# 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

  # Molecular pathology
  - Simultaneous tests of multiple markers
  - Cross-talk, auto-fluorescence removal => Color unmixing
  - Combined brightfield and fluorescent images

# Requirements from DICOM WG26

1. Display multi-spectral images as **true color** images.
2. **How to un-mix** multispectral input channels for deriving quantitative representations of individual biomarker intensities.
3. Display (un-mixed) multi-spectral images as **false color** images.

- Need to retain traceability

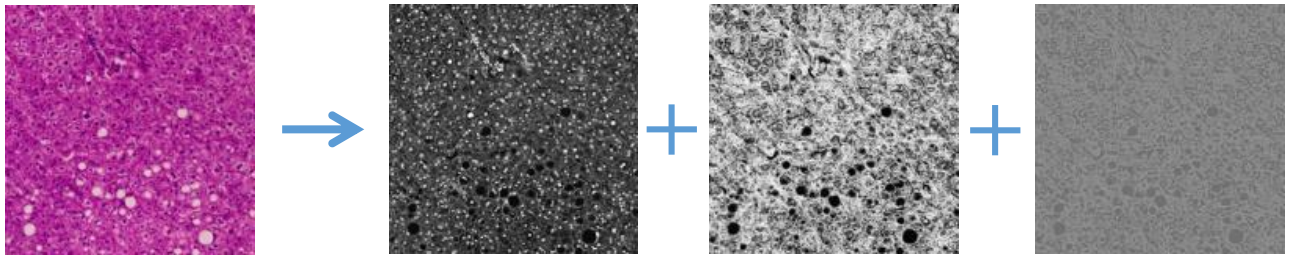
1&3: Color reproduction: ICC framework can make it!

**2: Unmixing: Not the issue of color reproduction:**

**Can we adopt ICC framework?**

# Estimating dye amount image

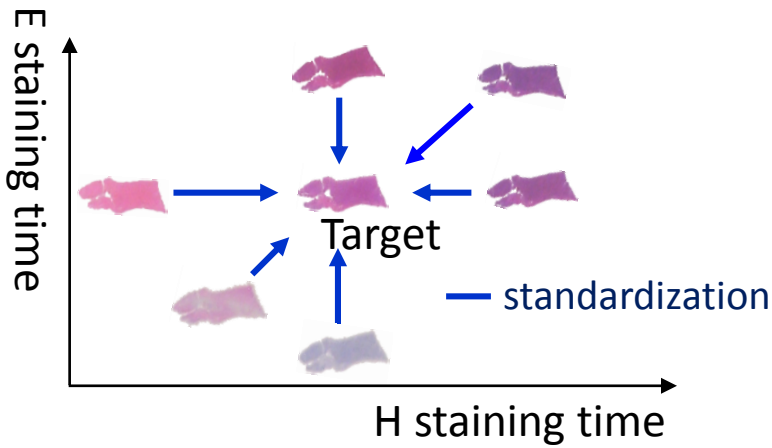
- Color unmixing
  - Estimation of dye amount image



HE stained image | H component -- nuclei | E component -- cytoplasm | Residual

Residual is not actual data

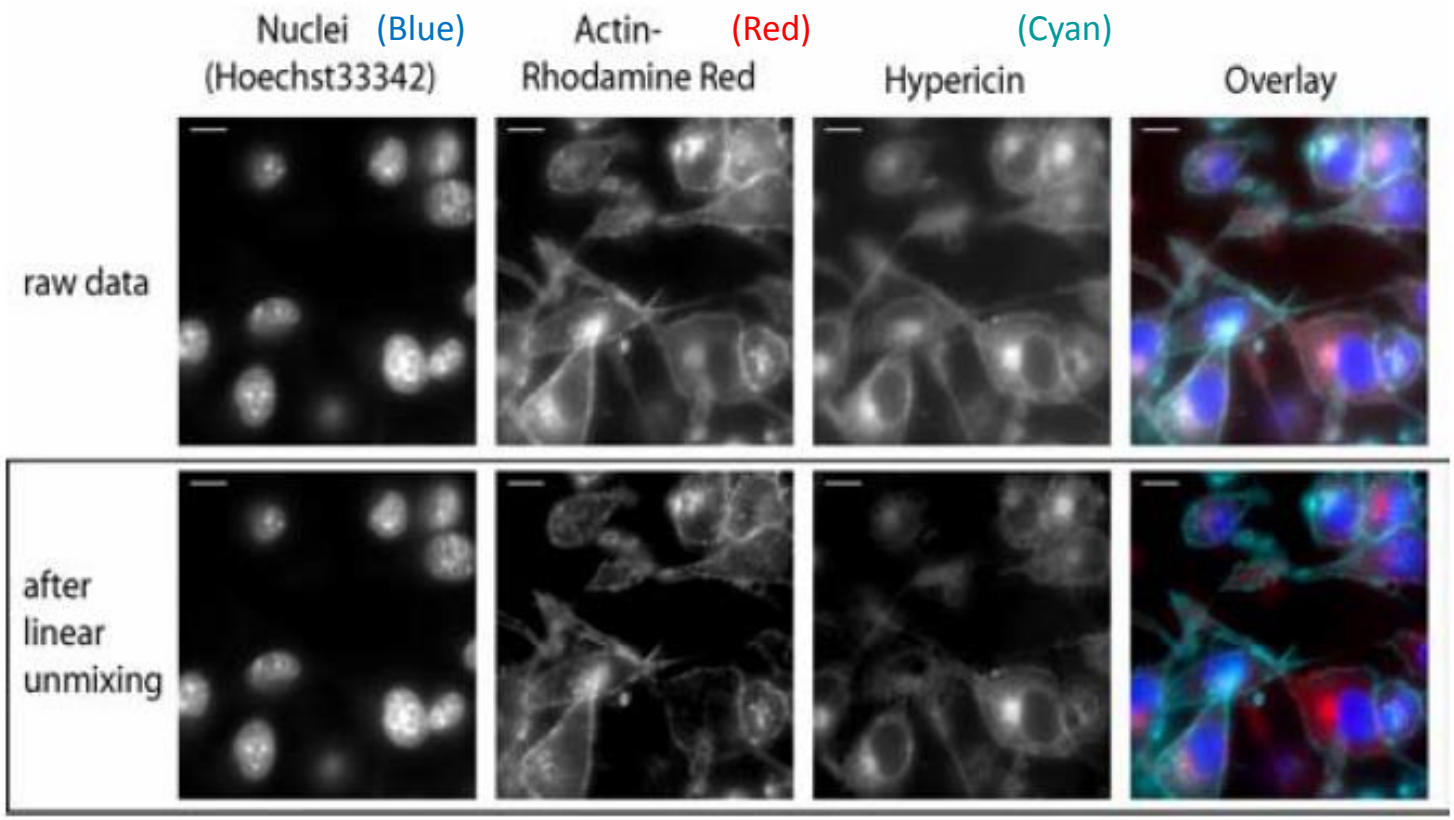
- Adjustment of staining strength
- Standardization of staining condition




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).

# Color unmixing for unwanted fluorescence removal



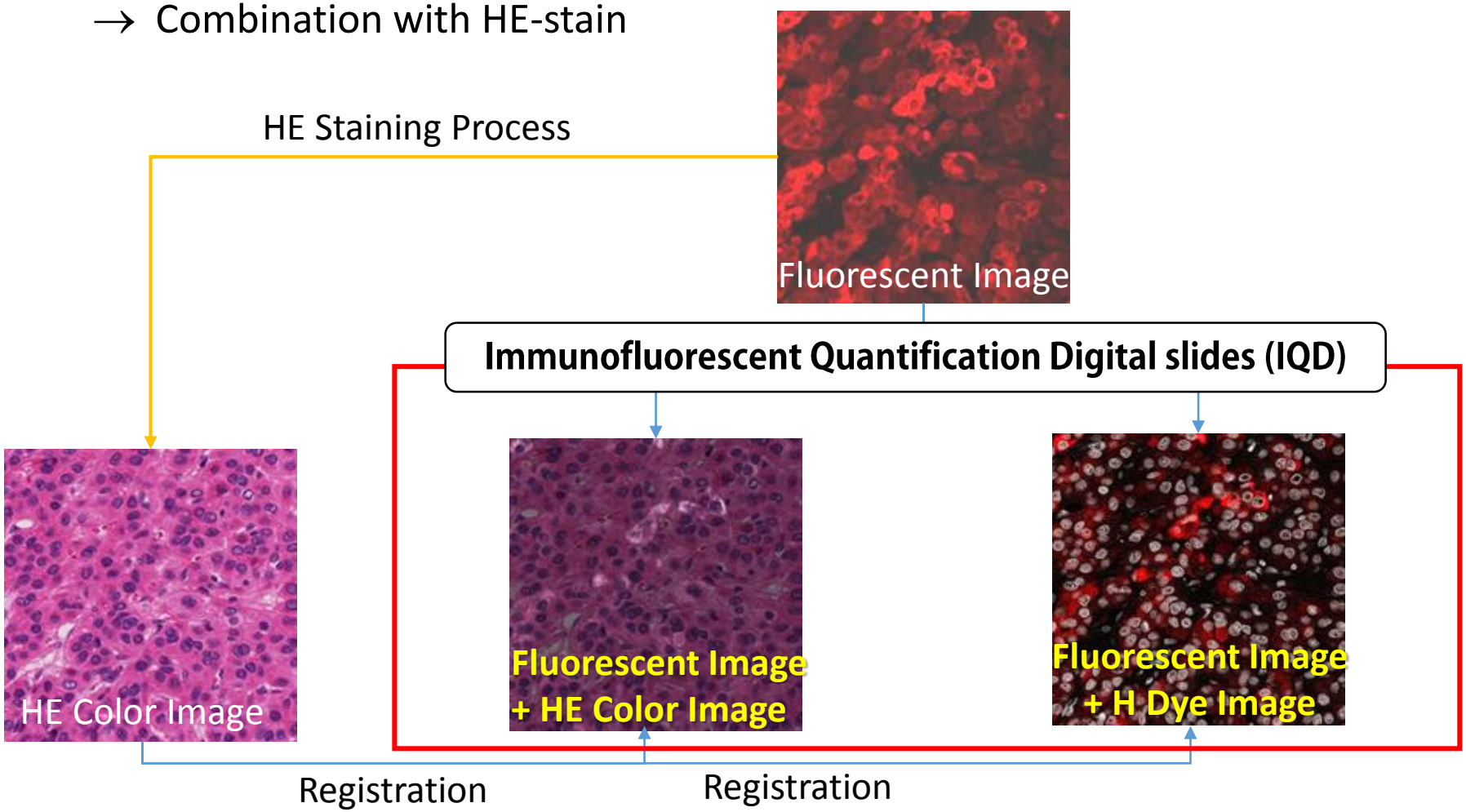

Modern Research and Educational Topics in Microscopy.
A. Méndez-Vilas and J. Díaz (Eds.)

# Combination of Fluorescent and HE-stain

In quantification / semi-quantification of marker expression

Identification of tumor, tissue, nuclei, or membrane is needed

→ Combination with 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.

# Solutions to color unmixing

- **Solution based on ICC v4**
  - Consider a virtual input device
    that can directly capture un-mixed biomarker images
  - Use DeviceLink profile:
    Connect Real device => Virtual device
  - Virtual Device Input Profile => Color rendering based on ICC

- **Solution based on ICC Labs**
  - RefICCLabs - - - ICC v5:
    Features suitable for multispectral
  - Proposal of "Material Connection Space" Profiles

# 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.