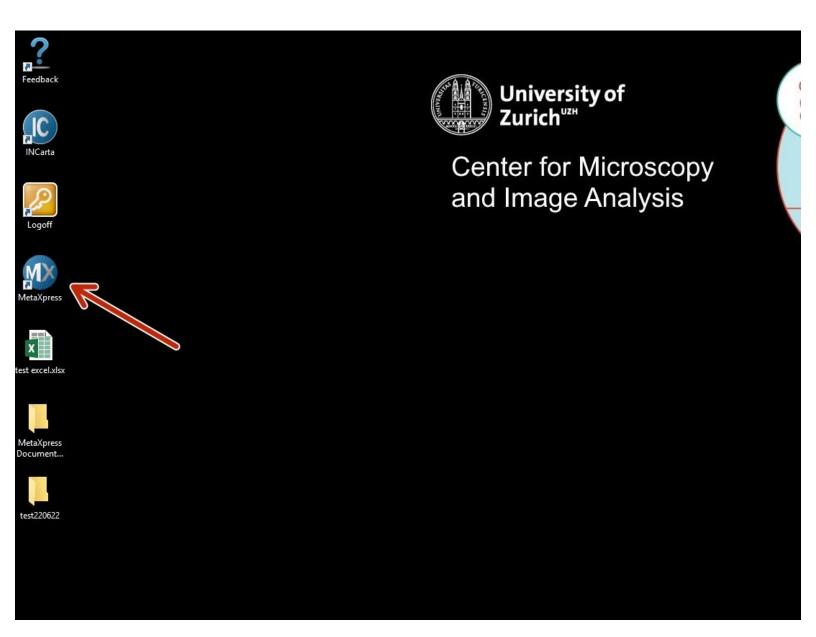


Counting nuclei with MetaXpress

Counting nuclei in HCS images acquired with the HCS - MD ImageXpress Confocal HT.ai and exporting the results.

Written By: Karin Seubert



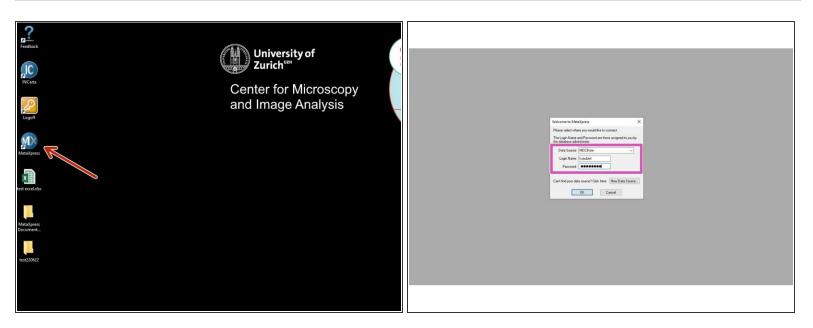
INTRODUCTION

This guide of the Center for Microscopy and Image Analysis explains how to count nuclei in HCS images acquired with the HCS - MD ImageXpress Confocal HT.ai and export the results.

NOTE 1: It is made for single-timepoint, multi-site, and multi-well acquisition for one channel (DAPI). Please adapt it to your own needs if you have different acquisition settings.

Note 2: Think beforehand how to design your plate. The data will be sorted by well A1-A12, B1-B12 etc.

Step 1 — Starting the software and login



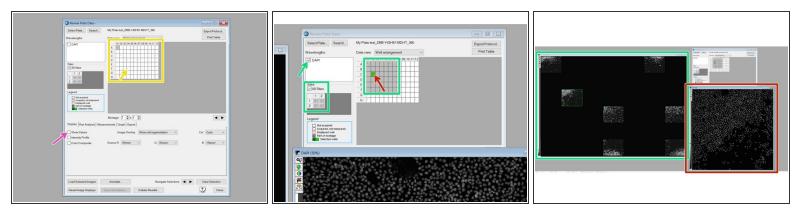
- Start the "MetaXpress" software either on Special VM B or on the dedicated Special MetaXpress VM.
- Login with the same name and password as on the machine.

Step 2 — Opening plate data

Process Log Meanuer Journal Cuttorn Module Screening Apps Window Help Am To D & S & S & To To Laboratoria (DB). Plate Data Uddie: (DB) Plate Data Uddie: (DB) Add Cutorn Module: (DB) Add Cutorn Model: (DB) Add Run Plate Statuse: (DB) Auto Run Plate Statuse: (DB)	Store for (c) Store for (c) <thstore (c)<="" for="" th=""> Store for</thstore>	Section for frame for an end of the section of
	Compare (M ((((((((((((((((((Non-Para Mill A papaloniti, Branis, Gradu, Gra

- Go to "Screening" and select "Review Plate Data [DB]".
- In the "Review Plate Data" window, click "Select Plate" to choose your plate to be analyzed.
- Your plates are first ordered according to the acquisition date. Double-click to open the folder.
- The different plates acquired on that day will appear below.
- "Select" will open the respective plate data.

Step 3 — Check your plate



- The plate format shows up. "-" indicates that these wells were imaged.
- If you have run an analysis on this plate before, you can hide the values (Low Pressure) by unclicking "Show values" in the "Display tab".
- For a montage, mark the corresponding wells, define the sites per well and select a channel for display.
- For a high-resolution image of one site in a well, right-click on the corresponding well.
 Please note that by right-click you will also automatically mark the cell for analysis.

Step 4 — Create your own analysis settings

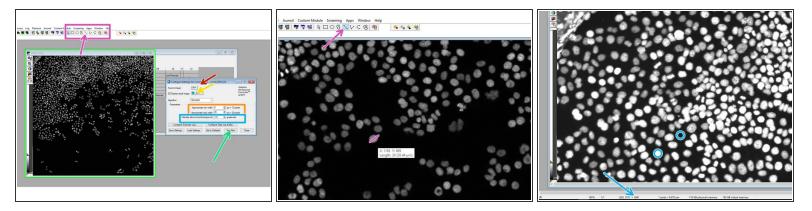
	teview Plate Data -									
🕜 Review Plate Data -	ect Plate Search	My Plate test_ZMB-Y4	2H91-MDHT_36	6	Export	t Protocol				
Select Plate Search My Plate test_ZMB:Y42H91-MDHT_366 Export Protocol	velengths:	Data view: Well arran	igement	~	Pri	nt Table				
Data view: Weil anangement V Print Table	DAPI	01 A Low Pressure		03 04	05 06 Low Pressure	07 01	8 09	10	11 12	
		В			LOWY IGSSOR			Low Pressure		
A Low Pressure Low Pressure		C	Low Pressure						Low Pressure	
C Low Pressue Low Pre-	es	E		Low Pressure	L	.ow Pressure		Low Pressure		
Stee E Low Pressue Low Pressue	All Stes	F		Low Pressure	L	ow Pressure		LOWTIEssuie		
Al Stes F Low Pressure Low Pressure	1 2	G								
1 2 6 Low Pressure Low Pressure re		H Low Pressure					Low Pressure		Low Pressure	
	gend									
Lopend I toolined	Not acquired									
Acquired, not measured	Acquired, not measured Displayed well									
Displayed well Pert of nontage	Part of montage			•						
Selected webs	Selected wells						Edit List og Bener State St	f Settings for Co	ount Nuclei	
Montage: 24 (plx 16 (p)						EVER DE LE	Settings:			
		Montage: 24 🛊 x 16	•			< ►	СН			Close
Davday Run Analysis Measurements (Graph Export	play Run Analysis Me	asurements Graph E	port				count_DAPI			New Settings
Analysis dell'exemption	shole - Ourse Muslars				Configure Sett	ings	count_DAPI			Rename Settings
Cell Scongy Court Nodes Courte	ngs: count_DAPI_K	5		V Edt List	Create Custom		count_DAPI			
Setting (-Oldermulae) description: (Mut Wavelength Cell Scoring> description: (Mut Wavelength Cell Scoring) description: (Mut Wavelength Cell Scoring) des	- Langever and a second s					-	count_FITC		1	Delete Settings
Agregates 20x	cription:					0	count_Texa count_TRIT			Configure Settings
katja_count_text1 Run on all wells					Run on all		Ide nuclei	L_KS		Import Settings
Run on selection					Hun on all	WG83	ns_count_D	API		mpon serangs
I Log into the database Run on displayed ate					Run on sel	ection				Export Settings
				.og into the databas			Description:			
Laad Selected Images Annotate Navigate Selections			Mr	.og into the databas	e Run on displa	ayed site				^
Reset image Displays Save Annotations Celular Results Colore	ad Selected Images	Annotate	N	avigate Selections	d D Clear S	Selection				
	an encode mages			angute conclusions						~
	eset Image Displays	Save Annotations	Cellular Results.		?	Close				
			_							

- In this example, a previous analysis has been performed on the plate.
- Select your channel to be displayed and analyzed.
- In the "Run Analysis" tab, under "Analysis" select "<Count Nuclei>".
- If you already have saved settings, you may proceed to the next step.

A Please do not overwrite other users settings!

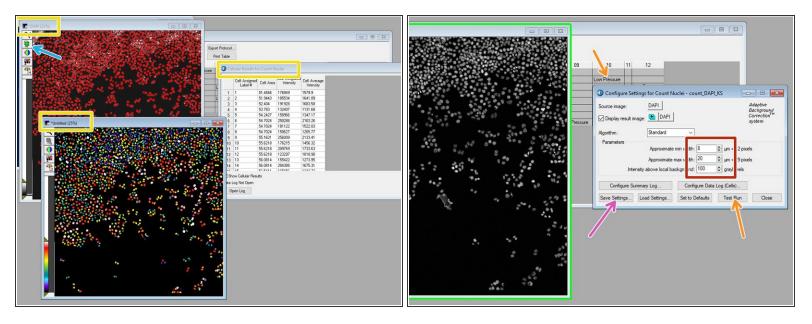
- To create your own settings, click on "Edit List" and "New Settings" with your specification.
- Click on "Configure Settings" to adjust the segmentation parameters.

Step 5 — Adjust your segmentation settings - 1



- Choose your channel for the segmentation: "DAPI".
- Choose how to display the results (we recommend "New").
- In the drawing toolbar, choose "Line" to measure the width of a few nuclei by clicking on one end of the cell and by placing the mouse over the diameter and getting the distance.
- Measure a few cells and use these values for the "Approximate min./max. width" for segmentation.
- Hover over regions of cells and background and check the intensity in order to choose the segmentation parameter "Intensity above local background".
- Click "Test Run" to test the input settings.

Step 6 — Adjust your segmentation settings - 2

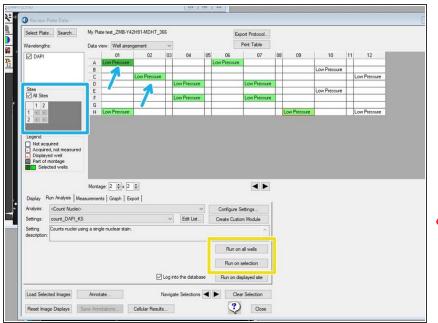


- When the analysis is done for your test image, an overlay of the segmentation will be shown in red together with your grayscale image. Additionally, a color-coded segmentation image and cell-bycell results will be shown.
- Show/hide the overlay to judge the quality of the results.
- If necessary, go back and adjust the input parameters to improve the segmentation.
- If you are happy with the result, click "Save Settings".

It might be worth checking for a few images whether the input parameters work, especially when the size of the nuclei or the intensity greatly vary within your plate.

 In order to check a different image of the same plate, go to the "Review Plate Data" tab and right-click on a different well. Do a "Test Run" again and judge the result.

Step 7 — Run the analysis on the plate.



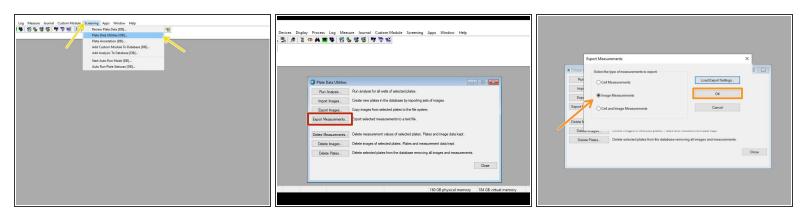
- You can now run the segmentation algorithm either on the whole plate or on on a selection.
 - If you want to run on a selection, select the corresponding wells by right-click (will appear green) and choose the sites per well.
- If you re-run an algorithm on a plate (for the same wells), the old data can be overwritten. However, it might still be accessible for export (c.f. step 9).
- Running the algorithm on a whole plate will take a while. When you open the plate again in the "Review Plate Data", you will see the entry "Low Pressure", which indicates that for this well an analysis has been performed.

Step 8 — Review the segmentation and display the results as a heatmap

	foreir Park Base foreir Park Base foreir Park foreir Park

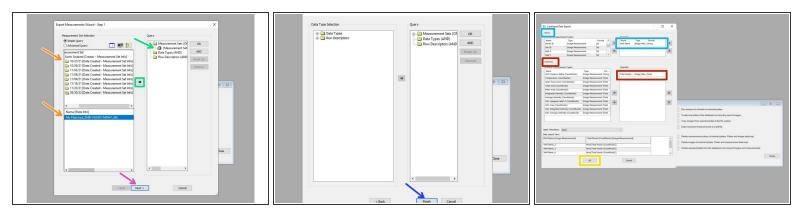
- In the "Display tab", choose for "Image Overlay": "Show cell Segmentation".
- Use this button to show/hide the segmentation and judge the quality of the algorithm.
- Go to the "Measurement tab" and choose the "Analysis" (if multiple were run on the same plate).
- Choose the type of "Analysis" to be displayed.
 - (i) E.g. "Total nuclei (CountNuclei)" gives the total number of nuclei determined by the "CountNuclei" algorithm.
 - (i) Note: "CO2 Pressure Status" is the default, therefore "Low Pressure" as default value after analysis.
 - If you choose one site per well, the corresponding value for the site will be shown. If you choose all sites, an average (not total!) will be shown.
- Click "Show Heat Map" to display your results as a heat map.

Step 9 — Exporting measurement data - 1



- For data export, go to "Screening" and select "Plate Data Utilities [DB]".
- Select "Export Measurements".
- Select "Image Measurements" and click "OK".
 - (i) "Image Measurement" will give you the measurements per image (e.g. total/average of nuclei), but will not provide information about the individual cells.

Step 10 — Exporting measurement data - 2



- The measurements are first sorted according to analysis date for the plate (Measurement Set) and then for the plate (Name [Plate Info]).
- Either double-click on the measurement or use the arrow to make it appear in the "Query" on the right.
- Click "Next".
- Click "Finish".
- In "Rows" select "Well Name" with the Type "Image Measurement" by double-clicking so it appears on the right.
- In "Columns" double-click on "Total Nuclei" to get the number of nuclei per image.
- Click "OK".

Step 11 — Exporting measurement data - 3

Export as text file	>	<					
Measurement Sets that will be exported:	aje 🖻						
Name [Measurement Set File Name Count Nuclei [ID_38] Count Nuclei				Export Measurement Set Summary Destination Filename: Clubersik seubert/Deskt	oplastor	×	
Destination		-		Name (Messurement Set Into) Count Nuclei	Fie Name		
C\Users\k.seubert\Desktop\	Browse			Save Summary.	Close (//		
File Option				Export all measurements to	one file		
Export all measurements to one file				File (set		Plate Data Utilities	0
File		Plate Data Utilitie	s	Export Options ATF (Data and selected Me Tab Delimited (Data only)	asurement sets properties)	Run Analysis Import Images Export Images	Run analysis for all wells of selected plates. Create new plates in the database by importing sets of images. Copy images from selected plates to the file system.
Export Options		Run Analysis	Run analysis fo	Save Export Settings	OK Cencel	xport Measurements.	Export selected measurements to a text file.
OATF (Data and selected Measurement sets properties)		Import Images	Create new plat				Delete measurement values of selected plates. Plates and image data kept.
Tab Delimited (Data only)		Export Images	Copy images fr			Delete Images	Delete images of selected plates. Plates and measurement data kept. Delete selected plates from the database removing all images and measurements.
Save Export Settings OK	Cancel	xport Measurement	s Export selected				
		elete Measuremen	s Delete measure				
		Delete Images	Delete images				
		Delete Plates	Delete selected				

- Select your export folder.
- Check "Export all measurements to one file" and define a File Name.
- Select "Tab Delimited (Data only)".
- Click "OK".
- You will get a notification that the export was successful.

Step 12 — Optional - Quick analysis in Excel - 1

	#X(2%&)@%%##000#0	#X[2%\$\$7]@@=%%%**000##
	Andre 👀 😫 🖓 • 🤆 • Paratanana v 👂 Seen Jarg	Ander 👀 🗄 🖓 🖉 🔹
		Bit Home Instructions from Apprication formation from Services View Help FootRM Models Game Prod.Ref Model Models Description Mail Home Instructions The Mail Home Instruction Service Instruction Services View Help FootRM Models Non-Amplitude The Mail Home Instruction Service Instruction Services View Help FootRM Models Non-Amplitude The Mail Home Instruction Services View Help FootRM Models The Mail Home Instruction Services View Help FootRM Models The Mail Home Instruction Services View Help FootRM Models The Mail Home Instruction Services View Help FootRM Models The Mail Home Instruction Services View Help FootRM Models The Mail Home Instruction Services View Help FootRM Models The Mail Home Instruction Services View Help FootRM Models The Mail Home Instruction Services View Help FootRM Models The Mail Home Instruction Services View Help FootRM Models The Mail Home Instruction Services View Help FootRM Models The Mail Home Instruction Services View Help FootRM Models The Mail Home Instruction Services View Help FootRM Models The Mail Home Instruction Services View Help FootRM Models The Mail Home Instruction Services View Help FootRM Models The Mail Home Instruction Services View Help FootRM Models The Mail Home Instruction Services View Help FootRM Models The Mail Home Instruction Services View Help FootRM Models The Mail Home Instruction Services View Help FootRM Models The Mail Home Instruction Services View Help FootRM Models The Mail Home Instruction Services View Help FootRM Models
Der heine bestehnen bestehnen des einen Bestehnen des Bestehnen B	Ord Standard Data Oversking Consultant Consultant Oversking Consultant Consultant Oversking Oversking Oversking Consultant Oversking Overs	Differential Spectration
Image: Control in the second	Image: Constraint of the state of	
Court 19 Court	Contribution of the second sec	
an Sama an	The Based map	

(i) We can provide you with a basic analysis template for Excel.

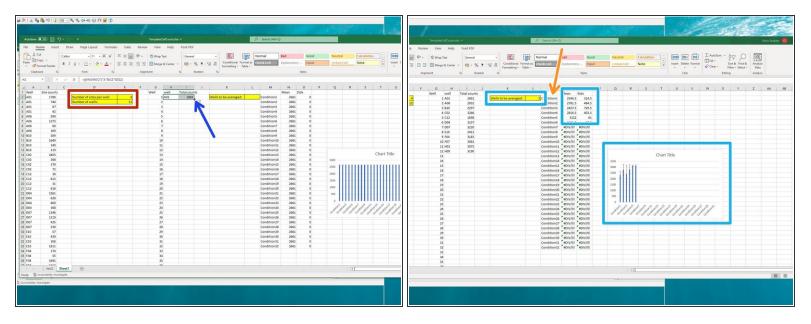
- In the "Data" tab, click "From Text/CSV".
 - Open the ".txt"-file generated in Step 9.
- Check whether the format is correctly recognized and click "Load".
- A new tab will appear with your well information and the number of detected nuclei.
 - If you have acquired multiple sites per well, the number of nuclei per site is displayed and therefore the well name listed repeatedly.

Step 13 — Optional - Quick analysis in Excel - 2

#× :2時間で10円-155,00000000000000000000000000000000000		#米 古海島沙 単田	■× 云陶陶均 ② Ⅲ
Andres 👀 🖗 🖓 - (* - 4 Despinisht Konstan v 😥 Sentisht (2010)		Ander 🐨 🔅 🖓 - () - a Destantidoretas - 😥 Santi (Al-Q)	Ander 🖅 🗄 🎷 - 👔 - 🗇 - Serbiddontes v 🖉 🖉 Serbiddont
file <u>Home</u> inset frae PageLapost Formáss bata Review Vew Help FostPDF		file theme meet draw PageLapost Formulas Data Raview View Help FoltPOF	No stome inset Oran Page Lapout Formulas Data Review View Help FockTOF
	Andreas (1)		
	S T U		

- To start referencing the data, go back to the "Sheet1", click on cell "A2" and type "=".
- Go to the **data sheet (test2)**, and click on the **first cell (A2) with a well name**, with the reference appearing in the formula bar.
 - Press Enter to make the reference.
- Repeat the same procedure for the counted nuclei by typing "=" in cell "B2" and referencing it to "C2" (Total Nuclei (CountNuclei)!) in data sheet.
- Mark Cells "A2&B2" and extend the referencing by grabbing and dragging the lower right corner of the marked cells down to the maximum number of sites measured.

Step 14 — Optional - Quick analysis in Excel - 3



• Specify the number of sites per well and number of wells.

(i) This will automatically fill cells H2 and J2.

- By grabbing the lower right corner of the two marked cells H2 and J2, drag to extend to your maximum well number.
- Type in the number of **wells to be averaged**, the corresponding mean and standard deviation will appear and the histogram will fill itself.
 - Default is average of >1 wells including standard deviation. If you want to change it, use all the tools that Excel has to offer.
- Here you can adjust your x-axis label.