Mitotic Count and Mitotic Figure Morphology Quick Reference Guide (QRG)

Introduction: Counting mitotic figures (MF) in tumors is one of the most widely used methods of predicting tumor behavior. The mitotic count (MC)* is a rapid, inexpensive test that can be performed by any pathologist, is part of many grading schemes, and aids in clinical prognostic decisions. The methods described in references 1 and 2 are summarized here. The methods are designed to be as detailed and as standardized as possible, however, there are practical issues that prevent full standardization. In these instances, explanations and/or disclaimers can be provided (see www.vcgp.org, referenced below).[1,2]

***Note**: "Mitotic index" is the incorrect term for this method. The term mitotic count should be used.

Counting Mitotic Figures:

1. **Determine the area of highest mitotic activity (e.g. hotspot)**: With glass slides or whole slide images (WSI), at a low magnification, scan the histologic section to identify hot spots of high mitotic activity. Choose viable cellular regions of the neoplasm. **Avoid regions that are cell poor** (e.g. hemorrhage, edema, necrosis, cysts, inflammation, autolysis). Use a magnification appropriate for identifying MF; most pathologists count at 400x magnification.

2. Within identified hotspots: **define a contiguous field of view (FOV) in an area of 2.37mm².** With a light microscope at 400x magnification with an ocular FN of 22 this is 10 FOVs.

3. Recommendation is to count both MF and atypical MF (AMF) in an area totaling 2.37 mm². **The total sum of MF + AMF in 2.37mm2 is the MC**.

4. Report MC as: Number within specified area* (example, 15 in 2.37mm^2)

***Note:** The term high-power field (HPF) is not a standard unit of area and therefore should not be used to report the MC.[1,5] This is especially important as digital pathology becomes more prevalent, HPF does not have any meaning in the context of whole-slide images (WSI).

What to Avoid when Counting Mitotic Figures:

1. DO NOT count mitotic-like-figures (MLF) or prophase

2. DO NOT report per 10 HPF, as HPF is not a standard unit of area (one HPF at the same magnification can vary up to 200% based on the FN of the ocular[1])

3. DO NOT report as mitotic index, as this is the incorrect term for this method. Report as mitotic count.

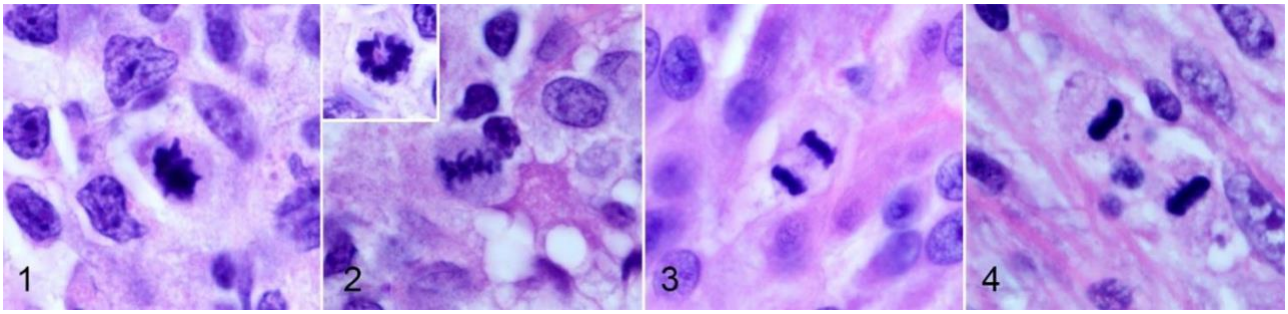
Practical Issues with Performing Mitotic Count:

- What if the MC is close to a prognostically significant "cut off"?
  - Recommendation:[1] Repeat the MC procedure in different hot spots (additional 2-5 if possible); report the highest MC.
  - Consider: Evaluate other (clinical, histological, immunohistochemical, molecular, etc.) parameters when the MC is close to a reported cutoff.
- What if the specimen size is smaller than 2.37 mm^2?
  - Recommendation:[1] Count all MF + AMF and report total sum in area counted (in mm^2).
  - Consider: Recut sections and/or /additional sections or extrapolate to approximate an area of 2.37mm^2. Add explanation or disclaimer detailing these steps.
- What if the specimen has numerous cell-poor spaces (vascular, ducts, acini, desmoplasia, tumor matrix, necrosis)?
  - Recommendation:[1] Skip over cell-poor spaces and resume contiguous field counting when viable tumor is present in FOV. May need to add small areas until an area equaling 2.37mm^2 is reached.

Mitotic Figure Morphology:

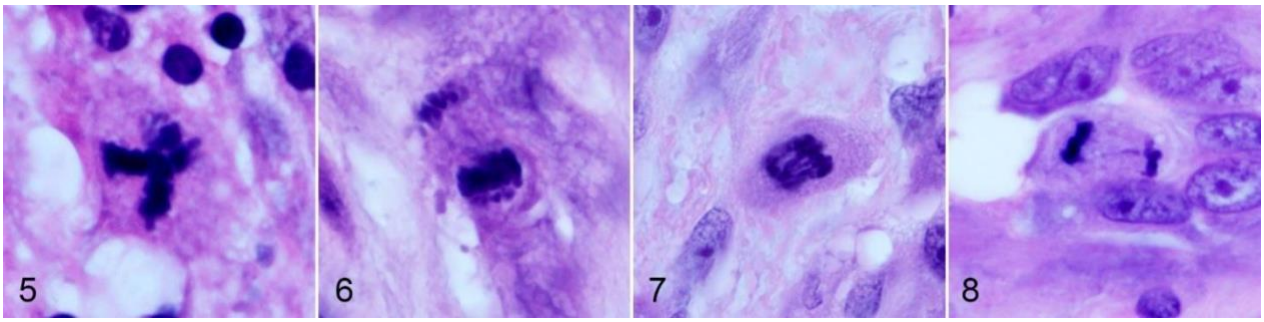
Mitosis is the process of karyokinesis while MF are the structures that can be identified with light microscopy. The morphologies of MF and AMF will vary with the phase of mitosis and the transitions between phases such that the nuclear chromatin comprises different shapes and staining characteristics.[2,3,4] MF are counted from prometaphase through telophase (count telophase as one). **MF have the following characteristics:**[2]

Normal MF:



Prometaphase	**Metaphase**	**Anaphase**	**Telophase (1 MF)**
Central dark aggregate	Linear or ring shaped	2 separated aggregates	Separated aggregates
Spikes/projections	Spikes/projections	Distances variable	Cleavage furrow

Atypical MF (AMF):



Multipolar More than 2 spindle poles in any phase	**Asymmetrical bipolar** Unequal size of chromosome clusters	**Chromosome Bridging** Chromosomes stretching from one cluster to opposite pole	**Chromosome Lagging** Fragments not in contact with cluster

Practical Issues: Differentiate MF from Mitotic-Like-Figures (MLF)[2,3]

1. Surface contour: MLF are smooth, MF have projecting rods/spikes.
2. Context: In areas of poor cell preservation, MLF may be more likely. In areas of high mitotic activity with good cell preservation, MF may be more likely.
3. Cytoplasm color (not reliable). MF tend to have more amphophilic/basophilic cytoplasm (increased RNA), MLF tend to have more eosinophilic cytoplasm.

SELECTED REFERENCES:

1. Meuten, D.J et al. Mitotic Count Guideline, version 1.0. Veterinary Cancer Guidelines and Protocols. http://vetcancerprotocols.org. Accessed on 10/8/21.
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3. Tvedten H. Atypical mitoses: morphology and classification. Veterinary Clinical Pathology. 2009;38: 418-420.
4. Donovan TA, Moore FM, Bertram CA, et al. Mitotic Figures-Normal, Atypical, and Imposters: A Guide to Identification. Vet Pathol. 2021;58: 243-2576.
5. Bonert M, Tate AJ. Mitotic counts in breast cancer should be standardized with a uniform sample area. Biomed Eng Online. 2017;16: 28.