

Software Guide

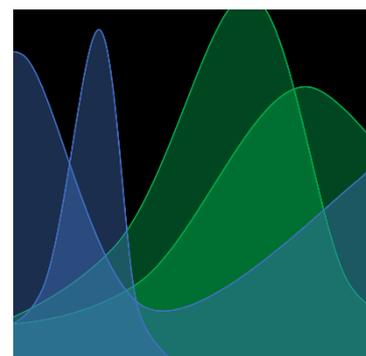
Fluorescence Viewer

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[1] SYNENTEC GmbH



INTRODUCTION

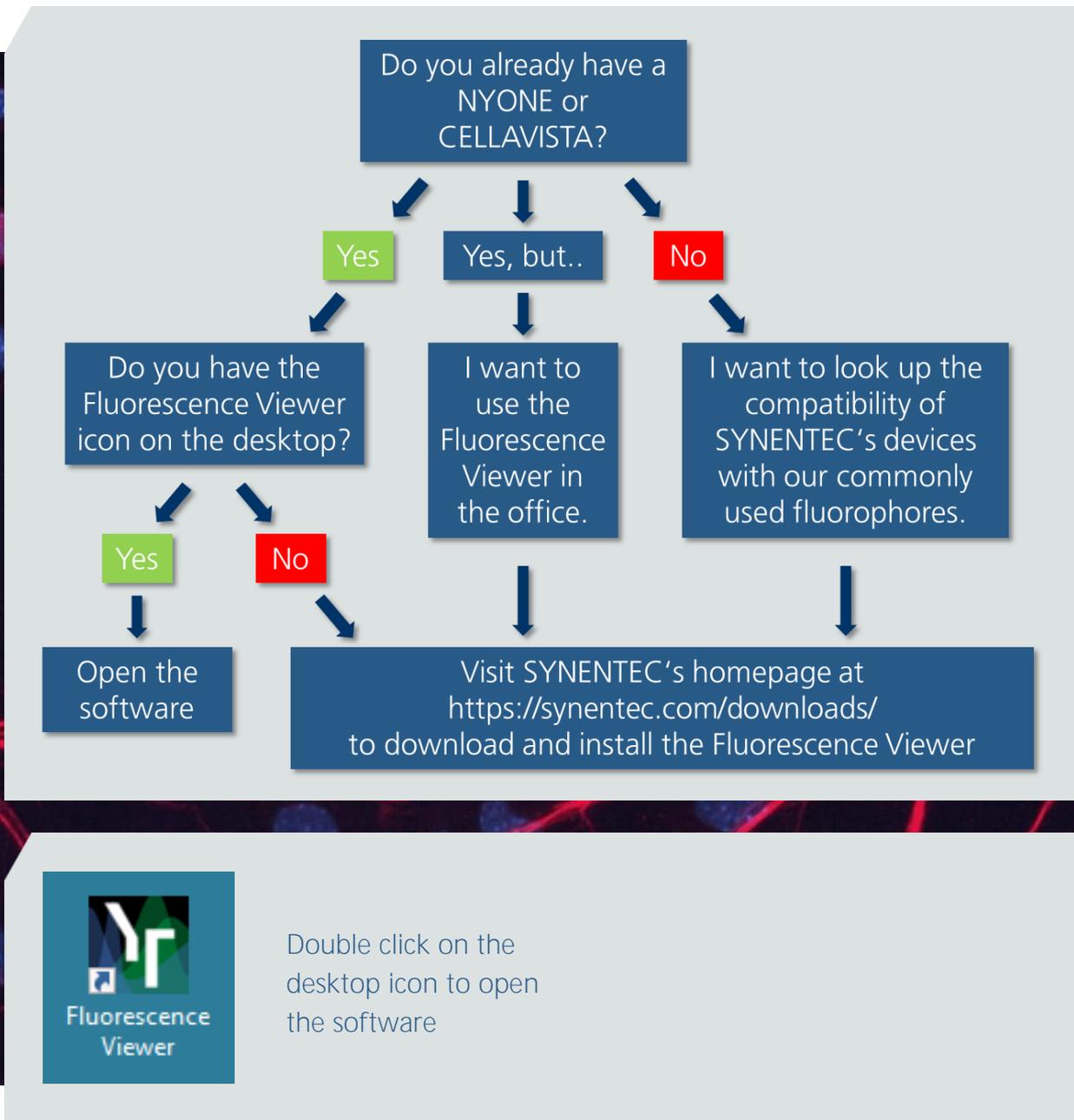
The Fluorescence Viewer is a standalone tool designed by SYNENTEC GmbH. It can help in the planning of fluorescence assays for NYONE[®] and CELLAVISTA[®] by quickly visualizing fluorescence spectra from various fluorophores, dyes and proteins as well as the fluorescence imaging capabilities and settings of our devices.



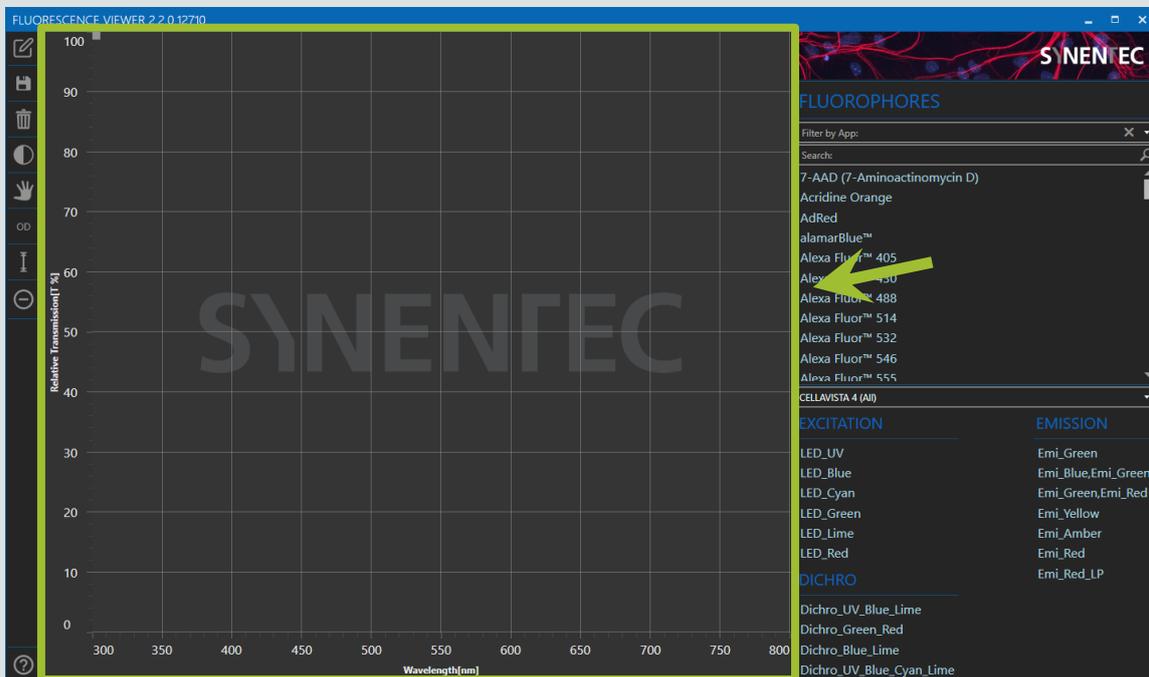
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1 GETTING STARTED



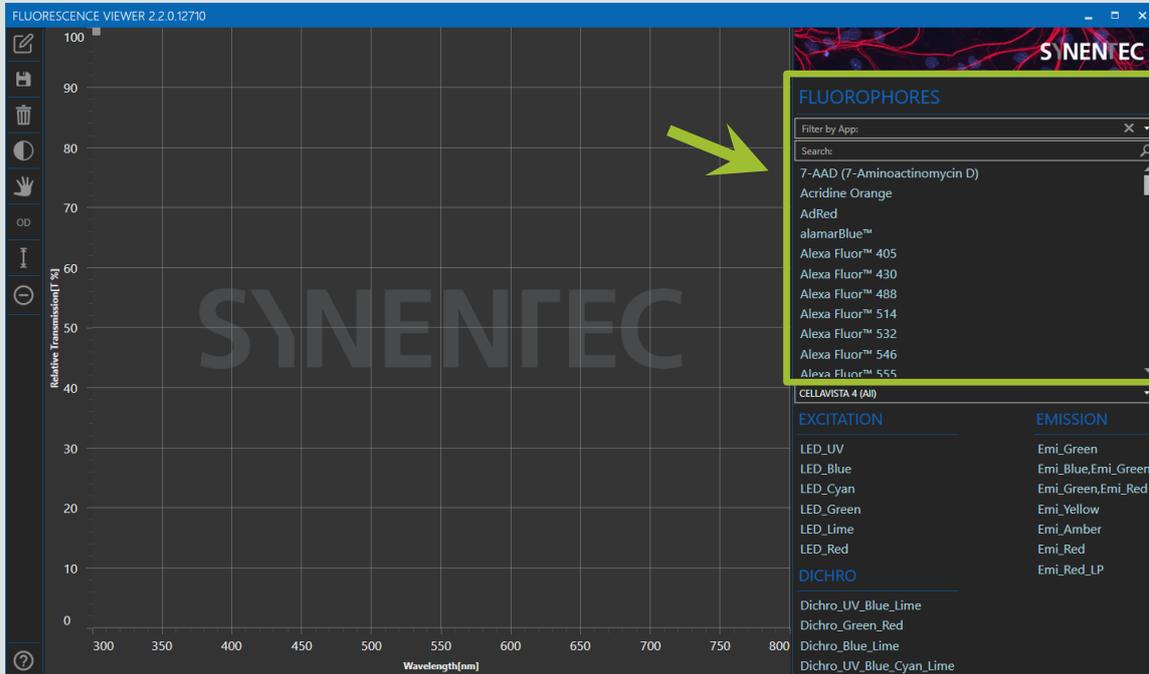
2 CHART



- The chart in the middle displays the relative transmission [%] per wavelength [nm] of the fluorophores, excitation LEDs, dichroic mirrors and emission filters selected on the right hand side of the software
- X- and Y-axis are automatically scaled depending on the selections
- To zoom in and out, use the mouse wheel
- The axes can be moved left/right or up/down with the double arrow that appears when the cursor is positioned over the specific axis
- A double click within the chart resets the scaling

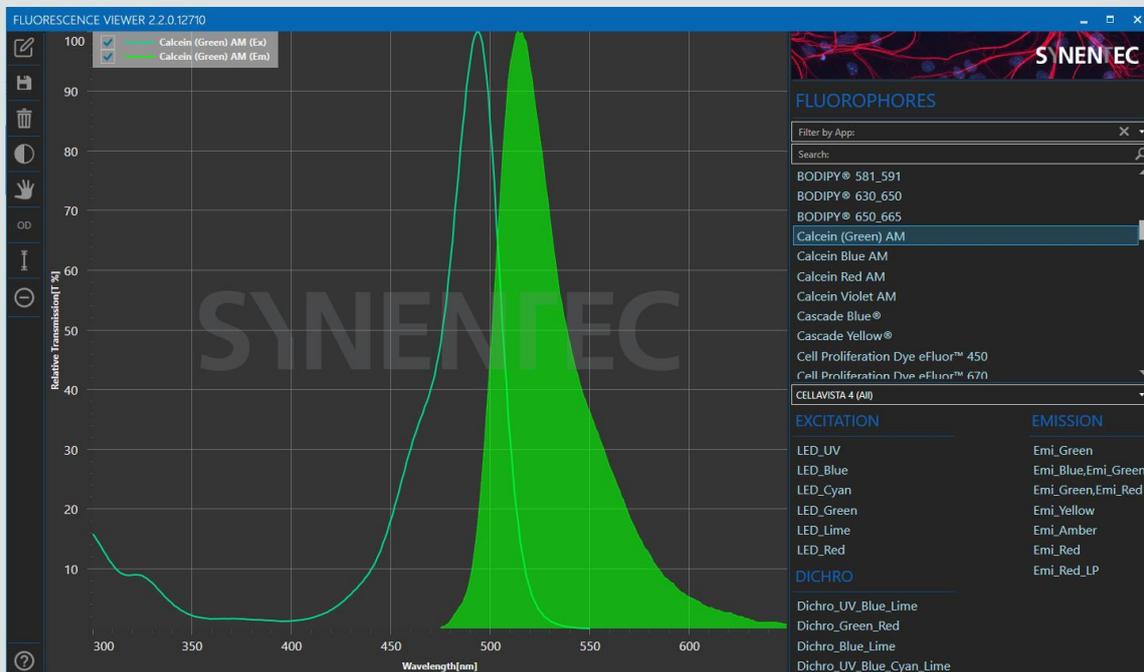
3 RIGHT HAND SIDE

3.1 Fluorophores



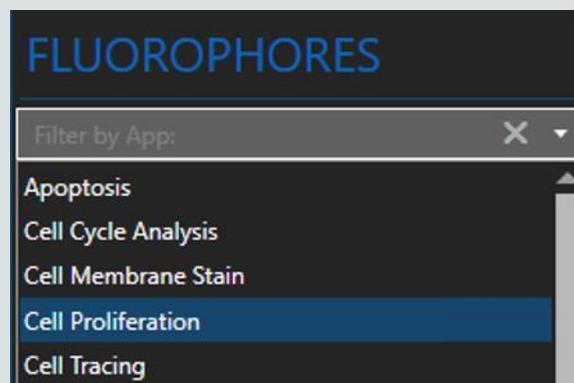
- Click on a fluorophore to select
- A tooltip box shows more information when the cursor is moved over the fluorophore, e.g. the application for which it is commonly used

- The excitation and emission spectra of the selected fluorophore are displayed in the chart. The Fluorescence Viewer will scale to fit the fluorophore spectra.

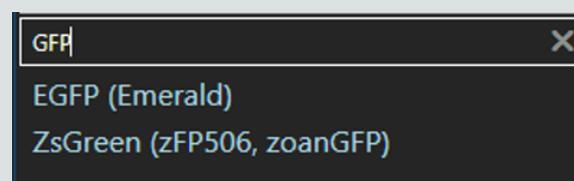


- The unfilled graph shows the excitation and the filled graph the emission
- To deselect a fluorophore, click on it again

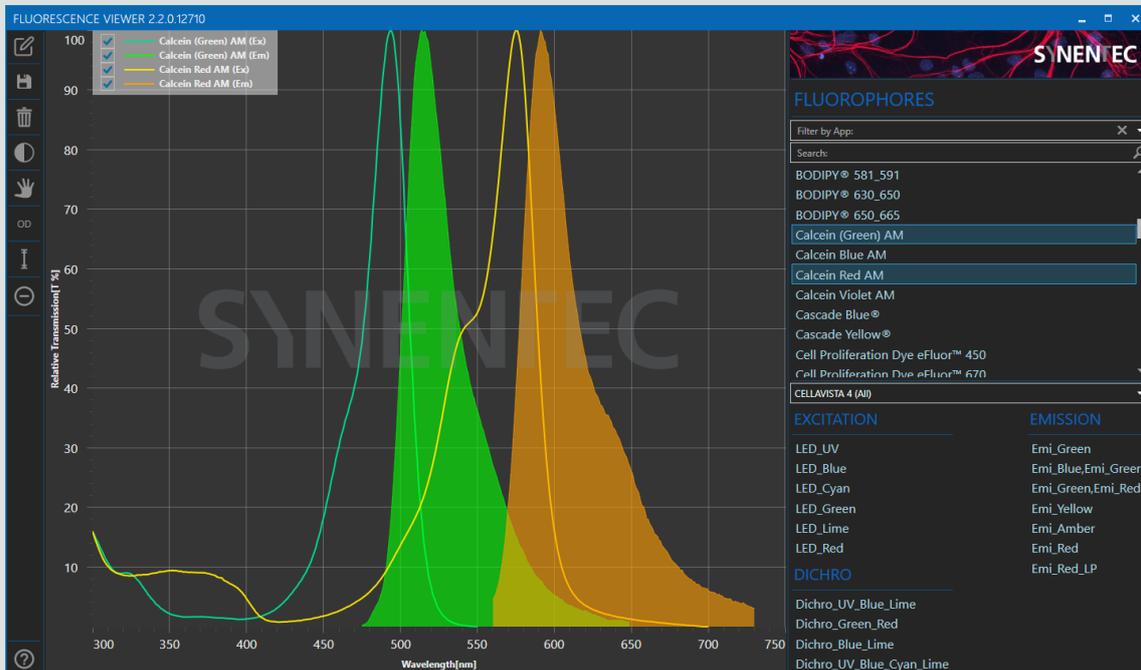
- With "Filter by App" it is possible to display only fluorophores of one specific application, e.g. "Cell Proliferation"
- To clear the app selection, click on the "X" on the "Filter by App" drop-down menu



- The search bar can be used to search for a specific fluorophore, dye or protein
- Previously added fluorophores remain selected during the search

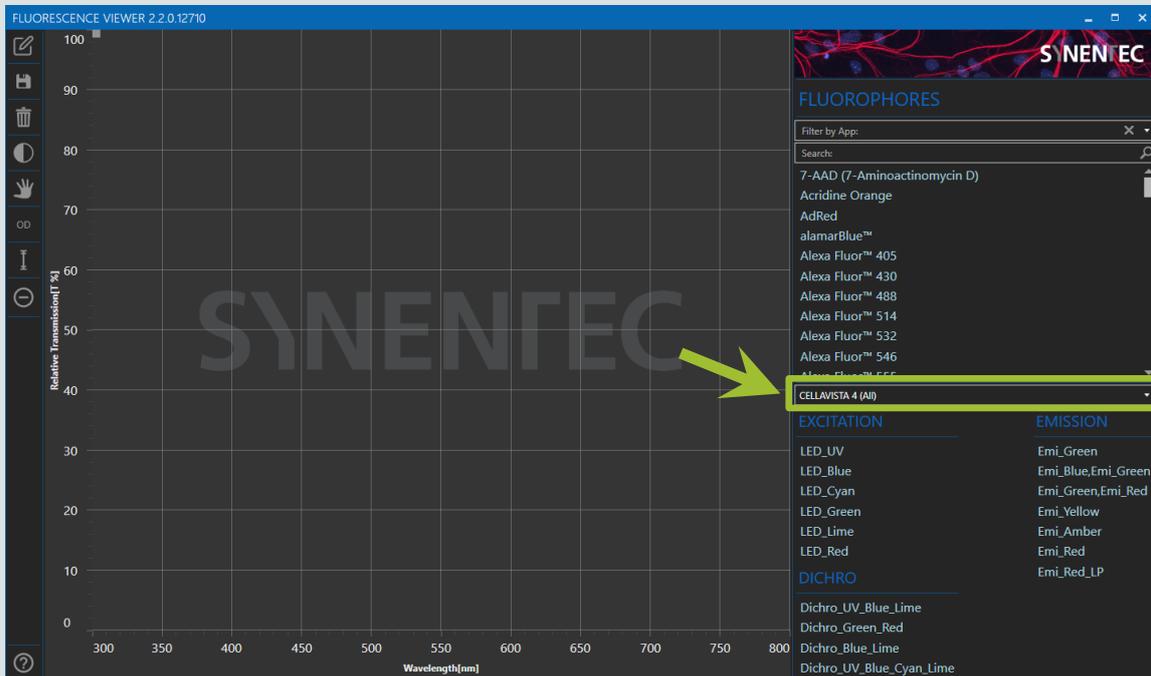


- Multiple fluorophores are selectable

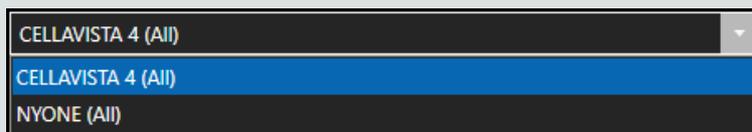


- In the legend at the top left, excitation and emission of each fluorophore can be selected and deselected separately
- The chart can be cleared via the bin symbol on the left hand side (p. 11, chapter 4.3)

3.2 Device



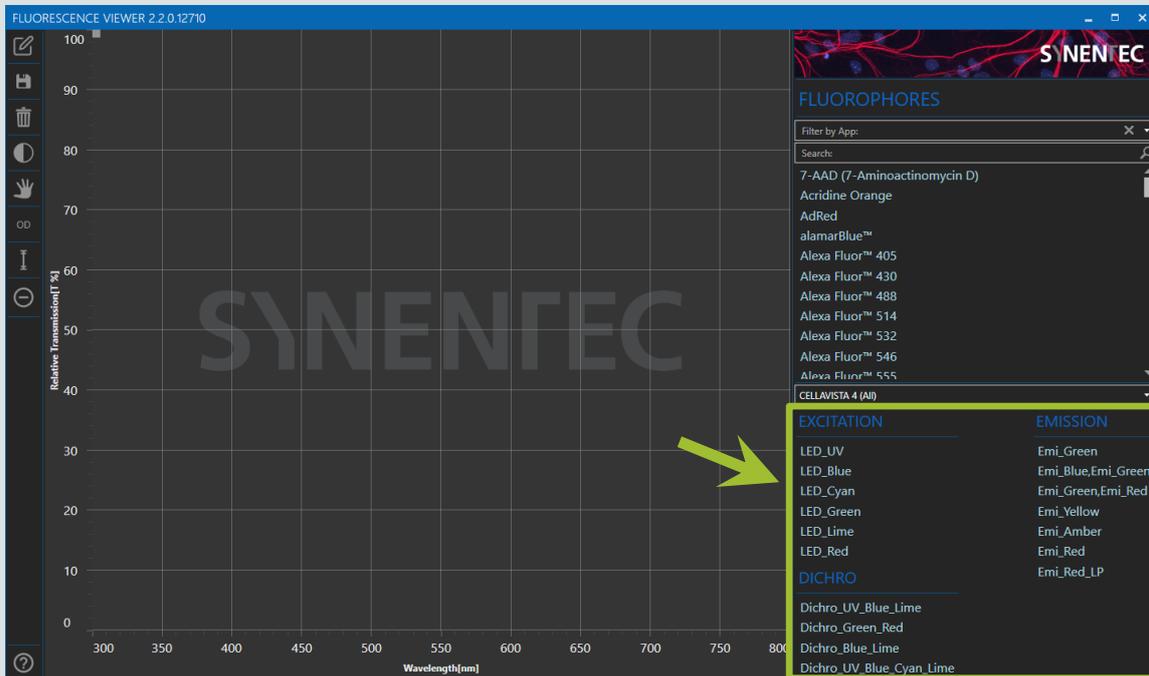
- Open drop-down menu to choose between SYNENTEC's devices: CELLAVISTA 4 or NYONE
- The CELLAVISTA has 6 fluorescence channels, the NYONE 4



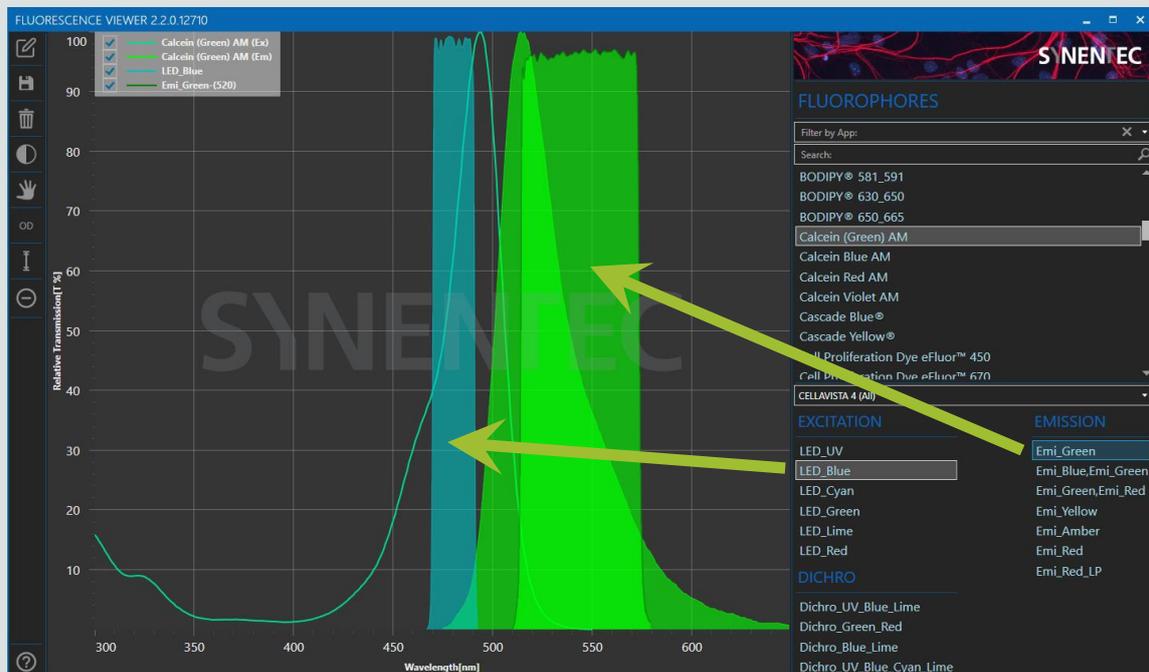
CELLAVISTA 4 (All)
EXCITATION
LED_UV
LED_Blue
LED_Cyan
LED_Green
LED_Lime
LED_Red

NYONE (All)
EXCITATION
LED_UV
LED_Blue
LED_Lime
LED_Red

3.3 Excitation & Emission



- Excitation: List of the standard built-in LEDs
- Emission: List of the most frequently used emission filters



- Dichros: The ones that are built into the actual device depend on the available LEDs, e.g. a blue excitation LED requires a "Dichro_Blue"

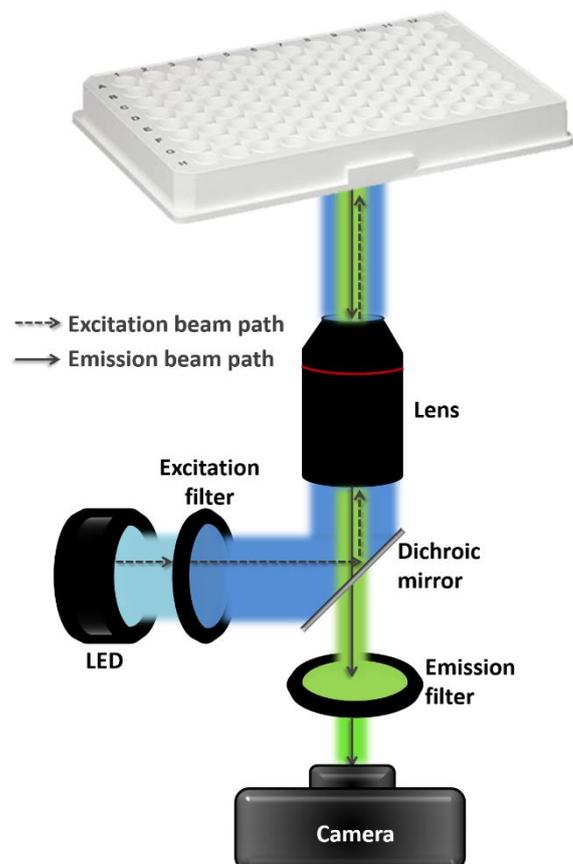
- LEDs, filters and dichros can also be hidden and shown with the legend
- Various combinations are possible, depending on the requirements and applications
- If you already have a device, it is useful to know which filters are installed to plan fluorescence assays using our Fluorescence Viewer
- Deselect all LEDs and filters with the trashcan symbol on the left hand side (p. 11, chapter 4.3)

Excitation Filter

The light of an LED first passes through an excitation filter to clean up the excitation wavelengths. This cleaned excitation spectrum is displayed as an excitation curve when an LED is selected on the right side.

Emission Filter

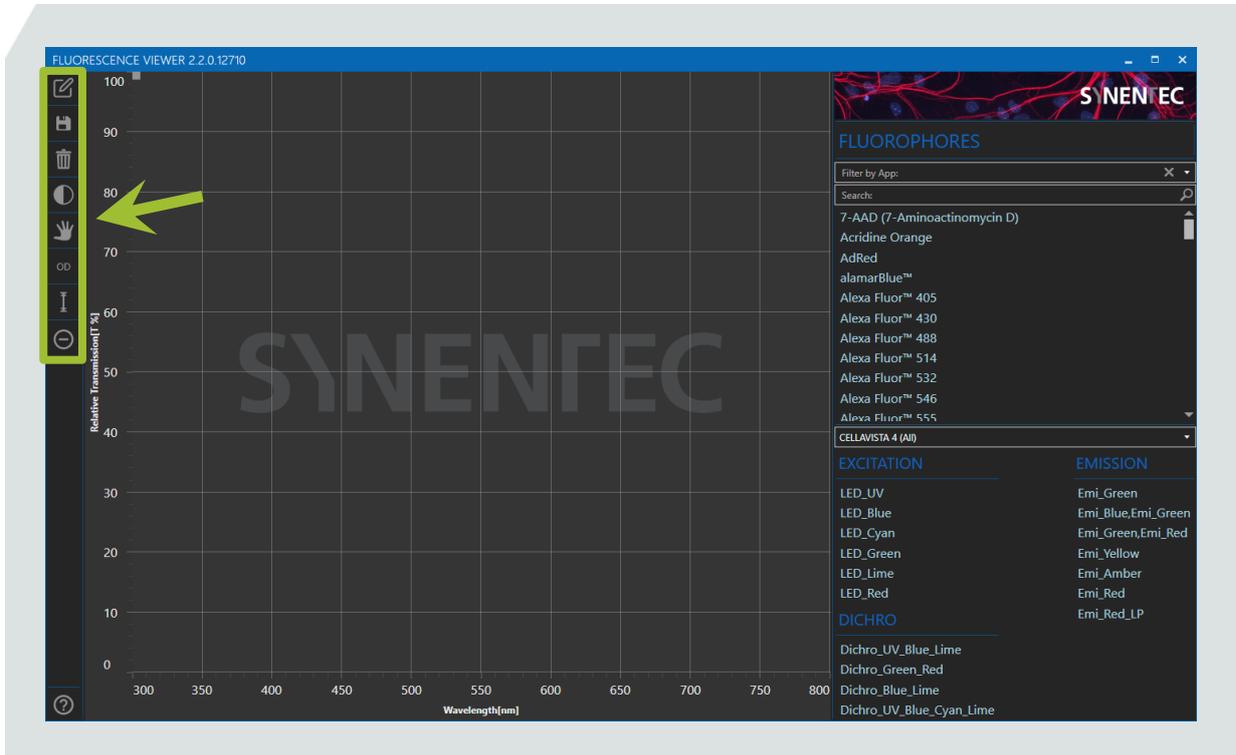
An emission filter ensures that only the light emitted by the fluorophores within the sample is transmitted to the camera whereas the remaining excitation and scattered light is blocked by the filter. The filled graph, which is displayed in the chart when an emission filter is selected, shows the wavelength range of light that can pass through the filter. Through an "Emi_Green" only emitted light from the green wavelength range can pass, here from about 515 to 574 nm. A few of the listed EMISSION filters are named e.g. "Emi_Blue, Emi_Green". Those filters are commonly called polychroic filters and are combined filters through which light from two or more different wavelength ranges can pass (e.g. blue 410 – 454 nm and green 507 – 554 nm). Using the checkboxes in the legend, a certain spectra of the polychroic filter can be hidden if it becomes confusing when planning assays or saving charts.



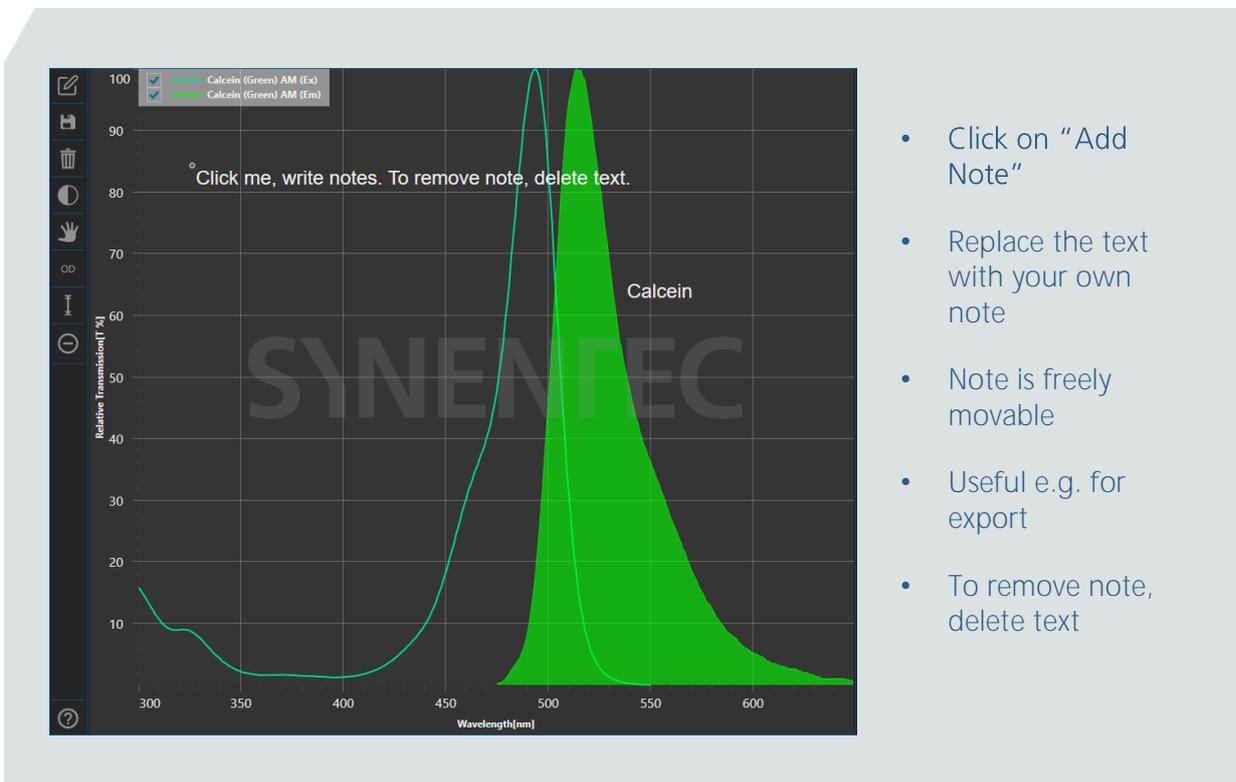
Dichro/Dichroic Mirror

A dichroic mirror is a filter that reflects light of a specific shorter wavelength, but allows another longer wavelength to pass. It reflects the excitation light in the beam path towards the sample, but lets the emitted light of longer-wavelength pass through to the emission filter towards the camera. The chart of the Fluorescence Viewer shows the filter property of the dichro, the wavelength range in which the emitted light can pass. The name of the dichro e.g. "Dichro_Blue" shows which excitation light can be reflected towards the sample, i.e. for which LED it needs to be chosen.

4 LEFT HAND SIDE: EDITING MENU

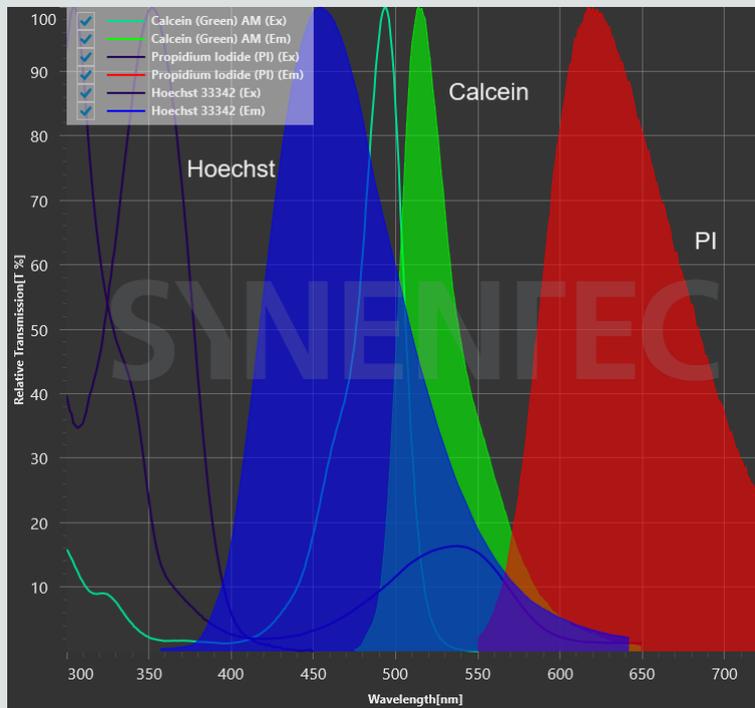


4.1 Add a Note to Chart



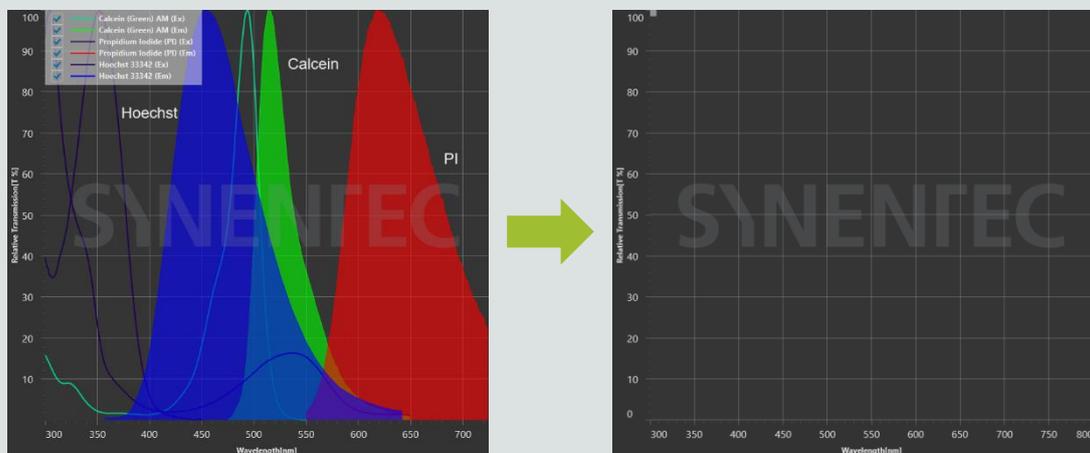
- Click on "Add Note"
- Replace the text with your own note
- Note is freely movable
- Useful e.g. for export
- To remove note, delete text

4.2 Save Chart



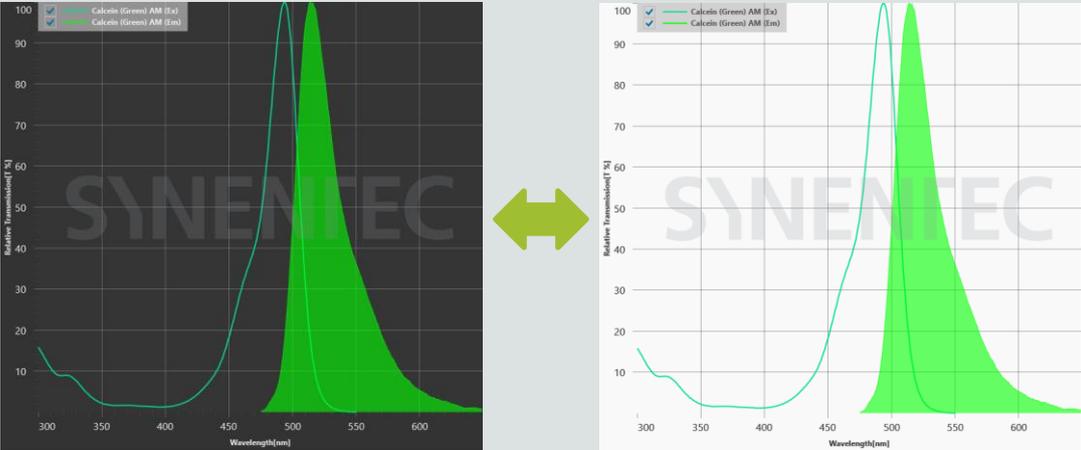
- Export chart as PNG
- Save image with spectra, LEDs, dichros, filters, notes, vertical slices

4.3 Clear Chart



- Click on bin symbol to delete all selected spectra, LEDs, dichros, filters, notes, vertical slices

4.4 Switch Chart to Dark-Light Mode

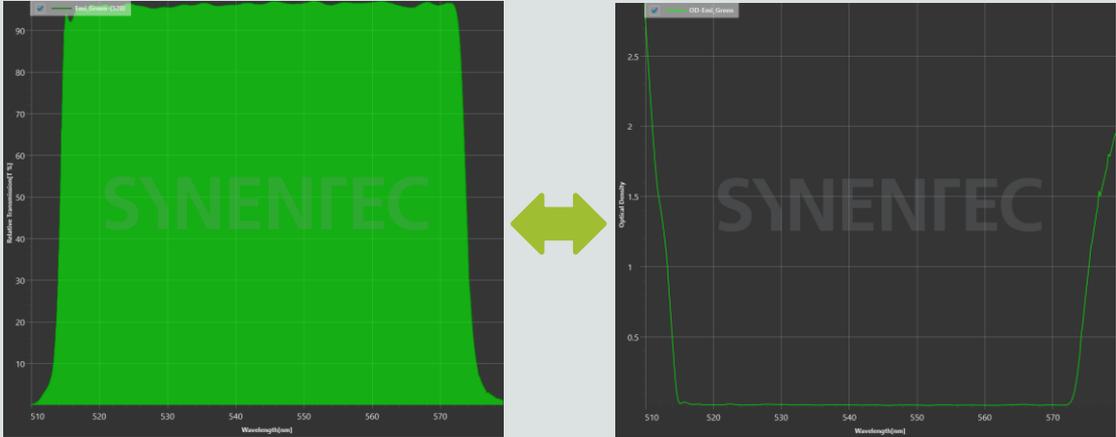


- Use the button to change the chart's background from grey to white
- Useful e.g. for printing the chart
- When mode is selected, symbol becomes blue

4.5 Enable Pan Mode

- Use the hand symbol to move the diagram right, left, up and down
- A double-click on axis label deletes changes
- When mode is selected, symbol becomes blue
- Click again to disable pan mode

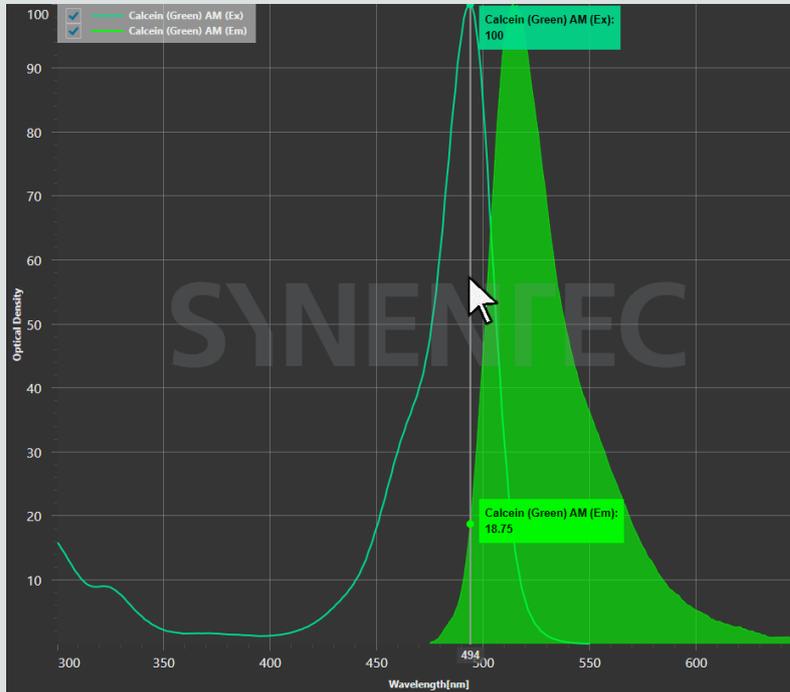
4.6 Enable Optical Density for Filters OD



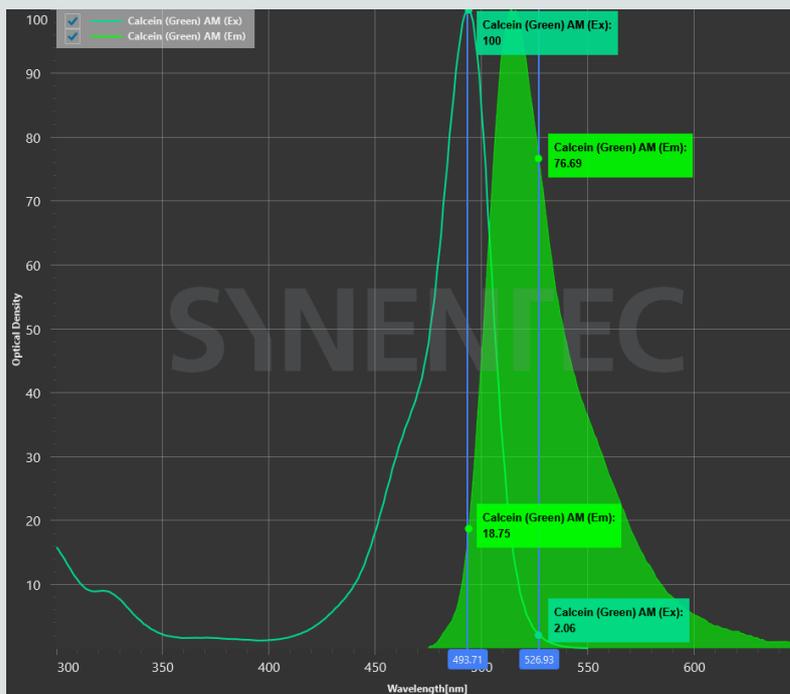
- Enable OD to change from the relative transmission to the optical density of a filter
- When mode is selected, symbol becomes blue
- Click again to show relative transmission again

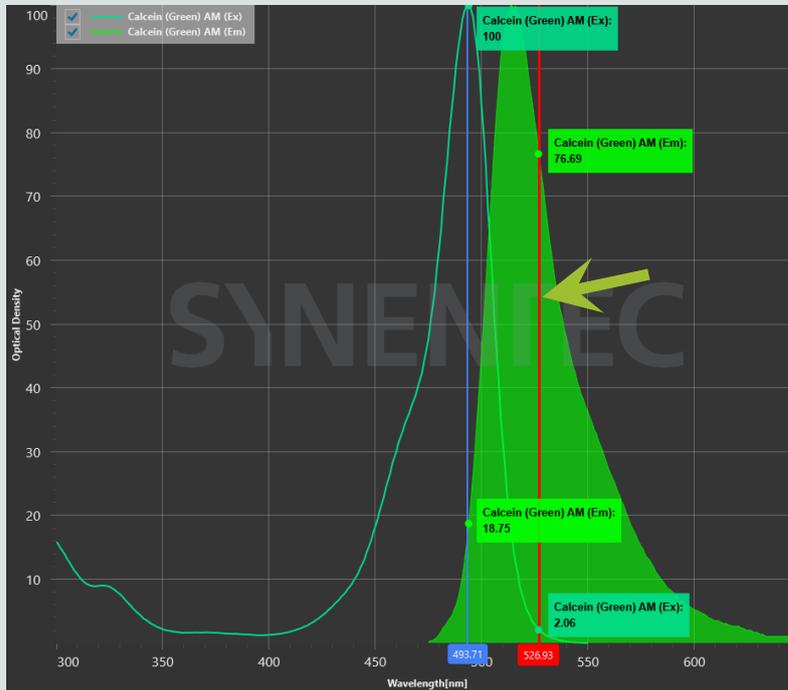
The Optical Density is the logarithmic ratio of light falling on a filter to the light transmitted through the filter. The higher the OD, the less light goes through the filter or the higher the OD, the better the blocking. So an optical density of 0 means that the selected wavelength can pass the filter completely.

4.7 Add a Vertical Slice



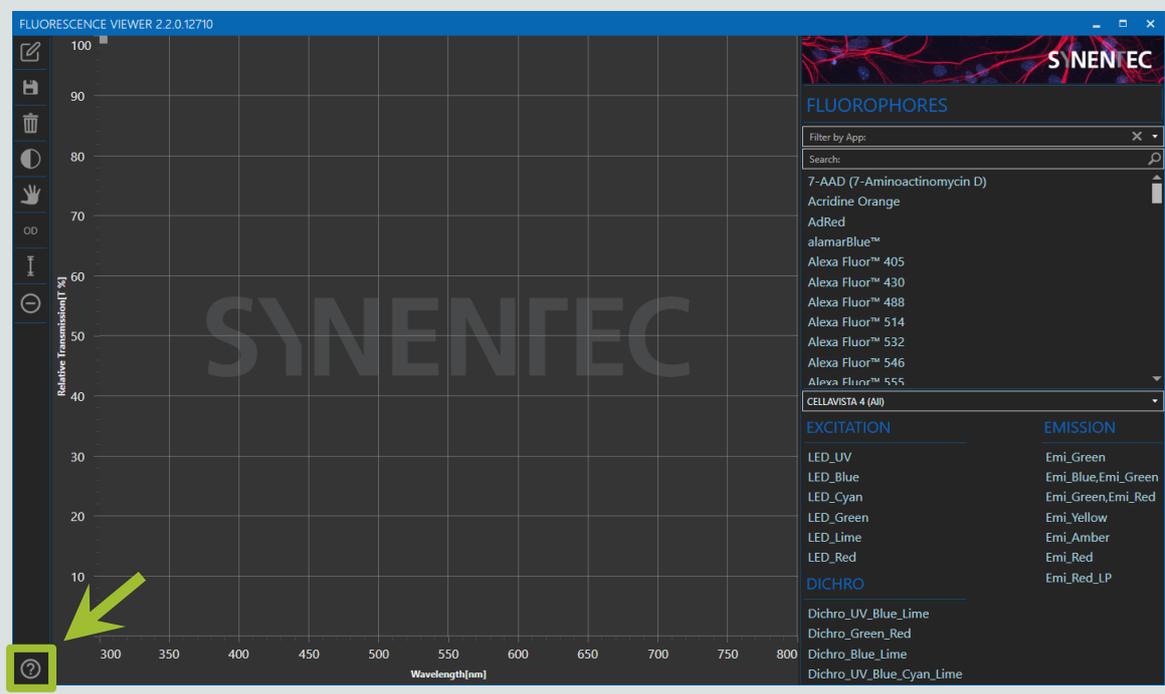
- Click on “Add a Vertical Slice” button
- Cursor over the chart
- Click to fix vertical slice
- Vertical slice shows intersections with curves as boxes with names and values
- The x-axis shows the wavelength at which the slice is located
- To add a second slice click on the button again and on the position in the chart



4.8 Remove Vertical Slice 

- Click on the vertical slice which should be deleted
- Marked vertical slice becomes red
- Click on the remove vertical slice button on the left hand side

5 HELP



FLUORESCENCE VIEWER 2.2.0.12710

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FLUOROPHORES

Filter by App: X

Search:

- 7-AAD (7-Aminoactinomycin D)
- Acridine Orange
- AdRed
- alamarBlue™
- Alexa Fluor™ 405
- Alexa Fluor™ 430
- Alexa Fluor™ 488
- Alexa Fluor™ 514
- Alexa Fluor™ 532
- Alexa Fluor™ 546
- Alexa Fluor™ 555

CELLAVISTA 4 (All)

EXCITATION	EMISSION
LED_UV	Emi_Green
LED_Blue	Emi_Blue,Emi_Green
LED_Cyan	Emi_Green,Emi_Red
LED_Green	Emi_Yellow
LED_Lime	Emi_Amber
LED_Red	Emi_Red

DICHRO

- Dichro_UV_Blue_Lime
- Dichro_Green_Red
- Dichro_Blue_Lime
- Dichro_UV_Blue_Cyan_Lime

- Click on the question mark in the lower left corner of the software to open the Fluorescence Viewer guide

Your preferred fluorophore is not available in the software? LEDs and filters do not quite fit your standards in the lab? Or do you need application support or technical service for your SYNENTEC system or just want to have more information about our products, please get in touch.

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