

Please come to the **Research in Progress Seminar Series**

**WONDERFUL WORLD OF TECHNOLOGY**

event featuring

**Chander Raman, PhD**

**J. Stewart New (Dr. Kearney's Lab)**

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**Using the ImageStream to Study the Immune System**

**Thursday, November 6, 2014,**

**12:00 – 1:00 PM**

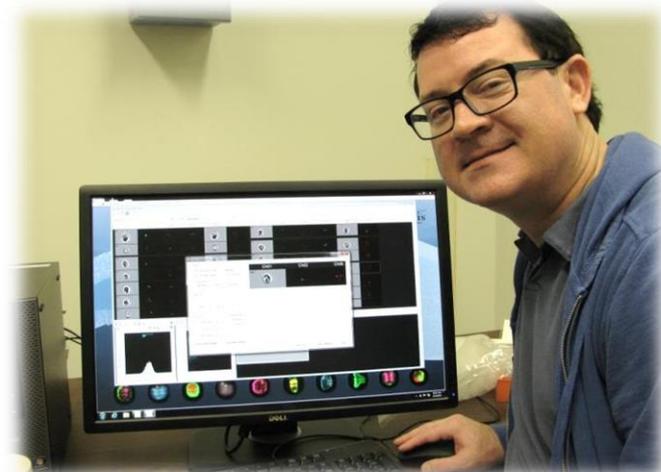
**SHEL 515, 1825 University Blvd.**

Raw data welcome!

You provide the science, and we'll provide the food.

# The Amnis ImageStream Imaging Flow Cytometer

is ready for you to use in **SHEL Rm 130A**



Please contact [Shawn Williams \(tzaron@uab.edu\)](mailto:tzaron@uab.edu) or call 934-7403 to set up an appointment.

- The Amnis ImageStream is acquired via a successful [Veterans Administration Shared Equipment Evaluation \(ShEEP\) Program](#) application (Dr. Louis J. Dell'Italia, MD, ACOS, Research and Development)
- It is operated by the Analytic Imaging and Immunoreagents Core of the Rheumatic Diseases Core Center ([P30-AR048311](#)).

# USING THE IMAGE STREAM TO STUDY PHAGOCYTOTIC AND KILLING POTENTIAL IN MACROPHAGES

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J. Stewart New

Laboratory of Dr. John Kearney

UAB Dept. of Microbiology

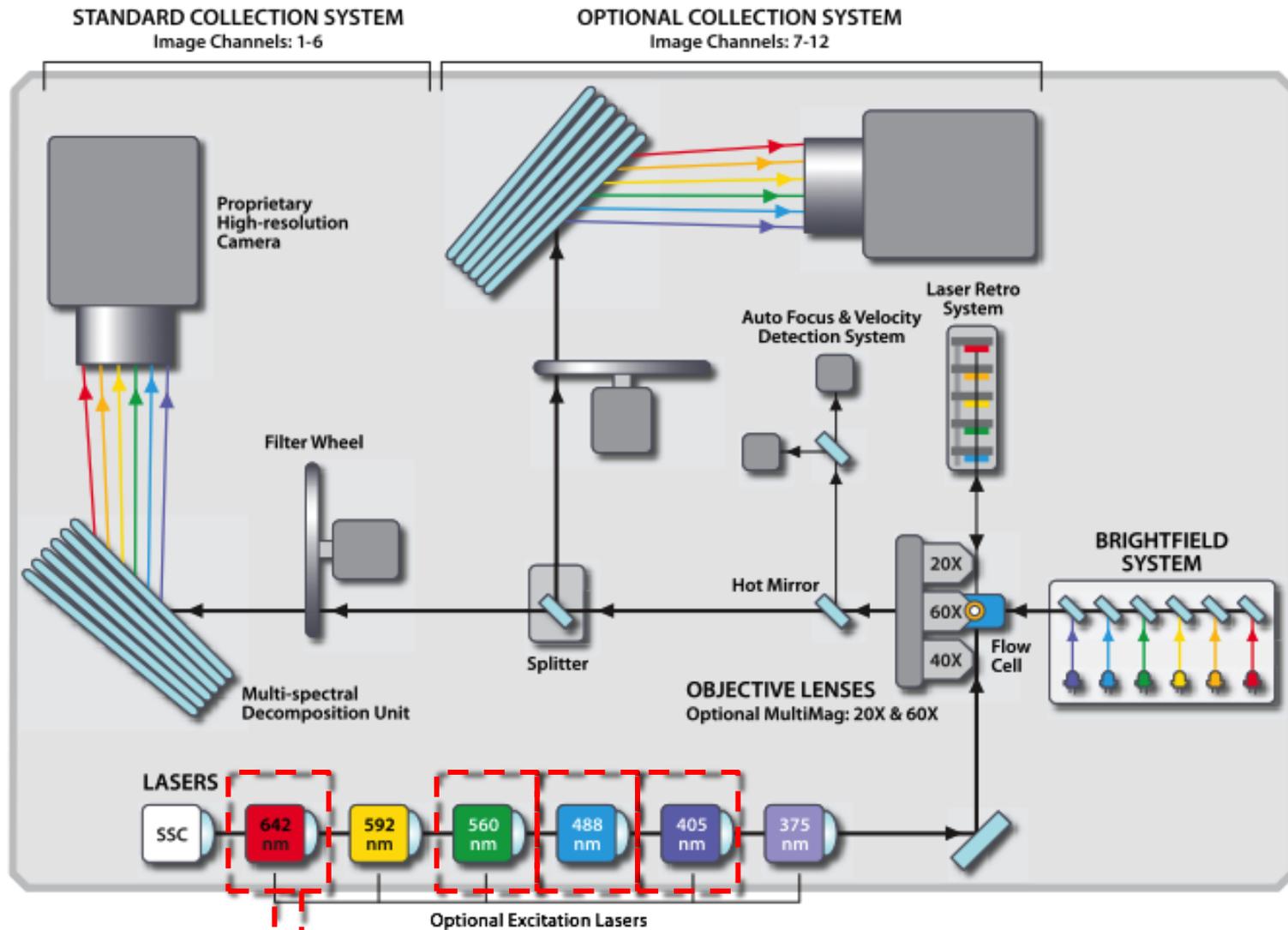
# Amnis ImageStream<sup>x</sup> Mark II



Please visit the  
Amnis website @

<https://www.amnis.com/imagestream.html>

# Optic Setup



Lasers on the SHEL 130A ImageStream Mark II

		Excitation Laser (nm)									
Ch	Band (nm)	375	405	488	561	592	642	730	785	Used	Ch
1	435-505 (457/45)	BRIGHTFIELD									1
2	505-560 (527/65)		FITC, AF488, GFP, YFP, DyLight488, PKH67, Syto13, SpectrumGreen, LysoTrackerGreen, MitoTrackerGreen,								2
3	560-595 (577/35)		PE, PKH26, Cy3, DSRed, CellMask/CellTracker/SY TOX Orange		PE, AF546, DyLight550, PKH26, DSRed, Cy3, SpctmOrange						3
4	595-640 (610/30)		PE-TxRed*, ECD*, PE- AF610*, 7AAD*, PI*, RFP, QD625*, eFluor625*		AF568*, DyLight594*, PE-TxRed*, ECD*, PE- AF610*, RFP, mCherry*						4
5	640-745 (702/86)		PE-Cy5*, PE-AF647*, PerCP*, PerCP-Cy5.5*, eFluor650* FuraRedlo		PE-Cy5*, PE- AF647*						5
6	745-780 (762/35)		PE-Cy7*, PE-AF750*, Draq5*		PE-Cy7*, PE- AF750*, Draq5*			SSC			6
7	435-505 (470/70)	AF350, DAPI, Hoechst, PacBlue, DyCycle Violet	DAPI, Hoechst, PacBlue, CascadeBlue, AF405, eFluor405, DyLight405, CFP, LIVE/DEAD Violet								7
8	505-570 (537/65)	PacOrange, QD525*	PacOrange, CascadeYellow, AF430, BDHorizonV550								8
9	570-595 (582/25)	BRIGHTFIELD									9
10	595-640 (610/30)	QD625*, eFluor625*	QD625*, eFluor625*				TexRed*, AF594*, DyLight594*, T exRed*, mCherry*				10
11	642-745 (702/86)	QD705*, eFluor650*	QD705*, eFluor650*				APC, AF647, AF660, Cy5, DyLight649, DRAQ5*	AF647, AF660, AF680, APC, Cy5, DyLight649, DyLight680, Draq5* PE- AF647*, PE-Cy5*, PerCP*, PerCP-Cy5.5*			11
12	745-780 (762/35)	QD800*	QD800*				APC-Cy7, APC-AF750, APC-H7, APC- eFluor750	APC-Cy7, APC-AF750, APC-H7, APC- eFluor750, Cy7, AF750, DyLight750, PE-Cy7*, PE-AF750*	AF750, Cy7, DyLight750	SSC	12

## Recommended dyes

(based on optimal excitation and detection channels) are in boldface.

\*Many dyes will excite by more than one laser, and this can increase cross camera compensation.

\*\*Channel bandpass may change depending on which laser are on during an experiment. Values listed are assuming 405, 488, and 642 laser excitation.

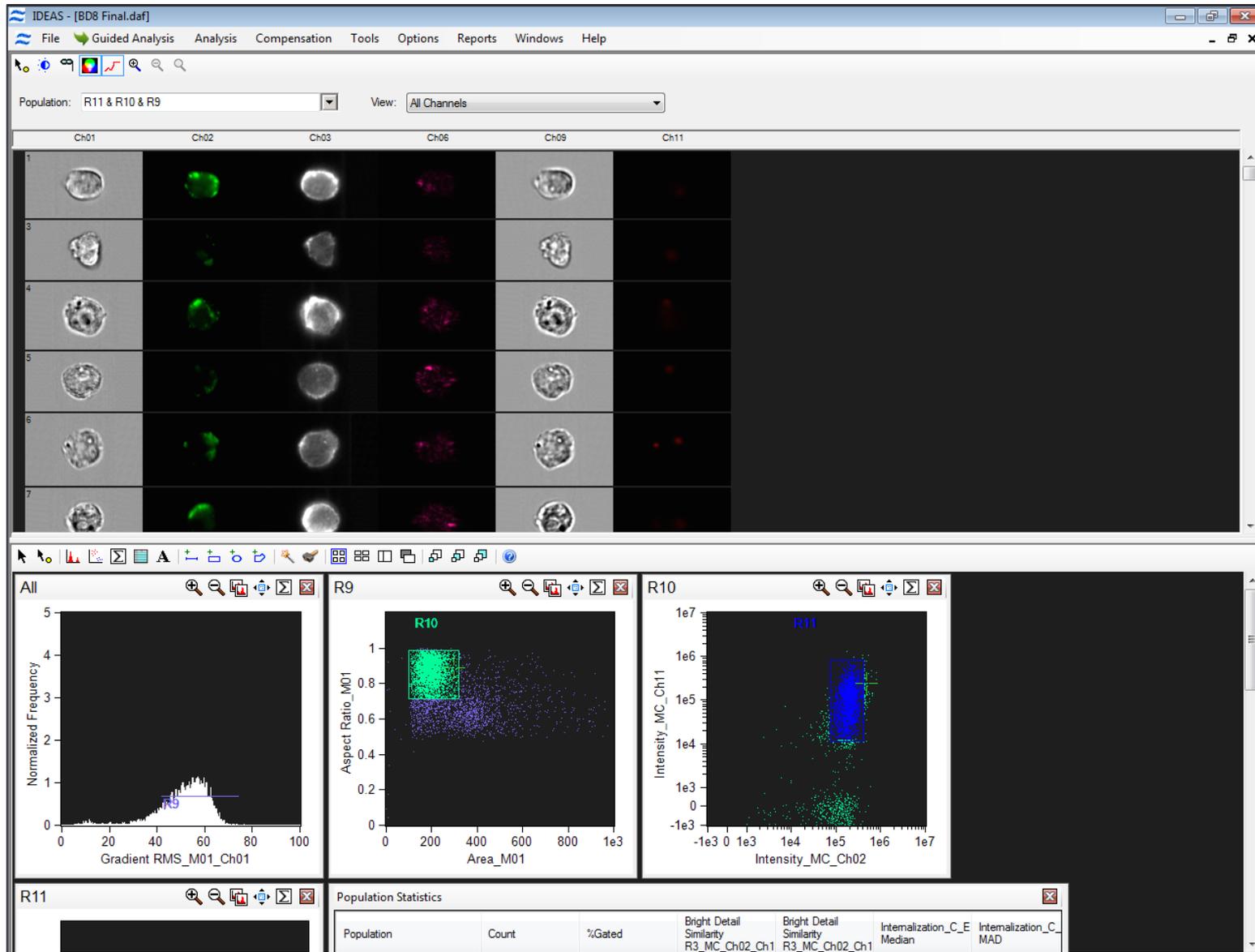
1 laser (488): ideal dyes are AF488, PE, PE-TxRed, PE-Cy5, PE-Cy7, SSC-Ch12,

2 laser (488,642): ideal dyes are AF488, PE, PE-TxRed, SSC-Ch6, and AF647, APC-Cy7

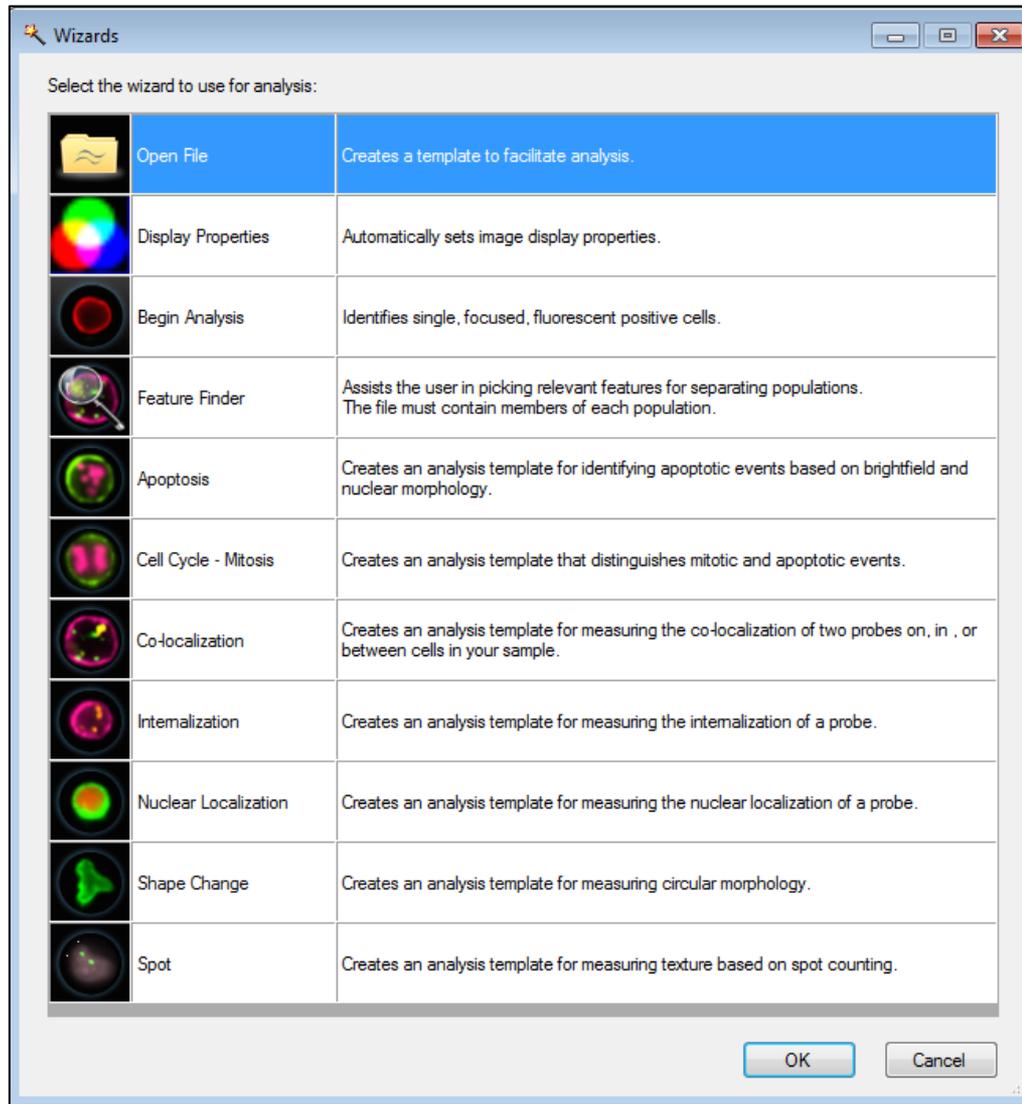
3 laser (405,488,642): ideal dyes are AF488, PE, PE-TxRed, SSC-Ch6, and DAPI, AF647, APC-Cy7

 Available lasers in SHEL Rm 130A

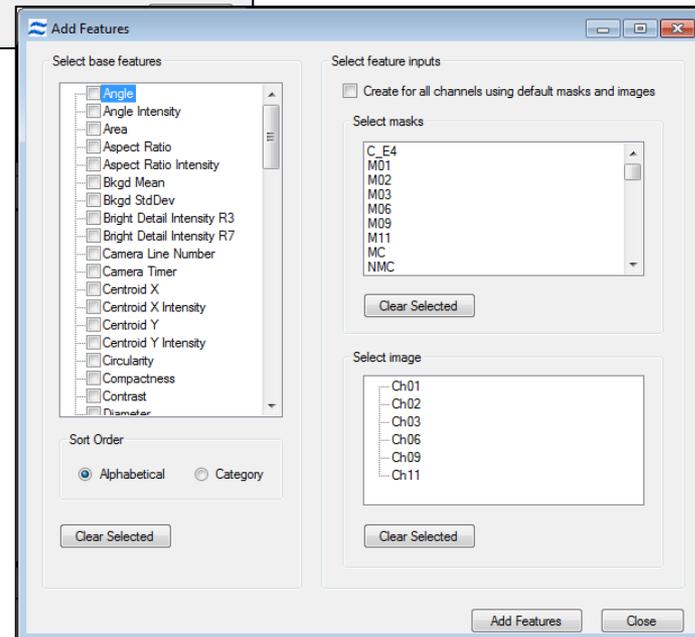
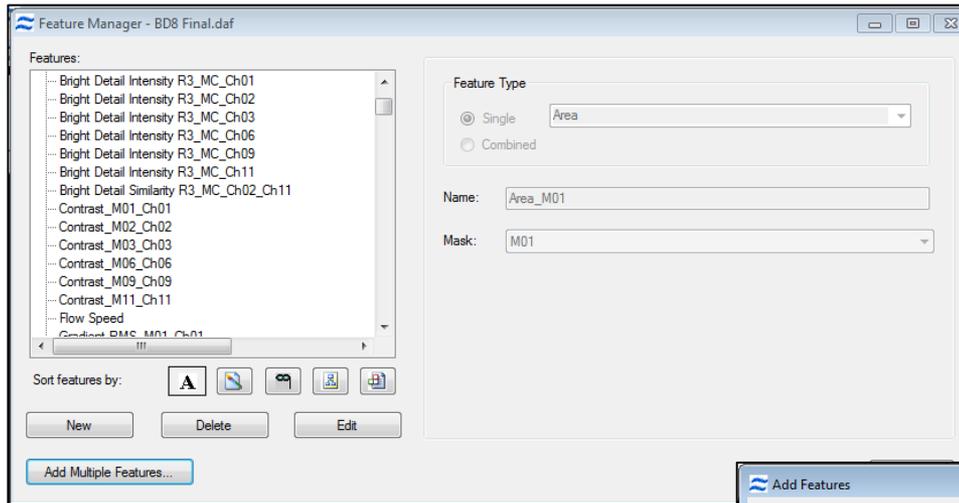
# IDEAS- Analysis software for the Amnis Image Stream



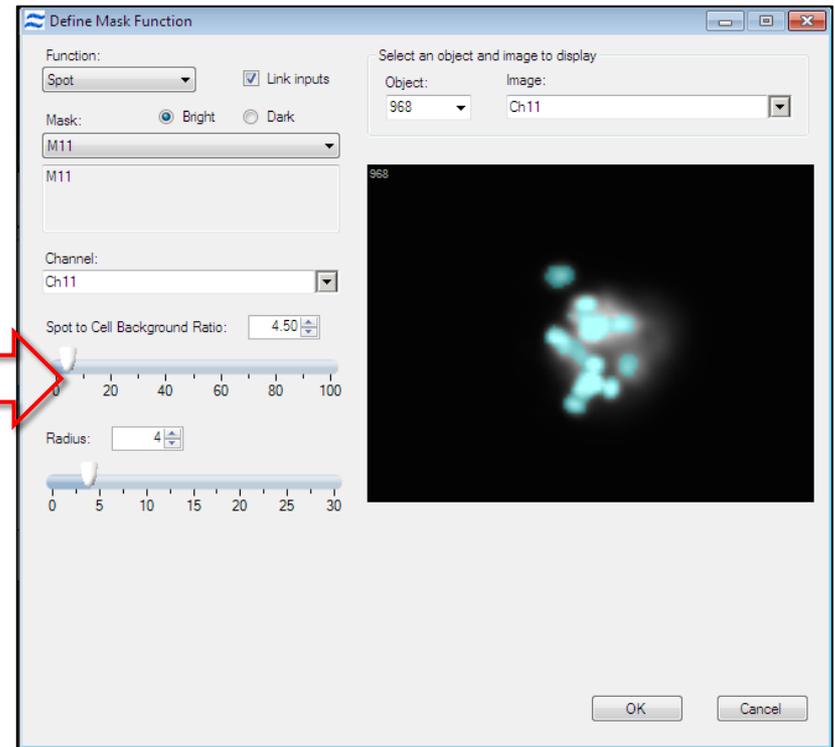
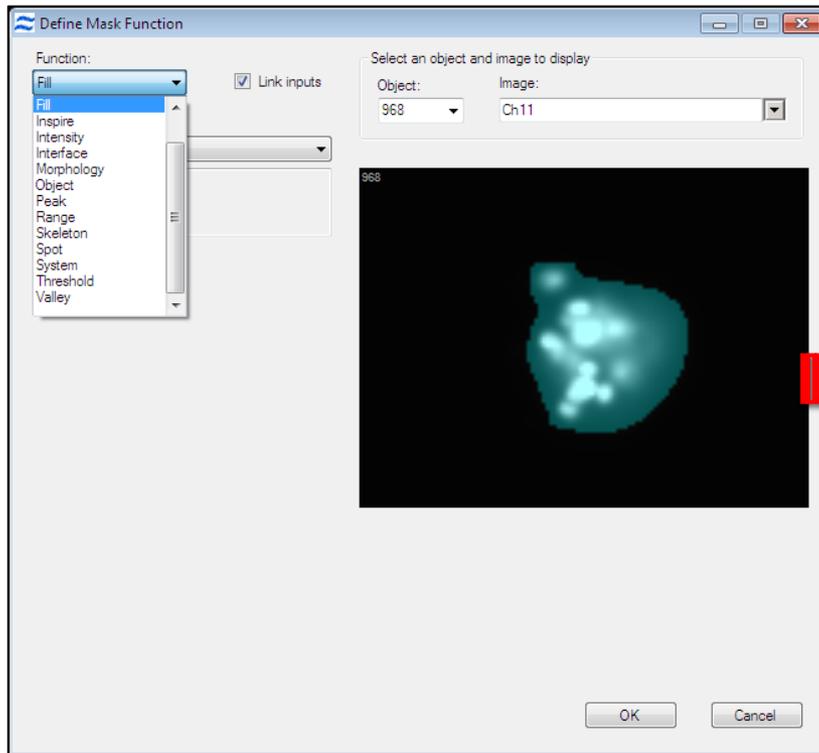
# Analysis Wizards in IDEAS



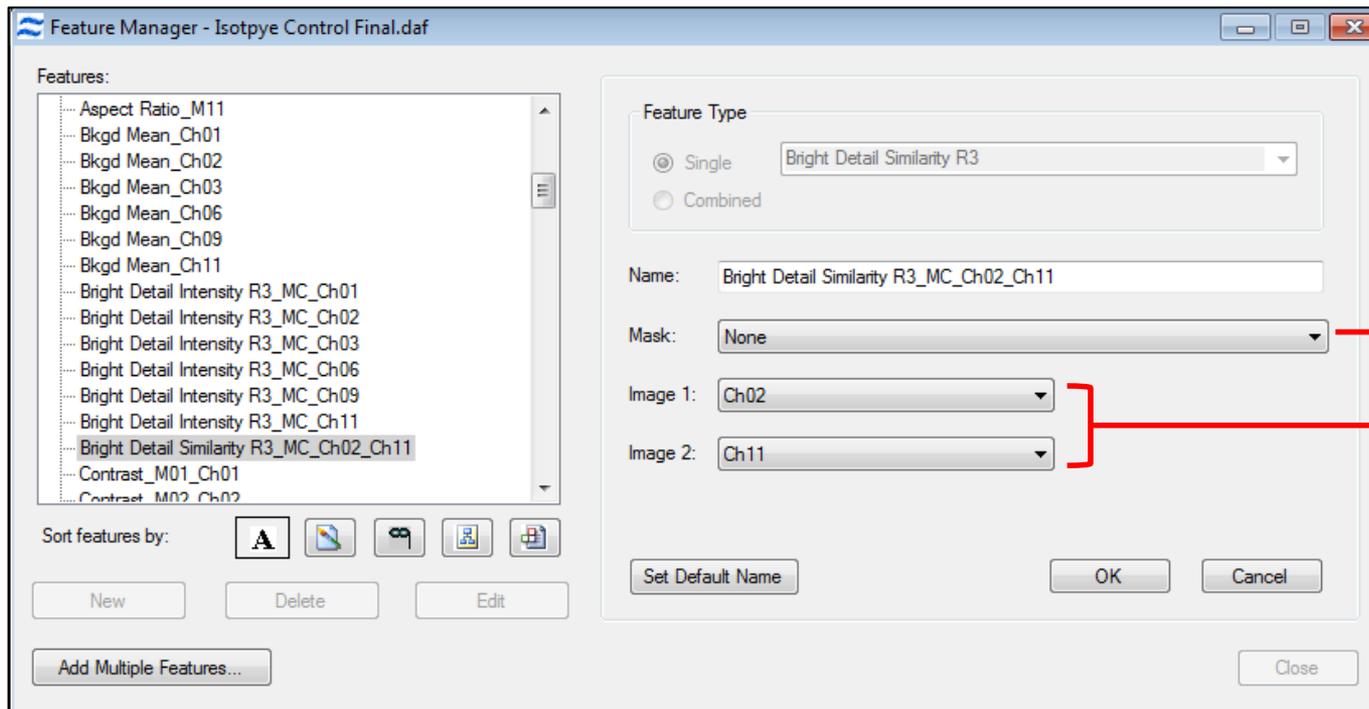
# Working with Features in IDEAS



# Adjusting the “Spot Mask”



# The Bright Detail Similarity Feature



Select Mask  
Channels for  
colocalization

# How to find and download the IDEAS ImageStream Analysis Software?

1. Please go to <https://www.amnis.com/login.html>
2. Please create a log-in account
3. Log-in
4. The title page will be as shown
5. Please select the software downloads link
6. You are in the download page to download the software and many other tutorial and educational materials.

The screenshot shows the Amnis website customer portal. At the top, there is a navigation bar with links for Home, PRODUCTS, APPLICATIONS, DISCIPLINES, RESOURCES, COMPANY, SUPPORT, and ACCOUNT. A search bar and contact information (Toll Free (800) 730-7147) are also visible. The main content area features a 'Customer' welcome message, a 'Knowledgebase' search bar, and a 'Subject Areas' list. The 'Software downloads' link is highlighted with a red box and a red arrow pointing to it from the text in step 5. Other links in the 'Subject Areas' list include 'How to use the knowledge base', 'Steps 1-3 FlowSight', 'Tutorials FlowSight', 'Steps 1-3 ImageStreamX (ISX and MKII)', 'Step 4 Data analysis for all instruments -pdfs', 'Tutorials - Data Analysis', 'Customer training presentations', 'Protocols and Tips', 'Publication Notes', 'IS 100 Resources', 'Service Procedures FlowSight', 'Service Procedures ImageStreamX', 'Amnis Travel Stipend -link', and 'Dropbox for file uploads >20MB -link'. On the right side, there are sections for 'Personal Profile', 'Customer Support', 'Desktop Sharing', 'Received from Amnis', 'Sent to Amnis', and 'Upload a File'.

# A Wonderful World of Technology Event:

## Using Image Stream to Evaluate Activation and Translocation of Signaling Molecules

Chander Raman, Ph.D.

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*November 6, 2014*

**UAB** MEDICINE

CLINICAL IMMUNOLOGY & RHEUMATOLOGY



# Acknowledgements

**Carlos Mier (UNAM)**

**Shawn Williams**

**Yanna Ding**

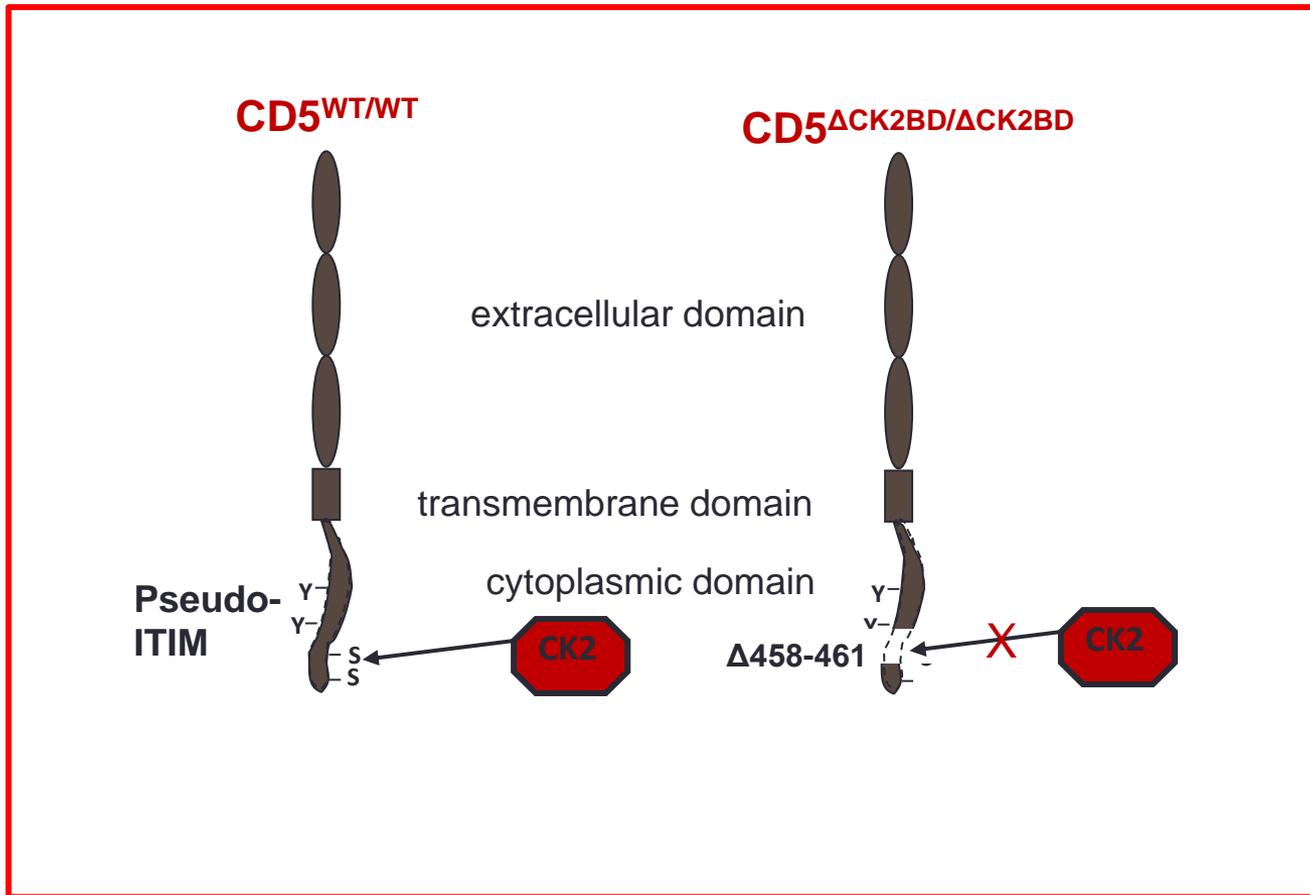
## **Funding:**

**NIH (NIAID, NINDS),**

**NIH grants to RDCC**

**AR048311, AI27667**

# Does absence of CD5-CK2 signaling alter TCR induced activation in thymocytes?



**CD5<sup>ΔCK2BD/ΔCK2BD</sup>**



+

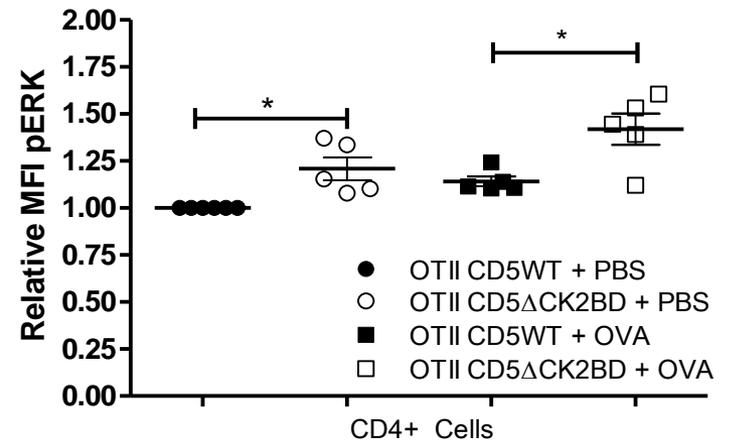
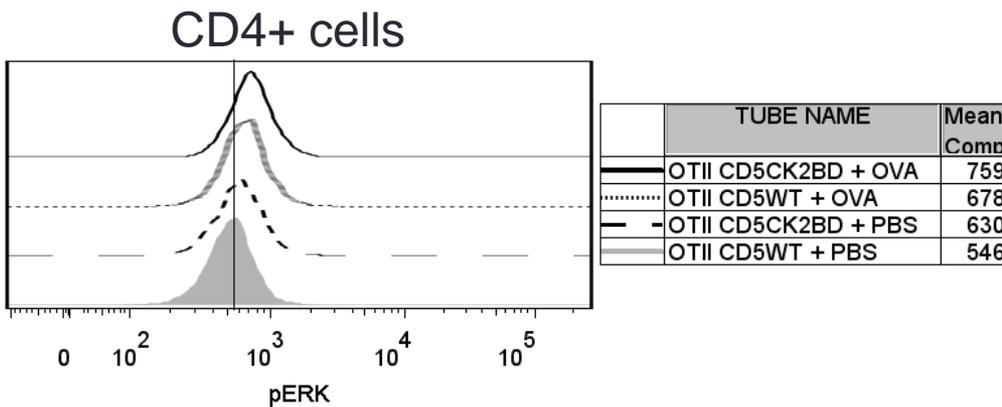
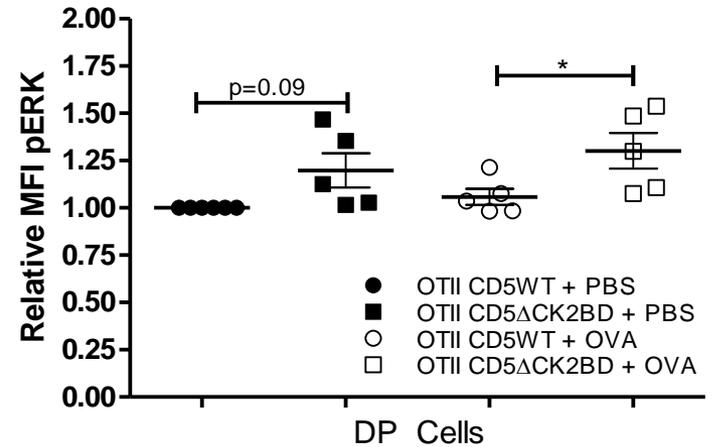
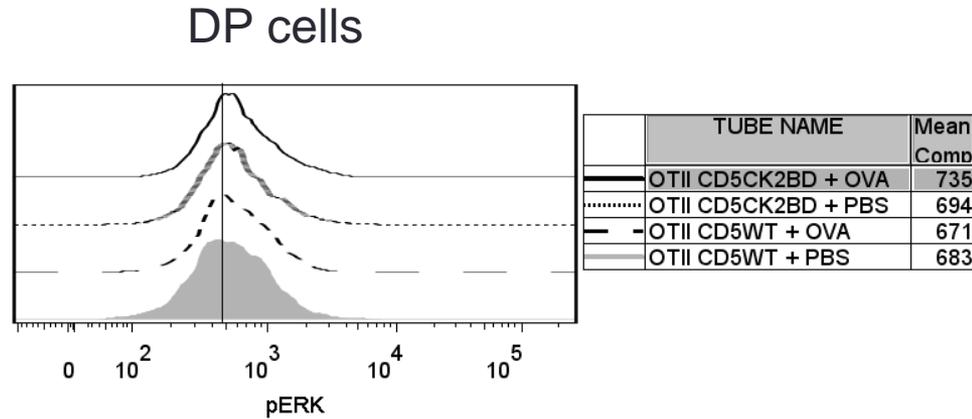
**OTII TCR<sup>OVA</sup>**



**OTII.CD5<sup>ΔCK2BD/ΔCK2BD</sup>**



# Basal and OVAp induced active ERK is elevated in DP cells and CD4 SP thymocytes from CD5-CK2 signaling deficient mice

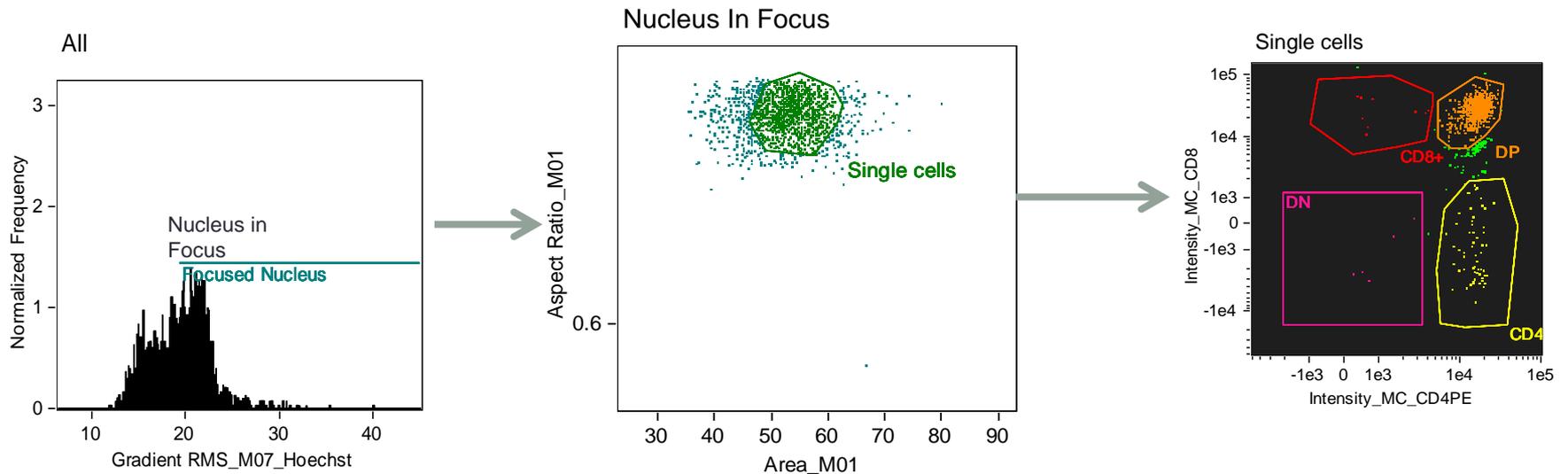


**Flowcytometry provided a first step analysis to determine if there are differences in levels of active ERK.**

**Questions: Is the active translocated to the nucleus and are there differences in cytoplasmic vs nuclear pERK between WT and CD5-mutant?**

**This can be quantitatively evaluated using ImageStream**

# ImageStream Analysis (PhosphoProtein Analysis)



1) Choose the Nucleus that are in Focus

2) Choose the Single Cells by Using Area and Aspect Ratio

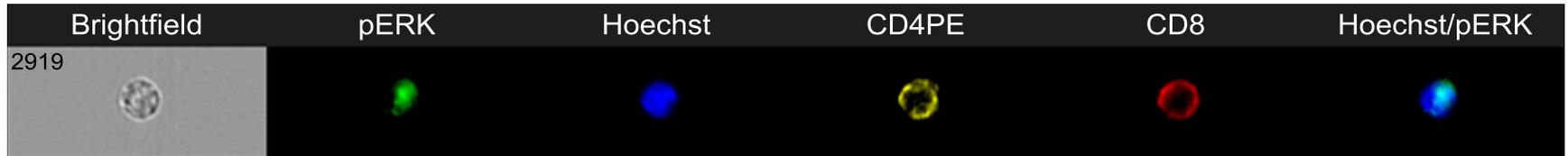
3) Gating of subpopulations)

# pERK: Imagestream Analysis

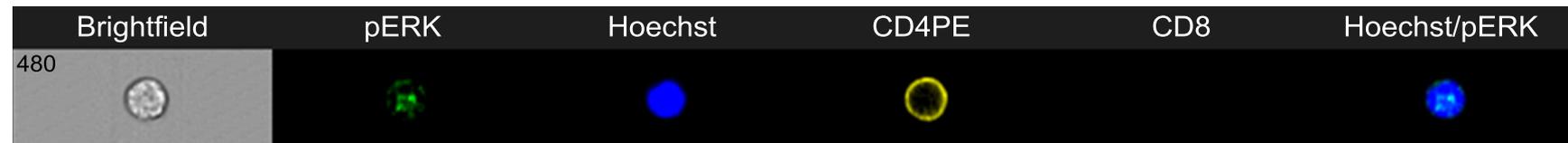
## OTII CD5WT DP Cells



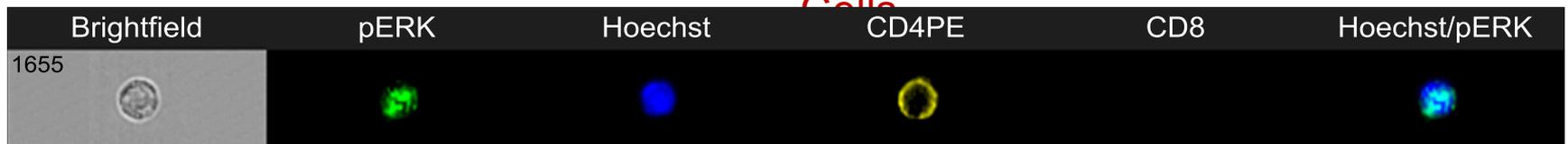
## OTII CD5 $\Delta$ CK2BD DP Cells



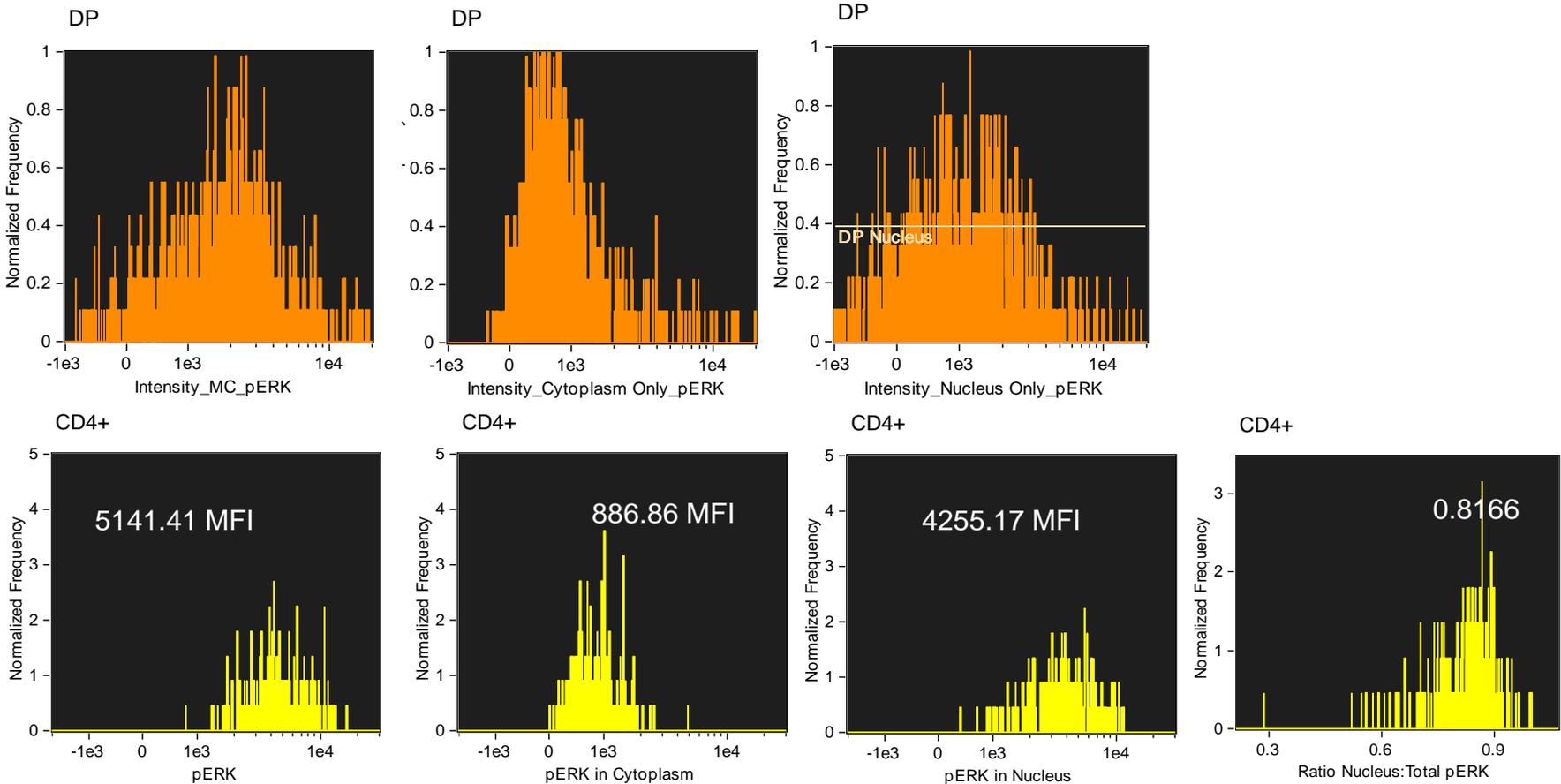
## OTII CD5WT CD4+ Cells



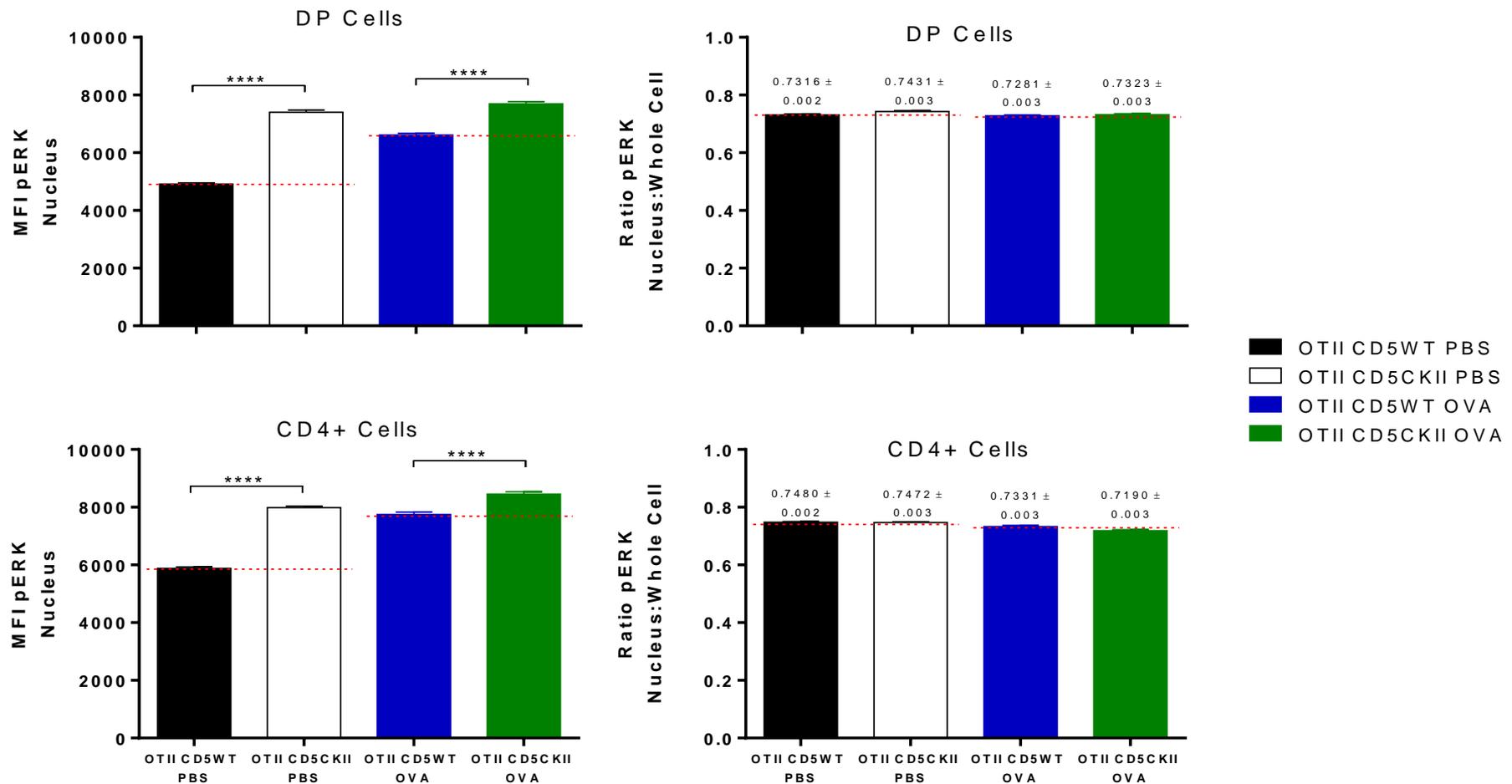
## OTII CD5 $\Delta$ CK2BD CD4+ Cells



Using masks to define the cytoplasm and nucleus, we can determine the MFI of pERK in nucleus, cytoplasm and whole cell.



# Elevated pERK in nucleus of CD5-CK2 signaling deficient CD4+ thymocytes



- Although pERK levels are different, ratio of nuclear to whole cell is not different.
- Ova immunization induces greater increase of pERK in nucleus of WT DP thymocytes