

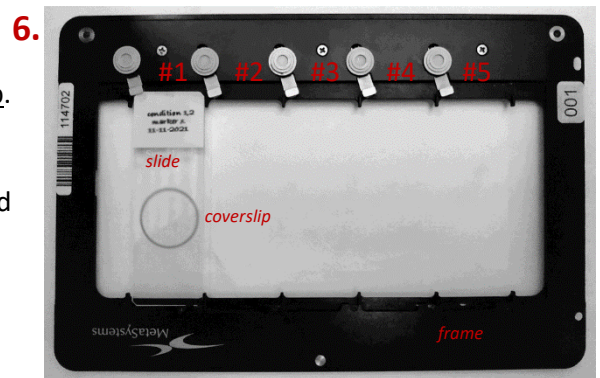
## Start system

1. Start the PC, located behind monitor
  - o Microscope system start automatically with PC.
2. Check if the light source is ready
  - o Lamphouse connected at the back of the microscope.
  - o Blue LED is continuously on means ready, blinking is warming up or cooling down.
3. Log in to windows account: Metasystems
  - o 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
  - o These were provided during your introduction to the system.



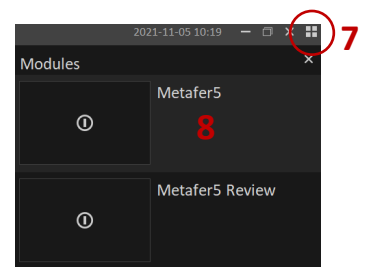
## Loading frame(s)

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



## Initialize microscope & robotic arm

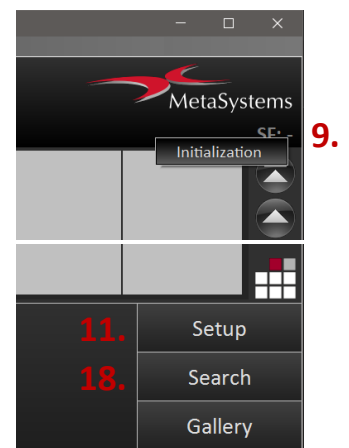
7. Open Module tab with the small squares sign.
8. Choose Metafer5
  - o XY Stage will calibrate automatically.
9. Right mouse click on SF- and choose initialization
  - o Robot arm will move towards the SlideFeeder
  - o **Make sure the space around the robotic arm is free** then, confirm warning is software.



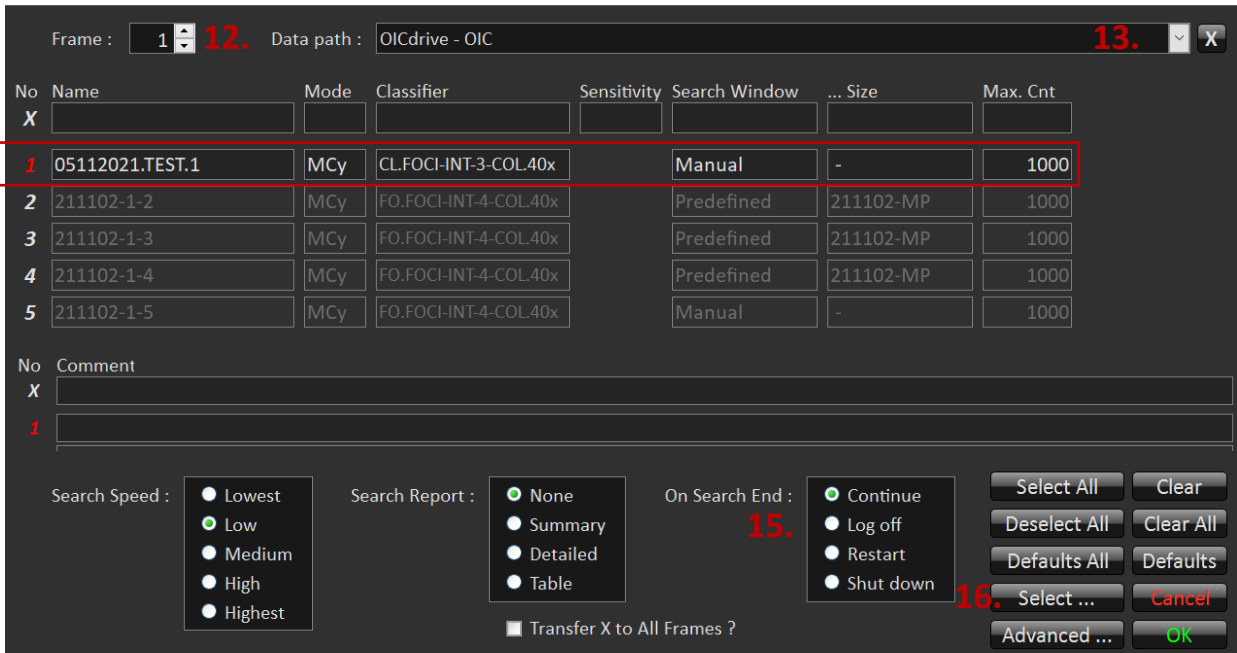
## Mode: MetaCyte

*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.*

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



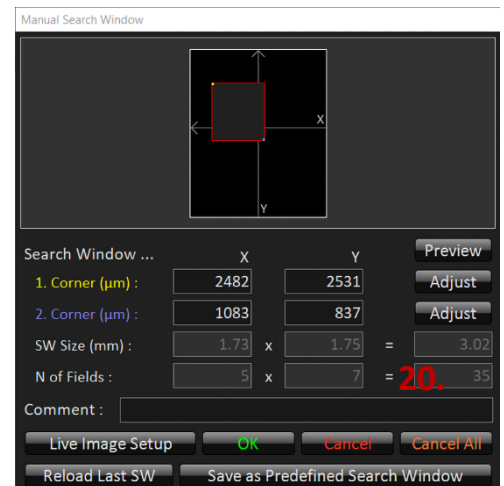
Metafer5 · MetaCyte · Slide Setup



12. Put in the frame number containing slides, loaded in the slide feeder
  - o When multiple frames are loaded, repeat step (14.) per frame.
13. Datapath by default is linked to your Neon account.
14. Prepare settings per slide
  - o Click on the position numbers that contain slides (Click white cross select all positions)
    - Red is active.
  - o Set a name per slide
    - Use a dot to create a subfolder (2 dots max.)
    - Name can't contain any spaces
  - o Mode is set to MCy.
  - o Select a Classifier
    - Classifier is specific to your experiment and objective.
  - o Search Window is set to manual
    - Setting up a new predefined region is possible later in the setup.
  - o 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.
15. On Search End
  - o Choose *Continue*.
  - o For overnight experiments choose *Shut Down*.
16. Show overview of the included slides of all frames in the slide feeder, with button *Select*
  - o Red is active, white is inactive.
17. Confirm with *OK*.
18. Click *Search* in main window
  - o The frame is loaded to the microscope's stage.

## Manual search window

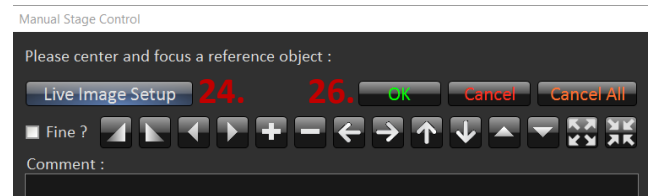
19. Define a rectangular area with the second mouse on the desk (red ball) controlling the stage
  - o Left button to select *corner 1*, yellow, left top.
  - o Right button to select *corner 2*, purple, right bottom.
20. *N of Field* shows size of the grid.
21. Selected area can be used in Slide Setup
  - o Click *Save As Predefined Search Window*.
  - o Choose *Cancel All*, and go back to Slide setup **(11.)**
  - o Possible to define only 1 area per slide.
22. When Slide Setup is loaded with the newly predefined area confirm with *OK*.



**21.**

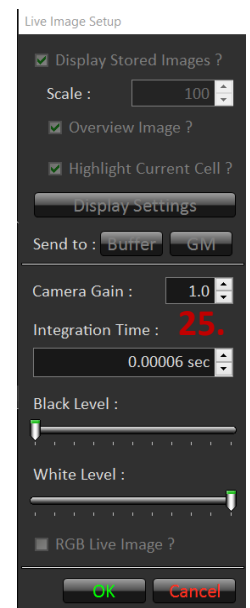
## Find focus

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



## Start Search

28. Search (actual acquisition) is started
  - o 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.
  - o Selected cells by the classifier are auto-contrasted, image looks different from overview.
29. When Search is finished, close Metafer5 main window.



## End of session

30. Remove slides from the frame when the robotic arm is at rest.
31. Check the agenda if a next session is booked
  - o [www.erasmusoic.nl](http://www.erasmusoic.nl) > OIC Scheduler button > AREA: OIC services > Metafer\_775.
  - o The agenda is booked for the time you need to set up the system.
  - o Acquisition will typically run over night.
32. Leave the complete system on for the next user.
33. When there is no user in the following 3 hours:
34. Turn the PC off, microscope system will shut down along automatically.

## Mode: AutoCapt for Metaphase spread

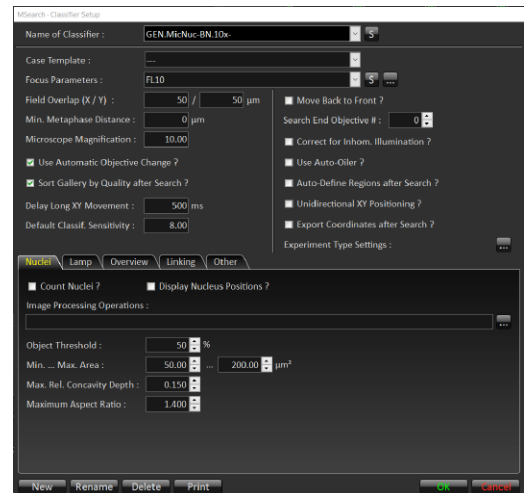
Automatically captures and exports high-quality images of objects detected by Metafer

35. Start AutoCapt Mode via menu bar: *Mode > AutoCapt*
36. Click Setup (11.) and set Classifier
37. Continue with Search (18. to 28.)

## Mode: Micro nuclei (MSearch)

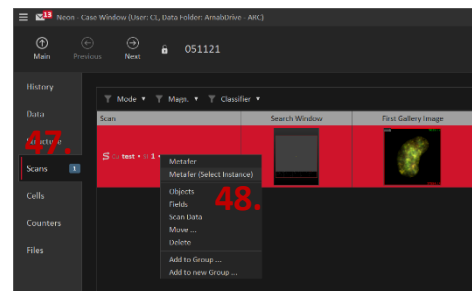
Automatically finds and counts micronuclei in specimen from the cytokinetic block micronucleus assay.

38. Start MSearch Mode via menu bar: *Mode > MSearch > Fluorescence*
39. Click Setup (11.)
40. MSearch Setup has one Classifier
  - o GEN.MicNuc-BN.10x is read only
41. Confirm Classifier setup with OK
42. Start Search in main window (18. to 28.)
43. Acquisition will start.



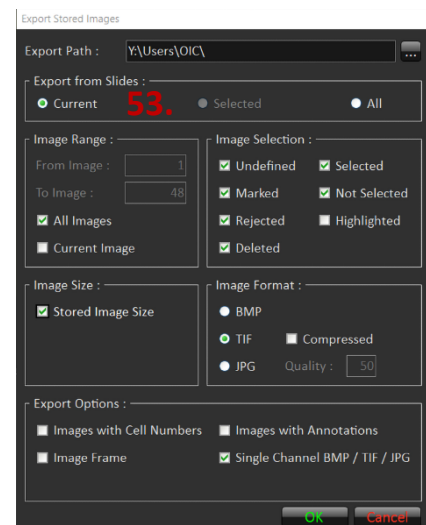
## Review/Analyse data:

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



## Create Training data

49. Choose Metafer review (7.)
50. Open Search Fields via menu bar: *Training > Search Fields*
51. Setup is set with an Analysis Classifier
  - o Wait for analysis to finish.
52. Export images via menu bar: *Slide > Export Stored Images*.
53. Export Path is Y:\User\[your name].
54. Select *Current* for exporting images from current slide only.
55. Confirm with OK
  - o Wait for export to finish.



## Training on created data

56. Choose Metafer review **(7.)**
57. Load Scan **(47. - 48.)**
  - 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.)
58. Choose Setup **(11.)**
  - Select a *Classifier* for analysis in the Setup Window.
  - Confirm with *OK*.

## Scan whole slide without classifier

59. Start like MetaCyte mode **(10.)**
60. Open Setup window in Metafer5
  - Use classifier **(14.)** 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 really 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.

61. Open Record Field via menu bar: *Training > Record Fields*
62. Select area as described at **(19.)**
63. Focus as described at **(23.)**
64. Data will be saved on network drive Y:\MSTRAIN
  - Folder name is equal to name of slide



## Offline workstation

- Microscope and PC in Ee-775 should be active when using the offline workstation
- When working on the offline workstation (Ee2173) the classifiers are different
- Ask the OIC or Calvin Lo to get the right classifier 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 Calvin Lo or call 31105.

For other question contact us via:

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