to work without registration for a period of 30 days (button "Continue unregistered") or purchase and register your copy of medeaLAB Count & Classify (choose the "Register" button).

For online registration you will need a valid email address and internet access. Follow the four steps in the "Register medeaLAB" dialog.

2.2. Image sources

medeaLAB supports the following image sources: Still image sources:

- Image files (BMP-, JPG-, PNG-, TIF-Format)
- Image acquisition via TWAIN/WIA interface (used by digital cameras and scanners)
- Canon Powershot cameras (models supporting remote capture and camera configuration: A620, A640, S80, S3 IS, S5 IS)
- Canon EOS Digital SLR cameras (all models supporting remote capture and camera configuration)

Video sources:

- Analog video cameras or VCR (using MATROX frame grabbers)
- FireWire (IEEE1394) cameras (if WDM driver is provided)
- USB cameras (if WDM driver is provided)
- Prosilica Gigabit Ethernet cameras
- Video files (AVI or MPEG)

3. First steps

In the following three sections we want to show you how easy it is to achieve good measurement results with the medeaLAB system.

- We will present examples for the following tasks:
 - Counting particles (3.1)
 - Classifying seeds as germinated (3.2)
 - Determining length parameters of fibers (3.3)

3.1. Object count



3.1.1 Load an image

To load an image into medeaLAB you may:

- open an image file from hard disk
- acquire an image via the TWAIN interface from a scanner or digital camera or
- capture an image from a video source.

To open an image file choose "File" – "Open" from the menu and select "Fertilizer_Pellets.JPG" in the Tutorial directory.

If you want to see the whole image scaled into the image window uncheck the option "View" –"Direct view (1:1)".

For the moment we do not calibrate the image to display real measurement units, but we will use pixel units.

3.1.2 Adjust threshold

There are two ways to adjust the gray level or color threshold values for object detection in the medeaLAB system:

- Automatic adjustment
- Manual adjustment using preview

In most cases you will only need the automatic method. This is also a good starting point if you want to do fine-tuning using the manual method afterwards.

1. Choose the **Options / Thresholds** menu command and indicate whether the **Object Appearance** is **Bright** (against a dark background), **Dark** (against a bright background), **Middle** (against a dark and bright background) or **Dark/Bright** (background is in the middle). The correct setting is important because it is the only indication for the system which objects it should search for.

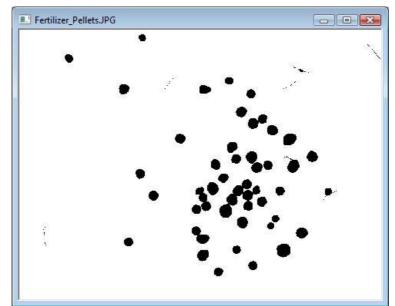
In our current sample image the objects are **Dark.**

olor Threshold	s					
ĥ	owerlevel			high leve	əl	
Red 🔳	0	▶ 80	4			▶ 240
Green 🔳		104	4			▶ 240
Blue 🔳		106	4			240
Automatic bject Appeara			. Dealed	-> < -	Pee	<>
bject Appeara		dle C	Dark/	-><- 'Bright	Pse	<> udo Liv
bject Appeara	nce		Dark/ Learn	'Bright	F	

2. Click the **Automatic** button to set the thresholds automatically.

3. Click the **Preview** button to check the binarized image in the image window. The pellets should appear black on white background in preview mode.





Preview mode is ended by clicking the **Preview** button again.

In our example the automatic thresholding should work without manual adjustments (done with the two arrow buttons right to the Automatic button).

3.1.3 Count objects

Choose "Image" – "Search objects" from the menu to start a measurement and a table of the objects found will be shown (in the titlebar of the table the number of objects found is displayed, in our case 51).

All objects counted are marked with a cross and a number, objects excluded are displayed with dimmed contours:



(displayed in "Direct View" – see "View" menu)

Click on a table row and the corresponding object is marked by a rectangle in the image (you can also click on an object in the image to select an entry).



			Index	PosX	PosY	Area [µm ²]	Form	Border	
		N	13	462	264	1660.00	1.15	False	
		1	14	818	265	1540.00	1.23	False	
		1. And 1.	15	1170	261	1.00	15.60	False	
 s / 1		+#	16	1023	285	1207.00	1.13	False	
			17	981	365	1684.00	1.14	False	
			18	1051	408	3030.00	2.09	False	
	٠.	(19	1118	447	1739.00	1.16	False	
•	120		20	1194	487	2396.00	1.20	False	
			21	711	484	1489.00	1.14	False	
	0		22	940	522	1714.00	1.17	False	
		•	23	1294	564	1776.00	1.13	False	
	0		24	1027	567	2048.00	1.14	False	
	554		25	958	574	1356.00	1.15	False	
			26	1210	606	2341.00	2.76	False	
			27	866	599	1541.00	1.13	False	
		•	28	1098	603	1316.00	1.13	False	
			29	1050	613	1714.00	1.14	False	
			30	534	641	1361.00	1.15	False	
			31	901	659	1388.00	1.21	False	
			32	973	750	12065.00	7.22	False	
			33	854	706	2184.00	1.15	False	

If you select the object with index 32, you will see that adjacent objects are counted as one object, which is definitely not what we want.

3.1.4 Conglomerate cutting

In order to improve the count result medeaLAB has an option to separate adjacent objects.

Click into the image window (activate the window) to switch to the image menu items, then choose "Options" – "Object filter" – "Conglomerate cutting". Select "Convex Defect Analysis" and set the "Sensitivity" value to 0 or 1.

Conglomerate	Cutting			X
⊂ Off	Convex Defect	Analysis	C Watershed	
Sensiti	vity			7
			1	
				-
	ОК	Cano	:el	

Repeat the measurement by choosing "Image" – "Search objects". Now the objects are separated and the count result will be 66.

However, this is not the correct number of fertilizer pellets, because also small objects like fibers were counted.

3.1.5 Object Form Parameters

In order to improve the count result medeaLAB has additional filter options.

In our example you may filter unwanted objects out by their form or area:

The desired objects are round, i.e. they have a form factor near 1. Elongated or jagged objects would have higher form factor values.

Also, the pellets have pixel areas over 700 pixels, the fibers have areas below 200 pixels. You may verify this, if you sort the objects by area: Click on the "Area" header of the table (object with index 42 is the smallest pellet):



Index	PosX	PosY	Area [µm²]	Form	Border	
1	1531	1	1.00	5.09	True	11
6	1569	98	1.00	1.99	False	
15	1170	261	1.00	15.60	False	E
5	1561	87	4.00	5.75	False	
4	1550	76	4.00	14.50	False	
12	656	245	5.00	131	False	
11	1190	251	6.00	172	False	
3	1542	67	6.00	15.33	False	
64	1352	745	7.00	35.65	False	
66	109	884	7.00	10.23	False	
63	1395	721	8.00	60.52	False	
54	1178	566	8.00	8.95	False	
7	1584	117	45.00	14.64	True	
44	112	929	58.00	31.28	False	
53	1194	572	63.00	7.88	False	
9	1244	180	176.00	19.19	False	
42	1111	872	724.00	1.17	False	
2	543	35	755.00	1.14	False	
10	1177	0.11	705.00	4.4.5	F 1	

In order to filter the objects activate the image window again and choose "Options" – "Form parameter" from the menu, check the "Area" parameter, set the value in the "Min" column to 200 and set a checkmark in the "Action" column (i.e. activate this filter). Now the dialog should look like this:

ect	Nucleus		& Model		
	ct Paramete meter	er Min	Action	Max	Action
A	rea	200	V +	500000	
F	omfactor	1	1	5	- 23
L	ength	0.1	8	100	- 23
V	Vidth	0.1	2	100	- 28
] C	olor (Avg.)	00-00-00		FF-FF-FF	59
H	lomogenity	0		1	10
] C	onvexity	0		1	5
H	loles	0	8	0	-8
A	ppendix	5	×		
] Hi	ude t Boundary sfault	Load		Save	

Repeat the measurement by choosing "Image" – "Search objects". Now the fiber objects are excluded and the count result will be 50, which is the exact result.



3.2. Object Classification

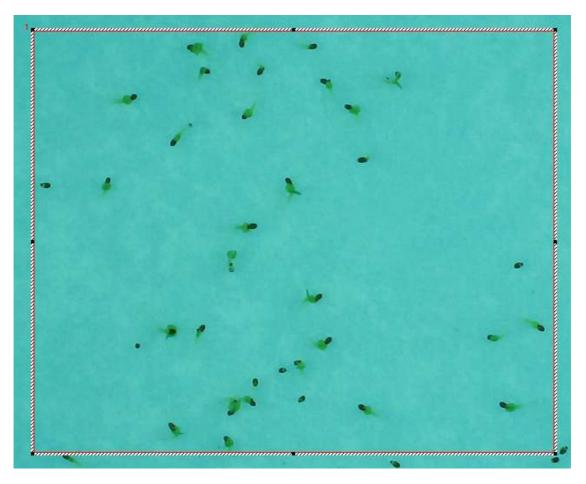
medeaLAB also has features for automatic classification of objects.

First you have to teach the system which objects are belonging to which class. During this selection process a statistical classification scheme is established, which will be used in subsequent automatic classifications.

We will demonstrate these abstract processes as follows:

Example: Classifying seeds as germinated

Have a look at this image taken with a 7 Megapixel camera ("Germinating_Seeds.JPG" in the Tutorial directory). Some of the small seeds are already germinated:



First we have to decide which object features are characteristic for germinated seeds and select them for evaluation.

We clearly see that germinated seeds differ from other objects (dirt and ungerminated seeds) in

- area (they have grown)
- form (irregular shapes)
- color (greenish appendices)



oject	Nucleus	Parameter &	& Model		
	ct Paramete meter	er Min	Action	Max	Action
V A	rea	1	E -	500000	-
V F	ormfactor	1	19	5	20
L	ength	0.1	8	100	33
N I	Vidth	0.1	22	100	- 28
0 C	olor (Avg.)	00-00-00		FF-FF-FF	50
回 H	lomogenity	0	0	1	-2
C	onvexity	0	8	1	-
H	oles	0	8	0	-18
A	ppendix	5	*		
	ude t Boundary efault	Load		<u>S</u> ave	

Therefore in the **Form Parameters** dialog we check at least "Area", "Formfactor" and "Color". We do not have to set lower or upper feature limits (**Action**), but you should check **Exclude -Hit Boundary**, because it is not possible to classify partial objects.

Now we should execute a test for object detection using the menu command **Image /** Search objects.

If the objects desired are not marked correctly and listed in the table please check for the right **Thresholds** and retry.

The next step is to teach the system which objects belong to a class. Activate the image window and open the **Classes** dialog using **Options / Class definition** on the menu.

1	Class name:	0		¥	Color
	Add	Modify		Delete	-
eatures:					
Index I	Name	Min	Max	: Avg	Dev
•		III			
< [m			
∢ [Included I	features:	····		Available f Area Color Blue Color Gree Color Ree Form Length	eatures:
	eatures:	>		Area Color Blue Color Gree Color Red Form	eatures:

At the first time no class is defined, so type a name for your new class in the **Classname** field and click Add. As a default the "Area" feature is added automatically to the **Included features** list for the new class. You now may add features characteristic for your objects from the **Available features** list with the \leftarrow - button (or remove from the **Included features** list with the \rightarrow button). In our example we add "Form" and "Color Green" besides Area. At least these features should have been selected for evaluation in the **Form Parameters** dialog before. You are free to select more features for evaluation in the result table but only the three features mentioned will be used for classification.



ndex:	Class name	¢			
	Germinate	d		•	Color
	Add	Modify	D	elete	-
eatures	¢				
Index	Name	Min	Max	Avg	Dev
1	Area	0.00	0.00	0.00	0.00
	Color Green	0.00	0.00	0.00	0.00
	Form	0.00	0.00	0.00	0.00
	d features:	m		Available fi	eatures:
Include Area				Color Blue	
Include Area Color G		····>			eatures:
Include Area Color G Form	reen	>	Learn	Color Blue Color Red Length Roundnes Width	eatures:

Select the features you want to teach (i.e. modify its statistical descriptors Min, Max, Avg, Dev) by clicking on them in the **Features** list with the CTRL key pressed and switch to the Learn Mode by clicking the button **Learn**.

The dialog should now appear like in the picture on the left and a message appears in the **Status** line indicating the "Learn Mode".

Now click on objects in the image which belong to the "Germinated" class and watch the statistical descriptors change. You will not have to select all of the objects, but it is a good idea to include objects representing the whole variety of the "Germinated" seeds class (e.g. with short and long primary root).

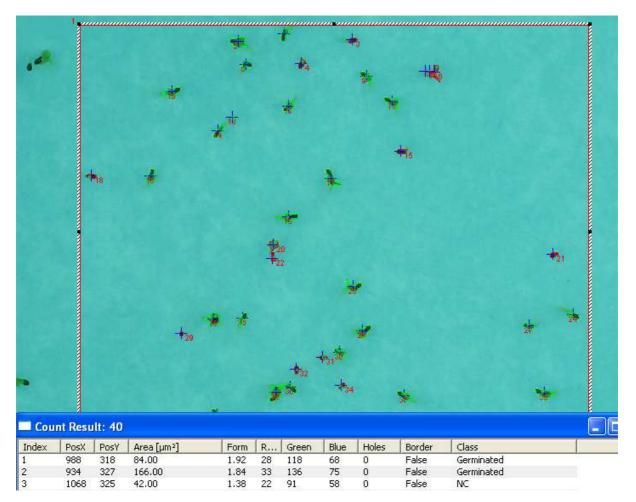
After teaching enough objects leave the Learn Mode by clicking **Learn** again and the Status message will disappear.

You may set all descriptor values of the current class to 0.0 using the **Reset** button, if you have to teach this class again from the beginning.

If you execute the menu command **Image / Search objects** again now, you will notice the new column "Class" in the result table and some of the objects should be classified as "Germinated" (objects not fitting into any of your classes are displayed as NC = Not Classified).

In the picture below "Germinated" objects are marked with a green border, which was selected as the color for this class (using the **Color** button right to the **Classname** in the **Classes** dialog)





Strategies for optimal classification results:

If germinated seeds are not classified correctly as "Germinated" (but e.g. as "NC"), add these objects to the "Germinated" class in Learn Mode.

If objects that do not belong to the "Germinated" class are misclassified as "Germinated", try the following:

- 1. Assign more objects to the "Germinated" class in order to improve the statistical descriptors for this class
- 2. Create and teach also classes for the objects not classified (NC). This will lower the risk of misclassification, because an object is assigned to the class to which it fits best.



3.3. Skeleton Object Model

Application: Measuring the mean length of fibers

For length measurement of fibrous structures we have to change from the "Contour" to the "Skeleton" object model.

First load the image "Synthetic_Fibres.JPG" from the Tutorial directory.

We will need other threshold values for this image. The image loaded is also suitable for automatic thresholding, but it will take a little time as the image has a size of 3200×2400 pixels.

Like in the chapter **Adjust threshold** choose "Options" – "Thresholds", select Object Appearance "Dark", press "Automatic" and the system will try to separate the objects from the background.

You should control the automatic separation: Choose "Options" – "Thresholds" and click "Preview".

If the automatic separation from background was successful (black objects on white <u>background in "Preview" mode</u>) choose "Options" – "Form parameter" from the menu.

	Nucleus		and the men		
Obje	ct Paramete	er			
Para	meter	Min	Action	Max	Action
A	rea	500		500000	
E F	omfactor	1	14	5	- 2
VL	ength	200	12	1000	2
V 🔽	Vidth	0.1	8	100	- C
C	olor (Avg.)	00-00-00	æ	FF-FF-FF	
III H	lomogenity	0	10	1	3
C	onvexity	0		1	-
VH	loles	10	V +	0	-
A 🗐	ppendix	5			
	ude It Boundary efault	Load	1	Save	

On the "Object" tab check Object Parameters "Length" and "Width".

The Object Parameter "Holes" ensures that areas totally enclosed by fibers are included in the segmentation process.

Ensure that also "Hit Boundary" (under Exclude) is checked, because we want to measure only the length of fibers visible as a whole.

Then select the "Parameter & Model" tab.



ject Nucleus	Paramete	er & Model
Object Parame	ter	
Isthmus Po	pints	
Convex A	nalysis	
Concave /	Analysis	
Split Conto	Dur	
Cluster	0	Maximum Distance
Merge Cor	ntour	
Object Model		
Contour		
Edge Obj	ect	
Ellipse		
Skeleton		
Contour S	Segment	

Here you should switch to **Object Model** "Skeleton". Segmentation using the object model "Skeleton" will lead to objects with a width of one pixel, which makes length measurements easier.

Start the measurement as usual by choosing "Image" – "Search objects". After calculation the following results will be displayed for each fiber object:

Length:	path of maximum length within the object
Tot. Length:	length of all paths of the object
Width:	estimate for fiber width
Tips:	number of end points
Nodes:	number of branching points

Note that short branchlets are automatically filtered out and therefore do not contribute to length and tip count results.

These short branches often occur due to pollutions or during skeleton segmentation of thick object parts and are not relevant for object geometry.

If you scroll to the end of the count results table, you will find the sum of all fiber lengths and also the sum of all tips (end points) found. From this we can calculate the mean fiber length if we take into account that each fiber has two tips. In our example:

$$\frac{\sum fibrelength}{\sum tips/2} = \frac{31661}{366/2} = 173 \text{ pixel.}$$

Mean fiber length =

This of course is not an exact result but an estimate, because the random orientation of adjacent fibers leads to a wrong tip count in some cases.



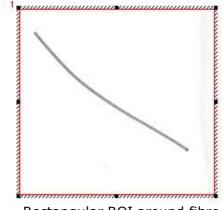
3.4. Regions Of Interest

You can also measure single fibers using ROIs (Regions Of Interest). medeaLAB supports several types of ROIs, which you can select on the toolbar:

Rectangular ROI	
Parallelogram ROI	\diamond
Elliptical/Circle ROI	0
Freehand ROI	0
Delete current ROI	×

To create a ROI of type rectangle move the cursor to the upper left corner, press the left mouse button and drag the cursor to the lower right corner. After releasing the mouse button you may also move or modify the ROI by dragging.

All measurement and filter functions of medeaLAB will be executed inside the Regions Of Interest only.



Rectangular ROI around fibre

Repeat the measurement by choosing "Image" – "Search objects". Now only the fiber object within the RIO is counted.

You may now measure other fibers by moving the ROI to another place (close the result table first, otherwise clicking the mouse will select objects, but not ROIs). It is also possible to create more than one ROI.

All parameters (also ROIs) are stored if you leave the program and restored on program launch.

The measurement methods described above are only a small subset of the possibilities of the medeaLAB system. You are free to try out your own samples. If you have any questions or problems with your application don't hesitate to send us your images together with a short description (email address info@medelab.de or visit http://www.medealab.de).