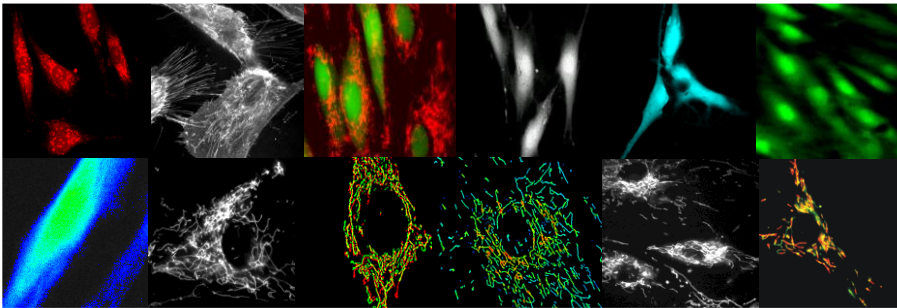




Standardized quantification of mitochondrial morphology and function in living patient fibroblasts using microscopy

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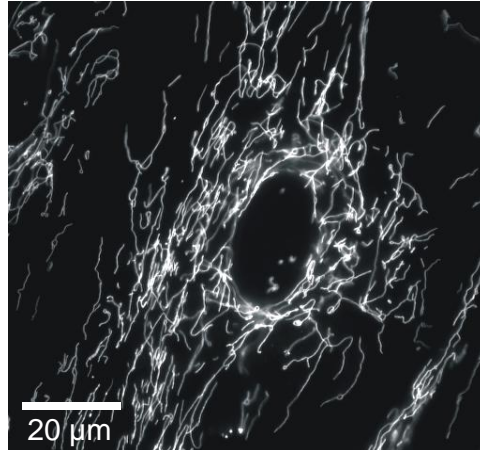
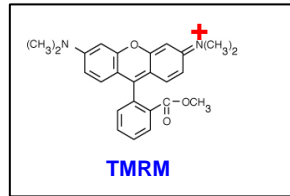
How to quantify mitochondrial morphology in primary human skin fibroblasts?

- Living cells
- Unbiased
- High-content
- Reproducible
- Automated
- Standardized





Staining mitochondria in living cells using the fluorescent cation TMRM



$$\text{PMF} = \Delta\psi - \frac{2.303 \cdot R \cdot T \cdot \Delta pH}{F}$$

$$\Delta\psi (\text{mV}) = \frac{2.303 \cdot R \cdot T}{zF} \log(C_m / C_{\text{cyt}})$$

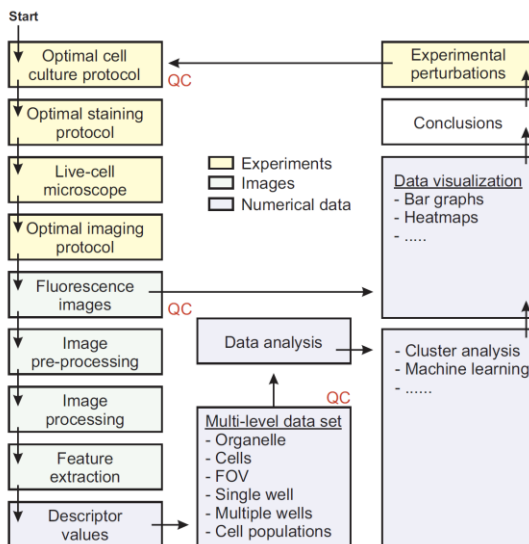
$$C_m = C_{\text{ext}} \exp[-(\Delta V + \Delta\psi)zF/RT]$$

$$\Delta\psi = -60 \cdot \log(7.6 \cdot F_{\text{mit}} / F_{\text{nuc}})$$

Koopman et al., Methods, 2008



Automated high-content analysis of mitochondrial morphology: Overall strategy



Standardization

- Cell culture
- Cell staining
- Cell imaging
- Image processing
- Image quantification

Quality control

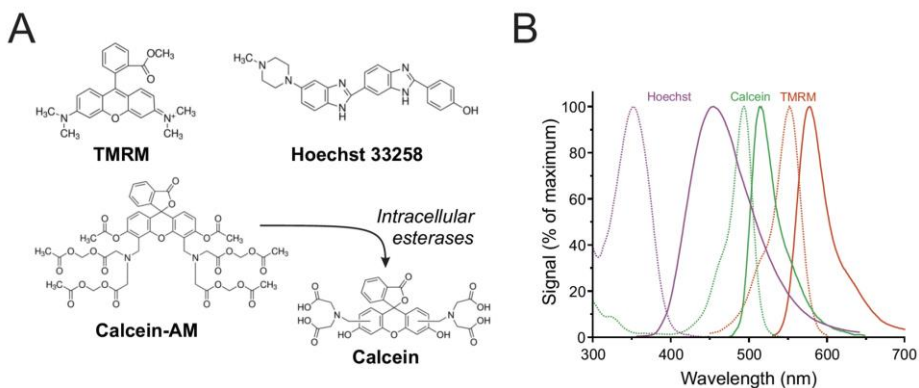
- Cell culture (visual)
- Passage number
- During data analysis

Performing quality control

- Automated detection and removal of unreliable experiments
- Measure, communicate & control process variation

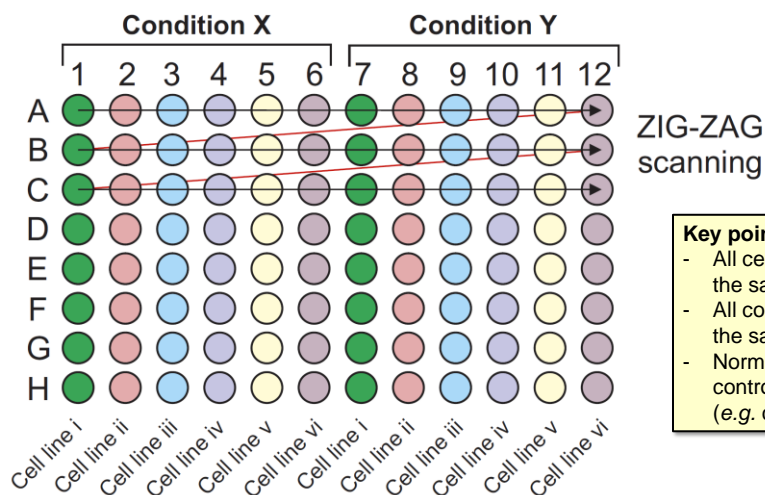
Iannetti et al., Int. J. Cell Biol. Biochem., 2015

Automated high-content analysis of mitochondrial morphology: Reporter molecules



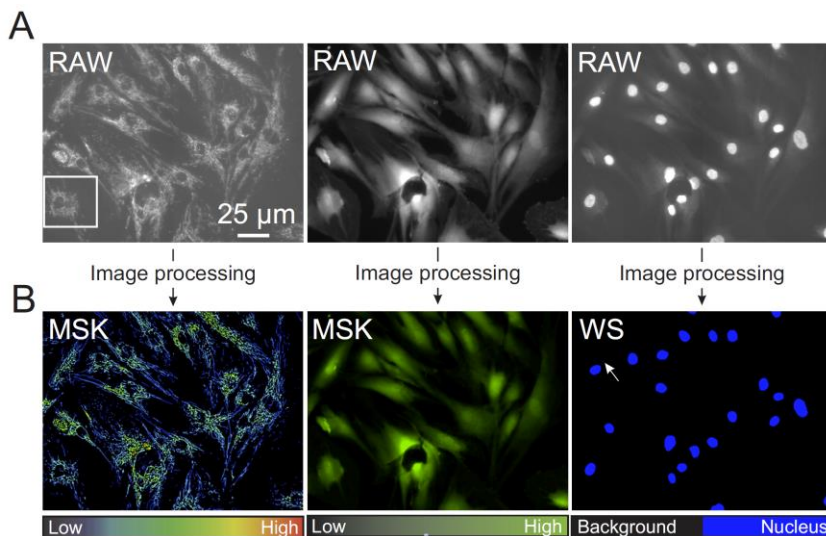
Iannetti et al., Nature Protocols, 2016

Automated microscopy analysis: Layout of the 96 well plate



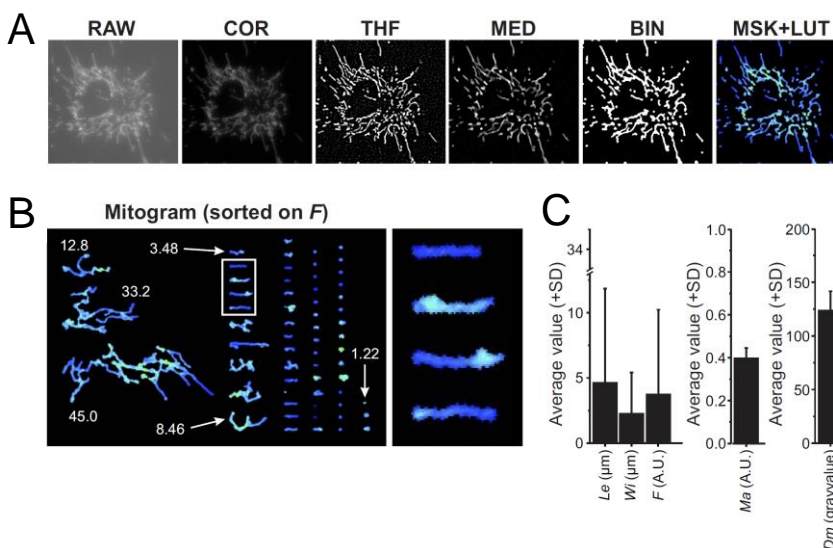
Iannetti et al., Nature Protocols, 2016

Automated high-content analysis of mitochondrial morphology: Image processing



Iannetti et al., Nature Protocols, 2016

Automated high-content analysis of mitochondrial morphology: Image processing



Iannetti et al., Nature Protocols, 2016

44 descriptors extracted from the images = information

Table 3 | Descriptors extracted from the images using the HCS protocol

No.	Reported	Description	Symbol	Definition/measurement of descriptor ^a
1	AREA	Area polygon	AP	The area of the polygon that defines the object's outline (pixels)
2	AREA	Area box	AB	The area between the area of an object and the area of its bounding box
3	FWHM	Aspect	AR	Ratio between major axis and minor axis of an ellipse equivalent to object measure of circularity/roundness
4	FWHM	Area ratio	AR	Ratio between area and perimeter of an object
5	FWHM	Box Area	AR	Ratio between width and height of object's bounding box
6	FWHM	Box Height	AR	Height of the object's bounding box (pixels)
7	FWHM	Box Width	AR	Width of the object's bounding box (pixels)
8	FWHM	Cent	AR	Centroid of the object
9	FWHM	Density mean	DM	Maximum density inside object (grey values)
10	FWHM	Density max	DM	Average density of object (grey values) average mitochondrial intensity of TMRM signal
11	FWHM	Density min	DM	Minimum density inside object (grey values)
12	FWHM	Density std dev	DM	Standard deviation of density inside object (grey values)
13	FWHM	Diameter max	DM	Length of longest line passing two points of object's outline and passing through the object's centroid
14	FWHM	Diameter mean	DM	Average length of diameters measured at 2 degree intervals and passing through the object's centroid
15	FWHM	Diameter min	DM	Length of shortest line passing two points of object's outline and passing through the object's centroid
16	FWHM	First max	DM	Longest first (coldest) length (pixels)
17	FWHM	First min	DM	Shortest first (coldest) length (pixels)
18	FWHM	First ratio	DM	Ratio between first (coldest) length (pixels) and second (coldest) length (pixels)
19	FWHM	Ratio	DM	Ratio between first (coldest) length (pixels) and second (coldest) length (pixels)
20	FWHM	Length	AR	Mean distance (largest length) along the major axis of the object (pixels)
21	FWHM	Margination	AR	The distribution of density between the center of an object and the edge of the object
22	FWHM	Perimeter	AR	Length of the object's perimeter
23	FWHM	Perimeter center	AR	Perimeter of the object's center
24	FWHM	Perimeter edge	AR	The perimeter of the object's edge
25	FWHM	Perimeter ratio	AR	Ratio of perimeter center to perimeter edge
26	FWHM	Perimeter	AR	Perimeter of the object's edge
27	FWHM	Radius Max	AR	Maximum distance between object's center and outline (pixels)
28	FWHM	Radius Min	AR	Minimum distance between object's center and outline (pixels)
29	FWHM	Radius Ratio	AR	Ratio between maximum "Radius max" and "Radius min"
30	FWHM	Roundness	AR	Perimeter of object (pixels) squared divided by area of object (pixels)
31	FWHM	Width	AR	Mean distance (largest length) along the minor axis of the object (pixels)
32	FWHM	Area ratio	AR	Ratio between area and perimeter of an object
33	FWHM	Object area	AR	Total area of the object (pixels)
34	FWHM	Area ratio	AR	Ratio between area and perimeter of an object
35	FWHM	Area ratio	AR	Ratio between area and perimeter of an object
36	FWHM	Area ratio	AR	Ratio between area and perimeter of an object
37	FWHM	Area ratio	AR	Ratio between area and perimeter of an object
38	FWHM	Area ratio	AR	Ratio between area and perimeter of an object
39	FWHM	Area ratio	AR	Ratio between area and perimeter of an object
40	FWHM	Area ratio	AR	Ratio between area and perimeter of an object
41	FWHM	Area ratio	AR	Ratio between area and perimeter of an object
42	FWHM	Area ratio	AR	Ratio between area and perimeter of an object
43	FWHM	Area ratio	AR	Ratio between area and perimeter of an object
44	FWHM	Area ratio	AR	Ratio between area and perimeter of an object

^aAdapted from the definitions in the Image Pro Plus software. Where the unit measurement is not differently specified between the brackets after the definition column, it is Arbitrary Units (A.U.). The first 31 descriptors (orange) were used for Principal Component Analysis (Blanchet et al., 2015).
The symbol "F" is derived from the descriptor name "Formfactor".

Iannetti et al., Nature Protocols, 2016

TMRM image (33 descriptors):

- Mitochondrial aspect ratio (*AR*; a measure of mitochondrial length).
- Average mitochondrial TMRM signal (*DM*; a semi-quantitative measure of $\Delta\psi$).
- Mitochondrial "roundness" (or Formfactor *F*; a combined measure of mitochondrial length and degree of branching).

Calcein image (2 descriptors):

- Total integrated intensity (*Ca/OD*) \rightarrow Calcein uptake
- Cell area (*CaSum*) \rightarrow Cell density/confluence

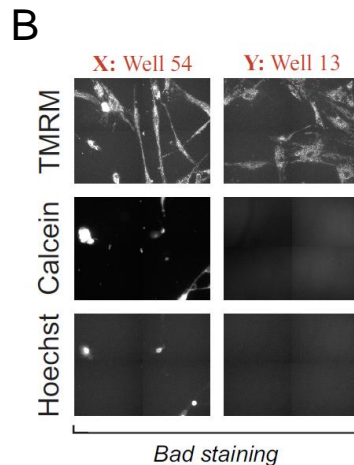
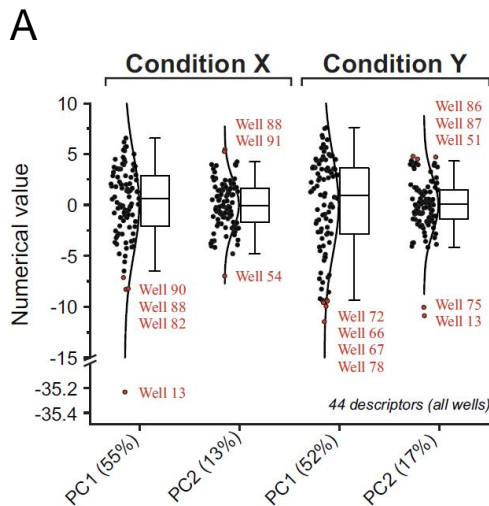
Hoechst image (3 descriptors):

- Total number of nuclei (*Nn*).
- Average area of the nucleus (*An* in pixels; can be expressed in μm^2 as well).
- Nuclear "roundness" (*Fn*).

Derived parameters (6 descriptors):

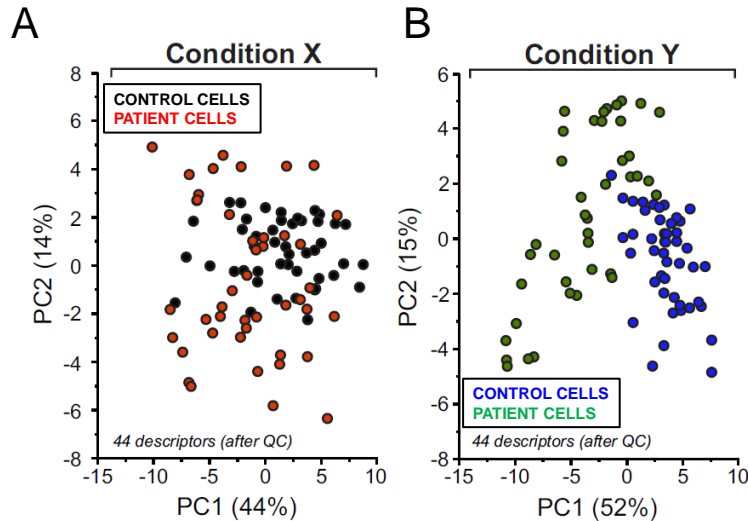
- Cell size (*Casum/Nn*; in pixels; can be expressed in μm^2 as well).
- Mitochondrial size (*Am*; in pixels; can be expressed in μm^2 as well).
- Mitochondrial mass (*Mm*; corrected for cell size!).
- Number of mitochondria per cell (*Nc*; corrected for cell size!).

Automated high-content analysis of mitochondrial morphology: Quality control



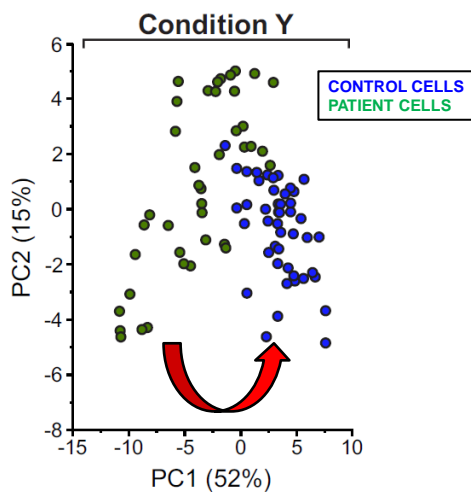
Iannetti et al., Nature Protocols, 2016

Automated high-content analysis of mitochondrial morphofunction: PCA



Iannetti et al., Nature Protocols, 2016

For what do we use the high-content approach?



Reversal of the patient phenotype:

- Small-molecule testing in drug development
- Metabolic manipulation
- Genetic intervention
 - Knockdown
 - Overexpression
- Fundamental insights into mitochondrial morphofunction regulation

Iannetti et al., Nature Protocols, 2016