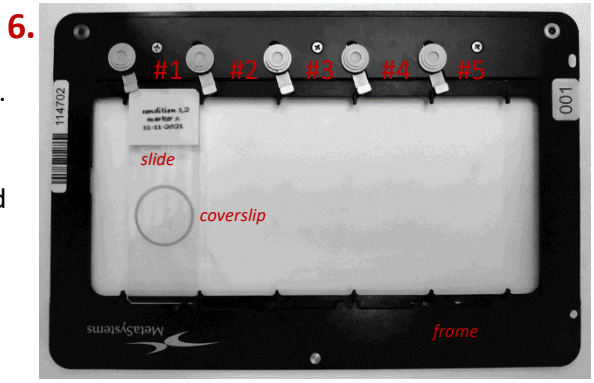
Start system

1. Start the PC, located behind monitor
   - Microscope system start automatically with PC.
2. Use light source at 50%
   - Lamp housing is located on the floor.
   - Wait for 5-10 minutes for the lamp to warm up
   - Press the button Shutter, and increase to 050 with button UP
3. Log in to windows account: Metasystems
   - For the password: check your notes or ask again.
4. Start Neon via shortcut on desktop.
5. Log in with your Neon username and password
   - These were provided during your introduction to the system.

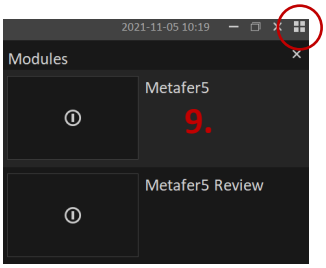
Loading frame(s)

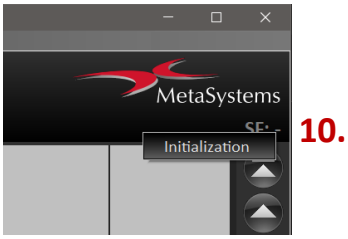
6. Fill the empty frame(s) from the SlideFeeder.
   - 5 Slides (or less) per frame.
   - Every slide can contain <u>only 1 mounted coverslip</u>.
   - The first slide should be positioned left (Furthest from the number of the frame.)
   - The marker side of the slide should be positioned at the clamp of the frame.
   - Frame is placed in the slide feeder, <u>not</u> onto the microscope stage.
   - Orientation of frame in SlideFeeder as depicted here, frame number is visible from outside.
   - The position in the frame corresponds to the order in the *Slide Setup* within the software.



Initialize microscope & robotic arm

7. Be aware of the position of the metal pin on the Zeiss microscope stand
   - In: Lightpath to ocular
   - Out: Lightpath to camera
8. Open Module tab with the small squares sign.
9. Choose Metafer5
   - XY Stage will calibrate automatically.
10. Right-mouse-click on SF- and choose initialization
    - Robot arm will move towards the SlideFeeder
    - **Make sure the space around the robotic arm is free** then, confirm warning is software.

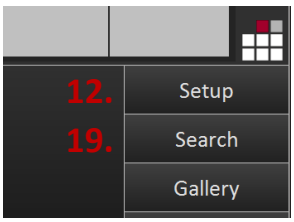


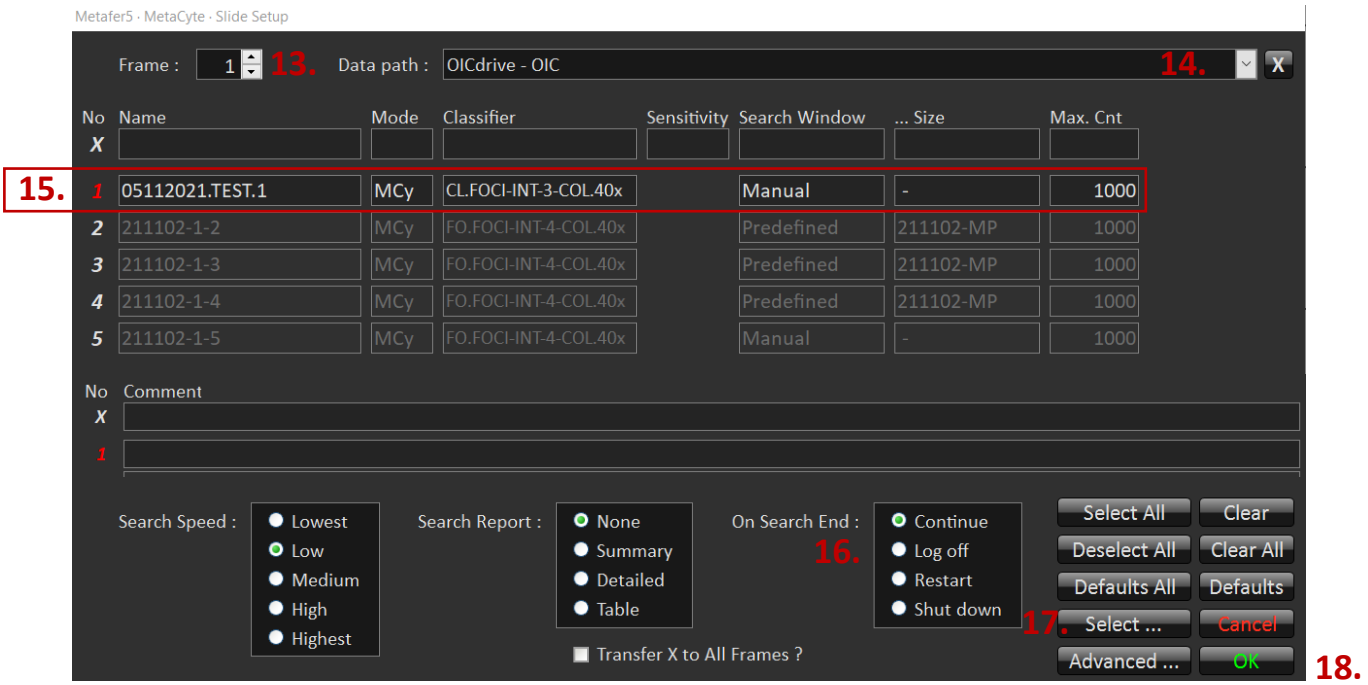


Mode: MetaCyte

The detection and analysis of more than 250 cell and staining features in single cell preparations and tissue sections. Results of analysis are stored together with images of single cells.

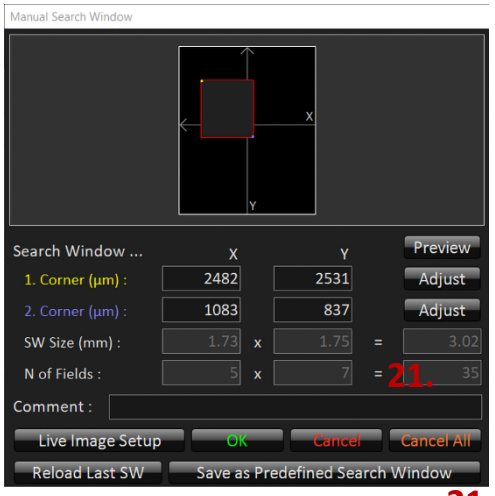
11. Start MetaCyte *Mode* via menu bar: *Mode* > *MetaCyte*.
12. Click Setup
    - Proceed in *Slide Setup* window.





13. Put in the frame number containing slides, loaded in the slide feeder
    - When multiple frames are loaded, repeat step **(13.)** per frame.
14. Datapath by default is linked to your Neon account.
15. Prepare settings per slide
    - Click on the position numbers that contain slides (Click white cross select all positions)
      - Red is active.
    - Set a name per slide
      - Use a dot to create a subfolder (2 dots max.)
      - Name <u>can't contain any spaces</u>
    - Mode is set to MCy.
    - Select a Classifier
      - Classifier is specific to your experiment and objective.
    - Search Window is set to manual
      - Setting up a new predefined region is possible later in the setup.
    - Max count
      - Max number of nuclei to be imaged per slide that meet criteria of classifier.
      - If this number isn't met, area of Search Window is limiting factor.
    - For multiple slides, this information can be put in the top fields of the white cross and passed to the activated lines below when clicking there.
16. On Search End
    - Choose *Continue*.
    - For overnight experiments choose *Shut Down*.
17. Show overview of the included slides of all frames in the slide feeder, with button *Select*
    - Red is active, white is inactive.
18. Confirm with *OK*.
19. Click *Search* in main window
    - The frame is loaded to the microscope's stage.

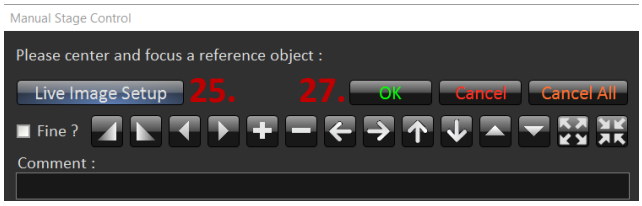
Manual search window



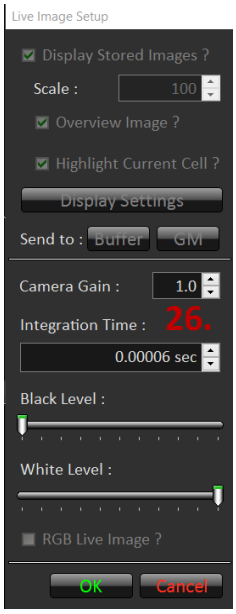
21.

20. Define a rectangular area with the second mouse on the desk (red ball) controlling the stage
    - Left button to select *corner 1*, yellow, left top.
    - Right button to select *corner 2*, purple, right bottom.
21. *N of Field* shows size of the grid.
    - This value is of importance if you use Record Fields for direct image export, you can use this to estimate the total time of acquisition.
22. Selected area can be used in Slide Setup
    - *Click Save As Predefined Search Window*.
    - Choose *Cancel All*, and go back to Slide setup **(12.)**
    - Possible to define only 1 area per slide.
23. When Slide Setup is loaded with the newly predefined area confirm with *OK*.

Find focus



24. Find focus via *Manual Stage Control*.
25. Click *Live Image Setup* to show live view
    - Use the black focus wheels on microscope stand.
26. Change parameters in Live Image Setup window to visualize sample
    - Adjust if image is too bright/dim to focus.
    - *Camera Gain*: 10.
    - *Integration Time:* 0.005 sec
      (change while live)
    - These parameters are only used for manually finding of focus reference point.
27. Confirm with *OK* once you have focus.
28. Confirm message to prepare microscope for Search.



Start Search

29. Search (actual acquisition) is started
    - The acquisition will continue until the predefined number of cells are found that meet the requirements of the selected classifier or the complete area is acquired.
    - Selected cells by the classifier are auto-contrasted, image looks different from overview.
30. When Search is finished, close Metafer5 main window.

End of session

31. Remove slides from the frame when the robotic arm is at rest.
32. Check the agenda if a next session is booked
    - www.erasmusoic.nl > OIC Schedular button > AREA: OIC services > Metafer_775.
    - The agenda is booked for the time you need to set up the system.
    - Acquisition will typically run over night.
33. Leave the complete system on for the next user.
34. When there is no user in the following 3 hours:
35. Turn off the PC, microscope system will shut down along automatically.

Manually determine integration time for classifier per channel

Exposure time can be set as fixed or can be determined by auto exposure in your classifier
Fixed is a faster method, but needs to be determined manually

36. The position of the metal pin on the microscope stand must point out, for light to camera
37. Select the desired channel via *Filters > Color*
38. If not already open the shutter via Filters > Fluorescence > Open
39. Right-mouse click in the image (left panel) to get the live acquisition window
40. Camera gain should be 5.0, integration time ranging from 0.03 – 1.0 second
41. Use these values to setup your classifiers fixed integration time

Mode: AutoCapt for Metaphase spread

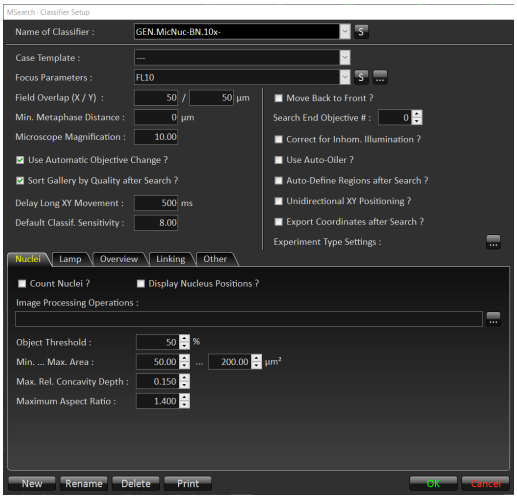
Automatically captures and exports high-quality images of objects detected by Metafer

42. Start AutCapt *Mode* via menu bar: *Mode > AutoCapt*
43. *Click Setup **(12.)** and set* Classifier
44. Continue with Search **(13. to 28.)**

Mode: Micro nuclei (MSearch)

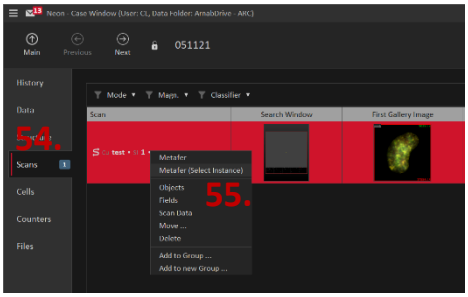
Automatically finds and counts micronuclei in specimen from the cytokinetic block micronucleus assay.

45. Start MSearch *Mode* via menu bar: *Mode > MSearch > Fluorescence*
46. Click Setup **(12.)**
47. MSearch Setup has one Classifier
    - GEN.MicNuc-BN.10x is read only
48. Confirm Classifier setup with OK
49. Start Search in main window **(13. to 28.)**
50. Acquisition will start.

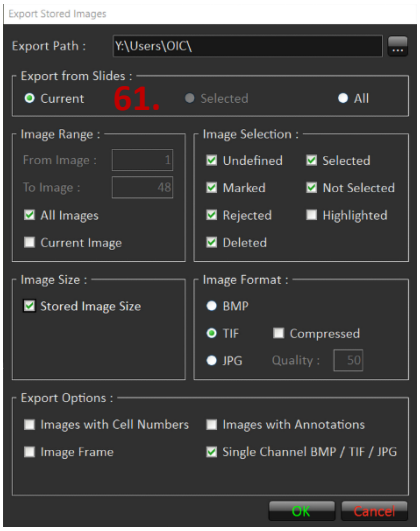


Review/Analyse data:

51. Data can be reviewed during an active Metafer5 session of another user.
52. Log off current user from Neon will not stop acquisition.
    - Via the current username shown in the top right of the main window
53. Log in to your Neon account.
54. Go to *Cases*
    - Select your experiment with a double click.
55. Select desired *Scan*
    - Right mouse click, choose *Metafer (Select instance).*



Export Training data



56. Choose Metafer review **(9.)**
57. Open Search Fields via menu bar: *Training > Search Fields*
58. *Setup is set with an* Analysis Classifier
    - Wait for analysis to finish.
59. *Export images via menu bar: Slide > Export Stored Images.*
60. Export Path is Y:\User\[*your name*].
61. Select *Current* for exporting images from current slide only.
62. Confirm with *OK*
    - *Wait for export to finish.*

Training on created data

63. Choose Metafer review **(9.)**
64. Load Scan **(54. - 55.)**
    - Main window with area & grid with individual nuclei is shown.
    - Performing training is only possible on the original data (not with suffix A, B, C, etc.)
65. Choose Setup **(12.)**
    - Select a *Classifier* for analysis in the Setup Window.
    - Confirm with *OK.*

Scan whole slide without classifier

66. Start like MetaCyte mode **(10.)**
67. Open Setup window in Metafer5
    - Use classifier **(15.)** specific for recording image only.
    - Images will be saved as tiff-format for each channel.
    - *Field Number* in the selected area is the limiting factor here, *Max Count* should be high e.g. 1000.

Record fields

This low-level high-throughput method is faster, because the lack of a classifier.
No training file available, only image files.

68. *Select fluorescent MSearch mode via: Mode > MSearch > Fluorescence*
69. *Via Setup* **(11.)** enable the slides for acquisition and desired classifier.
70. Open Record Field via menu bar: *Training > Record Fields*
71. Select area as described at **(20.)**
72. Focus as described at **(24.)**
73. Data will be saved on network drive Y:\MSTRAIN
    - Folder name is equal to name of slide
    - Remember to remove the files from this drive after acquisition/analysis/processing.

Offline workstation

- Microscope and PC in Ee-775 should be active when using the offline workstation
- When working on the offline workstation (Ee988a) the classifiers are different
- Ask the OIC to get the right classifier available on the workstation.

Solutions to possible errors

- **Z position is out of range**
  - At the touch screen of the microscope stand
  - Select Microscope tab
  - Choose pos. 6 (no objective)
  - Conforming error message with *OK*
- **Slide does not exist**
  - After confirming settings of Slide Setup, error message about non existing slide
  - Possible spaces or invisible illegal characters in name of slide(s)
  - Retype complete name of slide(s)

CONTACT INFORMATION

For direct support at the microscope contact the OIC via telephone number 35813**.**
For other question contact us via:

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