
# EUCLOCK
## Entrainment of the Circadian Clock

2006 - 2011

An Integrated Project funded by the 6th Framework Programme
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# 01 FROM LAB TO LIFE

Till Roenneberg, EUCLOCK Coordinator

Life on earth is embedded in regular daily changes of the environment. Chronobiology investigates how organisms adapt to these predictable changes. All organisms (including humans) have evolved biological clocks that produce self-sustained rhythms even in constant conditions where their internal days approximate but rarely match 24 hours (hence, *circa diem*, fused to *circadian*). These clocks synchronise their internal days to the cyclic environment by an active process, called entrainment, predominantly using light as a synchroniser (zeitgeber). The pathways and mechanisms that enable every cell within an organism to generate an internal, circadian day have been a focus of rhythms research for some decades.

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The genetic basis of the body clock makes individuals different “chronotypes” – the colloquial “larks” and owls”. Modern life frequently disrupts the entrainment of the human circadian clock, when we travel, for example, across time zones and suffer from jetlag, when we have to work in shifts rotating through the 24 hours of the day, or simply by having to wake up with the help of an alarm clock on workdays. Modern humans expose themselves to very weak zeitgebers due to pre-

dominantly living inside, where light intensities are almost 1.000-fold lower than outside. As a result, the distribution of chronotypes in the population becomes later and broader, so that the clocks in extreme early and late chronotypes are meanwhile up to 12 hours apart. The consequences of frequently disrupting our body clocks and our sleep have serious effects on our health, ranging from chronic sleep disturbances and memory deficits, obesity, diabetes and other metabolic diseases, to higher risks of developing cancers.

In October 2004, 18 European chronobiologists got together for three days in a beautiful house in Franconia, Germany. In splendid isolation,they worked on a grant proposal with the aimto consolidate and strengthen the excellent European Chronobiology tradition with a new initiative. The product of these three, hard-working days led to establishing the EUCLOCK consortium, subsequently funded by the EU, which had its kick-off meeting in January 2006.

While working out the mechanisms of the circadian clock in constant conditions was the main endeavour of chronobiology research over the past decade, we still knewlittle of how the body clock is entrainedin nature with its "noisy" light signals. EUCLOCK's aim was therefore to understand how biological clocks entrain in the real world. Eventually, a consortium of 150 scientists (including principal investigators, post-docs and students) working in 29 European laboratories from 12 countries (including Russia and the Philippines) and 5 small companies was formed to investigate "Entrainment of the circadian clock".

The strength of chronobiology is that it approaches understanding the mechanisms of the biological clock in a highly interdisciplinary fashion. In keeping with this tradition, EUCLOCK was built on four pillars (sub-projects) each using different "substrates" for their experiments: humans, mice, flies, and 'genes and principles'. To apply the insights of chronobiological laboratory research to everyday life situations of humans, new instruments and algorithms had to be developed that allow measurement of the body clock, for example in shift-workers. We developed devices that can measure light (intensity and spectral composition) that we receive at the level of the eye over the course of the day and year in our daily lives – an important factor in determining daily timing. Humans and mice were submitted to light of different spectral composition to investigate what kind of light was most efficient in entraining our body clocks, leading to important information for improving indoor lighting. Mice and flies had to live like human shift-workers to investigate this most extreme violation of our body clocks – the results revealed frightening consequences. Flies were held for the first time under natural conditions outside of the laboratory, where they were exposed to daylight and moonlight and to the natural daily temperature differences. Clocks and their light inputs were artificially reconstructed from artificially constructed genes in cellular systems to understand how molecules work together to entrain the circadian machinery. We searched for and found many new genes that form the basic components of the circadian clock and the organisation of sleep in the most diverse organisms. New model organisms for circadian research were developed, for example yeast, which is the prime model organisms for understanding the molecular machinery of cells but had not yet been successfully recruited for circadian research. The work of the four sub-projects is summarised in more detail in the following parts of this brochure.

The scientific impact of the consortium is tremendous. EUCLOCK's scientists published almost 300 papers during the five project-years (and many are still to come). Many of these publications appeared in high-ranking

international journals, such as Nature, Science, Cell and PNAS, with a cumulative impact factor around 1.700. (This scientific impact is equivalent to publishing more than 50 Nature or Science papers.) EUCLOCK's impact on the field of Chronobiology included the education of young colleagues. A faculty of close to 50 senior scientists taught 151 students in five Summer Schools, organised in the Czech Republic, Hungary, Estonia, Poland and India.Over the past years EUCLOCK's scientists reported their work at more than 40 international meetings.

The EUCLOCK consortium developed an internet-based Information System (EUCLIS) that allows scientists to share data and other information, to search for publications written by the pioneers of the field that are not readily accessible in common databases. EUCLIS also contains a growing library displaying the history of chronobiology – facsimiles of old documents, photos, films and interviews. EUCLIS will still continue to expand its content and features over the coming years and will serve the field as a rich depository of chronobiological information for many years to come.

EUCLOCK's productive years will sadly end in June 2011, but its impact on European Rhythms Research is unprecedented. Never before have so many of our colleagues formed such a large team, working together towards the common goal, i.e., to understand how circadian clocks entrain and how they affect sleep. With increasing awareness of nature's complexity, modern science depends on teamwork. The collaborative spirit established in EUCLOCK will determine how European chronobiologists work together over the next decades – freely exchanging methods, results and ideas. Here is a typical example of this spirit. EUCLOCK's geneticists had found a new gene that regulates individual sleep duration in humans; they asked members of the fly team whether it was possible to verify these findings in another species. Within months, this gene had been manipulated in flies, showing that it modulates sleep duration across species boundaries.

# 02 ENTRAINMENT IN HUMANS

## In humans, adequate entrainment fine-tunes our physiology and ensures high performance during wake and recuperative sleep during the night.

The overall aim of EUCLOCK is to understand mechanisms of synchronisation (entrainment) to the 24-h light-dark cycle. All biological and psychological functions have an optimal time of day that is orchestrated by the biological clock in the hypothalamus. In humans, adequate entrainment fine-tunes our physiology and ensures high performance during wake and recuperative sleep during the night. Misalignment of sleep schedules with the biological clock, for example by imposed shift work schedules, causes a form of 'dysentrainment'. This can result in increases in the risks of errors and accidents, loss of productivity, and long-term health problems (increased propensity for sleep disturbances, depression, gastrointestinal disorders, cancer, decreased immune responses, and life span). Individuals with early and late chronotypes ("larks" and "owls") can suffer a degree of misalignment in adjusting to societal pressures which may result in chronic "social jetlag" that impacts on the quality of life. Thus, we focused on developing new methods, from molecular to physiological, to measure characteristics of "real life" entrainment – and dysentrainment – in humans.

We developed innovative techniques to measure the circadian periodicity of clock genes in human skin fibroblasts and the circadian phase of clock genes in buccal mucosa and blood leucocytes, which well reflect the function of the central biological clock. These methods can be used to look at peripheral clocks in shift work, sleep and psychiatric disorders, and may even develop into diagnostic tools for circadian pathologies.


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QUARTZ

The effects of light on biological clocks and other functions are mediated by the recently discovered photopigment melanopsin (most sensitive to blue wavelength light) interacting with rods and cones in the eye. Our studies looked for circadian rhythms in photoreceptor sensitivity, used highly sensitive hormonal assays, advanced mathematical modeling of photoreceptor responses, and specifically designed systems for measuring pupillary light reflex and lens density. The older population exhibited a reduced blue light transmission through the aged lens, a factor which might contribute to sleep-wake cycle problems. Ocular pathologies such as glaucoma that lead to degeneration of melanopsin photoreceptors can also alter the response of the circadian timing system to light. We developed a wireless device, the "LightWatcher", worn at the level of the eye for continuously monitoring spectral composition and intensity of light exposure. The data provided insights into our daily lifestyle-related light exposure (or lack of), and the possible consequences for health in relation to entrainment and dysentrainment.This research has directly led to the design of lighting technologies and adaptive lighting strategies for health and well being in workplaces, homes, schools and retirement homes.

A major effort was invested in designing and developing a non-invasive device for ambulatory monitoring of a large number of physiological variables over multiple 24-hour periods ("ClockWatcher"). Intensive testing in the habitual environment and under controlled laboratory conditions led to a data set that used multivariate regression modeling to predict circadian phase with greater accuracy than conventional approaches had been previously able to do.Both ClockWatcher and LightWatcher have been tested in simulated shift work. They are being commercially developed and have application in sleep-wake cycle disorders. A simplified, highly sensitive, validated melatonin assay kit was developed for use in home saliva collections, and is now on the market. Precisely identifying circadian phase will allow accurately targeted countermeasures (such as light) to avoid or reduce the negative consequences of dysentrainment.

Field shift work studies provided empirical data to improve parameters of a shift work model incorporating individual chronotype.The shift-work model is the first of its kind and has enormous implications for improving health, well being, and productivity when applied to optimise individual shift-work rotations in the industrial context.

THE SHIFT-WORK MODEL IS THE FIRST OF ITS KIND AND HAS ENORMOUS IMPLICATIONS FOR IMPROVING HEALTH, WELL BEING, AND PRODUCTIVITY...


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ALARM

# 03 ENTRAINMENT IN MICE

The mouse, with its vast knowledge base in genetics and circadian organization, was a prominent study system. In the five years of EUCLOCK our view of the mouse circadian system changed incisively. At the outset, a central brain pacemaker in the *suprachiasmatic nuclei* (SCN), was thought to both generate a ca 24-h cycle in activity-rest and other rhythms, and synchronize these with night and day. Six years later, it has become clear that the SCN signals time-of-day to endogenous circadian oscillators elsewhere throughout the body. These oscillators generating, *e.g.*, behavioural activity and rest, and rhythms in liver physiology, maintain much autonomy and can adopt different phase positions relative to the SCN. The work focused on three subsystems: (1) the retina, (2) the SCN and (3) rhythms 'downstream' fom the SCN.

**Retina.** We unraveled the pathway through which information on day and night is obtained in the mouse retina. This was made possible by developing a mouse cell line expressing the key photopigment melanopsin (OPN4). It revealed a cascade of four proteins in the signaling pathway. This work may well provide a basis for understanding entrainment disturbances in humans.

**SCN.** A major joint effort addressed the question how the SCN cope with changing daylengths in the course of the year. We found evidence that coding for daylength is a property of the neuronal network of the SCN, and a direct reflection of the synchrony in this network. Synchrony among electrical firing rhythms of SCN neurons

is high in short winter days and low in summer. This is also seen as increased summer variation in phase of gene expression rates between SCN subunits. How the interaction between pacemaker neurons ("pacers") generates this adjustment to photoperiod was theoretically clarified. In the model developed, individual pacers each go through a circadian cycle of charge and discharge. Their activation through cells signalling light and other active pacers explains many SCN properties. A second set of questions addressed the role of specific genes that make the pacers rhythmic in the first place. New roles for clock proteins were detected, such as the role of PER2 as a co-regulator of nuclear receptor mediated transcription. New genes were found, such as *FBXL3*, involved in the degradation of CRY proteins, and in the control of the circadian period.

**Body rhythms.** A two-year field study led to the surprising conclusion is that the feeding activity of mice is not at all restricted to the night. Mice showed predominant diurnal feeding in summer at high population density. To understand this, we let mice in the lab work for their food by rewarding running wheel revolutions with food pellets. This induced a spontaneous shift of activity from the night into the day – voluntary shift work. The SCN retained its normal phase. Activity is probably generated by a separate oscillator outside the SCN.

Another focus has been on clocks in the liver, which are under control of both the SCN and feeing. EUCLOCK produced a strain of mice with conditionally active hepatocyte clocks. In this strain we identified 40 genes with a strongly oscillating expression in the absence of functional liver clocks. These include *Per2* and temperature-sensitive genes encoding heat shock proteins and cold-inducible RNA-binding proteins. PER2 and proteins whose accumulation is regulated by body temperature rhythms may thus have a prime function in conveying SCN-driven signals to hepatocyte oscil-

lators. Other proteins involved in liver entrainment by feeding were also identified. Finally we developed rat fibroblast lines expressing an *mPer2* promoter-driven luciferase reporter, to study cellular mammalian clocks. They showed that DNA damage can reset the circadian oscillator in a dose- and time-dependent manner. Ionizing radiation also resets behavioural rhythms in a time-of-day dependent manner in living mice.

These new insights in the circadian system of mice – with (a) a central SCN clock sharply tuned to the external day, and (b) separate body oscillators under gentle control of this SCN, but with the capacity to flexibly adjust to metabolic needs – provides a wholly new conceptual framework for understanding the consequences of human shiftwork.

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# 04 ENTRAINMENT IN FLIES

Our studies of natural entrainment of flies in the wild in northern and southern European locations revealed a number of novel observations that require a major re-assessment of the interpretation made of both circadian behaviour and clock gene expression from laboratory experiments.

Our studies of natural entrainment of flies in the wild in northern and southern European locations revealed a number of novel observations that require a major re-assessment of the interpretation made of both circadian behaviour and clock gene expression from laboratory experiments. Mimicking nature in the laboratory also uncovered some findings that could be relevant to human entrainment, for example, flies whose clock ticks genetically quickly, have particular problems entraining to long summer days, whereas flies with slowly ticking clocks show poor entrainment under short days. People with fast clocks (Familial Advanced Sleep Phase Syndome) might therefore be expected to have more sleep problems in the summer, while those with DSPS (Delayed Sleep Phase Syndrome) may experience entrainment defects in the winter.

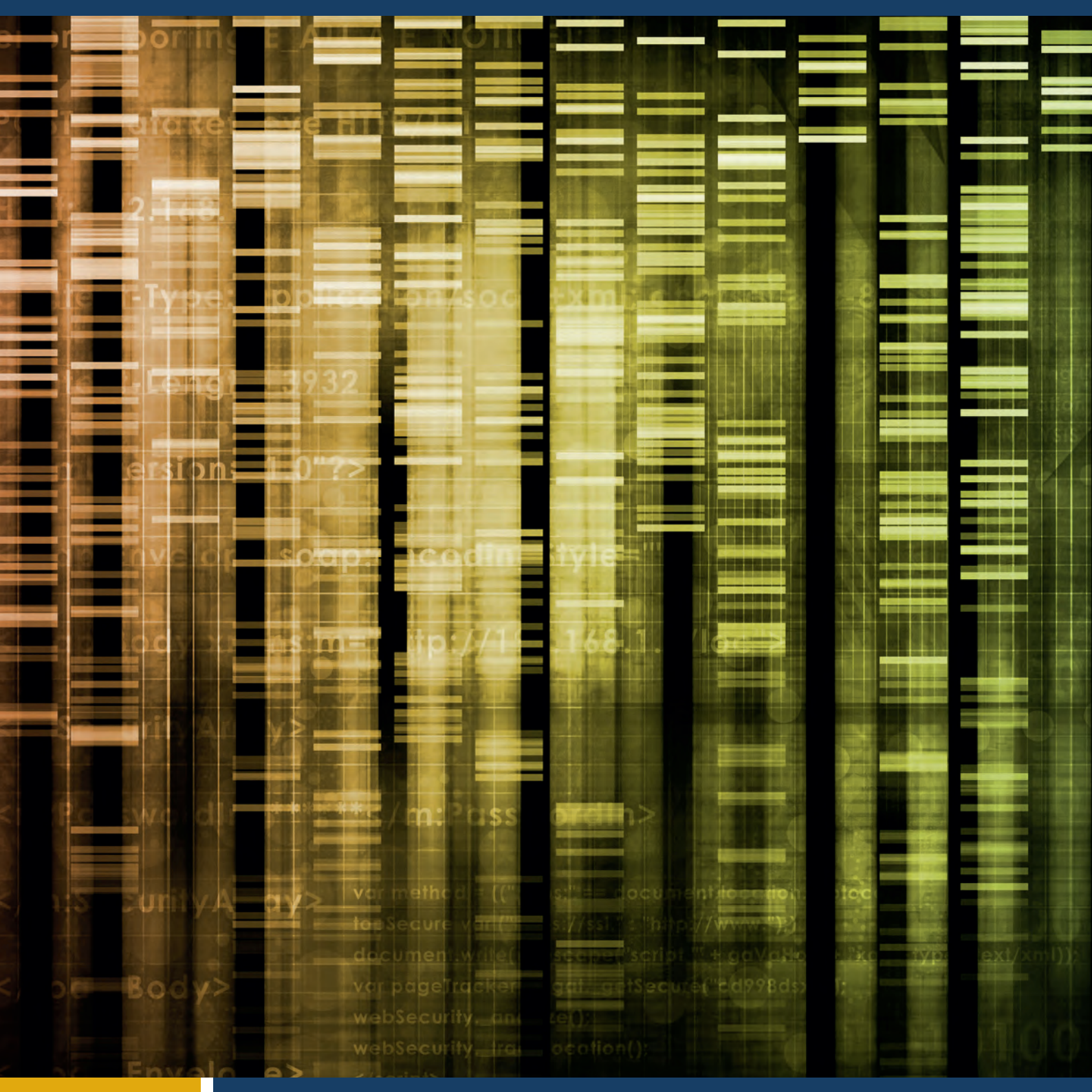
We also observed the evolution of a new clock gene variant that had spread across Europe because it affects the clock in a way that is particularly adapted to the seasonal environments observed on this continent. This new mutation in the timeless gene is about 3000

years old, and has spread from its point of origin in southern Italy, northwards to Scandinavia. Again, this finding may be relevant to human migrations, because Europe was originally populated by hominids from Africa. Thus clock genes that were more adapted to the seasons and the exotic photoperiods observed in the northern hemisphere, would be more likely to have been selected in human populations, a hypothesis that could be tested by comparing the circadian behaviour of sub-Saharan Africans to northern Europeans under seasonal challenges.

The roles of light and temperature on clock neurons were dissected with the finding that some of the 150 clock neurons are more responsive to the former, and others to the latter stimuli. Thus clock neurons are not all equal, something that could also be relevant to the mammalian clock neurons (30,000 of them). We were also able to dissect the complex signalling pathways that are relevant for circadian light responses, and the interactions among the different clock neurons in the way they processed light information. We demonstrated that the photopigment, Rhodopsin 6, and the glutamate neurotransmitter system play important roles in circadian photoreception. We also discovered that the brain senses circadian temperature information indirectly, and this process is mediated by a gene called nocte that is expressed in the stretch receptors (the chordotonal organs) of the periphery. This major finding may be quite general for the insects and the arthropods.

Finally, we developed a shiftwork model with flies. We observed that flies with rotating shiftwork patterns lived shorter lives, some of their behavioural responses were affected, and that their brains showed unusual patterns of degeneration compared to control flies. Clearly this finding is of general relevance to human shiftworkers, and the fly model may be extremely useful in future for designing rational interventions that may alleviate the physical and behavioural problems associated with this pattern of work.

THIS MAJOR FINDING MAY BE QUITE GENERAL FOR THE INSECTS AND THE ARTHROPODS.



# 05 NOVEL CLOCK GENES AND PRINCIPLES

By the start of the EUCLOCK project, the molecular mechanism of the circadian clock in virtually all of our model genetic systems was known as a feedback loop of proteins that regulate their own transcription. Yet, the regular, stepwise expansion of of this simple model with new components, and the discovery of key processes in the clock such as phosphorylation, indicated that the feedback loop falls short of the full mechanistic picture. In EUCLOCK, we supported projects to elaborate additional clock mechanisms by applying old and new genetic methods in the context of circadian entrainment protocols.

New clock-regulating genes were discovered in mice, Drosophila and human cells. In some cases, traditional mutant screen methods were combined with novel entrainment screens; in other cases, genome-wide, high throughput methods were implemented. We even exploited wild type populations of Drosophila! As a result,

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novel genes reported in EUCLOCK include F-box proteins, novel clock-associated kinases and the Trp channels, which are important for temperature entrainment in flies.

EUCLOCK also served as a starting point for a new consortium of human geneticists. The major genetics cohorts in Europe and Brazil were phenotyped for the timing of sleep wake behaviour. Through meta-analyses, this work led to discoveries of genetic associations concerning sleep duration and sleep latency. The unique nature of the EUCLOCK consortium allowed for experiments to be performed – very rapidly – in the fruit fly, showing that the sleep duration-associated gene from the human studies has a similar function in these non-vertebrates. The insertion of the timing of sleep into this massive genotyping network will reap benefits for years to come.

The EUCLOCK consortium also supported investigations using the powerful cell biology model system, S. *cerevisiae*, which is not typically in the circaian research portfolio. On one hand, it was shown that by imposing characteristic entrainment protocols, systematic entrainment could be demonstrated. On the other hand, yeast was used as a cellular vessel in which to construct circuits for emulating circadian properties, a unique synthetic biology approach to understanding molecular mechanisms of sub-networks.

In EUCLOCK, we were also challenged to probe clock principles. Our annual meetings stimulated much discussion and helped us in challenging old dogma. A high point in the search for new priniciples came with the publication of a completely novel hypothesis concerning entrainment in 2010.

The dedicated funding that EUCLOCK provided paired with a new philosophy (study the clock under entrainment!) has proven to be a powerful stimulus for discovery and new ideas. The European emphasis on collaboration and cooperation has stimulated an energetic and productive atmosphere that contributes to high quality chronobiology research worldwide.

# 06 EUCLIS

EUCLIS (EUCLOCK Information System) provides current chronobiological information curated by researchers from EUCLOCK, with the support of other members of the two largest professional societies, EBRS (European Biological Rhythms Society) and SRBR (Society for Research on Biological Rhythms). Two unique features of EUCLIS are the Chronohistory and ChronoCollection components. Chronohistory is a special, annotated collection of slideshows, images and essays about individuals and events associated with the development of Chronobiology as a field. It was developed in close collaboration with SRBR's Chronohistory Committee, headed by Anna Wirz-Justice, and is accessible for EUCLIS registered users through an interactive, graphical timeline. ChronoCollections, a part of the Clock References module, is a unique repository of papers and materials of pioneers of Chronobiology, which to date have largely not been available in digital form. Links in Chronohistory point to this unique and valuable resource currently containing works of 12 renowned chronobiologists including of course those of J. Aschoff and C. Pittendrigh.

EUCLIS adapted an advanced architecture used in the pioneering HepatoSys liver cell systems biology project to the needs of the chronobiology community. This framework combines a database with a digital library and flexibly segments the system into modules. Current modules include: **Clock Experiments**, which stores experiment information and cata and provides a visualization tool for time series data; **Clock KnowledgeBase**, a digital library containing the module for common components used by **Clock Experiments** and **Clock Models** as well as external information access; **Clock Genes**, which is a catalogue of genes associated with circadian rhythms of model organisms; **Clock References**, a repository of bibliographic references; **Clock Classes**, a repository of course materials for chronobiology related classes; and **Clock Tools**, a repository of software tools used in chronobiology for analyzing and simulating experimental data. EUCLIS also hosts **Clock Images**, which contains images gathered by chronobiologists for the use of the EUCLOCK community. EUCLIS also provides a venue for documenting events within the community through **Clock Museum**, as well as a venue for documenting academic linkages in **Clock Family Trees**. Central services are provided by the Clock Registered User Services.

**EUCLIS** was developed by a joint team from the University of the Philippines Diliman and LMU Munich. It can be accessed at http://www.bioinfo.mpg.de/euclis/

# 07 EUCLOCK SUMMER SCHOOL FOR CHRONOBIOLOGY 2006–2010

## INTENSIVE COURSE FOR PHD STUDENTS

**All schools were organised by**

**EUCLOCK Summer School Officer:**
Menno Gerkema
(University of Groningen)

**EUCLOCK management:**
Susanne Kantermann
(Ludwig-Maximilians-University)

**Advisory Board:**
Till Roenneberg
(Ludwig-Maximilians-University)

Martha Merrow
(University of Groningen)

Menno Gerkema
(University of Groningen)

**In the last five years over 200 persons (151 students) attended the EUCLOCK Summer Schools for Chronobiology and participated in classes on:**

- Ecology and Evolution of rhythms
- Basic principles of entrainment
- Genetic approaches
- Clocks and theeye
- Pacemaker physiology
- Human clocks and circadian rhythms
- Photoperiodism
- Output physiology
- Sleep approaches
- Light and clocks
- Pacemaker organization in mammals
- Modelsystems for new genes
- Output regulation
- Timing of human behavior
- Clocks in insects
- Daily human rhythms

*Trest Chateau, Czech Republic,*
*from 26th August to 2nd September 2006*

**Local organisers:**
Alena Sumova and Helena Illnerova
(Acad. Institute of Physiology, Prague)

**34 participants**
25 students (15 female/ 10 male) from 9 European countries, Russia and the Philippines

# EUCLOCK SUMMER SCHOOL 2006

# EUCLOCK SUMMER SCHOOL 2007

*Matrahaza, Hungary,*
*from 29th September to 6th October 2007*

**Local organisers:**
Ferenc Nagy and Laszlo Kozma-Bognar
(Biological Research Centre, Szeged)

**39 participants**
25 students (20 female/ 5 male) from 6 European countries, Russia and the Philippines

*Laulasmaa, Estonia,*
*from 7th to 14th June 2008*

**Local organisers:**
Andres Metspalu and Merike Leego
(Estonian Biocentre, Tartu)

**42 participants**
29 students (24 female/ 5 male)
from 11 European countries and USA

# EUCLOCK SUMMER SCHOOL 2008

# EUCLOCK SUMMER SCHOOL 2009

*Przegorzaly, Poland,*
*from 4th to 11th July 2009*

**Local organisers:**
Elzbieta Pyza
(Jagiellonian University, Krakow)

**35 participants**
27 students (16 female/ 11 male)
from 6 European countries and Australia


*Bangalore, India,*
*from 2nd to 9th October 2010*

**Local organisers:**
Vijay Sharma Kumar and Sheeba Vasu
(Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore)

**45 participants**
34 students (23 female/ 21 male) from 6 European countries, India, the Philippines and USA

# EUCLOCK SUMMER SCHOOL 2010


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# 08 PROJECT INFO

## EUCLOCK - ENTRAINMENT OF THE CIRCADIAN CLOCK

AN INTEGRATED PROJECT FUNDED BY THE EUROPEAN COMMISSION

**Acronym:** EUCLOCK
**Contract Number:** LSHM-CT-2006-018741
**Starting date:** 01.01.2006
**Duration:** 5.5 years
**Budget:** 16,036,195 €
**EU-contribution:** 12,299,389 €
**Scientific Officer:** Patrik Kolar

EUCLOCK is a large European wide research network that has been launched in January 2006. This project aims at the investigation of the circadian clocks in single cells and in humans. Behaviour, physiology, and biochemistry are temporally structured and characterised by daily oscillations. These cycles are not simply driven by external changes as light/dark or warm/cold. They are controlled by endogenous clocks that are prevalent in the most diverse organisms, from cyanobacteria to humans. These circadian clocks are synchronised to the outside world by a process called entrainment, which is generated by rhythmic environmental signals, called 'ZEITGEBERS'. EUCLOCK researchers are precisely interested in how these circadian clocks are synchronised to their specific cyclic environment. Therefore, EUCLOCK combines the expertise of 34 chronobiologists from 29 institutions in 11 European countries. EUCLOCK's budget is more than 16 million EUR over 5.5 years, of which 12 millions are a contribution by the European Union.

EUCLOCK researchers utilise the most advanced methods of functional genomics and phenomics in order to compare genetic model organisms and humans. For example, the prerequisites for large-scale, non-invasive research on human entrainment in the field will be developed. The first animal models for shift-work will be created. In analogy to 20% of Shift Workers in the population, flies and mice will be exposed to

'shift work' schedules. They will be active and fed out of phase with their natural rhythms. The ensuing „dys-entrainment" will be investigated at levels from genes to behavior. This work aims at providing insights for the prevention of the negative consequences of human shift-work.

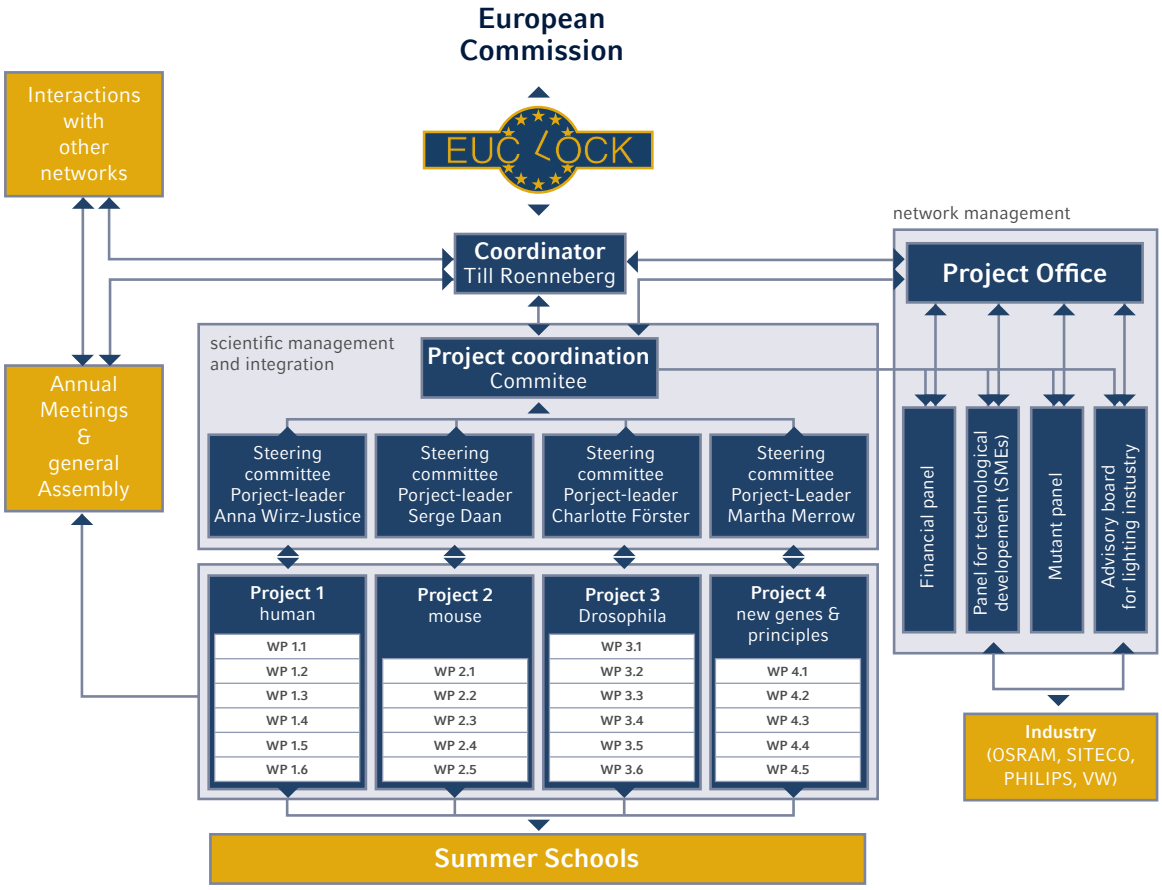
Furthermore, new genetic components that control the circadian clock and its entrainment will be identified in animals and in humans.


New tools will be developed and new circadian model organisms will be explored. These will enable the field of chronobiology to exploit the advantages of systems biology research on circadian timing to be performed and integrated at the level of the genome, the proteome, and the metabolome.

The innovations of EUCLOCK are predestined to shape the future of circadian research.

EUCLOCK is divided into four Sub-Projects that each manage specific Research activities and implement EUCLOCK's objectives:

**SP1: Humans**
(Anna Wirz-Justice, University of Basel)
**SP2: Mice**
(Urs Albrecht, University of Fribourg)
**SP3: Flies**
(Charlotte Helfrich-Förster, University of Regensburg)
**SP 4: Novel clock genes and principles**
(Martha Merrow, University of Groningen)


European Commission
EUCLOCK
Interactions with other networks
Annual Meetings & general Assembly
Coordinator
Till Roenneberg
network management
Project Office
scientific management and integration
Project coordination
Commitee
Steering committee Porject-leader Anna Wirz-Justice
Steering committee Porject-leader Serge Daan
Steering committee Porject-leader Charlotte Förster
Steering committee Porject-Leader Martha Merrow
Financial panel
Panel for technological developement (SMEs)
Mutant panel
Advisory board for lighting instustry
Project 1 human
WP 1.1
WP 1.2
WP 1.3
WP 1.4
WP 1.5
WP 1.6
Project 2 mouse
WP 2.1
WP 2.2
WP 2.3
WP 2.4
WP 2.5
Project 3 Drosophila
WP 3.1
WP 3.2
WP 3.3
WP 3.4
WP 3.5
WP 3.6
Project 4 new genes & principles
WP 4.1
WP 4.2
WP 4.3
WP 4.4
WP 4.5
Industry (OSRAM, SITECO, PHILIPS, VW)
Summer Schools

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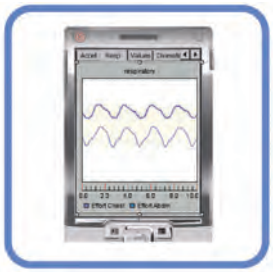
# PRODUCTS
# CLOCKWATCHER

# ClockWatcher

## WEARABLE WIRELESS PHYSIOLOGICAL MONITORING CONTINUOUSLY DURING SLEEP, WORK AND PLAY LONG TERM MONITORING FOR RESEARCH AND CLINICAL CARE

**Specifications**

- True ECG
- 2 channel Respiratory Effort
- 3-axis body position
- High-resolution activity
- Nasal pressure, snoring
- Temperature, Light and Sound
- Oximetry and Pulse wave
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- Sample rates up to 250 Hz.
- Pocket size monitor
- Data transmission via WiFi or 2G/3G

**Contact**

Personal Health Institute international
Rijswijkstraat 141e, 1062 ES
Amsterdam, The Netherlands

M: +31 6 4610 4625
T: +31 20 7071231
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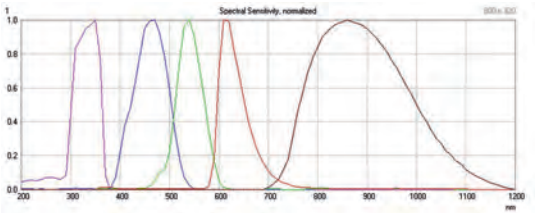
# PRODUCTS
## **LIGHTWATCHER** DATA LOGGER

Object-Tracker

The **'LightWatcher'** data logger is a miniaturized portable data acquisition system that measures and records a unique combination of 10 environmental variables that are important in the study of human performance, of biological rhythms, and of the effect of the environment on biological systems.




This small device measures and records **light irradiance** in 5 spectral bands (UV, Red, Green, Blue, IR), acceleration in 3 axis (**actimeter**) and **temperature**. The measurement of illuminance (Lux), barometric pressure and relative humidity are available as an option. The optical axis of the data logger points in longitudinal direction. An opaque window from PTFE protects the photo sensors. The data logger measures 20 x 10 x 50 mm and has a weight of only 12 grams.

The data logger is powered by a rechargeable Lithium Polymer battery, which is charged via the USB bus. One battery charge enables a stand-by time of up to 3 months and an operational time of days to weeks, depending on the selected recording rate.

The sampling and recording rate can be set to a value from 0.5 seconds to 30 minutes. A fast recording rate is available for the acceleration variables. Data are stored in Flash memory. Storage capacity is sufficient to record 1 data record per second for 18 hours, of 1 data record per minute for 6 weeks. Recording is toggled on / off with a single push button (push for 2 seconds). This push button serves also as an **event marker** if pushed for 0.5 seconds. A small LED and a sounder provide feedback to the user.

The device has a USB-2 data interface. Via this interface and a dedicated software program (OT-Sensor), the sensor can be configured for the particular monitoring task, and recorded data can be downloaded to the PC for display, analysis and archiving.

## TECHNICAL SPECIFICATIONS

| Variable | Description |
|---|---|
| Size | 20 mm x 50 mm x 10 mm |
| Mass | 12 grams |
| Power | Battery or via USB bus, 100 mA max. |
| Battery | Li-Polymer battery, 3.7 V, 100mAh |
| Charge time | 1.5 hours |
| Data memory | 4 MByte Flash |
| Recording cap. | 64,000 data records |
| Recording rate | 2 samples / sec. to 1 sample / 60 min.<br>32 samples / sec. for acceleration |
| Data interface | USB-2 |
| Download time | ~ 6 minutes for 64,000 data records |
| Variables | Irradiance: R,R,G,B,UV<br>Acceleration, 3-axis: -5.0 to +5.0 g<br>Temperature: -50 to +80 deg C<br>Barometric Pressure: 0 - 1500 hPa<br>Relative Humidity: 10 to 98 % |
| Real time clock | +- 10 ppm, +- 10 second/day |
| Stand-by time | ~ 3 month |
| Anatomical mounts | 1) Eyeglasses<br>2) Headset, to be worn on head<br>3)Armband, to be worn on upper arm<br>4) Necklace |

## ACCESSORIES

Figure 1: Eyeglass

Four types of anatomical mounts are currently available for studies with human subjects:

The **Eyeglass** anatomical interface is based on fashionable eyeglasses with zero diopter plastic glasses (several model available). The data logger is attached to one of the sidebars with double-sided adhesive tape. This mount keeps the optical axis of the data logger well aligned with the principal viewing direction of the subject.

The **Headset** anatomical mount is a light-weight mounting platform for the data logger and optional auxiliary devices. The headset is manufactured from stainless steel, has foam rubber padded temporal cushions, and a low weight of 25 grams. The headset guarantees comfort even if worn all day long. It provides good fixation of

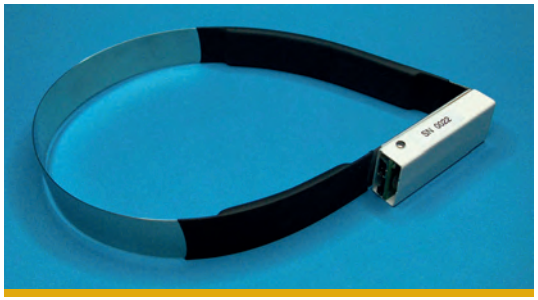


Figure 2: Headset

the sensor relative to the head during typical office work and low to medium impact physical activity, and keeps the optical axis of the data logger well aligned with the principal viewing direction of the subject.

Figure 3: Armband

The **Armband** anatomical interface attaches the data logger to the upper or lower arm. The Armband is manufactured from Neoprene, cushioned on the inside, and very comfortable to wear. The circumference of the armband can be easily adjusted. The data logger itself is securely fixed to the Armband with elastic bands. With this mount the optical axis of the data logger will point primarily in frontal direction.


Figure 4: Necklace

The **Necklace** mount provides an easy, comfortable way to wear the data logger. The optical axis of the data logger will point primarily in vertical direction.

The data logger is supplied in a plastic transport box together with useful accessories such as a dark calibrator, a wall charger, and a USB cable.


Figure 5: Subject wearing an Armband. Transport box with accessories

## SOFTWARE

The sensor is supplied with a license of the **OT-Sensor** software program. OT-Sensor is running under MS-Windows and provides all functions that are necessary to work with the sensor, i.e., sensor configuration, real time data acquisition, data download, data display, data storage, data retrieval, and the creation of reports. Prior to use, the program must be activated with a license key. The user interface of the program is organized in sections.

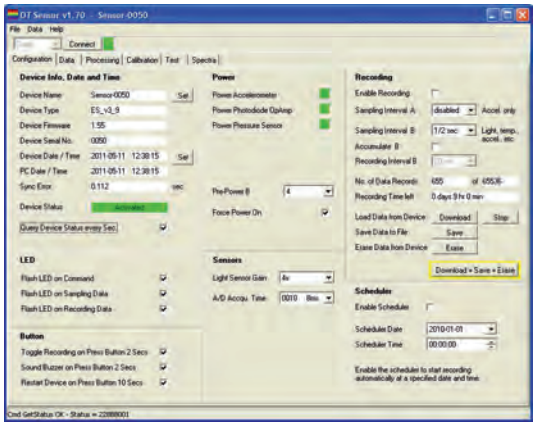



Figure 6: Screen of OT-Sensor program, section 'Configuration'

Section 'Configuration' displays important device properties and provides controls to set key device parameters such as date and time, data sampling and recording rates, and power settings. All configuration settings are stored in the flash memory of the data logger and will be remembered even if the device is not powered. Recorded data may be downloaded from the data logger to the PC, and saved to a file in binary and/or text format. A scheduler is available that can be configured to start data recording at a defined date and time.

Section 'Data' displays acquired data in numerical format and in a graph. The user can select a particular variable from the list of variables (left side) and inspect the time course of the selected variable in an associated graph (right side). The x- and y- axis of the graph can be easily adjusted to the particular requirement of the user. A utility is provided to acquire and display data in real-time.

Section 'Calibration' contains utilities that support the calibration process of the data logger. Calibration coefficients are calculated automatically based on a list of calibration points (i.e., measured value / true value data pairs) that must be provided by the user. All calibration coefficients are stored in the data logger.

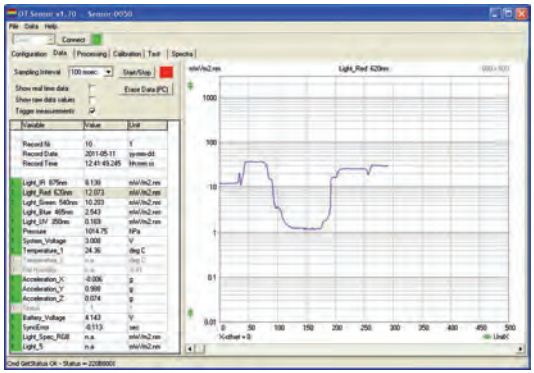
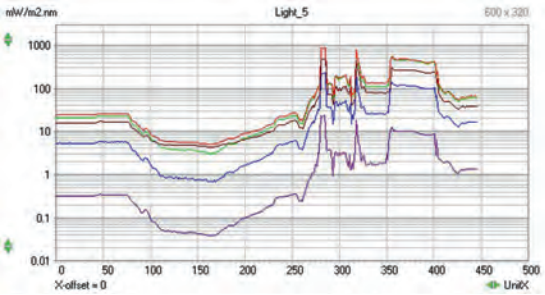
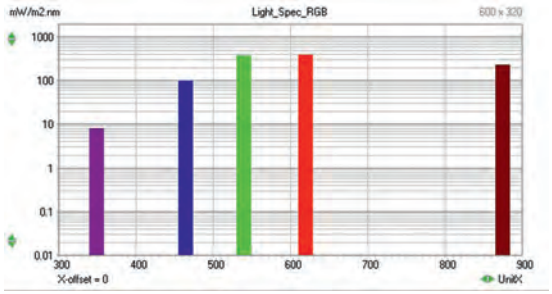


Figure 7: Screen of OT-Sensor program, section 'Data'



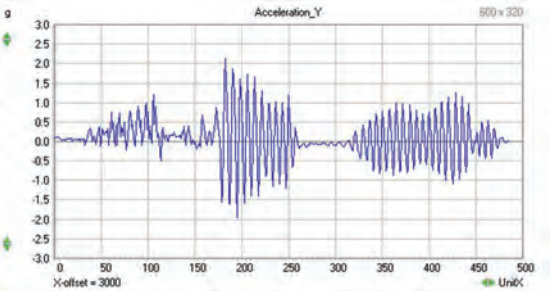
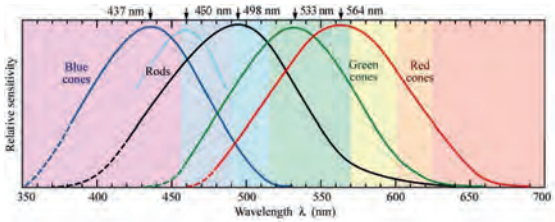




Figure 8: Graph of light irradiance in 5 spectral bands (UV, Blue, Green, Red, IR). Graph of acceleration measurement.

## APPLICATION AREAS

**Chronobiology research, occupational health:**
Our data loggers monitor a unique combination of variables that are of key importance in chronobiology research and occupational health related research. Test subjects can comfortably wear our data loggers for periods up to several weeks to collect extensive datasets that contain information on light irradiance, subject activity, and other environmental variables.




**Architecture:**
Modern energy efficient buildings require energy-efficient lighting through an appropriate combination of day lighting and various types of electric light sources. Recent insights into the effect of spectral composition of illumination on circadian rhythm, alertness, cognitive performance and mood suggest to strongly consider the spectral composition of illumination during lighting design. Our data logger measures light irradiance in 5 spectral bands and is therefore an attractive tool to support lighting design in modern buildings.

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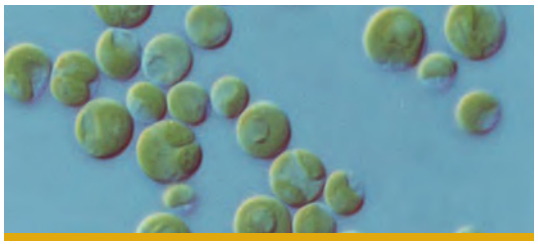
This development was supported by ESA Contract No. 19373/05/NL, and EU Contract No. 18741 (EuClock).

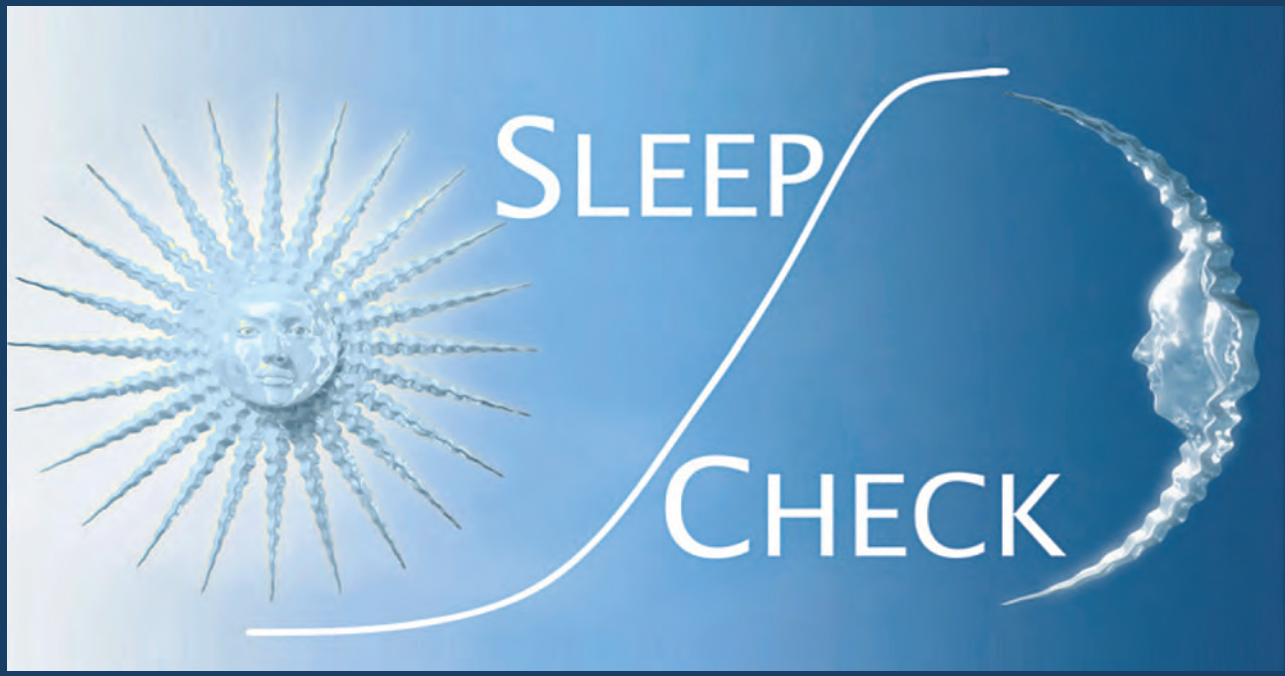
**Space research:**
Our data loggers monitor a unique combination of variables that are important in many life science and physics experiments. Scientists place our data loggers in experiment containers and transport containers to monitor the specimen / sample and its environment throughout the whole space mission (experimenter's lab --> transport --> launch --> storage --> experiment --> fixation --> storage --> reentry --> transport back). The data logger may also be used to monitor experiments during parabolic flights and inside centrifuges.



**Metrology:**
The environmental data logger has been successfully flown on a stratospheric balloon up to an altitude of 25000 m.

**Sports, training assist device:**
Our data loggers have been applied in various sports disciplines (track running, cross country running, skiing, tennis, glider plane flight, moto-cross) as a training assist device. In a typical setup, several data loggers are attached to body segments and/or sports equipment, and acceleration profiles together with environmental variables are monitored. The analysis of recorded data reveals interesting details of the athlete's patterns of movement (left-right symmetry, change due to fatigue or during recovery from injury, and more), which may support the athlete to improve his/her technique, to avoid injury, to monitor his/her progress during training, and to compare his/her patterns of movement with the ones of other athletes.

# PRODUCTS
# SLEEP CHECK



# IN VITRO DIAGNOSTIC DETERMINATION OF CIRCADIAN RHYTHM SLEEP DISORDERS

**Sleep Check Concept Comprises:**

Saliva Collection Tool Allowing Comfortable Sample Collection at Patient's Home

Assessment of the Biological Circadian Sleep Rhythm by Defining the Individual Melatonin Onset (DLMO)

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# PRODUCTS
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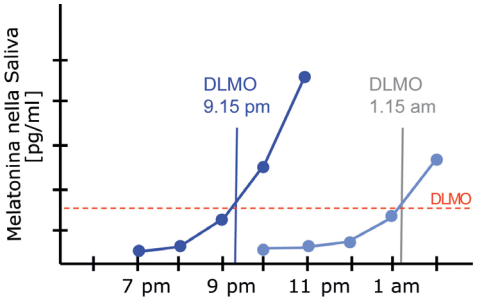
| Test System | RIA | RIA | ELISA | ELISA |
| Sample Vol. | 800 µl Sample | 800 µl Sample | 200 µl Sample | 10 µl Sample |
| Sample preparation | EXTRACTION (column) | DIRECT | PRETREATMENT | DIRECT |
| Time to results | 24 h | 24 h | 24 h | 4.5 h |
| Standard range | 0.5 - 50 pg/ml | 0.5 - 50 pg/ml | 0.6 - 25 pg/ml | 0.8 - 40 ng/ml |
| Sensitivity LOQ<br>LOB | 0.9 pg/ml<br>0.3 pg/ml | 0.9 pg/ml<br>0.2 pg/ml | 1.6 pg /ml<br>0.5 pg/ml | 1.5 ng/ml<br>0.14 ng/ml |
| Order code | RK-MEL2<br>200 tests | RK-DSM2<br>200 tests | EK-DSM<br>96 wells | EK-M6S<br>96 wells |




## SLEEP CHECK

Outpatient concept to assess the individual biological melatonin onset (DLMO). The most comfortable way for the assessment of the individual DLMO is to follow the salivary melatonin concentration during the evening.

Experience with a partial melatonin profile, composed of 5 saliva samples collected hourly showed a high success rate for the DLMO determination.

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