Enabling Biomarker Testing in Clinical Trials

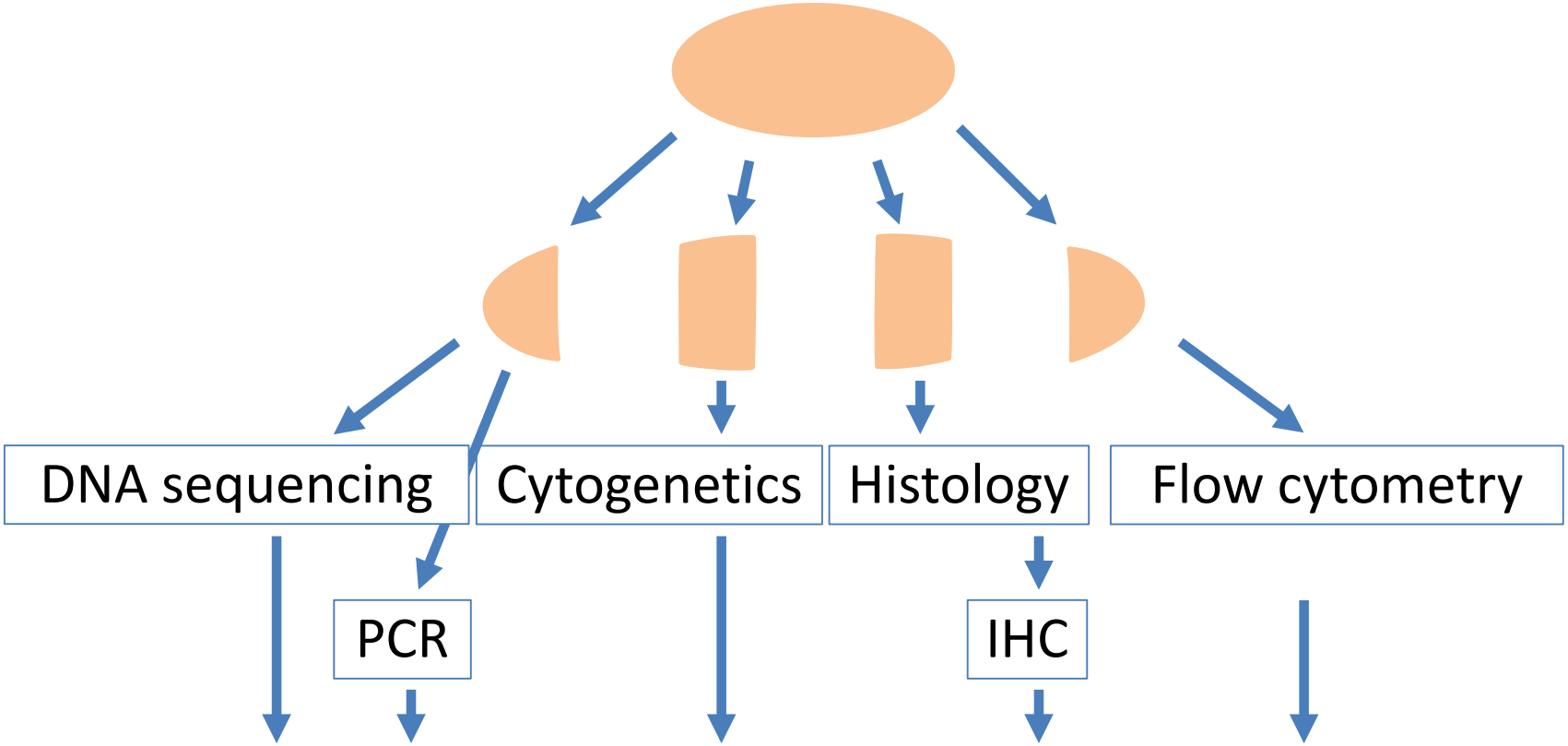
Lois Shepherd & David LeBrun

Tumour Tissue Data Repository (TTDR) &
Queen's Laboratory for Molecular Pathology (QLMP)

Canadian Cancer Trials Group &
Queen's Department of Pathology and Molecular Medicine
Kingston, Ontario

Provenance of archived samples

Tissue allocation



Integrated report synthesizes primary data and results from various assays into a single, concise, integrated report that provides comprehensible, clinically-actionable information

Formalin-fixed, paraffin-embedded (FFPE) tissue “blocks”

Macroscopic examination and “blocking”

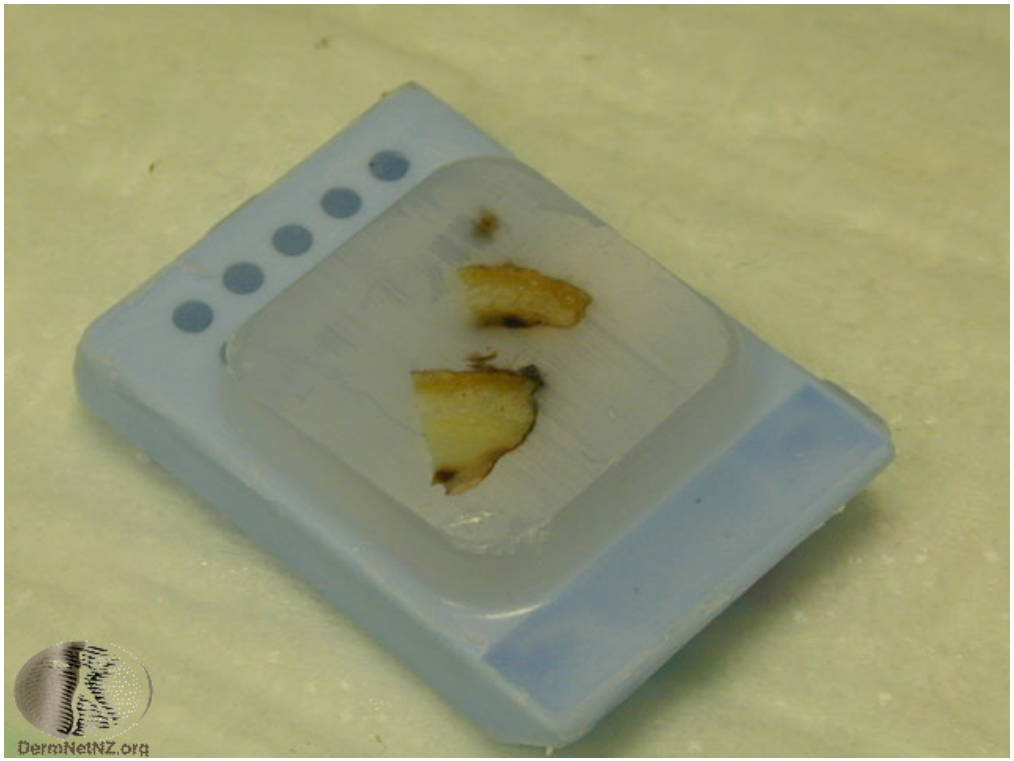
Tissue processing

Embedding in paraffin

Histological sectioning

Staining and coverslipping

Formalin-fixed, paraffin-embedded (FFPE) tissue “blocks”

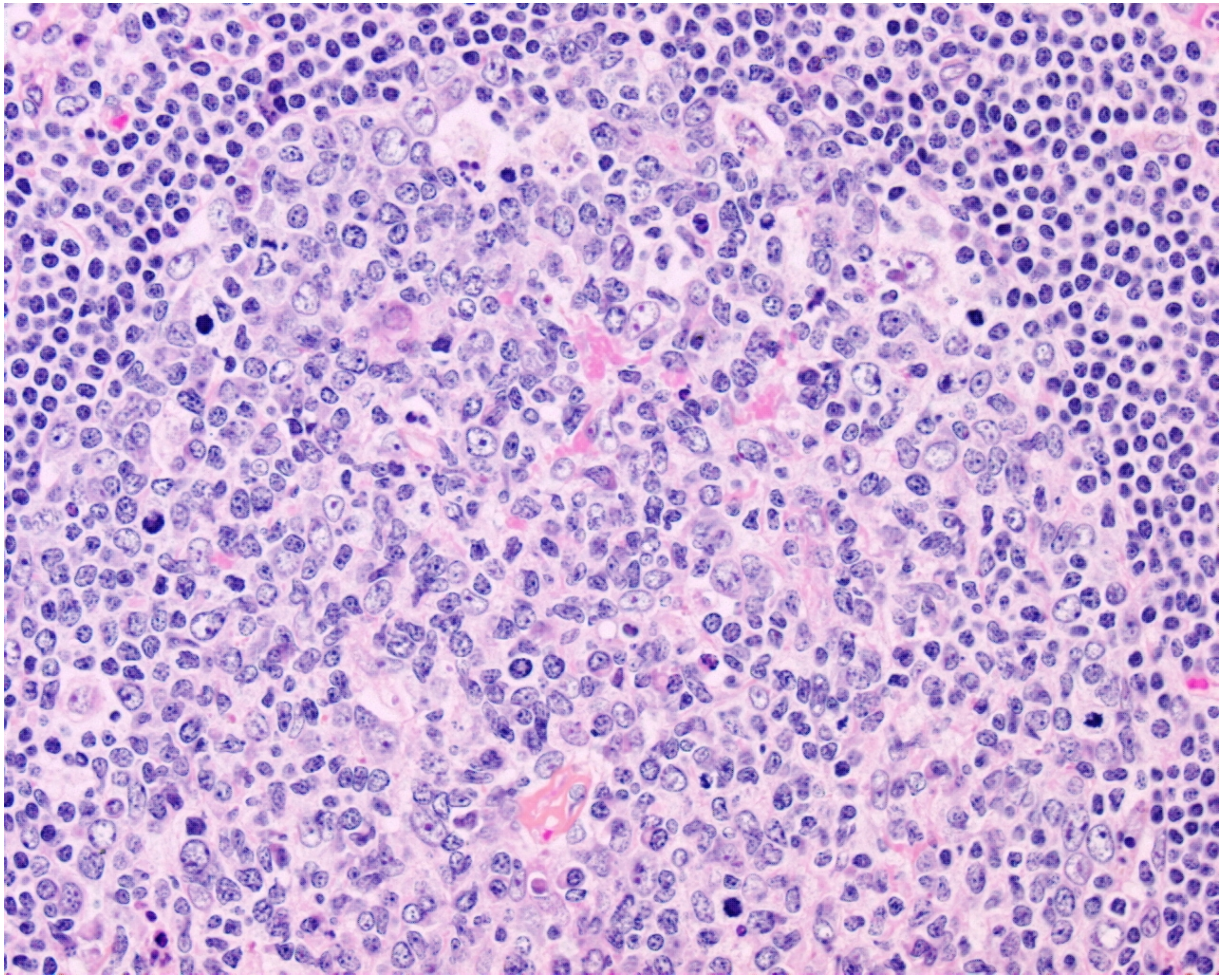


Archived tissue blocks

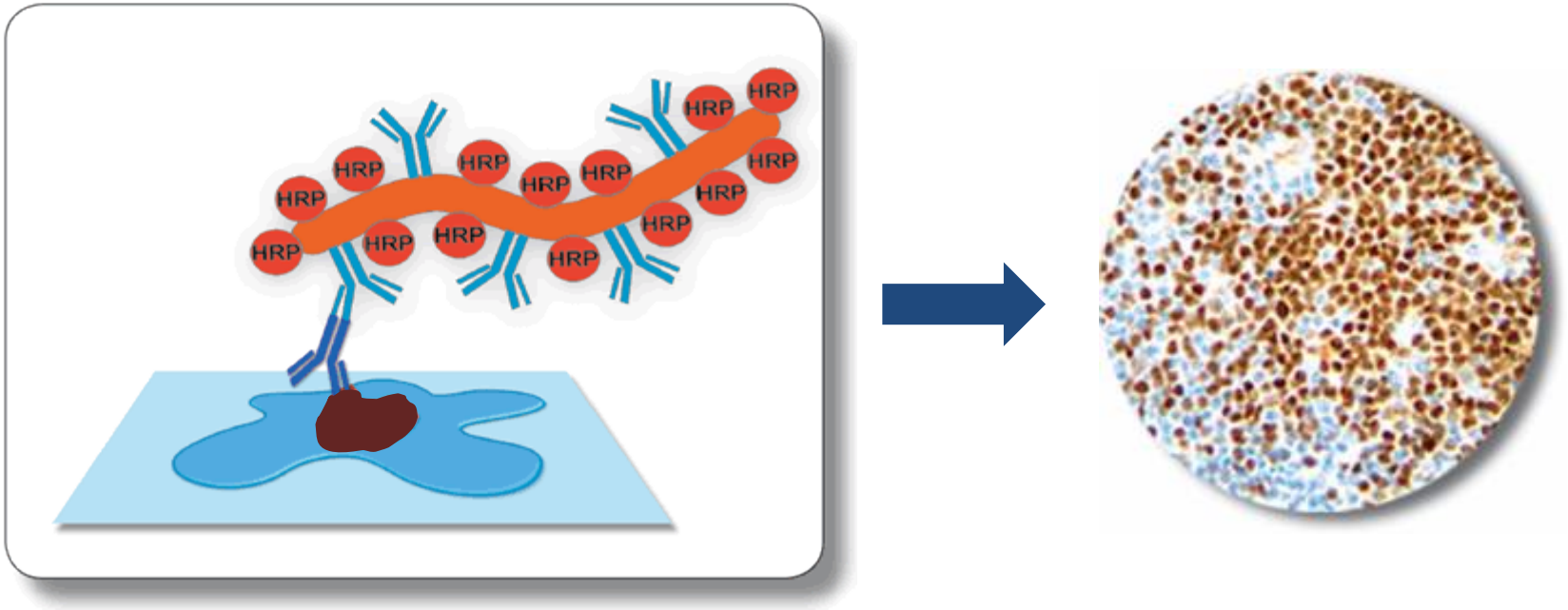
Uses of human tissue samples

- Tissue morphology
- Protein
- DNA
- RNA

Morphology is still the most important consideration in routine pathological diagnosis

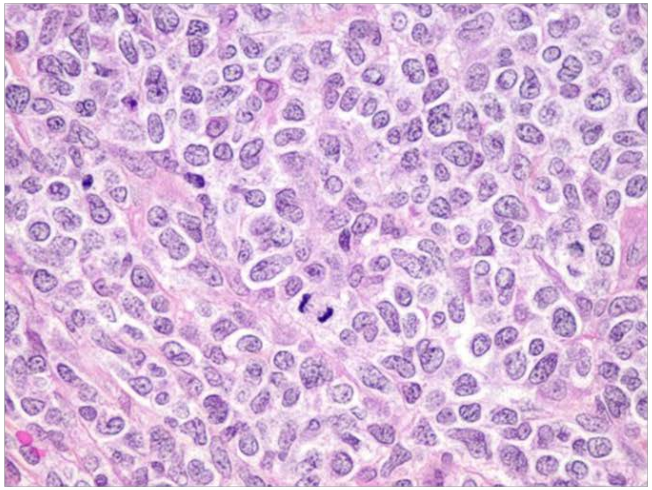


Immunohistochemistry (IHC)

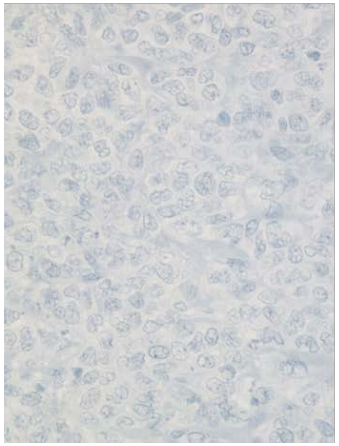


Alex Klimowicz

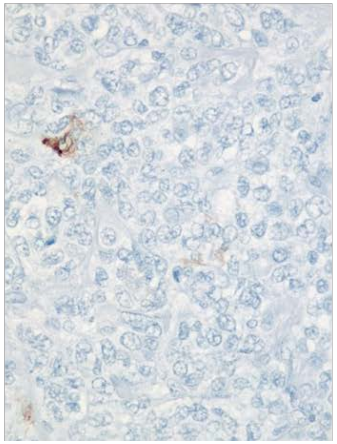
Immunohistochemistry (IHC)



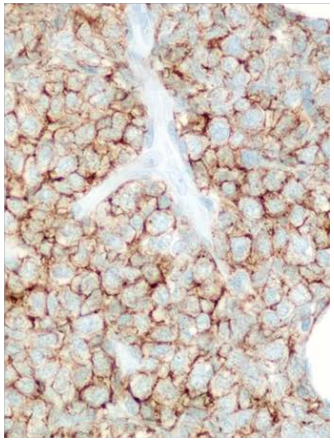
Keratin



S-100



CD45



Immunohistology at KGH

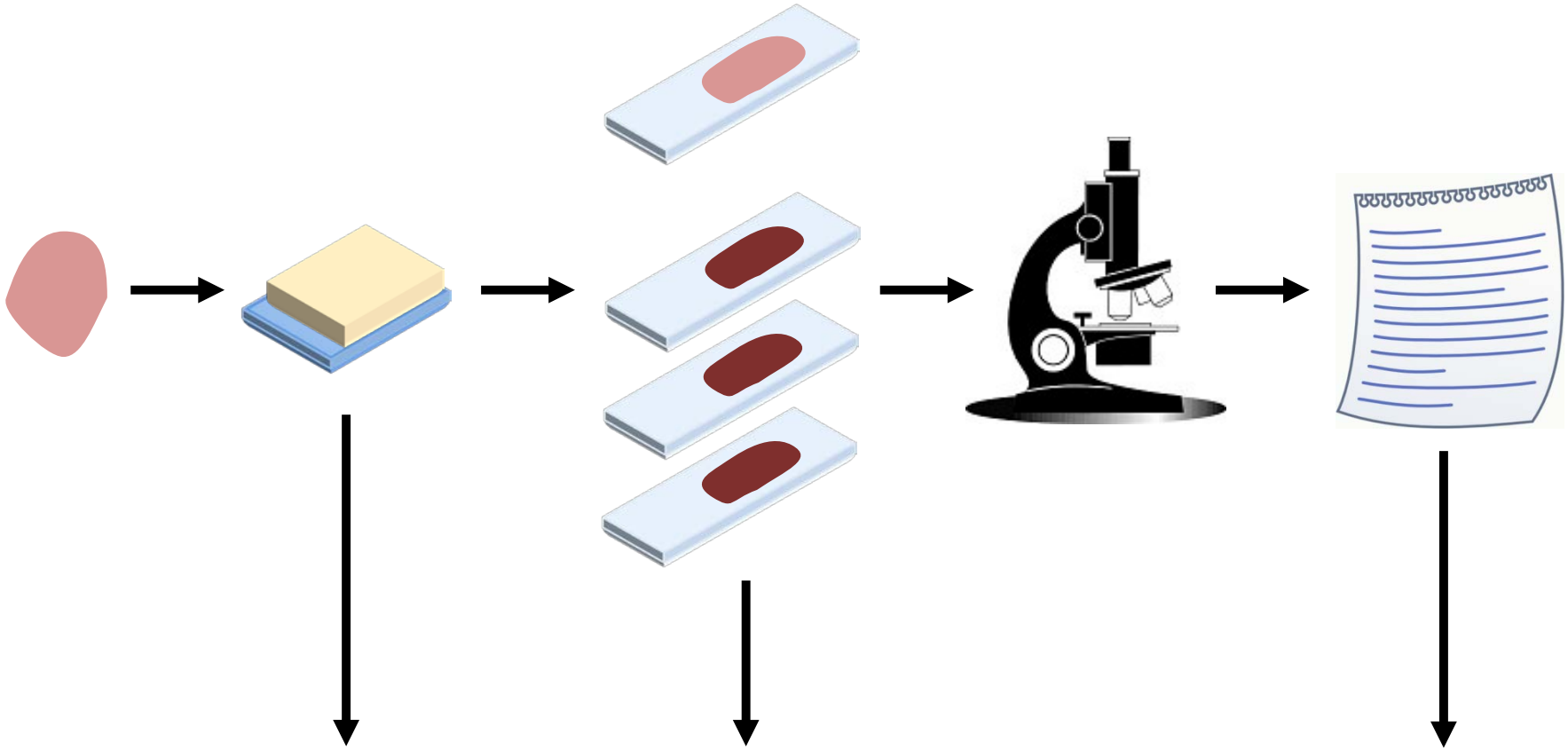
KGH Histo Lab

MARKER	CONTROL	MARKER	CONTROL	MARKER	CONTROL	MARKER	CONTROL
AAT	121	CD79a	CON 3	HEPPAR1	CON 1	PIN 4	400 Chromoplex
abCRY	235	CD99	CON 3	HER2	SISH / 179	PIT1	PIT CON
ACP [redacted]	400	CD117	CON 3	HMB45 [redacted]	145	PLAP	122
ACT	CON 3	CD138	CON 3	HPL	158	PMS2	CON 4
ACTH	PIT CON	CDX2	129	HSV1	131	PROL	PIT CON
AFP	121	CEA	CON 1	HSV2	131	PSA [redacted]	400
ALK	380	CHROM	CON 2	IGA	CON 3	SACROGLYCAN	Normal muscle
AMACR [redacted]	400	CK5	CON 1	IGE	CON 3	S100	CON 1
AMY A		CK7	CON 1	IGG	CON 3	S100 [redacted]	CON 1
APP	250	CK8/18	CON 1	IGM	CON 3	SMMS	CON 1
aSU	PIT CON	CK19	CON 1	IDH1	280	SMA	CON 1
aSYN	205	CK20	CON 1	INHIBIN	120	SOMATO	
bAMY	250	CK-PAN	CON 1	INSULIN	CON 2	SV40T	396
BCL2	CON 3	CMV	Commercial	KAPPA	CON 3	SYN	CON 2
BCL6	CON 3	c-MYC	CON 6	KAPPA-BM	330 decal	TAU	CON 2
Beta-Catenin	CON 1	CYCLIN D1	CON 3	KI67	CON 3	TDP43	240
C5B-9		DES	CON 1	LAMBDA	CON 3		
CALC	108	DOG1	CON 5	LAMBDA-BM	330 decal	TDT	370
CALR	CON 1	DYSFERLIN	Normal muscle	LH	PIT CON	THYRO	106
CAM5.2	CON 1	DYSTROGLYCAN	Normal muscle	MAMA	SISH / 179	TIA-1	144
CAVEOLIN	Normal muscle	DYS1	Normal muscle	MART [redacted]	145	TTF1	106
CD2	CON 3	DYS2	Normal muscle	MBP	240	TTR	102
CD3	CON 3	EBV	103	MCT	CON 1	UBIQ	CON 2
CD4	CON 3	ECAD	CON 1	MEROSIN	Normal muscle	VIM	CON 1
CD5	CON 3	EMA	CON 1	MITF	145	WILMS	150
CD8	CON 3	EMERIN	Normal muscle	MLH1	CON 4	VWF	CON 3
CD10	CON 3	ER	175	MPO	CON 1		
CD11c	390	ER brain		MSA	CON 1		
CD15	CON 3	EBER 3 slides	103	MSH2	CON 4		
CD20	CON 3	FACTOR 13a	104	MSH6	CON 4		
CD21	CON 3	FIBRIN	CON 3	MUR	CON 3		
CD23	CON 3	FRACTIN	202	Myogenin	125		
CD25	CON 3	FSH	PIT CON	Myoglobin	152		
CD30	CON 3	GASTRIN	147	NAPSIN A	106		
CD31	CON 3	GATA-3	SISH/179	NF	CON 2		
CD33	CON 3	GCDFP	177	NSE	CON 2		
CD34	CON 3	GFAP	CON 2	P16	110		
CD43	CON 3	GH	PIT CON	P40	CON 106		
CD45	CON 3	GLUC	CON 2	P53	153		
CD56	CON 3	GpA	CON 3	P57	157		
CD61	CON 3	HBcAg	51	P63	CON 1		
CD68	CON 3	HBsAg	51	PAX5	CON 3		
		HCG	122	PAX8	CON 3		
				PgR	175		

Optimizing and validating IHC

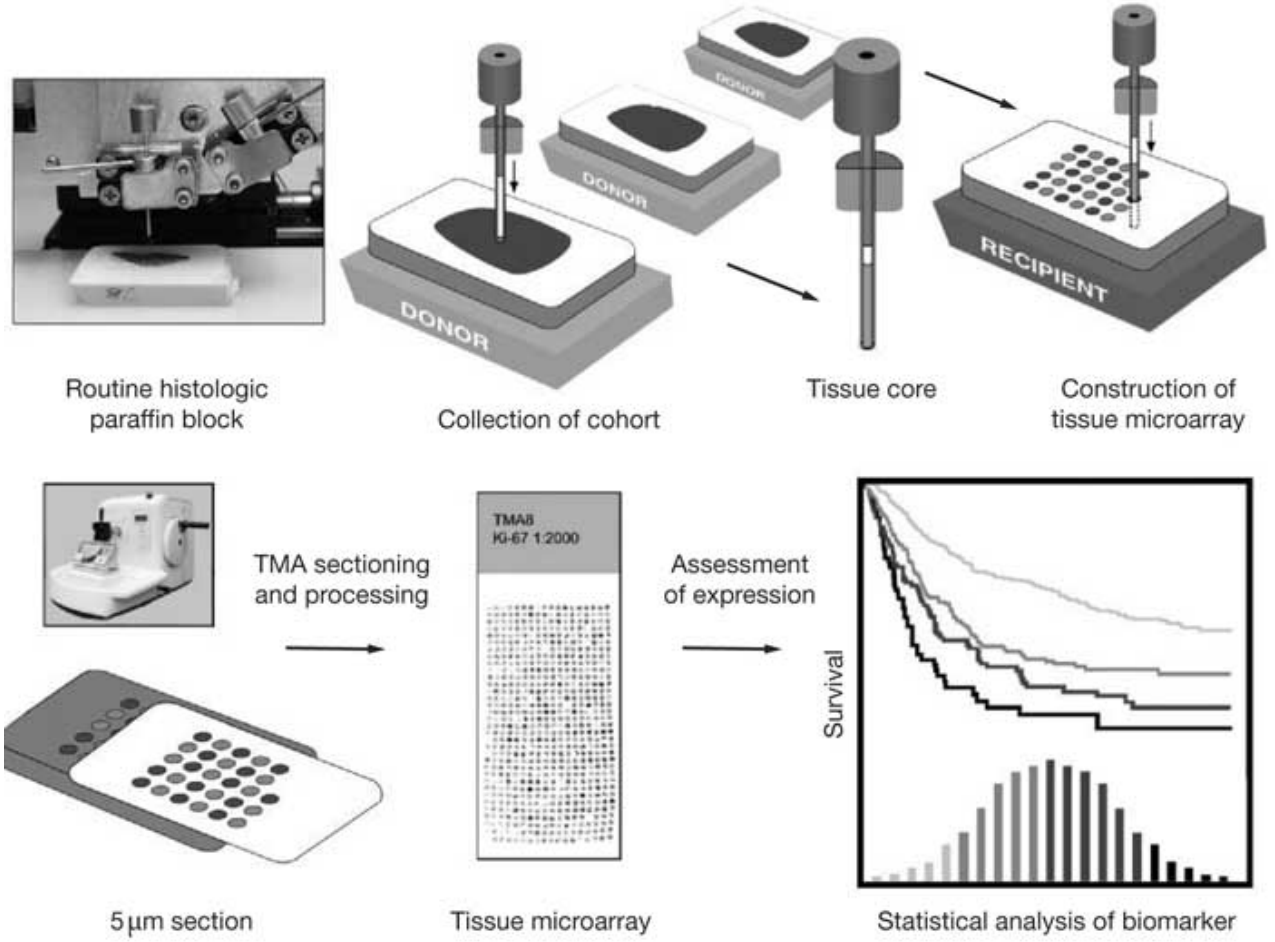
- Identify control tissues or cell lines
- Incorporate control tissues into a small TMA
- Choose an antibody
- Optimize antigen retrieval and IHC protocol
- Validate the optimized IHC assay

Diagnostic histopathology workflow

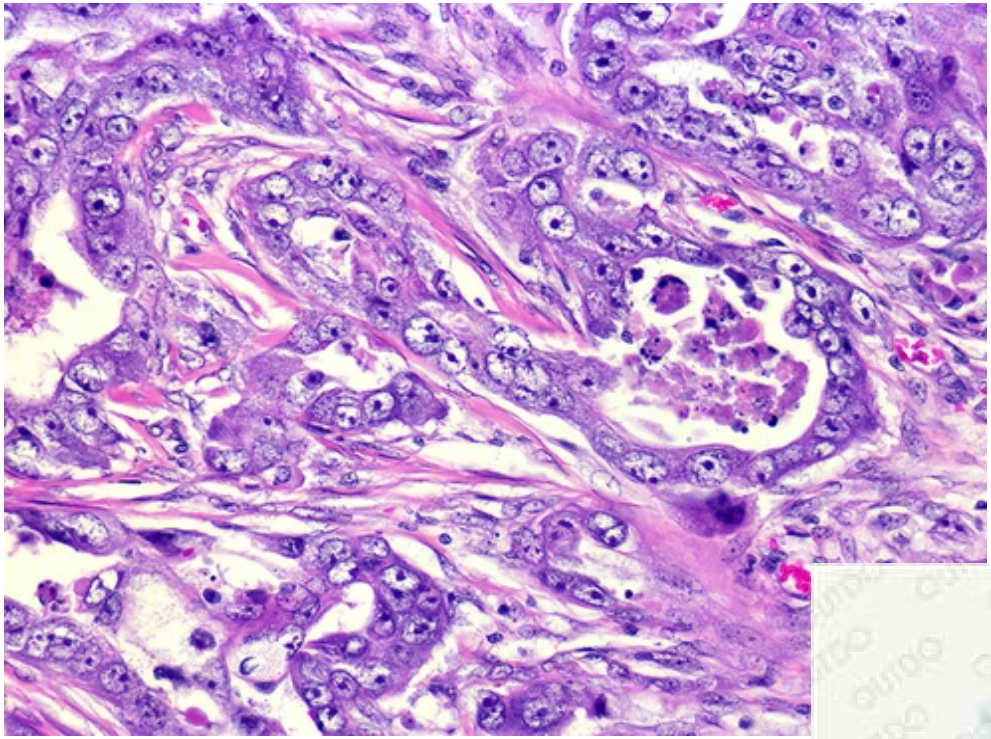


Archived and potentially available for retrieval

Tissue microarrays



Nature Clinical Practice Oncology (2004) **1**, 104-111



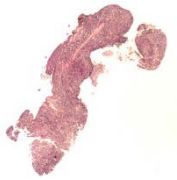
Tissue Microarray

TMA facilitates hundreds
of samples to be analysed
at one time

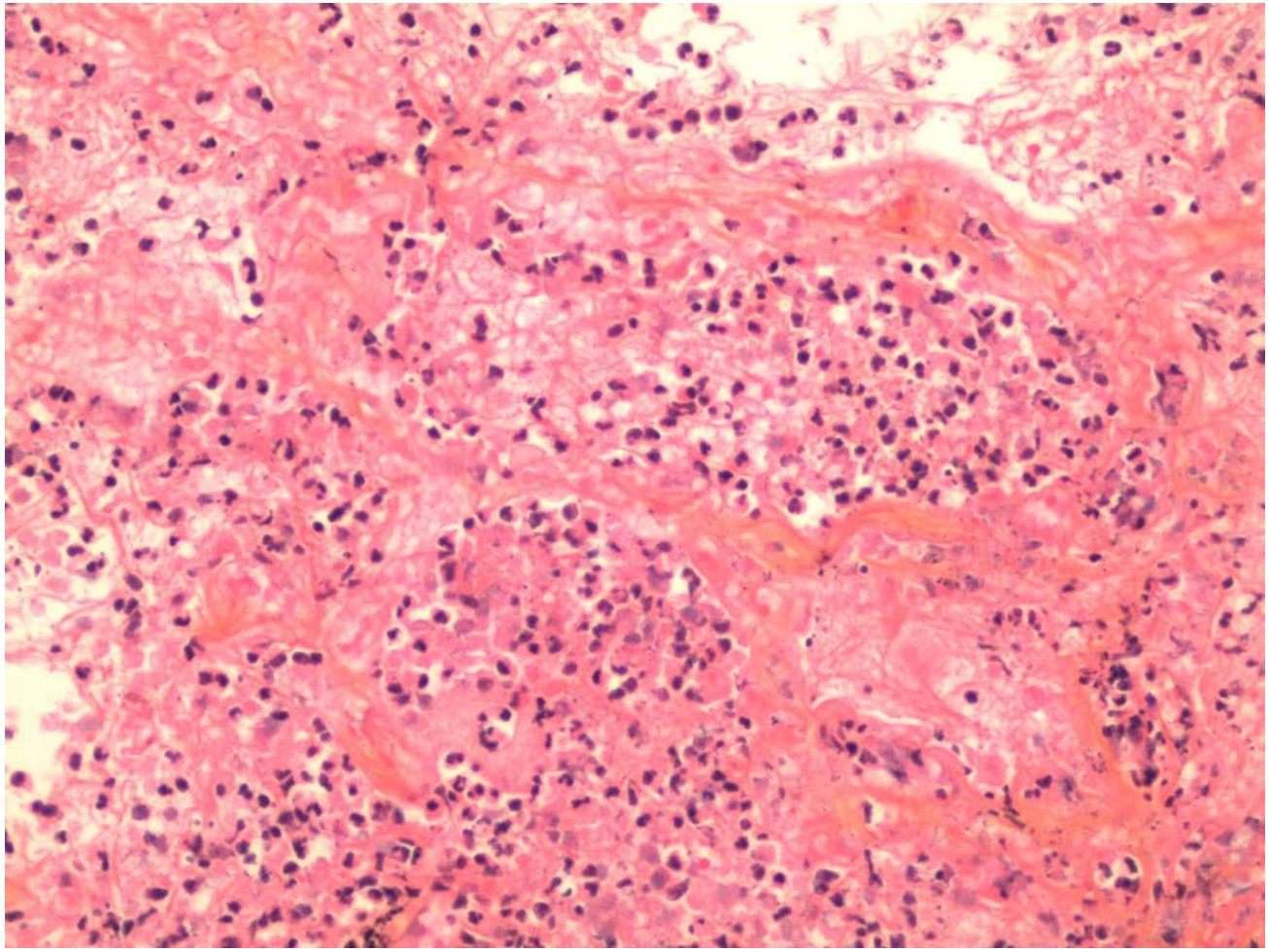
Steps in TMA construction

1. Retrieve all histology slides
2. Pathologist reviews slides in order to:
   - Confirm diagnosis and determine sample size
   - Choose best block
   - Circle representative area for subsequent tissue harvest
3. Retrieve paraffin blocks corresponding to marked slides
4. Transfer circle from slide to block
5. Harvest tissue cores and transfer to recipient block

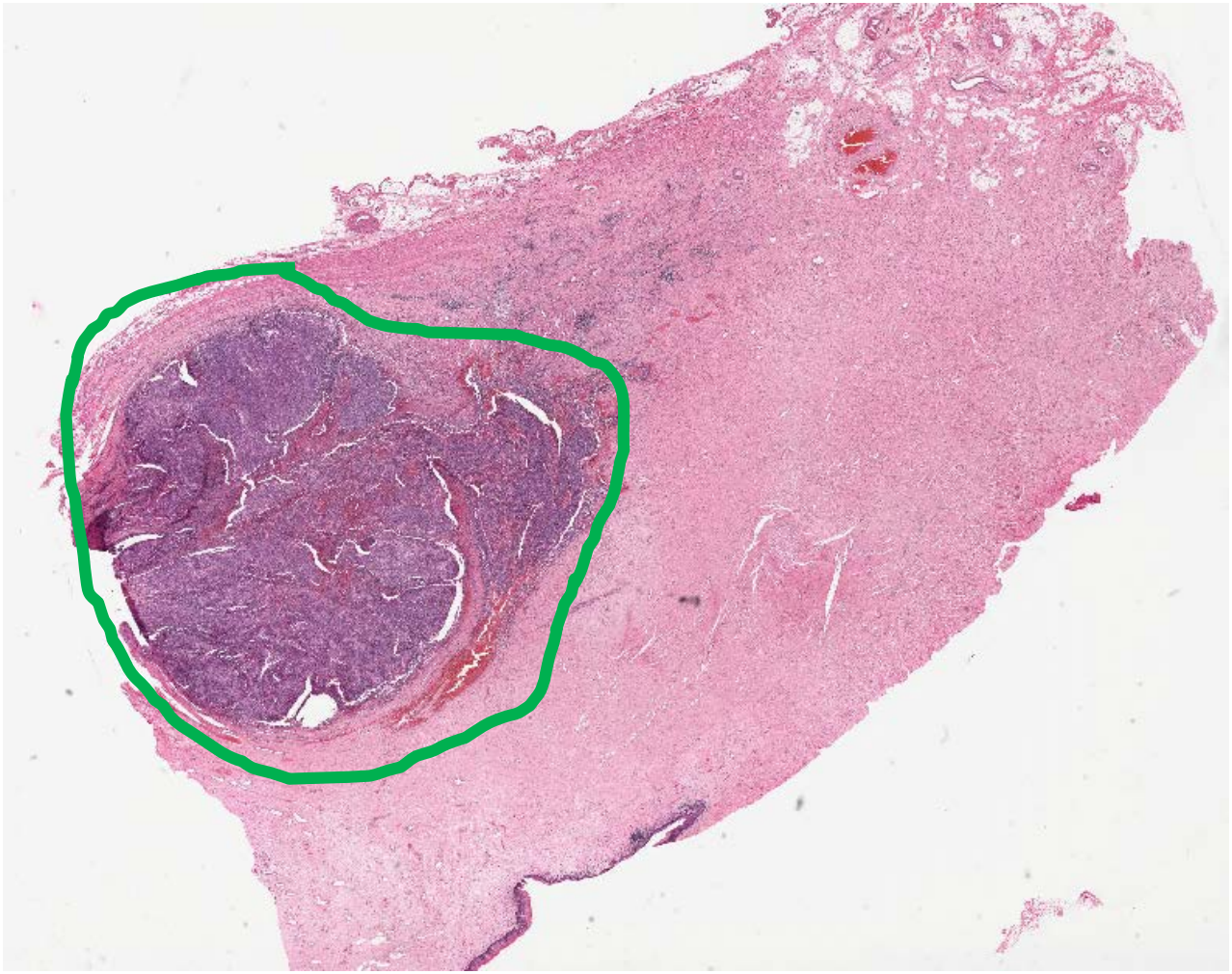
Small sample



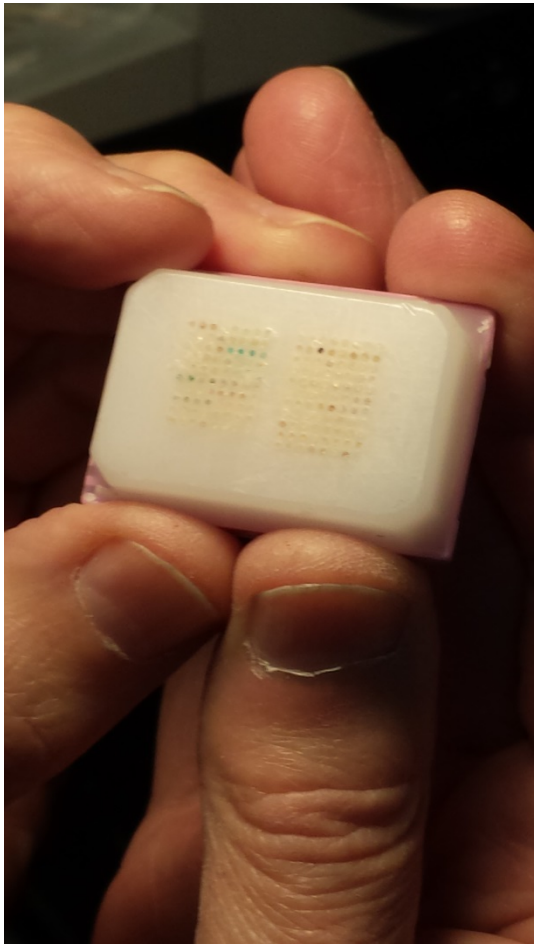
Necrotic sample



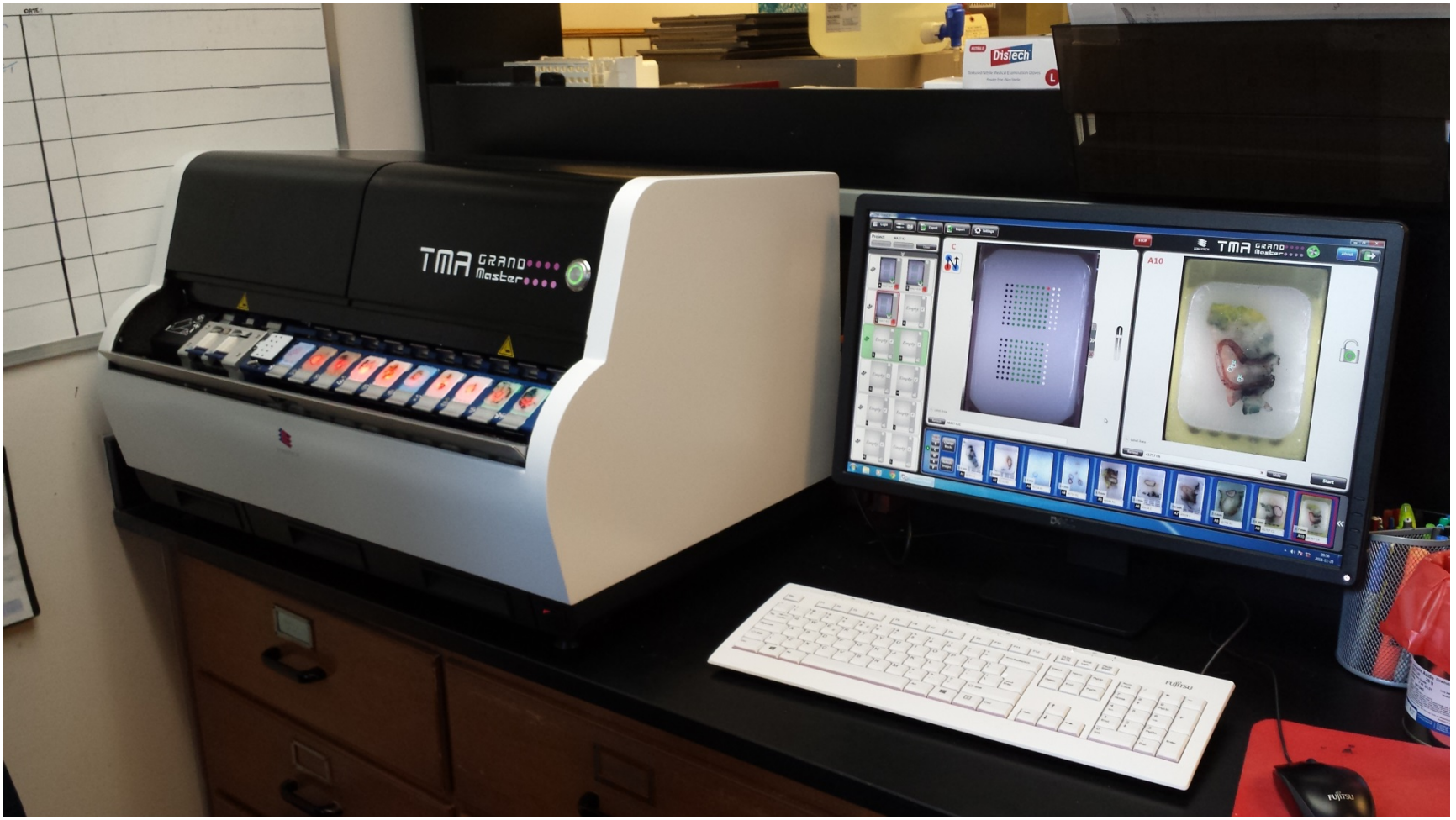
Intra-sample heterogeneity



Lee Boudreau in the TTDR/QLMP

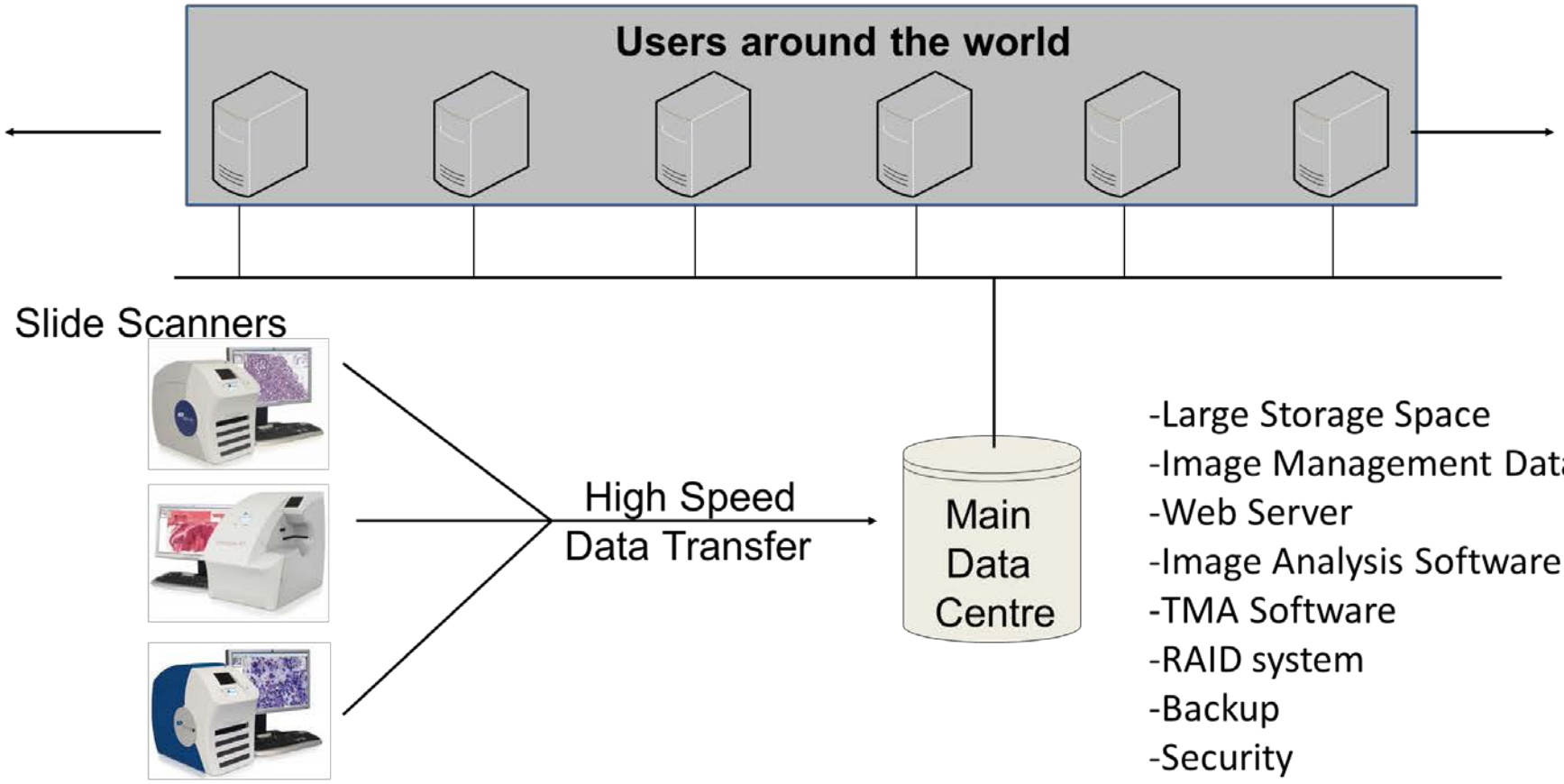


TMA Grand Master

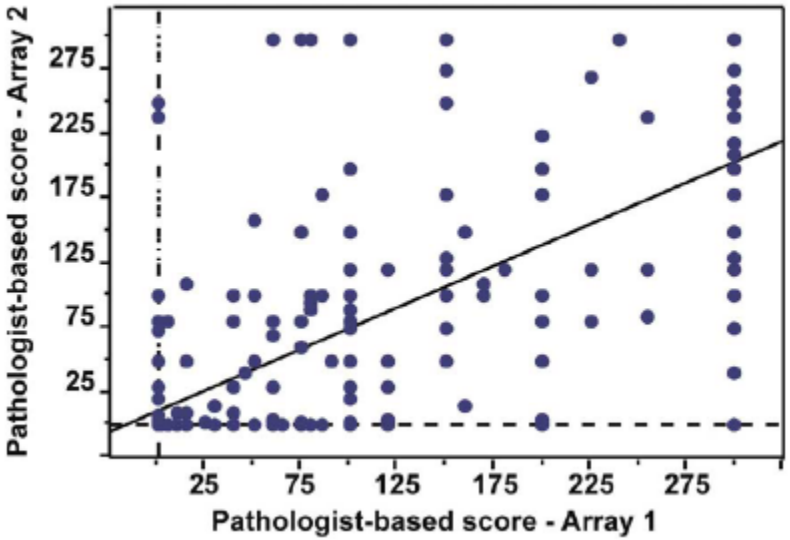


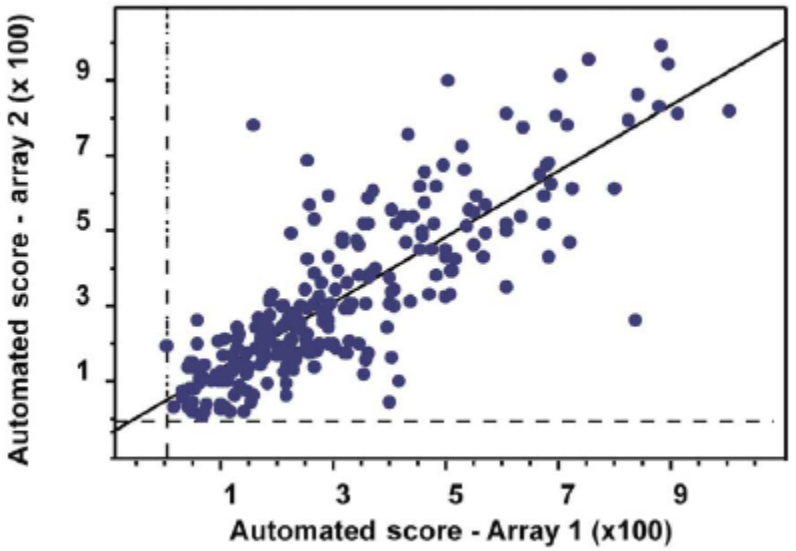
Digital Slide Scanning

High-resolution digital slide scanning and web hosting for bright-field or multi-channel immunofluorescence microscopic images with Leica Aperio slide scanners.



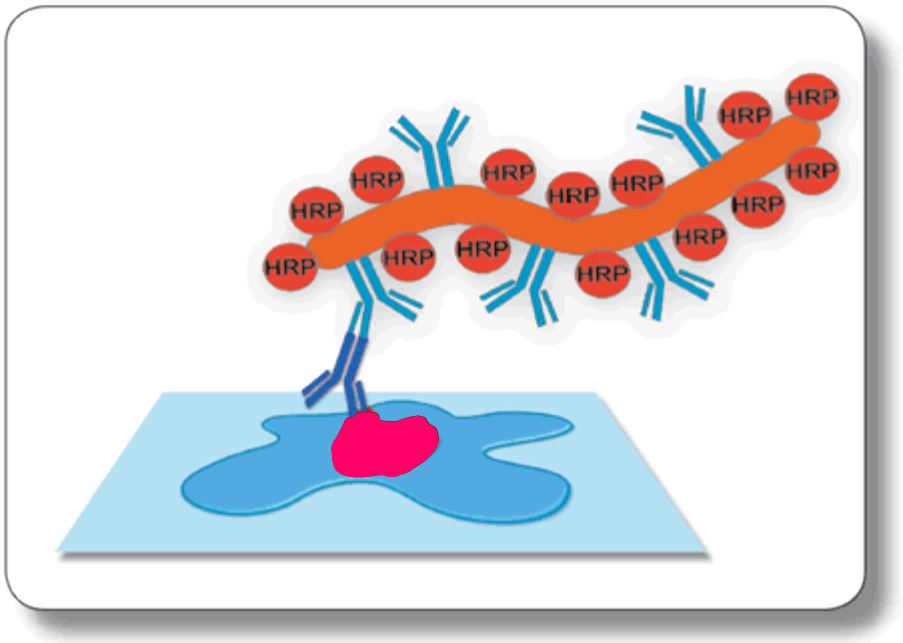
Scoring of ER staining by pathologist versus AQUA®

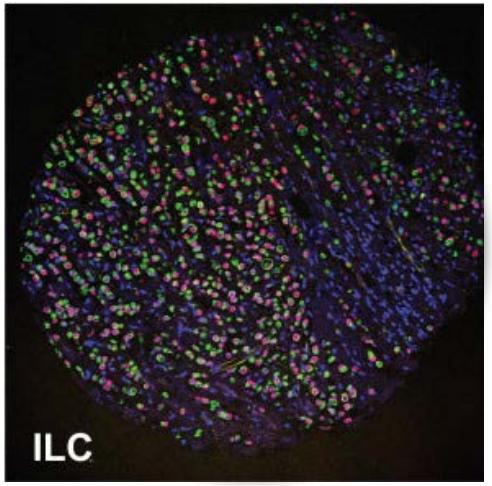




Nature Medicine 8:1323, 2002

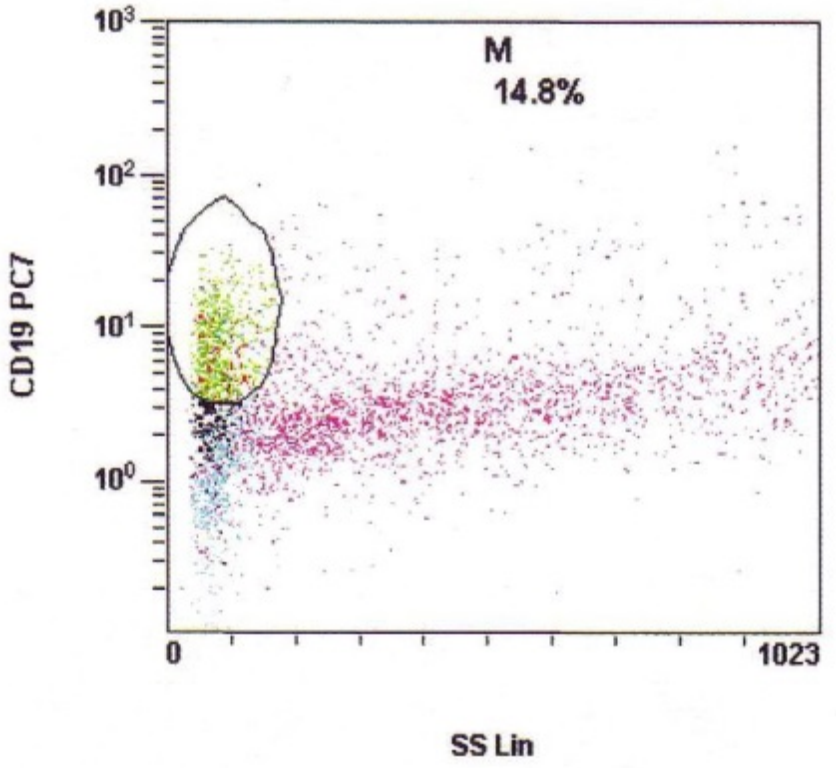
Immunofluorescence histology

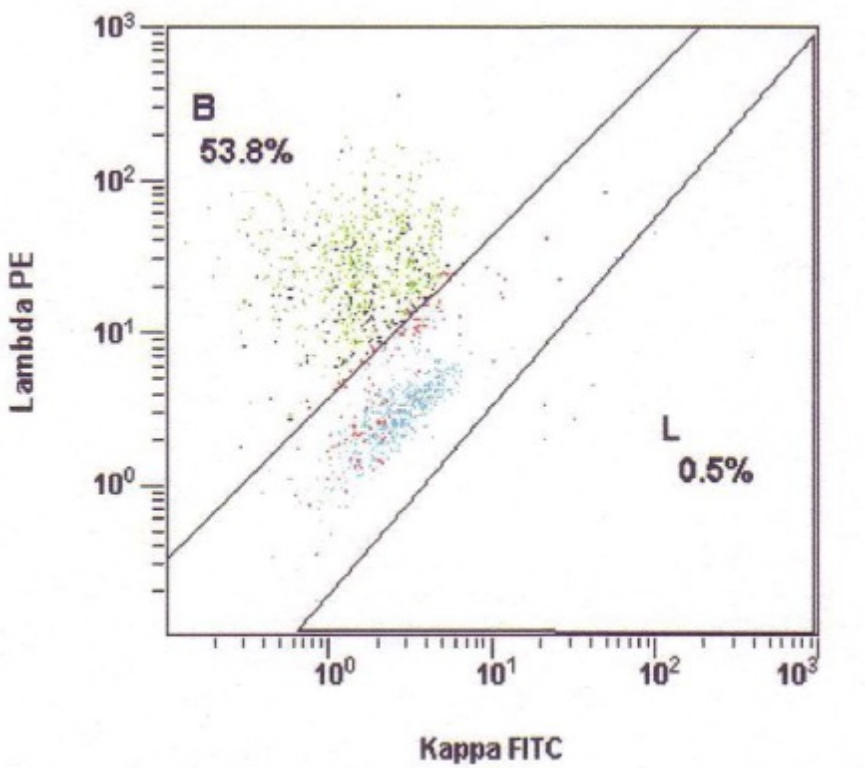




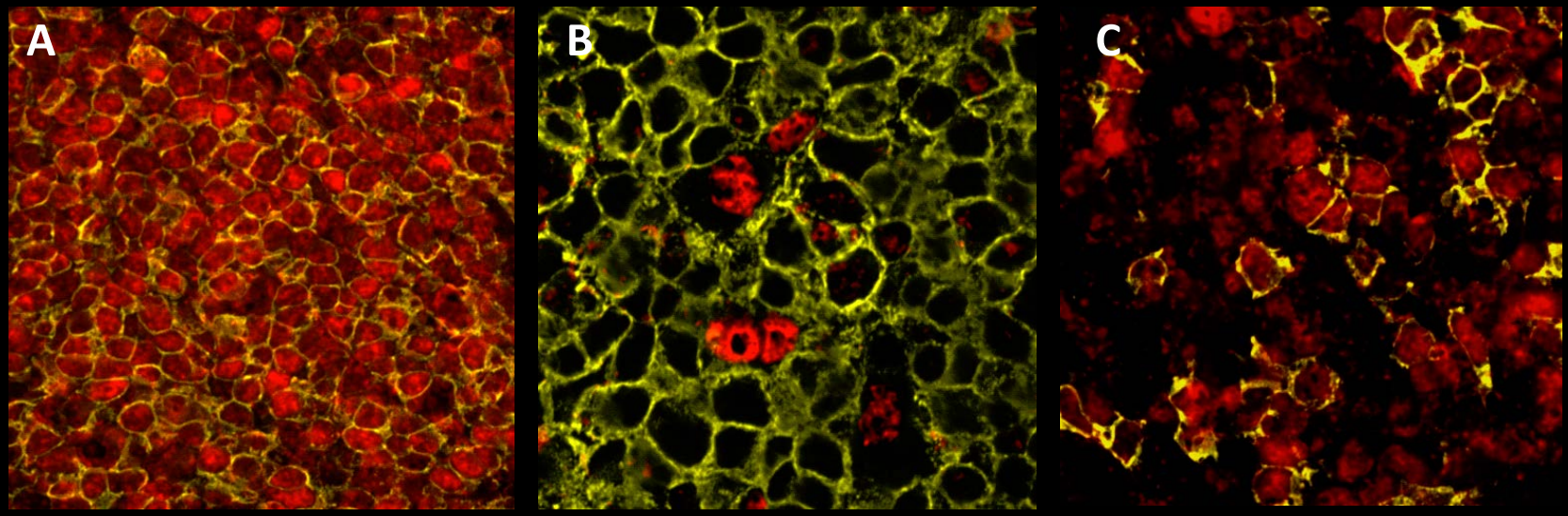
Alex Klimowicz

Fluorescence is quantitative and amenable to multiplexing



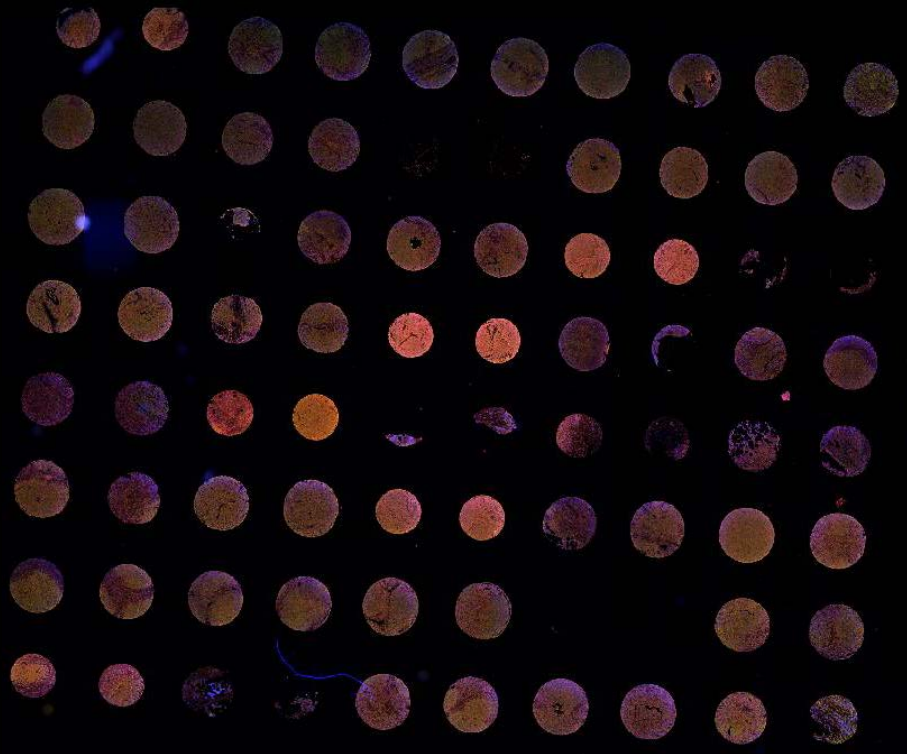


BMI1 in Hyperplastic Tonsil



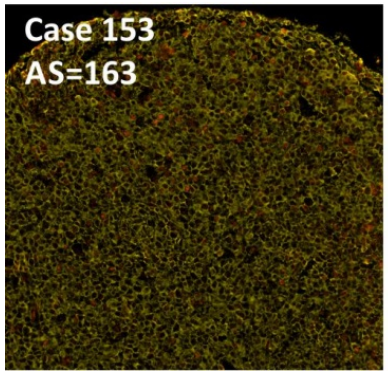
CD20 BMI1

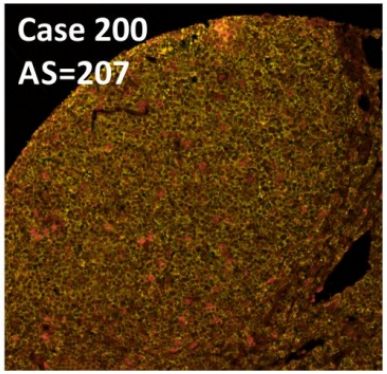
BMI1 in Follicular Lymphoma TMA

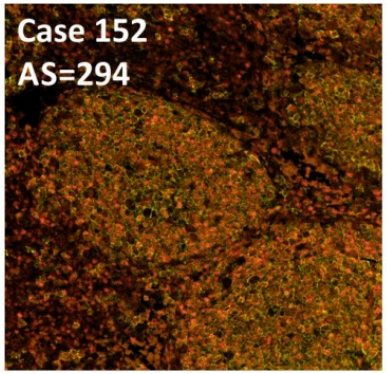


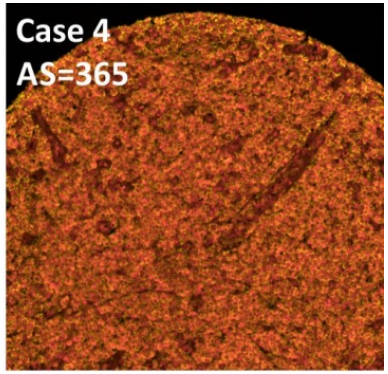
CD20 BMI1 DAPI

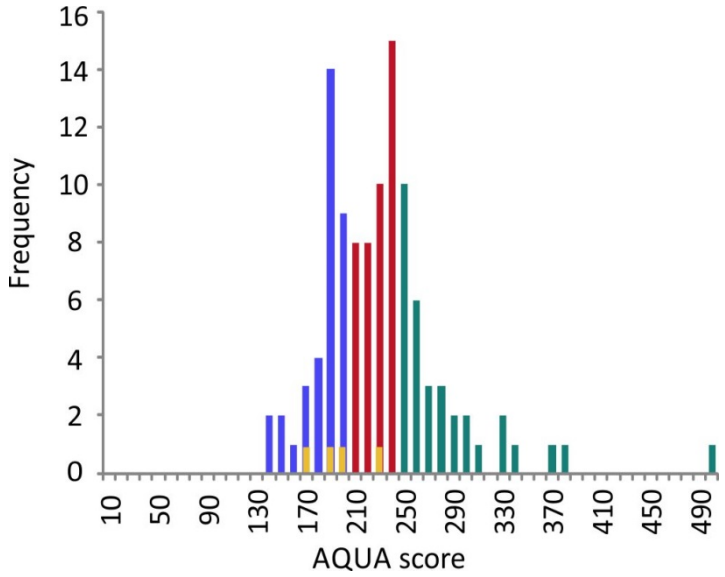
BMI1 in Follicular Lymphoma

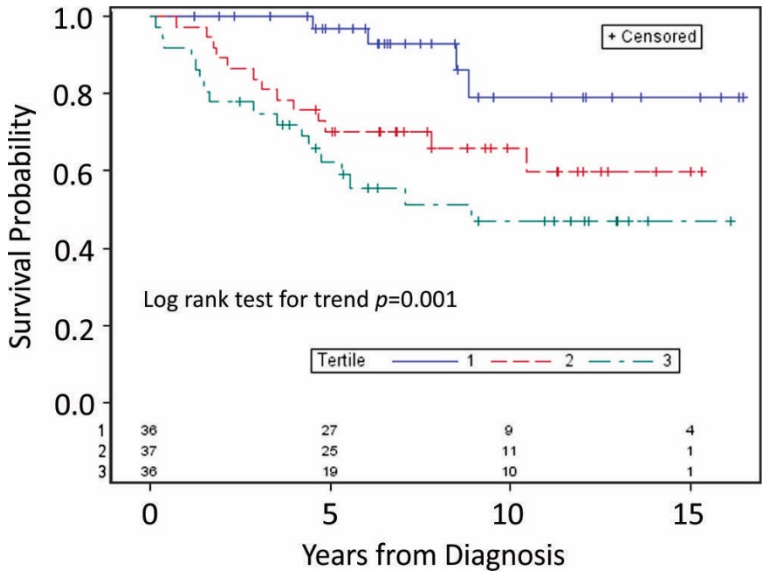




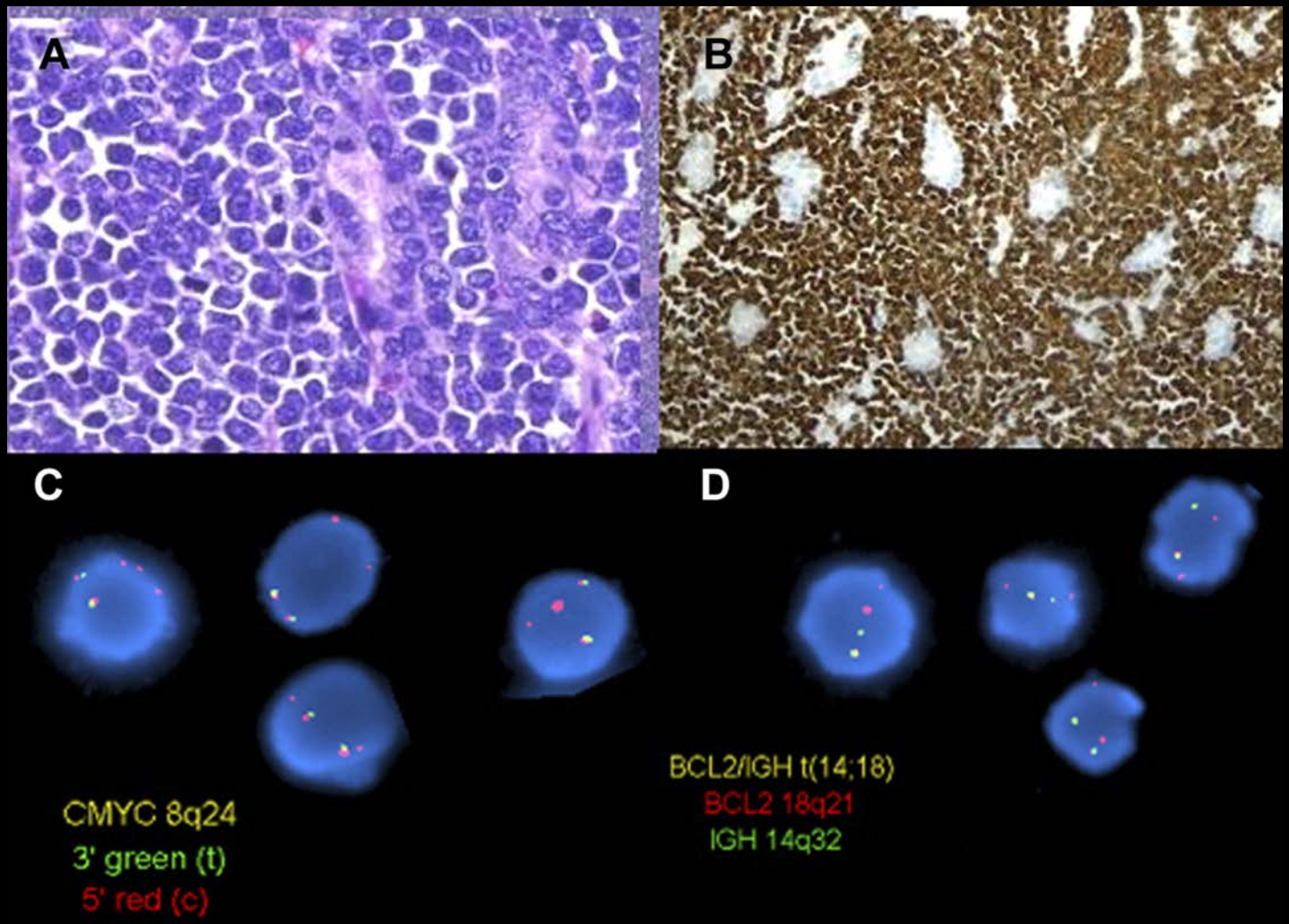




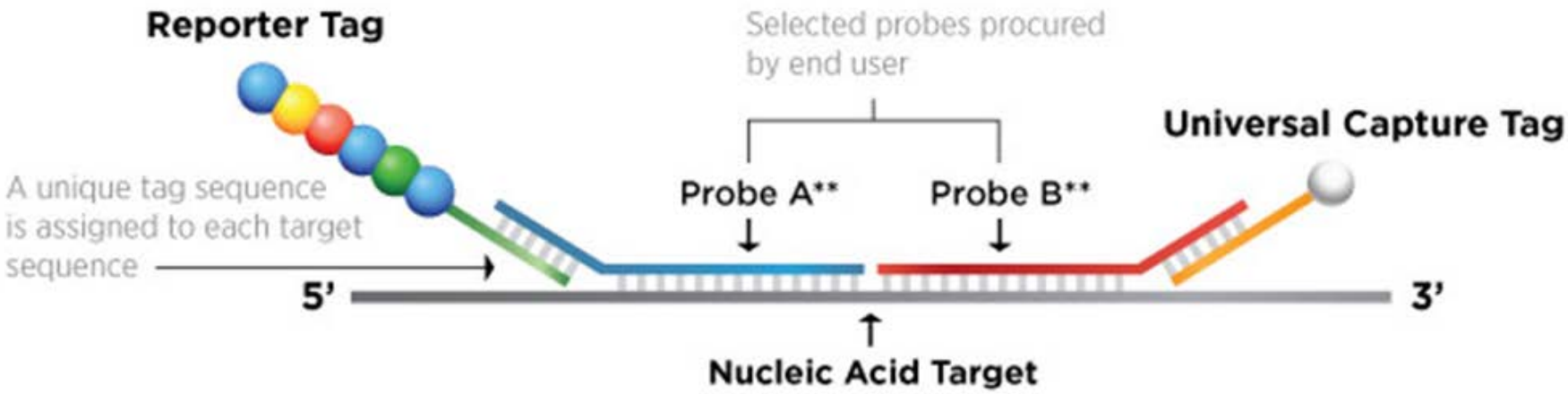




FISH


A
B
C
D
CMYC 8q24
3' green (t)
5' red (c)
BCL2/IGH t(14;18)
BCL2 18q21
IGH 14q32

NanoString


Reporter Tag
Selected probes procured by end user
Universal Capture Tag
A unique tag sequence is assigned to each target sequence
Probe A**
Probe B**
5'
3'
Nucleic Acid Target

Uses of human tissue samples

- Tissue morphology
- Protein
- DNA
- RNA

QLMP / TTDR

- Slide or block retrieval
- Conventional histology
- TMA construction, sectioning and staining
- Optimization of IHC or IF (KGH or investigator's Abs)
- Digital slide scanning
- Web hosting / web-based annotation
- Image analysis with AQUA® or HALO®
- Electron microscopy
- NanoString for gene expression profiling
- RNA/DNA extraction and QA for sequencing or other applications

Queen's Laboratory for Molecular Pathology

Queen's University

- About Us
- Microarray & RNA/DNA
- Tissue Microarray
- IHC & FISH
- Electron Microscopy
- Histology
- Slide Scanning
- Plastination

WELCOME

FACULTY

CONTACTS

Welcome

Queen's Laboratory for Molecular Pathology

Welcome to the Queen's Laboratory for Molecular Pathology (QLMP) website. The Laboratory is part of the Department of Pathology and Molecular Medicine located in Queen's University.

The QLMP seeks to:

1) Provide rapid and economic advanced pathology services to researchers, students, clinicians, and private sector groups.
2) Promote productive interactions between scientists, clinicians, pathologists and others by supporting research projects of common interest

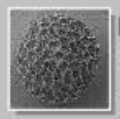
Expertise and Dedication

Tissue Microarray
The QLMP employs TMA technology and has created blocks with over 300 cores.

Digital Slide Scanning
The Aperio slide scanner is used routinely for high resolution scans and image analysis.

Gene Microarray
The gene expression micorarray facility features the Agilent microarray platform, and offers expertise in nucleic acid extraction.

IHC and FISH
The QLMP carries out both manual and automated IHC staining, as well as FISH.



Electron Microcopy & Histology
Fixing, emdedding, sectioning, staining, and photography are all performed by the QLMP.

BFFs…

- Clinician
- Pathologist
- Basic scientist
- Biostatistician
- Bioinformatician
- Technician

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Correlative Science / Tumour Bank

The NCIC Clinical Trials Group (NCIC CTG) established the Tumour Tissue Data Repository (TTDR) in 1997 to support Correlative Science with the assistance of Rhone Poulenc Rorer (now Sanofi-Aventis). The NCIC CTGTTDR contains unique disease specific collections which are linked to an associated clinical dataset. This represents a "real" tumour bank in that tissue is collected from institutions across Canada and the world, catalogued and housed centrally in the Department of Pathology and Molecular Medicine at Queen's University.

Our Mission

To establish a collection of specimens stored in house and linked with clinical trials dataset. Access to this tissue permits the assessment of prognostic factors in determining the outcome of disease, the assessment of predictive factors to various chemotherapeutic agents and treatment regimens, and to facilitate the understanding of the basic biological and genetic mechanisms of cancer.

Profile

The NCIC CTG TTDR has taken part in collection of tissue for over 120 clinical trials. The central coordinating office for the Bank is located in Kingston, Ontario. Participating centres include cancer care organizations, academic health science centres, community hospitals, and smaller individual practices. These centres were initially in Canada but more recently samples have been collected worldwide.

What is a biorepository or biobank?

"a large collection of biological or tissue samples
amassed for research purposes"
- population based, disease specific, site specific
- real or virtual
- availability of material communicated through CCTG website

A powerful tool in research and over the last two decades has provided support for genomics and personalised or precision medicine

If you collect specimens for research purposes, you are a biobank

A biorepository associated with cancer clinical trials is a composite of…

- Tumour: collected in the context of a clinical trial
- Tissue: whole blood, plasma, serum, urine, bone marrow, circulating tumour cells, cfDNA (liquid biopsy)
- Derivatives: DNA, RNA, Tissue Microarrays, proteins
- Data: well described and validated clinical data including demographic information, patient and disease characteristics, therapy, outcome measures, adverse event profiles, quality of life

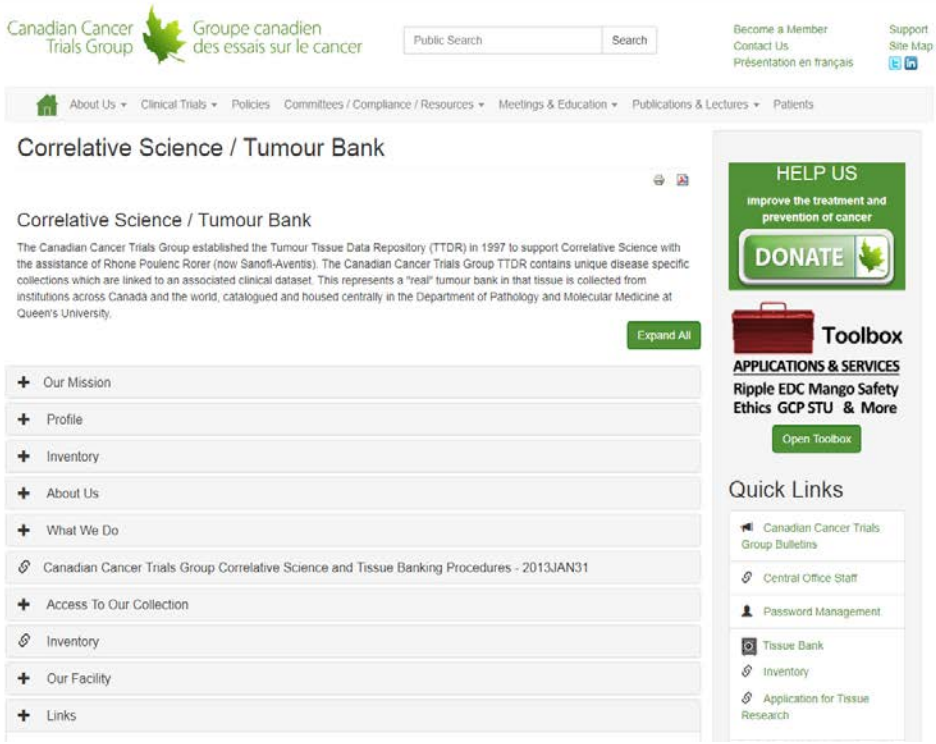
Tumour Tissue Data Repository (TTDR) of the CCTG

A national resource of clinical trial associated tissue on more than 120 trials providing material to the research community to better understand the biology of cancer and to impact ongoing and future patient care

- Tumour tissue > 18,500 patients
- Plasma >8,500 patients
- Serum >13,500 patients
- ~ 300,000 individual samples banked and matched with quality clinical data

Housed within the Queen's Laboratory for Molecular Pathology (QLMP) in the Department of Pathology and Molecular Medicine

www.ctg.queensu.ca



Required Operational Practices for a Tissue Bank ... ROPs

- Governance
- Ethics approval
- Privacy, Facility design and Security
- Informed consent
- Access and Release processes
- Quality management system and process improvement
- Education and Training
- Biospecimen storage and retrieval
- Equipment
- Safety
- Disaster Plan

Collection Kits and Laboratory manuals

MA.32 Kit Assembly Instructions

In a biohazard bag

- Lab Guard* Reclosable Biohazard Bag (Cat. No. 56766-370, 6 x 9 inch)

Place the following components:

1) 1 Lavender top tube (K2 EDTA 10.8 mg, Cat. No. BD367863, 6.0 mL)
2) 2 Red top tubes (Serum, Cat. No. BD367815, 6.0 mL)
3) 3 Light Green top tubes (PST Gel and Lithium Heparin, 83 Units, Cat. No.BD 367962, 4.5 mL)
4) 4 disposable transfer pipettes (Cat. No. VWR 414004-002, 5 mL sterile)
5) 14 Cryogenic vials (Cat. No. 82050-206, 2.0 mL, sterile)

- Collection tubes best suited for planned correlative studies and future research
- Collection volumes correspond to those described in patient informed consent form
- Components for fluid collection, labeling, processing and storage are usually supplied

CCTG TTDR Facility

- “The freezer farm”
- All samples carefully received, catalogued, assigned a unique tumour bank ID number, stored and inventory tracked.

Process for Access to Samples for Correlative Studies

- Appropriate research hypothesis, study design and statistical consideration
- Proven investigator experience with validated methodology
- Budget for sample preparation, shipping and funding for research
- Ethics approval at research institution
- Statistical analyses to be conducted by the CTG statistical centre
- Investigator Agreement to be signed before the release of tissue

Publication Standards for Biomarker Studies

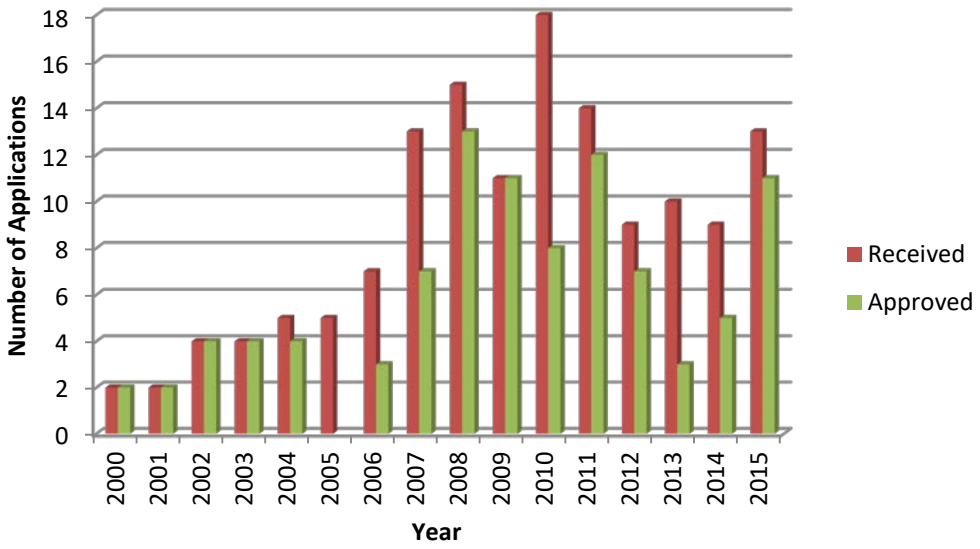
- REMARK, JNCI, August 2005

"Reporting Recommendations for Tumour Marker Prognostic Studies"

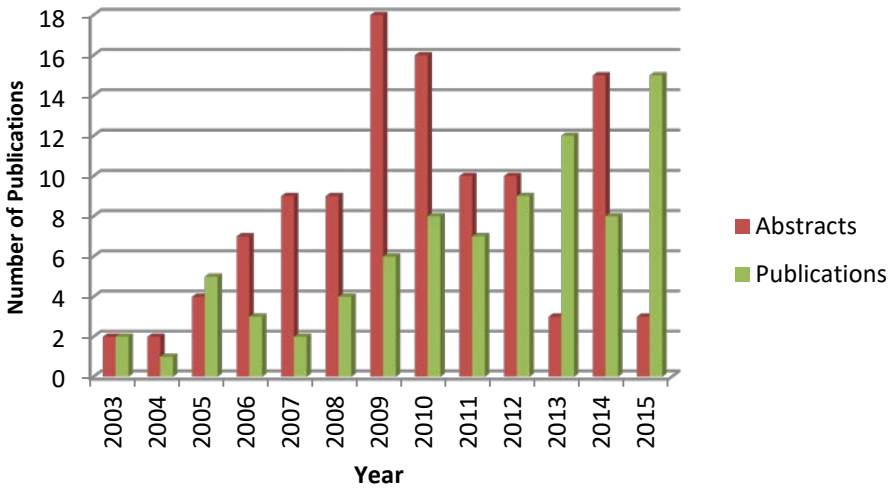
- REMARK BJC and PLoS Medicine, 2012

- Use of Archived Specimens for Prognostic and Predictive Markers, JNCI, November 2009

Applications to the CSTB to access material



CSTB Publications and Abstracts



What is the value of the Tumour/Tissue Bank?

- Molecular genetic information on tumours is a critical component of basic, applied, and drug development research
- Access to this tissue permits the assessment of prognostic factors, predictive factors to therapeutic agents and treatment regimens
- Facilitates the understanding of the basic biological and genetic mechanisms of cancer.
- Crucial in the development of "targeted" and specific therapy. This information is vastly enriched, more valuable, and powerful when associated with a clinical trials database.