

1 **APPLIED MICROBIOLOGY AND BIOTECHNOLOGY**

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4 **Supplementary Material**

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6 **Selective elimination of bacterial faecal indicators in the**  
7 ***Schmutzdecke* of slow sand filtration columns**

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36 **Running title:** Microbial communities in slow sand filtration systems

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39 **Keywords:** Wastewater reuse, bacteria removal, ecology of slow sand filtration,

40 *Schmutzdecke*

41 **Figure S1**

42 Schematic drawing (A) and photograph (B) of the laboratory-scale slow sand filter columns.

43 Dimensions are in cm; *a* = supernatant, *b* = sand bed, *c* and *d* = gravel beds.

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45 **Figure S2**

46 Photograph of the *Schmutzdecke* of sand column C1 (A). CLSM micrographs of the top layer

47 (B) and a representative section from about the middle (C) of the *Schmutzdecke*. Staining:

48 bacterial biomass (Syto 9, green); EPS glycoconjugates (AAL-lectin, red); algae

49 (autofluorescence of Chlorophyll a; blue); cyanobacteria (autofluorescence of phycobillins,

50 pink); surface of mineral particles and silica skeletons of diatoms (reflection, white).

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52 **Figure S3**

53 CLSM micrographs from the bottom zone of the *Schmutzdecke* (A), 5 cm sand (B), 10 cm

54 sand (C), and 25 cm sand (D) of C1. Green: bacterial biomass (Syto 9); Red: EPS

55 glycoconjugates (AAL-lectin); Blue: algae (autofluorescence of Chlorophyll, chlA); White:

56 mineral surface (reflection); grid size = 50  $\mu\text{m}$  for A-C and 42  $\mu\text{m}$  for D.

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66 **Table S1.** Diversity of bacterial 16S rRNA and microeukaryote 18S rRNA gene T-RFLP  
 67 fingerprints over filter compartments in sand filter columns C1, C2, and C3.

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Sample	Shannon index ( $H'$ )					
	C1		C2		C3	
	Bacteria	Eukaryota	Bacteria	Eukaryota	Bacteria	Eukaryota
Feed-water	3.52	1.71	3.52	1.71	3.52	1.71
Supernatant	3.53	2.01	3.46	1.94	3.6	2.25
<i>Schmutzdecke</i>	3.82	2.98	3.68	2.81	3.76	2.58
Sand (5cm)	3.99	2.25	3.96	2.47	4.08	2.55
Sand (10cm)	3.95	1.92	3.95	1.51	4.1	2.72
Sand (25cm)	3.78	2.22	3.84	2.1	3.94	2.32
Water (5cm)	3.65	2.08	3.32	1.64	3.63	2.35
Water (10cm)	3.55	2.08	3.42	2.08	3.63	2.29
Water (25cm)	3.5	1.97	3.47	2.01	3.44	1.98
Effluent	3.8	2.1	3.59	1.8	3.6	1.25

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**Table S2** 18S rRNA clone libraries of the inflow, *Schmutzdecke* , and effluent of sand filter column C1.

<b>Sequence identifier</b>	<b>Full name Taxonomic lineage (all Eukaryota)</b>
IN_euk__A01	Uncultured alveolate <i>SAR;Alveolata;</i>
IN_euk__A02	Uncultured alveolate <i>Eukaryota; SAR;Alveolata;</i>
IN_euk__A03	<i>Vorticellidae</i> <i>SAR;Alveolata;Ciliophora;Intramacronucleata;Conthreep;Oligohymenophorea;Peritrichia;</i>
IN_euk__A04	<i>Vorticellidae</i> <i>SAR;Alveolata;Ciliophora;Intramacronucleata;Conthreep;Oligohymenophorea;Peritrichia;</i>
IN_euk__B01	<i>Cercozoa</i> <i>SAR;Rhizaria;Cercozoa;Cercomonadidae;Cercomonas;</i>
IN_euk__B02	Uncultured alveolate <i>SAR;Alveolata;</i>
IN_euk__B03	Uncultured alveolate <i>SAR;Alveolata;</i>
IN_euk__B04	Uncultured alveolate <i>SAR;Alveolata;</i>
IN_euk__C02	Uncultured alveolate <i>SAR;Alveolata;</i>
IN_euk__C03	<i>Heteromita sp.</i> <i>Rhizaria; Cercozoa; Cercomonadida; Heteromitidae;</i>
IN_euk__C04	Uncultured alveolate <i>SAR;Alveolata;Protalveolata;Perkinsidae; A31;</i>
IN_euk__D01	Uncultured alveolate <i>SAR;Alveolata;</i>
IN_euk__D02	<i>Monas sp.</i> <i>SAR;Stramenopiles;Chrysophyceae;Ochromonadales;Paraphysomonas;</i>
IN_euk__D03	<i>Vorticellidae</i>



IN\_euk\_\_D04 SAR;*Alveolata*;*Ciliophora*;*Intramacronucleata*;*Conthreep*;*Oligohymenophorea*;*Peritrichia*;  
*Vorticellidae*

IN\_euk\_\_E01 SAR;*Alveolata*;*Ciliophora*;*Intramacronucleata*;*Conthreep*;*Oligohymenophorea*;*Peritrichia*;  
Uncultured alveolate

IN\_euk\_\_E02 SAR;*Alveolata*;  
*Stramenopiles*

IN\_euk\_\_E03 SAR;*Stramenopiles*;*Chrysophyceae*;  
Uncultured alveolate

IN\_euk\_\_E04 SAR;*Alveolata*;  
Uncultured fungus

IN\_euk\_\_F01 *Opisthokonta*;*Nucleomycea*;*Fungi*;*Chytridiomycota*;*Chytridiomycetes*;*Rhizophydiales*;  
Uncultured alveolate

IN\_euk\_\_F02 SAR;*Alveolata*;  
Uncultured alveolate

IN\_euk\_\_F03 *Opisthokonta*;*Nucleomycea*;*Fungi*;*Chytridiomycota*;*Chytridiomycetes*;*Rhizophydiales*;*Rhizophyidium*;  
Uncultured eukaryote

IN\_euk\_\_F04 *Vorticellidae*  
SAR;*Alveolata*;*Ciliophora*;*Intramacronucleata*;*Conthreep*;*Oligohymenophorea*;*Peritrichia*;

IN\_euk\_\_G01 SAR;*Alveolata*;  
Uncultured alveolate

IN\_euk\_\_G02 SAR;*Alveolata*;  
Uncultured alveolate

IN\_euk\_\_G03 *Heteromita sp.*  
*Rhizaria*;*Cercozoa*;*Cercomonadida*;*Heteromitidae*;

IN\_euk\_\_G04 SAR;*Alveolata*;  
Uncultured alveolate

IN\_euk\_\_H01 SAR;*Alveolata*;  
Uncultured alveolate

IN\_euk\_\_H02 SAR;*Alveolata*;  
Uncultured alveolate

IN\_euk\_\_H03 Uncultured eukaryote  
*Opisthokonta;Nucleomycea;Fungi;Chytridiomycota;Chytridiomycetes;Rhizophydiales;*

IN\_euk\_\_H04 Uncultured eukaryote  
*Opisthokonta;Nucleomycea;Fungi;Chytridiomycota;Chytridiomycetes;Chytridiales;*

SD\_euk\_\_A05 *Oxytrichidae*  
*SAR;Alveolata;Ciliophora;Intramacronucleata;Spirotrichea;*

SD\_euk\_\_A06 Uncultured eukaryote  
*Opisthokonta;Nucleomycea; LKM11;*

SD\_euk\_\_A07 Uncultured alveolate  
*SAR;Alveolata;*

SD\_euk\_\_A08 Uncultured eukaryote  
*Opisthokonta;Nucleomycea; LKM11;*

SD\_euk\_\_B05 Uncultured eukaryote  
*Opisthokonta;Nucleomycea;LKM11;*

SD\_euk\_\_B06 Uncultured eukaryote  
*Opisthokonta;Nucleomycea;LKM11;*

SD\_euk\_\_B07 Uncultured eukaryote  
*Opisthokonta;Nucleomycea;LKM11;*

SD\_euk\_\_B08 *Fragilariaceae*  
*SAR;Stramenopiles;Diatomea;Coscinodiscophytina;Fragilariales;*

SD\_euk\_\_C05 *Bacillariaceae*  
*SAR;Stramenopiles;Diatomea;Bacillariophytina;Bacillariophyceae;Nitzschia;*

SD\_euk\_\_C06 *Peritrichia*  
*Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea;*

SD\_euk\_\_C07 *Eurotiales*  
*Opisthokonta;Nucleomycea;Fungi;Dikarya;Ascomycota;Pezizomycotina;Eurotiomycetes;Eurotiomycetidae;Eurotiales;Trichocomaceae;*

SD\_euk\_\_C08 Uncultured eukaryote  
*Opisthokonta;Nucleomycea;*

SD\_euk\_\_D05 *Fragilariaceae*  
*SAR;Stramenopiles;Diatomea;Coscinodiscophytina;Fragilariales;*

SD\_euk\_\_D06 Nematode

SD\_euk\_\_D07 *Opisthokonta;Holozoa;Metazoa;Animalia;Nematoda;Chromadorea;Monhysteridae;*  
 Uncultured eukaryote  
*Opisthokonta;Nucleomyces ;LKM11;*

SD\_euk\_\_D08 *Bacillariaceae*  
*SAR;Stramenopiles;Diatomea;Bacillariophytina;Bacillariophyceae;Nitzschia ;*

SD\_euk\_\_E06 Uncultured eukaryote  
*Opisthokonta;Nucleomyces ;LKM11;*

SD\_euk\_\_E07 *Cercozoa*  
*SAR;Rhizaria;Cercozoa;Thecofilosea ;*

SD\_euk\_\_E08 *Bacillariaceae*  
*SAR;Stramenopiles;Diatomea;Bacillariophytina;Bacillariophyceae;Nitzschia;*

SD\_euk\_\_F05 *Fragilariaceae*  
*SAR;Stramenopiles;Diatomea;Coscinodiscophytina;Fragilariales ;*

SD\_euk\_\_F06 Uncultured eukaryote  
*Opisthokonta;Nucleomyces ;LKM11;*

SD\_euk\_\_F07 Uncultured eukaryote  
*Opisthokonta;Nucleomyces;Fungi ;*

SD\_euk\_\_F08 Uncultured eukaryote  
*Opisthokonta;Nucleomyces ;LKM11;*

SD\_euk\_\_G05 Uncultured eukaryote

SD\_euk\_\_G06 *Fragilariaceae*  
*SAR;Stramenopiles;Diatomea;Coscinodiscophytina;Fragilariales ;*

SD\_euk\_\_G07 Uncultured eukaryote  
*Opisthokonta;Nucleomyces ;LKM11;*

SD\_euk\_\_G08 *Parachela*  
*Opisthokonta;Holozoa;Metazoa;Animalia;Tardigrada;Hypsibiidae;*

SD\_euk\_\_H05 Uncultured eukaryote  
*Opisthokonta;Nucleomyces ;LKM11;*

SD\_euk\_\_H06 Uncultured eukaryote  
*Opisthokonta;Nucleomyces;*

SD\_euk\_\_H07 Uncultured eukaryote

SD\_euk\_\_H08 *Opisthokonta;Nucleomycea;*  
*Cercozoa*  
*SAR;Rhizaria;Cercozoa;Thecofilosea;*

EF\_euk\_A09 subclass *Stichotrichia*  
*SAR;Alveolata;Ciliophora;Intramacronucleata;Spirotrichea;*

EF\_euk\_A10 Uncultured fungus  
*Opisthokonta;Nucleomycea ;LKM11;*

EF\_euk\_A11 *Viridiraptor sp.*  
*Rhizaria; Cercozoa; Glissomonadida; Viridiraptoridae;*

EF\_euk\_A12 *Colpodella sp.*  
*SAR;Alveolata;Protalveolata;Colpodellida;Colpodella;*

EF\_euk\_B09 Uncultured fungus  
*Opisthokonta;Nucleomycea;Fungi;Chytridiomycota;Chytridiomycetes;Chytridiales;*

EF\_euk\_B10 *Ciliophora*  
*SAR;Alveolata;Ciliophora;Intramacronucleata;Conthreep;*

EF\_euk\_B11 Uncultured fungus  
*Opisthokonta;Nucleomycea ;LKM11;*

EF\_euk\_B12 *Ciliophora*  
*SAR;Alveolata;Ciliophora;Intramacronucleata;Conthreep;Prostomatea;Prorodon;*

EF\_euk\_C09 *Cercomonadidae*  
*SAR;Rhizaria;Cercozoa;Cercomonadidae;Cercomonas;*

EF\_euk\_C11 *Viridiraptor sp.*  
*Rhizaria; Cercozoa; Glissomonadida; Viridiraptoridae;*

EF\_euk\_C12 *Viridiraptor sp.*  
*Rhizaria; Cercozoa; Glissomonadida; Viridiraptoridae;*

EF\_euk\_D09 Uncultured eukaryote  
*SAR;Alveolata;Ciliophora;Intramacronucleata;Conthreep;*

EF\_euk\_D10 *Diplolaimelloides sp.*  
*Opisthokonta;Holozoa;Metazoa;Animalia;Nematoda;Chromadorea;*

EF\_euk\_D11 *Oxytrichidae*  
*SAR;Alveolata;Ciliophora;Intramacronucleata;Spirotrichea;*

EF\_euk\_D12 *Oxytrichidae*  
SAR;Alveolata;Ciliophora;Intramacronucleata;Spirotrichea;

EF\_euk\_E10 *Oxytrichidae*  
SAR;Alveolata;Ciliophora;Intramacronucleata;Spirotrichea;

EF\_euk\_E11 Uncultured Euamoebida  
*Amoebozoa;Discosea;Flabellinia;Dactylopodida;*

EF\_euk\_E12 *Oxytrichidae*  
SAR;Alveolata;Ciliophora;Intramacronucleata;Spirotrichea;

EF\_euk\_F10 *Viridiraptor sp.*  
*Rhizaria; Cercozoa; Glissomonadida; Viridiraptoridae;*

EF\_euk\_F11 *Cercomonadida*  
SAR;Rhizaria;Cercozoa;Cercomonadidae;Cercomonas;

EF\_euk\_F12 *Tetrahymena sp.*  
SAR;Alveolata;Ciliophora;Intramacronucleata;Conthreep;Oligohymenophorea;Hymenostomatia;Tetrahymena;

EF\_euk\_G09 *Oxytrichidae*  
SAR;Alveolata;Ciliophora;Intramacronucleata;Spirotrichea;

EF\_euk\_G10 *Oxytrichidae*  
SAR;Alveolata;Ciliophora;Intramacronucleata;Spirotrichea;

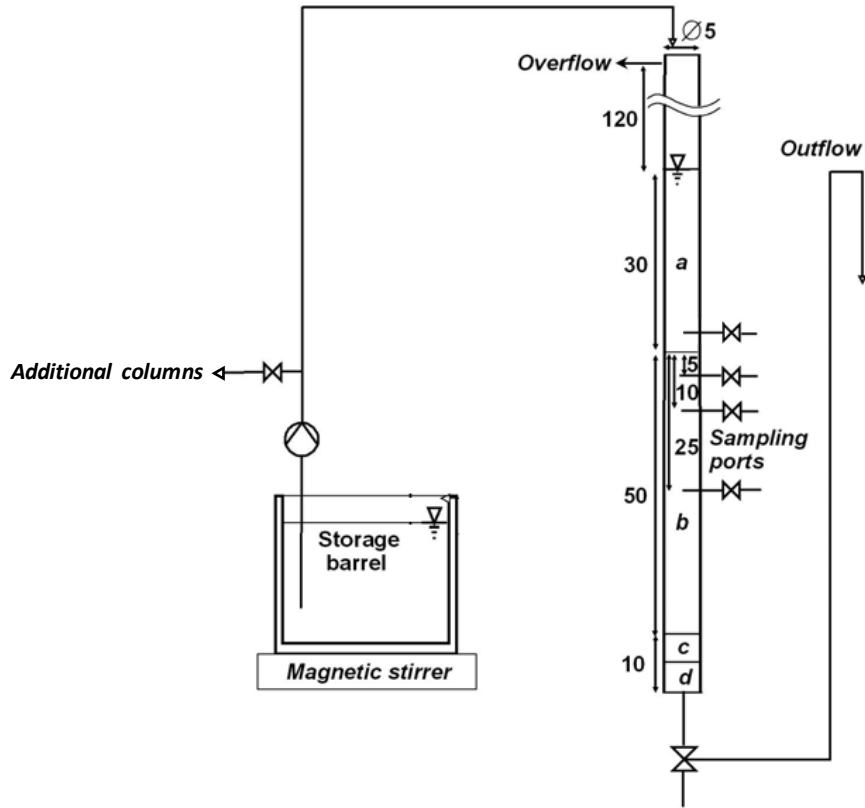
EF\_euk\_G11 *Oxytrichidae*  
SAR;Alveolata;Ciliophora;Intramacronucleata;Spirotrichea;

EF\_euk\_G12 *Diplolaimelloides sp.*  
*Opisthokonta;Holozoa;Metazoa;Animalia;Nematoda;Chromadorea;*

EF\_euk\_H11 *Oxytrichidae*  
SAR;Alveolata;Ciliophora;Intramacronucleata;Spirotrichea;

EF\_euk\_H12 *Oxytrichidae*  
SAR;Alveolata;Ciliophora;Intramacronucleata;Spirotrichea;

A



B



Figure S1



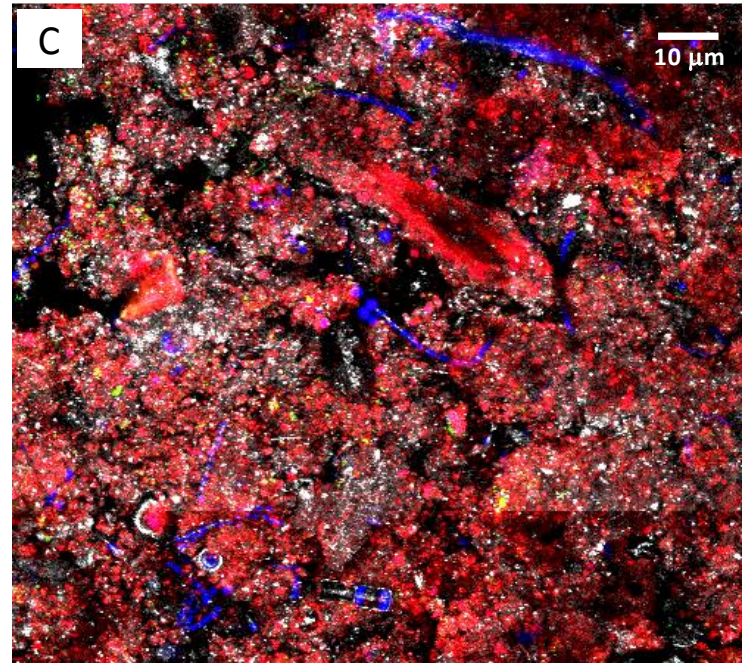
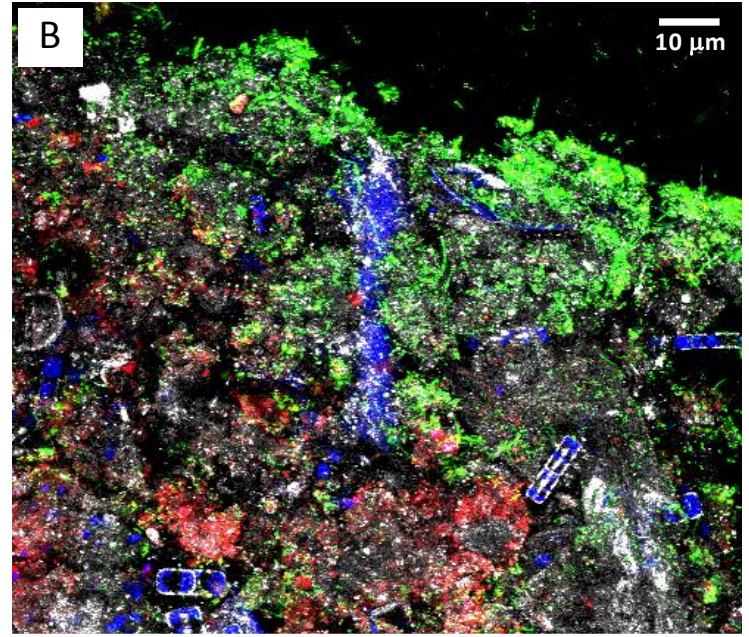


Figure S2



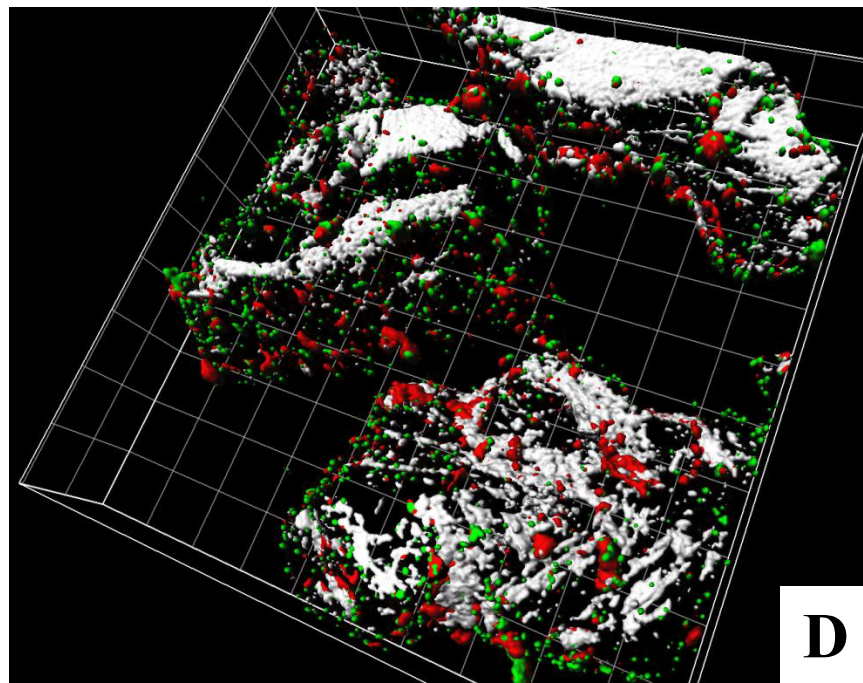
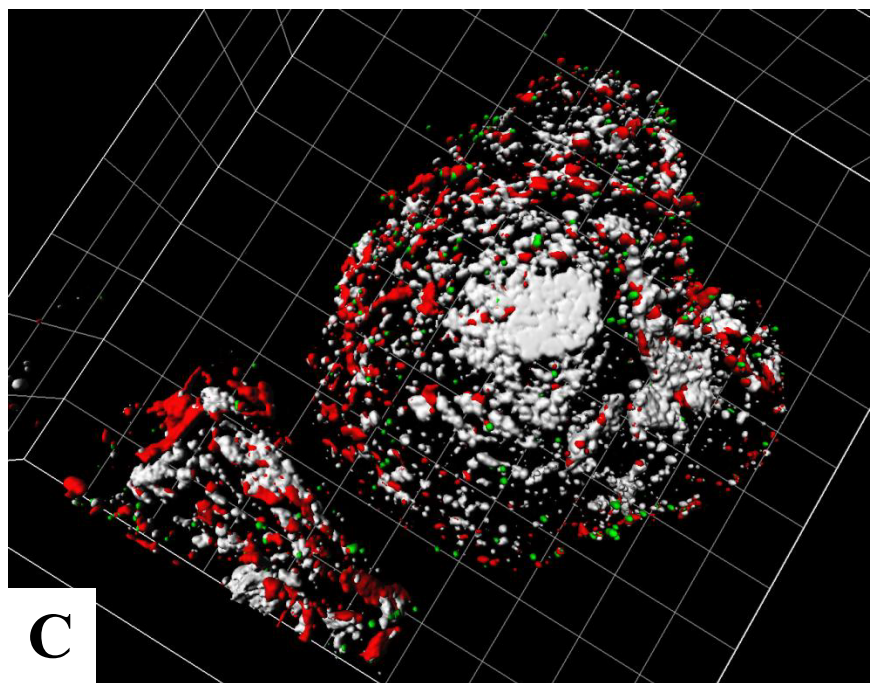
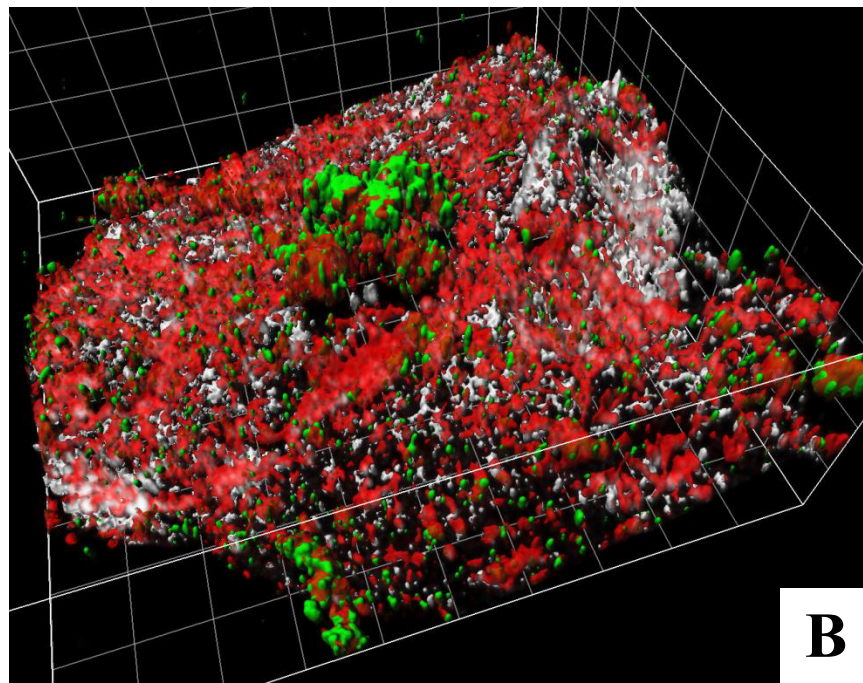
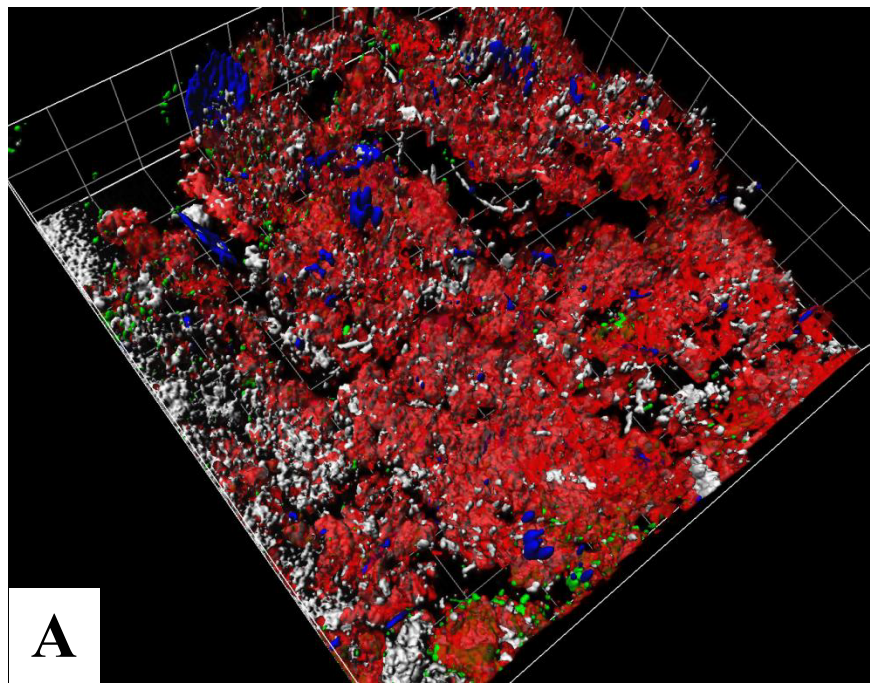


Figure S3