Robust Quantitation of Cellular Probe Localization

ImageStream Layout

detector

dichroic filter stack

velocity detector

autofocus

cells in flow

brightfield illuminator

laser
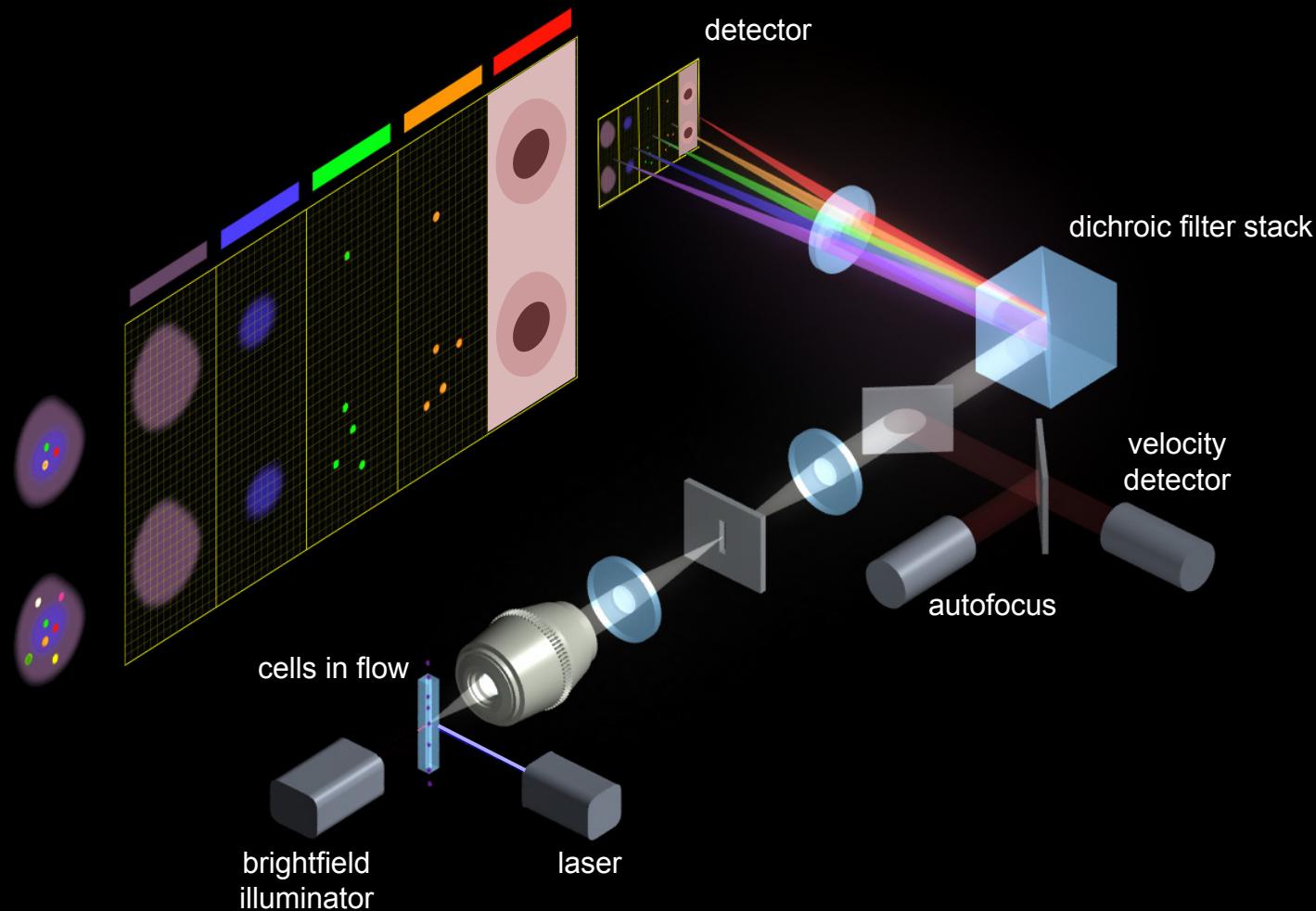
Principle of Image Similarity Analysis


Untranslocated
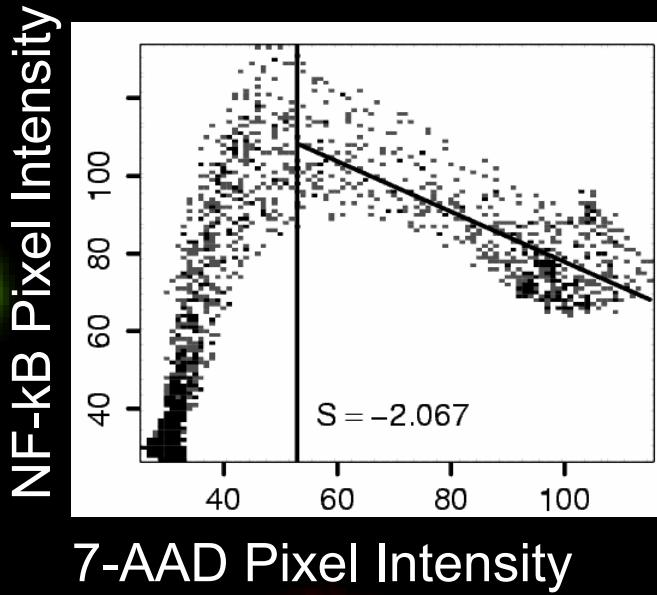
Translocated
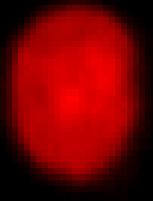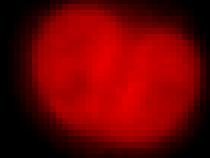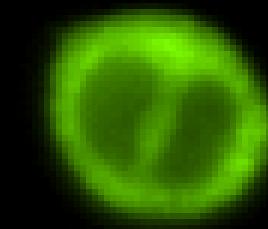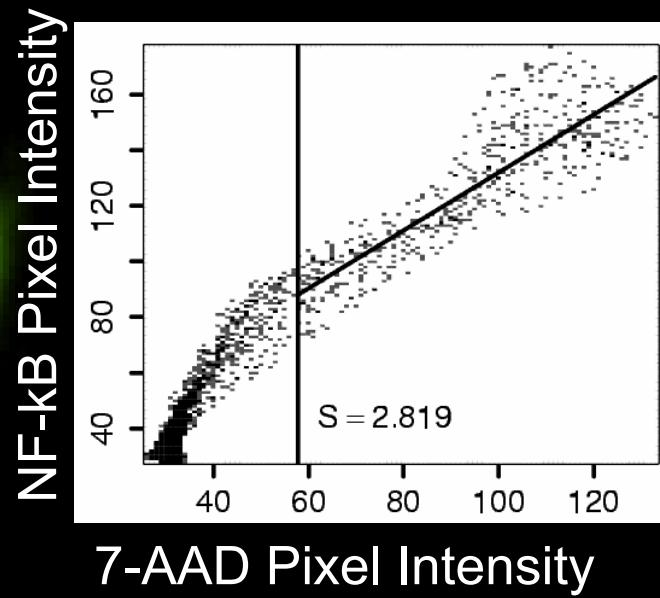
Pearson's Correlation Coefficient

$$\rho = \frac{\sum_i (x_i - X)(y_i - Y)}{\sqrt{\sum_i (x_i - X)^2 \sum_i (y_i - Y)^2}}$$

Similarity is a logarithmic rescaling of Pearson's Correlation Coefficient

$$S = \ln\left(\frac{1+\rho}{1-\rho}\right)$$

Nuclear Translocation Using Similarity
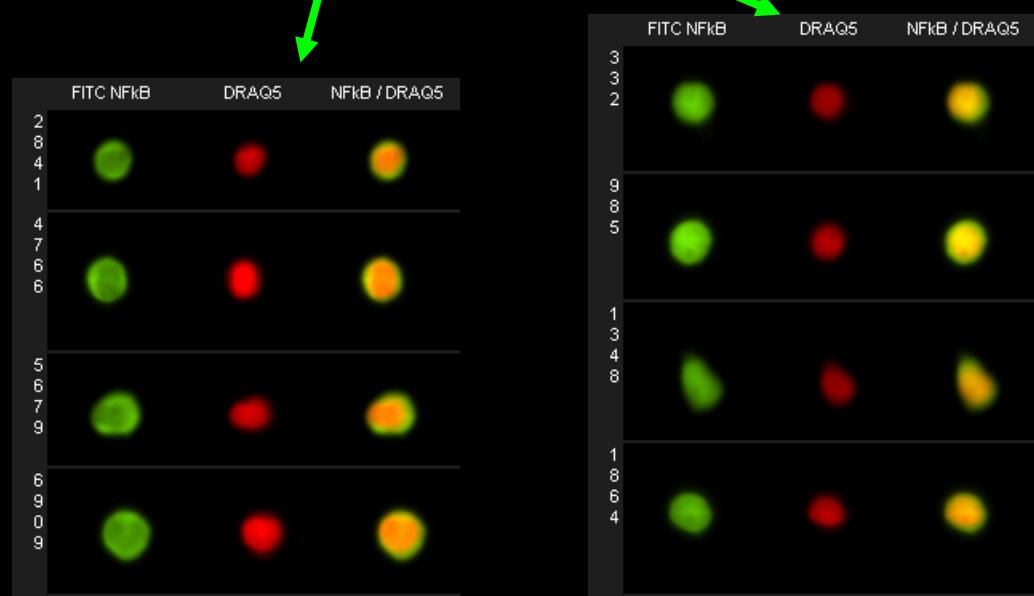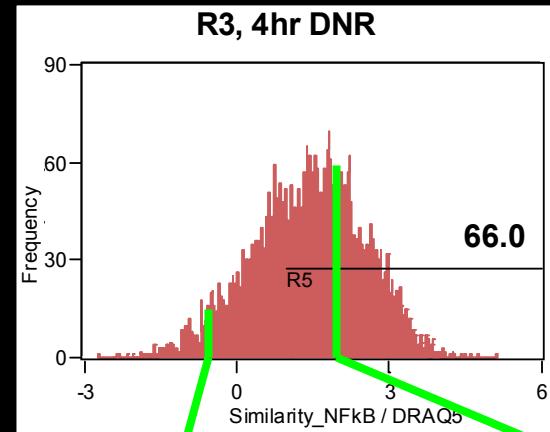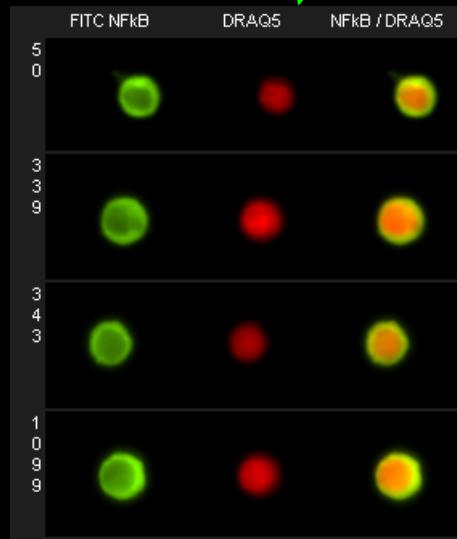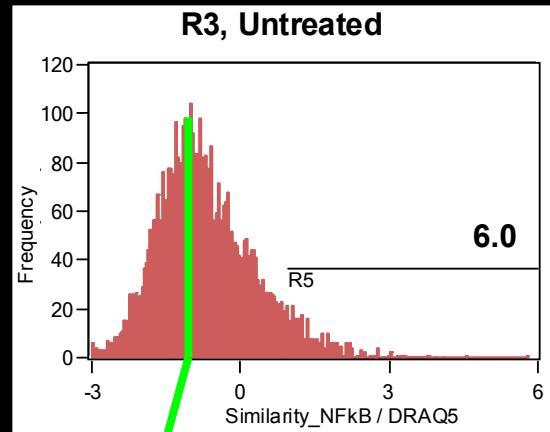
Receptor Capping Quantitation

RTX CD45 Composite

RTX / CD45
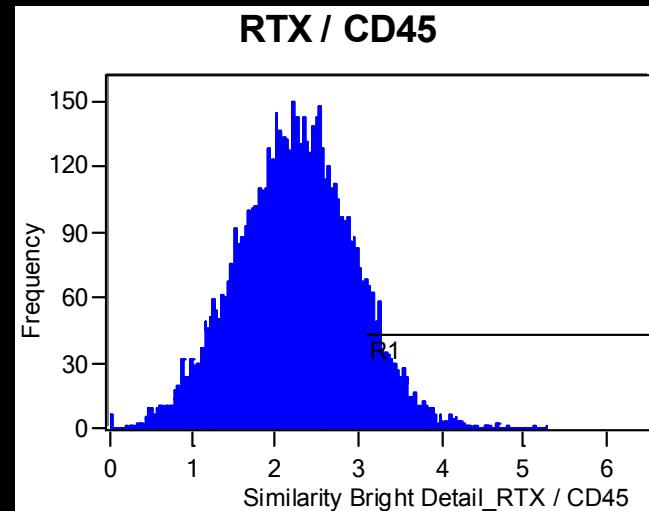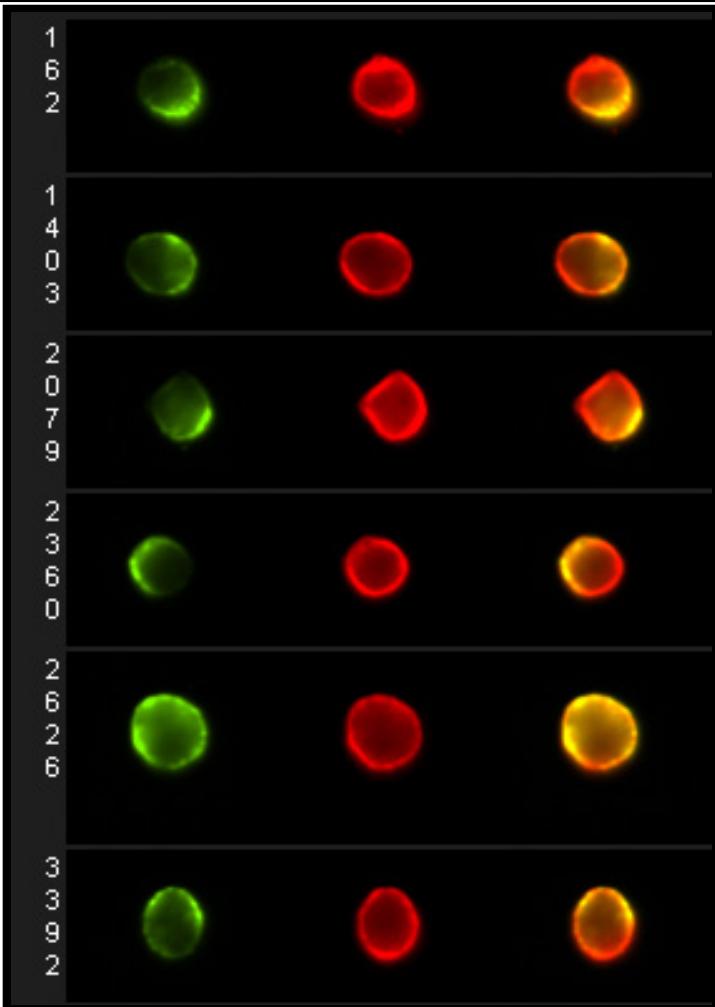
Raji cells in serum

Actively cap with AF488 RTX Counterstain with PE anti-CD45

Measure similarity between fluorescent images

Data produced in collaboration with Dr. Paul Beum and Dr. Ronald Taylor, University of Virginia School of Medicine

Receptor Co-Localization



RTX   C3b   Composite

RTX / C3b
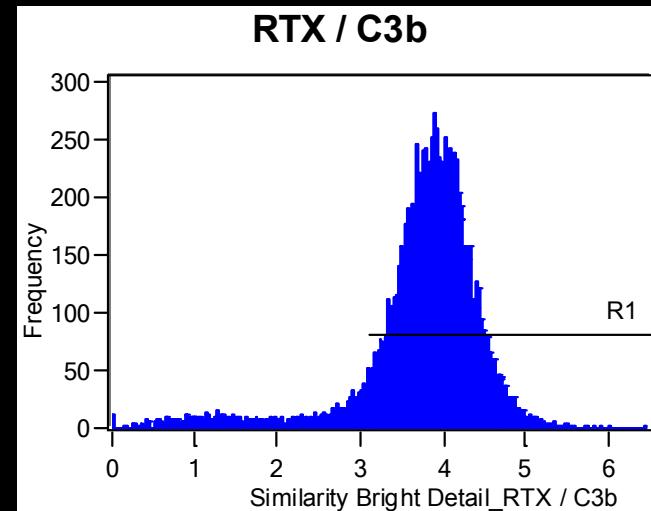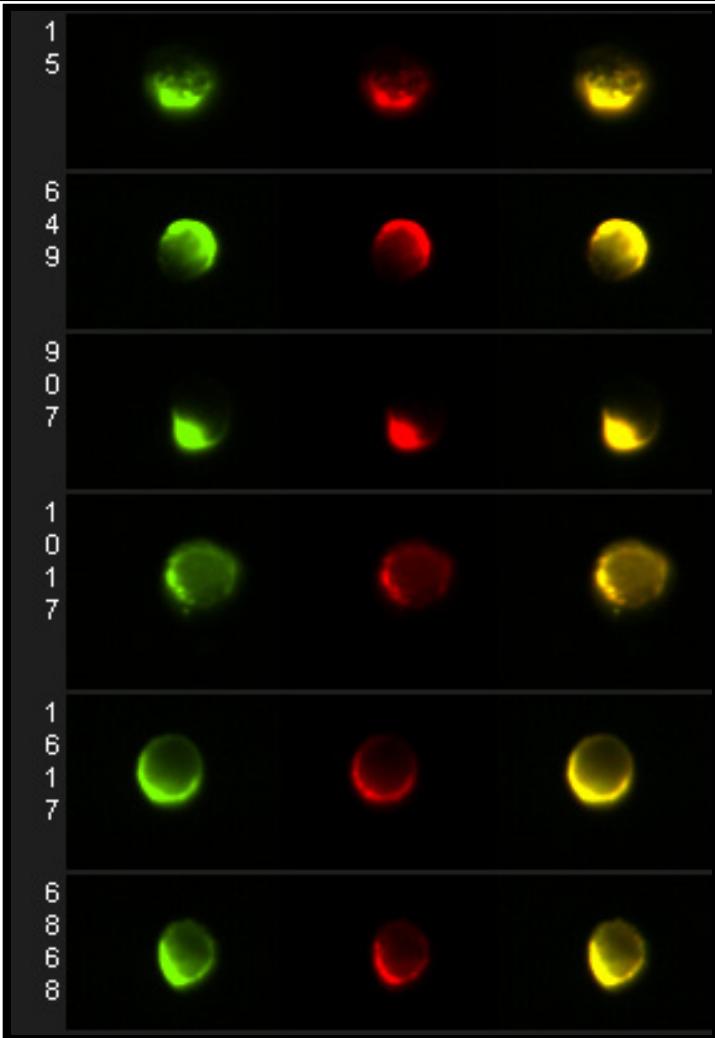
Raji cells in serum

Actively cap with AF488 RTX Counterstain with PE anti-C3b

Measure similarity between fluorescent images

Data produced in collaboration with Dr. Paul Beum and Dr. Ronald Taylor, University of Virginia School of Medicine

Subcellular Organelle Co-localization
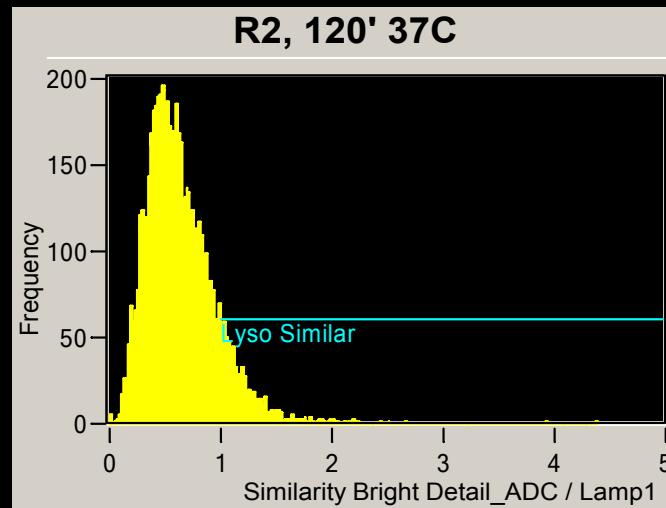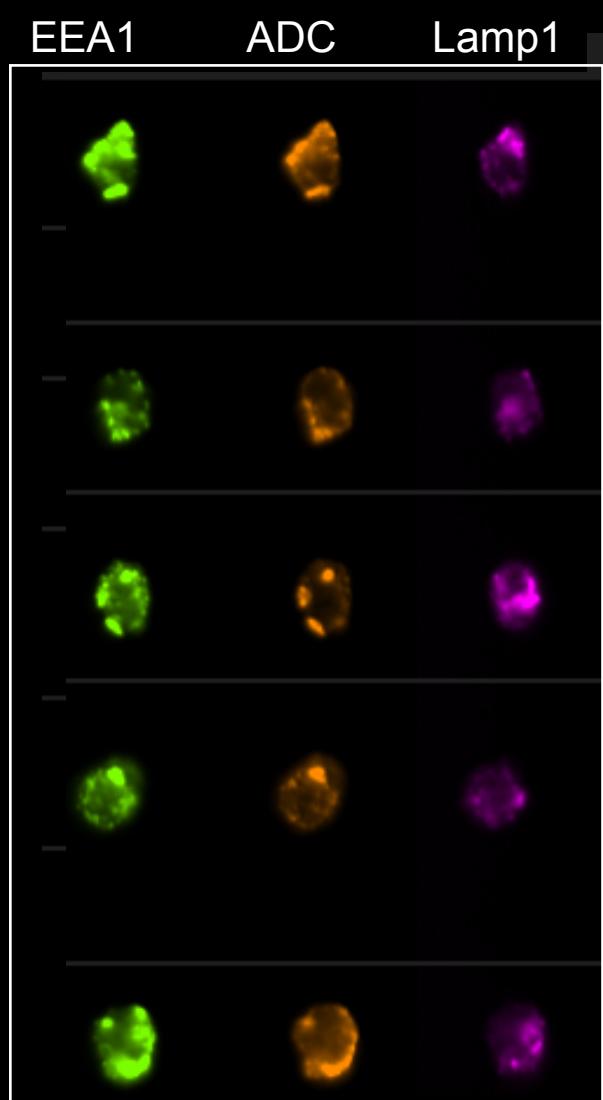
EEA1: FITC

Antibody drug conjugate: PE

Lamp1: Pe-Cy5
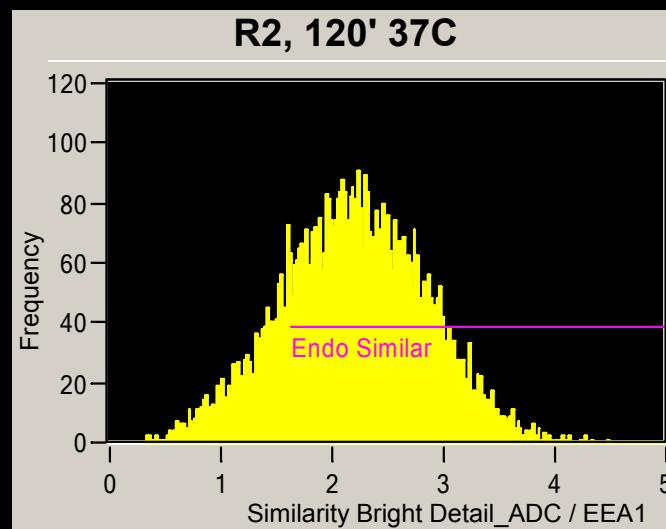
The ADC initially co-localizes to the endosome. It later co-localizes to the lysosome.