



WISCONSIN
UNIVERSITY OF WISCONSIN-MADISON

Wei Huang, MD

Pathology TRIP Laboratory

**WHAT CAN TRIP
IHC/TISSUE IMAGING LAB
OFFER?**

Tissue Imaging

- Nuance System (Perkin Elmer)
 - A manual multispectral imaging system (one slide capacity)
 - It enables imaging of multiple molecular markers in tissue sections for both fluorescence and brightfield, even when they are co-localized
 - Nuance imaging software can eliminate autofluorescence, unmixed co-localized signals for quantification and make weak signals visible and quantifiable by using a spectral library
 - It also enables quantifying co-localized signals (e.g., percentage of double positivity, etc) and selecting regions of interest (ROI) for analysis

Tissue Imaging

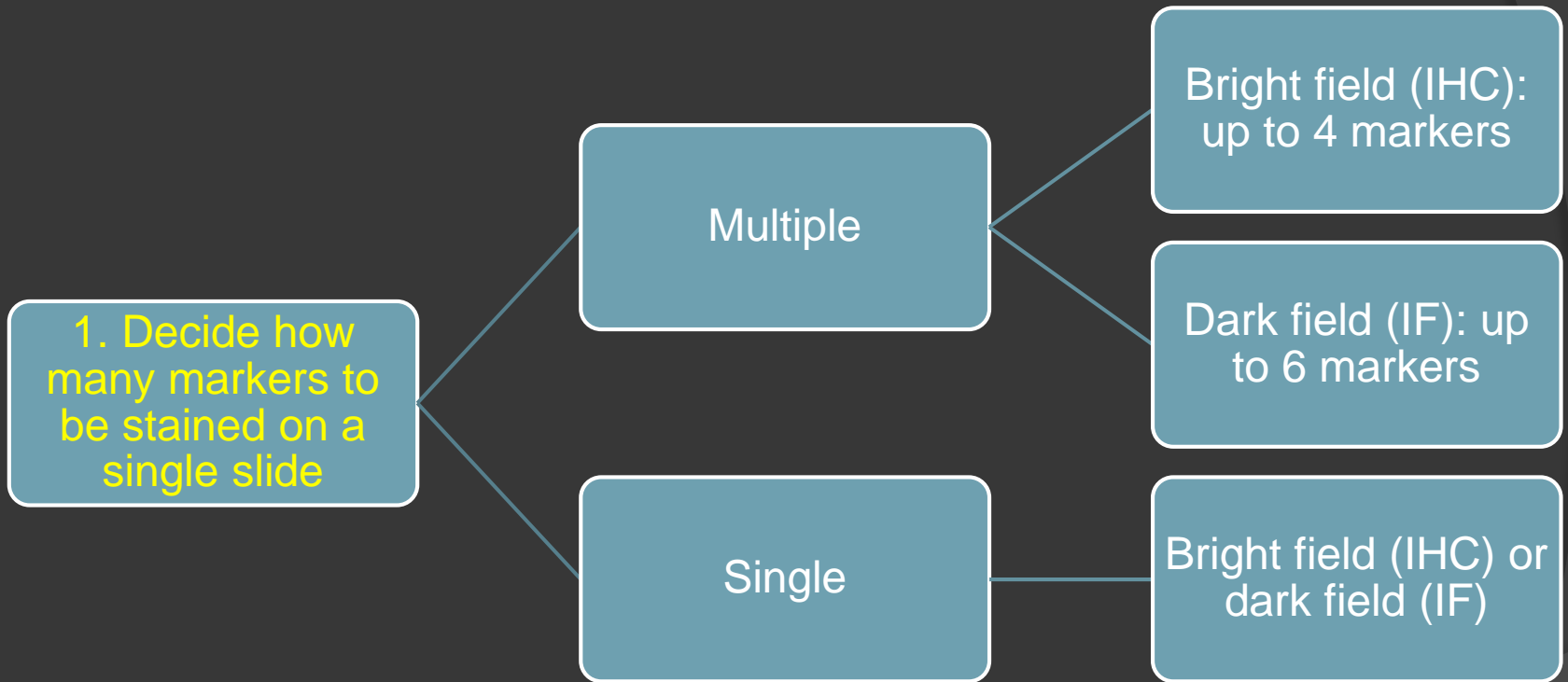
- Vectra System(Perkin Elmer)

- Is the most advanced instrument for extracting proteomic and morphometric information from tissue microarray or intact tissue sections
- Vectra merges automated slide-handling, multispectral imaging technology, and unique pattern-recognition-based image analysis (inForm software) into a powerful system for biomarker discovery and clinical studies
- This system accurately measures protein expressions and morphometric characteristics in distinct tissue regions of interest or on whole slides
- Sections can be labeled with either immunofluorescent (IF) or immunohistochemical (IHC) stains, or in situ hybridization (ISH or FISH) or with conventional stains such as H&E and trichrome
- With IF or IHC, single or multiple proteins or molecular markers (mRNA or DNA) can be measured on a per-tissue, per-cell, and per-cell-compartment (eg. nuclear, cytoplasmic) basis, even if those signals are spectrally similar, are in the same cell compartment or are obscured by autofluorescence
- Objects or structures of interest on H&E sections can be identified and counted with inForm software
- Vectra™ processes up to 200 slides in a single run or analyzes every core in a tissue microarray (TMA)

Instrument (Software) Key Features

	Nuance™ (Perkin Elmer)	Vectra™ (Perkin Elmer)
Brightfield	√	√
Fluorescence	√	√
TMA slide scanning	√ manual	√ automated
Whole section slide scanning	√ manual, single slide capacity	√ automated, 200 slide capacity
Multiplexing analysis	√ up to 10 channels	√ up to 10 channels
Autofluorescence removal	√	√
Spectral library tool	√	√
Software for analysis	Nuance and inForm	Nuance and InForm
<u>Project application:</u>		
Biomarker quantification	√	√
co-localization quantification	√	√
Per-tissue analysis (epithelium vs. stroma)	√ (by manual drawing)	√ (automated)
Subcellular quantification		√
Per-cell data		√
Tissue structure (vessel, glomerulus, etc) counting		√
Data format	continuous	Both ordinal and continuous

Workflow for Biomarker Quantification using Vectra Imaging System



2. Optimize
target
antibodies



Use vendor
suggested
tissue first



Test on the
intended
tissue(s)
(breast,
prostate,
skin, etc)



Run on
intended
experimental
slides
(TMAs)

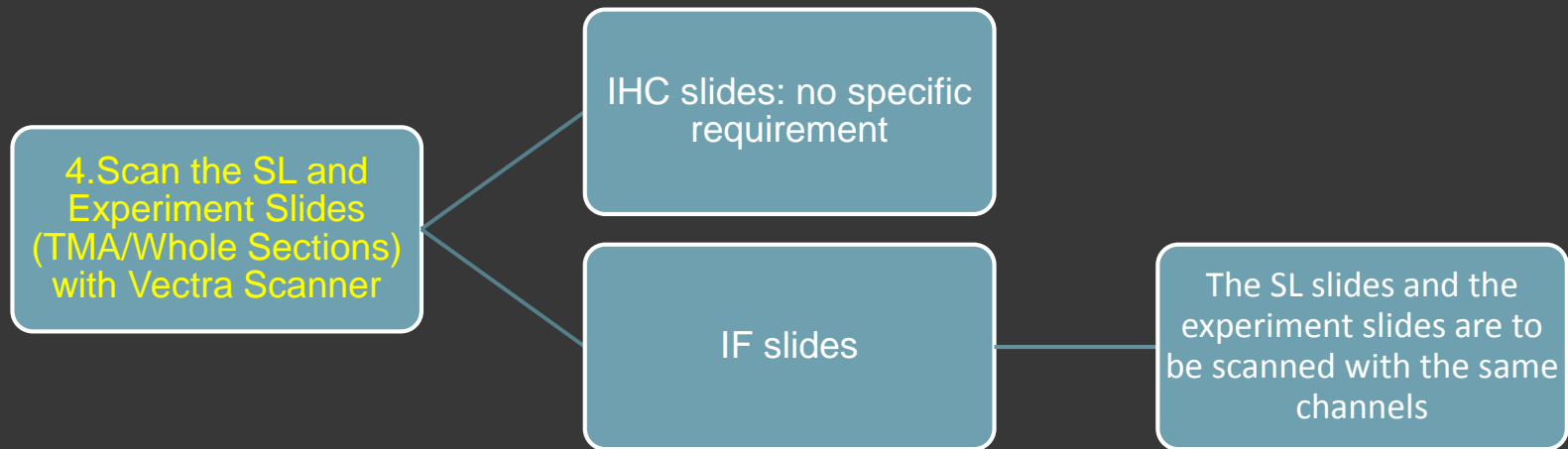
3. Build a spectral library (SL):
use a common working
antibody, e.g., AE1/AE3,
vimentin, Ki-67, etc.
One dye per slide

IF-SL: tissue specific (due to
autofluorescence)

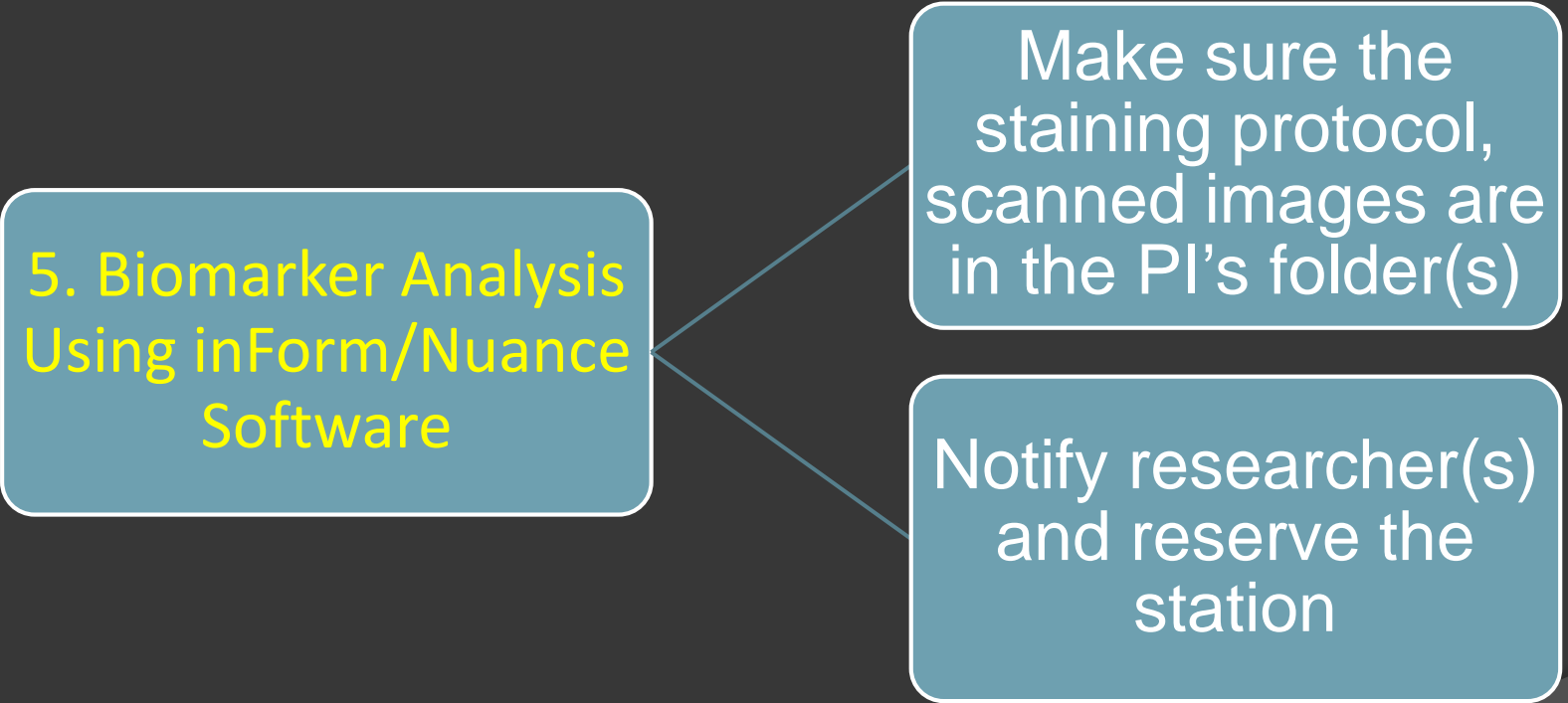
of slides = # of intended
markers + 1 nuclear
counterstain + 1 unstained
slide (without any
counterstain)

IHC-SL: not tissue specific

of slides = # of intended
markers + nuclear
counterstain (hematoxylin)



5. Biomarker Analysis Using inForm/Nuance Software



Make sure the staining protocol, scanned images are in the PI's folder(s)

Notify researcher(s) and reserve the station