Framework for Multispectral Imaging Application to digital pathology

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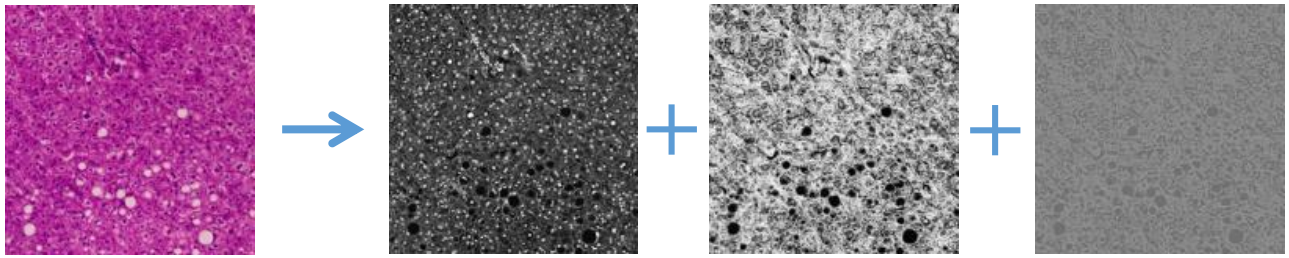
Multispectral imaging in pathology

- Brightfield
  - Object detection, segmentation
  - Color unmixing – Stain amount image
  - Digital adjustment of staining strength
  - Digital staining

- Fluorescence
  - Simultaneous imaging of multiple markers
  - Cross-talk, auto-fluorescence removal
  - Combined brightfield and fluorescent images

Estimating dye amount image

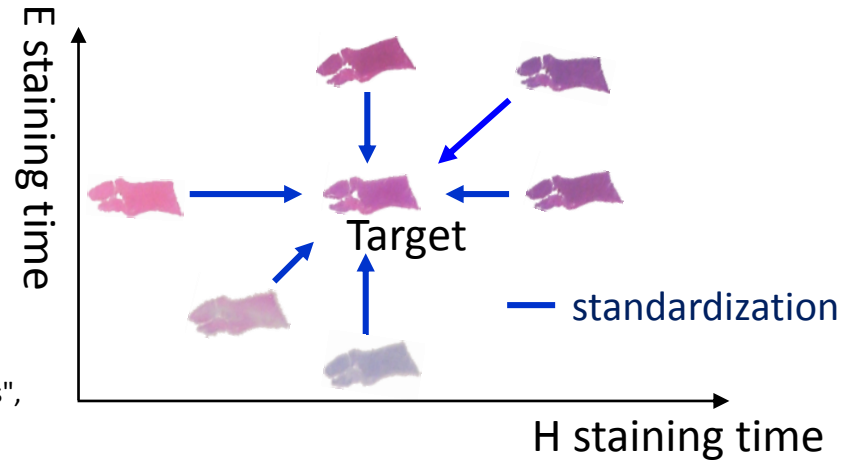
- Color unmixing
  - Estimation of dye amount image



HE stained image → H component + E component + Residual

Residual is not actual data

- Adjustment of dye amount
- Standardization of staining condition



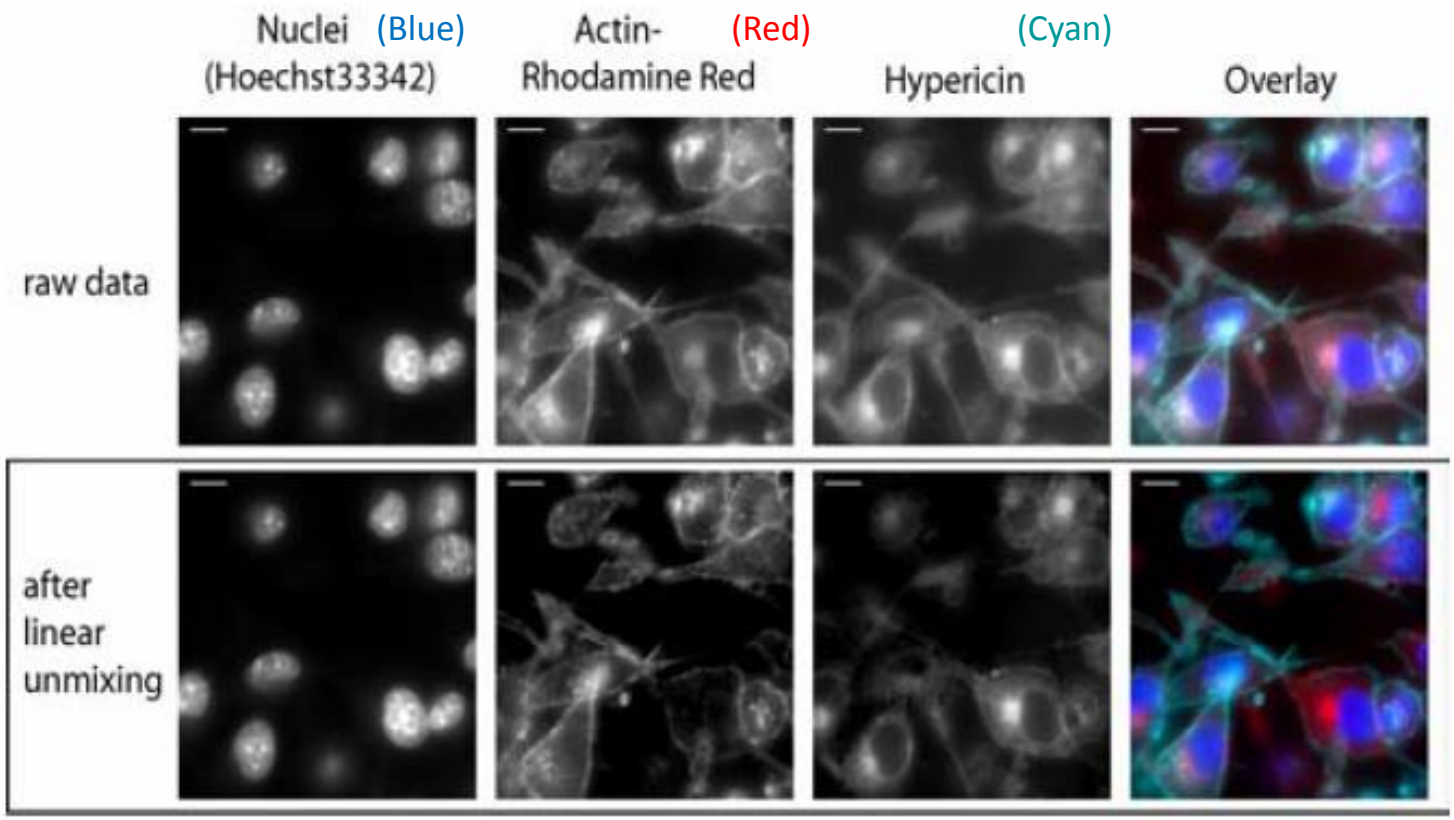
K. Fujii, M. Yamaguchi, N. Ohyama, K. Mukai, "Development of support systems for pathology using spectral transmittance: the quantification method of stain conditions", Proc. SPIE 4684, 1516 (2002)

T. Abe, Y. Murakami, M. Yamaguchi, N. Ohyama, Y. Yagi “Color correction of pathological images based on dye amount quantification,” Opt. Rev., 12, (4), 293-300 (2005).

Fluorescent imaging for molecular pathology

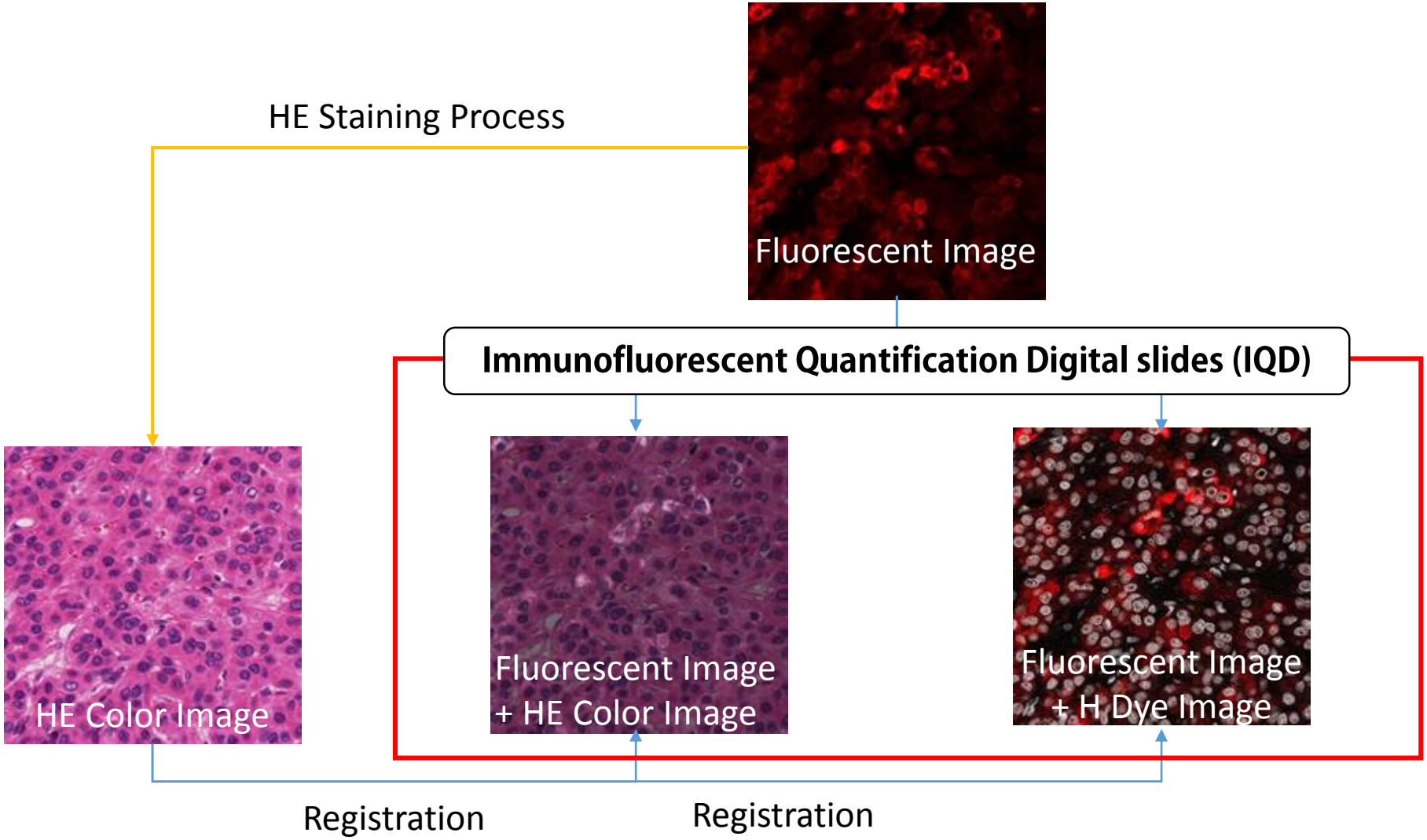
- Necessary to test various biomarkers
  - → Multispectral imaging with using multiple fluorescent dyes
- Crosstalk problem
  - Crosstalk between different fluorescent dyes
  - Auto-fluorescence
  - → Color unmixing
- Quantification / semi-quantification of marker expression,
  - Identification of tumor, tissue, nuclei, or membrane needed
  - → Combination of fluorescent and H- or HE-stain images

Color unmixing for unwanted fluorescence removal



Modern Research and Educational Topics in Microscopy.
A. Méndez-Vilas and J. Díaz (Eds.)

Combination of Fluorescent and HE-stain



A. Hashiguchi et al 'Using immunofluorescent digital slide technology to quantify protein expression in archival paraffin-embedded tissue sections' *Pathol Int* 2010; 60: 720–5.

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Requirements from DICOM WG26

1. Ability to define how to display multi-spectral images as true color visible light images.

   Color reproduction: ICC framework can make it!

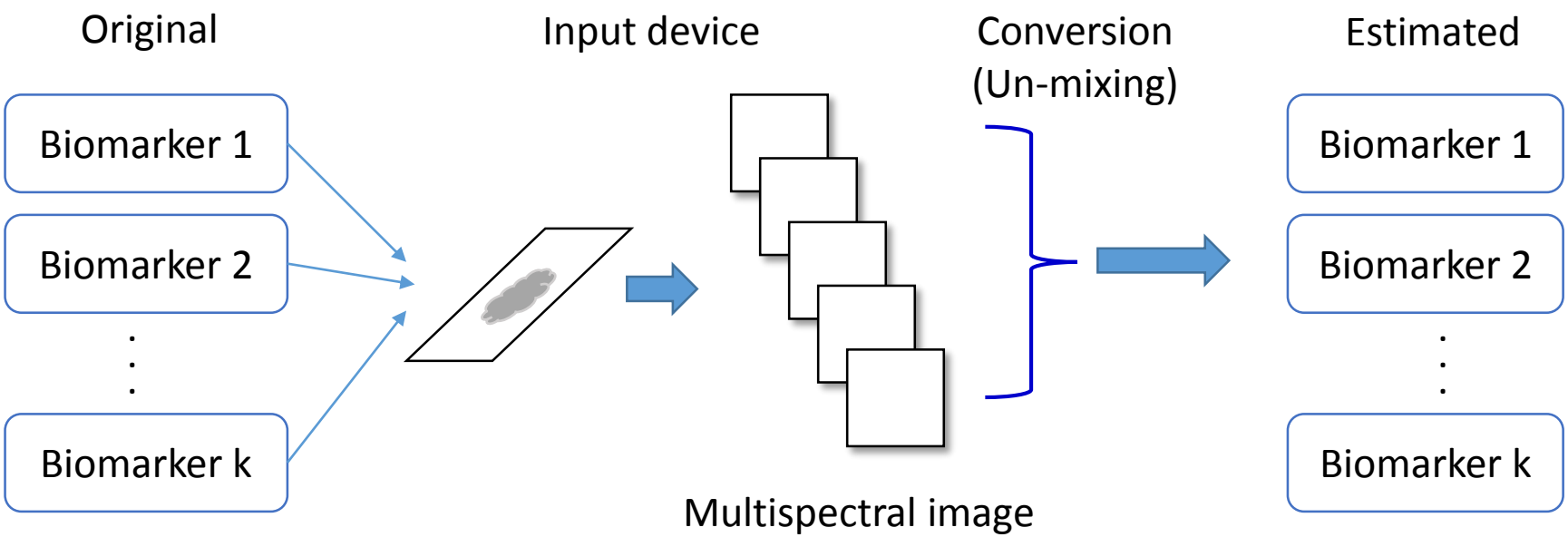
2. Ability to define how to un-mix multispectral input channels for the purpose of deriving quantitative representations of individual biomarker intensities.

   Not the issue of color reproduction: PCS is useless…
   Can we adopt ICC framework?

3. Ability to define how to display (un-mixed) multi-spectral images as pseudo color images.

   Color reproduction: ICC framework can make it!

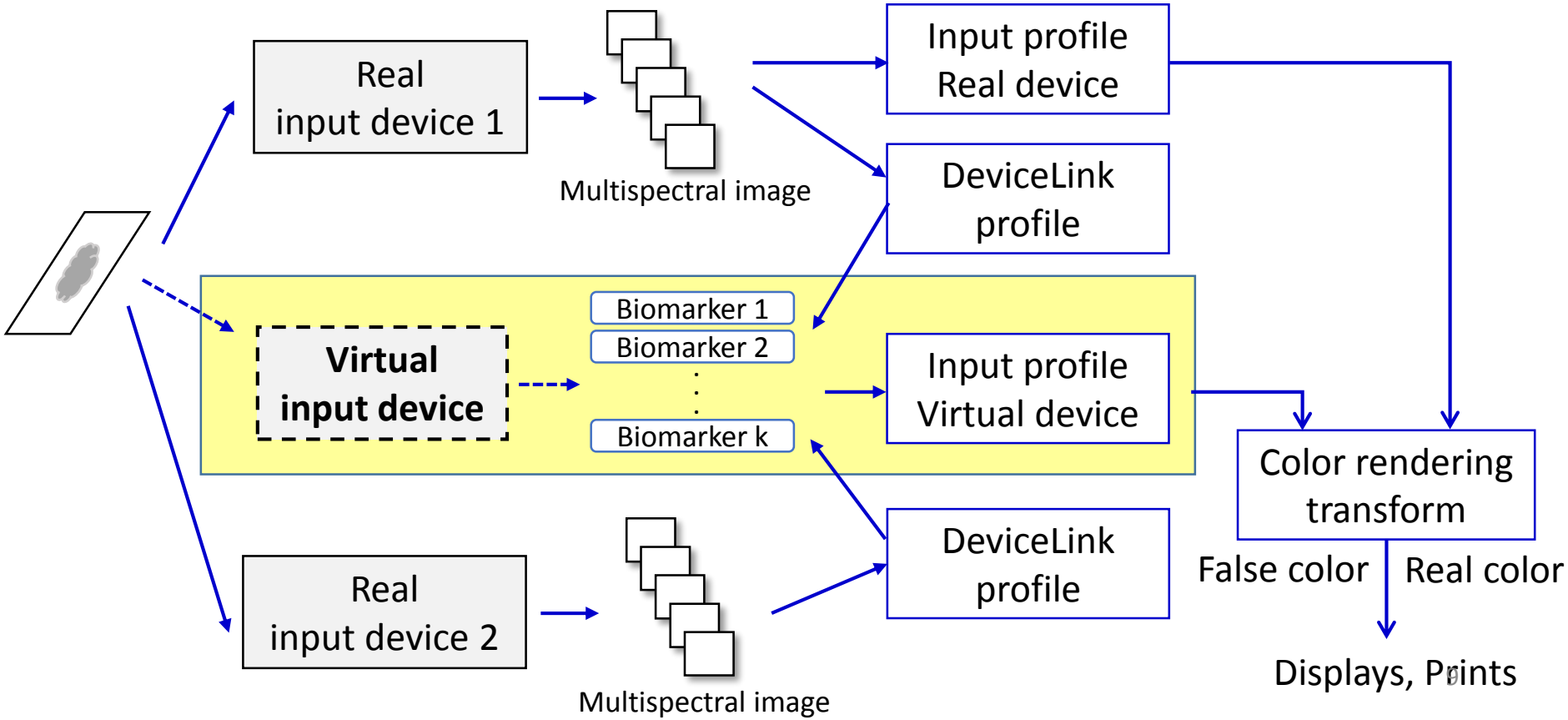
General model for multispectral un-mixing



PSA, Ki-67, CK-19
HER2, ER, PgR, …

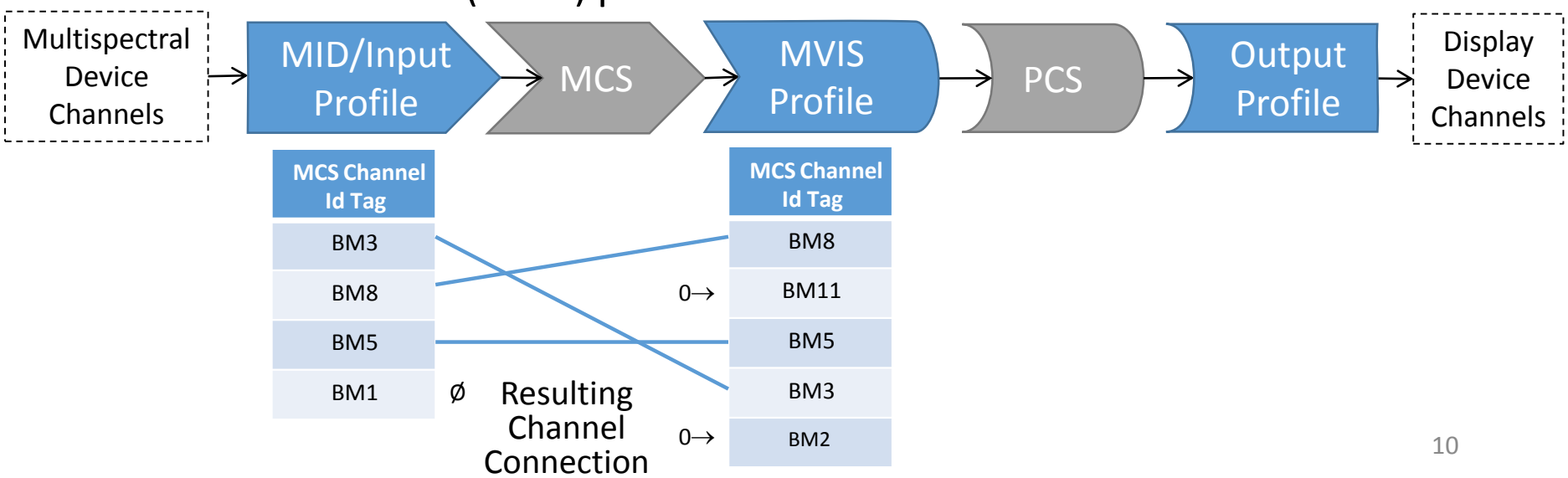
Solution to color unmixing by ICC v4

- Consider a virtual input device
  that can directly capture un-mixed biomarker images
- Use DeviceLink profile



Solution to color unmixing by ICC Labs

- RefICCLabs - - - ICC v5
  - Spectral profile
  - Calc element
  - …
- Proposal of "Material Connection Space" Profiles
  - MCS connection allowed between source biomarker Material Identification (MID) and destination biomarker Material Visualization (MVIS) profiles



For reference:
Requirements in the Technical Report draft “Multispectral format” of CIE TC8-07

http://www.multispectral.org

- Basic
  - Platform independent
  - Lossless compression (ZIP, LZW, etc.)
  - Lossy compression (JPEG, Wavelets, etc.), independent for each channel
  - geolocation information (e.g., latitude and longitude of the corner pixels)
  - High Accuracy: many channels, many pixels, many bits
  - Ability to store raw data such as naked camera outputs, DC bias values, camera gain
  - Unevenly sampled spectral signal
  - Royalty free
  - File size limitation
  - Archiving Application (details)
- Highly desirable
  - Ability to store uncompressed data
  - Geometric registration information including pin cushion model along with a grid of registration control points
  - Possibility to store company specific information (native tags)
  - Storage compatible with Lab, RGB possible (e. g., L, a, b, ch4, ch5, ch6, ...)
  - Reconstruction matrix stored as
  - Simple usage, easy spectral reconstruction (e.g. just multiply the channels by a matrix in order to yield the spectra)
  - All information in one file
  - Units of the data (e.g. reflectance (per cent for fraction) or radiance (watts/sq-meter, etc.))
  - Planar packing available
  - Industrial Application (simple reconstruction)

Next step

- Consider adoption of ICC v4 keeping in mind the upper compatibility in v5.
- Documentation for implementation to DICOM.
- Investigate the advantage and feasibility of ICC v5 application.