

SCA® Quick User's Guide for Research 6.5

English Version

SCA[®] - Sperm Class Analyzer[®] SCA[®] Quick User's Guide for Research

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EC – DECLARATION OF CONFORMITY

We herewith declare that the following In Vitro Diagnostic Medical device meet the transposition into national law Royal Decree 1662/2000, the provisions of Council Directive 98/79/EC of 27 October 1998 In vitro diagnostic directive. All supporting documentation is retained at the premises of the manufacturer.

Manufacturer:	MICROPTIC S.L. Av. Josep Tarradellas, 8, 1º 6ª 08029 - Barcelona (Spain)
Generic Name:	Sperm Analyzer
Product Name:	Sperm Class Analyzer - SCA (CASA system)
Classification:	Not listed A or B, Article 9 – (Self declare)
Directives:	In vitro diagnostic Directive (Annex III - Self declare) 98/79/EC
Classification:	Other General Device
Date:	30/03/2020
Standards:	ISO 9001:2015 (Quality Management) ISO 13485:2016 (Design, Production, Installation and Servicing) EN 62304:2006/A1:2015 (Medical Device Software) EN 62304:2006/AC2008 (Medical Device Software, update) EN ISO 15223-1:2016 (MD symbols) EN ISO 18113-3:2009 (IVD labelling) EN 61010-2-101:2017 (Safety for IVD) EN ISO 14971:2012 (Risk Management to Medical Devices) EN 62366:2008 (Usability)

Responsible person, position in company:

Eduard Sánchez Mallol, CEO

Signature:

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CASA SPERM CLASS ANALYZER

SCA® Quick User's Guide



This is a condensed manual for beginners. It does not substitute the full manual that is included with the SCA® installation disk.

1 SCA® Sample Management

1.1 Executing the application



1. To start the application, click on the SCA[®] evolution icon displayed on the desktop.

2. The system comes with a user account called **Administrator** that doesn't need any password. To login into the system just select **Administrator** user and press OK.

- 1.2 Customize the application
- 1.2.1 Create new users and roles



1. It is possible to create new users and assign a role (user profile) to each one. To see the different roles go to menu Administration > Roles.

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2. By default the system comes with three Roles (Analyst, Administrator and Viewer). You can add more roles or change the permissions (full access, read-only or no access).



3. To insert new Users go to the menu Administration > Users.

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4. Press the **Add** button to add new users or select a user from the table to modify their roles, name or password.

1.2.2 Modify/add labels

It is possible to modify all the labels in the SCA[®] program (words used on the program) and also add new description to the fields describing the sample data (options available for a field).

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(Labels

1. Change any word of the program by going to the menu *Administration* > *Labels* and a window with two columns will be displayed.

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2. The left column contains the default text of the labels, the right column contains the customized text of the labels that will be shown and can be modified with the preferred terms.

Another way to modify a word is with a double click on the text at any window of the program or with right click on the text and selecting the "Change word" option.

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3. Change/add words to the options in the fields of the macro/microscopic data by clicking on the menu **Settings** you will see the list of the fields shown in the main screen.

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4. Select the specific term you want to modify and a window will open that allows you to add new terms (for example if you click "Appearance" you will see that by default there are only "Normal" and "Abnormal" you can add for example, "Reddish", "Yellow", "White"). The existing options can also be modified.

Another way to manage the options of a field is with a right click on the text of the field (in the macro/microscopic data) and selecting the "Edit items" option.

1.3 Create a new sample

When you run the application, the default is to begin a new analysis. The reference number will always be the following one from the last sample reference number (you can alter this if you want and use a new one).

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1. To select an existing patient/animal: Insert the CHN (Clinical History Number) / Code (ID number) and press the Search button (magnification glass button).

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2. If the patient/animal doesn't exist in the database, press the Add Patient/Animal button and write any details about the patient/animal. The CHN/Code and Name/ID is obligatory, all other fields are optional.

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3. Fill all other fields of the macro/microscopic data window.



4. If you want to create more new samples, press the **New sample** button. A new tab will be added on the top of the window.

1.3.1 Panel of recently added samples

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1. On the right side of the screen the recently added samples are displayed. This panel is offering quick access to these samples by clicking on the reference number in case the user wants to recall a recent analysis.

1.3.2 Searching a sample



1. You can search any existing sample with the option **Search sample** in the Sample Management screen.

Searching can be done by inserting the following criteria:

- CHN (Clinical History Number) of the patient / Code of the animal (ID number) or sample reference.
- Patient/animal Name.
- Analysis date.
- Analyst.
- Centre.

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2. Press the **Search** button to see all the analysis carried out on a selected sample. You may sort the results of the analysis by pressing the header of the column.

2 SCA® Analysis Programs



1. Once the patient/animal and sample data has been entered click on the button **SCA® Programs** and select the program you want to open. You can also enter the programs by clicking the icons from the results tab.

Analysis programs available:



Automatic analysis programs:

- SCA® Motility and concentration
- SCA® Morphology
- SCA® DNA Fragmentation
- SCA® Vitality
- SCA® Acrosome reaction



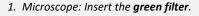
Manual analysis programs:

- SCA[®] Manual counter

2.1 SCA® Motility and concentration

2.1.1 Steps to follow







- 2. Condenser:
- Human and most animal species: Select **phase contrast** (Ph1).
- Rat: Select **pseudo phase contrast** (Ph2 or Ph3).



3. Place the specimen (using Leja slide or Makler chamber) on the microscope stage.

Objective:

- Human: Use the **10x positive phase** contrast objective.
- Most animal species: Use the 10x <u>negative</u> phase contrast objective.
- Rat: Use the **4x** brightfield objective.

4. Focus the image in the microscope and then direct the image to the camera in order to see the image on the computer screen.

SCA® Quick User's Guide for Research

5. Open the **SCA® Motility** module clicking on "Programs" button and then on "SCA® Motility".

6. Select the desired configuration and press **Enter** in the configuration window.

7. Check the current configuration (menu "Configuration" > "View/Edit").









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- 8. Observe the main analysis properties:
- Species: Select the specie
- Calibration:
 - 10x (human and most animal species)
 - 4x (rat)
- Capture method:
 - Ph+ (human: positive phase contrast)
 - Ph- (most animal species: negative phase contrast)
 - Pseudo (rat: pseudo phase contrast)
- Disposable: Leja, Makler, etc.

Press the "Save as default" button.

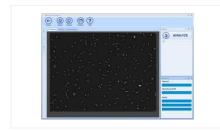


9. In main window, you will find information about the sample volume and dilution. Write the dilution if there has been any, if not leave at "0".

For the option "Post-treatment" consult the SCA® manual.

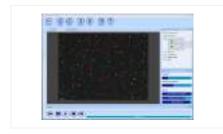


10. Press the **Analyze** button in order to display a live image on the screen.



11. Focus the image on the screen and reach the best level of contrast (adjust microscope illumination in order to achieve a homogeneous background similar to the background colour of the window).

Then capture the fields required by clicking the **Analyze** button (the software will advise you when enough fields have been captured).

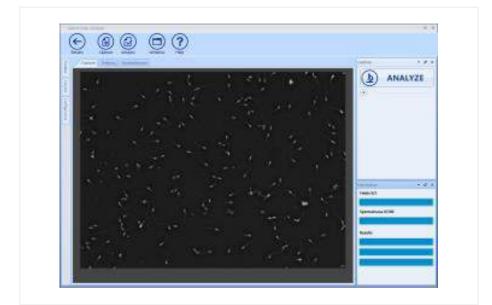


12. After capturing a sufficient number of fields click the **Results** button.



13. The main window will show the general results.

Analysis window main applications:



- Analyze: Click to start the analysis.
- Capture: View live image (start the capture).
- Analysis: View the last captured field (analysis).
- Results: Click to go back to the general results screen.
- Windows: Show/hide other panels in this window.

Note: For more in depth details about the Analysis functions please refer to the SCA® complete manual (SCA® installation disk)

Results window:



1. Classification based on progression



2. Classification based on velocity and sort groups



3. Kinematic values



4. Fields comparison

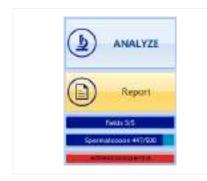


5. Spermatozoa details

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6. Data summary tool

Reports:



1. Open the Report by clicking the **Report** button. It is also possible to create reports from the Sample Management: Close the analysis program, save the analysis into the database and click the Reports option from the Sample management.

Report example:



2.2 SCA® Morphology

2.2.1 Steps to follow



1. Microscope: Insert the **blue filter**.



2. Condenser: Select the **brightfield position** (A or O) depending on the microscope.



3. Place the specimen (fixed and stained with SpermBlue) onto the microscope stage.

Objective for <u>standard</u> camera:

- Human and some animal species like dog or horse: Use the 100x objective.
- Most animal species: Use the 60x objective.

Objective for high resolution camera:

- Human and some animal species like dog or horse: Use the **60x objective**.
- Most animal species: Use the 40x objective.

4. Focus the image in the microscope and then direct the image to the camera in order to see the image on the computer screen.

5. Open the SCA® Morphology module

by clicking on "Programs" button and then select **SCA® Morphology**.

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- 6. Select the desired configuration and press **Enter** in the configuration window.

7. Check the current configuration (menu "Configuration" > "View/Edit").







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- 8. Observe the main analysis properties:
- Species: Select the specie
- Calibration for <u>standard</u> camera:
 - 100x (human and some animal species like dog or horse)
 - 60x (most animal species)
- Calibration for <u>high resolution</u> camera:
 - 60x (human and some animal species like dog or horse)
 - 40x (most animal species)
- Stain:
 - Human: Sperm Stain, Diff-Quik, SpermBlue, etc.
 - Animal: SpermBlue
- Criteria:
 - Humano: WHO, Kruger, Adaptative or Customized
 - Animal: Standard, Adaptative or Customized

Press the "Save as default" button.



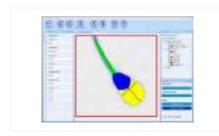
9. Press the **Analyze** button in order to see a live image.



10. Focus the image on the screen and reach the best level of contrast (adjust microscope illumination in order to achieve a homogeneous background similar to the background colour of the window).

Then capture the fields required clicking the **Analyze** button (the software will advise you when a sufficient number of fields have been captured).

Note For more in depth details about the Analysis functions please refer to the SCA® complete manual (SCA® installation disk)



11. Double click on the sperm head to visualize the individual sperm details.



12. After capturing the required number of fields click the **Results** button.



General results:



1. Results of general defects

13. The main window will show the

general results.



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3. Morphology classification

2. Sort groups classification

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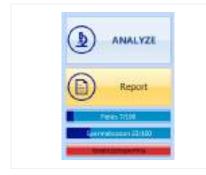
5. Fields comparison

6. Spermatozoa details

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7. Data summary tool

Reports:



1. Open the Report by clicking the **Report** button. It is also possible to create a report from the **Sample Management**: Close the analysis program, save the analysis into the database and click the Reports option from the **Sample management**.

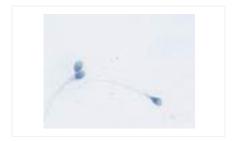
Report example:



2.2.2 Examples of correct and incorrect images

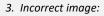


- 1. Correct image:
- Clean background
- Light stain
- Optimum illumination control
- Separated spermatozoa



- 2. Incorrect image:
- Two spermatozoa heads very close together.

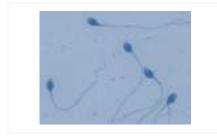




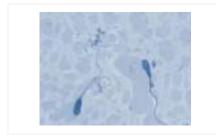
Background with a lot of detritus. Dirty background



- 4. Incorrect image:
- Too light an image (some of the midpieces cannot be analysed)



- 5. Incorrect image:
- Too dark a field (not enough contrast)



- 6. Incorrect image:
- Overstained (acrosome cannot be detected)



- 7. Incorrect image:
- One spermatozoon's head is very close together with the tail of another.

2.3 SCA® DNA Fragmentation

2.3.1 Steps to follow

Sample preparation: For the sample preparation and the interpretation of the DNA fragmentation analysis results, it is necessary to follow the steps described in the preparation protocol provided with the DNA Fragmentation kit.



1. Microscope: Insert the green filter.



2. Condenser: Select the **brightfield** position (A or O) depending on the microscope.



3. Place the specimen (previously prepared with the DNA Fragmentation kit) onto the microscope stage.

Objective: Use the **20x objective**.

4. Focus the image in the microscope and then direct the image to the camera in order to see the image on the computer screen.

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5. Open the **SCA® DNA** module by clicking on the "Programs" button and then on **SCA® DNA**.

6. Select the desired configuration and

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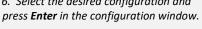
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7. Check the current configuration

(menu "Configuration" > "View/Edit").



SCA mobility

SCA dna

SCA Vitality

SCA Actosome

SCA counter

SCA morphology



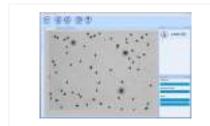
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- 8. Observe the main analysis properties:
- Species: Select the specie
- Calibration: 20x
- Capture method: Brightfield
- Disposable:
 - Halosperm (human)
 - Halomax (animal)
 - GoldCyto DNA (human and animal)

Press the "Save as default" button.



9. Press the **Analyze** button in order to see a live image on the computer screen.



10. Focus the image on the screen and reach the best level of contrast (adjust microscope illumination in order to achieve a homogeneous background similar to the background colour of the window)

Then capture the fields required by clicking the **Analyze** button (the software will advise when a sufficient number of fields have been captured).

Note For more in depth details about the Analysis functions please refer to the SCA[®] complete manual (SCA[®] installation disk)

11. After capturing the required fields click the **Results** button.



12. The main window will show the general results.

Results window:



1. Average results of the fields analysed



2. Sort groups classification



3. Fields comparison

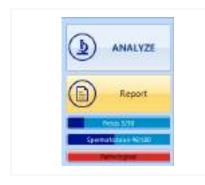
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4. Spermatozoa details

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5. Data summary tool

Report:

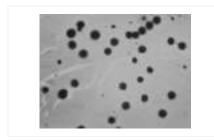


1. Open the report by clicking the **Report** button. It is also possible to create a report from the **Sample Management**: Close the analysis program, save the analysis into the database and click the Reports option from the **Sample management**.

Report example:



2.3.2 Examples of incorrect images



Incorrect image:

- Not enough space between the sperms.
- Background too dark and dirty.

Note: Check the analysis properties. The selection of the limits permits different analysis, for total halo area (selected) or for halo + core area (not selected). The choice depends on the staining (if the sperms are stained too dark, you don't see the core and it's better to capture for total halo area).

2.4 SCA® Vitality

2.4.1 Steps to follow

Sample Preparation: Follow the protocol of the FluoVit (fluorescence) or BrightVit (brightfield) kit.



1. Place the specimen (previously stained with FluoVit or BrightVit kit) on the microscope stage.

FluoVit:

- Microscope: Use fluorescence
- Fluorescence filter: DAPI filter (EX 330-380, EM 420, DM 440)
- Objective: Use the **20x objective**

BrightVit:

- Microscope: Use brightfield

Objective: Use the 20x objective



2. Focus the image in the microscope and then direct the image to the camera in order to see the image on the computer screen.



3. Open the SCA® Vitality module clicking on "Programs" button and then on SCA® Vitality.



4. Select the desired configuration and press **Enter** in the configuration window.

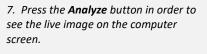


5. Check the current configuration (menu "Configuration" > "View/Edit").

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- 6. Observe the main analysis properties:
- Species: Select the specie
- Calibration: 20x
- Capture method: Fluorescence (FluoVit) or brightfield (BrightVit)
- Disposable: FluoVit or BrightVit

And press the "Save as default" button.



8. Focus the image on the screen and reach the best level of contrast (adjust microscope illumination in order to achieve a homogeneous background similar to the background colour of the window).

Then capture the fields required by clicking the **Analyze** button (the software will advise when a sufficient number of fields have been captured).

Note For more in depth details about the Analysis functions please refer to the SCA® complete manual (SCA® installation disk)

9. After capturing the required number of fields click the Results button.

10. The main window will show the general results.







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Results window:



1. Percentage of living and dead spermatozoa



2. Sort groups classification

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3. Fields comparison



4. Spermatozoa details



5. Data summarization tool

Report:



1. Open the report by clicking the **Report** button. It is also possible to create the report from the **Sample Management**: Close the analysis program, save the analysis into the database and click the Reports option from the **Sample management**.

Report example:



2.5 SCA® Acrosome reaction

2.5.1 Steps to follow

Sample Preparation: Follow the protocol of the FluoAcro kit (fluorescence).



1. Place the specimen (previously stained with FluoAcro kit) on the microscope stage.

Microscope: Use fluorescence.

Fluorescence filter: Dual band filter (EX 387/480, EM 433/530, DM 403/502)

Objective: Use the **40x plan fluor objective**.



2. Focus the image in the microscope and then direct the image to the camera in order to see the image on the computer screen.



3. Open the SCA® Acrosome module clicking on "Programs" button and then on SCA® Acrosome.



4. Select the desired configuration and press **Enter** in the configuration window.

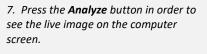


5. Check the current configuration (menu "Configuration" > "View/Edit").

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- 6. Observe the main analysis properties:
- Species: Select the specie
- Calibration: 40x
- Capture method: Fluorescence
- Disposable: FluoAcro

Press the "Save as default" button.



8. Focus the image on the screen and reach the best level of contrast (adjust microscope illumination in order to achieve a homogeneous background similar to the background colour of the window).

Then capture the fields required by clicking the **Analyze** button (the software will advise when a sufficient number of fields have been captured).

Note For more in depth details about the Analysis functions please refer to the SCA® complete manual (SCA® installation disk)

9. After capturing the required number of fields click the Results button.

10. The main window will show the general results.









Results window:



1. Percentage of intact and reacted spermatozoa



2. Sort groups classification

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3. Fields comparison

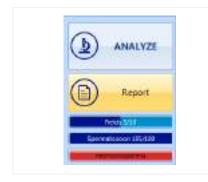
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4. Spermatozoa details

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5. Data summarization tool

Report:



1. Open the report by clicking the **Report** button. It is also possible to create the report from the **Sample Management**: Close the analysis program, save the analysis into the database and click the Reports option from the **Sample management**.

Report example:



2.6 SCA® Manual Counter

SCA® Manual Counter is an application that facilitates many types of manual cell counting. It allows you to create and arrange suitable configurations based upon your own personal counters.

The counter already has some analysis set as defaults (motility, morphology, vitality, DNA fragmentation) but you can also create new counters for different analysis or different cells.

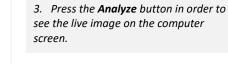
2.6.1 Steps to follow



1. Open the SCA[®] Counter module by clicking on "Programs" button and then on SCA[®] Counter.



2. Select the counter you wish to use from the available counter list and press *Enter*.

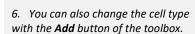


4. Focus the image and adjust the illumination to suite.

5. Use the mouse by clicking on the chosen cell. Use the buttons on the left of the screen to choose different cells.

The total number of the selected cell will be increased automatically.

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7. After counting the required number of cells click the **Results** button.



8. The main window will show the general results.

Note For more in depth details about the Analysis functions please refer to the SCA® complete manual (SCA® installation disk)

Results:



1. General Results



2. Fields comparison

2.6.2 Manual counter configuration window



1. In order to create a new counter for the analysis of other types of cells, click the **New** button from the **Configuration** menu.

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2. Insert a name for the new configuration.



3. Fill in the fields with the cell names that you need to count (features) and press "Save as default".

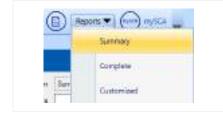
3 SCA[®] Results and Reports

Once you have all the analysis completed you can see the results or print several reports. From the "SCA® Sample management", select the results tab:

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1. **Reports buttons**: These buttons allow you to open the final results report (summary report, complete report and customized report). The button on the right allow you to display the images from the different analysis.



2. All reports can also be opened from the **Reports** option on the top right part of the menu.