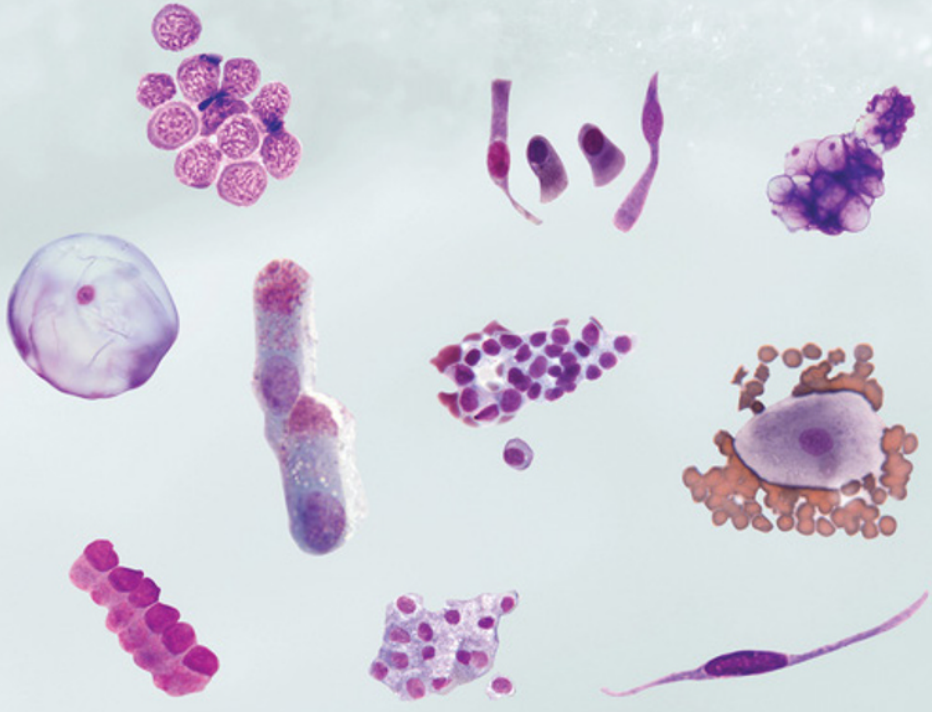
Normal Cell Morphology in Canine and Feline Cytology

AN IDENTIFICATION GUIDE

WRITTEN AND TRANSLATED BY
LORENZO RESSEL



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Edition History
Original title:
Principi d'identificazione morfologica in citologia nel cane e nel gatto – seconda edizione
© 2017, Poletto Editore srl, Via Corridoni 17, 20080 Vermezzo (Milan), Italy

Registered Offices
John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, USA
John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

Editorial Office
9600 Garsington Road, Oxford, OX4 2DQ, UK

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Library of Congress Cataloging-in-Publication Data

Names: Ressel, Lorenzo, 1979– author.
Title: Normal cell morphology in canine and feline cytology : an identification guide / written and translated by Lorenzo Ressel.
Other titles: Principi di identificazione morfologica in citologia nel cane e nel gatto. English
Description: Hoboken, NJ : Wiley, 2018. | Translation of: Principi di identificazione morfologica in citologia nel cane e nel gatto. 2010. | Includes index. | Description based on print version record and CIP data provided by publisher; resource not viewed.
Identifiers: LCCN 2017025396 (print) | LCCN 2017027036 (ebook) | ISBN 9781119278917 (pdf) | ISBN 9781119278900 (epub) | ISBN 9781119278894 (pbk.)
Subjects: | MESH: Dog Diseases–pathology | Cat Diseases–pathology | Cytodiagnosis–veterinary | Cells–cytology
Classification: LCC SF 991 (ebook) | LCC SF 991 (print) | NLM SF 991 | DDC 636.7/0896–dc23
LC record available at https://lccn.loc.gov/2017025396

Cover design by Wiley
Cover images: courtesy of Lorenzo Ressel

Set in 10/11 pt Optima LT Std by SPi Global, Pondicherry, India

10 9 8 7 6 5 4 3 2 1

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Foreword

Cytology for students, for clinicians or for diagnosticians? I'm sure this question often crossed the mind of the author, Dr. Lorenzo Ressel (a passionate devotee of the discipline, who I have the honor to call a colleague and friend), when he was thinking about the content, style and recipients of the present book, and hoping this would be the first in a long series.

There is a parallel universe, which belongs to the 'infinitely small', that is hidden and elusive, which is only unraveled by the use of a microscope, and that represents an irresistible call for those who are lucky to consider a passion and a job the very same thing.

Very easy to read, compact, useful and complete, this book sets as its first goal, for the student to draw the morphology of the cells in the mind, as they appear in the reality of that microscopic universe, and therefore to build solid foundations for their quick and secure identification. In the same way, the use of this book is also recommended to the professional cytologist, since it provides the instruments to 'scratch' the mnemonic rusts, which sometimes may compromise the ability to interpret and describe.

I followed the progressive development of this work and appreciated Lorenzo's efforts and commitment in his search for meticulous precision and attention to detail, as well as his enthusiasm during the realization of the book.

I'm sure now the answer to my first question is: 'cytology for those who love cells'.

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Introduction

The book you hold in your hands is not a classic text of diagnostic cytology of the dog and cat. The starting point is not the needle and the goal is not the diagnosis, but it continues to 'go in circles' around cells.

Due to this particular feature, it could be considered as something preparatory to diagnostic cytology activity and, predominantly, it has been precisely designed for this purpose: to give a comprehensive but original approach to the study of normal cells for the veterinary student interested in diagnostic veterinary cytology, hoping to fill the gap between the first year courses on cell biology, and final year's clinical pathology rotations. I think, however, this book will also find a place close to the microscope of the novice practising veterinary cytologist, when having 'easy-to-use' information to hand is the key to correct interpretation and diagnosis.

A first chapter, '*Cellular biology and cytological interpretation: the philosophy behind the system*', discusses the principles of morphological identification, trying to clarify the relationship between shapes, patterns and colours and the associated interpretation of cell origin and behaviour.

The second chapter, '*Distribution of cells in tissues and organs*', aims at clarifying which cells can typically be sampled from the different tissues and organs. Figures showing the location of different cell types in the context of the histological structures of organs guide the reader to an easy identification.

The third chapter, '*Cytotypes*', is the heart of the book: different cell types from the various organs and tissues are presented as 'identification sheets', arranged in alphabetical order. The cells' characteristics are systematically described in this chapter.

Chapter 4, '*Cytoarchitectures*', classifies the different morphologies that groups of cells form (or maintain from the original tissue arrangement) when sampled and subsequently smeared over the slide.

The fifth chapter, '*Background*', discusses the non-cellular material that may be observed alongside cells, and, in some cases, can be peculiar to a particular cytotype.

The sixth chapter, '*Morphological alterations of cells*', introduces the different cellular morphological alterations which can be observed in different pathological changes, such as degeneration and disturbances of tissue growth.

At the end of the book, instead of a traditional index, there is a unique '*Visual index*', in which the cytotypes (previously described in the third chapter) are presented together, to scale, to give the reader a quick, visual identification approach.

Cellular biology and cytological interpretation: the philosophy behind the system

Shape and observation

Aside from the mere pleasure of observation, an activity that is in its own way rather satisfying, the ability to extract information from the object observed is based on the axiom that different shapes and colours (of the object observed) correspond to different information.

This concept is at the heart of diagnostic cytology. The person who observes the cells on the slide (the cytologist) can use the morphological features of the cell observed (shape and colour) to classify it and interpret its characteristic biological behaviour.

Morphology, identity and behaviour

If properly interpreted, the different shapes and colours of a cell can provide information about its metabolism and differentiation. Indeed, specific chromatic features of the cytoplasm may indicate a particular cell's metabolic condition. Moreover, certain visible structures can tell us that a cell is dividing (e.g. the presence of a mitotic figure), or that it is undergoing phagocytosis (e.g. the presence of material within the cytoplasm). There are also morphologies that suggest no immediate functional interpretation. Such morphologies are 'structural' and connected to a specific type of cell (e.g. the polylobed nucleus of *neutrophils*).

It is also true that certain cell types, due to their ability to carry out a highly specialized and predetermined function (differentiation), have 'acquired' certain morphological features that make them unique and recognizable from other cells. Examples are *plasma cells*, which, due to their constant protein synthesis, display an intensely blue cytoplasm, or, *macrophages*, whose vacuole-containing cytoplasm is a distinctive feature, as well as an expression of phagocytosis.

This goes to show how from a plethora of shapes one can understand both the 'type of cell being observed', and, at times, 'what it is doing'.

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First Edition. Written and translated by Lorenzo Ressel.

Identity and interpretation

The observation of cellular morphology allows classification of cells into different 'cytotypes', i.e. it enables the cytologist to classify them into a specific category. In diagnostic cytology, it is common to classify cells into three morphological families: epithelial, mesenchymal and discrete, otherwise known as round cells.

Traditionally, these three morphological groups are defined as follows.

- Epithelial cells – usually large and round or polygonal in shape, with readily identifiable cellular margins. These cells usually form clusters, which are aggregates of cells that establish contact by means of membrane-to-membrane adhesion.
- Mesenchymal cells – usually of medium size, they appear elongated, spindle-shaped or pleomorphic. These cells may form aggregates of cells through interposition of matrix.
- Discrete cells – usually small and round, they do not establish contact with each other.

With some obvious exceptions, this classification system is of great diagnostic value, hence, the above-mentioned terms will be referred to several times throughout this book. Within these categories, subtle differences in shape, size, presence or absence of certain structures, location of the nucleus and other significant areas often allow classification of cells into specific 'cytotypes', in other words a cell that *Homo sapiens* has dignified with a name and surname. For example, the ability to selectively identify various cytotypes is crucial to tests such as differential and absolute cell counts, which often provide valuable diagnostic information.

Behaviour and interpretation

The adaptability of cells to outside stimuli or modifications of the environment (hormones, maturative stimuli, etc.) induces the same cytotype to modulate the specific morphological features (shape and identity) it 'normally' displays as it adapts to a new function. The evaluation of these changes, which are compared to the normal morphology of the cytotype (defined by its shapes and colours), allows a higher level of understanding compared to the more basic cellular identification: the cell's metabolic status. This, in turn, has important repercussions on cytological diagnosis, especially in the field of oncology. The variation of these features within a specified *range* will be considered within the normal limits of the phenotype, but a phenotype that is particularly active or reactive will exhibit morphological features far beyond such limits.

Knowledge and interpretation

Each of the features observed in a cell provides specific information that can help both cytotype identification and functional assessment. These features and their biological significance are discussed here individually, in detail. The combination of more features characterizing different cell types will be dealt with in Chapter 3, Cytotypes. The various morphological features observed have been divided into

cellular morphologies, nuclear morphologies, cytoplasmic morphologies and supercellular morphologies (those shapes that are determined by the connections between cells).

Cellular morphologies

'Cellular morphology' refers to the set of morphological features (shape and colour) of a cell as a whole. The features considered are:

- size;
- shape;
- nuclear:cytoplasmic ratio;
- presence of certain specialized structures.

Size of cells

The size of cells can vary greatly. It ranges from an erythrocyte 6 or 7 μm in diameter, up to a rhabdomyocyte of several hundred microns. Except for those cells whose size can vary greatly because of their specific activities (for example, macrophages can increase their size due to the accumulation of phagocytosed material), the size of a cell is often a useful tool to identify the cytotype.

Usually, very large cells in normal conditions are actually *syncytia*: several cellular bodies merging into one unique cytotype, which is characterized by the presence of multiple nuclei in the cytoplasm.

Cellular shape

The shape of a cell, determined by its margins, can provide valuable information about its classification. In general, a well-defined and repeatable cellular shape is given by its cytoskeleton, which determines the shape when the cell itself is originally located within the tissue and/or organ of origin. On the contrary, so-called *pleomorphic* cells indicate a more plastic cytotype. They will therefore feature a less rigid morphology, which is not characteristic or indicative of the original tissue. Consequently, when a cell is classified as pleomorphic, it is assumed that, within the cytotype to which it belongs, it will not have a specific and repeatable shape.

There are several cells that, within a given cytotype, maintain a typical morphology. A possible classification by shape would include round, ovoid, columnar, fusiform/spindle, cubic, polygonal, star-shaped and pear-shaped cells (Figure 1).

Nuclear:cytoplasmic (or nucleus to cytoplasm) ratio

The nuclear:cytoplasmic ratio determines how much cellular area is occupied respectively by the nucleus and the cytoplasm. A cell whose nucleus occupies almost the entire cellular area is typically at an immature cellular stage. During maturation, cytoplasmic structures (capable of performing different functions), gain space and tend to equalize such area (1:1 ratio) or exceed it. However, there are cases in which cells considered mature retain a high nuclear:cytoplasmic ratio (for example, mature lymphocytes). This feature has, in physiological terms, a major

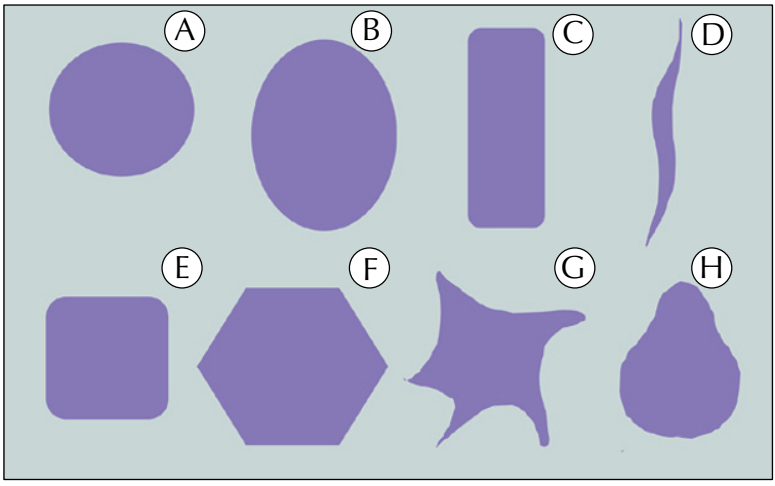

Figure 1 - Schematic representation of the most important cellular morphologies: round (A), ovoid (B), columnar (C), fusiform/spindle (D), cuboidal (E), polygonal (F), star-shaped (G) and pear-shaped (H).

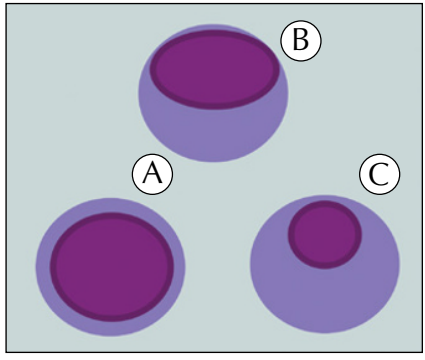

Figure 2 - Schematic representation of the nuclear:cytoplasmic ratio: high (A), equal/intermediate (B) and low (C).

impact on the identification of the cytotype. The implications of this morphological feature with regard to pathological conditions will be dealt with in Chapter 6, Morphological alterations of cells.

The nuclear:cytoplasmic ratio is typically classified as *high* (the nucleus takes up most of the cellular area), *equal/intermediate* (nucleus and cytoplasm occupy approximately the same amount of cellular area) and *low* (the cytoplasm takes up most of the cellular space) (Figure 2). Classifications using numerical values are less typical.

Specialized cellular structures

Some cells have specialized cellular structures, a sign of their specific functional differentiation. Specialized cellular structures are cilia, flagella, microvilli, basal plates, etc. (for more information, see Chapter 3, Cytotypes). These are proof of cellular differentiation, and are thus crucial features for recognizing a cytotype.

Nuclear morphologies

'Nuclear morphologies' are those morphological features that affect the nuclear and subnuclear structures (e.g. chromatin and nucleolus). The following characteristics of the nucleus are examined:

- shape;
- position;
- number;
- chromatin patterns;
- nucleolus;
- mitotic figures.

Shape of the nucleus

The nucleus can take different shapes within different cells. This criterion is extremely useful for identification of a particular cytotype. There are nuclear morphologies that are typically associated with a particular cytotype and are not known to be correlated with a particular metabolic state. Generally, they remain unchanged within a cell despite stress of a metabolic nature (e.g. in mature *neutrophils*, the lobulation is generally not lost in cases of stress).

Morphological changes of the nucleus (belonging to a single family of cells) are often a sign of cell maturation. Also in this case, it seems only natural and strategic to classify consecutive and well-identifiable maturation stages by using different names (*cytotypes*) despite merely being different 'ages' of the same cell (e.g. *myeloid* and *erythroid* cells). And that is how we go back to the starting point: the morphology of the nucleus helps to identify cytotypes.

There are several nuclear morphologies: round, oval, spindle-like, kidney-shaped, horseshoe-shaped, indented, bilobed, S-shaped, convoluted and polylobed (Figure 3).

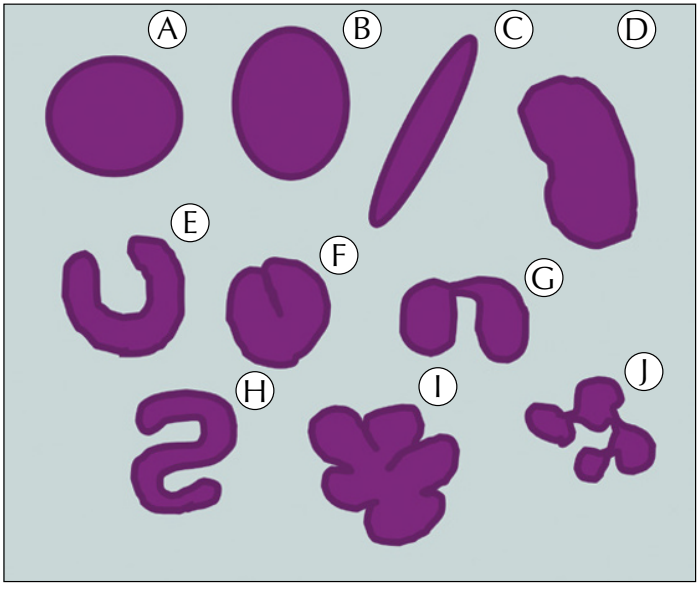

Figure 3 - Schematic representation of nuclear shapes: round (A), ovoid (B), fusiform (C), kidney-shaped (D), horseshoe-shaped (E), indented (F), bilobed (G), S-shaped (H), convoluted (I) and polylobate (J).

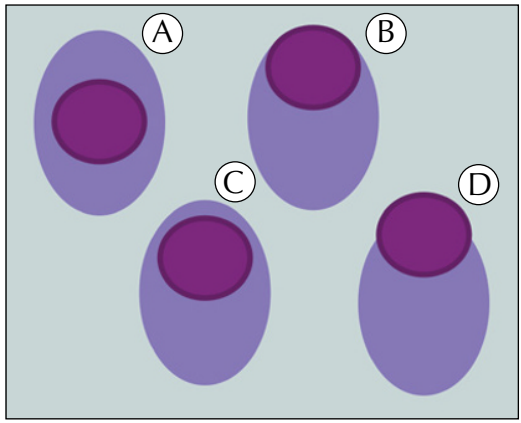

Figure 4 - Schematic representation of the cell nucleus position inside the cell: central (A), paracentral or subterminal (B), peripheral (C) and 'punched out' (D).

Location of the nucleus

The location of the nucleus is another strong indicator when determining a cytotype. The reason why the nucleus is located nearer one of the margins of the cell, and not in the centre, has to do with the fact that the cell has a so-called 'functional polarity', which leads it to amass its cytoplasmic structures on one side, resulting in the formation of a 'pole'.

The shift of the nucleus to the periphery of the cell, can by either due to a constituent cytoplasmic structure increasing in size (rough endoplasmic reticulum and the Golgi apparatus in *plasma cells*) or the result of a material waiting to be expelled (sebum granules in a *sebocyte*) or material that has been phagocytosed (grains of haemosiderin in the *haemosiderophage*).

The positions of the nucleus can be classified into central, paracentral or subterminal, peripheral and punched out (Figure 4).

Number of nuclei

Usually, cells have a single nucleus. However, there are special cases in which there may exist binucleate, multinucleate as well as anucleate cells (Figure 5). In mammals, an example of anucleate cell is the *erythrocyte*, in which extreme differentiation has led to the loss of the nucleus. Physiologically, multinucleation occurs when different cells of the same type merge (syncytium), however, it may also occur in conditions of inefficient cytokinesis (plasmodium). A syncytium is clearly the result of a particular cellular activity of some cytotypes (e.g. *the inflammatory giant cell*). Imperfect cytokinesis is more frequently observed in the context of pathological changes. It is a mechanism through which binucleate, rather than multinucleate, cells are typically formed. This mechanism may represent an atypical character, which will be dealt with later in this book (see Chapter 6, Morphological alterations in cells).

Chromatin patterns

Chromatin is a collection of genetic material (DNA) of cells. It also includes proteins which assist supercoiling and structural maintenance of DNA. During the resting