

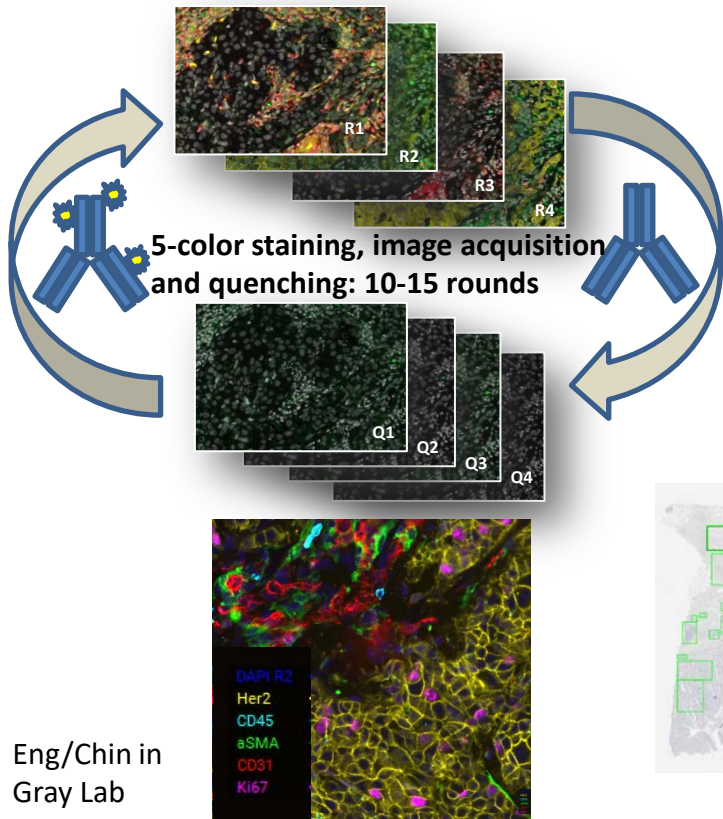
# Leveraging OME tools in highly multi-channel imaging

*suggestions to make it even better*

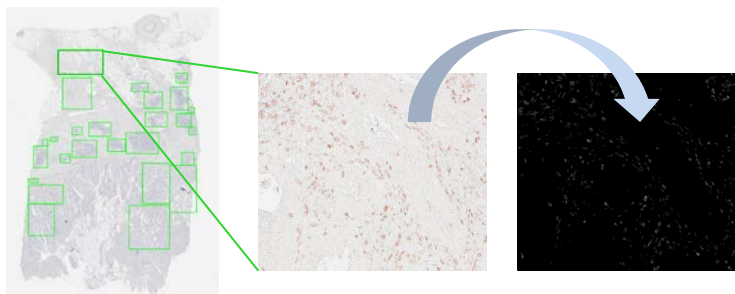
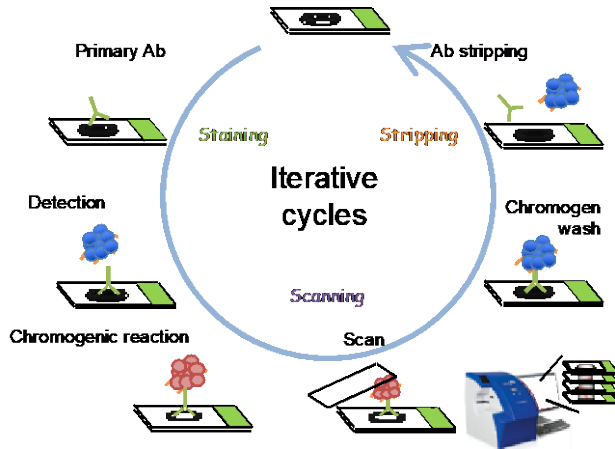
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# Highly multiplex imaging approaches

## Cyclic Immunofluor. ((t-)CyclIF)



## Multiplex IHC (mIHC)



Betts/Sivagnanam in Coussens Lab

## CyclIF-like:

GE/Cytiva Cell-DIVE  
Cell IDx UltraPlex  
Miltenyi MICS

## mIHC-like

UltiVue InSituPlex

## Oligo-based:

CODEX  
PCL Ab-Oligo  
DNA/RNA PAINT

## Mass-spec-based:

MIBI  
Hyperion/CyTOF

# How OME tools are helping now

- Bio-Formats (+ Glencoe) tools: importing/converting
  - dealing with all those odd-ball formats out there
- OME-TIFF & model: capable file/metadata format
  - tiles/pyramids, N channels, exchange standards, single-file,
- OMERO: storing/organizing/accessing/visualizing
  - multi-channel viz, access from analysis software,

# Bio-Formats – better?

- Community: pressure our vendors to move to standard formats and STOP releasing new proprietary formats – make full standard formats support a condition of a sale
- OME & Glencoe teams: please maintain and expand support for proprietary formats

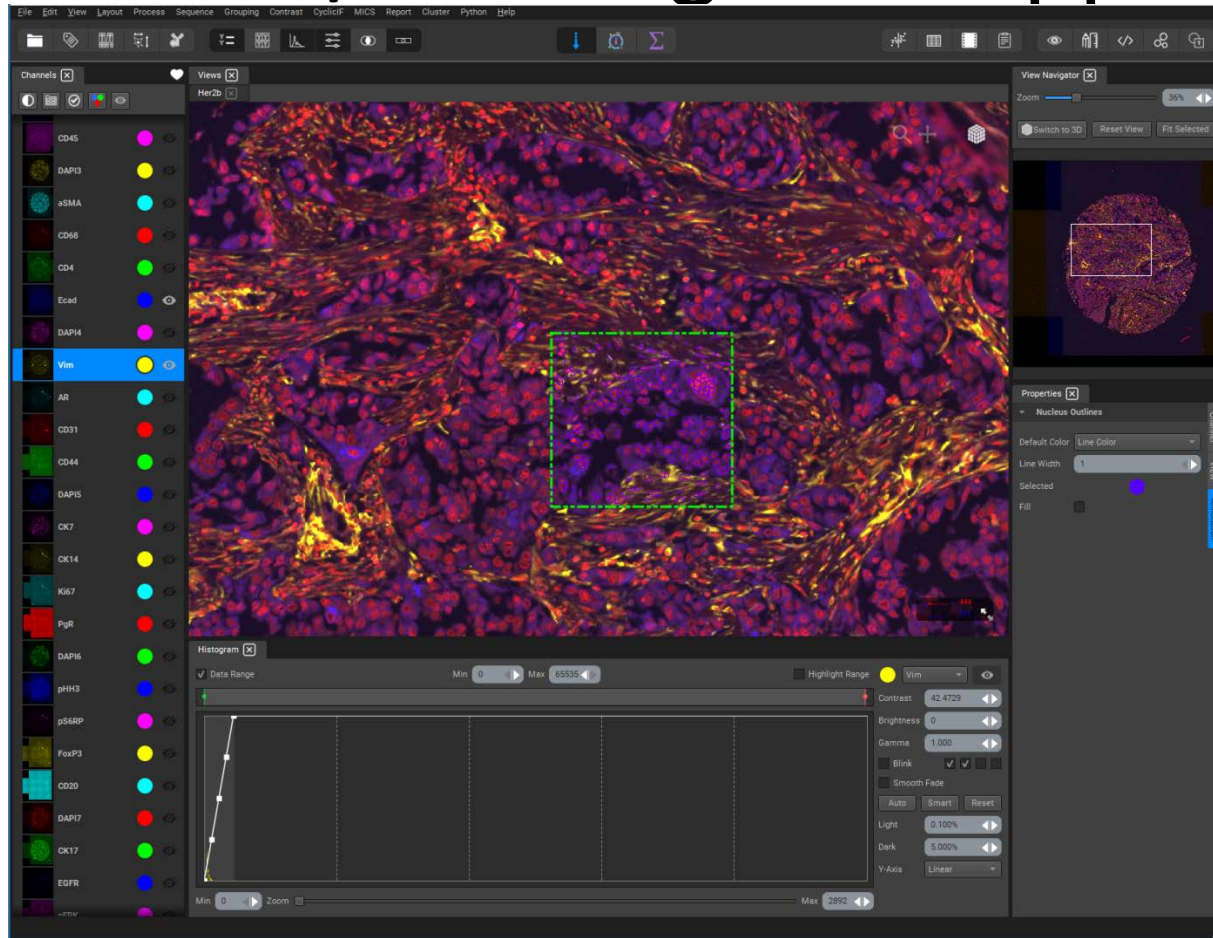
# OME-TIFF/OME-XML model – better!

- Compression – better lossless and lossy
  - decent fast lossy may be adequate for some WSI images – current JPEG2K is not very effective
- Revive OME Files (C++ library) and use that to provide full high-performance Python support
- Next generation containers and metadata model
  - Josh Moore and Caterina Strambio talks!!

# OMERO – better!!

- Full function HTTP/RESTful access as full alternative to ZeroC/ICE APIs
  - Avoid ICE requirements on clients; make C++ clients on Windows possible
  - Avoid problematic firewall issues
  - Permit horizontal scaling
- How?
  - Make deployment of micro-services standard; use ms-pixelbuffer for bulk access
  - Full libraries (Python first) based on the above

# QiTissue – C++/Qt integrated app for CyCIF



# OMERO (2) – better!!

- Add visualization/interaction for per-channel annotations
  - OME-XML model supports it but needs better definition
- Improve organization abilities
  - Further develop the (ROI) folders concept?
  - Improved search (beyond Lucene) and result display

	A	B	C	D	E	F	G	H	I
	Method	Channel	Cycle	Filter_Set	Marker_Name	Fluorophore	Vendor	Catalogue_Number	Dilution
1	t-CyCIF	1	1	DAPI	DNA		Cell Signaling Technology	40825	1:20000
2	t-CyCIF	2	1	FITC	Autofluorescence-488nm		Thermo Fisher	A-21208	1:2000
3	t-CyCIF	3	1	Cy3	Autofluorescence-555nm		Thermo Fisher	A-31572	1:2000
4	t-CyCIF	4	1	Cy5	Autofluorescence-647nm		Thermo Fisher	A-31571	1:2000
5	t-CyCIF	5	2	DAPI	DNA (2)		Cell Signaling Technology	40825	1:20000
6	t-CyCIF	6	3	FITC	CD3		Abcam	ab11089	1:500
7	t-CyCIF	7	3	Cy3	Na/K ATPase		Cell Signaling Technology	40825	1:20000
8	t-CyCIF	8	2	Cy5	Control-647nm		Thermo Fisher	A-31571	1:2000
9	t-CyCIF	9	3	DAPI	DNA (3)		Cell Signaling Technology	40825	1:20000
10	t-CyCIF	10	3	FITC	CD3		Abcam	ab11089	1:500
11	t-CyCIF	11	3	Cy3	Na/K ATPase		Cell Signaling Technology	40825	1:20000
12	t-CyCIF	12	3	Cy5	CD45RO		Dako	M0742	1:200
13	t-CyCIF	13	4	DAPI	DNA (4)		Cell Signaling Technology	40825	1:20000
14	t-CyCIF	14	4	FITC	Antigen K167		Thermo Fisher	A-31571	1:2000
15	t-CyCIF	15	4	Cy3	Pan-cytokeratin		Thermo Fisher	A-31571	1:2000
16	t-CyCIF	16	4	Cy5	Aortic smooth muscle actin		Thermo Fisher	A-31571	1:2000
17	t-CyCIF	17	5	DAPI	DNA (5)		Cell Signaling Technology	40825	1:20000
18	t-CyCIF	18	6	FITC	CD3		Abcam	ab11089	1:500
19	t-CyCIF	19	6	Cy3	CD3		Abcam	ab11089	1:500
20	t-CyCIF	20	6	Cy5	CD3		Abcam	ab11089	1:500
21	t-CyCIF	21	6	DAPI	DNA (6)		Cell Signaling Technology	40825	1:20000
22	t-CyCIF	22	6	FITC	CD20		Thermo Fisher	A-31571	1:2000
23	t-CyCIF	23	6	Cy3	CD68		Cell Signaling Technology	40825	1:20000
24	t-CyCIF	24	6	Cy5	CD8a		Thermo Fisher	A-31571	1:2000
25	t-CyCIF	25	7	DAPI	DNA (7)		Cell Signaling Technology	40825	1:20000
26	t-CyCIF	26	7	FITC	CD168		Thermo Fisher	A-31571	1:2000
27	t-CyCIF	27	7	Cy3	FOXp3		Thermo Fisher	A-31571	1:2000
28	t-CyCIF	28	7	Cy5	PD-L1		Thermo Fisher	A-31571	1:2000
29	t-CyCIF	29	8	DAPI	DNA (8)		Cell Signaling Technology	40825	1:20000
30	t-CyCIF	30	8	FITC	E-cadherin		Thermo Fisher	A-31571	1:2000
31	t-CyCIF	31	8	Cy3	Vimentin		Cell Signaling Technology	40825	1:20000
32	t-CyCIF	32	8	Cy5	CDX-2		Abcam	ab11089	1:500
33	t-CyCIF	33	9	DAPI	DNA (9)		Cell Signaling Technology	40825	1:20000
34	t-CyCIF	34	9	FITC	Lamin-A/B/C		Cell Signaling Technology	40825	1:20000
35	t-CyCIF	35	9	Cy3	Desmin		Abcam	ab11089	1:500
36	t-CyCIF	36	9	Cy5	CD31		Abcam	ab11089	1:500
37	t-CyCIF	37	10	DAPI	DNA (10)		Cell Signaling Technology	40825	1:20000
38	t-CyCIF	38	10	FITC	CD31		Cell Signaling Technology	40825	1:20000
39	t-CyCIF	39	10	Cy3	Intigen K167 (2)		Thermo Fisher	A-31571	1:2000
40	t-CyCIF	40	10	Cy5	Collagen		Thermo Fisher	A-31571	1:2000
41	t-CyCIF	41	10	Cy5	Collagen		Thermo Fisher	A-31571	1:2000