Proteins preservation and analysis standardization

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Research Topics of the TUM lab for Experimental Pathology in Munich

- Development and validation of molecular biomarkers
- Improvement of tissue quality for diagnosis and research
- Intratumoral heterogeneity of human cancers
- Quantitative (phospho)protein analysis of tissue samples

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Protein analysis -> Proteomics

Why do we study proteins?

Proteins

- are the chief actors within the cell
- expression levels can be monitored in cells and tissues
- can be diagnostic markers **and** targets for therapy

BUT

- protein analysis is often complex
- no information about mutations

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Challenges for protein compared to nucleic acid analysis in human tissues

- More than 1 million proteins estimated (compared to about 22.000 human genes)
- More than 300 posttranslational modifications known (phosphorylation, glycosylation, acetylation…)
- Wide dynamic range for protein abundances: 10^{10} (for mRNA: 10^{4})
- No protein amplification method available (comparable to PCR)
- Detection methods (only a few very good antibodies are available)
- Sensitivity (fM to aM needed, like ELISA)

Impact of tissue proteomics for cancer management

- Diagnosis, molecular classification
- Tumor biology
- Drug target evaluation
- Defining patient subgroups for therapy
- Response evaluation
- Molecular Imaging

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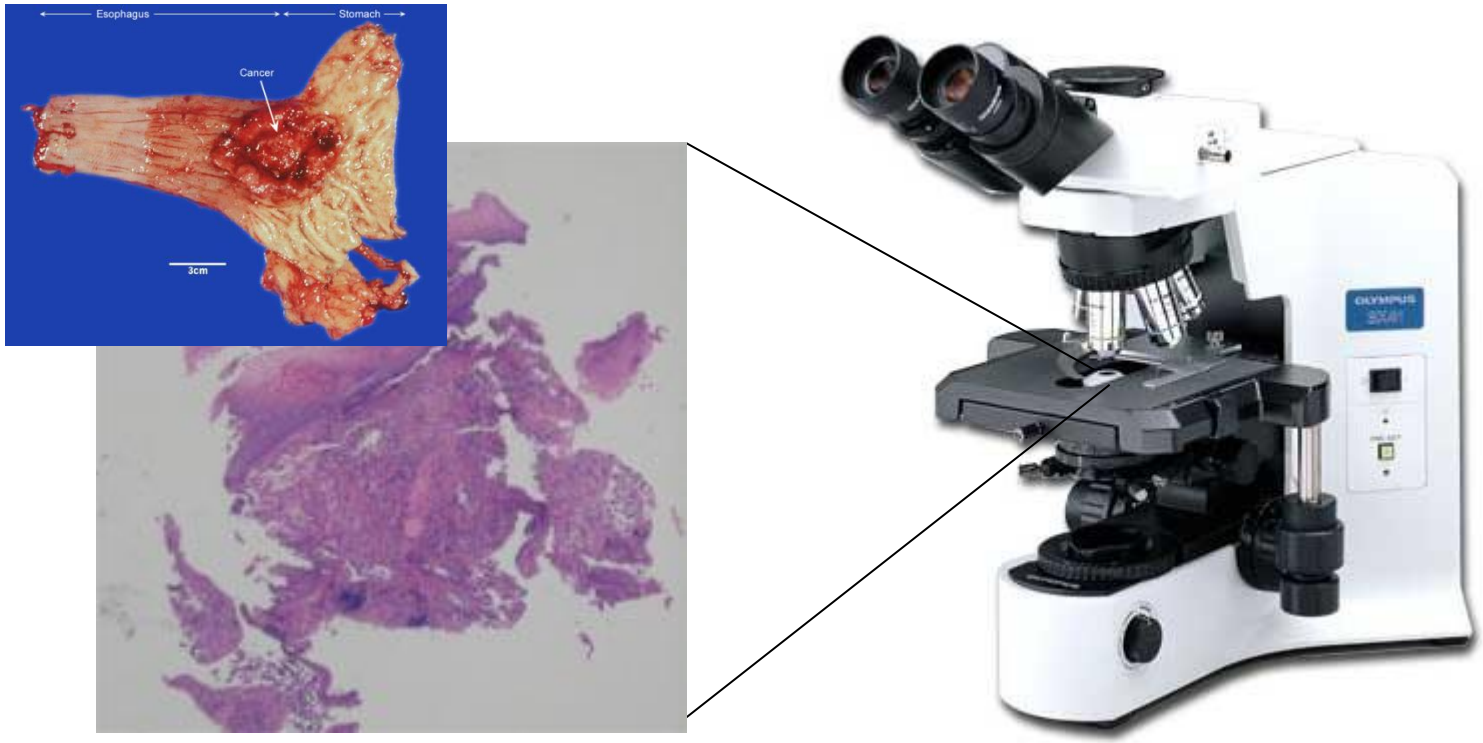
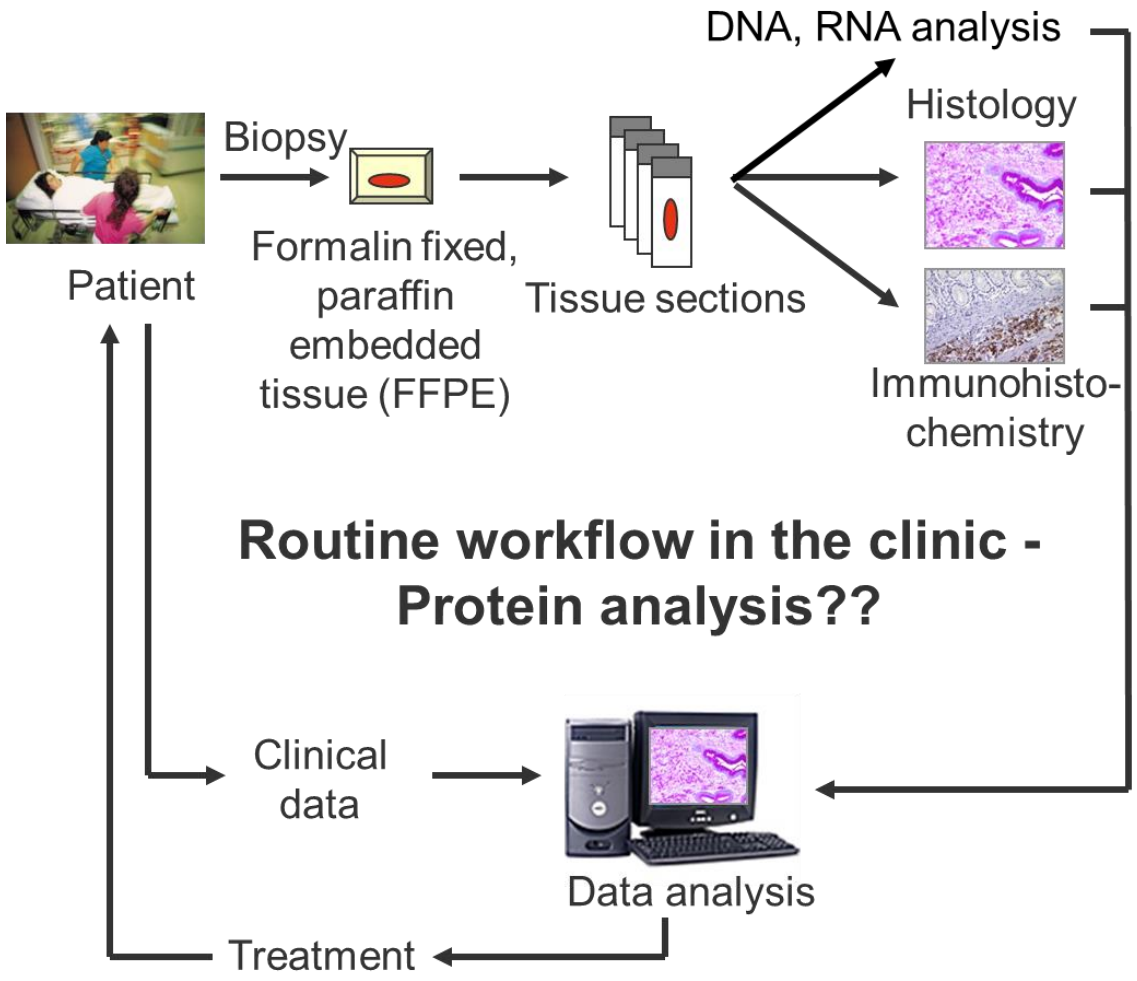
Gold standard for tissue diagnostic: histology



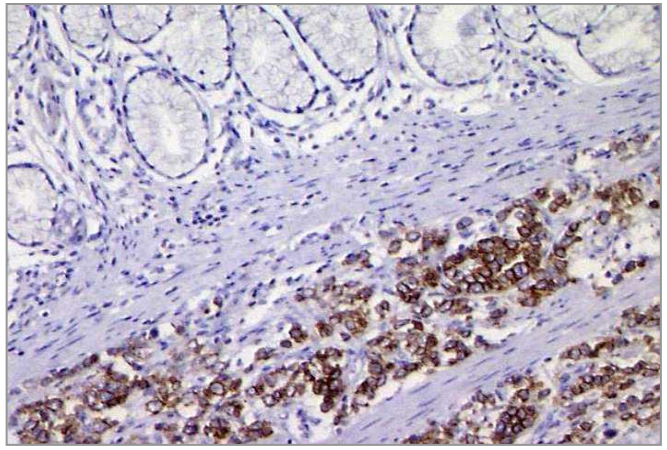
Current status for tissue proteomics in the clinic



Proteomic analysis in the clinic
- formalin fixed tissues vs. frozen tissues -

Routine: Formalin Fixed Tissue

- routine use in the clinic (histology, immunohistochemistry)



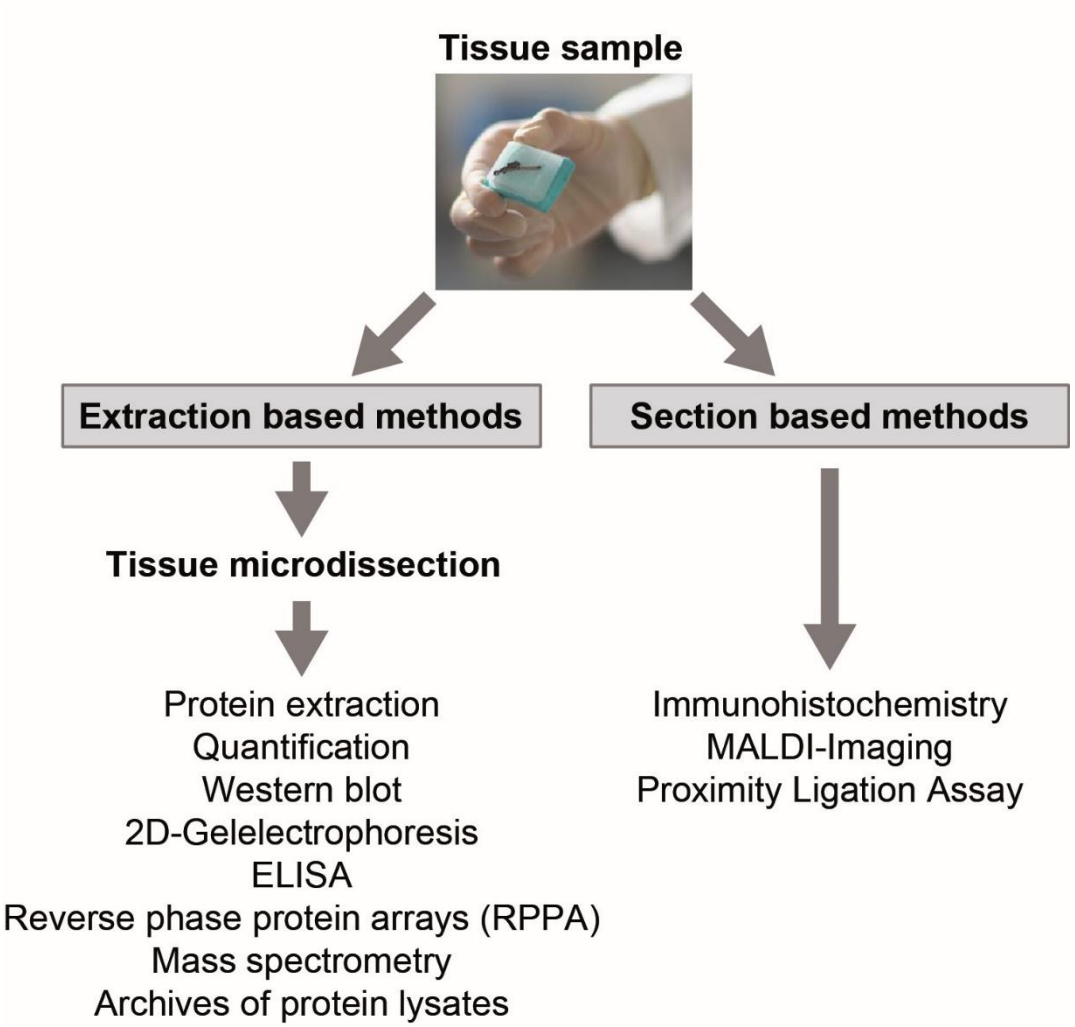
Research: Fresh Frozen Tissue

- has been used for protein microarrays, Western blot, 2D gel-electrophoresis, mass spec

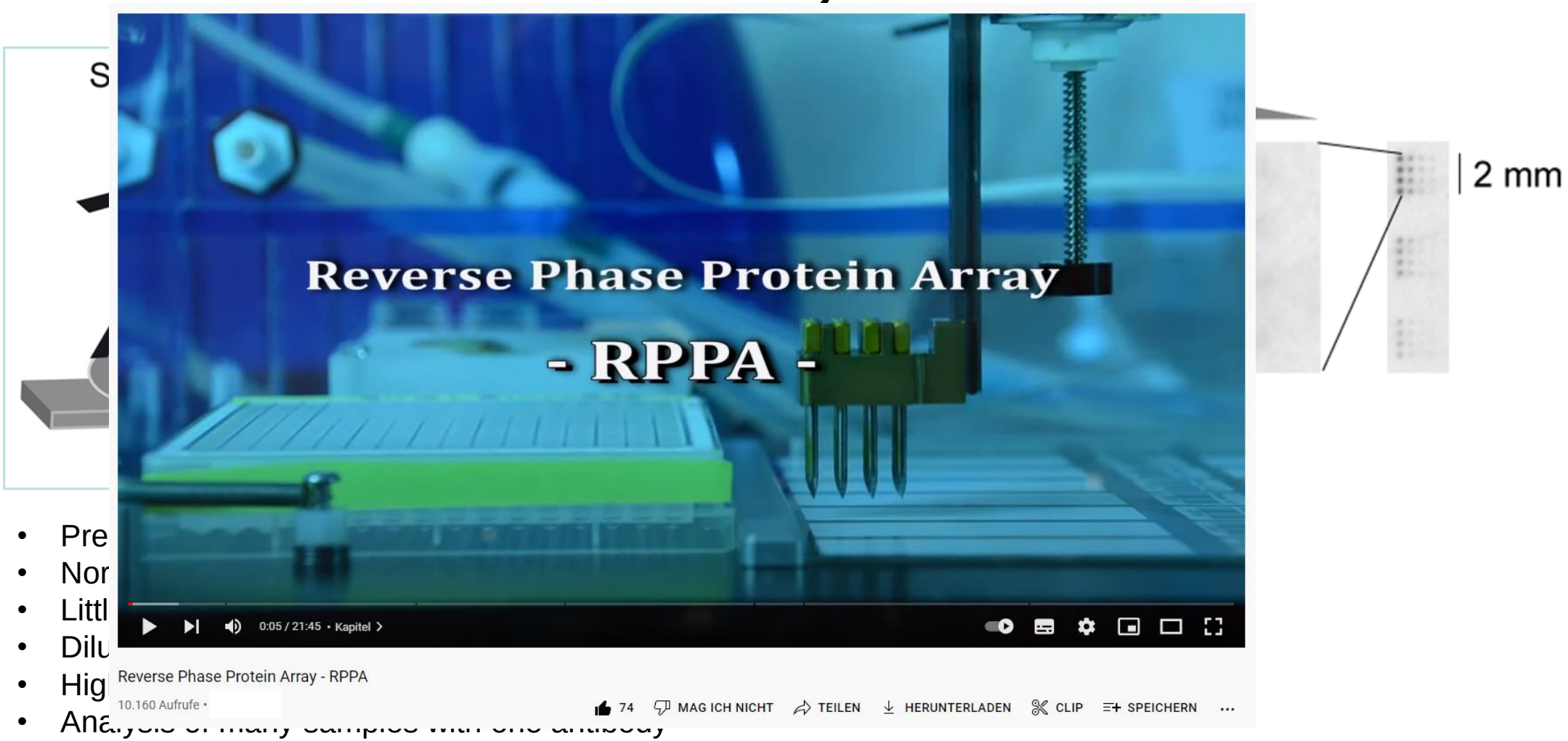
BUT

- large numbers are difficult to obtain
- expensive to store
- difficult to process
- will not be routinely used in the clinic

Protein analysis of clinical tissues



Reverse Phase Protein Array



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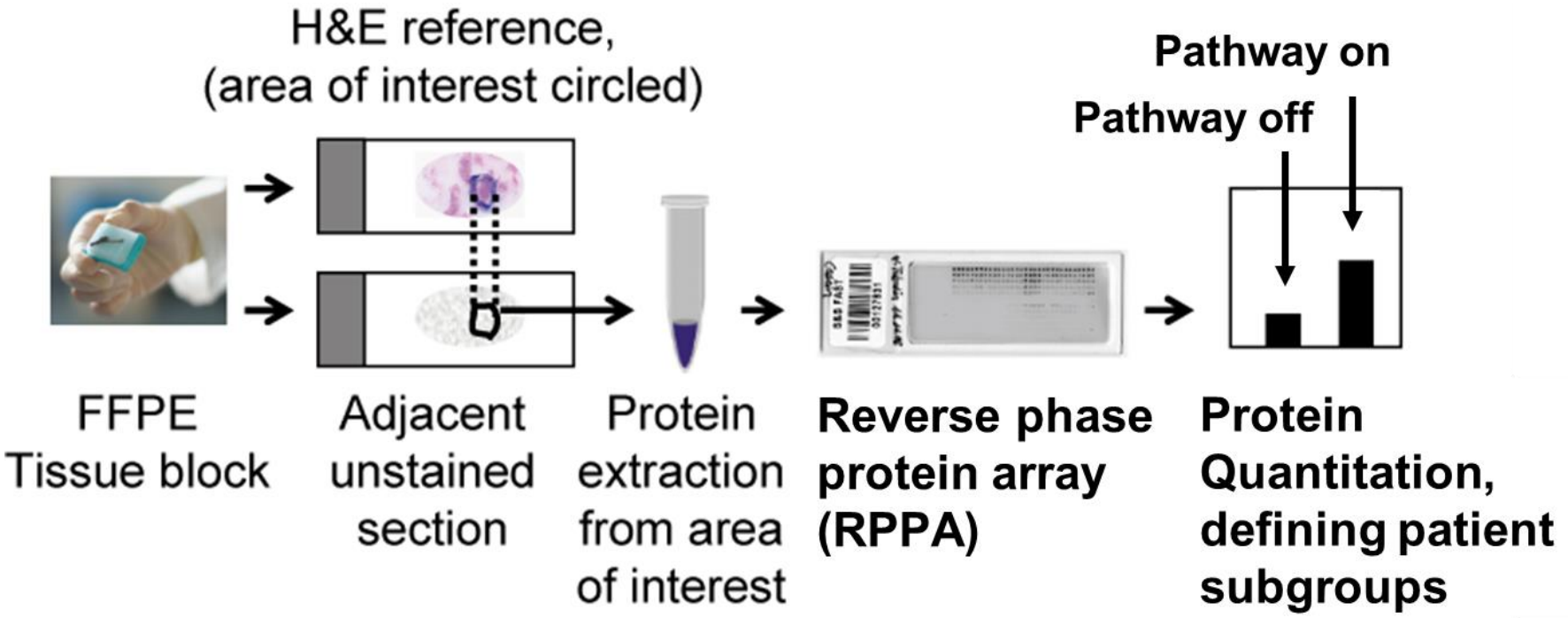
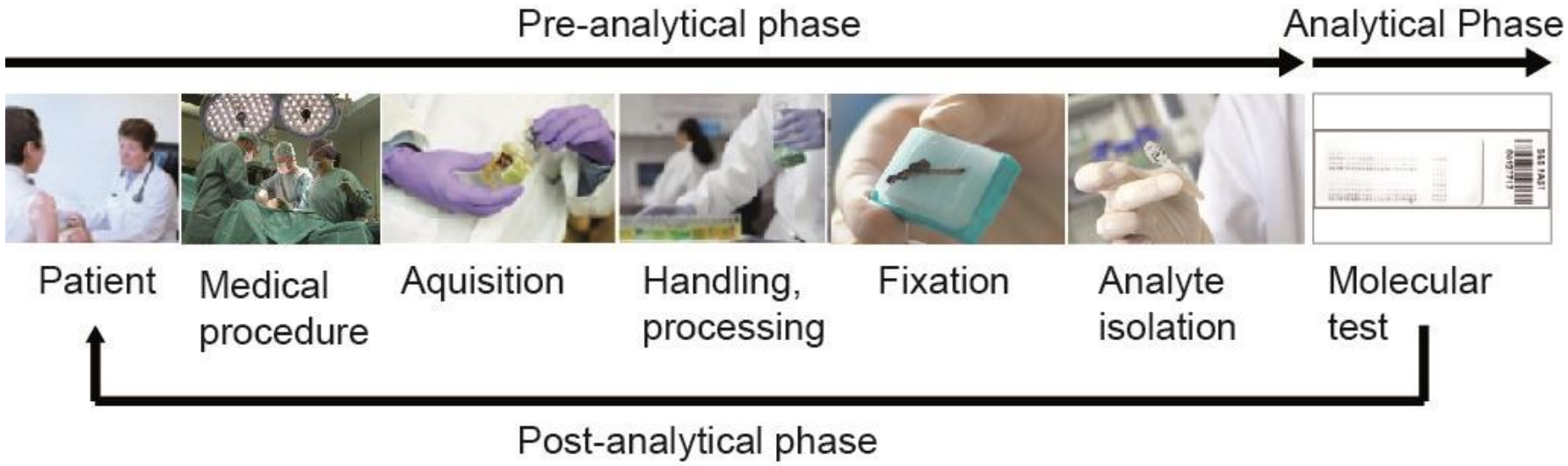
- Automatization possible (IHC stainer compatible)

Recommended workflow for RPPA analysis of clinical tissue samples



Protein analysis of clinical tissue samples - Consider the entire workflow!



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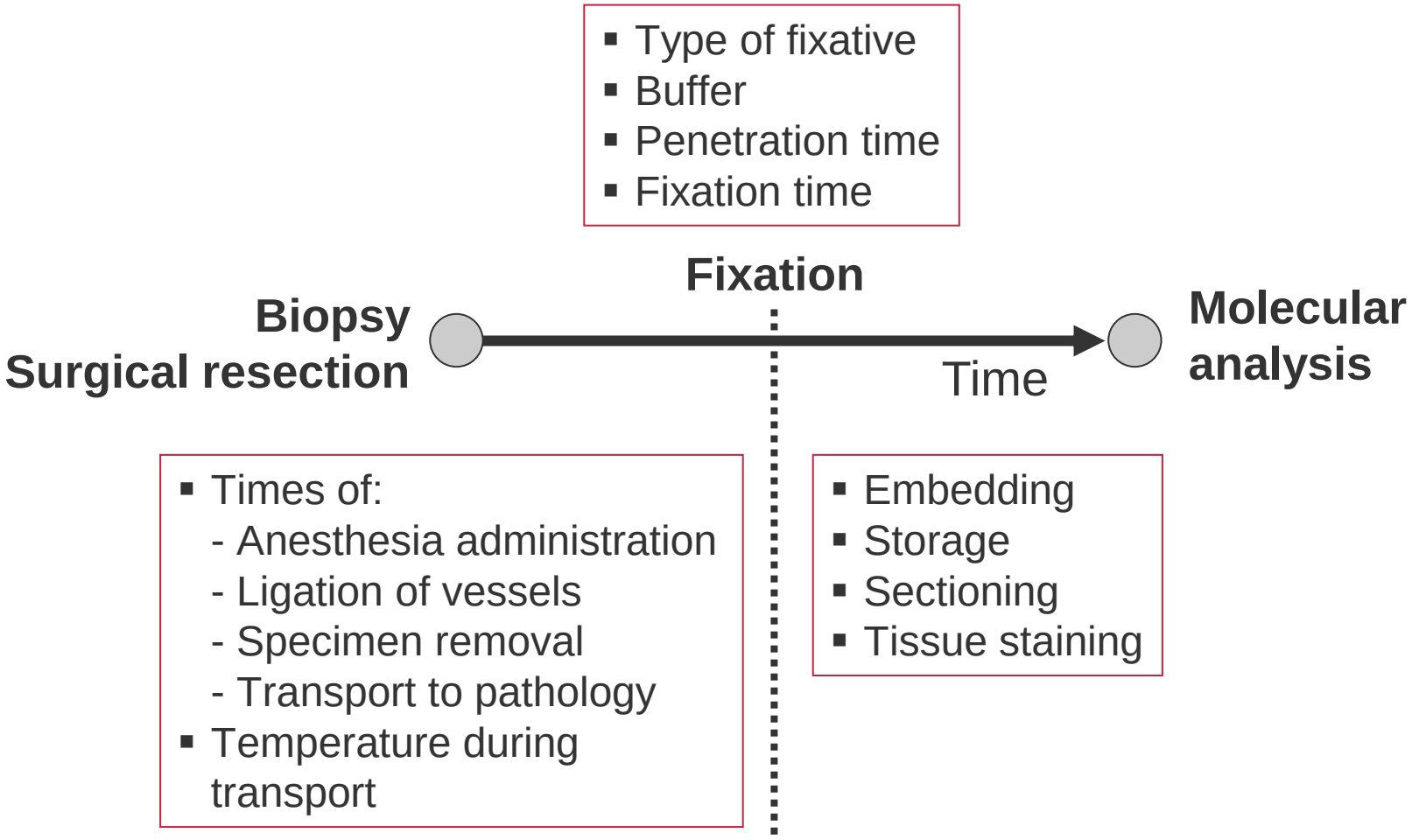
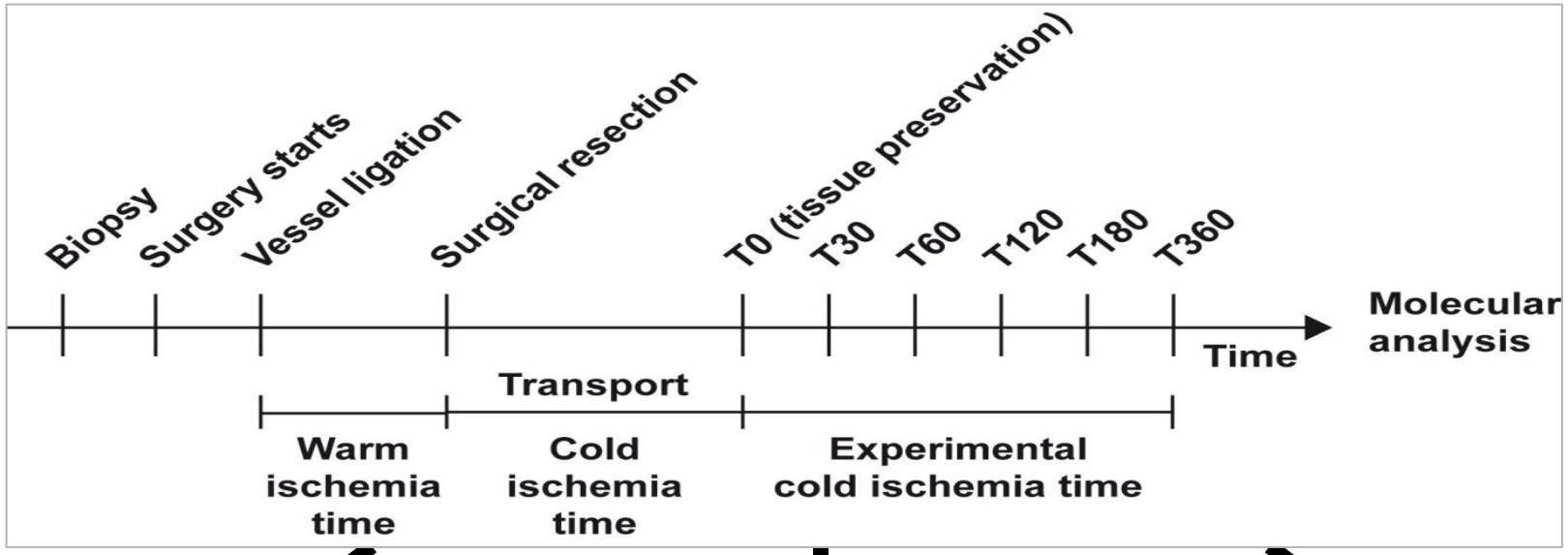
Problem for biomarker analysis: pre-analytical variables during tissue processing



Standards for the pre-analytical phase

Identifying the critical steps during tissue processing



SPIDIA

RNA analysis

Protein analysis

Metabolome analysis

European Committee for Standardization (CEN)

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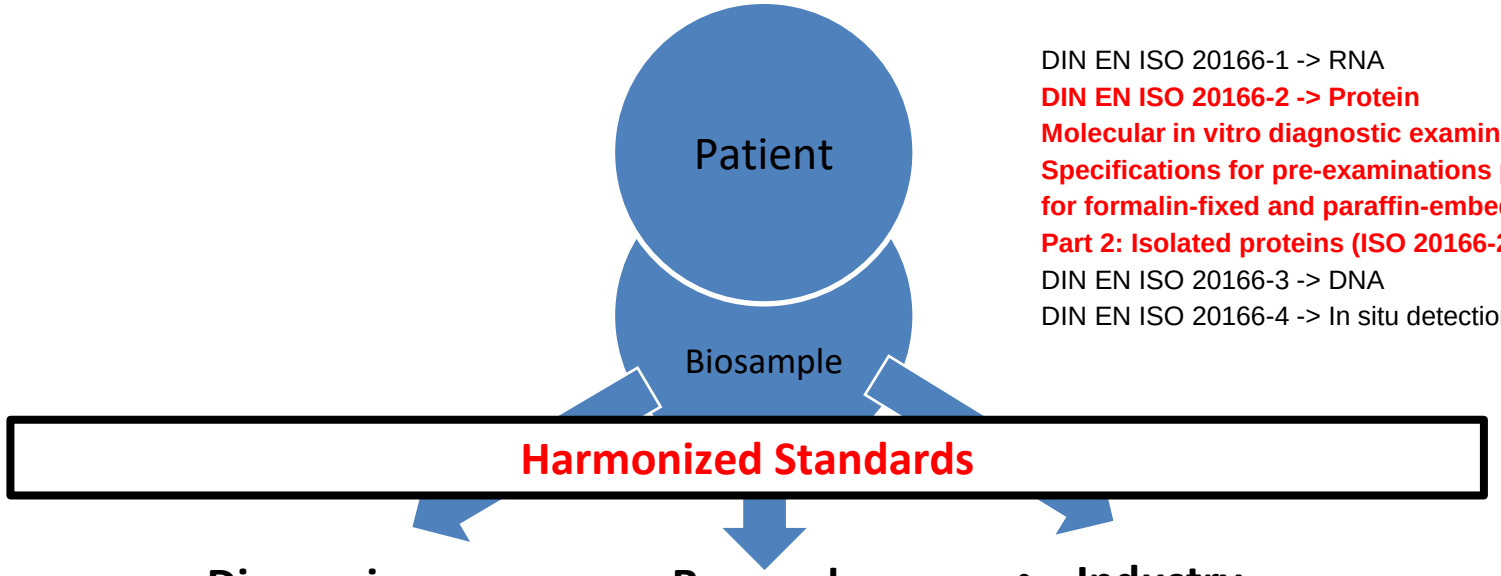
Aim: Harmonized Standards for different workflows



DIN EN ISO 20166-1 -> RNA
DIN EN ISO 20166-2 -> Protein
Molecular in vitro diagnostic examinations – Specifications for pre-examinations processes for formalin-fixed and paraffin-embedded (FFPE) tissue – Part 2: Isolated proteins (ISO 20166-2:2018)
DIN EN ISO 20166-3 -> DNA
DIN EN ISO 20166-4 -> In situ detection techniques

- **Diagnosis**
- **Therapy**
- **Clinical data**

- **Research**
- **Biomarkers**

- **Industry**
- **Assay development**
- **Diagnostics, drugs**

Hospital/Doctor´s office (routine workflow)

Biobanks

Clinical Trials

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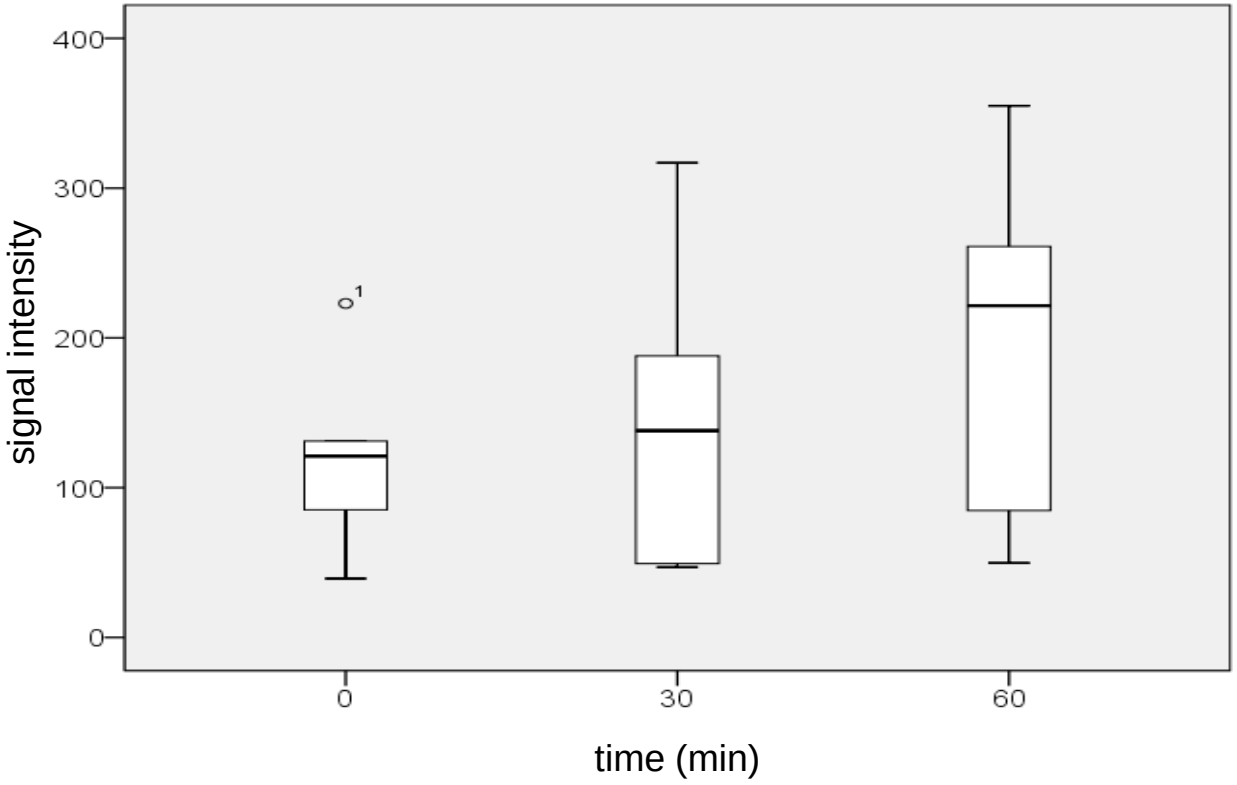
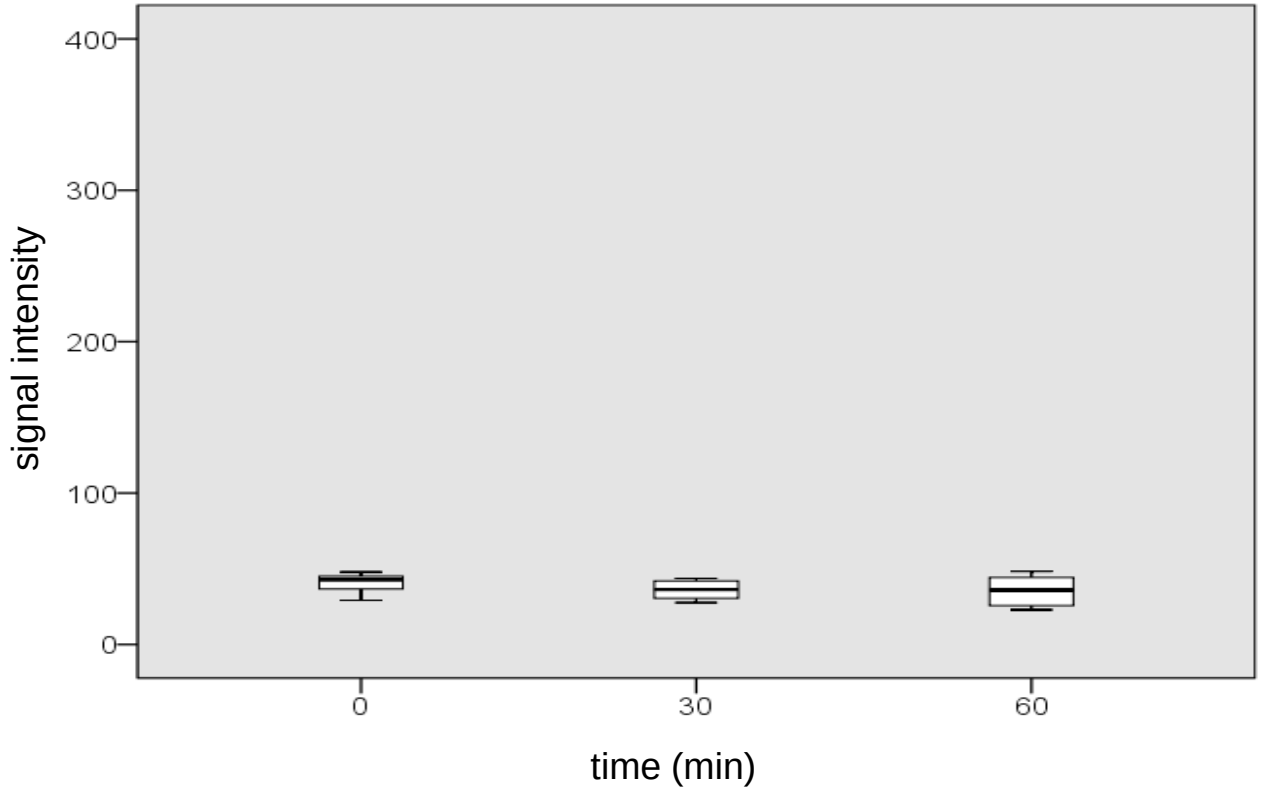
Interpatient variability

(HCC)

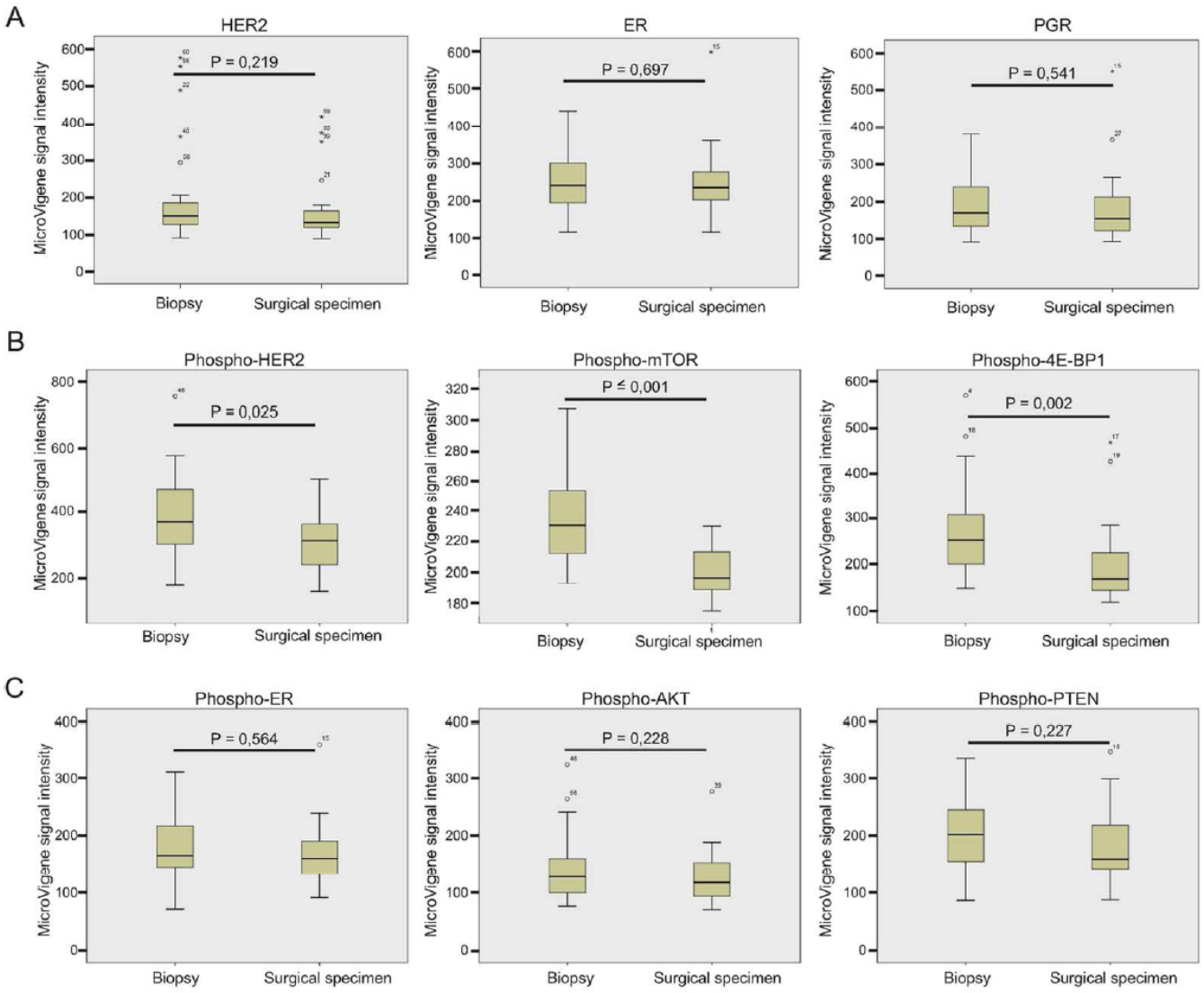
ß-Catenin



PTEN



Biopsy vs. Resection specimen: do not mix results



Examples

Proteins
No difference

Phosphoproteins
Biopsy higher levels

Phosphoproteins
No difference

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Summary

- Proteins are complex
- Isolated proteins are not routinely used in the clinical workflow
- Methods for protein analysis can be used for FFPE tissues
- Pre-analytical phase needs to be improved - variations of protein and phosphoprotein profiles
- Harmonized standards for different workflows

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