Life Marker Chip
and its Detection Technologies

Professor Mark Sims
University of Leicester
Professor David Cullen
Cranfield University

© M R Sims 2011

Life Marker Chip
and its Detection Technologies

Contents:

- LMC Consortium
- Mission Overview
- Science Objectives
- LMC Detection Technologies
- LMC Design
- LMC Current Status and Progress

© M R Sims 2011

Life Marker Chip
ESA/NASA ExoMars Rover Mission 2018

LMC is a collaboration between University of Leicester, Cranfield University, Imperial College London, Magna Parva Ltd, Surrey Satellites Technology Ltd., with science involvement from Open University, University of Aberdeen along with industries and scientists from Netherlands, Italy, Germany, Sweden, Norway and USA. UK funding is from UKSA/STFC.

Principal Investigator: Professor Mark Sims
University of Leicester
Deputy Principal Investigator: Professor David Cullen
Cranfield University

© M R Sims 2011

Acknowledgements (major partners)

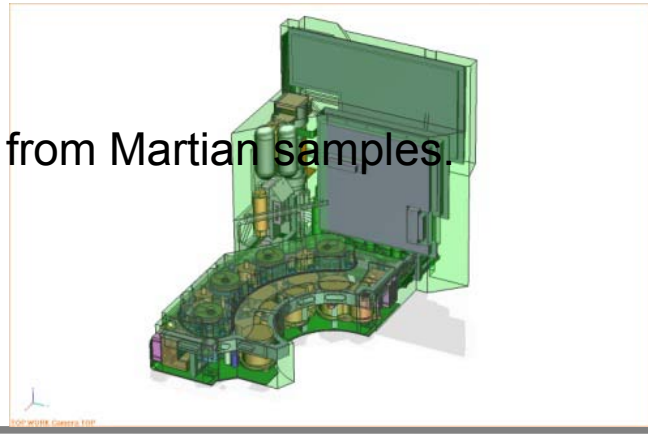
University of Leicester
Cranfield University
Imperial College London
magnaparva
Science & Technology Facilities Council
UK SPACE AGENCY
LIONIX
KAYSER ITALIA
esa
Dutch Space an EADS Astrium company

... plus many others

© M Sims 2011

Life Marker Chip (LMC)

- ESA/NASA ExoMars 2018 Rover Mission
- LMC Key Science Objectives
  - To detect organic molecules in a low temperature (20˚C) liquid extract of Martian regolith and/or crushed rock obtained by ExoMars drill (up to 1-2m below surface).
  - To interpret detected organic molecules within categories of extinct and extant life, abiotic and Earth-derived contamination.
- Enabling instrument capabilities
  - Low-temperature (20˚C) liquid extraction of organics from Martian samples.
  - Multiplexed immunoassay for up to 25 different organic molecular targets on each liquid extract.
  - pH measurement of a water/sample mixture prior to extraction. TBC.



© M R Sims 2011

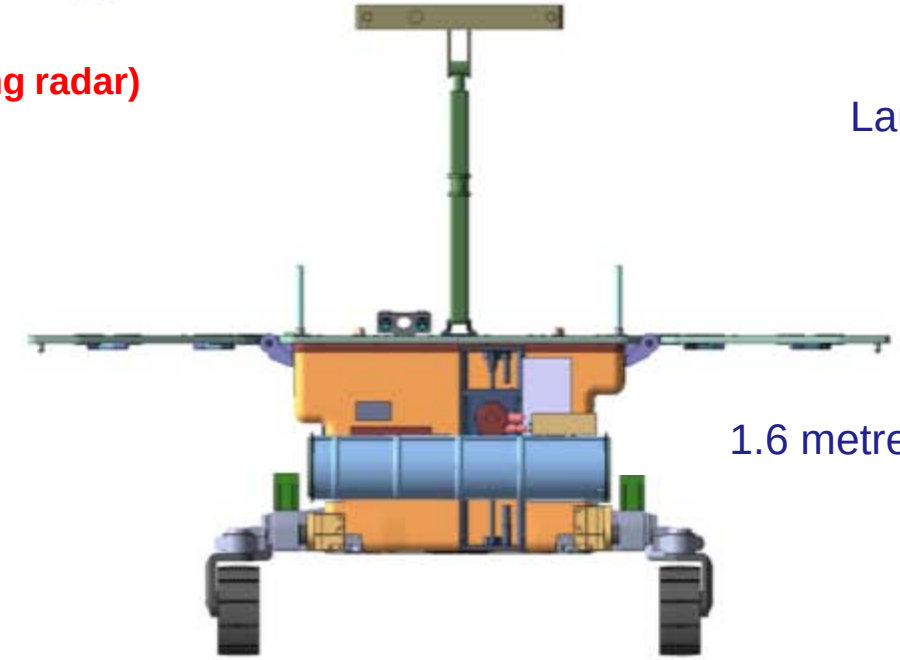
ExoMars Rover (coming into 2011)

Instruments

- PanCam
- **WISDOM (ground penetrating radar)**
- Ma-Miss
- MicroOmega
- **MOMA (GC/LD-MS)**
- RAMAN
- Mars-XRD
- **Life Marker Chip (LMC)**
- CLUPI

Other features

- **Drill (to ~2m)**

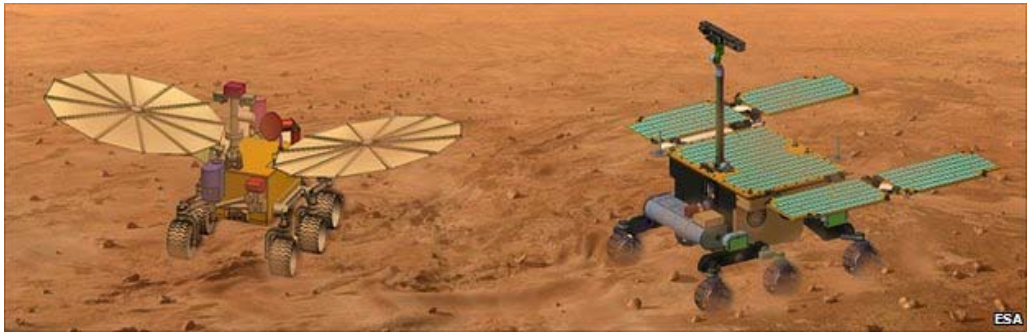


Launch scheduled for 2018

1.6 metres tall

Recent and Current Mission Status

- Joint Mars Exploration Programme (JMEP) between NASA and ESA
  - **2016 ExoMars mission – orbiter and ESA EDL demonstrator**
  - **2018 ExoMars mission – dual rover ESA ExoMars and NASA MAX-C delivered by modified NASA Sky Crane EDL (as per Mars Science Laboratory Curiosity Launch Nov 2011)**



- Spring 2011 - cost concerns in ESA and NASA
  - **Mismatch between the ESA cost cap, the programme's scope, and its estimated Cost at Completion (CAC)**
  - **NASA technical and budget difficulties to support their commitments for the Joint Mars Exploration Programme (JMEP). Decadal Review.**
- Has lead to a single rover (under design) that fulfils ExoMars objectives and caches samples for return in the future. Decision on whether mission proceeds is due October 2011

© M R Sims 2011

LMC Science Objectives

- Detection of organic molecules in Martian rocks and regolith at ppm-ppb
  - Detect organics in suitable samples – *e.g.* sedimentary, evapourite, … deposits
  - Measure organic content vs. depth
  - Detection signs of extinct and extant Life (with a emphasis on Earth-like Life)

- LMC target - biomarkers and control markers
  - *Extinct Life – preservation / diagenetic products of ancient life (geo-molecules)*
  - *Extant Life – short lived products of present life (bio-molecules)*
  - *Abiotic organics – examples of meteoritic in-fall, preservation / diagenetic products of early Mars organics inventory*
  - *Spacecraft contamination markers – mainly micro-organism markers*
  - *Assay control markers – for example synthetic organic molecules*

- See next slide for full list
- Uses immuno-assay techniques (with bespoke antibodies as molecular receptors) for organic detection and contains its own liquid based extraction system
- LMC central to astrobiology / ExoMars and other Mars mission science objectives
- Complementary with other ExoMars Pasteur organics instrument suite – *i.e.* Raman, MOMA/GC-MS

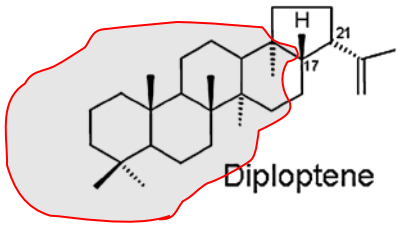
© M R Sims 2011

Target Biomarkers

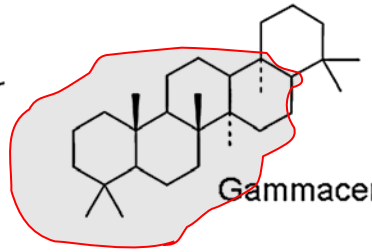
Extant		Extant		Meteoritic	
1	ATP	26	Melanoidins	47	Napthalene
2	Phosphoenolpyruvate	27	Sediment/cell extracts: 1. Acid mine drainage	48	Coronene
3	Acetyl phosphate	28	Sediment/cell extracts: 2. Methanogens	49	Pyrene
4	cyclic AMP	29	Sediment/cell extracts: 3. Cyanobacteria	50	1,3 Dimethylbenzene
5	Generic pyrimidine base	30	Sediment/cell extracts: 4. Mars Energy Users	51	1,4 Dimethylbenzene
6	Generic purine base	31	Sediment/cell extracts: 5. Extract/abiotic mix	45	Generic amino acid
7	DNA			52	isovaline
8	Nicotinamide (generic NAD, NADP)	Extinct		53	a-aminoisobutyric acid
9	Flavin (isoalloxazine ring)	32	Generic isoprenoid	54	Generic aromatic carboxylic acid
10	Fe-S centres	33	Pristane	55	Experimental abiotic
11	Quinones	18	Phytane		
12	Generic carotenoid	34	B-carotane	Contaminants	
13	Phycocyanin	35	Tetramethyl benzenes	56	Generic fungal
14	Thiol Esters	36	Tetramethyl cyclohexanes	20	Teichoic Acid
15	Generic porphyrin	37	Squalane	21	LPS
16	Chaperons	38	Generic ABC terpane	57	Staphylococcus
17	ATP Synthase	39	Generic hopane	58	Streptococcus
18	Phytane	40	Gammacerane	59	Bacillus
19	Fatty acids (1 or 2)	41	Generic diasterane	60	Micrococcus
20	Teichoic Acid	42	Generic sterane	61	Pseudomonas
21	LPS	43	Generic porphyrin (ancient)	62	Dipicolinic acid
22	Ectoine	44	Generic Straight-chain	63	Hydrazine (or equivalent fuel marker)
23	Trehalose	19a,b	2 individual Fatty Acids		
24	Squalene	45	Generic amino acid		
25	Diploptene	46	Quaternary carbon alkane		

Table result of LMC Mars Biomarker Workshop (Leicester, UK - May 2006) see *Astrobiology 7, 4, 578-604*

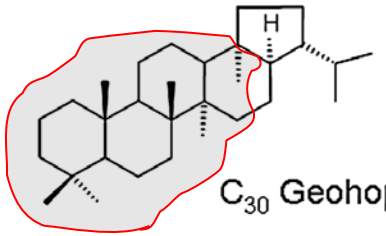
© M R Sims 2011



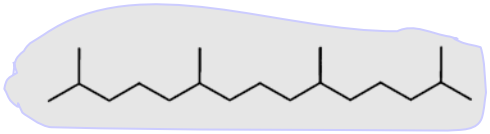
Diploptene



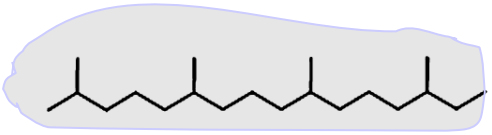
Gammacerane



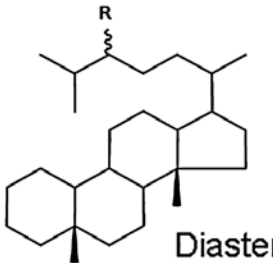
C_{30} Geohopanoid



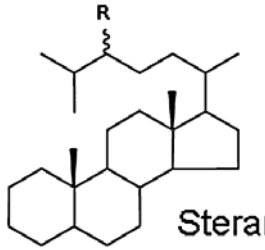
Pristane



Phytane

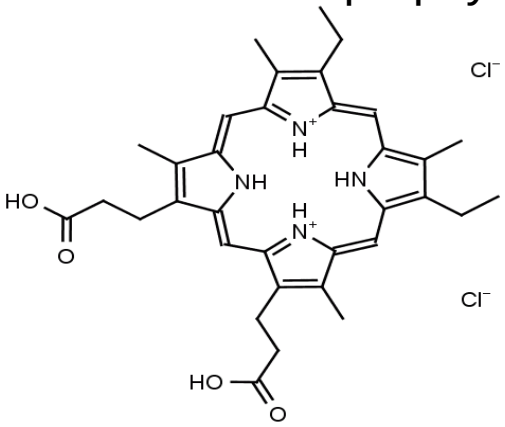


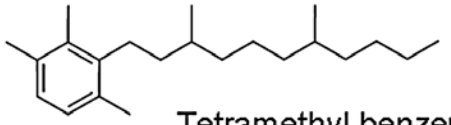
Diasteranes



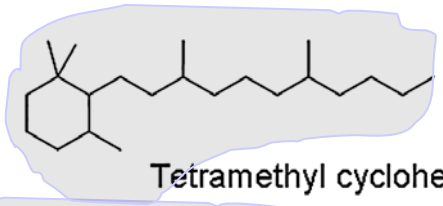
Steranes

Protoporphyrin IX

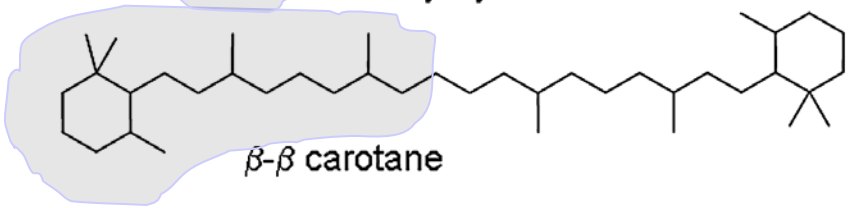




Tetramethyl benzene



Tetramethyl cyclohexane

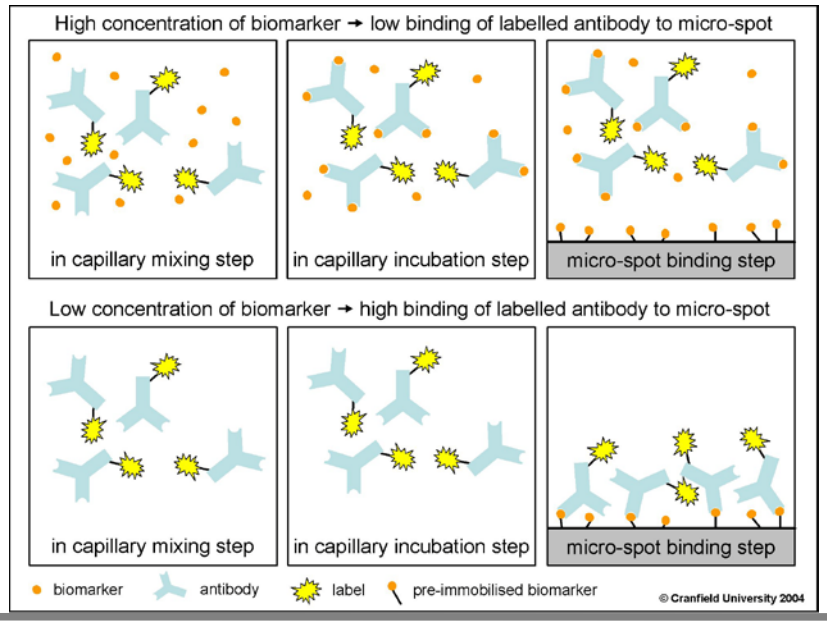


β-β carotane

© M Sims 2011

LMC Detection Technologies : I

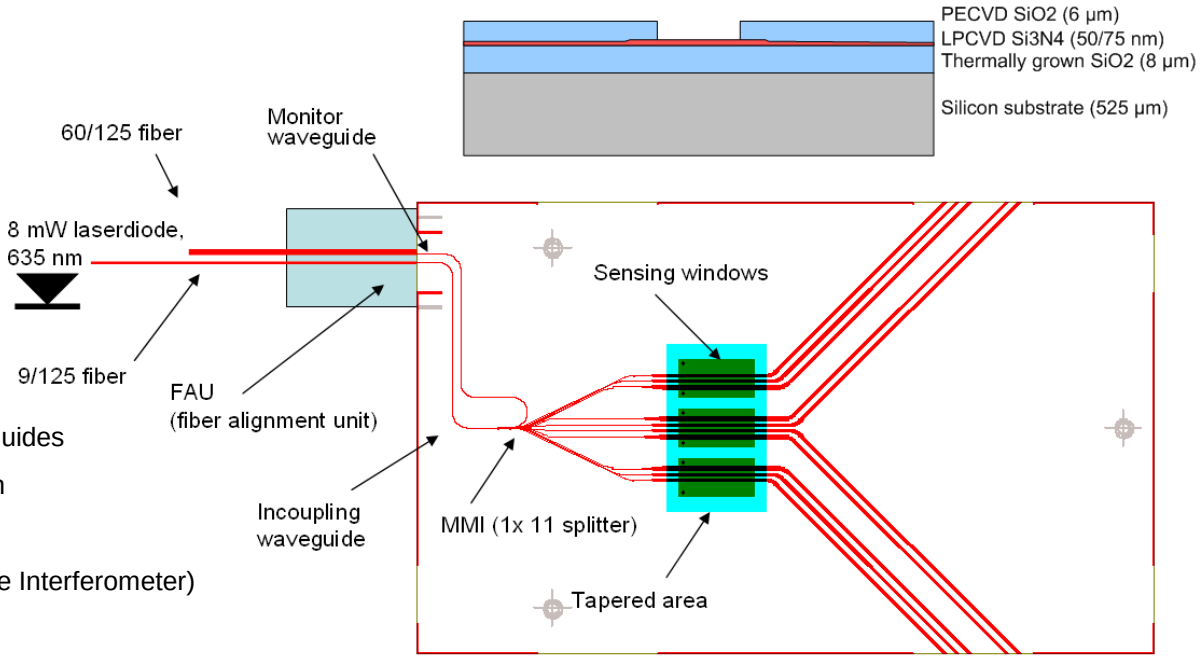
- Molecular Receptors
  - "lock and key" target molecule and receptor
  - Antibodies current receptor of choice
  - Get an immune response which generates antibodies against targets injected into animals
  - Very high sensitivities possible ~ppm to ppb
  - Other receptors possible e.g. apatmers (nucleic acid sequences), lipocalins (receptors for transport of small molecules around body)
  - "Inhibition" format thought to be best for small molecules



© M R Sims 2011

LMC Detection Technologies : II

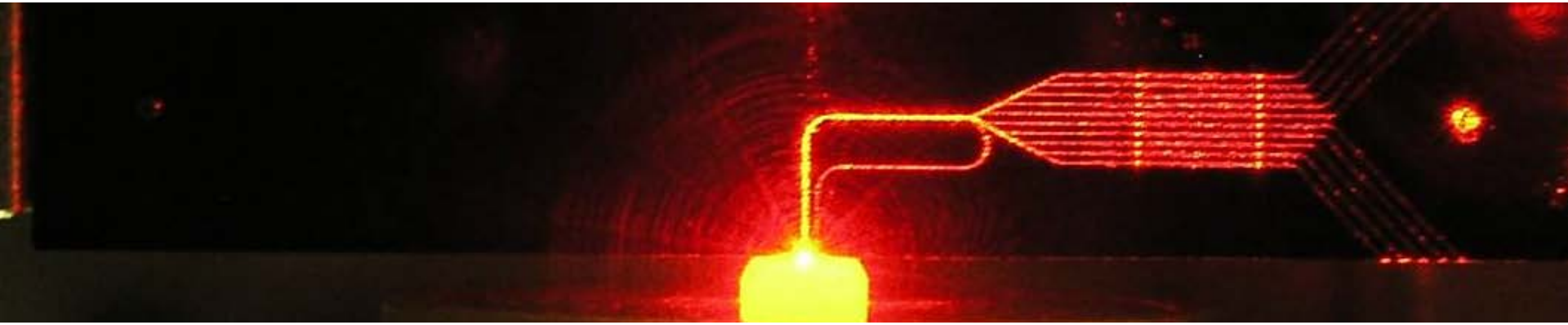
- Readout Technologies
  - Use fluorescence using 635nm Laser
  - Fluorophores excited via evanescent field from a waveguide
  - Silicon Nitride Waveguides including a Splitter
  - Target spots in an array
  - Image array using coherent fibre optics and a relay optics system onto a cooled CCD detector.

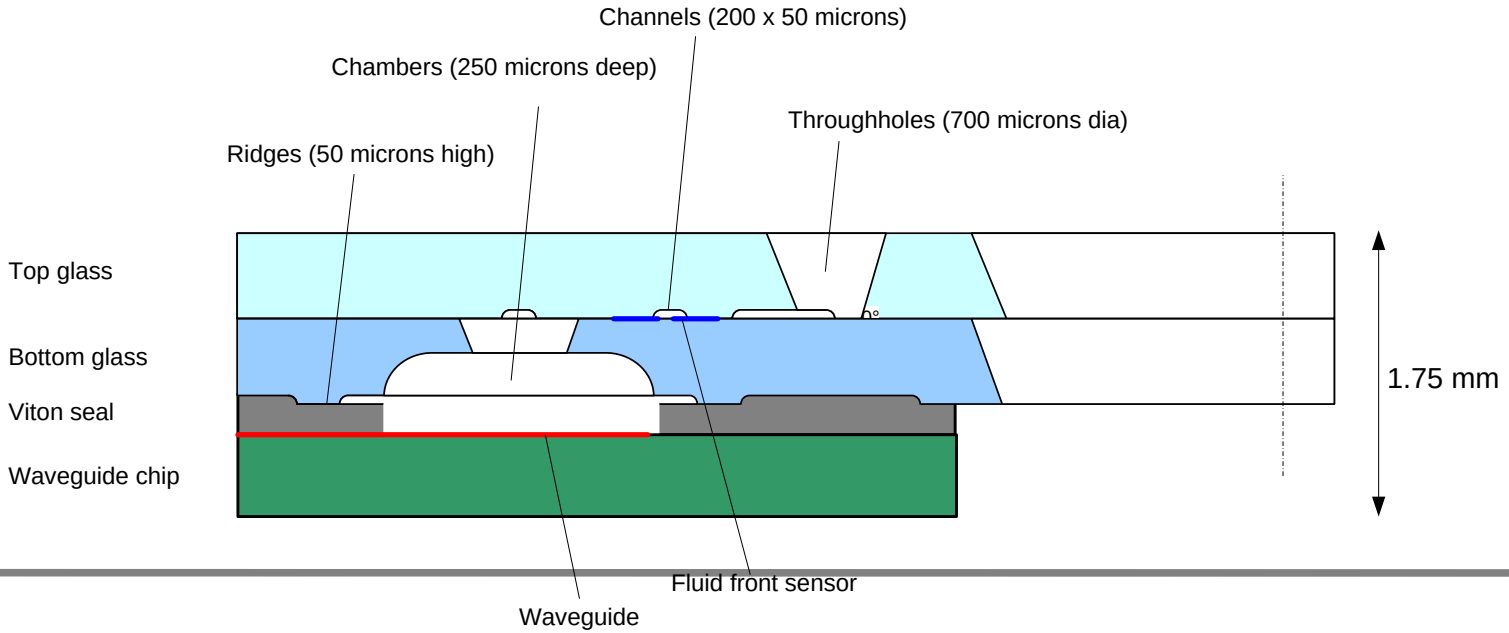


- Single mode transport waveguides
- Optimise Antibody Interaction
- Minimise bending losses
- 1x11 MMI Splitter (Multi Mode Interferometer)
- Optimise Coupling losses

© M R Sims 2011

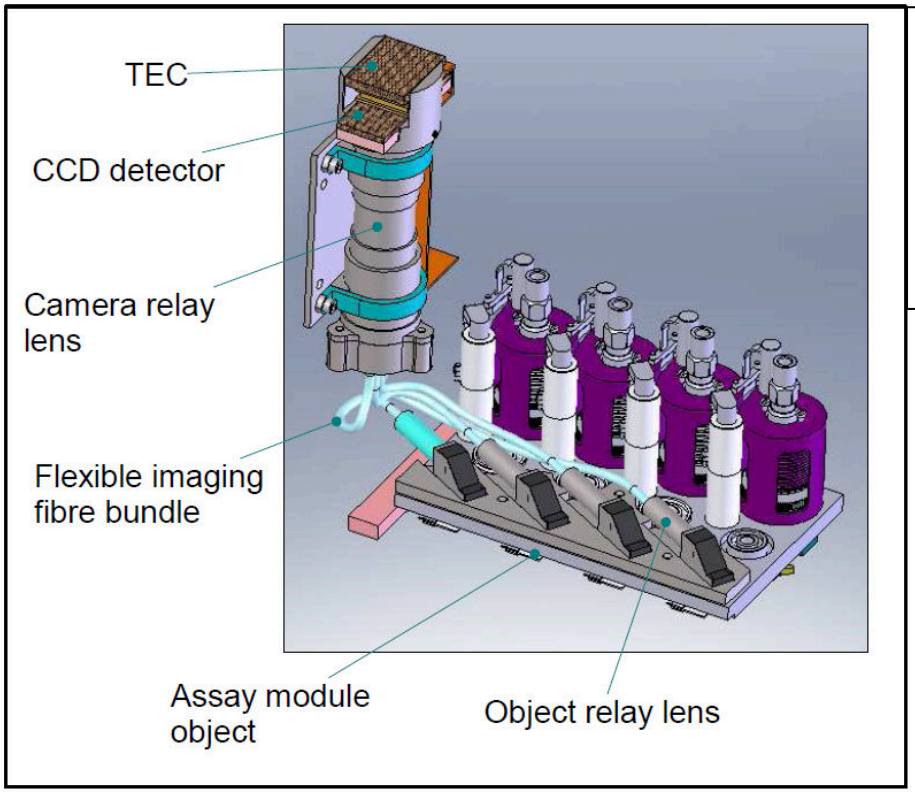
LMC Detection Technologies : III
Fluidic Chip and Waveguide Splitter





© M R Sims 2011

LMC Detection Technologies : IV
Relay Optics



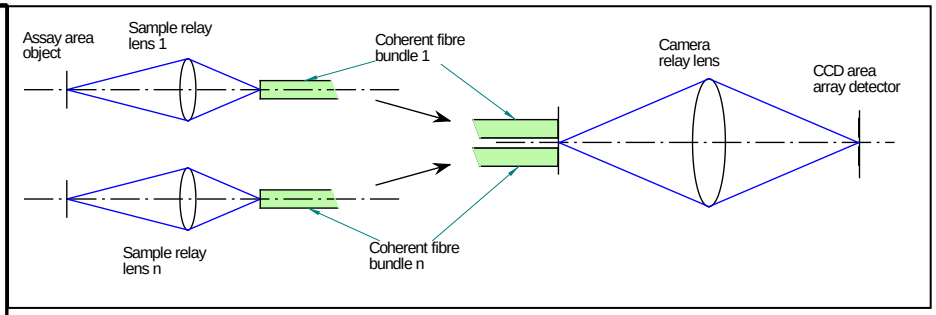


Figure 2.1: LMC Optical System Configuration

© M R Sims 2011

LMC Detection Technologies : V

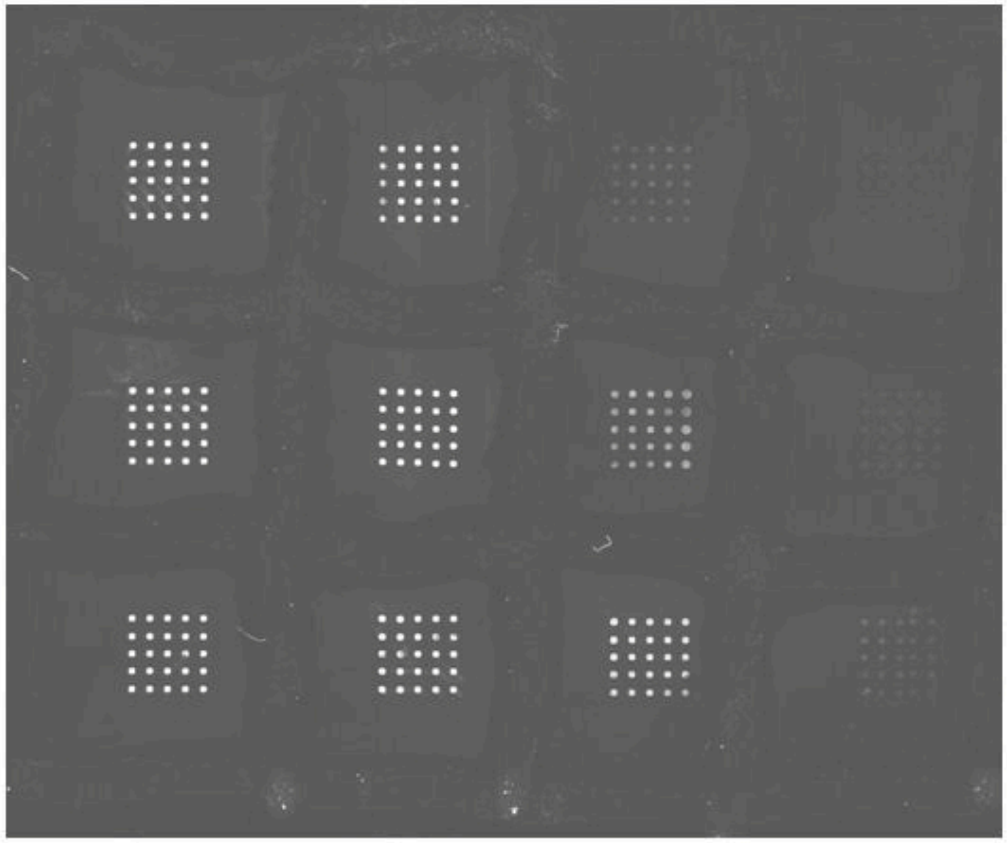
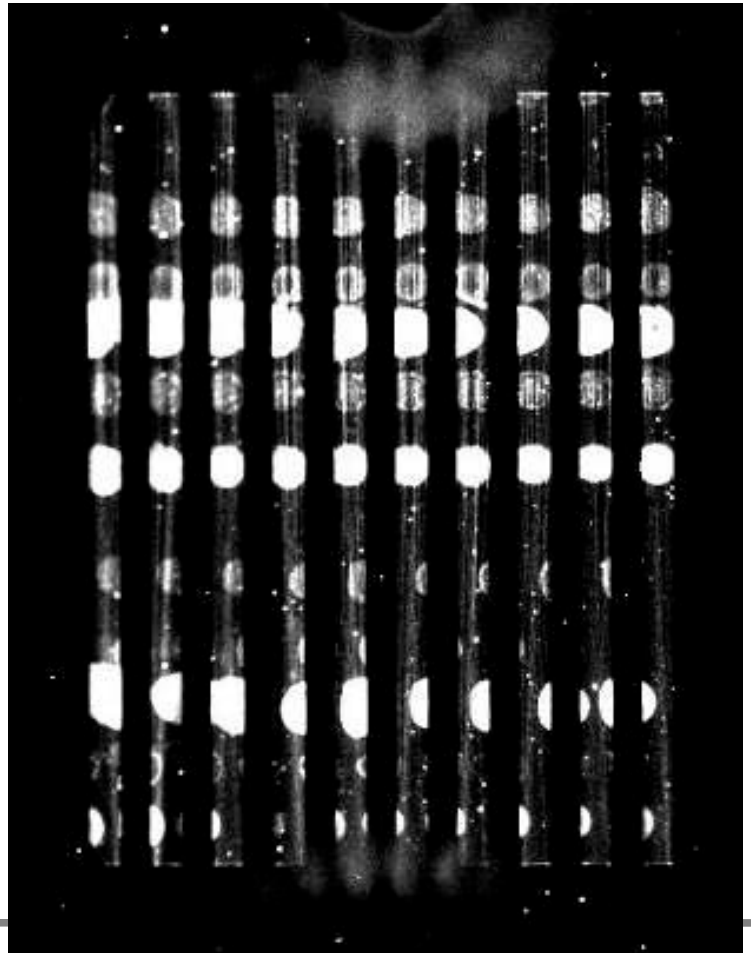


Figure 1: Example fluorescent image of high-throughput LMC microarray assay for benzo[a]pyrene. Twelve separate 5x5 microarrays with each array covered during assay with a separate drop of common LMC assay reagents, including fluorescently labelled antibody, but each with different concentration of free benzo[a]pyrene – highest concentration top right (1.125µg/ml) with two-fold dilution series of free benzo[a]pyrene on other microarrays to lowest concentration in bottom left (0.55ng/ml). Faint grid markings between arrays are a manually applied hydrophobic ink to stop the drops of assay reagents mixing. Each array measures 1.6mm x 1.6mm.

© M R Sims 2011

After assay image of optical waveguide LMC device showing multiplexed antibody binding



© M Sims 2011

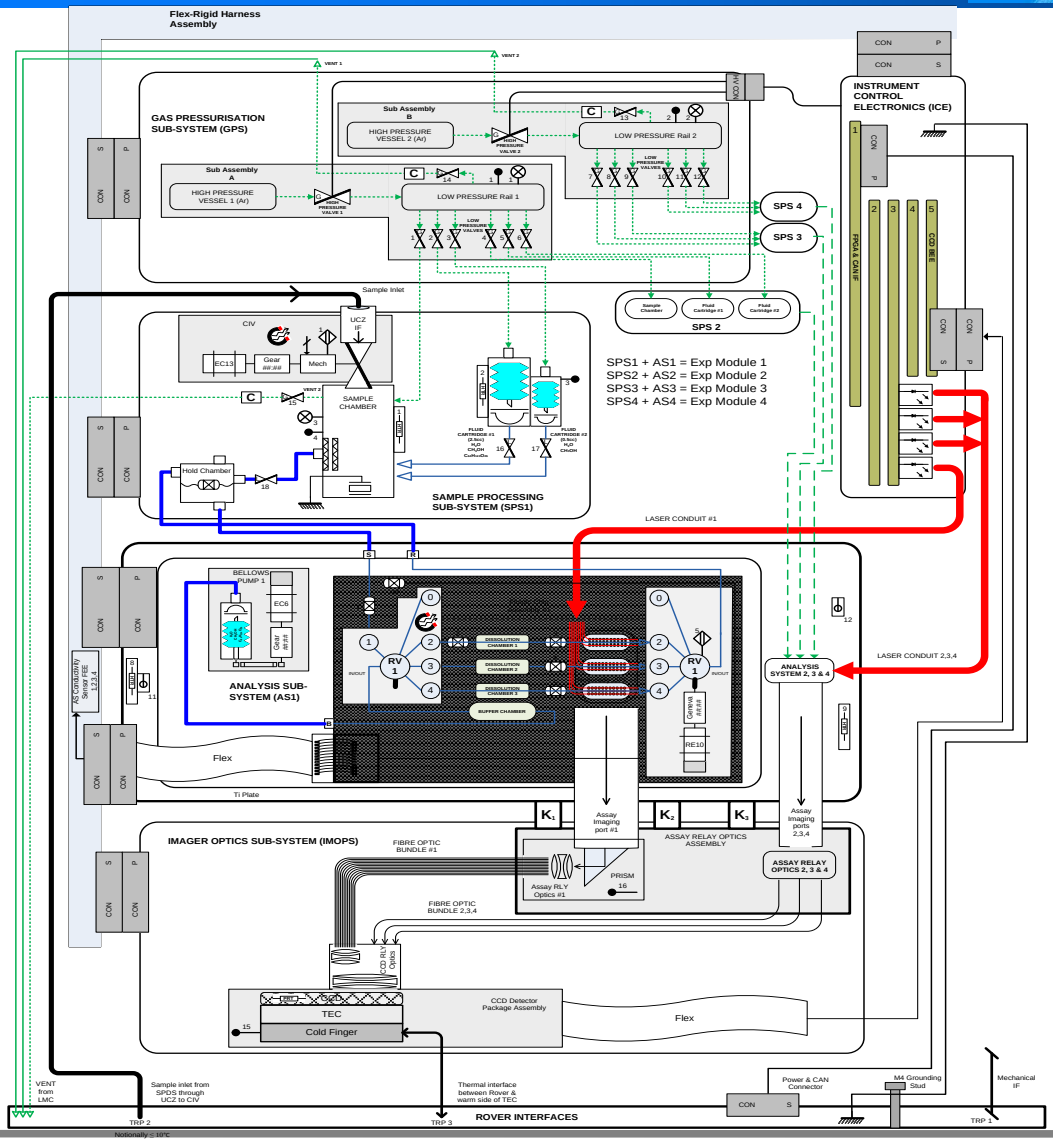
LMC Design Requirements

- Requirements
  - Single use assays
  - Assays to be run under Earth-like conditions (sensitivity calibration)
    - Temperature
    - Pressure
  - All fluidics and systems integral to instrument
  - Must operate without intervention
  - All fluids contained without leakage
  - Must be able to survive mission (particle) radiation requirements
  - Minimum size, power (~50W max), mass (~5kg)
  - High sensitivity possible ~ppm to ppb
  - Work with other instruments (as a screen e.g. XRD, GC-MS, Raman)
  - Must meet contamination and Planetary Protection requirements

- Implications
  - = > 4 Samples Only!
  - Sealed Fluidic Containers only opened once on Mars
  - Gas Pressurisation System
  - Integral Sample Processing System
  - Need to assemble in ultra-clean aseptic (sterile) environment

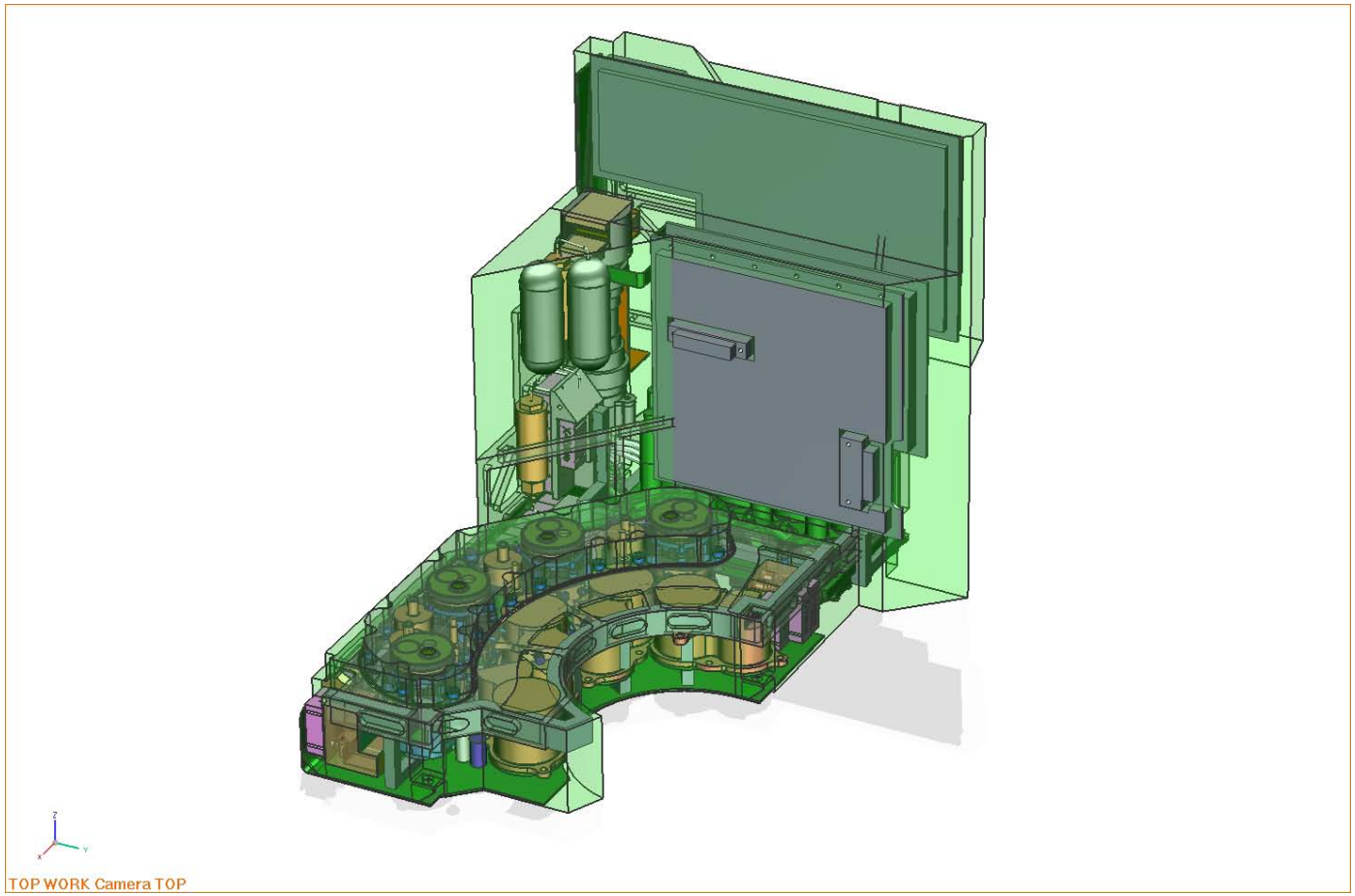
© M R Sims 2011

LMC FM System Design (as of July 2010)



© M R Sims 2011

LMC FM Design (as of July 2010)



© M R Sims 2011

LMC Current Status and Progress: I

- Instrument Development and Engineering
  - Detailed design of instrument sub-systems underway
  - Instrument packaging into allowed envelope underway (real-time concurrent engineering!)
  - Leading to definition of instrument interfaces to rover

- Assay Development
  - Underway, some antibodies exist, but having problems in getting recognition of small molecules by antibodies produced in animals.
  - Multiple antibody library types now under investigation from synthetic libraries to different animal derived types (camels, sheep ,mice)
  - Aptamers (nucleic acid based molecular receptors) and Lipocalins being looked as an alternative backup approach
  - Small molecule antibodies have been produced in past for targets such as hopane, however are no longer available due to limited amounts produced

© M R Sims 2011

LMC Current Status and Progress: II

- Sample Extraction
  - Have successfully demonstrated extraction of polar and non-polar molecules from spiked samples into patented solvent mix with reasonable efficiencies compatible with instrument performance.
  - Tests with real samples are about to start.

- Largest Development Risks
  - Assays
  - Instrument envelope
  - Electronics and internal software
  - Schedule. FM delivery end 2014.

- Terrestrial Spin-offs
  - Several being actively pursued, solvent mix (petrochemicals), sample processing (environmental and medical samples)

© M R Sims 2011

LMC Assay Readiness Level (ARL) status table
Status date: 8th August 2011
Author: D. Cullen

LMC Assay Status (as of August 2011)

			ARL	Antibody acquisition approach chosen	Antibody acquisition approach started	Antibody in appropriate form for assay development	Assay demonstrated in LMC microplate format	Cross reactivity measured, ready for potential FM use in LMC microplate format	Tested LMC microarray chip based format (e.g. non-OWG surface) in simple multiplex	Tested LMC microarray chip Based format on OWG's (ABB or BEB in simple multiplex (ideally with analogue samples if available)	Tested LMC microarray chip Based format on OWG's in FM multiplex format (ideally with analogue samples if available)	Integrated into EM/FM
				ARL 1	ARL 2	ARL 3	ARL 4	ARL 5	ARL 6	ARL 7	ARL 8	ARL 9
1	Abiotic	Benzo(a)pyrene	7	Y	Y	Y	Y	Y	Y	Y		
2	Abiotic	Pyrene	6	Y	Y	Y	Y	Y	Y			
3	Abiotic	BTEX (benzene, toluene, ethyl benzene, xylenes)	3	Y	Y	Y						
4	Abiotic	Napthalene ~~Fluorene~~	2	Y	Y							
5	Abiotic	α-aminoisobutyric acid (AIB)	1	Y								
6	Abiotic	Phenanthrene ~~Anthracene~~	3	Y	Y	Y						
7	Abiotic	Mellitic acid	1	Y								
8	Abiotic / Extant	D-α amino acids (D-cysteine)	2	Y	Y							
9	Abiotic / Extant	L-α amino acids (L-cysteine)	2	Y	Y							
10	Abiotic / Extant	D-α amino acids (D-tyrosine)	5	Y	Y	Y	Y	Y				
11	Abiotic / Extant	L-α amino acids (L-tyrosine)	5	Y	Y	Y	Y	Y				
12	Abiotic / Extant	Generic purine base (xanthine)	1	Y								
13	Abiotic / Extant	Generic pyrimidine base	0									
14	Extant	GroEL	7	Y	Y	Y	Y	Y	Y	Y		
15	Extant	Homoserine lactone (N-Acyl homoserine lactone)	4	Y	Y	Y	Y					
16	Extant	Lipopolysaccharides (LPS)	4	Y	Y	Y	Y					
17	Extant	Lipoteichoic acid (LTA)	4	Y	Y	Y	Y					
18	Extant	Cyclic adenosine monophosphate (cAMP)	6	Y	Y	Y	Y	–	Y			
19	Extant	ATP synthase	3	Y	Y	Y						
20	Extant	Scytonemin (melanoidins)	1	Y								
21	Extant	Isoalloxazine (flavin)	0									
22	Extant	Phycocyanobilin (phycobilins)	0									
23	Extant	Ubiquinone-1 (quinones)	0									
24	Extinct	Squalene	3	Y	Y	Y						
25	Extinct	Squalane	3	Y	Y	Y						
26	Extinct	5β sterane (coprostane)	2	Y	Y							
27	Extinct	5α sterane	2	Y	Y							
28	Extinct	β,β Carotane	2	Y	Y							
29	Extinct	Generic isoprenoid (phytane)	2	Y	Y							
30	Extinct	Generic straight chain alkane	2	Y	Y							
31	Extinct	Hopanoid	2	Y	Y							
32	Extinct	Porphyrin	2	Y	Y							
33	Contamination	Dipicolinic acid	2	Y	Y							
34	Contamination	Bacillus spores	3	Y	Y	Y						
35	Contamination	Micrococcus	2	Y	Y							
36	Contamination	Staphylococcus	2	Y	Y							
37	Control	Atrazine	7	Y	Y	Y	Y	Y	Y	Y		
38	Control	Fluorescein	7	Y	Y	Y	Y	Y	Y	Y		

© M R Sims 2011