# Exact Smart design that counts







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# Safety Information

To avoid damage or malfunction of the CytoSMART™ Exact:

- Be sure to set this device on a firm, stable and horizontal surface.
- Shut down the device if it produces smoke, a strange odour or unusual noise. Continued use may lead to fire. Unplug the USB cable immediately.
- Do not connect the usb cable in ways other than those mentioned in this manual.
- If water or other liquid enters the device, discontinue use immediately. Continued use may lead to fire. Unplug the USB cable immediately.
- Do not attempt to repair or remove the outer case of the device.
- Never attempt to repair, disassemble or modify this device yourself, tampering with this product may result in injury or fire.
- Do not drop the device or allow objects to fall onto it.
- Do not allow the device to be submerged in liquid.
- Do not place heavy objects on top of this device and never stand or lean on this device. Failure to follow this may cause the equipment fall or collapse, which results in possible breakage and/or injury.
- Do not autoclave this device.
- Do not perform the sterilization cycle of an incubator with the device inside.
- Do not use acetone to clean the device.



## Automated cell counter Instruction Manual

## 1. Introduction

#### **Technology to Make Your Work Easier**

Thank you for purchasing the CytoSMART™ Exact. Soon you will be automatically counting cells like never before.

The CytoSMART™ Exact is a benchtop automated image-based cell counter, which uses state of the art optics and image analysis software. Its innovative design combines the best qualities of manual cell counting with those of automated image based cell counting. With this, the Exact can accurately and precisely measure cell concentrations and viability - live, dead and total cells - using the standard trypan blue technique. It really is that simple.

#### **How Easy the CytoSMART™ Exact Works**

The CytoSMART™ Exact system can be installed within several minutes and requires minimal training. Many procedures are similar to manual cell counting.

The Exact uses a standard hemocytometer to analyse cell samples. Images from the cell sample are obtained by the Exact and the Exact App software analyses these images in the cloud. We use sophisticated image analysis algorithms for this, to produce accurate cell counts within a few seconds.

You directly obtain your cell concentration on the results page, but can also look it up later on the CytoSMART<sup>TM</sup> Cloud.

#### **Operating Range**

The Exact can be used to determine cell concentrations of mammalian cells. The system works within a cell size range between 5 - 70  $\mu$  m. Expect accurate results for concentration samples within a range of  $5.0\cdot10^5$  -  $1.0\cdot10^7$  cells/mL.



#### **Instrument Overview**

The hardware of the CytoSMART™ Exact is shown below (fig. 1):

- 1. **Light arc** Contains the light source for imaging
- **2. USB cable** For connecting the device via USB, which is also the power source for the device
- 3. Optical window Cover glass where the hemocytometer is placed on
- **4. Adapter plate** Used to guide the hemocytometer across the optical window
- 5. Focus knob Allows for changing the focus of the image

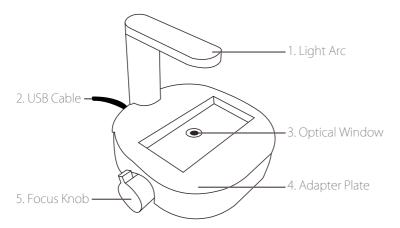


Fig. 1. Instrument overview of the CytoSMART™ Exact.

## Automated cell counter Instruction Manual

# 2. Getting Started

Of course you cannot wait to unbox the CytoSMART™ Exact. Inside the box you will find the following items (fig. 2):

- 1. Manual and Quick Starter Guide
- 2. CytoSMART™ Exact
- 3. License key
- 4. Adapter plate for hemocytometer
- 5. Hemocytometer with two cover slips
- 6. Download link to the Exact App software

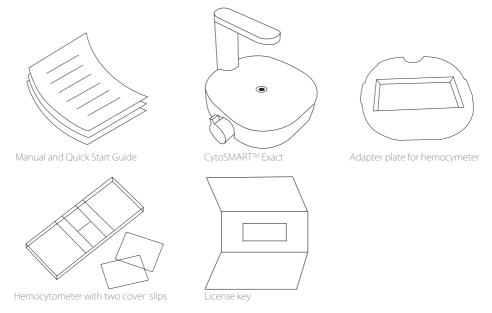


Fig. 2. CytoSMART™ Exact package overview.

Remember, if you ever have any questions regarding your purchase, please visit <a href="www.cytosmart.com/support">www.cytosmart.com/support</a> or contact our support staff via <a href="support@cytosmart.com">support@cytosmart.com</a>. We are happy to help you out.



# 3. Setting Up the Exact

It is really easy to set up the CytoSMART™ Exact. All you need are these items:

- A Windows computer with Windows 7 or higher, internet access (preferably via a LAN-cable) and a USB port (preferably USB 3.0)
- The CytoSMART™ Exact
- The download link for the CytoSMART™ Exact App software
- A license key to activate the device within the software

#### Steps to setup

- 1. Open an internet browser and download the Exact App, using the download link provided. If no link has been provided, please contact your sales representative or contact our support staff via <a href="mailto:support@cytosmart.com">support@cytosmart.com</a>.
- 2. When the download is finished, double click the file to install the Exact App on the Windows computer.
- 3. After the installation has completed, an icon for the CytoSMART™ Exact App will be visible on the desktop (fig. 3). Be sure to remove any wrapping before first time use of the CytoSMART™ Exact. Also clean the device with a damp lint free cloth and IPA or EtOH (70%).
- 4. Start the Exact App by double clicking the icon on the desktop. A notification to connect the device will appear (fig. 4).
- 5. Next, connect the cleaned CytoSMART™ Exact to the Windows computer using the USB cable. Connect via USB 3.0 port, if possible. This way the device will perform best.
- 6. When the CytoSMART™ Exact is connected, the green LED will activate in the light arc. This indicates that the device is ready for use.



Fig. 3. The Exact App icon.

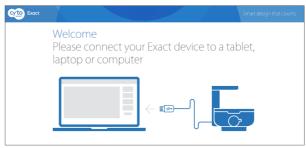


Fig. 4. Connect the device notification.



#### Registration of the device

Before you can use the Exact, you need to register. By clicking on the 'Register a new user' button (fig. 5), you will be led to the register form (fig. 6). Please fill in all the details and click 'Sign up'.



Fig. 5. Creating an account

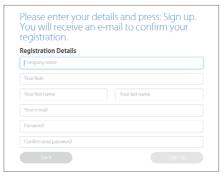


Fig. 6. Register form to sign up.

The next step is to register the device. In the product box you will find the license key for this device. Enter the license key that is provided within the box in the field 'License' and click 'Register' (fig. 7). Congratulations, you have now successfully registered and activated your Exact!



Fig. 7. Register a device

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## Error-code: multiple customer environments

Is your personal account linked to more than one company? In that case, you will see the comment box as shown in figure 8 after signing in with your email address and password. Select the desired environment that you would like to use from the dropdown menu and click 'Select'. This will lead you to the right environment to start your project.

#### Signing In

If your device is already activated and you have already an account, you can easily sign in. Fill in your email address and password and click 'Log in' (fig. 9).

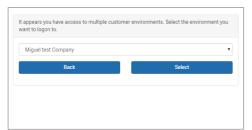




Fig. 8. Select environment

Fig. 9. Signing in

## Error-code: wrong account or company

If your personal account is not connected to the environment of the device, you will see a warning (fig. 10). This means that the device is already activated and registered to a company, but your personal account is not linked to this company.

This account is not connected to the environment for this device. Please login with an account that has access to the environment of 'CytoSMART test' or create a new account

Fig. 10. Warning: account not connected to environment



#### **Start a Project**

With the Exact automated cell counter you can perform a single count or multiple counts within a project. The Exact App software automatically stores the results within a project, which you can later on view in the CytoSMART<sup>TM</sup> cloud. Exactly like your lab journal.



Fig. 11. Create a project

After selecting 'create a new project' (fig. 11), you will be led to the "Start a project" page (fig. 12). Next to the project owner details, which are already filled in, you can also fill in the email address of collaborating users like your colleagues. Name the project, and give it a description. The final step is to assign a colored icon to the project. This helps you to find your project easily, based on the color of the icon.



Fig. 12. Start a project

## Automated cell counter Instruction Manual

#### **Start a Cell Count**

To start a cell count, you need to create an experiment. First you select the project that your experiment belongs to using the drop-down menu (fig. 11). If the cell count doesn't belong to any of the shown projects, you can click 'Create a new project' to create a new project.

Next, give the cell count a name. Our advice is to always include the date of the cell count into the name to easily find the experiment later on. After entering the details, click 'Start cell count' (fig. 13) and you are ready to count your cells!



Fig. 13. Start cell count

#### **Adapter Plate placement**

You can use the adapter plate to guide the hemocytometer during image acquisition of the sample. We recommend to place the adapter plate on the CytoSMART™ Exact, but this is not required to perform a cell count. Using the adapter plate will produce the most reliable results, as it allows for precise and repeatable handling of the hemocytometer.

To install the adapter plate:

- Make sure that both the CytoSMART™ Exact and adapter plate are clear of any debris.
   Note: Use a lint free wipe to clean the device, if needed.
- Carefully slide the notch of the adapter plate around base of the light arc (fig. 14).
- Push the adapter plate downwards, until it clicks into place on-top of the CytoSMART™ Exact (fig. 15).



Fig. 14. Sliding the adapter plate into place.

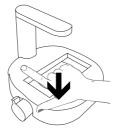


Fig. 15. Carefully press the adapter place downwards.



- Make sure the adapter plate is placed correctly. If it is placed incorrectly, this can lead to false results (fig. 16).
- To remove the adapter plate, carefully pull it upwards near the base of the light arc. Then carefully slide the plate from the CytoSMART™ Exact.

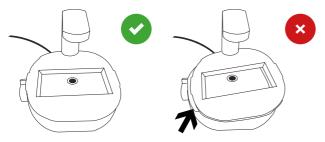


Fig. 16. Reference images for correct adapter plate placement.

#### **Sample Loading**

A hemocytometer is used for cell sample analysis. Most common hemocytometers are compatible with the CytoSMART™ Exact. This is because these counting chambers have a similar counting chamber depth of 0.1 mm.

Other good news, the CytoSMART $^{\text{m}}$  Exact does not require the counting grid - essential for manual counting - to be included to perform a cell count. Therefore, you can also use disposable counting slides, if they have a counting chamber depth of 0.1 mm.

Load the hemocytometer with a cell sample, according to your loading protocol. We recommend loading  $10\,\mu\text{L}$  of the sample in the counting chamber by using a  $10\,\mu\text{L}$  or  $20\,\mu\text{L}$  pipet. The CytoSMART<sup>TM</sup> Exact operates within a concentration range of  $5\cdot 10^5$  -  $1\cdot 10^7$  cells/mL. If the cell solution is out of this concentration range, use Phosphate Buffered Saline (PBS) or medium to resuspend the cells in an appropriate volume. Otherwise you may obtain false results.

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#### **Live/Dead Sample Loading**

To perform a live/dead analysis, we recommend to use the standard trypan blue technique. Mix a minimum of 20  $\mu$ L sample with 20  $\mu$ L trypan blue by using a 20  $\mu$ L pipet. Next load 10  $\mu$ L of the sample mixture into the hemocytometer.

Remember to correct the results when a dilution is used, as the CytoSMART™ Exact does not correct for this automatically.

#### **Hemocytometer Placement**

The optical window of the CytoSMART™ Exact needs to be clean. You can check this by monitoring the live screen. If any debris is present, you will see it in the Exact App.

If any debris is visible, clean the optical window using lint free wipes before placing the hemocytometer.

When the sample is loaded in the counting chamber and the adapter plate has been installed, you can place the hemocytometer on the CytoSMART™ Exact. Place the hemocytometer in the adapter plate.

## Automated cell counter Instruction Manual

# 4. Performing Cell Count

After placing the loaded hemocytometer on the CytoSMART™ Exact, you can see an image of the cell sample in the live screen (fig. 17).

#### **Focusing of the Cells**

Use the focus knob to adjust the focus of the image. Turn the knob clockwise or counter clockwise to focus. On the tablet or pc you will see the focus change when you turn the focus knob. Make sure to have the correct focus to get the best results.

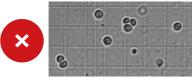
For proper analysis, you should be seeing a contrast: living cells having bright centers and dark edges (fig. 18). To achieve this, you need to set the focus just above the cell using the focus knob.



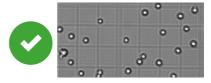
Fig. 17. Live screen of the Exact App

We recommend to try multiple focus levels to determine which focus setting to use.

**Important:** The image seen on the live screen is a digital magnification of the field of view, to allow for better focusing. The unmagnified image can be seen by clicking on the magnifying glass (1 in fig. 17)









#### **Performing a Count**

When the focus is set, you are ready to perform a count. To obtain your cell count, follow the protocol below:

- 1. Make sure that the hemocytometer is placed correctly in the adapter plate.
- 2. Check the image to see if the focus is correct correct it if needed and select 'Count' from the start screen (fig. 17).
- 3. The Exact App will now analyse the image and present the results. Within the analysed image, you will see green and red circles (1 in fig. 19). Green circles represent living cells, red circles are dead cells. Next to the image, the number of (live and dead) cells is displayed (2) together with experiment data (3).
- 4. In this window you can also add notes (4). These will be included in the CytoSMART™ Exact cloud environment.
- 5. If you want to perform another cell count, simply press 'Start new cell count' (5).



Fig. 19. Overview of the result page

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# 5. Connecting to the cloud

One of the coolest features of this device is that the experiment results can be accessed through the CytoSMART™ Cloud. If you want to access the cloud, the following are required:

- PC, tablet or another device with a web browser.
- Active internet connection.
- CytoSMART™ Cloud login details.

A two-year subscription to the CytoSMART™ Cloud is included with the CytoSMART™ Exact. After starting an experiment, the captured images will be stored in the cloud. To allow for this, make sure the system has an active internet connection. All time logs in the cloud are set to GMT.

#### **Confirmation Email**

After starting an experiment, a confirmation email is sent that contains a link to the cloud (fig. 20).



Fig. 20. Confirmation email.

**Important:** When you did not receive a confirmation email, please check the spam folder. Alternatively, go to <a href="https://cloud.cytosmart.com/">https://cloud.cytosmart.com/</a> to log in and view your projects and experiments.



This link will lead you to the CytoSMART™ Cloud login page (fig. 21). Use your credentials created earlier to log in. In case you have multiple customer environments, choose which environment you would like to log on to (fig. 22)



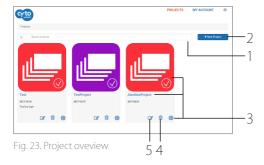


Fig. 21. Cloud login page

Fig. 22. Choose an environment to log on to

#### **Project Overview**

After logging in to the CytoSMART™ Cloud, you will get access to an overview of all projects (fig. 23). Navigating the portal is pretty simple. If you have multiple projects in an account, use the 'Search Projects' bar (1) to quickly search for a project. To create a new project, select 'New Project' (2). This is similar to when beginning a new project, as described in § 3. It is not possible to start an experiment from the cloud.



You can get to your data easily. To access a project and all its assigned experiments, select the projects corresponding 'Project Icon', 'Project Title' or 'Arrow Icon' (3).

To delete a project, select the 'Waste Bin Icon' (4). Select the 'Pencil Icon' (5) to edit project details. This allows you to change the project name and its description.

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#### **Experiment Overview**

After selecting a project (3), you will get access to an overview of all experiments assigned to that project (fig. 24). In the top, you can see an overview of the project data (6). As with the project overview, use the 'Search' bar (7) to find a specific experiment within a project.



Fig. 24. Overview of all experiments assigned 9 10

Select a specific experiment by clicking on its 'Experiment Image', 'Experiment Title' or the 'Arrow Icon' (8). This will bring up the experiment page of that specific experiment.

To edit details of an experiment, select the 'Pencil Icon' (9). This allows you to change the name and description of an experiment. To delete an experiment entirely, select the 'Waste Bin Icon' (10).



#### **Experiment Results**

After you select a specific experiment, the data collected during that experiment will be presented in the CytoSMART™ experiment page (fig. 25). Below, you will find an overview of the panes in this Experiment Page.



Fig. 25. Experiment page

#### **Overview:**

- 1. Analyzed image.
- 2. Experiment pane.
- 3. Notes Pane.
- 4. Logbook Pane.

## **Analyzed image**

The analyzed image shows live cells within green circles and dead cells within red circles

### **Experiment Pane**

The experiment pane provides an overview of the experiment i.e. project start date, owner, number of cells, and viability.

#### **Notes Pane**

Notes that you have added after counting the cells are shown here. You can also add additional information (e.g. passage number, experiment conditions, etc.) regarding your experiment. This information will be stored in the database with your experiment, including a date and time-stamp.

## **Logbook Pane**

The logbook pane shows a log of all the changes made to the experiment.

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# 6. Cleaning and Troubleshooting

#### **Cleaning and Maintenance**

Lucky for you, the CytoSMART™ Exact requires minimal cleaning for reliable operation. Unplug the USB cable before cleaning the device. Use a damp lint free wipe to clean the outer case with IPA or EtOH (70%).

If you see dust particles on the optical window, remove them by cleaning with a lint free wipe.

**Important:** Do not autoclave the CytoSMART™ Exact, this can result in damage to the electronics

The CytoSMART™ Exact does not need regular maintenance. Do not perform any repairs or service on the device yourself, but contact CytoSMART™ if a problem should arise via <a href="maintenance">support@cytosmart.com</a>.

#### **USB** device does not connect

The CytoSMART™ Exact can be connected via USB to various Windows computers, such as PC, laptop or tablet. When receiving an error message (fig. 26), that the computer cannot communicate with USB devices, make sure that the USB cable is properly connected



Fig. 26. USB not connected



Also, with certain mobile Windows computers, such as laptops or tablets, connectivity problems with the CytoSMART™ Exact may arise. This is often due to low battery. To resolve this please try the following:

- Make sure the mobile Windows computer is connected to a working power source.
- Make sure the power source is functioning properly, e.g. check by plugging in another electrical device).
- Switch off the Windows computer. Unplug and reconnect all cables and reboot the computer.

Note, when using a hub connector, older versions – e.g. non-powered USB hubs - might not provide enough power for the CytoSMART™ Exact to operate properly.

#### Which Hemocytometers Can I Use With the CytoSMART™ Exact?

The CytoSMART™ Exact is compatible with most common hemocytometers. Compatible hemocytometers must have a counting chamber depth of 0.1 mm. Certain disposable counting slides are also compatible with the CytoSMART™ Exact. For any disposables to be compatible they must have a chamber depth of 0.1 mm.

#### No Cells Are Visible

This may be due to cell concentration of the sample being below  $5 \cdot 10^5$  cells/mL, which is below the operating range of the CytoSMART<sup>TM</sup> Exact (fig. 27). To resolve this, pellet the solution by centrifuging and resuspend the cells in a smaller volume.

Another cause may be that the bulk sample solution has been left to stand for some time. Cells have then gravitated towards the bottom of the vial. By pipetting the suspension up and down before loading the hemocytometer, you should obtain a homogenous cell suspension.

WARNING! This count is below our optimum counting range  $(5.0 \times 10^5 \text{ cells/mL})$ 

Fig. 27. Warning message: not enough cells to count

#### Too many cells

This may be due to cell concentrations being above  $1 \cdot 10^7$  cells/mL (fig. 28). Dilute your cells suspension to obtain a lower concentration and count again.

WARNING! This count is above our optimum counting range (1.0 x 10^7 cells/mL)

Fig. 28. Warning message: too many cells to count

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#### **Cell Clumps**

Cells can aggregate into clumps, leading to false results when analyzed (fig. 29). When cell clumps are visible, prepare a new sample. By gently resuspending the stock cell suspension aggregates can be broken down.

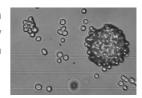


Fig. 29. Cell clump example.

#### **Bubbles**

Whilst looking at the images in the Exact App, you could see bubbles (figure 30). They are recognizable as big black circles.



Fig. 30. Air bubble example.

Bubbles can lead to false results, as they influence the distribution of cells and the volume inside the counting chamber. The cause for bubbles is often due to incorrect pipetting. Thus, make sure no air is included during pipetting a new sample.

#### **Debris**

When looking at the images from the Exact App, you could see (cell) debris (figure 31). This may be caused by not having properly cleaned the hemocytometer. To resolve this, properly clean the hemocytometer before loading a new sample.



Fig. 31. Debris present in the counting chamber.



# 7. Technical Specification

Operating Environment

Cable length

Counting range (cells/mL)	$5 \cdot 10^5 - 1 \cdot 10^7$	
Counting range (µm)	10 - 70 μm	
Minimal System Requirements	Windows 7 or higher 2.4 GHz i5 CPU 4 GB of Ram 128 GB of free drive space USB 3 connection Internet connection	
Weight	1.0 kg	
Power Supply	AC 100-240V, 2A, 10W, 50/60Hz	
Field of View	2.0 x 1.5 mm	
Magnification	200x	
Image Resolution	2048x1536	
Exported Formats	PNG	
Light Source	LED, wavelength 550 nm	
Camera	5 MP CMOS	
Optical Filters	No optical filters	
Unit Dimensions	122x122x125 (LxWxH)	
Data Storage	Available upon request	

5-40 °C, 20-95% humidity

with extra USB3 cable.

750 mm with option to extend

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# 8. Warranty

The CytoSMART™ Exact is covered by warranty - if the device is properly used and installed - for a period of one year (1 year) from the date of purchase. If any defects occur during this warranty period, CytoSMART™ will repair or replace the defective parts at its discretion without charge.

The following defects, however, are specifically excluded:

- 1. Defects caused by improper operation.
  - 2. Damage caused by accident or misuse.
  - 3. Damage caused by autoclavation of the device.
  - 4. Damage caused by the sterilization cycle of an incubator.
  - 5. Repair or modification done by anyone other than CytoSMART™ or an authorized agent.
  - 6. Damage caused by substituting alternative parts.
- 7. Use of fittings or spare parts supplied by anyone other than CytoSMART™.
  - 8. Damage caused by disaster.
- 9. Corrosion caused by improper solvent, sample or treatment of the device.

CytoSMART<sup>TM</sup> cannot be held responsible for damage incurred during shipment of a defective instrument. It is recommended that you save the original packing material in which the instrument was shipped. This warranty is limited to the replacement of defective products.

For any inquiry or request for repair service, please visit us at <a href="https://www.cytosmart.com/support">www.cytosmart.com/support</a> or contact our support staff via <a href="mailto:support@cytosmart.com">support@cytosmart.com</a>.



# 9. CytoSMART™ Care and Ordering Information

Would you like to purchase extended warranty for the CytoSMART™ Exact? Or do you need disposable counting slides? These products are available separately from CytoSMART.

Cat. No.	Description	Contents
GAA-1002	25 Glass Disposable Counting Slides	25 slides
GAA-3004	2-chamber disposable counting slides	100 slides (200 counts)
GAA-1004	2-chamber disposable counting slides	250 slides (500 counts)
GAA-2004	2-chamber disposable counting slides	500 slides (1.000 counts)
GAA-4004	2-chamber disposable counting slides	1.000 slides (2.000 counts)
HDA-1003	CytoSMART™ Care - Exact, option 1	Extended warranty for 1 year
HDB-1003	CytoSMART™ Care - Exact, option 2	Extended warranty for 2 year
HDC-1003	CytoSMART™ Care - Exact, option 3	Extended warranty for 5 year

## Automated cell counter Instruction Manual

NOTES	



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# Support

Visit <u>www.cytosmart.com</u> for more information, support or sign-up for our newsletter. If you have any question regarding a CytoSMART™ product, please contact our support staff via <u>support@cytosmart.com</u> or call us at +31 (0)88 20 32 200.

If you require technical assistance, our support staff is available to answer your phone-call, email or live-chat during weekly office hours (Central European Time, UTC/GMT +1 hr.)

#### Disclaimer:

For research use only. Not for use in diagnostic procedures.

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