
Localized molecular analysis of lung cancer tissue sections using the microfluidic probe

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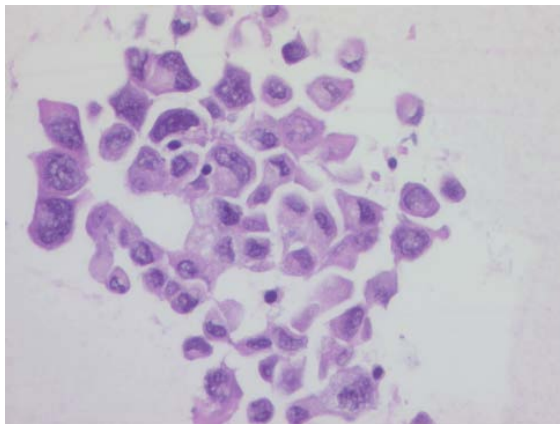
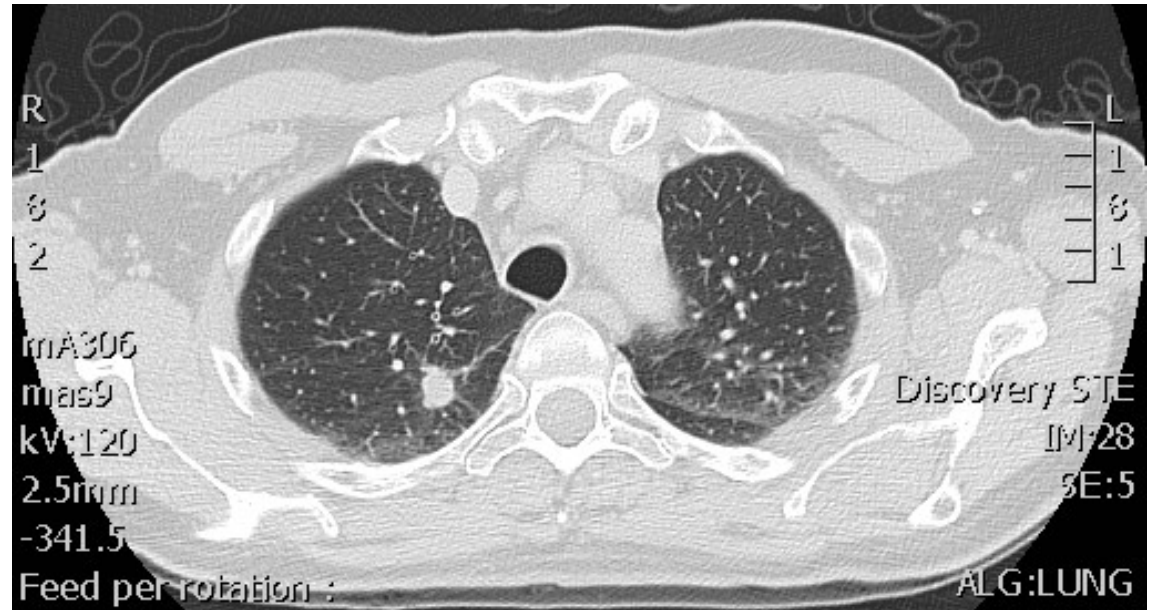
University Hospital
Zurich



**Institute of
Surgical Pathology**

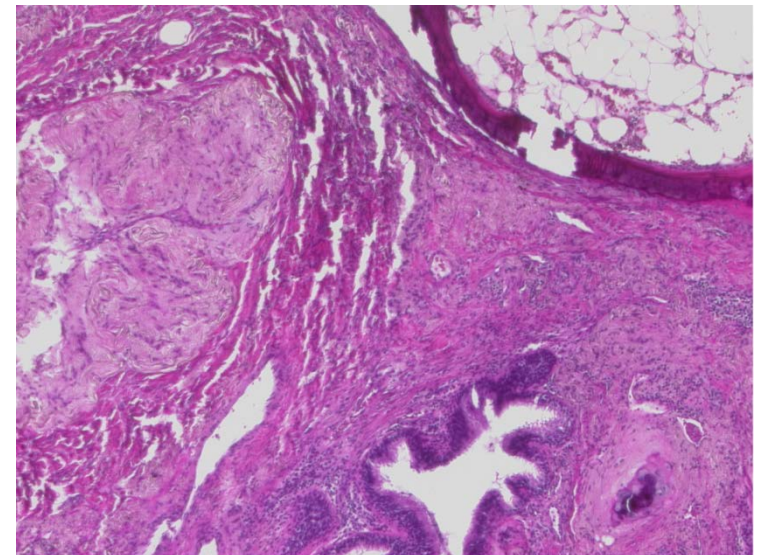
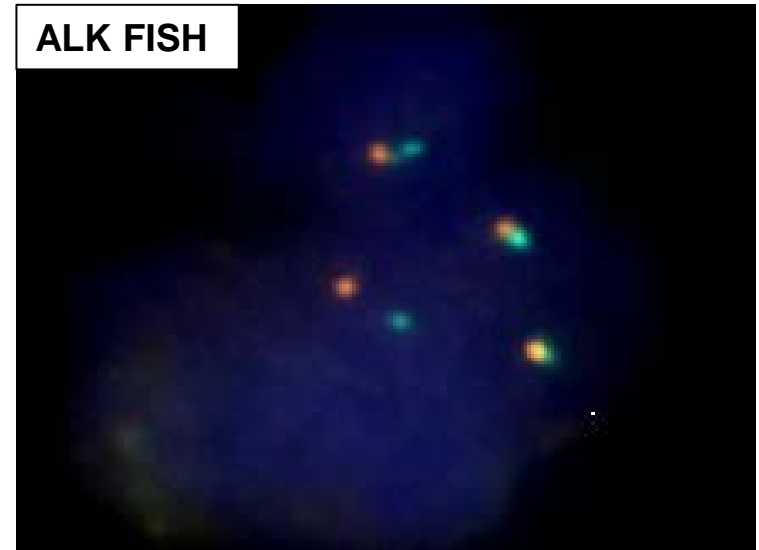
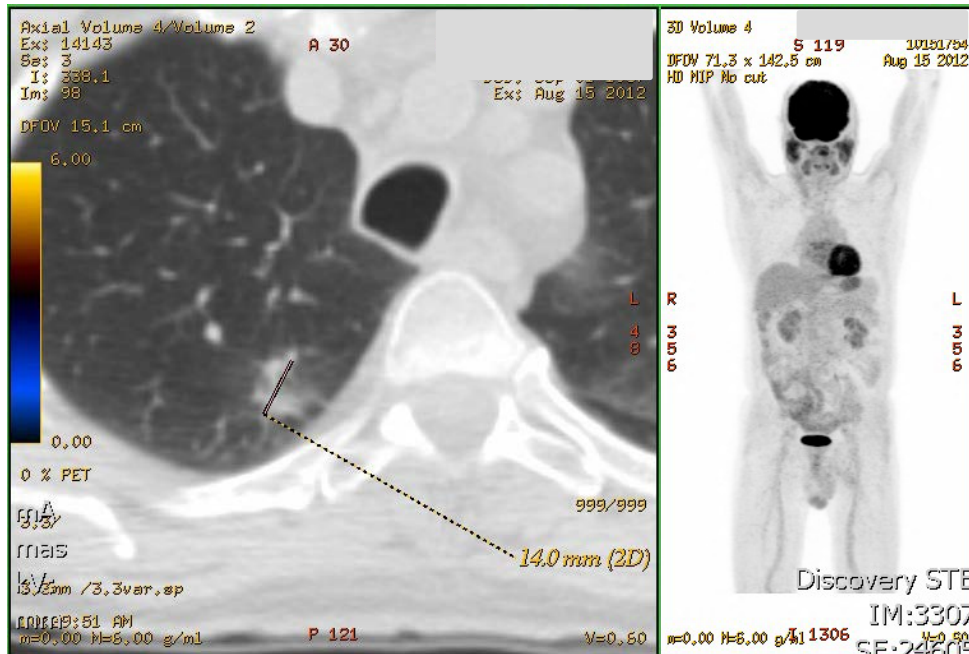


Personalized targeted therapy in lung cancer



- Oct 2011: Male, 75y, nicotine 4 py, stopped 40 yrs ago
- Poorly diff. adenocarcinoma of the right upper lung lobe, widespread lymph node metastasis
- TNM 7th edition: cT4 cN3 cM0, UICC stage 3b
- Nov 2011 - Dec 2011: 3 cycles cisplatin, pemetrexed and bevacizumab → no response

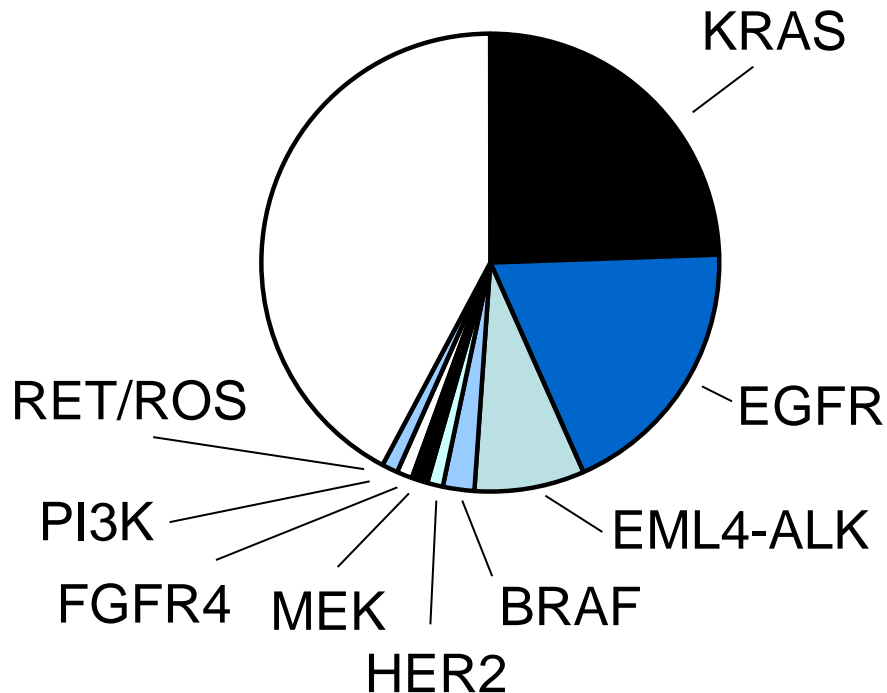
Complete metabolic and histologic remission



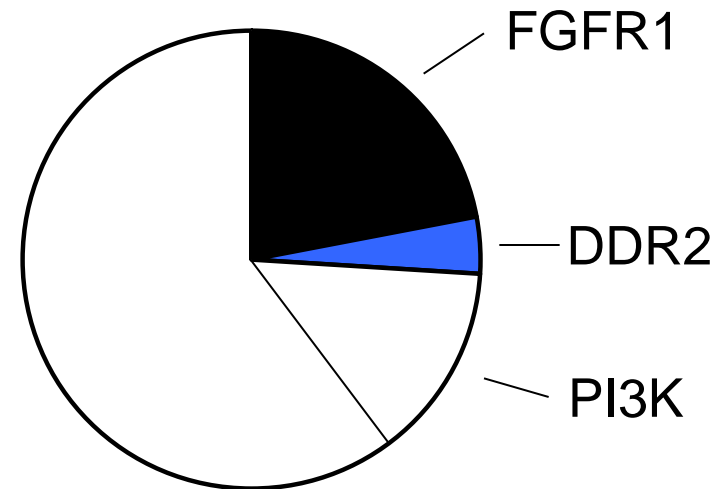
- Molecular pathology: No EGFR mutation, break-apart in ALK gene, >95% of cells
- Mar 2012: Crizotinib (ALK + ROS-1 inhibitor)
- Mar 2013: Complete remission, scarring fibrosis, dyspnea at >2000m altitude

Predictive biomarkers in lung cancer

Adenocarcinoma



Squamous cell carcinoma

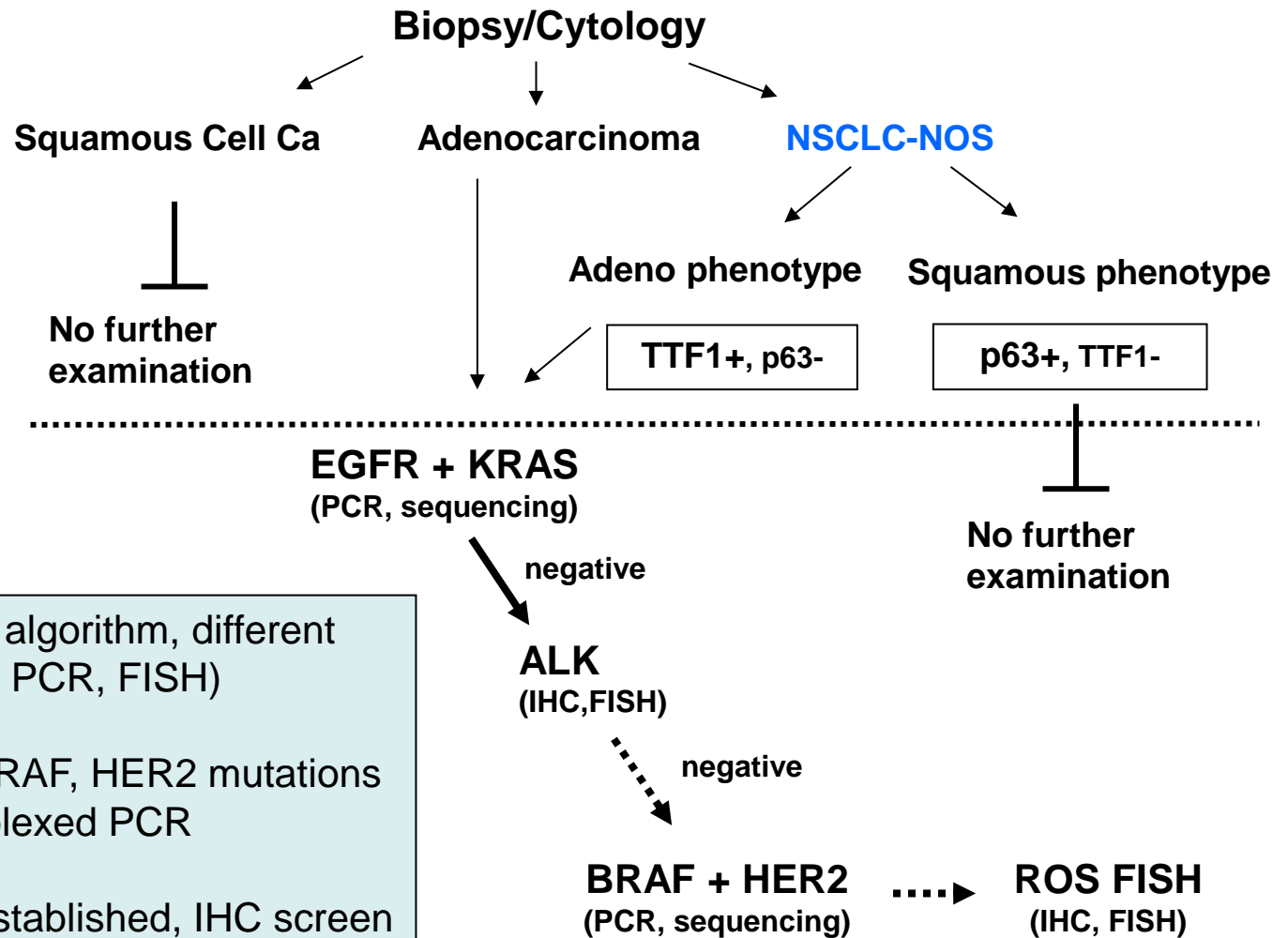


Alterations mutually exclusive ?

EGFR: Epidermal growth factor receptor
 KRAS: Kirsten rat sarcoma viral oncogene homolog
 EML4-ALK: Echinoderm microtubule-associated protein-like 4, anaplastic lymphoma kinase
 BRAF: v-Raf murine sarcoma viral oncogene homolog B1
 HER2: Human epidermal growth factor receptor 2

FGFR1/4: Fibroblast growth factor receptor 1/4
 DDR2: Discoidin domain receptor 2
 PI3K(-CA): Phosphatidylinositol-3 kinase, catalytic subunit alpha
 MEK: Mitogen-activated protein kinase kinase
 RET/ROS: RET proto-oncogene, C-ros oncogene 1

Mol path work-up for lung cancer in Switzerland

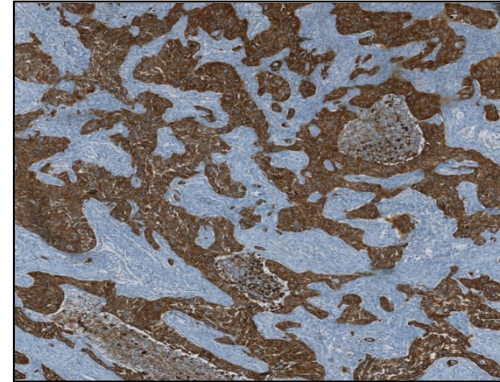
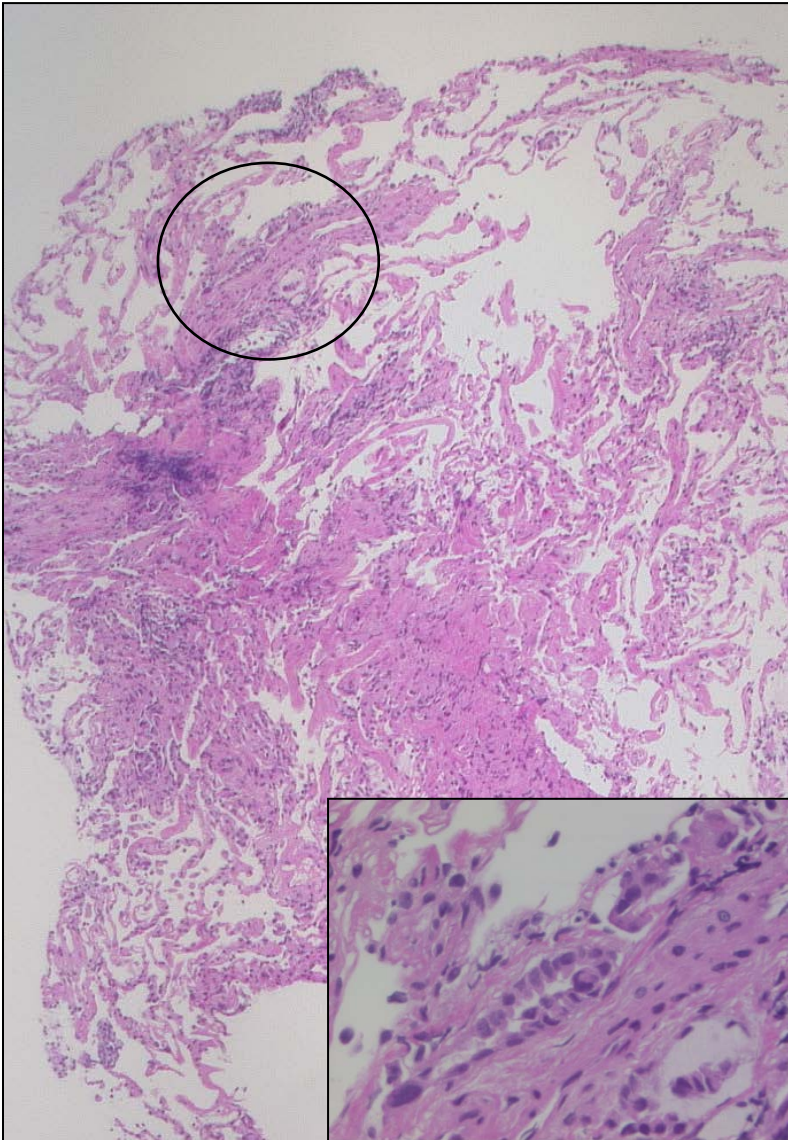


- Time consuming algorithm, different techniques (IHC, PCR, FISH)
- EGFR, KRAS, BRAF, HER2 mutations by parallel multiplexed PCR
- Multi-FISH not established, IHC screen

Current main issues in molecular pathology

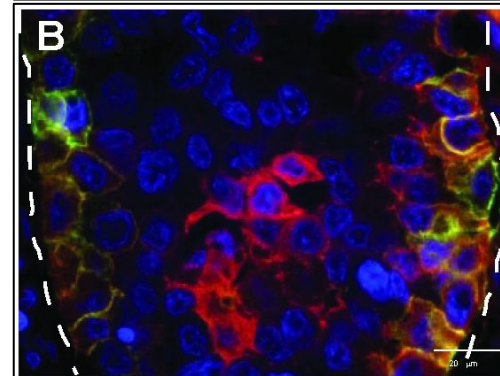
- **Single predictive driver mutation versus “systems pathology”**
 - Oncogenic addiction (EGFR-HER1, HER~~2~~, HER~~3~~, HER~~4~~)
 - EGFR downstream kinome, multikinase inhibitors
- **Tumor heterogeneity**
 - Different histosubtypes, e.g. lepidic, acinar, micropapillary, papillary and solid adenocarcinoma
 - Carcinoma: Tumor epithelia and stroma (tumor microenvironment)
 - Tumor invasion front versus tumor center
- **Limited amount of tumor cells**
 - Only biopsy/FNA in case of palliative treated cT4 cN3 tumor
 - Bronchial cryobiopsy may yield up to 50'000 cells

Localized analysis biopsy vs. surgical specimen



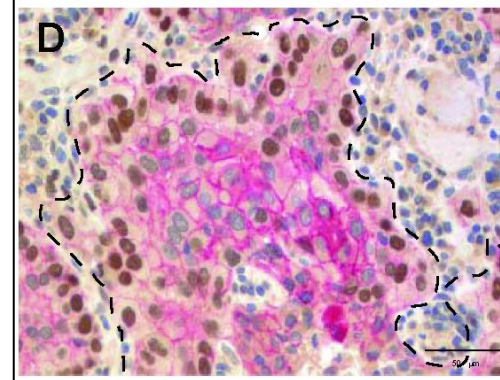
Cytokeratin IHC

Ratio tumor cells
versus stroma
40% to 60%



Epithelial-
mesenchymal
transition EMT
invasion front

E-cadherin
L1CAM



Double IHC:
E-cadherin
Slug

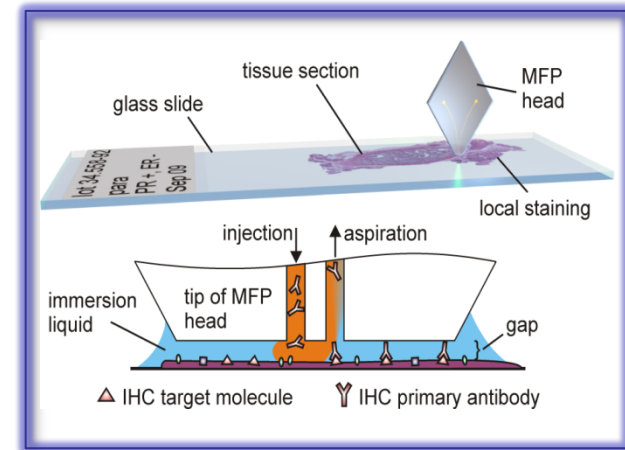
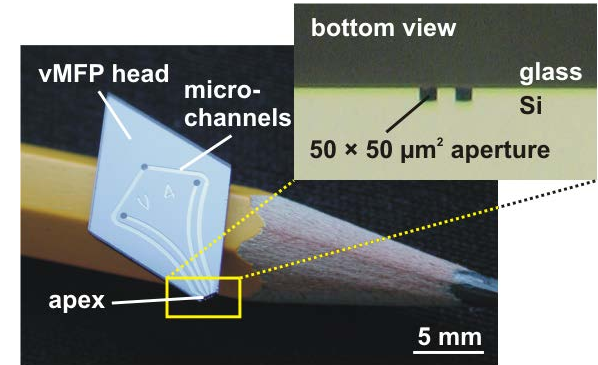
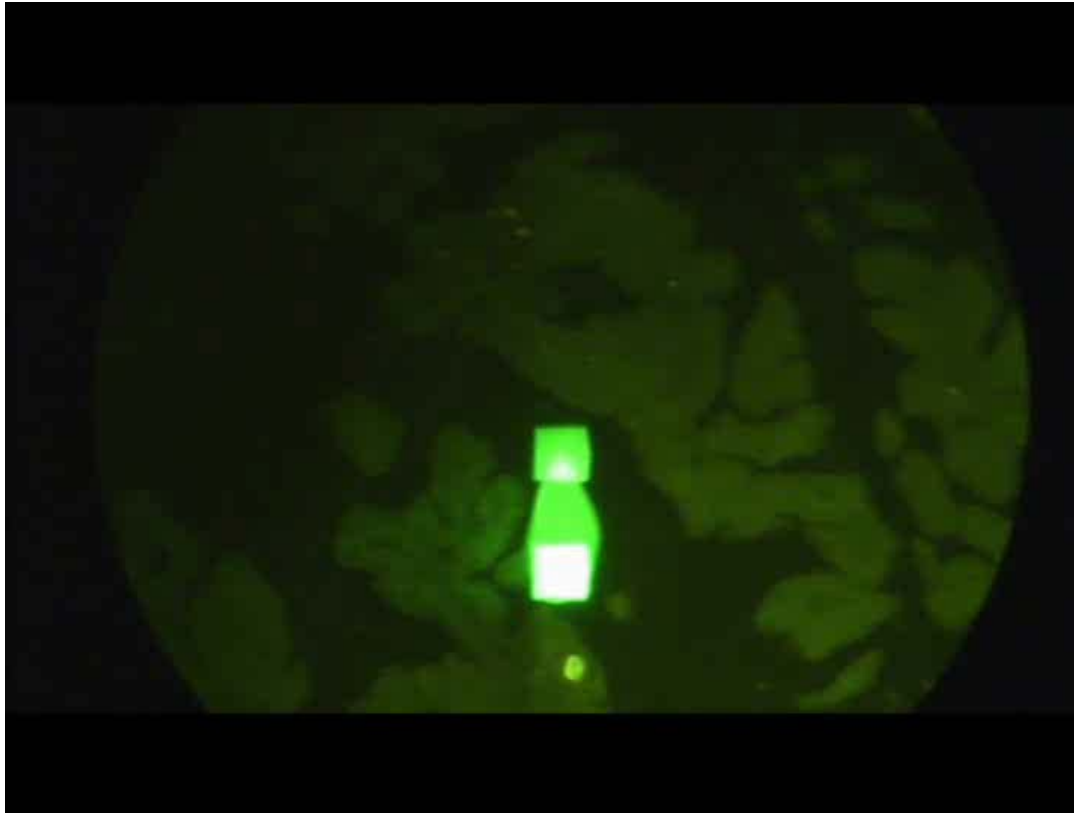
Vision for TF-IBM



- To develop a multifunctional tool to aid researchers and pathologists to investigate cells and critical tissue samples accurately for disease diagnostics and predictive genomic alterations.
- **Objective**
 - Ability to perform spatial and localized genetic profiling.
 - Visualize multiple marker stainings at high resolution.
 - More data from less sample.
- **Framework:** Convergence of two areas of expertise at ISP-USZ and IBM-Zurich, combining Pathology and MFP technology.

Microfluidic probe (MFP) technology

The MFP precisely shapes liquid in order to localize (bio)chemical events on surfaces.

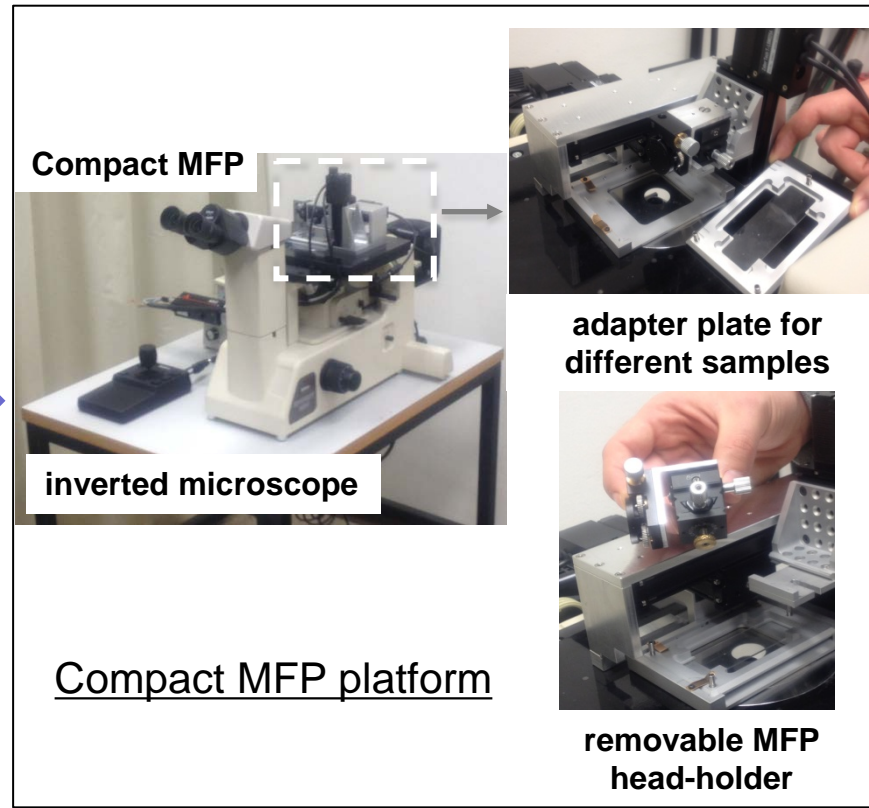
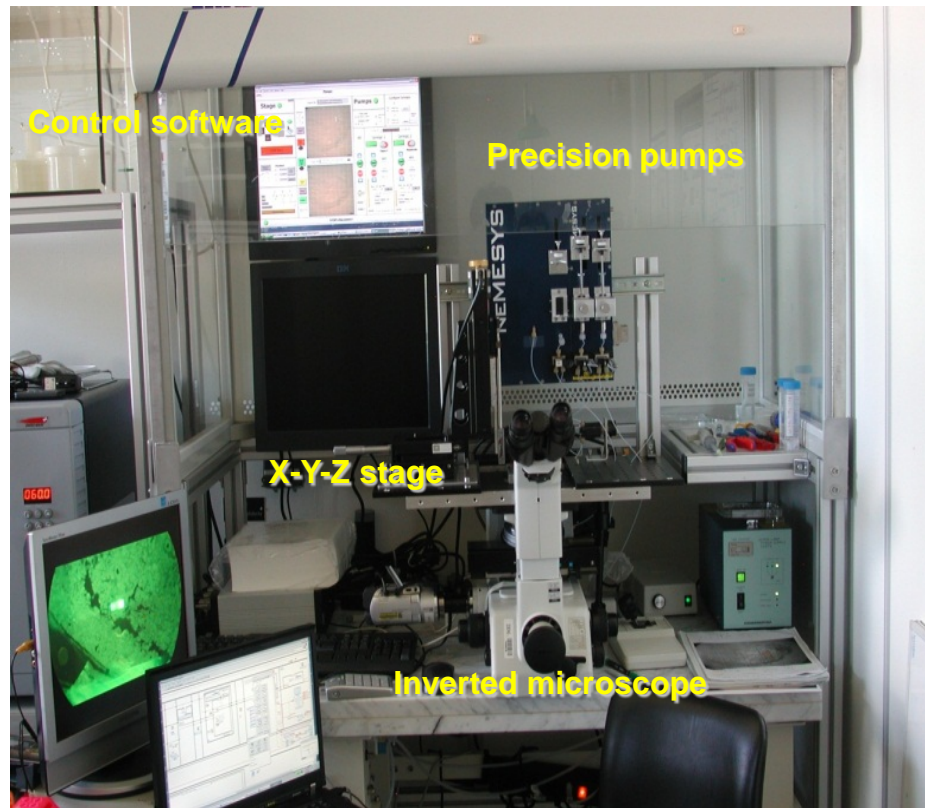


The liquid is confined in 100 picoliter volume over the tissue section and has a footprint of 100 μm²

G. Kaigala, R. Lovchik, U. Drechsler, E. Delamarche, "A vertical microfluidic probe," *Langmuir*, 2, 2011, 5686–5693.

D. Juncker, H. Schmid, E. Delamarche, "Multifunctional microfluidic probe," *Nature Materials*, 4, 2005, 622–628.

MFP instrumentation



As part of the TF project, IBM-Zurich will

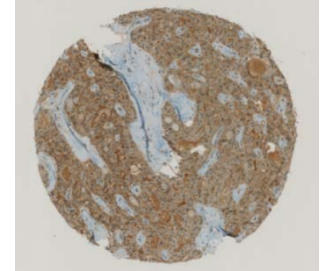
- Help set-up the MFP platform at ISP/USZ
- Provide general support for the MFP technology
- Provide existing microfabricated MFP heads and new designs
- Provide access to the MFP platform at IBM-Zurich and host the PhD student

Planned activities

- **Development of new protocols for localized tissue analysis:**
 - DNA extraction
 - Micro-immunohistochemistry (μ -IHC)
- **Provide multi-modal analysis of scarce amount of tumor cells:**
 - Biopsies and cell blocks of fine needle aspiration
 - Genetic alterations in tumor cells vs. stroma and at the invasion front
- **Student training:**
 - PhD student, transferring technology from IBM to ISP
 - Masters thesis student from ETHZ-Bioengineering (Aditya Kashyap)
- **Dissemination:**
 - Conferences, workshops, peer-reviewed publications, journal clubs

BRAF und DDR2

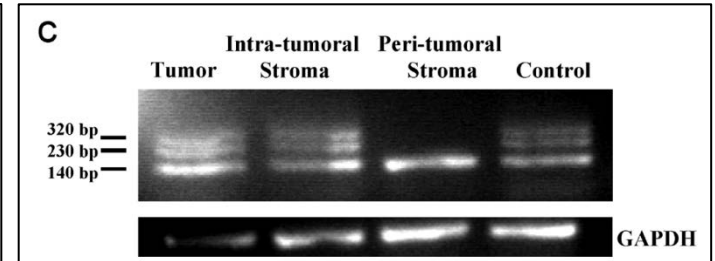
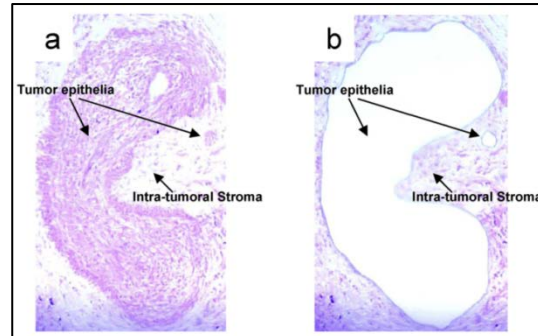
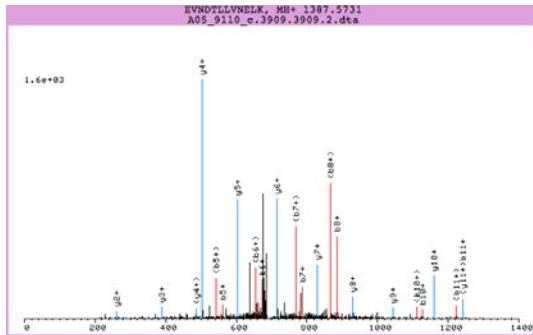
- **BRAF = v-Raf murine sarcoma viral oncogene homolog B1**
 - Mutations in up to 5% of lung adenocarcinoma
 - 50% V600E mutation in exon 15
 - Predominantly in micropapillary lung adenocarcinoma
 - BRAF sensitizes cancer cells to TGF- β induced EMT
 - Specific inhibitor vemurafenib (70% RR in melanoma)
- **DDR2 = discoidin domain receptor 2**
 - Mutations in 4% of lung squamous cell carcinoma
 - Membranous DDR2 is a direct collagen receptor
 - TGF- β promotes increase of type I collagen and DDR2
 - DDR2 siRNA k.o. inhibits collagen induced EMT
 - Specific inhibitors like dasatinib and sorafenib



IHC BRAF
V600E mut

Relation to other enrichment techniques

1. Macrodissection by punch core
2. Macrodissection by scalpel scratching
3. Laser capture microdissection (LCM)

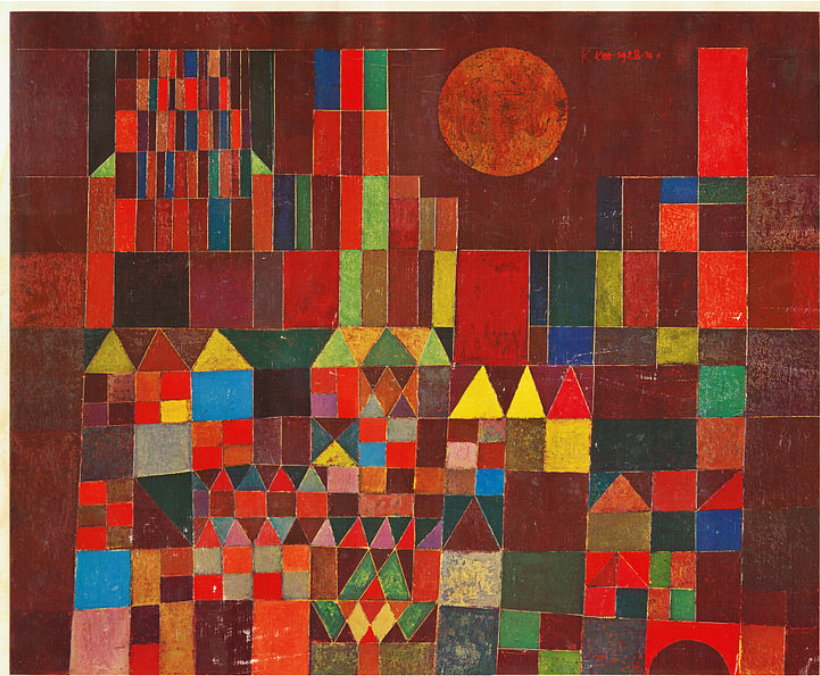
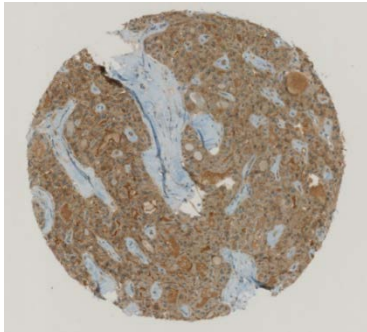


Soltermann et al. Cancer Cytopathol 2008, Morra et al. Lung Cancer 2012

Strengths of the microfluidic probe (MFP)

- Aqueous-based. No drying, freezing, organic solvents, boiling, burning
- *Reversible stainings following cell removal on defined area with 1 device*

Combined localized molecular analysis



Burg und Sonne - Paul Klee, 1928

Genomic DNA mutation

- Single exon sequencing
- Next generation sequencing

Genomic translocations

- Fluorescent in-situ hybridization

Single protein marker

- Immunohistochemistry

Protein profile

- Mass spectrometry

RNA

- In-situ hybridization

Personnel

Aditya Kashyap

USZ

Alex Soltermann

Peter Schraml

IBM

Govind Kaigala

Emmanuel Delamarche

Thanks!



SystemsX

The Swiss Initiative in Systems Biology



krebsliga schweiz

ligue suisse contre le cancer

lega svizzera contro il cancro



**University of
Zurich** ^{UZH}