Recent application of high throughput molecular techniques to aquatic environments has identified a huge reservoir of genetic and metabolic diversity. However in many cases inadequate sampling regimes limits our understanding of ecosystem dynamics. To help microbial ecologists overcome this bottleneck, we have developed a small, autonomous, open source in situ water sampling and archival device. The Sample Filtration and Archiving instrument has been designed to operate underwater where it will collect and filter 24 user defined time-stamped water samples of between ~250-1000 ml onto standard 0.2 µm membrane filters. Following filtration, a stabilization buffer is dispensed onto the filter to preserve the DNA in the captured biological material. Upon retrieval of the instrument the filters are available for downstream interrogation in the laboratory using a range of molecular techniques. To assess sample preservation in the instrument, water samples from Lake Mendota WI, USA were treated with the chemical preservatives RNAlater, ethanol and isopropanol and tested using Automated Ribosomal Intergenic Spacer Analysis. Initial results show samples can be preserved for up to 1 week without appreciable loss of DNA quality. For field evaluation, the prototype instrument has been packaged into a 27 cm (diameter) by 61 cm (length) pressure vessel with a total weight of ~18 kg. The system is powered by 2 laptop style batteries, and uses a peristaltic pump and rotary valve to filter water through re-usable 47 mm filter holders. In its current configuration, the instrument has a typical filtration rate of ~80 ml min⁻¹. The system is controlled by an open source Arduino microcontroller that is coupled to a custom electronic control shield that is responsible for interfacing with the instruments subsystems. To facilitate its construction by the user, the instrument has been designed using predominately commercially available or easily manufactured parts. With the exception of the external pressure vessel (which can be purchased commercially), laboratories that have basic electronic and machining expertise will be able to construct and operate the base instrument. Following successful field tests, all information relating to the construction and operation of the instrument is to be made freely available to the research community under an open source license. The instrument will therefore enable increased sampling regimes without the need for personnel to be deployed in the field, particularly during dangerous or inconvenient sampling periods. Similarly, it is hoped that the open source nature of the system will enable end user groups to evolve the system overtime by highlighting improved designs or novel applications.

A great bane in research and public health is the time and cost required to prepare and analyze environmental samples for the presence of microbial toxins. Current methods often require the use of HPLC, mass spectrometry, and other techniques not suitable to insitu deployment. We are developing biotechnology based methods for detecting and quantifying microbial toxins. Initial proof-of-concept experiments were carried out using microcystin-LR. We are also using anatoxin-a as an early target molecule. Several variations of the technique are being investigated, using ssDNA aptamers and antibody mimetics. ssDNA aptamers exhibit greater stability than traditional ssRNA aptamers but have greater binding efficiencies than dsDNA aptamers. They can be synthesized in large quantities cheaply either chemically or using PCR. Systematic Evolution of Ligands by Exponential Enrichment is used to evolve the aptamers to the targets of interest. To reduce the quantity of toxic and expensive substances used, we have conjugated the targets to magnetic beads, facilitating retrieval and reuse. The second approach involves the in vitro evolution of proteins to bind the targets. We are using the lipocalin fold (anticalin) which is known to be well suited for binding small molecules. These proteins can bind with dissociation constants in the nanomolar range and have many desirable characteristics lacking in antibodies. Anticalins are small (~20kDa), retain function at high temperatures (>60°C), and are easily expressed at high levels in bacterial or cell free systems. Anticalins are selected using the in vitro approach of ribosome display, avoiding the complexity of in vivo approaches such as phage display or bacterial display. Currently we are investigating the use of quantum dots for detecting and
quantifying the targets of interest. Quantum dots are orders of magnitude brighter than traditional fluorescent dyes. This yields the conclusion that a less sensitive detector can be used to provide acceptable resolution. We are developing a basic fluorometer using off the shelf components which will be used with a detection cell containing immobilized aptamers. This will be part of a larger system utilizing our automated sampling device. Quantum dots are well suited for multiplexing due to their sharp emission lines. Our approaches can be used to bind many molecules of interest including small molecules, proteins, and nucleic acids. The aim is to create a modular system which can be easily customized to fit the needs of the scientist using it.

503A  Studying bacterial swarming motility using atomic force microscopy
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Understanding the molecular mechanisms underlying bacterial swarming motility requires studying the factors that induce and control flagella expression in swarming cells. Moreover, bacterial flagella are currently recognized to mediate a number of functions besides their important role in motility, including acting as attachment organelles, secretory systems, and as stimuli of the innate immune response. Therefore, the analysis of flagella and other cell surface components has become an important challenge, especially in Gram-positive bacteria. Different methodologies are available for visualizing bacterial flagella using either optical or electronic microscopy, but none of these techniques combine versatility and easiness, with quantitative and high-resolution information. Here, we report an atomic force microscopy (AFM)-based approach for the fast imaging of bacterial phenotypes (cell shape, flagella expression) in swarming motility studies, focusing on the Gram-positive, rod-shaped, spore-forming bacterium Bacillus thuringiensis. This bacterium is an entomopathogen used worldwide as bioinsecticide which shares important genetic background with other two human pathogens, that is Bacillus anthracis, the etiological agent of the lethal disease anthrax, and Bacillus cereus, the food contaminant and opportunistic human pathogen. Because B. thuringiensis is safe for humans, it represents an ideal model to study swarming in this group of microorganisms with biomedical significance. B. thuringiensis sv. israelensis cells were inoculated on energy-rich media containing increasing agar concentrations. Following swarming assays (2 days), the cell morphology and the amount of flagella were directly observed by AFM imaging in air. Consistent with the macroscopic swarming behavior, cells harvested from the rim of colonies spreading on soft agar (0.35%) were hyperflagellated, elongated and arranged in chains. Increasing the agar concentration (up to 1.5%) led to much lower amounts of flagella and to shorter rod-shaped cells, a finding consistent with the slower swarming motility of the cells. Cells taken from colony centers on soft and hard agar surfaces were generally non-flagellated, rod-shaped, rarely arranged in chains, and exhibited sporulation and lysis. We next addressed the question as to whether cell-aging and nutrient limitation modulate the morphology of swarming cells, by observing B. thuringiensis sv. israelensis cells harvested from the rim and center of colonies developed on soft agar after 10 days of inoculation. AFM-imaging showed that aging of swarming-cells leads to a loss of flagella and to endospore formation. In addition, we found that aged cells developed at higher agar concentrations displayed complex colony architectures reminiscent of biofilms. Our results sustain previous findings for Bacillus subtilis indicating that flagellum formation is a prerequisite for biofilm formation in early stages. This study shows that AFM imaging can readily discriminate between swarming and non-swarming cells, and quantify their morphological details, thus offering an important tool to study the dynamics of bacterial populations.

504A  The gut microbiotassay – a high-throughput real-time PCR chip combined with next generation sequencing
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During the last decade it has become evident that there is a relation between certain medical conditions and the composition of the gut microbiota. To get a better understanding of this complex interaction it is important with high-throughput methods which are sensitive and specific but also informative. Many methods can be used to try to define and characterize the gut microbiota. Here we designed an assay consisting of twenty-four different primer systems targeting the most common bacterial groups of the intestine on different hierarchical levels. The aim of this study was to implement and test this assay with the high-throughput real-time PCR chip “Access Array 48.48” from Fluidigm. The chip executes 2304 individual reactions in parallel and afterwards it is possible to harvest the
amplicons for next-generation sequencing. This approach gives a taxonomical overview of the gut microbiota, hence the name: ‘the gut microbiotassay’.

The assay was tested on fifteen different bacterial type strains each functioning as target for one or more of the primer systems. In this way the sensitivity and the specificity of the primers were assessed. Next the assay was tested on complex ecosystems by extracting DNA from luminal content from small and large intestine, respectively. A 454-barcode library was added to the samples, and incorporated in the amplicons. Subsequently the amplicons were harvested, and any PCR bi-products were removed in a purification step. Finally detailed information on the bacterial composition for each sample was obtained with the Roche 454 GS FLX sequencing.

The gut microbiotassay had a high specificity and sensitivity, detecting from 50 down to at least 0.05ng/μl when tested on dilution series of pure cultures of bacterial type strains. When applied to complex ecosystems it demonstrated distinct quantities of bacteria in the different gut sections, with the highest number found in colon as expected. From the sequence data it was evident that primer systems targeting lower taxonomical levels, contributed with a higher resolution, revealing species that primer system targeting higher taxonomical levels could not detect. At the same time the results for the different primer systems confirmed one another, as some bacteria were detected on various phylogenetic levels, but all in line with their respective taxonomical classification.

The gut microbiotassay in combination with next generation sequencing both provides a quantitative measure in terms of Cq-values achieved from the real-time PCR, as well as the deeper information obtained from next-generation sequencing of the amplicons. It is quick to perform and offers a high-throughput at a relatively low cost. These features make the gut microbiotassay worth considering, when choosing between current methods used to characterize the gut microbiota.

551B A novel fluorescent reporter system for monitoring and identifying RNase III activity and its target RNAs
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Bacteriophage vectors for achieving single-copy gene expression linked to a colorogenic reporter assay have been used successfully for genetic screening applications. However, the limited number of cloning sites in these vectors, combined with the requirement for lac- strains and the time- and/or media-dependence of the chemical-based colorimetric reaction, have limited the range of applications for these vectors. An alternative approach using a fluorescent reporter gene such as green fluorescent protein (GFP) or GFP derivatives could overcome some of these technical issues and facilitate real-time monitoring of promoter and/or protein activity. Here, we report the development of a novel translational bacteriophage fusion vector encoding enhanced GFP (eGFP) that can be incorporated into the chromosome as a single-copy gene. We identified a Bacillus promoter (BP) that is stably expressed in Escherichia coli and drives ~6-fold more expression of eGFP than the T7 promoter in the absence of inducer. Incorporating this BP and RNase III target signals into a single system enabled clear detection of the absence or downregulation of RNase III activity in vivo, thereby establishing a system for screening and identifying novel RNase III targets in a matter of days. An RNase III target signal identified in this manner was confirmed by post-transcriptional analysis. We anticipate that this novel translational fusion vector will be used extensively to study activity of both interesting RNases and related complex or to identify or validate targets of RNases that are otherwise difficult to study due to their sensitivity to environmental stresses and/or autoregulatory processes.

505A Gold-ISH for linking microbial phylogeny and metabolic activities by using nanoSIMS
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Linking prokaryotic phylogeny and metabolic activities at single cell resolution with culture-independent techniques is one of the big concerns for environmental microbiology. Upon recent advancement of isotopical measurement technology, probing with stable isotopes and detection of it at single cell resolution by using NanoSIMS for understanding microbial eco-physiology, especially in relation to carbon and nitrogen assimilation has been more popular technique nowadays. Additionally, SISMISH,
EL-FISH, and HISH have been described as techniques for simultaneous isotopic measurements and phylogenetic identification, thus the way of linking function and phylogeny. While all above methods employ halogen elements for probing, the use of halogen elements sometimes has heavy background signal depending on environments. In this study, we tried to employ gold, which has higher ionization yield compared with halogen elements, and is also relatively lower natural abundance. Furthermore, the signal of gold can be increased in inorganically by gold enhancement, coaggregation of gold ions. We focus on ultra-small particle, undecagold, which is consisted of 11 Au atoms and its diameter is only 0.8 nm. We directly labeled undecagold to oligonucleotide via activated linker arms, and here we present applicability of undecagold-labeled probes for the identification of single cells by using nanoSIMS. Thiol-linked oligonucleotides were successfully labeled with mono-maleimide functionalized undecagold. Hybridization buffer was prepared as generally used, but hybridization and washing temperatures were reduced to 40 ºC and 42 ºC, respectively. After confirming the specific hybridization of probes under epifluorescent microscope, the samples were subjected to nanoSIMS analysis. For the proof-of-concept, purely cultivated \textit{E. coli} cells, labeled with $^{13}$C, and \textit{M. maripaludis} cells, non-labeled, were mixed and detected using the EUB338 probe. The undecagold-derived Au signals detected by nanoSIMS were only found from $^{13}$C-labeled \textit{E. coli} cells. Further experiment was conducted using a granular sludge sample. The Desulfovibrionales, targeted by the SRB385 probe, is known as lactate-utilizer under sulfate reducing environments. After granular sludge was incubated with $^{13}$C-labeled lactate and sulfate, lactate-utilizing microorganisms were found under nanoSIMS analysis, and gold signals indicated \textit{Desulfovibrionales} is the main lactate-utilizer in this sludge community. These results indicated that undecagold-labeled probes can be used for in situ hybridization study with nanoSIMS for linking microbial phylogeny and metabolic activities in microbial ecosystems.

506A Flow cytometric analysis of freshwater viruses
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Flow cytometry has been successfully used to analyze viruses in aquatic systems since the last decade. Although there are detailed protocols to enumerate aquatic viruses, this area remains partly unexplored as the majority of previous studies have focused on marine ecosystems, which have noticeable differences from freshwater ecosystems. Concerns about obtaining accurate and reproducible estimates of virus counts in freshwater led us to examine procedures for counting viruses in freshwater by flow cytometry. In this paper, we set out to detect and enumerate free viruses in natural freshwater samples collected from the Hai River using flow cytometry combined with optimization of the filtration /fixation /storage /staining /dilution. The natural virus communities through 0.1 μm pore size filters lost a group of viruses compared to samples through 0.22 μm pore size filters and unfiltered samples. The total virus counts of the sample through 0.22 μm pore size filter was slightly decreased compared to the unfiltered sample, but no virus groups were missing. It was observed that the number of viruses in samples decreased in proportion to the added glutaraldehyde, but fixation has a clear positive effect on the discrimination of virus groups. There was no obvious difference between samples with or without freezing in liquid nitrogen. The high staining temperature is crucial to prevent from underestimating the natural virus abundance. Dilution in Milli-Q water provides lower instrument background and better results for discrimination of virus groups. The lowest bacterial concentration is 4.04 X 10^4 counts mL^{-1} and R^2 of the trend line is 0.99. In conclusion, the conditions producing optimal counting results were filtered through sterile syringe filters with 0.22 μm pore size followed by fixation with glutaraldehyde (0.25 % final concentration) for 15 min at 4 ºC and storage at –80 ºC after freezing in liquid nitrogen. Na$_2$EDTA (5 mM) was added to samples immediately before staining, and samples were then incubated with 5 μL mL^{-1} SYBR Green I at 80 ºC for 10 min in the dark. Samples were diluted in Milli-Q water prior to analysis. Analysis of freshwater viruses by FCM will be a useful tool to help us better understand the diversity and community structure of freshwater viruses and yield more information on the role of viruses in freshwater ecosystem.
One of the current limitations of methods used in environmental microbiology is the lack, or loss, of spatial information which is essential to the understanding of interactions operating over the micrometer range within and between microbial communities. The architecture of natural microbial communities in different natural substrates including soils, the rhizosphere and within plant tissues describes the distribution of microbes with respect to physical features as well as their complex interactions. This type of spatial information is important in a range of environmental research. Here we propose a new method to sample microbial communities which maintains the spatial information. This uses chemically and biologically-inert PET (polyethylene terephthalate) films, which can be placed in different environments to allow the development of natural microbial communities and then recovered for analysis. We first show, using two model soil and rhizosphere Pseudomonas spp. (P. fluorescens SBW25 and P. putida KT2440), that bacteria are able to attach and detach from PET films in vitro. Bacteria attached to the films were neither dead nor inactive, as viable cells could be recovered and biofilms developed in liquid microcosms. PET films buried in soil microcosms were rapidly colonised by microorganisms and were readily investigated by fluorescent microscopy, confocal laser scanning microscopy (CLSM), and fluorescent in situ hybridization (FISH). Furthermore, micro-X-ray computed tomography (µCT) was able identify PET films buried in soil microcosms, suggesting that film positions could be mapped in 3D with respect to pore spaces, aggregates and other structural features. The PET film sampling method was also tested in the rhizosphere of rapeseed (Brassica napus) where interactions between GFP-tagged SBW25 and the native soil microbial community were studied, and the colonization of tomato (Solanum lycopersicum) roots by an artificial biocontrol community. Finally, it was used to study the endophytic microbial communities of bamboo (Phyllostachys atrovaginata) stems. In this work microbial colonies were isolated by a Micro Beam micro-dissection of the PET films, and these were then used to allow the identification of bacterial species. The PET film method enables the use of modern imaging and micro-dissection techniques coupled with molecular technologies, allowing an comprehensive investigation of the architecture of non-disturbed microbial communities from a variety of different environments.
The developed methodology was validated by comparison to traditional dedicated and simultaneous biomolecular isolation methods. In order to access the robustness of our method and to prove the broad applicability of the methodology, we further applied it to microbial consortia of biotechnological, environmental and medical interest as well as on mammalian tissues.

Importantly, the new methodology will allow exploitation of the inherent heterogeneity and dynamics within microbial consortia through spatial and temporal sampling of these systems to allow later statistical deconvolution of community-wide, population-wide and individual-level processes using the generated omic data. This approach has the potential to identify associations between distinct community members and biomolecules which may provide pointers towards unravelling previously unknown metabolic processes. Finally, by providing a standardized workflow, the methodology lays the foundation for comparative eco-systematic studies of different natural microbial consortia in the future.

509A Natural microbial community remediates oil in Australian hydrocarbon contaminated coastal sediment
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Taxonomic and metabolic shifts within the microbial community of a hydrocarbon contaminated beach sample were assessed using a combination of classical molecular tools and next generation sequencing methods. Microcosm experiments compared remediation of unamended samples to those amended with nutrients and surfactants. The unamended, natural samples reduced Total Petroleum Hydrocarbon concentrations over a 4 week period by 100%. Biotreatment did not confer any additional benefits to hydrocarbon contamination. Metagenomic analysis was used to determine shifts in taxa between the hydrocarbon contaminated beach sample and two uncontaminated controls prior to microcosm experiments. A fundamental shift in taxa was seen, with an overrepresentation of Pseudomonadales, Actinomycetales, Rhizobiales, Alteromonadales, Oceanospirillales and Burkholderiales in the contaminated sample. In addition to taxonomy, a relative overrepresentation of metabolic processes including aromatic compound metabolism, nitrogen metabolism and stress response were observed in the hydrocarbon contaminated beach sample. These differences suggest the natural community rapidly remediated the Total Petroleum Hydrocarbons without the addition of nutrients and surfactants. Obtaining a broader understanding of the structure and function of microbial communities involved in bioremediation is particularly important given their exploitation value in the cleanup of contaminants.

509B Community isotope arrays for sensitive resolution of substrate-species linkages in natural microbial communities
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The ability to study the ecophysiology of microorganisms without cultivation is crucial to advancing our understanding of the structure and function of complex microbial communities, and is of particular interest for identifying species that are capable of degrading hazardous chemicals within the context of wastewater treatment and bioremediation. In this work, community isotope arrays (CIArrays) are presented as a novel substrate-species technique that combines the strength of nucleic acid based stable isotope probing (SIP) as a discovery tool and that of isotope arrays for rapid, high-throughput and sensitive detection. To achieve higher sensitivity and specificity compared to 16S ribosomal RNA (rRNA) based isotope arrays, and also enable detection of hitherto unknown microorganisms, CIArrays are constructed with heterogeneous metagenome fragments (roughly 40 kbp in length) directly obtained from the sample under investigation through cloning. To demonstrate the feasibility of this approach, the CIArray was applied for identification of phenol-degrading microorganisms in an activated sludge reactor treating (synthetic) coke oven wastewater under anoxic conditions. Hybridization with DNA from sample amended with radiolabeled (14C-)substrate indicated that phenol
degraders were abundant in the reactor, with partial sequencing and taxonomic assignment of the clones suggesting that they belonged to the Gammaproteobacteria. To verify the CIArray data, sample was also incubated with $^{13}$C-phenol and clone-specific DNA in density-resolved fractions quantified by real-time quantitative PCR (that is, by SIP). Complete concordance between the CIArray and SIP analyses was observed for all probes with positive signal on the CIArray and several randomly selected negative probes. Importantly, this comparison also revealed that the CIArray technique was more sensitive than SIP, which is a crucial strength as it permits incubations to be performed with lower substrate concentrations and/or shorter incubation times. A 16S rRNA gene clone library prepared from the ‘heavy’ DNA fraction from the $^{13}$C-phenol-amended sample revealed two operational taxonomic units distantly related to various marine Gammaproteobacteria (90–92% sequence similarity) as main phenol degraders, in qualitative agreement with the CIArray-enabled identification. Meta-analysis based on previous rRNA-SIP analyses of the reactor community indicated that marine Gammaproteobacteria, probably derived from the seawater that is used to dilute the wastewater prior to treatment, constitute a stable phenol-degrading guild in the reactor. Overall, this study demonstrates the utility of CIArrays for linking microbial community structure and function, and also provides evidence for the existence of hitherto unknown marine Gammaproteobacteria capable of degrading phenol under anoxic conditions. The CIArray could be streamlined with high-throughput sequencing to add a crucial functional component to current metagenomic studies. Using whole genome probes, which is being enabled by recent advances in high-throughput whole genome amplification from single cells, could further extend the utility of this technique and provide a valuable approach for function-targeted single cell sequencing.