


UniversitätsKlinikum Heidelberg

Where do we truly need tissue imaging and analysis in diagnostic (molecular) pathology and biomarker research?

- the pathologist's viewpoint -

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Conventional histopathologic diagnosis

Where does tissue imaging analysis makes sense?

Molecular Diagnostics and Biomarker Research

What are tissue based biomarkers?

Why are they needed?

Which ones are currently under development or in clinical use?

Why and where do we need digital tissue imaging in this field?

Which algorithms are actually needed most?

Tissue imaging and analysis in routine diagnostics will be succesfull when……

- it saves time
- it saves money
- it improves patient treatment

Conventional histopathologic diagnosis

Conventional tissue based diagnosis

In the future digital imaging will replace the conventional microscope as the primary tool for histopathological diagnosis.

Currently no role in disease typing (purely experimental).

Image analysis could be helpful in those cases where quantification must be done in routine diagnostic pathology.

This includes several fields:

1. *Quantification of fibrosis* — *liver and heart diseases*
2. *Evaluation of inflammatory infiltrate* — *infectious diseases and transplant pathology*
3. *Quantification of growth pattern* — *lung adenocarcinoma*

Requirements for automated evaluation

Digital quantification must be based on already established schemes of histopathological staging and grading of diseases because….

- These schemes are usually extensively validated
- These schemes are well accepted in diagnostic pathology
- Quantification must be possible by the use of different platforms

Digital quantification may provide

…higher accuracy, objectivity, reproducibility and therefore may improve patient classification and finally patient treatment

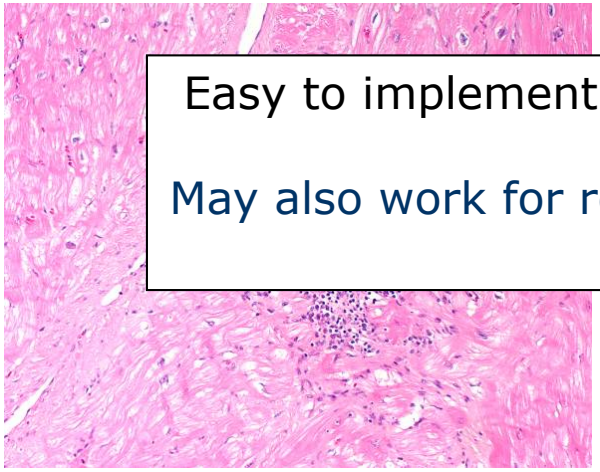
……for example……

Example: Heart biopsy diagnostics

Myocarditis

	Diagnosis	Dallas Classification (Histopathology) Infiltrate	Myocytolysis	Edema	WHF-Classi-fication
1st biopsy	Active myocarditis	+	+	+	≥ 14 cells/mm²
	Borderline myocarditis	+	–	–	≥ 14 cells/mm²
2nd biopsy	Ongoing myocarditis	+	+	+	≥ 14 cells/mm²
	Resolving/healing myocarditis	+	–	–	≥ 14 cells/mm²
	Resolved myocarditis	–	–	–	< 14 cells/mm²

Inflammatory cells: CD3+ CD68+



Easy to implement digital evaluation in routine diagnostics

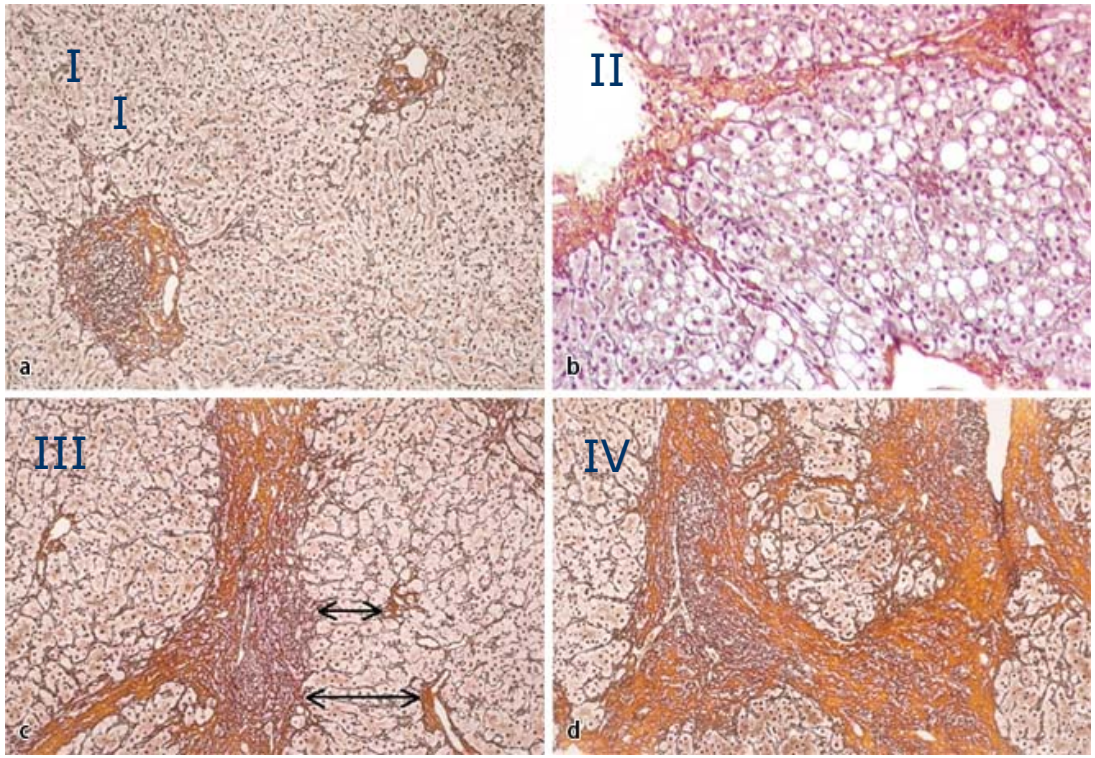
May also work for rejection, Sprue, lymphocytic colitis ……

CD3

Example: Liver fibrosis/cirrhosis

Staging (Desmet)

Stage I: Portal fibrosis
Stage II: Incomplete septa
Stage III: Complete septa, architectural disarray
Stage IV: Complete cirrhosis



…..must work on biopsies, as well

Longerich et al., 2008

Conclusion

Digital analysis as a supplement to conventional tissue evaluation in diagnostic pathology may have a role in increasing the objectivity in the application of established evaluation schemes.

Will not be chaeper, will probably spare time, will be more accurate and therefore may improve patient treatment

The implementation of novel evaluation procedures based on the use of certain „computer algorithms" will usually not result in a widespread application of those methods.

Molecular Diagnostics and Biomarker Research

Types of biomarkers

- diagnostic, prognostic and **predictive** biomarkers have to be distinguished.

to improve

- precision and reproducibility of diagnoses
- determination of the individual prognosis
- prediction of response to therapy
- probability of disease recurrence/metastases

} individualised therapy

NIH: Biomarkers Definitions Working Group, Clin Pharmacol Ther 2001;69:89-95;
Ludwig JA, Weinstein JN. Nat Rev Cancer 2005;5:845-56.

Which patient with a given disease should be treated?

Observation:
Almost all old and new therapeutics are only functional in a subgroup of patients

Consequence > Overtreatment

1. Side effects
   - Novel therapeutics comparatively low
   - „Old" therapeutics comparatively high

2. Costs
   - Novel therapeutics comparatively expensive
   - Conventional therapeutics comparatively cheap

Prediction of response

Central question:

Is it possible to predict response to (targeted) therapy prior to treatment?

If the answer is yes, how?

➢ ***Development of predictive biomarkers***

Possible factors which may influence response:

Presence/Abundance of target protein
Amplification of target gene (often influences expression)
Functionality of target
Factors not directly interconnected with target but which may influence/interact with the functionality of the target

Predictive tissue based biomarkers for targeted cancer therapy

Tumour type	Biomarker	Potential clinical use
Breast	Steroid hormone receptors	Select hormone therapy
Breast	HER2	Select trastuzumab use
Breast	Oncotype Dx gene profile	Assess prognosis; select chemotherapy
Colon	KRAS mutation status	Guide EGFR-specific antibody use
Colon	Microsatellite instability	Assess prognosis or utility of 5-fluoruracil adjuvant treatment
Non-small cell lung	EGFR mutation	Guide selection or use of EGFR tyrosine kinase inhibitors
Non-small cell lung	ERCC1	Select platinum-based chemotherapy
Glioblastoma	MGMT methylation	Guide temozolomide use
Melanoma	BRAF V600E mutation	Select therapy

EGFR, epidermal growth factor receptor; ERCC1, excision repair cross-complementation group 1; HER2, also known as ERBB2; MGMT, methyl guanine methyltransferase.

Schilsky et al., Nat Rev Drug Discovery, 2010

Types of biomarkers

Protein expression as determined by Immunhochistochemistry

Gene amplification as determined by FISH/SISH

Translocations as determined by FISH

Mutations as determined by sequencing

Methylation as determined by sequencing

Tissue biomarker expression

Gene amplification

Expression of target protein

Usually evaluated by immunohistochemistry on tissue slides

Several examples already in clinical use:
Her2 expression in breast cancer prior to Trastuzumab
Her2 expression in gastric cancer prior to Trastuzumab
ERCC1 expression in lung cancer prior to platinum based chemotherapy

Several others under development:
RRM1 expression prior to gemcitabine based chemotherpy
EpCAM expression prior to Adecatumumab

Objective evaluation of tissue biomarker expression: The dilemma

Attempts to quantify, standardize, and correlate semi(quantitative) marker expression with outcome have so far been insufficient and none of even the already applied tissue biomarkers have been properly va

The evaluation "by eye" is not obje

Therefore:

- a definition of standards (to be u
- an objective determination of thr
- a validation of the most appropri
- a determination of the influence
  quantification and influence on p

……..are urgently needed!

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Requirements for automated evaluation

Algorithms must be available for either membranous (Her2,EpCAM), nuclear (ERCC1) and cytoplasmic (RRM1) antigens.

Digital analysis should be able to discern tumor cells and only to evaluate these cells.

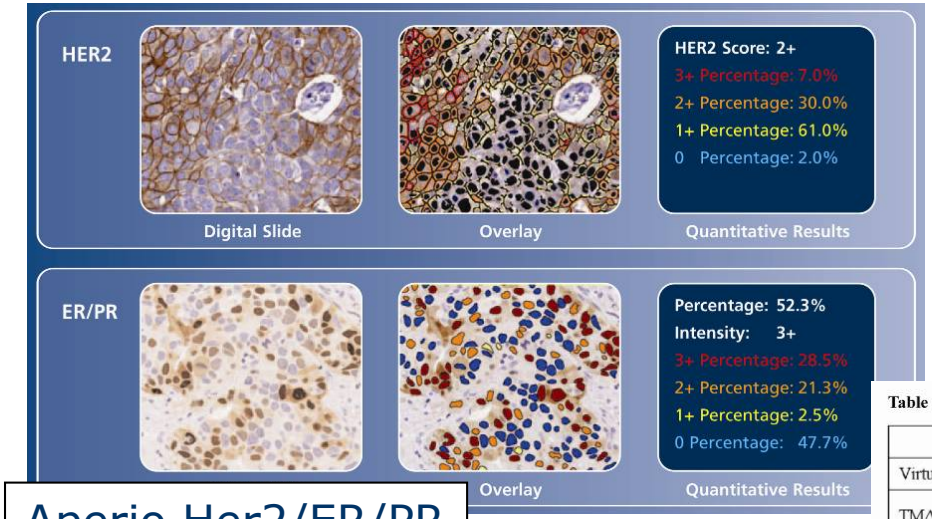
Digital analysis must be able to give the percentage of positive cells as well as the staining intensity to cope with a multitude of different evaluation algorithms.

The respective analysis should be as fast and as automated as possible.

The ideal situation would be just to scan the stained slide and to get back a relative expression niveau in tumor tissue.

A standard IH evaluation by an experienced pathologist needs 5 min and is cheap but may be inaccurate and therefore may result in suboptimal patient treatment.

Expression of target protein



Aperio Her2/ER/PR

For nuclear/membranous expression pretty good algorithms are available.

For cytoplasmic expression, not yet

FDA/EMEA clearance in Germany not mandatory.

Method must be evaluated in ring trials (in Germany: QuiP/DGP)

Table 1. Image analysis systems available for virtual slides

Product name	Manufacturer
Virtual slide scanning and automatic image analysis	
TMAscore	Bacus Labs/Olympus America http://www.olympusamerica.com/seg_section/seg_vm.asp
ScanScope Image Analysis Toolbox	Aperio http://www.aperio.com/imageanalysis/image-analysis.asp
MIRAX HistoQuant	3DHistech http://www.3dhistech.com/en/article/quantitative-histology
PATHIAM™ RUO	BioImagene http://www.bioimagene.com/pathiam.html
ACIS	Dako http://www.dakousa.com/index/prod_search/prod_groups.htm?productcatcaid=43
Ariol	Genetix http://www.genetix.com/en/systems/ariol
CytoVision	Genetix http://www.genetix.com/en/systems/cytovision
Image analysis software and static picture solutions	
TissueMap	Definiens http://www.definiens.com/definiens-tissuemap_134_7_10.html
Tissue Image Analysis	SlidePath http://www.slidepath.com/php/products-imageanalysis.php
iCyte, Laser Scanning Cytometer	CompuCyte
AQUA	HistoRx http://www.historx.com/AquaNew
Nuance™	Cambridge Research & Instrumentation, Inc (CRi) http://www.cri-inc.com/products/nuance.asp

Rojo et al., 2009

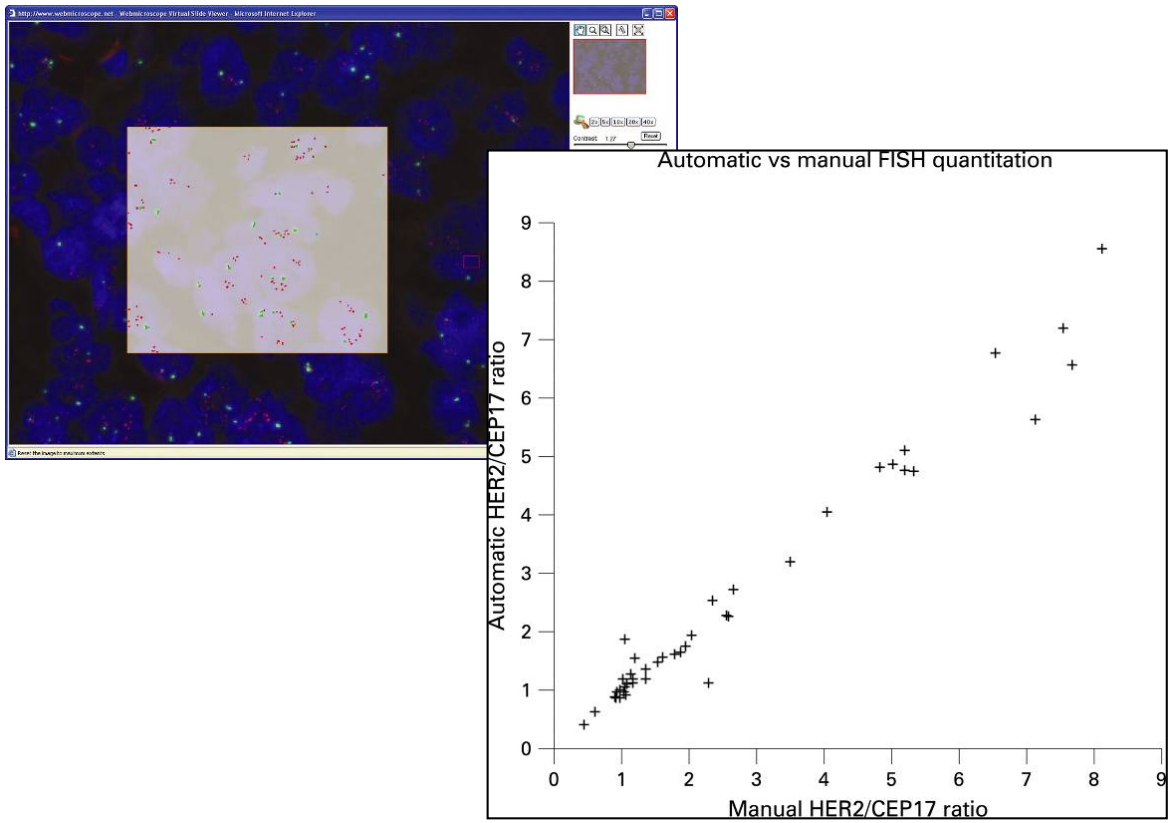
Amplification of target gene

Usually evaluated by fluorescence or chromogen in situ hybridization on tissue slides

Several examples already in clinical use:
Her2-Amplification in breast cancer prior to Trastuzumab
Her2-Amplification in gastric cancer prior to Trastuzumab

Several others under development:
EGFR-Amplification in colorectal and lung cancer

Amplification of target gene



Her2

FISH/CISH/SISH evaluation tools are available and reliable

Automated evaluation methods are not in daily routine use for Her2 (high level amplification)

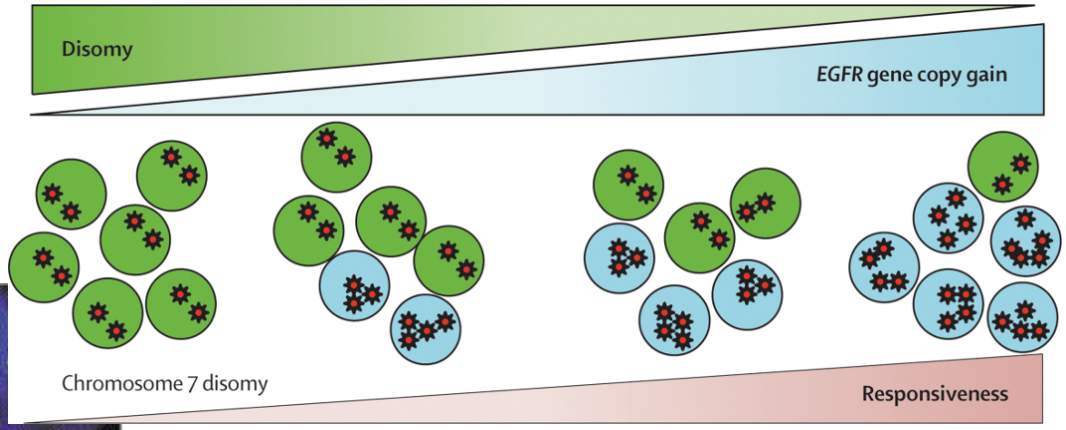
A standard FISH evaluation by an experienced pathologist needs 15 min and is cheap but again may be inaccurate and therefore may result in suboptimal patient treatment.

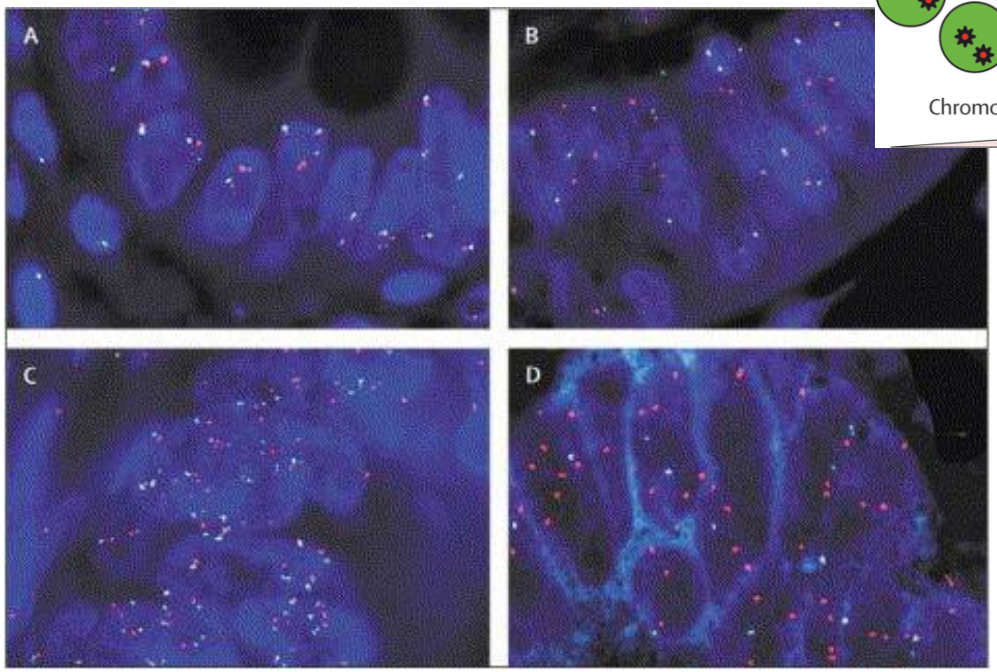
Konsti et al., 2008

Amplification of target gene

Low level amplification often poses problems in manual evaluation.

It is accepted that EGFR gene copy number correlates with response to anti EGFR-treatment in colorectal and lung cancer.





However, not one cutoff defined has been validated in a subsequent study

Automated detection of the exact gene copy number may help in this regard

Moroni et al. Lancet Oncol, 2005, 2008

Conclusion

Digital tissue image analysis for the determination of biomarker expression/gene amplification may have a role in

1. Increase objectivity and standardization in the evaluation of already established biomarkers

2. Improve tresholds for therapy selection for already established biomarkers

3. Determine tresholds for novel biomarkers

Automaten methods must be:

1. Evaluated against conventional scoring „by hand"

2. Supply a couple of informations (eg intensity/percentage of positive cells) to fit in already established evaluation algorithms

3. Prior to use: Ring trial validated

Will not spare time, will not be cheaper but may dramtically increase the quality of patient treatment, may even result in novel diagnostic tests

Mutations/Promotor Methylations

Usually mutations as well as promotor hypermethylations are detected by sequencing (conventional/pyrosequencing).

Several examples already in clinical use:
KRAS mutations in colorectal cancer prior to Cetuximab/Panitumumab
EGFR mutations in lung cancer prior to Gefitinib/Erlotinib
MGMT promotor hypermethylations in Gliomas prior to Temozolomide
Kit-Mutations in GISTs prior to Imatinib

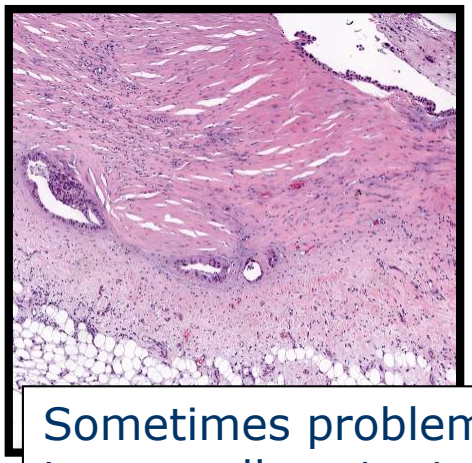
Several others under development:
BRAF-Mutations in colorectal cancer
PIC3CA-Mutations in colorectal cancer

Is image analysis needed in this regard?

Mutation detection – how is it done

Tumor areas were marked on H&E slides

Tumor areas were prepared from 2 subsequent unstained slides



Sometimes problematic (e.g. neoadjuvant treatment), tumor cell content must be evaluated

Validity of sequencing data is dependent on tumor cell content

Colorectal

	Percentage of tumor cells ≤10%	>10%	p-value
Sanger Sequencing			0.022
mutated	0 (0%)	108 (43.2%)	
non mutated	8 (100%)	142 (56.8%)	
Array			0.135
mutated	1 (14.3%)	103 (45.6%)	
non mutated	6 (85.7%)	123 (54.4%)	
Melting curve			0.077
mutated	0 (0%)	77 (42.1%)	
non mutated	5 (100%)	106 (57.9%)	
Pyrosequencing			0.292
mutated	0 (0%)	51 (38.3%)	

Automated detection of tumor cells may replace manual selection of dissection areas and increase the accuracy and objectivity in the evaluation of tumor cell content prior to mutational screening and thereby may allow for a better interpretation of sequencing results.

Weichert et al., 2010

Conclusion

Digital tissue image analysis prior to molecular testing may

1. Increase the objectivity in the evaluation of tumor cell content

2. Determine better treshold levels

Ideally automated tumor detection may be coupled to laser microdissection.

Will spare time, will be cheaper, increase the quality of patient treatment

Thanks!

Manfed Dietel

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Frederick Klauschen

…….and you for your attention!