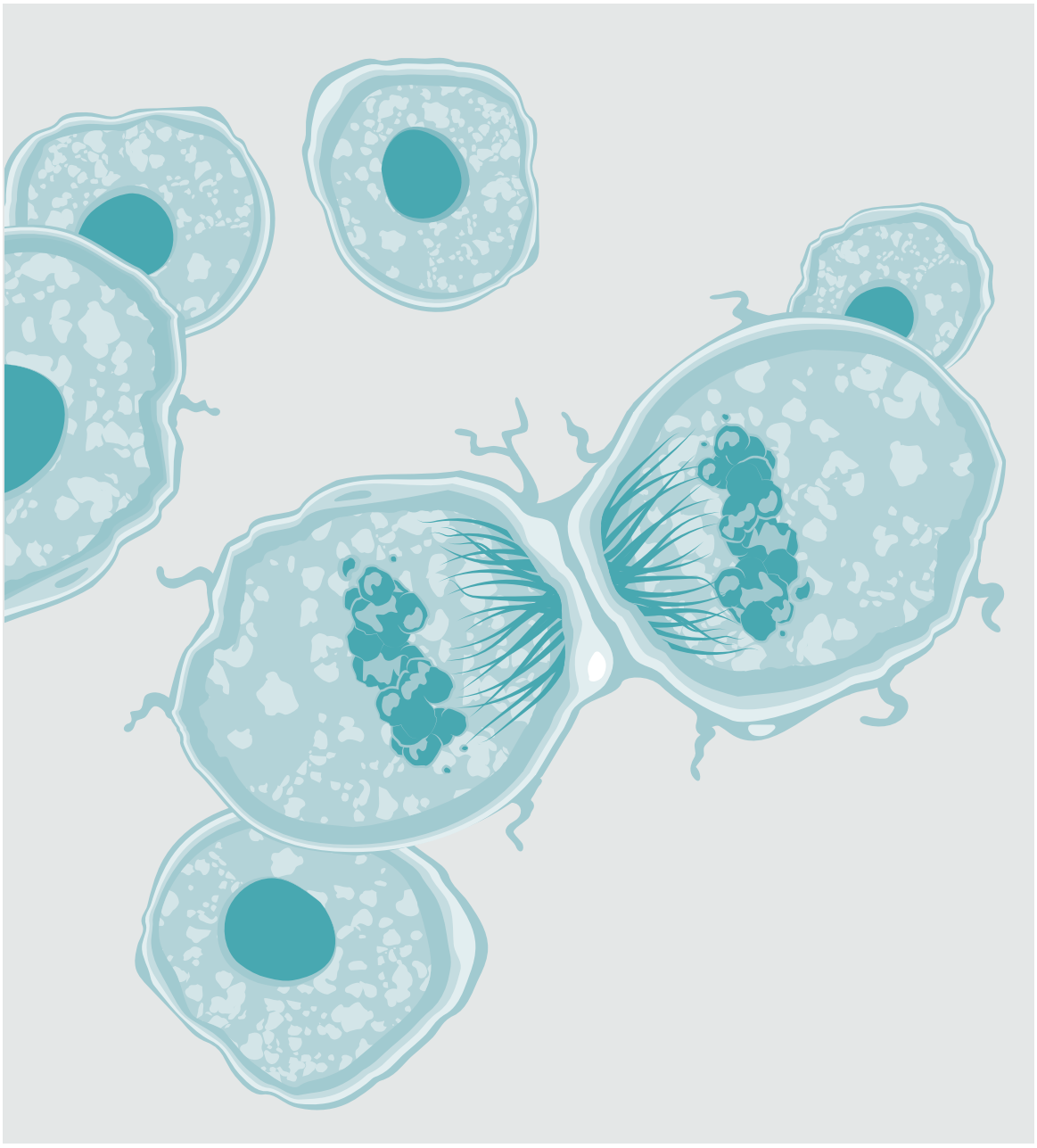
The cell proliferation guide



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The cell proliferation guide

All of the tools and techniques you need to stain and score cell proliferation.

Cell proliferation can be used to assess normal cell health, to measure responses to toxic insult, or as a prognostic and diagnostic tool in several cancers. The available markers typically look at DNA levels or synthesis, cellular metabolism, or proliferation-specific proteins.

This guide highlights the most common methods to mark and score cell proliferation.

Contents

Identifying proliferating cells

Below are some of the best methods used to study cell proliferation. We've highlighted in teal our recommended techniques for each method type.

For investigating cell proliferation in fixed samples, we suggest using Ki67 because it is well-established and highly-cited across both the basic and clinical research areas. MCM-2, another proliferation marker, is steadily gathering data around its use a prognostic marker in certain cancers, making this something to pay attention to as the research continues. For live cells, EdU is the preferred choice.

Method	Marker	Use and benefits	Limitations	Products
DNA synthesis	**BrdU**	Immunoassay to quantify cells in G1, S, and G2/M Trace cell cycle kinetics	Requires DNA denaturation, impairing co-staining and disrupting DNA morphology Complex protocol	**BrdU (5-bromo-2'-deoxyuridine) (ab142567)** **Anti-BrdU antibody [BU1/75 (ICR1)] (ab6326)** **Anti-BrdU antibody - Proliferation Marker (ab1893)**
	IdU & CldU	Immunoassay to study DNA replication fork progression rates, stability or origin firing Two dyes (against IdU and CldU) allow more complex experiments than with a single dye	Requires DNA denaturation, impairing co-staining and disrupting DNA morphology Complex protocol	**Idoxuridine (ab142581)** **Anti-BrdU antibody [BU1/75 (ICR1)] (ab6326)** **Anti-IdU antibody [32D8.D9] (ab181664)**
	EdU	Immunoassay to quantify cells in G1, S, and G2/M Trace cell cycle kinetics Simple protocol, without DNA denaturation	Can be expensive	**5-Ethynyl-2'-deoxyuridine (5- EdU) (ab146186) BDP FL alkyne (ab146583)** **EdU Proliferation Kit (iFluor 488) (ab219801)**

	MTT	Biochemical assay to indirectly quantify proliferating (respiring) cells Simple method	Toxic to cells Insoluble in water – needs to be dissolved in a solvent Endpoint measure only Metabolic assays may not accurately represent changes in cell growth	**Thiazolyl blue tetrazolium bromide (MTT) (ab146345)**
Cellular metabolism	**XTT**	Biochemical assay to indirectly quantify proliferating (respiring) cells Simple method More sensitive than MTT	Sensitivity varies Metabolic assays may not accurately represent changes in cell growth	**XTT sodium salt (ab146310)**
	WST-1	Biochemical assay to indirectly quantify proliferating (respiring) cells Simple method More sensitive than MTT and XTT	Metabolic assays may not accurately represent changes in cell growth	**WST-1 Cell Proliferation Reagent (ready to use) (ab155902)**
	PCNA	Immunoassay to detect cells mainly in late G1 and S phases. Prognostic value in some cancers	Scoring is subjective Can be less sensitive and specific than Ki67 methods	**Anti-PCNA antibody [PC10] (ab29)**
Proliferation proteins	**Ki67**	Immunoassay to detect cells in G1, S, G2 and M Prognostic and diagnostic value in some cancers Huge body of supporting evidence	Scoring is subjective Can be less sensitive and specific than MCM-2 in some cancers.	**Anti-Ki67 antibody (ab15580)** **Anti-Ki67 antibody [SP6] (ab16667)**
	MCM-2	Immunoassay to detect cells in G1, S, G2 and M Prognostic and diagnostic value in some cancers	Scoring is subjective	**Anti-MCM2 antibody (ab4461)** **Anti-MCM2 antibody [EPR4120] (ab108935)**

DNA synthesis

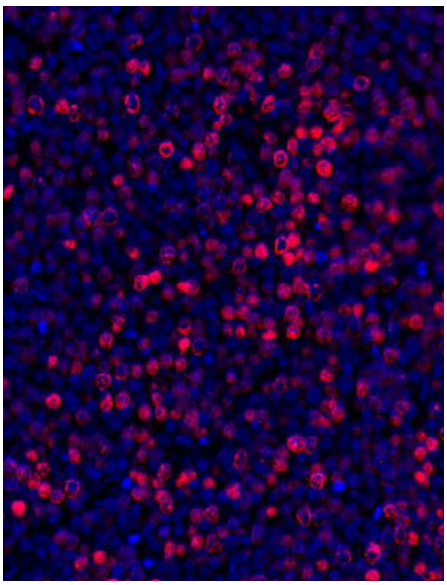
The most reliable and accurate method of assessing cell proliferation is a measurement of DNA-synthesizing cells. This relies on incubating live cells with compounds capable of being incorporated into newly synthesized DNA. These compounds can then be detected with a reporter.

Thymidine analogs are the compound of choice to be incorporated into DNA, substituting thymidine during DNA replication. However, it is important to be aware that these thymidine analogs can lead to mutations and DNA damage in some instances and thereby affect the cycle cycle[1,2].

This method is suitable for immunohistochemistry (IHC), immunocytochemistry (ICC), ELISA, flow cytometry, and some multiplex assays. Combining IdU and ClDU allows for time course studies when studying DNA replication by sequential labeling.

- Accurate and reliable
- High-and low-throughput options
- x Protocol can be lengthy
- x DNA denaturation prohibits subsequent co-staining experiments (not a concern with EdU)

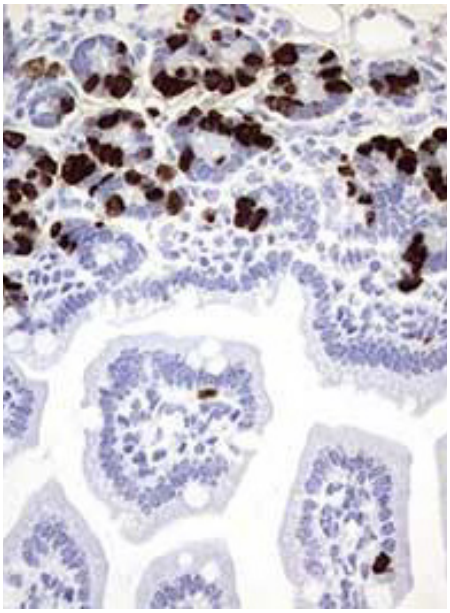
BrdU



- 5-bromo-2'-deoxyuridine (BrdU) is a thymidine analog that is incorporated into newly synthesized DNA
- Labels proliferating and daughter cells
- Detected by staining with anti-BrdU antibodies
- Can be used to accurately quantify the percentage of cells in G1, S, and G2/M, and trace cell cycle kinetics
- Requires DNA denaturation (DNase, heat, or acid) to allow antibody access to BrdU
- This disrupts DNA morphology and can destroy recognition antigens, impairing subsequent co-staining procedures

Opposite
Immunohistochemical analysis of formalin/PFA-fixed paraffin-embedded sections of Ramos cell line xenograft tissue sections using an anti-BrdU antibody **(ab1893).**

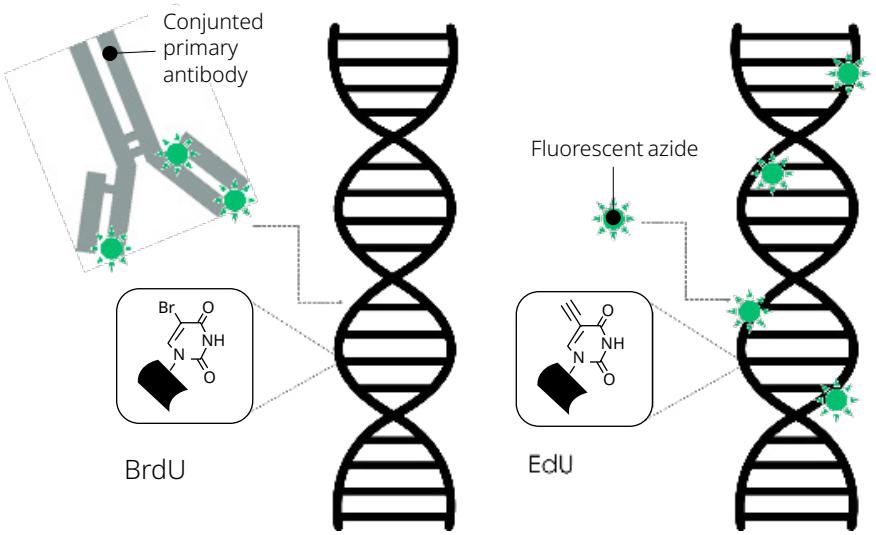
IdU and CldU



- 5-Iodo-2′-deoxyuridine (IdU) and 5-chloro-2′-deoxyuridine (CldU) are both thymidine analogs that are incorporated into newly synthesized DNA
- Labels proliferating and daughter cells
- Ideal for time course studies
- Can be used to study DNA replication fork progression rates, stability, or origin firing by sequential labeling with CldU and IdU
- Detected by staining with anti-BrdU or anti-IdU antibodies
- Note that anti-BrdU antibodies cross-react with CldU (but not IdU) and some with IdU (but not CldU). These should not be used in conjunction with BrdU
- Requires DNA denaturation (DNase, heat, or acid) to allow antibody access to BrdU
- This disrupts DNA morphology and can destroy recognition antigens, impairing subsequent co-staining procedures

Opposite
Immunohistochemical analysis of paraffin-embedded colon tissue from IdU injected mouse, labeling IdU with an anti-IdU [2F8] antibody (ab187742).

EdU



- 5-ethynyl-2′-deoxyuridine (EdU) is a thymidine analog that is incorporated into newly synthesized DNA
- Labels proliferating and daughter cells
- Can be used to accurately quantify the percentage of cells in G1, S, and G2/M
- Unlike BrdU, IdU, and CldU, EdU detection used 'click' chemistry rather than the addition of a detection antibody
- EdU's ethynyl group covalently crosslinks with a fluorescent azide (eg an Alexa Fluor®), which is small enough to diffuse freely through native tissues and DNA
- DNA does not need to be denatured, meaning EdU can be used in subsequent co-staining experiments
- Simplified protocol due to lack of antibody and denaturation steps

Above
BrdU assays (left) needs the DNA to be denatured in order to allow an anti-BrdU primary antibody access to the BrdU molecule. EdU assays (right) rely on 'click' chemistry, in which the fluorescent azide can freely bind the EdU molecule.

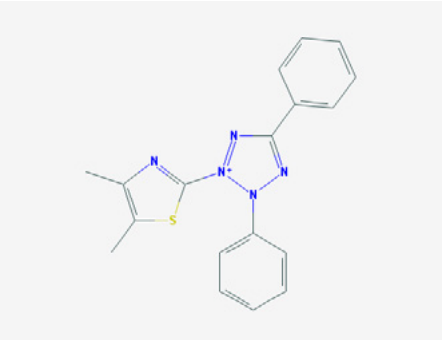
Cellular metabolism

Rather than looking at DNA synthesis, it is possible to assay cell proliferation by measuring the metabolic activity of your cells in culture via tetrazolium salts. These salts form a dye when present in a metabolically active environment. The resulting color change of the media can be quantified in a spectrophotometer, giving an indication of the extent of proliferation.

Although sensitive, some of these salts are insoluble in normal culture medium, and the dye crystals often need to be dissolved in a solvent like DMSO or isopropanol. However, others are soluble in culture medium and nontoxic.

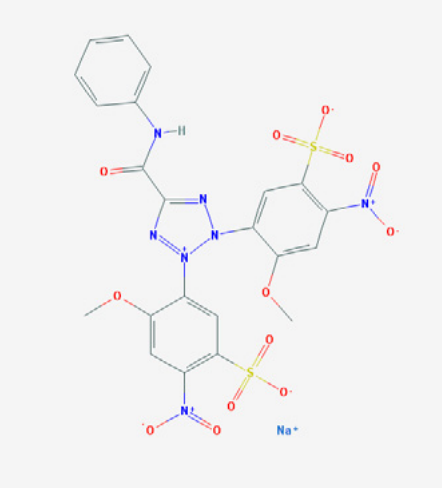
- Accurate to varying degrees
- High- and low-throughput options
- Protocol is simple
- x Some dyes require toxic solvents
- x Metabolic assays may not accurately represent changes in cell growth

MTT



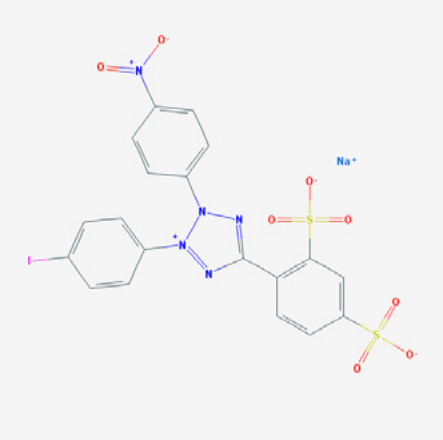
- 2-(4,5-Dimethyl-2-thiazolyl)-3,5-diphenyl-2H-tetrazolium bromide (MTT)
- MTT is soluble in water
- Respiring cells convert MTT to a purple formazan dye
- Resulting dye is insoluble in water
- Primarily an endpoint measurement due to needing to dissolve the dye crystals in a solvent

XTT



- 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide (XTT)
- XTT is soluble in water
- Respiring cells convert the XTT to an orange colored formazan dye
- Resulting dye is soluble in water
- No solubilization required prior to quantification
- Sensitivity equal to or better than that of MTT

WST-1



- Water-soluble tetrazolium salt-1 (WST-1)
- Respiring cells convert WST-1 to a dye that is measured at OD420–450
- Resulting dye is soluble in water
- More sensitive than MTT, XTT or MTX
- Assay can be performed in the sample microtiter plate
- No additional steps like washing harvesting or solubilization

Proliferation proteins

Another method to study cell proliferation is by looking at specific proteins that are expressed in proliferating cells, but absent from non-proliferating cells. This requires the use of specific primary antibodies against the antigens expressed during proliferation.

These antigens are typically expressed in the perinuclear or nuclear interior regions across all cell cycle phases except G0, making them excellent cellular markers for proliferation. Ki67 a very popular proliferation marker and is routinely used in pathology labs due to its diagnostic and prognostic power in cancer. PCNA is another common marker, yet multiple studies have shown that Ki67 is more sensitive and specific when evaluating cell proliferation in tumors from various origins[3–6]. A maker growing in prominence is MCM-2, and recent work suggests this may be a better choice for cancer prognoses than Ki67 and PCNA[7,8].

However, much of the data is inconclusive regarding the 'best' maker of proliferation, especially in a clinical context.

These immunoassays are excellent for fixed tissue samples and analysis by IHC.

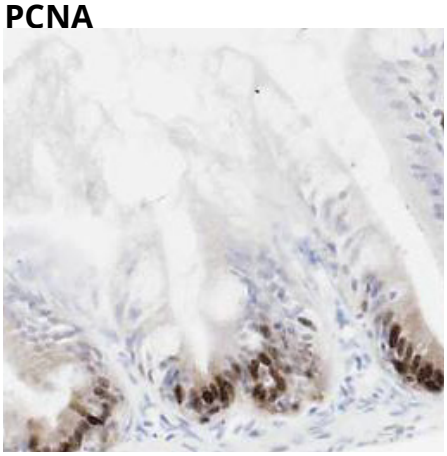
- Accurate and reliable
- Large body of supporting data
- Clinical diagnostic and prognostic value in some cases

x Limited high-throughput options

x Scoring of results can be subjective

x Conflicting data around the 'best' marker of cell proliferation in a clinical setting

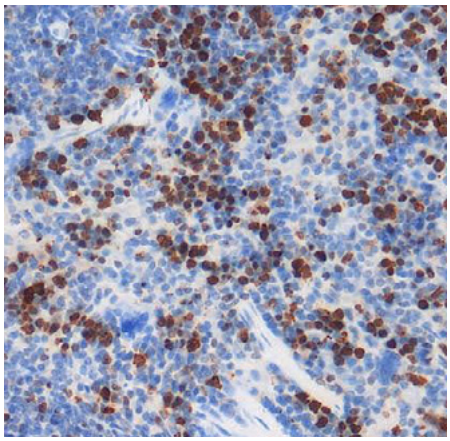
PCNA



- Proliferating cell nuclear antigen (PCNA) is expressed mainly in late G1 and S, to a lesser extent in S and G2, and low or absent in G0 and early G1
- Widely used general cell proliferation marker[9]
- Reported prognostic significance in certain cancers
- Results relate only the number of proliferating cells, not the rate of proliferation

Opposite
*Immunohistochemical analysis of frozen sections from adult zebrafish intestine, labeled with an anti-PCNA antibody [PC10] (***ab29***).*

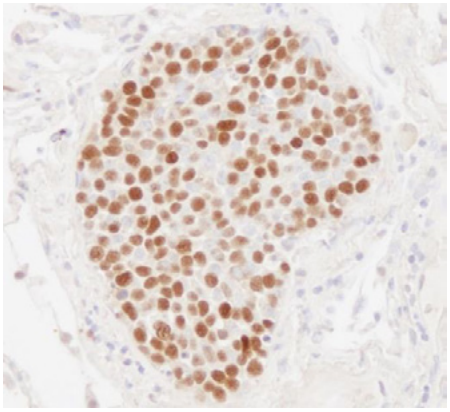
Ki67



- Ki67 nuclear antigen is expressed in the cell cycle phases G1, S, G2, and M, but is absent in G0
- Ki67 index is widely used as a tumor marker in research and pathology
- Prognostic and diagnostic value in many cancers[9]
- Ki-67 index correlates with the course of neoplastic disease and can be used to assess patient survival and cancer progression
- Results relate only the number of proliferating cells, not the rate of proliferation
- Often more specific than PCNA[6]

Opposite
*Immunohistochemical analysis of formalin/PFA-fixed paraffin-embedded sections from mouse spleen, labeled using an anti-Ki67 antibody (***ab15580***).*

MCM-2



- MCM-2 plays a major role in DNA replication during G1, and is expressed throughout all phases except for G0
- Widely used as a proliferation marker
- Prognostic value in certain cancers
- May be a better than Ki67 to evaluate the progression of some cancers in certain cases[7,8,10]

Opposite
Immunohistochemical analysis of formalin/PFA-fixed paraffin-embedded sections from human small cell lung cancer tissue, labeled with an anti-MCM2 antibody (**ab4461**).

Scoring proliferating cells

Scoring the extent of proliferation is especially important in a clinical setting. The percentage of Ki67-postive cells, for example, can be used to score the severity and course of cancer. There are several techniques available for use with the proliferation proteins methods, each with their own strengths and limitations. We've highlighted in teal our recommended technique for scoring cell proliferation via IHC.

Method	Time (minutes)	Practicality	Accuracy	Extra costs
'Eye-balling'	<1	Highest	Very low	None
Eye-counting on a microscope	~5	Low	High	None
Manual counting from an image	~10	Very high	Highest	None-to-moderate (high-quality camera and printer)
Automated counting: microscope	~5	Low	Moderate	High
Automated counting: software	~3	Moderate (requires knowledge of software plugins)	Moderate	None

Modified from Reid et all. (2015)[11].

'Eye-balling'

This involves looking at a slide under a microscope, typically at a relatively low power (x10 objective), and estimating the percentage of proliferation-positive cells. This does not involve any counting of individual cells.

While this method is widely used, quick, cheap, and advocated by some guideline papers, it remains a generally inaccurate method.

Eye counting with a microscope

This method consists of 'real-time' counting of proliferation-positive cells under a microscopic at an intermediate power (x20 objective), focusing on identified 'hot spots' (areas containing a large number of proliferation-positive cells).

This method can involve the use of grids and other counting tools frequently seen in pathology labs. However, even with the aid of such tools, this method can lead to errors due to counting the same proliferation-positive cells more than once.

Manual counting of camera-captured/digital image

Like eye counting with a microscope, this is a manual process but involved looking at either a printout or a screen capture of a section previously visualized with the microscope. This is typically done under low power (x10 objective). Reviewers then manually mark proliferation-positive cells on a physical print-out, or on the screen using simple software.

Counting this manner is very convenient and allows reviewers to easily avoid duplicate scoring.

Automated counting

This is divided into using an automated counting microscope, and using software, such as ImageJ, to analyze captured images. Both methods automatically score proliferation-positive cells from manually-selected hot spots.

Using software to manually count proliferation-positive cells requires either knowledge of plugin design (for software like ImageJ) or dependence on external programs hosted online (eg from the National Institutes of Health website).

Automatic counting microscopes can often require extensive calibration, and some struggle to score partial staining. These are also very expensive.

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