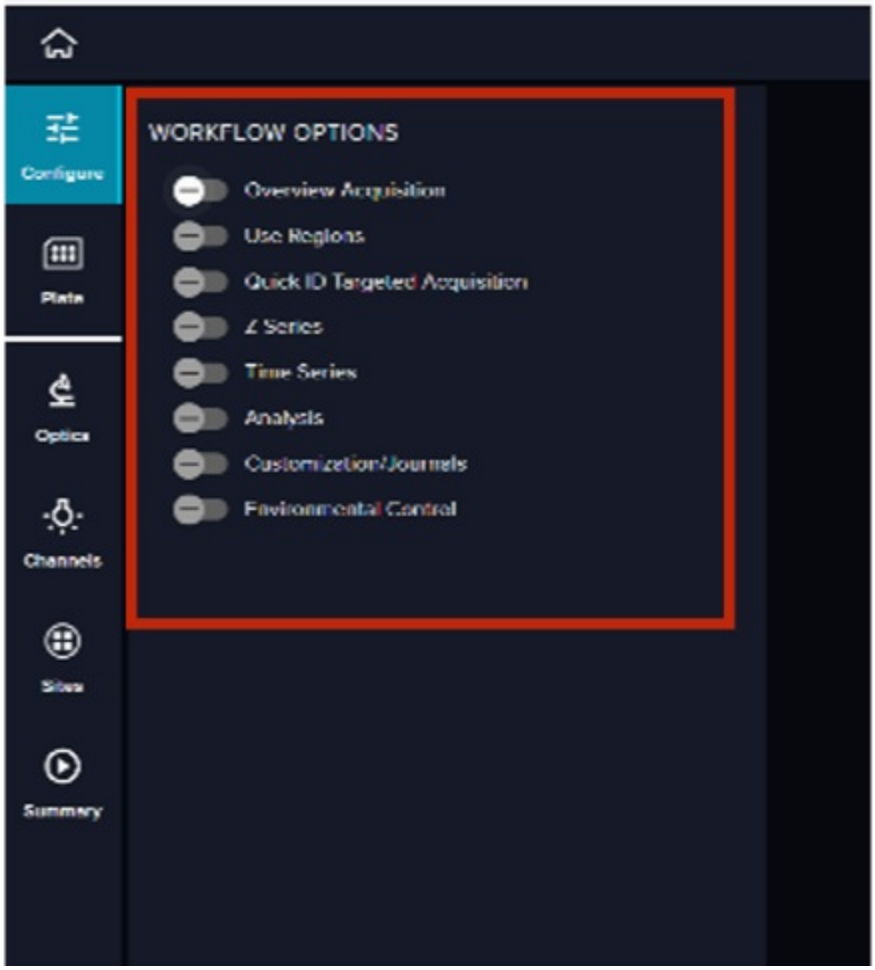
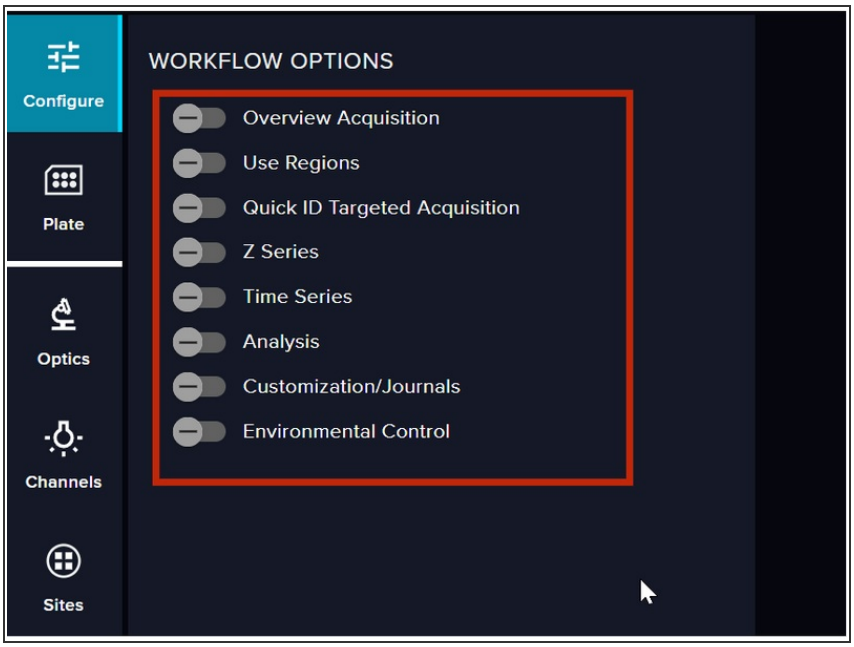
MD ImageXpress HCS.ai - 2. targeted acquisition

Set up the acquisition parameters for imaging plates and slides.

Written By: Dayra Lorenzo

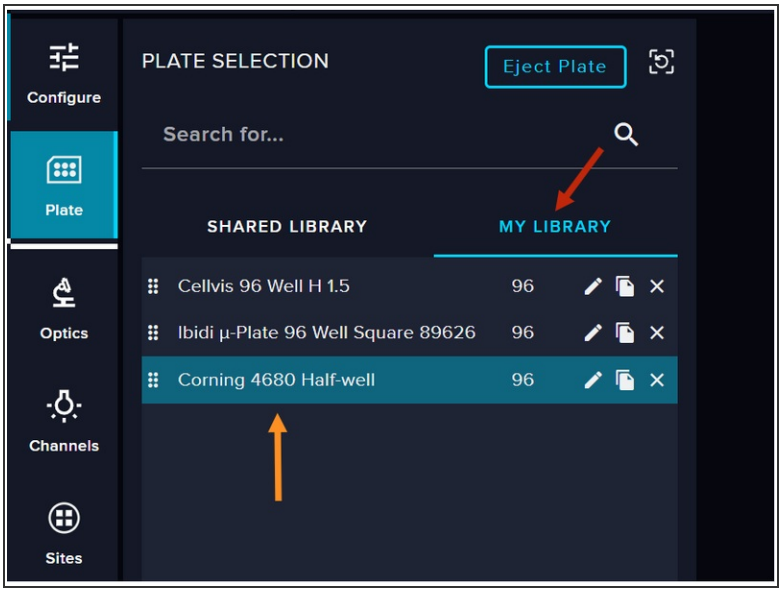


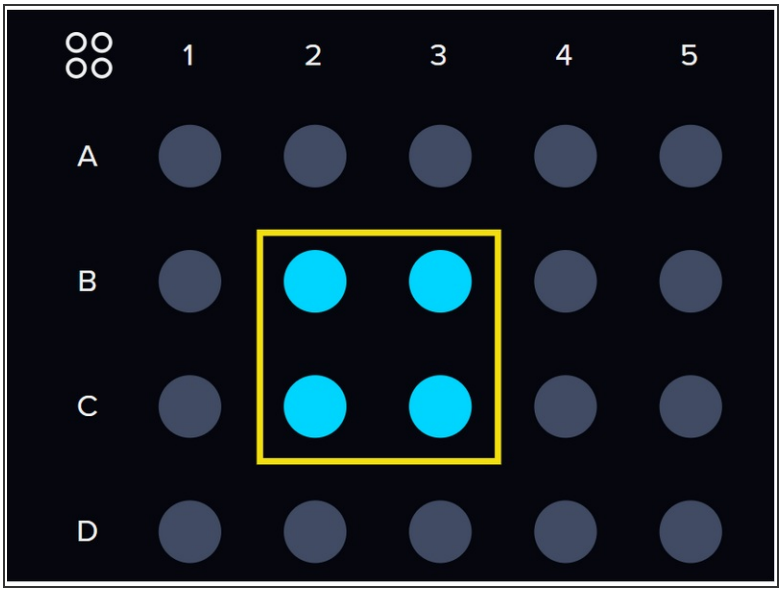
Step 1 — Overview acquisition: Workflow Options



- Leave all toggles unchecked for the overview acquisition.

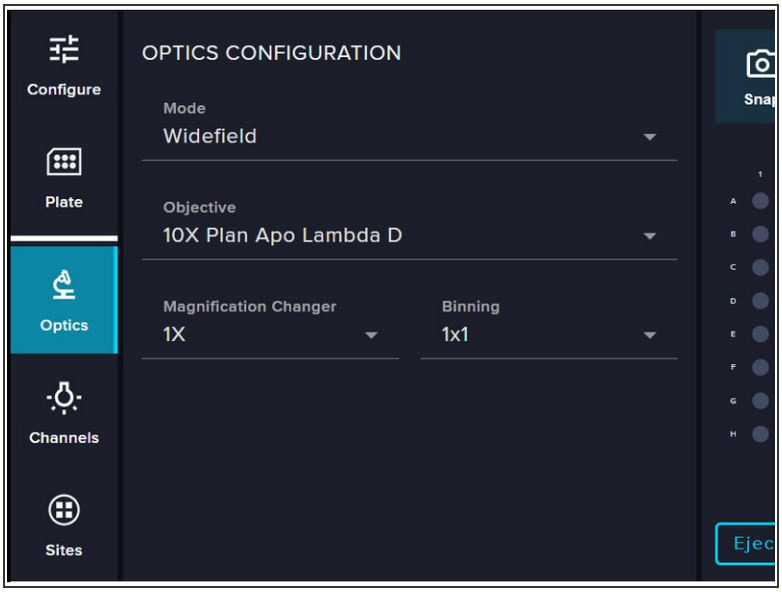
Step 2 — Overview acquisition: Plate Configuration

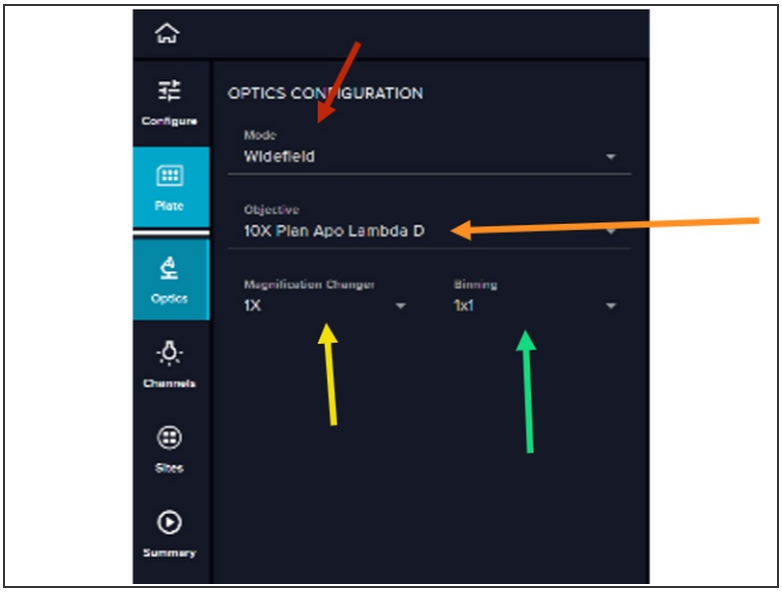




- Go to your **Plate library**
- Select the plate configuration you are using
- Select the fields you want to include in the overview scan

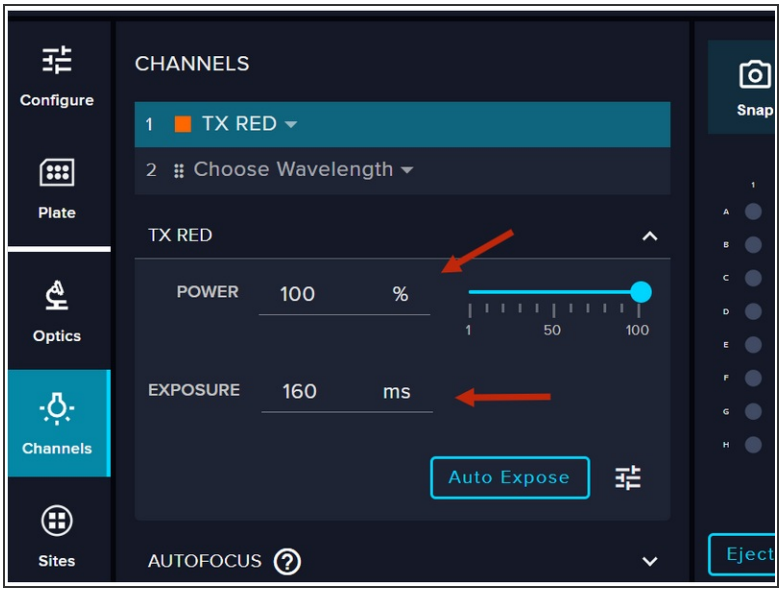
Step 3 — Overview acquisition: Optics Configuration





- For overview acquisition, **Widefield** mode is recommended over spinning disk. It allows faster acquisition through lower exposure times and is generally sufficient for detecting targets of interest.
- The **10× Plan Apo Lambda D** objective offers the best trade-off between image resolution, speed, and data size.
- Set the **Magnification Changer** to **1×**.
- Set **Binning** to **1×1**.

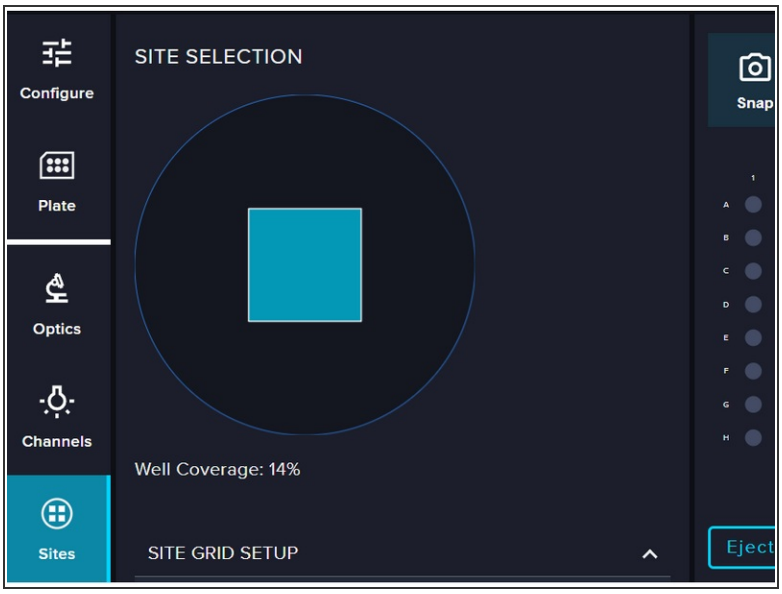
Step 4 — Overview acquisition: Channel Settings

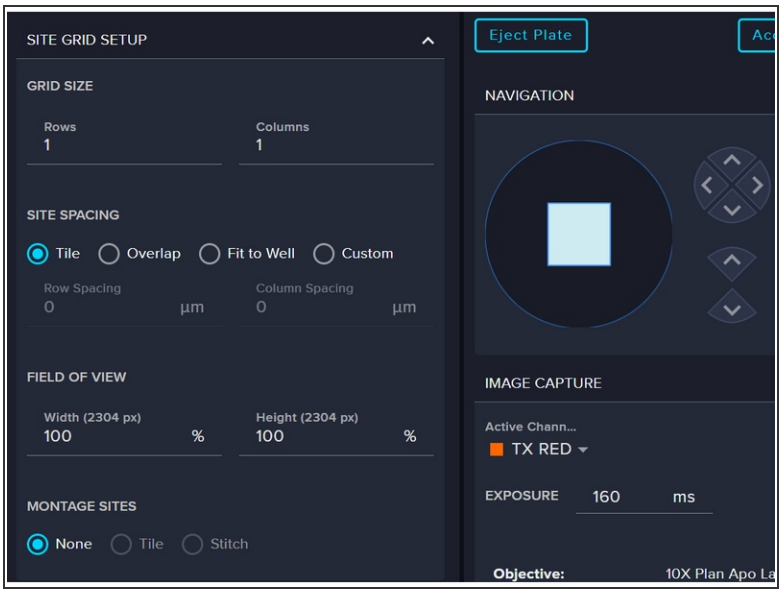




- Only one channel can be used for target selection during the analysis. Additional channels can be set up if desired, but they will not be used further in the workflow.
- Adjust the **laser power** and/or **exposure time** until your signal falls within the optimal range for your sample.

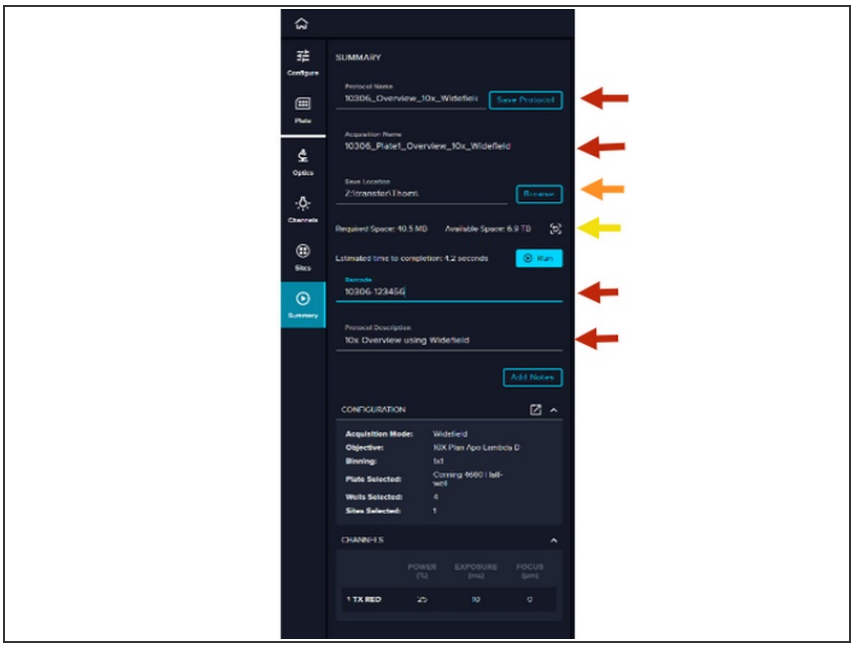
Step 5 — Overview acquisition: Site Selection





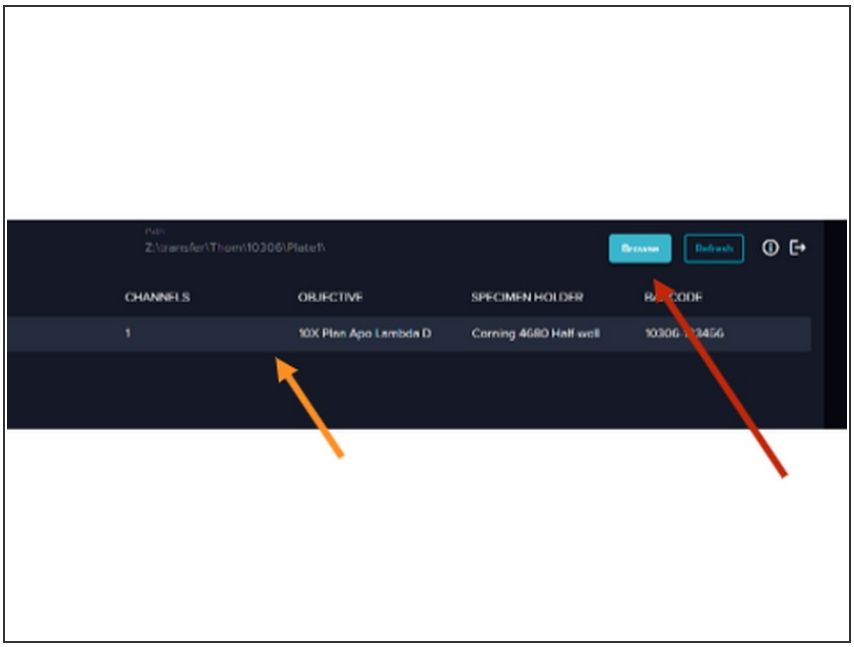
- Set up the sites you want to scan.

Step 6 — Overview acquisition: Summary



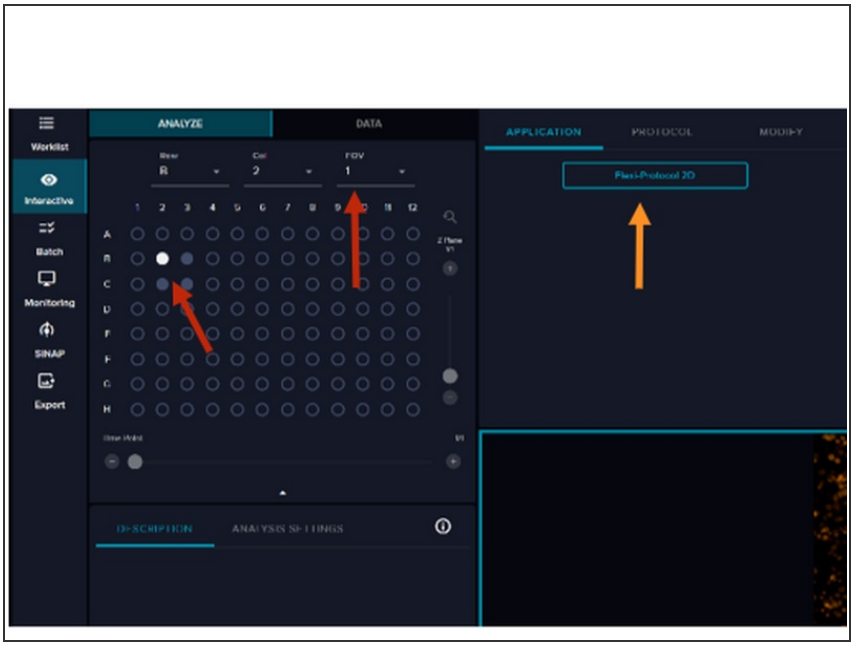
- Give the protocol and acquisition informative names.
- Save your data to your data folder on the **Z: drive** for easy access from the **ZMB MetaExpress VM**.
- Be mindful of the data size.

Step 7 — Target detection: Load data into IN Carta



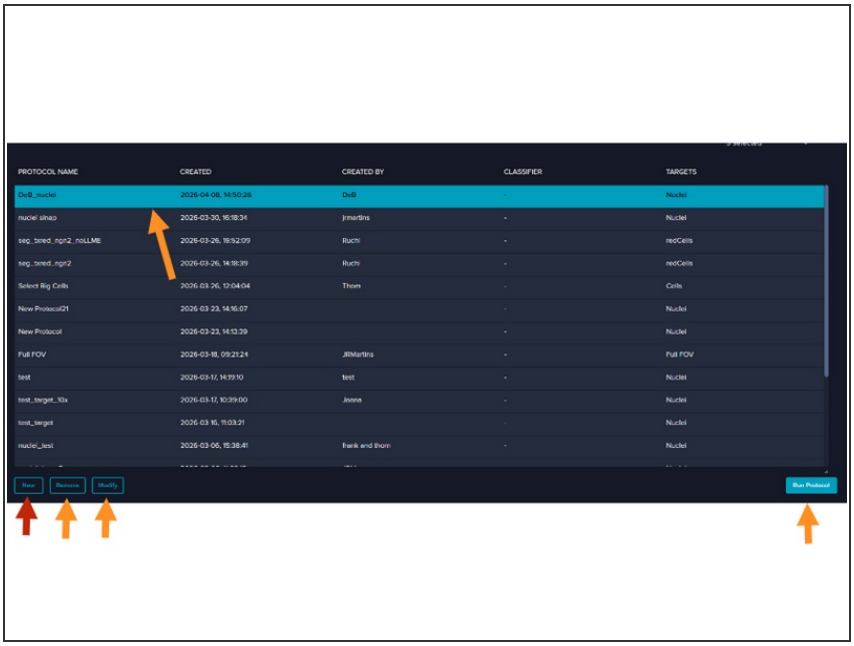
- **Browse** to the acquisition data in your data folder on the **Z: drive**.
- Select your data

Step 8 — Target detection: Position and Protocol type



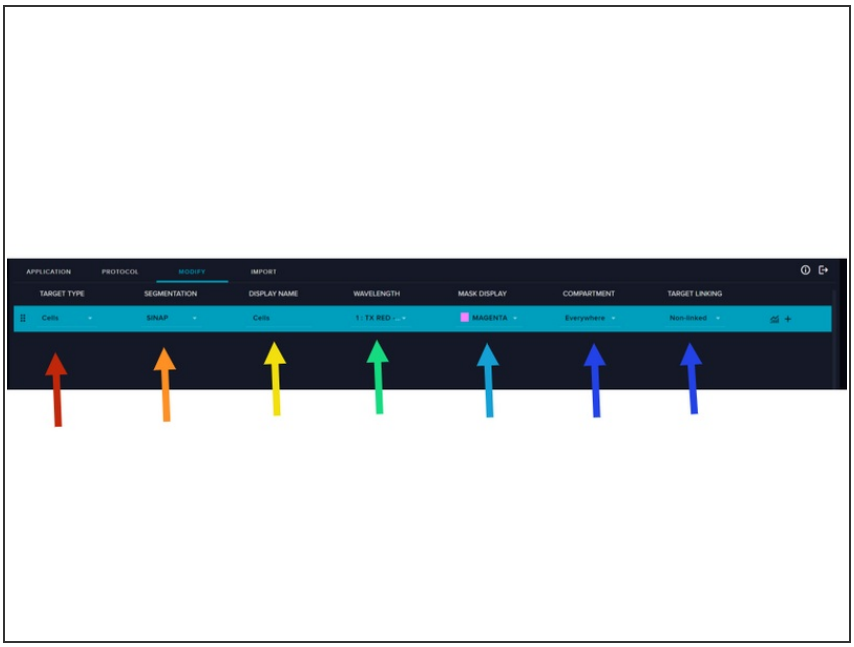
- Select the **position** and **field of view** (FOV) for target selection.
- Select the **FlexiProtocol 2D**

Step 9 — Target detection: Create detection protocol



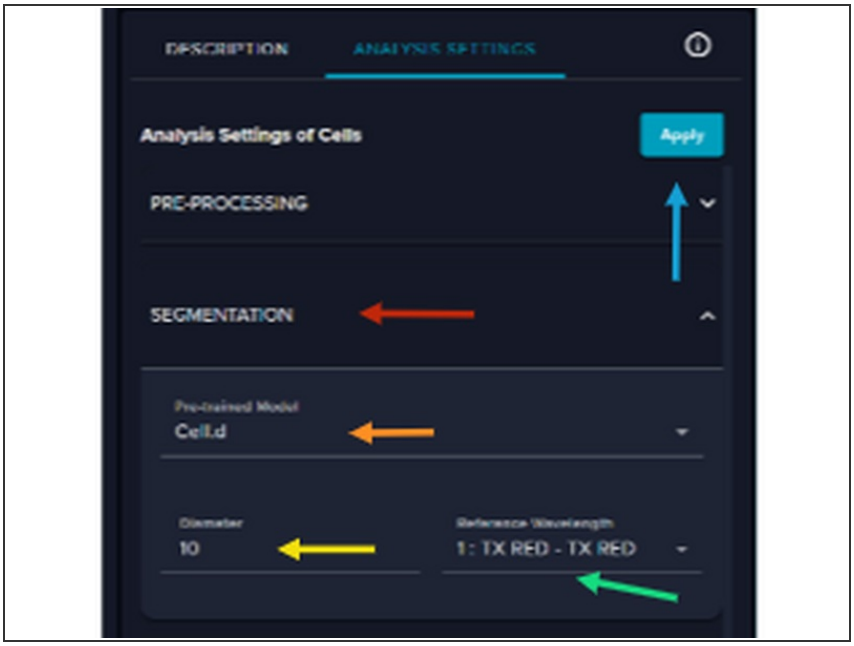
- Press **New** to create a new detection protocol.
- If you have already created a protocol, you can select it from the list to rename, modify, or run it.

Step 10 — Target detection: Segmentation type



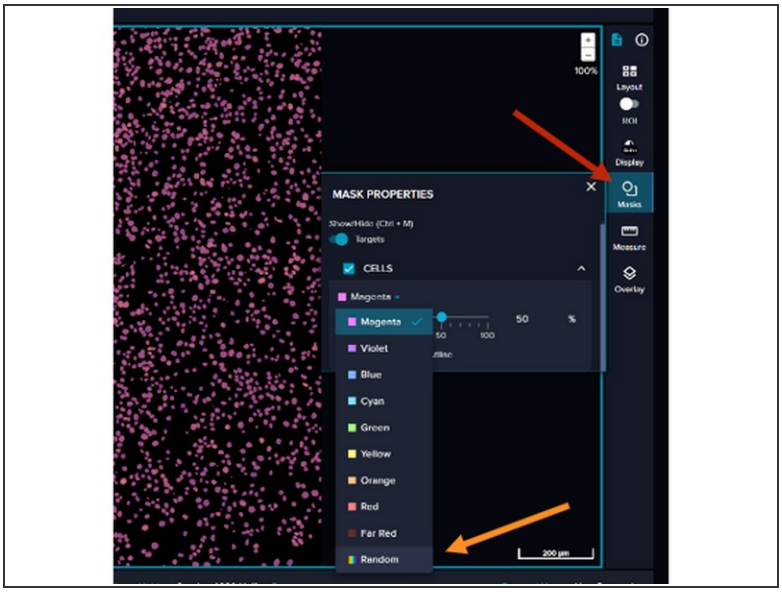
- Select **Cells** as the target type.
- The **SINAP** segmentation mode works well for most use cases.
- Give your targets a descriptive name.
- Select the channel to be used for segmentation.
- Select the color for the segmentation masks.
- Set the compartment to **Everywhere** and target linking to **Non-linked**.

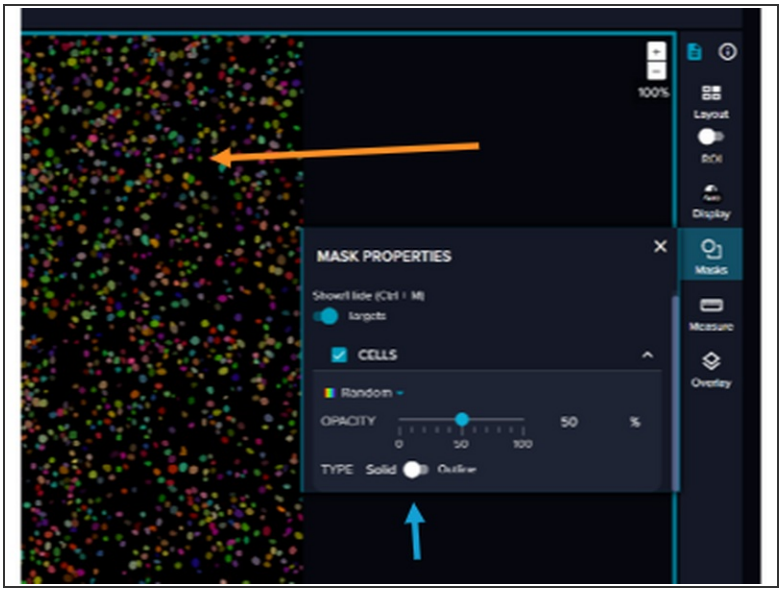
Step 11 — Target detection: Segmenting targets



- Configuring the segmentation settings properly, under **Analysis Settings**, has the biggest effect on your target detection.
- Several segmentation options are available. We recommend **Cell.d**, which generally gives good results.
- **Cell.d** also lets you provide a rough estimate of the object size you are selecting.
- Make sure the correct channel is selected for segmentation.
- Press **Apply** to test the settings.

Step 12 — Target detection: Evaluating the segmentation

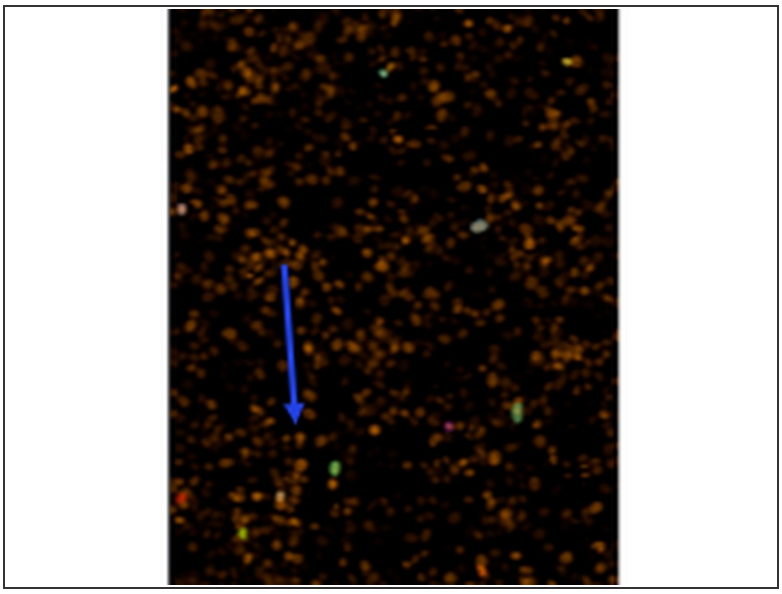




- Click **Masks** to adjust mask settings.
- Switch to **Random** color to better distinguish individual segmented objects.
- Change the mask type from **Solid** to **Outline** for a clearer view of underlying cells.

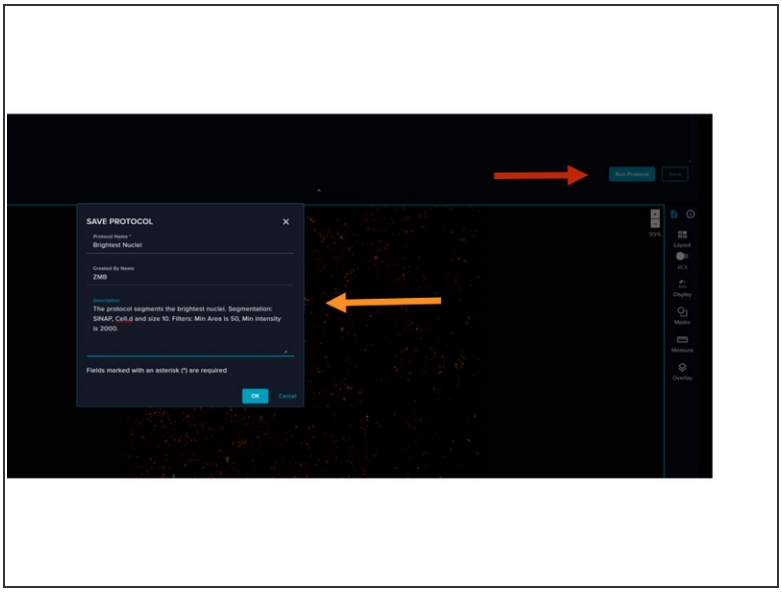
Step 13 — Target detection: Filtering the segmentation

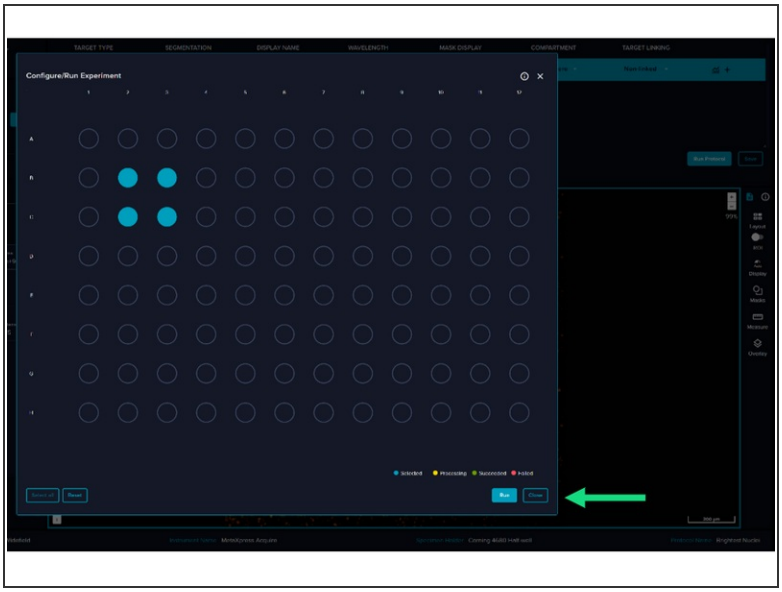




- Under **Filter** in the **Analysis Settings**, the segmented objects need to be further defined.
- Refine the segmentation by filtering cells by size and intensity
- Make sure the correct channel is selected.
- Suppress overexposed signals and cells touching the image border.
- Under **Advanced**, define the minimum number of pixels required between the border and segmented objects.
  - Only a few segmented objects should remain. These will be used as your targets.

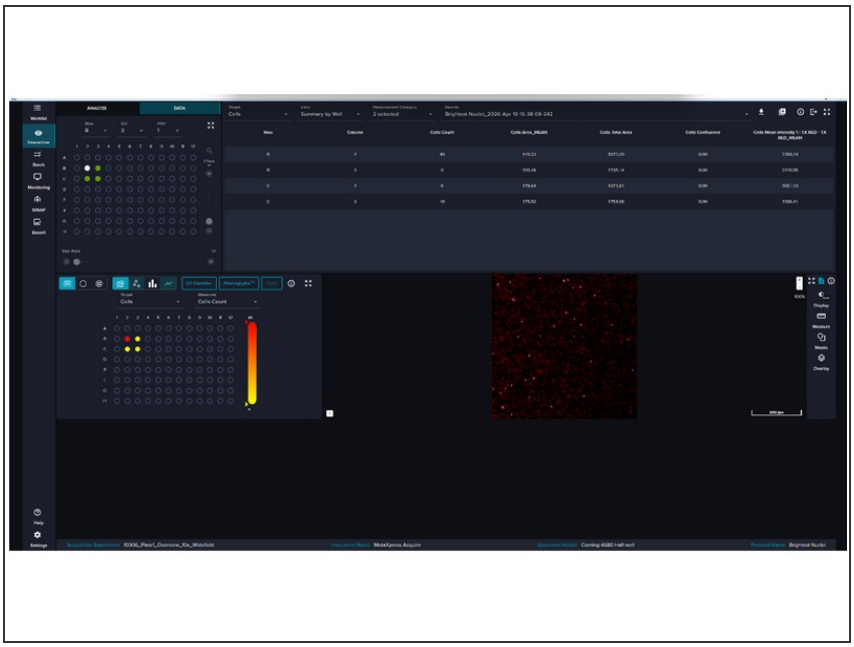
Step 14 — Target detection: Running the analysis





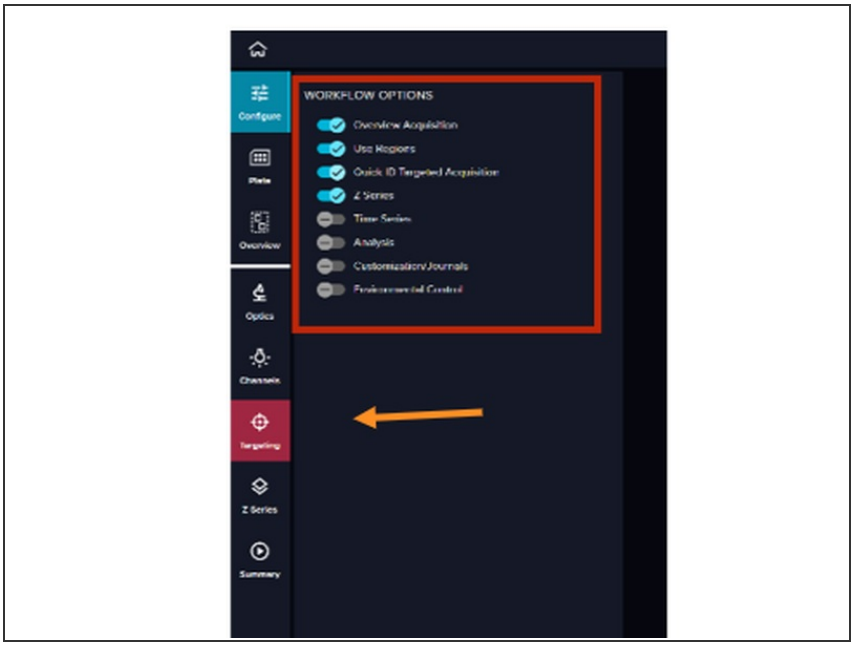
- Press **Run Protocol**
- Save your protocol with a descriptive name and an informative description
- Select the well you want to run the analysis on.
- Start the analysis.

Step 15 — Target detection: Analysis finished



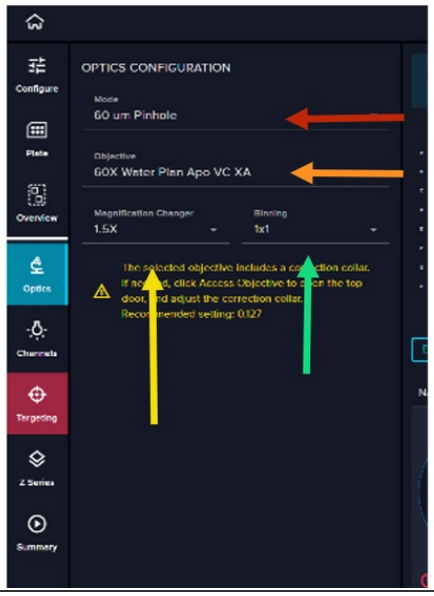
- The analysis is finished, and the results are shown here. You can now move on to target acquisition.

Step 16 — Target Acquisition: Configuration



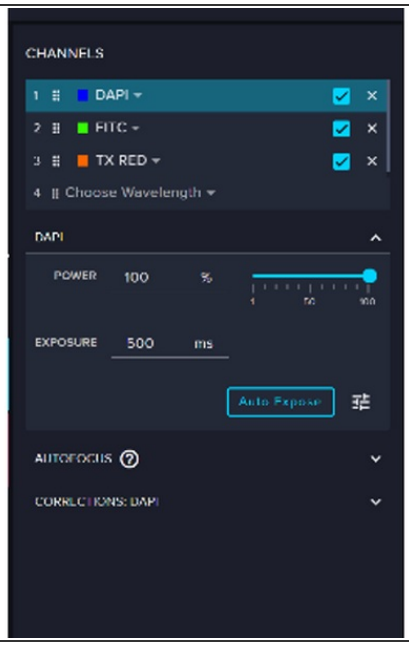
- Select **Overview Acquisition**, **Use Regions**, **Quick ID Targeted Acquisition**, and **Z-Series**.
- The **Targeting** button will appear in the sidebar.

Step 17 — Target Acquisition: Optics Configuration



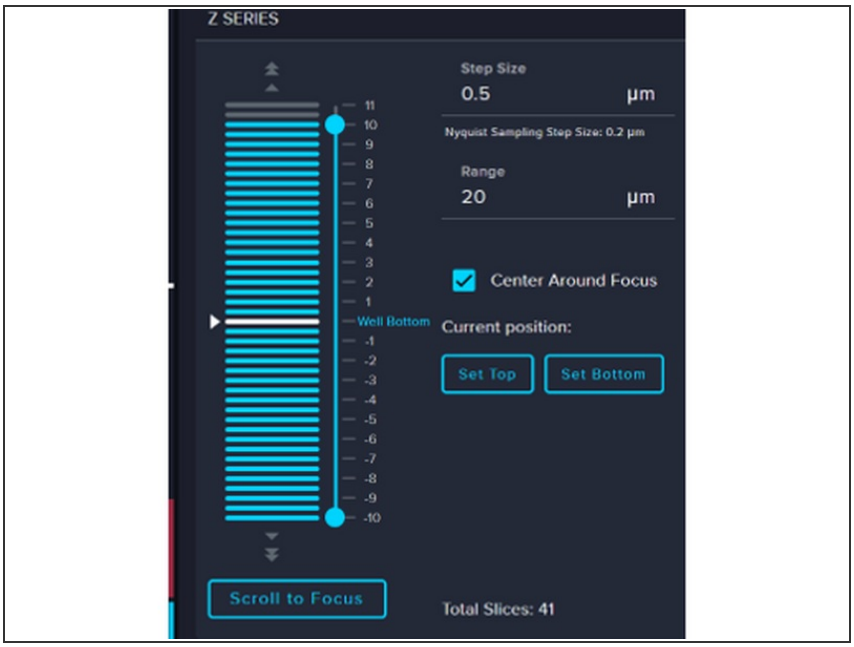
- For target acquisition, switch to a higher resolution.
- Select the **60 µm Pinhole** spinning disk.
- Select the **60× Plan Apo VC XA Water objective.**
- Set the **Magnification Changer** to 1.5×
- Leave **binning** at 1×1.

Step 18 — Target Acquisition: Channel configuration



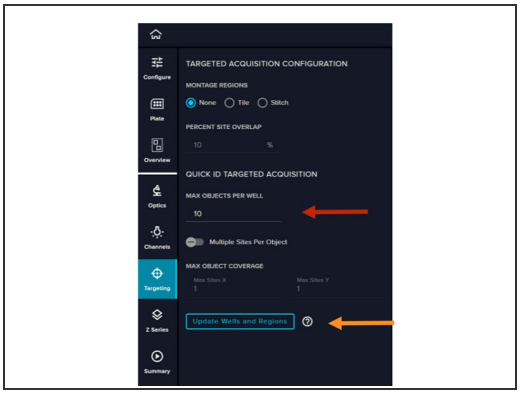
- Setup the channels of interest

Step 19 — Target Acquisition: Z-series



- Set up the Z-series **Before** you move to **Targeting**

Step 20 — Target Acquisition: Loading the targets

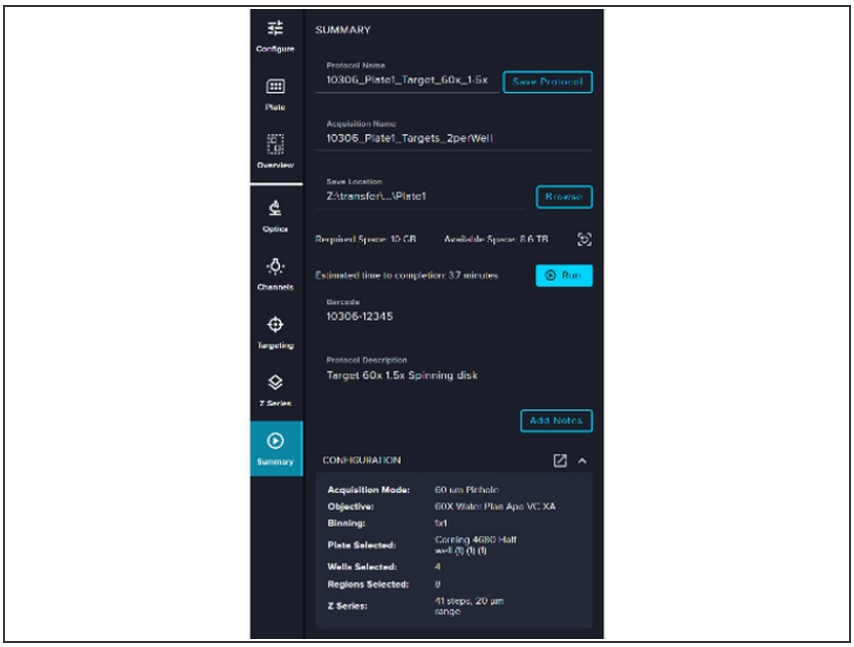






- Select the **maximum number of objects** per well you want to load.
- Load the objects using the **Update Wells and Regions** button.
- Navigate to the **experiment** folder in your data folder on the **Z: drive**.
- Select the analysis that has been done with IN Carta
- The targets will load into the **Navigation** window, where you can also delete overlapping areas.

Step 21 — Target acquisition: Summary



- Give your protocol and data appropriate names
- Run your protocol.