

Biomarker discovery with IHC (immunohistochemistry)



WuXi Oncology & Immunology Unit



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Facility and services

■ Instrument platforms

- Automated Station/ Leica Autostainer XL
- Vectra, PerkinElmer (caliper)
- Fluorescence Microscope/Axio Imager A2 & Nikon NI-U

■ Tissue Bank

- PDX/CDX tumors

■ Technology platforms

- H&E
- Regular IHC, IF
- Opal multiplex IHC
- TMA (tissue microarray)
- Sirius-Red staining
- MASSON trichrome staining



Automated Station



Vectra, PerkinElmer(caliper)



Fluorescence Microscope

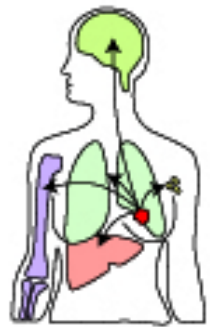
Workflow of IHC/IF for tumor tissues

Surgical resection
of tumors

Tissue processing
and section

Staining with
antibodies

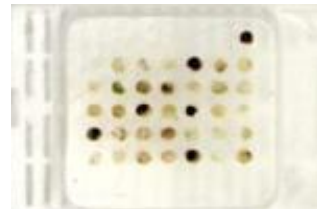
Take photos and analysis



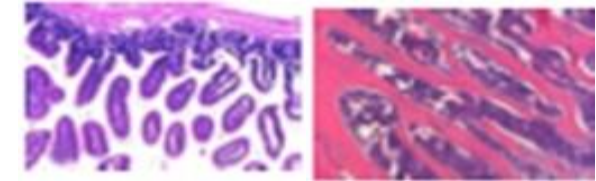
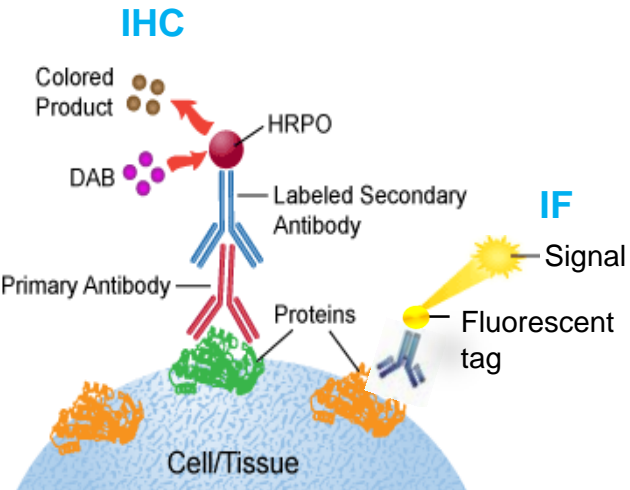
In vivo efficacy
study



FFPE blocks

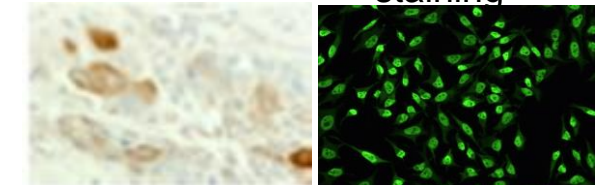


TMA block



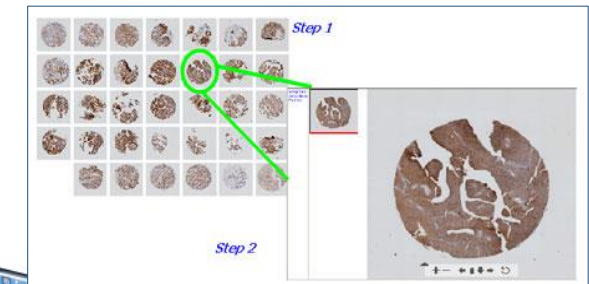
H&E

Special
staining



IHC

IF



TMA



Standard validation procedures for IHC

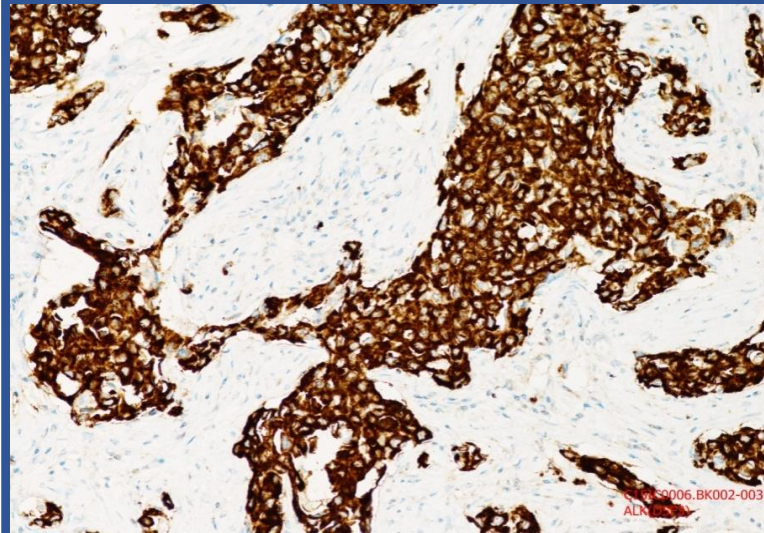
■ Sample fixation and processing

- Cut and trim the tissue samples at ≤ 5 mm thickness to ensure proper fixation
- Put the samples in NBF immediately, fix for 24 to 36 h at room temperature

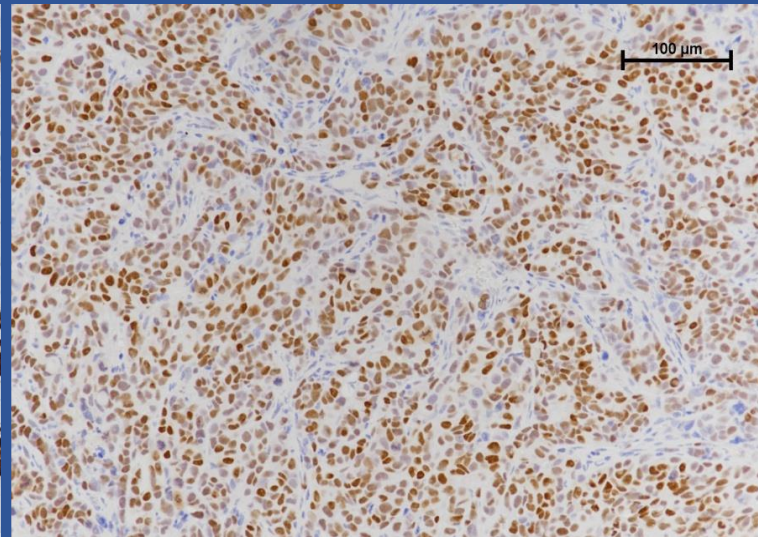
■ Standard validation procedures for IHC

- Proper positive and negative controls for target of interest
- Isotype and/or blank control
- Optimized primary antibody concentration by dilution
- Consistent dyeing time optimized for individual antibody and tissue
- Physiological-relevant location/expression level for each marker based on pathology expertise

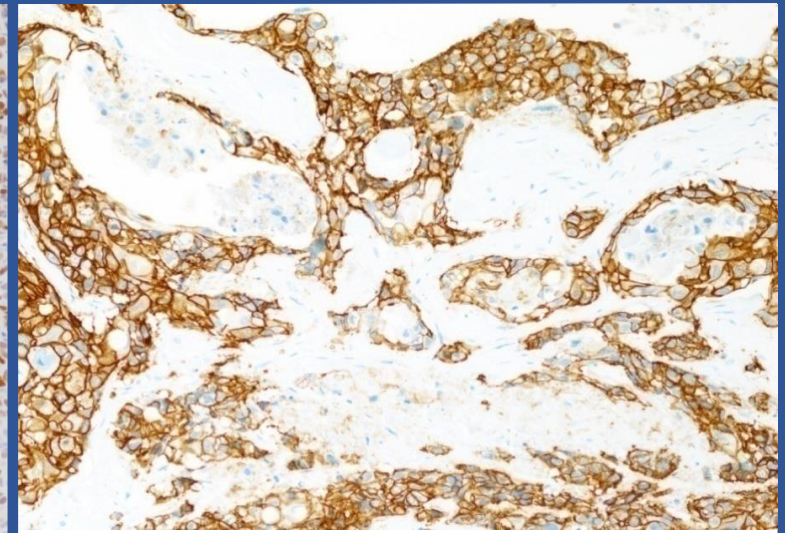
Representative images of regular IHC stained with anti-human antibodies



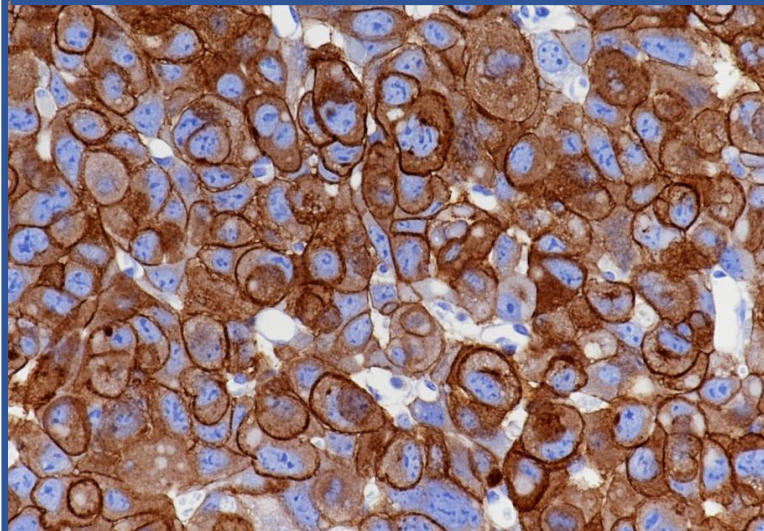
Patient lung cancer, ALK



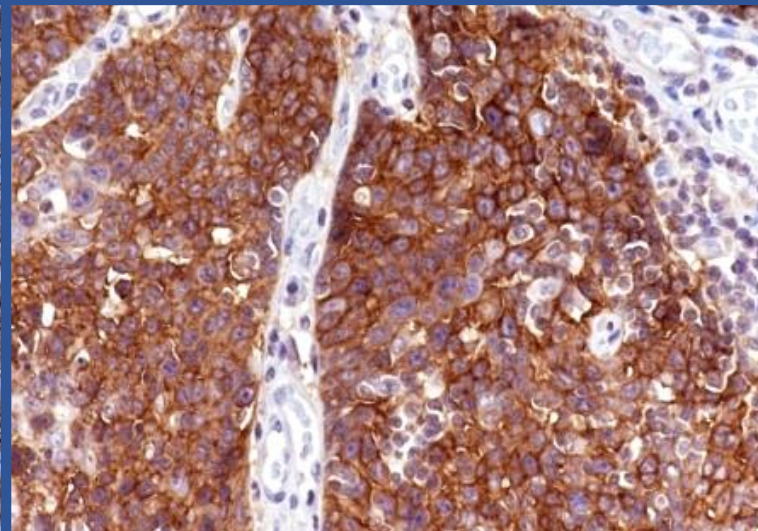
MCF-7 CDX, ER



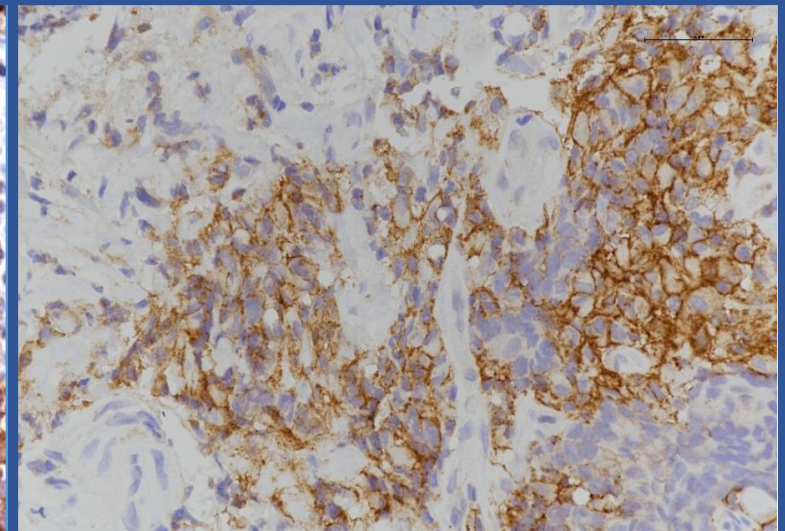
Patient breast cancer, Her2



H441 CDX, c-Met



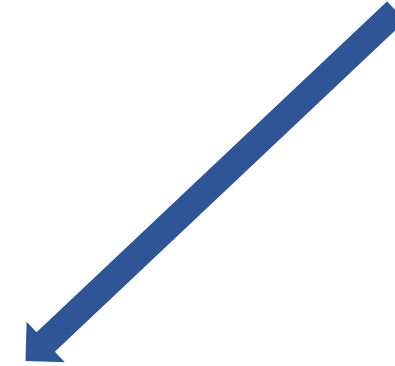
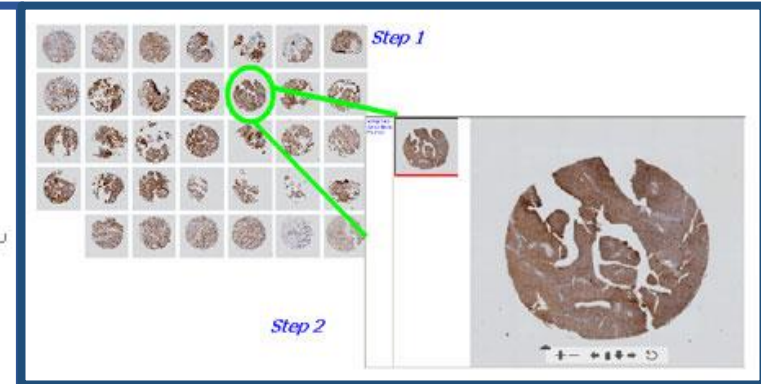
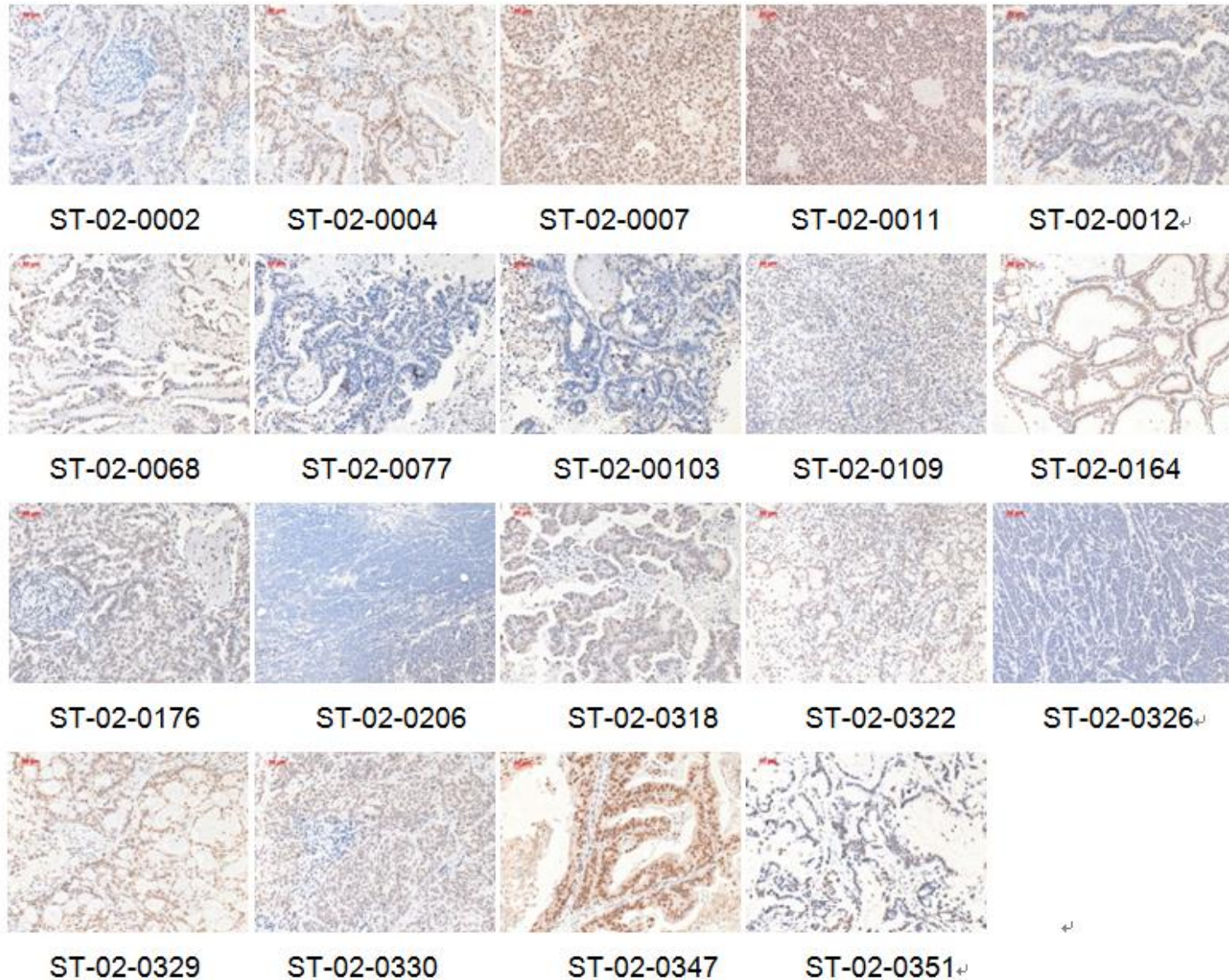
Patient esophageal cancer, EGFR



Patient colorectal cancer, PD-L1

Tissue Microarray Platform

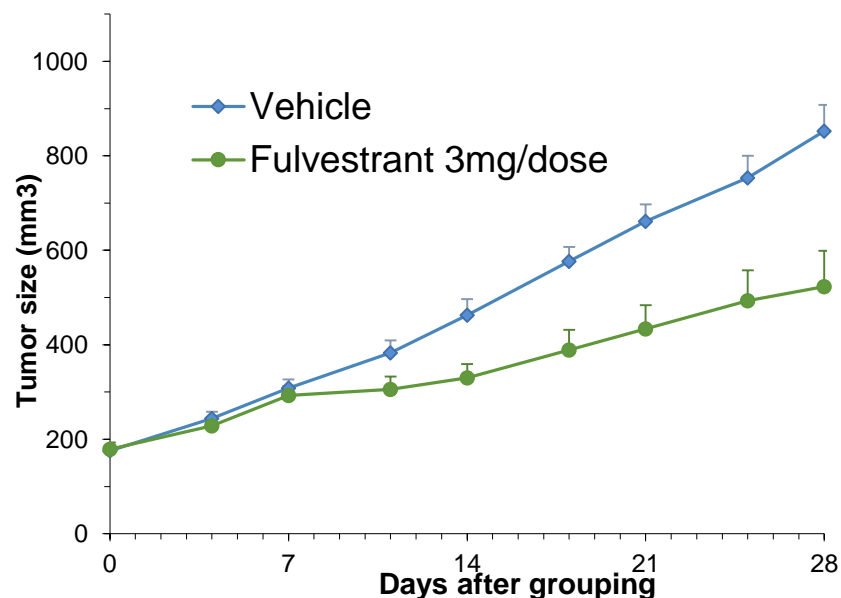
Images of regular IHC stained with anti-PAR antibody on gastric cancer TMA block



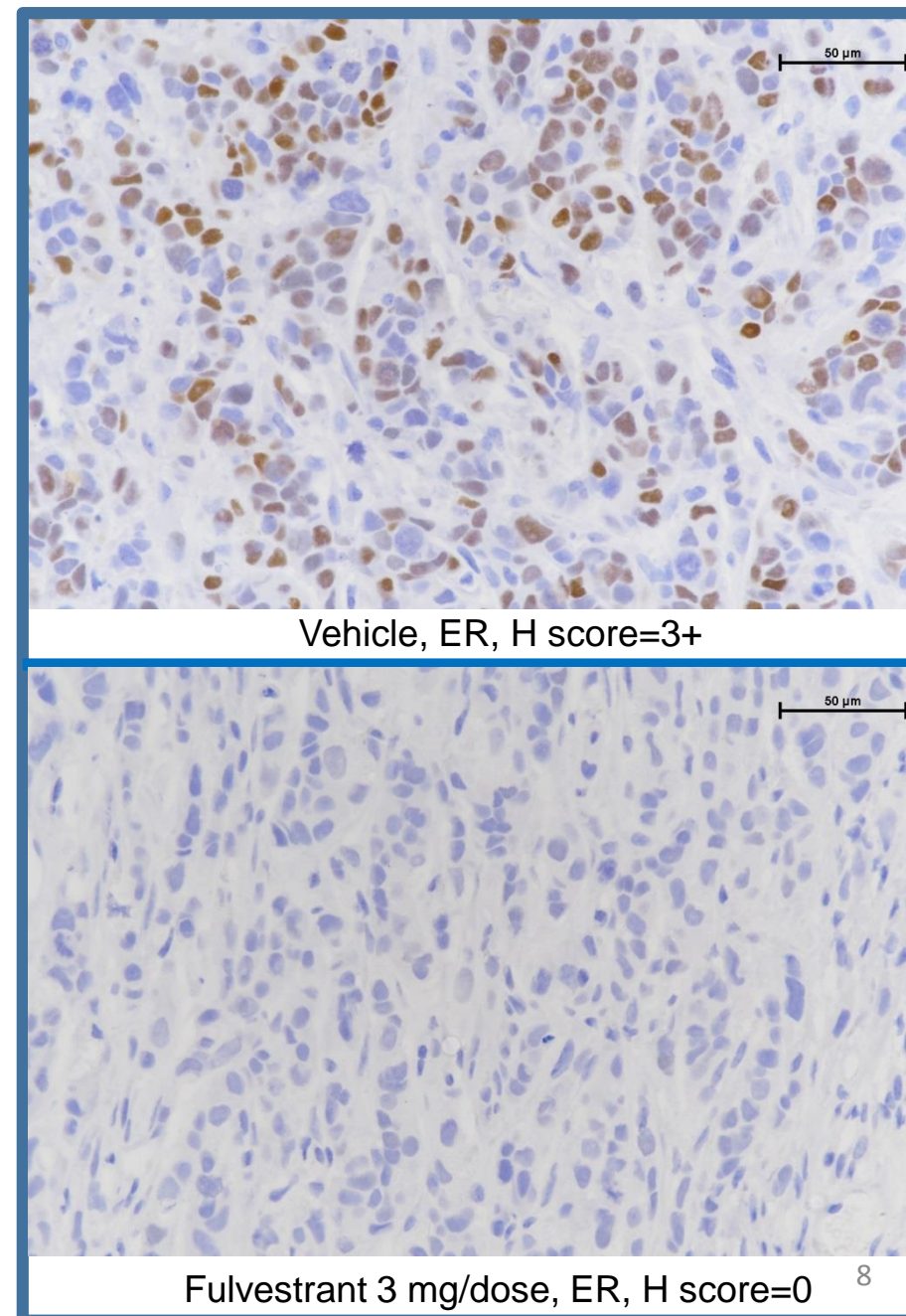
Case study: Fulvestrant treatment attenuated ER expression in T47D xenograft tumor

Consistency in the results between IHC and the *in vivo* efficacy study.

T-47D breast cancer CDX model

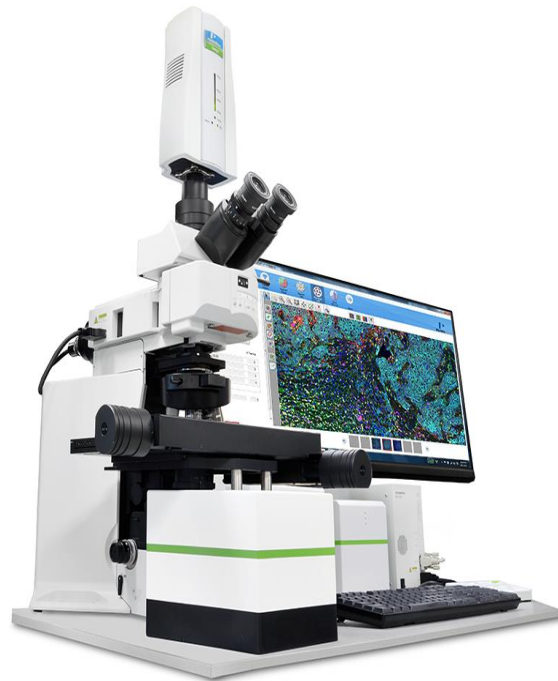


Tumor growth was inhibited in the fulvestrant treatment group. In addition, fulvestrant inhibited ER receptor dimerization and accelerated degradation of ER. Thus few ER positive cells were observed in compound treated xenograft tumor.

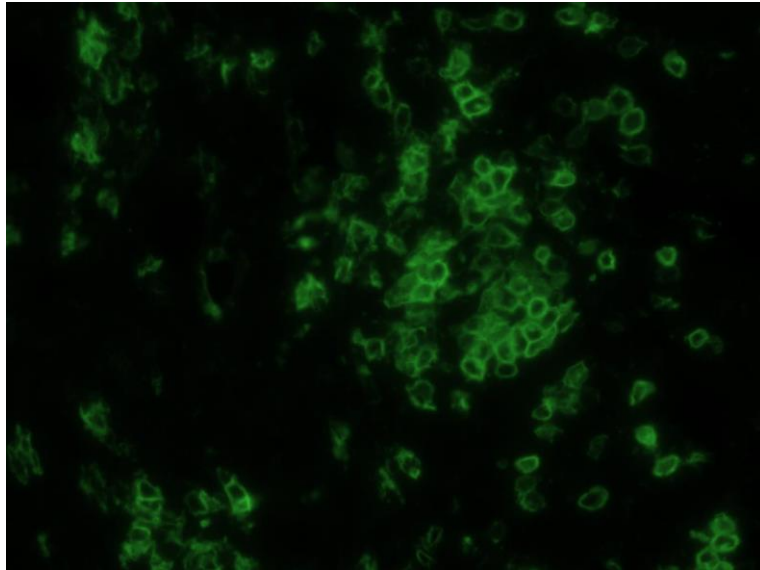


Opal multiplex IHC platform

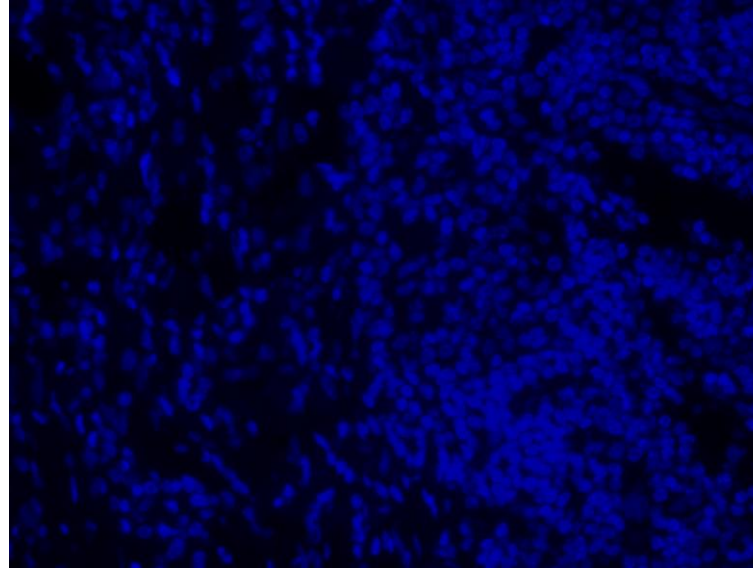
- ❖ Measure multiple tissue biomarkers simultaneously
- ❖ Use the best primary antibodies, regardless of species—with no crosstalk
- ❖ Retain spatial cellular context that is lost when using other methods
- ❖ Get more information from scarce samples
- ❖ Scan six slides for one time (including traditional tissue and TMA) in no matter bright or dark field
- ❖ The powerful software analyze images promptly and accurately



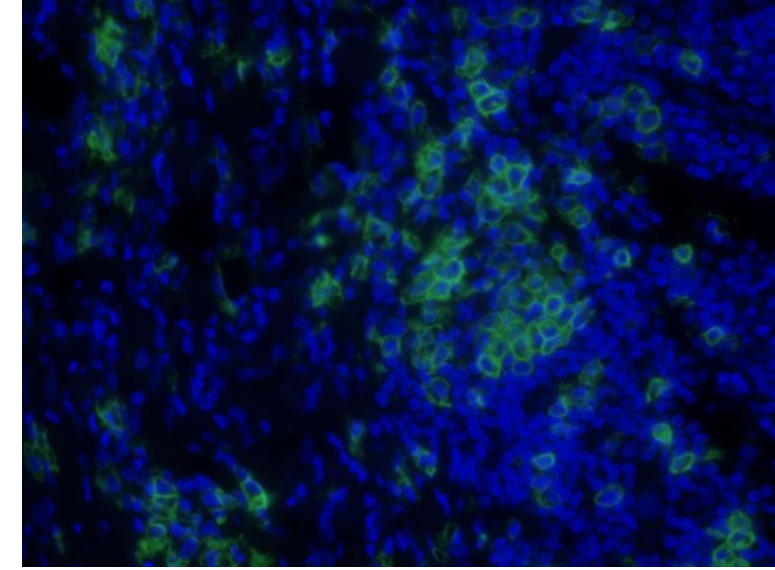
Score for FITC signal on 400x magnification images



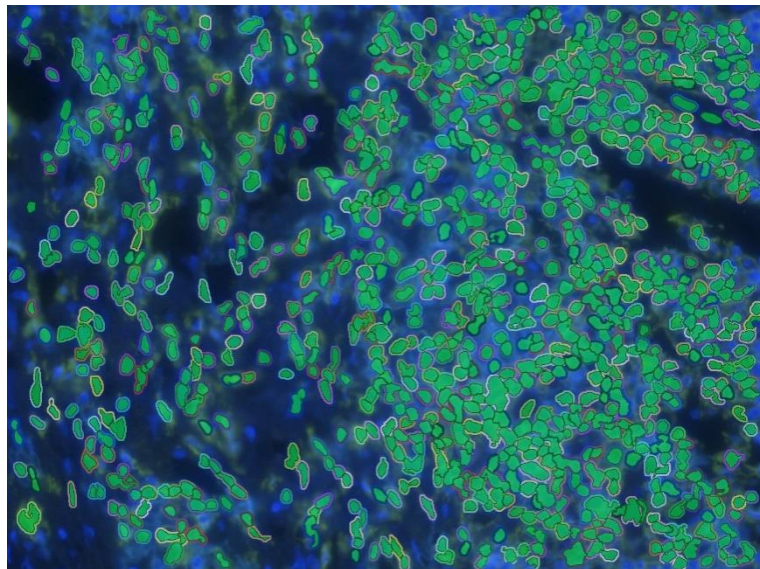
FITC



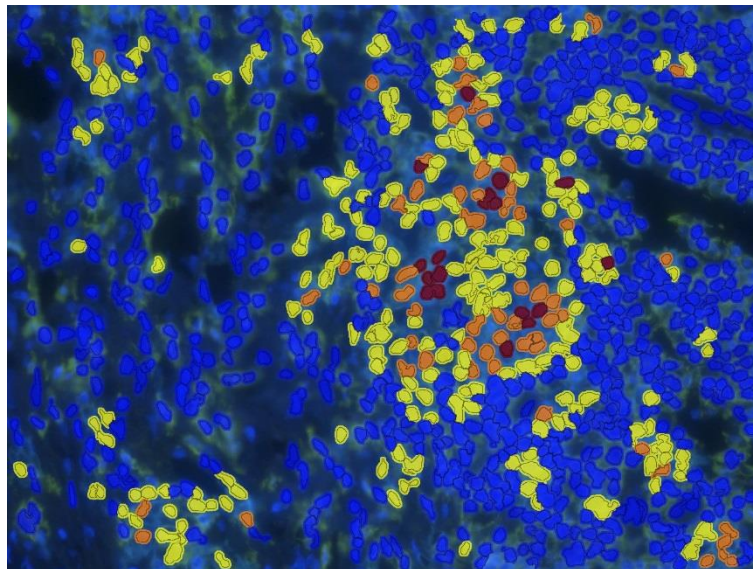
DAPI



Composite image

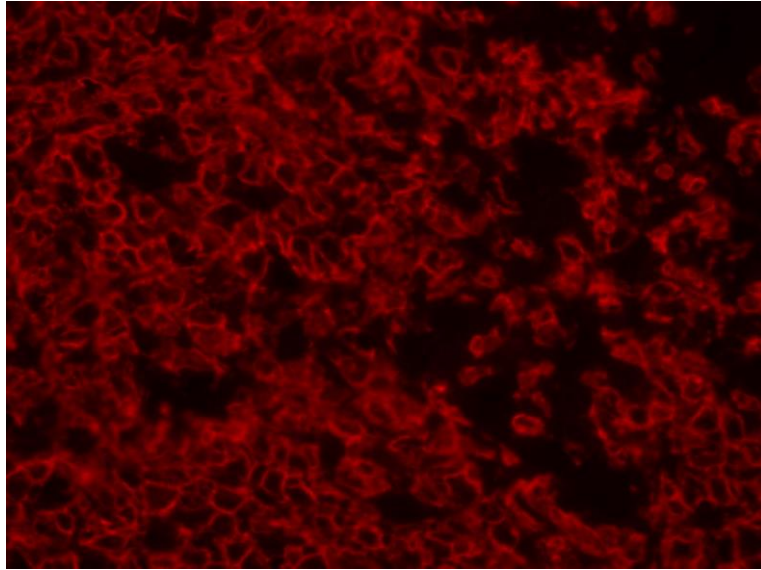


Cell segmentation

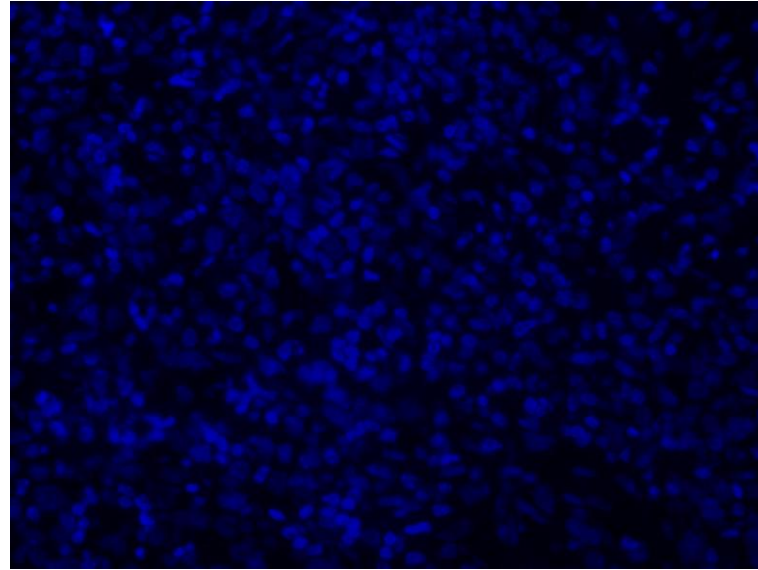


Blue: score=0	67.61%
Yellow: score=1+	23.79%
Orange: score=2+	6.71%
Deep brown: score=3+	1.88%

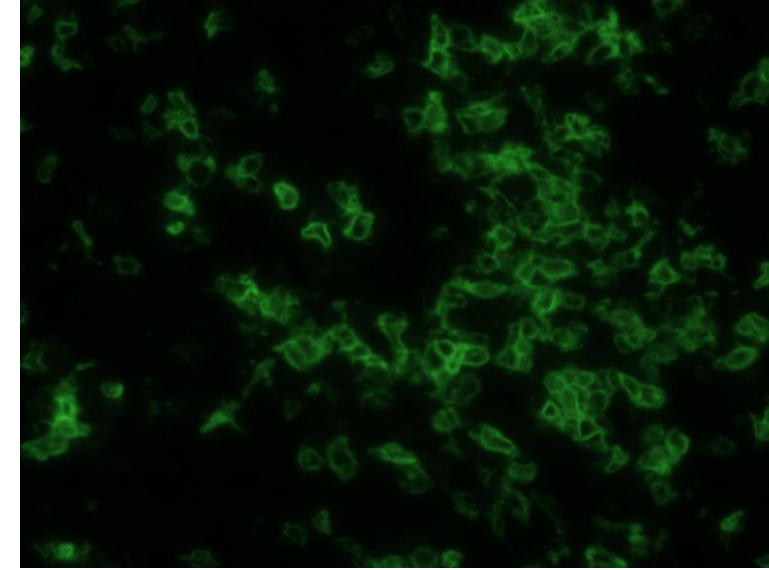
Co-expression of FITC and TRITC signal on 400x magnification images



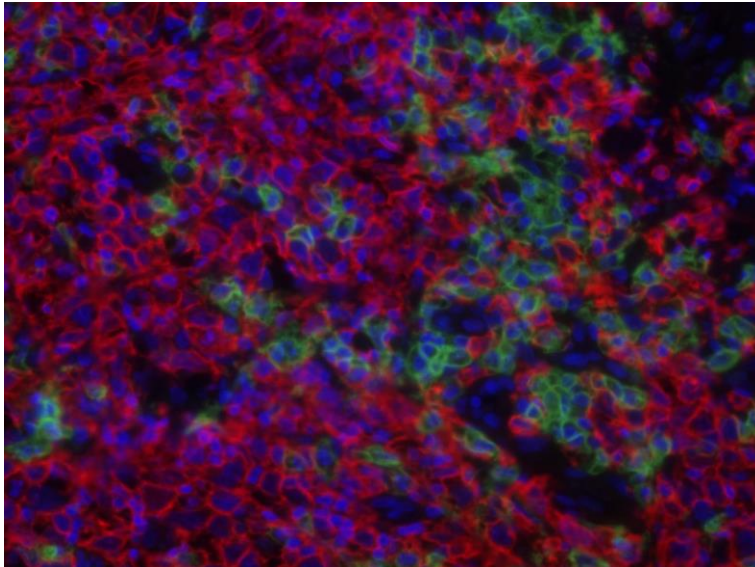
TRITC



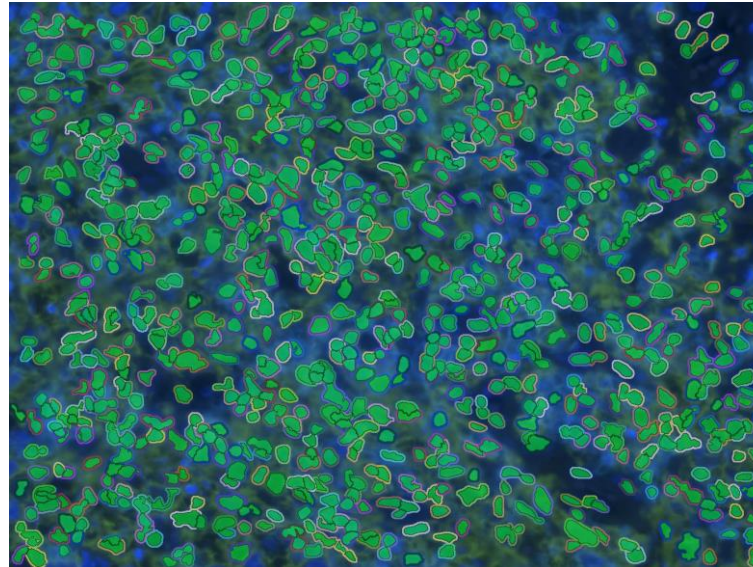
DAPI



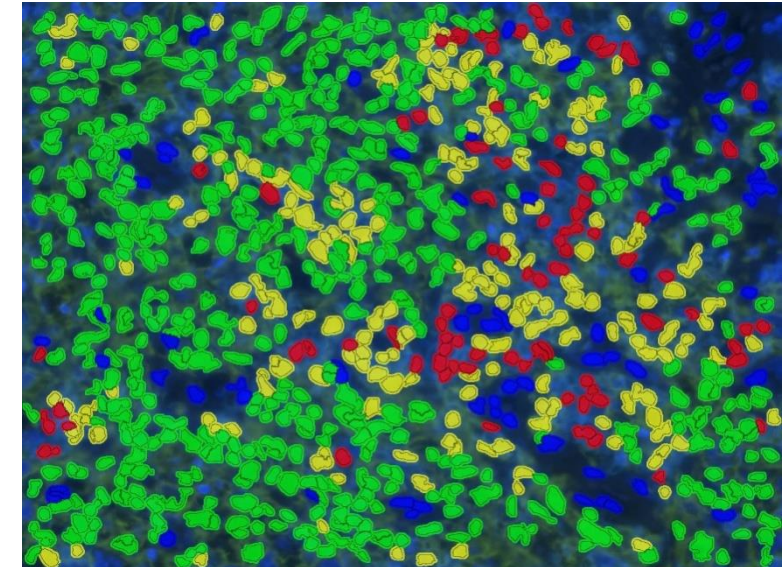
FITC



Composite image



Cell segmentation



Blue: Double negative 7.96%
Red: FITC single positive 9.89%

Green: TRITC single positive 58.99%
Yellow: Double positive 23.16%



OUR COMMITMENT

Improving Health. Making a Difference.

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