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# Deriving Element-specific Assimilation Rate of single cells from isotope ratios acquired with nanoSIMS

Hryhoriy Stryhanyuk, Federica Calabrese  
Florin Musat, Hans H. Richnow, Niculina Musat

The 7<sup>th</sup> International NanoSIMS user meeting

22-24 August 2017 Leipzig, Germany



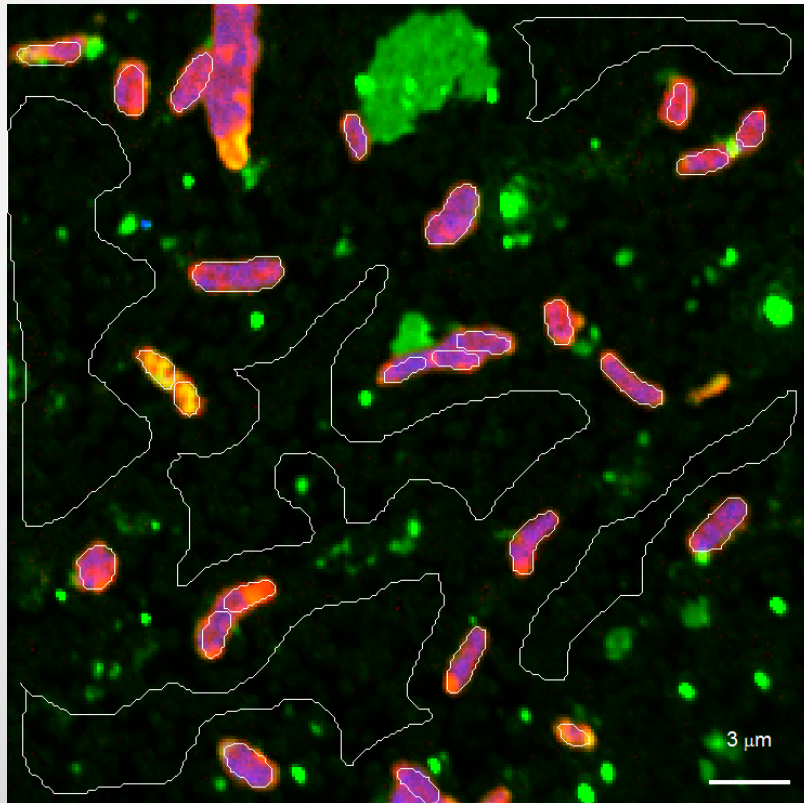
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ProVIS - Centre for  
chemical microscopy

# SIP-FISH-nanoSIMS experiment



nanoSIMS

Marine microbial community



## Metabolic activity

**<sup>13</sup>C-** Stable Isotope Probing

**<sup>32</sup>S-** biomass

## Identity

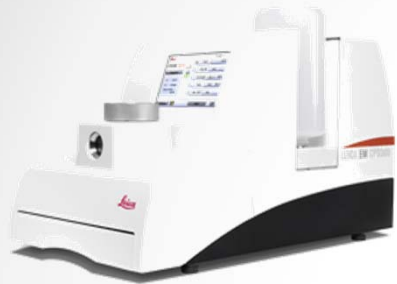
**<sup>19</sup>F-** HISH / FISH

## Quantitative Analysis

- **to be quantified:**
  - element-specific **Assimilation Rate**
  - microbial-mediated processes
  - global element cycling etc.
- **to be detected with nanoSIMS:**
  - Changes of **isotope ratio** (<sup>13</sup>C/<sup>12</sup>C; <sup>15</sup>N/<sup>14</sup>N) of microbial single cells
- **to be considered:**
  - **assimilation** of isotope labelled substance
  - **dilution/loss** of label during preparation

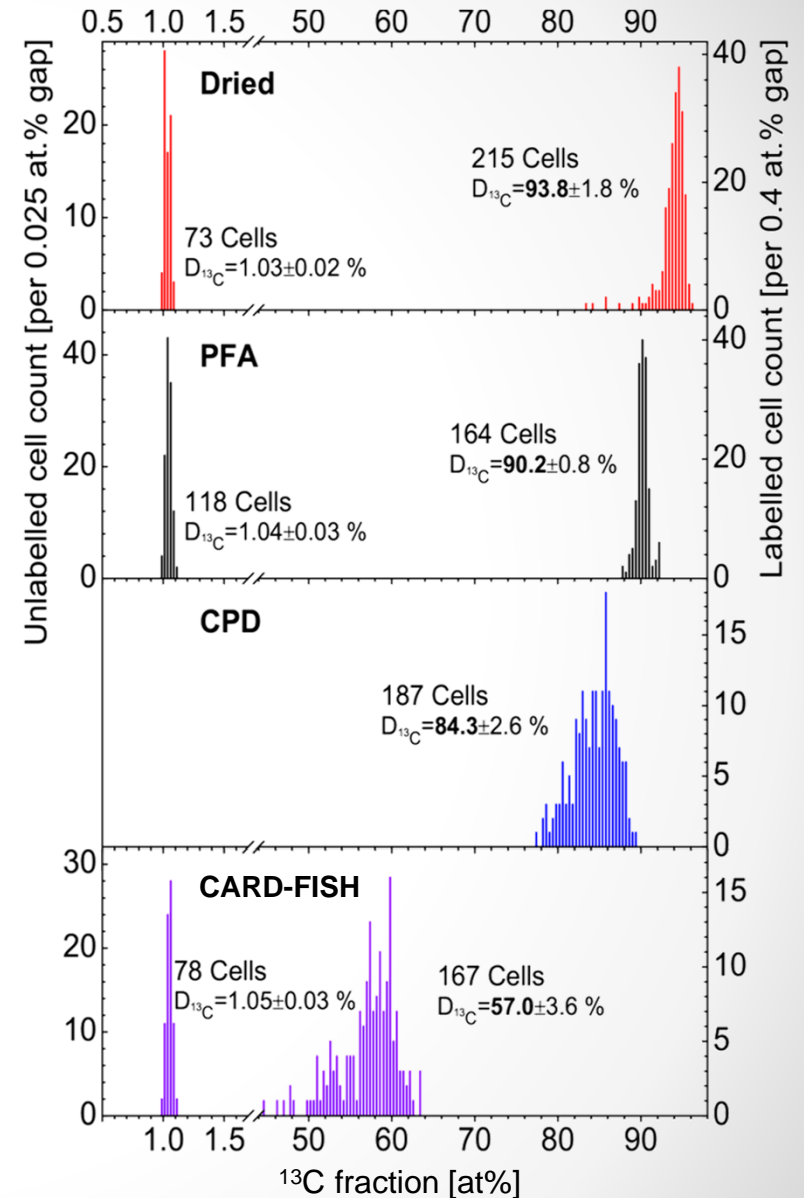
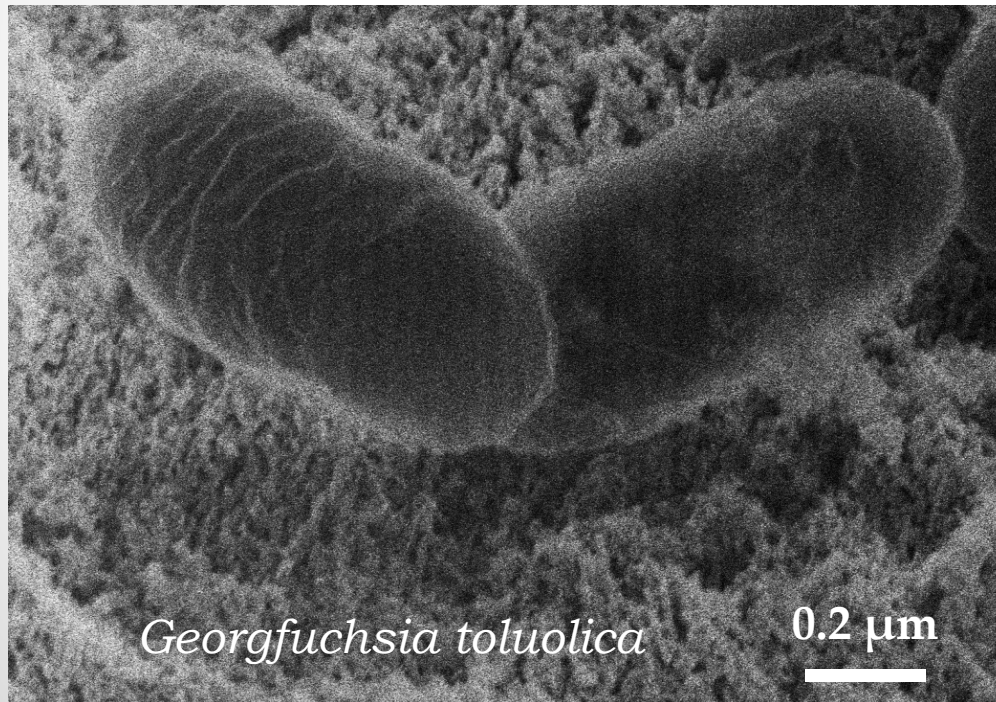
# Dilution of $^{13}\text{C}$ isotope label

## Critical Point Drying (CPD)



LEICA CPD 300 a

- PFA fixation
- $\text{H}_2\text{O} \leftarrow \text{C}_2\text{H}_5\text{OH}$
- $\text{C}_2\text{H}_5\text{OH} \leftarrow \text{CO}_2$



# Restoration of initial $^{13}\text{C}$ fraction

$$K = \frac{{}^{12}\text{C}_{Ch} + {}^{13}\text{C}_{Ch}}{{}^{13}\text{C} + {}^{12}\text{C}}$$

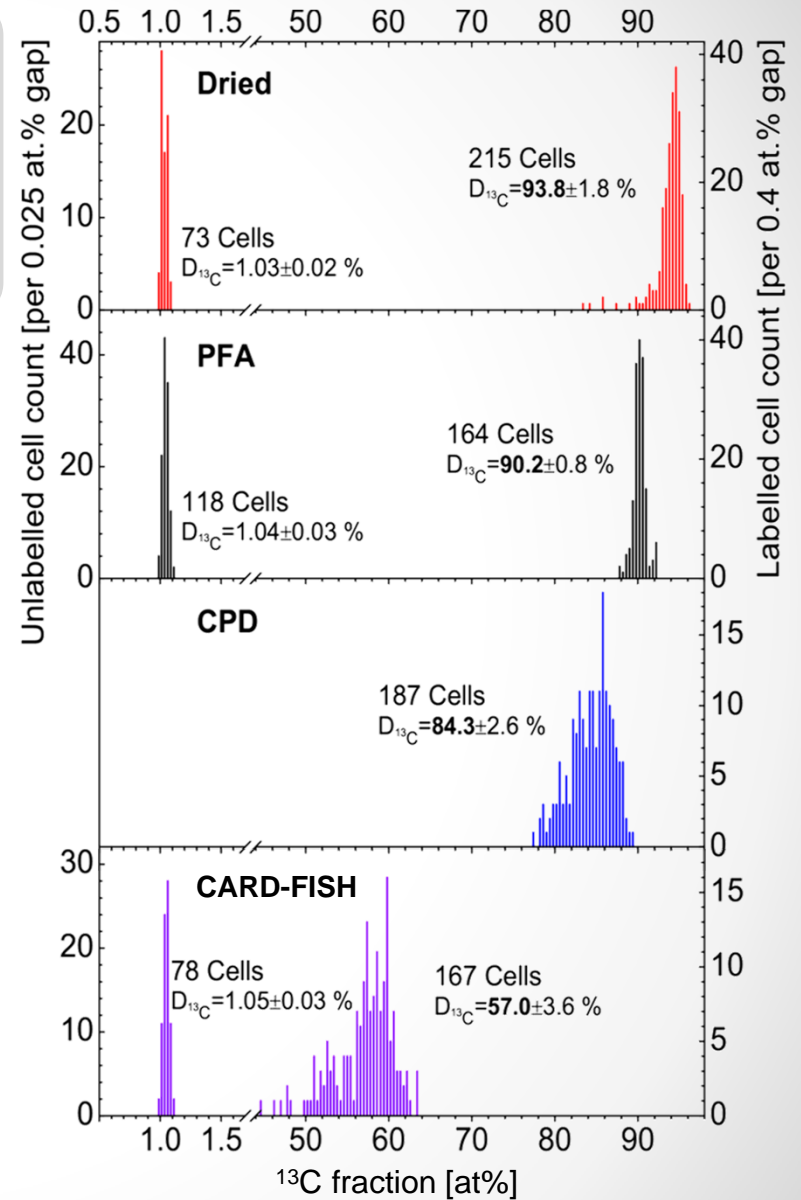
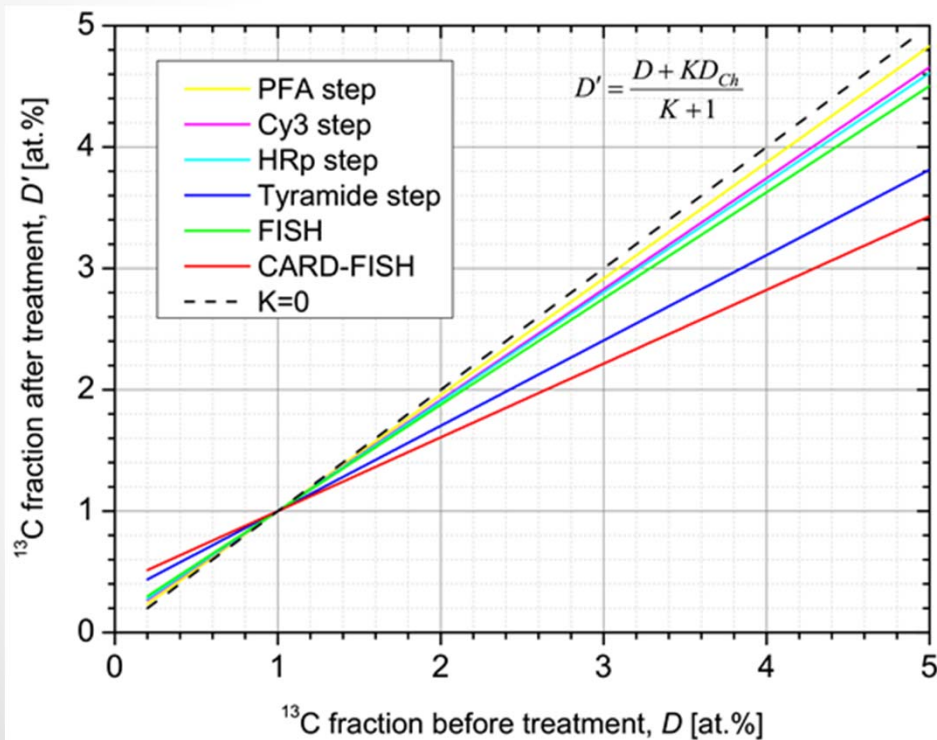
$$D = \frac{{}^{13}\text{C}}{{}^{13}\text{C} + {}^{12}\text{C}}$$

$$R = \frac{{}^{13}\text{C}}{{}^{12}\text{C}}$$

$$R' = \frac{{}^{13}\text{C} + {}^{13}\text{C}_{Ch}}{{}^{12}\text{C} + {}^{12}\text{C}_{Ch}}$$

$$D = \frac{R}{R+1}$$

$$D = D' + K(D' - D_{Ch})$$



N.Musat, H.Stryhanyuk, P.Bombach et al., SAM (2014) Vol 37, 267



# Restoration of initial $^{13}\text{C}$ fraction

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$$D = \frac{R}{R+1}$$

$$D = D' + K(D' - D_{Ch})$$

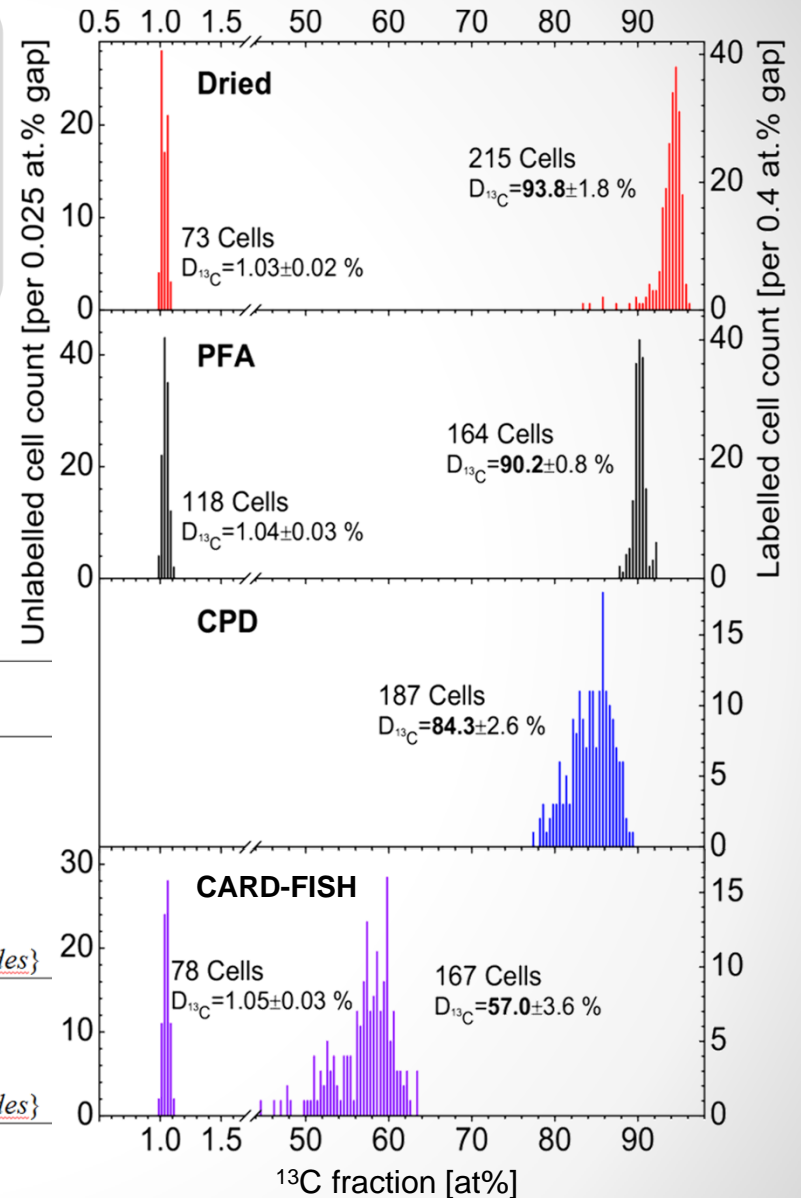
$$K = \frac{R - R'}{(1 + R)(R' - D_{Ch}(R' + 1))}$$

$$K' = \frac{K}{1 + K}$$

Treatments	introduced C $K \pm \Delta K$ ( $K' \pm \Delta K'$ ) [at.%]	initial ratio, $R$	final ratio, $R'$
<i>Single steps</i>			
PFA	4.4±1.4 (4.2±1.3)	$R=16.3 \pm 2.9$ { <i>dried</i> }	$R'=9.3 \pm 0.8$ { <i>PFA</i> }
Cy3	9.4±7.4 (8.6±6.3)	$R=9.3 \pm 0.8$ { <i>PFA</i> }	$R'=4.8 \pm 1.8$ { <i>Cy3</i> }
HRp	10.9±4.8 (9.8±3.9)	$R=9.3 \pm 0.8$ { <i>PFA</i> }	$R'=4.4 \pm 1.0$ { <i>HRp</i> }
Tyramides	42.2±9.5 (29.7±4.7)	$R=4.4 \pm 1.0$ { <i>HRp</i> }	$R'=1.4 \pm 0.2$ { <i>Tyramides</i> }
<i>Complex treatment</i>			
FISH	14.2±7.8 (12.3±6.0)	$R=16.3 \pm 2.9$ { <i>dried</i> }	$R'=4.8 \pm 1.8$ { <i>Cy3</i> }
CARD FISH	64.6±8.8 (39.2±3.2)	$R=16.3 \pm 2.9$ { <i>dried</i> }	$R'=1.4 \pm 0.2$ { <i>Tyramides</i> }

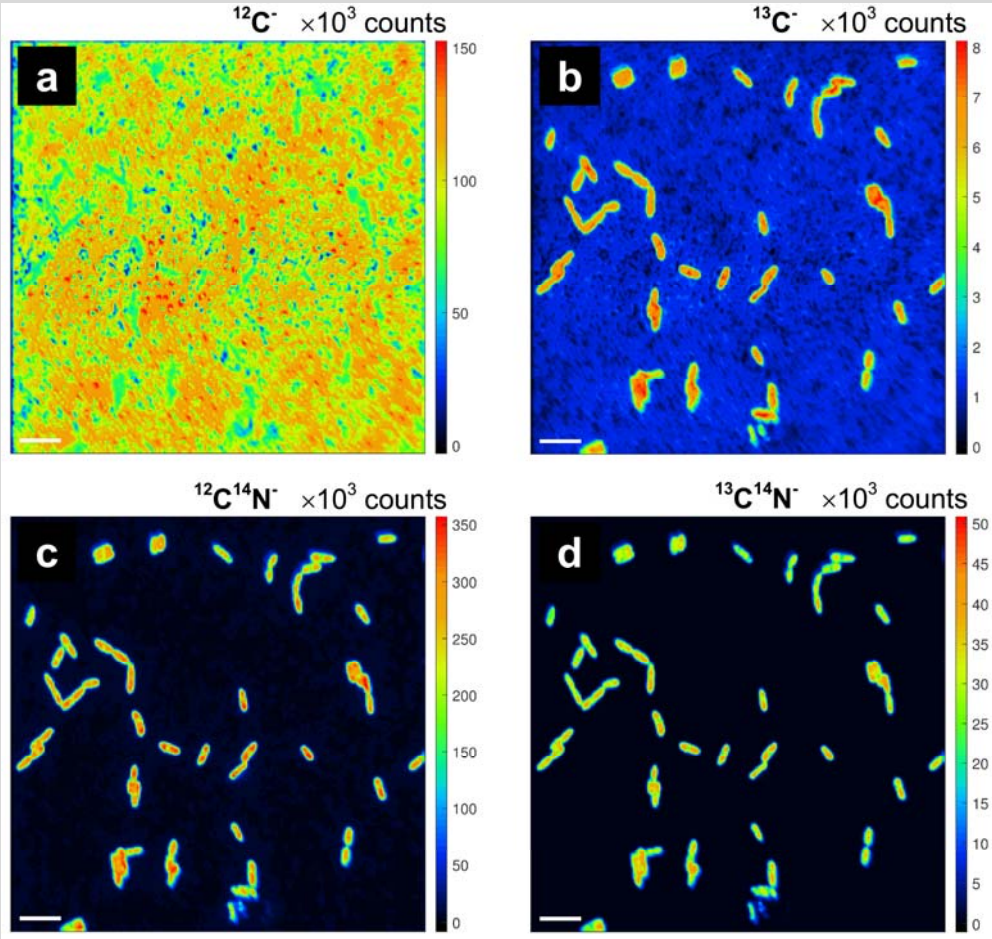
{*Comment*} shows the preparation stage considered as an initial or a final one.

N.Musat, H.Stryhanyuk, P.Bombach et al., SAM (2014) Vol 37, 267



# nanoSIMS data

## Ion yield maps



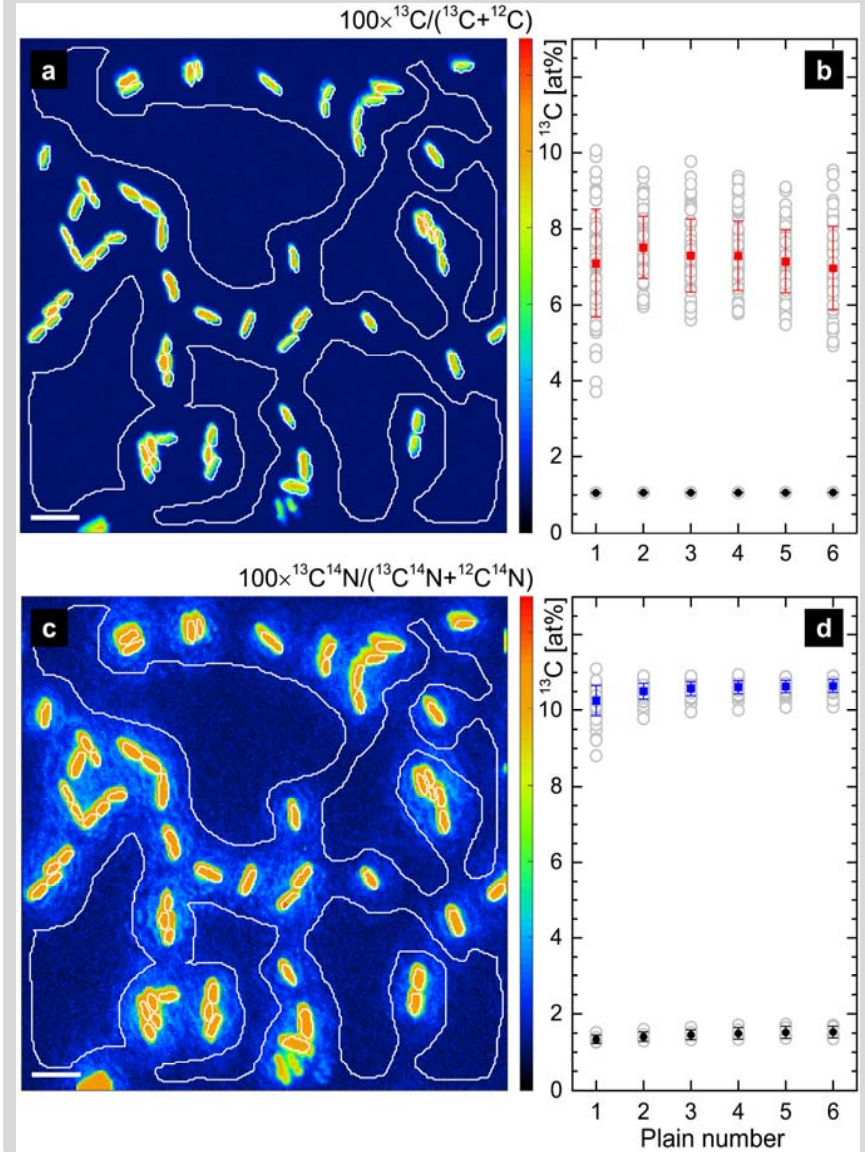
FoV  $40 \times 40 \mu\text{m}^3$

**Look@NanoSIMS**

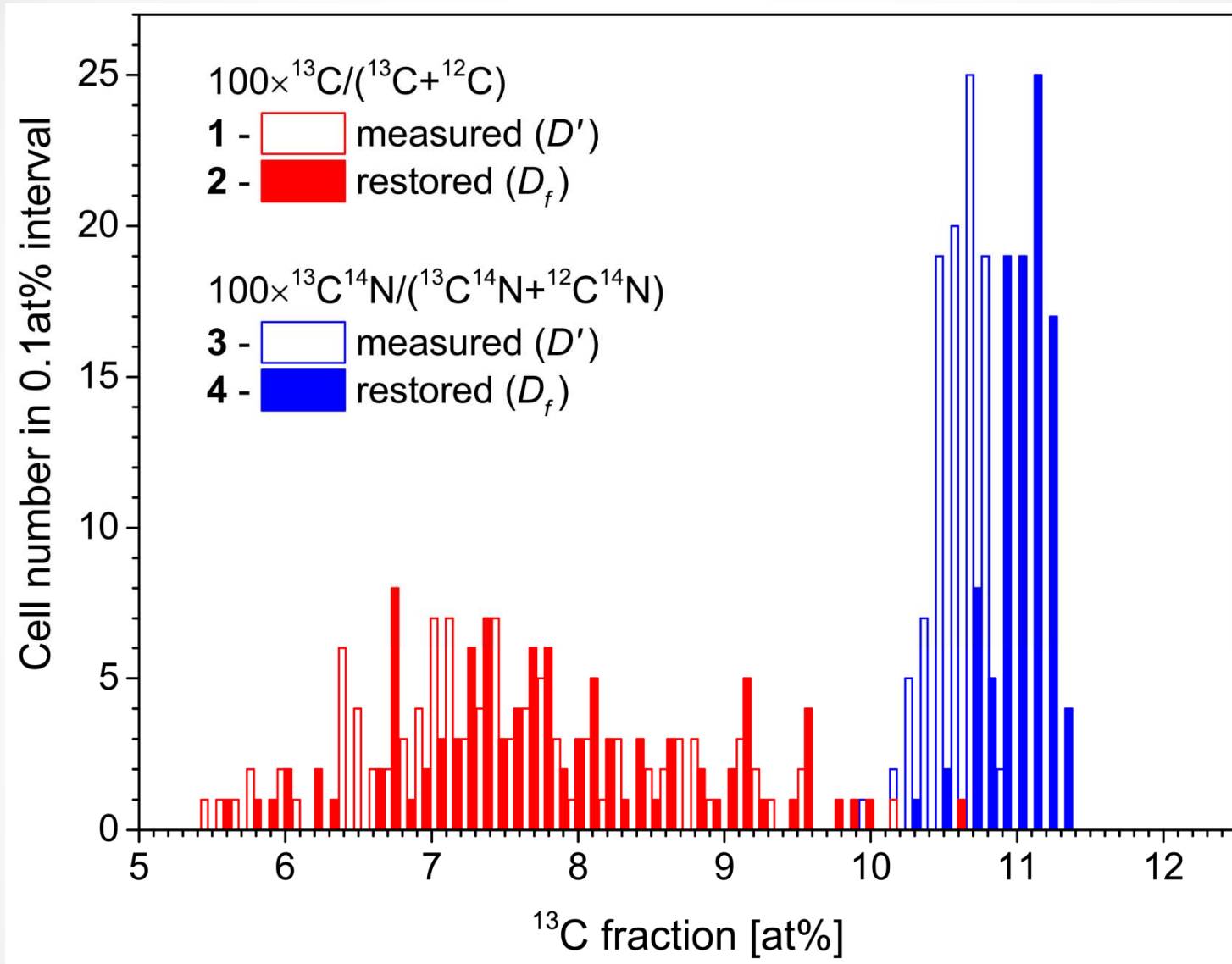
L. Polerecky et al., *Environ. Microbiol.* (2012) Vol 14, 1009

Stryhanyuk, Calabrese, Musat et al., (2017) in preparation

## $^{13}\text{C}$ Fraction

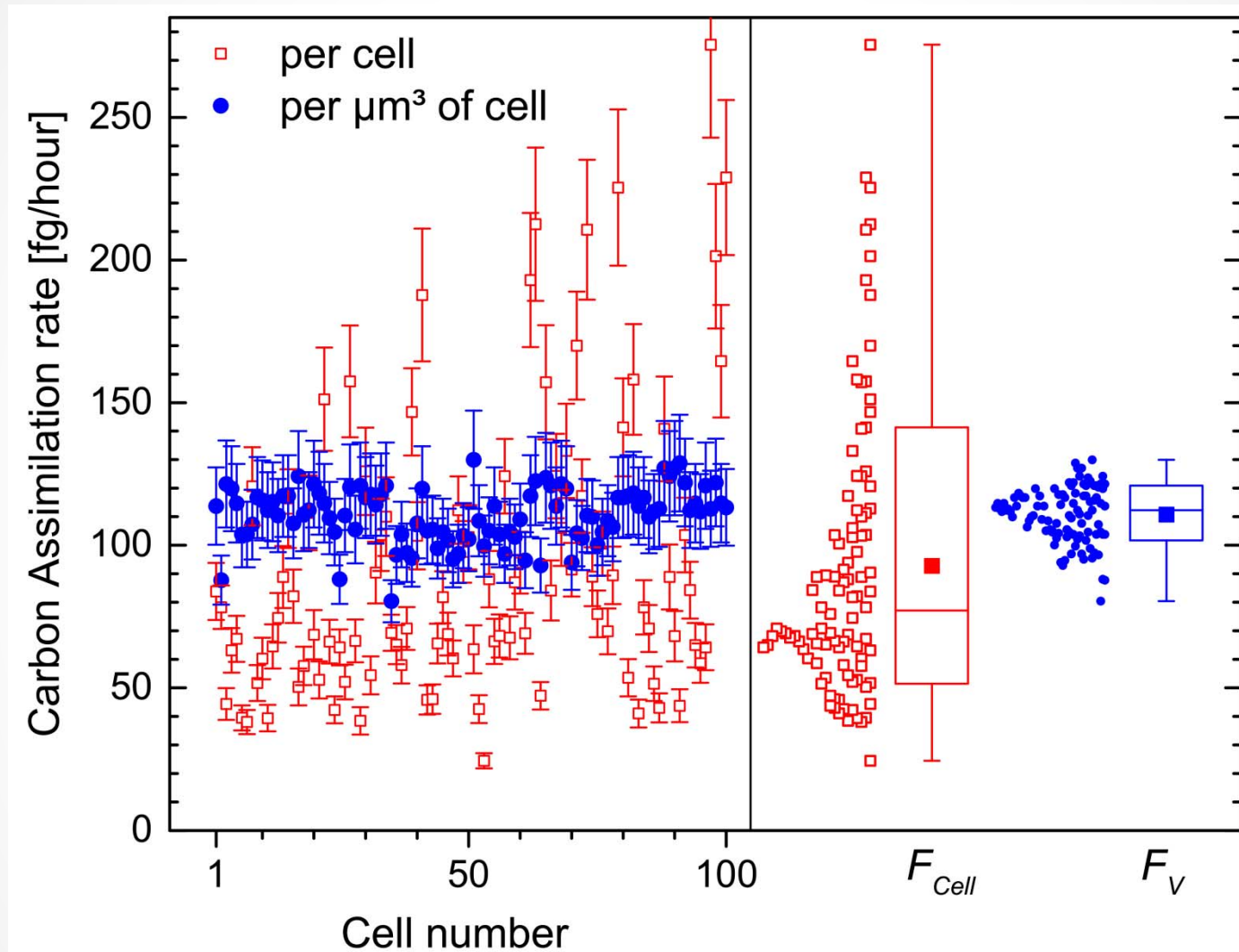


# Restoration of initial $^{13}\text{C}$ fraction



Stryhanyuk, Calabrese, Musat et al., (2017) in preparation

# Assimilation rate calculation



Stryhanyuk, Calabrese, Musat et al., (2017) in preparation





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# Thank you for your attention

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