Electronic Supplementary Information

Ruthenium red: a highly efficient and versatile cell staining agent for single-cell analysis using inductively coupled plasma time-of-flight mass spectrometry

Wen Qin[a], Hans-Joachim Stärk[a], Thorsten Reemtsma[a,b*]

[a] Department of Analytical Chemistry, Helmholtz Centre for Environmental Research – UFZ, Permoserstrasse 15, 04318, Leipzig, Germany

[b] Institute of Analytical Chemistry, University of Leipzig, Linnéstrasse 3, 04103, Leipzig, Germany

* Correspondence: thorsten.reemtsma@ufz.de

Table Index

Figure Index

Figure S1 The color difference between the cell pellets of yeast cells (*Saccharomyces cerevisiae*) with and without RR staining. Left: without RR staining, right: with RR staining.

Figure S2 An example of using ImageJ Fiji software to process a photo of *Saccharomyces cerevisiae* cells. The software automatically recognizes cells as particles in the background and draw their outlines to generate size data, such as area values.

Figure S3 Cell microphotography of yeast cells (*Saccharomyces cerevisiae*) before (a) and after (b) RR staining. White bar stands for 100 μm.

Figure S4 Ru distribution in cell populations from SC-ICP-TOF-MS measurements: *Saccharomyces cerevisiae* strain 1 and 2, and *Scenedesmus vacuolatus*. Each distribution is fitted by a normal distribution curve (line in the graph).

Figure S5 The cell size distribution (cell area value) of the three studied cell strains. The data derives from optical analysis based on micrographic observation.

Table S1 The components of Schatzmann medium (for yeast cells). Quantity of each substance is given that is required for composing 1 liter medium. pH = 5.4 - 5.5.

Substance	Quantity
Glucose	30.09 g
$(NH_4)_2SO_4$	4.49 g
$(NH_4)_2HPO_4$	1.85 g
Sodium citrate × 2 H_2O	6.62 g
$MgSO_4$ × 7 H_2O	0.345 g
$MnSO_4$ × H_2O	9 mg
$ZnSO_4$ × 7 H_2O	8.9 mg
$CuSO_4$ × 5 H_2O	2.5 mg
$CaCl_2$ × 2 H_2O	0.426 g
KCl	0.9 g
$FeCl_3$	9.1 mg
Myo-inositol	0.06 g
Calcium pantothenate	0.06 g
Thiamine Hydrochloride	6.1 mg
Pyridoxine hydrochloride	1.5 mg
Biotin	3 mg

Table S2 The cell area values of the three studied cell samples calculated according to the method given in the "Methods and Materials" section, including cell number, average, median, maximum and minimum.

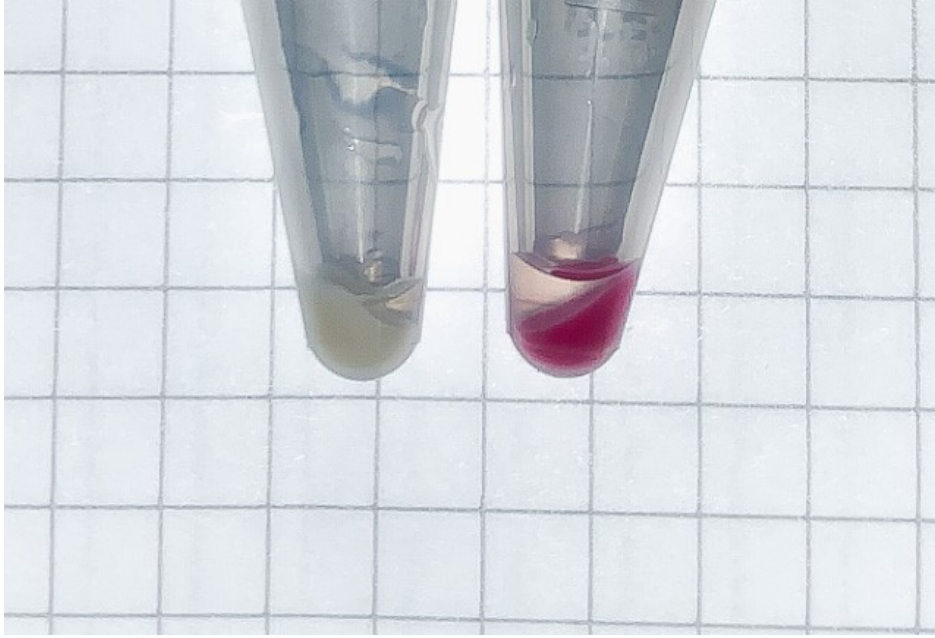| Parameter	*Saccharomyces cerevisiae* strain 1	*Saccharomyces cerevisiae* strain 2	*Scenedesmus vacuolatus*
Analyzed cell number	834	690	1835
Average	35.3	24.8	22.3
Median	35.3	23.6	18.7
Maximum	82.6	110.4	166.4
Minimum	0.2	0.4	0.4

Figure S1 The color difference between the cell pellets of yeast cells (*Saccharomyces cerevisiae*) with and without RR staining. Left: without RR staining, right: with RR staining.
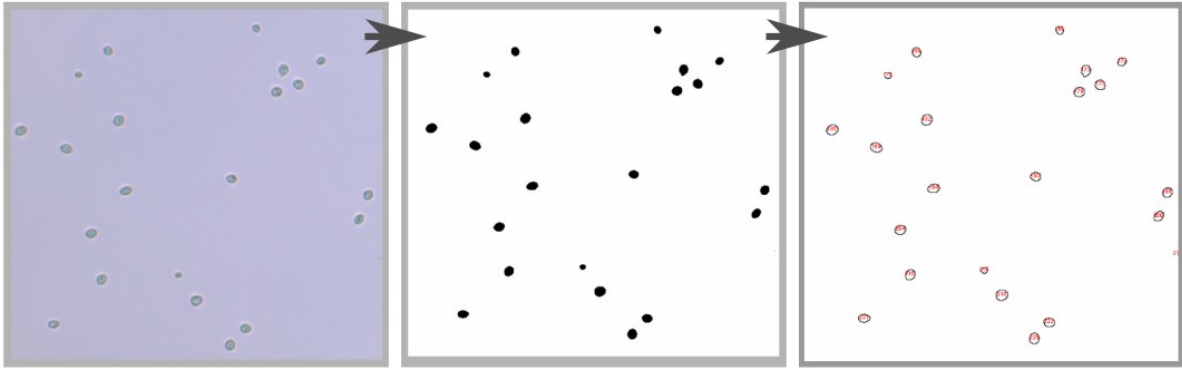
Figure S2 An example of using ImageJ Fiji software to process a photo of *Saccharomyces cerevisiae* cells. The software automatically recognizes cells as particles in the background and draw their outlines to generate size data, such as area values.
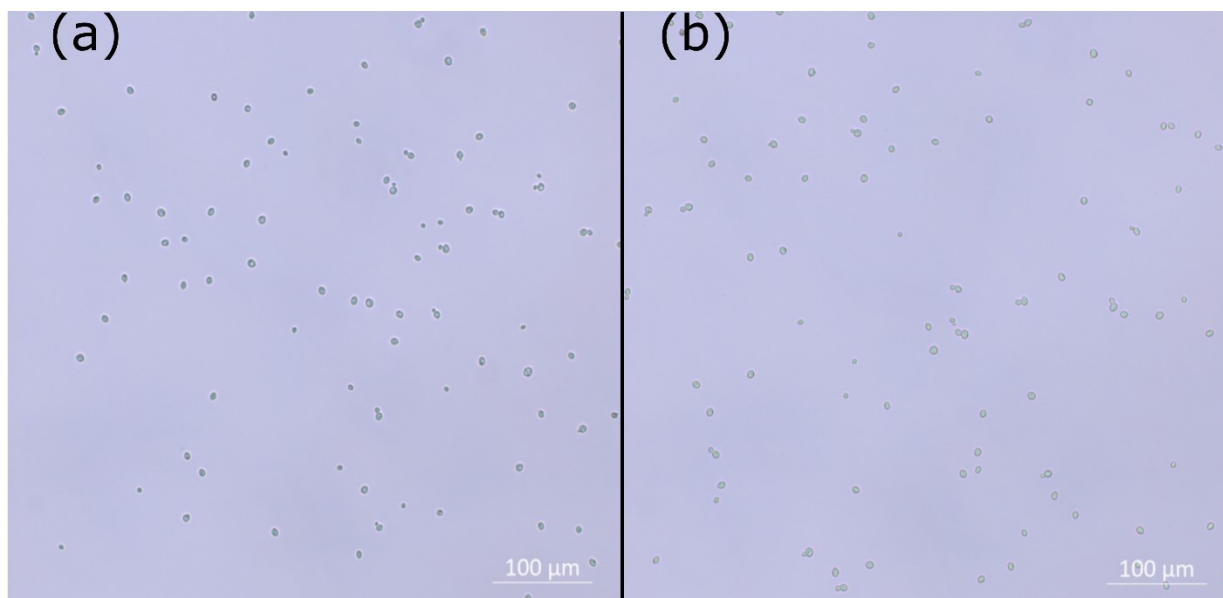
Figure S3 Cell microphotography of yeast cells (*Saccharomyces cerevisiae*) before (a) and after (b) RR staining. White bar stands for 100 μm.
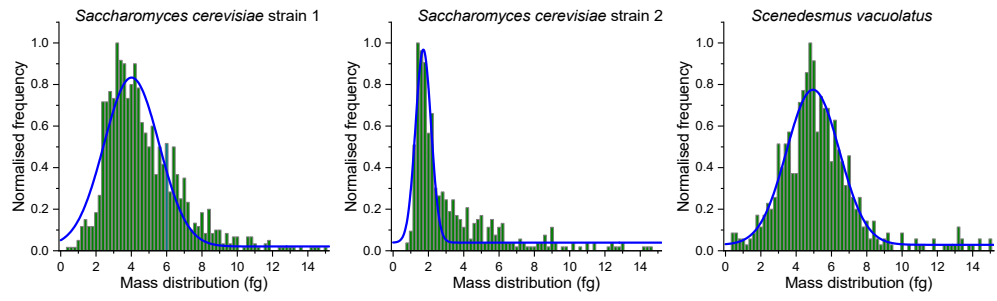
Figure S4 Ru distribution in cell populations from SC-ICP-TOF-MS measurements: *Saccharomyces cerevisiae* strain 1 and 2, and *Scenedesmus vacuolatus*. Each distribution is fitted by a normal distribution curve (line in the graph).
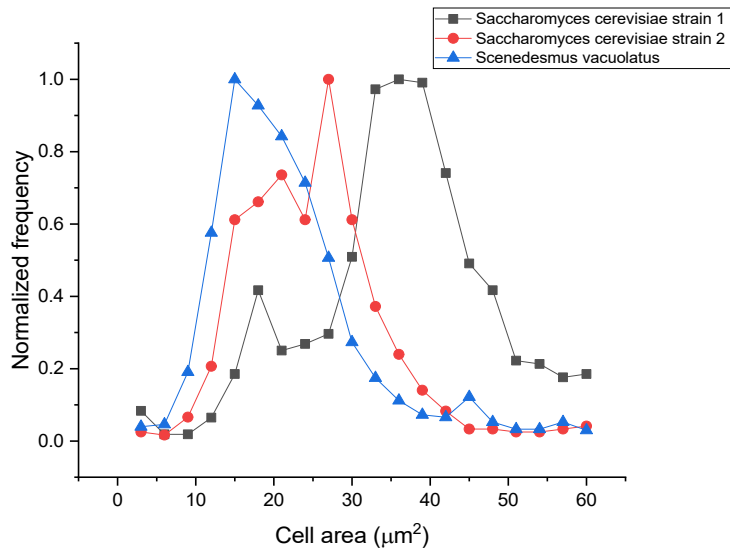
Figure S5 The cell size distribution (cell area value) of the three studied cell strains. The data derives from optical analysis based on micrographic observation.