

Quantification of the cellular localization of AR in prostate CTCs (3D analysis)

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Imaging Setup

- Images are acquired in 3D stacks of about 35 planes (Shin Tasaki)
- Manual crop selects the ROI (region of interest) in Androgen Receptor (AR) channel (movie shown)
- For each plane the nuclear area is selected based on computer-based segmentation of the DAPI channel
- The exact localization of DAPI is also being done automatically in the cropped area

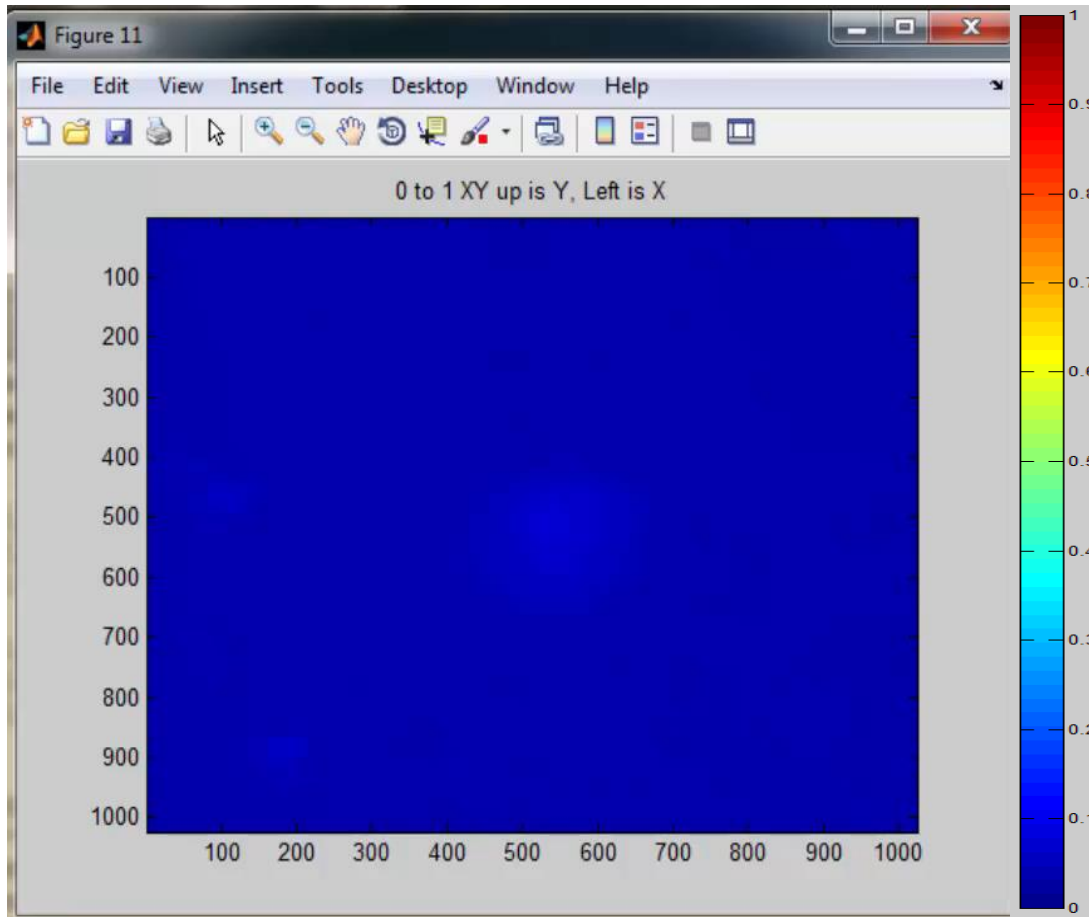
Analysis in 3D

- Automated segmentation of DAPI is done using Unimodal Thresholding (Rosin 2001)
- Automated segmentation of AR is done based on probabilistic approach using the intensity histograms (REF)
- The AR nuclear localization is calculated as:

$$Ratio = \frac{\sum_{i=1}^n \sum_{j=1}^m I(i, j) \cdot M_{DAPI}(i, j)}{\sum_{i=1}^n \sum_{j=1}^m I(i, j) \cdot M_{AR}(i, j)}$$

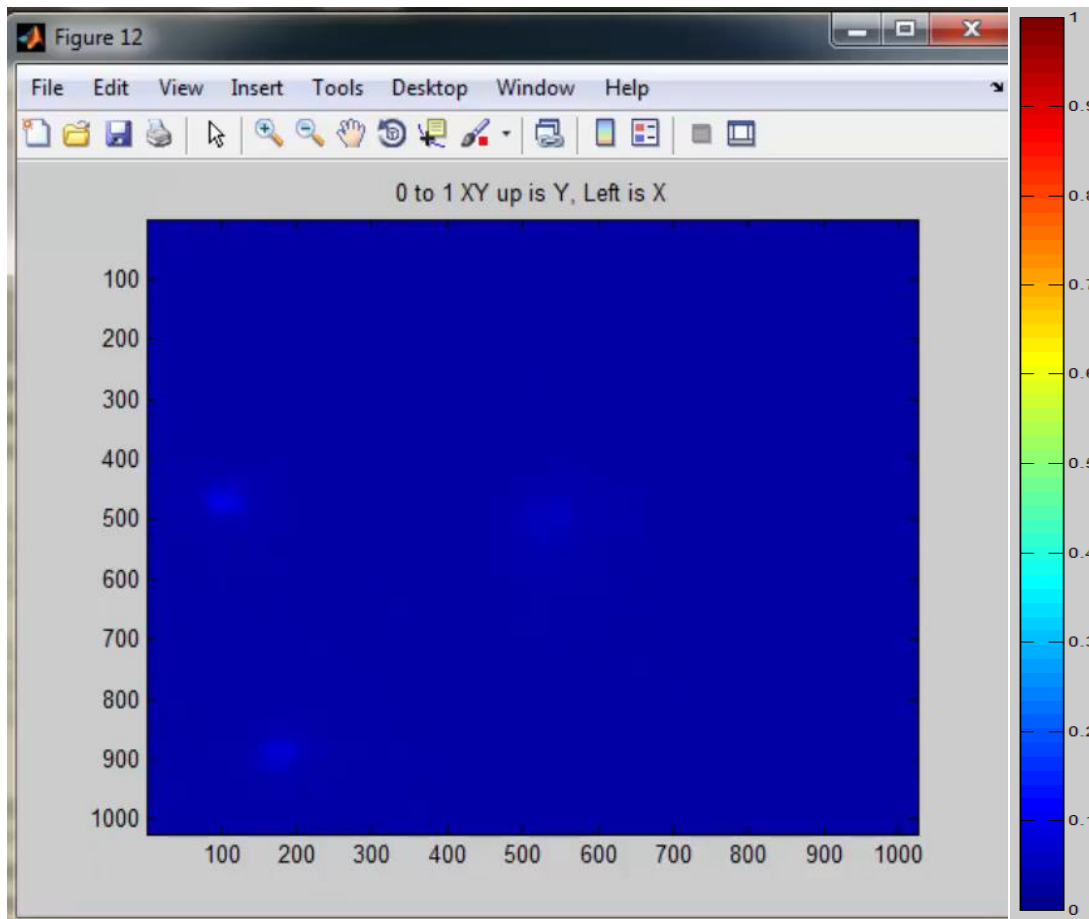
- Where:
 - $I(x, y)$ shows the intensity value of the AR image in pixel (x,y)
 - M_{DAPI} shows the automatically selected mask for DAPI (1 if it is selected and 0 if not)
 - M_{AR} is calculated similarly and defines the AR area (1 if it is selected and 0 if not)

Movies showing the raw 3D stack images for the AR channel



This movie was generated from CTC 103-02
(x=1822, y=5846)

Movies showing the raw 3D stack images for the DAPI channel



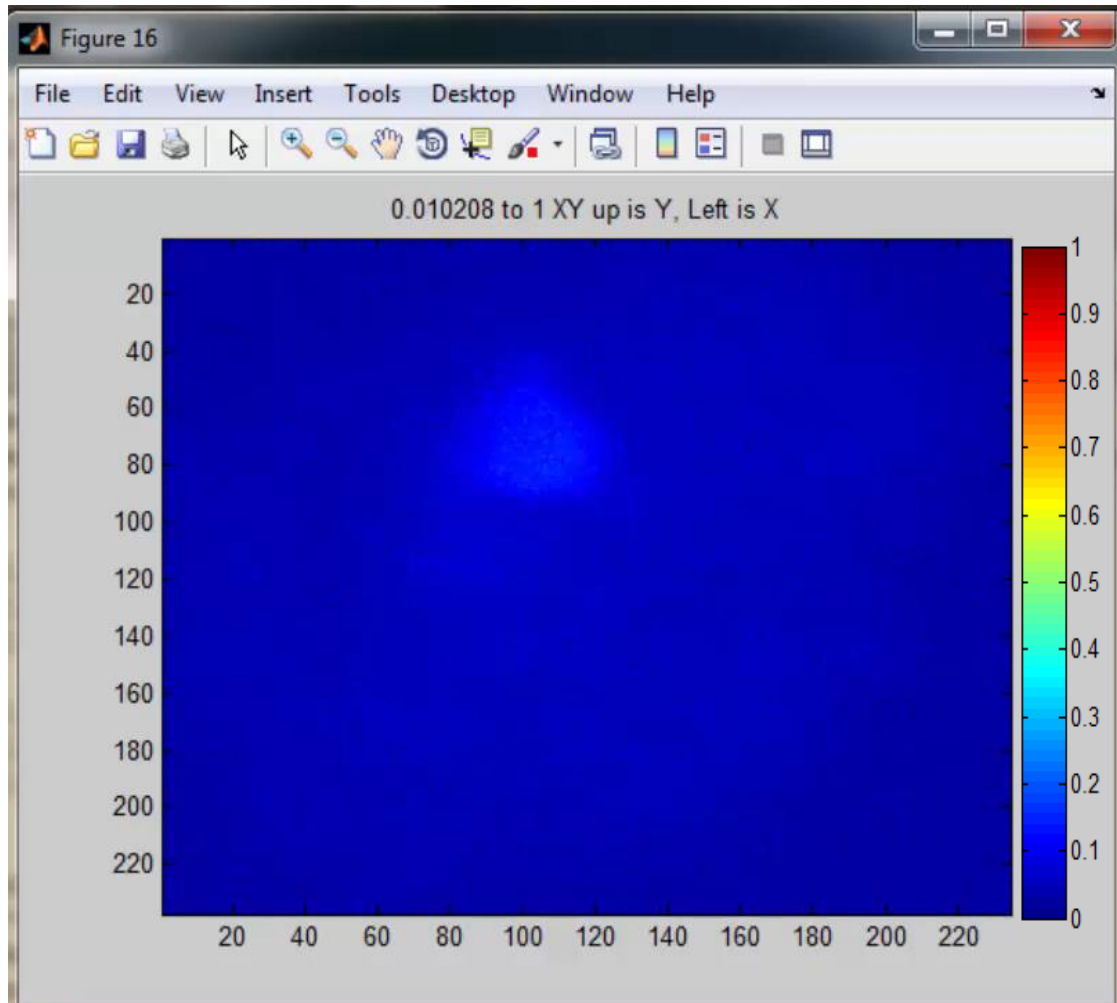
This movie was generated from CTC 103-02
(x=1822, y=5846)

Selecting the Region of Interest (ROI) based on the AR channel



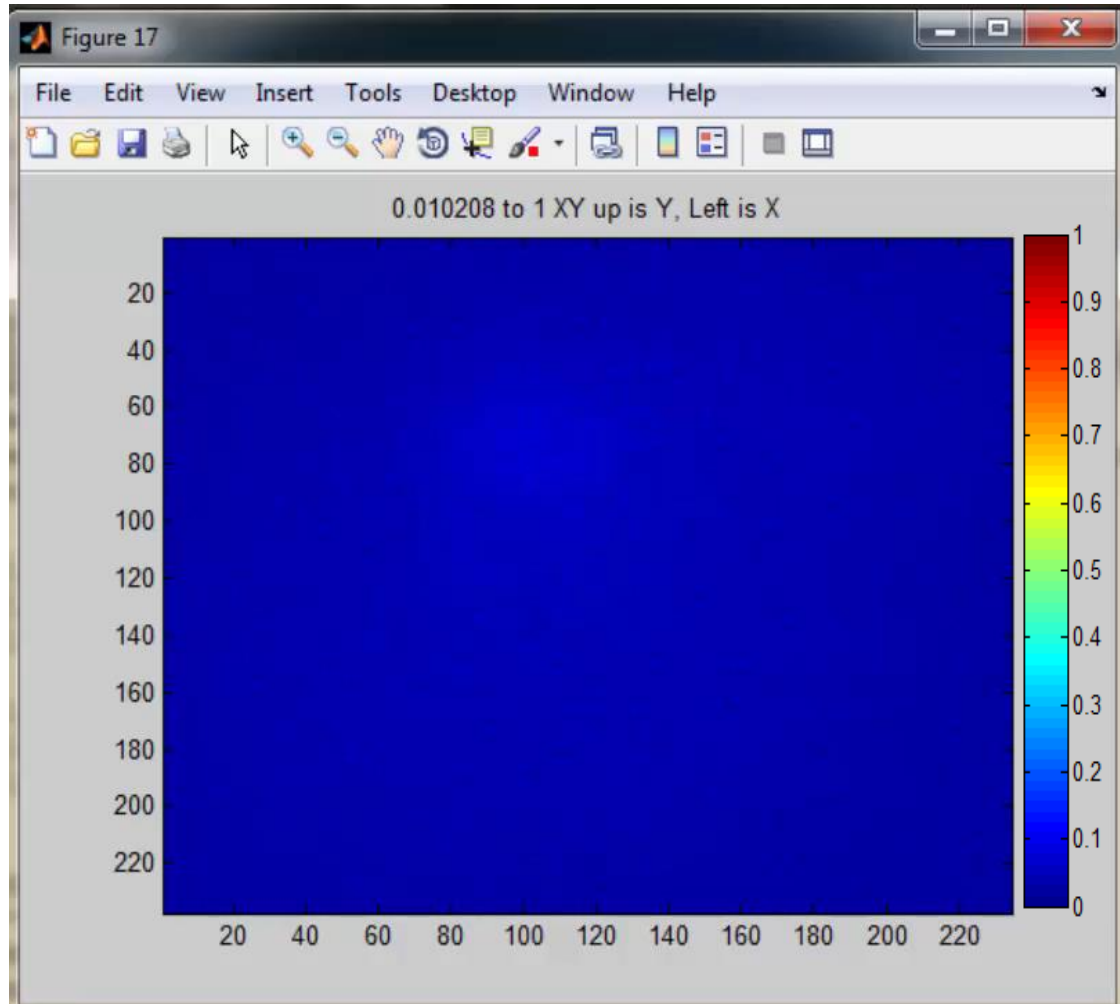
This movie was generated from CTC 103-02
(x=1822, y=5846)

Movie showing the evolution of the AR signal through the 3D stack of 35 AR images for the selected ROI



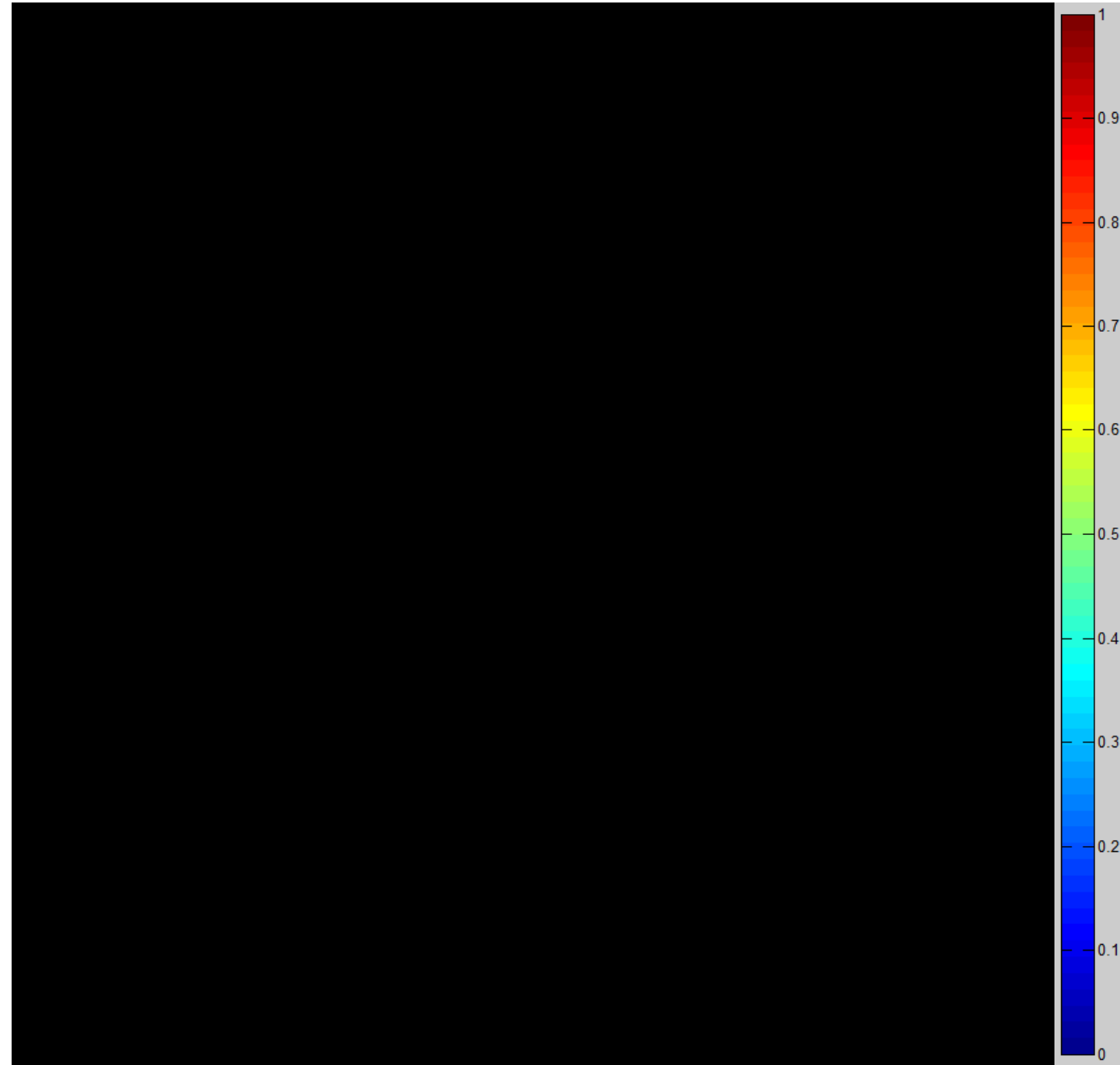
This movie was generated from CTC 103-02
(x=1822, y=5846)

Movie showing the evolution of the DAPI signal through the 3D stack of 35 AR images for the selected ROI



This movie was generated from CTC 103-02
(x=1822, y=5846)

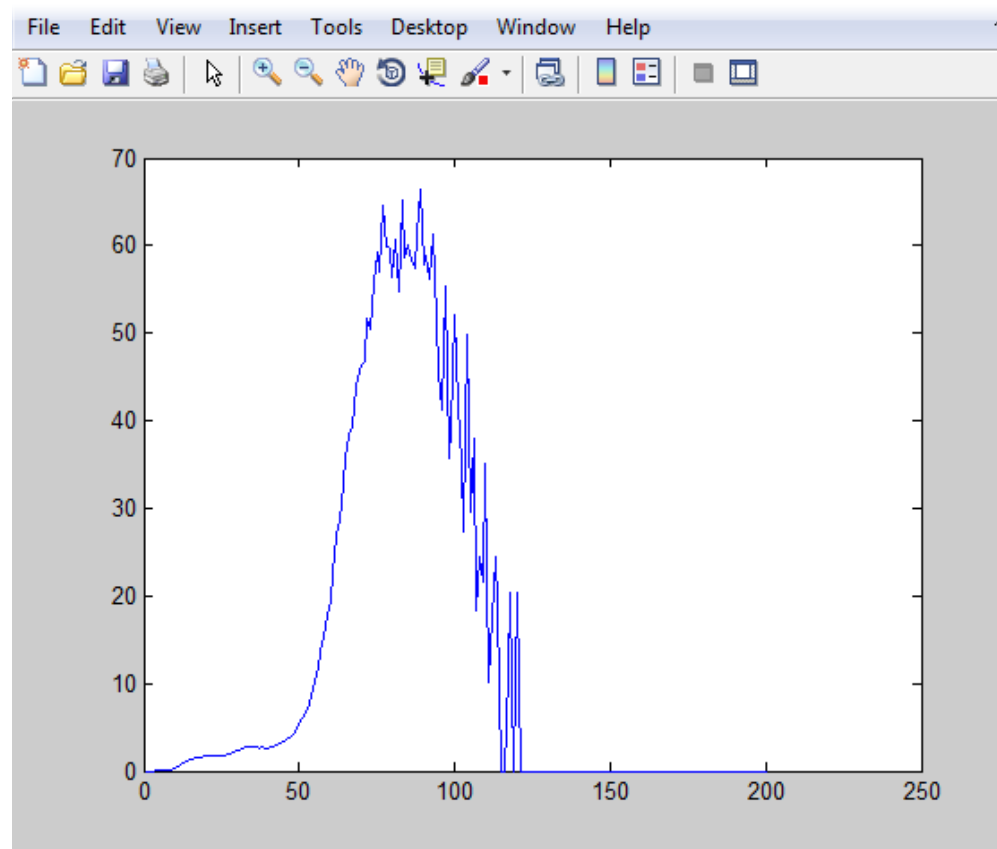
Examples of segmentation and quantification of the AR nuclear accumulation for CTCs from clinical study 9740 (movie)



Results: Percentage of AR nuclear accumulation

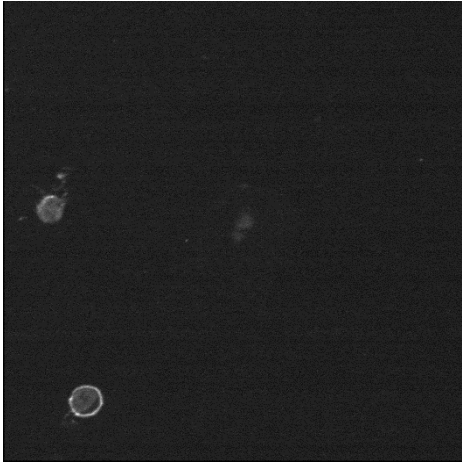
Coverslip 103-01-5335-5170	16.23%
Coverslip 103-02-1822-5846	14.99%
Coverslip 104Ficoll01-4280-7528	45.65%
Coverslip 107-01-2796-5801	28.23%
Coverslip 107-01-2871-4474	42.77%
Coverslip 167-1164-27303D	69.96%
Coverslip 167-1598-45933D	50.77%
Coverslip 167-3171-20633D	51.78%
Coverslip C55D8sick(1)-1032-5267	30.51%
Coverslip C5D8sick(1)1998-6222	28.43%

Backup slides: Method for AR segmentation

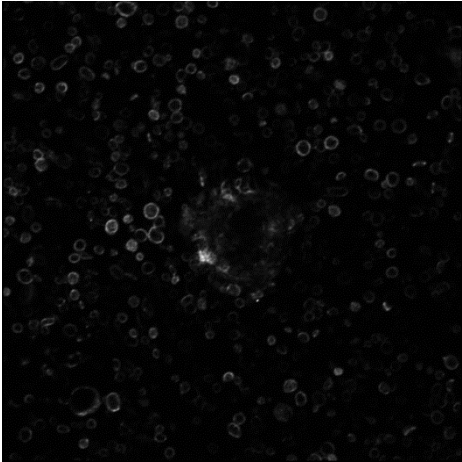


Normalized Histogram of Cropped Area
Reflect the probability of being Foreground
Normalized Histogram of the Whole Image

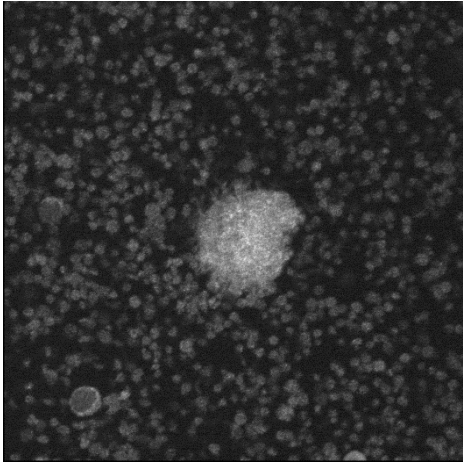
The extend of the AR label shows the cellular area (CTC 103-02-1822-5846)



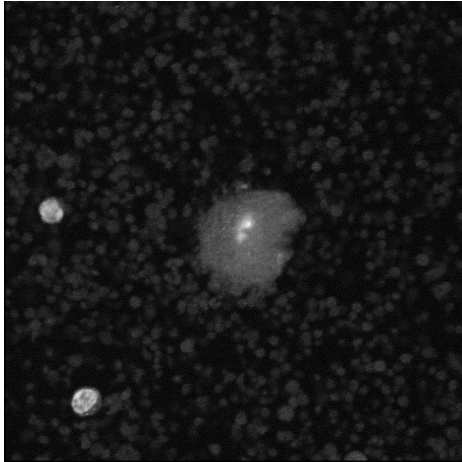
CD45



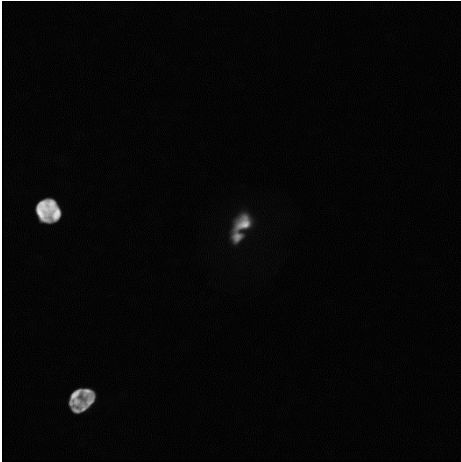
Tubulin



PSMA

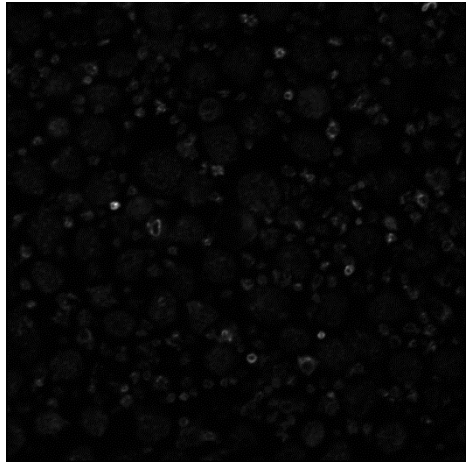


AR

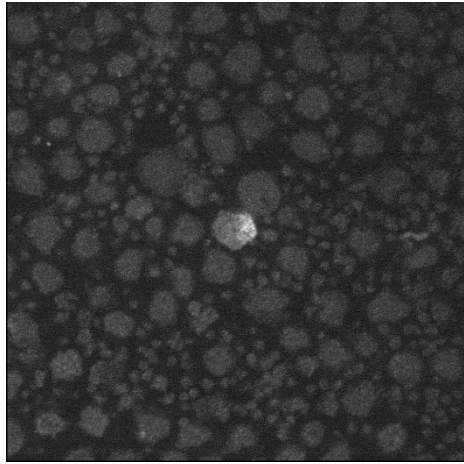


DAPI

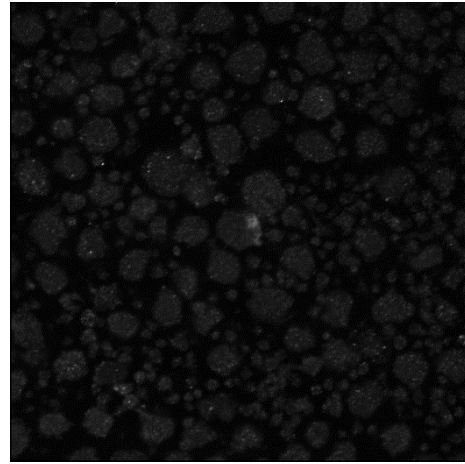
The extend of the AR label shows the cellular area (CTC C5D8sick(1) 1522-3452)



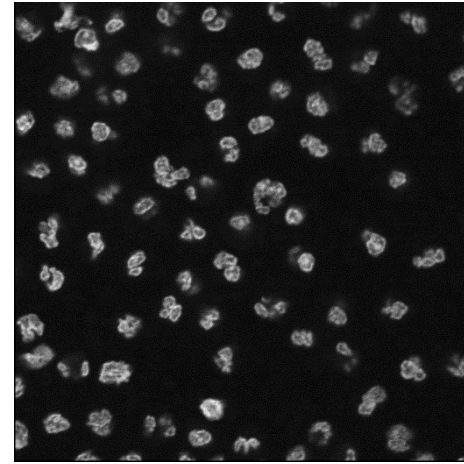
Tubulin



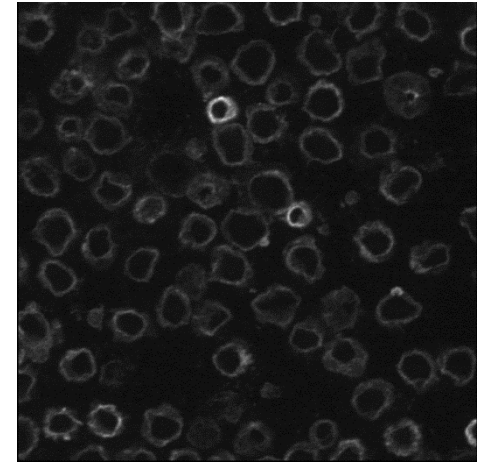
PSMA



AR



DAPI



CD45