

Supplemental Material for:

Bacterial community complexity in the phyllosphere penalises specialists over generalists

Schlechter, Rudolf O.^{1,2†}, Remus-Emsermann, Mitja N. P.^{1,2†}

¹ Institute of Microbiology and Dahlem Centre of Plant Sciences, Department of Biology, Chemistry, Pharmacy, Freie Universität Berlin, Berlin, Germany

² School of Biological Sciences and Biomolecular Interaction Centre and Bioprotection Research Core, University of Canterbury, Christchurch, 8011, New Zealand

† Correspondence: r.schlechter.jahn@fu-berlin.de, m.remus-emsermann@fu-berlin.de

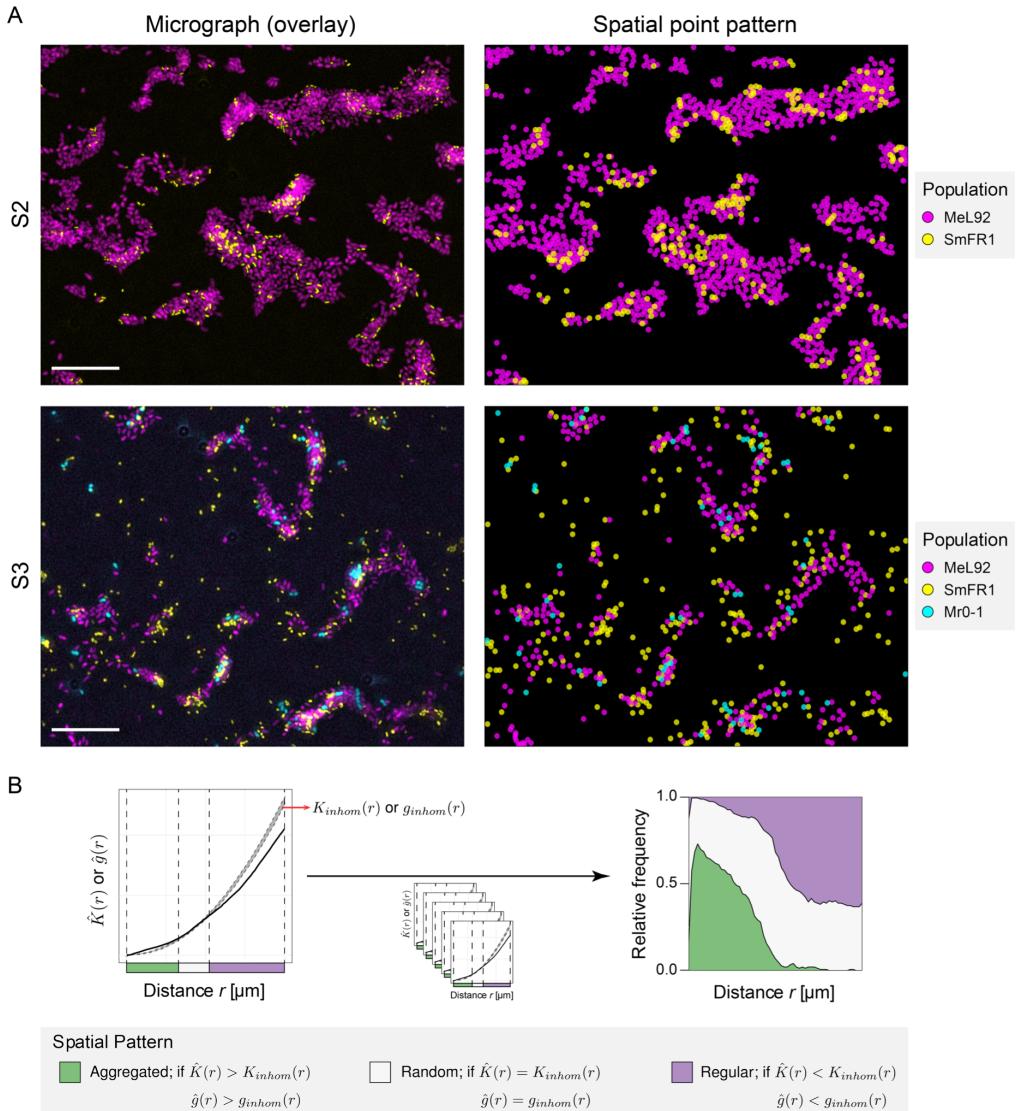


Figure S1. Cell segmentation and spatial point pattern analysis.

(A) *left*: Representative field of views of bacterial populations detected in an S2 (MeL92 and SmFR1) and S3 community (MeL92, SmFR1 and Mr0-1); *right*: corresponding spatial point pattern after cell segmentation and mapping of the cells in a micrograph. Thus, every point in the spatial point pattern represents the centre of mass of a bacterial cell. (B) For every point pattern, at a given distance r , either $\hat{K}(r)$ for intra-population or $\hat{g}(r)$ for inter-population spatial point patterns was estimated. A spatial aggregation pattern (green) was defined if $\hat{K}(r)$ or $\hat{g}(r)$ was greater than the upper limit of a Monte Carlo simulation envelope of the corresponding null models ($K_{inhom}(r)$ or $g_{inhom}(r)$, respectively). A random spatial pattern (grey) was defined if $\hat{K}(r)$ or $\hat{g}(r)$ fell within the Monte Carlo simulation envelope of the estimators. A regular or segregation pattern (purple) was defined if $\hat{K}(r)$ or $\hat{g}(r)$ was less than the lower limit of the Monte Carlo simulation envelope of the estimators. The frequency of each spatial pattern at a given distance was calculated based on the number of replicates and plotted for each tested condition.

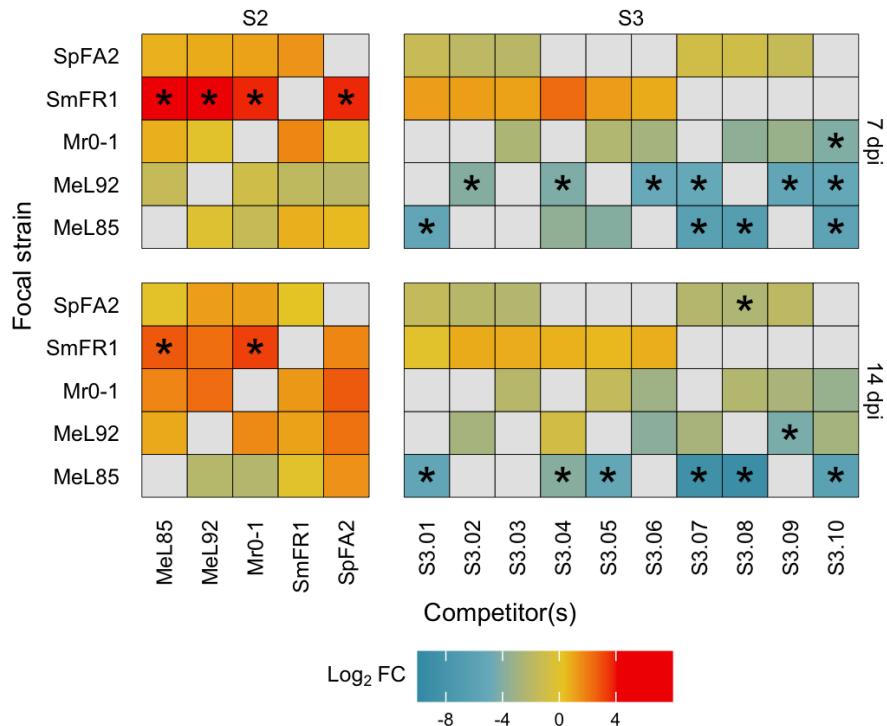


Figure S2. Bacterial population densities per strains and in each community. Log₂ fold change (Log₂ FC) of bacterial populations [CFU gFW⁻¹] in the presence of a single competitor (S2), or within S3. Fold changes were calculated from the median CFU of a strain in competition relative to the near-isogenic control. Asterisk indicates an adjusted *P*-value < 0.05, from a one-sample Wilcoxon test ($\mu = 0$, representing the near-isogenic control).

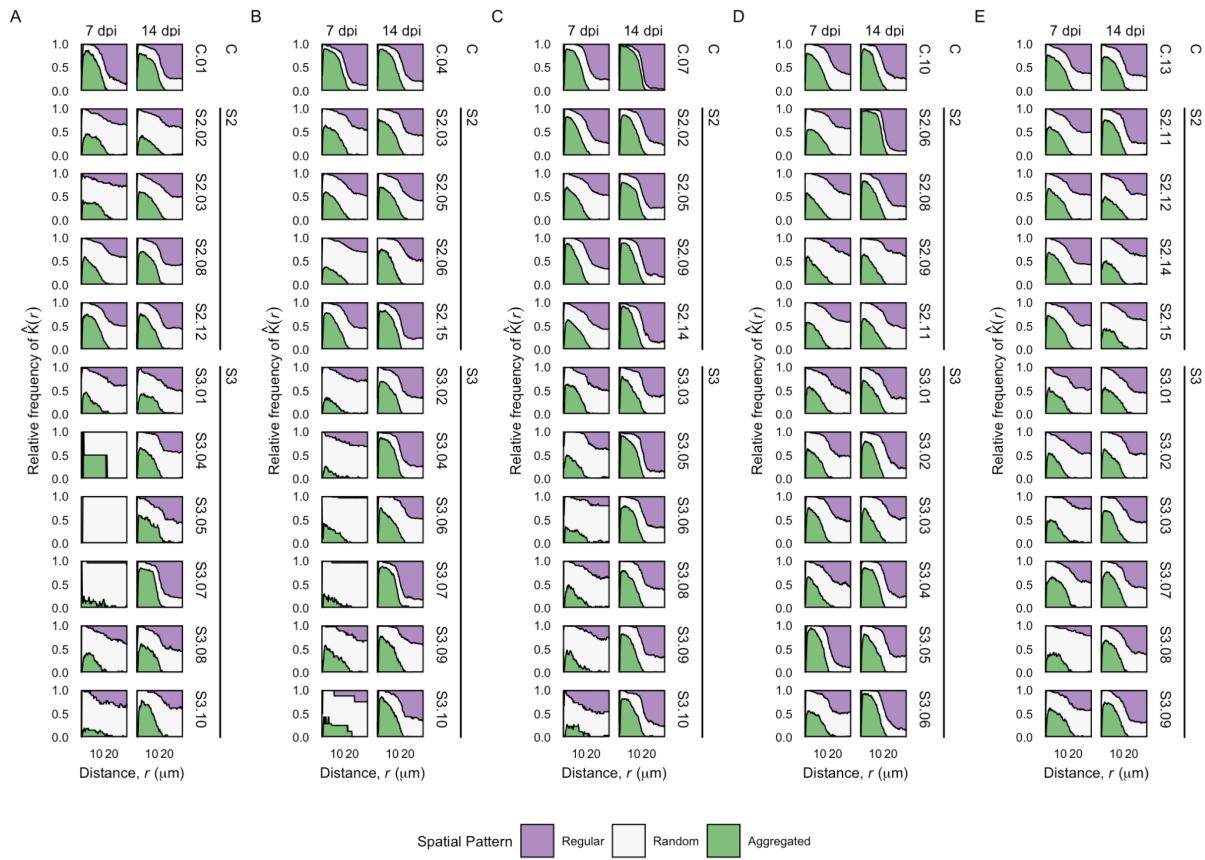


Figure S3. Intraspecific frequency plots for each strain.

Relative fractions of a spatial pattern (regular, random, aggregated) from Ripley's K -estimates for a bacterial population compared to the null model. (A) MeL85, (B) MeL92, (C) Mr0-1, (D) SmFR1, (E) SpFA2. Plots are grouped by community complexity (C, S2, S3) and time point. Refer to Table 2 in the main text for the identity of each strain in each combination.

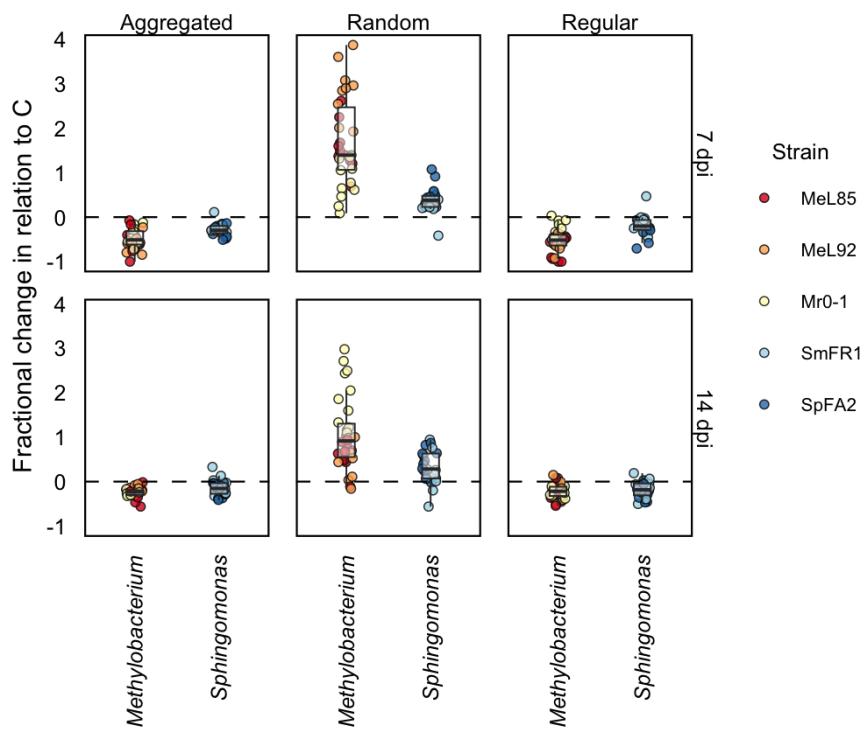


Figure S4. Fractional change of a spatial pattern within bacterial taxonomic groups.

Fractional change of a spatial pattern (aggregated, random, regular) over time of *Methylobacterium* and *Sphingomonas* relative to the corresponding near-isogenic control. Individual strains are highlighted within each group.

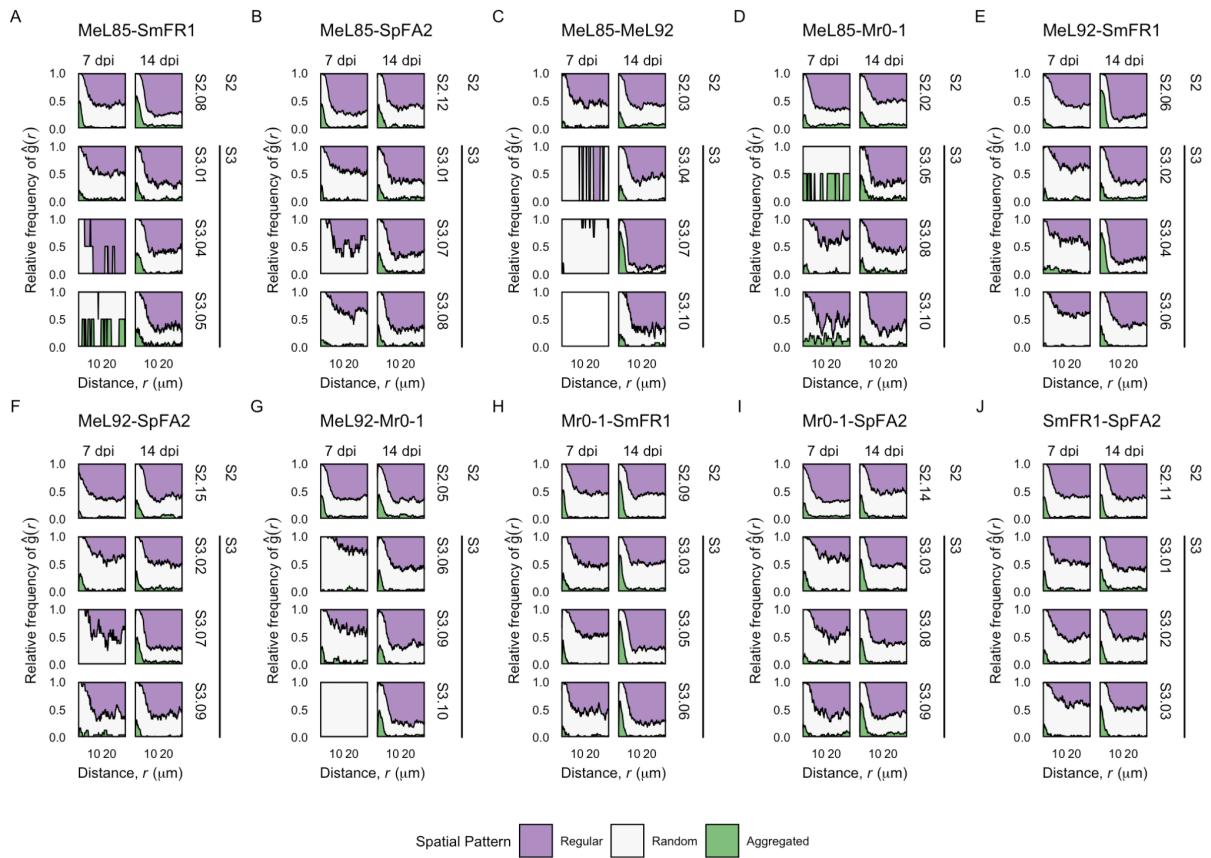


Figure S5. Interspecific frequency plots for each species pair.

Relative fractions of a spatial pattern (regular, random, aggregated) based on pair cross correlations between a bacterial pair compared to the null model. Frequency plots are grouped by bacterial pairs in S2 and S3, and time point. Species pair [Taxa pair]: (A) MeL85-SmFR1 [MS], (B) MeL85-SpFA2 [MS], (C) MeL85-MeL92 [MM], (D) MeL85-Mr0-1 [MM], (E) MeL92-SmFR1 [MS], (F) MeL92-SpFA2 [MS], (G) MeL92-Mr0-1 [MM], (H) Mr0-1-SmFR1 [MS], (I) Mr0-1-SpFA2 [MS], (J) SmFR1-SpFA2 [SS]. Refer to Table 2 in the main text for the identity of each strain in each combination.

Table S1. Strain selection within SynComs

Treatment	Community	Strain	Fluorescence	Resistance	Selective media
C	C.01	MeL85	mSc	GmR	R2A + Gm
		MeL85	sYFP2	KmR	R2A + Km
	C.02	MeL92	mSc	GmR	R2A + Gm
		MeL92	mTq2	KmR	R2A + Km
	C.03	Mr0-1	mSc	GmR	R2A + Gm
		Mr0-1	mTq2	KmR	R2A + Km
	C.04	SmFR1	mSc	GmR	NA + Gm
		SmFR1	mTq2	TcR	NA + Tc
	C.05	SpFA2	mSc	GmR	NA + Gm
		SpFA2	mTq2	TcR	NA + Tc
S2	S2.01	MeL85	mSc	GmR	R2A + Gm
		Mr0-1	mTq2	KmR	R2A + Km
	S2.02	MeL85	mSc	GmR	R2A + Gm
		MeL92	mTq2	KmR	R2A + Km
	S2.03	MeL92	mSc	GmR	R2A + Gm
		Mr0-1	mTq2	KmR	R2A + Km
	S2.04	SmFR1	mSc	GmR	NA + Gm
		MeL92	mTq2	KmR	R2A + Km
	S2.05	MeL85	mSc	GmR	R2A + Gm
		SmFR1	mTq2	TcR	NA + Tc
	S2.06	Mr0-1	mSc	GmR	R2A + Gm
		SmFR1	mTq2	TcR	NA + Tc
	S2.07	SpFA2	mSc	GmR	NA + Gm
		SmFR1	mTq2	TcR	NA + Tc
	S2.08	MeL85	mSc	GmR	R2A + Gm
		SpFA2	mTq2	TcR	NA + Tc
	S2.09	Mr0-1	mSc	GmR	R2A + Gm
		SpFA2	mTq2	TcR	NA + Tc
	S2.10	MeL92	mSc	GmR	R2A + Gm
		SpFA2	mTq2	TcR	NA + Tc
S3	S3.01	SmFR1	mSc	GmR	MM Proline + Gm
		SpFA2	sYFP2	GmR	MM Serine + Gm
		MeL85	mTq2	KmR	MM MeOH
	S3.02	SmFR1	mSc	GmR	MM Proline + Gm
		SpFA2	sYFP2	GmR	MM Serine + Gm
		MeL92	mTq2	KmR	MM MeOH
	S3.03	SmFR1	mSc	GmR	MM Proline + Gm
		SpFA2	sYFP2	GmR	MM Serine + Gm
		Mr0-1	mTq2	KmR	MM MeOH
	S3.04	SmFR1	mSc	GmR	MM Glucose + Gm

		MeL85	sYFP2	KmR	MM Fructose + Km
		MeL92	mTq2	KmR	MM MeOH + Km
S3.05	SmFR1	mSc	GmR	MM Glucose + Gm	
	MeL85	sYFP2	KmR	MM Fructose + Km	
	Mr0-1	mTq2	KmR	MM MeOH + Km	
	SmFR1	mSc	GmR	MM Glucose + Gm	
S3.06	MeL92	sYFP2	GmR	MM MeOH + Gm	
	Mr0-1	mTq2	KmR	MM MeOH + Km	
	MeL85	mSc	GmR	MM MeOH + Gm	
S3.07	SpFA2	sYFP2	GmR	MM Glucose + Gm	
	MeL92	mTq2	KmR	MM MeOH + Km	
	Mr0-1	mTq2	KmR	MM MeOH + Km	
S3.08	MeL85	mSc	GmR	MM MeOH + Gm	
	SpFA2	sYFP2	GmR	MM Glucose + Gm	
	Mr0-1	mTq2	KmR	MM MeOH + Km	
S3.09	MeL92	mSc	GmR	MM MeOH + Gm	
	SpFA2	sYFP2	GmR	MM Glucose + Gm	
	Mr0-1	mTq2	KmR	MM MeOH + Km	
S3.10	MeL85	mSc	GmR	MM Fructose + Gm	
	MeL92	sYFP2	GmR	MM MeOH + Gm	
	Mr0-1	mTq2	KmR	MM MeOH + Km	

Table S2. Strain used in conjugation experiments

Recipient strain	Donor strain	Phenotype(s)
<i>Sphingomonas melonis</i> Fr1	<i>E. coli</i> S17-1 (pMRE-Tn5-153)	Yellow fluorescence (sYFP2) Kanamycin resistance
<i>Sphingomonas phyllosphaerae</i> FA2	<i>E. coli</i> S17-1 (pMRE-Tn5-143)	Yellow fluorescence (sYFP2) Gentamicin resistance
<i>Sphingomonas phyllosphaerae</i> FA2	<i>E. coli</i> S17-1 (pMRE-Tn5-161)	Cyan fluorescence (mTurquoise2) Tetracycline resistance
<i>Methylobacterium radiotolerans</i> 0-1	<i>E. coli</i> S17-1 (pMRE-Tn5-145)	Red fluorescence (mScarlet-I) Gentamicin resistance
<i>Methylobacterium radiotolerans</i> 0-1	<i>E. coli</i> S17-1 (pMRE-Tn5-153)	Yellow fluorescence (sYFP2) Kanamycin resistance
<i>Methylobacterium radiotolerans</i> 0-1	<i>E. coli</i> S17-1 (pMRE-Tn5-161)	Cyan fluorescence (mTurquoise2) Tetracycline resistance
<i>Methylobacterium</i> sp. Leaf85	<i>E. coli</i> S17-1 (pMRE-Tn5-145)	Red fluorescence (mScarlet-I) Gentamicin resistance
<i>Methylobacterium</i> sp. Leaf85	<i>E. coli</i> S17-1 (pMRE-Tn5-153)	Yellow fluorescence (sYFP2) Kanamycin resistance
<i>Methylobacterium</i> sp. Leaf85	<i>E. coli</i> S17-1 (pMRE-Tn5-151)	Cyan fluorescence (mTurquoise2) Kanamycin resistance
<i>Methylobacterium</i> sp. Leaf92	<i>E. coli</i> S17-1 (pMRE-Tn5-145)	Red fluorescence (mScarlet-I) Gentamicin resistance
<i>Methylobacterium</i> sp. Leaf92	<i>E. coli</i> S17-1 (pMRE105)	Yellow fluorescence (sYFP2) Gentamicin resistance
<i>Methylobacterium</i> sp. Leaf92	<i>E. coli</i> S17-1 (pMRE-Tn5-151)	Cyan fluorescence (mTurquoise2) Kanamycin resistance

Table S3. Summary of microscopy data.

SynCom	Time of sampling [dpi]	Community	Indep. Exp.	nFOV	C0 [# cells]	C1 [# cells]	C2 [# cells]	Total [# cells]	Average [# cells/nFOV]
C	7	C.01	e1	90	15719	64023	n.a.	79742	886.0
		C.01	e2	10	128	1	n.a.	129	12.9
		C.02	e1	203	24665	23976	n.a.	48641	239.6
		C.03	e1	296	39755	25258	n.a.	65013	219.6
		C.04	e1	192	18484	12200	n.a.	30684	159.8
		C.04	e2	160	12739	23925	n.a.	36664	229.2
		C.05	e1	210	11242	12336	n.a.	23578	112.3
		C.05	e2	64	44230	17742	n.a.	61972	968.3
	14	C.01	e1	152	26928	9233	n.a.	36161	237.9
		C.01	e2	80	23541	7415	n.a.	30956	387.0
		C.02	e1	183	19205	20823	n.a.	40028	218.7
		C.02	e2	81	13392	1095	n.a.	14487	178.9
		C.03	e1	155	45063	32429	n.a.	77492	499.9
		C.04	e1	104	17371	7771	n.a.	25142	241.8
		C.04	e2	81	7642	10811	n.a.	18453	227.8
		C.05	e1	121	16234	9300	n.a.	25534	211.0
		C.05	e2	70	7517	9763	n.a.	17280	246.9
S2	7	S2.01	e1	253	10175	45356	n.a.	55531	219.5
		S2.01	e2	61	2836	11121	n.a.	13957	228.8
		S2.02	e1	143	3705	10468	n.a.	14173	99.1
		S2.02	e2	81	570	1873	n.a.	2443	30.2
		S2.03	e1	207	24405	18589	n.a.	42994	207.7
		S2.03	e2	130	6798	3411	n.a.	10209	78.5
		S2.04	e1	203	6454	27234	n.a.	33688	166.0
		S2.04	e2	163	9800	23432	n.a.	33232	203.9
		S2.05	e1	226	3308	17081	n.a.	20389	90.2
		S2.05	e2	81	14871	12478	n.a.	27349	337.6
		S2.06	e1	250	36811	13552	n.a.	50363	201.5
		S2.06	e2	81	20591	9408	n.a.	29999	370.4
		S2.07	e1	251	14674	21637	n.a.	36311	144.7
		S2.07	e2	80	14563	6963	n.a.	21526	269.1
		S2.08	e1	187	3729	13363	n.a.	17092	91.4
		S2.08	e2	80	13990	11083	n.a.	25073	313.4
		S2.09	e1	300	28038	64385	n.a.	92423	308.1
		S2.09	e2	164	16273	5759	n.a.	22032	134.3
		S2.10	e1	220	26148	22799	n.a.	48947	222.5
		S2.10	e2	163	22234	1934	n.a.	24168	148.3
	14	S2.01	e1	155	8458	45264	n.a.	53722	346.6
		S2.01	e2	80	5947	15804	n.a.	21751	271.9
		S2.02	e1	154	7890	27869	n.a.	35759	232.2
		S2.02	e2	60	6679	2962	n.a.	9641	160.7
		S2.03	e1	151	15246	20711	n.a.	35957	238.1

		S2.03	e2	160	22661	71162	n.a.	93823	586.4
		S2.04	e1	78	2011	10340	n.a.	12351	158.3
		S2.04	e2	80	38332	11958	n.a.	50290	628.6
		S2.05	e1	149	19727	25319	n.a.	45046	302.3
		S2.05	e2	80	20104	21108	n.a.	41212	515.2
		S2.06	e1	154	39837	15431	n.a.	55268	358.9
		S2.06	e2	81	35256	15338	n.a.	50594	624.6
		S2.07	e1	113	23671	20683	n.a.	44354	392.5
		S2.07	e2	80	14687	6337	n.a.	21024	262.8
		S2.08	e1	65	7479	12583	n.a.	20062	308.6
		S2.08	e2	80	23042	10364	n.a.	33406	417.6
		S2.09	e1	80	34940	8851	n.a.	43791	547.4
		S2.09	e2	80	16125	12825	n.a.	28950	361.9
		S2.10	e1	80	16312	4944	n.a.	21256	265.7
		S2.10	e2	80	15255	4784	n.a.	20039	250.5
		S3.01	e1	100	16524	6274	4650	27448	274.5
		S3.01	e2	30	1634	2311	7	3952	131.7
		S3.02	e1	80	5491	5907	2520	13918	174.0
		S3.02	e2	80	11250	4081	2920	18251	228.1
		S3.03	e1	80	13448	3868	17250	34566	432.1
		S3.03	e2	77	13476	2054	2862	18392	238.9
		S3.04	e1	81	14759	118	1305	16182	199.8
		S3.04	e2	61	6758	8	636	7402	121.3
		S3.05	e1	81	65369	81	13398	78848	973.4
		S3.05	e2	60	16609	25	1693	18327	305.5
		S3.06	e1	81	7453	1002	982	9437	116.5
		S3.06	e2	60	10187	483	2876	13546	225.8
		S3.07	e1	62	496	1643	395	2534	40.9
		S3.07	e2	61	186	6830	276	7292	119.5
		S3.08	e1	78	3473	2983	4094	10550	135.3
		S3.08	e2	21	5	347	250	602	28.7
		S3.09	e1	64	1253	1206	2008	4467	69.8
		S3.09	e2	61	542	7422	1725	9689	158.8
		S3.10	e1	41	1033	161	2164	3358	81.9
		S3.10	e2	13	99	534	0	633	48.7
		S3.01	e1	81	10496	5378	12927	28801	355.6
		S3.01	e2	60	10432	8820	2494	21746	362.4
		S3.02	e1	80	23956	9518	19330	52804	660.1
		S3.02	e2	60	14333	5609	3504	23446	390.8
		S3.03	e1	81	10756	6064	13026	29846	368.5
		S3.03	e2	60	8556	10008	34935	53499	891.7
		S3.04	e1	80	13537	5659	10177	29373	367.2
		S3.04	e2	60	11874	3298	14564	29736	495.6
		S3.05	e1	80	13675	470	27362	41507	518.8
		S3.05	e2	60	15034	2427	19273	36734	612.2
		S3.06	e1	80	54483	4974	15092	74549	931.9
		S3.06	e2	60	11573	9679	11861	33113	551.9
		S3.07	e1	80	20166	8322	22215	50703	633.8

S3.07	e2	60	507	14232	13617	28356	472.6
S3.08	e1	80	2718	8688	23567	34973	437.2
S3.08	e2	61	4809	7009	3567	15385	252.2
S3.09	e1	81	5032	9833	25215	40080	494.8
S3.09	e2	60	1010	9160	8604	18774	312.9
S3.10	e1	50	2722	3286	8123	14131	282.6
S3.10	e2	59	26	15367	12899	28292	479.5

e1: Independent experiment #1

e2: Independent experiment #1

nFOV: Number of field of views