Introducing a data-standard for fluorescence microscopy: increasing data quality and fidelity for biological measurements

2018 OME Annual Users Meeting

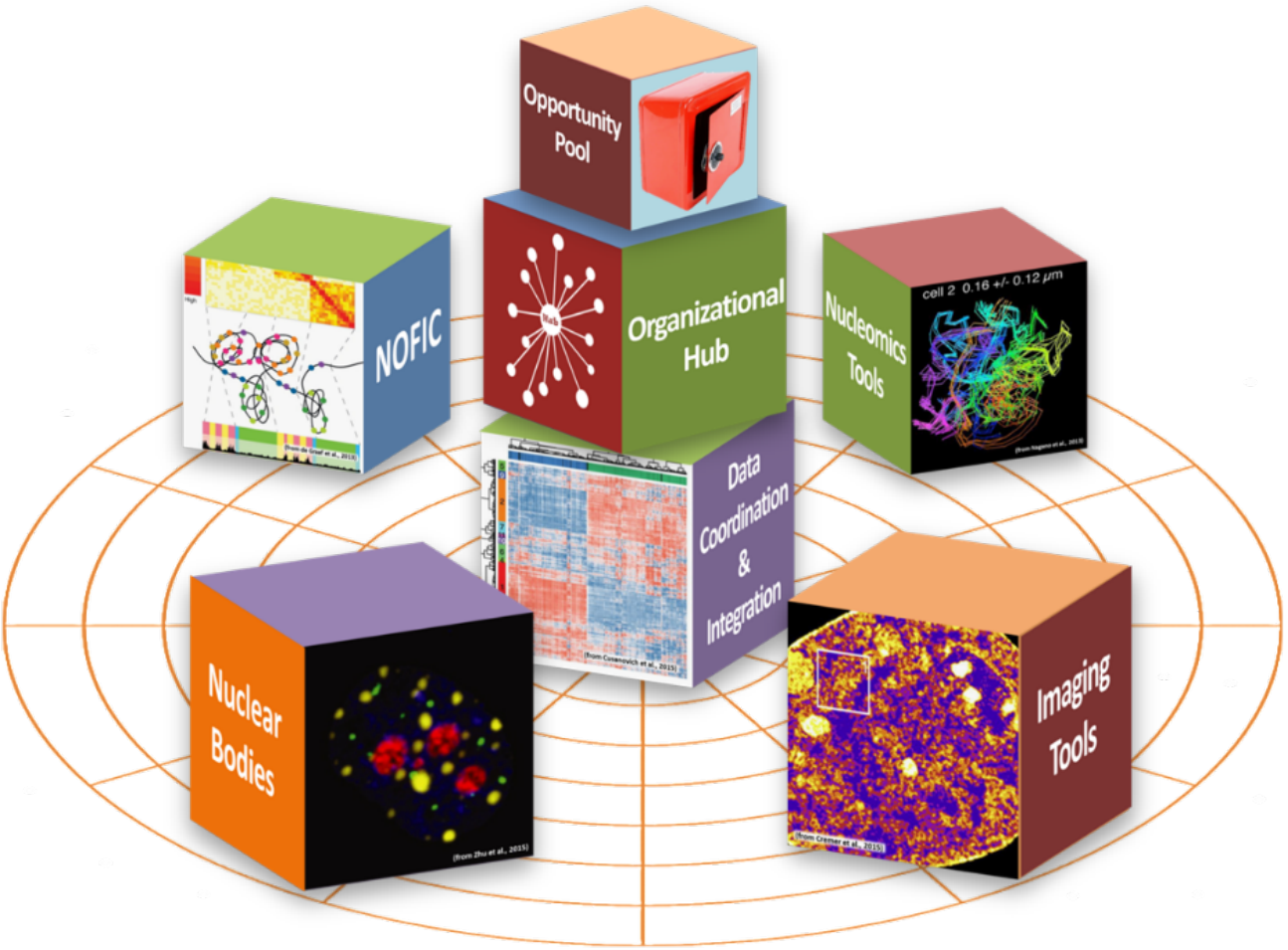
University of Dundee, May 30th 2018

David Grunwald Ph.D.

UMASS University of Massachusetts Medical School

4D Nucleome

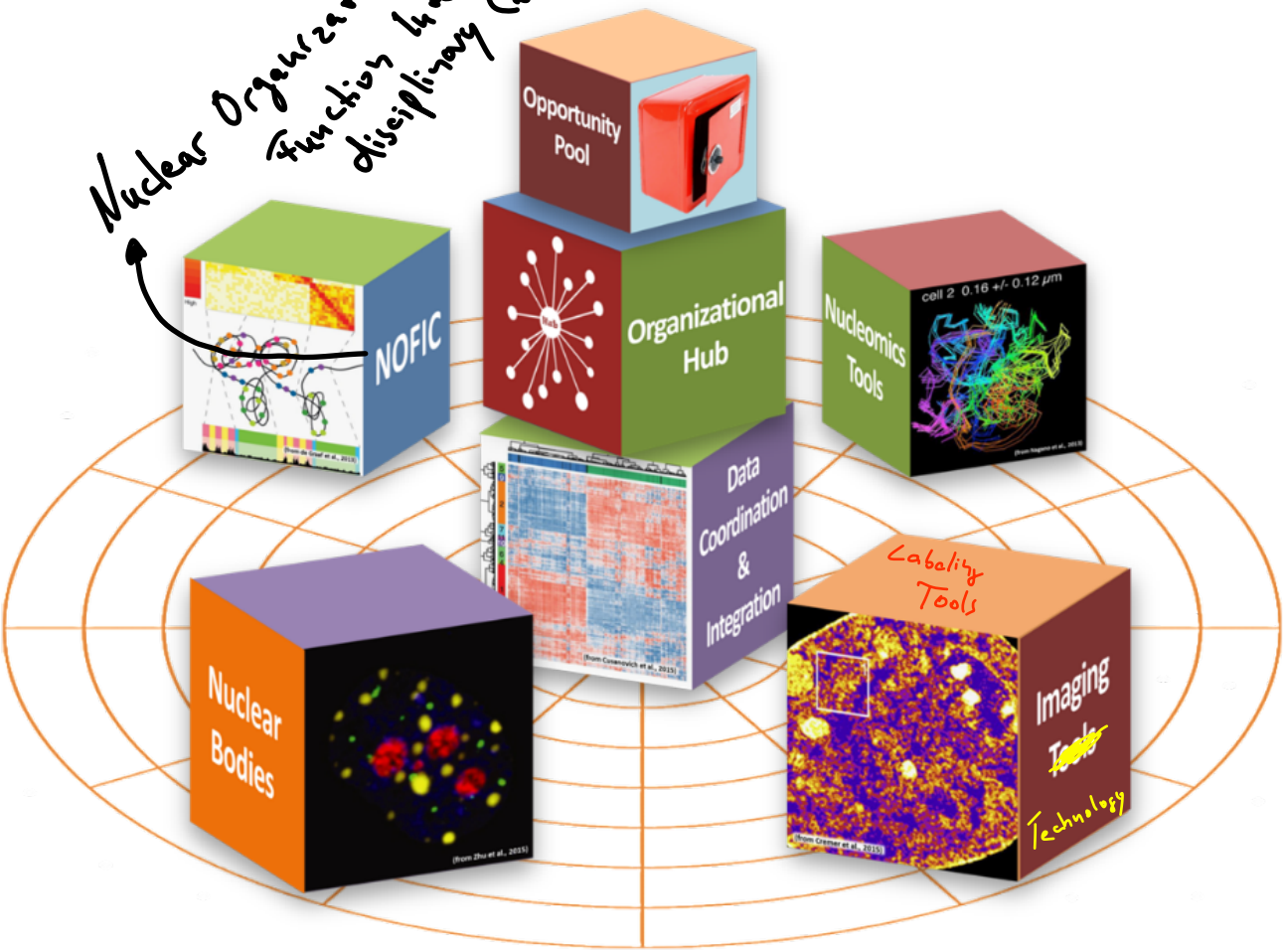
http://www.4dnucleome.org/
https://commonfund.nih.gov/4dnucleome/index



4DN aims to understand:

- Nuclear organization in space and time
- Nuclear organization in gene expression and cellular function
- Nuclear organization in development and disease

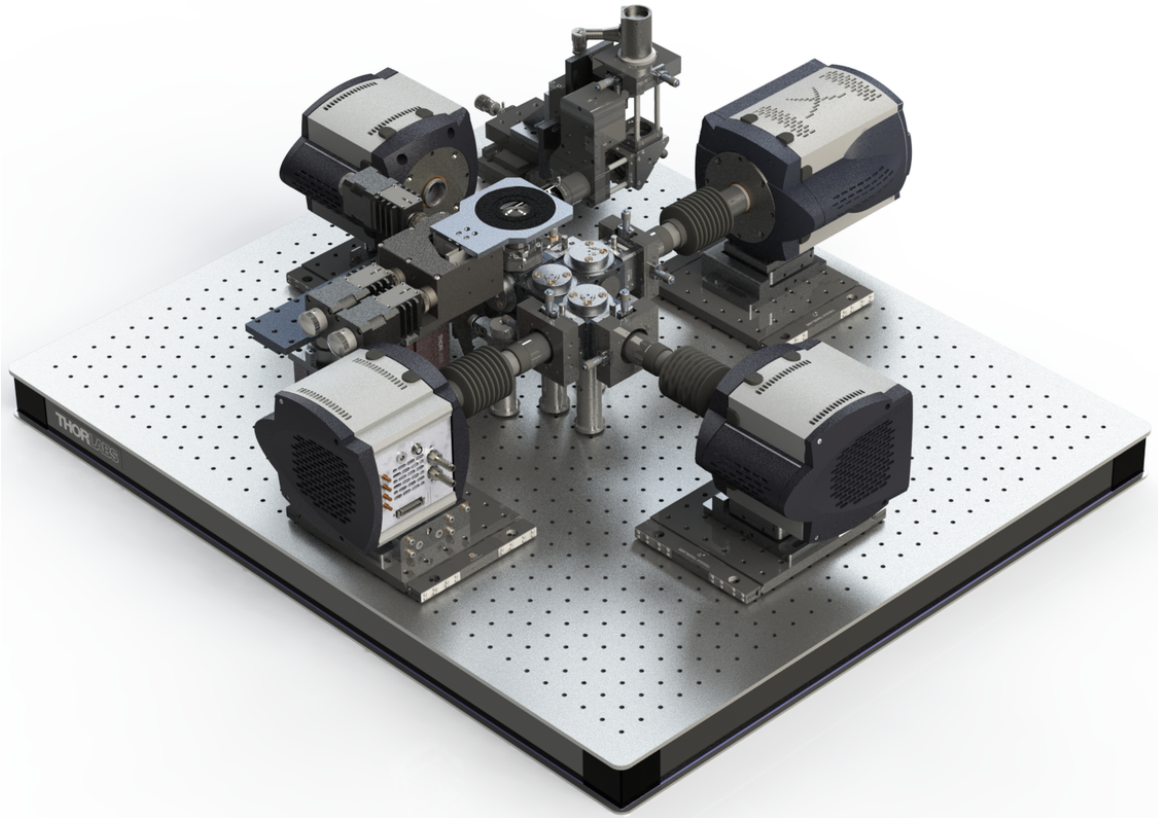
Nuclear Organization + Function labs - disciplinary Collaboration...



"Molecular" Resolution needed

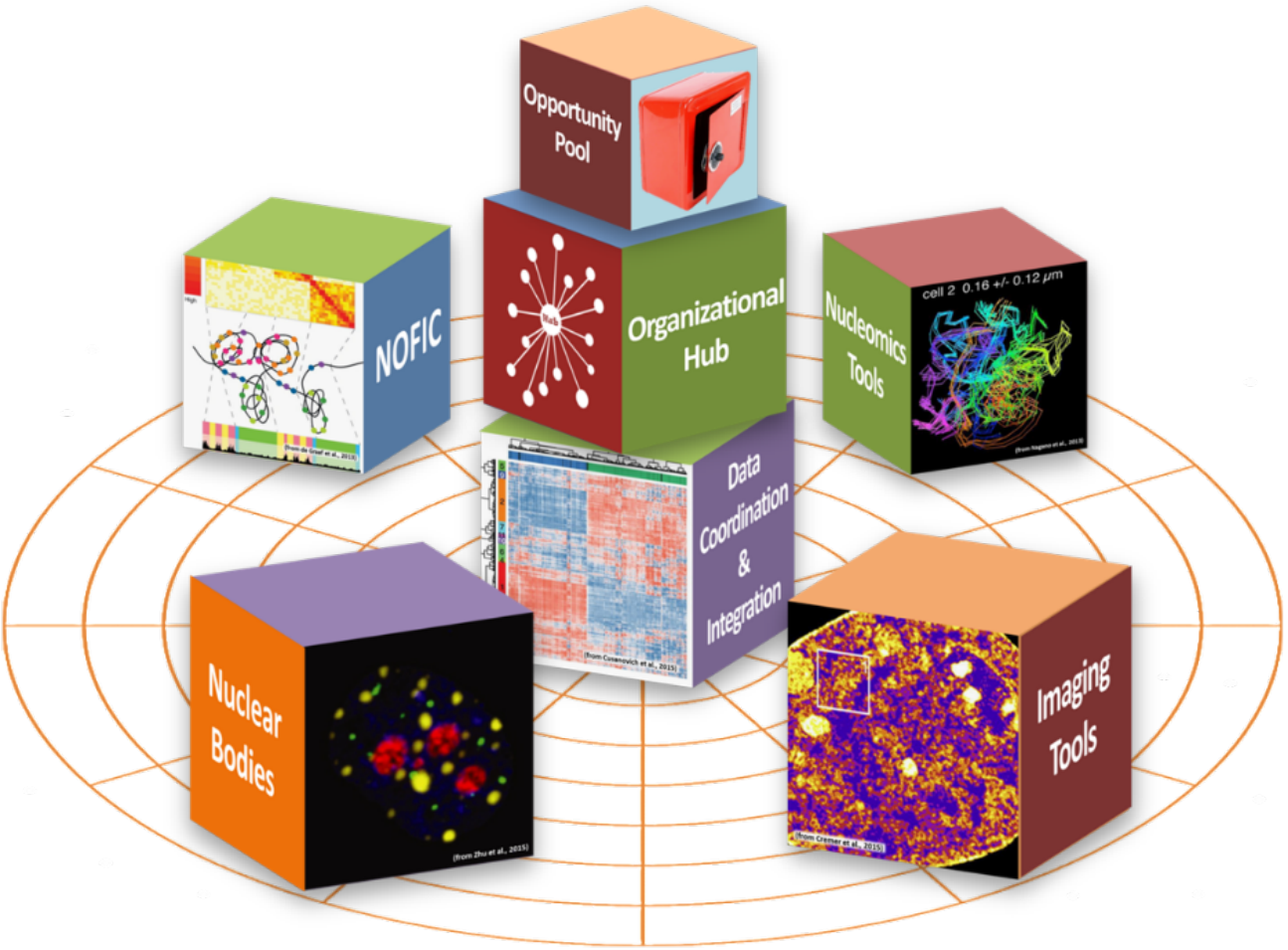
4DN aims to understand:

- Nuclear organization in space and time
- Nuclear organization in gene expression and cellular function
- Nuclear organization in development and disease



4D Nucleome

http://www.4dnucleome.org/
https://commonfund.nih.gov/4dnucleome/index



4DN aims to understand:

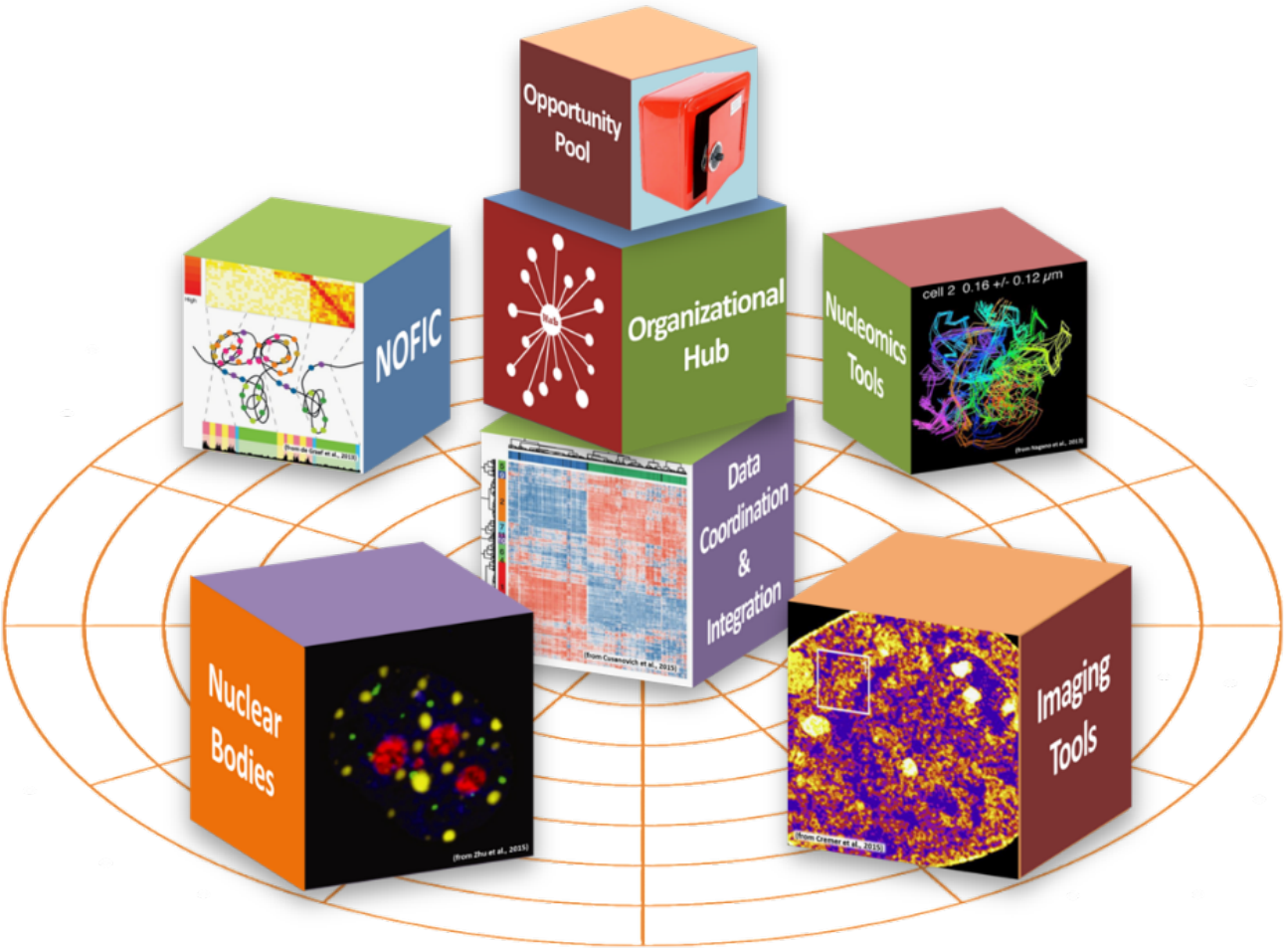
- Nuclear organization in space and time
- Nuclear organization in gene expression and cellular function
- Nuclear organization in development and disease

Deliverables:

- Community standards and metrics
- Next generation tools
- Reference maps of the 3D genome
- Predictive models of structure/function relationships
- Biological validation through controlled disruption of nuclear architecture and single-cell imaging
- Understanding poorly-characterized nuclear structures and nuclear bodies

4D Nucleome

http://www.4dnucleome.org/
https://commonfund.nih.gov/4dnucleome/index



4DN aims to understand:

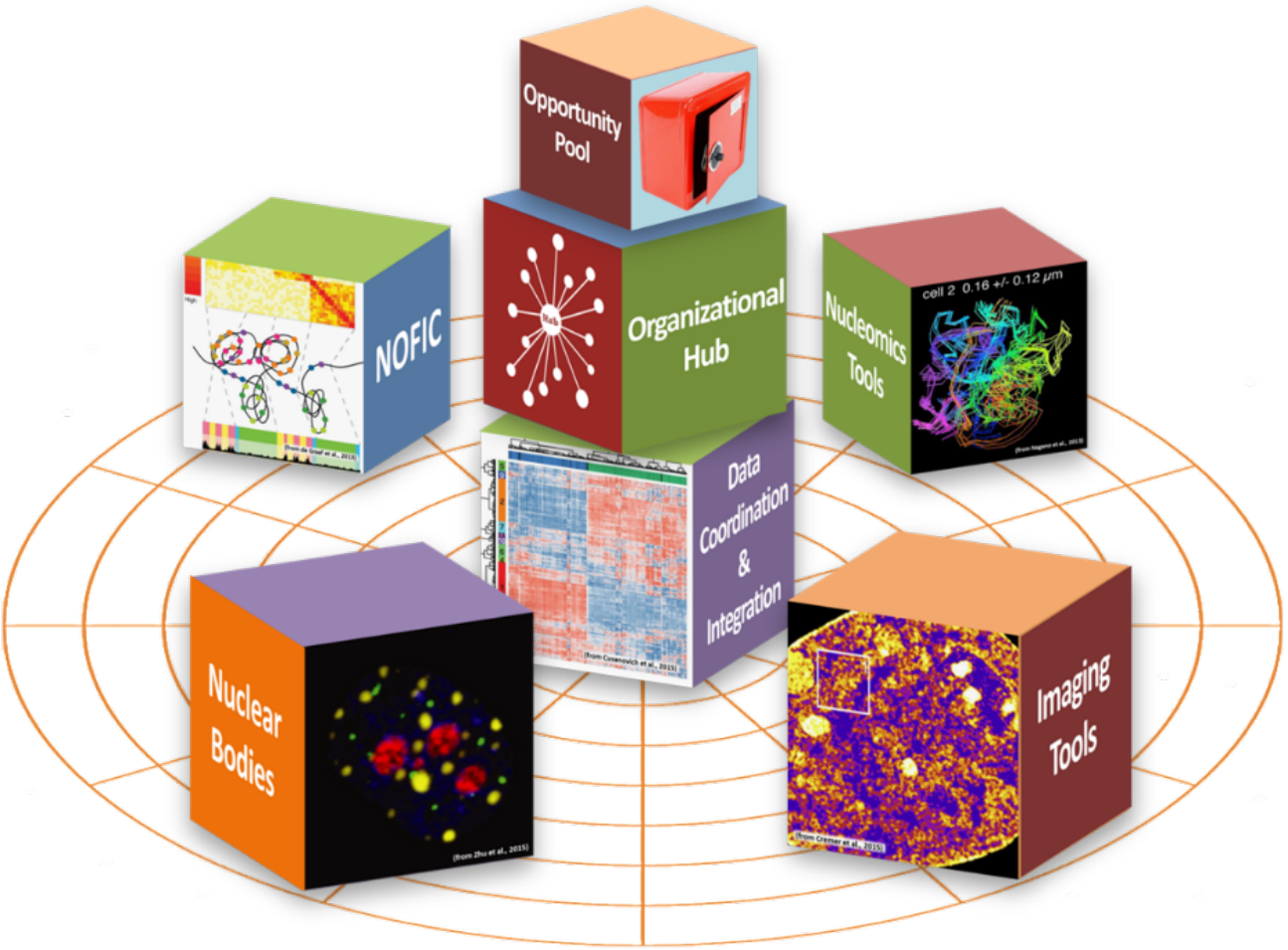
- Nuclear organization in space and time
- Nuclear organization in gene expression and cellular function
- Nuclear organization in development and disease

Deliverables:

- Community standards and metrics
- Next generation tools
- Reference maps of the 3D genome
- Predictive models of structure/function relationships
- Biological validation through controlled disruption of nuclear architecture and single-cell imaging
- Understanding poorly-characterized nuclear structures and nuclear bodies

4D Nucleome

http://www.4dnucleome.org/
https://commonfund.nih.gov/4dnucleome/index



4DN aims to understand:

- Nuclear organization in space and time
- Nuclear organization in gene expression and cellular function
- Nuclear organization in development and disease

Deliverables:

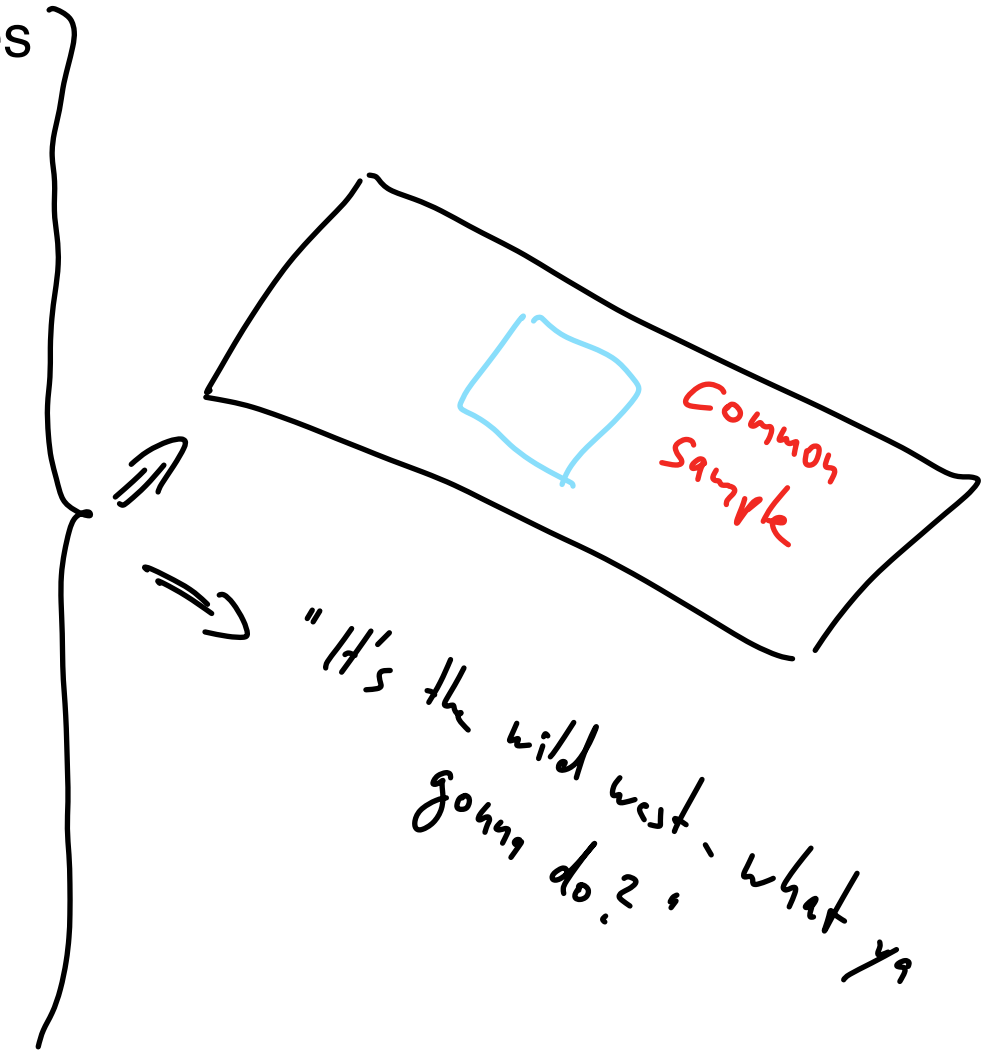
- Community standards and metrics [think ENCODE = Meta data in Genomics]
- Next generation tools
- Reference maps of the 3D genome
- Predictive models of structure/function relationships
- Biological validation through controlled disruption of nuclear architecture and single-cell imaging
- Understanding poorly-characterized nuclear structures and nuclear bodies

Starting Point 2016

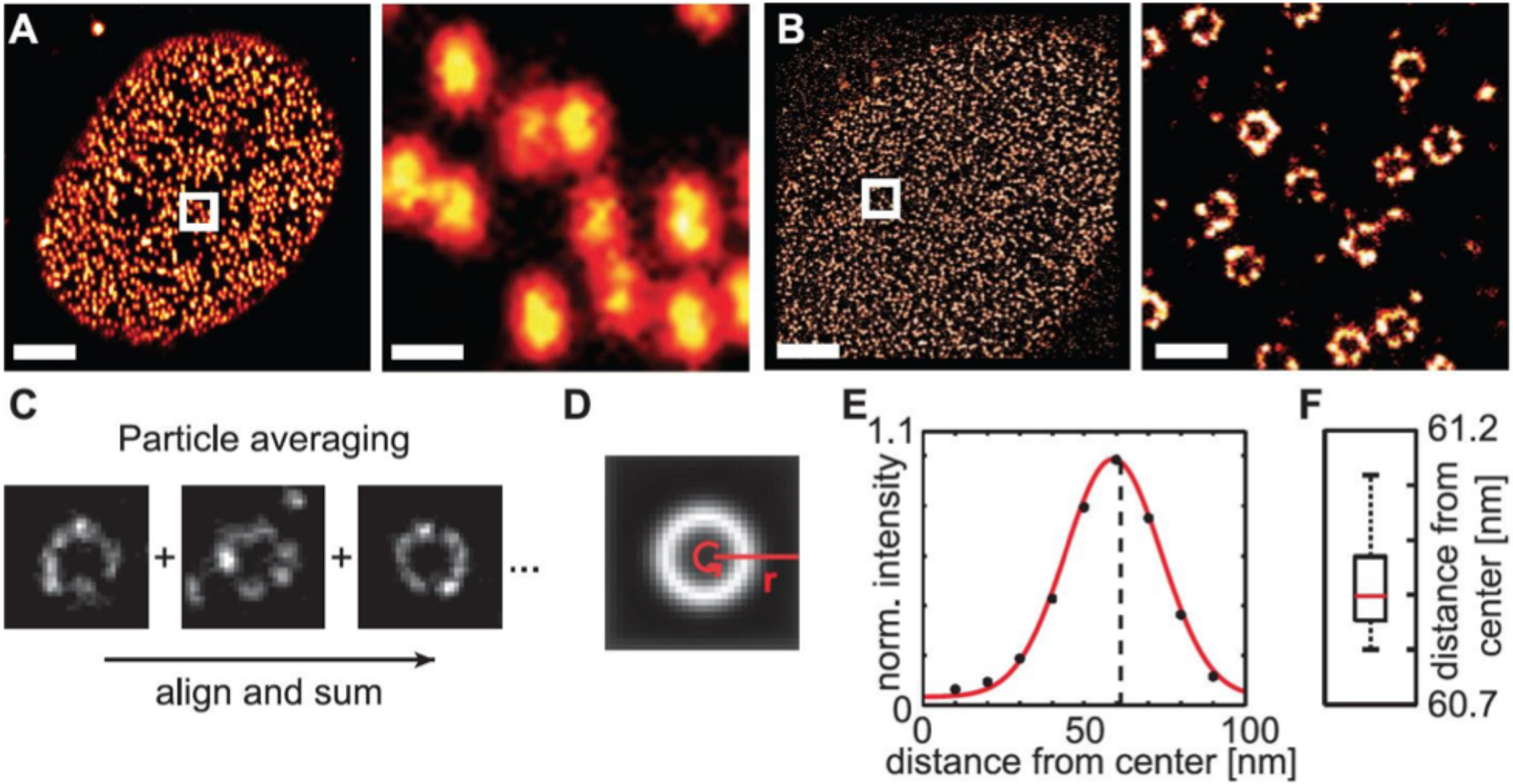
- Large number of imaging techniques
- Multiple platforms per technique
- Frequent custom builds or modifications
- Large performance variance within same product line
- Largely different protocols
- No metadata standard
- Data Coordination and Integration Center working off ENCODE, *little imaging experience*

Starting Point 2016

- Large number of imaging techniques
- Multiple platforms per technique
- Frequent custom builds or modifications
- Large performance variance within same product line
- Largely different protocols
- No metadata standard
- Data Coordination and Integration Center working off ENCODE, *little imaging experience*



Make Imaging Data Comparable - The Common Sample



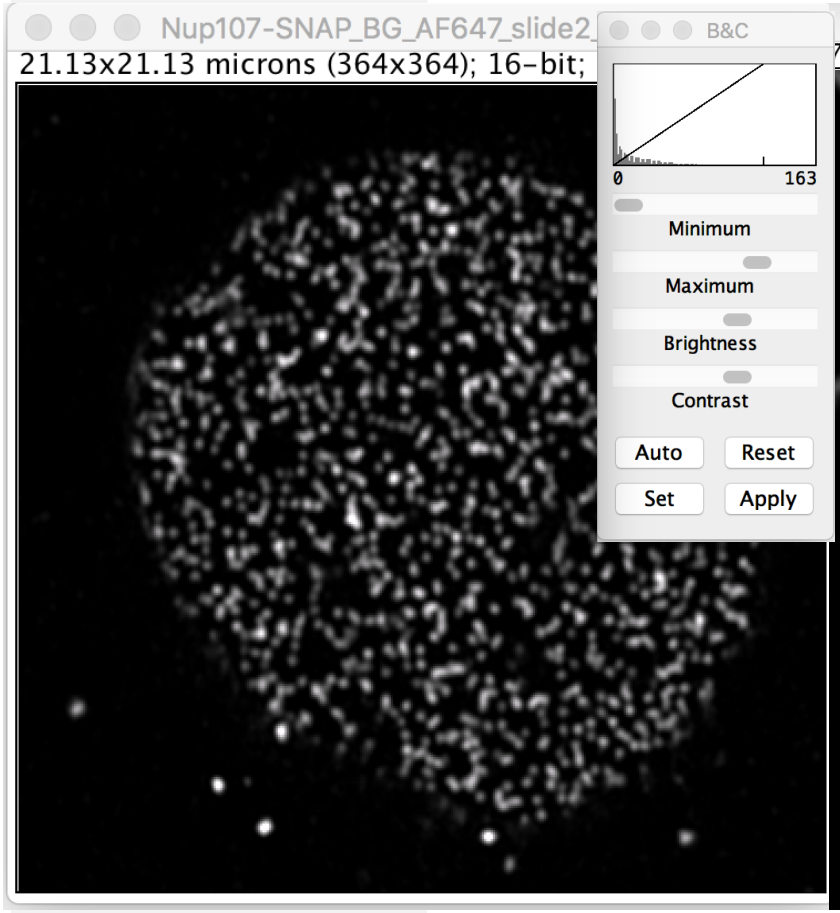
Szymborska, et al., *Science* 341: 655-658, 2013

The Common Sample

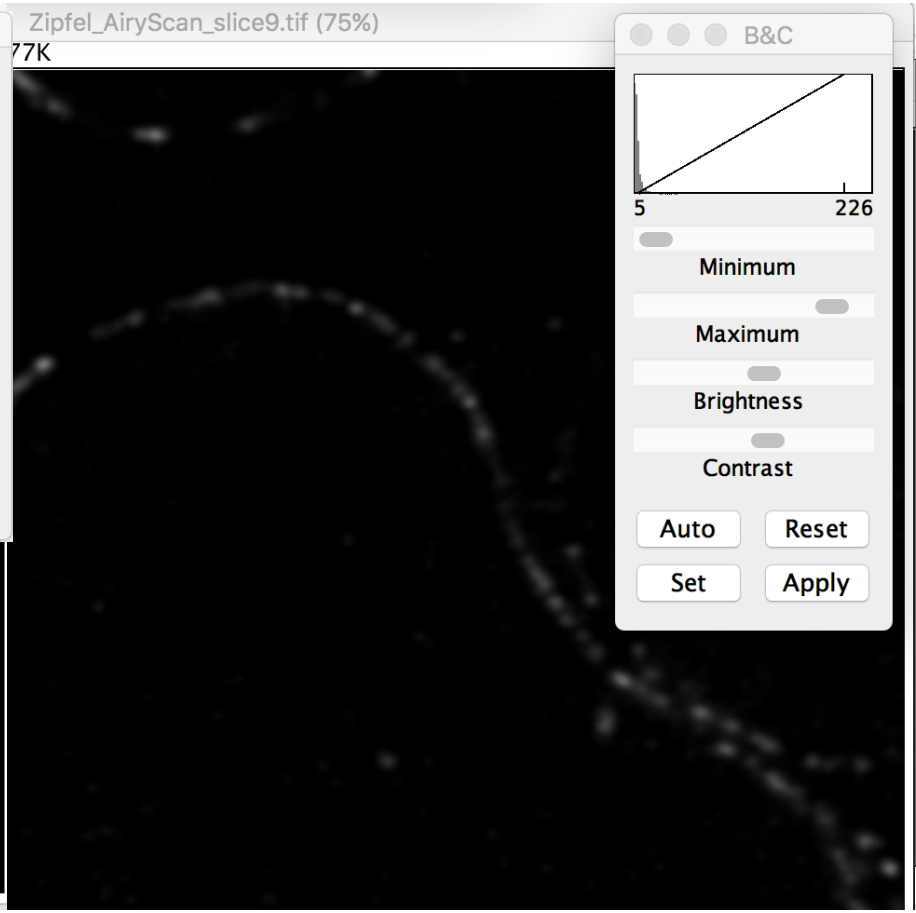
Introduction

Standardized Slide

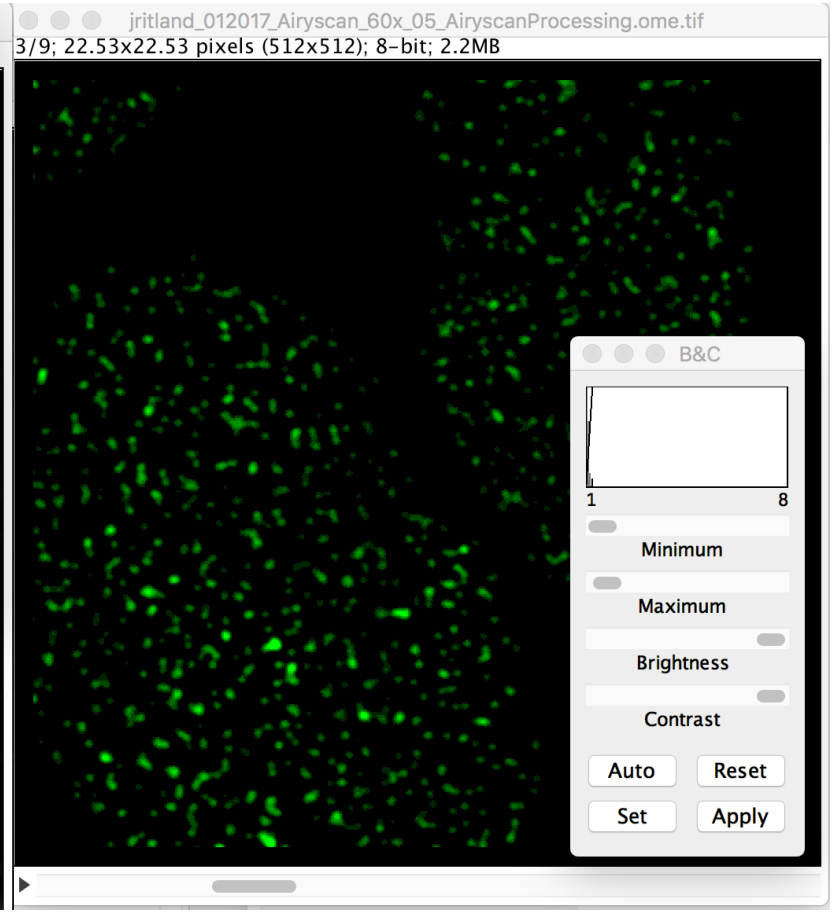
Example: Airy Scan



EMBL – Airy Scan processed



Cornell – Airy Scan Processed



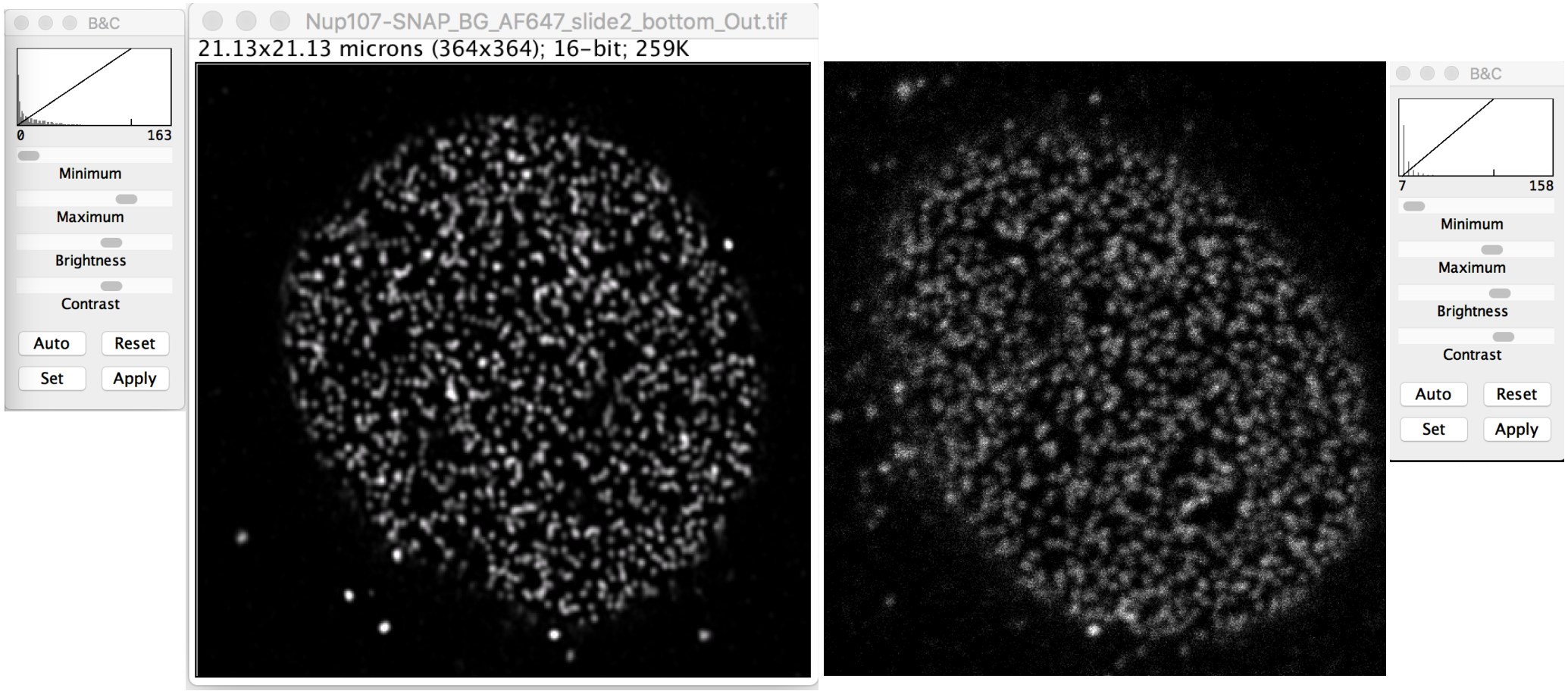
Fred Hutch – Airy Scan processed

The Common Sample

Introduction

Standardized Slide

Example: Airy Scan vs. confocal

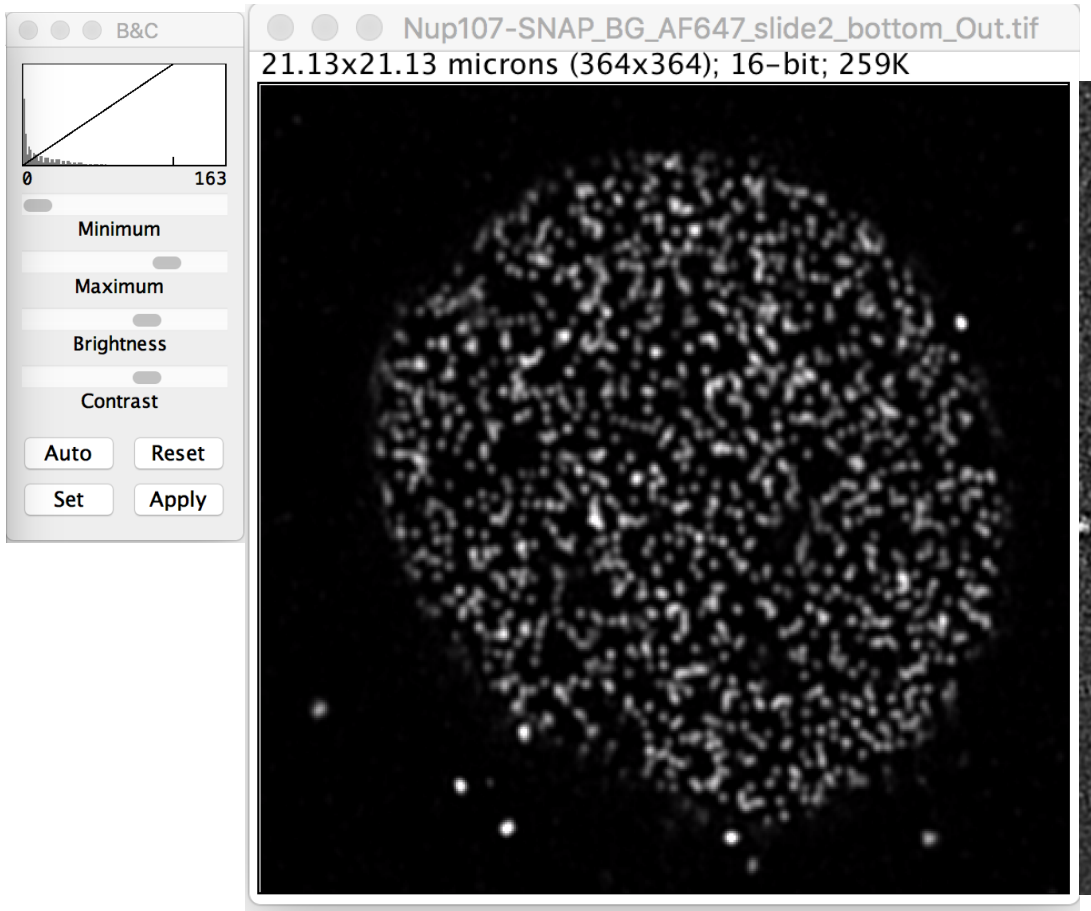


EMBL – Airy Scan processed

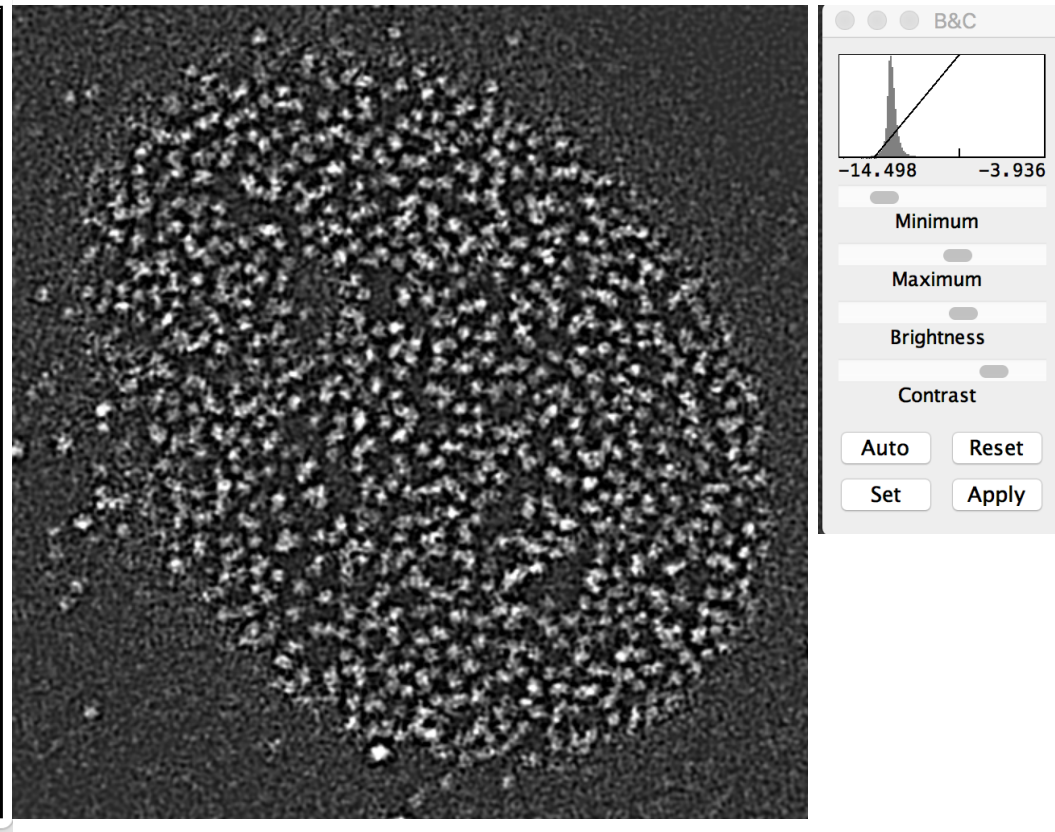
Yale – Confocal

The Common Sample

Example: Airy Scan vs. confocal

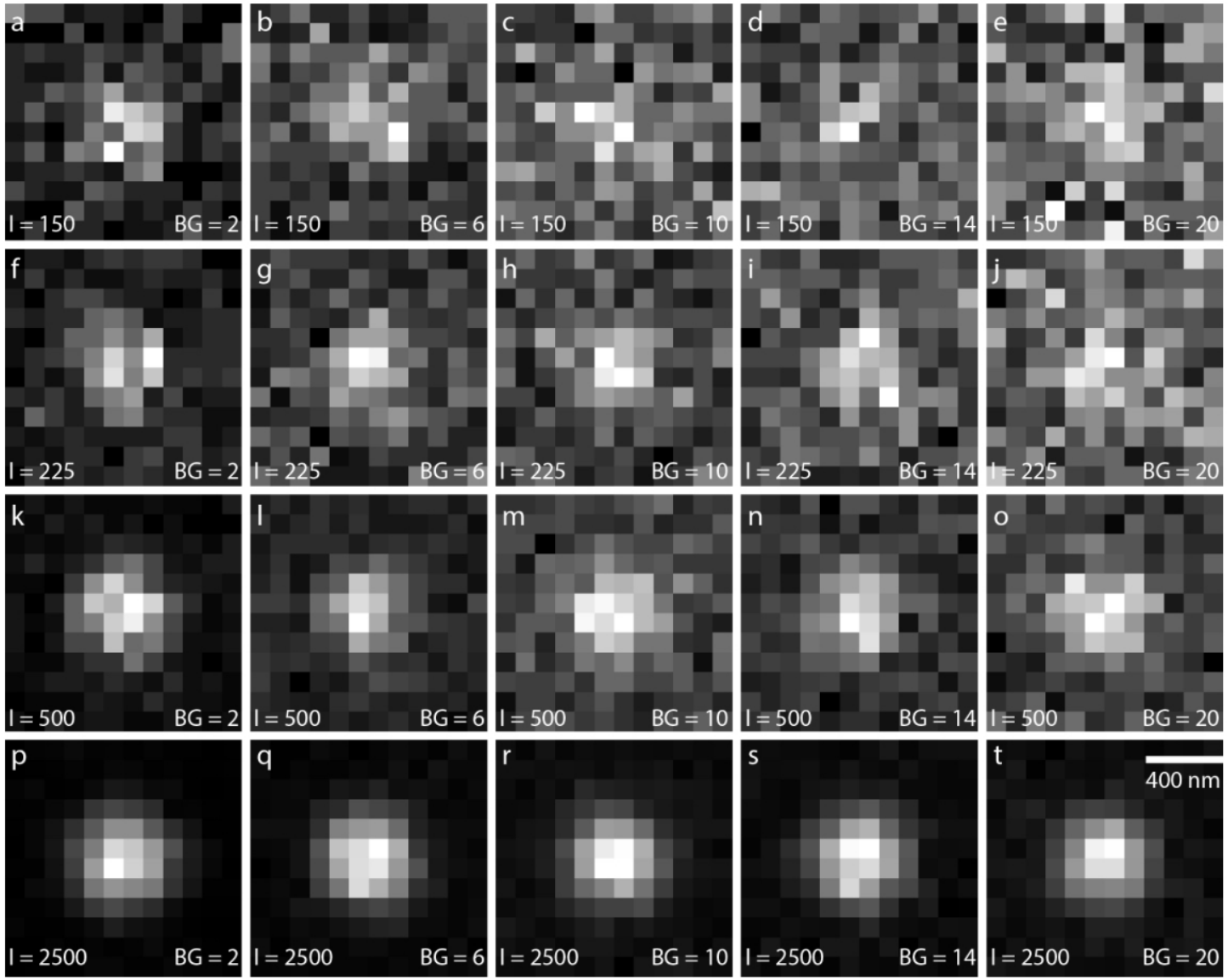


EMBL – Airy Scan processed



Yale – Confocal processed

The Common Sample & The Common Problem:



Is it signal?

The Common Sample & Meta-Data

Introduction

Standardized Slide

Example: Metadata documentation

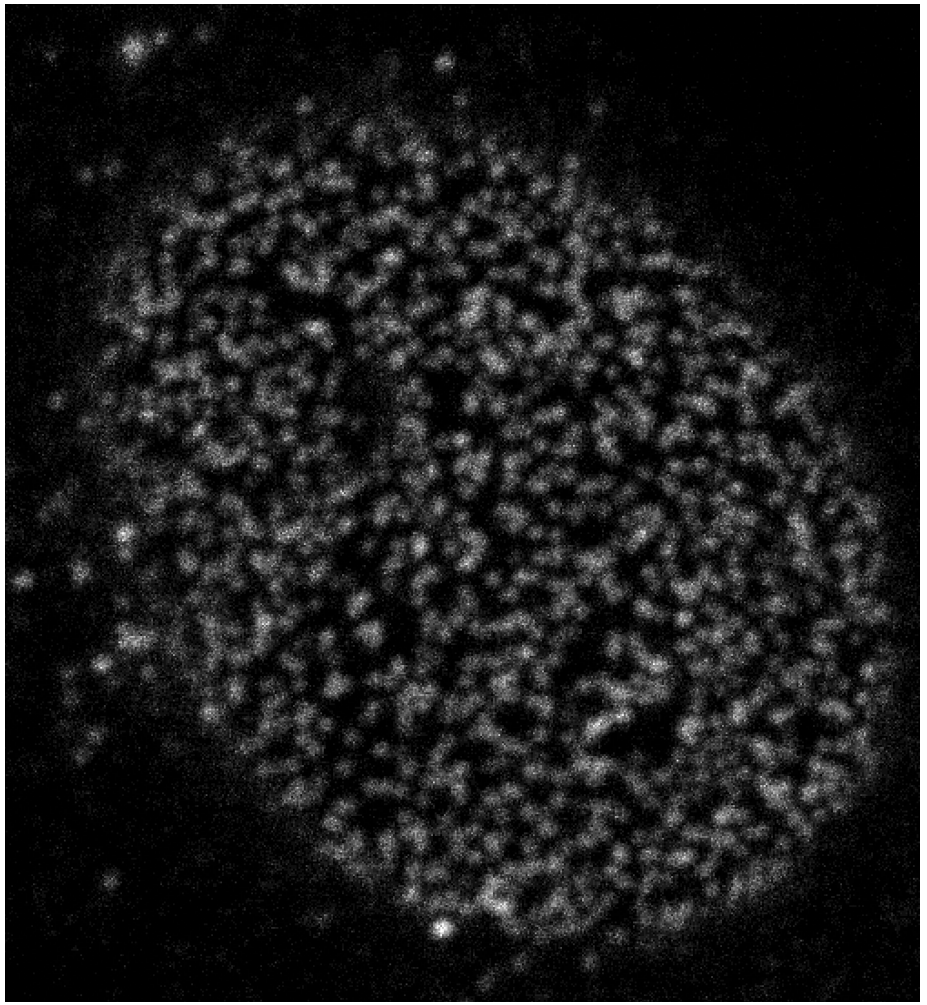
Zeiss Airy EMBL | Olympus Fluoview 1000 | Leica SPE

SIM reconstruction
Cornell

Microscope settings
Cornell

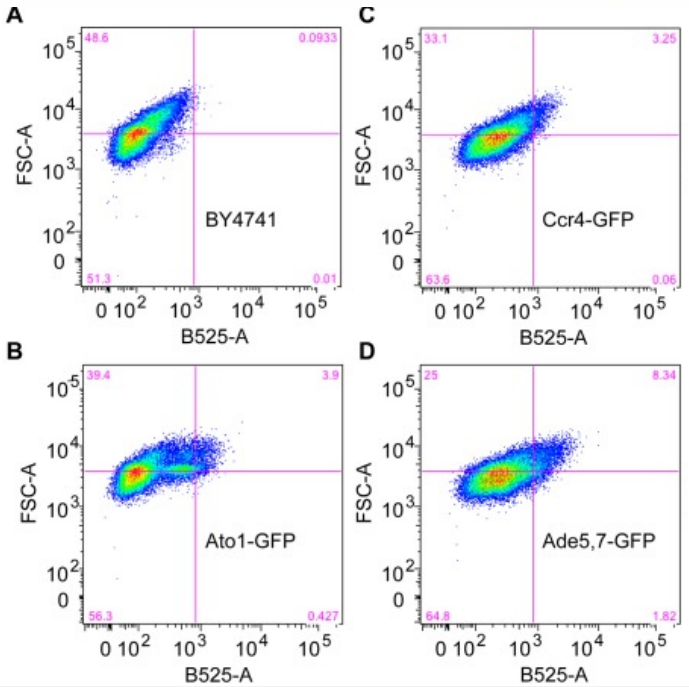
Sample information
Cornell

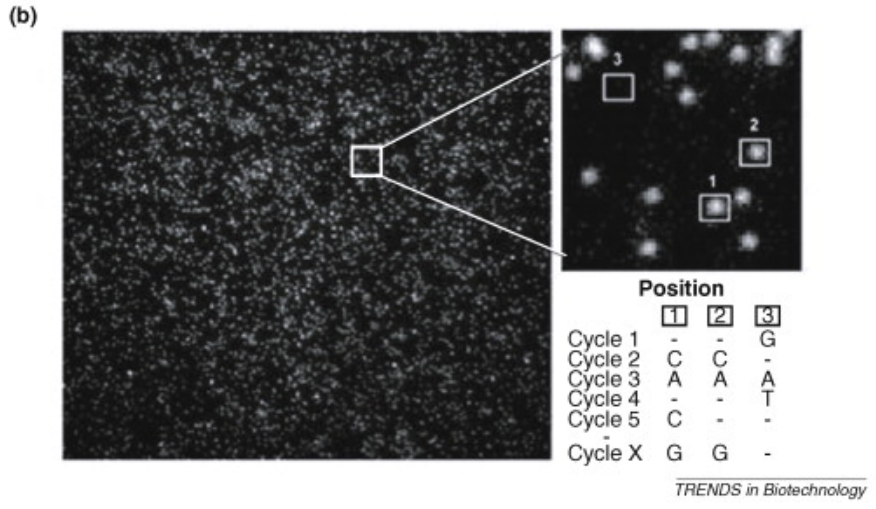
Is where a measurement error in an image?



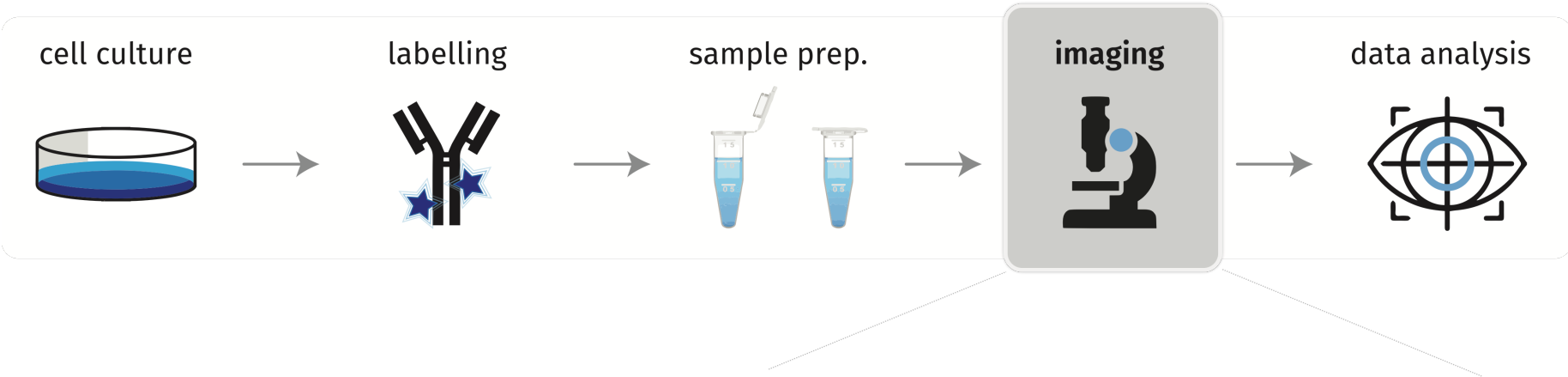
Is where a measurement error in an image?

Is where a measurement error in an image?





Calibrate Imaging Data (Photons)



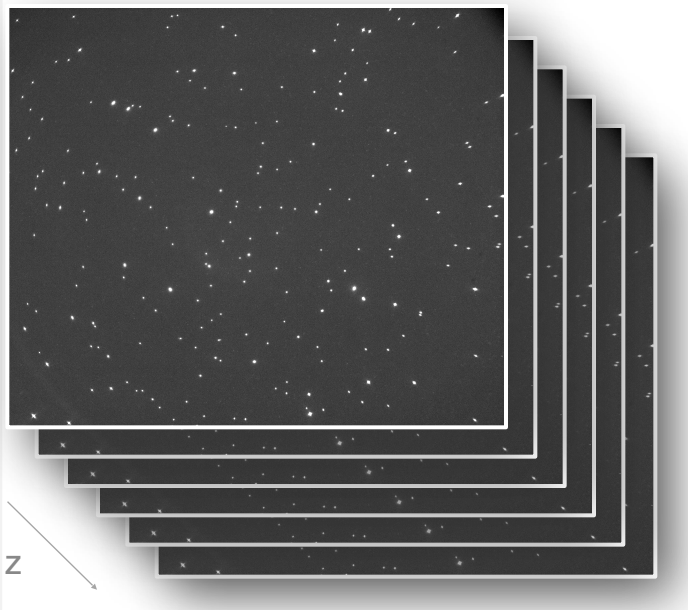
1. Microscope performance
2. Imaging parameters

Calibrate Imaging Data (Photons)

1. Microscope performance
"Image Quality"

Calibrate Imaging Data (Photons)

- Introduction
- Standardized Slide
- Optical Quality

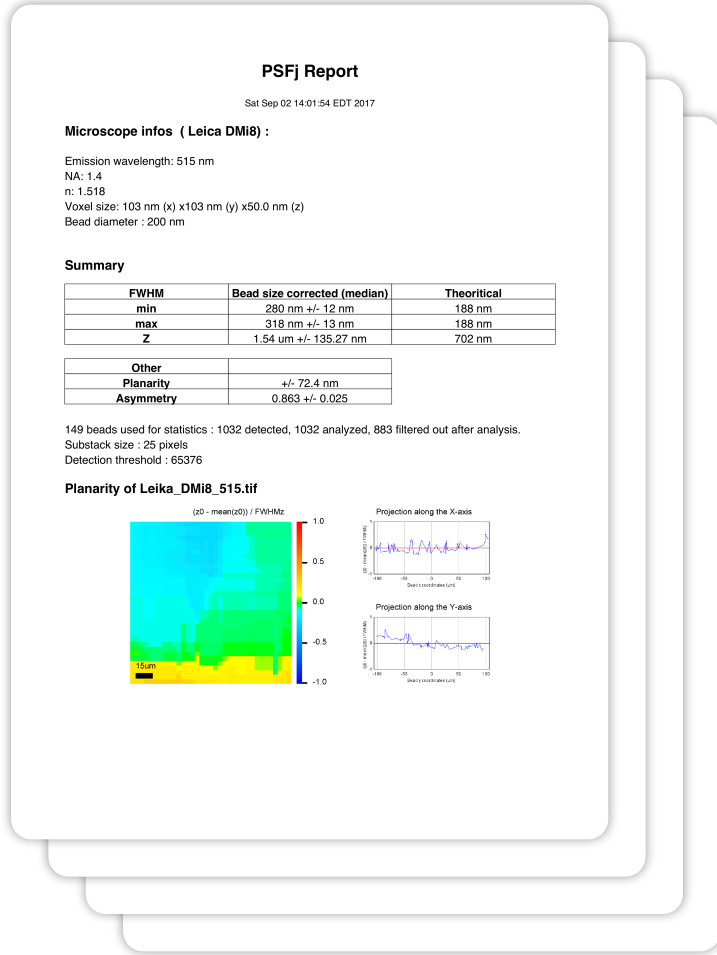


PSFj

Know your fluorescence microscope

KnopLab

PSFj was created by the Knop lab and
is available at *http://www.knoplab.de/psfj*



PSFj Report

Sat Sep 02 14:01:54 EDT 2017

Microscope infos (Leica DMi8) :

Emission wavelength: 515 nm
NA: 1.4
n: 1.518
Voxel size: 103 nm (x) x103 nm (y) x50.0 nm (z)
Bead diameter : 200 nm

Summary

FWHM	Bead size corrected (median)	Theoritical
min	280 nm +/- 12 nm	188 nm
max	318 nm +/- 13 nm	188 nm
Z	1.54 um +/- 135.27 nm	702 nm

Other	
Planarity	+/- 72.4 nm
Asymmetry	0.863 +/- 0.025

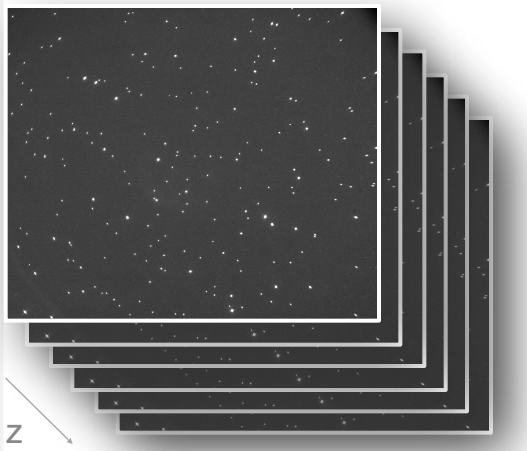
149 beads used for statistics : 1032 detected, 1032 analyzed, 883 filtered out after analysis.
Substack size : 25 pixels
Detection threshold : 65376

Planarity of Leika_DMi8_515.tif

Calibrate Imaging Data (Photons)

- Introduction
- Standardized Slide
- Optical Quality

On going project, used to guide DCIC image upload

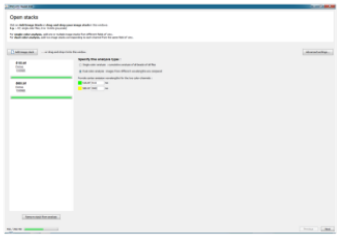


PSFj – short protocol

(PSFj is a Java program, which was developed from Knop Lab (download source: http://www.knoplab.de/psfj/)). A PSFj manual is provided at: http://www.knoplab.de/psfj/online-manual/ where all shown pictures were taken from.)

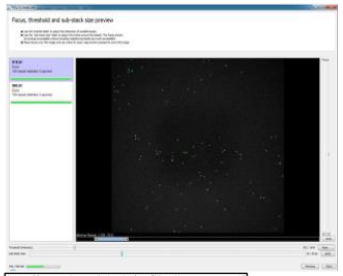
Data you have to provide:

- Image stacks, one file per color in .tif(f) grayscale 8 or 16-bit.
  - If your operating microscope software does not provide an option to save your z-stacks in the necessary format, use ImageJ or FIJI to convert them. Pay attention not to lose data, while converting!
- Step size of your z-stack
- Numerical aperture (NA) of the used objective
- Refractive index of the used objective oil or coverglas
- Objective magnification
- Pixel size of the detector of the used camera
- Size of the used beads



1) After PSFj was started, load your image stacks. When the 2nd stack (color) is loaded choose dual- color analysis and enter the emission wavelengths appropriate to the stack files.
Depending on the excitation wavelength (ex) you find for TetraSpeck™ Fluorescent Microspheres from Thermo Fisher Scientific, which we use, the emission wavelength (em) ex/em = 365⁄430 nm (blue), 505⁄515 nm (green), 560⁄580 nm (orange), and 660⁄680 nm (dark red).
The Advanced settings offer you the opportunity to use 3D localization. To safe your time you should not choose that for the first run.

2) Enter the requested data. Notice in case you have to change names or anything else on that side you have to delete the *.ini files in your working directory and restart the whole process or make these changes manually in an editor.

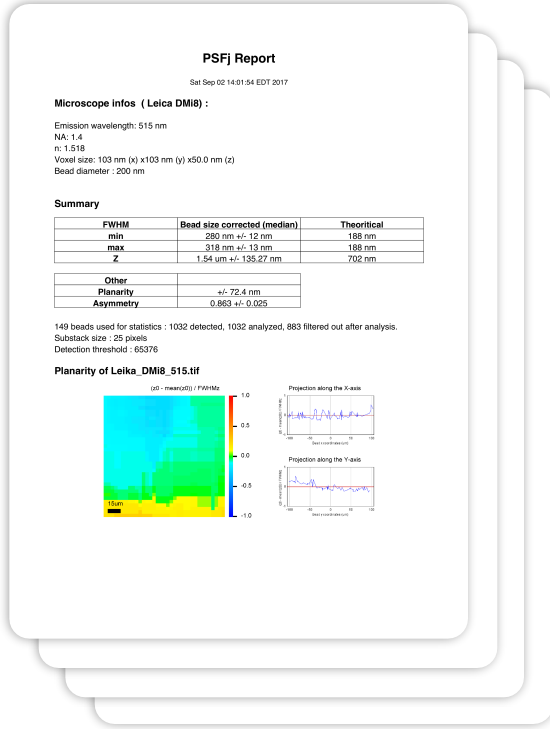


3) The analysis screen gives you the opportunity to inspect your beat pictures, adjust the focal plan (right slider), the background (slider at the bottom of bead picture), the intensity (slider under the picture) and the stack size (slider at the bottom).
The analysis results are dependent from the number of observed beats, that means you have to maximize them. To secure good results make sure, that you optimize the image to noise ratio, while taking the stack images!

4) The result page gives you different report outputs usually the quick PDF report is good to get a idea, how your microscope is performing, but you have also the opportunity to inspect all single beads.
If you run more than one analysis round with the same stacks make sure you rename the report. PSFj will overwrite the last one, without asking.
If you have optimized your setting, rerun PSFj and set the advanced setting to 3D, that will enhance your results.

For more detail have a lock at the PSFj manual

http://www.knoplab.de/psfj/online-manual/



PSFj Report

Sat Sep 02 14:01:54 EDT 2017

Microscope infos (Leica DMi8) :

Emission wavelength: 515 nm
NA: 1.4
n: 1.518
Voxel size: 103 nm (x) x103 nm (y) x50.0 nm (z)
Bead diameter : 200 nm

Summary

FWHM	Bead size corrected (median)	Theoritical
min	280 nm +/- 12 nm	188 nm
max	318 nm +/- 13 nm	188 nm
Z	1.54 um +/- 135.27 nm	702 nm

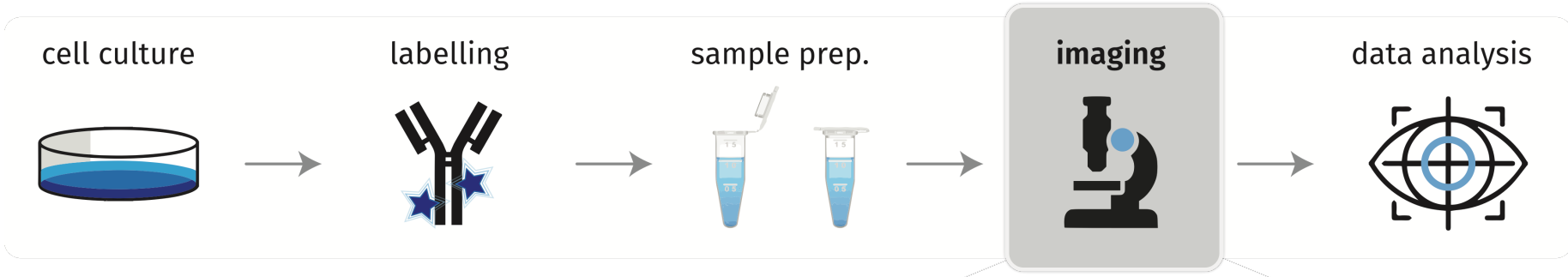
Other	
Planarity	+/- 72.4 nm
Asymmetry	0.863 +/- 0.025

149 beads used for statistics : 1032 detected, 1032 analyzed, 883 filtered out after analysis.
Substack size : 25 pixels
Detection threshold : 65376

Planarity of Leika_DMi8_515.tif

https://sites.google.com/a/4dnucleome.org/4d-nucleome-wiki/working-groups-list/imaging-data-standards-wg/microscope-calibration-project/optical-calibration-documents

Calibrate Imaging Data (Photons)

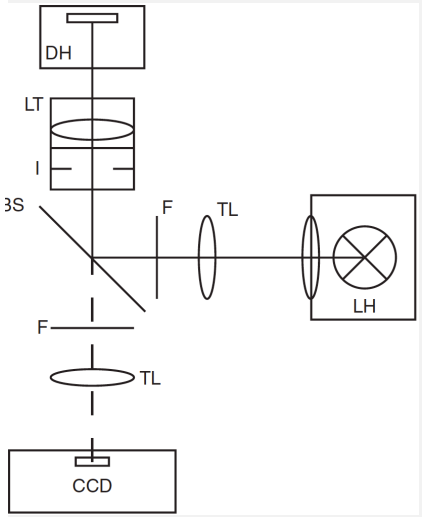


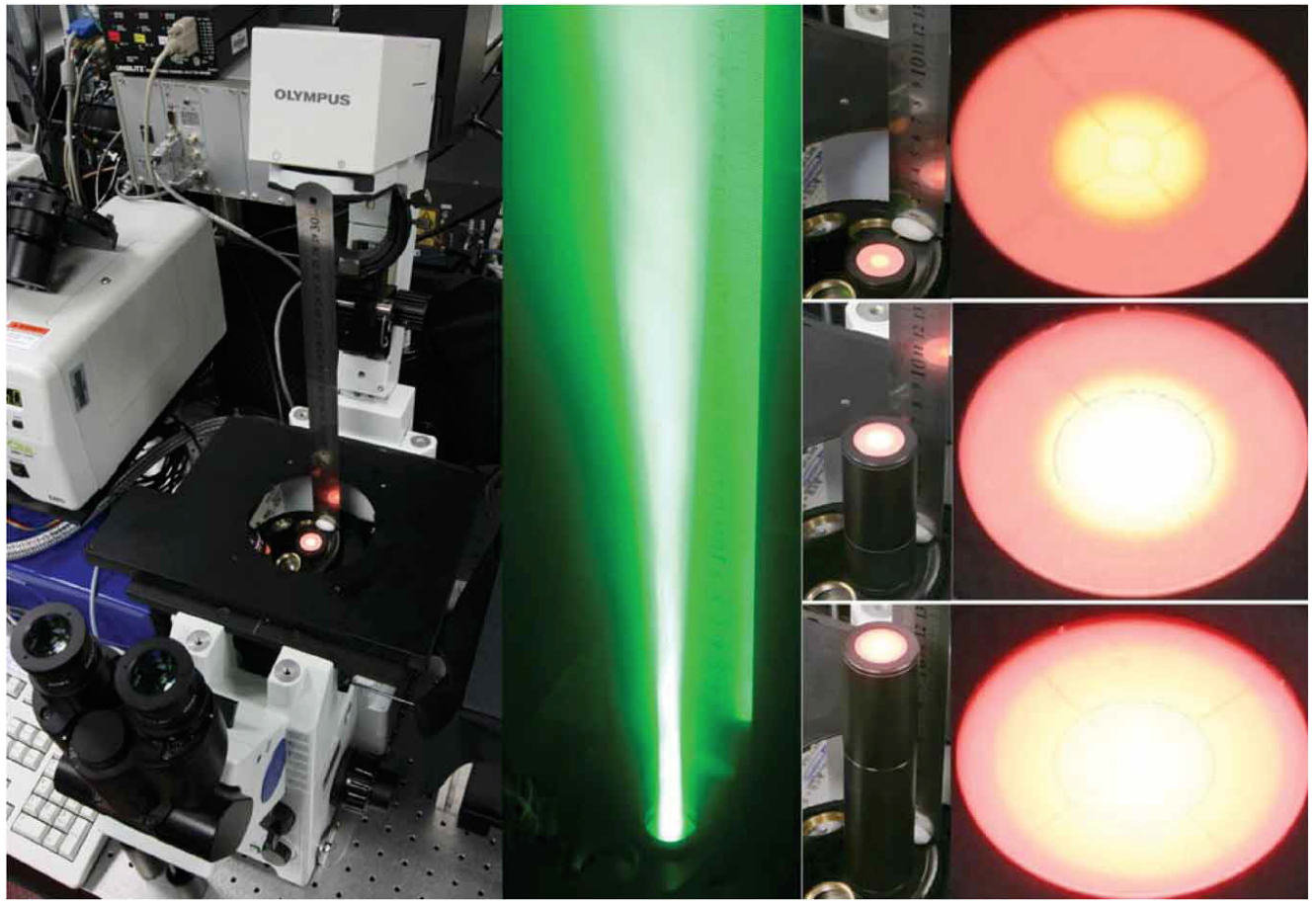
1. Microscope performance
"Image Information Content"

- Hypothesis: A microscope can be fully described as a 'photon in' – 'photon out' counter
- Can compare a wide range of microscopes at the raw data level
- Noise models for most detectors exist, but measuring power is to complex for 'normal' labs – even many physics labs

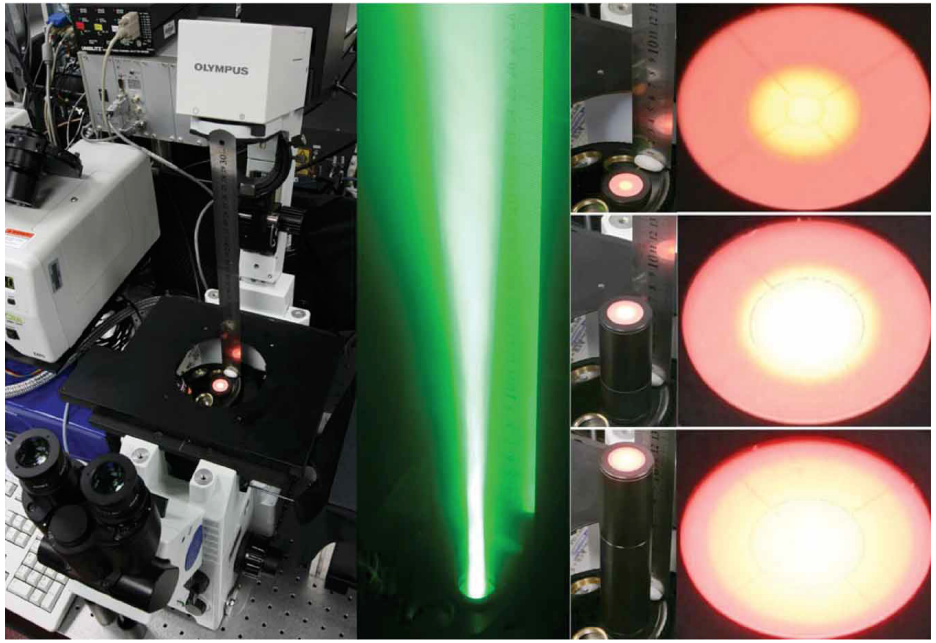
Calibrate Imaging Data (Photons)

- Introduction
- Standardized Slide
- Optical Quality
- ‘Photonic’ Quality





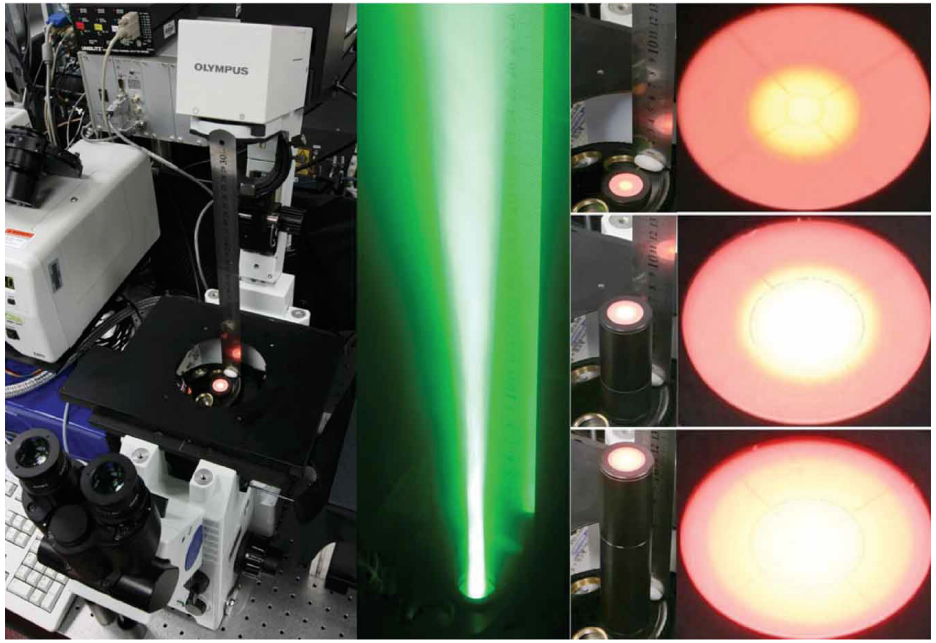
Calibrate Imaging Data (Photons)



What if ... we had a device with these features:

- Fits in objective mount
- Measures light (power) from lamp, laser, …
- Is a calibrated light source,
  - multi-color,
  - homogenous profile
  - linear intensity ramp
  - Possibly with minimal user input

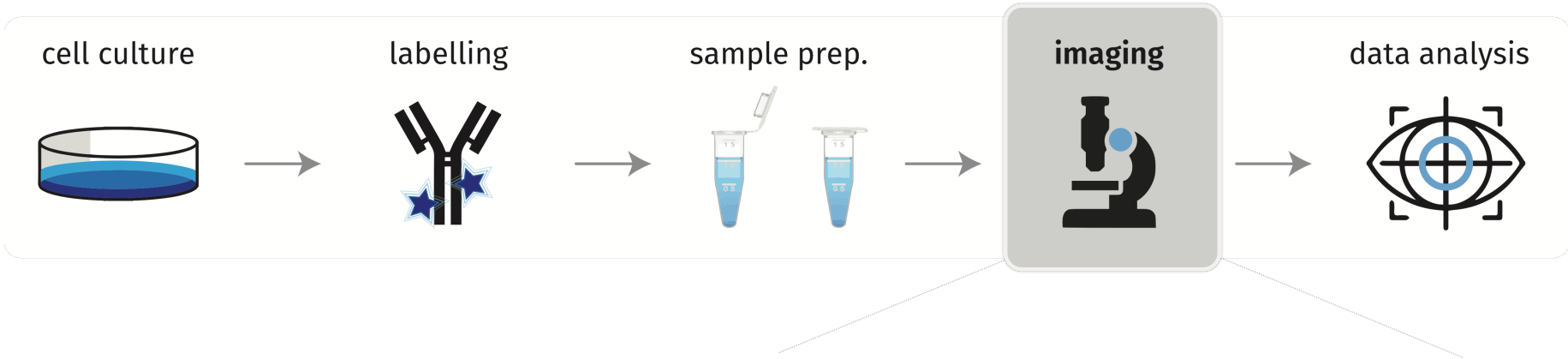
Calibrate Imaging Data (Photons)



What if ... we had a device with these features:

- Fits in objective mount
- Measures light (power) from lamp, laser, …
- Is a calibrated light source,
  - multi-color,
  - homogenous profile
  - linear intensity ramp
  - Possibly with minimal user input

Calibrate Imaging Data (Documentation)



1. Microscope performance
2. Imaging parameters

Calibrate Imaging Data (Documentation)

- Introduction
- Standardized Slide
- Optical Quality
- ‘Photonic’ Quality
- Meta Data

Zeiss Airy EMBL | Olympus Fluoview 1000 | Leica SPE

SIM reconstruction
Cornell

Microscope settings
Cornell

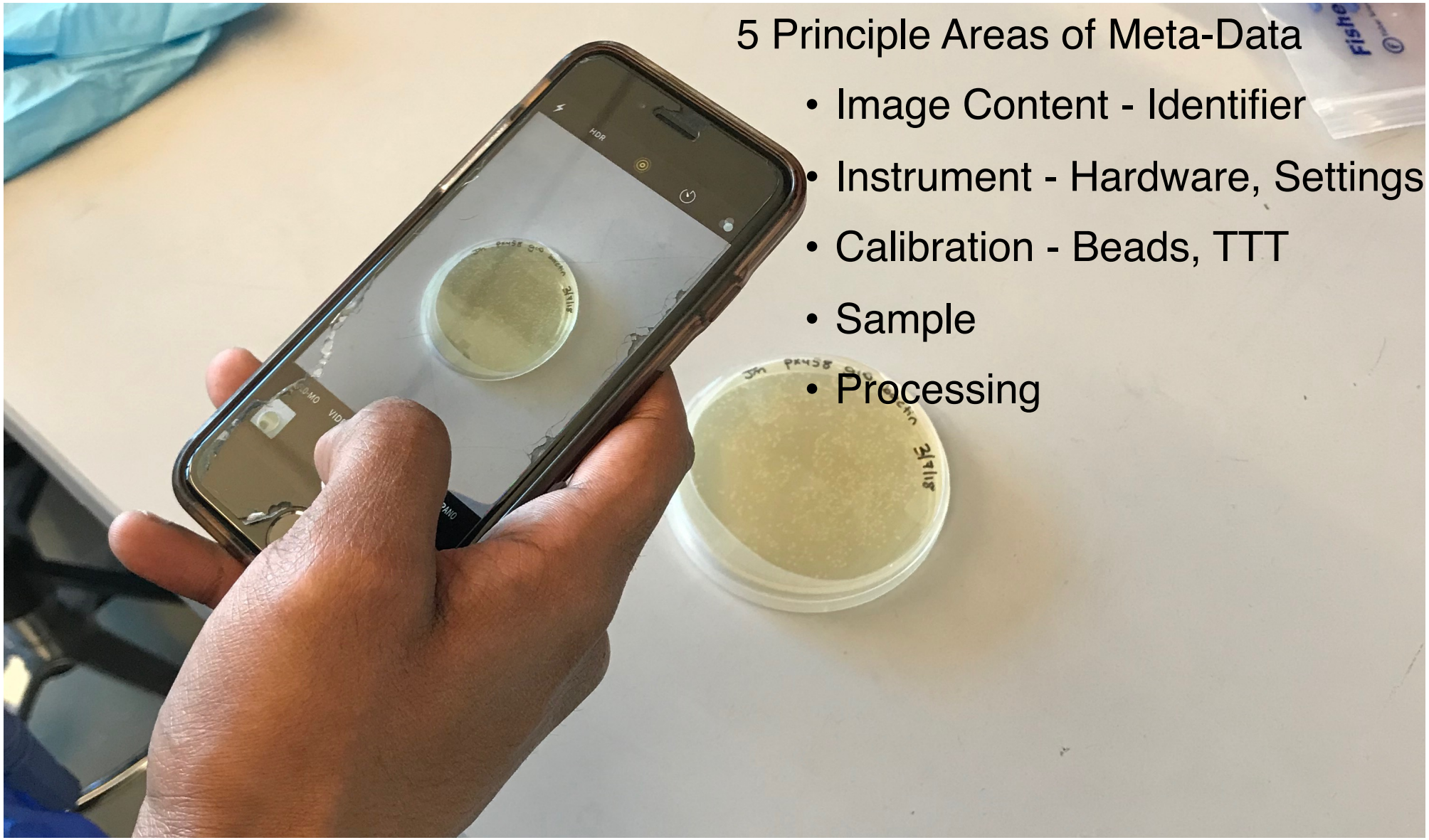
Sample information
Cornell

Intend Matters - Not All Images are Equal

- Introduction
- Standardized Slide
- Optical Quality
- ‘Photonic’ Quality
- Meta Data

Intend Matters - Not All Images are Equal

- Introduction
- Standardized Slide
- Optical Quality
- ‘Photonic’ Quality
- Meta Data

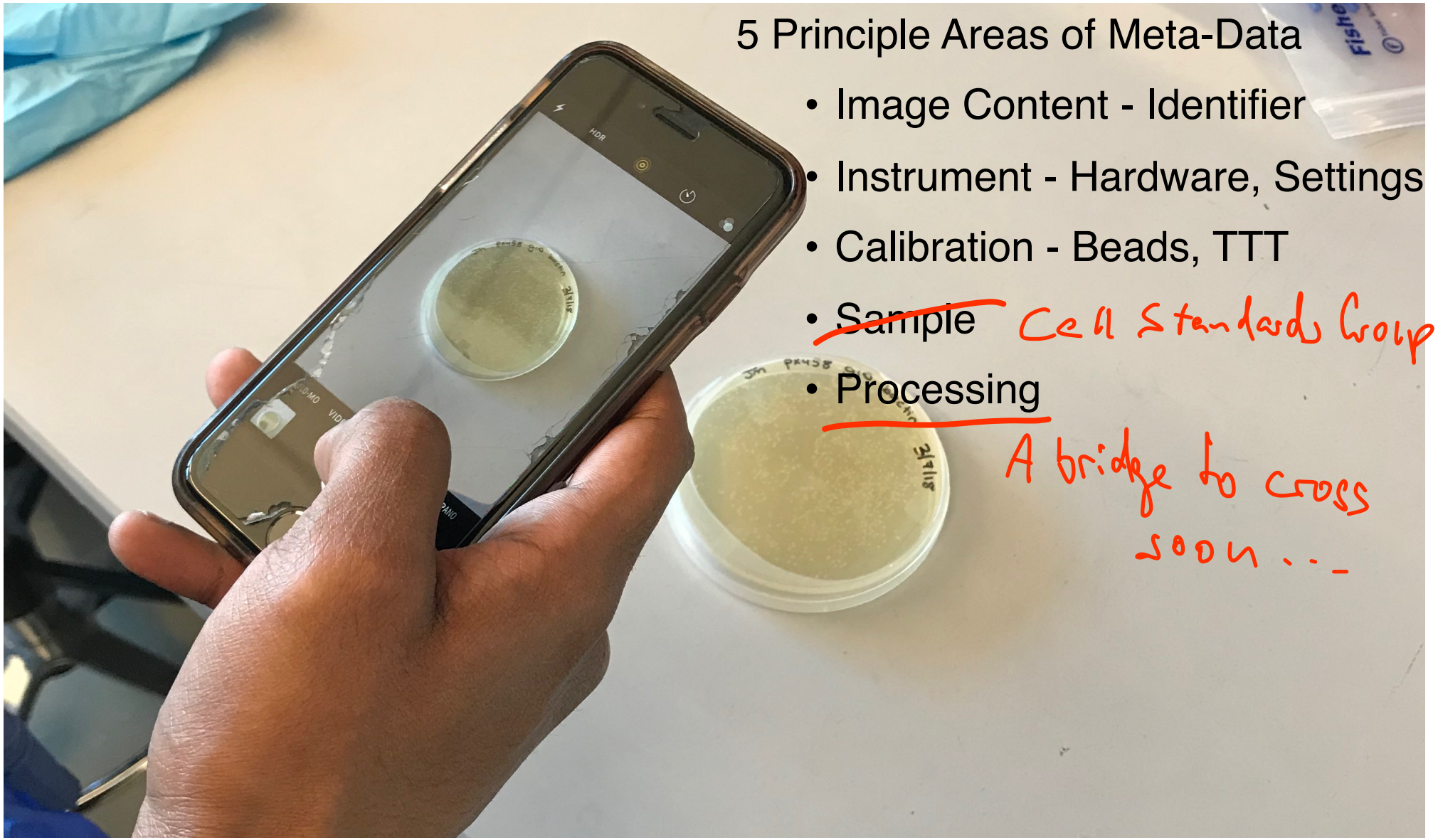


5 Principle Areas of Meta-Data

- Image Content - Identifier
- Instrument - Hardware, Settings
- Calibration - Beads, TTT
- Sample
- Processing

Intend Matters - Not All Images are Equal

- Introduction
- Standardized Slide
- Optical Quality
- ‘Photonic’ Quality
- Meta Data



5 Principle Areas of Meta-Data

- Image Content - Identifier
- Instrument - Hardware, Settings
- Calibration - Beads, TTT
- ~~Sample~~ Cell Standards Group
- Processing

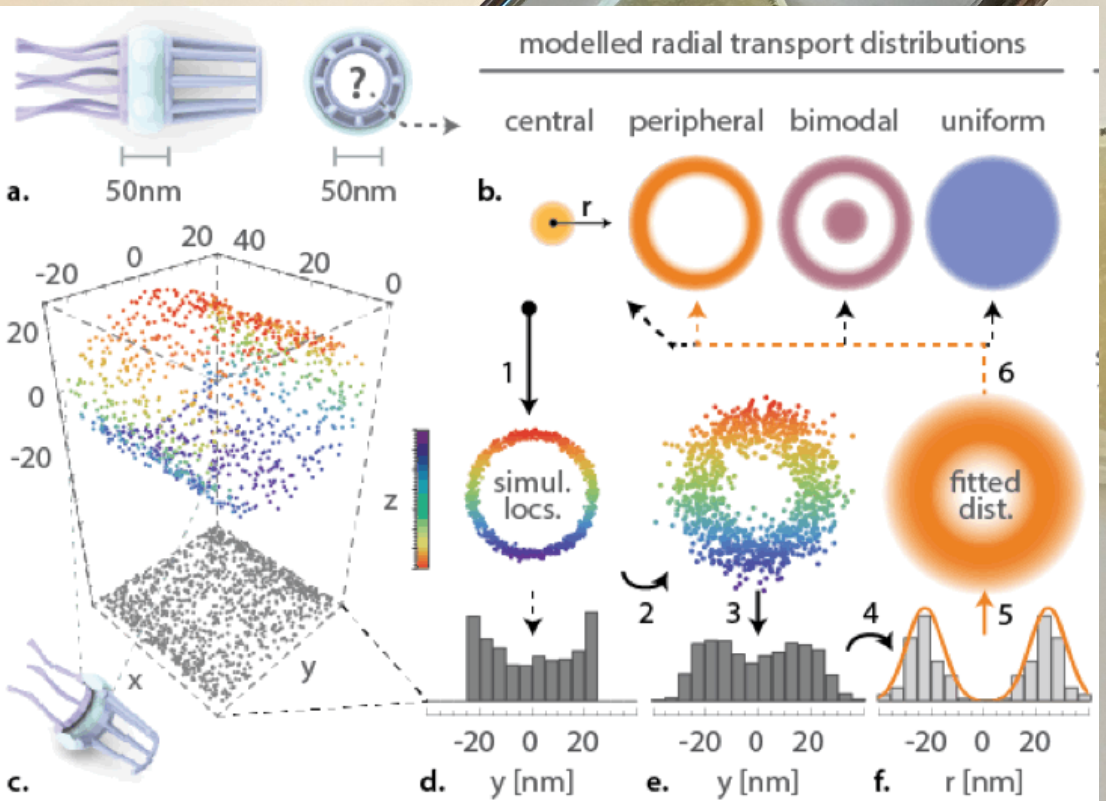
A bridge to cross soon...

Intend Matters - Not All Images are Equal

5 Principle Areas of Meta-Data

- Image Content - Identifier
- Instrument - Hardware, Settings
- Calibration - Beads, TTT
- ~~Sample~~ Cell Standards Group
- Processing

A bridge to cross soon...



Calibrate Imaging Data (Documentation)

- Not all Images are Equal (in intend)
- Tier System

Tier name	Description	Example Experiments	**Required metadata** (examples)
D1	**Descriptive 1** Show large-scale effects	- Transfection control - live/dead test	- microscope type - labeling technique - scale bar
D2	**Descriptive 2** Feature counting / confirmation	- cell counting - large domain/foci counting	*D1 requirements* + pixel size + illumination source
A1	**Analytical 1** Count small objects in cells	- single-molecule FISH - distance measurements	*D2 requirements* + excitation power + exposure / gain settings
A2	**Analytical 2** Limited signal / adv. processing	- SM-tracking in live cells - super-resolution	*A1 requirements* + PSF fitting algorithm used + photon statistics
P	**Pioneering** New, unproven technology	- new technique - new gold-standard	*A2 requirements* + replicate statistics + ...

https://docs.google.com/spreadsheets/d/1P5BwH2xnNQJSdivNxaAq6KpiFMFL6hwRxubh8bkLQFo/edit#gid=0

The 4 + 1 Tier System

- Descriptive 1
  - Large scale effects (e.g. Transfection Control)
  - Normally taken only once
  - Typically single image or image stack
  - Minor supporting evidence to manuscript or project
- Descriptive 2
  - Count features that are identifiable without any doubt, some statistical analysis
  - Taken in replicates as single images/stacks or image multi-dimensional time series
  - Not diffraction limited
  - Confirm or support other data

Meta-Data for Descriptive 1 Level

Category	Metadata key	Description
Image	pixel size	the dimensions of pixels in the image
Image	z-sampling	Distance between optical planes
Image	time step	the time step between images / volumes in a series
Image	time stamp	The time at which the image was taken
Image	unique ID	A unique ID which identifies this image, even if file names change
Image	imager	The person who acquired the image
Image	Channel encoding	Order in file, e.g. t,z,ch1-n

Category	Metadata key	Description
Instrument	Brand	Manufacturer, development
Instrument	Objective	Brand
	Objective	N.A.
	Objective	Magnification
	Objective	special designation (e.g. TIRF, …)
Instrument	Fluorescence light source(s)	Manufacturer
	Fluorescence light source(s)	Type
	Fluorescence light source(s)	center wavelength
	Detector(s)	Camera/ Confocal/etc..
Instrument	Modality	WF, confocal, TIRF etc…

Category	Metadata key	Description
Sample	preparer	The person who prepared the sample
Sample	preparation date	
Sample	cell line / tissue type	
Sample	labelling	What is labelled in each color channel
Sample	protocol	a link to the protocol used for sample preparation
Sample	fixation	the fixation method used
Sample	embedding	The embedding medium

Additional Meta-Data for Descriptive 2 Level (D1 +)

Category	Metadata key	Description
Instrument	Objective	Immersion Medium
Instrument	Filter Sets	Ch 1:
	Filter Sets	Ch 2:
	Filter Sets	Ch n:
Instrument	Detector(s)	Manufacturer
Instrument	integration time	the length of time the image is exposed to light for a single frame

Category	Metadata key	Description
Sample	embedding	Refractive index of embedding medium (nominal)

The 4 + 1 Tier System

- Analytical 1 (This is the smFISH level)
  - Small objects are counted, advanced analysis possible (e.g. fitting, noise models)
  - Typically fixed cells, or non-diffraction limited live cell imaging
  - Data reproducible within narrow margin, comparability between labs wanted
  - Major data set in project, e.g. production level
- Analytical 2
  - Live cell tracking, any advanced analysis
  - 'Super-resolution' imaging
  - Data should be fully portable
  - Experiments aim at correlating Chromatin Capture and imaging data

Additional Meta-Data for Analytical 1 Level (D2 +)

Category	Metadata key	Description
Instrument	Objective	working distance
	Objective	Field Number
Instrument	Fluorescence light source(s)	Power output nominal
	Fluorescence light source(s)	Lasers/diodes per channel
	Fluorescence light source(s)	Coupling (e.g. fiber, light guide, direct)
Instrument	Filter Sets	Manufacturer(s)
Instrument	camera model	Specific model, chip, etc.
Instrument	camera serial #	Camera serial number

Category	Metadata key	Description
Calibration	dark value	the value reported for no incoming light
Calibration	photometric conversion	conversion factor to convert incoming counts to photons
Calibration	read noise	the amplitude of read noise
Calibration	illumination intensity	Light intensity/power at the sample
Calibration	camera dark map	an image describing the pixel by pixel variations in dark level

Category	Metadata key	Description
Calibration	camera read map	an image describing the pixel by pixel variations in read noise
Calibration	3D multi-color bead stack	
Calibration	PSFJ report	
Calibration	Test Tube	Other Test Tube parameters

Additional Meta-Data for Analytical 2 and Pioneering Level (A1 +)

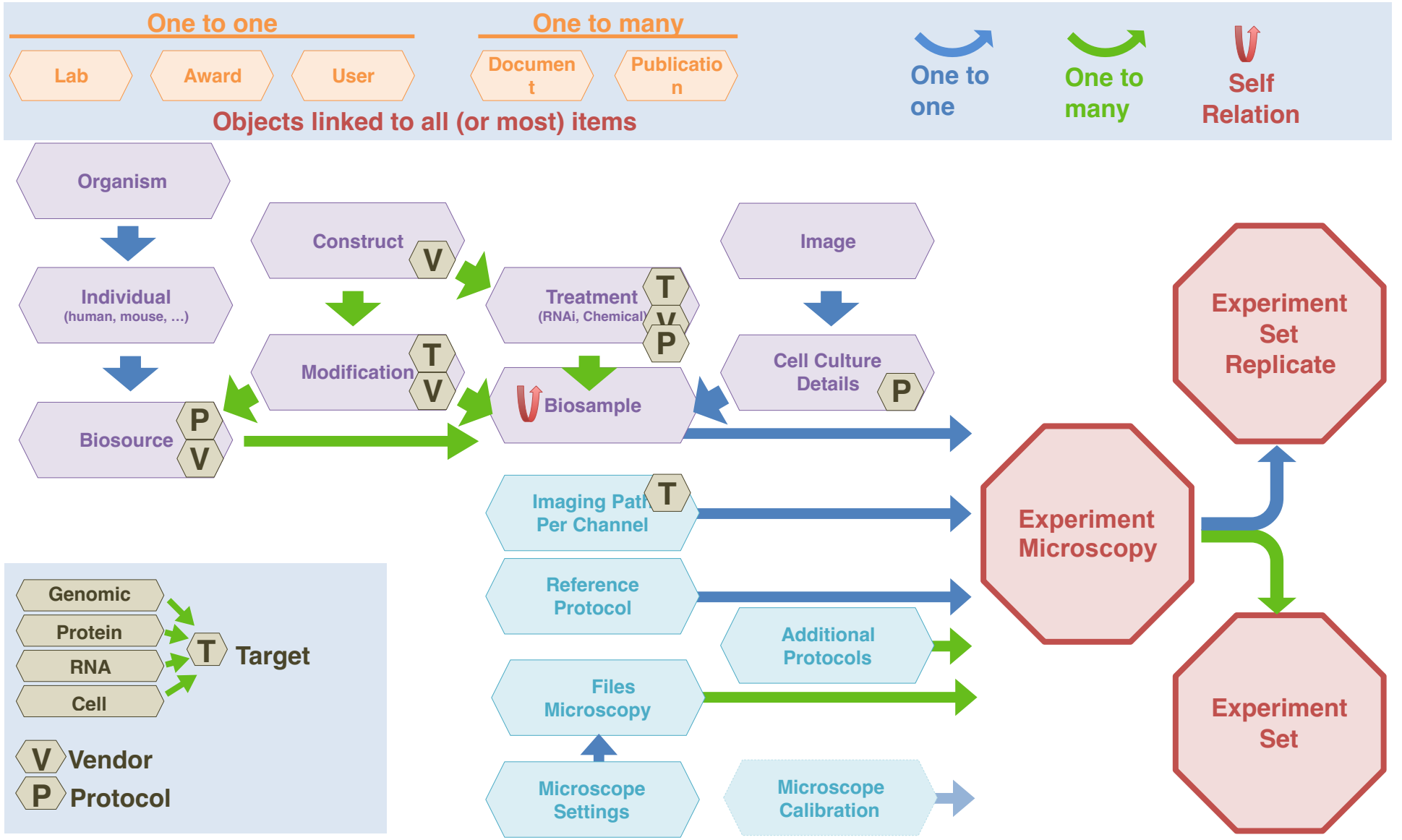
Category	Metadata key	Description
Instrument	Objective	Immersion Refractive Index (measured)
Instrument	Fluorescence light source(s)	Coupling (e.g. fiber, light guide, direct)
Calibration	***Imaging standard***	***e.g. Argolight, Geller MRS4, DNA origami***
Sample	embedding	Refractive index of embedding medium (measured)

The 4 + 1 Tier System

- Pioneering
  - New Technologies
  - New Gold Standards
  - Image Analysis not yet fully established
  - Meant to be analyzed in multiple contexts
  - Meant to be analyzed by multiple groups inside and outside of the 4DN consortium

Microscopy Experiment Structure

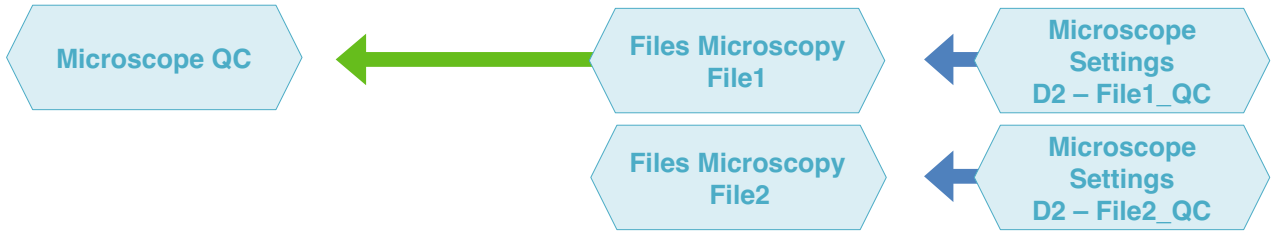
- Introduction
- Standardized Slide
- Optical Quality
- ‘Photonic’ Quality
- Meta Data
- DCIC image data portal



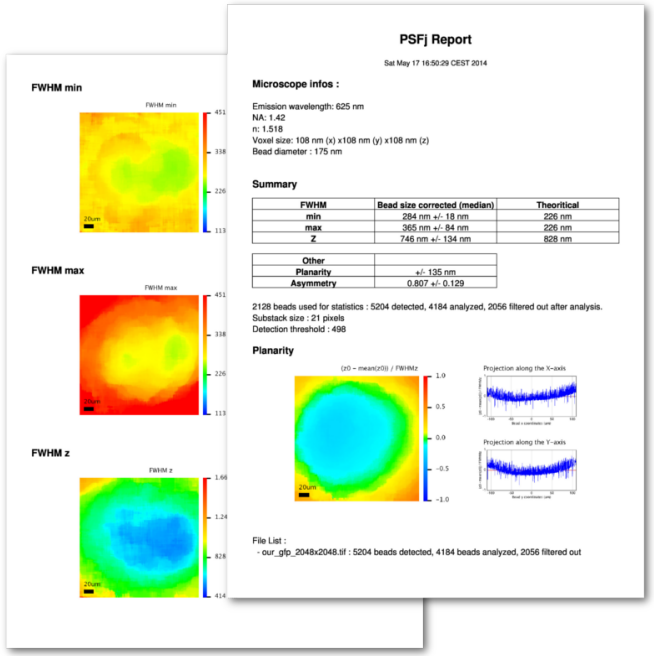
Calibrate Imaging Data (Beads Example)

- Introduction
- Standardized Slide
- Optical Quality
- ‘Photonic’ Quality
- Meta Data
- DCIC image data portal

Monthly checks for microscope by Z stack images of fluorescent beads analyzed by PSFj



Description	**DCIC QC March 2018**
bead_diameter	200
Magnification	100
objective_na	1.49
pixel_size	6.5
refractive_index	1.52
z_plane_distance	0.1
files_in_set	File1, File2
Attachment	PSFj Result PDF



Imaging Path

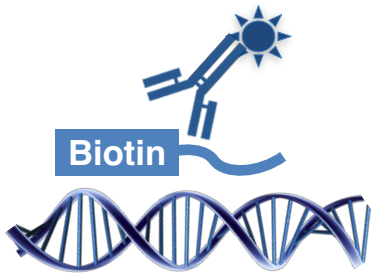
- Introduction
- Standardized Slide
- Optical Quality
- ‘Photonic’ Quality
- Meta Data
- DCIC image data portal

Solution for condensing information spread out in Biosource, Biosample and Experiment protocols

Experiment

- Biosample
- Labeling Path
- Protocol
- Files

CH01	Target-Probes-Label
CH02	Target-Probes-Label
CH03	Target-Probes-Label

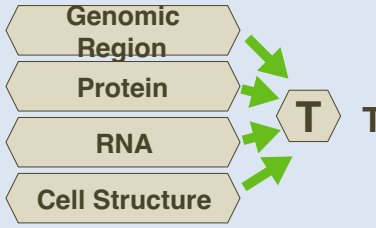


Label: Atto647N

Labeled Probe: Anti Biotin Antibody

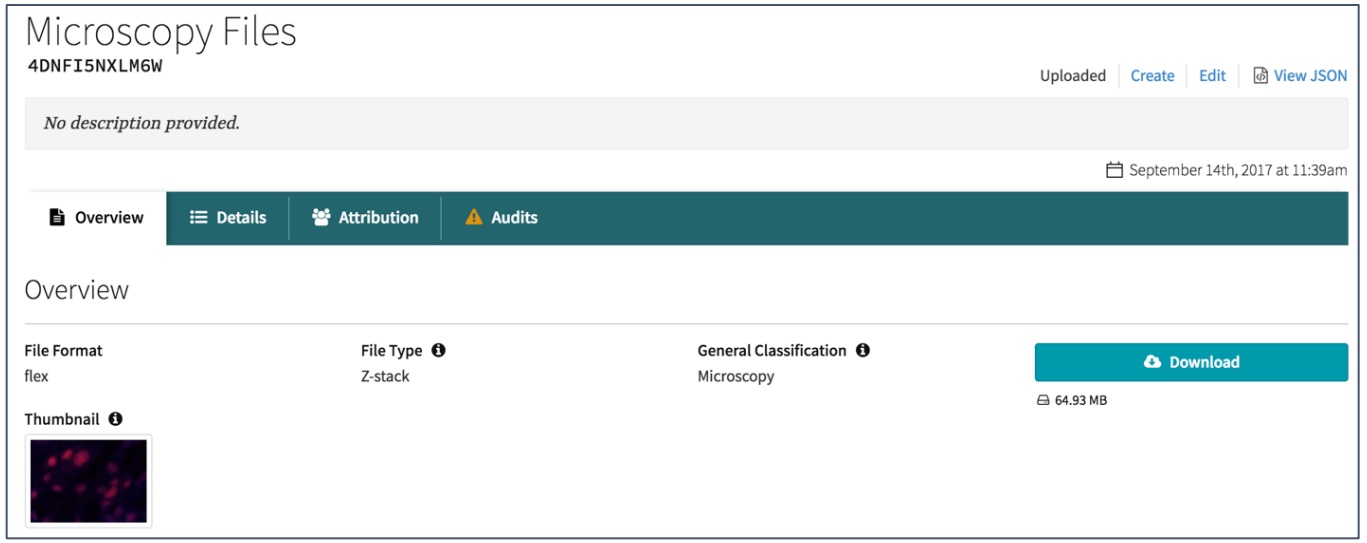
Other Probes: Biotinylated RNA probe set

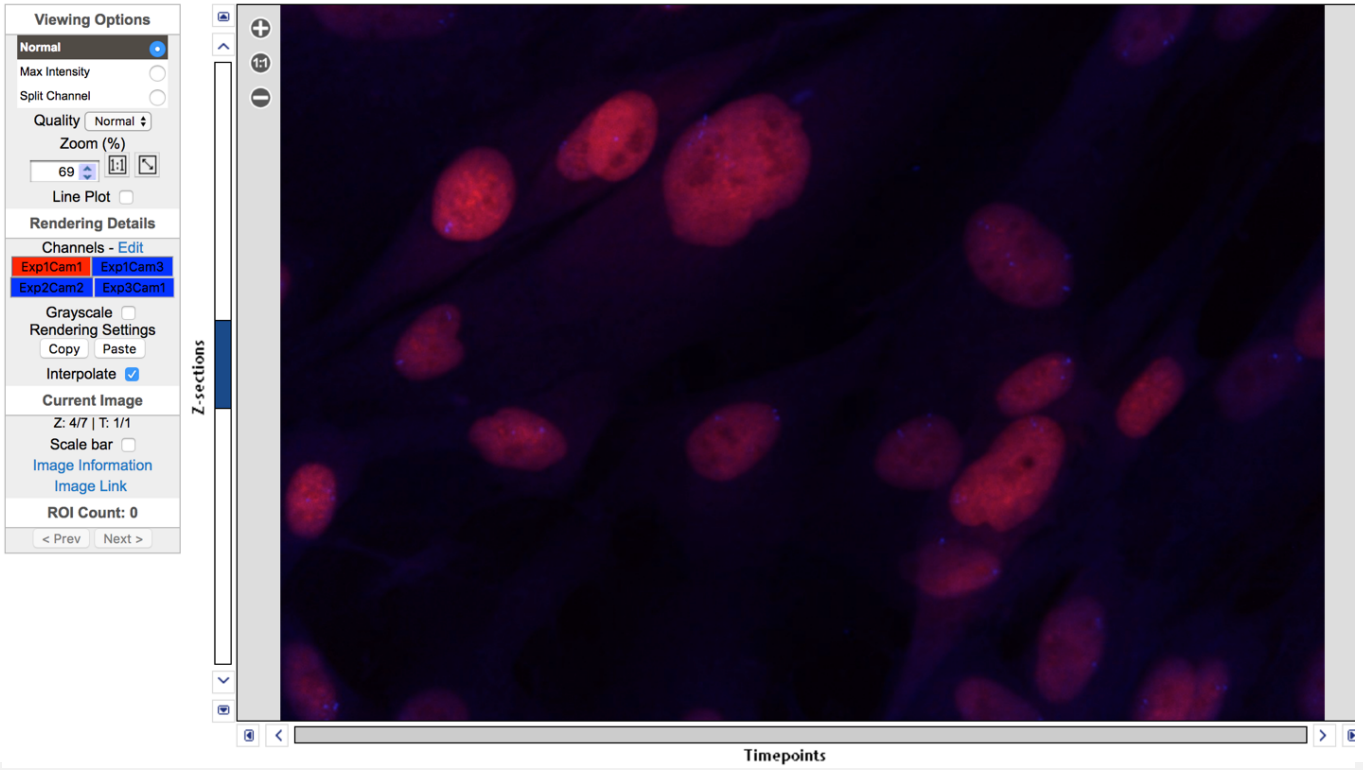
Target : LADF gene



Genomic Region
genome_assembly
chromosome
start_coordinate
end_coordinate
location_description
start_location
end_location

- Introduction
- Standardized Slide
- Optical Quality
- ‘Photonic’ Quality
- Meta Data
- DCIC image data portal





4DN Imaging Experiments

- Introduction
- Standardized Slide
- Optical Quality
- ‘Photonic’ Quality
- Meta Data
- DCIC image data portal

Microscopy Experiments **DNA-FiSH on HFF-hTERT**

DNA-FiSH | **4DNEXAZENHCT**

Released To Project | Clone | Create | Edit | View JSON

October 11th, 2017 at 5:36pm

Source Publication >

Elizabeth Finn et al. (2017) Heterogeneity and Intrinsic Variation in Spatial Genome Organization
Elizabeth Finn, Gianluca Pegoraro, et al., *bioRxiv* 2017

Properties

Experiment Type
DNA-FiSH

Follows SOP
No

Biological Sample
4DNBSMYCWH9U

Biosources
HFF-hTERT - 4DNSRIX5KE1M

Modifications summary
None

Treatments summary
None

Imaging Paths

ch00	Chromosomes targeted by DAPI	405nm
ch01	GRCh38:1:61560944-61746907 targeted by Cy5 labeled BAC	640nm
ch02	GRCh38:1:2370445-2570720 targeted by Alexa568 labeled BAC	568nm
ch03	GRCh38:1:12708720-12865744 targeted by Alexa488 labeled BAC	488nm

Microscopy Technique
CLSM

Microscope QC Analysis
None

Experiment Sets | Raw Files | Attribution | Details | Audits

40 Raw Files

Data Submission Process

- Introduction
- Standardized Slide
- Optical Quality
- ‘Photonic’ Quality
- Meta Data
- DCIC image data portal

Data Submission with Excel Workbooks

	A	B	C	D	E	F	G	H	I	J	K
1	#Field Name:	aliases	description	attachment	bead_diameter	files_in_set	magnification	objective_na	pixel_size	refractive_index	z_plane_distance
2	#Field Type:	array of string	string	object	number	array of Item:FileMicroscopy	number	number	number	number	number
3		grunwald_lab:sample_image_qc_1	Example imaging qc	final_report.pdf	200	grunwald_lab:sample_image_1, grunwald_lab:sample_image_2	100	1.49	6.5	1.52	0.1

FileSetMicroscopeQc | FileMicroscopy | MicroscopeSettingD2

	A	B	C	D	E	F	G
1	#Field Name:	aliases	description	*file_format	file_type	microscope_settings	filename
2	#Field Type:	array of string	string	string	string	Item:MicroscopeSetting	string
3		grunwald_lab:sample_image_1	MicroscopeQC Image 1	tiff	z-stack	grunwald_lab:sample_microscope_qc_d2_1	/Users/koray/Github/Submit4DN/david/515.tiff
4		grunwald_lab:sample_image_2	MicroscopeQC Image 2	tiff	z-stack	grunwald_lab:sample_microscope_qc_d2_2	/Users/koray/Github/Submit4DN/david/580.tiff

FileSetMicroscopeQc | FileMicroscopy | MicroscopeSettingD2

	A	B	C	D	E	F	G	H	I	J	K	L
1	#Field Name:	aliases	description	ch00_detecto	ch00_detecto	ch00_filter_a	ch00_filter_s	ch00_integra	ch00_light_s	ch00_light_s	ch00_light_s	ch01_detec
2	#Field Type:	array of string	string	string	string	string	string	string	number	string	string	string
3		grunwald_lab:sample_microscope_qc_d2_1	Test microscope tier D2 info	leica A002	spectral detector		BP 530-585 F	1 sec	406	Leica argon la	laser	
4		grunwald_lab:sample_microscope_qc_d2_2	Test microscope tier D2 info	leica A002	spectral detector		BP 365/12 FT	1 sec	510	Leica argon la	laser	

FileSetMicroscopeQc | FileMicroscopy | MicroscopeSettingD2

Copy paste rows and columns

Use formulas for enumerations and concatenation

Data Submission Process

- Introduction
- Standardized Slide
- Optical Quality
- ‘Photonic’ Quality
- Meta Data
- DCIC image data portal

Uploading Excel Workbook

```
koray — -bash — 80×24
koray@TecRef-LT-E49055:~$ pip install submit4dn
```

```
koray@TecRef-LT-E49055:~/Desktop/Gianluca$ import_data 160302_Misteli_Submission_V3_test.xls
Running on:       https://data.4dnucleome.org/
Submitting User:  4dndcic@gmail.com
Submitting Lab:   /labs/4dn-dcic-lab/
Submitting Award: /awards/1U01CA200059-01/

##############   DRY-RUN MODE   ################
Since there are no '--update' or '--patchall' arguments, you are running the DRY-RUN validation
The validation will only check for schema rules, but not for object relations
##############   DRY-RUN MODE   ################

PROTOCOL(1)                :  0 posted / 0 not posted       0 patched / 1 not patched, 0 errors
PUBLICATION(0)             :  0 posted / 0 not posted       0 patched / 0 not patched, 0 errors
BIOSOURCE(0)               :  0 posted / 0 not posted       0 patched / 0 not patched, 0 errors
GENOMICREGION(7)           :  0 posted / 0 not posted       0 patched / 7 not patched, 0 errors
TARGET(7)                  :  0 posted / 0 not posted       0 patched / 7 not patched, 0 errors
BIOSAMPLECELLCULTURE(1)    :  0 posted / 0 not posted       0 patched / 1 not patched, 0 errors
BIOSAMPLE(1)               :  0 posted / 0 not posted       0 patched / 1 not patched, 0 errors
MICROSCOPESETTINGA1(1)     :  0 posted / 0 not posted       0 patched / 1 not patched, 0 errors
```

Acknowledgements

- **Grunwald Lab**
  - Max Huisman
  - Mathias Hammer
- **DCIC**
  - Burak Alver
  - Koray Kirli
- **NIH**
  - Richard Conroy
  - John Satterlee
  - Judy Mietz
- **External**
  - Caterina Strambio de Castillia
- **'Lock-in' Gang**
  - Brian English
  - Warren Zipfel
  - Robert Singer
  - Jörg Bewersdorf
  - David Baddeley
  - Joan Ritland-Politz
  - Mathias Hammer
  - Max Huisman
  - David Grunwald
- **Imaging Work Group of 4DN**
  - *Joan Ritland* (co-chair)
  - Robert Singer (co-chair)
  - David Grunwald (co-chair)
  - Lock-in Gang
  - Gianluca Pegoraro
  - Jonas Ries
  - And *all other members*