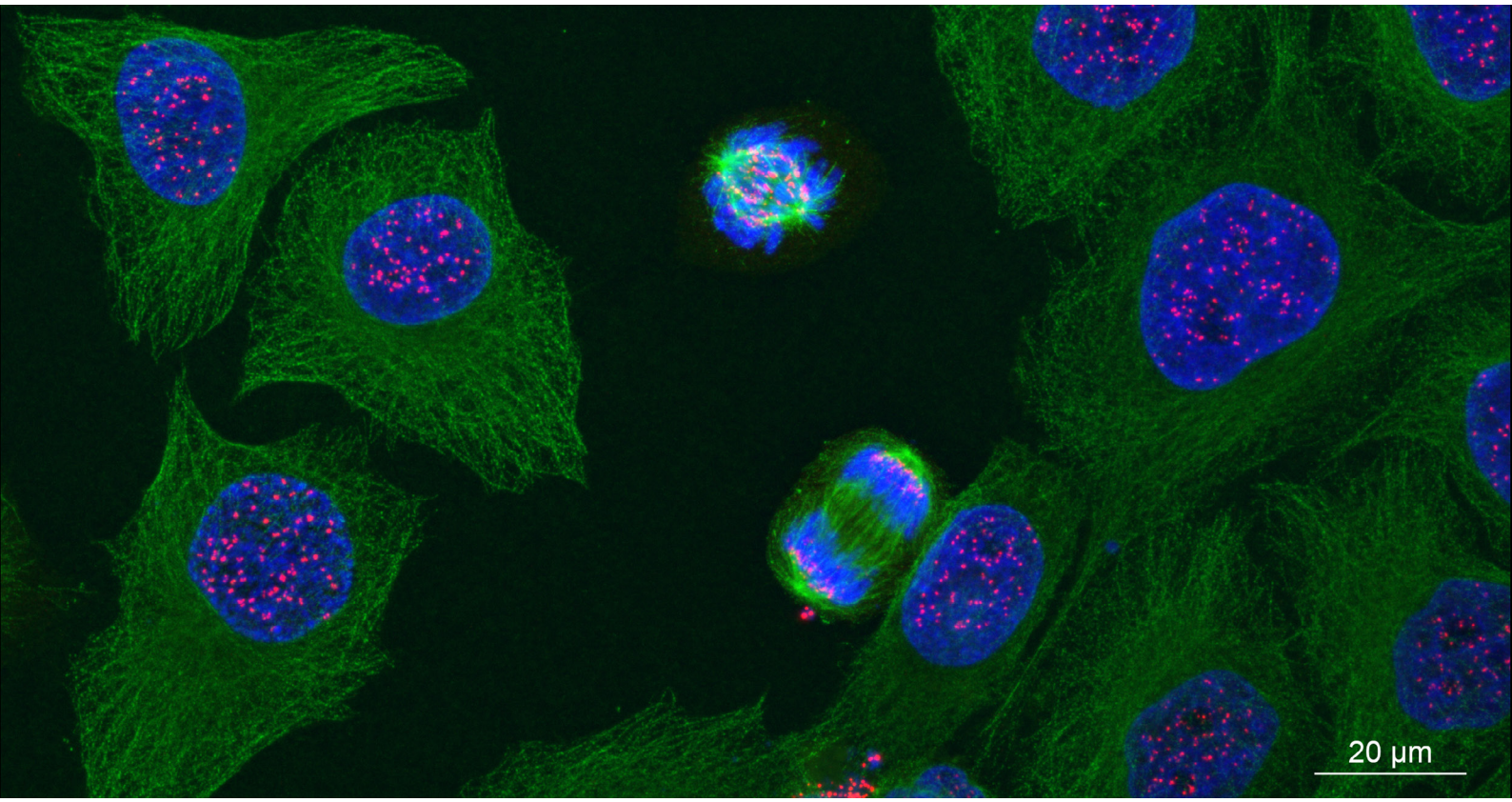
Application Note

The most commonly used immortal cell lines

An Introduction



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Seeing beyond

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Immortal cell lines are often used in research in place of primary cells. They offer several advantages, such as they are cost effective, easy to use, provide an unlimited supply of material and bypass ethical concerns associated with the use of animal and human tissue. Cell lines also provide a pure population of cells, which is valuable since it provides a consistent sample and reproducible results. Cell lines have revolutionized scientific research and are being used in vaccine production, testing drug metabolism and cytotoxicity, antibody production, study of gene function, generation of artificial tissues (e.g., artificial skin) and synthesis of biological compounds e.g., therapeutic proteins. This educational guide by ZEISS will introduce you to the basics of working with cell lines including interesting background information and additional useful resources.

> ***The time has come for us to culture our cells better and move from 2D to 3D models and attempt to more closely replicate the environment in which cells normally reside and then change the environment to mimic disease." [1]***

Human primary cells vs. immortal cell lines – a comparison

To study the development of cancer, test cancer treatments or the toxicity of compounds or drugs, researchers routinely use cell lines as a model for healthy or diseased tissue. The decision whether to use human primary cells or immortal cell lines depends on different factors:

Human primary cells are taken directly from the tissue of healthy donors, organ donation, surgical specimens, fetal tissues or post-mortem donors and afterwards kept in culture. They have the same morphology and phenotype as their original source but may also implicate difficulties. Primary cells in culture often have a limited number of replication cycles and die after a certain lifespan. As they age, they show morphological and functional changes. Also the source of human primary cells is limited as one might not be able to get extra material from the same donor which means that researchers can't repeat their experiments on identical cells or use the same cells for extended studies and long-term experiments. Plus, different types of cells need different media to grow and survive.
If you are interested in more details about primary human cell lines see Richter et al., Frontiers in Cell and Developmental Biology, 2021. [2]

Immortal cells for the most part overcome these issues. Researchers who plan to investigate basic biological processes, manipulate cellular functions, establish new methods or perform preliminary screenings tend to choose immortalized cell lines as they offer an easy, inexpensive, and stable platform. They are cultured in special vessels such as Petri dishes, flasks or multiwell plates in a controlled environment for longer periods of time. Culture media containing nutrients and optional supplements provide the necessary conditions for optimized cell growth. Immortal cells replicate indefinitely, making sure that there is always a constant supply of quickly growing cells for experiments available. Immortal cells were first discovered in the 1950s with the best-known HeLa cell line. The cell biologist George Otto Gey took a cancer cell from Henrietta Lacks, allowed that cell to divide, and found the culture survived indefinitely if given nutrients and a suitable environment. As the original cells continued to mutate there are now many strains of HeLa commercially available, all derived from the same single tumor cell.

Figure 1 *Statue of Henrietta Lacks. Copyright: University of Bristol [3]*

	Human Primary Cells	Immortal Cell Lines
Origin & Availability	Isolated from healthy or cancer tissue, limited availability	Derived from primary cell culture, commercially available
Characteristics & Applications	Genetic components stay the same as original tissue to study the biology of cells & tissue, pharmaceutical & drug tests	Uniform genetics across cells, consistent & reproducible results for vaccine & antibody production and to investigate gene functions
Culture & Handling	Require special media & adjusted culture conditions, difficult handling	Standard media & culture conditions, easy handling
Morphology & Phenotype	Healthy cell morphology, maintain original phenotype for limited time	Lack of key morphology features, changes in phenotype
Genome & Senescence	Genetically stable, limited self-renewal in culture	Altered genomic content, can be grown & expanded
Reproducibility & Relevance	Low reproducibility, high relevance in vivo	High reproducibility, low relevance in vivo

Table 1 *Comparison of primary cell culture vs. immortal cell lines*

Types of immortal cell lines

Animal cell lines and human cell lines are frequently used in the labs. The main difference is that the animal cell may have different sizes of genomes depending on the species whereas the human cell has 3 billion base pairs in its genome. Also, the number of protein-coding genes in the genome of an animal cell depends on the species while the human genome consists of 20,000 to 25,000 protein-coding genes.

Researchers use animal cells to examine a large range of disease mechanisms and assess novel therapies. Besides disease models, many animal cells are relied on for bioindustrial uses such as recombinant protein expression, virus production, pathogen detection, and toxicity screening. Animal cells can also provide insights into areas of developmental biology, intracellular signaling, and genetic evolution.

Animal cell lines	Human cell lines
CHO (Chinese hamster ovary)	HeLa (cervical cancer)
COS-7 (green monkey kidney)	SH-SY5Y (neuroblastoma)
Vero (green monkey kidney epithelial)	HEK 293 (embryonic kidney)
MDCK (Madin-Darby canine kidney)	MCF-7 (breast cancer)
Sf9 insect epithelial cells	H1, H9 (Embryonic stem cells)

Table 2 *Overview of selected animal and human cell lines*

Selected Examples

HeLa cell line

The HeLa cell line is the first and also the most famous immortal cell line. This cell line is named after Henrietta Lacks, an African-American woman that died of cancer on October 4, 1951. In February 1951, cervical cancer cells were taken from her and put into culture. This cell line was found to be remarkably durable and proliferating, dividing nearly endlessly. HeLa cells were used to develop the famous polio vaccine, and they continue to be the most widely used cell line in research labs worldwide.

Use

Though the initial use was in cancer research, HeLa cell lines have been used in more than 100,000 scientific publications on a range of topics including cancer, cell biology, genetics, and infectious diseases. HeLa cells have contributed to many medical breakthroughs and led to nearly 11,000 patents. They even led to discoveries that have merited Nobel prizes.

Highlights of medical breakthroughs enabled by HeLa cells:

- Development of polio vaccine: the polio vaccine was already developed in the early 1950's by Jonas Salk, but he struggled to find a way to test in field trials. In 1952, HeLa cells were evaluated as an ideal source of host cells.
- Study of leukemia
- Improved cell culture practices: during the mass production and distribution of HeLa cells for polio vaccine testing, the leading researchers at the Tuskegee University developed new cell culture protocols including the use of incubators and new shipment solutions.
- Chromosome counting: in 1953, a lab in Texas accidentally mixed up cell lines enabling the researchers to see and count each chromosome clearly in the HeLa cells they were working with. Following this discovery, Tijo and Levan developed a technique for staining and counting chromosomes, demonstrating that human somatic cells have 23 pairs of chromosomes. Today we know that deviations from 23 chromosome pairs are associated with various genetic diseases.
- Genome mapping: Harris and Watkins created the first human-animal hybrids in 1965, by fusing HeLa cells with mouse cells.
- Human papilloma virus (HPV) vaccine: in the 1980's, Henrietta's cells were found to contain HPV-18 by Harald zur Hausen leading to the development of HPV vaccines.

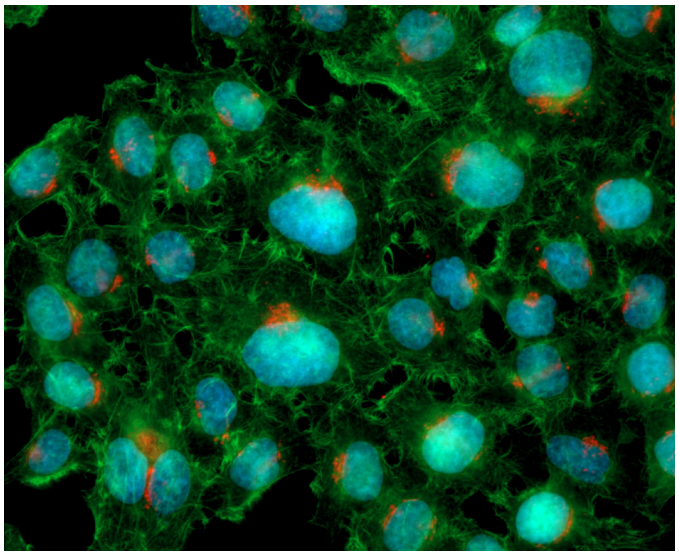

Figure 2 *Fixed cultured HeLa cells. Blue: DNA (DAPI), green: F-actin (phalloidin-Alexa Fluor 488), red: trans Golgi network (TGN-Alexa Fluor 561).*

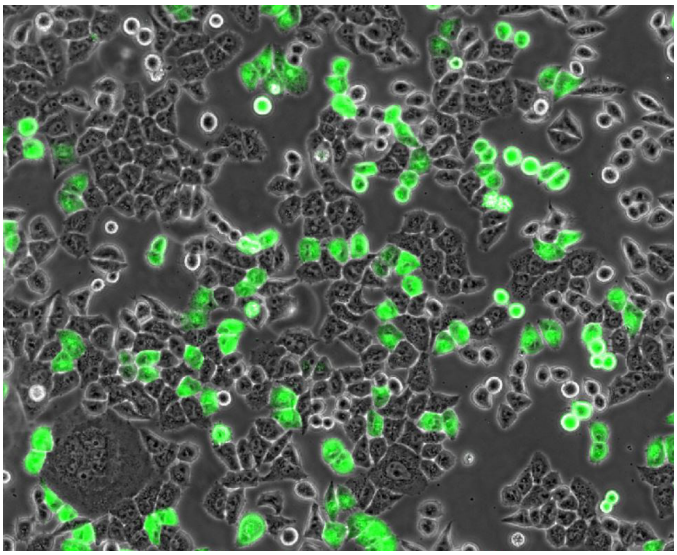

Figure 3 *HeLa cells transfected with GFP Expressionplasmid*

History

The Johns Hopkins Hospital in Baltimore was the hospital Henrietta Lacks visited after she had felt a lump in her womb. It was built in 1889 for the sick and poor and the only major hospital in the region that treated black patients. It was not uncommon for these patients to be used as research subjects; the doctors considered this fair compensation for their otherwise free treatment. Patients were not always told that their cases were used in research.

Henrietta's gynaecologist Wesley Telinde had developed a theory on cervical cancer, which, if proven right, could drastically reduce deaths from cervical cancer. Almost everybody in the field was sceptical about his ideas, but he was convinced that carcinoma in situ was an early stage of the more deadly invasive carcinoma, instead of a distinct type that only needed milder treatment. He proposed that carcinoma in situ should be treated in the same aggressive way as invasive cancers, to prevent it from spreading. Telinde wanted to prove his theory by showing that carcinoma in situ and invasive carcinoma behave the same way in the laboratory. He gave a sample of Henrietta's cervical carcinoma to George Otto Gey, who ran a tissue culture research laboratory with his wife, Margaret. The cells not only remained alive but divided, doubling their numbers every 24 hours. The first human immortal cell line was a reality. Soon, other scientists became interested, and George Gey sent vials to labs all over the planet.

HEK 293

HEK 293 are human embryonic epithelial cells from the kidney, originally isolated and grown by the Dutch biologist Alex van der Eb in the early 1970s. Frank Graham incorporated adenoviral genes Ad5 into the cells – allowing them to produce very high levels of recombinant proteins. As a result, they got a low maintenance, rapidly dividing, robust cell line with a good reputation for post-translational modification of its heterologous expressed proteins. Their cell doubling time is about every 36 hours. They can be cultured in suspension or as a monolayer and have been widely used in cell biology research for many years, because of their reliable growth and propensity for transfection. However, if they are cultured for an extended period of time their health degrades. This can affect growth rate and translation efficiency, so they tend to become less reliable in terms of experimental results. But, because they grow quite rapidly and are simple to keep, researchers just start a new passage. If there is the need of a cell line that can produce large amounts of recombinant proteins, or if you're a cell culture beginner looking for a low-maintenance cell line, HEK293 might be a good choice.

Use

Because of their advantages and versatility, they are the second most widely used cell line after HeLa. HEK 293 is the cell line of choice

- in transient and stable transformation experiments,
- for protein expression and production,
- in electrophysiological experiments,
- for transfection studies,
- to produce therapeutic proteins and viruses for gene therapy.

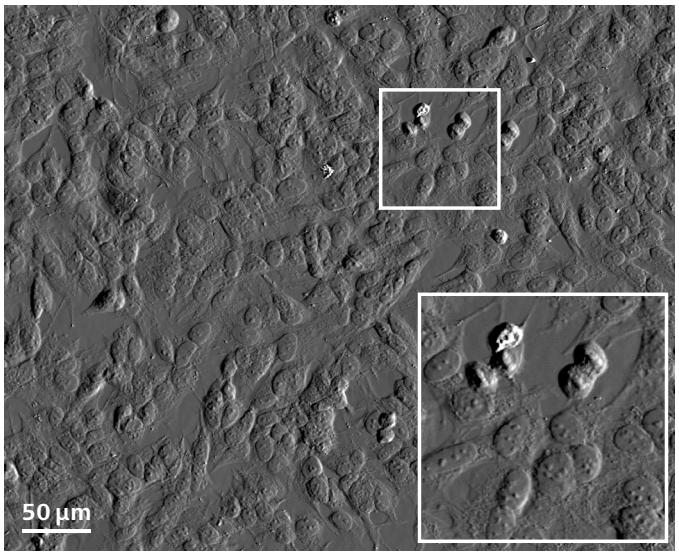

Figure 4 *SH-SY5Y cells cultured on a 384 microwell plate. Five channel image at a single position using Plan-Apochromat 20×/0.95; EDF from Z-stack; phase gradient contrast, overlay image. Sample courtesy of P. Denner, Core Research Facilities, German Center of Neurodegenerative Diseases (DZNE), Bonn, Germany.*

SH-SY5Y

SH-SY5Y is a human derived cell line used in scientific research. The original cell line, called SK-N-SH, from which it was sub-cloned was isolated from a bone marrow biopsy taken from a four-year-old female with neuroblastoma.

Use

SH-SY5Y cells are often used as in vitro models of neuronal function and differentiation. They are adrenergic in phenotype but also express dopaminergic markers and, as such, have been used to study Parkinson's disease, neurogenesis, and other characteristics of brain cells.

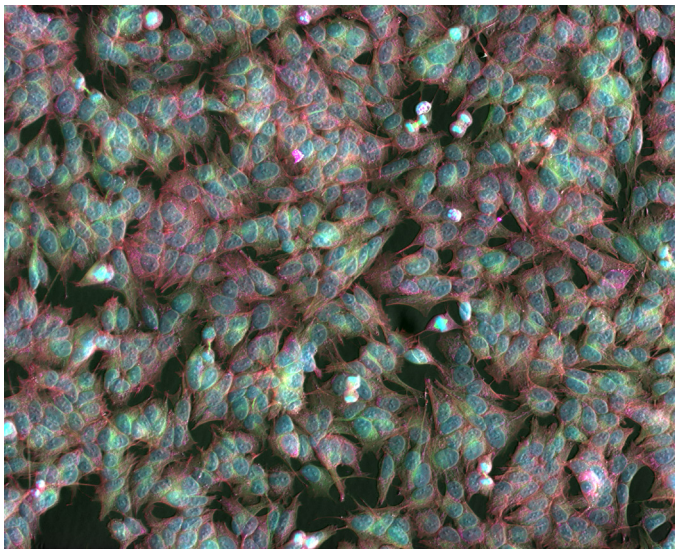

Figure 6 *SH-SY5Y cells cultured on a 384 microwell plate. Five channel image at a single position using the 20x / 0.95 objective. (Extended depth of focus from Z-stack) Hoechst – Chromatin, anti-alpha-tubulin antibody FITC for alphas tubulin (green), Phalloidine for actin (red), MitoTracker deepRed for Mitochondria (purple). Sample courtesy of P. Denner, Core Research Facilities, German Center of Neurodegenerative Diseases (DZNE), Bonn, Germany.*

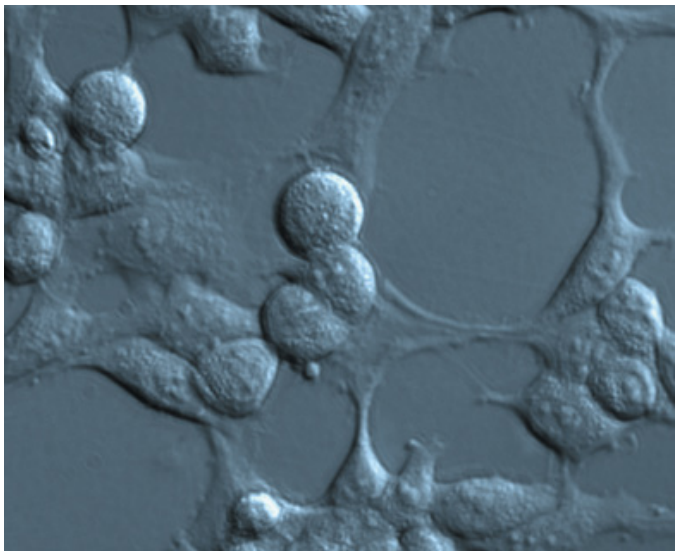

Figure 5 *HEK cells, PlasDIC; Sample courtesy of C. Lücking, Institut für Neurogenetik, Klinikum Großhadern, Germany*

CHO

CHO (Chinese hamster ovary) cells are an epithelial cell line derived from the ovary of the Chinese hamster. The Chinese hamster used in biomedical research is traditionally classified as *Cricetulus griseus*.

Figure 7 *Chinese Hamster; allocricetulus – stock.adobe.com*

The original cell line was created in the 1950s. The Chinese hamster has served as a model host for culture because of its small size, short gestation period and **low mammalian chromosome number**, while being able to maintain long-term, stable gene expression and provide high yields of proteins. The Chinese hamster has a low incidence of spontaneous and endogenous viral infections. CHO can exist both as adherent or suspension cells in culture.

Use

CHO has become an important mammalian cell line used for the industrial production of glycosylated therapeutic proteins and in cytogenetic toxicity assays.

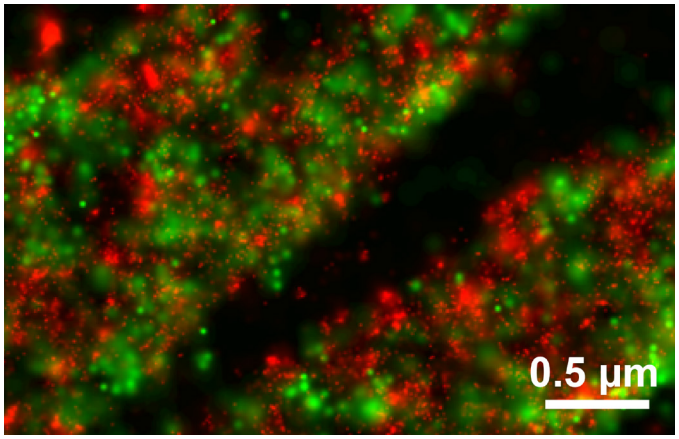

Figure 8 *Dual Color PALM of CHO cells (Chinese Hamster Ovary cells). Courtesy of H. Shroff & H. Hess, HHMI Janelia Farm, Ashburn, USA.*

COS-7

These cells are derived from kidney cells of African green monkey and also known as non-steroidogenic cells. There were established by Professor Yakov Gluzman in 1981. The acronym "COS" is derived from the cells being CV-1 (simian) in Origin, and carrying the SV40 genetic material. Three COS lines were created (COS-1, COS-3 and COS-7), of which two are commonly used (COS-1 and COS-7). In culture, COS-7 cells characteristically display adherent growth to glass and plastic surfaces and are fibroblast-like. The combination of fibroblastic-like growth and virus susceptibility make COS-7 a great choice for transfection experiments for DNA plasmids and mutations to the SV40 virus. The SV40 virus continues to be utilized due to its putative involvement in human cancers.

Figure 9 *Green Monkey* – Chlorocebus aethiops*; David Havel – stock.adobe.com*

Use

COS-7 cells are often used by biologists when studying the monkey virus SV40 and in transfection experiments to produce recombinant proteins for molecular biology, biochemistry, and cell biology research.

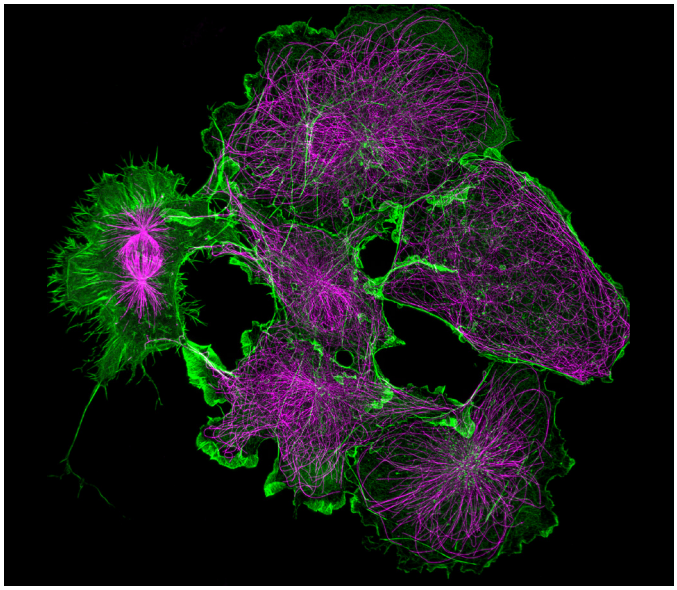

Figure 10 *COS cells labeled for actin (green) and microtubules (magenta). Sample courtesy of C. Leterrier, Institute of NeuroPhysiopathology, Faculty of Medical and Paramedical Sciences, Marseille, France*

MDCK

Madin-Darby canine kidney (MDCK) cells are a model mammalian cell line used in biomedical research. Following the initial isolation in 1958 of epithelial cells from the kidney tubule of an adult Cocker Spaniel dog by Stewart H. Madin and Norman B. Darby, Jr., the cell line bearing their name was employed primarily as a model for viral infection of mammalian cells. It is one of few cell culture models that is suited for 3D cell culture and multicellular rearrangements known as branching morphogenesis.

Use

MDCK cells are used for a wide variety of cell biology studies including

- cell polarity,
- cell-cell adhesions (termed adherens junctions),
- collective cell motility,
- as well as responses to growth factors.

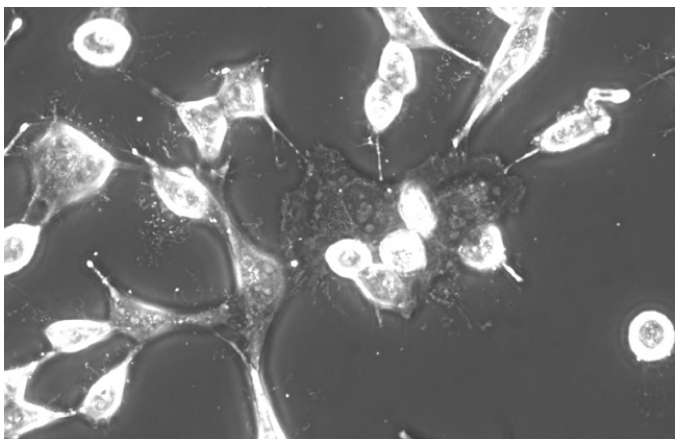

Figure 11 *DIC, MDCK cells (dog) – thick cell areas are displayed better using negative phase contrast. Courtesy of R. Nitschke, Life Imaging Center, University of Freiburg, Germany.*

References

[1] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5414769
[2] https://www.frontiersin.org/articles/10.3389/fcell.2021.711381/full
[3] https://www.bristol.ac.uk/research/impact/stories/hela-cells

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