
High-throughput diagnostic system for tissue-based personalized cancer therapy

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Fraunhofer

Who we are



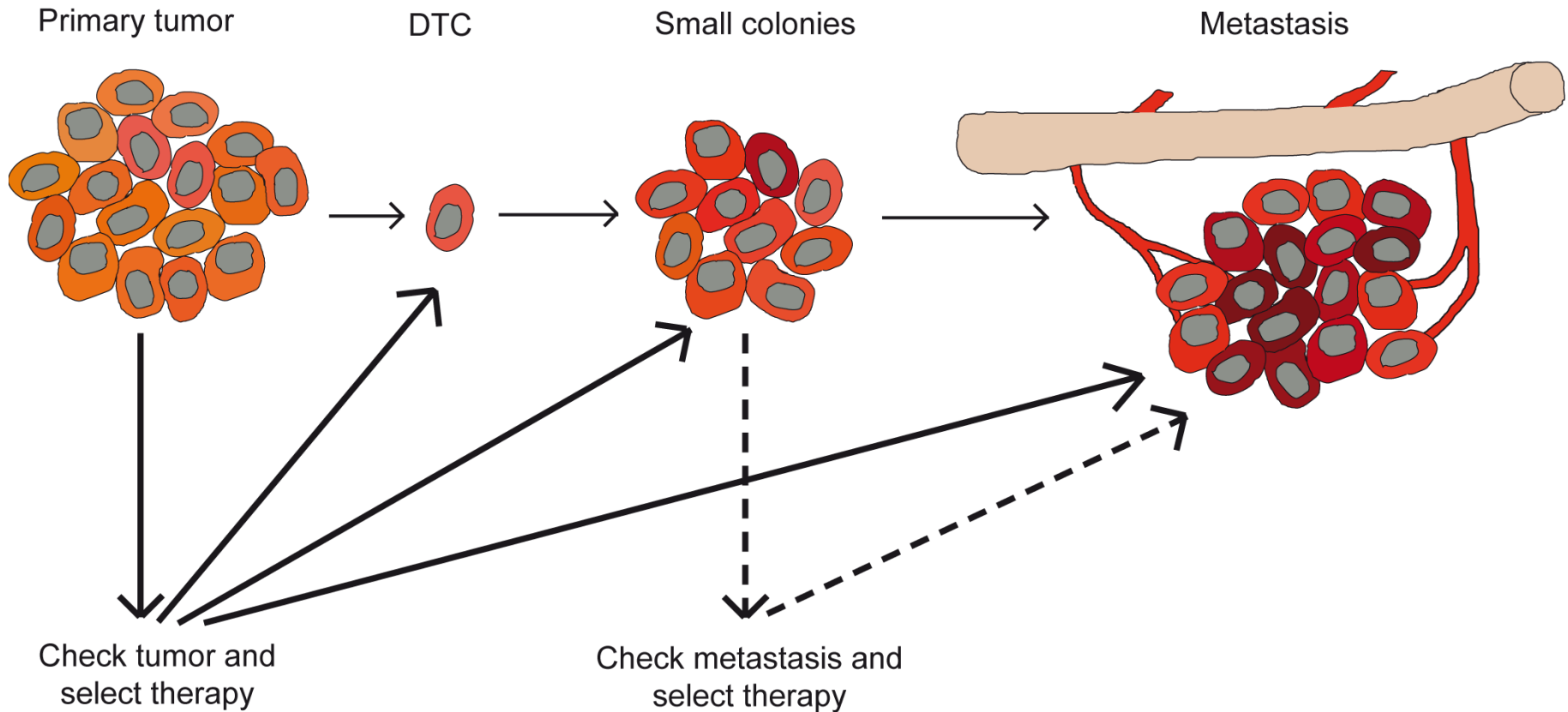
Europe's leading applied research organization with 24,000 employees, 2.1B€ annual budget and more than 20,000 patents.

Fraunhofer is home to leading experts in AI, healthcare visualization, diagnostic research and computational medicine.



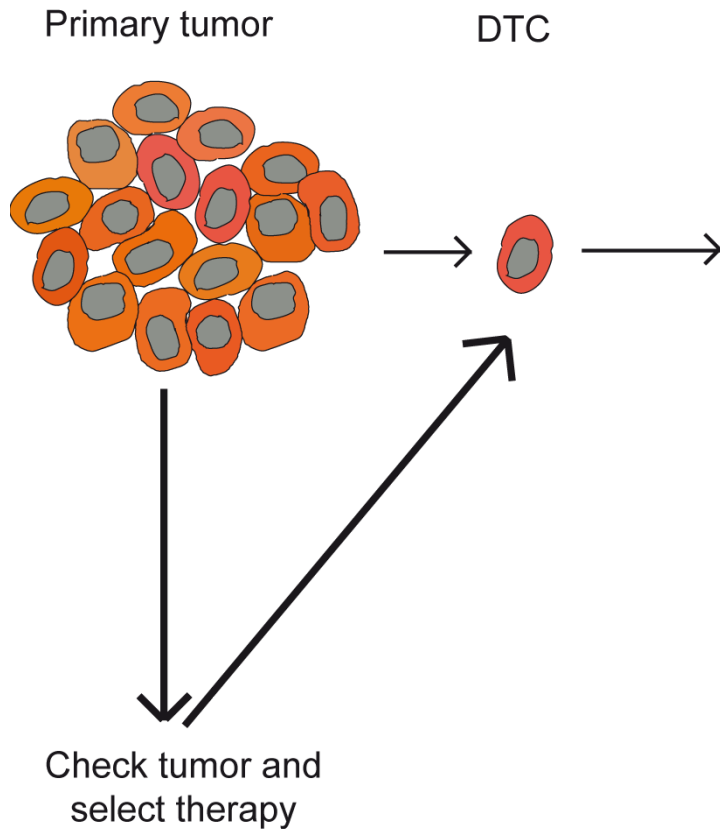
Where we stand today

Personalized Therapy – Hoping for improvement



Where we stand today

Personalized Therapy – Hoping for improvements

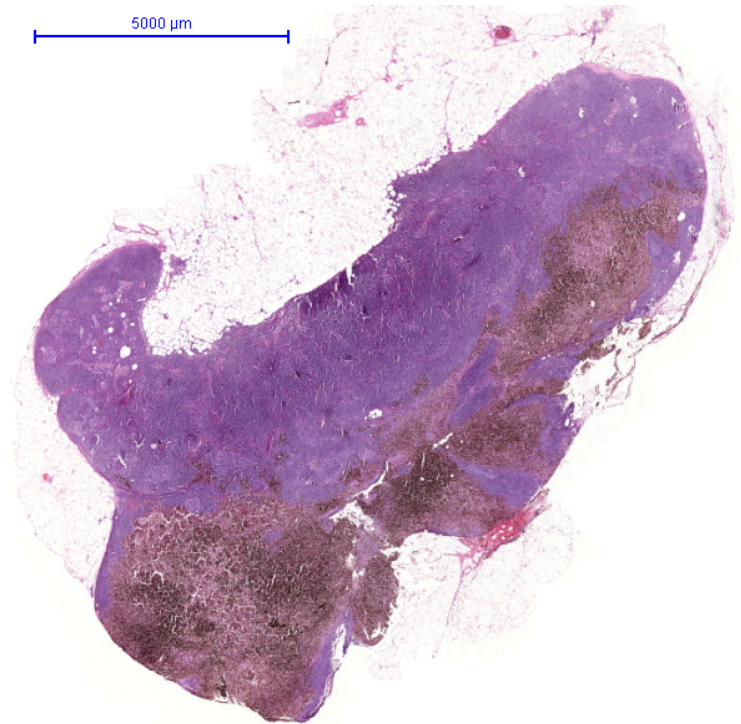
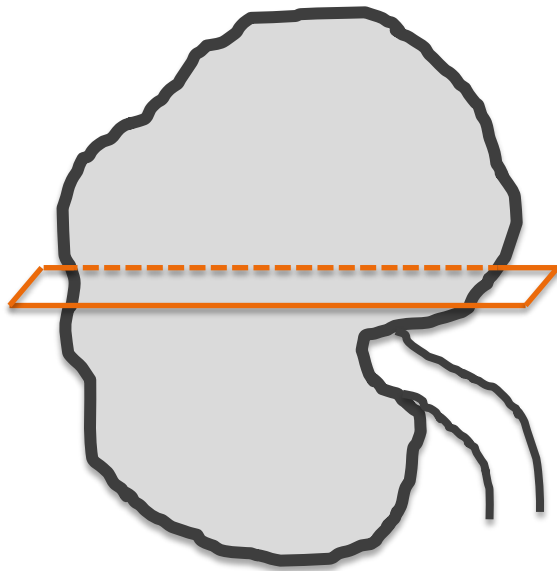


Goal:

We need to improve therapy for our patients by analysing the target cells. Possibly DCCs are more sensitive to drugs than metastases.

Where we stand today

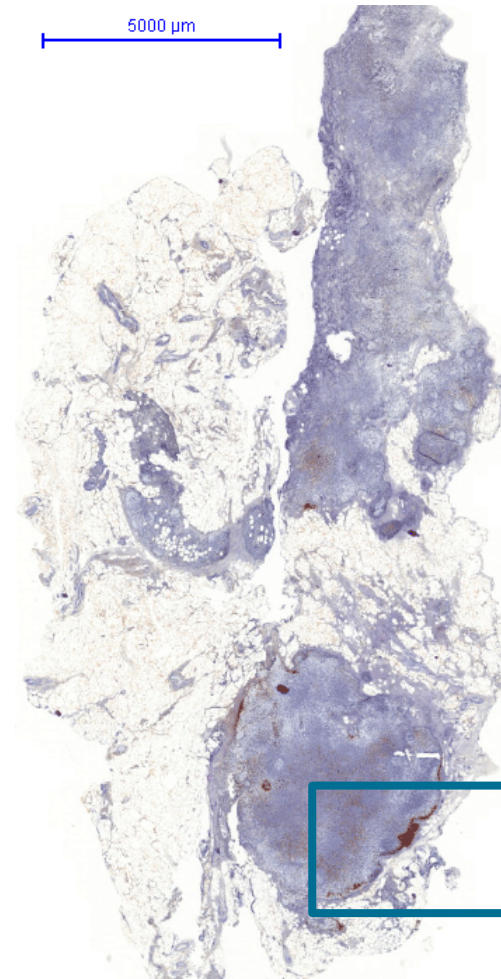
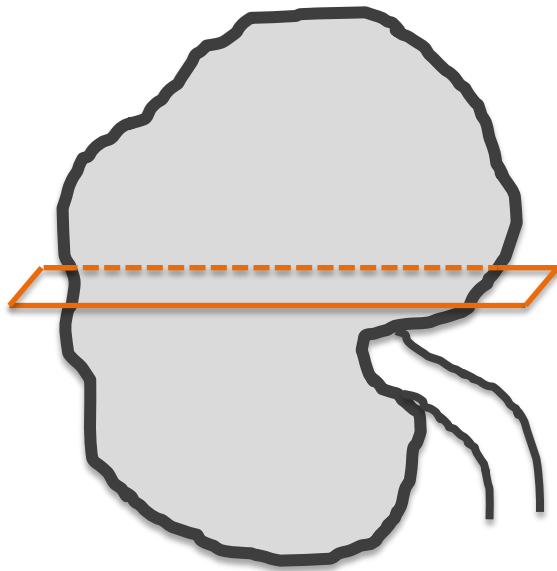
Lymph node staging



Werner-Klein et al., Nature Communications 2018

Where we stand today

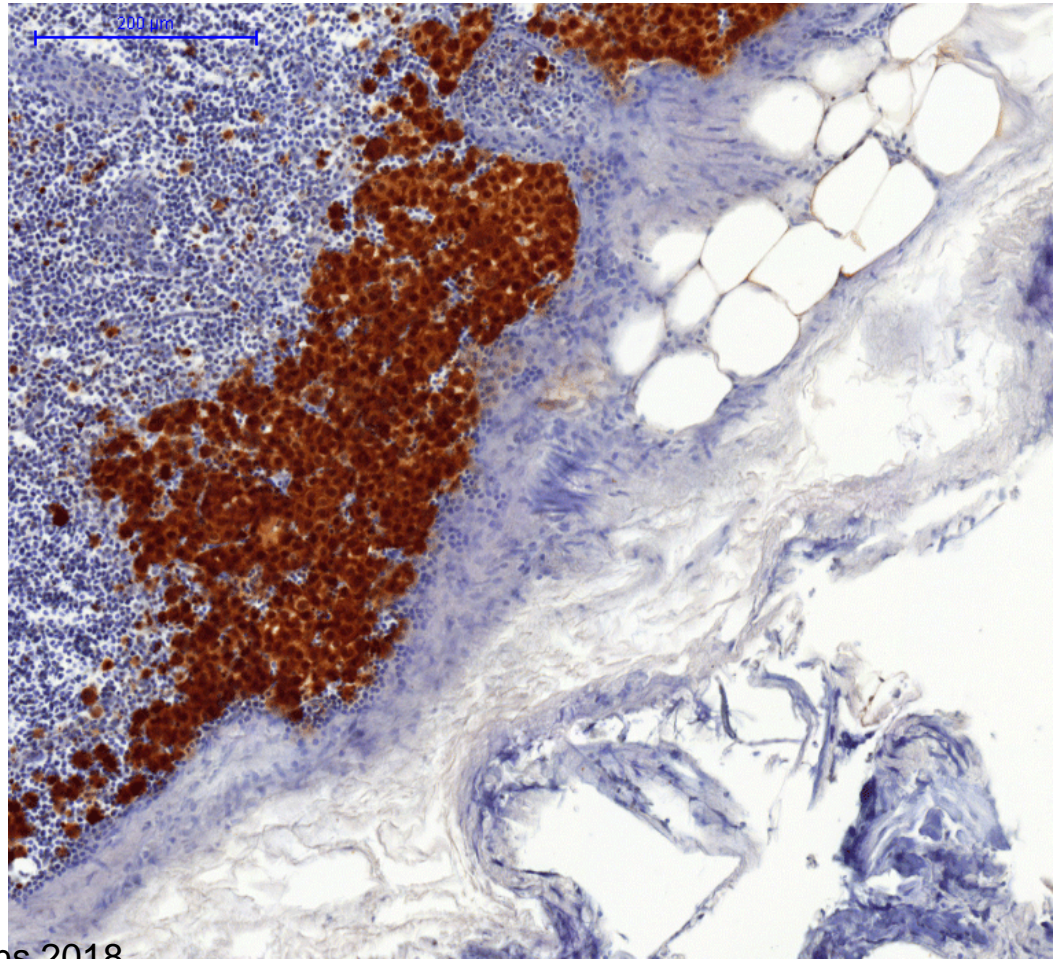
Lymph node staging



Werner-Klein et al., Nature Communications 2018

Where we stand today

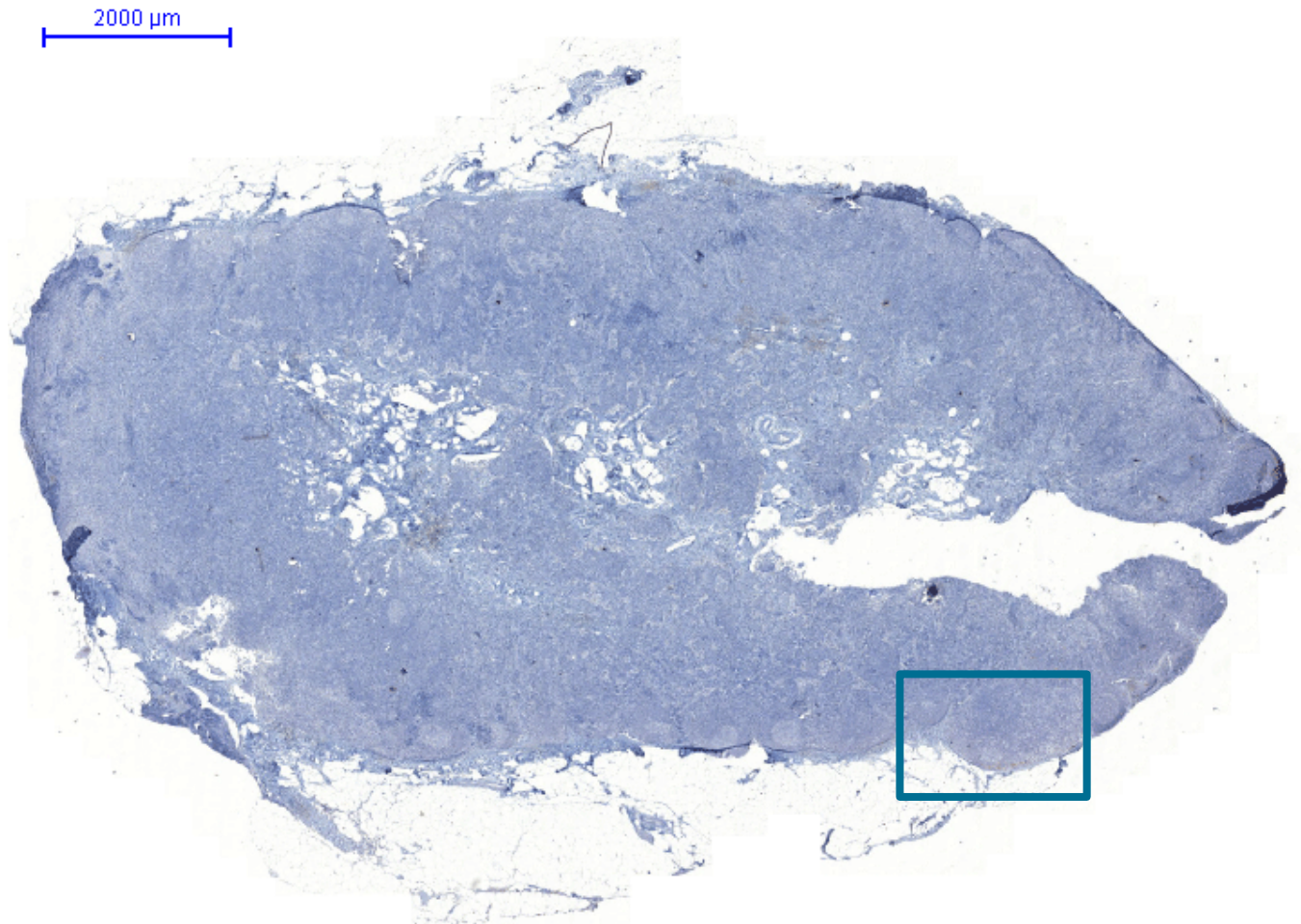
Lymph node staging



Werner-Klein et al., Nature Communications 2018

Where we stand today

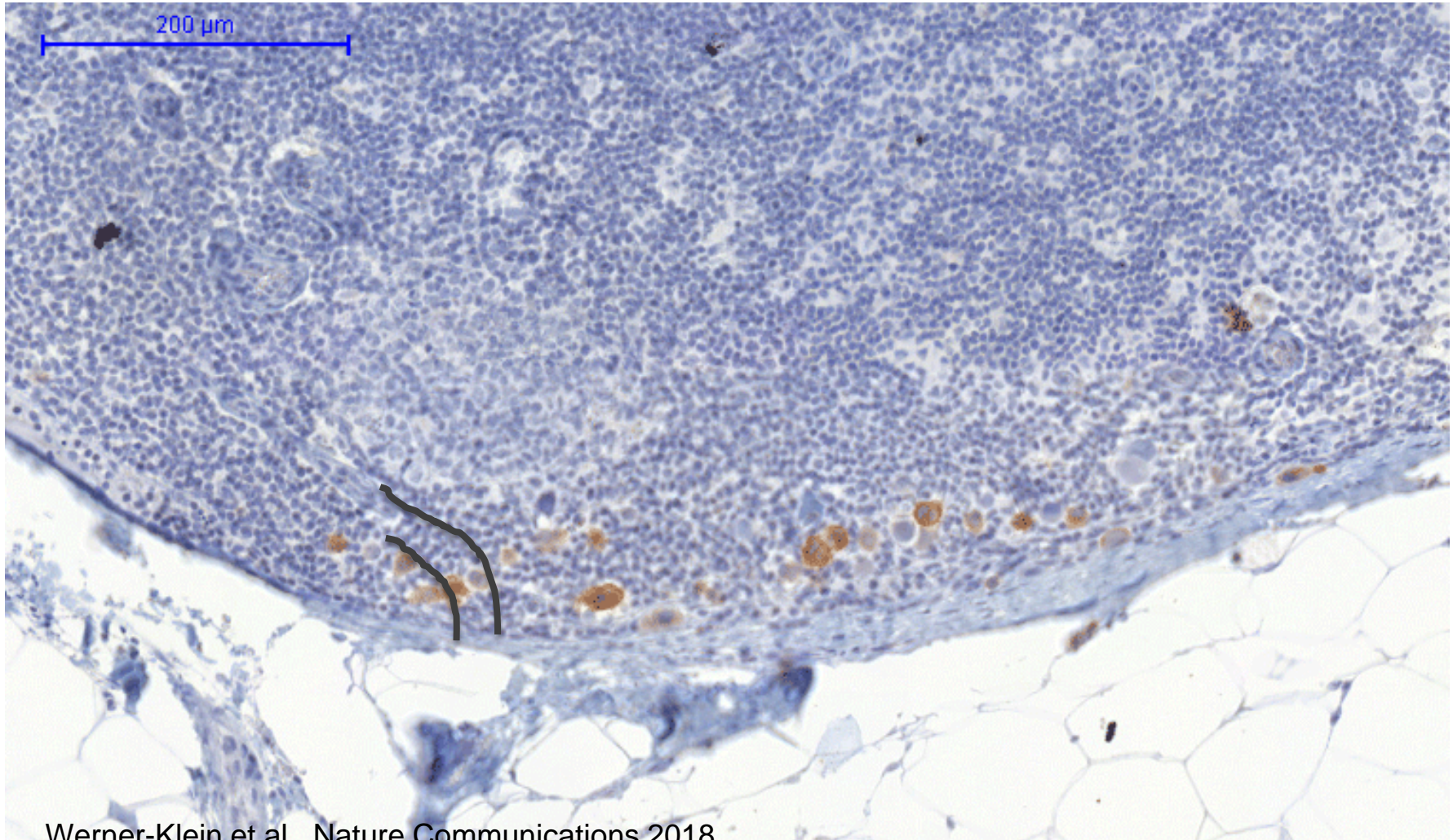
Lymph node staging



Werner-Klein et al., Nature Communications 2018

Where we stand today

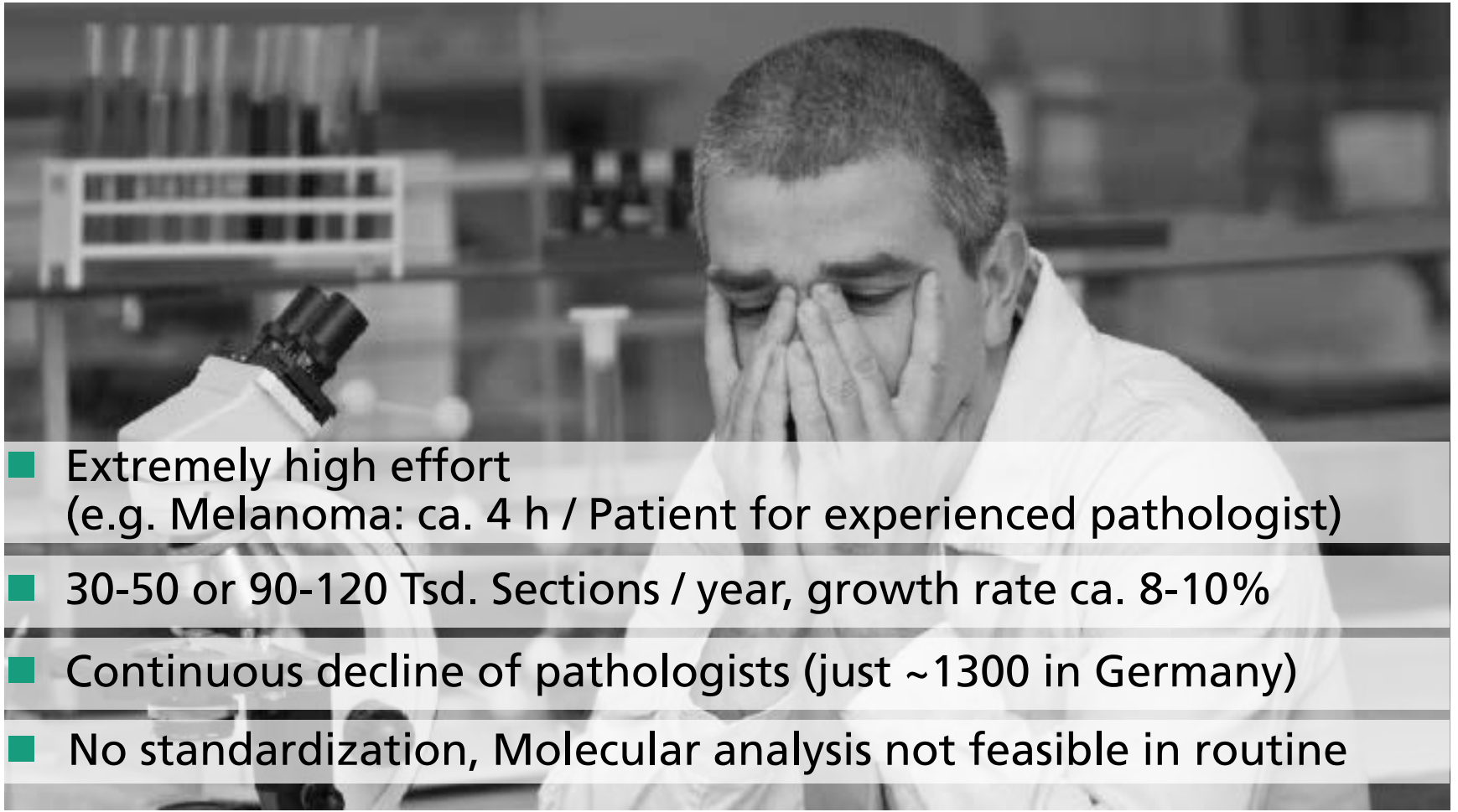
Lymph node staging



Werner-Klein et al., Nature Communications 2018

Where we stand today

Lymph node staging

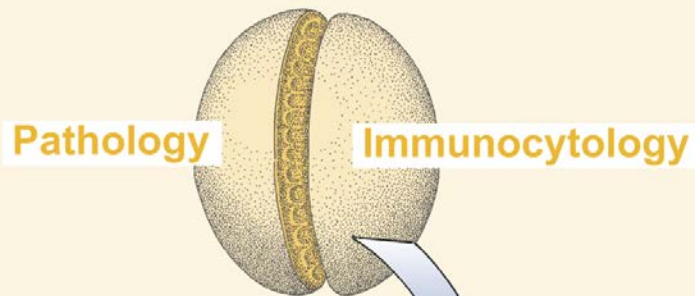


- Extremely high effort
(e.g. Melanoma: ca. 4 h / Patient for experienced pathologist)
- 30-50 or 90-120 Tsd. Sections / year, growth rate ca. 8-10%
- Continuous decline of pathologists (just ~1300 in Germany)
- No standardization, Molecular analysis not feasible in routine

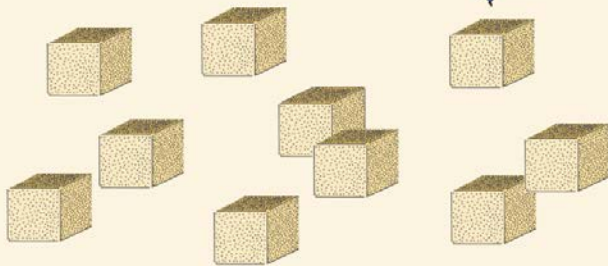
Our approach - a paradigm shift in diagnostics

From tissue sections.... to single cell analysis

Lymph node is bivalved

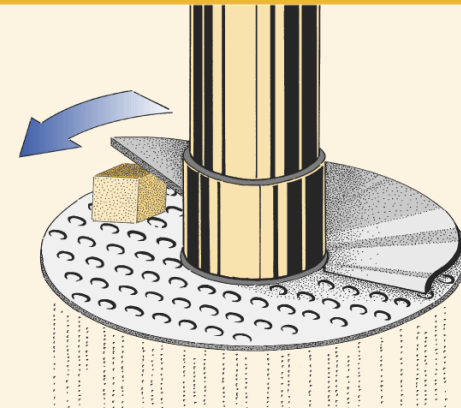


Tissue pieces 1 mm

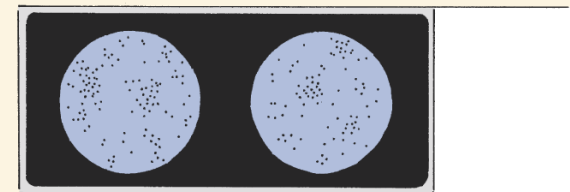


Ulmer et al., Plos Medicine 2014

Mechanical disaggregation



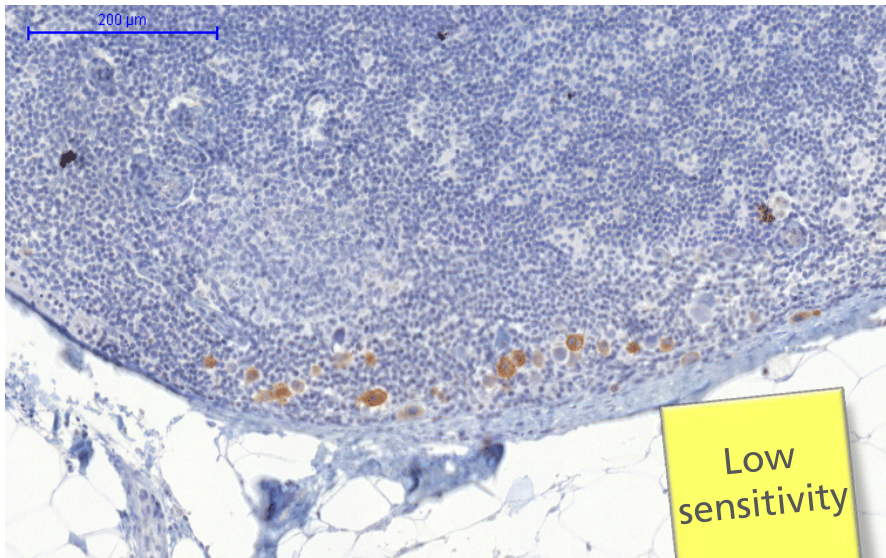
Single cell immunostains



Our approach - a paradigm shift in diagnostics

Single cell analysis from lymph nodes

Histopathology on FFPE tissue



Low
sensitivity

139/1027 patients positive (14%)

Single cell analysis



High
sensitivity

525/1027 patients positive (51%)

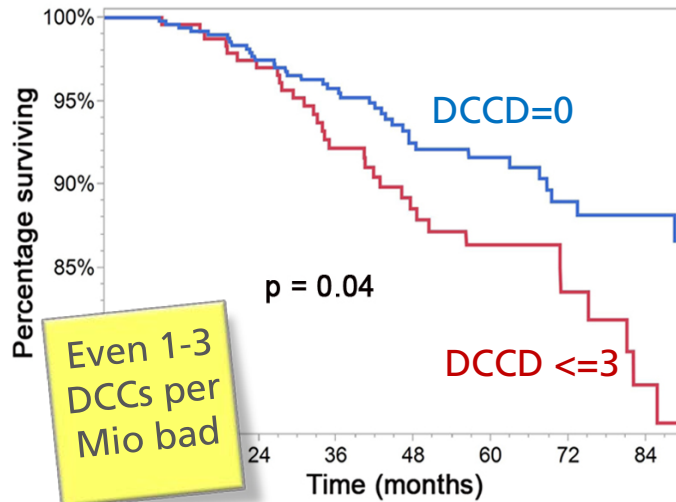
gp100 staining
(Melanocytic marker)
for detecting DCCs

Ulmer et al., Plos Medicine 2014

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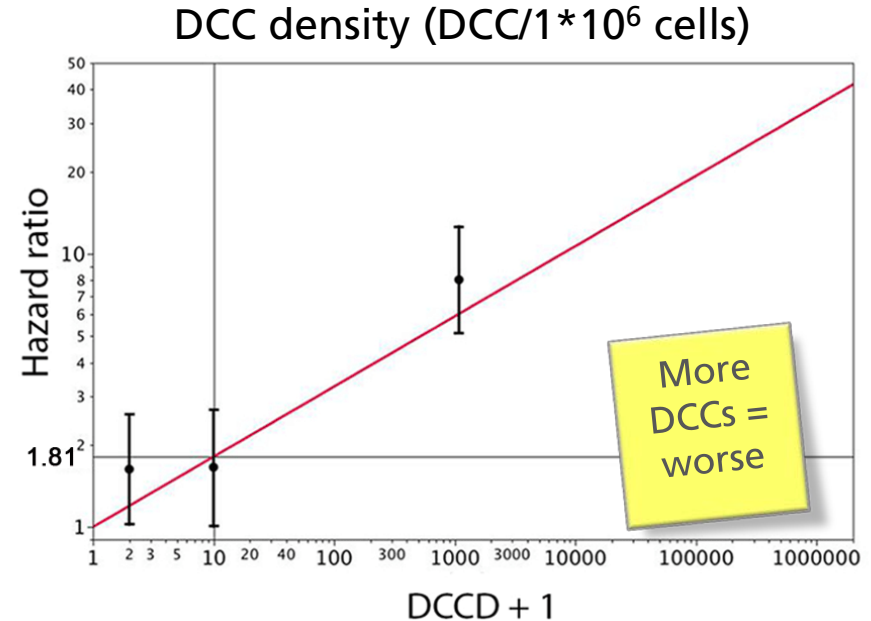
Our approach - a paradigm shift in diagnostics

Single cell analysis from lymph nodes



Number of patients at risk (at the corresponding months)

Risk group	0	30	60	90
--- DCCD=0	502	402	165	55
--- 0<DCCD≤3	249	205	98	27



risk of death doubles with every 10x increase of the amount of DCCs.

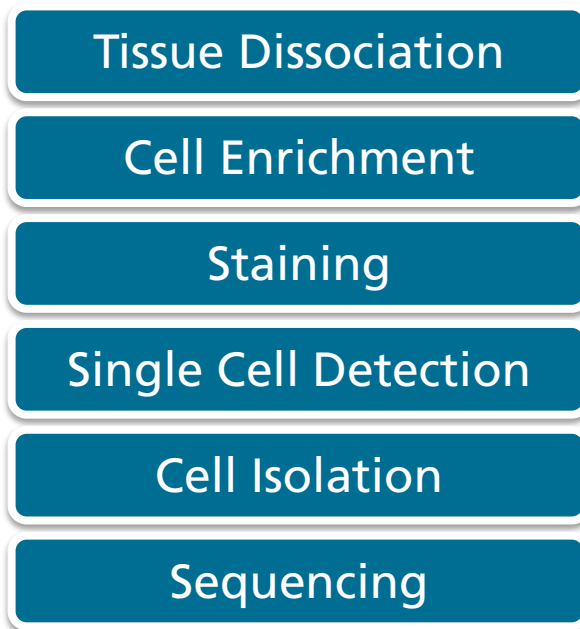
Ulmer et al., Plos Medicine 2014

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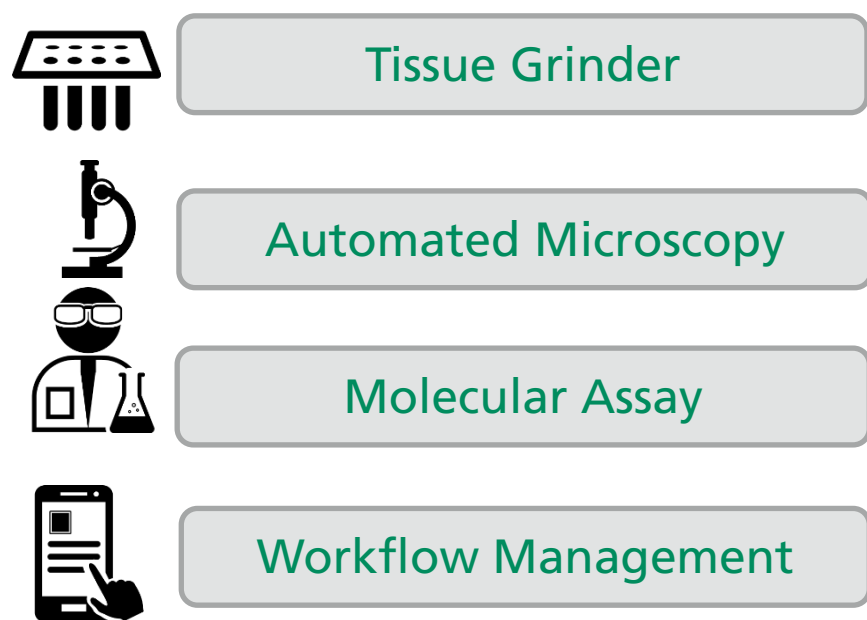
Our approach - a paradigm shift in diagnostics

Towards automating single cell analysis

Workflow



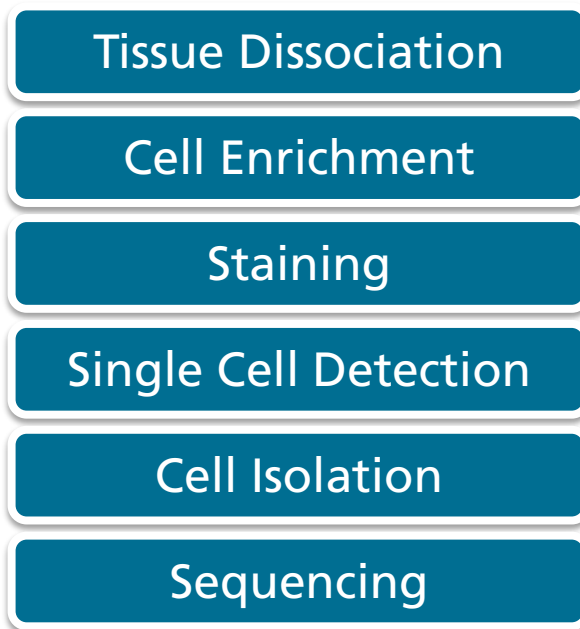
Fraunhofer Innovations



Our approach - a paradigm shift in diagnostics

Towards automating single cell analysis

Workflow



Fraunhofer Innovations



Tissue Grinder



Automated Microscopy



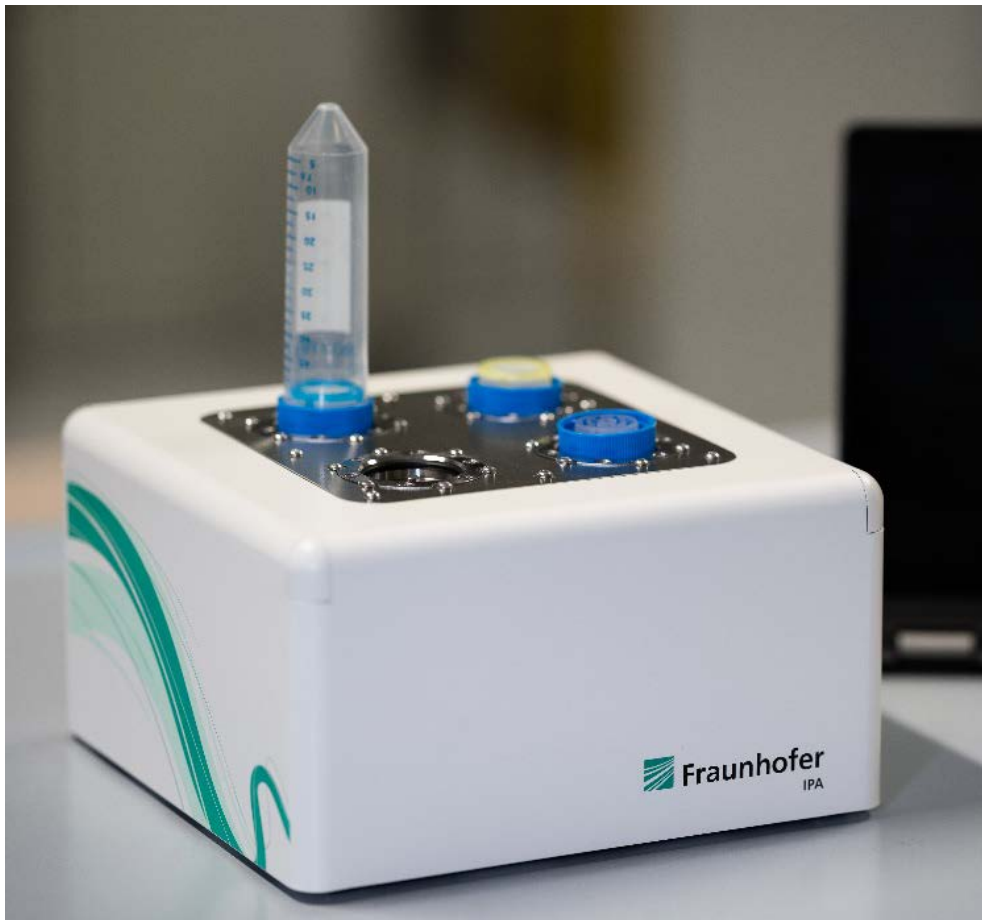
Molecular Assay



Workflow Management

TissueGrinder

Enabling automated, enzyme-free tissue dissociation

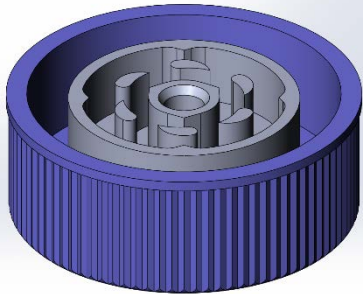
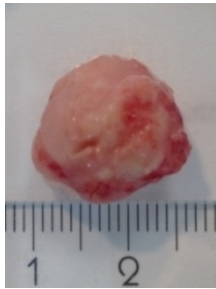


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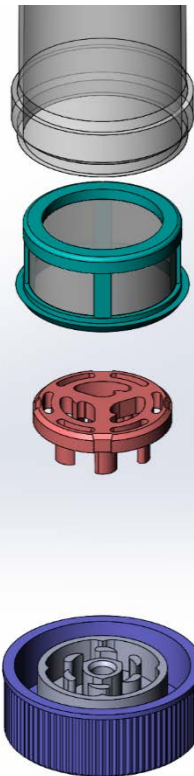
TissueGrinder

Function and Workflow

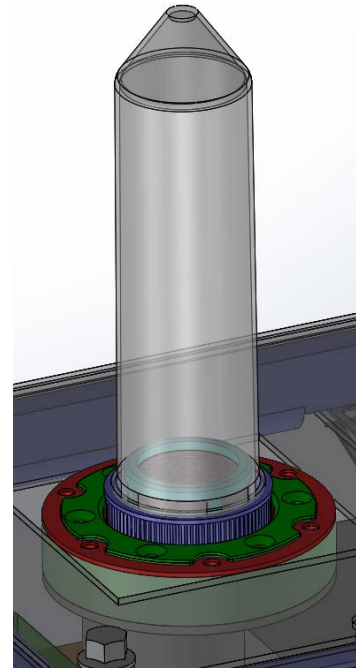
1. Tissue sample loading
in Grinding Tube



2. Assembling of
Grinding Tube



3. Efficient dissociation
by intelligent process
control



4. Subsequent
centrifugation in
closed sterile system



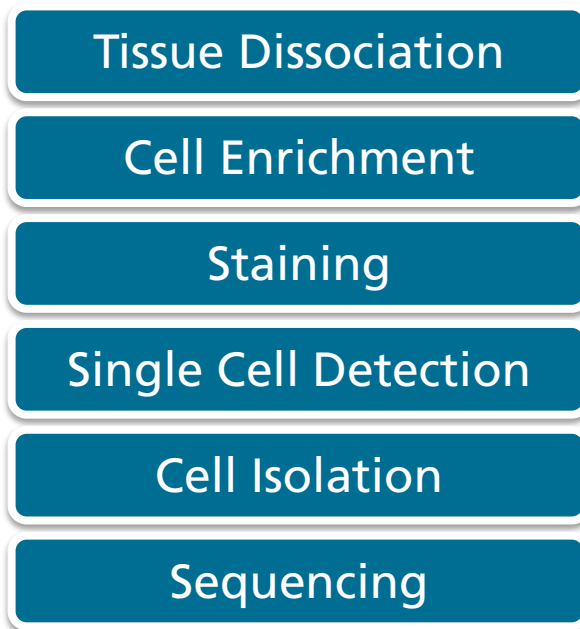
Ready-to-use
single cell
suspension

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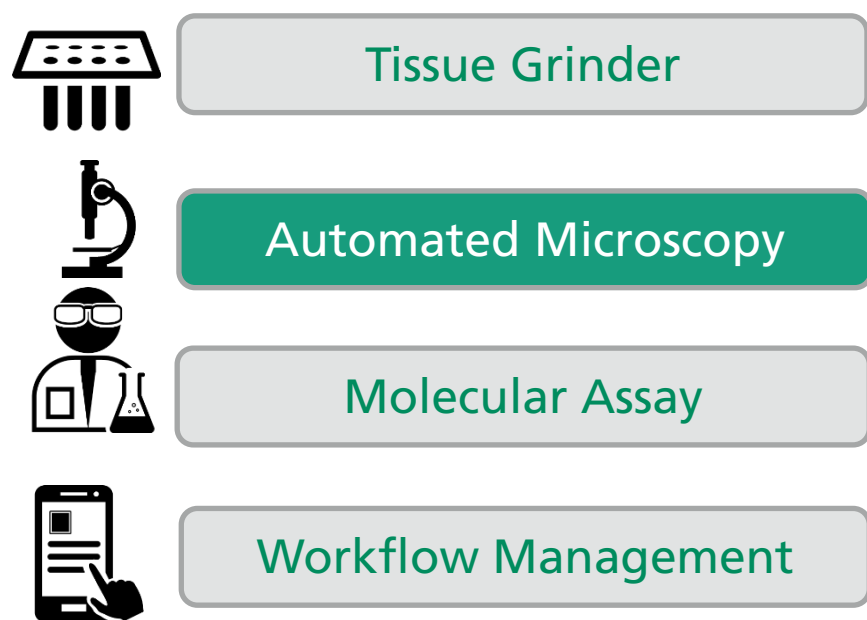
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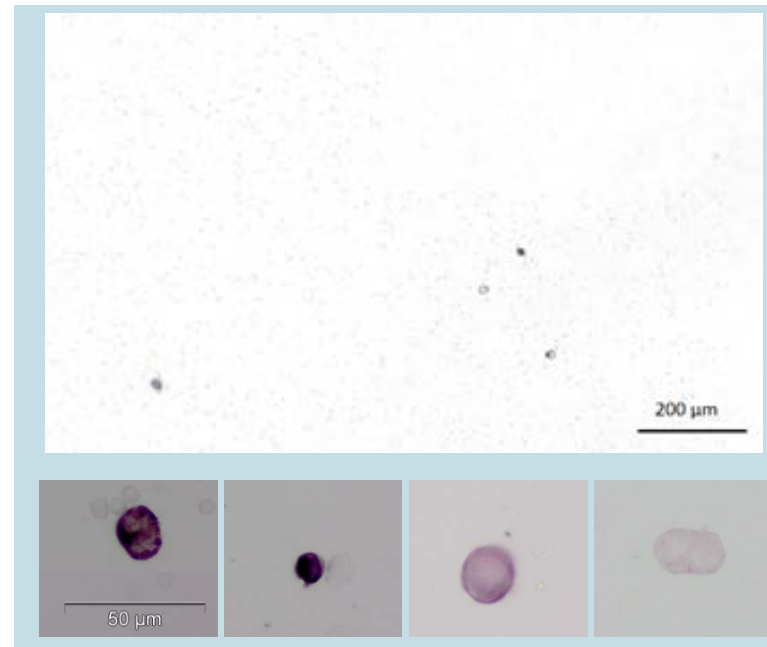
Automated Microscopy

Scanning and analysis

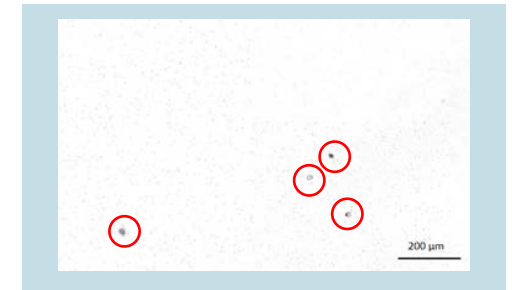
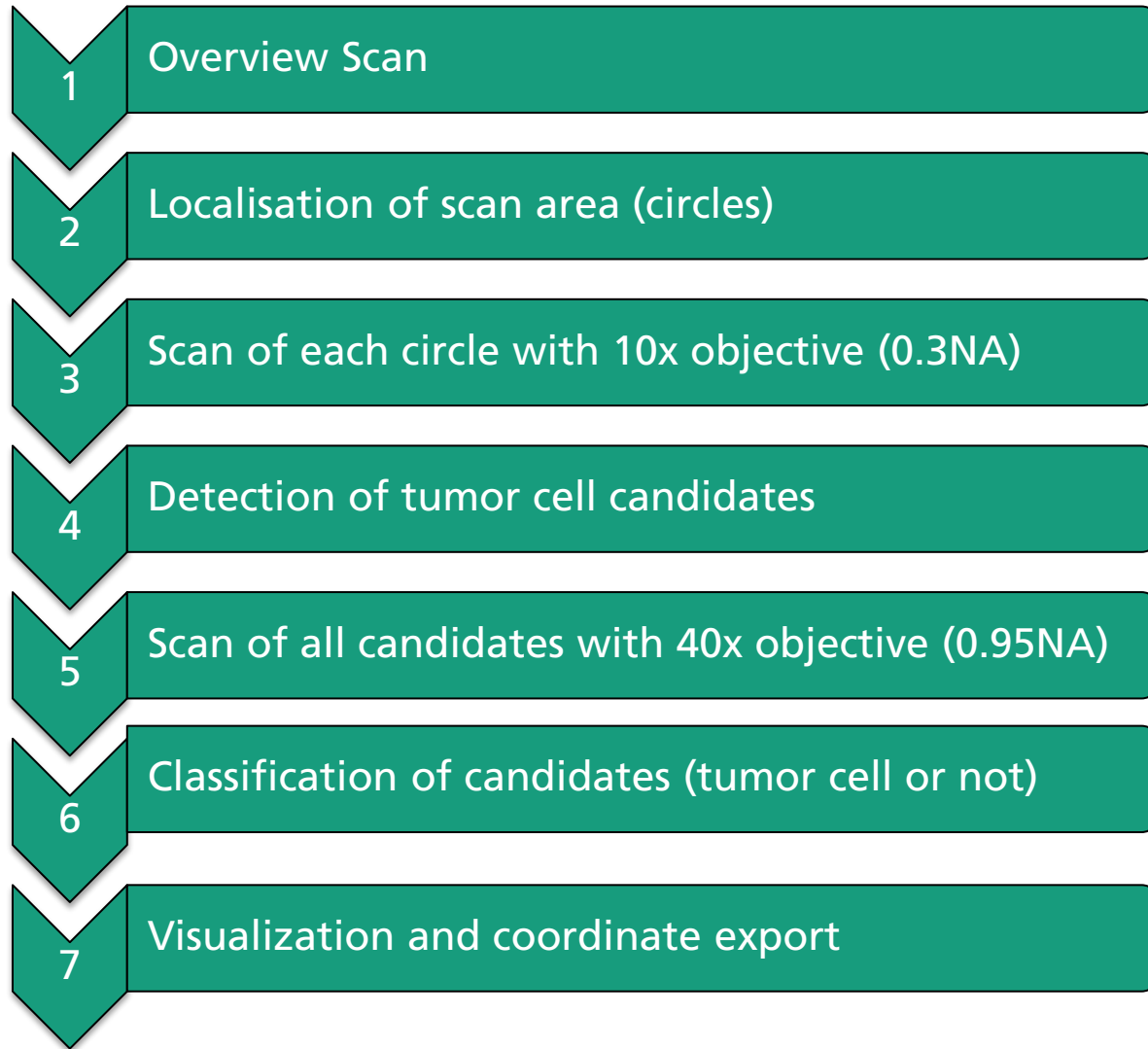
- Automated scan process
- Tumor cell detection and visualization
- Export of tumor cell coordinates



SCube Scanning platform



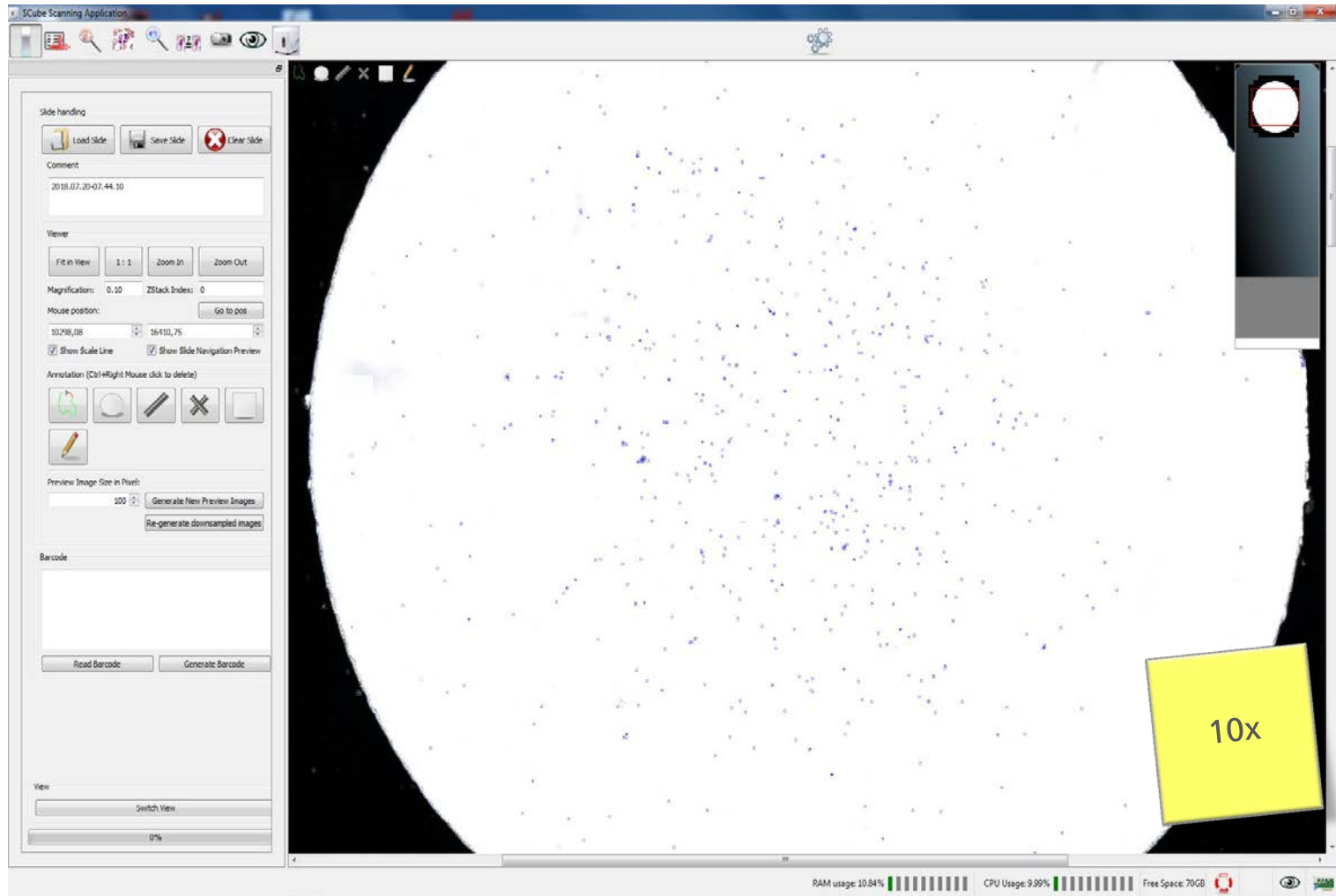
Automated Microscopy Scan process



10.2; 9.1
19.3; 6.5
24.5; 12.6
.....

Automated Microscopy

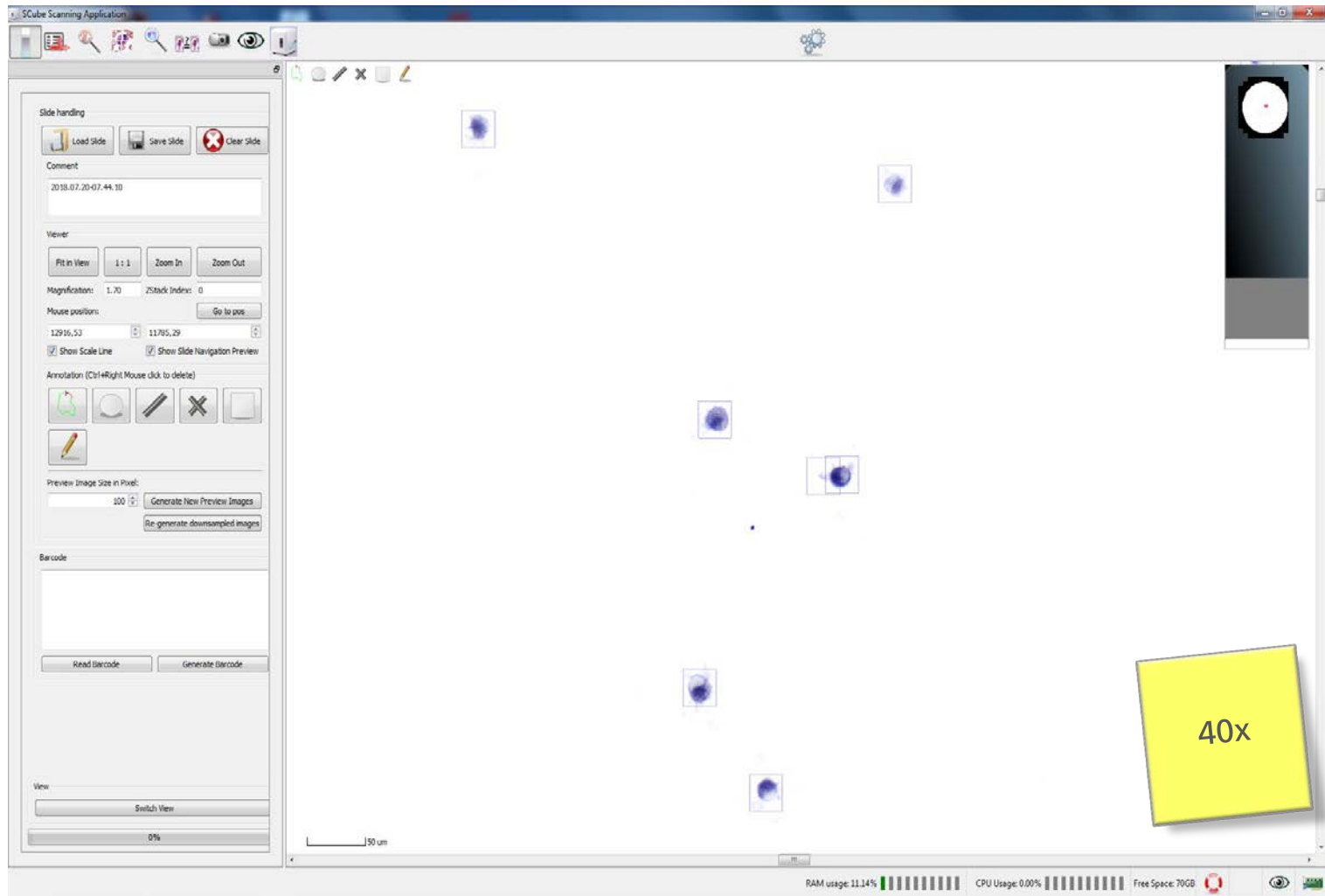
Automated Detection of DCCs in chopped lymph nodes



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Automated Microscopy

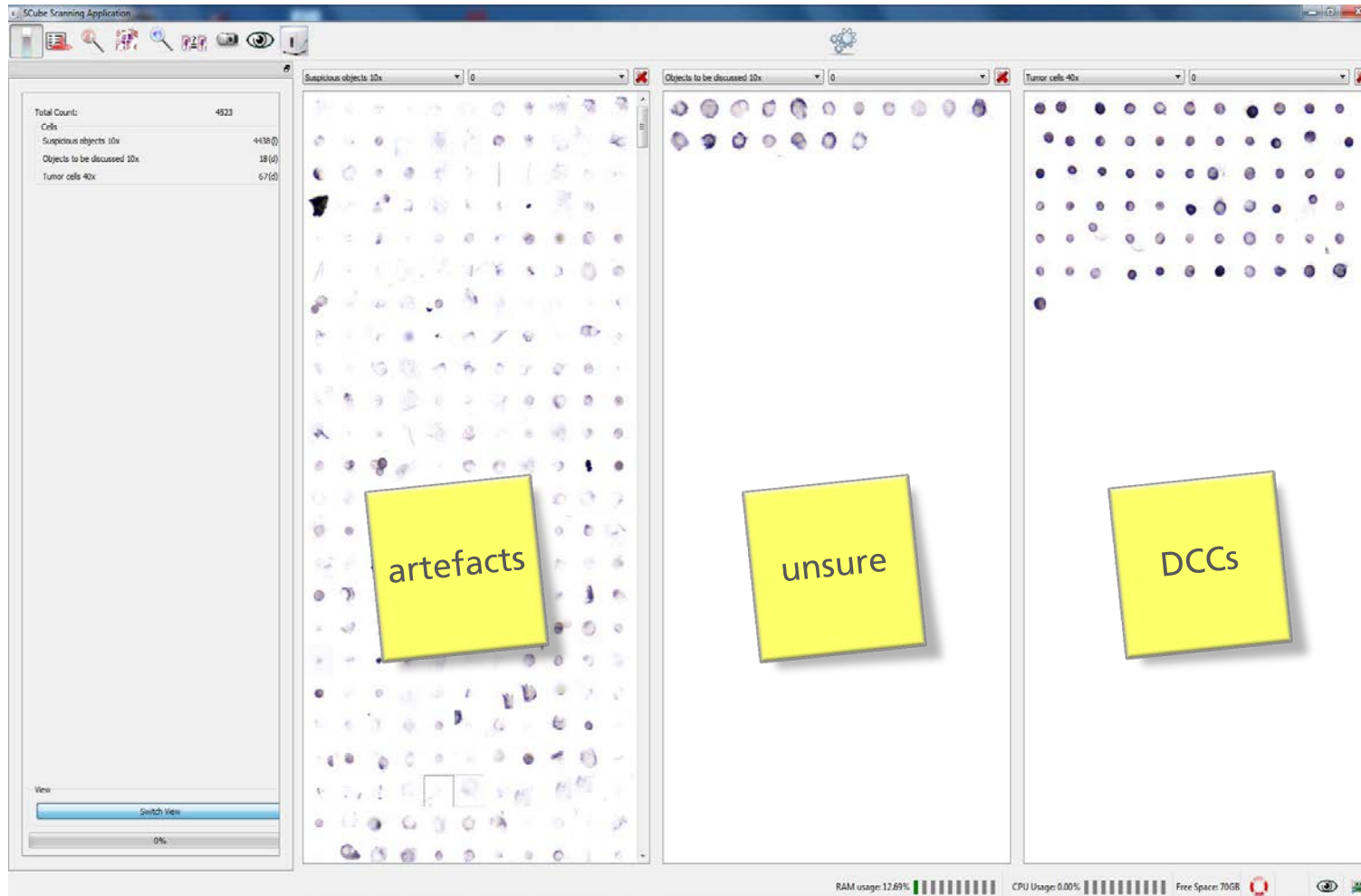
Automated Detection of DCCs in chopped lymph nodes



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Automated Microscopy

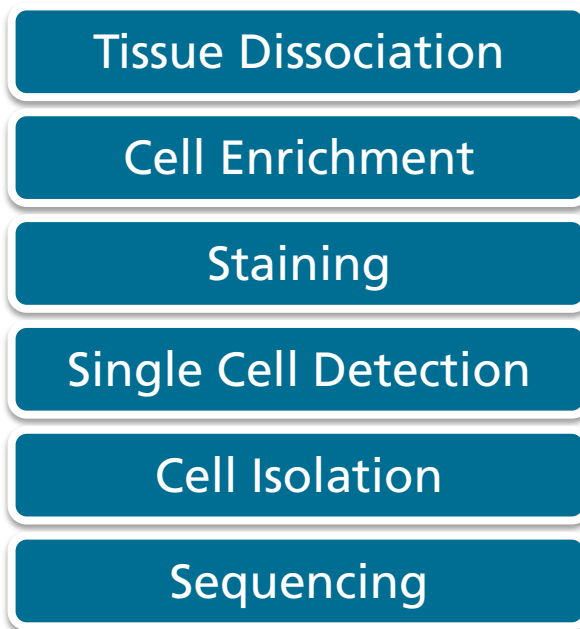
Classification of candidate objects



Our approach - a paradigm shift in diagnostics

Towards automating single cell analysis

Workflow



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Tissue Grinder



Automated Microscopy



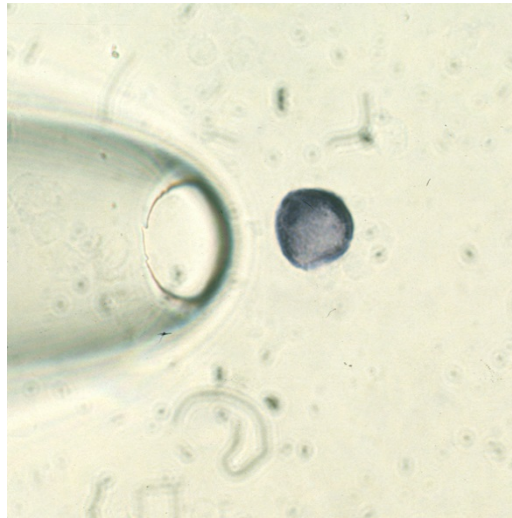
Molecular Assay



Workflow Management

Molecular Assay

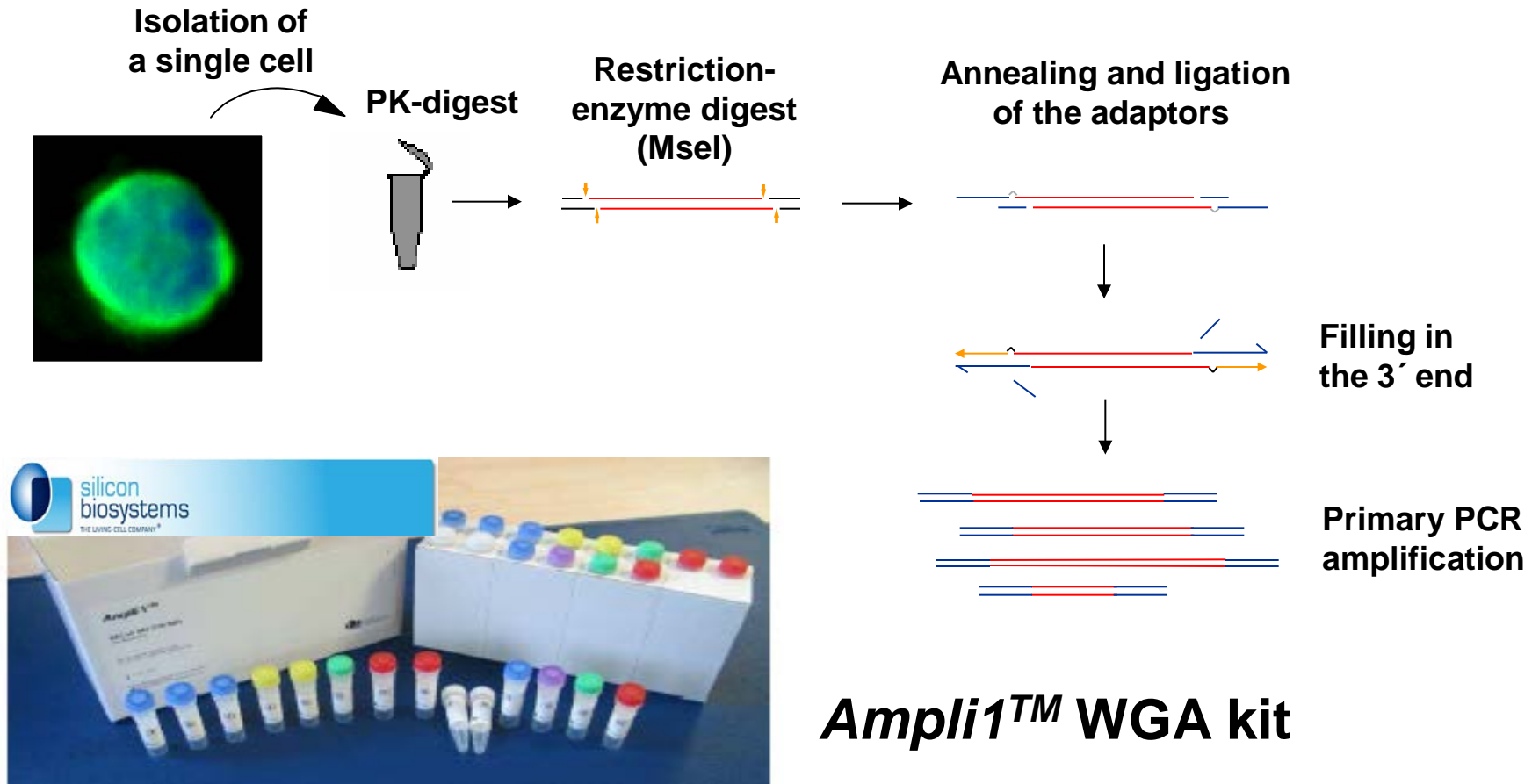
Single cell analysis from lymph nodes



⇒ Molecular single cell analysis identifies somatic mutations and CNVs correlated with lymph node colonization and poor prognosis

Molecular Assay

Deterministic single cell Whole Genome Amplification (WGA)



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Klein et al., PNAS 1999

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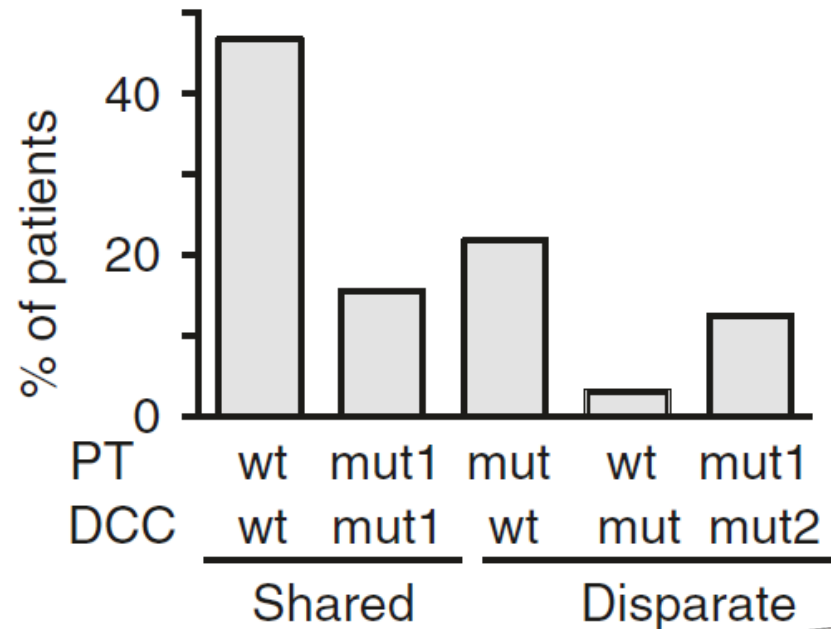
Molecular Assay

Compare primary melanoma to single lymph node cells

Paired samples:

	BRAF		NRAS	
PT	18/53	34%	7/47	15%
DCC	13/86	15%	9/84	11%
<i>p</i> value	0.012		0.580	

BRAF
melanoma
mutation
detected
also in DCCs



Mutations
often not
shared
between PT
and DCC

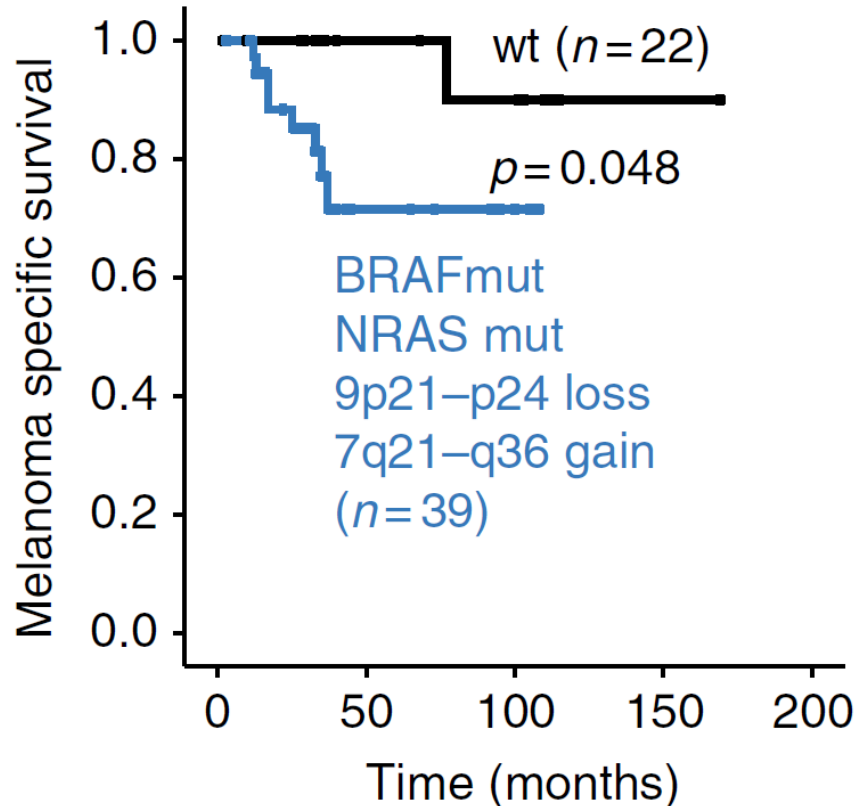
- ⇒ Single cells in lymph node are genetically distinct from primary melanomas
- ⇒ Dissemination occurred before fixation of mutation in PT
- ⇒ early DCCs are genetically immature and acquire critical alterations after homing to a distant site and thereby gain the ability to form a colony

Werner-Klein et al., Nature Communications 2018

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Molecular Assay

Identification of a molecular colonization signature



- ⇒ Presence of at least one of these mutations increases risk of death
- ⇒ Molecular single cell analysis can identify patients with risk of systemic progression

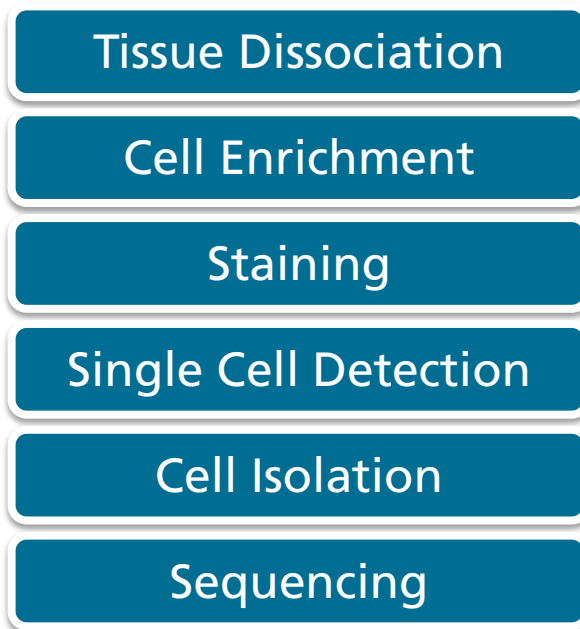
Werner-Klein et al., Nature Communications 2018

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Our approach - a paradigm shift in diagnostics

Towards automating single cell analysis

Workflow



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Tissue Grinder



Automated Microscopy



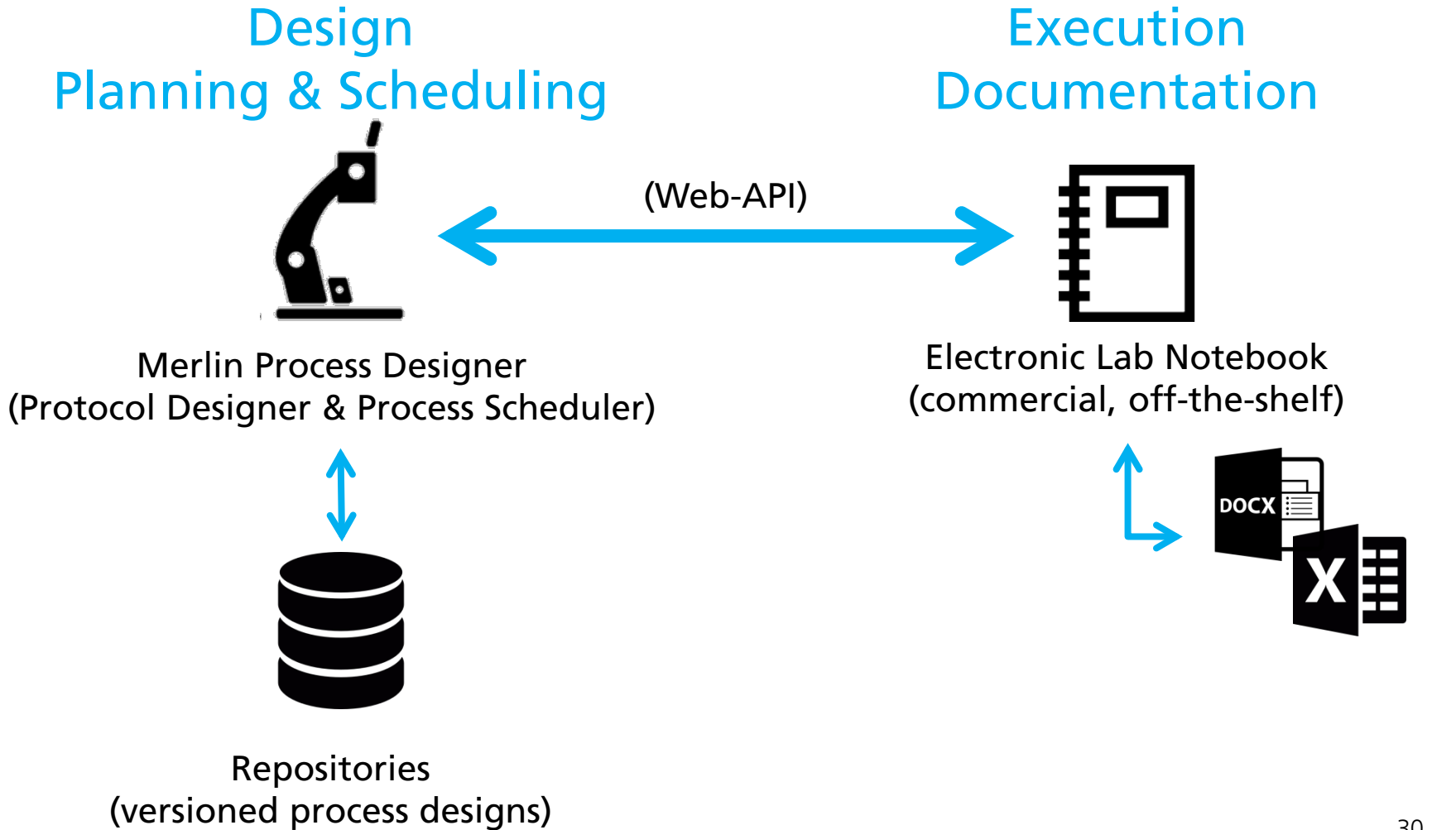
Molecular Assay



Workflow Management

Workflow Management (Merlin)

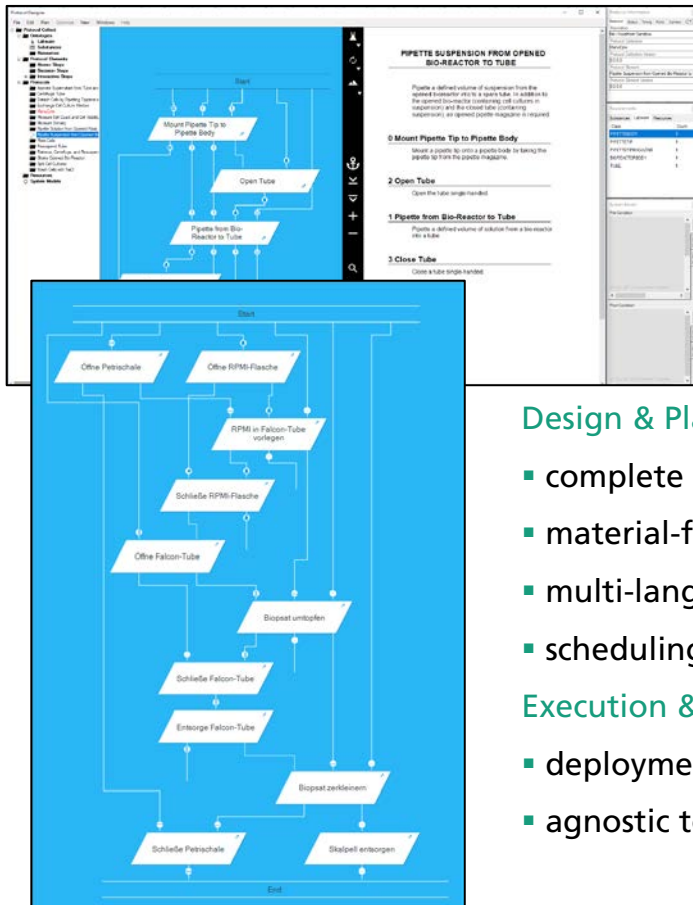
Software Interaction Concept



Workflow Management (Merlin)

Fact Sheet

Merlin Process Designer



**Export
Workflows
Experiments**

Electronic Laboratory Notebook
(e.g., R-Space, sciNote, or others!)



Design & Planning

- complete and comprehensive representation of lab workflows
- material-flow-based process modeling notation
- multi-language and versioning support
- scheduling of experiments

Execution & Documentation

- deployment of robust, established electronic laboratory notebooks
- agnostic to specific products and vendors *)

*) as long as they support modern web-based APIs and a sufficient set of capabilities

Summary and Conclusion

From sections to single cell diagnostics

We work on a new paradigm for lymph node diagnostics

Benefits

- ✓ Higher sensitivity compared to conventional sections diagnostic
- ✓ Modular approach for highest flexibility
- ✓ Improved stratification
- ✓ Reduced cost

Fraunhofer Innovations



Tissue Grinder



Automated Microscopy



Molecular Assay



Workflow Management